STEROIDS OF THE SPIROSTAND AND FUROSTAN SERIES FROM PLANTS OF THE GENUS *Allium*.

II. THE STRUCTURE OF ALLIOSPIROSIDE B FROM *Allium cepa*

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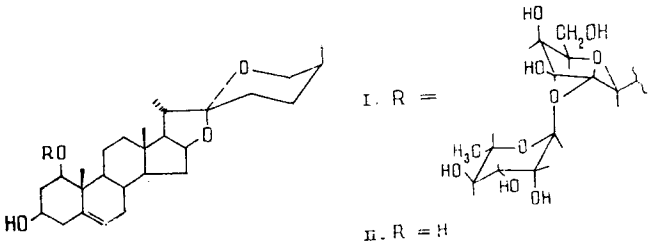
A new steroid glycoside – alliospiroside B (I) – has been isolated from the collective fruit of *Allium cepa* L. On the basis of chemical transformations and with the aid of physicochemical measurements it has been established that compound (I) has the structure of (25S)spirost-5-ene-1β,3β-diol 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranoside. Compound (I) $C_{39}H_{62}O_3$, mp 200-202°C (from ethanol). $[\alpha]_D^{20}$ −110.9 ± 2° (c 1.01; pyridine) was obtained by extracting the collective fruit of *A. cepa* with ethanol folowed by the column chromatographic separation of the combined glycosides on silica gel. The acid hydrolysis of (I) gave (25S)-ruscogenin (II), $C_{27}H_{42}O_4$, mp 189-191°C, $[\alpha]_D^{23}$ −104.1 ± 2° (c 0.98; pyridine). The ^1H and ^{13}C NMR spectra are given for both compounds and the IR spectrum for compound (I).

We have previously described steroid glycosides – alliospiroside A and alliofuroside A – isolated from the collective fruit of *Allium cepa* L. (family Liliaceae) [1]. In the present paper we give a proof of the structure of new steroid glycoside – alliospiroside B (I).



From its positive color reaction with vanillin/phosphoric acid [2] and its characteristic absorption in the IR region [3, 4], compound (I) was assigned to derivatives of the (25S)spirostan series.

The acid hydrolysis of alliospiroside B (I) led to the genin (II) found to be identical with an authentic sample of (25S)-ruscogenin [1, 5, 6].

The methanolysis of compound (I) and the subsequent analysis of the products by GLC [7] showed that the alliospiroside B (I) molecule contained L-rhamnose and D-galactose residues in a ratio of 1:1.

The structure of the carbohydrate chain of glycoside (I) and the position of its attachment to the aglycone were established on the basis of ^1H and ^{13}C NMR spectra (Tables 1 and 2).

The PMR spectrum of glycoside (I) was interpreted by the systematic application of selective homonuclear double resonance (beginning with the anomeric protons) and also in comparison with the spectrum of the genin (II) (Table 1).

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TABLE 1. Chemical Shifts of the Protons (δ, ppm) and SSCCs (J, Hz) in the PMR Spectra of Alliospiroside B (I) and of (25S)-Ruscogenin (II) (C_5D_5N)

