Chem. Pharm. Bull. 45(10) 1694—1696 (1997) Vol. 45, No. 10

Structures of a New Type of Indoloditerpene, Petromindole, and a New Asterriquinone Derivative, PM-53, from the Ascostromata of *Petromyces muricatus*

Mikiko OOIKE,[a] Koohei NOZAWA,[a] Shun-ichi UDAGAWA,[b] and Ken-ichi KAWAI*,[a]

Faculty of Pharmaceutical Sciences, Hoshi University,[a] 2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan and Nodai Research Institute, Tokyo University of Agriculture,[b] 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156, Japan.

Received April 7, 1997; accepted June 4, 1997

A new type of indoloditerpene, petromindole (1), and a new asterriquinone derivative, PM-53 (2), were isolated from the ascostromata of *Petromyces muricatus*, along with bisindolylbenzenoids, petromurins A (3)—D (6). The structure of 1 was suggested by spectroscopic data and confirmed by an X-ray crystallographic analysis, while that of 2 was determined by analyses of the various NMR spectra.

Key words *Petromyces muricatus*; indoloditerpene; petromindole; bisindolylbenzenoid; asterriquinone

In the course of a search for indoloditerpenes in sclerotium- or ascostromata-producing fungi using Ehrlich's reagent,[1)] we previously reported the isolation of an anti-insecticidal indoloditerpene, nominine (originally isolated from the sclerotia of *Aspergillus nomius* KURTZMAN *et al.*[2)]), from the ascostromata of *Petromyces alliaceus* MALLOCH *et* CAIN, along with bicoumarins.[3)] It is, therefore, of great interest to examine the profile of sclerotial metabolites of *Petromyces muricatus* UDAGAWA *et al.*, which characteristically produces pale yellow sclerotioidal bodies that may be converted slowly into ascostromata. As a result of an investigation of the metabolites, four bisindolylbenzenoids, petromurins A (**3**) to D (**6**),[4)] were isolated from the ascostromata of *P. muricatus* strain IMI 368521, a fungus isolated from grassland soil of the Philippines.[5)] Further investigation of the above extract afforded two new compounds, *i.e.*, petromindole (**1**), which showed a blue coloration with Ehrlich's reagent,[1)] and PM-53 (**2**), a pigment with a deep blue color. The structure determination of **1** and **2** is described here.

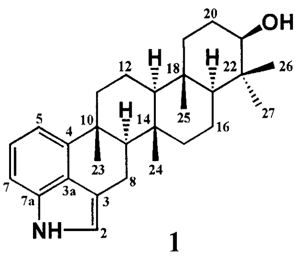
Structure of Petromindole (1)

The molecular formula of **1** was confirmed to be $C_{28}H_{39}NO$ by high-resolution electron-impact ionization mass spectrometry (EI-MS). A positive coloration with Ehrlich's reagent,[1)] and the fragment ion observed at m/z 130 $[(C_9H_8N)^+]$ in the EI-MS suggested the presence of an indole moiety in **1**. Compound **1** showed signals due to five tertiary methyl groups (δ 0.69, 0.85, 0.86, 1.02, and 1.08) and one secondary hydroxyl group [δ 4.27 (d, J=5.2 Hz, OH) and 2.95 (ddd, J=9.5, 5.2, and 4.5 Hz)] in the ^{1}H-NMR spectrum. The three protons observed at δ 6.67 (br d), 6.94 (dd), and 7.03 (br d) may be assigned to the adjacent protons of the benzene ring of the indole moiety, whereas the proton at δ 6.87 (br s) should be assigned to the proton at C-2 in the indole. Therefore the presence of a 3,4- or 3,7-disubstituted indole structure in **1** was assumed. Analysis of the ^{1}H-detected heteronuclear multiple-quantum coherence *via* direct coupling (HMQC) and the heteronuclear multiple bond connectivity by 2D multiple quantum NMR (HMBC) spectra of **1** suggested the indicated structure (**1**).

