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Studies on the Metabolites of *Penicillium diversum* var. *aureum*. I

YASUO FUJIMOTO,* EIZO YOKOYAMA, TATSUYUKI TAKAHASHI, JUN UZAWA, NOBUHISA MOROOKA, HIROSHI TSUNODA and TAKASHI TATSUNO

The Institute of Physical and Chemical Research, Wako-shi, Saitama 351-01, Japan

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Three new trihydroxytetralones (**1**, **2** and **3**) have been isolated from the culture broth of *P. diversum* var. *aureum* together with three known naphthalenones (sclerone, isosclerone and juglone). The structure of **1** was determined on the basis of its chemical properties and spectroscopic analyses of the monomethyl ether (**4**) and dimethyl ether (**5**).

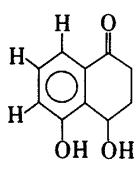
Keywords——*Penicillium diversum* var. *aureum*; trihydroxytetralone; metabolite anti-tumor activity

P. diversum var. *aureum* has frequently been isolated from fodder. However, there are no reports on the metabolites of this fungi to our knowledge. We now describe here the isolation and structural elucidation of the major metabolites of *P. diversum* var. *aureum*.

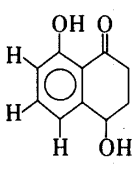
P. diversum var. *aureum* was grown in Czapek-yeast medium at 25 °C. After three weeks, the mycelia were separated by filtration and then dipped into acetone. The acetone solution was concentrated under reduced pressure to leave a dark brown solid. The solid was recrystallized from chloroform to give reddish brown crystals, which were identified as herqueinone[1] by comparison of the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and infrared (IR) spectra with those of an authentic specimen. From the culture broth, three new trihydroxytetralones (**1**, **2** and **3**) were isolated together with the known naphthalenone derivatives, sclerone,[2] isosclerone[3] and juglone,[4] by charcoal column chromatography, silica gel chromatography and finally high performance liquid chromatography (HPLC). The structures of the new naphthalenones were determined from their physicochemical properties as follows.

Compound **1** is a phenolic compound, because it gave a positive ferric chloride test. The acetylation of **1** gave a complex mixture, but the reaction of **1** with diazomethane gave a mixture of a monomethyl ether (**4**) and a dimethyl ether (**5**). ^{1}H-NMR decoupling experiments on **4** suggested the presence of the partial structures A and B.

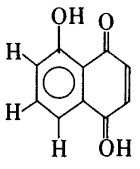
Acetylation of **4** with acetic anhydride-pyridine yielded 1,4-diacetoxy-5-methoxynaphthalene (**6**). A consideration of the results of acetylation and the ^{1}H-NMR decoupling



