Studies on Fungal Products. Part 19.[1] Isolation and Structure of a Novel Indoloditerpene, Emindole SA, from *Emericella striata*

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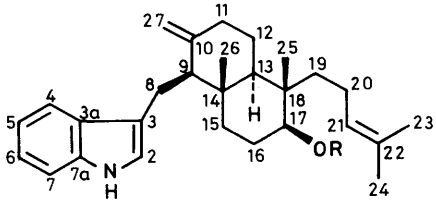
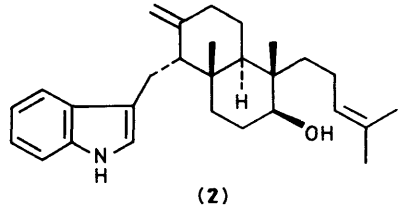
A novel compound designated emindole SA (**1**), $C_{28}H_{39}NO$, isolated from the mycelium of *Emericella striata*, was identified by spectroscopic and chemical investigation. Emindole SA is a novel type of indoloditerpene and is a diastereoisomer of emindole DA (**2**), isolated from *Emericella desertorum*.

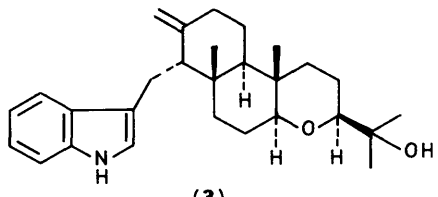
Recently we reported[2] the isolation of a new type of indoloditerpene, represented by emindoles DA (**2**) and DB (**3**), from the mycelium of *Emericella desertorum* Samson & Mouchacca, strain CBS 653.73. Paxilline (**4**), an another type of indoloditerpene, was also isolated from this fungus as a minor component[3] and from *Emericella striata* (Rai, Tewari, & Mukerji) Malloch & Cain, Strain 80–NE–22, as one of the main components.[4] In the course of a subsequent search for compounds related to (**2**)—(**4**), a novel indoloditerpene (**1**), designated emindole SA,[5] was isolated from the mycelial extract of the same strain of *E. striata*. The identification of emindole SA (**1**) is reported here.



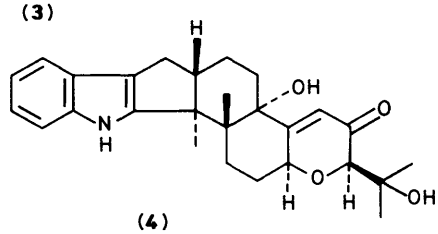
(**1**) R = H
(**5**) R = Ac
(**6**) R = COCH(Ph)Et



(**2**)



(**3**)



(**4**)

Results and Discussion

Emindole SA (**1**), an amorphous powder, $[\alpha]_D$ +32.0°, gave a molecular ion at *m/z* 405 in electron impact (e.i.) and at *m/z* 406 in field desorption (f.d.) mass spectrometry. On acetylation, it afforded a monoacetate (**5**), m.p. 102 °C, $C_{30}H_{41}NO_2$. The molecular formula of (**1**) was confirmed as $C_{28}H_{39}NO$, the same as that of emindole DA (**2**), by high resolution e.i. mass spectrometry of (**1**) and elemental analysis of (**5**). A positive colouration with Ehrlich's reagent (brownish green)[6] and a prominent fragment ion at *m/z* 130 $[(C_9H_8N)^+]$ in the e.i. mass spectrum of (**1**) suggested the presence of an indolylmethyl group. ^{1}H N.m.r. signals of four aromatic protons of the indole moiety in (**1**) appeared at δ 7.113, 7.167, 7.313, and 7.620, similar to those of emindole DA (**2**) (Table). The ^{1}H n.m.r. signal at δ 6.893 for (**1**), and that at δ 6.887 for (**2**), were assigned to 2-H of the indole moiety. These results confirmed the presence of an indol-3-ylmethyl group in (**1**) as well as in (**2**). The ^{13}C n.m.r. signals of the indol-3-ylmethyl group in (**1**) were closely similar to those of (**2**) (Table).

