Isolation and Structures of Indoloditerpenes, Possible Biosynthetic Intermediates to the Tremorgenic Mycotoxin, Paxilline, from *Emericella striata*[1]

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Together with paspaline (**7**), two indoloditerpenes designated dehydroxypaxilline (**5**), $C_{27}H_{33}NO_3$, and emindole SB (**6**), $C_{28}H_{39}NO$, were isolated from the mycelium of *Emericella striata*. The structures of dehydroxypaxilline (**5**) and emindole SB (**6**) were determined on the basis of spectroscopic investigation of these compounds. It is very interesting that emindole SB (**6**), paspaline (**7**), and dehyroxypaxilline (**5**) were isolated from the same fungus, *E. striata*, on consideration of the biogenesis of paxilline (**1**) which had also been isolated from the same fungus as one of the main components.

Recently we reported the isolation of a tremorgenic mycotoxin, paxilline (**1**), from the mycelial extract of *Emericella striata* (Rai, Tewari, and Mujkerji) Malloch and Cain, strain 80-NE-22,[2] as one of the major metabolites, and from *E. desertorum* Samson and Mouchacca, strain CBS 653.73,[3] as a minor component. During our search for compounds related to (**1**), novel indoloditerpenes, *i.e.*, emindoles DA (**2**)[4,5] and DB (**3**),[5] and emindole SA (**4**).[4,6] from *E. desertorum* and *E. striata*, respectively, were isolated. Recently, two compounds related to the biosynthetic intermediates to (**1**) designated dehydroxypaxilline (**5**) and emindole SB (**6**) were isolated along with paspaline (**7**), which was originally isolated from the mycelium of *Claviceps paspali* Stevens and Hall,[7] from the non-polar fraction of the mycelial acetone extract, and the structures of compounds (**5**) and (**6**) are reported in this paper.

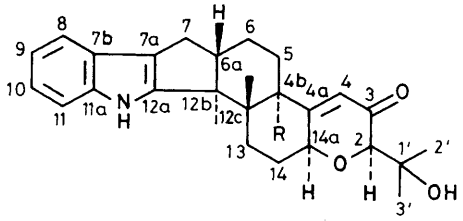
Results and Discussion

The molecular formulae of dehydroxypaxilline (**5**) and emindole SB (**6**) were confirmed as $C_{27}H_{33}NO_3$ and $C_{28}H_{39}NO$, respectively, by electron impact ionization (e.i.) mass spectrometry and by elemental analysis or high-resolution mass spectrometry. A positive colouration for van Urk's reagent (yellowish green)[8] and the fragmentation ion at *m/z* 130 $[(C_9H_8N)^+]$ in the e.i. mass spectra of compounds (**5**) and (**6**) suggested the presence of an indole moiety in both compounds. The ^1H n.m.r. signals of four aromatic protons of the indole moiety appeared at δ_H 7.091 (2 H), 7.305 (1 H), and 7.441 (1 H) in (**5**), and at δ_H 7.059 (2 H), 7.268 (1 H), and 7.408 (1 H) in (**6**). These data were almost superimposable on those of paxilline (**1**) and paspaline (**7**), which were also isolated from the same fungus, including the coupling patterns. The proton at C-2 of

Table. ^{13}C N.m.r. chemical shifts of paxilline (**1**), emindole SB (**6**), and related compounds in $CDCl_3$

