COMMUNICATIONS TO THE EDITOR

Benaphthamycin, a New Dihydrobenzo[*a*]naphthacenequinone Antibiotic from *Streptomyces* sp. HKI-0057

Sir:

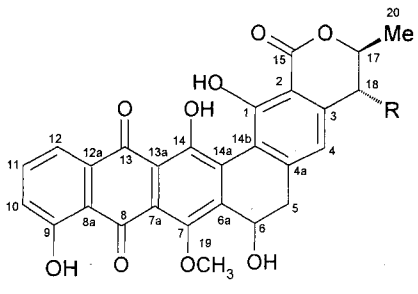
A new representative of the dihydrobenzo[*a*]naphthacenequinone antibiotics, benaphthamycin (**1**, $C_{27}H_{20}O_{10}$), was isolated from cultures of *Streptomyces* sp. HKI-0057 (obtained from an Australian soil sample). Its structure is closely related to WS79089A[1] (**2**), which was coisolated from the same strain. In this paper we report on the isolation, physico-chemical properties, structure elucidation and biological activity of this new antibiotic.

The fermentation of the strain was carried out for 96 hours at 26°C in a 30 liters fermentor using glucose 3%, oatmeal 2%, $CaCl_2$ 0.3%, $CaCO_3$ 0.2%, yeast extract 0.3%, $FeSO_4 \cdot 7H_2O$ 0.054%, $MnCl_3 \cdot 4H_2O$ 0.06%, pH 6.5 (aeration: 30 liters per minute). For pigment isolation, culture broth and mycelium were separated and the latter extracted overnight by 10 liters of methanol. Afterwards the extract was evaporated and the aqueous residue was reextracted by 5 liters of ethyl acetate. The culture broth was extracted twice by 10 liters of ethyl acetate and the combined extracts were dried over Na_2SO_4 and evaporated to dryness. The residue (4.8 g) was subjected to column chromatography on Silica gel 60 (Merck, 0.063~0.1 mm), and elution occurred by a gradient of *n*-hexane/ethyl acetate. Two reddish fractions with Rf 0.9 (WS79089A (**2**, Fig. 1)[1]; 160 mg) and Rf 0.6 (**1**, 140 mg) upon TLC (silica gel sheets, Merck; $CHCl_3$/MeOH; 9/1) were obtained. Final purification was accomplished by preparative HPLC using a binary gradient of water to acetonitrile (95:5 to 5:95, 30 minutes, column 25 mm × 250 mm; Spherisorb RP_{18} ODS-2). The physico-chemical properties of the new antibiotic **1** are summarized in Table 1.

Structure elucidation was based on mass spectrometric and NMR spectroscopic measurements. The electrospray mass spectrum of **1** displayed *m/z* 503.2 ($[M-H]^-$) in the negative ion mode. The elemental composition of **1** was suggested by *m/z* 504.10360 (M^+; calcd. 504.10565 for $C_{27}H_{20}O_{10}$) in the electron impact mass spectrum. The chemical constitution (Fig. 1) and shift assignment (Table 2) of **1** (1,6,9,14,18-pentahydroxy-7-methoxy-17-methyl-8,13,15-trioxo-17-oxa-naphthaceno[1,2-9]-isochroman) was concluded from two-dimensional NMR experiments such as COSY, NOESY, HSQC and HMBC (Fig. 2). The observation of a NOESY cross peak between 9-OH and 19-CH_3 settled the position of these residues on the same side of the *p*-quinone framework. Of pivotal importance were the two heteronuclear $J^4_{C,H}$ correlations of 4-H to C-14a and C-15, respectively, which were obtained in a HMBC experiment optimized on a small 2 Hz coupling constant. The relative *trans* configuration of 18-OH and 20-CH_3 was concluded from the strong NOESY cross peak between 18-H and 20-CH_3, which was significantly greater than the corresponding NOE between 18-OH and 20-CH_3. The present NMR data do not provide evidence of the relative configuration of the hydroxyl group at C-6.

