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ISOLATION AND CHARACTERIZATION OF YAMOGENIN FROM *BALANITES AEGYPTIACA*

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Abstract—Infra-red spectrophotometric analysis showed 1 per cent total steroidal sapogenin in the root, 0·7 per cent in the stem bark and 1·2 per cent in the soft fruit wall of *Balanites aegyptiaca* Del., on a moisture-free basis. Elemental analysis of the sapogenin and its derivatives as well as mass, i.r. and NMR spectroscopy data show that the major sapogenin in these plant parts is yamogenin.

INTRODUCTION

BALANITES AEGYPTIACA Del. is a tree widely distributed in the drier areas of East, West and North-East Africa. Related species occur in India and South Africa. Marker[1] reported 0·5 per cent diosgenin in the "plant" and this sapogenin was later isolated from the fruit pericarp,[2] kernel[3] and from the leaf.[4] The present work covers isolation of the major sapogenin of the root, the soft fruit wall (epicarp and mesocarp) and the stem bark, which also contain saponin.[5]

RESULTS

A routine total sapogenin assay, using the method of Brain *et al.*[6] indicated 1·0 per cent total sapogenin in the root, 0·7 per cent in the stem bark and 1·2 per cent in the soft fruit wall of *B. aegyptiaca*[7]. In all cases, the i.r. spectrophotometric analysis showed that 25β-sapogenin was predominent (ratio 2:1).

The major sapogenin from the root (isolated by column chromatography) co-chromatographed with diosgenin in TLC and was similarly stained red by a solution of antimony trichloride in hydrochloric acid. Its i.r. spectrum showed strong absorbance peaks, like diosgenin at 867, 900, 917, 985, 1050, 2900 cm^{-1}, but the absorbance at 917 cm^{-1} was stronger than at 900 cm^{-1}—unlike diosgenin—indicating a 25β-sapogenin.

The presence of a strong absorbance peak at 3500 cm^{-1} in an i.r. spectrum of this sapogenin in nujol indicated a free hydroxy group. Its mass spectrum was similar to that of diosgenin with the mass peak at $m/e = 414$. The root sapogenin was recrystallized from acetone to constant melting point and TLC purity, giving clusters of white (opaque) needles,

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[1] R. E. MARKER, R. B. WAGNER, P. R. ULSHAFER, D. P. J. GOLDSMITH and C. H. RUOF, *J. Amer. Chem. Soc.* **65**, 1248 (1943).
[2] V. V. DHEKNE and B. V. BHIDE, *J. Indian Chem. Soc.* **28**, 588 (1951).
[3] I. P. VARSHNEY and K. M. SHAMSUDDIN, *Arch. Pharm.* **295**, 401 (1962).
[4] A. A. M. DAWIDAR and M. B. E. FAYAZ, *Phytochem.* **8**, 261 (1969).
[5] R. G. ARCHIBALD, *Trans. Roy Soc. Trop. Med. Hyg.* **27**, 207 (1933).
[6] K. R. BRAIN, F. R. Y. FAZLI, R. HARDMAN and A. B. WOOD, *Phytochem.* **7**, 1815 (1968).
[7] On dry weight basis.

m.p. 184–185°. The acetate and 5α,6β-dichloro derivatives indicated that yamogenin was present.

Stem Bark

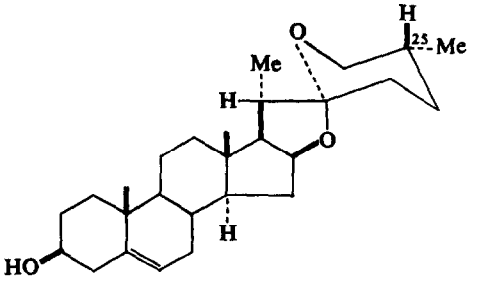
The sapogenin isolated from the stem bark afforded cluster crystals of fine needles (from acetone) which co-chromatographed with the root's major sapogenin, on TLC. It did not depress the melting point of the yamogenin obtained from the root and the i.r. spectra of both sapogenins were identical.

