Journal of Chromatography, 390 (1987) 439–443
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 290

Note

High-performance liquid chromatographic study of the photochemical oxidation of vasicine and its analogues*

B. K. CHOWDHURY* and S. K. HIRANI

Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria (Nigeria)

and

D. NGUR

Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria (Nigeria)

(First received October 28th, 1986; revised manuscript received November 25th, 1986)

Vasicine and vasicinone, the two alkaloids of *Adhatoda vasica* Nees, are known to possess interesting biological activities[1–4]. Some vasicine and vasicinone analogues have also been found to possess uterine stimulating and bronchodilatory activities[5–7]. Mehta *et al.*[8] reported that vasicine undergoes autoxidation to vasicinone when exposed to bright daylight or sunlight. Recently, during an investigation of the detection and quantitation of vasicine and vasicinone in *Adhatoda vasica* by high-performance liquid chromatography (HPLC), Brain and Thapa[9] studied the degradation of vasicine in various solvents under normal laboratory lighting conditions and continuous UV irradiation at 365 nm.

In course of our investigations on the biologically active compounds related to vasicine and vasicinone we observed that in non-polar solvents not only vasicine but also its benzene-substituted analogues were oxidized by direct sunlight to the corresponding vasicinone analogues. In view of the interesting biological activities associated with vasicine and its analogues, it was of interest to develop a method to study the oxidation of this group of compounds. We report here an HPLC method which involves the ion-pairing technique and uses an internal standard for quantitation. The method has also been found useful for the detection and quantitation of vasicine and vasicinone in *Adhatoda vasica*.

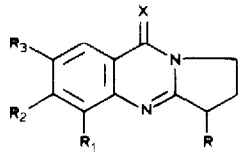
EXPERIMENTAL

Samples and reagents

Vasicine (I), vasicinone (II), vasicine analogues (III, IV) and vasicinone analogues (V–VII) were used.

* Part 5 in the series "Vasicine and related compounds".

0021-9673/87/$03.50



I R = OH; R_1 = R_2 = R_3 = H; X = H_2
II R = OH; R_1 = R_2 = R_3 = H; X = O
III R = R_1 = H; R_2 = R_3 = OCH_3; X = H_2
IV R = OH, R_1 = R_2 = OCH_3; R_3 = H; X = H_2
V R = R_1 = H, R_2 = R_3 = OCH_3; X = O
VI R = OH, R_1 = R_2 = OCH_3; R_3 = H; X = O
VII R = R_1 = R_2 = H; R_3 = Br; X = O

Vasicine and vasicinone were isolated from an ethanolic extract of the leaves of *Adhatoda vasica* Nees using the method described by Mehta *et al.*[8]. Compounds IV and V were prepared following the procedures reported in the literature[5,7]. Compound III was prepared by the condensation of 6-aminoveratraldehyde with 4-aminobutyraldehyde using the method reported earlier[7], but taking precautions that the reaction product was not exposed to sunlight during work-up. Compound VI was prepared by the oxidation of IV with 30% hydrogen peroxide[8]. The bromo derivative (VII) was prepared by the condensation of 5-bromoanthranilic acid and 4-aminobutyric acid with phosphorus pentoxide in boiling *m*-xylene[10]. All the compounds were identified by spectral methods (UV, IR, MS and ^{1}H NMR).

Analytical grade reagents were used. Methanol was distilled once and water was distilled twice before use. The solvents were prefiltered through a Millipore Type HA 0.45-μm filter and degassed in an ultrasonic bath.

Solutions

Separate solutions of compounds I–VII were prepared by dissolving 3 mg of each compound in 30 ml of chloroform. Parts of the stock solutions of compounds I, III and IV were exposed to direct sunlight and the photochemical reaction was monitored by HPLC every 2–5 h. The solution of compound VII was used as a stock solution of the internal standard.

Apparatus and conditions

HPLC was conducted with a Waters Millipore 2504 liquid chromatograph and a μBondapak C_{18} Radial-Pak cartridge (particle size 10 μm, 10 cm × 8 mm I.D.). The column effluent was monitored at 254 nm with a sensitivity setting at 0.05 a.u.f.s. using a Waters Assoc. Model 441 absorbance detector. Methanol–water (70:30) containing 1 ml of PIC B-6 (= hexane sulfonic acid; final concentration in mobile phase, 0.005 *M*) (Cat. No. 85140, Waters Assoc.) was used as the mobile phase at a flow-rate of 0.8 ml/min. Bromovasicinone (VII) was used as the internal standard in all cases.

