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Note

Thin-layer chromatography of acetyl derivatives of soyasapogenols A, B, C, D and E

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Recently, there has been great interest in saponins owing to their antinutritional and physiological properties^{1,2}. Aglycones of saponins from soybean, alfalfa and ladino clover have been separated by paper³, thin-layer⁴ and gas-liquid chromatography⁵. This paper describes the conditions for the separation of acetyl derivatives of soyasapogenols A, B, C, D and E by thin-layer chromatography (TLC). The acetyl derivatives are easy to prepare and their separation is better than that of soyasapogenols.

EXPERIMENTAL

Materials

Pre-coated with silica gel 60 (E. Merck, Darmstadt, G.F.R.) ready-for-use plates were used. Saponins were isolated from soybean var. *Warszawska* according to the method of Birk *et al.*⁶. The saponins were hydrolyzed with 2 N HCl in 50% methanol by boiling for 15 h. Standard soyasapogenols A, B, C, D and E were separated on a silica gel column (70-270 mesh; Macherey, Nagel U Co. Düren, G.F.R.), using benzene-absolute ethanol (96:4) as eluent. Each of the isolated standard soyasapogenols was purified by repeated crystallization.

Preparation of acetyl derivatives

Each of the standard soyasapogenols B, C, D and E was treated with a mixture of acetic anhydride-pyridine (1:1) and left overnight at room temperature. Soyasapogenol A and the saponin obtained by hydrolysis of the soybean saponin were acetylated by boiling with the same mixture for 3 h. The acetyl derivatives of the standards were purified by crystallization.

Thin-layer chromatography

Chromatograms were developed with three solvent systems: 1, hexane-benzene-acetone (50:45:5); 2, hexane-diethyl ether (65:35); 3, cyclohexane-acetone (90:10). The developed chromatograms were dried, sprayed with acetic anhydride-conc. sulphuric acid-ethanol (1:1:10)⁷ (Liebermann-Burchardt reagent) and heated at 110° until the spots were visible.

RESULTS AND DISCUSSION

Acetylation makes it possible to achieve good separation of all the soyasapogenols including C and D which are difficult to resolve. Up to now only one solvent system for their separation was known⁴. Table I presents the actual R_F values for the acetyl derivatives of soyasapogenols A, B, C, D and E in three solvent systems. The colours of the acetyl derivatives of the soyasapogenols sprayed with Liebermann-Burchardt reagent are as follows: A, cherry red and brown; B, cherry red and brown; C, cherry red and violet; D, brown; E, violet. Among the three solvent systems hexane-benzene-acetone (60:45:5) gave the best resolution. The other two solvents give tailing spots of soyasapogenol E. The results of the development of standard acetyl soyasapogenols A, B, C, D and E, and acetyl derivative of the soya sapogenins (S), are illustrated in Fig. 1.

TABLE I

R_F VALUES OF ACETYL DERIVATIVES OF SOYASAPOGENOLS A, B, C, D AND E IN THREE SOLVENT SYSTEMS

Solvent	R_F				
	A	B	C	D	E
Hexane-benzene-acetone (50:45:5)	0.23	0.42	0.63	0.57	0.33
Hexane-diethyl ether (65:35)	0.17	0.34	0.55	0.48	0.28
Cyclohexane-acetone (90:10)	0.23	0.39	0.59	0.54	0.34

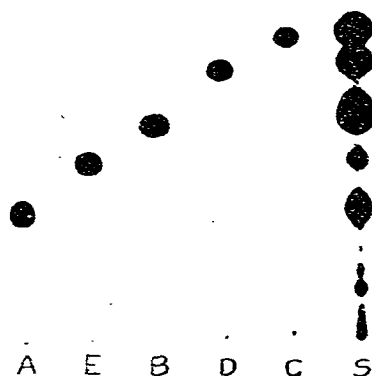


Fig. 1. TLC chromatogram of standard acetyl soyasapogenols A, B, C, D and E and acetyl derivative of soya sapogenins (S). Solvent: hexane-benzene-acetone (50:45:5).

REFERENCES

- 1 Y. Birk, in Liener (Editor), *Toxic Constituents of Plant Foodstuffs*, Academic Press, New York, London, 1969, p. 169.
- 2 P. R. Cheeke, J. H. Kinzell and M. W. Pedersen, *J. Anim. Sci.*, 46 (1977) 476.
- 3 B. Gestetner, *J. Chromatogr.*, 13 (1964) 259.
- 4 S. Shany, Y. Birk, B. Gestetner and A. Bondi, *J. Sci. Food Agr.*, 21 (1970) 131.
- 5 M. Jurzysta and A. Jurzysta, *J. Chromatogr.*, 148 (1978) 517.
- 6 Y. Birk, A. Bondi, B. Gestetner and I. Ishaaya, *Nature (London)*, 197 (1963) 1089.
- 7 E. Stahl (Editor), *Thin-Layer Chromatography, A Laboratory Handbook*, Springer, Berlin, Heidelberg, New York, 1969, p. 855.