Studies on New Antitumor Antibiotics, Leptofuranins A, B, C and D

II. Physicochemical Properties and Structure Elucidation

YOICHI HAYAKAWA, KIN-YA SOHDA and HARUO SETO

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

(Received for publication May 27, 1996)

The structures of new antitumor antibiotics, leptofuranins A, B, C and D were elucidated to be as shown in Fig. 1 by NMR spectral analysis including a variety of two-dimensional techniques. The leptofuranins are novel leptomycin-related substances containing a tetrahydrofuran ring. Leptofuranins C and D were revealed to be in tautomeric isomerism and their relative stereochemistries were analyzed by NOESY experiments.

In the preceding paper[1], we have described the fermentation, isolation and biological activities of new antitumor antibiotics, leptofuranins A, B, C and D, as well as the taxonomy of the producing organism, *Streptomyces tanashiensis* 3007-H1. This paper describes the physicochemical properties and structure elucidation of the leptofuranins (Fig. 1).

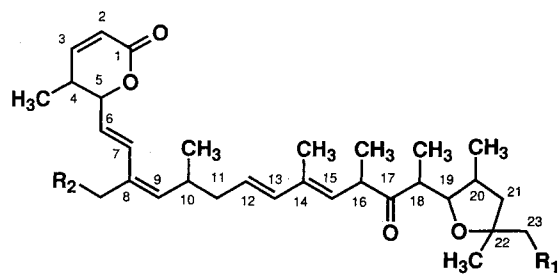
Physicochemical Properties

The physicochemical properties of the leptofuranins are summarized in Table 1. The high resolution FAB-MS established the molecular formulae of leptofuranins A, B, C and D as $C_{32}H_{48}O_5$, $C_{33}H_{50}O_5$, $C_{32}H_{46}O_5$ and $C_{33}H_{48}O_5$, respectively. Each leptofuranin exhibited IR absorption peaks due to hydroxyls (3450~3460 cm^{-1}) and carbonyls (1720 cm^{-1}).

Structure Elucidation

The ^{13}C NMR spectrum of leptofuranin A confirmed the presence of 32 carbons. A heteronuclear multiple-quantum coherency (HMQC)[2] experiment established all one-bond ^1H-^{13}C connectivities as shown in Tables 2 and 3. A COSY experiment revealed five spin networks to generate partial structures **I** to **V** (Fig. 2). The heteronuclear multiple-bond correlation (HMBC)[3] spectrum displayed ^1H-^{13}C long-range couplings from 8-CH_3 to C-7, C-8 and C-9, and from 6-H, 7-H and 9-H to C-8, indicating the connection between partial structures **I** and **II** *via* C-8 as shown in Fig. 2. ^1H-^{13}C long-range correlations from 22-CH_3 to C-21, C-22 and C-23, and from 21-H and 23-H_2 to C-22 established the linkage between partial structures **IV** and **V** *via* C-22 (Fig. 2). Partial structures **II**, **III** and **IV** were connected as shown

Fig. 1. Structures of leptofuranins A, B, C and D.



Leptofuranin A	**R_1 = CH_2OH**	**R_2 = H**
Leptofuranin B	**R_1 = CH_2OH**	**R_2 = CH_3**
Leptofuranin C	**R_1 = CHO**	**R_2 = H**
Leptofuranin D	**R_1 = CHO**	**R_2 = CH_3**

Table 1. Physicochemical properties of leptofuranins A, B, C and D.

	A	B	C	D
Appearance	Colorless oil	Colorless oil	Colorless oil	Colorless oil
Optical rotation	$[\alpha]_D^{24}$ −62° (*c* 0.29, MeOH)	$[\alpha]_D^{24}$ −67° (*c* 0.19, MeOH)	$[\alpha]_D^{20}$ −98° (*c* 0.17, MeOH)	$[\alpha]_D^{20}$ −77° (*c* 0.40, MeOH)
Formula	$C_{32}H_{48}O_5$	$C_{33}H_{50}O_5$	$C_{32}H_{46}O_5$	$C_{33}H_{48}O_5$
FAB-MS *m/z*	513.3580	527.3737	511.3394	525.3593
$(M+H)^+$ calcd.	513.3611	527.3702	511.3423	525.3580
UV λ_{max} nm (ε) in MeOH	239 (25,500)	239 (27,600)	242 (42,500)	242 (45,100)
IR ν_{max} cm^{-1}	3460, 1720	3460, 1720	3450, 1720	3450, 1720

Table 2. ^{13}C NMR assignments for leptofuranins A, B, C and D in $CDCl_3$.

