CYTOSAMINOMYCINS, NEW ANTICOCCIDIAL AGENTS PRODUCED BY *Streptomyces* sp. KO-8119

II. STRUCTURE ELUCIDATION OF CYTOSAMINOMYCINS A, B, C and D

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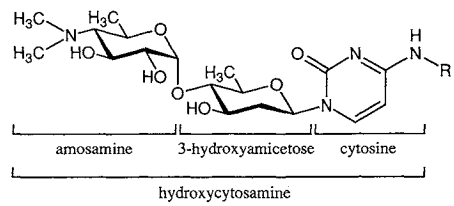
(Received for publication February 14, 1994)

Structures of novel anticoccidial antibiotics, cytosaminomycins A, B, C and D, were elucidated by NMR studies. Cytosaminomycins were shown to be nucleoside antibiotics related to oxyplicacetin. Their carboxylic acid moieties bonded to the cytosine residue were different from that of oxyplicacetin. The carboxylic acids contained in cytosaminomycins A, B, C and D were (*E*)-3-(methylthio)acrylic acid, 4-methylaminobenzoic acid, 3-methylcrotonic acid, and tiglic acid, respectively.

Cytosaminomycins A, B, C and D (**1**~**4**, Fig. 1) are novel anticoccidial antibiotics isolated from the cultured broth of *Streptomyces amakusaensis* KO-8119[1]. Here, we report on the structural elucidation of **1**~**4** by NMR studies.

The molecular formulae of **1**, **2**, **3** and **4** were established as $C_{22}H_{34}N_4O_8S$, $C_{26}H_{37}N_5O_8$, $C_{23}H_{36}N_4O_8$ and $C_{23}H_{36}N_4O_8$ by HRFAB-MS, respectively[1]. IR spectra of **1**~**4** showed amide absorbances at 1560 and 1640~1660 cm^{-1}. Although the UV spectra were not same, the IR spectra resembled each other. The UV and IR spectra of **2** were very similar to oxyplicacetin (**5**)[2]. Therefore, **1**~**4** were presumed to be the amicetin group.

Fig. 1. Structures of cytosaminomycins A, B, C and D (**1**~**4**) and oxyplicacetin (**5**).



Chemical shifts in the ^1H and ^{13}C NMR of **1**~**4** are shown in Table 1. Their ^{13}C-^1H correlations were studied by HMQC. The ^1H and ^{13}C NMR spectra of **1** are shown in Figs. 2 and 3. Compound **1** had 4 methyl, 1 methylene, 13 methine and 3 quarternary carbon signals in the DEPT spectra. The HMQC revealed one carbon signal (δ 41.4) that correlated with two methyl protons (δ 2.46, 6H), which showed that the carbon signal was derived from two carbons. Thus all 22 carbons were assigned. Four partial structures **a**, **b**, **c** and **d** for **1** were elucidated by ^1H-^1H COSY (Fig. 4). Their connectivity was established by HMBC as shown below (Fig. 5).

The long-range coupling between 5″-H (δ 3.58)

Table 1. ^{1}H and ^{13}C NMR assingments for **1**, **2**, **3** and **4**.

