A rapid paper chromatographic method for separation and identification of soybean sapogenols*

The biological effects of soybean saponins have been studied in this laboratory**. In order to differentiate between the various soybean saponins, a simple and rapid method was required for the separation and identification of the sapogenols of which these saponins are composed.

The aglucone moiety of soybean saponins is known to consist of four sapogenols, designated A, B, C, and D, respectively (OCHIAI *et al.*[1] and MEYER *et al.*[2]). The procedures described by these authors are, however, intended for a preparative separation of the sapogenols and have been found to be too lengthy for analytical purposes. DIECKERT *et al.*[3], using glass-paper chromatography for establishing the relationship between groundnut sapogenols and soybean sapogenols, achieved a satisfactory separation between the soybean sapogenols only when the paper was impregnated with silicic acid.

Soybean saponins were prepared from ether-extracted soybean meal by a method developed in this laboratory[4]. The saponins were subjected to acid hydrolysis in ethanolic hydrochloric acid, as described by DJERASSI *et al.*[5]. The resulting sapogenols were precipitated by adding water to the hydrolysate. The precipitate was dissolved in a small amount of chloroform and chromatographed on Whatman No. 1 and No. 3MM papers by ascending and descending techniques. Several solvent systems were examined and it was found that the water-free system of petrol ether–chloroform–acetic acid, recommended by SANNIÉ *et al.*[6] for the separation of steroid sapogenols, provided an excellent medium also for the separation of the triterpenoid soybean sapogenols. Preliminary experiments showed that the most suitable ratio of the solvents is petrol ether–chloroform–acetic acid (100 : 10 : 2.5) and that better resolution can be achieved with Whatman No. 3MM than with Whatman No. 1 paper. After the completion of the chromatographic run the papers were dried in a hood and then developed with a saturated solution of $SbCl_3$ in chloroform, as described by COULSON[7]. Although in some chromatograms five distinct spots could be identified, in most cases the differentiation was difficult because of considerable "tailing" of the spots.

This major obstacle was overcome by introducing the horizontal, circular chromatography technique. Whatman No. 3MM papers, 20 cm × 20 cm, were spotted with 5 λ samples 1.5 cm from the center and 2 cm apart, and a paper wick was introduced into the center of the paper. The latter was put above an open petri dish (15 cm diameter) leaning on the rim with the wick immersed in the solvent (25 ml) and then covered with the other part of the dish. When the solvent reached the rim of the petri dish, the paper was taken out and treated as described above. As can be seen in Fig. 1, the tailing effect was completely eliminated and satisfactory separation into five distinct zones could be achieved. The R_F values of the hydrolysate constituents were compared to those of the chemically defined soybean sapogenols A, B, C and D***.

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* Part of a thesis to be submitted to the Hebrew University of Jerusalem in partial fulfilment of the requirements for the Ph.D. degree.

** Supported by Grant No. FG-Is-112 from the U.S. Department of Agriculture.

*** Kindly donated by Prof. O. JEGER of The Federal Institute of Technology, Zürich.

When a mixture of sapogenols C and D was chromatographed some overlapping was observed even when the minimal amount of sapogenol (4–5 μg/spot) necessary to produce a distinct colour reaction with $SbCl_3$ was used. This difficulty was overcome by substituting hexane (b.p. 68–70°) for petrol ether in the solvent system. As shown

