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STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES FROM PLANTS OF THE GENUS Allium. XXI. STRUCTURE OF ALLIOSPIROSIDE A AND ALLIOFUROSIDE A FROM Allium cepa

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Two new steroid glycosides have been isolated from the generative organs (pericarps and peduncles) of Allium cepa L.: alliospiroside A and alliofuroside A. According to chemical transformations and spectral characteristics, alliospiroside A has the structure of (25S)-spirost-5-ene-1β,3β-diol 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside]. The structure of (25S)-furost-5-ene-1β,3β,22α,26-tetraol 20-O-β-D-glucopyranoside 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside] has been established for alliofuroside A.

Continuing a study of the steroids of the spirostan and furostan series from plants of the genus Allium [1], we have investigated the generative organs (pericarps and peduncles) of Allium cepa L. (garden onion, Uzbekestan variety "Karatal") after the elimination of the ripened seeds. From the total extractive substances, six steroid glycosides present in In the present paper we describe proofs of the structures of the two glycosides present in the largest amount, which we have called alliospiroside A (I) and alliofuroside A (IIa).

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

The hydrolysis of alliospiroside A (I) gave the aglycon (III), the acetylation of which with acetic anhydride in pyridine led to the diacetate (IV). The physicochemical constants and spectral indices of products (III) and (IV) permitted the genin (III) to be identified as (25S)-ruscogenin [5, 6].

The methanolysis of glycoside I followed by analysis of the sugars by GLC [7] showed that the carbohydrate moiety of alliospiroside A (I) included one L-arabinose residue and one L-rhamnose residue.

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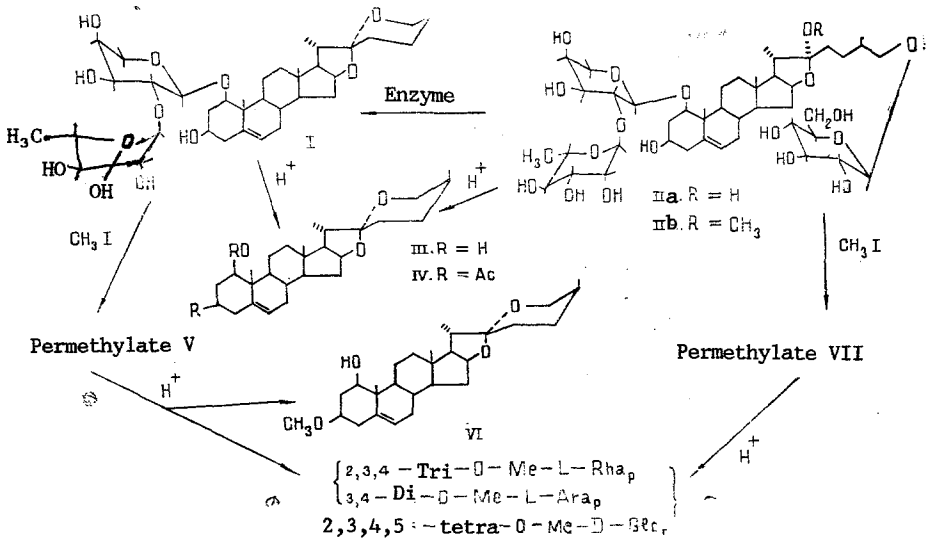

The Hakomori methylation [8] of the bioside (I) gave the permethylate (V). The completeness of methylation was checked by the absence from the IR spectrum of absorption bands characteristic for hydroxy groups and by the presence in the mass spectrum of this ether of the peak of a molecular ion with m/z 189 and 349 show that under the action of electron impact there is an elimination of the fragments of tri-O-methylrhamnose and of a methylated rhamnose-arabinose bioside. Consequently, alliospiroside A is a monodesmosidic diglycoside where L-rhamnoside occupies the terminal position.

After the acid hydrolysis of permethylate (V), the monomethyl ether of (25S)-ruscogenin (VI) (M^+ 444) and the combined methylated sugars were isolated from the reaction mixture. 2,3,4-Tri-O-methyl-L-rhamnopyranose and 3,4-di-O-methyl-L-arabinopyranose were identified by TLC and GLC [9]. Thus, in the alliospiroside A (I) molecule, the L-rhamnose residue is attached to the hydroxy group at C-2 of the L-arabinose residue.