Emindole SA acetate (**5**) showed ^{1}H n.m.r. signals at δ 2.046 assigned to the methyl protons of an aliphatic acetoxy group. The C*H*OH signal at δ 3.597 (1 H, dd) of (**1**) was shifted downfield to δ 4.862 (1 H, dd) after acetylation. These results suggested the presence of one secondary alcohol group in emindole SA (**1**). ^{1}H N.m.r. signals at δ 1.606 (3 H, br s), 1.677 (3 H, br s), and 5.094 (1 H, br t) corresponded to ^{13}C n.m.r. signals at δ 21.59 (t), 124.76 (d), 131.18 (s), 17.65 (s), and 25.70 (s). These were assigned to a 3-methylbut-2-enyl group. The signals of two aliphatic tertiary methyl groups (Table) were observed at δ 0.812 and 0.873 in the ^{1}H n.m.r. spectrum and at δ 16.94 and 14.98 in the ^{13}C n.m.r. spectrum, respectively (Table). Signals at δ 4.840 and 4.719 were assigned to the exocyclic methylene group. Other ^{1}H n.m.r. signals were due to aliphatic methylene and methine groups. It was clear that the functional groups of emindole SA (**1**) were the same as those of emindole DA (**2**).

The planar structure of emindole SA (**1**) was confirmed mainly by the two-dimensional (2D) homonuclear ^{1}H–^{1}H and heteronuclear ^{1}H–^{13}C correlation spectra (Figures 1 and 2). It is clear that (**1**) is a diastereoisomer of emindole DA (**2**). The ^{1}H and ^{13}C n.m.r. signals (Table) were closely similar, except for nuclei near C-9.

The stereochemistry of (**1**) was determined from 2D homonuclear ^{1}H–^{1}H nuclear Overhauser effect (n.O.e.) correlation spectra (Figure 3). N.O.e.s were observed between the proton bearing the hydroxy group (17-H) and 13-H and between 13-H and 9-H. This suggested that 13-H was in a 1,3-diaxial relationship with 17-H and 9-H. An n.O.e. was observed between one of the two protons at C-16 (consequently assigned as an axial proton) and both 25-H (Me) and 26-H (Me), and also between 26-H and one of the two protons at C-8. Therefore each of the two methyl groups (25-H and 26-H) was in a 1,3-

