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TRITERPENE GLYCOSIDES OF PATRINIA INTERMEDIA

II. The Structure of the Carbohydrate Chain of Patrinoside C_1

V. G. Bukharov, V. V. Karlin, and V. A. Talan

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It has been reported previously [1] that patrinoside C_1 is a trioside in which a trisaccharide consisting of L-rhamnose, D-glucose, and D-xylose is attached to the hydroxyl of oleanolic acid.

In the present paper, we report the establishment of the structure of the sugar chain of patrinoside C_1. To determine the sequence of addition of the monosaccharide residues in the trisaccharide, we carried out the stepwise hydrolysis of patrinoside C_1 on the cation-exchanger KU-2. It was found that the optimum conditions for the performance of the reaction are a temperature of 100° C and a time of 20 hr. A study of the reaction mixture showed that in this case the hydrolysis products are present in equal amounts. It is customary to isolate fragments of the carbohydrate chain and to deduce its structure from them. We isolated the glycosides. This approach has a number of advantages: in the first place, it permits a simpler and more reliable determination of the sequence of addition of the monosaccharides in the chain; in the second place, by means of a measurement of the specific rotation of the intermediate glycosides, it permits a determination of configurations of all the glycosidic centers and, moreover, shows the presence of branching in the carbohydrate chain.

By preparative chromatography on silica gel, from the reaction mixture we obtained—in addition to the initial patrinoside C_1—oleanolic acid, a monooside (I), and a bioside (II). Since, on subsequent acid hydrolysis, (I) yielded the aglycone and L-rhamnose, the monooside (I) is a rhamnoside of oleanolic acid. Similarly, D-glucose and L-rhamnose were identified in the hydrolysate from the bioside (II). Thus, this glycoside is a D-gluco-L-rhamnoside of oleanolic acid. Hence, patrinoside C_1 is a D-xylo-D-gluco-L-rhamnoside. To determine the sequence of the bonds and the dimensions of the oxide ring in the monosaccharides, patrinoside C_1 was methylated by Kuhn's method [2]. It was found that in all cases when it was possible to cause the reaction to take place vigorously, the yield of methylated glucoside with a 2-hr reaction time was, as a rule, 40-50%, and in rare cases 60-80%. Separation of the completely methylated product from the unmethylated fraction was easily achieved on a silica gel column. The completeness of the methylation was checked by IR spectroscopy (from the absence of absorption in the 3200-3600-cm^{-1} region). The subsequent acid hydrolysis of the full methyl ether of patrinoside C_1 and separation of the mixture of methylated sugars on silica gel (with monitoring by paper chromatography) yielded trimethyl-D-xylose, which once again confirmed the terminal position of the xylose in the chain. The choice between 2,3,4- and 2,3,5-trimethyl-D-xylose was made in favor of the former on the basis of the following facts. The specific rotation of the trimethyl-D-xylose isolated agreed satisfactorily with that for 2,3,4-trimethyl-D-xylose [3]. The chromatographic behavior (R_g values) in various systems was completely identical with that for an authentic sample of 2,3,4-trimethyl-D-xylose. When the material isolated and an authentic sample were heated with 47% hydrobromic acid, one and the same demethylation products were obtained.

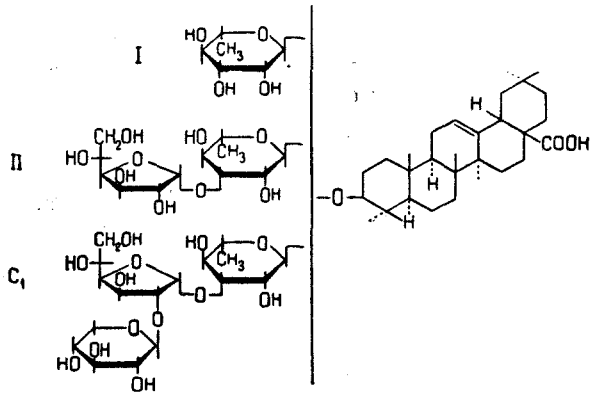
The glucose was isolated in the form of the trimethyl ether, the fact that this was a glucose derivative being shown by demethylation. The following variants of trimethylglucose are possible: 2,3,4-, 2,3,6-, 2,4,6-, and 3,4,6-trimethyl-D-glucose in the pyranose form, 2,3,5-, 2,3,6-, 2,5,6-, and 3,5,6-trimethyl-D-glucose in the furanose form. All trimethylglucoses with the pyranose ring are excluded since they have high positive specific rotations. Among the negatively rotating trimethylglucoses with a furanose ring, the choice became 3,5,6-trimethyl-D-glucose. Only in this trimethylglucose is there a free α-diol grouping, and therefore it must give a positive color reaction with Bonner's reagent [4], as was observed in our case. This specific rotation of the glucose derivative isolated was similar to that reported in the literature [5] for 3,5,6-trimethyl-D-glucose.

