Studies on Fungal Products. Part 17.[1] Isolation and Structures of Novel Indoloditerpenes, Emindoles DA and DB, from *Emericella desertorum*: *X*-Ray Molecular Structure of Emindole DA Acetate

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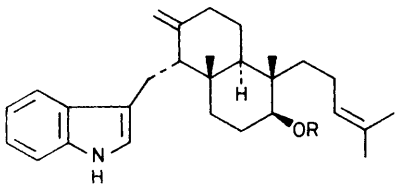
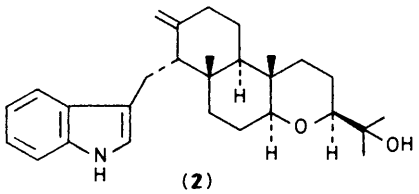
Together with tremorgenic mycotoxin, paxilline (**3**), and bicoumarins, desertorins A, B, and C, two new compounds designated emindoles DA (**1**), $C_{28}H_{39}NO$, and DB (**2**), $C_{28}H_{39}NO_2$, have been isolated from the mycelium of *Emericella desertorum*. The structures of emindoles DA (**1**) and DB (**2**) have been determined on the basis of the spectroscopic and chemical investigations of these compounds and an *X*-ray crystallographic study of emindole DA acetate (**4**). Emindoles DA (**1**) and DB (**2**) are novel indoloditerpenes biogenetically related to paxilline (**3**).

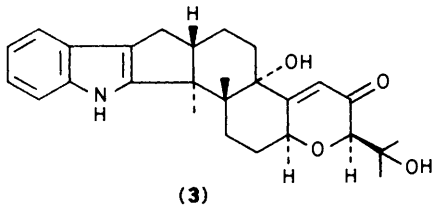
Recently we reported[2] the isolation of the novel bicoumarins, desertorins A, B, and C, as main components and the tremorgenic mycotoxin, paxilline (**3**), as a minor metabolite from the mycelium of *Emericella desertorum* Samson & Mouchacca, strain CBS 653.73. In the course of successive searching for the compounds related to (**3**) by the above fungus, two new indoloditerpenes (**1**) and (**2**) designated emindoles DA[3] and DB, respectively, were isolated from the mycelial hexane extract. The structural elucidation of emindoles DA (**1**) and DB (**2**) is mainly reported in this paper.



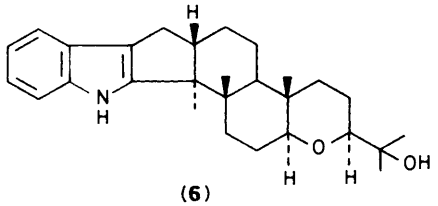
(**1**) R = H
(**4**) R = Ac
(**5**) R = $COCHCH_2CH_3$



(**2**)



(**3**)



(**6**)

Results and Discussion

The molecular formula of emindole DA (**1**), m.p. 146—147 °C, $[\alpha]_D$ −30.7°, and emindole DB (**2**), m.p. 165.5—166.5 °C, $[\alpha]_D$ −86.3°, were confirmed as $C_{28}H_{39}NO$ and $C_{28}H_{39}NO_2$, respectively, by electron impact ionization (e.i.) mass spectrometry and by elemental analyses. A positive colouration for Ehrlich's reagent (greenish red)[4] and the strong fragmentation ion at *m/z* 130 $[(C_9H_8N)^+]$ in the e.i. mass spectrum of (**1**) and (**2**) suggested the presence of an indol-3-ylmethyl group, *e.g.* a 3-substituted indole moiety, in both compounds. The ^1H n.m.r. signals for the four aromatic protons of the indole entity appeared at δ_H 7.087, 7.162, 7.325, and 7.563 for (**1**) and at δ_H 7.089, 7.163, 7.324, and 7.571 for (**2**). The ^1H n.m.r. signals at δ_H 6.887 for (**1**) and at δ_H 6.888 for (**2**) were characteristic of the α-H of the indole entity split by the indole NH.[5] The ^{13}C n.m.r. spectra of (**1**) and (**2**) (Table 1) were also well explained by the presence of an indol-3-ylmethyl entity.

On acetylation, emindole DA (**1**) afforded a monoacetate (**4**), m.p. 142.5—143.5 °C, $[\alpha]_D$ −9.0°, $C_{30}H_{41}NO_2$, which showed ^1H n.m.r. signals at δ_H 2.062 assigned to the methyl protons of an aliphatic acetoxy group. The ^1H n.m.r. signal of the proton attached to the carbon bearing an hydroxy group at δ_H 3.618 (1 H, dd) in (**1**) shifted downfield to δ_H 4.863 (1 H, dd) after acetylation. These results suggested the presence of one secondary alcohol in emindole DA (**1**). The ^1H n.m.r. signals at δ_H 1.671 (3 H, br s), 1.715 (3 H, br s), and 5.162 (1 H, br t) appeared in (**1**). These were assigned to $CH_2CH{=}C(Me)_2$. The signals of two aliphatic tertiary methyl groups appeared at δ_H 0.827 and 0.984. The signals at δ_H 4.156 and 4.510 were assigned as those of the *exo*-methylene protons of the double bond ($C{=}CH_2$). The other ^1H n.m.r. signals were due to aliphatic methylene and methine groups. Two dimensional (2D) homonuclear ^1H-^1H and heteronuclear ^1H-^{13}C shift correlation spectra suggested the structure of emindole DA as (**1**). The assignments of ^{13}C n.m.r. signals of (**1**) are summarized in Table 1.

