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THE TRITERPENE GLYCOSIDES OF PATRINIA INTERMEDIA ROEM ET SCHULT

III. Structure of the Carbohydrate Chain of Patrinoside D_1

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In one of our preceding papers [1] we reported the isolation of patrinoside D, a pentaoside of oleanolic acid, which was first obtained by A. Ya. Khorlin and V. M. Ivanova [2]. In this paper, we give information concerning the structure of the carbohydrate chain of the glycoside D_1. To obtain the latter, partrinoside D was subjected to alkaline hydrolysis on Dowex-1 anion-exchange resin. The method of stepwise hydrolysis that we have developed [1] proved to be extremely convenient: cleavage takes place at room temperature and the sugar quantitatively split off from the carboxy group is readily separated from the remaining acid glycoside. A determination of the molecular weight of the glycoside D_1 obtained showed that it is a tetraoside of oleanolic acid. When it was heated with mineral acids the hydrolysate was found to contain glucose, xylose, and rhamnose. Consequently, one of the monosaccharides appears twice in the carbohydrate chain. This question is generally resolved by a quantitative determination of the monosaccharide residues [2].

Monosaccharide glycoside	M_D, deg		Glycoside	M_D, deg	ΔC, deg	Form of the bond
	α	β				
Methyl D-xylopyranoside [9]	+253	−108	the trioside IV	+ 72	+ 30	β
			the bioside II	+ 42		
			the trioside III	−155		
Methyl L-rhamnopyranoside [10,11]	−111	+170			−197	β
			the bioside II	+ 42	−140	α
			the monoside I	+182	−182	β
Methyl D-glucopyranoside [12]	+312	− 64				
			Oleoanolic acid	+364		

We have shown the fact that xylose appears more than once by stepwise hydrolysis and methylation. In spite of the advantages of carrying out the hydrolysis with KU-2 ion-exchange resin, this method, which we have used previously, has a serious disadvantage—the long duration of the experiment. Consequently, in this case we carried out stepwise hydrolysis with 6% sulfuric acid in aqueous isopropanol. The separation of the mixture of intermediate glycosides formed from the sugars split off was carried out on an anion-exchange resin. The separation of the products was carried out by adsorption chromatography on silica gel. Oleanolic acid, a monoside (I), a bioside (II), two triosides (III) and (IV), and the initial patrinoside D_1 were isolated.

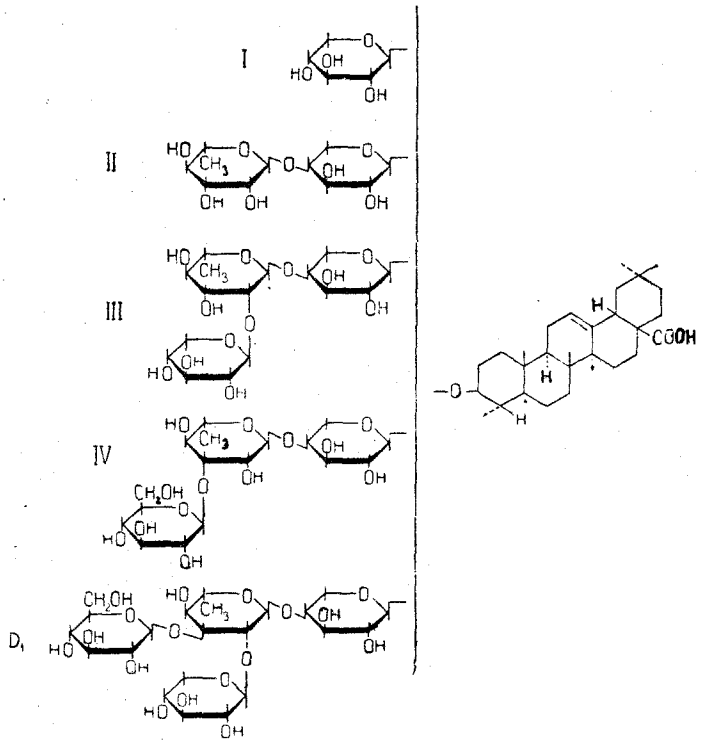
Subsequent acid hydrolysis showed that I contained D-xylose, II D-xylose and L-rhamnose, III D-xylose and L-rhamnose, and IV D-xylose, L-rhamnose, and D-glucose. These results show that the monoside I is a xyloside and the bioside II a rhamnoxyloside of oleanolic acid. The production of two triosides shows the presence of branching in the carbohydrate chain, while in the trioside III one of the monosaccharides is repeated. To clarify this point and also to establish the size of the oxide rings of the monosaccharides we methylated patrinoside D_1 by Kuhn's method [3]. The mixture of methylated monosaccharides obtained after acid hydrolysis was separated by adsorption chromatography on silica gel. The process was monitored by paper chromatography. The fractions enriched in the individual components were separated again. Four methylated monosaccharides were isolated. The tetramethyl-D-glucose was identical in its chromatographic behavior and specific rotation with an authentic sample of 2,3,4,6-tetramethyl-D-glucose. By demethylation with 47% hydrobromic acid, glucose was obtained. A trimethyl-D-xylose was identical in its R_g value of 0.94 [4] and its specific rotation with an authentic sample of 2,3,4-trimethyl-D-xylose; on demethylation it gave the same mixture of products (including xylose) as the authentic sample. The dimethyl-D-xylose agreed well in respect of its R_g value of 0.75 [4] and its specific rotation with 2,3-dimethyl-D-xylose. 2,4- and 3,4-dimethyl-D-xyloses were excluded both by their R_g values of 0.66 and 0.84 [4] and by their specific rotations. Furthermore, the latter gave a positive reaction on oxidation with periodate, which was not the case for our compound. L-Rhamnose was isolated in the form of a monomethyl derivative. Of the four possible monomethyl-L-rhamnoses (2-, 3-, 4-, and 5-), only the latter two have a 1,2,3-triol grouping and liberate formic acid on periodate oxidation [5], as our compound did. 5-Monomethyl-L-rhamnose is excluded since it possesses a specific rotation of opposite sign to that of the compound isolated. In its R_g value and specific rotation, the monomethylrhamnose isolated agrees well with 4-methyl-L-rhamnose [4,6,7].

