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TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS
XLVI. CHEMICAL TRANSFORMATION OF CYLOARTANES
II. GLYCOSYLATION OF ASKENDOSIDE D

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A method has been developed for the regioselective glycosylation of 20,24-epoxycycloartane-16β,25-diols. Two glycosides have been synthesized from askendoside D: askendoside D 16-O-acetate 25-O-α-L-rhamnopyranoside (V) and askendoside B 16,25-di-O-α-L-rhamnopyranoside (VI).

We are continuing syntheses based on cycloartane glycosides [1]. Selective glycosylation is the second direction of the transformation of cyclosieversigenin glycosides that we have developed. In this case, as well, the selective protection of the hydroxy groups has played the role of a factor ensuring the regiodirectivity of the introduction of a monosaccharide residue into the C25 and C-16,C25 positions.

For glycosylation we selected askendoside D, quantitatively the main glycoside of *Astragalus taschkendicus* Bunge, which possesses a number of important physiological activities [2, 3]. Glycosylation was performed by the Koenigs–Knorr method [4].

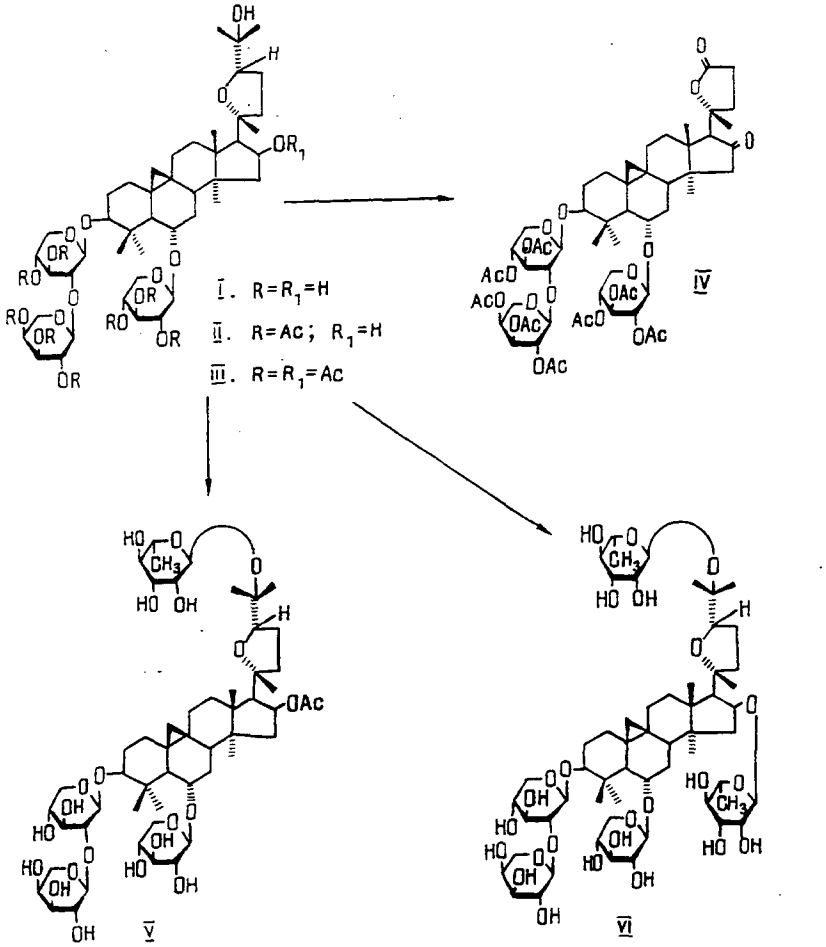

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TABLE 1. Chemical Shifts of the Carbon Atoms of glycosides (V) and (VI) (δ, ppm, C_5D_5N, 0 — TMS

