THE IDENTIFICATION OF DEPSIPEPTIDES BY CHEMICAL IONISATION MASS SPECTROSCOPY[1,2]

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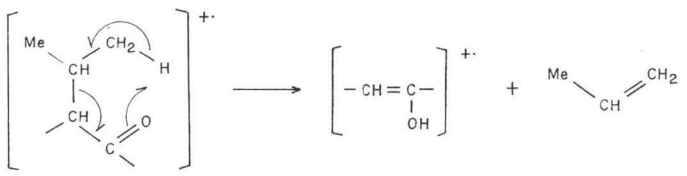
The chemical ionisation mass spectra of seven naturally occurring depsipeptides and some of their permethylated derivatives have been measured. The primary ionisation process involves an ester group and not an amide or other functionality. It probably occurs randomly when the molecule contains more than one ester link. Unlike electron impact mass spectra, those obtained under chemical ionisation conditions gave sequence information for all of the depsipeptides examined or their permethylated derivatives. A mechanism for the primary fragmentation is proposed.

The characterisation of complex antibiotic peptides[1] and depsipeptides[2] produced in fermentation broths has been helped by interpretation of their mass spectra. In certain cases[3] however, the mass spectra of such metabolites has only provided evidence of the molecular weight and elemental composition; information often compromised by the presence of congeners. Such ambiguities may arise by elimination of side chains by a mechanism[4] such as that shown in Scheme 1, or due to the low volatility of the metabolite(s) or due to high energies involved in the ionisation process. It seemed that some of these difficulties might be circumvented under conditions of low ionisation energy in the chemical ionisation source, and hence a representative set of depsipeptides have been examined by this method.

Scheme 1.



Methane or isobutane were used as sources of reactive ionic species. In both cases the most abundant ions in the spectra of all the depsipeptides were MH^+. In agreement with other work[5] the ions $MC_3H_3^+$ and $MC_4H_9^+$ were observed in the isobutane spectra but they were of low relative abundance (*e.g.* **I**, R=R′=H, R″=CHMe$_2$, X=O; *m/e* 654: *m/e* 692: *m/e* 710=100: 3.6: 0.07) and hence of less diagnostic value than the corresponding ions in the methane spectra. In Table 1, normalised values for the ions $(M-1)^+$, $(M+29)^+$, and $(M+41)^+$ in the latter spectra are given. The ratios are all very similar, and resemble those reported by MUNSON and FIELD[6] for simple esters which are thought to be characteristic of the 'basicity' of the reacting functional groups[7]. Thus, in the case of

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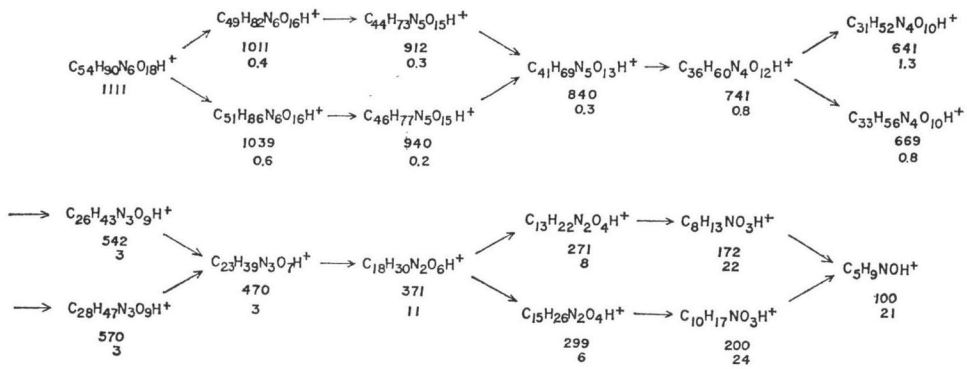
Table 1. Normalized abundances of $(M-1)^+$, $(M+29)^+$ and $(M+41)^+$ ions in the chemical ionisation mass spectra of cyclodepsipeptides using methane as the ionising gas. $MH^+=100$.

