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Note

Simplified preparation of the ginsenoside-Rh_2 minor saponin from ginseng

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Abstract

Condensation of the 12-*O*-acetylderivative of 20(S)-protopanaxadiol [dammar-24-ene-3β,12β,20(S)-triol] with tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of silver oxide in dichloroethane, followed by deprotection with sodium methoxide in methanol, results in formation of the 3-*O*-β-D-glucopyranosyldammar-24-ene-3β,12β,20(S)-triol identical with natural ginsenoside-Rh_2. The 12-*O*-acetyl-20(S)-protopanaxadiol is easily prepared from betulafolienetriol via the 3-keto-12-*O*-acetylderivative followed by $NaBH_4$ reduction. This comparatively simple five-step synthesis makes this hitherto rare ginsenoside relatively accessible. © 1997 Elsevier Science Ltd.

Keywords: Ginseng; Ginsenoside; Saponin; Birch; Cytotoxicity

1. Introduction

Two kinds of ginseng preparations have generally been used in oriental medicine, white ginseng and red ginseng. During the course of the elucidation of respective biological activities of red and white ginsengs it has been found that the minor saponin of red ginseng, ginsenoside-Rh_2, exhibits cytotoxic activities while other ginsenosides isolated from red and white ginsengs do not show cytotoxicity [1–3]. The ginsenoside-Rh_2 has been obtained by the mild hydrolysis of the ginsenosides-Rb_1,-Rc,-Rd in low yield [3].

The genuine sapogenins of the ginseng glycosides (= ginsenosides) are structurally similar to some chemical constituents of the birch leaves. Betulafolienetriol [dammar-24-ene-3α,12β,20(S)-triol] **1** isolated from birch leaves differs from the genuine sapogenin of ginseng glycosides, 20(S)-protopanaxadiol, in the configuration at C-3 only. For this reason triol **1** is used as relatively accessible starting material to prepare 20(S)-protopanaxadiol and its glycosides [4].

2. Results and discussion

The major disadvantage of condensation of 20(S)-protopanaxadiol with α-acetobromoglucose under Koenigs–Knorr conditions is the non-stereoselective location of glycosylation [4]. Generally the stereoselective location of glycosylation is accomplished in fairly good yields with partially protected com-

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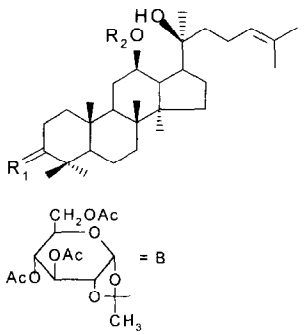
pounds. The tertiary hydroxyl group at C-20 in the dammarane type triols having a 12β-acetoxyl group is sterically hindered and therefore resistant towards glycosylation [4].



1 R_1 = ···OH, R_2 = H

2 R_1 = —OH, R_2 = Ac

3 R_1 = O, R_2 = H

4 R_1 = O, R_2 = Ac

5 R_1 = —O glcAc$_4$, R_2 = Ac

6 R_1 = — OB, R_2 = Ac

7 R_1 = — Oglc, R_2 = H

The 12-*O*-acetylderivative **2** of 20(S)-protopanaxadiol is the most convenient aglycon in stereoselective location of glycosylation of the hydroxyl group at C-3. However, both the direct acetylation of 20(S)-protopanaxadiol with acetic anhydride in pyridine and the deacetylation of the 3,12-di-*O*-acetyl-20(S)-protopanaxadiol with sodium methoxide in methanol gives the 3-*O*-acetyl-20(S)-protopanaxadiol only. The 12-*O*-acetate **2** is prepared from betulafolienetriol **1** by the following sequence of reactions. The triol **1** is oxidized to 3-ketoderivative **3** (yield 60–65%), which is acetylated with acetic anhydride in pyridine to give 3-keto-12-*O*-acetylderivative **4** quantitatively. Sodium borohydride reduction of the compound **4** in 2-propanol affords 12-*O*-acetate **2** (yield 93–95%).

Condensation of the 12-*O*-acetate **2** with tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of silver oxide and molecular sieves 4 Å in dichloroethane results in formation of the 3-*O*-β-D-glucopyranoside **5** (yield 47.7–50%). Moreover, the orthoester **6** (yield 8%), isomeric with the glucoside **5** is detected in the reaction mixture. A characteristic feature of sugar orthoesters is their sensitivity towards acids, which leads to their hydrolysis in aqueous solution. On acid treatment of the reaction mixture the orthoester **6** is decomposed to simplify the isolation of the glucoside **5**.

