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Progress in the Chemistry of Organic Natural Products

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L. Zechmeister

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The Trichothecenes and Their Biosynthesis

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Synthesis Pathways to *Erythrina* Alkaloids and *Erythrina* Type Compounds

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Contents

1. Introduction

The history of erythrina research begins at the end of the 19th century. During the last two decades of that time extracts from species of *Erythrina* have been found to exhibit curare-like neuromuscular blocking activities which are caused by alkaloids occurring therein (*1–4*).

It was Altamirano (*2*), who obtained a silky shining, crystallinic acetate as well as Greshoff (*3*) who already isolated several basic unspecified compounds. Because of their remarkable biological activity he suggested a systematic phytochemical examination of the genus *Erythrina*. But it still has taken at least half a century before this has been realized for the first time by Folkers. He has shown that more than fifty *Erythrina* species – as an example, *E. crista-galli* is shown in Plate 1 – are containing the typical alkaloids exhibiting the same curare-like activity reported earlier (*5*, *6*). Moreover, his group succeeded in isolating the first crystallized erythrinane alkaloid named erythroidine (**14**; Fig. 2) (*7*). Soon after numerous alkaloids have been isolated, *e.g.* erythramine (**8**) (*8*), erythraline (**3**) (*9*), erythratine (**9**) (*10*), erysodine (**6**), erysopine (**4**), and erysovine (**5**) (*11*). Another decade later the fundamental investigations of Prelog

Plate 1. Erythrina crista-galli L (Coral Tree)

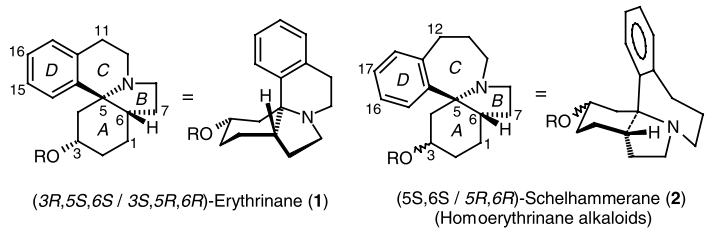


Fig. 1. Stereostructure and atom labeling of the *Erythrina* alkaloid frameworks

(*12*, *13*) and Boekelheide (*14*) have finally led to the correct structural framework of the erythrinane alkaloids (parent compound **1**, Fig. 1).

In the late 1960s ring C-homologues of erythrinane alkaloids have been anticipated from the biosynthetic pathway of certain alkaloids, which are known to be generated from 1-phenethyl-isoquinoline derivatives as precursors (*15*, *16*). Only a short time later such compounds named homoerythrinanes, homoerythrina alkaloids, or schelhammeranes indeed have been found in the plant kingdom (*17*) (parent compound **2**, Fig. 1).

Due to the increasing attraction and rapid extension in this field the *Erythrina* alkaloids have been regularly reviewed concerning occurrence, structure, analytic and spectral properties, biosynthesis, total synthesis, and biological activities covering the literature up to 1997. The most important reviews are cited in Refs. *18–24*.

The present contribution will give a brief classification of the *Erythrina* alkaloids, a compilation of new alkaloids isolated from 1997 to 2004 covering source, structure, analytical/spectral data, a new pathway of their biosynthesis, an overview of all the synthesis strategies hitherto known for the erythrinane alkaloids including several approaches to the homoerythrinane group, and finally a short review of their biological activities.

2. Structural Classification of *Erythrina* Alkaloids

The erythrina-type alkaloids are characterized by their unique tetracyclic spiroamine framework. They are generally classified into two main groups: Alkaloids predominantly possessing a 6-5-6-6-membered indoloisoquinoline core are called erythrinanes and those exhibiting a 6-5-7-6-membered indolobenzazepine skeleton are generally called schelhammeranes or homoerythrinane alkaloids (see Fig. 1).

Depending on the nature of the D ring both groups in turn may be subdivided into aromatic and non-aromatic alkaloids, the latter of which

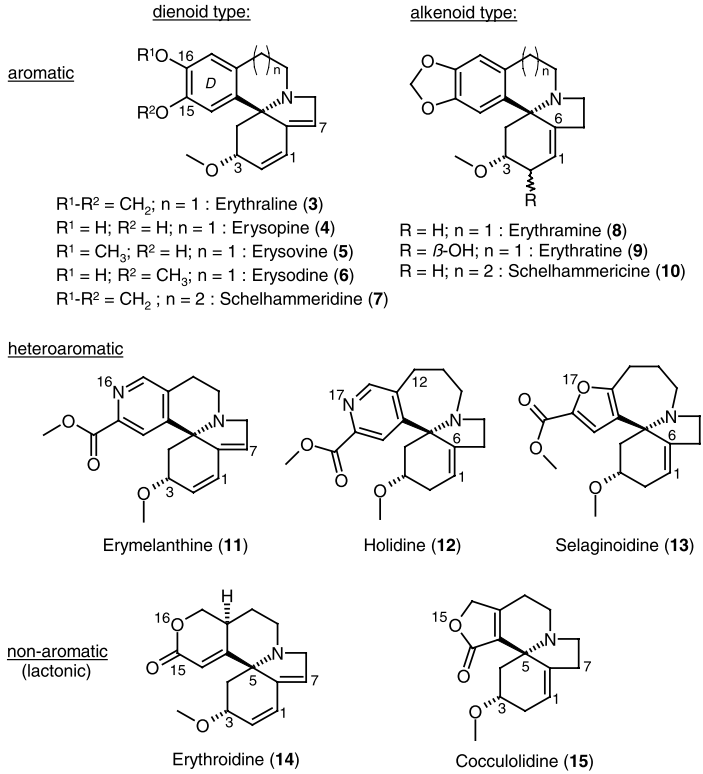


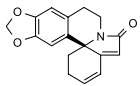
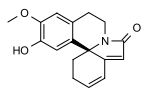
Fig. 2. General classification of *Erythrina* alkaloids: Dienoid and alkenoid type alkaloids and D ring modifications

including ring D oxa-compounds, are usually also called the lactonic alkaloids. In addition, in both series there have been isolated alkaloids containing a pyridyl instead of a phenyl unit, which are known as erymelanthine (**11**) and holidine (**12**) (16-azaerythrinane and 17-aza-homoerythrinane derivatives) belonging to two further different subtypes of these alkaloids (*25*, *26*) (see below and Fig. 2). Several D-*seco*-derivatives in the homoerythrinane group should also be mentioned (see *e.g.* **32**, Table 2).

Finally, the typical position and the number of olefinic bonds in the A and B ring have led to a further subdivision into dienoids and alkenoids in both alkaloid series. The former are characterized by a conjugated diene unit covering C atoms 1, 2, 6, and 7, while the latter possess only one double bond in the 1,6-position (see Fig. 2).

The aromatic erythrinanes and homoerythrinanes as the most important members of the *Erythrina* alkaloids show substitution patterns of

Table 1. New Erythrinane Alkaloids

Nr.	Trivial name(s)/ Formula/Structure	M.p./°C $\pm[\alpha]_D$/° $cm^2\,g^{-1}$	IR: $\bar{\nu}/cm^{-1}$ UV: λ_{max}/nm ($\log \varepsilon/mol^{-1}\,dm^3\,cm^{-1}$) MS: *m*/*z*	^1H NMR: δ/ppm ^{13}C NMR: δ/ppm	Natural source	Ref.
16	Erythrosotidienone $C_{17}H_{15}NO_3$ (281.31) 	250 –	2930, 2860, 1740, 1610, 1460, 1440, 1380, 1280–1260, 1160, 1130, 1075, 1040, 975, 960, 860–840, 730, 720, 675, 645, 610, 580 – 281 (5.0), 280 (22.5), 279 (100), 267 (7.5), 265 (7.5), 253 (17.5), 227 (2.5), 226, 199 (15.4), 174 (15.0), 167 (10.0), 133 (5.0), 81 (31.6), 20 (8.0), 77 (5.0), 76 (80.0), 56 (27.3), 55 (42.5)	6.02 (d, $J = 10$ Hz, 1-H), 5.94 (s, OCH_2O), 5.72, 6.02 (d, $J = 10$ Hz, 1-H), 5.94 (s, OCH_2O), 5.72 (m, 2-H), 3.63 (t, $J = 1.5$ and 4.5 Hz, 2H, 10-H), 3.27–1.87 (m, 6H) 184.0 (CO), 152.0 (C-16), 152.4 (C-15), 132.0 (C-2), 131.0 (C-1), 129.6 (C-7), 128.3 (C-12), 128.1 (C-13), 114.0 (C-17), 110.1 (C-14), 104.0 (C-6), 102.8 (OCH_2O), 68.1 (C-5), 40.3 (C-10), 32.0 (C-3), 30.4 (C-4), 23.2 (C-11)	*E. variegata* *Flowers*	(*33*)
17	Erythromotidienone $C_{18}H_{19}NO_3$ (297.35) 	172 –	2920, 2880, 1760, 1600, 1450, 1380, 1280–1260, 1250, 1170, 1110, 1075, 1055, 1025, 975, 960, 925, 885, 835, 800, 765, 730, 700 297 (5), 296 (8), 295 (11), 269 (19), 255 (30), 239 (8), 235 (10), 213 (16), 185 (16) 83, 81 (43)	7.32 (dd, $J = 2.5/9.0$ Hz, 16- or 15-H), 7.21 (d, $J = 2.7$ Hz, 14-H), 7.13 (d, $J = 2.3$ Hz, 17-H), 6.62 (s, 7-H), 6.34 (d, $J = 9.6$ Hz, 1-H), 6.02 (dd, $J = 2.5/7.5$ Hz, 2-H), 3.86 and 3.36 (2s, 2 OCH_3), 3.76–1.92 (m, 7H) ^{13}C not reported	*E. variegata* *Flowers*	(*33*)

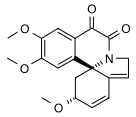
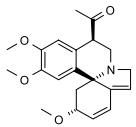
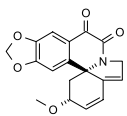
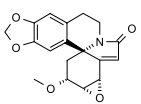
18	(+)-10,11-Dioxoerysotrine		1710, 1680, 1650, 1610	7.48 (s, 17-H), 7.17 (s, 14-H), 6.74 (dd, $J = 11.5/2.4$ Hz, 1-H), 6.00	*E. latissima*	(*34*)
		174–176	351 (3.54), 292 (sh, 3.62), 247 (4.17), 206 (4.38)	(d, $J = 11.5$ Hz, 2-H), 5.88 (br s, 7-H), 4.64 (br s, 8-H), 3.95 (16-OCH_3), 3.91 (s, 15-OCH_3), 3.64 (t, $J = 7.9$ Hz, 3-H), 3.23 (s, 3-OCH_3), 2.30 (d, $J = 7.9$ Hz, 4-H)		
	$C_{19}H_{19}NO_5$ (341.36)					
		+167.5	341 (M^+, 100), 310 (30), 282 (25), 257 (20)	181.8 (C-11), 159.7 (C-10), 153.5 (C-15), 149.5 (C-16), 141.7 (C-13), 138.0 (C-6), 132.6 (C-2), 124.9 (C-1), 124.2 (C-12), 121.2 (C-7), 111.1 (C-17), 106.4 (C-14), 76.1 (C-3), 70.8 (C-5), 56.9 (3-OCH_3), 56.7 (15- and 16-OCH_3), 54.7 (C-8), 49.9 (C-4)		
19	11-Acetylerysotrine		1760 ($COCH_3$), 1600 (C=C)	6.95 (s, 17-H), 6.82 (s, 14-H), 6.65 (d, $J = 10.0$ Hz, 2-H), 6.05 (d,	*E. stricta*	(*35*)
	$C_{21}H_{25}NO_4$ (355.43)	yellow oil	283.1 (3.7), 230.5 (4.2)	$J = 10.0$ Hz, 1-H), 4.74 (t, $J = 3.4$ Hz, 11-H), 4.05 (m, 3-H_{ax}), 3.94 (s, 16-OCH_3), 3.85 (s, 15-OCH_3), 3.68 (dd, $J = 13.5/3.5$ Hz, 10-H_{ax}), 3.32 (s, 3-OCH_3), 3.14 (dd, $J = 13.5/6.6$ Hz, 10-H_{eq}), 2.42 (dd, $J = 11.5/3.5$ Hz, 4-H_{eq}), 2.13 (s, $COCH_3$), 1.87 (t, $J = 11.5$ Hz, 4-H_{ax})		
			355 (M^+)			
		–		^{13}C not reported		

Table 1 (*continued*)

Nr.	Trivial name(s)/ Formula/Structure	M.p./°C $\pm[\alpha]_D$/° $cm^2\,g^{-1}$	IR: $\bar{\nu}$/cm^{-1} UV: λ_{max}/nm ($\log\varepsilon$/$mol^{-1}\,dm^3\,cm^{-1}$) MS: *m*/*z*	^{1}H NMR: δ/ppm ^{13}C NMR: δ/ppm	Natural source	Ref.
20	10,11-Dioxoerythraline		1710, 1680, 1650, 1610	7.41 (s, 17-H), 7.12 (s, 14-H), 6.68 (dd, $J = 10.3/2.2$ Hz, 1-H), 6.10	*E. bidwillii*	(*36*)
		amorph.	351 (3.53), 292 (sh, 3.62), 247	(d, $J = 1.5$ Hz, 1H, OCH_2O), 6.07		
	$C_{18}H_{15}NO_5$	solid	(4.17), 204 (4.32)	(d, $J = 1.5$ Hz, 1H, OCH_2O), 5.97		
	(325.32)			(d, $J = 10.3$ Hz, 2-H), 5.84 (br s, 7-H), 4.61 (br s, 2H, 8-H), 3.63 (m, 3-H), 3.24 (s, OCH_3), 2.25–2.31 (m, 2H, 4-H)		
						
		+254	325 (M^+, 100), 310 (12), 294 (46), 292 (34), 282 (37), 266 (83), 264 (63), 254 (11), 252 (15), 240 (27), 226 (26), 213 (18), 209 (21), 165 (13), 152 (22)	181.5 (C-11), 159.3 (C-10), 152.0 (C-16 or C-15), 148.0 (C-15 or C-16), 143.5 (C-13), 137.5 (C-6), 132.2 (C-2), 125.8 (C-12), 124.2 (C-1), 120.6 (C-7), 108.6 (C-17), 104.1 (C-14), 102.4 (OCH_2O), 75.5 (C-3), 70.5 (C-5), 56.5 (OCH_3), 54.2 (C-8), 49.6 (C-4)		
21	8-Oxoerythraline-epoxide	colourl. oil	1680	6.72 (s, 17-H), 6.55 (s, 14-H), 6.49 (s, 7-H), 5.98 (d, $J = 1.5$ Hz, 1H, OCH_2O), 5.94 (d, $J = 1.5$ Hz, 1H, OCH_2O), 4.24 (d, $J = 4.0$ Hz, 1-H), 3.83 (ddd, $J = 12.5/9.5/7.3$ Hz, 10-H_{ax}), 3.83 (d, $J = 4.0$ Hz, 2-H), 3.64 (m, 3-H), 3.57 (ddd, $J = 12.5/7.3/3.7$ Hz, 10-H_{eq}),	*E. bidwillii*	(*36*)
			289 (3.78), 205 (4.59)			
	$C_{18}H_{17}NO_5$					
	(327.34)	+94	327 (M^+, 53), 311 (14), 298 (30), 296 (22), 278 (14), 266 (14), 241 (100), 212 (43)			
						

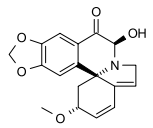
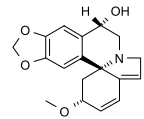
				3.41 (s, OCH_3), 3.09 (ddd, $J = 16.1/9.5/7.3$ Hz, 11-H_{ax}), 2.93 (ddd, $J = 16.1/7.3/3.7$ Hz, 11-H_{eq}), 2.36 (dd, $J = 11.7/5.1$ Hz, 4-H_{eq}), 1.59 (t-like, $J = 11.7$ Hz, 4-H_{ax})		
			CD: $\Delta\varepsilon$/mol^{-1} dm^3 cm^{-1} (λ/nm) = +3.51 (292), −17.31 (228), +20.76 (202)	170.2 (C-8), 155.0 (C-6), 147.2 (C-15 or C-16), 146.0 (C-16 or C-15), 130.0 (C-13), 129.7 (C-7), 128.0 (C-12), 109.8 (C-17), 105.7 (C-14), 101.3 (OCH_2O), 74.6 (C-3), 67.4 (C-5), 56.6 (CH_3), 52.9 (C-2), 48.7 (C-1), 37.4 (C-10), 32.9 (C-4), 27.5 (C-11)		
22	(+)-Erythbidin B	amorph. solid	3450, 1670, 1650	7.22 (s, 17-H), 6.88 (s, 14-H), 6.63 (dd, $J = 10.3/2.2$ Hz, 1-H),	*E. bidwillii*	(*37*)
	$C_{18}H_{17}NO_5$ (327.34)		205 (4.52), 243 (4.04), 291 (3.54)	5.99 (d, $J = 10.3$ Hz, 2-H), 5.98 (d, $J = 1.5$ Hz, 1H, OCH_2O), 5.94 (d, $J = 1.5$ Hz, 1H, OCH_2O), 5.76 (br s, 7-H), 5.26 (s, 10-H), 4.43 (d, $J = 17.6$ Hz, 1H, 8-H), 4.37 (dd, $J = 17.6/2.2$ Hz, 1H, 8-H), 4.05 (br s, OH), 3.72 (m, 3-H), 3.30 (s, OCH_3), 2.60 (dd, $J = 11.0/5.1$ Hz, 4-H_{eq}), 1.95 (t, $J = 11.0$ Hz, 4-H_{ax})		
		+148	327 (M^+, 100), 312 (23), 296 (74), 294 (34), 284 (6), 278 (13), 270 (7), 268 (12), 266 (14), 250 (5), 240 (7), 238 (7), 227 (6.5), 181 (6.7), 165 (8), 149 (10)			

Table 1 (*continued*)

Nr.	Trivial name(s)/ Formula/Structure	M.p./°C $\pm[\alpha]_D$/° $cm^2\,g^{-1}$	IR: $\bar{\nu}$/cm^{-1} UV: λ_{max}/nm ($\log \varepsilon$/$mol^{-1}\,dm^3\,cm^{-1}$) MS: *m*/*z*	^{1}H NMR: δ/ppm ^{13}C NMR: δ/ppm	Natural source	Ref.
			CD: $\Delta\varepsilon$/$mol^{-1}\,dm^3\,cm^{-1}$ (λ/nm) = +226 (286), +4.40 (252), −9.16 (218) (MeOH, c 3.06×10^{-5})	172.8 (C-11), 147.4 (C-15), 146.7 (C-16), 138.6 (C-6), 131.6 (C-2), 131.2 (C-13), 129.6 (C-12), 124.1 (C-1), 119.9 (C-7), 106.1 (C-17), 103.9 (C-14), 101.4 (OCH_2O), 76.1 (C-3), 71.7 (C-5), 67.7 (C-10), 56.4 (OCH_3), 54.1 (C-8), 39.5 (C-4)		
23	(+)-Epierythrinine $C_{18}H_{19}NO_4$ (313.35) 	amorph.[a] –	spectra not reported	^{1}H: differentiated signals for the *epi*-isomer: 7.04 (s, 17-H), 6.84 (s, 14-H), 6.54 (dd, 1-H), 6.03 (dm, 2-H), 5.94 (d, $J = 1.4$ Hz, OCH_2O), 5.91 (d, $J = 1.4$ Hz, OCH_2O), 5.72 (m, 7-H), 4.93 (dd, 11-H), 4.18 (m, 3-H), 3.71 (dd, 8-H), 3.64 (dm, 8-H), 3.47 (dd, 10-H), 3.37 (3-OCH_3), 3.16 (dd, 10-H), 2.68 (dddd, 4-H), 1.86 (dd, 4-H); position of coupling H-atoms Ha, Hb/*J* [Hz]: 1,2/10.1; 1,3/2.2; 4_{ax},3/10.6; 4_{eq},2/1.1; 4_{eq},3/5.5; 4_{eq},7/1.1; 4_{gem}/11.6; 8_{gem}/14.2; 8a,7/3.0; 10_{ax},11/10.0; 10_{eq},11/6.7; 10_{gem}/14.4 ^{13}C not reported	*E. caffra*	(*38*)

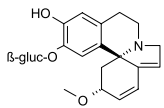
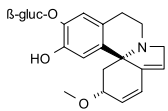
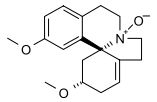
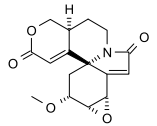
24	(+)-15β-D-Glucoerysopine $C_{23}H_{29}NO_8$ (447.49)  	150–152 (dark brown solid) +67.5	3415, 2920, 1507 279 (3.74), 222 (4.40), 206 (4.38) 447 (M^+, 80), 299 (80), 268 (100), 251 (70)	6.99 (s, 17-H), 6.75 (s, 14-H), 6.60 (dd, $J = 10.1/2.1$ Hz, 1-H), 6.02 (d, $J = 10.1$ Hz, 2-H), 5.79 (br s, 7-H), 4.74 (d, $J = 7.2$ Hz, 1′-H), 4.00 (m, 3-H), 3.95 (dd, $J = 11.0/3.9$ Hz, 6′-H_{ax}), 3.74 (m, 6′-H_{eq}) 3.61 (m, 8-H_{ax}), 3.48 (m, 4′-H), 3.48 (m, 2′-H), 3.45 (m, 8-H_{eq}), 3.40 (m, 5′-H), 3.40 (m, 10-H_{ax}), 3.34 (s, OCH_3), 3.30 (m, 3′-H), 2.91 (m, 11-H_{ax}), 2.91 (m, 10-H_{eq}), 2.67 (m, 11-H_{eq}), 2.52 (dd, $J = 10.6/5.7$ Hz, 4-H_{ax}), 1.78 (dd, $J = 10.9/10.9$ Hz, 4-H_{eq}) 147.6 (C-15), 145.8 (C-16), 143.3 (C-6), 133.9 (C-13), 132.2 (C-2), 125.4 (C-12), 124.7 (C-1), 122.6 (C-7), 118.1 (C-17), 114.6 (C-14), 103.8 (C-1′), 77.7 (C-3′), 77.1 (C-3), 77.0 (C-5′), 74.3 (C-2′), 70.8 (C-4′), 67.5 (C-5), 61.9 (C-6′), 57.1 (C-8), 55.9 (OCH_3), 44.4 (C-10), 41.3 (C-4), 24.3 (C-11)	*E. latissima*	(*39*)
25	(+)-16β-D-Glucoerysopine	158–160	3415, 2920, 1507	7.03 (s, 17-H), 6.79 (s, 14-H), 6.61 (dd, $J = 10.1/2.1$ Hz, 1-H), 6.04	*E. latissima*	(*39*)

Table 1 (*continued*)

Nr.	Trivial name(s)/ Formula/Structure	M.p./°C ±[α]$_D$/° cm^2 g^{-1}	IR: $\bar{\nu}$/cm^{-1} UV: λ_{max}/nm (log ε/mol^{-1} dm^3 cm^{-1}) MS: *m/z*	^{1}H NMR: δ/ppm ^{13}C NMR: δ/ppm	Natural source	Ref.
	$C_{23}H_{29}NO_8$ (447.49) 	(dark brown solid)	279 (3.74), 222 (4.40), 206 (4.38) 447 (M^+, 80), 299 (80), 268 (100), 251 (70)	(d, J = 10.1 Hz, 2-H), 5.79 (br s, 7-H), 4.78 (d, J = 7.2 Hz, 1′-H), 4.00 (m, 3-H), 3.95 (dd, J = 11.0/3.9 Hz, 6′-H$_{ax}$), 3.74 (m, 6′-H$_{eq}$) 3.61 (m, 8-H$_{ax}$), 3.48 (m, 4′-H), 3.48 (m, 2′-H), 3.45 (m, 8-H$_{eq}$), 3.40 (m, 5′-H), 3.40 (m, 10-H$_{ax}$), 3.34 (s, OCH_3), 3.30 (m, 3′-H), 2.91 (m, 11-H$_{ax}$), 2.91 (m, 10-H$_{eq}$), 2.67 (m, 11-H$_{eq}$), 2.52 (dd, J = 10.6/5.7 Hz, 4-H$_{ax}$), 1.79 (dd, J = 10.9/10.9 Hz, 4-H$_{eq}$)		
		+76.5		145.8 (C-15), 145.1 (C-16), 143.8 (C-6), 134.1 (C-13), 132.1 (C-2), 126.1 (C-12), 125.4 (C-1), 122.8 (C-7), 118.2 (C-17), 113.9 (C-14), 103.6 (C-1′), 77.7 (C-3′), 77.0 (C-3), 77.4 (C-5′), 74.2 (C-2′), 70.7 (C-4′), 67.5 (C-5), 61.8 (C-6′), 57.1 (C-8), 55.9 (OCH_3), 44.3 (C-10), 41.2 (C-4), 24.2 (C-11)		
26	Coculidine N-oxide	150–152	–	spectra not reported	*Cocculus laurifolius*	(*40*)
	$C_{18}H_{23}NO_3$ (301.39)	(HCl: 236–238)	287 (3.40), 225 (3.95), 205 (4.37)			

		–	301 (M^+, 3.4), 285, 284, 283			
27	(+)-8-Oxo-α-erythroidine epoxide	colourl. oil	1715, 1680 250 (sh, 3.69), 216 (4.23)	= 6.51 (s, 7-H), 6.09 (s, 14-H), 4.51 (dd, $J = 11.7/5.1$ Hz, 17-H_{eq}), 4.36 (m, 10-H_{eq}), 4.17 (dd, $J = 11.7/4.4$ Hz, 17-H_{ax}),	*E. poeppigiana*	(*41*)
	$C_{16}H_{17}NO_5$ (303.31)	+211	303 (M^+, 100), 287 (31), 274 (31), 272 (22), 271 (43), 255 (24), 244 (29), 242 (32), 231 (51), 217 (46)	4.05 (d, $J = 3.7$ Hz, 1-H), 3.64 (d, $J = 3.7$ Hz, 2-H), 3.55 (dd, $J = 11.0/5.1$ Hz, 3-H), 3.49 (s, OCH_3), 3.08 (ddd, $J = 13.2/13.2/3.7$ Hz, 10-H_{ax}), 2.76 (dd, $J = 13.2/5.1$ Hz,		
			CD (MeOH, c 3.30×10^{-5}): $\Delta\varepsilon$ +6.63 (263), −2.61 (228), +7.87 (208)	4-H_{eq}), 2.74 (m, 12-H), 1.94 (m, 11-H_{eq}), 1.73 (dddd, $J = 13.2/12.5/6.6/5.1$ Hz, 11-H_{ax}), 1.55 (dd, $J = 13.2/11.0$ Hz, 4-H_{ax})		

[a] Inseparable 79:21 mixture with the isomeric known alcohol (+)-erythrinine.

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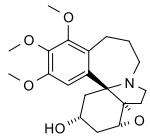
Table 2. New Homoerythrinane Alkaloids

Nr.	Trivial name(s)/ Formula/Structure	M.p./°C $\pm[\alpha]_D$/° $cm^2\,g^{-1}$	IR: $\bar{\nu}$/cm^{-1} UV: λ_{max}/nm ($\log \varepsilon$/$mol^{-1}\,dm^3\,cm^{-1}$) MS: *m*/*z*	^{1}H NMR: δ/ppm ^{13}C NMR: δ/ppm	Natural source	Ref.
28	1,6α-Epoxyrobustivine $C_{20}H_{27}NO_5$ (361.44) 	no data reported +99	3575, 3400, 2910, 2830, 1590, 1394, 1305 283 (3.11), 207 (4.55) 361 (M^+), 344, 181, 180, 167, 166 (100)	6.55 (15-H), 3.89, 3.83 and 3.79 (3 OCH_3), 3.79 (1-H), 3.46 (3-H), 3.46 (12-H), 3.46 (10-H), 3.32 (10-H), 3.02 (8-H), 2.83 (8-H), 2.57 (12-H), 2.50 (4-H_{eq}), 2.40 (2-H), 2.28 (7-H), 2.03 (2-H), 1.86 (4-H_{ax}), 1.80 (7-H), 1.76 (11-H), 1.58 (11-H) 151.81, 149.90, 141.42, 137.24, 129.44, 108.75 (C-15), 70.57 (C-5), 67.15 (C-6), 64.44 (C-3), 61.34 (OCH_3), 60.87 (OCH_3), 57.43 (C-1), 56.24 (OCH_3), 49.78 (C-10), 45.82 (C-8), 36.05 (C-4), 33.36 (C-2), 27.95 (C-7), 25.72 (C-12), 22.70 (C-11)	*Phelline comosa Labill. var. robusta (Baill.) Loesner*	(*42*)
29	18-De-O-methylholidine $C_{19}H_{25}NO_4$ (331.41)	140 (colourl. crystals)	3425, 3100 (s), 1595, 1480, 1440, 1360, 1325 276 (3.09), 208 (4.47); in alcaline solution: 293 (4.18)	6.39 (s, 15-H), 5.50 (m, 1-H), 3.87 (s, OCH_3), 3.51 (2m, 12-H_a, 10-H_a), 3.30 (m, 3-H), 3.25 (s and m, 3-OCH_3 and 10-H_b), 2.78 (dd and m, 4-H_{eq} and 8-$H_{a,b}$), 2.58 (m, 12-H_b and 2-H_a), 2.45 (m, 7-H_a), 2.30 (m, 7-H_b), 2.02 (m, 2-H_b), 1.76 (m, 11-H_a), 1.56 (dd and m, 2H, 4-H_{ax})	*Phelline comosa Labill. var. robusta (Baill.) Loesner*	(*42*)

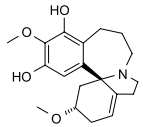
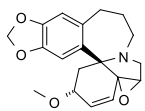
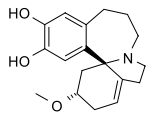
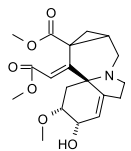
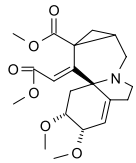
		+111	331 (M^+), 300, 273, 272, 180 (100)	147.0, 145.3, 141.7, 117.0, 110.8 (CH), 74.2 (C-3), 70.0 (C-5), 60.6 (OCH_3), 55.8 (3-OCH_3), 50.5 (C-10), 46.8 (C-8), 37.7 (C-4), 32.0 (C-2), 27.5 (C-7), 25.3 (C-12), 23.1 (C-11)		
30	Fortunine $C_{19}H_{21}NO_4$ (327.38) 	120–121.5	2980, 2930, 2890, 2820, 1610, 1500, 1480, 1350, 1325, 1298, 1229, 1090, 1030, 915	6.94 (s, 15-H), 6.57 (s, 18-H), 6.01 (br d, $J = 10.2$ Hz, 1-H), 5.85 (d, $J = 1.40$ Hz, OCH_2O), 5.75 (dd, $J = 10.2/2.0$ Hz, 2-H), 3.52 (ddd,	*Cephalotaxus fortunei*	(*43*)
		–	–	3-H), 3.29 (s, OCH_3), 3.22/2.62 (m, 11-$H_{a,b}$), 3.20 (m, 7-H), 2.85 (dd, $J = 12.7/4.2$ Hz, 8-$H_{a,b}$), 2.63 (m, 10-$H_{a,b}$), 1.80 (m, 11-$H_{a,b}$), 1.68–1.65 (br d, $J = 10.6$ Hz, 4-$H_{a,b}$)		
		–	327 (M^+, 75), 312 (100), 296, 284, 267, 254, 240, 228, 160, 152, 128, 115, 77	146.1 (C-17), 145.1 (C-16), 134.4 (C-13), 133.3 (C-2), 132.8 (C-14), 127.2 (C-1), 111.9 (C-18), 109.5 (C-15), 100.1 (OCH_2O), 76.3 (C-3), 69.3 (C-5), 68.9 (C-6), 60.1 (C-7), 56.2 (3-OCH_3), 55.5 (C-8), 49.7 (C-10), 35.4 (C-12), 31.7 (C-4), 29.1 (C-11)		
31	Cephalezomine M	colourl. solid	3360, 2930, 1670, 1510, 1200	6.75 (s, 15-H), 6.71 (s, 18-H), 6.05 (s, 1-H), 3.77 (m, 10-H_b), 3.51 (d, $J = 14.2$ Hz, 10-H_a), 3.35 (s, 8-H_b),	*Cephalotaxus harringtonia var. nana*	(*44*)

Table 2 (*continued*)

Nr.	Trivial name(s)/ Formula/Structure	M.p./°C $\pm[\alpha]_D$/° $cm^2 g^{-1}$	IR: $\bar{\nu}$/cm^{-1} UV: λ_{max}/nm ($\log \varepsilon$/$mol^{-1} dm^3 cm^{-1}$) MS: *m*/*z*	^{1}H NMR: δ/ppm ^{13}C NMR: δ/ppm	Natural source	Ref.
	$C_{18}H_{23}NO_3$ (301.39) 		286 (3.26), 233 (3.56), 217 (3.38)	3.28 (m, 3-H), 3.28 (m, 12-H_b), 3.23 (s, OCH_3), 3.17 (t, $J = 13.0$ Hz, 12-H_a), 2.84 (m, 4-H_b), 2.72 (m, 8-H_a), 2.72 (m, 7-$H_{a,b}$), 2.72 (m, 2-H_b), 2.08 (m, 2-H_a), 2.08 (m, 11-H_b), 1.94 (m, 11-H_a), 1.77 (t, $J = 11.4$ Hz, 4-H_a)		
		+64	302 ($M^+ + H$)	147.32 (C-17), 144.62 (C-16), 136.88 (C-6), 133.93 (C-13), 126.20 (C-1), 123.41 (C-14), 120.37 (C-18), 120.03 (C-15), 76.79 (C-5), 74.30 (C-3), 56.51 (OCH_3), 52.01 (C-10), 49.09 (C-8), 37.46 (C-4), 35.27 (C-12), 32.76 (C-2), 26.82 (C-7), 22.86 (C-11)		
32	2α-Hydroxy-lenticellarine $C_{19}H_{25}NO_6$ (363.41) 	no data reported	3560, 2960, 2950, 2860, 1732, 1710, 1680, 1610, 1510, 1452, 1445, 1370, 1280, 1260, 1207, 1150, 1106, 1080, 933, 750	6.88 (s, 15-H), 5.72 (br s, 1-H), 4.43 (dd, $J = 3.8$ Hz, 2-H), 3.92 (s, 16-OCH_3), 3.68 (m, 3-H), 3.47 (s, 17-OCH_3), 3.44 (dd, $J = 9.15$ Hz, 10-H_b), 3.34 (s, 3-OCH_3), 3.04 (dd, $J = 5.15$ Hz, 10-H_a), 2.96 (m, 8-$H_{a,b}$), 2.62 (t, $J = 4.0$ Hz, 4-H_{eq}), 2.48 (m, 7-$H_{a,b}$), 1.92 (dd, $J = 4.12$ Hz, 4-H_{ax}), 1.62 (br m, 11-H), 1.09 (dd, $J = 5.58$ Hz, 12-H_b), 0.94 (dd, $J = 5.9$ Hz, 12-H_a)	*Dysoxylum lenticellare*	(*45*)
			–			

			363 (M^+, 15), 348 (6), 346 (31), 345 (100), 332 (6), 305 (6), 181 (13), 165 (18), 137 (52)	^{13}C not reported		
33	2α-Methoxy-lenticellarine $C_{20}H_{27}NO_6$ (377.44) 	no data reported	2960, 2940, 2865, 1730 (C=O), 1690, 1630, 1510, 1480, 1440, 1370, 1334, 1280, 1260, 1205, 1150, 1100, 1080, 930, 740 –	6.90 (s, 15-H), 5.72 (br s, 1-H), 4.42 (d, $J = 3.8$ Hz, 2-H), 3.92 (s, 16-OCH_3), 3.82 (s, 2-OCH_3), 3.67 (m, 3-H), 3.46 (s, 17-OCH_3), 3.44 (dd, $J = 9.15$ Hz, 10-H_b), 3.33 (s, 3-OCH_3), 3.04 (dd, $J = 5.15$ Hz, 10-H_a), 2.96 (m, 8-$H_{a,b}$), 2.62 (dd, $J = 4.12$ Hz, 4-H_{eq}), 2.48 (m, 7-$H_{a,b}$), 1.90 (t, $J = 12.0$ Hz, 4-H_{ax}), 1.60 (m, 11-H), 1.07 (dd, $J = 5.0/5.8$ Hz, 12-H_b), 0.92 (dd, $J = 5.9$ Hz, 12-H_a)	*Dysoxylum lenticellare*	(*45*)
			377 (M^+, 28), 362 (5), 347 (31), 346 (100), 319 (12), 318 (19), 196 (5), 165 (15), 137 (58)	^{13}C not reported		

similar type. Thus, they mostly are oxygenated at atoms C3, C15, C16 and C3, C16, C17, respectively. Furthermore, there is a small group of C16-deoxygenated erythrinane alkaloids. The saturated parent compounds of both alkaloid series possess the *cis*-configuration of the A/B-moiety independently whether they have been obtained by transformation of natural alkaloids or by synthesis (*27–29*). The alkaloids are generally dextrorotatory and their absolute configuration at the spiro-atom C5 is (*S*) with respect to the basic framework (*17*, *22*, *24*, *30*, *31*). Due to an anomalous substitution pattern there are few exceptions, for instance wilsonine (**227**), with (*R*)-configuration of C5. Atom C3 of erythrinanes is always (*R*)-configured, that of homoerythrinanes, however, is also found exhibiting (*S*)-configuration. For an overview concerning structures, substitution patterns, and properties see *e.g.* refs. (*19*) and (*24*). Obviously, *Erythrina* alkaloids with *trans*-fused A/B rings do not naturally occur. Nevertheless, the *trans*-erythrinane skeleton has been synthesized by Mondon (*31*) and Desmaële (*32*).

3. New *Erythrina* Alkaloids

Up to the most recent reviews published in the years 1995/96 nearly 95 erythrinane and 68 schelhammerane type alkaloids were known (*19*, *21*, *24*). In this chapter all the new compounds reported in literature from 1995 to 2004 are listed including the natural source, structure, analytical and spectroscopical informations. All in all, there are 12 erythrinane and 6 homoerythrinane type compounds compiled in Tables 1 and 2.

4. Biosynthesis of *Erythrina* Alkaloids

4.1. Erythrinane Alkaloids

The first generally accepted pathway for the biosynthesis of erythrinane alkaloids was established by Barton's group. This route starts with the benzylisoquinoline (*S*)-norprotosinomenine (**34**) as the main precursor, which is cyclized by *para-para* phenol coupling to the neoproaporphine derivative (**35**). This in turn undergoes rearrangement yielding the symmetrical dibenzazonine **36**. Its hydrogenation product **37** is oxidized to the corresponding diphenoquinone (**38**). Finally, intramolecular Michael type addition proceeds to afford erysodienone (**39**) possessing the characteristic erythrinane skeleton (*46–48*). Both precursors, **34** and **37**, are naturally occurring compounds (*49*, *50*) (Scheme 1).

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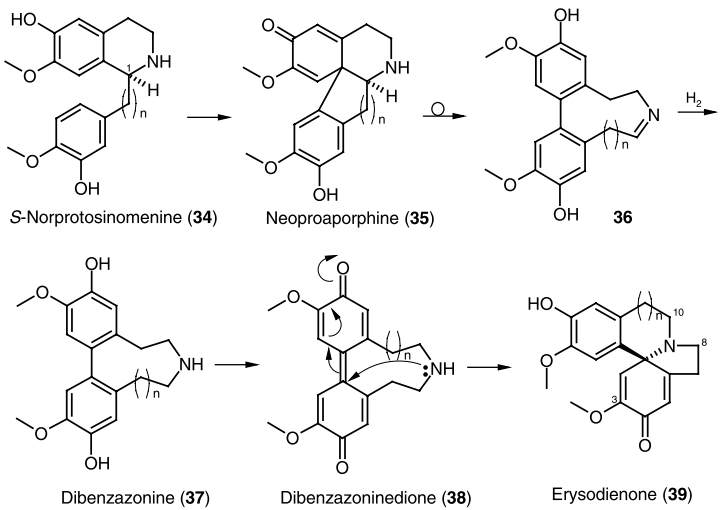
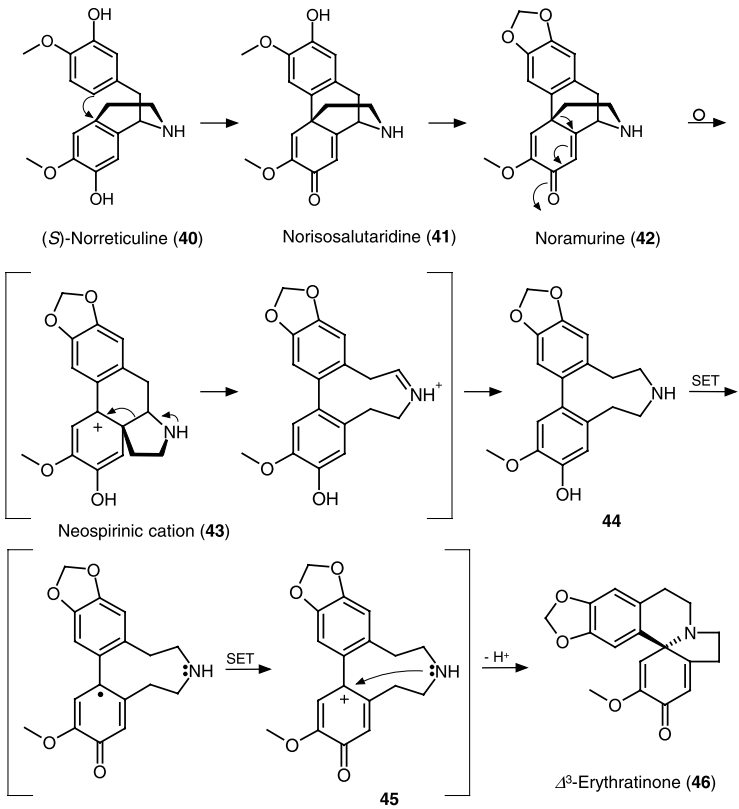


Scheme 1. Biosynthesis of erythrinane alkaloids according to Barton *et al*. (all formulae: n = 1) (*46–48*)

However, the relative small incorporation rate (0.1–0.25%; (*48*)) of the norprotosinomenine (**34**) into the alkaloid erythraline (**3**) and mainly the fact, that the majority of the important isoquinoline alkaloids, *e.g.* protoberberines, aporphines, bisbenzylisoquinolines, morphinanes, pavines, and benzophenanthridines, are biosynthetically derived from (*S*)-reticuline (**40**: NCH_3 instead of NH), have caused a reinvestigation of the biosynthesis pathway outlined in Scheme 1. Thus, in detailed investigations it has been unequivocally shown that (*S*)-norreticuline (**40**) in fact is also the most important biosynthetic precursor for the erythrinane alkaloids, and its incorporation rate exceeds by far that of the isomeric (*S*)-norprotosinomenine (**34**) previously found (7.9% *vs* 0.25% in the case of erythraline). Furthermore, the latter was not converted into any of the alkaloids in the reinvestigation concerned. These results required a new route and mechanism for the biosynthesis of the erythrinane alkaloids depicted in Scheme 2.

According to model reactions in this field previously carried out (*51*), the initial *para-para* coupling of (*S*)-norreticuline (**40**) should led – differently from the previous route – to the morphinandienone derivative norisosalutaridine (**41**) rather than to the neoproaporphine derivative (**35**). The latter, after generation of the benzo[1,3]dioxole function giving noramurine (**42**), can rearrange via the neospirinic ion (**43**) to the unsymmetrical dibenzazonine (**44**). Oxidation at the free phenolic unit



Scheme 2. New biosynthetic sequence of erythrinane alkaloids proposed by Zenk *et al*. (*52*)

proceeding through a SET mechanism can afford the diallylic cation (**45**), which is assumed to react with the nitrogen atom to generate Δ^3-erythratinone (**46**). A symmetrical intermediate of the diphenoquinone type (**38**) postulated previously could be excluded based on feeding experiments with ^{13}C-labelled precursors. Thus, *e.g.* using (*S*)-[1-^{13}C]-norreticuline a ^{13}C-enrichment should occur at C10 as well as at C8 of the isolated erythrinane alkaloid **39**. However, the enrichment has been observed exclusively at C10 (*52*). The subsequent steps leading to erythraline (**3**) are similar to those already established (*53*).

4.2. Homoerythrinane Alkaloids

The biosynthesis of homoerythrinane alkaloids has been proposed to proceed by the same pathway as that of the erythrinanes according to

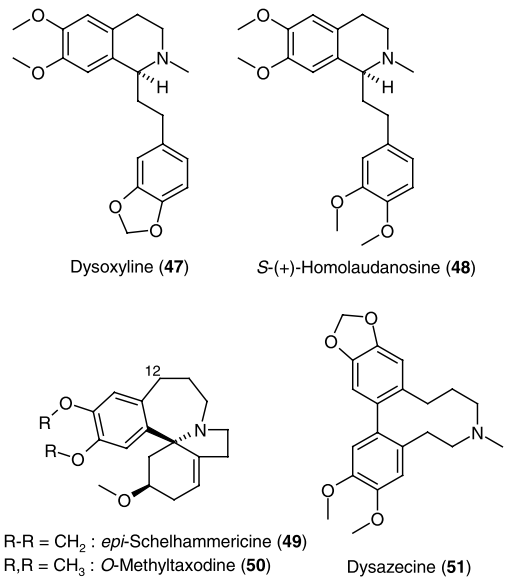


Fig. 3. Phenethylisoquinolines **47** and **48** isolated from plants, which produce homoerythrinane and dibenzazecine alkaloids **49**, **50**, and **51**

Scheme 1 starting from the homologous *S*(+)-1-phenethyltetrahydroisoquinoline precursor (**34**, n = 2) via the corresponding dibenzazecine (**37**, n = 2). Supports for these assumptions are based on the isolation of several naturally occurring phenethylisoquinolines, *e.g.* dysoxyline (**47**) and *S*(+)-homolaudanosine (**48**) from plants that produce the homoerythrinane alkaloids 3-epi-schelhammericine (**49**), *O*-methyltaxodine (**50**), and especially the dibenzazecine alkaloid dysazecine (**51**) possessing the same skeleton as the postulated biosynthetic intermediate (**37**, n = 2) (*22*) as shown in Fig. 3.

