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THE PRESENCE OF SEROTONIN IN THE EMBRYO OF *JUGLANS REGIA* ssp. FALLAX (DODE) POPOV

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The presence of serotonin has been detected in methanolic extracts of the embryo of *Juglans regia* ssp. *fallax* by means of chromatographical, spectrophotometrical, spectrophotofluorimetrical and histochemical methods. Serotonin has been present in the embryo in a quantity of 95 $\mu g \cdot g^{-1}$ fresh weight.

Introduction

The biogenic amine serotonin is a wide-spread in the plant and animal kingdom. Its activity in the Avena coleoptile test and longitudinal growth of roots is like that of plant growth hormone. The accumulated serotonin have been found in the vacuoles of the nettle stings (Collier and Cheser 1956, Regula and Devidé 1980), trichomes (Bowden et al. 1954) and in the lower epidermal cells of *Elaeagnus umbellata* (Regula 1972).

In this paper the presence of serotonin in the walnut *Juglans regia* ssp. *fallax* has been investigated.

Experimental

Embryos (10 g) of *Juglans regia* ssp. *fallax* were homogenized and extracted several times with cold methanol. After centrifugation the upper layer was discarded and the lower layer investigated on paper and thin layer chromatography. A part of this layer passed through the column of the cation exchanger Amberlite CG-50 in NH_4^+ form. After washing of the column with ammonium acetate, the basic substance was eluted with 1 N hydrochloric acid and determined spectrophotometrically and spectrofluorimetrically.

Results and Discussion

The Rf values of the basic substance in chromatography as well as the colour reactions with 1-nitroso-2-naphthol, ninhydrin acetic acid reagens and others (Table 1) have been identical with the authentic sample of serotonin. The identity of this substance has been confirmed also by measurement of UV spectra in neutral solution (maxima at 275 nm and 295 nm) and fluorescence in acidic solution (activation at 295 nm and fluorescence at 550 nm). The amounts of serotonin in the embryo was measured spectrocolorimetrically with 1-nitroso- 2-naphthol reagent at 535 nm. It has been established that serotonin is present in the embryo in a quantity of 95 $\mu g \cdot g^{-1}$ fresh weight.

The localisation of serotonin has been determined in embryo by the reaction with 6% p-dimethylaminobenzaldehyde in concentrated hydrochloric acid which gives in free hand sections of tissue a blue-green colour recognizable in the light microscope. Serotonin reacts in tissue also with 0.2% ninhydrin in 15% acetic acid giving a greenishblue fluorescence in U. V. light. Grose and co. (1983) have established that serotonin is synthesized by de novo formed adaptive enzymes during the later stage of the seed maturation.

Table 1. Rf values and colour reactions of the compound from extract and sample of serotonin

Substance	Paper chromatography Rf in solvent system				Thin layer chromatography		Reagents			
	1	2	3	4	5+	6+	I	II	III	IV
Substance	0.49	0.52	0.37	0.63	0.66	0.14	b.	b.	v.	v.
Serotonin	0.49	0.53	0.37	0.63	0.66	0.13	b.	b.	v.	v.

1. n-BuOH-AcOH-H_2O /60:15:25/
2. i-PrOH-NH_3-H_2O /10:1:1/
3. n-BuOH-EtOH-H_2O /4:1:1/
4. MeOH--BuOH-C_6O_6-H_2O /4:2:2:2/
5. i-PrOH-NH_3-EtAc /35:20:45/
6. $CHCl_3$-C_6H_6 /1:1/
+ SiO_2 G

I = Ehrlich
II = p-Dimethylaminocinnamaldehyde
III = 1-Nitroso-2-Naphthol
IV = Ninhydrin-Acetic Acid
b. = blue
v. = violet

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SAŽETAK

PRISUSTVO SEROTONINA U EMBRIJU ORAHA *JUGLANS REGIA* ssp. *FALLAX* (DODE) POPOV

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Embriji zrelih sjemenaka oraha *Juglans regia* ssp. *fallax* ekstrahirani su metanolom. Gornji masni sloj je nakon centrifugiranja odbačen, a donji istraživan kromatografijama na papiru i tankom sloju SiO_2. Dio ekstrakta je propuštan preko ionskog izmjenjivača amberlita CG-50, a solno-kiseli eluat u kojem se nalazio biogeni amin uziman je za spektrofotometrijska i spektrofluorimetrijska i kromatografska određivanja. Utvrđeno je da supstancija u ekstraktu po svojim karakteristikama odgovara autentičnom uzorku 5-hidroksitriptamina odnosno serotonina i da se nalazi u tkivu u koncentraciji od 95 $\mu g \cdot g^{-1}$ svježe tvari.

Lokalizacija serotonina u tkivu utvrđena je p-dimetilaminobenzaldehidom s kojim ovaj biogeni amin daje plavo obojenje, dok s ninhidrinom u octenoj kiselini daje zelenkasto-plavu fluorescenciju pod UV svjetloću.

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