TRITERPENE GLYCOSIDES OF HERNIARIA GLABRA. I

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Herniaria glabra L. (common burstwort) is a perennial herbaceous plant of the family Caryophyllaceae which has been little studied chemically. It is reported in the literature that it contains glycosides whose structure has not been established [1]. Thus, Hörhammer et al. isolated from this plant a saponin with mp 248–252° C possessing a pronounced hemolytic action. The authors suggested that the genin was quillaic acid.

We have studied H. glabra collected in the Tatar ASSR. Investigation of a methanolic extract of the whole plant on thin-layer chromatograms showed that it contains three glycosides, which we have called "glabrosides A, B, and C." The first of them is present in only small amount. By using, in one case partition chromatography on silica gel, and in the other gel filtration on Sephadex, the glycosides B and C were isolated in the individual state. On hydrolytic cleavage, they gave one and the same aglycone, but its properties, the constants of its derivatives, and its chromatographic behavior did not coincide with those for quillaic acid. The IR spectrum of the genin lacked the absorption band of an aldehyde group which is characteristic for quillaic acid.

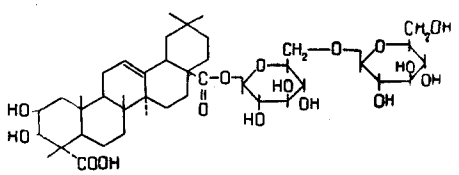
The glycone is oxidized by potassium periodate, forms a diacetyl derivative, and, according to titration, contains two carboxyl groups. On the basis of the results obtained and also by a direct comparison with an authentic sample, it was identified as medicagenic acid.

According to their molecular weights, glabroside B is a bioside and glabroside C a trioside of medicagenic acid. In addition to the aglycone, on acid hydrolysis the first glycoside gave two molecules of glucose, and the second glycoside gave D-glucose, D-fucose, and L-rhamnose.

The sugar chains in the glycosides are attached to a carboxyl of medicagenic acid, since the cleavage of the glabrosides to the aglycone also takes place on heating with alkali (substitution of only one carboxyl group, since the initial glabrosides are acidic and form monomethyl esters on treatment with diazomethane). It is known that in medicagenic acid only the carboxyl group in position 17 is capable of ring-closure to form a lactone. The glabrosides do not form lactones under lactonization conditions, and consequently the carbohydrate chains must be attached to this carboxyl group.

In order to determine the structure of the carbohydrate chains the completely etherified methyl ethers of the glabrosides were prepared. For this purpose, they were treated in the cold in a mixture of methyl iodide and dimethylformamide with sodium hydride [2]. In contrast to Kuhn's method, in this way it is possible by a single treatment to obtain the permethylated glycosides rapidly and in good yield (70–80%).

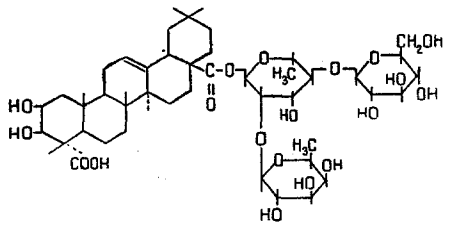
On subsequent hydrolysis, the permethylated glabroside B yielded 2,3,4-tri-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-glucose. Calculation of the configuration of the glycosidic centers by Klyne's method showed that both glucoses are connected by β-glycosidic bonds. The complete structure of glabroside B can be shown as follows.



Permethylated glabroside C was cleaved by acids to form 2,3,4,6-tetra-O-methyl-D-glucose 2,3,4-tri-O-methyl-D-fucose, and 3-O-methyl-L-rhamnose. The composition of the methylated sugars showed that the rhamnose is the center of the branching. To determine the position of the glucose and fucose bonds, partial hydrolysis of glabroside C on KU-2 ion-exchange resin was carried out, and the intermediate glucose-free progenin was isolated.

The completely etherified methyl ether of the latter yielded 2, 3, 4-tri-O-methyl-D-fucose and 3,4-di-O-methyl-L-rhamnose on hydrolysis. Consequently, the glucose is attached to the fourth and the fucose to the second hydroxyl of the L-rhamnose, while, according to calculations by Klyne's method, only the L-rhamnose has the α-configuration of the glycoside bond.

On the basis of what has been said above, glabroside C can be ascribed the following structural formula.



