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THE PRESENCE OF SEROTONIN IN THE EMBRYO OF BLACK WALNUT (*JUGLANS NIGRA*)

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The presence of serotonin was determined in acidic extracts of the embryio of Black walnut (*Juglans nigra*) which were investigated chromatographically and spectrophotometrically. Serotonin was detected in protein bodies of the embryo's tissue by histochemical reactions.

Introduction

Serotonin is a well known biogenic amine in vertebrates and some invertebrates. It is also synthesized in plants, such as some species of nettles (Collier and Chesher 1956, Regula 1970, 1974, Regula and Devidé 1980) in leaves of *Shepherdia argentea* (Regula and Devidé 1979), and in edible plants such ananas (Bruce 1960, West 1960, Foy and Parratt 1961, Regula 1977) in bananas (Cartier et al. 1958, Udenfriend et al. 1959, Foy and Parratt 1960), in tomato (West 1959, Udenfriend et al. 1959, Regula 1977), and walnut (Kirberg and Braun 1961, Bergman et al. 1970, Grosse 1982, Grosse et al. 1983) and in Manchurian walnut (Regula 1985). Serotonin may have the function of plant hormone (Niaussat et. al. 1958) with protective effects against X-rays in growing roots of broad bean (Lozeron et al. 1965). The formation of serotonin during the ripening of the seed might have the role of a detoxification mechanism for poisonous ammonia (Grosse 1982), and its quantity changes during the germination of the seed (Lembeck and Skofitsch 1984). The present paper describes the detection of serotonin in Black walnut.

* The paper is dedicated to Prof. Zvonimir Devidé on his 65th anniversary.

Table 1. Rf values and colour reactions of the *compound* from extract and sample of 5-hydroxytryptamine

Substance	Paper chromatography Rf in solvent system*					Thin layer chromatography			Reagents					
	1	2	3	4	5	6+	6++	7+	I	II	III	IV	V	VI
Substance	0.48	0.52	0.36	0.62	0.09	0.66	0.80	0.13	b.	b.	b.	v.	v.	v.
5-Hydroxy-tryptamine	0.48	0.52	0.36	0.63	0.08	0.66	0.80	0.13	b.	b.	b.	v.	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_6H_6-H_2O (4 : 2 : 2 : 2)
5. Dest. H_2O
6. i-PrOH-NH_3-EtAc (35 : 20 : 45)
7. $CHCl_3$-C_6H_6 (1 : 1)

\+ SiO_2 G
++ Al_2O_3 G

I = Ehrlich's
II = p-Dimethylaminocinnamaldehyde
III = Xanthydrol
IV = 1-Nitroso-2-Naphthol
V = Ninhydrin
VI = Ninhydrin-Acetic Acid
b. = blue
v. = viclet

Experimental

Embryos of Black walnut were homogenized and extracted with 0.1 M HCl. After centrifugation, the acidic supernatant was alkanized (pH 9.5—10) with Na_2CO_3. Serotonin was extacted by shaking the alkaline solution with n-butanol. The organic phase was evaporated under mild conditions (+33 °C) and the residue was dissolved in 1 ml of methanol and passed through a column of cation exchanger Amberlite CG-50 in NH_4^+ form. The basic substance was eluted with 1 N HCl. Serotonin was detected in acidic effluent by chromatography, spectrophotometry and spectrofluorimetry.

Results and Discusion

Two indolic compounds were observed in the extracts of the embryo of Black walnut. One of these was tryptophan. The Rf values of the other substance on chromatography, as well as colour reactions with 1--nitroso-2-naphthol, ninhydrin-acetic acid and other reagents (Table 1.) were identical with those of the authentic sample of serotin. The identity of this substance was also confirmed by measurements of U. V. spectra in neutral solution (max. 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 extracts were measured spectrocolorimetrically with 1-nitroso-2-naphthol reagent at 535 nm. It was established that serotonin was present in the embryo in an amount of 180 $\mu g \cdot g^{-1}$ fresh weight. Serotonin could not be identified in the leaves and the bark. As established by Grosse (1982) serotonin is synthesized by de novo formed enzymes during the maturation of the seed. Tryptamine as a possible precursor of serotonin hasn't been noticed.

The localization of serotonin in protein bodies of the embryo was detected by histochemical reactions with 6% p-dimethylaminobenzaldehyde in conc. HCl on thin sections of the tissue giving a blue-green colour in the light microscope, and with ninhydrin-acetic acid reagent giving a greenishsblue fluorescence in U. V. light (Regula 1985).

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SAŽETAK

PRISUSTVO SEROTONINA U EMBRIJU CRNOG ORAHA (*JUGLANS NIGRA*)

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Zreli embriji crnog oraha *(Juglans nigra)* homogenizirani su i ekstrahirani 0.1 M kloridnom kiselinom. Ekstrakti su upareni i zaluženi s Na_2CO_3 na pH 9.5 i izmućkavani n-butanolom. Butanolski ekstrakt je uparen do suha, a ostatak otopljen u metanolu i propuštan kroz ionski izmjenjivač

amberlit CG-50 u NH_4^+ obliku. Serotonin je određivan u eluatu kromatografskim, spektrofotometrijskim i spektrofluorimetrijskim metodama. Utvrđeno je da embrio sadržava serotonin u količini od 180 $\mu g \cdot g^{-1}$ svježe tvari.

Lokalizacija serotonina u proteinskim tjelešcima embrija utvrđena je histokemijskim reakcijama p-dimetilaminobenzaldehidom u konc. solnoj kiselini i ninhidrin-octenom kiselinom s kojom daje plavo obojenje odnosno zelenkasto plavu fluorescenciju pod UV svjetlošću.

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