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ISOLATION OF ESTRONE FROM MOGHAT ROOTS AND FROM POLLEN GRAINS OF EGYPTIAN DATE PALM

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Abstract—The estrogenic substance in the roots of moghat (*Clossostemon bruguieri*, Sterculiaceae) from Iraq, and in the pollen grains of date palm (*Phoenix dactylifera*, Egyptian variety, Samani) has been identified as estrone. Estrone was isolated from the two sources as the *p,p'*-nitrophenylazobenzoyl derivative. Cholesterol was also isolated from moghat roots.

INTRODUCTION

ESTRONE has been identified in seeds of *Elaeis guineensis*,[1] of *Phoenix dactylifera*,[2,3] and of *Punica granatum*.[4] No previous work, however, has been carried out on moghat, commonly used by women in Egypt after delivery as a hot beverage, and a possible source of estrone. Another local source of oestrogen is the pollen grain of the Egyptian date palm, extracts of which are used to produce fertility in women.[5–7] Earlier, El-Ridi[6,7] obtained an extract which had estrogenic activity, but he could not separate the estrogenic hormone. Later, Bennett *et al.*[2] were able to isolate estrone by TLC from pollen of date palm (*Phoenix dactylifera* L.) growing in California. We have therefore set out to examine the oestrogen of moghat and to confirm, or otherwise, the presence of estrone in the Egyptian date palm (variety Samani).

RESULTS

A reversed phase partition chromatographic method (see Experimental) for separating sex hormones as the *p,p'*-nitrophenylazobenzoyl esters (Table 1) was applied to the isolation of estrone from moghat roots and from pollen grains of date palm.

A phenolic fraction was obtained from the moghat root extract. By acid hydrolysis[3] and extraction with ether, then by reaction with *p,p'*-nitrophenylazobenzoyl chloride, it gave a derivative identical to that of estrone azoyl ester. Upon alkaline hydrolysis of this derivative, estrone was obtained (proved by m.p., mixed m.p.; color reactions,[8] u.v. and i.r. spectra).

The sterols of moghat root extract were isolated by solvent extraction. One was identified as cholesterol, its purity being checked by i.r. spectrum, and the formation of *p,p'*-nitrophenylazobenzoyl ester. The second and major component was a mixture of sitosterols, as indicated by its i.r. and mass spectra.

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TABLE 1. ANALYSES OF SEX HORMONE AZOATES

Ester of	M.p.	Found (%)			Formula	Required (%)		
		C	H	N		G	H	N
Estriol (triazoate)*	170	67·0	4·1	12·0	$C_{57}H_{45}O_{12}N_9$	66·8	4·4	12·3
α-Estradiol (diazoate)†	220	67·6	5·0	11·0	$C_{44}H_{38}O_8N_6$	67·9	4·9	10·8
α-Estradiol (monoazoate)†	218	—	—	8·2	$C_{31}H_{31}O_5N_3$	—	—	8·0
β-Estradiol (diazoate)†	249	67·9	4·8	11·3	$C_{44}H_{38}O_8N_6$	67·9	4·9	10·8
β-Estradiol (monoazoate)‡	235	70·2	6·0	8·1	$C_{31}H_{31}O_5N_3$	70·8	6·0	8·0
Estrone (azoate)*	240	71·0	5·3	8·2	$C_{31}H_{29}O_5N_3$	71·1	5·6	8·0
Pregnanediol (diazoate)†	291	68·0	6·0	10·0	$C_{47}H_{50}O_8N_6$	68·3	6·1	10·2
Pregnanediol (monoazoate)*	289	70·3	7·9	7·6	$C_{34}H_{43}O_5N_3$	71·2	7·6	7·3
Equilin (azoate)*	270	71·2	5·2	7·9	$C_{31}H_{27}O_5N_3$	71·4	5·2	8·1

* Recrystallized from benzene–acetone (1:1 v/v).
† Recrystallized from benzene–alcohol (1:1 v/v).
‡ Recrystallized from alcohol–acetone (1:1 v/v).

