Notes

A NEW ANSAMYCIN ANTIBIOTIC, NAPHTHOMYCIN H FROM A *STREPTOMYCES* SPECIES Y-83,40369

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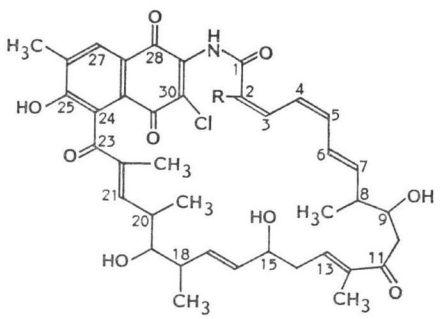
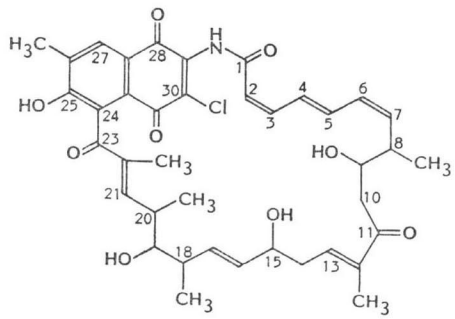
From the fermentation products of a *Streptomyces* culture number Y-83,40369, we have isolated naphthomycin A (**1**)[1,2] as a minor constituent and a new compound designated naphthomycin H (**2**); a light yellow crystalline substance analyzed as $C_{39}H_{44}ClNO_9$, mp 150°C (dec) as the major constituent. Naphthomycin H (**2**) is shown to be a geometrical isomer of naphthomycin B (**3**)[3] by extensive ^1H NMR double-resonance studies.

The organism was isolated from a soil sample collected near Dharchula, Himachal Pradesh, India. A spore suspension from a slant of the strain was inoculated into 100 ml of the seed culture medium consisting of glucose 1.5%, soyabean meal 1.5%, corn steep liquor 0.5%, $CaCO_3$ 0.2%, NaCl 0.5% in distilled water (pH 7.0) in 500-ml Erlenmeyer flasks and cultured for 3 days at 27°C on a rotary shaker. A second stage seed culture was carried out in a 15-liter fermentor containing 10 liters of the seed culture medium at 27°C for 1 day under aeration (7 liters/minute) and agitation (170 rpm). This inoculum was used in a 150-liter fermentor containing 100 liters of a medium consisting of starch 1.0%, glucose 1.0%, malt extract 0.75%, peptone 0.75%, NaCl 0.3%, $MgSO_4 \cdot 7H_2O$ 0.1%, $CuSO_4 \cdot 5H_2O$ 0.0007%, $FeSO_4 \cdot 7H_2O$ 0.0001%, $MnCl_2 \cdot 4H_2O$ 0.0008% and $ZnCl_2 \cdot 7H_2O$ 0.0002% in distilled water (pH 7.0). The fermentation was performed for 3 days at 27°C under aeration of 70 liters per minute and agitation of 130 rpm. Desmophen was added as antifoaming agent.

The filtered fermentation broth (90 liters, pH 6.78) was adjusted to pH 7.0 with 2 N NaOH and extracted twice with 15 liters of EtOAc. EtOAc extracts were evaporated under vacuum to dryness to get a residue weighing 35.8 g. The mycelium (3.9 kg) was extracted twice with 5 liters of acetone each time. The combined acetone extracts were concentrated and diluted with water to a 5-liter volume whose pH was adjusted to 7.0 and extracted twice with 5 liters of EtOAc. The combined EtOAc extracts were evaporated under vacuum to dryness to obtain 16.0 g of the crude antibiotic. The crude materials obtained from the filtered broth and the mycelium were combined. Part (36 g) of this material (51.8 g) was subjected to silica gel (mesh size 60~100) column chromatography. Elution with 5% MeOH in $CHCl_3$ gave bioactive material (14.6 g). This was subjected to a



Naphthomycin A (**1**) R = CH_3
Naphthomycin H (**2**) R = H



Naphthomycin B (**3**)

Table 1. Comparison of prominent physical and spectroscopic properties of naphthomycins A, B and H.

