Structure of Kifunensine, a New Immunomodulator Isolated from an Actinomycete[1)]

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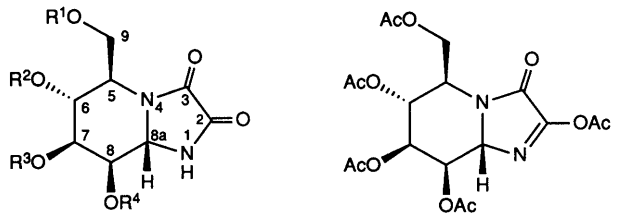
The structure of kifunensine, a new immunomodulator produced by a strain of *Kitasatosporia*, has been established as 1 on the basis of chemical and physicochemical evidence and X-ray crystallographic analysis. Kifunensine is unique both in its novel structure, containing a 4,5-dioxoimidazolidine ring included in the bicyclic framework, and in its potent immunomodulating activity. It is a representative of a new class of 1,5-iminopyranoses.

Keywords immunomodulator; α-mannosidase inhibitor; *Kitasatosporia kifunense*; polyhydroxylated piperidine; 4,5-dioxoimidazolidine; kifunensine; X-ray analysis

As part of a continuing program to screen for immunologically active compounds of microbial origin, a search was undertaken for substances having activity to restore mitogenic responses depressed by immunosuppressive factors of tumors. Kifunensine was isolated as such an immunoregulatory substance from an actinomycete, *Kitasatosporia kifunense* No. 9482.[2)] This compound was also found to possess an inhibitory activity against α-mannosidase.[1)] In this paper we report the structural elucidation of this natural product.

Kifunensine (**1**) was isolated as colorless prisms, mp > 280 °C, $[\alpha]_D$ +58.0° (c=0.1, H_2O). The molecular formula ($C_8H_{12}N_2O_6$) of **1** was established by elemental analysis and fast atom bombardment mass spectra (FAB-MS). The infrared (IR) spectrum showed absorption bands ascribable to hydroxy groups (3330, 3240, 3195 cm^{-1}) and carbonyl functions (1740, 1727, 1710 cm^{-1}).

The carbon nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I) showed eight carbon signals, of which two were observed in the sp^2 region (δ 164.2 (s), 162.8 (s)) and the remainder (six carbons) in the sp^3 region (δ 62.7 (t), 73.9 (d), 73.8 (d), 71.8 (d), 66.0 (d), 61.3 (d)), being assigned to two carbonyls (C-2 (or C-3), C-3 (or C-2)), one methylene (C-9), and five methines (C-8, C-7, C-6, C-8a, C-5). The chemical shifts of the C-9 methylene and three of the five methines (C-8, C-7, C-6), which all resonated at relatively low field, suggest that they bear hydroxy groups.



kifunensine(**1**): R^1=R^2=R^3=R^4=H

3: R^1,R^2=R^3,R^4=>CMe_2

2

Chart 1

Acetylation of **1** with Ac_2O in pyridine gave the pentaacetate **2**, whose proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Table II) showed five acetyl methyl signals at δ 2.60, 2.23, 2.10, 2.05, and 2.04. The relatively low chemical shift of one of these acetyl groups (δ 2.60) suggests this acetyl group to be an enol acetate. In the ultraviolet (UV) spectrum of **2**, a strong absorption band was observed at 232 nm (ε = 10000), revealing that **2** has an α,β-unsaturated carbonyl function and accordingly the original compound **1** has an enolizable α,β-dicarbonyl system.

