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).

## LITERATURE CITED


1. A. L. Takhtadzhyan, The Life of Plants [in Russian], Moscow, Vol. 6 (1982), p. 169.
2. A. V. Kamernitskii, N. K. Abubakirov, M. B. Gorovits, Yu. S. Vollerner, N. E. Voishvillo, I. G. Reshetova, and V. A. Paseshnichenko, The Chemistry of the Spirostanols [in Russian], Moscow (1986), pp. 8-37.
3. E. Stahl, Thin Layer Chromatography, 1st English edition, Academic Press, New York (1965), p. 491.
4. R. Kasai, M. Okihara, I. Asakawa, K. Mizutani, and K. Tanaka, Tetrahedron, 35, 1427 (1979).
5. M. E. Wall, C. R. Eddy, M. L. McClennan, and M. E. Klumpp, Anal. Chem., 24, 1337 (1952).
6. C. R. Eddy, M. E. Wall, and M. K. Scott, Anal. Chem., 25, 266 (1953).
7. S. D. Kravets, Yu. S. Vollerner, M. B. Gorovits, A. S. Shashkov, and N. K. Abubakirov, Khim. Prir. Soedin., 188 (1986).
8. C. Altona and C. A. G. Hasnoot, Org. Magn. Reson., 13, No. 6, 417 (1980).
9. S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, J. Am. Chem. Soc., 100, No. 11, 3331 (1978).
10. T. T. Gorovits, Khim. Prir. Soedin., 263 (1970).


# STEROIDS OF THE FUROSTAN AND SPIROSTAN SERIES FROM Nolina microcarpa

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


G. V. Shevchuk, Yu. S. Vollerner, A. S. Shashkov, and V. Ya. Chirva



UDC 547.918:547.926



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.



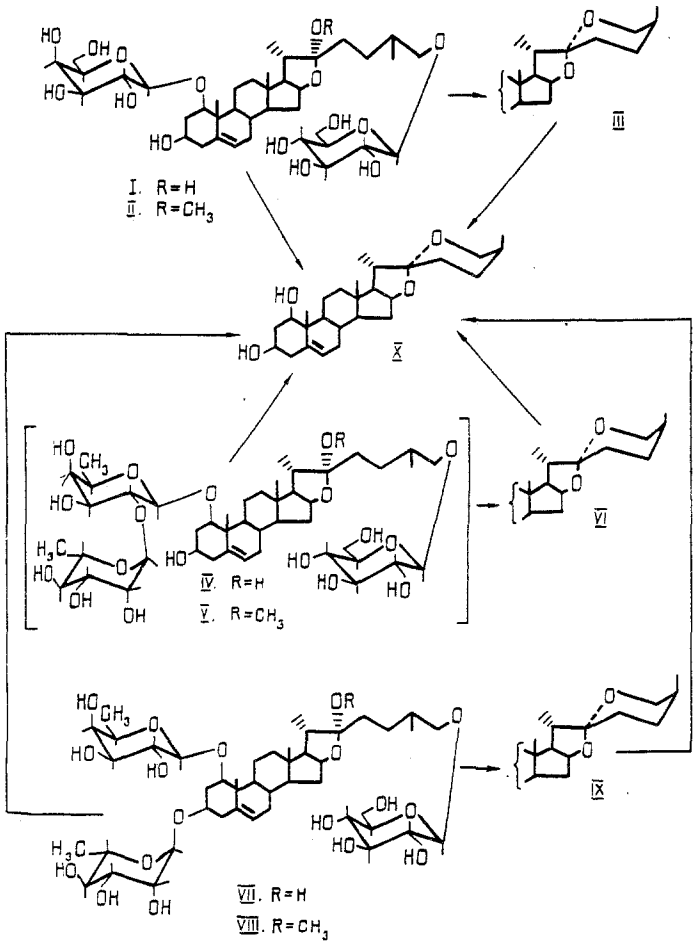
M. V. Frunze Simferopol' State University. Institute of Chemistry of Plant Substances, Uzbek Academy of Sciences, Tashkent.


Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 678-686, September-October, 1991. Original article submitted December 13, 1990.



0009-3130/91/2705-0597$12.50

On continuing the separation of the butanolic fraction of the total extractive substances of the epigeal part of Nolina microcarpa S. Wats. [1], we isolated a mixture of glycosides, which were detected in the form of spots with close $R_f$ values. Both components were colored green by vanillin/phosphoric acid [2, 3], and red by the Ehrlich reagent [4]. On being heated in water they underwent quantitative transitions to more, and on being heated in methanol to less, polar compounds. This showed that the compounds isolated belonged to derivatives of the furostan series.




The mixture described was subjected to acid and enzymatic hydrolysis. Acid hydrolysis led to an aglycon that was identified as (25S)-ruscogenin (X) [5]. Enzymatic hydrolysis led to the formation of three glycosides of the spirostan series (III), (VI), and (IX). Analysis by the GLC method of the products of the methanolysis of glycosides (III), (VI), and (IX) showed that compound (III) contained one D-galactose residue, while the (VI) and (IX) molecules contained D-fucose and L-rhamnose residues in a ratio of 1:1. As was to be expected, (25S)-ruscogenin was identified (by TLC) among the methanolysis products in all three cases.

In their physicochemical constants and spectral characteristics, biosides (VI) and (IX) were identical with glycosides B and C isolated from the epigeal part of Liriope platiphylla (family Liliaceae) [6], having the structures of the 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-fucopyranoside] and the 1-O-β-D-fucopyranoside 3-O-α-L-rhamnopyranoside, respectively, of (25S)-spirost-5-ene-1β,3β-diol. In addition, a glycoside having the structure of the latter has been isolated from Liriope spicata [7].

The monoside (III), which has been called nolinospiroside D, has not been described previously. A comparative analysis of the $^{13}C$ NMR spectra of the genin (X) and the glycoside (III) (Table 1) showed the attachment of the D-glucose residue to C-1 of the aglycon ($\Delta_{C-1}$ = +5.61, $\Delta_{C-2}$ = −5.97 ppm). From the PMR spectrum of nolinospiroside D followed the β-configuration of the glycosidic center and the pyranose form of the carbohydrate ring [8]. Thus, monoside (III) has the structure of (25S)-spirost-5-ene-1β,3β-diol 1-O-β-D-galactopyranoside.

TABLE 1. Chemical Shifts of the Carbon Atoms (ppm, 0 – TMS) of Nolinofurosides D (I) and F (VIII), of Nolinospirosides D (III), E (VI), and F (IX), and of (25S)-Ruscogenin (X) ($C_5D_5N$)

