Structure Determination of MS-444; a New Myosin Light Chain Kinase Inhibitor

YUMIKO AOTANI and YUTAKA SAITOH*

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.,
3-6-6 Asahimachi, Machida, Tokyo 194, Japan

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MS-444 is a novel myosin light chain kinase inhibitor, isolated from the culture broth of *Micromonospora* sp. KY7123. The structure of MS-444 was determined to be 5,8-dihydroxy-3-methyl-(9*H*)-naphtho[2,3-c]furan-4-one by means of spectral analysis.

MS-444 (Fig. 1)[1], produced by *Micromonospora* sp. KY7123, is a compound which works as a inhibitor for myosin light chain kinase. In the previous paper[2], fermentation, purification, and biological properties of MS-444 were reported. In this paper, we report its structure determination.

Physico-chemical Properties

The physico-chemical properties of MS-444 are summarized in Table 1. MS-444, obtained as yellow needles, didn't show clear melting point, and decomposed around 155°C. The molecular weight (230) and the molecular formula ($C_{13}H_{10}O_4$) of MS-444 were determined by EI-MS and HREI-MS respectively, and confirmed by ^1H and ^{13}C NMR spectra. The strong broad IR band at 3388 cm^{-1} indicated the presence of phenolic hydroxyl groups. Furthermore, a hypsochromic shift in base suggested the existanse of hydroquinone moiety. The absorptions at 1620 cm^{-1} (C=O) and 1604 cm^{-1} (C=C) suggested the presence of intramoleculary-hydrogen-bonded carbonyl group. The optical rotation could not be measured because of the coloring.

Fig. 1. Structure of MS-444.

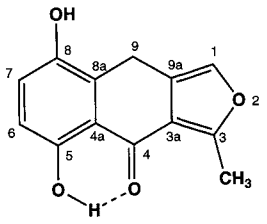


Table 1. Physico-chemical properties of MS-444.

MP (°C)	155 (dec.)
HREI-MS	230.0596 (Observed, M^+)
	230.0579 (Calculated for $C_{13}H_{10}O_4$)
Molecular formula	$C_{13}H_{10}O_4$
UV λ_{max}^{MeOH} nm (ε)	
(in neutral and acidic	203 (17,000), 245 (12,000)
solution)	319 (6,000), 387 (3,800)
(in basic solution)	203 (34,000), 228 (14,000)
	315 (7,000), 431 (2,500)
IR_{KBr} ν_{max} cm^{-1}	3388, 1620, 1604, 1564, 1473, 1327, 1300, 1273, 1257

Structure Determination

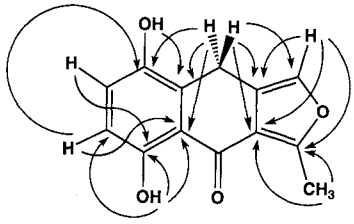
^{13}C NMR spectrum of MS-444 gave thirteen signals which is consistent with the molecular formula determined above. DEPT experiments showed the presence of one methyl (14.6 ppm), one methylene (20.4 ppm), and three sp^2 methines (116.4 ppm, 124.6 ppm, 138.0 ppm). The other eight quaternary carbon signals were assigned to be one carbonyl carbon (189.7 ppm), three oxygen-bearing aromatic carbons (147.8 ppm, 158.6 ppm, 159.2 ppm), and four aromatic carbons (117.5 ppm, 118.2 ppm, 122.9 ppm, 128.0 ppm). In ^1H NMR spectrum, except carbon-bonded protons, two hydroxy-protons were observed and one of which was hydrogen-bonded (12.6 ppm). These data agree well with the above properties.

Two aromatic protons (6.69 ppm, 7.13 ppm) were assumed to be in ortho position from the 8.8 Hz coupling. In proton-nondecoupling ^{13}C NMR, a 206 Hz C-H coupling was observed for ^{13}C that resonates at 138.0 ppm, which indicates the presense of furan ring moiety.

The C-H bonding were assigned by the analysis of proton selective decoupling ^{13}C NMR spectra. The whole structure was established by COLOC (Correlation of Long Range Coupling) experiment shown in Fig. 2. The ^1H and ^{13}C NMR signal assignments are summarized in Table 2.

After the construction of the structure, one coupling

Fig. 2. ^{1}H-^{13}C long-range couplings (COLOC experimemts) of MS-444.



Arrows are directing from H to C.

Table 2. ^{13}C and ^{1}H NMR chemical shifts (δ, ppm) of MS-444 in acetone-d_6.

Position	^{13}C		^{1}H
1	138.0	d	7.53 (1H, t, J=1.7Hz)
3	159.2	s	
3a	117.5	s	
4	189.7	s	
4a	118.2	s	
5	158.6	s	
6	116.4	d	6.69 (1H, dt, J=8.8,0.9Hz)
7	124.6	d	7.13 (1H, d, J=8.8Hz)
8	147.8	s	
8a	128.0	s	
9	20.4	t	3.93 (2H, dd, J=1.7,0.9Hz)
9a	122.9	s	
3-CH_3	14.6	q	2.68 (3H, s)
5-OH			12.60 (1H, s)
8-OH			8.28 (1H, s)

observed in proton homodecoupling experiment was found to be a strange result; the 0.9 Hz coupling observed between 6-H and 9-Hs is unusual for such protons that have 6 bonds between them. We assume that 9-Hs are somehow stereo-positioned in the optimal way for this long-range ^{1}H-^{1}H coupling.

Discussion

MS-444 possesses a unique 4(9*H*)-naphtho[2,3-c]furanone structure. This structure was found in two synthesis reports[3,4] as intermediates of aromatic ortho diketones. And while we were preparing this manuscript 5-hydroxy-3-methy-derivative was isolated from Cape aloe[5]. But there are no previous reports for any biological activities. As MS-444 has a potent bioactivity[2], the structure will be able to act as a lead for new drugs.

Experimental

Melting point was determined with a Yanagimoto melting point apparatus. EI-MS spectrum was measured with a HITACHI M-80B mass spectrometer. IR spectrum was taken on a JEOL JIR-RFX3001 spectrometer. NMR spectra were recorded on a Bruker AM400 spectrometer using TMS as an internal standard.

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