# CHEMISTRY OF BLEOMYCIN. XI*

## THE STRUCTURES OF THE TERMINAL AMINES

Sir:

In the previous paper[1], we presented six new components of bleomycin, demethyl $A_2$, $A_{2'}$-a, $A_{2'}$-b and $A_{2'}$-c, $B_{1'}$ and $B_6$. Of these components, the terminal amines of demethyl $A_2$, $A_{2'}$-a and $A_{2'}$-b have been described in our review on bleomycin[2]. Recently the total structure of bleomycin was proposed.[3] The components differ only in the terminal amine structure. In this communication, the structures of the terminal amines are presented (Table 1), thus describing the new bleomycins.

Bleomycin $A_1$, $A_2$, demethyl $A_2$, $A_{2'}$-a, $A_{2'}$-b and $A_{2'}$-c, $A_5$, $A_6$, $B_{1'}$, $B_2$ and $B_4$ were isolated in sufficient amount for chemical study. They were individually hydrolyzed with 6 N HCl at 105°C for 24 hours. The terminal amines were easily isolated by passage through Dowex 1×2 ($OH^-$) column, since they were found in the effluent, while the amino acid components were adsorbed on the resin. The terminal amines were precipitated with picric acid and purified by recrystallization. The terminal amines of $A_1$, $A_2$, demethyl $A_2$, $A_{2'}$-a, $A_{2'}$-b and $A_{2'}$-c, $A_5$, $A_6$ and $B_2$ were respectively identified as 3-methylsulfinylpropylamine[2], 3-aminopropyldimethylsulfonium salt[4], 3-methylthiopropylamine[2], 1, 4-diaminobutane[4], 1, 3-diaminopropane[2], histamine, spermidine[4], spermine[4] and agmatine[4] by elemental analysis, chromatographic and spectroscopic comparisons with authentic samples.

Table 1. The terminal amines of bleomycins

| Bleomycins | Terminal amines |
|---|---|
| $A_1$ | $NH_2$-$(CH_2)_3$-SO-$CH_3$ |
| Demethyl $A_2$ | $NH_2$-$(CH_2)_3$-S-$CH_3$ |
| $A_2$ | $NH_2$-$(CH_2)_3$-$\overset{+}{S}$=$(CH_3)_2$ $X^-$ |
| $A_{2'}$-a | $NH_2$-$(CH_2)_4$-$NH_2$ |
| $A_{2'}$-b | $NH_2$-$(CH_2)_3$-$NH_2$ |
| $A_{2'}$-c | $NH_2$-$(CH_2)_2$-(imidazole ring) |
| $A_5$ | $NH_2$-$(CH_2)_3$-NH-$(CH_2)_4$-$NH_2$ |
| $A_6$ | $NH_2$-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-$NH_2$ |
| $B_{1'}$ | $NH_3$ |
| $B_2$ | $NH_2$-$(CH_2)_4$-NH-C(=NH)-$NH_2$ |
| $B_4$ | $NH_2$-$(CH_2)_4$-NH-C(=NH)-NH-$(CH_2)_4$-NH-C(=NH)-$NH_2$ |

The structural relation of the terminal amines of $A_1$, $A_2$ and demethyl $A_2$ suggested the possible interconversion of bleomycins $A_1$, $A_2$ and demethyl $A_2$.

$$A_2 \underset{CH_3I}{\overset{\triangle}{\rightleftarrows}} \text{demethyl } A_2 \xrightarrow{H_2O_2} A_1$$

Copper-chelated bleomycin demethyl $A_2$ was methylated with methyl iodide in methanol at 42°C for 48 hours to afford bleomycin $A_2$ in good yield, while pyrolysis of $A_2$ under reduced pressure at 100°C for 24 hours yielded demethyl $A_2$ in70 % yield. Treatment of demethyl $A_2$ with one equivalent of hydrogen peroxide in aqueous solution at 0°C for 5 minutes afforded $A_1$ without any side reactions.

