Absolute Structure of Panaxytriol

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Diastereomeric mixture at C-3 of (9R,10R)-panaxytriol acetonide (3) and (9S,10S)-panaxytriol acetonide (4) were enantioselectively acetylated to give (3R)-acetates (3a-Ac, 4a-Ac) and (3S)-alcohols (3b, 4b) by enzyme mediated-acetylation using CHIRAZYME and vinyl acetate. A comparison of the 1H-NMR spectra of their α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) esters. The alcohols 2a and 2b were converted into MTPA esters (2a', 2b') with R-(−)-MTPA chloride, respectively. A comparison of the 1H-NMR spectrum of 2a' with that of 2b' revealed that the vinyl proton signals [δ 5.37, 5.53 (H-1), δ 5.83 (H-2)] of 2b' appeared at higher fields than those [δ 5.43, 5.62 (H-1), δ 5.92 (H-2)] of 2a'. Thus, the absolute configurations at C-3 of the esters 2a and 2b were assigned as R and S, respectively, based on the general rules of the MTPA method. The optical purities of 2a and 2b were more than 99%, respectively, which were estimated on the basis of the 1H-NMR spectra of 2a' and 2b'. The above results showed that only the alcohol (2a) having R-configuration was enantioselectively acetylated by the action of CHIRAZYME and vinyl acetate (Chart 1).

Next, we tried enantioselective acetylation of the diastereomeric mixture at C-3 of (9R,10R)-panaxytriol acetonide (3) and (9S,10S)-panaxytriol acetonide (4). Treatment of 3 with CHIRAZYME L-2,C3 and vinyl acetate gave a mixture of an acetate (3a-Ac) and an alcohol (3b) which was separated by HPLC. The obtained acetate (3a-Ac) was then hydrolyzed with CHIRAZYME L-2,C2 to afford an alcohol (3a). Similarly, the acetonide (4a) was also separated into the alcohols (4a, 4b) via CHIRAZYME catalyzed enantioselective acetylation and hydrolysis (Chart 2). The compounds (3a, 3b, 4a, 4b) were identical in comparison of their 1H- and 13C-NMR spectra.

**Key words** panaxytriol; panax species; polyacetylene; absolute configuration; enzyme mediated-acetylation

**Notes**

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In order to determine the absolute configuration at C-3 by the application of modified Mosher method, the alcohols (3a, 3b, 4a, 4b) were converted into (R)- and (S)-MTPA esters, respectively. As shown in Chart 3, the signals due to H2-1 and H-2 of 3a-(R)-MTPA (3a') and 4a-(R)-MTPA (4a') appeared at higher fields than those of 3a-(S)-MTPA (3a") and 4a-(S)-MTPA (4a"), respectively, thereby suggesting the stereochemistry at C-3 of 3a and 4a to be R configuration. On the other hand, the signals due to H2-1 and H-2 of 3b-(R)-MTPA (3b') and 4b-(R)-MTPA (4b') appeared at lower fields than those of 3b-(S)-MTPA (3b") and 4b-(S)-MTPA (4b''), respectively. Thus, the absolute configuration at C-3 of 3b and 4b could be assigned as S. The (3R)- and (3S)-hydroxy-9,10-acetonides (3a, 3b, 4a, 4b) were treated with MeOH–2 N HCl to give four sorts of synthetic panaxytriols, respectively. All the compounds obtained here showed the same 1H- and 13C-NMR spectra.

As shown in Table 1, the optical rotation value of natural panaxytriol was identical with that of synthetic (3R,9R,10R)-panaxytriol. Thus, the absolute configuration of natural panaxytriol could be concluded as 3R,9R,10R. The former result might be caused by erroneous movement of polarmeter.

**Table 1. The Optical Rotation Values of the Synthetic Panaxytriol and Natural Panaxytriol**

<table>
<thead>
<tr>
<th>Configuration</th>
<th>[α]D (in CHCl3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R,9R,10R</td>
<td>−18.6° (c=0.43)</td>
</tr>
<tr>
<td>3S,9R,10S</td>
<td>47.3° (c=0.79)</td>
</tr>
<tr>
<td>3R,9S,10S</td>
<td>−47.3° (c=0.80)</td>
</tr>
<tr>
<td>3S,9S,10S</td>
<td>17.6° (c=0.67)</td>
</tr>
<tr>
<td>Natural panaxytriol</td>
<td>−18.4° (c=0.33)</td>
</tr>
</tbody>
</table>

Experimental

The 1H- and 13C-NMR spectra were measured on a JEOL JNM-EX900 and a JEOL JNM-α 300 spectrometer in CDCl3 containing tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on a JEOL JMS-D 300 instrument. Waco-gel C-200 was used for silica gel column chromatography. The optical rotations were measured on a JASCO DIP-370 polarimeter. Senshu pack (PEGASIL Silica 60-5, 10 mm; TMS) was used for silica gel column chromatography.

