NISAMYCIN, A NEW MANUMYCIN GROUP ANTIBIOTIC FROM *Streptomyces* sp. K106

Sir:

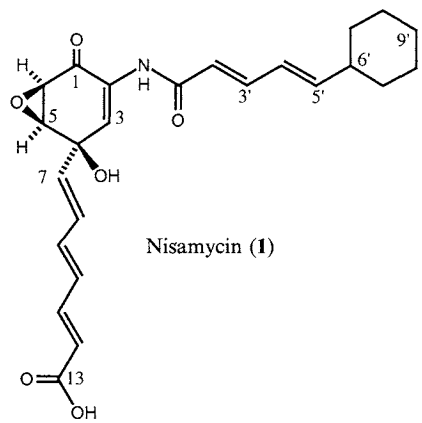
In the course of a screening program for new antibiotics, we found a new manumycin group antibiotic named nisamycin (**1**) in the culture broth of the strain K106. This strain was isolated from the soil sample collected in Sakai city, Osaka prefecture, Japan and identified as a genus *Streptomyces* sp. In this paper, the fermentation, isolation, structure and biological properties of **1** are reported (Fig. 1).

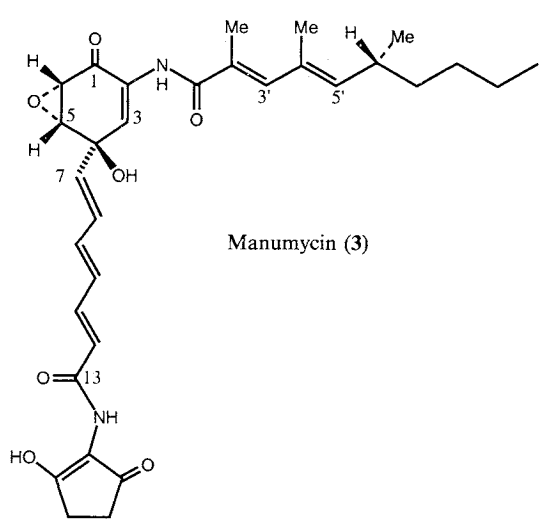
A slant culture of the strain K106 was inoculated into a 500-ml flask containing 100 ml of the medium (glycerol 1%, NZ Amine type A 0.25%, yeast extract 0.1%, meat extract 0.1%, pH 7.0) and the flask was incubated at 28°C for 2 days on a reciprocal shaker. Fifteen ml portion of this culture was transferred to a 5-liter Erlenmeyer flask contaning 1.4 liters of the same medium. The fermentation was carried out at 28°C for 2 days on a shaking machine. Antimicrobial activity was determined by the agar diffusion paper-disc method using *Bacillus subtilis* IFO 12210 as a test organism.

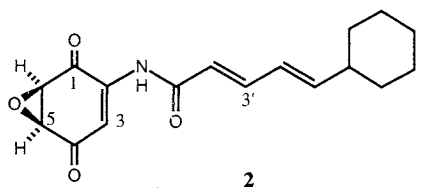
1 can be extracted with ethyl acetate at pH 3 from the broth filtrate and further purified by silica gel chromatography, preparative TLC and Sephadex LH-20 column chromatography, as summarized in Fig. 2. **1** was obtained as pale yellow powder. It was soluble in methanol, acetone, ethyl acetate, chloroform and dimethyl sulfoxide, but insoluble in water and *n*-hexane. Melting point of **1** was 122~124°C with decomposition. The optical rotation of **1** was $[\alpha]_D^{25}$ −143° (*c* 0.35, EtOH). Positive FAB-MS gave *m/z* 426 $(M+H)^+$, 448 $(M+Na)^+$. Molecular formula of **1** was determined as $C_{24}H_{27}NO_6$ by HRFAB-MS (Found 426.1918, Calcd for $C_{24}H_{28}NO_6$ 426.1917). The UV spectrum showed an absorption maxima at 278 nm (ε 32,100) and 305 nm (ε 22,600) in MeOH. The IR spectrum exhibited absorption peaks at 3346, 1694, 1668, 1615, 1521 and 1005 cm^{-1}. The existence of a carboxyl group in **1** was suggested by the IR absorption (3346, 1694 cm^{-1}) and the ^{13}C NMR signal (δ_C 171.2).

