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STEROIDAL CONSTITUENTS OF THE AERIAL PARTS OF *DIOSCOREA* AND *TAMUS* SPECIES

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Abstract—In both the stems and leaves of 8 *Dioscorea* species and one *Tamus* species examined, β-sitosterol, stigmasterol and cholesterol were detected, the sterol yield always being higher in the leaves than the stem. With the exception of *D. batatas* and *D. cotinifolia*, which were sapogenin free, diosgenin was detected in the stems and leaves of all the species examined. Yamogenin was found in both the stems and leaves of *D. sylvatica* *D. deltoidea* and *T. communis*, but was detectable only in the leaves of *D. prazeri*, *D. floribunda* and *D. belizensis*. No yamogenin was detected in *D. composita*. The total sapogenin yield was from 6 to 40 times greater in leaves than stems.

INTRODUCTION

THE GENUS *Dioscorea* is of considerable economic importance as a source of the steroidal sapogenin, diosgenin, which is used commercially as a precursor of the pharmacologically active steroids. Many different steroidal compounds have been isolated from the tubers, the part of the plant that is used commercially, but little work has been done on the constituents of the aerial parts. Marker and Lopez,[1] working with *D. mexicana*, isolated neo-kammogenin and neo-yuccagenin from the leaves. *D. spiculiflora* has been investigated extensively, and the sterols β-sitosterol (24α-ethyl-Δ^5-cholesten-3β-ol), stigmasterol (24α-ethyl-$\Delta^{5,22}$-cholestadien-3β-ol), and cholesterol (Δ^5-cholesten-3β-ol)[2] and the sapogenins diosgenin (25α-Δ^5-spirosten-3β-ol), yamogenin (25β-Δ^5-spirosten-3β-ol) and kryptogenin (Δ^5-cholestene-3β, 26-diol-16,22-dione)[3] have been isolated from the aerial parts. In a recent paper, lanosterol (4,4,14α-trimethyl-$\Delta^{8,24}$-5α-cholestadien-3β-ol), cholesterol and diosgenin were reported in the aerial parts of *D. deltoidea* and *D. sylvatica*.[4] In our study, the steroidal constituents of the stems and leaves of many of the *Dioscorea* species of major and potential economic importance were examined, as well as *Tamus communis*, a member of a different genus of the Dioscoreaceae, and two non-sapogenin containing *Dioscorea* species.

RESULTS

Preliminary chromatographic examination of extracts of *Dioscorea* and *Tamus* leaves and stems produced, with the majority of extracts, two major tangerine spots after spraying with antimony trichloride solution and heating, the colour gradually turning purple on further heating. The spot with the higher R_f value was present in all the extracts examined, co-chromatographed with the common phytosterols and was concluded to be sterol. The spot

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[1] R. E. MARKER and J. LOPEZ, *J. Am. Chem. Soc.* **69**, 2375 (1947).
[2] D. F. JOHNSON, R. D. BENNETT and E. HEFTMANN, *Science* **140**, 198 (1963).
[3] R. D. BENNETT, E. HEFTMANN, W. H. PRESTON, JR. and J. R. HAUN, *Arch. Biochem. Biophys.* **103**, 74 (1963).
[4] E. A. BAKER, J. T. MARTIN and A. P. WILSON, *Ann. Appl. Biol.* **58**, 203 (1966).

with the lower R_f value was present in all the extracts with the exception of *D. batatas* and *D. cotinifolia*. This spot co-chromatographed in all 8 solvent systems used with diosgenin/yamogenin. The 2-way chromatograms of all the extracts examined showed the presence of several other compounds, in particular ones giving tangerine and purple spots of high R_f value. (Fig. 1). By co-chromatography with reference sapogenins in the different solvent systems used, none of these spots corresponded with any of the reference compounds. Many, however, corresponded with the breakdown compounds of diosgenin.[5] Similarly, the purple and tangerine spots of high R_f value co-chromatographed with compounds produced by refluxing sterols and sapogenins with acid. The purple spot co-chromatographed with 25α-spirosta, 3,5-diene and also with the major products from the acid-treated sterols, which