A phenolic fraction was also isolated from Egyptian date palm pollen by extraction with ether, methyl alcohol and acetone. Acid hydrolysis,[3] re-extraction with ether and reaction with *p,p'*nitrophenylazobenzoyl chloride gave a derivative identical with estrone azoyl ester. Upon alkaline hydrolysis of the azoyl derivative, authentic estrone was obtained.

EXPERIMENTAL

Plant Sources

Dry moghat roots, imported from Iraq, were purchased at the local market. Pollen grains of Egyptian date (*Phoenix dactylifera*, variety Samani) were collected at the end of April 1967 from Alexandria, Egypt.

Preparation of Sex Hormone Azoyl Derivatives

The sex hormone (0·25 mmole) was dissolved in dry benzene (20 ml) and an appropriate amount of *p,p'*-nitrophenylazobenzoyl chloride (0·37 mmole for monohydric sex hormones, 0·74 mmole for dihydric compounds, etc.) in 500 ml of dry benzene and anhydrous pyridine (1 ml) were added. After keeping at room temperature in the dark for 2 weeks, the solution was filtered, the filtrate was washed with 20% H_2SO_4, re-filtered, washed successively with water, and dried. The benzene extract was concentrated to *ca.* 100 ml and adsorbed onto an Al_2O_3 column. The main lower band on the column was eluted with benzene and the eluate evaporated to dryness. The residue was washed with hot alcohol and then recrystallized from benzene–alcohol, benzene–acetone or alcohol–acetone (1:1, v/v). In case of diazoates and triazoates, an upper band of partially azoylated esters remained strongly adsorbed on the column. This was eluted with acetone and identified as a monoazoate ester in case of dihydric sex hormones, and as a mixture of di- and mono-azoyl esters in case of trihydric sex hormones. Table 1 lists the derivatives prepared with their analytical data.

Hydrolysis of the Azoates and Recovery of the Free Hormones

A benzene solution of the azoyl ester is added to an equivalent amount of 2% NaOH in EtOH. After keeping for 12 hr at room temperature in the dark, the mixture was washed with water, the aqueous layer acidified with 25% H_2SO_4 and re-extracted with ether. The ethereal layer was washed with water, sodium bicarbonate and again with water. The ether was evaporated and the residue crystallized to give the pure hormone in quantitative yield.

Separation of Azoyl Esters of Sex Hormones by Adsorption Chromatography

A solution of 1 mg of α-etradiol diazoate and 1 mg of testosterone azoate in 2 ml of benzene–light petroleum ether (1:1) was passed through an Al_2O_3 column without pressure. All the colouring matter was adsorbed as a deep-red zone, about 2 mm thick at the top of the adsorbant. Development with 0·25% ethanol in petroleum ether caused it to separate into two zones, and the lower zone of diazoate was easily eluted. Elution of the upper zone of monoazoate was accomplished with 1% alcohol in petroleum ether. Other pairs of azoyl esters of estriol and estradiol, pregnane diol and estriol were completely separated using this method.

Separation of Monoazoates and Triazoates by Reversed Phase Partition Chromatography

A mixture of nitromethane and nonane (4:1 v/v) was shaken thoroughly and then left to separate into two layers. A tube (20 cm long, 2 cm in dia.) was half-filled with the nitromethane layer. A 1:1 w/w mixture (10 g) of the other layer and kieselguhr impregnated with dimethyloxosilane was made into a slurry with the nitromethane layer (20 ml) and added to the column. When the column was drained, the mixture of esters (2–3 mg) dissolved in the nitromethane layer was added. The chromatogram was then developed with the nitromethane layer under reduced pressure. The different azoates were eluted separately and collected in the usual way. A mixture of estrone and estriol azoyl esters were completely separated whereas a mixture of estrone and estradiol esters was only partially separated.

