CADEGUOMYCIN, A NOVEL NUCLEOSIDE ANALOG ANTIBIOTIC

II. IMPROVED PURIFICATION, PHYSICOCHEMICAL PROPERTIES AND STRUCTURE ASSIGNMENT

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Cadeguomycin, a new nucleoside analog antibiotic, has been purified as colorless needle crystals by recycling preparative HPLC. The antibiotic, $C_{12}H_{14}O_7N_4$, mp 231~239°C (dec.), FD-MS: m/z 326 (M^+); is a weakly acidic substance, showing UV $\lambda_{max}^{H_2O}$ (ε) 232 (19677), 272 (6881) and 298 nm (7607), and IR ν_{max}^{KBr} 1650 (C=O) and 3420 (NH or OH) cm^{-1}. The UV spectrum is similar to other pyrrolo[2,3-*d*]pyrimidines. The structure of cadeguomycin, 2-amino-3,4-dihydro-4-oxo-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid, has been elucidated by ^1H NMR and ^{13}C NMR in comparison with other pyrrolo[2,3-*d*]pyrimidines and their nucleosides.

In the preceding paper[1], taxonomy of the cadeguomycin-producing organism, and isolation and purification of the antibiotic from the culture filtrate have been described. Various chromatographic methods hardly separate cadeguomycin from tubercidin. The isolation and purification of cadeguomycin have been successfuly performed by recycling preparative HPLC. We here report an improved purification procedure, physicochemical properties and structure assignment of cadeguomycin, a new nucleoside analog antibiotic. The biological activity will be published elsewhere.

Improved Purification Method

Cadeguomycin was isolated and purified by using recycling preparative HPLC from the culture filtrate of *Streptomyces hygroscopicus* strain IM7912T.

The fermentation broth (90 liters) was, after removal of the mycelial cake by filtration, adsorbed on an Amberlite XAD-8 column and eluted with methanol, or alternatively was applied to an Amberlite IRA-400 column and eluted with 0.2 N HCl, followed by neutralization with Amberlite IRA-45. In either case, the eluate was condensed to about 200 ml, which was then subjected to reverse phase preparative HPLC (15~23 μm C_{18}/LRP-1) with 30% methanol plus 1% acetic acid. Tubercidin passed through the column faster than cadeguomycin. Fine needle crystals (12.8 mg) of cadeguomycin were obtained, following the XAD-8 column, by recycling HPLC with changing elution solvents as shown in Fig. 1. Cadeguomycin obtained in the initial isolation procedure[1] was used as a standard.

Physicochemical Properties of Cadeguomycin

Pure cadeguomycin was obtained as colorless needle crystals, which melted at 231~239°C with decomposition. The compound was adsorbed by Amberlite IRA-400 (Cl^-) but not by IRA-45, IR-120B or IRC-50, suggesting that it is a weakly acidic substance. The free acidic form displayed good solubility

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Fig. 1. Isolation and purification of cadeguomycin.

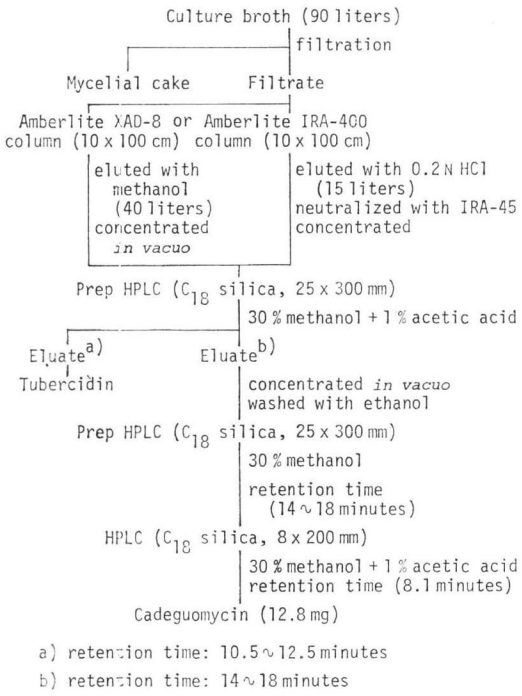


Fig. 2. UV absorption spectrum of cadeguomycin.

