on 10 g. of Alcoa alkaline alumina. The fractions eluted with benzene (0.142 g.) were combined and crystallized from petroleum ether to give 0.113 g. (76% yield), m.p. 173–177°. Successive recrystallizations from acetone–petroleum ether, acetone–methanol, and twice from petroleum ether gave colorless prisms, m.p. 181–183°, $\lambda_{max}^{95\%\ EtOH}$ 250 mμ (ε 31,700).

Anal. Calcd. for $C_{39}H_{54}O_4$: C, 83.25; H, 7.70. Found: C, 83.2; H, 7.7.

3α-Acetoxy-11β-hydroxy-Δ^{16}-5β-androstene-18,20-dialdehyde 11β,18-Lactol Methyl Ether (XXXVI).—Chamber B of the Rubin ozonolysis apparatus[38] was charged with 8 ml. of methylene dichloride, the apparatus was then cooled in a Dry Ice–acetone-bath, and ozone was admitted until the solvent was saturated. Chamber A of the apparatus was then charged with a solution of 100 mg. of the diolefin XXXV, m.p. 173–177°, prepared as described directly above, in 1 ml. of methylene dichloride and 8 ml. of anhydrous methanol; then the solution of ozone was transferred from chamber B into chamber A by the application of a positive pressure of nitrogen. The mixture was stirred magnetically at −70° for 9 min. and was then allowed to warm to 0° (ice-bath) for 2 min. Four-tenths gram of zinc dust and 2 ml. of glacial acetic acid were immediately added, and the solution was stirred at 0° for 1.5 hr., during which period three additional 0.4-g. portions of zinc and 2-ml. portions of acetic acid were added. The mixture was filtered, the filtrate neutralized with sodium bicarbonate solution and the mixture extracted with ether. The combined organic layers were washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue dissolved in methylene dichloride, dried again over anhydrous sodium sulfate and concentrated. This crude oily dialdehyde was immediately dissolved in 35 ml. of benzene, about 15 mg. of anhydrous crystalline piperidine hydroacetate was added, and the mixture was stirred at room temperature for 4 hr. Ether was added, and the solution was washed with water, saturated brine, dilute acetic acid, saturated sodium bicarbonate solution, again with brine and finally dried over anhydrous sodium sulfate. The residue obtained on evaporation of the solvent under reduced pressure was chromatographed on 15 g. of Florisil. The fractions eluted with 30% ether in benzene through ether amounted to 25 mg. of glassy material which crystallized on trituration with ether to give 18.7 mg. of colorless crystals, m.p. 202–204°, after sintering at 190°; $\lambda_{max}^{95\%\ EtOH}$ 238–239 mμ (ε 9,600); $\lambda_{max}^{CHCl_3}$ 3.4–3.45 μ (aldehyde C—H), 5.85 (acetate C=O), 5.95 (unsaturated aldehyde C=O). On slow crystallization from ether the substance was obtained as colorless needles having the same melting point.

Anal. Calcd. for $C_{23}H_{32}O_5$: CH_3O, 7.99. Found: CH_3O, 7.9.

Acknowledgments.—We wish to express our thanks to the following agencies who provided the major financial assistance to this project: the U. S. Public Health Service, the National Science Foundation, the Wisconsin Alumni Research Foundation, the Sterling–Winthrop Research Institute and the Upjohn Company.

We are grateful to Dr. C. M. Suter and his staff at the Sterling–Winthrop Research Institute for supplying us with generous quantities of intermediates.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES, LOS ANGELES 24, CALIF.]

Mold Metabolites. IX. Contribution to the Elucidation of the Structure of Althiomycin[1]

BY DONALD J. CRAM, OLOF THEANDER,[2] HERB. JAGER AND M. K. STANFIELD

RECEIVED NOVEMBER 2, 1962

The structure of the mold metabolite, althiomycin (probable formula $C_{27}H_{28}O_{10}N_8S_3$), has been investigated. Methanolysis (acid-catalyzed) of the substance gave 4-methoxy-Δ^3-pyrrolin-2-one, whose structure was established by degradation and synthesis. Strong acid hydrolysis of the amorphous products of the methanolysis provided thiazole-4-carboxylic acid, identified through its physical properties and by comparison with an authentic sample. When subjected to a Moore and Stein cation exchange resin column, the acid hydrolysate of althiomycin was demonstrated to contain cysteine, ammonia and an unknown and non-identified amino acid. The presence of a cysteine unit was confirmed through identification of cysteic acid in the hydrolysate of the oxidation product of the original antibiotic. When treated with acetic anhydride and pyridine, althiomycin gave an acetylated degradation product (acetylalthiomycin) whose probable formula is $C_{18}H_{17-19}O_6N_5S_2$. Hydrolysis and spectral studies demonstrated that acetylalthiomycin contained the same structural units identified as hydrolysis products of althiomycin itself. Strong acid hydrolysis of acetylalthiomycin gave carbon dioxide, ammonia, formic acid, acetic acid and hydrogen sulfide as volatile products. A provisional structure for acetylalthiomycin is considered.

A new sulfur-containing antibiotic was isolated at the Upjohn Company in 1957 from culture no. 116a of an unspecified mold.[3] In the same year, a group of Japanese investigators[4] reported the isolation of althiomycin, and comparison of this material with that of the Upjohn group demonstrated the identity of the two materials.[3]

Characterization.[3]—Althiomycin by isothermal distillation exhibited a molecular weight of 708, and the formula $C_{27}H_{28}O_{10}N_8S_3$ is the most consistent with the analytical data. The substance contains two methoxyl groups and no terminal methyl groups. The pure material, m.p. 180–181.6° dec., gave a rotation of $[\alpha]^{25}D$ +37.8° (*c* 2, 1:1 95% ethanol–methylene chloride). The antibiotic gave negative ninhydrin, biuret, ferric chloride, Sakaguchi, Benedict, anthrone, Molisch and Wegand tests, and positive Tollens and Tommila tests. Titration of the material with 2 *N* hydrochloric acid produced a well defined end point at pK_a 11.2, the equivalent weight being 256, but the antibiotic underwent decomposition during the titration, and a degradation product was probably involved.[3]

Methanolysis of Althiomycin.—Methanolysis of althiomycin in 0.25 *N* hydrogen chloride in methanol at 25° gave a solution which when neutralized with a strong anion exchange resin produced a precipitate (64% by weight, labeled fraction A) and a water-soluble fraction. From the latter was obtained 17% by weight of a paper chromatographically pure crystalline material, which was recrystallized first from ethanol–benzene and then from benzene to give 10% by weight of white prisms.

This compound was demonstrated to be 4-methoxy-Δ^3-pyrrolin-2-one (I), by the following experiments. Elemental analysis and molecular weight determination established $C_5H_7O_2N$ as the molecular formula. The substance (m.p. 133–134°) sublimed readily at 90° and 0.2 mm., was optically inactive, and formed a very unstable hydrogen chloride adduct. Methoxyl determination demonstrated the presence of one such group. Its ultraviolet spectrum (95% ethanol) contained two λ_{max}, one at 210 mμ (ε 23,000) and a second at 279 mμ (ε 9). The infrared spectrum (potassium bromide disk) contained a large number of well defined bands: 3150 cm.$^{-1}$ (NH or OH group); 1660, 1635 and 1610 cm.$^{-1}$ (associated with an unsaturated lactam structure).

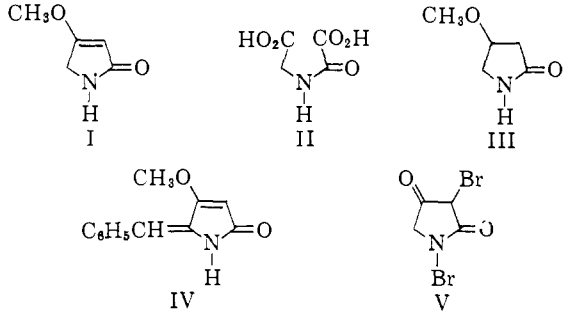
(1) The authors wish to express their appreciation to the Public Health Service for a grant which partially supported this investigation. They also wish to thank the Upjohn Company for a generous supply of althiomycin.

