Isolation of a New Tremorgenic Indoloditerpene, 1′-*O*-Acetylpaxilline, from *Emericella striata* and Distribution of Paxilline in *Emericella* spp.[1)]

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The distribution of a tremorgenic mycotoxin, paxilline (1), was investigated in 19 species belonging to the genus *Emericella*. It was found that *Emericella desertorum*, *E. foveolata*, and *E. striata* produced paxilline (1). A new type of indoloditerpene, emindole DA (4), was also found in *E. quadrilineata*. A new tremorgenic indoloditerpene, 1′-*O*-acetylpaxilline (3), was isolated from the mycelium of *E. striata*. Its structure was established on the basis of spectroscopic investigations.

Keywords *Emericella*; *Emericella striata*; *Emericella desertorum*; *Emericella foveolata*; *Emericella quadrilineata*; paxilline; 1′-*O*-acetylpaxilline; emindole DA; tremorgenic mycotoxin; indoloditerpene

Paxilline (**1**) was first isolated from *Penicillium paxilli* BAINIER, strain ATCC 26601, isolated from insect-damaged pecans.[2,3)] Cole *et al.* reported[2)] that severe (intermittent) tremors were induced in cockerels and mice upon oral administration of **1** at 25 mg/kg. Cockrum *et al.* reported[4)] that only a North American isolate (the above strain) of *P. paxilli* produced **1**, whereas South Australian isolates (FRR 1973 and FRR 2196) produced verruculogen, a different type of tremorgenic mycotoxin. Paxilline (**1**) was also isolated from *Acremonium lorii* LATCH *et al.*[5)] as a biosynthetic precursor of loritrem B (**2**), which causes a neurotic syndrome affecting sheep.[6)] Recently we reported the isolation of **1** from *Emericella striata* (RAI, TEWARI *et* MUKERJI) MALLOCH *et* CAIN, strain 80-NE-22,[7)] as a major metabolite, and from *E. desertorum* SAMSON *et* MOUCHACCA, strain CBS 653.73,[8)] as a minor component. The above results prompted us to investigate the distribution of paxilline (**1**) in *Emericella* spp. In the course of this study, a new compound designated 1′-*O*-acetylpaxilline (**3**) was isolated from the mycelial extract of the above strain of *E. striata*.

Paxilline (**1**) was detected on a thin layer chromatography (TLC) plate by spraying van Urk's reagent[9)] (green for **1**; detection limit, 40 μg per mycelium from one Roux flask) from *E. foveolata* HORIE, strain IFM 42015, and *E. striata*, strain IMI 163899, among 19 species (21 strains) of the genus *Emericella* tested. Although **1** was isolated from *E. desertorum* as a minor metabolite in the case of large-scale culture in Czapek-Dox medium,[8)] **1** could not be detected from the same strain when it was cultivated in Raulin-Thom medium. This may be because of the difference of the culture medium or the limitation of the detection of **1** by TLC. The distribution of **1** is not wide in *Emericella* spp., but it is interesting that three species of *Emericella* produced **1** because only two species (*P. paxilli* and *A. lorii*) have previously been found to produce **1**. Emindole DA (**4**), which is a novel type of indoloditerpene originally isolated from *E. desertorum*,[10)] was also detected from *E. quadrilineata* (THOM *et* RAPER) C. R. BENJAMIN, strain IFM 42006 and 42021.

The molecular formula of 1′-*O*-acetylpaxilline (**3**) was confirmed to be $C_{29}H_{35}NO_5$ by electron impact (EI) mass spectrometry and elemental analysis. A positive coloration (green) with van Urk's reagent[9)] suggested the presence of an indole moiety in **3**. The proton nuclear magnetic resonance (^{1}H-NMR) signals of four aromatic protons of the indole appeared as multiplets at δ 7.07 (2H), 7.29 (1H), and 7.43 (1H). These data were closely similar to those of **1** and dehydroxypaxilline (**5**), which were isolated from the same fungus,[11)] including the coupling patterns. The above results and carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals (Table I) were consistent with the presence of a 2,3-disubstituted indole moiety in 1′-*O*-acetylpaxilline (**3**).

