STRUCTURE OF RK-397, A NEW OXO PENTAENE ANTIBIOTIC

HIROYUKI KOSHINO, KIMIE KOBINATA, KIYOSHI ISONO† and HIROYUKI OSADA

The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan
†Department of Marine Science, Tokai University, Orido, Shimizu, Shizuoka 424, Japan

(Received for publication June 25, 1993)

RK-397 is a new antibiotic produced by *Streptomyces* sp. 87-397. The antibiotic showed antitumor, antifungal and antibacterial activities. The studies of taxonomy, fermentation, isolation, physico-chemical properties and biological activities are described in the preceding paper[1]. In this paper, we report the structural determination of RK-397.

RK-397 is a yellow powder with mp 157~163°C (dec) and has the molecular formula $C_{35}H_{56}O_{10}$ which was determined by HRFAB-MS (*m/z* 637.3930, MH^+). The UV spectrum exhibited characteristic absorption of the oxo pentaene chromophore, λ_{max} (in MeOH) 360 nm, and suggested that RK-397 belongs the oxo polyene macrolide group of antibiotics, such as mycoticins[2], roxaticin[3], roflamycoin[4] and dermostatins[5]. The ^1H NMR spectrum (Table 1) showed the ten olefinic protons of the oxo pentaene moiety, as well as two protons of an isolated olefin. The ^{13}C NMR and DEPT spectrum (Table 1) exhibited the signals of one carbonyl carbon, three methyl carbons, eight methylene carbons and twenty-three methine carbons which consist of twelve olefinic, nine oxygenated and two ordinary methine carbons. Two partial structures, C-1~C-12 and C-27~C-35, were assigned by ^1H-^1H COSY and ^{13}C-^1H COSY. The stereochemistry of the six double bonds was determined as all *E* from the large coupling constants (J=14.9 Hz for the pentaene moiety and J=15.7 Hz for H-28 and H-29). The remaining portion (C-13~C-26) contains seven methylenes (45~48 ppm) and seven oxygenated methines (65~69 ppm). These ^{13}C chemical shifts suggested that the presence of a skipped polyol for the C-13~C-26 portion[6,7]. Careful analyses of ^1H-^1H HOHAHA and HMBC[8] spectra in addition to ^1H-^1H COSY allowed full assignment of ^1H and ^{13}C NMR signals. Further, the 2D ^{13}C-^1H HOHAHA spectrum was measured and this data supported the assignment of the crowded proton signals for the skipped polyol portion (Table 2). In the HMBC spectrum summarized in Fig. 1, ^1H-^{13}C long range couplings from H-2, H-3 and H-31 to the carbonyl carbon (C-1 at 169.07 ppm) were observed. The chemical shift of the methine proton H-31 (4.78 ppm) indicated that this methine is acyloxy methine. Consequently, the planar structure of RK-397 (**1**) was determined to be the 32-membered macrolide depicted in Fig. 2.

Table 1. ^{13}C (150 MHz) and ^1H NMR (600 MHz) data for RK-397 (**1**)[a,b].

