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TRITERPENE GLYCOSIDES OF ALFALFA
VIII. MEDINOSIDE E FROM THE LEAVES

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From the leaves of Medicago sativa *L. (Leguminosae) we have isolated the glycoside soyasapogenol B — medinoside E. Medinoside E has the structure of olean-12-ene-3β,22β,24-triol 3-O-[O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranuronoside].*

A study of the triterpene glycosides of the roots of alfalfa (lucerne) (*Medicago sativa*) L. (*Leguminosae*) has led us to the determination of the structures of ten compounds out of the 13 that have been detected [1]. The saponin contents of the roots of alfalfa gathered in May and December amounted to 1.5 and 1.9%, respectively. A comparison of the saponins of the roots with the saponins of the epigeal part by thin-layer chromatography (TLC) showed the absence of identical compounds. The qualitative compositions of the leaves and of the stems did not differ.

In order to study the triterpene glycosides of the epigeal part, we isolated the total saponins from alfalfa leaves (0.16% on the weight of the air-dry raw material). With the aid of TLC, in the saponins we detected ten compounds and have designated them in accordance with their polarity as medinosides A-J. After the column chromatography of the saponins and rechromatography of the fractions it was possible to isolate five substances — B, D, E, H, and I. The present paper is devoted to the determination of the structure of one of the main components of the saponins — medinoside E (I).

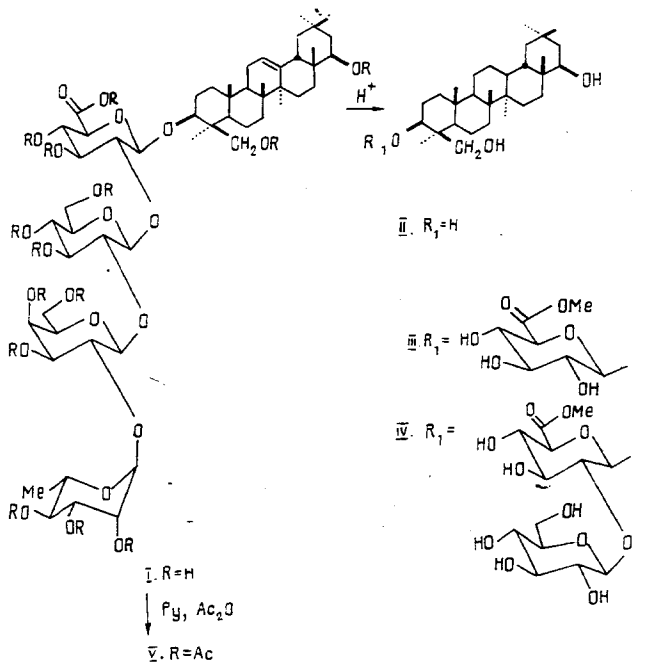

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TABLE 1. Chemical Shifts of the ^{13}C Atoms in Compounds (I), (II), (III), and (IV) (δ, ppm; 0 — TMS; C_5D_5N)

Genin moiety

C atom	Compound				C-атом	Compound			
	I	II	III	IV		I	II	III	IV
1	38.7	39,4	38,7	38,6	16	28,8[a]	29,2	28,7[a]	28,7[a]
2	26.6[d]	28,5	26,9	26,7	17	38,1	38,2	38,0	38,0
3	91,7	80,5	89,1	90,9	18	45,5	45,9	45,3	45,3
4	43,8	43,4	44,5	43,8	19	46,9	47.2	46,8	46,8
5	56,3	56,8	56,1	56,2	20	31,0	31,0	30,9	31,0
6	18,7	19,4	18,9	18,7	21	42,5[c]	42,5	42,3[c]	42,4[c]
7	33,4[b]	34,0	33,4[b]	33,4[b]	22	75,7	75,9	75,6	75,6
8	40,1	40,4	40,0	40,0	23	22,9	23,6	23,3	22,6
9	47,9	48,5	47,8	47,8	24	63,6	64,7	63,2	63,4
10	36,5	37,4	36,6	36,5	25	15,6	16,5	15,5	15,7
11	24,2	24,4	24,1	24,1	26	17,1	17,4	17,1	17,0
12	122,5	122,8	122,5	122,4	27	25,9	25,8	25,7	25,8
13	145,0	145,1	144,9	144,9	28	28,8[a]	28,9	28,7[a]	28,7[a]
14	42,5[c]	42,8	42,3[c]	42,4[c]	29	33,4[b]	33,1	33,4[b]	33,4[b]
15	26,6[d]	26,7	26,5	26,5	30	21,3	21,0	21,2	21,2

C-atom	Compound						
	I				III	IV	
	GlcA	Glc	Gal	Rha	GlcA	GlcA	Glc
1	105,5	102,4	102,0[g]	102,0[g]	105,5	105,1	104,8
2	79,3	78,7[f]	78,7[f]	72,5	75,3	81,6	75,8
3	77,2	77,4	77,5	72,7[e]	77,4	77,1	78,3
4	72,7[e]	71,3	69,5	74,0	73,2	72,6	69,9
5	77,7	78,1	77,0	69,9	77,9	78,1	78,5
6	173,9	61,8	61,5	19,0	170,4	170,4	61,6
$COOCH_3$	—				52,1	52,2	

Notes. The assignment of the signals in the spectra of compounds (I), (III), and (IV) was made by comparison with literature information [2, 4]. The signals denoted by the letters A, B, C, D, E, F, and G are superposed on one another.

