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IC202A, a New Siderophore with Immunosuppressive Activity Produced by *Streptoalloteichus* sp. 1454-19

II. Physico-chemical Properties and Structure Elucidation

MASATOMI IIJIMA, TETSUYA SOMENO, MASAHIDE AMEMIYA, RYUICHI SAWA†, HIROSHI NAGANAWA†, MASAAKI ISHIZUKA* and TOMIO TAKEUCHI

Institute for Chemotherapy, M.C.R.F.,
18-24 Aza-Motono, Miyamoto, Numazu-shi, Shizuoka 410-0301, Japan
† Institute of Microbial Chemistry, M.C.R.F.,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan

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IC202A (**1**) was isolated from the culture filtrate of *Streptoalloteichus* sp. 1454-19. The structure of **1** was determined by spectral analysis including a variety of two-dimentional NMR and FAB-MS experiments. IC202A is a ferrioxamine-related compound containing a butylidene *N*-oxide function.

In the course of our screening program for low molecular weight immunomodulators, we found that *Streptoalloteichus* sp. 1454-19 produced a new immunosuppressant, IC202A (**1**). In the preceding paper[1], we have described the fermentation, isolation, and biological activity of IC202A, as well as the taxonomy of the producing strain. This paper describes the structure elucidation of IC202A.

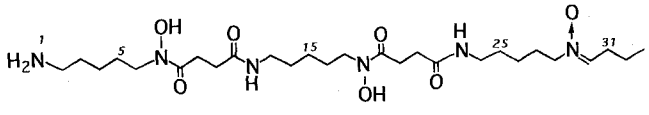
Results and Discussion

IC202A (**1**) was obtained as a hygroscopic powder. The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was determined to be $C_{27}H_{52}N_6O_7$ by HRFAB-MS [Calcd. for $C_{27}H_{53}N_6O_7$: m/z 573.3976, Found: m/z 573.3994 $(M+H)^+$] and ^{13}C NMR spectral data. These physico-chemical properties and NMR spectral data of **1** suggested that **1** was related to a ferrioxamine siderophore compound. **1** was positive to $FeCl_3$, indicating the presence of a hydroxamate (–N(OH)–CO–) moiety[2] in the molecule.

In the ^{13}C NMR spectrum, all 27 carbons were visible and the multiplicities were determined by DEPT experiments. The DEPT and HMQC experiments revealed the presence of one methyl, twenty-one methylenes, one olefinic methine, and four carbonyl carbons.

From the ^1H-^1H COSY and HMBC experiments, the partial structure (Fig. 2) containing a butylidene *N*-oxide moiety was determined as follows. ^1H-^1H COSY spectrum of **1** revealed spin networks from terminal methyl protons (H-33, δ_H 0.95) to an olefinic proton (H-30, δ_H 7.31). In the HMBC spectrum, correlations were revealed between relevant carbons (C-30 to C-33) as shown in Fig. 2. Additionally, the olefinic proton at δ_H 7.31 (H-30) coupled to the other methylene carbon (δ_C 66.7). Judging from the chemical shifts of the methylene [^{13}C (δ_C 66.7) and ^1H (δ_H 3.83)], this methylene (C-28) was inferred to be adjacent to either an oxygen or nitrogen atom. Since the methylene protons (H-28) were coupled to the sp^2 carbon (C-30) in the HMBC spectrum, an imino function must be inserted between C-28 and C-30. Furthermore, a higher-field

Fig. 1. Structures of IC202A (**1**) isolated from *Streptoalloteichus* sp. 1454-19 and deferoxamine (**2**).



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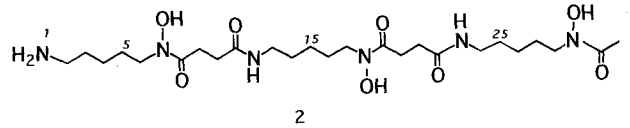

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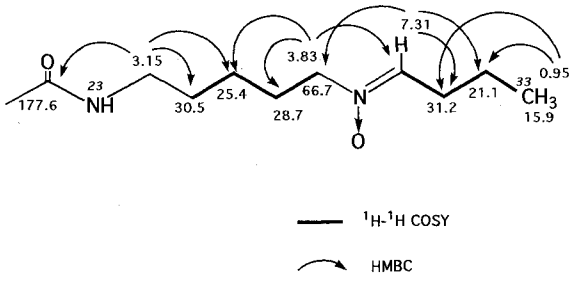
Table 1. Physico-chemical properties of IC202A.

