Communications to the Editor

NEW ANSAMYCIN ANTIBIOTICS, NAPHTHOQUINOMYCINS A AND B, INHIBITORS OF FATTY ACID SYNTHESIS IN *ESCHERICHIA COLI*

Sir:

Several inhibitors of fatty acid synthesis have recently been noted as a new group of antibiotics which show selective toxicity against procaryotes. Thus, thiolactomycin[1~3] selectively inhibits the fatty acid synthetase of *Escherichia coli* (type II) but has little effect on that of mammalian tissues (type I)[4~6]. On the other hand, cerulenin inhibits both type I and type II synthetases[4,7].

In order to find new inhibitors of the fatty acid synthetase of *E. coli* (type II), we employed a fatty acid synthetase assay system comprising fraction A and the acyl carrier protein (ACP), which was prepared by essentially the same method as described by MAJERUS *et al.*[8] from *E. coli* K12 except that the ACP preparation was used without further purification by DEAE-cellulose and DEAE-Sephadex chromatography. The activity of the fatty acid synthetase was determined by the radioactive assay method described by KAWAGUCHI *et al.*[9], which measured the incorporation of [2-^{14}C]malonyl-Co A into the fatty acid fraction in the presence of acetyl-Co A and NADPH.

Three active compounds were isolated from the culture filtrate of *Streptomyces* strain No. S-1998. These antibiotics belonging to the ansamycin group were named naphthoquinomycins A, B and C.

The strain No. S-1998 was cultivated on a rotary shaker at 27°C for 5 days in 5-liter Erlenmeyer flasks containing 1 liter of a medium consisting of glycerol 3.0%, corn steep liquor 1.0%, dry yeast 0.3%, NaCl 0.5% and $CaCO_3$ 0.35%. The filtered fermentation broth (3 liters, pH 7.1) was adjusted to pH 3.0 with HCl and extracted with EtOAc. The organic layer was concentrated *in vacuo* and subjected to silica gel column chromatography. The active fraction eluted with $CHCl_3$ - MeOH (100: 2) was evaporated *in vacuo* to give a crude material, which was

Table 1. Physico-chemical properties of naphthoquinomycins A, B and C.

	Naphthoquinomycin A	Naphthoquinomycin B	Naphthoquinomycin C
Nature	Yellow powder	Brownish yellow powder	Brownish yellow powder
MP °C (dec)	173~182	171~180	162~169
$[\alpha]_D^{23}$ (*c* 0.05, $CHCl_3$)	+212°	+541°	+218°
Molecular formula	$C_{40}H_{47}NO_{10}$	$C_{40}H_{47}NO_9S$	$C_{39}H_{44}ClNO_9$
FD-MS (*m/z*) $(M+H)^+$	702	718	706
$(M+Na)^+$	724	740	728
High resolution FAB-MS (*m/z*)			
$(M+H)^+$ Found	—	718.3047	706.2767
Calcd	—	718.3050	706.2783
UV λ_{max} nm (log ε)			
MeOH	233 (4.31), 302 (4.20)	232 (4.31), 306 (4.19), 580 (2.74)	232 (4.34), 305 (4.25), 380 (sh, 3.55), 580 (2.98)
0.01 N NaOH - MeOH	233 (4.32), 303 (4.23), 400 (sh, 3.54)	232 (4.30), 308 (4.19), 410 (sh, 3.46), 580 (2.90)	233 (4.34), 303 (4.25), 400 (sh, 3.57), 575 (2.85)
0.01 N HCl - MeOH	232 (4.30), 276 (4.18), 298 (4.17)	233 (4.28), 295 (4.15)	227 (4.35), 283 (4.21), 300 (4.23), 365 (sh, 3.59)
IR ($CHCl_3$) cm^{-1}	3510, 3370, 2930, 1630, 1610 (sh), 1480, 1340, 1320	3500, 3360, 2970, 1650, 1620 (sh), 1470, 1335, 1300	3510, 3360, 2980, 1660, 1620, 1475, 1315, 1305

Table 2. ^{1}H NMR of naphthoquinomycins (400 MHz, $CDCl_3$).

