STRUCTURES OF THE NOVEL ANTHRAQUINONE ANTITUMOR ANTIBIOTICS, DC92-B AND DC92-D

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The structures of the new anthraquinone antitumor antibiotic DC92-B and its photodegradation product DC92-D were determined by their spectral studies. DC92-B and D are pluramycin antibiotics, related to hedamycin which possess a characteristic ring F and a side chain containing a *cis*-epoxide and terminal dimethyl.

DC92-B (**1**) and DC92-D (**2**) were isolated from the culture broth of *Actinomadura* sp. DO92,[1] and **2** was also obtained by photodegradation of **1**. They are active against P388 leukemia and sarcoma 180 in mice and against Gram-positive bacteria. We wish to report their anthraquinone structures possessing a characteristic ring F and a side chain in this paper.

Physico-chemical properties of DC92-B (**1**) and DC92-D (**2**) are summarized in Table 1. **1** and **2** were orange and dark red powders, respectively, and soluble in chloroform, MeOH, acetone and EtOAc but insoluble in water and *n*-hexane. **1** and **2** were labile under basic conditions and daylight. Although **1** and **2** were adsorbed on usual reversed phase supports such as C-18, C-8, C-2 and CN silica gel, separation and purification of **1**, **2** and their derivatives could be performed in the dark with a rapid treatment of silica gel centrifugal chromatography using organic

Fig. 1. Structures of DC92-B (**1**) and D (**2**).

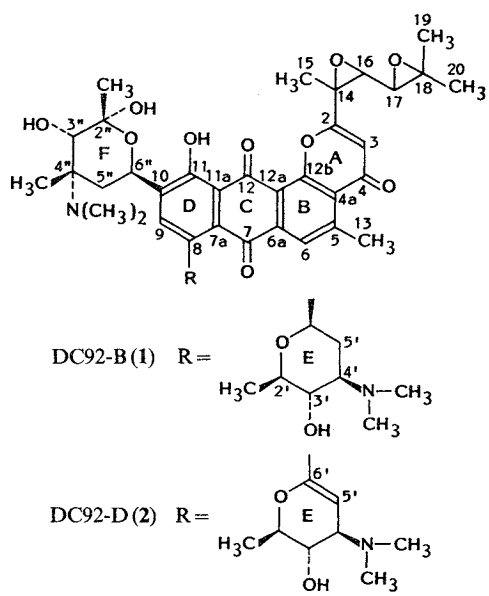


Table 1. Physico-chemical properties of DC92-B and DC92-D.

	DC92-B (**1**)	DC92-D (**2**)
Appearance	Orange powder	Dark red powder
MP (°C, dec)	140~145	134~138
Molecular formula	$C_{42}H_{52}N_2O_{12}$	$C_{42}H_{50}N_2O_{12}$
SI-MS	777 (M+1), 778 (M+2), 779 (M+3)	775 (M+1), 776 (M+2), 777 (M+3)
$[\alpha]_D^{25}$ (*c* 0.1, MeOH)	+349°	+90°
UV λ_{max}^{MeOH} nm (ε)	244 (39,000), 264 (sh, 26,000), 424 (7,600)	243 (38,000), 264 (sh, 28,000), 384 (sh, 6,000), 424 (6,500)
IR ($CHCl_3$) cm^{-1}	3450, 1657, 1632, 1587, 1465, 1422, 1380, 1308, 1257, 1076	3440, 1660, 1632, 1590, 1468, 1443, 1421, 1380, 1368, 1310, 1253, 1073
Rf value[a]	0.54	0.20

[a] HPTLC, NH_2 F_{254s}, Merck; toluene-acetone (6:4).

Table 2. ^{1}H NMR data for DC92-B (**1**), D (**2**) and hedamycin (400 MHz, $CDCl_3$).