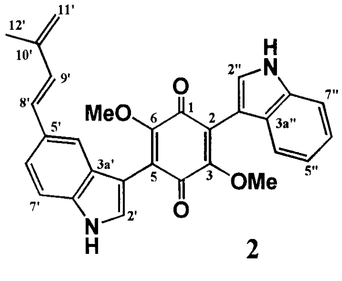
In order to confirm the structure of petromindole (**1**), an X-ray crystallographic analysis was undertaken. The compound was crystallized from acetone as colorless plates. The crystal structure of **1** was established to be as shown in Fig. 1. The molecules of **1** are mainly packed by van der Waals forces and no hydrogen bonding was observed. The relative structure of petromindole was consequently established to be as shown in **1**.

Structure of PM-53 (2)

The molecular formula of PM-53 (**2**) was confirmed to be $C_{29}H_{24}N_2O_4$ by high-resolution EI-MS (m/z 464.1735). The bathochromic coloration (dark blue, absorption at 614 nm in the UV spectrum) and the observation of the ion peak at m/z 466 (M+2) in the EI-MS suggested the presence of a benzoquinone moiety in the molecule of **2**.[6)] It was concluded that **2** has two methoxy groups (δ 3.59 and 3.62), one 3-methyl-1,3-butadienyl residue [δ 6.66 (1H, br d), 6.87 (1H, br d), 4.99 (1H, br s), 5.09 (1H, br s),



1



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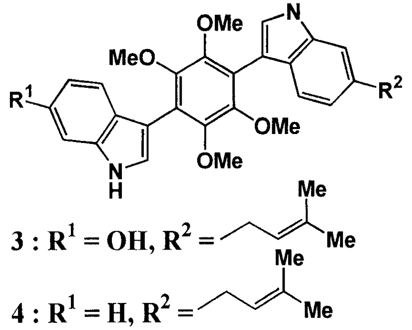

5 : R^1 = OH, R^2 = H

6 : R^1 = OH, R^2 = OMe

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* To whom correspondence should be addressed.

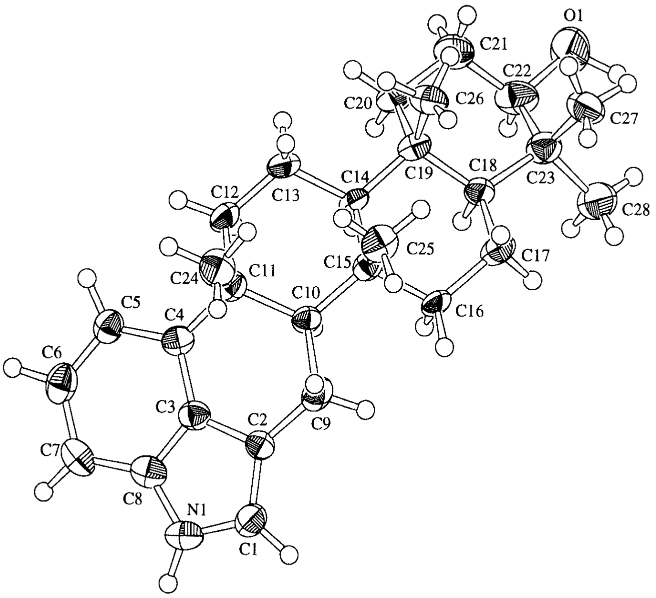

Fig. 1. Crystal Structure of Petromindole (**1**) with Thermal Ellipsoids of 50% Probability

and 1.92 (3H, s)], and two indole residues: 3,5- or 3—6-disubstituted [δ 7.45 (br s), 7.49 (br s), 7.37 (dd), 7.40 (d), and 11.46 (NH)] and 3-substituted [δ 7.50 (br s), 7.43 (br d), 7.05 (br t), 7.12 (br dd), 7.44 (br d), and 11.47 (NH)], from the analysis of the ^{1}H-NMR spectrum and the decoupling experiments. The ^{13}C-NMR spectrum (Table 1) indicated that the above two methoxy groups and the two indole residues are connected to the benzoquinone residue. Therefore **2** should be 3,6-dimethoxy-2,5-bis-indolyl-1,4-benzoquinone.

