DETECTION OF BIOCHEMICAL INTERMEDIATES BY MASS FRAGMENTOGRAPHY: MESCALINE AND TETRAHYDROISOQUINOLINE PRECURSORS

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Received 25 November 1970
Original figures received 11 January 1971

1. Introduction

One may conveniently differentiate between two different stages in the formation of an alkaloid: 1) the diversion of a primary metabolite, such as an amino acid, by enzymatic reactions e.g. decarboxylations, oxidations, methylations, from the primary pathways towards the complete alkaloid skeleton – the protoalkaloid; 2) the further biochemical conversion of the protoalkaloid to other alkaloids. So far, little is known about the intermediates between the amino acids and the completed alkaloid skeletons. Major reasons for the limited knowledge of the early intermediates are their often low concentration and chemical instability, combined with extraction and separation problems. In general, the determination of biochemical reaction sequences is often complicated by the fact that the intermediates are quickly metabolized and seldom accumulate in readily detectable quantities. Suitable methods for the detection of these intermediates necessarily involves selective separation and sensitive identification techniques. As discussed earlier [1, 2], some of these requirements can be met by the technique of gas chromatography–mass spectrometry. This technique still involves a considerable amount of gas chromatographic and mass spectrometric work. We are at present interested in ring closure mechanisms for tetrahydroisoquinoline alkaloids in cacti [1, 3], and it occurred to us, that the search for precursors of this ring system might be facilitated by the use of the new development "mass fragmentography". The present work indicates that "mass fragmentography", introduced by Hammar, Holmstedt and Ryhage [4] and with the later modifications by Hammar and Hessling [5], is potentially useful for the sensitive identification of biochemical intermediates.

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2. Experimental

T. cuzcoensis Br & R was obtained from W. Haage, Erfurt, DDR and *Marginatocereus marginatus* (DC.) Backbg. and other cacti from K. Edelmann, Reeuwijk, The Netherlands.

Alkaloids were extracted and separated into phenolic and nonphenolic compounds as described [1].

Also details for gas chromatography and gas chromatography (5% SE-30, Gas Chrom P)–mass spectrometry have been published previously [1]. *N*-Methyl-3-methoxy-4-hydroxyphenethylamine was prepared by methylation of 3-methoxy-4-benzyloxyphenethylamine [7] and subsequent debenzylation [7]. The principles of mass fragmentography and the technical details have been described [4, 6] and with later modifications by Hammar and Hessling [5]. Further informations are given in legends to figs. 1–3. The compounds described in the legends have been identified also by gas chromatography–mass spectrometry.

3. Results and discussion

In mass fragmentography, the mass spectrometer is used as a gas chromatographic detector which continously monitors 1–3 selected mass numbers of compounds eluted from the gas chromatographic column [5, 6, 8]. In fig. 1A is shown a mass fragmentogram of 3-methoxytyramine (mass spectrometric details in legend to fig. 1) focusing upon mass numbers 137 and 138. The fragment m/e 137 is formed by benzylic cleavage of the phenethylamine and the one mass unit higher fragment (m/e 138) is the product of a McLafferty rearrangement [1, 7].

The ions m/e 137 and 138 are brought alternately into focus [6, 8] and the intensities recorded as a function of time (fig. 1A). The relative intensities of the fragments when recorded, do not necessarily have to be those found in the mass spectrum, but can be altered [5] to give peaks of more comparable and more readily estimated size e.g. fig. 1B, where the intensity of the m/e 167 fragment is doubled compared to the m/e 168 fragment.

So far, during our work on the biosynthesis of cactus alkaloids all precursors have been found to be phenolic amines [3, 4]. Thus, a number of cacti were investigated mainly for the presence of phenolic amines. With the knowledge that the likely potential precursors were phenethylamines with a varying substitution pattern of methoxy- and hydroxy-groups, and that such compounds yield highly predictable major fragments from benzylic cleavage, a first test should involve the search for fragments of m/e 137, 138 from hydroxy-methoxyphenethylamines (fig. 1A) and m/e 167, 168 from hydroxy-dimethoxyphenethylamines (fig. 1B).

