THE KAPURIMYCINS, NEW ANTITUMOR ANTIBIOTICS PRODUCED BY *STREPTOMYCES*

PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE DETERMINATION

MAYUMI YOSHIDA, MITSUNOBU HARA, YUTAKA SAITOH and HIROSHI SANO

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

(Received for publication May 18, 1990)

The kapurimycins A1, A2 and A3 were revealed to be new antitumor antibiotics with molecular formula of $C_{27}H_{26}O_9$, $C_{26}H_{24}O_9$ and $C_{27}H_{24}O_9$, respectively. The structures of the kapurimycins were determined by NMR spectroscopic analysis. The kapurimycins are new class of polycyclic microbial metabolites having the tetrahydroanthra-γ-pyrone skeleton and the β,γ-unsaturated δ-keto carboxylic acid structure. The individual components of the kapurimycins differ from one another in the side chain at the pyrone ring of the molecule.

The new antitumor antibiotics, the kapurimycins, have been found in the culture of *Streptomyces* sp. DO-115.[1] The active complexes were separated into two minor and one major components, named A1, A2 and A3. The kapurimycins were active against bacteria, particularly Gram-positive organisms, and were cytotoxic toward cultured mammalian cells. Among the individual components of the kapurimycins, kapurimycin A3 exhibited strongest antibacterial and cytotoxic activities and showed a potent antitumor activity against murine leukemia P388 *in vivo*. The details of the producing organism, fermentation, isolation and biological activities of the kapurimycins are reported in the previous paper.[1] This paper describes physico-chemical properties and structure determination of these new antitumor antibiotics.

Results

Physico-chemical Properties

The physico-chemical properties of the kapurimycins are summarized in Table 1. Kapurimycins are yellow compounds which are soluble in MeOH, chloroform, EtOAc, slightly soluble in EtOH, hardly soluble or insoluble in acetone, *n*-hexane and water. The compounds are readily decomposed in DMSO. The antibiotics showed positive color reactions with ferric chloride, vanillin, bromcresol green and iodine vapor but negative with 2,4-dinitrophenylhydrazine, ninhydrin and Dragendorff reagent. The individual components of kapurimycins have nearly identical UV absorption maxima, indicating that their chromophore would be the same or very closely related to each other. Diagnostic IR absorption bands can be seen at 3460~3420, 1740~1720 and 1650~1615 cm^{-1}, suggesting the presence of hydroxyl and carbonyl functions in the molecule.

EI-MS of kapurimycin A1 gave parent ion at *m/z* 494 (M^+) and HREI-MS gave $(M-CO_2)^+$ at *m/z* 450.1659 ($C_{26}H_{26}O_7$ calcd 450.1642), indicating a molecular formula of $C_{27}H_{26}O_9$ for kapurimycin A1. Kapurimycin A2 gave a parent mass at 480 (M^+) and $(M-CO_2)^+$ at *m/z* 436.1557 ($C_{25}H_{24}O_7$ calcd 436.1521), indicating a molecular formula of $C_{26}H_{24}O_9$ for kapurimycin A2. The parent mass of

Table 1. Physico-chemical properties of kapurimycins.

	A1	A2	A3
Appearance	Yellow powder	Yellow powder	Yellow powder
$[\alpha]_D^{25}$ (*c* 0.1, MeOH)	−217°	−237°	−349°
UV λ_{max}^{MeOH} (nm)	216, 262, 375, 390	216, 265, 375, 392	216, 262, 375, 390
IR (KBr) cm^{-1}	3440, 2960, 1740, 1650, 1618, 1425, 1365	3420, 3250, 2970, 1720, 1650, 1615, 1420, 1360	3460, 2950, 1742, 1650, 1620, 1426, 1370
Molecular formula	$C_{27}H_{26}O_9$	$C_{26}H_{24}O_9$	$C_{27}H_{24}O_9$
SI-MS (*m/z*) $(M+H)^+$	495	481	493
EI-MS (*m/z*) (M^+)	494	480	492
HREI-MS (*m/z*) $(M-CO_2)^+$			
Observed	450.1659	436.1557	448.1526
Calcd	450.1642	436.1521	448.1520
TLC[a] (Rf)			
Solvent A	0.48	0.48	0.40
Solvent B	0.20	0.16	0.10

[a] Silica gel sheet (Merck Art. No. 5715).
Solvent A: $CHCl_3$ - MeOH - AcOH (20 : 1 : 0.1).
Solvent B: Toluene - acetone (7 : 3).

Table 2. ^1H NMR chemical shift assignments and coupling data of kapurimycins A1, A2 and A3 in MeOH-d_4.

