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Three New Types of Indoloditerpenes, Emindole PA—PC, from *Emericella purpurea*. Revision of the Structure of Emindole PA

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Three new type indoloditerpenes, emindoles PA (1), PB (2), and PC (3), were isolated from the mycelium of *Emericella purpurea* along with the sesterterpenes variecolol and variecolactone, and the dicyanide derivatives epurpurins A to C. The structures of 1—3 were confirmed by the spectroscopic investigation. The structure of emindoles PA (1) was revised from our preliminary report. Emindoles PA (1), PB (2), and PC (3) are the indoloditerpenes having a new type of carbon skeleton.

Key words *Emericella purpurea*; indoloditerpene; emindole PA; emindole PB; emindole PC

Two new sesterterpenes, variecolol and variecolactone,[1)] which showed bluish coloration by spraying modified Ehrlich's reagent,[2)] were isolated along with valiecolin from *Emericella purpurea* Samson & Mouchacca IFO 30849 that was isolated from Egyptian desert soil.[3)] Recently, new 1,4-di-(4-hydroxyphenyl)-2,3-dicyano-1,3-butadiene derivatives, designated epurpurins A to C,[4)] were also isolated. Three new compounds with reddish coloration by spraying modified Ehrlich's reagent,[2)] designated emindoles PA (**1**),[5)] PB (**2**) and PC (**3**), were isolated from the above fungus. The structure determination of **1**, **2** and **3** are reported in this paper.

The molecular formulae of **1** and **3** were confirmed as $C_{33}H_{47}NO_2$ by high resolution electron-impact ionization (EI) mass spectrometry. A positive coloration with modified Ehrlich's reagent (reddish purple)[2)] and the fragment ion at *m/z* 130 $[(C_9H_8N)^+]$ in the EI mass spectra suggested the presence of an indole moiety in **1** and **3**. The ^{1}H-NMR spectra of **1** and **3** (Table 1) were similar to that of emindole DB (**4**), which had been isolated from *E. desertrum* Samson & Mouchacca,[6)] except for the appearance of the ^{1}H-NMR signals assigned as 1,1-dimethyl-2-propenyl residue [δ 6.12 (1H, dd), 5.13 (1H, dd), 5.15 (1H, dd), and 1.53 (6H, s) for **1** and **3**] and that of the vinylic proton [δ 5.53 (br d) for **1** and **3**] instead of those of the *exo*-methylene (δ 4.18, 4.51) in **4**. The presence of four aromatic protons [δ 7.50 (br d), 7.04 (td), 7.10 (td), and 7.26 (br d) in **1** and δ 7.50 (br d), 7.04 (br t), 7.11 (ddd), and 7.26 (br d) in **3**], the lack of the proton at C-2 in the indole moiety [δ 6.89 in **4**], and the strong fragment ion of *m/z* 198 $[(C_{14}H_{16}N)^+]$ in EI mass spectra in **1** and **3** showed the presence of an (2-(1,1-dimethyl-2-propenyl)indol-3-yl)methyl group. In a preliminary report,[5)] we reported the structure of emindole PA (**1**) as **1′** as shown in Chart 1, from the analysis of the ^{1}H–^{1}H and ^{1}H–^{13}C shift correlation (COSY) spectra and the ^{1}H–^{13}C long-range shift correlation (COLOC) spectrum. The ^{1}H-detected heteronuclear multiple-quantum coherence *via* direct coupling (HMQC) and heteronuclear multiple bond connectivity by 2D multiple-quantum NMR (HMBC) (Fig. 1) spectra of emindole PA have been carefully analysed. One of two methyl groups observed at δ 1.53, which was assigned as the olefinic methyl group in structure **1′** in the preliminary report,[5)] was not correlated to the olefinic carbon at δ 113.9 (C-15), whereas these two methyl protons were correlated with C-2 (δ 139.4) of the indole moiety and with C-29 (δ 146.5) of the 1,1-dimethyl-2-propenyl residue in the HMBC spectrum. Two methyl groups at δ 1.04 and 1.34 were correlated with each of the carbons (δ 25.5, 23.3) in the HMBC spectrum, indicating that two methyl groups were connected to one carbon at δ 39.8 (C-9). From the above results and further analysis of the HMBC spectrum of **1**, the planar structure of emindole PA should consequently be revised as **1** shown in Chart 1. The planar structure of emindole PC (**3**) was also determined as the same as emindole PA (**1**), from the detailed analyses of the HMQC and HMBC spectra.