(2) This author wishes to thank the Rockefeller Foundation for a post-doctoral fellowship whose tenure was devoted to this investigation.

(3) T. E. Eble and G. B. Whitfield, private communication.

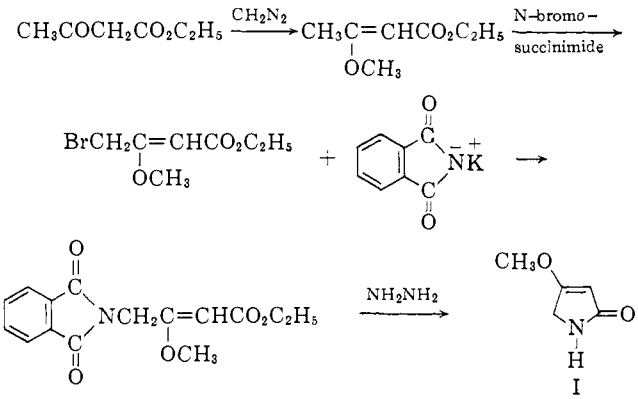
(4) H. Yamaguchi, Y. Nakayama, K. Takeda and K. Tawara, *J. Antibiotics* (Japan), **10A**, 195 (1957).

Oxidation of I with acidic hydrogen peroxide gave ammonia, glycine, formic acid and a dicarboxylic acid whose structure was demonstrated to be N-(carboxymethyl)-oxamic acid (II) by comparison with an authentic sample. Ozonolysis of I in acetic acid followed by hydrolysis gave glyoxylic acid and glycine, as demonstrated by comparison of their derivatives with authentic samples. Reduction of I with hydrogen and Raney nickel gave the corresponding dihydro derivative III, whereas condensation of the substance with benzaldehyde provided IV. Bromination of I with bromine water gave a disubstitution product whose properties are consistent with structure V. The dibromide readily liberated iodine from potassium iodide, was strongly reducing, and formed a hydrazone, unlike the starting material. The dibromide could not be formed by reaction of I with bromine in carbon tetrachloride. These facts are uniquely accounted for by structures I through V.

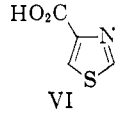


Since lactam I was produced from althiomycin by a mild methanolysis reaction, and since I contains a methoxyl group, the question arises as to the genesis of the methoxyl group in I. The possibility that the methoxyl was introduced during methanolysis was set aside by the fact that compound I was produced by ethanolysis of althiomycin.

Synthesis of 4-Methoxy-Δ^3-pyrrolin-2-one (I).—The structure of compound I was confirmed by a rational synthesis of the substance (see formulas). The over-all yield amounted to 0.2%, and comparison of the authentic material with that obtained by degradation of althiomycin proved the two specimens identical in all respects.



Products of Hydrolysis of Fraction A.—Fraction A (precipitate obtained upon acidification of the methanolysis liquors of althiomycin) was a light tan, noncrystalline powder. This material was soluble in pyridine and acetic acid, but was only very slightly soluble in water, lower aliphatic alcohols, ether and benzene. The material readily dissolved in dilute hydrochloric acid, but was precipitated when neutralized with an anion exchange resin. The complex mixture of materials produced by heating this material with 6 *N* hydrochloric acid was chromatographed on cellulose to give after purification (crystallization and sublimation) a 3% yield by weight of thiazole-4-carboxylic acid (VI) whose structure was confirmed by comparison with an authentic specimen.[5] The properties of the corresponding acid chloride and amide prepared from the acid obtained from althiomycin corresponded to those reported for the corresponding acid chloride and amide of thiazole-4-carboxylic acid.



VI

The presence of cysteine and glycine in the above hydrolysate was demonstrated by paper chromatography.

Acetylalthiomycin.—When stirred with acetic anhydride and either pyridine or sodium acetate at room temperature, acetylalthiomycin was obtained as either white plates or needles (polymorphs). The compound was demonstrated to be homogeneous to countercurrent distribution analysis.[6]

Elemental analysis, acetyl and methoxyl determinations indicates an empirical formula (probable molecular formula) of $C_{18}H_{17-19}O_6N_5S_2$. Attempts at molecular weight determination failed (Rast and isothermal distillation) due to insolubility and the tendency of the compound to polymerize. The compound is soluble in polar organic solvents such as dimethylformamide and dimethyl sulfoxide, and less soluble in the lower alcohols, acetic acid, the chlorinated hydrocarbons, and is insoluble in water and non-polar organic solvents. Tests for the sulfhydryl group were negative, and the substance reacted with aqueous base. The substance is optically inactive.

Methanolysis of acetylalthiomycin with dry hydrogen chloride gave a complex mixture of products among which 4-methoxy-Δ^3-pyrrolin-2-one was identified by paper chromatographic comparisons. Acid hydrolysis of the acetyl compound gave carbon dioxide in undetermined amounts, 1.58–1.78 moles of volatile acid (mixture of acetic and formic acid) and 0.69 to 1.00 mole of ammonia (these estimates were based on $C_{18}H_{17}O_6N_5S_2$ as the molecular formula). The non-volatile fraction from hydrolysis was shown by paper chromatography to be a complex mixture, in which the probable presence of thiazole-4-carboxylic acid was demonstrated, along with a strongly reducing substance.

Pyrolysis of acetylalthiomycin gave carbon dioxide as the only recognized product. The acetyl compound was oxidized with performic acid, and then submitted to amino acid analysis. Cysteic acid was positively identified, which indicates the presence of cysteine in the acetyl derivative. No volatile aldehyde or ketone was produced on ozonolysis, the substance could not be catalytically reduced with platinum and hydrogen in acetic acid, and attempts at Raney nickel desulfurization resulted in intractable mixtures.

Ultraviolet Absorption Spectra.—Table I records the ultraviolet absorption maxima for althiomycin, acetylalthiomycin, 4-methoxy-Δ^3-pyrrolin-2-one and thiazole-4-carboxylic acid. The spectral shift from acid to base for both althiomycin and acetylalthiomycin indicates that the absorbing system functions as an acid–base, although both substances decomposed in basic solution. The absorbing system in althiomycin and acetylalthiomycin resemble one another but not in detail.

(5) H. Erlenmeyer and C. J. Morel, *Helv. Chim. Acta*, **28**, 362 (1945). The authors wish to thank Professor Erlenmeyer for his kindness in providing the authors with this material.

(6) The authors wish to thank Dr. T. E. Eble for performing this analysis.

The sum of the spectra of the two degradation products 4-methoxy-Δ^3-pyrrolin-2-one and thiazole-4-carboxylic acid fall far short of accounting for the spectra of either althiomycin or acetylalthiomycin, which absorb at much higher intensities, but at not widely different wave lengths.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRAL DATA FOR ALTHIOMYCIN AND ITS DEGRADATION PRODUCTS

Compound	Solvent	$\lambda_{max.}$ mμ	$10^4 \times \epsilon$[a]
Althiomycin	95% ethanol	223	6.0
		240–245(sh)[b]	~4.0
		286(b)[c]	1.4
Althiomycin	95% ethanol	224	5.7
	0.01 *N* in H_2SO_4	235–240(sh)[b]	4.8
		290(sh)[b]	~2.0
Althiomycin	95% ethanol	238	3.6
	0.01 *N* in NaOH	310	2.0
Acetylalthiomycin	95% ethanol	222	4.1
		243	4.0
		~270(sh)[b]	~2.3
		~285(b sh)[b,c]	~1.7
Acetylalthiomycin	95% ethanol	222	4.1
	0.01 *N* in H_2SO_4	243	4.0
		~270	~2.3
		~290(b sh)[b,c]	~1.5
Acetylalthiomycin	95% ethanol	225	3.9
	0.01 *N* in NaOH[d]	~275(sh)	~0.84
		~283(sh)	~0.74
4-Methoxy-Δ^3-pyrrolin-2-one	95% ethanol	~210	~2.3
		279	0.0094
Thiazole-4-carboxylic acid	95% ethanol	231–232	.72
	0.01 *N* in H_2SO_4	~275–285	~ .03
Thiazole-4-carboxylic acid	95% ethanol	~225	~ .79
	0.01 *N* in NaOH	283	.17

[a] ε was calculated assuming a molecular weight of 722 for althiomycin, and 465 for acetylalthiomycin. [b] sh = shoulder. [c] b = broad. [d] Spectra changes rapidly with time; data apply to spectra taken immediately after dissolution.

