SAPURIMYCIN, NEW ANTITUMOR ANTIBIOTIC PRODUCED BY *STREPTOMYCES*

STRUCTURE DETERMINATION

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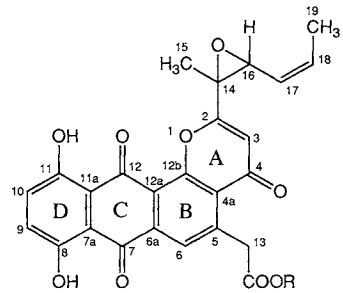
The structure of a new anthra-γ-pyrone antitumor antibiotic sapurimycin was determined by the spectral studies of its methyl ester. Sapurimycin has the same anthra-γ-pyrone skeleton as pluramycin, but is distinctly different because of the absence of sugars on the D ring and possessing a carboxylmethyl group on C-5.

Sapurimycin (**1**, Fig. 1) was isolated from the fermentation broth of *Streptomyces* sp. DO-116[1]. It was active against Gram-positive bacteria *in vitro* and effective against leukemia P388 and sarcoma 180 in mice. The producing organism, fermentation, isolation and biological properties of **1** were reported in the preceding paper[1]. In this paper, we wish to describe the structure determination of **1**.

The physico-chemical properties of **1** are summarized in Table 1. **1** was obtained as a red powder, which was soluble in EtOAc and slightly soluble in chloroform and MeOH. **1** was very unstable in DMSO and readily decomposed in it. The molecular formula was determined as $C_{25}H_{18}O_9$ based on the HRFAB-MS. The SI-MS spectrum showed pseudo molecular ion peaks $(M+1)^+$, $(M+2)^+$, $(M+3)^+$, characteristic of quinone compounds[2]. The UV spectrum suggested that **1** contains a 1,4-dihydroxy-naphthoquinone nucleus as the chromophore[3,4]. The IR spectrum showed characteristic absorptions attributable to hydroxyl (3430 cm^{-1}), carboxyl (3700~2400 and 1734 cm^{-1}), and quinone carbonyl (1650~1615 cm^{-1}) groups, respectively.

The NMR study of **1** was difficult because of its insufficient solubility in conventional solvents, and instability in DMSO, as mentioned above. After investigation of derivatization of **1**, the methyl ester (**2**) was selected as the stable and easily soluble derivative, which was used for NMR examination described below.

Fig. 1. Structures of sapurimycin (**1**) and its methyl ester (**2**).



1 R=H
2 R=CH_3

Structure of a Side Chain

Analysis of ^1H-^1H couplings in ^1H NMR of **2** (Table 3) revealed the $ABMX_3$ spin system composed of 19-H_3 (1.84 ppm), 18-H (5.85 ppm), 17-H (5.15 ppm) and 16-H (3.92 ppm). The coupling constant (J=11.2 Hz) between the two olefinic protons (17-H and 18-H) together with the observed NOE (Fig. 3) between them implied Z-configuration of the double bond. The bonds from C-16 through

Table 1. Physico-chemical properties of sapurimycin (**1**) and its methyl ester (**2**).

	Sapurimycin (**1**)	Methyl ester (**2**)
Appearance	Red powder	Red powder
MP (°C)	145~149	
$[\alpha]_D^{24}$	+270° (*c* 0.012, $CHCl_3$)	+370° (*c* 0.011, $CHCl_3$)
Molecular formula	$C_{25}H_{18}O_9$	$C_{26}H_{20}O_9$
HR-MS (*m/z*)	FAB-MS:	EI-MS:
Obsd:	463.1063 $(M+1)^+$	476.1070 (M^+)
Calcd:	463.1029 for $C_{25}H_{19}O_9$	476.1105 for $C_{26}H_{20}O_9$
MS (*m/z*)	SI-MS:	EI-MS:
	465 $(M+3)^+$, 464 $(M+2)^+$, 463 $(M+1)^+$	476 (M^+), 458, 445
UV λ_{max}^{MeOH} nm (ε)		
Neutral	242 (63,000), 264 (sh, 29,000), 380 (5,700), 506 (11,000)	242 (47,000), 262 (sh, 23,000), 362 (5,000), 511 (7,000)
Alkaline	247 (56,000), 348 (6,800), 600 (15,000)	
IR (KBr) cm^{-1}	3700~2400, 3430, 1755, 1734, 1654, 1629, 1585, 1454, 1224	3438, 1739, 1658, 1627, 1585, 1454, 1375, 1309, 1227, 1171
Solubility		
Soluble:	EtOAc, $CHCl_3$-MeOH	EtOAc, $CHCl_3$, MeOH, CH_3CN
Slightly soluble:	$CHCl_3$, MeOH, CH_3CN	
Insoluble:	H_2O, *n*-hexane	H_2O, *n*-hexane
TLC (Rf)	0.32[a], 0.58[b]	0.89[a], 0.37[b]