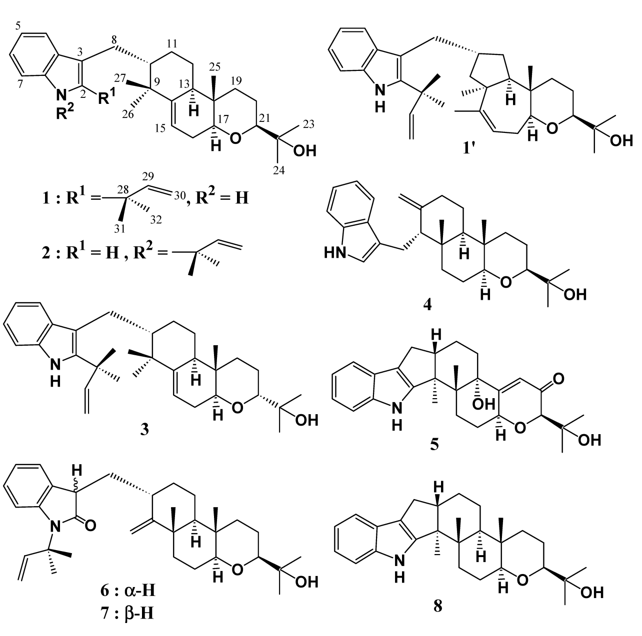

Chart 1

The stereochemistry of **1** was determined from the difference ^{1}H–^{1}H nuclear Overhauser enhancement (NOE) spectrum (Fig. 2). When one of the methyl protons at C-9 observed at δ 1.04 was irradiated, 4.6 and 5.2% of the NOE of the proton signal at 10-H [δ 1.65 (br t)] and one of the methylene protons at C-8 [δ 3.06 (dd)], respectively, along with 10.9 and 1.7% of the NOE of 15-H [δ 5.53 (br d)] and 4-H [δ 7.50 (br d)], respectively, whereas 7.7 and 5.2% of the NOE of 13-H [δ 1.90 (br d)] and another proton of the methylene at C-8 [δ 2.64 (dd)], respectively, were observed along with

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Table 1. ^{1}H- and ^{13}C-NMR Assignments for Emindoles PA (**1**), PB (**2**) and PC (**3**) in $CDCl_3$

