MENOXYMYCINS A AND B, ANTITUMOR ANTIBIOTICS GENERATING ACTIVE OXYGEN IN TUMOR CELLS

Sir:

In oxygen-containing environments, cellular metabolism results in the production of several potentially toxic oxygen-derived molecular species. Accordingly, all normal cells have various defense systems against such active oxygen species. However, several tumor cells are known to have lost part of these defense systems[1]. Thus, it could be expected that substances generating active oxygen might show selective cytotoxicity against such tumor cells. In the course of our screening for antitumor antibiotics generating active oxygen in tumor cells, *Streptomyces* sp. KB10 was found to produce two new antibiotics designated menoxymycins A and B.

The producing organism was cultivated on a rotary shaker at 27°C for 7 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of glycerol 2.0%, molasses 1.0%, casein 0.5%, Polypepton 0.1% and calcium carbonate 0.4% (pH 7.2). The broth filtrate (2 liters) was extracted with EtOAc and the organic layer was back-extracted with 0.01 N HCl. After the pH was adjusted to 7.0, the aqueous layer was extracted with EtOAc. The extract was subjected to preparative silica gel TLC with $CHCl_3$-MeOH-29% NH_4OH (200:20:1). Three active fractions thus obtained were individually purified by Sephadex LH-20 column chromatography with $CHCl_3$-MeOH (1:1). One of the fractions was identified as medermycin (lactoquinomycin)[3~5] based on its physicochemical properties and NMR spectrum. The remaining two fractions gave the new substances, menoxymycins A (3 mg) and B (6 mg), as yellow powders.

The physico-chemical properties of menoxymycins A and B are summarized in Table 1. From high-resolution FAB-MS (*m*-nitrobenzyl alcohol matrix), the molecular formulae of menoxymycins A and B were determined to be $C_{24}H_{27}NO_9$ and $C_{25}H_{31}NO_9$, respectively. Their UV and visible spectra indicated the presence of a 8-hydroxynaphthoquinone chromophore[5]. The IR spectrum of menoxymycin A exhibited absorption peaks due to hydroxyls (3425 cm^{-1}), a γ-lactone (1770 cm^{-1}) and quinone carbonyls (1660 and 1645 cm^{-1}). Menoxymycin B contained the same moieties (3475, 1665 and 1645 cm^{-1}) and an ester carbonyl (1740 cm^{-1}) in place of a γ-lactone.

As summarized in Tables 2 and 3, the ^1H and ^{13}C NMR spectra of menoxymycin A were very similar to those of medermycin except for the significant down-field shifts observed on both protons and carbons adjacent to 3′-N. Since the difference in their molecular formulae corresponded to one oxygen atom, menoxymycin A was determined to be medermycin *N*-oxide as shown in Fig. 1.

The ^1H-^1H COSY spectrum of menoxymycin B revealed a spin system from 1′-H to 6′-H. Protons on C-1′ to C-5′ displayed large vicinal coupling constants (Table 2) and were required to be in a pyranose ring with all equatorial substituents. Two equivalent *N*-methyls (δ_H 2.32) were ascribed to a dimethylamino residue at C-3′ from their ^1H-^{13}C long-range coupling observed in a heteronuclear multiple-bond correlation (HMBC)[6] experiment. A C-glycosidic linkage between this pyranose residue and an aromatic ring was established by the ^{13}C

Table 1. Physico-chemical properties of menoxymycins A and B and medermycin.

	Menoxymycin A	Menoxymycin B	Medermycin[5]
Appearance	Yellow powder	Yellow powder	Yellow powder
MP (°C)	168~173 (dec)	93~97 (dec)	151~159 (dec)
Molecular formula	$C_{24}H_{27}NO_9$	$C_{25}H_{31}NO_9$	$C_{24}H_{27}NO_8$
HRFAB-MS (*m/z*)			
Found:	474.1758 $(M+H)^+$	490.2163 $(M+H)^+$	458 $(M+H)^+$
Calcd:	474.1764	490.2077	
$[\alpha]_D^{21}$	+232° (*c* 0.10, MeOH)	+239° (*c* 0.11, MeOH)	+316.9° (*c* 0.2, MeOH)
UV λ_{max} (ε) nm			
in MeOH	215 (49,500), 249 (13,600), 418 (5,400)	215 (41,300), 252 (11,300), 427 (5,600)	215 (37,600), 254 (10,700), 432 (4,800)
in 0.01 N NaOH-MeOH	261 (12,000), 276 (12,000), 540 (5,000)	225 (34,400), 262 (11,000), 279 (11,000), 551 (5,300)	222 (32,200), 262 (8,680), 273 (8,640), 558 (4,980)
IR ν_{max} (KBr) cm^{-1}	3425, 1770, 1660, 1645	3475, 1740, 1665, 1645	1790, 1665, 1650

Table 2. ^{1}H NMR data for menoxymycins A and B and medermycin in $CDCl_3$.

