TRITERPENE GLYCOSIDES OF PATRINIA SCABIOSOFOLIA. I

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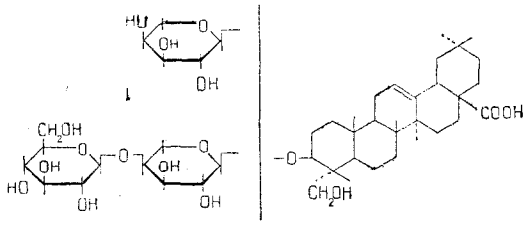
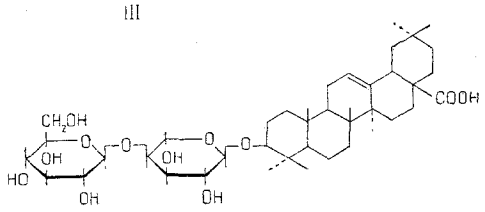
As one of us has shown [1], a methanolic extract of the roots of Patrinia scabiosofolia Fisch. et Link contains 12 substances giving a characteristic coloration with antimony trichloride.

This communication gives the results of an investigation of the low-molecular-weight glycosides and of a determination of their structure. By the method described previously, we obtained two extracts: one containing the low-polarity components of the mixture and the other the more polar components [1]. The latter was separated on a column of silica gel. The fractions enriches in the individual components were repurified. In this way we isolated and identified oleanolic acid, hederagenin, and β-sitosterol β-D-glucopyranoside. In addition to the free aglycones, we isolated three glycosides which we have called scabiosides A (I), B (II), and C (III).

Scabioside A is cleaved by acids into L-arabinose and hederagenin. This glycoside is acidic in nature and can be titrated with alkali. Its molecular weight corresponds to a monoside of hederagenin. From what has been said and from the results of methylation it follows that scabioside A is hederagenin 3-Larabopyranoside. Scabioside B hydrolyzes to form L-arabinose, D-glucose, and oleanolic acid. Both monosaccharide residues are attached to the hydroxyl of the aglycone, since the initial glycoside has its carboxyl group free. In order to establish which of the monosaccharides is attached directly to the genin, we carried out the stepwise hydrolysis of scabioside B and isolated an intermediate monoside (IV) which was cleaved by acid hydrolysis into the aglycone and arabinose.



III



II

Further information on the structure of the carbohydrate chain was obtained by the methylation of scabioside B. The full methyl ether obtained by Kuhn's method [2] decomposed on hydrolysis forming 2, 3, 4, 6-tetra-O-methyl-D-glucose and 2, 3-di-O-methyl-L-arabinose. The facts presented show that scabioside B is oleanolic acid 3-O-D-glycopyranosyl-(1 → 4)-L-arabopyranoside.

Scabioside C is cleaved by acids to form L-arabinose, D-glucose, and hederagenin. The carbohydrate chain is attached to the hydroxyl of the hederagenin, since the glycoside is acidic and titrates with alkali. The results of methylation show that the structure of the sugar chain in this glycoside is the same as in scabioside B and that it is hederagenin 3-O-D-glucopyranosyl-(1 → 4)-L-arabopyranoside (see formula).

In all the glycosides obtained, the configurations of the glycosidic centers were calculated by Klyne's rule [3] (table).

The contributions to [M] by each glycosidic center were determined from the molecular rotation differences, and

these were then compared with the values of the molecular rotations of methyl α- and β-L-arabinosides and -D-glucosides given in the literature.

Substance	M	$[\alpha]_D^{20}$, deg	$[M]_D^{20}$, deg	Form of the bond
β-Sitosterol glucopyranoside	576.83	−19±3	−109±17	—
Scabioside A	604.8	+38±3	+230±18	—
Scabioside B	750.9	− 7±3	− 52±23	—
Monoside IV	588.8	+38±3	+224±17	—
Scabioside C	766.19	+15±3	+115±22	—
β-Sitosterol	441.83	−37±3	−163±13	—
Hederagenin	472.7	+80±3	+378±14	—
Oleanolic acid	456.7	+82±3	+374±14	—
Contribution to $[M]_D$ of D-glucose in β-sitosterol glucopyranoside	—	—	+ 54	β
Contribution to [M] of L-arabinose in A	—	—	−148	α
Contribution to [M] of D-glucose in B	—	—	−276	β
Contribution to [M] of L-arabinose in IV	—	—	−150	α
Contribution to [M] of D-glucose in C	—	—	−150	β
Methyl α-L-arabopyranoside [4]	164.1	+ 17.3	+27.8	—
Methyl β-L-arabopyranoside [5]	164.1	+245.5	+394	—
Methyl α-D-glucopyranoside [6]	194.2	+158.9	+309	—
Methyl β-D-glucopyranoside [6]	194.2	− 34.2	−66.4	—

