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ESTROGENS IN DEVELOPING BEAN (*PHASEOLUS VULGARIS*) PLANTS

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Abstract—Estrogens first appear at the period of flower bud formation and then reach maxima at the time of flower bud development and of pod formation. Four estrogen-like substances were found in the extracts from the flowering beans.

INTRODUCTION

IN ANIMALS, estrogens are synthesized by degradation of cholesterol to 17-ketosteroids. The oxidation of the C-19 methyl group of androstenedione and aromatization of ring A result in the formation of estrogens.[1] An analogous degradation of cholesterol to androstenedione is carried out by microorganisms.[2] There are also data showing that steroid biosynthesis in higher plants in many ways resembles that in animals[3] which, in connection with proved occurrence of endogenous steroid hormones such as estriol,[4] estrone,[5–9] androstanetriol,[10] or ecdysterone[11] in plants, suggests that steroidal hormones occur in all living organisms.

It has been known that steroid hormones affect the growth and development of plants[12–17] and that the presence of estrogens increases the content of both gibberellins and auxins in plant tissues.[18–21] The purpose of this work is to examine quantitative changes in estrogen content during the course of bean plant development.

RESULTS AND DISCUSSION

Quantitative investigations on the content of estrogen-like substances in the developing bean plants showed that these compounds appear as the flower buds emerge, reaching

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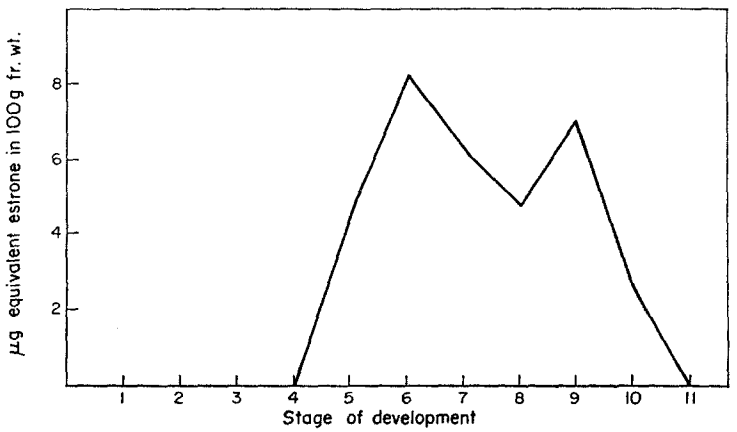

FIG. 1. ESTROGEN CONTENT IN THE DEVELOPMENT OF BEAN PLANTS.

Development stages: 1, dry seeds; 2, seeds with pierced coats; 3, seedlings in the period of rapid growth; 4, the formation of the second node of the stem; 5, the formation of the flower buds; 6, the enlargement of the flower buds; 7, full size flower buds; 8, young flowers (newly opened flower buds); 9, the falling of the petals (the pod about 15 mm long); 10, pods of about 60 mm long; 11, the period of ripe seeds.

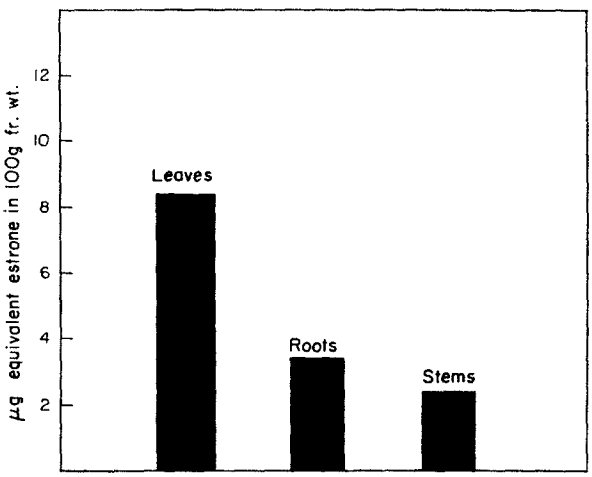

FIG. 2. ESTROGEN CONTENT IN DIFFERENT BEAN ORGANS.

afterwards two maxima in the period of flower bud development and of pod formation (Fig. 1). Estrogens were absent from ripe seeds and young bean seedlings.

Since it is difficult to establish the exact site of the biosynthesis of estrogen-like compounds, whole plants were taken for the investigations. In order to find out their distribution, the content of estrogens were determined separately in the roots, stems and leaves. The investigations were carried out on flowering plants (stage 7). The results show that comparatively the greatest quantity of estrogen-like substances are in the leaves, although their presence was also established in roots and stems (Fig. 2).