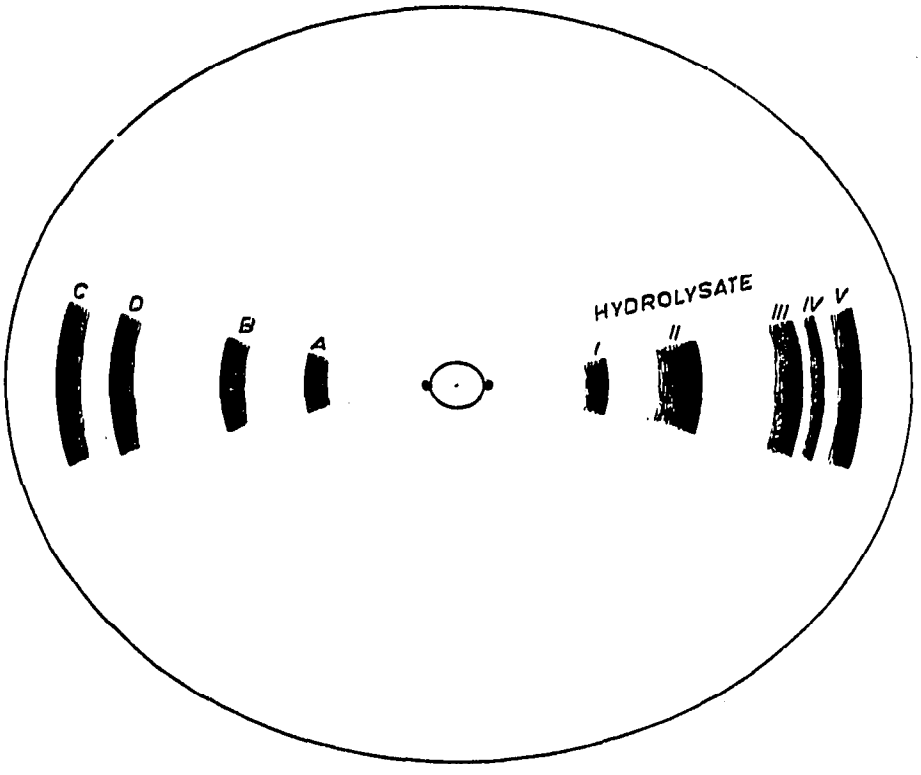

Fig. 1. Circular paper chromatography of soybean-sapogenols A, B, C, D and of the acid hydrolysate of soybean saponins. Paper: Whatman No. 3MM. Solvent: hexane–chloroform–acetic acid (100:10:2.5). Developing reagent: $SbCl_3$ in chloroform.

in Fig. 1, the latter modification of the solvent brought about a very clear separation between the different sapogenols. It should be pointed out that this separation was obtainable only in the range of 5–20 μg/spot. The chromatogram of the soybean saponin hydrolysate contains an additional zone (R_F 0.76), which stains green with $SbCl_3$, but is not detectable when the paper is dipped in a mixture of acetic anhydride–sulfuric acid (1:1). The chemical nature of this compound has not yet been established.

The R_F values of chromatograms performed in the two solvent mixtures are summarized in Table I. It is obvious from Table I that in the solvent which contains hexane, the difference between the R_F values of sapogenols C and D is greater, whereas the difference between the R_F values of sapogenols A and B does not change appreciably. Very good agreement was found between the R_F values of the components of the hydrolysate, and those of sapogenols A, B, C and D.

The use of circular paper chromatography enables separation between different sapogenols after a very short distance of migration of the solvent (6 cm), which is completed within 10 minutes. The high volatility of the solvent makes possible complete drying in 5 min; including the staining process, the chromatography of the soybean sapogenols can be accomplished in less than one hour.

TABLE I

R_F VALUES OF SOYBEAN SAPOGENOLS AND OF ACID HYDROLYSATES OF SOYBEAN SAPONINS

Compound	*R_F values*		*Colour with $SbCl_3$*
	Petrol ether–chloroform–acetic acid	*Hexane–chloroform–acetic acid*	
Soya-sapogenol A	0.32	0.27	brown
Soya-sapogenol B	0.50	0.47	violet
Soya-sapogenol C	0.71	0.86	violet
Soya-sapogenol D	0.67	0.73	violet
Zone I hydrolysate	0.32	0.26	brown
Zone II hydrolysate	0.50	0.47	violet
Zone III hydrolysate	0.65	0.73	violet
Zone IV hydrolysate	0.67	0.76	green
Zone V hydrolysate	0.70	0.85	violet

Since soybean sapogenols are frequently found in the saponins of other legumes[3,8,9], it is hoped that the described procedure may also be applicable in their analyses.

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Received May 3rd, 1963