But until now there is no experimental evidence for this route concerned, which possibly – with regard to that of the erythrinane alkaloids – will demand a revision.

5. Syntheses of *Erythrina* Alkaloids and *Eyrthrina* Type Compounds

Because of their unique structures and their biological activities there has been much interest in the total synthesis of *Erythrina* alkaloids. Thus numerous synthesis approaches to the tetracyclic framework, mainly to

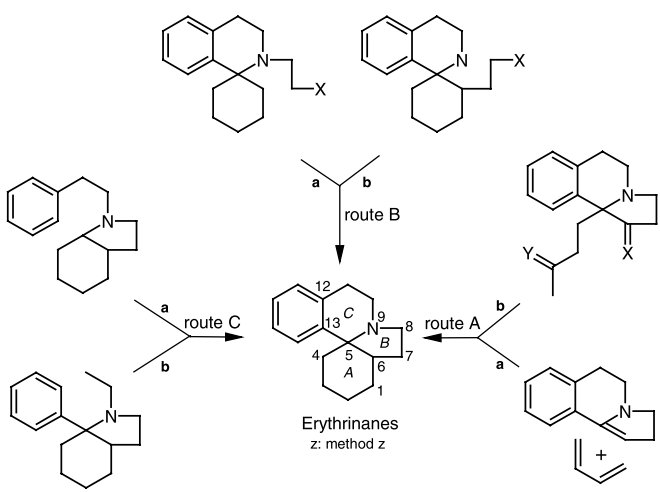
that of the erythrinanes, have been developed until now. Contrarily, only few routes have been reported for the synthesis of the ring C-homologous schelhammeranes.

5.1. Methodical Classification

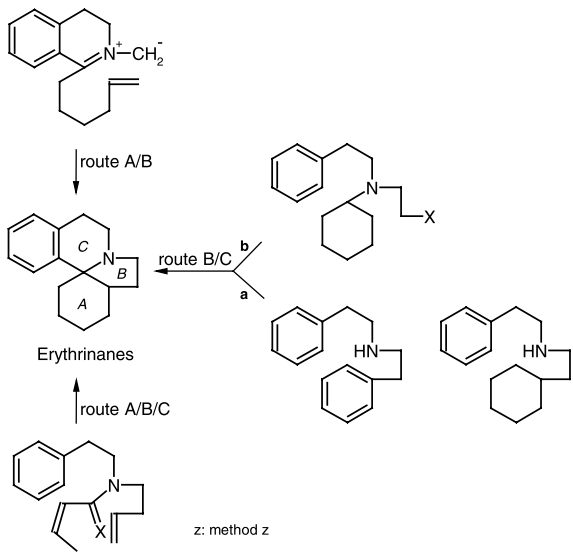
A practical classification of all syntheses especially concerning the erythrinane ring system is based on the idea which of the three alicyclic rings A, B, or C is completed in the final step. Thus in principle two alternatives are conceivable:

– The completion of one of the rings mentioned (in the following called "route A, B, or C"
or
– the sequential or simultaneous formation of more than one cycle *e.g.* A/B, A/C, B/C in the final or A/B/C in one step, correspondingly called "route A/B, B/C" *etc*. (Schemes 3 and 4).

Depending on the bond(s) to be formed several subtypes result from both cases as outlined in Schemes 3 and 4: *e.g.* ring C formation can be achieved using method (a) or method (b) [= route C(a) or C(b)]. Accordingly, two different pathways are available for the simultaneous construction of rings B and C [= route B/C(a) and B/C(b)].



Scheme 3. Strategies for the synthesis of aromatic erythrinane alkaloids (5.2.1.): Generation of one alicyclic ring in the final step



Scheme 4. Strategies for the synthesis of aromatic erythrinane alkaloids (5.2.2.): Generation of more than one alicyclic ring by sequential or tandem cyclization in the final or in one step

In this classification C-homoerythrinane syntheses remain out of consideration, since the construction of their framework is far more difficult than that of the erythrinanes. Nevertheless, several useful approaches to this alkaloid group are included in this section (see below).

Concerning the non-aromatic erythrinane alkaloids, only one single total synthesis has been achieved until now. Thus, the synthesis of (±)-cocculolidine (**15**, Fig. 2) has been completed in 0.42% overall yield through 21 steps (*54*). Furthermore, several synthesis approaches to ring D oxaerythrinane frameworks are known (*19*).

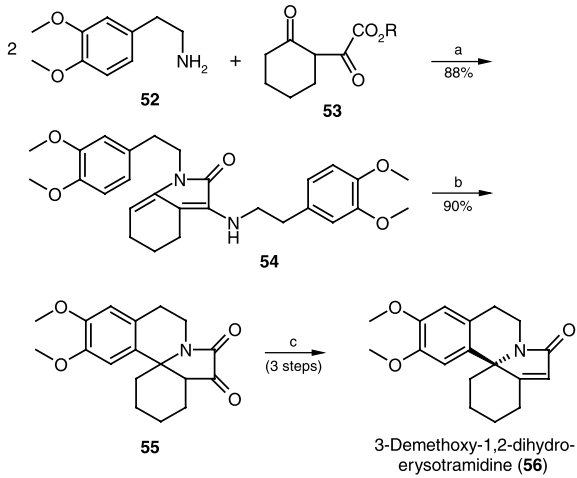
5.2. Erythrinanes

5.2.1. Final Formation of One Ring

5.2.1.1. Ring C (Route C)

5.2.1.1.1. Cyclization of N-Phenethylhydroindole Derivatives (Route C(a))

Generation of a C5 quaternary center – frequently in form of an acyliminium ion – and acid catalyzed reaction with the aromatic atom C13 – related to the Pictet-Spengler reaction – is a widespread method to



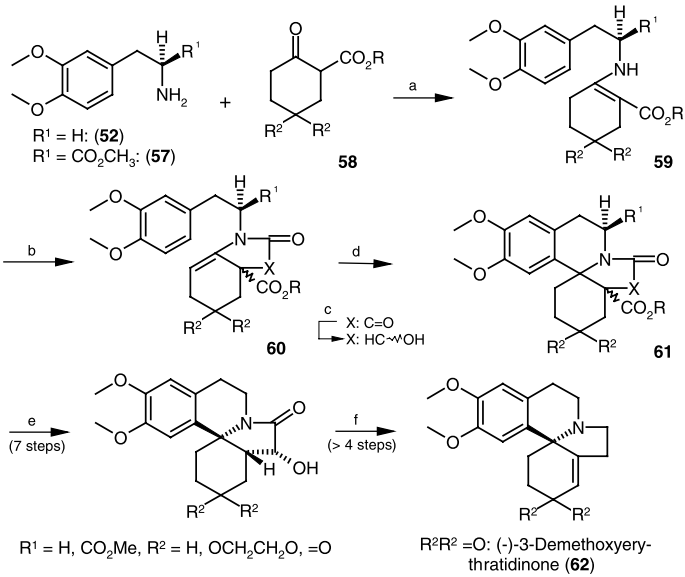
Scheme 5. Synthesis of 3-demethoxy-1,2-dihydroerysotramidine (**56**) (*56*, *57*): a) 180°C in xylene/N_2; b) dil. $H_3PO_4/CH_3OH/\Delta$; c) *i*: H_2/Raney-Ni/NaOH in EtOH; *ii*: *p*-TsCl in pyr/0°C; *iii*: refl. collidine

synthesize the erythrinane skeleton. This approach has often been realized with the simultaneous formation of ring B (s. below, route B/C(a)), especially in the early investigations (*20*, *55*).

A clean C(a) route represents one of Mondon's syntheses starting from two equivalents homoveratrylamine (**52**) and cyclohexanoyl glyoxylic acid (**53**) to afford the *N*-phenethylhydroindole derivative (**54**) in high yield. Intramolecular cyclization with phosphoric acid gives the 7,8-dioxoerythrinane (**55**), which can be transformed to 3-demethoxy-1,2-dihydroerysotrine (**56**) (*56*, *57*).

A related general pathway (Scheme 6), based on a subsequent introduction of the α-dicarbonyl moiety starts from the enamines **59** generated from homoveratrylamine (**52**) and 2-oxocyclohexane carboxylic derivatives **58**. They are reacted with oxalylchloride yielding also hydroindole precursors **60** (X=CO), which on immediate treatment with a Lewis acid or after reduction to the carbinol (X=CHOH) have been cyclized to the corresponding highly functionalized erythrinanes **61** in excellent yields (*58–60*). From a chiral phenethylamine, *e.g.* the (*S*)-(+)-dimethoxyphenylalanine methylester (**57**) the hydroindole derivatives **60** ($R^1 = CO_2CH_3$) have been diastereoselectively prepared by a similar sequence (*de* = 50–60%). After elevating the diastereomeric excess to *de* = 82% by a kinetic resolution, enantiotype erythrinane alkaloids such as (−)-3-demethoxyerythratidinone (**62**) are available (*60*).

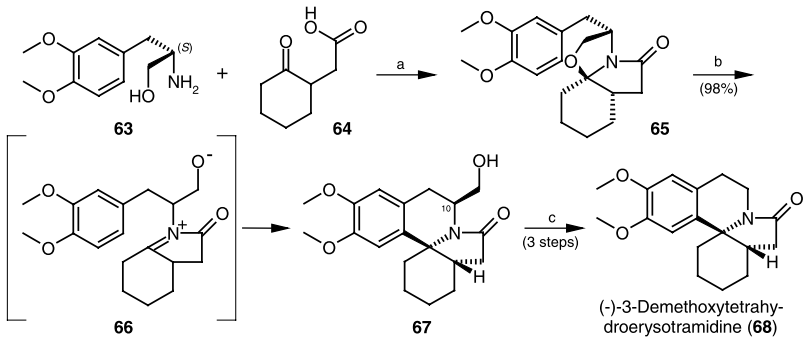
References, pp. 56–62



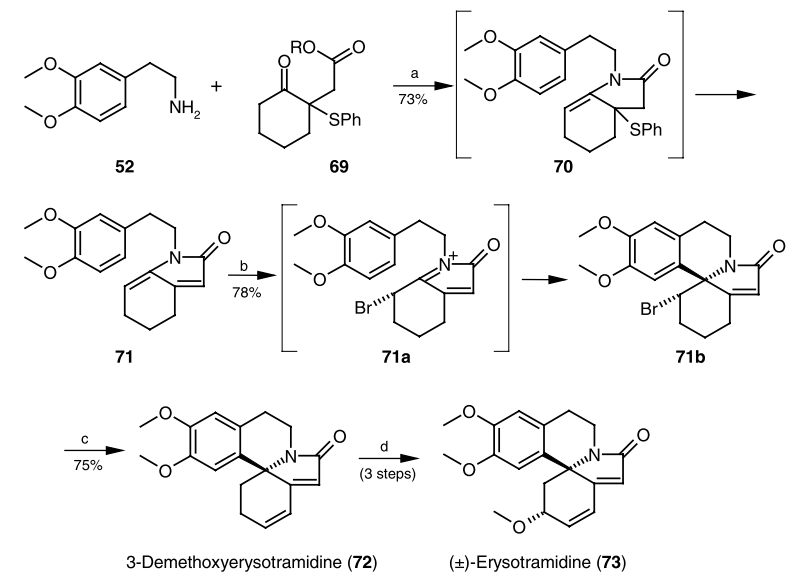
Scheme 6. Synthesis of (−)-3-demethoxyerythratidinone (**62**) (*58–60*): a) neat at 110°C or in EtOH at 100–150°C/sealed tube; b) $(COCl)_2$ in benzene or Et_2O/0 to −15°C; c) $NaBH_4$ in EtOH/0°C; d) $BF_3 \cdot Et_2O$ in CH_2Cl_2/20°C or refl.; e) 7 steps and f) 4 steps (*60*)

A more efficient asymmetric approach to the erythrinane core has been achieved by utilizing a bicyclic lactam template of Meyers. In the present case condensation of racemic cyclohexanoylacetic acid (**64**) with the chiral benzylaminoethanol **63** stereoselectively gives the required tricyclic lactam **65**, which on treatment with titanium tetrachloride has been cyclized – via the *N*-acyliminium intermediate **66** – to the desired erythrinane derivative **67** with 98% yield. Finally, the hydroxymethyl auxiliary group at the 10-position has been readily removed by an established three-step procedure affording the chiral (−)-3-demethoxy-tetrahydroerysotramidine (**68**) (*61*) (Scheme 7).

An interesting pathway concerning the generation as well as the reactivity of the required incipient *N*-acyliminium ion **71a** represents the NBS-promoted cyclization of the phenethylhydroindolinone precursor **71** (Scheme 8). This reaction markedly depends on the polarity of the solvent and only proceeds to give the desired cyclization product **71b** when acetonitrile has been used; in methylene chloride or tetrahydrofuran the cyclization of **71** → **71b** does not occur. Precursor **71** is available from homoveratrylamine (**52**) and the phenylsulfanyl cyclohexanoyl acetic acid ester **69** via the angularly substituted bicyclic lactam **70**. Finally, **71b**



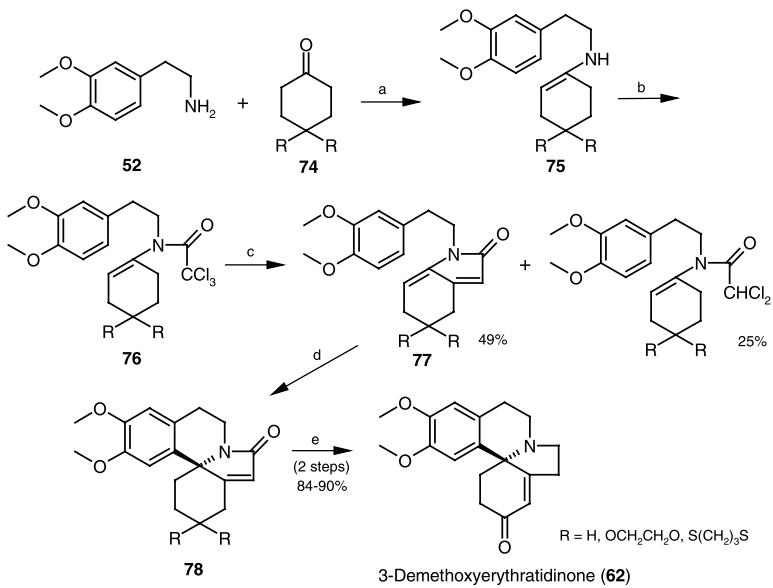
Scheme 7. Synthesis of (−)-3-demethoxytetrahydroerysotramidine (**68**) (*61*): a) toluene/Δ; b) $TiCl_4$ in CH_2Cl_2/−78°C; c) *i*: Dess-Martin periodane in CH_2Cl_2; *ii*: $Rh(PPh_3)_2(CO)Cl$/1,3-bis(diphenylphosphino)propane/xylene/Δ; *iii*: H_2/Pd–C, EtOH



Scheme 8. Synthesis of (±)-erysotramidine (**73**) (*62*): a) TFA/xylene/160°C; b) NBS in CH_3CN; c) DBU/refluxing xylene; d) *i*: SeO_2/HCO_2H; *ii*: CH_3COCl/EtOH; *iii*: CH_3I/KOH in THF

has been dehydrohalogenated yielding 3-demethoxyerysotramidine (**72**), which affords (±)-erysotramidine (**73**) in three additional steps (*62*).

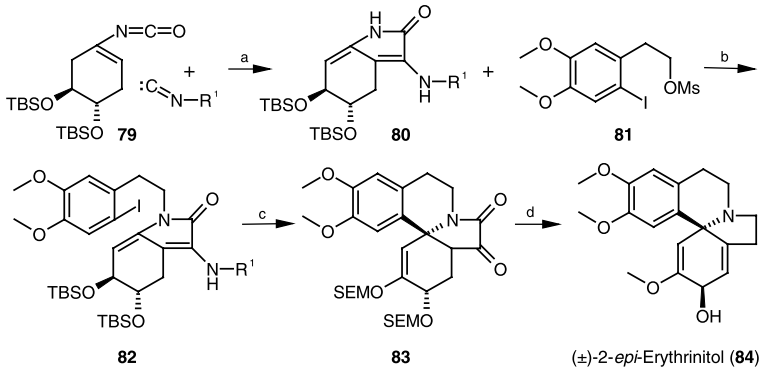
Suitably functionalized hydroindole precursors have been efficiently generated from alkenyltrichloroacetamides **76**, which undergo radical cyclization promoted by nickel powder providing the hydroindolone **77**



Scheme 9. Synthesis of 3-demethoxyerythratidinone (**62**) (*63*): a) in toluene, Dean-Stark; b) Cl_3CCOCl/Et_3N in toluene, 0°C; c) Ni/AcOH in 2-propanol/refl.; d) *p*-TsOH in benzene/refl.; e) $R = S(CH_2)_3S$, *i*: $LiAlH_4/AlCl_3$ in THF/Et_2O, 0°C; *ii*: *N*-chlorsuccinimide/$AgNO_3$ in CH_3CN/H_2O

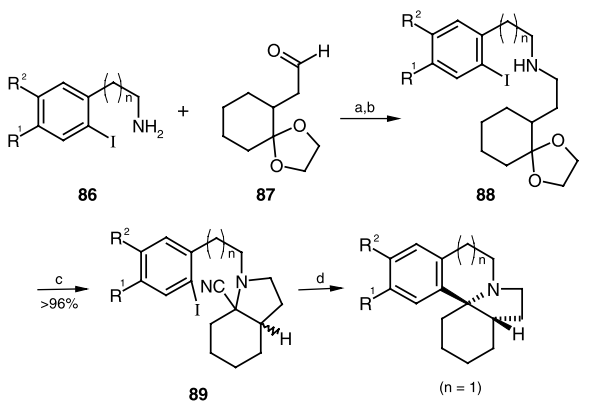
(Scheme 9). On treatment with *p*-toluene sulfonic acid in refluxing benzene they afford the 8-oxo-$\Delta^{6,7}$-erythrinane **78** with at least 84% yield. Further two steps involving the reduction of the amide moiety and deprotection of the ketone with concomitant migration of the double bond lead to 3-demethoxyerythratidinone (**62**) (*63*). The required educts **76** are readily available by reaction of homoveratrylamine (**52**) and cyclohexanones **74** via enamine **75** followed by N-acylation with trichloroacetylchloride.

Besides this widespread pathway based on the intermediate *N*-acyliminium ion, several other established methods have been applied to construct the C5/C13 bond of the erythrinane skeleton. Thus, the Heck reaction has proved to be an attractive approach to the target compounds. The synthesis reported by Rigby (Scheme 10) starts with a smooth [1 + 4] cycloaddition of certain isocyanides to the vinyl isocyanate **79** affording the required hydroindolone **80**. Then the iodoarene moiety has been installed by *N*-alkylation with the phenethylmesylat **81** giving the N-alkylated precursor **82**. Cyclization of **82** under Heck conditions yields the expected 7,8-dioxoerythrinane **83** as a single diastereomer, which then has been converted to (±)-2-*epi*-erythrinitol (**84**) in twelve additional steps (*64*).



Scheme 10. Synthesis of (±)-2-*epi*-erythrinitol (**84**) (*64*): a) R^1: *c*-hexyl, in CH_3CN/20°C; b) NaH in DMF; c) *i*: TBAF in THF; *ii*: SEMCl/*i*-Pr_2NEt; *iii*: $Pd(OAc)_2$/(*o*-tol)$_3$P, TEA in CH_3CN/H_2O; d) 12 steps, overall yield 7.2% (*64*)

A new diastereoselective route to aromatic *cis*-erythrinanes represents the combined intramolecular Strecker and Bruylants reactions of the phenethyl-cyclohexanylethylamines **88** (Scheme 11). Deprotection of the carbonyl function and addition of potassium cyanide causes the Strecker reaction to give the angularly substituted hydroindole derivatives **89** in nearly quantitative yields. Then the Bruylants reaction is

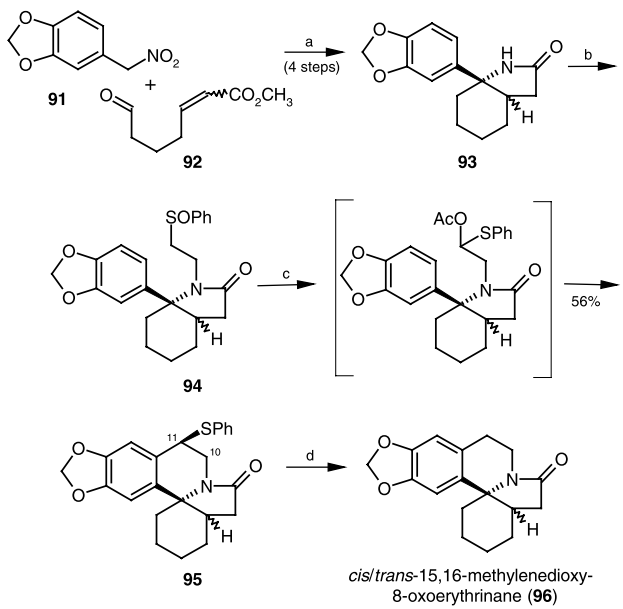


Scheme 11. Synthesis of erythrinanes (**1**, **90**, **91**; n = 1) (*29*): a) equimol. educts in toluene/Dean-Stark; b) $NaBH_4$ in CH_3OH/refl.; c) in 2 molequiv. 2 *N* HCl/H_2O, 60°C, then addition of 2 molequiv. KCN/H_2O at 20°C; d) 1.05 molequiv. 2 *M* *i*-PrMgCl in THF at −50° to +60°C

started with *i*-propylmagnesium chloride providing the parent tetracycles **1**, **90**, and **91** with 70–80% yields. Contrarily, the corresponding C-ring homologue schelhammeranes (*e.g.* **2**) are not available on this route. The required educts **88** have been easily prepared by reductive N-alkylation of iodophenethylamines **86** with cyclohexanylacetaldehyde (**87**) (*29*).

5.2.1.1.2. Cyclization of Angulary Arylated Hydroindole Derivatives (Route C(b))

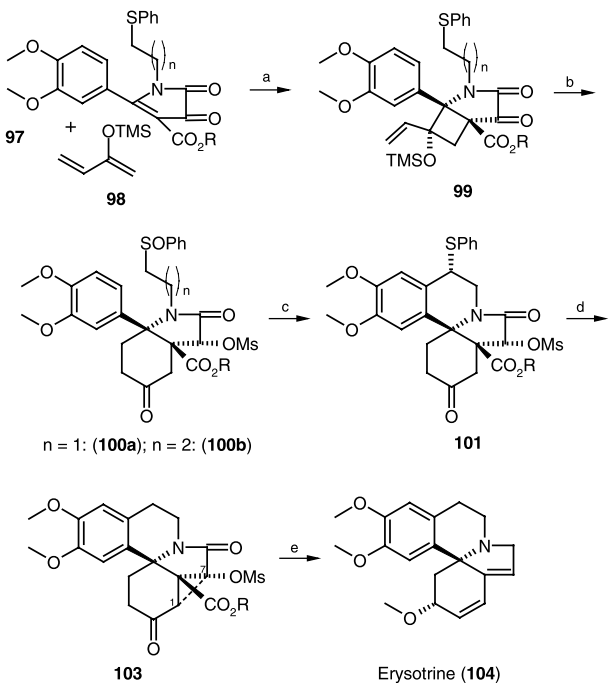
Alternatively, C-ring formation has been achieved by connecting the atoms C11 and C12 of the erythrinane core. This route principally involves the generation of angularly arylated hydroindoles, their N-alkylation with an appropriate C_2-unit followed by the final cyclization step. Thus the hydroindole **93**, accessible from 5-(nitromethyl)-1,3-benzodioxole (**91**) and the oxoheptenoic acid ester **92**, has been reacted in a hetero Michael addition with phenyl vinylsulfoxide providing the required N-substituted precursor **94**. The latter in turn has been cyclized in a Pummerer reaction to



Scheme 12. Synthesis of *cis*- and *trans*-15,16-methylenedioxy-8-oxo-erythrinane (**96**) (*32*): a) *i*: Amberlyst A-21 in Et_2O; *ii*: $EtO_2CN^-SO_2N^+Et_3$ in benzene/Δ; *iii*: $NaBH_3CN$ in AcOH/CH_3OH; *iv*: H_2/Raney-Ni in CH_3OH, then refl. in toluene; b) CH_2=CH-SOPh/$[(CH_3)_3Si]_2NNa$ in THF/$-78°$ to 20°C; c) Ac_2O/refl., then $SnCl_4$ in CH_2Cl_2; d) Bu_3SnH/AIBN in toluene/Δ

afford a *cis*/*trans* mixture of the 11-thiophenylether **95** as main product together with some sulfur-free 10,11-dehydrogenated material. Desulfuration with tri-*n*-butyl-stannane/azodiisobutyronitrile (AIBN) leads to 15,16-methylenedioxy-8-oxoerythrinane (**96**). Starting from the diastereomeric pure hydroindoles **93** this sequence is useful to prepare both stereomers of the erythrinane **96** (*32*) (Scheme 12).

Similar phenylhydroindole precursors have been prepared more efficiently by a strategy (Scheme 13), which generally allows the construction of the A ring of the erythrinane as well as of the homoerythrinane core. The basic feature of this pathway involves the Diels-Alder addition, or alternatively, the [2 + 2] photocycloaddition of functionalized 1,3-butadienes to yield suitably substituted phenylpyrroles. This reaction followed by 1,3-anionic rearrangement preforms at least the A/B-moiety of the alkaloid frameworks. In the actual case the [2 + 2] cycloaddition of the diene **98** to the dioxophenylpyrrole **97** possessing a sulfanylethyl



Scheme 13. General synthesis of erythrinanes (**104**) (*65*): a) $h\nu$ in DME/0°C; b) *i*: $NaBH_4$ in CH_3OH/−30°C; *ii*: TBAF in THF/−30°C; *iii*: MsCl in pyr/20°C, then $NaIO_4$ in $H_2O/CH_3OH/CH_2Cl_2$, 0°C; c) TFAA in CH_2Cl_2/20°C; d) *i*: Bu_3SnH/AIBN in toluene/Δ; *ii*: DBU in toluene/Δ; e) according to Ref. (*76*)

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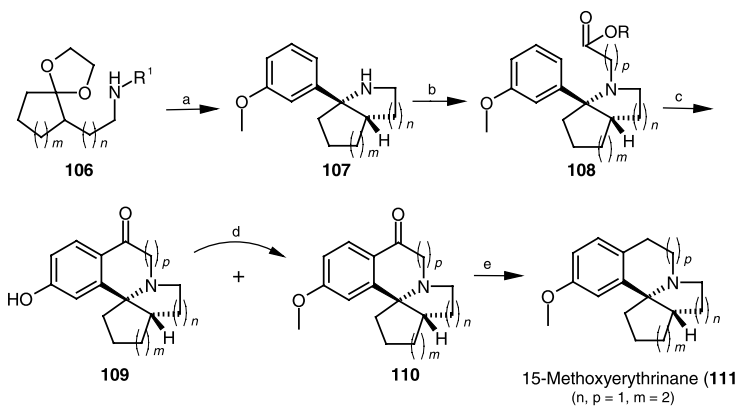
unit at the nitrogen atom, affords the vinylcyclobutane derivative **99** in high yield. The 1,3-rearrangement followed by transformations of the sulfanyl- and the C3-carbonyl groups provide the phenylhyroindoles **100**, which in turn have been cyclized – analogously to Scheme 12 – by Pummerer reaction giving the erythrinane derivative **101**. This can be converted to the 1,7-cycloerythrinane derivative **103** which allows to generate the alkaloid erysotrine (**104**) (*65*). The same route is applicable to prepare the corresponding homoerythrinane alkaloids (see Scheme 37).

Both, the photo- and the Diels-Alder cycloadditions have been also extended to pyrroloisoquinolines representing one of the most efficient strategies for the construction of the erythrinane as well as the homo-erythrinane cores according to route A(a) (see below *e.g.* Schemes 19 and 39).

An new alternative route for the synthesis of erythrinanes using the Friedel-Crafts acylation for C-ring completion is outlined in Scheme 14. The arylhydroindoles **107** (m = 2, n = 1), diastereoselectively prepared from the corresponding cycloalkanoylalkylamines **106** by Strecker and subsequent Bruylants reaction (m = 2; n = 1), have been N-alkylated with bromoacetic acid ester yielding the required precursor **108**, which on treatment with trifluoromethanesulfonic acid cyclizes to a separable mixture of the *cis*-11-oxoerythrinanes **109** and **110**. Realkylation of the phenolic group in **109** with diazomethane affords the methoxyketones **110** in low to moderate total yields. Contrarily, an attempted cyclization of the precursor lacking an activating *para* substituent in the aromatic



Scheme 14. Synthesis of 15-methoxyerythrinane (**111**) (*28*): a) *i*: 2 *N* HCl/H_2O, then $KCN/H_2O/20°C$; *ii*: ArMgX/THF, then toluene/Δ; b) $BrCH_2CO_2Et/Na_2CO_3$ in $CHCl_3/\Delta$; c) $F_3CSO_3H/110°C$; d) excess CH_2N_2 in $Et_2O/CH_3OH/20°C$; e) H_2/Pd in $Ac_2O/HClO_4$, 60°C, $6.5 \cdot 10^6$ Pa

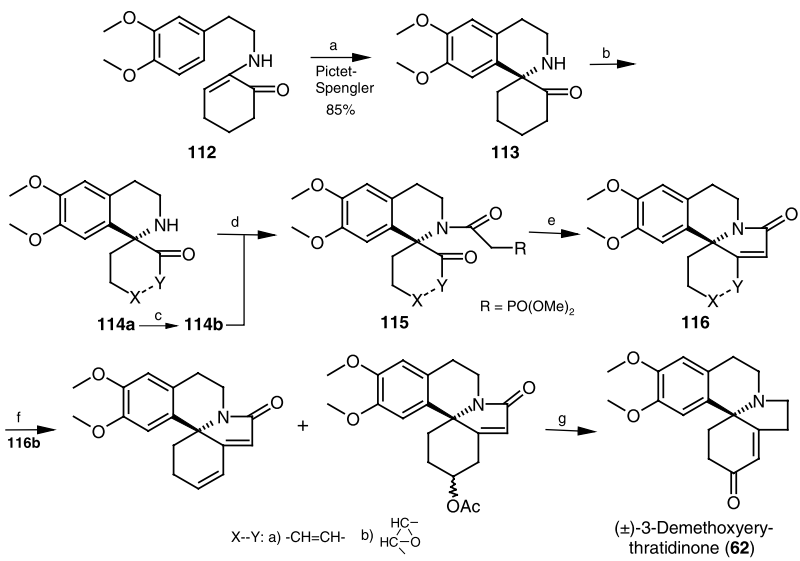
unit failed. The same is true for educts possessing dihydroxylated aromatic moieties, *e.g.* dimethoxy or methylenedioxy functions. Finally, the carbonyl group is catalytically removed to obtain the parent 15-methoxyerythrinane (**111**). The sequence can be extended to the synthesis of related ring homologue frameworks, *e.g.* to schelhammerane (see Scheme 38), B-homoerythrinane (m, n = 2; p = 1), A-norerythrinane (m, n, p = 1), and to the hitherto unknown A-norschelhammerane (m, n = 1; p = 2) using the corresponding educts as shown in Scheme 14 (*28*).

5.2.1.2. Formation of Ring B (Route B)

Various strategies have been examined to complete the erythrinane framework by B ring generation in the last step, starting from appropriate C5-spiroisoquinolines as depicted in Scheme 3 [route B(a) or B(b)].

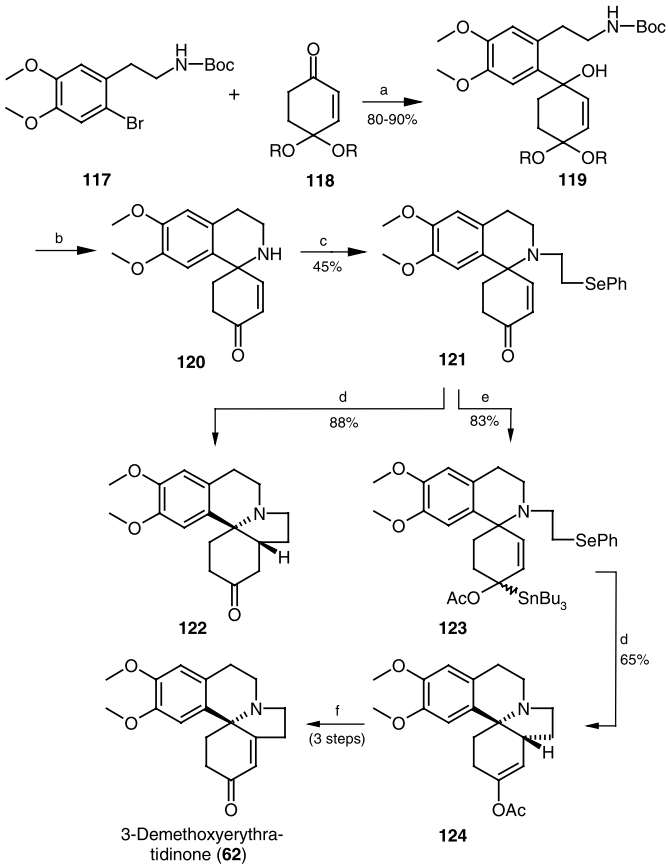
5.2.1.2.1. Cyclization of N-Substituted C5-Spiroisoquinoline Derivatives (Route B(a))

The spiroamine **113** conveniently available by a Pictet-Spengler like cyclization of the enamine **112** (*66*) has been alternatively converted to



Scheme 15. Synthesis of (±)-3-demethoxyerythratidinone (**62**) (*67*): a) TFA/Δ; b) *i*: $(tBuO)_2CO$ in $CHCl_3$/refl.; *ii*: LDA/PhSeCl in THF; *iii*: $NaIO_4$ in EtOH; *iv*: TFA in CH_2Cl_2; c) H_2O_2/NaOH in CH_3OH; d) $(MeO)_2P(O)–CH_2COCl$/pyr in CH_2Cl_2; e) aqueous KOH/benzene/20°C; f) Zn/AcOH/Ac_2O; g) *i*: AlH_3 in THF; *ii*: Swern oxidation

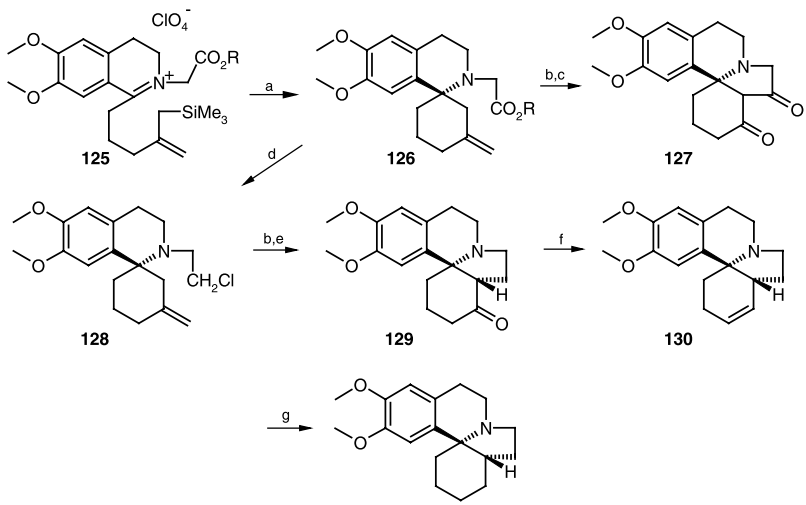
the corresponding α,β-unsaturated ketone **114a** or by an additional step to the epoxide **114b** followed by N-acylation with dimethyl phosphonoacetyl chloride providing the phosphonates **115a** and **115b**. Intramolecular Wadsworth-Emmons reaction smoothly gives the erythrinanes derivatives **116a** and **116b** in good yields. The latter has been transformed to (±)-demethoxyerythratidinone (**62**) by several further steps involving the reductive cleavage of the epoxide moiety, the $LiAlH_4$ reduction of the amide unit, and Swern oxidation of the hydroxy function with concomitant migration of the double bond (*67*) (Scheme 15).



Scheme 16. Synthesis of (±)-demethoxyerythratidinone (**62**) (*68*): a) KH/THF/25°C, then *n*-BuLi in THF/−78°C; b) *i*: $(CH_3)_3SiOTf$ in CH_2Cl_2/−78°C; *ii*: DBU; c) $PhSeCH_2CHO/NaBH_3$ CN in THF/MeOH; d) Bu_3SnH/AIBN in benzene; e) *i*: Bu_3SnLi in Et_2O/−78°C; *ii*: Ac_2O/Et_3N/DMPA in CH_2Cl_2; f) *i*: CH_3Li in THF; *ii*: PhSeCl/−78°C; *iii*: $NaIO_4$ in THF/H_2O

Starting with a spiroaminoketone **120** related to **113** (Scheme 15) the B ring construction has been performed via a Michael like free radical cyclization route (Scheme 16). Thus, **120** has been reductively alkylated with phenylselenoacetaldehyde affording the N-alkylated precursor **121**, which gives in the presence of tri-*n*-butylstannane/AIBN the 2-oxoerythrinane **122** in high yield. Contrarily, treatment of **121** with tri-*n*-butyllithium-thiostannane followed by acetanhydride leads to an 1:1 mixture of the diastereomeric acetoxystannanes **123**, which have been radically cyclized under the same conditions yielding the erythrinane enolester **124**. This can be converted to 3-demethoxyerythratidinone (**62**) in a three step sequence (*68*). The educt **120** has been readily prepared by transformation of the teriary alkohol **119**, which is available from the N-protected 2-bromohomoveratrylamine **117** and the enone ketal **118** (*69*).

Another alternative of this pathway offers the use of the C5-spiroisoquinoline **126** accessable from the dihydroisoquinolinium salt **125** by photocyclization (Scheme 17). Thus, the B ring can be completed by intramolecular Claisen condensation as well as by intramolecular C-alkylation giving the 1,7-dioxo- and the 1-oxoerythrinanes **127** and **129**. Removal of the CO-group via the $\Delta^{1,2}$-derivative **130** affords the *cis*-dimethoxyerythrinane (**90**) (*70*).



Scheme 17. Synthesis of 15,16-dimethoxyerythrinane (**90**) (*70*): a) *hν*/CH_3OH; b) OsO_4/$NaIO_4$ in dioxane/H_2O; c) NaOEt in EtOH/0°C; d) *i*: $LiAlH_4$ in Et_2O/0–25°C; *ii*: MsCl/Et_3N in Et_2O/25°C; e) DBU in THF/60°C; f) *i*: $LiAlH_4$ in THF/0° to 25°C; *ii*: MsCl/Et_3N in Et_2O/THF; *iii*: DBU in THF/80°C; g) H_2/Pd in THF/25°C/10^5 Pa

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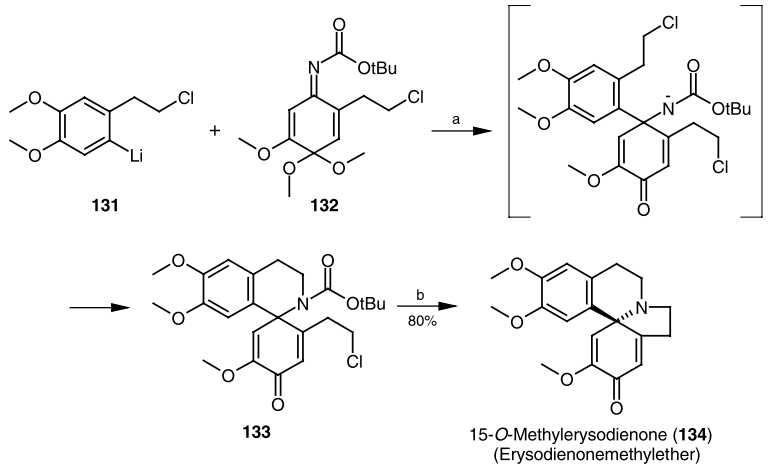


Scheme 18. Synthesis of 15-*O*-methylerysodienone (**134**) (*71*): a) −78°C to reflux temp.; b) F_3CCO_2H/*p*-Ts OH/20°C, then KOH in H_2O/THF

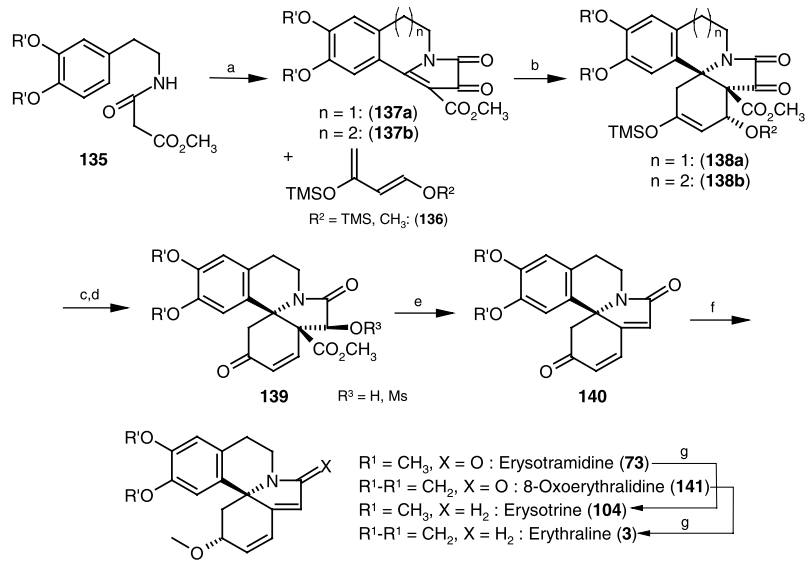
5.2.1.2.2. Cyclization of C6-Substituted C5-Spiroisoquinoline Derivatives (Route B(b))

The final B ring formation by intramolecular N-alkylation requires a C_2 side chain attached to the A ring of the C5-spiroisoquinoline educt, *e.g.* **133**. This has been similarly obtained as the related educt **119** (Scheme 16) by reaction of the lithiated phenethyl derivative **131** with the N-protected quinone imide ketal **132**. After deprotection the crude amine undergoes cyclization to form the 15-*O*-methylerysodienone (**134**) in 80% yield (*71*) (Scheme 18).

5.2.1.3. Formation of Ring A (Route A)

5.2.1.3.1. Cycloaddition to Pyrroloisoquinolines (Route A(a))

Formation of the A ring to complete the tetracyclic erythrinane core has been achieved starting from appropriate pyrroloisoquinolines. The most important routes are based – as already mentioned above (Scheme 13) – on the Diels-Alder reaction, or alternatively, on a [2 + 2] photocycloaddition between O-functionalized 1,3-butadiene derivatives, preferably 1,3-bis (trimethylsilyloxy)butadiene or 1-methoxy-3-trimethylsilyloxybutadiene (**136**), and dioxopyrroloisoquinolines (**137a**) (Scheme 19). Thus, the Diels-Alder method regio- and stereoselectively gives the angularly

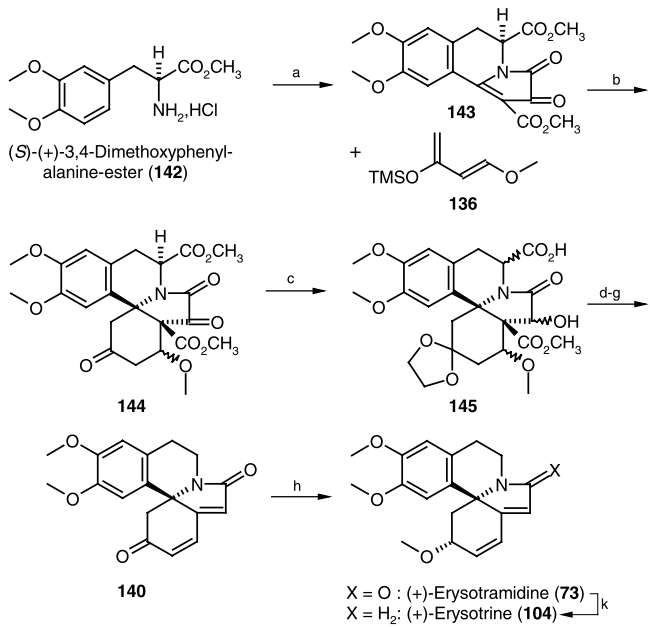


Scheme 19. Synthesis of dienoid type erythrinanes (**3**, **73**, **104**, **141**) (*72*): a) *i*: PPE in $CHCl_3$/reflux; *ii*: $(COCl)_2$ in Et_2O/0°C; b) sealed tube/Δ; c) $LiBH_4$ in THF/−60°C, then HCl/THF/reflux; d) MsCl/pyr, 20°C; e) $MgCl_2$ in DMSO/Δ, sealed tube; f) *i*: Al(*i*-PrO)$_3$ in *i*-PrOH/reflux, PTLC of 3-OH-isomers; *ii*: KOH/Et_4NBr/CH_3I in THF, 20°C; g) AlH_3 in THF/20°C

6-carboxylated erythrinanediones **138a** in high yields. These are closely related to those obtained according to route C(a), *e.g.* **61** (Scheme 6) and can be preferably transformed via the intermediates **139** and **140** to the 1,6-dienoid type erythrinanes **3**, **73**, **104**, and **141**. The educts **137a** are readily prepared by the reaction of a phenethylamine with chloroformylacetat providing the amides **135** followed by sequential Bischler-Napieralski reaction and oxalylation (*72*).

Starting from 3,4-dimethoxyphenyl-L-alanine ester (**142**) the sequence of Scheme 20 has been proved to be suitable for the preparation of enantiomeric products via **144** → **145** → **140**, the latter represents a valuable educt to natural alkaloids, *e.g.* (+)-erysotrine (**104**) or (+)-erysotramidine (**73**) (*73*, *74*).

