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STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES FROM Nolina microcarpa

1. STRUCTURES OF NOLINOSPIROSIDE C AND NOLINOFUROSIDES A AND C

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In addition to the known steroid sapogenin (25S)-ruscogenin (I), three new glycosides have been isolated from the leaves of Nolina microcarpa S. Wats. (family Dracaenacea), and the following structures are suggested for them: (25S)-spirost-5-ene-1β,3β-diol 1-O-β-D-fucopyranoside (nolinospiroside C, II), (25S)-furost-5-ene-1β,3β,22α,26-tetraol 1-O-β-D-fucopyranoside (nolinofuroside A, III), and (25S)-furost-5-ene-1β,3β,22α,26-tetraol 1-O-β-D-fucopyranoside 26-O-β-D-glucopyranoside (nolinofuroside C, V).

Among plants of the Dracaenaceae family there are a few saponin-bearing ones [1, 2]. However, not one of the species of the genus Nolina belonging to this family has been studied as a producer of steroids of the spirostan and furostan series. In a methanolic extract of the leaves of Nolina microcarpa S. Wats. growing on the southern coast of the Crimea as a decorative plant we have established the presence of at least ten substances, most of which were assigned to derivatives of the spirostan and furostan series.

The present paper is devoted to proofs of the structures of three previously undescribed steroid glycosides. They have been called nolinospiroside C (II) and nolinofurosides A (III) and C(V).

On TLC, compounds (I) and (II) were revealed with vanillin/phosphoric acid in the form of yellow spots [2, 3] and were not stained by Ehrlich's reagent [4]. Their IR spectra contained in the 800-1000 cm^{-1} region a series of bands corresponding to the absorption of a (25S)-spiroketal grouping [5, 6].

Substances (III) and (V) were isolated in the form of mixtures – (III and IV) and (V and VI), respectively. The products of the color reactions of compounds (III)-(VI) with vanillin/phosphoric acid had a green coloration, and those with Ehrlich's reagent a red coloration. Heating aqueous solutions of the mixtures (II, IV) and (V, VI) led to the chromatographically homogeneous glycosides (III) and (V). Their IR spectra each contained a weak broadened band at 915 cm^{-1}. When solutions of the mixtures (III, IV) and (V, VI) in absolute methanol were heated they were transformed into the less polar components (IV) and

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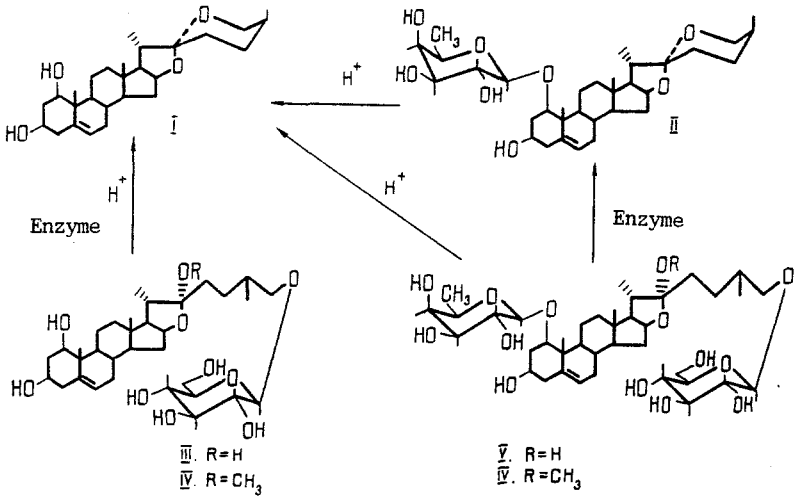
TABLE 1. Chemical Shifts of the ^{13}C Carbon Atoms of (25S)-Ruscogenin (I) Nolinospiroside C (II), and Nolinofurosides A (III) and C (V) (ppm, 0 – TMS; C_5D_5N)

