THE STRUCTURE OF DECILORUBICIN

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The structure of a new anthracycline antibiotic, decilorubicin was determined by a combination of chemical conversion, degradation and spectral interpretation, and the studies of this structure are reported in detail.

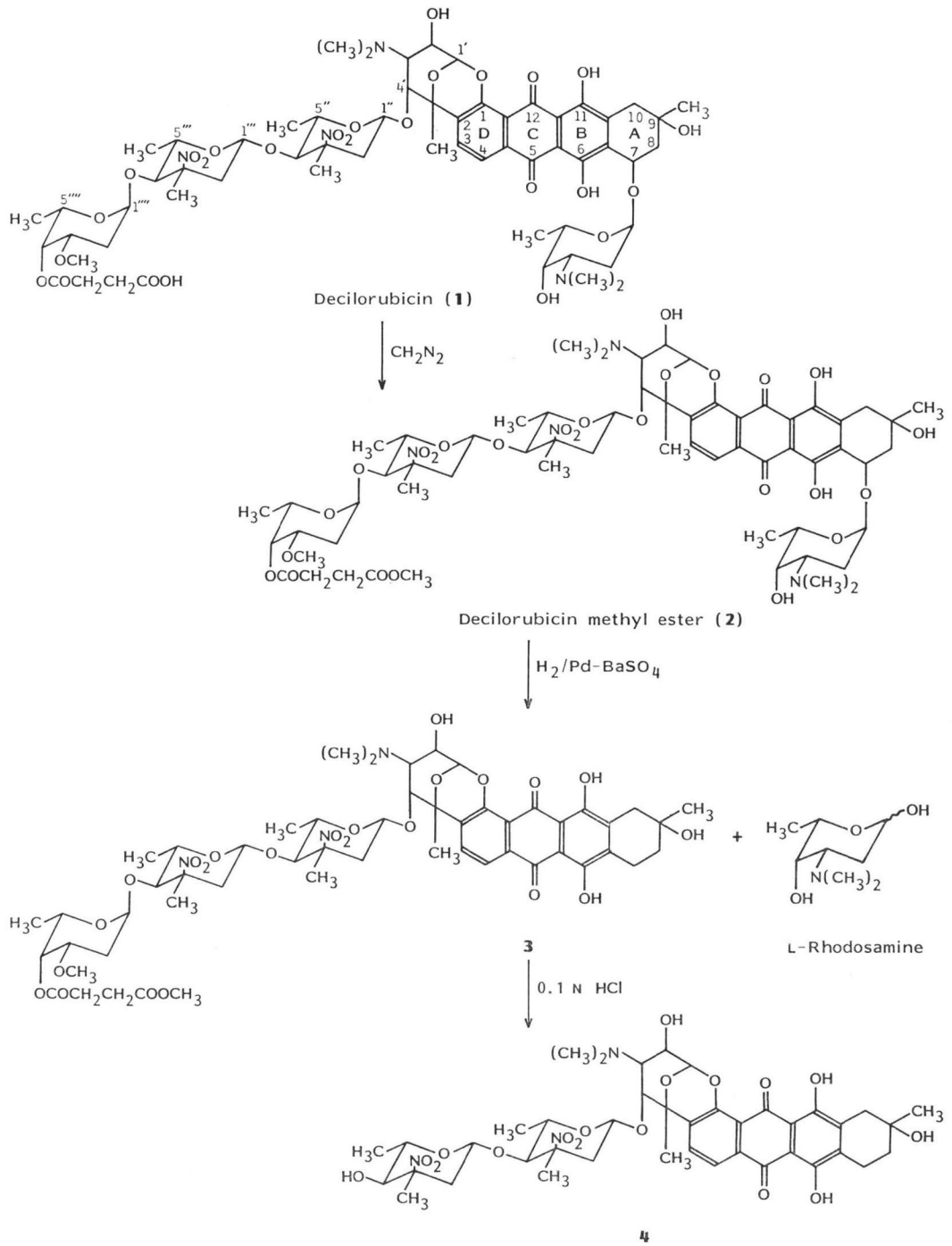
Decilorubicin is an antitumor antibiotic produced by *Streptomyces virginiae* MF266-g4. In a previous paper[1] we reported on isolation and properties of decilorubicin together with its provisional structure. We have already reported the structures of decilonitrose and 4-*O*-succinyl-L-diginose, the sugar moieties of this antibiotic.[2] In this communication we report a complete structure of decilorubicin (**1**), except the absolute stereochemistry of the aglycone.

A molecular formula of $C_{60}H_{82}N_4O_{26}$ was secured by mass spectrometry [*m/z* 1,275 $(MH)^+$] and elementary analysis. The ultraviolet spectrum of **1** is quite similar to those of anthracycline antibiotics, daunomycin[3] and adriamycin.[4] Its visible spectrum has the three maxima at 476, 496 and 536 nm, which move to 560 and 597 nm in an alkaline solution. The infrared spectrum indicates the presence of hydroxyl (3480 cm^{-1}), carbonyl (1735 cm^{-1}), and anthraquinone-α-hydroxyl (1620 cm^{-1}) and nitro (1545 cm^{-1}) groups. A band at 1620 cm^{-1} is typical of a ketone group hydrogen-bonded to a perihydroxyl group in an anthraquinone.[5] These data pointed to a 1,4,5-trihydroxyanthraquinone structure.[6] Both anthraquinone carbonyl groups seem to be hydrogen-bonded, since there are no band near at 1670 cm^{-1} in the infrared spectrum.[5] Futhermore, both carbonyl groups appear to be hydrogen-bonded, as by the chemical shifts at δ 186.5 and 186.2 in the ^{13}C NMR spectrum.[7,8]

