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SHORT COMMUNICATION

IDENTIFICATION OF ESTRONE IN POMEGRANATE SEEDS

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Abstract—Estrone was isolated from pomegranate seeds and its identity was confirmed by thin-layer chromatography in three solvent systems and by four color reactions. Three derivatives were prepared and had the same chromatographic characteristics in three solvent systems as the corresponding derivatives from authentic estrone. The biological potency of this material was also comparable to that of estrone. Pomegranate seeds are the richest plant source of steroidal estrogens yet found.

INTRODUCTION

RECENTLY Jacobsohn *et al.*,[1] in a paper entitled "The Absence of Steroid Estrogens in Plants", reported that they were unable to repeat Butenandt's isolation of estrone[2] from palm kernel press cake. In view of the questionable nature of the only other reports[3–5] on the subject, they concluded that more direct evidence was needed to demonstrate the presence of steroidal estrogens in plants. To clarify this question, we have reinvestigated the reported estrogenic activity of two plants, date palm[4,5] and pomegranate.[6] In the first case we found that estrone is present in both the seeds[7,8] and pollen[8] of the date palm, *Phoenix dactylifera* L. We have now isolated estrone from pomegranate seeds, thus providing further evidence for the occurrence of steroidal estrogens in plants.

RESULTS

Thin-layer chromatography (TLC) of an acidic, ketonic fraction isolated from pomegranate seeds indicated that it contained a minor component corresponding to estrone. This material (Compound A) was isolated by preparative TLC in Systems 1 and 2, Table 1, yielding 1·5 mg. Compound A had the same mobility as estrone in three solvent systems (see Table 1).

* A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Work conducted under a co-operative agreement with the California Institute of Technology.

[1] G. M. JACOBSOHN, M. J. FREY and R. B. HOCHBERG, *Steroids* **6**, 93 (1965).
[2] A. BUTENANDT and H. JACOBI, *Z. Physiol. Chem.* **218**, 104 (1933).
[3] B. SKARZYNSKI, *Nature* **131**, 766 (1933).
[4] M. S. EL RIDI and M. A. WAFA, *J. Royal Egypt. Med. Assoc.* **30**, 124 (1947).
[5] A. HASSAN and M. A. WAFA, *Nature* **159**, 409 (1947).
[6] A. SHARAF and S. A. R. NIGM, *J. Endocrinol.* **29**, 91 (1964).
[7] E. HEFTMANN, S.-T. KO and R. D. BENNETT, *Naturwissenschaften* **52**, 431 (1965).
[8] R. D. BENNETT, S.-T. KO and E. HEFTMANN, *Phytochem.* **5**, 231 (1966).

TABLE 1. COMPARISON OF R_f VALUES ON SILICA GEL G PLATES OF COMPOUND A AND DERIVATIVES WITH AUTHENTIC ESTRONE AND CORRESPONDING DERIVATIVES

Compound	Solvent system* 1	2	3
Compound A	0·61	0·46	0·54
Estrone	0·62	0 46	0·55
Compound A acetate	0·63	0·72	0·80
Estrone acetate	0·63	0·71	0·80
Compound B	0·50	0·29	0·42
Estradiol	0·50	0·29	0·42
Compound B acetate	0·62	0·74	0·83
Estradiol diacetate	0·62	0·74	0 83

* System 1, cyclohexane–ethyl acetate (1:1); System 2, dichloromethane–acetone (47:3); System 3, dichloromethane–methanol (24:1).

Furthermore, Compound A and estrone gave identical colors on TLC plates with the following spray reagents: Fast Black Salt K, which couples with phenolic compounds;[9 10] Zimmerman reagent, which gives a characteristic color with 17-ketosteroids;[10] Liebermann–Burchard reagent, a nonspecific steroid test;[10] and 50% sulfuric acid, which gives typical color and fluorescence responses with various steroids.[11]

A portion of Compound A was treated with lithium aluminum hydride in ether, and the reduction product thus obtained (Compound B) had chromatographic mobilities in three solvent systems corresponding to estradiol, the reduction product of estrone (Table 1). The

TABLE 2. COMPARISON OF ESTROGENIC ACTIVITIES OF COMPOUND A AND ESTRONE*

Treatment	Total dose (μg)	Body weight (g)	Uterus weight (mg)
Estrone	0·25	16±1†	35±2†
Estrone	0·125	17±1	17±1
Compound A	0·25	16±1	30±2
Compound A	0·125	16±1	15±1
Control‡	—	14±1	8±1

* Samples were dissolved in sesame oil and injected subcutaneously into ovariectomized female NIH strain mice (10 per group) daily for 5 days, with autopsy on day 6.
† Standard error.
‡ Sesame oil only.

[9] E. HEFTMANN, *Science* **111**, 571 (1950).
[10] B. P. LISBOA and E. DICZFALUSY, *Acta Endocrinol.* **43**, 545 (1963).
[11] E. HEFTMANN, S.-T. KO and R. D. BENNETT, *J. Chromatog.* **21**, 490 (1966).

color and fluorescence of Compound B on spraying with 50% sulfuric acid were also identical to those of estradiol.

For further identification, the acetates of Compound A and Compound B were prepared by treatment with pyridine–acetic anhydride (1:1). These derivatives had chromatographic mobilities corresponding to estrone acetate and estradiol diacetate, respectively, in three solvent systems (Table 1).

Finally, a bioassay of Compound A indicated a biological potency comparable to that of pure estrone (Table 2).

DISCUSSION

Estrogens have previously been isolated from plant sources in the following estimated yields (mg/kg): estriol from willow flowers, 0·11;[3] estrone from palm kernel press cake, 0·36;[2] estrone from date palm seeds, 0·40;[7, 8] and estrone from date palm pollen, 3·3.[8] Since pomegranate seeds contain 17 mg of estrone per kg, they represent the richest plant source of estrogens yet found.

EXPERIMENTAL

Pomegranate seeds (*Punica granatum*, var. *nana*) were obtained from Mistletoe Sales, Santa Barbara, California.* Thin-layer chromatographic techniques were as described previously,[12] except that silver membrane filters† (0·45 μ pore diameter) were used for the elution of zones. All chromatograms were run on Silica Gel G plates purchased from Analtech, Inc., Wilmington, Del.

A ketonic fraction (25 mg) was obtained from 88 g of pomegranate seeds by the procedure previously described.[8]

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* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

† Selas Flotronics, Spring House, Penn.

[12] R. D. BENNETT and E. HEFTMANN, *Arch. Biochem. Biophys.* **112**, 616 (1965).