Hypoxylonols A and B, Novel Reduced Benzo[*j*]fluoranthene Derivatives from the Mushroom *Hypoxylon truncatum*

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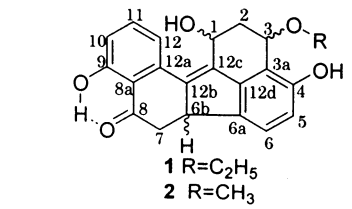
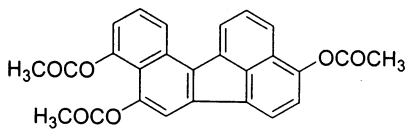
Two novel reduced benzo[*j*]fluoranthene derivatives, hypoxylonols A (3-ethoxy-1,4,9-trihydroxy-1,2,3,6b-tetrahydrobenzo[*j*]fluoranthen-8(7*H*)-one) (**1**) and B (3-methoxy-1,4,9-trihydroxy-1,2,3,6b-tetrahydrobenzo-[*j*]fluoranthen-8(7*H*)-one) (**2**), were isolated from the fruiting bodies of the mushroom *Hypoxylon truncatum*. The structures of these compounds were established on the basis of 1D and 2D NMR spectroscopic data.

In our search for bioactive compounds from mushrooms, we have reported the isolation of ganoderic acids A, B, G, and H and compound C6 as the antinociceptive compounds from CH_2Cl_2 extract of *Ganoderma lucidum* (Leyss. Ex Fr.) Karst. (Ganodermataceae).[1] In this paper, we report the isolation and structure determination of hypoxylonols A (**1**) and B (**2**) from the fruiting body of *Hypoxylon truncatum* (Schweinitz:Fries) J. H. Miller (Xylariaceae). Until now, eight compounds featuring this ring system from natural sources have been reported in the literature.[2−5] Bulgarein induced mammalian topoisomerase I-mediated DNA cleavage in vitro[2] and (6b*S*,7*R*,8*S*)-7-methoxy-4,8,9-trihydroxy-1,6b,7,8-tetrahydro-2*H*-benzo[*j*] fluoranthen-3-one inhibited anti-CD28-induced IL-2 production and Ab1 tyrosine kinase with IC_{50} values of 400 and 60 nM, respectively.[5]

The dried fruiting bodies of *H. truncatum* were extracted with $CHCl_3$. The $CHCl_3$ extract was subjected to separation and purification by silica gel column chromatography and RP HPLC to obtain hypoxylonols A (**1**) and B (**2**).



1 R=C_2H_5
2 R=CH_3



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Hypoxylonol A (**1**) had a molecular formula $C_{22}H_{20}O_5$ as established by HREIMS. The IR spectrum of **1** showed absorption bands at 3350 and 1625 cm^{-1}, indicating the presence of hydroxyl and carbonyl groups, respectively. The NMR data for **1** and **2** are summarized in the Experimental Section. The ^1H and ^{13}C NMR and HMQC spectra of **1** showed the presence of one methyl; three methylenes, one of which was attached to oxygen; three methines, two of which were attached to oxygen; five olefinic methines; nine olefinic quaternary carbons, two of which were attached to oxygen; one carbonyl carbon; and three hydroxy groups, one of which was chelated (δ_H 12.60). From the $^1H-^1H$ COSY spectrum of **1**, it was possible to establish the proton sequences from H-1 to H-3, H-5 to H-6, H-6b to H-7, and H-10 to H-12. HMBC correlations of H-2 to C-12c, H-3 to C-3a and C-12d, H-5 to C-3a, C-4, and C-6a, and H-6 to C-12d established the presence of a tetrahydronaphthalene ring. From the HMBC correlation of δ_H 3.65 and 3.80 (O-CH_2CH$_3$) to δ_C 71.6 (C-3), an ethoxy group was positioned at C-3. HMBC correlations of H-6b to C-12b, H-7 to C-8 and C-8a, H-10 to C-8a and C-9, H-11 to C-12a, and H-12 to C-12b established the presence of another tetrahydronaphthalene ring. The HMBC correlation of OH-9 (δ_H 12.60) to δ_C 116.8 (C-10) placed the hydrogen-bonded hydroxyl at C-9. The HMBC correlation of H-6b to C-6a defined connectivity between C-6a and C-6b. The remaining two olefinic quaternary carbons C-12b and C-12c must form the double bond, considering the molecular formula. Thus, the structure of hypoxylonol A (**1**) was determined to be as shown.

