# Structure of Althiomycin, A Highly Modified Peptide Antibiotic

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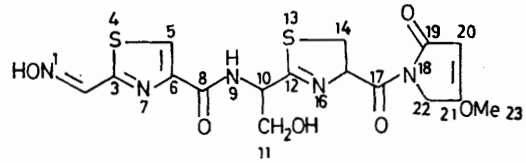
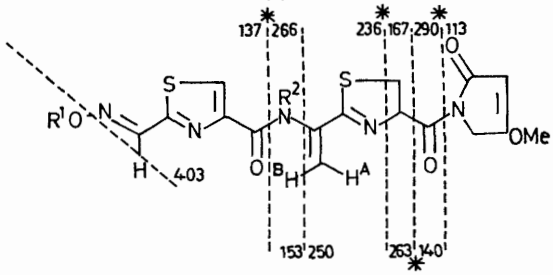
*Summary* The structure of the sulphur containing antibiotic althiomycin has been established by a detailed analysis of spectral data and chemical degradative evidence.

ALTHIOMYCIN was first isolated[1] from *Streptomyces althioticus* and later shown[2] to be identical with matamycin[3] from *Streptomyces matensis*. The antibiotic inhibits the growth of both Gram-positive and negative bacteria and possesses low toxicity. An initial investigation[4] determined the partial peptide nature of the molecule and identified $NH_3$, $CO_2$, cysteine, thiazole-4-carboxylic acid, and 4-methoxy-$\Delta^3$-pyrrolin-2-one as hydrolysis products,† but no definitive molecular formula or structure was assigned to the antibiotic. Our general interest[5] in modified peptide antibiotics, particularly those containing amino-acid residues at higher oxidation levels, led us to re-investigate this compound. Electron impact mass spectrometry failed to give a molecular ion for althiomycin (**1**) but showed peaks at *m/e* 421 ($M^+ - H_2O$) and 403 ($M^+ - 2H_2O$). The field desorption spectrum gave a strong quasi-molecular ion ($M^+ + 1$) at 440 confirming the assigned molecular formula. Chemical dehydration of althiomycin was readily achieved with acetic acid at room temperature to give anhydroalthiomycin (**2**). Attempted acetylation of (**1**) with either acetic anhydride–sodium acetate or acetic anhydride–pyridine also resulted in the loss of water yielding *O*-acetylanhydroalthiomycin (**3**).

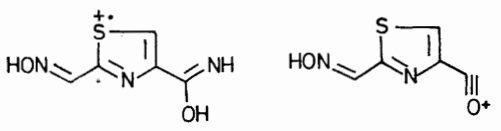
The mass spectral fragmentation patterns for (**1**)—(**3**) were virtually identical, the principle pathways stemming from the 403 ion ($M^+ - 2H_2O$ for althiomycin). Using the sulphur atoms as markers and knowing the basic units of althiomycin, the elemental mass maps of (**1**)—(**3**) were analysed in terms of the structures and sequence of the basic units shown. Those processes asterisked were further characterised by metastable ions and the appropriate mass shifts were observed in the spectra of the deuteriated derivatives (**4**)—(**6**). In addition, all the spectra contained a strong signal at *m/e* 171, while althiomycin showed a peak at 155. These ions which were not derived from the *m/e* 403 signal were assigned the structures (**7**) and (**8**) respectively and hence located one of the water molecules.



(1)



(2) $R^1 = R^2 = H$
(3) $R^1 = COMe$, $R^2 = H$
(4) $R^1 = H$; $R^2 = D$
(5) $R^1 = COMe$; $R^2 = D$
(6) $R^1 = COCD_3$; $R^2 = H$



(7) (8)

NH-Gly-Cys-Ser-Cys-Gly-OH

(9)

Althiomycin (**1**) and anhydroalthiomycin (**2**) are weakly acidic and their u.v. spectra show a bathochromic shift in

† The identity of these hydrolysis products has been confirmed; in addition small but significant amounts of serine and glycine have been detected. The cysteine has been shown to possess the D (*S*) configuration.

alkali (286 to 293 nm) not observed for (**3**), which possesses a strong carbonyl i.r. absorption at 1780 $cm^{-1}$ not present in (**1**) and (**2**) but characteristic of an *O*-acetyloxime.‡ The $^{13}C$ n.m.r. and $^{1}H$ n.m.r. spectra of (**1**)—(**3**) are consistent with the proposed structures (see Table) and provide further strong evidence for the thiazoline system.

The antibiotic contains a number of unusual structural features. The thiazoline[6] and thiazole[7] systems, both presumably derived from cysteine, have been observed in other naturally occurring peptides, but the pyrrolinone and oxime structures are unique. However, hydroxamic acid-containing peptides are common microbial products[8] and a simple oxime has been isolated[9] from a *Streptomyces*. The possible derivation of (**1**) from the linear pentapeptide precursor (**9**) is noteworthy in relation to the biosynthesis of other microbial peptides.

*Added in proof:* After the submission of this communication we were informed that Japanese workers had come to the same conclusion concerning the structure of althiomycin; H. Sakakibara, H. Naganawa, M. Ohno, K. Maeda, and H. Umezawa, *J. Antibiotics* (*Japan*), 1974, **11**, 899.

