RESPINOMYCINS A1, A2, B, C AND D, A NOVEL GROUP OF ANTHRACYCLINE ANTIBIOTICS

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION

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Respinomycins A1, A2, B, C and D were revealed to be novel anthracycline antibiotics with molecular formulae of $C_{51}H_{72}N_2O_{20}$, $C_{43}H_{58}N_2O_{15}$, $C_{35}H_{43}NO_{14}$, $C_{36}H_{45}NO_{14}$ and $C_{51}H_{70}N_2O_{22}$, respectively. Their structures were determined by means of ^1H-^1H COSY, ^{13}C-^1H COSY and HMBC spectra. The structure of the aglycone of respinomycins was unambiguously determined by LSPD experiments and NOESY. The common skeleton of respinomycins is a new type and is distinguished from that of the nogalamycin group.

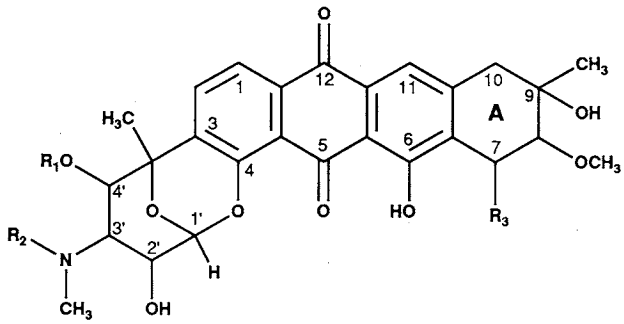
In the preceding paper,[1] we have reported the isolation and biological activities of novel antibiotics respinomycins A1, A2, B, C and D produced by *Streptomyces xanthocidicus*. Respinomycins A1 and A2 induced the terminal differentiation of human leukemia K-562 cells. Especially, respinomycin A2 showed a high differentiation induction rate. Unlike the other respinomycins, respinomycin A2 showed a strong anti-phage activity against actinophage B of *Streptomyces griseus* and only a weak antimicrobial activity against Gram-positive bacteria. On the contrary, respinomycin D showed the strongest cytotoxicity among these antibiotics. The common skeleton of respinomycins is a new type and distinguished from that of the nogalamycin group such as nogalamycin,[2] arugomycin,[3] decilorubicin,[4] viriplanin[5] and avidinorubicin.[6] In this paper, we report the physico-chemical properties and the structure determination of respinomycins A1, A2, B, C and D (Fig. 1). The absolute configurations remain to be determined.

Results and Discussion

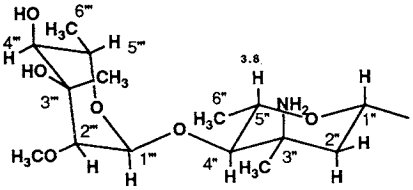
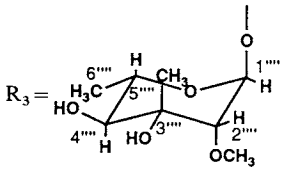
Physico-chemical Properties

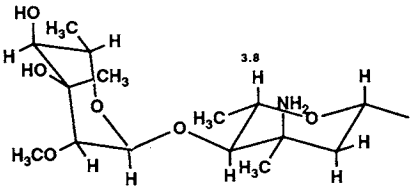
The physico-chemical properties of respinomycins are summarized in Table 1. Respinomycins are yellow powders and are optically active. The molecular formulae were established to be $C_{51}H_{72}N_2O_{20}$ for respinomycin A1, $C_{43}H_{58}N_2O_{15}$ for respinomycin A2, $C_{35}H_{43}NO_{14}$ for respinomycin B, $C_{36}H_{45}NO_{14}$ for respinomycin C, and $C_{51}H_{70}N_2O_{22}$ for respinomycin D on the basis of SI-MS, HRFAB-MS and the total number of carbons detected by ^{13}C-^1H COSY. The UV and visible absorption peaks were observed at approximately 230, 265, 290, 400, 415, and 440 nm and the last three peaks shifted to 520 nm in an

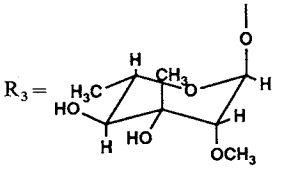
Fig. 1. The structure of respinomycins A1, A2, B, C and D.

