IDENTIFICATION OF SIMPLE NATURALLY OCCURRING INDOLES BY MASS SPECTROMETRY*†

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Abstract—Mass spectra for twenty 3-substituted indoles are presented and the distinctive features discussed. These spectra are useful in identifying naturally occurring indoles in quantities of 1 μ g or less.

INTRODUCTION

NATURALLY occurring indoles are frequently identified by methods unsuited to rigorous structure proof. Ultraviolet, fluorescence or phosphorescence spectra as well as partition coefficients and colour reactions give only incomplete evidence of compound identity. Only small samples of natural indoles are usually available and many conventional chemical and physical methods cannot be used. Because of this, a combination of chemical reactions on a microscale and chromatography has been worked out for more positive identification of some plant indoles¹ and microtechniques have also been applied in recording i.r. spectra of small amounts of 3-indoleacetic acid.^{2, 3}

Mass spectrometry seems to have advantages in identifying natural indoles. Only microgram samples are needed with a direct sample introduction system and valid structural deductions may often be made even when the sample is impure, since characteristic and abundant fragment ions are formed. When reference spectra are available, access to authentic material is not necessary. This method is also useful for labile compounds since mass spectra, unaffected by thermal degradation of the sample, can usually be obtained when the sample is introduced directly into the ionization chamber of the mass spectrometer.

Mass spectral characteristics of a variety of simple indoles with different substituents were recently thoroughly investigated.⁴ Mass spectrometry has been used to aid in the identification of some simple natural indoles: 3-indoleacetic acid derivatives,⁵⁻⁷ trypto-phol,^{8,9} 3-indolelactic acid,¹⁰ neoglucobrassicin¹¹ and several tryptamine and tryptophan

- † Part IV in the series "Indoles and Auxins", for Part III see Ref. 27.
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derivatives and related indole bases.¹²⁻¹⁸ Standard spectra or information on some tryptamine and tryptophan derivatives are also available, ¹⁹⁻²⁶ and the mass spectral identification of simple indoles as 2.4.7-trinitro-9-fluorenone complexes is under study in these laboratories.^{27, 28} Much of the early work on the mass spectrometry of indoles, initiated by Beynon, has been reviewed.4, 29-33

RESULTS

All indole compounds investigated (Figs. 1-4) vielded a molecular ion. As noted by other workers, compounds of the type indole-3-CH₂R give an abundant and characteristic fragment ion at m/e 130. This ion was usually very intense, often the base peak, and frequently accounted for most of the ion current.

The structure of the m/e 130 ion is probably either (I) or (II) formed by fragmentation β to the pyrrole ring. As far as the authors are aware, there is no direct evidence to favour either structure. Work with ¹³C-labeled 2-methylindole indicated that the quinolinium structure was involved in this case.³⁴



SCHEME I.

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Fig. 3.



With indole-3-CH₂R compounds substituted on the benzene moiety or on the nitrogen the ion 129 + R' was observed (Fig. 2d; cf. Refs. 5-7, 13, 14, 16-18) indicating the substituent on the indole ring. The corresponding ion is also prominent with tryptophan reduced in the benzene ring³⁵ (dihydro-m/e 132; tetrahydro-m/e 134) and 2-(2',4'-dinitrophenylthio)-derivatives of natural indoles.³⁶

Compounds of the structure indole-3-CH₂-CH₂-R" usually yield m/e 143 ions as well as an intense m/e 130 peak. This ion is of low intensity in compounds where R" = -OH (Fig. 1d) or -NH₂ (cf. Ref. 28) and about 10% as abundant as the base peak where R" = -COOH or -CH₂COOH (Figs. 3e, 3a). It is more abundant when R" = -NHCH₃ or -N(CH₃)₂ (cf. Refs. 13, 15, 16, 18, 25) and becomes the base peak in N-acetyltryptamine derivatives (cf. Refs. 22, 27).

3-Indoleacetyl- N^{ω} -diamino acid derivatives and peptides (Figs.4a, 4b; cf. Ref. 37) yielded an abundant ion at m/e 157 as well as the m/e 130 base peak. This ion is insignificant in simple amides, esters and the free acid (Figs. 1c, 1e and 1a); it is present in two nitrophenyl esters of 3-indoleacetic acid and becomes quite abundant in 3-indoleacetic anhydride and 3-indoleacetic acid N-hydroxysuccinimide ester.

As observed by Powers,⁴ 3-indolecarboxylic acid and 3-indolecarboxaldehyde give simple spectra showing the molecular ion and loss of —OH or —H to give an ion of m/e144. 5-, 6- and 7-hydroxy-3-indolecarboxylic acids give an analogous ion while the 4-hydroxy-3-indolecarboxylic acid loses water to give C₉H₅NO₂^{+, 38} 3-Indolyl aryl and alkyl ketones behave similarly in giving the corresponding ion.^{4, 39}

EXPERIMENTAL

Standard Indoles

3-Indoleacetaldehyde 2,4-dinitrophenylhydrazone, m.p. 200°, was prepared from the 3-indoleacetaldehyde sodium bisulphite addition compound.⁴⁰ The 2,4-dinitrophenyl derivative of tryptamine was prepared by the method of Oikawa *et al.*⁴¹ m.p. 221–222° (reported m.p. 215°). (Found: C, 59·00; H, 4·27; N, 17·22. Calc. for $C_{16}H_{14}N_4O_4$: C, 58·89; H, 4·33; N, 17·17%.) Previously prepared samples of 3-indoleacetyl-lysine⁴² and -ornithine³⁷ were used. All other compounds were obtained commercially.

Mass Spectrometry

70 Volt spectra were recorded with a Consolidated Electrodynamics Corporation 21–110B instrument using the standard probe for direct introduction of the sample, except where otherwise indicated. Other operating conditions are reported in Table 1.

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These data are being deposited with the Mass Spectrometry Data Centre, A.W.R.E., Aldermaston, Berkshire.

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/		Source temp.
Code	Name	(°Č)
1a	3-Indoleacetic acid	124
b	3-Indoleacetonitrile	Ž00*
с	3-Indoleacetamide	141
d	Tryptophol	204*
e	Ethyl 3-indoleacetate	202*
2a	3-Indolecarboxaldehyde	122
ь	3-Indolecarboxylic acid	141
с	Gramine	91
d	Serotonin	232
e	3-Indoleacrylic acid	169
3a	3-Indole-y-butyric acid	111
b	3-Indole-L-lactic acid	129
С	Tryptophan	235
d	3-Indolepyruvic acid	179
e	3-Indole-β-propionic acid	111
4a	3-Indoleacetyl-N ⁸ -L-ornithine	274
b	3-Indoleacetyl-N ^ϵ -L-lysine	269
c	3-Indoleacetaldehyde 2,4-dinitrophenylhydrazone	223
d	2,4-Dinitrophenyltryptamine	217
e	3-Indoleacetaldehyde	150†

TABLE 1. COMPOUNDS AND MASS SPECTROMETRY CONDITIONS

* By indirect introduction, reservoir temperature 200°.
† By direct introduction as 2,4,7-trinitro-9-fluorenone complex ²⁸ using a wide-range controlled temperature sample probe;⁴³ sample temperature 85°.

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