GLYCOSIDES OF PATRINIA SIBIRICA. II

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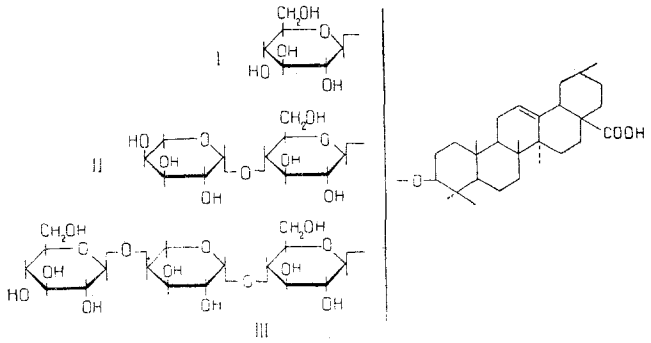
In a previous paper [1] we reported the isolation of two glycosides, sibirosides A and C, and suggested a total structural formula for the first one. It was also shown that sibiroside C is a hexaoside of hederagenin, whose carbohydrate moiety includes two monosaccharide units of arabinose and four of glucose. On being heated with dilute alkalies or on contact with an anion-exchanger it decomposes into an acid glycoside, desacylsibiroside C (III) (formula I). Inasmuch as there is a difference of 350 units between their molecular weights, it follows that the carboxyl of the original glycoside is joined to a trisaccharide. The other carbohydrate chain, consisting also of three monosaccharide units, is joined to the hederagenin hydroxyl. Upon further hydrolytic cleavage by dilute mineral acids, desacylsibiroside C decomposes into hederagenin, D-glucose and L-arabinose.



Densitometric measurements carried out on the paper chromatograms of the hydrolysate obtained showed that, in the carbohydrate chains of desacylsibiroside C, there are two glucose molecules to one of arabinose. In order to establish the sequence in which the monosaccharide links were attached, recourse was had to a partial hydrolysis of desacylsibiroside C and the isolation of the intermediate monoside (I) and the bioside (II) of hederagenin (see formula I). Since by acid hydrolysis I is split into aglycone and glucose, and II into aglycone, glucose, and arabinose, it is obvious that glucose is combined directly with the hydroxyl group of hederagenin, followed then by arabinose, and finally again by glucose at the end of the chain.

A further insight into the structure of the carbohydrate chain was provided by the results of methylation. The total methyl ester of desacylsibiroside C was synthesized by Kuhn's procedure [2], using IR-spectroscopy to check the completeness of methylation. The permethylate was decomposed by acids and the hydrolysate was submitted to paper chromatography. Using known markers, 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose and 2,3-di-O-methyl-L-arabinose were identified.

Gas-liquid chromatography confirmed the composition of the methylated carbohydrates. The permethylate was submitted to methanolysis and the mixture of methyl glycosides of methylated sugars was analyzed. The results mentioned previously did not permit the size of the oxide ring of the arabinose to be determined unambiguously. The formation of 2,3-dimethyl-L-arabinose may be explained in two ways: either the arabinose is in the pyranose form with a terminal glucose attached to its fourth hydroxyl group, or the arabinose has a furanose ring and is linked by the fifth hydroxyl. To settle this question the total methyl ester of the bioside (II), obtained by the partial hydrolysis of deacylsibiroside C, was synthesized. After subsequent hydrolysis of the permethylate, arabinose with a pyranose ring was identified.

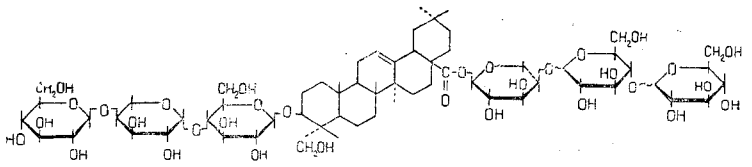
The total methyl ester of the original sibiroside C was obtained by an analogous procedure. Upon hydrolytic decomposition it yielded the same set of methylated sugars as the permethylate of desacylsibiroside C. This proves that the trisaccharides linked to the hydroxyl and carboxyl groups of hederagenin have a similar structure.

