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## THE PRESENCE OF SEROTONIN IN *BLUMENBACHIA CONTORTA* HOOK. FIL.

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The presence of serotonin was detected in methanolic extracts of the leaves and stem of the species *Blumenbachia contorta*, which was investigated chromatographically and spectrophotometrically. By histochemical reactions it was established that serotonin is located in the stings of the leaves or stem.

### Introduction

In the last thirty years serotonin was detected in about forty plant species or varieties. Very often serotonin is localized in the trichomes which cover the pods of *Mucuna pruriens* (Bowden et al. 1954), in the stings of nettle */Urtica dioica/* (Collier and Chesher 1956), *U. Pilulifera* and some other species of the genus *Urtica* (Regula and Devidé 1980), in lower epidermal cells of the leaves of *Elaeagnus umbellata* (Regula 1972), in fruits of tomato (West 1958, 1959, Udenfriend et al. 1959, Schneider 1972), banana (Waalkes et al. 1958, Foy and Parratt 1960), in seeds of walnut (Kirberger and Braun 1961, Bergman et al. 1970, Grosse et. al. 1983, Lembeck and Skofitsch 1984) and in Manchurian and Black walnut (Regula 1985, 1986).

### Experimental

Stems with leaves were taken for investigation during the stage of flowering. Leaves and stems were homogenized with cold methanol in a blender. Plant material was extracted five times with the same solvent at low temperature (+5°). The extracts were concentrated under mild

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

Substance	Paper chromatography Rf in solvent system				Thin layer chromatography		Reagents		
	1	2	3	4	5 <sup>++</sup>	5 <sup>+</sup>	I	II	III
Substance	0.47	0.28	0.24	0.50	0.28	0.26	v.	v.	v.
Tryptophan	0.47	0.29	0.24	0.50	0.29	0.26	v.	v.	v.
1. n-BuOH-AcOH-H <sub>2</sub> O	/60:15:25/						I = Ehrlich		
2. i-PrOH-NH <sub>3</sub> -H <sub>2</sub> O	/10:1:1/						II = p-N, N-dimethylaminocinnamaldehyde		
3. n-BuOH-EtOH-H <sub>2</sub> O	/4:1:1/						III = Ninhydrin		
4. MeOH-BuOH-C <sub>6</sub> O <sub>6</sub> -H <sub>2</sub> O	/4:2:2:2/						v. = violet		
5. i-PrOH-NH <sub>3</sub> -EtAc	/35:20:45/						+ Al <sub>2</sub> O <sub>3</sub> G ++ SiO <sub>2</sub> G		

Table 2. 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 <sup>+</sup>	6 <sup>+</sup>	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 <sub>2</sub> O	/60:15:25/						I = Ehrlich			
2. i-PrOH-NH <sub>3</sub> -H <sub>2</sub> O	/10:1:1/						II = p-Dimethylaminocinnamaldehyde			
3. n-BuOH-EtOH-H <sub>2</sub> O	/4:1:1/						III = 1-Nitroso-2-Naphthol			
4. MeOH-BuOH-C <sub>6</sub> O <sub>6</sub> -H <sub>2</sub> O	/4:2:2:2/						IV = Ninhydrin-Acetic Acid			
5. i-PrOH-NH <sub>3</sub> -EtAc	/35:20:45/						b. = blue			
6. CHCl <sub>3</sub> -C <sub>6</sub> H <sub>6</sub> + SiO <sub>2</sub> G	/1:1/						v. = violet			

conditions (+33° C). Plant pigments in partially concentrated extracts were removed by petroleum ether. Concentrated extracts were passed through a column of an Amberlite CG-50 cation exchanger in NH<sub>4</sub><sup>+</sup> form. Columns were washed with 0.02 N ammonium acetate and the basic substance eluted with 1 N hydrochloric acid. Ammonium acetate effluent was concentrated to dryness and the residue dissolved in methanol and applied on columns of a Dowex 50-X4 in H<sup>+</sup> form. Columns were eluted with water and then with 2N NH<sub>4</sub>OH. Indolic substances were identified by paper and thin layer chromatography as well as by spectrophotometry and fluorimetry.

