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Note

Synthesis of ginsenoside Rg_3, a minor constituent of Ginseng *Radix*

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Abstract

Glycosylation of 12β-acetoxy-dammar-24-en-3β,20(*S*)-diol (**4**), with hepta-*O*-acetyl-α-sophorosyl bromide (**5**) under catalysis by Ag_2CO_3 or Ag_2O afforded a chromatographically unseparated mixture of the α- and β-linked octaacetates **6** and **7** in an approximately 2.5:1 ratio. After deprotection and chromatographic purification, the free α- (**8**) and β-glycosides (**9**) were obtained. Sophoroside **9** was identical in all respects with ginsenoside Rg_3, the minor component of Ginseng *Radix rubra*. All compounds were fully characterized by ^1H and ^{13}C NMR spectroscopy.

Keywords: α-Acetobromosophorose; Ginsenoside Rg_3; Ginseng *Radix rubra*

Ginseng, the famous plant drug, has been used as an expensive traditional medicine in oriental countries for more than five thousand years. Ginseng and its crude extracts have a tranquillizing action on the central nervous system, elevate blood pressure, protect against physical and chemical stress, and have other actions [1]. After many twists and turns, it was determined that the major active components of ginseng are glycosides of triterpenes of the dammarane type. To date, about thirty glycosides have been isolated from roots of *Panax ginseng*. The isolation of the individual compounds in a pure state from crude extracts, especially minor components, is often tedious. Due to the biological activity and low content of some of the dammarane glycosides in ginseng, this preparation is of interest. In particular, this includes the 20(*S*)-ginsenoside Rg_3, a minor component isolated from Ginseng *Radix rubra* [2]. One route to ginsenoside Rg_3 is the mild acidic hydrolysis of ginsenosides Rb_1, Rb_2, Rc, and Rd, but a mixture of 20(*R*,*S*) epimers is formed [3]. We now describe the first synthesis of 20(*S*)-ginsenoside Rg_3.

For the synthesis of ginsenoside Rg_3, 12β-acetoxy-dammar-24-en-3β,20(*S*)-diol (12-acetoxy-protopanaxadiol, **4**) was chosen as glycosyl acceptor and hepta-*O*-acetyl-α-sophorosyl bromide (α-

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acetobromosophorose, **5**) as glycosyl donor (Scheme 1). The 12-*O*-acetyl derivative of 20(*S*)-protopanaxadiol **4** is the most convenient aglycon for the regioselective introduction of a carbohydrate residue onto the C-3 position, because the tertiary C-20 hydroxyl group is sterically hindered by 12β-acetoxy group [4]. 12-Acetoxy-protopanaxadiol (**4**) was synthesized from 20(*S*)-dammar-24-en-3α,12β,20-triol (betulafolienetriol, **1**) in three steps. Betulafolienetriol, isolated from the leaves of birch [5], was oxidized with Sarett reagent to give the 3-ketone **2**. The acetyl derivative **3**, prepared from **2**, was reduced with $NaBH_4$ to give 12-acetoxy-protopanaxadiol (**4**) [4].

12-Acetoxy-protopanaxadiol (**4**) was condensed with α-acetobromosophorose (**5**) [6] in dichloromethane in the presence of silver carbonate at room temperature (Koenigs–Knorr conditions). Chromatography on silica gel, and elution with light petroleum–acetone gave a chromatographically homogeneous product (57% overall yield). The use of silver oxide as an acceptor of hydrogen bromide gave a comparable result to that of silver carbonate (49.5% overall yield). Inspection of the ^1H NMR spectrum of product suggested that the latter was a mixture of the α- and β-linked octaacetates **6** and **7** (Scheme 1). Indeed, in the region of β-anomeric protons, there are three characteristic doublets at δ 4.44, 4.69, and 4.60 (Fig. 1). The coupling constants ($J \approx 7$ Hz) for these doublets, agreed with a β-configuration for the corresponding residues. The intensities of the first

