SPECTROPHOTOMETRIC DETERMINATION OF THE ALKALOIDS OF THE β-CARBOLINE SERIES IN THE BARK OF Elaeagnus angustifolia

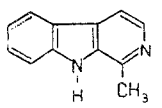
A. G. Nikolaeva, A. P. Prokopenko, and P. E. Krivenchuk

UDC 615.43:535.243+547.944/945

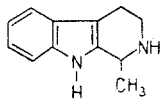
Alkaloids of the β-carboline series have previously [1-4] been isolated from the bark of Elaeagnus angustifolia L. (Russian olive).

This paper describes a spectrophotometric method we have developed for the determination of the amount of 1-methyl-β-carboline (harman) and 1-methyl-1,2,3,4-tetrahydro-β-carboline (tetrahydroharman) in preparations and in the bark of this plant.

Several absorption maxima are characteristic for the UV spectrum of harman ($\lambda_{max}^{C_2H_5OH}$ 235 nm, 249, 288, 336, 348 nm), and two for tetrahydroharman ($\lambda_{max}^{C_2H_5OH}$ 224, 280 nm). The spectrophotometric determination of the content of harman is most conveniently performed at 288 nm (specific absorption coefficient 1180 ± 1.5) and that of tetrahydroharman at 280 nm (specific absorption coefficient 400 ± 0.8). It has been found that harman in a concentration of 0.1-1 mg per 100 ml and tetrahydroharman in concentrations of 0.5-2.4 mg per 100 ml obey the Bouguer–Lambert–Beer law of light absorption.



harman



tetrahydroharman

In order to evaluate the accuracy of the method, we determined the amount of the alkaloids studied in preparations. The mean relative error is between ±0.82 and ±1.05%, which shows that the method is fairly accurate (Table 1).

The alkaloids were extracted exhaustively from the plant raw material with absolute ethanol. The contents of harman and tetrahydroharman were determined after their separation by TLC.

The experimental investigation showed that in the budding period the predominant alkaloid in the plant is tetrahydroharman, and then its amount gradually decreases and it reaches a minimum in the fruit-bearing phase; for harman there is an increase in its amount up to the end of the vegetation period (Table 2).

EXPERIMENTAL

The work was carried out with samples of harman having mp 234-235°C (from C_6H_6) and of tetrahydroharman with mp 177-178°C (from C_6H_6). The sorbent for TLC was alumina (Brockmann activity grade II). The layer of sorbent was deposited manually at a standard thickness of 0.6 mm, and chromatography was carried out in the methanol–chloroform (1 : 32) system. The optical densities were measured on an SF-4A spectrophotometer in quartz cells with a layer thickness of the solution of 1 cm at the appropriate wavelength.

Zaporozhe Medical Institute. Khar'kov Chemical and Pharmaceutical Scientific-Research Institute.
Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 708-711, November-December, 1970. Original article submitted July 17, 1970.

TABLE 1. Results of a Quantitative Determination of Harman and Tetrahydroharman Separately after TL Chromatography

Expt. No.	Taken, mg/100 ml	Found, mg/100 ml	Yield, %	Metrologic characteristics
	Harman			
1	0,5	0,488	97,6	$\bar{x} = 97{,}05$
2	0,5	0,488	97,6	$\sigma = \pm 0{,}64$
3	0,5	0,483	96,6	$\sigma_{\bar{x}} = \pm 0{,}32$
4	0,5	0,482	96,4	$I_p = \pm 1{,}02$
				$A = \pm 1{,}05$
	Tetrahydroharman			
1	1,0	0,962	96,2	$\bar{x} = 95{,}8$
2	1,0	0,952	95,2	$\sigma = \pm 0{,}49$
3	1,0	0,962	96,2	$\sigma_{\bar{x}} - \pm 0{,}245$
4	1,0	0,956	95,6	$I_p = \pm 0{,}78$
				$A = \pm 0{,}82$

TABLE 2. Results of a Determination of the Alkaloids in the Bark of the Russian Olive According to the Vegetation Phases

Sample of raw material No.	Weight, g	Phase of development of the plant	Found, mg		Found, %	
			harman	tetrahydroharmans	harman	tetrahydroharmans
1	10,0	Budding, 4.28.69	0,252	2,125	0,0126	0,106
2	10,0	Flowering, 5.26.69	0,250	1,612	0,0122	0,0812
3	10,0	After flowering, 6.20.69	0,531	0,982	0,0264	0,0496
4	10,0	Fruit-bearing, 8.30.69	0,572	0,705	0,0284	0,0356

Determination of the Alkaloids in a Powder. Portions (10 mg, accurately weighed) of harman and tetrahydroharman were each dissolved in 10 ml of absolute ethanol. One ml of the tetrahydroharman solution and 0.5 ml of the harman solution were deposited in a continuous band on plates of adsorbent and were chromatographed. After the solvent front had traveled 16 cm from the starting line, the chromatograms were observed in UV light and the zones of the alkaloids under investigation were marked. Harman had a bright blue fluorescence and tetrahydroharman a dark blue fluorescence.

The layer of sorbent was collected and the alkaloids were eluted with 10 ml of absolute ethanol. The solutions were filtered through a No. 4 glass filter and the optical densities of the solutions were measured at wavelengths of 288 nm (harman) and 280 nm (tetrahydroharman). As blank an extract obtained after the absolute ethanol treatment of an equal amount of alumina from the plate was used.

Determination of the Alkaloids in the Bark of the Russian Olive. Ten grams (accurately weighed) of the comminuted bark of the Russian olive (at the appropriate phase of development of the plant) was exhaustively extracted with absolute ethanol. The extract was transferred quantitatively to a 200-ml measuring flask. Ten ml of the resulting solution was deposited in several portions on a plate of adsorbent and chromatographed. The zones of the alkaloids under consideration were marked out in UV light and collected in receivers, and the alkaloids were eluted with 25 ml of ethanol, the solutions being filtered through a No. 4 glass filter and their optical densities being measured against an ethanolic eluate of the pure adsorbent as described above. Each of four samples was measured four times.

The percentage contents of harman and tetrahydroharman in the raw material (the calculations being performed on the absolutely dry weight of the raw material) were calculated from the formula

$$X = \frac{D_{288(280)} \cdot a \cdot b}{E^{1\%}_{1\,cm} \cdot l \cdot p \cdot V},$$

where $D_{288(280)}$ is the optical density of the harman and tetrahydroharman solutions at 288 and 280 nm, respectively;

a is the volume of extract, ml;

b is the volume of ethanol used to elute the individual alkaloids from the chromatogram, ml;

l is the thickness of the layer, cm;

p is the weight of the sample, g;

V is the amount of solution deposited on the alumina, ml.

SUMMARY

A spectrophotometric method for the quantitative determination of the alkaloids of the β-carboline series (harman and tetrahydroharman) in preparations and in the root of Elaeagnus angustifolia L. at various phases of development of the plant has been developed.

LITERATURE CITED

1. P. S. Massagetov, ZhOKh, 16, 139, 1946.
2. G. P. Men'shikov, E. L. Gurevich, and G. A. Samsonova, ZhOKh, 20, No. 10, 1927, 1950.
3. T. F. Platonova, A. D. Kuzovkov, and P. S. Massagetov, ZhOKh, 26, No. 11, 3220, 1956.
4. A. G. Nikolaeva, I. V. Terent'eva, P. E. Krivenchuk, and A. P. Prokopenko, KhPS [Chemistry of Natural Compounds], 6, 493, 1970.