Two New Sesterterpenes from the Ascomycetous Fungus *Emericella purpurea*

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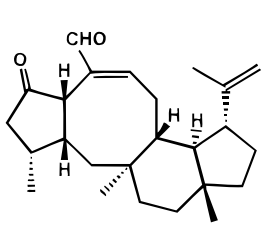
Two new sesterterpenes, designated variecolol (**2**) and variecolactone (**3**), were isolated from the mycelium of *Emericella purpurea*, along with a sesterterpene, variecolin (**1**), which was recently isolated from *Emericella variecolor* as an angiotensin II receptor binding inhibitor, and an indoloditerpene, emindole PA. The structures of **2** and **3** were confirmed by spectroscopic investigation and chemical correlation with **1**, and the direct *X*-ray analysis of **3**, respectively.

During a search for fungal indoloditerpenes, three components, one with a reddish coloration and the other two bluish when sprayed with modified Ehrlich's reagent,[1] were detected on TLC of a mycelial CH_2Cl_2 extract of *Emericella purpurea* Samson & Mouchacca (Eurotiaceae) (IFO 30849). The former compound was an indoloditerpene, emindole PA,[2] whereas one of the other compounds was identical with variecolin (**1**), a sesterterpene recently isolated from *E. variecolor* Berkeley & Broome and shown to be an angiotensin II receptor binding inhibitor.[3] The third compound was a new sesterterpene designated variecolol (**2**). Further purification of the extract using low-pressure liquid chromatography (LPLC) yielded another new sesteterpene, designated variecolactone (**3**), having an orange coloration on spraying with modified Ehrlich's reagent.[1] We now report the structure determination of **2** and **3**.



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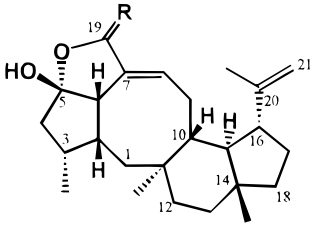

2 : R = H_2
3 : R = O

The molecular formulas of variecolol (**2**) and variecolactone (**3**) were determined by HREIMS as $C_{25}H_{38}O_2$ and $C_{25}H_{36}O_3$, respectively. The ^{1}H NMR signals of **2** were similar to those of variecolin (**1**), except for the appearance of two signals (δ 4.45 and 4.62) instead of the aldehyde proton at δ 9.13 in **1**. The signals of **3** were also similar to those of **1**, except for the absence of the aldehyde proton in **1**. The vinylic proton signal at δ 6.97 in **1** was shifted upfield (δ 5.44) in **2**, but this proton showed almost the same chemical shift (δ 6.96) in **3**. From comparison of the molecular ion peaks in EIMS of **2** and **3** with **1**, it was clear that **2** and **3** had two more hydrogens and one more oxygen, respectively, than **1**. Therefore, we assumed that **2** and **3** had a carbinol group and a carboxylate residue, respectively, instead of the aldehyde residue in **1**.

Crystals of variecolactone (**3**) grew as colorless plates (MeOH) suitable for X-ray single crystallographic analysis. The crystal structure of **3** was established to be as shown in Figure 1. Two molecules of **3** existed independently in an asymmetric unit. These two molecules are tightly packed by two sets of hydrogen bonding between the oxygen atoms of the lactone carbonyl and the hydroxyl groups of the hemiketal [O(3)−H−O(2a) (2.923 Å) and O(3a)−H−O(2) (2.866 Å)]. Conformations of two molecules of **3** are quite similar, including the eight-membered rings, from the comparison of the torsion angles. Bond lengths and angles are not significantly different from those expected.[4] The relative structure of variecolactone was consequently established as shown in **3**.

To confirm the structure of **2**, **1** was treated with sodium borohydride to obtain **2**. The IR absorption maxima at 3400 cm^{-1} in the spectrum of **2** suggested the presence of a hydroxyl group, and no absorption maxima for a carbonyl group was observed. A carbonyl signal appeared at δ 219.9 in the ^{13}C NMR spectrum of **1**, which was assigned to a five-membered ring ketone carbon, and a signal at δ 194.3 was assigned to an aldehyde carbon. Both signals disappeared in the spectrum of **2**. Instead of these, a signal of a carbon bearing an oxygen (δ 73.5), correlated with two protons at δ 4.45 and 4.62, and that of a carbon bearing two oxygen functions (δ 119.7) were observed in the spectrum of **2**. These results suggested that the carbinol group had formed a hemiketal with the five-membered ring ketone in **2**. From those results, and the detailed analysis of the homonuclear and heteronuclear shift correlated NMR spectra of **3**, the relative structure of variecolol, except for the chirality of the hemiketal, was determined as shown in **2**.

This is the second report of sesterterpenes having the same carbon skeleton as variecolin (**1**). The absolute configurations of **1**−**3** have not yet been determined.

