Signal Transduction Inhibitors, Hibarimicins A, B, C, D and G Produced by *Microbispora*

II. Structural Studies

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The structure of hibarimicins A, B, C, D and G which are inhibitors for tyrosine specific protein kinase are determined using spectroscopic techniques. Hibarimicins described in this report consist of a common aglycon and six deoxyhexoses. The aglycon contains a highly oxidized naphtylnaphthoquinone as a chromophore. Among them, hibarimicin B was identical with angelmicin B.

In the preceding paper[1], we have reported isolation and biological properties of new tyrosine kinase inhibitors, hibarimicins. This paper describes structure elucidation of hibarimicins A, B, C, D and G. These components have a common aglycon and the differences among them can be seen in the variation of terminal carbohydrates. The aglycon contains a highly oxidized naphtylnaphthoquinone as a chromophore.

Results and Discussion

Physico-chemical properties of hibarimicins A, B, C, D and G (They are abbreviated as HbA, HbB, HbC, HbD and HbG, respectively) were summarized in the preceding paper[1]. UV and visible spectra of hibarimicins were identical each other. The solution colors were red under neutral and acidic conditions but it turned to green under alkaline condition. In alkaline methanol, an absorption band at 511 nm in neutral methanol disappeared and new bands appeared at 614 and 647 nm.

IR spectra of hibarimicins were also very similar each other. Two kinds of carbonyl bands were detected at 1705 and 1620 cm^{-1}.

These spectroscopic behaviors suggested that the chromophore of hibarimicins was the same phenolic quinone. In addition, the ^{1}H and ^{13}C NMR data indicated that all hibarimicins contained carbohydrate moieties. ^{13}C and ^{1}H NMR data were summarized in Tables 1, 2 and 3.

Structure of Hibarimicin B

Structural study was carried out on HbB in detail at first, and then structures of HbA, C, D and G were determined by comparing their spectroscopic data with those of HbB. We describe herein the structural determination of HbB with its stereochemical aspect, which was not elucidated in our previous paper on angelmicin B[2,3].

FAB-MS spectra of HbB gave $[M]^+$, $[M+H]^+$ and $[M+Na]^+$ at *m/z* 1725, 1726, and 1748, respectively. ^{13}C NMR spectrum (complete ^{1}H decoupled) in $CDCl_3$ showed eighty five signals including twenty four sp^2 carbons and sixty one sp^3 carbons. Six carbonyl carbons and six anomeric methine carbons were observed. The molecular formula of HbB was determined to be $C_{85}H_{112}O_{37}$ based on MS, ^{1}H and ^{13}C NMR spectra.

Carbohydrate Moiety of Hibarimicin B

NMR analysis of HbB indicated that it contained six sugar units consisting of three kinds of deoxy sugars in

Table 1. ^{13}C chemical shifts (ppm) of hibarimicins in $CDCl_3$.

No.	HbA	HbB	HbC	HbD	HbG	No.	HbA	HbB	HbC	HbD	HbG
1	152.07	152.05	152.09	152.05	152.03	1'	187.84	187.82	187.84	187.82	187.84
2	107.92	107.93	107.94	107.91	107.89	2'	125.54	125.62	125.65	125.59	125.46
3	153.33	153.32	153.36	153.30	153.30	3'	158.40	158.40	158.41	158.40	158.37
4	138.56	138.56	138.56	138.55	138.53	4'	184.83	184.82	184.82	184.83	184.80
5	135.47	135.44	135.48	135.44	135.43	5'	116.28	116.28	116.29	116.31	116.28
6	111.98	111.97	112.01	111.97	111.96	6'	150.91	150.90	150.94	150.88	150.88
7	139.36	139.34	139.34	139.35	139.30	7'	147.97	147.98	147.99	147.90	147.90
8	27.79	27.79	27.83	27.79	27.79	8'	67.87	67.85	67.89	67.88	67.85
9	44.50	44.51	44.54	44.51	44.46	9'	55.78	55.79	55.81	55.76	55.74
10	76.19	76.18	76.23	76.23	76.19	10'	77.00	77.03	77.16	77	76.99
11	70.76	70.75	70.80	70.74	70.70	11'	75.34	75.34	75.35	75.31	75.30
12	86.59	86.60	86.63	86.59	86.62	12'	85.37	85.44	85.36	85.49	85.47
13	79.44	79.44	79.47	79.44	79.41	13'	82.73	82.69	82.79	82.68	82.64
14	77.18	77.19	77.20	77.2	77.14	14'	85.73	85.73	85.74	85.70	85.68
15	203.44	203.44	203.45	203.45	203.37	15'	195.55	195.54	195.59	195.48	195.49
16	110.55	110.54	110.57	110.55	110.52	16'	124.86	124.84	124.90	124.83	124.80
17	164.35	164.34	164.38	164.34	164.33	17'	157.24	157.23	157.26	157.22	157.20
18	108.37	108.37	108.41	108.37	108.34	18'	112.99	112.97	113.01	113.01	112.97
19	37.13	37.11	37.14	37.13	37.08	19'	34.22	34.25	34.19	34.25	34.25
20	18.04	18.00	18.04	18.01	18.00	20'	16.61	16.58	16.61	16.58	16.58
21	15.14	15.12	15.15	15.12	15.14	21'	14.87	14.88	14.88	14.90	14.91
3-OMe	60.91	60.87	60.90	60.89	60.88	3'-OMe	60.79	60.79	60.80	60.79	60.78
4-OMe	61.15	61.12	61.16	61.14	61.14						
DG1	98.64	98.63	98.63	98.65	98.66	DG1'	98.96	98.95	98.97	98.95	98.92
DG2	34.80	34.81	34.85	34.81	34.79	DG2'	35.29	35.29	35.32	35.29	35.25
DG3	67.27	67.27	67.29	67.25	67.25	DG3'	67.14	67.12	67.16	67.12	67.10
DG4	72.82	72.83	72.86	72.83	72.78	DG4'	72.59	72.59	72.64	72.59	72.55
DG5	65.17	65.15	65.19	65.15	65.14	DG5'	65.04	65.00	65.05	65.02	65.00
DG6	17.87	17.85	17.88	17.85	17.83	DG6'	17.68	17.66	17.69	17.66	17.66
AM1	103.26	103.24	o103.3	103.24	103.09	AM1'	103.32	103.24	o103.27	103.13	103.09
AM2	30.54	a30.53	30.56	30.53	f30.45	AM2'	30.45	a30.38	30.51	30.34	f30.32
AM3	29.46	29.43	29.46	29.43	29.65	AM3'	29.93	29.49	29.86	29.70	29.65
AM4	78.78	78.75	78.78	78.75	79.71	AM4'	79.44	78.88	79.47	79.86	79.85
AM5	75.43	75.42	75.45	75.42	75.10	AM5'	75.06	75.21	75.15	74.91	74.91
AM6	18.14	18.12	n18.13	p18.12	g18.13	AM6'	18.09	18.09	n18.12	p18.09	g18.10
X1	98.96	98.91	98.97	98.95	100.13	Y1'	103.01	98.91	103.04	100.12	100.13
X2	24.83	24.81	24.85	24.81	31.67	Y2'	29.62	24.81	30.91	31.68	31.67
X3	27.79	27.79	27.83	27.79	h69.72	Y3'	31.93	27.79	31.22	69.81	h69.77
X4	78.61	78.62	78.64	78.61	i79.30	Y4'	77	78.62	71.35	79.35	i79.34
X5	66.75	66.75	66.77	66.75	k62.77	Y5'	75.81	66.68	75.83	62.80	k62.81
X6	14.66	14.64	14.66	14.64	j14.28	Y6'	15.22	14.64	n18.06	14.26	j14.25
X7	210.59	210.59	210.58	210.61	m209.99	Y7'	210.35	210.60	-	209.98	m209.99
X8	25.06	e25.04	25.06	25.06	27.45	Y8'	28.63	e25.01	-	27.45	27.45

X=AT for HbA,B,C and D, X=AX for HbG

Y=βAT for HbA, Y=AT for HbB, Y=AM" for HbC, Y=AX for HbD and G

a-p ; interchangeable pairs.

Table 2. ^{1}H chemical shifts (ppm) of hibarimicins in $CDCl_3$.

