A NOVEL ANTIBIOTIC, NAPHTHOPYRANOMYCIN

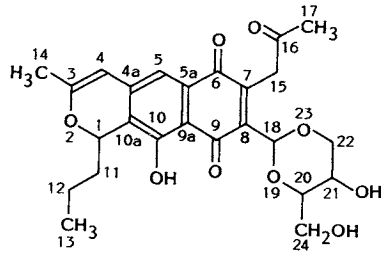
Sir:

In the course of our screening program for new antitumor substances, we isolated a novel antibiotic naphthopyranomycin (Fig. 1) from the mycelium extract of an actinomycete. Here, we report the fermentation, purification, structure elucidation and biological activities of naphthopyranomycin.

The producing organism identified as a *Streptomyces* sp. was isolated from soil sample collected at Quito in Ecuador. The fermentation was carried out in a 500-ml Erlenmeyer flask containing 100 ml of medium with following composition; potato starch 3.0%, soya flake 1.5%, yeast extract 0.2%, corn steep liquor 0.5%, NaCl 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCO_3$ 0.3%, $CoCl_2 \cdot 6H_2O$ 0.0005%. The pH of the medium was adjusted to 7.2 before sterilization. The seed culture was inoculated to the flasks, and the fermentation was carried out at 27°C for 5 days on a rotary shaker.

Naphthopyranomycin (**1**) was isolated according to the scheme as shown in Fig. 2, and showed the

Fig. 1. The total structures of naphthopyranomycin (**1**), anhydro-5-deoxyfusarubin (**2**) and anhydro-5-deoxyfusarubin acetate (**3**).



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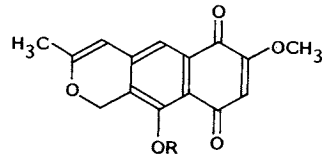


2 R=H
3 R=Ac

Fig. 2. Isolation scheme of naphthopyranomycin.

Mycelium cake (10 liters)
- added 70% acetone (2 liters)
- concentrated to small volume to remove acetone
- extracted with EtOAc (1 liter × 2)

EtOAc layer (2 liters)
- concentrated *in vacuo*

Silica gel column (150 ml)
- eluted with $CHCl_3$ - MeOH (50 : 1)

Active fractions
- concentrated *in vacuo*

Preparative TLC (Kieselgel 60 F254 0.5 mm)
- developed with $CHCl_3$ - MeOH (15 : 1)

Active band
- extracted with $CHCl_3$ - MeOH (1 : 1)

Crude naphthopyranomycin
- concentrated *in vacuo*

Sephadex LH-20 column (200 ml)
- developed with $CHCl_3$ - MeOH (1 : 1)

Active fractions
- concentrated *in vacuo*

Naphthopyranomycin (6.5 mg)

Table 1. Physico-chemical properties of naphthopyranomycin.

Appearance	Red powder
MP (dec)	131 ~ 133°C
Molecular formula	$C_{25}H_{28}O_9$
FD-MS m/z (M^+)	472
Analysis	Calcd: Found:
C	63.55 64.10
H	5.97 6.15
UV λ_{max} nm (ε)	
0.01 N HCl - MeOH	240.8 (12,200), 302.4 (15,200), 470.8 (3,700)
0.01 N NaOH - MeOH	264.4 (14,300), 296.4 (15,700), 534.4 (3,200)
IR ν (KBr) cm^{-1}	3400, 1700, 1640, 1600, 1390, 1295

Fig. 3. Partial structures of naphthopyranomycin.

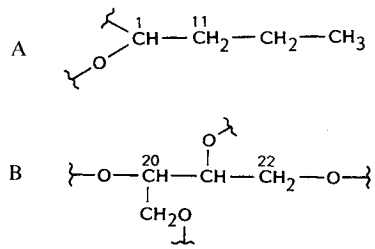


Table 2. ^{13}C NMR and ^{1}H NMR spectral data of naphthopyranomycin (**1**), anhydro-5-deoxyfusarubin (**2**) and anhydro-5-deoxyfusarubin acetate (**3**)[a].

Position	1		3	2
	δ_C	δ_H	(δ_C)	(δ_H)
1	73.1 d	5.65 (dd, 3.5, 9.5)	63.5 t	5.24 (s)
3	158.6 s		156.1 s	
4	99.9 d	5.60 (s)	100.6 d	5.62 (s)
4a	139.7 s		144.7 s	
5	114.9 d	7.16 (s)	111.4 d	7.43 (s)
5a	131.0 s		132.7 s	
6	184.4 s		179.8 s	
7	144.7 s[b]		160.0 s	
8	140.6 s[b]		118.3 d	6.04 (s)
9	186.4 s		183.3 s	
9a	112.5 s		120.1 s	
10	157.0 s	12.20 (OH)	138.7 s	12.25 (OH)
10a	122.6 s		125.8 s	
11	34.9 t	1.5~1.6[c], 1.96[c]		
12	18.1 t	1.4~1.6[c]		
13	13.3 q	0.96 (t, 7.0)		
14 (3-methyl)	20.4 q	1.96 (s)	19.8 q	1.96 (s)
15	41.9 t	4.05 (d, 16.0), 4.11 (d, 16.0)		
16	205.1 s			
17	29.8 q	2.18 (s)		
18	94.9 d	6.08 (s)		
20	82.1 d	3.62 (ddd, 6.3, 9.0, 9.0)		
21	62.1 d	3.97 (ddd, 4.5, 9.0, 10.5)		
22	71.2 t	3.60 (dd, 10.0, 10.5), 4.25 (dd, 4.5, 10.0)		
24	62.2 t	3.83 (dd, 9.0, 10.6), 3.90 (dd, 6.3, 10.6)		
15-*O*-Methyl			56.3 q	3.94 (s)