	Compound from fraction A (Naphthomycin A)	Naphthomycin B	Naphthomycin H*
MP °C (dec)	>190	155~160	150
UV (MeOH) nm	230, 276, 400	235, 307, 360 (sh)	228, 302, 360
NaOH shift, nm	230, 293, 424	235, 297, 345 (sh), 434	216, 236 (sh), 298, 428
IR (KBr) cm^{-1}	3448, 1689, 1639, 1290, 1053, 901, 725	IR chart given in ref 3	3546, 3448, 1667, 1626, 1600, 1471, 1299, 1198, 746
^1H NMR ($CDCl_3$): δ coupling constants in Hz	0.82, 0.95, 1.1 (d, J=6 Hz, 3H each), 1.72, 2.05, 2.14 (s, 3H each), 2.2 (m, 2H), 2.3 (m, 2H), 2.38 (s, 3H), 2.51 (dd, J=8, 16 Hz, 1H), 2.7 (m, 1H), 2.86 (s, 1H, OH), 3.08 (d, J= 9 Hz, 1H), 3.28 (dd, J=3, 17 Hz, 1H), 3.55 (m, 1H), 3.9 (br, 2H, 2×OH), 4.0 (m, 1H), 5.42 (dd, J=8, 16 Hz, 1H), 5.48 (dd, J=10, 16 Hz, 1H), 5.55 (dd, J=8, 16 Hz, 1H), 5.95 (dd, J=2, 10 Hz, 1H), 6.10 (d, J=10 Hz, 1H), 6.12 (d, J=10 Hz, 1H), 6.5 (m, 1H), 6.64 (m, 1H), 6.78 (dd, J=6, 8 Hz, 1H), 7.96 (s, 1H), 8.05 (s, 1H, NHCO), 9.85 (s, 1H, OH)	0.82 (d, J=7 Hz, 3H), 0.97 (d, J=6.5 Hz, 3H), 1.19 (d, J=7 Hz, 3H), 1.70 (s, 3H), 2.04 (d, J=2 Hz, 3H), 2.21 (m, 1H), 2.29 (s, 1H, OH), 2.30 (m, 2H), 2.39 (s, 3H), 2.72, 2.73 (m, 1H each), 2.77 (dd, J=5, 17 Hz, 1H), 3.00 (dd, J=4, 17 Hz, 1H), 3.11 (dd, J=2.5, 9 Hz, 1H), 3.66 (m, 1H), 3.68 3.89 (2s, 2H, 2×OH exchangeable), 4.10 (ddd, J=2, 7, 9 Hz, 1H), 5.39 (dd, J=11 Hz, 1H), 5.51 (dd, J=9.5, 15 Hz, 1H), 5.68 (dd, J= 7, 15 Hz, 1H), 5.91 (dd, J=2, 8 Hz, 1H), 5.95 (d, J=11 Hz, 1H), 6.20 (dd, J=11 Hz, 1H), 6.60 (dd, J=11 Hz, 1H), 6.74 (dd, J=11, 15 Hz, 1H), 6.75 (overlapped, 1H), 6.81 (dd, J=11, 15 Hz, 1H), 7.98 (s, 1H), 8.16 (s, 1H, NH exchangeable), 9.64 (s, 1H, OH exchangeable)	0.82 (d, J=6.5 Hz, 3H), 0.96 (d, J=6.5 Hz, 3H), 1.20 (d, J=7 Hz, 3H), 1.71 (s, 3H), 2.03 (d, J=1.5 Hz, 3H), 2.20 (m, 1H), 2.30 (m, 3H), 2.39 (s, 3H), 2.62 (dd, J=6.5, 16.5 Hz, 1H), 2.65 (s, 1H, OH exchangeable), 2.7 (m, 1H), 3.11 (dd, J=2.5, 10 Hz, 1H), 3.15 (dd, J=3.5, 17 Hz, 1H), 3.57 (ddd, J=3, 6.5, 9.5 Hz, 1H), 3.63, 3.65 (2s, 2H, 2×OH exchangeable), 4.04 (dt, J=5, 7.5 Hz, 1H), 5.47 (dd, J=9.5, 15 Hz, 1H), 5.56 (dd, J= 10.5, 15 Hz, 1H), 5.63 (dd, J=7.5, 15 Hz, 1H), 5.93 (dd, J=1.5, 10 Hz, 1H), 6.02 (d, J=11.5 Hz, 1H), 6.28 (dd, J=11, 11 Hz, 1H), 6.36 (dd, J=11, 11 Hz, 1H), 6.5 (dd, J= 10.5, 15 Hz, 1H), 6.72 (dt, J=1, 6 Hz, 1H), 6.98 (dd, J=11, 11 Hz, 1H), 7.98 (d, J=1.5 Hz, 1H), 8.0 (s, 1H, NH exchangeable), 9.78 (s, 1H, OH exchangeable)
TLC (SiO_2) Rf**	0.57***	0.47***	0.41

* Stepwise addition of benzene was done to resolve various peaks in the NMR and to obtain the values of the coupling constants.

** Solvent; EtOAc - AcOH (20: 0.1), precoated plate, E. Merck #5554.

*** Previous work did not indicate any Rf values in this TLC system.

second silica gel (mesh size 200~300) column chromatography and eluted with benzene with increasing concentrations of EtOAc. This resulted in two fraction — fraction A (0.19 g) and fraction B (1.2 g) — which were found to be bioactive.

Fraction A, on further purification on a Sephadex LH-20 column in MeOH, gave naphthomycin A (**1**) (69 mg). It was characterized by the identity of its physical and spectroscopic properties with those reported for naphthomycin A in the literature (mp, IR, UV, ^1H NMR; Table 1)[1,3].

