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Effect of estradiol-17β, estrone and estriol on the endogenous auxins content in plants*

JAN KOPCEWICZ

Although much information has been gathered on the biochemistry of steroids in animals, the problems of biosynthesis, metabolism and role of plant steroids remain quite unexplored (Heftmann and Mosettig 1960; Heftmann 1963).

There are, however, data showing that steroid metabolism in plants resembles in many ways that in animals (Heftmann and Mosettig 1960; Bennett et al. 1963; Johnson et al. 1963; Hügel et al. 1964; Johnson et al. 1964; Bennett and Heftmann 1966), and this in connection with the proved occurrence of endogenous steroid hormones of estrone (Butenadt and Jacobi 1933; Skarżyński 1933; ElRidi and Wafa 1947; Hassan and Wafa 1947; Heftmann et al. 1965; Bennett et al. 1966; Heftmenn et al. 1966), ecdysterone (Heftmann et al. 1968) or androstanetriol (Zalkow et al. 1964) types, suggests the important role of these substances in plants.

By now it is already known that steroid hormones have a flower promoting effect on plants (Chouard 1937; Czygan 1962), and it has been suggested that the flower hormone may have a steroid (Hendricks 1960; Bonner et al. 1963) or an isoprenoid (Bonner et al. 1963) structure. It was also pointed out that the inhibitor Tris-(2-diethyl-aminoethyl)-phosphate-trihydrochloride (SK&F 7997-A_3), which blocks the synthetic pathway between mevalonic acid and cholesterol, particularly in the conversion of lanosterol into zymosterol in animal systems (Holmes and Di Tullio 1962) suppresses floral induction in *Xanthium*, *Pharbitis* and *Lolium* (Bonner et al. 1963). Also Leshem (1967) has demonstrated that flower development and rooting of broccoli curd cuttings were promoted by various concentrations of steroids and arrested by the steroid biosynthesis inhibitor SK&F 7997-A_3. Löve and Löve (1945) have found that steroidal hormones could produce male or female flowers on *Melandrium dioecum* at will when either androgens or estrogens were applied to the stems before flowering. Löve and Löve (1945) claimed also that steroid hormones, especially estrogens, stimulate germination of seeds and cell division in the roots of *Melandrium dioecum*. It was also shown that cortisone and hydrocortisone stimulate the growth of *Ervum lens* (Ratsimamanga and Diot 1959) and other plants (Donnet et al. 1960).

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All these data pointing to the important physiological role of steroid hormones in the processes of plant growth and development, do not explain, however, the mechanism of their action.

In previous papers (Kopcewicz 1969 a, b, c) it has been shown that some steroids, namely estrone and estradiol-17β enhance the growth of the dwarf pea and that the stimulation of pea seedlings growth by estrone is connected with a simultaneous increase of endogenous gibberellins content in the plants. The positive influence of estrogens on the flowering of plants growing under noninductive conditions (Kopcewicz 1970 a) has also been demonstrated. This paper is a continuation of earlier studies. Their purpose was to investigate the mechanism of action of steroidal hormones in plants. A preliminary report of this work has appeared elsewhere (Kopcewicz 1970 b).

MATERIAL AND METHODS

Pea seeds (*Pisum sativum* var. Cud Kelwedonu) were germinated and cultivated in sterile sawdust under red light at 20—22°C. After six days seedlings were selected and treated with 10 μl aliquots of estradiol-17β, estrone or estriol correnspondinig to 0.1 μg of hormone per plant. The estrogens were dissolved in ethanol — 0.05% Tween 80 4:1 v/v. Controls were treated with a 10 μl drop of ethanol—Tween 80. The investigations were carried out on pea seedlings deprived of cotyledons just before treatment (Table 1, Figs. 1, 2 — stage 0) and 24, 48, 72 and 96 hours after application of the steroids.

Pine seeds (*Pinus silvestris* L.) were soaked for 36 hours in a 0.1% water solution of Tween 80 (controls) or in this solution with addition of 100 mg/l. estradiol-17β, estrone or estriol. Then, the seeds were germinated in sterile sawdust under long-day conditions (16 hrs, fluorescent tubes daylight intensity about 3500 lux) at room temperature. Material for experiments was taken at the following stages of germination: 1) seeds germinated 48 hours, 2) seeds with pierced coat, 3) seedlings at the primary stage of cotyledon development, 4) seedlings with free cotyledons.

