Phytochemistry, 1971, Vol. 10, pp. 887 to 889. Pergamon Press. Printed in England.

SHORT COMMUNICATION

THE RIPE FRUITS OF *BALANITES ORBICULARIS* AS A NEW SOURCE OF DIOSGENIN AND YAMOGENIN

ROLAND HARDMAN and C. N. WOOD*

Pharmacognosy Group, School of Pharmacy, Bath University of Technology, Bath BA2 7AY

(*Received* 29 *June* 1970)

Abstract—The dried ripe fruits of *Balanites orbicularis* Sprague (Balanitaceae) were easily broken and the fruit wall gave about 1% of a mixture of diosgenin and yamogenin along with sterols, tentatively identified as sitosterol, stigmasterol and cholesterol (trace). Incubation with water caused an increase of 20–40% in this sapogenin yield. The seed gave similar results along with 40% of a fixed oil.

INTRODUCTION

THE STEROIDAL sapogenins diosgenin and yamogenin are the most important raw materials for the production of pharmaceutical steroids. Commercially attractive quantities of these sapogenins have been demonstrated in the fruits of *Balanites aegyptiaca* and *B. roxburghii*.[1,2] These fruits have a thick woody endocarp and, prior to the extraction of the seed, mechanical removal of this layer is necessary and somewhat troublesome.[2] In contrast the fruits of *B. orbicularis*, a spiny savanna bush indigenous to East Africa,[3] have the merit of a relatively thin endocarp so that the fruits can be easily crushed and processed. The present work reports their steroid content.

RESULTS AND DISCUSSION

The sapogenin was afforded by acid hydrolysis of the defatted plant tissue.[4] TLC examination of the crude sapogenin obtained from the fruit wall and from the seed, showed the presence of 3β-hydroxysapogenin, sterol, sterol ester and spirostadiene. A methanolic extract of the crude sapogenin afforded 0·91% of recrystallized sapogenin from the fruit wall and 1·10% from the seed both yields being calculated on a moisture free basis. The sapogenin was identified by direct comparison with an authentic mixture of diosgenin and yamogenin[5] by co-chromatography before and after derivatization (4 solvents, TLC and GLC), i.r. and NMR analysis. Comparison of the absorbance data in the region 800–1000 cm^{-1} with standard calibration curves[4] showed that the sapogenin of m.p. 195° from the fruit wall, comprised 63% of diosgenin (25α) and 37% of yamogenin (25β). This ratio was confirmed by the strength of the NMR signal at 3·9δ (a doublet of doublets characteristic of yamogenin). The seed sapogenin, m.p. 210°, comprised 87% of diosgenin and 13% of

* Present address: Division of Pharmacognosy and Forensic Science, Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow, C.1, Scotland.

[1] A. A. M. DAWIDAR and M. B. E. FAYEZ, *Phytochem.* **8**, 261 (1969).
[2] R. HARDMAN and E. A. SOFOWORA, *Econ. Bot.* in press.
[3] I. R. DALE and P. J. GREENWAY, *Kenya Trees and Shrubs*, p. 531, Buchanan Kenya Estates in assoc. with Hatchards, London (1961).
[4] K. R. BRAIN, F. R. Y. FAZLI, R. HARDMAN and A. B. WOOD, *Phytochem.* **7**, 1815 (1968).
[5] R. HARDMAN and E. A. SOFOWORA, *Phytochem.* **9**, 645 (1970).

yamogenin. Since TLC examination of the petroleum extracts of the fruit wall and of the seed, obtained before acid hydrolysis, did not reveal any free sapogenin, the sapogenin must have been present in the fruit part in the form of the glycoside.

The petroleum extracts and the sapogenin extracts obtained from the fruit wall and from the seed also contained sterol fractions which were shown, using GLC, to consist of over 60% sitosterol with stigmasterol and trace quantities of cholesterol. The crushed seed afforded 39% of fixed oil by petroleum extraction in a soxhlet for 24 hr.

It has been shown that incubation of dried plant material with water at 37° effects an increase in its sapogenin yield.[6] The maximum sapogenin yields from the powdered fruit wall and from the powdered partially defatted seed were obtained after incubation for 12 hr and increases of 37% and 26%, respectively, over the non-incubated controls were secured (Fig. 1). The yields did not significantly fall back during incubations up to 72 hr. Little change was observed in the ratio of diosgenin to yamogenin in the fruit wall but the proportion of yamogenin in the seed tissue fell during incubation. TLC examination of the sapogenin isolated after incubation showed the presence of only diosgenin and yamogenin. Repeated experiments with the same batch of powdered fruit wall demonstrated that incubation was a variable process but in each experiment an increase in the total yield was achieved ranging from 20 to 40%.

