TRITERPENE GLYCOSIDES OF ALFALFA.

II. MEDICOSIDES C

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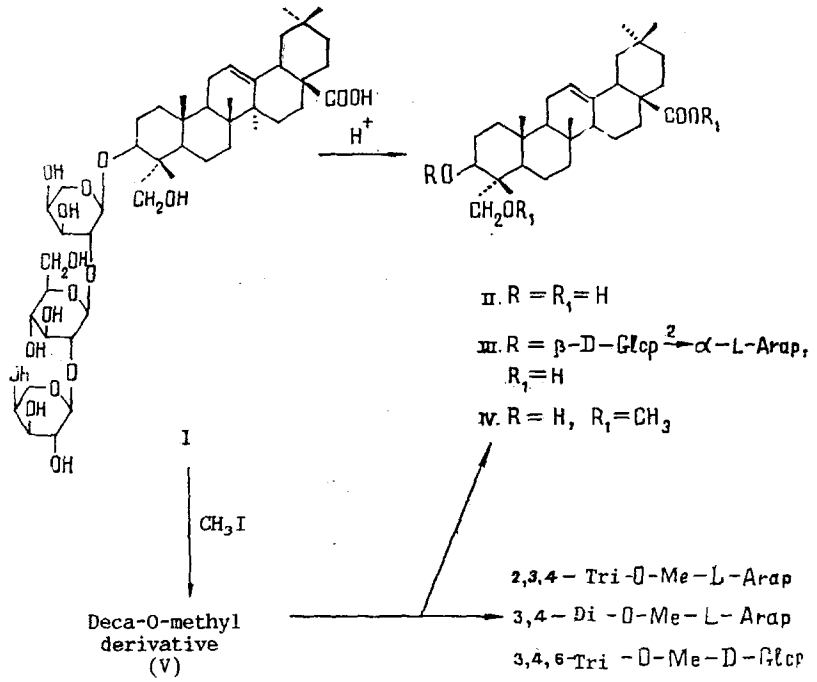
The roots of the plant Medicago sativa (family Fabaceae) have yielded, in addition to the medicoside G described previously, two more triterpeneglycosides, caulosaponin B and the new glycoside medicoside C. The latter has the structure of hederagenin 3-O-[α-L-arabinoyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-α-L-arabinpyranoside].

Continuing the study of the triterpene glycosides of Medicago sativa L. (family Fabaceae), from the roots of the plants we have isolated two compounds earlier designated as substances B and C [1]. Hederagenin (II) was isolated from the products of the acid hydrolysis of both substances. In the carbohydrate fraction of the hydrolysates, D-glucose and L-arabinose were detected by thin-layer chromatography (TLC). It was shown with the aid of gas-liquid chromatography (GLC) [2] that compounds B (III) and C (I) contained D-glucose and L-arabinose in ratios of 1:1 and 1:2, respectively.



In its physicochemical constants and spectral characteristics, glycosides B (III) was identical with hederagenin 3-O-[β-D-glucopyranosyl)-(1 → 2)-α-L-arabinopyranoside]. This diglycoside was first isolated from Caulophyllum robustum Maximim (family Berberidaceae) and was described under the name of caulosaponin B [3]. It was subsequently found in Caltha silvestris Worosch. (family Ranunculaceae) as calthoside D [4], in Akebia quinata Decne (family Lardizabalaceae) as saponin C [5] and as saponin P_F [6].

Glycoside C proved to be new and we have called it medicoside C (I). The partial hydrolysis of medicoside C led to hederagenin (II) and a progenin (III) identical with glycoside B (scheme). To determine the position of the L-arabinose residue, medicoside was methylated by Hakomori's method [7]. Acid hydrolysis of the deca-O-methyl derivative

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(V) obtained (M^+ 1038) led to the 23,28-dimethyl derivative of hederagenin (IV). It was established by the GLC method [8] that the carbohydrate chain of compound (V) consisted of residues of 2,3,4-tri-O-methyl-L-arabinopyranose, 3,4,6-tri-O-methyl-D-glucopyranose, and 3,4-di-O-methyl-L-arabinopyranose.

The facts given indicate that medicoside is a monodesmoside with an unbranched carbohydrate chain and a terminal L-arabinopyranose residue attached to the D-glucose residue at C-2". A calculation of molecular rotation differences [9] led to the conclusion that the terminal L-arabinose residue was attached to D-glucose residue by an α-glycosidic bond. The PMR spectra of medicoside C (I) and of the deca-O-methyl derivative (V) (Table 1) showed the signals of anomeric protons with spin-spin coupling constants of 5.0-7.5 Hz, which confirmed the β configuration of the D-glucopyranose residue and the α configuration of the L-arabinopyranose residue [10].