Thus, the results of stepwise hydrolysis and methylation show that the carbohydrate chain of patrinoside D_1 has branching at L-rhamnose and the terminal sugars are D-xylose and D-glucose.

In order to determine to what hydroxyl of the L-rhamnose the terminal xylose is bound, methylation of the triose III was carried out. On its subsequent hydrolysis, 2,3,4-trimethyl-D-xylose, 2,3-dimethyl-D-xylose, and 3,4-dimethyl-L-rhamnose were identified by paper chromatography [4]. It can be seen from this that the terminal xylose is connected to the second hydroxyl of rhamnose. Consequently, in the trioside IV the glucose must be connected with the third hydroxyl of the rhamnose.

Configuration of the glycosidic centers was determined from the difference in molecular rotations between the trioside IV and the bioside II, between the trioside III and the bioside II, between the bioside II and the monoside I, and between the monoside I and oleanolic acid by Klyne's method [8].

As can be seen from the table, the first xylose is connected with the aglycone by a β-, the rhamnose with this xylose by an α-, and the terminal xylose and glucose with the rhamnose by β-glycosidic bonds. Thus, the structures of the intermediate glycosides I–IV and of patrinoside D_1 are expressed by the formulas



Experimental

Type M paper of the Volodarskii Leningrad mill and silica gel of type KSK were used for chromatography. The following systems of solvents were used: 1) chloroform–ethanol (10 : 1); 2) ethyl acetate–ethanol–water (10 : 2 : 3); 3) chloroform–ethyl acetate–ethanol (24 : 6 : 5); 4) butan-1-ol– acetic acid–water (5 : 1 : 4); 5) benzene–butan-1-ol–pyridine–water (1 : 5 : 5 : 2); 6) chloroform–ethyl acetate–ethanol (24 : 6 : 8); 7) benzene–acetone–water (5 : 5 : 1); 8) benzene–chloroform (1 : 2); 9) butan-1-ol–ethanol–water (5 : 1 : 4); 10) benzene–chloroform–methanol (6 : 12 : 1); and 11) chloroform–methanol (60 : 1).

Alkaline hydrolysis of patrinoside D. A solution of 3.12 g of patrinoside D in 10 ml of water was transferred to a column of 50 g of Dowex-1 × 2 100/200 anion exchange resin in the OH form. After a day, it was washed with 1.5 *l* of distilled water and the eluate was evaporated to dryness to give 0.38 g of a sirup of the sugars that had been split off. Then the column was washed with 300 ml of 10% acetic acid in methanol. The eluate was evaporated in vacuum and the residue of patrinoside D_1, 2.6 g, was recrystallized from 80% ethanol, mp 240–242° C $[\alpha]_D^{20}$ −10 ± 3° (c 5.0; methanol).

Found, %: C 59.28; H 8.36; C 59.43; H 8.43. Calculated for $C_{52}H_{84}O_{20} \cdot H_2O$, %: C 59.70; H 8.28.

