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

component of the glucoside and the lipid fractions, but tended to accumulate in mature tissues. Stigmasterol, the C-22 unsaturated isomer, accumulated in young leaves and immature flower buds. The proportions of campesterol were generally similar in most organs with the exception of the roots where larger quantities were detected. It was interesting to note that concentrations of cholesterol glucoside were highest in the younger organs, and it is possible that these tissues are the sites of sterol metabolism to the cardenolides and sapogenins.

It is evident that biosynthesis of sterols occurs in the free form, and that either glucoside or ester formation occurs selectively at a stage when biosynthesis is complete, suggesting that a segregation of biological roles could lie behind this enzymatic selection. Free phytosterols have been implicated in the structure of cell and organelle membranes in association with phospholipids (Ansell & Hawthorne 1964; Evans 1971), and it has been suggested by Kemp, Goad & Mercer (1967) that ester sterols represent an intercellular transportation form. The areas of sterol requirement in the mature plant are the actively growing areas, and the export of sterols from mature leaves at the base of the plant could satisfy a heavy sterol requirement. This would involve the phloem transportation of sterols, possibly as the more hydrophilic glucosides, via the stem to the young leaves and developing inflorescence. The high phytosterol glucoside concentrations of these organs add weight to this suggestion.

REFERENCES

ANSELL, G. B. & HAWTHORNE, J. N. (1964). *Phospholipids, Chemistry, Metabolism and Function;* chapt. 10, Amsterdam: Elsevier.
EVANS, F. J. (1971). Ph.D. Thesis, University of London.
KEMP, R. J., GOAD, L. J. & MERCER, E. I. (1967). *Phytochem.*, **6**, 1609–1615.

The characterization of alkaloid D, a new alkaloid from *Euonymus europaea* L., as armepavine

D. W. BISHAY*, Z. KOWALEWSKI* AND J. D. PHILLIPSON
**Department of Pharmacognosy, Medical Academy, Poznan, Poland; Department of Pharmacy, Chelsea College, University of London, Manresa Road, London, S.W.3, U.K.*

Five new alkaloids have recently been isolated from *Euonymus europaea* L. (Celastraceae) growing in Poland (Bishay & Kowalewski, 1971). These alkaloids are not identical with those reported to be present in *E. europaea* (Doebel & Reichstein, 1949; Pailer & Libiseller, 1962; Libiseller & Preisinger, 1962) and have been named alkaloid A, alkaloid B, alkaloid C, alkaloid D and alkaloid E.

A study of ultraviolet, infrared, nmr and mass spectrometric data suggested the possible identity of alkaloid D with the known alkaloid armepavine [1-(4′-hydroxybenzyl)-2-methyl-1,2,3,4-tetrahydro-6,7-dimethyoxyisoquinoline]. The R_F values of alkaloid D and an authentic sample of armepavine were found to be identical in several t.l.c. systems and the m.p. of authentic armepavine oxalate was not depressed by admixture with the oxalate of alkaloid D. Hence this alkaloid is considered to be identical with armepavine. The ORD curve of the alkaloid shows that it is *R*-(−)-armepavine.

REFERENCES

BISHAY, D. W. & KOWALEWSKI, Z. (1971). *Herba Polonica*, **17**. In the press.
DOEBEL, K. & REICHSTEIN, T. (1949). *Helv. chim. Acta*, **32**, 592–597.
LIBISELLER, R. & PREISINGER, A. (1962). *Mh. Chem.*, **93**, 417–419.
PAILER, M. & LIBISELLER, R. (1962). *Ibid.*, **93**, 403–416.