C atom	Compound				C atom	Compound		
	I	II	III	V		II	III	V
1	78,12	84,00	78,19	83,98				
2	43,99	38,16	43,98	38,11			β-D-Fucose	
3	68,15	68,07	68,19	68,21	1	102,60		102,53
4	43,63	43,81	43,64	43,81	2	72,13		72,25
5	140,36	139,68	140,42	139,72	3	75,32		75,46
6	124,41	124,73	124,53	124,85	4	72,46		72,59
7	32,36	32,44	32,39	32,09	5	71,25		71,30
8	33,02	33,09	33,03	33,10	6	17,46		17,46
9	51,39	50,69	51,45	50,60				
10	43,63	42,92	43,64	42,94			β-D-Glucose	
11	24,25	23,84	24,26	23,88	1		105,21	105,17
12	40,64	40,46	40,62	40,63	2		75,28	75,26
13	40,26	40,26	40,62	40,63	3		78,53	78,48
14	56,99	57,12	56,97	57,09	4		71,78	71,78
15	32,47	32,03	32,76	32,75	5		78,67	78,63
16	81,22	81,25	81,19	81,25	6		62,88	62,88
17	63,04	62,99	64,15	64,04				
18	16,71	16,87	16,81	16,41				
19	13,96	14,88	13,99	14,91				
20	42,53	42,51	40,78	40,80				
21	14,94	14,88	16,49	14,91				
22	109,75	109,76	110,78	110,82				
23	26,45	26,43	37,24	37,17				
24	26,26	26,25	28,38	28,34				
25	27,59	27,58	34,49	34,48				
26	65,10	65,08	75,47	75,46				
27	16,36	16,35	17,51	17,07				

(VI), respectively. The above facts permit compound (II) to be assigned to the steroids of the (25S)-spirostan series, and (III)-(VI) to steroids of the furostan series. There was no doubt that substances (III) and (V) were glycosides of 22-OH furostanols, and (IV) and (VI) their 22-O-methyl ethers [2]. According to its physicochemical constants and spectral characteristics, spirostan (I) was identical with (25S)-ruscogenin [7].



As a result of the analysis of the products of the methanolysis of glycosides (II), (III), and (V) by the GLC method it was shown that the molecule of nolinospiroside C contained one D-fucose residue and that of nolinofuroside A one D-glucose residue, while the nolinofuroside C molecule contained D-glucose and D-fucose residues in a ratio of 1:1. This was also shown by their NMR spectra (Tables 1 and 2) [8, 9]. In all three cases, (25S)-ruscogenin (I) was identified among the methanolysis products. The aglycon (I) was also obtained after the acid hydrolysis of the monosides (II) and (III) and the bioside (V).

The enzymatic cleavage of glycosides (III) and (V) was carried out with the freeze-dried gastric juice of the grape snail Helix pomatia. The products of enzymatic hydrolysis proved to be identical with the genin (I) and the monoside (II), respectively.

TABLE 2. Chemical Shifts (δ, ppm, 0 – TMS; C_5D_5N) and Spin–Spin Coupling Constants (J, Hz) of the Protons of (25S)-Ruscogenin, Nolinospiroside C (II), and Nolinofurosides A (III) and C (V)

Proton	Compound			
	I	II	III	V
CH_3-18	0,91 s	0 80 s	0,86 s	0,84 s
CH_3-19	1,32 s	1,16 s	1,23 s	1,20 s
CH_3-21	1,11 d $J_{21,20}$=7,0	1,01 d $J_{21,20}$=7,0	1,20 d $J_{21,20}$=7,0	1,20 d $J_{21,20}$=6,5
CH_3-27	1,07 d $J_{27,25}$=7,0	1,01 d $J_{27,25}$=7,0	0,94 d $J_{27,25}$=6,5	0,97 d $J_{27,25}$=6,5
1a	3,77 dd $J_{1a,2a}$=11,5 $J_{1a,2e}$=4,0	3,70 dd $J_{1a,2a}$=11,5 $J_{1a,2e}$=4,5	3,68 dd $J_{1a,2a}$=11,5 $J_{1a,2e}$=4,3	3,77 dd $J_{1a,2a}$=11,5 $J_{1a,2e}$=4,0
3a	3,92 m $J_{3a,2a}$=$J_{3a,4a}$=11,0 $J_{3a,2e}$=$J_{3a,4e}$=5,5	3,81 m	3,80 m	3,86 m
6	5,60 br.d $J_{6,7}$=6,0	5,53 br.d $J_{6,7}$=5 5	5,48 br.d $J_{6,7}$=5,5	5,52 br.d $J_{6,7}$=5,0
16	4,51 dt $J_{16,15}$=8,0 $J_{16,15'}$=6,0 $J_{16,17}$=8,0	4,44 m	4,87 m	4,89 m
26	3,35 br.d	3,29 br.d	3,42 dd $J_{26,25}$=7,0	3,42 dd $J_{26,25}$=6.5
26′	4,05 dd $J_{26',25}$=2,7 $J_{26',26}$=11,0	3,99-dd $J_{26',25}$=4,0 $J_{26',26}$=11,5	3,99 dd $J_{26',25}$=5,0 $J_{26',26}$=9,0	4,02 dd $J_{26',25}$=6,0 $J_{26',26}$=9,5
			β-D-Glucose	
1			4,70 d $J_{1,2}$=7,5	4,74 d $J_{1,2}$=7,5
2			3,92 dd $J_{2,3}$=8,8	3,96 dd $J_{2,3}$=9,0
3			4,16 t $J_{3,4}$=8,8	4,21 t $J_{3,4}$=9.0
4			4,06 t $J_{4,5}$=8,8	4,15 t $J_{4,5}$=9,0
5			3,85 m	3,88 m
6			4,22 dd $J_{6,5}$=5,5 $J_{6,6'}$=11,5	4,30 dd $J_{6,5}$=5,5 $J_{6,6'}$=11,5
6′			4,43 dd $J_{6',5}$=2,5	4,48 dd $J_{6',5}$=2,5
		β-D-Fucose		β-D-Fucose
1		4,64 d $J_{1,2}$=8,0		4,69 d $J_{1,2}$=7,5
2		4,21 dd $J_{2,3}$=9,0		4.28 dd $J_{2,3}$=9,0
3		3,99 dd $J_{3,4}$=3 5		4,04 dd $J_{3,4}$=3,5
4		3,95 m		4,00 dd $J_{4,5}$=1,5
5		3,69 m		3,68 dq $J_{5,6}$=6,5
6		1,50 d $J_{6,5}$=6,5		1,52 d $J_{6,5}$=6 5