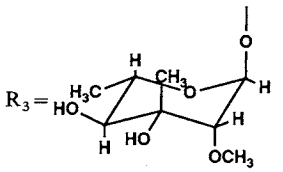


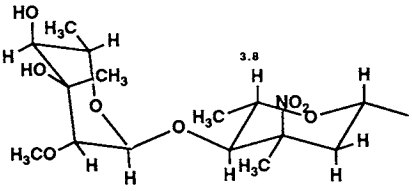
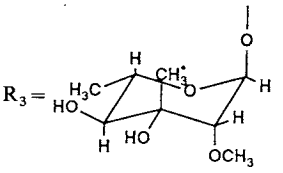
S2 S1 S3

A1: R_1 =  $R_2 = CH_3$ R_3 = 

A2: R_1 =  $R_2 = CH_3$ $R_3 = H$

B: $R_1 = H$ $R_2 = H$ R_3 = 

C: $R_1 = H$ $R_2 = CH_3$ R_3 = 

D: R_1 =  $R_2 = CH_3$ R_3 = 

alkaline solution. These observation indicated the presence of a hydroxyanthracycline chromophore in respinomycins. The IR spectra of respinomycins indicated the presence of hydroxyl groups (approximately 3400 cm^{-1}) and hydroxyanthraquinone (approximately 1670 and 1625 cm^{-1}). The IR spectrum of respinomycin D showed an absorption band ascribed to nitro group at 1545 cm^{-1}. This observation is

Table 1. Physico-chemical properties of respinomycins.

	A1	A2	B	C	D
Appearance	Yellow powder	Yellow powder	Yellow powder	Yellow powder	Yellow powder
MP (dec)	>250°C	>202°C	>181°C	>190°C	>210°C
$[\alpha]_D^{18}$	+174° (*c* 0.28, $CHCl_3$)	+213° (*c* 0.1, $CHCl_3$)	+207° (*c* 0.1, $CHCl_3$)	+187° (*c* 0.09, $CHCl_3$)	+191° (*c* 0.31, $CHCl_3$)
FAB-MS (*m/z*)	1,033 $(M+H)^+$, 1,055 $(M+Na)^+$	843 $(M+H)^+$, 865 $(M+Na)^+$	702 $(M+H)^+$	716 $(M+H)^+$, 754 $(M+K)^+$	1,063 $(M+H)^+$
High-resolution FAB-MS (*m/z*)	$C_{51}H_{72}N_2O_{20}$ 1,033.4761 $(M+H)^+$, Δ 0.4 mmu	$C_{43}H_{58}N_2O_{15}$ 843.3942 $(M+H)^+$, Δ 2.6 mmu	$C_{35}H_{43}NO_{14}$ 702.2777 $(M+H)^+$, Δ 1.5 mmu	$C_{36}H_{45}NO_{14}$ 716.2952 $(M+H)^+$, Δ 3.3 mmu	$C_{51}H_{70}N_2O_{22}$ 1,063.4491 $(M+H)^+$, Δ −0.8 mmu
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) in MeOH	220 (114.4), 265 (64.8), 290 (sh, 24.0), 395 (sh, 23.2), 415 (26.4), 440 (sh, 20.8)	230 (390), 265 (310), 290 (sh, 85), 400 (sh, 105), 415 (120), 440 (sh, 97.9)	230 (485), 262 (320), 285 (sh, 115), 395 (sh, 110), 415 (135), 435 (sh, 110)	230 (360), 265 (235), 285 (sh, 80), 400 (sh, 90), 415 (105), 440 (sh, 85)	230 (261.5), 265 (161.5), 285 (sh, 61.5), 400 (sh, 61.5), 415 (71), 440 (sh, 57.7)
IR (KBr) cm^{-1}	3410, 2910, 1675, 1630, 1580, 1410, 1255, 1095, 965, 940	3450, 2950, 1670, 1625, 1580, 1420, 1245, 1090, 1065, 1040	3420, 2940, 1670, 1625, 1580, 1415, 1245, 1080, 960	3340, 2940, 1670, 1625, 1580, 1420, 1250, 1085, 960	3420, 2940, 1660, 1630, 1580, 1545, 1420, 1250, 1090, 960

consistent with the difference between the molecular formula of respinomycin A1 and that of respinomycin D.

Respinomycins are soluble in MeOH, DMSO, $CHCl_3$, EtOAc and acidic H_2O, but insoluble in neutral or alkaline H_2O and hexane. They give positive reactions with anisaldehyde-H_2SO_4 and I_2 but are negative to a ninhydrin test.

