Communications to the Editor

ISOLATION AND CHARACTERIZATION OF ATRAMYCIN A AND ATRAMYCIN B, NEW ISOTETRACENONE TYPE ANTITUMOR ANTIBIOTICS

Sir:

During the course of our screening program for new antitumor antibiotics, an actinomycete identified as *Streptomyces atratus* BY90, isolated from Tomakomai greenfield, Hokkaido, Japan, was found to produce new isotetracenone type antibiotics active against mouse leukemia P388. In this communication the isolation, characterization and structural elucidation of the new compounds named atramycin A and atramycin B are reported.

The producing organisms, *S. atratus* BY90 was cultivated at 27°C in a 60-liter jar fermenter with agitation rate of 300 rpm and air flow of 15 liters/minute. The medium used consisted of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and $CaCO_3$ 0.4% (pH 7.0).

After fermentation for 72 hours, the mycelial cake was collected by centrifugation from 30 liters of the fermentation broth and extracted twice with acetone (each 2.5 liters). The extract was concentrated to a small volume and the aqueous residue was extracted three times with EtOAc. The solvent layer was concentrated to dryness and the residue was subjected to stepwise silica gel column chromatography (EtOAc followed by EtOAc - MeOH, 15 : 1). The active fraction was concentrated and subjected to silica gel column chromatography ($CHCl_3$ - MeOH - NH_4OH, 200 : 10 : 1). Subsequent Toyopearl HW-40 column chromatography (MeOH) gave two active fractions. The first fraction was concentrated to give a yellow powder, which was identified as rubiginone A_2 (5-deoxy derivative of rubiginone B_2, see Fig. 3) which has been reported by OKA *et al.*[1,2]. The second active fraction was concentrated to dryness to give a powder, which was further separated to give two active bands by preparative silica gel TLC (EtOAc - MeOH, 15 : 1). These two bands corresponding to atramycins A (Rf 0.60) and B (Rf 0.54) were separately scraped off and extracted with EtOAc followed by evaporation to dryness. These two fractions were finally purified by Sephadex LH-20 column chromatographies ($CHCl_3$ - MeOH, 1 : 1) to give atramycins A (30 mg) and B (6 mg). Their physico-chemical properties are as follows.

Atramycin A: MP 146~151°C (dec); $[\alpha]_D^{25}$ +63° (*c* 0.5, $CDCl_3$); UV λ_{max} nm (ε) 225 (22,000), 262 (25,700), 399 (5,700) in MeOH, 210 (21,300), 254 (29,700), 316 (8,200), 468 (4,300) in alkaline MeOH. HRFAB-MS of atramycin A showed the molecular ion at *m/z* (M+H) 469.1501 corresponding to the molecular formula $C_{25}H_{24}O_9$ (calcd for $C_{25}H_{25}O_9$, 469.1499).

Atramycin B: MP 136~141° (dec); $[\alpha]_D^{25}$ +55° (*c* 0.5, $CHCl_3$); UV λ_{max} nm (ε) 209 (19,800), 262 (23,200), 352 (3,100) in MeOH. HRFAB-MS of atramycin B showed the molecular ion at *m/z* (M+H) 453.1644 corresponding to the molecular formula $C_{25}H_{24}O_8$ (calcd for $C_{25}H_{25}O_8$, 453.1549).

The UV spectra of atramycins A and B suggested that these two compounds belonged to the isotetracenone group[3].

The ^1H NMR spectrum of atramycin A is shown in Fig. 1.

^1H NMR analysis based on COSY spectral data revealed the presence of 1 unit of rhamnose (1'-H to 5'-CH_3 in Table 1). The rhamnose moiety was also supported by the ^{13}C NMR spectral data summarized in Table 1. In agreement with this conclusion, acid hydrolysis of atramycin A (70 mg, 2N HCl, at 85°C, 5 minutes) gave L-rhamnose (19 mg) and ochromycinone[4,5] which was identified by ^1H and ^{13}C NMR spectral analysis.

In the ^1H NMR spectrum of atramycin A, three contiguous aromatic protons appeared as an AMX type signal (7.662 (dd, J=1.0 and 8.3 Hz, 9-H), 7.696 (dd, J=1.0 and 7.6 Hz, 11-H), 7.798 ppm (dd, J=7.6 and 8.3 Hz, 10-H)). Another aromatic methine (6.980 ppm, 5-H) was observed as a broad singlet.

In addition to these signals, ^1H-^1H COSY and ^1H-^{13}C COSY experiments revealed $-CH_2-CH(CH_3)-CH_2-$ as a partial structure of atramycin A.

