CHEMISTRY OF BLEOMYCIN. XXVIII PREPARATION OF DEGLYCO-BLEOMYCIN BY MILD ACID HYDROLYSIS OF BLEOMYCIN

Sir:

During biosynthetic studies of bleomycin, we isolated deglyco-bleomycin, the aglycone of bleomycin, from the culture filtrate[1,2]. However, the amount was too small to study further. If the aglycone was sufficiently available, it would be useful in order to solve the controversial question of the structure of bleomycin Fe(II)-complex[3,4]. Deglyco-bleomycin was also our primary target for the synthesis of bleomycin. We have been successful both in the preparation of deglyco-bleomycin by hydrolysis of bleomycin and by total chemical synthesis. In this communication, the isolation of deglyco-bleomycin from the mild acid hydrolysate of bleomycin is described.

During our structural studies of bleomycin, information on the nature of the chemical bonds in the bleomycin molecule has been accumulated[5]. For example, under acidic conditions several bonds such as carboxamides, glycosides and some peptide bonds are easily hydrolyzed, and mild alkali treatment readily liberates the sugar part by β-elimination resulting in the formation of the dehydrohistidine residue. At an elevated temperature the C-N-C bonds around the secondary amine of bleomycin are easily cleaved by competitive β-elimination.

Attempts to obtain deglyco-bleomycin by oxidative degradation of the sugar part such as with lead tetraacetate, chromic anhydride-pyridine, oxygenation with palladium catalyst, *etc.*, failed due to concomitant degradation of the peptide part. Therefore, the acidic degradation was again examined to obtain the aglycone, because it is already known that the glycosidic bond connecting the sugar and peptide parts is one of the most acid-sensitive bonds in bleomycin. Preliminary tests suggested that mild acid hydrolysis of bleomycin could provide us with the desired aglycone.

One gram of bleomycin B2 copper-complex was dissolved in 50 ml of 6 N HCl. The solution was kept at 20°C for 40 hours. The reaction mixture was adjusted to pH 7.5 with 5 N NaOH under ice-cooling and kept for 2 hours at room temperature to restore the N to O acyl migration which occurred during the acid treatment. The solution was passed through a 500 ml column of Amberlite XAD-2 to remove the resulting sodium chloride, the liberated sugar, and unadsorbed peptide fragments. Elution with 80% methanol containing 0.002 N HCl afforded 723 mg of a mixture of the degradation products and the unreacted starting material. The mix-

Fig. 1. Elution profile of a mild acid hydrolysate of bleomycin B2.
Hydrolysate 9 mg (see text), CM-Sephadex C-25 column 12.6 mm×800 mm, 0.05 N sodium phosphate of pH 6.8→1 M NaCl linear gradient (500 ml/500 ml), elution 50 ml/hour.

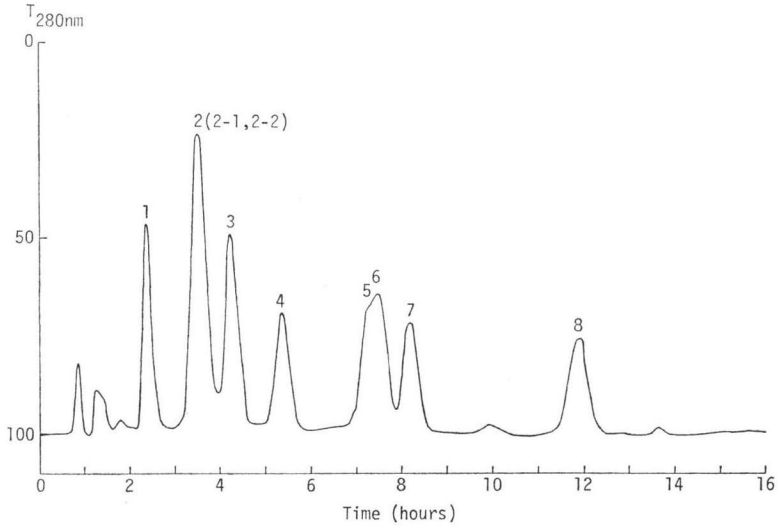
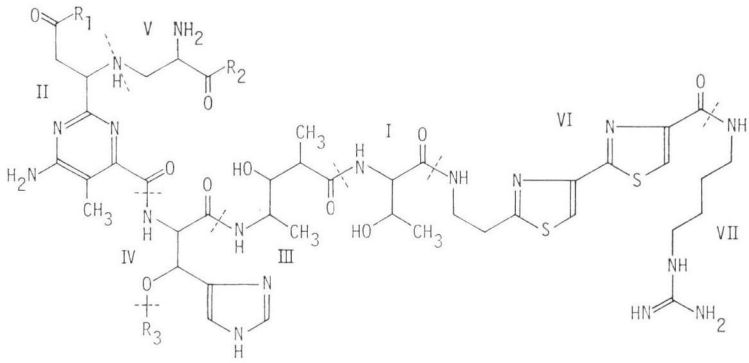


Fig. 2. Structures of the mild acid hydrolysates of bleomycin B2.