Structure of Respinomycin A1

The structure of the aglycone in respinomycin A1 was deduced from ^1H-^1H COSY, ^{13}C-^1H COSY and heteronuclear multiple bond correlation (HMBC) spectra. Tables 2 and 3 summarize the assignments from ^1H NMR and ^{13}C NMR spectra of respinomycins, respectively. In the HMBC spectrum, long range C-H correlations depicted in Figs. 2 and 3 were observed. As shown in Fig. 2, a pair of *ortho*-coupled protons at δ 7.5 and 7.9 are correlated to their relevant *meta* carbons (δ 134.7, 153.5; 119.5, 131.6, respectively). The former is coupled to a quaternary carbon (δ 77.2) and the latter to one of the quinone carbonyl carbons (δ 182.2). In addition, a singlet proton at δ 7.49 coupled to its relevant *meta* carbons (δ 115.1, 128.9) is coupled to the same quinone carbonyl carbon (δ 182.2). The proton is also coupled to a methylene carbon (δ 40.8). The corresponding methylene protons (δ 2.8, 3.05) are coupled to three carbons (δ 23.5, 68.2, 145.1). A benzylic oxymethine proton at δ 5.0 is also correlated to the last two carbons (δ 68.2, 145.1). In addition, the same proton is coupled to two carbons (δ 128.9 and 163.2). A doublet proton at δ 3.48 coupled with the benzylic proton at δ 5.0 is correlated to the quanternary carbon at δ 68.2. As shown in Fig. 3, an anomeric proton at δ 6.04 is coupled to the aromatic carbon at δ 153.5. The coupling data from ^1H-^1H COSY revealed the correlation among the three protons (δ 4.32, 2.78 and 4.2). A methyl proton at δ 1.68 is coupled to two carbons at δ 78 and 131.6. From these data, the structure of aglycone of respinomycin A1 was determined as shown in Fig. 4. Moreover, the unique skeleton of respinomycin A1 was confirmed by NOESY and Long-range Selective Proton Decoupling (LSPD) experiments. Nuclear

Table 2. 400 MHz ^{1}H NMR spectral data of respinomycins.

Position	A1	A2	B	C	D
1	7.9 (d, J=8.4 Hz)	7.88 (d, J=8.4 Hz)	7.9 (d, J=8.06 Hz)	7.9 (d, J=8.0 Hz)	7.94 (d, J=8.7 Hz)
2	7.5 (d, J=8.4 Hz)	7.48 (d, J=8.4 Hz)	7.55 (d, J=8.06 Hz)	7.54 (d, J=8.0 Hz)	7.57 (d, J=8.7 Hz)
7	5.0 (d, J=2.1 Hz)	2.93 (dd, J=4.8, 18.8 Hz), 3.11 (dd, J=4.8, 18.8 Hz)	5.0 (d, J=2.2 Hz)	5.0 (d, J=2.2 Hz)	5.02 (d, J=2.2 Hz)
8	3.48 (d, J=2.1 Hz)	3.47 (t, J=4.8 Hz)	3.5 (d, J=2.2 Hz)	3.49 (d, J=2.2 Hz)	3.52 (d, J=2.2 Hz)
OCH_3	3.61 (s)	3.49 (s)	3.63 (s)	3.63 (s)	3.62 (s)
10	2.8 (d, J=17.5 Hz)	2.84 (d, J=17.5 Hz)	2.85 (d, J=17 Hz)	2.84 (d, J=16.8 Hz)	2.86 (d, J=17.6 Hz)
13	1.35 (s)	1.37 (s)	1.35 (s)	1.35 (s)	1.37 (s)
1′	6.04 (d, J=3.8 Hz)	6.06 (d, J=3.5 Hz)	5.85 (d, J=3.4 Hz)	5.86 (d, J=4 Hz)	6.05 (d, J=3.7 Hz)
2′	4.32 (dd, J=3.8, 12.2 Hz)	4.3 (dd, J=3.5, 11.8 Hz)	3.87 (dd, J=3.4, 10 Hz)	4.15 (dd, J=4, 9.9 Hz)	4.33 (dd, J=3.7, 12 Hz)
3′	2.78 (dd, J=12.2, 3.6 Hz)	2.76 (dd, J=11.8, 3.1 Hz)	2.4 (dd, J=10, 3.4 Hz)	2.16 (dd, J=9.9, 3.3 Hz)	2.77 (dd, J=12, 2.9 Hz)
4′	4.2 (d, J=3.6 Hz)	4.2 (d, J=3.1 Hz)	3.7 (d, J=3.4 Hz)	3.78 (d, J=3.3 Hz)	4.26 (d, J=3.1 Hz)
6′	1.68 (s)	1.66 (s)	1.8 (s)	1.8 (s)	1.68 (s)
N-CH_3	2.43 (s×2)	2.46 (s×2)	2.28 (s)	2.43 (s×2)	2.46 (s×2)
1″	5.17 (dd, J=2.6, 9.9 Hz)	5.15 (dd, J=1.9, 9.9 Hz)	—	—	5.54 (dd, J=2.9, 9.4 Hz)
2″	1.62 (dd, J=9.9, 13.8 Hz), 1.95 (dd, J=2.6, 13.8 Hz)	1.63 (dd, J=9.9, 13.95 Hz) 1.95 (dd, J=1.9, 13.95Hz)	—	—	1.87 (dd, J=9.4, 15 Hz), 2.64 (dd, J=2.9, 15 Hz)
3″-CH_3	1.3 (s)	1.29 (s)	—	—	1.8 (s)
4″	3.16 (d, J=10.5 Hz)	3.16 (d, J=9.8 Hz)	—	—	3.39 (d, J=9.7 Hz)
5″	3.95 (m)	3.93 (m)	—	—	3.8 (m)
6″	1.3 (d, J=6.7 Hz)	1.33 (d, J=6.7 Hz)	—	—	1.38 (d, J=6.6 Hz)
1‴	4.96 (d, $J\leq$1 Hz)	4.97 (d, $J\leq$1 Hz)	—	—	4.97 (d, $J\leq$1 Hz)
2‴	3.22 (d, $J\leq$1 Hz)	3.22 (d, $J\leq$1 Hz)	—	—	3.22 (d, $J\leq$1 Hz)
2‴-OCH_3	3.48 (s)	3.48 (s)	—	—	3.47 (s)
3‴-CH_3	1.33 (s)	1.33 (s)	—	—	1.23 (s)
4‴	3.4 (d, J=8.4 Hz)	3.41 (d, J=9.8 Hz)	—	—	3.36 (d, J=9.7 Hz)
5‴	3.8 (m)	3.77 (m)	—	—	3.8 (m)
6‴	1.38 (d, J=6.7 Hz)	1.29 (d, J=6.7 Hz)	—	—	1.39 (d, J=6.7 Hz)
1⁗	5.6 (d, $J\leq$1 Hz)	—	5.6 (d, $J\leq$1 Hz)	5.6 (d, $J\leq$1 Hz)	5.65 (d, $J\leq$1 Hz)
2⁗	3.15 (d, $J\leq$1 Hz)	—	3.16 (d, $J\leq$1 Hz)	3.17 (d, $J\leq$1 Hz)	3.17 (d, $J\leq$1 Hz)
2⁗-OCH_3	3.57 (s)	—	3.58 (s)	3.58 (s)	3.57 (s)
3⁗-CH_3	1.17 (s)	—	1.2 (s)	1.2 (s)	1.2 (s)
4⁗	3.43 (d, J=8.4 Hz)	—	3.44 (d, J=8.5 Hz)	3.44 (d, J=9.5 Hz)	3.43 (d, J=9.4 Hz)
5⁗	3.78 (m)	—	3.8 (m)	3.8 (m)	3.83 (m)
6⁗	1.28 (d, J=6.7 Hz)	—	1.37 (d, J=6.1 Hz)	1.37 (d, J=6.0 Hz)	1.34 (d, J=6.7 Hz)