The infrared (IR) absorption maximum of **3** at 1728

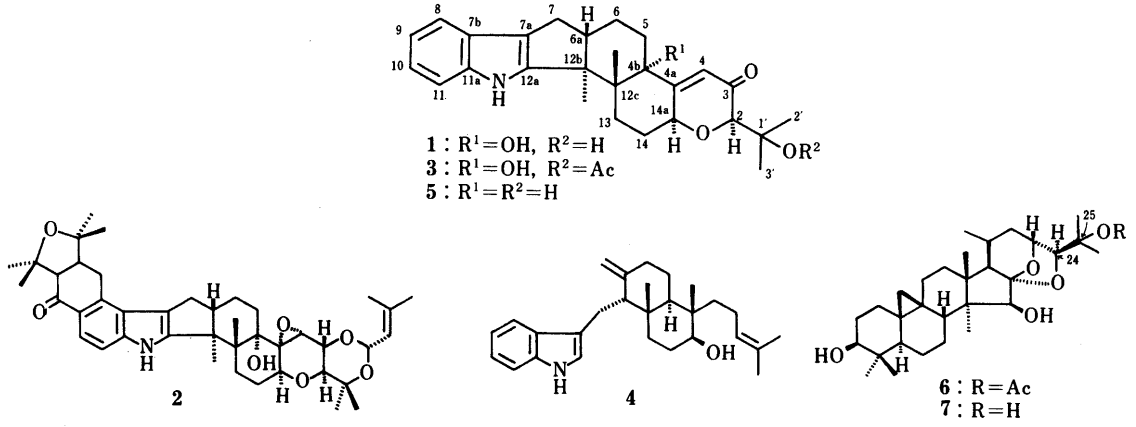

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of Paxilline (1) and Related Compounds in $CDCl_3$

Carbon No.	1[a]	3	5
C-2	83.16	80.43	82.69
C-3	199.50	195.31	198.24
C-4	119.16	120.07	121.86
C-4a	169.45	166.21	168.27
C-4b	76.78	77.37	42.69
C-5	28.58[b]	28.05[b]	25.61[b]
C-6	27.34[b]	28.37[b]	30.05[b]
C-6a	49.57	49.54	49.01
C-7	33.62	34.23	32.86
C-7a	116.68	117.49	118.56
C-7b	124.94	125.15	125.07
C-8	118.18	118.52	118.64
C-9	119.16	119.73	119.82
C-10	120.02	120.64	120.87
C-11	111.61	111.52	111.64
C-11a	139.92	139.81	140.19
C-12a	152.15	151.79	149.25
C-12b	50.80	50.85	50.57
C-12c	43.04	43.14	42.48
C-13	20.98[b]	20.94[b]	24.15[b]
C-14	27.16[b]	27.22[b]	27.35[b]
C-14a	72.79	72.94	74.84
C-1′	72.38	81.98	72.53
C-2′	24.19	22.84	24.27
C-3′	26.61	23.86	26.80
12b-Me	16.20	16.21	14.65
12c-Me	19.37	19.73	16.30
OCOMe		22.31	
OCOMe		170.92	

a) Measured in $CDCl_3$ containing a little $(CD_3)_2SO$. *b*) Assignments may be reversed.

cm^{-1}, which was not present in the spectrum of **1**, suggested the presence of an ester. The ^{13}C-NMR signals at δ 22.31 and 170.92 in **3** were assigned to the carbons of an acetyl group. The ^{1}H-NMR spectrum of **3** was similar to that of paxilline (**1**), except for the appearance of the signal of methyl protons of the aliphatic acetoxyl group at δ 2.04 and the downfield shift of the signal due to a proton attached to the carbon bearing an ether oxygen from δ 3.68 (d) in **1** to δ 4.79 (d) in **3**. The acetylation of the tertiary hydroxy group caused a downfield shift of the β-proton signal: the signal of the methyl protons of *tert*-butyl acetate were observed at 0.23 ppm downfield from that of *tert*-butanol, although no shift was observed in the methyl group signals between iso-propyl acetate and iso-propanol.[12] The signal of 24-H of 25-*O*-acetylcimigenol (**6**) was observed at 0.5 ppm downfield from that of cimigenol (**7**).[13] The above results suggested that one tertiary hydroxyl in **1** was replaced by an acetoxyl group in **3**.

