

STUDIES IN MASS SPECTROMETRY<sup>1</sup>

SPORIDESMIN AND SPORIDESMIN B

J.S. Shannon, Division of Coal Research, C.S.I.R.O.

P.O. Box 175, Chatswood, N.S.W., Australia.

(Received 1 March 1963)

The structure of sporidesmin ( $C_{18}H_{20}Cl N_3O_6S_2$ ) (I), a metabolic product of the mould Pithecoces chartarum, has recently been determined both by chemical<sup>2</sup> and X-ray<sup>3</sup> methods. A second product sporidesmin B (II) from the same mould was also described<sup>2</sup>. This present communication presents the results of the mass spectroscopic study of I and II from which a structure has been assigned to II.

Both I and II are characterized by very low volatility and extreme thermal instability, especially when in contact with surfaces of either metal or enamel inlet systems of the mass spectrometer. These difficulties were obviated and mass spectra obtained by inserting the compounds directly into the ion source and carefully heating them on the fringe of the ionizing electron beam. If I or II were heated by this method a little above their critical temperature of volatilization an intense peak at  $m/e$  256 due to sulphur ( $S_8^+$ ) appeared, followed by a series of seven others at  $m/e$  224 ( $S_7^+$ ), 192 ( $S_6^+$ ) ..... 32 ( $S_1^+$ ) and the peaks due to ion fragments of I or II gradually disappeared. The absence in the spectra of the sulphur peaks was used as an indication of volatilization of I or II without decomposition.

<sup>1</sup>Part V. For Part IV, see J.S. Shannon, C.G. Macdonald and J.L. Courtney,

Tetrahedron Letters 4, 173 (1963).

<sup>2</sup>R. Hodges, J.R. Ronaldson, A. Taylor and E.P. White, Chem. & Ind. (in press).

<sup>3</sup>A. McL. Mathieson, Tetrahedron Letters 2, 81 (1963).

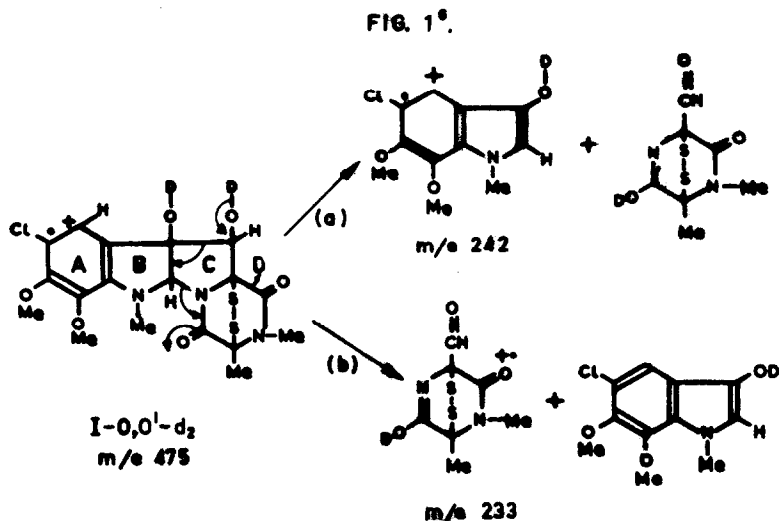
The mass spectra of I and II each contained pairs of molecular peaks at  $m/e$  475, 473 and at  $m/e$  459, 457 respectively, and the ratios of the heights in each pair were equal to the isotopic abundance ratio  $Cl^{37}/Cl^{35}$  indicating the presence of one chlorine atom<sup>4</sup>. Further, when deuterium oxide was admitted into the ion source<sup>5</sup> two hydrogens in I and one hydrogen in II were exchanged. This result, when considered in conjunction with the difference of sixteen in molecular weight between I and II, suggested that the latter contained one and the former two hydroxyl groups.

Both the spectra of I and II had strong M-64 and metastable peaks corresponding to the single step loss of 64 mass units from the respective molecular ions due to the elimination of  $S_2$  of disulphide bridges.

The spectrum of I contained a strong pair of peaks at  $m/e$  241, 243 of relative intensity consistent with the presence of one chlorine atom and a weak peak at  $m/e$  232. In the spectrum of I- $O, O^1-d_2$  the peaks at  $m/e$  241, 243 were resolved into two pairs; the more intense of these were shifted to  $m/e$  242, 244 while the other pair remained at their original values. The peak at  $m/e$  232 was shifted to  $m/e$  233. Thus cleavage across ring C by two different mechanisms was indicated; the first of these (Fig. 1) involved a hydrogen transfer from the secondary hydroxyl group and the second (Fig. 2) a hydrogen transfer from the tertiary hydroxyl group on to, in both instances, a carbonyl oxygen atom in ring D. Models showed that both of these transfers were feasible.