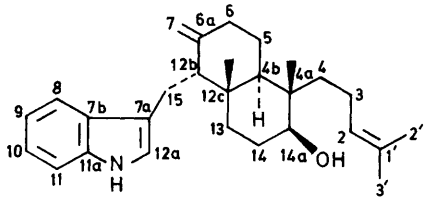
Carbon[a]	(1)[b]	(2)	(3)	(5)	(6)	(7)
C-2	83.16	124.94	85.85	82.69	124.68	85.86
C-3	199.50	21.89	37.81	198.24	21.42[c]	37.81
C-4	119.16	37.54	24.85	121.86	37.54	25.39[c]
C-4a	169.45	41.12	36.25	168.27	39.30	36.65
C-4b	76.78	39.07	45.43	42.69	39.94	46.56
C-5	28.58[c]	23.03	22.19[c]	25.61[c]	22.77[c]	22.04[c]
C-6	27.34[c]	30.83	30.74	30.05[c]	27.47[c]	27.61[c]
C-6a	49.57	136.25	136.26	49.01	49.80	48.87
C-7	33.62	110.20	110.22	32.86	33.67	33.98
C-7a	116.68	115.57	115.60	118.56	118.31	118.32
C-7b	124.94	127.64	127.66	125.07	125.17	125.26
C-8	118.18	118.88	118.90[d]	118.64	118.41	118.46
C-9	119.16	118.94	118.93[d]	119.82	119.55	119.60
C-10	120.02	121.63	121.61	120.87	120.43	120.48
C-11	111.61	111.00	111.02	111.64	111.38	111.52
C-11a	139.92	148.10	148.03	140.19	140.04	140.16
C-12a	152.15	121.90	121.87	149.25	150.82	150.90
C-12b	50.80	58.61	58.85	50.57	53.11	53.09
C-12c	43.04	37.92	38.48	42.48	41.24	40.15
C-13	20.98[e]	34.58	34.84	24.15[e]	25.17[e]	24.74[e]
C-14	27.16[e]	27.75	21.95[e]	27.35[e]	27.47[e]	22.08[e]
C-14a	72.79	73.95	84.75	74.84	77.33	84.81
C-1′	72.38	131.21	71.95	72.53	132.22	72.05
C-2′	24.19	17.70	23.80[e]	24.27	17.44	23.85
C-3′	26.61	25.74	26.14	26.80	25.71	26.18
4a-Me		16.91	13.45		16.41	12.77
12b-Me (C-15)	16.20	23.27	23.29	14.65	14.60	14.63
12c-Me	19.37	23.18	23.85[e]	16.30	19.16	20.04

[a] Numberings of the related compounds correspond to those of (**1**). [b] Measured in $CDCl_3$ containing a little $(CD_3)_2SO$. [c–e] Assignments may be reversed.

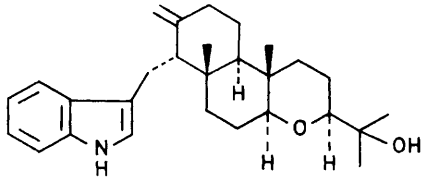


(1) R = OH

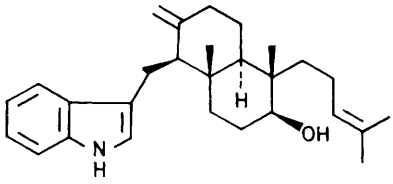
(5) R = H



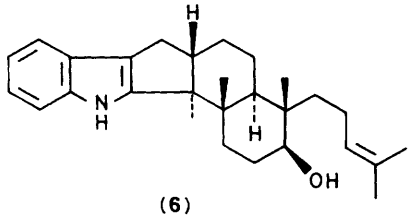
(2)



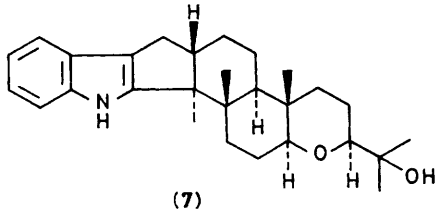
(3)



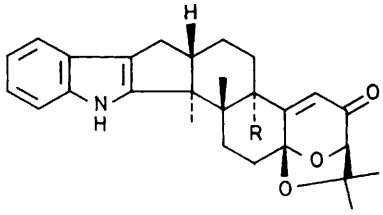
(4)



(6)



(7)



(8) R = OH

(9) R = H

the indole moiety, which appeared as a doublet at δ_H 6.887 in emindole DA (**2**) and at δ_H 6.893 in emindole SA (**4**), was not observed in compounds (**5**) and (**6**). The indole NH was observed at δ_H 7.881 in (**5**) and at δ_H 7.752 in (**6**). The above results and ^{13}C n.m.r. spectra (Table) were explicable by assuming the presence of a 2,3-disubstituted indole moiety in dehydroxypaxilline (**5**) and emindole SB (**6**).