Spectra recorded at 400 MHz in $CDCl_3$. Chemical shifts in ppm referenced to TMS at 0 ppm as internal standard. s=singlet, d=doublet, dd=double of doublet, m=multiplet.

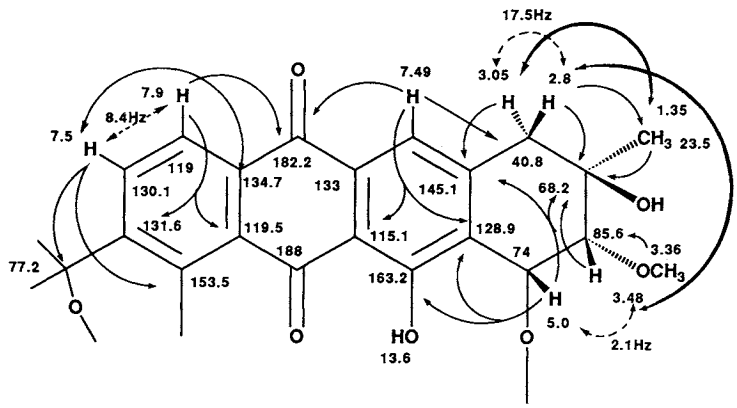
Overhauser effects (NOEs) between 11-H and 10-CH_2, and 2-H and 6′-CH_3 were observed. Irradiation of the aromatic proton H-11 or H-1 changed the triplet signal at δ 182.2 of C-12 to a doublet, and the coupling constants of both long range C-H were 3.7 Hz. Furthermore, weak W-type coupling between 11-H and C-5, and 1-H and C-5 were observed (Fig. 4). These data clearly ruled out the possibility of the nogalamycin type skeleton. The relative configuration of aglycone was deduced from the NOESY data and the coupling

Table 3. 100 MHz ^{13}C NMR spectral data of respinomycins.

