STRUCTURAL STUDIES ON AVIDINORUBICIN, A NOVEL ANTHRACYCLINE WITH PLATELET AGGREGATION INHIBITORY ACTIVITY

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(Received for publication January 28, 1991)

Avidinorubicin (MW 1,214, $C_{60}H_{86}N_4O_{22}$) was isolated from the cultured broth of strain NR0576 (which was identified as *Streptomyces avidinii* STAPLEY *et al.*) by butyl alcohol extraction, Sephadex LH-20 column chromatography and preparative HPLC. Avidinorubicin inhibited thrombin-induced platelet aggregation with an IC_{50} being 7.9 μM and was determined to be a novel anthracycline possessing two units of a new aminosugar, avidinosamine, in place of two decilonitrose groups in decilorubicin.

In the course of our screening for unique microbial products with pharmacological activity, avidinorubicin (**1**) that exhibited platelet aggregation inhibitory activity was isolated from the cultured broth of *Streptomyces avidinii*. Avidinorubicin was considered to belong to the nogalamycin[1] group based on the presence of a unique anomeric proton in the ^1H NMR spectrum. After spectral analyses, avidinorubicin was determined to be a novel anthracycline containing two units of a new aminosugar, avidinosamine. This paper describes the taxonomy, fermentation, isolation, physico-chemical properties and structure elucidation of avidinorubicin.

Fermentation

The producing strain of avidinorubicin, NR0576, was isolated from a soil sample collected at Komono-cho, Mie Prefecture, Japan and identified as *S. avidinii* STAPLEY, MATA, MILLER, DEMNY and WOODRUFF[2]. A spore suspension of this strain (0.2 ml) was inoculated into a 500-ml baffled Erlenmeyer flask containing 100 ml of a medium consisting of glucose 2.0%, potato starch 2.0%, yeast extract 0.5%, toasted soya 2.0%, NaCl 0.25%, $ZnSO_4 \cdot 7H_2O$ 0.005%, $CuSO_4 \cdot 5H_2O$ 0.0005%, $MnCl_2 \cdot 4H_2O$ 0.0005%, $CaCO_3$ 0.32% and Nissan Disfoam 0.05%. The medium was adjusted to pH 7.0 before addition of $CaCO_3$. The inoculated flask was incubated on a rotary shaker at 27°C for 3 days at 190 rpm to make a seed culture. The resultant seed culture (12 ml) was transferred into a 3-liter baffled Erlenmeyer flask containing 600 ml of the same medium followed by incubation on a rotary shaker at 27°C for 3 days at 100 rpm. This seed culture (600 ml) was transferred into a 200-liter jar fermenter containing 120 liters of the same medium. The cultivation was carried out at 27°C for 6 days, agitated at 280 rpm and aerated at 150 liters per minute.

Isolation Procedure of Avidinorubicin

The isolation procedure for avidinorubicin (**1**) is outlined in Fig. 1. The inhibitor in the broth filtrate (250 liters) was extracted with 162 liters of butyl alcohol at pH 8.5. The extract was concentrated to dryness *in vacuo* and suspended in 2.5 liters of H_2O. This solution was extracted with a mixture of chloroform and methanol (5:1, 2.5 liters × 3) and the organic layer was concentrated to dryness *in vacuo*. The residue (18.6 g) was chromatographed on a silica gel column (Wakogel C-200, 50 mm × 500 mm) and developed with a mixture of chloroform, methanol and aqueous ammonia (3:1:0.1) successively to yield a crude powder of **1** (554 mg).

Further purification was carried out by preparative HPLC with a Capcell pak C_{18} column (15 mm × 250 mm) developed with a mixture of methanol and 25 mM aqueous Na_2HPO_4 solution (3:1). The eluate containing **1** was concentrated to a small volume and extracted with butyl alcohol at pH 8.5. The organic layer was concentrated to dryness *in vacuo*. The residue was chromatographed on a column of Sephadex LH-20 with methanol to give a reddish powder of **1** (150 mg).

Structure of Avidinorubicin

The selected physico-chemical properties of **1** are summarized in Table 1, and its ^1H and UV spectra are shown in Figs. 2 and 3, respectively. The molecular formula of **1** was determined to be $C_{60}H_{86}N_4O_{22}$ based on the HRFAB mass (obsd 1,215.5911, +9.9 mmu for $C_{60}H_{87}N_4O_{22}$ $(M+H)^+$) and NMR spectroscopic data. Its characteristic UV data suggested that **1** belonged to the anthracycline group, and the presence of a unique anomeric proton signal δ 6.50 strongly suggested that **1** was closely related to nogalamycin[1], decilorubicin[3], arugomycin[4] and viriplanin[5]. The latter three anthracyclines contain nitrosugars in the molecule as indicated by typical IR absorption at 1545 cm^{-1} and 1350 cm^{-1}. The IR spectrum of **1** (Fig. 4) was almost identical with that of decilorubicin except

Fig. 1. Isolation procedure of avidinorubicin.