No.	A	B	C	D	No.	A	B	C	D
1	164.1	164.1	164.1	164.1	18	45.8	45.8	46.0	46.0
2	120.0	120.1	120.1	120.1	19	81.5	81.3	80.6[a], 81.6[b]	80.6[a], 81.6[b]
3	151.4	151.3	151.4	151.4	20	34.6	34.6	34.6[a], 34.9[b]	34.6[a], 34.9[b]
4	33.4	33.5	33.5	33.6	21	47.9	47.9	46.5[a], 46.9[b]	46.5[a], 46.9[b]
5	81.1	81.3	81.2	81.3	22	81.8	81.8	78.4[a], 78.8[b]	78.4[a], 78.7[b]
6	123.4	122.8	123.4	122.8	23	41.3	41.3	56.0[a], 54.3[b]	56.0[a], 54.3[b]
7	130.7	129.8	130.7	129.8	24	60.0	60.0	202.4	202.4
8	129.4	135.5	129.4	135.4	4-CH_3	12.3	12.3	12.3	12.3
9	138.7	136.9	138.8	136.9	8-CH_3	20.4		20.4	
10	32.1	32.0	32.1	32.0	8-$\mathbf{CH_2}CH_3$		26.5		26.5
11	40.7	40.7	40.7	40.8	8-$CH_2\mathbf{CH_3}$		13.5		13.5
12	127.4	127.5	127.5	127.5	10-CH_3	20.5	20.6	20.6	20.6
13	135.4	135.4	135.5	135.4	14-CH_3	13.0	13.0	13.0	13.0
14	135.5	135.3	135.6	135.3	16-CH_3	16.6	16.6	16.7, 16.6	16.7, 16.6
15	129.3	129.3	129.4	129.3	18-CH_3	15.7	15.8	15.9, 15.8	15.9, 15.8
16	44.3	44.3	44.3	44.3	20-CH_3	15.4	15.4	15.5, 15.4	15.5, 15.4
17	212.9	212.9	213.3[a], 213.1[b]	213.3[a], 213.1[b]	22-CH_3	26.8	26.8	27.0[a], 28.9[b]	27.0[a], 28.9[b]

[a,b] Assignable to each of the tautomeric isomers.

Table 3. ^1H NMR data summary for leptofuranins A to D in $CDCl_3$ (δ_H (J=Hz)).

