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SHORT COMMUNICATION

# STEROID OESTROGENS IN PLANTS: RE-ESTIMATION OF OESTRONE IN POMEGRANATE SEEDS

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## INTRODUCTION

OESTRONE has been reported in several plants.[1-5] In particular pomegranate (*Punica granatum*, var. *nana*) seeds are said to have an oestrone content of 17 mg/kg. We have reinvestigated this claim using the extremely sensitive technique of competitive protein binding to determine the location and concentration of oestrone and oestradiol in the pomegranate.

## RESULTS

The extracts of pomegranate seeds were purified by preparative TLC and areas of similar polarity to oestrone and oestradiol were isolated. Several solvent systems were used in sequence and each stage was monitored by analytical TLC. The plates were sprayed with either a solution of Brady's reagent (2,4-dinitrophenylhydrazine) or 50% (v/v) sulphuric acid. The colours observed were compared with oestrone and oestradiol markers.

With Brady's reagent, a ketonic material was observed with $R_f$ similar to that of oestrone (solvent A, see Experimental). However, the plant extract reacted very rapidly (5 sec) at room remperature, whereas oestrone standards had to be heated at 100° for 5 min for a colour to develop. On extracting this band and re-running in a different system (solvent B), most of the Brady-positive material moved to a different $R_f$ when compared with oestrone.

When the plates (solvent A) were sprayed with 50% sulphuric acid the oestrone and oestradiol markers showed a spot of salmon pink colour which was a fluorescent green under UV. The extracts showed a spot of violet–blue colour which was faintly fluorescent but at a lower $R_f$ (0·40) than oestrone (0·44–0·46) in solvent C.

The regions corresponding to oestrone and oestradiol were eluted from unsprayed chromatograms and the extracts assayed by the competitive protein binding technique of Exley and Corker.[6] The results are shown in Table 1, where the figures for the whole pomegranate plant are also compared.

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TABLE 1. CONCENTRATIONS OF OESTRADIOL AND OESTRONE IN POMEGRANATE TISSUES DETERMINED BY COMPETITIVE PROTEIN BINDING

| Tissue | Concentration of oestrone ($\mu$g/kg) | Concentration of 17$\beta$-oestradiol |
|---|---|---|
| Seed | 4·0 | 0 |
| Root | 4·5 | 0 |
| Aerial parts | 8·7 | 0 |
| Flower | 2·5 | 0 |

10 g of tissue were extracted as described by Bennett *et al.*[4] and purified by TLC in at least two systems. Regions corresponding to oestrone and oestradiol were assayed. The method was checked for interference by plant extract by running known quantities (50 × 100 pg) of oestrone with the extracts The samples were found to assay within 2% of the controls (no extract added)

## DISCUSSION

We are unable to find $\beta$-oestradiol in pomegranate by the methods which we have used. Also, although we can detect oestrone by sensitive competitive protein binding techniques, the levels we have observed differ from those previously reported[5] by a factor of 4000. Since we obtained our seeds from the same source as Heftmann *et al.*[5] we presume that either oestrone levels vary considerably from year to year, or that our separation methods, particularly in conjunction with the very sensitive and specific competitive protein binding method, eliminate materials which might have been mistaken for oestrone by earlier techniques.

## EXPERIMENTAL

Pomegranate seeds (*Punica granatum* var. *nana*) were obtained from Mistletoe Sales, Santa Barbara, California. Seedlings were grown under the supervision of Mr. J K. Hulme of the University of Liverpool Botanical Gardens, Ness. Miniature trees (height 30–60 cm) were harvested: aerial parts (flowers separate) and root were extracted separately. All tissues were macerated in a Waring Blendor and hydrolysed either by acid saponification as described by Bennett *et al.*[4] or by alkaline saponification by heating under reflux with 6% ethanolic KOH for 3 hr. The hydrolysates were exhaustively extracted with acetone and methanol.

The combined extracts were then concentrated by rotary film evaporation and made alkaline with KOH and extracted with ether (three times). The ether was discarded The extracted aqueous phase was neutralized with HCl and extracted with EtOAc (three times) The combined extracts were combined and dried over sodium sulphate. The extracts were purified by preparative TLC and were separated into oestrone and oestradiol fractions. Different (usually two) solvent systems were used for sequential chromatography. Preparative TLC was performed on silica gel plates (silica gel H [Woelm]) using one of the solvent systems indicated below.

The tissues were also examined by reversing the order of hydrolysis and extraction The first extraction being with aqueous acetone, then acetone, then acetone–methanol The combined extracts were concentrated and hydrolysed as above. Steroids were recovered from silica (following chromatography) using ethyl acetate Solvents used were (a) EtOAc–hexane (1 1); (b) $CHCl_3$, (c) $CH_2Cl_2$–acetone (47·3); (d) EtOAc–pentane (2·3).