Communications to the Editor

MONAZOMYCIN B, A NEW MACROLIDE ANTIBIOTIC OF THE MONAZOMYCIN FAMILY

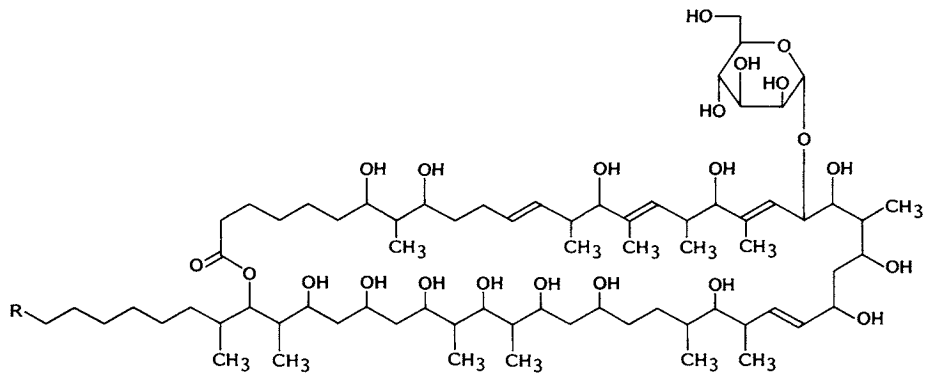
Sir:

54-Deamino-54-guanidinomonazomycin (**1**), a new member of the monazomycin[1] antibiotics, and monazomycin (**2**) were found in the culture filtrate of the microbial strain UC 8633. The producing strain was isolated from a soil sample collected in Indiana, U.S.A. and identified as a member of the genus *Streptoverticillium*. The antibiotics were isolated and their structures determined by NMR and MS studies. In this communication, the isolation, structure, and biological activities of **1** are reported.

Seed cultures of the producing organism were prepared in a medium containing Cerelose (25 g) and Pharmamedia (25 g) per liter of tap water. The medium was incubated at 28°C for 72 hours on a rotary shaker and used to inoculate the fermentation medium. The latter consisted of the following components: Lexein (15 g), Difco malt extract (10 g), Cerelose (10 g), soybean meal (10 g), and lactose (15 g) per liter of tap water; the pH was adjusted to 7.2 with 6 N NaOH prior to sterilization. Fermentations were conducted in New Brunswick 14-liter fermenters containing 10 liters of medium at 28°C, 250 rpm stirring speed and 2 liters/minute aeration rate, with foaming controlled by triggered addition of polyalkylene glycol. Fermentation tanks were inoculated with 500-ml seed culture.

The antibacterial activity was measured by agar diffusion assay using *Streptococcus pyogenes* UC 6055. The whole beer was filtered and the cake triturated with methanol. The methanolic extract was concentrated to aqueous solution and combined with the clear filtrate. The combined solution was used as the starting material for chromatography over Amberlite XAD-7. All bioactivity was adsorbed on the resin; the column was washed with water and then eluated batchwise with methanol - water (30 : 70), methanol - 0.05 N aqueous ammonium hydroxide (70 : 30) and methanol - 0.05 N aqueous acetic acid (70 : 30) solutions. The latter two batches were found to be bioactive. Analytical-TLC employing silica gel (Brinkman) eluted with chloroform - ethanol - water (25 : 30 : 5) indicated that basic methanol batch contained only one bioactive component (A) and the acidic methanol batch contained two bioactive components (A and B, Rf = 0.27 and 0.48, respectively). The batch containing A was further purified by preparative reverse phase column chromatography (E. Merck RP-8 column eluted with water - methanol gradient followed by methanol triethylamine (99 : 1)) to yield pure A. Pure B was obtained similarly by feeding the column with the batch containing A and B.

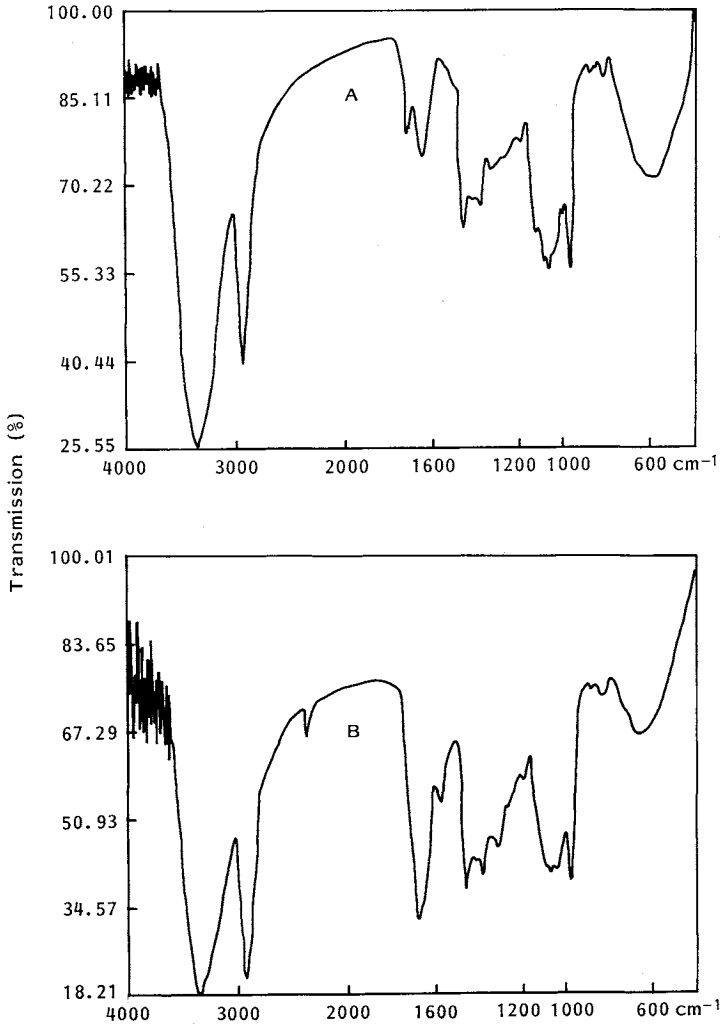
Antibiotics A and B were obtained as basic, hygroscopic, colorless powders, soluble in water, DMSO, and lower alcohols, but insoluble in less polar organic solvents. Only compound A showed



