Tetrahedron Letters No. 46, pp 4471 - 4474.
0040-4039/78/1108-4471$02.00/0

DIRECT ANALYSIS OF MUSHROOM CONSTITUENTS BY MASS SPECTROMETRY

G. A. McClusky and R. G. Cooks*
Department of Chemistry, Purdue University, W. Lafayette, IN 47907

A. M. Knevel
Department of Medicinal Chemistry, Purdue University, W. Lafayette, IN 47907

New mass spectrometric methodology[1] for structural elucidation of components present in complex mixtures is here applied to a species of mushroom, Helvella esculenta. This approach is shown to have the following characteristics which are important in natural products chemistry: (i) no preparation of the biological material is required, (ii) extremely small samples suffice, (iii) highly specific structure-related information is obtained for each natural product examined.

Helvella esculenta, considered a delicacy by some but reported by others to be toxic,[2] has been reported[3] to contain acetaldehyde-N-formyl-N-methylhydrazone (gyromitrin, 1). Isolation of a compound which has been shown polarographically to contain the azomethine moiety has also been reported.[4] We show that gyromitrin can be detected in the mushroom without extraction or chromatography and that a variety of related compounds can be obtained from the mushroom. Structures for these compounds are suggested.

For this study whole freeze-dried or fresh mushroom (1 to 40 mg) was loaded in a direct insertion probe and introduced into a chemical ionization source.[5] The isobutane mass spectrum showed peaks at m/z 99, 101, 102, 103, 127, 143, 167, and 183 corresponding to components of molecular weight 98, 100, 101, 102, 126, 142, 166, and 182, respectively. Each of these species in turn was selected by mass-analysis for further characterization. This was effected by dissociation in a high velocity collision with nitrogen target gas and subsequent measurement of the masses of the fragment ions. The resulting MIKE spectra[7] are then interpreted to provide structural information on the selected components.[9]

Figure 1 shows the MIKE spectrum of m/z 101 obtained from a total of 1 mg of mushroom. This sample gave spectra for three hours while the spectrum shown was recorded in six minutes. Thus, of the order of 10^{-4} to 10^{-5} g should suffice to obtain the highly specific information on individual components exemplified by Figure 1. The validity of the MIKES methodology is established by the identification of a known component of this mushroom by comparison to a spectrum obtained from an authentic sample of the same component. It can be seen in Figure 1 that the MIKE spectrum of m/z 101 from the mushroom is identical to that of authentic gyromitrin. The presence of gyromitrin in this mushroom sample was further confirmed by an accurate mass measurement (100.064 ± .003, $C_4H_8N_2O$ = 100.064) by electron impact high resolution mass spectrometry.

The interpretation of the MIKE spectrum in Figure 1 employs standard mass spectrometric arguments and is assisted by a knowledge of the gyromitrin structure. The major fragmentations of protonated gyromitrin are illustrated in Scheme 1. The most abundant fragment ions can be

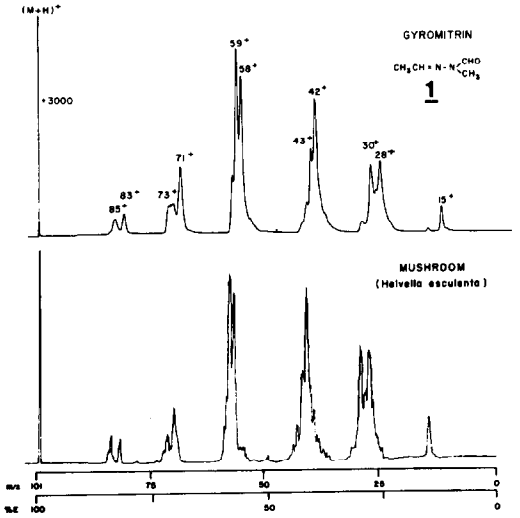
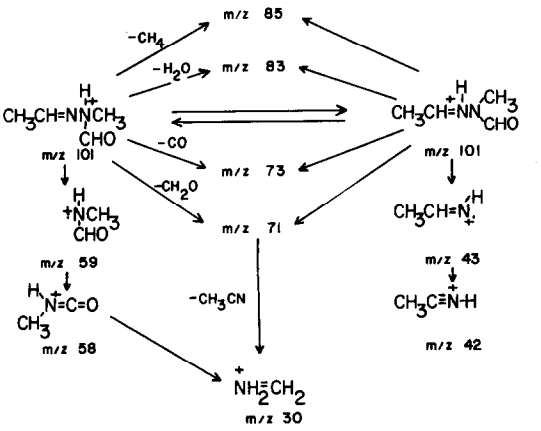


Figure 1. Comparison of the MIKE spectra of m/z 101 for authentic gyromitrin and mushroom sample.



Scheme 1. Some of the reactions of protonated gyromitrin.

accounted for by homolysis of the weak N-N bond with the charge residing on the protonated fragment ion. A subsequent hydrogen loss produces two very stable even-electron fragment ions of m/z 58 and 42. A number of fragmentation pathways involving the loss of stable neutral molecules (e.g., CH_4, H_2O, CO, CH_2O) are seen; they are particularly diagnostic of functional groupsand common to MIKE spectra of even-electron ions.[10] The electron impact mass spectrum of gyromitrin[11] is similar to the MIKE spectrum in that cleavage of the weak N-N bond is again important. The predominant fragmentation pathways involve N-N bond cleavage but this occurs

with hydrogen transfer so a neutral molecule is lost to produce ions of m/z 59 and 57.