Epicarp and Mesocarp (Soft Fruit Wall)

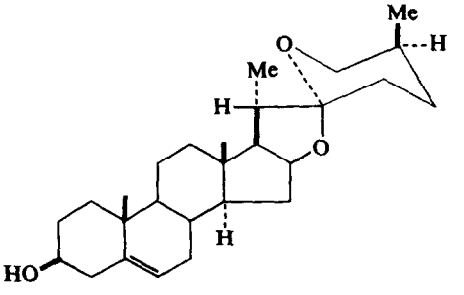
The white solid, m.p. 172–178°, obtained by column chromatography from the fruit wall, afforded white needles (from acetone) which did not depress the melting point of the root yamogenin. The i.r. spectra of this sapogenin was identical with that of yamogenin. It also formed an insoluble digitonide with digitonin.

DISCUSSION

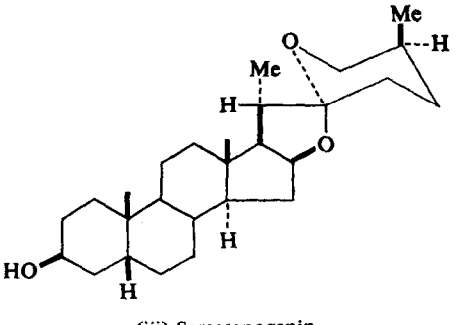
We are interested in the distribution of diosgenin (I) and its C_{25}-isomer yamogenin (II) in the tree as a whole as part of our general studies on the plant steroids. A preliminary "defatting" of the roots with dry (to limit removal of saponin) light petroleum removed unwanted material which interfered with the final isolation of sapogenin, while the preliminary incubation of the fruit wall before acid hydrolysis of the saponin removed resinous material and increased sapogenin yield.[8]



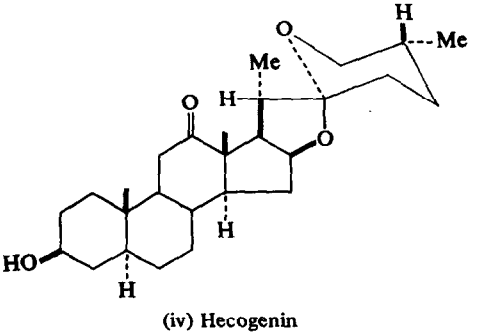
(i) Diosgenin



(ii) Yamogenin



(iii) Sarsasapogenin



(iv) Hecogenin

[8] E. A. SOFOWORA, Ph.D. Thesis, University of Nottingham (1967).

Isomers of the sapogenins have been reported to co-occur in most plant tissues.[9] Diosgenin and yamogenin, 25α- and 25β-epimers, respectively, occur together in, for example, tubers of *Dioscorea* spp. Hence the sapogenin isolated is a mixture of the two epimers and commonly the 25α-epimer (diosgenin) frequently predominates from such tubers. However, the i.r. spectrophotometric analyses of the sapogenin content of the root, stem bark and fruit wall of *Balanites aegyptiaca* showed that they contained a mixture of about 2 parts 25β- with 1 part 25α-sapogenin. After column chromatography, the major sapogenin of the root crystallized into fine needles from acetone, m.p. 184–185°. Elemental analysis of the fine needles obtained upon recrystallization from iso-propanol, however, indicated solvation with the latter solvent. This was supported by the presence of a doublet at 1·22 ppm ($J = 6H_2$) in its NMR spectrum.

Analytical and physical data on the major sapogenin from the root support the view that it is the 25β-epimer of diosgenin—i.e. yamogenin. The major sapogenin of the stem bark, and of the soft fruit wall, was also found to be yamogenin although diosgenin had been isolated from the pericarp by Dhekne and Bhide.[2] Yamogenin is not generally available. Consequently, diosgenin and the 25β-sapogenin, sarsasapogenin (III), were used as comparative standards for the i.r. and NMR investigation. On account of the integral, the splitting pattern and J values from its NMR spectrum, only one olefinic proton could be present in the sapogenin from the root. The quartet centred at 4·0 δ due to the C_{26} axial proton showed a large J value ~11 H_2 and a small one ~2H_2. This is identical with the situation described by Boll and Philipsborn[10] as obtaining in sarsasapogenin in support of an axial methyl group at C_{25}. Furthermore, the C_{27}-methyl group when equatorial appears[10] at 0·8 ppm, presumably together with the C_{18} methyl group and should therefore integrate to 3H in yamogenin but to 6H in diosgenin. As the only difference between diosgenin and yamogenin is the configuration about C_{25}, then two high field CH_3's are expected for diosgenin at 0·8 ppm but only one for yamogenin. A proton count in this region of the NMR spectrum of the root's major sapogenin supported yamogenin.

