A SULFATED GLYCOSIDE FROM THE PREPARATION "TRIBESTAN"

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The chemical composition and structures of the steroid glycosides forming the active principle of the preparation "tribestan," which is used for treating impotence and female infertility, have been studied. It has been shown that together with trillin, prosapogenin A of dioscin, trillarin, dioscin, gracillin, protodioscin, and protogracillin, tribestan contains a diosgenin rhamnoglucoside sulfated in position 4 of the glucose residue, the structure of which has been shown by chemical and physicochemical methods.

Medicinal preparations of natural origin are acquiring ever-increasing popularity at the present time. These include tribestan, which was created on the basis of the total glycosides from the epigeal part of puncture vine (Tribulus terrestris L.) containing mainly furostanols (about 45%), which is used for treating impotence and female infertility.

We have studied the steroid composition of tribestan and the chemical structures of the glycosides present in it. In addition to furostanol compounds, six spirostanols have been detected in it on the basis of the Sannié [1] and Ehrlich [2] reactions. The compounds have been called in order of increasing polarity, glycosides A-H, and they have been separated into individual substances by column chromatography on silica gel.

Thanks to the above-mentioned reactions and their IR spectra, which contained absorption bands characteristic for a spiroketal grouping, glycosides (I-VI) were assigned to the spirostanol compounds of the 25R series. The most polar glycosides, (VII) and (VIII), were of furostanol nature.

The hydrolysis of each of the compounds permitted the isolation of the aglycon, which for glycosides (I-VI) was identified from its physicochemical constant as diosgenin. In view of the fact that glycosides (VII) and (VIII) belong to the furostanol series, the aglycon of these compounds was (25R)-furost-5-en-3β,22α,26-triol. The carbohydrate moiety of glycoside A (I) contained only glucose, as was established as the result of analysis of a hydrolysate by gas, liquid, and paper chromatographies, while the methanolysis of the methylation products confirmed the presence in the chain of only a single glucose residue.

The results obtained indicated that the structure of glycoside (I) was identical with that of trillin [3], which was confirmed by the physicochemical constants of (I).

Making use of the above-mentioned reactions and, particularly, complete and stepwise hydrolysis, periodate oxidation, methylation, and methanolysis, and also GLC, ^{13}C NMR, IR, and UV spectroscopies, after the determination of the melting points and specific rotations of the individual compounds it was established that glycoside (II) was prosapogenin A of dioscin, (III) trillarin, (IV) dioscin, (V) gracillin, (VII) protodioscin, and (VIII) protogracillin [2, 4-6]. For glycoside (VI), rhamnose and glucose were detected in the carbohydrate chain by chromatographic methods. The IR spectrum showed the presence of a $R\text{-}SO_3$ group in the molecule, the position of which was established by direct and indirect methods.

The methylation and methanolysis of the initial (VI) led to methyl 3,4,6-tri-O-methyl-D-glucopyranoside as one of the products, which showed a bond in position 2 of the glucose with the other monosaccharide residue.

In the native glycoside subjected to preliminary acetylation, the sulfate group was replaced by a methyl group followed by methanolysis and deacetylation of the products. Me-4-

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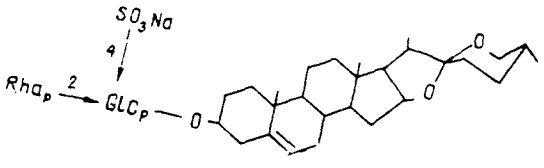
TABLE 1. Carbohydrate Parts of the PMR and ^{13}C NMR Spectra of Compound (VI)

Proton	δ, ppm	J, Hz	Atom	Chemical shift
Rha: H1	6,20d	$J_{1,2}=1,6$	C1	102,13
H2	4,75dd	$J_{2,3}=3,6$	C2	72,54
H3	4,56dd	$J_{3,4}=9,2$	C3	72,88
H4	4,31t	$J_{4,5}=9,2$	C4	74,27
H5	1,86dq	$J_{5,6}=6,1$	C5	69,68
H6	1,71d		C6	18,89
Glc: H1	4,88d	$J_{1,2}=8,1$	C1	100,19
H2	4,19dd	$J_{2,3}=8,9$	C2	78,35
H3	4,43t	$J_{3,4}=8,9$	C3	76,73
H4	5,1 dd	$J_{4,5}=10,0$	C4	81,35
5	3,82ddd	$J_{5,6}=2,8$	C5	76,34
H6, H6′	4,4 m	$J_{6,6'}=6,1$	C6	62,35