On the other hand, applying the [2 + 2] photocycloaddition method already described in Scheme 13, the pyrroloisoquinoline **137a** provides the related highly functionalized 2,8-dioxoerythrinane (**147a**) through the analogue cyclobutane intermediate **146**. Further transformation of **147a** leads to erysotramidine (**73**) and erysotrine (**104**) (*75*, *76*) as shown

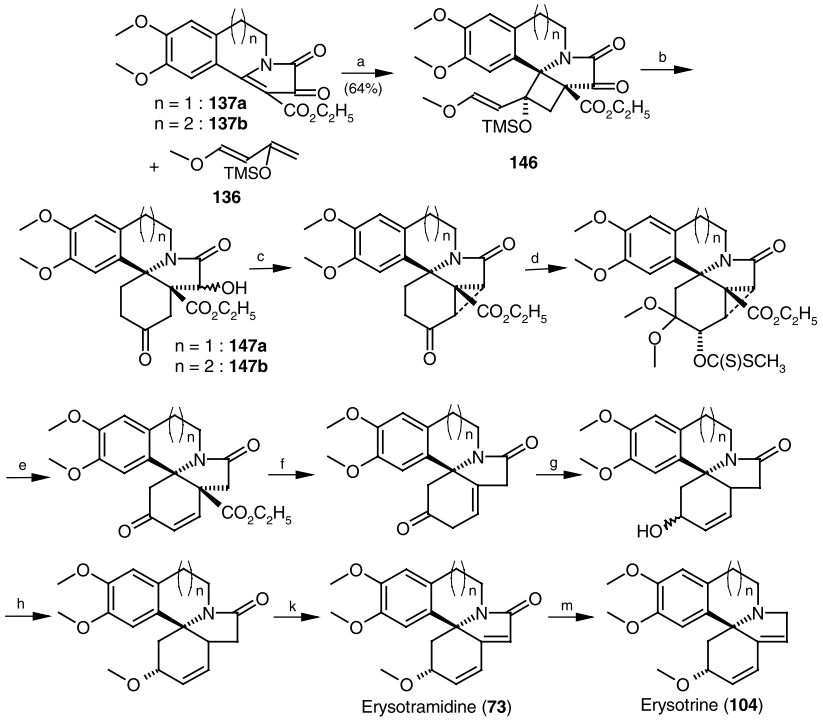


Scheme 20. Chiral synthesis of erythrinanes (**73**, **104**) (*73*, *74*): a) *i*: $ClCOCH_2CO_2CH_3$/K_2CO_3 in Et_2O/H_2O, −15° to 10°C; *ii*: PPE/100°C; *iii*: $(COCl)_2$ in Et_2O/CH_2Cl_2/0°C; b) 109 Pa in CH_2Cl_2/20°C, then treatment with SiO_2; c) *i*: ethyleneglycol/*p*-TsOH in benzene/reflux; *ii*: $NaBH_4$ in CH_3OH/0°C; *iii*: NaOH in CH_3OH/20°C; d) *i*: *N*-methylmorpholine, *i*-Bu-chloroformate in THF/−10°C; *ii*: *N*-hydroxypyridinethione-Na/Et_3N in THF/−10°C; *iii*: *hν*/*t*-BuSH in THF/0°C; e) HCl in THF/80°C, then PTLC of 7-OH-stereomers; f) MsCl/pyr; g) $CaCl_2$ in DMSO/Δ; h) *i*: $NaBH_4/CeCl_3$ in CH_3OH/20°C; *ii*: CH_3I/KOH/Et_4NBr in THF/20°C; k) $AlH_3/AlCl_3$ in EtOH/THF/0°C

in Scheme 21. In a similar manner the corresponding schelhammerane intermediates **147b** are accessable, when starting from pyrrolobenzazepines **137b** instead of the pyrroloisoquinolines, which open an approach to natural alkaloids (*cf.* Scheme 39 below) (*77*, *78*).

5.2.1.3.2. Intramolecular Aldol Condensation of Angularly Substituted Pyrroloisoquinoline (Route A(b))

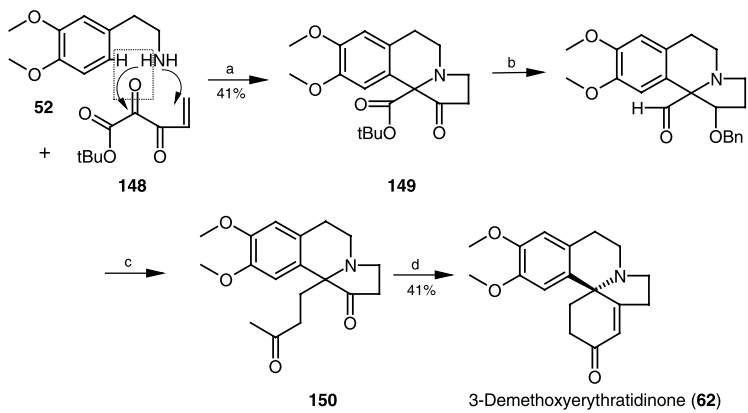
From the pyrroloisoquinoline carboxylic acid ester **149** (Scheme 22) generated from homoveratrylamine (**52**) and dioxopentenoic acid ester (**148**) by a combined Michael addition/condensation, the A ring has been constructed by an intramolecular aldol condensation of the diketone **150** affording 3-demethoxyerythratidinone (**62**) (*79*).



Scheme 21. Synthesis of erythrinanes (n = 1) (**73**, **104**) (*75*, *76*): a) *hν* in acetone/0°C; b) *i*: $NaBH_4$ in EtOH/0°C; *ii*: TBAF in THF/−30°C; *iii*: H_2/Pd in acetone; c) *i*: MsCl in pyr/20°C; *ii*: DBU in toluene/reflux; d) *i*: $PhSeCl/Et_2O \cdot BF_3$ in THF/reflux, then $Hg(ClO_4)_2$ in CH_3OH/0°C; *ii*: $NaBH_4$ in CH_3OH, then HCl; *iii*: NaH/imidazole/CS_2 in THF/reflux; e) Bu_3SnH/AIBN in toluene/reflux, then HCl in acetone/50°C; f) $CaCl_2$/3-ethylpentane-3-thiol in DMSO/Δ, sealed tube; g) *i*: DBU in benzene/Δ; *ii*: $NaBH_4/CeCl_3$ in CH_3OH/0°C, MPCL of 3-OH stereomers; h) *i*: NaH/imidazole/Bu_4NHSO_4/CH_3I/70°C; k) LDA/PhSeCl in THF, then $NaIO_4$ in CH_3OH/H_2O; m) AlH_3 in Et_2O/THF/20°C

5.2.2. Simultaneous Formation of More Than One Ring

Tandem and multicascade processes, more accurately also called domino reactions, belong to a growing group of reactions, which often allow an efficient regio- and stereoselective approach to complex compounds and ring systems in a single operation (*80*). This strategy also has been successfully applicated to construct the alicyclic rings A, B, and C of the erythrinane framework. Strategies reported in this field can be classified as depicted in Scheme 3. The final ring formation widely utilized in former as well as in recent investigations has been conducted



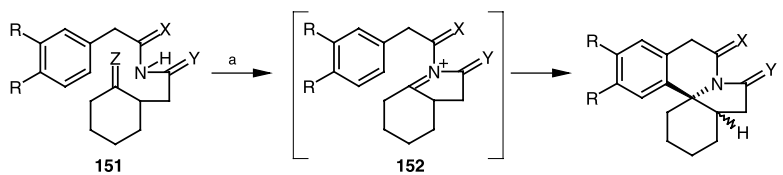
Scheme 22. Synthesis of 3-demethoxyerythratidinone (**62**) (*79*): a) in CH_2Cl_2, then $POCl_3$; b) *i*: $NaBH_3$ CN in AcOH/0° to 20°C; *ii*: NaH/BnBr in THF; *iii*: $LiAlH_4$ in Et_2O/0°C; c) *i*: $(MeO)_2P(O)CH_2COCH_3$/NaH in toluene/reflux; *ii*: H_2/Pd in acetone/$3.8 \cdot 10^5$ Pa, then H_2/Pd/catal. HCl in acetone; *iii*: $NaBH_4$ in CH_3OH/0°C; *iv*: $(COCl)_2$/DMSO in CH_2Cl_2/−78°C, then Et_3N/20°C; d) NaOH in CH_3OH/reflux

following route B/C. In contrast, only few syntheses have been accomplished hitherto based on the routes A/B and A/B/C.

5.2.2.1. Simultaneous Formation of Rings B and C (Route B/C)

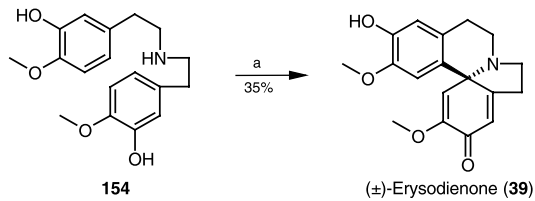
5.2.2.1.1. Cyclization of Secondary Diphenethyl- or (Cycloalkyl)ethyl-phenethylamine Derivatives (Route B/C(a))

The first syntheses of the erythrinane framework reported by Belleau (*81*, *82*) and Mondon (*83–86*) have been performed using cyclization of the isomeric ketalamides **151** with polyphosphoric acid via the assumed



R = H; X = O; Y = H_2; Z = O: 10-Oxoerythrinane (**153**)
R = OCH_3; X = H_2; Y = O; Z = $(R'O)_2$: Demethoxytetrahydroerysotramidine (**68**)
R = OCH_2O; X = H_2; Y = O; Z = $(R'O)_2$: 15,16-Methylenedioxy-8-oxoerythrinane (**96**)

Scheme 23. Synthesis of 8- and 10-oxoerythrinanes (**68**, **96**, **153**) (*83–86*): a) P_4O_{10}/PPA or PPA in varying concentrations, 20° to 100°C



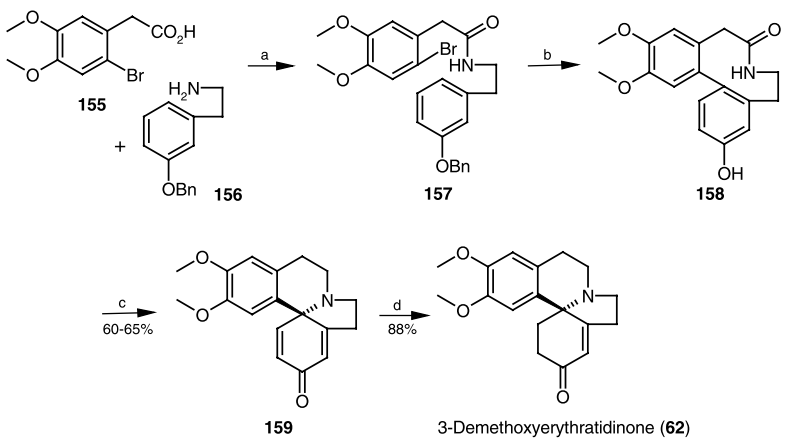
Scheme 24. Synthesis of (±)-erysodienone (**39**) (*87*, *88*): a) $K_3[Fe(CN)_6]/Na_2CO_3$ in H_2O/CH_2Cl_2

intermediate *N*-acyliminium ion (**152**) giving the oxoerythrinanes **68**, **96**, and **153** (Scheme 23).

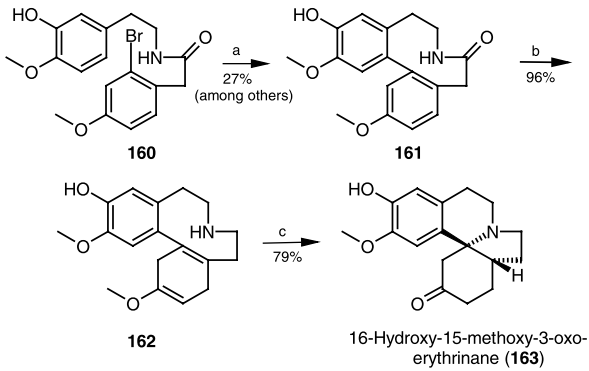
The same type of simultaneous C5/C13 and C5/C9 bond formations has been effected in several biomimetic syntheses starting from symmetrical phenolic bases **154**, which on oxidative coupling provide the expected (±)-erysodienone (**39**) (*87*, *88*) as shown in Scheme 24.

In a further biomimetic approach (Scheme 25), the phenolic educt **157**, related to **154** of Scheme 24, and conveniently available from the corresponding phenylacetic acid **155** and the phenethylamine **156**, first has been photochemically converted to the dibenzazocine **158** followed by oxidative cyclization to the corresponding 3-demethoxy-Δ^3-erythratidinone (**159**). This in turn can be selectively hydrogenated yielding 3-demethoxyerythratidinone (**62**) (*89*).

In this connection nonoxidative cyclization strategies are of general interest. They start also from diphenethylamines and involve a Birch



Scheme 25. Synthesis of 3-demethoxyerythratidinone (**62**) (*89*): a) in decaline/reflux; b) *i*: *hν* in NaOH/CH_3 OH; *ii*: $NaBH_4/Et_2O \cdot BF_3$ in THF; c) PbO_2 in benzene or $K_3[Fe(CN)_6]/NaHCO_3$ in $CHCl_3/H_2O$; d) H_2/Pd in EtOH/20°C, 10^5 Pa

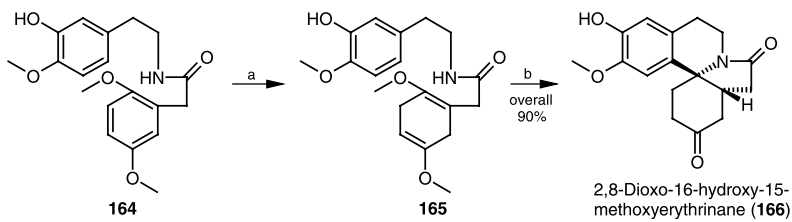


Scheme 26. Synthesis of 16-hydroxy-15-methoxy-3-oxoerythrinane (**163**) (*90*, *91*): a) $h\nu$ in $CH_3OH/NaOH$; b) *i*: $NaBH_4/Et_2O \cdot BF_3$; *ii*: Na/NH_3 in $CH_3OH/Et_2O/THF/-60°$ to 70°C; c) 10% H_2SO_4 in DMF/60°C

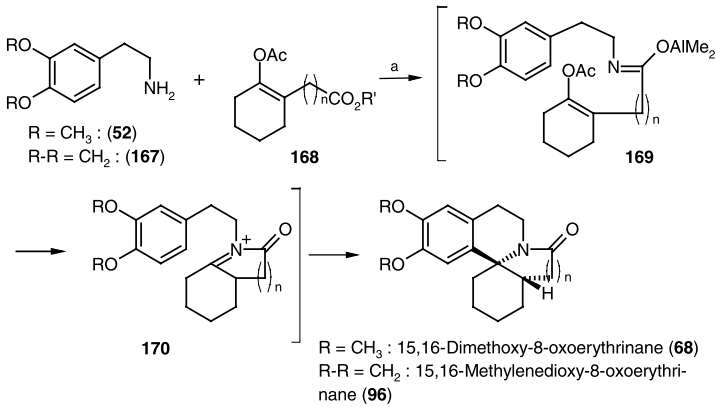
reduction as a main feature. Thus, the dibenzazocine **161**, obtained by photocyclization of **160**, gives the Birch 1,4-diene product **162**, which has been cyclized with diluted sulfuric acid affording the 16-hydroxy-15-methoxy-3-oxo-erythrinane **163** (Scheme 26).

On the other hand, the related educt **164** has been immediately reduced leading to the corresponding 1,4-cyclohexadiene **165**, which on treatment with acid provides the 2,8-dioxo-16-hydroxy-15-methoxyerythrinane (**166**) in 90% yield (*90*, *91*) (Scheme 27).

In a new attractive B/C(a) route the required N-disubstituted amine derivatives, *e.g.* the metalated carboxamides **169**, have been generated *in situ*, reacting at first the primary phenethylamins **52** or **167** with trimethylaluminum followed by the enolacetates of cyclohexanoyl carboxylic acids **168**. The intermediates **169** eliminate acetic acid affording the *N*-acyliminium ions (**170**), which cyclize to the desired erythrinanes **68** and **96**. Due to the typical ^1H NMR shift of 14-H given ($\delta = 6.93$ ppm (*31*)) the products should possess the B/C *cis*-configura-



Scheme 27. Synthesis of 2,8-dioxo-16-hydroxy-15-methoxyerythrinane (**166**) (*90*, *91*): a) Birch reduction; b) 10% H_2SO_4 in DMF

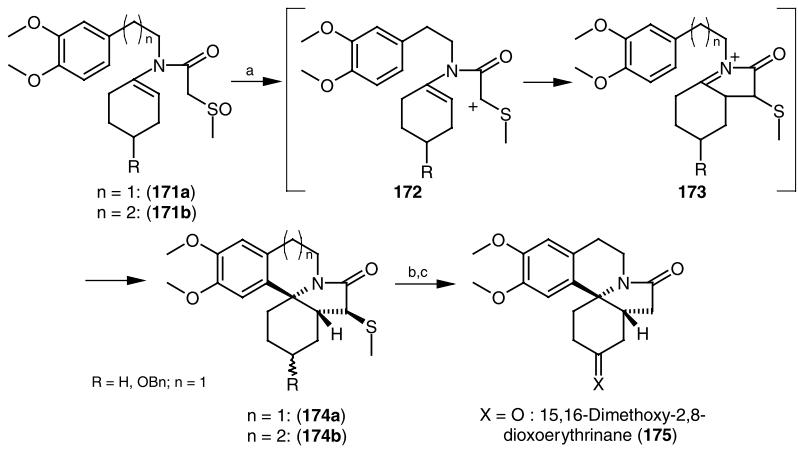


Scheme 28. Synthesis of 15,16-dioxygenated-8-oxo-erythrinanes (**68**, **96**; n = 1) (*92*): a) 2 equiv. of $Al(CH_3)_3$ in benzene/Δ

tion. This pathway of Scheme 28 opens an efficient approach to the erythrinane as well as to the B-homoerythrinane core (n = 1 or 2) in a single step (*92*).

5.2.2.1.2. Cyclization of Tertiary Cyclohexyl-ethyl-phenethyl-amide Derivatives (Route B/C(b))

Domino sequences especially induced by the Pummerer reaction are well known to be very useful for the construction of complex polycyclic



Scheme 29. Synthesis of oxoerythrinanes (**175**) (*94*): a) *p*-TsOH in CH_2Cl_2/reflux; b) Raney-Ni in EtOH; c) CrO_3 in pyr/CH_2Cl_2 (R = OBn)

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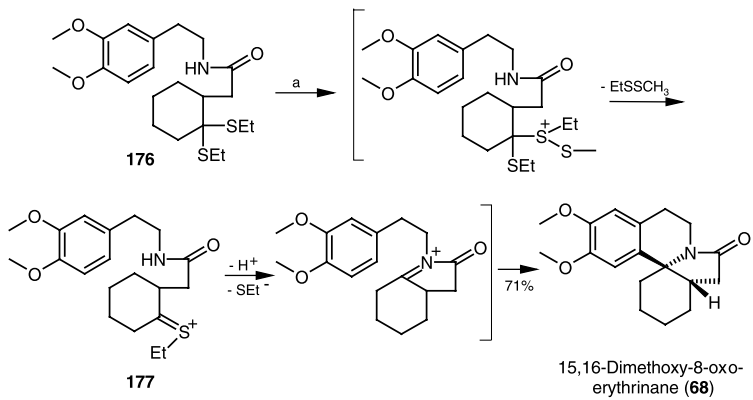
compounds (*cf. e.g.* (*93*)). They also have been successfully applied to the construction of the erythrinane core starting from relative simple building blocks. For instance, in a one step synthesis the sulfoxide **171a** (Scheme 29) undergoes sequential B/C ring cyclization via thionium/acyliminium intermediates **172** and **173** yielding 50–60% of the erythrinane derivative **174a**, which can be transformed to the 2,8-dioxo-derivative **175**. 15,16-Dimethoxy-8-oxo-erythrinane (X=H_2; **68**) is available using the same route (*94*).

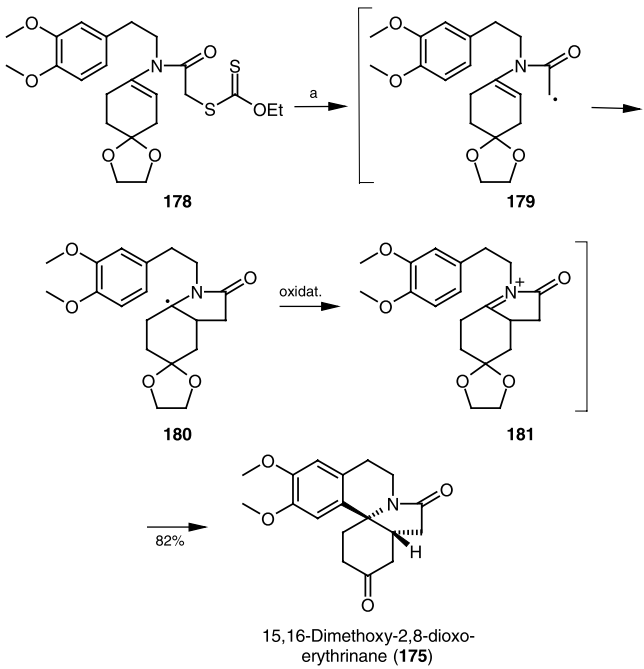
Since thionium ions, *e.g.* **177** also can be generated by treatment of thioacetals or thioketals with dimethyl(methylthio) sulfonium tetrafluoroborate (DMTSF), a similar cyclization sequence occurred when the amido thioketal **176** was used as an educt (Scheme 30). Starting from the educt **176** the ring formation according to route B/C(a) takes place giving 71% of 15,16-dimethoxy-8-oxoerythrinane (**68**) in one step (*95*).

Furthermore, sulfur containing compounds, especially α-S-functionalized acetamide derivatives are suitable precursors to assemble the erythrinane framework via a combined radical/acyliminium ion cyclization. Thus, treatment of the xanthate **178** (Scheme 31) with lauroyl peroxide causes B ring generation via the radicals **179** and **180**. After further oxidation of the latter forming the acyliminium ion **181**, catalytical amounts of *p*-toluenesulfonic acid induces the ring closure to the aromatic unit furnishing 15,16-dimethoxy-2,8-dioxoerythrinane (**175**) in 82% yield (*96*).

In the same manner the radical B ring formation also can be induced by manganese(III) acetate in the presence of copper(II) acetate or copper(II) triflate. Thus, the α-(methylthio) acetamide precursor **182**



Scheme 30. Synthesis of 15,16-dimethoxy-8-oxoerythrinane (**68**) (*95*): a) $[(CH_3)_2S^+SCH_3]BF_4^-$ (DMTSF) in CH_2Cl_2/reflux



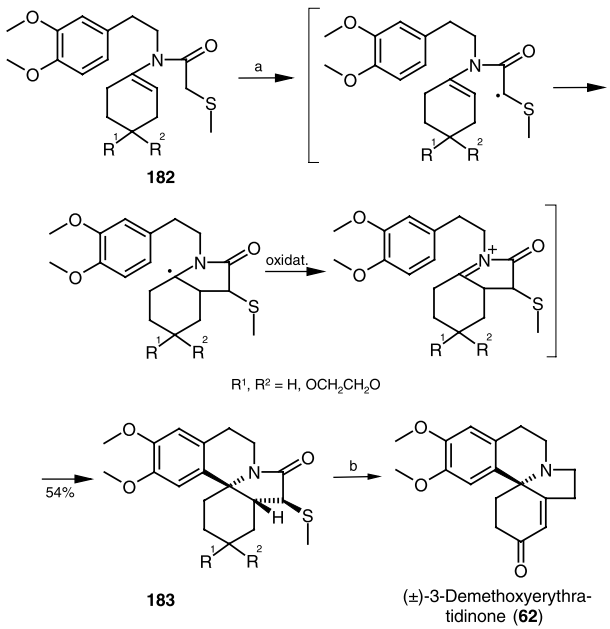
Scheme 31. Synthesis of 15,16-dimethoxy-2,8-dioxo-erythrinane (**175**) (*96*): a) lauroylperoxide in CH_2Cl_2/reflux, then catalytic amounts of *p*-TsOH/reflux

(Scheme 32) provides in only one step the erythrinane **183** (*97*), which allows the access to the naturally occurring (±)-3-demethoxyerythratidinone (**62**) (*98*).

5.2.2.2. Cyclization of N-Substituted 1-Acyldihydroisoquinolinium Derivatives (Route A/B)

Until now only one example is reported concerning a simultaneous A/B-ring construction for the synthesis of the erythrinane skeleton. The required key intermediate 1-acyldihydroisoquinoline **186** (Scheme 33) has been conveniently prepared by reaction of homoveratryl isonitrile **184** with appropriate carboxylic acid halogenides, as *e.g.* 5-hexenoyl chloride (**185**). On treatment of **186** with trimethylsilylmethyltriflate followed by cesium fluoride the intermediately generated azomethinylide **187** undergoes [3 + 2] cycloaddition affording the 4-oxoerythrinane **188** in 70% yield. In the same manner precursors involving an acetylenic dipolarophile, *e.g.* the 1-hexynoyldihydroisoquinoline **189** provide the corresponding $\Delta^{6,7}$-oxoerythrinane **190** in 42% yield (*99*).

References, pp. 56–62



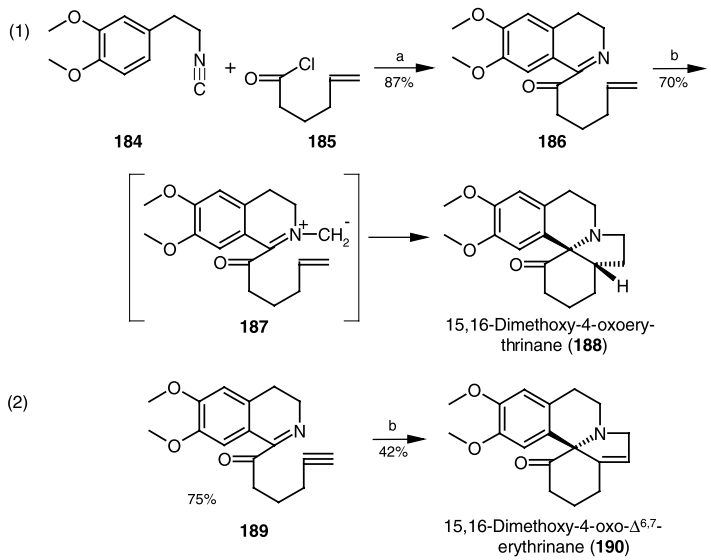
Scheme 32. Synthesis of (±)-3-demethoxyerythratidinone (**62**) (*97*, *98*): a) 6–10 equiv. $Mn(OAc)_3$, 1 equiv. $Co(OTf)_2$ in F_3CCH_2OH/reflux; b) i: $NaIO_4$ in H_2O/acetone/0°C, then $NaHCO_3$/toluene/reflux; *ii*: $LiAlH_4/AlCl_3$ in Et_2O/THF, −15°C; *iii*: HCl in acetone/reflux

5.2.2.3. Cyclization of a Highly Functionalized Homoveratrylimide (Route A/B/C)

Finally, there is also only a single report describing the sequential formation of the rings A, B, and C of the erythrinane framework in one step. Starting from the complex homoveratrylimide derivative **191** this triple cascade process involves – apart from the initial Pummerer reaction **191** → **192** – the Diels-Alder reaction **192** → **193** as well as the final acyliminium ion cyclization **194** → **195** providing the erythrinane **195** in 83% yield. This in turn could be converted to (±)-erysotramidine (**73**) by a sequence already reported (*76*) (Scheme 34). The requisite educt **191** has been smoothly prepared through six steps in 45% overall yield (*80*).

5.3. Homoerythrinanes

The construction of the C-homoerythrinane framework is far more difficult than that of the erythrinane, that is, methods developed for



Scheme 33. Synthesis of 4-oxoerythrinanes (**188**, **190**) (*99*): a) in CH_2Cl_2/25°C, then AgOTf in CH_2Cl_2/−20°C; b) $(CH_3)_3SiCH_2OTf$ in CH_2Cl_2/25°C, then CsF in 1,2-DME/65°C

the synthesis of erythrinanes are – apart from very few exceptions (s. below) – not automatically transferable to schelhammerane synthesis. For instance, the parallel approaches using several homologue educts or intermediates of the erythrinane synthesis, *e.g.* the *N*-arylpropyl-enamides **171b** as well as the corresponding dioxopyrrolobenzazepine **137b** failed to give the target compounds **174b** or **138b** at all or afford them only in less than 5% yield on acid-mediated cyclization or on Diels-Alder reaction with trimethylsilyloxybutadiene (*cf.* Schemes 19 and 29) (*59*, *100*, *101*). This substantial difference concerning the synthesis of both ring systems is attributed to the skewed nature of the sevenmembered nucleus. In this connection MMX force field calculations (PC Model (*102*)) are of interest revealing the parent *cis*-erythrinane to be essentially more stable than the corresponding C-homologue schelhammerane. Assuming the chair form of the benzocycloheptene core (*103*) and the same relative configuration (5*S*,6*S*)/(5*R*,6*R*) comparable to that of the erythrinane skeleton (*cf.* Fig. 1) a revised value of the strain energy difference has been found to be $\Delta E_S = 31.5$ kJ/mol (*104*) (*vs* 41.5 (*29*)).

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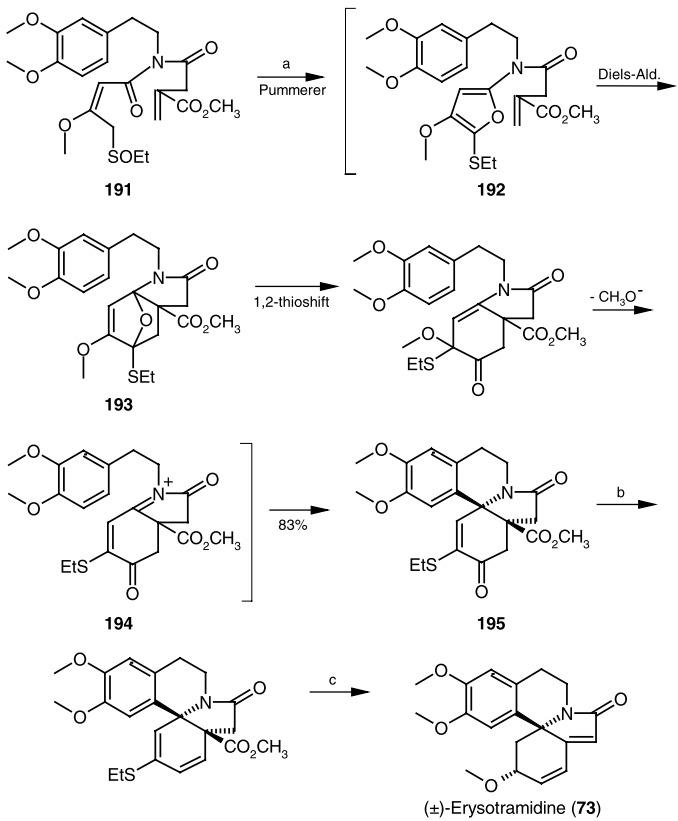
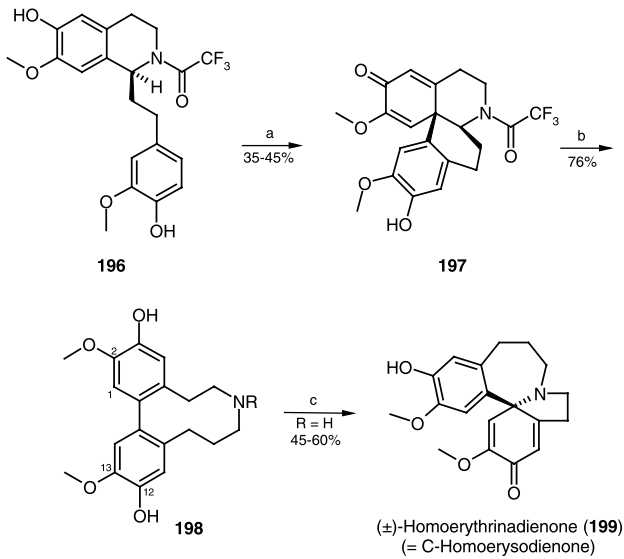


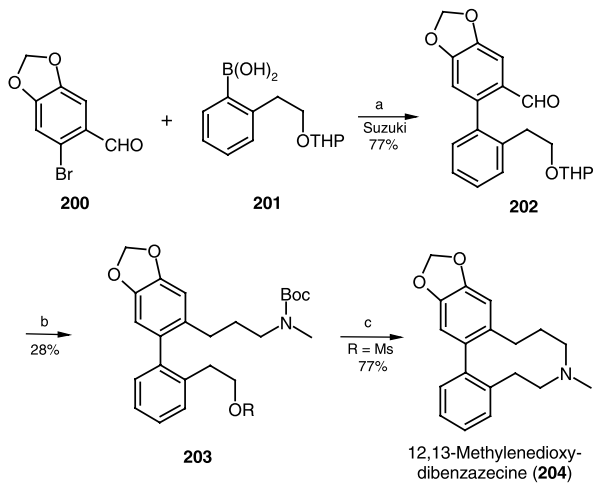
Scheme 34. Synthesis of (±)-erysotramidine (**73**) (*80*): a) $Et_3N/(F_3CCO)_2O/Et_2O \cdot BF_3$ in CH_2Cl_2/Δ; b) *i*: KH/PhNTf$_2$ in THF; *ii*: $(PPh_3)_2PdCl_2/NEt_3/HCO_2H$ in DMF/Δ; *iii*: $TiCl_4$ in AcOH; H_2O; c) transformation according to Ref. (*76*)

5.3.1. Biomimetic Routes

The first syntheses of the homoerythrinane framework are following the assumed biosynthetic route. Thus, the N-protected 1-phenethylisoquinoline **196** (Scheme 35) can be cyclized by a phenol oxidative procedure providing the tetracyclic naphthalenoisoquinoline **197**, a homologue of neoproaporphine (**35**) in 35–45% yield (*105*). This has been transformed to the dibenzazecine intermediate **198** by ring reopening. Finally, the N-deprotected free amine base can be oxidized giving homoerysodienone (**199**) (*106*, *107*). A diphenoquinone type intermediate **38** (Scheme 1; n = 2) is not available from the N-acylated base **198**.



Scheme 35. Synthesis of homoerysodienone (**199**) (*105–107*): a) $VOCl_3$ in CH_2Cl_2; b) *i*: *N* NaOH in CH_3OH; *ii*: $NaBH_4$ in EtOH; c) $K_3[Fe(CN)_6]/NaHCO_3$ in CH_2Cl_2



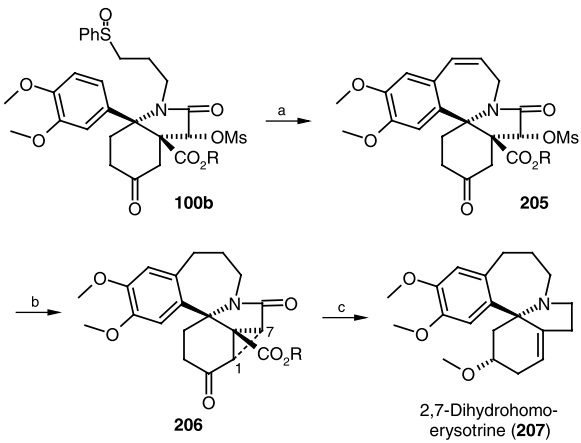
Scheme 36. Synthesis of 12,13-methylenedioxydibenzazecine (**204**) (*109*): a) $Pd(OAc)_2/PPh_3/NaHCO_3$ in dioxane/H_2O/reflux; b) *i*: $(EtO)_2P(O)CH_2CONHCH_3/(C_6H_{13})_4NI$ in NaOH; *ii*: Raney-Ni in $CH_3OH/10^5$ Pa; *iii*: $LiAlH_4$ in THF/reflux, then Boc_2O/Et_3N in CH_2Cl_2 ; c) *i*: AcOH in THF/H_2O/Δ, then $MsCl/Et_3N$ in CH_2Cl_2; *ii*: F_3CCO_2H/20°C, then *i*-Pr_2NEt in CH_3CN/reflux

References, pp. 56–62

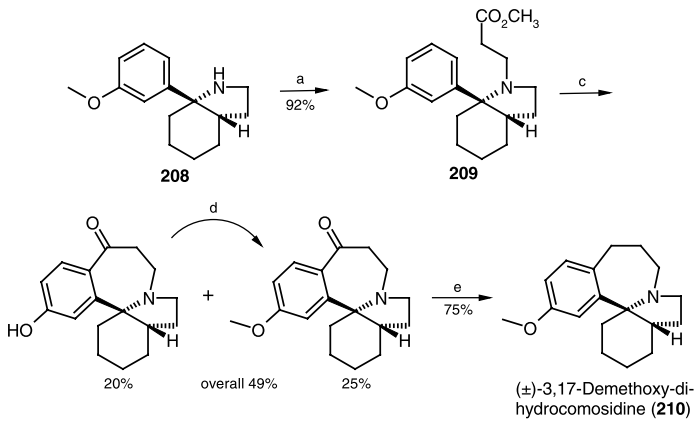
An analogous synthesis using dibenzazecine with unsymmetrically substituted aryl nuclei, *e.g.* the 3,12-dihydroxy-13-methoxy derivative (**198**, 2-H instead of 2-CH_3O; Scheme 35) is limited *a priori* because its inefficient preparation method leads to undesirable regioisomers (*108*). To overcome these limitations a Suzuki coupling procedure most recently developed should be particularly suitable for a more general approach to the mentioned dibenzazecine educts inclusively to their enantiomers, which offer an interesting new pathway to homoerythrinanes with flexible substitution patterns. Thus, 2-bromopiperonal (**200**) is reacted with the boronic acid **201** providing the Suzuki coupled biphenyl **202** (Scheme 36). Chain extension using the Wadsworth-Emmons method, followed by several transformation steps give the N-protected amine **203**, which can be cyclized to the target 12,13-methylenedioxydibenzazecine (**204**) (*109*).

5.3.2. Final C Ring Formation Starting from N-substituted Phenylhydroindoles

In contrast, the erythrinane synthesis based on the above mentioned cycloadditions of 1,3-butadienes to pyrrole derivatives is fully applicable to that of the ring C-homologue alkaloids. Thus, the phenylhydroindol **100b** prepared by [2 + 2] cycloaddition according to Scheme 13 cyclizes to yield the 2,8-dioxohomoerythrinane **205**, which then has been transformed via the 1,7-cyclointermediate **206** to 2,7-dihydrohomoerysotrine (**207**) (*110*) (Scheme 37).



Scheme 37. General synthesis of homoerythrinanes (**207**) (*110*): a) TFAA/TFA in CH_2Cl_2/20°C; b) *i*: DBU in toluene/Δ; *ii*: H_2/Pd in THF; c) according to Ref. (*77*)



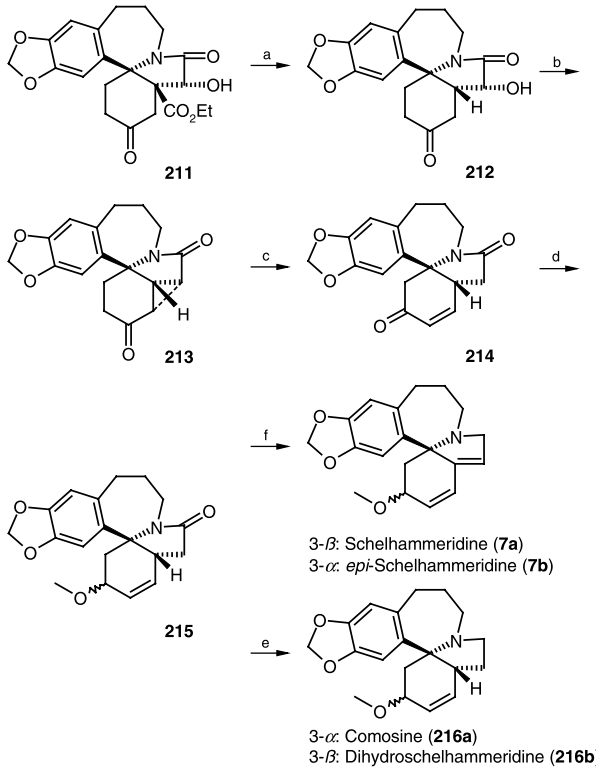
Scheme 38. Synthesis of (±)-3,17-demethoxy-1,2-dihydrocomosidine (**210**) (*28*): a) methyl acrylate in $CHCl_3$/50°C; c, d, e: reagents are the same as in Scheme 14

Furthermore, the formation of the C ring can be also accomplished following the Friedel-Crafts method already indicated in the erythrinane series (Scheme 14). Accordingly, the *cis*-arylhydroindole **208** has been alkylated with methyl acrylate providing the N-substituted precursor **209** in excellent yield, which after cyclization and consecutive removal of the carbonyl group affords the (±)-3,17-demethoxy-1,2-dihydrocomosidine **210** (Scheme 38). As already accentuated in the erythrinane series (Scheme 14) attempted approaches to the corresponding parent compound as well as to the 16,17-dioxygenated products also failed by this sequence (*28*).

5.3.3. A Ring Formation by [2 + 2] Photocycloaddition to Pyrrolobenzazepines

Similarly, replacement of the dioxopyrroloisoquinoline **137a** by the homologous dioxopyrrolobenzazepine **137b**, the synthesis of the erythrinane derivative **147a** by the [2 + 2] photocyclization method according to Scheme 21 could be successfully transferred to that of the dioxoschelhammerane **211** (Scheme 39). This in turn leads to the key compound, the α,β-unsaturated ketone **214** via the intermediates **212** and **213**, which – after generation of the 3-methoxy-derivative **215** – has been alternatively transformed to the 3-epimeric schelhammeridines **7a** and **7b** or to the corresponding 6,7-dihydro derivatives comosine (3α-**216a**) and dihydroschelhammeridine (3β-**216b**) (*78*).

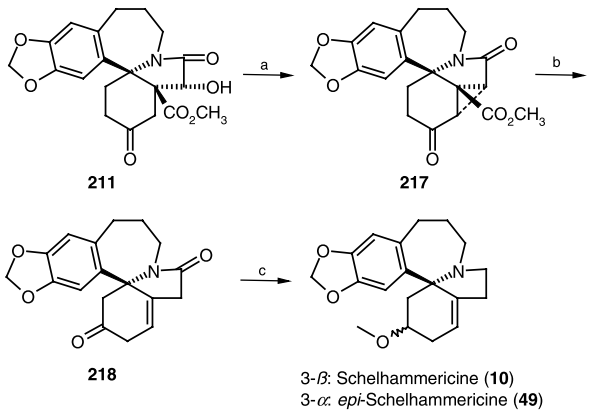
The synthesis of the 3-epimeric schelhammericines, being the first synthesis approach to a homoerythrinane alkaloid at all (*111*), is based



Scheme 39. Synthesis of schelhammeridines (**7**, **216**) (*78*); a) *i*: DMSO in Ac_2O; *ii*: ethyleneglycol/*p*-TsOH; *iii*: $MgCl_2/Et_3CSH$ in DMSO/Δ; *iv*: $NaBH_4$, then HCl in acetone; b) MsCl in pyr, then K_2CO_3 in CH_3OH; c) *i*: $PhSeCl/Et_2O \cdot BF_3$: *ii*: $Hg(ClO_4)_2$ in CH_3OH; *iii*: $NaBH_4$; *iv*: $NaH/CS_2/CH_3I$; *iw*: Bu_3SnH, then HCl in acetone; d) *i*: $NaBH_4/CeCl_3$ in CH_3OH, separation of 3α/3β-OH-stereomers; *ii*: $NaH/Bu_4NHSO_4/CH_3I$; e) $LiAlH_4/AlCl_3$, separate reduction of stereomers; f) *i*: *n*-BuLi/$(PhSe)_2$; *ii*: $NaIO_4$; *iii*: $LiAlH_4/AlCl_3$

on the same strategy concerning the intermediate **211** as shown in Scheme 40. This has been converted via the 1,7-cycloderivative **217** to the 2,8-dioxoschelhammerane **218**, which after consecutive stereoselective reduction, O-alkylation, and removal of lactam-oxygen affords the desired 3α- and 3β-methoxyschelhammericines **49** and **10** (*77*).

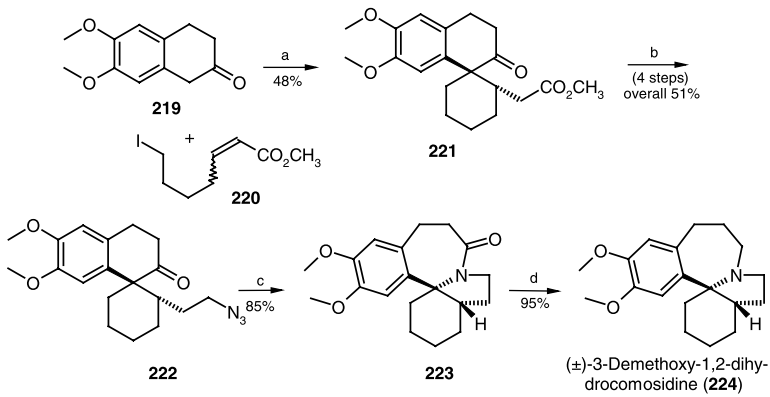
On the other hand, as already mentioned in the introduction to this section, the Diels-Alder addition of 1,3-butadienes to dioxopyrrolobenzazepine, *e.g.* according to reaction: **136** + **137b** → **138b** (Scheme 19) gives only very poor yields (<5%). This has been attributed to the marked conformational difference between the two dioxopyrroline dienophiles **137a** and **137b** (*77*).



Scheme 40. Synthesis of schelhammericines (**10** and **49**) (*77*, *111*): a) *i*: MsCl in pyr/20°C; *ii*: DBU in toluene/reflux; b) *i*: $PhSeCl/Et_2O \cdot BF_3$ in THF/reflux, then $Hg(ClO_4)_2$ in CH_3OH; *ii*: $NaBH_4$ in CH_3OH/THF, 20°C; *iii*: NaH/imidazole in THF/reflux, then CH_3I/CS_2; *iv*: Bu_3SnH/AIBN in toluene/reflux, then HCl in acetone; *iw*: $CaCl_2$ in DMSO/Δ; c) *i*: Bu_4NBH_4 or $NaBH_4/CeCl_3$ in CH_3OH/0°C; *ii*: NaH/imidazole/CH_3I/Bu_4NSO_4 in THF/reflux; *iii*: AlH_3 in THF/Et_2O/20°C

5.3.4. Simultaneous B Ring Formation/C Ring Expansion Starting from Spiro-2-tetralones

An original approach to the homoerythrinane framework represents the one pot tandem or domino alkylation-Michael addition sequence of 2-tetralones, followed by N-insertion via intramolecular



Scheme 41. Synthesis of (±)-demethoxy-1,2-dihydrocomosidine (**224**) (*112*): a) Cs_2CO_3 in DMF/20°C; b) *i*: TMSiOTf/Et_3N in CH_2Cl_2/20°C; *ii*: $LiAlH_4$; *iii*: MsCl; *iv*: NaN_3; c) neat TFA/30°C; d) $LiAlH_4$ in THF/20°C

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Schmidt rearrangement of an azido ketone. Thus, 6,7-dimethoxy-2-tetralone (**219**) has been reacted with methyl (*E*)-7-iodo-2-heptenoate (**220**) affording stereoselectively the C1-spiroketone **221** in 48% yield. After transformation of the latter to the azido derivative **222** the tetracyclic lactam **223** was obtained under ring expansion possessing the schelhammeridine core. Finally, $LiAlH_4$ reduction smoothly provides the (±)-3-demethoxy-1,2-dihydrocomosidine base **224** (*112*) (Scheme 41).

