STEROID SAPONINS

II.* GLYCOSIDES OF Tribulus terrestris

P. K. Kintya, É. D. Perepelitsa,
V. Ya. Chirva, and L. G. Kretsu

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The epigeal part of Tribulus terrestris L. (puncturevine), family Zygophyllaceae, contains substances causing photophobia when the herb is eaten by sheep.

Previously, from a hydrolyzate of an ethanolic extract of the plant by chromatography on a column of alumina there have been isolated diosgenin, ruscogenin, gitogenin, and 25D-spirosta-3,5-diene, the latter being an artifact [1]. In the same plant, G.B. Iskenderov has found diosgenin and ruscogenin [2]. In other literature sources there is information only of the presence of diosgenin in Tribulus terrestris [3, 4]. However, in none of these reports is there any information on the carbohydrate composition of the steroid-glycosides.

We have investigated the total saponins of this plant collected in Moldavia in the summer of 1971. In a methanolic extract there were five substances of glycoside nature which it was possible to separate on a column of alumina. The combustion of each substance gave a large amount of ash, which shows heavy contamination with mineral salts. Consequently, elementary analyses did not give useful information.

It was established by acid hydrolysis that all the compounds considered contain the same aglycone. In its melting point, specific rotation, IR spectrum, and mass-spectrometric decomposition, the sapogenin isolated was identical with diosgenin. A mixed sample of the aglycone with an authentic sample of diosgenin gave no depression of the melting point. No free sapogenins whatever were found in the methanolic extract.

The percentages of the saponins in the plant and their carbohydrate compositions are given in Table 1.

EXPERIMENTAL

Chromatography was performed with paper of type S (medium) of the Volodarskii Leningrad Mill, with KSK silica gel, and with neutral alumina (activity grade II). The sugars were revealed with aniline phthalate and p-anisidine, and the glycosides with conc. sulfuric acid. The IR spectra were taken on a UR-10 spectrometer, the melting points were determined on a Kofler block, and the mass spectrum of the aglycone was determined on an MKh-1303 instrument.

Isolation of the Total Saponins. After defatting with chloroform, 1.5 kg of the comminuted epigeal part of the plant was extracted with 70% aqueous methanol. The extracts were evaporated in vacuum, and the residue, dissolved in water, was repeatedly treated with butanol. In its turn, the organic extract was extracted with diethyl ether. The ethereal extracts were discarded. This gave 7 g of a dark brown extract.

TABLE 1

Saponin	Aglycone	Proportion of the total, %	Carbohydrate composition	Color on TLC
A	Diosgenin	0.4	Glucose	Gray-violet
B		0.6		
C		25	Glucose, rhamnose	Red
D		65	Glucose, arabinose, rhamnose	
E		9	Glucose, rhamnose	

Chromatographic Separation of the Saponins. The butanol extract was percolated through

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a layer of alumina in the chloroform–methanol–water (65 : 35 : 10) system. Of the total purified saponins, 5 g was deposited on a column of alumina (6 × 50 cm) and was eluted with the butan-1-ol–ethanol–water (10 : 2 : 5) system. Substances were isolated (see Table 1) which were named in order of increasing polarity A, B, C, D, and E.

Acid Hydrolysis of the Saponins. A. The combined saponins (1 g) were placed in a tube with 5% sulfuric acid, and the mixture was heated at 110°C for 8 h. Then the contents of the tube were extracted with diethyl ether. The ethereal extract was chromatographed on a column of alumina (ratio of substance to adsorbent 1 : 100). Elution was performed successively with the following solvents: petroleum ether, benzene, benzene–chloroform (1 : 1), chloroform, and chloroform–methanol (1 : 1). The pure chloroform eluted from the column the aglycone, which melted at 203-204°C after recrystallization from methanol. It gave no depression of the melting point with an authentic sample of diosgenin. Its IR and mass spectra coincided with those of the authentic sample, $[\alpha]_D^{25}$ –120° (c 1; chloroform). Yield 0.16 g.

B. Each glycoside (30 mg each) was hydrolyzed similarly. When the ethereal extracts were concentrated, aglycones were obtained which were identified by thin-layer chromatography in the chloroform–methanol (12 : 1) system. The sugars present in the filtrates were determined after neutralization with sodium carbonate by paper chromatography in the butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3) system (see Table 1).

SUMMARY

1. It has been established that the epigeal part of Tribulus terrestris L. contains five steroid saponins. Diosgenin was identified as the aglycone of all these compounds.

2. The carbohydrate compositions of the saponins have been determined.

LITERATURE CITED

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