| C-Atom | I | III | VI | VIII | IX | X |
|---|---|---|---|---|---|---|
| 1 | 83,88 | 83,73 | 84,11 | 83,77 | 83,86 | 78,12 |
| 2 | 38,09 | 38,02 | 37,99 | 35,89 | 35,93 | 43,99 |
| 3 | 68,17 | 68,17 | 68,37 | 73,64 | 73,57 | 68,15 |
| 4 | 43,84 | 43,78 | 43,95 | 39,78 | 39,42 | 43,63 |
| 5 | 139,73 | 139,81 | 139,85 | 138,51 | 138,42 | 140,36 |
| 6 | 124,89 | 124,70 | 124,78 | 125,73 | 125,77 | 124,41 |
| 7 | 32,10 | 32,07 | 32,16 | 32,07 | 32,05 | 32,36 |
| 8 | 33,12 | 33,14 | 33,23 | 33,10 | 33,09 | 33,02 |
| 9 | 50,57 | 50,55 | 50,76 | 50,72 | 50,64 | 51,39 |
| 10 | 42,97 | 42,98 | 42,97 | 43,04 | 43,03 | 43,63 |
| 11 | 23,96 | 23,88 | 24,05 | 23,88 | 23,89 | 24,25 |
| 12 | 40,64 | 40,46 | 40,58 | 40,62 | 40,46 | 40,64 |
| 13 | 40,14 | 40,32 | 40,32 | 40,42 | 40,26 | 40,26 |
| 14 | 57,04 | 57,11 | 57,31 | 57,05 | 57,08 | 56,99 |
| 15 | 32,76 | 32,47 | 32,50 | 32,48 | 32,47 | 32,47 |
| 16 | 81,28 | 81,31 | 81,33 | 81,47 | 81,29 | 81,22 |
| 17 | 64,00 | 63,04 | 63,14 | 64,49 | 63,03 | 63,04 |
| 18 | 17,02 | 16,81 | 16,85 | 16,78 | 16,79 | 16,71 |
| 19 | 14,94 | 14,91 | 14,83 | 14,65 | 14,65 | 13,96 |
| 20 | 40,84 | 42,61 | 42,60 | 40,64 | 42,55 | 42,53 |
| 21 | 16,45 | 14,91 | 15,07 | 16,20 | 14,86 | 14,94 |
| 22 | 110,84 | 109,81 | 109,84 | 112,84 | 109,84 | 109,75 |
| 23 | 37,25 | 26,52 | 26,50 | 31,06 | 26,46 | 26,45 |
| 24 | 28,38 | 26,30 | 26,28 | 28,27 | 26,26 | 26,26 |
| 25 | 84,52 | 27,64 | 27,63 | 34,56 | 27,60 | 27,59 |
| 26 | 75,41 | 65,18 | 65,17 | 75,02 | 65,13 | 65,10 |
| 27 | 17,53 | 16,37 | 16,36 | 17,54 | 16,36 | 16,36 |
| 22-O $CH_3$ | | | | 47,39 | | |
| | | | D-Glucose | | | |
| 1 | 105,24 | | | 105,12 | | |
| 2 | 75,31 | | | 75,28 | | |
| 3 | 78,55 | | | 78,43 | | |
| 4 | 71,80 | | | 71,93 | | |
| 5 | 78,68 | | | 78,66 | | |
| 6 | 62,91 | | | 63,03 | | |
| | | | D-Fucose | | | |
| 1 | | | 100,37 | 102,53 | 102,63 | |
| 2 | | | 76,83 | 72,26 | 72,23 | |
| 3 | | | 74,74 | 75,28 | 75,37 | |
| 4 | | | 73,33 | 72,53 | 72,53 | |
| 5 | | | 71,13 | 71,22 | 71,19 | |
| 6 | | | 17,18 | 17,36 | 17,43 | |
| | | | L-Rhamnose | | | |
| 1 | | | 101,63 | 99,97 | 99,96 | |
| 2 | | | 72,58 | 72,83 | 72,85 | |
| 3 | | | 72,73 | 72,83 | 72,85 | |
| 4 | | | 74,39 | 74,19 | 74,17 | |
| 5 | | | 69,29 | 70,07 | 70,12 | |
| 6 | | | 18,98 | 18,57 | 18,82 | |
| | | | D-Galactose | | | |
| 1 | 102,72 | 102,59 | | | | |
| 2 | 72,72 | 72,71 | | | | |
| 3 | 75,49 | 75,41 | | | | |
| 4 | 69,88 | 69,91 | | | | |
| 5 | 76,73 | 76,66 | | | | |
| 6 | 62,24 | 62,31 | | | | |

We showed by the TLC method that the products of the fermentation of (III), (VI), and (IX) were present in the total extractive substances, but in only trace amounts. The facts given above indicated that the native mixture of derivatives of the furostan series consisted of three pairs of glycosides: (I and II), (IV and V), and (VII and VIII). Repeated chromatography on ordinary and octadecylsilylated silica gels permitted the isolation of the mixtures (I and II) and (VII and VIII) free from other components. Nolinofuroside D (I) was obtained by heating the mixture of glycosides (I) and (II) in water, and the 22-O-methyl