The conclusions drawn also follow from an analysis of the PMR and ^{13}C NMR spectra of (25S)-ruscogenin (III) and of alliospiroside A (I).

With the aid of the spectral characteristics given below it has been possible to deduce the configurations of the glycosidic bonds, the conformations of the carbohydrate rings, and the position of attachment of the sugar chain to the aglycon. The PMR spectra of glycoside (I) and of (25S)-ruscogenin (III) were fairly well resolved in the region of the resonance of $-CH_2O-$ and > CHO groups (for an instrument with a working frequency of 250 MHz). A series of experiments with homonuclear double resonance in the ordinary and the differential variants permitted all the signals of the protons of these groups belonging to the genin and to the two sugar residues to be assigned with confidence (Table 1). The SSCC values given in Table 1 agree well with the idea that the carbohydrate moiety of alliospiroside A (I) is represented by L-rhamnopyranose and L-arabinopyranose residues [10]. Furthermore, the SSCC value of the anomeric protons of the L-rhamnose and L-arabinose residues indicate the α configurations of the glycosidic bonds [10].

The sequence of the linkage of the two sugar residues follows from an experiment with nuclear Overhauser effects (NOEs). The pre-irradiation of the H-1 proton of the L-rhamnopyranose residue caused an increase in the intensity of two signals – H-2 of the rhamnose residue and H-2 of the arabinose residue (5.1 and 6.6% in the difference NOE spectrum, respectively). The spatial propinquity of the H-1 proton of the L-rhamnopyranose residue and the H-2 proton of the L-arabinopyranose residue whows the existence of a 1 → 2 bond in these residues. This was confirmed by a shift of the signal of the C-2 atom of the L-arabinose residue in the ^{13}C NMR spectrum downfield by 3 ppm as compared with the value given in the literature [11].

The signals of the carbon atoms in the ^{13}C NMR spectrum of glycoside (I) included in the $-CH_2-$ and > CHO groups were assigned with the aid of the method of $^{13}C_i-\{^1H_i\}$ selective heteronuclear double resonance (Table 2). The large positive effect of glycosylation

TABLE 1. Chemical Shifts of the Protons of (25S)-Ruscogenin (III) and of Alliospiroside A (I) (C_5D_5N, δ, ppm, 0 is TMS)

Protons of the aglycon	(25S)-Roscogenin (III)	(25S)-Ruscogenin residue in (I)	Sugar protons	L-arabinose residue in (I)	L-rhamnose residue in (I)
CH_3-18	0.88 s	0,84, s	1	4,68, d., $J_{1,2}$ =6,5 Hz	6,28,d , $J_{1,2}$ =1,6 Hz
CH_3-19	1,32,s	1,41, s	2	4,53, d.d, $J_{2,3}$ =8,9 Hz	4,67,d d , $J_{2,3}$ =3,1Hz
CH_3-21	1,03,d., ^3J=7,5 Hz	1,07, d, ^3J=6,0 Hz		$J_{1,2}$ =6,5 Hz	$J_{1,2}$ =1,6 Hz
CH_3-27	1,03,d., ^3J=7,5 Hz	1,04,d., ^3J=6,0 Hz	3	4,1, m	4,57,d d ., $J_{3,4}$ =9,0 Hz
1	3. 0,d .d , J_{aa} =11,4 Hz	3, 0,d .d ., J_{aa} =11,4 Hz			$J_{2,3}$ =3,1 Hz
	J_{ae} =4,0 Hz	J_{ae} =4,0 Hz	4	4,1, m	4,26,t , $J_{4,5}$ =9,0 Hz
3	3,9 , tt , J_{aa} =10, Hz	3,52, t .t , J_{aa} =10,6 Hz	5	4,23,d . d, $J_{4\,5}$ =2,1 Hz	4,79,d q , $J_{5,6}$ =6,0 Hz
	J_{ae} =5,4 Hz	J_{a} =5,4 Hz		$J_{5,5*}$ =11,6 Hz	$J_{4,5}$ =9,0 Hz
6	5, 0,br.d.; ^3J=5,Hz	5,56 d, ^3J=5,6 Hz	5*	3,64, d .d , $J_{4,5*}$ =1,1 Hz	
16	4,49, d t , J_d = ,0 Hz	4,4 ,d t , J_d = ,0 Hz		$J_{5,5*}$ =11,6 Hz	
	J_t =7, Hz	J_t =7,6 Hz	CH_3—6		1,71,d , J= ,0 Hz
26*	4,03, dd , ^2J=10,8 Hz	4,02, d d, ^2J=11, Hz			
	^3J=2, Hz	^3J=2,6 Hz			
26	3,33, br.d.^2J =10,8 Hz	3,33,br.d , ^2J=11,8 Hz			