The ^{1}H-NMR spectrum of **1** (Table II) showed methylene signals at δ 4.02 (dd, J = 12, 9.5 Hz, H_e) and 3.86 (dd, J = 12, 4.5 Hz, H-f) and methine signals at δ 3.72 (dd, J = 9, 3 Hz, H_g), 4.11 (dd, J = 3.5, 3 Hz, H_d), 4.20 (dd, J = 3.5, 1 Hz, H_c), 4.41 (ddd, J = 9.5, 4.5, 1 Hz, H_b), and 5.12 (d, J = 9 Hz, H_a). An analysis of these signals in conjunction with a ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) experiment clarified the H–H relationships as shown in Fig. 1. A two-dimensional incredible natural abundance double quantum transfer (2D INADEQUATE) experiment[3)] (D_2O–NaOD) on **1** revealed C–C couplings between the

TABLE I. ^{13}C-NMR (100 MHz) Chemical Shifts (in ppm) for **1** and **2**[a)]

C	1[b)]	2[c)]
2	164.2 (s)[d)]	156.1 (s)[e)]
3	162.8 (s)[d)]	156.0 (s)[e)]
5	61.3 (d)	52.7 (d)
6	71.8 (d)	66.9 (d)
7	73.8 (d)	68.5 (d)
8	73.9 (d)	71.0 (d)
8a	66.0 (d)	62.9 (d)
9	62.7 (t)	59.7 (t)

a) Abbreviations given in parentheses denote signals observed in the off-resonance experiments. *b*) D_2O. *c*) $CDCl_3$. *d*, *e*) Assignments may be interchanged in each column.

TABLE II. ^{1}H-NMR (400 MHz) Chemical Shifts (in ppm), Multiplicities, and Coupling Constants (in Hz, in Parentheses) for **1**, **2**, and **3**

H	1[a)]	2[b)]	3[b)]
1-H			9.00 br s
5-H	4.41 ddd (9.5, 4.5, 1)	4.83 ddt (10, 5, 1.5)	3.59 ddd (11, 10, 5)
6-H	4.20 dd (3.5, 1)	5.08 dd (4, 1.5)	4.23 dd (11, 8)
7-H	4.11 dd (3.5, 3)	5.33 ddd (4, 3, 1.5)	4.36 dd (8, 8)
8-H	3.72 dd (9, 3)	5.02 dd (9, 3)	4.05 dd (8, 8)
8a-H	5.12 d (9)	5.91 d (9)	4.91 d (8)
9-H_a	4.02 dd (12, 9.5)	4.60 dd (12, 10)	3.80 dd (12, 10)
9-H_b	3.86 dd (12, 4.5)	4.29 dd (12, 5)	4.67 dd (12, 5)
2-OAc, 3H s		2.60	
6,7,8,9-O-Ac, each 3H s		2.23, 2.10, 2.05, 2.04	
7,8;6,9-Acetonides, each 3H s			1.57, 1.55, 1.48, 1.36

a) D_2O. *b*) $CDCl_3$.

carbons bonded directly to each other, disclosing the sequence of these carbons as shown in partial structures A and B (Fig. 2).

A reasonable connection of these partial structures A and B to partial structure C was obtained by analysis of the ^{1}H-NMR spectrum of the diacetonide **3**, which was prepared by treatment of **1** with 2,2-dimethoxypropane in the presence of TsOH. The spectrum showed methylene and methine proton signals at δ 4.67 (dd, J=12, 5 Hz, 9-H$_a$), 3.80 (dd, J=12, 10 Hz, 9-H$_b$), 4.05 (dd, J=8, 8 Hz, 8-H), 4.36 (dd, J=8, 8 Hz, 7-H), 4.23 (dd, J=11, 8 Hz, 6-H), 4.91 (d, J=8 Hz, 8a-H), and 3.59 (ddd, J=11, 10, 5 Hz, 5-H) (Table II). The spectrum further showed an exchangeable amide proton at δ 9.00 (br s, 1-H), between which and the C-8a proton a cross-peak was observed in the ^{1}H–^{1}H COSY spectrum ($CDCl_3$). These data indicated the bonding of the amide N to C-8a and thereby led to partial structure C. The two acetonide bonds are postulated to be between C-9 and C-6 and between C-7 and C-8 on the assumption that they are five- or six-membered rings. A reasonable cyclization of this partial structure through the remaining tertiary nitrogen atom (N-4) finally leads to structure **3** for the diacetonide and the structure for kifunensine is hence deduced to be **1**.