The terminal amine of $B_1$ could not be detected by ninhydrin reaction on paper and thin layer chromatograms. Behavior on CM-Sephadex chromatography and color reactions (ninhydrin and SAKAGUCHI: negative) suggested that $B_{1'}$ was the simple amide of bleomycinic acid. Ammonolysis of bleomycinic acid 3-benzoylaminopropyl ester[5] gave bleomycinic acid amide which was identical with bleomycin $B_{1'}$.

The terminal amine of $B_4$ (**1**) was isolated as a hygroscopic crystalline sulfate 270~272°C (dec.). Found: C, 30.95; H, 7.45; N, 24.14; O, 25.44; S, 11.90. Calcd. for $C_{10}H_{25}N_7 \cdot {}^3/_2H_2SO_4 \cdot {}^1/_2H_2O$ (MW 398.5): C, 30.06; H, 7.32; N, 24.55; O, 26.03; S, 12.04. VAN SLYKE nitrogen analysis, Found: 3.20%. Calcd. for presence of one amino group: 3.50 %. Potentiometric titration showed the presence of one mole of amino group (pKá 10.2, equivalent weight 377) and strongly basic functions over pKá 12. Compound **1** gave positive ninhydrin, SAKAGUCHI and DRAGENDORFF reactions. The NMR spectrum of **1** in deuterium oxide (external TMS reference) suggested the presence of two 1, 4-di-N-substituted tetramethylene carbon skeletons: $\delta$ 2.12 (multiplet, 8 protons), 3.56 (triplet, 2 protons) and 3.70 (multiplet, 6 protons). Hydrolysis of **1** with 1 N NaOH at 105°C for 45 hours gave 1, 4-diaminobutane, ammonia and a new amine (**2**). Compound **2** gave positive ninhydrin

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and negative SAKAGUCHI and DRAGENDORFF reactions. Compound **2** was isolated as the crystalline dihydrochloride, 171°C (dec.). Found: C, 38.72; H, 8.92; N, 19.70; Cl, 25.89. Calcd. for $C_6H_{22}N_4O \cdot 2HCl$: C, 39.27; H, 8.79; N, 20.36; Cl, 25.77. VAN SLYKE nitrogen analysis, Found: 9.70%. Calcd. for presence of two amino groups: 10.18%. The NMR spectrum suggested that it has a synmetrical structure: $\delta$ 2.07 (multiplet, 8 H), 3.48 (triplet, 4 H), 3.58 (triplet, 4 H). The IR spectrum (KBr) showed absorptions at 1645 and 1580 $cm^{-1}$. Further alkaline hydrolysis of 2 gave more than one mole of 1, 4-diaminobutane. DRAGENDORFF reaction is positive for 1, 3-disubstituted guanidine. So, **1** and **2** were determined to be 1-(4-aminobutyl)-3-(4-guanidinobutyl)-guanidine and N, N'-bis-(4-aminobutyl)-urea, respectively.

There are three basic functions through which spermidine, the terminal amine of $A_5$, could connect with bleomycinic acid. Bleomycin $A_5$ was treated with nitrous acid followed by acid hydrolysis. The deaminospermidine was isolated as the crystalline picrate, m.p. 166~167°C. One of the three structures, 4-(3-aminopropylamino)-butanol (**3**), 3-(4-aminobutylamino)-propanol (**4**) and 3-(4-hydroxybutylamino)-propanol (**5**), could be expected depending on the types of the linkage. Compound **5** was excluded because the paper electrophoretic behaviour indicated that the deaminospermidine is a dibasic amine. Compounds **3** and **4** were synthesized and the melting points of the picrates were 166~167°C and 153~154°C, respectively. The deaminospermidine was identical with **3** by chromatographic and IR spectroscopic comparisons with the synthetic material. Thus, the primary amino group on the trimethylene chain of spermidine is involved in the linkage. This finding suggested that transformation of $A_5$ to $A_{2'}$-b might be possible by enzyme degradation. Copper-free bleomycin $A_5$ was dissolved in 0.1 M phosphate buffer at pH 6.8 and incubated with freeze-dried cells of *Serratia marcescens* IAM-1223, which has been known to cleave spermidine into 1, 3-diaminopropane and 4-aminobutyraldehyde[6]. After incubation for 70 hours at 37°C, the product was purified by CM-Sephadex chromatography and identified with $A_{2'}$-b.

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