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STRUCTURE OF MILDIOMYCIN, A NEW ANTIFUNGAL NUCLEOSIDE ANTIBIOTIC

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Abstract—The chemical structure of mildiomycin (1) active against powdery mildews was determined by chemical degradations and physical analyses to be 2 - [(2*R*, 5*S*, 6*S*) - 2 - (4 - amino - 1,2 - dihydro - 5 - hydroxymethyl - 2 - oxopyrimidin - 1 - yl) - 5,6 - dihydro - 5 - L - serylamino - 2H - pyran - 6 - yl] - 5 - (3H⁺ - guanidino) - 2,4 - dihydroxyvalerate as shown in Chart 1.†

A new antifungal antibiotic mildiomycin (**1**) was isolated from the culture filtrate of *Streptoverticillium rimofaciens* B-98891.[1,2] Mildiomycin shows strong activity against powdery mildews on various plants and remarkably low toxicity in mammals and fishes.[2]

This report deals with the structure of **1** in detail[3] as shown in Chart 1.

In the preceding paper[2] the authors reported that **1** was a water-soluble basic antibiotic containing 5-hydroxymethyl cytosine as a pyrimidine base; m.p. > 300°, $[\alpha]_D$ + 100°, pKa' 2.8(–COO^-), 4.2(3–$NH\overset{+}{=}$), 7.2(2″–NH_3^+) and >12 (guanidine). **1** is readily soluble in water and shows positive color reactions with Sakaguchi, Greig-Leaback and ninhydrin reagents. **1** gave mildiomycin formic acid salt (**2**), 2″-N-monobenzoate (**3**) and 2′, 3′-dihydromildiomycin (**4**). The physico-chemical data of **1**–**4** revealed that the molecular formula of **1** is $C_{19}H_{30}N_8O_9 \cdot H_2O$ (532.53).[2] The IR spectrum of **1** showed the absorptions at 1650, 1000–1150 cm^{-1} like peptide analogues. The UV absorption at $\lambda_{max}^{pH\ 7}$ 271 nm (ϵ = 8,720) or $\lambda_{max}^{0.1NHCl}$ 280 nm (ϵ = 13,100) indicated the bathochromic shifts of 3–4 nm compared with those of cytidine. In the ^1H NMR spectrum (Table 1), two signals were observed at 7.61 ppm (s, H_6) and 4.42 (s, H_7) instead of two doublet signals in cytosine.[2]

On acidic hydrolysis in 2N HCl[2] or Amberlite IR-120 resin, **1** gave 5-hydroxymethyl cytosine (**5**) and L-serine (**6**) which were identified with the authentic samples of **5** and L-serine N-benzoate (**7**) derived from **6** by N-benzoylation. The ^{13}C NMR signals of **1** (Table 2) corresponding to those of **5** and **6** have been assigned.

The presence of a guanidyl group in **1** was established from degradation studies. **1** was hydrolysed in 0.2N NaOH to give ammonia and a ureido compound (**8**) showing negative Sakaguchi and positive *p*-dimethylaminobenzaldehyde (DAB) reagents. Another basic hydrolysis of **1** in saturated barium hydroxide afforded compounds, **5**, **6**, **8**, ammonia and urea (**9**) (Chart 1). A singlet signal at 158.0 ppm in the ^{13}C NMR spectrum of **1** can be assigned to the guanidyl carbon (Table 2).

The aliphatic moiety was unveiled by proton spin-decoupling studies of **2** (Fig. 1). On irradiation of a methine proton at 3.92 ppm (m, $H_{8'}$), methylene signals at 1.95 ppm (dq, $H_{7'}$) and 3.35 (dq, $H_{9'}$) collapsed two sets of AB quartet signals; only the 8′-proton signal was decoupled when each of the methylene protons was irradiated. As two methylenes among four in the ^{13}C NMR spectrum of **1** could be attributed to compounds **5** and **6**, two

†The numbering in Chart 1 is different from those in nomenclature.

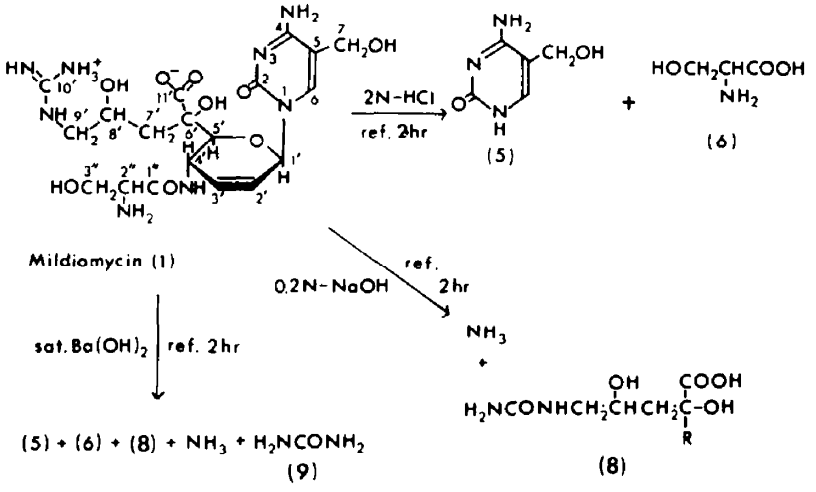


Chart 1.

Table 1. ^{1}H NMR spectra of mildiomycin and related compounds

Comp.	Solv.*1	Proton number											
		H_6	H_7	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{7'}$	$H_{8'}$	$H_{9'}$	$H_{2''}$	$H_{3''}$
1	W	7.61	4.42	6.43	6.05	5.88	4.88	4.24	1.95	3.93	3.36	3.58	3.82
2	W	7.62	4.46	6.43	6.14	5.85	4.92	4.23	1.95	3.93	3.35	4.20	4.05
3	W	7.96	4.63	6.58	6.28	6.05	5.08	4.47	2.08	4.10	3.34	4.76	4.17
	S	7.32	4.22	6.26	5.82	5.68	4.75	4.15	2.08	3.80	3.40	4.50	3.76
4	W	7.85	4.45	5.66	1.80	1.9-2.2	4.10	4.03	1.97	3.90	3.33	3.58	3.78
19	W			3.85	1.74	1.7-2.2	3.90	3.65	1.97	3.85	3.34	3.65	3.80
23	W			4.01	1.73	1.8-2.3	3.60	3.72	2.62	4.95	3.62		
25	W			4.17	1.6	1.9-2.2	3.20	3.57	2.10	3.90	3.35		
28	C			4.04	1.72	2.03	3.70	3.79	2.69	4.44	3.50		
29	W			4.10	1.70	1.9-2.2	3.20	3.97	2.54	3.60	3.25		
32	W			5.75	1.80	1.9-2.3	3.77	3.46	2.20	3.96	3.40		

*1 Solvent abbreviations: W: D_2O, S: DMSO-d_6, C: $CDCl_3$

Table 2. ^{13}C NMR spectra of mildiomycin and reference compounds in D_2O

Comp.	Carbon number																		
	C-2	C-4	C-5	C-6	C-7	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	C-7'	C-8'	C-9'	C-10'	C-11'	C-1''	C-2''	C-3''
Mildiomycin	157.9 (s)	165.9 (s)	107.9 (s)	142.2 (d)	58.4 (t)	81.0 (d)	126.8 (d)	133.8 (d)	44.1 (d)	80.8 (d)	79.5 (s)	39.2 (t)	67.9 (d)	48.1 (t)	158.1 (s)	178.7 (s)	175.1 (s)	57.1 (d)	64.5 (t)
5-Hydroxymethyl cytosine*	149.6 (s)	160.0 (s)	105.7 (s)	145.9 (d)	57.3 (t)														
L-Serine																	173.1 (s)	57.2 (d)	61.0 (t)
Sugar moiety of blasticidin S						80.6 (d)	126.9 (d)	133.7 (d)	47.5 (d)	78.5 (d)						175.6 (s)			
γ-Guanidino-β-hydroxy butyric acid											175.6 (s)	39.6 (t)	67.4 (d)	47.2 (t)	158.2 (s)				
γ-Amino-β-hydroxy butyric acid											179.1 (s)	43.2 (t)	66.4 (d)	45.0 (t)					