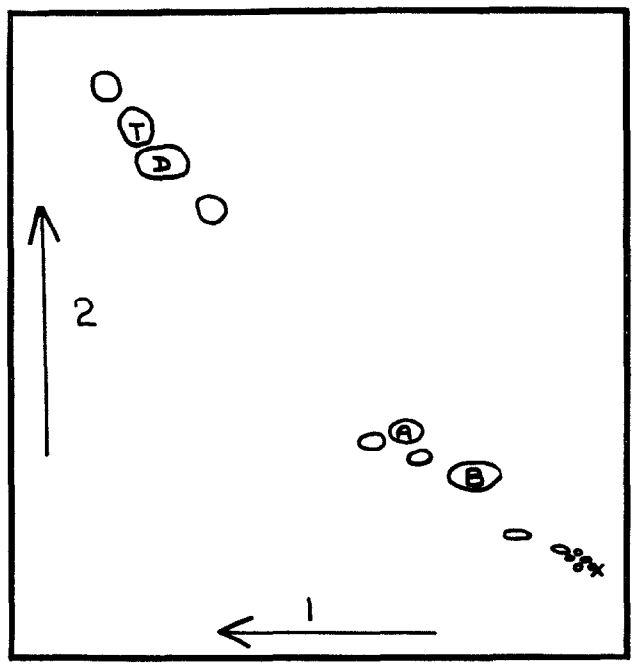

FIG. 1. TWO-WAY CHROMATOGRAM ON SILICA GEL G OF *D. composita* LEAF EXTRACT
Solvent systems: 1 n-hexane-ethyl acetate 3:1 v/v; 2 chloroform. A = sterol, B = sapogenin spot, D = diene, T = tangerine spot (after spraying with $SbCl_3$ in conc. HCl).

were probably dienes. The tangerine spots were produced also by the acid treatment of sterols such as stigmasterol and cholesterol.

The sterol band from the different extracts was separated by preparative TLC and examined by GLC both before and after acetylation. In all the extracts tested, β-sitosterol, stigmasterol and cholesterol were detected, β-sitosterol being the major component. The crude extracts were examined by TLC for the presence of lanosterol using co-chromatography with a reference sample, but it was not found to be a major constituent of any of the extracts. A spot with the same chromatographic characteristics as lanosterol was detected in the leaf and stem extracts of *D. sylvatica*, *D. cotinifolia* and *T. communis* and possibly in the extracts of *D. deltoidea*, *D. prazeri* and *D. batatas*, but was not detected in those of the other plants.

[5] G. BLUNDEN and C. T. RHODES, 27th Congrès International des Sciences Pharmaceutiques, Montpellier, September (1967).

The compound corresponding with the diosgenin/yamogenin reference solution was purified by preparative chromatography, acetylated and re-chromatographed on TLC with reference solutions of diosgenin and yamogenin acetates. With the exception of *D. batatas* and *D. cotinifolia*, which were sapogenin free, diosgenin was detected in the leaves and stems of all the species tested. Yamogenin was found in all the leaf extracts, with the exception of

TABLE 1. SAPOGENINS PRESENT IN *Dioscorea* AND *Tamus* AERIAL PARTS

Species		Diosgenin	Yamogenin
D. sylvatica	leaf	++	+++++
	stem	++	+++++
D. deltoidea	leaf	+++++	+
	stem	+++++	+
D. prazeri	leaf	+++++	++
	stem	+++++	−
D. belizensis	leaf	+++++	++
	stem	+++++	−
D. floribunda	leaf	+++++	+
	stem	+++++	−
D. composita	leaf	+++++	−
	stem	+++++	−
T. communis	leaf	+++++	+++++
	stem	+++++	++++

+++++ represents major component in each species. Relative proportion of minor compound represented by different number of + signs. − represents absence of compound.

TABLE 2. TOTAL SAPOGENIN YIELDS AS A PERCENTAGE, DRY WEIGHT, IN *Dioscorea* AND *Tamus* AERIAL PARTS

Species	No. of plants used	Date of collection	Sapogenin yield % Leaf	Sapogenin yield % Stem
D. sylvatica	2	November, 1966	0·15	0·004
D. deltoidea	4	November, 1966	0·48	0·012
D. prazeri	4	November, 1966	0·32	0·036
D. belizensis	2	November, 1966	0·17	0·005
D. floribunda	1	November, 1966	0·10	0·003
D. composita	1	May, 1967	0·28	0·010
T. communis	10	May, 1967	0·26	0·040

D. composita but was not detected in the stems of *D. composita*, *D. belizensis*, *D. floribunda*, or *D. prazeri*. With the exception of the *D. sylvatica* extracts and possibly the *T. communis* leaf extract, diosgenin was the major isomer in the stem and leaf of all species tested (Table 1).

The yields of sapogenin in the leaves and stems of the extracts, calculated as diosgenin, were determined using a densitometric TLC method.[6] The yield from the leaves was always

[6] G. BLUNDEN, R. HARDMAN and J. C. MORRISON, *J. Pharm. Sci.* **56**, 948 (1967).

greater than that from the stem, varying from 6 to 40 times as much (Table 2). The relative sterol yields were estimated from densitometer readings obtained from the sterol spots on thin layer chromatograms prepared from extracts of equal weights of leaf and stem. The sterol yield in the leaves was always higher than that in the stems, varying from $\frac{1}{3}$ to 5 times greater, but most leaf samples contained less than twice as much sterol as the stems of the same species.