**Diastereomeric Mixture at C-3 of Hept-1-ene-4,6-diyne-3-ol (2)**

2-Butyl methyl ether (1.57 mol/l) in hexane [28 ml (44 mmol)] was added dropwise to a stirred solution of diacetylene (5.0 ml) in THF (40 ml) at 40 °C. After 30 min, acrolein (5.0 ml) was added and stirring was continued for 3 h at the same temperature. The reaction mixture was quenched with saturated NH4Cl solution (20 ml) and then extracted with AcOEt (30 ml). The organic layer was washed with brine (30 ml×2), dried over MgSO4 and concentrated under reduced pressure to leave an oil, which was chromatographed on a silica gel column (hexane:AcOEt=10:1) to give 2 (1.84 g, 43.4%). The 1H-NMR δ: 2.24 (1H, s), 4.93 (1H, d, J=5.3 Hz), 5.28 (1H, d, J=11.4 Hz), 5.49 (1H, d, J=16.9 Hz), 5.95 (1H, d, J=5.3 Hz, 11.4, 16.9 Hz). CI-MS: m/z: 107 (M+1)

**Acetylation of 2 with Lipase (CHIRAZYME L-2,C3)**

Lipase (CHIRAZYME L-2,C3, 115.3 mg) and vinyl acetate (500 μl, 5.22 mmol) were added to a stirred solution of 2 (248 mg, 2.34 mmol) in tert-butyl methyl ether (8.0 ml) and the mixture was stirred overnight at room temperature. The reaction mixture was filtered with Celite and evaporated in vacuo. The residue was purified by HPLC to give 2a-Ac (70.5 mg, 20.4%) as an oil and 2b (29.6 mg, 11.9%) as an oil.

2a-Ac [(3R)-Hept-1-ene-4,6-diyne-3-ol Acetate]: 1H-NMR δ: 2.21 (3H, s), 2.24 (1H, s), 5.36 (1H, d, J=9.7 Hz), 5.49 (1H, d, J=15.4 Hz), 5.86 (1H, d, J=5.7, 9.7, 15.4 Hz), 5.89 (1H, d, J=5.7 Hz). CI-MS: m/z: 149 (M+1).

2b [(3S)-Hept-1-ene-4,6-diyne-3-ol]: [α]D +93.7° (c=0.71, CHCl3).

**Hydrolysis of 2a-Ac with Lipase (CHIRAZYME L-2,C2)**

Compound 2a-Ac (32.4 mg, 0.22 mmol) was dissolved in 0.5 ml of acetone and 4.5 ml of pH7.4 phosphate buffer, and then lipase (CHIRAZYME L-2,C2, 132 mg)
was added. The reaction mixture was stirred overnight at room temperature. The mixture was filtered with celite, and then extracted with AcOEt (30 ml). The organic layer was washed with brine (30 ml×2), dried over MgSO₄, and evaporated in vacuo to leave an oil. The residue was purified by HPLC to give 2a (5.7 mg, 24.6%) as an oil. 2a [3R]-Hept-1-ene-4,6-diyne-3-ol: [α]D₂₀ = 88.0° (ε = 0.95, CHCl₃). 2a [(3R)-Hept-1-ene-4,6-diyne-3-ol]: [α]D₂₀ = 88.0° (ε = 0.95, CHCl₃).

