STRUCTURE OF ALTHIOMYCIN

HERBERT A. KIRST, EDWARD F. SZYMANSKI, DOUGLAS E. DORMAN, JOHN L. OCCOLOWITZ, NOEL D. JONES, MICHAEL O. CHANEY, ROBERT L. HAMILL and MARVIN M. HOEHN

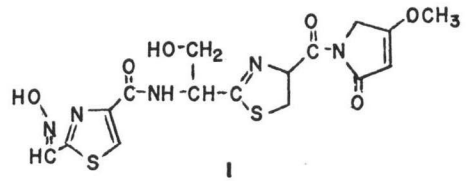
Lilly Research Laboratories, Eli Lilly and Company
Indianapolis, Indiana 46206, U.S.A.

(Received for publication January 7, 1975)

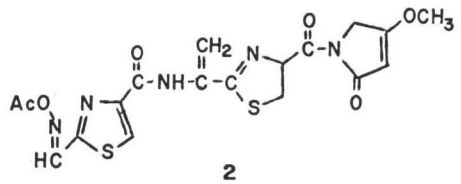
The structure of the antibiotic althiomycin has been determined by a combination of chemical and physical methods.

We wish to report the structure of althiomycin (**1**), a chemically-novel antibiotic produced in these laboratories by a strain of *Streptomyces chartreusis*.* Products identical to althiomycin have been isolated by several other groups[1~3], but an early chemical study did not yield the correct structure.[4] After our work had been completed, UMEZAWA, *et al.*, independently published the same structure for althiomycin.[5]

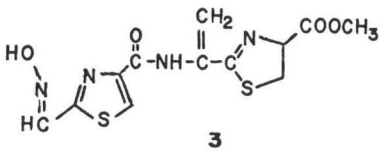
Elemental analysis of althiomycin suggested an empirical formula of $C_{16}H_{17}N_5O_6S_2$. Although the largest fragment observed in electron impact mass spectrometry was *m/e* 421 ($C_{16}H_{15}N_5O_5S_2$), field desorption mass spectrometry showed a quasi-molecular ion at *m/e* 440, confirming the molecular weight of 439**. Resonance signals for 17 protons and 16 carbon atoms in the proton and ^{13}C-nmr spectra further verified the empirical formula.



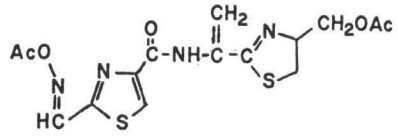
1
Althiomycin



2



3



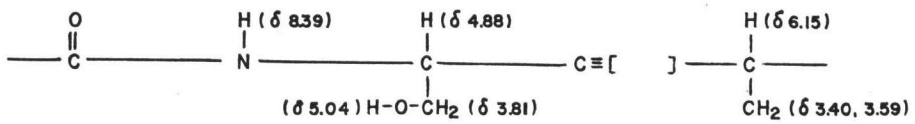
4

Althiomycin formed an acetate (**2**) during which elimination of an additional mole of water occurred simultaneously. Althiomycin in refluxing methanol yielded a methyl ester (**3**) and 4-methoxy-Δ^3-pyrrolin-2-one, the latter identified by spectral data compared with literature values[4]. 4-Methoxypyrrolinone was also isolated from reduction of althiomycin with sodium borohydride, along with a primary alcohol which formed a diacetate (**4**).

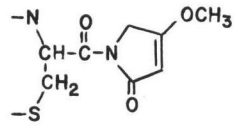
The proton nmr spectrum of althiomycin in DMSO-d_6 revealed one O-methyl group

* Taxonomy performed by Mr. R. E. KASTNER. This species differs from those species known to produce althiomycin.[1~3]

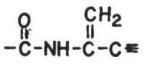
** We thank Mr. CARTER COOK of the University of Illinois for the field desorption mass spectrum.