* In CF_3COOD

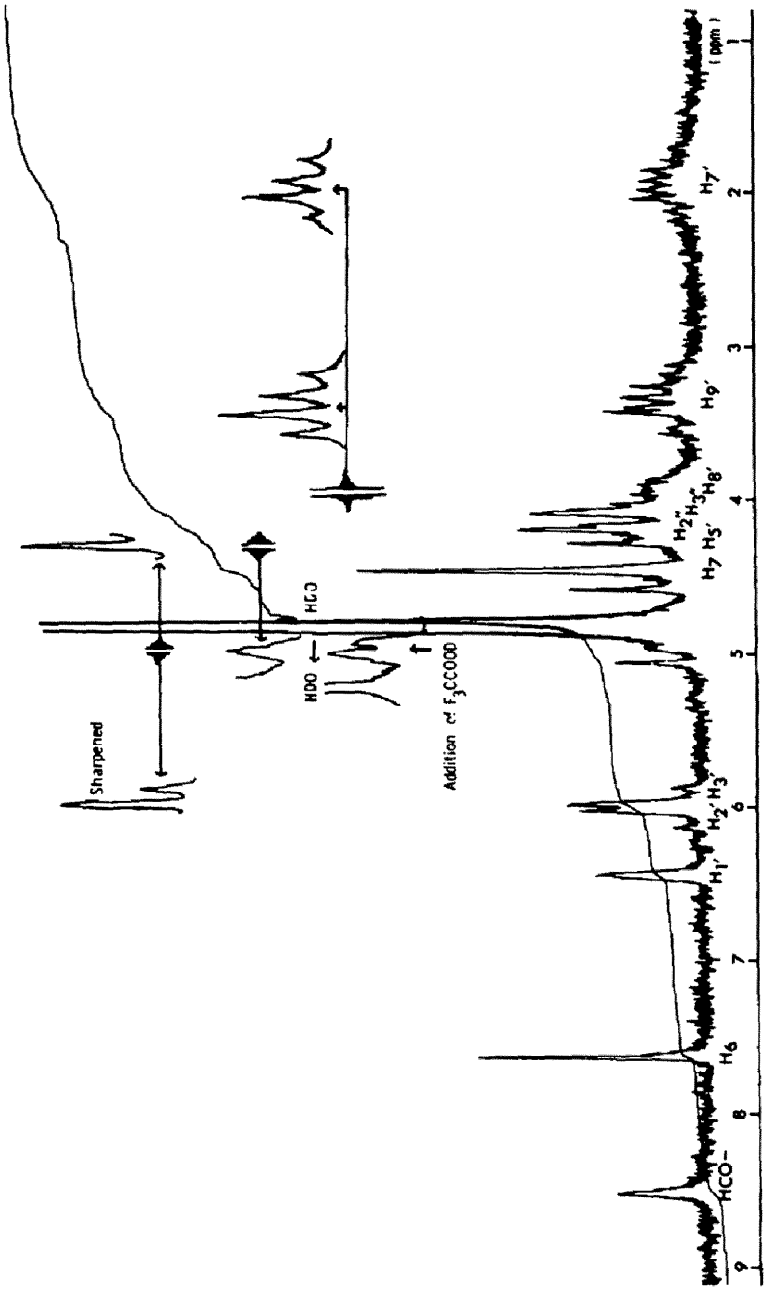


Fig. 1. ^{1}H NMR spectrum of 2.

residual methylene signals at 48.1 ppm and 39.2 should account for the N- ($C_{9'}$) and C- ($C_{7'}$) methylenes. These spectral data suggest the presence of $-N-CH_2-CH(O-)-CH_2-C-$formula in **2**. Seven nitrogen functions of the eight nitrogens in **1** were assigned to the parts of guanidine, serine and 5-hydroxymethyl cytosine, and therefore, nitrogen binding to $C_{9'}$-methylene was assumed to be the last nitrogen or the guanidyl group. The ^{13}C NMR spectra of D, L-γ-amino-β-hydroxybutyric acid (**10**) and its γ-guanidyl compound synthesized (**11**)[4] indicate the chemical shifts (Table 2). These spectral data evidenced that **1** has the following aliphatic moiety since corresponding four carbon signals except for the carboxyl carbon in **1** was closer to those of **11** than **10** and $C_{7'}$-methylene at 1.95 ppm in **2** was not adjacent to CO groups from corresponding signals at 2.50 ppm in **10**, 2.68 in **11** and 2.80 in blasticidin S[5] (**12**).

Furthermore, this aliphatic moiety was finally elucidated from a decomposition product. **1** was subjected to 6% periodate oxidation to give optically active γ-guanidino-β-hydroxybutyric acid which was identified with the racemate synthesized (**11**) except for specific rotation (Chart 2). The partial structure A was thus confirmed.

The presence of a 6-membered ring in **1** was suggested by the ^1H and ^{13}C NMR spectra of **1**, **2** and **4**. In the ^1H NMR spectrum of **1** (Table 1) an anomeric and two olefinic protons were observed at 6.43 ppm (s like, $H_{1'}$), 6.05 (d like, J = 10 Hz, $H_{2'}$) and 5.88 (d like, J = 10 Hz, $H_{3'}$) which were similar to those of the sugar moiety in **12**. In the ^1H NMR spectrum of **4** the anomeric proton shifted to 5.66 ppm (q, J = 10 and 2 Hz, $H_{1'}$) and newly occurring methylene signals were observed at 1.8 and 1.9–2.3 ppm (2H × 2, m, $H_{2'}$ and $H_{3'}$). The presence of two olefinic carbons at $C_{2'}$ and $C_{3'}$ was also supported by comparison of ^{13}C NMR spectra of **1** and **4** (Tables 2 and 3). These chemical shifts and splitting patterns revealed that there is a *cis* double bond and an anomeric proton at its adjacent position in **1**. When the proton signal at 5.88 ppm (d like, $H_{3'}$) was irradiated in the ^1H NMR spectrum of **2** (Fig. 1), the methine signal at 4.88 ppm (d like, $H_{4'}$) was sharpened. On irradiation of the 4'-proton signal, the doublet signal at 4.24 ppm (J = 10 Hz, $H_{5'}$) collapsed singlet. These data elucidated the partial structure B. The presence of a 6-membered ring is further supported by the ^1H NMR spectrum of cytosinine[6] (**13**) and the ^{13}C NMR data of the sugar moiety in **12**, which was determined by subtracting the signals of blastidic acid[7] (**14**) and cytosine from those of **12** (Chart 3 and, Tables 2 and 4).

