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COMBINED GAS CHROMATOGRAPHY AND MASS SPECTROMETRY OF AROMATIC β-CARBOLINES*

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SUMMARY

A method is described for the analysis of substituted aromatic β-carbolines employing gas chromatography combined with mass spectrometry (GC–MS). The spectra of the β-carbolines obtained by GC–MS are presented. Mass spectrometric patterns have been studied for their utility in the structural identification of biologically synthesized β-carbolines.

INTRODUCTION

Compounds with the β-carboline structural configuration have been of interest for some time because of the variety of actions which they evoke in biological systems. These actions include effects on enzymes[1–3], membrane translocation processes[4,5] and biogenic amine concentrations in brain and other tissues[6–8]. In addition, β-carbolines have psychotomimetic actions[9,10] and, when injected intraventricularly in very small doses, they increase the voluntary ingestion of ethanol by rats[11].

* The term β-carboline will be used to describe the class of heterocycles with which this paper is concerned. This is in conformity with the Ring Index usage. Other nomenclatures are also used to designate these compounds, *e.g.*, *Chemical Abstracts*' use of the term 9*H*-pyrido(3,4-*b*)indoles.

The presence of β-carbolines in plants[12,13] and animals[14] has raised questions about their biosynthesis. It has long been assumed that the nucleus arises in the plant by a Mannich-type (Pictet–Spengler) condensation of a tryptamine compound with an aldehyde[15] (Fig. 1). Such condensations have been carried out *in vitro*[16]. In animals, it is generally believed that the β-carboline nucleus is formed via a Pictet–Spengler condensation of an indolethylamine and an aldehyde[5]. A strong point in favor of this particular biosynthetic mechanism is the fact that direct condensation of aldehydes and biogenic indoleamines of biological significance has been reported to occur *in vitro* under conditions of temperature and pH similar to those existing in living tissues[4].

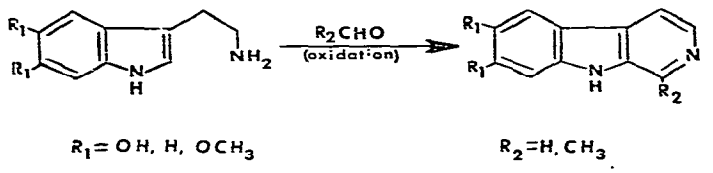

Fig. 1. Generalized reaction scheme for the formation of aromatic β-carbolines.

In order to study the biosynthesis of β-carbolines in human and animal tissues, there is a need for more specific and sensitive analytical methods than have been heretofore available. Existing methods, which involve ultraviolet and infrared spectroscopy, fluorimetry or spray-reagent treatment of thin-layer chromatograms, are deficient in terms of sensitivity, specificity and/or resolution.

This paper describes a sensitive gas–liquid chromatographic (GLC) method for the isolation and quantification of β-carbolines. GLC retention times and quantitative relationships are presented for four substituted aromatic β-carbolines together with their mass spectral characteristics.

EXPERIMENTAL

Reagents and materials

Reference samples of 1-methyl-β-carboline (harman), 6-methoxy-1-methyl-β-carboline (6-methoxyharman), 7-methoxy-1-methyl-β-carboline (harmine), 7-hydroxy-1-methyl-β-carboline (harmol) and spectral-grade methanol were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Dimethylchlorosilane was purchased from Pierce (Rockford, Ill., U.S.A.). OV-17 (3% on 100–120-mesh Gas-Chrom Q) and all other GLC materials were obtained from Applied Science Labs. (State College, Pa., U.S.A.). Additional solvents and inorganic chemicals were obtained from commercial sources at the maximal obtainable purity.

Standard solutions of reference β-carbolines were prepared by dissolving the compounds in 0.1 *N* formic acid in methanol. These GLC standard solutions were stable when stored in the dark at 4°.

Apparatus

GLC analyses were performed with a Varian Model 2700 gas chromatograph equipped with a flame-ionization detector. Coiled 6 ft. × 2.0 mm I.D. glass columns

were packed with 3% OV-17 on 100–120-mesh Gas-Chrom Q and operated isothermally at 246°. Dry helium, passed through a gas purifier (Applied Science Labs.), was used as the carrier gas at an initial pressure of 60 p.s.i., giving a flow-rate of 37 ml/min. The inlet temperature was 275° and the detector temperature was 300°.

Column conditioning included silylation of the glass column and packing with dimethylchlorosilane at 175° for 15 min. The column was then treated with three 25-μl injections of methanol. Finally, the column was conditioned at 300° for 1 h, then left overnight (16 h) at 340° with the detector side disconnected and carrier gas flow-rate maintained at 30 ml/min. This silylation–conditioning procedure was found to be essential for adequate and reproducible separations even with factory-silylated columns.