No.	C	H[c]
1	169.07	—
2	121.40	5.89 (d, 14.9)
3	146.84	7.30 (dd, 14.9, 11.7)
4	131.07	6.44 (dd, 14.9, 11.7)
5	142.84	6.70 (dd, 14.9, 11.3)
6	132.86	6.37 (dd, 14.9, 11.3)
7	139.30	6.51 (dd, 14.9, 10.9)
8	132.38	6.29 (dd, 14.9, 10.9)
9	137.12	6.40 (dd, 14.9, 10.9)
10	133.79	6.22 (dd, 14.9, 10.1)
11	134.19	5.85 (ddd, 14.9, 10.1, 5.3)
12	43.95	2.63 (m), 2.24 (ddd, 13.7, 10.1, 10.0)
13	69.35	3.96 (m)
14	46.92	1.68~1.59 (m)
15	67.53	3.82 (m)
16	48.03	1.70 (ddd, 13.7, 8.2, 4.8), 1.38 (ddd, 13.7, 8.9, 4.4)
17	66.36	3.89 (m)
18	45.34	1.46 (ddd, 14.1, 9.6, 3.2), 1.20 (ddd, 14.1, 10.1, 2.4)
19	65.09	4.07 (m)
20	47.37	1.33 (m), 1.27 (m)
21	68.98	3.94 (m)
22	46.29	1.33 (m), 1.25 (m)
23	68.55	4.04 (m)
24	46.37	1.44 (ddd, 14.1, 10.1, 8.4), 1.33 (m)
25	67.75	3.91 (m)
26	46.78	1.50 (ddd, 14.1, 10.1, 8.4), 1.31 (m)
27	71.56	4.22 (m)
28	133.57	5.53 (ddd, 15.7, 4.8, 1.6)
29	132.35	5.64 (ddd, 15.7, 5.2, 1.2)
30	37.48	2.64 (m)
31	81.93	4.78 (dd, 10.0, 2.6)
32	30.67	1.92 (dqq, 10.0, 6.9, 6.8)
33	19.15	0.98 (d, 6.9)
34	11.43	1.06 (d, 6.9)
35	20.24	0.88 (d, 6.8)

[a] Chemical shifts are given in ppm from TMS as internal standard.
[b] CD_3OD as solvent.
[c] Multiplicity and coupling constants (J in Hz) are given in parentheses.

Table 2. Cross peaks observed in the 2D ^{13}C-^{1}H HOHAHA experiment for RK-397 (**1**)[a,b].

C	H	C	H
C-4	3-H, 5-H	C-23	22-H, 24-H
C-5	6-H	C-27	26-H
C-7	6-H	C-28	29-H
C-9	10-H	C-30	34-H
C-10	9-H, 11-H	C-31	32-H, 33-H, 35-H
C-12	13-H	C-32	30-H, 31-H, 33-H, 35-H
C-14	15-H		
C-15	14-H, 16-H	C-33	35-H
C-17	16-H, 18-H	C-34	30-H
C-19	18-H, 20-H	C-35	33-H

[a] The ^{13}C-^{1}H HOHAHA spectrum was recorded in the absorptive mode, using mixing time 60 mseconds, by 600 MHz spectroscopy.
[b] Cross peaks of direct coupling ($^{1}J_{CH}$) were eliminated.

Fig. 1. Long range C-H coupling observed for RK-397 (**1**) in the HMBC experiment.

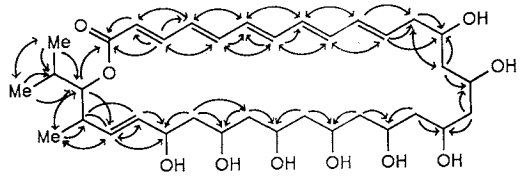
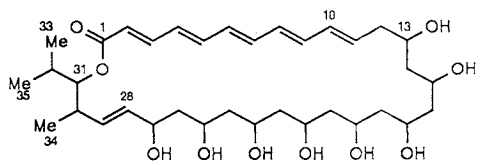
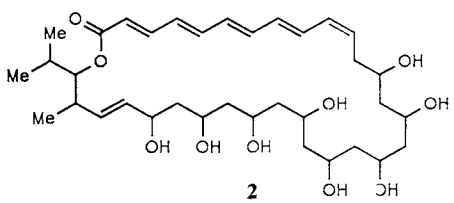


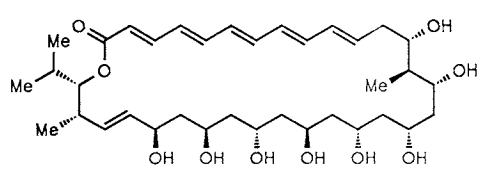
Fig. 2. Structures of RK-397 (**1**), 10,11-*cis* RK-397 (**2**) and mycoticin A (**3**).



