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TRIPENOID GLYCOSIDES OF ALFALFA. VII. MEDICOSIDES E AND F

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Two hederagenin glycosides — medicosides E and F — have been isolated from the roots of Medicago sativa *L. (Leguminosae). Medicoside E has the structure of hederagenin 28-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1→3)-β-D-xylopyranoside]. Medicoside F has the structure of hederagenin 28-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside].*

Continuing a study of the triterpene glycosides of alfalfa (lucerne) [1, 2], from the root of the plant we have isolated two minor glycosides which we have called medicosides E (I) and F (IV), according to their polarity in TLC. The substances were obtained as a result of the rechromatography of fractions isolated previously [2], at the same time as compounds A, C, G, I, J, and L.

Medicosides E (I) and F (IV) were subjected to acid hydrolysis, and, with the aid of a TLC comparison with an authentic sample, hederagenin was detected in the hydrolysates in both cases. The qualitative and quantitative compositions of the sugars in medicosides E and F were determined after the methanolysis of the glycosides, using the GLC of silyl derivatives of the methyl glycosides in a capillary column with the phase OV-101. The determination showed the presence of D-glucose and D-xylose in a ratio of 2:1 in medicoside E (I) and of D-glucose and L-arabinose in a ratio of 2:1 in medicoside F (IV).

Glycosides (I) and (IV) were methylated by Hakomori's method [3]. The permethylates obtained, (II) and (V), were subjected to methanolysis, and the methyl glycosides of the sugar methyl ethers were identified with the aid of GLC [4]. The permethylate (II) gave 2,3,4,6-tetra-O-methyl-D-glucopyranose and 2,4-di-O-methyl-D-xylopyranose, and permethylate (V) gave 2,3,4,6-tetra-O-methyl-D-glucopyranose and 3,4-di-O-methyl-L-arabinopyranose.

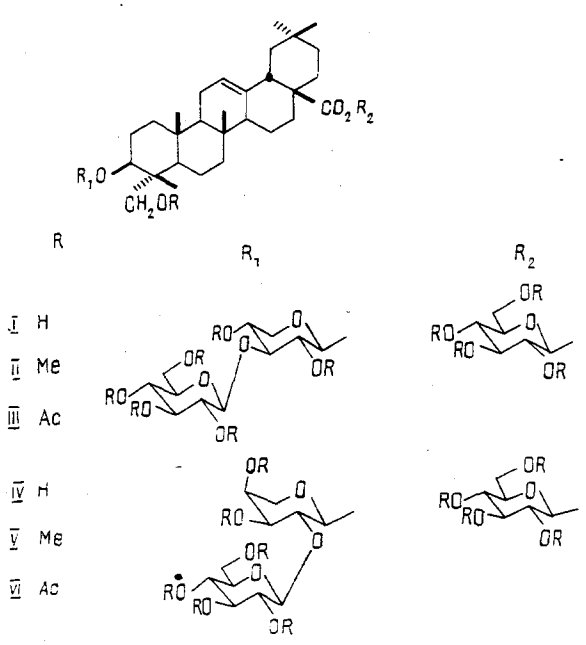

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TABLE 1. Chemical Shifts (δ, ppm); 0 — TMS), Multiplicities, and SSCCs (J, Hz) of the Protons of Medicoside E (I) in C_5D_5N and of the Peracetates of E (III) and F (VI)in $CDCl_3$