Assignment*	Naphthoquinomycin A		Naphthoquinomycin B		Naphthoquinomycin C		Naphthomycin H[13]	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
$CH_3C(20)$	0.84 d	6.0	0.81 d	6.5	0.82 d	6.5	0.82 d	6.5
$CH_3C(18)$	0.96 d	6.0	0.97 d	6.5	0.96 d	6.5	0.96 d	6.5
$CH_3C(8)$	1.22 d	6.0	1.21 d	6.5	1.21 d	6.0	1.20 d	7
$CH_3C(12)$	1.73 s		1.71 s		1.72 s		1.71 s	
$CH_3C(22)$	2.04 d	1.0	2.03 d	1.0	2.03 s		2.03 d	1.5
HC(18)	2.21 m		2.19 m		2.20 m		2.20 m	
$H_2C(14)$	2.30 m		2.29 dd	6.0/6.0	2.31 m		2.30 m	
HC(8)	2.30 m		2.34 m		2.31 m		2.30 m	
$CH_3C(26)$	2.38 s		2.38 s		2.39 s		2.39 s	
$CH_3SC(30)$	—		2.45 s		—		—	
$H_aC(10)$	2.69 dd	16.0/6.0	2.70 dd	16.5/5.5	2.62 dd	16.0/5.5	2.62 dd	16.5/6.5
OH	2.60 s		2.51 s		2.65 s		2.65 s	
HC(20)	2.69 m		2.70 m		2.70 m		2.7 m	
$H_bC(10)$	3.09 dd	16.0/4.0	3.07 dd	16.5/3.5	3.15 d	16.0	3.15 dd	17/3.5
HC(19)	3.18 dd	10.0/2.0	3.17 ddd	10.0/2.0/2.0	3.12 d	9.5	3.11 dd	10/2.5
HC(9)	3.58 br		3.59 m		3.59 br		3.57 ddd	9.5/6.5/3
OH	3.67 d	3.5	3.42 d	4.0	3.65 s		3.63 s	
OH	3.72 br		3.67 d	6.0	3.66 s		3.65 s	
HC(15)	4.04 m		4.06 m		4.05 m		4.04 dt	7.5/5
$CH_3OC(30)$	4.08 s		—		—		—	
HC(17)	5.44 dd	15.0/9.5	5.54 dd	15.0/8.0	5.47 dd	15.0/9.5	5.47 dd	15/9.5
HC(7)	5.56 dd	15.0/9.5	5.58 dd	15.0/9.0	5.57 dd	14.5/10.0	5.56 dd	15/10.5
HC(16)	5.63 dd	15.0/7.5	5.59 dd	15.0/5.0	5.63 dd	15.0/7.5	5.63 dd	15/7.5
HC(21)	5.88 dd	10.0/1.0	5.92 dd	10.0/1.0	5.94 d	10.0	5.93 dd	10/1.5
HC(2)	6.01 d	11.0	6.01 d	11.0	6.03 d	11.0	6.02 d	11.5
HC(5)	6.27 dd	11.0/11.0	6.29 dd	11.0/11.0	6.29 dd	11.0/11.0	6.28 dd	11/11
HC(4)	6.38 dd	11.0/11.0	6.48 dd	11.0/11.0	6.36 dd	11.0/11.0	6.36 dd	11/11
HC(6)	6.49 dd	15.0/11.0	6.49 dd	15.0/11.0	6.50 dd	14.5/11.0	6.5 dd	15/10.5
HC(13)	6.72 td	6.0/1.0	6.72 td	6.0/1.0	6.72 t	6.0	6.72 dt	6/1
HC(3)	6.93 dd	11.0/11.0	6.96 dd	11.0/11.0	6.98 dd	11.0/11.0	6.98 dd	11/11
HC(27)	7.91 d	1.0	7.94 d	0.5	7.97 s		7.98 d	1.5
NH	7.64 s		8.24 s		8.12 s		8.0 s	
OH	9.27 br		9.39 br				9.78 s	

* The assignments were performed by 2-D COSY, relayed COSY, decoupling experiments, C-H correlation and INEPT analysis.