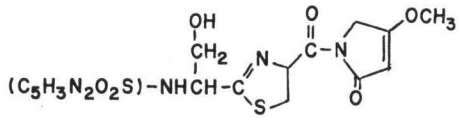
5



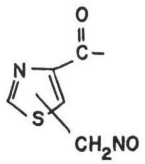
6



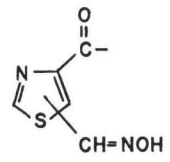
7



8



9



10

(singlet, 3.88 δ)* which, upon irradiation, led to a 19 % nuclear OVERHAUSER enhancement in a broad, one-proton singlet at δ 5.41; this latter resonance was significantly sharpened by irradiation of a two proton multiplet at δ 4.32. These absorptions were assigned to the 4-methoxypyrrolinone unit (*vide supra*). The first of three exchangeable protons found in the spectrum of the antibiotic occurred at δ 12.22 and was eventually assigned to the oxime proton (*vide infra*). The second was a doublet (J=8 Hz) at δ 8.39 which had the position and appearance of an amide proton. The third exchangeable proton appeared as a broad triplet at δ 5.04. Through a series of decoupling and exchange experiments, these latter two exchangeable protons were shown to fit into the molecule as indicated in partial structure **5**. Weak coupling (J=1.5 Hz) was also observed between the multiplet at δ 4.88 and the X-portion of the ABX system (δ_A=3.40, δ_B=3.59, δ_X=6.15 δ, J_{AB}=11, J_{AX}=8, J_{BX}=9.5 Hz). The remaining protons in the spectrum appeared as two low field doublets at δ 8.34 and 8.36 (J= 1 Hz).

Peracid oxidation[6] of althiomycin followed by acid hydrolysis yielded two amino acids, serine and cysteic acid; cysteine, itself, was isolated from hydrolysis without prior oxidation. Since the serine unit in structure **5** is obvious, the second ABX system was readily assigned to the cysteine protons. The loss of 4-methoxypyrrolinone in derivatives **3** and **4** was accompanied by sharp upfield shifts in the resonance position of the methine proton of the cysteine moiety; furthermore, in diacetate **4**, this resonance was coupled to two new protons. These observations indicated 4-methoxypyrrolinone was attached to the cysteine fragment by an imide linkage (**6**).

* All chemical shifts are measured in parts per million from internal tetramethylsilane.

The dehydration of the serine moiety (*e.g.*, **7**) was clearly observed in the proton nmr spectra of derivatives **2**, **3** and **4** by the appearance of two broad singlets for the terminal vinyl protons. The observation of unsaturated carbon resonances at 109 ($C=\underline{C}H_2$) and 134 ppm ($\underline{C}=CH_2$) in the off-resonance ^{13}C-nmr spectra played an instrumental role in this discovery. Chemical verification was obtained by ozonolysis of **2** to give formaldehyde, which was isolated as its 2,4-dinitrophenylhydrazone. Acetate **2** was also reduced with diborane in THF, yielding a new product containing a secondary methyl group and liberating alanine on acid hydrolysis.

Analysis of the mass spectral fragmentation pattern of althiomycin, coupled with the partial structures deduced from nmr spectra, suggested structure **8**. The observed doubling of numerous resonances in the nmr spectra was now readily explained by a racemized carbon atom attached to C-2 of the thiazoline ring[7]. Derivatives in which this carbon atom was sp^2 hybridized did not show this doubling phenomenon.

The nature of the $C_5H_3N_2O_2S$ moiety in **8** was further solved by analysis of the mass spectrum of methyl ester **3**. A prominent peak in the spectrum occurred at *m/e* 155 with the composition $C_5H_3N_2O_2S$; a metastable ion at *m/e* 104.1 indicated that the ion of mass 155 fragmented directly to *m/e* 127, which had the composition $C_4H_3N_2OS$ (155—CO). This information, coupled with the isolation of a small amount of thiazole-4-carboxylic acid[8] from acid hydrolysis of althiomycin*, suggested structure **9**. The mass spectrum of **3** after exchange with CH_3OD showed the peaks had shifted to *m/e* 156 and 128, indicating one exchangeable

Fig. 1. Conformation of methanolysis product **3** in the crystal. The thermal ellipsoids are drawn at the 50% probability level.