[a] Merck, Kieselgel 60 F_{254}: $CHCl_3$-MeOH-AcOH (200:10:1).
[b] Merck, HPTLC RP-18F_{254}: CH_3CN-H_2O (70:30).

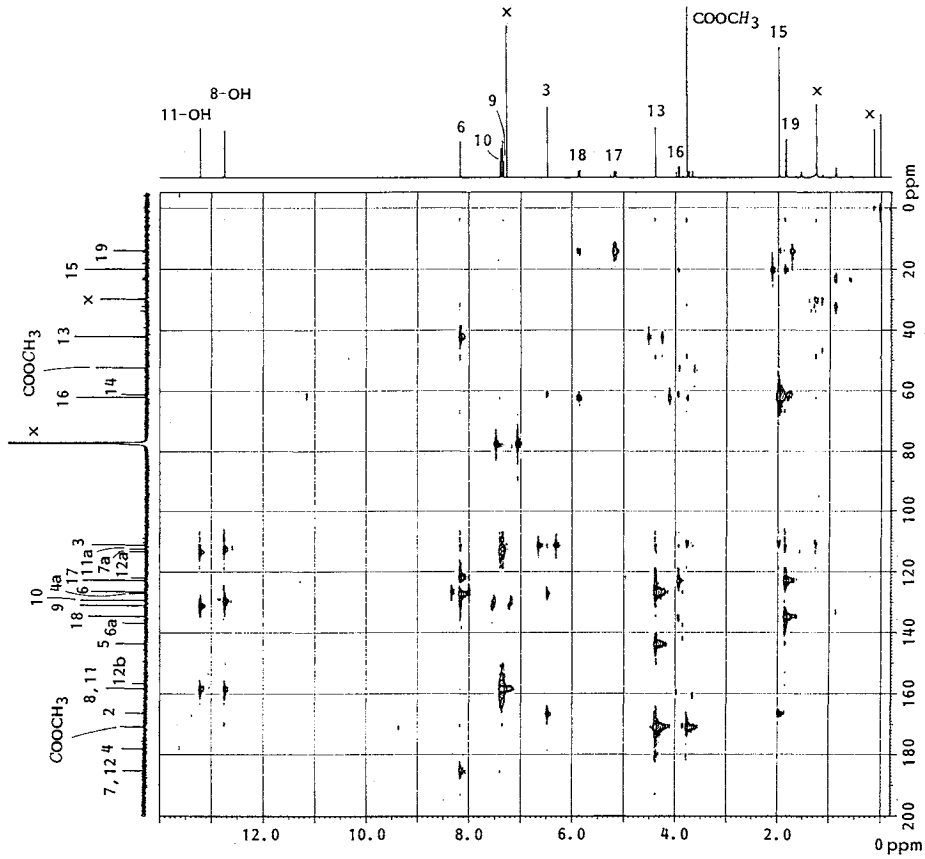
Table 2. ^{13}C NMR data of sapurimycin (**1**) and its methyl ester (**2**) (125 MHz).

