Studies on the Sesquiterpenoids of *Panax ginseng* C. A. MEYER. IV

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A tricarbocyclic sesquiterpenoid (1), isolated from the ethereal extract of the rootlets of *Panax ginseng* C. A. MEYER, showed almost identical nuclear magnetic resonance data to those reported for senecrassidiol (2). Detailed spectral analysis of 1 led us to revise the stereochemistry of 2.

Keywords senecrassidiol; tricarbocyclic sesquiterpenoid; *Panax ginseng*; Araliaceae; revised structure; NOE

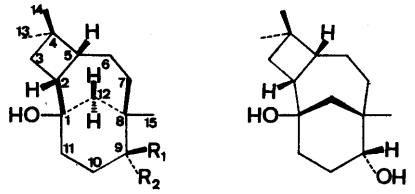
In our previous papers, we reported the isolation of some sesquiterpenoids from the ethereal extract of the rootlets of *Panax ginseng* C. A. MEYER.[1] Herein, we wish to describe the structural elucidation of a newly isolated sesquiterpene diol, **1**, from the neutral fraction.[2]

Compound **1**, $[\alpha]_D$ −14.1°, colorless crystals, mp 109—110 °C, had the molecular formula $C_{15}H_{26}O_2$, confirmed by high-resolution mass spectroscopy (HR-MS). The infrared (IR) spectrum showed hydroxyl absorption (3400—3600 cm^{-1}). The low-resolution MS of **1** showed the molecular ion peak at *m/z* 238 and fragment ion peaks at *m/z* 220 ($M^+ - H_2O$), 202 ($M^+ - 2H_2O$), 165 and 123 (base peak). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed three methyl signals at δ 0.94, 1.00 and 1.19 (each 3H, s), two methine protons at δ 2.48 (1H, m) and 3.33 (1H, br s), and signals due to the A and B parts of ABX system at δ 1.34 (1H, dd, J=16.2, 1.6 Hz) and 1.84 (1H, br d, J=16.2 Hz). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** showed two signals due to carbon a hydroxyl group (δ 74.1, d, and 72.5, s), six methylene carbon signals (δ 22.0, 27.0, 34.4, 35.7, 36.1 and 38.4), two methine carbon signals (δ 41.6 and 49.0) and two quaternary carbon signals (δ 33.9 and 37.5), but no sp^2 carbon signals. Therefore, this compound is a saturated tricarbocyclic sesquiterpene diol.

After an intensive survey of reported ^{1}H- and ^{13}C-NMR data, we noticed that the data of **1** closely resembled those of sescrassidiol (**2**), whose structure was proposed by Bohlmann and Ziesche mainly on the basis of the NMR spectra.[3] Furthermore, the results of a two-dimensional incredible natural abundance double quantum transfer experiment (2D-INADEQUATE) and 2D-NMR (^{1}H–^{13}C and long-range ^{1}H–^{13}C shift correlation spectra (COSY)) of **1** were consistent with the planar structure of **2**. Thus, these two compounds, **1** and **2**, might be identical. However, since we have isolated a series of compounds related to (−)-β-caryophyllene from *P. ginseng*,[1] we considered that the stereochemistry (*cis* junction of the A/B rings) of **1** should be carefully investigated.

The nuclear Overhauser effect (NOE) correlation spectrum (NOESY) of **4** showed NOE correlations between several protons (2-H and 14-H_3, and 5-H and 14-H_3),[4] and these were confirmed by the NOE experiments on **4**, that is, NOEs were observed between 14-H_3 and 2-H (9.8%), and between 14-H_3 and 5-H (7.9%). Thus, the cyclobutane ring was condensed in the *cis* mode.