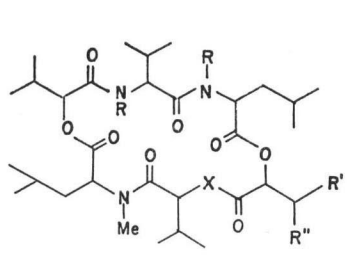
Cyclodepsipeptide	No. of atoms in ring	Normalised abundances of ions		
		$(M-1)^+$	$(M+29)^+$	$(M+41)^+$
Pimaydolide, **I**, R=R′=H, R″=Pr$^{\beta}$, X=O	18	6	7	6
Isariin, **VI**, R=H	19	10	7	4
Sporidesmolide I, **I**, R=H, R′=R″=Me, X=NH	18	10	15	9
Enniatin A, **II**	18	6	6	5
Valinomycin, **III**	36	5	5	4
Pithomycolide, **V**	17	1	6	3

Scheme 2.

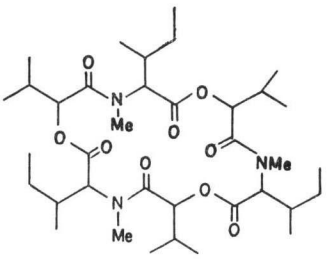


depsipeptides they indicate that the primary reaction occurs at an ester functionality and not *e.g.* at an amide. Support for this deduction was obtained from the mass spectrum of valinomycin (**III**). Here initial ionisation may occur either at a lactic acid residue or at an α-hydroxyisovaleric acid group, and the subsequent fragmentation following each of these ionisations will be different. This is shown in Scheme 2. If the assumptions are made that only reactions of the type: $X^+ \rightarrow (X\text{-}Y)^+ + Y$ occur, and that once the molecule becomes ionised there is an equal chance of fission at any amide or ester bond, the abundance of ions will be inversely proportional to their mass. The normalised abundances given in Scheme 2 fit this theory quite well if it is remembered that the ions *m/e* 840, 741, 470, and 371 are the products of two different fragmentation processes.

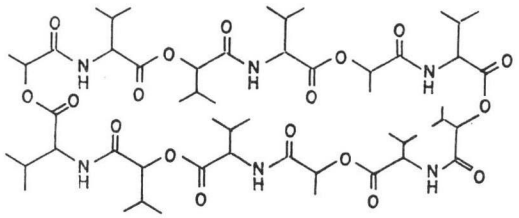
The ion reactions of the depsipeptides: isariin (**VI**)[8], enniatin A (**II**)[9], peptidolipin (**IV**)[10], pithomycolide (**V**)[11], pimaydolide (**I**, R=R′=H, R″=CHMe$_2$, X=O)[3], sporidesmolide I (**I**, R=H, R′=R″=Me, X=NH)[12] and valinomycin (**III**)[13] can be rationalised in terms of Scheme 3. A metastable ion indicating the reaction **VII**→**VIII** was observed in the cases of enniatin A, pimaydolide, and valinomycin. The characteristic reaction of the ion **IX** is a hydrogen transfer process, three variations of which are shown in Scheme 3. The evidence for this reaction is as follows. In the spectra of enniatin A (**II**), pimaydolide (**I**, R=R′=H, R″=CHMe$_2$, X=O), trimethylsporidesmolide I (R=R′=R″=Me, X=NMe), and valinomycin (**III**), the fragment ions which indicate the sequence of amino and hydroxy acid residues are one mass unit greater than the calculated ion weights. Secondly, in the spectrum of