The molecular formula of hypoxylonol B (**2**) was found to be $C_{21}H_{18}O_5$ (HREIMS). The molecular formula of **2** indicated CH_2 less than **1**. The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**. However, signals for an ethoxy group were not observed in ^1H and ^{13}C NMR spectra of **2**; methoxy group signals appeared (δ_H 3.46 and δ_C 56.6). Therefore, the structure of hypoxylonol B (**2**) was determined to be as indicated.

Acetylation of **1** was performed in an attempt to resolve the stereochemical assignments of carbons C-1, C-3, and C-6b by X-ray crystallographic analysis of a suitable acylated derivative of **1**. However, **3** was the isolated product without obtaining the prospective compound. Compound **3** was isolated as yellow prisms. Its molecular formula was established as $C_{26}H_{18}O_6$ by HREIMS (426.1106, calcd for $[M]^+$ 426.1103). The IR spectrum of **3** showed absorption bands at 1755 and 1200 cm^{-1}, indicating the presence of acetyl groups. The ^1H and ^{13}C NMR and HMQC spectra of **3** showed the presence of three methyl groups, nine olefinic methines, 11 olefinic quaternary carbons, three of which were attached to oxygen, and three carbonyl groups. From the $^1H-^1H$ COSY spectrum of **3**, it was possible to establish the proton sequences from H-1 to H-3, H-5 to H-6, and H-10 to H-12. The HMBC correlations of H-1 to C-12d, H-2 to C-12c, H-3 to C-3a and C-4, and H-5 to C-4 and C-6a established the presence of a naphthalene ring. The HMBC correlations of H-7 to C-8, C-8a, C-6b, and C-12b, H-10 to C-8a and C-9, and H-12 to C-8a, C-12a, and C-12b established the presence of another naphthalene

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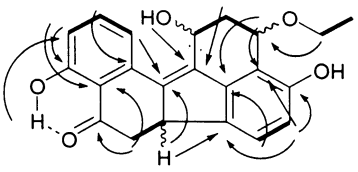

Figure 1. $^1H-^1H$ correlations (bold line) and HMBC correlations (arrows) of **1**.

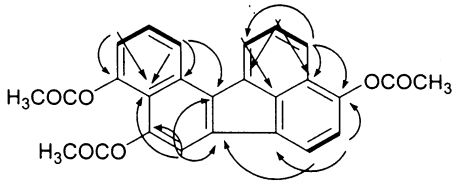

Figure 2. $^1H-^1H$ correlations (bold line) and HMBC correlations (arrows) of **3**.

ring. The HMBC correlation of H-6 to C-6b defines the connectivity between C-6a and C-6b. Thus, the structure of the reaction product was determined to be **3**.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanagimoto MP micromelting point apparatus and are uncorrected. Optical rotation, $[\alpha]^{20}_D$, data were recorded with a JASCO DIP-140 digital polarimeter. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. HR-mass spectra were obtained on a JEOL JMS-DX 302 spectrometer. ^1H and ^{13}C NMR spectra were run on JMN-LA 500 and GSX-400 spectrometers in acetone-d_6 with TMS as an internal standard. Chemical shifts are recorded in ppm (δ). IR spectra were obtained on a JASCO A-102 spectrometer. Silica gel 60 (Merck) was used for CC, and precoated silica gel plates (Merck, Kieselgel 60 F_{254} and DC-Fertigplatten RP-18 F_{254}) were used for TLC. HPLC was performed on a Sensyu flow system 3100-E equipped with a UVILOG 5IIIA UV detector at 254 nm. HPLC was performed with an ODS (Sensyu pak ODS-4251-D, 10ϕ × 250 mm) column.

Fungal Material. Fruiting bodies of *Hypoxylon truncatum* were collected in Tanashi, Tokyo, Japan, in October 1997. A voucher specimen has been deposited at the Department of Pharmacognosy and Phytochemistry, Meiji Pharmaceutical University. *H. truncatum* was identified by Dr. Kiyotaka Koyama.

Extraction and Isolation. The dried material (360 g) was extracted with $CHCl_3$ (1 L × 3) at room temperature. The extract was filtered, and the filtrate was concentrated under reduced pressure to yield 8.2 g of a residue. Column chromatography of the $CHCl_3$ extract (5.4 g) on silica gel and elution with a stepwise gradient of $CHCl_3-MeOH$ (1:0, 100:1, 10:1, 5:1, 1:1; 1.5 L each) gave five fractions. The 70% MeOH aqueous soluble part of fraction 3 (260 mg) was separated by reversed-phase HPLC (ODS, 90% MeOH) to give four fractions (3-1−3-4). Fraction 3-2 (121 mg) was fractionated by reversed-phase HPLC using CH_3CN-H_2O (1:1) as the solvent to afford hypoxylonols A (**1**) (17.7 mg) and B (**2**) (7.4 mg).