Combined gas chromatography–mass spectrometry (GC–MS) employed a Victoreen Model 4000 gas chromatograph coupled to a Hitachi–Perkin-Elmer RMS-4 single-focusing mass spectrometer operated with an ionization energy of 70 eV. The Victoreen gas chromatograph was connected to the mass spectrometer with a glass transfer line maintained at 300°. The Victoreen gas chromatograph was conditioned and operated in the manner described above for the Varian Model 2700. Perfluorokerosene (PFK) was used as a reference substance for calibrating the mass spectrometer.

The underivatized β-carbolines were dissolved in 0.1 *N* formic acid in methanol in concentrations of 50 pmoles/μl. Volumes of 1–10 μl of these solutions were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Gas chromatography

Fig. 2 is a gas chromatographic record demonstrating the separation of four substituted aromatic β-carbolines. The corresponding retention times were as follows: harman, 2.5; 6-methoxyharman, 4.37; harmine, 5.13; and harmol, 7.13 min. There is a linear detector response with concentrations varying from 55 to 550 pmoles (Fig. 3).

Mass spectrometry

Mass spectra of the GC effluents are shown in Figs. 4, 5, 6 and 7. The molecular ion (M^+) for each of the four compounds is the most abundant ionic species in each spectrum. As expected, the β-carboline nucleus and its 7-hydroxy derivative are stable in the mass spectrometer (Fig. 4, MS of harman; Fig. 5, MS of harmol).

Substitution of a methoxy group at the 6- or 7-position of the β-carboline nucleus yields an ion fragment at mass 197 in the mass spectrometer (Figs. 6 and 7). This fragment can be accounted for by the expected loss of a methyl group (mass 15) from each compound's molecular ion.

The spectra with masses of 197 and 212 do not by themselves distinguish between the 6- and the 7-methoxy-substituted aromatic β-carbolines (Figs. 6 and 7). However, these two compounds can be separated by means of the gas chromatograph (Fig. 2). Therefore, the identity of these derivatives can be established by correlating the relative retention times of the GC peaks with their individual mass spectra.

Thus, the identification and quantification of aromatic β-carbolines can be

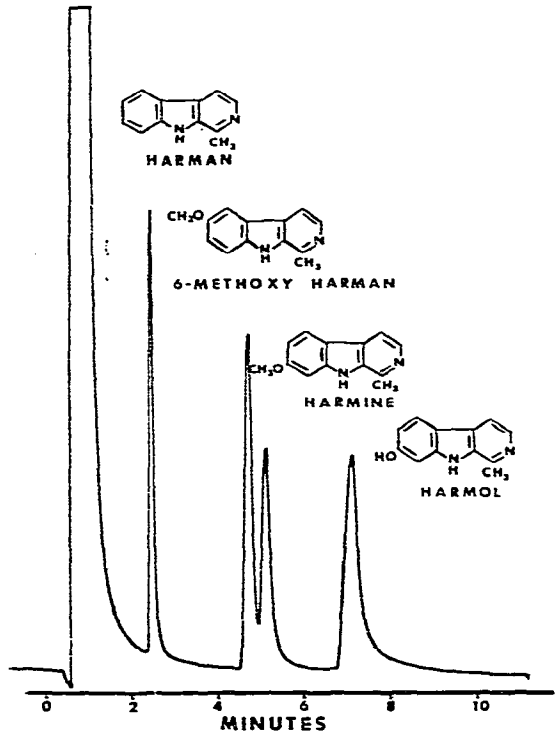

Fig. 2. Gas chromatographic record demonstrating the separation of four aromatic β-carbolines.

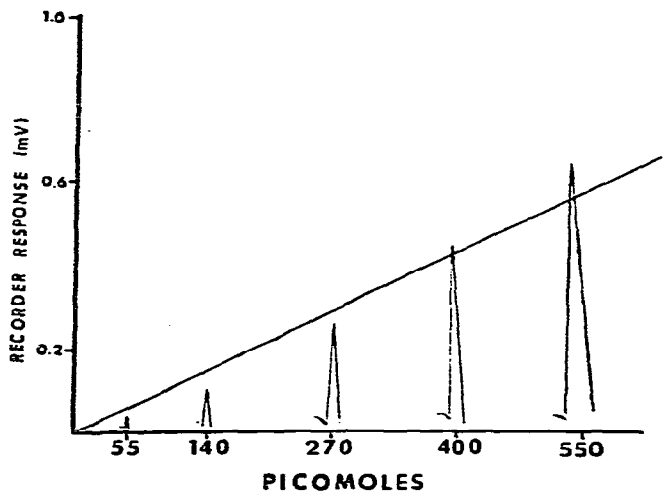

Fig. 3. Linear flame-ionization detector response of harman, which is representative of the aromat : β-carbolines.

