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Note

Gas–liquid chromatography of trimethylsilyl ethers of soya sapogenols and medicagenic acid

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During recent years a great interest in saponins of legume crop plants, especially soybean, alfalfa and ladino clover, has been shown[1]. Their sapogenins have been separated by paper[2] and thin-layer[3] chromatography, but no gas–liquid chromatographic (GLC) separation of the compounds has been reported. This paper describes the conditions for the GLC separation of soya sapogenols A, B, C, D and E, and medicagenic acid.

MATERIALS AND METHODS

Soya sapogenol standards

Saponins were isolated from soybean var. *Warszawska* according to the method described by Birk *et al.*[4]. The saponins obtained were hydrolysed with hydrochloric acid in methanol by boiling for 30 h (ref. 5). The mixture of soya sapogenols was separated on a silica-gel column (70–270 mesh, Macherey, Nagel & Co., Düren, G.F.R.) using benzene–absolute ethanol (96:4, v/v) as eluent. Each of the isolated soya sapogenols A, B, C and E was purified by repeated crystallisation.

Medicagenic acid standard

Saponins were isolated from alfalfa roots as described by Wall *et al.*[6]. The saponins were hydrolysed as above. The medicagenic acid was separated from the hydrolysate and purified by the method worked out in this laboratory[7]. The purity and authenticity of the isolated standard soya sapogenols and medicagenic acid were checked by TLC[3], melting points and mass spectrometry. Soya sapogenol D was obtained by the courtesy of Prof. Jeger from Zürich, Switzerland.

Soybean saponin aglycones

Soybean saponins were isolated from *Warszawska* var. as has been described for standard soya sapogenols except that hydrolysis was performed with 1 *N* sulphuric acid in water–dioxan (3:1, v/v) by boiling under reflux for 12 h (ref. 8).

Crystalline saponins were isolated from alfalfa seed of *Kleszczewska* var. by the method worked out in this laboratory[9]. The saponin was hydrolysed as above.

Crude saponin extract aglycones

Dried and ground alfalfa roots were extracted with 50% ethanol for 4 h. The extract, after evaporation of ethanol, was hydrolysed as above.

Reagents

Cholesterol from Byk-Mallinckrodt (Wesel, G.F.R.) served as the internal standard for gas chromatography.

For silylation N,O-bis-(trimethylsilyl)acetamide (BSA) and chlorotrimethylsilane (TMCS) (Pierce, Rockford, Ill., U.S.A.) were used.

Trimethylsilylation

The standards (1–3 mg) or aglycones (10 mg) were dissolved in 1 ml of a solution of BSA–TMCS (5:1.5). The solution was shaken for 30 sec and then allowed to stand for 4 h. Aliquots of 1–2 μl of the mixture were injected into the chromatograph.

Gas–liquid chromatography

The GLC analyses were performed on a Varian Aerograph Model 2440 equipped with two flame-ionization detectors. The column was a 6-ft. $\times$ 2 mm I.D. glass spiral. The packing material consisted of 3% OV-17 on 80–100 mesh Chromosorb W HP. The temperature of the oven, detector and injector port was 300°. The carrier gas was nitrogen at a flow-rate of 100 ml/min. Hydrogen and air flow-rates were adjusted to give maximum detector response.

RESULTS AND DISCUSSION

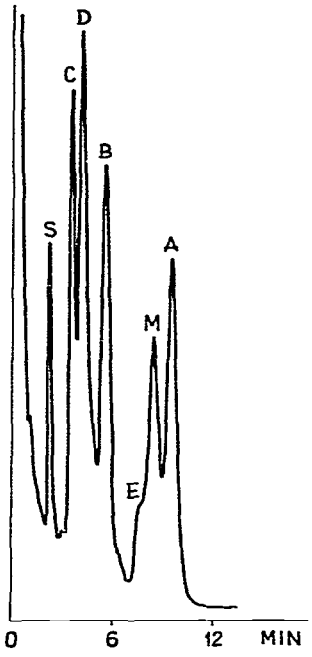
Soya sapogenols B, C, D and E react with the BSA–TMCS mixture in a few minutes. Complete silylation of soya sapogenol A takes place after about 2 h, but the TMS ether of medicagenic acid needs more time to form. Therefore, the time of 4 h was accepted to ensure complete silylation of all the components.

