Structure of Kifunensine, a New Immunomodulator Isolated from an Actinomycete

Hiroshi Kayakiri, Shigehiro Takase, Toshihiro Shibata, Masanori Okamoto, Hiroshi Terano, and Masashi Hashimoto*

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5-2-3 Tokodai, Tsukuba, Ibaraki 300-26, Japan

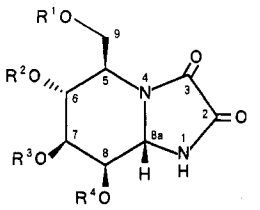
Toshiji Tada and Shigetaka Koda

Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan

Received April 24, 1989

Summary: The structure of kifunensine (1) isolated from an actinomycete as an immunomodulator has been determined on the basis of chemical and physical evidence and X-ray crystal analysis.

Sir: Glycosidase inhibitors have recently attracted considerable attention, because they are useful for investigating the structure–function relationships of glycoproteins. The latter play an important role in many biochemical processes including carbohydrate metabolic disorders,[1] viral infectivity,[2] cancer metastasis,[3] and immune response.[4] In the course of our screening program for immunologically active substances, kifunensine was isolated as a new immunomodulator[5] with α-mannosidase inhibitory activity[6] from an actinomycete, *Kitasatosporia kifunense* No. 9482. Herein we report the structure elucidation of this natural product as 1 on the basis of chemical and physical evidence and X-ray crystal analysis.

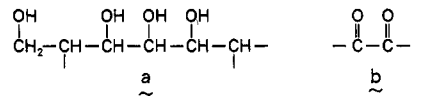


1 $R^1 = R^2 = R^3 = R^4 = H$

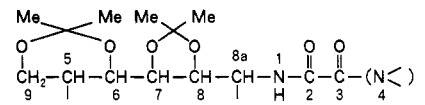
2 $R^1, R^2 = R^3, R^4 = >CMe_2$

Kifunensine (1) was isolated as colorless prisms:[7] $C_8H_{12}N_2O_6$ (FABMS, m/z 233 (M + H). 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); mp >280 °C; $[\alpha]_D$ +58° (*c* 0.1, H_2O); IR (KBr) 1740, 1727, 1710 cm^{-1}.

The ^{13}C NMR spectrum (D_2O) showed one methylene (δ 62.7 (t, C-9)), five methines (δ 73.9 (d, C-8), 73.8 (d, C-7), 71.8 (d, C-6), 66.0 (d, C-8a), 61.3 (d, C-5)), and two carbonyls (δ 164.2 (s, C-2 or C-3), 162.8 (s, C-3 or C-2)). The chemical shifts of the methylene (C-9) and three (C-8, C-7, and C-6) of the five methine carbons suggest that they bear hydroxy groups. The corresponding methylene and methine protons in the ^1H NMR spectrum (D_2O) were observed at δ 4.02 (dd, J = 12, 9.5 Hz, 9-Ha), 3.86 (dd, J = 12, 4.5 Hz, 9-Hb), 3.72 (dd, J = 9, 3 Hz, 8-H), 4.11 (dd, J = 3.5, 3 Hz, 7-H), 4.20 (dd, J = 3.5, 1 Hz, 6-H), 4.41 (ddd, J = 9.5, 4.5, 1 Hz, 5-H), and 5.12 (d, J = 9 Hz, 8a-H), respectively. The 2D INADEQUATE experiment[8] (D_2O–NaOD) revealed C–C couplings between the carbons bonded directly to each other, clarifying the serial linkages of these carbons as shown in partial structure a and b.



a b



c

Treatment of 1 with 2,2-dimethoxypropane in the presence of *p*-TsOH in DMF (room temperature, 15 h) gave diacetonide 2 (mp 275–278 °C dec; FABMS, m/z 313 (M + H); 82%), whose ^1H NMR spectrum ($CDCl_3$)[9] showed, in addition to the methylene and methine protons (δ 4.67 (dd, J = 12, 5 Hz, 9-Ha), 3.80 (dd, J = 12, 10 Hz, 9-Hb), 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)), an exchangable amide proton at δ 9.00 (br s, 1-H). In the ^1H–^1H COSY spectrum ($CDCl_3$), a cross-peak was observed between this amide NH and the C-8a proton, indicating the bonding of the amide N to C-8a and thereby leading to partial structure c. The two acetonide bonds between C-9/C-6 and C-7/C-8 are postulated on the assumption that they are five- or six-membered rings. A reasonable cyclization of this partial structure c through the remaining one nitrogen atom (N-4) finally leads to 2 for the full structure of the diacetonide and hence 1 for that of kifunensine itself (without stereochemistry).

