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Original scientific paper

PRESENCE OF SEROTONIN IN THE EMBRYO OF *JUGLANS REGIA* VAR. *MEMBRANICA*

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Serotonin (5-hydroxytryptamine) was established in methanolic extract of the mature embryo of *Juglans regia* var. *membranica*. The biogenic amine from the extract was separated on Amberlite CG-50 ion exchanger and detected by chromatographic, spectrophotometric and spectrofluorimetric methods. By these methods serotonin was detected in a quantity of 80 $\mu\text{g}\cdot\text{g}^{-1}$ fresh weight.

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

Introduction

Serotonin is wide spread in both the plant and the animal kingdom. Accumulated serotonin 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). In this way serotonin may have a protective role, like tannins against predators. 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 and Popović 1990). The present paper describes serotonin in *Juglans regia* L. var. *membranica* Carr.

Dedicated to Prof. Zvonimir Devidé on the occasion of his 70th birthday.

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

| Substance | Paper chromatography | | 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

Materials and Methods

Mature embryos of *Juglans regia* var. *membranica* were homogenized and extracted several times with methanol and acetone. The extract was concentrated and chromatographed on Whatman No 1 and thin layers of silica gel G and Al₂O₃ G (Table 1.). Part of the extract was passed through a column of an Amberlite CG—50 the ion exchanger in its NH₄⁺ 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 basic substance was carried out by a spectrophotometric method using 1-nitroso-2-naphthol and measuring the absorption at 535 nm.

Histochemical localisation 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 of 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 amounts 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 80 µg · g⁻¹ fresh weight.

The localisation of serotonin in the embryo was determined by 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 in the tissues also reacted with ninhydrin in acetic acid giving a greenish blue fluorescence in U. V. light. Grosse et al. (1983) have established that serotonin is synthesized by de novo formed adaptive enzymes during the later stage of seed maturation.

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SAŽETAK

PRISUTNOST SEROTONINA U EMBRIJU ORAHA *JUGLANS REGIA* var. *MEMBRANICA*

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Embriji zrelih sjemenaka oraha *Juglans regia* var. *membranica* ekstrahirani su metanolom i acetonom. Koncentrirani ekstrakt je istraživan kromatografijama na papiru i tankim slojevima SiO_2 G i Al_2O_3 G Dio ekstrakta je propuštán 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 serotoninu i da se nalazi u tkivu u koncentraciji od $80 \mu\text{g} \cdot \text{g}^{-1}$ svježe tvari. Lokalizacija serotoninu u tkivu utvrđena je p-dimetilaminobenzaldehidom s kojim ovaj biogeni amin daje plavo obojenje, dok s ninhidrinom u očnoj kiselini daje zelenkastoplavu fluorescenciju pod UV svjetlošću.

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