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ESTRONE IN *OLEA EUROPAEA* KERNEL

EL S. AMIN and A. R. BASSIOUNY
Biochemistry Department, Faculty of Science, Alexandria University, Egypt

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Key Word Index—*Olea europaea*; Oleaceae; olive; kernel; steroid; estrone.

Abstract—The estrogen of the olive kernel and of commercial oils has been investigated. A crystalline estrone has been isolated from olive kernel. An estrogen ester has been assayed in olive oil and a free estrone in corn oil.

INTRODUCTION

The olive tree (*Olea europaea*) is widely grown in Mediterranean climates and yields an edible fruit of value. It has been extensively studied chemically but no research had been carried out on its hormonal content.

In view of the universal use of olive oil, the present authors set out to see if any animal hormone was present in the kernel of olive and olive oil, and in other commercial oils.

RESULTS

A simple new method of isolation of estrone from natural oils, designed by El S. Amin, was used. It involves refluxing the oil directly with Girard's reagent T for 2 hr in EtOH and HOAc. Estrone was estimated physically by UV assay and colorimetrically using the Zimmermann method [1]. The isolated compound was identified by mp and mmp, UV, NMR and IR spectroscopy.

It was thus possible to separate the estrogenically active principle from the olive kernel in a pure state and to show it to be estrone. The 2 methods of assay gave approximately the same yield (8.4 mg estrone/240 g for UV assay and 8.1 mg/240 g for the Zimmermann method).

It was found also by UV assay that corn oil contains 4 μg estrone/100 ml oil, and olive oil contains 9 μg estrone ester/100 ml oil. Sesame oil, coconut oil, lettuce oil, lineseed oil, palm oil, arachis oil, cottonseed oil and soya bean oil were all found to be free of estrogens.

EXPERIMENTAL

Analysis of the kernel. 240 g finely ground kernel were treated according to Amin *et al.* [2] with 1 l. Me_2CO for 1 week, filtered, then with 1 l. MeOH for 1 week, filtered, then with 1 l. Et_2O for 2 weeks. The filtrates were separately concd at 50–60° at normal pressure to constant wt, yielding 12.1425, 46.2215 and 20.1241 g oil, respectively. The Me_2CO and Et_2O extracts showed a band with a UV max at 230–232 nm corresponding to the extrogen ester, while the MeOH extract gave 2 bands with max at 280 and 230 nm corresponding to free and estrogen ester, respectively. Aliquots of each extract were treated with Brown's colour reagent [3]. The Me_2CO and Et_2O extracts showed an absorption maximum at 420 nm corresponding to the estrogen ester, while the MeOH extract showed a peak at 516 nm, corresponding to the free estrone, and a peak at 420 nm, corresponding to the estrogen ester. Each extract was then refluxed for 2 hr with Girard's reagent T in EtOH/HOAc. The cooled soln was then diluted with 0.9 N $NaHCO_3$ soln to pH 6.5 and exhaustively extracted with Et_2O to remove non-ketonic compounds. The hydrazone formed was then acidified with 0.5 N HCl to pH 2 and left for 1 hr at room temp, then the liberated hormone was extracted with Et_2O and subjected to UV. Each extract showed λ_{max} at 280 nm, confirming the identity of isolated estrone. TLC of isolated estrone of each extract showed a single spot which migrated the same distance as pure estrone. This new method gave estrone as colourless crystals, mp and mmp 259° whose IR and NMR spectra were very similar to those of authentic estrone. Aliquots of isolated estrone of each extract were subjected to the Brown colour test. The absorption maxima were shifted to 516 nm, indicating free estrone. Also the isolated estrone was treated with Zimmermann reagent to give a colour with max at 520 nm, indicating free estrone, corresponding to 8.1 mg/240 g.

Investigation of estrogen in commerical oils. These oils were investigated by TLC, UV spectroscopy and saponification with 0.5 N alcoholic KOH. Olive oil was found only to contain estrogen ester, and gave a band with a max at 232 nm, before saponification, and at 280 nm, after saponification. It was examined by TLC. Aliquots of each oil were treated with Brown colour reagent. Olive oil only showed an absorption max at 420 nm, corresponding to the estrogen ester, while corn oil only showed a peak at 516 nm, corresponding to the free estrone, before and after saponification. TLC confirmed this result. The amount of hormone was calculated, after elution of saponified fractions from TLC of olive oil and corn oil, from a calibration curve prepared with pure estrone and was found to correspond to 9 μg estrone ester/100 ml olive oil, and 4 μg estrone/100 ml corn oil.

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