Trichocereus cuzcoensis, a cactus found to produce mescaline, was first investigated (fig. 1C). The mass fragmentograms were recorded at a paper rate of 2.5 cm/min in contrast to 1 cm/min for the simultaneously recorded gas chromatogram (total ion current). To facilitate direct comparison, retention times and numbers of each peak are shown. In the m/e 137, 138 mass fragmentogram, two peaks appear which may be hydroxy-methoxy-phenethylamines. Peak 1 was however, by gas chromatography–mass spectrometry (GLC–MS) identified as tyramine. Thus, m/e 137 is M^+ (molecular ion) and m/e 138 = M^{+1}. Peak 2 closely agrees with the mass fragmentogram (ret. time., intensities) of 3-methoxytyramine (fig. 1 A) and was also found to be identified (GLC–MS) with this compound. Peak 4, m/e 137, is derived from a fragment (see legend to fig. 1C) of mescaline.

The mass fragmentogram of m/e 167, 168 shows (fig. 1C) the molecular ion of 3-methoxytyramine (peak 2) and a major fragment of mescaline (peak 4). The only peak that contains both m/e 167 and 168 is peak 3 and with roughly the same intensities as in the standard (fig. 1B). GLC–MS also showed peak 3 to be 3,4-dimethoxy-5-hydroxyphenethylamine – a known tetrahydroisoquinoline precursor [3]. The latter compound was originally identified in peyote [2] and the detectability of the compound in the phenolic alkaloid fraction of peyote was reinvestigated with mass fragmentography (fig. 2).

The alkaloid fraction of peyote is, in contrast to *T. cuzcoensis*, quite complex with over thirty compounds present, most of them in the phenolic fraction (fig. 2). Mass fragmentography (m/e 167, 168) of the complex mixture readily reveals the similarity (confirmed by GLC–MS) of the rather small peak 3 in the gas chromatogram with reference 3,4-dimethoxy-5-hydroxyphenethylamine (fig. 1B). Gas chromatographic results indicated that peak 6 could be the isomer and mescaline precursor 3,5-dimethoxy-4-hydroxyphenethylamine, which has a very similar mass spectrum [1], but this is ruled out by the mass fragmentogram. The only compound with the proper mass spectral intensities is clearly compound 3.

Similarly, the mass fragmentogram: m/e 137, 138 (fig. 2) shows that only peak 2 can be a hydroxy-methoxyphenethylamine although the intensities in