<sup>4</sup>J.H. Beynon, "Mass Spectrometry and its Applications to Organic Chemistry". Elsevier, Amsterdam 1960, p.299.

<sup>5</sup>This method of producing O-deuterated derivatives "in situ" has been used successfully with a wide range of compounds. See J.S. Shannon, Aust. J. Chem. 15, 265 (1962); J.L. Courtney and J.S. Shannon, Tetrahedron Letters 1, 15 (1963); J.S. Shannon, C.G. Macdonald, and J.L. Courtney, Tetrahedron Letters 4, 173 (1963).



A metastable peak at  $m/e$  122.8 in the spectrum of I confirmed the occurrence of reaction (a). With the second mechanism the occurrence of the hydrogen transfer from the tertiary hydroxyl group was demonstrated by the shift of the peak at  $m/e$  169 in the spectrum of I to  $m/e$  171 in the spectrum of I-O-d<sub>2</sub>.

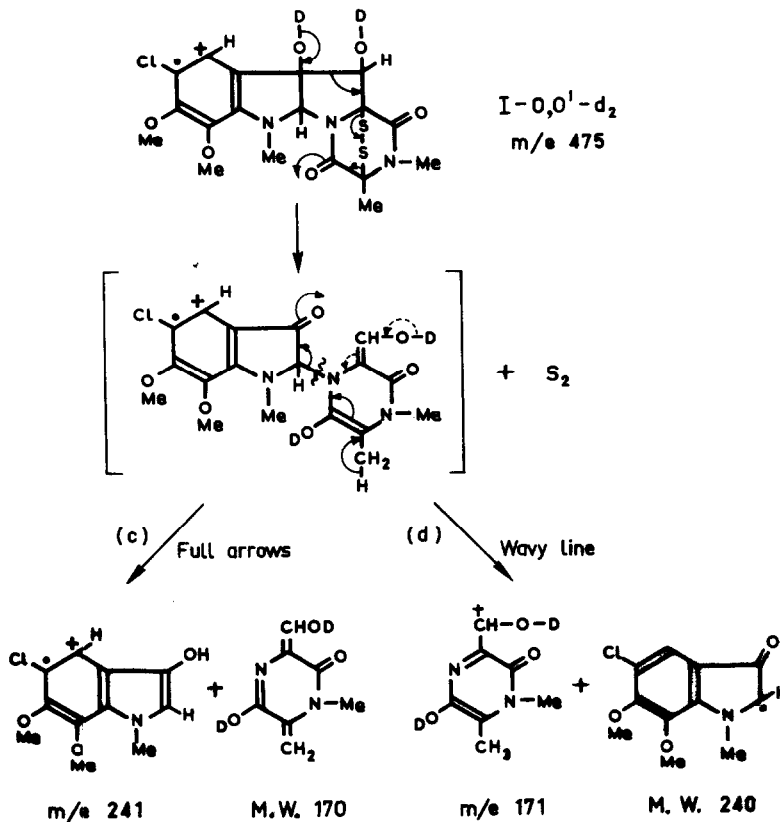
The hydrogen atom which must be transferred to produce ions of  $m/e$  241, 243 (reaction (c) Fig. 2) during the cleavage of the C-N bond of the proposed

6(i) For convenience of illustration localized charges are depicted whereas in fact they could be dispersed in the respective ions, see A.L. Wahrhaftig, in "Advances in Mass Spectrometry" (J.D. Waldron, ed.) p.274. Pergamon Press, London, 1959. Reactions (a) and (b) represent the two possible ways in which fragments of the formulae shown can be produced differing from one another according to which fragment becomes a radical ion and which a neutral molecule.

6(ii)  $m/e$  and N.W. values based on Cl = 35.

6(iii)  $\curvearrowright$  = 2 electron shifts.  $\curvearrowleft$  = 1 electron shift.