## Results and Discussion

Two indolic substances were detected in the extracts of *Blumenbachia contorta*. Tryptophan was found in the ammonia effluents from the Dowex column (Table 1.). The indole compound eluted with hydrochloric acid from the Amberlite column had R<sub>f</sub> values on paper and thin layer chromatography, and colour reactions were identical with those of an authentic sample of serotonin (Table 2.). 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 (activ. 295 nm and fluoresceice at 550 nm).

The amount of serotin was 3.10 µg · g<sup>-1</sup> fresh weight. Histochemical reaction with 6% p-dimethylaminobenzaldehyde in conc. HCl on thin sections of tissue gave a blue to blue-green colour in light microscope in the stings, and with ninhydrin-acetic acid reagent a greenish-blue 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.
- Bowden, K., Brown, B. and J. Batty, 1954: 5-Hydroxytryptamine, its occurrence in cowhage. *Nature* 174, 925—926.
- Collier, H., G. Chesher, 1956: Identification of 5-hödroxytryptamine in the sting of the nettle (*Urtica dioica*). *Brit. J. Pharmacol.* 11, 186—190.
- Foy, M., R. Parratt, 1960: A note on the presence of noradrenaline and 5-hydroxytryptamine in plantain (*Musa sapientum* var. *paradisica*). *J. Pharm. Pharmacol.* 12, 360—364.
- 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.
- Lembeck, F. and G. Skofitsch, 1984: Distribution of serotonin in *Juglans regia* seeds during ontogenetic development and germination. *Z. Pflanzenphysiol.* 114, 349—353.
- Regula, I., 1972: Identifikacija serotoninina u vrste *Elaeagnus umbellata* Thunb. *Acta Bot. Croat.* 31, 105—108.
- Regula, I., 1985: The presence of serotonin in the embryo of *Juglans Mandshurica* Maxim. *Acta Bot. Croat.* 44, 19—22.
- Regula, I., 1986: The presence of serotonin in the embryo of Black walnut (*Juglans nigra*). *Acta Bot. Croat.* 45, 91—95.
- Regula, I. and Z. Devidé, 1980: Presence of serotonin in some species of genus *Urtica*. *Acta Bot. Croat.* 39, 64—68.
- Schneider, E., R. Gibson and F. Wightman, 1972: The native indoles of Barley and Tomato shoots. *J. Exptl. Biol.* 23, 152—170.
- Udenfriend, S., W. Lovenberg and A. Sjoerdsma 1959: Physiologically active amines in common fruits and vegetables. *Arch. Biochem. Biophys.* 85, 487.
- Waalkes, P., A. Sjoerdsma C. Creveling, H. Weissbach and S. Udenfriend, 1958: Serotonin, norepinephrine and related compounds in Bananas. *Science* 127, 648.
- West, G., 1959: Tryptamines in Tomatoes. *J. Pharm. Pharmacol.* 11, 319.

SAŽETAK

PRISUTNOST SEROTONINA U VRSTE *BLUMENBACHIA CONTORTA* Hook. fil.

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Listovi i stabljike vrste *Blumenbachia contorta* homogenizirani su i ekstrahirani nekoliko puta metanolom. Djelomično koncentrirani ekstrakti su izmučkavani petrol-eterom i propuštani preko stupaca ionskih izmjenjivača Amberlita CG—50 i Dowexa-50. Triptofan i serotonin utvrđeni su kromatografijama na papiru i tankim slojevima, a posljednja supstancija i spektrofotometrijski i spektrofluorimetrijski. Serotonin je utvrđen u količini od  $3.10 \mu\text{g g}^{-1}$  tvari. Histokemijskim reakcijama utvrđeno je da je serotonin lokaliziran u dlakama žeravkama.

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