Absolute Structure of Panaxytriol

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Diastereomeric mixture at C-3 of (9*R*,10*R*)-panaxytriol acetonide (3) and (9*S*,10*S*)-panaxytriol acetonide (4) were enantioselectively acetylated to give (3*R*)-acetates (3a-Ac, 4a-Ac) and (3*S*)-alcohols (3b, 4b) by enzyme mediated-acetylation using CHIRAZYME and vinyl acetate, respectively. Hydrolysis of (3*R*)-acetate (3a-Ac, 4a-Ac) with CHIRAZYME and phosphate buffer afforded (3*R*)-alcohols (3a, 4a), respectively. Deprotection of panaxytriol acetonides (3a, 3b, 4a, 4b) gave panaxatriol and its isomers, respectively. Comparison of optical rotation values of the synthetic panaxatriols with that of the natural one confirmed that the absolute configuration of panaxytriol sould be 3*R*,9*R*,10*R*.

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

We have reported the synthesis of the diastereomeric mixtures at C-3 of (9*R*,10*R*)- and (9*S*,10*S*)-panaxytriols (**1**) starting from L-(+)- or D-(−)-tartaric acid diethyl ester and the absolute configuration of natural panaxytriol could be assigned as 3*R*,9*S*,10*S*.[1)] However, Kobayashi *et al.* and Lu *et al.* reported that the absolute configuration of natural panaxytriol should be 3*R*,9*R*,10*R* by circular dichroism (CD) analysis[2)] and synthetic method,[3)] respectively. On the other hand, Gurjar *et al.* reported that the absolute configuration of natural panaxytriol should be 3*R*,9*S*,10*S* by synthetic method.[4)] In order to clarify these conflicting reports, we synthesized four possible isomers [(3*R*,9*R*,10*R*), (3*R*,9*S*,10*S*), (3*S*,9*R*,10*R*), (3*S*,9*S*,10*S*)] of panaxytriol and revised the our former results to be 3*R*,9*R*,10*R*.

As preliminary experiments, we examined whether a diastereomeric mixture of acetylenic alcohol could be enantioselectively transformed into an acetate by enzyme mediated-acetylation[5)] using CHIRAZYME and vinyl acetate. A diastereomeric mixture of hept-1-ene-4,6-diyn-3-ol (**2**)[6)] corresponding to the C-1—C-7 moiety of panaxytriol was prepared by coupling reaction of diacetylene with acrolein. Treatment of **2** with vinyl acetate in the presence of CHIRAZYME L-2,C3 gave a mixture of an acetate (**2a**-Ac) and unreacted alcohol (**2b**) which was separated by high performance liquid chromatography (HPLC). The acetate (**2a**-Ac) was hydrolyzed with CHIRAZYME L-2,C2 and phosphate buffer (pH 7.4) to give an alcohol (**2a**). The absolute configurations of these alcohols (**2a**, **2b**) were investigated by analyses of the 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 ^{1}H-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.[7)] The optical purities of **2a** and **2b** were more than 99%, respectively, which were estimated on the basis of the ^{1}H-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 (9*R*,10*R*)-panaxytriol acetonide (**3**) and (9*S*,10*S*)-panaxytriol acetonide (**4**).[1)] 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 (**4**) was also separated into the alcohols (**4a**) and (**4b**) *via* CHIRAZYME catalyzed enantioselective acetylation and hydrolysis (Chart 2). The compounds (**3a**, **3b**, **4a**, **4b**) were identical in comparison of their ^{1}H- and ^{13}C-NMR spectra.