Found (by titration): mol. wt. 1010, 1040. Calculated: mol. wt. 1029.3. Acetate: mp 150–153° C, $[\alpha]_D^{20}$ −18 ± ± 3° (c 5.0; methanol).

Found, %: C 59.13; 58.93; H 7.22; 7.23. Calculated for $C_{72}H_{104}O_{30}$, %: C 59.20; H 7.30; monomethyl ether: mp 218–220° C, $[\alpha]_D^{20}$ −10 ± 3° (c 5.3; methanol).

Found, %: C 60.26, 60.30; H 8.52, 8.50. Calculated for $C_{53}H_{86}O_{20}$, %: C 59.90; H 8.38.

Stepwise hydrolysis of patrinoside D_1. A solution of 1.14 g of the substance in 120 ml of a 2:1 mixture of isopropanol and water was treated with 3.27 ml of concentrated sulfuric acid and heated in the boiling water bath for 30 min. The solution was neutralized with $Ba(OH)_2$, the precipitate of barium sulfate was filtered off, the filtrate was evaporated, the residue was dissolved in 40 ml of aqueous ethanol, and the solution was transferred to a column (3.5 × 30 cm) of Dowex-1 × 2 100/200 anion-exchange resin in the OH form. The resin was washed with 6 *l* of hot (50–60° C) water until the eluates were completely free from sugars (test for coloration with aniline phthalate) and with 1 *l* of 10% acetic acid in methanol. Evaporation of the methanolic solution gave 0.69 g of a mixture of glycosides which was separated on a column (2 × 45 cm) of silica gel, 60-ml fractions being collected. The eluates were examined by thin-layer chromatography in systems 1 and 2.

Fractions 1–5, eluted with system 3, contained oleanolic acid (0.13 g).

Fractions 6–7, eluted with system 3, contained 0.14 g of the monoside I, mp 215–218° C (from aqueous ethanol), $[\alpha]_D^{20}$ +30 ± 3° (c 1.1; ethanol).

Found, %: C 68.90; H 9.64. Calculated for $C_{35}H_{56}O_7 \cdot H_2O$, %: C 69.30; H 9.63.

After heating with 9% hydrochloric acid, the hydrolysate was found by paper chromatography in systems 4 and 5 to contain xylose.

Fractions 9 and 10 contained 0.11 g of the bioside II, mp 303–305° C (from aqueous ethanol). $[\alpha]_D^{20}$ +5.5 ± 3° (c 3.6; ethanol).

Found, %: C 65.25; H 9.06. Calculated for $C_{41}H_{66}O_{11} \cdot H_2O$, %: C 65.50; H 9.11. On hydrolysis, xylose and rhamnose were identified.

Fractions 11–13, eluted with system 6, contained 0.1 g of the trioside III, mp 217–220° C (from aqueous ethanol), $[\alpha]_D^{20}$ −18 ± 3° (c 2.4; ethanol).

Found, %: C 63.46; H 8.67. Calculated for $C_{46}H_{74}O_{15}$, %: C 63.70; H 8.53.

On hydrolysis, xylose and rhamnose were detected.

Fractions 14–16, eluted by the same system, contained 0.06 g of the trioside IV, mp 207–209° C (from aqueous ethanol), $[\alpha]_D^{20}$ +8.3 ± 3° (c 2.4; ethanol).

Found, %: C 63.40; H 8.16. Calculated for $C_{47}H_{75}O_{16}$, %: C 63.60; H 8.50. On hydrolysis, xylose, rhamnose, and glucose were found.

Fractions 17–19 contained 0.14 g of the initial patrinoside D_1.

Methylation of patrinoside D_1. A solution of 1.2 g of the substance in 40 ml of dimethylformamide was treated with 10 g of BaO and the mixture was heated to 90° C with vigorous stirring for 12 hr. After the addition of 11 ml of methyl iodide it was heated for another 9 hr. Then it was diluted with 200 ml of water, and the precipitate that deposited was filtered off and washed with 30 ml of chloroform, the filtrate was extracted with chloroform (5 × 100 ml), and the combined extracts were washed with $Na_2S_2O_3$ solution and with water and were evaporated. The methylation product was transferred to a column (2 × 10 cm) of silica gel and the following fractions were eluted: I, with 100 ml of benzene, 0.35 g; II, with 200 ml of chloroform, 0.5 g; III, with 50 ml of ether, 0.2 g.

Fraction II contained the fully methylated patrinoside D_1 as an amorphous powder with $[\alpha]_D^{20}$ −35.5 ± 3° (c 6.2; methanol). The IR spectrum lacked absorption bands of hydroxy groups (3400–3500 cm^{-1}).

