## THE STRUCTURE OF ALTHIOMYCIN[1]

Sir:

The unequivocal formulation of bisanhydroalthiomycin (**I**) prepared during a chemial study has been accomplished by an X-ray crystallographic study[2]. In this report, structural studies are described which have led to assignment of the (E)-N-[2-hydroxy-1-[4-[(4-methoxy-2-oxo-3-pyrroline-1-yl)carbonyl]-2-thiazoline-2-yl]ethyl]-2-[(hydroxyimino)-methyl]-4-thiazolicarboxamide structure (**II**) for althiomycin based on the X-ray results and chemical and spectroscopic evidence, showing that althiomycin is a unique sulfur-containing antibiotic with an aldoxime-group.

There were confusing data about the molecular formula of **II**, for instance $C_{15}H_{14}N_4O_6S_2$ by YAMAGUCHI, *et al.*[3] and $C_{27}H_{28}N_8O_{10}S_3$ by CRAM, *et al.*[4] The reason may be attributed to the instability of **II**. However, the formula $C_{16}H_{17}N_5O_6S_2$ is most consistent with the analytical data of purified **II**, mp 181~184°C (dec); $[\alpha]_D^{25}+25°$ (*c* 1.33, methylcellosolve), and the $^{13}$C-nmr spectrum (Table 1) of which confirmed the number of the carbon atoms.

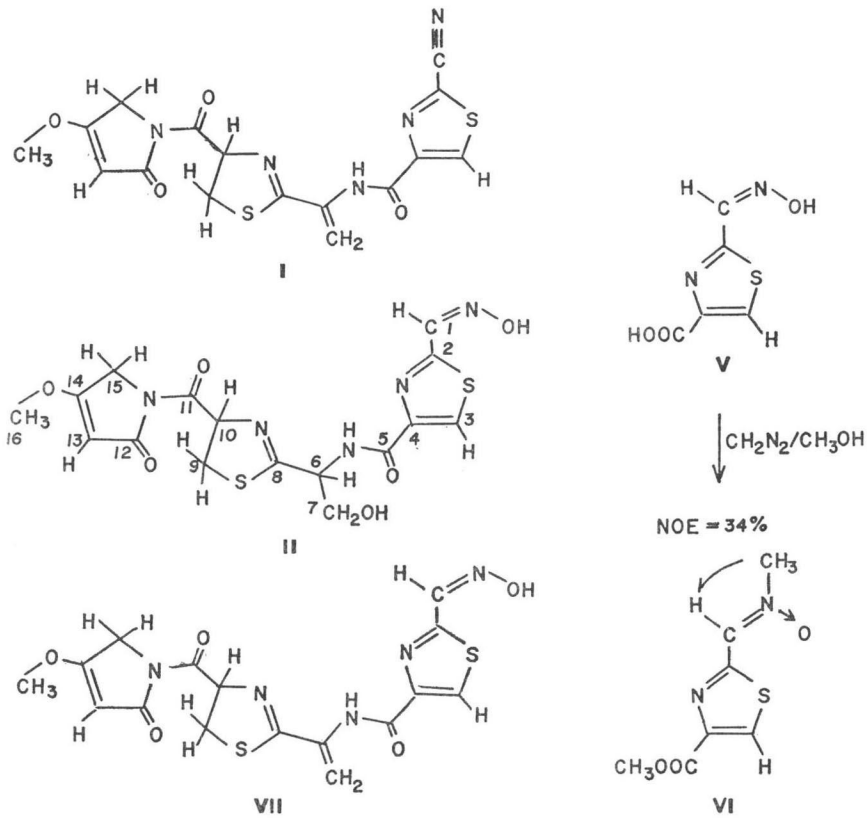
After oxidation of **II** with performic acid, the product was hydrolyzed with 6 N hydrochloric acid (105°C, 24 hours), affording DL-cysteic acid, DL-serine, glycine, thiazole-4-carboxylic acid[5], and ammonia. The results show that **II** is a modified peptide antibiotic. The base-catalysed hydrolysis (2 N NaOH, 60°C, 2 hours) of **II** afforded DL-serine, cystine, 4-methoxy-$\Delta^3$-pyrrolin-2-one (**III**)[4], 2-($\alpha$-aminoethyl)-thiazole-4-carboxylic acid (**IV**)[6], and a new thiazole compound $C_5H_4N_2O_3S$ (**V**, mp 256~259°C, $M^{+\cdot}$ 171.9953).

The new thiazole compound **V**, $\lambda_{max}$(EtOH) 223.5 nm ($\varepsilon$ 21300) and 287.5 nm ($\varepsilon$ 8100), became a key compound for the elucidation of the structure of **II**. The nmr spectrum showed two protons at $\delta$ 8.38 (s, aromatic proton) and 8.46 (s, aldoxime proton) and a hydroxyl proton at $\delta$ 12.18 (s). The presence of an aldoxime group was indicated by a positive test with TOLLENS reagent. 2-Deuterothiazole-4-deuterocarboxylic acid was obtained from **V** by treatment with 20% deutrium chloride in deuterium oxide proving that the aldoxime group was located at the C-2 position, since thiazole-4-carboxylic acid