Carbon No.	**1**		**2**	**3**	
	δ_C	δ_H (Hz)	δ_H (Hz)	δ_C	δ_H (Hz)
1-NH		7.82 br s			7.81 br s
2	139.4		7.02 br s	139.4	
3	110.5			110.5	
3a	130.4			130.4	
4	119.3	7.50 br d (7.9)	7.55[a] br d (7.3)	119.3	7.50 br d (7.9)
5	118.7	7.04 td (7.9, 0.9)	7.06 br t (7.3)	118.8	7.04 br t (7.9)
6	121.1	7.10 td (7.9, 0.9)	7.09 br t (7.3)	121.1	7.11 ddd (7.9, 7.0, 1.2)
7	110.2	7.26 br d (7.9)	7.48[a] br d (7.3)	110.2	7.26 dd (7.9, 0.9)
7a	134.3			134.4	
8	25.6	2.64 dd (14.0, 11.9)	2.26 dd (14.0, 11.0)	25.6	2.64 dd (14.3, 11.9)
		3.06 dd (14.0, 3.3)	3.08 br d (14.0)		3.05 dd (14.3, 3.4)
9	39.8			39.8	
10	46.5	1.65 br t (12.2)	1.70 (m)	46.7	1.65 m
11	26.8	1.27 m	1.30 (m)	26.9	1.25 m
		1.62 m	1.62 (m)		1.45 m
12	25.9	0.87 tdd (13.2, 12.8, 3.7)	0.92 (m)	25.9	0.88 dtd (13.1, 12.8, 3.4)
		1.62 m	1.63 (m)		1.58 m
13	45.5	1.90 br d (12.8)	1.90 (m)	45.8	1.95 br d (12.5)
14	147.0			147.7	
15	113.9	5.53 br d (6.1)	5.54 br d (6.1)	114.2	5.53 br d (6.1)
16	28.8	1.98 dddd (17.0, 10.4, 6.1, 2.2)	1.98 (m)	29.1	2.01 dddd (16.4, 10.4, 6.1, 1.8)
		2.07 ddd (17.0, 5.5, 1.5)	2.05 (m)		2.10 ddt (16.4, 5.5, 1.2)
17	79.8	3.21 dd (10.4, 5.5)	3.22 dd (10.4, 5.5)	73.2	3.64 dd (10.4, 5.5)
18	34.4			34.7	
19	36.3	1.25 m	1.30 (m)	33.4	1.39 m
		1.85 br ddd (12.8, 3.7, 3.4)	1.88 (m)		1.67 m
20	22.1	1.43 m	1.45 (m)	19.9	1.65 m
		1.47 dddd (13.2, 11.4, 10.8, 3.7)	1.45 (m)		1.72 m
21	83.9	3.13 dd (11.3, 2.7)	3.14 dd (10.4, 3.0)	79.1	3.52 dd (8.2, 5.2)
22	71.9			74.0	
22-OH		2.73 br s	2.70 br s		2.66 br s
23	23.7	1.15 s	1.16 s	26.5	1.20 s
24	26.0	1.16 s	1.19 s	25.4	1.18 s
25	13.2	0.71 s	0.70 s	16.1	0.75 s
26	25.5	1.34 s	1.35 s	25.5	1.34 s
27	23.3	1.04 s	1.01 s	23.3	1.03 s
28	39.2			39.2	
29	146.5	6.12 dd (17.4, 10.7)	6.15 dd (17.1, 11.0)	146.5	6.12 dd (17.4, 10.4)
30	111.7	5.13 dd (10.7, 0.9)	5.20 d (11.0)	111.7	5.13 dd (10.4, 0.9)
		5.15 dd (17.4, 0.9)	5.16 d (17.1)		5.15 dd (17.4, 1.2)
31	28.0	1.53 s	1.55 s	28.0	1.53 s
32	27.8	1.53 s	1.55 s	27.8	1.53 s

a) The signals may be reversed.

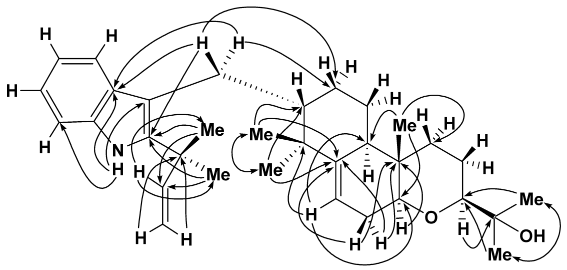

Fig. 1. The Main HMBC Correlations of Emindole PA (**1**)

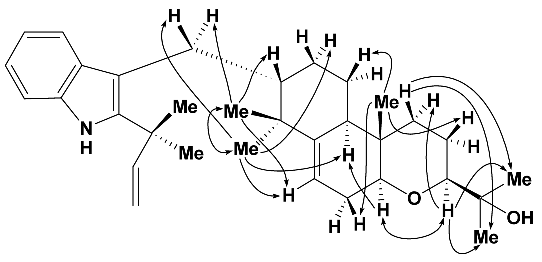

Fig. 2. The Selected NOE Correlations of Emindole PA (**1**)

1.8% of NOE' of 15-H, irradiating another methyl protons at C-9 (δ 1.34). Moreover, 7.7 and 8.0% of the NOE of 13-H and 21-H [δ 3.13 (dd)], respectively, were observed along with 4.4% of the NOE of one of the methylene protons at C-16 [δ 2.07 (ddd)], when H-17 [δ 3.21 (dd)] was irradiated. A NOE of 7.7% was observed at another proton of the methylene at C-16 [δ 1.98 (dddd)], when the methyl proton at C-18 (δ 0.71) was irradiated. The relative structure of emidole PA was consequently confirmed as **1**, as shown in Chart 1.

