STEROIDS OF THE SPIROSTAN AND FUROSTAN SERIES FROM Nolina microcarpa

IV. STRUCTURES OF NOLINOGENIN, NOLINOSPIROSIDE B, AND NOLINOFUROSIDE B

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A glycoside has been isolated from the plant Nolina microcarpa S. Wats. (family Dracaenaceae) which has been called nolinofuroside B and has the structure of 6β-methoxy-3β,5-cyclo-(25S)-furostan-1β,22α,26-triol 1-O-β-D-fucopyranoside 26-O-β-D-glucopyranoside (I). Enzymatic hydrolysis of the bioside (I) yielded nolinospiroside B (III) and nolinogenin (IV). The latter is 6β-methoxy-3β,5-cyclo-(25S)-spirostan-6β-ol, and glycoside (III) is its 1-O-β-D-fucopyranoside.

In preceding communications we have given proofs of the structures of five new glycosides of the furostan series and their spirostan analogues [1], and also of two natural sulfated glycosides of the pseudofuroastan series [2], from the leaves of the plant Nolina microcarpa S. Wats. (family Dracaenaceae). The present paper is devoted to a proof of the structure of a new furostanol glycoside – nolinofuroside B – isolated from the same source and also those of the nolinospiroside B (III) and nolinogenin (IV) obtained as the result of its enzymatic hydrolysis.

Compound (I) was isolated in the form of a mixture of two components, (I) and (II), with close R_f values the products of the color reactions (TLC) of which with vanillin/phosphoric acid were green and with Ehrlich's reagent red [3-5]. Heating an aqueous solution of compounds (I) and (II) led to a chromatographically homogeneous glycoside, while heating the same mixture in absolute methanol formed the less polar component (II). The above facts show that substance (I) was a 22-OH furostanol and substance (II) its 22-O-methyl ether [3].

Analysis of the products of the methanolysis of glycoside (I) by GLC showed that the nolinofuroside B molecule contained D-glucose and D-fucose residues in a ratio of 1:1, and this also followed from its PMR spectrum (Tables 1 and 2) [6, 7].

The enzymatic hydrolysis of bioside (I) gave a spirostan glycoside – nolinospiroside B (III) – and an aglycon, which we have called nolinogenin (IV). Substances (III) and (IV) were colored yellow by vanillin/phosphoric acid [4]. Their IR spectra contained absorption bands of a (25S)-spiroketal grouping [8, 9].

The nature of the mass-spectrometric fragmentation of the aglycon (IV) and the elementary compositions of the following ions – [M^+ 444 ($C_{28}H_{44}O_4$), M^+ – CH_3OH – 412 ($C_{27}H_{40}O_3$), a – 385 ($C_{25}H_{37}O_3$), b – 375 ($C_{23}H_{35}O_4$), c – 372 ($C_{24}H_{36}O_3$), d – 330 ($C_{22}H_{34}O_2$), f – 301 ($C_{20}H_{29}O_2$), g – 139 ($C_9H_{15}O$)] – showed that nolinogenin was a mono-O-methoxylated diol [10].

The structure of aglycon (I) was shown with the aid of NMR spectroscopy (double resonance; NOE – nuclear Overhauser effect; homo- and heteronuclear 2D spectroscopy; APT – attached proton test).

The presence in the strong field of the PMR spectrum of genin (IV) taken in C_5D_5N (see Table 1) of the signals of methylene protons at 0.66 and 1.73 ppm with the geminal spin-spin coupling constant J = 4.0 Hz convincingly showed the presence of a cyclopropane ring in the spirostan (IV) molecule [11].

In the PMR spectrum there were two signals with chemical shifts (CSs) of 4.21 and 2.73 ppm, corresponding to the resonances of protons geminal to oxygen-containing substituents. As a result of the observation of NOEs it was shown that these protons were spatially close

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TABLE 1. Chemical Shifts (δ, ppm, 0 – TMS) and Spin-Spin Coupling Constants (J, Hz) of the Protons of Nolinofuroside B (I), Nolinospiroside B (III), and Nolinogenin (IV) (C_5D_5N)