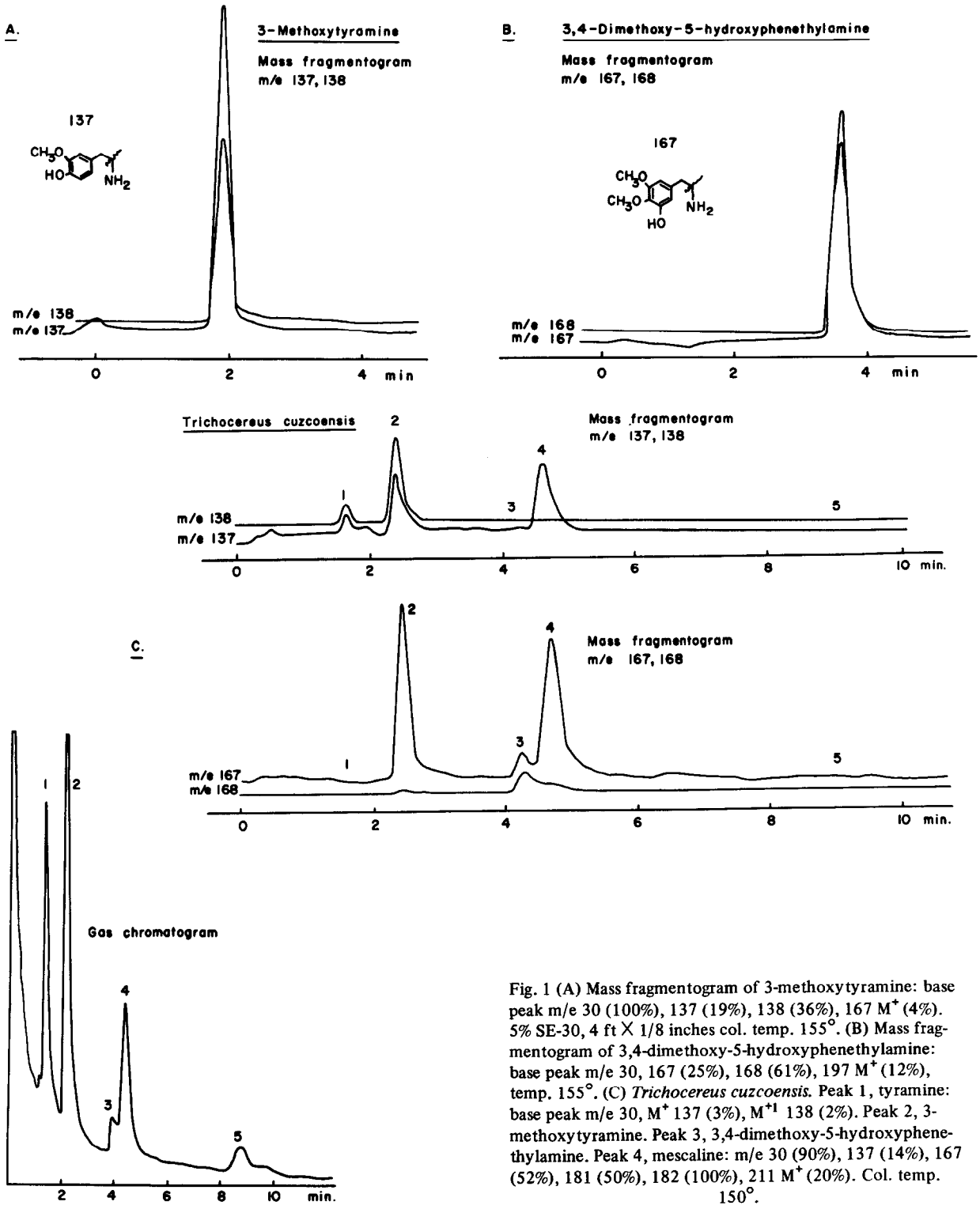

Fig. 1 (A) Mass fragmentogram of 3-methoxytyramine: base peak m/e 30 (100%), 137 (19%), 138 (36%), 167 M^+ (4%). 5% SE-30, 4 ft X 1/8 inches col. temp. 155°. (B) Mass fragmentogram of 3,4-dimethoxy-5-hydroxyphenethylamine: base peak m/e 30, 167 (25%), 168 (61%), 197 M^+ (12%), temp. 155°. (C) *Trichocereus cuzcoensis.* Peak 1, tyramine: base peak m/e 30, M^+ 137 (3%), M^{+1} 138 (2%). Peak 2, 3-methoxytyramine. Peak 3, 3,4-dimethoxy-5-hydroxyphenethylamine. Peak 4, mescaline: m/e 30 (90%), 137 (14%), 167 (52%), 181 (50%), 182 (100%), 211 M^+ (20%). Col. temp. 150°.