**2a [(3R)-Hept-1-ene-4,6-diyne-3-ol]: [α]D₂₀ = 88.0° (ε = 0.95, CHCl₃).** 2a was added to the solution of 2b (5.0 mg, 0.05 mmol) in pyridine (1.0 ml) and the stirring was continued overnight at room temperature. The mixture was diluted with AcOEt (30 ml) and then washed successively with 1N HCl (20 ml) and saturated NaHCO₃ solution, dried over MgSO₄, and evaporated in vacuo to leave an oil. The residue was purified by HPLC to give 2a' (6.4 mg, 42.2%). 1H-NMR δ: 2.26 (1H, s), 3.55 (3H, s), 5.43 (1H, d, J = 10.1 Hz), 5.62 (1H, d, J = 16.9 Hz), 5.92 (1H, ddd, J = 5.1, 10.1, 16.9 Hz), 6.07 (1H, d, J = 5.1 Hz), 7.41 (3H, m), 7.51 (2H, m). 13C-NMR: 23.5, 25.9, 27.0, 29.1, 29.6, 31.7, 32.9, 63.3, 66.5, 72.3, 76.8, 77.0, 78.1, 80.4, 108.7, 117.0, 136.1. CI-MS: m/z 360.2301 (M⁺). (S)-(−)-MTPA Ester of 2a (2a'). Five drops (large excess) of (R)-(−)-MTPA-Cl was added to a stirred solution of 2a (5.0 mg, 0.05 mmol) in pyridine (1.0 ml) and the stirring was continued overnight at room temperature. The mixture was diluted with AcOEt (30 ml) and then washed successively with 1N HCl (20 ml) and saturated NaHCO₃ solution, dried over MgSO₄, and evaporated in vacuo. The residue was purified by HPLC to give 2a' (6.4 mg, 42.2%). 1H-NMR δ: 2.26 (1H, s), 3.55 (3H, s), 5.43 (1H, d, J = 10.1 Hz), 5.62 (1H, d, J = 16.9 Hz), 5.92 (1H, ddd, J = 5.1, 10.1, 16.9 Hz), 6.07 (1H, d, J = 5.1 Hz), 7.41 (3H, m), 7.51 (2H, m). 13C-NMR: 23.5, 25.9, 27.0, 29.1, 29.6, 31.7, 32.9, 63.3, 66.5, 72.3, 76.8, 77.0, 78.1, 80.4, 108.7, 117.0, 136.1. CI-MS: m/z 360.2301 (M⁺). (S)-(−)-MTPA Ester of 2a (2a'). (S)-(−)-MTPA ester of 3a and 4a (3a', 4a'): 1H-NMR (CDCl₃) δ: 0.89 (3H, t, J = 7.2 Hz), 1.29 (10H, brm), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, ddd, J = 6.6, 17.7 Hz), 2.61 (1H, dd, J = 5.2, 17.7 Hz), 3.42 (3H, s), 3.77 (2H, m), 5.33 (1H, d, J = 10.0 Hz), 5.51 (1H, d, J = 17.1 Hz), 5.82 (1H, ddd, J = 5.5, 10.0, 17.1 Hz), 6.11 (1H, d, J = 5.5 Hz), 7.40 (3H, m), 7.52 (2H, m). (S)-(−)-MTPA ester of 3a and 4a (3a', 4a'): 1H-NMR (CDCl₃) δ: 0.89 (3H, t, J = 7.2 Hz), 1.29 (10H, brm), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, ddd, J = 6.6, 17.7 Hz), 2.61 (1H, dd, J = 5.2, 17.7 Hz), 3.42 (3H, s), 3.77 (2H, m), 5.42 (1H, d, J = 10.0 Hz), 5.60 (1H, d, J = 17.1 Hz), 5.92 (1H, ddd, J = 5.5, 10.0, 17.1 Hz), 6.10 (1H, d, J = 5.5 Hz), 7.40 (3H, m), 7.52 (2H, m). (R)-(−)-MTPA ester of 3b and 4b (3b', 4b'): 1H-NMR (CDCl₃) δ: 0.89 (3H, t, J = 7.2 Hz), 1.29 (10H, brm), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, ddd, J = 6.6, 17.7 Hz), 2.61 (1H, dd, J = 5.2, 17.7 Hz), 3.42 (3H, s), 3.77 (2H, m), 5.40 (1H, d, J = 10.0 Hz), 5.60 (1H, d, J = 17.1 Hz), 5.92 (1H, ddd, J = 5.5, 10.0, 17.1 Hz), 6.10 (1H, d, J = 5.5 Hz), 7.40 (3H, m), 7.52 (2H, m). (R)-(−)-MTPA ester of 3b and 4b (3b', 4b'): 1H-NMR (CDCl₃) δ: 0.89 (3H, t, J = 7.2 Hz), 1.29 (10H, brm), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, ddd, J = 6.6, 17.7 Hz), 2.61 (1H, dd, J = 5.2, 17.7 Hz), 3.42 (3H, s), 3.77 (2H, m), 5.34 (1H, d, J = 10.0 Hz), 5.51 (1H, d, J = 17.1 Hz), 5.82 (1H, ddd, J = 5.5, 10.0, 17.1 Hz), 6.11 (1H, d, J = 5.5 Hz), 7.40 (3H, m), 7.52 (2H, m). (R)-(−)-MTPA ester of 3b and 4b (3b', 4b') having the same absolute configurations at C-3 showed the same NMR spectral data in spite of the different absolute configurations at C-9 and C-10.