Proton	I	III	IV	IV*
H-1	4,32 m	4,29 m	4,21 d	4,06 d $J_{1,2}=6,5$
H-2			2,37 m $J_{2,2}=13,5$	2,25 m
H-2′			2,00 m	1,86 d $J_{2,2}=13,5$
H-3	0,94 m	0,93 m	0,98 m	0,89 ddd $J_{3,2}=8,0$ $J_{3,4}=4.0$
H-4	0,50ddd $J_{4,4}=4,5$ $J_{4,3}=8,0$	0,49 ddd $J_{4,4}=4.5$ $J_{4,3}=8,0$	0,66	0,55 ddd $J_{4,4}=4,0$ $J_{4,3}=8,5$ $J_{4,2}=1,5$
H-4′			1,73	1,43 t $J_{4',3}=4,0$
H-6	2,68 m	2,68 t $J_{6,7}=J_{6,7'}==2,5$	2,73	2,67 t $J_{6,7}=J_{6,7'}=3,0$
H-7			1,08 m $J_{7,7'}=12,5$	1,00 m
H-7′			1,95 m	1,89 m
H-8			2,19 dd $J_{8,7}=11,0$ $J_{8,7'}=3,0$	2,04 m
H-9			0,77 m	0,65 ddd J=6,0 J=10,5 J=16,0
			1,54	1,45 dd J=7,5 J=4,0
H-11				1,05
H-12			1,67	1,62 dt $J_{12,12'}=12,5$
H-12′				$J_{12',11}=3,5$
H-14				1,05 m
H-15		1,46 m	1.48 m	1,39 m
H-15′		2,08 m	2,10 m	2,06 m
H-16	4,97 m	4,56 dt $J_{16,15}=7,5$ $J_{16,15'}=6,5$ $J_{16,17}=7,5$	4,58	4,50dt $J_{16,15}=8,0$ $J_{16.15'}=6,0$
H-17		1,83	1,83	1,75 dd J=6,5 J=8,5
CH_3-18	0,91 s	0,86 s	0.87 s	0,78 s
CH_3-19	1,42 s	1,40 s	1,48 s	1,30 s
H-20				1,86 m
CH_3-21	1,31 d $J_{21,20}=7,0$	1,13 d $J_{21,20}=7.0$	1,12 d	1, 05 d $J_{21,20}=7,0$
H-23				1.35 m
H-24				1,32 m
H-24′			1.90	2,09 m
H-25			1.50 m	1,55 m
H-26e	3,46 dd $J_{26,26'}=9,5$ $J_{26,25}=7,0$	3,34br.d $J_{26,26'}=11,0$	3,34	3,31 dd $J_{26,26'}=11,0$ $J_{26,25}=2,0$
H-26a	4,04 dd $J_{26',25}=5,5$	4,04 br.d	4,04	3,98 dd $J_{26',25}=3,0$
CH_3-27	1,01 d $J_{27,25}=6,5$	1,04 d $J_{27,25}=7,0$	1,04	1,00 d $J_{27,25}=7.0$
6-OCH_3	3,24 s	3,23	3,33	3,28 s
	β-D-Fucopyranose			
1	4,53 d $J_{1,2}=7,5$	4,53 d $J_{1,2}=7,5$		
2	4,28 m	4,25 t $J_{2,3}=7,5$		
3	4,04 m	4,04 dd $J_{3,4}=3,5$		
4	4,03 m	4,02 m		
5	3,75 dq $J_{5,6}=6,5$	3,73 dq $J_{5,6}=6,5$		
6	1,54 d	1,53 d		

TABLE 1 (continued)

Proton	I	III	IV	IV*
1	β-D-Glucopyranose			
2	4,80 d $J_{1,2}$=7,5			
3	4,01 m			
4	4,24 m			
5	4,22 m			
6	3,94 m			
	4,38 dd $J_{6,6'}$=12,0 $J_{6,5}$=5,5			
6′	4,54 dd $J_{6',5}$=3,0			

*Solvent C_5D_5N–D_2O (4:1).