Position	A1	A2	B	C	D	Position	A1	A2	B	C	D
1	119.4	119.5	119.4	119.5	119.4	5′	77.2	77.2	75	75	76
2	130.1	130	131.1	131.2	130.2	6′	23	23	22.2	22	23
3	131.6	133.4	133.1	132.9	133	1″	99.2	99.2	—	—	99.5
4	153.5	153.4	153	152.7	153	2″	45	45	—	—	44.2
4a	119.5	119.5	119.4	119.3	119	3″	52	52	—	—	88.5
5	188	188	188.6	188.5	188	3″-CH_3	31.6	31.6	—	—	25.1
5a	115.1	114.1	115	115	115	4″	87.5	87.6	—	—	84.8
6	163.2	161	163.2	163	163	5″	70.1	70	—	—	69.1
6a	128.9	129.8	129	128.9	128.9	6″	18.7	18.7	—	—	18.7
7	74	25.3	74	74	74	1‴	99.4	99.2	—	—	100.4
8	85.6	81	85.6	85.6	85.6	2‴	84.8	85	—	—	84.7
8-OCH_3	58.6	58.9	58.6	58.6	58.6	2‴-OCH_3	59	57.2	—	—	59.1
9	68.2	70	69.2	69.2	68.7	3‴	79.2	72.1	—	—	72
10	40.8	41.3	40.9	40.7	40.7	3‴-CH_3	17.68	17.6	—	—	17.1
10a	145.1	144	145.8	145.6	145.3	4‴	75.8	75.8	—	—	75.8
11	119.1	119.3	119.9	119.4	119.2	5‴	67.9	68	—	—	68.7
11a	133.3	133.4	132.5	131.5	133.3	6‴	18.0	17.6	—	—	18.1
12	182.2	182.3	182	181.9	182	1⁗	101.6	—	101.7	101.6	101.6
13	31.9	31.6	23.6	23.6	23.6	2⁗	84.4	—	84.4	84.3	84.4
12a	134.7	134.7	134.7	134.6	134.7	2⁗-OCH_3	59.2	—	59.1	59.2	59.1
1′	96.7	96.6	96	96.2	96.5	3⁗	72	—	72	72	72
2′	66.2	66.3	69.5	67.9	66.2	3⁗-CH_3	17.3	—	17	17.3	17.2
3′	60.5	60.7	60.0	64.2	60.8	4⁗	76	—	76.3	76.3	75.8
N-CH_3	39	39.3	33	43.2	39.5	5⁗	68.2	—	68	67.9	67.9
4′	78	78.2	70.9	72.5	78.8	6⁗	17.7	—	18.1	18.1	17.7

Chemical shifts in ppm referenced to TMS at 0 ppm as internal standard.

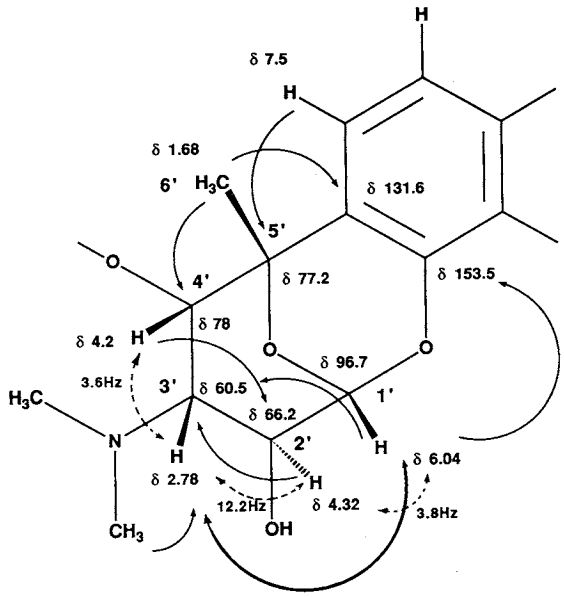
Fig. 2. 2D NMR experiments of respinomycin A1 and the relative configuration of ring A.



←→ ^1H-^1H coupling detected by COSY, ⟶ ^1H-^{13}C long range coupling, ⟷ NOE.

constants. The large coupling constant ($J_{2',3'} = 12.2$ Hz) between 2′-H and 3′-H showed that those protons exist as *trans diaxial*. NOE between 1′-H and 3′-H suggested the fused dimethylamino sugar moiety of the aglycone has the relative configuration as shown in Fig. 3. In the case of the A ring, NOEs between the higher field proton (δ 2.8) of C-10 methylene and C-8 methine proton at δ 3.48, and the lower field proton (δ 3.05) of C-10 methylene and C-9 methyl proton at δ 1.35 were observed, and the coupling constant

Fig. 3. 2D NMR experiments of respinomycin A1 and the relative configuration of the fused dimethylamino sugar.