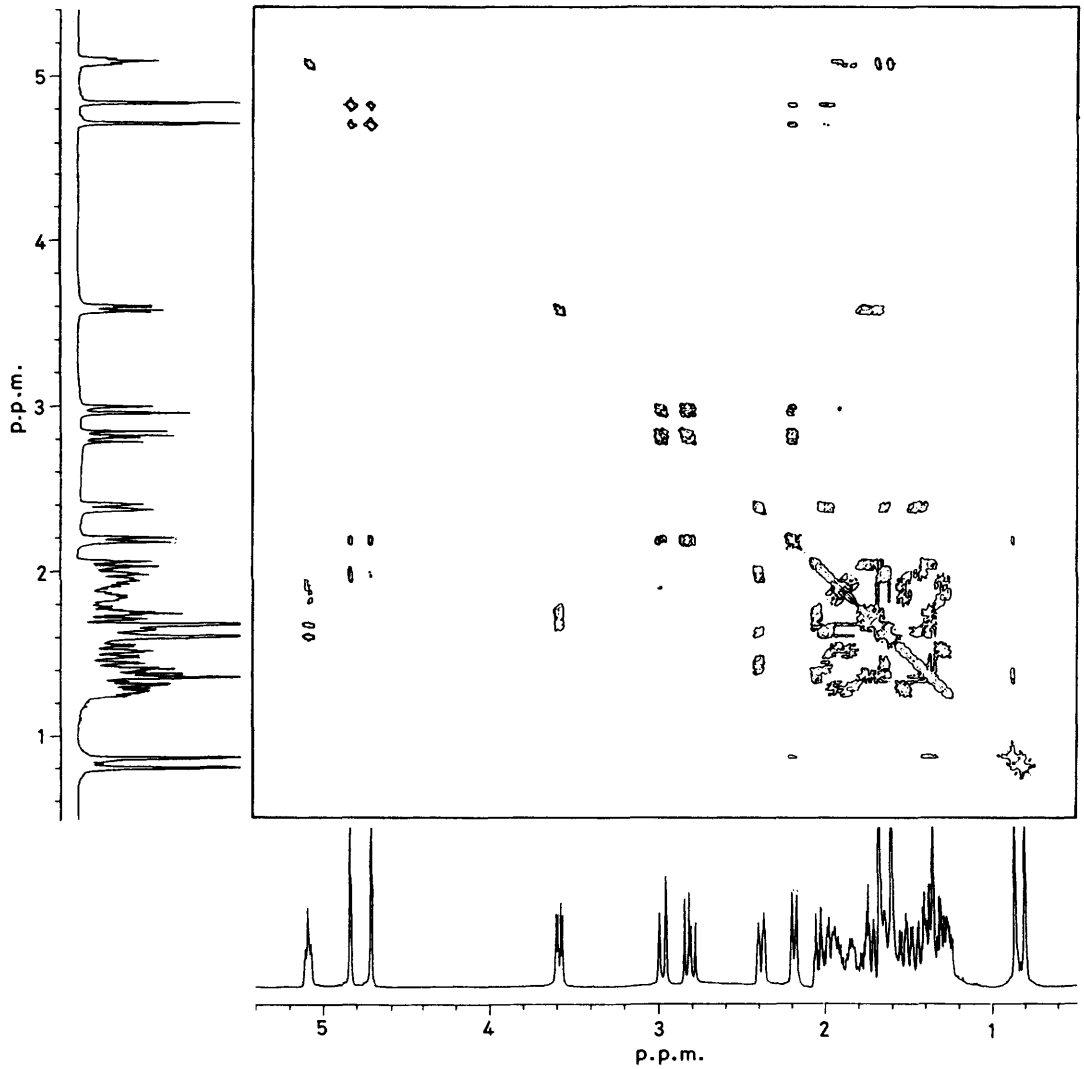


Figure 1. 2D ^1H–^1H Shift correlation spectrum of emindole SA (**1**) in the region δ_H 0.6—5.4

Table. N.m.r. chemical shifts (^1H and ^{13}C) of emindoles SA (**1**) and DA (**2**) in $CDCl_3$

	(1)		(2)			(1)		(2)	
Locant	δ_C	δ_H	δ_C	δ_H	Locant	δ_C	δ_H	δ_C	δ_H
1(NH)		7.882		7.866	15	37.39	2.050	34.58	1.325
2	121.69	6.893	121.90	6.887			1.35—1.43[a]		1.94—2.02
3	116.23		115.57		16	27.98	1.63—1.79	27.75	1.73—1.80
3a	127.66		127.64				1.63—1.79		1.73—1.80
4	118.70	7.620	118.88	7.563	17	73.27	3.597	73.95	3.618
5	119.06	7.113	118.94	7.087	18	41.36		41.12	
6	121.78	7.167	121.63	7.162	19	37.52	1.24—1.33	37.54	1.34—1.40
7	111.02	7.313	111.00	7.325			1.47—1.56		1.51—1.59
7a	147.94		148.10		20	21.59	1.80—1.95	21.89	1.96—2.06
8	19.63	2.983	23.27	3.132			1.80—1.95		1.96—2.06
		2.817		2.700	21	124.76	5.094	124.94	5.162
9	56.79	2.196	58.61	2.064	22	131.18		131.21	
10	136.13		136.25		23(Me)	17.65	1.606	17.70	1.671
11	37.92	1.987	30.83	2.284	24(Me)	25.70	1.677	25.74	1.715
		2.389		2.180	25(Me)	16.94	0.812	16.91	0.827
12	23.74	1.59—1.66	23.03	1.429	26(Me)	14.98	0.873	23.18	0.984
		1.36—1.47[a]		1.58—1.65	27	107.94	4.840	110.20	4.510
13	48.94	1.353	39.07	1.73—1.76			4.719		4.156
14	39.63		37.92						

[a] Assignments may be reversed.