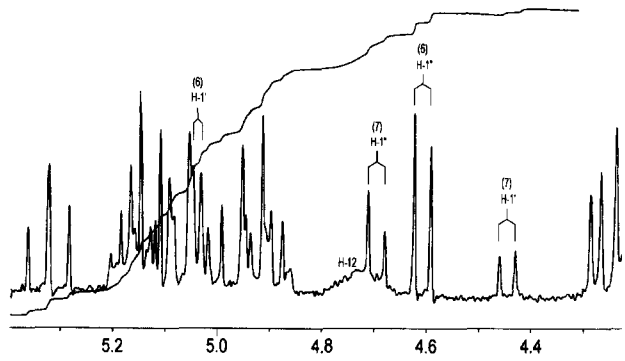
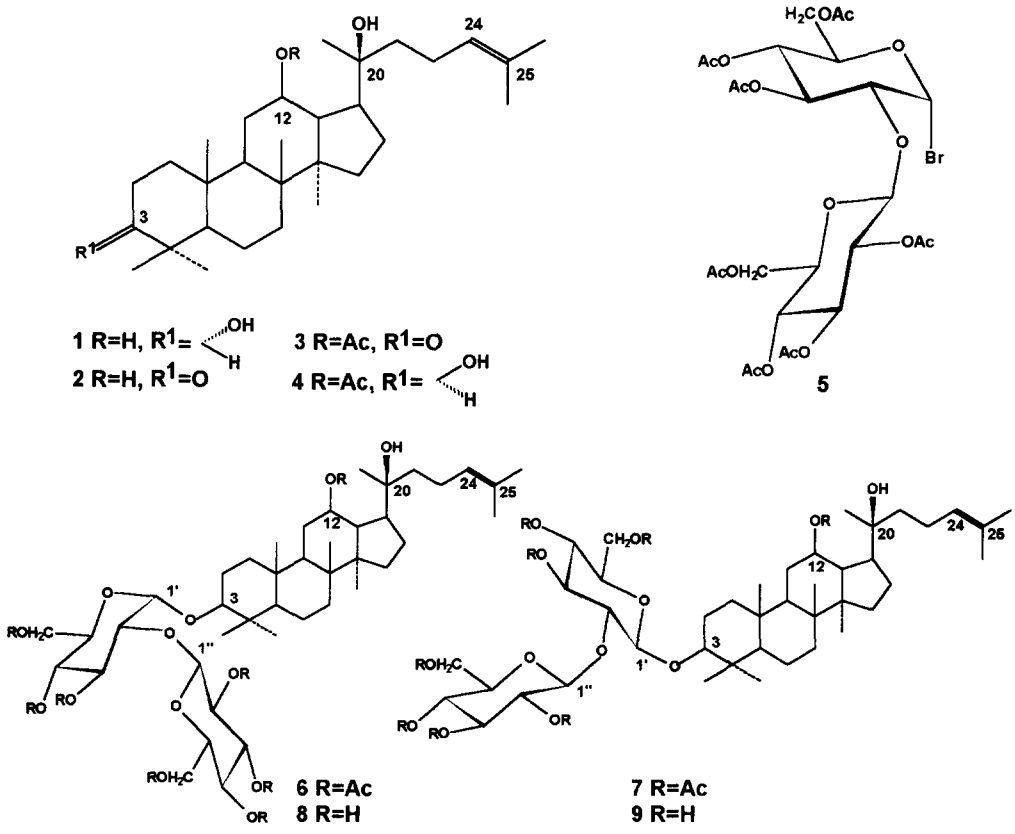


Fig. 1. Part of the ^1H NMR (250 MHz, in $CDCl_3$) of the mixture of α- and β-linked octaacetates **6** and **7** (a region of anomeric protons).



Scheme 1.

two signals are equal, indicating that the corresponding protons arise from β-sophoroside octaacetate (**7**). The signal at δ 4.60 arises from the β-anomeric proton H-1″ of the α-sophoroside's residue in glucoside (**6**). In the ^{1}H NMR spectra of α-glycosides, the anomeric proton signals are usually observed at $\delta >$ 5.0 with spin coupling values $J_{1',2'}$ in the range 3.7–5.0 Hz [4,6,8]. As it is shown in Fig. 1, the α-anomeric proton signal is overlapped by other carbohydrate resonances from compounds **6** and **7**. Thus the exact H-1′ signal position was measured by selective decoupling experiments on individual compounds.

The ratio of intensities of the anomeric proton signals at δ 4.60 and 4.44 (or 4.69) is equal to the ratio (≈ 2.5:1) of **6** and **7** in this mixture. In addition, in the region of methyl and acetyl group resonances, there are only three low intensity peaks from methyl groups and only three of the same intensity from acetyl groups. The intensity of each of these small signals was approximately one third of an ordinary CH_3 peak.

Deacetylation and chromatographic purification afforded the free α- (**8**) and β-sophorosides (**9**). The assignment of the ^{13}C NMR spectra of **8** and **9** was done by comparison of their spectra with those of related disaccharides and glycosides of protopanaxadiol, taking into account the effects of the change in configuration at C-1′ in **8** [4,7,8]. The spectral characteristics of **9** were identical to those of native 20(*S*)-ginsenoside Rg_3 [2].

Treatment of the free α- (**8**) and β-sophorosides (**9**) with acetic anhydride in pyridine resulted in the corresponding α- and β-octaacetates **6** and **7**. ^{1}H NMR analysis of pure octaacetates of α- (**6**) and β-sophorosides (**7**) confirmed our assumption about the ^{1}H NMR spectrum of the mixture.