Table 3. ^{13}C NMR of naphthoquinomycins (400 MHz, $CDCl_3$).

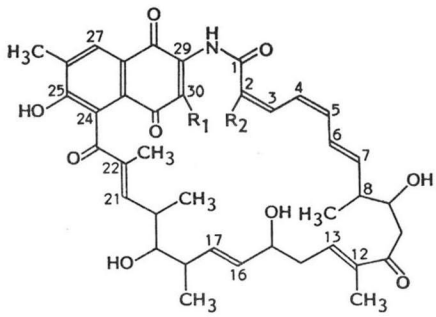
Assignment*			Naphthoquinomycin A δ (ppm)	Naphthoquinomycin B δ (ppm)	Naphthoquinomycin C δ (ppm)	Naphthomycin H[13] δ (ppm)
C=O region: Ketone			203.2	203.1	203.0	203.5
			201.3	201.5	201.2	201.6
Quinone			180.9	181.1	178.3	178.6
			180.4	178.0	177.7	178.1
Amide			165.3	164.4	164.6	165.0
sp^2 region: Singlets			159.8	160.1	161.1	161.2
			149.0	137.8	137.8	138.0
			137.8	137.4	137.5	137.6
			137.6	137.3	136.4	136.7
			133.8	136.5	134.4	134.5
			132.5	135.4	133.2	133.3
			123.5	132.6	132.4	132.5
			121.6	121.7	121.3	121.4
			119.8	120.0	119.8	119.9
Doublets		21	147.0	146.7	146.9	147.2
		13	142.3	142.4	142.3	142.5
		7	141.2	141.4	141.5	141.6
		16	137.0	136.2	136.6	136.8
		3	135.5	136.0	135.7	135.9
		5	135.1	135.4	135.4	135.5
		17	133.2	133.7	133.6	133.8
		27	130.4	130.6	131.1	131.2
		6	126.4	126.3	126.3	126.5
		4	123.5	123.6	123.2	123.4
		2	120.9	120.6	120.8	121.0
sp^3 region: CHO		19	75.9	76.4	76.4	76.3
		9	73.6	73.6	73.4	73.3
		15	71.6	71.6	71.9	71.8
CH		8	45.2	45.3	45.4	45.2
		18	41.8	42.0	41.9	41.7
		20	33.9	34.0	34.0	33.7
CH_2		10	40.6	40.6	40.8	40.6
		14	36.8	36.9	36.6	36.4
CH_3	$CH_3OC(30)$		59.7	—	—	—
	$CH_3SC(30)$		—	18.6	—	—
	$CH_3C(8)$		17.7	17.8	17.7	17.4
	$CH_3C(26)$		16.7	16.7	16.7	16.4
	$CH_3C(18)$		16.4	16.7	16.5	16.2
	$CH_3C(22)$		12.6	12.7	12.8	12.4
	$CH_3C(12)$		11.5	11.5	11.5	11.2
	$CH_3C(20)$		11.5	11.2	11.1	10.8

* The assignments were performed by C-H correlation and INEPT analysis.

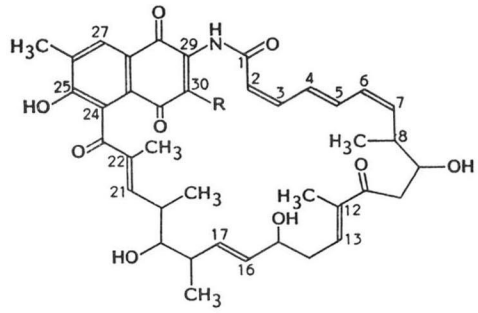
further purified by Sephadex LH-20 column chromatography developed with MeOH. The active eluate was evaporated to dryness to give a mixture (80 mg) of naphthoquinomycins A, B and C. These antibiotics were separated by HPLC monitored at 254 nm using a μBondapak Porasil B column (Waters Associates) and a mobile phase of MeOH - H_2O (60: 40). There were obtained 3 mg of naphthoquinomycin A, 4 mg of naphthoquinomycin B and 18 mg of naphthoquinomycin C.