On standing in acetic acid, N-benzoate of **8** (**15**)

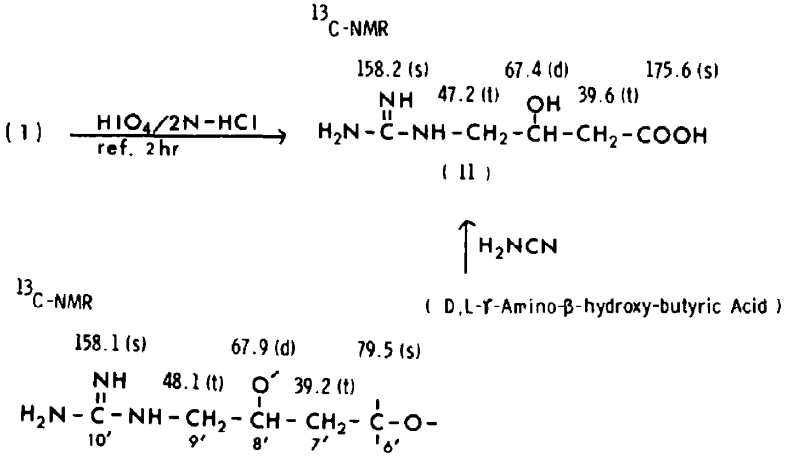


Chart 2.

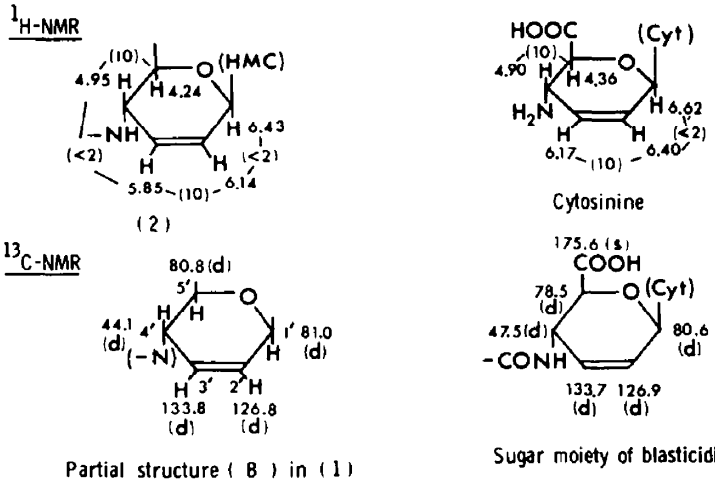


Chart 3.

Table 3. ^{13}C NMR spectra of mildiomycin and related compounds

Comp.	Carbon number																		
	C-2	C-4	C-5	C-6	C-7	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	C-7'	C-8'	C-9'	C-10'	C-11'	C-1"	C-2"	C-3"
2	157.3 (s)	165.3 (s)	107.9 (s)	142.3 (d)	58.2 (t)	81.0 (d)	127.0 (d)	133.5 (d)	44.3 (d)	80.7 (d)	79.3 (s)	39.1 (t)	67.6 (d)	48.1 (t)	157.9 (s)	179.0 (s)	168.1 (s)	55.8 (d)	60.9 (t)
3	148.8 (s)	159.2 (s)	107.2 (s)	144.6 (d)	57.3 (t)	80.6 (d)	125.0 (d)	133.4 (d)	43.7 (d)	80.6 (d)	78.4 (s)	39.0 (t)	66.0 (d)	48.2 (t)	157.9 (s)	177.0 (s)	172.7 (s)	57.7 (d)	61.9 (t)
4	157.4 (s)	165.6 (s)	107.3 (s)	141.7 (d)	58.5 (t)	83.9 (d)	30.1 (t)	30.3 (t)	45.9 (d)	83.4 (?)	80.0 (s)	39.7 (t)	68.2 (d)	48.0 (t)	158.0 (s)	178.9 (s)	174.9 (s)	57.2 (d)	64.4 (t)
31	155.2 (s)	164.1 (s)	108.3 (s)	142.1 (d)	58.0 (t)	80.5 (d)	129.2 (d)	129.2 (d)	44.6 (d)	77.3 (d)	N.D.*	36.6 (t)	76.5 (d)	44.6 (t)	158.1 (s)	177.0 (s)			
19						68.5 (t)	25.2 (t)	31.6 (t)	47.0 (d)	83.4 (d)	80.0 (s)	39.9 (t)	68.2 (d)	48.0 (t)	158.0 (s)	179.4 (s)	173.6 (s)	57.0 (d)	63.9 (t)
23						69.2 (t)	24.8 (t)	28.9 (t)	47.9 (d)	79.3 (d)	78.3 (s)	37.3 (t)	77.5 (d)	45.1 (t)	157.9 (s)	177.8 (s)			
25						67.9 (t)	25.6 (t)	33.3 (t)	49.4 (d)	86.1 (d)	80.4 (s)	40.7 (t)	68.9 (d)	48.3 (t)	158.1 (s)	179.8 (s)			
29						68.6 (t)	23.8 (t)	27.5 (t)	48.1 (d)	80.9 (d)	210.9 (s)	43.8 (t)	66.9 (d)	45.8 (t)	162.2 (s)				

* Not determined

Table 4. ^{13}C NMR spectra of blasticidin S and related compounds

Comp.	Carbon number																
	C-2	C-4	C-5	C-6	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'	C-1"	C-2"	C-3"	C-4"	C-5"	C-6"	C-7"
12	158.0 (s)	166.8 (s)	97.4 (d)	143.6 (d)	80.6 (d)	126.9 (d)	133.7 (d)	47.5 (d)	78.5 (d)	175.6 (s)	171.5 (s)	37.9 (s)	46.4 (t)	30.1 (t)	47.5 (d)	157.2 (s)	36.6 (q)
14											174.3 (s)	36.6 (t)	46.8 (d)	30.1 (t)	47.4 (t)	157.2 (s)	36.8 (q)
30					67.4 (t)	24.8 (t)	29.5 (t)	48.8 (d)	81.5 (d)	176.6 (s)	171.0 (s)	37.7 (t)	47.5 (d)	30.1 (t)	47.4 (t)	157.3 (s)	36.7 (q)

afforded a compound (**16**) whose IR spectrum indicated the strong absorption at 1770 cm^{-1} (Chart 4). The ^1H NMR spectrum did not show any acetyl Me signal and 8'–proton signal shifted to 4.60 ppm from 3.9 in **15**. When **16** was allowed to stand in ammonia, **15** was recovered completely. Acetylation of **15** gave 7-O-, 3''-O-diacetate (**17**) showing positive DAB. **8** was acylated to give (*p*-bromo) derivative of **15** (**18**). The IR spectra of **17** and **18** indicated the strong absorptions at 1770–1775 cm^{-1}. Compared with the ^1H NMR spectra of **17** and **18** in DMSO-d_6 two acetyl Me signals were observed at 2.00 ppm (3H × 2, s) and two methylene signals showed down-field shifts to 4.75 ppm from 4.20 (H_7) and to 4.40 ppm from 3.72 ($H_{3'}$). After treatment of **17** with ammonia–methanol the IR spectrum of the compound showed the absorption at only 1740 cm^{-1}.

These findings elucidated the presence of two free OH and one carboxylic acid in **8** because the 7- and 3''-OH groups were acetylated in **17** and the 8'-OH group provided a 5-membered lactone in **17** and **18**. And, it was found that serine and pyrimidine were binding at 1''-and N-1-positions, respectively, from the results of various acylations and UV spectra of the derivatives.

The combination of these fragments for the partial structure B was elucidated from the decomposition studies as described below.

