Prenylated Indole Alkaloid Derivatives from Marine Sediment-Derived Fungus *Penicillium paneum* SD-44

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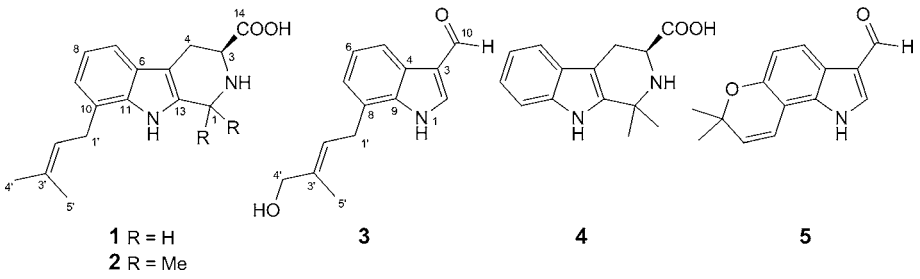
Three new prenylated indole alkaloids, including two β-carbolines, penipalines A and B (**1** and **2**, resp.), and one indole carbaldehyde derivative, penipaline C (**3**), as well as two known indole derived analogs, **4** and **5**, were isolated from the deep-sea-sediment derived fungus *Penicillium paneum* SD-44 cultured in a 500-l bioreactor. The structures of the new compounds were determined on the basis of 1D- and 2D-NMR spectroscopy, as well as by high-resolution mass spectrometry. The new compounds **2** and **3** showed potent cytotoxic activities against A-549 and HCT-116 cell lines.

Introduction. – Secondary metabolites from marine fungi, with diverse structures and promising bioactivities, have recently attracted great attention worldwide by marine chemists [1–4]. Indole alkaloids, which represent an important part of the marine-derived fungi metabolites, have diverse bioactive functions [5–8]. Our previous work revealed one novel triazole and two new quinazolinone alkaloids among the metabolites produced by the marine sediment-derived fungus *Penicillium paneum* SD-44, statically cultured in a rice medium [9]. During our ongoing exploration of new bioactive alkaloids from this strain by changing fermentation conditions, five new cytotoxic anthranilic acid derivatives were isolated from the AcOEt extract of the mycelia fermented in a 500-l bioreactor [10]. Further chemical studies of the liquid culture broth led to the isolation of three new prenylated indole alkaloids, including two β-carbolines, penipalines A and B (**1** and **2**, resp.), and one indole carbaldehyde derivative, penipaline C (**3**), as well as two known indole analogs **4** and **5**. Details of the isolation, structure elucidation, and biological activities are reported herein.

Results and Discussion. – *Structure Elucidation*. The decanted liquid culture broth was exhaustively extracted with AcOEt to yield an organic extract, which was fractionated by a combination of column chromatography (CC) with silica gel, reversed-phase (RP) silica gel *C18*, *Sephadex LH-20*, as well as semi-preparative HPLC to yield the five indole alkaloid derivatives **1**–**5**.

Compound **1** was isolated as yellowish solid. Its molecular formula was deduced as $C_{17}H_{20}N_2O_2$ from the HR-ESI mass spectrum (*m*/*z* 285.1611 ($[M+H]^+$; calc. 285.1603)), requiring nine degrees of unsaturation. In the ^{1}H-NMR spectrum, the splitting pattern and coupling constants of the aromatic H-atoms H–C(7), H–C(8), and H–C(9) (*Table*) implied the presence of a 1,2,3-trisubstituted benzene ring.