No.	**1**[a]		**2**[b]	
	^{13}C	^{1}H	^{13}C	^{1}H
Carboxylic acid moiety				
1	163.6 s		166.2 s	
2 (phenyl-1)	114.9 d	5.98 d (14.4)	120.1 s	
3 (phenyl-2,6)	148.7 d	7.89 d (14.4)	129.9 d×2	7.78 d×2 (8.8)
4 (phenyl-3,5)			111.5 d×2	6.59 d×2 (8.8)
Phenyl-4			153.2 s	
2-CH_3				
3-CH_3				
N-CH_3			29.9 q	2.88 s
S-CH_3	14.7 q	2.37 s		
Cytosine moiety				
2′	154.8 s		155.1 s	
4′	163.0 s		162.8 s	
4′-NH		9.42 br s		
5′	97.5 d	7.56 br s	97.4 d	7.61 br d (7.6)
6′	143.8 d	7.85 d (7.5)	143.7 d	7.83 d (7.6)
3-Hydroxyamicetose moiety				
1″	81.2 d	5.80 br d (10.2)	81.0 d	5.84 dd (2.0, 10.6)
2″	38.3 t	1.53 ddd (10.2, 11.0, 11.5), 2.54 br dd (3.5, 11.0)	38.0 t	1.52 ddd (10.6, 11.6, 12.6), 2.46 ddd (2.0, 4.9, 12.6)
3″	70.9 d	3.95 m	70.9 d	3.90 ddd (4.9, 9.0, 11.6)
4″	88.7 d	3.11 dd (9.0, 9.0)	89.1 d	3.08 dd (9.0, 9.0)
5″	74.1 d	3.58 dq (9.0, 6.0)	73.9 d	3.57 dq (9.0, 6.0)
6″	17.9 q	1.37 d (6.0)	17.9 q	1.36 d (6.0)
Amosamine moiety				
1‴	102.2 d	5.04 d (2.8)	102.2 d	5.01 d (3.8)
2‴	74.5 d	3.68 dd (2.8, 10.0)	74.3 d	3.66 dd (3.8, 9.2)
3‴	68.7 d	3.79 dd (10.0, 10.0)	68.7 d	3.75 dd (9.2, 9.8)
4‴	70.2 d	2.13 dd (10.0, 10.0)	70.2 d	2.11 dd (9.8, 10.2)
4‴-$N(CH_3)_2$	41.4 q×2	2.46 s×2	41.3 q×2	2.45 s×2
5‴	65.9 d	3.95 m	65.8 d	3.94 dq (10.2, 6.4)
6‴	19.6 q	1.24 d (5.2)	19.5 q	1.23 d (6.4)

No.	**3**[a]		**4**[a]	
	^{13}C	^{1}H	^{13}C	^{1}H
Carboxylic acid moiety				
1	165.5 s		167.9 s	
2 (phenyl-1)	117.7 d	5.80 s	131.8 s	
3 (phenyl-2,6)	159.0 s		135.3 d	6.65 q (6.7)
4 (phenyl-3,5)	27.8 s	1.93 s	14.5 q	1.83 d (6.7)
Phenyl-4				
2-CH_3			12.2 q	1.90 s
3-CH_3	20.5 s	2.22 s		
N-CH_3				
S-CH_3				
Cytosine moiety				
2′	154.7 s		154.6 s	
4′	162.7 s		162.3 s	
4′-NH		8.89 br s		8.50 br s
5′	97.0 d	7.54 d (7.5)	97.0 d	7.48 br d (7.6)
6′	143.8 d	7.82 d (7.5)	144.1 d	7.84 d (7.6)
3-Hydroxyamicetose moiety				
1″	81.1 d	5.82 dd (1.8, 10.8)	81.1 d	5.85 dd (2.1, 10.8)
2″	38.2 t	1.52 ddd (10.8, 10.8, 13.0), 2.52 ddd (1.8, 5.0, 13.0)	38.1 t	1.52 ddd (10.8, 12.2, 12.4), 2.50 ddd (2.1, 5.0, 12.4)
3″	70.9 d	3.94 m	70.9 d	3.93 m
4″	88.9 d	3.10 dd (8.6, 9.2)	89.1 d	3.09 dd (8.8, 9.2)
5″	74.0 d	3.58 dq (9.2, 6.2)	74.0 d	3.58 dq (9.2, 6.0)
6″	17.9 q	1.34 d (6.2)	17.9 q	1.36 d (6.0)
Amosamine moiety				
1‴	102.2 d	5.03 d (3.8)	102.2 d	5.03 d (3.6)
2‴	74.5 d	3.70 dd (3.8, 9.2)	74.5 d	3.70 dd (3.6, 9.2)
3‴	68.7 d	3.79 dd (9.2, 10.0)	68.6 d	3.78 dd (9.2, 10.0)
4‴	70.2 d	2.14 dd (9.6, 10.0)	70.3 d	2.13 dd (10.0, 10.0)
4‴-$N(CH_3)_2$	41.4 q×2	2.46 s×2	41.4 q×2	2.47 s×2
5‴	65.9 d	3.95 m	65.8 d	3.95 m
6‴	19.5 q	1.24 d (6.4)	19.5 q	1.25 d (6.1)

