STEROID SAPONINS

VIII. THE STRUCTURE OF AGAVE SAPONINS C' AND D FROM THE LEAVES OF *Agave americana*

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The detection in the leaves of *Agave americana* L. (century-plant agave) of nine steroid glycosides has been reported previously, and the chemical structure of agave saponin C has been established [1]. On repeated separation of the combined saponins and concentration, a new glycoside has been detected (in very small amounts) which has been called agave saponin C'. The present paper gives information on the chemical structure of another two saponins of this series – agave saponins C' and D.

To investigate their monosaccharide compositions and the nature of the genins, glycosides C' and D were subjected to acid hydrolysis. By paper chromatography (PC) and gas–liquid chromatography (GLC), galactose, glucose, and xylose were found in the hydrolyzate from saponin C', and, in addition, rhamnose in the case of glycoside D. The ratio of the monosaccharides was determined by the GLC of the acetates of the aldononitrile derivatives of the sugars – for saponin C' it was 1 :2 :1 and for D 1 :2 :1 :1.

In both cases, the aglycone was identified by its melting point, specific rotation, chromatographic mobility on thin-layer chromatography (TLC) in the presence of markers, and also by its IR and mass spectra as hecogenin. To determine the types of bonds between the monosaccharides in the carbohydrate chains, we methylated the saponins by Kuhn's method [2] followed by the methanolysis of the permethylated products. The methylated glycosides were identified by TLC and GLC in the presence of markers and also by comparison of the mass spectra of the partially methylated glycosides with those of substances known in the literature [3].

For agave saponin C' we found the following methyl glycosides: methyl 2,3,6-tri-O-methyl-D-galactoside, methyl 2,3,6-tri-O-methyl-D-glucoside, methyl 3,4,6-tri-O-methyl-D-glucoside, and methyl 2,3,4-tri-O-methyl-D-xyloside, and for saponin D methyl 2,3,6-tri-O-methyl-D-glucoside, methyl 2,3,6-tri-O-methyl-D-galactoside, and methyl 4,6-di-O-methyl-D-glucoside were also identified by NMR spectroscopy [4].

In order to determine the sequence of monosaccharides, compounds C' and D were subjected to partial hydrolysis. Each substance gave three progenins. The least polar progenin from both glycoside C' and from glycoside D gave galactose and hecogenin on hydrolysis.

The second progenin in order of polarity proved in each case to be a bioside the carbohydrate chain of which consisted of galactose and glucose. On methanolysis of the permethylated progenin, methyl 2,3,6-tri-O-methyl-D-galactoside and methyl 2,3,4,6-tetra-O-methyl-D-glucoside were identified. It follows from these results that in both cases the galactose is directly attached to the aglycone.

The third progenin, likewise common to both saponins, was a trioside with a carbohydrate chain consisting of galactose and glucose in a ratio of 1 :2 the physical constants and chromatographic mobilities of which coincided with those of the agave saponin C obtained previously [1].

When saponin D was hydrolyzed under mild conditions, in addition to the progenins described above a substance was obtained which proved to identical with saponin C'. The methanolysis of the permethylated product of this substance gave the same methylated glycosides as saponin C' itself. The production of methyl 3,4,6-tri-O-methyl-D-glucoside from the tetraoside in place of methyl 4,6-di-O-methyl-D-glucoside from the pentaoside shows that the xylose is attached to the glucose in position 2 and the rhamnose in position 3 in saponin D.

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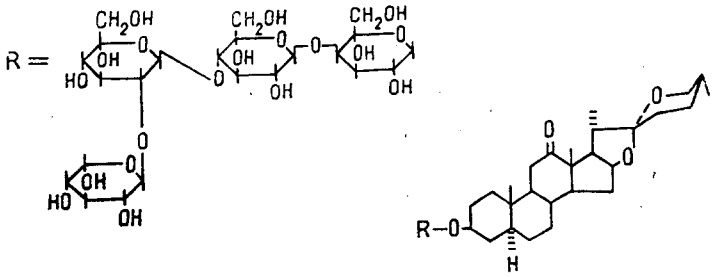
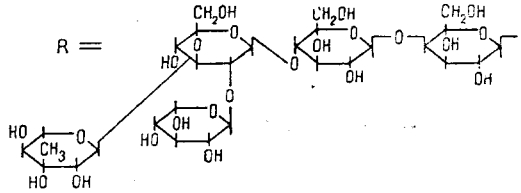
On periodate oxidation, only in the pentaoside did the glucose remain unchanged, which is confirmed by the results of the methylation of the glycosides. The configurations of the glycosidic centers are given in accordance with Klyne's rule [5]. On the basis of the results obtained, the structural formula for agave saponin C' is