The higher homologue of gyromitrin,[12] 3-methylbutanal-N-formyl-N-methylhydrazone (2), was identified from the fragmentations observed in the MIKE spectrum of m/z 143 derived from the mushroom (Figure 2). As in gyromitrin, the homolysis of the weak N-N bond is an important

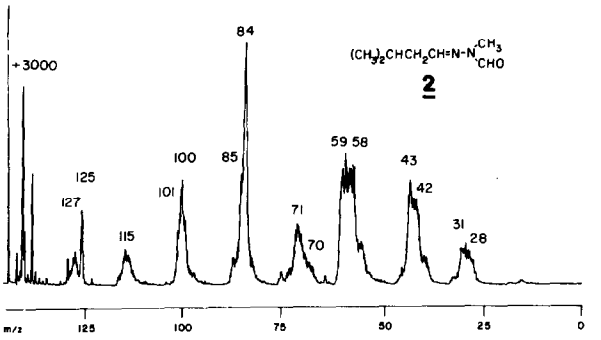


Figure 2. MIKE spectrum of m/z 143 from mushroom which is interpretated as proving the presence of butylgyromitrin.

fragmentation pathway and is followed by hydrogen loss to produce the stable even-electron ions of m/z 84 and 58. The same small stable neutral losses (e.g., CH_4, H_2O, CO) are again observed indicating the presence of the same functional groups. In addition, loss of propene and the isopropyl radical are strongly indicative of the 3-methylbutyl alkyl side chain.

Having established the utility of the MIKES mixture analysis methodology, we now focus on a set of unknown components in the mushroom. A striking feature of the MIKE spectra obtained from m/z 102, 103, and 99 (Table 1) on chemical ionization of the mushroom is the similarity

Table 1. MIKE spectra of mushroom components[a] at m/z 99, 102 and 103

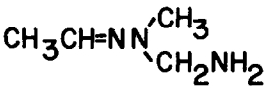
m/z 102: 86(45%), 85(95), 73(35), 72(60), 60(100), 58(50), 57(35), 56(20), 43(25), 42(40), 30(10), 29(12), 27(15)

m/z 103: 85(100%), 71(50), 57(55), 54(18), 52(16), 44(14), 43(16), 42(21), 32(6), 30(12)

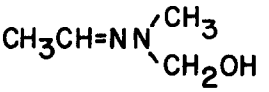
m/z 99: 82(75%), 71(38), 70(60), 69(87), 58(30), 57(60), 56(48), 55(60), 53(40), 44(28), 43(70), 42(100), 41(60), 39(40), 30(30), 29(25), 28(28), 27(26), 18(3), 15(6)

a) Spectra obtained on freeze-dried samples; relative intensities depended on probe temperature; this is a typical set of results.

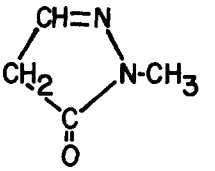
(e.g., intensity and distribution) of fragment ion multiplets in the lower half of the spectrum with the gyromitrin MIKE spectrum (compare Figure 1 and Table 1). This observation strongly suggests a structural relationship between gyromitrin and the components with apparent molecular weights of 101, 102, and 98. It is proposed that these components correspond to 3, 4, and 5,



3



4



5

respectively. Since the geminal aminohydrazine, 3, and the hydroxyhydrazine, 4, are not typically stable, they probably correspond to the gas phase reaction of N-methylacetaldehydrazone with formaldehyde or methylimine. It is less likely that 5 was derived by pyrolysis or a gas phase reaction. This preliminary result raises interesting questions regarding the biogenesis of azomethine compounds and provides further evidence of the usefulness of the MIKES technique in studying natural products.

Acknowledgment: This work was supported by the National Science Foundation (CHE 77-01295).

References

1. (a) T. L. Kruger, J. F. Litton, R. W. Kondrat and R. G. Cooks, Anal. Chem., 48, 2113 (1976); (b) R. Kondrat and R. G. Cooks, Anal. Chem., 50, 81A (1978).
2. P. H. List and P. Luft, Arch. Pharm. (Weinheim), 302, 143 (1969).
3. P. H. List and P. Luft, Tetrahedron Lett., 20, 1893 (1967).
4. M. Jemal, Ph.D. Thesis, Department of Medicinal Chemistry, Purdue University, 1976.
5. The compounds discussed here were evaporated from the probe over the temperature range 220-300°C.
6. Nominal pressure 2 x 10^{-5} Torr.
7. Mass-analyzed ion kinetic energy (MIKE) spectra; so named since kinetic energy measurements are used to infer the fragment ion masses.[8]
8. See J. H. Beynon, R. G. Cooks, W. E. Baitinger, J. W. Amy and T. Y. Ridley, Anal. Chem., 45, 1023A (1973).
9. For examples of such interpretation as applied to alkaloids, see T. L. Kruger, R. G. Cooks, J. L. McLaughlin and R. L. Raneiri, J. Org. Chem., 42, 4161 (1977); and R. W. Kondrat, R. G. Cooks and J. L. McLaughlin, Science, 199, 978 (1978).
10. (a) J. F. Litton, T. L. Kruger, R. G. Cooks, J. Amer. Chem. Soc., 98, 2011 (1976); (b) M. L. Sigsby, R. J. Day, and R. G. Cooks, submitted for publication.
11. H. Pyysalo and E. Honkanen, Acta. Chem. Scand. B, 30, 792 (1976).
12. H. Pyysalo, Naturwiss., 62, 395 (1975).

(Received in USA 16 August 1978)