HIGH RESOLUTION MASS SPECTRA OF MALFORMIN AND RELATED CYCLIC PEPTIDES

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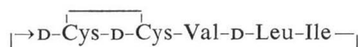
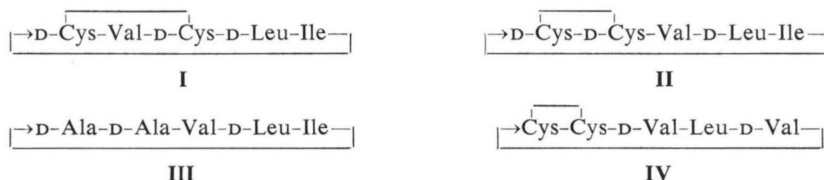
A comparison of the high resolution mass spectra of synthetic and natural preparations of malformin provided new evidence for the revised structure:



The interpretation of the mass spectra was aided by the examination of the spectra of the synthetic peptides, desthiomalformin and enantio-5-valine malformin.

Malformin, a metabolic product of several fungi, can cause severe curvatures on plants[1] and has antibacterial[2] and cytotoxic[3] properties as well. It was discovered by CURTIS[1], who, with his associates, isolated the active material in pure form[4] and determined its structure[5]. Mass spectra of members of the malformin family[6] were also reported[7]. These spectra were in general agreement with the proposed[5] structure of a cyclic pentapeptide disulfide (I, Fig. 1),

Fig. 1. I—The sequence originally proposed[5] for malformin;
II—The revised[8,9] sequence of malformin;
III—Desthiomalformin[12];
IV—Enantio-5-valine malformin[13]



although there were some unidentified ions that were considered to arise through rearrangements under the conditions of mass spectrometry. More recently, a revision of the structure of malformin became necessary. The revised structure (II, Fig. 1) was confirmed by synthesis[8,9]. The properties of the synthetic compound, including its biological activities, were indistinguishable from those of the natural product. Nevertheless, additional independent evidence for the correctness of the revised structure (II) seemed to be desirable.

It was not *a priori* obvious that mass spectra can provide the evidence sought. Homodetic[10] cyclic peptides do not follow the fairly simple fragmentation pattern of linear acylpeptide esters, or peptide lactones. In a ring formed only by peptide bonds, no distinct starting point of fragmentation is present and, therefore, instead of one series of sequence ions, several sets of fragments are generated. Furthermore, in addition to the bonds most frequently ruptured in

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linear peptides, namely the bonds between amino acid residues and the bonds connecting the carbonyl carbon and α-carbon in these residues, in homodetic cyclic peptides, fragmentation occurs also between α-carbons and the nitrogen atoms[11]. Because of these complications, we felt that a mere comparison of the high resolution mass spectra of natural and synthetic malformin preparations might not yield convincing evidence that could corroborate our revision of the structure. We expected, however, that an extension of the study to synthetic desthiomalformin[12] (**III**, Fig. 1) and to the synthetic, biologically active analog of malformin, enantio-5-valine malformin[13] (**IV**, Fig. 1) would enhance the probability of satisfactory interpretations.

The high resolution spectrum of the synthetic *cyclo*-pentapeptide desthiomalformin (**III**) revealed the molecular ion peak at *m/e* 467 (Table 1). Ions resulting from fragmentation of amino acid side chains were of minor importance and, for the sake of simplicity, these are not included in Table 1. The elimination of single amino acid residues led to moderately intense signals for ions of the $(M-NH \cdot CHR \cdot CO)^+$ type, while the loss of fragments with the general formula NH·CHR, described by MILLARD[11], was somewhat more pronounced. This was not limited to the residues with bulky side chains, as found by this author in a different cyclopeptide, although the NH·CHR fragments from valine, leucine and isoleucine were indeed the most abundant ones. The combination of elimination of NH·CHR and CO=NH led to fairly intense peaks corresponding to ions with the general formula $(M-NH \cdot CHR \cdot CONH)^+$. More importantly, intense signals were found for ions in which the mass of the molecular ion was diminished by NH·CHR·CO·NH·CHR′·CONH. These ions provided the desired information about the amino acid composition of the five possible tripeptide sequences. The tripeptides (Val, Leu_2), (Ala, Leu_2), (Ala, Val, Leu), (Ala_2, Leu) and (Ala_2, Val) allow only the sequence <u>*cyclo*—Ala—Ala—Val—Leu—Leu</u>, but do not permit a differentiation between leucine and isoleucine. Therefore, while only "leucine" was written, it has to be kept in mind that both peptides **II** and **III** contain one residue each of leucine and isoleucine. Furthermore, the data of the high resolution mass spectra gave no clue for the direction of the peptide chain.