Deprotection of the glucoside **5** gives the 3-*O*-β-D-glucopyranoside of 20(S)-protopanaxadiol **7** identical (physical constants and spectra) with natural ginsenoside-Rh$_2$.

3. Experimental

IR spectra were recorded with a Specord 75 spectrophotometer on solutions in $CHCl_3$. NMR spectra were recorded with a Bruker WM-250 spectrometer at 250 MHz for ^{1}H and 62.9 MHz for ^{13}C for solutions in $CDCl_3$ and C_5D_5N (internal Me_4Si) at 30°. Optical rotations were measured with a Perkin–Elmer 141 instrument, using a 10-cm cell at 20°. Melting points were determined on a Boetius table. Column chromatography was performed on KSK silica gel (120–150 mesh) using hexane–acetone (8:1 → 5:1). Compounds were checked for homogeneity by TLC on silica gel, using hexane–acetone (2:1, 3:2), benzene–$CHCl_3$–MeOH (6:4:1, 3:2:1) and detection by charring with H_2SO_4.

Dammar-24-ene-3α,12β,20(S)-triol **1** was isolated from an ethereal extract of the leaves *Betula pendula*, followed by chromatography on silica gel and crystallization from acetone: mp 195–196°, lit. 197–198° [5].

Dammar-24-ene-12β,20(S)-diol-3-one **3** was obtained by oxidation of **1** with chromic anhydride in pyridine (yield 60–65%): mp 196–198° (from acetone), lit. 196–199° [6].

12-O-Acetyl-dammar-24-ene-12β,20(S)-diol-3-one **4** was obtained by conventional acetylation of **3** with Ac_2O in pyridine. This was amorphous with ν_{max} 1600, 1700, 1720, 3535 cm^{-1}. ^{1}H NMR data: δ 0.94 (s, 3 H), 0.96 (s, 3 H), 1.04 (s, 6 H), 1.09 (s, 3 H), 1.14 (s, 3 H), 1.65 (s, 3 H), 1.72 (s, 3 H), 2.06 (s, 3 H, OAc), 3.04 (s, 1 H, OH), 4.73 (td, 1 H, *J* 10.0, 10.0, 5.0 Hz, H-12a), 5.17 (t, 1 H, *J* 6.3 and 6.3 Hz, H-24). Anal. Calcd for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 76.83; H, 10.32.

12-O-Acetyl-dammar-24-ene-3β,12β,20(S)-triol **2** was obtained by reducing **4** with $NaBH_4$ in 2-propanol under ice cooling. It was amorphous (yield 93–95%), $[\alpha]_D^{20}$ −6° (*c* 1.07, $CHCl_3$); ν_{max} 1598, 1720, 3535 cm^{-1}. ^{1}H NMR data: δ 0.78 (s, 3 H), 0.86 (s, 3 H), 0.96 (s, 3 H), 0.98 (s, 3 H), 1.02 (s, 3 H), 1.13 (s, 3 H), 1.64 (s, 3 H), 1.71 (s, 3 H), 2.05 (s, 3 H, OAc), 3.20 (dd, 1 H, *J* 4.9, 10.8 Hz, H-3a), 4.73 (td, 1 H, *J* 10.0, 10.0, 5.0 Hz, H-12a), 5.16 (t, 1 H, *J* 6.3 and 6.3 Hz, H-24). Anal. Calcd for $C_{32}H_{54}O_4$: C, 76.44; H, 10.83. Found: C, 76.53; H, 10.75.

Condensations of **2** *with α-acetobromoglucose in the presence of silver oxide.*—A mixture of the 12-*O*-acetate **2** (1.08 g, 2 mmol), silver oxide (1.4 g, 6 mmol), α-acetobromoglucose (2.47 g, 6 mmol), molecular sieves 4 Å (1.0 g) and dichloroethane (20 mL) was agitated at rt until the α-acetobromoglucose had reacted (TLC). The reaction mixture was then diluted with $CHCl_3$ and filtered. The solvent was evaporated and the residue was washed with hot

water to remove an excess of glucose derivatives. Silica gel column chromatography (8:1 *n*-hexane–acetone) gave **2** (133 mg, 12%), (5:1 *n*-hexane–acetone) orthoester **6** (124 mg, 8%) and glucoside **5** (853 mg, 47.7%).