Proton	DC92-B (**1**)	DC92-D (**2**)	Hedamycin[a]
3	6.53 (1H, s)	6.54 (1H, s)	6.46 (1H, s)
6	8.03 (1H, br s)	8.02 (1H, br s)	8.00 (1H, d, J=0.6 Hz)
9	8.34 (1H, br s)	7.84 (1H, br s)	8.33 (1H, s)
13	3.02 (3H, s)	3.02 (3H, d, J=0.5 Hz)	2.99 (3H, br s)
15	1.91 (3H, s)	1.91 (3H, s)	1.96 (3H, s)
16	3.18 (1H, d, J=5.0 Hz)	3.17 (1H, d, J=5.0 Hz)	3.32 (1H, d, J=4.7 Hz)
17	2.73 (1H, d, J=5.0 Hz)	2.74 (1H, d, J=5.0 Hz)	2.89 (1H, dd, J=4.7, 2.1 Hz)
18			3.11 (1H, dd, J=5, 2.2 Hz)
19	1.35 (3H, s)	1.34 (3H, s)	1.44 (3H, d, J=5.3 Hz)
20	1.08 (3H, s)	1.09 (3H, s)	
11-OH	13.88 (1H, br s)	13.68 (1H, br s)	
2′	*ca.* 3.6 (1H, dq)	4.33 (1H, dq, J=9.5, 6.3 Hz)	3.55 (1H, m)
3′	3.25 (1H, t, J=9.2 Hz)	3.66 (1H, dd, J=9.5, 8.4 Hz)	3.19 (1H, t, J=9 Hz)
4′	2.96 (1H, ddd, J=12.1, 9.2, 3.5 Hz)	3.49 (1H, dd, J=8.4, 2.1 Hz)	2.93 (1H, br)
5a′	*ca.* 1.4 (1H, br)	4.97 (d, J=2.1 Hz)	*ca.* 1.2 (1H)
5b′	*ca.* 2.2 (1H, br d)	—	*ca.* 2.5 (1H)
6′	5.44 (1H, dd, J=10.1, 1.4 Hz)	—	5.45 (1H, m)
2′-CH_3	1.45 (3H, d, J=6.1 Hz)	1.42 (3H, d, J=6.3 Hz)	1.43 (3H, d, J=5.9 Hz)
4′-$N(CH_3)_2$	2.37 (6H, s)	2.42 (6H, s)	2.32 (6H, s)
3″	3.55 (1H, s)	3.48 (1H, s)	3.35 (1H, br s)
5a″	*ca.* 1.4	1.33 (1H, dd, J=14.7, 11.5 Hz)	*ca.* 2.1 (1H, m)
5b″	*ca.* 2.6	*ca.* 2.6	*ca.* 2.7 (1H, m)
6″	5.50 (1H, br d, J=11.0 Hz)	5.46 (1H, br d, J=11.0 Hz)	5.45 (1H, m)
2″-CH_3	1.59 (3H, s)	1.55 (3H, s)	1.51 (3H, d, J=5.9 Hz)
4″-CH_3	1.12 (3H, s)	1.11 (3H, s)	0.71 (3H, s)
4″-$N(CH_3)_2$	2.61 (6H, br s)	2.60 (6H, br s)	2.22 (6H, s)
2″-OH	*ca.* 9.7 (1H, br)	*ca.* 9.7 (1H, br)	
2″-H			4.04 (1H, br q, J=6 Hz)

[a] 90 MHz, $CDCl_3$.[3]

eluent containing conc NH_4OH or with HPLC using aminopropyl silica gel column.

SI-MS of **1** and **2** showed pseudo-molecular ion peaks (M+1), (M+2) and (M+3), characteristic of quinone compounds.[2] The IR and UV spectra of them closely resembled those of pluramycin antibiotics. ^{1}H and ^{13}C NMR data of **1** were shown in Tables 2 and 3, respectively, compared with hedamycin.[3] Both spectra were extremely similar to each other except for the side chain moiety and ring F, implying that ring A through E of **1** is identical with that of hedamycin.

DC92-D (**2**) is found to be different from **1** only in the ring E structure by the comparison of ^{1}H and ^{13}C NMR spectra (detail is described in the later paragraph). The structure analyses of side chain moiety and ring F were performed using both data of **1** and **2**.

