

STRUCTURE OF A NOVEL Ca^{2+} AND CALMODULIN-
DEPENDENT CYCLIC NUCLEOTIDE
PHOSPHODIESTERASE INHIBITOR KS-619-1

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The structure of KS-619-1, a potent inhibitor of Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase, was determined to be 8,13-dioxo-3-(2-oxopropyl)-5,6,8,13-tetrahydro-1,7,9,11-tetrahydroxybenz[a]naphthacene-2-carboxylic acid by spectral studies of KS-619-1 and its methyl derivative.

KS-619-1 is a new inhibitor of Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase isolated from the culture broth of *Streptomyces californicus*. The fermentation, isolation and biological properties of KS-619-1 have been reported by MATSUDA *et al.*¹⁾ We wish to describe the structure determination of KS-619-1 in this paper.

KS-619-1 (**1**) was isolated as a dark red powder which melted at 198~200°C with decomposition. The electron impact mass spectrum (EI-MS) (m/z 475 ($M+1$)⁺) and the ¹³C NMR spectrum indicated that **1** had the molecular formula $\text{C}_{26}\text{H}_{18}\text{O}_9$. The IR spectrum (KBr) showed the presence of hydroxyl group (3380 cm^{-1}) and quinone function (1664, 1618 and 1594 cm^{-1}) which was further evidenced by ¹³C NMR signals at δ 189.4 and 181.6. The carbonyl absorption at 1697 cm^{-1} in the IR spectrum and the ¹³C NMR signals at δ 204.9 and 172.3 indicated the presence of a ketone and a carboxyl groups. The UV absorption spectra showed maxima at 225 nm ($E_{1\%}^{1\text{cm}}$ 816), 302 (587), 317 (542) and 470 (339)

Table 1. ¹H NMR data of **1** and **2** (400 MHz)^a.

Proton	1	2
4-H	6.38 (1H, s)	6.95 (1H, s)
5-H	ca. 2.7 (2H, m)	ca. 2.8 (2H, m)
6-H	ca. 2.8 (2H, m)	ca. 3.0 (2H, m)
10-H	6.58 (1H, d, $J=2.2$ Hz)	6.81 (1H, d, $J=2.5$ Hz)
12-H	7.15 (1H, d, $J=2.2$ Hz)	7.51 (1H, d, $J=2.5$ Hz)
14-H	9.08 (1H, s)	8.77 (s)
16-H	4.01 (2H, s)	3.74 (2H, s)
18-H	2.14 (3H, s)	2.21 (3H, s)
7-OH	12.51 (1H, br s) ^b	13.60 (1H, s)
9-OH	12.17 (1H, br s) ^b	
1-OCH ₃		3.65 (3H, s)
9-OCH ₃		4.05 (3H, s)
11-OCH ₃		4.00 (3H, s)
COOCH ₃		3.95 (3H, s)

^a **1** was measured in $\text{DMSO}-d_6$ and **2** was in CDCl_3 .

^b The assignments for these signals may be interchanged.

in methyl alcohol, and 242 nm ($E_{1\text{cm}}^{1\%}$ 877), 298 (540), 330 (721), 400 (250) and 515 (340) in 0.1 M sodium hydroxide - methyl alcohol. This was similar to that of K-259-2 which we have reported²³, suggesting the presence of same chromophore, 1,6,8-trihydroxyanthraquinone moiety.

As for the aromatic moiety of **1**, the ^{13}C NMR spectrum revealed eighteen sp^2 carbon signals except those of four carbonyl carbons, which were attributed to the anthraquinone moiety and a pentasubstituted benzene function (Table 2). The ^1H NMR spectrum of **1** showed two hydrogen bonded phenolic hydroxyls (δ 12.51 and 12.17) which correspond to 7-OH and 9-OH, and four aromatic protons at δ 9.08, 7.15, 6.58 and 6.38. The former three methines are assigned to 14-H, 12-H and 10-H, respectively, and the remaining one at δ 6.38 to the benzene function, by comparison with those of K-259-2. 14-H is observed in the low-field because it is close in space to the benzene ring. The ^1H NMR spectrum also show the presence of two adjacent methylene groups (δ *ca.* 2.7 and *ca.* 2.8) and one acetyl-methyl group (δ 2.14 (CH_3) and 4.01 (CH_2)).

The structure of **1** was further defined by long range selective proton decoupling (LSPD) experiments of tetramethyl derivative (**2**) prepared by methylation of **1** with diazomethane. Molecular formula of **2** was obtained to be $\text{C}_{30}\text{H}_{26}\text{O}_8$ by high-resolution mass spectroscopy (HR-MS). The ^1H and ^{13}C NMR data of **2** are presented in Tables 1 and 2, and the relevant results of the LSPD experiments are summarized in Fig. 1.