Protons of the aglycone	(25S)-Ruscogenin residue in (I)	(25S)-Ruscogenin (II)	Sugar protons	D-Galactose residue in (I)	L-Rhamnose residue in (I)
CH_3-18	0,85 s	0,88 s	H-1	4,74 д, $J_{1,2}$=7,5	6,34 д, $J_{1,2}$ = 1,6
CH_3-19	1,42 s	1,32 s	H-2	4,57 дд, $J_{2,3}$=9,0	4.70 дд, $J_{2,3}$ = 3.0
CH_3-21	1,05 d, ^3J=7 5	1,08 d, ^3J=7,5	H-3	4.14 дд, $J_{3,4}$ = 3,2	4,61 дд, $J_{3,4}$ = 9.0
CH_3-27	1,02 d, ^3J=7,5	1 03 d ^3J=7.5	H-4	4.45 дд, $J_{4,5}$=1,5	4.26 т, $J_{4,5}$ = 9,0
H-1	3,81 dd, J_{aa}=11,5 J_{ae}=4,3	3,80 dd, J_{aa}=11,4 J_{ae}=4,0	H-5	3,91 ддд, $J_{5,6*}$=6,6	4,86 дк, $J_{5,6}$ = 6,0
			H-6	4.44 дд, $J_{6,5}$=5.6	
H-3	3,83 m	3,96 tt, J_{aa}=10,6 J_{ae}=5,4	H-6*	4.31 дд, $J_{6*,6}$=-10.5	
			CH_3-6		1,72
H-6	5,50 br.d ^3J=5.7	5,60 br.d ^3J=5,6			
H-16	4.42 td, $J_д$ = 6,0 $J_т$ = 7,6	4,49 td $J_д$ = 6,0 $J_т$ = 7,6			
H-26'	4,15 dd, ^2J=11,0 ^3J = 2,6	4,03 dd ^2J=10,8 ^3J=2,6		d	d
H-26	3,33 br.d	3,33 br.d		dd	dd
				dd	dd
				ddd	t
				dd	dq
				dd	

Symbols: *s* – *singlet*; *d* – *doublet*; *t* – triplet; q – quartet; m – multiplet; br – broadened.

TABLE 2. Values of the Chemical Shifts of the Carbon Atoms of Alliospiroside B (I) and of (25S)-Ruscogenin (II) (C_5D_5N, δ, ppm, 0 = TMS)

Carbon atom	Compound I	Compound II	Carbon atom	Compound I
1	84,52	78,12	L-rhamnose	
2	38,08	43,95	1	101,73
3	68,32	68,15	2	72,67
4	43,95	43,61	3	72,67
5	139,66	140,37	4	74,39
6	124,95	124,35	5	69,40
7	33,25	33,02	6	19,15
8	32,17	32,35		
9	50,76	51,39		
10	42,99	43,61	D-galactose	
11	24,18	24,25	1	100,85
12	40,57	40,62	2	76,89
13	40,34	40,26	3	75,10
14	57,25	56,99	4	70,52
15	32,55	32,46	5	76,40
16	81,36	81,21	6	62,05
17	63,02	63,06		
18	17,01	16,63		
19	14,95	13,92		
20	42,63	42,54		
21	15,17	14,88		
22	109,89	109,76		
23	26,54	26,45		
24	26,35	26,23		
25	27,70	27,59		
26	65,21	65,11		
27	16,49	16,47		

It followed unambiguously from the values of the SSCCs for the protons of the sugar residues that the carbohydrate moiety of alliospiroside B (I) consisted of α-rhamnopyranose and β-galactopyranose residues [8]. The position of attachment of the rhamnose residue followed from an experiment with observation of nuclear Overhauser effects on the preirradiation of the anomeric proton of the L-rhamnose residue. In this experiment, an enhancement of the H-2 signals of the L-rhamnose and D-galactose residues (approximately 5% each in the difference NOE spectrum) was observed. This indicated substitution by the L-rhamnose residue of the D-galactose residue at the C-2 hydroxyl. The type of substitution of aglycone followed from an analysis of the ^{13}C spectrum of glycoside (I).

The ^{13}C NMR spectrum of alliospiroside B (I) was interpreted by the use of $^{13}C_i-\{^1H_i\}$ selective heteronuclear double resonance (Table 2); the assignment of the signals in the ^{13}C spectrum of (25S)-ruscogenin was made in accordance with the literature [6].

When the ^{13}C NMR spectra of (25S)-ruscogenin (II) and bioside (I) are compared, it can be seen that in the case of alliospiroside B (I) the signal of the C-1 atom is shifted downfield by 6.40 ppm (Table 2). The signal of the C-2 atom is shifted upfield by 5.87 ppm. The chemical shifts of the signals of the other carbon atoms have remained unchanged. This can be explained by the glycosylation effect arising on the addition of a sugar residue to the hydroxy group at C-1 of genin (II).

