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Note

Determination of hecogenin in *Agave sisalana* by gas–liquid chromatography

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The occurrence of steroidal saponins in *Agave sisalana* has previously been reported. The plant is harvested all the year round in tropical areas and used as a fibre source[1]. A major saponin in this plant, hecogenin [3β-hydroxy-(25*R*)-5α-spirostan-12-one], is a useful starting material for the manufacture of steroid hormones. The methods for the determination of hecogenin so far devised involve densitometry on a thin-layer chromatogram[2] and gas–liquid chromatography of the acetate[3] or trimethylsilyl derivative[4]. These methods, however, are not satisfactory with respect to feasibility and sensitivity. An urgent need to clarify the hecogenin contents of the crude sapogenin concentrates known as "coffee ground" prompted us to develop a reliable method for the quantitation of hecogenin.

EXPERIMENTAL

Materials

Authentic hecogenin was purchased from Steraloids (Pawling, N.Y., U.S.A.) and its purity was checked by thin-layer chromatography prior to use. Cholest-4-en-3-one (internal standard) was prepared in these laboratories. Trifluoroacetic anhydride and other chemicals were of analytical-reagent grade.

Gas chromatography

A Shimadzu Model 4BM gas chromatograph equipped with a hydrogen flame-ionization detector and a coiled glass column (1 m × 2 mm I.D.) was used. The column was packed with 1% XE-60 on Gas-Chrom Q (80–100 mesh). The detector and flash heater were kept at 230° and the column temperature was 215°. Nitrogen was used as the carrier gas at a flow-rate of 40 ml/min.

Gas chromatography–mass spectrometry

A Shimadzu Model 9000B gas chromatograph–mass spectrometer was used. A coiled glass column (2 m × 2 mm I.D.) was packed with 1% OV-1 on Gas-Chrom Q (80–100 mesh), and the flow-rate of carrier gas (helium) was 30 ml/min. The column temperature was 250° and the injection port and ion source were kept at 270°.

The accelerating voltage, ionization voltage and trap current were 3.5 kV, 70 eV and 60 μA, respectively.

Preparation of the trifluoroacetate

A sample (0.9–7.2 μg) was treated with trifluoroacetic anhydride (0.2 ml) and allowed to stand at room temperature for 10 min. After removal of the excess of reagent by means of a stream of nitrogen gas the residue was dissolved in chloroform (0.5 ml) containing a known amount of cholest-4-en-3-one (*ca.* 200 μg). A 1–2-μl volume of this solution was injected into the gas chromatograph. The retention times of hecogenin and cholest-4-en-3-one were 8.7 and 3.9 min, respectively.

Determination of hecogenin in crude sapogenin concentrates

A ground sample (100–339 mg) was extracted twice with 20 ml of methanol at 50°. The solid was removed by filtration and washed twice with 10 ml of cold methanol. The filtrate and washings were combined and concentrated under reduced pressure, and the whole volume was brought to 30 ml with methanol. An 1-ml aliquot of this solution was transferred into a tube, evaporated to dryness and submitted to gas chromatographic determination. Another 1-ml aliquot was similarly evaporated, and the residue was treated with 2 *N* hydrochloric acid at 90° for 1–3 h. The hydrolysate was extracted twice with 10 ml and once with 5 ml of ethyl acetate and the organic layer was dried over anhydrous sodium sulphate and evaporated to dryness. After addition of the internal standard to the extract the solution was subjected to gas chromatographic determination.

RESULTS AND DISCUSSION

Previous papers have described the gas–liquid chromatography of hecogenin in the form of the acetate[3] or trimethylsilyl ether[4]. These derivatives, however, are unsatisfactory with respect to volatility and sensitivity. Therefore, trifluoroacetylation was used as the derivatization method prior to gas chromatography.

Treatment of hecogenin with trifluoroacetic anhydride in the usual manner gave a single peak of the correct theoretical shape on the gas chromatogram. The structure of the resulting trifluoroacetate was characterized by gas chromatography–mass spectrometry. The mass spectrum (Fig. 1), exhibiting characteristic fragment ions (*m/e* 467, 454, 139, 115), provided unequivocal support for the assignment of the structure as hecogenin 3-trifluoroacetate[5].

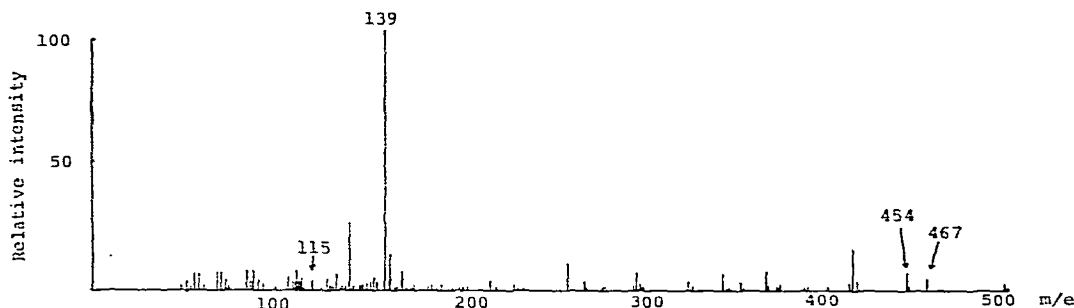

Fig. 1. Mass spectrum of trifluoroacetyl derivative of hecogenin in crude sapogenin concentrates.

A calibration graph was constructed by plotting the ratio of the peak height of hecogenin to that of the internal standard (cholest-4-en-3-one) against the weight ratio of the two; satisfactory linearity was observed in the range 0.9–7.2 μg of hecogenin. Quantitation of hecogenin in the crude preparation was then carried out. Initially, the content of conjugated hecogenin was measured by hydrolytic cleavage with hydrochloric acid followed by determination of the hydrolysate by gas chromagraphy. No substantial difference was found between the values obtained with and without prior hydrolysis. Hecogenin contents of crude sapogenin concentrates determined by the present method are given in Table I. The results are comparable with those previously reported by Cripps and Blunden[4]. It is of interest that the hecogenin content is dependent on the season when the plant is harvested.

TABLE I

HECOGENIN CONTENTS OF CRUDE SAPOGENIN CONCENTRATES DETERMINED BY THE PRESENT METHOD

*Sample**		*Hecogenin content***	
No.	*mg*	*mg*	*%*
1	100	32.2	32.2
2	152	46.5	30.6
3	120	23.5	19.6

* The three samples were obtained in different seasons.
** Mean value of three determinations.

It is hoped that the availability of the convenient and reliable method for the determination of hecogenin will provide a more precise knowledge of its content in *Agave sisalana* and related plants.

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