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TRITERPENE GLYCOSIDES OF ALFALFA.

III. MEDICOSIDE I

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On the basis of chemical transformations and with the aid of physicochemical results, the structure of glycoside I isolated from the roots of the plant Medicago sativa has been established as hederagin 3-O-[O-α-L-arabinopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside] 28-O-β-D-glucopyranoside. Compound (I), $C_{52}H_{84}O_{22}$, mp 210-212°C, $[\alpha]_D^{21}$ +38.4° (c 1.48; methanol). Acid hydrolysis of (I) led to hederogenin (II) – $C_{30}H_{48}O_4$, mp 326-330°C, $[\alpha]_D^{23}$ + 84.2° (c 0.19; pyridine. The Hakomori methylation of glycoside (I) yielded the permethylate (IV) – $C_{65}H_{110}O_{22}$ $[\alpha]_D^{23}$ +41.6° (c 1.79; methanol). The GLC analysis of the products of the methanolysis of compound (IV) showed the presence of 3,4,6-tri-O-methyl-D-glucopyranose, 2,3,4,6-tetra-O-methyl-D-glucopyranose, 3,4-di-O-methyl-L-O-arabinopyranose, and 2,3,4-tri-o-methyl-L-arabinopyranose. The alkaline hydrolysis of glycoside I gave compound (III) with mp 230-233°C, $[\alpha]_D^{21}$ +35.2° (c 0.21; methanol), which was identified as medicoside C. Details of the PMR spectrum are given for compound (IV) and of the IR spectrum for compound (I).

We have continued a study of the triterpene glycosides of Medicago sativa L. (family Fabaceae). The column chromatography on silica gel of the combined triterpene glycosides from a new batch of the roots yielded medicagenic acid 3-O-β-D-glucopyranoside (substance A), medicoside C (substance C) and medicoside G (substance G) [1, 2], and also three more polar compounds which we have called medicosides I, J, and L. In the present paper we consider the determination of the structure of medicoside I (I).

The acid hydrolysis of medicoside I led to hederagenin (II), which was identified from its physicochemical constants and IR spectrum. It was established with the aid of gas-liquid chromatography (GLC) that compound (I) contained D-glucose and L-arabinose residues in equimolar amounts.

The IR spectrum of glycoside (I) contained absorption bands due to an ester group, which showed the presence of an acyloside chain. The alkaline hydrolysis of the medicoside gave compound (III), the GLC analysis of which showed the presence of D-glucose and L-arabinose residues in a ratio of 1:2. The physicochemical constants, spectral characteristics, and, and the R_f value on a thin-layer chromatogram (TLC) showed the identity of glycosides (III) and native medicoside C, isolated previously [2].

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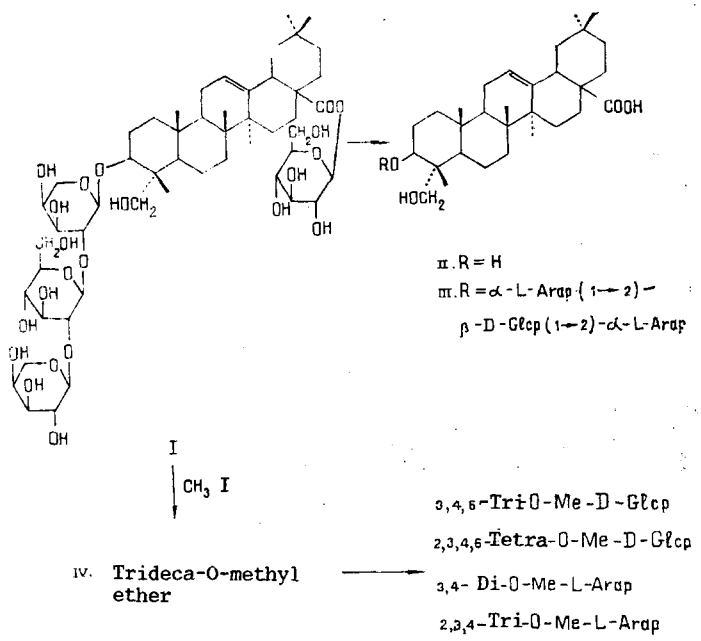

The Hakomori methylation [3] of medicoside I yielded the trideca-O-methyl ether (IV) (M^+ 1272). The PMR spectrum (C_5D_5N) of compound (IV) contained the doublets of four anomeric protons at 5.88 ppm (^3J = 6 Hz), 5.00 ppm (^3J = 5 Hz), 4.89 ppm (^3J = 5 Hz), and 4.65 ppm (^3J = 6 Hz). GLC analysis of the methanolysis products showed that the trideca-O-methyl ether (IV) contained 2,3,6-tri-O-methyl-D-glucopyranose, 2,3,4,6-tetra-O-methyl-D-glucopyranose, 3,4-di-O-methyl-L-arabinopyranose, and 2,3,4-tri-O-methyl-L-arabinopyranose residues.