The configurations of the glycosidic centers, the dimensions of the oxide rings, and the sites of attachment of the monosaccharide residues in the molecules of compounds (II), (III), and (V) were established by NMR methods. In the recording of the PMR spectra we used double homonuclear resonance and the observation of nuclear Overhauser effects (NOEs); in the ^{13}C NMR spectra we used the APT (attached-proton test).

The spin–spin coupling constants (SSCCs) of the anomeric protons of the glucose and fucose residues in the PMR spectra of glycosides (II), (III), and (V) ($J_{1,2}$ = 7.5-8.0 Hz, Table 2) showed the β-configurations of the corresponding glycosidic bonds and the pyranose forms of the oxide rings of the monosaccharides.

A comparison of the chemical shifts (CSs) of the carbon atoms of the genin (I) and of the monoside (II) (Table 1) showed the attachment of the sugar residue to C-1 of the aglycon (Δ_{C-1} = +5.88; Δ_{C-2} = −5.83 ppm). This was confirmed by the observation of an NOE showing the spatial propinquity of H-1 of the β-D-fucopyranose residue and H-1 of the aglycon. Consequently, nolinospiroside C (II) has the structure of (25S)-spirost-5-ene-1β,3β-diol 1-O-β-D fucopyranoside.

The results of the enzymatic hydrolysis of glycosides (III) and (V) and also a comparative analysis of the spectral characteristics of compounds (I), (II), (III), and (V) (Tables 1 and 2) convincingly showed that in the monoside (III) and in the bioside (V) a β-D-glucose residue was attached at C-26 of the aglycon, and in the bisdesmoside (V) a β-D-fucose residue at its C-1 atom. As well as by the effects of glycosylation (Table 1), this was confirmed by the observation of NOEs after the preirradiation of the anomeric protons of the sugar residues, which showed the propinquity of H-1 of the β-D-glucopyranose residue to the 2 H-26 atoms in (III) and in (V) and also of H1 of the β-D-furanopyranose residue to H-1 of the aglycon in the bioside (V). Thus, nolinofurosides A and C are, respectively, the 26-O-β-D-glucopyranoside and the 1-O-β-D-fucopyranoside; 26-O-β-D-glucopyranoside of (25S)-spirost-5-ene-1β,3β,22α,26β-tetraol.

EXPERIMENTAL

General Remarks. Thin-layer chromatography (TLC) was conducted on plates coated with silica gel L (Czechoslovakia) and silica gel KSK (Estonia). The conditions for performing column chromatography are given below. The following solvent systems were used: 1) chloroform–methanol [a) 20:1; b) 10:1] and 2) chloroform–methanol–water [a) 65:10:1; b) 65:15:2; c) 65:22:4].