Infrared Spectra.—The infrared spectra of the principal bands of althiomycin, acetylalthiomycin, 4-methoxy-Δ^3-pyrrolin-2-one and thiazole-4-carboxylic acid are recorded in Table II, along with tentative structural assignments.[7] Reference 7b was particularly rich in spectral data on complex amides, and 8 contains spectra of 78 thiazole derivatives.

The spectral comparisons in Table II suggest that particularly acetylalthiomycin and possibly althiomycin contain the 4-substituted thiazole ring system. The thiazole skeletal vibration[8] which appears at 1484 cm.$^{-1}$ of thiazole-4-carboxylic acid is present at 1485 cm.$^{-1}$ in althiomycin and 1487 cm.$^{-1}$ in the acetyl compound. The band at 1437 cm.$^{-1}$ in the thiazole acid, which is characteristic of a thiazole ring system,[8] could well correspond to the 1432 and 1425 cm.$^{-1}$ absorptions in althiomycin and the acetyl derivative. Similarly, the band at 1105 cm.$^{-1}$ in the thiazole acid is characteristic of 4-substituted thiazole compounds (1090–1135 cm.$^{-1}$),[8] and could be moved to 1123 and 1125 cm.$^{-1}$ in althiomycin and its acetyl derivative, respectively. Mijovic and Walker[8] report that, "The 4-monosubstituted thiazoles show the most characteristic behavior in this region (1050–930 cm.$^{-1}$), all showing a strong band in the 980–930 cm.$^{-1}$ region." The absorption of thiazole-4-carboxylic acid and acetylalthiomycin at 930 cm.$^{-1}$ are in accord with this statement, and the other bands in althiomycin and its acetyl

(7) (a) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958; (b) "The Chemistry of Penicillin," National Academy of Sciences, Princeton University Press, Princeton, N. J., 1949, and the references therein.

(8) M. P. V. Mijovic and J. Walker, *J. Chem. Soc.*, 3381 (1961).

TABLE II

INFRARED SPECTRAL DATA[a] FOR ALTHIOMYCIN AND ITS DEGRADATION PRODUCTS (CM.$^{-1}$)

Althiomycin	Acetylalthiomycin[b]	4-Methoxy-Δ^3-pyrrolin-4-one	Thiazole-4-carboxylic acid	Tentative assignment
..	3663	..	..	OH (Probable water in potassium bromide
3546	3571[c]	..	..	OH (
..	3448	3460	..	Free amide NH or bonded OH
3378	3367	..	..	Amide NH bonded or imine NH
..	..	3225	..	NH and/or CH
3195	3135	..	..	Amide NH bonded
3125	3106	3125	..	Vinyl or aromatic CH
3049	3040	3070	3090	Vinyl or aromatic CH
3003	3012	3005	3060	CH and/or carboxylic acid OH
2941	2959	2945	3000	CH
..	..	..	2500	
2882	..	..	..	
2874	2865	2860	..	CH_2
2825	2825	2845	..	CH_3CO
2778	..	..	..	
2625	..	2070	..	
..	1780	..	..	Strained >C=O
1720	1719	..	1716(sh)[d]	Imide or amide >C=O
1689	1687(sh)[d]	..	1684(sh)[d]	Amide >C=O
..	1679	1664	1669(b)[e]	Lactam or acid >C=O
1654	..	1639	1630(sh)[d]	Amide band
1624	1620	1614	1619(sh)[d]	>C=C<
..	1597	..	..	Amide band
1542	..	..	..	Amide band
..	1525	..	..	Amide band
1505	..	..	1507(sh)[d]	
1485	1487	..	1484	Thiazole skeletal vibration
1450	1455(sh)[d]	..	..	CH_2 and/or CH_3O
1440(sh)[d]	1440	1445	..	CH_2 and/or CH_3O
1432	1425	..	1437	Thiazole ring or allylic methylene
1415(sh)[d]	1392(sh)[d]	..	1390	Carboxyl or NH_2 group
1383[c]	1383(sh)[d]	..	..	
1362	1365(sh)[d]	1365	..	CH_2
..	1322	1340	1330	Thiazole ring
1314	1315	..	..	
..	..	..	1287(sh)[d]	NH
1265(sh)	..	..	1273	Carboxyl group
1252	1255	..	1255(sh)[d]	Carboxyl group
..	..	1235	..	
..	..	1215	..	
1193	1205(sh)[d]	..	1205	Carboxyl group
..	1195[c]	..	..	
1158	1170	1170	..	Ether group
1123	1125	..	1105	Monosubstd. thiazole ring
1075	..	..	..	
1053	1040	..	..	
1007	..	..	..	
995	1000	990	..	Vinyl hydrogen
975(sh)[d]	985(sh)[d]	..	..	
960(sh)[d]	957	..	..	
..	944	945	..	
..	930	..	930	4-Substd. thiazole
913	910(sh)[d]	905	..	Vinyl hydrogen
875(sh)[d]	..	..	882	4-Substd. thiazole
..	865	..	855	4-Substd. thiazole
830(sh)[d]	..	..	..	
..	825(sh)[d]	820	823	
808	815	..	..	
755	..	..	775	
..	725	720	735	
707	702	..	..	
694	690	..	..	
670	..	..	..	

[a] The spectra of althiomycin, acetylalthiomycin and 4-methoxy-Δ^3-pyrrolin-2-one were taken on a Beckman IR-4 spectrophotometer with lithium fluoride optics in KBr disks in the region from 2500 to 4000 cm.$^{-1}$. The total spectrum of thiazole-4-carboxylic acid and the remaining spectra of the other three compounds were taken in KBr disks on a Perkin–Elmer spectrophotometer, model 21, with sodium chloride optics. [b] Spectra taken on the polymorph which crystallized as plates. [c] Broad band which spans the wave numbers indicated. [d] sh = shoulder. [e] b = broad.

derivative that occur in this region could also be associated with a 4-substituted thiazole ring. The same could be said of the multitude of bands exhibited by the althiomycin and its acetyl derivative at 930–800 cm.$^{-1}$, since all 4-monosubstituted thiazoles that have been

TABLE III

NUCLEAR MAGNETIC RESONANCE DATA FOR ALTHIOMYCIN AND ITS DEGRADATION PRODUCTS

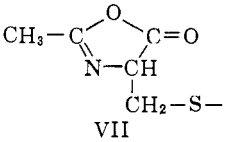
Compound	Solvent	τ	Peak multiplicity	No. H's	Possible assignment
4-Methoxy-Δ^3-pyrrolin-2-one[a]	$CDCl_3$	6.20	Singlet	3	CH_3O
		6.08	Singlet	2	$N—CH_2$
		4.93	Singlet	1	C=C—H
Thiazole-4-carboxylic acid[b]	$(CD_3)_2SO$	1.60	Partially resolved doublets	1	Aromatic H's
		0.92		1	
		0.45	Singlet	1	CO_2H
Acetylalthiomycin[a]	$CDCl_3$	7.75	Singlet	3	CH_3CO
		~6.3	Multiplet	2	CH_2
		6.10	Singlet	3	CH_3O
		5.67	Singlet	2	CH_2
		4.83	Singlet	1	C=C—H
		4.56	Singlet	1	C=C—H
		3.63	Triplet	1	O
		2.24	Singlet	1	‖ H—C—N
		1.55	(Unresolved) doublets	1	Aromatic H's
		1.41		1	
		0.00	Singlet	1	ArCH=N—
Althiomycin	$(CD_3)_2SO$[a]	7.50	Unresolved multiplet	13	
		6.55	Singlet?	13.9	
		6.13	Unsymmetrical Singlet?	5	
		5.68	Singlet	1.8	
		4.62	Singlet	1[c]	
		3.87	Triplet?	1[c]	
		1.67	Unresolved Doublet?	2.4	
Cysteine	CF_3CO_2H[d]	6.54	Doublet–doublet	2	CH_2
		5.22	Quadruplet	1	CH