The structure of PM-53 (**2**) was confirmed by detailed analysis of the HMBC spectrum (Table 1). The proton observed at δ 7.37 (dd, J = 8.4, 1.5 Hz) was correlated with the carbon at δ 135.5, a characteristic signal of carbon at the 7a′ position of an indole. The signal at δ 112.0, due to carbon bearing the proton at δ 7.40 (d), was assigned to C-7′, because the signal of hydrogen-bearing carbon at C-7 in the indole ring is generally observed around δ 110 ppm.[7] The carbon at C-7″ of the other indole was observed at δ 111.7. It is therefore confirmed that the 3-methyl-1,3-butadienyl residue was connected at C-5 of the indole ring. Therefore the structure of PM-53 was determined to be as shown in **2**.

Discussion

Petromindole (**1**) is an indoloditerpene having a novel carbon skeleton and is the first example of an indoloditerpene, in which the diterpene is connected to the C-3 and C-4 positions of the indole moiety. The carbon skeleton of this compound is similar to that of a pentacyclic triterpene, although the absolute stereochemistry of **1** has not been determined yet.

PM-53 (**2**) is one of the bisindolylbenzoquinone derivatives, so-called "asterriquinones".[8] This fungus, *P. muricatus*, produced large amount of bisindolylbenzenoids such as petromurin A (**3**)—D (**6**), but little of the corresponding quinones from the ascostromata.

Table 1. ^{1}H- and ^{13}C-NMR Signals of PM-53 (**2**) in $(CD_3)_2SO$

Carbon No.	δ_C	δ_H[a]	HMBC correlation[b]
1	179.8[c]		
2	160.9[d]		
2-OMe	60.5[e]	3.59[c]	5
3	116.9[f]		
4	179.9[c]		
5	161.4[d]		
5-OMe	60.7[e]	3.62[c]	2
6	117.4[f]		
1′ (NH)		11.46[d]	
2′	127.8	7.45 br s	3′, 3a′, 7a′
3′	105.2		
3a′	127.3		
4′	119.2	7.49 br s	6′, 7a′, 8′
5′	128.4		
6′	119.7	7.37 dd (8.4, 1.5)	4′, 7a′, 8′
7′	112.0	7.40 d (8.4)	3a′, 5′
7a′	135.5		
8′	130.4	6.66 br d (16.1)	6′, 10′
9′	128.4	6.87 br d (16.1)	5′, 10′, 11′
10′	141.9		
11′	115.9	4.99 br s	9′, 12′
		5.09 br s	9′, 12′
12′	18.5	1.92 s	10′, 11′
1″ (NH)		11.47[d]	
2″	127.5	7.50 br s	3″, 3a″
3″	104.7		
3a″	126.7		
4″	120.2	7.43 br d (8.4)	3a″, 5″, 6″
5″	119.3	7.05 br t (8.4)	3a″, 7″
6″	121.3	7.12 br dd (8.4, 8.0)	7″, 7a″
7″	111.7	7.44 br d (8.0)	6″, 7″
7a″	135.6		