In order to determine the exact structure of emindole DA (**1**) including its stereochemistry, an *X*-ray structure analysis of emindole DA acetate (**4**) was undertaken. Crystals of emindole DA acetate were grown in acetone solution as prisms. Two

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

Carbon No.[a]	(1)	(2)	(6)
C-2	121.90	121.87	150.90
C-3	115.57	115.60	118.32
C-3a	127.64	127.66	125.26
C-4	118.88	118.90[b]	118.46
C-5	118.94	118.93[b]	119.60
C-6	121.63	121.61	120.48
C-7	111.00	111.02	111.52
C-7a	148.10	148.03	140.16
C-8	23.27	23.29	14.63
C-9	58.61	58.85	53.09
C-10	136.25	136.26	48.87
C-11	30.83	30.74	27.61[b]
C-12	23.03	22.19[c]	22.04[b]
C-13	39.07	45.43	46.56
C-14	37.92	38.48	40.15
C-15	34.58	34.84	24.74[b]
C-16	27.75	21.95[c]	22.08[b]
C-17	73.95	84.75	84.81
C-18	41.12	36.25	36.65
C-19	37.54	24.85	25.39[b]
C-20	21.89	37.81	37.81
C-21	124.94	85.85	85.86
C-22	131.21	71.95	72.05
C-23	17.70	23.80[d]	23.85
C-24	25.74	26.14	26.18
C-25	16.91	13.45	12.77
C-26	23.18	23.85[d]	20.04
C-27	110.20	110.22	33.98

[a] Numberings of the related compounds correspond to those of (**1**). [b–d] The assignments may be reversed.

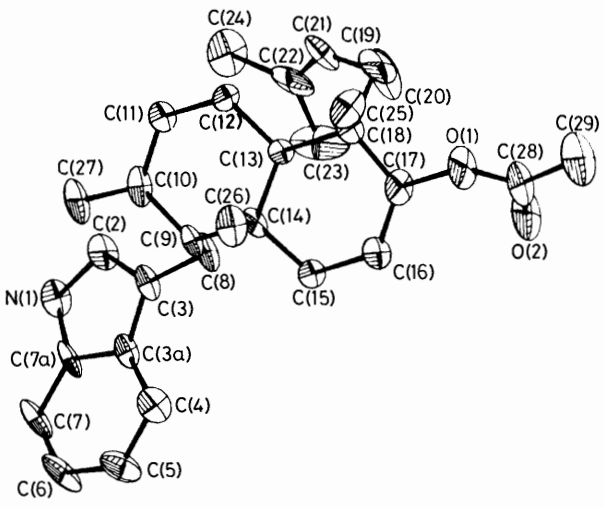


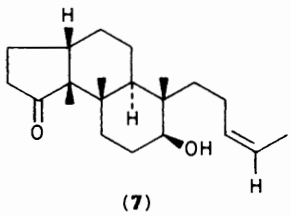
Figure Perspective view of one molecule of the crystal structure of emindole DA acetate (**4**) with thermal ellipsoids at 50% probability

molecules of (**4**) existed independently in an asymmetric unit. The relative structure of emindole DA acetate was established as shown in the Figure and in structure (**4**). The present *X*-ray analysis of (**4**) established the structure of emindole DA as (**4**) or its enantiomer in accordance with the chemical correlation. The bond lengths and angles for (**4**) are listed in Tables 2 and 3, respectively (see Experimental section). These values are not significantly different from the expected ones. The conformations of two molecules in the asymmetric unit are closely similar to each other. The deviations of the carbon atoms within the cyclohexane rings from the best planes formed by each ring (Table 4) (see Experimental section) indicate that both rings form slightly deformed chair forms. The molecules in the crystal are mainly packed through the van der Waals interactions; no hydrogen bonding was observed.

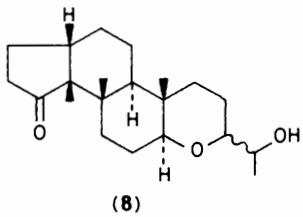
With the proposed relative configuration of emindole DA as shown in (**1**) (or its enantiomer), the chirality of the 17 hydroxy group, and thus the absolute configuration of emindole DA, was determined by the 'partial resolution' method of Horeau.[6] Esterification of (**1**) with racemic 2-phenylbutyric anhydride and pyridine, proceeded smoothly, leading quantitatively to the 17-*O*-2-phenylbutyrate (**5**). The mass spectrum of the ester (**5**) (M^+, 557) indicated the presence of monoester. The recovered 2-phenylbutyric acid had $[\alpha]_D^{20}$ −13.2° (benzene). Emindole DA (**1**) must, therefore, have the 17*S*-configuration[6,7] and, consequently, the absolute configuration as depicted in (**1**).

The molecular formula of emindole DB (**2**) has only one more oxygen atom than those of emindole DA (**1**), but (**2**) was not changed by acetylation. Therefore two oxygen functions of (**2**) must be tertiary alcohols and/or ethers. The ^1H n.m.r. signals of (**2**) were similar to those of (**1**), except for the following features: the olefinic methyl proton signals (δ_H 1.671 and 1.715) in (**1**) were shifted upfield (δ_H 1.178 and 1.199) in (**2**); appearance of two new signals assigned to the protons attached to the carbons bearing an oxygen function appeared at δ_H 3.065 and 3.231 in (**2**); disappearance of the olefinic proton (δ_H 5.162) and the proton attached to the hydroxy group bearing carbon (δ_H 3.618) in (**1**). The assignments of the ^{13}C n.m.r. signals of (**2**) (Table 1) were confirmed on the basis of the chemical shifts, the multiplicity of signals, and 2D homonuclear ^1H-^1H and heteronuclear ^1H-^{13}C shift correlation spectra. The carbon signals near the indole entity in (**2**) were closely similar to those in (**1**), whereas those around the tetrahydropyran ring in (**2**) were superimposable on those in paspaline (**6**).[8] The above results, considering the molecular formula of (**2**), suggested that cyclization occurred between the double bond and the hydroxy group of (**1**) to afford (**2**).

