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Studies on the Metabolites of *Penicillium diversum* var. *aureum*. II. Synthesis and Cytotoxic Activity of Trihydroxytetralones

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(±)-2,4,5- and 2,4,8-Trihydroxy-1-tetralones (**2**, **9**, **3**, **11**) were synthesized from juglone, and their cytotoxic activities against Yoshida sarcoma cells were examined. The trihydroxytetralones were more cytotoxic than dihydroxytetralones. While the configuration of the hydroxyl groups in the alicyclic ring scarcely affected the cytotoxicity against Yoshida sarcoma cells, the position of the hydroxyl groups in the aromatic ring seem to be important. The 2,4,8-trihydroxytetralones (**3**, **11**) were more cytotoxic than the 2,4,5-trihydroxy derivatives (**2**, **9**).

Keywords——*Penicillium diversum* var. *aureum*; trihydroxytetralone; dihydroxytetralone; cytotoxic activity

In the previous paper,[2)] we reported the isolation and structural elucidation of three new trihydroxy-1-tetralones (**1**, **2**, **3**) produced by *Penicillium diversum* var. *aureum*. In this paper, we describe the synthesis and the cytotoxicity of trihydroxytetralones and related compounds against Yoshida sarcoma cells in tissue culture.

O OH HO OH **1**

O OH OH OH **2**

OH O OH OH **3**

Chart 1

Synthesis of Hydroxytetralones

Firstly, 2β,4α,5-trihydroxy-1-tetralone (**9**) and 2α,4α,5-trihydroxy-1-tetralone (**2**) were synthesized as follows. Reduction of juglone (**4**)[3)] with lithium aluminum hydride gave a mixture of (±)-isosclerone (**5**)[4)] and (±)-sclerone (**6**).[5)] The direct separation of this mixture was found to be difficult even by high performance liquid chromatography (HPLC). Thus, the mixture, without purification, was treated with 2,2-dimethoxypropane in the presence of a catalytic amount of camphorsulfonic acid (CSA) to give an acetonide (**7**) along with unchanged **5** (separated by silica gel column chromatography). Compound **7** was then hydrolyzed with 5% HCl to give (±)-sclerone (**6**). Oxidation of **7** with oxodiperoxymolybdenum–hexamethylphosphoric triamide ($MoO_5 \cdot HMPA$)[6)] gave a mixture of the 2β-hydroxy-ketone (**8a**) and the 2α-hydroxy-ketone (**8b**) in a ratio of 3:1. These products were separated by HPLC. The configurations of the hydroxyl groups in **8a** and **8b** were confirmed from the coupling constants of H-2 (dd, J=2.4, 4.9 Hz in **8a**, J=4.6, 13.2 Hz in **8b**) and H-4 (dd, J=4.9, 11.9 Hz in **8a**, J=4.6, 11.5 Hz in **8b**). Hydrolysis of **8a** and **8b** gave 2β,4α,5-trihydroxy-1-tetralone (**9**) and 2α,4α,5-trihydroxy-1-tetralone (**2**), respectively. Re-

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moval of the acetonide group of **8a** caused a dramatic change of the coupling constants of H-4 (br t, $J=4.4$ Hz in **9**). This suggested that ring inversion from the stereostructure A to B occurred in the course of hydrolysis. In contrast, the ring conformation remained unchanged in the transformation of **8b** to **2**.