On hydrogenation of **1** a compound (**19**) was obtained besides **4** and **5** (Chart 5). **19** showed the pKa' values at 7.2 (2''-NH_3^+) and 3.45 (–COO^-), and no absorption in the UV spectrum. In the ^1H NMR spectrum of **19** the signals of pyrimidine base and anomeric proton disappeared and a new methylene signal occurred at 3.85 ppm (2H, m). The corresponding methylene signal in the hydrogenolysis product of **12** (**20**)[5] was observed at 3.95 ppm (2H, m). The ^{13}C NMR data in the 6-membered ring was in good accord with those of **20** (Tables 3 and 4). These data indicated that **19** was a hydrogenolysis product of **1**. On basic hydrolysis **19** gave a ureido compound which was benzoylated to give N-benzoate (**21**). **21** showed the absorption at 1770 cm^{-1} in the IR spectrum, but no pKa' value at 4–11. On acetylation, **21** afforded 3''-O-, 10'-N-diacetate (**22**) showing negative DAB. The IR spectrum of **22** indicated the absorption at 1780 cm^{-1} (5-membered lactone), 1740 (OAc) and 1700 (NAc). The ^1H NMR spectrum showed the signals at 9.40 (s, C_{10}–NH–), 8.63 (t, C_9–NH), 7.85 (d, C_3–NH–) and 7.50 (d, C_4–NH) in acetone-d_6, and 1.97 (3H, s, –NAc) and 2.03 (3H, S, –OAc) in DMSO-d_6.

When **19** was vigorously hydrolysed by 3N HCl, a lactone dihydrochloride (**23**) was obtained together with L-serine (**6**) (Chart 5). **23** gave a monohydrochloride (**24**) or a free base (**25**). The pKa' value of **25** was measured at 2.8 (–COO^-), 8.6 (new NH_3^+) and >12 (guanidine). The ^{13}C NMR data of **25** showed clearly the lack of the serine part (Table 2). **19** showed no absorption at 1680–1800 cm^{-1} in the IR spectrum and the chemical shift at 173.6 ppm in the ^{13}C NMR spectrum so that the acid amide in **19** was cleaved by acid to give a primary amine. In the ^{13}C NMR spectrum of **25** the signals at $C_{3'}$ and $C_{5'}$ showed down-field shifts[7] by 4.4 and 6.8 ppm, respec-

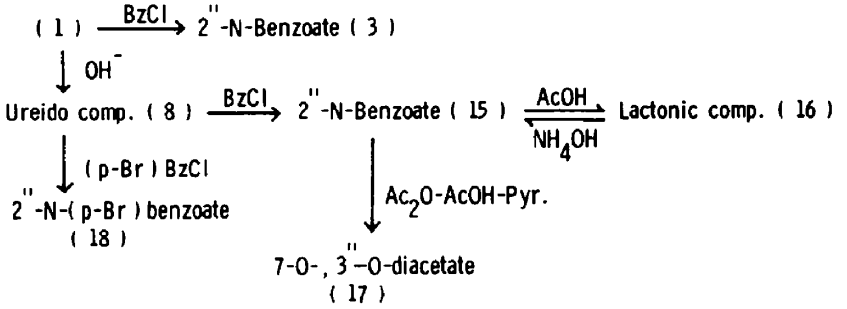


Chart 4.

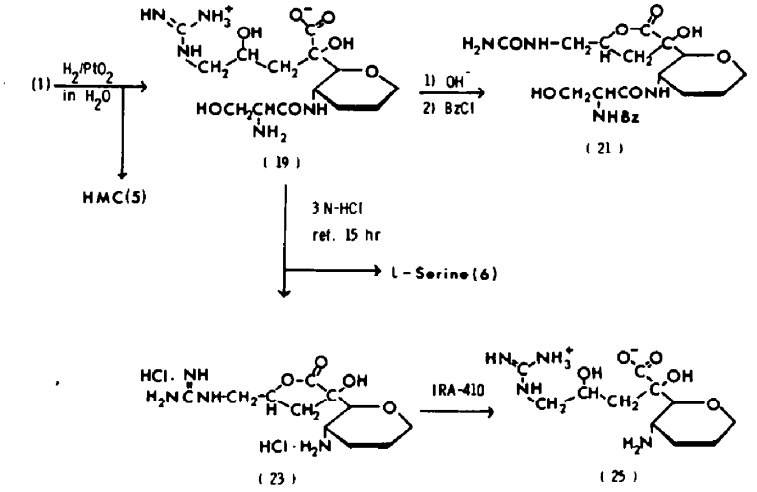


Chart 5.

tively, when compared with those of **23** (Table 3). Thus, the primary amine should be located at 4'.

The IR spectrum of **23** showed a strong absorption at 1770 cm^{-1} but that of **25** showed no absorption. The ^{13}C NMR signal of $C_{8'}$ showed a down-field shift to 77.5 ppm in **23** from 68.9 ppm in **25**. In the ^1H NMR spectrum of **25** the signals at 3.75 ppm (m, $H_{8'}$) and 1.95 (m, $H_{7'}$) showed down-field shifts at 4.95 ppm and 2.62 (d like) in **23** whose signals were determined by proton spin-decoupling studies (Fig. 2). These findings revealed that (1) a 5-membered lactone occurred at $C_{8'}$, (2) the binding location of carboxylic acid was at $C_{6'}$ of the quarternary carbon and (3) the ^{13}C NMR signal at 178.7 ppm in **1** should be assigned to carboxylic acid.

Finally, α-hydroxy carboxylic acid structure was determined (Chart 6). The chemical shifts of non-protonated carbon at $C_{6'}$ are about 80 ppm in mildiomycin related compounds (Table 3), and therefore, the binding moiety are assumed not to be –CH–O– or –C–O, but to be –C–O– or –C–O–. The following experiments were carried out to distinguish between these binding moieties.

On basic hydrolysis **25** afforded a ureido compound (**26**) and ammonia. The crystals showing positive DAB possessed pka' values at 7.75 (4'-NH_2) and 2.9 (-COO^-). **26** showed characteristic absorptions at 2700 cm^{-1} (-COOH) in the IR spectrum and the peaks at *m/e* 256 (M^+-18(NH_3)) and 211 (256-45 (-COOH)) in the mass spectrum. On acetylation **26** gave 4'-N-, 8'-O-, 10'-N-triacetate (**27**) which showed only one pKa' value at 2.8 (COO^-) and broad absorptions at 1700–1740 cm^{-1} (Ac) in the IR spectrum. On methylation, **27** gave a monomethyl ester (**28**). In the IR spectrum of **28** the absorption of ester was clearly observed at 1740 cm^{-1}. The ^1H NMR spectrum in deuterated chloroform showed the signals at 3.80 ppm (3H, s, $-COOCH_3$) and 2.15 (3H×3, S, 2Ac) (Table 1). These products and spectral data elucidated the presence of the carboxylic acid.

On oxidation by lead tetraacetate, **26** gave carbon dioxide and a ketonic compound (**29**). In the IR spectrum the absorption of an isolated CO group was newly observed at 1720 cm^{-1}. The ^1H NMR data showed down-field shifts to 3.97 ppm in **29** from 3.8 in **26** at $H_{5'}$ and to 2.54 ppm in 29 from 2.02 in 26 at $H_{7'}$. In the ^{13}C NMR spectrum of **29** the signal of an isolated CO group newly appeared at 210.9 ppm ($C_{6'}$) instead of the signals at 80.4 ppm ($C_{6'}$) and 179.8 ($C_{11'}$) in **25**. 4'-N-acetate of **29** was reduced to give a hydroxy product at $C_{6'}$ (**30**). The proton spin-decoupling studies of **30** revealed the following; when the 7'-methylene signal was irradiated, the methine signals at 4.25 ppm (m, $H_{6'}$) and 4.03 (m, $H_{8'}$) collapsed a doublet (J = 7 Hz) and a doublet-like. On irradiation of the methine proton at 3.62 ppm (q, $H_{5'}$), the $H_{6'}$-methine proton collapsed a doublet-like. From these reaction patterns and spectral data the binding moiety of α-hydroxy-carboxylic acid was finally confirmed.

The deseryl derivative of **1** was obtained as follows; on oxidation of **1** with potassium periodate the product showed no pKa' at 7.2 and negative ninhydrin reaction. After treatment with alkaline hydrolysis, the compound was hydrolysed by 2N H_2SO_4 to give the compound (**31**) as dihydrochloride (Chart 7).