A further insight into the structure of the trisaccharide combined with the carboxyl group was afforded by the reductive decomposition of the permethylate of sibiroside C. The methylated trisaccharide which was split off by reduction with lithium aluminum hydride was submitted to hydrolysis with hydrochloric acid. In the hydrolysate only two reducing methylated monosaccharides–2,3,4,6-tetra-O-methylglucose and 2,3,6-tri-O-methylglucose–were identified, while 2,3-di-O-methylarabinose was not detected. Consequently the arabinose is directly linked to the carboxyl and underwent reduction. The corresponding 2,3-dimethylarabitol was detected in the paper chromatogram by means of periodate [3].

The configuration of the glycosidic centers in the trisaccharide combined with the hydroxyl group of hederagenin was calculated according to Klyne [4] (see table).

Substance	M	$[\alpha]_D$, degrees	$[M]_D$, degrees	Bond type
Desacylsibiroside C	929.1	−30±3	−279±28	—
Bioside (II)	766.1	+40±3	+306±23	—
Monoside (I)	634.7	−41±3	−260±19	—
Hederagenin	472.7	+80±3	+378±14	—
$[M]_D$ contribution of D-glucose in deacylsibiroside C	—	—	−585	β
$[M]_D$ contribution of L-arabinose in (II)	—	—	+566	β
$[M]_D$ contribution of D-glucose in (I)	—	—	−638	β
Methyl α-D-glucopyranoside (5)	194.2	+158.9	+309	—
Methyl β-D-glucopyranoside (5)	194.2	− 34.2	−66.4	—
Methyl α-L-arabopyranoside (6)	164.1	+ 17.3	+27.8	—
Methyl β-L-arabopyranoside (7)	164.1	+245.5	+394	—

The configuration of the glycosidic center of the arabinose combined with carboxyl will be similar to the configuration for glycoside A, inasmuch as the two glycosides are probably biogenetically related. Taking this circumstance into account, the complete structure of the glycoside may be represented as follows:



EXPERIMENTAL

Silica gel brand ASK, Volodarskii Leningrad factory paper, and solvent systems (1) butan-1-ol–acetic acid–water (4 : 1 : 5), (2) ethyl acetate–pyridine–water (2 : 1 : 2) and (3) butan-1-ol–ethanol–water (5 : 1 : 4) were employed in the chromatographic analysis.

Alkaline hydrolysis of sibiroside C. Exactly 0.3 g of the substance was dissolved in 5 ml of water and introduced int into a column (2 × 8 cm) filled with Dowex 1 × 4 ion-exchange resin (in the OH^- form) and allowed to stand for one day. Then the resin was washed with 3.6 *l* of water and 300 ml of 3% acetic acid. After evaporation of the solvent, 0.14 g of sugars and 0.133 g of deacylsibiroside C were obtained. This latter was introduced into a column (0.8 × 20 cm) filled with silica gel and was eluated with the ethyl acetate-methanol-water (10 : 2 : 3) system. After recrystallization from aqueous methanol, the product melted at 245–248° C, $[\alpha]_D^{20}$ − 30° (c 2.1; pyridine).

Found, %: C 60.66; H 8.60; mol. wt. 903 (by titration). Calculated for $C_{47}H_{76}O_{18}$, %: C 60.76; H 8.26; mol. wt. 929.1.

The product obtained (0.05 g) was dissolved in 10 ml of a methanol–water (2 : 1) mixture containing 5% of conc H_2SO_4, and was heated for 5 hr in a tube at 100° C. The reaction mixture was neutralized with barium hydroxide, the precipitate was filtered off and the solution was evaporated under vacuum. D-glucose and L-arabinose were identified in the residue by paper chromatography in systems 1 and 2. Densitometric measurement of the paper chromatogram in an EPJ-65 automatic densitometer established that the glucose and arabinose were in the ratio 2 : 1.

Stepwise hydrolysis of desacylsibiroside C. 0.6 g of the glycoside was dissolved in 20 ml of a mixture of isopropyl alcohol and water (2 : 1), containing 5% of H_2SO_4, and was heated on a boiling water-bath for 35 min. The

reaction mixture was neutralized with barium hydroxide, the barium sulfate was eliminated by centrifuging, 100 ml of water was added to the solution, and it was extracted with chloroform (4 × 30 ml). The chloroform extracts were combined, washed with water, and evaporated to dryness. The residue (0.45 g) was placed in a column (1.5 × 40 ml) with silica gel and eluated, 40-ml fractions being collected. Fractions 1–2 were eluted with chloroform; fractions 3–10 with the chloroform–ethyl acetate (1 : 1) system; fractions 11–15 with chloroform–methanol (10 : 1); and fractions 16–19 with methanol. Fractions 3–6 contained 0.095 g of hederagenin; fractions 7–9, 0.055 g of a mixture of aglycone and the monoside (I); fraction 10, 0.02 g of a mixture of the monoside (I) and the bioside (II); fractions 11–14, 0.09 g of the pure bioside (II); fraction 15, 0.03 g of the original glycoside; and fractions 16–19, 0.025 g of a mixture of sugars.