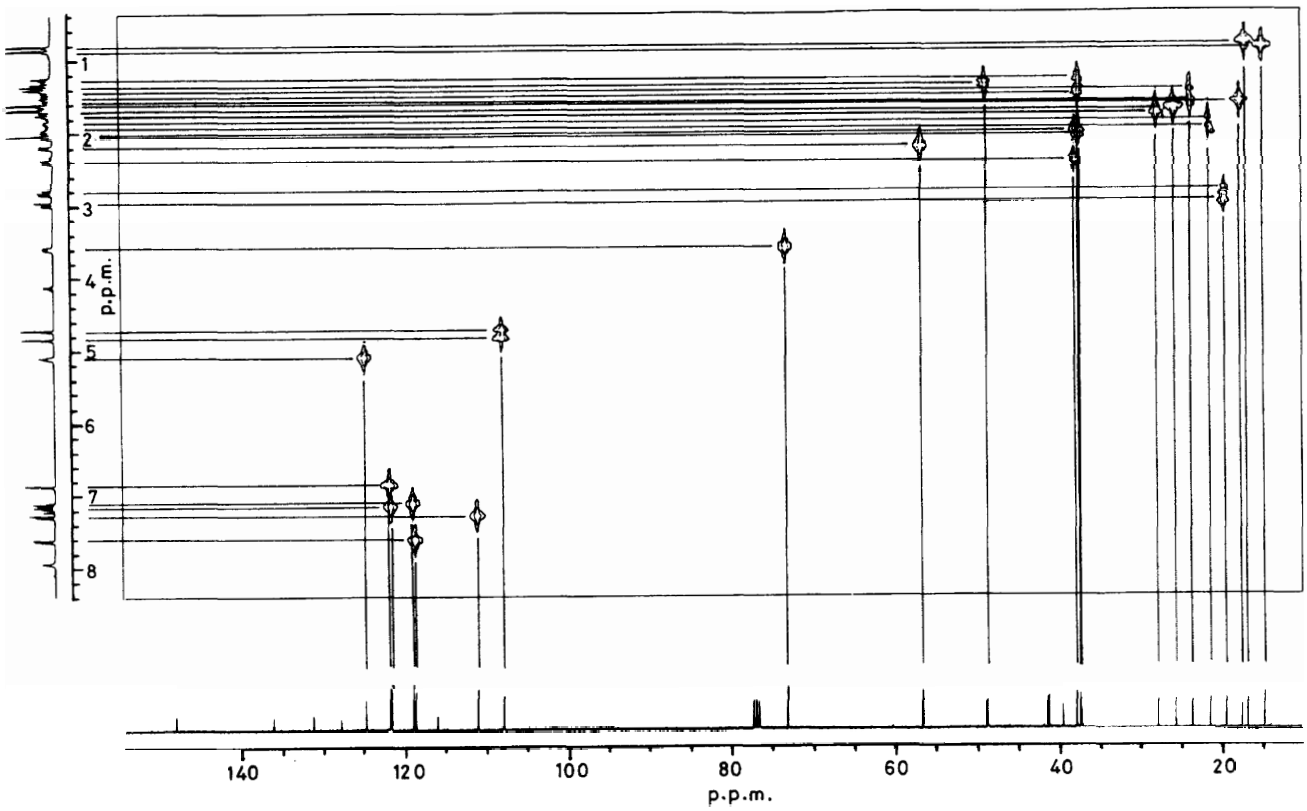
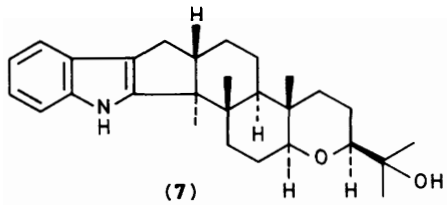


Figure 2. 2D ^{1}H–^{13}C Shift correlation spectrum of emindole SA (**1**)

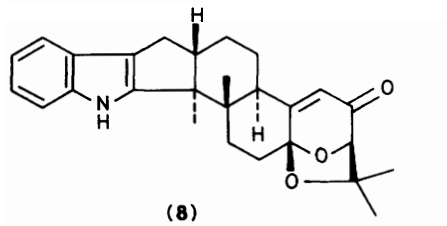
diaxial relationship with the axial proton at C-16. Furthermore, the C-26 carbon signal (δ 14.98) in (**1**) was observed to change into a double doublet ($^{3}J_{C,H}$ 6.1 and 5.3 Hz) on selective irradiation at the frequency of both 9-H (δ 2.196) and the equatorial proton at C-15 (δ 2.050), and the C-25 methyl carbon signal (δ 16.94) was observed to change into a double doublet ($^{3}J_{C,H}$ 5.3 and 4.6 Hz) on selective irradiation at the frequency of the two protons at C-19 (δ 1.285 and 1.515). These facts confirmed that the methyl groups at C-25 and C-26 in (**1**) were both in a *trans*-diaxial relationship with 13-H, in view of the C,H-coupling constants. From the foregoing results, the relative stereochemistry of emindole SA was confirmed as that shown (**1**).

The chirality of the 17-hydroxy group in emindole SA (**1**), and thus the absolute configuration of (**1**), was determined by the 'partial resolution' method of Horeau.[7] Esterification of (**1**) with racemic 2-phenylbutyric anhydride and pyridine proceeded smoothly, leading quantitatively to the 17-(2-phenylbutyrate) (**6**). The recovered 2-phenylbutyric acid had $[\alpha]_D$ −9.0° (in benzene). Emindole SA (**1**) must therefore have the 17*S*-configuration[7,8] and consequently the absolute configuration was confirmed as that depicted (**1**).