Broth filtrate
- adjusted to pH 8.5
- extracted with BuOH

BuOH layer
- concd under reduced pressure
- dissolved in H_2O
- extracted with $CHCl_3$-MeOH (5:1)

Organic layer
- concd under reduced pressure

SiO_2 column chromatography
- eluted with $CHCl_3$-MeOH-aq NH_4OH (3:1:0.1)

Preparative-HPLC
- Capcell pak C_{18}
- eluted with MeOH-aq Na_2HPO_4 (3:1)
- flow rate 8.0 ml/minute

Active fractions
- concd under reduced pressure
- adjusted to pH 8.5
- extracted with BuOH

BuOH layer
- concd under reduced pressure
- dissolved in MeOH

Sephadex LH-20 column chromatography
- eluted with MeOH

Avidinorubicin

Table 1. Physico-chemical properties of avidinorubicin (**1**).

Appearence	Reddish powder
Molecular formula	$C_{60}H_{86}N_4O_{22}$
MW	1,214
HRFAB-MS	Obsd 1,215.5911 +9.9 mmu error for $C_{60}H_{87}N_4O_{22}$ $(M+H)^+$
UV λ_{max}^{MeOH} nm (ε)	237 (28,300), 255 (21,000), 294 (6,200), 390 (3,000), 498 (10,000)
$\lambda_{max}^{MeOH+NaOH}$ nm (ε)	238 (28,700), 254 (30,600), 294 (6,000), 366 (4,700), 563 (11,000), 600 (10,500)
IR ν_{max} (KBr) cm^{-1}	3450, 1750, 1640, 1600, 1440, 1220, 1180, 1100~1000
MP	206~209°C (dec)
$[\alpha]_D^{24}$ (c 0.1, MeOH)	+523°

Fig. 2. 500 MHz ^{1}H NMR spectrum of avidinorubicin in pyridine-d_5.

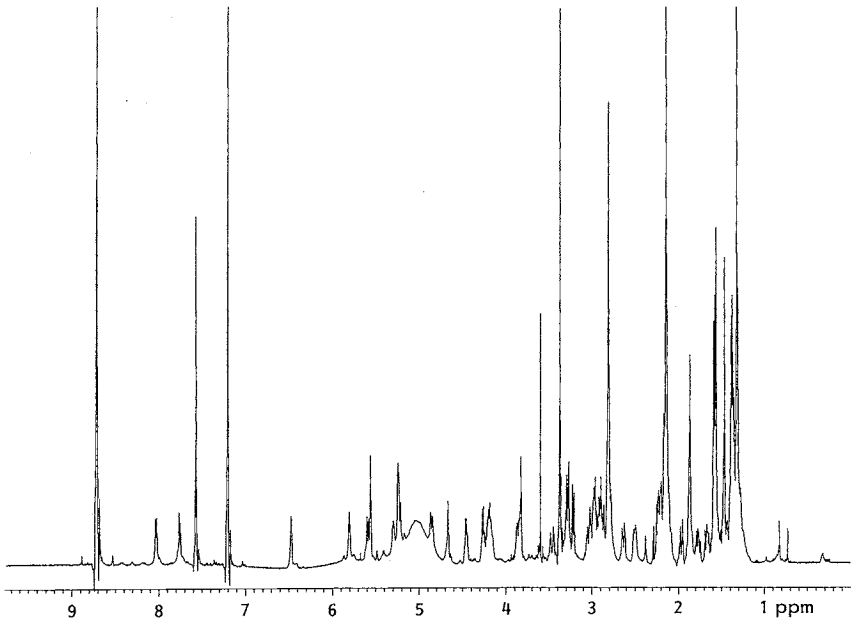
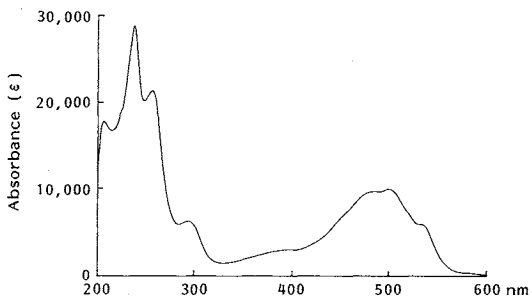


Fig. 3. UV spectrum of avidinorubicin in MeOH.



for the absence of the bands derived from nitro substituents. Consequently, we compared directly ^{1}H and ^{13}C NMR spectra of **1** with those of decilorubicin. As shown in Table 2, ^{1}H and ^{13}C chemical shifts of **1** are quite similar to those of decilorubicin except for the following protons and carbons adjacent to nitro group: I) C-3″-CH_3 (δ 2.00) and C-3‴-CH_3 (δ 1.92) proton signals of decilonitrose in decilorubicin are shifted upfield to δ 1.60 and 1.35, respectively, in **1**; II) C-3″ (δ 89.7) and C-3‴ (δ 89.5) carbons attached to a nitro group are shifted to δ 52.2 and 52.2 in **1**. Taking into consideration of the chemical shifts of the 3″ and 3‴ positions and the molecular formula ($C_{60}H_{86}N_4O_{22}$ = decilorubicin $-2NO_2+2NH_2$), the structure assignment of **1** is consistent with a compound with amino groups in place of the corresponding nitro groups in decilorubicin as shown in Fig. 5.

