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Note

Analysis of Fenugreek sapogenins by gas–liquid chromatography

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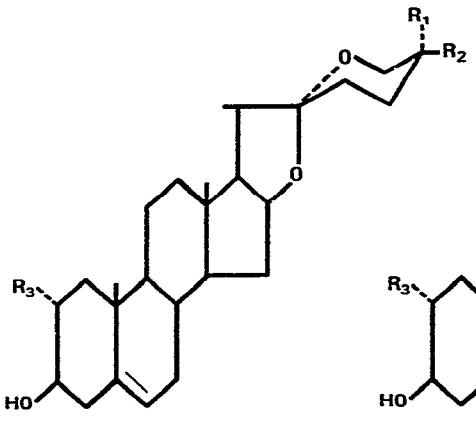
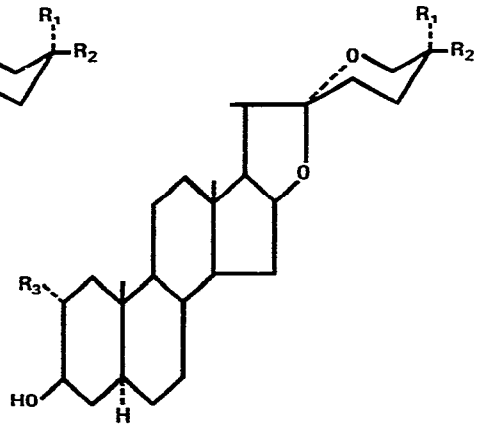
The seed of the Fenugreek plant (*Trigonella foenum-graecum*) is a potentially useful source of diosgenin (I), a widely-used raw material for steroid manufacture.





	R_1	R_2	R_3	
I	H	CH_3	H	Diosgenin
II	CH_3	H	H	Yamogenin
V	H	CH_3	OH	Yuccagenin
VI	CH_3	H	OH	Lilagenin

	R_1	R_2	R_3	
III	H	CH_3	H	Tigogenin
IV	CH_3	H	H	Neotigogenin
VII	H	CH_3	OH	Gitogenin
VIII	CH_3	H	OH	Neogitogenin

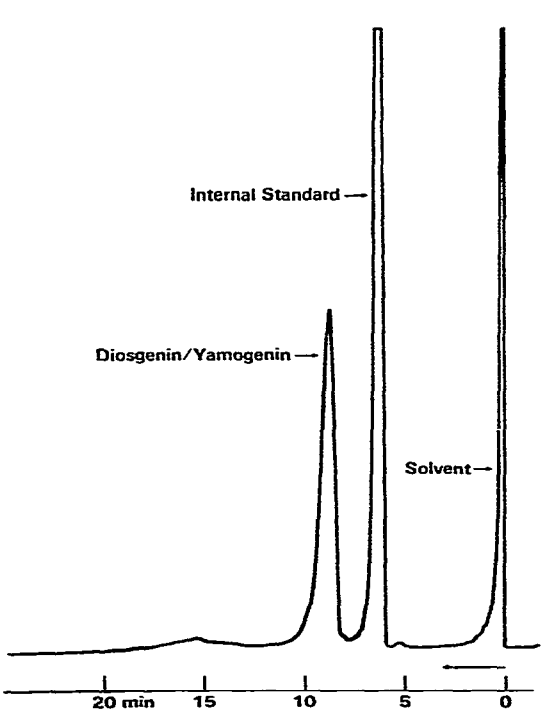
Assay of the crude steroidal sapogenins obtained by acid hydrolysis of the plant material presents some problems, however, because together with diosgenin a considerable amount of the C_{25}-epimer yamogenin (II) is found, from which it can only be separated with some difficulty. In addition, there is usually some of the 5α-dihydro analog tigogenin present (III) plus the polar sapogenins gitogenin (VII) and yuccagenin (V).

