TRITERPENE GLYCOSIDES OF ALFALFA.

V. MEDICOSIDE H

A. É. Timbekova, M. F. Larin, M. R. Yagudaev, and N. K. Abubakirov

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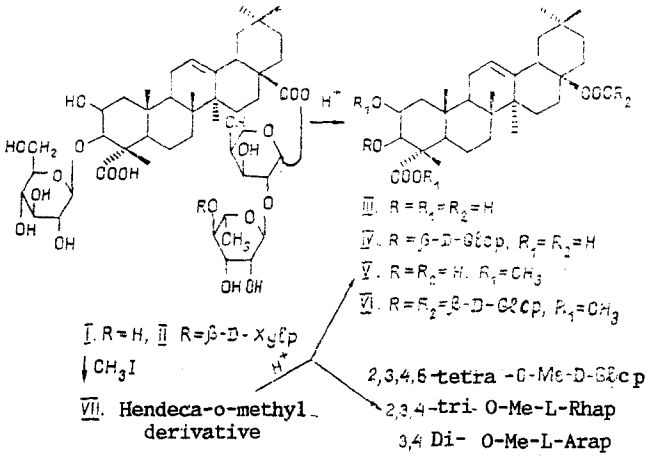
A new triterpene glycoside – medicoside H – has been isolated from the roots of Medicago sativa L. (Fabaceae), and on the basis of chemical transformations and spectral characteristics its structure has been established as medicagenic acid 3-O-β-D-glucopyranoside 28-O-[O-α-L-rhamnopyronosyl-(1 → 2)-β-L-arabinopyranoside].

Continuing a study of the triterpene glycosides of alfalfa (Medicago sativa L. (Fabaceae) [1-4], we have established the structure of a new compound isolated from the roots of this plant. Substance (I), which has been called medicoside H, was isolated as the result of the repeated rechromatography of fractions obtained previously [3] together with substances A, C, G, I, J, and L.

Under the action of mineral acids, medicoside H (I) formed medicagenic acid (III) and medicagenic acid 3-O-β-D-glucopyranoside. On the basis of the results of an analysis of the monosaccharides by the GLC method [5] it was established that compound (I) contained residues of D-glucose, L-arabinose, and L-rhamnose in equimolar ratio.

The Hakomori methylation [6] of medicoside H gave a hendeca-O-methyl derivative (VII) (M^+ 1096). The qualitative composition of the methylated monosaccharides obtained by the methanolysis of the ester (VII), determined by Aspinall's method [7] included 2,3,4,6-tetra-O-methyl-D-glucopyranose, 2,3,4-tri-O-methyl-L-rhamnopyranose, and 3,4-di-O-methyl-L-arabinopyranose.

The PMR spectrum of the ester (VII) showed the doublet signals of three anomeric protons; of D-glucopyranose at 4.39 ppm (^3J = 7 Hz), of L-rhamnopyranose at 5.47 ppm (^3J = 2 Hz), and of L-arabinopyranose at 6.09 ppm (^3J = 3 Hz).



The permethylate (VII) was subjected to acid hydrolysis and from the reaction products the 2,23-di-O-methyl derivative of medicagenic acid (V) was isolated.

On the basis of what has been said above, medicoside H is a bisdesmosidic glycoside. The D-glucose residue is attached at C-3 and a residue of the disaccharide O-L-rhamnopyranosyl-(1 → 2)-L-arabinopyranoside at C-28 of medicagenic acid. The spin–spin coupling con-

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TABLE 1. Chemical Shifts of the Carbon Atoms of Medicosides H (I), J (II), and G (VI), and of Medicagenic Acid 3-O-β-D-Glucopyranoside (IV) (C_5D_5N, δ, ppm, 0 – TMS)

C Atom	Compound I	II	IV	VI	C Atom		Compound I	II	IV	VI
Aglycon 1	44,12	44,40	44,14	44,14	3-O-Glcp	1	105,25	105 38	105,35	105,14
2	70,29	70,41	70,25	70,08		2	75,14*	75,37	75,12	74 96
3	86,05	86,30	85,96	85,85		3	78,28**	78,40*	78,32*	78,10*
4	52,81	53,02	52,81	52,70		4	71,48	71,76	71,49	71,28
5	52,70	52,56	52,43	52,26		5	78,28**	78,40*	78,32*	78,10*
6	21,02	21,36	21,17	21,00		6	63,12	62,89	62,66	62,45
7	33,17*	33,26*	33,25*	32,98*	28-O-Arap	1	93,52	93,59		
8	40,26	40,53	40,13	40,18		2	75,14*	75,54		
9	48,70	48,90	48,60	48,58		3	66,26[a]	66,13[a]		
10	36,83	36,99	36,82	36,66		4	70,29[a]***	70,03[a]		
11	23,68**	24,15	23,77**	23,55**		5	63,70	63,03		
12	122,65	123,14	122,65	122,65	Rhap	1	101,36	101,24		
13	144,24	144,41	144,79	143,98		2	72,90	72,74		
14	42,28	42,46	42,24	42,13		3	72,60	72,05		
15	28,10	28,38	28,21	28,10		4	73,80	84,33		
16	23,68**	23,88	23,77**	23,55**		5	70,29***	68,75		
17	47,28	47,40	46,57***	46,79		6	18,53	18,46		
18	41,93	41,90	41,92	41,59	Xylp	1		107,26		
19	46,31	46,46	46,57**	46,09		2		76,13		
20	30,85	31,01	30,92	30,65		3		78,69		
21	34,21	34,32	34,22	33,95		4		71,08		
22	33,17*	32,86	33,25*	32,98*		5		67,58		
23	180,84	181,07	180,60	180,71	28-O-Glcp	1				95,60
24	14,12	14,33	14,18	14,07		2				73,88
25	16,88	17,02	16,78	16,78		3				78,65
26	17,33	17,57	17,32	17,32		4				70,90
27	26,07	26,25	26,20	26,04		5				79,08
28	176,28	176,39	180,11	176,32		6				62,07
29	33,17*	33,26*	33,25*	32,98*						
30	23,68**	23,38	23,77**	23,55**						