This assignment was confirmed by degradation experiments of **1** (Fig. 6). Hydrolysis of **1** (40% HCOOH, 80°C, 1 hour) gave **3** (MW 813, FAB mass 814 $(M+H)^+$, $[\alpha]_D^{24}$ +412° (*c* 0.1, MeOH), mp 138~142°C) and two sugar derivatives. The carbohydrate fragments were determined to be L-rhodosamine and 4-succinyl-L-diginose based on their NMR and FAB mass spectral data, and $[\alpha]_D$ values[6,7].

The MW of **3** (HRFAB-MS, obsd 814.3760, −0.3 mmu for $C_{41}H_{56}N_3O_{14}$ $(M+H)^+$), could easily be explained by cleavage of the rhodosamine and 4-succinyldiginose moieties from **1**. Upon comparison of the ^{13}C NMR spectrum of **1** with that of **3**, glycosidation shifts were observed at C-7 (δ 69.1 for **1** vs. 62.6 for **3**) and C-4‴ (δ 85.4 for **1** vs. 77.5 for **3**) in **1**. These results indicated that the sugar units were removed from C-7 and C-4‴ in **1** during hydrolysis.

Hydrogenolysis of **1** over Pd-$BaSO_4$ gave **2** (MW, 1,041) and rhodosamine. This result indicated that

Table 2. NMR chemical shifts of avidinorubicin, **3** and decilorubicin in pyridine-d_5 (δ ppm).

	Avidinorubicin		**3**		Decilorubicin	
1	157.5		157.4		154.6	
2	139.7		139.1		ND	
3	131.2	7.75 (d, J=8.3)[a]	131.0	7.75 (d, J=8.3)	131.1	7.90 (d, J=8)
4	119.2	8.03 (d, J=8.3)	118.8	8.02 (d, J=8.3)	118.9	8.05 (d, J=8)
4a	134.8		134.6		ND	
5	181.8		186.7		ND	
5a	111.9		111.7		111.8	
6	154.5		154.5		157.4	
6a	138.0		ND		ND	
7	69.1	5.55 (br s)	62.6	5.50 (br s)	68.8	5.57 (br s)
8	41.2	2.65 (d, J=13), 2.25[b]	41.5	2.52 (d, J=13), 2.01 (dd, J=13, 3)	41.7	2.22[b], 2.65 (d, J=13)
9	67.4		68.2		67.5	
10	38.6	3.45 (d, J=18), 2.85[b]	38.7	3.56 (d, J=17), 2.82 (d, J=17)	38.5	2.88 (d, J=18), 3.47 (d, J=18)
10a	120.1		120.0		120.4	
11	157.0		156.5		156.2	
11a	111.2		111.2		111.4	
12	186.7		186.7		ND	
12a	137.2		ND		ND	
13	32.0	1.45 (s)	30.4	1.54 (s)	32.0	1.55 (s)
1′	99.0	6.50 (d, J=3)	98.8	6.43 (d, J=3)	98.8	6.46 (d, J=3)
2′	68.1	4.88 (dd, J=11, 3)	67.9	4.84 (dd, J=11, 3)	68.0	4.85 (dd, J=11, 3)
3′	61.6	3.34[b]	61.4	3.29 (dd, J=11, 1.5)	61.6	3.30 (dd, J=11, 2)
4′	80.7	4.70 (d, J=1.5)	80.9	4.68 (d, J=1.5)	81.3	4.70 (br d, J=2)
5′	78.0		78.1		77.8	
6′	23.2	1.88 (s)	23.2	1.87 (s)	21.5	1.78 (s)
3′-$N(CH_3)_2$	40.3	2.84 (s)	40.4	2.80 (s)	40.5	2.80 (s)
1″	100.9	5.58 (d, J=9.5)	100.7	5.68 (br d, J=9)	100.2	5.90 (d, J=9)
2″	45.9	1.78 (dd, J=14, 9.5), 2.12[b]	45.4	2.27 (d, J=13), 1.81 (dd, J=13, 9)	42.0	2.18 (dd, J=14, 9), 2.97 (d, J=14)
3″	52.2		52.9		89.7	
4″	87.9	3.20 (d, J=10)	85.2	3.38 (d, J=10)	82.3	3.78 (d, J=10)
5″	70.8	4.20 (dq, J=10, 6.5)	70.6	4.30 (dq, J=10, 6.5)	70.2	4.21 (dq, J=10, 6)
6″	18.9	1.37 (d, J=6.5)	18.7	1.43 (d, J=6.5)	18.4	1.33 (d, J=6)
3″-CH_3	30.3	1.60 (s)	30.9	1.56 (s)	24.8	2.00 (s)
1‴	100.0	5.27 (d, J=9.5)	99.8	5.35 (d, J=9)	99.8	5.55 (d, J=9)
2‴	45.9	1.65 (dd, J=14, 9.5), 1.95 (d, J=14)	44.8	2.15 (d, J=13), 1.71 (dd, J=13, 9)	42.0	1.88 (dd, J=14, 9), 2.56 (d, J=14)
3‴	52.2		52.5		89.5	
4‴	85.4	3.28 (d, J=10)	77.5	3.32 (d, J=10)	83.8	3.66 (d, J=10)
5‴	70.8	4.13 (dq, J=10, 6.5)	71.3	4.24 (dq, J=10, 6.5)	70.2	4.18 (dq, J=10, 6)
6‴	19.0	1.40 (d, J=6.5)	19.1	1.58 (d, J=6.5)	18.5	1.35 (d, J=6)
3‴-CH_3	32.0	1.35 (s)	31.5	1.40 (s)	24.8	1.92 (s)
1⁗	101.9	5.30 (br s)			102.1	5.35 (br s)
2⁗	29.2	ND			30.0	ND
3⁗	73.4	ND			73.3	3.70 (ddd, J=11.5, 6, 2.5)
4⁗	71.8	5.77 (br s)			71.7	5.85 (br s)
5⁗	66.6	4.28 (q, J=6.5)			66.8	4.33 (q, J=6)
6⁗	17.0	1.33 (d, J=6.5)			17.0	1.37 (d, J=6)
3⁗-OCH_3	56.0	3.38 (s)			55.9	3.25 (s)
Suc.-CO	173.3				173.4	
Suc.-CH_2	30.0	3.00[b]			30.0	ND
Suc.-CH_2	30.0	3.00[b]			30.0	ND
Suc.-COOH	175.4				172.7	