These structures were further extended to give partial structures as shown in Fig. 2 by heteronuclear multiple-bond correlation[6] (HMBC). Thus, ^{13}C-^1H-long range couplings from 9-H to C-7a, from 10-H to C-8 and C-11a, and from 11-H to C-7a and C-12 revealed the connectivity including the AMX type signal mentioned above and a carbonyl carbon as indicated in Fig. 2 (A). In the same way,

Fig. 1. 500 MHz ^{1}H NMR spectrum of atramycin A in CD_3OD.

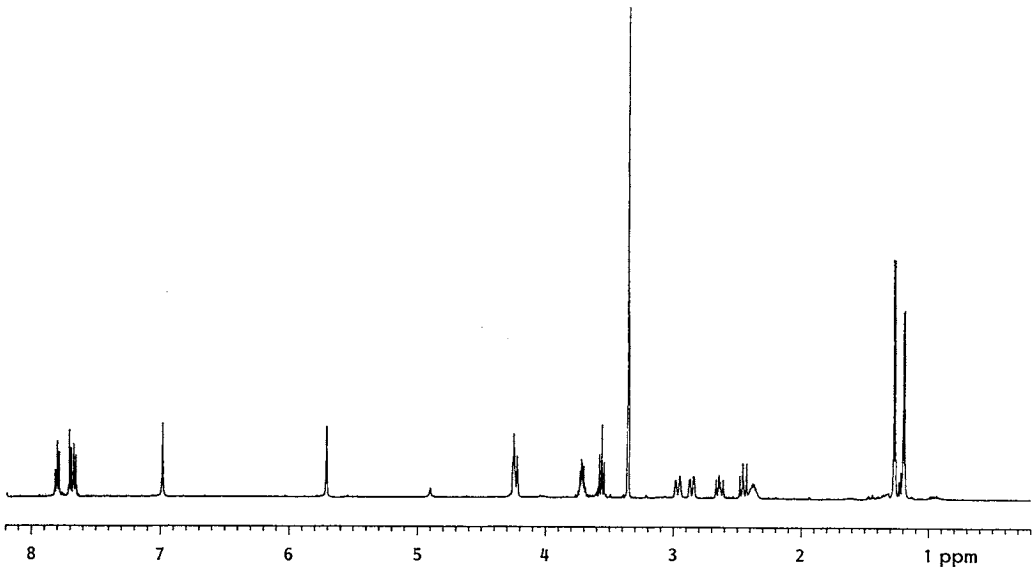


Table 1. ^{1}H and ^{13}C NMR spectral data of atramycins A and B (500 and 125 MHz, respectively, in CD_3OD).

	Atramycin A			Atramycin B		
	δ_H		δ_C	δ_H		δ_C
1			200.2			201.1
2	2.451	(dd, 10.2, 15.5),	48.8	2.480	(dd, 10.5, 15.8),	48.5
	2.854	(ddd, 1.0, 4.0, 15.5)		2.863	(ddd, 1.0, 5.1, 15.5)	
3	2.369	(m)	31.6	2.359	(m)	32.2
3-CH_3	1.193	(d, 6.3)	21.5	1.178	(d, 5.7)	21.5
4	2.637	(dd, 11.0, 16.2),	39.3	2.607,	(dd, 10.5, 16.5),	39.0
	2.964	(dd, 4.0, 16.2)		2.953	(dd, 4.0, 16.5)	
4a			154.0			151.4
5	6.980	(br s)	122.1	7.464	(d, 8.5)	134.6
6			165.3	8.015	(d, 8.5)	130.4
6a			119.2			136.2
7			188.7			182.0
7a			122.1			122.9
8			157.9			157.5
9	7.662	(dd, 1.0, 8.3)	123.1	7.577	(dd, 1.0, 8.5)	123.3
10	7.798	(dd, 7.6, 8.3)	136.9	7.714	(dd, 8.4, 8.5)	136.2
11	7.696	(dd, 1.0, 7.6)	121.6	7.637	(dd, 1.0, 8.4)	121.7
11a			138.7			138.8
12			185.9			185.6
12a			138.9			136.2[a]
12b			128.2			136.0[a]
1′	5.704	(d, 1.5)	100.3	5.628	(d, 1.5)	100.5
2′	4.244	(dd, 1.5, 3.8)	71.9[a]	4.276	(dd, 1.5, 3.8)	71.9[b]
3′	4.223	(dd, 3.8, 9.5)	72.0[a]	4.233	(dd, 3.8, 9.5)	72.1[b]
4′	3.556	(dd, 9.5, 9.5)	73.7	3.564	(dd, 9.5, 9.5)	73.8
5′	3.712	(m)	71.5	3.713	(m)	71.4
5′-CH_3	1.268	(d, 6.0)	18.0	1.265	(d, 6.2)	18.0

δ ppm (J = Hz).

[a,b] Signals may be exchangeable in each column.

Fig. 2. Partial structures of atramycin A as revealed by NOEs and 2D NMR analyses.

