THE STRUCTURE OF BAUMYCINS A1, A2, B1, B2, C1 and C2

Sir:

In a previous paper, the isolation and physicochemical properties of new antitumor antibiotics, baumycins A1, A2, B1, B2, C1 and C2 were reported[1]. We now wish to report on their proposed structures. They are daunomycin analogues containing glycoside moieties.

Mild acid hydrolyses of baumycins A1 and A2 with 1% sulfuric acid (32°C, 30 minutes) gave daunomycin which was identified by direct comparison of their IR, UV, NMR, CD and TLC, and by mixed melting point with an authentic sample. This indicates that baumycins A1 and A2 consist of daunomycin and an unidentified moiety.

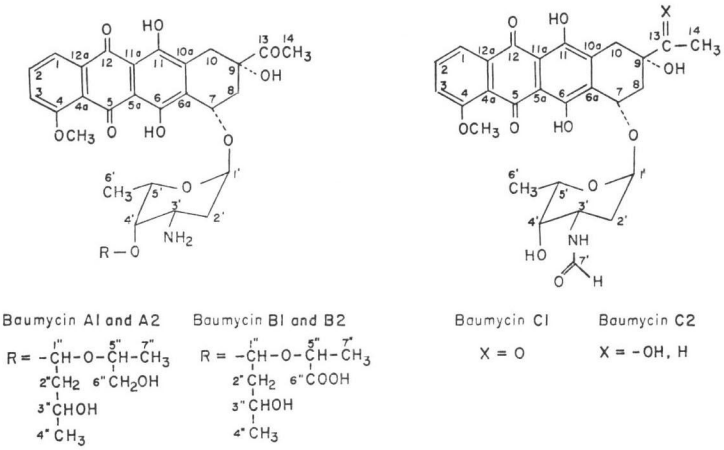
The ^{13}C-NMR spectra (CMR, in $CDCl_3$) of baumycins A1 and A2 indicated the presence of 34 carbons in both compounds. Off-resonance decoupled spectrum of baumycin A2 showed the presence of four C-methyls, one O-methyl, five methylenes, eight methines, one quaternary

Table 1. ^{13}C-Chemical shift-assignments*

C	Daunomycin $CDCl_3$, 0.13 M	Baumycin A1 $CDCl_3$, 0.07 M	Baumycin A2 $CDCl_3$, 0.11 M	Baumycin B1 $CDCl_3$-CD_3OD 0.11 M	Baumycin B2 $CDCl_3$-CD_3OD 0.08 M	Baumycin C1 $CDCl_3$ 0.07 M
1	118.5	118.5	118.6	119.3	119.3	118.3
2	135.8	135.8	135.7	136.5	135.6	135.5
3	119.8	119.9	119.9	120.2	120.0	119.6
4	161.2	161.2	161.2	161.6	161.5	160.7
6	(156.6)	(156.5)	(156.6)	(156.7)	(156.6)	(156.2)
11	(155.9)	(155.9)	(155.9)	(155.7)	(155.5)	(155.5)
5	(186.6)	(186.7)	(186.7)	(187.0)	(186.9)	(186.2)
12	(186.9)	(187.0)	(186.9)	(187.3)	(187.3)	(186.7)
4a	120.9	121.0	121.0	121.0	120.9	120.7
12a	135.5	135.6	135.6	135.8	136.4	135.3
5a	(111.4)	(111.4)	(111.4)	(111.7)	(111.7)	(111.1)
11a	(111.2)	(111.3)	(111.2)	(111.5)	(111.5)	(111.3)
10a	(134.5)	(134.4)	(134.4)	(134.8)	(134.7)	(133.7)
6a	(134.6)	(134.5)	(134.4)	(135.0)	(136.2)	(134.2)
7	69.7	69.9	69.8	70.4	70.2	70.0
8	(34.9)	(36.3)	(34.9)	(35.9)	(35.9)	(35.0)
9	76.9	76.9	76.9	76.6	76.5	76.5
10	(33.3)	(34.9)	(34.9)	(33.0)	(32.9)	(33.3)
13	212.0	212.4	215.0	213.0	213.0	211.8
14	24.8	24.9	24.8	24.7	24.6	24.8
4-OCH_3	56.7	56.7	56.7	57.0	56.8	56.5
1′	101.1	101.2	101.3	100.1	100.1	100.4
2′	32.6	33.4	33.4	30.0	29.6	29.9
3′	46.4	45.9	46.3	46.2	46.7	44.2
4′	67.4	82.0	76.2	77.7	74.5	66.9
5′	70.8	64.2	64.3	64.0	64.1	69.3
6′	17.0	17.1	16.8	16.6	17.1	16.7
7′						160.3
1″		106.7	101.6	106.9	102.1	
2″		45.9	43.4	44.8	42.6	
3″		75.9	73.4	75.1	73.1	
4″		23.4	23.5	23.7	23.6	
5″		68.0	68.0	66.7	67.0	
6″		66.9	66.7	180.6	179.9	
7″		18.1	17.6	20.3	18.7	