Table 2. (Continued)

	Avidinorubicin		3	Decilorubicin	
1''''	100.9	5.27 (br s)		101.6	5.23 (br d, $J=3$)
2''''	32.0	1.30[b]		30.0	2.20[b]
3''''	67.0	2.50 (m)		61.0	2.68 (m)
4''''	67.4	3.85 (br s)		67.1	3.90 (br s)
5''''	68.1	4.48 (q, $J=6.5$)		68.0	4.46 (q, $J=6$)
6''''	17.8	1.60 (d, $J=6.5$)		17.6	1.59 (d, $J=6$)
3''''-$N(CH_3)_2$	42.2	2.15 (s)		42.0	2.15 (s)

[a] Multiplicity and coupling constant (Hz) are in parentheses.
[b] J value could not be determined.
ND: Not determined.

Fig. 4. IR (KBr) spectrum of avidinorubicin.

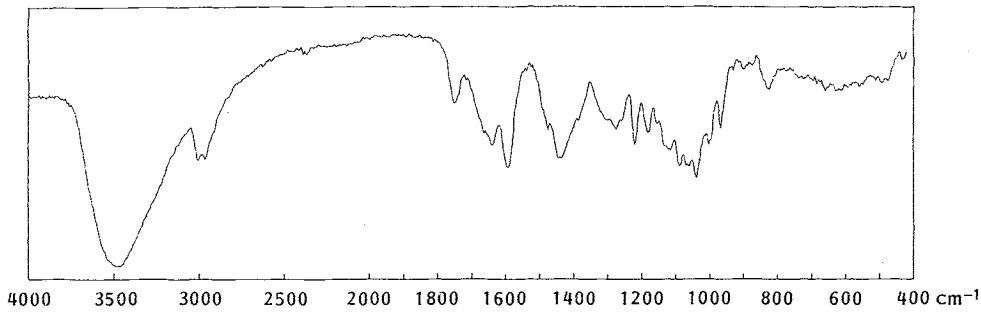


Fig. 5. Structure of avidinorubicin (**1**).