On the other hand, cross peaks between 2-H and one proton of the methano bridge part (C-12) were observed in the ^{1}H–^{1}H COSY spectra of **1** and **3**. From the inspection of Dreiding models, these observations are explicable in terms of long-range coupling *via* W-interaction between the 2- and 12α-H in **1** and **3** rather than **2**. To elucidate the relative geometry between 2-H and the methano bridge part (C-12), NOE difference experiments were undertaken using **3**[3] (Fig. 1).[5] Irradiation at the frequency of the 14-H_3 signal caused an enhancement of the intensity of the 2-H signal, as well as an ambiguous enhancement of the intensity of the 5-H signal. Next, irradiation of 13-H_3 signal caused an enhancement of the intensity of the 6-H signal, whose relative configuration is assigned to be α from the inspection of Dreiding models based on the NOE findings mentioned above in **3** and **4**. Furthermore, irradiation of 15-H_3 caused an enhancement of the intensity of this 6α-H. These observations indicate that the 13- and 15-methyl groups are spatially close to 6α-H. Other NOE findings are indicated by the double-headed arrows in the structure in Fig. 1. From these results, the relative geometry between 2-H and the methano bridge part was elucidated to be anti. On the other hand, the epimer (**5**) of **1** was obtained by the lithium aluminium hydride (LAH) reduction of **3**, and the secondary hydroxyl group of **1** should be α-oriented, taking the reduction mechanism from **3** to **5** into consideration. This was supported by the observation of long-range coupling between 9β-H and 12β-H in the ^{1}H–^{1}H COSY spectrum of **1**. Therefore, the stereochemistry of compound **1** should be represented by the formula **1**. This is different from the proposed structure **2**, and the previous reported structure **2** should be revised.



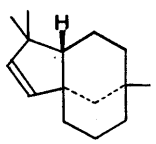


Chart 1

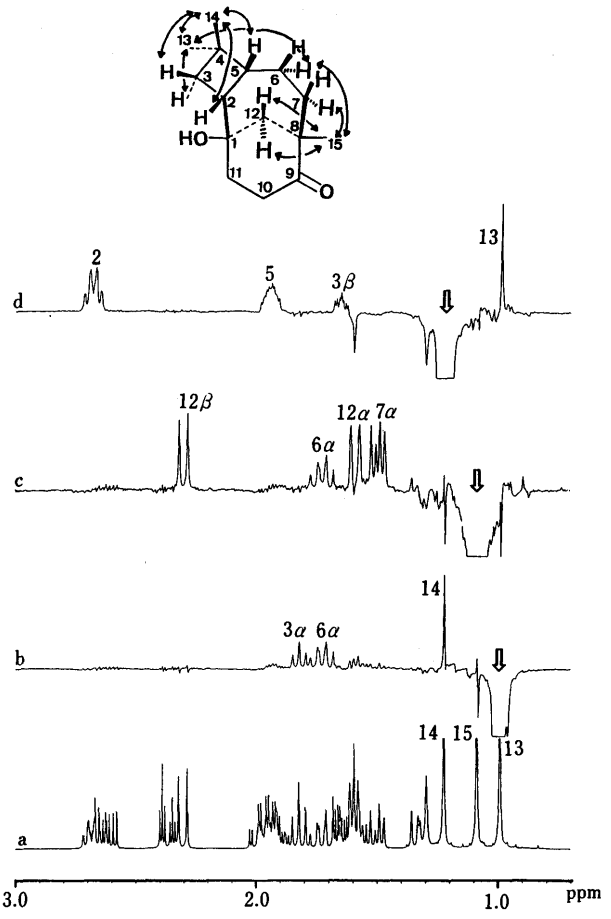


Fig. 1. NOE Spectra of **3** (400 MHz)

(a) Off-resonance decoupled spectrum; (b), (c), (d) NOE difference spectra between off- and on-resonance (shown by arrow: ⇩) saturated.

On treatment of **4** with concentrated H_2SO_4 in anhydrous ether under conditions similar to those employed in the rearrangement of (−)-β-caryophyllene,[6] compound **6**, $[\alpha]_D$ −30.3°, and compound **7**, $[\alpha]_D$ −25.0°, were obtained as major products. Compound **6** was identical with clovene by direct comparison with an authentic sample prepared by the reported method from (−)-β-caryophyllene in respect of mass (MS), IR, ^{1}H-NMR spectral data and retention time on capillary gas chromatography (GC).[6-8b] On the other hand, compound **7** and its derivative (**8**) were shown to be identical with clovanol and clovanone, respectively, by comparing the spectral data with reported values.[8] Therefore, the absolute structure of compound **1** can be represented by the formula **1**.