Benaphthamycin (**1**) displays moderate narrow-spectrum activity against Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 during the agar diffusion plate assay[2]. Cytopathic effect was observed against L-929 mouse fibroblast cells, K-562 human leukaemia cells and HeLa cells with approximately the same ID_{50}

Fig. 1. Chemical constitution of benaphthamycin (**1**) and WS79089A (**2**)[1] from *Streptomyces* sp. HKI-0057.



1 R = OH
2 R = H

Table 1. Physico-chemical properties of benaphthamycin (**1**).

Appearance	Reddish microcrystals
Chemical formula	$C_{27}H_{20}O_{10}$
HREI-MS *m/z*	Calcd. 504.10565
	Found 504.10360
$[\alpha]_D^{25}$	−125.8° (*c* 0.1, MeOH)
UV λ_{max} nm (ε) in MeOH	475 (14.000)
IR ν_{max} cm^{-1}	800, 920, 953, 981, 1036, 1065, 1134, 1180, 1250, 1299, 1364, 1412, 1451, 1613, 1660, 2930, 3440
Rf (TLC)*	0.60

* Silica gel; $CHCl_3$/MeOH 9:1.

Table 2. ^{1}H and ^{13}C-NMR chemical shifts of **1** (in DMSO-d_6, δ in ppm relative to internal TMS).

Position	δ_C	δ_H	Postion	δ_C	δ_H
1	158.3 s	11.79 (s)	10	124.7 d	7.40 (dd, 7.7, 1.8)
2	105.7 s		11	136.6 d	7.81 (t, 7.7)
3	143.7 s		12	118.5 d	7.78 (dd, 7.7, 1.8)
4	117.3 d	7.12 (s)	12a	132.5 s	
4a	146.2 s		13	188.0 s	
5	38.3 t	2.86 (dd, 16.1, 3.0)	13a	114.8 s	
		3.11 (dd, 16.1, 2.5)	14	157.3 s	13.96 (s)
6	58.7 d	5.22 (ddd, 4.2, 3.0, 2.5)	14a	129.6 s	
6-OH		5.29 (d, 4.2)	14b	117.8 s	
6a	144.5 s		15	168.8 s	
7	151.2 s		17	80.1 d	4.63 (dq, 6.0, 7.6)
7a	122.3 s		18	67.1 d	4.59 (dd, 6.1, 7.6)
8	186.9 s		18-OH		6.21 (d, 6.1)
8a	116.8 s		19	62.5 q	3.94 (s)
9	161.5 s	12.89 (s)	20	17.8 q	1.42 (d, 6.0)

Abbreviations: s: singlet, d: doublet, t: triplet, q: quartet.

Fig. 2. Instructive C, H long range couplings and nuclear Overhauser effects as detected in the HMBC and NOESY spectra of **1**.

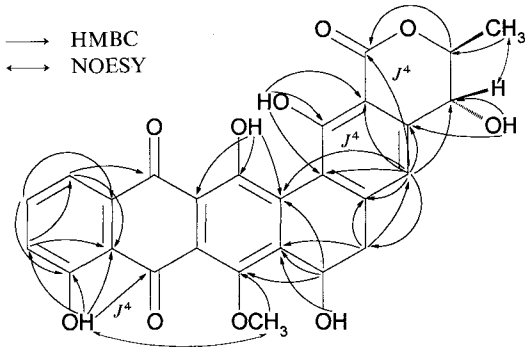
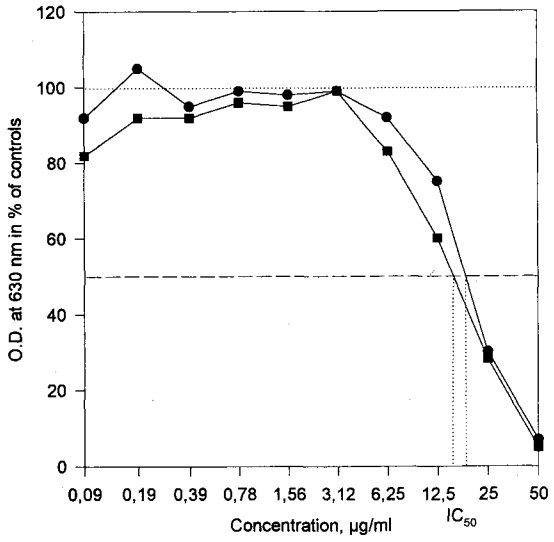