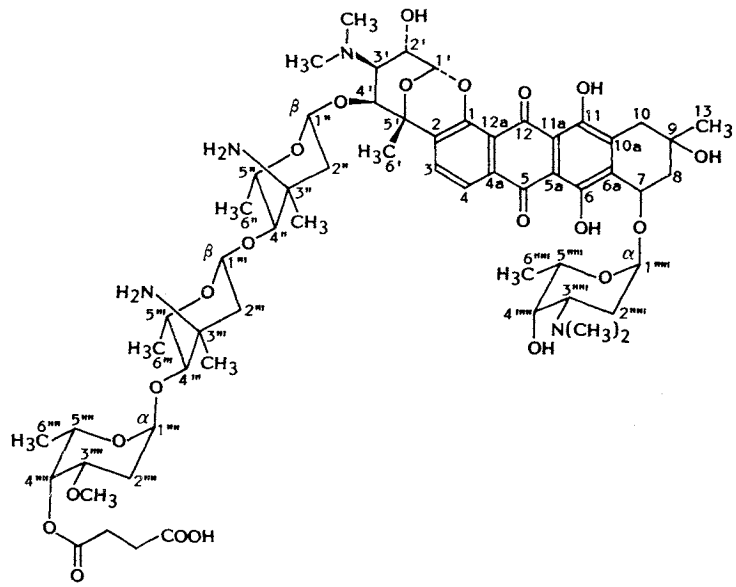
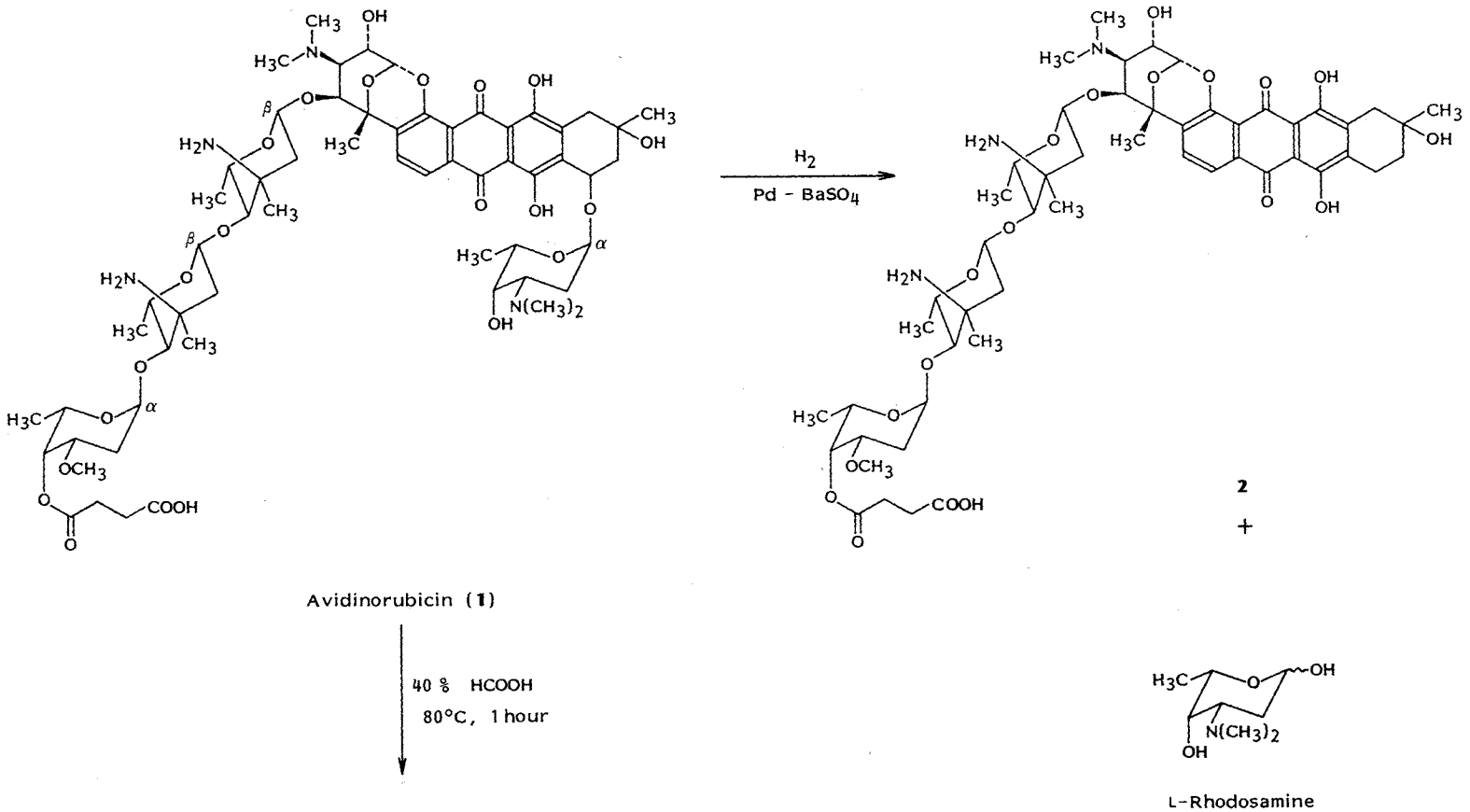
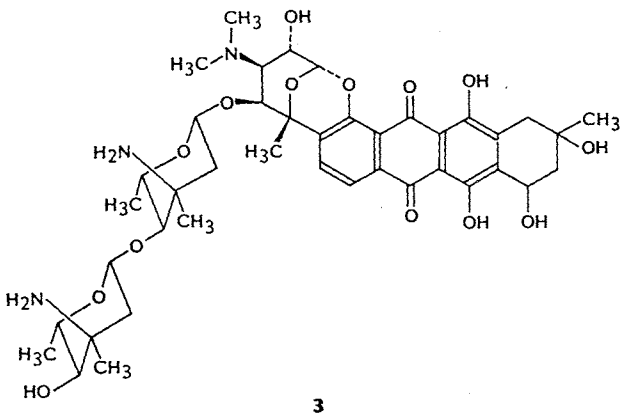
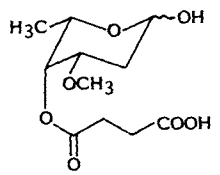