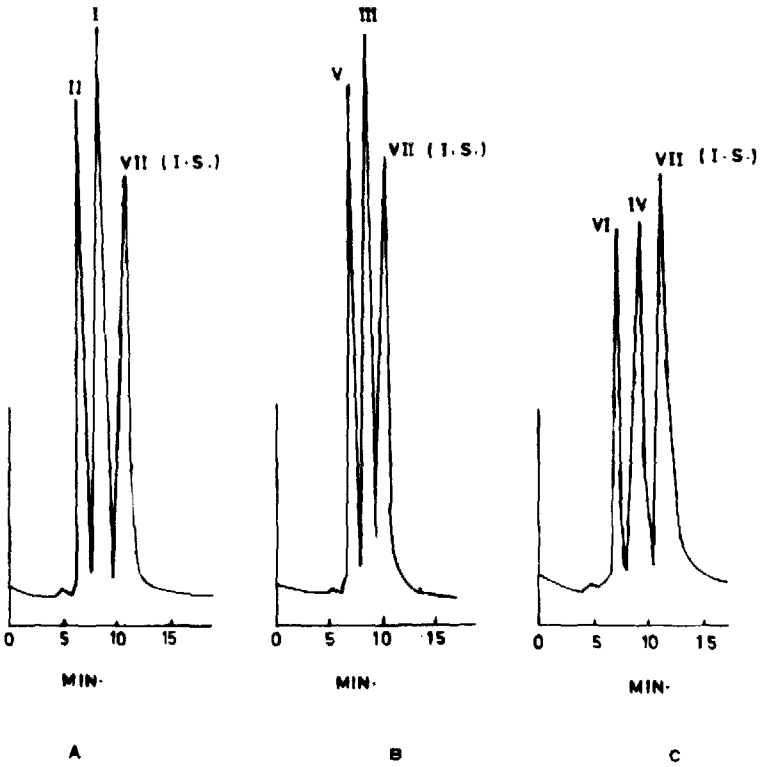


Fig. 1. HPLC profiles of (A) I, II and VII (I.S.); (B) III, V and VII (I.S.), and (C) IV, VI and VII (I.S.). Conditions: column, μBondapak C_{18} Radial-Pak cartridge; mobile phase, methanol–water (70:30) containing 1 ml of PIC B-6; flow-rate, 0.8 ml/min; detection, 254 nm; sensitivity, 0.05 a.u.f.s.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of vasicine (I), vasicinone (II) and the internal standard (VII, I.S.). The separation of III, V and the VII (I.S.), and of IV, VI and VII (I.S.) are also shown in Fig. 1. Reversed-phase chromatography using the ion-pairing agent gave good and reproducible separations. Initial attempts at separation using methanol–water in various proportions resulted in good separations of vasicine and vasicinone. The vasicine analogue was also well separated from the corresponding vasicinone analogue. However, vasicine and its analogues appeared as broad tailing peaks not suitable for quantitative work. In addition, the analysis time was long.

Vasicine and its analogues, being more basic, could be chromatographed successfully by ion pairing with an oppositely charged molecule, thus improving the peak shape and decreasing the retention time. Vasicinone and its analogues are less basic and their retention is therefore unaffected by changes in the concentration of the mobile phase ion-pairing agent.

A plot of the ratio of the peak height of vasicine to that of the internal standard against the amount of vasicine was linear over the range of 0.01–0.10 mg. Linear

TABLE I

OXIDATION OF VASICINE AND ITS ANALOGUES IN CHLOROFORM BY DIRECT SUNLIGHT

Time (h)	*Content of vasicine and its analogues as % of initial*			*% Yield of the corresponding oxo derivatives*		
	I	*III*	*IV*	*II*	*V*	*VI*
4	89.5	70.4	78.8	9.0	24.8	11.7
6	73.3	30.3	55.0	22.4	56.2	38.2
10	58.0	3.5	34.2	33.5	80.3	55.1
15	40.2		10.5	48.5		76.2
20	25		3.2	63.6		79.8
25	10.3			78.1		
30	5.1			82.2		