Experimental

General procedures are the same as in the previous report[1b] except for the NMR spectrometers. ^{1}H- and ^{13}C-NMR spectra were measured with JEOL FX-200, GSX-270 and GSX-400 spectrometers. COSY and NOE difference spectra were measured with the JEOL GSX-400 spectrometer. NOEs and the NOESY spectrum were measured with a Bruker AM-400 spectrometer.

Isolation The ether extracts and neutral fractions of *P. ginseng* were obtained as described in the previous paper.[1b] The fractions eluted with acetone[1b] were further chromatographed on a silica-gel column using solvents of increasing polarity from benzene to acetone. Fractions containing **1** were further subjected to preparative high performance liquid chromatography (HPLC) (solvent, hexane–isopropanol (9 : 1, v/v); flow rate, 2.0 ml/min) to give crude **1**. Purification of **1** was carried out by repeated preparative HPLC (solvent, hexane–isopropanol (95 : 5, v/v); flow rate, 2.5 ml/min) to give a crystalline substance, which was recrystallized from hexane–ether mixture to give **1** (138 mg).

Compound 1 Colorless crystals, mp 109—110 °C. $[\alpha]_D^{22}$ −14.1° (c=0.99, $CHCl_3$). IR ($CHCl_3$): 3400—3600, 2950, 1460, 1375, 1365 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 0.94 (3H, s, 13-H_3), 1.00 (3H, s, 15-H_3), 1.19 (3H, s, 14-H_3), 1.34 (1H, dd, J=16.2, 1.6 Hz, 12α-H), 1.84 (1H, br d, J=16.2, 12β-H), 2.48 (1H, m, 2-H), 3.33 (1H, br s, 9-H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 22.0 (t, C-6), 24.4 (q, C-13), 27.0 (t, C-10), 28.7 (q, C-14), 30.0 (q, C-15), 33.9 (s, C-4), 34.4 (t, C-3), 35.7 (t, C-11), 36.1 (t, C-7), 37.5 (s, C-8), 38.4 (t, C-12), 41.6 (d, C-3), 49.0 (d, C-5), 72.5 (s, C-1), 74.1 (d, C-9). MS m/z (% rel. int.): 238 (M^+, 1), 220 (2), 205 (2), 202 (4), 165 (29), 123 (100), 109 (22), 95 (26), 81 (21), 67 (19), 55 (38), 41 (51). HR-MS m/z: M^+ Calcd for $C_{15}H_{26}O_2$ 238.193. Found: m/z 238.194. The ^{1}H- and ^{13}C-NMR, and mass spectra, and $[\alpha]_D$ value were in good agreement with those reported for **2**.[3]