The ^1H n.m.r. spectrum of dehydroxypaxilline (**5**) was closely similar to that of paxilline (**1**), except for the upfield shift, δ_H 4.826 (br t) in (**1**) to δ_H 4.268 (br dd) in (**5**), of the proton attached to the carbon bearing the ether oxygen. Compound (**5**) had only one less oxygen atom than paxilline (**1**), and so it was assumed that one hydroxy group in (**1**) was replaced by a proton in (**5**). The strong fragmentation ion at *m/z* 361 [$(M - Me_2CO)^+$], the same as in (**1**), suggested the presence of a 1-hydroxy-1-methylethyl group in the molecule. The assignments of the ^{13}C n.m.r. chemical shifts of compound (**5**) were carried out on the basis of the signal multiplicity and on a comparison with those of compound (**1**), and are listed in the Table. The carbon signal at δ_C 76.78 assigned to C-4b bearing the hydroxy group in (**1**) was changed to a signal at δ_C 42.69, which appeared as a doublet in the off-resonance spectrum of compound (**5**). The other ^{13}C n.m.r. signals differing from compounds (**1**) and (**5**) were those of carbons at C-4, C-5, C-6, C-12a, C-13, C-14a, and the 12c-methyl group, which were near C-4b. The above results confirmed that dehydroxypaxilline was the dehydroxy derivative at C-4b of paxilline (**1**), as shown in structure (**5**). The c.d. spectra of compounds (**1**) and (**5**) showed maxima at 237 (−), 297 (+), and 338 (+) nm, and at 238 (−), 294 (+), and 336 (+) nm, respectively, and the absolute configuration of dehydroxypaxilline (**5**) should therefore be the same as that of paxilline (**1**).

The ^1H n.m.r. signals at δ_H 1.632 (3 H, br s), 1.693 (3 H, br s), and 5.115 (1 H, br t) in emindole SB (**6**) corresponded to the ^{13}C n.m.r. signals at δ_C 17.44 (q), 25.71 (q), 132.22 (s), and 124.68 (d). These data suggested the presence of a 4-methylpent-3-enyl group in compound (**6**). The signals of three aliphatic tertiary methyl groups were observed at δ_H 0.808, 1.008, and 1.086 in the ^1H n.m.r. spectrum and at δ_C 14.60, 16.41, and 19.16 in the ^{13}C n.m.r. spectrum. The signal observed at δ_H 3.568 (1 H, dd), which corresponded to the carbon signal at δ_C 77.32, was assigned to the proton attached to the carbon bearing an equatorial secondary alcohol. The other ^1H n.m.r. signals were due to aliphatic methylene and methine protons. The assignments of the ^{13}C n.m.r. signals of compound (**6**) (Table) were confirmed on the basis of the chemical shifts and multiplicity of the signals,

and by comparison with the signals of emindoles DA (**2**) and SA (**4**), and paspaline (**7**). The carbon signals of the decalin ring near the indole moiety in compound (**6**) were closely similar to those in (**7**), whereas the side-chain signals and those of the pyran ring in (**6**) were superimposable on those in (**2**) or (**4**). The above results, considering the molecular formula of compound (**6**), suggested that cyclization of emindole SB (**6**) at the double bond by epoxidation and attack of the hydroxy group afforded paspaline (**7**).

The ^{13}C n.m.r. signals which were greatly shifted from emindole SB (**6**) to paspaline (**7**) were those for carbons C-2, C-3, C-4, C-4a, C-4b, C-14, C-14a, C-1′, C-2′, and the 4a-methyl group, the shift values of which were calculated as +38.02, −16.39, +12.15, +2.65, −6.62, +5.39, −7.48, +59.26, −6.41, and +3.64, respectively, consistent with those from emindole DA (**2**) to emindole DB (**3**) (Table). The above facts confirmed the relative structure of emindole SB as (**6**). The absolute configuration of compound (**6**) has not yet been determined, but it was assumed to be as shown in structure (**6**) because of the co-occurrence of paxilline (**1**), emindole SA (**4**), dehydroxypaxilline (**5**), and paspaline (**7**) with the same basic configuration.

