CHEMICAL STUDIES ON BLEOMYCINS. I

THE ACID HYDROLYSIS PRODUCTS OF BLEOMYCIN A_2

Sir:

Isolation and properties of bleomycin group of anti-tumor antibiotics were described by H. UMEZAWA *et al.*[1,2]. In a previous paper[2], degradation during purification was considered possible and each bleomycin component was purified and designated bleomycin At-n. However, further studies have confirmed no such decomposition occurs. Therefore, each bleomycin component is now designated without that. In this communication structures of the acid hydrolysis products of bleomycin A_2[2], one of the major components of bleomycins, are reported.

Bleomycin A_2 was hydrolyzed with 6 N HCl at 105°C for 20 hr in a sealed tube. The hydrolysate contained at least seven ninhydrin-positive products. Each component was isolated by ion-exchange resin chromatography (Dowex 50 W×4), and was designated as the compounds I, II, III, IV, V, VI and VII in order of the elution. The relative mobility (Rm-value) of high voltage paper electrophoresis and Rf-value of paper chromatography of the compounds are listed in Table 1.

Table 1. Relative mobility (Rm-value) of paper electrophoresis and Rf-value of paper chromatography of the acid hydrolysis products of bleomycin A_2

Product	Rm-value*	Rf-value**	Ninhydrin reaction***	
			room temp.	100°C
I	0.67	0.26	purple	purple
II****	0.71	0.14	purple	yellow
III	0.95	0.50	none	purple
IV	1.14	0.11	purple	brown
V	1.38	0.11	purple	purple
VI****	0.60	0.48	none	brown
VII	2.48	0.17	none	purple

* Toyo filter paper No. 51, buffer solution; formic acid - acetic acid - water (25 : 75 : 900), Rm-value of alanine=1.

** Toyo filter paper No. 51, solvent; *n*-butyl alcohol - acetic acid - water (4 : 1 : 2), descending.

*** 0.2% ninhydrin in acetone containing 5% pyridine, freshly prepared.

**** showed UV absorption on paper chromatogram.

Compound I was identified as L-threonine by its chromatographic behavior, elemental analysis, infrared absorption spectrum and optical rotation.

Compound II was crystallized from aqueous alcohol. It darkens at *ca.* 200°C. It has the molecular formula* $C_9H_{12}N_4O_4 \cdot H_2O$. Potentiometric titration showed the presence of two basic groups of pK'a 9.2 and *ca.* 3.4 and two acidic groups of pK'a *ca.* 3.4 and <2; the equivalent weight was 263 (calcd. 258). The ultraviolet absorption spectrum of II showed two maxima at 234 mμ ($\log \varepsilon = 3.9$) and 274 mμ ($\log \varepsilon = 3.7$) in aqueous solution. Studies of the structure of II are now in progress.

Compound III was crystallized from aqueous butanol. It melts at 144 ~ 146°C, $[\alpha]_D^{23} + 10.7°$ (c 7.25, water). It has the molecular formula $C_6H_{13}NO_3 \cdot {}^1/_2H_2O$. The n.m.r. spectrum** of III showed the presence of two C-CH_3 groups at δ 1.26 (ppm) (doublet, J=7 cps) and 1.30 (doublet, J=7 cps) and three methine groups at 2.44 (double quartets, J=7 cps and 10 cps), 3.54 (double quartets, J=7 cps and 2.5 cps) and 3.83 (double doublets, J=10 cps and 2.5 cps). This indicates that III has the partial structure $CH_3\text{-}\underset{X}{CH}\text{-}\underset{Y}{CH}\text{-}\underset{Z}{CH}\text{-}CH_3$. The potentiometric titration showed the presence of one of each amino (pK'a 10.2) and one carboxyl (pK'a 3.4) group with an equivalent of 168 (calcd. 156). The elemental analysis suggested that III had an alcohol group as a substituent which was confirmed by formation of an acetyl derivative (1735 cm^{-1}). To explain the chemical shifts of III adequately, it must have the structure: 4-amino-3-hydroxy-2-methyl-*n*-valeric acid.