Table 1. Desthiomalformin ($C_{23}H_{41}N_5O_5$)

Mass found	Rel. int.[b] (%)	Interpretation[a] (ions)
468.3196	27.74	MH and ^{13}C isotope of M
467.3095	22.75	M
466.3017	2.74	M−H
451.3117	2.17	MH−OH
450.3066	7.25	M−OH
424.3063	0.83	M−HNCO
424.2759	1.48	M−NHCHR (Ala)
397.2507	3.38	MH−NHCHR (Val)
396.2366	0.71	M−NHCHR (Val)
383.2315	3.12	MH−NHCHR (Leu)
382.2228	5.55	M−NHCHR (Leu)
396.2755	1.09	M−Ala
369.2504	0.58	MH−Val
368.2381	1.90	M−Val
367.2369	1.05	M−(Val+H)
354.2297	4.66	M−Leu
381.2584	12.76	M−(Ala·NH)
353.2311	14.57	M−(Val·NH)
339.2125	37.04	M−(Leu·NH)
310.2257	4.81	(Val, Leu_2)−NH
282.1945	12.06	(Ala, Leu_2)−NH
268.1799	6.94	(Ala, Val, Leu)−NH
240.1451	17.88	(Ala_2, Leu)−NH
239.1371	16.42	(Ala_2, Leu)−NH_2
226.1299	10.96	(Ala_2, Val)−NH

[a] Leu stands for both Leu and Ile.

[b] The most abundant ion (100%) with *m/e* 86.0963 corresponds to Leu−CO+H; the next most intense peak (69.73%) with *m/e* 72.0813 was interpreted as $H_2N-{}^+CH-CH(CH_3)_2$ from valine.

As anticipated, the spectrum of the synthetic pentapeptide disulfide **IV** was more complex (Table 2). A general tendency for the loss of S, SH, H_2S, and particularly of H_2S_2 could by clearly discerned. The fragmentation along the peptide backbone was superimposed on the elimination of the disulfide

Table 2. Enantio-5-valine malformin ($C_{22}H_{37}N_5O_5S_2$)

Mass found	Rel. Int. (%)	Interpretation[a] (ions)
547.1921	0.68	M+S
516.2278	2.34	M+H
515.2240	6.54	M
498.2144	0.40	M−OH
484.2556	0.67	M+H−S
483.2509	0.94	M−S
482.2438	0.85	M−HS
481.2348	0.65	$M-H_2S$
452.2861	0.18	$M+H-S_2$
451.2793	0.35	$M-S_2$
450.2726	1.23	$M-HS_2$
449.2662	1.42	$M-H_2S_2$
384.1788	0.20	(Cys−H, Val, Leu, Dha+H)
350.1936	0.54	(Dha, Dha, Leu, Val)
337.1924	0.40	(Dha, Dha, Val, Val)+H
336.1838	0.42	(Dha, Dha, Val, Val)
310.2160	1.30	(Val, Val, Leu)−H
238.1110	0.56	(Dha, Dha, Val)+H
237.1050	1.32	(Dha, Dha, Val)
213.1602	1.36	(Leu, Val)+H
212.1563	1.65	(Leu, Val)
211.1438	9.01	(Leu, Val)−H
169.0978	1.85	(Dha, Val)+H
167.0808	1.17	(Dha, Val)−H
138.0405	0.75	(Dha, Dha)
444.1532	0.32	M−NH·CH·R (Val)
378.1849	0.14	$M-H_2S_2$−NH·CH·R (Val)
397.1980	0.16	(Cys, Val, Val, Leu) −NH−H
387.1240	0.13	(Cys, Cys, Val, Val)−NH
366.2361	0.39	(Dha, Leu, Val, Val) −NH+H
321.1660	0.47	(Dha, Dha, Val, Val)−NH
296.2104	0.24	(Val, Val, Leu)−NH
267.1683	1.02	(Val, Dha, Leu)−NH+H
223.1043	1.22	(Dha, Dha, Val)−NH+H
197.1420	1.44	(Val, Leu)−NH
154.0831	3.87	(Dha, Val)−NH+H
378.1849	0.18	CO·(Dha, Dha, Val, Leu)
338.2031	1.71	CO·(Val, Val, Leu)−H
308.1654	0.70	CO·(Dha, Val, Leu)+H
265.1080	0.27	CO·(Dha, Dha, Val)
239.1324	2.15	CO·(Val, Leu)−H
276.0866	0.24	(Cys, Cys, Val)−CO+H
185.1648	1.32	(Leu, Val)−CO+H
112.0661	4.73	(Dha, Dha)−CO+2H
111.0562	5.33	(Dha, Dha)−CO+H
110.0521	1.60	(Dha, Dha)−CO
169.1441	1.19	(Leu, Val)−CO−NH
143.0788	2.99	(Cys, Val)−CO−NH+H
126.0906	6.06	(Dha, Val)−CO−NH+H