In the ^{13}C NMR spectrum the signals of the serine part disappeared. The presence of the lactone ring was assigned by the strong absorption at 1770 cm^{-1} in the IR spectrum and the down-field shift at 2.61 ppm (2H, sex. $H_{7'}$) in the ^1H NMR spectrum. The sugar moiety was also obtained by acidic hydrolysis. **4** gave two decomposition products, **32** and **33** by refluxing in 3N HCl. The ^1H NMR of the minor hydrolysate (**32**) showed the anomeric proton signal at 5.75 ppm (s like) instead of the 1'-methylene signal in **25**. The major one (**33**) was identified with the dihydro derivative of **31** (Chart 7).

As for the absolute configuration of the 6-membered ring, the stereochemistry of $H_{4'}$ and $H_{5'}$ should be diaxial by the coupling constant of $J_{4',5'}$ = 10 Hz in **2**. Also the stereochemistry of H_1, was assigned axial from $J_{1',2'}$ = 10 and 2 Hz in **4**. Thus, three bulky groups in the ring should reasonably be all equatorial. Only two sterically stable stereostructures of β-D or α-L could be permitted among all possible isomers of dihydropyran as shown in Chart 8. Since these formula are in relation of the mirror image, Cotton effect of the CD spectrum in B_{2u} band[8] must show the opposite sign to each other. The absolute configuration of blasticidin S and gougerotin has been determined as β-D.[9,10] Mildiomycin compounds showed negative Cotton effect quite similar to those of the model compounds (Chart 8), indicating that the 6-membered

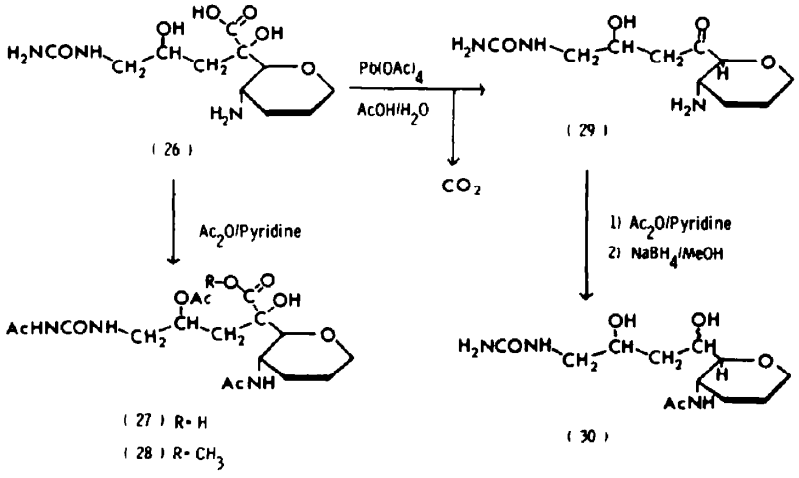


Chart 6.

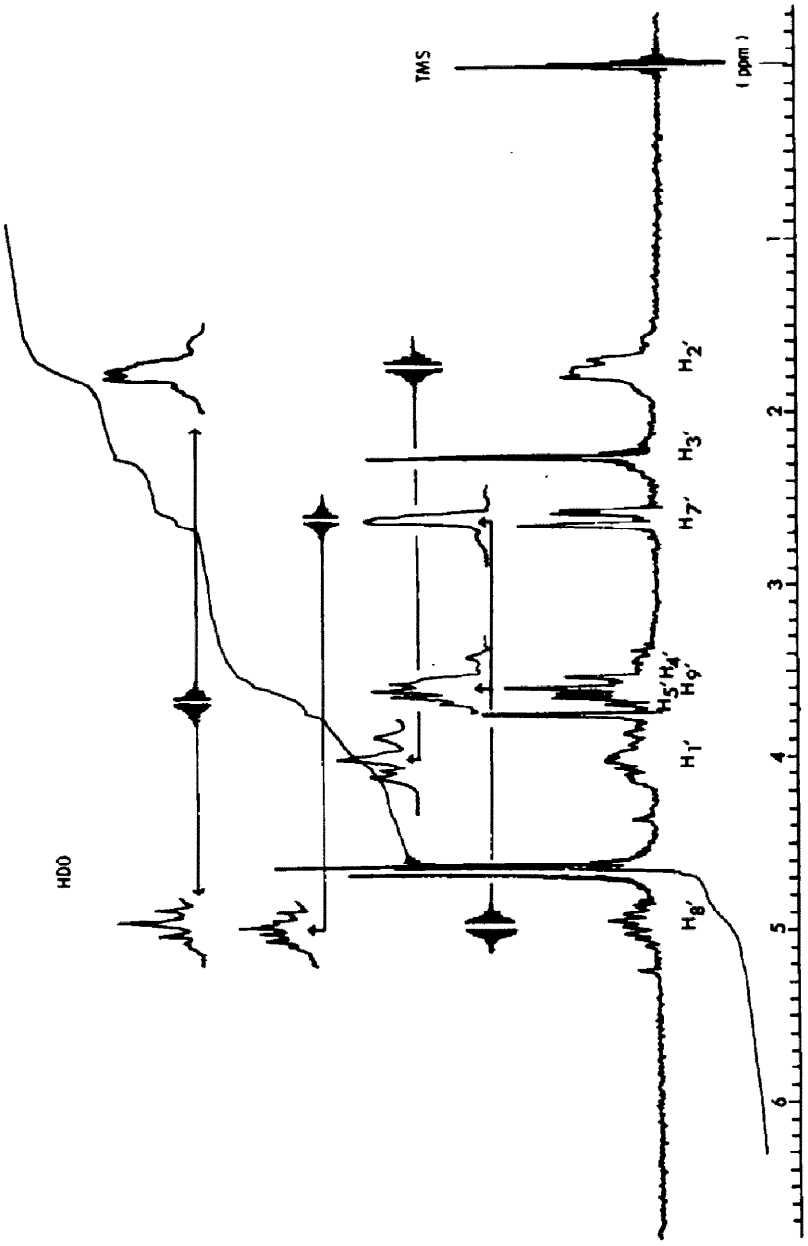


Fig. 2. ^{1}H NMR spectrum of 23.

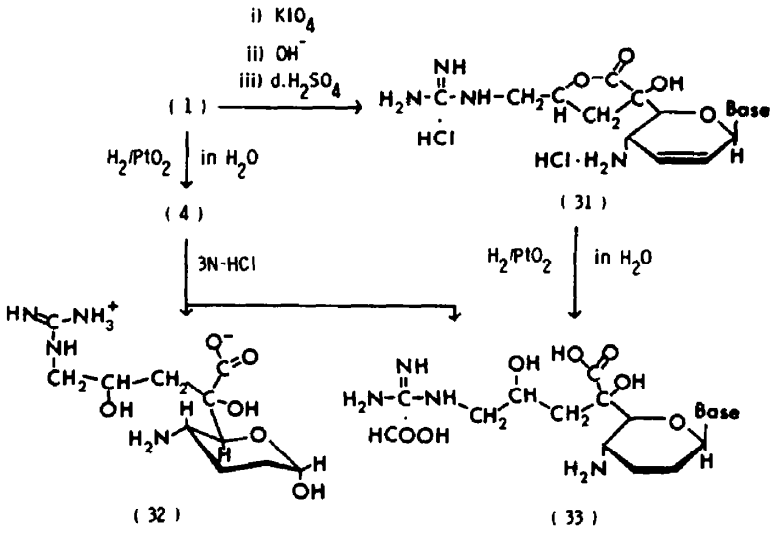
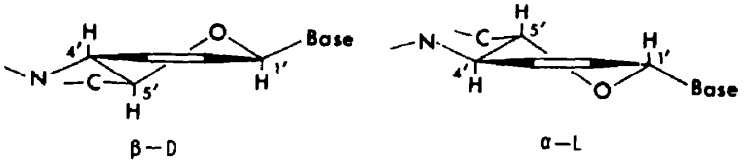


Chart 7.



