A NEW ANTITUMOR COMPLEX, WF-1360, WF-1360A, B, C, D, E AND F

SUMIO KIYOTO, YOSHIO KAWAI, TAKESHI KAWAKITA, EIKO KINO,
MASAKUNI OKUHARA, ITSUO UCHIDA, HIROKAZU TANAKA,
MASASHI HASHIMOTO, HIROSHI TERANO,
MASANOBU KOHSAKA, HATSUO AOKI
and HIROSHI IMANAKA†

Exploratory Research Laboratories,
†Planning and Development Group,
Fujisawa Pharmaceutical Co., Ltd.,
2-1-6 Kashima, Yodogawa-ku, Osaka 532, Japan

(Received for publication December 14, 1985)

A complex of the new antitumor antibiotics (WF-1360, WF-1360A, B, C, D, E and F) was produced by *Rhizopus* sp. No. F-1360. Structural studies of these compounds suggested that they were novel 16-membered-ring lactones having an oxazole ring in their structures. WF-1360 was found to be identical with rhizoxin (**1**) and WF-1360B, C, E and F were determined to be homologues of **1** with structures **2**, **3**, **4** and **5**, respectively.

These compounds were cytotoxic when tested on P388 leukemia cells *in vitro*. WF-1360 was highly active against leukemia L1210 and melanoma B16. They also exhibited potent antifungal activities, but weak antimicrobial activities against some Gram-positive or negative bacteria.

In our screening program for antitumor compounds, *Rhizopus* sp. No. F-1360 was found to produce a complex of antitumor antibiotics, which were extracted from the fermentation broth and separated into seven components, one major component (WF-1360)[1,2] and six minor components (WF-1360A, B, C, D, E and F).

The present paper describes the production, isolation, physico-chemical properties, structures and biological activities of these compounds.

Fermentation

A loopful of slant culture of *Rhizopus* sp. No. F-1360 (ATCC 20577, or FERM P-5362) was inoculated into a seed medium (100 ml) containing corn starch 1%, glycerol 0.5%, gluten meal 1%, dried yeast 1%, corn steep liquor 1% and $CaCO_3$ 1% (pH 6.5), poured into a 500-ml Erlenmeyer flask and cultured at 30°C for 48 hours at 250 rpm using a rotary shaker.

Fermentation studies were carried out in tank fermentors. A seed culture was shaken in the above mentioned Erlenmeyer flasks and then transferred at the rate of 0.5% to 300 liters of the same medium in a 500-liter stainless steel fermentor, which was agitated at 200 rpm at 30°C for 24 hours. Further, total volume of thus obtained seed medium was inoculated into 3,000 liters of a production medium containing glycerol 5%, soybean meal 3%, cotton seed flour 1% and $CaCO_3$ 2% in a 4,000-liter stainless steel fermentor was operated at 30°C for 90 hours under aeration of 2,500 liters/minute and agitation of 90 rpm.

The antitumor activity level in the fermentation broth was assayed by antimicrobial activity against *Penicillium chrysogenum* ATCC 10002 and cytotoxic activity against P388 murine leukemia cells in tissue culture.

Isolation and Purification

The fermentation broth (3,000 liters) was filtered with the aid of diatomaceous earth (60 kg). The mycelial cake was extracted with 4,700 liters of ethyl acetate. The ethyl acetate layer was separated, washed with 2,000 liters of aqueous sodium bicarbonate (0.5%) and concentrated *in vacuo* to a volume of 10 liters. After dehydration with anhydrous sodium sulfate, 3 liters of *n*-hexane were added to the concentrate to give a precipitate which contained the active fractions. The crude powder thus obtained was dissolved into a small amount of chloroform and applied to a 3-liter silica gel chromatographic column. After developing with 6 liters of chloroform, the column was eluted with a mixture of chloroform - methanol (50: 1). Seventy-five grams of WF-1360 was obtained as a purified powder.

