Hydrogenation of Dihydroisoalantolactone with Raney Nickel. To a solution of 0.25 g of DIA in 20 ml of ether alcohol was added 0.5 ml of freshly prepared Raney nickel. The reaction was conducted at 60°C for 3 h. The solution was filtered and was diluted with water (1:1). The precipitate was separated off and was recrystallized from aqueous acetone (1:1) and dried under vacuum over P_2O_5 at 50-60°C for 4 h. This gave 0.1 g of tetrahydroalantolactone.

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STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES FROM Nolina microcarpa

III. STRUCTURE OF NOLINOFUROSIDES G AND H

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Two new glycosides which have been called nolinofurosides G(I) and H(III), have been isolated from the leaves of Nolina microcarpa. Nolinofuroside G is the sodium salt of 26-β-D-glucofuranosyloxy-(25S)-furost-5,20(22)-diene-1β, 3β-diol 1-sulfate, and nolinofuroside H is the sodium salt of 1-β-D-fucopyranosyloxy-26-β-D-glucopyranosyloxy-(25S)-furost-5,20(22)-dinen-1β-3β-ol 3-sulfate.

In preceding communications we have described proofs of the structures of five new glycosides of the furostan series and their spirostan analogs isolated from the leaves of Nolina microcarpa S. Wats. (family Dracaenacea) [1]. The present paper is devoted to a determination of the structures of the most polar components of the butanolic fraction of the total extractive substances from this plant – nolinofurosides G (I) and H (III).

Compounds (I) and (III) were colored green by vanillin phosphoric acid, and red by the Ehrlich reagent (TLC), which is characteristic of glycosides of the furostan series [2-4]. In contrast to furostan glycosides decribed previously, which were isolated in the form of mixtures of 22-OH derivatives and their 22-O-methyl ethers [1], substances (I) and (III) were isolated in the individual state. In the "fingerprint" region their IR spectra differed substantially from the IR spectra of glycosides of the furostan series]5].

The ^{13}C NMR spectrum of compound (I) lacked a signal in the 109-112 ppm region corresponding to the C-22 resonance of steroids of the furostan series [6] and contained signals at 152.47 and 103.73 ppm (Table 1). According to the APT (attached proton test), both singlets were due to the resonance of quaternary carbon atoms.

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TABLE 1. Chemical Shifts of the Carbon Atoms of Nolinofurosides G (I) and H (III) and of the products of their Desulfation (II and IV) ppm 0 – TMS, C_5D_5N