Hypoxylonol A (1): yellow powder; mp 173−174 °C; $[\alpha]^{20}_D$ −10° (*c* 0.3, acetone); UV (acetone) λ_{max}(log ϵ) 211 (3.90), 319 (sh, 3.63), 325 (3.74), 388 (4.02) nm; IR (KBr) ν_{max} 3350, 1625, 1455, 1360, 1220, 1060 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 12.60 (1H, s, O*H*-9), 8.41 (1H, s, O*H*-4), 7.54 (1H, t, J = 7.8 Hz, H-11), 7.51 (1H, dd, J = 7.8, 1.5 Hz, H-12), 7.31 (1H, dd, J = 7.8, 0.7 Hz, H-6), 6.83 (1H, dd, J = 7.8, 1.5 Hz, H-10), 6.73 (1H, d, J = 7.8, H-5), 5.53 (1H, dddd, J = 8.3, 6.2, 4.4, 2.1 Hz, H-1), 5.10 (1H, dd, J = 6.1, 3.4 Hz, H-3), 4.18 (1H, d, J = 6.2 Hz, O*H*-1), 4.08 (1H, dddd, J = 13.9, 5.5, 2.1, 0.7 Hz, H-6b), 3.80, 3.65 (each 1H, dq, J = 9.2, 7.0 Hz, OCH_2CH_3), 3.39 (1H, dd, J = 16.3, 5.5 Hz, H-7), 2.36 (1H, ddd, J = 13.0, 6.1, 4.4 Hz, H-2), 2.29 (1H, dd, J = 16.3, 13.9 Hz, H-7), 2.20 (1H, ddd, J = 13.0, 8.3, 3.4 Hz, H-2), 1.22 (3H, t, J = 7.0 Hz, OCH_2CH_3); ^{13}C NMR (acetone-d_6, 100 MHz) δ 205.8 (s, C-8), 163.3 (s, C-9), 155.2 (s, C-4), 145.0 (s, C-12d), 139.3 (s, C-12a), 138.7 (s, C-12c), 137.7 (s, C-12b), 137.0 (d, C-11), 136.7 (s, C-6a), 123.8 (d, C-6), 120.4 (d, C-12), 119.2 (s, C-3a), 116.8 (d, C-10), 115.6 (s, C-8a), 114.1 (d, C-5), 71.6 (d, C-3), 64.5 (t, OCH_2CH_3), 63.4 (d, C-1), 49.7 (d, C-6b), 43.6 (t, C-7), 39.8 (t, C-2), 15.9 (q, OCH_2CH_3); EIMS m/z 364 $[M]^+$ (19), 318 (57), 300 (100); HREIMS m/z 364.1317 $[M]^+$ (calcd for $C_{22}H_{20}O_5$, 364.1311).

Hypoxylonol B (2): yellow powder; mp 188 °C; $[\alpha]^{20}_D$ −28° (*c* 0.5, acetone); UV (acetone) λ_{max}(log ϵ) 211 (3.81), 318 (sh, 3.29), 323 (3.41), 386 (3.81) nm; IR (KBr) ν_{max} 3400, 1630, 1455, 1365, 1325, 1225, 1075, 810 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 12.59 (1H, s, O*H*-9), 8.38 (1H, s, O*H*-4), 7.54 (1H, t, J = 7.7 Hz, H-11), 7.51 (1H, dd, J = 7.7, 1.7 Hz, H-12), 7.32 (1H, dd, J = 8.0, 0.7 Hz, H-6), 6.83 (1H, dd, J = 7.7, 1.7 Hz, H-10), 6.75 (1H, d, J = 8.0, H-5), 5.51 (1H, dddd, J = 8.8, 6.4, 4.6, 2.3 Hz, H-1), 4.98 (1H, dd, J = 6.0, 3.4 Hz, H-3), 4.17 (1H, d, J = 6.4 Hz, O*H*-1), 4.08 (1H, dddd, J = 13.9, 5.5, 2.3, 0.7 Hz, H-6b), 3.46 (3H, s, OCH_3), 3.39 (1H, dd, J = 16.4, 5.5 Hz, H-7), 2.41 (1H, ddd, J = 13.1, 6.0, 4.6 Hz, H-2), 2.29 (1H, dd, J = 16.4, 13.9 Hz, H-7), 2.15 (1H, ddd, J = 13.1, 8.8, 3.4 Hz, H-2); ^{13}C NMR (acetone-d_6, 100 MHz) δ 206.1 (s, C-8), 163.5 (s, C-9), 155.2 (s, C-4), 145.3 (s, C-12d), 139.4 (s, C-12a), 138.9 (s, C-12c), 138.0 (s, C-12b), 137.1 (d, C-11), 137.0 (s, C-6a), 124.1 (d, C-6), 120.8 (d, C-12), 119.1 (s, C-3a), 117.0 (d, C-10), 115.8 (s, C-8a), 114.3 (d, C-5), 73.1 (d, C-3), 63.6 (d, C-1), 56.6 (q, OCH_3), 50.0 (d, C-6b), 43.8 (t, C-7), 39.8 (t, C-2); EIMS m/z 350 $[M]^+$ (38), 318 (86), 300 (100); HREIMS m/z 350.1151 $[M]^+$ (calcd for $C_{21}H_{18}O_5$, 350.1154).

