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CHANGES IN THE SAPOGENIN AND OIL CONTENT OF *BALANITES AEGYPTIACA* SEEDS DURING THE EARLY STAGES OF GERMINATION

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Abstract—Before the emergence of the radicle during the germination of *Balanites aegyptiaca* seeds, a sudden fall in both the oil and total sapogenin content occurred. Although the oil content fell continuously, the total sapogenin content rose to a maximum in 5 days due to the biosynthesis of sapogenin by the germinating seedlings. The proportion of 25α- and 25β-sapogenin present remained fairly constant during the first 9 days of germination.

INTRODUCTION

Balanites aegyptiaca Del. (Zygophyllaceae) is an evergreen tree found in the tropical savannah forests and the subtropics. Its fruit is a plum-like drupe with a fleshy edible fruit pulp and a stone which contains the oval-shaped oily seed. The seed yields up to 50 per cent w/w of a golden-yellow, edible, fixed oil. Marker[1] reported 5 g/kg of steroidal sapogenins in this plant and diosgenin has been reported in the seed by other workers.[2,3] Both diosgenin and its C_{25} isomer—yamogenin, in a ratio of 3:1, have been found in the seed by us.[4]

Studies on the biogenesis of steroidal sapogenins have been made on monocotyledons, e.g. *Dioscorea* and *Agave* spp., and extended to dicotyledons, e.g. *Digitalis* (where they co-occur with cardenolides) and *Lycopersicon* spp. (where they occur as steroidal alkamines). In order to test whether the biosynthetic pathways proposed for the steroidal sapogenins are universally valid, it is necessary to carry out investigations in diverse plants producing these compounds. The dicotyledonous seeds of *B. aegyptiaca* begin to germinate after 2–3 days' incubation, in the dark, with water at 30° (a temperature similar to that of the natural habitat of the plant). The present investigation examines the possibility of using these seeds in studying the biosynthesis of the steroidal sapogenin present in the seed (1·7 per cent w/w), bearing in mind that changes in sterols[5] and sapogenins[6] contents occur in the seeds of other genera during germination.

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[1] R. E. MARKER, R. B. WAGNER, P. R. ULSHAFER, D. P. J. GOLDSMITH and C. H. RUOF, *J. Am. Chem. Soc*, **65**, 1248 (1943).
[2] M. L. DUTTA, *Pharm. Acta Helv.* **29**, 260 (1954).
[3] I. P. VARSHNEY and K. M. SHAMSUDDIM, *Arch. Pharm.* **295**, 401 (1962).
[4] E. A. SOFOWORA, Ph.D. Thesis, University of Nottingham (1967).
[5] D. S. INGRAM, B. A. KNIGHTS, I. J. MCEVOY and P. MCKAY, *Phytochem.* **7**, 1241 (1968).
[6] A. AKAHORI, F. YASUDA, I. OKUNO, M. TAGANI and T. OKANISHI, *Phytochem.* **8**, 45 (1969).

RESULTS AND DISCUSSION

Five groups of forty seeds (surface sterilized and of known weight) were incubated (1–9 days) at 30° in the dark with sterile water for germination to take place. After incubation, each group was filtered prior to the estimation of its oil and sapogenin content. An estimation of the oil and sapogenin content of a weighed sample of the unincubated seeds served as control. There was a latent period of 2½–3 days, after which the radicles emerged. The results (Table 1) indicate a 7 per cent drop in the dry weight during the latent period resulting from the metabolism of food reserves. Allowance was made for loss in weight during germination in the sapogenin and oil content (Table 1, columns 8 and 9); even so, an initial drop in sapogenin content on the first day was followed by a steady rise, to a maximum on the fifth day of incubation, thus suggesting biosynthesis of sapogenin during germination. That biosynthesis of sapogenin took place during this time was later confirmed when labelled precursors

TABLE 1. CHANGES IN THE SAPOGENIN AND OIL CONTENT OF *Balanites aegyptiaca* SEEDS DURING GERMINATION

Period of incubation	Oil content (%)*	Sapogenin content: Total (%)*	Sapogenin content: % Proportion 25α-	Sapogenin content: % Proportion 25β-	Dry wt.† ratio	% Germination‡	Corrected§ sapogenin content	Corrected‖ oil content
0	51·0	1·47	72	28	1	0	1·47	51·0
1	52·6	1·31	72	28	0·96	0	1·26	50·5
3	53·0	1·53	71	29	0·93	45	1·42	49·3
5	48·0	1·54	74	26	0·94	60	1·45	45·1
7	45·5	1·47	72	28	0·93	70	1·37	42·3
9	43·6	1·32	72	28	0·94	65	1·24	41·9

* On dry wt. basis.