No.	HbA	HbB	HbC	HbD	HbG	No.	HbA	HbB	HbC	HbD	HbG
1OH	9.696	9.700	9.700	9.701	9.700	X6	0.951	b 0.946	0.952	0.951	a 1.027
6	7.382	7.379	7.381	7.383	7.375	X8	2.249	c 2.245	2.225	2.248	2.373
8a	3.038	3.038	3.035	3.038	3.030	6'OH	12.220	12.215	12.2	12.221	12.216
8b	3.770	3.768	3.700	3.771	3.752	8'	5.833	5.836	5.830	5.832	5.825
9	2.691	2.693	2.690	3.689	2.684	9'	2.645	2.645	2.642	2.649	2.642
10	4.229	4.223	4.231	4.230	4.218	10'	4.162	4.160	4.153	4.165	4.153
11	4.155	4.151	4.158	4.156	4.145	11'	4.114	4.098	4.085	4.1	4.091
11OH	4.673	4.680	4.677	4.672	4.561	11'OH	4.808	4.822	4.844	4.698	4.705
12	3.77	3.755	3.755	3.756	3.752	12'	3.951	3.954	3.95	3.9-4.0	3.95
13OH	1.969	1.974	1.955	1.958	1.977	14'OH	4.372	4.375	4.361	4.371	4.379
14OH	3.949	3.934	3.930	3.927	3.958	17'OH	13.945	13.945	13.945	13.955	13.948
17OH	14.971	14.980	15.0	14.970	14.970	19'a	1.1	1.09	1.1	1.09	1.1
19a	1.52	1.52	1.52	1.5	1.505	19'b	1.9	1.84	1.8	1.83	1.8
19b	1.7	1.68	1.68	1.66	1.65	20'a	1.0	0.99	0.99	0.99	1.0
20a	1.15	1.15	1.15	1.18	1.15	20'b	1.33	1.33	1.33	1.33	1.3
20b	1.37	1.37	1.37	1.348	1.35	21'	0.781	0.789	0.780	0.789	0.781
21	0.873	0.872	0.872	0.872	0.867	3'-OMe	3.951	3.950	3.947	3.951	3.943
3-OMe	3.972	3.973	3.973	3.973	3.966	DG1'	5.344	5.350	5.339	5.349	5.335
4-OMe	3.874	3.873	3.874	3.875	3.877	DG2'a	1.953	1.95	1.95	1.953	1.950
DG1	5.429	5.428	5.431	5.431	5.410	DG2'b	2.406	2.405	2.403	2.404	2.400
DG2a	1.959	1.956	1.95	1.96	1.960	DG3'	4.0	3.995	4.0	4.0	3.99
DG2b	2.432	2.431	2.430	2.431	2.424	DG3'OH	3.39	3.382	3.400	3.36	*
DG3	4.03	4.03	4.03	4.05	4.025	DG4'	3.127	3.125	3.125	3.126	3.12
DG3OH	3.72	3.727	3.73	3.719	*	DG4'OH	2.496	2.500	2.488	2.480	2.51
DG4	3.167	3.167	3.165	3.166	3.16	DG5'	3.690	3.685	3.686	3.68	3.680
DG4OH	2.496	2.497	2.488	2.48	2.51	DG6'	1.147	1.150	1.144	1.156	1.138
DG5	3.820	3.820	3.819	3.818	3.819	AM1'	4.444	4.460	4.429	4.486	4.477
DG6	1.294	1.294	1.295	1.294	1.281	AM2'a	1.75	1.76	1.719	1.6-1.7	1.8
AM1	4.529	4.524	4.527	4.529	4.540	AM2'b	1.85	1.88	1.860	1.92	1.9
AM2a	1.7	1.68	1.7	1.68	1.7	AM3'a	1.6	1.63	1.553	1.78	1.7
AM2b	1.9	1.92	1.9	1.92	1.95	AM3'b	2.3	2.20	2.28	2.3	2.3
AM3a	1.6	1.63	1.650	1.6-1.7	1.7	AM4'	3.273	3.260	3.212	3.297	3.289
AM3b	2.2	2.20	2.2	2.2	2.3	AM5'	3.512	3.527	3.492	3.543	3.5
AM4	3.260	3.260	3.261	3.260	3.289	AM6'	1.345	1.331	1.320	1.348	e 1.344
AM5	3.535	3.535	3.533	3.535	3.5	Y1'	4.706	4.940	4.501	5.094	5.084
AM6	1.332	1.331	1.333	1.332	e 1.341	Y2'a	1.9-2.0	1.70	1.582	1.875	f 1.878
X1	4.939	4.940	4.939	4.938	5.084	Y2'b	1.9-2.0	2.03	1.87	2.341	g 2.341
X2a	1.701	1.70	1.7	1.703	f 1.887	Y3'a	1.9-2.0	1.50	1.46	3.616	h 3.620
X2b	2.030	2.03	2.03	2.029	g 2.351	Y3'b	1.9-2.0	2.22	2.06	-	-
X3a	1.5	1.50	1.5	1.51	h 3.608	Y3'OH	-		-	3.760	i 3.75
X3b	2.2	2.22	2.22	2.2	-	Y4'	-	-	3.28	-	-
X3OH	-	-	-	-	i 3.726	Y4'OH	4.306	d 3.541	*	4.001	j 3.997
X4OH	3.523	d 3.527	3.521	3.515	j 4.008	Y5'	3.601	4.275	3.28	4.626	k 4.621
X5	4.278	4.275	4.278	4.278	k 4.615	Y6'	1.043	b 0.952	1.300	1.039	a 1.036
						Y8'	2.439	c 2.252	-	2.385	2.380

X=AT for HbA, HbB, HbC and HbD, X=AX for HbG

Y=βAT for HbA, Y=AT for HbB, Y=AM for HbC, Y=AX for HbD and HbG

* ; could not be observed due to broadening.

a - k ;interchangeable pairs

Table 3. ^{1}H-^{1}H coupling constants (Hz) of hibarimicins in $CDCl_3$.

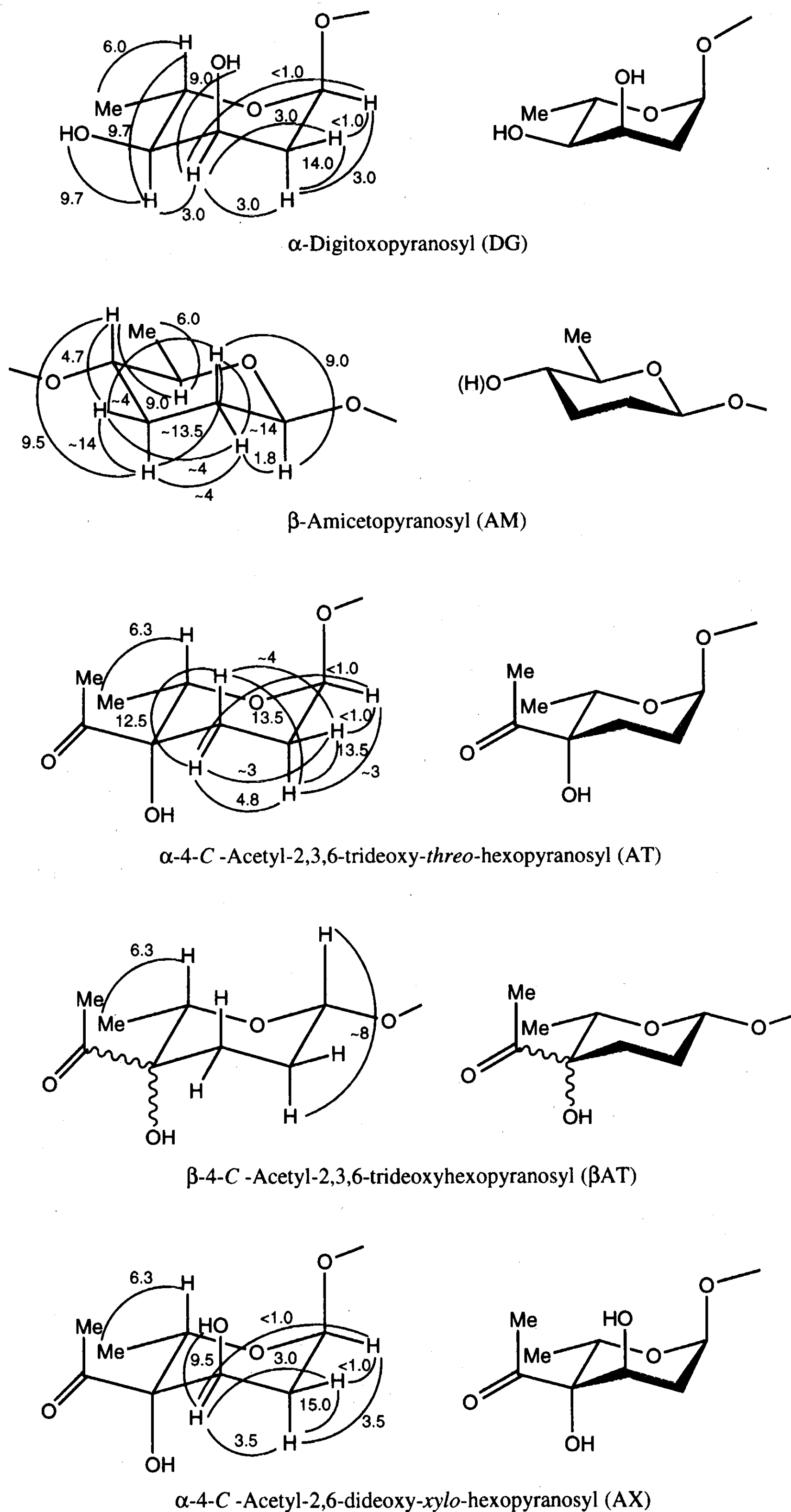
West	HbA	HbB	HbC	HbD	HbG
J8a,8b	17.5	17.5	17.5	17.5	17.5
J8a,9	6.3	6.3	6.3	6.3	6.3
J8b,9	12.5	12.5	12.5	12.5	12.5
J9,10	5.3	5.3	5.3	5.3	5.3
J10,11	9.8	9.8	9.8	9.8	9.8
J11,12	8.8	8.8	8.8	8.8	8.8
J11,OH	0	0	0	0	0
J20,21	7.2	7.2	7.2	7.2	7.2
DG					
J1,2a	3.0	3.0	3.0	3.0	3.0
J1,2b	<1.0	<1.0	<1.0	<1.0	<1.0
J1,3	<1.0	<1.0	<1.0	<1.0	<1.0
J2a,2b	14.0	14.0	14.0	14.0	14.0
J2a,3	3.0	3.0	3.0	3.0	3.0
J2b,3	3.0	3.0	3.0	3.0	3.0
J3,OH	9.0	9.0	9.0	ca.8	9.0
J3,4	3.0	3.0	3.0	3.0	3.0
J4,OH	9.7	9.7	9.7	9.7	9.7
J4,5	9.7	9.7	9.7	9.7	9.7
J5,6	6.0	6.0	6.0	6.0	6.0
AM					
J1,2a	9.0	9.0	9.0	9.0	9.0
J1,2b	1.8	1.8	1.8	1.8	1.8
J2a,2b	ca.14	ca.14	ca.14	ca.14	ca.14
J2a,3a	ca.13.5	ca.13.5	ca.13.5	ca.13.5	ca.13.5
J2a,3b	ca.4	ca.4	ca.4	ca.4	ca.4
J2b,3a	ca.4	ca.4	ca.4	ca.4	ca.4
J2b,3b	ca.4	ca.4	ca.4	ca.4	ca.4
J3a,3b	ca.14	ca.14	ca.14	ca.14	ca.14
J3a,4	9.5	9.5	9.5	9.5	9.5
J3b,4	4.7	4.7	4.7	4.7	4.7
J4,5	9.0	9.0	9.0	9.0	9.0
J5,6	6.0	6.0	6.0	6.0	6.0
X					
J1,2a	<1.0	<1.0	<1.0	<1.0	<1.0
J1,2b	ca.3	ca.3	ca.3	ca.3	3.5
J1,3	<1.0	<1.0	<1.0	<1.0	<1.0
J2a,2b	13.5	13.5	13.5	13.5	15.0
J2a,3a	ca.4	ca.4	ca.4	ca.4	3.0
J2a,3b	ca.3	ca.3	ca.3	ca.3	-
J2b,3a	13.5	13.5	13.5	13.5	3.5
J2b,3b	4.8	4.8	4.8	4.8	-
J3a,3b	12.5	12.5	12.5	12.5	-
J3',OH	-	-	-	-	9.5
J5,6	6.3	6.3	6.3	6.3	6.3

