THE STRUCTURE OF THE SULFUR-CONTAINING CHROMOPHORE OF PHLEOMYCIN, AND CHEMICAL TRANSFORMATION OF PHLEOMYCIN TO BLEOMYCIN

Sir:

Phleomycins were discovered by UMEZAWA *et al.* in 1956 as anti-bacterial antibiotics[1,2]. In 1962, it was found that phleomycins inhibited animal tomors with a high therapeutic index[3,4]. In 1964, IKEKAWA *et al.* reported that phleomycins were effectively separated into their components by CM-Sephadex C-25 column chromatography[5]. The components were named phleomycins A, B, C, D_1, D_2, E, F, G, H, I, J, and K, and could be classed in two groups by UV absorption. One included phleomycins C, D_2, and F, and the other included phleomycins D_1, E, G, H, and I. The former showed a stronger UV absorption maximum at about 295 nm than the latter. There was no description of the UV absorption of A, B, J, and K.

In 1966, bleomycins were discovered by UMEZAWA *et al.* after an intensive search for phleomycin-like antibiotics[6,7]. Bleomycins were also effectively separated into their components by CM-Sephadex C-25 column chromatography[7]. They all showed UV spectra essentially the same as that of phleomycins C, D_2, and F. The UV absorption of phleomycins D_1, E, G, H, and I, we call the phleomycin-type UV absorption, and the UV absorption of all bleomycins and phleomycins C, D_2, and F, we call the bleomycin-type UV absorption. Comparative degradation studies indicated that phleomycin D_2 is the same as bleomycin B_2 and phleomycin F is bleomycin B_4. These results are inconsistent with the former assignment[7].

In this communication, the structural relationships are elucidated, and the chemical transformation of phleomycin to bleomycin is presented.

Phleomycin D_1, which has phleomycin-type UV absorption, was hydrolyzed with 6 N HCl at 105°C for 18 hours. In Table 1, the acid hydrolyzate of phleomycin D_1 is compared to bleomycin B_2. In the hydrolyzate of phleomycin D_1 there were six amine components among seven which were found in the hydrolyzate of bleomycin B_2. The only one missing component was compound **VI**, which contributes mostly to the UV absorption maximum at about 295 nm of bleomycin.

These results suggested that there should be present a substitute for **VI** in the molecule of phleomycin D_1. The finding of β-alanine (**VI'**-a) in the hydrolyzate of phleomycin D_1 supported this speculation, because **VI** might be biosynthesized from one mole of β-alanine and two moles of cysteine[11].

The NMR studies showed that there were four protons between $\delta=7\sim10$ ppm in the spectrum of bleomycin B_2 (in D_2O, reference: external TMS). They were assigned to the 2- and 4-protons of the imidazole of **IV** ($\delta=7.94$ and 8.82, sensitive to pH change and coupled to each other with $J<1.0$ Hz), and the two ring protons of the bithiazole of **VI** ($\delta=8.44$ and 8.60 ppm, singlet). On the other hand, there were only three protons in this region of the spectrum of phleomycin D_1. Two of them were assigned to the ring protons of **IV** ($\delta=8.04$ and 9.17 ppm, sensitive to pH change and coupled with $J<1.0$ Hz). The presence of the single proton at

Table 1. Components found in the acid hydrolyzate of phleomycin D_1 and bleomycin B_2

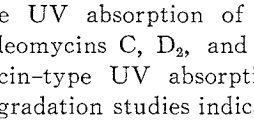
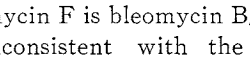
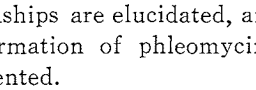
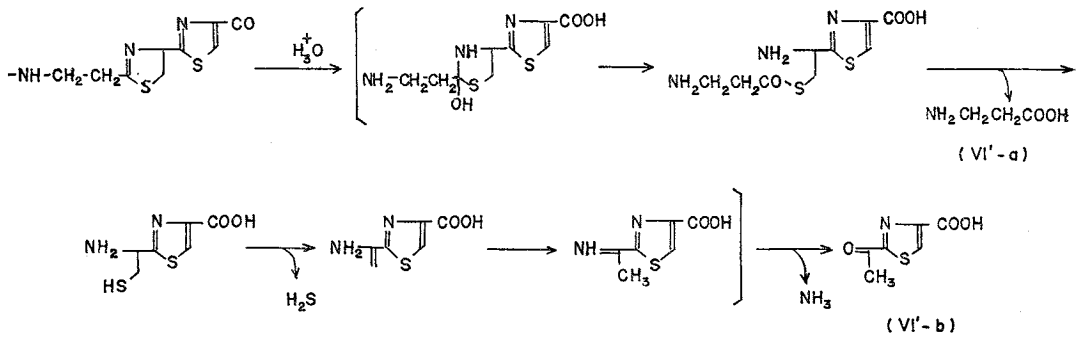
		Phleomycin D_1	Bleomycin B_2
I	$CH_3CH(OH)CH(NH_2)COOH$ 8)	+	+
II	 9)	+	+
III	$CH_3CH(NH_2)CH(OH)CH(CH_3)COOH$ 8)	+	+
IV	 10)	+	+
V	$NH_2CH_2CH(NH_2)COOH$ 8)	+	+
VI	 11)	−	+
VII	$NH_2CH_2CH_2CH_2CH_2NH\text{-}C(=NH)\text{-}NH_2$ 12)	+	+
VI'-a	$NH_2CH_2CH_2COOH$	+	−

Fig. 1.