1. Experimental

General procedures.—All melting points were determined with a Boethius apparatus and are uncorrected. Optical rotations were measured at 20 °C with a Perkin–Elmer 141 polarimeter for MeOH or $CHCl_3$ solutions. Column chromatography was performed on Silica Gel L (Chemapol, Czechoslovakia) 40/100 (μ). Precoated Silica Gel 60F-254 E. Merck® plates were used for TLC, and detection was performed by spraying with a 10% soln of concd sulfuric acid in methanol followed by heating. All NMR experiments were run on a Bruker WM-250 instrument operating at 250 MHz for ^{1}H, and at 62.9 MHz for ^{13}C, using $CDCl_3$ or pyridine-d_5 as solvent and Me_4Si as an internal reference (δ 0). Elemental analysis was performed with a Perkin–Elmer 240.

12β - Acetoxy - dammar - 24 - en - 3β, 20(S) - diol (**4**).—(i) *Oxidation of betulafolienetriol* (**1**): Sarett reagent was prepared from dry pyridine (17.5 mL, 220 mmol) and CrO_3 (10.56 g, 106 mmol) in accordance with procedures described by Ratcliffe and Rodehorst [9]. Betulafolienetriol (**1**) (18.89 g, 41 mmol) in CH_2Cl_2 (300 mL) was added to a stirred CH_2Cl_2 soln (200 mL) of pyridine–CrO_3 complex. The reaction mixture was stirred at room temperature for about 5 h and monitored by TLC (3:2 hexane–acetone) every 30 min. A small volume of MeOH was added to decompose an excess of CrO_3 and the reaction mixture was purified by percolation through a short column of alumina (100 g). The volatiles were removed under vacuum. Methanol (30 mL) was added and evaporated from the residue thrice. The semisolid was triturated with hot MeOH (80 mL). After cooling, the precipitate was separated by filtration. The product (12.2 g, 65%) proved to be identical with the authentic *dammar-24-en-12β,20(S)-diol-3-one* (**2**) by comparisons of their IR-spectra and by TLC [10]. ^{1}H NMR ($CDCl_3$): δ 0.90 (s, 3 H), 0.98 (s, 3 H), 1.03 (s, 3 H), 1.05 (s, 3 H), 1.08 (s, 3 H), 1.21 (s, 3 H), 1.65 (br s, 3 H), 1.70 (br s, 3 H), 3.61 (ddd, 1 H, $J_{12,11a}$ 10.0, $J_{12,13}$ 9.7, $J_{12,11e}$ 5.0 Hz, H_a-12), 5.18 (m, 1 H, H-24); ^{13}C NMR ($CDCl_3$): δ 217.8 (3), 70.5 (12), 73.8 (20), 125.1 (24), 131.3 (25).

From the ^{1}H NMR spectrum, the resulting solid (**2**) appeared to contain a small amount of impurity (less than 5%) and therefore was used in the second stage without purification.

(ii) *Acetylation of 12,20-dihydroxy-3-ketone* **2**: Compound **2** (12.0 g) was acetylated with Ac_2O (36 mL) in pyridine (60 mL) at room temperature for 15 h. After the usual work up, *12β-acetoxy-dammar-24-en-20(S)-ol-3-one* (**3**) (11.8 g) was obtained. NMR data [4] showed the product **3** was slightly impure and was used in the third stage without purification.

(iii) *Reduction of 12-acetoxy-20-hydroxy-3-ketone* **3**: Crude product **3** (11.5 g) was reduced with $NaBH_4$ (1.3 g) in *i*-PrOH (200 mL). After working up in the usual way, the residue was eluted from a column of silica gel with light petroleum–acetone (15:1 → 9:1). The fractionation, which was monitored by TLC (3:2 hexane–acetone), gave the amorphous product (9.7 g, 84%), $[\alpha]_D^{20}$ −6.1° (*c* 1.07, $CHCl_3$), which proved to be identical with the authentic 12-acetoxy-protopanaxadiol (**4**) by comparisons of IR- and ^{1}H NMR spectra, and by TLC [4].