Table 1. $^{13}$C-NMR Spectra of althiomycin and monoanhydroalthiomycin

| Carbon[a] | Althiomycin | | Monoanhydroalthiomycin | |
|---|---|---|---|---|
| | Chemical shift[b], ppm | Multiplicity on off-resonance | Chemical shift[b], ppm | Multiplicity on off-resonance |
| 1 | 145.8 | doublet | 145.6 | doublet |
| 2 | 165.5 | singlet | 165.7 | singlet |
| 3 | 127.4 | doublet | 128.3 | doublet |
| 4 | 162.8 | singlet | 161.0 | singlet |
| 5 | 171.4 or 172.6 | singlet | 170.5 or 170.9 | singlet |
| 6 | 55.1, 54.9[c] | doublet | 136.4 | singlet |
| 7 | 63.5 | triplet | 109.7 | triplet |
| 8 | 152.6 | singlet | 152.1 | singlet |
| 9 | 35.5 | triplet | 36.3 | triplet |
| 10 | 79.4, 79.1[c] | doublet | 78.7 | doublet |
| 11 | 171.4 or 172.6 | singlet | 170.5 or 170.9 | singlet |
| 12 | 176.5 | singlet | 172.5 | singlet |
| 13 | 96.0 | doublet | 95.9 | doublet |
| 14 | 180.3 | singlet | 180.4 | singlet |
| 15 | 49.0 | triplet | 49.0 | triplet |
| 16 | 60.2 | quartet | 60.2 | quartet |

[a] For the numbering, see structure **II**. All of the assignments of any given carbons are tentative.
[b] Downfield from internal TMS.
[c] See footnote 2*on p. 899.




was formed from **II** with 6 N hydrochloric acid. Conclusive evidence for the presence of the aldoxime-group in **V** was obtained by the reaction of **V** with diazomethane. It was found that two moles of diazomethane reacted with **V** in methanol to afford the corresponding nitrone **VI** (mp 196~198°C, $M^+$ 200.0237). The nmr spectrum showed two methyl groups at $\delta$ 3.86 ($COOCH_3$) and 3.96 ($=N(CH_3)\rightarrow O$), and two protons at 8.51 (aromatic proton) and 8.77 ($-\overset{H}{C}=N<$). The mass spectrum showed the characteristic fragmentation of a nitrone, containing an N-oxide moiety[7], at *m/e* 184 ($M^{+\cdot}-O$), 183 ($M^{+\cdot}-OH$) and 42 ($CH_3-\overset{+}{N}\equiv CH$).

Generally, *syn*-aldoximes give mostly the O-methyl derivative, whereas antialdoximes give a much higher proportion of nitrone[7,8] with methyl iodide or dimethyl sulfate in basic media. There is no complete investigation of the reaction of aldoximes and diazomethane. Therefore. the reaction of *m*- and *p*-chlorobenzaldoximes of known configuration with diazomethane was investigated, and *syn*- and *anti*-isomers gave the corresponding O-methyl and nitrone derivatives, respectively, in almost quantitative yields. It should be mentioned here that the reaction proceeds smoothly only in the presence of methanol in ether. On the other hand, althiomycin (**II**) was easily dehydrated by heating in butanol (60°C, 75 hours, 95% yield) to yield monoanhydroalthiomycin (**VII**), mp 211~212°C (dec); $[\alpha]_D^{25}+55.3°$ (*c* 1.0, DMSO). The nmr spectrum of **VII** showed clearly that the dehydration occurred at the serine moiety ($HOCH_2-\overset{C}{\underset{N}{CH}}\rightarrow CH_2=\overset{C}{\underset{N}{C}}$). Furthermore, **VII** was easily dehydrated by acylation with N-bromoacetoxysuccinimide to give bisanhydroalthiomycin (**I**), mp 222~224°C (dec); $M^+$ 403, $[\alpha]_D^{25}=0$, ir 2235 $cm^{-1}$ (CN), showing the change $-\overset{H}{C}=NOH\rightarrow CN$ and complete

racemization. The reaction was originally conducted with a view to adding a heavy atom for X-ray study, although **I** itself turned out to be a suitable compound. The facile conversion of the aldoxime group of **VII** to the cyano group ($-C(H)=N-OH \rightarrow CN$) also supports the *anti*-configuration of the aldoxime group[9].

Since **III** is mono-functional*1 and only the carboxyl group of the thiazole **V** is available for peptide linekage, **III** and **V** must be located at the terminal positions. However, an unambiguous formulation of **II** using other hydrolysis products was not achieved at this stage, and the differentiation between a chain- and a cyclic-structure was considered to require many additional experiments. The problem was solved by the X-ray study as shown in the accompanying paper.[2] Compound **IV** is reasonably explained as being formed from the thiazoline-serine moiety of **II** after dehydration followed by isomerization.

Finally, investigation of the two asymmetric centers of serine and cysteine was carried out, and D-cysteic acid was obtained after methanolysis of **VII** followed by performic acid oxidation and hydrolysis with 6N hydrochloric acid, but serine was always found in the racemic form even under the condition where L-serine holds the stereochemistry. Therefore, cysteine has the S-configuration and the serine originally exists in the racemic form or might be racemized during either isolation or hydrolysis processes.*2

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(Received June 17, 1974)

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*1 This compound afforded glycin in good yield with 6N HCl.

*2 The $^{13}$C-nmr spectrum of **II** shows evidence for the existence of two diastereoisomers in equal amounts as expected from the SR-configuration at C-6 and the S-configuration at C-10, and this has been confirmed by $^{13}$C-nmr spectroscopy of **VII** which shows singlet peaks for the corresponding carbons.

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