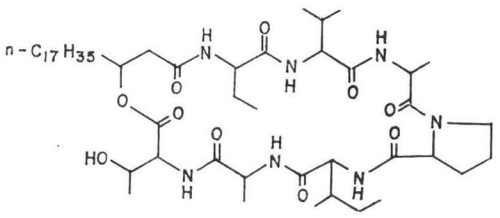
I



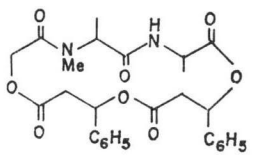
II



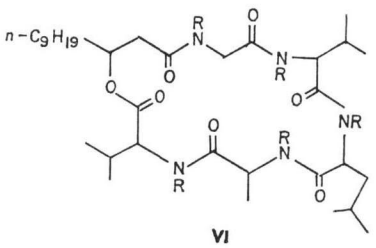
III



IV



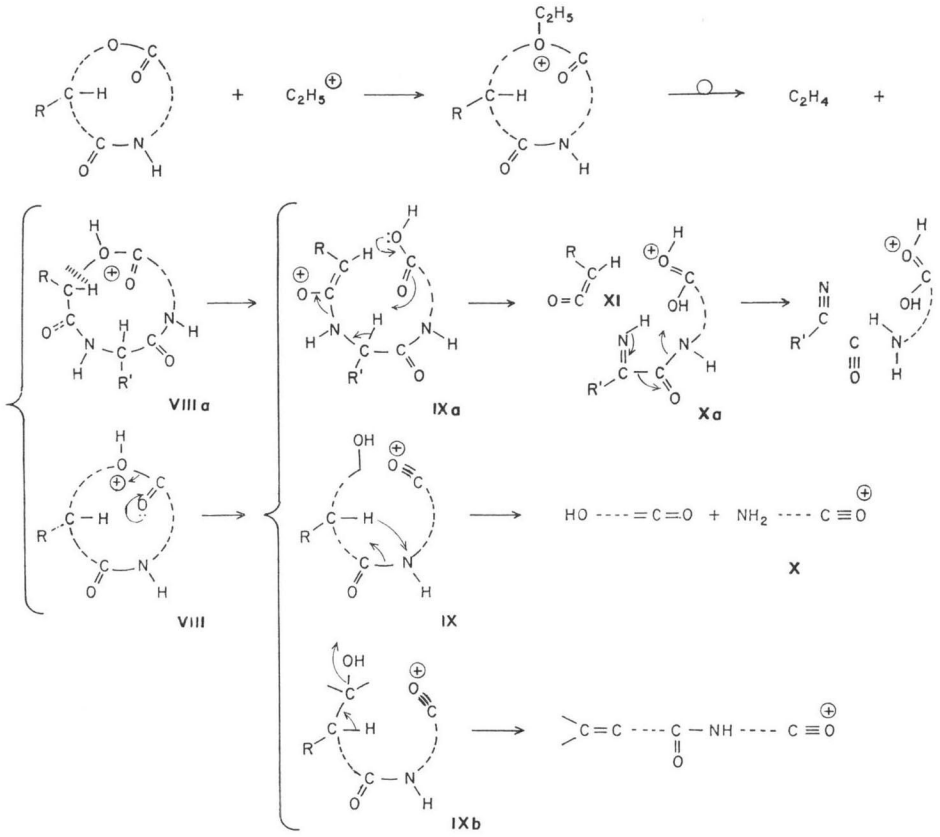
V



VI

dimethylpimaydolide (**I**, R=Me, R′=H, R″=CHMe$_2$, X=O) where tetradeuteriomethane was used as the ionising gas, the ions: $(M+C_3{}^2H_5)^+$, $(M+C_2{}^2H_5)^+$, and $(M+{}^2H)^+$ were observed, their ratios being the same as the ratios of the corresponding ions when methane was used as the ionising gas. However, the fragment ions *m/e* 582, 455, 355, 242, and 115 did not contain deuterium and their normalised abundances were the same as those found in the spectrum where the reagent ions were derived from methane. Thus the deuteron on the cationic oxygen of species **VIII** is not retained in the fragmentation of the species **IX**, but a proton (which cannot be an amide proton) is shifted as shown in Scheme 3. If the transfer reaction shown in **IX** occurred at random with all other ester and amide groups in the ion there would necessarily be produced ions of high mass that proceed to fragment in the usual way, thus giving sequence information. Such reactions were observed in the

Scheme 3.