East	HbA	HbB	HbC	HbD	HbG
J8',9'	<0.5	<0.5	<0.5	<0.5	<0.5
J9',10'	3.8	3.8	3.8	3.8	3.8
J10'11'	8.0	8.0	8.0	8.0	8.0
J11',12'	7.5	7.5	7.5	7.5	7.5
J11',OH	0	0	0	0	0
J20',21'	7.2	7.2	7.2	7.2	7.2
DG'					
J1',2'a	3.0	3.0	3.0	3.0	3.0
J1',2'b	<1.0	<1.0	<1.0	<1.0	<1.0
J1',3'	<1.0	<1.0	<1.0	<1.0	<1.0
J2'a,2'b	14.0	14.0	14.0	14.0	14.0
J2'a,3'	3.0	3.0	3.0	3.0	3.0
J2'b,3'	3.0	3.0	3.0	3.0	3.0
J3',OH	9.0	9.0	9.0	9.0	9.0
J3',4'	3.0	3.0	3.0	3.0	3.0
J4',OH	9.7	9.7	9.7	9.7	9.7
J4',5'	9.7	9.7	9.7	9.7	9.7
J5',6'	6.0	6.0	6.0	6.0	6.0
AM'					
J1',2'a	9.0	9.0	9.0	9.0	9.0
J1',2'b	1.8	1.8	1.8	1.8	1.8
J2'a,2'b	ca.14	ca.14	ca.14	ca.14	ca.14
J2'a,3'a	ca.13.5	ca.13.5	ca.13.5	ca.13.5	ca.13.5
J2'a,3'b	ca.4	ca.4	ca.4	ca.4	ca.4
J2'b,3'a	ca.4	ca.4	ca.4	ca.4	ca.4
J2'b,3'b	ca.4	ca.4	ca.4	ca.4	ca.4
J3a,3b	ca.14	ca.14	ca.14	ca.14	ca.14
J3'a,4'	9.5	9.5	9.5	9.5	9.5
J3'b,4'	4.7	4.7	4.7	4.7	4.7
J4',5'	9.0	9.0	9.0	9.0	9.0
J5',6'	6.0	6.0	6.0	6.0	6.0
Y					
J1',2'a	ca.8	<1.0	9.0	<1.0	<1.0
J1',2'b	*	ca.3	1.8	3.5	3.5
J1',3'	*	<1.0	-	<1.0	<1.0
J2'a,2'b	*	13.5	13.5	15.0	15.0
J2'a,3'a	*	ca.4	ca.13	3.0	3.0
J2'a,3'b	*	ca.3	ca.4	-	-
J2'b,3'a	*	13.5	ca.4	3.5	3.5
J2'b,3'b	*	4.8	ca.4	-	-
J3'a,3'b	*	12.5	ca.14	-	-
J3',OH	-	-	-	9.5	9.5
J3'a,4	-	-	ca.10	-	-
J3'b,4	-	-	ca.4	-	-
J4',5'	-	-	9.0	-	-
J5',6'	6.3	6.3	6.0	6.3	6.3

X=AT for HbA, HbB, HbC and HbD, X=AX for HbG
Y=βAT for HbA, Y=AT for HbB, Y=AM" for HbC, Y=AX for HbD and HbG
* ; not determined due to peak overlapping
Coupling constants of H-19, H-19', H-20 and H-20' are also not determined.

Fig. 1. ^{1}H-^{1}H coupling constants, structures and abbreviations of deoxyhexose residues in hibarimicins.

Absolute configurations are unknown.



pairs. ^{1}H-^{1}H COSY, HOHAHA and ^{1}H-^{1}H coupling constants (Fig. 1) suggested that two kinds of them were a pair of β-amicetosyl (2,3,6-trideoxy-β-*erythro*-hexopyranosyl) (AM) and a pair of α-digitoxosyl (2,6-dideoxy-α-*ribo*-hexopyranosyl) (DG) residues.

NMR signals of the third duplicate sugars (AT) were almost identical each other. Both anomeric protons of the pair were observed at 4.940 ppm as a broad doublet and both anomeric carbons were observed at 98.91 ppm as a single peak. COSY spectrum suggested that each anomeric carbon was linked to the methylene sequence ($-CH_2-CH_2-$, AT2, 3 and AT2′, 3′), respectively. HMBC spectrum at 270 MHz showed the correlation between the anomeric protons (4.940 and 4.940 ppm) and oxymethine carbons (66.75 ppm, C-AT5 and 66.68 ppm, C-AT5′) to which methyl groups (14.64 ppm, AT6 and AT6′) were attached. HMBC correlations suggested the connection of two quarternary carbons (78.62 ppm, C-AT4 and C-AT4′) with C-AT3/3′, C-AT5/5′, acetyl groups and hydroxy groups as summarized in Fig. 2. Large deuteration shifts for C-AT4 (−110 ppb) and C-AT4′ (−136 ppb) were induced by the addition of deuterium oxide, indicating the attachment of hydroxy groups to the quarternary carbons. AT and AT′ were, therefore, determined as 4-*C*-acetyl-2,3,6-trideoxyhexopyranosyl residues. Coupling constants $J_{AT1,AT2ax}=$ *ca.* 3 Hz, $J_{AT1,AT2eq}<1.0$ Hz, $J_{AT1',AT2'ax}=$ *ca.* 3 Hz, $J_{AT1',AT2'eq}=<1.0$ Hz indicated that both anomeric configurations were α.

Relative stereochemistry at AT4, 5 and AT4′, 5′ was determined by comparing NMR spectra of a synthetic methyl 4-*C*-acetyl-2,3,6-trideoxy-α-D-*threo*-hexopyranoside with that of the anomeric mixture of AT methyl glycosides prepared from hibarimicin mixture. Methyl 4-*C*-acetyl-2,3,6-trideoxy-α-D-*threo*-hexopyranoside was prepared according to Brimacombe's protocol[4]. An anomeric mixture of natural AT methyl glycosides was obtained by methanolysis of HbB in 3% methanolic hydrogen chloride, followed by purification with preparative silica-gel TLC and reversed phase HPLC.

^{1}H NMR spectra of the synthetic sugar was identical with that of the α methyl glycoside prepared from HbB. Thus, AT was determined to be 4-*C*-acetyl-2,3,6-trideoxy-*threo*-hexopyranose. Absolute configuration of

Fig. 2. Selected COSY, HMBC and NOE correlations of the carbohydrate moieties in hibarimicin B.