Signals of two of four methyl protons [δ 1.28 (6H) in **1**] were shifted downfield to δ 1.43 (3H) and 1.65 (3H) in **3**. The assignments of the ^{13}C-NMR signals of **3** were carried out on the basis of the signal multiplicity and the comparison of the signals of **1** and **5**, and are listed in Table I. The C-1′ signal (δ 72.38 in **1**) was greatly shifted downfield to δ 81.98 in **3** compared with that of **1**. The other ^{13}C-NMR signals noticeably shifted between **1** and **3** were those of three carbons in the pyran ring and two carbons in the side chain. Three of the above carbons, *i.e.* C-2, C-2′, and C-3′, were α carbons with respect to C-1′. The above results suggested the position of the acetoxyl group in **3**. Finally, 1′-*O*-acetylpaxilline (**3**) was derived from paxilline (**1**) by acetylation with acetic anhydride and 4-dimethylaminopyridine in pyridine, and so the relative structure of **3** was confirmed. The circular dichroism (CD) of **3** showed maxima at 244 (−), 268 sh (−), 296 (+), and 338 (+) nm, which were almost the same as those at 237 (−), 276 sh (−), 296 (+), and 338 (+) nm in **1**. Therefore the absolute configuration of 1′-*O*-acetylpaxilline was established as being the same as in paxilline (**1**), as shown in structure **3**.

1′-*O*-Acetylaxilline (**3**) also caused tremors at the concentration of 3.125 mg/kg (i.v.) in mice. The tremorgenicity of **3** was almost as strong as that of paxilline (**1**), but **3** characteristically caused opisthotonus in mice along with tremors. The detail will be published elsewhere.

Experimental

Melting points were determined on a Yamagimoto micro-melting point apparatus and are uncorrected. IR and ultraviolet (UV) spectra were recorded on a JASCO IR-810 spectrometer and a Hitachi 124 spectrometer, respectively. EI mass spectra (MS) were obtained on a JEOL JMS-D 300 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM-GX 400 spectrometer at 399.78 MHz and at 100.43 MHz, respectively, using tetramethylsilane as an internal standard. CD curves were determined on a JASCO J-40 spectrometer. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low pressure liquid chromatography (LPLC) 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). TLC was conducted on pre-coated Kieselgel 60 F_{254} (Art. 5715; Merck).

Detection of Paxilline (1) in *Emericella* spp. The isolates, listed below, were each grown at 25 °C for 21 d in a Roux flask on modified Raulin-Thom medium (250 ml). The dried mycelium was extracted with acetone, and the evaporated extract was dissolved in chloroform (2 ml), then 10 μl of the solution was developed by TLC with bezene–acetone (10 : 1, v/v). The spot of **1** was detected by the use of van Urk's reagent (detection limit: 0.2 μg).