[a] Taken on Varian associates A-60, analytical n.m.r. spectrophotometer at 60 megacycles. [b] Taken on a Varian Associates V-4300 high resolution n.m.r. spectrophotometer at 40 megacycles. The relative areas under the peaks were estimated using a planimeter. [c] The number of hydrogens were approximated by a planimeter and the smallest peak (τ 4.62) was assumed to have an area equivalent to one proton. [d] The multiplicity of the signal is such that the doublet due to the methylene group adjacent to the α-hydrogen is further split by the sulfide hydrogen. The α-hydrogen signal appears to be split by the NH_3 group attached to the same carbon atom. These data and interpretation are taken from F. A. Bovey and G. V. D. Tiers, *J. Am. Chem. Soc.*, 81, 2870 (1959), and O. Jardetyky and C. D. Jardetyky, *J. Biol. Chem.*, **233**, 383 (1958).

examined[8] usually showed two to four bands at these wave lengths.

The infrared spectral comparisons offer no direct evidence for the presence of the 4-methoxy-Δ^3-pyrrol-2-one ring system in althiomycin or acetylalthiomycin. However, the band by which this ring system would be attached to the larger molecules is hydrolytically very labile, a fact which suggest that the conjugated system of the pyrrolone might be extended in the larger molecules, and therefore modified spectroscopically. All three molecules contain a number of strong bands in the double bond region attributable to amide, imide or lactam absorptions.

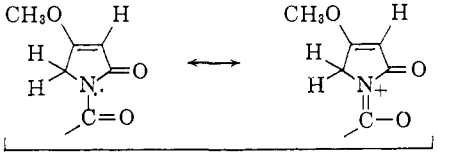
The band in acetylalthiomycin at 1780 cm.$^{-1}$ deserves special comment. Relatively few groups absorb at this high a frequency: β-lactams, oxazolones and five-membered cyclic imides.[7b] Since oxazolones are formed in the medium used to convert althiomycin to acetylalthiomycin, the band at 1780 cm.$^{-1}$ in acetylalthiomycin might be due to this ring system (VII).



VII

Nuclear Magnetic Resonance Spectra.—Table III contains the nuclear magnetic resonance spectra of althiomycin,[9] acetylalthiomycin,[9] 4-methyl-Δ^3-pyrrol-2-one,[9] thiazole-4-carboxylic acid and cysteine. In the spectrum of acetylalthiomycin, 17 hydrogens are accounted for of the 17–19 suggested by the empirical formula. Unfortunately, the spectrum of althiomycin was not integrated, and was too poor to allow any meaningful quantitative estimates to be made. The compound decomposes slowly in dimethyl sulfoxide and is insoluble in the usual solvents used in n.m.r. spectral measurements.

The spectrum of the pyrrolone is distinct, and gives the expected three sharp singlets with areas in the ratio of 3:2:1, which correspond to the methoxyl, methylene and vinyl hydrogens, respectively. These three singlets are clearly visible with the same relative intensity in the spectrum of acetylalthiomycin, but the three bands (τ = 6.10, 5.67 and 4.83) are shifted to lower τ-values by 0.1 to 0.4 τ unit. These lower values reflect lower shielding of the hydrogens in the pyrrolone ring system attached to the larger molecule than in the simple heterocycle, an effect to be expected from a part



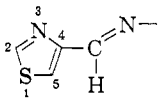
VIII

structure such as VIII. The carbonyl group attached to nitrogen in VIII withdraws electrons from nitrogen, and decreases the shielding for the ring system, particularly for the methylene group attached to nitrogen (which shows the greatest decrease in τ-value). The

(9) The authors are indebted to Drs. L. F. Johnson and D. P. Hollis of Varian Associates for these spectra.

same three peaks are found in althiomycin itself at about the same τ-values as in acetylalthiomycin.

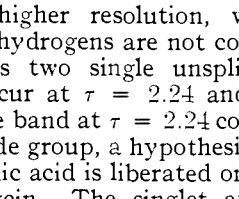
The two ring hydrogens of thiazole-4-carboxylic acid absorb at τ = 1.60 (—N=CH—S—) and 0.92 (—S—CH=C<), and occur as partially resolved doublets. The unresolved doublets found at τ = 1.55 and 1.41 in the spectrum of acetylalthiomycin are probably due to the same hydrogens (at C-2 and C-5, respectively), the hydrogen at C-5 being more shielded in acetylalthiomycin than in thiazole-4-carboxylic acid itself, and therefore absorbing at higher τ-values. Such a condition might apply if acetylalthiomycin contains the part structure IX. The low yield of thiazole-4-



IX

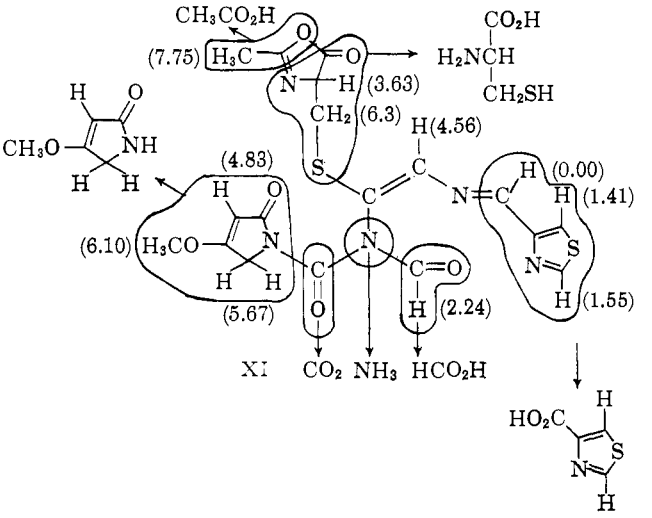
carboxylic acid by acid hydrolysis of acetylalthiomycin coupled with the fact that a strongly reducing substance was formed suggests the possibility that thiazole-4-carboxaldehyde was the initial product, and the acid was produced by air oxidation. Part structure IX also provides an explanation of the singlet at τ = 0.00, which could be associated with the hydrogen of the —CH=N— group. Most of these bands at low τ-values are absent in althiomycin, which suggests that the thiazole ring is somewhat modified in the antibiotic itself.

All of the other bands in acetylalthiomycin can be provisionally assigned. The singlet at τ = 7.75 is attributed to either an acetamido group or to the methyl group of oxazolone part structure VII. The unsymmetrical multiplet of two hydrogens at $\tau \sim 6.3$ may very well be due to the methylene of an incorporated cysteine unit (τ = 6.54 in cysteine itself). The multiplicity might arise from environmental dissimilarity of the two hydrogens, each environment providing a different chemical shift. If so, and if the energy of their spin–spin coupling interaction (J) is similar to δ, the simple rules for prediction of splitting no longer apply.[10] If the cysteine–oxazolone residue VII is responsible for this absorption, the α-hydrogen of the cysteine residue should occur as a triplet. The only triplet in acetylalthiomycin is found at τ = 3.63, which is very low for an aliphatic proton. This triplet shows small irregularities indicative of possible further splitting under higher resolution, which would be expected if the β-hydrogens are not completely equivalent. This leaves two single unsplit hydrogen absorptions that occur at τ = 2.24 and 4.56 to be accounted for. The band at τ = 2.24 could be associated with the formamide group, a hypothesis consistent with the fact that formic acid is liberated on acid hydrolysis of acetylalthiomycin. The singlet at 4.56 occurs at τ-values associated with vinyl hydrogens, and part structure X is suggested as being responsible for this band. This part structure leaves the vinyl hydrogen unsplit, as required by the spectrum.



