Journal of Chromatography, 245 (1982) 138–140
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,951

Note

Quantitative high-performance liquid chromatographic method for the estimation of hecogenin and tigogenin in the leaves and sapogenin concentrates of *Agave sisalana*

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(Received April 5th, 1982)

Hecogenin [3β-hydroxy-(25*R*)-5α-spirostan-12-one], obtained commercially from the juice of *Agave sisalana* Perrine leaves, is used for the production of corticosteroids. Large scale production of the sapogenin concentrates known as "coffee grounds", from which hecogenin is extracted, is probably confined, at present, to Tanzania, although pilot scale production is being undertaken in other countries. *A. sisalana* leaves yield several steroidal sapogenins[1] of which, after hecogenin, tigogenin [(25*R*)-5α-spirostan-3β-ol] is the most abundant. The ratio of hecogenin to tigogenin is important commercially and the proportion of tigogenin should be as low as possible.

A routine and reliable analytical method for the estimation of hecogenin and tigogenin in extracts of *A. sisalana* leaves was developed using gas–liquid chromatography (GLC)[2], but it was necessary to acetylate the sapogenins prior to analysis. Similarly, Higgins[3] published a method using high-performance liquid chromatography (HPLC) but, as only an ultraviolet detector was available, the benzoate derivatives of the sapogenins had to be prepared. Recently, Tal and Goldberg[4] showed that good separation could be achieved between hecogenin, tigogenin and diosgenin [(25*R*)-spirost-5-en-3β-ol] by HPLC and that the use of a differential refractometer enabled the sapogenins to be used without preparing derivatives. As a result we have developed a reliable method for the rapid quantitative analysis of hecogenin and tigogenin which does not require the formation of a derivative prior to assay.

EXPERIMENTAL

Chromatography

A Waters Assoc. HPLC system was used consisting of a Model M6000 A pump, a U6K injection valve fitted with a 2-ml loop and a R401 differential refractometer connected to a Philips PM8252 chart recorder. Analyses were performed on a Waters Assoc. μBondapak C_{18} column (30 cm × 3.9 mm I.D.) using acetonitrile–water–methanol–chloroform (73:20:6:1) as the mobile phase at a flow-rate of 1 ml/min. All solvents used were of HPLC grade.

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0021-9673/82/0000–0000/$02.75

Standardisation procedure

Standard solutions of hecogenin and tigogenin (10 mg/ml) were prepared in chloroform. From these solutions a series of dilutions were prepared so that concentrations of 8, 6, 5, 4, 2 and 1 mg/ml were obtained. From each solution 10-μl samples were injected into the apparatus. The areas under the peaks produced by hecogenin and tigogenin were calculated and standard curves were plotted of peak area as a function of the corresponding amount of sapogenin.

Extraction and assay of A. sisalana samples

Dry, powdered *A. sisalana* leaf samples (10 g) obtained from Tanzania, Kenya and Angola were extracted by the method described by Blunden *et al.*[1] to yield 10 ml final extract in chloroform.

"Coffee grounds" samples from Tanzania and Kenya were extracted with chloroform for 4 h, the extracts evaporated to dryness, the residues redissolved in chloroform and made up to a known volume.

RESULTS AND DISCUSSION

Good separation is achieved between hecogenin (capacity factor, $k' = 1.6$) and tigogenin ($k' = 6.7$) by HPLC. A linear relationship is found between sapogenin weight and area under the peak for both hecogenin and tigogenin in the weight range 10–80 μg. The correlation coefficient, r, for both hecogenin and tigogenin is 0.99.

Examination of the *A. sisalana* leaf extracts and the extracts of "coffee grounds" showed that the peaks derived from hecogenin and tigogenin were separated from those produced by other components of the extract. In addition, rockogenin [(25*R*)-5α-spirostane-3β,12β-diol] has a shorter retention time ($k' = 1.1$) than hecogenin and can be readily detected if present in the extract. Rockogenin has been found in samples of "coffee grounds" prepared on a pilot plant scale on several sisal plantations[5].

The quantities of hecogenin and tigogenin in the *A. sisalana* leaf and "coffee grounds" extracts were calculated by comparing the areas under the peaks produced by the sapogenins present with the standard curves. Good correlation was obtained

TABLE I

COMPARATIVE RESULTS OF THE TIGOGENIN AND HECOGENIN CONTENTS OF *A. SISALANA* LEAF AND "COFFEE GROUNDS" SAMPLES WHEN DETERMINED BY THE HPLC AND GLC METHODS[2]

Sample	*Sapogenin content (%)*			
	HPLC method		*GLC method*	
	Hecogenin	*Tigogenin*	*Hecogenin*	*Tigogenin*
Young leaves, Tanzania	0.34	0.2	0.38	0.3
Kenya	0.47	0.2	0.43	0.2
Mature leaves, Angola	1.16	0.1	1.09	0.1
"Coffee grounds", Tanzania	16.0	0.6	16.3	0.6
Kenya	25.2	3.3	24.7	4.0

between the results from the HPLC analytical method and the GLC method of Cripps and Blunden[2] (Table I).

The major advantage of this new assay method for hecogenin and tigogenin in crude extracts is the speed and ease of the operation. The use of a differential refractometer enables the sapogenins to be estimated without the need of preparing derivatives, which is time consuming, and so allows many samples to be analysed in a day.

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