Experimental Section

General Experimental Procedures. EIMS were taken with a JEOL JMS-MS600W spectrometer. IR spectra were recorded on a JASCO IR-810 spectrophotometer. ^{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 TMS as an internal standard. Column chromatography was performed using Kieselgel 60 (Art. 7734, Merck). LPLC was performed with a Chemco Low-Prep 81-M-2 pump and glass column (200 × 10 mm) packed with Si gel CQ-3 (30−50 μm, Wako). TLC was conducted on precoated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected by spraying with modified Ehrlich's reagent[1] and then heating.

Isolation of Metabolites from *Emericella purpurea*. *E. purpurea*, strain IFO 30849, was cultivated in potato−dextrose medium (5 L) using 20 Roux flasks at 25° for 28 days. The dried mycelium (21 g) was extracted with CH_2Cl_2, and the

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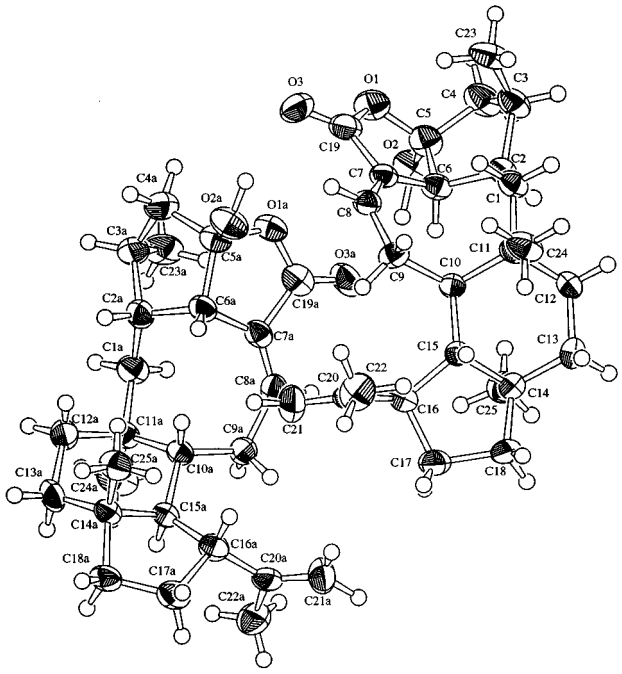

Figure 1. ORTEP drawing of molecular structure of variecolactone (**3**) with 50% thermal ellipsoids

organic layer was dried over Na_2SO_4 and then evaporated *in vacuo*. The extract obtained (1.7 g) was chromatographed on Si gel (Merck Si gel 60; 70−230 mesh) with C_6H_6−EtOAc (30:1) followed by repeated LPLC with C_6H_{14}−EtOAc (9:1) and/or $CHCl_3$ to obtain variecolin (**1**) (122 mg), ergosterol (40 mg), variecolactone (**3**) (4 mg), and variecolol (**2**) (12 mg).

Variecolol (2): colorless amorphous powder; IR ν_{max} (KBr) 3400 (OH) cm^{-1}; ^1H NMR δ ($CDCl_3$) 0.81 (3H, d, J = 7 Hz, 3-Me), 0.85 (3H, s, 14-Me), 0.90 (3H, s, 11-Me), 0.94 (1H, m, 12-H), 1.00 (1H, br d, J = 14 Hz, 1-H), 1.21 (1H, br dd, J = 11, 10 Hz, 13-H), 1.33 (1H, br ddd, J = 14, 10, 6 Hz, 17-H), 1.38−1.52 (4H, m, 13-H, 15-H, 18-H_2), 1.68 (3H, br s, 20-Me), 1.76 (1H, dd, J = 14, 13 Hz, 1-H), 1.90 (1H, br d, J = 13 Hz, 4-H), 1.93 (1H, m, 10-H), 1.97 (2H, m, 12-H, 17-H), 2.08 (1H, m, 9-H), 2.10 (1H, dd, J = 13, 3 Hz, 9-H), 2.16 (1H, qdd, J = 7, 7, 5 Hz, 3-H), 2.37 (1H, ddd, J = 11, 11, 5 Hz, 16-H), 2.41 (1H, br dd, J = 13, 7 Hz, 4-H), 2.67 (1H, br ddd, J = 13, 11, 5 Hz, 2-H), 3.37 (1H, br d, J = 11 Hz, 6-H), 4.55 (1H, br d, J = 12 Hz, 19-H), 4.58 (1H, br s, 21-H), 4.62 (1H, dddd, J = 12, 2, 2, 2 Hz, 19-H), 4.69 (1H, br s, 21-H), 5.44 (1H, m, 8-H); ^{13}C NMR δ ($CDCl_3$) 15.5 (q, 3-Me), 18.2 (q, 14-Me), 19.5 (q, 20-Me), 22.7 (q, 11-Me), 29.0 (t, C-9), 30.3 (t, C-17), 34.7 (t, C-12), 35.4 (t, C-13), 38.6 (d, C-3), 38.7 (s, C-11), 38.8 (d, C-2), 39.7 (d, C-10), 40.1 (t, C-18), 41.5 (t, C-1), 43.5 (s, C-14), 45.3 (t, C-4), 48.2 (d, C-15), 48.6 (d, C-16), 53.8 (d, C-6), 73.5 (t, C-19), 109.6 (t, C-21), 119.7 (s, C-5), 120.6 (d, C-8), 135.7 (s, C-7), 151.1 (s, C-20); EIMS m/z 370 $[M]^+$ (21), 355 $[M - Me]^+$ (7), 352 $[M - H_2O]^+$ (8); HREIMS m/z $[M]^+$ 370.2871 (calcd for $C_{25}H_{38}O_2$, 370.2872).