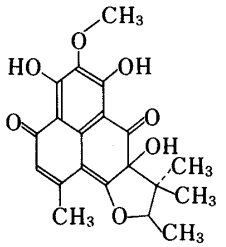
sclerone



isosclerone



juglone



herqueinone

Chart 1

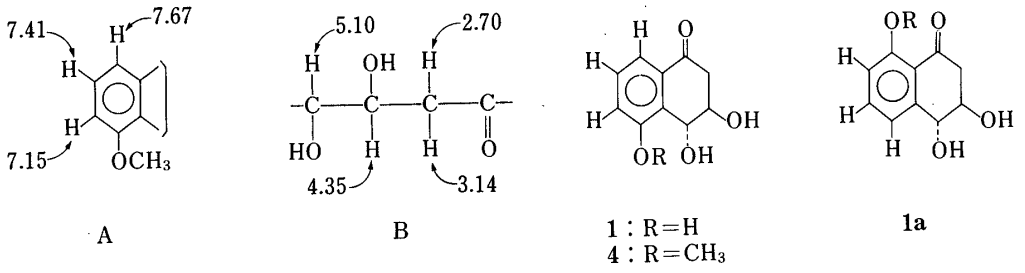


Chart 2

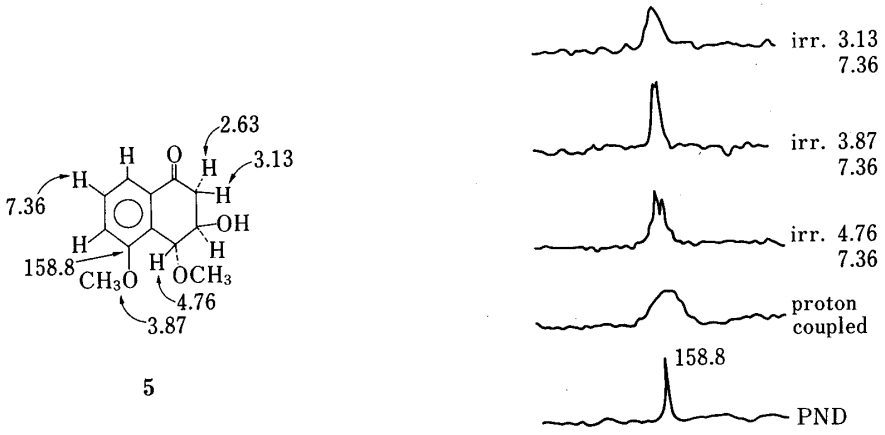


Fig. 1

experiments on **4** indicated that there are two possibilities (**1** and **1a**) for the structure of **1**. Finally, the structure of **1** was confirmed by ^{13}C–$\{^1H\}\{^1H\}$ long range selective proton decoupling (LSPD) experiments on the dimethyl ether (**5**), which is more readily soluble in $CDCl_3$ than the monomethyl ether (**4**). On selective irradiation of the methyl proton at δ 3.87 and the aromatic proton at δ 7.36, the multiplet ^{13}C signal at δ 158.8 appeared as a doublet. This signal was also decoupled and appeared as a quartet by selective irradiation of the protons at δ 4.76 and 7.36, as shown in Fig. 1.

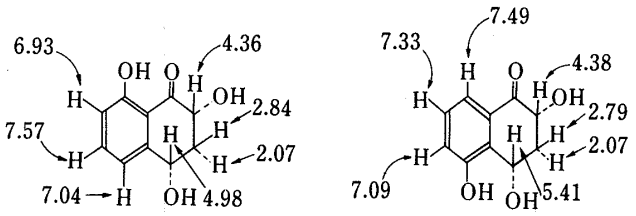
Thus, the structure of **1** was assigned as 3,4,5-trihydroxyl-1-tetralone. The stereochemistry of **1** was confirmed by referring to the ^1H-NMR data for 2-methyl-1-tetralol (**7**)[5] and 3-methyl-4,8-dihydroxy-1-tetralone (**8**).[6] The coupling constants between H-1 and H-2 in *trans*-**7** and that between H-4 and H-3 in *trans*-**8** were 6.5 and 8.0 Hz, while those in *cis*-**7** and in *cis*-**8** were 3.0 and 2.5 Hz, respectively. In the case of compound **1**, the coupling constant between H-3 and H-4 was 6.6 Hz. Thus, the 3- and 4-hydroxyl groups in **1** should be in a mutually *trans* configuration.

The structures of **2** and **3** were determined as follows. Compound **2** showed the molecular ion peak at *m/z* 194 (M^+) ($C_{10}H_{10}O_4$). The structure of the alicyclic ring in **2**, including the relative stereochemistry, was confirmed by ^1H-NMR decoupling experiments. Irradiation of the signals at δ 2.07 and 2.84 decoupled the signals at δ 4.36 and 4.98 simultaneously, suggesting the presence of a 1,3-diol system. Furthermore, the coupling constants of H-2 (J= 5.3 and 13.2 Hz) and H-4 (J=4.8 and 11.4 Hz) showed that both hydroxyl groups were equatorial. The IR spectrum of **2** showed the presence of a hydrogen-bonded carbonyl group (1630 cm^{-1}) and moreover the patterns of the aromatic proton signals in the ^1H-NMR spectrum of **2** were very similar to that of isosclerone, thereby suggesting that the hydroxyl group in the aromatic ring should be placed at the C-8 position. Thus, the structure of **2** was assigned as 2,4,8-trihydroxy-1-tetralone.

