Cytoxazone: A Novel Cytokine Modulator Containing a 2-Oxazolidinone Ring Produced by *Streptomyces* sp.

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Received September 22, 1998

It is well established that the induction of humoral or cellular response is influenced by the development of distinct subsets of $CD4^+$ T cells.[1] The Th1 cell subset produces predominately IL-2, GM-CSF, INF-γ, and TNF-β (type 1 cytokines) and is involved in delayed-type hypersensitivity reactions, whereas the Th2 cell subset secretes IL-4, IL-5, IL-6, IL-10, and IL-13 (type 2 cytokines), which are important factors for B cell growth and differentiation to Ig secretion. The imbalance of cytokine production by $CD4^+$ T cells leads to a wide variety of immunological disorders, i.e. allergy, progressive lymphoproliferation, and severe immunodeficiency. Skin and lung biopsies from allergic patients indicate that the pivotal cells in the allergic site are the Th2 cells.[2] Treatments effectively suppressing the function or the differentiation of these allergen-specific Th2 cells will most likely provide efficient ways to intervene in Ig-mediated allergic diseases. In the course of screening for chemical immunomodulators that inhibit the type 2 cytokine production in Th2 cells, we found cytoxazone (**1**) containing a 2-oxazolidinone ring, which is rare in microbial metabolites, as a novel cytokine modulator produced by *Streptomyces* sp.[3] Cytoxazone (**1**) shows a cytokine-modulating activity by inhibiting the signaling pathway of Th2 cells, but not Th1 cells. We report herein mainly the structure elucidation of **1** based on NMR, CD spectra, and X-ray crystallographic experiments.

Results and Discussion

The molecular formula of cytoxazone (**1**) was determined to be $C_{11}H_{13}NO_4$ by HRFAB-MS (*m*/*z* 224.0958 (M + H)$^+$, +3.5 mmu error) and elemental analysis, which was consistent with the data of ^{1}H NMR and ^{13}C NMR spectra. The ^{13}C NMR spectrum confirmed the presence of eleven carbons, which were classified into one carbonyl carbon (δ 158.73 s, C-2), six aromatic carbons (δ 129.25 s, C-7; δ 128.00 d, C-8 and C-12; δ 113.65 d, C-9 and C-11; and δ 158.97 s, C-10), and four aliphatic carbons (δ 56.17 d, C-4; δ 80.03 d, C-5; δ 61.02 t, C-6; and δ 55.07 q, O*C*H$_3$). The HMQC spectrum established all one-bond ^{1}H−^{13}C correlations as summarized in Table 1. The ^{1}H NMR data showed a proton spin network representing 4-H (δ 4.89 d, J = 8.3 Hz), 5-H (δ 4.68 ddd, J = 8.3, 7.8, 3.9 Hz), and 6-H (δ 2.95 dd, J = 11.7, 7.8 Hz and δ 2.97 dd, J = 11.7, 3.9 Hz). ^{1}H−^{13}C couplings from a pulse-field gradient (PFG) HMBC experiment established the total structure of **1**. The long-range correlations between aromatic protons and carbons revealed a 1,4-disubstituted benzene ring. The following long-range correlations revealed that *p*-methoxyphenyl group was linked to C-4 (δ 56.17): 4-H (δ 4.89)/C-7 (δ 129.25), C-8 (δ 128.00), and C-12 (δ 128.00); 5-H (δ 4.68)/C-7 (δ 129.25); and 8-H (δ 7.14) and 12-H (δ 7.14)/C-4 (δ 56.17). The amide proton (δ 8.04 br s) and methine proton (4-H, δ 4.89) showed ^{1}H−^{13}C long-range couplings to C-2 (δ 158.73), indicating the formation of a 2-oxazolidinone ring. Moreover, the 2-oxazolidinone ring was confirmed by PFG ^{1}H−^{15}N HMQC experiments at 25 °C; the amide proton at 8.04 ppm was correlated to the nitrogen at 68.30 ppm ($^1J_{NH}$ = 94.0 Hz).[4,5] We also analyzed the chemical shift of the nitrogen in the molecule (*S*)-4-phenyl-2-oxazolidinone; the amide proton at 8.17 ppm was correlated to the nitrogen at 67.00 ppm,[5] which supported the 2-oxazolidinone structure of **1**.