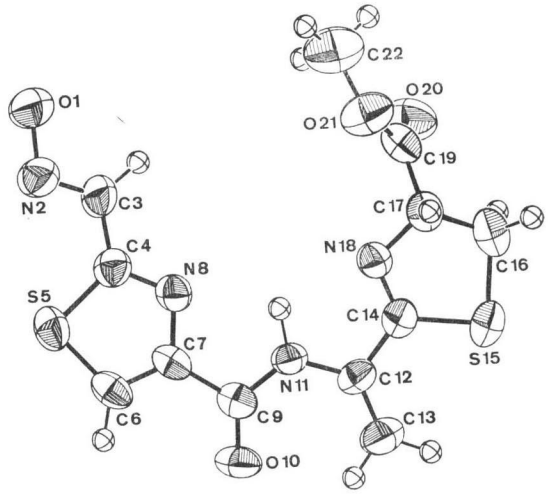


Table 1. Heavy atom coordinates $\times 10^4$ for methanolysis product **3**. Atoms are numbered as in Fig. 1.

Atom	X	Y	Z
O (1)	3936	5022	9530
N (2)	4394	4450	9082
C (3)	4187	3921	7374
C (4)	4623	3294	6728
S (5)	5397	3218	8192
C (6)	5504	2379	6279
C (7)	4967	2208	4726
N (8)	4460	2720	4931
C (9)	4876	1508	2780
O (10)	5308	1032	2524
N (11)	4292	1505	1432
C (12)	4040	939	−441
C (13)	4285	265	−964
C (14)	3427	1187	−1850
S (15)	2962	582	−4218
C (16)	2298	1265	−4156
C (17)	2644	2034	−3372
N (18)	3215	1881	−1500
C (19)	2214	2593	−1746
O (20)	1804	2413	36
O (21)	2372	3304	−2340
C (22)	2043	3908	−617

* The 2-carboxaldehyde oxime substituent was lost under these conditions (6 N HCl, reflux), as was also found by other workers.[5]

proton in the CH_2NO moiety of **9** and implying structure **10**. An oxime had been suspected in althiomycin from the presence of a titratable group (pK_a=11.2) and the spectral characteristics of acetylated derivatives (CH_3CO at δ 2.25, ν_{CO}=1780 cm^{-1}). Chemical evidence was obtained by the isolation of hydroxylamine as the oxime of *p*-nitrobenzaldehyde after acid hydrolysis of **3**.

The position of the oxime substituent was determined and the rest of the structure verified by X-ray diffraction of methyl ester **3**. This compound crystallized from methanol as colorless needles in the space group $P2_12_12_1$, with four molecules in a unit cell having the dimensions a=20.387±0.002 Å, b=17.454±0.002 Å, and c=4.459±0.001 Å. The density measured by flotation was 1.43 g cm^{-3}, while that calculated for $C_{12}H_{12}N_4O_4S_2$ (molecular weight 340.4) was 1.45 g cm^{-3}. The intensities of 1035 independent reflections were measured on a four-circle automated diffractometer using monochromated copper radiation.

The positions of the two sulfur atoms were located from an E^2-1 map. An E map calculated using the phases of the sulfur atoms showed the positions of the rest of the non-hydrogen atoms. Refinement by the least-squares method, using anisotropic temperature factors, brought the R factor down to 0.057. A difference electron density map calculated at this point showed the positions of 11 of the 12 hydrogen atoms, with only the oxime hydrogen atom remaining undetermined. Further least-squares refinement on all atoms gave a final R value of 0.044. The coordinates of the non-hydrogen atoms are listed in Table 1 and the conformation of the molecule is shown in Fig. 1.

As expected for the *syn* configuration of the oxime group in ester **3**, irradiation of the oxime proton (δ 12.27, DMSO-d_6) led to an 18 % nuclear OVERHAUSER enhancement in the doublet assigned to the carboxaldehyde oxime proton (δ 8.34, J=1 Hz) without affecting the doublet of the 5-thiazole proton (δ 8.45, J=1 Hz). Similarly, irradiation of the oxime proton of althiomycin led to an 11 % N.O.E. in the doublet at δ 8.36, thereby establishing the *syn* configuration for the oxime group in althiomycin*.