6. Pharmacology

The biological activity of extracts of *Erythrina* species is known for a long time. Thus, concentrated extracts were used as arrow poisons by the natives of South America, as antidote against strychnine, or as hypnoticum and epilepticum (*4*). On the other hand when an alcoholic seed extract from *Erythrina americana* was applied to dogs in different doses an activity similar to that of d-tubocurarine was already observed in 1877 (*2*, *113*) and confirmed much later (*7*, *114–118*).

The first crystalline pharmacologically active alkaloid was isolated from *Erythrina americana* mentioned above (*7*) and was called erythroidine. This name had already been used by Altamirano referring, however, to the unknown constituents of the plant (*114*). Further analytical investigations revealed the material isolated to be a mixture of isomeric alkaloids, which were subsequently named α- and β-erythroidines (**14** and **225**, see Figs. 2 and 4).

Between 1940 and 1950 the systematic examination of more than fifty *Erythrina* species showed that all the alkaloids isolated produce effects similar to curare alkaloids (*116*, *119*), which had been used as adjuvant in surgical anaesthesia (*120*).

A considerable number of properties, biological effects, and applications attributed to extracts from various *Erythrina* species has been

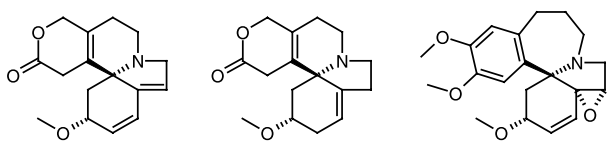

Fig. 4. Chemical structures of several *Erythrina* alkaloids with special pharmacological properties (*cf.* pages 26–27)

described. Thus, they are used in indigeneous medicine as eyewashes, dressings for open wounds, for pain relieving in joints, or as calmers and relaxants (*121*). Antiasthmatic, diuretic, and hypnotic properties have also been reported. Besides their smooth curare like muscle relaxation the total alkaloids from *E. variegata* have been found CNS depressant and anticonvulsive, furthermore they increase the pentobarbital hypnosis, and inhibit the acetylcholine-induced spasm. However, these alkaloids do not have any analgesic, antipyretic, antiinflammatory, laxative, and diuretic effects. Extracts from *E. velutina*, and *E. suberosa* exhibit spasmolytic and antineoplastic activities (*19*, *21*, *33*, *39*, *122–124*). In recent investigations crude extracts of *E. americana* as well as its pure constituents β-erythroidine (**225**) and dihyro-β-erythroidine (**226**; Fig. 4) have been found to diminish the aggressive behaviour of rats using diazepam as a control. These effects are attributed to an interaction between the cholinergic and GABAergic system. Additionally, the lethal doses LD_{50} of the extracts as well as those of the pure alkaloids have been determined (*121*).

From all the pure alkaloids tested α- and β-erythroidine exhibit the highest activity (*121*). They have been assumed to be the principles responsible for the hypnotic activity of the extracts of the flowers of *E. americana* (*19*). β-Eyrthroidine (**225**) and its more potent 2,7-dihydro-derivative **226** have been used as muscle relaxant in numerous clinical applications (*114*, *120*). This activity is attributed to an antagonistic action of dihydro-β-erythroidine to nicotinic acetylcholine receptors, which is now well known and is frequently used in the experimental pharmacology (*19*, *125*, *126*).

Erysodine (**6**) has been found to be a competitive, reversible antagonist of nicotine-induced dopamine release. It is equipotent with dihydro-β-erythroidine (**226**) and may be a useful tool to characterize neuronal nicotinic acetylcholine receptors (*127*).

Very little is known about the pharmacological action of homo-erythrinanes. Indeed, they are occurring together with *Cephalotaxus* alkaloids and are biosynthetically and structurally closely related to them, but nevertheless, they seem to possess no antitumor properties like the latter. Wilsonine (**227**; Fig. 4), however, shows weak antileukemic effects in mice (*128*).

3-*Epi*-schelhammericine (**49**), and dyshomoerythrine (**10**: 18-OCH_3 instead of 18-H) are known to exhibit a strong molluscicidal activity; the latter is also active against some agriculturally important insect pests (*129*). Lenticellarine (**32**: 2-H instead of 2-OH) occurring in the same plant is less effective. 3-*Epi*-schelhammericine and dyshomoerythrine as

well as 2,7-dihydro-homoerysotrine (**207**) and 3-*epi*-12-hydroxyschelhammericine (**49**: 12-OH instead of 12-H) are considered to exhibit cardiovascular effects (*19*).

Finally, it should be mentioned that the curare alkaloids – as is generally known – are quaternary salts and due to their strong hydrophilicity they have to be administered parenterally. In contrast, the *Erythrina* alkaloids are all tertiary amines and therefore they are able to develop their pharmacological activities upon oral administration.

7. Concluding Remarks

The present review summarizes the work of the last fifty years in the field of the synthesis of *Erythrina* alkaloids and structurally related compounds. Their unique structure has attracted the attention of the synthesis chemists over a period of more than half a century until now. The numerous efforts devoted to the construction of the tetracyclic spiroamine framework have certainly led to original and impressive results, but with regard to today's standard and especially to an adequate supply of the promising compounds for a systematic pharmacological examination the total efficiency of their preparation yet leaves wishes in many cases.

While a great number of synthesis pathways providing the aromatic erythrinane type compounds is reported, comparatively much less approaches to the homoerythrinanes have been developed. This may be explained by the fact, that the erythrinane routes cannot be *a priori* applied to the synthesis of the corresponding C-homologues. Furthermore, asymmetric syntheses of the alkaloids are rare, and approaches to the nonaromatic and heteroaromatic compounds are lacking. There is certainly a need for shorter routes to the target compounds. For instance, domino reactions obviously represent the tool of choice for this purpose as it is demonstrated herein enabling the rapid construction of complex frameworks.

Not only because of their attractive structure, but also of their pharmacological potential, mainly that of the pure compounds, the synthesis of the *Erythrina* alkaloids will remain an important goal challenging the synthesis chemists in the forthcoming years. It is hoped, that this review along with the powerful arsenal of modern synthesis methods can stimulate the development of new efficient strategies to the *Erythrina* alkaloids and possibly to related new active agents.

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The Trichothecenes and Their Biosynthesis

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Contents

1. Introduction

The trichothecenes are a group of naturally-occurring sesquiterpenoid epoxides which show a broad range of biological activity. They are powerful inhibitors of eukaryotic protein synthesis, are phytotoxic, insecticidal and toxic to animals, and some are among the most toxic non-nitrogenous compounds known to man. Several are commonly found in cereal grains, and the potential health risk from contaminated animal feed and human food is a major factor in stimulating research into this

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group of compounds. Since the isolation of the macrolide mixture, glutinosin (*1*), was reported in 1946, the body of trichothecene literature has expanded to some 3000 publications.

This review lists the trichothecenes recorded in the literature up to Dec. 2000, together with their sources, coupled with a summary of the pathways involved in trichothecene biosynthesis, an area in which significant advances have been made during the past decade. The review contains both macrocyclic trichothecenes (''macrocycles''), previously recorded to Dec. 1991 (*1*), and non-macrocyclic trichothecenes, previously recorded to Dec. 1995 (*2*, *3*). Some omissions from these earlier lists have been included, and some errors corrected.

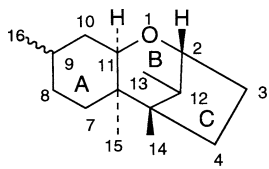
2. The Trichothecenes

2.1. Macrocyclic and Non-Macrocyclic Compounds

A total of 217 trichothecenes, based on the sesquiterpene skeleton (**1**) named trichothecane (*4*), which replaced the earlier (*5*) scirpane nomenclature[1], have now been reported from natural sources. They are made up of 133 (61%) non-macrocyclic and 84 (39%) macrocyclic compounds. Thus, 20 new non-macrocyclic trichothecenes have been isolated since 1995 and 17 new macrocycles since 1991.

Included in the total of non-macrocyclic trichothecenes are 35 (26%) trichoverroids (*6*) which have complex ester side chains at positions 4 and/or 15 of verrucarol (**2**; $R^1 = R^2 = R^5 = R^6 = H$, $R^3 = R^4 = OH$) and are biosynthetic precursors of, or shunt products from the biosynthesis of, the macrocycles. The remaining 98 non-macrocyclic compounds are designated ''simple'' trichothecenes. In the macrocycles two side chains of a trichoverroid are joined to form an 18-membered ring. As would be expected, the trichoverroids and the macrocycles are produced by the same organisms.

Also included in the 133 non-macrocyclic trichothecenes are one uncharacterised compound, seven compounds detected only in the mass



(**1**)

[1] Scirpane (= 12,13-epoxytrichothecane) nomenclature is still used in devising trivial names for new compounds.

spectrometer, and thus incompletely characterised, and three possible artefacts (see Notes to Tables 1–4). Included in the macrocycles are two possible artefacts (see Notes to Tables 5–7). If all the compounds about which there is some element of doubt are removed from the totals, the number of known naturally occurring trichothecenes is reduced to 204.

For tabulation, the non-macrocyclic trichothecenes have been subdivided into 12,13-epoxytrichothec-9-enes (103, 77%) (Table 1), 12,13-epoxytrichothec-9-en-8-ones (23, 17%) (Table 2), trichothec-9,12-dienes (5, 4%) (Table 3), and miscellaneous (2) (Table 4). Thus, all but five of the non-macrocyclic trichothecenes have the 12,13-epoxide group. The pair of diastereoisomers in Table 4 have a 9,10-epoxide and are the only non-macrocyclic compounds not to have the 9(10)-ene implicit in the name trichothecene.

Two non-macrocyclic trichothecenes, 8-deoxotrichothecinol A (Table 1: $C_{19}H_{26}O_5$), from *Holarrhena floribunda*, and miotoxin G (Table 1: $C_{29}H_{40}O_9$), from *Baccharis coridifolia*, are plant products (but see Notes to Tables 1–4).

Whilst the remaining non-macrocyclic trichothecenes are metabolic products of fungi, 36 macrocyclic trichothecenes (43%), the baccharinoids, have been isolated *only* from plants of the genus *Baccharis*, and are listed in Table 7. The remaining 48 macrocycles are fungal products, classified as verrucarins (12, 25%) [skeleton (**6**)] (Table 5), mainly C_{27} compounds; or roridins (36, 75%) [skeleton (**18**)] (Table 6), mainly C_{29} compounds. Among these macrocycles, verrucarins A and J, and roridins A, D, E, and H have also been obtained from *Baccharis* spp., as has the simple trichothecene diacetylverrucarol.

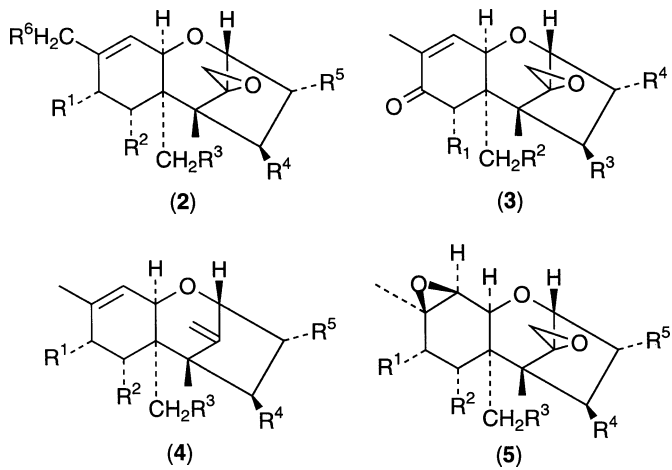


Table 1. Non-Macrocycles: 12,13-Epoxytrichothec-9-enes (**2**; $R^6 = H$)[a]

Formula	R^1	R^2	R^3	R^4	R^5	Trivial Name	Source[b,c]	References[d]
$C_{15}H_{22}O_2$	H	H	H	H	H	Scirpene	*T. roseum*, *F. culmorum*, *F. crookwellense*	*10*, *11*, *12*
						(12,13-Epoxytrichothecene)	*F. graminearum*, ***Spicellum roseum***	*12*, *13*
$C_{15}H_{22}O_3$	H	H	H	OH	H	Trichodermol	*M. roridum*, *Trichoderma polysporum*	*14*, *15*
						(Roridin C)	*S. cylindrospora*, *T. roseum*	*16*, *15*
							Memnoniella echinata, ***Spicellum roseum***	*17*, *13*
							Gliocladium virens	*18*
$C_{15}H_{22}O_3$	H	H	H	H	OH	Isotrichodermol	*F. crookwellense*, *F. graminearum*	*19*, *12*
							F. venenatum	*20*
$C_{15}H_{22}O_3$	OH[e]	H	H	H	H	8-Hydroxyscirpene	*F. sporotrichioides*	*21*
$C_{15}H_{22}O_4$	OH	H	H	H	OH	8-Hydroxyisotrichodermol	*F. crookwellense*, ***F. graminearum***	*19*, *22*
							F. sporotrichioides	*23*
$C_{15}H_{22}O_4$	H	OH	H	H	OH	7-Hydroxyisotrichodermol	*F. crookwellense*, ***F. graminearum***	*19*, *22*
$C_{15}H_{22}O_4$	OH	H	H	OH	H	Trichothecodiol	*T. roseum*	*10*
$C_{15}H_{22}O_4$	H	OH	H	OH	H	7-Hydroxytrichodermol	*M. roridum*	*24*
$C_{15}H_{22}O_4$	H	H	OH	OH	H	Verrucarol[f]	*S. atra*, *S. microspora*, ***F. sporotrichioides***	*25*, *25*, *26*
$C_{15}H_{22}O_4$	H	H	OH	H	OH	Isoverrucarol	*F. culmorum*, *F. sporotrichioides*	*27*, *28*
							F. oxysporum	*29*
$C_{15}H_{22}O_5$	OH	H	H	OH	OH	**Scirpen-3,4,8-triol**	***F. sporotrichioides***	*23*
$C_{15}H_{22}O_5$	H	H	OH	OH	OH	Scirpentriol[f,g]	*F. semitectum* (*roseum*), *F. sporotrichioides*	*30*, *31*
						(Scirpen-3,4,15-triol)	*F. sambucinum*, *F. camptoceras*	*32*, *33*
							F. acuminatum, *F. equiseti*, ***F. poae***	*34*, *34*, *35*
							F. venenatum	*36*
$C_{15}H_{22}O_6$	OH	H	OH	OH	OH	T-2 tetraol[f]	*F. sporotrichioides*, *F. acuminatum*	*37*, *38*
							(*heterosporum*), *F. acuminatum*, *F. poae*	*34*, *37*

$C_{15}H_{22}O_6$	OH	OH	OH	H	OH	Scirpen-3,7,8,15-tetraol	*F. graminearum*	*39*
$C_{17}H_{24}O_4$	H	H	H	OAc	H	Trichodermin	*Trichoderma viride*, *Trichoderma polysporum*	*40*, *15*
							S. cylindrospora, *Dendrostilbella* sp.	*16*, *41*
							Memnoniella echinata, ***Gliocladium virens***	*17*, *18*
$C_{17}H_{24}O_4$	H	H	H	H	OAc	Isotrichodermin	*F. graminearum* (*roseum*), *F. culmorum*	*42*, *27*
							F. crookwellense, *F. sambucinum*	*19*, *12*
							F. sporotrichioides, ***F. venenatum***	*23*, *20*
$C_{17}H_{24}O_5$	OH	H	H	H	OAc	8-Hydroxyisotrichodermin	*F. graminearum* (*roseum*), *F. crookwellense*	*43*, *19*
							F. culmorum, ***F. sporotrichioides***	*44*, *23*
$C_{17}H_{24}O_5$	H	OH	H	H	OAc	7-Hydroxyisotrichodermin	*F. graminearum* (*roseum*), *F. crookwellense*	*43*, *19*
							F. culmorum	*44*
$C_{17}H_{24}O_5$	H	H	OH	H	OAc	15-Deacetylcalonectrin	*F. culmorum* (*Calonectria nivalis*)	*45*
							F. graminearum (*roseum*), *F. sporotrichioides*	*42*, *28*
$C_{17}H_{24}O_5$	H	H	OAc	H	OH	3-Deacetylcalonectrin	*F. culmorum*, *F. crookwellense*, *F. graminearum*	*27*, *12*, *12*
$C_{17}H_{24}O_6$	H	H	OAc	OH	OH	15-Acetoxyscirpendiol	*F. equiseti* (*avenaceum*, *concolor*, *semitectum*)	*46*, *46*, *46*
							F. equiseti, *F. sambucinum* (*sulphureum*)	*46*, *47*
							F. sambucinum, *F. sporotrichioides*	*32*, *48*
							F. poae, ***Cylindrocladium floridanum***	*49*, *50*
							F. semitectum (*roseum*), ***F. venenatum***	*30*, *36*
$C_{17}H_{24}O_6$	H	H	OH	OAc	OH	4-Acetoxyscirpendiol[f]	*F. semitectum* (*roseum*), *F. sambucinum*	*51*
							(*sulphureum*), *F. sambucinum*, *F. camptoceras*	*47*, *32*, *33*
							Cylindrocladium floridanum, ***F. venenatum***	*50*, *36*
$C_{17}H_{24}O_6$	H	H	OH	OH	OAc	3-Acetoxyscirpendiol[f]	*F. sambucinum*, *F. camptoceras*	*52*, *33*
$C_{17}H_{24}O_6$	OH	OH	H	H	OAc	7,8-Dihydroxyisotrichodermin	*F. crookwellense*	*19*
$C_{17}H_{24}O_7$	OH	H	OAc	OH	OH	15-AcetylT-2 tetraol	*F. acuminatum* (*heterosporum*)	*38*
							F. acuminatum, *F. sporotrichioides*	*53*, *54* (*48*[h])

Table 1 (*continued*)

Formula	R^1	R^2	R^3	R^4	R^5	Trivial Name	Source[b,c]	References[d]
$C_{17}H_{24}O_7$	OAc	H	OH	OH	OH	8-AcetylT-2 tetraol	*F. sporotrichioides*, *F. acuminatum*	*54* (*48*[h]), *53*
$C_{17}H_{24}O_7$	OH	OH	OH	H	OAc	7,8-Dihydroxy-15-deacetylcalonectrin	*F. culmorum*	*55*
$C_{17}H_{24}O_7$	OH	H	OH	OAc	OH	NT-2	*F. sporotrichioides* (*solani*), *F. acuminatum*	*56*, *53*
$C_{19}H_{24}O_5$	–O–[i]		H	OR^7	H	Crotocin	*Cephalosporium crotocinigenum*, *T. roseum*	*57*, *58*
$C_{19}H_{26}O_4$	H	H	H	OR^7	H	Isocrotonyltrichodermol (8-Deoxotrichothecin)	*T. roseum*, *Spicellum roseum*	*59*, *60*
$C_{19}H_{26}O_5$	H	H	H	OR^7	OH	**8-Deoxotrichothecinol A**[j]	***Holarrhena floribunda***[k]	*61*
$C_{19}H_{26}O_5$	OH	H	H	OR^7	H	**Trichothecinol B**	***T. roseum***	*62*
$C_{19}H_{26}O_6$	OH	H	H	OR^7	OH	**Trichothecinol C**	***T. roseum***	*62*
$C_{19}H_{26}O_6$	H	H	OAc	OAc	H	Diacetylverrucarol[f]	*M. verrucaria*, *B. coridifolia*[l]	*63*, *64*
$C_{19}H_{26}O_6$	H	H	OAc	H	OAc	Calonectrin	*F. culmorum* (*Calonectria nivalis*)	*45*
							F. graminearum (*roseum*)	*43*
							F. sporotrichioides, *F. crookwellense*	*65*, *12*
$C_{19}H_{26}O_7$	H	H	OH	OAc	OAc	3,4-Diacetoxyscirpenol[f]	*F. sambucinum*	*52*
$C_{19}H_{26}O_7$	H	H	OAc	OH	OAc	3,15-Diacetoxyscirpenol[f]	*F. sambucinum*	*32*
$C_{19}H_{26}O_7$	H	H	OAc	OAc	OH	Diacetoxyscirpenol (4,15-Diacetoxyscirpenol)	*F. equiseti*, *F. equiseti* (*avenaceum*	*5*, *46*
							concolor, *semitectum*)	*46*, *46*
							F. equiseti (*roseum*), *F. graminearum*	*66*
							(*tricinctum*, *roseum*)	*66*, *66*
							F. sporotrichioides (*solani*, *tricinctum*)	*66*, *66*
							F. sporotrichioides, *F. sambucinum*	*66*
							(*sulphureum*, *roseum*), *F. sambucinum*	*47*, *51*, *67*

							F. poae (*tricinctum*), *F. graminearum*	*68* (*116*), *69*
							F. acuminatum, *F. lateritium*, *F. oxysporum*	*9a*, *70*, *71*
							F. oxysporum (*lateritium*), *F. solani*	*72*, *73*
							F. equiseti (*compactum*), *F. moniliforme*	*74*, *75*
							F. culmorum, *F. poae*, *F. avenaceum*	*12*, *76*, *69*
							F. crookwellense, *F. semitectum*	*77*, *78*
							F. venenatum	*79*
$C_{19}H_{26}O_7$	OH	H	OAc	H	OAc	8-Hydroxycalonectrin	*F. culmorum*, *F. graminearum* (*roseum*)	*27*, *43*
$C_{19}H_{26}O_7$	H	OH	OAc	H	OAc	7-Hydroxycalonectrin	*F. graminearum* (*roseum*), *F. culmorum*	*43*, *44*
$C_{19}H_{26}O_8$	OH	H	OAc	OAc	OH	Neosolaniol[m]	*F. sporotrichioides* (*solani*, *poae*, *tricinctum*)	*80*, *82*, *66*
							F. sporotrichioides, *F. graminearum*	*82*
							(*decemcellulare*, *roseum*)	*66*, *66*
							F. sambucinum (*roseum*), *F. acuminatum*	*51*
							(*sulphureum*), *F. equiseti*, *F. sambucinum*	*83*, *84*, *85*
							F. avenaceum, *F. semitectum*	*66*, *78*
							F. acuminatum, *F. solani*, *F. crookwellense*	*78*, *86*, *87*
							F. tumidum, *F. poae*, *F. oxysporum*	*88*, *37*
							(*lateritium*)	*84*
$C_{19}H_{26}O_8$	OAc	H	OH	OAc	OH	NT-1	*F. sporotrichioides* (*solani*, *tricinctum*)	*56*, *89*
							F. sporotrichioides, *F. acuminatum*	*90*, *53*
$C_{19}H_{26}O_8$	OAc	H	OAc	OH	OH	Acuminatin[f]	*F. acuminatum*, *F. equiseti* (*compactum*)[n]	*53*, *74*
$C_{19}H_{26}O_8$	OH	OH	OAc	H	OAc	7,8-Dihydroxycalonectrin	*F. graminearum* (*roseum*), *F. culmorum*	*42*[o], *91*
$C_{19}H_{26}O_8$	H	OH	OAc	OAc	OH	7-Hydroxydiacetoxyscirpenol	*F. oxysporum* (*lateritium*), *f. graminearum*	*84*
							(*roseum*)	*92*
$C_{19}H_{26}O_9$	OH	OH	OAc	OAc	OH	7,8-Dihydroxydia-cetoxyscirpenol	*F. oxysporum* (*lateritium*), *F. camptoceras*	*84*, *33*

Table 1 (*continued*)

Formula	R^1	R^2	R^3	R^4	R^5	Trivial Name	Source[b,c]	References[d]
$C_{20}H_{30}O_6$	OR^8	H	OH	H	OH	Sporotrichiol	*F. sporotrichioides*	*54*
$C_{20}H_{30}O_7$	OR^8	H	OH	OH	OH	T-2 triol[f]	*F. sporotrichioides*	*93*
$C_{20}H_{30}O_7$	OH	H	OR^8	OH	OH	15-IsovalerylT-2 tetraol[h]	*F. sporotrichioides*	*48*
$C_{20}H_{30}O_8$	OR^9	H	OH	OH	OH	3′-HydroxyT-2 triol	*F. acuminatum* (*heterosporum*)	*38*
$C_{21}H_{28}O_8$	H	H	OAc	OAc	OAc	Triacetoxyscirpene[f]	*F. sambucinum* (*sulphureum*)	*47*
							F. sambucinum, ***F. poae***	*32*, *35*
$C_{21}H_{28}O_9$	OAc	H	OAc	OAc	OH	9-Acetylneosolaniol	*F. chlamydosporium* (*tricinctum*)	*94*
							F. equiseti (*compactum*)	*74*
							F. sambucinum (*roseum*)	*51*
							F. sambucinum, *F. acuminatum*	*85*, *53*
							F. sporotrichioides. *F.* sp.	*95*, *96*
$C_{21}H_{28}O_{10}$	OAc	OH	OAc	OAc	OH		*F. equiseti*	*5*
$C_{21}H_{30}O_6$	H	H	OR^{10}	OH	H	Verrol	*M. verrucaria*, ***S. atra***	*97*, *98*
$C_{21}H_{30}O_8$[h]	OR^{11}	H	OAc	OH	OH		*F. sporotrichioides*	*48*
$C_{22}H_{30}O_8$[h,p]	OR^{12}	H	OAc	OH	OH		*F. sporotrichioides*	*48*
$C_{22}H_{30}O_9$	OR^{13}	H	OAc	OAc	OH	8-Propionylneosolaniol	*F. sporotrichioides*, *F. sambucinum*	*65*, *85*
$C_{22}H_{32}O_7$	OR^8	H	OAc	H	OH	4-DeacetoxyT-2 toxin	*F. sporotrichioides*	*90*
$C_{22}H_{32}O_8$	OR^8	H	OH	OAc	OH	15-DeacetylT-2 toxin[h]	*F. sporotrichioides*	*48*
$C_{22}H_{32}O_8$	OR^8	H	OAc	OH	OH	HT-2 toxin	*F. sporotrichioides* (*tricinctum*, *solani*, *poae*)	*99*, *66*, *100*
							F. sporotrichioides	*37*
							F. graminearum (*tricinctum*, *roseum*)	*66*, *66*
							F. acuminatum (*sulphureum*, *heterosporum*)	*83*, *38*

							F. acuminatum, *F. sambucinum*	*86*, *85*
							F. oxysporum, *F. equiseti*, *F. solani*	*71*, *86*, *86*
							F. culmorum, *F. graminearum*	*87*, *87*
							F. moniliforme, *F. poae*	*101*, *37*
$C_{22}H_{32}O_9$	OR^9	H	OAc	OH	OH	3′-HydroxyHT-2 toxin	*F. acuminatum* (*heterosporum*)	*38*
							F. sporotrichioides	*102*
$C_{23}H_{28}O_6$	H	H	H	OR^{14}	H	Harzianum A	*Trichoderma harzianum*	*103*
$C_{23}H_{30}O_5$	H	H	H	OR^{15}	H	Trichodermadiene	*M. verrucaria*	*104*
$C_{23}H_{30}O_{10}$	OAc	H	OAc	OAc	OAc	Diacetylneosolaniol[f]	*F. acuminatum*	*53*
$C_{23}H_{32}O_6$	H	H	H	OR^{16}	H	Trichodermadienediol A,B[q]	*M. verrucaria*, *M. roridum*, *S. atra*	*105*, *106*, *107*
$C_{23}H_{32}O_7$	H	H	OR^{10}	OAc	H	**4-Acetylverrol**	***Acremonium neo-caledoniae***	*108*
$C_{23}H_{32}O_7$[r]	H	H	H	OR^{16}	H	16-Hydroxytrichoderma-dienediol A,B[q]	*M. verrucaria*, *M. roridum*	*109*, *109*
$C_{23}H_{32}O_7$	H	H	OH	OR^{16}	H	Trichoverrol A,B[q]	*M. verrucaria*, *S. atra*, *S. albipes*	*105*, *110*, *111*
							S. kampalensis, *S. microspora*	*111*, *111*
$C_{23}H_{32}O_7$	H	H	OH	OR^{17}	H	Isotrichoverrol A,B[s]	*M. verrucaria*	*112*
$C_{23}H_{32}O_7$	H	H	OH	OR^{18}	H	**(2′E)-Isotrichoverrol A,B[s]**	***M. verrucaria***	*113*
$C_{23}H_{32}O_9$	OR^{11}	H	OAc	OAc	OH	8-Butyrylneosolaniol	*F. sporotrichioides*, *F. sambucinum*	*65* (*48*[h]), *85*
$C_{22}H_{32}O_9$	OR^{19}	H	OAc	OAc	OH	8-Isobutyrylneosolaniol	*F. sporotrichioides*	*65*
$C_{23}H_{34}O_{11}$	H	H	OAc	OR^{20}	OH	15-Acetoxyscirpendiol-4-β-glucoside	*F. sambucinum* (*sulphureum*)	*114*
$C_{24}H_{32}O_9$	OR^{12}	H	OAc	OAc	OH	8-Pentenoylneosolaniol[h,t]	*F. sporotrichioides*	*48*
$C_{24}H_{34}O_9$	OR^{12}	H	OAc	OAc	OH	8-*n*-Pentanoylneosolaniol	*F. sporotrichioides*	*115*
$C_{24}H_{34}O_9$	OR^8	H	OAc	OAc	OH	T-2 toxin	*F. sporotrichioides* (*tricinctum*, *solani*	*116*, *66*
							moniliforme, *poae*), *F. sporotrichioides*	*117*, *100*, *37*
							F. avenaceum (*roseum*), *F. semitectum*	*66*, *118*
							F. acuminatum (*sulphureum*, *heterosporum*)	*83*, *38*

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Table 1 (*continued*)

Formula	R^1	R^2	R^3	R^4	R^5	Trivial Name	Source[b,c]	References[d]
							F. equiseti, *F. sambucinum* (*roseum*)	*118*, *51*
							F. graminearum (*tricinctum*, *roseum*)	*66*, *66*
							F. culmorum, *F. acuminatum*, *F. poae*	*69*, *119*, *120*
							F. graminearum, *F. oxysporum*	*69*, *121*
							F. moniliforme, *F. sambucinum*	*75*, *85*
							F. crookwellense, *F. stilboides*, *F. solani*	*69*, *69*, *86*
							F. subglutinans	*101*
							Trichoderma viride (*lignorum*)	*99*
$C_{24}H_{34}O_9$	OR^8	H	OAc	OH	OAc	IsoT-2 toxin[f]	*F. sporotrichioides*, *F. graminearum*	*71*, *122*
$C_{24}H_{34}O_{10}$	OR^9	H	OAc	OAc	OH	3′-HydroxyT-2 toxin	*F. sporotrichioides*, *F. poae*, *F. oxysporum*	*102*, *123*, *71*
$C_{25}H_{36}O_9$	OR^8	H	OAc	OR^{13}	OH	4-PropionylHT-2 toxin	*F. sporotrichioides*	*21*
$C_{25}H_{36}O_9$	OR^{22}	H	OAc	OAc	OH	8-*n*-Hexanoylneosolaniol	*F. sporotrichioides*	*115*
$C_{26}H_{36}O_{10}$	OR^8	H	OAc	OAc	OAc	AcetylT-2 toxin[f]	*F. sporotrichioides* (*poae*)	*124*
							F. sporotrichioides, *F. graminearum*	*71*, *122*
$C_{29}H_{38}O_9$	H	H	OH	OR^{23}	H	Roridin L-2	*M. roridum*, *S. atra*	*125*, *107*
$C_{29}H_{38}O_9$	H	H	OH	OR^{24}	H	**(2′E)-Roridin L-2**[u]	***M. verrucaria***	*113*
$C_{29}H_{38}O_{10}$[r]	H	H	OH	OR^{23}	H	16-Hydroxyroridin L-2	*M. roridum*	*126*
$C_{29}H_{40}O_9$	H	H	OH	OR^{25}	H	**Miotoxin G**[v]	***B. coridifolia***	*127*
$C_{29}H_{40}O_9$	H	H	OR^{10}	OR^{16}	H	Trichoverrin A,B[q]	*M. verrucaria*, *S. atra*	*105*, *110*
$C_{29}H_{40}O_9$	H	H	OR^{10}	OR^{17}	H	**Isotrichoverrin A,B**[s]	***M. verrucaria***	*113*
$C_{29}H_{40}O_9$	H	H	OR^{10}	OR^{18}	H	(2″E)-Isotrichoverrin A,B[s]	*M. verrucaria*	*112*
$C_{29}H_{40}O_9$	H	H	OR^{10}	OR^{26}	H	**(2′E,4″Z)-Isotrichoverrin A,B**[s]	***M. verrucaria***	*113*
$C_{29}H_{40}O_9$	H	H	OR^{27}	OR^{17}	H	[iso]Trichoverrin C[w]	*M. verrucaria*	*112*
$C_{29}H_{40}O_9$	OH	H	OR^{10}	OR^{17}	H	**8-Hydroxyisotrichoverrin A**	***M. verrucaria***	*113*

$C_{31}H_{52}O_6$	H	H	OR^{28}	OR^{28}	OR^{28}	Palmitylscirpentriol[h]	*F. moniliforme*	*128*
$C_{31}H_{52}O_7$	OR^{29}	H	OR^{29}	OR^{29}	OR^{29}	PalmitylT-2 tetraol[h]	*F. moniliforme*	*128*
$C_{35}H_{46}O_{11}$	H	H	OR^{10}	OR^{23}	H	Trichoverritone	*M. roridum*	*126*

[a] New compounds and sources reported since Dec. 1995 are printed in bold type.
[b] *B* = *Baccharis*, *F* = *Fusarium*, *M* = *Myrothecium*, *S* = *Stachybotrys*, *T* = *Trichothecium*.
[c] Fusaria are named according to the scheme proposed by Booth (*7*), as extended and amended by Nelson *et al.* (*8*). Where reinvestigation has led to a change in identification (*9*), the original assignment is given in parentheses.
[d] The reference is to the first isolation from each source.
[e] 8β-OH.
[f] Known compound at time of isolation: previously obtained by chemical modification of other naturally-occurring trichothecenes.
[g] 3,4,15-Oxygenation is assumed unless stated otherwise.
[h] Ms identification only: not completely characterized.
[i] 7β,8β-Epoxide.
[j] Incorrectly named 8-dihydrotrichothecinol A.
[k] A West African shrub. The workers (*61*) could not exclude the possibility that this compound arose from fungal contamination of the plant material. The material tested negative for *Fusarium* spp.; however, the pattern of the *known* metabolic products isolated included the diterpenoids rosenonolactone, 6β-hydroxy-rosenonolactone, and rosololactone, and is typical of a *T. roseum* strain.
[l] The derivative, 4β,15-diacetoxy-10,13-cyclotrichothecan-9α,12-diol, $C_{19}H_{28}O_7$, also isolated from *B. coridifolia* (*53*) is presumed to be an artefact.
[m] Initially named solaniol (*80*), but later renamed neosolaniol (*81*).
[n] Initially believed to be the isomer NT1.
[o] The correct configuration for the 8-hydroxy substituent in this compound is α (*54*).
[p] Possible artefact by degradation of 3′-hydroxyHT-2 toxin.
[q] Diastereoisomers, epimeric at C-7′. Isomer A has C-7′S.
[r] $R^6 = OH$
[s] Diastereoisomers, epimeric at C-7′ The prefix "iso" indicates that C-6′ is R. In this series isomer A has C-7′R.
[t] Possible artefact by degradation of 3′-hydroxyT-2 toxin.
[u] Claimed, but not characterized.
[v] Possible artefact derived from the macrocycle miotoxin D by hydrolysis and relactonisation.
[w] Named trichoverrin C, but on the "iso" convention (notes) should be isotrichoverrin C.

Radicals for Tables 1–4

R^{7} = CO·CH$\overset{Z}{=}$CHMe

R^{8} = CO·CH_2·$CHMe_2$

R^{9} = CO·CH_2·C(OH)Me_2

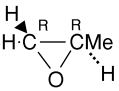
R^{10} = CO·CH$\overset{E}{=}$CMe·CH_2·CH_2OH

R^{11} = CO·C_3H_7

R^{12} = CO·C_4H_7

R^{13} = CO·Et

R^{14} = CO·CH$\overset{Z}{=}$CH·CH$\overset{E}{=}$CH·CH$\overset{E}{=}$CH·CO_2H

R^{15} = CO·CH$\overset{Z}{=}$CH·CH$\overset{E}{=}$CH· 

R^{16} = CO·CH$\overset{Z}{=}$CH·CH$\overset{E}{=}$CH·$\overset{6'\,S}{C}$HOH·$\overset{7'\,S,R}{C}$HOH·Me

R^{17} = CO·CH$\overset{Z}{=}$CH·CH$\overset{E}{=}$CH·$\overset{6'\,R}{C}$HOH·$\overset{7'\,R,S}{C}$HOH·Me

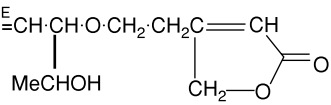
R^{18} = CO·CH$\overset{E}{=}$CH·CH$\overset{E}{=}$CH·$\overset{6'\,R}{C}$HOH·$\overset{7'\,R,S}{C}$HOH·Me

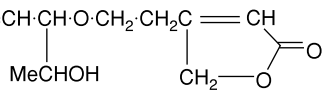
R^{19} = CO·$CHMe_2$

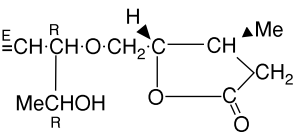
R^{20} = $C_6H_{11}O_5$

R^{21} = CO·C_4H_9

R^{22} = CO·C_5H_{11}

R^{23} = CO·CH$\overset{Z}{=}$CH·CH$\overset{E}{=}$CH· 

R^{24} = CO·CH$\overset{E}{=}$CH·CH$\overset{E}{=}$CH· 

R^{25} = CO·CH$\overset{Z}{=}$CH·CH$\overset{E}{=}$CH· 

R^{26} = CO·CH$\overset{E}{=}$CH·CH$\overset{Z}{=}$CH·$\overset{6'\,R}{C}$HOH·$\overset{7'\,R,\,S}{C}$HOH·Me

R^{27} = CO·CH_2·CMe$\overset{E}{=}$CH·CH_2OH

R^{28} = [H,H,$CO(CH_2)_{14}Me$]

R^{29} = [H,H,H,$CO(CH_2)_{14}Me$]

R^{30} = CO·CHOH·Me

R^{31} = CO·CH=CH·CH=CH·Me

R^{32} = CO·CH$\overset{E}{=}$CH·Ph

R^{33} = CO·$(CH_2)_{14}$Me

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Table 2. Non-Macrocycles: 12,13-Epoxytrichothec-9-en-8-ones (**3**)[a]

Formula	R^1	R^2	R^3	R^4	Trivial Name	Source[b,c]	References[d]
$C_{15}H_{20}O_4$	H	H	OH	H	*Trichothecolone*[e]	*T. roseum*, *F. moniliforme*, ***Holarrhena floribunda***[g]	*58*, *128*, *61*
$C_{15}H_{20}O_5$	H	OH	H	OH	7-Deoxyvomitoxin	*F. graminearum*	*129*
$C_{15}H_{20}O_6$	OH	OH	H	OH	Vomitoxin	*F. graminearum* (*roseum*), *F. graminearum*	*130*, *131*
					(4-Deoxynivalenol)	*F. culmorum*, *F. sporotrichioides*	*132*
						(*tricinctum*, *moniliforme*) *F. sporotrichioides*	*117*, *117*, *95*
						F. solani, *F. sambucinum*, *F. avenaceum*	*133*, *133*, *134*
						F. oxysporum, *F. equiseti*, *F. moniliforme*	*135*, *135*, *136*
						F. semitectum, *F. acuminatum*, *F. poae*	*134*, *87*, *87*
						F. crookwellense, *F. subglutinans*	*87*, *137*
						Microdochium nivale (*F. nivale*)	*117*
$C_{15}H_{20}O_6$	H	OH	OH	OH	7-Deoxynivalenol	*F. graminearum*, *F. camptoceras*	*138*, *33*
$C_{15}H_{20}O_7$	OH	OH	OH	OH	Nivalenol	*F. sporotrichioides* (*nivale*, *episphaeria*)	*139*, *66*
						F. sporotrichioides, *F. semitectum*, *F. sambucinum*	*87*, *78*
						(*sulphureum*), *F. equiseti*, *F. graminearum*	*78*, *78*, *66*
						F. crookwellense, *F. camptoceras*, *F. poae*	*140*, *33*, *141*
						F. culmorum, *F. solani*, *F. avenaceum*	*87*, *142*, *143*
						F. sambucinum, *F. oxysporum*	*143*, *143*
$C_{17}H_{22}O_5$	H	H	OAc	H	Acetyltrichothecolone[e]	*T. roseum*	*144*
$C_{17}H_{22}O_5$	H	H	H	OAc	8-Oxoisotrichodermin	*F. crookwellense*	*19*
$C_{17}H_{22}O_6$	H	OH	H	OAc	8-Oxo-15-deacetylcalonectrin	*F. graminearum* (*roseum*), *F. culmorum*	*42*, *27*
$C_{17}H_{22}O_7$	OH	OH	H	OAc	3-Acetylvomitoxin	*F. culmorum*, *F. graminearum*, *F. graminearum*	*145*, *146*
						(*roseum*), *F. semitectum*, *F. camptoceras*	*130*, *134*, *33*
						F. solani, *F. acuminatum*, *F. avenaceum*	*133*, *143*, *143*
						F. sambucinum, *F. oxysporum*	*143*, *143*
$C_{17}H_{22}O_7$	OAc	OH	H	OH	7-Acetylvomitoxin	*F. camptoceras*	*33*
$C_{17}H_{22}O_7$	OH	OAc	H	OH	15-Acetylvomitoxin	*F. graminearum*, *F. sporotrichioides*, *F. semitectum*	*147*, *86*, *134*
						F. crookwellense, *F. avenaceum*, *F. culmorum*	*87*, *87*, *87*
						F. equiseti, *F. poae*	*87*, *87*
$C_{17}H_{22}O_8$	OH	OH	OAc	OH	Fusarenone	*F. sporotrichioides* (*nivale*, *episphaeria*, *oxysporum*)	*148*, *66*, *66*

Table 2 (*continued*)

Formula	R^1	R^2	R^3	R^4	Trivial Name	Source[b,c]	References[d]
					(Fusarenone X)	*F. sporotrichioides*, *F. sambucinum* (*sulphureum*)	*87*, *78*
						F. equiseti, *F. semitectum*, *F. graminearum*	*78*, *78*, *66*
						F. culmorum, *F. crookwellense*, *F. poae*	*134*, *140*, *141*
						F. camptoceras, *F. acuminatum*, *F. sambucinum*	*33*, *87*, *134*
						F. avenaceum	*143*
$C_{18}H_{24}O_8$	OH	OH	H	OR^{30}	CBD_2	Fungus infected barley	*149*
$C_{19}H_{24}O_5$	H	H	OR^7	H	Trichothecin	*T. roseum*, *F. graminearum*, ***Holarrhena floribunda***[g]	*150*, *151*, *61*
$C_{19}H_{24}O_6$	H	H	OR^7	OH	**Trichothecinol A**	***T. roseum***, ***Holarrhena floribunda***[g]	*62*, *61*
$C_{19}H_{24}O_7$	H	OAc	H	OAc	8-Oxocalonectrin	*F. culmorum*	*27*
$C_{19}H_{24}O_8$	H	OAc	OAc	OH	8-Oxodiacetoxyscirpenol[e]	*F. sporotrichioides*, *F. crookwellense*, *F. culmorum*	*152*, *19*, *12*
$C_{19}H_{24}O_8$	OH	OAc	H	OAc	3,15-Diacetylvomitoxin[e]	*F. graminearum* (*roseum*), *F. culmorum*	*153*, *11*
$C_{19}H_{24}O_9$	OH	OAc	OAc	OH	4,15-Diacetylnivalenol	*F. equiseti*, *F. culmorum*, *F. oxysporum* (*lateritium*)	*5*, *145*, *84*
						F. sporotrichioides (*nivale*, *oxysporum*)	*154*, *66*
						F. crookwellense, *F. camptoceras*, *F. sambucinum*	*19*, *33*, *12*
						F. graminearum, ***F. poae***	*12*, *35*
$C_{21}H_{26}O_6$	H	H	OR^{31}	OH	**F-11703-1**	***Acremonium* sp.**	*155*
$C_{21}H_{26}O_7$	OH	H	OR^{31}	OH	**F-11703-2**	***Acremonium* sp.**	*155*
$C_{24}H_{26}O_5$	H	H	OR^{32}	H	Cinnamyltrichothecolone	*T. roseum*	*144*
$C_{31}H_{50}O_5$	H	H	OR^{33}	H	Palmityltrichothecolone[f]	*F. moniliforme*	*128*

[a] New compounds and sources reported since Dec. 1995 are printed in bold type.

[b] *B* = *Baccharis*, *F* = *Fusarium*, *M* = *Myrothecium*, *S* = *Stachybotrys*, *T* = *Trichothecium*.

[c] Fusaria are named according to the scheme proposed by Booth (*7*), as extended and amended by Nelson *et al.* (*8*). Where reinvestigation has led to a change in identification (*9*), the original assignment is given in parentheses.

[d] The reference is to the first isolation from each source.

[e] Known compound at time of isolation: previously obtained by chemical modification of other naturally-occurring trichothecenes.

[f] Ms identification only: not completely characterized.