X

Speculations Concerning Structure.—The data available on althiomycin are insufficient to allow speculation as to its total structure. Acetylalthiomycin has been much better characterized, and possible total structures can be written which serve to summarize the known facts concerning the compound. Structure XI serves this purpose, and points the way to further experiments. The numbers which appear associated with the hydrogens of structure XI identify possible assignments in the n.m.r. spectrum of acetylalthiomycin. In XI, the correspondence between the expected and found hydrolysis products is delineated.



Structure XI contains an oxazolone structure which incorporates the asymmetric carbon atom of a cysteine unit, yet acetylalthiomycin exhibited no optical activity. In the conversion of althiomycin to acetylalthiomycin, optical activity was lost, a fact consistent with the expected acidity (exchangeability) of this proton in structure XI. The extended conjugation of the side chain of the thiazole unit of XI could possibly account for the ultraviolet absorption spectrum of acetylalthiomycin. It should be emphasized that a large measure of fortuity would be involved should structure XI actually correspond to that of acetylalthiomycin.

Experimental

Characterization of Althiomycin.—Althiomycin, pure to Craig countercurrent distribution in methanol–chloroform–benzene–water (23:15:15:7), exhibited a distribution coefficient of 1.79.

Anal. Calcd. for $C_{27}H_{28}O_{10}N_8S_3$: C, 44.95; H, 3.91; O, 22.20; N, 15.54; S, 13.41; mol. wt., 722. Found: C, 44.52, 44.43, 45.50, 45.38; H, 3.90, 3.90, 3.78, 4.05; O, 21.60, 21.64; N, 14.12, 14.24, 14.44, 14.82, 14.54; S, 13.89, 12.73; mol. wt., 708 (isothermal distillation).

The substance exhibits low solubility in water and most organic solvents with the exception of dimethylformamide and dimethyl sulfoxide. The substance can be recrystallized from methylene chloride–95% ethanol (1:1), and stored as a stable crystalline compound, m.p. 180–181.6° dec., $[\alpha]^{25}D$ +37.8° (c 2% in 1:1 95% ethanol–chloroform). The substance gave no acetic acid in a terminal methyl determination, but in a methoxyl determination gave 7.67% CH_3O (theory for two methoxyls in $C_{27}H_{28}N_8O_{10}S$, 8.40%).

Methanolysis.—Althiomycin of approximately 95% purity (Craig distribution), 50 g., was dissolved in 1500 ml. of 0.25 N hydrogen chloride in methanol, and allowed to stand for 20 hr. The brown solution was filtered from 1.8 g. of insoluble inorganic material, and to the filtrate was added 500 ml. of water and an excess of Dowex 1-X8 (calculated on the amount of chloride). A yellowish precipitate formed, and the mixture was stirred for 15 minutes. The mixture was filtered, and the precipitate and ion exchange resin were separated by use of a sedimentation technique, followed by washing the resin on a 200-mesh screen. The precipitate was dried to give a tan powder, designated as fraction A.

The filtrates from the above separations were evaporated under reduced pressure during which periodically more tan powder (fraction A) was removed (filtration). Fraction A in entirety amounted to 30.6 g. After a volume of 200 ml. was reached, 400 ml. of ethanol was added, and the mixture was cooled. The red precipitate that separated was collected

(10) J. D. Roberts, "An Introduction to the Analysis of Spin-Spin Splitting in Nuclear Magnetic Resonance," W. A. Benjamin, Inc., New York, N. Y., 1961.

and washed with aqueous ethanol. The filtrates were evaporated further, ethanol was again added, the solution cooled, and a precipitate again collected. Two further cycles of the same kind provided 3.2 g. of a dark red powder labeled fraction B.

The solution after the last filtration was evaporated to dryness (no precipitate formed), and the slightly pink oil crystallized to give 8.30 g. of crude material. Two recrystallizations of the substance from ethanol–benzene and two more from benzene produced 4.80 g. of well formed white prisms of compound I. The weight-yield of I based on althiomycin free of inorganic material (48.2 g.) was approximately 10%. Based on a formula of $C_{27}H_{28}O_{10}N_8S_3$ for althiomycin, the yield of I was 64%.

Elution of the ion exchange resin with excess 2 *N* hydrochloric acid followed by a thorough washing with water gave eluates, concentration of which under vacuum gave 7.6 g. of a sirup (fraction C).

In a second experiment ethanolic hydrogen chloride was substituted for methanolic hydrogen chloride, and compound I was obtained in a 68% yield, m.p. 132–133°, undepressed by admixture with authentic material. Some advantage for separation of I from impurities after it once crystallized was gained by sublimation of I at low pressures.

Characterization of Compound I (4-Methoxy-Δ³-pyrrolin-2-one).—Compound I gave a m.p. 133–134°, sublimed at 90° at about 0.2 mm., was optically inactive, and showed no basicity on potentiometric titration.

Anal. Calcd. for $C_5H_7O_2N$: C, 53.08; H, 6.25; N, 12.38; CH_3O, 27.44; mol. wt., 113. Found: C, 53.55; 53.38; H, 6.11, 6.05, N, 12.30; CH_3O, 27.59; mol. wt., 123 (isothermal distillation).

Determinations of terminal and N-methyl groups showed them absent in I. When dry hydrogen chloride gas was passed into a solution of I in benzene, a crystalline, chlorine-containing product was obtained. *Anal.* Calcd. for $C_5H_7O_2N \cdot HCl$: Cl, 23.7. Found: Cl, 23.1. This substance was too unstable for crystallization and gave off hydrogen chloride when allowed to stand at room temperature. Treatment of a solution of this hydrogen chloride adduct of I with a strong cation-exchange resin regenerated I.

Dibromide of 4-Methoxy-Δ³pyrrolin-2-one (V).—A solution of 0.150 g. of I in 5 ml. of water was mixed with 18 ml. of saturated bromine water. The yellow color of the solution was dispelled by addition of a trace of I. The solution was extracted 15 times with 15 ml. of ether, and the combined ether extracts were dried and evaporated to give 0.242 g. of a white crystalline residue. Three recrystallizations of the material from benzene gave 0.145 g. of V, m.p. 143–144°, as colorless needles.

Anal. Calcd. for $C_4H_3O_2NBr_2$: C, 18.70; H, 1.18; O, 12.46; N, 5.45; Br, 62.21. Found: C, 18.68; H, 1.20; O, 13.23; N, 5.86; Br, 63.57.

A methoxyl determination indicated the absence of the group. In the infrared (in chloroform), the substance exhibited a strong band at 1720 cm.$^{-1}$ and a somewhat weaker one at 1730 cm.$^{-1}$; the other principal bands were found at 1255, 1770, 3180 and 3380 cm.$^{-1}$. No bands were found characteristic of either OH or NH. The substance spontaneously liberated iodine from starch–iodide paper, and gave an immediate reaction with 2,4-dinitrophenylhydrazine.

Benzal Derivative of 4-Methoxy-Δ³pyrrolin-2-one (IV).—The reaction is modeled after a similar one in the literature.[11] To a solution of 0.050 g. of I in 3 ml. of 4 *N* sodium hydroxide was added 0.050 g. of benzaldehyde dissolved in 1 ml. of methanol. The solution was heated to 100° for 8 minutes, 1 ml. of water was added, the solution cooled, and the colorless crystals that separated were collected and washed. The dried product, 0.085 g. (96%), had m.p. 182–183°, which after two recrystallizations from benzene was constant at 185–186°.

Anal. Calcd. for $C_{11}H_{11}O_2N$: C, 71.62; H, 5.52; N, 6.96. Found: C, 71.25; H, 5.66; N, 7.35.

4-Methoxy-2-pyrrolinone (III).—A mixture of 0.100 g. of I, 5 ml. of 70% aqueous ethanol and excess Raney nickel was heated at reflux for 2.5 hr. The mixture was filtered, evaporated under vacuum, and the residue was distilled at 70–80° at 1 mm. to give a colorless sirup, which crystallized when cooled. Three recrystallizations of the material from benzene and petroleum ether gave 0.070 g. (69%) of white needles, m.p. 53–54°.