[a]Assignment of the signals within a column ambiguous. Signals marked with asterisks are superimposed on one another.

stants (SSCCs) of the anomeric protons of the L-rhamnose and L-arabinose residues in the PMR spectrum of the ester (VII) permitted the conclusion that the L-rhamnose residue had the α-configuration and the L-arabinose residue the β-configuration.

The facts presented are in complete agreement with the ^{13}C NMR spectrum of medicoside H (I), the assignment of the signals of which was made by comparison with the analogous spectra of medicagenic acid 3-O-β-D-glucopyranoside (IV), medicoside G (VI) [1], and medicoside J (II) [4] (Table 1) and by comparison with literature information [8, 9].

Thus, medicoside H (I) has the structure of medicagenic acid 3-O-β-D-glucopyranoside 28-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside].

EXPERIMENTAL

General Observations. The following solvent systems were used: 1) chloroform–methanol–water (400:100:13); and 2) benzene–acetone [a – (5:1); b –(10:1); c – (20:1)].

Mass spectra were taken on a MKh-1310 instrument at an ionizing voltage of 50 V and a temperature of 180-200°C. PMR spectra were obtained on a Tesla BS-567A spectrometer in deuteropyridine with HMDS as internal standard, δ-scale. ^{13}C NMR spectra of (I) were taken on the Tesla BS-567A instrument, those of (II) on a Bruker AM-300 instrument, and those of (IV) and (VI) on a Jeol FX-90 Q instrument in deuteropyridine with TMS as internal standard under conditions of complete and incomplete decoupling of C–H interactions.

GLC was conducted on a Chrom-5 chromatograph. The sugars were chromatographed in the form of the trimethylsilyl ethers of the methyl glycosides [5] on a column (3 mm × 3.2 m) containing Chromaton N-AW-DMCS impregnated with 5% of the silicone phase SE-30. The thermostat temperature was 175°C, and the carrier gas was helium at a rate of 60 ml/min. The methyl ethers of the sugars were identified in the form of their methyl glycosides [7]. They were chromatographed on a column (3 mm × 1.2 m) containing 20% of poly(butane-1,4-diyl succinate) on Celite, 30-60 mesh, (phase 1) at a column temperature of 160°C and on a column of the same dimensions containing Chromaton N-AW-DMCS (0.100-0.125 mm) impregnated with 10% of the poly(phenyl ether) 5F4E (phase 2) at a column temperature of 180°C. The carrier gas was helium at a rate of 50 ml/min.

Isolation of medicoside H [substance H, (I)]. The column chromatography of the combined triterpene glycosides gave, in addition to the substances A, C, G, I, J, and L described in [3], fractions containing compounds G and H (8.05 g) and H and I (3.43 g). The repeated column rechromatography of the fractions in system 1 gave the following substances: medicoside G – 0.23 g; medicoside H – 1.28 g (0.0051% yield calculated on the air-dry raw material); and medicoside I – 0.15 g. The percentage amounts of G and I had been determined previously [3] from the results of single chromatography.

Medicoside H (I), $C_{47}H_{74}O_{19}$, mp 222-225°C (from butanol); $[\alpha]_D^{21}$ 0 ± 3° (c 0.96; methanol); ν_{max}^{KBr}, cm^{-1}: 3510-3330, 1745, 1265. PMR spectrum (C_5D_5N, ppm): 0.78, 0.83, 0.90, 1.08, 1.34, 1.73 (3H each, s 6 × CH_3); 1.50 3H, d, ^3J = 5 Hz, CH_3 of L-rhamnopyranose); 4.94 (1H, d, ^3J = 7 Hz, anomeric proton of D-glucopyranose); 5.28 (1H, broadened s, H-12); 5.63 (1H, s, anomeric proton of L-rhamnopyranose); 6.22 (1H, d, ^3J = 2 Hz, anomeric proton of L-arabinopyranose). Result of a determination of the amounts of sugars in compound (I) by the GLC method [5]: L-arabinose, L-rhamnose, and D-glucose in a ratio of 1.05:0.72:1.00.