Every time growth measurements were carried out on sixty pea or pine seedlings.

Auxins were determined in 20-g samples by the previously described method (Michniewicz and Kopcewicz 1968). The paper chromatography with isopropanol:ammonia:water 10:1:1 as solvent were used. The content of auxins was estimated by the Avena section straight growth test. The position of auxins was examined in UV light and compared with the position of Indole-3-acetic acid (IAA), Indole-3-acetinitrile (IAN) and Indole-3-pyruvic acid (IPyA) in the control chromatograms. The significance of differences in relation to the control was established by estimating LSD at p = 0.01. All experiments were repeated four times. The results are presented graphically. Each value (Fig. 1, 3) represents the total amount of auxins in the neighbouring sections of the chromatogram.

RESULTS AND DISCUSSION

As seen from Table 1 the pea seedlings treated with estradiol-17β, estrone and estriol were characterized by a more intensive growth as compared with the controls. No essential differences in the action of the particular substances were observed and 96 hours after application the plants treated with estrogens were about by 40 per cent taller than the control plants. The lack of differences in the action of the particular estrogens can be explained by the structural similarity of these compounds and by the possibility of conversion of one kind of estrogens into another in plant tissue.

Table 1

Effect of estrogens on the growth of the pea seedlings

Hours after application	Value in	Control	Estrone 0.1 μg/plant	Estradiol-17β 0.1 μg/plant	Estriol 0.1 μg/plant	LSD at P = 0.01
0	mm	39.8	39.8	39.8	39.8	
24	mm	46.8	49.6	50.1	48.9	3.5
	%	100	105.98	107.05	104.48	—
48	mm	57.5	64.2	67.3	66.1	4.2
	%	100	111.65	117.04	114.19	—
72	mm	67.2	86.9	89.1	87.8	3.8
	%	100	129.31	132.58	130.65	—
96	mm	72.1	98.4	102.5	99.9	4.1
	%	100	136.47	142.16	138.55	—

In the case of pine seedlings no influence of estrogens on growth was noted (Tab. 2). As pointed out, however, in the previous paper (Kopcewicz 1968) pine seedlings react, to a small extent, to growth substances, and for example from among

Table 2

Effect of estrogens on the growth of the pine seedlings

Stage of development	Value	Control	Estrone 100 mg/l	Estradiol-17β 100 mg/l	Estriol 100 mg/l	LSD at P = 0.01
1 *	mm	30.5	30.7	31.7	30.9	2.7
	%	100	100.65	103.93	101.31	—
2 **	mm	30.9	31.1	32.2	31.5	2.5
	%	100	100.64	104.20	101.94	—

* Seedlings at primary stage of cotyledon development.

** Seedlings with free cotyledons.

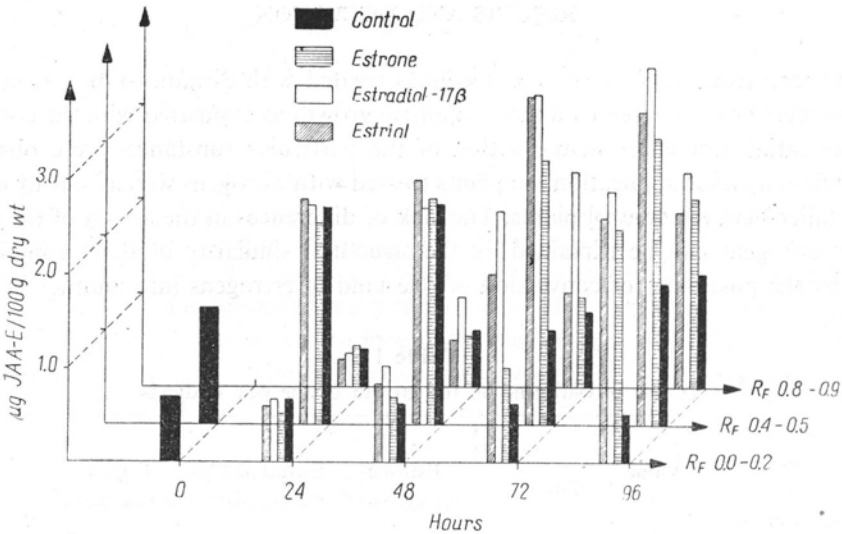

Fig. 1. Influence of estrogens on the content of various auxins in pea seedlings

the gibberellins A_1, A_3, A_4, A_7 and A_{13}, only gibberellin A_{13} stimulated slightly the growth of pine seedlings.