Compound **3** showed the molecular ion peak at *m/z* 194 (M^+) ($C_{10}H_{10}O_4$). A comparison

of the ^{1}H-NMR spectrum of **3** with those of **2**, **1** and sclerone showed that the substitution patterns of the alicyclic ring and the aromatic ring of **3** were similar to that of the alicyclic ring of **2**, and those of the aromatic rings of **1** and sclerone, respectively. Thus, the structure of **3** was assigned as 2,4,5-trihydroxy-1-tetralone.

Compounds **1**, **2** and **3** inhibited the growth of Yoshida sarcoma cells in tissue culture at 20—25 μg/ml. Details of the biological activities of these and related compounds will be published elsewhere.



2 3

Chart 3

Experimental

Melting points were determined on a Yanagimoto melting-point apparatus and are uncorrected. Spectral data were obtained on the following instruments: IR on a Shimadzu IR-430 in KBr; ^{1}H-NMR and ^{13}C-NMR on JEOL FX-400 and FX-100 instruments in $CDCl_3$ containing tetramethylsilane as an internal standard; mass spectra (MS) on a Hitachi RMU-6M.

Isolation of Herqueinone and Naphthalenone Derivatives——*P. diversum* var. *aureum* was grown in Czapek-yeast medium (10 l) at 25 °C. After three weeks, the mycelia were separated by filtration and then dipped into acetone (3 l). The acetone solution was concentrated under reduced pressure to leave a dark brown solid. The solid was recrystallized from chloroform to give reddish brown crystals (1.4 g), which were identified as herqueinone by comparison of the ^{1}H- and ^{13}C-NMR and IR spectra with those of an authentic specimen. Active charcoal (100 g) was added to the culture broth, which was allowed to stand for 1 h with occasional stirring. The active charcoal was filtered off and then washed with water. The charcoal adsorbates were extracted with acetone (3—4 l) and concentrated under reduced pressure to leave an acetone–water solution. The solution was extracted with ethyl acetate (500 ml × 3) and the combined organic layer was washed with brine, then dried over sodium sulfate. The ethyl acetate solution was concentrated *in vacuo* to leave an oil, which was subjected to silica gel chromatography (benzene : acetone = 4 : 1). The fractions showing a spot at *ca. Rf* 0.1 on thin layer chromatogram (benzene : acetone = 4 : 1) were combined and evaporated to give a solid, which was then recrystallized from acetone to afford 240 mg of 3,4,5-trihydroxy-1-tetralone (**1**). The mother liquor was concentrated *in vacuo* to give an oil. Purification of this oil by HPLC (Nucleosil 50-5, 8 × 300 mm, hexane : ethyl acetate = 1 : 1) gave *ca.* 15 mg of 2,4,8-trihydroxy-1-tetralone (**2**) and *ca.* 15 mg of 2,4,5-trihydroxy-1-tetralone (**3**). The fractions including less polar compounds were concentrated to leave an oily material which was separated by HPLC (Nucleosil 50-5, hexane : ethyl acetate = 2 : 1) to afford sclerone (*ca.* 10 mg), isosclerone (*ca.* 10 mg) and juglone (*ca.* 10 mg).

Herqueinone——mp 221—223 °C (from $CHCl_3$). MS: *m/z* 372 (M^+). $[\alpha]_D^{22}$ +453° (*c* = 0.02, $CHCl_3$). IR (KBr): 3250 (OH), 1662 (hydrogen-bonded C=O) cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 1.06 (3H, s), 1.52 (3H, s), 1.69 (3H, d, *J* = 6.8 Hz), 2.45 (3H, d, *J* = 1.1 Hz), 3.86 (3H, s), 4.69 (1H, q, *J* = 6.8 Hz), 4.92 (1H, s), 6.06 (1H, d, *J* = 1.1 Hz), 13.08 (1H, s), 14.86 (1H, s). ^{13}C-NMR (22.5 MHz, DMSO-d_6, δ): 15.9, 18.6, 23.7, 43.0, 59.8, 78.9, 95.9, 102.6, 103.2, 109.1, 122.7, 131.2, 138.9, 150.7, 161.9, 162.8, 178.5, 186.3, 196.9.