The UV and IR spectral data (Table 1) suggest that these antibiotics contain a naphthoquinone moiety, and the ^1H and ^{13}C NMR spectral data indicate that their structures are very similar to those of naphthomycins A[10,11], B[12], C[12] and

Fig. 1. Structures of naphthoquinomycins and naphthomycins.



Naphthomycin A $R_1=Cl$, $R_2=CH_3$
Naphthoquinomycin A $R_1=OCH_3$, $R_2=H$
Naphthoquinomycin B $R_1=SCH_3$, $R_2=H$
Naphthoquinomycin C $R_1=Cl$, $R_2=H$
(Naphthomycin H)



Naphthomycin B R=Cl
Naphthomycin C R=H

Table 4. Biological activities of naphthoquinomycins and naphthomycins.

	Inhibition of fatty acid synthesis (%)			Inhibition zone diameter (mm)*
	100 μg/ml	200 μg/ml	300 μg/ml	
Naphthoquinomycin A	33	60	74	16
Naphthoquinomycin B	42	66	80	17
Naphthoquinomycin C	36	61	75	28
Naphthomycin A	43	70	83	—
Naphthomycin B	31	57	71	—

* Antibacterial activity against *Bacillus subtilis* IAM 1026 (each 50 μg/paper disc).

H[13] (Fig. 1).

Comparison of the physico-chemical properties and the ^1H and ^{13}C NMR spectral data (Tables 2 and 3) between naphthoquinomycin C and the above known antibiotics leads to the conclusion that naphthoquinomycin C is identical with naphthomycin H[13].

The molecular formula of naphthoquinomycin B is established to be $C_{40}H_{47}NO_9S$ based on high resolution FAB mass spectral data, whereas that of naphthomycin H is $C_{39}H_{44}ClNO_9$[13]. The ^1H NMR spectrum (Table 2) of naphthoquinomycin B shows the presence of a new *S*-methyl signal at 2.45 ppm suggesting that the chlorine atom in naphthomycin H is replaced by an *S*-methyl group in naphthoquinomycin B. This conclusion is confirmed by comparison of the ^{13}C NMR spectra (Table 3); naphthoquinomycin B shows 7 methyl carbon signals, one of them at 18.6 ppm corresponding to an *S*-methyl carbon is missing in the spectrum of naphthomycin H.

In the ^1H NMR spectrum of naphthoquinomycin B, the coupling constants of the triene system ($J_{2,3}=11.0$, $J_{4,5}=11.0$, $J_{6,7}=15.0$ Hz) prove that C(2)=C(3) and C(4)=C(5) have *Z*- and C(6)=C(7) have *E*-configurations. The *E*-configuration is suggested for C(16)=C(17) ($J_{16,17}=15.0$ Hz). Since the chemical shifts and the coupling constants of the olefinic protons at C(13) and C(21) are nearly identical with those of naphthomycins A and H, and since the chemical shifts of the C(12) and C(22) methyl carbons are shifted upfield (11.5 and 12.7 ppm, respectively) by γ-effect, the configuration of both the double bonds, C(12)=C(13) and C(21)=C(22), are proposed to be *E* as shown in the structure (Fig. 1).

The ^1H and ^{13}C NMR spectral data of naphthoquinomycin A show the presence of a new methoxy signal at 4.08 ppm and 59.7 ppm, respectively, instead of the *S*-methyl signal of naphthoquinomycin B. These data and FD mass spectral data (*m/z* $(M+H)^+$ 702) of naphthoquinomycin A give the molecular formula as $C_{40}H_{47}NO_{10}$ indicating the replacement of the *S*-methyl group in naphthoquinomycin

B by a methoxy group in naphthoquinomycin A. The geometries of C(2)=C(3), C(4)=C(5), C(6)=C(7), C(12)=C(13), C(16)=C(17) and C(21)=C(22) in naphthoquinomycin A are *Z*, *Z*, *E*, *E*, *E* and *E*, respectively ($J_{2,3}$=11.0, $J_{4,5}$=11.0, $J_{6,7}$=15.0, $J_{16,17}$=15.0 Hz).