Measurements of the SSCCs between the anomeric protons and the corresponding carbon atoms of the carbohydrate residues in the ^{13}C NMR spectrum, taken under conditions of the retention of spin-spin interaction of the carbon atoms with protons, additionally confirmed the α configuration for the rhamnopyranose residue ($J_{C-1-H-1}$ = 170.9 Hz) and the β configuration for the galactopyranose residue ($J_{C-1-H-1}$ = 158.3 Hz) [9, 10]. The facts given show the $^4C_1$ conformation of the β-D-galactopyranose ring and the $^1C_4$ conformation for the α-L-rhamnopyranose ring.

Thus, bioside (I) has the structure of (25S)-spirost-5-ene-1β,3β-diol 1-O-[o-α-L-rhamnopyranosyl)-(1 → 2)-β-D-galactopyranoside].

EXPERIMENTAL

General Remarks. Silufol and type KSK silica (particle size <63 μ) containing 10% of gypsum were used for thin-layer chromatography (TLC), and KSK silica gel (particle size

63-100 and <63 μ for column chromatography. The following solvent systems were used: 1) chloroform–methanol–water [a)–(65:15:2); b) – (65:22:4)], and 2) chloroform – methanol [a) – (50:1); b) – (10:1)].

PMR spectra were taken on a WM-250 instrument (Bruker) using solutions of compounds (I) and (II) in pyridine-d_5 at 40°C. In the NOE experiment (difference variant) the intensity of the irradiated protons were taken as 100%. The observed NOEs were positive.

The ^{13}C NMR spectra were taken on a AM-300 instrument (Bruker) with a working frequency for carbon of 75 MHz at a temperature of 40°C. Other information is given in [1].

Isolation of Alliospiroside B (I). The extraction of the plant raw material and the preliminary treatment of the combined substances have been described in [1].

The combined water-insoluble glycosides (150 g) were chromatographed on silica gel columns in systems Ia and Ib. Fractions containing chromatographically homogeneous alliospiroside B (3.6 g) were collected. The total yield calculated on the weight of the air-dry raw material was 0.05%.

Alliospiroside B (I), $C_{39}H_{62}O_{23}$, mp 200-202°C (from ethanol; $[\alpha]_D^{20}$–110.9 ± 2° (c 1.01; pyridine). ν_{max}^{KBv} (cm^{-1}): 3500-3300 (OH); 990, 920 > 900, 850, 825 (spiroketal chain of the 25S series).

(25S)-Ruscogenin (II) from (I). Glycoside (I) (250 mg) was dissolved in 15 ml of 50% aqueous methanol containing 0.6 ml of concentrated sulfuric acid and the mixture was boiled for 8 h. The precipitate that deposited was separated off, and, after column chromatography (eluent: system 2a) and recrystallization from ethanol, 98 mg of the genin (II) was obtained; $C_{27}H_{42}O_4$, mp 189-191°C, $[\alpha]_D^{23}$ –104.1 ± 2° (c 0.98; pyridine), identical with an authentic sample of (25S)-ruscogenin.

Methanolysis of Alliospiroside B (I). Glycoside (I) (10 mg) was dissolved in 4 ml of absolute methanol containing 5% of hydrogen chloride, and the solution was boiled for 14 h. Then an equal volume of water was added to the reaction mixture and the aglycone that precipitated was filtered off and was shown to be identical with (25S)-ruscogenin (II) (TLC, system 2b). The filtrate was neutralized with silver carbonate and was then filtered and evaporated to dryness. L-Rhamnose and D-galactose were detected in a ratio of 1:00:0.90 by GLC (Chromaton N-AW impregnated with 5% of the silicone phase SE-30 [1]).

SUMMARY

From the collective fruit of Allium cepa L. (family Liliaceae) a new glycoside (alliospiroside B, has been isolated; it has the structure of (25S)-spirost-5-ene-1β,3β-diol 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-galacton pyranoside).

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