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Note

Thin-layer chromatographic separation of steroid glycosides from *Agava americana* L.

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In our earlier studies on steroidal saponins[1], we isolated several glycosides from *Agava americana* L., and these we designated agavasaponins A, B, C, C′, D, E, F and G. Glycoside G gave a positive reaction with Ehrlich's reagent[2], and we assumed that it was a mixture of furostan glycosides. All the glycosides yielded hecogenin on acid hydrolysis; this compound was identified by its m.p.[3], its infrared (IR) and mass spectrometric parameters and its chromatographic mobility.

Paper chromatography of the sugars obtained from the agavasaponin hydrolysates, and gas–liquid chromatography of their aldononitrile derivatives, showed the presence of galactose in agavasaponin A; galactose and glucose in the ratio 1:1 in agavasaponin B; galactose and glucose in the ratio 1:2 in agavasaponin C; galactose, glucose and xylose in the ratio 1:2:1 in agavasaponin C′; galactose, glucose, xylose and rhamnose in the ratio 1:2:1:1 in agavasaponin D; and the four sugars in the ratios 1:2:1:2 and 1:2:1:3 in agavasaponins E and F, respectively. Agavasaponin G contained the same sugars as the glycosides D, E and F.

The presence of furostanol and spirostanol glycosides in similar plants has been reported[5], and glycosides of these classes possess antitumour activity[6]. Until now, there have been no reports of separation of the saponins from *A. americana*; this paper presents complete data for the thin-layer chromatography (TLC) of ten saponins isolated from the leaves of this plant.

EXPERIMENTAL

Preparation of chromatoplates

Glass plates (17 × 13 × 0.3 cm) were coated with a layer of silica gel (Kieselgel G; Merck, Darmstadt, G.F.R.), then dried at room temperature and activated by heating at 110° for 1 h in an electric oven. In order to avoid edge effects, 5 mm of adsorbent was scraped off each side of the plates perpendicular to the starting line.

Application of samples and development

All the saponins were isolated or prepared in our laboratory. Samples were dissolved in methanol and applied to a starting line 1 cm above the bottom edge of

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TABLE I

R_F VALUES OF AGAVASAPONINS IN DIFFERENT SOLVENT SYSTEMS

Saponin	*Solvent system**				
	1	*2*	*3*	*4*	*5*
A	0.50	1.00	0.62	0.77	0.70
B	0.25	0.94	0.44	0.65	0.61
C	0.11	0.90	0.31	0.56	0.53
C′	0.06	0.77	0.20	0.45	0.46
D	0.04	0.72	0.17	0.43	0.44
E	0.03	0.60	0.11	0.36	0.35
F	0.02	0.54	0.07	0.32	0.30
G	0.00	0.48	0.04	0.26	0.26

* Solvent systems 1, 2, 3 and 4 consisted of chloroform–methanol–water in the ratios 13:5:2, 55:35:7, 13:6:2, and 65:35:7, respectively. System 5 was butanol–ethanol–water (5:1:1).

the plate using a capillary tube. After drying, the chromatogram was developed with one of the solvent systems listed in Table I.

Detection

After development, the plates were air-dried, sprayed with sulphuric acid and heated at about 90° with an IR lamp for 5 min.

Preparation of acetates

The mixed saponins (70 mg), 2 ml of pyridine and 2 ml of acetic anhydride were set aside overnight at room temperature, then the mixture was diluted to 10 ml with ice–water and extracted with chloroform.

RESULTS AND DISCUSSION

TLC is a versatile method for separating various kinds of complex mixture; its application to steroid glycosides has been known since 1963 (ref. 7). Most of our saponins were separated as free compounds, but three formed an inseparable mixture, which we designated saponin G. After acetylation, we separated the derivatives from saponin G by TLC using solvent systems 6 and 7 shown in Table II.

The R_F values of the agavasaponins in various solvent systems are shown in

TABLE II

R_F VALUES OF ACETYLATED COMPONENTS OF SAPONIN G

Component No.	*Solvent system**	
	6	7
1	0.75	0.55
2	0.61	0.46
3	0.41	0.27

* Solvent system 6 = chloroform–methanol (24:1); system 7 = benzene–dioxane–ethyl acetate (3:1:1).

Table I, and those of the saponins impossible to separate as free compounds are presented in Table II. It can be seen that the best separation of free saponins was achieved in system 4, and that of the acetates of saponin G in system 6.

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