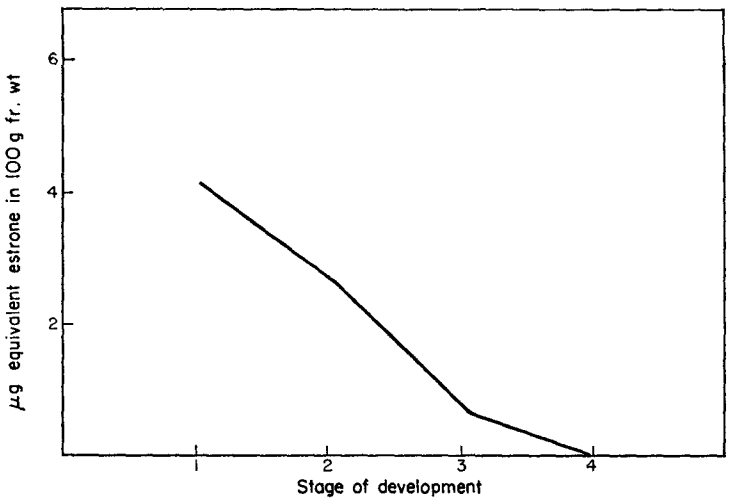

FIG. 3. ESTROGEN CONTENT IN THE DEVELOPMENT OF BEAN PODS.
Development stages: 1, pods about 45 mm long; 2, about 65 mm long; 3, about 95 mm long; 4, pods with completely ripe seeds.

The presence of estrogens was also established in the developing pods. The young pods with unripe seeds were characterized by the great content of estrogens, while in the period of seed-ripening the estrogens content considerably decreased (Fig. 3).

The properties of estrogen-like substances, extracted from two kilograms of flowering bean plants, were then compared with standard compounds by means of their mobilities in different solvents and their fluorescent colours. The results show the presence of four substances in the bean extracts with similar fluorescence to known estrogens. Factor D and estrone are similar in R_f and colour (Fig. 4, Table 1).

The inhibitor *Tris*-(2-diethylaminoethyl)-phosphate trihydrochloride (SK & F 7997-A_3) which is known to block the synthetic pathway between mevalonic acid and cholesterol, particularly at the conversion of lanosterol into zymosterol in animal systems, also suppresses floral induction in *Xanthium*, *Pharbitis* and *Lolium*.[22,24] Also, Leshem[15] has demonstrated that flower development of broccoli cuttings is promoted by various concentrations of steroids and inhibited by the steroid biosynthesis inhibitor SK & F 7997-A_3. Löve and Löve[25] found that steroidal hormones could produce male or female flowers on *Melandrium dioecum* if either androgens or estrogens are applied to the stems before flowering. It was also shown that steroid hormones have flower promoting effects on plants[12,13,15,17] and it has been proposed that the flower hormone may have a steroid[23,26] or an isoprenoid[23] structure.

The above results show the participation of steroidal substances in the floral induction and sex-expression in plants; however, their mechanism of action has not been explained

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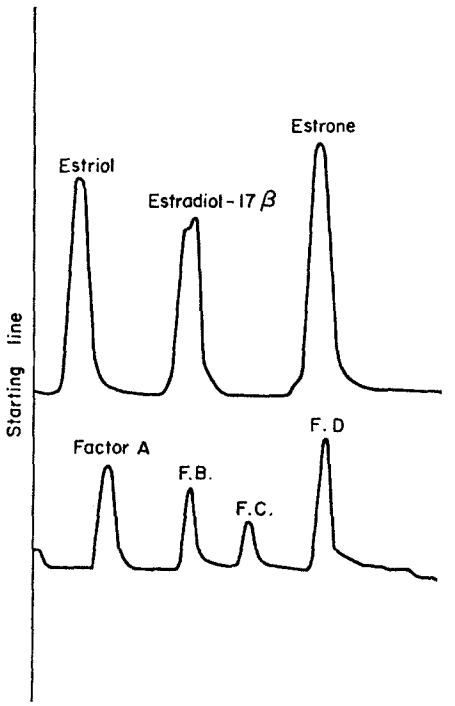

FIG. 4. DENSITOMETRY OF A THIN LAYER CHROMATOGRAM SHOWING THE SEPARATION OF STANDARD ESTROGENS AND ESTROGEN-LIKE COMPOUNDS FROM BEAN PLANTS.