Marker *et al.*[11] isolated yamogenin (m.p. 198–200° ex ether) from *Dioscorea bulbifera* and stated that a mixture of this sapogenin with diosgenin melted 10° lower. As much as 20° difference in melting points had also been reported by the same workers[11] for hecogenin (IV) and this was attributed to the possibility that plant sapogenins could exist in polymorphic forms. Our melting point for yamogenin (m.p. 184–185° ex acetone) was subsequently confirmed by the independent isolation of this sapogenin from *Trigonella foenum-graecum* seeds by Jefferies,[12] using the continuous development technique of Bennett and Heftmann[13] in the preparative TLC separation of yamogenin and diosgenin which co-occur in fenugreek seeds. In addition, a natural mixture (1:1) of these two isomers as isolated from *T. foenumgraecum* seeds by Brain,[14] using a steady-state counter-current extractor, melts at 198° (Kofler block).

EXPERIMENTAL

Materials

The roots and pieces of stem bark used were collected from a fully grown *Balanites aegyptiaca* tree in Moshi (Tanzania) at early fruiting stage and sent to us by air mail while still fresh. Upon receipt (4 days

[9] E. HEFTMANN, *Lloydia* **30**, 209 (1967).
[10] P. M. BOLL and W. V. PHILIPSBORN, *Acta Chem. Scand.* **19**, 1365 (1965).
[11] R. E. MARKER, R. B. WAGNER, P. R. ULSHAFER, E. L. WITTBECKER, D. P. J. GOLDSMITH and C. H. RUOF, *J. Amer. Chem. Soc.* **69**, 2167 (1947).
[12] T. M. JEFFERIES, Pharmacognosy Group, Bath University, personal communication (1969).
[13] R. D. BENNETT and E. HEFTMANN, *J. Chromatog.* **21**, 488 (1966).
[14] K. R. BRAIN, Pharmacognosy Group, Bath University, personal communication (1969).

later), they were chopped and sliced before drying at 50° for 12 hr. They were then milled to a fine powder, separately, using a Christie Norris disintegrator. The soft fruit wall (epicarp and mesocarp) was scraped from ripe fruits obtained from Nigeria (Kwara State). It was dried as above and powdered in a mortar.

Methods

Thin-layer chromatography was done on silica gel G plates as previously described[15] using either hexane: acetone (4:1) or benzene:alcohol (95 per cent) (9:1) as developing solvent. Melting points were determined on a Buchi apparatus and are corrected. Mass spectra were obtained from an M.S.9 double-focusing mass spectrometer, i.r. spectra from a Perkin–Elmer Model 137 spectrophotometer using a KBr disc (except otherwise stated). NMR spectra were determined in $CDCl_3$ in a Perkin–Elmer R10 spectrometer operating at 60 Mc/sec with tetramethylsilane as internal reference.

Isolation of the Major Sapogenin from Root

The powdered root, 170 g, was extracted for 24 hr in a soxhlet with dry ($MgSO_4$) light petroleum (b.p. 40–60°). The "defatted" powder was dried in a hot-air oven (80°) to remove excess solvent. It was then extracted to exhaustion with MeOH in a soxhlet apparatus to afford 40 g of a dark-brown, hygroscopic crude saponin which was hydrolysed by refluxing with 2 N HCl (600 ml) for 2 hr. The mixture was cooled, filtered and the acid-insoluble washed with H_2O before neutralizing with 20 ml of 10% NH_4OH. After it had drained, the acid-insoluble was dried in a hot-air oven (80°) for 4 hr. The dried residue was crushed in a mortar and extracted with light petroleum in a soxhlet for 2 days. Removal of the solvent, *in vacuo*, afforded 3·15 g of crude sapogenin.