[g] A West African shrub. The workers (*61*) could not exclude the possibility that this compound arose from fungal contamination of the plant material. The material tested negative for *Fusarium* spp.; however, the pattern of the *known* metabolic products isolated included the diterpenoids rosenonolactone, 6β-hydroxy-rosenonolactone, and rosololactone, and is typical of a *T. roseum* strain.

Table 3. Non-Macrocylces: Trichothec-9,12-dienes (**4**)[a]

Formula	R^1	R^2	R^3	R^4	R^5	Trivial Name	Source[b,c]	References[d]
$C_{19}H_{26}O_6$	H	H	OAc	OAc	OH	12,13-Deoxydiacetoxyscirpenol	*F. graminearum*	*156*
$C_{23}H_{30}O_4$	H	H	H	OR^{15}	H	12,13-Deoxytrichodermadiene	*M. verrucaria*	*97*
$C_{29}H_{40}O_8$	H	H	OR^{10}	OR^{16}	H	12,13-Deoxytrichoverrin A,B[e]	*M. verrucaria*	*157*
$C_{29}H_{40}O_8$	H	H	OR^{10}	OR^{18}	H	**12,13-Deoxy-(2′E)-isotrichoverrin B**	***M. verrucaria***	*113*

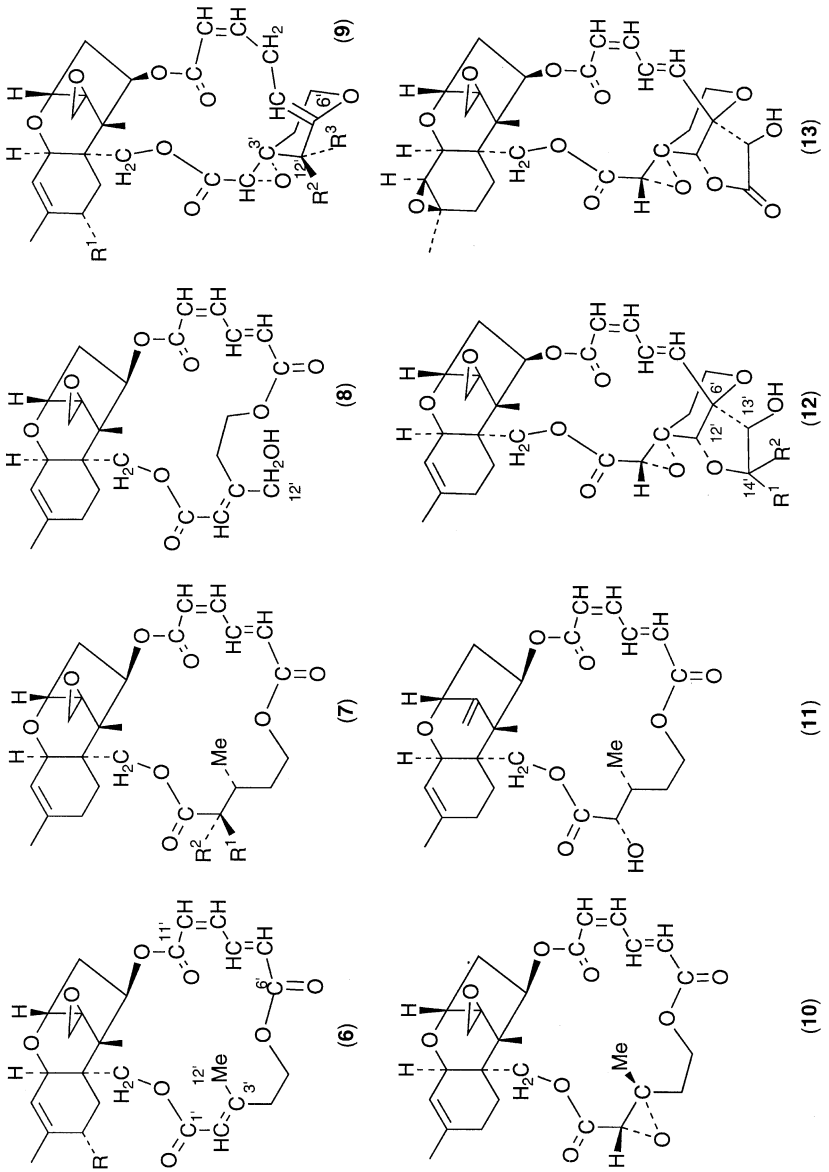
[a] New compounds and sources reported since Dec. 1995 are printed in bold type.
[b] *B* = *Baccharis*, *F* = *Fusarium*, *M* = *Myrothecium*, *S* = *Stachybotrys*, *T* = *Trichothecium*.
[c] Fusaria are named according to the scheme proposed by Booth (*7*), as extended and amended by Nelson *et al.* (*8*). Where reinvestigation has led to a change in identification (*9*), the original assignment is given in parentheses.
[d] The reference is to the first isolation from each source.
[e] Diastereoisomers, epimeric at C-7′. Isomer A has C-7′S.

References, pp. 113–130

Table 4. Non-Macrocycles: Miscellaneous (**5**)[a]

Formula	R^1	R^2	R^3	R^4	R^5	Trivial Name	Source[b,c]	References[d]
$C_{29}H_{40}O_{10}$	H	H	OR^{10}	OR^{17}	H	**9β,10β-Epoxyisotrichoverrin A,B**[e]	***M. verrucaria***	*113*

[a] New compounds and sources reported since Dec. 1995 are printed in bold type.
[b] *B* = *Baccharis*, *F* = *Fusarium*, *M* = *Myrothecium*, *S* = *Stachybotrys*, *T* = *Trichothecium*.
[c] Fusaria are named according to the scheme proposed by Booth (*7*), as extended and amended by Nelson *et al.* (*8*). Where reinvestigation has led to a change in identification (*9*), the original assignment is given in parentheses.
[d] The reference is to the first isolation from each source.
[e] Diastereoisomers, epimeric at C-7′ The prefix ''iso'' indicates that C-6′ is R. In this series isomer A has C-7′R.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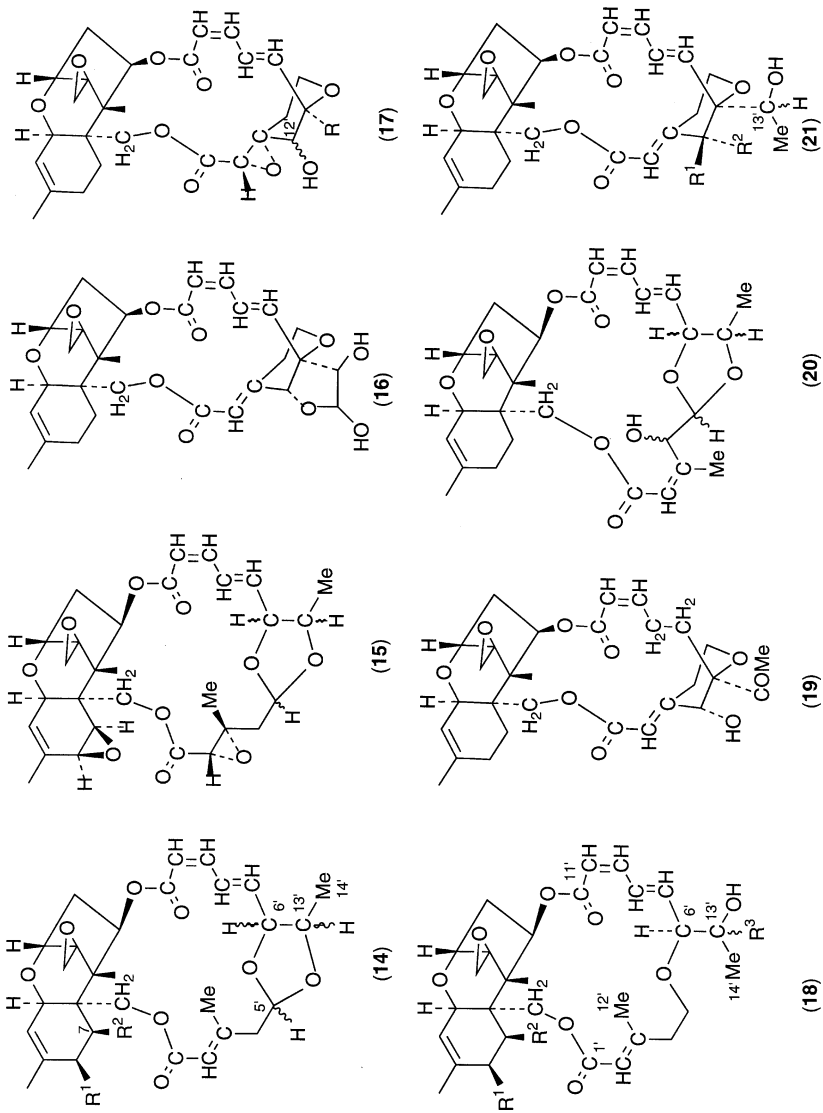

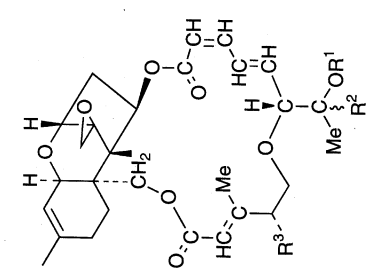

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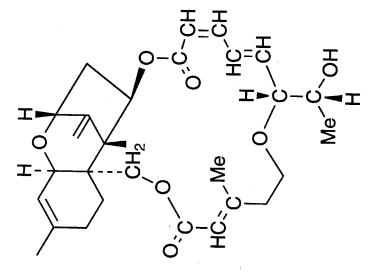

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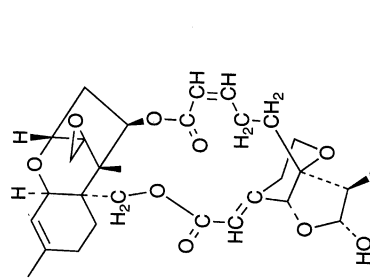

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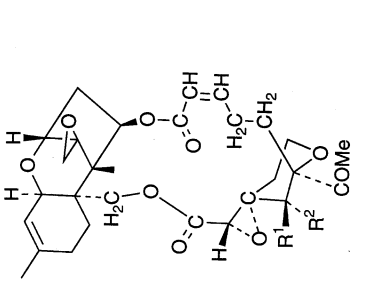

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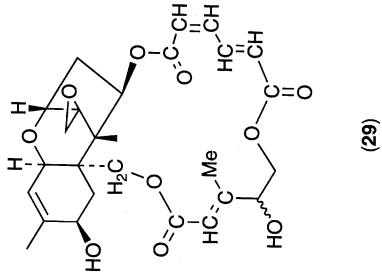

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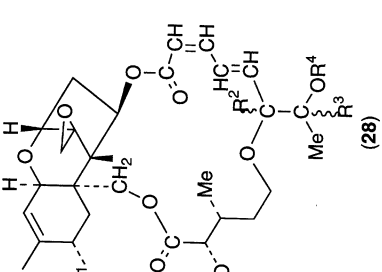

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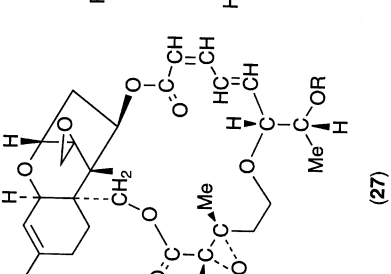

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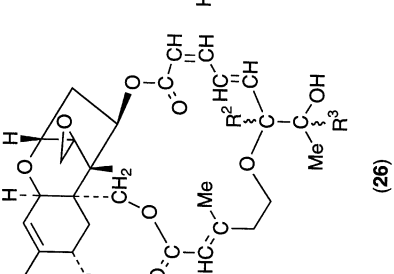

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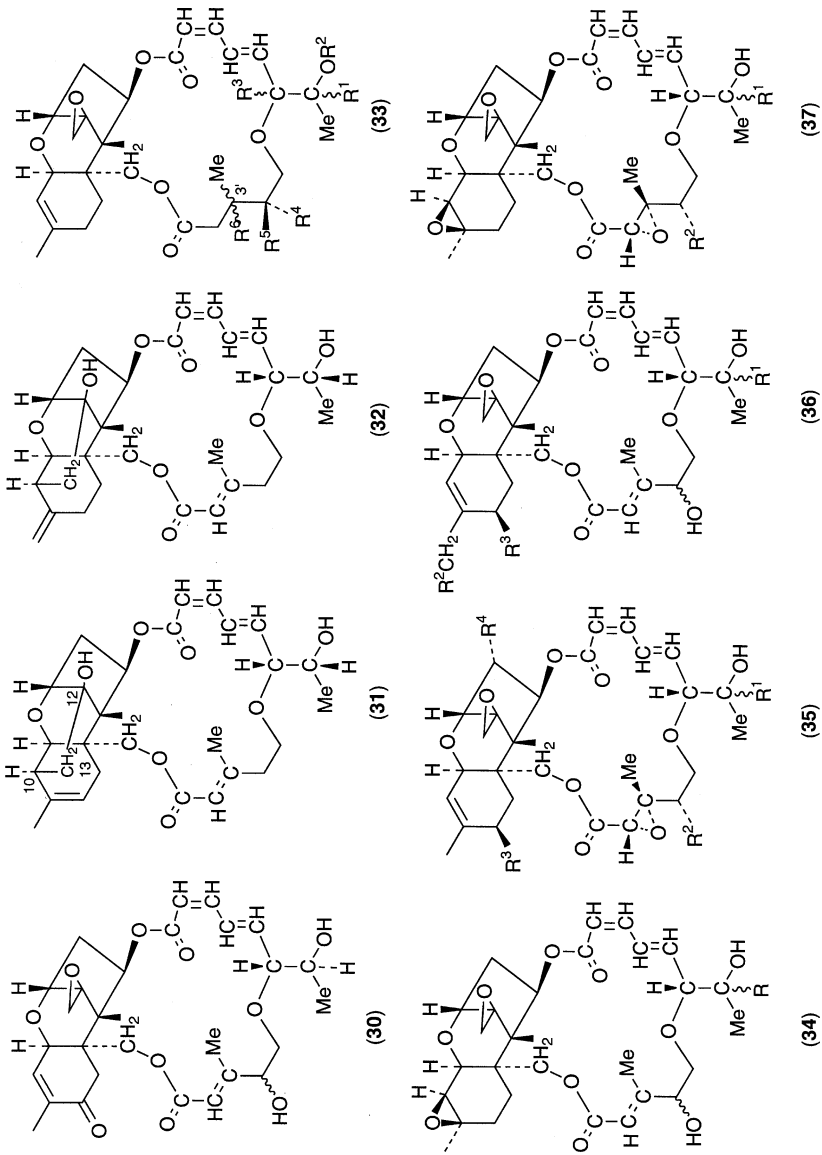

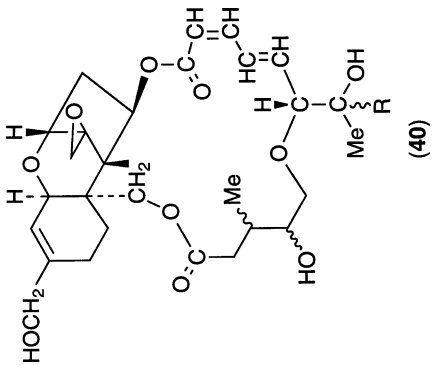

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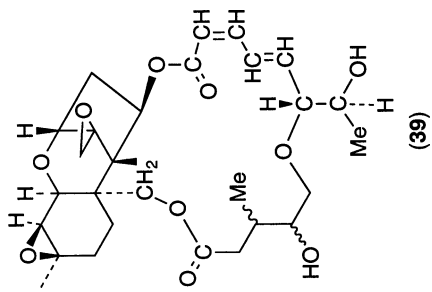

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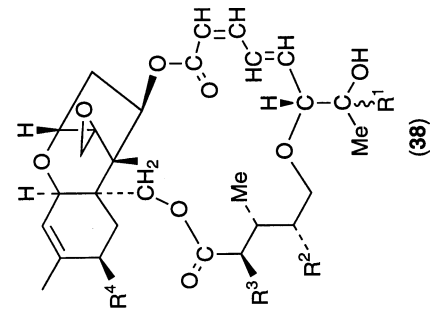


(38)

Table 5. Macrocycles: Verrucarins and Myrotoxins[a]

Formula	Trivial Name (synonym)	Structure	Source[b]	References[c]
$C_{27}H_{32}O_8$	Verrucarin J (Muconomycin B)[161] (Satratoxin C)[163]	(**6**; R = H)	*M. verrucaria*, *M. roridum* *S. atra*, *S. kampalensis*, *S. albipes* *S. microspora*, *B. coridifolia*, ***B. artemisioides*** ***Ceratopycnidium baccharidicola*** **Unidentified marine fungus**	*158*, *159* *160*, *111*, *111* *25*, *162*, *164* *164* *165*
$C_{27}H_{32}O_9$	2′-Dehydroverrucarin A	(**7**; R^1R^2 = O)	*M. roridum*	*166*
$C_{27}H_{32}O_9$	PD 113325[d]	(**8**)	*M. roridum*	*159*
$C_{27}H_{32}O_9$	Myrotoxin A	(**9**; $R^1 = R^2$ = H, R^3 = OH)	*M. roridum*	*168*
$C_{27}H_{32}O_9$	Myrotoxin C	(**9**; $R^1 = R^3$ = H, R^2 = OH)	*M. roridum*	*169*
$C_{27}H_{32}O_9$	Verrucarin B (SIPI-299-O)[171]	(**10**)	*M. verrucaria*, *M. roridum*, ***S. atra*** ***Phoma* sp.**	*14*, *14*, *170* *171*
$C_{27}H_{32}O_9$	Verrucarin L	(**6**; R = OH)	*M. verrucaria*	*172*
$C_{27}H_{34}O_8$	Verrucarin K[e]	(**11**)	*M. verrucaria*	*175*
$C_{27}H_{34}O_9$	Verrucarin A (Antibiotic Y379)[177] (Muconomycin A)[178] (SIPI-299-B)[171]	(**7**; R^1 = H; R^2 = OH)	*M. verrucaria*, *M. roridum*, *M. leucotrichum*, *B. coridifolia*, ***B. artemisioides*** ***Ceratopycnidium baccharidicola*** ***Acremonium neo-caledoniae*, *Phoma* sp.**	*14*, *14*, *176* *162*, *164* *164* *108*, *171*
$C_{29}H_{34}O_{10}$	Acetylverrucarin L	(**6**; R = OAc)	*M. verrucaria*, **Unidentified marine fungus**	*172*, *165*
$C_{29}H_{34}O_{11}$	Myrotoxin B	(**9**; R^1 = OAc, R^2 = H, R^3 = OH)	*M. roridum*	*168*
$C_{29}H_{34}O_{11}$	Myrotoxin D	(**9**; R^1 = OAc, R^2 = OH, R^3 = H)	*M. roridum*	*169*

[a] New compounds and sources reported since Dec. 1991 are printed in bold type.
[b] *B* = *Baccharis*, *C* = *Cylindrocarpon*, *M* = *Myrothecium*, *S* = *Stachybotrys*.
[c] The reference is to the first isolation from each source.
[d] Originally (*167*), incorrectly, named 12′-hydroxyverrucarin J.
[e] An earlier (*173*) bearer of this name was renamed roridin E (*174*).

Table 6. Macrocycles: Roridins and Roridin relatives[a]

Formula	Trivial Name (synonym)	Structure	Source[b]	References[c]
$C_{29}H_{32}O_{11}$	Roritoxin D	(**12**; $R^1R^2 = O$)	*M. roridum*	*179*
$C_{29}H_{32}O_{12}$	Roritoxin C	(**13**)	*M. roridum*	*179*
$C_{29}H_{34}O_9$	7β,8β-Epoxyroridin H	(**14**; $R^1R^2 = O$)	*C.* sp.	*180*
$C_{29}H_{34}O_{10}$	Diepoxyroridin H	(**15**)	*C.* sp.	*180*
$C_{29}H_{34}O_{10}$	Roritoxin A	(**16**)	*M. roridum*	*179*
$C_{29}H_{34}O_{10}$	Satratoxin F[d]	(**17**; R = COMe)	*S. atra*, *S. kampalensis*	*181*, *111*
$C_{29}H_{34}O_{10}$	**Isosatratoxin F**[d]	(**17**; R = COMe)	***S. atra***	*98*
$C_{29}H_{34}O_{11}$	Roritoxin B	(**12**; R^1R^2 = H, OH)	*M. roridum*	*179*
$C_{29}H_{36}O_8$	Roridin H[e]	(**14**; $R^1 = R^2 = H$)	*M. verrucaria*, *C.* sp., ***B. coridifolia***	*158*, *180*, *164*
$C_{29}H_{36}O_9$	7β,8β-Epoxyisororidin E	(**18**; $R^1R^2 = O$, $R^3 = \alpha H$)	*C.* sp.	*180*
$C_{29}H_{36}O_9$	Mytoxin B	(**19**)	*M. roridum*	*169*
$C_{29}H_{36}O_9$	Roridin J	(**20**)	*M. verrucaria*	*182*
$C_{29}H_{36}O_9$	Satratoxin H	(**21**; R^1 = H, R^2 = OH, C-13′ S)	*S. atra*, *S. kampalensis*, *S. microspora*	*163*, *111*, *25*
$C_{29}H_{36}O_9$	PD 113326 (*M* Isosatratoxin H)[167, 107]	(**21**; R^1 = H, R^2 = OH, C-13′ R)	*M. roridum*	*159*
$C_{29}H_{36}O_9$	Satratoxin H isomer[f] (*S* Isosatratoxin H)[107]	(**21**; R^1 = OH, R^2 = H)	*S. atra*	*183*
$C_{29}H_{36}O_9$	Satratoxin H isomer[f]	(**21**; R^1 = OH, R^2 = H)	*S. atra*	*160*
$C_{29}H_{36}O_{10}$	Mytoxin A	(**22**; R^1 = H, R^2 = OH)	*M. roridum*	*169*
$C_{29}H_{36}O_{10}$	Mytoxin C	(**22**; R^1 = OH, R^2 = H)	*M. roridum*	*169*
$C_{29}H_{36}O_{10}$	Satratoxin G[g]	(**17**; R = $\overset{13'}{C}$HOH·Me)	*S. atra*, *S. kampalensis*	*181*, *111*
$C_{29}H_{36}O_{10}$	**Isosatratoxin G**[h]	(**17**; R = $\overset{13'}{C}$HOH·Me)	***S. atra***	*107*
$C_{29}H_{36}O_{10}$	Vertisporin	(**23**)	*Verticinimonosporium diffractum*	*184*
$C_{29}H_{38}O_7$	**12,13-Deoxyroridin E**	(**24**)	***M. roridum***	*185*

Table 6 (*continued*)

Formula	Trivial Name (synonym)	Structure	Source[b]	References[c]
$C_{29}H_{38}O_8$	Roridin E (Satratoxin D)[186]	(**25**; $R^1 = R^3 = H$, $R^2 = \beta H$)	*M. verrucaria*, *M. roridum*, *S. atra*	*158*, *106*, *160*
			S. kampalensis, *S. microspora*	*111*, *25*
			B. coridifolia, *B. megapotamica*	*187*, *188*
			B. artemisioides, ***Cercophora areolata***	*164*, *189*
			Ceratopycnidium baccharidicola	*164*
$C_{29}H_{38}O_8$	**13′-Epiroridin E**	(**25**; $R^1 = R^3 = H$, $R^2 = \alpha H$)	***S. atra***, ***M. verrucaria***	*107*, *190*
$C_{29}H_{38}O_8$	Isororidin E	(**18**; $R^1 = R^2 = H$, $R^3 = \alpha H$)	*C.* sp., *M. verrucaria*, ***S. atra***	*180*, *6*, *107*
$C_{29}H_{38}O_8$	**13′-Epiisororidin E**	(**18**; $R^1 = R^2 = H$, $R^3 = \beta H$)	***S. atra***, ***M. verrucaria***	*107*, *190*
$C_{29}H_{38}O_8$	Roridin E-2[i]		*M. verrucaria*	*191*
$C_{29}H_{38}O_9$	8α-Hydroxyisororidin E[j] (**Isororidin K**)[190]	(**26**; $R^1 = H$, $R^2 = R^3 = \alpha H$)	*M.* sp., ***M. Verrucaria***	*192*, *190*
$C_{29}H_{38}O_9$	Roridin D	(**27**; $R = H$)	*M. roridum*, *B. megapotamica*	*158*, *188*
			B. coridifolia, ***B. artemisioides***	*193*, *164*
			Ceratopycnidium baccharidicola	*164*
$C_{29}H_{40}O_9$	Roridin A (Antibiotic X379)[177]	(**28**; $R^1 = R^4 = H$, $R^2 = R^3 = \beta H$)	*M. roridum*, *M. verrucaria*	*14*, *14*
			B. coridifolia, *B. megapotamica*	*187*, *194*
			Phomopsis leptostromiformis	*195*
			B. artemisioides	*164*
			Ceratopycnidium baccharidicola	*164*
$C_{29}H_{40}O_9$	Isororidin A	(**28**; $R^1 = R^4 = H$, $R^2 = \beta H$, $R^3 = \alpha H$)	*M. verrucaria*, ***Acremonium neo-caledoniae***	*196*, *108*
$C_{29}H_{40}O_9$	**6′-Epi-13′-epiroridin A**[k]	(**28**; $R^1 = R^4 = H$, $R^2 = R^3 = \alpha H$)	*M.* sp., ***M. verrucaria***	*192*, *190*
$C_{31}H_{40}O_9$	**Acetylroridin E**	(**25**; $R^1 = Ac$, $R^2 = \beta H$, $R^3 = H$)	***M. verrucaria***	*197*

$C_{31}H_{40}O_{10}$	Acetylroridin K	(**26**; R^1 = Ac, $R^2 = R^3 = \beta$H)	*M. verrucaria*	*6*
$C_{33}H_{44}O_{11}$	**YM-47524**	(**28**; R^1 = OCOCH = CHMe, $R^2 = \beta$H, $R^3 = R^4$ = H)	**Unidentified fungus**	*198*
$C_{33}H_{46}O_{11}$	**YM-47525**	(**28**; R^1 = $OCOCH_2CH_2Me$, $R^2 = \beta$H, $R^3 = R^4$ = H)	**Unidentified fungus**	*198*

[a] New compounds and sources reported since Dec. 1991 are printed in bold type.
[b] *B* = *Baccharis*, *C* = *Cylindrocarpon*, *M* = *Myrothecium*, *S* = *Stachybotrys*.
[c] The reference is to the first isolation from each source.
[d] Likely to be epimeric at C-12′, but the nmr evidence was inconclusive (*98*).
[e] Previously verrucarin H (*158*).
[f] Possibly C-13′ epimers.
[g] C-12′-αOH: the configuration at C-13′ is unknown.
[h] Nmr evidence suggests a C-12′-epimer: the configuration at C-13′ is unknown.
[i] Well characterized isomer of roridin E. The 2′-ene may have the Z configuration (*191*).
[j] Originally (*192*) believed to be 8β-hydroxyroridin E.
[k] Originally (*192*) believed to be roridin A (6′R, 13′R), but is now considered (*190*), on compelling nmr evidence, to be the (6′S, 13′S) isomer.

Table 7. Macrocycles: Baccharinoids[a]

Formula	Trivial Name (synonym)	Structure	Source[b]	Refs.[c]
$C_{27}H_{32}O_{10}$	Baccharinoid B25[d]	(**29**)	*B. megapotamica*	*199*
$C_{29}H_{36}O_{10}$	Baccharinoid B27	(**30**)	*B. megapotamica*	*199*
$C_{29}H_{38}O_8$	Miophytocen A[e]	(**31**)	*B. coridifolia*	*200*
$C_{29}H_{38}O_8$	Miophytocen B[e]	(**32**)	*B. coridifolia*	*200*
$C_{29}H_{38}O_9$	Miotoxin A	(**25**; $R^1 = H$, $R^2 = \beta H$, $R^3 = OH$)	*B. coridifolia*	*201*
$C_{29}H_{38}O_9$	Miotoxin B	(**33**; $R^1 = R^2 = R^3 = R^6 = H$, $R^4R^5 = O$)	*B. coridifolia*	*202*
$C_{29}H_{38}O_{10}$	**Miotoxin E**	(**33**; $R^1 = R^3 = \beta H$, $R^2 = H$, $R^4R^5 = O$, $R^6 = OH$)	*B. coridifolia*	*127*
$C_{29}H_{38}O_{10}$	Baccharinoid B9	(**34**; $R = \beta H$)	*B. megapotamica*	*199*
$C_{29}H_{28}O_{10}$	Baccharinoid B10	(**34**; $R = \alpha H$)	*B. megapotamica*	*199*
$C_{29}H_{38}O_{10}$	Baccharinoid B12	(**35**; $R^1 = \beta H$, $R^2 = R^3 = H$, $R^4 = OH$)	*B. megapotamica*	*199*
$C_{29}H_{38}O_{10}$	Baccharinoid B13	(**36**; $R^1 = \beta H$, $R^2 = H$, $R^3 = OH$)	*B. megapotamica*	*199*
$C_{29}H_{38}O_{10}$	Baccharinoid B14	(**36**; $R^1 = \alpha H$, $R^2 = H$, $R^3 = OH$)	*B. megapotamica*	*199*
$C_{29}H_{38}O_{10}$	Baccharinoid B16	(**36**; $R^1 = \alpha H$, $R^2 = OH$, $R^3 = H$)	*B. megapotamica*	*199*
$C_{29}H_{38}O_{10}$	Baccharinoid B17	(**37**; $R^1 = \beta H$, $R^2 = H$)	*B. megapotamica*	*199*
$C_{29}H_{38}O_{10}$	Baccharinoid B21	(**35**; $R^1 = \beta H$, $R^2 = R^4 = H$, $R^3 = OH$)	*B. megapotamica*	*199*
$C_{29}H_{38}O_{11}$	Baccharinoid B4 (Baccharinol)	(**35**; $R^1 = \alpha H$, $R^2 = R^3 = OH$, $R^4 = H$)	*B. megapotamica*	*203*
$C_{29}H_{38}O_{11}$	Baccharinoid B5 (Baccharin)	(**37**; $R^1 = \beta H$, $R^2 = OH$)	*B. megapotamica* *B. coridifolia* (?)	*204* *193*
$C_{29}H_{38}O_{11}$	Baccharinoid B6 (isoBaccharinol)	(**35**; $R^1 = \beta H$, $R^2 = R^3 = OH$, $R^4 = H$)	*B. megapotamica*	*203*
$C_{29}H_{38}O_{11}$	Baccharinoid B8 (isoBaccharin)	(**37**; $R^1 = \alpha H$, $R^2 = OH$)	*B. megapotamica*	*203*
$C_{29}H_{40}O_9$	Miotoxin D[f]	(**33**; $R^1 = R^3 = \beta H$, $R^2 = R^5 = R^6 = H$, $R^4 = OH$)	*B. coridifolia*	*205*
$C_{29}H_{40}O_9$	isoMiotoxin D[f]	(**33**; $R^1 = R^3 = \beta H$, $R^2 = R^5 = R^6 = H$, $R^4 = OH$)	*B. coridifolia*	*205*

$C_{29}H_{40}O_{10}$	**Miotoxin F**	(**33**; $R^1 = R^3 = \beta H$, $R^2 = H$, $R^4R^5 = H$, OH, $R^6 = OH$)	***B. coridifolia***	*127*
$C_{29}H_{40}O_{10}$	Baccharinoid B1	(**38**; $R^1 = \beta H$, $R^2 = R^4 = OH$, $R^3 = H$)	*B. megapotamica*	*206*
$C_{29}H_{40}O_{10}$	Baccharinoid B2	(**38**; $R^1 = \alpha H$, $R^2 = R^4 = OH$, $R^3 = H$)	*B. megapotamica*	*206*
$C_{29}H_{40}O_{10}$	Baccharinoid B3 (Baccharisol)	(**38**; $R^1 = \alpha H$, $R^2 = H$, $R^3 = R^4 = OH$)	*B. megapotamica* *B. coridifolia*	*206* *162*
$C_{29}H_{40}O_{10}$	Baccharinoid B7 (isoBaccharisol)	(**38**; $R^1 = \beta H$, $R^2 = H$, $R^3 = R^4 = OH$)	*B. megapotamica* *B. coridifolia*	*206* *162*
$C_{29}H_{40}O_{10}$	Baccharinoid B20	(**39**)	*B. megapotamica*	*199*
$C_{29}H_{40}O_{10}$	Baccharinoid B23	(**40**; $R = \beta H$)	*B. megapotamica*	*199*
$C_{29}H_{40}O_{10}$	Baccharinoid B24	(**40**; $R = \alpha H$)	*B. megapotamica*	*199*
$C_{31}H_{42}O_{11}$	Miotoxin C	(**33**; $R^1 = R^3 = \beta H$, $R^2 = Ac$, $R^4R^5 = H$, OH, $R^6 = OH$)	*B. coridifolia*	*202*
$C_{33}H_{44}O_{14}$	**Verrucarin A glucoside**	(**7**; $R^1 = H$, $R^2 = OC_6H_{11}O_5$)	***B. coridifolia***	*127*
$C_{35}H_{48}O_{13}$	**Roridin E glucoside**	(**25**; $R^1 = C_6H_{11}O_5$, $R^2 = \beta H$, $R^3 = H$)	***B. coridifolia***	*127*
$C_{35}H_{48}O_{14}$	**Roridin D glucoside**	(**27**; $R = C_6H_{11}O_5$)	***B. coridifolia***	*127*
$C_{35}H_{48}O_{14}$	**Miotoxin A 13′-glucoside**	(**25**; $R^1 = C_6H_{11}O_5$, $R^2 = \beta H$, $R^3 = OH$)	***B. coridifolia***	*127*
$C_{35}H_{50}O_{14}$	**Roridin A glucoside**	(**28**; $R^1 = H$, $R^2 = R^3 = \beta H$, $R^4 = C_6H_{11}O_5$)	***B. coridifolia***	*127*
$C_{35}H_{50}O_{15}$	**Miotoxin F glucoside**	(**33**; $R^1 = R^3 = \beta H$, $R^2 = C_6H_{11}O_5$, $R^4R^5 = H$, OH, $R^6 = OH$)	***B. coridifolia***	*127*

[a] New compounds and sources reported since Dec. 1991 are printed in bold type.
[b] *B* = *Baccharis*, *C* = *Cylindrocarpon*, *M* = *Myrothecium*, *S* = *Stachybotrys*.
[c] The reference is to the first isolation from each source.
[d] No evidence is presented (*199*) for an (E)-2′-ene. The chemical shift for C-12′ is consistent with the (Z) configuration.
[e] Possible artefact, derived from roridin E.
[f] C-3′ epimers.

The carbon skeleton (**9**) of the myrotoxins can formally be constructed from the verrucarin skeleton by formation of a C6′–C12′ bond, and this group of compounds is tabulated with the verrucarins. Likewise, the skeletons of the satratoxins, roritoxins, mytoxins and vertisporin can be obtained from the roridin skeleton by C6′–C12′ bond formation, and these compounds are tabulated with the roridins.

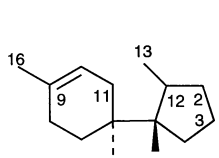
With the exception of verrucarin K (**11**) and 12,13-deepoxy-roridin E (**24**), which are 9(10),12(13)-dienes; and the miophytocens, which are 10,13-cyclo-trichothecanes, all the macrocycles have a 12,13-epoxide. The 9(10)-ene is replaced by a 9,10-epoxide in roritoxin C and in several baccharinoids.

2.2. Trichothecene Relatives

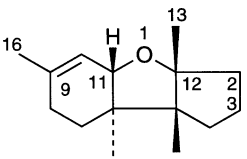
Some large scale and/or blocked fermentations with *Trichothecium roseum* or *Fusarium* spp. have yielded a number of metabolic products with structures closely related to the trichothecenes. These relatives, 49 in number (including 2 likely artefacts), an addition of six since 1995, are listed in Table 8 (structures: Scheme 1), together with their sources. They consist mainly of

(a) compounds with the tricho-9-ene skeleton (**41**)[2], some of which are intermediates in the biosynthesis of the trichothecenes, and
(b) from *Fusarium* spp., compounds with the 11-epiapo-trichothecene nucleus (**42**).

The *Fusarium* spp. metabolic products sambucinol (**81**) and its derivatives diacetylsambucinol (**82**) and 3-deoxysambucinol (**80**) can be regarded as 11-epi-12-epitrichothecenes but are more conveniently classified with the trichothecene relatives (Table 8), as is gramilaurone (**98**). These compounds are excluded from Tables 1–4 and the total of trichothecenes, as is the ''isotrichothecin'' (*233*) whose ^{13}C-nmr spectrum is indistinguishable from that of trichothecin.



(**41**)



(**42**)

[2] Biosynthetic numbering: no position 1.

Table 8. Naturally occurring trichothecene relatives[a]

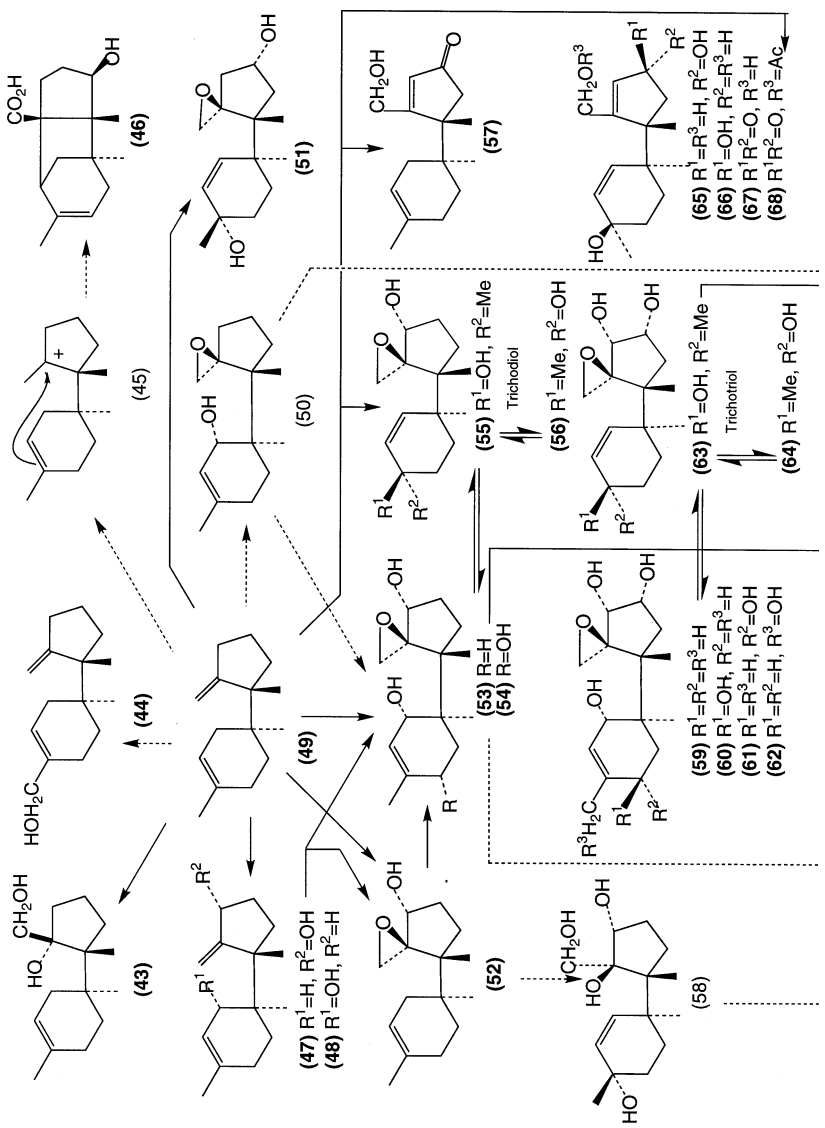
Formula	Trivial Name	Structure	Source[b]	Refs.
$C_{15}H_{20}O_3$	FS 3	**76**	*F. sambucinum*	*32*
$C_{15}H_{20}O_4$	**Loukacinol B**	**89**	***Holarrhena floribunda***[c]	*61*
$C_{15}H_{20}O_5$	**Loukacinol A**	**90**	***Holarrhena floribunda***[c]	*61*
$C_{15}H_{22}O_2$		**57**	*F. culmorum*	*207*
$C_{15}H_{22}O_3$	FS 1	**75**	*F. sporotrichioides*, *F. sambucinum*	*21*, *32*
$C_{15}H_{22}O_3$	FS 4	**67**[d]	*F. sambucinum*	*32*
$C_{15}H_{22}O_3$	3-Deoxysambucinol	**80**	*F. culmorum*, *F. graminearum*, *F. crookwellense*	*208*, *208*, *19*
$C_{15}H_{22}O_3$	Sambucinic acid	**46**	*F. sambucinum*	*209*
$C_{15}H_{22}O_3$	Sambucoin	**69**	*F. sambucinum*, *F. sporotrichioides*, *F. poae*	*210*, *65*, *12*
			F. culmorum, *F. graminearum*, *F. crookwellense*	*27*, *43*, *19*
$C_{15}H_{22}O_3$		**92**	*F. sporotrichioides*, *F. culmorum*, ***F. graminearum***	*211*, *211*, *22*
$C_{15}H_{22}O_3$	3-Dehydroapotrichodiol	**88**	*F. sporotrichioides*, *F. sambucinum*	*32*, *32*
$C_{15}H_{22}O_4$		**93**	*F. sporotrichioides*, ***F. graminearum***	*90*, *22*
$C_{15}H_{22}O_4$	8β-Hydroxysambucoin	**70**	*F. sporotrichioides*	*212*
$C_{15}H_{22}O_4$	8α-Hydroxysambucoin	**71**	*F. sporotrichioides*	*212*
$C_{15}H_{22}O_4$	Sambucinol	**81**	*F. sambucinum*, *F. sporotrichioides*, *F. culmorum*	*210*, *65*, *27*
			F. graminearum, *F. crookwellense*, ***F. venenatum***	*208*, *19*, *213*
$C_{15}H_{22}O_4$	Sporol	**91**[e]	*F. sporotrichioides*	*54* (*214*)
$C_{15}H_{22}O_7$	Gramilaurone	**98**[f]	*F. graminearum*	*215*
$C_{15}H_{24}$	Trichodiene	**49**	*T. roseum*, *F. sambucinum*, *F. sporotrichioides*	*216*, *217*, *218*
			F. culmorum, *Monascus purpureus*, ***S. atra***	*219*, *220*, *221*
$C_{15}H_{24}O$	**2α-Hydroxytrichodiene**	**47**	***F. culmorum***	*222*
$C_{15}H_{24}O$	11α-Hydroxytrichodiene	**48**	*F. sporotrichioides*	*223*

Table 8 (*continued*)

Formula	Trivial Name	Structure	Source[b]	Refs.
$C_{15}H_{24}O$	**16-Hydroxytrichodiene**[g]	**44**	***Nicotiana tabacum***[h]	*224*
$C_{15}H_{24}O_2$	Isotrichool	**52**	*F. culmorum*	*225*
$C_{15}H_{24}O_2$	Apotrichool	**85**	*F. culmorum*, ***F. sporotrichioides***	*226*, *26*
$C_{15}H_{24}O_3$	Apotrichodiol	**86**[i]	*F. culmorum*, *F. graminearum*, *F. crookwellense*	*208*, *208*, *19*
			F. sporotrichioides, *F. sambucinum*, ***F. venenatum***	*65*, *32*, *20*
$C_{15}H_{24}O_3$	3-epi-Apotrichodiol	**87**[i]	*F. culmorum*, *F. graminearum*, *F. crookwellense*	*208*, *208*, *19*
			F. sporotrichioides, *F. sambucinum*	*65*, *32*
$C_{15}H_{24}O_3$		**83**	***F. culmorum***	*228*
$C_{15}H_{24}O_3$		**100**	***F. culmorum***	*228*
$C_{15}H_{24}O_3$		**78**	*F. sporotrichioides*	*229*
$C_{15}H_{24}O_3$	FS 2	**65**[d]	*F. sporotrichioides*	*152*
$C_{15}H_{24}O_3$	3-epi-FS 2	**66**	*F. sporotrichioides*	*32*
$C_{15}H_{24}O_3$	Trichodiol[j]	**55**[k,l]	*T. roseum*	*230*
$C_{15}H_{24}O_3$	9-epi-Trichodiol[m]	**56**	*T. roseum*	*55*
$C_{15}H_{24}O_3$	Isotrichodiol	**53**[k]	*F. culmorum*	*219*
$C_{15}H_{24}O_3$		**51**[n]	*F. sporotrichioides*	*152* (*231*)
$C_{15}H_{24}O_4$	Trichotriol	**63**[k,o]	*F. sporotrichioides*, *F. culmorum*	*152*, *231*
$C_{15}H_{24}O_4$	9-epi-Trichotriol[p]	**64**	*F. sporotrichioides*, *F. culmorum*	*232*, *231*
$C_{15}H_{24}O_4$	Isotrichotriol	**59**[k]	*F. sporotrichioides*, *F. culmorum*	*223*, *55*
$C_{15}H_{24}O_4$	8α-Hydroxyisotrichodiol	**54**	*F. culmorum*	*231*
$C_{15}H_{24}O_5$	8β-Hydroxyisotrichotriol	**60**	*F. sporotrichioides*	*223*
$C_{15}H_{24}O_5$	8α-Hydroxyisotrichotriol	**61**	*F. sporotrichioides*	*223*
$C_{15}H_{24}O_5$	16-Hydroxyisotrichotriol	**62**	*F. sporotrichioides*	*223*
$C_{15}H_{26}O_2$		**43**[q]	*F. culmorum*	*225*

$C_{17}H_{24}O_4$	AcetylFS 4	**68**[d]	*F. sambucinum*	*207*
$C_{17}H_{26}O_4$		**79**	*F. sporotrichioides*	*229*
$C_{17}H_{26}O_5$		**94**	*F. sporotrichioides*	*90*
$C_{19}H_{26}O_6$	Diacetylsambucinol	**82**	*F. sporotrichioides*	*90*
$C_{19}H_{28}O_6$		**95**	*F. sporotrichioides*	*90*
$C_{19}H_{28}O_6$		**96**	*F. sporotrichioides*	*90*

[a] New compounds and sources reported since December 1995 are printed in bold type.
[b] *F* = *Fusarium*, *S* = *Stachybotrys*, *T* = *Trichothecium*.
[c] A West African shrub. The workers (*61*) could not exclude the possibility that this compound arose from fungal contamination of the plant material. The material tested negative for *Fusarium* spp.; however, the pattern of the *known* metabolic products isolated included the diterpenoids rosenonolactone, 6β-hydroxy-rosenonolactone, and rosololactone, and is typical of a *T. roseum* strain.
[d] Originally formulated (*152*, *32*, *207*) as the 9α-hydroxy epimer, but changed (*231*) on spectroscopic evidence.
[e] An earlier, incorrect, structure (*54*) has been renamed neosporol.
[f] As published, but a possible artefact. The analogous structure (**99**) could be derived from vomitoxin (**3**; $R^1 = R^2 = R^4 = OH$, $R^3 = H$) (*3*).
[g] Reported (*224*) as 15-hydroxytrichodiene (distinct numbering).
[h] Cell suspension culture of *N. tabaccum* transformed with a gene encoding trichodiene synthase from *F. sporotrichioides*.
[i] Initially (*208*) formulated as an apotrichothecene, but subsequently shown (*227*, *211*) to belong to the 11-epi series.
[j] Trichodiol A (**97**) is considered (*229*) to be an artefact.
[k] Some groups of workers, *e.g.* (*225*, *219*), consistently (and mistakenly) write this compound as the 12-epimer.
[l] Originally (*230*) formulated without assignment of configuration at position 9, but commonly written as the 9α-hydroxy compound. It was subsequently shown to be the 9β-hydroxy epimer (*231*).
[m] Originally (*55*) called 9β-trichodiol.
[n] The metabolite was believed (*152*) to be trichodiol. This conclusion has been criticised (*231*), but the metabolite does not have the newly assigned (*231*) 12-ene structure: It is most probably (**51**).
[o] Initially (*152*) written as the 9α-hydroxy epimer by analogy with the commonly written structure for trichodiol.
[p] Originally (*232*) called 9β-trichotriol.
[q] Or C-12 epimer.



