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ESTRONE IN *HYPHAENE THEBAICA* KERNEL AND POLLEN GRAINS

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Key Word Index—*Hyphaene thebaica*; Palmae; doum palm; estrone.

Abstract—The estrogen of the doum palm kernel and pollen grains has been isolated as the *p,p'*-nitrophenylazo benzoyl derivative and identified as estrone.

INTRODUCTION

THE DOUM PALM (*Hyphaene thebaica*) is so named from the word 'doum' which means 'permanence', in allusion to the persistence of the tree under abnormal conditions. It is almost as widely grown in Egypt as the date palm. It has been studied by many investigators;[1-13] however, no research had been carried out on its hormonal content.

In view of the discovery of estrone in the seeds of the date palm (*Phoenix dactylifera*) by Butenandt[14] and its subsequent isolation,[15] the present authors set out to see if this animal hormone was present in the kernel and pollen grains of the doum palm.

RESULTS

Two methods were used for the isolation of estrone: the first was performed essentially as reported by Heftmann *et al.*[16] and the second was evolved from one of Amin *et al.*[15] and incorporates a further purification and identification step involving PC. The estrone was estimated colorimetrically using an improved modification of the Kober color reaction.[17] The isolated compound was systematically purified by PC and formation of the *p,p'*-nitrophenylazobenzoyl ester and identified by m.p. and m.m.p., UV, NMR and IR spectroscopy and C, H determination.

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It was thus possible to separate the estrogenic active principle from the doum palm kernel and pollen grains in a pure state and to show that it was estrone. Both methods proved to be quite efficient giving approximately the same yield (5·13 mg estrone/kg for Method I and 5·25 mg/kg for Method II). However, the latter method gave a better and more detailed picture about the state of the estrogen in the different extracts before and after hydrolysis.

Further investigation is presently being undertaken on the estrogens of the pollen grains.

EXPERIMENTAL

Analysis of the kernel. In the present investigation two methods were used for the isolation of estrone: *Method I.* 1 kg finely ground kernel was hydrolysed according to Heftmann *et al.*[16] with 5 l. 3 N HCl for 3 hr, filtered and the residue washed acid-free with H_2O and air-dried overnight, yielding 150 g (15%). It was then refluxed with 1·5 l. MeOH for 24 hr, filtered, and the resulting oil concentrated under vacuum. The residue obtained from the first extraction was then refluxed with 1 l. acetone for 24 hr, filtered and the acetone distilled off. The air-dried residue weighed 46 g (0·46%). The combined extracts were partitioned between 2 N NaOH and Et_2O. The aqueous phase was acidified with HCl and reextracted with Et_2O. The Et_2O extract was washed with H_2O until neutral, dried and the Et_2O evaporated. It was then dissolved in C_6H_6, passed through a column of Al_2O_3 and cluted with C_6H_6, then with 0·5% EtOH in C_6H_6. The eluate was evaporated to constant wt, yielding 10·2496 g (1%). In ethanolic solution it showed UV_{max} at 280 and 288 nm. After quantitative treatment with Brown's color reagent[17] it showed a peak at 516 nm, corresponding to 5·13 mg estrone/kg. By using descending PC with the upper layer of C_6H_6–MeOH–H_2O (5:4:1) and 10% ethanolic phosphomolybdic acid as the spray, it was shown to migrate the same distance as pure estrone. *Method II.* 1 kg finely ground kernel was treated according to Amin *et al.*[15] with 3 l. Et_2O for 2 weeks, filtered, then with 3 l. MeOH for 1 week, filtered, then with 3 l. acetone for 1 week. The filtrates were separately concentrated under vacuum to constant wt, yielding 75·1447 g, 29·7256 g and 7·7066 g oil, respectively. The residue was then extracted with 3 l. boiling distilled H_2O. After filtration it was left in 3·5 l. of 1·5% Na_2CO_3 for 3 days and filtered. The residue was then refluxed for 3 hr with 3·5 l. of 4% NaOH and finally for 3 hr with 3·5 l. of 25% NaOH. These extracts were freed from proteins and polysaccharides, and then acidified with HCl and extracted with Et_2O, the Et_2O extract washed with H_2O until neutral, dried and evaporated. Aliquots of each extract were treated with Brown's color reagent.[17] The Et_2O and MeOH extracts showed an absorption maximum at 420 nm corresponding to the estrogen ester, while the acetone extract showed a peak at 516 nm, corresponding to the free estrone. The boiling H_2O, 1·5% Na_2CO_5, 4% NaOH and 25% NaOH extracts did not show any appreciable visible absorption. The amount of hormone was calculated from calibration curves prepared with pure estrone and estradiol benzoate and was found to correspond to 4·7 mg/kg estrone ester and 1·75 mg/kg free estrone. The Et_2O and MeOH extracts were then separately hydrolyzed with 3 N methanolic HCl eventually yielding 50·958 g and 21·541 g of a pale brown oil, respectively. Aliquots of the hydrolyzed Et_2O and MeOH extracts were subjected to the Brown color test. The absorption maxima were shifted to 516 nm, indicating free estrone, corresponding to 3·5 mg/kg. PC of the Et_2O ,MeOH and acetone extracts showed single spots which migrated the same distance as pure estrone. They showed UV_{max} at 280 and 288 nm.

TABLE 1. ESTROGEN CONTENT OF DOUM PALM EXTRACTS

Extract	Weight (g)		Estrogenic component (mg/kg)
(i) Method I:	Combined	10·260	5·13
(ii) Method II:	Ether	75·145	2·85
	Methanol	29·726	1·85
	Acetone	7·707	1·75
After hydrolysis:	Ether	50·958	2·10
	Methanol	21·541	1·40

Identification. From the combined extracts from Methods I and II (Table 1) about 8 mg crude estrone were isolated. The *p,p'*-nitrophenylazo benzoate[18] was purified by Al_2O_3 chromatography and by crystallization from C_6H_6–acetone (1:1) and obtained as red needles (6 mg) m.p. and m.m.p. 249°. Hydrolysis

[18] NUTTING, W. H., JEWELL, R. A. and RAPOPORT, H. (1970) *J. Am. Chem. Soc.* **35**, 505.

of the derivative eventually gave estrone as colourless crystals, m.p. and m.m.p. 259° whose NMR was very similar to that of authentic estrone. (Found: C, 79·86; H, 8·12. Calc. for $C_{18}H_{22}O_2$: C, 79·96; H, 8·20%.)

Analysis of the pollen grains. 12 kg air dried pollen grains and styles of the doum palm were kept under 4 l. MeOH in the dark for 1 week at room temp. After filtration, the MeOH was distilled off under vacuum to dryness, yielding 135 g (1·12%). 1 g of this extract was further purified by PC yielding 10 m of material with the same UV spectrum and R_f as estrone.