For the characterization of the major sapogenin in this crude material, the extract obtained during the first 3 hr of light petroleum extraction of crude sapogenin was used. This afforded crystalline sapogenin (2·7 g), TLC of which indicated one major compound with a slight impurity. The latter was removed by chromatographing the sapogenin (600 mg) on a neutral alumina (25 g, Brockman type H) column monitored with u.v. light while most of the greenish yellow major sapogenin band was eluted with benzene:$CHCl_3$ (1:1). The eluate (200 ml) afforded 300 mg of a TLC pure, white powder, m.p. 180–187°. This major sapogenin was further purified for analysis by recrystallization from both iso-PrOH and Me_2CO, m.p. 184–185°, (Found: C, 78·2; H, 10·33—sample exploded on heating—Calc. for $C_{27}H_{42}O_3$: C, 78·2; H, 10·21%) had $[\alpha]_D^{24}$ − 124° ($CHCl_3$) (Marker[11] reported m.p. 198–201° $[\alpha]_D^{25}$ − 123° for yamogenin). The acetate, from acetone, afforded transparent colourless prismatic crystals, m.p. 163–165° (Found: C, 74·9; H, 8·88—sample exploded on heating—Calc. for $C_{29}H_{44}O_4$: C, 76·3; H, 9·65%), had $[\alpha]_D^{24}$ − 124·6° ($CHCl_3$) (Marker[11] reported m.p. 180–182° $[\alpha]_D^{27}$ − 113° for yamogenin acetate). The *5α, 6β-dichloro derivative* of the acetate (by Morita's[16] method) afforded white (opaque) cluster crystals from MeOH, m.p. 182–184°. (Found: C, 65·6; H, 8·52; Cl, 14·2. $C_{27}H_{42}O_3Cl_2$ require: C, 66·0; H, 8·41; Cl, 13·5%.)

Isolation of the Major Sapogenin from the Stem Bark

The powdered stem bark, 50 g was refluxed with 2 N HCl (400 ml) for 2 hr. The mixture was cooled, filtered and the acid-insoluble material washed before neutralizing with 10 ml of 10% NH_4OH. After the residue had drained, it was dried in a hot-air oven for 4 hr and extracted with light petroleum for 24 hr in a soxhlet. Removal of the solvent, *in vacuo*, afforded 1·31 g of a resinous solid which, when warmed with 20 ml of light petroleum, deposited 0·5 g of a semi-crystalline solid on cooling. This solid was chromatographed on a neutral alumina (15 g, Brockman type H) column and eluted with benzene:$CHCl_3$ (7:3). The column was monitored with u.v. light and the eluate (200 ml) of the sapogenin band (fluorescing greenish yellow) which was collected afforded a white solid (246 mg), m.p. 174–178°. (Found: C, 78·0; H, 10·37. Calc. for $C_{27}H_{42}O_3$: C, 78·2; H, 10·21%.)

Isolation of the Major Sapogenin from the Soft Fruit Wall

The powdered fruit wall was incubated (37°) with H_2O (400 ml) for 7 days and conc. HCl (88 ml) was added to bring the mixture to 2 N before refluxing for 2 hr. It was then cooled, filtered, washed with H_2O and neutralized with 20 ml of 10% NH_4OH. The acid-insoluble residue was dried in a hot-air oven (80°) for 4 hr before it was exhausted with light petroleum. Upon removal of the solvent, *in vacuo*, a slightly resinous solid, 2·59 g, was obtained. This was purified by chromatography on neutral alumina (Brockman type H) column ×3, using benzene:$CHCl_3$ (1:1) as eluent until finally 407 mg of a white solid (m.p. 172–178°) was obtained from the sapogenin band. (Found: C, 77·8; H, 10·42. Calc. for $C_{27}H_{42}O_3$: C, 78·2; H, 10·21%.)

Preparation of Sapogenin Acetate and its 5α, 6β-Dichloro Derivative

Sapogenin, 100 mg, was acetylated with pyridine (1 ml) and acetic anhydride (1 ml). The crude acetate was recrystallized from acetone.

[15] G. BLUNDEN and R. HARDMAN, *J. Chromatog.* **15**, 273 (1964).
[16] K. MORITA, *Chem. Pharm. Bull.* **11**, 90 (1963).

The 5α,6β-dichloro derivative was prepared by the method of Morita[16] using a saturated solution of Cl_2 in CCl_4 at room temperature.

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