Boundaries between the labeled residues are indicated with broken lines.

Peak*	Compound	R_1	R_2	R_3
1	bisdeamido-BLM B2	OH	OH	sugar**
2-1	N^{II}-deamido-BLM B2	OH	NH_2	sugar
2-2	bisdeamido-deglyco-BLM B2	OH	OH	H
3	N^{II}-deamido-deglyco-BLM B2	OH	NH_2	H
4	N^{V}-deamido-BLM B2 (known)[5]	NH_2	OH	sugar
5	BLM B2 (starting material)	NH_2	NH_2	sugar
6	N^{V}-deamido-deglyco-BLM B2	NH_2	OH	H
7	deglyco-BLM B2 (aglycone)	NH_2	NH_2	H

* see Fig. 1.

** 2-O-(3-O-Carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl.

ture (705 mg) was separated by a CM-Sephadex C-25 column chromatography (310 ml) developed with linear gradient between 0.05 N sodium phosphate of pH 6.8 and 1 M NaCl. The eluate was monitored by UV absorption at 280 nm. There appeared more than 10 peaks (see Fig. 1). The components contained in the major peak fractions were isolated by desalting with Amberlite XAD-2. Each of the peaks 1, 3, 4, 7 and 8 fractions (see Fig. 1) contained a single component, and their weights were 77, 82, 57, 34 and 44 mg, respectively. The peak 2 fraction contained two components and peaks 5 and 6 overlapped. They were separated further by a CM-Sephadex C-25 column chromatography developed with linear gradient of sodium citrate buffer of pH 4.5 (0.05 N→1.0 N). The peak 2 was separated into peaks 2-1 (84 mg) and 2-2 (77 mg), and the peaks 5 and 6 were clearly separated to give 48 and 44 mg, respectively. The nine components thus isolated were obtained as their blue copper-complexes except for the peak 8 substance which was obtained in the metal-free form. After removal of the chelated copper by the EDTA method[6], their structures were elucidated by ^{13}C-NMR study[7,8].

The peak 5 substance was found to be bleomycin B2, the unreacted starting material, the peak 4 to be N^{V}-deamido-bleomycin B2*, an enzyme-inactivated product of bleomycin previously reported[9,10], and the peak 8 to be tripeptide S[11], the C-terminal tripeptide fragment of bleomycin B2. Components 1, 2-1, 2-2, 3 and 6 were found to be new degradation products. Their structures were assigned as bisdeamido-bleomycin B2, N^{II}-deamido-bleomycin B2, bisdeamido-deglyco-bleomycin B2, N^{II}-deamido-deglyco-bleomycin B2 and N^{V}-deamido-deglyco-

* Formerly, it was called merely deamido-bleomycin, but in this study another kind of deamido-bleomycin was obtained (*vide infra*). Therefore, to differentiate them, the former is called N^{V}-deamido-bleomycin and the latter is called N^{II}-deamido-bleomycin. The N^{V} and N^{II} indicate the carboxamides present in the amino acid components V and II moieties (see Fig. 2) of bleomycin, respectively.

Fig. 3. ^{1}H-NMR spectrum of deglyco-bleomycin B2 (in D_2O at 100 MHz, external TMS reference).

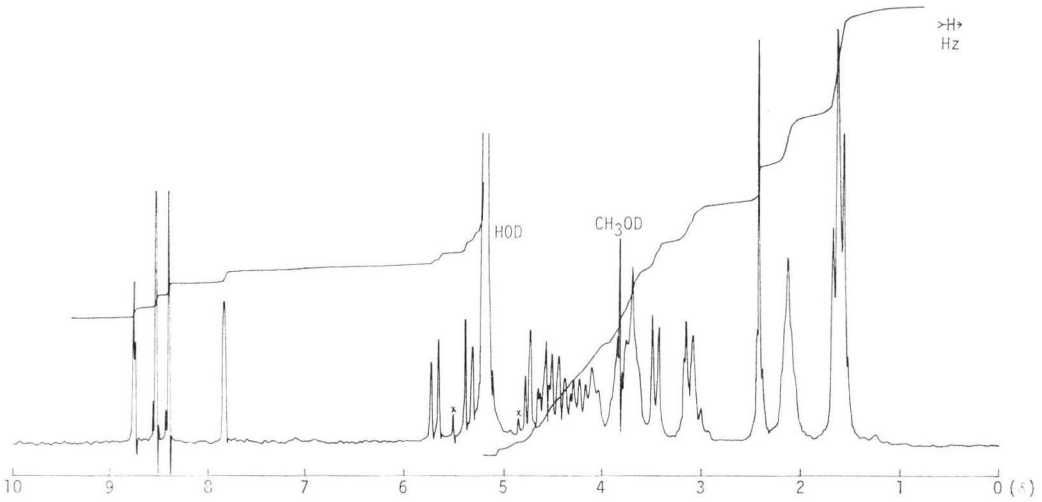


Table 1. ^{13}C-NMR chemical shifts of deglyco-bleomycin B2 and bleomycin B2.