TABLE 2. Chemical Shifts and Spin–Spin Coupling Constants (J, Hz) of the Protons of Nolinofurosides D (I) and F (VIII) and of Nolinospirosides D (III), E (VI), and F (IX)

| Protons of the aglycon | I | III | VI | VIII | IX |
|---|---|---|---|---|---|
| $CH_3$-18 | 0,87 s | 0,83 s | 0,85 s | 0,84 s | 0,82 s |
| $CH_3$-19 | 1,20 s | 1,22 s | 1,42 s | 1,13 s | 1,11 s |
| $CH_3$-21 | 1,18 d $J_{21,20}$=7,0 | 1,05 d $J_{21,20}$=7,0 | 1,06 d $J_{21,20}$=7,0 | 1,08 d J=7,0 | 1,03 d J=7,0 |
| $CH_3$-27 | 0,97 d $J_{27,25}$=7,0 | 1,04 d $J_{27,25}$=7,0 | 1,03 d $J_{27,25}$=7,0 | 1,01 d J=7,0 | 1,02 d J=7,0 |
| 1a | 3,85 m | 3,86 dd $J_{1,2a}$=11,5 $J_{1,2e}$=3,5 | 3,78 dd $J_{1,2a}$=11,5 $J_{1,2e}$=4,0 | 3,74 dd $J_{1,2a}$=12,0 $J_{1,2e}$=4,2 | 3,71 dd $J_{1,2a}$=11,5 |
| 3a | 3,98 m | 3,84 m | 3,88 m | 3,85 m | 3,78 m |
| 6 | 5,52 br. d J=5,5 | 5,56 br. d J=5,5 | 5,57 br. d J=6,0 | 5,51 br. d J=5,5 | 5,49 br. d J=5,5 |
| 16 | 4,50 m | 4,47 m | 4,47 m | 4,50 m | 4,48 m |
| 26 | 3,42 dd $J_{26,26'}$=9,0 $J_{26,25}$=6,5 | 3,33 br. d $J_{26,26'}$=11,5 | 3,32 br. d $J_{26,26'}$=11,0 | 3,48 dd $J_{26,26'}$=10,0 $J_{26,25}$=7,1 | 3,31 br. d $J_{26,26'}$=11,0 |
| 26′ | 4,02 m | 4,03 dd $J_{26',25}$=3,5 | 4,02 dd $J_{26',25}$=2,5 | 4,05 dd $J_{26',25}$=5,0 | 4,00 m |
| 22-OMe | | | | 3,23 s | |
| | D-Galactose | | | D-Fucose | |
| 1 | 4,80 d $J_{1,2}$=7,0 | 4,83 d $J_{1,2}$=7,5 | 4,66 d $J_{1,2}$=8,0 | 4,68 d $J_{1,2}$=7,8 | 4,64 d $J_{1,2}$=7,5 |
| 2 | 4,39 dd $J_{2,3}$=9,0 | 4,42 dd $J_{2,3}$=9,0 | 4,54 dd $J_{2,3}$=9,5 | 4,31 dd $J_{2,3}$=8,5 | 4,26 dd $J_{2,3}$=8,5 |
| 3 | 4,11 dd $J_{3,4}$=2,5 | 4,13 dd $J_{3,4}$=4,5 | 4,09 dd $J_{3,4}$=3,5 | 4,05 m $J_{3,4}$=4,0 | 4,02 m $J_{3,4}$=4,0 |
| 4 | 4,19 m | 4,59 br. d $J_{4,5}$=1,5 | 3,89 br. d $J_{4,5}$=1,0 | 4,42 m | 3,99 m |
| 5 | 4,00 m | 4,00 m | 3,65 d. q $J_{5,6}$=6,0 | 3,68 d. q $J_{5,6}$=7,0 | 3,64 d. q $J_{5,6}$=6,5 |
| 6 | 4,35 m | 4,53 dd $J_{6,6'}$=11,0 $J_{6,5}$=7,0 | 1,49 d | 1,54 d | 1,50 d |
| 6′ | 4,35 m | 4,40 dd $J_{6',5}$=5,5 | | | |
| | D-Glucose | | | D-Glucose | |
| 1 | 4,75 d $J_{1,2}$=7,5 | | | 4,63 d $J_{1,2}$=8,0 | |
| 2 | 3,97 dd $J_{2,3}$=6,5 | | | 4,04 dd $J_{2,3}$=9,0 | |
| 3 | 4,21 m | | | 4,28 t $J_{3,4}$=9,0 | |
| 4 | 4,17 m | | | 4,22 t $J_{4,5}$=9,0 | |
| 5 | 3,88 m | | | 3,96 m | |
| 6 | 4,50 dd $J_{6,5}$=3,5 $J_{6,6'}$=11,5 | | | 4,57 dd $J_{5,6}$=3,0 $J_{6,6'}$=12,6 | |
| 6′ | 4,32 dd $J_{6',5}$=5,0 | | | 4,39 dd $J_{6',5}$=4,7 | |
| | | | | *L*-Rhamnose | |
| 1 | | | 6,38 d $J_{1,2}$=1,5 | 5,55 br. s | 5,50 br. s |
| 2 | | | 4,75 dd $J_{2,3}$=2,5 | 4,55 br. s | 4,52 dd $J_{1,2}$=1,4 |
| 3 | | | 4,65 dd $J_{3,4}$=9,5 | 4,51 dd $J_{2,3}$=4,0 $J_{3,4}$=9,5 | 4,47 dd $J_{2,3}$=3,5 $J_{3,4}$=8,5 |
| 4 | | | 4,31 t $J_{4,5}$=9,5 | 4,25 m | 4,27 t $J_{4,5}$=8,5 |
| 5 | | | 4,91 d. q $J_{5,6}$=6,5 | 4,37 d. q $J_{4,5}$=10,3 $J_{5,6}$=6,0 | 4,28 d. q $J_{5,6}$=4,5 |
| 6 | | | 1,74 d | 1,69 d | 1,65 d |