TABLE 2. Aglycon Parts of the ^{13}C NMR Spectra of Compunds (II), (IV), and (VI)

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
II	37,69	30,35	78,22	39,15	141,10	121,91	31,99	31,87
IV	37,90	30,52	78,64	39,32	141,23	122,21	32,18	32,07
VI	37,67	30,77	78,44	39,12	141,04	121,98	32,00	31,87
Compound	**C-9**	**C-10**	**C-11**	**C-12**	**C-13**	**C-14**	**C-15**	**C-16**
II	50,48	37,31	21,26	40,05	40,64	56,83	32,36	81,31
IV	50,71	37,52	21,50	40,26	40,86	57,02	32,54	81,58
VI	50,45	37,67	21,26	40,04	40,64	56,81	32,48	81,35
Compound	**C-17**	**C-18**	**C-19**	**C-20**	**C-21**	**C-22**	**C-23**	**C-24**
II	63,06	16,47	19,57	42,16	15,14	109,49	32,48	2944
IV	63,17	19,80	19,80	42,38	15,38	109,85	32,70	29,59
VI	63,05	16,51	19,56	42,16	15,20	109,51	32,60	29,43
Compound	**C-25**	**C-26**	**C-27**					
II	30,74	67,05	17,48					
IV	31,92	67,31	17,69					
VI	30,77	67,06	17,58					

Mono-O-Me-D-Glcp was identified by GLC, which showed the presence of the sulfate group in position 4 of the glucose residue. This conclusion was confirmed by a comparison of the shifts for C-4 of sulfated and unsubstituted glucose in the ^{13}C NMR spectra. In the light of what has been said above, the structure of (VI) appears as:



The conclusion concerning the structure was confirmed completely by PMR and ^{13}C NMR spectra.

EXPERIMENTAL

General Observations. Thin-layer chromatography (TLC) was conducted on Silufol UV-254 plates. The revealing agents were concentrated sulfuric acid and the Sannié and Ehrlich reagents, and the following chromatographic systems of solvents were used: 1) chloroform–methanol–water (65:30:10), lower layer; (2) chloroform–methanol (9:1); 3) benzene–ether (7:3).

TABLE 3. Carbohydrate Parts of the ^{13}C NMR Spectra of Compounds (II), (IV), and (VI)

Compound	C-1	C-2	C-3	C-4	C-5	C-6
Glc: II	100,58	79,70	78,16	72,04	78,22	61,74
IV	100,58	79,27	77,98	78,61	78,06	61,8
VI	100,19	78,35	76,73	81,35	76,34	62,35
Compound	**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**C-6**
Rha: II	100,22	72,65	72,98	74,30	69,64	18,77
IV	103,16	72,70	73,10	74,10	70,82	18,78
	102,45	72,70	74,30	69,92	69,92	19,94
VI	102,13	72,54	72,88	74,27	74,68	18,80

Column chromatography was performed on silica gel L 100/250 μm in the above-mentioned solvent systems.

For paper chromatography we used the butanol–benzene–pyridine–water (5:1:3:3), upper layer system, with aniline phthalate as the revealing agent. GLC was performed on a Chrom-5 instrument. For the sugar derivatives we used a glass column 2.4 m long filled with 5% of XE-60 on Chromaton N-AW-HMDS. For the genins we used a glass column 1.2 m long filled with 3% of OF-1 on Chromaton Super and 5% of SE-3 on Chromaton N-AW-HMDS. The carrier gas was helium, and the temperature for the chromatography of the acetates of the aldononitrile derivatives of the sugars was 180-230°C. With programming of the temperature at 3°C/min and a rate of flow of the carrier gas of 60 ml/min. The temperature for the chromatography of the methyl glycosides was 140°C at rate of flow of the carrier gas of 45 ml/min. The chromatography of the genins was carried out at a thermostat temperature of 230-250°C with a rate of flow of carrier gas of 60 ml/min.

Mass spectra were taken on a MKh-1303 instrument, IR spectra on a UR-20 instrument (tablets with KBr), and PMR and ^{13}C NMR spectra on a Bruker AM-300 instrument at 30°C in Py-d_5. The PMR spectra were interpreted with the aid of the homonuclear double resonance procedure.