Collins Oxidation of 1 A solution of **1** (70 mg) in dry CH_2Cl_2 (3 ml) was added to the prepared CrO_3–pyridine complex (0.8 g in 10 ml of dry CH_2Cl_2) and stirred at room temperature for 15 min. After usual work-up, the oily substance was subjected to silica-gel chromatography followed by preparative HPLC (solvent, benzene–ethyl acetate (9 : 1, v/v)) to give a crystalline substance, which was recrystallized from hexane–ether mixture to give **3** (39 mg) as colorless crystals, mp 105—106 °C. $[\alpha]_D^{22}$ −82.4° (c=0.61, $CHCl_3$). IR ($CHCl_3$): 3400—3600, 2940, 1705, 1460, 1385 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.99 (3H, s, 13-H_3), 1.09 (3H, s, 15-H_3), 1.22 (3H, s, 14-H_3), 1.50 (1H, m, 7α-H), 1.59 (1H, br d, J=13.8 Hz, 12α-H), 1.74 (1H, m, 6α-H), 1.82 (1H, t, J=11.0 Hz, 3α-H), 2.30 (1H, d, J=13.8 Hz, 12β-H), 2.37 (1H, dt, J=17.2, 4.4 Hz, 10-H), 2.62 (1H, ddd, J=17.2, 12.6, 6.2 Hz, 10-H), 2.68 (1H, m, 2-H). ^{13}C-NMR ($CDCl_3$, 68 MHz) δ: 21.3 (t), 24.3 (q), 24.5 (q), 29.3 (q), 33.0 (t), 33.2 (t), 33.3 (s), 35.1 (t), 36.0 (t), 40.0 (t), 42.6 (d), 44.1 (s), 47.1 (d), 71.4 (s), 215.7 (s). MS m/z (% rel. int.): 236 (M^+, 2), 218 (17), 178 (6), 163 (22), 144 (42), 129 (31), 123 (100), 105 (26), 96 (37), 79 (26), 69 (28), 55 (55), 41 (84). HR-MS m/z: M^+ Calcd for $C_{15}H_{24}O_2$ 236.178. Found: m/z 236.175. The ^{1}H-NMR spectrum was in good agreement with that reported for **3**.[3]

Wolff–Kishner Reduction of 3 A solution of **3** (30 mg) in triethylene glycol (5 ml) containing KOH (80 mg) and 80% hydrazine hydrate (0.25 ml) was heated at 120 °C for 1.5 h followed by 180 °C for 2 h. The reaction mixture was treated in the usual way to give a residue, which was subjected to preparative HPLC (solvent, benzene–ether (9 : 1, v/v)). Recrystallization from hexane–ether gave **4** (16.5 mg) as colorless crystals, mp 96—97 °C. $[\alpha]_D^{22}$ −10.8° (c=0.93, $CHCl_3$). IR (KBr): 3300—3600, 2930, 1460, 1380, 1330, 1100, 1055, 1015 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 0.93 (3H, s, 13-H_3), 0.95 (3H, s, 15-H_3), 1.18 (3H, s, 14-H_3), 1.82 (1H, m, 5-H), 2.07 (1H, dt, J=13.2, 2.2 Hz, 12β-H), 2.47 (1H, m, 2-H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 20.7 (t, C-10), 23.2 (t, C-6), 24.4 (q, C-13), 28.9 (q, C-14), 33.1 (s, C-8), 33.8 (s, C-4), 34.5 (t, C-3), 35.2 (q, C-15), 36.6 (t, C-7), 40.6 (t, C-9), 41.5 (d, C-2), 42.8 (t, C-11), 44.8 (t, C-12), 48.9 (d, C-5), 73.0 (s, C-1). MS m/z (% rel. int.): 222 (M^+, 2), 207 (1), 204 (4), 166 (11), 149 (25), 123 (100), 95 (29), 55 (36), 41 (52). HR-MS m/z: M^+ Calcd for $C_{15}H_{26}O$ 222.198. Found: m/z 222.195.

LAH Reduction of 3 LAH (5 mg) was added to an ice-cooled solution of **3** (7.5 mg) in dry ether (2 ml), and the mixture was heated at reflux for 1 h. The reaction mixture was treated in the usual way to give a residue, which was subjected to silica-gel chromatography. Recrystallization from hexane–ether mixture gave **5** (5.7 mg) as colorless crystals, mp 122—124 °C. $[\alpha]_D^{22}$ −20.0° (c=0.40, $CHCl_3$). IR ($CHCl_3$): 3500—3650, 2950, 1460, 1380 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 0.95 (3H, s), 1.06 (3H, s), 1.19 (3H, s), 2.12 (1H, dd, J=13.9, 2.2 Hz), 2.49 (1H, m), 3.39 (1H, m, $W_{h/2}$=17.0 Hz). MS m/z (% rel. int.): 220 (M^+ − 18), 165 (12), 123 (100), 109 (24), 105 (23), 95 (26), 81 (25), 55 (33), 43 (30), 41 (55).