Elucidation of the structure of **1** was achieved by the detailed NMR spectral analyses and the comparison of the NMR data of **1** with those

Fig. 1. Structure of nisamycin (**1**), its oxidative product (**2**) and manumycin (**3**).







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of manumycin (**3**)[1], asukamycin[1], colabomycin[2] and alisamycin[3]. The structure of **1** was also confirmed by chromic acid oxidation of **1** to give an oxidative product **2** (Fig. 1).

Fig. 2. Isolation of Nisamycin.

Culture filtrate (16.8 liters)
↓ extracted with EtOAc at pH 3.0
Organic layer (20liters)
↓ concd *in vacuo*
Crude product (2.7g)
↓ silica gel column (400ml) eluted stepwise with Benzene-Acetone
Active fraction (Benzene:Acetone=8:2)
↓ silica gel column (200ml) eluted stepwise with $CHCl_3$-MeOH
Active fraction ($CHCl_3$-MeOH=95:5)
↓ preparative TLC ($CHCl_3$-MeOH =95:5, Rf=0.32) eluted with $CHCl_3$-MeOH (9:1)
Semi-pure nisamycin
↓ Sephadex LH-20 column (200ml) eluted with MeOH
Nisamycin (120mg)

^{13}C and ^{1}H NMR spectral data of **1** and their assignments are shown in Table 1. The ^{13}C NMR signals of **1** at δ_C 52.8 (C-6) and δ_C 57.3 (C-5) were attributed to an epoxide, whose protons were observed in ^{1}H NMR spectrum at δ_H 3.63 (H-6) and δ_H 3.70 (H-5). A long-range "W" coupling of the olefinic proton (H-3, J=2.1 Hz) with the epoxide proton (H-5) was detected by ^{1}H-^{1}H COSY spectrum. The ^{13}C NMR spectrum showed the presence of a conjugated ketone carbonyl carbon (C-1), an amide carbonyl carbon (C-1′), an olefinic carbon (C-3), and a tertiary carbiol carbon (C-4). It also showed an olefinic carbon connected with amide nitrogen (C-2). The NMR signals mentioned above were characteristic of manumycin group antibiotics. 2-Amino-3-hydroxycyclopent-2-enone moiety was also known to be component of manumycin group antibiotics. However, the NMR spectrum of nisamycin did not show any signals of 2-amino-3-hydroxycyclopent-2-enone moiety. This suggests that nisamycin lacks 2-amino-3-hydroxycyclopent-2-enone moiety in its structure.

Considering the chemical shifts of six carbons and eleven protons from C-6′ to C-11′, cyclohexyl group

Table 1. ^{13}C and ^{1}H NMR spectral data of nisamycin.

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$)	δ_H (C_5D_5N)
1	188.7 (s)		
2	127.8 (s)		
3	126.9 (d)	7.41 (d, 2.1)	8.20 (d, 2.1)
4	71.2 (s)		
5	57.3 (d)	3.70 (dd, 4.0, 2.1)	4.07 (dd, 3.7, 2.1)
6	52.8 (d)	3.63 (d, 4.0)	3.92 (d, 3.7)
7	136.6 (d)	5.82~5.93 (m)	6.17 (d, 15.0)
8	131.5 (d)	6.50~6.58 (m)	7.07 (dd, 15.0, 10.8)
9	139.4 (d)	6.50~6.58 (m)	6.71 (dd, 14.7, 10.8)
10	131.9 (d)	6.39 (m)	6.49 (dd, 14.7, 11.1)
11	145.7 (d)	7.20~7.37 (m)	7.73 (dd, 15.3, 11.1)
12	121.5 (d)	5.82~5.93 (m)	6.35 (d, 15.3)
13	171.2 (s)		
1′	165.4 (s)		
2′	120.8 (d)	5.82~5.93 (m)	6.54 (d, 15.0)
3′	144.5 (d)	7.20~7.37 (m)	7.60 (m)
4′	125.5 (d)	6.11~6.14 (m)	6.18 (m)
5′	151.0 (d)	6.11~6.14 (m)	5.94 (dd, 15.2, 6.9)
6′	41.1 (d)	2.08 (m)	1.88 (m)
7′	32.2 (t)		
8′	25.8 (t)	0.89~1.40 (m) 5-H[a]	0.77~1.40 (m) 5-H[a]
9′	26.0 (t)	1.75 (m) 5-H[a]	1.57 (m) 5-H[a]
10′	25.8 (t)		
11′	32.2 (t)		
NH		7.60 (br s)	9.66 (br s)

Chemical shifts in ppm from internal TMS. Coupling constants in J=Hz.
[a] Cyclohexyl ring protons from C-7′ to C-11′.