Proton	A1	A2	A3
3	6.32[a] (1H, s)[b]	6.34 (1H, s)	6.38 (1H, s)
6	7.51 (1H, s)	7.54 (1H, s)	7.55 (1H, s)
7	7.33 (1H, s)	7.36 (1H, s)	7.37 (1H, s)
8	6.15 (1H, dd, 7.0, 3.6)	6.18 (1H, dd, 6.8, 3.6)	6.19 (1H, dd, 6.8, 3.5)
9	2.30 (1H, m)	2.33 (1H, m)	2.33 (1H, m)
	2.40 (1H, m)	2.40 (1H, m)	2.40 (1H, m)
10	2.82 (1H, ddd, 18.1, 7.4, 5.0)	2.84 (1H, ddd, 18.1, 7.2, 5.0)	2.88 (1H, ddd, 18.1, 7.2, 5.0)
	3.04 (1H, ddd, 18.1, 8.5, 5.0)	3.04 (1H, ddd, 18.1, 8.7, 5.0)	3.05 (1H, ddd, 18.1, 7.5, 5.0)
13	4.17 (1H, d, 16.7)	4.19 (1H, d, 16.7)	4.21 (1H, d, 16.7)
	4.26 (1H, d, 16.7)	4.27 (1H, d, 16.7)	4.26 (1H, d, 16.7)
15	1.84 (3H, s)	1.84 (3H, s)	1.91 (3H, s)
16	3.24 (1H, t, 6.2)	3.23 (1H, t, 6.5)	4.01 (1H, d, 8.3)
17	1.50 (2H, m)	1.46 (1H, dq, 6.5, 7.5)	5.15 (1H, ddq, 11.1, 8.3, 1.7)
		1.55 (1H, dq, 6.5, 7.5)	
18	1.50 (2H, m)	0.98 (3H, t, 7.5)	5.82 (1H, dqd, 11.1, 7.1, 0.9)
19	0.86 (3H, t, 7.0)	—	1.82 (3H, dd, 7.1, 1.7)
8-OCOCH_3	2.17 (3H, s)	2.17 (3H, s)	2.15 (3H, s)

[a] Measured at 400 MHz; chemical shifts in ppm from TMS.
[b] Multiplicity, coupling constants (*J* = Hz) and peak area.

kapurimycin A3 was 492 (M^+) and $(M-CO_2)^+$ was 448.1526 ($C_{26}H_{24}O_7$ calcd 448.1520), indicating a molecular formula of $C_{27}H_{24}O_9$ for kapurimycin A3. These molecular formulae set the kapurimycins apart from any known antitumor antibiotics.

Structure Determination

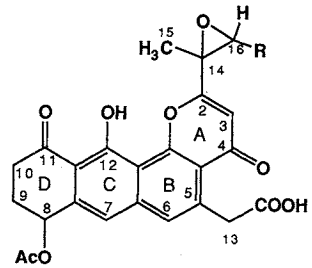
The structures of the kapurimycins were determined by ^1H and ^{13}C NMR studies including 2D NMR experiments. Assignment of the protons to the relevant carbons was made by the HETCOR experiments and the quaternary carbons were located by the long range HETCOR (COLOC) experiments. The ^1H and ^{13}C NMR data are shown in Tables 2 and 3.

Table 3. ^{13}C NMR chemical shift assignments for kapurimycins A1, A2 and A3 in MeOH-d_4.

Carbon	A1	A2	A3	Carbon	A1	A2	A3
2	166.8[a] s[b]	166.9 s	166.6 s	12	165.5 s	165.6 s	165.5 s
3	111.9 d	111.9 d	111.5 d	12a	114.6 s	114.7 s	114.7 s
4	180.4 s	180.5 s	181.0 s	12b	158.8 s	158.9 s	158.9 s
4a	121.0 s	121.0 s	120.9 s	13	42.8 t	42.9 t	42.7 t
5	138.4 s	138.5 s	138.2 s	14	60.9 s	61.1 s	62.2 s
6	129.6 d	129.7 d	129.7 d	15	20.2 q	20.2 q	19.8 q
6a	141.9 s	141.9 s	141.9 s	16	67.6 d	68.7 d	62.9 d
7	118.1 d	118.2 d	118.1 d	17	30.7 t	22.3 t	124.1 d
7a	141.9 s	141.9 s	141.9 s	18	20.3 t	10.9 q	134.8 d
8	70.5 d	70.6 d	70.7 d	19	14.0 q	—	13.7 q
9	28.7 t	28.7 t	28.7 t	8-OCOCH$_3$	171.9 s	172.1 s	172.0 s
10	35.3 t	35.3 t	35.2 t	8-OCOCH$_3$	21.1 q	21.1 q	21.1 q
11	205.6 s	205.7 s	205.7 s	13-COOH	175.6 s	175.8 s	175.5 s
11a	113.0 s	113.0 s	113.0 s				

[a] Measured at 100 MHz; chemical shifts in ppm from TMS.
[b] Multiplicity was determined by DEPT data.