Further, signals of four Me, two CH_2, two CH groups (one olefinic), and five quaternary C-atoms (one C=O) were also observed in the ^{1}H- and ^{13}C-NMR spectra. Among them, the deshielded sp^3 CH_2 and CH C-atoms resonating at δ(C) 40.4 and 55.9, respectively, were deduced to be nitrogenated. Comprehensive analyses of the NMR data, and the characteristic absorption bands at 221 and 279 nm in the UV spectrum suggested that compound **1** should be a β-carboline derivative [11]. The observed COSY correlations between the sp^3 H-atoms H–C(3) and H–C(4), and the HMBCs from these H-atoms to the C=O C-atom evidenced the position of the COOH group at C(3) (*Fig.*). Additionally, the characteristic signals for a isopentenyl moiety were detected in the 1D-NMR spectra of **1**. This observation was verified by the COSY correlation between CH_2(1′) and H–C(2′), and by the HMBC from the two and Me(5′) to the olefinic C-atoms C(2′) and C(3′). On the other hand, the HMBC cross-peaks from H–C(1′) to the aromatic C-atoms C(9), C(10), and C(11), and from H–C(2′) to C(10) unambiguously indicated the position of the isopentenyl moiety as C(10). The configuration at the stereogenic center (C(3)) in **1** was tentatively deduced as (*S*) by comparison of its optical rotation ($[\alpha]_D^{25} = -22.9$ ($c = 0.40$, MeOH)) with that of the lycopedine-1 [11][12]. Thus, the structure of **1** was assigned as (−)-(3*S*)-2,3,4,9-tetrahydro-8-(3-methylbut-2-en-1-yl)-1*H*-β-carboline-3-carboxylic acid, named penipaline A.

Compound **2**, a yellowish powder, had the molecular formula $C_{19}H_{24}N_2O_2$ as deduced from the HR-ESI mass spectrum (*m*/*z* 313.1911 ($[M+H]^+$; calc. 313.1915)), implying nine degrees of unsaturation. Comparison of the NMR data of **2** with those of **1** indicated that the structures of these two compounds are very similar, except that one N-bound CH_2 group (C(1); δ(C) 40.4) in **1** was replaced by a quaternary C-atom (δ(C)

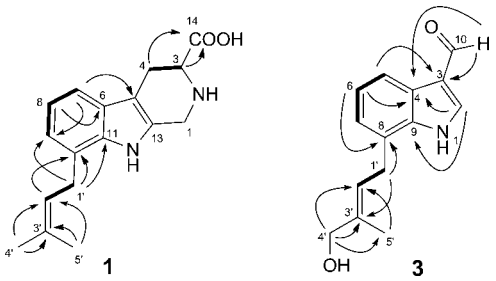


Figure. *Key HMBCs* (arrows) *and COSY correlations* (bold lines) *of compounds* **1** *and* **3**

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of Compounds* **1**–**3**. δ in ppm, J in Hz. Assignments were corroborated by $^{1}H,^{1}H$-COSY, HSQC, and HMBC experiments. Arbitrary atom numbering as indicated in the *Formulae*.

Position	**1**[a])		**2**[a])		**3**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	4.30 (*d*, *J* = 15.5) 4.27 (*d*, *J* = 15.5)	40.4 (*t*)	–	54.6 (*s*)	–	–
2	–	–	–	–	8.09 (*s*)	139.4 (*d*)
3	3.85 (*dd*, 4.8, 10.8)	55.9 (*d*)	3.82 (*dd*, *J* = 4.6, 12.0)	53.2 (*d*)	–	120.5 (*s*)
4	3.17 (*dd*, *J* = 4.8, 15.9), 2.86 (*dd*, *J* = 10.8, 15.9)	22.7 (*t*)	3.13 (*dd*, *J* = 4.6, 15.9), 2.77 (*dd*, *J* = 12.0, 15.8)	23.6 (*t*)	–	125.9 (*s*)
5	–	106.5 (*s*)	–	105.6 (*s*)	7.10 (*d*, *J* = 7.5)	124.4 (*d*)
6	–	126.0 (*s*)	–	125.9 (*s*)	7.17 (*t*, *J* = 7.5)	123.9 (*d*)
7	7.27 (*d*, *J* = 7.7)	115.4 (*d*)	7.25 (*d*, *J* = 7.8)	115.5 (*d*)	8.00 (*d*, *J* = 7.5)	120.3 (*d*)
8	6.92 (*t*, *J* = 7.5)	119.0 (*d*)	6.93 (*t*, *J* = 7.5)	119.0 (*d*)	–	126.4 (*s*)
9	6.86 (*d*, *J* = 7.1)	120.5 (*d*)	6.85 (*d*, *J* = 7.1)	120.3 (*d*)	–	137.6 (*s*)
10	–	124.0 (*s*)	–	124.1 (*s*)	9.89 (*s*)	187.5 (*d*)
11	–	134.9 (*s*)	–	134.9 (*s*)	–	–
12	10.90 (*s*)	–	10.7 (*s*)	–	–	–
13	–	134.8 (*s*)	–	136.s (*s*)	–	–
14	–	169.8 (*s*)	–	169.8 (*s*)	–	–
15	–	–	1.64 (*s*)	25.8 (*q*)	–	–
16	–	–	1.76 (*s*)	25.9 (*q*)	–	–
1′	3.48 (*d*, *J* = 7.2)	29.0 (*t*)	3.52 (*d*, *J* = 6.0)	28.7 (*t*)	3.67 (*d*, *J* = 7.2)	29.9 (*t*)
2′	5.40 (*t*, *J* = 7.2)	122.0 (*d*)	5.42 (*t*, *J* = 6.7)	122.1 (*d*)	5.71 (*td*, *J* = 7.2, 1.3)	123.5 (*d*)
3′	–	132.0 (*s*)	–	131.8 (*s*)	–	138.1 (*s*)
4′	1.71 (*s*)	25.4 (*q*)	1.72 (*s*)	25.4 (*q*)	4.00 (*s*)	68.6 (*t*)
5′	1.71 (*s*)	17.6 (*q*)	1.72 (*s*)	17.6 (*q*)	1.80 (*s*)	13.9 (*q*)