It is notable that the C-9 stereoisomers (**1**) and (**2**)[2] were isolated as major components from fungi in the same genus. Compounds (**1**)—(**3**) are representatives of a new type of indoloditerpene and have basically the same configurations as paxilline (**4**). Acklin *et al.* have reported[9] that the indoloditerpenes paspaline (**7**) and pasplicine (**8**), tremorgenic mycotoxins isolated from *Claviceps paspali* Stevens & Hall,[10] are derived from tryptophan and geranylgeraniol, and that the migration of the carbon skeleton occurred through a five-membered ring intermediate in the course of the biosynthesis (Figure 4). The isolation of emindole SA (**1**) along with paxilline



(**7**)



(**8**)

(**4**) from *E. striata* suggested that there are two cyclization pathways for the diterpene moiety in this fungus at an earlier stage of biosynthesis. One pathway would give (**3**) through a route similar to that just described, and the other would give the new type of indoloditerpene [emindole SA (**1**)] by direct cyclization without migration of the carbon skeleton (Figure 4).

Experimental

M.p.s were determined with a Yanagimoto micro apparatus. Optical rotations were measured with a JASCO DIP-181 spectrometer. E.i. and f.d. mass spectra were taken with a JEOL JMS-D 300 spectrometer. U.v. spectra and i.r. spectra were recorded with a Hitachi 124 spectrophotometer and a Hitachi 215 and/or JASCO IR-810 spectrophotometer, respectively.