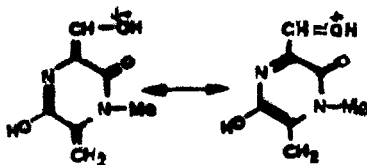
FIG. 2.



intermediate shown in brackets may originate from the methyl group (full arrows). The most favourable alternative, namely transfer of the O-deuterium (dotted arrows), would cause formation of ions  $m/e\ 242, 244$  unless there was random hydrogen rearrangement throughout the molecular ion prior to or during the C-N bond cleavage. The occurrence of such rearrangement was considered unlikely since the ion of  $m/e\ 171$ , formed by homolytic rupture of the C-N bond (reaction (d), Fig. 2), lost none of its deuterium during or before its formation, despite its having a higher appearance potential than the ions formed in reaction (c).

Besides the  $M-64$  peaks already referred to, the mass spectrum of II had other features in common with the spectrum of I which confirmed the close relationship of II with I. For example, the former also contained a pair of peaks at  $m/e$  241, 243 and a peak at  $m/e$  153 which corresponded to the peak at  $m/e$  169 in the spectrum of I. The difference in the values of the masses of the two peaks was equivalent to the extra hydroxyl group in the molecular ion of I. Because of the presence of the peak at  $m/e$  153 and the absence of a peak at  $m/e$  216, corresponding to  $m/e$  232 in the spectrum of I, the operation of the second mechanism for cleavage across ring C (Fig. 2) was indicated for II. Confirmatory evidence for this was obtained from the spectrum of II- $O-d$  in which the peaks at  $m/e$  241, 243 were not shifted, whereas the peak at  $m/e$  153 was shifted to  $m/e$  154, entirely consistent with the requirements of the second mechanism (Fig. 3).

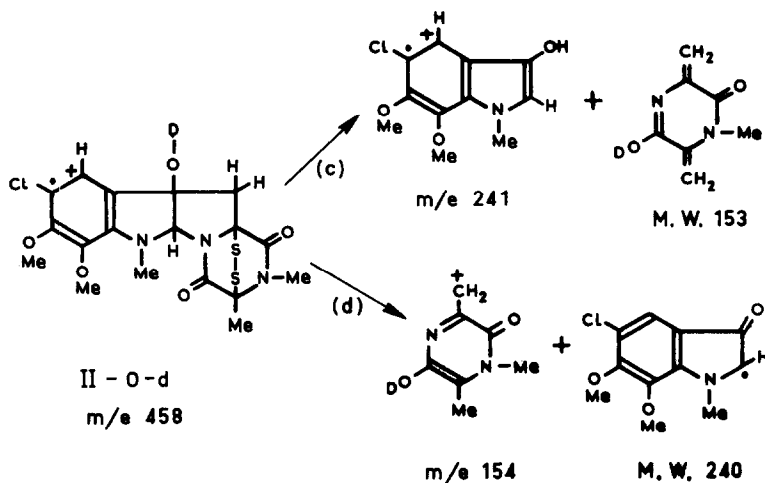
There was a minor peak at  $m/e$  168 and also at  $m/e$  240 in the spectrum of I (and at  $m/e$  170 and  $m/e$  240 in the spectrum of I- $O, O-d_2$ ) due to ions of the fragments which were produced as a neutral molecule and as a free radical respectively in the reactions illustrated in Fig. 2. A corresponding weak peak appeared at  $m/e$  240 in the spectrum of II but none at  $m/e$  152 corresponding to the peak at  $m/e$  168 in the spectrum of I; the presence of the additional hydroxyl group in I may have contributed to the resonance stabilization of the ion due to this latter peak; for example,



The ratio of the intensities of the peaks  $m/e$  153 :  $m/e$  241 was significantly higher in the spectrum of II than was the corresponding ratio  $m/e$  169 :  $m/e$  241 from the spectrum of I, which data were consistent with the

formation from II of the ion  $m/e$  241 by only one compared with two routes with I. Similarly the total abundance of the ion  $m/e$  241 with respect to its molecular ion was greater with I than with II. Thus the mass spectral data indicated not only that II was a de-oxy derivative of I, but also that the hydroxyl group present in I and not in II was the secondary group attached to ring C.

FIG. 3.



Acknowledgements - The author is grateful to Dr. R. Hodges, Dr. A. Taylor, and their colleagues at Ruakura Animal Research Station, Hamilton, New Zealand, for providing both stimulating discussions and the samples of sporidesmin and sporidesmin B. The investigation was made possible by the support and encouragement of Mr. H.R. Brown, Chief, Division of Coal Research, C.S.I.R.O.