Carbon No.	Chemical shift (multiplicity)[a]		Carbon No.	Chemical shift (multiplicity)[a]	
	Sapurimycin (**1**) ($CDCl_3+CD_3OD$)	Methyl ester (**2**) ($CDCl_3$)		Sapurimycin (**1**) ($CDCl_3+CD_3OD$)	Methyl ester (**2**) ($CDCl_3$)
C-2	166.7 (s)	166.1 (s)	C-12	185.28 (s)[b]	185.09 (s)[f]
C-3	110.9 (d)	110.0 (d)	C-12a	121.8 (s)	121.7 (s)
C-4	178.8 (s)	178.0 (d)	C-12b	156.7 (s)	156.5 (s)
C-4a	126.9 (s)	126.9 (s)	C-13	42.1 (t)	41.9 (t)
C-5	144.1 (s)	143.5 (s)	C-14	61.3 (s)	61.0 (s)
C-6	126.5 (d)	126.3 (d)	C-15	19.9 (q)	19.9 (q)
C-6a	137.0 (s)	136.7 (s)	C-16	62.2 (d)	62.0 (d)
C-7	185.30 (s)[b]	185.12 (s)[f]	C-17	122.6 (d)	122.6 (d)
C-7a	113.4 (s)[c]	113.2 (s)[g]	C-18	134.4 (d)	134.4 (d)
C-8	157.89 (s)[d]	158.20 (s)[h]	C-19	13.8 (q)	13.8 (q)
C-9	130.8 (d)[e]	130.8 (d)[i]	13-COOH	172.5 (s)	
C-10	129.1 (d)[e]	129.1 (d)[i]	13-$COOCH_3$		170.7 (s)
C-11	157.88 (s)[d]	158.20 (s)[h]	13-$COOCH_3$		52.2 (q)
C-11a	112.5 (s)[c]	112.3 (s)[g]			

[a] Chemical shifts in ppm from TMS.
[b~i] Assignments may be interchanged.

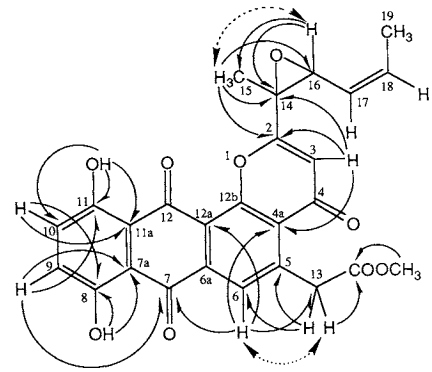
15-methyl group were demonstrated by the long range couplings between 16-H and C-14 and C-15, and 15-H_3 (1.97 ppm) and C-14 and C-16 in the heteronuclear multiple-bond correlation (HMBC) spectrum (Fig. 2). C-14 and C-16 exhibited the chemical shifts (61.0 and 62.0 ppm, respectively) characteristic of carbons in the epoxide group. A large C-H coupling constant ($J=174.9$ Hz) at 16 also suggested the existence of 14,16-epoxide. The observation of NOE between 15-H and 16-H showed *cis* configuration of the epoxide. Thus the side chain structure consisting of a 2,3-epoxy-hexa-4-en-

Fig. 2. HMBC spectrum of methyl ester (**2**) ($CDCl_3$, 500 MHz).



2-yl group was confirmed.

Fig. 3. HMBC and NOE data of methyl ester (**2**).
⟶ HMBC, ⟷ NOE.



Structure of B, C, D Rings

Diagnostic results of the HMBC experiment of **2** are shown in Fig. 3. A ^1H-^{13}C long range coupling network, starting from the hydrogen bonded phenolic proton (8-OH δ 12.73) through the *O*-coupled aromatic protons (9-H δ 7.33 and 10-H δ 7.38) to another hydrogen bonded phenolic proton (11-OH δ 13.21), was observed, confirming the ring D structure.

Another network was observed from the methoxy (δ 3.76) carbonyl through the isolated benzylic methylene (13-H_2 δ 4.38) to the isolated aromatic proton (6-H δ 8.15), which revealed the ring B structure having a methoxycarbonylmethyl group at 5. The long range couplings between the 7-quinone carbonyl (δ 185.12, tentatively assigned) and the 9-H(^4J),6-H(^3J) were observed, establishing that the B-C-D ring is a dihydroxynaphthoquinone system. The quaternary carbons at δ 136.7 and 156.5

Table 3. ^{1}H NMR data of sapurimycin (**1**) and its methyl ester (**2**) (500 MHz).