† Dry wt. ratio = $\frac{\text{dry wt. after incubation}}{\text{dry wt. before incubation}}$.

‡ Calculated from the proportion of seedlings with radicle length more than 1 mm.

§ % total sapogenin × dry wt. ratio.

‖ % Oil content × dry wt. ratio.

(acetate, malonate and mevalonate) were incorporated into diosgenin by the germinating seeds.[4] The initial fall in sapogenin content (on the first day of incubation) could suggest the use of some steroidal sapogenin for promoting germination, as reported for other steroids.[7–12] The corrected oil content fell steadily throughout the period of incubation as expected for a food reserve. There was, however, no significant change in the proportion of 25α, and 25β-sapogenins during the period investigated. Appreciable changes in the composition of the sapogenins have been reported in germinating seeds of *Dioscorea tokoro*.[6]

The time taken for the radicle to emerge during germination of *Balanites aegyptiaca* seeds varied but, usually, after 7 days' incubation (30°), the radicle had emerged from 70 per cent of them. The observed increase in steroidal sapogenin yield during incubation could have

[7] E. HEFTMANN, *Lloydia* **31**, 293 (1968).
[8] A. LOVE and D. LOVE, *Arkiv. Botan.* **32A (13)** (1945).
[9] H. FIEDLER, *Z. Botan.* **30**, 385 (1936).
[10] J. BONNER and G. AXTMAN, *Proc. Nat. Acad. Sci. U.S.* **23**, 453 (1937).
[11] F. KOGL and A. J. HAAGEN-SMITH, *Z. Physiol. Chem.* **243**, 209 (1936).
[12] G. INCESCU, *Acord. Rep. Populare-Romino Studii Cercetari. Endocrinol* **11**, 308 (1960).

been contributed therefore by either the seeds which had germinated or those which had not. This was investigated by incubating (30°) eighty seeds with water for 6 days, by which time the rise in sapogenin content should have taken place (Table 1). They were then filtered and grouped into those which germinated—namely (forty-seven) with the root 1 mm or more—and those which had not germinated—namely (thirty-three) with the root less than 1 mm long. Each group was dried, defatted and assayed for sapogenin separately. The data in Table 2 show that the germinating seeds contained more sapogenin per unit weight than those which had not germinated; thus supporting biosynthesis of sapogenin during germination. No significant change in the 25α- and 25β-sapogenin ratio occurred but an expected lower oil content was obtained for the germinating seeds. It is not clear whether some oil was metabolized, during germination, to synthesize steroidal sapogenins.

By using a microchemical stain ($SbCl_3$ in perchloric acid) modified for the detection of steroidal sapogenins in plant tissues directly,[4] sapogenins were found in the 5-day-old roots of *B. aegyptiaca*, a higher concentration being present at the growing tip. Steroidal sapogenins have also been isolated from older roots obtained from fully grown trees of *B. aegyptiaca*;

TABLE 2. COMPARISON OF THE OIL AND SAPOGENIN CONTENT OF *B. aegyptiaca* SEEDS WHICH GERMINATED AND THOSE WHICH DID NOT GERMINATE AFTER 5 DAYS' INCUBATION (30°)

		Sapogenin content		
			% Proportion	
Group of seed	Oil content (%)*	Total (%)*	25α-	25β-
Germinated	40·7	1·46	71	29
Non-germinated	54·7	1·06	75	25

* On dry wt. basis.

this forms the subject of a separate publication.[13] The presence of sapogenins in the young roots could be due to transportation of these compounds from the cotyledons (perhaps to stimulate growth in the root) and/or synthesis of these compounds in the root. Our investigation of the sapogenin yield from the cotyledons alone revealed a 13 per cent loss of sapogenin (from 1·74 per cent w/w to 1·50 per cent w/w) in the coytledons, after 5 days' incubation of the seeds. There was no change in the proportion of 25α- or 25β-sapogenin in the cotyledons but the oil content fell by 15 per cent. Considering that an overall rise (to a maximum) in sapogenin content of the germinating seeds (cotyledon and root) was obtained in 5 days under the same conditions (Table 1), these observations strongly pin-point the root as a site of synthesis for the steroidal sapogenins. The site of synthesis of sapogenin in *B. aegyptiac* palant is to be further investigated.