The inhibitory activity on fatty acid synthesis in *E. coli* and the antimicrobial activity of these naphthoquinomycins and naphthomycins are shown in Table 4.

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References

1) OISHI, H.; T. NOTO, H. SASAKI, K. SUZUKI, T. HAYASHI, H. OKAZAKI, K. ANDO & M. SAWADA: Thiolactomycin, a new antibiotic. I. Taxonomy of the producing organism, fermentation and biological properties. J. Antibiotics 35: 391~395, 1982
2) SASAKI, H.; H. OISHI, T. HAYASHI, I. MATSUURA, K. ANDO & M. SAWADA: Thiolactomycin, a new antibiotic. II. Structure elucidation. J. Antibiotics 35: 396~400, 1982
3) NOTO, T.; S. MIYAKAWA, H. OISHI, H. ENDO & H. OKAZAKI: Thiolactomycin, a new antibiotic. III. *In vitro* antibacterial activity. J. Antibiotics 35: 401~410, 1982
4) HAYASHI, T.; O. YAMAMOTO, H. SASAKI, A. KAWAGUCHI & H. OKAZAKI: Mechanism of action of the antibiotic thiolactomycin. Inhibition of fatty acid synthesis of *Escherichia coli*. Biochem. Biophys. Res. Commun. 115: 1108~1113, 1983
5) HAYASHI, T.; O. YAMAMOTO, H. SASAKI & H. OKAZAKI: Inhibition of fatty acid synthesis by the antibiotic thiolactomycin. J. Antibiotics 37: 1456~1461, 1984
6) NISHIDA, I.; A. KAWAGUCHI & M. YAMADA: Selective inhibition of type II fatty acid synthetase by the antibiotic thiolactomycin. Plant Cell Physiol. 25: 265~268, 1984
7) ŌMURA, S.: Cerulenin. *In* Methods in Enzymology. Vol. 72, *Ed.*, LOWENSTEIN, J. M., pp. 520~532, Academic Press Inc., New York, 1981
8) MAJERUS, P. W.; A. W. ALBERTS & P. R. VAGELOS: Acyl carrier protein from *Escherichia coli*. *In* Methods in Enzymology. Vol. 14, *Ed.*, LOWENSTEIN, J. M., pp. 43~50, Academic Press Inc., New York, 1969
9) KAWAGUCHI, A.; Y. SEYAMA, T. YAMAKAWA & S. OKUDA: Fatty acid synthase from *Brevibacterium ammoniagenes*. *In* Methods in Enzymology. Vol. 71, *Ed.*, LOWENSTEIN, J. M., pp. 120~127, Academic Press Inc., New York, 1981
10) WILLIAMS, T. H.: Naphthomycin, a novel ansa macrocyclic antimetabolite. Proton NMR spectra and structure elucidation using lanthanide shift reagent. J. Antibiotics 28: 85~86, 1975
11) KELLER-SCHIERLEIN, W.; M. MEYER, L. CELLAI, S. CERRINI, D. LAMBA, A. SEGRE, W. FEDELI & M. BRUFANI: Metabolites of microorganisms. 229. Absolute configuration of naphthomycin A determined by X-ray analysis and chemical degradation. J. Antibiotics 37: 1357~1361, 1984
12) KELLER-SCHIERLEIN, W.; M. MEYER, A. ZEECK, M. DAMBERG, R. MACHINEK, H. ZÄHNER & G. LAZAR: Isolation and structural elucidation of naphthomycins B and C. J. Antibiotics 36: 484~492, 1983
13) MUKHOPADHYAY, T.; C.M.M. FRANCO, G.C.S. REDDY, B. N. GANGULI & H. W. FEHLHABER: A new ansamycin antibiotic, naphthomycin H from a *Streptomyces* species Y-83,40369. J. Antibiotics 38: 948~951, 1985