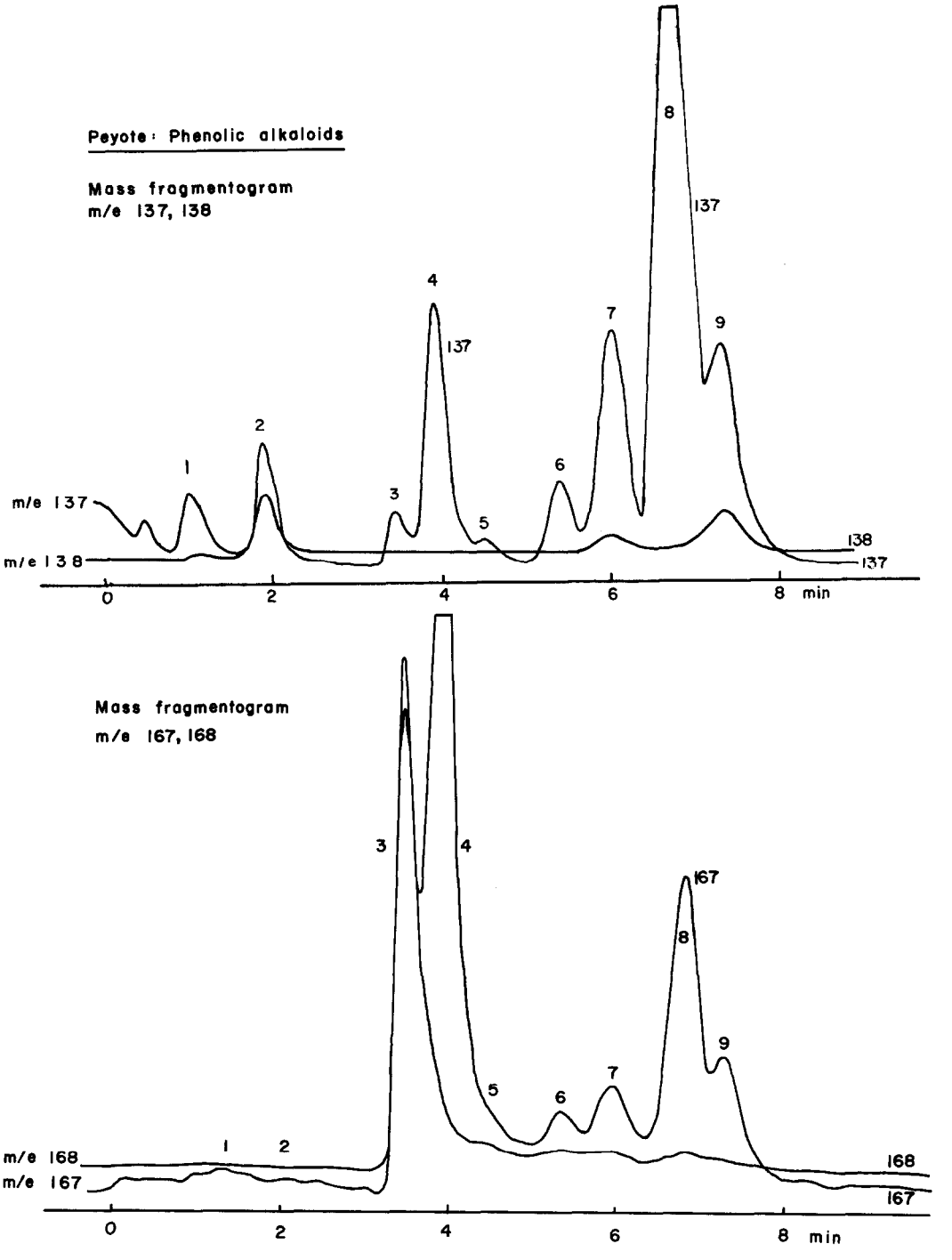

Fig. 2a. *Lophophora williamsii.* Mass fragmentograms of m/e 137, 138 and m/e 167, 168 in the phenolic alkaloid fraction of peyote. Peak 1, tyramine-hordenine. Peak 2, *N*-methyl-3-methoxy-4-hydroxyphenethylamine: base peak m/e 44, 137 (7%), 138 (22%), 181 M^+ (1%). Peak 3, 3,4-dimethoxy-5-hydroxyphenethylamine. Peak 4, mescaline. Peak 5, unknown. Peak 6, anhalidine. Peak 7–9, mixtures of several compounds. Col. temp. 155°.

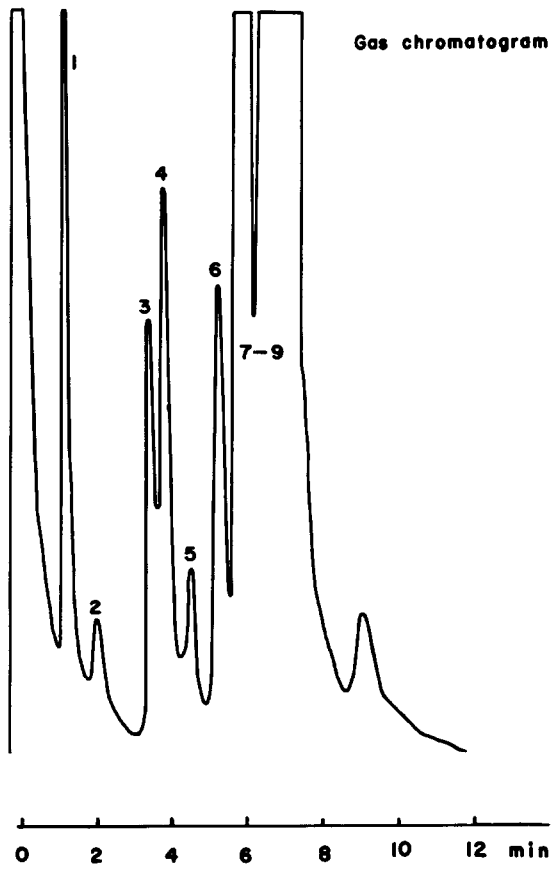

Fig. 2b. Gas chromatograph of m/e 137, 138 and m/e 167, 168 (see fig. 2a).

fig. 2 and fig. 1A are not quite the same. Further investigations by GLC–MS found compound 2 to be identical with synthetic *N*-methyl-3-methoxy-4-hydroxyphenethylamine, a compound previously not known from plants but a plausible alkaloid precursor.The mass fragmentogram (fig. 3) of *M. marginatus* (known to contain tetrahydroisoquinoline alkaloids) was run at the same sensitivity as in fig. 1C and well demonstrates the usefulness of mass fragmentography to prove the presence or in this case the absence of certain compounds.