Conversion of 1 to 3. To a solution of **1** (5.0 mg) in C_5D_5N (2 mL) was added Ac_2O (2 mL), and the reaction mixture was stirred for 12 h at room temperature. After 2 mL of H_2O was added, the mixture was concentrated under reduced pressure to yield a residue, which was passed through a silica gel short column eluted with $CHCl_3-MeOH$ (50:1). Compound **3** (4.1 mg) was crystallized ($CHCl_3-MeOH$ (10:1), 4 °C) from the eluate.

4,8,9-Triacetoxybenzo[*j*]fluoranthene (3): yellow prisms; mp 231−232 °C; UV (acetone) λ_{max}(log ϵ) 212 (4.11), 326 (4.55), 348 (3.69), 365 (3.92), 385 (4.07) nm; IR (KBr) ν_{max} 1755, 1430, 1365, 1200, 1165 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 8.78 (1H, dd, J = 8.6, 1.0 Hz, H-12), 8.63 (1H, d, J = 7.1 Hz, H-1), 8.14 (1H, d, J = 7.3 Hz, H-5), 7.97 (1H, d, J = 8.3 Hz, H-3), 7.90 (1H, s, H-7), 7.78 (1H, dd, J = 8.3, 7.1 Hz, H-2), 7.69 (1H, dd, J = 8.6, 7.6 Hz, H-11), 7.44 (1H, d, J = 7.3 Hz, H-6), 7.24 (1H, dd, J = 7.6, 1.0 Hz, H-10), 2.50 (3H, s, $-OCOCH_3$), 2.47 (3H, s, $-OCOCH_3$), 2.45 (3H, s, $-OCOCH_3$); ^{13}C NMR (acetone-d_6, 100 MHz) δ 170.1 ($-OCOCH_3$), 170.0 ($-OCOCH_3$), 170.0 ($-OCOCH_3$), 149.8 (C-4), 147.7 (C-9), 147.1 (C-8), 138.4 (C-6a), 137.5 (C-12c), 134.7 (C-6b), 133.9 (C-12a), 133.8 (C-12d)*, 133.1 (C-12b), 129.8 (C-2), 128.5 (C-11), 126.0 (C-1), 125.0 (C-3a) *, 123.7 (C-12), 123.1 (C-5)†, 123.0 (C-3)†, 122.1 (C-8a), 121.4 (C-6), 121.4 (C-10), 116.1 (C-7), 21.3 ($-OCOCH_3$), 21.3 ($-OCOCH_3$), 20.9 ($-OCOCH_3$) (*,† assignments bearing the same superscript may be interchanged); EIMS m/z 426 $[M]^+$ (25), 384 (23), 342 (63), 300 (100); HREIMS m/z 426.1106 $[M]^+$ (calcd for $C_{26}H_{18}O_6$, 426.1103).

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References and Notes

(1) Koyama, K.; Imaizumi, T.; Akiba, M.; Kinoshita, K.; Takahashi, K.; Suzuki, A.; Yano, S.; Horie, S., Watanabe, K.; Naoi, Y. *Planta Med.* **1997**, *63*, 224−227.
(2) Hashimoto, T.; Asakawa, Y. *Heterocycles* **1998**, *47*, 1067−1110.
(3) Edwards, R. L.; Lockett, H. J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2149−2155.
(4) Brauers, G.; Ebel R.; Edrada R.; Wray V.; Berg A.; Grafe U.; Proksch P. *J. Nat. Prod.* **2001**, *64*, 651−652.
(5) Wrigley S. K.; Ainsworth A. M.; Kau D. A.; Martin S. M.; Bahl S.; Tang J. S.; Hardicj D. J.; Rawlins P.; Sadheghi R.; Moore M. *J. Antibiot.* **2001**, *54*, 479−488.

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