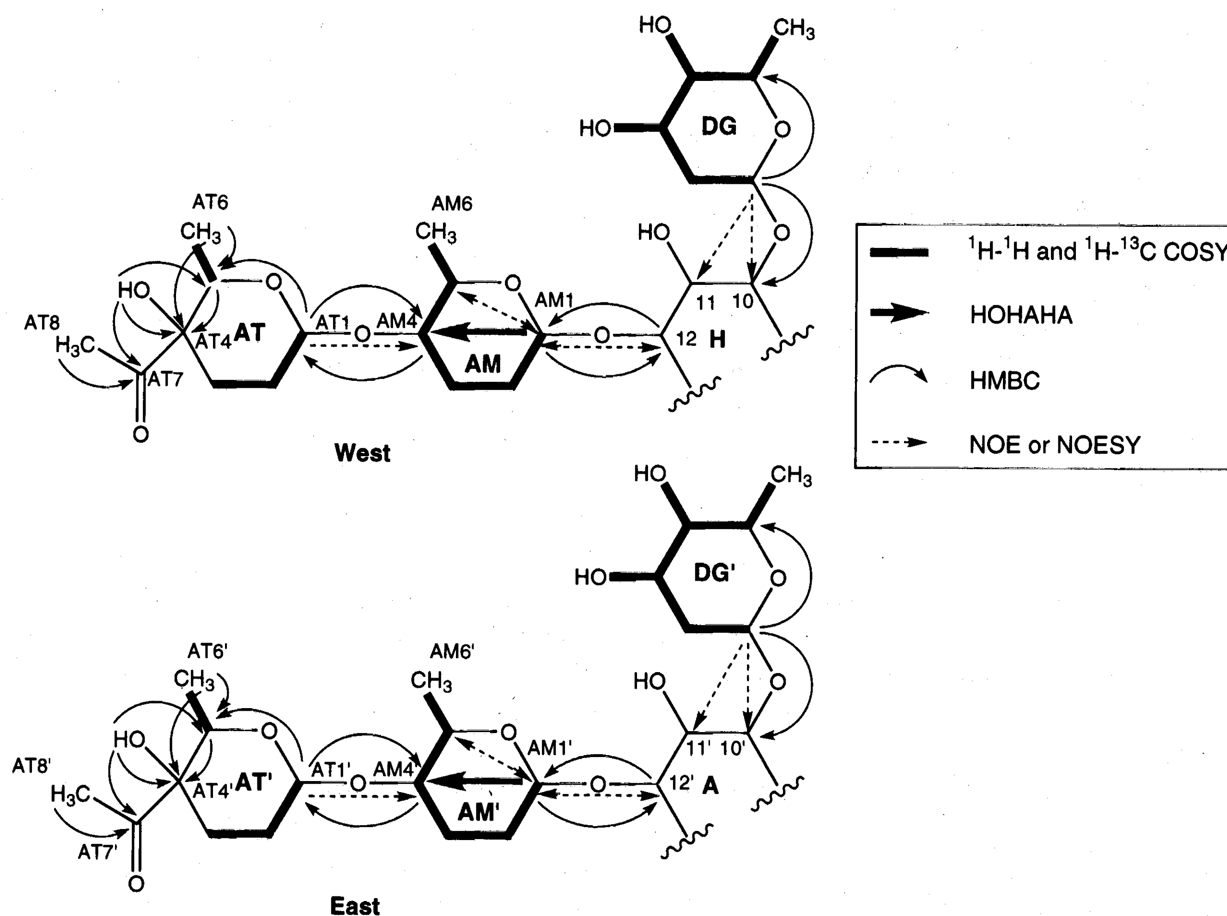
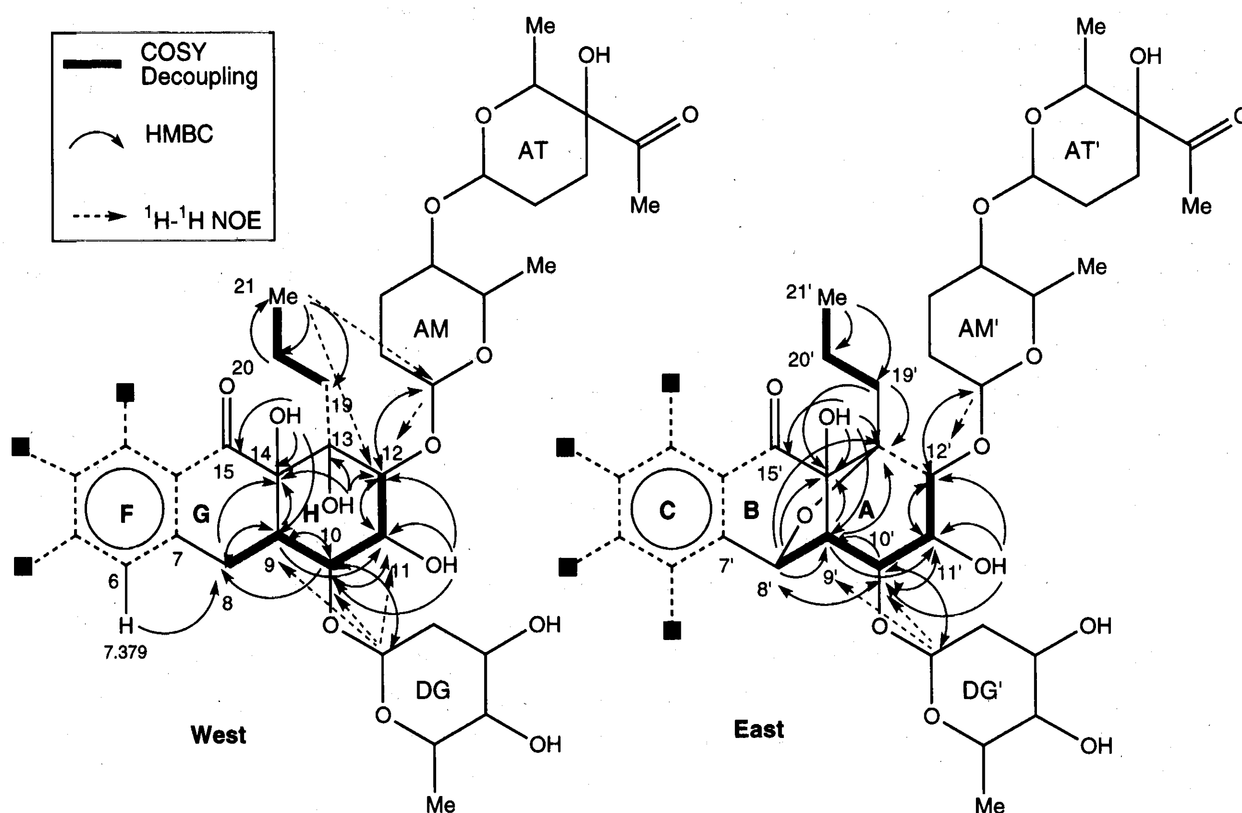


Fig. 3. COSY, HMBC and NOE correlations of hibarimicin B.



these sugars has not yet been elucidated. The positions of glycosidic linkage were confirmed by HMBC and NOE experiments. HMBC correlations were observed between H-AT1 and C-AM4, and between H-AT1′ and H-AM4′, and NOEs were observed at H-AM4 and H-AM4′ when H-AT1 and H-AT1′ were irradiated. These findings indicated that each AT was linked at O-AM4 and O-AM4′ (Fig. 2 and 3).

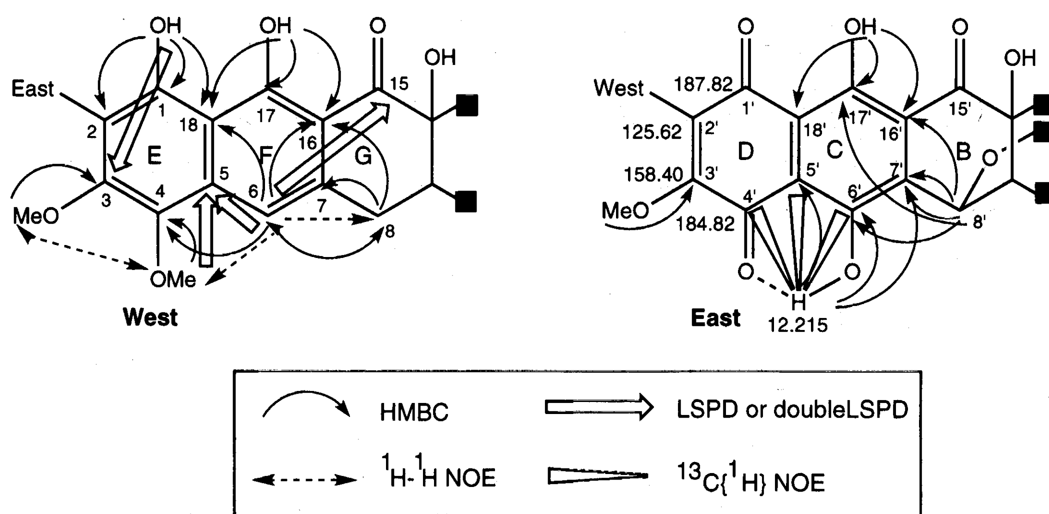
We concluded that the carbohydrate moieties in HbB were two α-digitoxosyl groups and two disaccharides, 4-*O*-(4-*C*-acetyl-2,3,6-trideoxy-α-*threo*-hexopyranosyl)-2,3,6-trideoxy-*β*-*erythro*-hexopyranosyl groups.

Aglycon of Hibarimicin B

The aglycon of HbB is composed of two similar units which we call the east and the west. COSY, CH-COSY, TOCSY and decoupling experiments for HbB indicated the presence of two propyl groups (C-19∼C-21 and C-19′∼C-21′) and two C_5 units (C-8∼C-12 and C-8′∼C-12′) as shown in Fig. 3. One of the C_5 units and one of the propyl units belong to the west aglycon, while the other ones belong to the east. First, the west part was analyzed.

HMBC spectrum indicated that two hydroxy quaternary carbons at 79.44 ppm (C-13) and 77.19 ppm (C-14) bonded to carbons at 86.60 ppm (C-12) and 44.51 ppm (C-9), respectively, to form ring H. The presence of hydroxyl groups on C-11 (70.75 ppm), 13 and 14 was supported by not only HMBC correlations as shown in Fig. 3 but also deuteration shift of the carbons; the deuteration shift of C-11, 13 and 14 was −132, −163, and −136 ppb, respectively. HMBC correlation also demonstrated the connection of C-14 with a carbonyl carbon at 203.44 ppm (C-15). The chemical shift of C-15 implied its bonding to an sp^2 carbon. HMBC correlations between H-AM1 and C-12, H-12 and C-AM1, and H-DG1 and C-10 indicated that amicetose (AM) and digitoxose (DG) units bonded to O-12 and O-10, respectively. We finally deduced that one of the propyl group was attached to C-13, even though no direct evidence was in hand, because no other possible position for the attachment was available. This was supported by weak NOEs of H-12 and H-AM1 under the irradiation of H-21 (Fig. 3). In addition, INADEQUATE spectrum of ^{13}C labeled hibarimicin B isolated from cultured broth fed with [$^{13}C_2$] sodium acetate proved C-13∼C19 linkage. The results of the feeding experiments will be discussed in a following paper.

Fig. 4. HMBC, NOE and LSPD correlations of hibarimicin B.



Three HMBC correlations between phenolic proton at 14.980 ppm (17-OH) and quaternary carbons at 108.37 (C-18), 164.5 (C-17), 110.54 ppm (C-16), and strong correlations between H-6 and C-16, 18 disclosed the structure of ring F. Another HMBC correlations between H-8b and C-6, 16 and a carbon at 139.34 ppm (C-7) suggested that ring H and F were linked together through C-8. It was speculated that C-16 bonded to a carbonyl C-15 to close ring G since the chemical shift of C-15 implied the presence of an adjacent sp^2 carbon, and the large chemical shift of OH-17 (14.980 ppm) could be explained by hydrogen bonding with a carbonyl oxygen at *peri*-position. The C-15$\sim$C-16 linkage was also supported by detection of a small coupling $^4J_{C\text{-}15,H\text{-}6}$ in low power selective double decoupling experiment $^{13}C\{^1H\}\{^1H\}$ (double LSPD); H-9 was irradiated during the experiment in order to simplify splitting pattern of C-15 and gain the peak height with or without irradiation of H-6. This simple and classical technique is still effective since such a small coupling usually can not be detected by HMBC experiment.