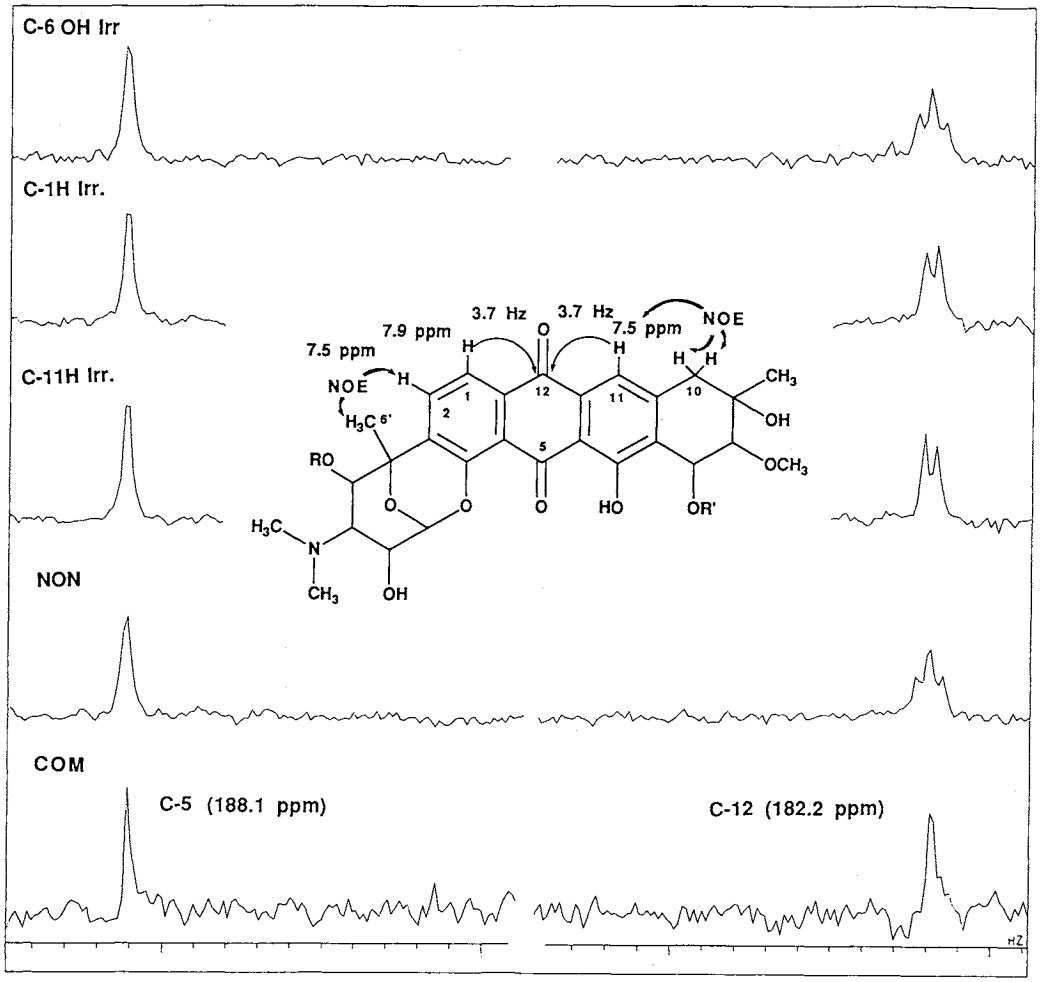


←--→ ^{1}H-^{1}H coupling, ⟶ ^{1}H-^{13}C long range coupling, ⟷ NOE.

between 7-H and 8-H was rather small ($J_{7,8}=2.1$ Hz). These observations suggested that the A ring possesses the relative configuration as shown in Fig. 2, and the proton at δ 2.8, methyl at C-9, methoxy at C-8 and glycoside at C-7 exist in *pseudo axial*, *pseudo axial*, *pseudo equatorial* and *pseudo axial*, respectively.

In ^{1}H NMR spectrum, three other anomeric protons at δ 5.6, 5.17 and 4.96 in addition to the anomeric proton of the aglycone were observed. From the chemical shifts, the coupling constants, NOE and HMBC data, the structures of the three sugars moieties were deduced as shown in Fig. 5 and Fig. 6. The amino sugar was determined to be 3-amino-2,3,6-trideoxy-3-*C*-methyl-*β*-*ribo*-hexopyranoside (S1), and the other two sugar units (S2 and S3) were both determined to be 6-dideoxy-3-*C*-methyl-2-*O*-methyl-*α*-*manno*-hexopyranoside. The amino sugar (S1) was a known sugar which had been synthesized as an intermediate of decilonitrose,[7,8] and more recently it was identified as a sugar component in avidinorubicin,[6] designated as avidinosamine and determined to be 3-amino-2,3,6-trideoxy-3-*C*-methyl-L-*ribo*-hexopyranoside. The ^{1}H and ^{13}C NMR data of the amino sugar in the literature are comparable with those of the amino sugar in respinomycin A1. The anomeric configuration of S1 was determined to be *β*, based on the large coupling constants which indicate the presence of *trans diaxial* proton; $J_{1'',2''ax}=9.9$ Hz, $J_{1'',2''eq}=2.6$ Hz. C-H long-range coupling between H-4′ and C-1″, and H-1″ and C-4′ reveal that S1 is linked at the C-4′ position. The neounits (S2 and S3) of the new sugar component have the same relative configuration as nogalose. The anomeric configurations of both S2 and S3 were estimated to be *α*, based on the small anomeric coupling constant, $J\leq 1$ Hz. C-H long-range couplings between H-7 and C-1‴, H-1‴ and C-7 showed that S3 was attached to the C-7 position (Fig. 6). In the same way, the attachment of S2 to C-4″ of S1 was determined by C-H long-range coupling detected by HMBC as shown in Fig. 5. The relative configurations of S2 and S3 were also confirmed by NOE data of respinomycin A2 and respinomycin B, respectively.