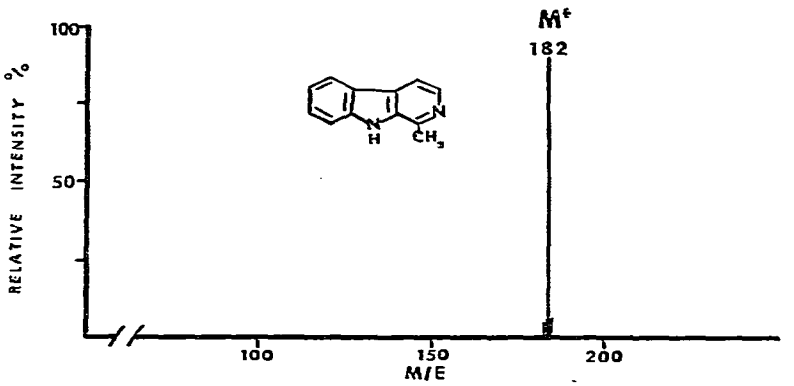

Fig. 4. Mass spectrum of harman. Minor ion fragments, including one with mass ($M^+ - 28$) formed by loss of H_2CN from the nucleus, are omitted from these mass spectrum records.

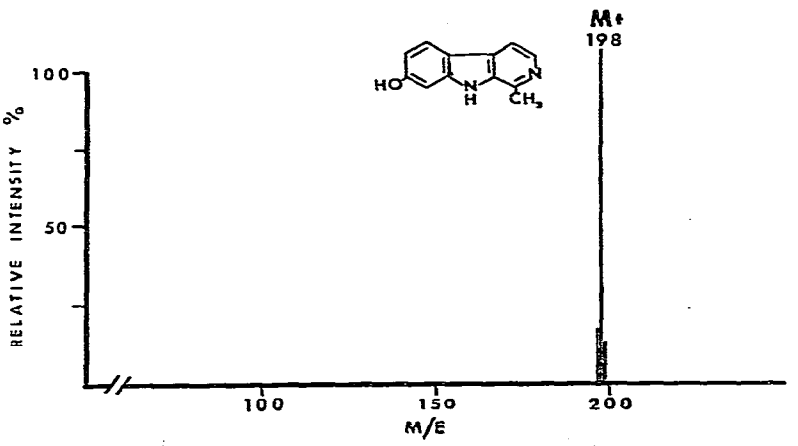

Fig. 5. Mass spectrum of harmol.

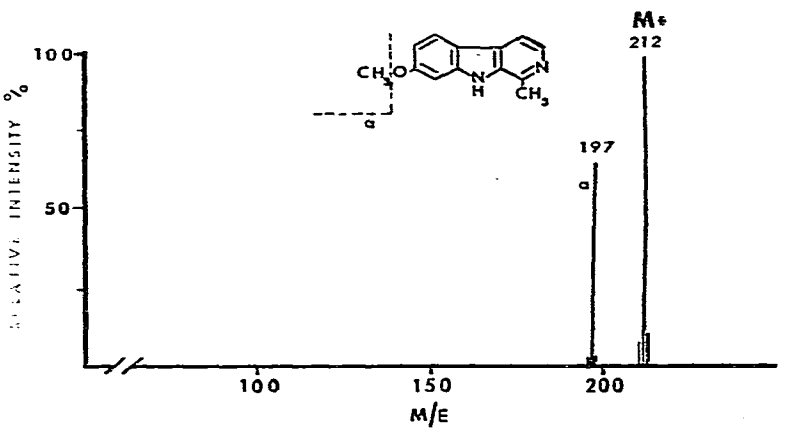

Fig. 6. Mass spectrum of harmine.

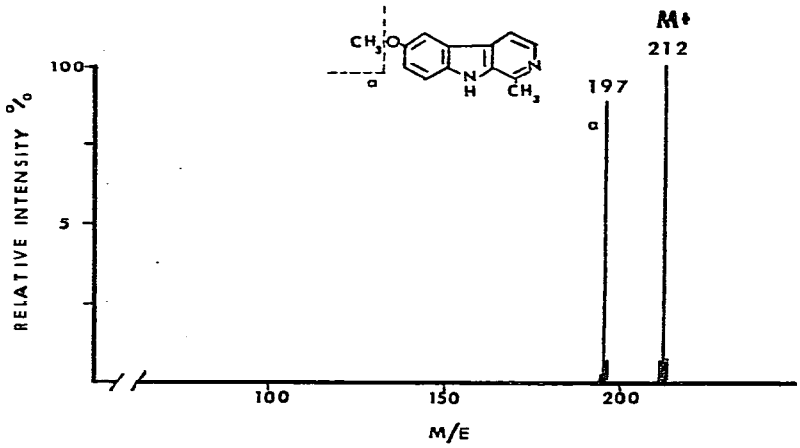

Fig. 7. Mass spectrum of 6-methoxyharman.

readily achieved by the use of combined GC–MS analyses. The fact that this type of analysis can readily be carried out with picomole amounts of these compounds should make the GC–MS methodology useful for studies of the biosynthesis, metabolism and pharmacological actions of β-carbolines in biological systems.

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