TABLE 2. Chemical Shifts of the ^{13}C Carbon Atoms of Nolinofuroside B (I), Nolinospiroside B (III), and Nolinogenin (IV) (C_5D_5N, δ, ppm. 0 – TMS)

C atom	I	III	IV	IV*	C atom	I	III
1	84,48	84,51	77,58	77,53		β-D-Glucose	
2	32,71	32,72	36,35	36,53			
3	20,93	20,89	21,20	21,99	1	105,13	
4	16,88	16,79	17,49	17,84	2	75,24	
5	49,51	49,51	49,32	49,94	3	78,38	
6	82,55	82,52	82,87	83,71	4	71,86	
7	35,73	35,78	35,74	36,12	5	78,62	
8	30,52	30,57	30,69	30,33	6	62,96	
9	50,29	50,24	49,77	50,58		β-D-Fucose	
10	36,51	36,43	36,34	37,17			
11	23,34	23,37	23,37	23,83	1	103,20	103,27
12	40,71	40,58	40,52	41,13	2	72,12	72,13
13	41,43	41,09	41,04	41,69	3	75,68	75,72
14	56,47	56,48	56,56	57,18	4	72,79	72,84
15	32,44	32,19	32,16	32,64	5	71,45	71,49
16	81,22	81,36	81,32	82,11	6	17,49	17,34
17	64,09	63,07	63,01	63,46			
18	16,88	16,79	16,78	17,28			
19	16,36	15,94	15,10	15,28			
20	40,71	42,58	42,53	43,26			
21	15,87	14,89	14,91	15,23			
22	110,69	109,81	109,75	110,82			
23	37,09	26,47	26,42	27,09			
24	28,36	26 24	26,22	26,67			
25	34,43	27,60	27,58	28,04			
26	75,37	65,21	65,14	66,10			
27	17,26	16,34	16,32	16,84			
6-OCH_3	56,47	56,48	56,56	57,09			

*Solvent C_5D_5N–D_2O (4:1).

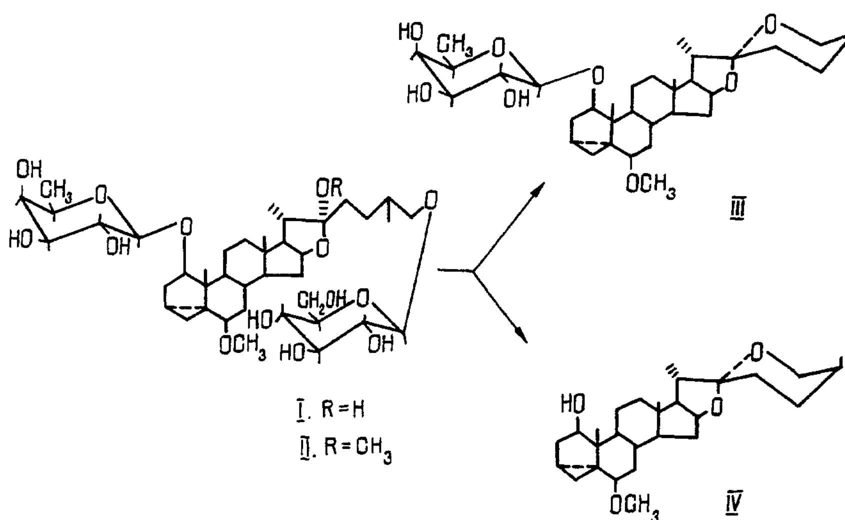
to the protons of the angular methyl group at C-10 (CH_3-19). Preirradiation of the protons of the methoxy group (singlet at 3.33 ppm) led to a change in the intensity of the signals with CSs of 2.73 and 1.95 ppm (Table 3). Moreover, preirradiation of the cyclopropane proton with a CS of 0.66 ppm led to the appearance in the difference NOE spectrum of the signal of a proton of a methoxy group and of a signal with the CS 2.73 ppm, which showed the spatial propinquity of the protons of the cyclopropane ring and of the methoxy group. The multiplicity of the signal with CS 2.73 ppm (triplet) with the constants J = 3.0 Hz showed that it was involved in spin-spin coupling with only two protons and had the equatorial orientation, which is possible only if an axial methoxy group is located at C-6. Consequently, the cyclopropane ring is formed by the C-3, C-4, and C-5 atoms.

When the proton with a CS of 4.21 ppm geminal to a hydroxy group was pre-irradiated, enhancements were observed of the signals of the protons with CSs of 2.37, 2.00, 1.54, and

TABLE 3. NOEs for Nolinogenin (IV)

NOE signal in the difference spectrum	Proton pre-irradiated		
	6-OCH$_3$	H-4	H-1
H-2			+
H-2			+
H-4	+		
H-4		+	
H-6	+		
6-OCH$_3$		+	
H-7e	+		
H-9			+
H-11e			+
CH$_3$-19	+		+

0.77 ppm assigned with the aid of two-dimensional homonuclear and heteronuclear spectroscopy to H-2, H-2', H-11e, and H-9, respectively. This is a proof of the localization of the hydroxy group at C-1 of the aglycon and of its β-orientation in the ring.