EXPERIMENTAL

Chromatography was carried out with silica gel of type ASK and paper of the Leningrad Volodarskii mill with the following systems of solvents: 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).

Chromatographic separation of the low-polarity extract. 4.2 g of the extract was deposited on a column (4.5 × × 20 cm) of silica gel and was subsequently eluted with the solvent systems chloroform (fractions 1–2, 40 ml each), chloroform–methanol (9:1) (fractions 3–16, 60 ml each), and chloroform–methanol (5:1) (fractions 17–19, 170 ml each). The process was monitored in a thin fixed layer of silica gel. Fractions 3–5 contained 0.82 g of material.

After crystallization from methanol, mp 306–308° C, $[\alpha]_D^{20}$ +74° (c 5; chloroform). From its chromatographic behavior and the results of a mixed melting point, the product was identical with an authentic sample of oleanolic acid. Fraction 6 (0.13 g) was recrystallized from methanol: mp 320–323° C, $[\alpha]_D^{20}$ +79° (c 3.6; pyridine). A mixture with an authentic sample of hederagenin gave no depression of the melting point. Acetyl derivative, mp 158–160° C; literature data, 156° C [7]. Methyl ester, mp 235–237° C; literature data, 240° C [7].

Fractions 8–10 (0.71 g) consisted of a mixture of β-sitosterol glucopyranoside and scabioside A; and fractions 11–19 (0.4 g) consisted of a mixture of scabiosides A, B, and C.

β-Sitosterol glucopyranoside. Fractions 8–10 were evaporated to dryness and extracted with 6 ml of methanol, and the residue was recrystallized from a mixture of methanol and chloroform. This gave 0.1 g of a compound with mp 276–279° C, $[\alpha]_D^{20}$ −19° (c 2.8; pyridine). The solution of 0.046 g of this product in a mixture of 3 ml of methanol, 1 ml of water, and 0.6 ml of HCl was heated in a sealed tube in the water bath for 5 hr. After cooling, the mixture deposited acicular crystals with mp 137–139° C, $[\alpha]_D^{20}$ −36° (c 2; chloroform), which gave a positive Liebermann-Burchard reaction for steroids. Their chromatographic behavior and a mixed melting point test showed that the crystals were identical with an authentic sample of β-sitosterol. The hydrolysate was shown by paper chromatography in systems 1 and 2 to contain D-glucose.

A mixture of this product (0.06 g), methyl iodide (2 ml), and barium oxide (0.5 g) in 3 ml of dimethylformamide was heated in a sealed tube in the boiling water bath for 30 hr. Then it was poured into 50 ml of a 1% solution of sodium thiosulfate and the mixture was extracted with chloroform (2 × 30 ml). After the separation of the solvent, the residue was heated with 1 ml of a 3% aqueous methanolic solution of HCl. In the hydrolysate 2, 3, 4, 6-tetra-O-methyl-D-glucose was identified by paper chromatography in system 3 together with an authentic sample.

Scabioside A. The methanolic extract from the preceding experiment was evaporated to dryness and the residue was recrystallized from aqueous methanol. A product with mp 216–219° C, $[\alpha]_D^{20}$ +38° (c 4.3; pyridine) was obtained. Found, %: C 67.64, 67.44; H 8.99, 9.1. Calculated for $C_{35}H_{56}O_8 \cdot H_2O$, %: C 67.48; H 9.3.

When the product was heated with HCl, L-arabinose was shown to be present in the hydrolysate by paper chromatography in systems 1 and 2 using the method described above. The aglycone that had deposited was identified as hederagenin. On titration with phenolphthalein, two 0.0588-g samples of the glycoside consumed 0.94 and 0.98 ml of 0.1 N caustic potash.

