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STEROIDS, PHTHALYL ESTERS AND HYDROCARBONS FROM *BALANITES WILSONIANA* STEM BARK

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Key Word Index—*Balanites wilsoniana*; Balanitaceae; sterols; diosgenin 3-glucoside; yamogenin; phthalyl esters; hydrocarbons.

Abstract—IR assay for sapogenin of *Balanites wilsoniana* revealed 0·2% in the root wood, 0·7% in the root bark, 0·3% in the stem bark, 1·4% in the fatty seed and 0·6% w/w in the leaf. The 25 α-epimers predominated in all parts except in the root wood. Six glucosides having diosgenin or yamogenin as aglycones were found and one was characterized as diosgenin 3 β-D-glucopyranoside from IR, MS and NMR studies. Cholesterol, stigmasterol, sitosterol, 25 α-spirosta-3:5-diene and 25-β-spirosta-3:5-diene were also present. Free diosgenin and dienes were detected in appreciable quantities in the fat from the bark. The 'unsaponifiable' fraction of this fat contained phthalyl esters (mainly dioctyl and dibutyl) and saturated hydrocarbons C_{10}–C_{32} (with C_{10}–C_{20} predominating), together with the above mentioned steroids.

INTRODUCTION

Balanites wilsoniana Dawe and Sprague is a forest tree attaining a height of up to 45 m. The flowers are greenish-yellow and the ripe ellipsoid fruits (10 × 5 cm) are yellow with a strong smelling pulp and a single seed enclosed in a fibrous woody endocarp. Although extensive phytochemical investigations have been carried out on *B. aegyptiaca*,[1,2] *B. orbicularis*[3,4] and *B. pedicellaris*[5] all of which have been shown to afford steroidal sapogenins, very little information is available concerning *B. wilsoniana*. This species is said to produce a 'gum'[6] and that the 'extracts of the plant have proved ineffective in experimental malaria'.[7]

Diosgenin and its 25 β-epimer, yamogenin are both accepted as raw materials for the synthesis of steroidal drugs and both epimers frequently co-occur in plants.[8] Their occurrence, in appreciable amounts, in the aerial parts of species of *Balanites* investigated by us led us to recommend the possible utilization of these plants as commercial sources of these sapogenins.[3,9,10] The present work reports the steroidal components of *B. wilsoniana*.

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RESULTS AND DISCUSSION

Table 1 shows the total sapogenin content of different parts of *B. wilsoniana*, with the highest concentration in the seed. 25-α-(*iso*)-Sapogenin predominates in all parts except the root wood. In *B. aegyptiaca*, however, it is the 25-β-(*neo*)-sapogenin which predominates.[2]

TABLE 1. SAPOGENIN CONTENT* OF *Balanites wilsoniana*

Plant part	Sapogenin yield Total % (w/w)	25-epimer α/B	Plant part	Sapogenin yield Total % (w/w)	25-epimer α/B
Root wood	0·19	0·78	Root bark	0·65	2·9
Stem wood	ND		Leaf	0·55	1·5
Stem bark	0·30	2·9	Seed	1·42†	12·0

* Estimated by IR analysis as described by Brain *et al.*[19]
† Estimated on the defatted seed.
ND—Not detectable by IR analysis.

Fractionation, by preparative TLC, of the crude sapogenin isolated from the acid treated bark afforded sapogenin, sterol and diene fractions. The sapogenins were shown to be diosgenin and yamogenin. TLC (system VIII) of the diene fraction showed that it contained 25-α- and 25-β-spirosta-3:5-dienes, which are considered to be artefacts. GLC of the sterol fraction following recrystallization from acetone, revealed cholesterol, stigmasterol and sitosterol, in the ratio 1:2:10.

The same sapogenins and dienes were found in the 'fat' obtained from the light petrol extract of the bark, *before* acid treatment, both in appreciable quantities. This suggests that diosgenin, yamogenin and their dienes occur free in this species. The occurrence of diene in this fraction was intriguing as this component had previously been attributed by several authors,[1,11–14] including ourselves, to be an artefact produced during acid treatment of diosgenin. The possibility that post-harvest enzymic processes in the bark might have produced this diene from free or bound diosgenin cannot, however, be overlooked in this case.

TLC (system I and II) of the saponin mixture isolated from the bark showed the presence of six glycosides, with R_fs 0·56, 0·29, 0·22, 0·12, 0·06, 0·00 (system I) and 0·86 (Saponin A), 0·29 (B), 0·11 (C), 0·07 (D), 0·04 (E), 0·00 (F) in System II. All the spots gave a red colour with $SbCl_3$. Acid (2 N H_2SO_4) hydrolysis of the saponin mixture afforded diosgenin, yamogenin and glucose only. The two less polar saponins (A and B) were obtained pure by continuous elution preparative TLC (system II). Saponin-A afforded prisms from MeOH–$CHCl_3$ (1:1) m.p. 242° (decomp). M 576·3680. $C_{33}H_{52}O_8$ requires: 576·3662. The acetate, needles m.p. 204–206° from methanol, showed 4 acetoxy protons in the NMR spectrum with the anomeric proton of the sugar appearing as a doublet at δ 4·54 ppm indicating a

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β-glucoside.[15,16] The 3-β-glucopyranoside structure of this saponin (A) was confirmed by synthesis from diosgenin and acetobromoglucose. Saponin B which was deliquescent was obtained in low yield. Its MW could not be determined accurately as it fragmented in the mass spectrometer even under mild conditions. It afforded diosgenin on acid hydrolysis.