Scheme 1 (*continued*)

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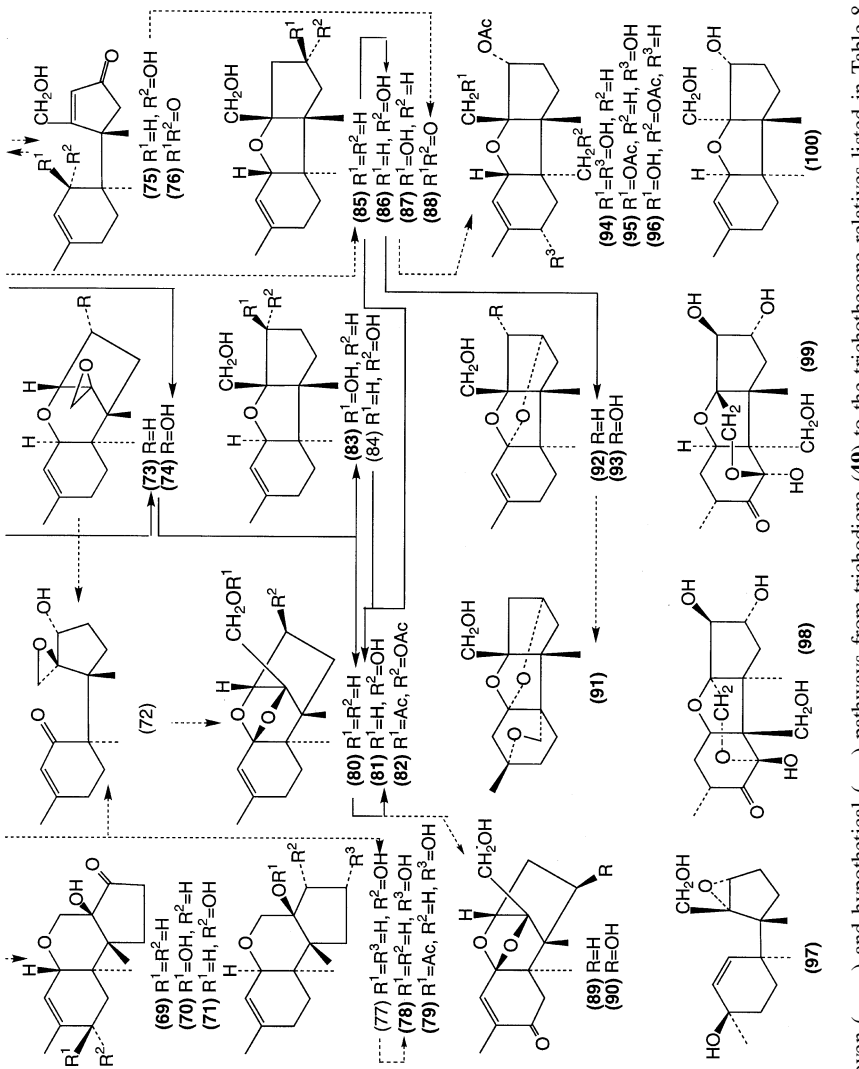


Scheme 1. Proven (——) and hypothetical (- - -) pathways from trichodiene (**49**) to the trichothecene relatives listed in Table 8. **(nn)** Isolated trichothecene relative. (nn) Hypothetical intermediate

2.3. Sources

The genus *Fusarium* is classified according to the scheme proposed by Booth (*7*) and later extended and modified by Nelson *et al.* (*8*, *9*), who, in the Sporotrichiella Section, accepted *F. chlamydosporium* but confined the name *F. tricinctum* to *F. tricinctum* sensu stricto. Nelson *et al.* (*9*) reexamined many of the known toxigenic *Fusarium* cultures and, in cases of mistaken identification, corrected the assignment. Where this has occurred the original assignment, or its equivalent in Booth's system of nomenclature, is placed in parentheses in Tables 1–4. Some new *Fusarium* sources claimed since this authoritative treatise (*9*) went to press (1981) have not been subjected to the same rigorous scrutiny.

F. compactum is equated with *F. equiseti* (*7*). The subspecies of *F. acuminatum*, *F. acuminatum* subsp. *armeniacum* (*234*) is not differentiated in the Tables. The morphological diversity of strains classified as *F. sambucinum* has led to their being divided into *F. sambucinum* sensu stricto, *F. torulosum* and *F. venenatum* (*235*). Strains classified as *F. torulosum* did not produce trichothecenes. Those classified as *F. sambucinum* sensu stricto produced mainly diacetoxyscirpenol, though some produced neosolaniol and T-2 toxin (*79*, *236*), but *F. venenatum* strains produced only diacetoxyscirpenol (*79*, *236*) and isotrichodermin (*20*) and their close relatives. In the Tables, "*F. sambucinum*" refers to *F. sambucinum* sensu lato. With the removal of *F. nivale* (Aarachnites Section) to the genus *Microdocium* (*237*), trichothecenes are produced by 20 *Fusarium* species drawn from 8 of the 12 Sections in Booth's scheme.

Stachybotrys atra, *S. alternans* and *S. chartarum* are synonymous (*238*) and *S. atra* is used. *Trichoderma lignorum* is equated with *T. viride* (*239*).

The phylogenetic relationship between *Spicellum roseum* and *Trichothecium roseum* has been examined by analysis of partial sequences of RNA subunits (*13*). Although the relationship was close, morphological differences supported the maintenance of separate genera for these sources. The genus *Memnoniella* is very close to *Stachybotrys* (*17*), but morphological differences are again sufficient for the two genera to be regarded as distinct.

Dendrodochium toxicum, a source of verrucarin A (*240*) and roridin A (*241*), is considered to be *Myrothecium verrucaria* (*242*).

The simple trichothecenes are products of *Acremonium*, *Fusarium*, *Myrothecium*, *Stachybotrys* and *Trichoderma* spp., and one species each of *Cephalosporium*, *Cylindrocladium*, *Dendrostilbella*, *Gliocladium*, *Memnoniella*, *Microdocium*, *Spicellum* and *Trichothecium*. *Trichothecium roseum* is, nevertheless, responsible for 12 metabolic products with

the trichothecene skeleton. *Acremonium*, *Cylindrocladium*, *Gliocladium*, and *Memnoniella* are new sources reported since 1995.

The trichoverroids and fungal macrocycles are products of *Myrothecium* and *Stachybotrys* spp., and one species each of *Acremonium*, *Ceratopycnidium*, *Cercophora*, *Cylindrocarpon*, *Phoma*, *Phomopsis*, and *Verticinimonosporium*. *Acremonium*, *Ceratopycnidium*, *Cercophora* and *Phoma* are new sources reported since 1991.

The shrub *Baccharis artemisioides* is a new source of the known fungal macrocycles verrucarins A and J, and roridins A, D and E, as is the *Baccharis* spp. endophyte *Ceratopycnidium baccharidicola* (*164*). *B. megapotamica* and *B. coridifolia* remain the only sources of the baccharinoids.

Of the 98 known simple trichothecenes, 82 have been obtained from *Fusarium* spp. This is, in part, a reflection of the interest shown in toxins produced by the grain pathogens *F. sporotrichioides* (45 trichothecenes) and *F. graminearum* (33 trichothecenes).

Trichodiene (**49**; Scheme 1) has been detected as a volatile product of *Monascus purpureus* and of *Stachybotrys atra*, but the trichothecene relatives (Table 8), including, possibly, the loukacinols (see note c), are essentially products of *Fusarium* spp. and *T. roseum*.

2.4. Oxygenation Pattern

The oxygenation pattern of the simple trichothecenes shows marked genus specificity. With very few exceptions products of *Fusarium* spp. show oxygenation at C-3α, which can be accompanied by additional oxygenation at positions 15, 8α, 7α and 4β. On the other hand, products from *Myrothecium*, *Stachybotrys* and *Trichoderma* spp. show oxygenation at C-4β, which can be accompanied by additional oxygenation at positions 15 or 8α, and, in the case of *Myrothecium*, position 16. *Trichothecium roseum* holds an intermediate position with oxygenation at C-4β accompanied by additional oxygenation at C-8α and C-3α.

8β-Hydroxyscirpene (Table 1: $C_{15}H_{22}O_3$) (from *F. sporotrichioides*) is the only known example of 8β-hydroxylation in the simple trichothecenes.

12,13-Epoxytrichothec-9-en-8-ones (Table 2) are often referred to as Type B trichothecenes [Group III on the chemical classification (*2*), based on ring A chemistry]; the remaining simple trichothecenes, without a keto group at C-8, are Type A (Groups I and II of the chemical classification).

Within a given *Fusarium* species, variation in the ability to effect 4-hydroxylation or acetylation at positions 3 and 15 is significant, and the classification of some *Fusarium* strains in the Discolor Section

(*F. sambucinum*, *culmorum*, *graminearum*, *crookwellense*) into chemotypes based on the hydroxylation pattern of the metabolites has been proposed (*243*). Within *F. graminearum* two chemotypes exist, producing either vomitoxin (4-deoxynivalenol) or nivalenol-related trichothecenes (*244*, *245*, *246*). An important factor in all these chemotypes is the geographical location from which the organism was isolated. However, in assessing this work it must be remembered that some strains of *F. graminearum* produce both nivalenol and vomitoxin and/or their acetyl derivatives.

The sequences within the nuclear ribosomal DNA (28S) of a number of *Fusarium* spp. have been examined with a view to determining the genetic relationship of the trichothecene producers (*247*). It was concluded that the phylogenetic placement of these correlated better with secondary metabolite data than with the current classification system based on morphology. There seems to be general agreement that the systematics of the Fusaria is in an unsatisfactory state, and that, in the long term, a new system, based on genetic constitution, will be introduced.

The oxygenation pattern of the trichothecene nucleus in the fungal macrolides is similar to that of the simple trichothecenes, but roritoxin C is a 9β,10β-epoxide and four of the five known products of an unidentified *Cylindrocarpon* sp. have a 7β,8β-epoxide. A 7β,8β-epoxide is also found in the simple trichothecene crotocin.

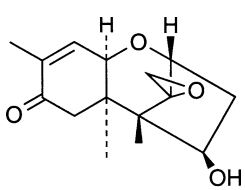
The baccharinoids (Table 7) are essentially roridins which have undergone further hydroxylation and/or epoxidation giving a substitution pattern found only rarely in the fungal macrocyclic trichothecenes. 3α-Hydroxylation, in baccharinoid B12, is unknown in other macrocycles, whilst 8β-hydroxylation occurs in ten baccharinoids, and 9β,10β-epoxidation in six. 16-Hydroxylation occurs in three baccharinoids but otherwise only in non-macrocyclic *Myrothecium* products. Baccharinoid B27 is the only macrocycle to have an 8-oxo group, a common feature among simple trichothecenes.

3. Biosynthesis

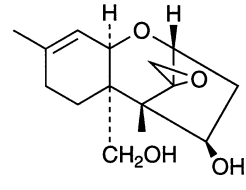
3.1. Simple Trichothecenes

3.1.1. Mevalonic Acid to Trichodiene (Scheme 2)

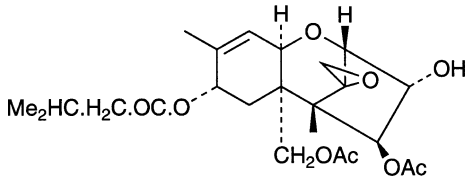
The early work on the biosynthesis of the trichothecene nucleus related to the formation of trichothecolone (**101**) in *Trichothecium roseum* and of derivatives of verrucarol (**102**) in *Myrothecium* spp. This work has been reviewed in detail (*248*). The more recent work has been



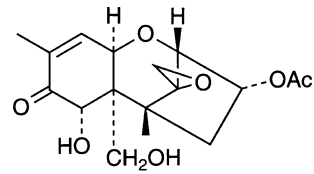
(**101**)



(**102**)



(**103**)



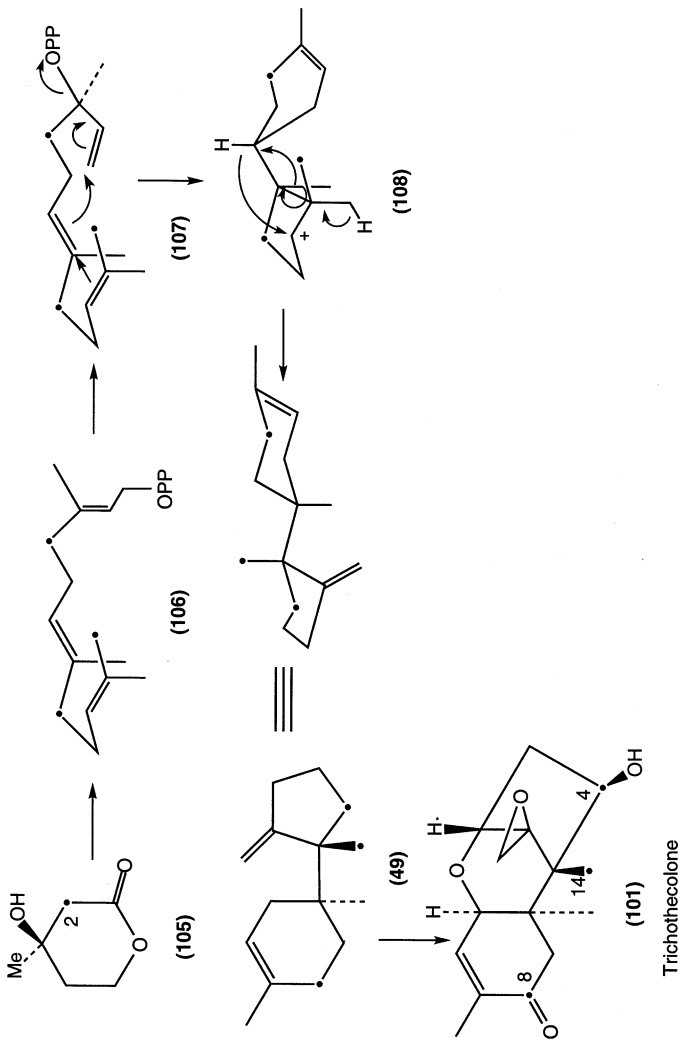
(**104**)

concerned mainly with the biosynthesis of T-2 toxin (**103**) by *Fusarium sporotrichioides* and of 3-acetylvomitoxin (**104**) by *F. culmorum* and *F. graminearum*.

Studies in which [2-^{13}C]-mevalonic acid lactone ("mevalonate") (**105**) was used as a precursor showed that three molecules were incorporated into trichothecolone (**101**), the label appearing at positions 4, 8, and 14 (*249*). The involvement of 2-*trans*-6-*trans*-farnesyldiphosphate (**106**) had been shown previously using ^{14}C-labelled material (*250*). The biosynthesis thus follows a standard sesquiterpene pathway from mevalonate through farnesyldiphosphate to nerolidyldiphosphate (**107**) (*251*, *252*), which is then cyclised by the enzyme trichodiene synthase to the bicyclic hydrocarbon trichodiene (**49**) (*253*). The evidence for the participation of nerolidyldiphosphate has been reviewed (*254*, *255*). None of the possible enzyme bound intermediates has been isolated from what is shown (**107**, **108**) as a concerted process involving a 1,4-hydride shift and two 1,2-methyl shifts (*256*).

Results consistent with the same biosynthetic pathway to trichodiene have been obtained with *Fusarium graminearum* and *F. culmorum*, initially with labelled acetate (*257*, *91*), and, conclusively, with [3,4-$^{13}C_2$]-mevalonate (*258*).

Trichodiene synthase has been obtained from *T. roseum* (*259*) and subsequently from *F. sporotrichioides* (*260*, *261*) and *F. sambucinum* (*261*); the *F. sporotrichioides* enzyme has been characterised. Isomerisation of farnesyldiphosphate to nerolidyldiphosphate is the rate-determining step in the reaction pathway (*262*). Factors affecting substrate recognition by the active site have been studied (*263*), and a number of amino-acid



Scheme 2. Biosynthesis of trichodiene (**49**) and trichothecolone (**101**) from mevalonic acid lactone (**105**), labelled atom •

residues important for the trichodiene synthase reaction have been identified (*264*, *265*).

The trichodiene synthase gene, *Tri5*, has been cloned from *F. sporotrichioides* (*266*), *F. sambucinum* (*267*), *F. graminearum* (*268*), and *F. poae* (*269*); sequence analysis showed a high degree of similarity. The *F. sporotrichioides* gene has been expressed in *E. coli* (*270*, *271*) and in tobacco, *Nicotiana tobaccum* (*272*). In *E. coli* the recombinant enzyme had properties closely resembling those of the native enzyme and acceptable yields of trichodiene were obtained: Only low yields of trichodiene were obtained in tobacco tissue. Using standard gene disruption techniques, *Tri5*$^-$ mutants have been obtained from plant pathogenic strains of *F. sambucinum* (*273*, *267*) and *F. graminearum* (*268*, *274*) in which trichothecene production is suppressed and the virulence of the strain is diminished. Wild-type virulence has been restored to *F. graminearum Tri5*$^-$ mutants by the reintroduction of *Tri5* (*274*). These results provide compelling evidence that trichothecene production contributes to the virulence of these strains.

Another important application arising from the work with trichodiene synthase has been the development of *Tri5* specific polymerase chain reaction-based assays for the detection of potential trichothecene-producing *Fusarium* species in pure culture and in contaminated grain (*269*, *275*, *276*).

3.1.2. Trichodiene to 12,13-Epoxytrichothecene and Isotrichodermol (Scheme 1)

Trichodiene (**49**), the last hydrocarbon intermediate in the pathway, has been isolated from *T. roseum*, from *Fusarium* spp., and also from *S. atra* (Table 8). Specific labelling experiments have shown it to be a precursor of trichodiol (**55**) and 12,13-epoxytrichothecene (**73**), in addition to trichothecolone (**101**), in *T. roseum* (*277*); it is also a precursor of isotrichodiol (**53**) (*219*) and 3-acetylvomitoxin (**104**) in *F. culmorum* (*278*). However, relatively little work has been done on this stage of the pathway to simple trichothecenes in *Myrothecium*, *Stachybotrys* and *Trichoderma* spp.

Much effort, using mainly *Fusarium* spp., has been devoted to the identification of the oxygenation, cyclisation and esterification steps which take place after the formation of trichodiene. Fermentations carried out on a large scale, or in the presence of enzyme inhibitors, such as ancymidol (*225*) or xanthotoxin (*219*), or with mutant strains (*223*), have yielded metabolic products [(**47**), (**52**), (**53**), (**59**): Table 8] which are proven intermediates. They are derived from trichodiene by plausible pathways involving allylic hydroxylation at positions 2α and 11α, fol-

lowed or accompanied by β-epoxidation of the 12-ene. The number of potential intermediates points to the operation of metabolic grids rather than unique pathways (*279*, *219*), but this aspect remains to be clarified.

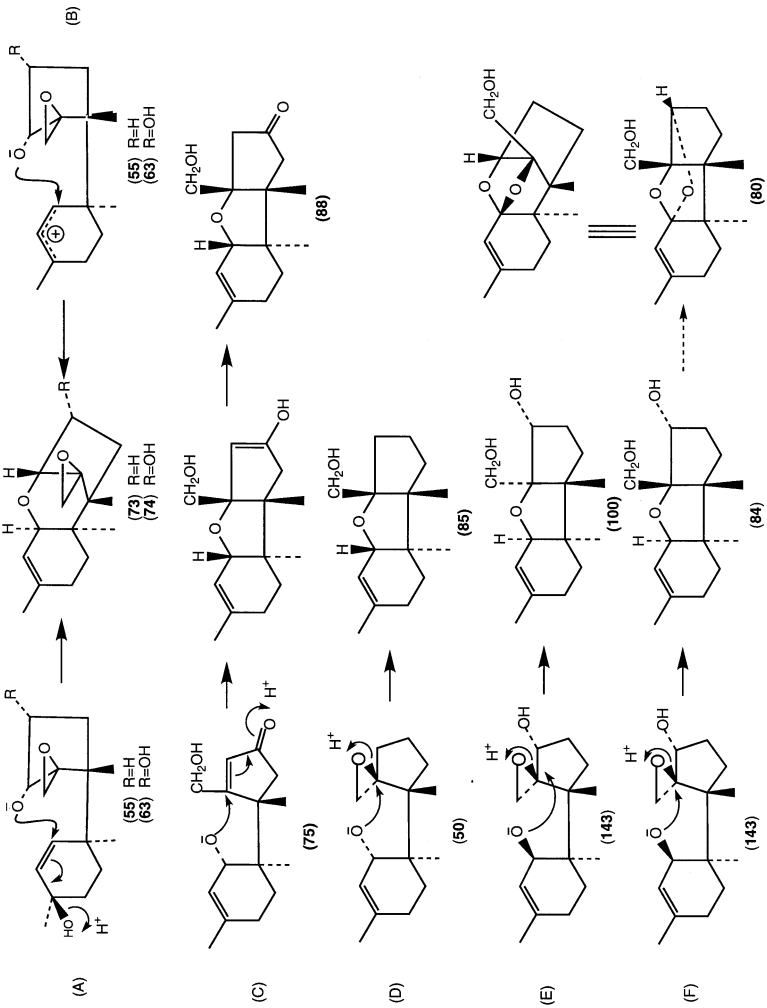
A cell-free enzyme system capable of epoxidizing the 12-ene of a trichodiene derivative has been obtained from *F. culmorum* (*280*). This system was also capable of effecting 3α-hydroxylation of the product, an interesting result since 3α-hydroxylation is commonly found amongst the trichothecene relatives.

The *Tri4* gene of *F. sporotrichioides* encodes a cytochrome P450 enzyme which catalyses the first oxidation step post trichodiene (*281*). Although the product was not characterised, it was generally assumed to be 2α-hydroxytrichodiene (**47**). This compound has since been isolated from *F. culmorum* (*222*) together with the compound thought likely to be the second step in this stage of the pathway, isotrichool (**52**) (*225*). Specifically-labelled 2α-hydroxytrichodiene was incorporated into 3-acetylvomitoxin but synthetic 12,13-epoxytrichodiene was not, showing that 2α-hydroxylation precedes epoxidation. Specifically-labelled isotrichool was also a precursor of 3-acetylvomitoxin. The pathway function, if any, of 11α-hydroxytrichodiene (**48**) has not been determined.

Isotrichodiol (**53**) and isotrichotriol (**59**) and their respective products of acid-catalysed allylic rearrangement, trichodiol (**55**) and 9-epitrichodiol (**56**), both isolated from *T. roseum*, and trichotriol (**63**) and 9-epitrichotriol (**64**), isolated only from *Fusarium* spp., are central to the pathway from trichodiene to the epoxytrichothecene nucleus. The conversion of trichodiol and trichotriol into 12,13-epoxytrichothecene (**73**) and isotrichodermol (**74**), respectively, proceeds spontaneously, though slowly, at acid pH (*152*) by mechanism (A), Scheme 3. However, the rapid *in vivo* incorporation indicates an enzyme-mediated process (*282*). An alternative proposal, with mechanism (B), has isotrichodiol and isotrichotriol as the true intermediates (*231*).

There is ample circumstantial evidence that the next step in *Fusaria* in the pathway to T-2 toxin and 3-acetylvomitoxin after trichodiol/isotrichodiol is 3α-hydroxylation to trichotriol/isotrichotriol. In feeding studies with a mutant strain of *F. sporotrichioides* in which this step was blocked, trichotriol, 9-epitrichotriol and isotrichotriol were all converted into T-2 toxin, but trichodiol was not (*231*), indicating that 3α-hydroxylation precedes the cyclisation step leading to the trichothecene nucleus.

Arising from this important conclusion it was suggested (*283*) that the trichothecenes should be divided into two groups based on the structure of the pathway intermediate immediately preceding cyclization, d-type (*e.g. Myrothecium* and *Trichothecium* metabolites) derived from trichodiol; and t-type (*e.g.* most *Fusarium* metabolites) derived



Scheme 3. Possible cyclisation mechanisms in the biosynthesis of trichothecenes and apotrichothecenes; the electronic representation is purely indicative, *e.g.* alcohol nucleophiles may act in their hydroxy rather than alkoxide forms

from trichotriol. Some doubt became attached to this attractive proposal, which was initially consistent with the known oxygenation pattern specificity, by the isolation from *T. roseum* of trichothecinols A (Table 2: $C_{19}H_{24}O_6$) and C (Table 1: $C_{19}H_{26}O_6$) which have 3α-hydroxylation.

There is no evidence that the 8- and 16-hydroxy substituted isotrichodiol and isotrichotriols isolated from *F. sporotrichioides* (Table 8) are, respectively, involved in the biosynthesis of 8- or 16-hydroxytrichothecenes.

3.1.3. Further Oxygenation and Esterification of the Trichothecene Nucleus (Scheme 4): Biosynthesis of Specific Metabolites

3.1.3.1. Trichothecolone

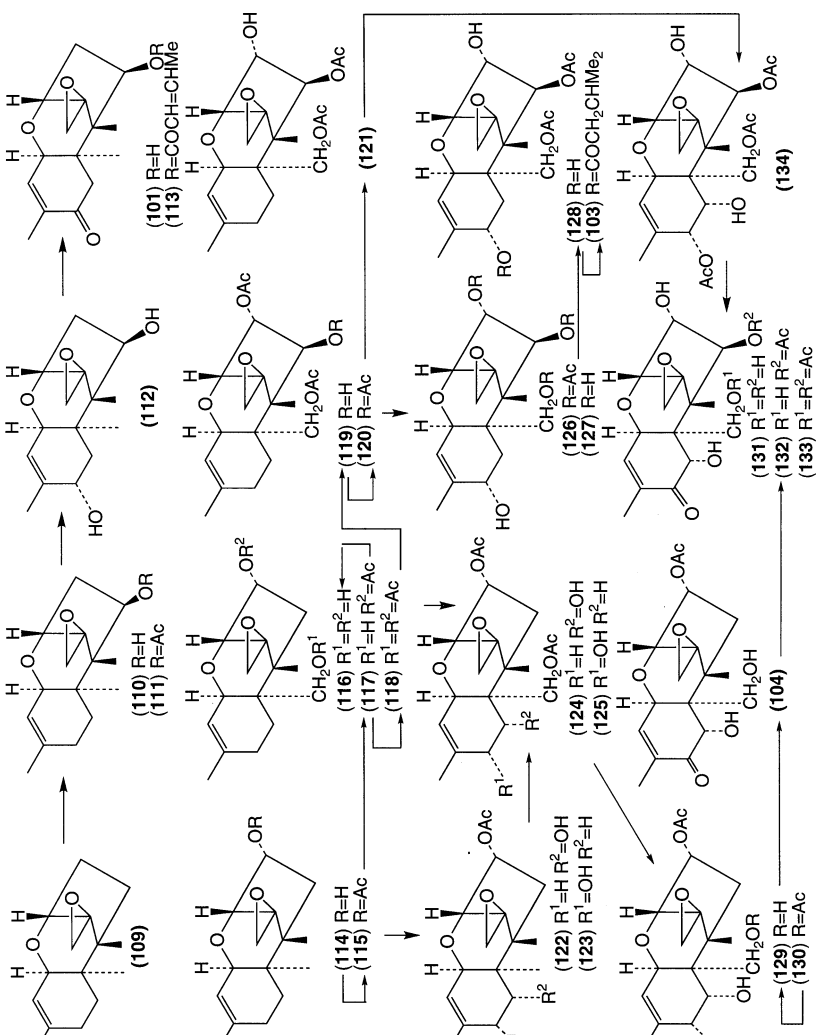
In *T. roseum* fermentations, experiments with specifically-labelled materials showed that both trichodermol (**110**) and trichothecodiol (**112**) were converted into trichothecolone (**101**) and thence to trichothecin (**113**) (*284*), indicating that 4-hydroxylation of 12,13-epoxytrichothecene (**109**) probably precedes 8-hydroxylation in the biosynthetic sequence: (**109**) → (**110**) → (**112**) → (**101**). The *T. roseum* metabolite (**109**) has always been assumed (*10*), but, to this reviewer's knowledge, never proved, to be a trichothecolone precursor.

3.1.3.2. Vomitoxin and Derivatives

Although isotrichodermol (**114**) [or its acetate isotrichodermin (**115**)] was found to be a precursor both of 3-acetylvomitoxin (**104**) in *F. culmorum* (*226*), and of T-2 toxin (**103**) using the blocked mutant strain (see above) of *F. sporotrichioides* (*232*), 12,13-epoxytrichothecene (**109**) was not (*285*)[3] (*232*). 12,13-Epoxytrichothecene was not converted into isotrichodermol by *F. culmorum* (*285*), and neither trichodermol (**110**) nor 15-hydroxy-12,13-epoxytrichothecene were precursors of T-2 toxin in *F. sporotrichioides* (*232*). These results reinforce the earlier conclusion (above) that 3α-hydroxylation of trichodiol precedes the cyclization step in these organisms. The conversion of isotrichodermol to isotrichodermin by an acetyltransferase is discussed in connection with the function of the resistance gene *Tri101* (Section 3.1.4.).

The steps in the conversion of isotrichodermin (**115**) to 3-acetylvomitoxin (**104**) follow logically from the pattern of secondary metabolites, common to *F. culmorum* and *F. graminearum*, but originally

[3] Correcting an earlier (*226*) statement to the contrary.



Scheme 4. Biosynthetic pathway to trichothecolone (**101**) from 12,13-epoxytrichothecene (**109**), and to 4,15-diacetoxyscirpenol (**121**), T-2 toxin (**103**), and 3-acetylvomitoxin (**104**) from isotrichodermol (**114**)

isolated from a *F. graminearum* fermentation (*42*). However, this pattern, consisting of 7-(**122**), 8-(**123**), and 15-hydroxyisotrichodermin (15-deacetylcalonectrin) (**117**), 7-(**124**), and 8-hydroxycalonectrin (**125**) and 7,8-dihydroxycalonectrin (**130**) gave no clue as to the sequence in which hydroxylation occurred. Kinetic pulse-labelling techniques identified 15-deacetylcalonectrin (**117**) as the first intermediate after isotrichodermin in *F. culmorum* (*44*). This step has been confirmed with a cell-free enzyme system (*286*); however, this system was not regiospecific and also hydroxylated isotrichodermin at positions 7 and 8.

7-(**122**), 8-(**123**), and 15-Hydroxyisotrichodermin (**117**), and calonectrin (**118**) are all proven precursors of 3-acetylvomitoxin (**104**) in *F. culmorum* (*44*), consistent with the operation of a metabolic grid. The 7,8-dihydroxy compounds (**129**) and (**130**), derivatives, respectively, of 15-deacetylcalonectrin and calonectrin, have both been shown to be precursors of 3-acetylvomitoxin, but the deacetylcalonectrin derivative (**129**) was not incorporated into dihydroxycalonectrin (**130**) (*287*). This result indicates a unique pathway for the last two steps in 3-acetylvomitoxin biosynthesis in *F. culmorum*, namely 15-deacetylation of dihydroxycalonectrin (**130**) followed by oxidation at C-8.

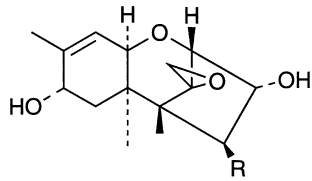
There is, nevertheless, evidence that this section of the pathway from isotrichodermin (**115**) to 3-acetylvomitoxin (**104**) is not as straightforward as depicted in Scheme 4, and, overall, at least one deacetylation/reacetylation step takes place at position 3 with the involvement of isoverrucarol (''dideacetylcalonectrin'') (**116**) (*288*) in the pathway. Esterases with a high specificity for the 3-position have been obtained from an unidentified *Fusarium* sp. (*289*) and from *F. sporotrichioides* (see below).

The emphasis of most of this work has been on 3-acetylvomitoxin biosynthesis. Both 15-acetylvomitoxin and vomitoxin are major metabolic products of some strains of *F. graminearum* (*147*), but the steps involved in the late stages of their biosynthesis have not yet been examined.

The *Tri11* gene of *Fusarium sporotrichioides* encodes a cytochrome P450 hydroxylase which converts isotrichodermin to 15-deacetylcalonectrin (*290*). Although the *Tri11* gene from *F. graminearum* has substantial identity with the *F. sporotrichioides* gene, and presumably encodes a P450 monooxygenase (*291*), the *F. culmorum* enzyme is not a hemoprotein and is not attached to P450 (*291*). *Tri11*$^-$ mutant strains, lacking a functional C-15 hydroxylase, accumulate the 8α-hydroxy-derivatives (**135**; R = H and OH) of isotrichodermol and scirpene-3,4-diol, respectively (*23*).

The genes and enzymes involved in the steps between calonectrin and the vomitoxin derivatives, particularly the final oxidation step which differentiates Type B from Type A compounds, have still to be identified.

References, pp. 113–130



(**135**)

3.1.3.3. T-2 Toxin

The *Tri3* gene of *F. sporotrichioides* encodes a 15-O-acetyl-transferase that acetylates the 15-OH of 15-deacetylcalonectrin (**117**) (*292*). This enzyme also acetylates the 3α-hydroxyl in a number of 12,13-epoxytrichothecene substrates. The C-15 acetylation step appears to be essential to the pathway to T-2 toxin and *Tri3*$^-$ mutants accumulate neither T-2 toxin nor the major co-metabolites 4,15-diacetoxyscirpenol (**121**) and neosolaniol (**128**).

It seems certain that the pathways to 3-acetylvomitoxin and T-2 toxin in *Fusaria* diverge at 15-deacetylcalonectrin; the next step on the T-2 toxin pathway, after 15-acetylation, is the 4β-hydroxylation of calonectrin to 3,15-diacetoxyscirpenol (**119**). Nothing is known about the genes and enzymes involved with C-4 hydroxylation in *Fusaria*. The gene *Tri7* controls the 4-OH acetylation step in *F. sporotrichioides* (*293*), giving triacetoxy-scirpene (**120**). The gene disruption mutant *Tri7*$^-$ did not produce T-2 toxin, but accumulated HT-2 toxin (4-deacetylT-2 toxin); and feeding studies with this mutant showed that only precursors containing a 4-acetyl group, *e.g.* 4,15-diacetoxyscirpenol (**121**), were converted to T-2 toxin, whilst those without this grouping, *e.g.* HT-2 toxin, were not.

C-4 acetylation is followed by 8α-hydroxylation to 3-acetylneosolaniol (**126**) and esterification at C-8. The *Tri1* gene controls C-8 hydroxylation in *F. sambucinum* (*294*), and *Tri8* is said to have a similar function in *F. sporotrichioides* (*295*, *293*). However, there is evidence that *Tri8* is involved in both oxidation and esterification steps at C-8. A *Tri8*$^-$ mutant strain did not produce T-2 toxin but accumulated 4,15-diacetoxyscirpenol, suggesting that *Tri8* is involved specifically in C-8 oxidation. Additionally though, this mutant was unable to effect esterification with the isovalerate moiety, and failed to convert T-2 tetraol (**127**) to T-2 toxin. A known T-2 toxin-deficient *F. sporotrichioides* strain, produced by UV mutagenesis, was able to effect this esterification.

4,15-Diacetoxyscirpenol (**121**), neosolaniol (**128**) and T-2 toxin (**103**) result from deacetylation of their respective 3-acetyl derivatives.

An esterase with higher affinity for the ester bond at the 3α position than at the 4β position and with some specificity for 3-acetylT-2 toxin has been partially purified and characterised from *F. sporotrichioides* (*296*).

The isovalerate moiety present in T-2 toxin is derived by decarboxylative transamination from L-leucine (*297*, *298*). Leucine limitation enhances the production of neosolaniol (**128**).

3.1.3.4. Nivalenol and Derivatives

By using a *Fusarium* strain known to be an abundant producer of diacetoxyscirpenol, 3-acetylvomitoxin (**104**) was converted to fusarenone (**132**) (*299*).

The sequence of hydroxylation steps leading to nivalenol (**130**) and its derivatives is unproven, but could follow logically from the pattern of secondary metabolites isolated from *F. equiseti* (*5*): 4,15-diacetoxyscirpenol (**121**) $\rightarrow$ the diol (**134**) $\rightarrow$ 4,15-diacetylnivalenol (**133**). The genes and enzymes concerned with these (hypothetical) oxidative steps have not been investigated. The diol (**134**) has not been reported from any other *Fusarium* spp.

The work outlined in Sections 3.1.3.2. and 3.1.3.3., above, deals mainly with the biosynthesis of 3-acetylvomitoxin by *F. culmorum* and of T-2 toxin by *F. sporotrichioides*; some strains of both these *Fusaria* produce nivalenol and derivatives, possibly by appropriate modification of the vomitoxin and T-2 toxin pathways.

3.1.4. Trichothecene Biosynthetic Gene Clusters

In *F. sporotrichioides* ten biosynthetic pathway genes, *Tri3–12*, are closely linked and form a gene cluster (*300*). Homologous gene clusters exist in *F. sambucinum* (*301*) and in *F. graminearum* (*302*, *293*). In an important new approach to the investigation of trichothecene diversity, the biosynthetic gene clusters from T-2 toxin- and vomitoxin-producing strains of *F. sporotrichioides* and *F. graminearum*, respectively, were subjected to comparative analysis of nucleotide sequence and genome organization and orientation (*293*). The *Tri7* gene from *F. graminearum* was found to include base deletions and insertions which would preclude translation of a functional protein, consistent with the absence of C-4 oxygenated products from this strain. Similar comparative analyses of Type A- and Type B-producing strains of *F. sporotrichioides*, and of vomitoxin- and nivalenol-producing chemotypes of *F. graminearum*, could yield interesting results.

References, pp. 113–130

The genes *Tri1* from *F. sambucinum* (*294*) and *Tri101* from *F. graminearum* (*283*) are not linked to the clusters. In addition to the six genes, *Tri3–5,7,8* and *11*, that control biosynthetic enzymes, whose function has been outlined above, the *F. sporotrichioides* and *F. graminearum* clusters contain one regulatory gene, *Tri6*, and one membrane facilitator gene, *Tri12*. *Tri9* and *Tri10* encode proteins whose function is not understood (*293*).

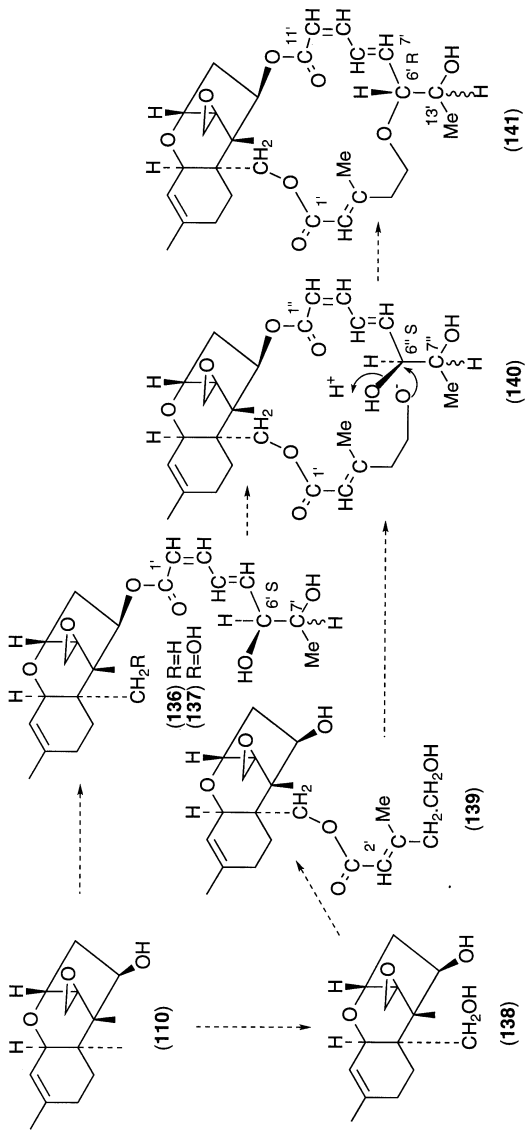
Tri6 encodes a zinc finger protein involved in the transcriptional regulation of the pathway genes concerned with trichothecene biosynthesis and binds with all but one of the known pathway genes (*303*). Disruption of *Tri6* resulted in a mutant that accumulated low levels of trichodiene but did not produce trichothecenes, was unable to convert the intermediates calonectrin, diacetoxyscirpenol and neosolaniol to T-2 toxin, and had greatly reduced transcription of *Tri4* (*304*). The *Tri6* homologs cloned from the Type B trichothecene producers *F. graminearum* and *F. crookwellense* were almost identical at the amino acid sequence level to *Tri6* from *F. sporotrichioides*, a Type A producer (*305*).

The *Tri12* gene encodes a protein involved in the specific extrusion of trichothecenes across the fungal plasma membrane, causing their accumulation in the substrate and thus participating in the self-defence mechanism of the producing organism (*306*). *Tri102* from *F. graminearum* (*302*) is identical with *Tri12*.

Tri101, a pathway gene not associated with the gene cluster, is also involved with resistance of the producing organism to toxic metabolites. It encodes an 3α-acetyltransferase, an esterification which significantly diminishes the toxicity, including phytotoxicity (*307*), of a trichothecene, and has been cloned from both *F. graminearum* (*283*, *308*) and *F. sporotrichioides* (*309*). More recent work with *Tri101*$^-$ mutants has shown that *Tri101* converts isotrichodermol to isotrichodermin and is required for the biosynthesis of T-2 toxin by *F. sporotrichioides* (*310*). The results are consistent with the hypothesis that much of the pathway to T-2 toxin involves 3-acetylated intermediates (*283*, *310*).

3.2. Trichoverroids and Macrocyclic Trichothecenes (Scheme 5)

The genes controlling the initial stages of the biosynthetic pathway to the verrucarol moiety of the macrocyclic trichothecenes have been studied in *Myrothecium roridum* (*311*). In so far as the pathways overlap, the pathway outlined above for simple trichothecenes is followed, and clustered genes *MRTri4–6*, similar to *Tri4–6* and with the same function,



Scheme 5. Possible pathways from trichodermol (**110**) to roridin E (**141**, 13′-*β*H)

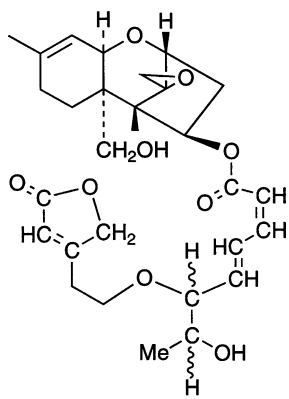
have been cloned. There are differences, however, and the protein specified by *MRTri6* is almost twice the size of the analog from *F. sporotrichioides*.

The sequence of the hydroxylation and esterification steps in *Myrothecium* spp. fermentations between trichodermol (**110**) and the C-7′ diastereoisomeric trichoverrins (C-6″S) (**140**) and isotrichoverrins (C-6″R) is unclear and has still to be tested with specifically labelled compounds. The pattern of secondary metabolites which could be intermediates, verrucarol (**138**), verrol (**139**), the trichodermadienediols (**136**) and the trichoverrols (**137**), again suggests a metabolic grid rather than a unique pathway.

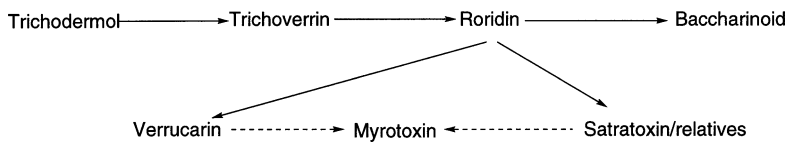
The six-carbon moiety attached to C-15 in verrol and the trichoverrins is mevalonate-derived (*248*), and after cyclisation to form the macrolide, the 2′-ene can undergo hydrogenation, α-epoxidation, or hydration. The ethylenic double bonds in the acetate-polymalonate-derived eight-carbon moiety attached to C-4 in the trichoverroids occur as Z or E (see Table 1), but are always 7′ (E), 9′(Z) in the macrolide.

Ring closure of the trichoverrins occurs with inversion of configuration at position 6′. In most roridins and all baccharinoids C-6′ is R; the corresponding centre in the trichoverrins is S. Exceptionally, isororidin E, 13′-epiiso-roridin E and 6′-epi-13′-epiroridin A have C-6′S, but the *M. verrucaria* strains which produce them also produce isotrichoverrins with C-6′R (*113*). Trichoverrin B has been proved to be a precursor of roridin A (**28**; $R^1 = R^4 = H$, $R^2 = R^3 = \beta H$) and verrucarin A (**7**; $R^1 = H$, $R^2 = OH$) (*105*).