TABLE 3

| Glycoside | Monosaccharides (ratio of the components) | | | |
|---|---|---|---|---|
| | D-glucose | D-galactose | D-fucose | L-rhamnose |
| I | 1,00 | 0,96 | — | — |
| III | — | 1.00 | — | — |
| VI | — | — | 1,00 | 0,94 |
| VIII | 1,00 | — | 0.98 | 0,96 |
| IX | — | — | 1,00 | 0.98 |

ether of nolinofuroside F (VIII) by heating the mixture of (VII) and (VIII) in absolute methanol. The completeness of the transitions was shown by NMR spectroscopy (Tables 1 and 2).

The PMR spectrum of glycoside (I) lacked the signal of the protons, and the $^{13}C$ NMR spectrum that of the carbon atom, of a methoxy group. In the $^{13}C$ NMR spectra of substances (I) and (VIII), C-22 resonated at 110.84 and 112.84 ppm, respectively, which agrees well with literature information [9]. The products of the methanolysis of compounds (I) and (VIII) were subjected to GLC analysis, which showed that the carbohydrate moiety of glycoside (I) consisted of D-glucose and D-galactose residues in a ratio of 1:1. In substance (VIII), D-glucose, D-fucose, and L-rhamnose residues were found in a ratio of 1:1:1.