3-O-(2′,3′,4′,6′-Tetra-O-*acetyl-β*-D-*glucopyranosyl)-12*-O-*acetyldammar-24-ene-3β,12β,20(S)-triol* **5** had mp 223–225° (from EtOH); $[\alpha]_D^{20}$ −19° (*c* 1.0, $CHCl_3$): ν_{max} 1598, 1720, 1750, 3535 cm^{-1}. ^1H NMR data: δ 0.74 (s, 3 H), 0.85 (s, 3 H), 0.90 (s, 3 H), 0.94 (s, 3 H), 1.00 (s, 3 H), 1.13 (s, 3 H), 1.65 (s, 3 H), 1.71 (s, 3 H), 2.00–2.09 (s, 15 H, 5 × OAc), 3.08 (dd, 1 H, *J* 4.9 and 10.8 Hz, H-3a), 3.69 (m, 1 H, H-5′), 4.12–4.24 (m, 2 H, 2 × H-6′), 4.54 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.73 (td, 1 H, *J* 10.0, 10.0 and 5.0 Hz, H-12a), 5.04 (m, 2 H, H-2′, H-4′), 5.16 (t, 1 H, *J* 6.5 and 6.5 Hz, H-24), 5.20 (t, 1 H, *J* 9.5 and 9.5 Hz, H-3′). Anal. Calcd for $C_{46}H_{72}O_{13} \cdot 0.5H_2O$: C, 65.61; H, 8.74. Found: C, 65.43; H, 8.42.

3-[3″,4″,6″-tri-O-*Acetyl-1″,2″-(ethylidene-1″-yl)-α-glucopyranose]-12*-O-*acetyldammar-24-ene-3β, 12β,20(S)-triol* **6** had $[\alpha]_D^{20}$ +10° (*c* 1.0, pyridine); ν_{max} 1600, 1720, 1750, 3535 cm^{-1}. ^1H NMR data: δ 0.78 (s, 3 H), 0.85 (s, 3 H), 0.95 (s, 3 H), 0.98 (s, 3 H), 1.00 (s, 3 H), 1.13 (s, 3 H), 1.64 (s, 3 H), 1.71 (s, 3 H), 1.73 (s, 3 H, CH_3 at ethylidene group), 2.00–2.11 (s, 12 H, 4 × OAc), 3.08 (m, 1 H, H-3a), 4.03–4.32 (m, 4 H, H-2″, H-5″, 2 × H-6″), 4.73 (td, 1 H, *J* 10.0, 10.0 and 5.0 Hz, H-12a), 4.89 (m, 1 H, H-4″), 5.18 (m, 2 H, H-24, H-3″), 5.69 (d, 1 H, $J_{1'',2''}$ 5.0 Hz, H-1″). Anal. Calcd for $C_{46}H_{72}O_{13} \cdot 0.5H_2O$: C, 65.61; H, 8.74. Found: C, 65.38; H, 8.78.

A mixture of the 12-*O*-acetate **2** (8.46 g, 16.8 mmol), silver oxide (13.9 g, 51 mmol), α-acetobromoglucose (20.84 g, 51 mmol), molecular sieves 4 Å (2.0 g) and dichloroethane (100 mL) was agitated for 3 h at rt. The reaction mixture was then diluted with $CHCl_3$ and filtered. The solvent was evaporated and the residue was washed with water containing a few drops of mineral acid to decompose the orthoester **6** isomeric with glucoside **5**. After working up in the usual way, the crude product was subjected to column chromatography. From the fractions eluted with 8:1 *n*-hexane–acetone, **2** (3.225 g, 38.1%) was obtained. Elution with 5:1 *n*-hexane–acetone afforded glucoside **5** (7.04 g, 50%).

Deacetylation of **5** was performed in methanolic 1 M NaOMe to give the 3-*O*-β-D-glucopyranosyldammar-24-ene-3β,12β,20(S)-triol **7**, mp 224–225° (from aq MeOH), lit. 224–225° [3]; $[\alpha]_D^{20}$ +8° (*c* 1.0, pyridine). ^{13}C NMR data: δ 62.9 (C-6′), 70.8 (C-12), 72.0 (C-4′), 73.4 (C-20), 75.7 (C-2′), 78.2 (C-5′), 79.2 (C-3′), 89.2 (C-3), 106.9 (C-1′), 125.2 (C-24), 130.1 (C-25). Anal. Calcd for $C_{36}H_{62}O_8 \cdot 1.5CH_3OH$: C, 67.12; H, 10.21. Found: C, 67.00; H, 9.93.

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