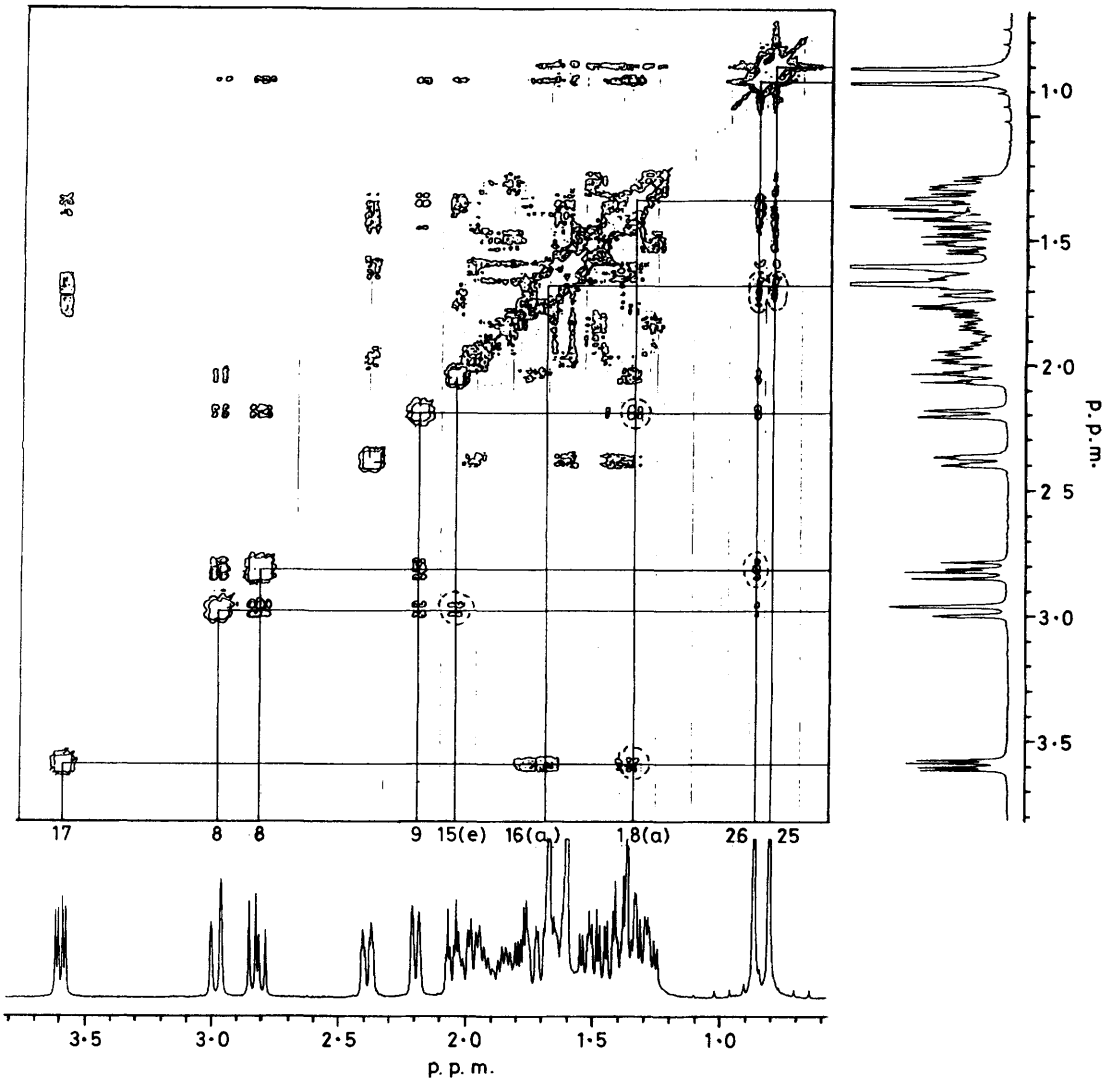


Figure 3. 2D ^1H–^1H N.O.e. correlation spectrum of emindole SA (**1**) in the region δ_H 0.6—3.8; dashed circles indicate n.O.e.-correlated peaks

N.m.r. spectra [^1H (399.65 MHz) and ^{13}C (100.40 MHz)] were taken with a JEOL JNM-GX 400 spectrometer (tetramethylsilane as internal standard). C.d. curves were determined with a JASCO J-40 spectrophotometer. Column chromatography was performed on Kieselgel 60 (Art. 7734; Merck). Low pressure liquid chromatography (l.p.l.c.) was performed with a Chemco Low-Prep pump 81-M-2 and glass column (30—50 μm; Wako). T.l.c. was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck). Spots on t.l.c. plates were detected by their absorption under u.v. light, and/or by spraying with Ehrlich's reagent.

Isolation of Emindole SA (**1**) *from* Emericella striata.—*Emericella striata*, strain 80–NE–22, was cultivated at 30 °C for 21 days in Czapek-Dox medium (50 l). The dried mycelia (660 g) were pulverized and extracted with acetone at room temperature. The extract (26 g) was chromatographed on silica gel with benzene–acetone (100:1 v/v). Part of this fraction was purified by l.p.l.c. [hexane–ethyl acetate (8:1, v/v)] to give emindole SA (**1**) (106 mg). Since compound (**1**) was not very stable, the other part was purified after acetylation (described later) by l.p.l.c. in benzene to obtain emindole SA acetate (**5**) (1.06 g).

Emindole SA (**1**) was obtained as an amorphous powder, m.p. 58—60 °C; $[\alpha]_D^{15}$ +32.0° (*c* 0.79 in MeOH); *m/z* 405.303 [M^+ ($C_{28}H_{39}NO$) requires 405.303, 4%, e.i.], 387 [$(M - H_2O)^+$, 5], and 130.066 [$(C_9H_8N)^+$ requires 130.066, 100]; *m/z* 406 [$(M + 1)^+$, 100%, f.d.]; $\lambda_{max.}$(MeOH) 224 (log ε 4.62), 276sh (3.76), 283 (3.81), and 291 nm (3.78); $\nu_{max.}$(KBr) 3 550, 3 420, 3 300 (OH, NH), 1.450, and 740 cm^{-1}; $\delta_H(CDCl_3)$ 0.812 [3 H, s, 25-H (Me)], 0.873 [3 H, s, 26-H (Me)], 1.24—1.33 (1 H, m, 19-H), 1.353 (1 H, m, 13-H), 1.35—1.43 (1 H, m, 15- or 12-H), 1.36—1.47 (1 H, m, 12- or 15-H), 1.47—1.56 (1 H, m, 19-H), 1.59—1.66 (1 H, m, 12-H), 1.606 [3 H, br s, 23-H (Me)], 1.677 [3 H, br s, 24-H (Me)], 1.63—1.79 (2 H, m, 16-H), 1.80—1.95 (2 H, m, 20-H), 1.987 (1 H, br dd, *J* 13.9 and 5.3 Hz, 11-H), 2.042 (1 H, s, 17-OH), 2.050 (1 H, ddd, *J* 13.8, 3.4, and 3.4 Hz, 15-H), 2.196 (1 H, br d, *J* 10.7 Hz, 9-H), 2.389 (1 H, ddd, *J* 13.9, 2.5, and 2.5 Hz, 11-H), 2.817 (1 H, dd, *J* 15.4 and 10.7 Hz, 8-H), 2.983 (1 H, br d, *J* 15.4 Hz, 8-H), 3.597 (1 H, dd, *J* 11.4 and 4.8 Hz, 17-H), 4.719 (1 H, br s, 27-H), 4.840 (1 H, br s, 27-H), 5.094 (1 H, br t, *J* 6.9 Hz, 21-H), 6.893 (1 H, d, *J* 2.2 Hz, 2-H), 7.113 (1 H, br dd, *J* 8.1 and 7.4 Hz, 5-H), 7.167 (1 H, br dd, *J* 8.1 and 7.4 Hz, 6-H), 7.313 (1 H, br d, *J* 8.1 Hz, 7-H), 7.620 (1 H, br d, *J* 8.1 Hz, 4-H), and 7.882 [1 H, br s, 1-H (NH)] (^{13}C n.m.r. signals in the Table).