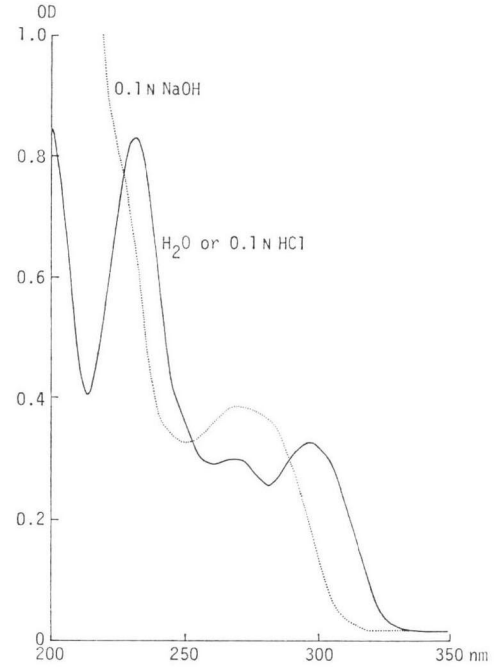
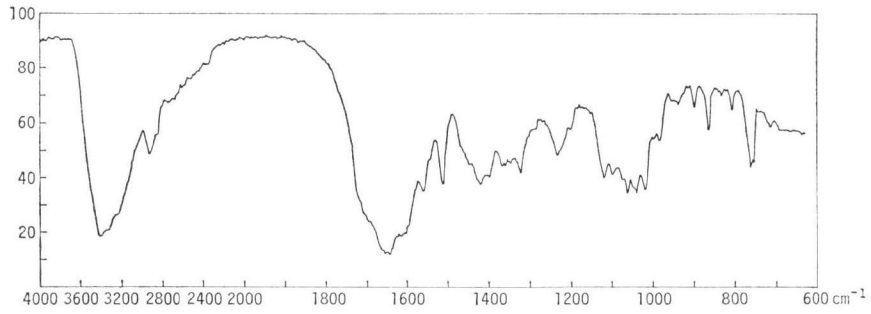


Fig. 3. IR spectrum of cadeguomycin (KBr).



in dimethyl sulfoxide (>10 mg/ml); moderate solubility in methanol (*ca.* 2.5 mg/ml) and water (*ca.* 1.3 mg/ml); and very slight solubility in ethanol, ethyl acetate and chloroform. The antibiotic was stable in acidic, neutral and alkaline solutions; no significant change in biological activity was detected when it was held at pH 2~10 and heated at 100°C for 5 minutes.

The field desorption mass spectrum (FD-MS) revealed the molecular ion peak at *m/z* 326 (M^+). The UV spectra showed maxima at 232 nm (ε 19677), 272 nm (ε 6881) and 298 nm (ε 7607) in H_2O or in 0.1 N HCl, and a maximum at 268 nm (ε 9175) in 0.1 N NaOH (Fig. 2). The IR spectrum (KBr) was consistent with the presence of carbonyl (1650 cm^{-1}), and NH or OH group (3420 cm^{-1}) (Fig. 3). ^{13}C NMR in Me_2SO-d_6, and ^1H NMR in Me_2SO-d_6, D_2O and CD_3OD are presented in Tables 1~4.

The elemental analysis was as follows:

Found:	C 43.57, H 4.29, N 16.17.
Calcd. for $C_{12}H_{14}O_7N_4$:	C 44.17, H 4.33, N 17.17.

Table 1. 100 MHz ^{13}C NMR of cadeguomycin in Me_2SO-d_6.

Carbon	Chemical shift* (ppm)	Multiplicity**	Carbon	Chemical shift (ppm)	Multiplicity
C-2	153.32	s	C-1′	86.17	d
C-4	161.39	s	C-2′	74.14	d
C-5	110.96	s	C-3′	70.47	d
C-6	125.64	d	C-4′	85.27	d
C-7a	152.18	s	C-5′	61.29	t
C-4a	96.31	s			
C-8	162.67	s			

* TMS (0 ppm) was used as an internal standard.
** s=singlet, d=doublet, t=triplet.

Table 2. 400 MHz ^1H NMR of cadeguomycin in Me_2SO-d_6.