On the basis of the facts presented, medicoside C (I) has the structure of hederagenin 3-O-[α-L-arabinopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside].

EXPERIMENTAL

For general observations, see [1]. The following solvent systems were used: 1) chloroform–methanol–water (a – (65:23:4); b–(35:8:1)); 2) chloroform–methanol (a – (50:1); b – (20:1)); 3) benzene–acetone (5:1); and 4) butanol–methanol–water (5:3:1). Sugars were chromatographed in a thin layer of silica gel impregnated with a 0.3 M solution of sodium dihydrogen phosphate, and on Silufol plates.

Isolation of the Glycosides. The fractions containing glycosides B and C [1] were rechromatographed on a column using system 1a. Substances B and C were isolated (50 mg, 0.001%, and 750 mg, 0.002%, respectively, calculated on the air-dry raw material).

Hederagenin 3-O-[β-D-Glucopyranosyl-(1 → 2)-α-L-arabinopyranoside] (Substance B, III). $C_{41}H_{66}O_{13}$, mp 249-251°C (from methanol $[\alpha]_D$ + 41.2 ± 2° (c 0.56; dimethyl sulfoxide). According to the literature [4]: mp 248-253°C (from butanol), $[\alpha]_D$ +39.2 ± 0.5° (c 0.96; dimethyl sulfoxide).

Medicoside C (Substance C, I). $C_{46}H_{74}O_{17}$, mp 227-229°C (from methanol), $[\alpha]_D^{21}$ +34.0 ± 2° (c 0.41; ethanol); ν_{max}^{KBr} (cm^{-1}): 3200-3590 (OH); 1710 (C=O of a carboxy group).

Hederagenin (II) from (I) and (III). A solution of 250 mg of medicoside C (I) in 50 ml of methanol was treated with 0.5 ml of a 20% solution of sulfuric acid in ethanol, and the mixture was heated on the boiling water bath for 5 h. Then 50 ml of water was added to the solution and the methanol was distilled off. The precipitate that had deposited was filtered off, washed with water, and dried. Then it was chromatographed on a column using system 2a. This led to the isolation of 25 mg of hederagenin (II), $C_{30}H_{48}O_4$, mp 330-333°C, $[\alpha]_D^{21}$ + 84.0 ± 2° (c 0.20; pyridine).

The filtrate was neutralized with barium carbonate and the precipitate was separated off, after which D-glucose and L-arabinose were detected in the residual solution by TLC with authentic samples in system 4.

The hydrolysis of 20 mg of substance B (III) was carried out in the same way, with the isolation of 5 mg of hederagenin, which was shown to be identical with an authentic sample from its R_f value in system 2b. D-Glucose and L-arabinose were detected in the hydrolysate.

Hederagenin 3-O-[β-D-Glucopyranosyl-(1 → 2)-α-L-arabinopyranoside] (III) from (I). A solution of 210 mg of medicoside C (III) in 40 ml of a 0.5% solution of sulfuric acid in methanol was heated on the boiling water bath for 1 hour and was then diluted with 40 ml of water and the methanol was distilled off. The precipitate that had deposited was filtered off and washed with water and the residue (95 mg) was chromatographed in a column in system 1b. This gave 40 ml of caulosaponin B [3] (calthoside D [4]) with mp 247-249°C (from methanol), m/z (%): $[\alpha]_D^{21}$ +43.4±2° (*c* 0.50; dimethyl sulfoxide).

The Deca-O-methyl Derivative (V) from (I). A solution of 50 mg of medicoside C (I) in 5 ml of dimethyl sulfoxide was treated with 50 mg of sodium hydride. The mixture was stirred for 1 hour, and then 1 ml of methyl iodide was added dropwise over 10 min and stirring was continued for another hour. Then the reaction mixture was poured into 20 ml of a 2% solution

TABLE 1. Chemical Shifts of the Protons of Medicoside C (I) and of Its Derivatives (δ, ppm, O − HMDS)