Isolation of the Individual Glycosides. A solution of 50 g of the preparation tribestan in system 2 was deposited on a column. By elution successively with systems 2 and 1, the total material was separated into mixtures containing few components which were rechromatographed and, 10-ml fractions being collected, individual glycosides were obtained:

Compound	mp, °C	$[\alpha]_D^{20}$, deg
I. Trillin	275	−103
II. Prosapogenin A of dioscin	237–240	−99
III. Trillarin	258–260	−93
IV. Dioscin	273–276	−115
V. Gracillin	291–293	−88
VI. Sulfated prosapogenin A of dioscin	310–312	−120
VII. Protodioscin	191–196	−79
VIII. Protogracillin	236–238	−51

PMR spectra were recorded of all the compounds except (I).

Proof of the Structure of Glycoside (VI) from Tribestan. Acid cleavage. A solution of 200 mg of (VI) in 5 ml of methanol was treated with 5 ml of 10% H_2SO_4 in MeOH, and the mixture was heated in a sealed tube at 105°C for 24 h. The aglycon, which had deposited as a precipitate, was filtered off, washed with diethyl ether, and crystallized from ethanol mp 201°C $[\alpha]_D^{20}$ − 129° (c 1.0; methanol). ν_{max}^{KBr}, cm^{-1}: 987, 925, 905, 825 (25R-spiroketal).

Partial Hydrolysis. A solution of 50 mg of (VI) in 15 ml of 1% H_2SO_4 was heated in the water bath at 80°C for 30 min. This gave (I) (10 mg; mp 272°C, $[\alpha]_D^{20}$ − 101°) and (II) (17 mg; mp 256-258°C; $[\alpha]_D^{20}$ −92°).

Methylation and Methanolysis. Compound (VI) (10 mg) was methylated by Hakomori's method until the permethylated compound had been obtained, and this was subjected to methanolysis with a mixture of 72% $HClO_4$ and methanol (1:10). After neutralization with an anion-exchange

resin and evaporation, GLC in the presence of markers permitted the identification of methyl 2,3,4-tri-O-methyl-L-Rha$_p$ and methyl 3,4,6-tri-O-methyl-D-Glc$_p$.

Acetylation. A mixture of 30 mg of (VI), 3 ml of pyridine, and 5 ml of acetic anhydride was left at room temperature for 36 h. The resulting peraceate of (VI) was methylated by Hakamori's method and the product was hydrolyzed in methanol and deacetylated with 5% NaOH in MeOH, and the proudcts were analyzed by GLC. Methyl 4-mono-O-methyl-D-Glc$_p$ was identified.

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TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS

XXXII. CYCLOCARPOSIDE FROM Astragalus coluteocarpus

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A new triterpene glycoside of the cycloartane series, which has been called cyclocarposide, has been isolated from the epigeal part of the plant Astragalus coluteocarpus Boiss. (Leguminosae). The structure of cyclocarposide has been established on the basis of chemical transformations and spectral characteristics as 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 6-O-α-L-rhamnopyranoside 3-O-α-D-xylopyranoside.

Continuing investigations of cycloartane methylsteroids and their glycosides from plants of the genus Astragalus (Leguminosae), we have begun a study of Astragalus coluteocarpus Boiss. [1]. In a methanolic extract of epigeal parts of this plant, in various solvent systems on TLC, five products of triterpenoid nature were the most outstanding, and these have been designated in order of increasing polarity as substances 1-5. Substance 4 was isolated by column chromatography of the purified total material obtained from a methanolic extract of the epigeal part of A. coluteocarpus. This substance, of glycosidic nature, proved to be new and we have called it cyclocarposide (I). The present paper is devoted to a proof of the structure of this glycoside.

The PMR spectrum of glycoside (I), containing two one-proton doublets of an AB system at 0.10 and 0.30 ppm and the signals of seven methyl groups in the strong field permitted this compound to be assigned to the triterpenoids of the cycloartane series [2, 3]. An absorption band at 3055 cm^{-1} in the IR spectrum of cyclocarposide due to the stretching vibrations of the methylene group of the cyclopropane ring is in harmony with this conclusion.

The Smith degradation [4] of cyclocarposide gave the genin (II), which was identified as cyclosieversigenin [3].

It was shown by the GLC method that cyclocarposide contains D-xylose and L-rhamnose residues in a ratio of 1:1. This was also shown by the ^{13}C and ^{1}H NMR spectra of glycoside (I) in which the signals of two anomeric carbon atoms at 107.49 and 103.90 ppm (Table 1) and of two anomeric protons at 4.60 and 5.15 ppm, respectively, were readily traced.

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