The relative stereochemistry of **1** was presumed on the following grounds (Fig. 3). In the ^{1}H-NMR spectrum of **1**, nuclear Overhauser effect (NOE) was observed between 8a-H and 9-H$_a$, suggesting that 8a-H and the C9-hydroxymethyl group are 1,3-diaxial and hence that the piperidine ring of **1** takes a chair form. Supposing this is correct, the fact that the vicinal coupling constant of 8-H and 8a-H was large (J=9 Hz) suggests these protons to be in *trans* diaxial relationship. Since the coupling constant between 7-H and 8-H was small (J=3 Hz), 7-H was assigned to be equatorial. Regarding the relative configuration of the remaining C-6, an NOE experiment was undertaken on the diacetonide **3**. In this experiment, NOE was observed between 6-H and 8a-H in **3**, suggesting that the piperidine ring takes a boat form in which these protons exist on the same side of the piperidine ring. In the ^{1}H-NMR spectrum of **3**, the coupling constants between the two vicinal protons are all relatively large (5-H and 6-H, 11 Hz; 6-H and 7-H, 8 Hz; 7-H and 8-H, 8 Hz; 8-H and 8a-H, 8 Hz). All these protons are therefore presumed to be quasi-axial, in agreement with the conclusion derived from the above discussion. The relative stereochemistry of kifunensine has thus been deduced to be as shown in **1**.

The presumed structure of **1** was finally established by X-ray crystallographic analysis using crystals of kifunensine itself obtained from water. A perspective drawing of the

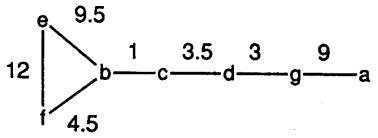


Fig. 1. ^{1}H–^{1}H Relationships and Coupling Constants (in Hz) in **1**

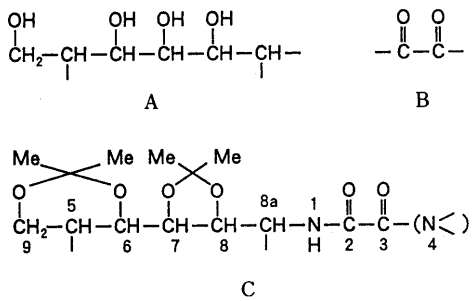


Fig. 2. Partial Structures of **1** and **3**

TABLE III. Atomic Coordinates and Thermal Parameters with e.s.d.'s (Å^2) in Parentheses

Atom	x	y	z	B_{eq}/B_{iso}
N1	0.2443 (3)	0.4465 (5)	0.0351 (3)	1.7
C2	0.4163 (4)	0.4671 (5)	0.0705 (4)	1.5
C3	0.4770 (4)	0.3539 (5)	0.2195 (3)	1.4
N4	0.3370 (3)	0.2615 (4)	0.2497 (3)	1.3
C5	0.3288 (4)	0.1240 (5)	0.3752 (3)	1.3
C6	0.1833 (4)	0.1949 (5)	0.4536 (3)	1.5
C7	0.0146 (4)	0.2328 (6)	0.3387 (3)	1.4
C8	0.0425 (4)	0.3824 (6)	0.2144 (3)	1.5
C8a	0.1817 (4)	0.3016 (5)	0.1338 (3)	1.4
C9	0.3135 (4)	−0.0944 (5)	0.3193 (4)	1.6
O10	0.5114 (4)	0.5533 (5)	−0.0012 (3)	2.3
O11	0.6243 (3)	0.3511 (5)	0.2926 (3)	2.1
O12	0.4676 (3)	−0.1562 (4)	0.2728 (3)	2.0
O13	0.2282 (3)	0.3805 (5)	0.5318 (3)	2.1
O14	−0.0493 (3)	0.0483 (4)	0.2684 (3)	1.8
O15	−0.1085 (3)	0.4057 (5)	0.0984 (3)	2.6
H1	0.173 (7)	0.513 (10)	−0.048 (6)	3.5
H5	0.446 (6)	0.131 (9)	0.456 (6)	2.0
H6	0.166 (6)	0.084 (9)	0.532 (6)	1.8
H7	−0.074 (6)	0.294 (9)	0.398 (6)	2.1
H8	0.076 (7)	0.526 (10)	0.268 (6)	2.8
H8a	0.131 (6)	0.174 (9)	0.072 (6)	2.0
H9a	0.207 (6)	−0.112 (10)	0.226 (5)	2.3
H9b	0.291 (6)	−0.191 (9)	0.407 (6)	2.4
H12	0.473 (7)	−0.085 (11)	0.182 (6)	3.6
H13	0.353 (7)	0.380 (13)	0.600 (6)	4.1
H14	−0.115 (8)	−0.006 (11)	0.338 (7)	4.1
H15	−0.204 (7)	0.401 (11)	0.147 (6)	3.7