C atom	V	VI	C atom	V	VI	C atom	V	VI
1	31.81	31.67	23	27.64	27.55 [a]	6-O-β-D-Xylp residue		
2	28.13 [a]	28.03	24	82.50	82.72	1	105.48	105.87
3	87.59	87.65	25	77.92	77.90	2	75.32	75.23
4	42.65	42.56	26	22.60*	22.68*	3	78.27	78.15
5	51.81	51.81	27	25.96*	25.39*	4	71.04	70.89
6	77.49	77.51	28	19.70	19.38	5	66.85	66.94
7	32.87 [b]	32.95	29	28.13 [a]	27.55 [a]	16-O-α-L-Rhap residue		
8	43.24	43.00	30	16.40	16.29	1		104.51
9	21.21	21.30	3-O-β-D-Xylp residue			2		72.68
10	30.00	29.91	1	105.28	105.16	3		73.56 [c]
11	26.35	26.55	2	83.60	83.46 [b]	4		73.73
12	32.87 [b]	32.84	3	77.11	77.31	5		70.53
13	44.93	45.44	4	70.98	71.02	6		18.13
14	46.62	46.24	5	67.05	66.41	25-O-α-L-Rhap residue		
15	46.84	46.98	α-L-Arap residue			1	96.01	95.83
16	76.06	83.46 [b]	1	106.71	106.55	2	72.73	72.30
17	57.85	60.11	2	73.62 [c]	73.56 [c]	3	73.49	73.09
18	21.52	20.45	3	74.23	74.18	4	73.62 [c]	74.11
19	26.83	25.73	4	69.13	69.04	5	69.54	69.46
20	85.78	86.48	5	66.57	66.88	6	18.69	18.64
21	23.28	23.41				CH_3	19.85	
22	37.64	39.97				COO	169.98	

*Signals marked with the same letters are superposed upon one another and those with asterisks have been assigned uncertainly.

The selective acetylation of askendoside D (I) with acetic anhydride in pyridine enabled askendoside D nonaacetate (III), which has been described previously, to be obtained [1]. Condensation of the nonaacetate (III) with acetobromorhamnose was carried out in dichloroethane in the presence of mercury cyanide and 4 Å molecular sieve under a current of nitrogen. Saponification of the reaction products with a 1% methanolic solution of potassium hydroxide at room temperature for 3 h led to glycoside (V).

The IR spectrum of compound (V) had absorption bands characteristic for an ester group. The PMR spectrum of the glycoside (V) obtained, containing a three-proton singlet at 2.04 ppm, showed that one acetyl group was retained in the molecule of glycoside (V). The proton geminal to the acetoxy group resonated at 5.57 ppm in the form of a multiplet. The chemical shift of this signal permitted the assumption that it referred to H-16 and, consequently, the acetyl group was located at C-16. We confirmed this conclusion by a double homonuclear resonance experiment. The preirradiation of the proton geminal to the acetoxy group (5.57 ppm) led to the transformation of the H-17 signal resonating at 2.58 ppm into a singlet.

The ^{13}C NMR spectrum was in complete agreement with the facts given and showed the L-rhamnopyranoside residue in this glycoside had the α-configuration and the $^1C_4$ conformation (Table 1).

Thus, glycoside (V) was 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 16-O-acetate 3-O-[O-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranoside] 6-O-β-D-xylopyranoside.

The octaacetate (II) was likewise obtained by the acetylation of askendoside D with acetic anhydride in pyridine. The IR spectrum of acetate (II) showed the presence of a hydroxy group. The PMR spectrum of this product included the signals of eight acetyl groups. Consequently, compound (II) was an octaacetate, and, in all probability, contained free hydroxyls at

C-16 and C-25. To confirm the structure of the octaacetate (II), it was oxidized with the Jones reagent [5]. This led to the ketolactone (IV), the IR spectrum of which lacked tha absorption band of hydroxy groups, while the absorption bands of a γ-lactone and a ketone in a five-membered ring were overlapped by the broad band of ester groups. As was to be expected, in the PMR spectrum of the ketolactone (IV) we traced the signals of five methyl groups, while the H-17 signal had been converted into a singlet, which was observed at 3.00 ppm. The experimental facts given show that the ketolactone (IV) was 3β,6α-dihydroxy-16-oxo-20R,25-norcycloartane-20,24-olide3-O-[O-α-L-arabinopyranosyl(1→2)-β-D-xylopyranoside]6-O-β-D-xylopyranoside octaacetate and thereby confirmed the conclusion concerning the structure of the octaacetate (II).

The condensation of the octaacetate (II) with acetobromorhamnose under the conditions given above followed by saponification of the reaction products with 1% methanolic potassium hydroxide led to the glycoside (VI). In its PMR spectrum, the resonance lines of the methyl groups (1.59 and 1.66 ppm) of the two L-rhamnopyranoside residues and of their anomeric protons (5.14 and 5.59 ppm) stood out clearly from the signals of the askendoside B residue. In agreement with this, the ^{13}C NMR spectrum, contained, in addition to the signals of the askendoside D residue, the signals of the carbon atoms of two L-rhamnopyranoside residues. The chemical shifts of the carbon atoms of the residues of the newly added monosaccharides showed its α-configuration and $^1C_4$ conformation.