Anal. Calcd. for $C_5H_9O_2N$: C, 52.15; H, 7.89; CH_3O, 26.96. Found: C, 52.58; H, 7.93; CH_3O, 26.82.

Ozonolysis of 4-Methoxy-Δ³-pyrrolin-2-one (I).—A stream of ozone was introduced at 25° into a solution of 0.100 g. of I dissolved in 20 ml. of glacial acetic acid containing 10 drops of water. The stream of ozone was interrupted when a trap containing acidic potassium iodide placed after the reaction vessel liberated iodine. The resulting solution was mixed with 20 ml. of 6 *N* hydrochloric acid and heated to 100° for 30 minutes. The solution was evaporated to dryness and diluted to 4 ml. with water. Paper chromatography and electrophoresis showed the main products to be glycine and glyoxylic acid, the latter giving a very characteristic black color with silver nitrate spray in acetone (followed by ethanolic sodium hydroxide) and a light yellow color with a 2,4-dinitrophenylhydrazine spray.

From part of the solution was prepared a 2,4-dinitrophenylhydrazone derivative (60% over-all yield), m.p. 190–191°, undepressed with that derivative of authentic glyoxylic acid.

A second part of the solution was passed through a small column of cation-exchange resin (Dowex-50, H^+), and the glycine was eluted with 2 *N* hydrochloric acid. The eluate was evaporated to dryness and recrystallized from aqueous ethanol to give a 78% over-all yield of glycine hydrochloride, m.p. 181–183°, undepressed by admixture with an authentic sample.

Oxidation of 4-Methoxy-Δ³-pyrrolin-2-one (I) with Hydrogen Peroxide.—Many preliminary experiments that involved paper chromatographic and electrophoretic methods provided the following procedure for the oxidation. To a solution of 0.400 g. of I dissolved in 10 ml. of water was added 3 ml. of 30% hydrogen peroxide. The solution was kept at 94° for 1.5 hr. under a condenser. After 20 minutes a gas was evolved, and a yellow color appeared, which subsequently disappeared. The solution was cooled, and the *p*H of 1 to 2 was adjusted to 7 by addition of sodium bicarbonate. A few mg. of catalase was added to destroy the excess hydrogen peroxide, and the solution was left at 25° for 4 hr. The solution was passed through a cation exchange resin column (Dowex-50, H^+), and the eluate and washings were evaporated to a sirup (fraction I) under vacuum at 35°, and the distillate (fraction II) was collected at −40°. The column was eluted with 2 *N* hydrochloric acid, the eluate evaporated to dryness (vacuum), and the residue was dissolved in water and passed through a column of anion-exchange resin (Dowex-1, OH^-). The alkaline column eluate contained ammonia (R_f-value and decomposition point of its hydrochloride). The column was eluted with 2 *N* hydrochloric acid, the eluate was evaporated in vacuum and the residue was dried over P_2O_5 and then NaOH to give 0.109 g. of a crystalline product. Recrystallization of the material from aqueous ethanol gave white crystals of glycine hydrochloride, m.p. 183–184°, not depressed by admixture with authentic material. Conversion of the material to its methyl ester gave the hydrochloride of glycine methyl ester, m.p. 174–175°, undepressed by admixture with an authentic sample.

Fraction II was continuously extracted with ether for 2 days. The ether phase was shaken with an excess of a weak anion-exchange resin (Dowex-3, free base), and the acid was recovered from the resin as ammonium salt by treatment with ammonia. The salt was quantitatively transformed into the free acid by passing the solution through a microcolumn of cation exchange resin (Dowex 50). The acid was titrated with standard sodium hydroxide (0.157 meq. end-point) and the resulting solution evaporated and dried to give 0.011 g. of salt. The data provided an equivalent weight for the acid of 48 (±5), a fact consistent with its being formic acid (46). The free acid was regenerated, extracted into ether, isolated and shown to possess the same R_f value as formic acid on dimethyl sulfoxide impregnated paper with hexane as the mobile phase.

Fraction I was evaporated (vacuum) and finally lyophilized to give 0.460 g. of a partly crystalline sirup. An aliquot of the sirup was extracted with ether and crystallized to give 0.080 g. of soft white hygroscopic crystals (probably a monohydrate) of N-(carboxymethyl)-oxamic acid (II), m.p. 64–66°, undepressed on admixture with an authentic sample (m.p. 65–66°) prepared by liberation of the free acid from its calcium salt.[12] The free acid from the degradation experiment was dried for 2 days over P_2O_5 at 40° to give material with no definite melting point, but a good analysis for II.

Anal. Calcd. for $C_4H_5O_5N$: C, 32.66; H, 3.43; N, 9.52. Found: C, 32.42, 32.81; H, 3.75, 3.69; N, 9.48, 9.64.

The synthetic acid and that obtained in the oxidation were further shown to be identical by paper chromatography (R_f value of 0.31 in acetic acid–*n*-butyl alcohol–water, 1:2:3:4) on Whatman No. 1 paper, detected with methyl red spray as indicator). The dimethyl esters[13] of the two samples of II were also prepared, and demonstrated to possess identical infrared spectra in chloroform solution.

Synthesis of 4-Methoxy-Δ³-pyrrolin-2-one (I).—Ethyl 4-methoxycrotonate was prepared by the method of Arndt, *et al.*,[14] on a 31-g. scale in 28% yield, b.p. 90–95°. The material was shown by vapor-phase analysis to be about 80% of the desired ether and 20% of ethylenoxide XII. This mixture was brominated directly with N-bromosuccinimide by a recorded method[15]

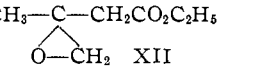
(11) H. Plieninger and M. Decker, *Ann.*, **598**, 198 (1956).

(12) A. Kraemer, *Ber.*, **39**, 4385 (1906).

(13) Diazomethane was used on II from the natural source, and the method of W. Kerp and K. Unger [*ibid.*, **30**, 579 (1897)] was employed for preparation of authentic material.

(14) F. Arndt, L. Loewe, T. Serge and L. Türegrün, *ibid.*, **71**, 1640 (1938).

(15) D. G. F. R. Kostermans, *Rec. trav. chim.*, **70**, 79 (1951).



XII

on a 31-g. scale to give 25 g. of ethyl 4-bromo-3-methoxycrotonate, $n^{25}D$ 1.4944 (lit.[16] $n^{19}D$ 1.4988). This material was shown by vapor phase chromatography to be contaminated with 5% of XII.

The bromo ester was converted to ethyl 4-phthalimido-3-methoxycrotonate as follows.[17] Bromo ester (22.2 g.) and 18.5 g. of N-potassium phthalimide were dissolved in 150 ml. of isoamyl ether, and stirred vigorously at reflux temperature for 2 hr. The dark brown, hot solution was filtered, and the inorganic precipitate was washed twice with 5 ml. of hot isoamyl ether. The filtrate was cooled, and the slightly brown precipitate collected. The semicrystalline solid was extracted with two 100-ml. portions of boiling benzene, the filtered benzene solutions were concentrated to 60 ml., and diluted with 180 ml. of hot isoamyl ether. When cooled, nearly colorless thin plates separated, which when recrystallized twice from methanol gave 3.18 g. (11%) of colorless crystals of desired ester, m.p. 131–132.4°.

Anal. Calcd. for $C_{15}H_{15}O_5N$: C, 62.27; H, 5.22. Found: C, 62.36; H, 5.44.