The relative stereochemistry between 4-H (δ 4.89) and 5-H (δ 4.68) was determined as *cis*-configuration from the coupling constant (J = 7.8 Hz), which was consistent with that of the vicinal methine protons of *cis*-4-ethyl-2-oxazolidinone-5-carboxylic acid synthesized from diethyl tartrate in a several steps.[6] In difference NOE experiments, significant NOEs were observed between 4-H and 5-H. Furthermore, the relative configuration of **1** was confirmed by the application of X-ray crystallographic analysis as shown in Figure 2. The presence of an intermolecular hydrogen bond between N−*H* and O(16) was revealed and each cytoxazone molecule was coordinated with a single H_2O molecule at the O(14).

The absolute configurations were determined on the basis of the comparison of CD spectra with authentic compounds, (*S*)- and (*R*)-4-phenyl-2-oxazolidinone. The sign of Cotton effects of (*S*)- and (*R*)-4-phenyl-2-oxazolidinone gave negative and positive at around 220 nm, respectively, indicating that the sign of the Cotton effects was governed by the stereochemistry of C-4 in the 2-oxazolidinone ring. The sign of Cotton effects of **1** at the same region was positive, indicating that the stereochemistry of C-4 in **1** was the *R* configuration. Thus structure of **1** was determined to be (*4R, 5R*)-5-(hydroxymethyl)-4-(4-methoxyphenyl)-2-oxazolidinone. The absolute configuration of **1** was also supported by its enantioselective synthesis by Sakamoto et al.[7]

Cytoxazone (**1**) significantly inhibited the production of IL-4, IL-10, and IgG induced by pokeweed mitogen (PWM)-treatment in whole spleen cells of normal mice, whereas it did not affect the PWM-induced GM-CSF

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10.1021/jo981922b CCC: $18.00
Published on Web 01/05/1999

Table 1. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR Spectral Data for Cytoxazone (1) in DMSO-d_6

position	^{13}C[a]	^{1}H[b]	HMBC correlations (H to C)
2	158.73 s		
4	56.17 d	4.89 (1H, d, 8.3)	C-2, C-5, C-6, C-7, C-8, C-12
5	80.03 d	4.68 (1H, ddd, 8.3, 7.8, 3.9)	C-4, C-6, C-7
6	61.02 t	2.95 (1H, dd, 11.7, 7.8)	C-4, C-5
		2.97 (1H, dd, 11.7, 3.9)	C-4, C-5
7	129.25 s		
8, 12	128.00 d	7.14 (2H, d, 8.3)	C-4, C-9, C-10, C-11
9, 11	113.65 d	6.92 (2H, d, 8.3)	C-7, C-8, C-10, C-12
10	158.97 s		
NH		8.04 (1H, br.s)	C-2, C-4, C-5
OH		4.84 (1H, br)	
OCH_3	55.07 q	3.74 (1H, s)	C-10

[a] Chemical shifts are given in ppm as a reference of 39.50 ppm for DMSO. [b] Chemical shifts are given in ppm as a reference of 2.49 ppm for DMSO.

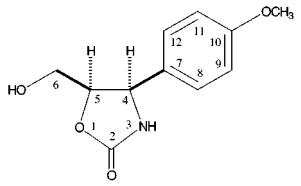

Figure 1. Structure of cytoxazone (**1**).

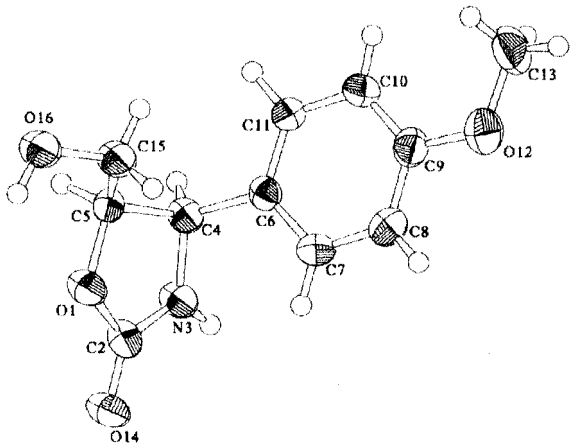

Figure 2. ORTEP view of cytoxazone (**1**).

production.[3] These results suggest that **1** interferes with the cytokine production via the inhibition of signaling pathway of Th2 cells, but not Th1 cells.