Medinoside E (I) was subjected to methanolysis, and, with the aid of GLC, D-glucuronic acid, D-glucose, D-galactose, and L-rhamnose were detected in the products in a ratio of 1:1:1:1. After the partial acid hydrolysis of (I), soyasapogenol B (II) and progenins (III) and (IV) were isolated from the hydrolysate. Soyasapogenol B (II) was identified from its melting point and ^1H and ^{13}C NMR spectra [2, 3] the assignment of the chemical shifts of the signals in these spectra being made with the use of the method of homonuclear double resonance and J-modulation.

A comparison of the ^{13}C NMR spectra of compounds (II), (III), (IV), and (I) (Table 1) revealed a downfield shift of the signal of the third carbon atom of the aglycon by 8-10 ppm, showing the substitution of soyasapogenol B (II) at the third position. The spectrum of progenin (III) had, in addition, the signals of the carbon atoms of a D-glucopyranuronosyl residue esterified by a methyl group. The spectrum of compound (IV) contained, in addition to the signals of the aglycon, the signals of a methyl D-glucopyranosyluronate residue and of a D-glucopyranosyl residue. A consideration of the parameters of the PMR spectra of these compounds in the carbohydrate part (Table 2) confirmed the presence in compound (III) of a methyl glucopyranosyluronate residue in the C1 conformation, and in compound (IV) of a methyl D-glucopyranosyluronate residue and of a D-glucopyranosyl residue, both likewise in the C1 conformation. When the H1 proton of the D-glucuronic acid residue

TABLE 2. Chemical Shifts of the Protons of Progenins (III) and (IV) and of the Peracetate (V) (δ, ppm, 0 — TMS; C_5D_5N), Multiplicity and SSCC (J, Hz)

Genin moiety

Positions of the protons	Compound		
	III	IV	V
12	5.33 t (3.5)	5.32 t (3.5)	4,64 t (3.5)
24	4.34 d (11.3)		
24′	3.64 д (11.3)		
22	3.70 dd (6.5; 3.4)		
3	3.59 dd (11.5; 5.0)		
18	2.37 dd (13.7; 3.4)	2,37 dd (13.8; 3.5)	2.22 dd (13.8; 3.5)
CH_3 groups	1.53s; 1.28c; 1.26s; 1.19c; 1.01s; 1.00c; 0.88c	1.38s; 1.26s 1.24s; 1.19s 1.00s; 0.79s; 0.78s	1.25s; 1.14s 1.07s; 1.00s; 0.96s; 0.90s; 0.81s

Carbohydrate moiety

Positions of the protons	Compound						
	III	IV		V			
	GlcA	GlcA	Glc	GlcA	Glc	Gal	Rha
1	5.02 d (8.0)	3.88 d (8.0)	5.46 d (7.5)	5.09 d (5.0)	4.86 d (8.0)	4.83 d (8.0)	5.02 s
2	3.80 t (8.0)	4.08 t (8.0)	4.01 dd (8.5; 7.5)		3.72 dd (9.5; 8.0) 5.27 т (9.5)	3.87 dd (10.0; 8.0)	4.98 d (3.5)
3	4.17 t (8.0)	4.23 t (8.0)	4.11 t (8.5)		4.98 т (9.5)	5.00 dd (10.0; 3.5)	5.15 dd (10.0; 3.5)
4	4.38 t (8.0)	3.35 t (8.0)	4.29 t (8.5)			5.40 d (3.5)	5.00 t (10.0)
5	4.51 d (8.0)	4.42 d (8.0)	3.70 dt (8.5; 2.3)				3.95 dd (10.0; 6.3)
6	—	—	4.33	—	4.14 dd (12.5; 2.5)		1.07 d (6.3)
6′	—	—		—	4,22 dd (12.5; 4.5)		
$COOCH_3$	3.73 s	3.76 s	—	—	—	—	—

in compound (III) was irradiated, a NOE was observed on H2, H3, and H5 of the D-glucuronic acid residue and H3 of soyasapogenin B. Irradiation of the H1 atom of the D-glucose residue in compound (IV) caused a response of the second, third, and fifth protons of the D-glucose residue and of the second proton of the D-glucuronic acid residue. Consequently, progenin (III) was the 3-O-(methyl β-D-glucopyranosiduronate) of soyasapogenol B, and progenin (IV) the 3-O-[O-[β-D-glucopyranosyl-(1→2)-(methyl β-D-glucopyranosiduronate)] of soyasapogenol B.