This phthalimidic ester was converted to 4-methoxy-Δ^3-pyrrolin-2-one as follows.[18] The ester (2.6 g.) and 0.55 g. of hydrazine hydrate were dissolved in 25 ml. of methanol, and held at reflux for 1 hr. under nitrogen. The resulting solution was brought to *p*H 7 with 1 *N* hydrochloric acid solution, filtered, and the filtrate was concentrated under vacuum to 2 ml. The brown sirup was extracted 3 times with 10 ml. of chloroform, and the chloroform layers were washed twice with 2 ml. of sodium sulfate-saturated water. The organic layer was dried, and evaporated to dryness under vacuum at 25°. Sublimation of the residue at 110° and 1 mm. over a period of 6 hr. gave 0.60 g. of nearly colorless sublimate. After 4 recrystallizations of this material from methanol–ether mixtures, 0.130 g. of 4-methoxy-Δ^3-pyrrolin-2-one (I) was obtained, m.p. 129–131.4°, undepressed by admixture with material isolated from althiomycin. The ultraviolet and infrared spectra of synthetic and "natural" specimens of I were identical in all respects, and both samples exhibited the same R_f values (0.50) in paper chromatograms (system *n*-butyl alcohol saturated with water on Whatman No. 1 paper).

Isolation of Thiazole-4-carboxylic Acid (VI) from Fraction A Obtained by Methanolysis of Althiomycin.—The tan powder obtained as fraction A was further hydrolyzed as follows. The material, 4.5 g., was heated at reflux with about 75 ml. of 6 *N* hydrochloric acid for 24 hr. The solution was filtered from a black residue and evaporated to approximately 25 ml. Ethanol (25 ml.) and 4 g. of Norit were added. The mixture was heated to 95° for 20 min., filtered, and the cake was washed with 50% aqueous ethanol. The filtrates were evaporated to dryness (vacuum) to give 2.92 g. of solid, which was submitted to chromatographic separation on a 5 by 56 cm. cellulose column prepared by wet packing with the lower phase of an *n*-butyl alcohol–acetic acid–water mixture, 3:0–9:4. The column was equilibrated with the upper phase of the solvent system, and the solid put on the column dissolved in a small amount of the upper phase. The upper phase was used as eluent, with methyl orange as a guide to indicate the solvent front. The eluate was collected in 300-ml. fractions. Paper chromatograms were run on each fraction in the upper layer of acetic acid–*n*-butyl alcohol–water, 1:4:5, with sprays of ninhydrin and silver nitrate in acetone (followed by ethanolic sodium hydroxide). Compound VI appeared in fraction 3 (0.322 g. of solid) and to a lesser extent in 4 (0.135 g. of solid) and possessed an R_f value of 0.65 on Whatman No. 1 paper. Fraction 3 was heated (0.1 mm.) and VI sublimed in a nearly pure state, wt. 0.155 g., at 3.4% of the weight of fraction B. Paper chromatography of the residue showed that more material was trapped in the residue. Recrystallization of the sublimate from methyl ethyl ketone gave white needles, m.p. 195.5–196°, undepressed by admixture with an authentic sample[5] of thiazole-4-carboxylic acid.

Anal. Calcd. for $C_4H_3O_2NS$: C, 37.20; H, 2.36; N, 10.37; S, 24.87. Found: C, 37.64, 37.64; H, 2.33, 2.57; N, 10.51, 10.06; S, 24.52.

This acid exhibited an equivalent weight of 124 (theory 129) and a pK_a of about 3.8. Infrared spectra of the two specimens of the acid were identical.

A sample of VI obtained from fraction A was converted to its acid chloride (thionyl chloride method),[5] and gave m.p. 85–86°, lit.[5] 85°. The acid chloride was treated with ammonia to give thiazole-4-carboxamide, m.p. 151–152°, lit.[5] 150°.

Acetylalthiomycin.—A mixture of 5 g. of 95% pure (Craig distribution) althiomycin, 50 ml. of pyridine and 20 ml. of acetic anhydride was stirred at 25° for 18 hr. (complete solution occurred after 1 hr.). The dark solution was poured onto ice, and a sticky dark brown precipitate appeared. The mixture was evaporated to dryness under vacuum at room temperature, and the yellow-brown solid was dissolved in methylene chloride containing 10% ethanol. The solution was treated with Norit, filtered, and the solution was evaporated to the cloud point. The yellow solid that separated was twice recrystallized from the same mixed solvent to give 1.73 g. of very light yellow plates, m.p. 183–186° dec. The filtrate from the last recrystallization gave a second crop of very soft white needles (like cotton), 0.27 g., m.p. 187–189° dec. These two specimens appear to be polymorphs. They give slightly different infrared spectra as KBr disks, but identical spectra in chloroform. Methylene chloride as recrystallizing solvent favors plates, whereas ethanol favors needles. The crystal forms were interconverted through employment of the proper solvent.

Anal. Calcd. for $C_{18}H_{19}O_6N_5S_2$: C, 46.46; H, 4.12; O, 20.63; N, 15.04; S, 13.75; CH_3O, 6.67; CH_3CO, 9.25. Calcd. for $C_{18}H_{17}O_5N_5S_2$: C, 46.65; H, 3.69; O, 20.71; N, 15.11; S, 13.81; CH_3O, 6.69; CH_3CO, 9.28. Found: C, 46.63, 46.48, 45.77, 45.99, Av. 46.22; H, 3.70, 3.91, 4.12, 3.86, Av. 3.90; O, 21.94; N, 14.70, 14.35, 15.61, 13.06, 12.88, 15.32, 14.33, 14.48, 15.90, Av. 14.51; S, 13.58; CH_3O, 6.55; CH_3CO, 9.46.

The compound is soluble in pyridine, dimethyl sulfoxide, and dimethylformamide; less soluble in ethanol, acetic acid and chloroform; and is insoluble in water, benzene and carbon tetrachloride. Prolonged heating of the compound in any solvent caused decrease in the melting point and formation of an amorphous solid. The compound decomposed rapidly in the presence of either acid or base. The nitroprusside test for mercapto groups was negative. In dimethylformamide, the compound exhibited no optical activity (α_{obs}^{25} 0.00° at 1 dm., λ 546 mμ) as a 1% solution, and its solution darkened rapidly. No optical activity was observed in a 5% solution in dimethyl sulfoxide (α_{obs}^{25} 0.00° at 1 dm., λ 546 mμ). An attempt to carry out a Rast molecular weight determination resulted in extensive decomposition of the compound. The isothermal distillation method in methylene chloride led to polymerization of the compound.

Methanolysis of Acetylalthiomycin.—This reaction was carried out by the same procedure employed for althiomycin itself except that the scale was greatly reduced (0.36 g. of acetylalthiomycin), and instead of isolating 4-methoxy-Δ^3-pyrrolin-2-one (I) and thiazole-4-carboxylic acid (VI), their presence was demonstrated through use of paper chromatography at the appropriate stages, with authentic specimens of the two compounds as standards. The former substance (I) gives a characteristic R_f value of 0.67 on Whatman No. 1 paper with acetic acid–1-butanol–water (1:4:5), the upper layer, as developer, and an equally characteristic immediate brown-yellow spot with a spray of silver nitrate in acetone followed by sodium hydroxide in ethanol. The latter compound (VI) was demonstrated to be present in the acid hydrolysate of fraction B obtained from methanolysis of acetylalthiomycin. Thiazole-4-carboxylic acid (both authentic material and material from acetylalthiomycin) exhibited an R_f value of 0.65 on paper chromatograms run with the same solvent system and spray (see above). A characteristic brown spot appears slowly after several minutes after the paper was sprayed with the silver nitrate–alkaline solutions. Two other components with R_f values of 0.12 and 0.46 also reacted with the spray, but much more rapidly.

Acid Hydrolysis of Acetylalthiomycin.—Acetylalthiomycin (0.46 g.) was heated with 75 ml. of 5 *N* sulfuric acid (100°). As the compound dissolved, bubbles formed on the surface of the crystals. The gas formed was demonstrated not to be a volatile aldehyde or ketone by appropriate experiments with dimedon. When passed through an aqueous solution saturated with barium hydroxide, the gas caused a precipitate of barium carbonate, which demonstrated the gas to be carbon dioxide. The yellow sulfuric acid solution was steam distilled after being heated at reflux for 1.5 hr. A total of 65 ml. of distillate was collected before a fresh portion of distillate became neutral.

Titration of the distillate to a phenolphthalein end point with standard base indicated a 178% yield of one volatile acid residue. A test of the acidic distillate with lead acetate demonstrated the absence of sulfide. The original solution was made basic and further steam distilled until the distillate was neutral, and titration of the total distillate with standard acid revealed a 100% yield of one volatile basic residue. Use of Nessler reagent identified this volatile base as ammonia. Tests of the basic distillate for sulfide (lead acetate) were negative.