No.	A	B	C	D
2	5.98 dd (9.8, 0.9)	5.99 dd (9.8, 1.0)	6.00 dd (9.8, 1.0)	5.99 dd (9.8, 1.0)
3	6.94 dd (9.8, 5.8)	6.94 dd (9.8, 5.7)	6.95 dd (9.8, 5.8)	6.94 dd (9.8, 5.8)
4	2.51 m	2.52 m	2.52 m	2.52 m
5	5.00 m	5.00 m	5.02 m	5.00 m
6	5.66 dd (15.7, 6.7)	5.71 dd (16.0, 6.7)	5.68 dd (15.5, 6.8)	5.70 dd (16.0, 7.0)
7	6.74 d (15.7)	6.64 d (15.8)	6.75 d (15.5)	6.64 d (16.0)
9	5.24 d (9.8)	5.22 d (10.0)	5.25 d (10.0)	5.22 d (10.0)
10	2.70 m	2.68 dq (10.0, 6.9)	2.70 m	2.67 m
11	2.05 m	2.07 m	2.06 m	2.06 m
12	5.56 dt (15.6, 7.3)	5.56 dt (15.6, 7.3)	5.57 dt (15.5, 7.2)	5.56 dt (15.0, 7.3)
13	5.97 d (15.6)	5.97 d (15.6)	5.98 d (15.5)	5.97 d (15.0)
15	5.10 d (10.0)	5.11 d (10.3)	5.13 d (10.0)	5.12 d (10.0)
16	3.71 m	3.72 dq (10.3, 6.5)	3.72[a] dq (10.0, 6.8), 3.74[b] dq (10.0, 6.8)	3.72[a] dq (10.0, 6.8), 3.74[b] dq (10.0, 6.8)
18	2.71 m	2.74 dq (5.0, 7.3)	2.70[a] m, 2.75[b] m	2.70[a] m, 2.75[b] m
19	4.04 dd (9.8, 5.0)	4.05 dd (9.8, 5.0)	4.08[a] dd (9.8, 5.0), 4.03[b] dd (9.8, 5.0)	4.07[a] dd (10.0, 5.0), 4.02[b] dd (10.0, 5.0)
20	2.33 m	2.34 m	2.42[a] m, 2.36[b] m	2.42[a] m, 2.36[b] m
21	2.01 m, 1.46 m	2.01 m, 1.47 m	2.03[a] m, 1.48[a] t (4.0), 2.08[b] m, 1.45[b] t (4.0)	2.03[a] m, 1.48[a] t (4.0), 2.08[b] m, 1.45[b] t (4.0)
23	1.98 m, 1.45 m	1.98 m, 1.46 m	2.56[a] m, 2.51[a] m, 2.63[b] m, 2.43[b] m	2.56[a] m, 2.51[a] m, 2.63[b] m, 2.43[b] m
24	3.90 m, 3.64 m	3.91 m, 3.65 m	9.80[a] t (2.8), 9.78[b] dd (3.3, 2.3)	9.79[a] t (2.8), 9.77[b] dd (3.3, 2.3)
4-CH_3	1.04 d (7.2)	1.06 d (6.7)	1.06 d (7.2)	1.05 d (7.2)
8-CH_3	1.80 s		1.81 d (1.0)	
8-$\mathbf{CH_2}CH_3$		2.18 m		2.18 m
8-$CH_2\mathbf{CH_3}$		1.04 t (7.6)		1.03 t (7.2)
10-CH_3	0.93 d (6.8)	0.94 d (6.9)	0.94 d (6.8)	0.94 d (6.8)
14-CH_3	1.81 s	1.81 s	1.82 d (1.5)	1.81 d (1.5)
16-CH_3	1.10 d (6.8)	1.11 d (6.5)	1.13 d (6.8)	1.11 d (6.8)
18-CH_3	1.21 d (7.2)	1.22 d (7.3)	1.24[a] d (6.9), 1.25[b] d (6.8)	1.23[a] d (6.5), 1.24[b] d (6.8)
20-CH_3	0.72 d (7.5)	0.73 d (7.0)	0.70[a] d (7.2), 0.73[b] d (7.2)	0.70[a] d (7.2), 0.73[b] d (7.2)
22-CH_3	1.30 s	1.31 s	1.28[a] s, 1.36[b] s	1.27[a] s, 1.36[b] s
24-OH	3.38 br	3.38 br		

[a,b] Assignable to each of the tautomeric isomers.

Fig. 2. COSY, HMBC and NOESY data summary for leptofuranin A.

Bold lines show ^{1}H spin networks, solid arrows show ^{1}H-^{13}C long-range correlations and dashed arrows show NOEs.