Proton	Menoxymycin A	Menoxymycin B	Medermycin[5]
1	5.07 q (6.5)*	5.02 q (6.9)	5.08 q (7.0)
3	4.69 dd (4.8, 2.8)	4.35 dt (2.5, 6.5)	4.69 dd (5.1, 2.9)
4	5.26 d (2.8)	4.66 d (2.5)	5.25 d (2.9)
6	7.72 d (7.5)	7.67 d (7.7)	7.71 d (7.8)
7	7.92 d (7.5)	7.87 d (7.7)	7.91 d (7.8)
11	2.98 dd (16.5, 4.8),	2.84 d (6.5)	2.97 dd (17.6, 5.1),
	2.70 d (16.5)		2.69 d (17.6)
1-Me	1.57 d (6.5)	1.56 d (6.9)	1.57 d (7.0)
9-OH	12.30 br s	12.33 br s	12.2 br s
12-OMe		3.75 s	
1′	4.95 dd (9.6, 2.0)	4.86 dd (10.7, 2.0)	4.87 dd (10.9, 2.0)
2′	2.59 ddd (11.5, 3.5, 2.0),	2.25 ddd (12.4, 3.5, 2.0),	2.26 ddd (12.4, 3.8, 2.0),
	1.43 ddd (11.5, 11.5, 9.6)	1.28 ddd (12.4, 12.4, 10.7)	1.30 ddd (12.5, 12.4, 10.9)
3′	3.68 ddd (11.5, 8.5, 3.5)	2.74 ddd (12.4, 9.0, 3.5)	2.78 ddd (12.5, 9.5, 3.8)
4′	3.74 dd (8.5, 8.0)	3.18 dd (9.0, 9.0)	3.20 dd (9.5, 8.9)
5′	3.64 dq (8.0, 5.6)	3.52 dq (9.0, 6.0)	3.53 dq (8.9, 6.2)
6′	1.47 d (5.6)	1.42 d (6.0)	1.43 d (6.2)
3′-Me_2	3.29 s	2.32 s	2.34 s
	3.25 s		

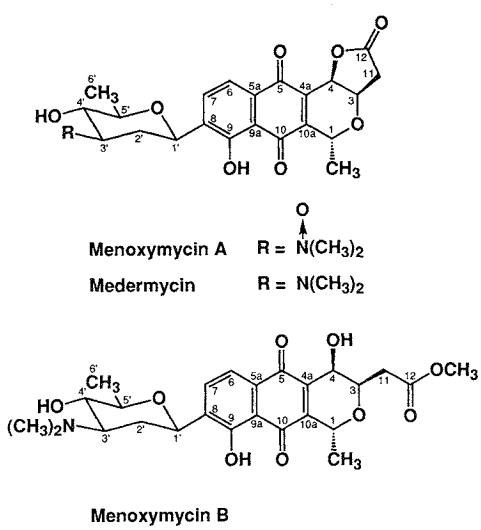
* δ_H, multiplicity, coupling constant (Hz).

Table 3. ^{13}C NMR data for menoxymycins A and B and medermycin.

Carbon	Menoxymycin A[a]	Menoxymycin B[b]	Medermycin[c]
1	66.2	67.1	66.3
3	68.5	67.6	66.5
4	66.4	59.3	68.7
4a	135.4	141.2	134.9
5	181.1	182.7	180.8
5a	130.4	130.3	129.7
6	119.8	119.2	119.6
7	133.8	133.2	133.5
8	136.9	138.1	138.6
9	157.8	157.5	157.7
9a	114.3	114.2	114.0
10	188.4	189.3	187.8
10a	149.6	146.7	149.2
11	36.9	35.5	37.0
12	173.9	171.9	173.5
1-Me	17.8	17.7	18.8
12-OMe		51.8	
1′	72.9	72.0	72.2
2′	29.7	28.3	28.2
3′	75.9	66.8	67.2
4′	71.3	71.3	71.5
5′	77.8	77.4	77.6
6′	18.5	17.6	18.9
3′-Me_2	58.4, 52.7	40.0	40.3

[a] δ_C in $CDCl_3$; [b] in $CDCl_3$ - CD_3OD (10 : 1); [c] in $CDCl_3$[5].