Fig. 4. LSPD experiments of respinomycin A1 (400 MHz, $CDCl_3$).

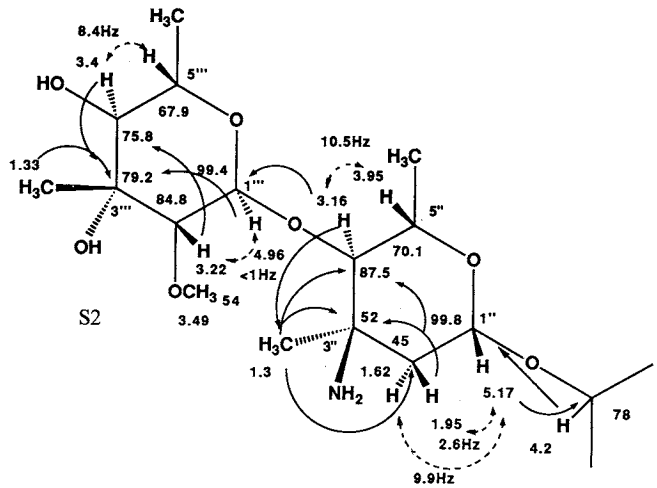


COM: complete decoupling, NON: nondecoupling, C-11 H Irr: irradiation at δ 7.5 ppm, C-1 H Irr: irradiation at δ 7.9 ppm, C-6 OH Irr: irradiation at δ 13.8 ppm.

Structure of Respinomycin A2

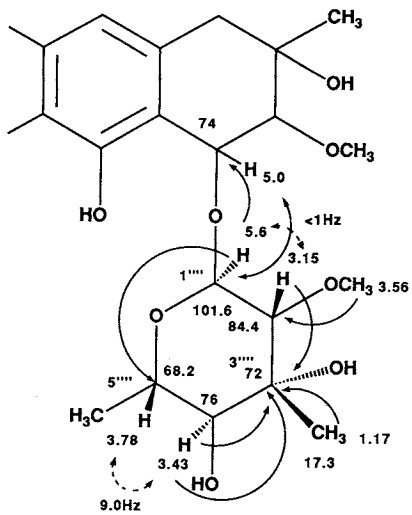
The structure of respinomycin A2 was established by a comparison with respinomycin A1. As shown in Tables 2 and 3, ^1H and ^{13}C NMR chemical shifts of respinomycin A2 are quite similar to those of respinomycin A1 except for the following protons and carbons in the A ring: 1) The benzylic oxymethine proton (C-7-CH) at δ 5.0 observed in respinomycin A1 is replaced by the benzylic methylene protons (C-7-CH_2) at δ 2.93 and 3.11 in the case of respinomycin A2, 2) the benzylic carbon (C-7) shifts upfield from δ 74 for respinomycin A1 to δ 25.3 for respinomycin A2. These chemical shifts and coupling pattern in the A ring suggested that respinomycin A2 is a 7-deoxy derivative of respinomycin A1. This consideration was further supported by ^1H-^1H COSY, ^{13}C-^1H COSY and HMBC spectra of respinomycin A2. The relative configurations of S1 and S2 were determined by the chemical shift data, the coupling constant data and the NOE data. The anomeric configuration of S2 was estimated to be α from the anomeric coupling constant and confirmed by the data of NOEs between 3‴-CH_3 and 2‴-H/5‴-H

Fig. 5. 2D NMR experiments of respinomycin A1 and the relative configuration of S1 and S2 of respinomycin A1.



⟶ ^{1}H-^{1}H coupling, ⟶ ^{1}H-^{13}C long range coupling.

Fig. 6. 2D NMR experiments of respinomycin A1 and the relative configuration of S3 of respinomycin A1.



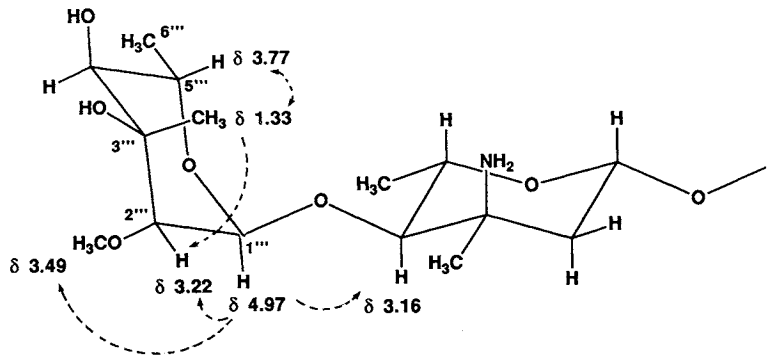
⟶ ^{1}H-^{1}H coupling, ⟶ ^{1}H-^{13}C long range coupling.

but not between 3‴-CH_3 and 1‴-H, and NOEs between 1‴-H and 2‴-H/2‴-OCH_3 as shown in Fig. 7.