The facts given permit the conclusion that medicoside I is a bisdesmosidic glycoside of hederagenin containing four sugar residues. Two L-arabinose residues and one D-glucose residue form a carbohydrate chain attached at C-3 of the aglycon as in medicoside C (III). The acyloside moiety of medicoside I is represented by a D-glucose residue. The signal of the anomeric proton of the latter in the PMR spectrum of the permethylate (IV) corresponds to the resonance at 5.88 ppm with ^3J = 6 Hz, which shows the β configuration of the anomeric center of the D-glucopyranose residue at C-28 [4].

Thus, medicoside I has the structure of hederagenin 3-O-[O-α-L-arabinopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside) 28-O-β-D-glucopyranoside.

EXPERIMENTAL

General Remarks. Silufol plates were used for TLC, and type L silica gel (Czechoslovakia) (63/100) for column chromatography. The triterpenoids were detected on the plates by spraying them with a 25% methanolic solution of tungstophosphoric acid followed by heating at 100°C for 5-10 min. The following solvent systems were used: 1) chloroform–methanol–water (65:23:4); 2) chloroform–methanol [a (10:1); b (50:1); c (100:1)]; and 3) benzene–acetone [a (5:1); b (20:1)].

PMR spectra were taken on a Tesla BS-567A instrument with HMDS as internal standard, δ scale.

GLC was performed on a Biokhrom-1 chromatograph using a capillary column (15 m) with the liquid phase OV-101. The temperature of the column was 150°C, that of the evaporator 180°C, and that of the detector 180°C, and the carrier gas was helium at a rate of flow of 4 ml/min. Sugars were chromatographed in the form of the trimethylsilyl esters of methyl glycosides, the relative retention times (T_{res}) were calculated in relation to methyl β-D-glucopyranoside. Methylated sugars were chromatographed in the form of their methyl glycosides and the trimethylsilyl ethers of partially methylated methylglycosides were also chromatographed under the same conditions; the relative retention times were calculated in relation to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside.

Isolation of the Glycosides. Alfalfa roots collected in December were dried and brought to the form of shavings. The raw material (25 kg) was treated with choroform at room temperature three times, and was then exhaustively extracted with hot methanol. The methanolic extract was evaporated to dryness. The dry residue was dissolved in water and the solution was extracted repeatedly with n-butanol. The butanolic extracts were combined and washed with water. After the solvent had been distilled off they yielded 480 g of purified combined triterpene glycosides (1.9%; here and below, yields are given on the air-dry raw material). The combined material obtained was transferred to a column and was eluted with chloroform and then with system 1. Elution with system 1 gave in the individual form the following substances (denoted in accordance with the chromatogram of an extraction performed previously [1]: A – 1.19 g (0.0048%); C – 2.49 g (0.0100%); G – 3.29 g (0.0132%); I – 5.78 g (0.0231%); J – 2.59 g (0.0104%); and L – 2.91 g (0.0120%). Caulosaponin B (substance B) was not obtained in this new extraction.

Medicoside I (substance I, (I)). $C_{52}H_{84}O_{22}$, mp 210-212°C $[\alpha]_D^{21}$ + 38.4 ± 2° (c 1.48; methanol. ν_{max}^{KBr} (cm^{-1}): 3580-3240, 1740, 1265.

GLC Analysis of the Sugar Residues in Medicoside I (I). A solution of 5 mg of glycoside (I) in 5 ml of a 7% solution of hydrogen chloride in absolute methanol was boiled for 5 h. Then it was neutralized with Ag_2CO_3, the precipitate was filtered off, and the filtrate was evaporated. The residue was dissolved in 0.8 ml of pyridine, and to the resulting solution were added 0.2 ml of hexamethyldisilazane and 0.1 ml of trichlorosilane. The mixture was left for 12 h, and then the solvent was distilled off and the residue was dissolved in hexane and applied to a chromatograph. The sugars were identified by comparison with authentic samples. The mixture was found to contain D-glucose (T_{rel} = 1.00; 0.91) and L-arabinose (T_{rel} = 0.20; 0.19) in a ratio of 1.0:0.9.

Hederagenin (II) from (I). A solution of 1.550 mg of medicoside I in 30 ml of a 2% solution of sulfuric acid in methanol was boiled for one hour. Then the reaction mixture was diluted with 30 ml of water and the methanol was distilled off. The precipitate that deposited (260 mg) was transferred to a column and, on elution with system 2b, 115 mg of hederagenin (II) was obtained: $C_{30}H_{48}O_4$, mp 326-330°C (from chloroform–methanol (50:1)), $[\alpha]_D^{23}$ +84.2 ± 2° (c 0.19; pyridine).