DISCUSSION

Little variation was found in the steroidal constituents of the aerial parts of the 8 *Dioscorea* and one *Tamus* species examined. The main sterols detected in the leaves and stems of all the species examined were β-sitosterol, stigmasterol and cholesterol, the same 3 compounds having previously been found in *D. spiculiflora* by Johnson, Bennett and Heftmann.[2] Baker, Martin and Wilson[4] reported the presence of cholesterol in *D. deltoidea* and *D. sylvatica*, but by their methods, cholesterol would not have been differentiated from many other common sterols. In our study, both the species examined by these workers were shown to contain β-sitosterol and stigmasterol in addition to cholesterol. On examination of the sterol fraction by GLC, a peak was always obtained with a retention time between that of cholesterol and stigmasterol. This peak had the same retention time as a component present in the β-sitosterol reference solution and may well be 5α-stigmastanol, which is stated to occur with the sitosterols.[7] Before the sterol fraction was examined by GLC, it was necessary to free it from sapogenins, as in the system used, diosgenin had the same retention time as stigmasterol and diosgenin acetate the same retention time as stigmasterol acetate.

Diosgenin was found to be present in the leaves and stems of all the saponin-containing species examined. Yamogenin was present in a number of the tested samples, but it was of interest that, although present in the leaves of *D. prazeri*, *D. floribunda* and *D. belizensis* it was not detected in the stem. Moreover, in *T. communis* the relative proportion of yamogenin to diosgenin was higher in the leaf than the stem. The presence of yamogenin in *T. communis* was in contrast with the findings of Held and Vágujfalvi,[8] but the presence or absence of the two isomers and their relative proportions, may depend on the time of year, the age of the plant or other factors. Bennett *et al.*[3] detected both diosgenin and yamogenin in the aerial parts of *D. spiculiflora*, yamogenin being the predominant isomer. The presence of kryptogenin in *D. spiculiflora* may or may not be an artifact. It was absent when the aerial parts were hydrolysed with aqueous instead of ethanolic hydrochloric acid.[9–11] Baker, Martin and Wilson reported the presence of diosgenin in *D. deltoidea* and *D. sylvatica*.[4] Their methods would not differentiate between diosgenin and yamogenin, but in our study, both isomers were found in these two species, yamogenin being the predominant sapogenin in *D. sylvatica*. The absence of yamogenin from *D. composita* leaves and stems was consistent with the results obtained by Wall and co-workers[12] from the tubers of this species.

Little attempt was made to determine the nature of the other antimony trichloride positive spots on the 2-way chromatograms. Many of the spots can be accounted for as decomposition products of the sterols and sapogenins, either produced during the acid treatment of the plant material or subsequent storage of the extract. The major tangerine and purple spots

[7] C. W. SHOPPEE, *Chemistry of the Steroids*, 2nd edition, Butterworths, London, p. 76 (1964).
[8] G. HELD and D. VÄGUJFALVI, *Bot. Közlem.* **52**, 201 (1963).
[9] R. E. MARKER and J. LOPEZ, *J. Am. Chem. Soc.* **69**, 2386 (1947).
[10] G. BLUNDEN and R. HARDMAN, *J. Chromatog.* **15**, 273 (1964).
[11] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 577 (1965).
[12] M. E. WALL, J. J. WILLAMAN, T. PERLSTEIN, D. S. CORRELL and H. S. GENTRY, *J. Am. Pharm. Assoc. Sci. Ed.* **46**, 155 (1957).

of high R_f value, which were present in chromatograms prepared from all the extracts, were shown to be produced by acid treatment of the sterols and sapogenins, the major purple spot including 25α-spirosta, 3,5-diene.

The analytical results showed that the sapogenin yields of the leaves were consistently higher than those of the stems. The sterol yields were also higher in the leaves, although not to such a marked degree. The densitometer assay results were often liable to greater than normal errors due to the interference of plant pigments and other antimony trichloride positive spots, particularly in the case of *D. composita* and *D. floribunda*.

In studies on the biosynthesis of *Dioscorea* sapogenins it was shown that cholesterol was converted to diosgenin and kryptogenin.[11] Cholesterol is present in *Solanum tuberosum*, which also contains sapogenins having 27 C atoms.[2] It is perhaps worthy of note that cholesterol was found to be present in both *D. batatas* and *D. cotinifolia*, although neither contained sapogenins. The lack of these compounds in these species may therefore be due to the absence of the necessary enzyme systems rather than the absence of the necessary precursors.