Symbols. s) singlet; d) doublet; t) triplet; q) quartet; m) multiplet; br.) broadened. Asterisks denote axial protons.

TABLE 2. Chemical Shifts of the Carbon Atoms of (25S)-Ruscogenin (III), of Alliospiroside A (I), and of the 22-O-Methyl Ether of Alliofuroside A (IIb) (C_5D_5N, δ, ppm, 0 is TMS)

Carbon atom	Compound			Carbon atom	Compound	
	III	I	IIb		I	IIb
1	78,12	85,65	83,81	L-Rhannose		
2	43,95	37,45	37,31	1	101,70	101,73
3	68,15	68,28	68,37	2	72,67	72,66
4	43,61	43,91	43,98	3	72,58	72,66
5	140,37	139,64	139,73	4	74,30	74,35
6	124,35	124,76	124,85	5	69,46	69,54
7	33,02	33,22	33,28	6	19,04	19,10
8	32,35	32,08	32,16	L_Arabinose		
9	51,39	50,49	50,58	1	100,43	100,57
10	43,61	42,98	43,07	2	75,26	75,31
11	24,25	24,12	24,16	3	75,90	75,97
12	40,62	40,42	40,42	4	70,10	70,20
13	40,26	40,25	40,68	5	67,35	67,45
14	56,99	56,90	56,90	D-Glucose		
15	32,46	32,46	32,56	1		105,16
16	81,21	81,28	81,51	2		75,31
17	63,06	62,91	64,40	3		78,53
18	16,63	16,78	16,78	4		71,90
19	13,92	14,91	15,20	5		78,68
20	42,54	42,52	40,68	6		63,02
21	14,88	15,10	17,69			
22	109,76	109,78	112,88			
23	26,45	26,47	31,18			
24	26,23	26,26	28,32			
25	27,59	27,61	34,62			
26	65,11	65,11	75,10			
27	16,47	16,36	17,69			
22-OCH_3			47,48			

(+ 5.5 ppm) for the C-1 atom determined by the difference in the chemical shifts of the signals of this atom in the glycoside (I) and in the genin (III) shows the attachment of the sugar residue to the hydroxy group at C-1 of the genin [6, 11].

The SSCCs between the anomeric proton and the anomeric carbon atom observed is the ^{13}C NMR spectrum of bioside (I) taken under the conditions of the preservation of the spin–spin coupling of carbon atoms with protons definitively confirm the configurations of the glycosidic centers of both pyranoses. An SSCC of 170.9 Hz indicates the axial orientation of the OR group at C-1 in 1-rhamnopyranose (the α configuration of the glycosidic center in the case of the $^1C_4$ conformation of the ring), and the SSCC of 158.7 Hz found for the L-arabinopyranose residue is characteristic for the equatorial orientation of the OR group at C-1 in the latter (α configuration of the glycosidic center in the case of the $^4C_1$ conformation of the ring [12, 13]).

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

Glycosides (Ia) and (IIb), having close R_f values, were isolated in the form of a mixture. The IR spectrum of the mixture of substances (IIa) and (IIb) lacked the absorption characteristic for compounds of the spirostan series but had a weak broad band at 900 cm^{-1}. Glycosides (IIa) and (IIb) gave a positive Ehrlich color reaction [13], and with vanillin/phosphoric acid [2] they were revealed in the form of green spots. These results permitted substances (IIa) and (IIb) to be assigned to the glycosides of the furostan series.

On the basis of the chromatographic mobilities of the compounds under consideration, it may be suggested that glycoside (Ia) is a 22-hydroxyfurostan and substance (IIb) is its 22-O-methyl ether.