LiAlH₄ → ; $Me_2C(OMe)_2$ / CSA → ; LDA-MoO_5·HMPA → ; 1) DHP-CSA 2) LDA-MoO_5·HMPA ; 5% HCl

4 5 6 7 8a : 2β-OH 8b : 2α-OH

10a : 2β-OH 10b : 2α-OH 11 3 9 2

Chart 2

A → B

Chart 3

Secondly, 2β,4α,8-trihydroxy-1-tetralone (**11**) and 2α,4α,8-trihydroxy-1-tetralone (**3**) were prepared as follows. The hydroxyl groups of **5** were protected as tetrahydropyranyl ethers and the compound obtained was then oxidized with MoO_5·HMPA to give an inseparable mixture of 2β-hydroxy- and 2α-hydroxytetralones (**10a** and **10b**). This mixture, without purification, was treated with 5% HCl to hydrolyze tetrahydropyranyl ethers to afford **11** and **3**, separation of which was performed by reversed-phase HPLC, followed by normal-phase HPLC. The proton nuclear magnetic resonance (^{1}H-NMR) spectral data of **11** [4.94 (ddd, $J=3.2$, 5.1, 12.9 Hz, H-2), 5.06 (dd, $J=2.7$, 5.4 Hz, H-4)] and **3** [4.31 (dd, $J=5.1$, 11.7 Hz, H-2), 4.94 (dd, $J=5.1$, 11.7 Hz, H-4)] indicated that their stereostructures are similar to those of **9** and **2**, respectively.

Cytotoxicity of Hydroxytetralone

The effects of various compounds on the growth of Yoshida sarcoma cells were investigated as follows. Cells were suspended in MEM medium (Eagle) containing 15% calf serum. Aliquots (1 ml) of cell suspension (approximately 20×10^4 cells/ml) were transferred into vials. After addition of a compound to be tested at the concentration indicated, the cells were incubated at 37 °C for 2—3 d and then viable cells were counted. The cytotoxicity was decided by comparing the viable cell number with that of the control.

As shown in Table I, dihydroxytetralone derivatives (**5**, **6** and **7**) inhibited the growth of Yoshida sarcoma cells by 34—66% at the concentration of 50 μg/ml. The effects of the trihydroxytetralones (**2**, **3**, **9** and **11**) and their derivatives (**8a** and **8b**) were slightly more

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Table I. Growth Inhibition of Yoshida Salcoma Cells in Tissue Culture

Sample	Concentration (μg/ml)	Inhibition (%)	Sample	Concentration (μg/ml)	Inhibition (%)
5	100	49	**2**	100	94
	50	41		50	88
	25	16		25	54
6	100	50	**9**	100	100
	50	34		50	81
	25	31		25	71
7	100	70	**3**	100	100
	50	66		50	100
	25	62		25	100
8b	100	97	**11**	100	98
	50	94		50	95
	25	62		25	92
8a	100	100			
	50	87			
	25	57			

potent than those of the dihydroxy derivatives. While the configurations of the hydroxyl groups in the alicyclic ring scarcely affected the growth inhibition of Yoshida sarcoma cells, the positions of the hydroxyl group in the aromatic ring seemed to be important. Although the mode of action of hydroxytetralones against tumor cells is not yet clear, it is interesting that 2,4,8-trihydroxy-1-tetralones are more potent than 2,4,5-trihydroxy derivatives.

Studies on the fungicidal activity and the cytotoxicity of hydroxytetralones against other tumor cells are in progress.

Experimental

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

Synthesis of (±)-Isosclerone (5) and (±)-Sclerone (6)——A tetrahydrofuran (THF) solution (40 ml) of juglone (2 g) was added dropwise to a stirred suspension of lithium aluminum hydride (3.4 g) in absolute ether (300 ml) and stirring was continued overnight. The reaction mixture was carefully poured into ice-cold 5% HCl solution (500 ml) and extracted with ether (150 ml × 3). The combined organic layer was washed with brine and then dried over sodium sulfate. Evaporation of the solvent left an oily material, which was dissolved in 2,2-dimethoxypropane (100 ml) containing a catalytic amount of CSA. The solution was stirred at room temperature for 30—40 min, then poured into saturated salt solution (150 ml), and the whole was extracted with ether (100 ml × 2). The ether solution was dried over sodium sulfate and then concentrated under reduced pressure to leave an oily material, which was subjected to silica gel column chromatography (hexane : ethyl acetate = 2 : 1) to give 0.81 g of (±)-isosclerone (**5**), 0.25 g of (±)-

