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SEROTONIN IN THE TISSUE OF *LOASA VULCANICA* ED. ANDRÉ*

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Introduction

Serotonin has up to now been detected in only about thirty plant species and it could be considered very rare in plant kingdom. This indole amine occurs in leaves, stems and seeds of the species *Urtica* (Collier and Cheshier 1956, Regula 1970, 1974, Regula and Devidé 1980), in edible fruits of banana (*Musa sapientum*) (Cartier et al. 1958, Foy and Parratt 1961, Waalkes et al. 1958), in green vegetative organs and fruits of tomato (*Lycopersicum esculentum*) (West 1958, 1959, Udenfriend et al. 1972), and also in pineapple (*Ananas comosus*) fruit (Bruce 1960, West 1960, Foy and Parratt 1961) and the leaves of the crown (Regula 1977).

In this study the presence of serotonin and other hydroxyindoles in *Loasa vulcanica* were investigated. This species belongs to the order *Passiflorales*, which also comprises watermelon (*Colocynthis citrulus*) the only species of the order whose tissue in vitro can hydroxylate tryptophan to 5-hydroxytryptophan (Dannenburg and Liverman 1957).

Experimental

Stems with leaves of *Loasa vulcanica* (cultivated in the Botanical Garden, Faculty of Science, University of Zagreb) were homogenized and extracted with cold methanol at low temperature (278 K). Extracts were

* This paper is dedicated to Professor Zvonimir Devidé on the occasion of his 60th birthday with gratitude for his never failing, critical advice.

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

Substance	Paper chromatography						Thin layer chromatography		Reagents						
	1	2	3	4	5	R _f in solvent system*	6 ⁺	6 ⁺⁺	7 ⁺	I	II	III	IV	V	VI
5-Hydroxytryptamine	0.48	0.52	0.36	0.62	0.09		0.66	0.80	0.13	b.	b.	b.	v.	v.	v.
	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															

concentrated under mild conditions (306 K and 2400 Pa). Pigments and other lipophilic substances were removed by shaking the extracts with petroleum ether. The extracts were then passed through a column of ion exchanger Amberlite CG-50 in NH_4^+ form. The column was washed with 0.02 mol dm^{-3} ammonium acetate and the basic substances eluted with 1 mol dm^{-3} hydrochloric acid.

Indolic substances were detected and identified by paper and thin layer chromatography as well as by spectrophotometry and spectrofluorimetry (Oates 1961, Iskrić et al. 1969).

Results and Discussion

In the acidic effluent from the Amberlite column a compound was detected which reacted positively with Ehrlich's reagent and 1-nitroso-2-naphthol, and gave the colour and fluorescence characteristic of tryptamines under UV-light after reaction with ninhydrin-acetic acid. The colour reactions and Rf values of this substance on paper and thin layer chromatography (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). It was established that serotonin was present in leaves and stems in a quantity of $2.54 \mu\text{g g}^{-1}$ fresh weight.

Histochemical reactions with 6% (w/v) p-dimethylaminobenzaldehyde in conc. hydrochloric acid on the sting gave a characteristic blue-green colour given by the authentic sample of 5-hydroxytryptamine.

Conclusion

In methanolic extracts of green vegetative organs of *Loasa vulcanica* the presence of serotonin was proved by chromatography, spectrophotometry and spectrofluorimetry. Positive histochemical reaction inside the sting demonstrated that 5-hydroxytryptamine was localised also in these parts of the plant.

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S A Ž E T A K

SEROTONIN U TKIVU BILJKE *LOASA VULCANICA* ED. ANDRÉ

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Stabljike s listovima vrste *Loasa vulcanica* su homogenizirane i ekstrahirane s hladnim metanolom. Ekstrakti su upareni pod blagim uvjetima i izmuckavani petrol eterom i propušteni preko kolone ionskog izmjenjivača Amberlita CG-50. Serotonin je dokazan kromatografijama na papiru, tankim slojevima, spektrofotometrijom i spektrofotofluorimetrijom. Histokemijskom reakcijom je utvrđeno da se taj biogeni amin nalazi u dlakama žeravkama u najvećoj količini. Mjerenjima je ustanovljeno da zeleni vegetativni organi sadržavaju 2,54 µg serotonina po gramu svježeg tkiva.

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