a) Coupling patterns and coupling constants (Hz in parentheses) are also indicated. *b*) Correlation from the proton to carbons are indicated. *c*—*f*) Assignments may be reversed.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. EI-MS were taken with a JEOL JMS-D-300 spectrometer. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^{1}H-NMR and ^{13}C-NMR were recorded on a JEOL Lambda-500 (^{1}H, 500.00 MHz; ^{13}C, 125.43 MHz) or a JEOL Lambda-600 (^{1}H, 600.05 MHz; ^{13}C, 150.80 MHz) spectrometer, using tetramethylsilane as an internal standard. Coupling patterns are indicated as follows: singlet = s, doublet = d, triplet = t, quartet = q, multiplet = m, and broad = br. Circular dichroism (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 Chemco Low-Prep 81-M-2 pump and a glass column (200 × 10 mm) packed with silica gel CQ-3 (30—50 μm, Wako). HPLC was performed with a Senshuu SSC-3160 pump (flow rate, 3 ml/min) and a YMC-Pack SIL-06 pre-packed column (300 × 10 mm), equipped with a Shimamura YAD-883 RI detector. TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected on the basis of their absorption or fluorescence under UV light, or by spraying modified Ehrlich's reagent and then heating.[1]

Isolation of Petromindole (1) and PM-53 (2) from *Petromyces muricatus* *P. muricatus*, strain IMI 368521, was cultivated in 480 Petri dish plates (i.d. 90 mm) containing 25 ml of Czapek-yeast extract agar (CYA) medium in each dish at 35 °C for 21 d. The fresh ascostromata were collected and extracted with $CHCl_3$ at room temperature. The evaporation residue (1.8 g) was dissolved in $CHCl_3$ (30 ml) and the soluble portion was chromatographed on silica gel. Elution with cyclohexane–EtOAc (5 : 1) followed by repeated purification by LPLC [cyclohexane–acetone (5 : 1)] gave petromindole (**1**) (3 mg), and elution with cyclohexane–EtOAc (2 : 1) followed by repeated purification by LPLC and HPLC [cyclohexane–acetone (2 : 1)] gave PM-53 (**2**) (3 mg).

Petromindole (**1**): Colorless plates (from acetone); van Urk's reagent, blue; mp >300 °C. EI-MS *m/z* (%): 405.3034 (M^+, 405.3032 for $C_{28}H_{39}NO$, 6), 387 [$(M-H_2O)^+$, 100]. UV $\lambda_{max}^{dioxane}$ nm (log ε): 226 (4.24), 282 (3.65), 293 (3.56). IR ν_{max}^{KBr} cm^{-1}: 3500, 3400 (OH, NH). ^1H-NMR [$(CD_3)_2SO$] δ: 0.69 [3H, s, 27-H_3 (Me)], 0.70 (1H, m, 17-H), 0.79 (1H, br d, J=11.9 Hz, 13-H), 0.85 [3H, s, 25-H_3 (Me)], 0.86 [3H, s, 26-H_3 (Me)], 0.90 (1H, m, 16-H), 0.93 (1H, m, 15-H), 1.02 [3H, s, 24-H_3 (Me)], 1.08 (3H, s, 23-H_3 (Me)], 1.08 (1H, m, 19-H), 1.40 (1H, dd, J=12.5, 3.3 Hz, 9-H), 1.42—1.59 (6H, m), 1.65 (1H, m, 12-H), 1.69 (1H, m, 16-H), 1.94 (1H, br d, J=12.5 Hz, 15-H), 2.43 (1H, m, 11-H), 2.60 (1H, br dd, J=14.7, 12.5 Hz, 8-H), 2.85 (1H, dd, J=14.7, 3.3 Hz, 8-H), 2.95 (1H, ddd, J=9.5, 5.2, 4.5 Hz, 21-H), 4.27 (1H, br d, J=5.2 Hz, 21-OH), 6.67 (1H, br d, J=7.0 Hz, 5-H), 6.87 (1H, br s, 2-H), 6.94 (1H, dd, J=8.0, 7.0 Hz, 6-H), 7.03 (1H, d, J=8.0 Hz, 7-H), 10.43 (1H, br s, 1-NH). ^{13}C-NMR [$(CD_3)_2SO$] δ: 15.5 [C-27 (Me)], 16.2 [C-25 (Me)], 17.2 [C-24 (Me)], 17.3 (C-12), 17.7 (C-19), 17.8 (C-8), 24.6 [C-23 (Me)], 27.1 (C-20), 27.9 [C-26 (Me)], 36.8 (C-18), 37.1 (C-10), 37.9 (C-14, C-16), 38.1 (C-11), 38.4 (C-22), 41.9 (C-15), 54.8 (C-17), 57.3 (C-9), 60.6 (C-13), 76.7 (C-21), 107.7 (C-7), 109.7(C-5), 111.4 (C-3), 117.6 (C-2), 121.7 (C-6), 124.8 (C-3a), 133.5 (C-7a), 144.0 (C-4). CD ($c=4.4\times10^{-4}$, dioxane) $\Delta\varepsilon^{20}$ (nm): +0.7 (274).