3,4,5-Trihydroxy-1-tetralone (1)——mp 199—200 °C (dec.). $[\alpha]_D^{22}$ −14.2° (*c* = 2.5, MeOH). IR (KBr): 3100—3200 (OH), 1662 (C=O), 1578 cm^{-1}. MS: *m/z* 194.0546 (M^+), $C_{10}H_{10}O_4$. ^{1}H-NMR ($(CD_3)_2CO$, 90 MHz, δ): 2.62 (1H, dd, *J* = 7.9, 16.3 Hz; H-2), 3.02 (1H, dd, *J* = 3.5, 16.3 Hz; H-2), 4.27 (1H, m; H-3), 5.14 (1H, d, *J* = 6.6 Hz; H-4), 7.14 (1H, dd, *J* = 1.7, 7.5 Hz; H-6), 7.26 (1H, t, *J* = 7.5 Hz; H-7), 7.44 (1H, dd, *J* = 1.7, 7.5 Hz; H-8). ^{13}C-NMR (CD_3OD, 22.5 MHz, δ): 43.1 (C-2), 68.3 (C-3), 71.6 (C-4), 118.3 (C-6), 122.2 (C-8), 128.8 (C-4a), 129.9 (C-7), 133.7 (C-8a), 157.9 (C-5), 198.7 (C-1).

2,4,8-Trihydroxy-1-tetralone (2)——mp 141—143 °C. $[\alpha]_D^{26}$ −18.6° (*c* = 0.086, MeOH). IR (KBr): 3400—3000 (OH), 1630 (C=O), 1580 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 90 MHz, δ): 2.07 (1H, ddd, *J* = 11.4, 11.9, 13.2 Hz; H-3), 2.84 (1H, ddd, *J* = 4.8, 5.3, 11.9 Hz; H-3), 4.36 (1H, dd, *J* = 5.3, 13.2 Hz; H-2), 4.98 (1H, dd, *J* = 4.8, 11.4 Hz; H-4), 6.93 (1H, dd, *J* = 2.0, 8.3 Hz; H-7), 7.04 (1H, dd, *J* = 2.0, 8.3 Hz; H-5), 7.57 (1H, t, *J* = 8.3 Hz; H-6).

2,4,5-Trihydroxy-1-tetralone (3)——mp 139—141 °C. $[\alpha]_D^{26}$ −36.3° (c=0.062, MeOH). ^{1}H-NMR ($(CD_3)_2CO$, 90 MHz, δ): 2.07 (1H, ddd, J=10.8, 11.9, 13.6 Hz; H-3), 2.79 (1H, ddd, J=4.6, 5.1, 11.9 Hz; H-3), 4.38 (1H, dd, J=5.1, 13.6 Hz; H-2), 5.41 (1H, dd, J=4.6, 10.8 Hz; H-4), 7.09 (1H, dd, J=2.0, 7.7 Hz; H-6), 7.33 (1H, t, J=7.7 Hz; H-7), 7.49 (1H, dd, J=2.0, 7.7 Hz; H-8).

Sclerone——MS m/z: 178 (M^+), $C_{10}H_{10}O_3$. ^{1}H-NMR ($CDCl_3$, 90 MHz, δ): 1.95—3.10 (4H, m), 5.35 (1H, dd, J=4.5, 9.0 Hz; H-4), 7.09 (1H, dd, J=1.8, 8.0 Hz; H-6), 7.30 (1H, t, J=9.0 Hz; H-7), 7.53 (1H, dd, J=1.8, 8.0 Hz; H-8).

Isosclerone——MS m/z: 178 (M^+), $C_{10}H_{10}O_3$. ^{1}H-NMR ($CDCl_3$, 90 MHz, δ): 2.1—3.2 (4H, m), 4.90 (1H, dd, J=4.2, 6.8 Hz; H-4), 6.87 (1H, dd, J=1.1, 7.9 Hz; H-7), 7.01 (1H, dd, J=1.1, 7.9 Hz; H-5), 7.50 (1H, t, J=7.9 Hz; H-6).