[a] The base peak (100 %) is a fragment with m/e 72.0824 ($C_4H_{10}N$, Val−CO+H).

bridge and the concomitant formation of dehydroalanine (Dha, $-NH-\overset{\overset{CH_2}{\|}}{C}-CO-$) residues. These residues were recognized as constituents of several fragment ions. The mass 547 (515+32) probably corresponds to a trisulfide which might have originated from collisions between H_2S_2 with still intact molecules of **IV**, or from a small amount of trisulfide in the synthetic product. The characteristic appearance of the ions corresponding to dehydroalanyl-dehydroalanyl and of (Val_2, Leu), together with the absence of fragments corresponding to (Dha, Leu), (Val, Val) and to (Dha_2, Leu), would have allowed an unequivocal sequence assignment, if the sequence would not have been known before.

The experience gained through the examination of the spectrum of **IV** was helpful in the interpretation of the spectra of synthetic and natural malformin (Table 3) since, apart from the configuration of individual residues, malformin is the next higher homolog of compound **IV**. Because of the presence of both leucine and isoleucine in malformin, the mass spectra in themselves were not sufficient for the determination of the structure. Yet, the spectra, and particularly the presence of a fragment corresponding to Dha-Dha, prove the crucial point of the structure revision[8,9]: that the two half cystine residues are next to each other in the sequence. Also, the presence of fragment ions corresponding to (Val, Leu) or (Val, Ile), (Dha, Val), (Leu, Ile), (Val, Leu, Ile) and to (Dha_2, Val), and particularly the excellent agreement between the spectra of the natural and synthetic products are all in harmony with the revised structure (**II**) of malformin.*

* For a preliminary account of this study cf. BODANSZKY, M.; J. HENES, S. NATARAJAN, G. L. STAHL & R. L. FOLTZ: Cyclic pentapeptides related to malformin. Polymer Preprints 16-2: p. 133, 1975.

Table 3. Malformin (natural) ($C_{23}H_{39}N_5O_5S_2$)

Mass found	Rel. int. (%)	Interpretation[a] (ions)
561.2090	0.12	M+S*
530.2473	2.68	M+H
529.2432	6.24	M
512.2279	0.46	M−OH*
498.2679	0.47	M+H−S
497.2610	0.54	M−S
496.2552	0.68	M−HS
495.2500	0.14	M−H_2S
465.2909	0.17	M−S_2
464.2893	0.97	M−HS_2
463.2795	0.67	M−H_2S_2
350.1983	0.26	(Dha, Dha, Leu, Val)
324.2250	2.90	(Val, Leu, Leu)−H
282.1874	0.09	(Dha, Val, Leu)+H
281.1785	0.15	(Dha, Val, Leu)
280.1715	0.28	(Dha, Val, Leu)−H
238.1221	0.13	(Dha, Dha, Val)+H
237.1189	1.01	(Dha, Dha, Val)
225.1543	1.27	(Leu, Leu)−H
213.1562	0.43	(Leu, Val)+H
212.1476	1.85	(Leu, Val)
211.1431	11.06	(Leu, Val)−H
181.0971	0.11	(Leu, Dha)−H
169.0980	2.17	(Dha, Val)+H
167.0822	0.81	(Dha, Val)−H
139.0507	0.43	(Dha, Dha)+H
444.1428	0.50	M−NH·CH·R (Leu)*
401.1460	0.11	(Cys, Cys, Val, Leu)−NH*
338.2094	0.28	(Dha, Dha, Val, Leu) −NH+3H*
336.1887	0.14	(Dha, Dha, Val, Leu) −NH+H
267.1710	0.73	(Dha, Val, Leu)−NH−2H*
223.1049	0.25	(Dha, Dha, Leu)−NH+H