Positions of the protons	Compound		
	I	III	VI
3		3.37 dd J=11.0 J=5.0	3.40 dd J=10.3 J=5.0
12	5.40 t J=3.5	5.29 t J=3.5	5.29 t J=3.5
18	3.15 dd J=14.0 J=4.5	2.80 dd J=14.0 J=4.5	2.78 dd J=13.6
23		3.88 d J=11.3 3.69 d J=11.3	J=4.5
CH_3	0.85 s; 0.87 c;	0.66 c; 0.69 c;	0.68 c; 0.70 c;
groups	0.90 s; 0.94 c; 1.08 s; 1.18 c	0.87 c (CH_3X2) 0.89 c; 1.06 c	0.74 c; 0.88 c; 0.90 c; 1.07 c
	3-O-Xyl		3-O-Ara
1	4.93 d $J_{1,2}$=7.0	4.29 d $J_{1,2}$=7.1	4.35 d $J_{1,2}$=6.9
2	3.95 t $J_{2,3}$=7.0	4.91 dd $J_{2,3}$=9.0	3.86 dd $J_{2,3}$=9.4
3	3.97 t $J_{3,4}$=7.0	3.80 t $J_{3,4}$=9.0	4.92 dd $J_{3,4}$=3.6
4	4.21 ddd $J_{4,5e}$=5.0 $J_{4,5a}$=10.5	4.85 ddd J4,5e=3,4 $J_{4,5a}$=8,5	5.08
5a	3,51 t $J_{5a,5e}$=10.5	3.23 dd $J_{5a,5e}$=11.8	
5e_	4,01 t		
		Glc	
1	5.17 d $J_{1,2}$=8.0	4.58 d $J_{1,2}$=7.9	4.67 d $J_{1,2}$=8.0
2	4.00 t $J_{2,3}$=8.0	4.89 dd $J_{2,3}$=9.3	4.88 dd $J_{2,3}$=9.8
3	4.18 t $J_{3,4}$=8.0	5.06 t $J_{3,4}$=9.3	5.12 dd $J_{3,4}$=7.5
4	4.09 dd $J_{4,5}$=8.8	5.10 t $J_{4,5}$=9.3	5.10 dd $J_{4,5}$=9.8
5	3.96	3.77	3.74
6	4.47 dd $J_{6',6}$=11.8 $J_{5,6}$=2.5	4.05 dd $J_{6',6}$=12.3 $J_{5,6}$=2.5	4.02 dd $J_{6',6}$=12.5 $J_{5,6}$=2.5
6'	4.24	4.25 dd $J_{5,6'}$=4.2	4.30 dd $J_{5,6'}$=4.3
		28-O-Glc	
1	6.24 d $J_{1,2}$=8.0	5.54 d $J_{1,2}$=7.8	5.54 d $J_{1,2}$=7.8
2	4.13 t $J_{2,3}$=8.0	5.18 dd $J_{2,3}$=9.0	5.19 dd $J_{2,3}$=9.3
3	4.18 t $J_{3,4}$=8.0	5.15 t $J_{3,4}$=9.0	5.16 t $J_{3,4}$=9.3
4	4.25 dd $J_{4,5}$=6.5	5.13 dd $J_{4,5}$=9.4	5.05 t $J_{4,5}$=9.3
5	3.97	3.66 ddd $J_{5,6}$=2.5 $J_{5,6'}$=4.1	
6	4.40 dd $J_{6',6}$=12.0 $J_{5,6}$=2.1	4.32	
6'	4.32 dd $J_{6',6}$=12.0 $J_{5,6'}$=4.4	4.01	

By acetylation with acetic anhydride in pyridine, compounds (I) and (IV) were converted into the peracetates (III) and (VI), respectively. The compounds were studied with the aid of NMR spectroscopy. The signals of the protons were assigned by the use of homonuclear double resonance experiments. To assign the signals in the ^{13}C NMR spectrum, we employed the J-modulation procedure and literature information [5]. The PMR spectra of the peracetates (III) and (VI) (Table 1) showed the signals of the protons of two fully acetylated D-glucopyranose residues. In each of the two spectra, the anomeric proton of one glucose residue was shifted downfield (5.54 ppm), showing the attachment of this sugar at the carboxy group of the aglycon. In the spectrum of compound (III) moreover, the signals of the protons of a D-xylopyranose residue substituted in the third position were observed. A gem-acyl (ring) proton is, as a rule, shifted to a field weaker than 4.5 ppm, while the H-3 signal of the D-xylopyranose residue was present at 3.80 ppm, showing substitution in this position. The chemical shifts of the protons of the L-arabinose residue observed in the spectrum of compound (VI) had values corresponding to an arabinopyranose residue substituted in the second position. The spin-spin coupling constants (SSCCs) of the carbohydrate protons in these spectra corre-

TABLE 2. Chemical Shifts of the Carbon Atoms of Medicoside E (I) (δ, ppm; 0 — TMS; C_5D_5N)

C atom	I	C atom	I	C atom	I
1	38.9	19	46.3		Glc
2	26.2	20	30.9	1	105.8
3	81.9	21	34.1	2	75.6
4	43.6	22	32.6	3	78.8
5	47.6	23	64.2	4	71.7
6	18.2	24	13.7	5	78.4
7	32.9	25	16.3	6	62.6
8	40.1	26	17.6		28-O-Glc
9	48.3	27	26.2	1	95.9
10	37.0	28	176.6	2	74.2
11	24.0	29	33.2	3	79.4
12	123.1	30	23.8	4	71.2
13	144.3		3-O-Xyl	5	79.0
14	42.2	1	106.5	6	62.3
15	28.4	2	74.4		
16	23.5	3	88.4		
17	47.1	4	69.7		
18	41.8	5	66.6		

sponded to the C1 conformation of the D-glucopyranose, D-xylopyranose, and L-arabinopyranose residues. Signals at 3.37 and 3.40 ppm in the PMR spectra of compounds (III) and (VI) were assigned to the third proton of hederagenin substituted by a glycosidic bond in the C(3)−OH position.

The facts given above agree completely with the ^{13}C NMR spectrum of medicoside E (I) (Table 2). The C-3 signal of hederagenin is shifted downfield by 8.2 ppm, and the C-28 signal is also shifted downfield, by 3.8 ppm, as compared with the analogous signals in the spectrum of hederagenin itself [5]. The C-3 signal of the D-xylose residue has also undergone a downfield shift by 10 ppm relative to the analogous signal of unsubstituted D-xylose [6].