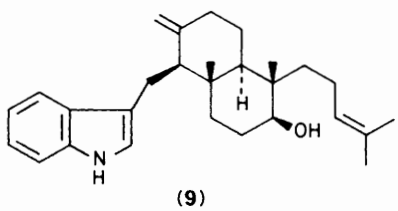
In the course of the synthesis of optically active paspaline (**6**), Smith and Mewshaw recently reported[9] that compound (**7**) was easily cyclized by treatment of *m*-chloroperbenzoic acid (MCPBA) in dichloromethane to give the pyranyl alcohol (**8**).



(**7**)



(**8**)



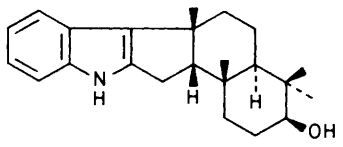
(**9**)

Thus, emindole DA (**1**) was treated with MCPBA by the same procedure to afford emindole DB (**2**) with the same optical rotation. Therefore, the structure of emindole DB was established as (**2**), except for the stereochemistry at C-21.

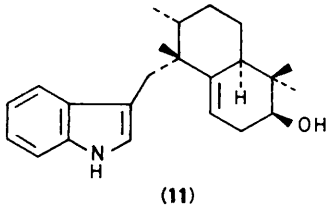
The two cyclohexane rings of emindole DA (**1**) were clearly in the chair conformation as evidenced by the results of the *X*-ray analysis as mentioned above. The three six-membered rings of

emindole DB (**2**) would also be in the chair form. Two protons attached to the carbons bearing the same secondary ether oxygen in (**2**) appeared at δ_H 3.065 (dd, *J* 11.6 and 4.0 Hz) and 3.231 (dd, *J* 12.0 and 2.9 Hz), which showed a nuclear Overhauser effect (n.O.e.) in the 2D homonuclear ^1H-^1H n.O.e. correlated spectrum of (**2**). The above fact indicated that both protons were attached in a 1,3-diaxial configuration to the tetrahydropyran ring according to Karplus' rule concerning the relation between vicinal couplings and dihedral angles.[10] Therefore the chirality of the carbon at C-21 was determined as that of *S*-configuration, and consequently the structure of emindole DB was confirmed as shown in (**2**) including the absolute stereochemistry.

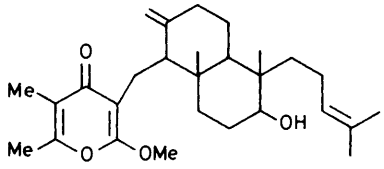
It is interesting that the stereoisomer at C-9 of emindole DA (**1**), emindole SA (**9**), which was reported in our preliminary paper,[3] was isolated from the fungus in the same genus: *Emericella striata*. Compounds (**1**), (**2**), and (**9**) are a new type of indoloditerpene and have basically the same configurations as (**3**) and (**6**), although the absolute structure of (**9**) has not been determined yet. Recently indolosesquiterpenes, *e.g.* polyveoline (**10**)[11] and polyalthenol (**11**),[12] were isolated from higher plants of *Annonaceae*: *Polyalthia suaveolens* Engl. & Diels and *Polyalthia oliveri* Engl., respectively. These compounds also have an indole entity which is directly bonded to a prenyl group. Kimura *et al.*[13] reported the isolation of colletochin (**12**) from a plant pathogenic fungus, *Colletotrichum nicotianae* Averna-Saccá, which had the same carbon skeleton as emindoles DA (**1**), DB (**2**), and SA (**9**). Compound (**12**) is the biosynthetic intermediate to a phytotoxin, colletotrichin (**13**), from *C. nicotianae*[14] and *Colletotrichum capsici* (Sydow) Butler & Bisby.[15]



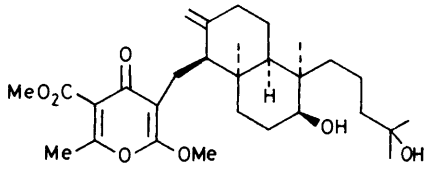
(**10**)



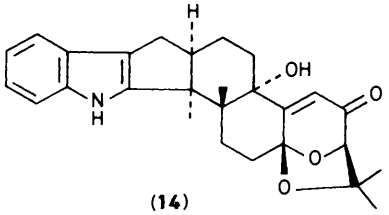
(**11**)



(**12**)



(**13**)



(**14**)

W. Acklin *et al.*[16] reported that indoloditerpenes such as paspaline (**6**) and paspalicine (**14**) were derived from tryptophan and geranylgeraniol in *Claviceps paspali* Stevens & Hall and that migration of the carbon skeleton resulted in an intermediate having a five-membered ring in the diterpene entity in close proximity in the indole entity during the biosynthesis. It is interesting that emindoles DA (**1**) and DB (**2**) were isolated along with paxilline (**3**), a minor metabolite, from *Emericella desertorum*, in consideration of the biogenesis of indoloditerpenes. There seems to be two pathways for the cyclization of the diterpene entity in *E. desertorum* at the earlier stage of biosynthesis. The minor pathway would give (**3**) *via* a route similar to that described above. The other may give the new type of indoloditerpene, emindoles DA (**1**) and DB (**2**), by direct cyclization and without the migration of the carbon skeleton. The details of the structural elucidation of emindole SA (**9**) will be published in the near future.

Experimental

M.p.s were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 spectrometer. E.i. mass spectra were taken with a JEOL JMS-D 300 spectrometer. U.v. spectra and i.r. spectra were recorded on a Hitachi 124 spectrophotometer and a Hitachi 215 spectrophotometer, respectively. ^1H (399.65 MHz) and ^{13}C (100.40 MHz). N.m.r. spectra were taken with a JEOL JNM-GX 400 spectrometer using tetramethylsilane as internal standard. C.d. curves were determined on a JASCO J-40 spectrophotometer. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low pressure liquid chromatography (l.p.l.c.) was performed on a Chemco Low-Prep pump 81-M-2 and glass column (200 × 10- or 20-mm) packed with silica gel CQ-3 (30—50 μ; 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 Ehrlich's reagent.