54-Deamino-54-guanidinomonazomycin (**1**) R = NHC(=NH)NH$_2$

Monazomycin (**2**) R = NH$_2$

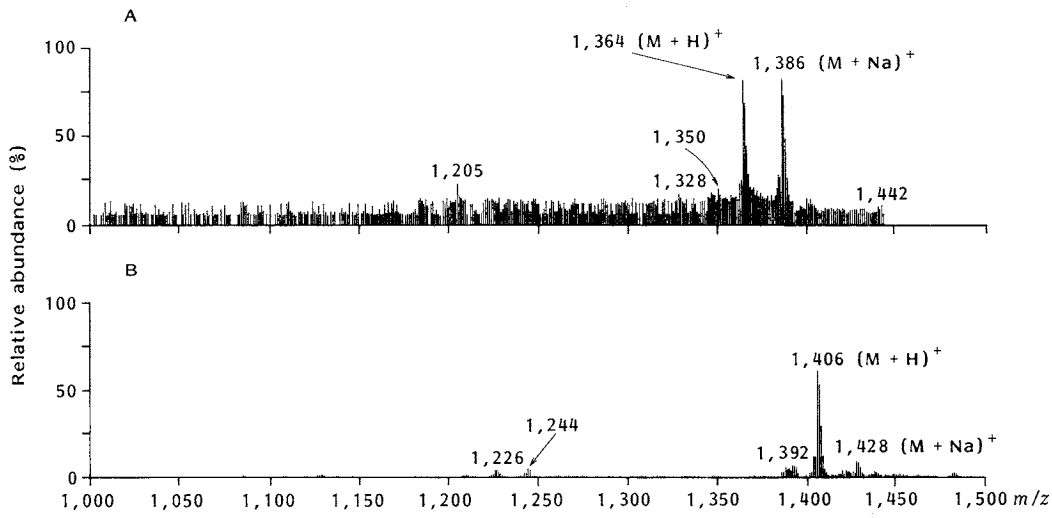
Fig. 1. IR spectra of monazomycins A (1) and B (2) (ZnSe/FMIR).



a positive reaction to ninhydrin on a silica gel TLC plate suggesting that A has a primary amino group. Both A and B exhibit only end absorption in the UV region. The IR spectra of A and B are shown in Fig. 1. The great similarities between the two spectra suggest that A and B are structurally related. However, extra absorption peaks in the 1700 to 1550 cm^{-1} region were observed in the B spectrum. Search of IR spectra of known antibiotics resulted in the identification of A as monazomycin, an antibiotic isolated from *Streptoverticillium mashuense*[1,2] whose structure was elucidated later as a 48-membered lactone[3]. FAB mass spectrometric analysis of A gave the protonated molecular ion peak, $(M+H)^+$, at *m/z* 1,364 (Fig. 2), completely in agreement with the reported formula of monazomycin[3] ($C_{72}H_{133}NO_{22}$). Also, the ^{13}C NMR spectrum of A was virtually identical to that previously reported[3].

After identifying A as monazomycin, FAB-MS (Fig. 2) and ^{13}C NMR spectroscopy (results not shown) methods were used to identify B. The ^{13}C NMR spectrum of B is very similar to that of A except that B has an extra quaternary signal at 158.7 ppm which is characteristic of an N-C(=X)-Y type carbon (where X and Y are heteroatoms). The FAB-MS analysis of B (Fig. 2) gave the protonated molecular ion peak, $(M+H)^+$, at *m/z* 1,406 which is 42 mass units higher than that of A. The molecular formula of B is therefore deduced as $C_{73}H_{135}N_3O_{22}$ from the above mass spectroscopic analysis, elementary analysis and the number of peaks in the ^{13}C NMR spectrum. The molecular formula difference between A and B, the fact that B does

Fig. 2. FAB-Mass spectra of monazomycins A (1) and B (2) (m/z < 1,000 not shown).



not have an amino functional group, and the appearance of the 158.7 ppm signal in the ^{13}C NMR spectrum lead to the conclusion that B possesses a guanidino functional group. Furthermore, this guanidino group is connected to the rest of the molecule through C-N linkage at the C-54 position. This later conclusion is deduced from the fact that the methylene carbons of A and B have almost identical chemical shift values in their respective ^{13}C NMR spectra. It is well established that the replacement of an amino group with a guanidino group does not affect the neighboring carbon chemical shift values. For example, the δ carbon of arginine has an almost identical chemical shift value as that of δ carbon of ornithine[4]. Based on the NMR and MS data we concluded that B was 54-deamino-54-guanidinomonazomycin. We propose to name the parent compound monazomycin A and the new derivative monazomycin B.

Monazomycins A and B exhibit similar antimicrobial activity against a variety of Gram-positive bacteria and fungi by disc diffusion assay. Neither compound, however, protected mice infected with *S. pyogenes* UC 152 when it was administered subcutaneously at doses as high as 25 mg/kg. The antibiotic was also found to be toxic when it was administered intraperitoneally to mice (ACT_{50}: 12 mg/kg). The IC_{50} against L1210 cells is 0.92 μg/ml.

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