On comparing the corresponding CSs in the ^{13}C NMR spectra of nolinogenin (IV) and of 6-methoxy-3,5-cycloergostane [12], satisfactory agreement was observed for the C-3, C-4, C-5, and C-6 atoms and for the carbon atom of the methoxy group.

Thus, the structure of nolinogenin has been established as 6β-methoxy-3β,5-cyclo-(25S)-spirostan-1β-ol.

The spin-spin coupling constants of the anomeric protons of the D-glucose and D-fucose residues in the PMR spectra of glycosides (I) and (III) ($J_{1,2}$ = 7.5-8.0 Hz; see Table 1) show the β-configurations of the corresponding glycosidic bonds and the pyranose forms of the oxide rings of the monosaccharides [6].

A comparison of the chemical shifts of the genin (IV) and of the spirostanol glycoside (III) (see Table 2) showed the attachment of the β-D-glucopyranose residue at C-1 of the aglycon (Δ_{C-1} = +6.93; Δ_{C-2} = −3.63 ppm). Consequently, nolinospiroside B (III) is 6β-methoxy-3β,5-cyclo-(25S)-spirostan-1β-ol β-D-fucopyranoside.

The results of the enzymatic hydrolysis of nolinofuroside B and also a comparative analysis of the spectral characteristics of compounds (I), (III), and (IV) confirmed that in glycoside (I) the β-D-glucopyranose residue was attached at C-26 and the β-D-fucopyranose residue at C-1 of the aglycon. In addition, observance of nuclear Overhauser effects showed the spatial propinquity of the H-1 atom of the β-D-glucopyranose residue and the two H-26 atoms of the aglycon.

Thus, nolinofuroside B (III) has the structure of 6β-methoxy-3β,5-cyclo-(25S)-furostan-1β,22α,26-triol 1-O-β-D-fucopyranoside 26-O-β-D-glucopyranoside.

EXPERIMENTAL

General Remarks. We used the following solvent systems for chromatography: 1) chloroform–methanol: a) (50:1); b) (20:1); and 2) chloroform–methanol–water (65:10:1).

PMR and ^{13}C NMR spectra were taken for a solution of substance (IV) in $C_5D_5N–D_2O$, and for solutions of substances (I), (III), and (IV) in pyridine-d_5, on a Bruker WM-250 instrument. The COSY-45 spectrum was taken by a standard procedure of the programs supplied with the ASPECT 3000 computer (Bruker). The heteronuclear COSY C–H spectrum was taken by the standard XH CORRD procedure. In the recording process we used the delays D1 (relaxation delay, 1 s), D3 0.0032 (the optimum for a direct constant of 150 Hz), and D4 = 1/2 D3. A matrix with dimensions of 4 K × 256 W was employed, which ensured a resolution of better than 5 Hz per point along the axis of proton chemical shifts. In the transformation of the spectra we used a sinusoidal function with zero shift in both dimensions. The experiments with nuclear Overhauser effect were conducted on a Bruker WM-250 instrument for solutions of the substances in pyridine-d_5. Other information is given in [1].

Nolinofuroside B (I). After repeated chromatography in system 2 of the fractions enriched with glycoside (I) and rechromatography on microdisperse silica gel, 0.16 g of a mixture of substances (I) and (II) was obtained. A solution of 80 mg of this mixture in 50 ml of water was heated at 60°C for 18 h. The solvent was distilled off, giving the amorphous glycoside (I), $C_{40}H_{66}O_{14}$, $[\alpha]_D^{22}$ −44.2 ± 2° (c 1.50; pyridine). ν_{max}^{KBr}, cm^{-1}: 910 (weak broadened band), 3200-3600 (OH). LSIMS mass spectrum $(M + 2Na - H)^+$ 815. Yield 0.003% (yield of the mixture of a glycoside of the furostan series and its 22-O-methyl ether calculated on the weight of the freshly gathered plant).