Proton	Chemical shift* (ppm)		Multiplicity**	Coupling constant (Hz)
2-NH_2	6.72	2H	br.s	
3-NH	11.67	1H	br.s	
6-H	7.78	1H	s	
8-COOH	14.32	1H	br.s	
1′-H	5.87	1H	d	$J_{1',2'}=6.8$
2′-H	4.28	1H	ddd	$J_{2',2'OH}=6.8$
3′-H	4.03	1H	ddd	$J_{3',3'OH}=4.2$
4′-H	3.83	1H	ddd	
5′-H_2	3.50	1H	ddd	$J_{5',5'OH}=5.8$
	3.57	1H	ddd	$J_{5',5'OH}=5.8$
2′-OH	5.31	1H	d	$J_{2'OH,2'}=6.8$
3′-OH	5.08	1H	d	$J_{3'OH,3'}=4.2$
5′-OH	5.05	1H	t	$J_{5'OH,5'}=5.8$

* TMS (0 ppm) was used as an internal standard.
** s=singlet, br.s=broad singlet, d=doublet, ddd=double double doublet, t=triplet

Structure Assignment for Cadeguomycin

FD-MS of cadeguomycin showed its molecular weight of 326. The elemental analysis together with the result of FD-MS indicated the molecular formula $C_{12}H_{14}O_7N_4$, which was confirmed by ^1H NMR and ^{13}C NMR in Me_2SO-d_6.

The aglycon moiety of cadeguomycin was estimated as $C_7H_5O_3N_4$ (MW 193) from its spectral data, possessing functional groups: –COOH, $-NH_2$ and –OH, which were indicated by its ^1H NMR (Table 2) and ^{13}C NMR (Table 1) in Me_2SO-d_6. The UV absorption spectrum of cadeguomycin (Fig. 2) was similar to those of 7-deazaguanine (2-amino-4-oxo-pyrrolo[2,3-*d*]pyrimidine)[2], 7-methyl-7-deazaguanine (2-amino-5-methyl-4-oxo-pyrrolo[2,3-*d*]pyrimidine)[3] and 7-cyano-7-deazaguanosine (pre Q_0 nucleoside; the precursor of nucleoside Q)[4] in neutral solution. The fragment ion peak at *m/z* 194 (aglycon+1) in FD-MS of cadeguomycin, and comparatively lower chemical shift of its ^1H NMR signal due to 1′-H indicated that cadeguomycin has an *N*-glycoside bond[5~10]. However, the corresponding aglycon has not been obtained by acid hydrolysis, which could be attributed to the pyrrolo[2,3-*d*]pyrimidine structure of the aglycon moiety[11~13]. Summarizing the above discussion, the aglycon of cadeguomycin was presumed to possess a tri-substituted pyrrolo[2,3-*d*]pyrimidine structure (probably 7-deazaguanine-

Table 3. 400 MHz ^{1}H NMR of cadeguomycin in D_2O.

Proton	Chemical shift (ppm)*		Multiplicity**	Coupling constant (Hz)
6-H	7.78	1H	s	
1′-H	5.98	1H	d	$J_{1',2'}=5.6$
2′-H	4.58	1H	dd	$J_{2',1'}=5.6$, $J_{2',3'}=5.4$
3′-H	4.34	1H	dd	$J_{3',2'}=5.4$, $J_{3',4'}=3.6$
4′-H	4.18	1H	ddd	$J_{4',3'}=3.6$, $J_{4',5'}=3.2$, 4.1
5′-H_2	3.86	1H	dd	$J_{5',4'}=3.2$, $J_{5',5'}=12.7$
	3.79	1H	dd	$J_{5',4'}=4.1$, $J_{5',5'}=12.7$

* *t*-Butanol (1.23 ppm) was used as an internal standard.
** s=singlet, d=doublet, dd=double doublet, ddd=double double doublet.

Table 4. 400 MHz ^{1}H NMR of cadeguomycin in CD_3OD.