The data presented in Table 3 show that the oil content fell steadily during elongation of the radicle while sapogenin content in the seedling increased up to 30 mm radicle length. The final fall in sapogenin content in the 30–80-mm rooted seedling, together with a stationary oil content at this level, suggests a preferential metabolism of the steroidal sapogenins at this stage.

[13] R. HARDMAN and E. A. SOFOWORA, *Phytochem.* **9**, 645 (1970).

EXPERIMENTAL

Material and Methods

Whole seeds (av. wt. 0·5 g) from freshly opened nuts of ripe fruits of *Balanites aegyptiaca* from Nigeria (Kwara State) were used. In order to minimize sampling error, only seeds weighing 0·45–0·60 g were used. Quantitative estimations were performed in duplicate and a mean of the results taken.

Surface Sterilization of Seeds

Seeds were surface sterilized before incubation by shaking them first with bromine water in a stoppered flask for 1 min. Then they were rinsed with sterile water before being shaken with H_2O_2 (10 vols.) for 15 min. They were finally rinsed thoroughly with sterile water.

Estimation of Moisture and Oil Content

Moisture determination was done by an oven drying method, estimating the loss in weight after heating at 105° for 16 hr. Oil content was determined by extracting a weighed sample of crushed seed with light petroleum (b.p. 40–60°) in a soxhlet for 24 hr. The solvent was then removed *in vacuo* and the oil weighed.

Estimation of Steroidal Sapogenin Content

Because of the relatively high oil content (about 50%) of the seed which interfered with the i.r. spectrophotometric analysis of sapogenins,[4] material to be assayed for sapogenin was first defatted as above. The defatted seed was dried in a hot-air oven (50°) to remove traces of solvent and ground in a mortar until all the particles passed through a British Standard sieve No. 40. About 5 g of this powder, accurately weighed, was then refluxed with 2 N HCl (100 ml) for 2 hr. It was cooled, filtered and the insoluble matter was washed with water (100 ml), before neutralization with 100 ml of 5% w/v NH_4OH. When the residue had drained, it was dried in a hot-air oven at 60° for 16 hr before it was extracted in a soxhlet with light petroleum (b.p. 40–60°) to exhaustion (normally 24 hr).

The solvent was removed *in vacuo* and the crude sapogenin re-dissolved in $CHCl_3$ (10 ml). The amount of sapogenin in $CHCl_3$ solution was estimated by i.r. spectrophotometric analysis, using the method of Brain *et al.*[14] A Hilger H.800 double-beam recording i.r. spectrophotometer with rock-salt prism was used to produce the spectra under the following conditions: 1 mm path length cell, slit 550 μ at 900 cm^{-1}, Autoslit 25, Gain 7, Damping 4, scan speed 33 min/rev.

Investigation of Sapogenin Yield from Cotyledons during Germination

Forty seeds were surface sterilized and incubated (30°), in pairs, in 10 ml of sterile water contained in each of 20 × 100 ml sterile plugged conical flasks for 5 days. They were then filtered and the cotyledons separated. The cotyledons were dried at 80° for 24 hr and their weight was noted before estimating their moisture, oil and sapogenin content. For comparison, the cotyledons removed from 20 g of seeds were crushed and an estimation of the moisture, oil and sapogenin content was made.

TABLE 3. VARIATION OF SAPOGENIN AND OIL CONTENT WITH RADICLE LENGTH IN GERMINATING SEED OF *B. aegyptiaca*

Radicle length† (mm)	Sapogenin content			Oil content (%)*
	Total (%)*	% Proportion		
		25α-	25β-	
Less than 1	1·03	75	25	51·6
1–10	1·31	77	23	48·8
10–30	1·38	77	23	45·4
30–80	1·11	76	24	45·3

* On dry wt. basis.
† After 7 days' incubation at 30°.

[14] K. R. BRAIN, F. R. Y. FAZLI, R. HARDMAN and A. B. WOOD, *Phytochem.* 7, 1815 (1968).

Comparison between Radicle Length and Sapogenin Content of Germinating Seeds

One hundred and twenty seeds were surface sterilized and transferred, in pairs, into sterile water (10 ml) contained in each of 60 × 100 ml sterile plugged conical flasks. The seeds were then incubated for 7 days at 30° for germination to take place. The resulting seedlings were filtered and sorted into groups on the basis of radicle length (see Table 3). The total dry weight of the seedlings in each group was estimated, together with the sapogenin and oil content, after drying them in a hot-air oven (80°) for 2 hr (Table 3).

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