⟶ ^{13}C-^{1}H long range couplings. ⟿ NOEs.

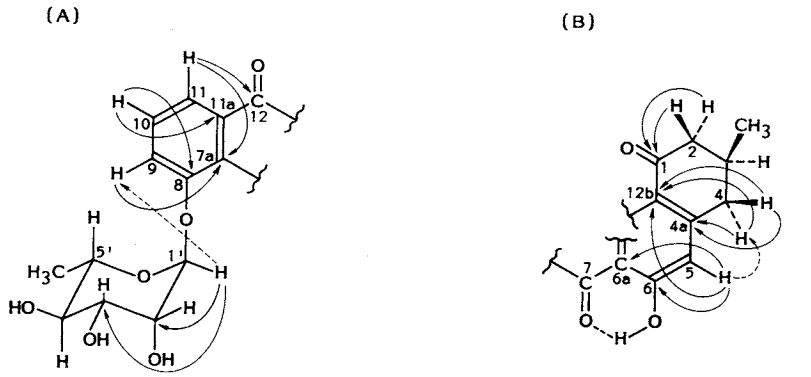
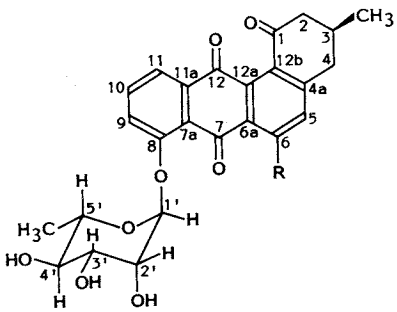
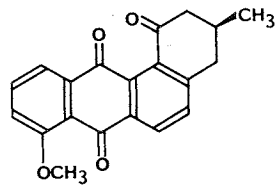


Fig. 3. Absolute structures of atramycins A, B and rubiginone B_2.



Atramycin A R=OH
Atramycin B R=H



Rubiginone B_2

^{13}C-^{1}H-long range couplings from 2-H to C-1, from 4-H to C-4a and C-12b, and from 5-H to C-6, C-6a and C-12b revealed partial structures of atramycin A including the $-CH_2-CH(CH_3)-CH_2-$ system in Fig. 2 (B). In atramycin A, the signal of C-7 (188.7 ppm) was observed at a lower field than that of C-12 (185.9 ppm) due to the hydrogen bonding to a hydroxyl group at C-6.

The presence of the isotetracenone moiety in atramycin A revealed by the UV absorption enabled to connect the two partial structures to give the aglycone part of Fig. 3.

NOEs of atramycin A were observed between 1′-H (5.704 ppm, rhamnose anomeric proton) and 9-H (7.662 ppm, dd, aromatic methine proton), and 5-H (6.980 ppm, br s, aromatic methine proton) and 4-H_{eq} (2.964 ppm). These NOEs revealed that the rhamnose residue was connected to C-8 carbon of the chromophore and that the hydroxyl group was connected to C-6 carbon.

The stereochemistry at C-1′ of the rhamnose moiety was determined to be α based on the magnitude of the ^{13}C-^{1}H coupling constant of the anomeric carbon (172 Hz)[7].

These NMR studies established the planar structure of atramycin A as shown in Fig. 3.

The ^{1}H NMR spectra of atramycin A and atramycin B are very similar each other except that the singlet aromatic proton (5-H) in atramycin A was replaced by an AB coupling system in atramycin B (7.464 (d, J=8.5 Hz, 5-H), 8.015 ppm (d, J=8.5 Hz, 6-H)). This spectral feature together with the molecular formula of atramycin B containing one less oxygen of atom than atramycin A suggested that the hydroxyl group on C-6 of atramycin A is replaced by a proton in atramycin B as shown in Fig. 3. The higher field shift of C-6 carbon in atramycin B (from 165.3 ppm in A to 130.4 ppm) well explained this structural modification.

Since the optical rotation value of ochromycinone (8-*O*-demethyl derivative of rubiginone B_2[2]), $[\alpha]_D^{25}$ +199° (*c* 1.0, $CHCl_3$)) obtained by hydrolysis of atramycin B (*vide supra*) is similar to that of rubiginone B_2 (literature[1] $[\alpha]_D^{25}$ +78° (*c* 0.5,

$CHCl_3$)), the absolute stereochemistry at C-3 of atramycins A and B can be reasonably assumed to be *S* as shown in Fig. 3.

Atramycins A and B inhibited the growth of P388 murine leukemia cells (IC_{50} 4.5 and 9.8 μg/ml, respectively). Investigations on other biological activities of them are now in progress.

Acknowledgments

This work was supported in a part by a Grant-in-Aid for Cancer Research, The Ministry of Education, Science and Culture, Japan.

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(Received March 13, 1991)

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