1



2



3

Table 3. ^{1}H NMR data (400 MHz) for 10,11-*cis* RK-397 (**2**)[a,b].

No.	H[c,d]
2	5.94 (d, 15.1)
3	7.33 (dd, 15.1, 11.7)
4	6.46 (dd, 15.1, 11.7)
5	6.75 (dd, 15.1, 11.2)
6	6.41 (dd, 14.7, 11.2)
7	6.60 (dd, 14.7, 11.2)
8	6.37 (dd, 14.7, 11.2)
9	6.70 (dd, 14.7, 11.7)
10	6.25 (dd, 11.7, 11.7)
11	5.72 (ddd, 11.7, 11.7, 3.9)
12	2.83 (ddd, 12.7, 11.7, 2.0), 2.33 (m)
13	4.14 (m)
27	4.20 (m)
28	5.52 (m)
29	5.55 (m)
30	2.61 (m)
31	4.76 (dd, 9.7, 1.4)
32	1.95 (m)
33	0.97 (d, 6.8)
34	1.05 (d, 6.9)
35	0.86 (d, 6.9)

[a] Chemical shifts are given in ppm from TMS as internal standard.
[b] CD_3OD as solvent.
[c] Multiplicity and coupling constants (J in Hz) are given in parentheses.
[d] Assignments of 14-H~26-H were not determined. Signals of 15, 17, 19, 23-H, and 25-H were observed at 4.25~3.60 ppm and signals of 14, 16, 18, 20, 22, 24-H and 26-H were observed at 1.90~1.10 ppm.

RK-397 was labile to light and converted to some geometrical isomers. One of the isomers, compound **2**, was partially purified and analyzed by NMR spectroscopy. Compound **2** and RK-397 (**1**) have the same molecular formulas, which were determined by HRFAB-MS. The ^{1}H NMR spectrum (Table 3) of **2** was similar to that of RK-397, but some difference was observed in the signals of the pentaene moiety. Sequential assignment of protons of **2** from the doublet signal of H-2 at 5.94 ppm was carried out by ^{1}H-^{1}H COSY. The vicinal coupling constant between H-10 and H-11 was $J=11.7$ Hz, and the stereochemistry of this double bond was assigned to *cis* configuration. Other double bonds were assigned to *trans* stereochemistry from large coupling constants. The structure of compound **2** was thus determined to be the 10,11-*cis* isomer of RK-397. Recently, fulongmycin[9] which has the planar structure of 10,11-*cis* mycoticin was reported.

RK-397 (**1**) is a new member of the oxo polyene

macrolide antibiotics which include mycoticins A(**3**) and B[2], roxaticin[3], roflamycoin[4], dermostatins A and B[5], fulongmycin[9], flavofungins[10], and surgumycin[11]. The configurations of the oxo polyene antibiotics, roxaticin[3] and mycoticins[12~14] were determined by X-ray analysis and synthesis, respectively. The planar structure of RK-397 corresponds to 14-demethyl-mycoticin A. The NMR data for RK-397 gave some information about the stereochemistry, but the determination of the stereochemistry for the polyol portion of RK-397 needs further experiments[15]. The NMR data for vicinal coupling constants (Table 1) indicated that the all *E* conjugated pentaene is extended *s-trans* conformation and the conformation of terminal portion (C-28~C-35) is the same as that of mycoticin A ($J_{29,30}=5.4$, $J_{30,31}=2.5$, $J_{31,32}=10.0$ Hz for **3**)[15] and roxaticin[3].

Acknowledgments

We are grateful to Mr. Y. ESUMI and Ms. Y. ITO of RIKEN for HRFAB-MS measurements. We also thank Dr. J. UZAWA and Dr. M. URAMOTO of RIKEN for helpful discussions and encouragement.

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