When an aqueous solution of the mixture of (IIa) and (IIb) was heated, the chromatographically homogeneous alliofuroside A (IIa) was obtained. When the same mixture was boiled in absolute methanol, the 22-O-methyl ether of alliofuroside A (IIb) was formed. The presence of a methoxy group in compound (IIb) was confirmed by a three-proton singlet at 3.24 ppm in its PMR spectrum and a signal at 47.48 ppm in its ^{13}C NMR spectrum. The native furostan compound is probably the glycoside (IIa). The methyl derivative (IIb) was apparently formed during its chromatography and repeated rechromatography on columns of silica gel in methanol-containing solvent systems.

Thus, glycosides (IIa) and (IIb) readily pass into one another, and in proving the structure of alliofuroside A (IIa), the mixture of (IIa) and (IIb) was used.

Complete acid hydrolysis of the mixture (IIa, b) gave the genin (III), which was identified as (25S)-ruscogenin [5, 6].

Gas–liquid chromatography of the products of the methanolysis of the glycosides (IIa,b) showed the presence of L-rhamnose, L-arabinose, and D-glucose residues in a ratio of 1:1:1.

The enzymatic hydrolysis of the combined triosides (IIa, b) with a complex enzyme from the snail Helix plectotropis led to a bioside identical with alliospiroside A (I).

The Hakomori methylation of the mixture of glycosides (IIa, b) gave the permethylate (VII) the IR spectrum of which contained no band characteristic of hydroxy groups. In the mass spectrum of (VII), in addition to the peak of the molecular ion with m/z 1042, there were the peaks of ions characterizing the ejection of carbohydrate fragments: a tetra-O-methylglucose (m/z 219), a tri-O-methylrhamnose (m/z 189), and a methylated rhamnose-arabinose bioside (m/z 349).

The acid hydrolysis of the permethylate (VII) gave a mixture of methylated sugars. 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 were identified by TLC and GLC [9].

The facts given have permitted us to consider that alliofuroside A (IIa) is a glycoside of the furostan series to the C-1 atom of the genin of which is attached a bioside consisting of terminal rhamnose connected to the hydroxy group at C-2 of an arabinose residue. The terminal glucose residue is attached to the hydroxy group at C-26 of the aglycone.

The changes in the spectral characteristics of the carbon atoms of rings E and F in the ^{13}C NMR spectra on passing from spirostan (I) to furanostan (IIc) (Table 2) agree well with literature figures [14, 15].

The $J_{C_1-H_1}$ SSCC of the D-glucose residue (156.2 Hz) and the chemical shift of the signal of the C-26 atom at 75.10 ppm in the ^{13}C NMR spectrum of the glycoside (II) show that the D-glucose residue is attached to the hydroxy group, at C-26 by a β-glycosidic bond. This also follows from the SSCC of the doublet of the anomeric proton of 2,3,4,6-tetra-O-methyl-D-glucopyranose in the NMR spectrum of the permethylate of alliofuranoside A (VII) (J = 8.0 Hz). As was to be expected, the SSCCs of the anomeric protons of the sugar residues present at C-1 amounted to 1.7 Hz (L-rhamnose) and 6.8 Hz (L-arabinose), which indicates the α configurations of the glycosidic bonds. The $J_{C_1-H_1}$ constants of the sugar residues under discussion in the ^{13}C NMR spectrum of glycoside (IIb) are identical with those observed in the ^{13}C NMR spectrum of alliospiroside A (I).

Consequently, alliofuroside A (IIa) is (25S)-furost-5-ene-1β,3β,22α,26-tetraol 26-O-β-L-glucopyranoside 1-O-[O-α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranoside.

EXPERIMENTAL

General Observations. Thin-layer chromatography was performed on a fixed layer of type KSK silica gel (particle size < 63 μm) containing 10% of gypsum, and also on Silufol plates (Czechoslovakia). For column chromatography we used type KSK silica gel (particle size < 63 and 63-100 μm). The following solvent systems were used: 1) chloroform–methanol–water: a) (65:15:2), b) (65:22:4), c) 65:30:6), d) (65:35:8); 2) chloroform–methanol: a) (10:1), b) (50:1); and 3) benzene–acetone (2:1).

Steroids of the spirostan series were detected with vanillin/phosphoric acid [2], furostan glycosides with the Ehrlich reagent [13], and sugar derivatives with o-toluidine salicylate.