The sequence of C-6$\sim$C-7$\sim$C-8 was also supported by NOE experiment; signal enhancements were observed for H-8a and H-8b when H-6 was irradiated.

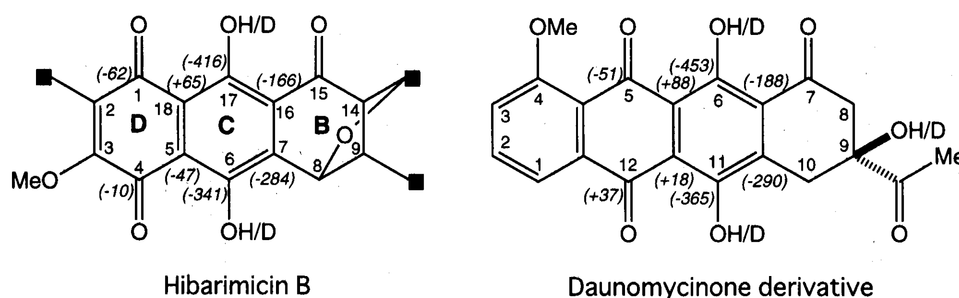
HMBC correlations between a phenolic OH at 9.700 ppm (HO-1) and C-18/C-1 (152.05 ppm)/C-2 (107.93 ppm) suggested that ring F was fused with another aromatic ring. Structure of the ring, which we call E, was elucidated by HMBC, double LSPD and NOE experiments summarized in Fig. 4. Double LSPD experiments revealed crucial long range CH couplings such as $^4J_{OH\text{-}1,C\text{-}3}$, $^4J_{OCH3\sim4,C\text{-}5}$ and $^2J_{H\text{-}6,C\text{-}5}$. Connection between C-3 and C-4 was speculated by careful NOE experiment; a signal of methoxy protons at CH_3–O-3 (3.973 ppm) was enhanced when methoxy protons at CH_3–O-4 (3.873 ppm) were irradiated.

Thus, the planar structure of the west part of the aglycon was established (Fig. 3, 4) though a substitution group at C-2 was not clear at this stage.

The structure of the east aglycon was determined as follows. A C_5 unit (C-8'$\sim$C-13') and a propyl group (C-19'$\sim$C-21') in the east aglycon were confirmed by COSY, TOCSY and decoupling experiments (Fig. 3). Coupling between C-9' and C-8' in the C_5 unit was not observed by COSY and TOCSY since $J_{8',9'}$ was very small. Decoupling experiment irradiating H-9', however, revealed the small coupling (less than 0.5 Hz) between H-8' and H-9'. This small coupling will be discussed after in this section.

HMBC correlations demonstrated that a hydroxy quaternary carbon C-14' connected with C-9', a carbonyl C-15' (195.54 ppm), and a quaternary oxycarbon C-13 to which the propyl group (C-19'$\sim$C-21') was linking (Fig. 3). The chemical shift of the carbonyl C-15' implied its bonding to an sp^2 carbon. HMBC correlation between H-8' and C-13' and chemical shifts of C-8' and C-13' suggested the presence of an ether bridge between C-8' and C-13'. NOE and HMBC spectra indicated that the other digitoxose unit (DG') and amicetose unit (AM') were attached to O-10' and O-12', respectively. Although no direct evidence was obtained, the connection between C-12' and C-13' was speculated in consideration of the

Fig. 5. Deuteration shift (ppb, ±15 ppb) on ^{13}C chemical shift of hibarimicin B and 7-oxo derivative of daunomicinone.



coupling constants $J_{9',10'}$, $J_{10',11'}$ and $J_{11',12'}$ and NOE between H-8′ and H-11′. The coupling constants will be discussed in this section later. Chemical shifts of H-9′, 10′, 11′ and 12′, and the coupling constants ($J_{9',10'}$, $J_{10',11'}$ and $J_{11',12'}$) were similar to those of H-9, 10, 11 and 12 on ring H, suggesting the structural similarity between rings A and H.

The partial structure of ring C was determined to be per-substituted *p*-dihydroxybenzene by analysing HMBC correlations summarized in Fig. 4. Correlations between H-8′ and C-6′, 7′, 8′, 9′ indicated that the linkage between rings A and C through C-8′. The chemical shift of carbons in ring C suggested the connection of C-16′ with C-15′, which was finally confirmed by comparison with daunomycinone derivative (see below).

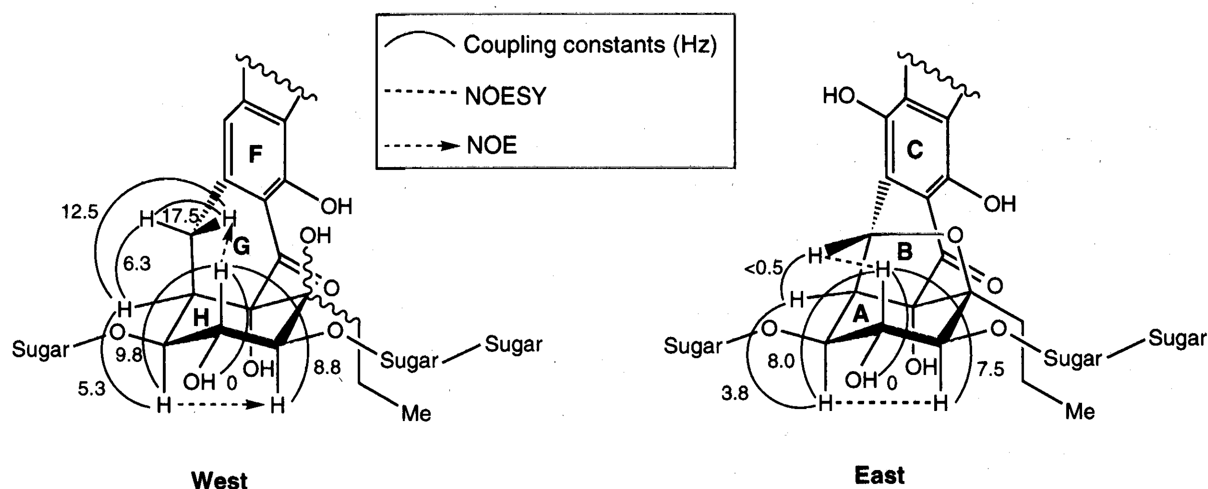
There were five carbon signals which were not assigned at this stage. They were two quinone carbonyls (C-1′, 4′), a quaternary aryl carbons (C-2′) and a methoxylated aryl carbon (CH_3O-C-3′). The chemical shift of ring C carbons indicated the substitution of quinone carbonyls (184.82 and 187.82 ppm) at C-5′ and C-18′. The two remaining carbon (C-2′ and CH_3O-C-3′) supposed to comprise ring D based on the comparison of their chemical shifts and those of related compounds such as xanthomegnin and viomellein[5]. In order to confirm the substitution pattern on ring C, we compared ^{13}C NMR of HbB with that of 7-oxo derivative of daunomycinone which had a similar ring system. The derivative was prepared by methanolysis of daunorubicin hydrochloride followed by the oxidation with pyridinium dichromate. The chemical shifts of C-5a, 6, 6a, 7, 10a, 11 and 11a of the 7-oxo derivative corresponded to those of C-18, 17, 16, 15, 7, 6 and 5 of HbB, respectively. C-7 and 16 of HbB showed unexpected large deuteration shift by the addition of deuterium oxide to the $CDCl_3$ solution although hydroxy groups were not directly attached to these carbons (Fig. 5). Similar deuteration shift was observed again for the corresponding carbons C-10a and 6a of the daunomycinone derivative. These findings supported the validity of our proposed structure.

The position of the methoxy group on ring D was elucidated by comparison of chemical shifts of the quinone carbonyls with those of analogous compounds. In 2-methoxy-1,4-naphtho- or anthraquinone system such as xanthomegnine and viomellein, the reported chemical shift[5] of α-methoxylated carbonyl is usually smaller than that of β-methoxylated one. Therefore, we determined that C-2′ and methoxylated C-3′ were bonded to the carbon at 187.82 ppm and 184.82 ppm, respectively. It was confirmed that the carbonyl carbon at 184.82 ppm was C-4′ and the latter was C-1′ by the observation of hetero NOE of C-4′ (184.82 ppm) under the irradiation of HO-6′ (12.215 ppm). This experiment was carefully conditioned to avoid a saturation transfer by chemical exchange because the irradiated nucleus was an exchangeable phenolic proton.

Thus, the planar structure of the east aglycon was established. Its carbon framework was identical with that of the west unit. The only difference was the oxidation state. We then concluded that the east and the west units were joined together at C-2 (ring E) and C-2′ (ring D) since no other combination was possible.

Coupling constants of the protons on ring A and H, and NOE between H-8a and H-11, and H-8′ and H-11′ confirmed the stereochemistry of the rings as shown in Fig. 6. In the east unit, an ether bridge between C-8′ and C-13′ and ring A formed a bicyclo[3,2,1]octane system which constrains the dihedral angle between H-8′ and H-9′ to be *ca.* 90°. A very small coupling $J_{8',9'}$ reflected this structure. A relative configuration at C-13 is not clear now. The relation of the stereochemistry of rings A and H, and the absolute configurations are now under

Fig. 6. Selected ^{1}H-^{1}H coupling constants, NOE correlations and the relative configurations of hibarimicin B.



investigation.

In conclusion, the structure of hibarimicin B was determined as shown in Fig. 11 and angelmicin B was identical with HbB stereochemically.

