STEROID GLYCOSIDES OF THE LEAVES OF Yucca aloifolia

M. M. Benidze, O. D. Dzhikiya, T. A. Pkheidze,
É. P. Kemertelidze, and A. S. Shashkov

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Two new steroid glycosides have been isolated from the leaves of aloe yucca and their structures have been established. Glycosides B and C are tigogenin penta- and hexaosides. Glycoside B, which we have called yuccaloeside B is (25R)-5α-spirostan-3β-ol 3-{[O-β-D-glucopyranosyl-(1→2)]-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}, and glycoside C, which we have called yuccaloeside C is (25R)-5α-spirostan-3β-ol 3-{[(O-β-D-glucopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→2)]-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}.

The presence of five steroid glycosides, A, B, C, D, and E in the leaves of Yucca aloifolia (aloe yucca), family Agavaceae, has been established. The isolation and investigation of glycoside A, which we have called yuccaloeside A has been described previously [1]. In the present paper we give information to establish the structure of glycosides B and C. Both compounds gave a positive Matthews reaction and a negative one with the Ehrlich reagent, which showed their spirostan nature [2, 3]. The IR spectra of both glycosides had absorption bands in the 856, 900, 920, and 990 cm^{-1} regions that are characteristic for a spiroketal grouping [4].

As a result of the complete acid hydrolysis of glycoside B and C, it was established that they were tigogenin derivatives and contained rhamnose, galactose, and glucose residues in the carbohydrate moiety in ratios of 1:1:3 and 1:1:4, respectively.

A preliminary analysis of the ^{13}C NMR spectrum of glycoside B showed all the lines of the aglycon residue when it was compared with the spectrum of tigogenin [5]. So far as concerns the carbohydrate moiety of the molecule, the ^{13}C NMR spectrum unambiguously showed the presence of five carbohydrate residues in it (the number of signals in the region of resonance of anomeric carbon atoms) (Table 1). Analysis of the spin-spin coupling constants of the carbon atoms of the protons $^1J_{H1-C1}$ (2-D spectrum) showed that of the five carbohydrate residues, one had an axial substituent at C-1 and the other four an equatorial substituent (169 Hz and 160 Hz, respectively).

In the PMR spectrum of the glycoside in the region of resonance of the anomeric protons, likewise, five signals were observed. The values of the SSCCs $^3J_{H1-H2}$ showed that four of the carbohydrate residues had a β-gluco- or β-galactopyranose or an α-arabinopyranose configuration and one residue an α-mannopyranose configuration. The successive application of the method of selective homonuclear resonance in the difference variant enabled the positions of all the key signals of the pyranoses to be found for each of the monosaccharide residues and the SSCCs through 3 bonds (Table 2). Analysis of the SSCC values showed the presence of three residues with a β-gluco configuration, one residue with a β-galacto configuration, and one with an α-rhamno configuration.

The sequence of the linkages of the carbohydrate residues and the types of substitutions in them followed from an analysis of a number of nuclear Overhauser effect (NOE) spectra. The preirradiation of the H1 proton of the rhamnopyranose A residue led to the appearance in a difference NOE spectrum of signals from H2 of the same residue and from H4 of the

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N. G. Kutateladze Institute of Pharmacochemistry of the Georgian SSR Academy of Sciences, Tbilisi. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 537-542, July-August, 1987. Original article submitted February 24, 1986; revision submitted March 5, 1987.

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TABLE 1. ^{13}C NMR Chemical Shifts of the Carbohydrate Residues in Glycoside B (pyridine, δ, ppm, 40°C)

Carbon atom	Monosaccharide residue				
	A	B	C	D	E
1	102,93	104,32	105,02	102,69	104,87
2	72,62	75,57	81,49	73,32	75,47
3	72,81	76,88	88 15	75,73	78,14
4	74,01	78,70	70,81	80,22	71,29
5	70,64	77,57*	77,35*	76,25	78,74
6	18,62	61,43	62,58	60,89	63,15

*The assignment may be reversed.