Juglone——The IR and ^{1}H-NMR spectra of juglone isolated from the culture broth were identical with those of an authentic specimen obtained from Tokyo Kasei Co., Ltd.,

Methylation of 3,4,5-Trihydroxy-1-tetralone——An ether solution of diazomethane was added to a solution of **1** (300 mg) in methanol (3 ml) until no more nitrogen gas evolved. After completion of the reaction, the solution was concentrated *in vacuo* to leave an oil which was chromatographed on silica gel(benzene : acetone = 4 : 1) to give a dimethyl ether (**5**: 47 mg) and a monomethyl ether (**4**: 250 mg).

Monomethyl Ether (4)——mp 172—174 °C (from acetone). MS m/z: 208 (M^+), $C_{11}H_{12}O_4$. IR (KBr): 3330—3380 (OH), 1673 (C=O), 1575 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 90 Hz, δ): 2.44 (1H, d, J=3.1 Hz; 3-OH), 2.70 (1H, dd, J=8.8, 16.4 Hz; H-2), 3.14 (1H, dd, J=3.7, 16.4 Hz; H-2). 3.65 (1H, d, J=2.0 Hz; 4-OH), 3.96 (3H, s; OCH_3), 4.37 (1H, m; H-3), 5.10 (1H, dd, J=2.0, 6.0 Hz; H-4), 7.15 (1H, dd, J=1.3, 8.1 Hz; H-6), 7.41 (1H, dd, J=7.6, 8.1 Hz; H-7), 7.67 (1H, dd, J=1.3, 7.6 Hz; H-8).

Dimethyl Ether (5)——Oil. MS m/z: 222 (M^+), $C_{12}H_{14}O_4$. ^{1}H-NMR ($CDCl_3$ + D_2O, 90 MHz, δ): 2.63 (1H, ddd, J=1.3, 3.1, 17.4 Hz; H-2), 3.13 (1H, dd, J=2.9, 17.4 Hz; H-2), 3.47 (3H, s; C-4-OCH_3), 3.87 (3H, s; C-5-OCH_3), 4.49 (1H, m; H-3), 4.76 (1H, dd, J=1.3, 3.5 Hz; H-4), 7.10 (1H, dd, J=1.4, 8.0 Hz; H-6), 7.36 (1H, dd, J=7.6, 8.0 Hz; H-7), 7.61 (1H, dd, J=1.4, 7.6 Hz; H-8). ^{13}C-NMR ($CDCl_3$, 22.5 MHz, δ): 41.4 (C-2), 55.9 (C-5-OCH_3), 57.7 (C-4-CH_3), 67.8 (C-3), 72.6 (C-4), 115.9 (C-6), 118.8 (C-8), 127.9 (C-4a), 129.4 (C-7), 133.2 (C-8a), 158.8 (C-5), 196.0 (C-1).

Acetylation of the Monomethyl Ether (4)——A mixture of **4** (10 mg), pyridine (1.0 ml) and acetic anhydride (0.2 ml) was stirred at room temperature overnight, then poured into cold water (30 ml) and extracted with ethyl acetate (20 ml × 3). The organic layer was washed with brine (20 ml × 2) and dried over sodium sulfate. Evaporation of the solvent gave an oil, which was purified by preparative thin layer chromatography (silica gel, chloroform : acetone = 3 : 1) to provide 6.5 mg of 1,4-diacetoxy-5-methoxynaphthalene (**6**).

1,4-Diacetoxy-5-methoxynaphthalene (6)——^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 2.37 (3H, s), 2.45 (3H, s), 3.93 (3H, s; OCH_3), 6.88 (1H, dd, J=1.0, 7.3 Hz; H-6), 7.05 (1H, d, J=8.3 Hz; H-3), 7.23 (1H, d, J=8.3 Hz; H-2), 7.42 (1H, t, J=7.3 Hz; H-7), 7.46 (1H, dd, J=1.0, 7.3 Hz; H-8).

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References and Notes

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