It is likely that the trichoverroid roridin L-2 (**142**) and its close relatives arise by hydrolysis and relactonisation of the corresponding, unknown, 12′-hydroxyroridin E analogues.



(**142**)



Scheme 6. Possible biosynthetic pathway to the macrocyclic trichothecenes

C-7′-diastereoisomeric 12,13-deoxytrichoverrins have been isolated from *M. verrucaria* but whether these 12-enes are precursors of verrucarin K, or whether deoxygenation takes place after the formation of the macrolide is unknown. Likewise, a possible relationship between 8α-hydroxyisotrichoverrin A (Table 1: $C_{29}H_{40}O_{10}$) and verrucarin L is unproven.

A plausible sequence based on inspection of the structural formulae, and in which the roridin skeleton holds a central position, is shown in Scheme 6. The scheme assumes that the pathway in *Baccharis*, or its endophyte (see below), is essentially the same as in *Myrothecium*, and that all the fungal producers of macrocyclic trichothecenes use the same pathway.

The biosynthesis of the macrocyclic trichothecenes in *Baccharis* remains controversial. It was initially believed (*188*) that the baccharinoids were the products of a plant-fungus interaction in which fungal macrocycles were taken up by the plant and further modified. This hypothesis was withdrawn (*162*) following the failure to demonstrate any fungal involvement either from the immediate soil environment or from examination by electron microscopy of the plant tissues. It was concluded (*162*, *312*) that *Baccharis* synthesized macrocyclic trichothecenes *de novo*. Some doubts about this conclusion have resulted as a result of the isolation (*313*) from *B. coridifolia* of an endophyte, *Ceratopycnidium baccharidicola*, and the demonstration (*164*, *314*) that this organism is a source of verrucarins and roridins (see Tables 5 and 6), all of which are known metabolic products attributed to *B. coridifolia*. However, none are baccharinoids, suggesting that the original plant-fungus interaction hypothesis (*188*) could have been correct.

3.3. Trichothecene Relatives (Scheme 1)

This account deals only with the 11-epimeric apotrichothecenes and with sambucinol (**81**). A more extended account covering all the trichothecene relatives derived from trichodiene is contained in Ref. *3*.

Of the naturally occurring apotrichothecenes, compounds in the 11-epi-series are the most numerous, but little work has been done on their biosynthesis. The *F. sambucinum* metabolites FS 1 (**75**) and FS 4 (**67**),

which bear the same relationship to each other as do isotrichodiol and trichodiol, are possible precursors of dehydroapotrichodiol (**88**) and hence of the 3-epimeric apotrichodiols (**86**) and (**87**) by mechanism C, Scheme 3.

Alternatively, by mechanism D, the hypothetical intermediate (50) could lead to apotrichool (**85**) directly. Apotrichool was converted by *F. culmorum* into apotrichodiol (**86**) (*207*).

The apotrichothecene (**83**) is the product from the acidcatalysed trichothecene-apotrichothecene rearrangement of 12,13-epoxytrichothecene (**73**) and could be an artefact. The 12-epi-apotrichothecene (**100**) is believed to arise from the hypothetical intermediate 11-epiisotrichodiol (**143**: Scheme 3), and by mechanism E (*228*) which involves epoxide-opening with retention of configuration. Isotrichodiol and its 11-epimer should be interconvertible via the common allylic cation.

The nature of the biosynthetic pathway to sambucinol (**81**) is controversial. Trichodiene and isotrichool (**52**) are proven precursors in *F. culmorum* (*225*), as are apotrichool (**85**) (*226*) and 3-deoxysambucinol (**80**) (*285*). Another precursor is 2α,13-dihydroxyapotrichothecene (**84**) (*227*), which has not yet been isolated as a natural product but which should be readily obtainable from 11-epiisotrichodiol (**143**) by mechanism F, Scheme 3. Apotrichodiol (**86**) is converted photochemically in solution at room temperature into the acetal (**92**) (*211*), a likely precursor of sporol (**91**). Similar photochemical reactions with 2α-hydroxyapotrichothecenes, which could lead to deoxysambucinol (**80**) (mechanism F), have not been reported.

12,13-Epoxytrichothecene (**73**) has also been identified as a precursor of sambucinol (*226*), but the evidence for this has been criticised (*211*). There is no evidence for the participation of the 11-ketone (**72**) in the pathway from isotrichool to sambucinol (*227*).

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Melanin, Melanogenesis, and Vitiligo

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1. Melanin

1.1. Introduction

Melanins, pigments of diverse origin and chemical function, have been subjects of interest for a long time (Aristotle, in ‘‘Historia Animalia’’, 315 B.C.). These natural cosmetics of skin, hair, and feathers usually occur in the form of insoluble fine granules in certain dendritic cells

of the epidermis. The term melanin ($\mu\varepsilon\lambda\alpha\varsigma$ = black) is, however, misleading and confusing since not all biogenetically related pigments are black. The melanogenic enzyme, tyrosinase, is known to catalyze the biosynthesis of not only black but also red to brown, or even yellow pigments, *e.g.* pheomelanins (*208*, *266*). On the other hand, the fascinating colors in the feathers of birds, skins of reptiles and fishes and the blue eyes in animals are optical phenomena due to diffraction, light absorption and scattering, interference, that are produced by melanin granules either in combination or not, with other pigmentary colors (*94*, *156*) and by complex formation of the granules with heavy metals (*21*, *156*).

Skin pigmentation is a natural phenomenon, and has substantial protective, social, and cosmetic significance, and so, melanin and melanogenesis is of obvious interest and importance for researchers. Earlier, it was believed that the colour of black skin was due to insoluble granular pigments derived from bile (*175*). The first concept of the origin and biogenesis of melanin came at the end of the nineteenth century with recognition of tyrosinase, an enzyme capable of transforming tyrosine into a black product in *Russula nigricans* (*9*). Later, a similar enzyme was also found in other plants and in tissues of various invertebrates and vertebrates (*221*). However, investigation of the black products was not an easy task and rather discouraging, due to their rigid behavior towards solubility, either chemically or enzymatically. But melanin research changed and proceeded dramatically with the identification and demonstration of melanocytes, the pigment synthesizing specialized cells, in the epidermis (*10*). It has now been generally accepted that both natural and synthetic melanins are products of a tyrosinase-tyrosine reaction and in modern terminology melanins are described as complex polymeric indole alkaloids derived from 5,6-dihydroxyindole, the latter compound originating from phenylalanin, tyrosine and 5,6-dihydroxyphenylalanin (*70*).

Melanins, although superficially inert, possess some biologically significant physicochemical properties by acting as radical scavengers, ion-exchangers, metal-binder, *etc.* (*55*). Extensive studies of the chemical reactivities of melanin and putative melanin precursors have led researchers into many areas of scientific research the results of which have been reviewed (*55*, *70*, *92*, *123*, *184*, *211*, *259*, *264*, *265*).

In the present article, an attempt has been made to give not only a brief account of melanin and melanogenesis but also to discuss the present state of research on vitiligo, an idiopathic disease of pigmentary disorder of the skin.

2. Chemistry of Melanin

Melanin pigmentation is determined mainly by two chemically distinct but biologically related types of pigments. One of these consists of the dark insoluble eumelanins and the other of the alkali soluble phaeomelanins, both of them originating from a common precursor, *i.e.* tyrosine. There exist, however, certain hybrid pigments in the epidermal tissues that possess structural features as well as chemical and physical properties of both eu- and phaeomelanins (*210*). Swan (*259*) in an earlier article of this series has presented a comprehensive account of the chemistry of melanin which covered references up to 1974. Since the present paper highlights vitiligo, a very short discussion of melanin chemistry, emphasizing especially the eumelanins, is given here.

2.1. Isolation and Analysis

The most salient point of melanin chemistry is the isolation of the pigment polymer, occurring *in vivo* or produced *in vitro*, as a single chemical compound of definite composition. The intractable nature of the material, the concomitant protein residue, metal ions, and the hydrated state of the sample are the main difficulties in obtaining the pigment in pure form.

Two methods generally used for the extraction of eumelanins from native sources involve removal of all other components of the tissue by prolonged digestion with conc. HCl at room temperature or boiling with 6 *M* HCl (*70*, *184*). However, this drastic treatment of eumelanins in 6 *M* HCl may cause some changes in pigment structure and composition (*8*, *119*). To avoid these difficulties mild processes have been developed, and in some cases, *e.g.* eye-melanin, sepiomelanin, these can be isolated with minimal damage by mechanical separation of the pigment granules followed by a short treatment with 0.5 *N* HCl at room temperature and extensive sonication in deionized water (*8*). Purification of melanosomes from melanoma tissues by sucrose density gradient ultracentrifugation after digestion with detergents or proteolytic enzymes, *e.g.* pronase and papain, at neutral pH (*210*) is another mild process. Whether these mild treatments can efficiently remove proteins and other impurities bound to the pigment granules is not clear and thus, in keeping with these studies and other limitations, variation has been noticed in the analytical values of melanins from different sources (*211*). In fact, the analyses reflect the average composition of mixed polymers. However, all melanins from animal sources and *in vitro* tyrosinase melanins contain, as a rule, more than

8% nitrogen, and the use of the molar ratio of carbon and nitrogen (C/N) of the pigment is now preferred for characterization purposes (*119*).

2.2. Solubilization

A melanin is considered completely solubilized if the solution does not scatter light. So far only two approaches for solubilization have been successful, one of which involves treatment of the pigment with Solulene 100 (0.1 *M* solution of dimethyl-*n*-dodecyl-*n*-undecyl ammonium hydroxide in toluene; incubation for 2.5 hr at 75°C). The mechanism of this solubilization process is unknown and degradation of the pigment cannot be ruled out (*186*, *281*). The second approach involves treatment of naturally occurring melanosomes and synthetic melanins with a dilute solution of H_2O_2 at pH 9–10. Hydrogen peroxide oxidation in mild alkaline media first solubilizes melanin with no obvious structural change followed by decomposition of the excess H_2O_2 (Pt-black, catalase) in the second stage. Melanin precipitates from this solution under acid conditions but readily redissolved in basic media. Such melanin retains the chromatic characteristics of the intact pigment by its color and ESR signal but accounts for only 60–62% of the weight of the starting materials (*281*). Repeated treatment of this precipitated melanin with H_2O_2 results in no further significant weight loss. It appears that melanin, whether native or synthetic, may comprise two major fractions, only one of which is responsible for the color and ESR signal (*281*).

Melanin solubilization provides an opportunity for determination of molecular weights, and using various methods the molecular weights have been found to range between 1100–6000 irrespective of pigment origin (*70*, *281*).

2.3. Protein Content

Native melanin is often conjugated with protein through sulfur linkages and thus forms a melanoprotein complex. From the amount of amino acids (numbering 18) removed by hydrolysis, it has been calculated that protein constitutes about 10% of the weight of melanoprotein (*204*). Conjugation could occur either by addition of cystein residues to the pigment polymer to form a sulfur-linked melanoprotein, or by oxidation of a melanosomal protein, containing a terminal tyrosine residue. The presence of sulfur in the protein-free eumelanins and the formation of cysteic acid and taurine by peracetic acid oxidation of sepiomelanin support the former mechanism (*204*).

There is no direct evidence for the alternative mode of conjugation of the protein through a peptide linkage although oxidation of tyrosine containing peptides by tyrosinase was reported, first by Bu'Lock and Harley-Mason (*30*) and later by Yasunobu *et al.* (*284*) and Rosel *et al.* (*225a*) using model peptides.

Benathan and Wyler (*8*) found that native sepiomelanin loses on treatment with hot 6 *M* HCl, most of the ''proteic'' component during the first 15 min, the remaining part being removed in about an hour. Analysis of the solubilized fraction revealed a mixture of amino acids (11%) along with a small amount of glucosamine. The result indicates that a major portion of protein is loosely bound to the pigment granules.

2.4. Carboxylic and Phenolic Function

The ratio of carboxylic and phenolic groups in native melanin has been found to be 1.1 which is reduced to 0.8 after removal of protein with acid (*8*, *288*). Significant differences are also noticed between enzymatic (0.5) and autooxidative (1.7) dopamelanin, the latter possessing the highest proportion of carboxylic groups (*8*, *288*) and the longer the oxygenation time the higher the carboxyl content as evidenced by acid decarboxylation. According to Swan and Waggott (*257*), during *in vitro* melanogenesis, H_2O_2 is generated, which attacks the 5,6-indolequinone moieties and gives rise to carboxylate pyrrolic units. The same mechanism also seems to be operative *in vivo* (*204*).

2.5. Chemical Degradation

The chemical degradation of both eu- and pheomelanin has been studied, mainly by three methods. Although the yield of products in most experiments is very low and varies significantly for melanins of different origin, the results appear particularly useful in leading to an understanding of the monomeric units present in the pigment structure.

2.5.1. Reductive Methods

Reduction of sepiomelanin in ethanol at 150°C with hydrogen in the presence of a palladium catalyst yielded 5,6-dihydroxyindole (DHI) (*184*), while mild treatment of sepiomelanin or dopamelanin with sodium borohydride in 0.1 *N* NaOH results in the formation of some

5,6-dihydroxyindole-2-carboxylic acid (DHICA), the latter arising presumably from incorporation of some pigment precursors in the melanin granule (*75*). Degradation with hydriodic acid (*120*, *199*) was found to be a specific method for identification of phaeomelanins; aminohydroxyphenylalanine, the degradation product identified by HPLC, is characteristic for melanins derived from 5-*S*-cysteinyldopa. The reaction follows both reductive and hydrolytic processes.

2.5.2. Oxidative Methods

Alkali fusion of sepiomelanin at 300° yielded DHI, DHICA, 4-methylcatechol and 5,6-dihydroxyindole-4,7-dicarboxylic acid identified by paper chromatography (*204*). These products indicate the presence of indole units in sepiomelanin some of which are linked through positions 4 and 7. The formation of catechol was ascribed to the presence of dopachrome units in the natural pigment (*204*). Moreover, when the pigment was boiled with 4% aqueous NaOH solution pyrrole-2,3,5-tricarboxylic acid (PTCA) was obtained in about 1% yield. This substance arises presumably from hydrolysis of terminal carboxylated pyrrole units linked through a carbonyl group to the other structural units (*184*, *204*). Similar results were obtained by degradation of other natural and synthetic pigments (*184*, *204*).

A recent study of sodium hydroxide degradation of both natural and synthetic melanins has revealed the formation of two different components; one (more stable under the reaction conditions used) absorbing in the visible region, and a second absorbing in the UV region. It was postulated that the former is composed of stakes of planar monomer units and that the latter represents the ''core'' of the polymer, providing protective function against harmful UV radiation (*70*).

Oxidation of sepiomelanin with potassium permanganate gave four pyrrolic acids, pyrrole-2,3,5- and 2,3,4-tricarboxylic acid, pyrrole-2,3-dicarboxylic acid (PDCA), and 2,3,4,5-pyrroletetracarboxylic acid (*184*, *204*). On the other hand, the yield and number of PDCA increased when decarboxylated sepiomelanin was oxidized with permanganate. The origin of these pyrrolic acids was interpreted as resulting from the oxidative breakdown of various types of DHI units in the pigment backbone (*184*).

2.5.3. Pyrolytic Methods

Natural black (human hair, bovine eyes) and synthetic tyrosine-, dopa-, dopamine-melanins were investigated by Curie point pyrolysis–

gas chromatography–mass spectrometry (*79a*). The pigments were characterized in terms of the different ratios of the degradation products aromatic hydrocarbons, phenols, catechols, pyrroles, and indoles.

2.6. Spectroscopic Studies

Melanins have been investigated spectrophotometrically by different authors (*70*, *259*) but the results are of little value in elucidating the structure of the pigment molecule.

2.6.1. UV and IR Spectroscopy

The UV-visible range spectrum of melanin shows high absorption but no definite bands. However, using very dilute KBr pellets, the finger print region allowed characterization of some IR bands as being associated with protonation and deprotonation of titrable groups at different pH and binding of iron to various chelating functional groups and permitted comparison of natural and synthetic melanins (*70*).

2.6.2. NMR Spectroscopy

Chedekel *et al.* (*59a*) examined the ^{13}C NMR spectrum of enzymatically produced melanin and identified the benzylic carbon of L-dopa as the C-3 carbon in the dihydroxyindole repeating unit. In melanin formed by autooxidation, however, the C-3 carbons were included in a pyrrolidone as well as a pyrrole ring and a carbonyl carbon. Eumelanins produced in a similar way from DHI showed no presence of carbonyl-containing structural units. These results strongly suggest that the polymerization step involves the 4 and 7 positions of the indole ring. Moreover, NMR studies of melanins can differentiate samples depending on the source and different functional groups present in the eumelanins can be identified (*59a*).

2.6.3. X-Ray Defraction Study

More detailed information on the structure and dynamic of melanin-polymers were obtained from X-ray defraction studies through the introduction of a high energy resolution technique, *i.e.* Rayleigh scattering of Mössbauer radiation (RSMR) (*70*). Although the spectrum is affected by the contribution of water coordinated to the melanin, some informative peaks have been identified. Thus, the main peaks correspond to the average bond lengths (C–C, C=O, C–N) in the monomer units (1.45 A), to distances between next-nearest neighbors (2.4 A), to the perpendicular

interlayer spacing between indole planes (3.4 A), and to the distances between atoms in adjacent layers occupying different positions in each monomer unit (4.4 A). The dynamics of the system are typical of a layer structure characterized by large anisotropies in the bonding forces (*70*).

2.6.4. ESR Study

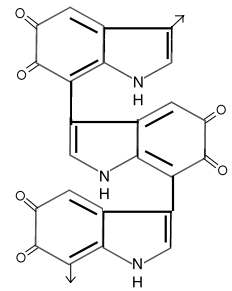
Melanins possess properties of radicals thus exhibiting paramagnetism, and eumelanins were the first biological molecules studied by electron spin resonance (ESR) spectroscopy (*16*, *64*, *236*). The ESR spectra of both natural and synthetic melanins are very similar and include a featureless signal with a line width of about 4–6 G, g-value 2.004, and no hyperfine coupling. The spin concentration is very low, within the range of $4–10 \times 10^{17}$ spins/g. Semiconductor models and charge transfer complexes through the stacked monomer units of the eumelanin polymer have been proposed to account for such an unusual type of stable radical property (*98a*, *b*, *169*). ESR measurements of hydrated melanin suspensions on melanin polymers at neutral pH reveal that their spin concentrations are reversible, temperature dependent, and largely controlled by an intragranular equilibrium involving quinone, hydroquinone, and semiquinone units in the polymers (*62*). The ESR signals exhibited by these macromolecules have been used successfully to study the photobiophysical properties of melanins (*234*). Further, attempts have been made to correlate the free radical properties of melanins with their chemical structure, biosynthesis, and physiological role in cells and tissues (*236*).

2.7. Structure of Melanin

Another striking feature of melanin chemistry is the structure of the pigment polymer. The two principal approaches employed for elucidating the structure of these complex molecules were (i) biosynthetic and (ii) analytical. Interestingly, while the first approach provided information on the ultimate monomeric precursors of eu- and pheomelanin, the analytical one was significant in developing methods for shedding light on the elemental composition, functional groups, and structural features of both natural and synthetic melanins.

2.7.1. Melanin as Homopolymer

The fundamental work of Raper (*216*) on the *in vitro* enzymatic synthesis of melanin, using tyrosinase, tyrosine and oxygen, was the first synthetic approach investigating melanogenesis and the structure of



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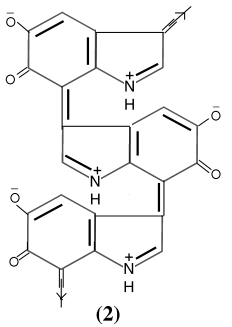

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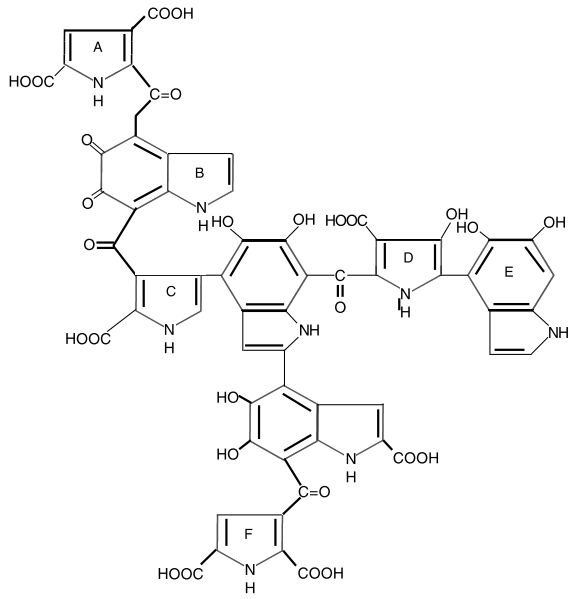


Fig. 1. Partial polymeric structure of eumelanin

melanin. He concluded that, in the formation of this pigment polymer from 5,6-dihydroxyindole (DHI), quinone formation is a probable first step (*217*). Mason (*163*) found that the oxygen consumption and CO_2 evolution during the oxidative conversion of 5,6-dihydroxyphenylalanin (dopa) to the dopachrome stage were consistent with formation of DHI. He described melanin as a "homopolymer", arising by repeated self-condensation of DHI. Later, in support of this view, it was suggested by other workers (*7*, *30*) that for melanin formation from indole-5,6-quinone by a self-condensation process, an unsubstituted 3-position was essential, together with a free position at either C-4 or C-7. A steric-free coplanar structure **1**, with a 3–7 linkage was proposed, the mesomeric form of which with extensive conjugation, **2**, could account for the general light absorption of the polymer. The occasional cross links at the 4–7, 2–4 (or 7) positions, leading to an irregular three-dimensional polymer, might explain the extreme insolubility of melanin (*30*).

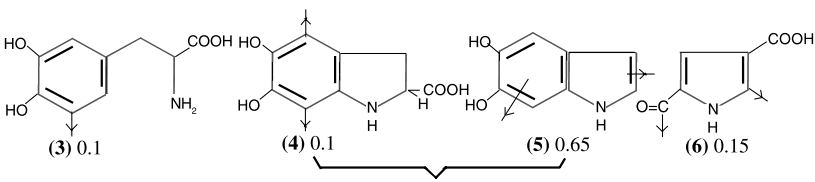
2.7.2. Melanin as Poikilopolymer

An analytical approach to studying the structure of melanin, by Nicolaus and co-workers (*184*, *204*) led to the concept of melanin as a

complex macromolecule or mixture of macromolecules, built from heterogeneous units and linked by more than one type of bonds that are not easily hydrolyzed. The type of linkages, which bind the units, are not known. According to Nicolaus (*184*), an unsubstituted 3-position is not essential, melanin being rather formed by random copolymerization of different highly reactive intermediates formed during the melanization process. The extent to which these monomeric units could contribute to the formation of the pigment polymer, depends upon the chemical and biological environment of the reaction site. Moreover, when free radicals are formed, they could be trapped into the macromolecules and in the end, the irregularity increases further, from the formation of the pyrrol units by the peroxidative cleavage of some labile indole units (Fig. 1) (*259*). The concept of melanin as a ''poikilopolymer,'' was later, confirmed through studies, conducted *in vivo* and *in vitro*, with labelled precursors (*109*, *128*, *222*, *258*).

2.7.3. Melanin as Bipolymer

Swan and Waggott (*257*), on the basis of their various experimental results, suggested that autooxidative dopa-melanin consists in the main of four types of units: 10% of uncyclised units, which are diphenolic (**3**), 10% of indoline-carboxylic acid type (**4**), 65% of indole type (**5**), and 15% of pyrrole type (**6**). Moreover, of the units of types **4** and **5** taken together, one half are quinonoid and the other half are diphenolic; 0.5 H_2O was included per average polymer unit.



2.7.4. Biophysical Model of Melanin Structure

The bioelectronic band structure of an indole-5,6-quinone polymer, with an exceptional electron-accepting ability from the lowest empty band in the bonding energy region, was first theoretically calculated by Pullman and Pullman (*212*). The stable free radical properties of the pigment polymers and their role as a sunscreen for biologically harmful quanta (*168*), and their implication in various neurological and psychiatric

disorders (*137*), suggested that natural melanins have some significant physiological functions. McGinness (*167*), by applying the theory of Mott (*176*) dealing with the electronic structure of amorphous materials, suggested a model in which the melanin granules act as hypothetical solid state devices that might assume many physiological functions (*168*). Both natural and synthetic melanin act as an amorphous semiconductor threshold switch with a rise in conductivity of melanin under applied voltage. Switching occurs reversibly at potential gradients comparable to gradients existing in some biological systems. The threshold switching in melanins and melanosomes, a rather exotic property of amorphous semiconductors, was studied and according to McGinness *et al.* (*169*) it can occur at biologically attainable electrical field strength. The relation between electronic properties and cellular functions of melanosomes was assumed to be a nonlineal energy transduction device operating by a phonon-electron coupling mechanism (*168*). Phonon-electron coupling plays an important role in amorphous semiconductor theory and according to McGinness *et al.* the coupling of phonons (vibrational modes of macromolecular structure) to an electronically excited state might proceed in both directions and may be particularly efficient in melanin (*169*). It was suggested, further, that the lack of a threshold for ESR signals at low temperature is due to mobility gaps in the amorphous band model as compared with the quantum mechanical model of conduction in amorphous solids (*167*, *176*). Evidence for a band model of melanin and its phonon-electron coupling mechanism was also provided by other workers (*69*, *173*).

Many biophysical properties of melanin have been studied using these new ideas of melanin structure, *e.g.* the absorption and dispersion of sound waves in melanins. It has been observed that hydrated melanins and melanosomes are exceptional ''black'' materials with respect to ultrasound absorption (*130*). Melanins are bioelectrets, *i.e.* they can store electrical charge and/or polarization (*25*, *81*). Studies on the storage of electrical charge and depolarization in both hydrated natural (epithelium-choroid complexes) and synthetic melanins have revealed a possible role as transconductors for the melanin granules (*25*). Further, melanins have been found in such non-illuminating areas as the brain and the inner ear (*83*) and neuromelanin has been hypothesized to be a component of bioelectronic mechanisms in brain function (*137*). According to McGinness (*170*) phonon-electron coupling may lead to interaction between melanins and pigmented neurons (the fundamental functional units of nervous tissues) which could explain the functional significance of neuromelanin in the brain as well as in the inner ear.

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2.7.5. Structure of Phaeomelanin

Little is known about the structure of phaeomelanins. Although the presence of 1,4-benzothiazine units in these pigment polymers is generally accepted, the other postulates, *i.e.* incorporation of benzothiazole and tetrahydroisoquinoline units into the pigment backbone, need further investigations (*59*).

2.8. Synthesis of Melanin

Synthetic melanins are obtained by biomimetic oxidation reactions using known precursors. So far, four different methods for melanin synthesis have been reported, *i.e. in vitro* enzymatic, autooxidative, electrochemical, and photochemical methods. Of these, the first two have been generally used, for large scale preparations of the pigment polymers and have been reviewed elsewhere (*70*, *211*). The latter two methods which are discussed here, have been used effectively to understand the mechanism of the melanization process in biological systems.

2.8.1. Electrochemical Synthesis

Electrochemical mechanistic studies of melanin are an outcome of melanin research in 1980. Various authors have employed these methods using various catecholamines and related compounds as substrates (*32*, *106*, *224*, *285*, *286*, *287*). These studies have not only confirmed the validity of Raper-Mason's scheme of melanogenesis (see page 158; Fig. 5) but also provided information regarding the mechanism of the chemical steps that occur in the early stages of the melanization process, the identification of each electron-transfer process, and the determination of the rate constants of non-oxidative reactions.

Using a selective amperometric detector in combination with liquid chromatography, the effect of pH on the sequence of events that occur during electrooxidation of catecholamine as well as the quantitative estimation of catecholamines has been studied (*129*). Thus, the cyclic voltammogram in IM $HClO_4$ (pH range 0.60–6.82 at 15, 20, 25, and 30°C) shows only peaks corresponding to the catechol-quinone redox couple at pH 0.6, since protonation of the amino group prevents the cyclization steps (Fig. 2). At pH 6.36, appearance of a new redox couple indicates the formation of cyclic products, *i.e.* the respective dihydroindole derivative in the reaction mixture. This step is of particular importance in biological systems since the oxidized form of catecholamines is a major factor in determining substrate toxicity that results from

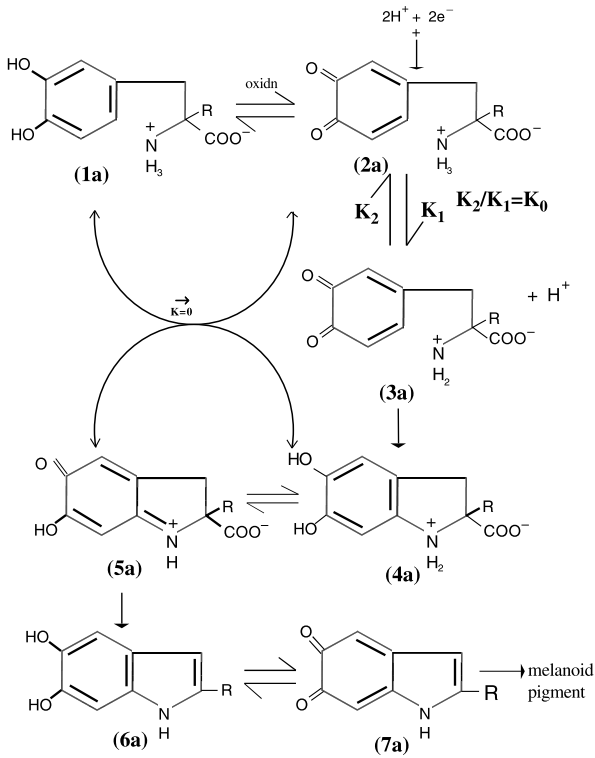

Fig. 2. ECC mechanism of electrochemical oxidation of catecholamines

competitive reactions of the oxidized chatecholamines with the sulfydryl groups of some essential enzymes. Thus the fast cyclizing N-methyl substituted catecholamines are less toxic than the unsubstituted ones which cyclize more slowly (*287*, *209*).

At an even higher pH (>7.68), the absence of a cathodic peak permits an estimate of the half line of the corresponding quinone as being on the order of tens of milliseconds (*287*); simultaneous darkening around the anode is considered evidence for melanin formation by electrooxidation. The overall reaction sequence in the electrochemical process is, however, very slow involving only a few monolayers.

A theoretical design of an enzymatic chemical mechanism (ECC), both on kinetic evidence and considering its pH dependence, has been suggested for electrochemical oxidation of chatecholamines (*32*, *224*, *286*). Thus, α-methyldopa (**1a**) (Fig. 2) first undergoes a two-electron oxidation to α-methyldopa-quinone (**2a** $\leftrightarrow$ **3a**), which then cyclizes to

α-methylcyclodopa (**4a**). A further two-electron exchange (**4a** + **2a** $\rightarrow$ **5a** + **1a**) then occurs to yield α-methyldopachrome (**5a**) which is fairly stable in solution but is further converted to 5,6-dihydroxy-2-methylindole (**6a**) by a decarboxylative rearrangement.

No direct electron transfer between melanin particles suspended in aqueous buffers and electrodes has been observed. This permitted studies of charge-transfer processes between the chlorpromazine cation radical and catecholamines spectroelectrochemically in order to determine the biological function of chlorpromazine (*164*).

2.8.2. Photochemical Synthesis

Catecholamines are thermodynamically and photochemically unstable compounds. This property has been utilized in the photochemical synthesis of melanin (*74*, *277*). Thus, a dilute solution of adrenaline, isoprenaline and noradrenaline saturated with oxygen on irradiation (254 nm) gives the corresponding aminochromes in 65, 56, and 35% yield, respectively. Longer irradiation produces melanins (*74*). Studies of the action spectrum confirmed the excited state of the catecholamine as the primary factor in the transformation processes. N-substituted catecholamines have been found to react more rapidly than the corresponding N-unsubstituted ones (*74*).

Photooxidation experiments in the presence of azide radicals using pulse radiolysis have revealed that dopa and cysteinyldopa yield first unstable semiquinones which disproportionate to a quinone-quinol complex. The quinones rapidly decay to more stable products, *i.e.* dopaquinones produce dopachromes and cysteinyldopa-quinones rearrange to benzothiazine isomers (*136*). Further investigations of the oxidation of various melanin precursors, both under physiological conditions (phosphate buffer, pH 7), and in organic solvents, *e.g.* methanol, were performed (*77*) to study the molecular mechanism of the immediate pigment darkening, *i.e.* natural skin tanning. While experiments in aqueous media showed significant competition between the primary photochemical and autooxidative processes, in methanol, a complex mixture of products was obtained.

3. Characteristic Biophysicochemical Properties of Melanin

Natural melanins possess some distinctive physicochemical properties of biological importance. For example, in biological systems,

photoprotection of skin against the harmful effect of quanta is believed to be one of the major functions of melanin pigmentation (*197*, *198*). In the eye, melanin acts as light screen and strongly resists light adaptation (*81*, *93*). Characteristically, the reactions of insoluble melanins are heterogeneous, involving both the surface and the interior of the pigment molecules, thus exhibiting their bifunctional mode of action (*112*). Since the present paper includes a discussion of vitiligo, the etiology of which is still unknown, it is appropriate to discuss briefly some biophysicochemical properties of melanin.

3.1. Interaction of Melanin with Light

3.1.1. Melanin in UV and Visible Light

Melanin shows a relatively structureless spectrum in the ultraviolet and visible range which intensifies with decreasing wavelength (*18*). Basing their arguments on amorphous semiconductor theory, McGinness *et al.* (*169*) suggested that the absorbed light is not radiated but is instead captured and converted to rotational and vibrational energy (photon-phonon coupling) and that such photon capture is available for any energy level from the UV through the visible and into the IR region. Hence, melanin can be considered black not just in the visible region. An expression for optical density (OD) derived from amorphous semiconductor theory can be stated as follows:

$$\mathrm{OD}z^{1,2} = KE_0^{1,2}(Z - 1)$$

where Z equals $1.242/\lambda E_0$ (dimensionless), E_0 is the optical bandgap in eV, λ the vacuum wavelength in μm, z a dimensionless independent variable, and K a constant. This relationship appears to hold for both eu- and phaeomelanin.

3.1.2. Melanin in the Photoprotection of Skin

Melanin can effectively protect skin from solar radiation and UV light by dissipating light energy either as heat (*168*, *169*) or in a chemical reaction which results in the consumption of molecular oxygen (*232*), or by scavenging active oxygen species, *e.g.* superoxide (*133*), and singlet oxygen (*237*). These phenomena are studied by ESR spectroscopy and spin trapping methods (*133*).

In biological systems, superoxide and H_2O_2 are formed in small quantities by normal processes and both of these species produce harmful

effects in tissues. While cell defense mechanisms are adequate to remove these active oxygen species under normal conditions, with exposure to UV light, their concentration increases and thus the function of melanin as an *in situ* quencher is important for the protection of skin (*133*). However, on continuous exposure to UV radiation, melanin itself may become energetically overloaded into a toxic state thus augmenting the radiative damage to cells (*207*). There exists evidence for phaeomelanin's role in sunlight-induced skin cancer (*57*, *58*).

The process of photoinduced interaction of melanin with molecular oxygen is accompanied by photobleaching (*232*), and phaeomelanin has been found to be more photolabile and susceptible to photodegradation on prolonged photolysis (*58*).

3.1.3. Melanin as Light Screen in the Eyes

The melanin granules of the eye generate free radicals when irradiated with visible light. The rapid generation of free radicals in light and their subsequent decay in the dark, coupled with the proximity of the melanin granules in the rods and cones of the eye, suggests that melanin plays a role in the visual process which is more important than the mere absorption of stray light (*65*).

The stimulation of a visual receptor in a vertebrate eye by an intense flash of light generates a fast electrical response, the early receptor potential (RP). A similar response in the pigment epithelium-choroid complex (PE–CC) of the eye was observed by Brown (*26*) which unlike the early RP is photostable and resistant to light adaptation. The PE–CC consists of the cell layers immediately behind the retina which are densely pigmented with melanin. Brown concluded that the photopigment involved in the PE–CC response was related to the visual pigments.

Ebrey and Cone (*81*) reexamined the phenomenon using isolated PE–CC layers and found that the amplitute of the major peak of the PE–CC response was proportional to the energy of the stimulus flash. They also studied the action spectrum of the PE–CC response using whole-eye preparations and found that the early RP which is photolabile to continuous light dominated the entire response recorded in the spectrum. At the far end, when steady light fully bleaches the visual pigments, the spectrum of the whole-eye response changes shape and a new response is observed with a flat action spectrum which is similar to that obtained from isolated PE–CC. The authors concluded that the photopigments for these responses absorb all the wavelengths of light equally well, and that the melanins present in the PE–CC cells are primarily responsible for generating at least part of the PE–CC response. This new electrical

response of the PE–CC is, however, fundamentally different from the early RP which depends on visual pigments.

3.2. Melanin and Its Redox Function

One of the most characteristic properties of melanins is their ability to exist in both the oxidized quinone and reduced quinol forms by acting either as electron acceptors or electron donors in reaction with reducing and oxidizing agents respectively, thus exhibiting their dual functionality (*33*). These processes merely involve the reversible exchange of two electrons and two protons (*33*, *112*, *138b*).

To examine changes in the oxidation state of melanins, ESR techniques have been used extensively while to determine the concentration changes of the reagents (oxidants, reductants), spectrophotometric or electrochemical methods have been used (*236*). Owing to the presence of carboxyls and phenolic groups in melanins, positively charged reagents have been found to react much faster than anions or neutral species, especially in basic media (*138a*). Generally reduction of both synthetic and natural melanins results in a lighter color and changes in the ESR spectra. The populations and the role of semiquinone states, assumed to be responsible for the characteristic ESR signals, have been studied for all types of melanins (*236*).

Both reduction and oxidation processes have been found to be biphasic. Thus, in kinetic studies of the reduction of synthetic D,L-dopa melanin with Ti^{3+} and oxidation with Fe^{3+}, respectively, a fast electron-exchange step was followed by a slow second step (*112*). The biphasic character of the electron-exchange processes has been interpreted as being due to a difference in the reaction mechanisms involving the surface and the core of the melanin granules (*112*). Using the oxidation-reduction capacities obtained for the fast electron-exchange processes, one-fourth of the indole units were found at the particle surface. Assuming the same fast rate of electron exchange in both the oxidation and reduction, respectively, the slow diffusion of the reagent (Ti^{3+}, Fe^{3+}, and H^{+}) in and out of the melanin particle is believed to control the rate of the second phase (*112*).

The electron-exchange properties of melanins have been studied with a number of especial reagents in order to understand the reaction mechanism as well as the role of melanin redox properties in biological systems. The processes have been found to be strongly irradiation dependent (both by visible and UV light). Thus, nitroxide radicals are reversibly reduced by melanins in the dark, and the redox equilibria are altered on irradiation (*233*). Similarly, other processes in living systems

(especially the processes involving dihydronoradrenaline, dihydrophenylnoradrenaline, and cytochrome) have been found directly linked to the redox properties of melanins (*99*).

Of particular significance to biological systems is the reaction of melanins with oxygen and the effect of external factors on this reaction. Thus, pH, illumination with visible light, temperature, and catalase have been studied in detail. Melanins were studied in their native, reduced, oxidized, and methylated forms and the reactions were monitored *via* ESR (*231*). The rates of oxygen uptake were, generally, higher with illumination and over the pH range 5.5–11.9. In addition to direct electron-exchange properties, melanins act as electron-transfer agents. Thus, some synthetic melanins have been found to accelerate the oxidation of dihydronoradrenaline with $Fe(CN)_6^{3-}$ (*99*).

3.3. Binding Complexation and Medicinal Aspects of Melanin

Metal cations and organic species carrying positive charges possess strong affinity for melanins (*139*, *140*), and binding complexation which occurs through ion-exchange and/or hydrophobic interaction mechanisms have biological significance (*17*, *278*). These ESR characteristic phenomena have been studied to explain medicinal aspects which may involve the affinity and binding of various molecules to melanins, such as the toxicity of drugs, the malignancy of melanoma cells, and *substantia nigra* (*141*, *158*, *181*). For example, a pathological pigmentation often occurs in the skin of the patients taking large doses of chlorpromazine (*5*). It has been suggested that chlorpromazine acts on the autonomic nervous system by blocking the production of pigment-lightening factors such as melatonin (*283*). According to some authors, white light produces free radicals from chlorpromazine, and the formation of a stable charge-transfer complex with melanin is the explanation for this chemical hyperpigmentation (*5*, *141*).

Studies of metal binding by both native and synthetic melanins have provided data that not only confirm the ion-exchange properties of melanins but also reveal the relative affinities of metals for melanins (*139*). Thus, similar to other ion-exchangers, the affinity for melanin increases with the valency of the cation and atomic number of the element (*i.e.* $Cs^+ \gg Li^+$; $Ba^{2+} \gg Mg^{2+}$). However, the exceedingly high affinity found for Pb^{2+} when compared with similar divalent ions suggests the possible involvement of other factors though protein present in native melanins plays a minor role in the binding of metals (*139*).

Binding studies combined with ESR spectroscopy have revealed the mechanism of the interaction of metal ions with melanins which indicates the formation of a chelate complex between di- and trivalent diamagnetic metal ions and *o*-semiquinone radical centers on the pigment polymer (*85*, *124*). This interaction often results in an increase of total free radical concentration. Furthermore, the binding capacity varies for melanins of different origins with the number of reactive sites (*230*).

The cation exchange mechanism has also been observed in the binding of organic molecules (mostly bases and often positively charged) to melanins (*140*). A systematic structure-affinity study was reported for a series of heterocyclic compounds and synthetic D,L-melanin. The relative affinities measured from absorption in pH 7 phosphate buffer, showed that π-electron system, basicity, and steric factor are the main determining factors for the affinity towards melanin (*113*, *140*). These results are consistent with the stability of charge-transfer complexes between the organic molecule (as donor) and melanin (as acceptor). This structure-affinity relationship can be used for the development of drugs which may selectively target melanocytes (*e.g.* melanoma cells), or drugs with low toxicity that are not accumulated in melanin-containing tissues, such as the eyes.

3.4. Use of Melanin for Defence

The melanin of sepia is found in the ink sac of cuttlefish. Aristotle in his book ''Historia Animalia'' mentioned that ''instead of intestines molluscs have an organ known as the *mytin* where a black substance is found which is especially abundant in cuttlefish; they put forth this black substance when frightened, particularly the cuttlefish''. The ink has considerable staining power, and is alkaline and odorless. When fresh it disperses readily in water and is partly soluble in alkali, but insoluble in acid. This biological function of melanin appears to be defensive but the mechanism is uncertain (*270*). However, like other pigments, melanin can be utilized for camouflage purposes in insects and fishes.

4. Melanogenesis

4.1. Melanogenesis *in vivo*

Epidermal melanin synthesis is a multistage process involving fast or slow reactions, some of which are enzyme catalyzed, others requiring only oxygen. The process is controlled by pH, temperature, redox

potential, activity of the enzyme and the presence of inhibitors. The overall reaction occurs, within a genetically controlled biochemical environment, in specialized cells called melanocytes. Apart from melanin granules, melanocytes contain unique organelles, ''premelanosomes'' and ''melanosomes'' in which the biosynthesis of melanin occurs (*89*).

4.1.1. Melanocytes

Melanocytes are dendritic cells which are wedged between the basal cells of the epidermis. They derive from neural crest (*i.e.* at a high point of action of the nervous system) and have complex structures mainly made up of protein and various oxydase systems, including tyrosinase (*160*).

The biosynthesis of melanin has been studied by electron microscopy in epidermal melanocytes after *in vivo* ultraviolet irradiation with either dopa or tyrosine as substrate (*114*). The melanogenic enzyme, originating from minute polypeptide particles (50–100 Å), is transferred into the Golgi area where it condenses to membrane-limited vesicles to produce ''protyrosinase''. There, protyrosinase is incorporated into a structural protein matrix containing filaments that have a distinctive periodicity. The unit represents a ''premelanosome'' (*95*, *123*), which possesses intense enzyme activity but contains no melanin (*271*). The protyrosinase then becomes active (*i.e.* becomes tyrosinase), biosynthesis of melanin begins, and the unit is called a ''melanosome''. In their development stages these melanosomes move from the cytoplasm of the melanocyte into dendritic processes (*i.e.* branching protoplasmic processes that produce impulses toward the body of a nerve cell); their melanin content increases while their enzyme activity decreases (*89*, *267*), and at the end, when the melanosomes no longer possess enzyme activity, melanin is formed entirely by nonenzymatic polymerization, fills the entire organelle and obscures its (melanin) internal structure. Melanin is transferred to the basal dendritic cells, the keratinocytes. Each melanocyte supplies several keratinocytes with melanins (1:36) thus forming with them an epidermal melanin unit (*89*).

Morphologically, melanocytes and melanin granules from various sources are very similar. However, a definite correlation exists between the size and morphology of the melanocytes of human skin, and their pigment forming activities, *e.g.*, dark color skin contains larger and uniformly more highly reactive dendritic melanocytes than caucasoid skin (*88*, *255*), whereas the melanocytes of caucasoid skin, when not exposed to sunlight, are highly variable in dopa reactivity (*213*).

Although the melanosome theory has been accepted in general, some authors support a mitochondrial origin of the melanin granules (*282*).

The chemical compositions of melanosoma and mitochondria have been determined and compared, using differential and density-gradient techniques (*241*). The data show that melanosomal fractions contain a high percentage of Zn and a low content of RNA and phospholipid-P as compared with mitochondrial fractions. The lack of succinoxidase and glutamate oxidase activities support the idea that melanosomes are subcellular particles, different from mitochondria, and contain a particular specialized metabolic pathway through which tyrosine or dopa is converted into melanin (*239*).

4.1.2. The Characteristics of the Enzyme

The traditional view of enzymatic melanogenesis, expressed by different authors (*88*, *145*, *242*), holds that tyrosinase is the melanogenic enzyme, and studies on *in vitro* melanogenesis using mushroom tyrosinase have been considered as valid for mammalian melanogenesis as well, although the enzymes isolated from different sources show qualitative differences (*27*, *126*).

