UDC 547.597+547.918

THE TRITERPENE GLYCOSIDES OF PATRINIA INTERMEDIA ROEM ET SCHULT

IV. Structure of the Carbohydrate Chains of Patrinosides C and D

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Khimiya Prirodnykh Soedinenii, Vol. 5, No. 2, pp. 89-93, 1969

In preceding papers [1, 2] we have given information on the structure of the carbohydrate chains of patrinosides C_1 and D_1. Since these glycosides are not only present in the plant but can also be obtained by the alkaline hydrolysis of the corresponding glycosides C and D, the structure of the carbohydrate chains attached to the hydroxyl of oleanolic acid in these glycosides is clear. The structures of the saccharide chains attached to the carboxyl of the aglycone has remained obscure.

Glycoside	$[M_D]$, deg		Possible associations	ΔC, deg
	α	β		
Methyl D-glucofuranoside [5]	+230	−150	α-pyr + α-fur + α-pyr	+854
			α-pyr + α-fur + β-pyr	+464
			α-pyr + β-fur + β-pyr	+ 98
Methyl D-glucopyranoside [6]	+312	− 64		
			β-pyr + β-fur + β-pyr	−284
			β-pyr + β-fur + β-pyr	−364
Patrinoside C	−346		β-pyr + β-fur + β-pyr	−260
Patrinoside C_1	− 86			
Patrinoside D	−303		β-pyr + β-fur + β-pyr	−201
Patrinoside D_1	−102			

The present paper gives a solution of this problem. For this purpose it was necessary to split off the trisaccharide attached to the carboxyl. We first attempted this by alkaline hydrolysis. However, the experiments proved unsuccessful in spite of all precautions. Even when hydrolysis was performed at room temperature on an anion-exchange resin previously washed with water almost to pH 7 and by evaporation at low temperature the trisaccharides split off underwent change. Consequently, we prepared full methyl ethers of patrinosides C and D which were then split by reduction with lithium aluminum hydride [3]. On subsequent acid hydrolysis of the reduced methylated trisaccharides we obtained the same set of three methylated monosaccharides in each case. The tetramethylglucose was identical in its chromatographic behavior, specific rotation, and boiling point with an authentic sample of 2,3,4,6-tetramethyl-D-glucose. The demethylation of the sample isolated and the authentic sample gave identical degradation products.

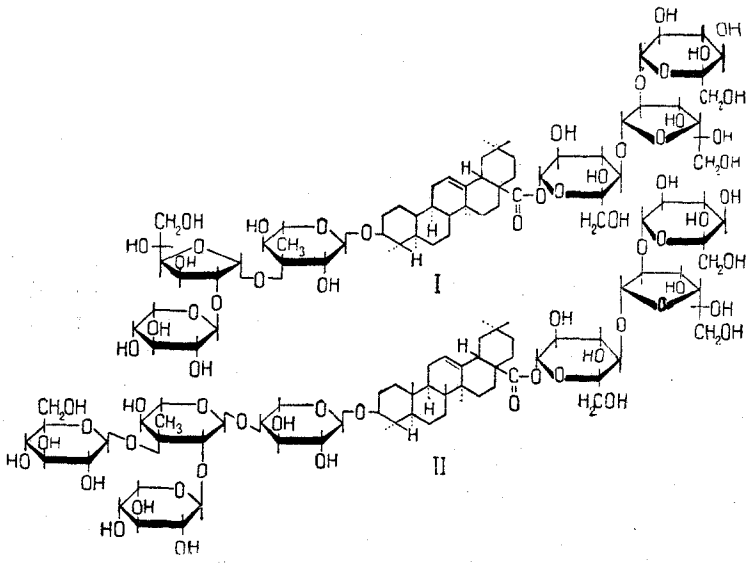
The trimethylglucose agreed completely in its specific rotation, R_g value, and the results of demethylation and of periodate oxidation (positive test for a free α-diol grouping) with 3,5,6-trimethyl-D-glucofuranose, which we have isolated previously from the trisaccharide chain of patrinoside C_1 [1].

The trimethylsorbitol obtained as a result of the reduction of the glucose attached directly to the carboxyl of the aglycone, agreed in its chromatographic behavior with an authentic sample of 2,3,6-trimethyl-D-sorbitol. Just like the latter, the sorbitol isolated has a free α-diol grouping and is, therefore, oxidized by periodate. A chromatographic study of the products of the oxidation of the sample isolated and the authentic sample showed that they gave identical products.