Found, mol. wt. 625 and 600. Calculated for $C_{35}H_{56}O_8$: 604.8.

Full methyl ether. A mixture of 0.058 g of scabioside A, 2 ml of methyl iodide, 1 g of barium oxide, and 3 ml of dimethylformamide was heated in a sealed tube in the water bath for 30 hr. The reaction mixture was diluted with 50 ml of water, the product was extracted with chloroform (2 × 30 ml), and the extracts were washed with sodium thiosulfate solution and with water and were evaporated to dryness. The residue was transferred to a column of silica gel (1.5 × 15 cm) and was eluted first with chloroform and then with methanol. The permethylated product (0.03 g) obtained after the evaporation of the chloroform was dissolved in a mixture of 0.7 ml of methanol, 0.3 ml of water, and 0.13 ml of conc HCl and was heated for 5 hr. The reaction mixture was diluted with 10 ml of butanol and evaporated in vacuum. The residue was transferred to a flanged flask, and the 2, 3, 4-trimethylarabinose was distilled at 160–170° C/2 mm. This gave a syrup, $[\alpha]_D^{20}$ +16.8°; (c 1.8, chloroform). Literature data: $[\alpha]_D^{20}$ +16.4° [5]. On a paper chromatogram in system 3, the substance appeared in the form of a spot with R_g 0.83. Literature data: R_g 0.83 [8]. The residue was recrystallized from aqueous methanol, giving 23-methoxyhederagenin with mp 227–229° C.

Chromatographic separation of the polar extract. 18.6 g of the extract was transferred to a column of silica gel (4.5 × 36 cm) and was eluted with chloroform–methanol (9 : 1) (fractions 1–5, 60 ml each), chloroform–methanol (5:1) (fractions 6–14, 120 ml each), and ethyl acetate–methanol–water (10 : 2 : 3) (fractions 15–19, 180 ml each). The process was monitored in a thin fixed layer of silica gel. Fractions 2–5 (2.35 g) consisted of a mixture of oleanolic acid, hederagenin, and β-sitosterol glucopyranoside, while fractions 6–14 (4 g) contained mainly scabiosides B and C, and fractions 15–19 (1.8 g) were enriched in polar glycosides.

The material from fractions 6–14 was transferred to a column of silica gel (2.5 × 45 cm) and was eluted with the chloroform–methanol (9 : 1) system, 30-ml fractions being collected. Fractions 4 and 5 (0.73 g) contained scabioside B, and fractions 10–12 (0.3 g) contained scabioside C in the pure state.

Scabioside B. After the recrystallization of fractions 4 and 5 from aqueous methanol, a compound with mp 210–212° C, $[\alpha]_D^{20}$ −7° (c 7.0; pyridine) was obtained.

Found, %: C 65.22; H 8.93. Mol. wt. 759 (alkalimetry). Calculated for $C_{41}H_{66}O_{12}$, %: C 65.57; H 8.85. Mol. wt. 750.9.

The product (0.1 g) was hydrolyzed by being heated in aqueous ethanolic solution with 5% HCl by the method described above. The hydrolysate was shown by paper chromatography in systems 1 and 2 to contain L-arabinose and D-glucose. The genin that deposited proved to be identical with oleanolic acid.

Partial hydrolysis. A solution of 0.02 g of scabioside B in 1 ml of a mixture of isopropanol and water (2 : 1) containing 5% of H_2SO_4 was separated into six parts which were placed in tubes and heated in the boiling water bath for 20, 25, 35, 40, and 45 min, respectively. After neutralization with barium hydroxide, the hydrolysates were studied by thin-layer chromatography on silica gel in the chloroform–methanol (20 : 3) system. The largest amount of intermediate glycoside was formed after 25–30 min. To obtain a supply of it, 0.06 g of scabioside B was heated as described above. The reaction mixture was diluted with 20 ml of water and the product was extracted with chloroform (2 × 15 ml), after which the extract was washed with water to neutrality and was evaporated. The residue was transferred to a column of silica gel (1.5 × 14 cm) and eluted with 30 ml of chloroform (giving 0.015 g of hederagenin) and with 30 ml of a mixture of chloroform and ethyl acetate (1 : 1) (giving 0.015 g of the monoside IV). The following fractions, eluted by chloroform–methanol (5 : 1), contained a mixture of the monoside IV and the initial scabioside, and the subsequent fractions contained unchanged scabioside B.