The ^{1}H-NMR signals of **3** were very similar to those of **1**, except for the chemical shifts of H-17 [δ 3.21 for **1** and δ 3.64 (dd) for **3**] and H-21 [δ 3.13 for **1** and δ 3.52 (dd) for **3**], and the coupling constants of 21-H [11.3 and 2.7 Hz for **1** and 8.2 and 5.2 Hz for **3**]. This fact suggested that **3** should be an epimer of **1** at C-21. The difference NOE spectrum of **3**

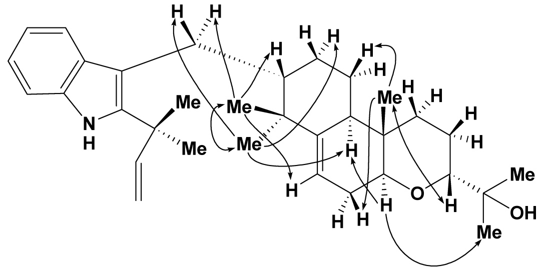

Fig. 3. The Selected NOE Correlations of Emindole PC (**3**)

(Fig. 3) showed correlations similar to that of **1**, except for 1.4% of NOE on two methyl protons of isopropyl residue (δ 1.18, 1.20) irradiating at H-17 [δ 3.64 (dd)] and 4.1% of NOE at 21-H [δ 3.52 (dd)], irradiating the methyl proton at C-18 (δ 0.75). From the above result, the relative structure of emidole PC was consequently confirmed as **3**, as shown in Chart 1. The absolute configurations of **1** and **3** have not yet been determined, but it is assumed that the absolute structure of **1** and **3** might be as shown in Chart 1, taking into consideration that indoloditerpenes, ever isolated from fungal sources, *i.e.* emindole DB (**4**) and the tremorgenic mycotoxin, paxilline (**5**)[6] *etc.* have the same absolute configuration.

The molecular formula of emindole PB (**2**) was confirmed as $C_{33}H_{47}NO_2$, which is the same as those of **1** and **3**, by high resolution EI mass spectrometry. A positive coloration with modified Ehrlich's reagent (reddish purple)[2] and the fragment ion at *m/z* 130 [$(C_9H_8N)^+$] in the EI mass spectrum suggested the presence of an indole moiety in **2**. The ^{1}H-NMR spectrum of **2** was closely similar to that of emindole PA (**1**), except for the appearance of the proton at C-2 [δ 7.02 (br s)] in the indole moiety, which was the same as that of **4** [δ 6.89 (br s)], and the disappearance of the NH proton [δ 7.82 (br s) for **1** and δ 7.81 (br s) for **3**]. This result suggested that the 1,1-dimethyl-2-propenyl residue [δ 6.15 (1H, dd), 5.20 (1H, d), 5.16 (1H, d), and 1.55 (6H, s)] in **2** was attached at N-atom, not at C-2. From the comparison of the ^{1}H- and ^{13}C-NMR spectra with **1** and **2** and the analysis of the ^{1}H–^{1}H COSY spectrum of **2**, a planar structure for the emindoles PB (**2**) was assumed, although it could not be confirmed because of the low yield and the unstableness of **2**. The stereochemistry of **2** was assumed to be the same as **1** rather than **3** based on a comparison of the chemical shifts of the diterpene moiety and the coupling constants of 21-H [11.3 and 2.7 Hz for **1**; 10.4 and 3.0 Hz for **2**; 8.2 and 5.2 Hz for **3**] in the ^{1}H-NMR spectra (Table 1).