Subsequently, the silica gel column was eluted with a mixture of chloroform - methanol (20: 1). This eluate was concentrated *in vacuo* and fractionated by high performance liquid chromatography (HPLC). The active fractions were separated into three parts by using an octadecyl-substituted silica gel ODS column (5×30 cm, Waters Associates, Milford, MA) and acetonitrile - water (1: 1) as the mobile phase. Retention times, detected by refractive index at a flow rate of 1.5 ml/minute, of fraction (1) (3 liters), fraction (2) (3 liters), and fraction (3) (4 liters) are approximately 7.5, 23 and 59 minutes, respectively.

Further, the fraction (1) described above was concentrated *in vacuo*, applied to a steel column (2×15 cm) packed with silica gel (YMC-sil, Shimakyu Co., Ltd., Japan) and eluted with a mixture of chloroform - methanol (100: 1) at a flow rate of 15 ml/minute. Fractions A and B, containing WF-1360A and B, were collected. Retention times of WF-1360A and B, detected by ultraviolet absorption at 254 nm, were 35 and 53 minutes, respectively.

Fig. 1. Isolation of WF-1360, WF-1360A, B, C, D, E and F.

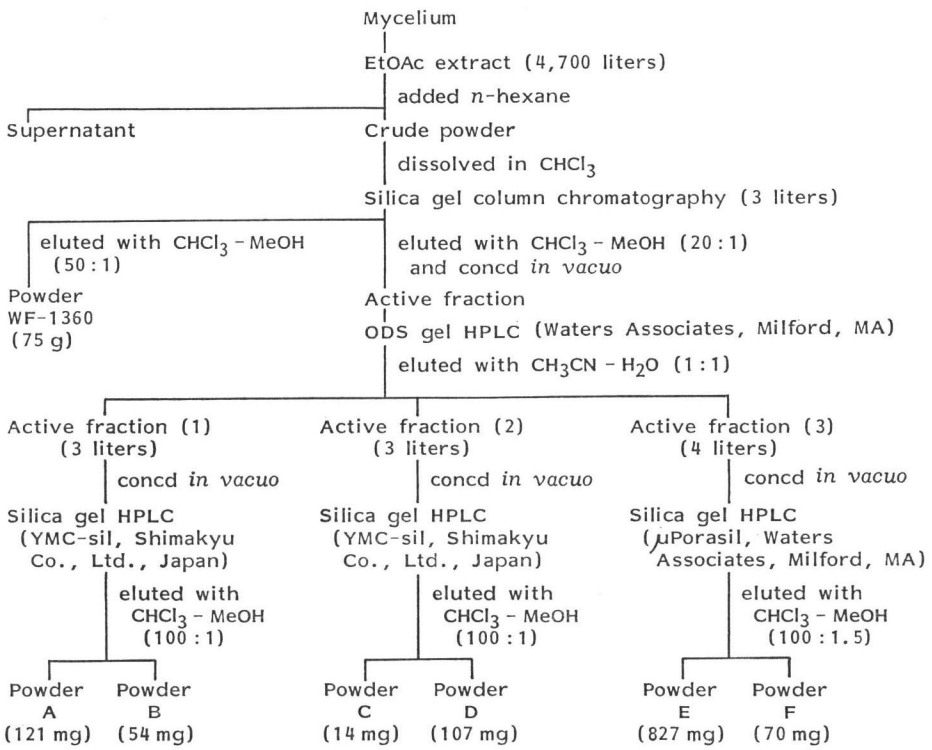


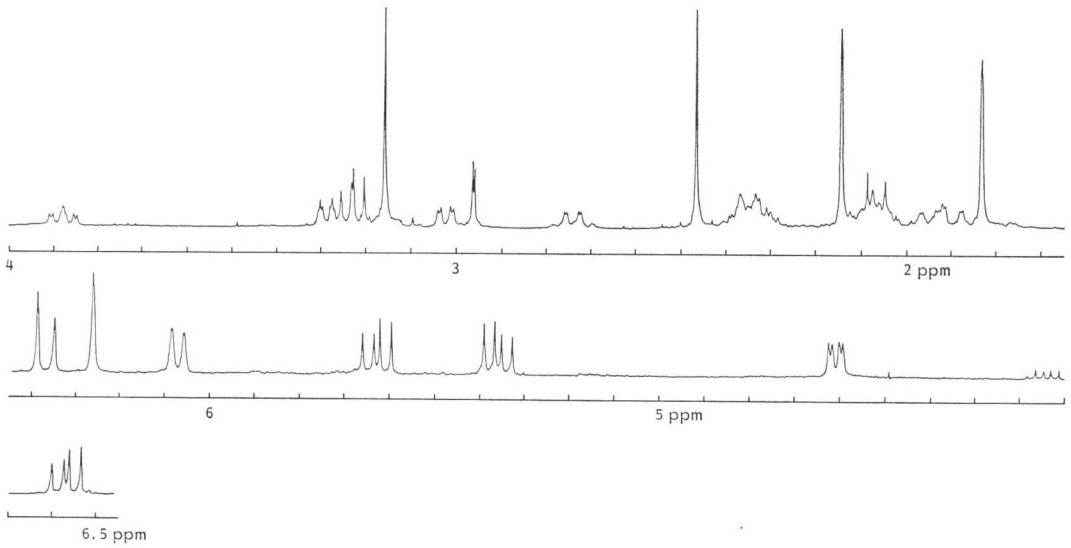
Table 1. Physico-chemical properties of

	WF-1360	WF-1360A	WF-1360B
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder
Specific rotation	$[\alpha]_D^{20}+140°$ [a] (c 1, $CHCl_3$)		$[\alpha]_D^{23}+110°$ (c 1.9, $CHCl_3$)
UV λ_{max}^{MeOH} nm (ε)	296 (41,000), 308 (51,600), 324 (38,200)		296 (42,500), 308 (53,000), 323 (40,800)
IR ν_{max} cm^{-1} [b]	3450, 2960, 2920, 1730, 1575, 1445, 1377, 1190, 1107, 1077, 1045, 980	3430, 3000, 2940, 1720, 1580, 1445, 1380, 1150, 1115, 1080, 1050, 970	3450, 3000, 2940, 1720, 1580, 1450, 1380, 1175, 1110, 1080, 1050, 975
MS (m/z, M^+)	EI: 625	FD: 641	EI: 595
Elemental *Anal*			
Calcd for	$C_{35}H_{47}O_9N$: C 67.18, H 7.57, N 2.24		$C_{34}H_{45}O_8N\cdot H_2O$: C 66.54, H 7.72, N 2.28
Found	C 67.01, H 7.56, N 2.22		C 65.99, H 7.52, N 2.28
Molecular formula	$C_{35}H_{47}O_9N$	$C_{36}H_{51}O_9N$	$C_{34}H_{45}O_8N$
TLC (Rf)			
Silica gel plate $CHCl_3$ - MeOH (20: 1)	0.48	0.25	0.32

[a] This value was somewhat different from the reported value $[\alpha]_D^{24}$ +201° (c 0.5, MeOH)[3], which was later

[b] WF-1360: KBr disk. WF-1360A, B, C, D, E and F: $CHCl_3$ solutions.

Fig. 2. ^1H NMR spectrum



The fraction (2) was concentrated *in vacuo* and subjected to HPLC in substantially the same method as the treatment of the fraction (1) as mentioned above. The fractions WF-1360C (150 ml) and D (200 ml) were collected. Retention times of WF-1360C and D detected by UV absorption at 254 nm, are 23 and 43 minutes, respectively.