Fraction B was further purified by column

Table 2. ^{13}C NMR of naphthomycin H in $CDCl_3$ (67.9 MHz) in ppm.

C=O region:	Ketone C atoms 203.5, 201.6 Quinone C atoms 178.6, 178.1 Amide C atoms 165.0
Sp^2 region:	161.2, 147.2, 142.5, 141.6, 138.0, 137.6, 136.8, 136.7, 135.9, 135.5, 134.5, 133.8, 133.3, 132.5, 131.2, 126.5, 123.4, 121.4, 121.0, 119.9
Sp^3 region:	(CH-O, CH-, CH_2-, CH_3-) 76.3, 73.3, 71.8, 45.2, 41.7, 40.6, 36.4, 33.7, 17.4, 16.4, 16.2, 12.4, 11.2, 10.8

Table 3. ^1H NMR decoupling experiments of naphthomycin H (270 MHz, $CDCl_3$).

Irradiation (ppm)	δ ppm H(X)[a]	Changed (ch) to J (Hz)	Assignment H(X)
6.98	6.36 dd	d/11	HC(4)
	6.02 d	s	HC(2)
6.72	2.30 m	ch	H_2C(14)
	1.71 d	s	H_3C-C(12)
6.50	6.28 dd	d/11	HC(5)
	5.56 dd	bd/10.5	HC(7)
6.02	6.98 dd	d/11	HC(3)
5.93	2.70 m	dq/2.5, 7	HC(20)
	2.03 d	s	H_3C-C(22)
4.04	5.63 dd	d/15	HC(16)
	2.30 m	ch	H_2C(14)
3.57	3.15 dd	d/18	H_2C(10)
	2.62 dd	d/18	
	2.30 m	ch	HC(8)
1.22	2.30 m	ch	HC(8)
0.96	2.20 m	dd/10, 10	HC(18)
0.82	2.70 m	dd/3, 10	HC(20)

[a] Coupling constants in Hz: 2/3=11.5, 3/4=11, 4/5=11, 5/6=11, 6/7=15, 7/8=10.5, 9/10a=6.5, 9/10b=3, 10a/b=18, 15/16=7.5, 16/17=15, 17/18=9.5, 18/19=10, 19/20=2.5, 20/21=10, 21/22 CH_3=1.5.

chromatography using TLC grade silica gel-H (without binder) and eluted with 4% MeOH in $CHCl_3$. The active material thus obtained was finally purified by preparative TLC using $CHCl_3$ - MeOH (95: 5) as a developer. This compound upon crystallization from a petroleum ether - EtOAc mixture yielded naphthomycin H (**2**). The elemental analysis and FAB mass spectrometry [$(M+H)^+$ 706] suggested the molecular formula of **2** as $C_{39}H_{44}ClNO_9$. Further ^{13}C NMR (Table 2) confirmed the presence of 39 carbons. Based on its spectroscopic properties, the new antibiotic, naphthomycin H was clearly a geometrical isomer of naphthomycin B.

In order to arrive at the geometry of various double bonds in naphthomycin H (**2**) extensive decoupling experiments were carried out (see Table 3). The signals due to olefinic protons other than the triene system are assigned as follows: The doublet of doublets at 5.47 (H-C(17)) and 5.63 (H-C(16)) ppm belong to a *trans* disubstituted double bond ($J_{16,17}$=15 Hz). A doublet of doublets at 5.93 ppm ($J_{21,22Me}$=1.5 Hz, $J_{20,21}$=10 Hz) shows an allylic coupling with a methyl group (2.03 ppm) and a vicinal coupling with a methine proton (2.7 ppm) and is therefore assigned to HC(21). The signal of HC(13) appears as a multiplet at 6.72 ppm. Since the chemical shifts and coupling constants of olefinic protons at C(13) and C(21) are nearly identical with those of naphthomycin A the double bonds C(12)=C(13) and C(21)=C(22) have been tentatively assigned *E*-configuration[2,3]. The coupling constants of the triene system in naphthomycin H ($J_{2,3}$=11.5, $J_{4,5}$=11, $J_{6,7}$=15 Hz) prove that C(2)=C(3) and C(4)=C(5) have *Z*-geometry, whereas the other double bond C(6)=C(7) has *E*-geometry as shown in the structure (**2**). The comparative biospectrum of the naphthomycins H, A and B are presented in Table 4.

Table 4. Antimicrobial activity of naphthomycins *in vitro*.

Microorganism	Zone diameter (mm)		
	H	A	B
Staphylococcus aureus 209 P	17	14	15
Streptococcus faecalis	12	9	10
Micrococcus luteus	18	12	16
Bacillus subtilis	15	9	12
Alcaligenes faecalis	12	—	10
Candida albicans	18	11	16
Penicillium italicum	16	14	15
Aspergillus niger	17	13	14
Saccharomyces cerevisiae	10	—	10
Fusarium nivale	15	—	20

Naphthomycin (1 mg/ml).

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