A comparative analysis of the magnitudes of the chemical shifts (CSs) of the carbon atoms in the $^{13}C$ NMR spectra of compounds (I), (III), (VIII), (IX), and (X) (Table 1) indicated that in bioside (I) the centers of glycosylation were C-1 and C-26. Trioside (VIII) was a trisdesmoside and bore carbohydrate residues at C-1, C-3, and C-26. The results of enzymatic hydrolysis unambiguously determined the localization of the D-glucose residue (at C-26). The sites of attachment of the D-fucose and L-rhamnose residues in glycoside (VIII) were shown through the observation of nuclear Overhauser effects (NOEs). The pre-irradiation of H-1 of the D-fucose residue caused an increase in the intensity of the H-1 signal of the aglycon. The spatial propinquity of H-1 of the L-rhamnose residue and H-3 of the aglycon was shown by the same method. The pyranose form of the monosaccharide residues in the molecules of the bioside (I) and the trioside (VIII) followed from the SSCCs of the protons of the sugar residues geminal to hydroxy and methyl groups in the PMR spectra of these compounds (Table 2) [8].

The SSCCs of the H-1 atoms showed the β-configuration of the glycosidic centers of the D-glucopyranose and D-fucopyranose residues ($J_{1,2}$ = 7.5-8.0 Hz) and the α-configuration of C-1 of the L-rhamnose residue ($J_{1,2}$ = 1.5 Hz). Thus, compound (I), which has been called nolinofuroside D, is (25S)-furost-5-ene-1β,3β,22α,26-tetraol 1-O-β-D-galactopyranoside 26-O-β-D-glucopyranoside. Nolinofuroside F (VII) has the structure of (25S)-furost-5-ene-1β,3β,22α,26-tetraol 1-O-β-D-fucopyranoside 26-O-β-D-glucopyranoside 3-O-α-L-rhamnopyranoside.

We did not succeed in isolating nolinofuroside E (IV) as an individual compound. However, it followed from the results obtained that this glycoside was (25S)-furost-5-ene-1β,3β,22α,26-tetraol 26-O-β-D-glucopyranoside 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside].

## EXPERIMENTAL

General Remarks. The following solvent systems were used for chromatography: 1) chloroform–methanol (20:1); 2) chloroform–methanol–water [a) (65:15:2); b) (65:22:4); c) (65:30:6)]; and 3) methanol–water (3:2).

LSIMS mass spectra were taken on an M-80 A mass spectrometer (Hitachi) fitted with an ion source working in the regimes of EI and SIMS mass spectrometry, with an M-0003 data-processing system. As the bombarding beam of primary ions we used $Xe^+$ with an energy of 8 keV, the temperature of the ionizing chamber being 30°C, the accelerating voltage 3 kV, and the range of mass numbers from 100 to 1500 m. u.

Other information has been given in [1]. The same paper describes the extraction of the plant, the preliminary treatment of the total extractive substances and the conditions of performing column chromatography.

Chromatography was also conducted on a column containing the silica gel Silasorb C-18 (particle size 10-20 μm) filled by the "dry" method. The rate of flow of eluent (system 3) was 3 ml/min, the pressure at the inlet to the column being 30 ± 1 kPa.

Isolation of the Total Glycosides of the Furostan Series. An enriched fraction consisting of six glycosides (I, II, IV, V, VII, and VIII) was isolated with the use of systems 2b and c. The weight of the fraction was 6.00 g. Yield 0.133%, calculated on the weight of the freshly gathered plant.

Nolinofuroside D (I). After the repeated chromatography of the above-mentioned total glycosides in systems 2b and c and 3, a mixture of nolinofuroside D and its 22-O-methyl ether (II) containing no other components was obtained. Heating the mixture of compounds (I) and (II) (250 mg) in 100 ml of water at 60°C for 18 h led to the formation of the individual glycoside (I). $C_{39}H_{64}O_{15}$ (amorphous), $[\alpha]_D^{20}$ −60 ± 2° (c 1.00; pyridine). $\nu_{max}^{KBr}$, $cm^{-1}$: 910 (weak broadened band), 3200-3600 (OH). $(M - 18)^+$ 754.