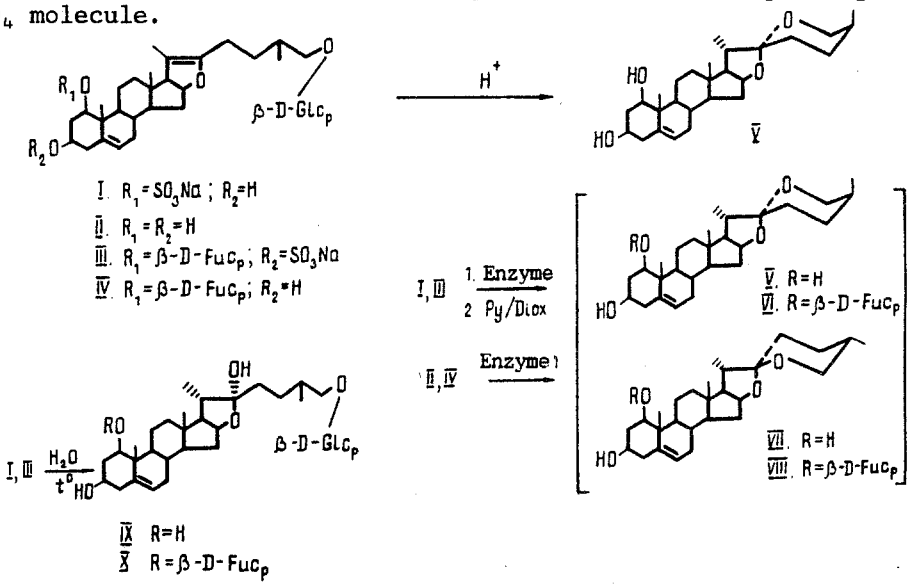
C-atom	I	II	III	IV	C-atom	I	II	III	IV
						D-Glucose			
1	85,17	78,44	83,78	83,96					
2	39,67	43,99	32,52	38,92	1	105,07	105,21	105,29	105,31
3	67,97	68,21	75,28	68,23	2	75,16	75,26	74,85	75,34
4	43,53	43,67	40,52	43,93	3	78,26	78,67	78,22	78,61
5	138,78	140,52	138,25	139,81	4	71,95	71,94	71,94	71,81
6	125,57	124,42	126,25	124,89	5	78,53	78,22	78,56	78,70
7	32,41	32,52	32,81	32,91	6	62,98	64,04	63,01	62,95
8	33,78	33,82	33,88	33,85					
9	49,96	51,45	50,55	50,58		D-Fucose.			
10	43,18	43,29	43,26	43,29					
11	23,91	24,45	23,78	24,06	1			102,53	102,66
12	40,57	40,52	40,52	40,34	2			72,22	72,32
13	43,18	43,62	42,84	42,93	3			75,28	75,51
14	55,09	55,38	55,40	55,49	4			72,64	72,66
15	32,86	32,84	32,19	32,20	5			71,18	71,34
16	84,57	84,59	84,62	84,65	6			17,47	17,56
17	64,84	64,94	64,81	64,84					
18	14,45	14,44	14,65	14,63					
19	14,78	13,94	14,65	14,97					
20	103,73	103,73	103,91	103,92					
21	11,76	11,79	11,90	11,90					
22	152,47	152,56	152,40	152,40					
23	23,72	23,75	23,78	23,78					
24	31,49	31,52	31,53	31,54					
25	34,74	34,84	34,85	34,88					
26	75,16	75,26	75,34	75,34					
27	17,24	17,25	17,28	17,30					

TABLE 2. Chemical Shifts (δ, ppm, 0 – TMS, C_5D_5N) and Spin–Spin Coupling Constants (J, Hz) of the Protons of Nolinofurosides G (I) and H (III) and of the Products of their Desulfation (II and IV)

Protons of the aglycon	I	II	III	IV
CH_3-18	0,69 s	0,78 s	0,64 s	0,60 s
CH_3-19	1,28 s	1,34 s	1,03 s	1,11 s
CH_3-21	1,53 s	1,58 s	1,48 s	1,47 s
CH_2-27	0,99 d $J_{27,25}=7,0$	1,01 d $J_{27,25}=7,0$	0,95 d $J_{27,25}=7,0$	0,94 d $J_{27,25}=7.0$
H-1a	4,77 m	3,80 m		
H-3a	3,90 m	3,96 m		
6	5,57 br.d $J_{6,7}=5,0$	5,61 br.d $J_{6,7}=5,5$	5,48 br.d $J_{6,7}=5,6$	5,46 br.d $J_{6,7}=5-5$
16	4,80 m	4,80 m		
26	3,45 m	3,45 d.d $J_{26,26}=10.0$ $J_{26,25}=7,0$	3,39 d.d $J_{26,26}=10.0$ $J_{26,25}=7.0$	3,39 d.d $J_{26,26}=10,0$ $J_{26,25}=7,2$
26	3,93 m	4,05 d.d $J_{26,25}=6.0$	4,04 br.d	3,97 d.d $J_{26,25}=6,5$
	β-D-Glucoe			
1	4,79 d $J_{1,2}=7,5$	4,82 d $J_{1,2}=8,0$	4,75 d $J_{1,2}=8,0$	4,72 d $J_{1,2}=8,0$
2	4,00 m	3,95 dd $J_{2,3}=9,5$	3,95 t $J_{2,3}=8,0$	3,93 t $J_{2,3}=8,0$
3	4,22 m	4,25 m	4,21 t $J_{3,4}=8,0$	4,15 t $J_{3,4}=8,0$
4	4,22 m	4,25 m	4,13 t $J_{4,5}=8,0$	4,08 t $J_{4,5}=8,0$
5	4,00 m	3,95 m	3,90 m	3,84 m
6	4,35 dd $J_{6,6}=12,0$ $J_{6,5}=5,0$	4,40 $J_{6,6}=11,5$ $J_{6,5}=5,0$	4,23 m	4,21 dd $J_{6,5}=6,0$
6	4,51 $J_{6,5}=2,5$	4,56 br.d	4,47 dd $J_{6,6}=12,0$ $J_{6,5}=2,5$	4,44 dd $J_{6,6}=12,0$ $J_{6,5}=2,0$
	β-D-Fucose			
1			4,58 d $J_{1,2}=8,0$	4.65 d $J_{1,2}=8,0$
2			4,22 t $J_{2,3}=8,0$	4 24 t $J_{2,3}=8,0$
3			4,17 t $J_{3,4}=8,0$	4,03 dd $J_{3,4}=7,0$
4			3,99 br.d	3,99 br.d
5			3,60 q $J_{5,6}=6,5$	3,64 q $J_{5,6}=7,0$
6			1,48 d	1,46 br.s