Found, %: C 63.84; H 8.72. Calculated for $C_{63}H_{106}O_{20}$, %: C 63.60; H 8.96.

Hydrolysis of methylated patrinoside D_1. A mixture of 2.07 g of substance, 100 ml of methanol, and 7 ml of concentrated hydrochloric acid was boiled for 5 hr. After this, 40 ml of water was added and the mixture was heated for another 5 hr. The methanol was distilled off in vacuum, the precipitate of methyl oleanolate that deposited (0.6 g) was filtered off and washed with 30 ml of water, and the filtrates were combined and evaporated in vacuum. The resulting sirup of 1.5 g of a mixture of methylated monosaccharides was then separated on a column (2 × 30 cm) of silica gel, the process being monitored by thin-layer chromatography on silica gel in system 7.

Fraction I, eluted with 700 ml of benzene, contained 35 mg of methyl oleanolate; II, eluted with system 8, contained 0.13 g of 2,3,4,6-tetramethyl-D-glucose; bp 80–85° C/0.1 mm; $[\alpha_j]_D^{20}$ +88 ± 3° (c 6.0; acetone). According to the literature—$[\alpha]_D$ + 83.9° (acetone) [3]; R_g 1.0 (system 9). Its chromatographic behavior was identical with that of an authentic sample of 2,3,4,6-tetramethyl-D-glucose. On heating with 47% hydrobromic acid, in addition to other demethylation products, D-glucose was identified by paper chromatography in systems 9 and 4. Fraction III, eluted with

1000 ml of system 10, contained 1.07 g of material, which was reseparated. Elution was carried out with system 11 and 45-ml fractions were collected.

Fractions I–II contained a trimethylxylose with a small amount of a tetramethylglucose. By reseparation, 0.1 g of 2,3,4-trimethyl-D-glucose was obtained with bp 110–115° C/0.5 mm; $[\alpha]_D^{20}$ +53 ± 3° (c 2.5; chloroform) and $[\alpha]_D^{20}$ +63 ± ± 3° (c 4.5; water) R_g 0.94 (in system 9). According to the literature, $[\alpha]_D$ +55.8° (chloroform), $[\alpha]_D$ +64.5° (water) [14]. Demethylation of the trimethylxylose isolated and of an authentic sample of 2,3,4-trimethyl-D-xylose gave identical cleavage products, including xylose. Fraction III–IV contained 0.38 g of material the reseparation of which yielded 2,3-dimethyl-D-xylose; $[\alpha]_D^{20}$ +51 ± 3° (c 2.3; chloroform) and $[\alpha]_D^{20}$ +52 ± 3° (c 1.1; water), R_g 0.75 (in system 9). According to the literature, $[\alpha]_D$ + 70° (water) [4,15]. Fractions IX–XIII contained 0.19 g of material; after rechromatography, 4-monomethyl-L-rhamnose was isolated: $[\alpha]_D^{20}$ +13 ± 3° (c 2.3; methanol), R_g 0.56 (in system 9). According to the literature $[\alpha]_D$ +12.9° (methanol), R_g 0.57 [4,6]. The qualitative reaction for a 1,2,3-triol grouping was positive [5].

Methylation of the trioside (III). To a solution of 10 mg of the substance in 1.5 ml of dimethylformamide were added 1.5 ml of methyl iodide, 0.5 g of BaO, and a small crystal of $Ba(OH)_2 \cdot 8H_2O$, and the mixture was heated in a tube at 100° C for 13 hr. Then it was poured into 5 ml of an aqueous solution of $Na_2S_2O_3$, the precipitate was filtered off and washed with 5 ml of water and 10 ml of chloroform, and the filtrate was extracted (4 × 5 ml) with chloroform. The combined extracts were washed with water (3 × 3 ml) and evaporated, the residue was dissolved in 2 ml of methanol, 0.12 ml of concentrated hydrochloric acid was added, and the mixture was heated at 100° C in a tube for 11 hr. After this, 2 ml of water was added and the mixture was heated for another 3 hr. The precipitate was filtered off, the filtrate was evaporated to dryness, and the residue was dissolved in 0.5 ml of ethanol and subjected to paper chromatography. On elution with system 9, three spots were found on the chromatogram with R_g values of 0.94 (2,3,4-trimethyl-D-xylose), 0.84 (3,4-dimethyl-L-rhamnose), and 0.74 (2,3-dimethyl-D-xylose).

Conclusions

It has been established that patrinoside D_1 is the

β-D-glucopyranosido(1 → 3)
β-D-xylopyranosido(1 → 2) > α-L-rhamnosido(1 → 4)-β-D-xylopyranoside (1 → 3) of oleanolic acid

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