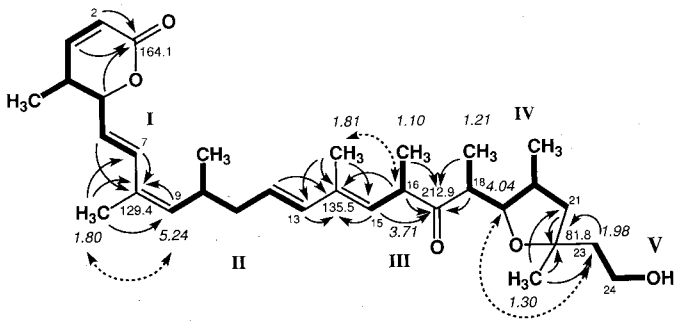
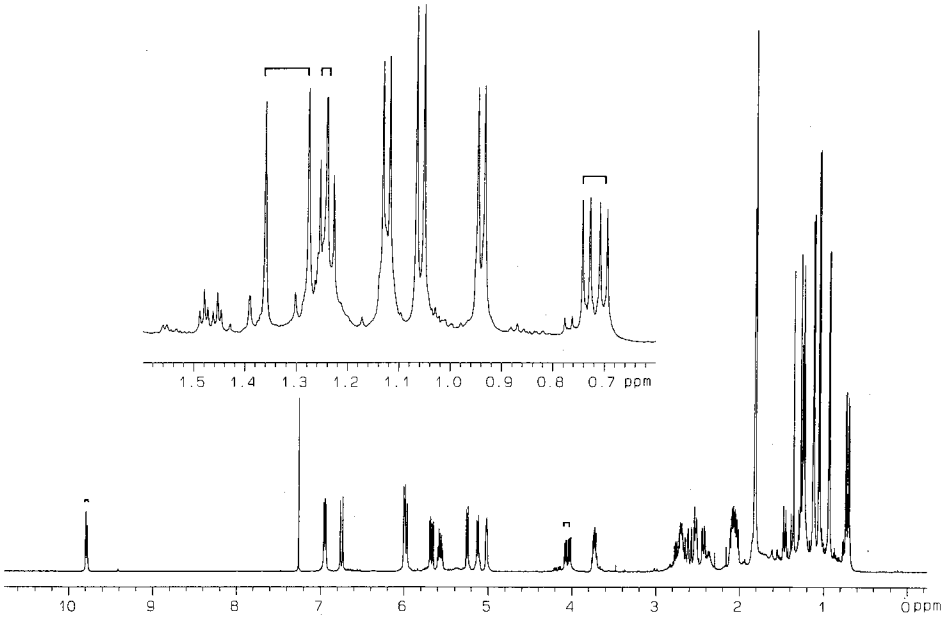


Fig. 3. ^{1}H NMR spectrum of leptofuranin C in $CDCl_3$.

Marks are put on the spectrum for typical twin peaks due to the tautomers.



in Fig. 2 by ^{1}H-^{13}C long-range couplings from 14-CH_3 to C-13, C-14 and C-15, from 12-H, 13-H and 16-H to C-14, and from 15-H, 16-H, 18-H, 16-CH_3 and 18-CH_3 to a ketone carbonyl carbon (C-17). In addition, a δ-lactone ring was constructed from ^{1}H-^{13}C long-range correlations from 2-H, 3-H and 5-H to an ester carbonyl carbon (C-1). An NOE observed between 19-H and 23-H (δ 1.98) revealed the presence of a tetrahydrofuran ring representing C-19$\sim$C-22 to establish the structure of leptofuranin A except for its stereochemistry.

The geometrical configurations of C-6 and C-12 were determined to be 6*E* and 12*E* by $J_{6\sim7}=15.7$ Hz and $J_{12\sim13}=15.6$ Hz. A lower-field chemical shift for 8-CH_3 (δ_C 20.4) and a higher-field chemical shift for 14-CH_3 (δ_C 13.0) indicated 8*Z* and 14*E* configurations, which were confirmed by NOEs observed between 9-H and 8-CH_3, and between 16-H and 14-CH_3 (Fig. 2). These data established the planar structure of leptofuranin A as shown in Fig. 1.

The ^{13}C and ^{1}H NMR spectra of leptofuranin B were very similar to those of leptofuranin A. Leptofuranin B, however, contained an ethyl group in place of 8-CH_3 in leptofuranin A (Tables 2 and 3) resulting in a downfield shift for C-8 by 6.1 ppm. The substitution of the ethyl group at C-8 in leptofuranin B was further confirmed by COSY, HMQC and HMBC experiments (data not shown).