The stereochemistry of 1 was deduced as follows. Kifunensine (1) showed, in the ^1H NMR spectrum, a large value (J = 9 Hz) for the vicinal coupling constant of 8-H and 8a-H, suggesting that these protons are in trans diaxial relationship. The vicinal coupling constant of 5-H and 6-H in 2 was also large (J = 11 Hz), although, in 1, the corresponding coupling constant was small (J = 1 Hz). These facts suggest that, in 2, 5-H and 6-H are trans diaxial: the conformation of 1 differs from that of 2, the corresponding protons being equatorial–equatorial in 1. The configurations of 7-H and 8-H in 1 are presumed, by considering

(1) Truscheit, E.; Frommer, W.; Junge, B.; Muller, L.; Schumidt, D. D.; Wingender, W. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 744.
(2) Leigh, D. A. *J. Antimicrob. Chemother.* **1988**, *22*, 271 and references cited.
(3) (a) Sasak, U. W.; Ordovas, J. M.; Elbein, A. D.; Berninger, R. W. *Biochem. J.* **1985**, *232*, 759. (b) Truqnan, G.; Rousset, M.; Zweibaum, A. *FEBS Lett.* **1986**, *195*, 28. (c) Humphries, M. J.; Matsumoto, K.; White, S. L.; Olden, K. *Cancer Res.* **1986**, *46*, 5215.
(4) (a) Kino, T.; Inamura, N.; Nakahara, K.; Kiyoto, S.; Goto, T.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1985**, *38*, 936. (b) Humphries, M. J.; Matsumoto, K.; White, S. L.; Olden, K. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1752. (c) Dennis, J. W. *Cancer Res.* **1986**, *46*, 5131.
(5) Iwami, M.; Nakayama, O.; Ternao, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1987**, *40*, 612. Kifunensine had been tentatively designated FR900494 in that paper.
(6) An α-mannosidase inhibitory activity: IC_{50} 1.2×10^{-4} M against α-mannosidase (Jack bean). Details will be reported in due course.
(7) A repeated experiment for isolation of kifunensine provided a sample in a higher state of purity, and the physical data reported in the original paper (mp 120–136 °C dec, $[\alpha]_D$ +33° (*c* 0.1, H_2O)) should be corrected to those in the text.
(8) Turner, D. L. *J. Magn. Reson.* **1982**, *49*, 175.
(9) ^{13}C NMR data of 2: δ ($CDCl_3$) 158.0 (s, C-2 or C-3), 156.7 (s, C-3 or C-2), 48.3 (d, C-5), 70.2 (d, C-6), 75.9 (d, C-7), 79.6 (d, C-8), 65.4 (d, C-8a), 61.3 (t, C-9).

0022-3263/89/1954-4015$01.50/0

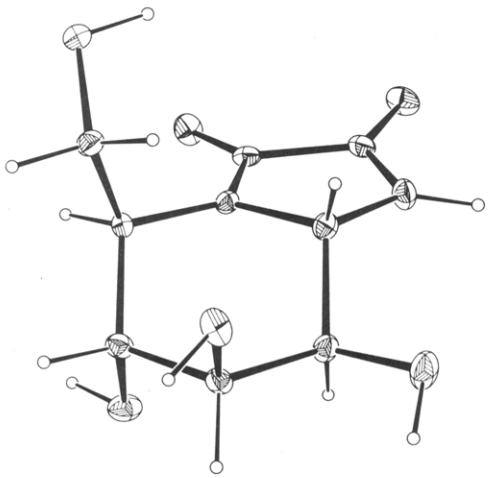


Figure 1. Molecular structure of 1 by an ORTEP drawing.

the fact that 1 showed inhibitory activity against α-mannosidase,[6] to be the same as those of mannose. The relative stereochemistry of kifunensine has thus been deduced to be as shown in 1.