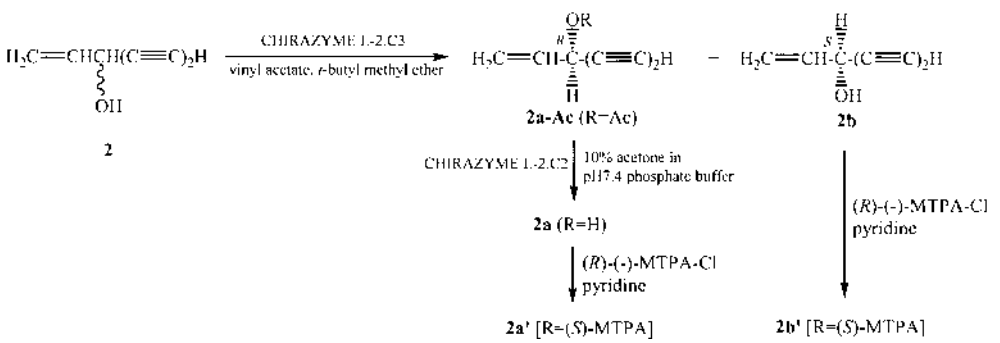

Chart 1

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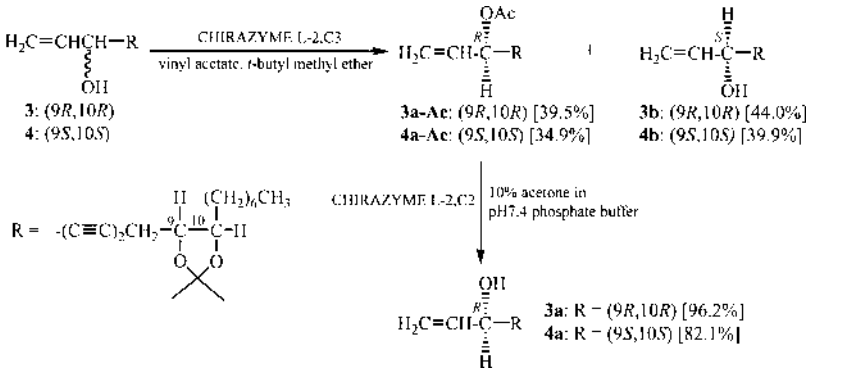
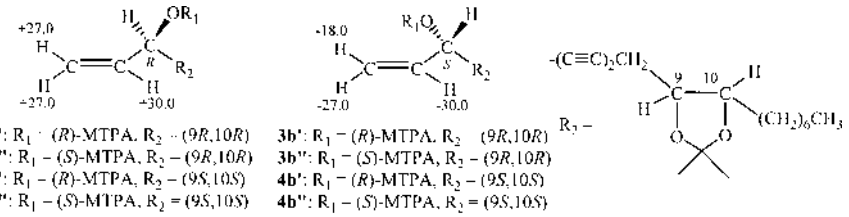


Chart 2



Δδ (= $\delta_S - \delta_R$) values obtained from the MTPA esters of **3a(3a', 3a'')**, **3b(3b', 3b'')**, **4a(4a', 4a'')** and **4b(4b', 4b'')**. Δδ values are expressed in Hz.

Chart 3

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

Configuration	$[\alpha]_D$ (in $CHCl_3$)
3*R*,9*R*,10*R*	−18.6° (*c*=0.43)
3*S*,9*R*,10*R*	47.3° (*c*=0.79)
3*R*,9*S*,10*S*	−47.3° (*c*=0.80)
3*S*,9*S*,10*S*	17.6° (*c*=0.67)
Natural panaxytriol	−18.4° (*c*=0.33)

In order to determine the absolute configuration at C-3 by the application of modified Mosher method,[8] the alcohols (**3a**, **3b**, **4a**, **4b**) were converted into (*R*)- and (*S*)-MTPA esters, respectively. As shown in Chart 3, the signals due to H_2-1 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 H_2-1 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.[9] Thus, the absolute configuration at C-3 of **3b** and **4b** could be assigned as *S*. The (3*R*)- and (3*S*)-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 ^{1}H- and ^{13}C-NMR spectra.

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

Experimental

The ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-EX90 and a JEOL JNM-α 300 spectrometer in $CDCl_3$ 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, 10ϕ×250 mm) column was used for HPLC. CHIRAZYME L-2,C3 and L-2,C2 were purchased from Boehringer Mannheim.

Diastereomeric Mixture at C-3 of Hept-1-ene-4,6-diyn-3-ol (2) *n*-BuLi (1.57 mol/l) in hexane [28 ml (44 mmol)] was added dropwise to a stirred solution of diacetylene (5.0 ml, 8.0 mg/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 NH_4Cl solution (20 ml) and then extracted with AcOEt (30 ml). The organic layer was washed with brine (30 ml×2), dried over $MgSO_4$ 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%) as an oil. **2**: ^{1}H-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, ddd, *J*=5.3, 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, 5.42 mmol) was 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 [(3*R*)-Hept-1-ene-4,6-diyn-3-ol Acetate]: ^{1}H-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, ddd, *J*=5.7, 9.7, 15.4 Hz), 5.89 (1H, d, *J*=5.7 Hz). CI-MS: *m/z*: 149 $(M+1)^+$.

2b [(3*S*)-Hept-1-ene-4,6-diyn-3-ol]: $[\alpha]_D$ +93.7° (*c*=0.71, $CHCl_3$). ^{1}H-NMR δ: 2.24 (1H, s), 4.93 (1H, d, *J*=5.3 Hz), 5.28 (1H, d, *J*=10.1 Hz), 5.49 (1H, d, *J*=15.6 Hz), 5.95 (1H, ddd, *J*=5.3, 10.1, 15.6 Hz). ^{13}C-NMR δ: 63.2, 67.3, 69.0, 70.3, 74.8, 117.4, 135.6. CI-MS: *m/z*: 107 $(M+1)^+$.

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 $MgSO_4$ 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 [(3*R*)-Hept-1-ene-4,6-diyn-3ol]: $[\alpha]_D$ −88.0° (*c*=0.95, $CHCl_3$).