A gas–liquid chromatographic assay for diosgenin in dioscorea root has been described[1] in which the acid hydrolysis products are chromatographed without derivatization. This works well when diosgenin is substantially the only sapogenin present, but it fails to separate the C_{25}-epimers from each other or from their 5α-dihydro

analogs, and the more polar 2α,3β-dihydroxy steroids (V–VIII) are decomposed on the column. Diosgenin and yamogenin may each be determined by IR spectrophotometry after column chromatography to remove interfering components[2,3] but this method does not allow determination of the tigogenin/neotigogenin content. A generally applicable method which would allow simultaneous determination of all the above-mentioned compounds without elaborate sample preparation would therefore be of considerable interest.

EXPERIMENTAL

Analyses were performed on a Varian Model 2440 gas chromatograph. Glass columns were used exclusively (6 ft. × 6 mm O.D. × 3 mm I.D.), packed with Chromosorb Q (100–120 mesh) coated with the following stationary phases: 2% SE-30 for free sapogenins; 3% OV-17 for trimethylsilyl (TMS) ethers; 3% QF-1 for trifluoroacetates. TMS ethers were prepared by dissolving the sapogenin (10 mg) in a mixture of pyridine (0.5 ml) and BSTFA (1 ml) and warming to 60° until the reaction was complete. For the preparation of trifluoroacetates, the sapogenin was dissolved in a mixture of chloroform (0.5 ml) and trifluoroacetic anhydride (0.5 ml). After 15 min at room temperature, the solution was evaporated to dryness in a stream of



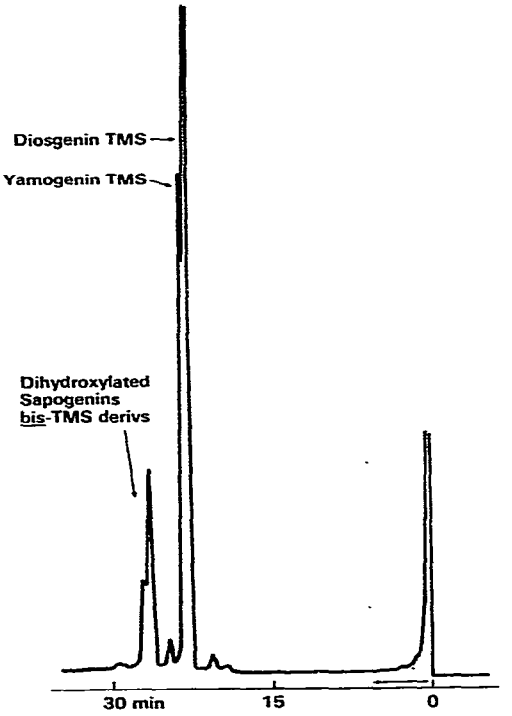


Fig. 1. Analysis of crude diosgenin with 5α-cholestan-3β-ol as internal standard and no derivatization. Stationary phase, 2% SE-30; column temperature, 250°.

Fig. 2. Crude diosgenin after trimethylsilylation. Stationary phase, 3% OV-17; temperature programmed from 200–260° at 4°/min with 5-min preprogram hold.

nitrogen at room temperature and the residue redissolved in chloroform prior to injection.

RESULTS

As shown in Fig. 1, there was no appreciable separation of diosgenin and yamogenin when the extract was not derivatized, and the more polar sapogenins decomposed. Silylation improved the separation, and also gave peaks for the polar components (Fig. 2), but tigogenin and diosgenin had identical retention times under these conditions. The best separation was obtained when the trifluoroacetates were chromatographed on QF-1 (Fig. 3), when eight compounds could be detected, which were evidently arranged in four neo–iso pairs. These were identified where possible by comparison with authentic samples (diosgenin, tigogenin, and gitogenin), and the remainder by gas chromatographic–mass spectral[4] data. Additional proof of the presence of the neo epimers came from an equilibration experiment. After refluxing for 8 h in ethanolic HCl[5], the peaks ascribed to the neo-sapogenins were all greatly diminished (Fig. 4) because of conversion to the iso-forms. The first eluting peak in Fig. 4 is due to a competing side reaction, the dehydration of the 5-en-3-ol components to give 3,5-dienes. The relative amounts of the various sapogenins found in the sample illustrated in Fig. 3 are given in Table I.