Gas–liquid chromatography (GLC) was performed on a Chrom-5 chromatograph. Monosaccharides were chromatographed in the form of the trimethylsilyl ethers of the corresponding methyl glycosides [7] on a column (4 mm × 3 m) of Chromaton N-AV containing 5% of the silicone phase SE-30 (phase 1). Thermostat temperature 190°C; carrier gas helium; rate of flow of gas 45 ml/min. The methyl glycosides of the methylated sugars were obtained by boiling the methyl ethers in anhydrous methanol containing 5% of HCl for 4 h. The compounds obtained were chromatographed on a column (3 mm × 1.2 m) containing Celite impregnated with 20% of poly(butane-1,4-diyl succinate) (phase 2) at a thermostat temperature of 160°C with helium as the carrier gas at a rate of flow of 50 ml/min. The retention times (T_{rel}) for the

methylated methyl glycosides were calculated in relation to the retention time of methyl 2,3,4,6-tetra-O-methyl-β-D-methyl glucopyranoside [9].

Mass spectra were taken on a MKh-1310 instrument, IR spectra on a UR-20 instrument in KBr or Nujol, and NMR spectra (^1H and ^{13}C) on a VM-250 instrument (Bruker) in $CDCl_3$ or C_5D_5N using TMS as standard.

Isolation of Alliospiroside A (I) and Alliofuroside A (IIa). The pericarps and fruit stems (35 kg) of Allium cepa L., variety "Karatal," cultivated in the Tashkent province, after the separation of the ripe seeds, were exhaustively extracted with ethanol. Elimination of the ethanol gave a total amount of about 4.5 kg of the combined extractive substances. The resinous residue was suspended in water and was extracted successively with hexane and n-butanol. The hexane fraction contained none of the group of compounds under investigation (TLC). The butanolic extracts were evaporated to a resinous state, and portions of this material were suspended in water with vigorous stirring. The insoluble residue, *a*, was separated off by decanting the supernatant liquid. This liquid was exhaustively extracted with n-butanol, and the solvent was evaporated off to dryness. The residue was dissolved in ethanol, and the glycosides were precipitated with acetone – precipitate b. As a result, ∿ 800 g of water-insoluble glycosides, *a*, and ∿ 300 g of water-soluble glycosides, b, were obtained.

Precipitate *a* (150 g) was chromatographed on a column of silica gel (with systems 1a and 1b as eluents). Fractions containing the chromatographically homogeneous alliospiroside A (IIa) (7.7 g) were collected.

After the combined material b (100 g) had been chromatographed in systems 1c and 1d, 53.4 g of a mixture of alliofuroside A (IIa) and its 22-O-methyl ether (IIc) was obtained.

The total yield calculated on the weight of the air-dry raw material was 0.12% for the alliospiroside A and 0.46% for the alliofuroside A and its methyl ether.

Alliospiroside A (I). $C_{38}H_{60}O_{12}$, mp 186-189°C (from ethanol), $[\alpha]_D^{25}$ – 106.8 ±2° (c 100; pyridine). ν_{max}^{KBr} (cm^{-1}): 850, 905 < 930, 990 (spiroketal chain of the 25S series), 3300-3500 (OH). For the PMR spectrum, see Table 1).

(25S)-Ruscogenin (III) from (I). A solution of 500 mg of glycoside (I) in 100 ml of 50% aqueous methanol containing 4 ml of concentrated sulfuric acid was heated at the boil for 8 h. The precipitate that deposited was filtered off, and recrystallization from ethanol yielded 195 mg of genin (III), $C_{27}H_{42}O_4$, mp 190-192°C, $[\alpha]_D^{21}$ – 105.6 ±2° (c 1.10; pyridine). ν_{max}^{KBr} (cm^{-1}): 812, 845, 862, 883, 905 < 930, 984, 998 (spircketal chain of the 25S series), 3350-3400 (OH). For the PMR spectrum, see Table 1, M^+ 430.

Literature figures for (III) [6]: mp 194-196°C, 212-214°C; $[\alpha]_D^{18}$ –108°.