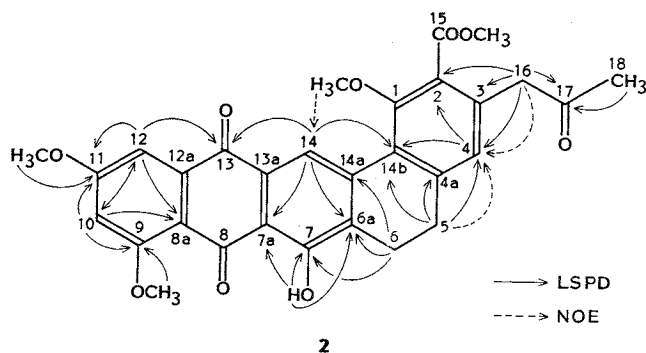
Anthraquinone moiety of **2** was evidenced by the following observations: The methine protons 12-H and 14-H are both coupled to quinone carbonyl C-13 ($^3J_{\text{C-H}}$), and the former correlates with the methine proton 10-H through *meta* coupling. 10-H is coupled ($^2J_{\text{C-H}}$) with C-9 and C-11 which show couplings ($^3J_{\text{C-H}}$) with methoxyl protons at δ 4.05 and 4.00, respectively, demonstrating the arrangement from C-9 to C-14. The hydrogen bonded phenolic hydroxyl proton signal at δ 13.60 is coupled to the carbons C-6a and C-7a which, respectively, are coupled to 14-H, indicating the presence of the phenolic hydroxyl group at C-7.

The observation of a long range coupling between the methylene protons at δ *ca.* 3.0 (6-H) and C-6a establishes the connection between C-6 and C-6a. The methylene signal at δ *ca.* 2.8 (5-H) show a long range coupling with C-14b, C-4 ($^3J_{\text{C-H}}$) and C-4a, and the methine proton at δ 6.95 (4-H) is coupled to C-14b, indicating the connection between C-5 and C-4a and between C-4a and C-4. The arrangement from C-4

Table 2. ^{13}C NMR data for **1** and **2** (100 MHz).

Carbon	1	2
1	164.7	156.6
2	116.5	128.3
3	140.5	134.1
4	120.1	126.2
4a	142.1	143.2
5	28.2	28.7
6	20.2	20.5
6a	131.4	134.1
7	157.8	159.3
7a	112.4	115.3
8	189.4	187.6
8a	109.0	115.4
9	164.4	163.1
10	107.7	104.7
11	165.6	165.4
12	108.7	104.0
12a	135.4	138.0
13	181.6	182.3
13a	130.0	130.7
14	119.9	118.4
14a	141.4	138.4
14b	118.3	125.3
15	172.3	168.4
16	49.9	48.3
17	204.9	205.0
18	29.8	29.7
1-OCH ₃		62.0
9-OCH ₃		56.7
11-OCH ₃		56.1
COOCH ₃		52.5

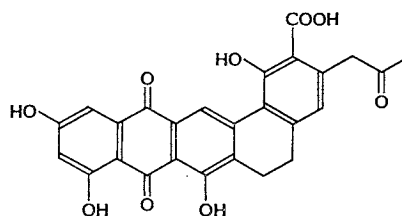
1 was measured in $\text{DMSO}-d_6$ and **2** was in CDCl_3 .

Fig. 1. LSPD and NOE experiments of **2** (400 MHz, CDCl₃).

through C-5 is also assisted by the observation of the nuclear Overhauser effect (NOE, 15%) from 5-H to 4-H. The long range coupling between 14-H and C-14b indicates the bondage between C-14a and C-14b, which explains the low-field chemical shift of 14-H (δ 8.77) as the anisotropic effect of benzene moiety. Acetyl-methyl group is confirmed by the observation of the long range coupling between 18-methyl protons and 17-carbonyl carbon and between 16-methylene protons and C-17. 16-H also couples with C-2, C-4 (${}^3J_{C-H}$) and C-3 (${}^2J_{C-H}$), and 16-H exhibits the NOE (18%) to 4-H, which reveals the bonding position of acetyl-methyl group at C-3.

The NOE from the methoxyl protons at δ 3.65 to 14-H (27%) indicates that the methoxyl group is attached to C-1. Therefore, the remaining methoxycarbonyl group is concluded to be attached to C-2.

From these findings, the structure of KS-619-1 was determined as Fig. 2.

Fig. 2. Structure of KS-619-1 (**1**).

Experimental

${}^1\text{H}$ and ${}^{13}\text{C}$ 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. Mass spectra were measured 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 F₂₅₄.

Methylation of **1**

To a solution of KS-619-1 (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 stood for 2 hours at room temp. The residue obtained after evaporation *in vacuo* was purified by preparative TLC developing with toluene - (CH₃)₂CO (5:1) to give a pale yellow powder of **2** (6 mg). EI-MS m/z 530 (M⁺), 488, 335, 285, 169; HREI-MS calcd for C₃₀H₂₆O₉: 530.1574, found: 530.1571; IR(CHCl₃) 3400, 1728, 1715, 1670, 1624, 1595 cm⁻¹; UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ) 230 (39,000), 302 (28,000), 317 (26,000), 470 (16,000).

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References

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