Isolation of the Estrogen of Moghat

Powdered moghat roots (10 kg) were extracted with petroleum ether (b.p. 60–70°, 30 l.) in a soxhlet for 30 hr. The petroleum ether extract was concentrated to give a crystalline substance (3·5 g) and an orange fluorescent oil (12 g).

When the oil was dissolved in ethanol and treated with Brown's reagent,[8] it gave the same max. (at 420–425 nm) as that given by α-estradiol dipropionate, indicating the presence of an esterified hormone. The oil was subjected to acid hydrolysis[3] in 3 N methanolic HCl (70 ml); the hydrolysate was extracted with ether, the ether extract washed with water, then extracted with 2 N NaOH. The aqueous layer was acidified with conc. HCl and re-extracted with ether. The ethereal layer was washed with water, 9% $NaHCO_3$, again with water, and then dried to a constant weight (0·3 g). The ether extract in ethanol showed λ_{max} at 280–282 nm and when treated with Brown's reagent[3] the max. was shifted to 515–520 nm, (authentic estrone behaved similarly). The ether extract (0·3 g) was fractionated with light petroleum in the cold (b.p. 30–50°) into soluble (0·2 g) and insoluble (100 mg) fractions. The insoluble fraction was dried, mixed with dry benzene (10 ml) then treated with *p,p'*-nitrophenylazobenzoyl chloride (0·1 g) and pyridine (10 ml) and kept in the dark at room temperature for 2 weeks. The solution was concentrated, pyridine and free acid were removed by filtration, the filtrate washed with 20% H_2SO_4 and again filtered. The filtrate was washed with water, 1 N NaOH, and water and then placed on alumina column. Of the two coloured bands produced, the lower red band was eluted with benzene, the eluate evaporated, the residue washed with ethanol in the cold and dried, giving a red crystalline material (20 mg). This derivative was subjected to partition chromatography on kieselguhr as described above. Elution of the main lower band with the nitromethane layer and recrystallization of the recovered material from acetone–benzene (1:1 v/v) an orange red crystalline substance (10 mg) was obtained; m.p. and mixed m.p. with authentic estrone azoyl ester was 249°. The i.r. spectrum was also identical with that of estrone azoyl ester. On alkaline hydrolysis, it gave a material having estrogenic activity; its m.p. and mixed m.p. with estrone was 259°, and it was identical to estrone in its i.r. spectrum.

Isolation of Cholesterol from Moghat Roots

The sun-dried roots were powdered and extracted in a soxhlet with petroleum ether (60–70°) for 30 hr. The extract was evaporated to a very small volume and kept at room temperature overnight. A crystalline substance (3·5 g) which separated out was recrystallized from ethanol giving white plate-like crystals. When warmed with acetone–ethanol (1:1 v/v) and immediately cooled, crystals (2·5 g) with m.p. 150–160° were obtained. It seemed to be a mixture of sitosterols from i.r. and mass spectral analysis. After removing the first crop of crystals, a second crop (2 mg) separated out, having m.p. and mixed m.p. (149°) with an authentic specimen of cholesterol. The i.r. spectrum was also similar to that of cholesterol. Final proof was obtained by transformation into the *p,p'*-nitrophenylazobenzoyl derivative, which was identical in m.p. (178°) and i.r. spectrum to the authentic derivative.

Isolation of Estrone from Date Palm Pollen

Air-dried pollen grains (735 g) of Egyptian date palm, variety Samani, were kept under peroxide-free ether (4 l.) in the dark at room temperature for 2 weeks. After filtration, the pollen grains were re-extracted with methyl alcohol (4 l.) and a third extraction with acetone (4 l.) was carried out under similar conditions. These extracts, when worked up as for the moghat extracts (see above) eventually yielded 14 mg of the *p,p'*-nitrophenylazobenzoyl ester of estrone, which was identified as before. This yielded, on hydrolysis, estrone identical in every way with authentic material.