EXPERIMENTAL

Chromatography was carried out on Leningrad "M" and Schleicher und Schüll No. 2043 papers, on KSK silica gel, and on Sephadex G-25 with the following solvent systems: 1) butan-1-ol saturated with water, 2) butan-1-ol–acetic acid–water (5 : 1 : 4), 3) benzene–butan-1-ol–pyridine–water (10 : 50 : 30 : 30), 4) ether–ethyl acetate (3 : 1), 5) chloroform–ethyl acetate (3 : 1), and 6) butan-1-ol–ethanol–water (5 : 1 : 4).

Extraction of the plant. After preliminary defatting with chloroform, 1.7 kg of the air-dried plant was exhaustively extracted with methanol at the boil. This gave 250 g of extract, which was dissolved in 1.2 *l* of water and extracted with butan-1-ol (7 × 500 ml). After the solvent had been distilled off, 80 g of substance was left.

Glabroside B. The butanol extract (80 g) was deposited on a column of silica gel (1.5 kg) and eluted with butan-1-ol saturated with ammonia, 5-*l* portions being collected. Fractions 5–8 (18 g) were enriched in glabroside B. They were transferred to a column (3.3 × 50 cm) of silica gel and eluted with system 1; 0.35-*l* fractions were collected. After treatment with KU-2 cation-exchanger, fractions 4–14 contained chromatographically homogeneous glabroside B (2.5 g). Yield 0.2% of the wight of the raw material. Mp 240–245° C (from butan-1-ol), $[\alpha]_D^{20}$ +20° (c 1.0, pyridine). Mol wt 770 (by titration).

Found, %: C 58.15; H 7.97. Calculated for $C_{42}H_{66}O_{16} \cdot 2H_2O$., % C 58.50; H 8.18.

The acetate had mp 163–165° C (from aqueous ethanol), $[\alpha]_D^{20}$ +17° (c 2.5, chloroform).

Found, %: C 59.69; H 6.86. Calculated for $C_{60}H_{84}O_{25}$. %: C 59.80; H 6.98.

Acid hydrolysis of glabroside B. A solution of 0.55 g of glabroside B in 15 ml of 5% HCl was heated at 80–90° C for 6 hr. The precipitate was filtered off and dried in vacuo over P_2O_5. The filtrate was neutralized with AV-17 anion-exchange resin. D-Glucose was identified in it by paper chromatography (systems 2 and 3). The precipitate was deposited on a column of silica gel (12 g) and eluted in system 4. This gave 0.22 g of a product with mp 347–349° C (from ethanol), $[\alpha]_D^{20}$ + 113° (*c* 1.2, ethanol). Mol wt 505 (alkalimetry).

The aglycone gave no depression of the melting point with an authentic sample of medicagenic acid. The acetate had mp 206° C, $[\alpha]_D^{20}$ + 86° (*c* 1.1, chloroform). The acetate of the methyl ester had mp 222–224° C (from ethanol), $[\alpha]_D^{20}$ + 74° (*c* 1.0, chloroform). Literature data: genin, mp 340-350° C, $[\alpha]_D^{20}$ + 111° (ethanol) [3], acetate, mp 206–207° C, $[\alpha]_D^{20}$ + 87 ± 3° (chloroform) [3]; acetate of the methyl ester, mp 220–225° C, $[\alpha]_D$ + 73 ± 3.5° (chloroform) [4]. The results of analysis for the genin and its derivatives corresponded to the calculated figures.

Alkaline hydrolysis of glabroside B. A solution of 0.1 g of glabroside B in 10 ml of 5% KOH was heated in a tube at 90° C for 6 hr. The tube was opened and the contents were neutralized with KU-2 cation exchanger and extracted with butan-1-ol (5 × 30 ml). The extract was evaporated and the residue was crystallized from ethanol. The resulting product was identical with medicagenic acid in its constants and chromatographic behavior.

Permethyl ether of glabroside B. With stirring, a solution of 0.1 g of glabroside B in a mixture of 10 ml of

dimethylformamide and 2.5 ml of CH_3I was treated with 50 mg of NaH in two portions. After the evolution of hydrogen ended, the reaction mixture was heated to the boiling point of the methyl iodide and stirred for another hour. The reaction product was diluted twofold with water and was extracted with chloroform (3 × 20 ml). The extract was washed with water and dried over $MgSO_4$. The solvent was evaporated off to give 90 mg of crude product, which was transferred to a column of silica gel (15 g). The pure product was eluted in system 5. Yield 70 mg, $[\alpha]_D^{20} + 16^\circ$ (*c* 1,1; chloroform).