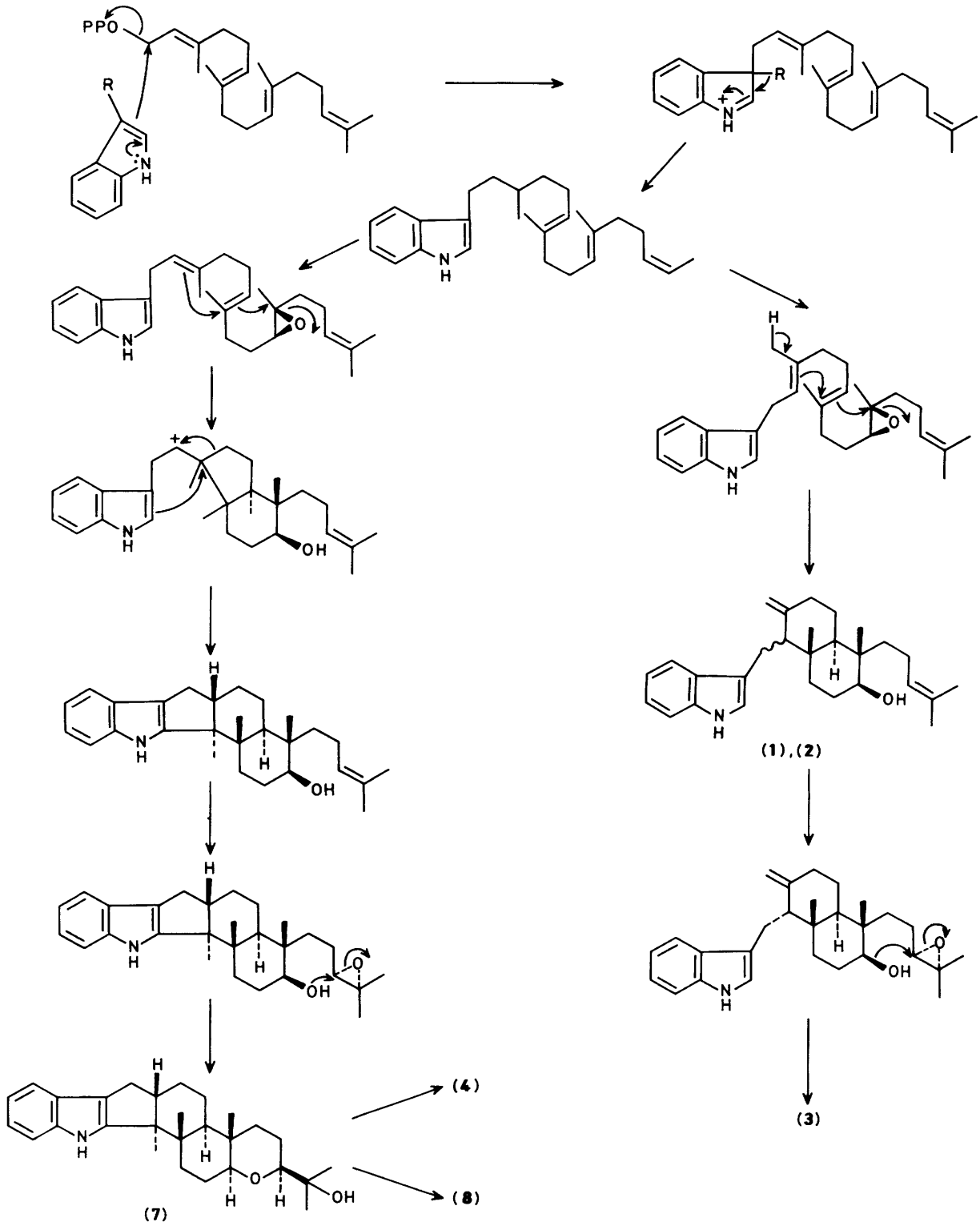


Figure 4. Possible biosynthetic pathway for emindoles and paxilline

Acetylation of Emindole SA (**1**).—Emindole SA (**1**) (150 mg) dissolved in pyridine (0.5 ml) containing acetic anhydride (0.5 ml) was kept overnight at room temperature. The mixture was poured into ice–water and extracted with chloroform. The extract was evaporated and the residue purified by l.p.l.c. (benzene) to afford *emindole SA monoacetate* (**5**) (140 mg) as leaflets (from methanol), m.p. 102 °C (Found: C, 78.9; H, 9.2; N, 3.05. $C_{30}H_{41}NO_2$·0.5H_2O requires C, 78.9; H, 9.3; N, 3.05%); *m/z* 477 (M^+, 8%, e.i.) and 130 [$(C_9H_8N)^+$, 100]; $\lambda_{max.}$(EtOH) 224 (log ε 4.61), 275sh (3.86), 282 (3.88), and 290sh nm (3.85); $\nu_{max.}$(KBr) 3 420, 3 330 (NH), 1 715, 1 695 (OAc), 1 450, 1 265, and 735 cm^{-1}; $\delta_H(CDCl_3)$ 0.892 [3 H, s, 25- or 26-H (Me)], 0.897

[3 H, s, 26- or 25-H (Me)], 1.13—1.31 (2 H, m), 1.40—1.67 (4 H, m), 1.568 [3 H, br s, 23-H (Me)], 1.659 [3 H, br s, 24-H (Me)], 1.68—1.89 (3 H, m), 1.89—2.10 (3 H, m), 2.046 (3 H, s, 17-OAc), 2.219 (1 H, br d, *J* 10.5 Hz, 9-H), 2.381 (1 H, ddd, *J* 11.2, 3.2, and 3.2 Hz, 11-H), 2.827 (1 H, dd, *J* 15.4 and 10.5 Hz, 8-H), 2.980 (1 H, br d, *J* 15.4 Hz, 8-H), 4.735 (1 H, br s, 27-H), 4.842 (1 H, br s, 27-H), 4.862 (1 H, dd, *J* 11.5 and 4.9 Hz, 17-H), 5.020 (1 H, br t, *J* 7.1 Hz, 21-H), 6.903 (1 H, d, *J* 2.2 Hz, 2-H), 7.130 (1 H, ddd, *J* 7.9, 7.9, and 1.0 Hz, 5-H), 7.176 (1 H, ddd, *J* 7.9, 7.6, and 1.2 Hz, 6-H), 7.326 (1 H, br d, *J* 7.6 Hz, 7-H), 7.623 (1 H, br d, *J* 7.9 Hz, 4-H), and 7.867 [1 H, br s, 1-H (NH)].