Isolation of Emindoles DA (**1**) *and DB* (**2**) *from* Emericella desertorum.—*Emericella desertorum*, strain CBS 653.73, was cultivated at 28 °C for 15 days in Czapek-Dox medium (50 l). The dried mycelia (395 g) were pulverized and extracted with hexane and chloroform successively. The mycelial hexane extract (24.0 g) was chromatographed on silica gel with benzene–ethyl acetate (3:1, v/v) to obtain two fractions. The less polar fraction was recrystallized from benzene–hexane to give emindole DA (**1**) (1.20 g), and the more polar fraction was rechromatographed on silica gel with benzene–ethyl acetate (25:1, v/v) to give emindole DB (**2**) (340 mg). From the fraction slightly less polar than ergosterol of the mycelial chloroform extract (15.0 g), emindole DA (**1**) (110 mg) was also isolated.

Emindole DA (**1**) was obtained as prisms (from benzene–hexane), m.p. 146—147 °C; $[\alpha]_D^{15}$ −30.7° (*c* 2.32 in MeOH) (Found: C, 83.1; H, 9.9; N, 3.4. $C_{28}H_{39}NO$ requires C, 82.9; H, 9.7; N, 3.5%); *m/z* 421 (M^+, 7%, e.i.) and 130 [$(C_9H_8N)^+$, 100]; $\lambda_{max.}$(MeOH) 224 (log ε 4.22), 277 sh (3.75), 283 (3.79), and 291 nm (3.75); $\nu_{max.}$(KBr) 3 550, 3 400, 3 280 (NH, OH), 1 450, 1 430, and 740 cm^{-1}; $\delta_H(CDCl_3)$ 0.827 (3 H, s, Me), 0.984 (3 H, s, Me), 1.28—1.50 (3 H, m), 1.50—1.66 (3 H, m), 1.671 [3 H, br s, $=C(Me)_2$], 1.715 [3 H, br s, $=C(Me)_2$], 1.73—1.80 (3 H, m), 1.93—2.10 (4 H, m), 2.180 (1 H, dd, *J* 12.7 and 3.2 Hz), 2.284 (1 H, ddd, *J* 13.4, 13.1, and 5.4 Hz), 2.700 (1 H, dd, *J* 14.4 and 10.3 Hz), 3.132 (1 H, dd, *J* 14.4 and 3.7 Hz), 3.618 (1 H, dd, *J* 8.6 and 6.9 Hz, CH_2CHOH), 4.156 (1 H, br s, $C=CH_2$), 4.510 (1 H, br s, $C=CH_2$), 5.162 (1 H, br t, *J* 7.1 Hz, $CH_2CH=C$), 6.887 (1 H, d, *J* 2.0 Hz), 7.087 (1 H, br t, *J* 7.8 Hz), 7.162 (1 H, br dd, *J* 8.1 and 7.8 Hz), 7.325 (1 H, br d, *J* 8.1 Hz), 7.563 (1 H, br d, *J* 7.8 Hz), and 7.866 (1 H, br s, NH); c.d. (*c* 3.4 × 10^{-3} in MeOH) $[\theta]_{229}$

Table 2. Bond lengths (Å) for emindole DA acetate (**4**) with estimated standard deviations in parentheses

O(1)–C(17)	1.467 (9)	O(1′)–C(17′)	1.471(9)
O(1)–C(28)	1.314(10)	O(1′)–C(28′)	1.351(9)
O(2)–C(28)	1.189(11)	O(2′)–C(28′)	1.198(10)
N(1)–C(2)	1.388(10)	N(1′)–C(2′)	1.384(9)
N(1)–C(7a)	1.375(9)	N(1′)–C(7a′)	1.369(9)
C(2)–C(3)	1.375(10)	C(2′)–C(3′)	1.358(9)
C(3)–C(3a)	1.443(9)	C(3′)–C(3a′)	1.439(8)
C(3)–C(8)	1.477(9)	C(3′)–C(8′)	1.485(9)
C(3a)–C(4)	1.406(9)	C(3a′)–C(4′)	1.394(9)
C(3a)–C(7a)	1.401(9)	C(3a′)–C(7a′)	1.416(9)
C(4)–C(5)	1.390(11)	C(4′)–C(5′)	1.409(11)
C(5)–C(6)	1.412(12)	C(5′)–C(6′)	1.408(12)
C(6)–C(7)	1.366(12)	C(6′)–C(7′)	1.352(12)
C(7)–C(7a)	1.392(10)	C(7′)–C(7a′)	1.404(10)
C(8)–C(9)	1.556(9)	C(8′)–C(9′)	1.555(8)
C(9)–C(10)	1.520(9)	C(9′)–C(10′)	1.503(8)
C(9)–C(14)	1.584(8)	C(9′)–C(14′)	1.578(8)
C(10)–C(11)	1.483(10)	C(10′)–C(11′)	1.518(9)
C(10)–C(27)	1.327(11)	C(10′)–C(27′)	1.337(10)
C(11)–C(12)	1.538(10)	C(11′)–C(12′)	1.536(10)
C(12)–C(13)	1.564(9)	C(12′)–C(13′)	1.556(9)
C(13)–C(14)	1.565(8)	C(13′)–C(14′)	1.563(8)
C(13)–C(18)	1.554(9)	C(13′)–C(18′)	1.550(8)
C(14)–C(15)	1.535(9)	C(14′)–C(15′)	1.524(8)
C(14)–C(26)	1.544(10)	C(14′)–C(26′)	1.548(9)
C(15)–C(16)	1.541(9)	C(15′)–C(16′)	1.538(9)
C(16)–C(17)	1.506(10)	C(16′)–C(17′)	1.522(10)
C(17)–C(18)	1.532(10)	C(17′)–C(18′)	1.544(9)
C(18)–C(19)	1.583(12)	C(18′)–C(19′)	1.586(10)
C(18)–C(25)	1.539(12)	C(18′)–C(25′)	1.548(10)
C(19)–C(20)	1.571(14)	C(19′)–C(20′)	1.546(13)
C(20)–C(21)	1.487(13)	C(20′)–C(21′)	1.553(13)
C(21)–C(22)	1.335(11)	C(21′)–C(22′)	1.319(12)
C(22)–C(23)	1.494(15)	C(22′)–C(23′)	1.530(16)
C(22)–C(24)	1.501(13)	C(22′)–C(24′)	1.506(14)
C(28)–C(29)	1.508(15)	C(28′)–C(29′)	1.514(12)