[a] Solvent: $CDCl_3$.
[b] Solvent: $CDCl_3+CD_3OD$. The coupling constants (Hz) are in parentheses.

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

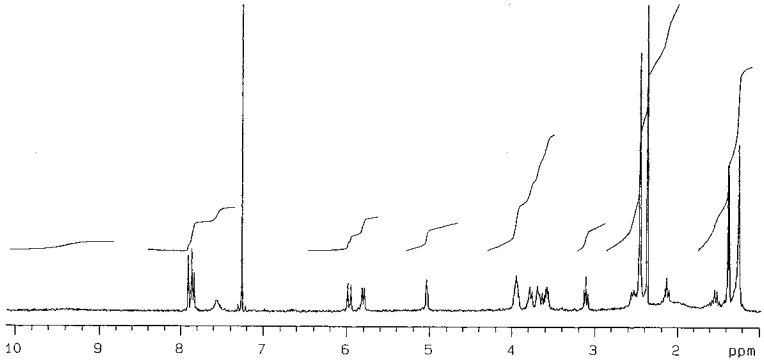


Fig. 3. ^{13}C NMR spectrum of **1**.

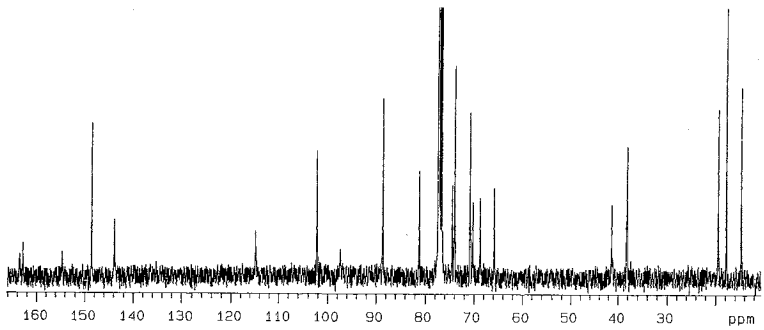


Fig. 4. Partial structures **a**~**d** of **1** elucidated by ^{1}H-^{1}H COSY.

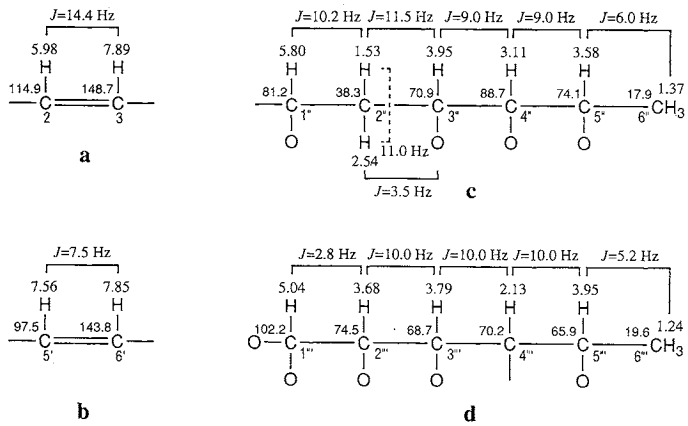
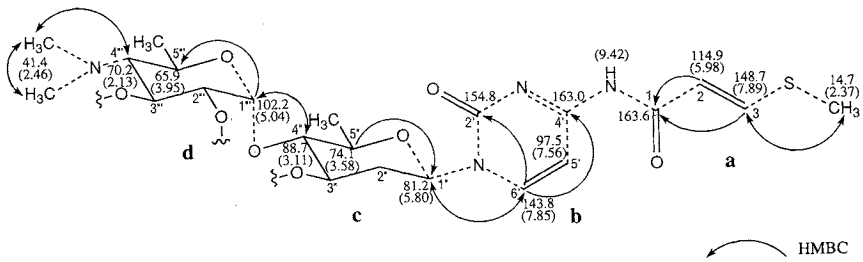