Emidoles PA (**1**), PB (**2**), and PC (**3**) are a new type of indoloditerpenes bearing a 1,1-dimethyl-2-propenyl residue at C-2 or N-1 in the indole moiety. It is very interesting that indoloditerpenes having similar carbon skeletons as **1**—**3**, anthcolorins A—H (**6** for A, **7** for B), were recently isolated from *Aspergillus versicolor* (VUILLEMIN) TIRABOSCHI separated from *Anthocidaris* sea urchin.[7] Acklin *et al.* reported[8] that indoloditerpenes such as paspaline (**8**) and paxilline (**5**) were derived from tryptophan and geranylgeraniol with skeletal rearrangement from a five membered-ring to a six membered-ring during the biosynthetic process in *Claviceps paspali* STEVENS & HALL. Anthcolorins A (**6**) and B (**7**) had the same carbon skeleton as paspaline (**8**) without the further ring closure between C-2 and C-9, whereas in emindoles PA (**1**)—PC (**3**) the further migration of the methyl group from C-12 to C-9 occurred.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. EI-MS were taken with a JEOL JMS-MS600W spectrometer. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL Lambda-500 (^{1}H, 500.00 MHz; ^{13}C, 125.43 MHz) spectrometer, using tetramethylsilane as an internal standard. CD curves were determined on a JASCO J-600 spectropolarimeter. Column chromatography was performed using Kieselgel 60 (Art. 7734, Merck). Low-pressure liquid chromatography (LPLC) was performed with a FMI pump RP-SY and glass column (350×25 mm) packed with Silica gel CQ-3 (30—50 μm, Wako). HPLC was performed with a Senshu SSC-3160 pump (flow rate, 4 ml/min) and Senshu-Pak PEGASIL Silica 60-5 (300×10 mm), equipped with a Shimamura YRD-883 RI detector. TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected by UV light at 254 nm and/or by spraying with modified Ehrlich's reagent and then heating.[2]

Isolation of Emindoles PA (1)—PC (3) from *Emericella purpurea* *E. purpurea* IFO 30849 was cultivated in potato-dextrose medium (5 l) using 20 Roux flasks at 25 °C for 28 d. The dried mycelium (21 g) was pulverized and extracted with CH_2Cl_2 at room temperature and the organic layer was dried over Na_2SO_4 and then evaporated *in vacuo*. The extract obtained (1.7 g) was chromatographed on silica gel with C_6H_6–EtOAc (30 : 1) followed by the repeated purification by LPLC with C_6H_{14}–EtOAc (20 : 1) and/or $CHCl_3$ and then HPLC to obtain emindoles PA (**1**) (4.7 mg), PB (**2**) (0.8 mg), and PC (**3**) (1.4 mg).

Emindole PA (**1**): Colorless amorphous powder. Ehrlich's reagent: reddish violet. EI-MS *m/z* (%): 489.3605 (M^+, 489.3606 for $C_{33}H_{47}NO_2$, 6), 471 [$(M-H_2O)^+$, 1], 420.2850 [$(M-C_5H_9)^+$, 420.2900 for $C_{28}H_{38}NO_2$, 6], 198.1253 [$(C_{14}H_{16}N)^+$, 198.1281 for $C_{14}H_{16}N$, 100], 130.0664 [$(C_9H_8N)^+$, 130.0657 for C_9H_8N, 12]. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 227 (4.45), 285 (3.83), 292 (3.80). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3400 (OH), 1700, 1460. CD $\Delta\varepsilon$ (nm): −4.1 (228), +1.6 (290). The ^{1}H- and ^{13}C-NMR signals are summarized in Table 1.

Emindole PB (**2**): Colorless amorphous powder. Ehrlich's reagent: reddish violet. EI-MS *m/z* (%): 489.3614 (M^+, 489.3606 for $C_{33}H_{47}NO_2$, 6), 471 [$(M-H_2O)^+$, 14], 329 (8), 285 (18), 198.1245 [$(C_{14}H_{16}N)^+$, 198.1281 for $C_{14}H_{16}N$, 64], 130.0664 [$(C_9H_8N)^+$, 130.0657 for C_9H_8N, 76], 57 (C_4H_9, 100). The ^{1}H-NMR signals are summarized in Table 1.

Emindole PC (**3**): Colorless crystalline powder (from ether), mp 238—240 °C. Ehrlich's reagent: reddish violet. EI-MS *m/z* (%): 489.3605 (M^+, 489.3606 for $C_{33}H_{47}NO_2$, 30), 471 [$(M-H_2O)^+$, 13], 198 [$(C_{14}H_{16}N)^+$, 74], 130 [$(C_9H_8N)^+$, 100]. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 229 (4.13), 285 (3.56), 292 (3.53). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3400 (OH), 1700, 1460. CD $\Delta\varepsilon$ (nm): −2.2 (231), +0.9 (291). The ^{1}H- and ^{13}C-NMR signals are summarized in Table 1.

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