Fig. 3. Concentration response curves of L-929 cells after 72 hours incubation with benaphthamycin (**1**) and coproduced WS79089A (**2**)[1] from *Streptomyces* sp. HKI-0054.

■: **1**; ●: **2**. ····· controls, ––– 50% inhibition.



values ranging from 13 to 21 µg/ml. Fig. 3 shows the inhibitory effect of **1** and the coproduced WS79089A (**2**)[1] on L-929 cell cultures.

Dihydrobenzo[*a*]naphthacenequinone-type antibiotics such as pradimicins[3,4] or benanomicins[5] are usually produced by *Actinomadura* sp. as glycosylated representatives. Mostly they harbour 5*S*,6*S*-dihydroxy functions which are 6-*O*-monoglycosylated. Pradimicin Q[6] is known as a 5-monohydroxy derivative and the WS79089 compounds[1] contain a single hydroxyl group at C-6. Dihydrobenzo[*a*]naphthacenequinones attracted much interest, in the past, due to the fungicidal activity of benanomicins and pradimicins, involving a lectine-like mode of action[3,4]. Moreover, inhibition of the human immunodeficiency virus (HIV)[7], of α-glucosidase[6] and the endothelin converting enzyme[1] suggested this polycyclic aromatic structures as novel leads for the development of new drugs. Due to the presence of hydroxyl groups at C-6 and C-18 benaphthamycin (**1**) appears as a promising starting material for semisynthetic modifications.

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References

1) Tsurumi, Y.; N. Ohhata, T. Iwamoto, N. Saigemaisu, K. Sakamoto, M. Nishikawa, S. Kiyoto & M. Okuhara: WS79089A, B and C, new endothelin converting enzyme inhibitors isolated from *Streptosporangium roseum* No. 79089. J. Antibiotics 47: 619~630, 1994
2) Anonymous: Deutsches Arzneibuch 9th Edition. pp. 47 and 424, Deutscher Apothekerverlag Stuttgart, 1986
3) Sawada, Y.; K. Numata, T. Murakami, H. Tanimichi, S. Yamamoto & T. Oki: Calcium-dependent anticandidal action of pradimicin A. J. Antibiotics 43: 715~721, 1990
4) Ueki, T.; K. Numata, Y. Sawada, T. Nakajima, Y. Fukugawa & T. Oki: Studies on the mode of antifungal action of pradimicin antibiotics. I. Lectin-mimic binding of BMY-28864 to yeast mannan in the presence of calcium. J. Antibiotics 46: 149~161, 1993
5) Kondo, S.; S. Gomi, D. Ikeda, M. Hamada & T. Takeuchi: Antifungal and antiviral activities of benanomicins and their analogs. J. Antibiotics 44: 1228~1236, 1991
6) Sawada, Y.; T. Ueki & T. Tsuno: Manufacture of the α-glucosidase inhibitor pradimicin Q with Actinomadura. US 5091418, 25.2.1992
7) Tanabe, A.; H. Nakashima, O. Yoshida, N. Yamamoto, O. Teumyo & T. Oki: Inhibitory effect of new antibiotic, pradimicin A on infectivity, cytopathic effect and replication of human immunodeficiency virus *in vitro*. J. Antibiotics 41: 1708~1710, 1988