Fig. 1. Structures of menoxymycins A and B and medermycin.



chemical shift of C-1′ (δ 72.0) and long-range correlations from H-1′ to C-7 and C-8. This aromatic moiety was expanded to the 8-hydroxynaphtoquinone chromophore based on long-range couplings from 6-H to C-5, C-8 and C-9a, from 7-H to C-5a and C-9, and from 1′-H to C-7 and C-8. A low-field chemical shift (δ 12.33) for a phenolic hydroxyl (9-OH) indicated its hydrogen bond to one of the quinone carbonyls (C-10) to establish the partial

Fig. 2. Partial structures of menoxymycin B derived from COSY and HMBC.

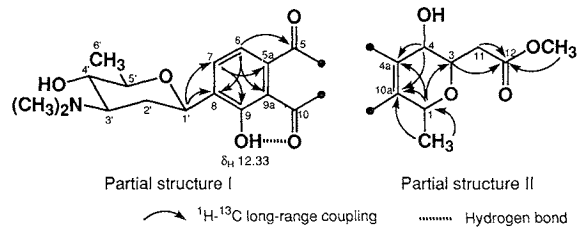


Table 4. Effect of dithiothreitol (DTT) on the cytotoxicity of menoxymycins A and B (IC_{50}, μM).

		KB	N18-RE-105
Menoxymycin A	−DTT	0.86	0.14
	+DTT (250 μM)	8.2	2.4
Menoxymycin B	−DTT	0.22	0.023
	+DTT (250 μM)	1.3	0.34

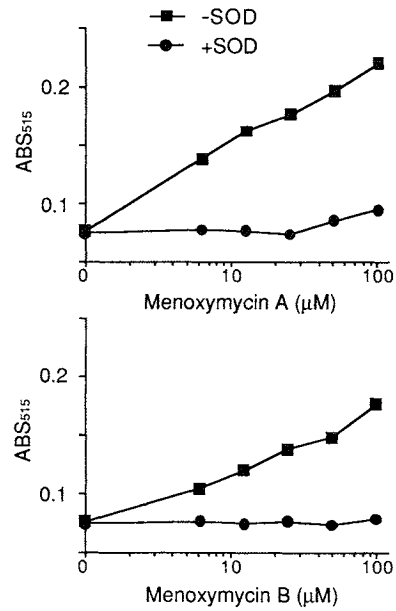
structure I as shown in Fig. 2.

In the remaining part, a COSY experiment identified two separate proton spin systems (3-H, 4-H and 11-H_2; 1-H and 1-CH_3). A dihydropyrane moiety was constructed by ^{1}H-^{13}C long-range couplings from 1-H to C-3, C-4a and C-10a, from 4-H to C-4a and C-10a, and from 1-CH_3 to C-1 and C-10a. Long-range correlations from 3-H, 11-H and 12-OCH_3 to C-12 revealed the presence of a carbomethoxy group located on C-11. These data established partial structure II as shown in Fig. 2.

Menoxymycin B was converted into medermycin[3~5] by treatment with 0.02 N H_3PO_4 at 50°C for 1 hour, thereby establishing the connection between partial structures I and II to determine the structure of menoxymycin B as shown in Fig. 1. TLC analysis identified menoxymycin B together with medermycin in the EtOAc extract of the fresh broth filtrate. Under these conditions, medermycin could not form menoxymycin B. Therefore, menoxymycin B is believed to be a microbial metabolite and not an artifact.

Since the cytotoxicity caused by active oxygen species can be prevented by antioxidants, we examined the effect of dithiothreitol (DTT) on the cytotoxicity of the menoxymycins by using KB human epidermoid cancer cells and N18-RE-105 neuronal cells[2], which are known to be vulnerable to oxygen stress. Menoxymycins A and B showed cytotoxicity against both cell lines, and their cytotoxic activities were reduced by addition of 250 μM DTT as shown in Table 4. The generation of superoxide radicals in N18-RE-105 cell lysate by the menoxymycins was examined by measuring

Fig. 3. Superoxide radical generation in N18-RE-105 cell lysate by menoxymycins A and B.



reduction of nitro blue tetrazolium (NBT)[7]. As shown in Fig. 3, menoxymycins A and B dose-dependently generated superoxide radicals, which were not observed in the presence of 130 μg/ml of superoxide dismutase (SOD). These results suggest that the cytotoxicity of the menoxymycins is related to the generation of active oxygen species in the cells. Further studies on the antitumor activity of the menoxymycins are in progress.

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