Glycosylation of 12-acetoxy-protopanaxadiol with α-acetobromosophorose.—(i) *Condensation in the presence of silver carbonate*: A solution of 12-acetoxy-protopanaxadiol **4** (0,502 g, 1 mmol) and α-acetobromosophorose **5** (1.75 g, 2.50 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 15 h in the presence of freshly prepared Ag_2CO_3 (0,746 g, 2,70 mmol) in the dark. The mixture was filtered and concd under reduced pressure. The residue was chromatographed on silica gel using 6:1 → 2:1 petroleum–acetone as eluent, to yield a product with R_f 0.46 (2:1 hexane–acetone) (0.638 g, 57%). A soln of the product (0.638 g) in MeOH (3 mL) was kept at room temperature in the presence of MeONa (three drops of 1 N soln in MeOH) for 15–20 h and reaction monitored by TLC (3:1 $CHCl_3$–MeOH, satd with H_2O). The reaction mixture was neutralized with Amberlite IR 120 (H^+) and filtered. The filtrate and methanol washings were taken to dryness. The residue (0.439 g) was chromatographed using 15:3 $CHCl_3$–MeOH, satd with H_2O, as eluent to yield pure **8** and **9**.

(*12β,20*(S)-*Dihydroxy-dammar-24-en-3*-O-*yl*)-*α-sophoroside* (**8**) (280 mg, 35.7%): mp 196–198 °C (MeOH–H_2O); $[\alpha]_D^{20}$ +73.5° (*c* 1.0, MeOH); ^1H NMR (C_5D_5N): δ 5.25 (d, 1 H, $J_{1'',2''}$ 7.6 Hz, H-1''), 5.67 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1'); ^{13}C NMR (C_5D_5N): δ 97.5 (1'), 106.2 (1''), 85.4 (3), 70.9 (12), 72.9 (20), 126.1 (24), 130.3 (25); Anal. Calcd for $C_{42}H_{72}O_{13}$: C, 64.26; H, 9.25. Found: C, 64.46; H, 9.30.

(*12β,20*(S)-*Dihydroxy-dammar-24-en-3*-O-*yl*)-*β-sophoroside* (ginsenoside Rg_3, **9**) (109 mg, 13.9%): mp 192–194 °C (MeOH–H_2O); $[\alpha]_D^{20}$ +8.5° (*c* 1.0, MeOH); ^1H NMR (C_5D_5N): δ 4.95 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1'), 5.39 (d, 1 H, $J_{1'',2''}$ 7.3 Hz, H-1''); ^{13}C NMR (C_5D_5N): δ 104.7 (1'), 105.6 (1''), 88.9 (3), 70.9 (12), 72.9 (20), 126.1 (24), 130.4 (25); Anal. Calcd for $C_{42}H_{72}O_{13}$: C, 64.26; H, 9.25. Found: C, 64.43; H, 9.31.

(ii) *Condensation in the presence of silver oxide*: Glycosylation of 12-acetoxy-protopanaxadiol (0,502 g, 1 mmol) with α-acetobromosophorose (1.75 g, 2.50 mmol), in the presence of freshly prepared silver oxide (0.626 g, 2.70 mmol), was carried out as described above to give a mixture of two octaacetates **6** and **7** (0.554 g, 49.5%). After deacetylation in the usual way, the residue (0.374 g) was chromatographed as already described above to yield pure **8** (239 mg, 30.5%) and **9** (100 mg, 12.8%).

Acetylation of sophorosides **8** *and* **9**.—Compounds **8** and **9** (100 mg) were acetylated with Ac_2O (1 mL) in pyridine (2 mL) at room temperature for 15 h. After the usual work up, the corresponding octaacetates **6** and **7** were obtained.

(*12β-Acetoxy-20*(S)-*hydroxy-dammar-24-en-3*-O-*yl*)-*3',4',6',2'',3'',4'',6''-hepta*-O-*acetyl-α-sophoroside* (**6**): mp 113–116 °C (petroleum–ether); $[\alpha]_D^{20}$ +32.8° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.60 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, H-1''), 5.04 (d, ^1H, $J_{1',2'}$ 3.7 Hz, H-1'); ^{13}C NMR ($CDCl_3$): δ 96.8 (1'), 101.2 (1''), 87.5 (3), 76.7 (12), 73.7 (20), 125.3 (24), 131.0 (25); Anal. Calcd for $C_{58}H_{88}O_{21}$: C, 62.12; H, 7.91. Found: C, 62.24; H, 7.98.

(*12β-Acetoxy-20*(S)-*hydroxy-dammar-24-en-3*-O-*yl*)-*3',4',6',2'',3'',4'',6''-hepta*-O-*acetyl-β-sophoroside* (**7**): mp 110–113 °C (petroleum–ether); $[\alpha]_D^{20}$ −7.45° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.44 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.69 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1''); ^{13}C NMR ($CDCl_3$): δ 100.7 (1''), 103.5 (1'), 91.0 (3), 76.6 (12), 73.8 (20), 125.4 (24), 131.2 (25); Anal. Calcd for $C_{58}H_{88}O_{21}$: C, 62.12; H, 7.91. Found: C, 62.29; H, 7.96.

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