Communications to the Editor

NEW ANTITUMOR ANTIBIOTICS, ANGUINOMYCINS A AND B

Sir:

During the course of our screening program for new antitumor antibiotics, an actinomycete was found that produced two previously unreported antibiotics, which were named anguinomycins A and B. Both of these compounds were highly cytotoxic to murine P388 leukemia cells (IC_{50}: 0.1~0.2 ng/ml) and displayed potent antitumor activity in mice.

The producing organism, *Streptomyces* sp. R2827, was cultivated at 27°C for 5 days in a 50-liter jar fermentor containing 25 liters of a medium consisting of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and calcium carbonate 0.4% (pH 7.0).

The mycelial cake obtained from the cultured broth (50 liters) was extracted with acetone. After being concentrated *in vacuo*, the extract was partitioned between butyl acetate and water at pH 2.0. The organic layer was evaporated and then subjected to silica gel column chromatography. The active fraction eluted with $CHCl_3$ - MeOH (20:1) was chromatographed again on a silica gel column with hexane - EtOAc (1:1) and the active eluate was applied to a Sephadex LH-20 column. Development of the column with MeOH gave a mixed fraction of anguinomycins A and B. Isolation of these compounds was carried out by semi-preparative HPLC over C-18 silica gel. Two antibiotic fractions eluted with MeOH - 0.1 M $AcONH_4$ (3:1) were separately collected and evaporated *in vacuo*, followed by lyophilization to give 10 mg of anguinomycin A and 40 mg of anguinomycin B in pure form.

The physico-chemical properties of anguinomycins A and B are as follows:

Anguinomycin A: Colorless viscous oil; $[\alpha]_D^{27}$ −139° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 233 (784), 297 (32); IR ν_{max}^{KBr} cm^{-1} 3450 (OH), 1700 (C=O); field desorption mass spectrometry (FD-MS) *m/z* 513 $(M+H)^+$.

Anguinomycin B: Colorless viscous oil; $[\alpha]_D^{27}$ −130° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 233 (720), 296 (30); IR λ_{max}^{KBr} cm^{-1} 3450 (OH), 1700 (C=O); FD-MS *m/z* 527 $(M+H)^+$.

The above mass spectral data and the ^{13}C and ^1H NMR data (Tables 1 and 2) for anguinomycins A and B agree with the molecular formulae $C_{31}H_{44}O_6$ and $C_{32}H_{46}O_6$, respectively.

Table 1. ^{13}C NMR data for anguinomycins A and B and leptomycin B.

Position	Anguinomycin A	Anguinomycin B	Leptomycin B[2)]
1	170.8 s	171.2 s	171.3 s
2	117.3 d	117.4 d	117.1 d
3	160.0 s	160.0 s	160.9 s
4	45.5 t	45.5 t	45.7 d
5	33.5 d	33.5 d	33.6 d
6	74.2 d	74.1 d	74.2 d
7	46.7 d	46.8 d	47.0 d
8	215.2 s	215.1 s	214.9 s
9	45.7 d	45.7 d	45.7 d
10	128.2 d	128.1 d	128.0 d[a]
11	136.4 s	136.4 s	136.5 s[b]
12	135.3 d	135.2 d	135.3 d
13	127.9 d	127.9 d	128.2 d[a]
14	40.7 t	40.8 t	40.9 t
15	32.3 d	32.2 d	32.2 d
16	139.0 d	137.1 d	136.9 d
17	129.5 s	135.4 s	135.6 s[b]
18	130.9 d	130.1 d	130.2 d
19	125.4 d	124.7 d	122.8 d
20	78.7 d	78.9 d	81.5 d
21	30.1 t	30.0 t	33.6 d
22	144.8 d	144.8 d	151.6 d
23	121.6 d	121.5 d	120.0 d
24	164.2 s	164.2 s	164.4 s
3-CH_3	18.5 q	18.5 q	16.0 q
5-CH_3	13.7 q	13.6 q	13.6 q[c]
7-CH_3	12.6 q	12.6 q	20.9 q
9-CH_3	16.1 q	16.1 q	13.0 q[c]
11-CH_3	13.1 q	13.0 q	18.5 q
15-CH_3	20.8 q	20.8 q	13.0 q[c]
17-CH_3	20.4 q		
17-CH_2CH_3		26.4 t, 13.4 q	26.6 t, 13.5 q[c]
21-CH_3			12.3 q[c]

Chemical shifts in ppm are given in $CDCl_3$ using TMS as an internal standard.

Assignments for anguinomycins A and B are based on chemical shift data and 2D C-H correlation spectral analysis.

[a~c] Assignment of these signals may be interchanged.[2)]

Table 2. ^{1}H NMR data for anguinomycins A and B and leptomycin B.

