A NEW ANTITUMOR ANTIBIOTIC, DIOXOLAMYCIN

Sir:

A new antibiotic with weak cytostatic activity has been isolated from the cultured broth of *Streptomyces filamentosus* MC521-C5 which had been reported to be a requinomycin-producing strain.[1] We named this antibiotic dioxolamycin, because it contains a dioxolane ring.

The strain MC521-C5 was shake-cultured at 27°C in a medium containing potato starch 2.0%, glucose 2.0%, soybean meal 2.0%, yeast extract 0.5%, NaCl 0.25%, $CaCO_3$ 0.32%, $CuSO_4 \cdot 5H_2O$ 0.0005%, $MnCl_2 \cdot 4H_2O$ 0.0005%, $ZnSO_4 \cdot 7H_2O$ 0.0005% (adjusted to pH 7.4 prior to sterilization). The fermentation was stopped after 27 hours and the fermented broth was filtered.

The filtrate (34 liters) was charged on a column of activated carbon (ϕ5 cm×50 cm) and the antibiotic was eluted with 50% aqueous acetone. After evaporation of the acetone under reduced pressure, the residual powder (70 g) was subjected to silica gel column chromatography (ϕ 3.5 cm×100 cm), using ethyl acetate to afford the pure antibiotic. Recrystallization from hot ethyl acetate gave colorless crystals (114.5 mg) of dioxolamycin.

Dioxolamycin had no antibacterial activity at concentrations of less than 100 μg/ml, but it showed a moderate cytostatic activity against cultured L-1210 leukemia cells *in vitro* (IC_{50} 30~32.5 μg/ml). The LD_{50} value was over 200 mg/kg (mice, iv). Dioxolamycin did not show a significant activity against L-1210 leukemia ascites tumor *in vivo*.

The physico-chemical properties of dioxolamycin are as follows: mp 214~215°C; $[\alpha]_D^{25}$ −53.1° (*c* 0.36, MeOH); λ_{max}^{MeOH} 218 nm ($E_{1cm}^{1\%}$ 316.5); IR (KBr) cm^{-1} 3460, 3440, 3310, 2910, 2890, 1710, 1690, 1670, 1635, 1600; FD-MS *m/z* 258 (M+1). From the elemental analysis and the FD mass spectral data we deduced its molecular formula to be $C_{11}H_{15}NO_6$. *Anal* Calcd for $C_{11}H_{15}NO_6$: C 51.36, H 5.88, N 5.45, O 37.31 (Found: C 51.42, H 5.99, N 5.19, O 37.40). It is soluble in methanol, ethanol, acetone, ethyl acetate and chloroform, and almost insoluble or insoluble in toluene, benzene and water. ^1H NMR and ^{13}C NMR spectral data of dioxolamycin are shown in Table 1.

Crystals grown in a mixed solvent of chloroform, ethanol and methanol with volume ratios 10: 2: 0.5 were used for the X-ray diffraction study. The lattice dimensions and intensity data were collected on a Philips X-ray diffractometer using a small crystal with dimensions 0.6×0.15×0.05 mm. Crystal data: $C_{11}H_{15}NO_6$, MW 257.2, Orthorhombic $P2_12_12_1$, a=8.344(4), b=17.079(9), c=8.260(4) Å, V=1177.1 Å^3, Z=4, D_{cal}=1.452 gcm^{-3}, μ for CuKα=9.72 cm^{-1}.

Of the total of 1,495 reflections within the 2θ range of 6° through 156°, 1,315 were observed as above the 2σ(I) level. The structure was solved by direct methods using the Multan pro-

Table 1. NMR chemical shift values* of dioxolamycin.

Position	^{13}C NMR (100 MHz, $CDCl_3$ - CD_3OD, 10: 1)	^1H NMR (400 MHz, $CDCl_3$ - CD_3OD, 10: 1)	
	δ (ppm)	δ (ppm)	J (Hz)
1	73.4 (d)	4.68 (-H)	~1.5, 1.8, 3.5, 9.0
2	134.3 (d)	7.18 (-H)	1.5, 1.8, 2.5
3	129.8 (s)		
4	34.9 (t)	2.60, 2.84 (-CH_2)	~1, ~1.5, 1.5, 20.0 2.5, 3.5, 5.0, 20.0
5	62.4 (d)	4.52 (-H)	~1, 2.0, 5.0
6	81.7 (d)	3.62 (-H)	2.0, 9.0
8	108.2 (s)		
10	173.9 (s)	3.76 (-OCH_3)	
11	52.2 (q)		
12	22.9 (q)	1.62 (-CH_3)	
13	166.6 (s)		

* TMS was used as the internal reference.

Fig. 1. The molecular structure of dioxolamycin.

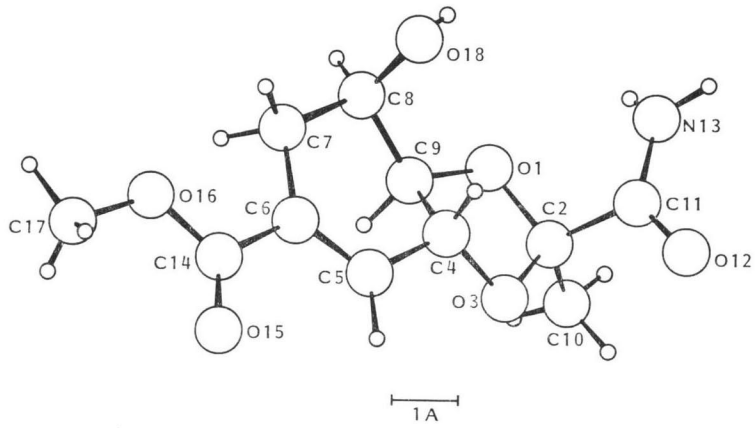
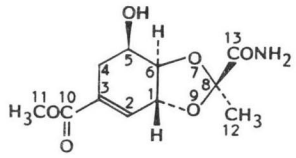


Fig. 2. The structure of dioxolamycin.



gram and refined by block diagonal least-squares methods to an R value of 0.045*. Hydrogen atoms were located on the difference electron density map and their positional parameters along with isotropic temperature factors were included in the refinement. Fig. 1 shows the molecular structure. The absolute configuration was not determined in X-ray diffraction study. The bond lengths and bond angles are quite normal for the chemical structure. The molecules are linked together through O18–H---O15 and N13–H---O12 hydrogen bonds of the lengths 2.906(4) and 3.070(5) Å, respectively.

Therefore, the structure of dioxolamycin was determined to be methyl [1*R*,5*R*,6*R*,8*S*]-8-carbamoyl-5-hydroxy-8-methyl-7,9-dioxabicyclo-[4,3,0]non-2-ene-3-carboxylate or its enantiomer. Since acid hydrolysis of dioxolamycin gave (−)-4-*epi*-shikimic acid,[2] $[\alpha]_D^{28}-72°$, it was determined that the absolute configuration corresponds to the first of these possibilities and is that represented in Figs. 1 and 2.

* The list of atomic parameters has been sent to Cambridge Crystallographic Data Centre and will be compiled in the Date-file. F_o and F_c table may be obtained from one of the authors (H. NAKAMURA) upon request.

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