Experimental

Production and Isolation of Althiomycin

Streptomyces chartreusis was grown in 150-liter tanks at 30°C for 48~72 hours in a modified NZ-amine A, starch medium. The broth filtrate was extracted with ethyl acetate, and the extract was concentrated. The oily residue was dissolved in a small volume of dimethylacetamide, diluted with 50 volumes of chloroform, and applied to a silica gel column packed in chloroform. After elution of several impurities with chloroform, the desired product was eluted with ethyl acetate.

The sample of althiomycin used in this study was proven identical to authentic samples by thin-layer chromatography, infrared, nmr and mass spectrometry, mixed melting point and X-ray powder pattern. Althiomycin was crystallized from acetone-water, m.p. 173~175°C (dec), $[\alpha]_D^{25}$+45.2° (c 1.03, 1 : 1 $CHCl_3$-EtOH), pK_a (66 % DMF) 11.2, λ_{max}^{EtOH} 222 nm (ε=36,600), 236 (28,300), 284 (8,200); $\lambda_{max}^{0.1N\ NaOH}$ 235 (22,800), 312 (11,500); IR (KBr) 3530, 3365, 1720, 1690, 1657, 1650, 1620 cm^{-1}; ^{13}C (DMSO-d_6) ppm 34.8, 48.1, 54.2, 59.2, 62.2, 77.6, 94.4, 125.2, 143.2,

* This conclusion is opposite that proposed previously.[5] However, the earlier conclusion was based upon a derivative obtained upon basic hydrolysis, during which isomerization may have occurred.

149.7, 159.8, 162.6, 168.3, 169.6, 173.3, 177.2.

Anal. Calcd. for $C_{16}H_{17}N_5O_6S_2$: C, 43.73; H, 3.90; N, 15.94; S, 14.59.
Found: C, 43.94; H, 3.72; N, 16.01; S, 14.26.

Acetylation

Althiomycin (500 mg) was treated with acetic anhydride (1.43 ml) in dry pyridine (10 ml) at 25°C for 20 hours. The crude product was crystallized from methyl ethyl ketone to give colorless crystals of **2** in 60% yield; m.p. 178~179°C (dec); λ_{max}^{EtOH} 220 nm (ε=35,800), 243 (31,700), 280 sh (17,000); IR ($CHCl_3$) 3340, 1780, 1725, 1690, 1625 cm^{-1}; parent *m/e* 403 (M−60); nmr ($CDCl_3$)δ 10.00 (s, 1, NH), 8.55 and 8.27 (d, J=1 Hz, 2), 6.76 and 5.45 (s, 2, C=CH_2), 6.39 (m, 1, C$\underline{H}$CH_2S), 5.18 (s, 1, C$\underline{H}$=C−OCH_3), 4.34 (m, 2, CH_2N), 3.91 (s, 3, OCH_3), 3.85, 3.55 (m, 2, CH_2S), and 2.25 (s, 1, CH_3CO).

Anal. Calcd. for $C_{18}H_{17}N_5O_6S_2$: C, 46.65; H, 3.70; N, 15.11; S, 13.84.
Found. C, 46.37; H, 3.63; N, 14.87; S, 13.91.

Methanolysis

Althiomycin (2 g) was refluxed in methanol (80 ml) for 80 hours; on cooling, methyl ester **3** precipitated as colorless needles in 41% yield; m.p. 194~196°C (dec); pKa (66% DMF) 11.2; λ_{max}^{EtOH} 222 nm (ε=22,700), 264 (20,600); $\lambda_{max}^{0.1N\ NaOH}$ 237 (18,400), 290 (17,200); IR (KBr) 3356, 1754, 1688 cm^{-1}; parent *m/e* 340; nmr (pyridine-d_5) δ 14.4 (s, 1, OH), 10.37 (s, 1, NH), 8.50 and 8.36 (d, J=1 Hz, 2), 7.12 and 5.57 (s, 2, C=CH_2), 5.47 (m, 1, C$\underline{H}$COOCH_3), 3.78, 3.68 (m, 2, CH_2S) and 3.65 (s, 3, OCH_3).