Enzymatic Hydrolysis of Nolinofuroside B (I). A solution of 100 mg of glycoside (I) in 50 ml of water was treated with 30 mg of the freeze-dried gastric juice of the snail *Helix pomatia*, and the reaction mixture was stirred at 38°C for 18 h. The resulting suspension was evaporated to dryness, the residue was dissolved in chloroform–methanol (1:1), and the solution was filtered. Chromatography in chloroform and then in system 1b (TLC in systems 1a and 1b) yielded 20 mg of nolinogenin (IV) and 25 mg of nolinospiroside B (III).

Nolinospiroside B (III). $C_{34}H_{54}O_8$, $[\alpha]_D^{24}$ −21.7 ± 2° (c 0.85; pyridine). ν_{max}^{KBr}, cm^{-1}: 860, 910 < 930, 990 (spiroketal chain of the 25S series), 3200-3600 (OH). M^+ 590.

Nolinogenin (IV). $C_{28}H_{44}O_4$. ν_{max}^{KBr}, cm^{-1}: 860, 910 < 930, 990 (spiroketal chain of the 25S series), 3200-3600 (OH). Mass spectrum, m/z (%): 444 (M^+; 7.6), 412(4.2), 385(5,6), 375(10.3), 372(27.5), 330(10.2), 315(7,9), 301(40.1), 139(100).

The PMR spectra of compounds (I), (III), and (IV) are given in Tables 1 and 2.

Methanolysis of Glycoside (I). A solution of 10 mg of compound (I) in 3 ml of absolute methanol containing 5% of hydrogen chloride (3 ml) was heated at the boil for 14 h. Then the reaction mixture was cooled, diluted with an equal volume of water, filtered, and evaporated to dryness, and the residue was silylated [13]. The trimethylsilylated derivatives of methyl glycosides were chromatographed as described in [1].

It was established that nolinofuroside B includes D-glucose and D-fucose residues in a ratio of 1.00:0.95.

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SYNTHESIS OF 24-EPITEASTERONE

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Starting from ergosterol, the synthesis of the brassinosteroid 24-epiteasterone has been achieved by the use of a new scheme for introducing a 3β-hydroxy-6-keto group as the result of the Birch reduction of the corresponding 5α-hydroxy-Δ^7-3,6-dione.

One of the brassinosteroids of green tea Thea sinensis [Camellia sinensis viridis] is teasterone (I) [1]. This phytohormone has a fairly simple structure and, although it is substantially less active than brassinolide, may find practical use in agriculture as a plant growth regulator. Undoubted interest is also presented by its isomer 24-epiteasterone (II). It must be mentioned that brassinosteroids with the (R)-configuration of the 24-methyl group such as 24-epibrassinolide or (24R)-castasterone have recently been detected in plants [2]. In view of this one may expect the presence of compound (II) in natural sources. This hypothesis has served as an incentive for the first synthesis of 24-epiteasterol (II) from ergosterol (III), which we have now accomplished.

It is known [3] that the oxidation of ergosterol (III) with chromic acid forms the 5α-hydroxy-3,6-diketone (IV). We obtained this compound with a yield of 22% when the oxidation was performed in acetone. In the following stage, with the aim of saturating the 7(8)-double bond and hydrogenolyzing the 5-hydroxy group, the steroid so obtained was subjected to Birch reduction with an excess of lithium in a mixture of liquid ammonia and hexamethylphosphorotriamide. It was found that in this case the Birch reduction took place in a rather complex fashion. The main products, which we isolated with yields of 12 and 13%, respectively, were the steroids (V) and (VI) (see top of following page).

We established the structures of compounds (V) and (VI) by analyzing their spectra. Thus, the IR spectrum of the main product, of the Birch reduction of the hydroxydione (IV) contained the bands of a saturated C=O bond and of an OH group at 1710 and 3460 cm^{-1}, respectively. In the CD spectrum of the hydroxyketone (VI) the maximum of a negative Cotton effect was observed at 285 nm, which is characteristic for a 6-ketosteroid with a trans-A/B linkage. From this it was possible to conclude that, in the Birch reduction of compound (IV) with the formation of (VI), not only the saturation of the 7(8)-double bond and the hydrogenolysis of the 5α-hydroxy group but also the reduction of the 3-keto group to a 3-hydroxy group had taken place. In the PMR spectrum of compound (VI), the position (δ 3.58 ppm) and form of the signal of the methine proton geminal to the 3-hydroxy group were char-

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