Structure of the Side Chain

It is known in pluramycin antibiotics that extension of conjugation from the pyrone ring (ring A) into the olefin side chain at C-2 causes a red shift by 5~6 nm around 270 nm in UV spectrum,[3,4] and in the IR spectrum, lowers the frequency of the pyrone carbonyl group, resulting in a broad absorption band together with the absorption of olefinic double bond and the quinone carbonyls in the region of 1650~1600 cm^{-1}.[3] In the comparison of UV and IR spectra of **2**, hedamycin and kidamycin, **2** exhibited

Table 3. ^{13}C NMR data for DC92-B (1), D (2) and hedamycin (100 MHz, $CDCl_3$).

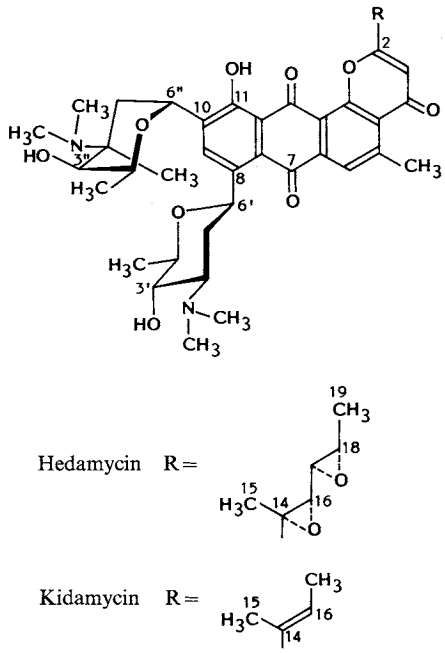
Carbon	DC92-B (1)	DC92-D (2)	Hedamycin[a]	Carbon	DC92-B (1)	DC92-D (2)	Hedamycin[a]
2	164.9	164.9	166.3	16	63.6	63.6	63.9
3	110.9	111.1	110.0	17	59.9	59.8	55.4
4	178.3	178.3	178.7	18	58.2	58.2	51.8
4a	126.0	126.1	126.2	19	19.7	19.7	17.2
5	150.0	150.3	149.7	20	24.1	24.2	
6	126.15	126.1	125.9	2′	77.5	76.7	77.3
6a	137.5	137.3	137.3	3′	71.6	69.2	71.9
7	183.2	181.0	183.1	4′	67.4	66.1	67.4
7a	126.16	128.8	125.8	5′	28.7	95.5	28.3
8	140.0	129.4	140.2	6′	75.1	156.5	75.2
9	132.5	136.4	133.1	2′-CH_3	18.8	17.7	18.9
10	138.6	138.7	138.6	4′-$N(CH_3)_2$	40.2	40.9	40.4
11	159.3	159.8	159.8	2″	100.0	100.0	67.3
11a	115.6	116.3	116.1	3″	78.9	79.0	70.9
12	187.8	188.0	188.0	4″	59.0	59.0	57.3
12a	119.1	119.0	119.2	5″	43.1	43.3	33.7
12b	156.0	156.1	156.0	6″	62.9	62.6	69.6
13	24.2	24.2	24.1	2″-CH_3	25.2	25.2	17.6
14	58.6	58.6	57.7	4″-CH_3	14.3	14.4	12.3
15	19.9	19.8	14.5	4″-$N(CH_3)_2$	41.1	41.0	36.8

[a] 25 MHz, $CDCl_3$.[3]

Table 4. UV data for DC92-D (2), hedamycin and kidamycin.

	λ_{max}^{MeOH} nm
DC92-D (2)	212, 243, 264s, 425
Hedamycin	213, 244, 264s, 434
Kidamycin	216, 243, 270s, 434

Fig. 2. Hedamycin and kidamycin.



a UV absorption band at 264 nm which is same as that of hedamycin but shorter by 6 nm in wave length than that of kidamycin (Table 4). Compound **2** also showed two clear absorption bands of carbonyl groups as in the case of hedamycin but not kidamycin (Fig. 3). These results suggested that **2**, like hedamycin, does not have a double bond at an adjacent position of C-2.