In the PMR spectrum of the glycoside (I) (Table 2), the protons of the four methyl groups resonated in the form of three singlets and one doublet, while in the steroids of furostan series two methyl groups are secondary and two are tertiary. The features mentioned also appear in the NMR spectra of nolinofuroside H (see Tables 1 and 2). Such spectral features are characteristic of derivatives of the $\Delta^{20(22)}$-furostan (pseudofurostan) series [7-10]. Natural representatives of this group of steroids have been described in [7, 8] and in earlier papers [5, 9-12] the prdoucts of the transformations of the genins and glycosides of the furostan series. Among the products of the methanolysis and acid hydrolysis of compound (I) we identified (25S)-ruscogenin (V) [13] and D-glucose, and in the case of glycoside (III) D-fucose, in addition. Quantitative analysis of the monosaccharides showed that substances (I) and (III) were mono- and biosides, respectively. We obtained the same results after acid cleavage of nolinofurosides A and C [1].

The considerably higher polarity of the monoside (I) and the bioside (III) is a consequence of the fact that the molecules of these glycosides contain polar residues. In the IR spectrum of nolinofuroside G and that of nolinofuroside H there is a strong broad band at 1200-1300 cm^{-1}. In the electron-impact mass spectrum of each of them there is a peak with m/z 64 (SO_2 [14] and in the LSIMS spectra the peaks of ions corresponding to the splitting out of a $NaHSO_4$ molecule.



When a solution of barium chloride was added to the hydrolysates of compound (I) and (III) a precipitate formed. Sodium was detected in the ash after the combustion of substances (I) and (III). Thus, glycosides (I) and (III) were the sodium salts of pseudofurostanol sulfates.

Sulfuric esters of steroids of the spirostan and furostan series form a comparatively small group of compounds [15-19]. No sulfates of pseudofurostanols have been described previously.

Glycosides (I) and (III) were desulfated by heating in a mixture of anhydrous pyridine and anhydrous dioxane [15-18]. On methanolysis, the desulfation products (II) and (IV) formed the same aglycon and monosaccharides as the initial substances.

By the enzymatic cleavage of a bisdesmosidic glycoside of a pseudofurostandiol, Liang et al. [7] obtained the monodesmosidic degluco analog with a free hydroxy group at C-26. On the enzymatic hydrolysis of the monoside (II), the reaction product was an aglycon practically identical with (25S)-ruscogenin (V) so far as concerned its chromatographic behavior and its IR and mass spectra. However, the NMR spectra showed that the substance obtained was not an individual compound.

The fermentation of glycoside (I) with subsequent desulfation also gave a mixture of the genins (V) and (VII). The same rections with the biosides (III) and (IV) led to a mixture of glycosides (VI) and (VIII) identical with nolinospiroside C [1] according to R_f values and IR and mass spectra.