TABLE 2. Parameters of the PMR Spectrum of the Carbohydrate Moiety of Glycoside B (pyridine, 40°C)

Residue	Chemical shifts (δ, ppm) and SSCCs (J, Hz)					
	H1	H2	H3	H4	H5	H6
A	5,72 d $J_{1,2}=1,7$	4 61 dd $J_{2,3}=3,1$	4,49 dd $J_{3,4}=9,1$	4,31 t $J_{4,5}=9,1$	4,87 dq $J_{5,6}=6,0$	1,78 d
B	5,27 d $J_{1,2}=7,5$	3,93 dd $J_{2,3}=8,6$	4,07 t $J_{3,4}=8,6$	4,30 dd $J_{4,5}=9,4$	3,79 m	—
C	5,09 d $J_{1,2}=7,6$	4,32 dd $J_{2,3}=8,8$	4,16 t $J_{3,4}=8,8$	3,72 t $J_{4,5}=8,8$	3,78 m	—
D	4,85 d $J_{1,2}=7,5$	4,39 dd $J_{2,3}=8,7$	4,09 dd $J_{3,4}=3,0$	4,56 dd $J_{4,5}=1,2$	4,0 m	—
E	5,55 d $J_{1,2}=7,5$	4,03 dd $J_{2,3}=8,6$	4,12 t $J_{3,4}=8,6$	4,23 dd $J_{4,5}=9,0$	3,85 m	—

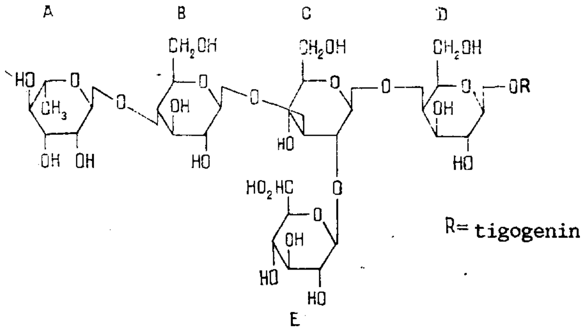
glucopyranose residues. When the H1 proton of the glucopyranose B residue was preirradiated, signals from H2, H3, and H5 of residue B and from H3 of the glucopyranose residue C appeared in the difference spectrum. The preirradiation residue C led to the appearance of signals from H2, H3, and H5 of this residue and H4 of the galactopyranose D residue. The preirradiation of the glucopyranose E residue was accompanied by the appearance in the difference NOE spectrum of signals from H2, H3, and H5 of residue E and H2 of residue C. Finally, the preirradiation of H1 of residue D led to the appearance of signals from H2, H3, and H5 of residue D and a multiplet signal with shift of 3.90 ppm.

In the difference selective homonuclear resonance spectrum obtained on saturating the resonance multiplet with a chemical shift of 3.90 ppm, a number of signals were detected in the 1.2-1.6 ppm region, which showed that this multiplet must be assigned to one of the protons of the genin. It follows from the series of experiments mentioned that the carbohydrate moiety of the glycoside is represented by the oligosaccharide [α-L-Rhap(1→4)-β-D-Glcp(1→3)]-[β-D-Glcp(1→2)]-β-D-Glcp(1→4)-β-D-Galp.

The complete assignment of the three signals in the proton spectrum of the carbohydrate moiety of the molecule permitted an unambiguous assignment of the ^{13}C signals in the carbon spectrum using the $^{13}C_i$–{$^{1}H_i$} heteronuclear double resonance method (see Table 1). In particular, saturation of the resonance multiplet with a chemical shift of 3.90 ppm led to the appearance in the carbon spectrum of a singlet signal with a chemical shift of 77.84 ppm. Taking the effect of glycosylation into account, the signal can be assigned only to the C-3 atom of the aglycon and, thus, the aglycon is glycosylated by residue D at C-3, as was confirmed by a comparison of the ^{13}C NMR spectrum of glycoside B and that of tigogenin taken under identical conditions [5].

The assignment of the signals of the hydroxymethyl groups of the pyranoses was made by a comparison of the ^{13}C NMR spectra of the glycoside under investigation and of the glycoside neoprazerigenin A [6], in which there is a frament of four carbohydrate residues similar to those present in the glycoside B under investigation.

Thus, an analysis of the NMR spectra has enabled the structure of the glycoside B to be established independently of chemical methods and permits us to assign the following formula to it:



In the ^{13}C NMR spectrum of glycoside C, six additional signals appeared in the resonance region of 60-105 ppm; all the signals of the aglycon had remained unchanged (having the same chemical shifts as in the case of glycoside B).