As described in this work, **1** possesses a unique structure containing a 2-oxazolidinone ring. It is worthwhile noting that few compounds containing a 2-oxazolidinone ring isolated from microorganisms have been reported such as cinodine γ2 from *Nocardia* and coumadine γ2 from *Saccharopolyspora*.[8,9] Furthermore, it is well-known that the macrolides such as rapamycin and FK506 are potent immunosuppressants that inhibit signal transduction pathways required for T cell activation and growth. FK506 interferes with Ca^{2+}-dependent signaling event that couples T cell antigen receptor occupancy to transcription of the genes encoding IL-2 and several other cytokines. In contrast, rapamycin inhibits IL-2-stimulated T-cell proliferation by blocking cell cycle progression from late G1 into S phase.[10] Cytoxazone (**1**) is different from known immunomodulators such as FK506 and rapamycin in respects of the structure and the biological activity. It should be a useful tool for understanding the signaling pathways in Th2 cells. Further studies on the biological activities in vitro and in vivo are in progress.

Experimental Section

General Procedures. Fast atom bombardment mass spectrum (FAB-MS) was measured on a JEOL JMS DSX-300. ^{1}H and ^{13}C NMR spectra were taken on a JEOL JNM-A600 spectrometer with DMSO-d_6 and chemical shifts are recorded in δ values.

Fermentation Conditions, Extraction, and Isolation. See our previous report.[3]

Cytoxazone (1). Cytoxazone (**1**) was obtained as colorless crystals and soluble in MeOH, DMSO, and EtOAc, but only slightly in *n*-hexane and H_2O. The R_f value in solvent system $CHCl_3$−MeOH (10:1) was 0.42: mp 118−121 °C; $[\alpha]_D$ −71° (*c* 0.1, MeOH, 23 °C); IR (KBr) ν_{max} 3475, 3250, 2950, 1715, 1615, 1515, 1400, 1250, 1050, 995 cm^{-1}; UV (MeOH) λ_{max} nm (ϵ) 225 (19600), 277 (2710), 284 (sh, 2300); CD λ (MeOH) 220 nm ($\Delta\epsilon$ +1.5); ^{1}H and ^{13}C NMR data, see Table 1; HRFAB-MS *m*/*z* 224.0958 $(M + H)^+$ (calcd for $C_{11}H_{13}NO_4$, 224.0923). Anal. Found: C, 54.90; H, 6.29; N, 5.80. Calcd for $C_{11}H_{13}NO_4 \cdot H_2O$: C, 54.77; H, 6.27; N, 5.81.

X-ray Analysis. X-ray crystal analysis was performed with a single crystal (colorless, 0.40 × 0.29 × 0.14 mm) obtained from a solvent of MeOH−H_2O (30:70). X-ray diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated Mo−Kα radiation. The structure was solved by direct methods and all non-H atoms were refined anisotropically by full-matrix least-squares techniques. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation. The crystal data were as follows: $C_{11}H_{13}NO_4 \cdot H_2O$, monoclinic, $P2_1$, a = 6.1468(5), b = 7.4644(5), c = 12.9684(8) Å, β = 92.917(6)°, Z = 2, R = 3.8%, R_w = 3.7% for 1473 independent reflections.

Acknowledgment. This work was supported in part by Grants for Multibioprobes and for Biological Defense Network (RIKEN), and a Grant from the Ministry of Education, Science, Sports and Culture, Japan. We are grateful to Dr. Phillip C. C. Liu (The Michigan Medical School) for the preparation of this manuscript.

Supporting Information Available: X-ray structure report for cytoxazone (**1**) (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO981922B

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