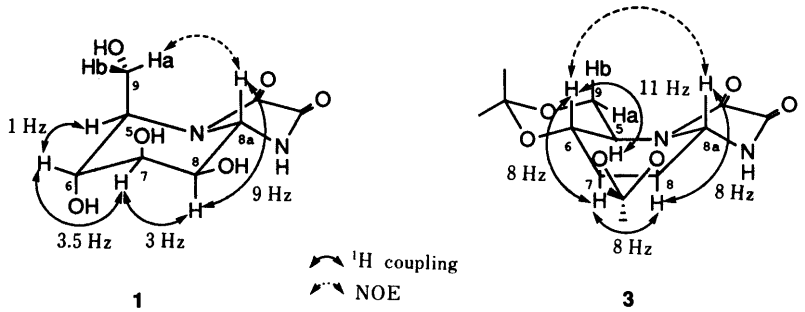


Fig. 3. ^{1}H-NMR Coupling Constants and NOE in **1** and **3**

TABLE IV. Bond Lengths (Å) and Angles (°) with Their e.s.d.'s in Parentheses

Bond length (Å)			
N1–C2	1.344 (5)	N1–C8a	1.458 (5)
N1–H1	0.95 (7)	C2–C3	1.520 (5)
C2–O10	1.226 (5)	C3–N4	1.343 (4)
C3–O11	1.219 (5)	N4–C5	1.457 (4)
N4–C8a	1.466 (5)	C5–C6	1.539 (5)
C5–C9	1.529 (5)	C5–H5	1.06 (6)
C6–C7	1.535 (5)	C6–O13	1.425 (5)
C6–H6	1.05 (6)	C7–C8	1.538 (5)
C7–O14	1.421 (5)	C7–H7	1.04 (6)
C8–C8a	1.531 (5)	C8–O15	1.426 (5)
C8–H8	1.087 (7)	C8a–H8a	1.05 (6)
C9–O12	1.428 (5)	C9–H9a	1.07 (7)
C9–H9b	1.05 (6)	O12–H12	0.95 (7)
O13–H13	1.05 (9)	O14–H14	0.96 (7)
O15–H15	0.94 (8)		
Bond angle (°)			
C2–N1–C8a	112.2 (3)	C2–N1–H1	124 (4)
C8a–N1–H1	124 (4)	N1–C2–C3	106.5 (3)
N1–C2–O10	128.8 (4)	C3–C2–O10	124.6 (3)
C2–C3–N4	105.8 (3)	C2–C3–O11	125.3 (3)
N4–C3–O11	128.9 (3)	C3–N4–C5	127.4 (3)
C3–N4–C8a	112.8 (3)	C5–N4–C8a	119.7 (3)
N4–C5–C6	108.2 (3)	N4–C5–C9	110.8 (3)
N4–C5–H5	109 (3)	C6–C5–C9	114.9 (3)
C6–C5–H5	108 (3)	C9–C5–H5	105 (3)
C5–C6–C7	112.2 (3)	C5–C6–O13	110.5 (3)
C5–C6–H6	108 (3)	C7–C6–O13	106.5 (3)
C7–C6–H6	111 (3)	O13–C6–H6	109 (3)
C6–C7–C8	110.8 (3)	C6–C7–O14	109.9 (3)
C6–C7–H7	108 (3)	C8–C7–O14	109.1 (3)
C8–C7–H7	109 (3)	O14–C7–H7	110 (3)
C7–C8–C8a	109.6 (3)	C7–C8–O15	111.9 (3)
C7–C8–H8	108 (3)	C8a–C8–O15	106.0 (3)
C8a–C8–H8	113 (3)	O15–C8–H8	109 (3)
N1–C8a–N4	101.9 (3)	N1–C8a–C8	114.8 (3)
N1–C8a–H8a	111 (3)	N4–C8a–C8	108.4 (3)
N4–C8a–H8a	114 (3)	C8–C8a–H8a	107 (3)
C5–C9–O12	110.6 (3)	C5–C9–H9a	111 (4)
C5–C9–H9b	111 (3)	O12–C9–H9a	110 (4)
O12–C9–H9b	109 (3)	H9a–C9–H9b	106 (5)
C9–O12–H12	107 (4)	C6–O13–H13	113 (5)
C7–O14–H14	103 (4)	C8–O15–H15	107 (5)