Of the six signals in the region of resonance of anomeric protons of carbohydrate residues, five have a constant $^1J_{H1-C1}$ of 159-163 Hz and one a constant of 168.5 Hz. Thus, the additional glucopyranose residue has the β-configuration of the glycosidic bond (since the rhamnopyranose residue, the chemical shift of the C-1 atom of which coincides with that of the C-1 atom of the rhamnopyranose residue in the spectrum of glycoside B, has the α-configuration of the glycosidic bond).

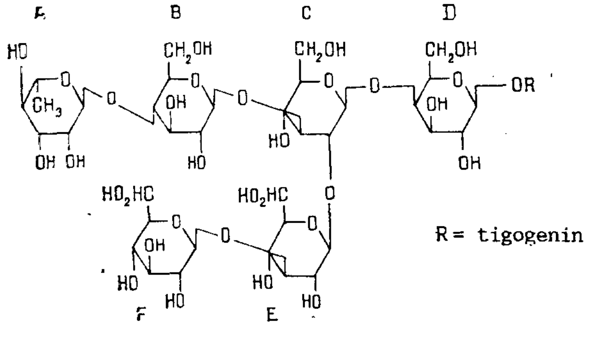
The position of attachment of the new residue was easily determined from the appearance of an initial signal with a chemical shift of 88 ppm (Table 3). Such a weak-field position of the signal is characteristic for the C-3 atom of a glucopyranose residue substituting a hydroxyl of a pyranose having the β-D-configuration [7].

In glycoside B, there are free hydroxyls at the C-3 atoms in residues B and E. The appearance of a strong-field signal with a chemical shift of 68.65 ppm in the spectrum of glycoside C, relating to the C-4 atom of a glucopyranose residue with a free hydroxyl, undoubtedly indicates substitution at the C-3 atom of residue E, and this upfield shift is due to the β-effect of glycosylation.

Thus, glycoside C differs from the preceding glycoside B by the presence of one more glucopyranose residue glycosylating residue E at the hydroxyl attached to the C-3 atom.

The positions of the signals of the additional glucopyranose residue were found by comparison with the spectrum of the nonreducing residue of methyl β-laminariboside [7]. The positions of the signals of the C-2—C-4 atoms of residue E in the spectrum of glycoside C likewise correspond to the positions of the signals of methyl β-laminariboside [7]. The chemical shifts of the carbon atoms of the other pyranose residue in the spectrum of glycosides B and C are close in value (see Tables 1 and 3).

Thus, glycoside C can be ascribed the following structure:



EXPERIMENTAL

Silica gel of type KSK was used for chromatography. GLC analysis was performed on a Chrom-5 instrument using a column containing 5% of XE-60 on Chromaton N-AW HMDS, FID with helium as the carrier gas at a rate of flow of 40 ml/min with the following temperatures: column, 210°C; evaporator, 250°C; detector, 270°C. IR spectra were taken on a UR-20 instrument in paraffin oil.

TABLE 3. ^{13}C Chemical Shifts of the Carbohydrate Residues in Glycoside C (pyridine, δ, ppm, 40°C)

Carbon atom	Monosaccharide residue					
	A	B	C	D	E	F
1	102.70	104,18	105 44	102,70	104.01	104.65
2	72,59	75.41	80,86	73,20	74.82	75,63
3	72,74	76,66	88,24	75,63	87,98	78.60
4	73,98	78.44	70.77	81,32	69,65	71,76
5	70,52	77,23	77,47	75,63	79,84	78,07
6	18.53	61.44	62.70	67,90	63,09	62,35

PMR and ^{13}C NMR spectra were taken on WP-250 and AM-300 instruments (Bruker) with working frequencies for protons and for carbon of 250 and 63 MHz and of 300 and 75 MHz, respectively, using solutions of the glycosides in pyridine at various temperatures (the Tables give the results for a temperature of 40°C). In the experiments with selective homonuclear double resonance a modified procedure for the difference variant of this type of spectroscopy was used in which the power of the saturated radiofrequency field was considerably lower (by a factor of 4-6) than that necessary for the complete suppression of the spin-spin interaction of protons. This procedure permits a considerable increase in the selectivity of an experiment.

As is known, the incomplete decoupling of spin-spin interaction leads to a distortion of the signal of the interacting proton to form an irregular multiplet with a relatively low peak intensity of the components. The addition of such a multiplet in counterphase to the undistorted multiplet characteristic for the undecoupled signal gives, in the difference spectrum, an almost undistorted undecoupled signal with a normal multiplicity of the SSCC and even a "roof effect."