Developing system: light petroleum–$CHCl_3$–MeOH (40:10:3). Spray reagent: 70% H_2SO_4.

TABLE 1. R_fS, COLOURS AND FLUORESCENCES OF ESTROGENS AND ESTROGEN-LIKE COMPOUNDS FROM BEAN PLANTS

Substances	R_f (×100)				Colour† in visible light	Fluorescence‡
	1*	2	3	4		
Estriol	8	8	21	71	violet	yellow
Estradiol-17β	25	43	42	85	yellow-orange	yellow-green
Estrone	45	55	66	96	yellow-orange	yellow-green
Factor A	13	11	16	68	yellow-orange	yellow-green
Factor B	23	29	28	78	violet	dark yellow
Factor C	31	36	39	79	pink	pink
Factor D	43	53	64	97	yellow-orange	yellow-green

* Solvents: 1. light petroleum–$CHCl_3$–MeOH (40:10:3), 2. washed $CHCl_3$–AcOH (10:1), 3. 10% MeOH in benzene, 4. Acetone–CH_2Cl_2 (3:7).

† TLC, 70% H_2SO_4.

‡ TLC, 70% H_2SO_4, u.v.-light.

hitherto. It is possible that on the molecular level the action of steroids is likely to be the same on both plant and animal cells.[3]

The present paper reveals that there is increased estrogen biosynthesis in the period of plant flowering. These results confirm those of Bennett *et al.*[27] who treating *Haplopappus heterophyllus* plants with mevalonic acid-2-^{14}C and found that the radioactive phenolic steroids were found only in the flowering plants. These data, together with the known effects of inhibitors of steroid biosynthesis on flowering, lead to the hypothesis that, in order for the flowering to take place, a suitable level of steroidal hormones is necessary. The participation of steroidal hormones in the regulation of flowering is also indicated by their relationship to such important flowering regulators as gibberellins.[18,19] It seems possible, then, that estrogens constitute one of the components of the hypothetical flowering hormone, florigen.[28]

EXPERIMENTAL

Plant Material

The bean seeds (*Phaseolus vulgaris* L. var. 'Saxa') were germinated in sterile sawdust. After 7 days the seedlings were selected, planted in soil and cultivated in long day conditions (16 hr, daylight fluorescent tubes, intensity about 4500 lx) at 24–26°. Investigations were carried out on the whole bean plants deprived of the pods, and separately on the pods only.

Analytic Procedures

Estrogens were determined in 100 g samples. The chilled material was homogenized with hot methanol and the homogenate was filtered. The filter cake was extracted in a Soxhlet with benzene–MeOH (3:1) for 6 hr. This extract and the filtrate previously obtained were combined and evaporated to dryness. The residue was refluxed with a mixture of benzene–HCl–H_2O (1:1:2) for 3 hr. The benzene layer was separated and the aqueous layer was extracted with CH_2Cl_2. The benzene and CH_2Cl_2 fraction were combined and evaporated. The residue was taken up in benzene–*n*-BuOH (3:1) and extracted with 2% $NaHCO_3$. $NaHCO_3$ extract was discarded and benzene fraction was extracted with 1N NaOH. This extract was immediately acidified with HCl to pH 3. The mixture was then extracted with CH_2Cl_2 and the extracts were combined, filtered and evaporated to dryness. The residue was dissolved in a small volume of acetone–MeOH (1:1) and the whole extract was streaked on aluminium oxide G (type E) TLC plates and developed to 10 cm long strips in light petroleum–$CHCl_3$–MeOH (40:10:3). The estrogen zone (R_f 0·05–0·45) was removed and eluted with acetone–MeOH (1:1) and then rechromatographed in CH_2Cl_2–acetone (7:3). The zone corresponding to estrogens (R_f 0·65–0·98) was removed, eluted and the eluate evaporated to dryness. The residue was redissolved in EtOH and the estrogens were determined by the Kober colour reaction.[22] The extinction was measured in a Specol Spectrocolorimeter at 474, 515 and 556 nm, against similarly treated reagents blanks in 10 mm glass cells. The readings were corrected for unspecific background colour by applying $E_{corr.} = 2E_{515} - (E_{474} + E_{556})$.[22]

The estrogen content was expressed as μg equivalent of estrone in 100 g fr. and dry wt. The results are recorded graphically only as regards fresh weight. Data referring to dry weight were essentially identical.

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