The ^{1}H and ^{13}C NMR signals for leptofuranin C in $CDCl_3$ partially appeared as twin peaks in the area ratio of 1:1 (Fig. 3, Tables 2 and 3). In addition, HPLC

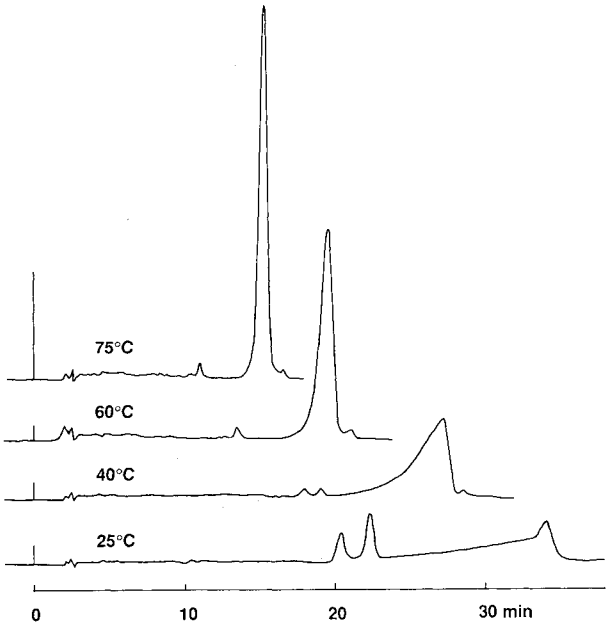
analysis of leptofuranin C by using an ODS column with 70% acetonitrile at room temperature showed several broad peaks, which collapsed to a sharp single peak at 75°C as shown in Fig. 4, indicating the presence of tautomeric isomers in $CDCl_3$.

A COSY experiment revealed five spin networks to generate partial structures **VI** to **X** for one of the isomers (a) as shown in Fig. 5. The other isomer (b) gave the same partial structures including *E* configurations for C-6 and C-12. The HMBC spectrum displayed ^1H-^{13}C long-range couplings for one of the tautomeric isomers (a) as shown in Fig. 5 to construct the structure of leptofuranin C except for a tetrahydrofuran ring, which was established by an NOE observed between 19-H and 22-CH_3. The 8*Z* and 14*E* configurations were determined by NOEs observed between 9-H and 8-CH_3, and between 16-H and 14-CH_3, respectively (Fig. 5). Although the same ^1H-^{13}C long-range correlations were with the other isomer (b), NOEs were observed between 19-H and 23-H (δ 2.63), and between 20-CH_3 and 22-CH_3, but not between 19-H and 22-CH_3. These data indicate that this tautomerism is due to 22-epimerization by such a mechanism as shown in Fig. 6.

A pair of tautomeric isomers was also detected in the ^{13}C and ^1H NMR spectra of leptofuranin D (Tables 2 and 3). From the spectral similarity, leptofuranin D appeared to be an 8-ethyl derivative of leptofuranin C, which was confirmed by COSY, HMQC, HMBC and NOESY experiments (data not shown).

The planar structures of leptofuranins A to D thus obtained are summarized in Fig. 1. The leptofuranins are new members of the leptomycin family including leptomycins[4], kazusamycins[5], anguinomycins[6,7] and leptolstatins[8], which contain a carboxyl group, a hy-

Fig. 4. HPLC analysis of leptofuranin C at various temperatures.



Column: PEGASIL-ODS (i.d. 4.6 mm × 250 mm, Senshu Scientific Co.), solvent system: 70% acetonitrile, flow rate: 1 ml/minute, detection: UV 254 nm.

Fig. 6. Relative stereochemistry for the tetrahydrofuran ring of leptofuranin C tautomers and a plausible mechanism of the tautomerism.