The GLC separation of standard TMS ether soya sapogenols and TMS ether medicagenic acid is shown in Fig. 1. Retention times relative to cholesterol were as follows: soya sapogenol C, 1.59; D, 1.91; B, 2.55; E, 3.55; A, 4.32; and medicagenic acid, 3.82.

When medicagenic acid is absent (see Fig. 2) all the analysed soya sapogenols are well separated on the column. But if medicagenic acid is present in the sample it is not well-separated from soya sapogenol E, although the two compounds can be distinguished.

Fig. 2 shows aglycones of soybean saponin. Soya sapogenols A, B, C and E and an unidentified component, X, can be seen on the chromatogram. All the compounds were also identified by TLC. In contrast to the literature[5,8], soya sapogenol D was not found among the soybean sapogenins.

As can be seen from Fig. 3, the same soya sapogenols except for A were identified in alfalfa seed sapogenins but in different quantitative ratios. Compound X was also found. All these components have been previously found in alfalfa seed saponins[10]. In a crude saponin extract of alfalfa roots, medicagenic acid was the



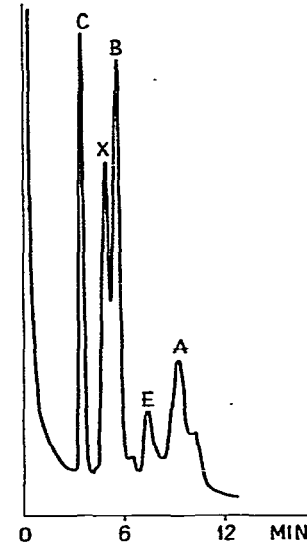
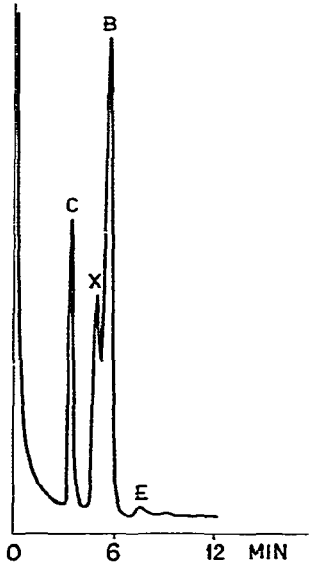


Fig. 1. Gas–liquid chromatogram of standard soya sapogenols A, B, C, D and E, and medicagenic acid (M). S, cholesterol standard.

Fig. 2. Gas–liquid chromatogram of soybean saponin aglycones. ×, unidentified compound. Identification of other peaks as in Fig. 1.



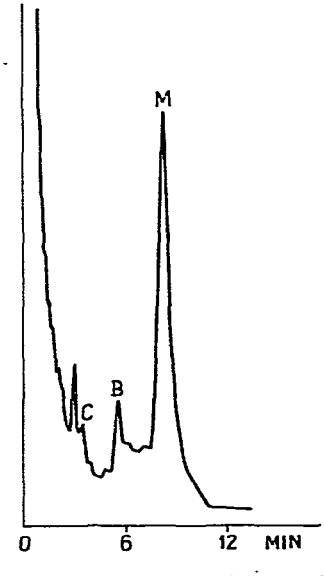


Fig. 3. Gas–liquid chromatogram of crystalline saponin aglycones from alfalfa seed. ×, unidentified compound. Identification of other peaks as in Fig. 1.

Fig. 4. Gas–liquid chromatogram of crude saponin extract aglycones from alfalfa root. Identification of peaks as in Fig. 1.

dominant component (Fig. 4). Soya sapogenols B and C were also found but in small quantities.

Component X, which is of sapogenin nature, was found in all the saponin hydrolysates. It was also isolated but its structure has not yet been established.

This method can be successfully applied to various plant sapogenin separations and also to crude saponin extract hydrolysates.

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