Hibarimicin A

Mass number of molecular ions of HbA was identical with that of HbB ($m/z=1725$). UV and IR spectra of HbA were also very similar to those of HbB. DEPT experiment showed that numbers of methyl, methylene, methine and quaternary carbons of HbA were same as those of HbB. The molecular formula of HbA was determined to be $C_{85}H_{112}O_{37}$ based on MS, ^{1}H and ^{13}C NMR spectra.

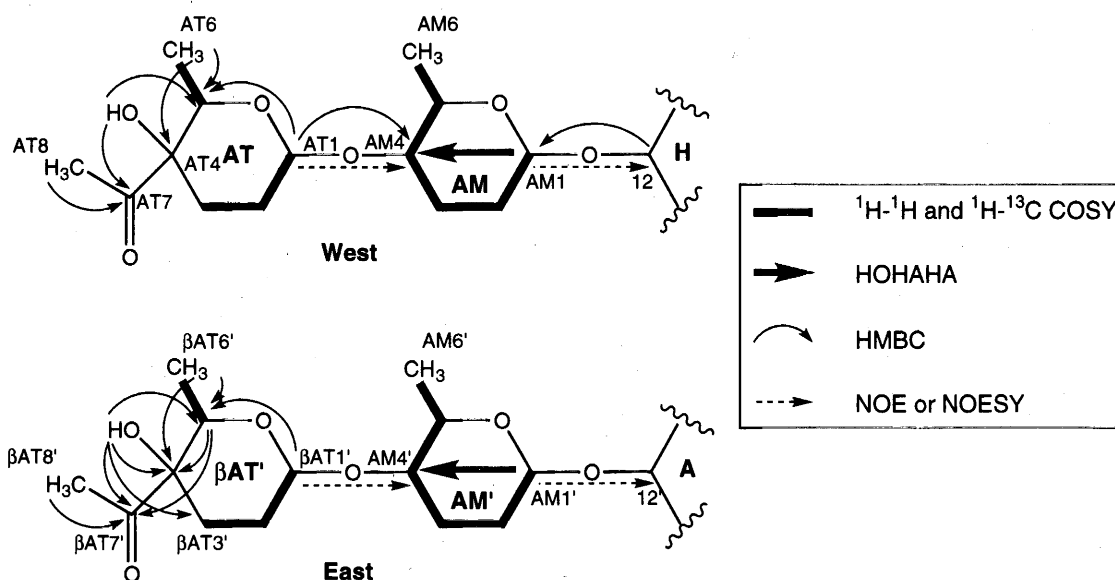
Most of ^{13}C signals of HbB corresponded to those of HbA in close proximity (within ± 0.1 ppm) except for carbons of *C*-acetylhexose (AT′) and a part of amicetose units (AM′) in HbB. Although carbon signals of HbA corresponding to AT′ in HbB were observed, differences of the chemical shifts between the carbon signals of HbB and HbA ranged from 0.24 up to 4.81 ppm. The differences were less than 0.08 ppm for AM1′, 2′ and 6′, while the differences were 0.15~0.56 ppm for AM3′, 4′ and 5′ which were closer to AT′ unit in HbB. These data strongly suggested that the aglycon of HbA and HbB was the same, and HbA was a regio- or stereoisomer of AT′ moiety of HbB.

Six anomeric protons were observed in the ^{1}H NMR spectrum of HbA. Four of them were identical with H-DG1, H-DG1′, H-AM1 and H-AT1 in HbB. COSY, CH-COSY and HMBC data also indicated that the structure of above four monosaccharides were preserved in HbA. It seemed that one of remainder anomeric protons at 4.444 ppm corresponded to H-AM1′ because coupling pattern was identical with H-AM1′ of HbB and the difference of the chemical shift between them was very small (0.016 ppm). The chemical shift perturbation may be explained by structural changes of adjacent AT′ moiety. The last anomeric proton of an unknown monosaccharide was observed at 4.706 ppm.

COSY, CH-COSY and HMBC correlations (Fig. 7) indicated that the anomeric proton connected to an ethylene $-CH_2-CH_2-$ unit and a methylated oxymethine $CH_3-CH-O-$ unit through an oxygen atom. The ethylene unit and the oxymethine were connected by a quaternary carbon (77 ppm) to which an acetyl group and a hydroxyl group were attached. The anomeric configuration was determined to be β since one of the coupling constants between the anomeric and adjacent methylene protons was *ca.* 8 Hz. Thus, we determined that the unknown monosaccharide was 4-*C*-acetyl-2,3,6-trideoxy-*β-threo*- or *erythro*-hexopyranose (βAT). The configuration of C-bAT4′ has not yet been determined.

NOE, NOESY and HMBC experiments suggested that α and βAT residues were linked to O-AM4 and O-AM4′, respectively, and AM and AM′ residues were linked to O-12 on ring H and O-12′ on ring A, respectively (Fig. 7); when anomeric protons of βAT and αAT were individually irradiated, NOE was observed at H-AM4′ (3.273 ppm) and H-AM4 (3.260 ppm), respectively. Difference between the chemical shifts of H-AM4 and H-AM4′ was very small but they were clearly distin-

Fig. 7. Selected COSY, HMBC and NOE correlations of the disaccharides moieties in hibarimicin A.



guished from each other. Spin-spin correlations between H-AM4 and H-AM1 (4.529 ppm), and between H-AM4′ and H-AM1′ (4.444 ppm) were proved by HOHAHA experiments. NOE between H-AM1 and H-12, and between H-AM1′ and H-12′ suggested that AM and AM′ units were attached to O-12 and O-12′, respectively (Fig. 7).

The structure of HbA was thus determined as shown in Fig. 11, based on the analysis mentioned above.

Hibarimicin C

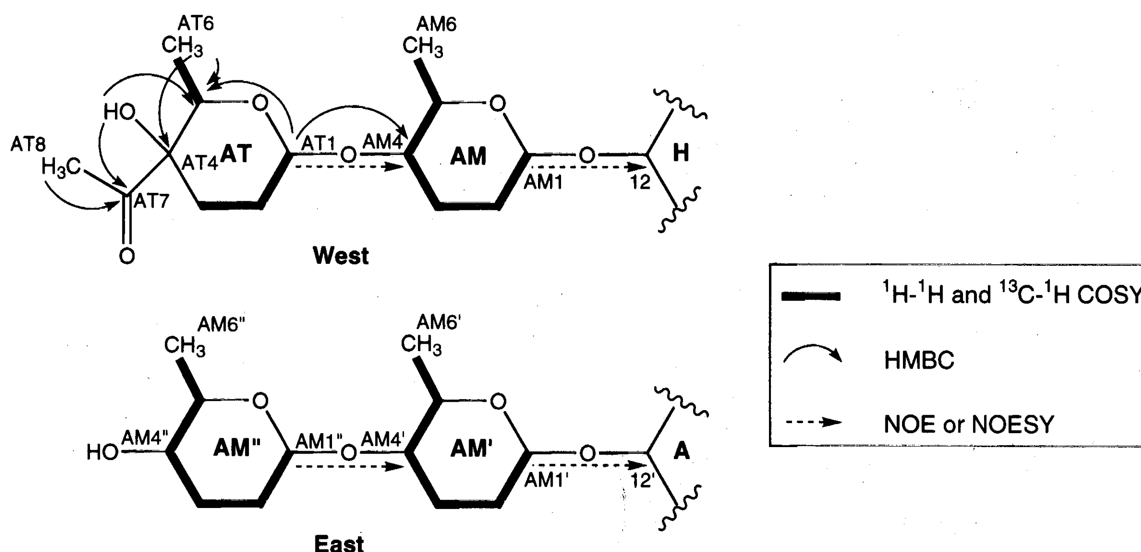
UV and IR spectra of HbC were identical with those of HbB. FAB-MS spectra showed peaks at $m/z=1683$ $[M]^+$, 1684 $[M+H]^+$ and 1706 $[M+Na]^+$ indicating that molecular weight of HbC was smaller than that of HbB by 42 m.u.. ^{13}C NMR and DEPT spectra of HbC showed the lack of an acetyl group on AT or AT′ residue in HbB. Furthermore, in the spectra of HbC, a signal corresponding to a quaternary oxycarbon C-AT or C-AT4′ in HbB was not observed, and an oxymethine carbon was observed at 78.62 ppm instead of the C-AT or C-AT4′. The molecular formula of HbC was determined to be $C_{83}H_{110}O_{36}$ based on the MS and NMR spectra.

^{1}H NMR spectrum of HbC supported the difference in the terminal carbohydrate moiety. The spectrum showed six anomeric protons. Four of them were identical with signals of H-DG1, H-DG1′, H-AT1 and H-AM1 of HbB while a signal corresponding to H-AM1′ was slightly shifted. A signal of an anomeric proton, whose coupling pattern was identical with H-AM1, was newly observed at 4.501 ppm instead of H-AT1′ of HbB. COSY, CH-COSY and HMBC correlations for the carbohydrate moiety (Fig. 8), indicated that HbC contained another 2,3,6-trideoxyhexopyranosyl residue (AM″) instead of AT′ residue in HbB. An orientation of the hydroxyl group at AM4″ was determined to be equatorial since a large coupling (*ca.* 10 Hz) between H-AM4″ and H-AM3″a was observed though a precise value was not obtained because of second order splitting. Configuration of AM5″ was not directly determined because the chemical shift of H-AM4″ and H-AM5″ were too close for first order analysis. If the stereochemical relationship between AM4″ and AM5″ was *threo*, the methyl group (AM6″) and the hydroxyl group at AM4″ would be oriented equatorial and axial, respectively. In this conformation, $J_{AM3''a,AM4''}$ should be much smaller than that we observed. Therefore, we determined that the monosaccharide was 2,3,6-trideoxy-*erythro*-hexopyranose (amicetose). The anomeric configuration was determined to be β because $J_{AM1'',AM2a''}$ was 9.0 Hz.