In summary, biochemical reaction sequences, whether in vivo or in vitro, usually contain predictable intermediates. With a knowledge of mass spectrometry, it is possible to predict also certain typical features in their mass spectra. By the use of mass fragmentography, one can specifically and with high sensitivity [5, 6] look for the presence of these compounds. The technique is particularly useful for minor constituents in complicated mixtures (figs. 1C, 2, 3), since there is no need, as in GLC–MS, for complete resolution unless there is considerable

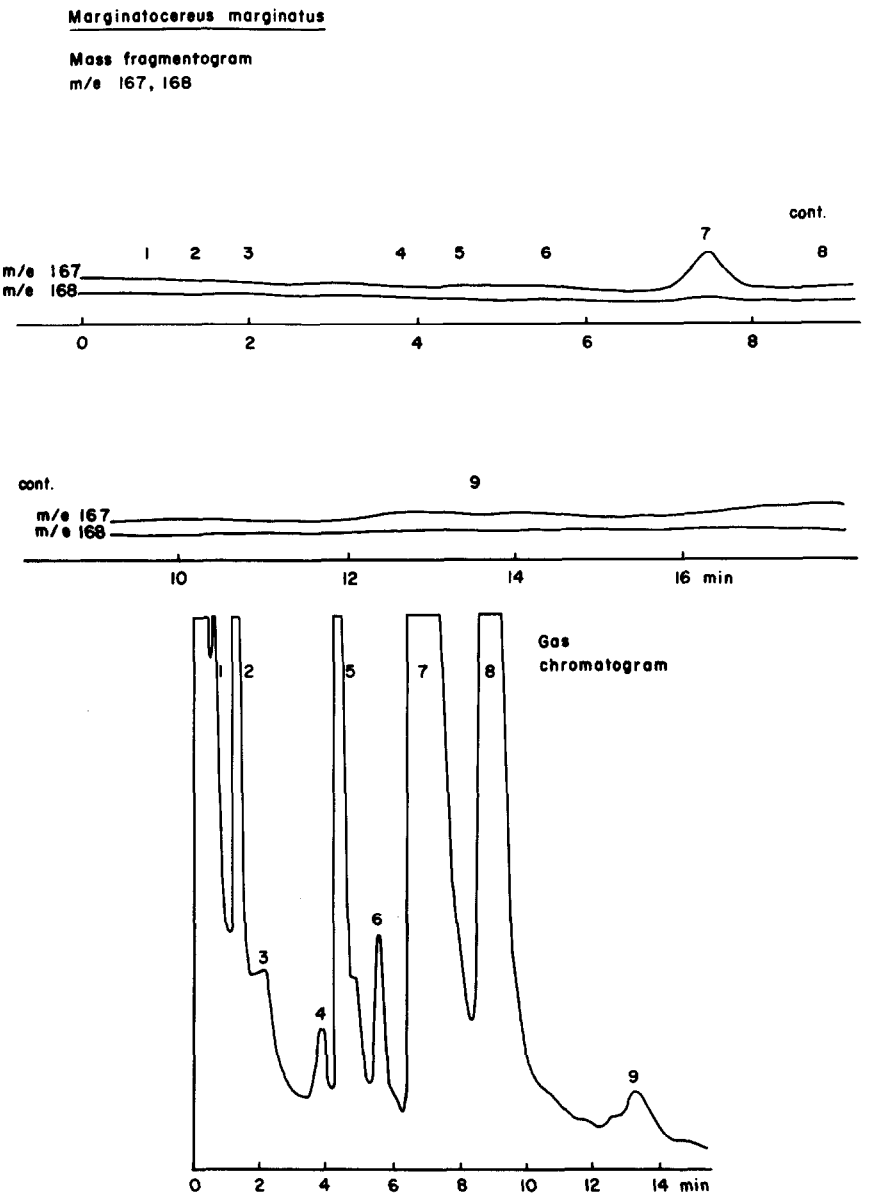

Fig. 3. *Marginatocereus marginatus.* Peak 1–6, 8–9 unknowns. Peak 7, lophocerine: base peak m/e 192, 167 and 168 (less than 1%), 249 M^+ (2%). Col. temp. 165°.

interference of the same fragment derived from another compound. The usefulness of mass fragmentography to pin-point key intermediates is amply demonstrated.

Acknowledgements

Support by (S.A.) Swedish Natural Science Research Council, (J.E.L.) the National Institute of Mental Health (MH 12007), the National Institute

of General Medical Sciences (GM 13978) and the Swedish Medical Research Council (B 70–40 X–2629) is gratefully acknowledged.

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