The acetylation of medinoside E (I) with acetic anhydride in pyridine gave the peracetate (V). In the PMR spectrum of compound (V) (Table 2), the signals of the protons were assigned with the use of the homonuclear double resonance procedure. The signals of the secondary protons of the D-glucose and D-galactose residues were present in a relatively strong field, while the remaining protons resonated at values exceeding 4.5 ppm. Thus, the second positions of the D-glucose and D-galactose residues were not acetylated but were the positions of attachment of sugars. The spin-spin coupling constants (SSCCs) of the protons of the D-galactose and L-rhamnose residues in the PMR spectrum of compound (V) corresponded to the C1 conformation of the D-galactose residue and the 1C conformation of the L-rhamnose residue.

On the basis of what has been said, medinoside E has the structure of olean-12-ene-3β,22β,24-triol 3-O-[O-α-L-rhamnopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranuronoside].

EXPERIMENTAL

For General Remarks, see [1]. For chromatography we used the following solvent systems: 1) chloroform–methanol–water [a) 71:25:4; b) 61:32:7; c) 60:15:2] and 2) chloroform–methanol [a) 50:1; b) 25:1; c) 10:1].

^1H NMR spectra were measured on a Bruker WM-250 instrument, and ^{13}C NMR spectra on a Bruker AM-300 instrument.

Isolation of the Glycosides. Alfalfa leaves gathered in April and dried in the shade (14 kg) were extracted with boiling water for 3 h. The substances were exhaustively extracted from the aqueous extract with n-butanol. The butanolic extracts were combined, washed with water, and evaporated. The dry residue was dissolved in methanol, the solution was filtered, and the filtrate was evaporated. The residue so obtained (90.3 g) was dissolved in 200 ml of methanol and was precipitated with 2 liters of acetone. The precipitate was filtered off and washed with acetone. This gave 22.6 g (0.16%; here and below the yield is calculated on the weight of the air-dry material) of total saponins. The saponins (22 g) were chromatographed on a column, with elution by system 1a. As a result, 10 fractions were obtained, containing substances A, AB, B, BC, C, CD, D, DE, DEF, and EF.

When elution was continued with system 1b, three fractions were obtained which contained substances GH, I, and IJ. Fractions 1-10 were rechromatographed in system 1c and fractions 11-13 were rechromatographed in system 1a. This gave the following substances: B (0.33 g; 0.0024%), D (0.28 g; 0.0020%), E (0.58 g; 0.0041%), H (0.22 g; 0.0016%), I (0.51 g; 0.0036%).

Medinoside E (Substance E, I), $C_{54}H_{88}O_{23}$, mp 218-221°C (methanol), ν_{max}^{KBr}, cm^{-1}: 3600-3200, 3000-2890, 1725-1705, 1615, 1415, 1385, 1250. The result of GLC for compound (I) was as follows: GlcA:Glc;Gal:Rha = 1.1:1.0:1.2:1.2.

Soyasapogenol B (II) and Progenins (III) and (IV) from Medinoside E (I). A solution of 310 mg of compound (I) in 35 ml of 1% methanolic sulfuric acid was boiled for 10 h and was then diluted with water and the methanol was distilled off. The precipitate that had deposited was filtered off and washed with water. The residue (270 mg) was chromatographed on a column in system 2a. This gave 8 mg of soyasapogenol B (II), $C_{30}H_{50}O_3$ with mp, 253-255°C (methanol). PMR-spectra ($C_5S_5N_1\delta_1$, ppm): 5.32 (H-12, f_1, ^3J = 3.6 Hz), 4.41 (H-24′, d), ^2J = 10.6 Hz, 3.66 (H-22, dd, $^3J_1$ = 7.0 Hz, $^3J_2$ = 3.4 Hz), 3.64 (H-24′, dd, ^2J = 10.4 Hz, ^{3} J = 1.2 Hz), 3.58 (H-3, ddd, $^3J_1$ = 11.2 Hz, $^3J_2$ = 4.9 Hz, $^3J_3$ = 1.2 Hz), 2.32 (H-18, dd, $^3J_1$ = 13.1 Hz, $^3J_2$ = 3.2 Hz), 1.48 (CH_3-23, s), 1.21 (CH_3-30, s), 1.21 (CH_3, s), 1.15 (CH_3-28, s), 1.01 (CH_3, s), 0.96 (CH_3-29, s), 0.95 (CH_3, s).

On continuing elution with system 2b, we also obtained 19 mg of the the methyl 3-O-β-D-glucopyranosiduronate of soyasapogenol B, $C_{37}H_{60}O_9$, mp 240-243°C (chloroform–methanol (25:1)), $[\alpha]_D^{28}$ — 20.9 ± 2° (c 0.85; methanol).

Elution with system 2c gave 35 mg of soyasapogenol B 3-O-[O-β-glucopyranosyl-(1→2)-(methyl β-D-glucopyranosiduronate)], $C_{43}H_{70}O_{14}$, mp 229-232°C (chloroform–methanol, 10:1), $[\alpha]_D^{28}$ 0 ± 3° (c 0.96; methanol).

The Peracetate (V) from (I). Compound (I) (90 mg) was acetylated with acetic anhydride in pyridine as described in [1]. The reaction product (143 mg) was chromatographed on a column with elution by system 2a. This gave 100 mg of the peracetate (V). In its UV spectrum there was no absorption in the region of hydroxy groups.

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