Position	Anguinomycin A	Anguinomycin B	Leptomycin B[2)]
1	5.68 s	5.66 s	5.68 s
4a	2.20 dd (J=13.0, 5.9)	2.19 dd (J=13.0, 7.6)	2.21 dd
4b	1.90 dd (J=13.0, 8.7)	1.89 dd (J=13.0, 8.7)	1.90 dd
5	1.73 m	1.72 m	1.75 m
6	3.58 dd (J=6.0, 4.9)	3.57 dd (J=6.4, 4.9)	3.58 t
7	2.83 dq (J=6.0, 7.1)	2.81 dq (J=6.4, 7.0)	2.83 m
9	3.65 dq (J=10.3, 6.7)	3.64 dq (J=10.1, 6.6)	3.67 m
10	5.09 d (J=10.3)	5.07 d (J=10.1)	5.08 d
12	6.01 d (J=15.4)	5.99 d (J=15.6)	6.00 d
13	5.59 dt (J=15.4, 7.3)	5.57 dt (J=15.6, 7.2)	5.59 m
14	2.08 m 2H	2.07 m 2H	2.09 t 2H
15	2.67 m	2.64 m	2.67 d
16	5.26 d (J=9.6)	5.22 d (J=9.8)	5.23 d
18	6.72 d (J=15.7)	6.60 d (J=15.7)	6.65 d
19	5.71 dd (J=15.7, 6.9)	5.74 dd (J=15.7, 7.1)	5.72 dd
20	4.98 dt (J=6.9, 7.6)	4.95 dt (J=7.1, 7.5)	5.00 dd
21	2.47 m 2H	2.45 m 2H	2.53 m
22	6.90 dt (J=9.8, 4.2)	6.89 dt (J=9.8, 4.2)	6.95 d
23	6.06 dt (J=9.8, 1.7)	6.04 dt (J=9.8, 1.7)	6.00 d
3-CH_3	2.10 s 3H	2.09 d 3H (J=0.9)	2.13 s 3H
5-CH_3	0.79 d 3H (J=6.8)	0.77 d 3H (J=6.7)	0.79 d 3H
7-CH_3	1.15 d 3H (J=7.1)	1.13 d 3H (J=7.0)	1.15 d 3H
9-CH_3	1.13 d 3H (J=6.7)	1.11 d 3H (J=6.6)	1.14 d 3H
11-CH_3	1.82 d 3H (J=1.0)	1.80 d 3H (J=0.9)	1.82 d 3H
15-CH_3	0.96 d 3H (J=6.8)	0.95 d 3H (J=6.7)	0.97 d 3H
17-CH_3	1.81 d 3H (J=0.9)		
17-CH_2CH_3		2.17 m 2H	2.20 q 2H
17-CH_2CH_3		1.02 t 3H (J=7.5)	1.05 t 3H
21-CH_3			1.07 d 3H

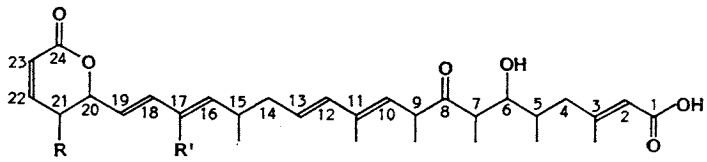
Chemical shifts in ppm are given in $CDCl_3$ using TMS as an internal standard.
Coupling constants in Hz are given in parentheses.

Comparisons of the ^{13}C and ^{1}H NMR spectral data for anguinomycin B with those previously reported for leptomycin B (Tables 1 and 2)[1,2] show that these compounds are very similar. In the ^{13}C NMR spectrum of anguinomycin B, a new methylene signal was observed at δ 30.0 replacing the methine (δ 33.6, C-21) and methyl (δ 12.3) signals in the spectrum of leptomycin B. In addition, the upfield shifts observed on C-20 (δ 81.5→78.9) and C-22 (δ 151.6→144.8) and the close similarity of the remaining signals indicate that anguinomycin B is the 21-demethyl derivative of leptomycin B. The ^{1}H NMR spectrum of anguinomycin B supports this formulation, exhibiting signals at δ 6.89 (dt, J=9.8 and 4.2 Hz, 22-H), 6.04 (dt, J=9.8 and 1.7 Hz, 23-H), 4.95 (dt, J=7.1 and 7.5 Hz, 20-H) and 2.45 (2H, m, 21-H) corresponding to a 6-substituted 5,6-dihydro-2-pyrone moiety.

The structure of anguinomycin A was determined by comparison of its spectral data with those of anguinomycin B. The absence of signals in the ^{13}C and ^{1}H NMR spectra of anguinomycin A for the C-17 ethyl group of anguinomycin B and the presence of a new signal (δ_C 20.4, δ_H 1.81) for a vinylic methyl group indicate that anguinomycin A is the C-17 methyl analog of anguinomycin B. Similarly, HAMAMOTO *et al.* reported that in leptomycin A[1,2] the C-17 ethyl group in leptomycin B is replaced by a methyl group.