cases of enniatin A, pimaydolide, trimethylsporidesmolide I, and valinomycin. The rearrangement **VIIIa**→**IXa** is included for completion, to show an alternative mode of lactone rupture. The ion **IXa** may fragment to species such as **Xa** or by similar processes to analogues of **Xa** one or more amino/hydroxy acid units smaller (in such cases presumably neutral species HN=CHR′ and CO are lost in addition to the ketene **XI**). The ionic products of such reactions thus give sequence information. This mode of fragmentation was uncommon in the spectra of the depsipeptides reported here, but is of high probability in other cases[14]. However, the molecular architecture may be such that there is a high probability of the hydrogen transfer reaction proceeding in a specific direction. An obvious case (and one which clearly differentiates this group of depsipeptides) is that of depsipeptides containing β-hydroxy acid residues, where the reaction shown of **IXb** has a high probability. This reaction involves the loss of the proton acquired in the ionisation process, hence further fragmentation gives abundant ions of correct ion weight. Such reactions were observed in the spectra of peptidolipin (**IV**), pentamethylisariin (**VI**, R=Me), and pithomycolide (**V**). There remain the examples of sporidesmolide I (**I**, R=H, R′=R″=Me, X=NH) and isariin (**VI**, R=H). In both cases the charge is retained on a fragment **X** of low molecular weight, clearly identified in the case of isariin as the hydroxy acid residue. It is interesting that methylation of the peptide NH groups of isariin reduces the probability of this reaction to the point that the characteristic fragmentation of this type of depsipeptide predominantes.

Experimental

Chemical ionisation mass spectroscopy was done using a MS-9 mass spectrometer (Associated Electrical Industries Ltd.) whose source was modified as described by VARENNE *et al.*[15] All samples were introduced directly into the source. Depsipeptides were permethylated as described by RUSSELL *et al.*[3] No precise mass measurements were made: molecular formulae given are assumed. The data is presented in the format: *m/e* value, normalized abundance ($MH^+ = 100$), assumed molecular formula, and in the case of pimaydolide, the number of protons exchanged with ^2H_2O in the source.

Enniatin A (II)[9]

Enniatin A was obtained from cultures of *Fusarium sambucinum* (HLX 316)* as described.[16] Mass spectral data was acquired at a source temperature of 220°C, methane pressure in the source, 0.5 torr and a repeller voltage of 5 volts. Hereafter the source conditions used for each compound are given in the order, and as defined in the previous sentence. 682, 100, $C_{36}H_{63}N_3O_9H^+$; 582, 0.1, $C_{31}H_{55}N_3O_7H^+$; 455, 0.2, $C_{24}H_{42}N_2O_6H^+$; 355, 0.02, $C_{19}H_{34}N_2O_4H^+$; 228, 3.2, $C_{12}H_{21}NO_3H^+$; 128, 0.07, $C_7H_{13}NOH^+$.

Isariin (VI)[8]

Isariin was a gift from Dr. L. C. VINING. 200°, 0.5 torr, 20 volts. 638, 100, $C_{33}H_{59}N_5O_7H^+$; 595, 19, $C_{30}H_{53}N_5O_7H^+$; 198, 24, $C_{12}H_{22}O_2^+$. Pentamethylisariin (VI, R=Me) 190°, 0.5 torr, 10 volts. 708, 100, $C_{38}H_{69}N_5O_7H^+$; 577, 65, $C_{32}H_{57}N_4O_5^+$; 492, 127, $C_{28}H_{50}N_3O_4^+$; 365, 164, $C_{21}H_{37}N_2O_3^+$; 252, 52, $C_{15}H_{26}NO_2^+$; 181, 8, $C_{12}H_{21}O^+$.

Peptidolipin (IV)[10]

Peptidolipin was a gift from Dr. G. MICHEL. 260°, 0.5 torr, 20 volts. Data is given for the C_{20} homologue. 948, 100, $C_{50}H_{89}N_7O_{10}H^+$; 829, 15, $C_{46}H_{81}N_6O_7^+$; 758, 7, $C_{43}H_{81}N_6O_7^+$; 645, 3, $C_{37}H_{65}N_4O_5^+$; 548, 28, $C_{32}H_{58}N_3O_4^+$; 477, 13, $C_{29}H_{53}N_2O_3^+$; 378, 36, $C_{24}H_{44}NO_2^+$.