Absolute Configuration of Emindole SA (**1**).—A solution of emindole SA (**1**) (30 mg, 0.074 mmol) and 2-phenylbutyric anhydride (0.148 mmol) in pyridine (0.40 ml) was kept at room temperature overnight. The excess of anhydride was destroyed by adding water (0.05 ml) and stirring the mixture vigorously for 30 min. The mixture was neutralized with M/11 NaOH (2.4 ml) and extracted with chloroform. The organic phase was washed with water, dried (Na_2SO_4), and evaporated. The residual ester (**6**) contained no starting material (t.l.c.).

The combined aqueous sodium hydroxide layer was acidified with 4M HCl and extracted with dichloromethane to yield 2-phenylbutyric acid (36 mg), $[\alpha]_D^{20}$ −9.0° (*c* 1.79 in benzene) (theoretical $[\alpha]_D$ −32.1°).[8] The optical yield therefore was 28% (−), on the basis of an esterification yield of 100%.

Acknowledgements

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References

1 Part 18, N. Kawahara, S. Nakajima, M. Yamazaki, and K. Kawai, *Chem. Pharm. Bull.*, in the press.
2 K. Nozawa, S. Nakajima, K. Kawai, and S. Udagawa, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1689.
3 K. Nozawa, H. Seya, S. Nakajima, S. Udagawa, and K. Kawai, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1735.
4 H. Seya, K. Nozawa, S. Udagawa, S. Nakajima, and K. Kawai, *Chem. Pharm. Bull.*, 1986, **34**, 2411.
5 K. Nozawa, S. Udagawa, S. Nakajima, and K. Kawai, *J. Chem. Soc., Chem. Commun.*, 1987, 1157.
6 R. A. Heacock and M. E. Mahon, *J. Chromatogr.*, 1965, **17**, 338.
7 A. Horeau, *Tetrahedron Lett.*, 1961, 506.
8 A. Horeau and J. K. Sutherland, *J. Chem. Soc. C*, 1966, 247.
9 W. Acklin, F. Weibel, and D. Arigoni, *Chimia*, 1977, **31**, 63.
10 Th. Fehr and W. Acklin, *Helv. Chim. Acta*, 1966, **49**, 1907.

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