Table 3. Bond angles (°) for emindole DA acetate (**4**) with estimated standard deviations in parentheses

C(17)–O(1)–C(28)	119.7(6)	C(17′)–O(1′)–C(28′)	119.2(6)
C(2)–N(1)–C(7a)	109.5(6)	C(2′)–N(1′)–C(7a′)	108.3(6)
N(1)–C(2)–C(3)	109.2(6)	N(1′)–C(2′)–C(3′)	110.9(6)
C(2)–C(3)–C(3a)	106.2(6)	C(2′)–C(3′)–C(3a′)	105.9(6)
C(2)–C(3)–C(8)	127.9(6)	C(2′)–C(3′)–C(8′)	128.3(6)
C(3a)–C(3)–C(8)	125.9(6)	C(3a′)–C(3′)–C(8′)	125.8(5)
C(3)–C(3a)–C(4)	133.3(6)	C(3′)–C(3a′)–C(4′)	133.7(6)
C(3)–C(3a)–C(7a)	107.8(6)	C(3′)–C(3a′)–C(7a′)	107.3(5)
C(4)–C(3a)–C(7a)	118.8(6)	C(4′)–C(3a′)–C(7a′)	119.0(6)
C(3a)–C(4)–C(5)	118.2(7)	C(3a′)–C(4′)–C(5′)	118.4(7)
C(4)–C(5)–C(6)	121.2(8)	C(4′)–C(5′)–C(6′)	120.4(8)
C(5)–C(6)–C(7)	121.5(8)	C(5′)–C(6′)–C(7′)	122.5(8)
C(6)–C(7)–C(7a)	117.0(7)	C(6′)–C(7′)–C(7a′)	117.1(7)
N(1)–C(7a)–C(3a)	107.3(6)	N(1′)–C(7a′)–C(3a′)	107.6(6)
N(1)–C(7a)–C(7)	129.3(7)	N(1′)–C(7a′)–C(7′)	129.7(6)
C(3a)–C(7a)–C(7)	123.4(6)	C(3a′)–C(7a′)–C(7′)	122.6(6)
C(3)–C(8)–C(9)	111.7(5)	C(3′)–C(8′)–C(9′)	112.5(5)
C(8)–C(9)–C(10)	109.6(5)	C(8′)–C(9′)–C(10′)	110.7(5)
C(8)–C(9)–C(14)	115.5(5)	C(8′)–C(9′)–C(14′)	115.5(5)
C(10)–C(9)–C(14)	109.3(5)	C(10′)–C(9′)–C(14′)	108.5(5)
C(9)–C(10)–C(11)	115.1(6)	C(9′)–C(10′)–C(14′)	115.0(5)
C(9)–C(10)–C(27)	120.7(7)	C(9′)–C(10′)–C(27′)	122.4(6)
C(11)–C(10)–C(27)	124.1(7)	C(11′)–C(10′)–C(27′)	122.6(6)
C(10)–C(11)–C(12)	113.1(6)	C(10′)–C(11′)–C(12′)	111.1(6)
C(11)–C(12)–C(13)	109.1(5)	C(11′)–C(12′)–C(13′)	110.9(5)
C(12)–C(13)–C(14)	111.2(5)	C(12′)–C(13′)–C(14′)	111.1(5)
C(12)–C(13)–C(18)	111.8(5)	C(12′)–C(13′)–C(18′)	111.6(5)
C(14)–C(13)–C(18)	116.8(5)	C(14′)–C(13′)–C(18′)	116.5(5)
C(9)–C(14)–C(13)	108.2(5)	C(9′)–C(14′)–C(13′)	109.2(4)
C(9)–C(14)–C(15)	110.4(5)	C(9′)–C(14′)–C(15′)	110.4(4)
C(9)–C(14)–C(26)	105.0(5)	C(9′)–C(14′)–C(26′)	105.4(5)
C(13)–C(14)–C(15)	109.6(5)	C(13′)–C(14′)–C(15′)	108.3(5)
C(13)–C(14)–C(26)	114.5(5)	C(13′)–C(14′)–C(26′)	113.9(5)
C(15)–C(14)–C(26)	109.0(5)	C(15′)–C(14′)–C(26′)	109.7(5)
C(14)–C(15)–C(16)	111.6(5)	C(14′)–C(15′)–C(16′)	112.0(5)
C(15)–C(16)–C(17)	108.6(6)	C(15′)–C(16′)–C(17′)	108.2(5)
O(1)–C(17)–C(16)	108.0(6)	O(1′)–C(17′)–C(16′)	109.5(5)
O(1)–C(17)–C(18)	106.9(6)	O(1′)–C(17′)–C(18′)	106.1(5)
C(16)–C(17)–C(18)	116.1(6)	C(16′)–C(17′)–C(18′)	114.4(6)
C(13)–C(18)–C(17)	107.2(5)	C(13′)–C(18′)–C(17′)	107.4(5)
C(13)–C(18)–C(19)	110.8(6)	C(13′)–C(18′)–C(19′)	109.6(5)
C(13)–C(18)–C(25)	113.6(6)	C(13′)–C(18′)–C(25′)	113.7(5)
C(17)–C(18)–C(19)	108.7(6)	C(17′)–C(18′)–C(19′)	109.8(5)
C(17)–C(18)–C(25)	109.0(6)	C(17′)–C(18′)–C(25′)	110.7(6)
C(19)–C(18)–C(25)	107.4(6)	C(19′)–C(18′)–C(25′)	105.7(6)
C(18)–C(19)–C(20)	117.2(8)	C(18′)–C(19′)–C(20′)	117.8(7)
C(19)–C(20)–C(21)	112.0(8)	C(19′)–C(20′)–C(21′)	114.0(8)
C(20)–C(21)–C(22)	128.9(8)	C(20′)–C(21′)–C(22′)	125.7(8)
C(21)–C(22)–C(23)	119.8(8)	C(21′)–C(22′)–C(23′)	120.2(9)
C(21)–C(22)–C(24)	123.8(8)	C(21′)–C(22′)–C(24′)	120.4(9)
C(23)–C(22)–C(24)	116.4(8)	C(23′)–C(22′)–C(24′)	119.3(9)
O(1)–C(28)–O(2)	123.8(8)	O(1′)–C(28′)–O(2′)	124.0(7)
O(1)–C(28)–C(29)	110.4(8)	O(1′)–C(28′)–C(29′)	108.8(7)
O(2)–C(28)–C(29)	125.7(9)	O(2′)–C(28′)–C(29′)	127.0(7)