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sclerone acetonide (7) and 0.10 g of juglone. A mixture of 7 (3 mg) and 5% HCl (500 μl) in THF (500 μl) was stirred at room temperature for 1 h. After completion of the reaction, the mixture was extracted with ethyl acetate (10 ml $\times$ 3). The combined organic layer was washed with brine and dried over sodium sulfate. Evaporation of the solvent gave ($\pm$)-sclerone (6: 2 mg). 5: mp 98—100 °C (ether–hexane). MS m/z: 178 (M^+), 160 ($M^+ - H_2O$), 150 ($M^+ - C_2H_4$). ^{1}H-NMR δ: 1.89 (1H, d, J=5.1 Hz, –OH), 2.20 (1H, m, H-3), 2.35 (1H, dq, J=4.6, 8.3 Hz, H-3), 2.65 (1H, ddd, J=4.6, 8.3, 17.8 Hz, H-2), 3.01 (1H, ddd, J=4.6, 8.3, 17.8 Hz. H-2), 4.92 (1H, ddd, J=4.6, 5.1, 8.3 Hz, H-4), 6.93 (1H, d, J=7.9 Hz, H-7), 7.02 (1H, d, J=7.9 Hz, H-5), 7.50 (1H, t, J=7.9 Hz, H-6). The ^{1}H-NMR data of 5 were identical with those of natural isosclerone. 6: mp 100—102 °C (ether–hexane). MS m/z: 178 (M^+), 160 ($M^+ - H_2O$), 150 ($M^+ - C_2H_4$), ^{1}H-NMR δ: 1.83 (1H, d, J=5.4 Hz, –OH), 2.19 (1H, m, H-3), 2.34 (1H, m, H-3), 2.65 (1H, ddd, J=4.6, 8.3, 17.8 Hz, H-2), 3.00 (1H, ddd, J=4.6, 8.3, 17.8 Hz, H-2), 5.30 (1H, ddd, J=4.6, 5.4, 8.3 Hz, H-4), 7.05 (1H, dd, J=1.7, 8.0 Hz, H-6), 7.25 (1H, t, J=8.0 Hz, H-7), 7.53 (1H, dd, J=1.7, 8.0 Hz, H-8). The ^{1}H-NMR data of 6 were identical with those of natural sclerone. 7: mp 143—145 °C (ether–hexane). MS m/z: 218 (M^+), 160 ($M^+ - C_3H_6O$), 131 ($M^+ - C_4H_7O_2$). ^{1}H-NMR δ: 1.61, 1.63 (3H each, s, $CH_3 \times 2$), 2.08 (1H, dq, J=4.4, 12.0 Hz, H-3), 2.41 (1H, m, H-3), 2.60 (1H, ddd, J=4.9, 14.4, 18.0 Hz, H-2), 2.83 (1H, ddd, J=2.4, 4.4, 18.0 Hz, H-2), 5.01 (1H, dd, J=4.5, 11.6 Hz, H-4), 7.01 (1H, dd, J=1.0, 7.9 Hz, H-6), 7.30 (1H, t, J=7.9 Hz, H-7), 7.54 (1H, dd, J=1.0, 7.9 Hz, H-8).