22-O-Methyl Ether of Nolinofuroside F (VIII). The mixture of glycosides (VII and VIII) was chromatographed in solvent systems 2b and c and 3. The individual product (VIII) was obtained by heating of compounds (VII and VIII) (300 mg) in 100 ml of absolute methanol at 60°C for 18 h. The ether (VIII) was obtained as an amorphous substance, $C_{46}H_{76}O_{18}$, $[\alpha]_D^{18}$ −54 ± 2° (c 1.01; pyridine). $\nu_{max}^{KBr}$, $cm^{-1}$: 910 (weak broadened band), 3200-3600 (OH). LSIMS $(M + Na)^+$ 939.

Acid Hydrolysis of a Mixture of Nolinofurosides D, E, and F and Their 22-O-Methyl Ethers (I and II; IV and V; and VII and VIII). The total mixture of glycosides of the furostan series (0.20 g) was dissolved in 50 ml of a 4% aqueous solution of sulfuric acid, and the reaction mixture was heated at 90°C for 8 h. The resinous deposit formed was separated off by the decantation of the supernatant liquid and was washed with water. After column chromatography in system 1 and recrystallization from methanol, 0.05 g of (25S)-ruscogenin (X) was obtained, $C_{27}H_{42}O_4$, mp 188-190°C, $[\alpha]_D^{18}$ −96 ± 2° (c 1.10; pyridine). According to the literature: mp 190-192°C, $[\alpha]_D^{21}$ −105.5° [5].

The chromatographic mobility (TLC, system 1) and the spectral characteristics (IR, mass, and NMR spectra) of the sample of the aglycon (I) obtained corresponded to the parameters of an authentic specimen.

Enzymatic Hydrolysis of a Mixture of Nolinofurosides D, E, and F and Their 22-O-Methyl Ethers (I and II; IV and V; and VII and VIII). A solution of the mixture of glycosides D, E, and F (1.00 g) in 300 ml of water was treated with 200 mg of the freeze-dried gastric juice of the snail Helix pomatia, and the reaction mixture was stirred at 38°C for 20 h. The resulting suspension was evaporated to dryness, and a solution of the residue in chloroform–methanol (1:1) was filtered. The filtrate was evaporated and the residue was subjected to chromatography in system 2a followed by rechromatography of the individual fractions in the same solvent system. This gave 70 mg of nolinospiroside D (III), 40 mg of (25S)-spirost-5-ene-1β,3β-diol 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-O-β-D-fucopyranoside], and 200 mg of (25S)-spirost-5-ene-1β,3β-diol 1-O-β-D-fucopyranoside 3-O-α-L-rhamnopyranoside (IX).

Nolinospiroside D (III), $C_{33}H_{52}O_9$, mp 198-200°C (from methanol), $[\alpha]_D^{20}$ −48.0 ± 2° (c 1.50; pyridine). $\nu_{max}^{KBr}$, $cm^{-1}$: 860, 910 < 930, 990 [spiroketal chain of the (25S) series], 3200-3600 (OH). $M^+$ 592.

(25S)-Spirost-5-ene-1β,3β-diol 1-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside] (VI), $C_{39}H_{62}O_{12}$, mp 199-202°C (from methanol, $[\alpha]_D^{20}$ −86.0 ± 2° (c 1.01; pyridine). According to the literature, [6]: mp 201-203°C (from methanol); $[\alpha]_D^{19}$ −89.6°.

(25S)-Spirost-5-ene-1β,3β-diol 1-O-β-D-fucopyranoside 3-O-α-L-rhamnopyranoside, $C_{39}H_{62}O_{12}$, mp 227-228°C (from methanol); $[\alpha]_D^{20}$ −99.0 ± 2° (c 1.01; pyridine). According to the literature [6]: mp 225-227°C; $[\alpha]_D^{19}$ −103.8°.

For the NMR spectra of glycosides (I), (III), (VI), (VIII), and (IX) and of the genin (X), see Tables 1 and 2.