Variecolactone (3): colorless plates (MeOH); mp 249−251 °C; UV λ_{max} (MeOH) (log ϵ) 232 nm (3.78); IR ν_{max} (KBr) 3400 (OH), 1735 (−COO−) cm^{-1}; ^1H NMR δ ($CDCl_3$) 0.69 (3H, d, J = 8 Hz, 3-Me), 0.87 (3H, s, 14-Me), 0.91 (3H, s, 11-Me), 0.99 (1H, ddd, J = 14, 3, 3 Hz, 12-H), 1.09 (1H, br d, J = 14 Hz, 1-H), 1.24 (1H, m, 18-H), 1.37 (1H, m, 17-H), 1.51 (5H, m, 1-H, 13-H_2, 15-H, 18-H), 1.70 (3H, br s, 20-Me), 1.98 (2H, m, 12-H, 17-H), 2.13 (3H, m, 4-H, 9-H, 10-H), 2.22 (2H, m, 3-H, 4-H), 2.39 (1H, ddd, J = 11, 11, 6 Hz, 16-H), 2.76 (1H, ddd, J = 14, 3, 3 Hz, 9-H), 2.78 (1H, m, 2-H), 3.59 (1H, dd, J = 10, 2 Hz, 6-H), 4.63 (1H, br s, 21-H), 4.71 (1H, br s, 21-H), 6.96 (1H, m, 8-H); ^{13}C NMR δ ($CDCl_3$) 16.3 (q, 3-Me), 18.5 (q, 14-Me), 19.7 (q, 20-Me), 22.1 (q, 11-Me), 30.1 (t, C-9), 30.3 (t, C-17), 34.7 (t, C-12), 35.5 (t, C-13), 38.3 (d, C-3), 39.0 (d, C-10), 39.3 (s, C-11), 40.1 (d, C-2), 40.2 (t, C-18), 41.2 (t, C-1), 43.8 (s, C-14), 45.1 (t, C-4), 48.3 (d, C-15), 48.4 (d, C-16), 52.1 (d, C-6), 110.8 (t, C-21), 115.8 (s, C-5), 125.6 (s, C-7), 144.9 (d, C-8), 150.8 (s, C-20), 171.3 (s, C-19); EIMS m/z 384 $[M]^+$ (21), 369 $[M - Me]^+$ (7), 366 $[M - H_2O]^+$ (8); HREIMS m/z $[M]^+$ 384.2718 (calcd for $C_{25}H_{36}O_3$, 384.2716).

Reduction of Variecolin (1) with Sodium Borohydride. The suspended solution of $NaBH_4$ (10 mg) with **1** (20 mg) in MeOH (2 mL) was stirred at room temperature for 15 min. The reaction mixture was poured into ice-H_2O and extracted with $CHCl_3$, and the extract dried over Na_2SO_4. After evaporation of solvent, the residue was purified by LPLC using $CHCl_3$ to afford **2** (5 mg), which was identical with naturally occurring variecolol by comparison of the ^1H NMR and IR spectra, TLC behavior, and mixed melting point.

Structure Determination of Variecolactone (3) by X-ray Diffraction. Variecolactone (**3**) was crystallized from MeOH to give plates (mp >300 °C). Diffraction intensities were collected from a crystal of dimensions 0.50 × 0.30 × 0.10 mm on a Rigaku AFC-7 four-circle diffractometer. Of the total 3636 reflections (complete for $2\theta < 120°$), 3322 reflections satisfied the criterion $F > 3\sigma(F)$, and only these were used in the solution and refinement of the structure.

Crystal Data: $C_{25}H_{36}O_3$, M = 384.56, orthorhombic, space group $P2_12_12_1$, a = 21.843(2), b = 22.804(1), c = 8.577(2) Å, V = 4272.4 (8) $Å^3$, Z = 8, D_C = 1.196 gcm^{-3}, $F(000)$ = 1680, 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[5] and expanded using Fourier techniques (DIRDIF 92).[6] The final refinement was done by the full-matrix least-squares method. Anisotropic thermal parameters were used for all nonhydrogen atoms, and the hydrogen atoms were fixed. The refinement converged to R (R_W) 0.058 (0.065).[4]

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(4) Crystallgraphic data for variecolactone (**3**) have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk].
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