Fig. 5. Structure of **1** elucidated by HMBC.



and C-1″ (δ 81.2) proved the alignment of C-1″ ~ O ~ C-5″. Therefore, partial structure **c** should be cyclized to form a sugar and the sugar was 3-hydroxyamicetose[2,3].

The HMBC of the partial structure **d** showed coupling between 1‴-H (δ 5.04) and C-5‴ (δ 65.9), suggesting another sugar moiety. Two methyls ($N(CH_3)_2$, δ_C 41.4 and δ_H 2.46) were coupled, which proved they were attached to same atom. Their chemical shifts and the couplings between their carbons and 4‴-H (δ 2.13) and between their protons and C-4‴ (δ 70.2) indicated an *N*-dimethyl residue bonded to C-4‴. These data revealed the partial structure **d**, namely amosamine[2,4]. Moreover, couplings were observed between 1‴-H (δ 5.04) and C-4″ (δ 88.7), and between 4″-H (δ 3.11) and C-1‴ (δ 102.2). Therefore, 3-hydroxyamincetose proved to be bonded to amosamine. The coupling constants of the protons of **c** and **d** were reasonable for the conformation of 3-hydroxyamicetose and amosamine, respectively and showed similar values to those previously described[2,3].

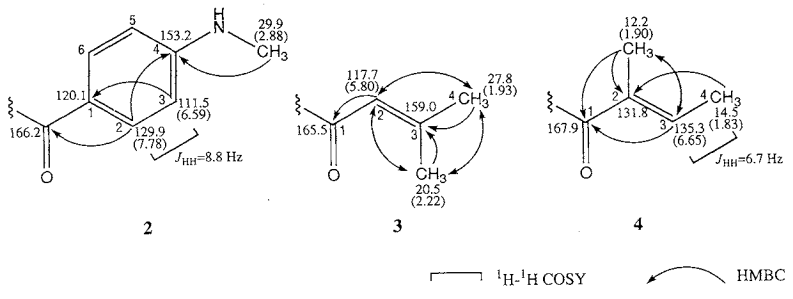
Both 3-hydroxyamicetose and amosamine are components of oxamicetin and oxyplicacetin. Comparing their NMR spectra[2,3] with **1**, partial structure **b** was elucidated as a part of cytosine. Consequently, **b**, **c** and **d** comprised hydroxycytosamine just same as did oxamicetin and oxyplicacetin.

The remaining atoms, C_4H_5OS, were assumed to bond to the 4′-NH of cytosine. C-1 (δ 163.6) had couplings with 2-H (δ 5.98) and 3-H (δ 7.89), suggesting the connectivity of C-1 to **a** (–C2=C3–). Couplings between the remaining methyl (δ_C 14.7, δ_H 2.37) and C-3 (δ 148.7), or 3-H were also observed. The carbon chemical shift of the methyl was more suitable for an *S*-methyl than an acetyl, and thus the alignment of –C1–C2=C3–S–CH_3 was established. The configuration of the olefin bond of partial structure **a** was suggested as *E* by the coupling constant of $J_{2H,3H}$ (14.4 Hz), and finally the carboxylic acid moiety was elucidated as (*E*)-3-(methylthio)acrylic acid.