Methanolysis of Glycosides (I), (III), (VI), (VIII), and (IX). Samples of these compounds (10 mg each) were dissolved in 5% hydrogen chloride (3 ml each). The solutions were heated at the boil for 14 h. Then the reaction mixtures were cooled, diluted with an equal volume of water, and filtered. In all five cases, (25S)-ruscogenin was identified in the

precipitates (TLC, system 1). The filtrates were neutralized with silver carbonate, filtered, and evaporated to dryness, and the residue was silylated. The trimethylsilyl derivatives of the methyl glycosides were chromatographed as described in [1]. The results of the analysis are presented in Table 3.

For compound (III), methanolysis and GLC were repeated in the presence of 3 mg of L-rhamnose as internal standard.

## LITERATURE CITED


1. G. V. Shevchuk, Yu. S. Vollerner, A. S. Shashkov, and V. Ya. Chirva, Khim. Prir. Soedin, 672 (1991).
2. E. M. Stahl, Thin Layer Chromatography, 1st English edition, Academic Press, New York (1965).
3. A. V. Kamernitskii, N. K. Abubakirov, M. B. Gorovits, Yu. S. Vollerner, N. E. Voishvillo, I. G. Reshetova, and V. A. Paseshnichekov, The Chemistry of the Spirostanols [in Russian], Nauka, Moscow (1986).
4. R. Kasai, M. Okihara, I. Asakawa, K. Mizutani, and O. Tanaka, Tetrahedron, 35, 1427 (1979).
5. S. D. Kravets, Yu. S. Vollerner, A. S. Shashkov, and N. K. Abubakirov, Khim. Prir. Soedin., No. 2, 188 (1986).
6. Y. Watanabe, S. Sanada, Y. Ida, and J. Shoji, Chem. Pharm. Bull., 31, 1980 (1983).
7. W. Lin, Z. Wang, and H. Q. Liang, Yaoxue Xuebao, 24, No. 10, 749 (1989); Chem. Abstr., 112, 73,873 (1990).
8. C. Altona and C. A. G. Haasnoot, Org. Magn. Res., 13, No. 6, 417 (1980).
9. Y. Hirai, T. Konishi, S. Sanada, Y. Ida, and J. Shoji, Chem. Pharm. Bull., 30, No. 10, 3476 (1982).


# TRITERPENE GLYCOSIDES OF Hedera taurica

## VII. STRUCTURES OF TAUROSIDES A AND D FROM THE LEAVES OF CRIMEAN IVY


V. I. Grishkovets, N. V. Tolkacheva, A. S. Shashkov, and V. Ya. Chirva



UDC 547.918:543.422



A mixture of the β-D-glucopyranosides of stigmaterol and β-sitosterol, and also the new triterpeneglycoside echinocystic acid 3-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside] has been isolated from the leaves of Crimean ivy.


In the present paper we describe the establishment of the structures of taurosides A and D from the leaves of Crimean ivy, Hedera taurica Carr. Their isolation, together with other weakly polar glycosides, has been described previously [1].

The purification of tauroside A, which proved to be a mixture of two glycosides, was carried out by chromatographing its acetate on silica gel with subsequent deacetylation and additional purification. The proof of the structure of the chromatographically inseparable glycosides was achieved in the following way. In an acid hydrolysate of tauroside A, by the TLC method, we identified glucose and an aglycon coinciding in its mobility both with β-sitosterol and with stigmasterol, which are also chromatographically inseparable. The electron-ionization mass spectrum of tauroside A was typical for sterols and their derivatives [2]. In it it was possible to identify the peak of a molecular ion $M^{+\cdot}$ with m/z 574, which corresponds to the molecular mass of a stigmasterol glucoside, and also fragmentary ions


M. V. Frunze Simferopol' State University.


Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 686-689, September-October, 1991. Original article submitted November 27, 1990; revision submitted May 23, 1991.



0009-3130/91/2705-0603$12.50