The third sugar—L-rhamnose—was isolated in the form of a dimethyl derivative. Decisive importance in the selection from the three (2,3-, 3,4-, and 2,4-) most probable dimethylrhamnoses was attached to the periodate oxidation of the carbohydrate chain of the glycoside C_1. It was found that only the rhamnose remained undestroyed. This is possible only where the rhamnose lacks a free α-diol grouping (three hydroxy groups occupied). Consequently, the dimethyl ether isolated is 2,4-dimethyl-L-rhamnose [6].

Thus, it follows from what has been said above that patrinoside C_1 is D-xylopyranosido(1 → 2)-D-glucofuranosido(1 → 3)-L-rhamnosido(1 → 3)oleanolic acid.

To establish the configuration of the glycosidic centers, we calculated the difference in the molecular rotations between patrinoside C_1 and the bioside (II), between the bioside (II) and the monooside (I), and between the monooside (I) and oleanolic acid by Klyne's rule [7].

As can be seen from the table, the xylose is connected to the glucose by a β-, the glucose to the rhamnose by an α-, and the rhamnose to the aglycone by an α-glycosidic bond. Consequently, the structures of the intermediate glycosides (I) and (II) and patrinoside C_1 are expressed by the following formulas:



Experimental

Paper of type M 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–methanol–water (10 : 2 : 3); 3) 1-butanol–acetic acid–water (5 : 1 : 4); 4) 1-butanol–acetic acid–water (5 : 4 : 1); 5) benzene–acetone–water (5 : 4 : 1); 6) ethyl acetate saturated with water; and 7) 1-butanol–ethanol–water (5 : 1 : 4). The sugars were revealed with aniline phthalate and p-anisidine, and the glycosides and their derivatives with antimony trichloride and concentrated sulfuric acid.

Glycosides of the monosaccharides	$[M]_D$, deg		Glycosides	$[M]_D$ deg	Δ C deg	form of the bond
	α	β				
Methyl D-xylopyranoside [8]	+253	−108	Patrinoside C_1	−86	−316	β
			Bioside II	+230	+442	α
Methyl D-glucofuranoside [9]	+230	−150	Monooside (I)	−212	−576	α
			Oleanolic acid	+364		
Methyl-L-rhamnopyranoside [10]	−111	+170				

Stepwise hydrolysis of patrinoside C_1. A mixture of 0.2 g of patrinoside C_1, 2 g of KU-2 ion-exchange resin (in the H^+ form), and 10 ml of a mixture of n-propanol and water (2 : 1) was boiled in the water bath for 20 hr. The resin was filtered off and washed with 10 ml of the above-mentioned mixture, and the filtrate was evaporated in vacuum. The residue was transferred to a column of 100 g of silica gel and eluted with chloroform (50-ml fractions) and then with ethyl acetate (20-ml fractions). The fractions were monitored on plates of silica gel in systems 1 and 2. The fractions eluted with chloroform (I–V) contained oleanolic acid and those eluted with ethyl acetate (VII–VIII) contained the monooside (12 mg), decomp. 255–259° C (from aqueous ethanol); $[\alpha]_D^{20}$ −35 ± 3° (c 2.2; ethanol). Found, %: C 71.50; H 9.67. Calculated for $C_{36}H_{58}O_7$, %: C 71.75; H 9.68.

When the monooside was heated with 5% sulfuric acid, rhamnose was identified in the hydrolysate by paper chromatography (systems 3 and 4). Fraction IX was a bioside (24 mg) with mp 229–231° C (from aqueous ethanol), $[\alpha]_D^{20}$ +30 ± 3° (c 2.7; ethanol). Found, %: C 65.74; H 8.64. Calculated for $C_{42}H_{68}O_{12}$, %: C 65.80; H 8.97.

On hydrolysis by the method described above, rhamnose and glucose were found.

Methylation of patrinoside C_1. Twenty grams of a ground barium oxide was added to a solution of 4.5 g of the substance in 40 ml of dimethylformamide and the mixture was heated to 90° C with vigorous stirring for 6 hr. Then another 2 g of BaO and, in drops, 15 ml of methyl iodide were added. After the beginning of the reaction, which was accompanied by vigorous effervescence and darkening, the reaction mixture was stirred and heated for another 8 hr. The precipitate was filtered off and the filtrate was treated with 300 ml of water and extracted with chloroform (5 × 300 ml). The chloroformic extracts were washed with $Na_2S_2O_3$ solution and with water and were evaporated. The residue was transferred to a column of silica gel (2 × 20 cm).