(*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 1 N HCl (20 ml) and saturated $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated *in vacuo.* The residue was purified by HPLC to give **2a′** (6.4 mg, 42.2%). ^{1}H-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)

(*S*)-(−)-MTPA Ester of 2b (2b′) The reaction was carried out in a similar manner as above. **2b′** (48.7%). ^{1}H-NMR δ: 2.28 (1H, s), 3.59 (3H, s), 5.37 (1H, d, *J*=10.1 Hz), 5.53 (1H, d, *J*=16.9 Hz), 5.83 (1H, ddd, *J*=5.1, 10.1, 16.9 Hz), 6.10 (1H, d, *J*=5.1 Hz), 7.41 (3H, m), 7.51 (2H, m).

Acetylation of 3 with Lipase (CHIRAZYME L-2,C3) The reaction was carried out in a similar manner as described in acetylation of **2** with lipase. **3a**-Ac (20.1 mg, 39.5%) and **3b** (19.8 mg, 44.0%).

3a-Ac [(3*R*,9*R*,10*R*)-Heptadec-1-ene-9,10-isopropylidenedioxy-4,6-diyn-3-ol Acetate]: ^{1}H-NMR δ: 0.87 (3H, t, *J*=7.0 Hz), 1.29 (10H, br, m), 1.41 (6H, s), 1.58 (2H, m), 2.10 (3H, s), 2.58 (1H, dd, *J*=6.6, 17.7 Hz), 2.60 (1H, dd, *J*=5.1, 17.7 Hz), 3.75 (2H, m), 5.33 (1H, d, *J*=9.7 Hz), 5.53 (1H, d, *J*=15.6 Hz), 5.85 (1H, ddd, *J*=4.0, 9.7, 15.6 Hz), 5.90 (1H, d, *J*=4.0 Hz), High-resolution MS: *m*/*z* 360.2301 $(M)^+$ (Calcd for $C_{22}H_{32}O_4$).

3b [(3*S*,9*R*,10*R*)-Heptadec-1-ene-9,10-isopropylidenedioxy-4,6-diyn-3-ol]: ^{1}H-NMR δ: 0.89 (3H, t, *J*=7.2 Hz), 1.29 (10H, br, m), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, dd, *J*=6.6, 17.7 Hz), 2.61 (1H, dd, *J*=5.2, 17.7 Hz), 3.77 (2H, m), 4.91 (1H, d, *J*=5.5 Hz), 5.24 (1H, d, *J*=10.0 Hz), 5.46 (1H, d, *J*=17.1 Hz), 5.94 (1H, ddd, *J*=5.5, 10.0, 17.1 Hz), ^{13}C-NMR δ: 14.0, 22.6, 23.5, 25.9, 27.0, 27.4, 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* 319 $(M+1)^+$.

Hydrolysis of 3a-Ac with Lipase (CHIRAZYME L-2,C2) The reaction was carried out in a similar manner as described in hydrolysis of **2a**-Ac with lipase. **3a** (15.0 mg, 96.2%).

(*R*)-(+)-MTPA and (*S*)-(−)-MTPA Esters of Panaxytriol Acetonide and Its Isomers (*R*)-(+)- and (*S*)-(−)-MTPA esters of panaxytriol acetonide and its isomers were prepared in a similar manner as described in the preparation of *S*-(−)-MTPA esters of **2a** and **2b**.

(*R*)-(+)-MTPA-ester of **3a** and **4a** (**3a′**, **4a′**): ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.2 Hz), 1.29 (10H, br m), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, dd, *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″**): ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.2 Hz), 1.29 (10H, br m), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, dd, *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′**): ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.2 Hz), 1.29 (10H, br m), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, dd, *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).

(*S*)-(−)-MTPA ester of **3b** and **4b** (**3b″**, **4b″**): ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.2 Hz), 1.29 (10H, br m), 1.40 (6H, s), 1.60 (2H, m), 2.59 (1H, dd, *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).

References and Notes

1) Satoh M., Takeuchi N., Fujimoto Y., *Chem. Pharm. Bull.*, **45**, 1114—1116 (1997).
2) Kobayashi M., Mahmund T., Umezome T., Wang W., Murakami N., *Tetrahedron*, **53**, 15691—15700 (1997).
3) Lu W., Zheng G., Gao D., Cai J., *Tetrahedron*, **55**, 7157—7168 (1999).
4) Gurjar M. K., Kumar V. S., Rao B. V., *Tetrahedron*, **55**, 12563—12576 (1999).
5) Naoshima Y., Kamezawa M., Kimura T., Okimoto F., Watanabe M., Tachibana H., Ohtani T., *Recent Res. Devel. Org. & Bioorg. Chem.*, **4**, 1—16 (2001).
6) Takahashi M., Yoshikura M., *Yakugaku Zasshi*, **86**, 1053—1056 (1966).
7) Sullivan G. R., Dale J. A., Mosher H. S., *J. Org. Chem.*, **38**, 2143—2147 (1973).
8) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc.*, **113**, 4092—4096 (1991).
9) The MTPA esters (**3a′**, **4a′**, or **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.