+8 270 and $[\theta]_{272}$ +1 080. The ^{13}C n.m.r. signals are summarized in Table 1.

Emindole DB (**2**) was obtained as prisms (from benzene–hexane), m.p. 165.5—166.5 °C, $[\alpha]_D^{22}$ −86.3° (*c* 0.48 in MeOH) (Found: C, 80.0; H, 9.5; N, 3.3. $C_{28}H_{39}NO_2$ requires C, 79.8; H, 9.3; N, 3.3%); *m/z* 421 (M^+, 6%, e.i.) and 130 [$(C_9H_8N)^+$, 100]; $\lambda_{max.}$(EtOH) 224 (log ε 4.58), 277sh (3.85), 283 (3.89), and 291 nm (3.84); $\nu_{max.}$(KBr) 3 540, 3 300 (OH, NH), 1 440, 1 430, 1 090, and 740 cm^{-1}; $\delta_H(CDCl_3)$ 0.865 (3 H, s, Me), 1.002 (3 H, s, Me), 1.178 [3 H, s, $HOC(Me)_2$], 1.199 [3 H, s, $HOC(Me)_2$], 1.20 (1 H, m), 1.33—1.50 (4 H, m), 1.58—1.79 (4 H, m), 1.860 (1 H, ddd, *J* 12.6, 4.2, and 3.1 Hz), 2.00—2.08 (2 H, m), 2.195 (1 H, br d-like), 2.286 (1 H, m), 2.697 (1 H, br s, OH), 2.715 (1 H, dd, *J* 14.3 and 10.2 Hz), 3.065 (1 H, dd, *J* 11.6 and 4.0 Hz, CH_2CHO), 3.127 (1 H, dd, *J* 14.3 and 3.5 Hz), 3.231 (1 H, dd, *J* 12.0 and 2.9 Hz, CH_2CHO), 4.175 (1 H, br s, $C{=}CH_2$), 4.513 (1 H, t, *J* 2.2 Hz, $C{=}CH_2$), 6.888 (1 H, d, *J* 2.2 Hz), 7.089 (1 H, br dd, *J* 8.0 and 7.8 Hz), 7.163 (1 H, br t, *J* 8.0 Hz), 7.324 (1 H, br d, *J* 8.0 Hz), 7.571 (1 H, br d, *J* 7.8 Hz), and 7.900 (1 H, br s, NH). The ^{13}C n.m.r. signals are listed in Table 1.

Acetylation of Emindole DA (**1**).—Emindole DA (**1**) (70 mg) was dissolved in pyridine (0.3 ml) containing acetic anhydride (0.3 ml) and the solution was kept overnight at room temperature. The mixture was poured into ice–water and extracted with chloroform. The evaporated extract was purified by l.p.l.c. with benzene–ethyl acetate (25:1, v/v) as solvent system followed by recrystallization from hexane to yield *emindole DA monoacetate* (**4**) (56 mg) as needles, m.p. 142.5—143.5 °C; $[\alpha]_D^{22}$ −9.0° (*c* 0.51 in MeOH) (Found: C, 80.9; H, 9.4; N, 3.1. $C_{30}H_{41}NO_2$ requires C, 80.5; H, 9.2; N, 3.1%) *m/z* 447 (M^+, 4% e.i.) and 130 [$(C_8H_9N)^+$, 100]; $\lambda_{max.}$(EtOH) 224 (log ε 4.61), 277sh (3.91), 283 (3.93), and 291 nm (3.86); $\nu_{max.}$(KBr) 3 400 (NH), 1 710 (CO_2), 1 450, 1 255, and 740 cm^{-1}; $\delta_H(CDCl_3)$ 0.892 (3 H, s, Me), 0.998 (3 H, s, Me), 1.216 (1 H, ddd, *J* 14.8, 11.7, and 5.2 Hz), 1.27—1.39 (2 H, m), 1.446 (1 H, ddd, *J* 13.0, 13.0, and 4.9 Hz), 1.56—1.62 (1 H, m), 1.628 [3 H, br s, $=C(Me)_2$], 1.695 [3 H, br s, $=C(Me)_2$], 1.75—1.83 (3 H, m), 1.87—1.97 (1 H, m), 2.062 (3 H, s, OAc), 1.98—2.15 (3 H, m), 2.175 (1 H, br d, *J* 13.2 Hz), 2.287 (1 H, ddd, *J* 13.2, 13.2, and 5.8 Hz), 2.694 (1 H, dd, *J* 14.1 and 10.3 Hz), 3.133 (1 H, dd, *J* 14.1 and 3.8 Hz), 4.167 (1 H, br s, $C{=}CH_2$), 4.519 (1 H, t, *J* 2.0 Hz, $C{=}CH_2$), 4.863 (1 H, dd,