PM-53 (**2**): Deep blue crystalline powder , mp 240 °C (dec.) (from hexane–CH_2Cl_2). EI-MS *m/z* (%): 466.1897 (M+2, 466.1893 for $C_{29}H_{26}N_2O_4$, 17), 464.1735 (M^+, 464.1736 for $C_{29}H_{24}N_2O_4$, 25). UV $\lambda_{max}^{dioxane}$ nm (log ε): 219 (4.42), 274 (4.44), 308 (4.21), 614 (3.14). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1700, 1660, 1640 (C=O). ^1H-NMR [$(CD_3)_2SO$] δ: 1.92 (1H, s, 12′-H), 3.59 (3H, s, OMe), 3.62 (3H, s, OMe), 4.99 (1H, br s, 11′-H), 5.09 (1H, br s, 11′-H), 6.66 (1H, br d, J=16.1 Hz, 8′-H), 6.87 (1H, br d, J=16.1 Hz, 9′-H), 7.05 (1H, br t, J=8.0 Hz, 5″-H), 7.12 (1H, br dd, J=8.4, 8.0 Hz, 6″-H), 7.37 (1H, dd, J=8.4, 1.5 Hz, 6′-H), 7.40 (1H, d, J=8.4 Hz, 7′-H), 7.43 (1H, br d, J=8.4 Hz, 4″-H), 7.44 (1H, br d, J=8.0 Hz, 7″-H), 7.45 (1H, br s, 2′-H), 7.49 (1H, br s, 4′-H), 7.50 (1H, br s, 2″-H), 11.46 (1H, br s, NH), 11.47 (1H, br s, NH).

Structure Determination of Petromindole (1) by X-Ray Diffraction Analysis Petromindole (**1**) was crystallized from acetone as plates (mp >300°). Diffraction intensities were collected from a crystal of dimensions 0.30 × 0.25 × 0.07 mm on a Rigaku AFC-7 four-circle diffractometer. Of the total of 1828 reflections (complete for $2\theta < 120°$, 1770 satisfied the criterion $F > 3\sigma(F)$ and only these were used in the solution and refinement of the structure. Crystal Data: $C_{28}H_{39}NO$, Mr=405.62, monoclinic, space group $C2$, a=13.682(3), b=6.184(3), c=26.302(4) Å, β=93.48(2)°, V=2221(1) $Å^3$, Z=4, D_C=1.213 g·cm^{-3}, $F(000)$=888, Cu$K\alpha$ X-radiation (graphite monochromator), λ=1.54178 Å.

Structure Solution and Refinement The structure was solved by direct methods using SHELX 86[9] and expanded using Fourier techniques (DIRDIF 92).[10] The final refinement was done by the full-matrix least-squares method. Anisotropic thermal parameters were used for all non-hydrogen atoms and the hydrogen atoms were fixed. The refinement converged to $R(R_W)$ 0.070 (0.065).[11]

Acknowledgements We are grateful to Dr. N. Kawahara of the National Institute of Health Sciences for the NMR measurements. We are also grateful to Mrs. T. Ogata, Mrs. Y. Kawada and Mr. Komiya of Hoshi University for NMR and mass measurements, and elemental analysis. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 07672296) from the Ministry of Education, Science, Sports and Culture, Japan.

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