NEW COMPONENTS OF BLEOMYCIN

Sir:

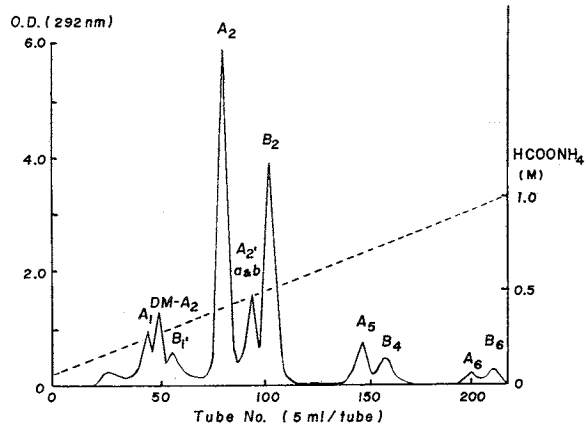
Streptomyces verticillus, cultured in a medium composed of soybean meal, glucose, millet jelly, corn steep liquor and mineral salts, produces bleomycin A_2 and B_2, two major components, and other minor components. Eleven bleomycins ($A_1 \sim A_6$, $B_1 \sim B_5$) were reported initially[1]. Six new components have been since isolated are described in this communication.

A 10 g sample of copper-chelated bleomycin mixture, Lot No. B-66019 Fl, manufactured by Nippon Kayaku Co. Ltd., was subjected to CM-Sephadex C-25 column chromatography, developed by a linear gradient of ammonium formate. The elution pattern is shown in Fig. 1. The eluate from 0.20~0.24 M ammonium formate gave 530 mg of bluish powder after desalting. It contained two components, A_1 and a new component, designated demethyl A_2, because of its structure described in the succeeding report. They were separated by column chromatography on Dowex 50W X4 pretreated with 0.2 M pyridine-acetate buffer at pH 4.6. Bleomycin A_1 and demethyl A_2 were eluted successively with 0.2 M pyridine-acetate buffer at pH 5.5. The yield of demethyl A_2 was 312 mg. It gave negative ninhydrin and SAKAGUCHI reactions.

The eluate from 0.25~0.27 M ammonium formate gave 135 mg of bluish powder, which was subjected to the same ion-exchange resin chromatography described above. A new component, B_1' (83 mg), was obtained. Its chromatographic behavior was similar to B_1, but B_1' gave a negative SAKAGUCHI reaction, while B_1 is positive.

Fig. 1. CM-Sephadex C-25 column chromatography of bleomycin

Sample: 50 mg (Lot No. B-66019 F1)
Column: ϕ 12 mm, h 885 mm (100 ml)
Elution: linear gradient method (0.05~1.0 M $HCOONH_4$)
Flow rate: 20 ml/hour



The fraction between A_2 and B_2 (0.45~0.48 M $HCOONH_4$) yielded 480 mg of two new components which have very similar properties.

Table 1. Properties of new bleomycin components

Component	TLC (Rf)			PPC (Rf) (d)	HVE (Rm) (e)	Potency (*Myc.* 607) (f) u/mg
	(a)	(b)	(c)			
Demethyl A_2	0.80	0.78	0.72	0.71	0.64	2,300
$A_{2'}$-a	0.70	0.65	0.49	0.82	0.88	1,488
$A_{2'}$-b	0.70	0.65	0.46	0.82	0.88	1,543
$A_{2'}$-c	0.71	0.82	0.53	0.71	0.84	847
$B_{1'}$	0.80	0.76	0.55	0.73	0.65	685
B_3	0.47	0.39	0.51	0.52	0.94	3,627
A_3	0.13*	—	—	0.85*	—	—
B_1	0.77*	—	—	0.70*	—	—
B_5	0.52*	—	—	0.70*	—	—

(a) Silicagel G, MeOH-10 % $AcONH_4$ (1:1), (b), Silicagel G, MeOH-10 % $AcONH_4$-10 % NH_4OH (10:9:1), (c) Avicel SF, *n*-PrOH-pyridine-AcOH-water (15:10:3:12), (d) Toyo No. 51, 10 % NH_4Cl (bioautography by *B. subtilis*), (e) Toyo No. 51, HCOOH-AcOH-water (25:75:900), 3000 volts, 40 min., Rm (Alanine: 1.0). Detection: UV light absorption and/or coloration with rubeanic acid, (f) Cylinder-agar plate method, standard (Cu-free-A_2 base: 1,000 u/mg). (*) cited from reference 1).

The two, $A_{2'}$-a and $A_{2'}$-b, were separated by CM-Sephadex C-25 column chromatography repeated three times. First eluted was 28 mg of $A_{2'}$-a, followed by 76 mg of $A_{2'}$-b. They showed positive ninhydrin and negative SAKAGUCHI reactions.

The eluate from 0.93~0.98 M ammonium formate contained 55 mg of a new component, B_6. It gave positive SAKAGUCHI and negative ninhydrin reactions. It was differentiated from B_5 by paper chromatography (Table 1).

The eluate between A_2 and B_2 from CM-Sephadex column chromatography of another preparation of bleomycin mixture (Lot No. B-72056 F 1) contained another new component, $A_{2'}$-c. It was separated from $A_{2'}$-a and $A_{2'}$-b, by a column chromatography on CM-Sephadex C-25 pretreated with 0.05 M sodium bicarbonate, developed with a linear gradient of sodium bicarbonate. Component $A_{2'}$-c was eluted at the concentration of 0.13 M while a mixture of $A_{2'}$-a and $A_{2'}$-b was eluted at 0.25 M. Component $A_{2'}$-c gave strongly positive PAULI and negative ninhydrin and SAKAGUCHI reactions. Components $A_{2'}$-a, $A_{2'}$-b and $A_{2'}$-c were differentiated from A_3 by thin-layer chromatography (Table 1).

The properties of new components are shown in Table 1. Their chemical structures are detailed in the succeeding report together with the other components of bleomycin.

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1) UMEZAWA, H.; Y. SUHARA, T. TAKITA & K. MAEDA: Purification of bleomycins. J. Antibiotics, Ser. A 19: 210~215, 1966