[a]) Recorded in (D_6)DMSO. [b]) Recorded in CD_3OD.

54.6), and two more Me groups were present in **2**. This observation implied that the H-atoms at C(1) in **1** was replaced by two Me groups in **2**, which was verified by the HMBC data from the two Me groups to C(1). The configuration at C(3) was also determined as (*S*) by comparison of the optical rotation ($[\alpha]_D^{20} = -15.7$ ($c = 0.30$, MeOH)) with those of the reported analogs [11][12]. Thus, the structure of **2** was determined as (−)-(3*S*)-2,3,4,9-Tetrahydro-1,1-dimethyl-8-(3-methylbut-2-en-1-yl)-1*H*-*β*-carboline-3-carboxylic acid, named penipaline B.

Compound **3** was obtained as a colorless powder. The HR-ESI mass spectrum provided the molecular formula $C_{14}H_{15}NO_2$, indicating eight degrees of unsaturation. Comprehensive analysis of the ^{1}H- and ^{13}C-NMR spectra of **3** revealed the presence of one CHO, one olefinic Me, and one O-bearing CH_2 group, one C=C bond, and four aromatic H-atoms. The spin-coupling patterns suggested a trisubstituted indole system with three adjacent H-atoms [13]. Detailed comparison of the 1D-NMR data of **3** (recorded in CD_3OD) with those of 7-(3-methylbut-2-enyl)-1*H*-indole-3-carbaldehyde indicated that both structures were very similar, except that one olefinic Me group in the latter was replaced by an O-bearing CH_2 group in **3** [14]. This deduction was verified by the HMBC data as shown in the *Figure*. The key HMBCs from CH_2(1′) and CH_2(4′) (δ(H) 4.00) to the olefinic C-atoms C(2′) and C(3′), respectively, as well as to the Me(5′) group further supported the oxygenated substitution at C(4′). The NOE correlation CH_2(4′)/H–C(2′) indicated the (*E*) configuration of the C=C bond. Thus, the structure of **3** was established as 7-[(2*E*)-4-hydroxy-3-methylbut-2-en-1-yl]-1*H*-indole-3-carbaldehyde, named penipaline C.

In addition to **1**–**3**, two known compounds, (−)-(3*S*)-2,3,4,9-tetrahydro-1,1-dimethyl-1*H*-*β*-carboline-3-carboxylic acid (**4**) [15] and 1,7-dihydro-7,7-dimethylpyrano[2,3-*g*]indole-3-carbaldehyde (**5**) [16], were also isolated and identified.

Biological Activity. The new compounds **1**–**3** were tested for their cytotoxic activities against the two tumor cell lines A-549 and HCT-116. The results indicated that compounds **2** and **3** were active against both cell lines. The IC_{50} values of **2** and **3** against A-549 were 20.44 and 21.54 μM, respectively, while those against HCT-116 were 14.88 and 18.54 μM.