and for agave saponin D



EXPERIMENTAL

Chromatography was performed with silica gel L40/100μ and FN-3 chromatographic paper. The mass spectra were taken on an MKh-1303 instrument and the NMR spectra on a RS-60 instrument. GLC was performed on a Khrom-4 chromatograph (Czechoslovakia) with helium as the carrier gas and glass columns containing 5% of XE-60 on Chromaton N-AW-HMDS (0.125-0.250 mm), V_{He} = 50 ml/min. The following solvent systems were used: 1) chloroform–methanol–water (55 : 35 : 7); 2) benzene–ethanol (9 : 1); 3) benzene–acetone (2 : 1); 4) benzene–pyridine–butanol–water (1 : 3 : 5 : 3); 5) chloroform–methanol (9 : 1).

The silica gel thin-layer chromatograms were revealed with sulfuric acid and the paper chromatogram with aniline phthalate.

Preparation of the Individual Glycosides. The combined saponins (3 g) were chromatographed on a column of silica gel in solvent system 1, 10-ml fractions being collected. The separation was monitored by TLC in the same system. This gave 100 mg of saponin C' with mp 200-204°C $[\alpha]_D^{20}$ −54° (c 1.1; CH_3OH), and 500 mg of compound D with mp 298-300°C, $[\alpha]_D^{20}$ −60° (c 1.33; CH_8OH).

Hydrolysis of the Glycosides. Saponins C' and D (30 mg each) were each dissolved in 3 ml of H_2SO_4 and the mixtures were heated in sealed tubes at 110°C for 12 h. The hydrolyzates obtained were filtered from the precipitate that had deposited and were passed through Dowex-8 resin. The eluates were evaporated to 0.1 ml and part of this residue was subjected to paper chromatography. For agave saponin C', galactose, glucose, and xylose were found and for D the same sugars with the addition of rhamnose. The dried hydrolyzate in each case was treated with 1 ml of pyridine and 1.05 g of hydroxylamine hydrochloride, and the mixture was heated at 90°C for 1 h. After cooling, 1 ml of acetic anhydride was added and the mixture was heated at 90°C for another 1 h. Then the reaction mixture was dissolved in water and extracted with chloroform. The chloroform layer was evaporated at 35°C and analyzed by GLC. For saponin C', galactose, glucose, and xylose were detected (1 : 1.85 : 0.9), and for saponin D the same sugars with the addition of rhamnose (1 : 1.85 : 0.9 : 1).

The precipitates after the filtration of the hydrolyzates were purified preparatively on plates of silica gel in system 5. The aglycones obtained had the same melting point, 244-247°C, $[\alpha]_D^{20}$ + 12° (c 0.83; $CHCl_3$) and a molecular peak with m/e 430 (mass spectrometry). Literature data for hecogenin: 345-247°C, $[\alpha]_D^{20}$ + 10° [6].

Methylation of Agave Saponins C' and D. In each case, 200 mg of the glycoside was converted into the permethylated compound [2], and this was purified on a column of silica gel in system 2 and heated with 70% $HClO_4$ in methanol (1 : 10) at 110°C for 5 h, after which the reaction mixture was diluted with water and filtered from the precipitate that had deposited. The filtrate was neutralized and evaporated. The methyl glycosides were separated by chromatography on silica gel in system 3. Saponin C' yielded methyl 2,3,6-tri-O-methyl-

D-glucoside, methyl 2,3,6-tri-O-methyl-D-galactoside, methyl 3,4,6-tri-O-methyl-D-glucoside, and the completely methylated xyloside, which were identified by GLC in the presence of markers. Methyl 2,3,6-tri-O-methyl-4-O-CD_3-D-glucoside and methyl 2,3,6-tri-O-methyl-4-O-CD_3-D-galactoside were obtained by Hakomori's method [7] from the partially methylated methyl glucoside and methyl galactoside and were subjected to mass-spectrometric analysis [3].

