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THE PRESENCE OF SEROTONIN IN THE
EMBRYO OF *JUGLANS*
MANDSHURICA MAXIM.

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In acidic extracts of the embryo of *Juglans mandshurica* Maxim. which were investigated chromatographically and spectrophotofluorimetrically the presence of serotonin was stated. In leaves serotonin could not be detected.

Introduction

Serotonin (5-HT) does not occur only in animals but also in variety of plants as in some species of nettles (Collier et al. 1956, Regula 1970, 1974, Regula and Devidé 1980), in leaves of *Shepherdia argentea* (Regula and Devidé 1979) and of *Loasa vulcanica* (Regula 1981). Serotonin is present in edible fruits e. g. in bananas and tomatoes (Udenfriend et al. 1959, West 1959), ananas (Bruce 1960, West 1960). Among the edible fruits the walnut (*Juglans regia*) embryo contains the highest amount of 5-HT known at present (Kirberger and Braun 1961, Bergman et al. 1970, Grosse et al. 1983). During the germination and ontogenetic development of embryo the quantity and distribution of serotonin are changing (Lembeck et al. 1984). The knowledge of serotonin levels of edible plants is of importance in avoiding possible misdiagnosis of malignant carcinoid tumors.

Serotonin may have the function of plant hormone since it causes curvature in the *Avena* coleoptile test (Niaussat et al. 1958). Serotonin also affects permeability of plasma membrane (Pickles et al. 1955) and concerns protective effects against X-rays in growing roots of *Vicia faba* (Lozeron et al. 1965). Furthermore serotonin may be the end product of the detoxification of ammonia derived from deamination of amino acids in plants (Grosse 1982).

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
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 ₂ O				(60 : 15 : 25)					I = Ehrlich's					
2. i-PrOH-NH ₃ -H ₂ O				(10 : 1 : 1)					II = p-Dimethylaminocinnamaldehyde					
3. n-BuOH-EtOH-H ₂ O				(4 : 1 : 1)					III = Xanthidrol					
4. MeOH-BuOH-C ₆ H ₆ -H ₂ O				(4 : 2 : 2 : 2)					IV = 1-Nitroso-2-Naphthol					
5. Dest. H ₂ O									V = Ninhydrin					
6. i-PrOH-NH ₃ -EtAc				(35 : 20 : 45)					VI = Ninhydrin-Acetic Acid					
7. CHCl ₃ -C ₆ H ₆				(1 : 1)					b. = blue					
+ SiO ₂ G									v. = violet					
+ Al ₂ O ₃ G														

Experimental

Embryos of *Juglans mandshurica* were homogenized and extracted with 0.1 mol HCl. After centrifugation acidic supernatant was alkalinized (pH 9.5–10) with Na_2CO_3 . Serotonin was extracted by shaking alkaline solution with n-butanol. Organic phase was evaporated under mild condition (+ 33°C and 18 mm Hg) and residue was dissolved in 1 ml of methanol and passed through a column of cation exchanger Amberlite CG-50 in NH_4^+ form. The column was washed with 0.02 mol ammonium acetate and the basic substance eluted with 1 mol HCl.

Results and Discussion

In the extracts of the embryos of *Juglans mandshurica* we noticed only one basic indole compound. The Rf values of this substance on paper and thin layer 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 serotonin. The identity of this substance was confirmed also by measurements of UV spectra in neutral solution (max. 275 nm and 295 nm) and fluorescence in strong acid (activation at 295 nm and fluorescence at 550 nm). The amounts of serotonin in the embryo was measured spectrophotometrically with 1-nitroso-2-naphthol reagent at 535 nm. It was established that serotonin was present in the embryo in a quantity of 251 $\mu\text{g} \cdot \text{g}^{-1}$ fresh weight. In the leaves and bark 5-HT could not be identified. As quoted by Grosse (1982) serotonin is synthesized by de novo formed enzymes and accumulates in protein bodies during the later stage of the seed (*Juglans regia*) maturation when cotyledons becomes storage organs. Serotonin is synthesized as a second compound for ammonia detoxification.

Localization of serotonin was detected in embryo by histochemical reaction with 6% p-dimethylaminobenzaldehyde in conc. HCl on thin sections of tissue giving a blue-green colour in the light microscope. Serotonin of tissue also reacts with 0.2% ninhydrin in 15% acetic acid giving a greenishblue fluorescence in U. V. light.