The structures of anguinomycins A and B were thus established as shown in Fig. 1. Previously, four antibiotics belonging to this family were reported, namely leptomycin A, leptomycin B[1,2] (elactocin),[3,4] kazusamycin[5~7] (hydroxyelactocin)[3,4] and PD 124,895.[8] Since each of these compounds possesses a C-21 methyl group, anguinomycins A and B are new members of this family.

Fig. 1. Structures of anguinomycins A and B and leptomycins A and B.



Anguinomycin A	R=H,	R′=CH_3
Anguinomycin B	R=H,	R′=CH_2CH_3
Leptomycin A	R=R′=CH_3	
Leptomycin B	R=CH_3,	R′=CH_2CH_3

Table 3. Effects of anguinomycins A and B on Lewis lung carcinoma in mice.

Sample	Dose (μg/kg/day)	T/C (%)	Cured mice
Anguinomycin A	62.5	145	2/6
	31.3	159	0/6
	15.6	148	0/6
	7.8	126	0/6
Anguinomycin B	62.5	50	3/6
	31.3	110	5/6
	15.6	201	3/6
	7.8	165	1/6

Treatment schedule: Tumor cells (1×10^6) were inoculated intraperitoneally on day 0. Mice were given intraperitoneal injections of samples on days 1~5.

T/C: The ratio of mean survival days of the treated group divided by that of the control group. Cured mice were excluded from the calculation of T/C.

Table 4. Effects of anguinomycins A and B on P388 leukemia in mice.

Sample	Dose (μg/kg/day)	T/C (%)	Cured mice
Anguinomycin A	100	113	0/6
	50	147	0/6
	25	137	0/6
	12.5	116	0/6
Anguinomycin B	50	140	0/6
	25	137	0/6
	12.5	121	0/6
	6.25	108	0/6

Treatment schedule: Tumor cells (1×10^6) were inoculated intraperitoneally on day 0. Mice were given intraperitoneal injections of samples on days 1~9.

T/C: The ratio of mean survival days of the treated group divided by that of the control group.

Anguinomycins A and B showed antitumor activities against murine Lewis lung carcinoma and P388 leukemia as summarized in Tables 3 and 4, respectively. When anguinomycin B was administered intraperitoneally on days 1~5 at a dose of 31.3 μg/kg/day into mice bearing Lewis lung carcinoma, 5 out of 6 mice were cured. Anguinomycin B appears to be a potent antitumor agent against murine solid tumor.

YOICHI HAYAKAWA
KAZUYOSHI ADACHI
NOBUYASU KOMESHIMA

Pharmaceutical Laboratory,
Kirin Brewery Co. Ltd.,
Miyahara, Takasaki 370-12, Japan

(Received March 27, 1987)

References

1) HAMAMOTO, T.; S. GUNJI, H. TSUJI & T. BEPPU: Leptomycins A and B, new antifungal antibiotics. I. Taxonomy of the producing strain and their fermentation, purification and characterization. J. Antibiotics 36: 639~645, 1983
2) HAMAMOTO, T.; H. SETO & T. BEPPU: Leptomycins A and B, new antifungal antibiotics. II. Structure elucidation. J. Antibiotics 36: 646~650, 1983
3) SCHAUMBERG, J. P.; G. C. HOKANSON & J. C. FRENCH: The structures of the antitumor antibiotics, PD 114720 and PD 114721. J. Chem. Soc. Chem. Commun. 1984: 1450~1452, 1984
4) TUNAC, J. B.; B. D. GRAHAM, W. E. DOBSON & M. D. LENZINI: Novel antitumor antibiotics, CI-940 (PD 114,720) and PD 114,721. Taxonomy, fermentation and biological activity. J. Antibiotics 38: 460~465, 1985
5) UMEZAWA, I.; K. KOMIYAMA, H. OKA, K. OKADA, S. TOMISAKA, T. MIYANO & S. TAKANO: A new antitumor antibiotic, kazusamycin. J. Antibiotics 37: 706~711, 1984

6) KOMIYAMA, K.; K. OKADA, H. OKA, S. TOMISAKA, T. MIYANO, S. FUNAYAMA & I. UMEZAWA: Structural study of a new antitumor antibiotic, kazusamycin. J. Antibiotics 38: 220~223, 1985
7) KOMIYAMA, K.; K. OKADA, Y. HIROKAWA, K. MASUDA, S. TOMISAKA & I. UMEZAWA: Antitumor activity of a new antibiotic, kazusamycin. J. Antibiotics 38: 224~229, 1985
8) HURLEY, T. R.; R. H. BUNGE, N. E. WILLMER, G. C. HOKANSON & J. C. FRENCH: PD 124,895 and PD 124,966, two new antitumor antibiotics. J. Antibiotics 39: 1651~1656, 1986