The NMR spectra of these sugars were taken [4]. The methyl 2,3,6-tri-O-methyl-D-glucoside and -galactoside had the following chemical shifts of the CH_3 groups (ppm): at C_2 3.15, C_3 3.44, C_6 3.49 in CCl_4 relative to HMDS as internal standard.

The methanolysis of permethylated saponin D gave methyl 2,3,6-tri-O-methyl-D-glucoside, methyl 2,3,6-tri-O-methyl-D-galactoside, methyl 4,6-di-O-methyl-D-glucoside, and the completely-methylated xyloside and rhamnoside. The latter two sugars were identified by GLC. The methyl 2,3,6-tri-O-methyl-D-glucoside, methyl 2,3,6-tri-O-methyl-D-galactoside, and methyl 4,6-di-O-methyl-D-glucoside were identified mass spectrometrically [3] and by NMR spectroscopy. In the NMR spectrum of methyl 4,6-di-O-methyl-D-glucoside signals were observed at 3.46 and 3.49 ppm corresponding to CH_3 groups at C_4 and C_6.

Partial Hydrolysis of the Glycosides. In each case, 300 mg of the saponin, C' or D, in 10 ml of 2% H_2SO_4 was heated in the water bath for 6 h. The precipitate produced was filtered off, dissolved in system 1, and chromatographed on a column of silica gel in the same system. In addition to hecogenin (traces) and the initial saponins, three progenins were obtained from each glycoside.

Progenin 1, yield 40 mg, 220-223°C $[\alpha]_D^{20}$ − 113° (c 1.01; dimethylformamide), the least polar, gave hecogenin and galactose on hydrolysis.

Progenin 2, yield 70 mg, mp 260-264°C, $[\alpha]_D^{20}$ −70° (c 1.01; dimethylformamide), gave galactose and glucose in a ratio of 1 : 1 on hydrolysis.

Progenin 3, yield 100 mg, mp 275-279°C, $[\alpha]_D^{20}$ −55° (c 1.0; dimethylformamide). After hydrolysis, galactose and glucose were found (1:2 :1).

On milder hydrolysis (1% H_2SO_4 in the water bath, 40 min), saponin D yielded yet another substance which had the same R_f as glycoside C', mp 200-204°C, $[\alpha]_D^{20}$ −54° (c 1.1; CH_3OH). The methylation of this progenin followed by methanolysis gave the same methyl glycoside as saponin C'.

Qualitative Periodate Oxidation. In each case, 30 mg of agave saponin, C' or D, was dissolved in 10 ml of H_2O, and then 70 mg of $NaIO_4$ was added and the mixture was left at room temperature in the dark for three days. Then two drops of ethylene glycol was added, the mixture was extracted with butanol, and the extract was evaporated to dryness. The residue was hydrolyzed with 3% H_2SO_4 at 105°C for 8 h. The hydrolyzate was chromatographed on paper in system 4. In the case of saponin D, glucose was detected.

SUMMARY

The complete structures of agave saponins C' and D have been established: they are a tetraoside and a pentaoside, respectively, of hecogenin.

LITERATURE CITED

1. P. K. Kintya, V. A. Bobeiko, and A. P. Gulya, Khim. Prirodn. Soedin., 104 (1975).
2. R. Kuhn and H. Trishman, Chem. Ber., 96, 284 (1963).
3. N. K. Kochetkov et al., The Chemistry of Carbohydrates [in Russian], Moscow (1967), p. 73.
4. D. Gagnair and L. Odier, Carbohyd. Res., 11, 33 (1969).
5. W. Klyne, Biochem. J., 47, xli (1950).
6. S. Harkishan and P. Wilfred, Indian J. Chem., 2, 297 (1964).
7. S. Hakomori, J. Biochem., Tokyo, 55, 205 (1964).