Tyrosinase is a copper-containing glycoprotein that carries a coupled binuclear copper active site capable of catalyzing two distinct reactions: (i) hydroxylation of tyrosine to dihydroxyphenylalanin, *i.e.* dopa (cresolase activity), and (ii) subsequent two-electron oxidation to dopaquinone (catecholase activity) (*221*). Both reactions require oxygen and the enzyme, *i.e.* tyrosinase, in reduced cuprous form. Because of these two catalytic functions, tyrosinase is in modern terminology referred to as a "mixed function oxidase" (*157*). A mechanism (Fig. 3) for hydroxylation

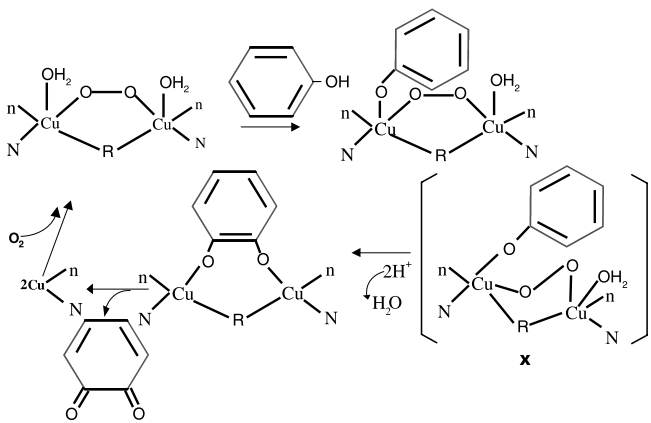

Fig. 3. Proposed mechanism of tyrosinase oxidation

and oxidation of phenolic substrates to *o*-quinones has been proposed which states that during the *o*-hydroxylation process an intermediate complex X is formed which involves one molecule of oxygen and two neighboring cuprous atoms attached to a protein chain. The formation of such a structure depends on the relative position of the copper atoms, hence the ''cresolase activity'' would be sensitive to any variation in the configuration of the protein chain (*221*, *274*).

The unique property of this enzyme is that the product of the first monoxygenation step, *o*-diphenol, serves as the electron donor for the reduction of the cupric ions with formation of the corresponding *o*-quinone (*161*, *274*). The products formed by catecholase activity from tyrosine are extremely reactive and undergo intermolecular reactions to form indole derivatives which subsequently polymerize to melanin.

The tyrosine-tyrosinase reaction is characterized by a variable lag period (*145*), which can be shortened by addition of a catalytic amount of dopa or related compounds. However, there is no time lag with dopa as substrate, and it seems very likely that dopa induces a conformational change in normal tyrosinase and activates the reduction of tyrosinase, thereby acting as a cofactor in the melanization process (*145*). When tyrosinase is present in low concentration as in nonirradiated skin, this lag period is prolonged markedly, whereas in skin exposed *in vivo* to ultraviolet light (*87*), in epidermal sheets (*260*), and in hair bulbs (*88*), the tyrosine-tyrosinase reaction is readily detectable. The presence of tyrosinase in human epidermis was established in 1950 (*86*).

Okun and his associates (*187*) suggest that ''peroxidase'' rather than copper-dependent tyrosinase, mediates the conversion of tyrosine to melanin in the presence of dopa as cofactor. However, some results obtained in the course of investigations on melanogenesis by other authors contradict their concept (*111*, *280*).

Tyrosinase activation has been found to be inhibited by its own substrate *in vitro* and this inhibition-mechanism has been studied (*110*, *125*, *268*).

4.1.3. Regulation of Melanogenesis

Biochemical analyses of the regulation of pigmentation and proliferation have largely been confined to the population of melanoma cells grown in culture. These studies have revealed that in addition to these tyrosinase-calalyzed steps (*200*) various non-melanosomal regulatory factors are involved in the pathway for melanin biosynthesis (*244*). A short discussion of these factors is given in the following.

4.1.3.1. Physiological Factors

Elevation of temperature increases tyrosinase activity, shortens the induction period and stimulates melanin formation within physiological limits. The enzyme activity is optimal in the pH range 6.7–7.2 during *in vitro* reactions (*183*). The temperature responses of melanin-stimulating hormones have also been studied (*226*).

4.1.3.2. Organic Sulfur Compounds

Organic sulfur compounds are known to inhibit *in vitro* melanin synthesis (*209*), and it was suggested that thiol groups form strong bond with copper, inactivate tyrosinase and thus inhibit melanin formation (*91*, *225*). It has now been confirmed that dopaquinone, generated *in situ* by tyrosinase catalyzed oxidation of dopa, reacts with cysteine forming cysteinyldopas (*117*). Hyperpigmentation after UV irradiation proceeds by a marked decrease in glutathione reductase activity and a decrease in reduced glutathione which is an inhibitor of melanin synthesis (*118*). In fact, glutathione reductase activity and reduced glutathione are lower in black than in caucasian skin (*120*). Furthermore, γ-glutamyl transpeptidase was found to be inactivated in the course of melanogenesis (*120*).

4.1.3.3. Metal Ions and Other Chemicals

Metals play an important role in melanin biosynthesis (*244*) and incorporation of various metal-ions into melanins, produced *in vivo* and *in vitro*, respectively, has long been known (*28*). During melanin formation under anaerobic conditions, certain metals (*e.g.* Zn, Fe, Mn *etc.*) catalyzed non-decarboxylating rearrangement of dopachrome (*190*). According to Chakraborty *et al.* (*50*), though autooxydation of dopa is a copper catalyzed reaction, it can equally be catalyzed by nickel, cobalt, and slightly by selenium also. However, lead inhibits melanogenesis (*263*).

Hydroquinone and its derivatives *p*-hydroxypropiophenone, pyridine derivatives, catechol, mercaptamine derivatives, etc., inhibit melanin formation by blocking tyrosinase activity (*56*, *61*).

4.1.3.4. Vitamins

Deficiency of pantothenic acid causes depigmentation while administration of folic acid increases hepatic storage of pantothenic acid as the Vitamin B-complex acts synergistically with folic acid in normal pigmentation (*269*). Ascorbic acid maintains optimum sulfhydryl levels in the body and keeps melanin in reduced form (*262*). Any change in the

dose levels of this acid (higher or lower), inhibits or accelerates tyrosinase activity (*47*). Thiamine increases the lag time for induction of the tyrosine-tyrosinase reaction and disrupts the equilibrium of dopa-dopaquinone autooxidation (*261*).

It has now been confirmed that cholecalciferol, Vitamin D_3, has a stimulatory effect on the melanocytes, due to the *de novo* synthesis of tyrosinase and transfer of melanin granules to the surrounding keratinocytes with elongation of their dendrites (*269*).

4.1.3.5. Hormones

The pituitary gland plays an important role in the hormonal control of melanin pigmentation (*142*). The highly reactive melanocyte stimulating hormone intermedine (MSH) secreted by this gland is not only able to darken the human skin (*150*), but has numerous biological activities in higher vertebrates. For example, it acts on the thyroid gland of the rabbit (*68*, *235*) and on the blood-aqueous humor barrier in the rabbit eye (*80*); α-MSH acts on the adrenal cortex (*102*), β-MSH acts on the central nervous system *etc.* (*134*, *154*). The hormone adrenocorticotropin (ACTH) secreted by the anterior lobe of the pituitary gland disperses melanin granules in the frog-melanophore and moderately stimulates epidermal melanocytes (*171*).

Tyrosinase activity decreases after hypophysectomy, but can be restored by chronic administration of pituitary hormones like prolactin, MSH, and ACTH to a greater or lesser extent (*131*). Prolactin can promote *in vivo* melanin synthesis not only by stimulating tyrosinase activity, but also by increasing the supply of available substrate (*131*). However, it is still unknown which of the pituitary hormones is responsible for hyperpigmentation in Addison's disease in man.

Melatonin (*N*-acetyl-5-methoxytryptamine) is a pineal gland hormone which aggregates melanin granules in the dermal melanophores and is capable of lightening the color of frog melanocytes by reversing the darkening action of MSH, ACTH, and caffeine (*116*, *149*). Its effect on the human and other mammalian melanocytes is, however, questionable (*253*).

Very small amounts of adrenalin and noradrenalin inhibit MSH action on skin pigmentation, but there is no direct evidence of their action on mammalian melanocytes (*151*, *174*).

Among sex hormones, the female hormones are strong stimulants of melanogenesis (*251*). The reports reveal that administration of small doses of estrogen to ovariectomized guinea pigs increases the melanin content of melanocytes in all skin regions examined, while the effect of

large doses on skin pigmentation is direct, and not *via* the pituitary gland (*11*, *250*). Small doses of progesterone slightly stimulate melanogenesis (*252*), while very large ones reduce the amount of free melanin (*250*). Interestingly, a mixture of estrogen and progesterone (in small doses), when given to ovariectomized guinea pigs, causes greater stimulation of melanogenesis than one produced by either hormone given alone in the same doses (*11*). The significance of sex hormones in the tanning of the skin of women has been examined by Hamilton *et al.* (*104*). Testosterone has been shown to increase skin pigmentation in the castrated and hypogonadal human male and in some other species (*10*, *73*).

Experiments with cyclic-AMP have revealed that in addition to an increase in melanin formation by increasing tyrosinase activity, it can also enhance the pigment transfer (*115*, *276*). Dibutyryl-cyclic-AMP has been found to induce melanin formation in the hair bulb of chinchilla mice (*115*). The roles of prostaglandin and cyclic nucleotides, such as cyclic-GMP, remain unclear for men at present.

The hormonal control on pigmentation may be summarized in Fig. 4.

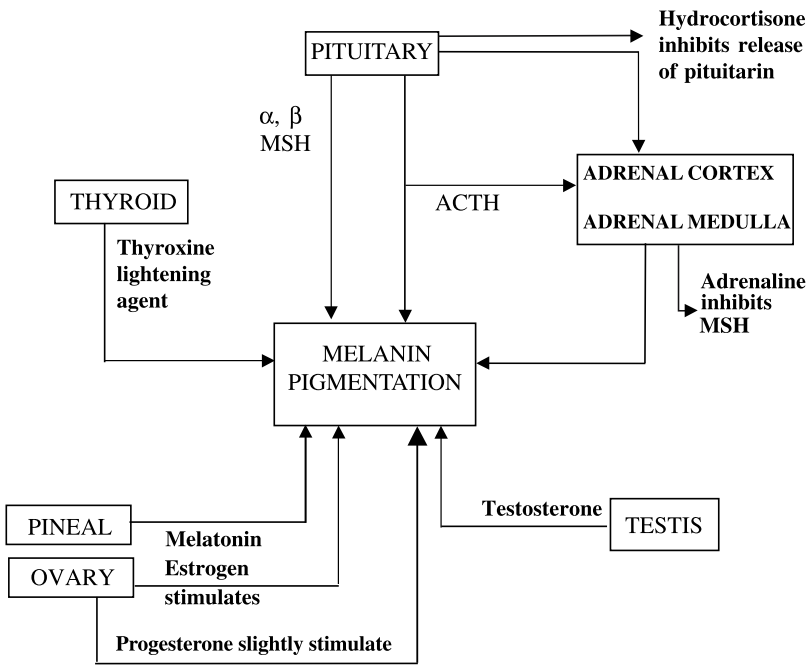

Fig. 4. Hormonal control of melanogenesis

References, pp. 171–185

4.1.3.6. Neural Influence

Although melanocytes are derived developmentally from the neural crest, the neural influence on human skin pigmentation is not clearly understood (*152*). According to some authors, vitiligo results from an increase in activity of the peripheral nerve endings in the skin (*24*). Neural influences of pigmentation have also been noticed in lower vertebrates where skin color changes rapidly (*102*, *194*).

4.1.3.7. Malpighian Cells

Melanocytes synthesize melanins and malpighian cells help in the transfer and distribution of the melanin granules in the epidermis, thus operating together as a single functioning unit in the process of skin pigmentation (*79*, *89*). According to Fitzpatrick *et al.* (*89*), malpighian cells control the rate of production of melanin granules by melanocytes, *i.e.* the production of melanin granules in melanocytes and their pool of malpighian cells.

4.1.3.8. UV Light

The role of UV light on the pigmentary system has been reported in detail by Quevedo (*214*). It has been documented that if a single exposure to UV light is administered and delayed tanning is studied, then increased enzyme activity, increased pigment formation, increased number of melanocytes and melanosomes, increased degree of melanization, and increased pigment transfer occur (*100*, *198*). The regulatory events at the operon nucleic acid level are, however, not yet clear.

4.2. Melanogenesis *in vitro*

The chemistry of melanins reveals that it is difficult to study these pigment polymers using conventional chemical and physical methods. On the other hand, by using known precursors biosynthetic studies under biologically relevant conditions can be used successfully to understand the chemistry of melanin, *i.e.* the identification of the intermediates, their reactivities, the formation of melanin, and its properties.

4.2.1. Enzymatic Melanin Synthesis

This biosynthetic approach was first exploited with remarkable success by Raper (*216*). Later, using advanced research techniques, biosynthetic studies of eumelanin polymers have revealed many facts regarding

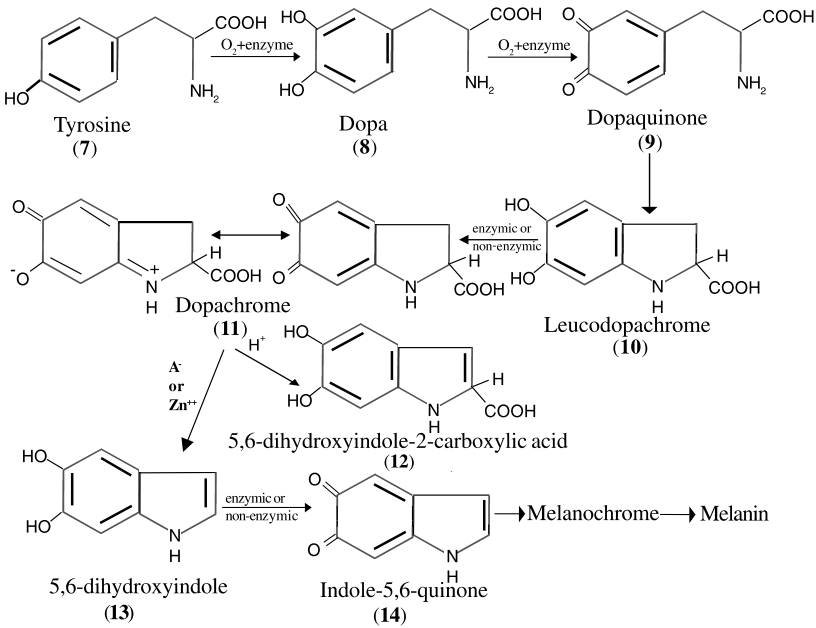

Fig. 5. The Raper-Mason scheme of melanogenesis

the diversity of the pigment origin, the heterogeneity of its structure, and its unique properties.

In the Raper-Mason scheme of melanin biosynthesis (Fig. 5) (*216*, *217*), tyrosine is enzymatically converted *via* dopa to dopaquinone. The subsequent oxidation steps leading to melanin formation depend upon the biochemical environment of the reaction site. However, the melanization process *in vitro* or *in vivo* has two important features; the rearrangement of dopachrome and the oxidative polymerization of 5,6-dihydroxyindoles leading to melanochrome.

4.2.1.1. Rearrangement of Dopachrome

For the rearrangement of dopachrome, one possible mechanism which has been accepted by different authors (*66*, *218*, *255*), involves a hydrogen shift from position 3 and the formation of a quinone methide followed by subsequent decarboxylation to **13**. Analysis of the analytical data from various laboratories (*66*, *108*, *202*) has shown that the yield of DHI *vs* DHICA is about 95 to 5, at a pH range from 3 to 8.5 which indicates that tyrosinase-catalyzed synthetic dopamelanin is made up mainly of DHI-derived units, as proposed by Mason (*163*). Hence, the

carboxyl content of dopamelanin must be due to peroxidative cleavage of indole units rather than to a random incorporation of carboxylated biosynthetic intermediates, a view which contradicts the concept of the poikilopolymer model of melanin structure (*259*).

The presence of certain metal ions, especially Cu, Fe, Zn *etc.*, in melanin biosynthesis, however, accelerates the non-decarboxylating rearrangement of dopachrome leading to the formation of DHICA rather than DHI (*50*, *190*).

Further, recent studies (*132*) have revealed the presence in melanocytes of a melanosomal protein different from tyrosinase, which has the ability to catalyze the rearrangement of dopachrome to DHICA. This enzymic reaction is highly stereospecific for normal L-dopachrome, is unaffected by metal chelators and has an optimal pH of about 6.8. Different names have been proposed for this enzyme, *i.e.* dopachrome conversion factor (*132*, *256*), dopachrome oxidoreductase (*143*), dopachrome isomerase (*201*), and dopachrome tautomerase (*4*). It is of interest that another enzyme named dopaquinoneimine conversion factor seems to exist which has the remarkable ability to catalyze the decarboxylative rearrangement of dopachrome to DHI rather than DHICA (*193*).

According to Pawelek *et al.* (*200*), the biosynthesis of melanin in Cloudman melanoma cells is a complex process and is regulated by three factors: (a) a dopamine conversion factor which converts dopamine to 5,6-dihydroxyindole (**13**), (b) a 5,6-dihydroxyindole conversion factor which catalyzes the conversion of 5,6-dihydroxyindole to melanin and is active when cells are exposed to melanotropin (MSH), and (c) a 5,6-dihydroxyindole blocking factor which restricts melanogenesis at the 5,6-dihydroxyindole stage. They have also shown that at least three steps in the Raper-Mason scheme of melanin formation from tyrosine are catalysed by tyrosinase (Fig. 6).

4.2.1.2. Polymerization of DHI

To understand the chemistry involved in formation of eumelanin studies dealing with the polymerization of DHI leading to melanochrome (first detected by Mason, 1948), are of great importance. According to Beer *et al.* (*7*), the broad absorption spectrum at 540–560 nm exhibited by melanochrome was more consistent with a DHI-dimer or oligomer, and they suggested that the 3- and 7-positions were involved in the oxidative coupling of DHI (*30*). Later, Swan (*258*) and Kirby *et al.* (*128*) showed on the basis of tracer studies that during melanin formation each of the various positions of the side chain and the benzene ring of dopa were involved to a similar extent.

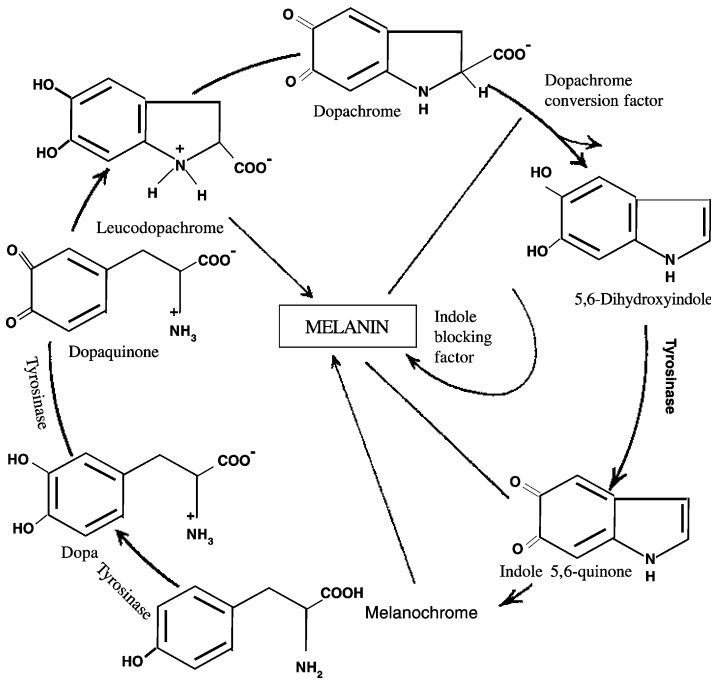

Fig. 6. Regulation of melanogenesis

Much later, Prota *et al.* (*78*), using improved procedures, isolated from a mixture of oligomers at the melanochrome stage three 2,2-, 2,4- and 2,7-dimers and three related trimers. They concluded that the dominant mode of coupling of DHI involved the 2- and 4-positions of the indole ring, with a minor contribution of C-7.

Kinetic experiments and pulse irradiation studies on the mechanism of DHI polymerization (*2*, *136*) revealed that the dominating species formed by DHI oxidation was the quinone methide and that coupling proceeds *via* oxygen-centered semiquinone radicals, which can also account for the complexity of the later stages of melanogenesis and the heterogeneity of melanin structure.

The oxidation of DHICA was also studied in the absence and presence of DHI, and two dimers (*191*) and one mixed dimer (*180*) were identified. The overall results obtained in the oxidation of DHI or DHICA at the melanochrome stage support the concept of eumelanin as an intimate mixture of homopolymers of DHI and DHICA, and copolymers of the two indole units in different proportions, the latter depending upon the ratio of formation of DHI and DHICA in the rearrangement of dopachrome.

References, pp. 171–185

4.2.2. Non-Enzymatic Melanin Synthesis: Model Reaction

It appears from the previous discussion that melanogenesis *in vivo* or *in vitro* is regulated by various factors. Hence, to study the dynamics of melanin formation (monooxygenase reaction, *i.e.* constructive metabolism) and breakdown (dioxygenase reaction, *i.e.* catabolism of melanin precursors), a non-enzymatic melanin synthesis from tyrosine and tryptophan, respectively, was devised by Roy *et al.* (*227*), using a prototype of a monooxygenase reaction, *i.e.* the Udenfriend reaction (Fe^{+2}/EDTA/ascorbic acid) (*272*).

4.2.2.1. The Udenfriend System: A Model for Mixed Function Oxidase

A truly enormous number of different enzymes has been characterized as mixed function oxidases (MFOs) by Mason (*162*) and as monooxygenases by Hayaishi (*107*). They can catalyze a wide variety of oxidation reactions including hydroxylation of aromatic and aliphatic compounds, epoxidation of olefins, oxidative decarboxylation, lactonization *etc.* (*104*). According to Hamilton (*105*), MFO-catalyzed reactions proceed *via* an ''oxene mechanism'' and there is now ample evidence that the ''oxinoid species'' is a reduced form of the enzyme (or an enzyme with a reduced cofactor, *e.g.* metal ions) which reacts with oxygen and the substrate either in one step or more than one (*273*).

Many MFO-catalyzed reactions possess characteristic similarities to model reactions and since these enzymatic reactions occur very fast and the intermediates are too short lived, non-enzymatic model systems have been developed to study oxene mechanism. One specific example is the Udenfriend system (*272*) which involves the hydroxylation of aromatic compounds under physiological conditions by a mixture of ascorbate, Fe(II), EDTA, and O_2. According to Hamilton (*105*), the intermediate complex **A** formed during the oxidation reaction (Fig. 7), is believed to be an oxinoid species which transfers electrons to the oxygen atom in the transition state and probably, at the same time, allows the transfer of singlet oxygen from complex **A** to substrate in one step so that diradical intermediates would not be necessary (*105*).

The possible function of an EDTA ligand is to protect the nucleophilic oxygen in **A** from intermolecular reactions although the system operates also without it, even though more slowly (*272*, *273*).

In many cases two metal ions can perform the function of the reducing agent; when oxygen is transferred to the substrate each metal ion is oxidized by one electron. This seems to be the explanation for many

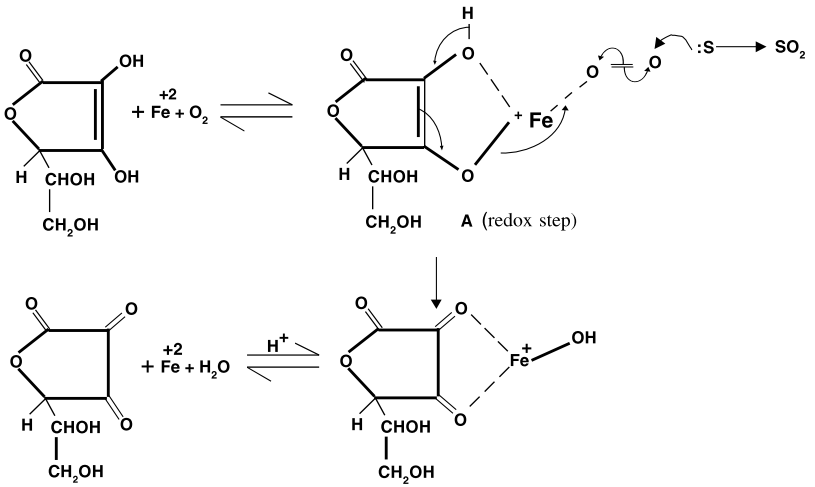

Fig. 7. Mechanism for the oxidation by Udenfriend system

model oxidation reactions as well as for enzyme-catalyzed oxidation reactions involving reduced metal ions (*96*, *273*). Hence, a similar mechanism can be written for the non-specific copper tyrosinase, in which the orthodiphenol intermediate takes the place of ascorbate (*274*, *275*). In fact the mechanism shown in Fig. 3 (see page 152) is very similar to the one in Fig. 7.

4.2.2.2. Melanin Formation Under Udenfriend Conditions

The biomimetic synthesis of melanin from indole accompanied by other biproducts using the Udenfriend reaction was first reported in 1981 (*227*). Since the obligatory formation of an indole skeleton during the synthesis of melanin from tyrosine may also arise from tryptophan with loss of the latter's three carbon side chain, Chakraborty *et al.* (*51*) proposed to study the participation of tryptophan in melanogenesis under Udenfriend conditions. For comparison, experiments with tyrosine as a substrate were also carried out. Thus, in an atmosphere of oxygen, a mixture of ascorbic acid, EDTA, $FeSO_4$, $7H_2O$, and L-tryptophan (L-tyrosine), in 0.1 *M* phosphate buffer, pH 6.7, was stirred for 240 min with tryptophan and 120 min with tyrosine, respectively. The black particles deposited on standing were collected by centrifugation, the aqueous solutions were extracted with ether, and the ether extracts were worked up by the usual procedures. The yield of the isolated constituents was, however, very low although reproducible.

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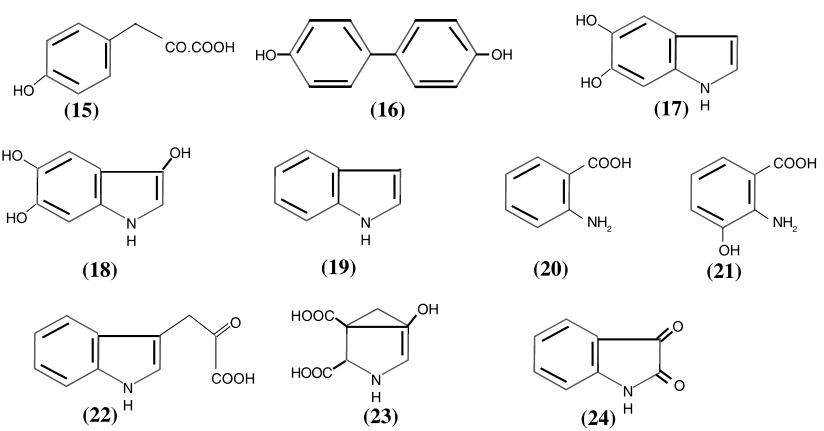
Both tryptophan and tyrosine furnished melanin. Similar results were also obtained by Allegri *et al.* (*3*), who studied melanin synthesis from tryptophan and tyrosine spectrophotometrically. From tyrosine, *p*-hydroxyphenylpyruvic acid (**15**), 4,4-dihydroxybiphenyl (**16**), 5,6-dihydroxyindole (**17**), and 3,5,6-trihydroxyindole (**18**) were obtained in addition to melanin and from tryptophan, 5,6-dihydroxyindole (**17**), indole (**19**), anthranilic acid (**20**), 3-hydroxyanthranilic acid (**21**), indolylpyruvic acid (**22**), 3-hydroxypyrrol-4,5-dicarboxylic acid (**23**), and isatin (**24**).

The results of this biomimetic melanin synthesis are significant. For example, isatin (**24**) as a monoamino oxidase inhibitor may aid higher catecholamine concentration favouring depigmentation (*1*). Oxidation products **20**, **21**, **23** formed from tryptophan could arise through a reaction resembling the *in vivo* dioxygenase reaction on the intermediates leading to 5,6-dihydroxyindole and melanin (*50*). Compounds **18** and **23** could be considered to be candidates for participation in detoxication mechanisms of **17** as indole itself is detoxicated through its 3-hydroxy derivative (*110*). Such a chemical transformation of 5,6-dihydroxyindole might relieve the cell from the cytotoxic effect of **17** (*110*).

Further, owing to deamination, *p*-hydroxyphenylpyruvic acid (**15**), a well-known inhibitor of tyrosinase, was produced from tyrosine. This product underwent further transformation to yield biphenyl **16**; while indolylpyruvic acid (**22**) and subsequently indole (**19**) were formed from tryptophan. Hence, in biological systems, the loss of the starting material, *i.e.* tyrosine, for melanin synthesis due to the formation of **15**, **16** and **18** may be counteracted by tryptophan, as an alternative substrate in the pathway of melanogenesis through **22** and **19**. However, such a replacement of substrate for melanin by tryptophan is not possible in



subjects with higher tryptophan pyrrolase (TP) activity, as occurs in vitiligo (*51*).

The overall results of the model reactions suggest that *in vitro* melanin synthesis is a complex process requiring the regulation of various participating reactions (*i.e.* mono-oxygenase/dioxygenase/deamination). Probably the formation of the end products is regulated by the kinetics of the reactions. The results may have relevance to the factors regulating melanin formation *in vivo* according to Pawelek (*200*).

5. Vitiligo

5.1. Introduction

Vitiligo refers to an idiopathic, usually progressive, cosmetic disfigurement of skin due to depigmentation that starts after birth and is not fatal (*188*, *189*). The earliest information regarding the disease is in the Ebers Papyrus (*90*) according to which vitiligo was treatable. In the sacred Indian volume ''Atharva Veda'' dating to 1400 B.C. the condition of ''Shweta Kustha'' (white leprosy) refers to vitiligo (*280*). ''Bohak'', ''bahah'', and ''baras'' are Arabic names (Koran – 3:48, 5:109) and ''kilas'' is the Buddhist name for vitiligo (624–544 B.C.).

India has the largest population suffering from vitiligo (1.7%) (*188*). The most characteristic histological picture of this disease is the depletion of skin melanin; the epidermis and dermis are otherwise normal. Since there are no specific biochemical features of the disease, rigid laboratory criteria for a diagnosis of vitiligo are lacking. It is uncertain whether vitiligo is one disease entity with a specific pathogenesis or a common expression of several different lesions.

Although, vitiligo is generally considered to be an acquired condition, several cases of ''congenital vitiligo'' have been described (*122*, *147*). A genetic predisposition is considered to be involved in this disease (*72*, *188*).

5.2. Melanocytotoxicity: Antimelanocyte-Antibodies Formation

Several hypotheses regarding the etiology of vitiligo have been reported, but none can totally explain the genesis of this disease (*19*, *144*, *185*, *188*, *189*, *215*). However, there are four prevailing theories of the pathogenesis of vitiligo which are briefly described in the following.

References, pp. 171–185

5.2.1. The Immune Hypothesis

Two immunological mechanisms could explain the pathogenesis of vitiligo (*155*). A primary disturbance in the immunologic system may result in autoimmunization with the formation of autoantibodies against an antigen of the melanogenic system. This causes melanocytotoxicity, or antimelanocyte-antibodies formation. An alternative mechanism is that there is some injury to the melanocytes which results in the release of a toxic antigenic substance so that formation of antimelanocyte-antibodies ensues and melanogenesis is inhibited (*29*, *155*, *182*).

5.2.2. The Neural Hypothesis

The neural hypothesis suggests that vitiligo results from the accumulation of some neurochemical mediator which reduces melanin formation (*148*, *152*). Both melanocytes and nerve cells are of neuroectodermal origin and both utilize tyrosine to produce an end product, melanin or catecholamine. The similarity of the structures of dopa and catechol suggests that a translation error of receptor sites controlling melanogenesis may cause the aberration of vitiligo (*29*, *152*).

5.2.3. The Self-Destruction Hypothesis

This hypothesis suggests that there are intermediates or metabolites in the melanin-synthesis pathway which, being unchecked, lead to melanosome destruction, *i.e.* melanocytes' dysfunction or death (*121*, *153*).

5.2.4. The Composite Hypothesis

The above three hypotheses are not mutually exclusive. An immunologic event may be secondary to cutaneous injury or neural stimulation may lead to overproduction of the toxic precursors in melanocytes with subsequent leakage of an aggressive immunologic process destructive to melanocytes (*188*).

Alternatively, melanin formation and destruction may be seen as a physiologically precarious balance process with a metastable equilibrium in those genetically disposed towards it. Overstimulation of neural elements, trauma, sunburn etc., may upset this homeostasis in favour of melanin destruction, again with incontinence of antigenic material and resultant immunologic melanocyte destruction (*35*, *144*, *189*).

Finally, it cannot be excluded that the primary event is a deficit in feedback control from keratinocytes to melanocytes, such that whatever mechanism genetically limits the number and packaging of melanosomes

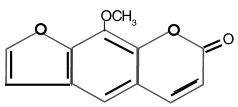
in keratinocytes, it becomes defective and dominant so as to ''turn off'' melanocytes completely (*123*). This too could implicate neural, immunologic and self-destruction factors.

Although, this composite hypothesis, like the other three hypotheses, lacks experimental support it appears to encompass more of the facts and abnormalities in vitiliginous patients.

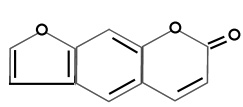
5.3. Chemotherapy of Vitiligo

5.3.1. Psoralens

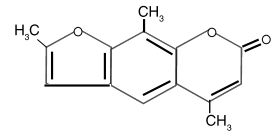
Chemotherapy of vitiligo begins with use of the indigenous drugs *Ammi majus* in Egypt (1948) and *Psoralea corylifolia* in India (1957). El Mofty (*82*) in Egypt successfully used the 8-methoxy derivative of psoralen **(8-MOP**; **25)** against vitiligo which was put on the market under the trade name Meladenin, while in India, Roychowdhury and Chakraborty (*228*) introduced psoralen (**26**) isolated from *Psoralea corylifolia* as a chemotheraputic agent for treatment of vitiligo.



Xanthotoxin (8-MOP)
(**25**)



Psoralen
(**26**)



Trimethylpsoralen (TMP)
(**27**)

Although psoralens do not constitute a magic bullet for the therapy of vitiligo, they are still being used as chemotherapeutic agents on a major scale. In Egypt and in America, 8-MOP has been applied topically or orally with UV irradiation, later known as PUVA therapy (*195*). In India, psoralen and 8-MOP have been used without UV irradiation in many cases of vitiligo, though the effect of sunlight after psoralen application has not been ruled out. Psoralen is considered a better therapeutic agent against vitiligo than 8-MOP because its lower toxicity requires lower doses (*46*, *238*). Trimethylpsoralen (**27**) has, however, been claimed to be better than either by Sehgal (*238*). The availability of pure psoralen in India and abroad is limited for want of standard plant materials.

5.3.2. Psoralen Action and UV Light

Furocoumarins are photodynamic substances which evoke various biological activities after photochemical activation. Thus, when psoralen is applied topically to mammalian skin and irradiated with light, it causes

formation of erythema and pigmentation. According to Pathak *et al.* (*196*) and Musajo *et al.* (*178*), the pigmentation caused by the psoralen group of drugs is due to photoactivation of the chemical species derived from these drugs. ESR studies have indicated that excitation of the psoralen molecule to the triplet state may lead to free radical formation, which most probably causes biological changes in the irradiated system (*196*).

Kinetic studies of the photoreaction between psoralen and DNA have been carried out. Cole (*63*) reported that interstrand cross-links are formed in native DNA by irradiation with 360 nm light in the presence of psoralen, and suggested that the cross-links may result from the reaction of an excited psoralen molecule with pyrimidine bases in opposite strands of the DNA duplex.

5.3.3. Psoralen Action on Melanogenesis

Pigmentation induced by psoralen drugs plus UV irradiation is evidently due to an increase of functioning melanocytes, increase in the number of melanosomes, increase in the activity of the enzyme, and in the transfer of melanin granules to malpighian cells (*196*).

Psoralen regenerates melanin on vitiliginous spots (*228*) and Chakraborty *et al.* (*43*) reported that psoralen, when applied topically and orally to *Bufo melanostictus*, enhanced melanin formation by accelerating the activity of the melanogenic enzyme tyrosinase (*43*). These results are consistent with the reciprocal relationship between melanization and tyrosinase activity in melanosomes, *i.e.* melanin granules, reported by Seiji *et al.* earlier (*240*). Tyrosinase activation with 8-MOP on vitiliginous skin was also reported by Rudowska (*223*). Lerner *et al.* (*146*), however, found that 8-MOP is without any effect on tyrosinase activation in isolated skin sections of *Xenopus leivis*. On toads, psoralen was more effective orally than topically which simulates the results when psoralen is applied to the skin of human vitiliginous subjects. The use of amphibian subjects like *Bufo melanostictus* as an experimental model is logical, based on observation of Burger and Van Oordt (*31*), as well as on the use of these species by other workers in their research on melanogenesis (*97*, *159*).

Studies of hypophysectomized todes by Indian workers revealed that psoralen in the absence of light stimulates tyrosinase activity in the skin and liver of toads (*13*, *14*). Carter *et al.* (*34*) found a 2- to 3-fold increase in tyrosinase activity as compared to psoralen alone after UV irradiation of Cloudman melanoma containing TMP. Borkovic *et al.* (*20*), on the basis of their experimental results on melanization with TMP, suggested that TMP may be used in a clinical setting without UV radiation for the

regeneration of melanin on vitiliginous spots. Stimulation of melanogenesis with the psoralen group of drugs in the absence of UV light was also reported by other workers (*45*, *159*, *172*, *228*).

It is pertinent here to mention that psoralen exhibits some biological functions which do not require photoactivation such as growth inhibitory properties (*245*), estrogenic activity (*6*), antifungal activity (*42*) and antibiotic activity (*44*). All these observations may be cited to support the concept of Chakraborty (*45*) that psoralen augments melanogenesis through deep seated biochemical events, even without UV irradiation, although additive or synergistic effects of photoactivation are not ruled out.

5.4. Abnormal Biochemical Parameters in Vitiligo

Since none of the hypothetical concepts, acquired or congenital, can totally explain the genesis of vitiligo and since it is well known that depletion of skin melanin is the main criterion for diagnosis of the disease, to get a better understanding of the etiology of the disease, a search for

Table 1. Abnormal biochemical parameters in vitiligo

Parameters	Vitiliginous subjects	References
1. Urinary indole profile	Larger excretion of 5-hydroxyindole acetic acid, 5-hydroxy tryptamine, and kynurenine are consistently present but indican is absent	*48*, *135*, *229*
2. Urinary anthranilic acid, 3-hydroxy anthranilic acid	Higher than normal	*48*, *229*
3. Blood- and skin-SH constant	Higher than normal	*135*
4. Skin lead and tin level	Increased	*50*, *263*
5. Skin nickel, cobalt, and copper level	Insignificant change after depigmentation	*50*
6. Urinary hydroquinone level	Decreased	*40*, *192*
7. Serum tyrosinase	Slightly increased	*39*, *49*
8. Serum tryptophan pyrrolase (TP) and indoleamine-2,3-dioxygenase (IOD) in skin and lever (dioxygenase activity)	Increased	*37*, *39*, *49*
9. Tyrosine aminotransferase (TAT), Tryptophan pyrrolase (TP), and corticosteroids (Parameters of stress conditions)	Increased	*52*, *148*, *203*, *205*

References, pp. 171–185

some biochemical abnormalities associated with the depigmentation in vitiligo was carried out. However, no definite biochemical abnormalities can be considered characteristic of vitiligo skin and it seems most likely that these scattered abnormalities are secondary to the primary process in vitiligo. In most cases reversal of these conditions has been observed during regeneration of pigment with psoralen.

According to Chakraborty *et al.* (*229*), these findings (Table 1) indicate the involvement of abnormal tryptophan metabolism in vitiligo. Lerner (*148*) suggested that stress conditions which are associated with higher TP, TAT, and corticosteroid levels in biological systems (*203*) could be a factor in the precipitation of vitiligo. Further, the activities of dioxygenases, like TP, IOD, associated with vitiligo are superoxide anion (a toxic species of oxygen) dependent (*84*). Hence, overproduction of this reactive oxygen species by varieties of cell stresses may be involved to play an etiological role in vitiligo (radical hypothesis) (*22*, *71*, *165*, *219*).

5.5. Status of Tryptophan in the Melanogenic System

The above results together with previous findings suggest that tryptophan could also be an alternative substrate for melanin synthesis. Chen and Chavin (*60*) used tryptophan as a substrate for goldfish tyrosinase while the incorporation of tryptophan in the melanin of Harding Passey mouse melanoma cells was reported by other authors (*67*). Tryptophan in a true melanin synthesizing system, *i.e.* in B16–F10 melanoma cells cultured in the presence of 2 *mM* tryptophan, increased melanin formation on the second day (*41*).

The conventional substrates, tyrosine and dopa, can stimulate tyrosinase activity as well as melanization of the cells (*166*, *206*, *248*, *249*). Positive regulation of tyrosinase by its precursors *in vivo* that require active protein synthesis was shown for the first time by Slominski *et al.* (*246*, *247*). Both L-tyrosinase and L-dopa stimulate tyrosinase at the level of translation (*248*). Depending on dose and time, L-dopa can both stimulate and inhibit tyrosinase mRNA expression (*249*). Recently, it has been found that tryptophan can stimulate new mRNA-dependent tyrosinase synthesis in B16 murine melanoma cells (*38*).

It has also been observed that tryptophan, like dopa, inhibits tyrosine hydroxylase and dopa oxidase activity of melanosomal tyrosinase and that its inhibitory mechanism differs from inhibition caused by non-substrate type compounds like cysteine and ascorbic acid (*36*). In fact, tyrosinase is inhibited by its own substrate *in vitro* and this inhibition mechanism differs from that caused by cysteine and ascorbic acid (*242*, *268*).

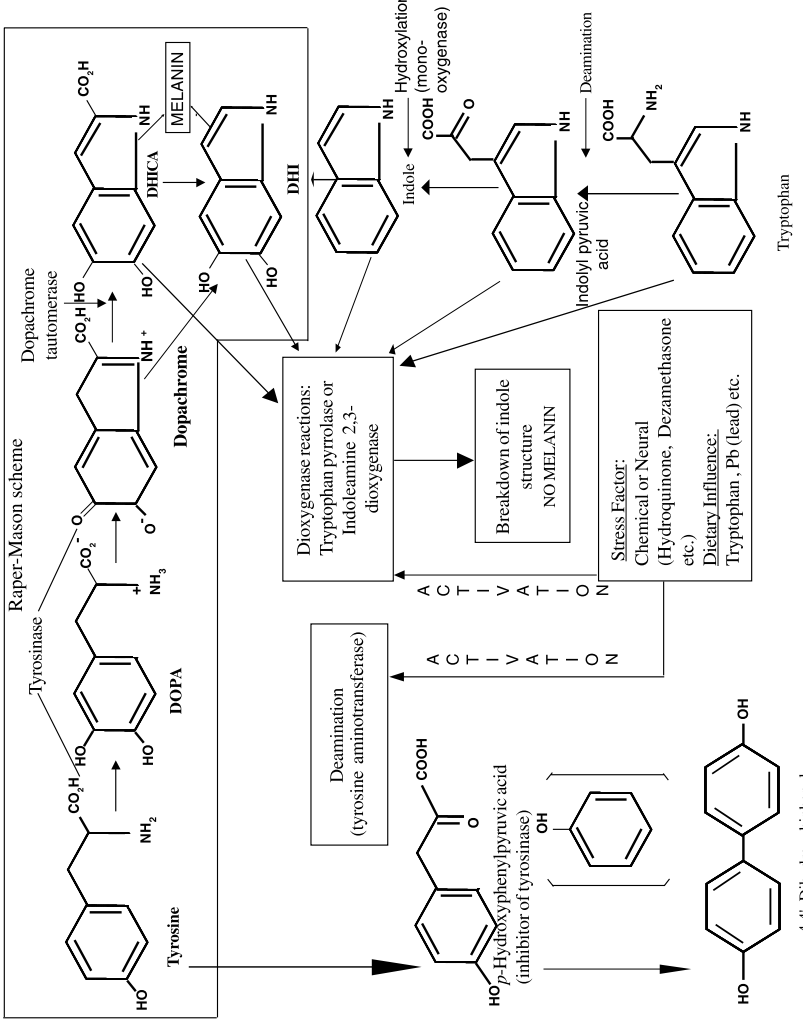


Fig. 8. Combined tyrosine and tryptophan participation for the synthesis of melanin and its catabolism in relation to vitiligo (modification of the Raper-Mason scheme)

All these results show the close resemblance of tryptophan to tyrosine and dopa, with respect to their role as melanin precursors as well as positive regulators of tyrosinase.

5.6. A Composite Hypothesis on Vitiligo

Because both tryptophan and tyrosine participate in melanogenic processes, the possible influence of different enzymes like tyrosinase, tryptophan pyrrolase (TP), indoleamine 2,3-dioxygenase (IOD), and tyrosine aminotransferase (TAT) on the regulation of melanin synthesis as it relates to vitiligo has been depicted in Fig. 8, which constitutes a modification of the Raper-Mason scheme for melanin synthesis (*53*, *54*).

Also in view of the results discussed earlier, it seems that inhibitors like hydroquinones, dexamethasone may increase the amount of catabolic enzymes associated with stress conditions as well as the *in vivo* release of phenyl radicals, facilitating depigmentation (*15*, *220*). Stress not only releases inhibitors of tyrosinase, *e.g.* catecholamines, but also induces TAT *in vivo* (*1*) causing deamination of tyrosine and yielding *p*-hydroxyphenylpyruvic acid, which is an inhibitor of tyrosinase. Stress has also a great effect on the immune system (*127*) and on brain-immune system interaction as well (*179*).

Therefore, the conjecture that foreign inhibitory substances introduced through diet (including nutritional factors) or otherwise may have a role in precipitating and promoting conditions favoring vitiligo may have some validity. The experimental results further suggest that autocytotoxic substances and also superoxide-mediated cell damage (*22*) may be factors in the impairment of melanogenic processes in vitiligo by immunologic or neurologic mediation (*29*), thus supporting the composite hypothesis (*144*, *189*) for the pathogenesis of vitiligo.

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