Found, %: C 64.95; H 8.69. Calculated for $C_{52}H_{86}O_{16}$, %: C 64.59; H 8.89.

The permethylate (70 mg) was heated with 10 ml of 2% HCl in methanol at 90° C for 6 hr. Then the reaction mixture was diluted twofold with water and heated for another hour. The hydrolysate was neutralized with AV-17 anion-exchange resin and evaporated. The mixture of methyl ethers of the sugars (2.2 mg) was separated preparatively on Schleicher und Schüll paper in system 6. This gave 9 mg of 2, 3, 4, 6-tetra-O-methyl-D-glucose with $[\alpha]_D^{20} + 78^\circ$ (*c* 1.12, acetone); literature data: $[\alpha]_D$ 83.9° (acetone) [5], and 7 mg of 2, 3, 4-tri-O-methyl-D-glucose with $[\alpha]_D + 66^\circ$ (1.0, methanol), R_g 0.85. Literature data: $[\alpha]_D + 69.1^\circ$ (methanol) [6], R_g 0.85 [7].

Attempt to obtain a lactone of glabroside B. A solution of 50 mg of glabroside B in 2 ml of conc acetic acid was treated with 2 ml of 47% HBr and the mixture was left at room temperature for 2 days and then diluted with 20 ml of water and heated for 5 hr. The precipitate was filtered off and dried. On chromatographic comparison with samples of medicagenic acid and its diacetyllactone, it coincided with the medicagenic acid.

Glabroside C. The aqueous solution, 16 g, after extraction with butan-1-ol was transferred to a column (5 × 65 cm) of Sephadex G-25 and was eluted with water, 100-ml fractions being collected. Fractions 1 and 2 contained chromatographically homogeneous glabroside C (1.1 g). Yield 0.8% of the weight of raw material, mp 254–257° C (from butan-1-ol). $[\alpha]_D^{20} + 23^\circ$ (*c* 1.45, pyridine), mol wt 997 (titration).

Found, %: C 56.83; H 7.95. Calculated for $C_{48}H_{76}O_{19} \cdot 3H_2O$, %: C 57.03; H 8.31.

The acetate of glabroside C had mp 174–176° C (from ethanol), $[\alpha]_D^{20} + 20^\circ$ (*c* 1.7, chloroform).

Found, %: C 59.58; H 6.94. Calculated for $C_{68}H_{96}O_{29}$, %: C 59.30; H 6.98.

The methyl ester of glabroside C was obtained by treating it with an ethereal solution of diazomethane, mp 228–231° C (from butan-1-ol), $[\alpha]_D^{20} + 21^\circ$ (*c* 1.3, pyridine).

Found, %: C 57.45; H 7.91. Calculated for $C_{49}H_{78}O_{19} \cdot 3H_2O$, %: C 57.42; H 8.31.

Acid hydrolysis of glabroside C. In a manner similar to that described above, 1.2 g of glabroside C yielded 0.4 g of medicagenic acid. The hydrolysate was shown by chromatography in systems 2 and 3 to contain D-glucose, D-fucose, and L-rhamnose. The densitometry of the chromatograms on an ERi-65 densitometer showed that the ratio of these substances was 1:1:1.

Alkaline hydrolysis of glabroside C. This was carried out in the same manner as for glabroside B. For 0.1 g of the substance, 40 mg of medicagenic acid was recovered.

Periodate oxidation of glabroside C. A solution of 50 mg of glabroside C in 1 ml of water was treated with 0.2 g of sodium periodate and 25 ml of acetate buffer (pH 3.6) and left at room temperature for a day. Then a few drops of ethylene glycol were added, the solution was evaporated, and the residue was heated with 2 ml of 5% HCl for 5 hr. After neutralization with AV-17 anion-exchange resin, L-rhamnose was identified in the hydrolysate by paper chromatography (systems 2 and 3).

Permethyl ether of glabroside C. This was obtained as described above. The reaction used 0.15 g of glabroside C, and 0.12 g of the completely etherified methyl ether with mp 89–93° C, $[\alpha]_D^{20} + 29^\circ$ (*c* 1.7, chloroform) was obtained.

Found, %: C 64.27; H 8.83. Calculated for $C_{59}H_{98}O_{19}$, %: C 63.78; H 8.83.