Fig. 1. Structure of the kapurimycins.



Kapurimycin A1 R=$-\overset{17}{C}H_2\overset{18}{C}H_2\overset{19}{C}H_3$
Kapurimycin A2 R=$-CH_2CH_3$
Kapurimycin A3 R=$-CH{=}CHCH_3$

Structure of Kapurimycin A3

Structure of Side Chain: The ^{13}C chemical shifts of C-14 (δ 62.2) and C-16 (δ 62.9) and the coupling constant (J_{CH} = 174 Hz) between C-16 and 16-H were characteristic for those of the epoxide. The hetero long range couplings between these carbons and 15-H$_3$ (δ_H 1.91) indicated that C-15 is linked at C-14 of the epoxide. ^1H NOESY experiments, which showed a cross peak between 15-H$_3$ and 16-H, suggested that 15-CH$_3$ and 16-H of the epoxide was in the *cis*-orientation. COSY experiments led to the propenyl (17-H δ 5.15, 18-H δ 5.82 and 19-H$_3$ δ 1.82) group being placed on C-16 for the kapurimycin A3. The two olefinic protons (17-H and 18-H) were shown to be in the *Z*-configuration based on the coupling constant (J = 11.1 Hz) together with the observation of NOE between them.

Structure of A Ring

In the ^1H NMR spectrum, a singlet olefinic proton was seen at δ 6.38 (3-H), which showed long range coupling to C-2 (δ 166.6) and C-4a (δ 120.9). These ^1H and ^{13}C chemical shifts, indicative of an attachment of an electron withdrawing group at C-3 position, are typical of those exhibited by γ-pyrone. The presence of γ-pyrone was also supported by the IR absorption bands at 1650~1615 cm^{-1} and was confirmed by comparison with published NMR data for pluramycin-type antibiotics.[2~5] These considerations enabled assignment of C-4 (δ 181.0) and C-12b (δ 158.9). Long range coupling between the quaternary carbon (C-14) of the epoxide and 3-H along with that between 15-H$_3$ and C-2 indicated the attachment point of the epoxide to be C-2 of the γ-pyrone (A) ring.

Structure of B, C, and D Rings

Remaining protons were a methyl group, three methylenes, and three methines. An AB quartet was

Fig. 2. Long range HETCOR data summary for kapurimycin A3.

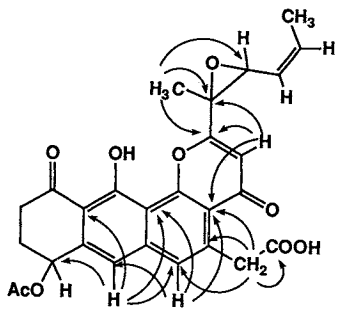
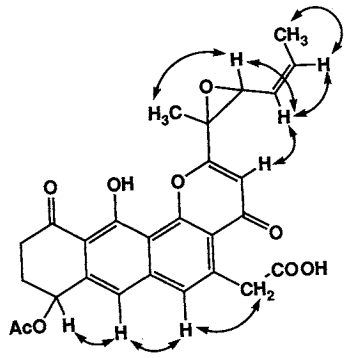


Fig. 3. NOE data summary for kapurimycin A3.



observed for 13-H_2 at δ 4.21, 4.26, which showed long range coupling to C-4a, C-5 (δ 138.2), C-6 (δ 129.7) and carboxylate carbon (δ 175.5). Thus, the carboxymethyl group was shown to attach at the C-5 position. COSY and HETCOR experiments identified 8-H (δ 6.19)–9-H_2 (δ 2.33 and 2.40)–10-H_2 (δ 2.88 and 3.05) spin system associated with C-8 (δ 70.7), C-9 (δ 28.7), C-10 (δ 35.2). Long range coupling between 10-H_2 and C-11 (δ 205.7) indicated the connectivity of 10-H_2 and C-11, and long range coupling between 7-H and C-8 confirmed the connectivity of this C_4 chain on C-7a. 8-H and methyl proton (δ 2.15) also exhibited long range coupling to the ester carbonyl (δ 172.0), which suggested the presence of acetoxy group at C-8. NOE cross peaks in NOESY experiments revealed the NOE network from 13-H_2 through 6-H, 7-H to 8-H, as shown in Fig. 3. The hetero long range coupling for 6-H and 7-H (Fig. 2) confirmed the B, C ring system. Remaining unsaturation is one and C-11 and C-11a should be connected to construct ring A. Thereby the remaining carbon was assigned as a C-12 phenolic carbon.

From all of above results, the structure of kapurimycin A3 was determined as shown in Fig. 1.