Compound	Positions of the protons						
	H-3	H-12	2H-23	H-1′	H-1‴	H-1″	CH_3 and OCH_3 groups
I	4,06 m*	5,25 m		5,05d×2 ^3J=6,3 Hz		4,29 d ^3J=5,0 Hz	0,79×3; 0,83×2; 1 05
II		5 38 m	4,04d* ^2J=10,0 Hz 3,59 d ^2J=10,0 Hz	—	—	—	0,81; 0 83; 0,85; 0,91×2; 1,10
III		5,30 m		4,97 d ^3J=7 5Hz	—	4,23 d ^3J=6 3 Hz	0 79×2; 0,87×3; 1,08
IV	3,88t ΣJ=18,0 Hz	5,26 m	3,50 d ^2J=10,0 Hz 3 07d ^2J=10 0 Hz	—	—	—	0,70; 0,74; 0,76; 0,80×2; 1,00 3,14; 3,58
V		[5,18 m]		[4,59d; 4 51 d ^3J=7,5 Hz		[4,30 d ^3J=5 0 Hz]	[0,64×2; 0,86×3; 1,07; 3,24×2; 3,31; 3,34; 3,39×2; 3,43; 3,50; 3,55; 3,58]

*The spectra were recorded in C_5D_5N or in $CDCl_3$ (indices given in square brackets). The signals marked with asterisks are superposed on one another. The signals of the methyl groups are singlets; for the other signals: d − doublet; t − triplet; m − multiplet.

of sodium thiosulfate and the reaction product was extracted with chloroform. The chloroform extracts were combined, washed with water, and evaporated. The dry residue (75 mg) was chromatographed in system 3. This yielded 41 mg of the amorphous deca-O-methyl derivative (V), $C_{56}H_{94}O_{17}$. There was no absorption in the hydroxy group region of the IR spectrum. Mass spectrum, m/z (%): 1038 (M^+ 0.03), 507(1), 483(48), 451(24), 423(9), 409(9), 391(9), 379(66), 365(18), 347(100), 315(15), 301(6); 269(15), 262(34), 220(18), 203(72), 189(37), 175(82), 157(20), 143(25), 115(25).

After methanolysis of compound (V), analysis of the methylated and partially methylated methyl glycosides obtained was carried out by GLC [8]. 2,3,4-Tri-O-methyl-L-arabinose, 3,4,6-tri-O-methyl-D-glucose, and 3,4-di-O-methyl-L-arabinose were detected.

The 23,28-Di-O-methyl Derivative of Hederagenin (IV) from (V). A solution of 24 mg of the deca-O-methyl derivative (V) in 9 ml of methanol was treated with 3 ml of a 20% solution of sulfuric acid in methanol, and the mixture was heated in the boiling water bath for 5 h. Then 15 ml of water was added to the solution, the methanol was evaporated off, and the precipitated that had deposited was filtered off, washed with water, and dried. The residue obtained (14 mg) was chromatographed on a column using chloroform. This led to the isolation of 7.9 mg of the 23,28-dimethyl derivative of hederagenin (IV), $C_{32}H_{52}O_4$, mp 190-192°C (from methanol), $[\alpha]_D^{21}$ +78.3 ± 2° (c 0.20; benzene). Mass spectrum, m/z (%): 500 (M^+, 84), 482(79), 440(76), 395(100), 263(100), 262(100), 203(99), 189(99), 187(100), 175(100), 173(80), 169(47), 161(71), 147(27), 133(91), 121(64), 119(64), 105(68). According to the literature [4]: mp 191-193°C (from ethanol), $[\alpha]_D^{20}$ +76 ± 3° (c 0.38; benzene).

SUMMARY

From the roots of Medicago sativa (family Fabaceae), in addition to the medicoside G described previously, two triterpene glycosides have been isolated: caulosaponin B and a new glycoside – medicoside C. The latter has the structure of hederagenin 3-O-[α-L-arabinopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside].

LITERATURE CITED

1. A. É. Timbekova and N. K. Abubakirov, Khim. Prir. Soedin., 451 (1984).
2. T. T. Gorovits, Khim. Prir. Soedin, 263 (1970).
3. T. Murakami, M. Nagasawa, S. Urayama, and T.Satake, Yakugaku Zasshi, 88, 321 (1968). Chem. Abs., 69, 67661 (1968).
4. L. I. Strigina, T. M. Remennikova, A. P. Shchedrin, and G. B. Elyakov, Khim. Prir. Soedin, 303 (1972).
5. R. Higuchi, K. Miyakava, and T. Kawasaki, Chem. Pharm. Bull. (Tokyo), 20, 1935 (1972).
6. R. Higuchi and T. Kawasaki, Chem. Pharm. Bull. (Tokyo), 24, 1021 (1976).
7. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
8. G. O. Aspinall, J. Chem. Soc., 1676 (1963).
9. W. Klyne, Biochem. J., 47, xli (1950).
10. J. M. van der Veen, J. Org. Chem., 28, 564 (1963).