Fig. 6. Degradation products of avidinorubicin.

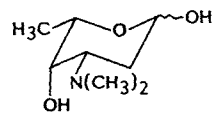




3



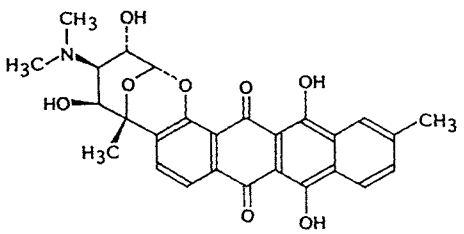
L-Succinyldiginose



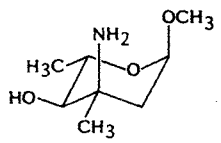
L-Rhodosamine

6 N HCl
110°C, 18 hours

5 % HCl - MeOH
110°C, 18 hours



Decilorone (**4**)



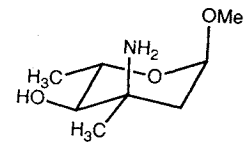
Methyl α-L-avidinosaminide (**5**)

rhodosamine was attached to C-7, and consequently 4-succinyldiginose was bound at C-4‴. The anomeric protons of the rhodosamine and diginose moieties in **1** (1⁗⁗⁗-H and 1⁗⁗-H, respectively), observed at δ 5.27 and 5.30 as broad singlets (Table 2), indicated that these glycosidic bonds had α-configurations.

Methanolysis of **3** (5% HCl in methanol, 110°C, 18 hours) yielded a methyl glycoside of a novel aminosugar **5**, (methyl α-L-avidinosaminide). **5** was determined to be methyl 3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranoside based on its FAB mass ($(M+H)^+$ 176) and ^1H and ^{13}C NMR spectroscopic studies (Table 3). The equatorial orientation of the methyl group at C-3 was elucidated by analyzing ^{13}C NMR chemical shifts and measuring NOEs. In most cases, the ^{13}C NMR chemical shifts of 3-axial methyl carbons of 3-*C*-methyl sugars are observed at higher field than those of 3-equatorial methyl carbons (Table 4)[8]. The ^{13}C NMR chemical shift of the methyl group at C-3 in **5** is similar to those of equatorial methyls of other 3-*C*-methyl sugars (Table 4) and those of decilonitroses in decilorubicin (Table 2). In addition, NOEs were observed between 3″-CH_3 and 4″-H, and 3‴-CH_3 and 4‴-H in NOESY experiments of **3**. Therefore, the 3-methyl substituent in **5** was determined to occupy an equatorial position. The absolute configuration of **5** was examined by TACu method[9]. The positive contribution ($\Delta[M]_{436(TACu)}$ +605°) of **5** suggested that this sugar belonged to the L-series. Therefore, avidinosamine in the sugar moiety of **3** was determined to be 3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranose.

Hydrolysis of **3** (6N HCl, 110°C, 18 hours) gave an aromatized aglycone, which was identified

Table 3. Physico-chemical properties of **5** (methyl α-L-avidinosaminide)[a].

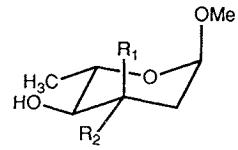


	^{13}C (ppm)	^1H (ppm) in CD_3OD
1	98.8	4.71 (br d, J=3.5)[b]
2	39.0	2.00 (d, J=15), 1.86 (dd, J=15, 3.5)
3	56.6	
4	75.0	3.24 (d, J=10)
5	65.5	3.66 (dq, J=10, 6.5)
6	18.3	1.18 (d, J=6.5)
3-CH_3	24.8	1.23 (s)
1-OCH_3	55.6	3.23 (s)

[a] MW 175 (FAB mass, m/z 176 $(M+H)^+$), $[\alpha]_D^{24}$ (c 0.085, MeOH) +103°, $[\alpha]_{435}^{24}$ (c 0.04, H_2O) +62.1°, $[\alpha]_{435}^{24}$ (c 0.04, tetraamminecopper (II) sulfate) +408°, $\Delta[M]_{TACu}$ +605°.