After a second purification, fractions 7–9 yielded the pure monoside (I), melting at 216–218 ° C, $[\alpha]_D^{20}$ – 41° (c 1.2; pyridine), which when heated with 5% HCl was decomposed into hederagenin and glucose. Fractions 11–14 were reprecipitated from their methanolic solution by acetone to obtain the pure bioside (II), melting at 198–200° C, $[\alpha]_D^{20}$ + 40° (c 0.8; pyridine). The bioside decomposed on acid hydrolysis into hederagenin, D-glucose, and L-arabinose.

Preparation of the total methyl ether of desacylsibiroside C. 0.15 g of the substance, 4 ml of methyl iodide, and 0.6 g silver oxide in 5 ml of dimethylformamide were heated for 10 hr in a tube in the water-bath. Then, in two portions, 0.2 ml of methyl iodide and 0.2 g of silver oxide were added, and the mixture was heated for another 5 hr. The reaction mass was poured into 50 ml water containing 0.3 g of sodium thiosulfate, and the product was extracted with chloroform (3 × 30 ml). After the solvent had been distilled off, the residue was introduced into a column (0.8 × 15 cm) of silica gel and the total methyl ester was eluted with 50 ml of chloroform. 0.075 g of product was obtained, the IR spectrum of which did not exhibit absorption bands corresponding to free hydroxyl groups.

Hydrolysis of the total methyl ether of deacylsibiroside C. A) 0.3 g of the product was dissolved in 1.5 of ml methanol containing 5% of hydrogen chloride, and was heated for 5 hr in a tube in the water bath. 0.5 ml of water was then added and the mixture heated for another 3 hr. Then, 30 ml of butyl alcohol was added to the reaction mixture, and the solvent was distilled off under vacuum. 2,3,4,6-Tetra-O-methylglucose (R_g 1.0), 2,3,6-tri-O-methylglucose (R_g 0.85) and 2,3-di-O-methylarabinose (R_g 0.66) were identified in the residue by means of paper chromatography in system 3, using known markers.

B) 0.035 g of the substance dissolved in 1.5 ml methanol containing 4% of hydrogen chloride was heated for 14 hr in an tube in the water bath. The solvent was distilled off under vacuum and the contents were distilled in a flanged flask at 140–160° C/2 mm. 0.015 g of a mixture of methyl ethers was obtained and was analyzed by gas-liquid chromatography on a Tsvet-1 apparatus with a flame-ionization detector. The analysis was carried out in a column (2 m, diameter 4mm) filled with Chromosorb W, on which 15% polyethyleneglycol adipate had been deposited.

The rate of flow of nitrogen was 30 ml/min. The retention time was measured with reference to methyl 2,3,4,6-tetra-O-methyl-β-D-glucoside, whose retention time was taken as unity. The chromatogram exhibited peaks with retention times 1 and 1.43 (β- and α-anomers of methyl 2,3,4,6-tetra-O-methyl-D-glucoside), 1.76 and 2 (α- and β-anomers of methyl 2,3-di-O-methyl-L-arabinoside) and 3.52 (methyl 2,3,6-tri-O-methyl-β-D-glucopyranoside) [8].

Total methyl ether of the bioside (II). 0.044 g of the bioside, 2 ml of methyl iodide, and 0.5 g of silver oxide in 3 ml of dimethylformamide were heated in a tube and treated further as described above. 0.025 g of the total methyl ether of the bioside was obtained. A portion of the product was heated with an aqueous-methanolic solution of 5% HCl. By means of paper chromatography in system 3, 2,3,4-tri-O-methyl-L-arabinose (R_g 0.83) and 2,3,6-tri-O-methyl-D-glucose were identified in the hydrolysate.