(25S)-Ruscogenin 1,3-Diacetate (IV) from (III). A solution of 170 mg of the genin (III) in 5 ml of anhydrous pyridine was treated with 2 ml of acetic anhydride, and the mixture was left at room temperature for 30 h. Then it was poured into water and the precipitate that deposited was filtered off. Recrystallization from methanol, yielded 140 mg of the diacetate (IV), $C_{31}H_{46}O_6$, mp 173-176°C, $[\alpha]_D^{20}$ – 91.0 ±2° (c 1.00; chloroform). ν_{max}^{KBr} (cm^{-1}): 810, 848, 861, 880, 910 < 930, 965, 998 spiroketal chain of the 25S series, 1250, 1735 (CH_3COO). PMR spectrum ($CDCl_3$, δ, ppm): 0.77 (3 H at C-18, s); 0.99 (3 H at C-21, d, ^3J = 6.0 Hz); 1.08 (3H at C-27, d ^3J = 6.0 Hz); 1.16 (3H at C-19, s) 2.02 (3 H of an acetate, s); 2.03 (3 H of an acetate, s); 3.30 (H at C-26, br.d, ^2J = 11.5 Hz); 3.95 (H at C-26, d.d, ^2J = 11.5 Hz; ^3J = 2.5 Hz); 4.42 (H at C-16, d.t, J_d = 6.0 Hz, J_t = 7.6 Hz); 4.66 (2 H at C-1 and C-3, m); 5.64 (H at C-6, br.d, ^3J = 5.0 Hz), M^+ 514.

Literature figures for (IV) [6]: mp 182-185°C; $[\alpha]_D^{19}$ –91.0°.

Methanolysis of Alliospiroside A (I). A solution of 15 mg of glycoside (I) in 3 ml of absolute methanol containing 5% of hydrogen chloride was heated at the boil for 14 h. After cooling and the addition of an equal volume of water, the aglycon that had separated out was filtered off and was shown to be identical with (25S)-ruscogenin (III) (TLC, system 2a). The filtrate was neutralized with silver carbonate, the solid matter was filtered off, and the solution was evaporated to dryness. L-Rhamnose and L-arabinose were detected by GLC (phase 1) in a ratio of 1.00:0.94.

Alliospiroside A Hexa-O-methyl Ether (V) from (I). Over 30 min. 1.0 g of sodium hydride was added to a solution of 1.0 g of glycoside (I) in 100 ml of dimethyl sulfoxide. The mixture

was stirred at room temperature for 1 h. After this, 15 ml of methyl iodide was added over 30 min, and the mixture was stirred for another 3 h. Then it was poured into 0.5 liter of water and was extracted with chloroform (6 × 50 ml). The combined chloroform extracts were washed with sodium thiosulfate solution, worked up in the usual way, and evaporated to dryness. The residue was chromatographed on a column (system 2b). Recrystallization of the main reaction product from methanol gave 910 mg of the hexa-O-methyl ether of alliospiroside A (V), $C_{44}H_{72}O_{12}$, mp 194-196°C, $[\alpha]_D^{20}$ − 82.1 ±2° (c 1.20; chloroform). ν_{max}^{Nujol} (cm^{-1}): 800, 835, 845, 860, 878, 898 < 920, 940, 960, 970, 990 (spiroketal chain of the 25S series); absorption bands of hydroxy groups were absent. PMR spectrum ($CDCl_3$, δ, ppm); 0.77 (3 H at C-18, s); 0.97 (3 H at C-21, d, ^3J = 6.0 Hz); 1.05 (3 H at C-19, s); 1.08 (3 H at C-27, d, ^3J = 6.0 Hz); 1.25 (3 H at C-6 of an L-rhamnose residue, d, ^3J = 6.0 Hz); 3.35-3.51 (6 × OCH_3); 4.31 (H at C-1 of an L-arabinose residue, d, ^3J = 6.8 Hz); 5.22 (H at C-1 of an L-rhamnose residue, d, ^3J = 1.7 Hz); 5.55 (H at C-6, br.d, ^3J = 5.0 Hz). Mass spectrum, m/z (%): 792 (M^+, 0.2), 349 (12), 189 (100).