The production of 2,3,6-trimethyl-D-sorbitol did not enable the size of the ring of the glucose attached directly to the carboxyl of the aglycone to be established unambiguously. There are two possibilities, i.e., the glucose has a pyranose ring and a 1-4 bond or a furanose ring and a 1-5 bond. The first possibility is the more probable, as is confirmed by a calculation of the configuration of the glycosidic centers in the trisaccharide. The calculation was carried out from differences in molecular rotations [3, 4]. First we calculated the contribution to the molecular rotation of the glycosides C and D provided by the trisaccharide. For this purpose we deducted the molecular rotation of patrinoside C_1 from the molecular rotation of patrinoside C and proceeded similarly with D and D_1. These magnitudes proved to have the same sign and fairly similar values: −260° and −200° C.

At least five variants of the combinations of α- and β-bonds in the trisaccharide, including the bond of the latter with the aglycone, are possible.

As can be seen clearly from the table, the best agreement of the contribution ΔC of the molecular rotation calculated from the figures found is obtained if the presence of only β-linkages and a single furanose ring in the trisaccharide is assumed. It follows that the trisaccharide attached to the carboxyl of the oleanolic acid in glycosides C and D is D-glucopyranosido(1 → 2)-β-D-glucofuranosido(1 → 4)-β-D-glucopyranose.



Thus, on the basis of the results given previously [1, 2] and in this paper, patrinoside C has structure I and patrinoside D structure II.

Experimental

Chromatography was carried out on type M paper of the Volodarskii Leningrad mill and on silica gel of type KSK. The following systems of solvents were used: 1) benzene—acetone—water (5 : 2 : 1) and 2) butan-1-ol—ethanol—water (5 : 1 : 4). The sugars were revealed with aniline phthalate and the sugar alcohols with $AgNO_3$ and with sodium periodate and benzidine [7].

Methylation of patrinoside C [8]. A solution of 5 g of the patrinoside in 240 ml of dimethylformamide was treated with 56 g of BaO and the mixture was stirred vigorously at 90° C for 4 hr. Then, at first, 5 ml of methyl iodide was added (leading to a vigorous reaction) followed, dropwise, by another 50 ml, the reaction mixture being kept at a uniform boil. After cooling, it was poured into 500 ml of water, the precipitate that deposited was filtered off, and the filtrate was extracted with chloroform (5 × 500 ml). The combined chloroform extracts were washed with 100 ml of aqueous $Na_2S_2O_3$ solution and with water and were evaporated to dryness. The residue was transferred to a column (2 × 10 cm) of silica gel and the following fractions were eluted: I, with 100 ml of benzene, 2.9 g; II, with 100 ml of chloroform, 0.5 g; III, with 100 ml of ethyl acetate—chloroform (1 : 1), 1.0 g; and IV, with 100 ml of ethyl acetate, 0.5 g. The IR spectra of fractions I and II lacked the absorption bands for a free OH group (3200–3600 cm^{-1} region); amorphous powder, $[\alpha]_D^{20}$ −24 ± 3° (c 5.0; methanol).

Found, %: C 60.80; H 8.19. Calculated for $C_{83}H_{142}O_{31}$, %: C 61.10; H 8.70.

Fractions III and IV were remethylated by the method described above. The total yield of the full methyl ether of patrinoside C after the experiment had been carried out twice was 3.9 g.

Reduction of the full methyl ether of patrinoside C. In drops, with stirring, a solution of 3.08 g of the product in 50 ml of absolute alcohol was added over 2 hr to a solution of 0.5 g of lithium aluminum hydride in 42 ml of absolute ether. After this, 50 ml of absolute benzene was added to the reaction mixture with the simultaneous removal of the ether by distillation, and the mixture was boiled for another 4 hr. Then, 0.3 g of lithium aluminum hydride was added and the mixture was left overnight. The excess of reducing agent and the complex were decomposed with 30 ml of water, the benzene layer was separated off, and the aqueous layer was extracted with 50 ml of water. The 3 g of solid residue obtained by the evaporation of the combined extracts was dissolved in 300 ml of ether and extracted with water (3 × 100 ml). Distillation of the ethereal solution yielded 2.4 g of a methylated trioside of erythrodiol, $[\alpha]_D^{20}$ −22 ± ± 3° (c 2.0; methanol).

Found, %: C 65.25; H 9.06. Calculated for $C_{55}H_{98}O_{15}$, %: C 65.60; H 9.45.

Distillation of the aqueous extracts yielded 0.57 g of the reduced methylated trisaccharide, bp 105–110° C/0.5 mm, $[\alpha]_D^{20}$ −9 ± 3° (c 1.9; ethanol).

Found, %: C 51.80; H 8.56. Calculated for $C_{28}H_{54}O_{16}$, %: C 52.00; H 8.41.