Medicagenic Acid (III) and Medicagenic Acid 3-O-β-D-glucopyranoside (IV) from (I). Medicoside H (250 mg) was dissolved in 25 ml of 0.5% methanolic sulfuric acid and the solution was heated at the boiling point of the solvent for 3 h. Then it was diluted with 25 ml of water and the precipitate that deposited was separated off. The residue (168 mg) was chromatographed on a column with elution by system 1. This gave 10 mg of medicagenic acid (III), $C_{30}H_{46}O_6$, mp 352-354°C from methanol), $[\alpha]_D^{21}$ +111.1 ± 2° (c 0.09; ethanol) and 80 mg of medicagenic acid 3-O-β-D-glucopyranoside, $C_{36}H_{56}O_{11}$, mp 288-290°C (from methanol), $[\alpha]_D^{21}$ +65.2 ± 2° (c 0.51; ethanol).

Hendeca-O-methyl Derivative (VII) from (I). Glycoside (I) (500 mg) was subjected to Hakomori methylation [6]. The reaction product (790 mg) was chromatographed on a column with elution by system 2b. This gave 303 mg of the amorphous hendeca-O-methyl derivative (VII), $C_{58}H_{96}O_{19}$, $[\alpha]_D^{21}$ 0 ± 3° (c 0.93; methanol). There was no IR absorption in the hydroxy group region. Mass spectrum, m/z (%): 1096 (M^+; 0.08), 861 (4), 815 (2), 801 (1), 746 (3), 703 (15), 702 (27), 644 (3), 541 (3), 527 (13), 513 (20), 512 (21), 483 (14),

481 (16), 470 (14), 469 (30), 468 (21), 467 (41), 453 (18), 437 (24); 436 (14), 435 (25), 423 (11), 409 (27), 408 (24), 407 (27), 377 (12), 350 (24); 349 (100), 318 (27), 317 (100), 311 (11), 309 (15), 285 (25), 281 (13), 265 (20), 257 (15), 256 (11), 255 (16), 253 (19), 249 (19), 248 (32), 247 (11), 233 (20), 221 (22), 219 (41), 205 (100), 204 (65), 203 (84), 202 (57), 201 (35), 199 (24), 191 (35), 190 (89), 189 (100), 188 (62), 187 (100), 185 (38), 175 (65), 173 (57), 171 (32), 169 (22), 163 (35), 161 (43), 159 (57), 157 (100), 155 (78).

PMR spectrum (C_5D_5N, δ, ppm): 0.80, 0.82, 0.95, 1.16, 1.24, and 1.63 (3H each, s, 6 × CH_3); 1.34 (3H, d, ^3J = 6 Hz, CH_3 of L-rhamnopyranose); 3.27, 3.29, 3.34, 3.36, 3.38, 3.42 × 3, 3.46, 3.50, 3.69 (11 × OCH_3); 4.39 (1H, d, ^3J = 7 Hz, anomeric proton of D-glucopyranose); 5.37 (1H, broadened s, H-12); 5.47 (1H, d, ^3J = 2 Hz, anomeric proton of L-rhamnopyranose); 6.09 (1H, d, ^3J = 3 Hz, anomeric proton of L-arabinopyranose).

Determination of the Methylated Monosaccharides in the Hendeca-O-Methyl Derivative (VII). A solution of 5 mg of compound (VII) in 5 ml of a 7% solution of hydrogen chloride in absolute methanol was boiled for 5 h. The reaction mixture was neutralized with silver carbonate and filtered. After the solvents had been distilled off from the filtrate, methylated sugars were determined by chromatography on two columns containing phases 1 and 2. The relative retention times (T_{rel}) were calculated in relation to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside. Phase, 1, T_{rel}: 0.42 (2,3,4-tri-O-methyl-L-rhamnopyranose); 1.00; 1.42 (2,3,4,6-tetra-O-methyl-D-glucopyranose); 2.10 (3,4-di-O-methyl-L-arabinopyranose). Phase 2, T_{rel}: 0.46 (2,3,4-tri-O-methyl-L-rhamnopyranose); 1.00; 1.50 (3,4-di-O-methyl-L-arabinopyranose); 1.00; 1.35 (2, 3, 4, 6-tetra-O-methyl-D-glucopyranose).

2,23-Di-O-methyl Derivative of Medicagenic Acid (V) from (VII). A solution of 165 mg of compound (VII) in 15 ml of 2% methanolic solution of sulfuric acid was heated for 5 h. Then it was diluted with 15 ml of water and the precipitate that deposited was separated off. The product (95 mg) was chromatographed on a column with elution by system 2c. This gave 54 mg of the amorphous 2,23-di-O-methyl derivative of medicagenic acid (V), $C_{32}H_{50}O_6$, $[\alpha]_D^{21}$ +98.0 ± 2° (c 0.50; chloroform). Compound (V) was also identified by TLC with an authentic sample in system 2a.

SUMMARY

A new triterpene glycoside – medicoside H – has been isolated from the roots of Medicago sativa L. (Fabaceae) and on the basis of chemical transformations and spectral characteristics its structure has been established as medicagenic acid 3-O-β-D-glucopyranoside 28-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside].

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