Pimaydolide (I, R=R′=H, R″=$CHMe_2$, X=O)

Pimaydolide was isolated from cultures of *Pithomyces maydicus* (HLX 694) as described[3]. 230°, 0.5 torr, 2 volts. 654, 100, d_2, $C_{34}H_{59}N_3O_9H^+$; 524, 7, d_2, $C_{28}H_{49}N_3O_6H^+$; 411, 11, d_2 (?), $C_{22}H_{38}N_2O_5H^+$; 312, 2, d_0, $C_{17}H_{29}NO_4H^+$; 212, 9, d_0, $C_{12}H_{21}NO_2H^+$.

Dimethylpimaydolide (I, R=Me, R′=H, R″=$CHMe_2$, X=O) 160°, 0.5 torr, 5 volts. 682, 100, $C_{36}H_{63}N_3O_9H^+$; 582, 3, $C_{31}H_{55}N_3O_7H^+$; 455, 13, $C_{24}H_{42}N_2O_6H^+$; 355, 12, $C_{19}H_{34}N_2O_4H^+$; 242, 7, $C_{13}H_{23}NO_3H^+$; 115, 3, $C_6H_{10}O_2H^+$.

Dimethylpimaydolide (I, R=Me, R′=H, R″=$CHMe_2$, X=O), 170°, 0.7 torr ($^2H_2O + C^2H_4$), 5 volts. 727, 4, $C_{36}H_{63}N_3O_9C_3^2H_5^+$; 715, 9, $C_{36}H_{63}N_3O_9C_2^2H_5^+$; 683, 100, $C_{36}H_{63}N_3O_9{}^2H^+$; $(M-1)^+$, 6; 582, 3, $C_{31}H_{55}N_3O_7H^+$; 455, 18, $C_{24}H_{42}N_2O_6H^+$; 355, 12, $C_{19}H_{34}N_2O_4H^+$; 242, 10, $C_{13}H_{23}NO_3H^+$; 115, 8, $C_6H_{10}O_2H^+$.

Pithomycolide (V)

Pithomycolide was isolated from cultures of *Pithomyces chartarum* (HLX 133) as described by RAHMAN *et al.*[11] 190°, 0.5 torr, 2 volts. 553, 100, $C_{30}H_{36}N_2O_8H^+$; 464, 63, $C_{27}H_{30}NO_6^+$; [405, 9, $C_{21}H_{28}N_2O_6H^+$] 316, 37, $C_{18}H_{22}NO_4^+$; 231, 6, $C_{14}H_{15}O_3^+$; 131, 41, $C_9H_7O^+$.

Sporidesmolide I (I, R=H, R′=R″=Me, X=NH)

Sporidesmolide I was isolated[12] from a culture of *P. chartarum* isolated in the Republic of South Africa by Dr. G. C. A. VAN DER WESTHUIZEN (HLX 1369). 200°, 0.25 torr, 20 volts. 639, 100, $C_{33}H_{58}N_4O_8H^+$; 527, 8, $C_{25}H_{42}N_4O_8H^+$; no other ions $> m/e$ 100 > 0.01. Trimethylsporidesmolide I (I, R=R′=R″=Me, X=NMe), 200°, 0.5 torr, 2 volts. 681, 100, $C_{36}H_{64}N_4O_8H^+$; 581, 8, $C_{31}H_{56}N_4O_6H^+$; 468, 7, $C_{25}H_{45}N_3O_5H^+$; 341, 11, $C_{18}H_{32}N_2O_4H^+$; 241, 12, $C_{13}H_{24}N_2O_2H^+$; 128, 15, $C_7H_{13}NOH^+$.

Valinomycin (III)

Valinomycin was a gift from Dr. J. C. MACDONALD. 200°, 0.5 torr, 5 volts. Mass spectral data given in Scheme 2.

* Accession number to the collection of cultures held at the Atlantic Regional Laboratory.

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