Reaction of decilorubicin with diazomethane gave a compound **2** which had the molecular formula $C_{61}H_{84}N_4O_{26}$. Its molecular formula and the NMR spectra revealed that the methyl ester of decilorubicin had been formed. This indicates that decilorubicin has a free carboxylic acid. Catalytic reduction of **2** under medium pressure (3.5 kg/cm^2) formed the degradation product **3** and L-rhodosamine.[9] The molecular formula of **3** derived from the elementary analysis and mass spectrum was $C_{53}H_{69}N_3O_{23}\cdot H_2CO_3$. The ^1H NMR and ^{13}C NMR spectra of **3** were very similar to those of **2**, taking into account the loss of L-rhodosamine. Mild acid hydrolysis of **3** with 0.1 N hydrochloric acid gave the degradation product **4**. The ^1H NMR and ^{13}C NMR spectra of **4** showed the loss of 4-*O*-succinyl-L-diginose methyl ester from **3**.

Methanolysis of **1** with 0.5 N hydrogen chloride in methanol formed two degradation products (**5** and **6**) and three glycosides (**7**, **8** and **9**). Analytical data and mass spectrometry of **5** showed that its molecular formula was $C_{41}H_{51}N_3O_{18}$. The ultraviolet and visible spectra, infrared spectrum, ^1H NMR and ^{13}C NMR spectra were very similar to those of decilorubicin, except for the absence of L-rhodosamine and 4-*O*-succinyl-L-diginose in **5**. The another product **6** has a molecular formula of $C_{30}H_{40}N_2O_{14}$ established by elementary analysis and mass spectrum, suggesting that it arises from **5** by loss of 1 mol

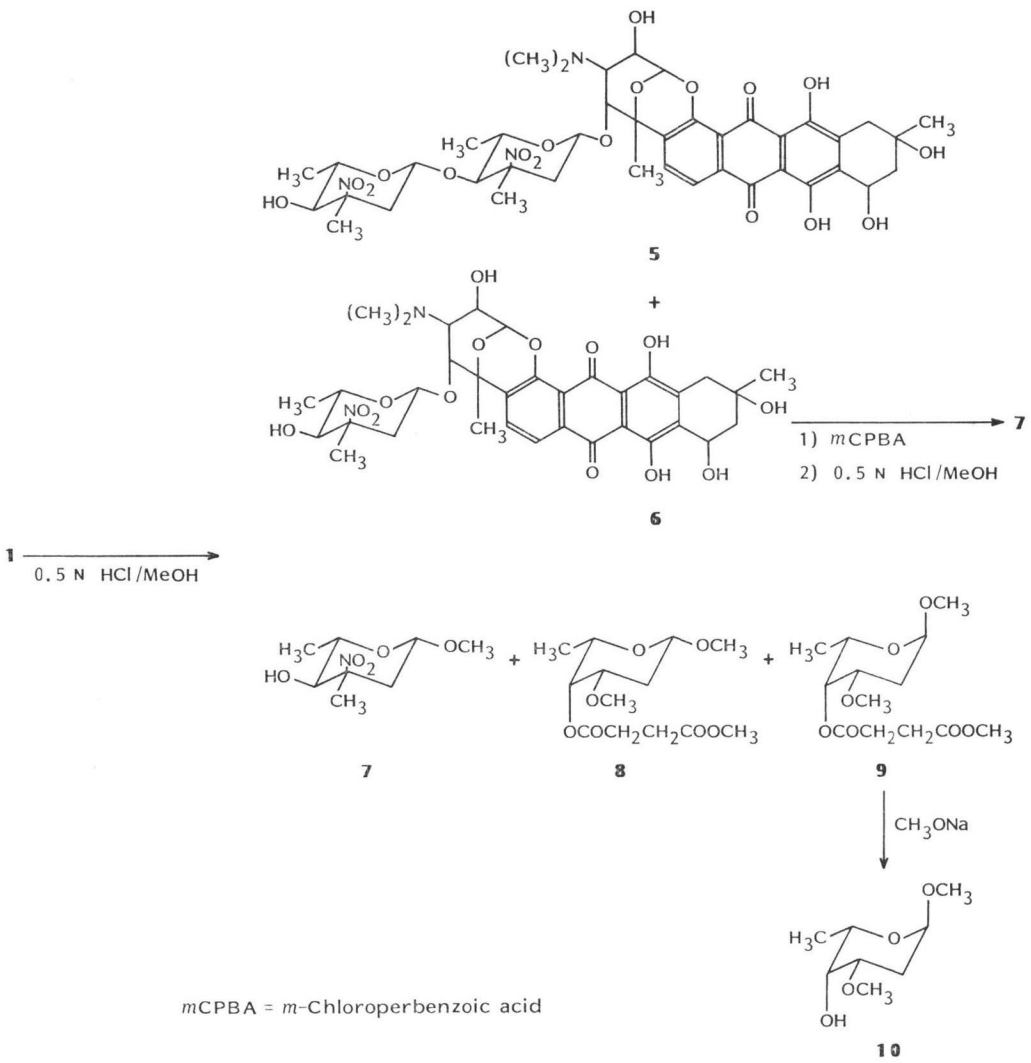
Scheme 1.