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Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2; 100–200 and 200–300 mesh; *Qingdao Haiyang Chemical Factory*), *Lobar LiChroprep RP-18* gel (40–63 mm; *Merck*), and *Sephadex LH-20* gel (*Pharmacia*). Anal. and semi-prep. HPLC: *Dionex* HPLC system equipped with a *P680 pump*, an *ASI-100* automated sample injector, a *TCC-100* column oven, and a *UV-DAD 340U* detector. UV Spectra: *Gold Spectrumlab 54* UV/VIS spectrophotometer (*Shanghai Lengguang Tech. Co.*); λ_{max} (log ε) in nm. ^{1}H- and ^{13}C-NMR Spectra: *Bruker Avance 500* spectrometer (at 500 and 125 MHz, resp.); chemical shifts, δ, in ppm rel. to Me_4Si as internal standard, *J* in Hz. Low- and high-resolution (LR and HR, resp.) ESI-MS: *VG Autospec-3000* mass spectrometer, in *m*/*z*.

Fungal Material. The procedures of isolation and identification of the fungal strain used in this experiment were described in [10].

Fermentation, Extraction, and Isolation. The procedure of fermentation was described in an earlier report. The fermented mycelia and broth were separated by centrifugation, and the liquid culture broth was exhaustively extracted with AcOEt to yield an org. extract (76.0 g). The extract was fractionated by SiO_2 vacuum liquid chromatography (VLC) with $CHCl_3$/MeOH from $0:1 \rightarrow 0:1$ to afford eight fractions, *Frs. 1–8*. *Fr. 3* (2.4 g), eluted with $CHCl_3$/MeOH 20:1, was further purified by CC (*Lobar LiChroprep C18*; MeOH/H_2O $2:8 \rightarrow 1:0$), and then subjected to a further CC (*Sephadex LH-20*; MeOH) to afford **3** (12.7 mg), **4** (14.2 mg), and **5** (11.2 mg). *Fr. 4* (4.1 g) was seperated by CC (*Lobar LiChroprep C18*; MeOH/H_2O $2:8 \rightarrow 1:0$), and then subjected to a further CC (*Sephadex LH-20*; MeOH) and semi-prep. HPLC (*Elite ODS-BP*, 10 μm; 10.0 × 300 mm; 3 ml/min) to yield **1** (t_R 18.5 min; 4.8 mg; 68% MeOH/H_2O), and **2** (t_R 21.3 min; 7.6 mg; 68% MeOH/H_2O).

Penipaline A (*=(−)-(3S)-2,3,4,9-Tetrahydro-8-(3-methylbut-2-en-1-yl)-1*H*-β-carboline-3-carboxylic Acid*; **1**). Yellowish solid. $[\alpha]_D^{25} - 22.9$ ($c = 0.40$, MeOH). UV (MeOH): 221 (4.31), 279 (3.53). ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 285.1611 ($[M+H]^+$, $C_{17}H_{21}N_2O_2^+$; calc. 285.1603).

Penipaline B (*=(−)-(3S)-2,3,4,9-Tetrahydro-1,1-dimethyl-8-(3-methylbut-2-en-1-yl)-1*H*-β-carboline-3-carboxylic Acid*; **2**). Yellowish powder. $[\alpha]_D^{25} = -15.7$ ($c = 0.30$, MeOH) UV (MeOH): 221 (4.35), 281 (3.57). ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 313.1911 ($[M+H]^+$, $C_{19}H_{25}N_2O_2^+$; calc. 313.1915).

Penipaline C (*=7-[(2*E*)-4-Hydroxy-3-methylbut-2-en-1-yl]-1*H*-indole-3-carbaldehyde*; **3**). Colorless powder. UV (MeOH): 213 (4.73), 249 (4.23), 258 (3.07), 302 (4.18). ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 230.1178 ($[M+H]^+$, $C_{14}H_{16}NO_2^+$; calc. 230.1176).

Cytotoxicity Assay. The cytotoxic activities against A-549 (human lung cancer), and RKO (human colon cancer) cell lines were determined by the MTT (= 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method [17].

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