Consequently, glycoside (VI) had the structure of 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-[O-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranoside] 16,25-di-O-α-L-rhamnopyranoside 6-O-β-D-xylopyranoside.

EXPERIMENTAL

For general observations, see [6]. The following solvent systems were used: 1) chloroform–methanol (100:1); and 2) chloroform–methanol–water (70:23:4).

The PMR spectra of compounds (II)-(IV) were taken on a Tesla BS-567 A instrument, and the ^1H and ^{13}C NMR spectra of the other substances on a Bruker AM 400 instrument. The ^{13}C NMR spectra were obtained with complete decoupling of C–H interactions, and also under the conditions of J-modulation (δ, ppm).

20R,24S-Epoxycycloartane-3β,6α,16β,25-tetraol 16-O-Acetate 3-O-[O-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl] 6-O-β-D-xylopyranoside (V) from (III). A solution in 10 ml of dichloroethane of 500 mg of the askendoside D nonaacetate (III) obtained in [1] was treated with 1 g of mercury cyanide and 1 g of 4 Å molecular sieve [7]. The mixture was stirred at room temperature for 30 min, and then 12.5 g of acetobromorhamnose in 5 ml of dichloroethane that had previously stood over 4 Å molecular sieve for 30 min was added. Stirring at room temperature was continued for 2 h with the passage of a gentle current of dry nitrogen to protect the mixture from atmospheric moisture. Monitoring was carried out by TLC in system 1. The reaction mixture was filtered, and the filtrate was diluted with 100 ml of chloroform and was washed successively with 20% potassium iodide solution, sodium hydrogen carbonate solution, and water. After this it was dried with anhydrous sodium sulfate and evaporated.

The dry residue was dissolved in 20 ml of methanol, and 20 ml of a 2% methanolic solution of KOH was added. After 3 h, the reaction mixture was neutralized with KU-2 cation-exchange resin and it was filtered and evaporated to dryness. The residue was chromatographed on a column of silica gel with elution by system 2. This gave 380 mg of glycoside (V) (the yield of products calculated on the theoretical yield of the last stage amounted to 89.6%).

Glycoside (V, $C_{53}H_{86}O_{22}$, mp 257-260° (from methanol), $[\alpha]_D^{28}$ −12.3±2° (c 0.81; pyridine), ν_{max}^{KBr}, cm^{-1}: 3600-3220 (OH), 1735; 1260 (ester group). PMR spectrum (C_5D_5N, 0–TMS): 0.10 and 0.59 (2H-19, d, ^2J = 4 Hz), 1.10; 1.32; 1.32; 1.32; 1.32; 1.36, 1.75 (7×CH_3, s), 1.59 (CH_3 L-rhamnose, d, ^3J = 5.5 Hz), 2.04 (CH_3COO at C-16, s), 2.58 (H-17, d, ^3J = 8 Hz), 3.33 (H-3, dd, $^3J_1$ = 12 Hz, $^3J_2$ 4 Hz), 4.75 and 4.80 (anomeric protons of D-xyloses, d, ^3J = 7.5 Hz), 5.14 (anomeric proton of L-arabinose, d, ^3J = 7.5 Hz), 5.57 (H-16, m), 5.63 (anomeric proton of L-rhamnose, br. s). For the ^{13}C NMR spectrum, see Table 1.

Askendoside D Octaacetate (II) from (I). Askendoside D (1.5 g) was acetylated with 7.5 ml of acetic anhydride in 7.5 ml of pyridine at room temperature. The reaction mixture was poured into ice water, and the precipitate was filtered off and washed with water. The products were chromatographed on a column with elution by system 1. This gave 2 g of the amorphous octaacetate (II), $C_{61}H_{90}O_{25}$, $[\alpha]_D^{24}$ 0 ± 3° (c 1.8; methanol), ν_{max}^{KBr}, cm^{-1}: 3560-3370 (OH), 3050 (CH_2 of a cyclopropane ring), 1760, 1260-1220 (ester groups). PMR spectrum (C_5D_5N. 0–HMDS: 0.06 and 0.38 (2H-19, d, ^2J = 4 Hz), 0.88; 1.11; 1.16; 1.26; 1.33; 1.44 (7 ×CH_3, s), 1.84; 1.84; 1.84; 1.84; 1.96; 2.02; 2.20; 2.29 (8×CH_3COO, s), 4.68; 4.82; 4.88 (each 1H, d, ^3J = 7 Hz, anomeric protons).