NOE between H-AM1″ and H-AM4′, and H-AM1′ and H-12 indicated that 4-*O*-(2,3,6-trideoxy-β-*erythro*-hexopyranosyl)-2,3,6-trideoxy-β-*erythro*-hexopyranosyl residue was attached to O-12′. We also observed NOE between H-AT1 and H-AM4, and H-AM1 and H-12, indicating that 4-*O*-(4-*C*-acetyl-2,3,6-trideoxy-α-*threo*-hexopyranosyl)-2,3,6-trideoxy-β-*eryhro*-hexopyranosyl residue was attached to O-12.

Therefore, the structure of HbC was determined as

Fig. 8. Selected COSY, HMBC and NOE correlations of the disaccharides moieties in hibarimicin C.



shown in Fig. 11.

Hibarimicin D

FAB-MS spectra of HbD showed peaks at $m/z=1741$ $[M]^+$, 1742 $[M+1]^+$ and 1764 $[M+Na]^+$ which were 16 m.u. larger than those of HbB, suggesting that HbD contained an additional oxygen atom, and its molecular formula was $C_{85}H_{112}O_{38}$. ^{1}H and ^{13}C NMR spectra also supported the molecular formula.

Most of ^{13}C signals of HbD were identical with those of HbB except for carbons of one of *C*-acetylhexose units, indicating that the structural difference between HbB and HbD was attributable to the structure of one of the terminal sugars. DEPT experiment revealed that a methylene carbon of AT or AT′ unit in HbB disappeared in the spectrum of HbD and an additional oxymethine (^{13}C 69.81 ppm, ^{1}H 3.616 ppm) was observed. In the ^{1}H NMR of HbD, six anomeric protons were observed, and five of them were identical with those of HbB, but an anomeric proton signal resonated at 5.094 ppm did not correspond to any ^{1}H signals of HbB.

COSY correlations (Fig. 9) starting from the anomeric proton at 5.094 ppm (H-AX1′) showed a partial structure O–(O–)CH–CH_2–CHOH–, in which the hydroxyl proton was confirmed by the addition of deuterium oxide. HMBC spectrum suggested that this C_3 unit and another unit CH_3–CH(OH)– were linked together through an oxygen atom. The other HMBC correlations also indicated that these two units were brigded by an acetylcarbinol to close a ring (Fig. 9). These data indicated that the sugar was 4-*C*-acetyl-2,6-dideoxyhexose. We abbreviated this sugar as AX.

Since coupling constants $J_{AX1',AX2'a}<1.0$, $J_{AX1',AX2'b}=3.5$, $J_{AX2a',AX3'}=3.0$ and $J_{AX2b',AX3'}=3.5$ were small, the anomeric configuration was α and the orientation of the hydroxyl group at AX3′ was axial. Orientation of the methyl group (AX6′) was not deduced from the coupling constant due to the lack of proton at AX4′. However, we concluded it was equatorial since if the methyl group adopted an axial configuration, three substituents, alkoxy, hydroxyl and methyl group in the sugar became 1,3,5-*syn*-triaxial relationship which was unfavorable thermodynamically, and the ring should be flipped. Thus, AX should be 4-*C*-acetyl-2,6-dideoxy-α-*xylo* or *ribo*-hexopyranose. Configuration at C-AX4′ could not be directly determined by NMR.

MATERN[6] has reported spectroscopic properties of methyl 4-*C*-acetyl-2,6-dideoxy-α-*xylo*-hexopyranoside prepared from quinocyclin B, an antibiotic produced by *Streptomyces aureofaciens*. In order to compare AX with Matern's glycoside, the α-methyl glycoside of AX was prepared by methanolysis of HbD. The ^{1}H NMR spectrum of AX methyl glycoside was identical with that of the reported glycoside prepared from quinocyclin B. Therefore, we concluded that AX was 4-*C*-acetyl-2,6-dideoxy-*xylo*-hexopyranose.

The glycosidic bonding position of AX was determined to be O-AM4′ by NOE and HOHAHA experiments; under the irradiation of H-AX1′, NOE was observed for a proton at 3.297 ppm (H-AM4′) to which magnetization

Fig. 9. Selected COSY, HMBC and NOE correlations of the carbohydrate moieties in hibarimicin D.

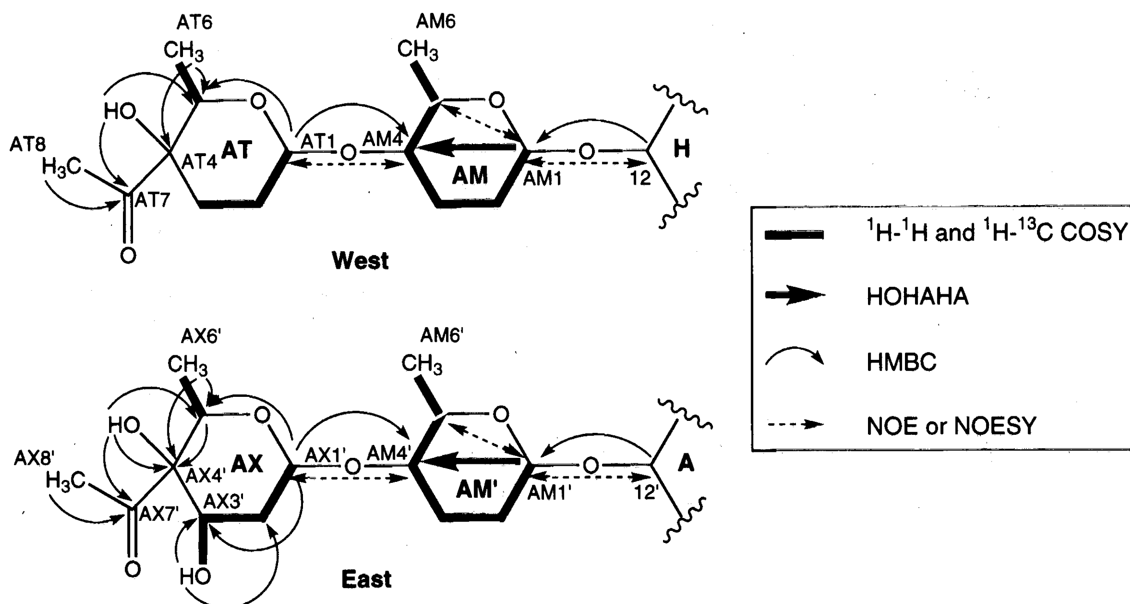
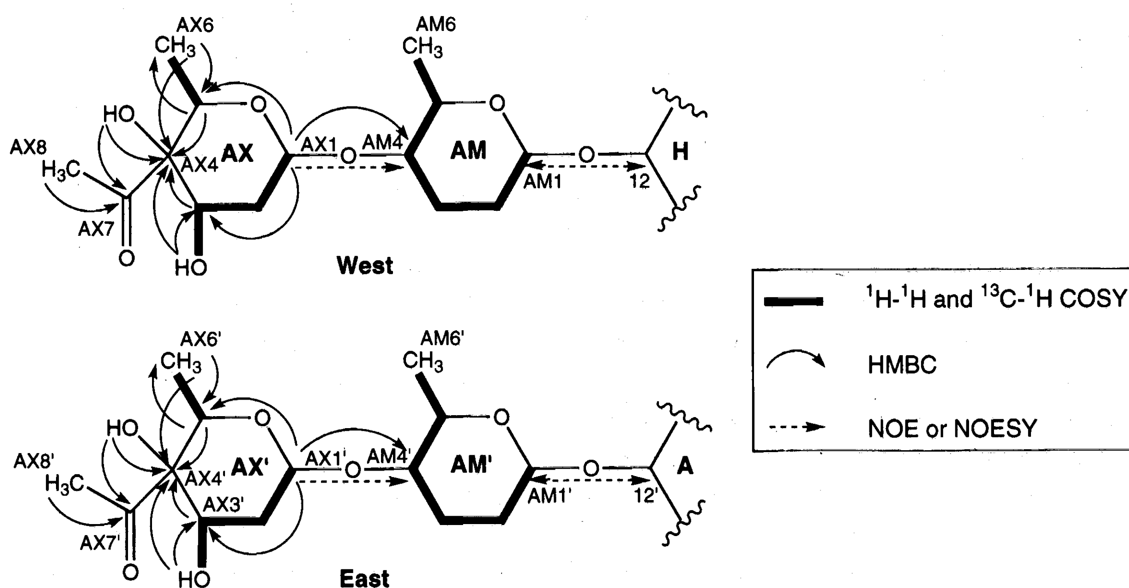


Fig. 10. Selected COSY, HMBC and NOE correlations of the carbohydrate moieties in hibarimicin G.



transfer was observed from an anomeric proton at 4.486 ppm (H-AM1′) by HOHAHA experiment. A correlation between the anomeric proton (H-AM1′) and H-12′ (ring A) was detected by NOESY experiment. These data indicated that 4-*O*-(4-*C*-acetyl-2,6-dideoxy-α-*xylo*-hexopyranosyl)-2,3,6-trideoxy-β-*erythro*-hexopyranosyl group was attached to O-12′.

We also observed NOEs between H-AT1 and H-AM4, and between H-AM1 and H-12 (H ring), and magnetization transfer from H-AM1 to H-AM4 in HOHAHA experiment (Fig. 9), indicating that 4-*O*-(4-*C*-acetyl-2,3,6-trideoxy-α-*threo*-hexopyranosyl)-2,3,6-trideoxy-β-*erythro*-hexopyranosyl group was attached to H-12.

Therefore, structure of HbD was determined as shown in Fig. 11.

