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Original scientific paper

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

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The presence of serotonin has been detected in methanolic extracts of the mature embryo of *Juglans sieboldiana* Maxim. The amine from the extract was separated on the Amberlite CG—50 ion exchanger and detected by chromatographic, spectrophotometric and spectrofluorimetric methods. By these methods serotonin was detected in a quantity of $20 \mu\text{g} \cdot \text{g}^{-1}$ fresh weight.

The localization of serotonin was detected in the embryo by a histochemical reaction with p-dimethylaminobenzaldehyde in HCl, or with ninhydrin in acetic acid.

Introduction

Serotonin is wide spread in both the plant and animal kingdom. This biogenic amine has been found in the vacuoles of nettle stings (Collier and Cheshier 1956, Regula and Devidé 1980) and in trichomes on the pods of *Mucuna pruriens* (Bowden et al. 1954). Serotonin also occurs in edible fruits or seeds such as pineapple (Bruce 1960), banana (Waalkes et al. 1958), tomatoes (West 1959, Regula 1977) and walnuts (Kirberger and Brown 1961, Bergman et al. 1970, Grosse et al. 1983, Regula 1989, Regula et al. 1991). The present paper described the first detection of serotonin in *Juglans sieboldiana*.

Table 1. Rf values and colour reactions of the compound from the plant extract and of authentic 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
Plant constituent		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	0.37	0.63	0.09	0.66	0.80	0.13	b.	b.	b.	v.	v.	v.
*1. n-BuOH-AcOH-H ₂ O	/60:15:25/									I = Ehrlich					
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					
										v. = violet					

+ Silica gel G

++ Al₂O₃ G

Material and Methods

Mature embryos (10 g) of *Juglans sieboldiana* were homogenized and extracted several times with methanol. The extract was concentrated, chromatographed on Whatman No 1 and thin layers of silica gel G and Al_2O_3 G (Table 1.). The extract was passed through a column of the Amberlite CG-50 ion exchanger in its NH_4^+ form. The column was washed with 0.02 M ammonium acetate and the basic substance was eluted with 1 N HCl and determined spectrophotometrically and spectrophotofluorimetrically. Quantitative determination of the amine was carried out by a spectrophotometric method using 1-nitroso-2-naphthol and measuring the absorption at 535 nm.

Histochemical localization was achieved by p-dimethylaminobenzaldehyde or ninhydrin-acetic acid on thin sections of the tissue.

Results and Discussion

The Rf values of the indolic substance in chromatography as well as colour reactions with 1-nitroso-2-naphthol, ninhydrin-acetic acid reagent and others (Table 1) were identical with those of an authentic sample of serotonin. The identity of this substance was also confirmed by measurement the U. V. 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 amount of serotonin in the embryo was measured spectrophotometrically with 1-nitroso-2-naphthol reagent. It was established that serotonin is present in the embryo, in a quantity of $20 \mu g \cdot g^{-1}$ fresh weight. In comparison with other species of the genus *Juglans* this amount is relatively low. Serotonin could not be identified in the leaves and the bark. As established by Grosse et al. (1983) serotonin is synthesized by de novo formed enzymes during the maturation of the seed. Tryptamine as possible precursor of serotonin hasn't been noticed.

The localization of serotonin in protein bodies of the embryo was detected by histochemical reaction with 6% p-dimethylaminobenzaldehyde in conc. hydrochloric acid which gives, in free hand sections of tissue a blue-green colour recognizable in the light microscope. Serotonin in the tissue also reacted with ninhydrin in acetic acid giving a greenish blue fluorescence in U. V. light.

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SAŽETAK

PRISUSTVO SEROTONINA U EMBRIJU ORAHA *JUGLANS SIEBOLDIANA* Maxim.

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Embriji (10 g) zrelih sjemenaka oraha *Juglans sieboldiana* usitnjeni su i ekstrahirani metanolom. Koncentrirani ekstrakt istraživani je kromatografijama na papiru i tankim slojevima. Ekstrakt je propuštan preko ionskog izmjenjivača Amberlita CG-50 u NH_4^+ obliku koji veže bazične supstancije ekstrakta, pa se naknadno eluiraju 1N solnom kiselinom. Solno kiseli eluat uziman je za spektrofotometrijska, spektrofluorimetrijska i kromatografska istraživanja. Utvrđeno je da supstancija u ekstraktu po svojim karakteristikama odgovara autentičnom uzorku serotonina (5-hidroksitriptamina) i nalazi se u koncentraciji od $20 \mu\text{g} \cdot \text{g}^{-1}$ 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 zelenkastoplavu fluorescenciju pod UV svjetlošću.

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