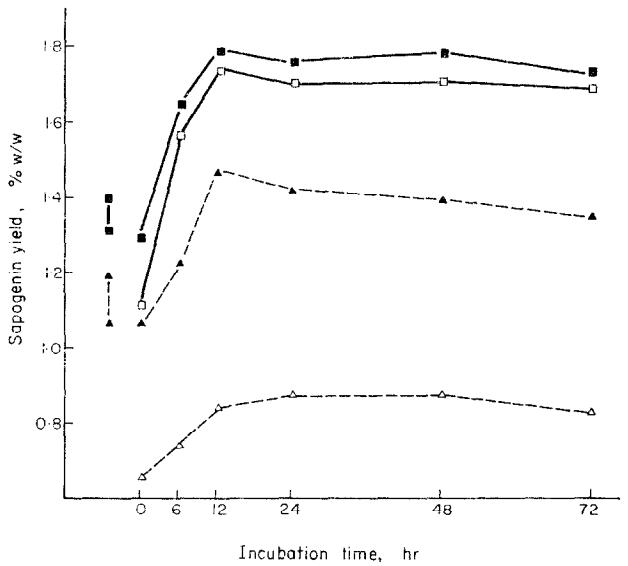

FIG. 1. THE EFFECT OF INCUBATION ON THE SAPOGENIN YIELD OF THE FRUIT PARTS OF *B. orbicularis* (■—■), Seed, total yield; (□—□), seed, diosgenin yield; (▲--▲), fruit wall, total yield; (△--△), fruit wall, diosgenin yield; ┃, minimum significant range of the total yields for the samples of seed; ┃, minimum significant range of the total yields for the samples of fruit wall. Infra-red assay. Results are calculated on a moisture free basis of the fruit part. Yields from the seed are calculated as the %, w/w of the undefatted seed.

[6] G. BLUNDEN, R. HARDMAN and W. R. WENSLEY, *J. Pharm. Pharmacol.* **17**, 274 (1965).

EXPERIMENTAL

The dried ripe fruits (av. wt. 2·1 g) obtained from the Conservator of Forests, Entebbe, Uganda were authenticated after consultation with the East African Herbarium in Nairobi. By light pressure the fruit wall was broken and the seed removed.

Powdered fruit wall, 1·24 kg, was extracted in a soxhlet for 24 hr with light petroleum (b.p. 40–60°) and the defatted tissue was refluxed with 2N HCl for 2 hr to afford crude sapogenin (3·65% on a moisture free basis) by the method of Brain *et al.*[4] After examination by TLC[7] this solid was extracted under reflux with three 80 ml quantities of 85% aq. MeOH. The bulked methanolic extracts afforded crystalline material which was recrystallized from ethanol to yield white needles of sapogenin, m.p. 195°. (Found: C, 78·9; H, 10·4%. Calc. for $C_{27}H_{42}O_3$: C, 78·2; H, 10·2%.) The sapogenin formed a monoacetate m.p. 188°. Using similar procedures the crude sapogenin from the seed afforded white needle crystals of sapogenin, m.p. 210° after recrystallization from acetone. (Found: C, 78·6; H, 10·1%.) All m.ps were determined on a Köfler block and are corrected. The unsaponifiable matter isolated from the petroleum extracts obtained above and from the mother liquors remaining after recrystallization of the crude sapogenin, from both the fruit wall and the seeds, were separately fractionated on 20 g alumina columns. The TMSi ethers of the four sterol fractions so obtained were investigated by GLC. Peaks were tentatively identified by co-chromatography with reference sterols. All GLC analyses were performed on a 5% XE60 on A.W., D.C.M.S. chromosorb W, stainless steel column; oven temperature 230° isothermal; on column injection at 240°; detector oven temperature 240°; carrier flow rate (N_2) 40 ml/min.

Prior to incubation with water at 37° in the dark, the crushed seed was partially defatted in a soxhlet for two hr with light petroleum (b.p. 40–60°) so as to reduce the fixed oil content to a level (10% of the partially defatted seed by exhaustion with light petroleum) which would not effect the i.r. assay. Duplicate samples, 5 g, of the powdered partially defatted seed and of the powdered fruit wall were used in the incubation experiments and the sapogenin was recovered and estimated by the i.r. method[4] (Fig. 1).

Acknowledgements—We are grateful to Mr. A. G. Kenyon of the Tropical Products Institute, London for obtaining the specimens of fruits and C. N. Wood thanks the Institute for his Research Studentship. We also thank the National Research Development Corporation for financial support.

[7] G. BLUNDEN and R. HARDMAN, *J. Chromatog.* **15**, 273 (1964).