Hydrolysis of the reduced methylated trisaccharide and separation of the methylated monosaccharides. A solution of 0.16 g of the product in a mixture of 22 ml of methanol and 0.13 ml of concentrated hydrochloric acid was boiled for 7 hr. Then 20 ml of water was added and the mixture was heated for another 3 hr and evaporated to dryness in vacuum. The resulting sirup was transferred to a column (2 × 50 cm) of silica gel and eluted with system 1, 20-ml fractions being collected.

Fractions 5–12 contained 44 mg of 2,3,4,6-tetramethyl-D-glucose, bp 79–80° C/0.1 mm; $[\alpha]_D^{20}$ +88 ± 3° (c 4.0; acetone), R_g 1.0 (system 2). Literature data: $[\alpha]_D$ +83.9° (acetone) [9, 10]. On being heated with 47% hydrobromic acid, the tetramethylglucose obtained and an authentic sample of 2,3,4,6-tetramethyl glucose gave identical demethylation products as found by paper chromatography.

Fractions 17–19 contained 45 mg of 3,5,6-trimethyl-D-glucose, $[\alpha]_D^{20}$ −29.6 ± 3° (c 1.0; methanol), R_g 0.90 (in system 2). Literature data: $[\alpha]_D$ −41.6° (chloroform), $[\alpha]_D$ −25.9° (water) [11].

Fractions 30–50 contained 38 mg of 2,3,6-trimethyl-D-sorbitol, $[\alpha]_D^{20}$ ±0 ± 3° (c 2.5; methanol), bp 150–155° C/0.5 mm, R_f 0.45–0.41; R_g 0.60 (in system 2). Its chromatographic behavior did not differ from that of an authentic sample of 2,3,6-trimethyl-D-sorbitol. A solution of 3 mg of the product in 5 ml of water was treated with 3 mg of HIO_4 and heated in the boiling water bath for 20 min. The mixture was cooled and the oxidation products were extracted with ether (3 × 5 ml). The ethereal extracts were combined and evaporated and the residue was deposited on a paper chromatogram. An authentic sample of 2,3,6-trimethyl-D-sorbitol was oxidized similarly. In both cases spots with R_f 0.89 and 0.97 (system 2) appeared on the chromatograms.

Methylation of patrinoside D. In a similar manner to that described above, after the experiment had been carried out twice 5.9 g of patrinoside D and 50 ml of methyl iodide in 170 ml of dimethylformamide, with 42 g of BaO and 2 g of $Ba(OH)_2 \cdot H_2O$, yielded 3.2 g of the fully methylated product in the form of an amorphous powder with $[\alpha]_D^{20}$ − −28 ± 3°; methanol).

Found, %: C 60.79; H 8.71. Calculated for $C_{90}H_{154}O_{35}$, %: C 60.45; H 8.60.

Reduction of the full methyl ether of patrinoside D. By the method described above, 2.7 g of the substance was reduced with 0.4 g of lithium aluminum hydride in 100 ml of absolute ether and 100 ml of absolute benzene. This gave 2.5 g of the methylated tetraoside of erythrodiol; amorphous powder, $[\alpha]_D^{20}$ −19 ± 3° (c 3.6; ethanol).

Found, %: C 64.47; H 9.23. Calculated for $C_{62}H_{105}O_{19}$, %: C 64.50; H 9.17.

The yield of reduced methylated trisaccharide was 0.23 g, bp 105–110° C/0.5 mm; $[\alpha]_D^{20}$ +10 ± 3° (c 3.8; chloroform).

Found, %: C 51.68; H 8.50. Calculated for $C_{28}H_{54}O_{16}$, %: C 52.00; H 8.41.

Hydrolysis of the reduced methylated trisaccharide and separation of the methylated monosaccharides. The hydrolysis was carried out by the method described, using 30 ml of methanol and 0.18 ml of concentrated hydrochloric acid for 0.36 g of the substance. A preliminary comparison of the hydrolysate with that obtained by the hydrolysis of the methylated trisaccharide from glycoside C showed a complete identity of their compositions.

The resulting mixture of monosaccharides was separated on a column (2 × 50 ml) of silica gel with elution by system 1, 20-ml fractions being collected. This gave 82 mg of 2,3,4,6-tetramethyl-D-glucose, 91 mg of 3,5,6-trimethyl-D-glucose, and 73 mg of 2,3,6-trimethyl-D-sorbitol.

Conclusions

1. It has been found that the trisaccharide residues attached to the carboxyl of the aglycone in patrinosides C and D have identical structures and configurations, the middle monosaccharide being glucofuranose.

2. The complete structures of patrinosides C and D have been established and the configurations of their glycosidic centers have been determined.

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31 July 1967

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