Acid-Catalyzed Rearrangement of 4 According to the previously outlined procedure,[1,6] **4** (15 mg) was treated with concentrated H_2SO_4 to give a two-component mixture which was subjected to silica-gel chromatography. The fractions eluted with hexane were further subjected to preparative HPLC (solvent, hexane; column temperature, −45 °C) to give pure **6** (5.2 mg) as a colorless oil. $[\alpha]_D^{22}$ −30.3° (c=0.33, $CHCl_3$) (lit.,[7] $[\alpha]_D^{27}$ −27.1° (c=3.13, $CHCl_3$)). IR (CCl_4): 3030, 2950, 1460, 1380, 1360 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 0.86 (3H, s), 0.95 (3H, s), 1.05 (3H, s), 5.28 (1H, d, J=5.7 Hz), 5.36 (1H, d, J=5.7 Hz). MS m/z (% rel. int.): 204 (M^+, 8), 189 (55), 175 (3), 161 (100), 133 (13), 119 (19), 105 (24), 91 (23), 77 (14), 55 (11), 41 (25). This product (**6**) was directly identified as (−)-clovene by comparisons of the IR, NMR, and MS spectra, $[\alpha]_D$ value, and retention time on GC with those of an authentic sample.[6-8b] The fractions eluted with hexane–ether (4 : 1) were further subjected to preparative HPLC (solvent, benzene–ether (9 : 1, v/v)) to give **7** (7.6 mg) as colorless crystals,

mp 99—100 °C (lit.,[8a] 97—98.5 °C) $[\alpha]_D^{22}$ −27.0° (c=0.22, $CHCl_3$) (lit.,[8a] $[\alpha]_D$ −24.0° (c=1.10, $CHCl_3$)). IR (KBr): 3200—3500, 2940, 1460, 1360, 1130, 1070 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 0.90 (3H, s), 0.97 (6H, s), 3.82 (1H, dd, J=9.6, 6.7 Hz). MS m/z (% rel. int.): 222 (M^+, 5), 204 (3), 189 (11), 166 (100), 123 (38), 95 (28), 85 (26), 55 (28), 41 (48). HR-MS m/z: M^+ Calcd for $C_{15}H_{26}O$ 222.198. Found: m/z 222.199. The IR, NMR, and mass spectra, and $[\alpha]_D$ value were identical with those reported for clovanol.[8]

Collins Oxidation of 7 According to the method described above, **7** (5 mg) was oxidized. After usual work-up, the oily substance was subjected to silica-gel chromatography followed by preparative HPLC (solvent, hexane–ether (9 : 1, v/v)) to give pure **8** (4.1 mg) as a colorless oil, $[\alpha]_D^{22}$ +19.2° (c=0.68, $CHCl_3$) (lit.,[8a] $[\alpha]_D$ +20.0° (c=2.23, $CHCl_3$)). IR ($CHCl_3$): 3010, 2950, 1725, 1460, 1370 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 0.89 (3H, s), 0.99 (3H, s), 1.07 (3H, s), 2.13 (1H, d, J=16.1 Hz), 2.28 (1H, d, J=16.1 Hz). MS m/z (% rel. int.): 220 (M^+, 40), 205 (9), 177 (5), 164 (8), 136 (100), 121 (39), 107 (33), 93 (40), 83 (82), 79 (32), 55 (25), 41 (55). HR-MS m/z: M^+ Calcd for $C_{15}H_{24}O$: 220.183. Found: 220.180.

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References and Notes

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2) Compound **1** was reported under the name of isocaryolanediol at the 32nd Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics, Miyazaki, October 1988.
3) F. Bohlmann and J. Ziesche, *Phytochemistry*, **20**, 469 (1981).
4) In the ^{1}H-NMR spectra of **1** and **3**—**5**, the 5-H signal was observed as a multiplet separated from other signals only in **4**. For this reason, **4** was used for the NOE experiments to obtain precise results.
5) Each signal was assigned on the basis of the ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra and inspection of the Dreiding model of **3**.
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