Communications to the editors

CHEMISTRY OF BLEOMYCIN. IV* THE STRUCTURE OF AMINE COMPONENT II OF BLEOMYCIN A₂

Sir:

We previously reported^{2,3)} the structures of six ninhydrin-positive products obtained by acid hydrolysis of bleomycin A₂. In this communication, unidentified compound II²⁾ is shown to be β -amino- β -(4-amino-6-carboxy-5-methylpyrimidin-2-yl)-propionic acid (II)**.

HOOC
$$N$$
 CH-CH₂-COOH
CH₃ N NH₂ (II)
NH₂ (II)

Compound II was obtained as a partially racemized mixture. It has the molecular formula $C_9H_{12}N_4O_4 \cdot H_2O$. On a cellulose thin-layer chromatogram using *n*-propyl alcohol - pyridine - acetic acid - water (15: 10:3:12 by volume), it gave two spots: Rf, 0.146 (main) and 0.250. The optical activity of the former was $[\alpha]_{365}^{20}$ +44.9° (c 0.7, 2 N HCl) and the latter was -45.0° . They gave the same UV, IR, and NMR spectra, and each of them was racemized in alkaline or acid solution. The ultraviolet absorption spectrum of II shows two maxima at $234 \text{ m}\mu$ $(\log \varepsilon 3.9)$ and $274 \,\mathrm{m}\mu \,(\log \varepsilon 3.7)$ in aqueous solution. The nmr spectrum of II in

Fig. 1. The NMR spectrum of IIb [100 M Hz, in D₂O, internal reference : TMS (suspension)]



deuterium oxide shows peaks at 2.41 (3H; singlet), 3.27 (2H; doublet, J=7 Hz) and 5.09 δ (1H; triplet, J=7 Hz) (internal reference; DSS). Potentiometric titration showed the presence of four dissociable groups (one at pKa' 9.2, three at less than 3.9). By refluxing a methanolic solution saturated with hydrogen chloride, II gave the dimethyl ester dihydrochloride (IIa) [1710 and 1735 cm⁻¹, two singlets at 3.81 (3H) and 4.09 δ (3H) in D₂O]. Compound II liberated 1.26 mole of nitrogen by VAN SLYKE's nitrogen analysis, losing the strongly basic function. These results indicate that two of the four dissociable groups are carboxy groups, one is an aliphatic primary amino group, and the other is a weak base.

Compound IIa was reduced by lithium borohydride in tetrahydrofuran and the reduced product (IIb) was obtained as the crystalline dihydrochloride. It has the molecular formula $C_9H_{18}N_4O_2Cl_2$. The nmr spectrum of IIb is shown in Fig. 1. The spectrum indicates that two hydroxy methyl groups were formed by reduction of carbomethoxy functions [5.51 (2H; singlet), and 4.48 δ (2H; triplet, J=6.5 Hz in D₂O]. It also indicates the presence of $-CH-CH_2-CH_2OH$ carbon chain [5.55 (1H; triplet, J=6.5 Hz), 2.98 (2H; quartet, J=6.5 Hz) and 4.48 δ (2H; triplet, J=6.5 Hz)].

From these results, partial structure of II was deduced as follows:

$$\left[\begin{array}{c} -CH_{3} \\ -COOH \\ -CH-CH_{2}-COOH \\ -CH-CH_{2}-COOH \\ \dot{N}H_{2} \end{array}\right]$$

The presence of a β -amino propionic acid moiety was supported further by mass spectrum of IIa, which shows a distinct peak at m/e 195 (M-CH₂COOCH₈).

The UV spectrum⁴ suggested that II would be a 4-aminopyrimidine derivative.

We synthesized three substitutional isomers of 4-aminopyrimidine which have two methyl and one carboxy groups. They

* Part III of this series is published in J. Antibiotics 22: 237~239, 1969.¹⁾

** The structure of compound II presented at the 166 th meeting of Japan Antibiotics Research Association, March 28, 1969 in Tokyo, should be revised as shown in this paper. served as model compounds for UV absorption. The scheme of the synthesis is as follows:



Details of the synthesis will be published in a separate paper. Ultraviolet absorption spectra of II and model compounds are shown in Fig. 2. The UV spectrum of II was similar to 4-amino-6-carboxy-2, 5-dimethylpyrimidine in dilute alkaline solution.

Compound II and 4-amino-6-carboxy-2,5dimethylpyrimidine were hydrogenated with platinum catalyst in dilute hydrochloric acid solution. The hydrogenated products were



immediately hydrolyzed with 6 N hydrochloric acid. From both hydrolyzates, DL-threomethylaspartic acid was isolated as crystals, which was identified by direct comparison of IR spectra with the synthetic material⁵.

Thus compound II from bleomycin is β -amino- β -(4-amino-6-carboxy-5-methylpyri-midin-2-yl)-propionic acid.



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