## The titrimetric assay of solasodine, a spirosolane of commercial importance from *Solanum laciniatum* Ait.

ROLAND HARDMAN AND T. G. WILLIAMS

*Pharmacognosy Group, School of Pharmacy, University of Bath, Claverton Down, Bath, BA2 7AY, U.K.*

Solasodine is a starting material for the steroid industry. It occurs in *Solanum laciniatum* as its 3-$\beta$-*O*-glycosides. These are most abundant in the green, unripe fruits. Existing assays for solasodine involve solvent extraction of the glycosides from dried plant material, after which, because the glycosides are not readily estimated, they are hydrolysed and the aglycone is separated and determined titrimetrically or colorimetrically (Birner, 1968). It has been shown that aqueous incubation of sapogenin affording plant material alone and with additives increases sapogenin yield (Hardman & Brain, 1971; Hardman & Wood, 1971). Therefore our assay has been designed to process powdered plant material in water. Extraction of glycosides from aqueous media presents problems; their hydrolysis *in situ* by the addition of HCl to the media was hence the first stage in the assay. Preliminary results are now reported.

Dried unripe fruit, 2·5 g, which had been partially defatted by 24 h continuous extraction with light-petroleum (40–60°), was refluxed for 3 h with 50 ml 2N HCl and cooled to 80°. Solution of ammonia (2 × 20 ml, s.g. 0·880) was added. After further cooling the mixture was filtered and the residue washed and then dried overnight at 60°. The residue with filter paper was extracted with $CHCl_3$ for 24 h in a soxhlet and the extract adjusted to 100 ml with solvent. Aliquots of this solution were titrated automatically with 0·01N $HClO_4$ in dioxan using a recording potentiometric titrator.

Preliminary experiments have shown that 65·5% of the solasodine liberated during the hydrolysis is dehydrated to solasodiene. The end point potentials of this and of solasodine are very similar and the titration does not distinguish between them. The results are therefore expressed as solasodine.

Using this method we have made preliminary investigations of the effects of fine powdering and of partial defatting on the assay and its reproducibility (Table 1).

Table 1. *Replicate assays of dried, unripe fruits of* S. laciniatum.

| Undefatted powder; 60% retained by a No. 30 sieve | Undefatted fine powder; 98% passed through a No. 30 sieve | Partially defatted fine powder | Light-petroleum extractive* |
|---|---|---|---|
| 2·34 | 3·60 | 3·00 | 0·32 |
| 2·61 | 3·47 | 3·01 | |
| 2·55 | 3·31 | 2·98 | |
| | | 3·00 | |
| | | 2·98 | |

Results expressed as % base calculated as solasodine with reference to the dried fat-containing fruits (moisture, 7·5% fat, 6·4%*).

REFERENCES

BIRNER, J. (1968). *J. pharm. Sci.*, **58**, 258–259.
HARDMAN, R. & BRAIN, K. R. (1971). *Phytochem.*, **10**, 519–523.
HARDMAN, R. & WOOD, C. N. (1971). *Ibid.*, **10**, 757–763.

## The determination of diosgenin and yamogenin in fenugreek seed by combined column chromatography and infrared spectrometry

ROLAND HARDMAN AND T. M. JEFFERIES

*Pharmacognosy Group, School of Pharmacy, University of Bath, Claverton Down, Bath, BA2 7AY, U.K.*

Acid hydrolysis of Moroccan seed of *Trigonella foenumgraecum*, L. (fenugreek), followed by extraction with light-petroleum 40–60° affords a mixture of the epimers diosgenin and yamogenin, 1%, with fixed oil, 6% and free sterol, sterol esters, spirostadienes, and gitogenin. We have separated diosgenin from yamogenin by preparative-t.l.c. and have described how they

may be determined individually in the presence of one another in all proportions by infrared spectrometry (Jefferies & Hardman, 1971). The degree of accuracy attained in the method can be achieved only in the absence of the fixed oil and other components of the crude extract, and we now have a routine procedure for their removal by column chromatography.