Table 4. Deviations of atoms from least-squares planes through the atoms within cyclohexane rings, with estimated standard deviations in parentheses

A ring			
C(9)	0.233(22)	C(9′)	0.235(25)
C(10)	−0.217(22)	C(10′)	−0.249(26)
C(11)	0.177(22)	C(11′)	0.189(26)
C(12)	−0.261(22)	C(12′)	−0.229(26)
C(13)	0.248(22)	C(13′)	0.249(25)
C(14)	−0.285(22)	C(14′)	−0.273(25)
C(8)*	1.770(22)	C(8′)*	1.770(25)
C(26)*	−1.823(23)	C(26′)*	−1.816(26)
C(27)*	−0.945(23)	C(27′)*	−1.015(26)
B ring			
C(13)	0.173(20)	C(13′)	−0.203(31)
C(14)	−0.227(20)	C(14′)	0.220(31)
C(15)	0.251(20)	C(15′)	−0.259(31)
C(16)	−0.261(20)	C(16′)	0.285(31)
C(17)	0.247(20)	C(17′)	−0.227(31)
C(18)	−0.193(20)	C(18′)	0.204(31)
O(1)*	−0.241(20)	O(1′)	0.300(31)
C(19)*	0.579(21)	C(19′)*	−0.545(31)
C(25)*	−1.695(21)	C(25′)*	1.714(31)
C(26)*	−1.739(21)	C(26′)*	1.744(31)

* These atoms are not included in the least-squares calculation.

J 8.3 and 7.8 Hz, CH_2CHOAc), 5.086 (1 H, br t, *J* 7.3 Hz, $CH_2CH{=}C$), 6.897 (1 H, d, *J* 1.9 Hz), 7.088 (1 H, br t, *J* 8.1 Hz), 7.163 (1 H, br dd, *J* 8.1 and 7.6 Hz), 7.327 (1 H, br d, *J* 8.1 Hz), 7.557 (1 H, br d, *J* 7.6 Hz), and 7.883 (1 H, br s, NH).

Acetylation of Emindole DB (**2**).—Emindole DB (**2**) (110 mg) was acetylated with acetic anhydride (1.0 ml) and pyridine (1.0 ml) in a similar manner to that described above. The purification by l.p.l.c. afforded starting material (80 mg), which was identified as emindole DB by t.l.c., i.r. and ^{1}H n.m.r. spectra.

Oxidation of Emindole DA (**1**) *with* m-*Chloroperbenzoic Acid.*—Emindole DA (**1**) (134 mg) was dissolved in dichloromethane (10 ml) and MCPBA (100 mg) was added. The solution was kept overnight at room temperature. The evaporated mixture was purified by l.p.l.c. using the solvent system benzene–ethyl acetate (20:1, v/v) to give emindole DB (**2**) (36 mg) and an unknown compound (15 mg). The former compound was identical with naturally occurring emindole DB as confirmed by comparison of its t.l.c. behaviour, i.r. and ^{1}H n.m.r. spectra, and optical rotations.

Absolute Configuration of Emindole DA (**1**).—A solution of emindole DA (**1**) (60 mg, 0.15 mmol) and 2-phenylbutyric anhydride (93 mg, 0.30 mmol) in pyridine (0.75 ml) was set aside at room temperature overnight. The excess of anhydride was destroyed by addition of water (0.1 ml) and vigorous stirring of the reaction mixture for 30 min. The mixture was neutralized with 1/11M NaOH (4.1 ml) and extracted with chloroform. The organic phase was washed with water, dried (Na_2SO_4), and evaporated. The residual ester (**5**) contained no starting material on t.l.c.

The combined sodium hydroxide layers were acidified with 4M HCl and extracted with dichloromethane to yield 2-phenylbutyric acid (61 mg), $[\alpha]_D^{20}$ −13.2° (*c* 3.06 in benzene) (theoretical $[\alpha]_D$ −32.1°).[7] The optical yield therefore was 41% (−), based on an esterification yield of 100%.

Structure Determination of Emindole DA Acetate (**4**) *by* X-*Ray Diffraction.*—Emindole DA acetate (**4**) was grown from methanol as prisms. Diffraction intensities were collected from a crystal of dimensions 0.5 × 0.5 × 0.2 mm on a Rigaku AFC-5 FOS four-circle diffractometer. Two molecules of (**4**) were

Table 5. Final atomic fractional co-ordinates with estimated standard deviations in parentheses