Appearance		Hygroscopic powder
Molecular formula		$C_{27}H_{52}N_6O_7$
HRFAB-MS (*m/z*)		
$(M+H)^+$	Calcd:	573.3976 (for $C_{27}H_{53}N_6O_7$)
	Found:	573.3994
UV λ_{max}nm(ε)		No maxima above 210nm
IR ν_{max}(KBr)cm^{-1}		3310, 3090, 2930, 2860, 1625, 1565, 1460, 1270, 1225, 1195, 1165, 735
TLC (Rf value)[a]		0.45[b]

[a] Silica gel TLC (Merck Art. 1.05715) : BuOH-BuOAc-MeOH-H_2O(2:2:1:1).
[b] Detected with I_2 vapor.

Fig. 2. Partial structure of IC202A obtained from ^{1}H-^{1}H COSY and HMBC experiments.



shift[3] of an azomethine carbon (C-30) and a lower-field shift of C-28 suggested that the nitrogen be oxidized. HRFAB-MS spectrum of **1** showed the fragment ion at *m/z* 557.4037 (Calcd. for $C_{27}H_{53}N_6O_6$: 557.4027), originating from the loss of one oxygen atom from the molecular ion, which supported the presence of *N*-oxide[3,4].

In the vicinity of the *N*-oxide functional group, spin networks from H-25 to H-28 were observed in the ^{1}H-^{1}H COSY spectrum. In the HMBC spectrum, a methylene at δ_H 3.15 (H-24) was coupled to C-25 (δ_C 30.5), C-26 (δ_C 25.4) and a carbonyl carbon (δ_C 177.6), resulting in a partial structure as shown in Fig. 2. As far as the remaining part of the structure is concerned, **1** showed two sets of signals due to succinyl (–CO–CH_2–CH_2–CO–) and diaminopentane (–N–$(CH_2)_5$–N–) moieties in the HMBC spectrum, although the complete assignment was obstructed by severe overlapping of proton signals.

It is well known that many microbes, including Gram-positive Nocardiae and Streptomycete, Gram-negative Pseudomonads[5] produce various types of ferrioxamines[6]. These ferrioxamines are comprised of alternate repeating units of ω-amino-α-hydroxyamino-alkanes. Since the structure of deferoxamine ($C_{25}H_{48}N_6O_8$, see **2** in Fig. 1), one of ferrioxamines, has been well established, the spectral data of **1** were compared with those of an authentic sample of deferoxamine. The direct comparison of ^{13}C NMR in D_2O revealed the following differences: 1) Disappearance of one carbonyl carbon at δ_C 176.2 (C-30) in **2** and appearance of sp^2 methine at δ_C 152.0 (C-30) was shown for **1**. 2) Two methylene signals at δ_C 31.2 (C-31) and δ_C 21.1 (C-32) were observed for **1**. 3) Down field shift of methylene carbon at δ_C 66.7 (C-28) was observed for **1** by comparing the relevant signal at δ_C 50.4 in **2**. All other signals agreed with each other to within 0.5 ppm. Therefore, the both compounds possess the same partial structure for the N-1 to C-27.

Assignments of ^{1}H and ^{13}C NMR of **1** and **2** are summarized in Table 2.

The structure of **1** thus obtained was further confirmed by FAB-B/E linked scan spectrum, which revealed the characteristic fragment peak of *m/z* 319 by cleavage at the hydroxyamide. FEISTNER *et al.*[6] demonstrated the relevant mass fragmentations using a variety of ferrioxamines [$NH_2(CH_2)m$-N(OH)CO$(CH_2)_2$CONH$(CH_2)n$-N(OH)CO$(CH_2)_2$CONH$(CH_2)o$-N(OH)–CO–R, $m=3$,

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of IC202A (**1**) and deferoxamine (**2**).