GLC of the hydrocarbon fraction (0·001% of the bark) from the unsaponifiable matter indicated that it contained *n*-alkanes (C_{10}–C_{30}) with the shorter chain lengths (C_{10}–C_{20}) constituting 75% of the total. Acid hydrolysis had resulted in the release of further hydrocarbons[5] from the kernels of *B. aegyptiaca*, *B. pedicellaris*, and *B. orbicularis*. The same treatment afforded a second crop (0·007% of bark) of hydrocarbons from this bark but the pattern of hydrocarbons present did not change.

The major component of the 'unsaponifiable' matter was a colourless semisolid separated by alumina column chromatography. Its IR spectrum showed absorptions at 1720, 1285, 1130 (aromatic ester), 1605, 1585, 1075, 1043 and 746 cm^{-1} (*ortho* substituted benzene ring). GLC showed it to be a mixture of several compounds in a homologous series although it appeared homogeneous on TLC (system II). The MS of its two major components (from GLC–MS) both had base peaks at *m/e* 149 which is characteristic of phthalates. From the cracking pattern, one was identified as dibutyl phthalate, with characteristic fragment ions at *m/e* 149 (base peak), 205, 223, and molecular ion at 278 in the MS. The second was dioctyl phthalate which gave no molecular peak but characteristic peaks at *m/e* 149 (base peak), 167 and 279. It is remarkable that there were phthalyl esters in the unsaponifiable fraction from this plant but these esters have been reported[17,18] in some umbelliferous plants; this, however, is the first report of their occurrence in the Balanitaceae. As phthalyl esters are notorious contaminants from extraneous sources, their presence was established by 3 separate extractions of the bark, in which contact with plastics was avoided and the solvents used were free of phthalates. The presence of phthalyl esters in this species may be of chemotaxonomic significance and is being further investigated in the Balanitaceae.

EXPERIMENTAL

Plant material. The morphological parts of *B. wilsoniana* were collected from full grown trees (identified by the Forest Research Department of Benin) in Sapoba forest reserve, Midwestern State, Nigeria. They were sun-dried and sent to England by air before powdering in a Christis Norris disintegrator.

Methods. M.ps are corrected. Sapogenin content of plant parts was estimated as described by Brain *et al.*[19] Acetates were prepared according to the method of Morita.[20]

TLC. Unless otherwise stated silica gel plates (250 nm) were used with the following solvent systems: I—EtOAc–EtOH (2:3); II—$CHCl_3$–MeOH–H_2O (13:7:5)–Lower phase; III—hexane–EtOAc (4:1); IV—*n*-BuOH–HOAc–Et_2O–H_2O (9:6:3:1); V—*iso*-PrOH–C_6H_5Me–EtOAC–H_2O (20:4:10:5); VI—*iso*-PrOH–H_2O (10:4); VII—$CHCl_2$–Et_2O (49:1); VIII—hexane-(*iso*-Pr)$_2O$ (39:1).

Preparative TLC. A slurry of silica gel nach Stahl $PF_{254+366}$ (240 g) in 560 ml of H_2O was spread over three 40 × 20 cm plates with the gap of the spreader adjusted to 2 mm. The plates were air dried for 15 hr before heating at 110° for 1·5 hr. Thickness of absorbent after drying was 1·8 mm. Bands were detected with I_2 vapour or in UV light.

Spray reagent. Sugars were detected on TLC by spraying with a mixture of diphenylamine 2% in acetone; aniline 2% in acetone; orthophosphoric acid 88% (9:9:2). Plates were heated at 110° for 5 min. Sugar PCs were dipped in aniline, 1% (1 ml) plus diphenylamine, 1% (9 ml) in acetone and phosphoric acid, 85% (2 ml) and the papers were then heated to 95–100°. For sapogenins and sterols, the TLC plates were heated at 105°

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for 5 min after spraying with a 300% w/v solution of $SbCl_3$ in conc. HCl. Saponins were detected by spraying with *p*-anisaldehyde–HOAc–H_2SO_4 (100:1:2) or $SbCl_3$. Plates were viewed in daylight and UV.