EXPERIMENTAL

The plant material used in this study was grown in a glasshouse at the University of Nottingham, with the exception of the *T. communis* which was collected from wild sources in Cornwall and *D. batatas* which was grown in a glasshouse, Portsmouth College of Technology. After collection, the aerial parts were dried in a circulating air oven at 65° for 16 hr. The leaves and petioles were then separated from the stems, and the two samples worked up separately. Extraction of the plant material was by the method of Blunden and Hardman,[13] which entailed disintegration in water of a known weight of plant material, of known moisture content, incubation for 24 hr at 37°, refluxing with 2N HCl for 2 hr, separation of the acid-insoluble material by filtration, washing with water, Na_2CO_3 and water until neutral. The dried acid-insoluble residue was extracted with light petroleum (40–60°) for 24 hr the extract evaporated to dryness, re-dissolved in $CHCl_3$ and made up to volume. This extract was examined by 2-way chromatography on air-dried thin-layers of silica gel G (Merck), 250 μ thick using *n*-hexane-ethyl acetate 3:1 v/v in the first direction and $CHCl_3$ in the second. Development was for 15 cm in each direction. The steroidal compounds were located by spraying with $SbCl_3$ in conc. HCl[14] and the colour developed by heating with a hot air blower.

Further 2-way chromatograms were prepared by running the plant extracts with reference compounds. The reference compounds used included cholesterol, lanosterol, diosgenin, yamogenin, kryptogenin, 25α-spirosta, 3,5,-diene and a range of other steroidal sapogenins. The solvent systems used included those described by Blunden and Hardman[10] and Bennett and Heftmann.[11]

The major sterol component of each extract was isolated by preparative TLC on silica gel G layers, 500 μ thick, using double development with n-hexane-ethyl acetate 3:1 v/v. The sterol band was located by spraying with Rhodamine 6G, scraped off the plate and eluted by maceration with $CHCl_3$ for 24 hr. The eluate was concentrated and a sample was examined by GLC using a Perkin–Elmer F11 Gas Chromatograph, fitted with dual 1·8 m × 6 mm glass columns, filled with silicone gum rubber E-301 on A.W.-DMCS Chromosorb G 80-100 mesh (D.E.-400) 2½:97½, injection temperature 220°, oven temperature 230°, and carrier gas nitrogen 55 ml/min. Prior to GLC examination the sterol extract was tested by TLC to show the absence of sapogenins. The sterol extract was acetylated by the method of Wall, *et al.*[15] and the sterol acetate separated by band chromatography as described above, except single development with dichloromethane-ether 97:3 v/v was used. The sterol acetates were examined by GLC as before.

The sapogenin band was separated from the same plates used for the isolation of the sterol band. After elution of the sapogenins, they were acetylated, and examined on air-dried layers of silica gel G (250 μ thick) by 2-way chromatography using dichloromethane-ether 97:3 v/v in the first direction and cyclohexane-ethyl acetate 4:1 v/v in the second.

The sapogenin yields in the stem and leaves of the *Dioscorea* and *Tamus* extracts were determined by the quantitative TLC method of Blunden, Hardman and Morrison[6] using a Vitatron densitometer. The method described double development of the chromatogram with n-hexane-ethyl acetate 4:1 v/v. This system was the one usually employed, but with some extracts, a modification had to be employed as a result of the quantity

[13] G. BLUNDEN and R. HARDMAN, *J. Pharm. Pharmacol.* **15**, 273 (1963).

[14] T. NAKAO, M. HIRAI and N. YOSHIZAWA, *Tokyo Jikeikai Ika Daigaku Zasshi* **73**, 1575 (1958), through *Chem. Abstr.* **53**, 22198 (1959).

[15] M. E. WALL, R. C. EDDY, M. L. MCCLELLAN and M. E. KLUMPP, *Anal. Chem.* **24**, 1337 (1952).

of plant pigment, the small sapogenin yields and the presence of other $SbCl_3$ positive spots. The modification entailed single development with *n*-hexane-ethyl acetate, followed by a second development in the same direction with $CHCl_3$.

Samples of β-sitosterol, stigmasterol, cholesterol and diosgenin/yamogenin were refluxed with 2N HCl for 2 hr, extracted with $CHCl_3$ and chromatographed on silica gel G layers using the solvent systems described above. The compounds produced as a result of the acid-treatment were noted and related to similar spots produced on chromatograms of the *Dioscorea* and *Tamus* extracts.

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