3β,6α-Dihydroxy-16-oxo-20R,25-norcycloartane-20,24-olide 3-O-[O-α-L-arabinoopyranosyl-(1→2)-β-D-xylopyranoside] 6-O-β-D-Xylopyranoside Octaacetate (IV) from (II). The octaacetate (II) (1.19 g) in 40 ml of acetone was treated with 1 ml of the Jones reagent [5], and the mixture was stirred and was then left at room temperature for 20 min. After this, the excess of oxidant was decomposed by the addition of 5 ml of methanol. The reaction mixture was poured into water and extracted with chloroform. The chloroform extract was washed with water and evaporated. The residue was chromatographed on a column with elution by system 1.

This gave 1 g of product (IV), $C_{58}H_{80}O_{25}$, mp, 150-152° (from ethanol), $[\alpha]_D^{27}$ −54.5±2° (c 0.55; methanol), ν_{max}^{KBr}, cm^{-1}: 1780-1740; 1250-1230 (C=O at C-16, γ-lactone and ester groups). PMR spectrum (C_5D_5N, 0−HMDS): 0.04 and 0.42 (2H-19, d, ^2J = 4 Hz), 1.01; 1.05; 1.12; 1.33; 1.33 (5×CH_3,s), 1.83; 1.85; 1.88; 1.97; 2.03; 2.19; 2.30 (8×CH_3COO, s), 3.00 (H-17, s), 4.70; 4.86; 4.92 (anomeric protons, d, ^3J = 6 Hz).

20R,24S-Epoxycycloartane-3β,6α,16β,25-tetraoß-O-[O-α-L-Arabinopyranosyl-(1→2)-β-D-xylopyranoside]16,25-Di-O-α-L-rhamnopyranoside 6-O-βD-Xylopyranoside (VI), from (II). A solution of 612 mg of askendoside D octaacetate in 10 ml of dichloroethane was treated with 1 g of mercury cyanide and 1 g of 4 Å molecular sieve [7]. The mixture was stirred at room temperature for 30 min, and then 0.6 g of acetobromorhamnose in 5 ml of dichloroethane that had previously stood over 4 Å molecular sieve for 30 min was added. Stirring at room temperature was continued for 2 h with the passage of a gentle current of dry nitrogen to protect the reaction mixture from atmospheric moisture. Monitoring was carried out by TLC in system 1. The reaction mixture was filtered and the filtrate was diluted with 100 ml of chloroform and was washed successively with 20% potassium iodide solution, sodium hydrogen carbonate solution, and water. After this it was dried with anhydrous sodium sulfate and evaporated. The dry residue was dissolved in 20 ml of methanol, and 20 ml of a 2% methanolic solution of KOH was added. After 18 h, the reaction mixture was neutralized with KU-2 cation-exchange resin and was filtered and evaporated to dryness. The residue was chromatographed on a column of silica gel with elution by system 2. This gave 500 mg of glycoside (VI) (the yield of product calculated from the overall theoretical yield in two stages was 80.4%).

Glucoside (VI), $C_{57}H_{94}O_{25}$, mp 209-210°C (from ethanol), $[\alpha]_D^{22}$ −7.4±2°(c 1.43; pyridine), ν_{max}^{KBr}, cm^{-1}:3550-3250 (OH), 3050 (CH_2 of a cyclopropane ring)> PMR spectrum (C_5D_5N, 0−TMS): 0.07 and 0.63 (2H-19, d,^2J = 4 Hz), 1.19; 1.27; 1.31; 1.34; 1.40; 1.45; 1.74 (d×CH_3,. s), 1.59 (CH_3 of L-rhamnose at C-16, d, ^3J = 6 Hz, 1.66 (CH_3 of L-rhamnose at C-25, d, ^3J = 6 Hz), 2.49 (H-17, d, ^3J = 8 Hz), 3.31 (H-3, dd, $^3J_1$ = 12 Hz, $^3J_2$ = 4 Hz), 4.75 and 4.80 (anomeric protons of D-xylopyranoses, d, ^3J = 7 Hz), 5.13 (anomeric proton of L-arabinose, d, ^3J = 7 Hz), 5.14 (anomeric proton of L-rhamnose at C-16, d, ^3J = 1.5 Hz), and 5.59 (anomeric proton of L-rhamnose at C-25, d, ^3J = 1.5 Hz). For the ^{13}C NMR spectrum, see Table 1.

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