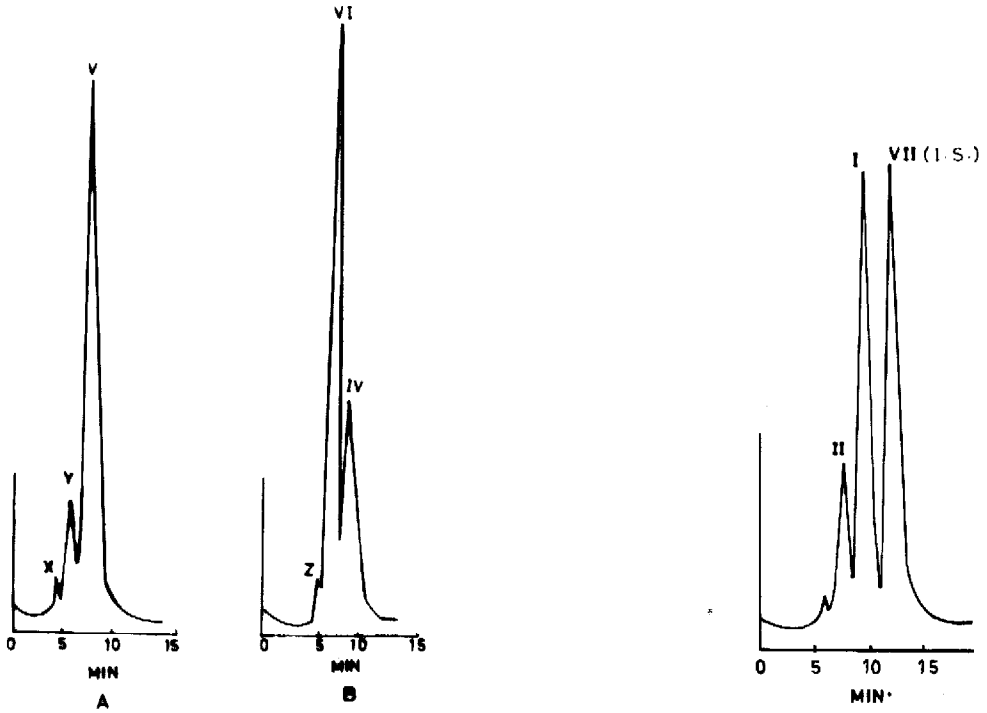


Fig. 2. HPLC profiles showing the degradation of (A) III and (B) IV after 12 h of exposure to sunlight. Peaks X, Y and Z indicate minor products formed during degradation. Other peaks and chromatographic conditions as in Fig. 1.

Fig. 3. HPLC profile of a crude extract (95% ethanol) of the leaves of *Adhatoda vasica* together with the internal standard. Chromatographic conditions and peak identification as in Fig. 1.

calibration plots were also obtained with vasicinone, the vasicine analogues III and IV and the vasicinone analogues V and VI. Mean standard deviations were less than ±5%.

Minimum detectable levels of these compounds were 5–10 ng.

Vasicine and its analogues III and IV in chloroform solutions were oxidized at different rates by direct sunlight to vasicinone and the corresponding vasicinone analogues, respectively, in high yields (Table I). However, two minor compounds were also detected in the degradation products of III, while compound IV showed the presence of one minor compound (Fig. 2). The vasicine analogues with methoxy substituents on the benzene ring were oxidized at much more rapidly than vasicine.

In order to investigate the applicability of this method to the quantitation of vasicine and vasicinone in plant extracts, a crude ethanol (95%, room temperature) extract of the leaves of *Adhatoda vasica* was examined (Fig. 3). The method was found to be useful for the detection and quantitation of vasicine and vasicinone in plant extracts.

In conclusion, HPLC provides a useful method for monitoring the photochemical degradation of the vasicine group of compounds. The present technique may be extended to the determination of any *in vivo* oxidation of vasicine and its analogues.

REFERENCES

1 A. H. Amin, D. R. Mehta and S. S. Samarth, *Prog. Drug Res.*, 14 (1970) 218.
2 O. P. Gupta, K. K. Anand, B. J. Ghattak Ray and C. K. Atal, *Indian J. Exp. Biol.*, 16 (1978) 1075.
3 V. P. Arya, *Drugs of the Future*, 6 (1981) 373.
4 H. L. Bhalla and A. Y. Nimbkar, *Drug Dev. Ind. Pharm.*, 8 (1982) 833.
5 R. L. Sharma, R. K. Gupta, B. K. Chowdhury, K. L. Dhar and C. K. Atal, *Indian J. Chem., Sect. B*, 187 (1979) 449.
6 V. P. Arya, *Drugs of the Future*, 6 (1981) 362.
7 B. K. Chowdhury, E. O. Afolabi, G. Osuide and E. N. Sokomba, *Indian J. Chem., Sect. B*, 24 (1985) 789.
8 D. R. Mehta, J. S. Naravane and R. M. Desai, *J. Org. Chem.*, 28 (1963) 445.
9 K. R. Brain and B. B. Thapa, *J. Chromatogr.*, 258 (1983) 183.
10 A. Chatterjee and M. Ganguly, *Phytochemistry*, 7 (1968) 307.