δ=8.60 (singlet) suggested that phleomycin D_1 contained only one thiazole ring.

From the above, we conclude that the phleomycin equivalent of **VI** should be 2-[2-(2-aminoethyl)-Δ^2-thiazolin-4-yl]-thiazole-4-carboxylic acid. This should yield 2-acetylthiazole-4-carboxylic acid (**VI'**-b) in the acid hydrolyzate of phleomycin D_1 (Fig. 1). So, the acid hydrolyzate was extracted with ethyl ether. The ether extract was concentrated and treated with chloroform and petroleum ether to yield crystals, m.p. 167~169°C. The molecular formula $C_6H_5NO_3S$ (M.W. 171) was established by elemental analysis and mass spectrometry (*m/e* 171). It accorded with the formula of 2-acetylthiazole-4-carboxylic acid. The UV absorption [$\lambda_{max}^{95\%\ EtOH}$ 283 nm (log ε 3.68)] was similar to that of 2-propionylthiazole-4-carboxylic acid[13] [$\lambda_{max}^{95\%\ EtOH}$ 282 nm (log ε 3.69)]. Methyl ester of **VI'**-b was derived by treatment with diazomethane, m.p. 80~82°C [lit.[14] 78~80°C]. The IR spectrum was identical with that of methyl 2-acetylthiazole-4-carboxylate[15]. Thus, 2-acetylthiazole-4-carboxylic acid is present in the acid hydrolyzate of phleomycin D_1.

To confirm the postulated partial structure, we oxidized phleomycin D_1 under mild condition, hoping to get compound **VI** from the acid hydrolyzate of the oxidized product. To the aqueous solution of phleomycin D_1 was added manganese dioxide. The suspension was stirred for two days at room temperature. The UV spectrum of the filtrate showed increased UV absorption at 295 nm. The filtrate was dried and then dissolved in 0.01 N HCl. In 0.01 N HCl solution at 27°C for 24 hours, unreacted acid-labile phleomycin was decomposed. However, the solution showed strong inhibitory activity against *Mycobacterium* 607. The bioactive substance was isolated by CM-Sephadex column chromatography. It showed bleomycin-type UV absorption. The acid hydrolyzate contained compound **VI**, but did not contain **VI'**-a and b. The oxidized phleomycin D_1, dehydrophleomycin D_1, was compared with bleomycin B_2. They showed almost the same activity (dehydrophleomycin D_1 2,680 u/mg, bleomycin B_2 2,720 u/mg) against *Mycobacterium* 607 by cup assay method (bleomycin A_2 free base: 1,000 u/mg). The chromatographic behavior was exactly the same: The Rf values on Avicel thin-layer chromatography was 0.66 (MeOH - 10% $AcONH_4$ - 10% NH_4OH, 10 : 9 : 1) and 0.56 (*n*-PrOH - pyridine - AcOH - H_2O, 15 : 10 : 3 : 12). They were not separable by CM-Sephadex C-25 column chromatograpy. The IR, UV and NMR spectra were identical, and hydrolyzates of the two compounds gave the same ninhydrin patterns after two-dimensional electrophoresis, (HCOOH - AcOH - H_2O, 25 : 75 : 900), and ascending paper chromatography (*n*-PrOH - pyridine - AcOH - H_2O, 15 : 10 : 3 : 12). The sugar component was analyzed by gas chromatography (SE-30 on Chromosorb W.AW) of the trimethylsilyl derivative of the methanolyzate of dehydrophleomycin D_1[16]. Dehydrophleomycin D_1 contains one mole each of glucose and 3-O-carbamoyl-mannose.

Thus, dehydrophleomycin D_1 is identical with bleomycin B_2. Similarly, dehydrophleomycin E was identified with bleomycin B_4.

Recently, two phleomycin-like antibiotics,

zorbamycin[17] and YA-56[18], were reported. Zorbamycin has phleomycin-type UV absorption, and the NMR spectrum in D_2O showed the presence of three protons in the low magnetic field ($\delta = ca$ 7.3, 8.0 and 8.2 ppm, reference) is not recorded), which was similar to that of phleomycin. Antibiotic YA-56 also has phleomycin-type UV absorption, and the acid hydrolyzate contains β-alanine (Y. ITO, personal communication). These data suggest that both antibiotics may have the same sulfur-containing chromophore, 2-[2-(2-aminoethyl)-Δ^2-thiazolin-4-yl]-thiazole-4-carboxylic acid, as that of phleomycin.

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