Auxins isolated from germinating seeds and growing seedlings of pea and pine occurred in the form of various compounds. In chromatograms from pea and pine extracts, they were localized in three different sections with R_F 0.0—0.2, 0.4—0.5,

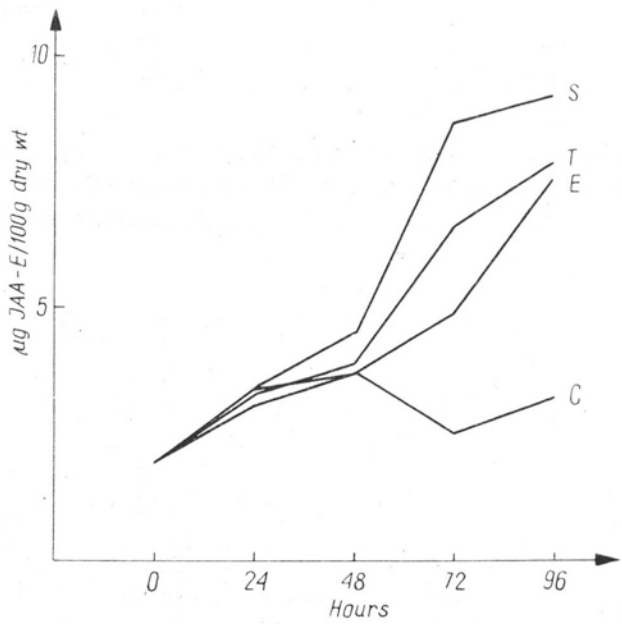

Fig. 2 Influence of estrogens on total content of auxins in pea seedlings

C — control, *S* — estradiol-17β, *E* — estrone, *T* — estriol

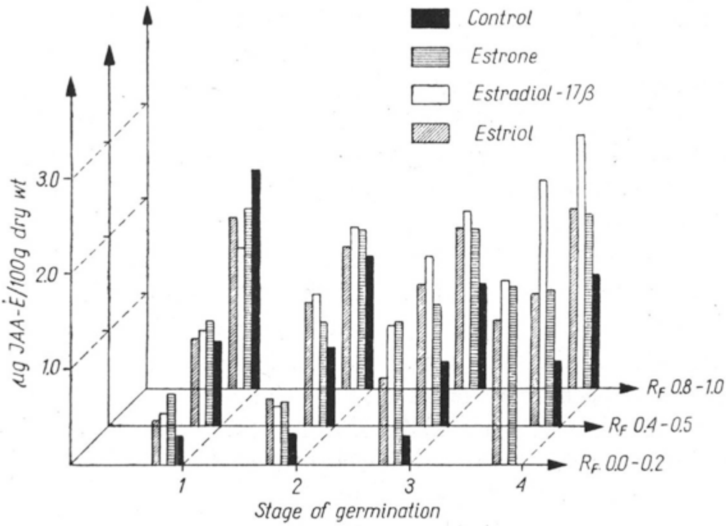

Fig. 3 Influence of estrogens on content of various auxins in pine seeds and seedlings

and 0.8—1.0 (Fig. 1, 3). Similar auxins were also found in extracts from herbaceous plants (Stowe and Thimann 1954) and in the seeds, buds, shoots and cones of *Pinus silvestris* (Michniewicz and Kopcewicz 1968, Kopcewicz 1969 d, e, f, Kopcewicz et al. 1969) and in the buds of *Pinus resinosa* (Giertych and Forward 1966). No detailed identification of these substances was undertaken. It may be,