Gas–liquid chromatography (GLC) was conducted on a Biokhrom-1 chromatograph. Monosaccharides were chromatographed in the form of trimethylsilyl ethers of methyl glycosides. A glass capillary column (0.25 mm × 50 m) filled with the phase OV-101 was used. The thermostat temperature was 160°C and the carrier gas helium at the rate of 4 ml/min. IR spectra were recorded on UR-20 instrument in tablets with KBr, and mass spectra on a MKh-1310 instrument at an ionizing voltage of 50-70 V; the temperature of the ion source was 180-260°C and the inlet temperature 100-250°C. PMR spectra were obtained on WM-250 and AM-300 instruments (Bruker), the solvent being C_5D_5N. 0 – TMS.

Analytical samples of the compounds described were dried in vacuum at room temperature or at the boiling point of methanol, benzene, or toluene.

Melting points were determined on a Boëtius stage and optical rotations on a Zeiss polarimeter in a tube 0.5 dm long.

Isolation and Purification of (25S)-Ruscogenin (I), Nolinospiroside C(II), and Nolinofurosides A and C (III and IV) and Their 22-O-Methyl Ethers (V and VI). Leaves of Nolina microcarpa were gathered in the Nikitskii botanical garden in December, 1988. The freshly gathered raw material (4.5 kg) was exhaustively extracted with 70% aqueous ethanol (6 × 6 liters). After the elimination of the alcohol, the resinous residue of extractive substances (1 liter) was diluted twofold with water, and the lipophilic components were extracted with hexane (4 × 0.3 liter). The hexane extract contained practically none of the desired substances. Then the aqueous solution was extracted with n-butyl alcohol (6 × 0.4 liter).

The combined butanolic extracts (150 g) were chromatographed on a column of silica gel KSK (particle size 10-100 μm) using a stepwise elution gradient (chloroform–methanol 10:1 → 1:1). The resulting fractions, enriched with compounds (I), (II), (III and IV), and (V and VI), were subjected to rechromatography on columns of silica gels L and KSK (particle size

63-100 μm). For the above-described chromatographic cycles we used glass columns filled by the "dry" method or with a suspension of the sorbent in chloroform, with the outflow of the eluate under the action of its intrinsic weight at the rate of 1-2 ml/min. This proved sufficient for obtaining the chromatographically homogeneous substances (I) and (II).

It was possible to isolate glycosides (III and IV) and (V and VI) in the form of mixtures free from pigments and other impurities after chromatography on Silpearl silica gel (Czechoslovakia; particle size 10-30 μm), Chromatography was conducted in stainless steel columns filled by the "dry" method. To overcome the resistance of the sorbent we used a modified liquid pump of the MS type (Czechoslovakia). The rate of flow of the eluent was 6 ml/min, the pressure at the column inlet being 10 ± 0.25 kPa.

(25S)-Ruscogenin (I). The fractions enriched with genin (I) were rechromatographed in system 1a. Recrystallization from methanol gave 0.20 g of (25S)-ruscogenin, $C_{27}H_{42}O_4$. mp 188-190°C, $[\alpha]_D^{20}$ −95 ± 2° (c 1.01; pyridine). According to the literature: mp 190-192°C, $[\alpha]_D$ −105 ± 2° [7]. The IR, mass, and NMR spectra of the sample obtained were practically identical with those given in the literature. Yield 0.004% (here and below, calculated on the weight of the freshly gathered plant).

Nolinospiroside C (II). The rechromatography of the relevant fractions was carried out in system 1b. Recrystallization from methanol gave 0.16 g of nolinospiroside C, $C_{33}H_{52}O_8$, mp 209-210°C, $[\alpha]_D^{20}$ −98 ± 2° (c 0.96; pyridine). ν_{max}^{KBr}, cm^{-1}: 860, 910 < 930, 990 [spiroketal chain of the (25S) series], 3200-3600 (OH). M^+ 576. Yield 0.003%.

Nolinofuroside A (III). Rechromatography of the fraction containing glycosides (III and IV) in system 2a gave 0.30 g of a purified mixture of (III and IV) containing no other components. After this mixture had been heated in 100 ml of water at 60°C for 18 h and the solvent had been driven off, the amorphous glycoside (III) was obtained: $C_{33}H_{54}O_{10}$ $[\alpha]_D^{20}$ −56 ± 2° (c 1.01; pyridine). ν_{max}^{KBr}, cm^{-1}: 915 (broadened low-intensity band), 3200-3600 (OH). $(M - H_2O)^+$ 592. Yield 0.006%.