Carbon*	Deglyco-BLM B2	BLM B2 (peptide part)	Difference	Carbon*	Deglyco-BLM B2	BLM B2 (peptide part)	Difference
I-CO	172.7***	172.7	0	IV-4	137.2	135.5	1.7
I-β-CH	67.9	67.9	0	IV-5	117.4	118.6	−1.2
I-α-CH	59.9	59.9	0	IV-β-CH	67.5	68.7	−1.2
I-CH_3	19.8	19.8	0	IV-α-CH	58.9	57.8	1.1
II-S-CO	176.9	177.0	−0.1	V-CO	172.2	172.1	0.1
II-R-CO	168.4	168.5	−0.1	V-α-CH	60.6	60.5	0.1
II-2	166.3	166.1	0.2	V-β-CH_2	47.9	47.9	0
II-4	165.1	165.5	−0.4	VI-CO	171.2	171.2	0
II-6	154.3	153.0	1.3	VI-2	163.6	163.6	0
II-5	112.1	113.1	−1.0	VI-2′	163.2	163.2	0
II-CH	53.3	53.3	0	VI-4	149.8	149.8	0
II-CH_2	41.0	40.9	0.1	VI-4′	147.7	147.7	0
II-CH_3	11.5	11.7	−0.2	VI-5′	125.4	125.5	−0.1
III-CO	178.3	178.3	0	VI-5	119.8	119.9	−0.1
III-β-CH	75.1	75.2	−0.1	VI-β-CH_2	39.8	39.9	−0.1
III-γ-CH	48.3	48.4	−0.1	VI-α-CH_2	32.8	32.9	−0.1
III-α-CH	43.6	43.4	0.2	VII-G	157.6	157.6	0
III-γ-CH_3	15.2	15.6	−0.4	VII-δ-CH_2	41.7	41.7	0
III-α-CH_3	13.3	12.7	0.6	VII-α-CH_2	39.8	39.9	−0.1
IV-CO	170.3	169.8	0.5	VII-β-CH_2**	26.7	26.7	0
IV-2	137.6	137.7	−0.1	VII-γ-CH_2**	26.3	26.3	0

* For the numbering, see Fig. 2 and reference 6.

** May be exchanged.

*** δ-value, internal dioxane as δ 67.4 (ppm).

bleomycin B2, respectively (see Fig. 2). The peak 7 substance was identified to be deglyco-bleomycin B2, the desired aglycone of bleomycin B2.

These structural studies indicate that under the above-described hydrolysis condition two carboxamides and the glycosidic bond connecting the sugar and peptide parts are most easily hydrolysed followed by the aminopeptide bond of the threonine moiety. The clear structure-retention time relationship is shown in Figs. 1 and 2[12].

In the IR spectrum of deglyco-bleomycin B2, there is no strong and broad absorption centered at 1050 cm^{-1}, a characteristic absorption attributed to the sugar moiety of bleomycin. The ^1H-NMR spectrum (Fig. 3) also indicates the absence of the sugar moiety, and all of the signals can be assigned by ^1H-^1H decoupling studies even in the 100 MHz spectrum[13].

The ^{13}C-NMR chemical shifts of deglyco-bleomycin B2 are compared with those of bleomycin B2 in Table 1, in which the chemical shifts of the sugar moiety of bleomycin are omitted. The chemical shifts of the six carbons in the side chain at the 2-position of the pyrimidine ring of the deglyco-bleomycin are the same as the corresponding shifts of bleomycin within 0.1 ppm of the difference. This indicates that the side chain of the aglycone has the same structure as that of the starting bleomycin[14]. Table 1 also shows that the chemical shifts of 18 carbons contained in the C-terminal tripeptide moiety (I, VI and VII in Table 1) are essentially same in both spectra. The chemical shifts of the β-hydroxyhistidine (IV) moieties, of which the hydroxy group is the connecting point to the sugar in bleomycin, were distinctly different between the deglyco-bleomycin and the original bleomycin. Besides the β-hydroxyhistidine moiety, some chemical shift changes were observed in the neighboring pyrimidine and 4-amino-3-hydroxy-2-methylpentanoic acid (III) moieties. These ^{13}C-NMR data indicate that the desired deglyco-bleomycin has been obtained by mild acid hydrolysis of bleomycin.

Deglyco-bleomycin A2, which is the primary target of our synthetic study, was also prepared in a similar manner to that described above. Deglyco-bleomycin A2 which was obtained by total chemical synthesis was identical in all respects examined[15]. The coordination chemistry of deglyco-bleomycin will appear in a separate paper.

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