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A STEROID GLYCOSIDE FROM THE SEEDS OF *Yucca macrocarpa*

V. I. Grishkovets,[1] P. A. Karpov,[2] S. V. Iksanova,[3] and V. Ya. Chirva[3]

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Plants of the genus *Yucca* L. (fam. Agavaceae) have long been known as sources of steroid glycosides. Schott's yucca, *Yucca macrocarpa* Engelm. (= *Y. torrei* Shafer) grows in south-west Texas in an area extending deep into Mexico [1] and has been successfully introduced into the Nikitskii botanical garden on the southern coast of the Crimea.

TLC analysis of alcoholic extracts of various organs of this plant has shown the presence of only trace amounts of steroid glycosides in the roots, a wider variety but a moderate level of glycosides in the leaves and flowers, and a very high content of them in the seeds (detection with the aid of tungstophosphoric acid and the Ehrlich reagent).

A high content (8—12%) of steroid glycosides in the seeds of *Yucca brevifolia* and *Y. arizonica* and an 8% yield of sarsasapogenin from the seeds of *Y. filifera* have been reported in the literature [2].

In the TLC analysis of an extract of ripe seeds of *Y. macrocarpa* we detected the presence of mainly two steroid glycosides, of which the more polar one greatly predominated, and also trace amounts of still more polar glycosides. The isolation of the main steroid glycoside (SG) was achieved by defatting the carefully ground seeds with hexane, followed by extraction with 80% isopropyl alcohol. Evaporation of the alcoholic extract gave a 20% total yield of unpurified glycosides. Crystallization of this mixture from ethanol enabled the main glycoside to be obtained in the practically pure state with a yield of 10%, and two recrystallizations of this permitted the complete elimination of a contaminating weakly polar glycoside.

By TLC, in a complete acid hydrolysate of the SG we identified glucose, galactose, and an aglycon close in chromatographic mobility to authentic specimens of smilagenin and tigogenin. The further determination of the structure of the SG was made with the use of ^1H and ^{13}C NMR spectroscopy.

In the low-field region of the PMR spectrum of the SG we easily identified two doublet signals of anomeric protons with spin-spin coupling constants of $J_{1,2}$ = 7—8 Hz, corresponding to the β-configuration of the glycosidic bonds of both monosaccharide residues.

The assignment of the signals in the ^{13}C NMR spectrum of the SG was made in the light of the chemical shifts of the signals of the C atoms of the main types of steroid glycosides [3] and on the basis of APT editing of the spectra with complete suppression of spin-spin interaction with protons. Here, in the 100—110 ppm region, in addition to the two signals of anomeric C atoms, there was a signal with δ 109.4 ppm, assigned to C-22 and characteristic for steroids of the spirostanol series [3].

On the basis of the chromatographic mobility of the progenin obtained by acid hydrolysis and the presence in the 60—80 ppm region of only four signals of aglycon C atoms (C-3, C-16, C-17, and C-26), besides the signals of the carbohydrate C atoms, it followed that rings *A, B, C, D, E,* and *F* had no additional OH groups and that there was no double bond at C-5 since there were no signals of olefinic C atoms in the low-field part of the spectrum. Consequently, the aglycon of the SG could be represented by smilagenin, sarsasapogenin, neotigogenin, or tigogenin, which differ by the configurations at C-5 and C-25; however, the first and last of these were excluded by the results of spectral analysis.

The *cis*-linkage of rings *A/B* (5β-H) in the aglycon of the SG followed from the absence in the regions around 44.5 and 55.5 ppm (free from the signals of other C atoms) of C-5 and C-9 signals, which are most sensitive to the configuration at C-5 and are characteristic for 5α-steroids. The signals of C-5 and C-9, together with others, were present at 35.7 and 39.2 ppm. Such a position of them is characteristic for 5β-steroids. The 25S- configuration in ring *F* followed from an analysis of the positions of the C-23—C-25 signals (all in the 28—31 ppm region for the 25R- configuration and in the 25—27 ppm region for the 25S- configuration). The 25S- configuration (axial position of the 27-CH_3 group) was additionally confirmed by an

1) Simferopol' State University; 2) State Nikitskii Botanical Garden, Yalta; 3) Institute of Organic Chemistry, National Academy of Sciences of the Ukraine, Kiev. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 828—830, November-December, 1998. Original article submitted August 11, 1998.

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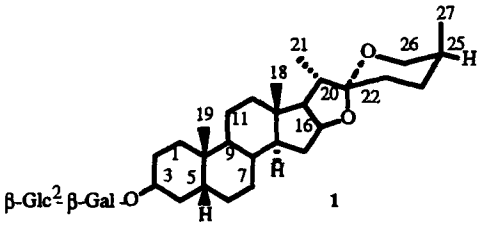
TABLE 1. Chemical Shifts of the Signals of the ^{13}C Atoms of Glycoside (1) (δ, ppm, 0 — TMS, C_5D_5N)

C atom	Chemical shift	C atom	Chemical shift	C atom	Chemical shift
Gal					
1'	102.0	1	30.7	15	30.7
2'	81.1	2	26.8	16	81.1
3'	75.2	3	76.1	17	62.7
4'	69.5	4	31.9	18	16.3
5'	76.2	5	36.7	19	23.7
6'	61.8	6	26.6	20	42.3
		7	26.6	21	14.5
Glc		8	35.4	22	109.4
1"	105.3	9	40.2	23	26.3
2"	74.8	10	35.0	24	26.0
3"	77.6	11	21.0	25	27.3
4"	71.6	12	40.2	26	64.9
5"	77.8	13	40.7	27	16.0
6"	62.8	14	56.4		

analysis of the multiplicity of the signal of the H-26 axial proton, readily identified in the PMR spectrum in the 3.3-3.5 ppm region and split into a doublet of doublets with constants of 10.5 and 2.5 Hz, which corresponds to the equatorial orientation of the proton at C-25 and, consequently, the axial orientation of the 27-CH_3 group. Thus, the aglycon of the SG is (5β,25S)-spirostan-3β-ol or sarsasapogenin and the SG has a carbohydrate chain at the C_3-OH group.

The sequence of monosaccharide residues in the carbohydrate chain was established by partial acid hydrolysis, as a result of which a glucose residue was split off (TLC analysis) and the progenin obtained contained a galactose residue (found by TLC after acid hydrolysis of the progenin). The type of bond between the monosaccharide residues was deduced from an analysis of the position of the C-2 signal of the galactose residue, which was absent from its usual position at about 73 ppm, free from signals of other glucose and galactose C atoms, and was found at 80.1 ppm because of a considerable downfield α-effect of glycosylation. In addition, the chemical shifts of the signals of the carbohydrate chain coincided with those given in the literature for the disaccharide fragment Glc-(1→2)-Gal [4].



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Thus, the steroid glycoside that was isolated is sarsasapogenin 3-O-β-*D*-glucopyranosyl-(1→2)-O-β-*D*-galactopyranoside (1), which has been isolated previously from *Cornus florida* [5].

^1H NMR spectrum of (1) (δ, ppm, 0 — TMS, C_5D_5N): 5.34 (d, $J_{1,2}$=7.8 Hz, H-1 Gal), 4.98 (d, $J_{1,2}$=7.5 Hz, H-1 Glc), 3.45 (dd, $J_{26a,26e}$=10.5 Hz, $J_{26a,25e}$=2.5 Hz, H-26a), 1.24 (d, $J_{27,25}$=6.3 Hz, 27-CH_3), 1.16 (d, $J_{21,20}$=6.6 Hz, 21-CH_3), 10.4 (s, 19-CH_3), 0.91 (s, 18-CH_3).

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