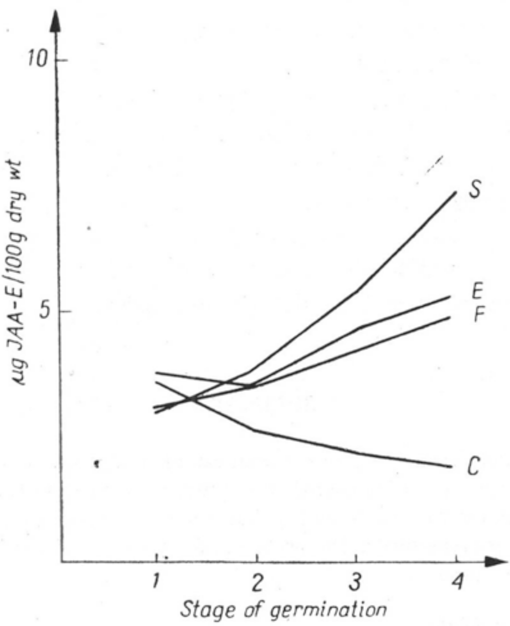

Fig. 4 Influence of estrogens on total content of auxins in pine seeds and seedlings

C – control, *S* – estradiol-17β, *E* – estrone, *F* – estriol however, mentioned that in control chromatograms IPyA, IAA, IAN with R_F^{-s} 0.0—0.2, 0.4—0.5, 0.8—1.0, respectively, were found.

As seen from the results, treatment of pine seeds and pea seedlings with estrogens increases the auxins content in both, especially in the case of estradiol-17β. Considerable differences were found 72 and 96 hours after application in the case of pea (Fig. 1, 2), in later stages of pine seeds germination and in developing pine seedlings (Fig. 3, 4).

The results obtained confirm the important physiological role of estrogens in plants. It thus appears that these hormones influence the content of both gibberellins and auxins in the tissue of plants, and this can be the cause of occurrence of many important metabolic reactions. It seems that the increase in auxins content under the influence of estrogens may result of the primary rise in gibberellins content and this as pointed out by many authors, can in turn increase the auxins activity (Michniewicz 1962; Kuraishi and Muir 1963) or decrease IAA-oxidase activity (Pilet and Wurglen 1958) in plant tissues.

Very little is know about the mode of action of steroids and a few theories have been proposed. According to one of them (Hechter and Lester 1960) steroid hormones regulate cell permeability, a property which would be of great importance in the electrolyte and water metabolism of animals and plants. Wilmer (1961) has recently advanced a theory based on the interaction of steroidal hormones with the lipids of cell membranes. According to other authors (Karlson 1963; Dorfman and Ungar 1965) steroids act directly on the chromosomes, relasing genetic information, and thereby regulate cell differentiation. In plants they could affect a variety of morphological changes such as flowering and sex expression. It seems also that at the molecular level the action of steroids is likely to be the same in both plant and animal cells (Heftmann 1967).

The effect of estrogens on the auxins or gibberellins content in plants, though it does not explain definitively the mechanism of steroidal hormones action in complicated physiological processes such as growth or flowering, it calls, however, attention to the existence of a relation between steroids and other groups of active substances in plants. It seems that the detection of these relationships is important because strict control of growth and development processes can be achieved in plants through the combined action of several regulatory substances.

SUMMARY

Pea seedlings and pine seeds were treated with estradiol-17β, estrone or estriol. It was found that treatment of pea seedlings and pine seeds with estrogens increases the auxins content in both. Considerable differences were found 72 and 96 hours after application in the case of pea and in developing pine seedlings respectively. The problem of mechanism of the action of steroidal hormones in plants is also discussed.

Department of Plant Physiology
Institute of Biology N. Copernicus University
Toruń, Poland

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Wpływ estradiolu-17β, estronu i estriolu na zawartość endogennych auksyn u roślin

Streszczenie

Siewki grochu (*Pisum sativum* odm. 'Cud. Kelwedonu') oraz nasiona sosny (*Pinus silvestris* L.) traktowano estradiolem-17β, estronem i estriolem. Stwierdzono, że traktowanie siewek grochu i nasion sosny estrogenami prowadzi do podwyższenia w nich zawartości auksyn. Wyraźny wpływ estrogenów na podwyższenie się zawartości auksyn stwierdzono w 72 i 96 godzin po aplikacji preparatów u grochu oraz w rozwijających się siewkach sosny. W pracy dyskutuje się problem mechanizmu działania hormonów sterydowych u roślin.

Zakład Fizjologii Roślin
Instytut Biologii Uniwersytetu M. Kopernika
w Toruniu