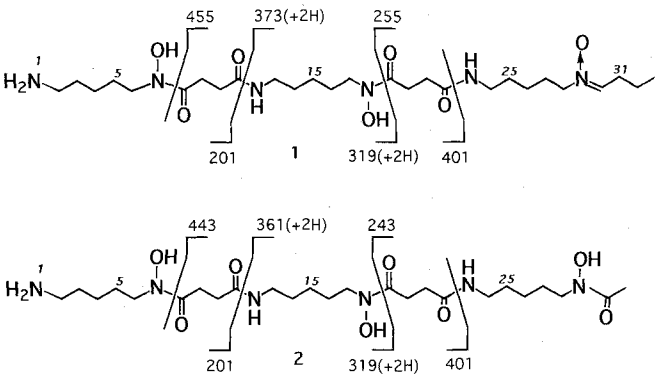
Carbon No.	**1** ^{13}C	**1** ^{1}H	**2** ^{13}C	**2** ^{1}H
2	42.1 t	2.98 (t,7.6)	42.0 t	2.98 (t,7.6)
3	29.1 t	1.65 (m)	29.0 t	1.65 (m)
4	25.4 t	1.50 (m)	25.3 t	1.48 (m)
5	28.0[a] t	1.65 (m)	28.0 t	1.65 (m)
6	50.4[b] t	3.65 (m)	50.4 t	3.60 (m)
8	176.6 s	-	176.5 s	-
9	30.3 t	2.80 (m)	30.3 t	2.80 (m)
10	33.1[c] t	2.50 (m)	33.1 t	2.50 (m)
11	177.6 s	-	177.5 s	-
13	42.0 t	3.15 (m)	41.9 t	3.16 (m)
14	30.6 t	1.50 (m)	30.4 t	1.50 (m)
15	25.8 t	1.30 (m)	25.7 t	1.30 (m)
16	28.2[a] t	1.65 (m)	28.0 t	1.65 (m)
17	50.6[b] t	3.65 (m)	50.4 t	3.62 (m)
19	176.6 s	-	176.6 s	-
20	30.3 t	2.80 (m)	30.3 t	2.80 (m)
21	33.2[c] t	2.50 (m)	33.1 t	2.50 (m)
22	177.6 s	-	177.5 s	-
24	41.8 t	3.15 (m)	41.9 t	3.16 (m)
25	30.5 t	1.51 (m)	30.4 t	1.50 (m)
26	25.4 t	1.29 (m)	25.7 t	1.30 (m)
27	28.7 t	1.83 (m)	28.0 t	1.65 (m)
28	66.7 t	3.84 (t,6.8)	50.4 t	3.60 (m)
30	152.0 d	7.31 (t,5.9)	176.2 s	-
31	31.2 t	2.42 (m)	21.9 q	2.14 (s)
32	21.1 t	1.55 (m)		
33	15.9 q	0.95 (t,7.5)		

[a~c] Chemical shifts with the same superscript may be transposed.

4, 5, $n=4$, 5 $o=4$, 5)]. As reported prominent fragment ions at m/z 201, 319 and 401 for the compounds (m and $n=5$) in ferrioxamines, both spectra of **1** and **2** also gave these characteristic ions (Fig. 3). This result defined the number m and n to be 5.

IC202A is a new member of the ferrioxamine family including linear trihydroxamates containing diaminopentane[7]. Within the family, however, IC202A is characterized as being unique due to the presence of a terminal butylidene *N*-oxide moiety . So far as we know, cribrochalinamine oxides A and B[3] are the only other natural products having a butylidene *N*-oxide in the molecule. Within the ferrioxamine group, IC202A is the first reported compound having an azomethine *N*-oxide moiety.

Fig. 3. FAB-B/E linked scan analysis of IC202A (**1**) and deferoxamine (**2**).



Experimental

Deferoxamine mesylate was purchased from Sigma-Aldrich Japan (Tokyo). IR spectra were measured on a Horiba FT-200 fourier transform infrared spectrometer and UV spectra were measured on a Hitachi 228A spectrometer.

^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra in D_2O were measured on a JEOL JNM-A-400 spectrometer. Chemical shifts are expressed in δ value (ppm) with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as an internal standard and coupling constants are given in J (Hz). FAB-MS spectra were measured on a JEOL JMS-SX102 spectrometer.

Acknowledgments

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