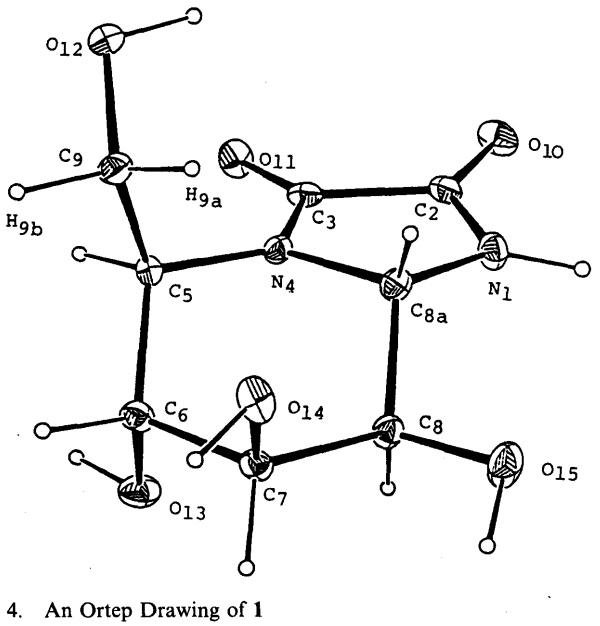


Fig. 4. An Ortep Drawing of **1**

structure of **1** is given in Fig. 3. Kifunensine was thus determined to have the structure **1** (relative stereochemistry). The absolute stereochemistry was presumed, by considering the fact that **1** showed inhibitory activity against α-mannosidase, to be the same as that of D-mannose and this was finally confirmed by a synthesis of **1** from D-mannosamine.[4]

The structural study described above thus revealed that kifunensine has a unique bicyclic structure **1** corresponding to a cyclic oxamide derivative of 1-amino-substituted mannojirimycin.[5,6] Synthetic studies of kifunensine and related compounds will be reported in subsequent papers in this series.

Experimental

General Procedures The melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. The elemental analyses were performed using a Yanaco MT-3 CHN CORDER. The optical rotations were measured with a JASCO DIP-140 digital polarimeter. The IR spectra were taken on a JASCO A-102 infrared spectrophotometer. The UV spectra were taken on a Hitachi 220A spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were taken on a Bruker AM 200 (200 MHz for ^{1}H-NMR) or Bruker AM 400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) NMR spectrometer with tetramethylsilane (TMS) or sodium 3-(trimethylsilyl)propionate-d_4 (TSP-d_4) as an internal standard, and chemical shifts were recorded in δ values. Multiplicities of ^{13}C-NMR signals were determined by the distortionless enhancement by polarization transfer (DEPT) method. Pulse programs of the standard Bruker software library were used for 2D experiments. The FAB-MS were taken on a VG Analytical ZAB-SE mass spectrometer. Column chromatography was run on Merck Silica gel 60 (70—230 mesh). Thin layer chromatography (TLC) was performed on glass plates precoated with Silica gel 60F_{254} (Merck).