For the confirmation of the presumed structure and determination of its stereochemistry, a single-crystal X-ray analysis was undertaken using crystals of 1: monoclinic, space group $P2_1$; unit cell a = 7.934 (2), b = 6.634 (1), and c = 8.933 (3) Å; β = 101.59 (3)°; V = 460.6 (2) Å^3; Z = 2, D_x = 1.68 g cm^{-3}. Intensities were measured with $2\theta/\omega$ scan mode using graphite-monochromated Mo Kα radiation (λ = 0.71069 Å). The structure was determined by the direct method (MULTAN 84) and successive block-diagonal least-squares and Fourier syntheses. Parameters were refined by using anisotropic temperature factors to R = 0.047 for 1273 reflections used ($F_o \geq 3\sigma(F_o)$). A perspective drawing of the structure of 1 is given in Figure 1. The structure of kifunensine was thus defined to be 1 (relative configuration). The absolute stereochemistry of 1 was presumed, on the basis of its biological activity (α-mannosidase inhibition), to be the D form.[10]

Kifunensine corresponds to a cyclic oxamide derivative of 1-amino-substituted mannojirimycin.[11,12] Because of its novel structure and interesting biological activity, kifunensine represents a unique 1,5-iminopyranose and provides a new insight into the chemistry and biochemistry of this class of compounds.

Supplementary Material Available: Details of the X-ray crystal analysis of 1 including tables of fractional coordinates, thermal parameters, bond lengths, and bond angles (5 pages). Ordering information is given on any current masthead page.

(10) Very recently, we have completed a synthesis of kifunensine from D-mannosamine, confirming its absolute structure. Details will be reported in due course.

(11) Mannojirimycin (nojirimycin B): Niwa, T.; Tsuruoka, T.; Goi, H.; Kodama, Y.; Itoh, J.; Inoue, S.; Yamada, Y.; Niida, T.; Nobe, M.; Ogawa, Y. *J. Antibiot.* **1984**, *37*, 1579.

(12) For a review on 1,5-iminopyranoses and 1,4-iminofuranoses, see: Fleet, G. W. J. *Spec. Publ. Royal Chem. Soc.* **1988**, No. 65, 149.

Studies on the Mechanism of the Asymmetric Epoxidation: A Ligand Variation Approach†

Paul R. Carlier and K. Barry Sharpless*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

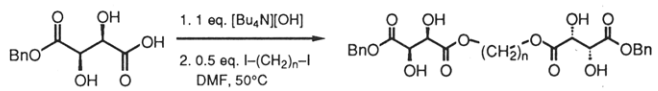
Received March 16, 1989

Summary: The application of linked bis-tartrate esters as ligands for the titanium-mediated asymmetric epoxidation is studied in order to gain information about the structure of the active catalytic species. The results obtained argue against the sole intermediacy of monomeric titanium–tartrate in the parent system.

Sir: Determination of the structure of the active species in any catalytic cycle is extremely difficult. The possibility of catalytic activity being derived solely from a minor species must be dealt with as unambiguously as is possible.[1] This paper details the synthesis of three linked bis-tartrate ligands and their use in epoxidation reactions to gain information about the structure of the active catalytic species in the titanium-mediated asymmetric epoxidation/kinetic resolution.[2]

The mechanism of the asymmetric epoxidation has been studied in detail in our laboratories.[3] A broad range of experimental data suggest that the predominant (~90%) species in solution generated by the addition of 1 equiv of dialkyl tartrate to a titanium alkoxide has a structure 1, analogous to the solid-state structure of a related derivative[4] (Figure 1). Kinetic studies support the hypothesis of epoxidation by such a species, and a stereochemical model has been developed to rationalize the observed sense and degree of asymmetric epoxidation and kinetic resolution.[5] However, one mechanistic possibility

Scheme I



3

4, n = 3, 51%

5, n = 4, 81%

6, n = 5, 84%

† This paper is dedicated to Dr. Günther Ohloff on the happy occasion of his 65th birthday.

(1) Extensive studies of the rhodium–bisphosphine catalyzed asymmetric hydrogenation of α-aminoacrylic acid derivatives revealed that the major product (>60:1) was derived from the minor (~9%) alkyl hydride intermediate (see: Halpern, J. *Science* **1982**, *217*, 401).

(2) (a) Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974. (b) Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237. (c) Hanson, R. M.; Sharpless, K. B. *J. Org. Chem.* **1986**, *51*, 1922. (d) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

(3) (a) Woodard, S. S. Ph.D. Dissertation, Stanford University, Stanford, CA, 1981. (b) Sharpless, K. B.; Woodard, S. S.; Finn, M. G. *Pure Appl. Chem.* **1983**, *55*, 1823. (c) Finn, M. G.; Sharpless, K. B. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1985; Vol. 5, Chapter 8. (d) Finn, M. G. Ph.D. Dissertation, Massachusetts Institute of Technology, Cambridge, MA, 1985. (e) Carlier, P. R.; Mungall, W. S.; Schröder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 2978. (f) Burns, C. J.; Martin, C. A.; Sharpless, K. B. *J. Org. Chem.* **1989**, *54*, 2826.

0022-3263/89/1954-4016$01.50/0