TABLE

| | Assignment of $^{13}C$ chemical shifts[a] Compound (**1**) | Compound (**3**) | | $^{1}H$ Chemical shifts[b] Compound (**1**) | Compound (**3**) |
|---|---|---|---|---|---|
| C-2 | 144·1 | 150·1 | H-2 | 1·63 s | 1·39 s |
| C-5 | 125·5 | 126·8 | H-5 | 1·67 s | 1·67 s |
| C-10 | 54·9 | 133·9 | H-10 | 4·95 m[d] | — |
| C-11 | 63·6 | 109·2 | H-11 | 6·00 d | $H_A$ 3·20 s; $H_B$ 4·51 s[e] |
| C-14 | 35·6[b] | 36·2[b] | H-14 | 6·38 m | 6·25 m |
| C-15 | 78·8[b] | 77·9[b] | H-15 | 3·72 m[d] | 3·75 m |
| C-20 | 94·9 | 94·3 | H-20 | 4·57 s | 4·79 s |
| C-22 | 48·8 | 48·5 | H-22 | 5·66 s | 5·64 s |
| C-23 | 59·7 | 58·4 | H-23 | 6·03 s | 6·07 s |
| | *Me*CO | 19·3 | H-9 | 1·50 d | −0·07 s |
| C-3 | 150·9 | 151·4 | | | |
| C-6 | 160·3 | 158·3 | | | |
| C-8 | 163·4 | 158·6 | | | |
| C-12 | 169·2 | 167·5 | | | |
| C-17 | 170·4 | 168·5 | | | |
| C-19 | 174·5 | 169·2 | | | |
| C-21 | 178·1 | 169·5 | | | |
| | MeCO | 176·7 | | | |

[a] The $^{13}C$ n.m.r. spectra were obtained using a Jeol PS100 pulsed FT spectrometer at 25·15 MHz in [$^{2}H_7$]-DMF (**1**) and $CDCl_3$ (**3**) against $Me_4Si$ as internal standard. Assignments were made on the basis of continuous wave decoupling experiments irradiating at the resonance frequencies of $Me_4Si$ and the proton at $\tau$ 3·20 in the n.m.r. spectrum of (**3**) and by comparison with simple model compounds. [b] The chemical shifts of C-14 and C-15 are highly characteristic of a cysteine residue in the thiazoline form. [c] The $^{1}H$ n.m.r. spectra were recorded at 220 MHz; decoupling experiments were conducted at 100 MHz. [d] Decoupling experiments revealed fine coupling (2 Hz) between H-10 and H-15; ethyl 2-methylthiazole-4-carboxylate exhibits similar long range coupling. [e] Irradiation at the resonance frequency of either proton $H_A$ or $H_B$ caused very significant enhancements in the signal strength of $H_B$ (55 ± 5%) and $H_A$ (50 ± 5%) respectively.

(*Received, 29th November* 1974; *Com.* 1446.)

‡ Althiomycin gives a positive Fehling and Tollens test. Attempts to detect hydroxylamine as a hydrolysis product were unsuccessful. The isolation of thiazole-4-carboxylic acid suggests the initial formation of a nitrile followed by hydrolysis and decarboxylation.

[1] H. Yamaguchi, Y. Nakayama, K. Takeda, K. Tawara, K. Maeda, T. Takeuchi, and H. Umezawa, *J. Antibiotics* (*Japan*), 1957, **A10**, 195; T. E. Eble and G. B. Whitfield, unpublished results quoted in reference 3.
[2] P. Sensi, personal communication.
[3] P. Sensi, R. Ballotta, and G. G. Gallo, *Antibiotics and Chemotherapy*, 1959, **9**, 76.
[4] D. J. Cram, O. Theander, H. Jaeger, and M. K. Stanfield, *J. Amer. Chem. Soc.*, 1963, **85**, 1430.
[5] B. W. Bycroft, *Nature*, 1969, **224**, 595; *J.C.S. Chem. Comm.*, 1972, 660; *J.C.S. Chem. Comm.*, 1972, 652.
[6] L. C. Craig, W. Konigsberg, and R. J. Hill in 'Amino Acids and Peptides with Antimetabolic Activity,' ed. G. E. Wolstenholme and C. M. O'Connor, Churchill, London, 1958, p. 226; W. H. White, F. McCapra, G. F. Field, and W. D. McElroy, *J. Amer. Chem. Soc.*, 1961, **83**, 2402; B. Anderson, D. C. Hodgkin, and M. A. Viswamitra, *Nature*, 1970, **225**, 233.
[7] M. Bodanszky, J. Fried, J. T. Sheehan, N. J. Williams, J. Alicino, P. I. Cohen, B. T. Keeler, and C. A. Birkhimer, *J. Amer. Chem. Soc.*, 1964, **86**, 2478; R. J. Clarke and J. Walker, *J. Chem. Soc.*, 1966, 1354; H. Umezawa, *Pure Appl. Chem.*, 1971, **28**, 665; S. Nakamura and H. Umezawa, *Chem. and Pharm. Bull.* (*Japan*), **14**, 981.
[8] H. Maehr, *Pure Appl. Chem.*, 1971, **28**, 603.
[9] P. H. Wiley, R. R. Herr, F. A. MacKellar, and A. O. Argondelis, *J. Org. Chem.*, 1965, **30**, 2330.