Consequently, medicosides E (I) and F (IV) are hederagenin 28-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1→3]-β-D-xylopyranoside] and hederagenin 28-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside], respectively.

EXPERIMENTAL

General Remarks. We used Silufol plates for thin-layer chromatography. The plates were revealed by spraying with a 25% alcoholic solution of tungstophosphoric acid and heating at 110°C for 10 min. For column chromatography we used type L 100/63 μ silica gel, with the following solvent systems: 1) chloroform−methanol−water (80:20:2.5); 2) chloroform−methanol (100:1); and 3) benzene−acetone (5:1).

TLC was conducted on a Biokhrom-1 chromatograph using a capillary column (50 m) with the liquid phase OV-101. The column temperature was 150°C, and the carrier gas helium at a rate of 4 ml/min. Sugars were chromatographed in the form of trimethylsilyl ethers of methyl glycosides [2]. Methylated sugars were chromatographed on a Chrom-5 chromatograph using a column (3 mm × 1.2 m) containing Chromaton N-AW with 10% of the polyphenyl ether 5F-4É. The column temperature was 180°C, and the carrier gas helium at a rate of 50 ml/min. Relative retention times (T_{rel}) were calculated in relation to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside [4].

^1H and ^{13}C NMR spectra were taken on WM-250 and AM-300 instruments, respectively.

Isolation of Medicosides E (Substance (E, I), and F (Substance F, IV). By the column chromatography of the total triterpene glycosides from the roots, in addition to substances A, C, G, I, J, and L, isolated previously [2], we also obtained fractions containing compounds C and E, and C, E, and F, in amounts of 0.91 and 14.16 g, respectively. The fractions were rechromatographed on a column with elution by system 1. This gave substances C (0.21 g), E (0.51 g), 0.0006%, and F (0.27 g, 0.0011%).

Medicoside E (substance E, I), $C_{47}H_{76}O_{18}$, $[\alpha]_D^{20}$ + 51.9 ± 2° (c 1.31; methanol, ν_{max}^{KBr} (cm^{-1}): 3600-3200, 3000-2870, 1740, 1640, 1470, 1385, 1270. The result of a determination of the sugars in compound (I) with the aid of GLC was as follows; Glc:Xyl = 2:1.

Medicoside F (substance F, IV), $C_{47}H_{76}O_{18}$, ν_{max}^{KBr} (cm^{-1}): 3600-3200, 3000-2860, 1740, 1650, 1465, 1390, 1260. The result of the determination of the ratio of the sugars in compound (IV) with the aid of GLC was as follows: Glc:Ara = 2:1.

The Permethylate (II) from (I). A solution of 50 mg of medicoside E (I) in 10 ml of dimethyl sulfoxide was treated with 50 mg of sodium hydride, the mixture was stirred for 2 h, and 1 ml of methyl iodide was added. Stirring was continued for another 3 h, and then the solution was poured into 20 ml of a 2% solution of sodium thiosulfate and the reaction product was extracted with chloroform. The chloroform extracts were combined, washed with water, and evaporated. The residue (100 mg) was chromatographed on a column in system 3. This gave 2.0 mg of the permethylate of E (II).

The Permethylate (V) from (IV). Medicoside F (IV) was methylated in a similar way to that described for medicoside E (I). From 50 mg of glycoside (IV) we obtained 2.3 mg of the permethylate of F (V).

GLC of the Permethylates (II) and (V). Solutions of the permethylates (II) (2.0 mg) and (V) (2.3 mg) in 2 ml of a 7% solution of HCl in absolute methanol were boiled for 5 h. Each solution was neutralized with silver carbonate and filtered. The filtrate was evaporated, and the residue was dissolved in hexane and chromatographed on a column of Chromaton N-AW impregnated with the polyphenyl ether 5F-4É. Result for the permethylate (II): T_{rel} = 0.70; 1.00 (2,4-di-O-methyl-D-Xyl); and 1.00, 1.32 (2,3,4,6-tetra-O-methyl-D-Glc). Result for the permethylate (V): T_{rel} = 1.00 (3,4-di-O-methyl-L-Ara); 1.00 and 1.34 (2,3,4,6-tetra-O-methyl-D-Glc).

The Peracetate (III) from (I). A solution of 100 mg of medicoside E (I) in 3 ml of pyridine was treated with 3 ml of acetic anhydride. The mixture was kept at room temperature for 24 h, the solvent was distilled off, and the dry residue (250 mg) was chromatographed on a column with elution by system 2. This gave 65 mg of the peracetate (III).

The Peracetate (VI) from (IV). Medicoside F (IV) was acetylated in a similar way to that described above for medicoside E [1]. From 100 mg of the glycoside (IV) we obtained 35 mg of the peracetate (VI).

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