Proton	Chemical shift (ppm)*		Multiplicity**	Coupling constant (Hz)
6-H	7.84	1H	s	
1′-H	6.00	1H	d	$J_{1',2'}=5.6$
2′-H	4.44	1H	dd	$J_{2',1'}=5.6$, $J_{2',3'}=5.4$
3′-H	4.25	1H	dd	$J_{3',2'}=5.4$, $J_{3',4'}=3.6$
4′-H	4.06	1H	ddd	$J_{4',3'}=3.6$, $J_{4',5'}=3.1$, 3.6
5′-H_2	3.74	1H	dd	$J_{5',4'}=3.6$, $J_{5',5'}=12.2$
	3.82	1H	dd	$J_{5',4'}=3.1$, $J_{5',5'}=12.2$

* TMS (0 ppm) was used as an internal standard.
** s=singlet, d=doublet, dd=double doublet, ddd=double double doublet.

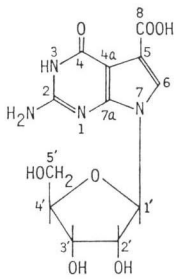
carboxylic acid). This was also supported by comparison of its ^{13}C NMR data with those of nucleoside antibiotics with pyrrolo[2,3-*d*]pyrimidine nucleosides: tubercidin, toyocamycin and sangivamycin[14], and of other pyrrolo[2,3-*d*]pyrimidines[3,15], and by comparison of ^{1}H NMR data of cadeguomycin with those of pre Q_0 nucleoside[16]. The ^{13}C NMR signals at δ 153.32 (s) and 161.39 (s) in the spectrum of cadeguomycin appeared at comparable positions with those of 2-amino-5-methyl-pyrrolo[2,3-*d*]-pyrimidine-4-one [δ 152.1 (C-2) and 159.4 (C-4)][3], indicating that $-NH_2$ and $-OH$ groups are linked to C-2 and C-4 of the pyrrolo[2,3-*d*]pyrimidine ring, respectively. The remaining $-COOH$ group may be, therefore, attached to 5 or 6 position of the aglycon moiety. The ^{13}C NMR signals at δ 125.64 (d) and 110.96 (s) of cadeguomycin were similar to those of sangivamycin [δ 126.09 (C-6) and 111.27 (C-5)][14], which has $-CONH_2$ group at C-5 position, suggesting that $-COOH$ is attached to C-5 of the aglycon moiety. The ^{1}H NMR signal at δ 7.78 in the spectrum of cadeguomycin in Me_2SO-d_6 (Table 2) was assigned as 6-H by comparison with the signal due to 6-H of pre Q_0 nucleoside in Me_2SO-d_6 (δ 7.88)[16], which proved the above suggestion. Thus the aglycon moiety of cadeguomycin was determined to be 2-amino-4-oxo-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid.

The ^{1}H NMR spectra in CD_3OD due to the sugar moiety of cadeguomycin (Table 4) and of tubercidin[1] resembled each other, suggesting that cadeguomycin possesses ribose as its sugar moiety. This was further supported by comparison of its ^{13}C NMR data with those reported with pyrrolo[2,3-*d*]pyrimidine nucleosides: tubercidin, toyocamycin and sangivamycin[14], and of guanosine[17]. The ^{1}H NMR signals due to the sugar moiety of cadeguomycin in Me_2SO-d_6 (Table 2) and in D_2O (Table 3) closely resembled those of pre Q_0 nucleoside[16] in Me_2SO-d_6 [δ 5.82 (1′-H), 4.20 (2′-H), 4.01 (3′-H),

3.81 (4′-H) and 3.55 (5′-H_2)] and in D_2O [δ 5.91 ($J_{1',2'}$=5.5 Hz, 1′-H), 4.51 ($J_{2',3'}$=5.4 Hz, 2′-H), 4.29 ($J_{3',4'}$=3.5 Hz, 3′-H), 4.14 ($J_{4',5'}$=~3.5 Hz, 4′-H) and 3.81 (5′-H_2); *t*-butanol was used as an internal standard], respectively. This strongly suggested that cadeguomycin has a β-D-ribofuranosyl structure as its sugar moiety. The glycosylation site at N-7 of cadeguomycin was assumed to be the same as tubercidin, toyocamycin, sangivamycin and pre Q_0 nucleoside.

Thus the structure of cadeguomycin was elucidated as shown in Fig. 4.

Fig. 4. Structure of cadeguomycin (7-carboxy-7-deazaguanosine).



2-Amino-3,4-dihydro-4-oxo-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid.

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