3-O-Methyl-(25S)-ruscogenin (VI) from (V). A solution of compound (V) (500 mg) in 100 ml of 50% aqueous methanol containing 4 ml of concentrated sulfuric acid was heated at the boil for 10 h. Then 100 ml of water was added, the methanol was distilled off, and the aglycon (VI) that precipitated was recrystallized from methanol. This gave 240 mg of substance (VI), $C_{28}H_{44}O_4$, mp 208-210°C, $[\alpha]_D^{20}$ − 139.8 ±2° (c 1.01; chloroform). ν_{max}^{KBr} (cm^{-1}): 810, 840, 855, 880, 900 < 920, 960, 970, 990 (spiroketal chain of the 25S series), 3510 (OH). PMR spectrum (C_5D_5N, δ, ppm): 0.88 (3 H at C-18, s); 1.04 (3 H at C-27, d, ^3J = 6.0 Hz); 1,09 (3 H at C-21, d, ^3J = 6.0 Hz); 1.24 (3 H at C-19, s); 3.18 (1 H at C-3, t.t, J_{aa} = 11.0 Hz, J_{ae} = 4.5 Hz); 3.30 (3 H, OCH_3, s); 3.33 (1 H at C-26, br.s, ^2J = 10.8 Hz); 3.69 (1 H at C-1, d.d.d, J_{aa} = 11.7 Hz, J_{ae} = 4.6 Hz, $J_{Hc-1-OH}$ = 6.0 Hz; 4.03 (1 H at C-26, d.d, ^3J = 2.6 Hz, ^2J = 10.8 Hz); 4.49 (1 H at C-16, d.t, J_t = 7.6 Hz, J_d = 6.0 Hz); 5.57 (1 H at C-6, br.d, ^2J = 5.7 Hz). Mass spectrum, m/z (%): 444 (M^+; 1.3), 414 (0.7), 385 (43), 375 (1), 372 (1.3), 330 (3), 315 (8), 139 (100).

The filtrate (aqueous solution of methylated sugars) was heated at 100°C for 6 h. After this, the reaction mixture was neutralized with the anion-exchange ÉDÉ 10 P and was evaporated to dryness. The following were identified by TLC (system 3) and GLC (phase 2):

	T_{rel}	T_{rel} [9]
2,3,4-Tri-O-Methyl-L-rhamnopyranose	0.43	0.46
3,4-di-O-Methyl-L-arabinopyranose	2.00	2.15

Alliofuroside A (IIa). A mixture of glycosides (IIa) and (IIb) (500 mg) was dissolved in 50 ml of water, and then 20 ml of acetone was added and the solution was boiled for 18 h. After the solvent had been driven off, the amorphous alliofuroside A (IIa) was obtained: $C_{44}H_{72}O_{18}$, mp 164-166°C, $[\alpha]_D^{20}$ −63.7 ±2° (c 1.11; pyridine); ν_{max}^{KBr} (cm^{-1}): 895 (weak broadened band), 3000-3500 (OH). The PMR spectrum lacked the signal of the protons of a methoxy group.

22-O-Methyl Ether of Alliofuroside A (IIb). The product (IIa-IIb) (500 mg) was dissolved in 50 ml of absolute methanol and the solution was heated at the boil for 18 h. After the methanol had been distilled off, the amorphous glycoside (IIb) was obtained; $C_{45}H_{74}O_{18}$, mp 160-171°C, $[\alpha_D^{20}$ − 75.3 ±2° (c 1.03; pyridine), ν_{max}^{KBr} (cm^{-1}): 895 (weak broadened band) 3300-3500 (OH). PMR spectrum (C_5D_5N, δ, ppm): 0.82 (3 H at C-18, s); 1.01 (3 H, secondary methyl group, d, ^3J = 6.0 Hz); 1.10 (3 H, secondary methyl group, d, ^3J = 6.0 Hz); 1.43 (3 H at C-19, s); 1.72 (3 H at C-6 of a L-rhamnose residue, d, ^3J = 6.0 Hz); 3.24 (3 H, methoxy group at C-22, s); 5.56 (H at C-6, d, ^3J = 5.0 Hz); 6.33 (H at C-1 of a L-rhamnose residue, br.s).

Hydrolysis and Methanolysis of Glycosides (IIa-IIb). A solution of 500 mg of the mixture of glycosides (IIa) and (IIb) in 100 ml of 4% aqueous sulfuric acid (v/v) was heated at 100°C for 8 h. The resulting precipitate was filtered off and was chromatographed on a column in system 2a. After the appropriate fractions had been recrystallized from ethanol, 200 mg of the genin (III) was obtained with mp 190-192°C, $[\alpha]_D^{20}$ −105.3 ±2° (c 1.19; pyridine), identical with an authentic sample of (25S)-ruscogenin.