R. Tschesche et al. [5] consider that the acid hydrolysis of a pseudofurostan glycoside first produces a cyclopseudo form and only then an aglycon of this spirostan series. Starting

from this, we assumed that compounds (VII) and (VIII) were cyclopseudo analogs of spirostans (V) and (VI), respectively. A convincing proof, in our view, of the hypothesis put forward was the formation of the genin (V), identical with an authentic sample of (25S)-ruscogenin in all its parameters when the mixtures (V)/(VII) and (VI)/(VIII) were treated with an acid.

When aqueous solutions of compounds (I) and (III) were heated, the glycosides (IX) and (X), identical with nonlinofurosides A and C [1], were obtained. This unambiguously determined the β-configurations of the glycosidic centers and the pyranose forms of the oxide rings of the monosaccharides, and also the positions of thir attachment to the aglycons (D-glucose at C-26, and D-fucose in bioside (III) at C-1).

The positions of the esterifying groups were established as the result of a comparative analysis of the ^{13}C NMR spectra (see Table 1) and the PMR spectra (see Table 2) of substances (I-IV). On passing from the desulfation product (II) to the sulfate (I) only the chemical shifts (CSSs) of C-1 and C-2 of the steroid part of the molecule underwent appreciable changes (Δ_{C-1} = +6.73; Δ_{C-2} = −4.32 ppm). A downfield shift of the signal of H-1 of the aglycon was observed in the PMR spectra (Δ_{H-1} = +0.97 ppm). Consequently, nonlinofuroside G (I) is the sodium salt of β-D-glucopyranosyloxy-(25S)-furost-5,20(22)-diene-1β-diol 1-sulfate.

The existence of α- and β-effects of esterification on the transition from glycoside (IV) to compound (III) showed the localization of the sulfate group at C-3 of the aglycon (Δ_{C-3} = +7.05; Δ_{C-2} = −6.41; Δ_{C-4} = −3.4 ppm). The value of Δ_{C-2} is due to the additivity of the β-effects of glycosylation and esterification. Thus, nolinofuroside H (III) is 1-β-D-fucopyranosyloxy-26-β-D-glucopyranosyloxy-(25S)-furost-5,20(22)-diene-3β-ol 3-sulfate.

EXPERIMENTAL

General Observations. The following solvent systems were used for chromatography: 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); d) (65:30:6); e) 65:35:8); and f) (65:40:10)].

Semiquantitative spectral analysis was performed on a STÉ-1 spectrograph. According to the results of the semiquantitative analysis, the amount of Na in the ash of compounds (I) and (II) was of the order of fractional numbers (%), while other elements were detected in trace amounts (<0.01%). Other information has been given previously [1].

Nolinofuroside G (I). Repeated rechromatography of the enriched fractions [1] in systems 2d and 2e gave 2.0 g of a glycoside $C_{33}H_{51}O_8 \cdot SO_3Na$ (amorph.), $[\alpha]_D^{20}$ −38±2° (c 1.01; pyridine), ν_{max}^{KBr} cm^{-1}: 1200–1300 (ester bond) 3200–3600 (OH).

LSIMS spectra: $(M + Na)^+$ 717; $(M + Na - NaHSO_4)^+$ 597; $(M + H)^+$ 695. Yield 0.066% (calculated on the weight of the freshly gathered plant).

Nolinofuroside H (III). Rechromatography of the appropriate fractions [1] was carried out in systems 2d, e, and f. This led to the isolation of 4.0 g of glycoside (III), $C_{39}H_{61}O_{13} \cdot SO_3Na$ (amorph.), $[\alpha]_D^{20}$ −42±2° (c 1.03; pyridine), ν_{max}^{KBr}, cm^{-1}: 1200–1300 (ester bond), 3200–3600 (OH).

LSIMS spectra: $(M + Na)^+$ 863; $(M + Na - NaHSO_4)^+$ 743; $(M + H)^+$ 841. Yield 0.088%.