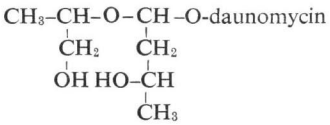
* In ppm (δ), TMS as internal reference at 25.16 MHz. Similar values in parentheses may be interchanged; their tentative assignments are given on the basis of the value of ref. 2.

Fig. 1.



carbon, three aromatic methines, nine substituted aromatic carbons, and three carbonyl carbons (Table 1). Thus, it has been shown that A2 has the following seven carbons in addition to daunomycin: two C-methyls (δ 17.6, 23.5), two methylenes (δ 43.4, 66.7), three methines (δ 68.0, 73.4, 101.6). The presence of these groups can also be shown by proton NMR (PMR, in $CDCl_3$) of A2 as follows: two methyls (δ 1.17, 1.23), two methylenes (δ 1.9~, 3.5), three methines (δ 3.85, 4.14, 4.93). The molecular formula of A1 and A2 were determined to be $C_{34}H_{43}NO_{13}$ (MW 673) on the basis of field desorption mass spectroscopy (FD-MS, *m/e* 673 or 674) and CMR (C_{34}). The treatment of A1 and A2 with acetic anhydride in pyridine (20°C, 96 hours) gave their peracetyl derivatives. PMR spectra of both compounds showed the acetyl groups at δ 1.89, 2.03, 2.05, 2.18, 2.19, 2.50, 2.51 in peracetylbaumycin A1, δ 1.93, 2.03, 2.09, 2.17, 2.22, 2.50, 2.51 in peracetylbaumycin A2, respectively. The signal at δ 2.19 in A1-peracetate and δ 2.22 in A2-peracetate can be assigned to the 14-CH_3 (Fig. 1). The methine signal at δ 3.85 and the methylene signal at δ 3.5 in A2 were shifted by acetylation to δ 5.44 and 4.0, respectively, suggesting that A2 has two hydroxyl groups in the seven carbon moiety described above. The same was suggested for A1.

Hydrogenolysis of A2 with $Pd/BaSO_4$ gave 7-deoxydaunomycinone (*m/e* 382.1037, calcd. for $C_{21}H_{18}O_7$, *m/e* 382.1051) and a sugar moiety. Treatment of the sugar moiety with acetic anhydride in pyridine afforded the tetraacetate (**I**). Although **I** was an anomeric mixture, the presence of four acetyl groups (δ2.0, 2.05, 2.1 and 2.1) and three methyl groups (δ 1.19*, 1.28, 1.29* and 1.29) in **I** was shown by its PMR. The irradiation of methylene region (at δ 2.1) caused the methine signals at δ 4.65 (triplet) and 5.44 (multiplet) to singlet and quartet, respectively. On irradiation at δ 5.44, the methyl signal at δ 1.29 changed to a singlet. The chemical ionization mass spectrum (CI-MS, methane as a reagent gas) of **I** showed the quasi-molecular ion at *m/e* 462, and fragment ions at *m/e* 300, 284 and 172. From the above-mentioned results, the unidentified moiety in baumycin A1 and A2 can be proposed to have the following unique acetal structure.



Comparison of the PMR spectra of A1, A2 and daunomycin indicated the 4′-methine proton of the daunosamine moiety at δ 3.90, 3.71, and 3.49, respectively. In the CMR spectra of A1, A2 and daunomycin, the signal assigned to 4′-C or the daunosamine moiety was observed at δ 82.0, 76.2 and 67.4, respectively. These lower field shifts in PMR and CMR spectra of baumycins suggest that the acetal moiety should be attached to 4′ of daunosamine moiety.

As described above, A2 had the same planar structure as A1. A1 and A2 are considered to be stereoisomers. Although it is not conclusive, CMR suggests that they are different in stereochemistry of 1″-C or 3″-C (Table 1).