CD spectrum	Compound		Compound	
	Blasticidin S	$[\theta]_{270}$ -12,900	(1)	$[\theta]_{273}$ -8,700
	Cytosinine	$[\theta]_{271}$ -9,500	(31)	$[\theta]_{273}$ -9,300
	Gougerotin	$[\theta]_{280}$ -2,700	(4)	$[\theta]_{285}$ -1,800

Chart 8.

ring should be β-D. The absolute configuration of 1 was thus assigned 1'R, 4'S, 5'S and 2"S.[11]

One of the interesting structural features of 1 is that the carboxy-guanidine-butyl group is bound to the unsaturated pyranoside with a C–C bond. In view of the biosynthesis of this antibiotic it provides another interesting problem whether the quarternary carbon ($C_{6'}$) originates from an amino acid or sugar as a precursor.

Structure-activity relationship of the above-mentioned antibiotics is also very interesting. 2',3'-Dihydro mildiomycin (4) stable to acid showed strong activity against *Rhodotolura rubra* and *Erysiphe graminis* on barley.[1] However, ureido (8), 2"-N-benzoyl (3), deseryl (31) and depyrimidine (32) compounds showed little activity.[12]

EXPERIMENTAL

The m.ps were measured by FT-5 (Mettler) at 3°/min. The specific rotations were also measured at concentration of 0.5–1.0 in water unless otherwise stated. The IR spectra were measured in KBr pellet. The δ values in the ^1H and ^{13}C NMR spectra using XL-100 (Varian) were recorded in ppm down-field from TMS. All spectra herein were measured in D_2O unless otherwise stated. In the ^{13}C NMR spectra dioxane was contained as an internal standard (67.4 ppm). Most of the samples herein contain water of crystallization or adhesion measured by thermo-gravimetric analysis although they were dried at 60° for 10 hr *in vacuo*. Blasticidin S and its related compounds described herein were prepared and their spectral data were measured under the same conditions for comparison studies.

Acidic hydrolysis of 1 (5, 6 *and* 7)

A suspension of 1 (10 g) in Amberlite IR-120 (H^+ form, 250 ml) was refluxed for 1 hr. The mixture was mounted on IR-120 (250 ml) and eluted with 0.5N and 2N HCl. The 2N HCl eluate was passed through Amberlite IR-45 and concentrated to afford 5 as colorless crystals.[2] The crude crystals were recrystallized from EtOH–water to give pure crystals (2.4 g). After treatment with IR-45 (750 ml) the 0.5N HCl eluate was applied to Amberlite IRA-410 (OH^- form, 100 ml) and eluted with 0.5N HCl. The eluate was evaporated to give crude 6 (1. g). To a soln of 6 in 5% $NaHCO_3$ (50 ml) was added benzoyl chloride (1.5 ml) and the whole was stirred at 25° for 3 hr. The mixture was chromatographed on activated charcoal and eluted with acetone:water (2:8) and (1:1). The eluate was concentrated to obtain colorless crystals of 2 (348 mg). By similar method the authentic L-serine N-benzoate (640 mg) was synthesized from commercial L-serine (1 g). 7: m.p. 159° (dec), m.m.p. 160° (dec), $[\alpha]_D^5$ +23.5°, UV: λ_{max}^{MeOH} 226 nm (ε = 10,600). (Found: C, 56.92; H, 5.10; N, 6.64.

Calc. for $C_{10}H_{11}NO_4$ (209.20): C, 57.41; H, 5.30; N, 6.70; O, 30.59%). The crystals were identical with the authentic sample in tlc and IR, MS and ^1H NMR spectra.

Basic hydrolysis of **1**

(1) A soln of **1** (20 g) in 0.2N NaOH (200 ml) was refluxed for 2 hr under N_2. The generating gas was trapped with N HCl (50 ml) which was concentrated to give colorless crystals of NH_4Cl (280 mg). The mixture was applied to Amberlite CG-50 (H^+ form, 500 ml) and eluted with 0.5% NH_4OH. The eluate was chromatographed on activated charcoal (500 ml) and eluted with acetone:water (2:8). The eluate was concentrated to give a colorless powder of **8** (11.2 g):$[\alpha]_D^{22}$ +83.0°, UV: $\lambda_{max}^{pH\ 7}$ 270 nm (ϵ = 7940) and $\lambda_{max}^{0.1N\ HCl}$ 279 (11,600). (Found: C, 43.11; H, 6.14; N, 18.03; O, 31.57; H_2O, 2.94. Calc. for $C_{19}H_{29}N_7O_{10}\cdot H_2O$ (533.52), C, 42.77; H, 5.86; N, 18.38; O, 32.99; H_2O, 3.38%).

(2) A soln of **1** (10 g) in saturated $Ba(OH)_2$ (500 ml) was refluxed for 2 hr under N_2. After excluding Ba salt the filtrate was applied to Dowex 50W × 2 (250 ml) and eluted with 0.5N HCl, 2N HCl and 2N NaOH. The effluent gave colorless crystals of urea (9, 133 mg). The 0.5N HCl eluate was applied to IRA-410 (100 ml) and eluted with 0.5N HCl. The residue of the eluate was benzoylated to give **7** (160 mg). **5** in the 2N HCl eluate was detected by tlc and HLC. The 2N NaOH eluate was chromatographed on activated charcoal (250 ml) to afford **8** (5 g). **9**: m.p. 134°, m.m.p. 134°. (Found: C, 20.32; H, 6.71; N, 46.77. Calc. for CH_4N_2O (60.06), C, 20.00; H, 6.71; N, 46.65; O, 26.64%).

Periodate oxidation of **1** (**11**)

A soln of **1** (5.3 g) in 6% HIO_4 (300 ml) and 2N HCl (250 ml) was refluxed for 2 hr. After treatment with IR-45 (250 ml) the mixture was applied to CG-50 (500 ml) and fractionated with 0.2 and 0.5% NH_4OH. The eluates were concentrated to afford optically active colorless crystals of **11** (252 mg): m.p. 250° (dec), m.m.p. 250.5° (dec), $[\alpha]_D^{27}$ −19.5° (in AcOH:0.05N HCl:MeOH (3:7:10)): (Found: C, 37.24; H, 7.02; N, 25.79. Calc. for $C_5H_{11}N_3O_3$ (161.11): C, 37.28; H, 6.85; N, 26.08; O, 29.79%). The crystals were also identified with the racemate of **11** synthesized in tlc, PPC, IR and ^1H NMR spectra.

Benzoylation of **8**

(1) A soln of **8** (5 g) in 5% $NaHCO_3$ (250 ml) was benzoylated by benzoyl chloride (4 ml) at 25° for 3 hr. After chromatography of activated charcoal (40 ml) **15** was obtained as a white powder (4.4 g): $[\alpha]_D^{22}$ + 60.8° (in 0.1N HCl), UV: $\lambda_{max}^{pH\ 7}$ 268 nm (ϵ = 9,100) and $\lambda_{max}^{0.1N\ HCl}$ 278 (13,000). (Found: C, 47.41; H, 5.69; N, 14.82. Calc. for $C_{26}H_{33}N_7O_{11}\cdot 2H_2O$ (655.65): C, 47.63; H, 5.69; N, 14.96; O, 31.72%).