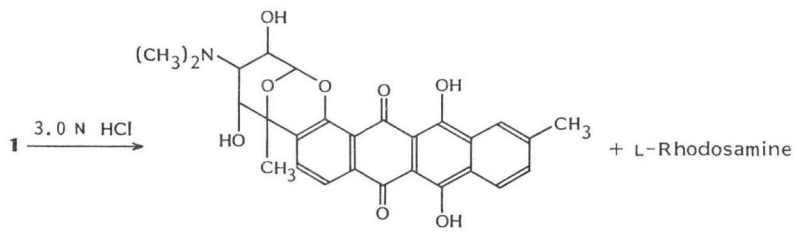
of decilonitrose. Structures of methyl β-decilonitroside (**7**), methyl 4-*O*-succinyl-α- and β-L-diginoside methyl esters (**8** and **9**) have been reported in our previous paper.[2] Oxidation of **6** with *m*-chloroperbenzoic acid followed by methanolysis with 1 N hydrogen chloride in methanol gave **7**.

Strong acid hydrolysis of **1** with 3 N hydrochloric acid gave a degradation product **11** designated as

Scheme 2.



Scheme 3.



decilorene and L-rhodosamine. The molecular formula of **11** as determined by analysis and mass spectrum is $C_{27}H_{25}NO_8$. This suggests that **11** arises from **1** by loss of rhodosamine, 4-*O*-succinyldiginose, 2 mol of decilonitrose and 1 mol of water. The electronic spectra are consistent with more unsaturation than is present in **1**. These data all indicate that the anthraquinone nucleus is attached linearly to a

Table 1. ^{13}C NMR chemical shifts.

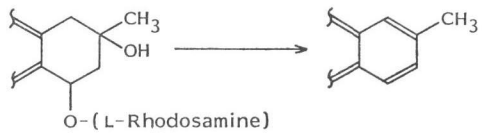
Carbon		2* ($CDCl_3$)	3* ($CDCl_3$)	5* ($CDCl_3$-CD_3OD)	6* ($CDCl_3$-CD_3OD)	11 ($CDCl_3$-CD_3OD)
	12	(185.8 s)	(185.5 s)	(186.3 s)	(185.9 s)	176.2 s
	5	(185.5 s)	(184.7 s)	(185.9 s)	(185.6 s)	173.7 s
	6	(156.8 s)	(156.9 s)	(156.6 s)	(156.3 s)	168.7 s
	11	(156.6 s)	(156.2 s)	(156.6 s)	(156.1 s)	168.4 s
	1	154.2 s	153.6 s	154.1 s	153.8 s	153.0 s
	2	137.1 s	138.7 s	137.5 s	137.1 s	131.3 s
	6a	(135.0 s)	(135.9 s)	(136.6 s)	(136.3 s)	129.2 s
	12a	(134.6 s)	(134.2 s)	(134.6 s)	(134.3 s)	119.5 s
	4a	134.1 s	133.6 s	134.3 s	134.1 s	133.7 s
	3	130.8 d	130.1 d	131.4 d	130.8 d	125.6 d
	10a	119.3 s	119.3 s	119.7 s	119.4 s	131.0 s
	4	118.7 d	118.5 d	119.5 d	119.0 d	125.6 d
	5a	(110.9 s)	(109.8 s)	(111.4 s)	(111.1 s)	(106.4 s)
	11a	(110.1 s)	(108.6 s)	(110.6 s)	(110.3 s)	(106.6 s)
	9	67.5 s	67.4 s	68.6 s	68.2 s	143.6 s
	7	65.8 t	20.7 t	61.5 d	61.0 d	118.4 d
	8	37.7 t	33.6 t	38.0 t	37.7 t	134.1 d
	10	39.7 t	37.2 t	40.4 t	39.8 t	125.5 d
	C-9-CH_3	30.0 q	30.2 q	30.3 q	30.2 q	22.1 q
	1′	97.9 d	97.4 d	98.0 d	97.6 d	97.3 d
	2′	68.5 d	68.7 d	67.7 d	67.5 d	68.4 d
	3′	61.7 d	61.3 d	62.6 d	62.4 d	62.7 d
	4′	80.5 d	81.0 d	80.5 d	80.4 d	75.4 d
	5′	77.0 s	76.9 s	77.6 s	77.3 s	76.4 s
	6′	23.2 q	22.9 q	23.2 q	23.0 q	22.4 q
	$N(CH_3)_2$	41.9 q	40.1 q	40.2 q	40.0 q	43.3 q
Decilonitrose	1″	(99.8 d)	(99.7 d)	(100.0 d)	99.6 d	
	2″	(42.0 t)	(41.8 t)	(42.6 t)	41.9 t	
	3″	(88.9 s)	(89.0 s)	(90.1 s)	89.8 s	
	4″	81.7 d	81.6 d	81.9 d	76.3 d	
	5″	(70.0 d)	(70.0 d)	(71.0 d)	71.1 d	
	6″	(18.6 q)	(18.0 q)	(18.7 q)	18.5 q	
	C-3″-CH_3	(25.3 q)	(25.3 q)	(25.5 q)	25.0 q	
Decilonitrose	1‴	(98.7 d)	(98.6 d)	(99.1 d)		
	2‴	(42.5 t)	(42.5 t)	(41.6 t)		
	3‴	(88.0 s)	(88.6 s)	(89.6 s)		
	4‴	84.1 d	84.1 d	75.3 d		
	5‴	(70.0 d)	(70.0 d)	(70.8 d)		
	6‴	(18.8 q)	(18.0 q)	(18.6 q)		
	C-3‴-CH_3	(25.0 q)	(25.1 q)	(24.8 q)		
4-*O*-Succinyl-L-diginose	1⁗	101.7 d	101.8 d			
	2⁗	31.6 t	31.6 t			
	3⁗	66.5 d	66.5 d			
	4⁗	67.6 d	67.5 d			
	5⁗	72.8 d	72.8 d			
	6⁗	16.6 q	16.6 q			
	C-3⁗-OCH_3	56.1 q	56.1 q			
	ester CH_3	51.8 q	51.8 q			
	ester CO	172.5 s	172.5 s			
	ester CO	171.8 s	171.8 s			
	$(CH_2)_2$	29.1 t	29.1 t			
L-Rhodosamine	1	101.2 d				
	2	29.7 t				
	3	59.7 d				
	4	71.5 d				
	5	66.5 d				
	6	17.0 q				
	$N(CH_3)_2$	39.6				