Silicagel for adsorption (Woelm), activity II, 6 g per 15 cm × 1 cm column was packed in hexane–ethyl acetate (9:1). The extract was prepared by refluxing 2·5 g whole seed with 100 ml 2N HCl for 2 h. The mixture was cooled and filtered and the residue was made alkaline with dilute ammonia solution before it was dried overnight at 70°. This material was extracted in a soxhlet for 24 h using light-petroleum and the solvent removed in a vacuum. The oily residue was transferred to the column using a total of 10 ml hexane–ethyl acetate (9:1). At a flowrate of 1 ml/min, 90 ml of the same solvent system was used to collect 85 ml (which contained all the unwanted material) and 3 × 5 ml fractions for a t.l.c. check. Then 55 ml hexane–ethyl acetate (3:1) was used to collect 40 ml containing diosgenin and yamogenin together, followed by 3 × 5 ml fractions for a t.l.c. check. All diosgenin and yamogenin residues from one column were then dissolved in 4 ml Analar $CHCl_3$ for infrared assay.

This procedure is suitable for up to 75 mg diosgenin and yamogenin sapogenins in the presence of up to 600 mg fixed oil, approximately three times the extractive from 2·5 g fenugreek seed. The recovery of diosgenin plus yamogenin sapogenin from the column was tested by using mixtures of pure sapogenin (30 mg) with 2N acid-treated fixed oil (250 mg) and gave recoveries of ±4% [coefficient of variation (c.v.) = 2·6%]. The reproducibility was tested by analysis of a crude extract (1% diosgenin plus yamogenin, ratio = 6:4) by twelve columns, and gave c.v. = 2·5% (diosgenin plus yamogenin), 1·3% (diosgenin) and 4·5% (yamogenin). Analysis of twelve × 2·5 g seed gave c.v. = 2·8% (diosgenin plus yamogenin), 2·2% (diosgenin) and 6·8% (yamogenin). For duplicate 2·5 g seed assays ($t$ at $P = 0{\cdot}05$), the range of error was found to be ±4·4% (total sapogenin), ±3·5% (diosgenin) and ±10·6% (yamogenin).

REFERENCE

JEFFERIES, T. M. & HARDMAN, R. (1971). *Planta Med.* In the press.

## The composition and distribution of phytosterols in *Digitalis purpurea* L.

F. J. EVANS

*Department of Pharmacognosy, School of Pharmacy (University of London), Brunswick Square, London, WC1N 1AX, U.K.*

A comparative study of the distribution of phytosterols of the lipid and glucoside fractions of mature flowering plants was undertaken. The plants were from the same clone, were harvested together and divided into leaf, flower, fruit, stem and root samples.

The phytosterols were generally distributed throughout the plants and small quantities of 7-ene precursors were also present in the lipid fractions, indicating that phytosterol biosynthesis is carried out in all tissues. The leaves were divided into three types according to size. It was evident that young leaves from beneath the inflorescence accumulated glucoside sterols, whilst mature leaves from the basal rosette contained greater quantities of lipid sterols. The proportions of 7-ene sterols were also greatest in mature leaves, indicating that these are the primary site of phytosterol biosynthesis.

In the flower buds and developing flowers high concentrations of both lipid and glucoside sterols were found. The quantities of lipid fraction sterols increased during maturation of the flowers, whilst the glucoside sterols remained at a constantly high concentration. In developing fruits phytosterols continued to accumulate, particularly during fruit ripening. The amounts of 5-ene and 7-ene sterols isolated from the stems and root were comparatively small. However, the amount of glucoside sterols isolated from the stems was higher than that of the roots, and was similar to the young leaves.

The 5-ene sterols of both fractions were found by g.l.c. to consist of β-sitosterol, stigmasterol, campesterol and cholesterol. Certain of the samples from the inflorescence also contained 24-methylene-cholesterol. β-Sitosterol at all times was found to be the major