(2) A soln of **8** (1 g) in 5% $NaHCO_3$ (50 ml) was benzoylated by (*p*-bromo) benzoyl chloride (1.5 g) at 25° for 5 hr. After treatment with the same method as (1) **18** was obtained as a white powder (600 mg): $[\alpha]_D^{25}$ + 85.4° (in MeOH), UV: $\lambda_{max}^{pH\ 7}$ 270 nm (sh, ϵ = 10,900). (Found: C, 45.06; H, 4.77; N, 13.64; Br, 12.33; H_2O, 1.85. Calc. for $C_{26}H_{30}N_7O_{10}\cdot Br\cdot H_2O$ (698.53): C, 44.70; H, 4.62; N, 14.04; O, 25.20; Br, 11.44; H_2O, 2.58%).

Lactonization of **15** (**16**)

A soln of **15** (300 mg) in AcOH (30 ml) was allowed to stand at 25° overnight. The residue of the soln was applied to prep tlc on silica gel HF_{254} and developed with BuOH:AcOH:H_2O (2:2:1). Main band was extracted with MeOH:water (1:1). The concentrate of the extract was chromatographed on activated charcoal (20 ml) and eluted with acetone:water (1:1). The eluate was concentrated to give a white powder of **16** (105 mg): $[\alpha]_D^{25}$ + 91.8°, UV: $\lambda_{max}^{pH\ 7}$ 270 nm (ϵ = 9,350) and $\lambda_{max}^{0.1N\ HCl}$ 279 (13,200). (Found: C, 50.58; H, 5.36; N, 15.74; H_2O, 2.17. Calc. for $C_{26}H_{31}N_7O_{10}\cdot H_2O$ (619.61): C, 50.40; H, 5.37; N, 15.83; O, 28.40; H_2O, 2.91).

A soln of **16** (20 mg) in 2% NH_4OH (5 ml) was allowed to stand at 25° for 4 hr. The soln was evaporated to dryness. The dried powder was identical with **15** by tlc and IR spectrum.

Acetylation (**17**, **22**, **27**)

(1) A suspension of **15** (800 mg) in Ac_2O, AcOH and pyridine (40 ml each) was stirred at 25° for 3 hr until becoming homogeneous and allowed to stand overnight. After removing the solvents the residue was subjected to prep tlc of silica gel HF_{254} and developed with BuOH:AcOH:H_2O (4:1:5 upper layer). The main band was extracted with BuOH. The extract was evaporated to give a white powder of **17** (195 mg): $[\alpha]_D^{25}$ +99.8° (in MeOH), UV: λ_{max}^{MeOH} 267 nm (sh, ϵ = 7,000). (Found: C, 51.36; H, 5.29; N, 12.73; H_2O, 3.08. Calc for $C_{30}H_{35}N_7O_{12}\cdot H_2O$ (703.69): C, 51.20; H, 5.30; N, 13.94; O, 29.56; H_2O, 2.56%).

(2) A soln of **21** (150 mg) in Ac_2O (5 ml) and pyridine (10 ml) was allowed to stand at 25° overnight. After BuOH extraction **22** was obtained as a white powder (130 mg): $[\alpha]_D^{23}$ +29.8° (in MeOH), UV: λ_{max}^{MeOH} 225 nm (ϵ = 12,300). (Found: C, 54.30; H, 5.86; N, 9.60. Calc. for $C_{25}H_{32}N_4O_{10}$ (548.57): C, 54.74; H, 5.88; N, 10.21; O, 29.17%).

(3) A suspension of **26** (440 mg) in Ac_2O (15 ml) and pyridine (30 ml) was stirred at 25° for 2 days until becoming homogeneous. After BuOH extraction the extract was chromatographed on activated charcoal (50 ml) to give a white powder of **27** (143 mg): $[\alpha]_D^{25}$ +14.6° (in MeOH). (Found: C, 49.52; H, 6.58; N, 9.63. Calc. for $C_{17}H_{27}N_3O_9$ (417.43), C, 48.91; H, 6.52; N, 10.07; O, 34.50%).

Hydrogenation of **1** (**4**, **5** *and* **19**)

A soln of **1** (12 g) in water (500 ml) was hydrogenated over PtO_2. Hydrogen uptake ceased to absorb 1.8 mole. The mixture was applied to CG-50 (400 ml) and eluted with 0.5%- and 1.0%-NH_4OH. The 0.5% NH_4OH eluate was concentrated and crystallized from water to afford **5** (1.6 g). The 1.0% NH_4OH eluate was chromatographed on activated charcoal (200 ml) and eluted with acetone:water (5:95), (1:9), (2:8) and (4:6). The eluates of (5:95) and (1:9) were concentrated to yield a white powder of **19** (3.9 g). The eluates of (2:8) and (4:6) were concentrated and crystallized from EtOH:water (1:1) to give colorless hexagonal plates of **4** (2.2 g): **4**: m.p. >300°, $[\alpha]_D^{22}$ ±0°, UV: $\lambda_{max}^{pH\ 7}$ 274.5 nm (ϵ = 9,020) and $\lambda_{max}^{0.1N\ HCl}$ 283 (13,000). (Found: C, 42.72; H, 6.31; N, 20.78; O, 29.57; H_2O, 3.50. Calc. for $C_{19}H_{32}N_8O_9\cdot H_2O$ (534.54): C, 42.70; H, 6.41; N, 20.96; O, 29.93; H_2O, 3.37%).

19: $[\alpha]_D^{23}$ −21.8°, UV: End absorption. (Found: C, 43.88; H, 7.44; N, 17.55; H_2O, 1.14. Calc. for $C_{14}H_{27}N_5O_7\cdot 1/4H_2O$ (381.88): C, 44.03; H, 7.26; N, 18.33; O, 30.38; H_2O, 1.18%).

Benzoylation of the basic hydrolysate of **19** (**21**)

A soln of **19** (300 mg) in 0.2N NaOH (3 ml) was refluxed for 2 hr. After neutralization the mixture was concentrated and the residue in 5% $NaHCO_3$ (30 ml) was benzoylated by benzoyl chloride (0.3 ml). The mixture was treated with the same method described above to obtain a white powder of **21** (170 mg). $[\alpha]_D^{23}$ +22.8° (c = 0.39), UV: $\lambda_{max}^{H_2O}$ 228 nm (ϵ = 11,600). (Found: C, 50.96; H, 6.18; N, 11.56; H_2O, 5.16. Calc. for $C_{21}H_{28}N_4O_8\cdot 3/2H_2O$ (491.51), C, 51.32; H, 6.36; N, 11.40; O, 30.92; H_2O, 5.49%).

Acidic hydrolysis of **19** (**7** *and* **23**)

A soln of **19** (3.9 g) in 3N HCl (120 ml) was refluxed for 15 hr. After neutralization with IR-45 the filtrate was applied to IRA-410 (150 ml) and eluted with 0.5N HCl. The effluent was chromatographed on activated charcoal (150 ml) and eluted with acetone: 0.05N HCl (1:1). The eluate was concentrated to give a white powder of **23** (2.12 g). The eluate of 0.5N HCl was neutralized and the concentrate was passed through activated charcoal (50 ml). The concentrated residue of the effluent was benzoylated by the same method to give colorless crystals of **7** (210 mg). **23**: $[\alpha]_D^{23}$ ±0°, UV: End absorption. (Found: C, 36.04; H, 6.45; N, 14.65; Cl, 18.92; H_2O, 4.90. Calc. for $C_{11}H_{20}N_4O_4\cdot 2HCl\cdot H_2O$ (363.25), C, 36.38; H, 6.66; N, 15.42; O, 22.02; Cl, 19.52; H_2O, 4.96%).