Anal. Calcd. for $C_{12}H_{12}N_4O_4S_2$: C, 42.34; H, 3.55; N, 16.46; S, 18.84.
Found: C, 42.17; H, 3.47; N, 16.61; S, 18.66.

Sodium Borohydride Reduction

Althiomycin (500 mg) was added to a solution of sodium borohydride (227 mg) in absolute ethanol (50 ml) and stirred at 25°C for 4 hours; the crude product was then treated with acetic anhydride (1.52 ml) in dry pyridine (10 ml) for 20 hours at 25°C. Crystallization from acetone-water afforded diacetate **4**; m.p. 145~147°C (dec); λ_{max}^{EtOH} 218 nm (18,200), 266 (15,500); IR ($CHCl_3$) 3370, 1779, 1739, 1684 cm^{-1}; parent *m/e* 336 (M−60); nmr ($CDCl_3$)δ 9.94 (s, 1, NH), 8.61 and 8.27 (d, J=1 Hz, 2), 6.76 and 5.42 (s, 2, C=CH_2), 4.87 (m, 1, methine), 4.49, 4.21 (m, 2, CH_2O), 3.51, 3.30 (m, 2, CH_2S), 2.25 and 2.11 (s, 6, CH_3CO).

Anal. Calcd. for $C_{15}H_{16}N_4O_5S_2$: C, 45.45; H, 4.07; N, 14.13; S, 16.18.
Found: C, 45.28; H, 4.02; N, 13.89; S, 16.13.

Acknowledgements

We thank T. K. Elzey and J. W. Paschal for nmr spectra, J. M. Gilliam for mass spectra, L.A. Spangle and associates for ir and uv spectra, G. M. Maciak and associates for microanalyses, N. E. Davis and associates for paper chromatography and bioautography, and L. D. Boeck, R. Maze and C. Carrell for fermentation and isolation work. We also thank Dr. E. H. Flynn of these laboratories and Prof. J. E. Baldwin of M.I.T. for helpful discussions.

References

1) Yamaguchi, H.; Y. Nakayama, K. Takeda, K. Tawara, K. Maeda, T. Takeuchi & H. Umezawa: A new antibiotic, althiomycin. J. Antibiotics, Ser. A10: 195~200, 1957
2) Sensi, P.; R. Ballotta & G. G. Gallo: Matamycin, a new antibiotic. II. Isolation and characterization. Antibiot. & Chemoth. 9: 76~80, 1959
3) Bergy, M. E. & C. DeBoer: Antibiotic. Garlandosus and process for preparing the same. U.S. Patent 3,642,984, Feb. 15, 1972
4) Cram, D. J.; O. Theander, H. Jager & M. K. Stanfield: Mold metabolites. IX. Contribu-

tion to the elucidation of the structure of althiomycin. J. Amer. Chem. Soc. 85: 1430~1437, 1963

5) SAKAKIBARA, H.; H. NAGANAWA, M. OHNO, K. MAEDA & H. UMEZAWA: The structure of althiomycin. J. Antibiotics 27: 897~899, 1974
6) MOORE, S.: On the determination of cysteine as cysteic acid. J. Biol. Chem. 238: 235~237, 1963
7) KONIGSBERG, W.; R. J. HILL & L. C. CRAIG: The oxidation and acid isomerization of bacitracin A. J. Org. Chem. 26: 3867~3871, 1961
HIROTSU, Y.; T. SHIBA & T. KANEKO: Synthetic studies on bacitracin. VII. Isomerization of amino acid components of thiazoline peptides. Bull. Chem. Soc. Japan 43: 1870~1873, 1970
8) ERLENMEYER, H. & C. J. MOREL: Structure chemical investigation. VIII. 4,5-Thiazoledicarboxylic and 4-thiazolecarboxylic acids. Helv. Chim. Acta 25: 1073~1077, 1942