Communications to the editor

CHEMISTRY OF BLEOMYCIN. V
REVISED STRUCTURE OF AN AMINE COMPONENT OF BLEOMYCIN A_2

Sir:

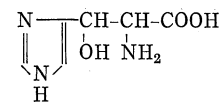
Previously[1], we reported that bleomycin A_2 yielded at least seven ninhydrin-positive products by acid hydrolysis. We assigned β-carboxy-histidine to one of the hydrolysis products, named compound IV in the former report[1]. Further studies have revealed that IV has the following properties unexpected from the proposed structure. On paper electrophoresis using 0.1 M pyridine-acetate buffer of pH 5.0 IV migrated to the cathode like a basic amino acid. Refluxing a methanol solution saturated with hydrogen chloride gave a monomethyl ester from IV. These results suggested that IV contains only one carboxyl group. Recently, bleomycin became more available for our studies, so we started to reinvestigate the structure of IV.

Compound IV was isolated from the acid hydrolysate by chromatography over a sulfonic acid resin (Dowex 50 W×4) using 0.2 M pyridine-acetate buffer of pH 5.1. The solvent was evaporated under reduced pressure and dried under high vacuum. The dried material was dissolved in water, and the pH of the solution was adjusted to 4.5 with hydrochloric acid. After decolorization with charcoal, the solution was acidified to pH 3.0 with hydrochloric acid and concentrated. By addition of ethyl alcohol to the concentrate crystalline IV hydrochloride was obtained. It was recrystallized with water and ethyl alcohol; $[\alpha]_D^{28}+40°$ (c 1, H_2O).

Elemental analyses showed that IV has the molecular formula $C_6H_9N_3O_3 \cdot HCl$ (MW 207.63) [Found: C 34.91, H 4.76, N 20.33, O 23.28, Cl 16.77. Calcd.: C 34.71, H 4.85, N 20.24, O 23.12, Cl 17.08.]. This formula suggested that IV should be hydroxy histidine. The compound decomposed at about 205°C, giving small amounts of glycine and histidine, detected by thin-layer chromatography and paper electrophoresis. Three dissociable groups (pKa′ <2.0, 5.5, 8.8) were shown by potentiometric titration and titration equivalent was 216. The NMR spectrum was taken in deuterium oxide solution using tetramethylsilane as the external reference ($\delta=0$). There are four protons at 9.16 δ (d*, J=1.4 cps), 7.90 δ (dd*, J=1.4 & 0.9 cps), 5.97 δ (dd*, J=0.9 & 3.6 cps), and 4.64 δ (d*, J=3.6 cps). (d*=doublet; dd*=doublet of doublets). This spectrum indicated that IV should be β-hydroxy-histidine. The protons at 9.16 δ and 7.90 δ can be assigned as the 2− and 4−(or 5−) protons of the imidazole ring, respectively. The chemical shift (5.97 δ) of the β-carbon proton is due to the hydroxyl and the imidazole groups on the β-carbon.

To prove the presence of vicinal hydroxy and amino groups, the oxazolidone derivative of IV was prepared by treatment with phosgene in aqueous potassium hydroxide solution at 5°C for 2 hours;[2] m.p. 238~240 °C (dec.), ν_{KBr} 1765 and 1740 cm^{-1} [Calcd. for $C_7H_7N_3O_4$: C 42.64, H 3.58, N 21.32. Found: C 42.67, H 3.65, N 20.94].

The structure of compound IV



Finally, the structure of IV was confirmed by synthesis through the following steps. 4-Hydroxymethylimidazole was first synthesized by heating D-fructose with ammonium hydroxide, basic cupric carbonate, and formaldehyde[3]. 4-Imidazolecarboxaldehyde was prepared from 4-hydroxymethylimidazole by oxidation with nitric acid[4]. The aldehyde was treated with copper glycinate in sodium carbonate solution[5], giving a mixture of racemic IV and the diastereoisomer. The NMR spectrum and thin-layer chromatography of the mixture showed that the major product was racemic IV and the diastereoisomer in the ratio 2.5 : 1. Racemic IV and the diastereoisomer were separated by cellulose thin-layer chromatography using

methanol-water-pyridine (8 : 2 : 0.4, volume) as the developing solvent. The Rf values were 0.19 and 0.13, respectively. Racemic IV was separated from the diastereoisomer by cellulose column chromatography using the same solvent system as above, and was crystallized as the mono-hydrochloride. Recemic IV decomposed at about 205 °C giving small amounts of glycine and histidine, and it had no optical rotation. The results of the elemental analysis agreed with the calculated values. [Found : C 35.15, H 4.89, N 20.27. Calcd.: C 34.71, H 4.85, N 20.24].

Natural and synthetic IV showed the same chromatographic behavior in all systems so far tested. The NMR spectrum was exactly the same as that of natural IV under the same conditions, while the NMR spectrum of the diastereoisomer was slightly different from that of IV : 9.14 δ (d, J=1.5 cps), 7.96 δ (dd, J=1.5 & 1.0 cps), 5.86 δ (dd, J=1.0 & 5.0 cps), and 4.48 δ (d=5.0 cps).

From the above results, the structure of IV was determined as β-hydroxy-histidine. The absolute configuration of natural IV is now being studied by X-ray crystallographic analysis of the hydrobromide.

On mild alkaline treatment of bleomycin A_2, the hydroxyl group of IV was eliminated to yield dehydrohistidine, and, at the same time, the sugar moiety was liberated. This result suggests that one of the most likely binding-sites of the sugar moiety of bleomycin A_2 is the hydroxyl group of IV.

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