Nolinofuroside C (V). A mixture of glycosides (V and VI) (8.00 g) was obtained by the repeated chromatography of the enriched fractions in systems 2b and c. A solution of the mixture of (V and VI) (300 mg) in 100 ml of water was heated at 60°C for 18 h. After evaporation of the solvent, the amorphous glycoside (V) was obtained: $C_{39}H_{64}O_{14}$, $[\alpha]_D^{20}$ −38 ± 2° (c 1.20; pyridine), ν_{max}^{KBr}, cm^{-1}: 915 (weak broadened band), 3200-3600 (OH) $(M - H_2O)^+$ 738. Yield 0.177% (here and in the preceding case the yield was calculated on the weight of the mixture of glycosides of the furostan series and 22-O-methyl ethers). For the NMR spectra of steroids (I), (II), (III), and (V), see Tables 1 and 2.

Methanolysis of Glycosides (II), (III), and (V). Samples of compounds (II), (III), and (V) (10.0 mg each) were dissolved in absolute methanol containing 5% of hydrogen chloride (3 ml in each case). The solutions were heated at the boil for 14 h. After cooling, the reaction mixtures were diluted with an equal volume of water and were filtered. (25S)-Ruscogenin was identified in the precipitates (TLC, system 1a). The filtrates were neutralized with silver carbonate and were evaporated to dryness. After silylation of the methyl glycosides, the trimethylsilyl derivatives were chromatographed (GLC) as described in the General Remarks section. It was established that the carbohydrate components of nolinospiroside C and nolinofuroside A were D-fucose and D-glucose, respectively, and those of nolinofuroside C were D-glucose and D-fucose in a ratio of 1.00:0.96. Then, for the fucoside (II) and the glucoside (III) all the operations described above were repeated, using D-glucose as internal standard in the first case and L-rhamnose in the second. Calculation showed that both compounds were monosides.

Acid Hydrolysis of Glycosides (II), (III), and (V). The monosides (II) (50 mg) and (III) (100 mg) and the bioside (100 mg) were each dissolved in 50% aqueous methanol containing 4% of sulfuric acid. The solutions were heated to the boil for 8 h. Each of the cooled reaction mixtures was treated with 50 ml of water, after which the methanol was distilled off. The aqueous suspensions were filtered, and the residues were washed with water, dried, and chromatographed on columns of silica gel in system 1a. Recrystallization of the appropriate fractions from methanol in each case gave (25S)-ruscogenin, shown to be identical with an authentic sample on the basis of physicochemical constants and IR, mass, and PMR spectra.

Enzymatic Hydrolysis of Glycosides (III) and (IV). Compounds (III) and (V) (100 mg each) were dissolved in water (100 ml), and the freeze-dried gastric juice of the grape snail, Helix pomatia, (20 mg in each case) was added, after which the solutions were stirred at 38°C for 20 h. Then the reaction mixtures were evaporated to dryness, the residues were dissolved in the chloroform–methanol (1:1) system, and the solutions were filtered. The filtrates were evaporated to dryness and the residues were chromatographed on silica gel columns, with the use of systems 1a and 1b, respectively, for purifying the products of the fermentation of glycosides (III) and (V). This gave, in the first case, 30 mg of (25S)-ruscogenin, and, in the second case, 35 mg of (25S)-ruscogenin 1-O-β-D-glucopyranoside. The reaction products were identified from their chromatographic mobilities in comparison with authentic samples and from their physicochemical constants and spectral characteristics (IR, mass, and PMR spectra).

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STEROIDS OF THE FUROSTAN AND SPIROSTAN SERIES FROM Nolina microcarpa

II. STRUCTURES OF NOLINOSPIROSIDE D AND NOLINOFUROSIDES D, E, AND F

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Proofs are given of the structures of two new glycosides of the furostan series isolated from the leaves of the plant Nolina microcarpa S. Wats. (family Dracaenaceae). Nolinofuroside D is (25S)-furost-5-ene-1β,3β,22α,26-tetraol 1-O-β-D-galactopyranoside 26-O-β-D-glucopyranoside (I), and nolinofuroside F is (25S)-furost-5-ene-1β,3β,22α,26-tetraol 1-O-β-D-fucopyranoside 26-O-β-D-glucopyranoside 3-O-α-L-rhamnopyranoside (VII). The latter was characterized as its 22-O-methyl ether (VIII). Nolinofuroside E (IV) has the structure of (25S)-furost-5-ene-1β,3β,22α,26-tetraol 26-O-β-glucopyranoside 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside], which followed from the structure of the fermentation product (VI). The products of the fermentation of the above-named compounds were present in the plant in only trace amounts. Only one of them – nolinospiroside D (III) – has not been described previously. This monoside of the spirostan series is (25S)-spirost-5-ene-1β,3β-diol 1-O-β-D-galactopyranoside.

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