Kifunensine (1) Colorless prisms, mp > 280 °C (H_2O), $[\alpha]_D$ +58.0° (c=0.1, H_2O). *Anal.* Calcd for $C_8H_{12}N_2O_6$: C, 41.38; H, 5.21; N, 12.06. Found: C, 40.94; H, 5.07; N, 11.78. IR (KBr): 3405, 3330, 3240, 3195, 2920, 1740, 1727, 1710, 1452, 1100, 1070, 1052, 1010 cm^{-1}. UV $\lambda_{max}^{H_2O}$ nm (ε): 226 (230). ^{1}H- and ^{13}C-NMR: see Tables I and II. FAB-MS m/z: 233 $(M+H)^+$.

Kifunensine Pentaacetate (2) Acetic anhydride (10 ml) was added to a suspension of **1** (1.73 g) in pyridine (20 ml) and the mixture was stirred at room temperature for 20 h. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in AcOEt and washed with 1N aqueous HCl, water, saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$, evaporated *in vacuo*, and purified by column chromatography (SiO_2 30 g, CH_2Cl_2–MeOH) to furnish **2** (2.92 g, 84%). **2**, amorphous solid, $[\alpha]_D$ −35.3° (c=0.6, $CHCl_3$). *Anal.* Calcd for $C_{18}H_{22}N_2O_{11}$: C, 48.87; H, 5.01; N, 6.33. Found: C, 48.61; H, 4.92; N, 6.20. IR ($CHCl_3$): 1778, 1750, 1432, 1368, 1250, 1210, 1142, 1058 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 232 (10000). ^{1}H- and ^{13}C-NMR: see Tables I and II. FAB-MS m/z: 433 $(M+H)^+$.

Kifunensine Diacetonide (3) A suspension of **1** (200 mg) in *N,N*-dimethylformamide (DMF) (5.0 ml) was treated with 2,2-dimethoxypropane (3.2 ml) and TsOH·H_2O (20 mg) at 60 °C for 12 h under an N_2 atmosphere. After neutralization with saturated aqueous $NaHCO_3$, the solvent was removed under reduced pressure and the residue was extracted with MeOH (15 ml). The extract was evaporated *in vacuo* and purified by column chromatography (SiO_2 10 g, $CHCl_3$–AcOEt) to afford the diacetonide **3** (223 mg, 83%). **3**, colorless fine crystals, mp 275—278 °C (dec., *n*-hexane–AcOEt), $[\alpha]_D$ −66.6° (c=0.5, MeOH). *Anal.* Calcd for $C_{14}H_{20}N_2O_6$: C, 53.84; H, 6.45; N, 8.97. Found: C, 53.87; H, 6.77; N, 8.99. IR ($CHCl_3$): 3415, 2993, 1754, 1419, 1375, 1100, 1074 cm^{-1}. ^{1}H-NMR: see Table II. FAB-MS m/z: 313 $(M+H)^+$.

X-Ray Analysis of 1 The crystals were recrystallized from water: $C_8H_{12}N_2O_6$, prisms, monoclinic, space group $P2_1$, a=7.934(2), b=6.634(1), c=8.933(3) Å, β=101.59(3)°, V=460.6(2) Å^3, Z=2, D_x=1.68 g/cm^3, μ=0.93 cm^{-1}. The X-ray diffraction intensity data from a selected crystal (0.15×0.05×0.05 mm) were obtained on a Rigaku AFC-5R diffractometer equipped with a rotating anode X-ray generator (50 KV–180 mA), using graphite-monochromated Mo$K\alpha$ radiation (λ=0.71069 Å). A total of 1461 independent reflections with $2\theta < 60°$ were collected in the ω–2θ scaling mode. The structure was solved by the direct

method using MULTAN 84 (Main *et al.*, 1984). The H atoms were located from difference Fourier syntheses. The refinement was carried out by the block-diagonal least-squares method with anisotropic thermal parameters for non H atoms and with isotropic thermal parameters for H atoms. The R factor was reduced to 0.047 using 1273 reflections with $F_o \geqq 3\sigma(F_o)$. The atomic parameters, bond lengths and bond angles are given in Tables III and IV.

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