Acklin *et al.*, reported[9] that indoloditerpenes like paspalinine (**8**) and paspalicine (**9**), which were originally isolated from *Claviceps paspali* Stevens and Hall as tremorgenic mycotoxins,[7] would have been derived from tryptophan and geranylgeraniol by cyclization and migration of the carbon skeleton, and that they isolated a compound which they considered to be emindole SB (**6**) as the intermediate to paspaline (**7**) and (**8**) but without any supporting data. It is very interesting that dehydroxypaxilline (**5**), emindole SB (**6**), and paspaline (**7**) were isolated from *E. striata* along with paxilline (**1**). The biogenesis of paxilline (**1**) in *E. striata* would be as follows: linear indologeranylgeraniol cyclizes, followed by migration of the carbon skeleton to give emindole SB (**6**), which is further cyclized, after epoxidation, to give compound (**7**). Paspaline (**7**) was changed into dehydroxypaxilline (**5**) by oxidation at C-3 and the overall loss of methane from C-4–C-4a, and then hydroxylation of compound (**5**) at C-4b gave paxilline (**1**). Hydroxylation at C-4b would have occurred after further cyclization of paspaline (**7**) in *C. paspali*, because paspalicine (**9**) was isolated from this fungus.[7]

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 spectrometer and a JASCO IR-810 spectrometer, respectively. ^1H (399.78 MHz) and ^{13}C (100.43 MHz) N.m.r. spectra were recorded on a JEOL JNM-GX 400 spectrometer, using tetramethylsilane as internal standard. C.d. curves were determined on a JASCO J-40 spectrometer. 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 a glass column (200 × 10 mm) packed with silica gel CQ-3 (30—50 μm; Wako). T.l.c. was conducted on pre-coated Kieselgel $60F_{254}$ (Art. 5715; Merck). Spots on t.l.c. were detected by their absorption under u.v. light, and/or by spraying van Urk's reagent.[8]

Isolation of Metabolites Related to Paxilline (**1**).—*E. 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 extracted with acetone at room temperature. The extract (26 g) was chromatographed on silica gel with benzene–acetone (50:1). After removal of ergosterol, the residue was rechromatographed on silica gel with benzene–ethyl acetate (5:1) to give two fractions. The less polar fraction was purified by repeated l.p.l.c. with the solvent system of hexane–ethyl acetate (10:1 and 5:1) to give paspaline (**7**) (118 mg). The more polar fraction was purified by repeated l.p.l.c. with the solvent system of chloroform–acetone (93:7 and 100:1) to obtain dehydroxypaxilline (**5**) (71 mg) and emindole SB (**6**) (52 mg).

Paspaline (**7**) was obtained as a crystalline powder (from methanol), m.p. 253—254 °C (lit.,[7] 254 °C); $[\alpha]_D^{20}$ −24° (*c* 0.38 in $CHCl_3$) [lit.,[7] −23° (*c* 0.36 in $CHCl_3$)]; *m/z* 421 (M^+, 76%), 406 [$(M - Me)^+$, 79], 362 [$(M - 59)^+$, 10], 182 (100), and 130 [$(C_9H_8N)^+$, 41]; $\delta_H(CDCl_3)$ 0.878 (3 H, s, Me), 1.015 (3 H, s, Me), 1.119 (3 H, s, Me), 1.176 (3 H, s, Me), 1.187 (3 H, s, Me), 1.35—1.84 (12 H, m), 1.938 (1 H, ddd, *J* 12.7, 12.7, and 3.7 Hz), 2.319 (1 H, dd, *J* 13.1 and 10.6 Hz), 2.663 (1 H, dd, *J* 13.1 and 6.6 Hz), 2.709 (1 H, br s, OH), 2.774 (1 H, m), 3.019 (1 H, dd, *J* 11.5 and 3.7 Hz, CH_2CHO), 3.209 (1 H, dd, *J* 12.0 and 2.9 Hz, CH_2CHO), 7.061 (2 H, m), 7.285 (1 H, m), 7.415 (1 H, m), and 7.850 (1 H, br s, NH). ^{13}C N.m.r. signals are summarized in the Table.