Table 2. Antimicrobial spectra of nisamycin.

Test organism	MIC (μg/ml)
Staphylococcus aureus IFO 3060	0.19
Bacillus subtilis IFO 12210	0.39
B. cereus IFO 3514	0.19
Arthrobacter globiformis IFO 12140	0.39
Micrococcus roseus IFO 3768	0.39
Escherichia coli K-12 IFO 3301	50
Pseudomonas aeruginosa IFO 3923	>100
Serratia marcescens IFO 12648	>100
Saccharomyces cerevisiae	>100
Candida albicans	>100
C. tropicalis IFO 0589	100
Aspergillus niger van Tieghem IFO 4416	100
Fusarium oxysporum IFO 5880	50

bonded to an olefinic carbon (C-5′) was deduced. Double bond linkages from C-2′ to C-5′ and from C-7 to C-12 were established by ^{1}H-^{1}H COSY spectral data; as the result, diene (C-2′ to C-5′) and triene structure (C-7 to C-12) were suggested, which were supported by the UV spectrum. The configuration of these double bonds were determined to be all *E* form by ^{1}H-^{1}H coupling constants in pyridine-d_5. A coupling behavior (6.9 Hz) between cyclohexyl ring proton (H-6′) and olefinic proton (H-5′) was detected by ^{1}H-^{1}H COSY spectrum.

The structural determination of **2** was made by ^{1}H, ^{13}C NMR spectral analyses [it's physico-chemical properties: colorless needles; mp 142~145°C; HREI-MS Found 301.1332, Calcd for $C_{17}H_{19}NO_4$ 301.1314; UV λ_{max} ($CHCl_3$) nm (ε), 270 (22,400), 336 (17,700); IR ν_{max} (KBr) cm^{-1}, 3282, 1671, 1632, 1605, 1505, 1000; $[\alpha]_D^{25}$ −50° (*c* 0.12, $CHCl_3$); CD $\lambda_{extreme}$ ($CHCl_3$) nm ($\Delta\varepsilon$), 375 (−9.37), 328 (+16.69), 266 (−6.34)].

The configuration at C-4 of **1** was determined by the exciton chirality method[4] which has been adopted for asukamycin, manumycin and colabomycin[2,5,6]. The configuration at C-5 and C-6 was determined by comparing the CD spectrum of **2** with those of antibiotic G7063-2 and chromic oxidative products of manumycin and colabomycin[5,6]. **1** indicated medium-strong negative CD couplet in chloroform (315 nm $\Delta\varepsilon$ −14.78, 261 nm $\Delta\varepsilon$ +15.24), which was consistent only with the 4*R*-configuration. Thus, **1** was shown to possess the same stereochemistry at C-4 as manumycin and the opposite of asukamycin and colabomycin. **2** containing oxirane ring gave two Cotton effects between 300 and 400 nm, which were same signs as that of antibiotic G 7063-2 and opposite to those of the chromic oxidative products of manumycin and colabomycin[2,6]. Therefore, the absolute configuration at C-5 and C-6 of **2** was determined to be 5*S*/6*R*. Since the degradation of **1** to **2** does not give any effects on the chirality at C-5 and C-6 of **1**, the configuration at C-5 and C-6 of **1** was assigned as C-5/*S*, C-6/*R*. As mentioned above, the absolute structure of **1** was determined as shown in Fig. 1. All proton and carbon signals of **1** were assigned by ^{1}H-^{1}H and ^{13}C-^{1}H COSY spectrum (Table 1).

Nisamycin mainly inhibits the growth of Gram-positive bacteria but shows a weak antifungal activity (Table 2). The MIC was measured by the agar dilution method using bouillon agar for bacteria and sabouraud agar for fungi and yeast. In addition, nisamycin displays a cytotoxic activity in the proliferation assay (IC_{50} 2.5 μg/ml) against B-16F melanoma cells.

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