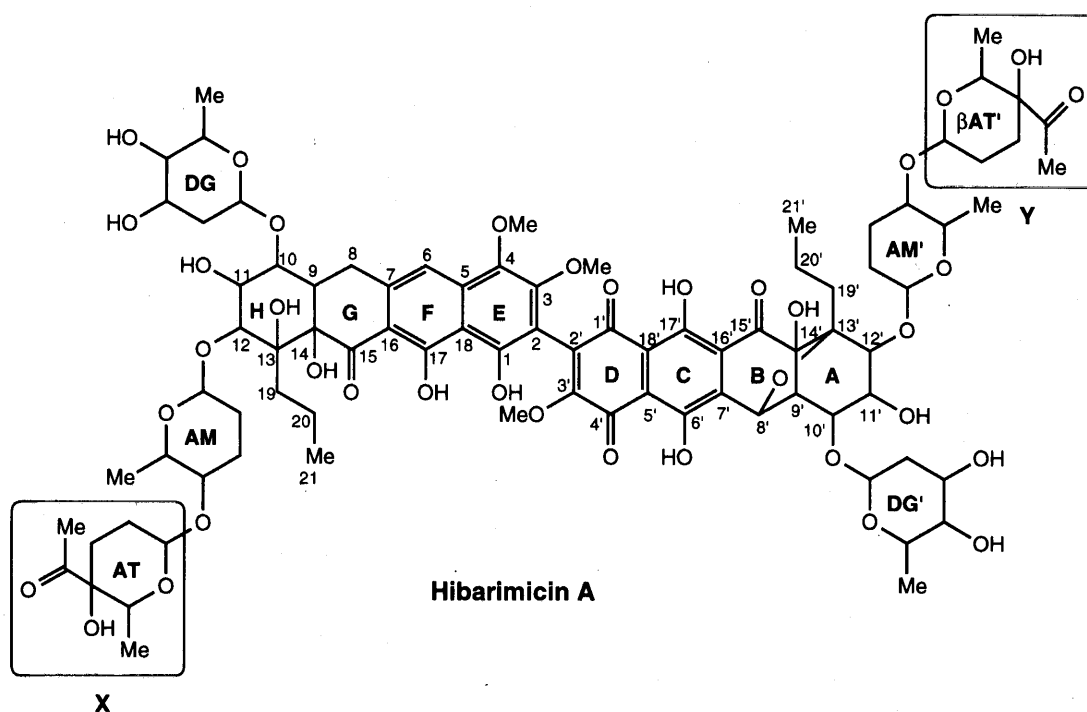
Hibarimicin G

UV and IR spectra of HbG were identical with those of HbB. FAB-MS spectra of HbG showed peaks at

m/*z*=1757 $[M]^+$, 1758 $[M+H]^+$, 1780 $[M+Na]^+$. These mass numbers were larger than those of HbB by 32 m.u., suggesting that HbG contained additional two oxygen atoms. The molecular formula of HbG was estimsted to be $C_{85}H_{112}O_{39}$ based on MS and NMR spectra. DEPT experiments also supported this formula; two methylene groups C-AT3 and C-AT3′ in HbB disappeared and additional two oxymethine were detected at 69.72 and 69.77 ppm in DEPT spectra of HbG. ^{13}C signals of HbG were identical with those of HbB except for AT and AT′ unit, and a part of AM and AM′ unit in HbB.

The ^{13}C signals which did not correspond to those of HbB were identical with those of the disaccharide residue, 4-*O*-(4-*C*-acetyl-2,6-dideoxy-*xylo*-hexopyranosyl)-2,3,6-trideoxy-*erythro*-hexopyranosyl group, in HbD. Duplicated ^{13}C signals corresponding to the disaccharide (AX-AM) residue were observed in HbG, indicating that both terminal AT residues in HbB were exchanged with AX residues in HbG. This structure was also supported by ^1H NMR. Anomeric region of ^1H NMR of HbG showed overlapped signals of H-AX1/H-AX1′ at 5.084 ppm and H-AX5/H-AX5′ at 4.615/4.621 ppm which were characteristic signals for AX. Other NMR data such as COSY, CH-COSY, HMBC, NOESY and NOE summarized in Fig. 10 also agreed with that structure.

Fig. 11. Structures of hibarimicins A, B, C, D and G.



	HibarimicinA	HibarimicinB	HibarimicinC	HibarimicinD	HibarimicinG
X	AT (Me, OH, O, Me)	AT (Me, OH, O, Me)	AT (Me, OH, O, Me)	AT (Me, OH, O, Me)	AX' (Me, OH, O, Me, OH)
Y	βAT' (Me, OH, O, Me)	AT' (Me, OH, O, Me)	AM" (Me, OH)	AX' (Me, OH, O, Me, OH)	AX' (Me, OH, O, Me, OH)

Abbreviations (AT, βAT, AM, AX and DG); see Fig. 1.

Therefore, the structure of HbG was determined as shown in Fig. 11.

In conclusion, we determined the structures of hibarimicins B, C, D, and G, specific inhibitor of *src* tyrosine kinase. Hibarimicin B is identical with angelmicin B isolated by us. The other components hibarimicin A, C, D and G are new compounds. Their absolute configurations are now under investigation and will be reported elsewhere.

Experimental

Analytical Methods

TLC analysis was carried out using Merck Silica-gel 60 F_{254} and RP-18 F_{254}. UV spectra of hibarimicins were measured in ethanol, ethanol - 0.1 M hydrochloric acid (9 : 1) and ethanol - 0.1 M aq. sodium hydroxide (9 : 1) on Shimadzu UV-160A UV-visible recording spectrometer. IR spectra were measured on Shimadzu IR-420 infrared spectrometer. Optical rotations were taken by HORIBA SEPA-300 high sensitive polarimeter. FAB-mass spectra were measured on JEOL JMS-HX110 or JMS-AX505 using *m*-nitrobenzyl alcohol as an matrix. NMR spectra were measured in chloroform-*d* at 400 and 270 MHz for ^{1}H, and 100.5 and 67.8 MHz for ^{13}C on JEOL JNM-GX400 and GX270 spectrometers.

Hibarimicins

Hibarimicin A, B, C, D and G were purified from fermentation broth of *Microbispora rosea* subsp. *hibaria* as described in previous paper[1].

Preparation and Purification of Methyl Glycoside of 4-*C*-Acetyl-2,3,6-trideoxyhexose from Hibarimicin

Crude hibarimicins mixture (70 mg) was dissolved in 3% methanolic hydrogen chloride (1.2 ml) at room temperature. After 2 hours, the solvent and hydrogen chloride were removed by flushing dry nitrogen stream and azeotropic evaporation with benzene. The residue was applied to preparative TLC (Merck Silica gel 60 F_{254}, 200 × 200 mm, toluene - ethyl acetate 3 : 2). A band (Rf = 0.42) was scratched off the plate and extracted with ethyl acetate (30 ml × 3). The extract was concentrated to a syrup, which was further purified by HPLC (column; SHISEIDO CAPCELL PAK C18 (S-5 mm, i.d. 4.6 × 300 mm), elution; methanol - water 4 : 5, flow rate; 0.7 ml/minute, detection; A220 nm). Concentration of the eluate gave an anomeric mixture of methyl 4-*C*-acetyl-2,3,6-trideoxy-D-*threo*-hexopyranosides.

Preparation of 9-Acetyl-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-7-oxonaphthacene-5,12-quinone

Daunorubicin (80 mg) was dissolved in 2.8% methanolic hydrochloric acid, and was kept for 4 hours at 50°C. After evaporation, trace of hydrochrolic acid was removed by azeotropic evaporation with benzene. The residue was applied to silica-gel column chromatography (toluene - ethyl acetate 1 : 1) to give daunomicinone (Rf = 0.31, toluene - ethyl acetate 1 : 1) 25.6 mg (45%).

To a solution of daunomycinone (12.8 mg) in dichloromethane (1 ml) was added pyridinium dichromate (30 mg). After 20 hours, the solvent was removed by evaporation and the residue was applied to preparative silica-gel TLC (toluene - ethyl acetate 1 : 1). A band of the oxidative product (Rf = 0.44) was scratched off the plate and was extracted with ethyl acetate.

The solvent was removed by evaporation to give 9-acetyl-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-7-oxonaphthacene-5,12-quinone (2.5 mg, 9.8%). The reaction conditions were not optimized. FAB-MS (positive) *m/z* 397 $[M+H]^+$, 419 $[M+Na]^+$, 353 $[M-43]^+$, ^{1}H NMR ($CDCl_3$) δ: 14.444 (1H, s, 6-OH), 13.088 (1H, s, 11-OH), 8.030 (1H, dd, $J_{1,3}=1.1$, $J_{1,2}=7.7$ Hz, H-1), 7.801 (dd, 1H, $J_{1,2}=7.7$ Hz, H-2), 7.431 (1H, dd, $J_{1,3}=1.1$, $J_{2,3}=8.4$ Hz, H-3), 4.093 (3H, s, 4-OCH_3), 3.720 (1H, s, 9-OH), 3.380 (1H, dd, $J_{8a,10a}<2.0$, $J_{10a,10b}=18.3$ Hz, H-10a), 3.313 (1H, d, $J_{10a,10b}=18.3$ Hz, H-10b), 3.143 (1H, d, $J_{8a,8b}=15.4$ Hz, H-8a), 2.789 (1H, dd, $J_{8b,10a}=2.0$ Hz, $J_{8a,8b}=15.4$ Hz, H-8b), 2.426 (3H, s, CH_3CO), ^{13}C NMR ($CDCl_3$) δ: 207.86 (C-13), 192.53 (C-7), 187.45, 186.42 (C5, 12), 161.19 (C-4), 157.45 (C-6), 153.54 (C-11), 140.17 (C-10a), 135.78 (C-2), 134.80 (C-1a), 127.26 (C-6a), 121.02 (C-4a), 119.77 (C-1), 119.05 (C-3), 115.25 (C-11a), 113.47 (C-5a), 78.25 (C-9), 56.75 (CH_3O-4), 47.93 (C-8), 32.83 (C-10), 24.04 (C-14).

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