Isolation of the Glycosides. The sum of the saponins from the air-dried leaves of the aloe yucca was isolated as described previously [1]. A butanolic extract containing glycosides B, C, D, and E was chromatographed on a column of silica gel. When the column was eluted with chloroform–methanol–water (65:35:8), glycosides B and C were isolated, and they were then recrystallized from methanol. From 40 mg of the mixture, the individual glycosides B and C were isolated in the form of white crystalline powders in amounts of 1.25 and 0.65 g, respectively.

Glycoside B had mp 308-311°C, $[\alpha]_D^{20}$ −99° (c 1.0; pyridine), and glycoside C had mp 260-264°C, $[\alpha]_D^{20}$ −81° (c 1.0; pyridine). IR spectra of glycosides B and C: λ_{max} 856, 900, 920, 990 cm^{-1}; both compounds gave a positive Matthews reaction and a negative reaction with the Ehrlich reagent [2, 3].

Hydrolysis of Glycosides B and C. Glycoside B and C (0.1 g each) were hydrolyzed in 2 N HCl (5 ml) at 100°C for 5 h. The aglycon that precipitated was filtered off, washed with distilled water, dried and recrystallized from methanol. In the first case, 0.03 g, and in the second case, 0.024 g of genin was obtained. By TLC in chloroform–ethanol (24:1), the genin was identified in comparison with an authentic sample of tigogenin (the Matthews reagent [3] being used for revealing the spots). In its physicochemical constants (IR spectra, melting point of the genins of its acetate), the sapogenin isolated coincided with tigogenin [8], a mixture with which gave no depression of the melting point.

In the carbohydrate moiety of the glycosides under investigation (after evaporation of the filtrate), by TLC in butanol–methanol–water (5:3:1) with markers, in both cases, glucose, galactose, and rhamnose were identified (the revealing agent being o-toluidine salicylate). The hydrolysates were reduced with sodium tetrahydroborate and were then acetylated in an Ac_2O/Py mixture [1]. The polyol acetates so obtained were identified by GLC in comparison with authentic samples. In both cases, rhamnitol, dulcitol, and sorbitol were identified, in a ratio of 1:1:3 for glycoside B and 1:1:4 for glycoside C.

CONCLUSION

Two new steroid glycosides, consisting of tigogenin penta- and hexaosides, have been isolated from the leaves of aloe yucca, and their chemical structures have been established.

Glycoside B, which we have called yuccaloeside B is (25R)-5α-spirostan-3β-ol 3-{[O-β-D-glucopyranosyl-(1→2)]-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}, and glycoside C, which we have called yuccaloeside C is (25R)-5α-spirostan-3β-ol 3-{[O-β-D-glucopyranosyl(1→3)-O-β-D-glucopyranosyl-(1→2)]-[O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}.

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MASS SPECTRA OF DITERPENE ALKALOIDS WITH THE HETISINE SKELETON

Yu. V. Rashkes, M. S. Yunusov,
E. G. Sirotenko, and Z. M. Vaisov

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An analysis of literature material has shown that the stability of the M^+ ions of bases with the hetisine skeleton decreases considerably when an OR group is present at C-1, C-6, or C-9. The directions of fragmentation are not monotypical and depend greatly on the positions of the oxygen substituents. A similar conclusion can be made from a study of the group and individual features of the breakdown of hetisine, nominine, talatisine, and their derivatives revealed with the aid of high-resolution mass spectrometry and MD spectra. An unusual property of these spectra is the formation of nitrogen-free fragments. The mass spectra of hetisine alkaloids of a new type — zeraconine and its N-oxide — have been characterized.

The interest in diterpene alkaloids that has been increasing in the last quinquennium has been accompanied by an increase in the number of publications on questions of isolating and demonstrating the structure of C_{20} bases with the hetisine skeleton (I) [1]. The volume of mass-spectrometric information contained in these publications is small, and there is practically no information on the laws of their fragmentation. Nor are there any special investigations in the literature of the mass spectra of alkaloids of this series, although the method has been used successfully in determining the structures of diterpene bases of other groups.

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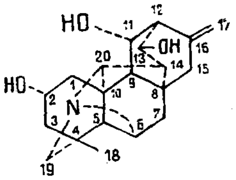

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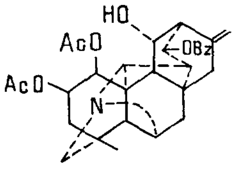

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