* Tentative assignments are given on the basis of the value of ref 10, 13.
Similar values in parentheses may be interchanged.

fourth ring (ring A) which is not aromatic. Many attempts to remove a possible amino sugar from **11**, even treatment with strong base at elevated temperature[10] were not successfull.

The ^{13}C NMR spectra of **2**, **3**, **5**, **6** and **11** are assigned as shown in Table 1. This was done on the basis of comparisons of these spectra with each other, off resonance decoupling, proton selective decoupling technique, theoretical considerations and comparison with models and the similar compound such as nogalamycin.[11] Two protons appearing in the spectrum of **1**, **2**, **3**, **4**, **5** and **6** as doublets (J=8.0 Hz) in the neighborhood of δ 7.65 and 7.93 must arise from aromatic protons which are *ortho* to each other. This establishes that the anthraquinone system has six substituents. As has already been shown, two of these are phenolic hydroxyl groups which must be attached to the same ring (B or D) and another two must be attached to the alternative ring (D or B). The ^{1}H NMR of **11** shows a doublet at 5.95 coupled with a proton with a chemical shift of δ 4.24. The latter is coupled with a proton at δ 2.49, which in turn is coupled with a proton appearing at δ 3.96. These signals are ones to be expected arising from an amino sugar. The ^{13}C NMR spectrum of **11** has one resonance at δ 96.9, which is that expected for an anomeric carbon of sugar. In addition, a group of four signals at δ 67~76 arise from carbon atoms substituted with oxygen or nitrogen as in an amino sugar. The ^{1}H NMR spectrum of **11** also shows the presence of a chemical shift as a singlet representing 6H at δ 2.61, suggesting the presence of a dimethylamino group attributed to the amino sugar.

In the ^{1}H NMR spectrum of **11** the resonance of one C-CH_3 group has shifted downfield to δ 2.58. Such shift must result from aromatization of the ring substituted by the methyl group. The appearance of two doublets and one doublet of doublet in the ^{1}H NMR spectrum in the aromatic region representing three new aromatic protons (δ 7.51, 7.72 and 8.01) with coupling constants shows that two of them are *ortho* to each other (δ 7.51 and 7.72, J=8.0 Hz) and two of them are *meta* to each other (δ 7.51 and 8.01, J=1.5 Hz). These data indicate that **11** could be produced by the aromatization of ring A with the loss of two molecules of water if two hydroxyl groups were present in that ring of the hydrolyzed intermediate of **1**. As NMR spectra indicate retention of that portion of the molecule attributed to the amino sugar, aromatization must have occurred in ring A. The result of hydrogenolysis of **2** mentioned already above[12] and ^{1}H NMR spectrum of **11** establish that the system shown as **12** is present in **1** and **2** and is converted to **13**. This conclusion is supported by ^{13}C NMR spectrum of **11** and the application of its proton selective decoupling technique (*vide post*).



12 **13**

NMR spectral data of **2**, **3**, **4**, **6** and **11** showed the presence of an amino sugar as shown in Table 2. A series of four carbon atoms (1′, 2′, 3′ and 4′) have protons which give rise to signals in the ^{1}H NMR spectrum. The H-1′ and H-2′ are coupled (e-e or e-a) and the latter is coupled with H-3′ (a-a) which in turn is coupled with H-4′ (a-e). The chemical shifts of the protons at C-2′ and C-4′ indicate that C-2′ and C-4′ have hydroxyl substituents. The chemical shift of H-1′ is that of an anomeric proton and that of H-3′ is consistent with substitution of $N(CH_3)_2$. The ^{13}C NMR spectrum of **11** is indicative of a five-carbon chain (C-1′ to C-5′) in a sugar moiety. There is no hydrogen on C-5′ since there is no indication of a proton at C-5′ in the ^{1}H NMR spectra and the signal attributed C-5′ in ^{13}C NMR spectra appears as a singlet. As mentioned above, the methyl group is attached to C-5′. Those NMR data establish the relative configuration of the first four carbon atoms except C-5′ of the amino sugar. The lack of a proton at C-5′ suggests that this carbon is attached to the anthraquinone ring. There are 8 oxygen atoms

Table 2. ^{1}H NMR spectra.