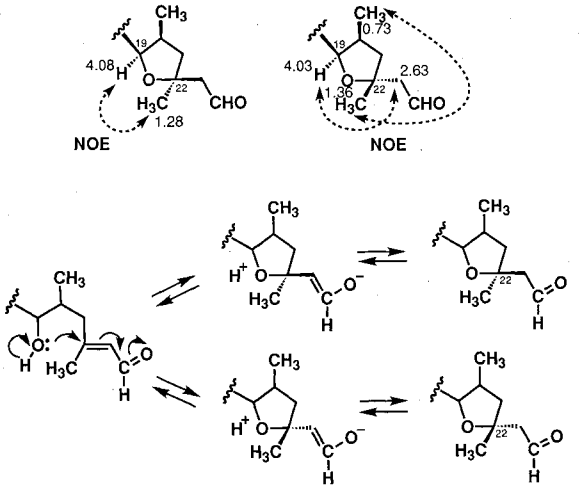
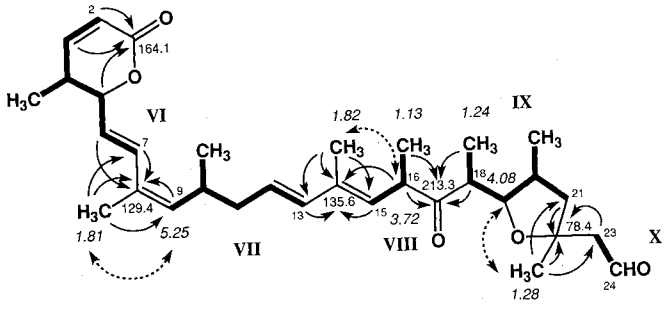


Fig. 5. COSY, HMBC and NOESY data summary for leptofuranin C.

Bold lines show ^1H spin networks, solid arrows show ^1H-^{13}C long-range correlations and dashed arrows show NOEs.



droxymethyl group or a methyl group at C-24 position. Among the leptomycin family, however, the leptofuranins are characterized as being unique derivatives because of the presence of an aldehyde group at C-24 in leptofuranins C and D as well as a tetrahydrofuran ring in common.

Experimental

Specific rotations were obtained on a JASCO DIP-371 spectropolarimeter at 589.6 nm. Mass spectra were measured on a JEOL HX-110 spectrometer in the FAB mode using *m*-nitrobenzyl alcohol as matrix and polyethylene glycol as internal standard. UV and visible spectra were recorded on a Hitachi U-3210 spectrophotometer. IR spectra were obtained on a JASCO A-102 spectrometer. NMR spectra were obtained on a JEOL JNM-A500 spectrometer with ^{1}H NMR at 500 MHz and with ^{13}C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard. HPLC analysis was carried out by using a PEGASIL-ODS column (i.d. 4.6 mm × 250 mm, Senshu Scientific Co.) and an SSC-2100 column oven (Senshu Scientific Co.) with 70% acetonitrile at a flow rate of 1 ml/minute.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, Japan.

References

1) HAYAKAWA, Y.; K. SOHDA, K. FURIHATA, T. KUZUYAMA, K. SHIN-YA & H. SETO: Studies on new antitumor antibiotics, leptofuranins A, B, C and D. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 49: 974~979, 1996
2) SUMMERS, M. S.; L. G. MARZILLI & A. BAX: Complete ^{1}H and ^{13}C assignments of coenzyme B_{12} through the use of new two-dimensional NMR experiments. J. Am. Chem. Soc. 108: 4285~4294, 1986
3) BAX, A. & M. F. SUMMERS: ^{1}H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986
4) HAMAMOTO, T.; H. SETO & T. BEPPU: Leptomycins A and B, new antifungal antibiotics. II. Structure elucidation. J. Antibiotics 36: 646~650, 1983
5) KOMIYAMA, K.; K. OKADA, H. OKA, S. TOMISAKA, T. MIYANO, S. FUNAYAMA & I. UMEZAWA: Structural study of a new antitumor antibiotic, kazusamycin. J. Antibiotics 38: 220~223, 1985
6) HAYAKAWA, Y.; K. ADACHI & N. KOMESHIMA: New antitumor antibiotics, anguinomycins A and B. J. Antibiotics 40: 1349~1352, 1987
7) HAYAKAWA, Y.; K. SOHDA, K. SHIN-YA, T. HIDAKA & H. SETO: Anguinomycins C and D, new antitumor antibiotics with selective cytotoxicity against transformed cells. J. Antibiotics 48: 954~961, 1995
8) ABE, K.; M. YOSHIDA, H. NAOKI, S. HORINOUCHI & T. BEPPU: Leptolstatin from *Streptomyces* sp. SAM1595, a new gap phase-specific inhibitor of the mammalian cell cycle. II. Physico-chemical properties and structure. J. Antibiotics 46: 735~740, 1993