[b] Multiplicity and coupling constant (Hz) are in parentheses.

Table 4. ^{13}C NMR chemical shifts of methyl 3-*C*-methyl-hexopyranosides (δ ppm in $CDCl_3$).



	R_1: NHAc[a] R_2: CH_3	CH_3[a] NHAc	NO_2[b] CH_3	CH_3[b] NO_2	NHOH[c] CH_3	NHOBz[c] CH_3	NHBz[c] CH_3
1	99.7	99.7	98.6	99.1	97.8	97.5	98.1
2	38.5	40.5	41.7	41.4	38.4	39.7	41.8
3	56.7	56.4	89.4	89.3	57.4	59.2	55.6
4	87.9	82.9	77.0	74.6	80.0	78.9	78.6
5	69.7	70.3	71.1	70.5	65.2	62.4	63.2
6	18.4	19.0	18.3	18.3	18.2	17.6	17.5
3-CH_3	24.7	20.0	25.2	18.1	23.3	23.9	24.0
1-OCH_3			56.5	56.5	55.0	55.3	55.4

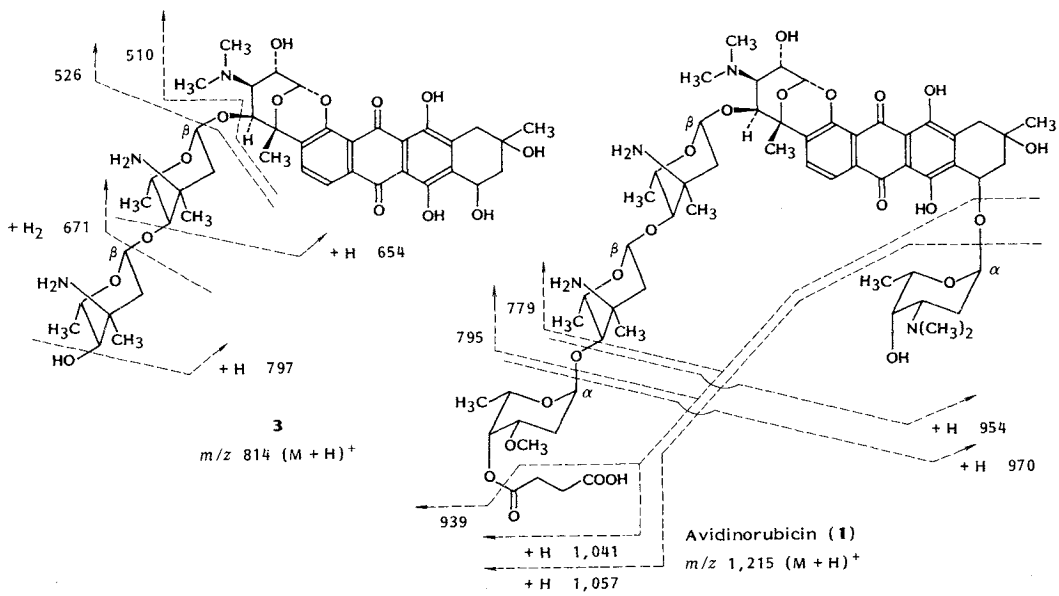
[a~c] Data taken from refs 7, 6, and 10, respectively.

Table 5. Comparison of NMR chemical shifts of **4** and decilorone (δ ppm, $CDCl_3$ - CD_3OD (9 : 1))

	4		Decilorone[a]	
1	152.3		153.0	
2	131.1		131.3	
3	125.6	7.49 (d, J=8)[b]	125.6	7.49 (d, J=8)
4	125.6	8.15 (d, J=8)	125.6	7.91 (d, J=8)
4a	133.6		133.7	
5	173.3		173.7	
5a	106.5		106.4	
6	169.5		168.7	
6a	129.6		129.2	
7	118.4	7.88 (d, J=8)	118.4	7.72 (d, J=8)
8	133.9	7.55 (dd, J=8, 1)	134.1	7.51 (dd, J=8, 1)
9	143.5		143.6	
10	125.4	8.10 (d, J=1)	125.5	8.01 (d, J=1)
10a	130.8		131.0	
11	169.0		168.4	
11a	106.5		106.6	
12	176.3		176.2	
12a	119.4		119.5	
13	21.8	2.53 (s)	22.1	2.58 (s)
1′	96.5	5.83 (d, J=3)	97.3	5.95 (d, J=4)
2′	67.0	4.32 (dd, J=11, 3)	68.4	4.24 (dd, J=10.5, 4)
3′	63.1	2.65[c]	62.7	2.49 (dd, J=10.5, 3.2)
4′	73.4	4.08 (br s)	75.4	3.96 (d, J=3.2)
5′	75.8		76.4	
6′	21.8	1.68 (s)	22.4	1.80 (s)
3′-$N(CH_3)_2$	42.9	2.65 (s)	43.3	2.58 (s)

[a] Data taken from ref 2.
[b] Multiplicity and coupling constant (Hz) are in parentheses.
[c] J value could not be determined.