Oxidation of ($\pm$)-Sclerone Acetonide (7)——A 15% hexane solution (890 μl, 2.0 eq) of butyl lithium was added dropwise to a mixture of THF (1.0 ml) and diisopropylamine (200 μl, 2.0 eq) with stirring at -70 °C under a nitrogen atmosphere. After 20 min, a THF solution (1.0 ml) of 7 (156 mg) was added dropwise and the reaction temperature was adjusted to -25 °C, then a solution of $MoO_5 \cdot$HMPA (797 mg, 3.0 eq) in THF (1.2 ml) was added in a single portion. After 30 min, the reaction mixture was quenched with saturated sodium sulfite solution (4 ml), warmed to room temperature, diluted with water (10 ml) and extracted with ether (30 ml $\times$ 3). The combined organic layer was washed with brine and dried over sodium sulfate. Evaporation of the solvent left an oil, which was subjected to silica gel column chromatography (hexane : ethyl acetate=2 : 1) to separate a mixture of the ketols (**8a** and **8b**) and the starting material (30 mg). The mixture of ketols was further separated by HPLC (Nucleosil 50-5, 8 $\times$ 300 mm, hexane : ethyl acetate=5 : 1) to give 2β,4α,5-trihydroxy-1-tetralone 4,5-acetonide (**8a**) (35 mg) and 2α,4α,5-trihydroxy-1-tetralone 4,5-acetonide (**8b**) (12 mg). **8a**: mp 90—92 °C (ethyl acetate–hexane). MS m/z: 234 (M^+), 216 ($M^+ - H_2O$), 176 ($M^+ - C_3H_6O$). ^{1}H-NMR δ: 1.62, 1.64 (3H each, s, $CH_3 \times 2$), 2.16 (1H, dt, J=4.9, 11.9, 11.9 Hz, H-3α), 2.67 (1H, ddd, J=2.4, 4.9, 11.9 Hz, H-3β), 2.80 (1H, s, –OH), 4.40 (1H, br s, H-2), 5.31 (1H, dd, J=4.9, 11.9 Hz, H-4), 7.05 (1H, dd, J=1.1, 8.2 Hz, H-6), 7.32 (1H, t, J=8.2 Hz, H-7), 7.52 (1H, dd, J=1.1, 8.2 Hz, H-8). **8b**: Oil. MS m/z: 234 (M^+), 216 ($M^+ - H_2O$), 176 ($M^+ - C_3H_6O$). ^{1}H-NMR δ: 1.64, 1.65 (3H each, s, $CH_3 \times 2$), 2.10 (1H, dt, J=11.5, 13.2 Hz, H-3α), 2.85 (1H, dt, J=4.6, 11.5 Hz, H-3β), 3.82 (1H, d, J=1.7 Hz, –OH), 4.41 (1H, ddd, J=1.7, 4.6, 13.2 Hz, H-2), 5.12 (1H, dd, J=4.6, 11.5 Hz, H-4), 7.06 (1H, dd, J=1.0, 7.0 Hz, H-6), 7.35 (1H, t, J=7.9 Hz, H-7), 7.65 (1H, dd, J=1.0, 7.9 Hz, H-8).

Preparation of 2β,4α,5-Trihydroxy-1-tetralone (9) and 2α,4α,5-Trihydroxy-1-tetralone (2)——The acetonides **8a** (8 mg) and **8b** (4 mg) were hydrolyzed with 5% HCl as described in the case of 7 and products were purified by HPLC (Nucleosil 50-5, 8 $\times$ 300 mm, hexane : ethyl acetate=1 : 1) to give **9** (6 mg) and **2** (3 mg) respectively. **9**: mp 146—148 °C (ethyl acetate–hexane). MS m/z: 194 (M^+), 176 ($M^+ - H_2O$), 147 ($M^+ - CH_3O_2$), 121 ($M^+ - C_3H_5O_2$). ^{1}H-NMR δ: 2.24 (1H, dt, J=4.4, 13.2, 13.2 Hz, H-3), 2.71 (1H, ddd, J=2.7, 4.4, 13.2 Hz, H-3), 3.44 (1H, br s, –OH), 3.79 (1H, br s, –OH), 4.90 (1H, br dd, J=4.4, 13.2 Hz, H-2), 5.40 (1H, br t, J=4.4 Hz, H-4), 7.19 (1H, d, J=7.8 Hz, H-6), 7.32 (1H, t, J=7.8 Hz, H-7), 7.53 (1H, d, J=7.8 Hz, H-8). **2**: Oil. MS m/z: 194 (M^+), 176 ($M^+ - H_2O$), 147 ($M^+ - CH_3O_2$), 121 ($M^+ - C_3H_5O_2$), ^{1}H-NMR δ: 2.11 (1H, ddd, J=11.0, 11.7, 13.7 Hz, H-3), 2.70 (1H, br s, –OH), 2.94 (1H, dt, J=5.2, 5.2, 11.7 Hz, H-3), 3.81 (1H, d, J=2.2 Hz, –OH), 4.31 (1H, ddd, J=2.2, 5.2, 13.7 Hz, H-2), 5.42 (1H, ddd, J=4.4, 5.2, 11.0 Hz, H-4), 7.17 (1H, dd, J=1.2, 7.8 Hz, H-6), 7.37 (1H, t, J=7.8 Hz, H-7), 7.61 (1H, dd, J=1.2, 7.8 Hz, H-8). The ^{1}H-NMR data of **2** were identical with those of the natural product.