The isolates used were as follows: *E. acristata* (FENNELL & RAPER) HORIE, CBS 119.55 and IFM 4230; *E. aurantiobrunnea* (ATKINS, HINDSON & RUSSELL) MALLOCH & CAIN, IMI 74895; *E. bicolor* CHRISTENSEN & STATES, RMF H2058; *E. corrugata* UDAGAWA & HORIE, IFM 4521; *E. dentata* (SANDHU & SANDHU) HORIE, IFM 42024; *E. desertorum* CBS 653.73; *E. foveolata*, IFM 42015; *E. fruticulosa* (RAPER & FENNELL) MALLOCH & CAIN, CBS 650.73A; *E. heterothallica* (KWON, FENNELL & RAPER) MALLOCH & CAIN, ATCC 16847; *E. navahoensis* CHRISTENSEN & STATES, RMF SD-7; *E. nidulans* (EIDAM) VUILLEMIN var. *lata* (FENNELL & RAPER) SUBRAMANIAN, CBS 492.65; *E. purpurea* SAMSON & MOUCHACCHA, CBS 754.74; *E. quadrilineata*, IFM 42006 and IFM 42021; *E. rugulosa* (THOM & RAPER) C. R. BENJAMIN, IFO 8629; *E. spectabilis* CHRISTENSEN, RMF H429; *E. striata*, IMI 163899; *E. sublata* HORIE, IFM 4553; *E. unguis* MALLOCH & CAIN, ATCC 16812; *E. variecolor* BERKELEY & BROOME, NHL 2881.

Isolation of 1′-*O*-Acetylpaxilline (3) *Emericella striata*, strain 80-NE-22, was cultivated at 30 °C for 21 d in Czapek-Dox medium (50 l). The dried mycelia (660 g) were extracted with acetone at room temperature. The acetone extract (26 g) was chromatographed on silica gel with benzene–acetone (50 : 1, v/v) followed by column chromatography with benzene–ethyl acetate (5 : 1, v/v). A fraction was further purified by repeated LPLC [hexane–ethyl acetate (10 : 1, v/v)] to give 1′-*O*-acetylpaxilline (**3**) (304 mg).

1′-*O*-Acetylpaxilline (**3**): Colorless needles, mp 262—265 °C (sublim.) from AcOEt. IR ν_{max}^{KBr} cm^{-1}: 3480, 3400 (OH, NH), 1728 (OAc), 1680 (–CO–). UV λ_{max}^{EtOH} nm (log ε): 231 (4.62), 280 (3.97), 290 sh (3.92). EI-MS *m/z* (%): 477 (100, M^+), 462 (75, $[M\text{-}Me]^+$), 417 (22, $[M\text{-}AcOH]^+$), 402 (25, $[M\text{-}AcOH\text{-}Me]^+$), 384 (20, $[M\text{-}AcOH\text{-}Me\text{-}H_2O]^+$), 182 (65), 130 (45, C_9H_8N). *Anal.* Calcd for $C_{29}H_{35}NO_5$: C, 72.93; H, 7.39; N, 2.93. Found: C, 73.07; H, 7.42; N, 2.81. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, s, Me), 1.32 (3H, s, Me), 1.43 (3H, s, Me), 1.50 (1H, m), 1.61 (1H, s, OH), 1.65 (3H, s, Me), 1.55—2.08 (6H, m), 2.04 (3H, s, OAc), 2.27 (1H, m), 2.44 (1H, dd, J = 13.1, 10.9 Hz), 2.73 (1H, dd, J = 13.1, 6.1 Hz), 2.80 (1H, m), 4.77 (1H, br t, J = 10.0 Hz, –CH_2–C<u>H</u>–O–), 4.79 (1H, d, J = 1.8 Hz, –C<u>H</u>–O–), 5.78 (1H, d, J = 1.8 Hz, –C=C<u>H</u>–), 7.07 (2H, m), 7.29 (1H, m), 7.43 (1H, m),

7.83 (1H, br s, NH). CD [θ] (nm): −80400 (244), −1090 (268 sh), +8260 (296), +3430 (338).

Acetylation of Paxilline (1) Paxilline (**1**) (110 mg) was acetylated overnight at room temperature with acetic anhydride (1 ml) and pyridine (1 ml) in addition to 4-dimethylaminopyridine (50 mg). The reaction mixture was poured into ice-water, and the collected precipitate was chromatographed on silica gel with benzene–acetone (100 : 1, v/v) followed by LPLC using cyclohexane–ethyl acetate (5 : 1, v/v) to give a monoacetate (18 mg). This acetate was identical with 1′-*O*-acetylpaxilline (**3**), based on a comparison of the ^{1}H-NMR and IR spectra and the TLC behavior, and mixed melting point determination.

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References and Notes

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