Fig. 7. Fragment ions observed in the FAB mass spectra of avidinorubicin and **3**.



to be decilorone[3] by comparison of physico-chemical properties (MW 491, FAB mass 492 $(M+H)^+$, $[\alpha]_D^{24}$ +850° (*c* 0.1, $CHCl_3$-MeOH, 9:1), mp 190~193°C (dec)) and NMR spectral data with an authentic sample (Table 5). Since the chemical shifts and coupling constants of the dimethyl aminosugar moiety (1'-H to 6'-H and C-3'-$N(CH_3)_2$) directly fused to the aglycone are similar to those of arugomycin but different from those of nogalamycin[10], the relative configuration of this aminosugar moiety was also confirmed to be 3,6-dideoxy-3-dimethylamino-galactopyranose with axial orientations for 2'-H ($J_{1',2'}=3$ Hz and $J_{2',3'}=11$ Hz) and 3'-H ($J_{2',3'}=11$ Hz and $J_{3',4'}=1.5$ Hz) and an equatorial orientation for 4'-H ($J_{3',4'}=1.5$ Hz).

The glycosidation shift observed at C-4' (δ 73.4 in **4** vs. 80.9 in **3**) indicated the sugar chain was attached to the C-4' position. In the ^1H and ^{13}C NMR spectra of **3**, a pair of signals assignable to the avidinosamine unit were observed (1''-H to 6''-H and 3''-CH_3, and 1'''-H to 6'''-H and 3'''-CH_3 in Table 2) and NOEs were detected between 4'-H and 1''-H, and 4''-H and 1'''-H in the NOESY spectrum of **3**. These results indicated the presence of glycosidic bonds between 1'' and 4', and 1''' and 4''. The configurations of these glycosidic bonds were determined to be β based on their large coupling constants ($J_{1'',2''}=J_{1''',2'''}=9.0$ Hz).

As a result, the structure of avidinorubicin was determined to be **1**, a compound with two amino groups in place of two nitro groups in decilorubicin. The fragmentation patterns observed in positive FAB mass spectrum of **1** and **3** (Fig. 7) also supported this structure. The determination of the absolute stereochemistry of the aglycone is being investigated.

Biological Properties

1 inhibited thrombin-, ADP- or collagen-induced aggregation in human platelet with IC_{50}s 7.9, 7.9, and 3.0 μM, respectively. This compound showed cytotoxicity against HeLa cells with IC_{50} value being 1.37 μg/ml, which was slightly weaker than those of arugomycin and decilorubicin. This may mean that the nitro or amino group in decilonitrose and avidinosamine do not play an important role in their biological activities. Detailed biological data will be reported elsewhere.

Discussion

Avidinorubicin, containing two units of a unique aminosugar was determined to be a novel anthracycline related to decilorubicin, and its aminosugar was determined to belong to the L-series.

On the assumption that the nitro group was formed biosynthetically from the amino group, avidinorubicin may be a precursor of decilorubicin. The decilonitrose moiety of decilorubicin had been determined to belong to the L-series by TACu method and synthetic approach by ISHII *et al.*[7]. On the other hand, the decilonitrose moiety of viriplanins was recently claimed to be of the D-series by analysis of the CD spectra of a dibenzoate derivative of the reduced product of decilonitrose by KIND *et al.*[11]. More recently, the L-configuration of decilonitrose was established unambiguously by synthesis of its methyl β-glycoside starting from L-rhamnose by NISHIMURA *et al.*[12]. It is, therefore, gratifying that the aminosugar moiety obtained from avidinorubicin also belongs to the L-series, supporting the L-configuration of the decilonitrose moieties in decilorubicin and arugomycin.

Acknowledgment

The authors wish to thank Dr. S. KONDO of Institute of Microbial Chemistry for a gift of decilorubicin and are also grateful to Dr. Y. NISHIMURA of Institute of Microbial Chemistry and Dr. K. ISHII of Kanebo Chemical Co., Ltd. for sending NMR spectra of decilorubicin derivatives. The authors are grateful for many useful suggestions by Drs. M. ARISAWA and T. OKUDA, and Mrs. K. FUJIMORI and Miss Y. ISHIWATA of Nippon Roche Research Center.

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