Structures of Respinomycin B and C

In the ^{1}H and ^{13}C NMR spectra of respinomycin B and C, peaks corresponding to signals of the sugar chain (S1 and S2) attached to C-4′ were absent, and the chemical shifts at C-4′ changed from δ 78 for respinomycin A1 to δ 70.9 and 72.5 for respinomycin B and C, respectively (Tables 2 and 3). These observations and the molecular formulae for respinomycin C ($C_{36}H_{45}NO_{14}$ = respinomycin A1 − S1 − S2) and respinomycin B ($C_{35}H_{43}NO_{14}$ = respinomycin A1 − S1 − S2 − CH_2) suggest the structures of respinomycin C and B to be as shown in Fig. 1. Furthermore, only three protons corresponding to N-CH_3 attached to C-3′ were observed in the ^{1}H NMR of respinomycin B. Upfield shifts of N-CH_3 and C-3′ in the ^{13}C NMR of respinomycin B also reasonably explained the structure of respinomycin B which has N-CH_3 group instead of CH_3-N-CH_3 group. Further evidence for the structures of respinomycin B and C was obtained from ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and HMBC. The anomeric configuration of S3 which was estimated to be α form from the small coupling constant ($J \leq 1$ Hz) of the anomeric proton was confirmed by the presence of NOEs between 3⁗-CH_3 and 5⁗-H/2⁗-H

Fig. 7. NOE experiments of respinomycin A2 and the relative configuration of S1 and S2 deduced from NOESY and differential NOE experiments.

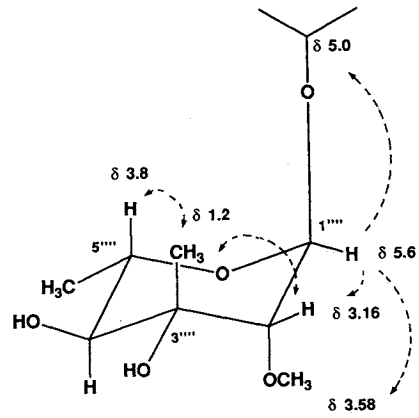


S2 S1

—→ NOE.

but not between 3''''-H and 1''''-H, and NOEs between 1''''-H and 2''''-H/2''''-OCH_3 as shown in Fig. 8.

Fig. 8. NOE experiments of respinomycin B and the relative configuration of S3 deduced from NOESY and differential NOE experiments.



S3

—→ NOE.

Structure of Respinomycin D

The IR absorptions at 1545 cm^{-1} of respinomycin D suggested the presence of nitro group. The molecular formula ($C_{51}H_{70}N_2O_{22}$ = respinomycin A1 $-NH_2+NO_2$) suggests that respinomycin D possesses a nitro sugar instead of an amino sugar. In the ^1H NMR spectrum, the similar coupling constants in the nitro sugar to those in the aminosugar (S1) suggest that the relative configuration of the nitrosugar is the same as that in S1. The ^{13}C NMR chemical shift (δ 25.1) at C-3''-CH_3 indicated that the methyl existed in *equatorial* position. In the ^1H and ^{13}C NMR spectra, the chemical shifts and coupling constants of nitro sugar moiety in respinomycin D agreed with those of decilonitroside in decilorubicin and arugomycin.[3,7,8] The configuration at C-1'' was determined to be β on the basis of the coupling constant data $J_{1'',2''eq}=2.9$ Hz, $J_{1'',2''ax}=9.4$ Hz (Tables 2 and 3). The gross structure was further confirmed by ^1H-^1H COSY, ^{13}C-^1H COSY and HMBC data as shown in Fig. 1.

In conclusion, the structures of the novel anthracycline antibiotics respinomycins A1, A2, B, C and D were determined as shown in Fig. 1. Respinomycin A2 which lacks both C-7 oxygen and the S3 moiety in ring A has strong induction activity of differentiation on human leukemia K-562 cells among respinomycins.[1] On the other hand, respinomycin D which possesses decilonitrose instead of avidinosamine was highly cytotoxic against K-562. It is interesting that such differences in the sugar components affect the biological activity profile of respinomycins.

Experimental

Spectroscopy

Optical rotations were determined on a Perkin-Elmer 241MC polarimeter. Melting points were taken on a Yanagimoto micro melting point apparatus. UV and IR spectra were measured on a Hitachi 220A spectrophotometer and a Shimadzu 27G recording IR spectrophotometer, respectively. Mass spectral data were analyzed by SI-MS on a Hitachi M-80 mass spectrometer and the high resolution FAB mass spectra were obtained by using a JEOL HX-110. ^{1}H and ^{13}C NMR spectra were recorded on JEOL GX-400 and GSX-500 instruments.

Respinomycins

Respinomycins A1, A2, B, C and D were isolated from culture broth and mycellium of *Streptomyces* sp. as described in preceding paper.[1)]

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