Another portion of the methyl ester was methylated and the methyl glycosides of the methylated sugars were analyzed by gas-liquid chromatography as described above. The chromatogram exhibited peaks with retention times of 1.03 corresponding to the β-anomer of methyl 2,3,4-tri-O-methyl-L-arabinoside and 3.50 corresponding to the β-anomer of methyl 2,3,6-tri-O-methyl-D-glucoside [8].

Preparation of the total methyl ether of sibiroside C. Methylation was applied to a mixture of 0.21 g of the glycoside, 3 ml of methyl iodide, 0.8 g of silver oxide and 4 ml of dimethylformamide. Methylation was performed in a tube as in the previous experiment. As a result, 0.09 g of total methyl ether in the form of an amorphous powder was obtained. It was submitted to hydrolysis and methanolysis by the method previously described. 2,3,4,6-tetra-O-methylglucose (R_g 1.0), 2,3,6-tri-O-methylglucose (R_g 0.85) and 2,3-di-O-methylarabinose (R_g 0.66) were

identified in the hydrolysate by paper chromatography using known samples.

Among the products of methanolysis the following substances were detected by gas-liquid chromatography, as previously described: the α- and β-anomers of methyl 2,3,4,6-tetra-O-methyl-D-glucoside and of methyl 2,3-di-O-methyl-L-arabopyranoside, as well as methyl 2,3,6-tri-O-methyl 2,3,6-tri-O-methyl β-D-glucopyranoside. The retention times were: 1 and 1.43; 1.76 and 2 and 3.52 respectively [8].

Reductive cleavage of the total methyl ether of sibiroside C by lithium aluminum hydride. 0.165 g of the substance was dissolved in 15 ml of absolute ether and gradually added to a suspension of 0.5 g of lithium aluminum hydride in 10 ml of absolute ether. The reaction mass was stirred at the boil for 2 hr. Then 5 ml of benzene was added drop by drop, with the ether being simultaneously distilled off. Then, 0.05 g of fresh lithium aluminum hydride was added and boiling was continued for another 5 hr. The excess of reducing agent was decomposed by the careful addition of 40 ml of a 3% acetic acid solution, and the product was extracted with chloroform (3 × 50 ml). After the solvent had been distilled off, there was left 0.14 g of solid residue, which was dissolved in 170 ml of chloroform and the solution was extracted with water (5 × 20 ml). After the water had been distilled off 0.04 g of reduced methylated trisaccharide was obtained, and evaporation of the chloroform yielded 0.12 g of reduced methylated desacylsibiroside C form. 0.01 g of methylated trisaccharide and 1 ml of a methanol–water (3 : 2) mixture containing 5% of HCl were heated for 7 hr in a sealed tube in the boiling water bath. The presence of 2,3,4,6-tetra-O-methylglucose (R_g 1.0) and of 2,3,6-tri-O-methylglucose (R_g 0.86) was detected in the hydrolysate by spraying the paper chromatograms with aniline phthalate. Some of the paper chromatograms, developed in system 3, were successively treated with a saturated solution of sodium periodate and then with a 0.1 M benzidine solution. 2,3-Dimethylarabitol (R_g 0.49) appeared as a white spot against a blue background.

CONCLUSIONS

It has been established that two trisaccharide chains are present in sibiroside C, one combined with the 3-hydroxyl and the other with the 28-carboxyl of hederagenin.

The first trisaccharide is β-D-glucopyranoside-(1-4)-β-L-arabopyranosido-(1-4)-β-D-glucopyranose, while the second is D-glycopyranosido-(1-4)-D-glucopyranosido-(1-4)-α-L-arabopyranose.

REFERENCES

1. V. G. Bukharov and V. V. Karlin, "Glycosides of Patrinia sibirica I," printed in this issue of KhPS [Chemistry [Chemistry of Natural Compounds], p. 60.
2. R. Kuhn, Ber., 88, 504, 1956; Ber., 89, 2514, 1956.
3. I. M. Hais and K. Macek eds., Paper Chromatography [Russian translation from the Czech], 725, Moscow, 1962.
4. W. Klyne, Biochem. J., 47, no. 4, XII, 1950.
5. E. Fischer, Ber., 28, 1156, 1895; T. Patterson and J. Robertson, J. Chem. Soc., 300, 1929.
6. E. Fischer, Ber., 26, 2400, 1893.
7. E. Purdie, Rose, J. Chem. Soc., 89, 1204, 1906.
8. G. O. Aspinal, J. Chem. Soc., 1676, 1963.

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