Position	Chemical shifts[a] (coupling constants)[b]				
	2 ($CDCl_3$)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**6** (Pyridine-d_5)	**11** ($CDCl_3$)
1′	6.25 (d, J=3.5)	6.17 (d, J=3.5)	6.14 (d, J=3.3)	6.48 (d, J=3.3)	5.95 (d, J=4.0)
2′	4.42 (d,d, J=3.5, 12)	4.37 (broad d,d)	4.39 (d,d, J=3.3, 12)	4.86 (d,d, J=3.3, 12)	4.24 (d,d, J=4.0, 10.5)
3′	2.91 (d,d, J=2.5, 12)	2.87 (broad d,d)	—	3.36 (d,d, J=2.3, 12)	2.49 (d,d, J=3.2, 10.5)
4′	4.30 (d, J=2.5)	4.33 (d, J=2.5 Hz)	4.31 (d, J=2.5)	4.82 (d, J=2.3)	3.96 (d, J=3.2)

[a] In ppm downfield from TMS. [b] In Hz.

in **11**, with two of them related as phenolic hydroxyls, and two as anthraquinone carbonyls. This leaves four oxygen atoms to be associated with the amino sugar, and C-1′ is a normal anomeric carbon atom and two hydroxyls are present, no oxygen remains to link C-5′ with the anthraquinone nucleus. Consequently, this must be a carbon-carbon bond. As NMR data show that C-1′ has an axial oxygen, geometrical consideration based on the conformation and configuration necessitate that C-5′ has an axial bond to the aromatic nucleus at a position *ortho* to the linkage with C-1′. These data limit the sugar stereochemistry to that of 3,6-dideoxy-3-dimethylamino-α-D-galactose or its α-L-isomer, but does not distinguish between them. As has already been mentioned above, an amino sugar must be attached to ring D. This conclusion is further supported by the proton selective decoupling technique of ^{13}C NMR spectra of **11** (*vide infra*). The amino sugar attachment is through either C-1 and C-2 or C-3 and C-4, since two protons of ring D appears as *ortho* to each other in ^{1}H NMR spectrum of **11**. However, it does not distinguish between them by chemical or physico-chemical method.

The proton selective decoupling technique clearly clarified all the carbon atoms in the ^{13}C NMR spectrum of **11**. The selective irradiation at the resonance of the proton attached to the carbon changed the shape and intensity of α and γ carbon mainly. The result is shown in Table 3. These data all pointed to the attachment of anomeric carbon of amino sugar to C-1 or C-4 of ring D through an *O*-glycosidic linkage and attachment of C-5′ of the amino sugar to C-2 or C-3 of ring D by carbon-carbon linkage.

Table 3. The proton selective decoupling in ^{13}C NMR spectrum of **11**.

Irradiated proton (δ in ^{1}H NMR)	Influenced carbon (δ in ^{13}C NMR)
H-3 (7.51)	C-1 (153.0), C-3 (129.9), C-4a (133.7)
H-4 (7.82)	C-2 (131.3), C-4 (125.6), C-4a (133.7), C-5 (173.7), 12a (131.0)
H-7 (7.6)	C-6 (168.7), C-7 (118.4), C-9 (143.6), C-10a (119.5)
H-8 (7.44)	C-6a (129.2), C-8 (134.1), C-10 (125.5)
H-10 (7.9)	C-10 (125.5), C-10a (119.5)
C-9-CH_3 (2.5)	C-9-CH_3 (22.1), C-9 (143.6)
H-1′ (5.9)	C-1 (153.0), C-1′ (97.3)
H-2′ (4.4)	C-2′ (68.4)
H-3′ (2.6)	C-3′ (62.7), $N(CH_3)_2$ (43.3)
H-4′ (4.1)	C-4′ (75.1)
C-5′-CH_3 (1.8)	C-2 (131.3), C-5′ (22.4)

In ppm (δ), obtained from $CDCl_3$-CD_3OD solutions containing TMS as internal reference.

The ^{13}C NMR spectrum of **5** demonstrates that it consists of two mol of decilonitrose and one mol of amino sugar attached to ring D and aglycone. Methanolysis of **5** gives compounds **6** and **7**, which are identified with authentic samples by comparison of Rf values on TLC and retention time of HPLC. In the ^{13}C NMR spectra, the signals of C-4'' are observed at δ 81.9 and 76.3 in **5** and **6**, respectively. These chemical shifts indicate that decilonitrose attaches to the C-4'' of **6**. The chemical shift (δ 5.50) and coupling constant (doublet of doublets, J=2.0 and 9.0 Hz) of anomeric proton of glycoside in the ^1H NMR spectrum of **5** indicate that the configuration at C-1 is β.

The ^{13}C NMR spectrum of **6** shows that it contains one mol of decilonitrose. In spite of very extensive attempts to isolate decilonitrose from **5** by acid and base treatment, this was never achieved. However, as has already been shown, oxidation of **6** with *m*-chloroperbenzoic acid followed by methanolysis gave **7**. The difference of chemical shifts of C-4' between **6** (δ 80.4) and **11** (δ 74.4) in the ^{13}C NMR spectra indicates that decilonitrose binds to C-4' of amino sugar attached to ring D. This conclusion further supported by the result of the proton selective decoupling technique of the proton attached to C-4' in the ^{13}C NMR spectra of **2** and **11**. According to chemical shift (δ 6.02) and coupling constants (doublet of doublets, J=2.0 and 9.4 Hz) at C-1 of glycoside in the ^1H NMR spectrum of **6**, the configuration of glycoside was determined as β.

Catalytic reduction of **2** with palladium on $BaSO_4$ caused partial cleavage of **2** into the 7-deoxy derivative **3** and L-rhodosamine, indicating L-rhodosamine attaches to C-7 position of aglycone. In the ^{13}C NMR spectrum of **3**, the signal attributed C-7 has shifted upfield from δ 65.8 to 20.7. These chemical shifts supported the above conclusion. In the ^1H NMR spectrum of **2**, the chemical shifts (δ 5.09) and the small coupling constant (half height of width of 7.5 Hz) of anomeric proton of rhodosamine pointed to α-configuration at C-1.