In the NMR spectra of **2**, two methine protons coupled to each other (δ_H 3.17 (d, J=5.0 Hz), δ_C 63.6 ($J_{C\text{-}H}$=179 Hz) and δ_H 2.73 (d, J=5.0 Hz), δ_C 59.8 ($J_{C\text{-}H}$=172 Hz)) were observed and assigned to protons bonded to two epoxide rings, respectively, from their chemical shifts and C-H coupling constants. Observation of the C-H long range couplings between 19-methyl (δ 1.35), 20-methyl (δ 1.09), 17-H (δ 2.73) and C-18 (δ 58.2) established a terminal epoxide moiety, and long range couplings between 16-H (δ 3.17) and C-14 (δ 58.6), and 15-methyl (δ 1.91) and C-16, C-14 and C-2 (δ 63.6, 58.6 and 164.9, respectively) revealed the other epoxide moiety

Fig. 3. IR spectra of DC92-D (**2**), hedamycin and kidamycin.

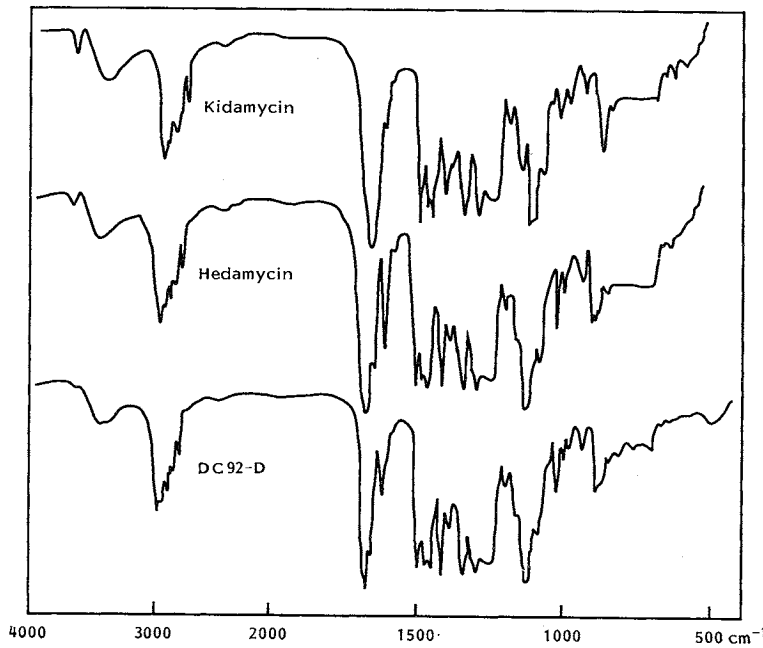


Fig. 4. LSPD and NOE experiments of DC92-D (**2**).

→ Long range coupling, ⇢ NOE.

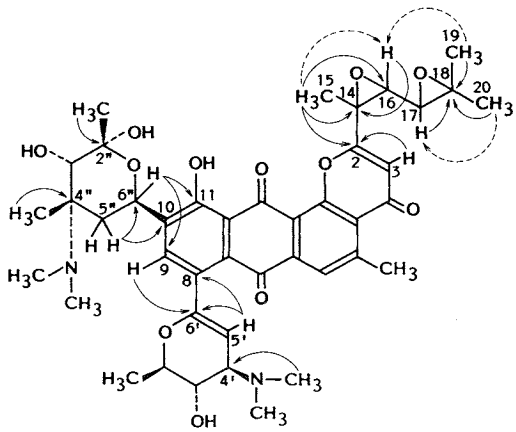
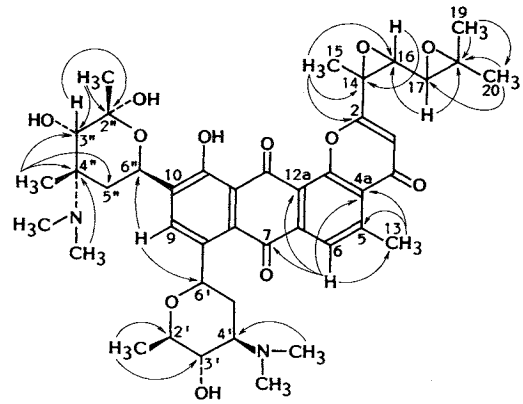


Fig. 5. COLOC experiment of DC92-B (**1**).

→ Long range coupling.



bonded to C-2 of ring A (Fig. 4). Similar long range couplings were observed in the correlation *via* long range coupling (COLOC) experiment of **1** (Fig. 5). Thus the side chain structure consisted of two epoxides having a terminal dimethyl group.

