STRUCTURE OF A NOVEL Ca^{2+} AND CALMODULIN-DEPENDENT CYCLIC NUCLEOTIDE PHOSPHODIESTERASE INHIBITOR K-259-2

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The structure of K-259-2, a potent inhibitor of Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase, was determined to be 3-(2*Z*-2-ethyl-2-butenyl)-1,6,8-trihydroxy-anthraquinone-2-carboxylic acid by chemical conversion and spectral studies.

K-259-2 is a new inhibitor of Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase isolated from the culture broth of *Micromonospora olivasterospora* K-259. Taxonomy of the producing organism, fermentation, isolation and biological properties of K-259-2 has been reported by MATSUDA *et al.*[1]. We wish to describe the structure determination of K-259-2 in this paper.

K-259-2 (**1**), mp 140~145°C (dec), was obtained as red needles, insoluble in chloroform, ethyl acetate and acetone, and soluble or slightly soluble in methyl alcohol, water, dimethyl sulfoxide and pyridine. It gave Rf values of 0.35 (butyl alcohol - ethyl alcohol - chloroform - concentrated ammonia, 4 : 5 : 2 : 4) and 0.56 (chloroform - methyl alcohol - ethyl alcohol - water, 5 : 2 : 2 : 1) on silica gel TLC.

The electron impact mass spectrum (EI-MS) of **1** exhibited a weak molecular ion at *m/z* 382. The IR spectrum (KBr) showed the presence of hydroxyl groups (3425 cm^{-1}) and carbonyl groups (1626 and 1562 cm^{-1}). The UV absorption spectrum showed maxima at 223 nm ($E^{1\%}_{1cm}$ 408), 292 (454) and 437 (70) in methyl alcohol, which was similar to that of 2-hydroxyaklavinone[2], suggesting the presence of the same trihydroxy chromophore, 1,6,8-trihydroxyanthraquinone, in **1**.

The ^1H and ^{13}C NMR spectra of **1** did not yield useful information because of signal broadening and its low solubility. Methylation of **1** with diazomethane in methyl alcohol at room temperature led to the trimethylate **2**, which was freely soluble in various organic solvent. Spectral analyses were performed using **2**. Since the molecular formula of **2** was determined as $C_{24}H_{24}O_7$ by high-resolution (HR) EI-MS, that of **1** was estimated as $C_{21}H_{18}O_7$.

The ^1H (in $CDCl_3$ and C_6D_6 solutions) and ^{13}C NMR data of **2** are presented in Tables 1 and 2. ^1H-^1H Decoupling and ^1H-^{13}C long range selective proton decoupling (LSPD) experiments demonstrated the presence of one 2-ethyl-2-butenyl group (ii) composed of one isolated methylene group, one ethyl group and one ethylidene function, one methoxycarbonyl group (iii) and one anthraquinone skeleton (i) bearing one hydroxyl group and two methoxyl groups (Fig. 1).

In the low field region of the ^1H NMR spectrum (in $CDCl_3$ solution), the signals of one hydrogen bonded phenolic hydroxyl proton (δ 13.09) and three aromatic protons (δ 7.78, 7.31 and 6.71) were observed. Since the doublet signals at δ 7.31 and 6.71 showed *meta* coupling to one another and the former had a long range coupling with a carbonyl carbon at C-10, these signals were assigned to 5-H

Table 1. ^{1}H NMR data of **1** and **2**[a].

Proton	1	2	
	DMSO-d_6+CD_3OD	$CDCl_3$	C_6D_6
4-H	7.36 (s)	7.78 (s)	7.68 (s)
5-H	6.39 (d, J=2.4 Hz)	7.31 (d, J=2.6 Hz)	7.49 (d, J=2.6 Hz)
7-H	6.36 (d, J=2.4 Hz)	6.71 (d, J=2.6 Hz)	6.60 (d, J=2.6 Hz)
12-H	4.38 (br s)	4.21 (br s)	4.46 (br s)
14-H	*ca.* 5.1 (m)	5.32 (m)	5.40 (qt, J=6.8, 1.7 Hz)
15-H	1.67 (br d, J=*ca.* 6 Hz)	1.69 (br d, J=6.7 Hz)	1.81 (br d, J=6.8 Hz)
16-H	*ca.* 1.7[b]	*ca.* 1.7 (m)[b]	2.02 (br q, J=7.4 Hz)
17-H	0.78 (t, J=7.3 Hz)	0.85 (t, J=7.2 Hz)	0.98 (t, J=7.4 Hz)
1-OCH_3		4.01 (s)[c]	3.14 (s)[c]
6-OCH_3		3.93 (s)[c]	3.08 (s)[c]
8-OH		13.09 (s)	13.49 (s)
$COOCH_3$		3.87 (s)[c]	3.52 (s)[c]

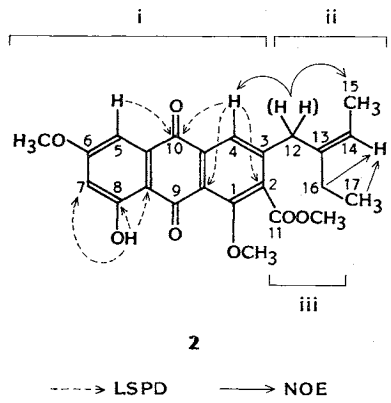
[a] **1** was measured at 100 MHz and **2** was at 400 MHz.
[b] This signal was overlapped with 15-H.
[c] The assignments for these signals within the same vertical column may be interchangeable.

Table 2. ^{13}C NMR data of **2** (100 MHz, $CDCl_3$).