The following fractions were eluted: I (1.2 g)—200 ml of benzene, $[\alpha]_D^{20}$ −23 ± 3° (c 2.6; methanol). Found, %: C 65.30; H 9.29. Calculated for $C_{56}H_{94}O_{16}$, %: C 65.60; H 9.25; amorphous powder.

The IR spectra had no absorption bands in the 3200–3600-cm^{-1} region characteristic for OH groups. II (0.48 g)—660 ml of chloroform; and III (3.04 g)—200 ml of ethyl acetate.

Fractions II and III were methylated again by the method described above. The total yield of the full methyl ether of patrinoside C_1 after the reaction had been performed twice was 3.20 g.

Hydrolysis of the full methyl ether of patrinoside C_1. A solution of 2.14 g of the substance in 100 ml of methanol and 6 ml of concentrated hydrochloric acid was boiled in the water bath for 9 hr. Then 40 ml of water was added and the mixture was heated for another 3 hr. The methanol was distilled off and the methyl ester of oleanolic acid which precipitated was filtered off and recrystallized from 90% ethanol, mp 199–201° C. After the filtrate had been evaporated to dryness in vacuum, 0.85 g of a syrup consisting of a mixture of methylated monosaccharides was obtained.

Separation of the methylated monosaccharides. The mixture obtained in the preceding experiment was transferred to a column (2 × 48 cm) of silica gel and was eluted in system 5 with 20-ml fractions being taken, monitoring being carried out in a thin fixed layer of silica gel in system 6.

Fractions I–II contained traces of methyl oleanolate, and fractions III–IV contained 88 mg of 2,3,4-trimethyl-D-xylose, bp 110–112° C/0.5 mm; $[\alpha]_D^{20}$ +53 ± 3° (c 7.0; chloroform) and $[\alpha]_D^{20}$ +62 ± 3° (c 5.0; water). Literature data: $[\alpha]_D$ +55.8° (chloroform), $[\alpha]_D$ +64.5° (water) [3]. R_g 0.94 in system 7. Literature data: R_g 0.94 [11].

The product (5–7 mg) was heated in a sealed tube with 0.5 ml of 47% hydrobromic acid at 100° C for 5–7 min, the reaction mixture was diluted with 10 ml of water and evaporated to dryness in vacuum, and the residue was dissolved in 2–3 drops of aqueous ethanol and deposited on a paper chromatogram. A sample of 2,3,4-trimethyl-D-xylose was demethylated similarly. After the chromatogram had been run in system 7, spots with R_g 0.94, 0.78 (dimethylxylose), 0.52 (monomethylxylose), and 0.18 (xylose) were obtained. After chromatographic purification, fraction V contained 37 mg of 3,5,6-trimethyl-D-glucose; $[\alpha]_D^{20}$ −37 ± 3° (c 3.1; chloroform) [5]. On demethylation by the method described above, glucose was identified. Fractions VII–VIII consisted of 57 mg of 2,4-dimethyl-L-rhamnose, bp 85–95° C/0.5 mm; $[\alpha]_D^{20}$ +44 ± 3° (c 2.9; methanol) and $[\alpha]_D^{20}$ +20 ± 3° (c 5.7; water); R_g 0.75 in system 7. Literature data: $[\alpha]_D$ +42° (methanol) [6].

Periodate oxidation of patrinoside C_1. A solution of 30 mg of the substance in 10 ml of an acetate buffer with pH 4.5 [12] was treated with 18 mg of periodic acid, and the reaction mixture was left in the dark for 3 days. Then the excess of periodic acid was destroyed by the addition of a few drops of ethylene glycol, the solvent was distilled off in vacuum, and the oxidation products were extracted with 5 ml of ethanol. The solvent was evaporated and the residue was dissolved in 5 ml of 5% hydrochloric acid and heated at 100° C for 4 hr. Then the reaction mixture was neutralized with Dowex 1 × 4 ion-exchange resin, the resin was filtered off and washed with aqueous ethanol (50 ml), and the filtrate was evaporated in vacuum to 0.1–0.2 ml and deposited on a paper chromatogram. When the chromatogram was run with system 7, only L-rhamnose was identified.

Conclusions

It has been established that patrinoside C_1 is β-D-xylopyranosido(1 → 2)-α-D-glucofuranosido(1 → 3)-α-L-rhamnopyranosido(1 → 3)oleanolic acid.

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Arbuzov Institute of Organic and Physical Chemistry, AS USSR