Both the volatile acid and base distillates contained material which reduced silver ion. Exhaustive extraction of the neutralized acid and base distillates with ether left the reducing compound in the aqueous layer.

In a second experiment, 1.003 g. of acetylalthiomycin was held at reflux with 50 ml. of 6 *N* sulfuric acid for 1.5 hr. The presence of evolved hydrogen sulfide during this period was demonstrated with filter paper dipped in lead nitrate and placed

(16) F. Kögl and O. A. D. DeBruin, *Rec. trav. chim.*, **69**, 729 (1950).

(17) This procedure is patterned after that of D. Langenbeck and H. Boser, *Ber.*, **84**, 526 (1951).

(18) This method was patterned after that of R. Ing and R. H. F. Manske, *J. Chem. Soc.*, 2348 (1926).

at the outlet of the reflux condenser. The solution was steam distilled until the distillate was neutral, and the total distillate was neutralized with excess barium hydroxide, filtered, and evaporated to dryness. The solid residue was treated with sulfuric acid, the barium sulfate was filtered, and 2 ml. of an ammonium sulfate solution was added to the filtrates, which were then subjected to paper chromatography with ethanol–ammonia solution–water (80:4:16) as solvent system. Comparisons were made with formic and acetic acids individually and together, in each case in the presence of ammonium sulfate. Both ninhydrin and the silver ion spray (for reducing substances) were employed. With ninhydrin spray, the R_f values for the formic and acetic acids both individually and as a mixture was 0.56 ± 0.02. The measured R_f value (ninhydrin) for the volatile acids from acetylalthiomycin was 0.56, the only other spot on the paper being due to ammonium sulfate near the origin. With basic silver ion spray, authentic acetic acid gave no spot, whereas formic acid or the acetic–formic acid mixture gave spots with an R_f value of 0.58. The volatile acids from acetylalthiomycin gave an R_f value of 0.58. These experiments demonstrate the presence of formic acid in the volatile acids from acetylalthiomycin.

Amino Acid Analyses of Althiomycin and Acetylalthiomycin.—The Moore and Stein method[19] for amino acid analysis was applied to hydrolysates of althiomycin and acetylalthiomycin.[20] These procedures, except for minor variations, were followed:

The sample (2 to 3 mg.) to be analyzed was hydrolyzed[19b] for 22 hr. in a sealed tube with redistilled 6 *N* hydrochloric acid (1 ml.). A constant temperature of 110° was maintained by suspending the tube above refluxing toluene. After cooling and drying in a vacuum desiccator over phosphorus pentoxide and sodium hydroxide, the sample was dissolved in water and 0.2 *M* sodium phosphate buffer, *p*H 6.5 (1 ml. total solution). The sample was then allowed to stand in air for 4 hours to oxidize cysteine to cystine before reacidification with *N* hydrochloric acid (0.06 ml.) and 0.2 *N* sodium citrate buffer, *p*H 2.2 (2 ml.). The resulting solutions were kept frozen until analyzed.

The most effective column material now in use is a strong cation exchange resin, Dowex 50-X4, hydraulically sieved, through 200 mesh. The pretreated resin[19a] as a slurry, resin–buffer (0.2 *N* sodium citrate–acetate, *p*H 5.09), was used to pack the water-jacketed column (0.9 × 150 cm.) under an air pressure of 10 cm. The column was washed overnight with 0.2 *N* sodium hydroxide under 10 cm. pressure. Before use, the column was equilibrated with 0.2 *N* sodium citrate buffer, *p*H 3.02, overnight until the *p*H of the eluate reached the *p*H of the developer. The column was mounted over a fraction collector actuated by an electronic drop counter calibrated to give 2-ml. fractions.

After introduction of the sample to the surface of the resin, the column was developed using the *p*H 3.02 buffer under 10-cm. pressure. The column was maintained at a temperature of 30° using a constant temperature bath with a water-circulating pump. The rate of elution was approximately 10 ml. per hour and was varied when necessary by a change in air pressure. After 21 to 24 hr. (210 to 240 ml.) the temperature was raised to 50°. A linear gradient system going from the 0.2 *N*, *p*H 3.02 buffer to a 2 *N*, *p*H 5.09 sodium citrate–acetate buffer (500 ml. of each) was started after 34 to 38 hours (340 to 380 ml.). A total of 1000 ml. of buffer was used to develop the columns.

The colorimetric detection procedure[19c] made use of the color reaction given by amino acids and ammonia with ninhydrin. The optical density of the solutions was determined using a Coleman Junior spectrophotometer at 570 mμ except for yellow solutions which were read at 440 mμ. The results given by various samples are presented in Table IV.

TABLE IV

RESULTS OF MOORE AND STEIN AMINO ACID ANALYSIS OF HYDROLYSATES

Sample[a]	Possible compound[b]	One residue recovd., %
Althiomycin (2.5 mg.)	Unknown (yellow)[c]	31
	Cystine[d,e]	37
	Ammonia[d]	217
	Unknown (red-violet)[f]	82
Althiomycin (2.47 mg.)	Cystine[d,e]	39
	Ammonia[d]	208
	Unknown (red-violet)[f]	95
Acetylalthiomycin (3.28 mg.)	Unknown (yellow)[c]	20
	Cystine[d,e]	26
	Ammonia[d]	115
	Unknown (red-violet)[f]	55
Oxidized acetylalthiomycin (2.23 mg.)	Cysteic acid[d]	70
	Unknown (yellow)[c]	6
	Ammonia[d]	236
	Unknown (red-violet)[f]	45

[a] Molecular weights assumed for purposes of calculation were: althiomycin, 722; acetylalthiomycin, 463; oxidized acetylalthiomycin, 511 (assumes that three oxygens were added). [b] Compounds listed in order of their elution. [c] Color yield presumed to be equal to that of proline, which produces a similar color. [d] Leucine was used as a standard reference, and correction was made for the varying color yield of each compound compared to leucine (ref. 19c). [e] Cysteine is air oxidized under the conditions of the experiment to cystine, which is analyzed as such. [f] Color yield based on leucine but not corrected to any amino acid relative to that of leucine.

All chromatograms showed many peaks which amounted to less than 10% of one residue. The unknown "yellow" peak gave the same color with ninhydrin as does proline, and appeared between the elution positions of proline and hydroxyproline. The unknown "red violet" peak was eluted where lysine would be expected. However, no evidence either by paper chromatography or electrophoresis could be obtained on these hydrolysates to support the possibility that lysine is present. The analytical procedure is particularly sensitive to contamination by ammonia, and the values obtained are undoubtedly high.

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The Oxidation and Reduction of Free Radicals by Metal Salts

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The oxidation and reduction of organic free radicals by metal salts has been studied. The organic products of oxidation are highly dependent on the anion associated with the metal moiety. The use of such ligands as chloride, bromide and thiocyanate results in the formation of the corresponding substituted product, such as ethyl chloride from ethyl radical and cupric chloride. Sulfate or perchlorate salts yield products of elimination such as ethylene from ethyl radical and cupric sulfate. The mechanism of these processes is discussed; the former has been termed *ligand transfer* and the latter *electron transfer*. In both cases the metal moiety is reduced to the lower valence state. Oxy and thiyl radicals are unaffected by cupric salts which are so effective with carbon free radicals. Reduction of free radicals is possible by such metal ions as chromous, titanous and vanadous. In these reactions, carbanion intermediates are formed. Based on these reactions, preparative procedures for 5-hexenoic acid and esters, heptenoic acids, 6-substituted hexanoic acids, 6-heptene-2-one and methoxy-*t*-butoxybutenes are developed.

Introduction

The oxidation and reduction reactions of organic free radicals promoted by metal salts have been recognized by Waters for some time.[1] Recently, Dainton[2] and Bamford[3] and their co-workers have reported some quantitative values for the rates of some of these reac-

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