Journal of Chromatography, 369 (1986) 258–260
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 952

Note

High-performance liquid chromatographic separation of carbazole alkaloids

B. K. CHOWDHURY* and S. K. HIRANI
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria (Nigeria)
and
P. BHATTACHARYYA
Department of Chemistry, Bose Institute, Calcutta 700009 (India)
(Received June 27th, 1986)

Recently we reported[1] the gas–liquid chromatographic (GLC) separation of carbazole alkaloids. The GLC method requires high temperatures for some alkaloids. It is therefore of interest to find an effective method of separation of this group of alkaloids using milder conditions. We report here the separation of C_{13}, C_{18} and C_{23} carbazole alkaloids at room temperature by high-performance liquid chromatography (HPLC). The method has been found to be useful for the detection of these alkaloids in plant extracts.

EXPERIMENTAL

Samples and reagents

Carbazole (I) and seven carbazole alkaloids[2] (II–VIII) were used.

Analytical grade reagents were used. Methanol and chloroform were distilled before use. Hexane was treated with conc. sulphuric acid and distilled. The solvents were prefiltered through a Millipore Type F-H 0.5-μm filter and degassed.

A mixture containing 1 mg of each sample was dissolved in methanol (30 ml) and aliquots (10 μl) were injected into the HPLC system.

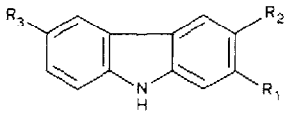
Apparatus and conditions

HPLC was conducted on a Waters Millipore 2504 liquid chromatograph with a μPorasil Radial-Pak cartridge (particle size 10 μm, 10 cm × 8 mm I.D.). The column effluent was monitored at 254 nm using a Waters Model-441 absorbance detector. The mobile phase flow-rate was 0.7 ml/min.

RESULTS AND DISCUSSION

Preliminary studies indicated that the carbazole alkaloids (I–VIII) could not be resolved satisfactorily on a μBondapak C_{18} Radial-Pak cartridge using methanol–water (8:2 or 7:3). However, resolution could be achieved on a μPorasil Radial-Pak cartridge using a mobile phase of hexane–chloroform (7.5:2.5). The sep-

0021-9673/86/$03.50

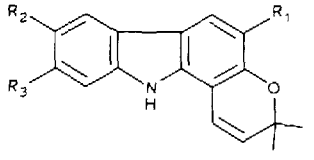


Carbazole (I) $R_1 = R_2 = R_3 = H$

3 Methylcarbazole (II) $R_1 = R_3 = H$; $R_2 = CH_3$

Glycozoline (III) $R_1 = H$; $R_2 = CH_3$; $R_3 = OCH_3$

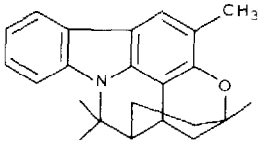
Glycozolidine (IV) $R_1 = R_3 = OCH_3$; $R_2 = CH_3$



Heptazolidine (V) $R_1 = OCH_3$; $R_2 = CH_3$; $R_3 = H$

Koenimbine (VI) $R_1 = CH_3$; $R_2 = OCH_3$; $R_3 = H$

Koenidine (VII) $R_1 = CH_3$; $R_2 = R_3 = OCH_3$



Murrayazoline (VIII)

aration is shown in Fig. 1. Better resolution of carbazole (I) and 3-methylcarbazole (II) could be obtained by decreasing the percentage of chloroform, but koenidine (VIII) took much longer to elute. For example, koenidine was eluted from the column in 1 h as a broad tailing peak when 20% chloroform in hexane was used as the mobile phase. It may be mentioned here that the order of elution of these alkaloids in HPLC was different from that obtained in gas chromatography[1].

The detection limits for these alkaloids were determined at 0.005 a.u.f.s. The limit for carbazole, 3-methylcarbazole, heptazolidine, koenimbine and koenidine was found to be 1–2 ng. This method can detect less than 1 ng of glycozoline, glycozolidine and murrayazoline.

In order to investigate the applicability of this method to the identification of carbazole alkaloids in plant extracts, the neutral fraction of a light petroleum (b.p. 40–60°C) extract of the root bark of *Glycosmis pentaphylla* (Retz) DC was examined using the present technique. It was observed that, besides glycozoline (III) and glycozolidine (IV), the two major carbazole alkaloids present in the plant, carbazole (I) and 3-methylcarbazole (II) could also be detected in the chromatograms. This is the first report of the detection of carbazole in a plant source. In addition, 3-methylcarbazole was detected for the first time in *Glycosmis pentaphylla*.

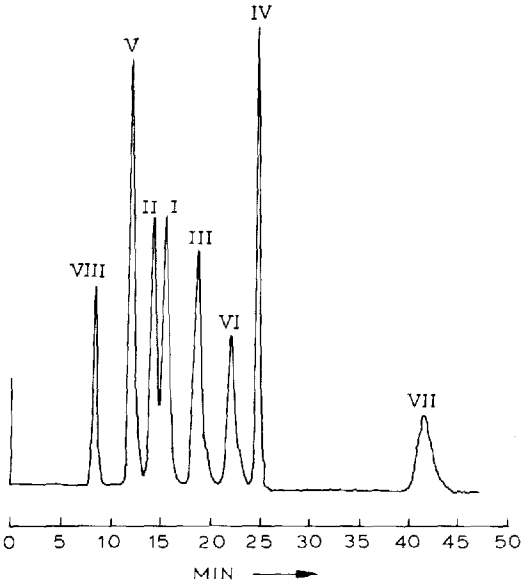


Fig. 1. HPLC chromatogram of carbazole (I) and seven carbazole alkaloids (II–VIII) on a μPorasil Radial-Pak cartridge using 25% chloroform in hexane; flow-rate 0.7 ml/min. Detection 254 nm; sensitivity 0.1 a.u.f.s.

In conclusion, HPLC provides a method for the resolution and identification of complex mixtures of carbazole alkaloids. The method may be extended for preparative or semipreparative separations.

REFERENCES

1 B. K. Chowdhury, A. Mustapha and P. Bhattacharyya, *J. Chromatogr.*, 329 (1985) 178.
2 D. P. Chakraborty, *Fortschr. Chem. Org. Naturst.*, 34 (1977) 299.