The methanolysis of glycosides (IIa-IIb) and the working up of the reaction mixture were carried out as described for alliospiroside A (I). L-Rhamnose, L-arabinose, and D-glucose were identified by GLC (phase 1) in a ratio of 1.08:1.04:1.00. The presence of (25S)-ruscogenin was shown with the aid of TLC (system 2a).

Enzymatic Hydrolysis of the Glycosides (IIa-IIb). A solution of 500 mg of the product (IIa-IIb) in 50 ml of water was treated with 0.5 ml of the complex enzyme from the snail Helix plectotropis, and the mixture was left at 36°C for 16 h. The precipitate that had formed was filtered off, washed with water, dried, and chromatographed on a column in systems 2a and 2b. Recrystallization of the main reaction products from ethanol yielded 290 mg of a glycoside with mp 186-189°C, $[\alpha]_D^{21}$ −104.9 ± 2° (c 1.06; pyridine), identical with the native alliospiroside A (I).

Methylation of the Glycosides (IIa-IIb). A mixture of compounds (IIa-IIb) (1.0 g) was methylated and the reaction mixture was worked up as described in the preparation of the hexa-O-methyl ether of alliospiroside A (V). After column chromatography in system 2b, 900 mg of the amorphous hendeca-O-methyl ether of alliofuroside A (VII) was isolated: $C_{55}H_{94}O_{18}$, $[\alpha]_D^{19}$ −45.9 ± 2° (c 1.03); chloroform). ν_{max}^{Nujol} (cm^{-1}) 895 (weak broadened band). Signals corresponding to the absorption of hydroxy groups were absent. PMR spectrum ($CDCl_3$, δ, ppm): 0.79 (3 H at C-18, s); 0.95 (3 H, secondary methyl group, d, ^3J = 6.0 Hz); 0.98 (3 H, secondary methyl group, d, ^3J = 6.0 Hz); 1.03 (3 H at C-19, s); 1.25 (3 H at C-6 of an L-rhamnose residue, d, ^3J = 6.0 Hz); 3.33-3.63 (signals of the protons of 11 methoxy groups); 4.19 (H at C-1 of a D-glucose residue, d, ^3J = 8.0 Hz); 4.33 (H at C-1 of an L-arabinose residue, d, ^3J = 6.7 Hz); 5.23 H at C-1 of an L-rhamnose residue, d, ^3J = 1.7 Hz); 5.55 (H at C-6, br.d, ^3J = 5.0 Hz). Mass spectrum, m/z (%): 1042 (M^+, 0.06), 349 (10), 219 (3), 189 (100).

Acid Hydrolysis of the Permethylate of Alliofuroside A (VII). A solution of 600 mg of compound (VII) in 50 ml of 50% aqueous methanol containing 4% of sulfuric acid (by volume) was heated at the boil for 15 h. The cooled solution was treated with 50 ml of water, the methanol was distilled off, and the resulting resinous precipitate was separated by the decantation of the supernatant liquid. The aqueous solution of methylated sugars was heated at 100°C for 6 h and was then cooled, neutralized with ÉDÉ 10 P anion-exchange resin and evaporated to dryness. The residue (360 mg) was chromatographed on a column in system 3. The methylated sugars obtained were identified by TLC (system 3) and GLC (phase 2):

	T_{rel}	T_{rel} [9]
2,3,4-Tri-O-methyl-L-rhamnopyranose	0.43	0.46
2,3,4,6-Tetra-O-methyl-D-glucopyranose	1.00:1.43	1.00
3,4-Di-O-methyl-L-arabinopyranose	2.04	2.15

SUMMARY

Two new steroid glycosides have been isolated from the generative organs (pericarps and fruit stems) of Allium cepa L. (family Liliaceae): alliospiroside A, which is (25S)-spirost-5-ene-1β,3β-diol 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinospyranoside, and alliofuroside A, which has the structure of (25S)-furost-5-ene-1β3β,22α,36-tetraol 26-O-β-D-glucopyranoside 1-O-[O-α-L-rhamnopy ranosyl-(1 → 2)-α-L-arabinopyranoside].

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