Fractionation of the sapogenins and sterols. The stem bark (1 kg) was exhausted with dried ($MgSO_4$) light petrol (b.p. 40–60°). The semi-solid pale yellowish 'fat' obtained, after removing the solvent *in vacuo* was set aside (Fraction A, 3·3% w/w). The defatted material was dried and refluxed with 2N HCl (5 l.) for 2 hr. After cooling, the mixture was filtered, washed and neutralized with 10% NH_4OH. The acid-insoluble matter was then dried at 80° for 16 hr before it was exhausted with light petrol by Soxhlet extraction and it afforded 10·6 g (1% w/w) of crude sapogenin. TLC (system III) showed spots corresponding to spirosta-3:5-dienes, sterols and diosgenin, which were isolated by preparative TLC (system III). Preparative TLC by continuous elution (system VII) of the extract from the 'diosgenin' band afforded equal amounts of diosgenin m.p. 210° and yamogenin m.p. 188–189°—authentication of both being by m.p., m.m.p. IR examination and preparation of their acetates. The sterols were fractionated on a Varian Aerograph gas chromatograph Model 1520 using dual 189 × 0·64 cm stainless steel columns coated with 5% XE60 on AW., DCMS chromosorb W. Column oven temp. isothermal at 235°. Injector temp. 235°, f.i.d. detector temp. 240°, H_2 flow rate 32 ml/min, recorder fitted with a disc integrator. Peaks were obtained at R_t 23, 31 and 36·5 min, which corresponded to cholesterol, stigmasterol and sitosterol in the ratio 1:2:10. IR spectroscopy (Nujol) of the diene fraction showed strong absorptions at 980, 915 and 900 cm^{-1} but no absorption near 3500 cm^{-1}. TLC (system VIII, S-chamber) showed two spots corresponding to 25 α- and 25β-spirosta-3:5-dienes. Both spots gave a red colour with $SbCl_3$ reagent in the cold.

Isolation of saponins. In a separate experiment, the stem bark, 1 kg was exhausted (2 days) with light petrol (b.p. 40–60°, dried over $MgSO_4$) in a Soxhlet. The 'defatted' material was dried and exhausted with 95% EtOH in a Soxhlet. The dark brown ethanolic extract was concentrated to 650 ml, after adding 200 ml Et_2O, it was set aside in a refrigerator for 3 days. The deposited solid was collected and the supernatant concentrated before more Et_2O (200 ml) was added. This procedure was repeated until no more solids separated on chilling. The total solids were bulked, triturated with Et_2O (2 × 200 ml), and finally with $CHCl_3$ (500 ml). The crude saponin thus obtained was dried (3·1 g). Preparative TLC (solvent II) (using iodine vapour to localize the zones) afforded saponin A and B separately.

The sugar content of saponins. The saponin mixture was refluxed with 2 N H_2SO_4 for 2 hr. The mixture was cooled and extracted with $CHCl_3$. This extract contained diosgenin and yamogenin as a 1:1 mixture. The neutralized ($BaCO_3$) aqueous hydrolysate showed only D-glucose by co-chromatography on TLC (systems IV and V) and PC (system VI, Whatman No. 4 paper).

Fractionation of the light petrol soluble extract of the stem bark—(fraction A). The light petrol extract from the barks (1 kg) was evaporated to dryness to afford 32 g (3·3%, w/w) of a yellowish semisolid which was refluxed with 200 ml of N/2 alcoholic KOH for 2 hr. 9·9 g of it (61% of the fat) was unsaponifiable. Preparative TLC (hexane) of this afforded a mixture of saturated hydrocarbons at the solvent front, with the rest of the material remaining at the origin. The alkane band was scraped off and extracted with $CHCl_3$ in a Soxhlet. The alkanes (dissolved in *n*-hexane) were analysed on a Varian Aerograph series 1520 gas chromatograph equipped with dual stainless steel columns (189 × 0·32 cm, 5% w/w SE30 on acid-washed DCMS chromosorb-W) temp. programmed from 100 to 260° at 2°/min. Injector temp. 270°, f.i.d. detector temp. 280°, carrier (N_2); 40 ml/min. Identification was based on chromatography with known standards and relative R_ts. The remainder of the unsaponifiable matter after removal of hydrocarbons was extracted from the plate with $CHCl_3$. The solvent was evaporated and the residue chromatographed on a column of alumina developed in hexane. Elution with hexane afforded a colourless semi solid of phthalyl esters. Further elution with $CHCl_3$ afforded diosgenin, yamogenin, 25-α and 25-β-spirosta-3:5-dienes, cholesterol, stigmasterol and sitosterol, all identified by TLC and GLC as described above.

GLC–MS of phthalyl esters. The hexane solution of phthalyl esters was resolved on a Perkin-Elmer Model 881 gas chromatograph linked through a Biemann separator to an MS902 mass spectrometer (ion source 220°; electron energy 70 eV, trap current 100 μA). GLC separation was on a 2·5% E301 on chromosorb G-AW DMCS (80–100 mesh). Stainless steel columns (189 × 0·32 cm) programmed from 200 to 250° at 8°/min. Injection temp. 270° f.i.d. detector temp. 270°. Carrier (He) flow 30 ml/min.

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