Atom	*x*	*y*	*z*
O(1)	0.455 4(42)	0.323 8	0.453 4(39)
O(2)	0.332 9(48)	0.352 2(74)	0.328 9(45)
O(1′)	0.008 9(45)	0.367 2(53)	1.017 6(41)
O(2′)	0.130 4(43)	0.398 0(57)	1.152 2(43)
N(1)	0.260 3(53)	0.384 4(62)	1.054 2(50)
N(1′)	0.208 6(56)	0.458 1(58)	0.418 9(52)
C(2)	0.285 9(66)	0.324 4(74)	0.984 1(61)
C(3)	0.307 7(51)	0.395 9(64)	0.919 3(52)
C(3a)	0.293 1(53)	0.505 3(62)	0.950 3(52)
C(4)	0.299 5(64)	0.611 1(70)	0.910 7(65)
C(5)	0.276 7(77)	0.700 9(75)	0.957 5(83)
C(6)	0.248 9(81)	0.686 9(83)	1.043 4(87)
C(7)	0.242 1(69)	0.584 7(80)	1.082 6(68)
C(7a)	0.263 8(58)	0.495 0(67)	1.034 1(54)
C(8)	0.338 3(52)	0.368 6(67)	0.833 3(53)
C(9)	0.429 6(50)	0.392 4(60)	0.873 5(50)
C(10)	0.476 0(54)	0.312 2(70)	0.965 4(52)
C(11)	0.479 0(68)	0.197 3(69)	0.928 8(59)
C(12)	0.510 2(71)	0.188 6(70)	0.835 6(64)
C(13)	0.460 5(55)	0.267 5(58)	0.738 7(53)
C(14)	0.464 6(51)	0.389 2(60)	0.780 2(52)
C(15)	0.415 4(60)	0.464 0(62)	0.682 9(59)
C(16)	0.441 6(68)	0.452 1(68)	0.584 8(63)
C(17)	0.430 7(60)	0.333 8(74)	0.547 1(56)
C(18)	0.478 8(62)	0.248 4(67)	0.633 6(59)
C(19)	0.451 4(99)	0.129 1(81)	0.584 5(77)
C(20)	0.361 9(100)	0.096 3(88)	0.556 6(79)
C(21)	0.351 6(79)	0.043 4(74)	0.652 6(73)
C(22)	0.292 0(72)	0.055 3(73)	0.687 2(76)
C(23)	0.224 8(94)	0.132 8(106)	0.628 0(134)
C(24)	0.286 8(89)	−0.006 9(105)	0.783 2(90)
C(25)	0.568 0(73)	0.260 2(95)	0.654 9(71)
C(26)	0.550 3(59)	0.436 0(81)	0.836 1(66)
C(27)	0.512 4(66)	0.346 5(91)	1.069 4(58)
C(28)	0.402 1(70)	0.336 3(86)	0.351 5(60)
C(29)	0.440 3(81)	0.318 5(126)	0.269 2(71)
C(2′)	0.172 1(63)	0.399 4(69)	0.479 4(58)
C(3′)	0.167 7(51)	0.465 2(61)	0.560 7(52)
C(3a′)	0.202 4(53)	0.568 8(59)	0.549 8(53)
C(4′)	0.216 2(68)	0.667 9(71)	0.608 2(67)
C(5′)	0.255 0(81)	0.753 5(77)	0.576 0(88)
C(6′)	0.278 3(78)	0.738 8(80)	0.486 3(85)
C(7′)	0.265 7(69)	0.643 6(82)	0.428 2(72)
C(7a′)	0.226 9(57)	0.558 4(66)	0.460 2(57)
C(8′)	0.135 7(53)	0.437 9(63)	0.646 0(54)
C(9′)	0.045 2(52)	0.465 8(54)	0.607 5(50)
C(10′)	−0.004 1(59)	0.395 4(66)	0.510 3(58)
C(11′)	−0.008 4(69)	0.274 5(66)	0.537 0(62)
C(12′)	−0.039 2(67)	0.260 1(63)	0.630 0(65)
C(13′)	0.010 1(52)	0.331 8(56)	0.732 6(53)
C(14′)	0.009 8(52)	0.455 6(55)	0.699 6(51)
C(15′)	0.061 1(60)	0.520 0(60)	0.803 0(54)
C(16′)	0.031 9(64)	0.505 5(69)	0.897 7(60)
C(17′)	0.037 9(56)	0.384 0(68)	0.928 4(54)
C(18′)	−0.012 7(60)	0.307 1(63)	0.832 7(58)
C(19′)	0.007 4(81)	0.182 4(81)	0.869 3(78)
C(20′)	0.095 5(100)	0.144 7(86)	0.907 5(87)
C(21′)	0.142 1(82)	0.144 8(75)	1.035 5(79)
C(22′)	0.203 1(78)	0.080 5(75)	1.091 5(90)
C(23′)	0.230 6(126)	−0.008 2(122)	1.031 1(131)
C(24′)	0.242 9(91)	0.088 5(113)	1.216 0(89)
C(25′)	−0.103 0(64)	0.321 1(82)	0.806 5(64)
C(26′)	−0.074 8(64)	0.507 3(70)	0.647 8(64)
C(27′)	−0.041 8(62)	0.436 6(81)	0.408 2(59)
C(28′)	0.060 5(69)	0.379 2(76)	1.124 3(58)
C(29′)	0.016 9(77)	0.353 8(95)	1.199 2(67)

packed in an asymmetric unit. Of the total 4 166 reflections (complete for $2\theta \leqslant 120°$), 3 784 satisfied the criterion $F \geqslant 3\sigma(F)$ and only these were used in the solution and refinement of the structure.

Crystal data. $(C_{30}H_{41}NO_2)_2$, M = 895.3, monoclinic, space group $P2_1$, a = 18.155 (25), b = 12.137 (13), c = 13.097 (12) Å, β = 113.07 (9)°, V = 2 655.2 Å^3, Z = 2, D_c = 1.12 g cm^{-3}, $F(000)$ = 976, Cu-K_α X-radiation (graphite monochromator), λ = 1.5405 Å.

Structure solution and refinement. The structure was solved by direct methods using MULTAN 80[17] and in the final refinement by block-matrix least-squares method; anisotropic thermal parameters were used for all non-hydrogen atoms. Isotropic thermal parameters were used for 67 hydrogen atoms. The contribution of the remaining 15 hydrogen atoms was ignored. The refinement converged to R 0.061 and R_w 0.060. The data were not corrected for the effects of absorption. Positional parameters are shown in Table 5, and bond lengths and angles are summarized in Tables 2 and 3. A list of anisotropic thermal parameters and torsion angles are available from the Cambridge Crystallographic Data Centre.*

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* For details of the Supplementary Publication Scheme, see Instructions for Authors (1988), *J. Chem. Soc., Perkin Trans. 1*, Issue 1.

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