2-β-D-Glucopyranosyloxy-(25S)-furost-5,20(22)-diene-1β,3β-26-triol (II) from (I). A solution of 500 mg of glycoside (I) in a mixture of anhydrous pyridine and anhydrous dioxane (4:1) was heated at 120°C for 3 h. Then the solvents were distilled off and the residue was chromatographed in system 2a. After recrystallization of the appropriate fractions from methanol, 160 mg of compound (II) was obtained. $C_{33}H_{52}O_9$, mp 110-112°. $[\alpha]_D^{20}$ −18 ± 2° (c 1.00; pyridine). Its IR spectrum lacked absorption at 1200-1300 cm^{-1}; M^+ 592.

1β-(β-D-Fucopyranosyloxy)-26-β-D-glucopyranosyloxy-(25S)-furost-5,20(22)-diene-3β-ol (IV) from (II). Glycoside (III) (500 mg) was desulfated as described above. The reaction product was chromatographed in system 26. Then crystallization from methanol gave 140 mg of substance (IV), $C_{39}H_{62}O_{13}$, mp 125-128°, $[\alpha]_D^{20}$ −25 ± 2° (c 1.10; pyridine). Its IR spectrum lacked a band at 1200–1300 cm^{-1}. M^+ 738. For the NMR spectra of compounds (I-IV), see Tables 1 and 2.

(25S)-Ruscogenin (V) from (I-IV). Glycoside (III) (50 mg) was hydrolyzed, and the reaction mixture was worked up as described in [1]. The reaction product was chromatographed in system A, giving 15 mg of the aglycon (V), $C_{27}H_{42}O_4$, mp 188-190°C (from methanol).

$[\alpha]_D^{20}$ −95.5 ± 2° (c 1.00; pyridine). According to the literature: mp 190-192°C, $[\alpha]_D^{21}$ −105.6° [13].

The enzymatic cleavage of glycosides (I-IV) (300 mg each) was carried out with the freeze-dried gastric juice of the snail Helix pomatia. The reaction mixtures were worked up as described in [1]. The products of the fermentation of compounds (II) and (IV) were chromatographed in systems 1a and 1b. After recrystallization from methanol, 180 g of the mixture of aglycons (V/VII) and 160 mg of a mixture of the glycosides (VI/VIII) were obtained. The products of the fermentation of glycosides (I) and (III) were desulfated, and the reaction mixtures were worked up as described above. As in the preceding case, mixtures of genins (V/VII) (50 mg) and of the monosides (VI/VIII) (40 mg) were obtained.

The mixtures of compounds (V/VII) and (VI/VIII) (20 mg each) were dissolved in 50% aqueous methanol containing 4% of concentrated sulfuric acid (10 ml in each case), and the reaction mixtures were boiled for 10 h. For the working up of the reaction mixtures after acid hydrolysis, see [1]. The reaction products were chromatographed in system 1a. In both cases the aglycon (V) (18 mg and 10 mg, respectively), identical with an authentic sample of (25S)-ruscogenin with respect to R_f, physicochemical constants, and IR, mass, and NMR spectra was obtained.

1β-(β-D-Fucopyranosyloxy)-26-β-D-glucopyranosyloxy-(25S)-furost-5-ene-1β-3β,22α-diol(X) from (III). A solution of 300 mg of glycoside (III) in 50 ml of water was heated at 90°C for 3 h. Then the solvent was distilled off, the residue was dissolved in chloroform–methanol (1:1) and the solution was filtered. The filtrate was dried and evaporated, after which the residue was chromatographed in system 2b. This gave 50 mg of the amorphous glycoside (X), identical with nolinofuroside C [1] with respect to R_f, $[\alpha]_D$, and IR, mass, and NMR spectra. When the operations described were repeated with glycoside (I) (15 mg), the product ws identified as nolinospiroside A (TLC, system 2a).

Methanolysis of the Glycosides (I), (II), (III), (IV), (VI/VIII), and (X). Samples of these compounds (10 mg each) were cleaved, and the reaction mixtures were worked up as described in [1]. It was shown that substances (I) and (II) were monoglucosides, (VI-VIII) monofucosides, and (III) and (IV) each contained one residue of d-glucose and one of D-fucose. In all cases, (25S)-ruscogenin (V) was identified among the methanolysis products (TLC, system 1a).

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