Carbon		Carbon	
1	159.5	10	182.5
2	132.0	10a	134.0[a]
3	144.1	11	167.1
4	108.1	12	32.8
4a	137.5[a]	13	138.2
5	106.7	14	117.8
6	165.5	15	13.6
7	107.5	16	27.4
8	165.2	17	12.2
8a	111.7	1-OCH_3	56.5
9	188.1	6-OCH_3	56.0
9a	125.6	$COOCH_3$	52.5

[a] These assignments may be interchangeable.

Fig. 1. NOE and LSPD experiments of **2**.



and 7-H, respectively. The singlet at δ 7.78 was also coupled with C-10 and was therefore assigned to 4-H. The observation of a long range coupling between the proton at δ 13.09 and C-7 suggested that the hydroxyl group is attached at C-8. The chemical shift values for C-5 and C-7 (δ 106.7 and 107.5, respectively) showed the presence of a methoxyl group at C-6. These results and the UV spectrum of **1** confirmed the anthraquinone skeleton (i).

A vinyl proton at δ 5.40 which was coupled with the vinyl-methyl proton at δ 1.81 exhibited a small coupling with methylene proton at δ 2.02 of the ethyl group, suggesting a sequence of C-15~C-17. A small coupling was also observed between the methylenes at 12-H and 16-H (δ 4.46 and 2.02, respectively), which indicated these methylenes were bonded to the same sp^2 carbon at C-13. Thus, the 2-ethyl-2-butenyl structure of ii (C-12~C-17) was established.

The presence of the 2-ethyl-2-butenyl group was further verified by the ^{1}H NMR spectrum of **3** obtained by catalytic hydrogenation of **2** (Fig. 2): The methylene protons 12-H, seen as a broad singlet at δ 4.21 in **2**, were observed as a broad doublet at δ 3.21 coulpled with methine 13-H. The terminal methyls were observed as an equivalent triplet at δ 0.84 (6H, t, J=7.0 Hz).

Fig. 2. Catalytic hydrogenation of **2**.

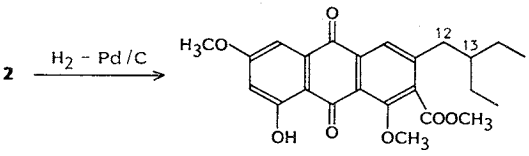


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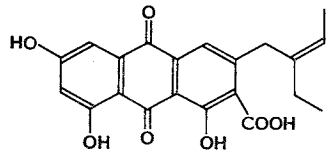
Fig. 3. Structure of K-259-2 (**1**).



K-259-2 (**1**)

The geometry of 13-14 double bond was confirmed to be *Z* from the findings of nuclear Overhauser effect (NOE) from 12-H to 15-H and from 16-H and 17-H to 14-H in the ^{1}H NMR spectrum of **2** in C_6D_6. NOE observed from 12-H to 4-H also indicated the linkage of C-12 to C-3. The remaining methoxycarbonyl group (iii) was concluded to be bonded to C-2.

From these findings, the whole structure of K-259-2 was determined as shown in Fig. 3.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Jeol JNM FX100 and Bruker AM400 spectrometer with TMS (0 ppm) as an internal standard. IR spectra were obtained using a Shimadzu IR-27G spectrometer. UV spectra were taken with a Hitachi 200-20 spectrometer. Low resolution EI-MS were measured on Jeol 01SG mass spectrometer and its HR-MS were on Hitachi M-80B mass spectrometer. Melting points were taken with a Yanagimoto micro melting point apparatus and were not corrected. Thin-layer chromatography (TLC) was performed on pre-coated plates, Merck Kieselgel 60 F_{254}.

Methylation of **1**

To a solution of K-259-2 (10 mg) in MeOH (2 ml), ethereal diazomethane (2 ml), generated from bis-(*N*-methyl-*N*-nitroso)terephthalamide (9 g) in ether (40 ml), was added and left to stand for 2 hours at room temp. After the reaction mixture was evaporated *in vacuo*, the residue was purified by preparative TLC developing with hexane - EtOAc (2:1) to furnish trimethylate **2** (7 mg), a pale yellow powder: TLC Rf 0.55 (hexane - EtOAc, 2:1); IR ($CHCl_3$) cm^{-1} 3440, 1739, 1672, 1627, 1575; UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 221 (339), 274 (330), 283 (334), 430 (64); EI-MS *m/z* 424 (M^+), 406, 395, 365; HREI-MS calcd for $C_{24}H_{24}O_7$: 424.1521, found: 424.1544.

Hydrogenation of **2**

2 (1 mg) in EtOAc (0.5 ml) was hydrogenated over 10% palladium on carbon catalyst (5 mg) for 3 hours at atmospheric pressure and room temp. After filtering off the catalyst, the solution was evaporated to give a pale yellow powder of **3** (1 mg). TLC Rf 0.55 (hexane - EtOAc, 2:1); EI-MS *m/z* 426 (M^+), 411, 395, 379, 365, 341; HREI-MS calcd for $C_{24}H_{26}O_7$: 426.1677, found: 426.1696.

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References

1) MATSUDA, Y.; K. ASANO, I. KAWAMOTO & H. KASE: K-259-2, a new inhibitor of Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase from *Micromonospora olivasterospora*. J. Antibiotics 40: 1092~1100, 1987

2) MATSUZAWA, Y.; A. YOSHIMOTO, N. SHIBAMOTO, H. TOBE, T. OKI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: New anthracycline metabolites from mutant strains of *Streptomyces galilaeus* MA144-M1. II. Structure of 2-hydroxyaklavinone and new aklavinone glycosides. J. Antibiotics 34: 959~964, 1981