FAB-MS of **1** exhibited the fragment ion peaks at *m/z* 174 (amosamine), 212 (cytosine+carboxylic acid), and 304 (amosamine+hydroxyamicetose), which was consistent with the structure of **1**. FAB-MS of **2**, **3** and **4** also showed the fragment ion peaks at *m/z* 174 and 304. So they seemed to contain amosamine and hydroxyamicetose. Respective fragment peaks at *m/z* 245, 194 and 194 were observed in **2**, **3** and **4** instead of the fragment peak *m/z* 212 in **1**, each of which corresponded to M−(amosamine+hydroxyamicetose). It suggested that these compounds differed from **1** in cytosine, or the carboxylic acid moieties.

^1H and ^{13}C NMR of **2**, **3** and **4** showed the signals of the hydroxycytosamine moiety (Table 1). Therefore, the structure of the carboxylic acid moiety was studied on those compounds below. As for **2**, ^1H-^1H COSY showed coupling between 2-H (and 6-H, δ 7.78) and 3-H (and 5-H, δ 6.59). Long-range couplings were observed between 2-H (and 6-H) and the carbonyl (δ 166.2), 2-H (and 6-H) and C-4

Fig. 6. Structures of carboxylic acid moiety of **2**, **3** and **4** elucidated by ^1H-^1H COSY and HMBC.



(δ 153.2), and 3-H (and 5-H) and C-1 (δ 120.1) in HMBC as shown in Fig. 6. These data suggested a benzoic acid structure. HMBC showed coupling between the NCH_3 (δ 2.88) and C-4, which revealed that the *N*-methyl was bonded to C-4. Thus the structure of the carboxylic acid moiety of **2** was elucidated as 4-methylaminobenzoic acid.

As for **3**, the structure of 3-methylcrotonic acid was elucidated by long-range couplings from 2-H (δ 5.80) to C-1 (δ 165.5), C-4 (δ 27.8) and 3-CH_3 (δ 20.5), from 4-H (δ 1.93) to C-2 (δ 117.7), C-3 (δ 159.0) and 3-CH_3, and from 3-CH_3 (δ 2.22) to C-2, C-3 and C-4. ^1H-^1H COSY showed coupling between 3-H (δ 6.65) and 4-H (δ 1.83) in **4**. Long-range couplings were observed from 3-H (δ 6.65) to C-1 (δ 167.9) and 2-CH_3 (δ 12.2), from 4-H (δ 1.83) to C-2 (δ 131.8), and from 2-CH_3 (δ 1.90) to C-1, C-2 and C-3 (δ 135.3). These data indicated that the structure was 2-methyl-2-butenoic acid. NOEs were observed between 3-H and 4-H and were not observed between 3-H and 2-CH_3 in differential NOE experiments. Thus the *E* stereochemistry was suggested for C-2. Moreover, the chemical shifts of the 2-CH_3 and C-4 of tiglic acid (*E* form) and angelic acid (*Z* form) were different. The former was δ 10.95 (2-CH_3) and δ 13.83 (C-4) and the latter was δ 20.22 (2-CH_3) and δ 15.93 (C-4)[5]. Those same chemical shifts of **4** were δ 12.2 and δ 14.5, which were similar to tiglic acid. Therefore, the structure of the carboxylic acid moiety of **4** was elucidated as tiglic acid.

The carboxylic acid moieties of all known amicetin group antibiotics are aminobenzoic acid or its derivatives. Thus **1**, **2** and **4** are the first compounds in the amicetin group that have aliphatic carboxylic acids.

Experimental

NMR spectra were recorded on a Valian XL-400 spectrometer in $CDCl_3$ (**1**, **3** and **4**) or $CDCl_3+CD_3OD$ (**2**). FAB-MS was recorded on JEOL JMS-DX300 or JMS-AX505 HA spectrometers.

Acknowledgment

We thank Ms. AKIKO HATANO and Ms. NORIKO SATO of School of Pharmaceutical Sciences, Kitasato University for measurements of NMR spectra, and also thank Ms. AKIKO NAKAGAWA and Ms. CHIKAKO SAKABE of School of Pharmaceutical Sciences, Kitasato University for measurements of mass spectra.

References

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