The geometry of epoxides was determined as 14-*cis*, 16-*trans*-oid from the observation of NOE between 15-methyl and 16-H, 19-methyl and 16-H, and 20-methyl and 17-H in **1** and **2** (Figs. 4 and 6). The configuration of the epoxide in pluramycin A and related compound were reported to be confirmed by ^{13}C NMR chemical shift, where 15-methyl group resonated at higher field (δ 13.6) in *trans*-epoxide than *cis*-epoxide (δ 19.2).[5] The chemical shift of 15-methyl group in **1** and **2** was observed at δ 19.9 and 19.8, respectively, which suggested *cis*-configuration for 14-epoxide of **1** and **2**. The 14-*cis*-epoxide and terminal

dimethyl group found in C-2 side chain are the first example among the pluramycin antibiotics.

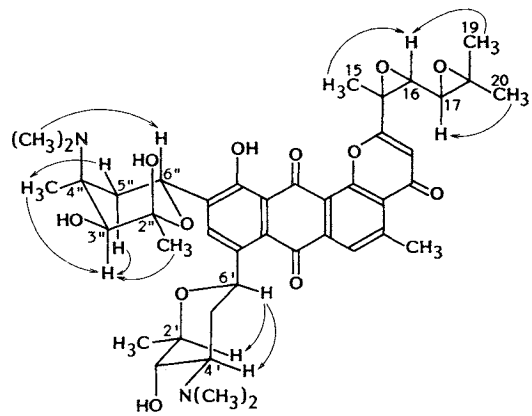
Structure of Ring E

Ring E of **1** is suggested to be identical with that of hedamycin from the comparison of their ^{1}H and ^{13}C NMR spectra (Tables 2 and 3, respectively) as described above. Spin decoupling and COLOC experiment of **1** (Fig. 5) revealed the planary structure and bonding position of ring E to ring D. Analysis of coupling constants and observation of NOE between 2'-H, 4'-H and 6'-H demonstrated its conformation as shown in Fig. 6.

Arrangement of all protons through 2'-methyl to 5'-H in ring E of **2** was determined by the ^{1}H NMR and decoupling experiments. 6'-H and one proton of 5'-H observed in ^{1}H NMR of **1** were absent in the ^{1}H NMR of **2** and shift of the other proton of 5'-H to low field was observed. Corresponding to the ^{1}H NMR behavior, sp^2 carbon signals of C-5' and C-6' in **1** changed to sp^3 ones in ^{13}C NMR of **2**. An long range selective proton decoupling spectrum (LSPD) experiment with **2** exhibited long range coupling between 5'-H and C-8, and 9-H and C-6' which also long range coupled with 5'-H. These facts suggested the existence of double bond at 5'. The position of the *N*-dimethyl group was confirmed to be 4' by LSPD experiment of **2**. The hydroxy group was confirmed to be located at 3' by the low field shift of 3'-H in its acetate (**3**) (Fig. 7). 2' through 4'-H were found to take all *trans*-diaxial arrangement from the coupling constants among them.

Fig. 6. NOESY experiment of DC92-B (**1**).

→ NOE.

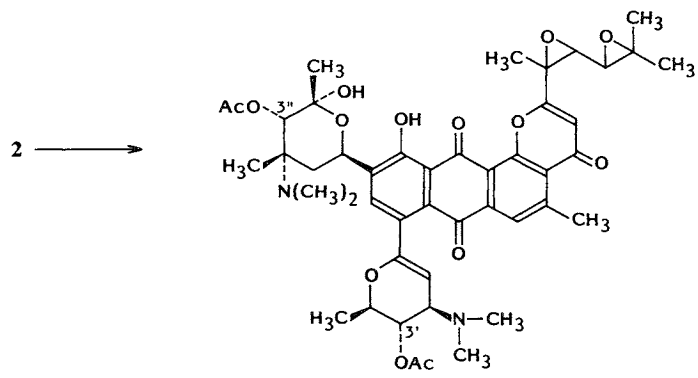


Structure of Ring F

^{1}H NMR data of ring F of **2** exhibited two tertiary methyl, one dimethylamino group, and one methine substituted by oxygen which coupled with one methylene. ^{1}H NMR data also showed one isolated methine which was demonstrated to be bonded to hydroxy group by the low field shift in its acetate (**3**). ^{13}C NMR suggested the existence of additional two quaternary carbons.