Monohydrochloride and free base of **23** (**24** *and* **25**)

(1) A soln of **23** (500 mg) in water (30 ml) was passed through IR-45 (50 ml). The effluent was evaporated to afford a white powder of **24** (427 mg): $[\alpha]_D^{23}$ ±0°. (Found: C, 40.38; H, 7.18; N, 16.64; Cl, 11.44. Calc. for $C_{11}H_{22}N_4O_5\cdot HCl$ (326.79): C, 40.43; H, 7.09; N, 17.15; O, 24.48; Cl, 10.85%).

(2) A soln of **23** (500 mg) in water (30 ml) was passed through IRA-410 (50 ml). The effluent was concentrated to yield a white

powder of **25** (367 mg): $[\alpha]_D^{23}$ −7.9°. (Found: C, 45.50; H, 7.65; N, 19.67. Calc. for $C_{11}H_{22}N_4O_5$ (290.33): C, 45.51; H, 7.64; N, 19.30; O, 27.55%).

Basic hydrolysis of **25** (**26**)

A soln of **25** (1.2 g) in 0.2N NaOH (12 ml) was refluxed for 2 hr under N_2. The generating gas was trapped with 0.2N HCl (25 ml) to give NH_4Cl (120 mg). The mixture was chromatographed on activated charcoal (150 ml) at pH 8. The eluate of acetone:water (5:95) was evaporated and the residue was crystallized from water to give colorless crystals of **26** (565 mg): m.p. 248° (dec), $[\alpha]_D^{23}$ +5.9°. (Found: C, 44.85; H, 7.29; N, 14.16. Calc. for $C_{11}H_{21}N_3O_6$ (291.31), C, 45.36; H, 7.27; N, 14.42; O, 32.95%).

Methylation of **27** (**28**)

To a soln of **27** (70 mg) in MeOH (7 ml) was added excess CH_2N_2 in Et_2O and the whole was allowed to stand at 25° overnight. The mixture was concentrated to afford a white powder of **28** (56 mg): $[\alpha]_D^{23}$ +14.1° (in MeOH). (Found: C, 50.03; H, 6.64; N, 9.51. Calc. for $C_{18}H_{29}N_3O_9$ (431.45), C, 50.11; H, 6.77; N, 9.74; O, 33.38%).

$Pb(OAC)_4$ *oxidation of* **26** (**29**)

To a soln of **26** (1.1 g) in water (23 ml) was added $Pb(OAc)_4$ (2.2 g) in AcOH (110 ml) during 40 min while stirring. The generating gas was trapped by 1% $Ba(OH)_2$. After 2 hr $BaCO_3$ accumulated was filtered and dried (342 mg). Thereafter, the mixture was allowed to stand at 25° overnight. The mixture was passed through IRA-410 (50 ml). The effluent was chromatographed on activated charcoal (50 ml) and eluted with acetone:water (5:95), (1:9) and (2:8). The eluates were concentrated to give a white powder of **29** (380 mg): $[\alpha]_D^{23}$ −1.2°, −3.4° (in MeOH). (Found: C, 47.06; H, 7.83; N, 16.04; H_2O, 3.64. Calc. for $C_{10}H_{19}N_3O_4 \cdot 1/2H_2O$ (254.29): C, 47.24; H, 7.93; N, 16.52; O, 28.31; H_2O, 3.54%).

$NaBH_4$ *reduction of* **29** (**30**)

A soln of **29** (260 mg) in Ac_2O (5 ml), AcOH (10 ml) and pyridine (10 ml) was allowed to stand at 25° overnight. The mixture was treated with the same method described above. 4′-N-Monoacetate of **29** was obtained as a white powder (145 mg). To a soln of the acetate (120 mg) in MeOH (6 ml) was added $NaBH_4$ (220 mg) in MeOH (6 ml) while stirring and the whole was allowed to stand at 25° overnight. The mixture was chromatographed on activated charcoal (20 ml) at pH 4.5 and eluted with acetone:water (2:8). The eluate was evaporated to give a white powder of **30** (40 mg): $[\alpha]_D^{27}$ +59.0°. (Found: C, 50.10; H, 7.68; N, 14.30. Calc. for $C_{12}H_{23}N_3O_5$ (289.33): C, 49.82; H, 8.01; N, 14.52; O, 27.65%).

Potassium periodate oxidation of **1** (**31**)

To a soln of **1** (6 g) in 0.05N HCl (600 ml) was added KIO_4 (2.8 g) and the mixture was stirred at 25° for 3 hr. After treatment by sat $Sr(OH)_2$ the filtrate was chromatographed on activated charcoal (600 ml) and eluted with acetone:water (2:8). After concentration of the eluate, a pale yellowish powder (3.1 g) was obtained. UV: $\lambda_{max}^{H_2O}$ 273 nm (ϵ = 8.270) and $\lambda_{max}^{0.1N\ HCl}$ 281 (12,700).

A soln of the powder obtained (2.5 g) in N NaOH (50 ml) was warmed at 60° for 2 hr. The mixture was chromatographed on activated charcoal (200 ml) at pH 4 and eluted with acetone:water (2:8). The eluate was evaporated to give a pale yellowish powder (1.3 g). A soln of the powder (1 g) in 2N H_2SO_4 (10 ml) was warmed at 85° for 5 hr. The mixture was treated with fine activated charcoal (1 g). The filtrate (150 ml) was passed through IRA-410 (20 ml). The effluent was chromatographed on IRC-50 (20 ml) and eluted with 0.5N HCl (100 ml). The eluate was passed through activated charcoal (20 ml) and the effluent was evaporated to afford a white powder of **31** (325 mg): $[\alpha]_D^{24}$ +26.9°, UV: $\lambda_{max}^{H_2O}$ 272 nm (ϵ = 8810) and $\lambda_{max}^{0.1N\ HCl}$ 280 (12,700). (Found: C, 37.39; H, 5.52; N, 19.07; Cl, 14.77; H_2O, 6.72. Calc. for $C_{16}H_{23}N_7O_6 \cdot 2HCl \cdot 2H_2O$ (518.38): C, 37.07; H, 5.64; N, 18.92; O, 24.69; Cl, 13.68, H_2O, 6.94%).

Acidic hydrolysis of (**32** *and* **33**)

A soln of **4** (5.5 g) in 3N HCl (110 ml) was refluxed for 15 hr. After treatment with IR-45 (150 ml), the filtrate was passed through IRA-410 (100 ml). The effluent was chromatographed on activated charcoal (200 ml) and eluted with acetone:water (5:95 and 2:8) and acetone: 0.5% HCOOH (1:1). The former eluate was evaporated to give a white powder of **32** (110 mg). The later eluate was evaporated to give a crude powder (2.2 g). The crude powder (1.5 g) was again chromatographed on activated charcoal (50 ml) and eluted with acetone:water (5:95 and 1:9). The eluate was concentrated to afford a white powder of **33** (450 mg). **32**: $[\alpha]_D^{28}$ −40.2°. UV: End absorption. (Found: C, 37.71; H, 7.71; N, 15.12. Calc. for $C_{11}H_{22}N_4O_6 \cdot 5/2H_2O$ (351.37): C, 37.60; H, 7.75; N, 15.95; O, 38.70%). **33**: $[\alpha]_D^{28}$ −11.8°, UV: $\lambda_{max}^{H_2O}$ 274 nm (ϵ = 6410) and $\lambda_{max}^{0.1N\ HCl}$ 283 nm (ϵ = 9660). (Found: C, 41.33; H, 6.64; N, 19.82. Calc. for $C_{16}H_{27}N_7O_7 \cdot HCOOH \cdot H_2O$ (493.50): C, 41.38; H, 6.33; N, 19.87; O, 32.43%).

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