By the comparison of ^{13}C NMR and ^1H NMR spectra of **3** with those of **5**, it was concluded that **3** contained one mol of 4-*O*-succinyldiginose. The ^{13}C NMR spectra of **3** and **5** show the signals of C-4' at δ 84.1 and δ 75.3, respectively. These chemical shifts indicate that 4-*O*-succinyldiginose attaches to C-4'''. The chemical shift (δ 4.99) and small coupling constant (broad triplet, half height width of 7.0 Hz) at C-1 of glycoside show that the configuration of glycoside is α.

In summary, the accumulated data from the present study points to a structure of decilorubicin as shown **1**. The absolute stereochemistry of the aglycone will be established by further structural studies.

Experimental

General

Melting points were determined with a Yamato apparatus and are uncorrected. UV spectra were determined on a Hitachi Model 200-10 and IR spectra on a Hitachi Model 260-10 spectrophotometer. Optical rotations were measured with a Parkin-Elmer 241 polarimeter. The ^1H NMR spectra were recorded with Varian XL-100, Varian EM-390, Bruker WM250 and Jeol GX-400 spectrometers. Chemical shifts are expressed in values (ppm) with tetramethylsilane as an internal standard. Proton-noise decoupled FT-^{13}C NMR spectra were taken at 25.2 MHz on a Varian XL-100 and at 100.4 MHz on a Jeol GX-400 spectrometer using tetramethylsilane as reference. The mass spectra were taken by a Hitachi RMU-6M mass spectrometer for electron-impact ionization or RMU-7M for field-desorption.

Decilorubicin (1)

This compound was prepared in pure form from a culture broth of *S. virginiae* MF266-g4 as reported previously.[1]

Decilorubicin Methyl Ester (**2**)

To a solution of decilorubicin (**1**, 79.0 mg) in a mixture of chloroform and methanol (9: 1) was added and ethereal solution of diazomethane (0.5 M, 0.5 ml), and the mixture was allowed to stand at room temperature for 30 minutes. Evaporation of the solvent gave a solid. The solid was dissolved in chloroform, and the solution was washed with water and dried over sodium sulfate. After filtration, the solvent was evaporated to give a pure solid (**2**, 76 mg): λ_{max}^{MeOH} 220, 235, 254, 290, 380, 476, 495, 533, 580 nm; mp 178~182°C (dec); $[\alpha]_D^{25}$ +462° (*c* 0.05, $CHCl_3$); mass spectrum *m/z* 1,289 (MH^+); IR (KBr) 3400, 2920, 1735 ($COCH_3$), 1620 (C=O), 1570 and 1545 (NO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.17 (3H, d, *J*=6.5 Hz, H-6''''), 1.31 (3H, d, *J*=6.0 Hz, Rhod-6), 1.38 (3H, d, *J*=6.0 Hz, H-6'''), 1.41 (3H, d, *J*=6.0 Hz, H-6''), 1.50 (3H, s, CH_3-9), 1.62 (3H, s, H-6'), 1.71 (3H, s, H-3'''), 1.79 (3H, s, H-3''), 2.11 (6H, s, Rhod-$N(CH_3)_2$), 2.56 (6H, s, $N(CH_3)_2$-3'), 2.91 (1H, dd, *J*=3.0 and 10.0 Hz, H-3'), 3.31 (3H, s, OCH_3), 3.33 (1H, d, *J*=9.0 Hz, H-4''), 3.49 (1H, d, *J*=9.0 Hz, H-4'''), 3.52 (1H, m, H-3''''), 3.98 (1H, m, H-5''), 4.08 (1H, m, H-5'''' and Rhod-5), 4.20 (1H, m, H-5'''), 4.30 (1H, d, *J*=3.0 Hz, H-4'), 4.42 (1H, dd, *J*=4.0 and 10.0 Hz, H-2'), 4.65 (1H, broad s, half height width of 9.0 Hz, H-7), 4.99 (1H, broad t, half height width of 7.5 Hz, H-1''''), 5.09 (1H, broad t, half height width of 7.5 Hz, Rhod-1), 5.28 (1H, broad s, H-4''''), 5.33 (1H, dd, *J*=2.0 and 9.5 Hz, H-1'''), 5.52 (1H, dd, *J*=2.0 and 9.5 Hz, H-1''), 6.29 (1H, d, *J*=4.0 Hz, H-1'), 7.58 (1H, d, *J*=8.0 Hz, H-3) and 7.92 (1H, d, *J*=8.0 Hz, H-4).

Anal Calcd for $C_{61}H_{84}N_4O_{26}$: C 56.83, H 6.57, N 4.35.
Found: C 56.15, H 6.70, N 4.23.

Hydrogenolysis of Decilorubicin Methyl Ester (**2**)

A solution of **2** (76 mg) in methanol (8 ml) was hydrogenated over 5% $Pd/BaSO_4$ (160 mg) at room temperature under a pressure of hydrogen (3.5 kg/cm^2) for 3 hours. The reaction mixture was filtered and evaporated to dryness. The residue was partitioned between water (50 ml) and chloroform (50 ml). The aqueous layer was evaporated and chromatographed on a column of silica gel with chloroform - methanol (9: 1) to give a colorless solid of L-rhodosamine (7.0 mg). Evaporation of the chloroform layer followed by chromatography on a column of silica gel with the same solvent system gave a red solid (58 mg), which was further purified by preparative TLC of silica gel to give a red solid (20 mg). Recrystallization from methanol gave red needles (**3**, 10 mg).