Medicoside C (III) from (I). A solution of 1.260 mg of medicoside I in 10 ml of water was treated with 320 mg of KOH, and the mixture was heated at 100°C for one hour. after which it was neutralized with KU-1 cation-exchange resin. The resin was filtered off, the filtrate was evaporated and the dry residue was chromatographed on a column in system 1. This gave 61 mg of the glycoside (III) with mp 230-233°C (from methanol), $[\alpha]_D^{21}$ + 35.2 ± 2° (c 0.21; methanol), the GLC of which(performed in a similar manner to the analysis of medicoside I) revealed the presence of D-glucose and L-arabinose residues in a ratio of 1.0:2.1. This glycoside was also identified as medicoside C [2] by its spectral characteristics and by TLC (system 1) in comparison with an authentic sample.

The Trideca-O-Methyl Derivative (IV) from (I). A solution of 1.950 mg of medicoside I in 100 ml of dimethyl sulfoxide was treated with 1 g of sodium hydride. The mixture was stirred for one hour, and then 15 ml of methyl iodide was added dropwise over 15 min and the mixture was stirred for another 2 h. Then it was poured into 400 ml of a 2% solution of sodium thiosulfate and the reaction product was extracted with chloroform. The chloroform extracts were evaporated. The dry residue was chromatographed on a column, and elution by system 3a yielded 180 mg of the amorphous trideca-O-methyl ether (IV), $C_{65}H_{110}O_{22}$ $[\alpha]_D^{23}$ + 41.6 ± 2° (c 1.79; methanol), M^+ 1272, in the IR spectrum of which there was no absorption in the region of hydroxy groups. PMR spectrum (C_5D_5N, ppm): 0.8-1.04 (6 × CH_3); 3.18-3.60 (13 × $OCH)_3$); 4.65 (1 H, d, ^3J = 6 Hz; anomeric proton of a D-glucopyranose residue): 4.89 (1 H, d, ^3J = 5 Hz; anomeric proton of the L-arabinose residue); 5.00 (1 H, d, ^3J = 5 Hz; anomeric proton of a L-arabinopyranose residue); 5.30 (1 H, broadened singlet, H-12); 5.88 (1 H, d, ^3J = 6 Hz; anomeric proton of the D-glucopyranose residue at C-28). PMR spectrum ($CDCl_3$, ppm): 0.64-1.30 (6 × CH_3); 3.25-3.58 (13 × OCH_3); 4.83 (1 H, d, ^3J = 5 Hz; anomeric proton of a D-glucopyranose residue); 4.60 (2 H, d, ^3J = 7 Hz; anomeric protons of L-arabinopyranose residues); 5.30 (2 H, m, anomeric proton of a D-glucopyranose residue and H-12).

GLC Analysis of the Methylated Monosaccharides Obtained from the Trideca-O-methyl Ether (IV). The analysis was performed similarly to that described for medicoside I. In compound

(IV) were detected residues of 3,4,6-tri-O-methyl-D-glucopyranose (T_{rel} = 2.04; 1.92); 2,3,4, 6-tetra-O-methyl-D-glucopyranose (T_{rel} = 1.26; 1.00); 3,4-di-O-methyl-L-arabinopyranose (T_{rel} = 0.96; 0.86); and 2,3,4-tri-O-methyl-L-arabinopyranose (T_{rel} = 0.58).

SUMMARY

The roots of Medicago sativa L. (family Fabaceae) have yielded a new triterpeneglycoside – medicoside I, for which the structure of hederagenin 3-O-[O-α-L-arabinopyranosyl)-(1 → 2)-O-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside] 28-O-β-D-glucopyranoside has been established.

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TRITERPENE GLYCOSIDES OF ALFALFA.

IV. MEDICOSIDE J.

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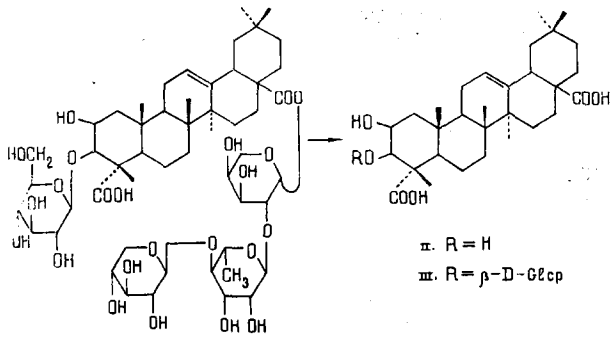
A new triterpene glycoside has been isolated from the roots of Medicago sativa L. (family Fabaceae) – medicoside J, and its structure has been established as a medicagenic acid 3-O-β-D-glucopyranoside 28-O-[O-β-D-xylopyranosyl-(1 → 4)-O-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside].

Continuing a study of the triperpene glycosides of Medicago sativa L (family Fabacaeae) [1-3], we have established the structure of medicoside J (I) isolated previously [3] – one of the main components of the saponin fraction of alfalfa roots.

The acid hydrolysis of glycoside (I) led to medicagenic acid (II). It was established with the aid of GLC that compound (I) contained D-glucose, D-xylose, L-arabinose, and L-rhamnose residues in a ratio of 1:1:1:1.



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