LSPD experiment of **2** exhibited long range

Fig. 7. Acetylation of **2**.



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coupling between 6″-H and C-9 and C-11 of ring D, and 5″-H and C-6″ and C-10, suggesting the arrangement of methine and methylene at positions 6″ and 5″, respectively, and the bonding position of ring F to ring D to be at 10. In COLOC experiment of **1**, long range couplings between one tertiary methyl (δ 1.12) and C-3″, C-4″ and C-5″, and between aminomethyl and C-4″ were observed, suggesting the presence of a tertiary carbon substituted by one methyl and one dimethyl amino group at 4″, and an adjacent 3″-hydroxymethine. Other long range couplings were observed between a second tertiary methyl (δ 1.59) and C-2″ and C-3″, and 3″-H and C-2″, that revealed a tertiary carbon substituted by one methyl and the remaining hydroxy group. The chemical shift of C-2″ at δ_C 100.0 was consistent with its hemiketal structure.

The conformation of ring F was demonstrated by a NOESY experiment with **1** (Fig. 6). NOE between 3″-H and one of the 5″-H revealed they were *trans*-diaxial protons, respectively. NOE between 4″-*N*-dimethyl and 6″-H (which was considered to be an axial proton by its large coupling constant) and between 4″-methyl and each 3″-H and equatorial 5″-H implied 4″-dimethylamino group was axial and the 4″-methyl group equatorial. Furthermore, observation of NOE between 2″-methyl and 3″-H revealed the 2″-methyl group is equatorial. Thus the structure of ring F containing hemiketal moiety was confirmed as shown in Fig. 6, which is the same configuration as hedamycin except for the 2″-hydroxy group but takes C1 chair conformation that is different from hedamycin, kidamycin (twisted form) and isokidamycin (1C chair form).[6]

Conclusion

Structures of **1** and **2** were determined as shown in Fig. 1. Their characteristic feature is in the C-2 side chain containing *cis*-epoxide and terminal dimethyl groups, and in the ring F possessing a unique hemiketal structure.

DC92-D (**2**) was obtained by irradiation in the daylight and thought to be an artifact. Similar photooxidation from hedamycin to photohedamycin A and from kidamycin to photokidamycin A has been reported by FREDENHAGEN and SÉQUIN.[7]

Experimental

^1H and ^{13}C NMR spectra were recorded on Jeol JNM FX100 and Bruker AM400 spectrometers with TMS (0 ppm) as an internal standard. Mass spectra were measured on a Hitachi M-80B mass spectrometer. IR spectra were obtained using a Shimadzu IR-27G spectrometer. UV spectra were measured on Hitachi 200-20 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. MP's were taken with a Yanagimoto melting point apparatus and were uncorrected. TLC was performed on pre-coated plates, Merck Kieselgel $60F_{254}$ and HPTLC NH_2 F_{254} visualized by UV (254 nm). HPLC was accomplished using a column of YMC-Pack NH_2 (YMC Co.). Centrifugal chromatography (Chromatotron, Harrison Research) was done on plates, Merck Kieselgel $60PF_{254}$.

Acetylation of DC92-D

Acetic anhydride was added to a solution of **2** (12 mg) in pyridine (1 ml), and the solution was stirred for 5 hours at room temperature. Water (0.5 ml) was added to the reaction mixture and it was allowed to stand for 30 minutes at room temperature. The solution was diluted with EtOAc and washed with water, dil HCl, $NaHCO_3$ solution and satd NaCl. EtOAc solution was dried over magnesium sulfate and evaporated *in vacuo*. The residue was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH, (20:1) to give **2** acetate (**4**, 5 mg).

Photooxidation of DC92-B

A solution of **1** (80 mg) in MeOH (20 ml) was stirred for 1 hour under daylight. The reaction mixture

was evaporated and the residue was subjected to silica gel centrifugal chromatography eluted by $CHCl_3$ satd by ammonia - MeOH (10 : 1) to give **2** (13 mg) and **1** (46 mg) as recovered starting material.

Acknowledgments

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References

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