3: λ_{max}^{MeOH} 218, 237, 255, 291, 370, 472, 496, 533 nm; mp 186~190°C (dec); $[\alpha]_D^{25}$ +230° (*c* 0.05, $CHCl_3$); mass spectrum *m/z* 1,116 (MH^+); IR (KBr) 3450, 2940, 2930, 1735 ($COCH_3$), 1615 (C=O), 1570 and 1540 (NO_2) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.14 (3H, d, *J*=6.5 Hz, H-6''''), 1.31 (3H, d, *J*=6.0 Hz, H-6'''), 1.37 (3H, d, *J*=6.0 Hz, H-6''), 1.50 (3H, s, CH_3-9), 1.63 (3H, s, H-6'), 1.70 (3H, s, CH_3-3'''), 1.79 (3H, s, CH_3-3''), 2.55 (6H, s, $N(CH_3)_2$), 3.33 (3H, s, OCH_3-3''''), 3.35 (1H, d, *J*=9.0 Hz, H-4'''), 3.50 (1H, d, *J*=9.0 Hz, H-4''), 3.70 (3H, s, $COCH_3$), 3.97 (1H, m, H-5'''), 4.09 (1H, m, H-5''''), 4.17 (1H, m, H-5''), 4.31 (1H, d, *J*=3.0 Hz, H-4'), 4.37 (1H, dd, *J*=~4 and ~10 Hz, H-2'), 4.99 (1H, broad t, half height width of 7.0 Hz, H-1''''), 5.29 (1H, broad s, H-4''''), 5.33 (1H, dd, *J*=2.0 and 9.5 Hz, H-1'''), 5.52 (1H, dd, *J*=2.0 and 9.5 Hz, H-1''), 5.52 (1H, d, *J*=4.0 Hz, H-1'), 7.54 (1H, d, *J*=8.0 Hz, H-3) and 7.83 (1H, d, *J*=8.0 Hz, H-4).

Anal Calcd for $C_{53}H_{69}N_3O_{23}\cdot H_2CO_3$: C 55.05, H 6.07, N 3.56.
Found: C 55.01, H 6.10, N 3.56.

Partial Methanolysis of **3**

To a solution of **3** (9.0 mg) in methanol (5 ml) was added 0.2 N hydrochloric acid (5 ml). The mixture was stirred at 60°C for 4.5 hours. On TLC of silica gel with chloroform - methanol (9: 1), the starting material (Rf 0.63) disappeared and the product (**4**, Rf 0.54) appeared. After neutralization with Amberlite IR-45 (OH^-) and filtration, the filtrate was evaporated to dryness and purified by preparative TLC of silica gel with the same solvent system to give a red solid (**4**, 3.8 mg): λ_{max}^{MeOH} 218, 235, 253, 290, 380, 478, 495, 534 nm; mp 174~176°C (dec); $[\alpha]_D^{25}$ +280° (*c* 0.05, $CHCl_3$); mass spectrum *m/z* 858 (MH^+); IR (KBr) 3450, 2940, 1620 (C=O), 1580 and 1545 (NO_2) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.40 (3H, d, *J*=6.0 Hz, H-6''), 1.42 (3H, d, *J*=6.0 Hz, H-6'''), 1.52 (3H, s, CH_3-9), 1.64 (3H, s, H-6'), 1.76 (3H, s, CH_3-3''), 1.77 (3H, s, CH_3-3'''), 2.50 (6H, s, $N(CH_3)_2$), 3.33 (1H, d, *J*=9.0 Hz, H-4'''), 3.42 (1H, d, *J*=9.0 Hz, H-4''), 3.64 (1H, m, H-5'''), 4.17 (1H, m, H-5''), 4.30 (1H, d, *J*=2.5 Hz, H-4'), 4.35

(1H, dd, J=3.3 and 12 Hz, H-2′), 4.73 (1H, dd, J=2.0 and 9.0 Hz, H-1‴), 5.50 (1H, dd, J=2.0 and 9.0 Hz, H-1″), 6.17 (1H, d, J=3.3 Hz, H-1′), 7.54 (1H, d, J=8.0 Hz, H-3) and 7.93 (1H, d, J=8.0 Hz, H-4).

Methanolysis of Decilorubicin (**1**)

A solution of **1** (2.0 g) in 0.5 N methanolic hydrogen chloride (200 ml) was refluxed for 4.5 hours. On TLC of silica gel with toluene - ethyl acetate (1: 1), the mixture showed four spots (Rf 0.0, 0.38, 0.42 and 0.60). The mixture was neutralized with silver carbonate, filtered and evaporated to dryness. The residue was chromatographed on a column of silica gel with toluene - ethyl acetate (30: 1). The first fraction contained methyl β-decilonitroside (**7**, 27.9 mg), and second fraction contained methyl 4-*O*-succinyl-α-L-diginoside methyl ester (**8**, 126.9 mg), and successive eluates containing methyl 4-*O*-succinyl-β-L-diginoside methyl ester (**9**, 36.7 mg) were collected. After elution of three glycosides, the column chromatography was developed with chloroform - methanol (1: 1) to give a crude solid. On TLC of silica gel with chloroform - methanol - water (78: 20: 2), the solid showed three spots of **5** (Rf 0.70), **6** (Rf 0.46) and methyl α (and β)-L-rhodosaminide (Rf 0.10). The solid was chromatographed on a column of Amberlite XAD-2 with 30% aqueous methanol. After elution of methyl α (and β)-L-rhodosaminide, the mixture of **5** and **6** was collected by the development with acetone - 0.001 N hydrochloric acid. The eluates was neutralized with Amberlite IR-45 (OH^-), filtered and evaporated to give a red solid. The solid was separated by the partition chromatography using a column of Sephadex G-25 (stationary phase/mobile phase: 0.1 M acetic acid saturated with 1-butanol/1-butanol saturated with 0.1 M acetic acid) into **5** (400 ~ 500 ml) and **6** (790 ~ 1,200 ml). Each eluates were neutralized with aqueous sodium hydroxide solution and evaporated to dryness. The residues were dissolved in a mixture of chloroform and methanol (9: 1), and the solutions were washed with water, dried over sodium sulfate and evaporated, respectively. The pure solids of **5** (154 mg) and **6** (289 mg) were obtained.

