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THE ISOLATION OF ESTRONE FROM APPLE SEEDS

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Abstract—The presence of estrone in apple seeds was demonstrated by TLC, gas chromatography, and the formation of the acetate derivative. The analyses per 100 g of Red Delicious (*Malus sylvestris*) apple seeds revealed an average of 13·0 μg of estrone, and for MacIntosh (*Malus sylvestris*) seeds, 10·1 μg of estrone.

INTRODUCTION

EVIDENCE for the presence of steroidal estrogens in plants has appeared several times in the literature. Skarzynski[1] reported finding estriol in female willow flowers in 1933, and in the same year Butenandt[2] isolated estrone from palm kernels. In 1947 Wafa *et al.*[3,4] isolated what they believed to be estrone from date palm pollen. More recently Bennett *et al.*[5] isolated estrone from date palm pollen and seeds. In 1966 Heftmann *et al.*[6] obtained estrone from pomegranate seeds and showed that the plant estrogen had a biological potency comparable to that of standard estrone by the mouse uterine bioassay. Also a radioactive phenolic material originating from mevalonic acid-2-^{14}C has been found in *H. heterophyllus* plants only in the flowering state.[7] We decided to further study plant steroids in apple seeds.

RESULTS

A portion of the Red Delicious apple seed ketonic extract on TLC in cyclohexane:ethyl acetate (1:1) gave a spot corresponding in mobility and color to co-chromatographed standard estrone after spraying with 50% H_2SO_4 in ethanol. It also gave a characteristic blue spot with the correct R_f after spraying with 10% $NaHCO_3$ followed by Folin's reagent. Also when run in CH_2Cl_2:acetone (7:3) the extract yielded a blue spot with the Folin's test with the same R_f as standard estrone. The ketonic extract applied to the gas chromatograph (column 205°) after TLC gave five distinct peaks, the third peak (*Rt* 8·2 min) had the same retention time as standard estrone. On a new column with a temperature of 235° the retention time for the estrone standard and that of the third extract peak was 3·6 min. An internal standard of estrone added to the extract gave one single large peak at the same retention time. Calculations from the peak areas of four gas chromatographic runs (range 9·6–15·4) showed that Red Delicious apple seeds contained 13·0 μg of estrone/100 g. The extract of the MacIntosh

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apple seeds without TLC purification was applied to the column and revealed five distinct peaks similar to those from the Red Delicious seeds, again the third having the same average retention time (27·0 min) as standard estrone. The acetate derivative of the extract estrone had a retention time of 37·9 min, the same as standard estrone acetate. Calculations from the peak areas of three runs (range 9·8–11·3) showed that MacIntosh apple seeds contained 10·1 μg of estrone/100 g.

DISCUSSION

The specific extraction procedure for the isolation of plant ketonic phenols employed successfully by Bennett[5] in the isolation of estrone, the identification of estrone by spraying after TLC, the identification and quantitation of estrone from two types of apple seeds on several different gas chromatographic columns under a variety of conditions, and the gas chromatographic identification of the acetate derivative of estrone, together with results previously reported,[1–7] leave little doubt that steroidal estrogens do occur in plants. Also in support of this finding is the isolation of cholesterol (385 μg/100 g) and progesterone (50 μg/100 g) from Red Delicious apple seeds,[8] and the identification of estrone from the seeds of Sorrell Broadleaved *Rumex acetosa*.[9] However, the physiological significance of estrone in apple seeds remains to be studied.

EXPERIMENTAL

Methods

Silica gel (Camag, DF-5) with fluorescent indicator was used for the preparation of 20 cm^2 TLC plates, with 0·3 mm thick adsorbent layers. Samples and standards were applied as spots and following development were transferred from the plate to a centrifuge tube, eluted with $CHCl_3$–methanol (1:1) and centrifuged. The gel was washed three times and the supernatant was evaporated to dryness under N_2 before application to the gas chromatograph. A Barber-Colman Model 5000 series gas chromatograph with a flame ionization detector and a U-shaped 6 ft × $\frac{1}{8}$ in. glass column was used. The estrone extract from the Red Delicious seeds was analyzed on a 1% SE-30, Anakrom ABS, 100/110 mesh column, and that from the MacIntosh seeds on a 3% SE-30 Chromosorb W, AW-DMCS, 80/100 mesh column. N_2 was maintained at 20 lb/in^2. Quantitative results and retention times were calculated by comparing extract peaks with that of a standard.

Materials

260 g of seeds were obtained from fresh Red Delicious apples, and 250 g from MacIntosh apples through the generosity of Dr. Herbert V. Marsh, Jr., Department of Plant and Soil Science, University of Massachusetts.

Isolation of Esterone from Seeds

The ketonic fraction was isolated according to the method of Bennett *et al.*[5] which included hydrolysis, Soxhlet extraction of lipids, column chromatography on neutral alumina of acidic material, Girard's separation, and preparative TLC. The residue from the ketonic fraction and standard estrone were applied to opposite corners of the plate and the chromatogram was developed in cyclohexane:ethyl acetate (1:1). One portion of the apple seed extract was located by spraying with 50% H_2SO_4 and heating, another was eluted from the plate and the remainder of the extract (not run on TLC) was acetylated overnight at room temperature with pyridine:acetic anhydride (1:1). The eluted and acetylated samples were analyzed by gas chromatography for estrone and estrone acetate.

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