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OCCURRENCE OF SOME INDOLES IN  
*SHEPHERDIA ARGENTEA* (PURSH) NUTT.

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## Introduction

Petrova et al. (1961, 1964) identified the arylalkylamine — 5-hydroxytryptamine (serotonin) in the bark of *Hippophaë rhamnoides*. Recently this amine has also been detected in the related species *Elaeagnus umbellata* (Regula 1972). Investigating alkaloids in *Shepherdia* species Ayer et al. (1970) detected tetrahydroharmol, 5- and 6-hydroxytryptamines in *Shepherdia canadensis*, while in *Shepherdia argentea* they could identify tetrahydroharmol only. Consequently it seemed to be interesting to reinvestigate the presence of biogenic amines in the last species.

## Experimental

Leaves and bark of *Shepherdia argentea* (cultivated in the Botanical Garden in Zagreb) were homogenized and extracted with a mixture of methanol-ethanol (1 : 1) at a temp. of +3° C. The extracts were concentrated under mild conditions (+33° C and 18 mm Hg). Plant pigments and lipophilic substances were removed by shaking the extracts with petroleum ether. Extracts were passed through a column of ion exchanger Amberlite CG-50 in  $\text{NH}_4^+$  form, and the column was eluted first with 0.02 N ammonium acetate (pH 7.5) and then with 1 N hydrochloric acid.

The ammonium acetate effluent was concentrated and the residue dissolved in methanol and put on a column of Dowex-50 X4 in  $\text{H}^+$  form. This column was eluted with water and then with 2 N  $\text{NH}_4\text{OH}$ .

Indolic substances were detected and identified by paper and thin layer chromatography and spectrophotofluorimetrically.

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

Table 2. Rf values and colour reactions of compound from extract and sample of Tryptophan

Substance	Paper chromatography Rf in solvent system*					Thin layer chromatography		Reagents		
	1	2	3	4	5	6 <sup>++</sup>	6 <sup>+</sup>	I	II	III
Substance	0.47	0.27	0.24	0.50	0.58	0.28	0.27	v.	v.	v.
Tryptophan	0.47	0.27	0.24	0.49	0.58	0.28	0.27	v.	v.	v.

\* 1. n-BuOH-AcOH-H<sub>2</sub>O (60 : 15 : 25)2. i-PrOH-NH<sub>3</sub>-H<sub>2</sub>O (10 : 1 : 1)3. n-BuOH-EtOH-H<sub>2</sub>O (4 : 1 : 1)4. MeOH-BuOH-C<sub>6</sub>H<sub>6</sub>-H<sub>2</sub>O (4 : 2 : 2 : 2)5. Dest. H<sub>2</sub>O6. i-PrOH-NH<sub>3</sub>-EtAc (35 : 20 : 45)

I = Ehrlich's

II = p-Dimethylaminocinnamaldehyde

III = Ninhydrin

v. = violet

+ Al<sub>2</sub>O<sub>3</sub> G++ SiO<sub>2</sub> G

## Results and Discussion

In the hydrochloric 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 reaction and Rf values of this substance showed the identity with the authentic sample of 5-hydroxytryptamine (Table 1.). The presence of serotonin was proved also by spectrophotofluorimetric measurements of the substance in strong acid (activation at 295 nm and corresponding fluorescence maximum at 550 nm). We found that serotonin was present in leaves in a quantity of 30.18  $\mu\text{g g}^{-1}$  fresh weight, while in the bark it occurred in a quantity of 6  $\mu\text{g g}^{-1}$  fresh weight.

The ammonia effluents from Dowex column of leave extract contained an indolic compound which reacted positively with Ehrlich's reagent, ninhydrin, bromcresol green and others indicating the presence of indolic structure and functional groups. The co-chromatography with an authentic sample of tryptophan showed that the substance from the extract was really tryptophan (Table 2.).

## Conclusion

In alcoholic extracts of the leaves of *Shepherdia argentea*, which were investigated chromatographically and spectrophotofluorimetrically, the presence of 5-hydroxytryptamine and tryptophan was stated. The same biogenic amine was identified in the bark, but in a smaller quantity.

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## References

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## S A Ž E T A K

NALAZ NEKIH INDOLA U VRSTE *SHEPHERDIA ARGENTEA* (PURSH) NUTT.

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Listovi i kora vrste *Shepherdia argentea* homogenizirani su i ekstrahirani nekoliko puta smjesom metanoletanola (1 : 1). Sirovi ekstrakti su izmučkanvani s petroleterom i propušteni preko kolona Amberlita CG-50 i Dowexa-50 X4. Indolski spojevi su identificirani kromatografijama i spektrofotofluorimetrijski. U ekstraktu listova utvrđeni su triptofan i 5-hidroksitriptamin ( $30.18 \mu\text{g g}^{-1}$  svježe tvari), dok je u ekstraktu kore utvrđena znatno manja količina tog biogenog amina ( $6 \mu\text{g g}^{-1}$  svježe tvari).

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