Mass found	Rel. int. (%)	Interpretation[a] (ions)
353.2297	0.27	CO·(Val, Leu, Leu)
352.2232	1.71	CO·(Val, Leu, Leu)−H
253.1528	0.27	CO·(Leu, Leu)−H*
239.1403	2.31	CO·(Val, Leu)−H
310.2156	0.46	(Dha+H, Dha+H, Leu, Val) −CO−NH+H
195.1142	0.43	(Dha, Dha, Val) −CO−NH+H
193.0954	0.23	(Dha, Dha, Val) −CO−NH−H
169.1433	0.14	(Leu, Val)−CO−NH
149.0227	2.69	(Cys·Cys)−CO−NH
140.1054	2.37	(Dha, Leu)−CO−NH
199.1797	0.24	M−(Cys·Cys, Val, CO)+H
141.1024	2.58	M−(H_2S_2)−(Dha, Leu, Leu, CO)+H
157.0960	0.31	CO·Leu·NH+H
155.0806	0.60	CO·Leu·NH−H
143.0810	5.74	CO·Val·NH+H
152.0690	0.21	(Dha, Val)−NH−H
112.0614	2.81	(Dha, Dha)−CO+2H
111.0523	3.52	(Dha, Dha)−CO+H
110.0461	1.66	(Dha, Dha)−CO
109.0389	0.14	(Dha, Dha)−CO−H
127.0878	0.90	Leu·NH−H
112.0759	0.53	Leu−H
114.0941	0.20	Leu+H
98.0607	1.92	Val−H
70.0301	1.93	Dha+H

[a] The base peak (100 %) was observed at *m/e* 86.0999. This corresponds to $C_5H_{12}N$, Leu−CO+H.

* In the spectrum of the synthetic sample of malformin, all the fragments in Table 3 were observed, with the exception of a few which are indicated with asterisks.

Experimental Part

Natural malformin was prepared by the purification of a crude product (received from Professor Roy W. Curtis) by chromatography on a silica gel column with water-saturated ethyl acetate as eluent[7]. Thin-layer plates of silica gel (with the same solvent) were used for the detection of malformin in the eluates. Fractions, in which only the higher molecular weight material was present, were pooled, the solvent removed *in vacuo* and the residue dried at 80°C and about 0.1 mm for 2 hours.

Synthetic malformin was prepared as described in refs. 8 and 9, and was purified by the method used for natural malformin (cf. above).

Desthiomalformin (**IV**) was a synthetic material[12]; it was purified by sublimation *in vacuo* at 270~280°C and 0.05 mm.

Spectra. The high resolution electron impact mass spectra were obtained using an A.E.I. MS-9 double-focusing mass spectrometer at an effective resolving power (M/ΔM) of approximately 15,000. The mass spectrometer was equipped with an SRIC CIS-2 ion source which can be used for either electron impact (EI) or chemical ionization (CI). For EI operation, no reagent gas is introduced into the ion source. However, the relatively closed construction of

the ionization chamber can result in high sample vapor pressures and therefore in some self-protonation which gives enhanced MH^+ ion abundances. The samples were introduced by means of a heated direct-insertion probe and heated until sample ions were observed (approximately 250°C). Perfluorotributylamine was used to provide reference ions. The spectra were recorded at a scan speed of 40 seconds per mass decade on digital magnetic tape with off-line computer processing of the high resolution data. Other significant operating parameters were: accelerating voltage, 8 KV; ionizing energy, 70 eV; ion repeller voltage, 24 V; and source temperature, 200°C.

Acknowledgments

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