5: λ_{max}^{MeOH} 218, 236, 253, 290, 475, 495, 534 and 577 nm; mp 181 ~ 185°C (dec); $[\alpha]_D^{25}$ +830° (c 0.02, $CHCl_3$); mass spectrum m/z 874 (MH^+); IR (KBr) 3440, 2950, 1620 (C=O), 1570 and 1545 (NO_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ - CD_3OD (*ca.* 9: 1)) δ 1.49 (3H, s, CH_3-9), 1.64 (3H, s, H-6′), 1.70 (3H, s, CH_3-3″), 1.79 (3H, s, CH_3-3‴), 2.49 (6H, s, $N(CH_3)_2$), 2.78 (1H, dd, J=3.0 and 12.5 Hz, H-3′), 2.84 (1H, m, H-5‴), 4.14 (1H, m, H-5″), 4.31 (1H, d, J=2.5 Hz, H-4′), 4.35 (1H, dd, J=3.0 and 12.5 Hz, H-2′), 5.06 (1H, dd, J=2.0 and 9.0 Hz, H-1‴), 5.08 (1H, broad s, H-7), 5.50 (1H, dd, J=2.0 and 9.0 Hz, H-1″), 6.03 (1H, d, J=3.0 Hz, H-1′), 7.63 (1H, d, J=7.5 Hz, H-3) and 7.95 (1H, d, J=7.5 Hz, H-4).

Anal Calcd for $C_{41}H_{51}N_3O_{18}$: C 56.35, H 5.88, N 4.81.
Found: C 56.14, H 6.02, N 4.73.

6: λ_{max}^{MeOH} 220, 235, 253, 290, 380, 475, 496 and 535 nm; mp 206 ~ 207°C (dec); $[\alpha]_D^{25}$ +442° (c 0.05, $CHCl_3$ - MeOH (1: 1)); mass spectrum m/z 701 (MH^+); IR (KBr) 3400, 2930, 1620 (C=O), 1575 and 1540 (NO_2) cm^{-1}; ^1H NMR (250 MHz, pyridine-d_5) δ 1.51 (3H, d, $J_{5'',6''}$=6.0 Hz, H-6″), 1.59 (3H, s, CH_3-9), 1.91 (3H, s, CH_3-3″), 1.93 (3H, s, H-6′), 2.15 (1H, dd, $J_{1'',2''ax}$=9.4, J_{gem}=15.0 Hz, H-2″ax), 2.82 (6H, s, $(CH_3)_2N$), 2.90 (1H, dd, $J_{1'',2''eq}$=2.0, J_{gem}=15.0 Hz, H-2″eq), 3.36 (1H, dd, $J_{2',3'}$=12.0, $J_{3',4'}$=2.3 Hz, H-3′), 3.69 (1H, d, $J_{4',5'}$=9.0 Hz, H-4″), 4.31 (1H, m, H-5″), 4.82 (1H, d, J=2.3 Hz, H-4′), 4.86 (1H, dd, J=3.3 and 12.0 Hz, H-2′), 5.57 (broad s, H-7), 6.02 (1H, dd, $J_{1'',2''ax}$=9.4, $J_{1'',2''eq}$=2.0 Hz, H-1″), 6.48 (1H, d, $J_{1',2'}$=3.3 Hz, H-1′), 7.89 (1H, d, J=7.5 Hz, H-3) and 8.08 (1H, d, J=7.5 Hz, H-4).

Anal Calcd for $C_{30}H_{40}N_2O_{14}$: C 58.28, H 5.75, N 4.00.
Found: C 58.18, H 5.54, N 3.93.

Decilorene (**11**)

A solution of **1** (90 mg) in 3 N hydrochloric acid (5 ml) was refluxed for 6 hours. The mixture was diluted with water (100 ml), and adjusted pH value to 7.5. The mixture was extracted twice with a solution of chloroform - methanol (9: 1, 200 ml). Evaporation of the extracts gave a crude red solid. The residue was purified by preparative TLC of silica gel with chloroform - methanol - 10% aqueous ammonium acetate (20: 15: 1) to give a red solid (**11**, 15.0 mg): λ_{max}^{MeOH} 201, 268, 467, 497, 535 and 557 nm; mp 186 ~ 190°C (dec); $[\alpha]_D^{25}$ +904° (c 0.05, $CHCl_3$); mass spectrum m/z 491 (M^+); IR (KBr) 3400, 1620 (sh), 1580 and 1510 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ - CD_3OD (*ca.* 9: 1)) δ 1.80 (3H, s, H-6′), 2.49 (1H, dd, J=3.2 and 10.5 Hz, H-3′), 2.58 (10H, broad s, CH_3-9, $N(CH_3)_2$ and H-3′), 3.96 (1H, d, J=3.2 Hz, H-4′), 4.24 (1H, dd, J=4.0 and 10.5 Hz, H-2′), 5.95 (1H, d, J=4.0 Hz, H-1′), 7.49 (1H, d, J=8.0 Hz, H-3),

7.51 (1H, dd, J=1.5 and 8.0 Hz, H-8), 7.72 (1H, d, J=8.0 Hz, H-7), 7.91 (1H, d, J=8.0 Hz, H-4) and 8.01 (1H, broad s, H-10).

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