Preparation of 2β,4α,8-Trihydroxy-1-tetralone (11) and 2α,4α-8-Trihydroxy-1-tetralone (3)——A mixture of **5** (990 mg), dihydropyran (1.5 ml, 1.5 eq) and a catalytic amount of CSA in methylene chloride (10 ml) was stirred at room temperature for 30 min, then poured into saturated sodium bicarbonate solution (15 ml), and the whole was extracted with ether (20 ml $\times$ 3). The combined organic layer was washed with brine and dried over sodium sulfate. Evaporation of the solvent gave ditetrahydropyranyl ether in quantitative yield. The ether (1.05 g) was oxidized with $MoO_5 \cdot$HMPA to give a mixture of ketols (**10a** and **10b**) as described in the case of **7**. The products were separated by silica gel column chromatography (hexane : ethyl acetate=3 : 1) to give the starting material (315 mg, 30%) and a mixture of ketols (405 mg, 38%), which could not be separated by normal-phase or reversed-phase HPLC. Thus, a part (50 mg) of this mixture was hydrolyzed with 5% HCl as described in the case of **7**. The crude products were separated by reversed-phase HPLC (ODS-2, 8 $\times$ 300 mm, acetonitrile : water=1 : 9), followed by normal-phase HPLC (Nucleosil 50-5, 8 $\times$ 300 mm, hexane : ethanol=20 : 1) to give **3** (3 mg) and **11** (14 mg). **3**: mp 114—117 °C (ethyl acetate–hexane). MS m/z: 194 (M^+), 176 ($M^+ - H_2O$), 150 ($M^+ - C_2H_4O$), 121 ($M^+ - C_3H_5O_2$). ^{1}H-NMR δ: 2.02 (1H, q, J=11.7 Hz, H-3α), 2.78 (1H, dt, J=5.1, 5.1, 11.7 Hz, H-3β), 4.31 (1H, dd, J=5.1, 11.7 Hz, H-2), 4.94 (1H, dd, J=5.1, 11.7 Hz, H-4), 6.88 (1H, d, J=7.8 Hz, H-7), 7.17 (1H, d, J=7.8 Hz, H-5), 7.51 (1H, t, J=7.8 Hz, H-6). The ^{1}H-NMR data of **3** were identical with those of the natural product. **11**: Oil. MS m/z: 194 (M^+), 176 ($M^+ - H_2O$), 150 ($M^+ - C_2H_4O$), 121 ($M^+ - C_3H_5O_2$). ^{1}H-NMR δ: 1.67 (1H, d, J=5.1 Hz, –OH), 2.05 (1H, br s,

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–OH), 2.23 (1H, dt, J=2.7, 12.9, 12.9 Hz, H-3β), 2.71 (1H, ddd, J=3.2, 5.4, 12.9 Hz, H-3α), 4.94 (1H, ddd, J=3.2, 5.1, 12.9 Hz, H-2), 5.06 (1H, dd, J=2.7, 5.4 Hz, H-4), 6.97 (1H, d, J=7.5 Hz, H-7), 7.00 (1H, d, J=7.5 Hz, H-5), 7.53 (1H, t, J=7.5 Hz, H-6).

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