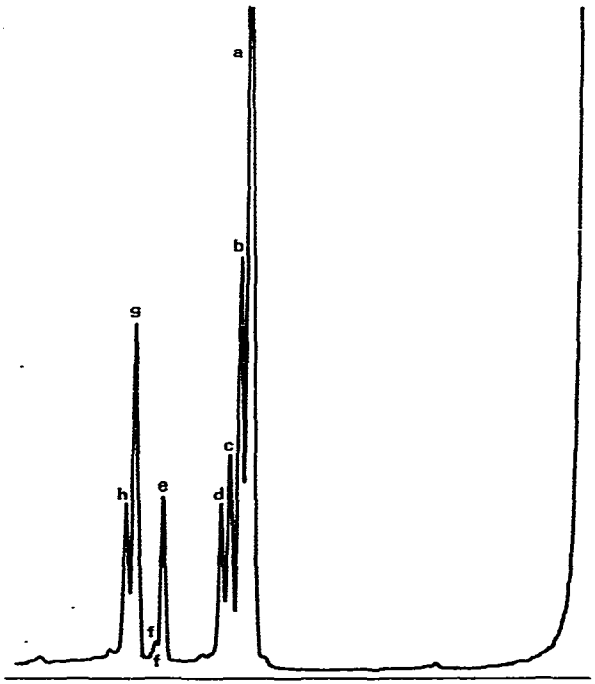


Fig. 3. Crude diosgenin after trifluoroacetylation. Stationary phase, 3% QF-1; column temperature programmed from 200–230° at 2°/min, with 20-min preprogram hold. a = diosgenin; b = yamogenin; c = tigogenin; d = neotigogenin; e = yuccagenin; f = lilagenin; g = gitogenin; h = neogitogenin.

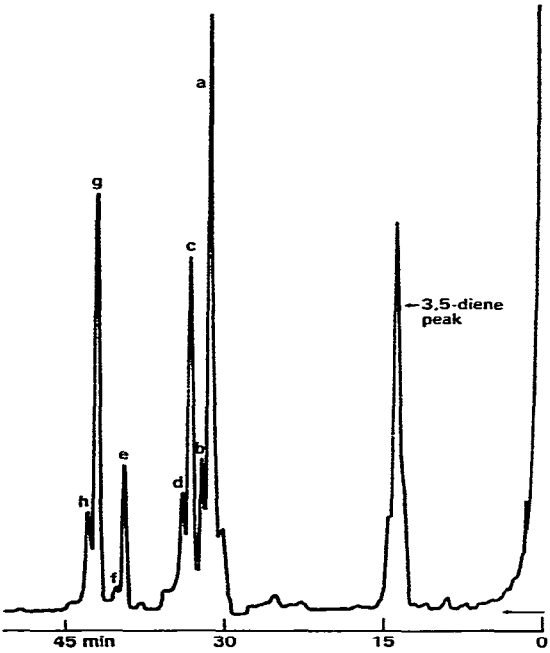


Fig. 4. Trifluoracetates of crude diosgenin, previously equilibrated in ethanolic HCl. Same conditions and peak identification as in Fig. 3.

TABLE I

SAPOGENIN CONTENT OF CRUDE DIOSGENIN FROM FENUGREEK

Compound	*Amount (%)*	*Type*
Diosgenin I	40.3	iso
Yamogenin II	16.1	neo
Tigogenin III	10.2	iso
Neotigogenin IV	7.8	neo
Yuccagenin V	5.2	iso
Lilagenin VI	0.6	neo
Gitogenin VII	12.0	iso
Neogitogenin VIII	6.6	neo

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