[a] Taken in $CDCl_3$.
[b] The assignments may be interchanged.
[c] Resonance in one-dimentional spectra obscured by overlapping signals.

physico-chemical properties as summarized in Table 1. The molecular formula of **1** was determined to be $C_{25}H_{28}O_9$ by using FD-MS and elemental analysis. IR and UV spectra of **1** were consistent with the naphthaquinone chromophore. In particular, the UV spectrum of **1** was closely similar to that of anhydro-5-deoxyfusarubin[1] (**2**) (Fig. 1) which was isolated as a red pigment from *Nectria haematococca* and that of umbrinomycin[2] whose structure has not been identified yet. Comparison of the ^{13}C NMR signals of **1** with those of acetate of **2** (**3**) (Table 2) revealed down field shifts for C-1 (δ 73.1 vs. δ 63.5) and C-8 (δ 140.6 vs. δ 118.3), and an upfield shift for C-7 (δ 144.7 vs. δ 160.0). These data indicated the structure of **1** was that of 1,7,8-tri-substituted **2**.

^{1}H-^{1}H and ^{1}H-^{13}C COSY experiments revealed the presence of two partial structures A and B in **1** (Fig. 3). The remaining four carbons in **1** were attributed to a singlet methyl (C-17, δ 29.8), an isolated methylene (C-15, δ 41.9), an acetal (C-18, δ 94.9) and a ketone (C-16, δ 205.1). The ^{1}H NMR spectra of the chromophore of **1** and **2** were very similar, but the singlet methylene proton (1-H, δ 5.24) in **2** was replaced by the double doublet methine proton (δ 5.65) in **1** which was coupled with methylene protons (11-H) in the partial structure A. Therefore, **1** was confirmed to possess a propyl group at C-1.

The connectivities of the chromophore and the partial structures were determined and confirmed by heteronuclear multiple-bond correlation (HMBC)[3] experiment as shown in Fig. 4. The HMBC experiment showed the long range couplings of 17-H (δ 2.18; singlet methyl) and 15-H (δ 4.05 and 4.11; isolated methylene) to C-16 (δ 205.1;

Fig. 4. Long range couplings observed by HMBC experiment.

⟶ Long range coupling.

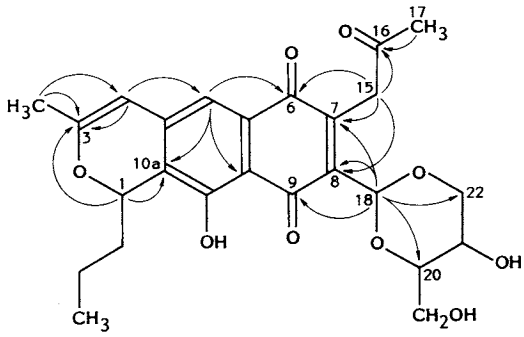
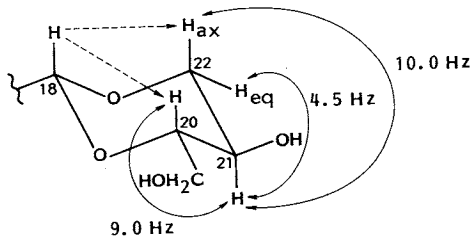


Fig. 5. Relative configuration of the 1,3-dioxan moiety.

⟶ NOE.



ketone), indicating the presence of acetonyl side chain. In addition, since 15-H was also coupled to C-6 (δ 184.4), C-7 (δ 144.7) and C-8 (δ 140.6), this acetonyl side chain was proved to attach to C-7. The long range couplings of 18-H (δ 6.08; acetal) to C-20 (δ 82.1) and C-22 (δ 71.2) revealed the presence of the 4-hydroxymethyl-5-hydroxy-1,3-dioxan moiety containing the partial structure B. Furthermore, the long range couplings of 18-H to C-7, C-8 and C-9 (δ 186.4) indicated that the 1,3-dioxan moiety was linking to C-8. On the basis of the above mentioned results, structure of **1** was determined as shown in Fig. 1.

The relative stereochemistry for the dioxan moiety was determined by coupling constants and NOE experiment as shown in Fig. 5. A large coupling constant between 20-H and 21-H (9.0 Hz) indicated that both of them were axially-oriented. 18-H was also proved to be the axial-orientation, because irradiation of 18-H enhanced the intensities of 20-H and 22-H_{ax} in the NOE study.

1 showed cytotoxicity against P388 leukemia cells (IC_{50} 0.3 μg/ml) and weak antimicrobial activity against Gram-positive bacteria.

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(Received December 11, 1991)

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