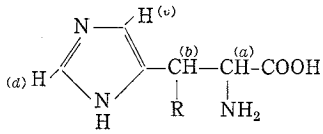
$$\underset{NH_2}{CH_3}\text{—}\underset{NH_2}{CH}\text{—}\underset{OH}{CH}\text{—}\underset{CH_3}{CH}\text{—}COOH \qquad \text{III}$$

* Analytical values for all the compounds described in this paper are consistent with the indicated formulas.

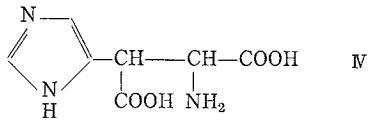
** N.m.r. spectra were observed in deuterium oxide on a Varian A-60 spectrometer using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal reference.

Table 2. The n.m.r. spectra of Compound IV and histidine

Compound		Chemical Shift (δ)			
		a	b (b')	c	d
IV (R=COOH)	in D_2O	4.15 (J_{ab}=4.5 cps)	5.32	7.17	7.77
	in 1N DCl	4.62 (J_{ab}=3.3 cps)	5.65	7.58	8.80
Histidine (R=$H^{(b')}$)	in D_2O	3.95	*ca.* 3.1	7.03	7.73
	in 1N DCl	4.58	*ca.* 3.6	7.60	8.83

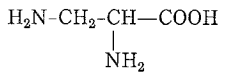


Compound IV was crystallized from aqueous alcohol as colorless needles. $[\alpha]_D^{22}$+ 30.5° (c 1.5, water), $[\alpha]_D^{22}$ + 73.1° (c 1.35, 1.2 N HCl). It has the molecular formula $C_7H_9N_3O_4 \cdot H_2O$. The potentiometric titration showed the presence of two basic groups of pK'a 9.4 and *ca.* 5.3 and two acidic groups of pK'a *ca.* 5.3 and <2. The equivalent was 222 (calcd. 217). It decomposed at 171°C giving histidine and carbon dioxide. A positive PAULY reaction (orange color)[3] suggested that IV might be β-carboxy-histidine, which was supported by n.m.r. spectra (Table 2).



IV

A mono-hydrochloride of V was crystallized from dilute hydrochloric acid and ethanol, m. p. 240°C. $[\alpha]_D^{29}$ + 7° (c 1.42, 5 N HCl). It has the molecular formula $C_3H_8N_2O_2 \cdot HCl$. It was identified as L-β-aminoalanine[4] by comparison of infrared absorption spectrum and optical rotation with those of the authentic sample.



V

Compound VI was slightly solubl in water andwas crystallized by neutralization of the hydrochloric acid solution with dilute sodium hydroxide. It did not decompose at 240°C. It has the molecular formula $C_9H_9N_3O_2S_2 \cdot H_2O$. The potentiometric titration showed the presence of one carboxyl (pK'a 2.8) and one amino (pK'a 9.2) group. The equivalent was 272 (calcd. 273). The ultraviolet absorption spectrum showed a maximum at 290 mμ (log ε=4.10) in a hydrochloric acid solution. X-ray crystallographic studies of VI are now in progress.

Compound VII was isolated as a crystalline picrate with m. p. 230° (dec.). It has the molecular formula $C_4H_{12}N_2 \cdot 2C_6H_3N_3O_7$. It was identified as putrescine di-picrate by comparison of the infrared absorption spectrum with that of an authentic sample.

$H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}NH_2$ VII

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References

1) UMEZAWA, H.; K. MAEDA, T. TAKEUCHI & Y. OKAMI: New antibiotics, bleomycin A and B. J. Antibiotics, Ser. A 19: 200~209, 1966.
2) UMEZAWA, H.; Y. SUHARA, T. TAKITA & K. MAEDA: Purification of bleomycins. J. Antibiotics, Ser. A 19: 210~215, 1966.
3) SMITH, I.: Chromatographic Techniques. p. 60. William Heinemann Medical Books Ltd., London, 1958.
4) GREENSTEIN, J. P. & M. WINITZ: Chemistry of the Amino Acids. Vol. III, p. 2463. John Wiley and Sons, Inc., New York, N. Y., 1961.