Proton No.	Chemical shift (multiplicity, J in Hz)[a]	
	Sapurimycin (**1**) ($CDCl_3+CD_3OD$)	Methyl ester (**2**) ($CDCl_3$)
3-H	6.47 (s)	6.47 (s)
6-H	8.18 (s)	8.15 (s)
9-H	7.34 (d, 9.3)[b]	7.33 (d, 9.4)[c]
10-H	7.39 (d, 9.3)[b]	7.38 (d, 9.4)[c]
13-H_2	4.40 (d, 16.6), 4.36 (d, 16.6)	4.38 (s)
15-H_3	1.99 (s)	1.97 (s)
16-H	3.96 (d, 8.5)	3.92 (dd, 8.4, 1.0)
17-H	5.15 (ddq, 11.2, 8.5, 1.8)	5.15 (ddq, 11.2, 8.4, 1.8)
18-H	5.86 (ddq, 11.2, 1.0, 7.1)	5.85 (ddq, 11.2, 1.0, 7.1)
19-H_3	1.85 (dd, 7.1, 1.8)	1.84 (dd, 7.1, 1.8)
8-OH		12.73 (s)[d]
11-OH		13.21 (s)[d]
13-COOH_3		3.76 (s)

[a] Chemical shifts in ppm from TMS.
[b~d] Assignments may be interchanged.

were assigned to C-6a and C-12b based on their chemical shifts, although no crosspeak related to these carbons was observed in the HMBC spectrum.

Structure of A Ring

The olefinic carbon (C-2 δ 166.1) was shown to be connected to both C-3 (δ 110.0) and C-14, based on the long range couplings with 3-H (δ 6.47) and 15-H. The chemical shift of C-2 was surprisingly low which suggested not only that oxygen was attached to C-2, but also the existence of an electron withdrawing group such as a carbonyl group at C-3. Up to then, only one carbonyl group (C-4 δ 178.0) was left unassingned which was appropriate to bond to C-3. Consideration of its molecular formula, C-2 and C-12b must be attached to the same oxygen. Bondage between C-3 and C-4a through 4-carbonyl was confirmed by the observation of the long range coupling between 3-H and C-4a. Thus the structure of A ring and arrangement of the side chain moiety and rings B, C and D were constructed, and whole structure of **2** was determined to be shown in Fig. 1.

Conclusion

The structure of **2** was determined and, consequently, the structure of sapurimycin (**1**) was determined as shown in Fig. 1. ^{1}H NMR signals of 13-H_2 in **1** were observed as the pair of doublets (δ 4.40 and 4.36) coupled with each other (J=16.6 Hz) as compared with the singlet (δ 4.38) in **2**. This indicates a hydrogen bond between 13-carboxyl group and carbonyl group at C-4, which inhibits free rotation of the C-5–C-13 bond.

1 possesses the same anthra-γ-pyrone skeleton as the pluramycin antibiotics[5~7], but is distinctly different becasue of the lack of *C*-glycosides on the D ring, and the presence of a carboxyl group on C-13. Both characteristics are also possessed by the kapurimycins[8,9] and SS43405D[10], but **1** is clearly different from these compounds.

The 13-carboxyl group is a β,γ-unsaturated-δ-ketocarboxylic acid moiety and it is thought to be labile to give decarboxylate especially in polar solvent such as DMSO, thus explaining the instability of **1** in DMSO. Similar decarboxylation reaction was observed in a DMSO solution of kapurimycins[9,11].

Experimental

General

NMR spectra were recorded on Bruker AM500 and AM400 spectrometers with TMS (δ 0 ppm) as an internal standard. HRFAB-MS spectrum was obtained on a Jeol JMS-SX102 spectrometer. SI-MS, EI-MS and HREI-MS spectra were obtained on a Hitachi M-80B spectrometer. IR spectra were measured with Jasco IR-810 and Jeol JIR-RFX3001 spectrometers. UV spectra were taken with a Hitachi 200-20 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. MP was taken with a Yanagimoto melting point apparatus and uncorrected. TLC was performed on precoated plates, Merck Kieselgel 60 F_{254} and HPTLC RP-18F_{254}.

Synthesis of Methyl Ester (2) of Sapurimycin (1)

To a solution of **1** (3.2 mg) in 0.2 ml $CHCl_3$-MeOH (5:1), an ether solution of diazomethane was added and stirred for 5 minutes at room temperature. The reaction mixture was evaporated *in vacuo*. The residue was chromatographed on silica gel (Wakogel C-200) column eluted by $CHCl_3$-MeOH (100:0.2) to give the methyl ester **2** (1.1 mg) in 33% yield.

The physico-chemical and NMR data are summarized in Tables 1, 2 and 3.

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