Dehydroxypaxilline (**5**) was obtained as leaflets (from methanol), m.p. 232—234 °C (Found: C, 77.05; H, 8.0; N, 3.3. $C_{27}H_{33}NO_3$ requires C, 77.29; H, 7.93; N, 3.34%); *m/z* 419 (M^+, 22%), 404 [$(M - Me)^+$, 24], 361 [$(M - Me_2CO)^+$, 59], 346 [$(M - Me_2CO - Me)^+$, 100], 182 (55), and 130 [$(C_9H_8N)^+$, 24]; $\lambda_{max.}$(EtOH) 231 (log ε 4.70), 280 (4.10), and 290sh nm (4.03); $\nu_{max.}$(KBr) 3 410 (OH, NH) and 1 660 cm^{-1} (C=O); $\delta_H(CDCl_3)$ 0.987 (3 H, s, Me), 1.240 (3 H, s, Me), 1.288 (3 H, s, Me), 1.300 (3 H, s, Me), 1.545 (1 H, m), 1.709 (4 H, m), 1.837 (1 H, m), 1.887 (1 H, m), 2.119 (1 H, ddd, *J* 13.4, 13.4, and 4.1 Hz), 2.299 (1 H, m), 2.409 (1 H, dd, *J* 13.2 and 10.3 Hz), 2.743 (1 H, dd, *J* 13.2 and 6.4 Hz), 2.810 (1 H, m), 3.734 (1 H, d, *J* 2.2 Hz, CHO), 4.226 (1 H, s, OH), 4.268 (1 H, br dd, *J* 10.0 and 7.8 Hz, CH_2CHO), 5.871 (1 H, br s, =CH), 7.091 (2 H, m), 7.305 (1 H, m), 7.441 (1 H, m), and 7.881 (1 H, br s, NH); c.d. (*c* 3.2 × 10^{-3} in MeOH); $[\theta]_{238}$ −7.2 × 10^4, $[\theta]_{277}$ +7.19 × 10^3, $[\theta]_{294}$ +7.82 × 10^3, and $[\theta]_{336}$ +7.82 × 10^3. ^{13}C N.m.r. signals are summarized in the Table.

Emindole SB (**6**) was obtained as an amorphous powder, *m/z* 405.3019 (M^+, $C_{28}H_{39}NO$ requires *M*, 405.3029, 87%), 390.2804 [$(M - Me)^+$, $C_{27}H_{36}NO$ requires *m/z* 390.2797, 51], 182.0975 ($C_{13}H_{12}N$ requires *m/z* 182.0970, 100), and 130.0655 [$(C_9H_8N)^+$, C_9H_8N requires *m/z* 130.0655, 45]; $\nu_{max.}$(KBr) 3 410 (OH, NH); δ_H 0.808 (3 H, s, Me), 1.008 (3 H, s, Me), 1.086 (3 H, s, Me), 1.24—1.50 (14 H, m), 1.632 (3 H, br s, $=CMe_2$), 1.693 (3 H, br s, $=CMe_2$], 2.320 (1 H, dd, *J* 12.9 and 10.3 Hz), 2.665 (1 H, dd, *J* 12.9 and 6.4 Hz), 2.732 (1 H, m), 3.568 [1 H, dd, *J* 8.3 and 6.7 Hz, $CH_2CH(OH)$], 5.115 (1 H, br t, *J* 7.0 Hz, $CH_2CH=$), 7.059 (2 H, m), 7.268 (1 H, m), 7.408 (1 H, m), and 7.752 (1 H, br s, NH). ^{13}C N.m.r. signals are summarized in the Table.

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