The permethylated product, 50 mg, was hydrolyzed in 5 ml of 2% HCl in methanol as described previously. The hydrolysate (35 ml) was separated preparatively on Schleicher und Schüll paper (58 × 60 cm) in system 6. This gave

6 mg of 2, 3, 4, 6-tetra-O-methyl-D-glucose with $[\alpha]_D^{20}$ + 79° (c 1.1, methanol), literature data: $[\alpha]_D$ + 83.9° (methanol) |5|; 10 mg of 2, 3, 4-tri-O-methyl-D-fucose with $[\alpha]_D^{20}$ + 133° (c 1.0, ethanol), Rg 0.88, literature data: $[\alpha]_D$ + 184° → + 128° (water) |8|, Rg 0.88 |9|; and 7 mg of 3-O-methyl-L rhamnose with $[\alpha]_D^{20}$ + 60° (c 1.0, ethanol), Rg 0.58, literature data: $[\alpha]_D$ + 57° (ethanol) |10|. The methyl glycoside of the latter was not oxidized by sodium periodate.

When all the methylated monosaccharides mentioned were demethylated by heating with 47.5% HBr, the corresponding monosaccharides were identified.

Partial hydrolysis of glabroside C. A solution of 0.5 g of the glycoside in 25 ml of water was treated with 5 g of KU-2 cation-exchange resin (in the H-form) and the mixture was heated at 90° C for 2 hr. The ion-exchange resin was filtered off and the solution was extracted with butan-1-ol. The butanol extract was evaporated (0.3 g), transferred to a column (1.5 × 15 cm) of silica gel, and eluted with system 1, 15-ml fractions being collected. Fractions 2–5 (50 mg) contained the chromatographically homogeneous progenin of glabroside C with mp 274–278° C (from butan-1-ol), $[\alpha]_D^{20}$ + 35° (*c* 1.0, pyridine). The progenin, 10 mg, was heated in 2 ml of 5% HCl for 5 hr. After neutralization of the hydrolysate, D-fucose and L-rhamnose were identified by paper chromatography in systems 2 and 3. The progenin (50 mg) was methylated as described above. After the methanolysis and hydrolysis of the progenin, preparative separation by paper chromatography yielded 8 mg of 2, 3, 4-tri-O-methyl-D-fucose and 7 mg of 3, 4-di-O-methyl-L-rhamnose with $[\alpha]_D^{20}$ + 8° (*c* 1.0, ethanol), R_g 0.84 (system 6). The test for oxidation with potassium periodate was positive. Literature data: $[\alpha]_D$ ± 0 → + 18.6° (ethanol) |11|, R_g 0.84 |7|.

CONCLUSIONS

From Herniaria glabra L. two triterpene glycosides, glabrosides B and C, have been isolated. We have shown that the first is the β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, and the second is the O-β-D-glucopyranosyl-(1→4)
O-β-D-fucopyranosyl-(1→2)⟩α-L-rhamnopyranoside-(1→17) of medicagenic acid.

REFERENCES

1. S. Szyszewska, Biul. Inst. rosl. leczn, 6, no. 2, 192, 1960. L. Horhammer, H. Wagner, and W. Probst, Nat., 47, 63, 1960.
2. U. E. Diner, F. Sweet, and R. R. Brown, Canad. J. Chem., 44, no. 13, 1591, 1966.
3. R. J. Morris and E. W. Hussey, J. Org. Chem., 30, no. 1, 166, 1965.
4. E. D. Walter, Y. K. Van Atta, C. R. Thompson, and W. D. Maclay, J. Am. Chem. Soc., 76, 2271, 1954.
5. W. N. Haworth, J. Chem. Soc., 2809, 1927.
6. J. C. Irwin and J. W. H. Oldham, J. Chem. Soc., 119, 1744, 1921.
7. I. M. Hais and K. Macek, Paper Chromatography [Russian translation], Moscow, p. 285, 1962.
8. O. T. Schmidt, W. Mayer, and A. Distelmaier, J. Am. Chem. Soc., 26, 555, 1943.
9. G. O. Aspinall, J. Chem. Soc., 1691, 1963.
10. O. T. Schmidt, E. Plakhenhorn, and F. Kubler, Ber., 75, 579, 1942.
11. P. S. Tipson and P. A. Levene, J. Biol. Chem., 130, 235, 1939.

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