* Two signals are due to the anomer.

Baumycins B1 and B2 have the same molecular formula, $C_{34}H_{41}NO_{14}$ and their mild acid hydrolyses gave daunomycin. On the basis of NMR analysis, the structures of baumycins B1 and B2 can be proposed as shown in Fig. 1. In the CMR spectra ($CDCl_3$-CD_3OD), it is clearly indicated that B1 and B2 have one carbonyl carbon at δ 180.6 and 179.9 in place of the methylene carbon in baumycin A group, and the same number of carbons. Hydrogenolysis with $Pd/BaSO_4$ also gave 7-deoxydaunomycinone and an anomeric mixture of the sugar moiety. PMR of the acetylated sugar moiety (**II**) indicated the presence of two acetyl groups: ($CDCl_3$, -$COCH_3 \times 2$, δ 1.93, 2.10, -CH-$CH_3 \times 3$, δ 1.18, 1.26, 1.48*, 1.45*), one acetyl group on the amino group and the other on the 1′ of the daunosamine moiety. The CI-MS spectrum of **II** showed the peak *m/e* 374 which indicated dehydration during acetylation. The IR spectrum of B1 (KBr) showed no absorption attributed to an ester group. Therefore, it is reasonable that the carbonyl carbon at δ 180.6 in CMR is due to a carboxyl group. To confirm the proposed structures of baumycins B1 and B2, methyl esters of both compounds were prepared by treatment with diazomethane. A sharp signal was observed at δ 3.74 in PMR ($CDCl_3$) of B1-methylester. Both derivatives gave the molecular ion peaks at *m/e* 701 and *m/e* 702 ($M^+ + 1$) in FD-MS spectra. The lower field shifts of 4′ carbon signals (δ 77.8, 74.5) in CMR of B1 and B2 indicated that the glycosidic linkage should be at 4′ of the daunosamine moiety. B1 and B2 seem to be stereoisomers of each other.

The studies on stereochemistry of baumycins A1, A2, B1 and B2 are in progress.

Baumycins C1 and C2 have the molecular formula $C_{28}H_{29}NO_{11}$ and $C_{28}H_{31}NO_{11}$, respectively. Acid hydrolyses (0.1 N HCl, 85°C, 30 minutes) gave daunomycinone (*m/e* 398) and dihydrodaunomycinone (*m/e* 400), respectively. The PMR spectrum of C1 ($CDCl_3$) showed that there are signals assigned to the daunomycinone moiety with additional signals of the sugar moiety at δ 5.44 (1′-H, broad, signal half height width; width 1/2 = 6 Hz), δ 1.8 ~ (2′-CH_2), δ 4.26 (3′-H), δ 6.34 (3′-NH, broad doublet J = 8.5), δ 3.63 (4′-H, width 1/2 = 9 Hz, which was reduced to 6 Hz by addition of D_2O), δ 4.21 (5′-H, overlapped with 3′-H and 4′-OH), δ 1.28 (6′-CH_3, doublet, J = 6.5 Hz), and δ 8.05 (slightly broad singlet, coupled with NH). Irradiation at δ 4.26 (3′-H) sharpened the signal at δ 3.63 (4′-H) and the signal at δ 6.34 (NH) to a slightly broad singlet. The broad signal at δ 8.05 sharpened on irradiation of NH at δ 6.34. The signal at δ 8.05 in PMR which corresponded to a signal at δ 160.3 in CMR suggested the presence of an N-formyl group at 3′ in the sugar moiety of C1. Hydrogenolyses of C1 and C2 give the same sugar moiety. The CI-MS spectra of the sugar moiety from C1 and C2 showed a quasi-molecular ion peak at *m/e* 176 which agrees with N-formyldaunosamine ($C_7H_{13}NO_4$).

The PMR spectrum of C2 in $CDCl_3$ - CD_3OD (6: 1) showed a methyl signal at δ 1.32 which coupled with a methine signal at δ 3.71. The other signals were the same as in C1.

Thus, the structures of C1 and C2 are proposed to be N-formyldaunomycin and N-formyldihydrodaunomycin, respectively. The stereochemistry of 13-position in the dihydrodaunomycinone moiety is not known. Finally, the structure of C1 was confirmed by direct comparison with the N-formyl derivative of daunomycin prepared by treatment of daunomycin base with one equivalent of formic acid and dicyclohexylcarbodiimide in chloroform (0°C, 10 minutes).

* Two signals are due to the anomer.

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