Structure of Kapurimycins A1 and A2

Molecular formulae of kapurimycins are listed in Table 1. Their difference are H_2 in A1 and A3, and CH_2 in A1 and A2, suggesting the difference in double bond and methylene.

Detailed NMR experiments including 2D NMR experiments were carried out on A1 and A2, in the similar manner as described above in A3. ^1H and ^{13}C NMR spectra of A1 and A2 were extremely similar to those of kapurimycin A3 except side chain moiety, where A1 was *n*-propyl (17-H_2 δ 1.50, 18-H_2 δ 1.50 and 19-H_3 δ 0.86) group, and A2 was ethyl (17-H_2 δ 1.46 and 1.55, and 18-H_3 δ 0.98) group. Thus, individual components of the kapurimycins differ from one another only in the substituents attached to C-16 position, as shown in Fig. 1.

Discussion

The structure of the novel antitumor antibiotics kapurimycins A1 through A3 were determined based on analysis of the spectroscopic data presented in this paper. The individual components of the kapurimycins contain the tetrahydroanthrapyrone structural portion in common but they are differentiated from each other in side chain at the pyrone ring. There are pluramycin-group antitumor antibiotics[2~5] that have an anthropyrone, but they are different from the kapurimycins in having amino sugars and quinone moiety. In addition, the kapurimycins contain the carboxylic acid at C-13 position. SS43405D[6] is an anthra-γ-pyrone antibiotic with carboxylic acid at C-13 but differs from the kapurimycins in having the quinone group at

C ring of the chromophore. Thus, the kapurimycins are new class of antitumor antibiotics which have not previously been reported.

The kapurimycins show a common loss of 44 amu in EI-MS with the resultant fragment ion indicating the loss of carbon dioxide from the carboxylic acid groups. The 13-carboxyl group composes a β,γ-unsaturated-δ-keto carboxylic acid moiety with 4-keto and 4a,5-double bond. It therefore follows that the mechanism of decarboxylation might be the analogous one to that for decarboxylation of β-keto carboxylic acid (malonic acid). That is a 8-membered cyclic process of elimination, in which hydrogen bonding plays an important role. It is not clear, however, how the β,γ-unsaturated δ-keto carboxylic acid moiety of the kapurimycins is involved in biological activities of these compounds. Studies on this point are in progress in our laboratory and will be published elsewhere.[7]

Experimental

NMR spectra were recorded on a Bruker AM400 spectrometer. Mass spectra were obtained on a Hitachi M-80B spectrometer. IR spectra were recorded on a Shimadzu IR-27G spectrometer. UV spectra were run on a Hitachi 200-20 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. TLC was performed on precoated plates, Merck Kieselgel 60 F_{254} and detected with UV light (254 nm).

Acknowledgment

We are grateful to Messrs. S. KAKITA and A. NAKAMURA for assistance with NMR experiments, Mrs. Y. YASUZAWA and Miss C. SIMAZAKI for assistance with MS measurements.

References

1) HARA, M.; T. MOKUDAI, E. KOBAYASHI, K. GOMI & H. NAKANO: The kapurimycins, new antitumor antibiotics produced by *Streptomyces*. Producing organism, fermentation, isolation and biological activities. J. Antibiotics 43: 1513~1518, 1990
2) MAEDA, K.; T. TAKEUCHI, K. NITTA, K. YAGISHITA, R. UTAHARA, T. ŌSATO M. UEDA, S. KONDŌ, Y. OKAMI & H. UMEZAWA: A new antitumor substance pluramycin. Studies on antitumor substances produced by actinomycetes. XI. J. Antibiotics, Ser. A 11: 75~81, 1956
3) KONDO, S.; T. WAKASHIRO, M. HAMADA, K. MAEDA, T. TAKEUCHI & H. UMEZAWA: Isolation and characterization of a new antibiotic, neopluramycin. J. Antibiotics 23: 354~359, 1970
4) FURUKAWA, M.; I. HAYAKAWA & G. OHTA: Structure and chemistry of kidamycin. Tetrahedron 31: 2989~2995, 1975
5) SÉQUIN, U.: The structure of the antibiotic hedamycin-II. Comparison of hedamycin and kidamycin. Tetrahedron 34: 761~767, 1978
6) MATSUMOTO, M.; K. NAGAOKA, T. ASAOKA, K. YOKOI & T. NAKAJIMA (S.S. Pharm.): Antibiotic SS43405D. Jpn. Kokai 139394 ('86), June 26, 1986 [CA 105: 224599b, 1986]
7) HARA, M.; M. YOSHIDA & H. NAKANO: Covalent modification and single strand scission of DNA by a new antitumor antibiotic kapurimycin A3. Biochemistry, in press