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References

- Bergman, L., W. Grosse, G. Ruppel, 1970: The formation of serotonin in *Juglans regia* L. *Planta*, 94, 47–59.
- Bruce, W., 1960: Serotonin in Pineapple. *Nature*, 188, 147–148.
- Collier, H., G. Chesher, 1956: Identification of 5-hydroxytryptamine in the sting of the nettle (*Urtica dioica*). *Brit. J. Pharmacol.* 11, 186–190.
- Grosse, W., 1982: Function of serotonin in seeds of Walnuts. *Phytochemistry*, 21, 819–822.
- Grosse, W., M. Karisch, P. Schröder, 1983: Serotonin biosynthesis and its regulation in seeds of *Juglans regia* L. *Z. Pflanzenphysiol.* 110, 221–229.
- Kirberger, E., L. Braun, 1961: Über das vorkommen von 5-hydroxytryptamin in der Walnus (*Juglans regia*). *Biochim. Biophys. Acta*, 49, 391–393.

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- Lembeck, F., G. Skofitsch, 1984: Distribution of serotonin in *Juglans regia* seeds during ontogenetic development and germination. *Z. Pflanzenphysiol.* 114, 349—353.
- Lozeron, H., A. Maggiora, 1965: A propos of chemical radioprotection. *Dermatologica*, 131, 28—40.
- Niaussat, P., H. Laborit, C. Dubois, M. Niaussat, 1958: Action de la sérotonine sur la croissance des jeunes plantules d'Avoine. *C.R.Soc. Biol.* 152, 945—947.
- Pickles, V., J. Sutcliffe, 1955: The effects of 5-hydroxytryptamine, indole-3-acetic acid, and some other substances, on pigment effusion, sodium uptake, and potassium efflux, by slices of red beetroot in vitro. *Biochim. Biophys. Acta*, 17, 244—251.
- Regula, I., 1970: 5-Hidroksitriptamin u ljutoj koprivi (*Urtica pilulifera* L.) *Acta Bot. Croat.* 29, 69—74.
- Regula, I., 1974: Kromatografska identifikacija serotonina u koprivi *Urtica pilulifera* var. *odartii* (L.) Aschers. *Acta Bot. Croat.*, 33, 89—91.
- Regula, I., 1981: Serotonin in the tissue of *Loasa vulcanica* ed. André. *Acta Bot. Croat.* 40, 91—94.
- Regula, I., Z. Devidé, 1979: Occurrence of some indoles in *Shepherdia argentea* (Pursh) Nutt. *Acta Bot. Croat.* 38, 41—44.
- Regula, I., Z. Devidé, 1980: The presence of serotonin in some species of genus *Urtica*. *Acta Bot. Croat.* 39, 47—50.
- Udenfriend, S., W. Lovenberg, A. Sjoerdsma, 1959: Physiologically active amines in common fruits and vegetables. *Arch. Biochem. Biophys.* 85, 487—489.
- West, G., 1959: Indole derivatives in Tomatoes. *J. Pharm. Pharmacol.* 11, 275—277.
- West, G., 1960: Carcinoid and Pineapples. *J. Pharm. Pharmacol.* 12, 768—769.

SAŽETAK

PRISUSTVO SEROTONINA U EMBRIJU VRSTE JUGLANS MANDSHURICA Maxim.

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Embriji zrelih sjemenaka vrste *Juglans mandshurica* Maxim. su homogenizirani i ekstrahirani 0.1 mol HCl. Ekstrakti su upareni i zaluženi s Na₂CO₃ na pH 9.5—10 i izmučavani n-butanolom. Butanolski ekstrakt je uparen gotovo do suha, a ostatak je otopljen u metanolu (2 ml) i propuštan kroz stupac ionskog izmjenjivača amberlita CG-50. Serotonin je identificiran i kvantitativno određen kromatografskim i spektrofotometrijskim metodama. Utvrđeno je da embrio sadrži 251 µg·g⁻¹ svježe tvari.

Lokalizacija serotonina u embriju utvrđena je histokemijskom reakcijom s p-dimetilaminobenzaldehidom u konc. HCl na preparate tkiva u kojoj serotonin daje modro obojenje. Serotonin u tkivu također reagira s 0.2% ninhidrinom u 15% octenoj kiselini dajući zelenkasto-plavu fluorescenciju pod UV svjetlošću.

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