After recrystallization from methanol, the monoside had mp 272–275° C, $[\alpha]_D^{20}$ +38° (c 1.9; pyridine). On acid hydrolysis it formed oleanolic acid and arabinose.

Full methyl ether. A mixture of 0.065 g of scabioside B, 3 ml of methyl iodide, 2 g of barium oxide, and 3 ml of dimethylformamide was heated in a sealed tube in the water bath for 14 hr. Then the product was worked up and purified as described above. This gave 0.045 g of the permethylated derivative. When it was heated with a 5% aqueous

ethanolic solution of HCl, the hydrolysate was shown by paper chromatography in system 3, using authentic reference samples, to contain 2,3,4,6-tetra-O-methyl-D-glucose (R_g 1) and 2,3-di-O-methyl-L-arabinose (R_g 0.63).

The permethylated product (0.03 g) was heated in 1 ml of absolute methanol containing 4% of hydrogen chloride in a sealed tube in the boiling water bath for 14 hr. The solvent was distilled off in vacuum to dryness and the methyl glycosides of the methylated sugars were distilled from a flanged flask in vacuum at 140–160° C/2 mm. The distilled mixture (0.01 g) was analyzed on a Tsvet-1 gas-liquid chromatograph with a flame ionization detector with respect to relative retention times. The analysis was carried out on a column (2 m long and 4 mm in diameter) filled with Chromosorb W upon which 15% of poly(ethylene glycol adipate) had been deposited. The rate of flow of nitrogen was 30 ml/min. The retention times were measured in relation to the retention time of methyl β-2,3,4,6-tetra-O-methyl-D-glucoside. The chromatograms had peaks with retention times of 1 and 1.46 (methyl β- and α-2,3,4,6-tetra-O-methyl-D-glucosides), and 1.73 and 2.08 (the anomeric methyl 2,3-di-O-methyl-L-arabinosides). Literature data: 1 and 1.43, and 1.76 and 1.93, respectively [9].

Scabioside C. After the recrystallization of fractions 10–12 from aqueous methanol, a product was obtained with mp 216–219° C, $[\alpha]_D^{20}$ +15.5° (c 6.4; pyridine).

Found, %: C 62.75; H 8.77. Mol. wt. 770. Calculated for $C_{41}H_{66}O_{13} \cdot H_2O$, %: C 62.75; H 8.74. Mol. wt. 766.9.

The scabioside (0.15 g) was heated with 5% aqueous ethanolic HCl as described above. The hydrolysate was shown by paper chromatography to contain L-arabinose and D-glucose. The aglycone that had precipitated was shown to be identical with hederagenin.

Full methyl ether. A mixture of 0.06 g of scabioside C, 3 ml of methyl iodide, 2 g of barium oxide, and 3 ml of dimethylformamide was heated in a sealed tube and was worked up as described above. This gave 0.045 g of permethylated product.

Part of the product obtained was hydrolyzed and part was subjected to methanolysis as described previously. In the hydrolysate, 2,3,4,6-tetra-O-methyl-D-glucose (R_g 1) and 2,3-di-O-methyl-L-arabinose (R_g 0.62) were identified by paper chromatography in system 3 with reference samples.

By gas-liquid chromatography as described above, methyl β- and α-2,3,4,6-tetra-O-methyl-D-glucosides (retention times 1 and 1.4) and methyl α- and β-2,3-di-O-methyl-L-arabinosides (retention times 1.73 and 2.08) were identified.

CONCLUSIONS

From the fraction of the feebly polar saponins of Patrinia scabiosofolia Fisch. et Link oleanolic acid, hederagenin, β-sitosterol, β-D-glucopyranoside, and hederagenin 3-α-L-arabopyranoside have been isolated.

The polar fraction has yielded two triterpene glycosides. It has been established that one of them is oleanolic acid 3-O-β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranoside, and the other is hederagenin 3-O-β-D-glucopyranosyl-(1 → 4)-α-L-arabopyranoside.

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