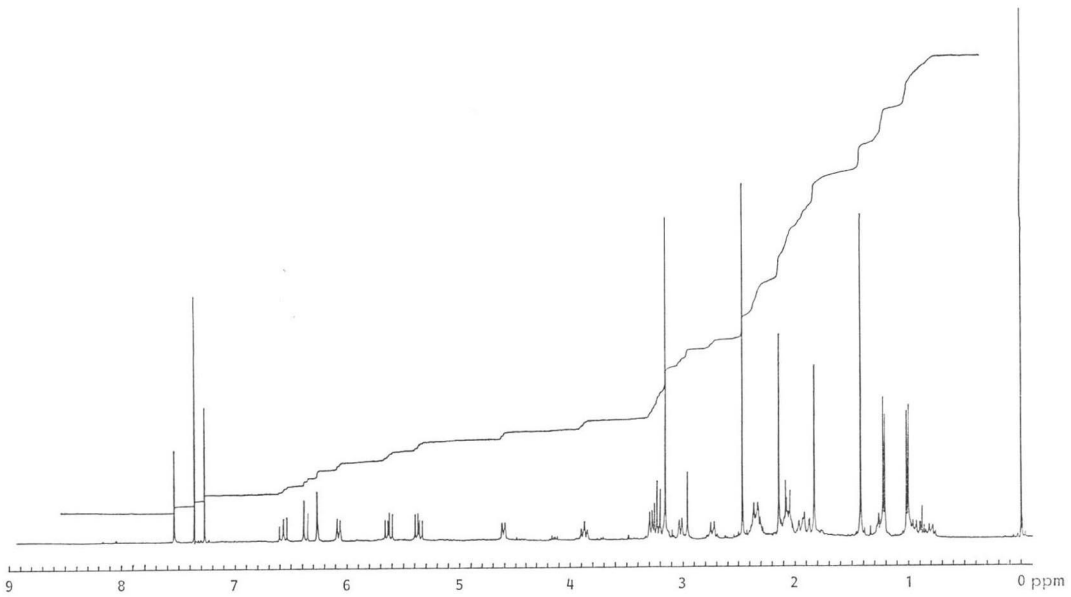
The fraction (3) was concentrated *in vacuo* and subjected to HPLC using a steel column (5×30 cm) packed with silica gel (μPorasil, Waters Associates, Milford, MA). Elution was carried out with a mixture of chloroform - methanol (100 : 1.5) at the flow rate of 150 ml/minute. Fractions WF-1360E (150 ml) and F (350 ml) were collected. The fraction F was further purified by HPLC by the same method as used for fraction (1) as mentioned above. The elution was carried out with a mixture of chloroform -

WF-1360, WF-1360A, B, C, D, E and F.

WF-1360C	WF-1360D	WF-1360E	WF-1360F
Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder
$[\alpha]_D^{23}$ +197°	$[\alpha]_D^{23}$ +57°	$[\alpha]_D^{23}$ +58°	$[\alpha]_D^{23}$ +97°
(*c* 0.2, $CHCl_3$)	(*c* 0.7, $CHCl_3$)	(*c* 1.6, $CHCl_3$)	(*c* 1.9, $CHCl_3$)
296 (39,300), 308 (48,200), 323 (36,700)		296 (42,800), 309 (53,200), 323 (40,000)	296 (35,900), 309 (45,000), 323 (33,000)
3430, 3000, 2940, 1720, 1580, 1450, 1380, 1170, 1080, 1050, 985, 975	3400, 2980, 2930, 1720, 1580, 1450, 1380, 1170, 1105, 1075, 1045, 970	3450, 3000, 2940, 1720, 1580, 1445, 1380, 1160, 1110, 1080, 1050, 970	3450, 3000, 2940, 1720, 1580, 1450, 1380, 1175, 1110, 1080, 1050, 985
EI: 579	FD: 595	FD: 655	EI: 609
$C_{34}H_{45}O_7N \cdot H_2O$:		$C_{37}H_{53}O_9N \cdot H_2O$:	$C_{35}H_{47}O_8N \cdot H_2O$:
C 68.32, H 7.93, N 2.34		C 65.95, H 8.23, N 2.08	C 66.96, H 7.87, N 2.23
C 67.62, H 7.71, N 2.27		C 66.18, H 8.02, N 2.14	C 67.01, H 7.85, N 2.30
$C_{34}H_{45}O_7N$	$C_{34}H_{45}O_8N$	$C_{37}H_{53}O_9N$	$C_{35}H_{47}O_8N$
0.32	0.40	0.41	0.54

corrected to +155.5° (*c* 0.8, MeOH)[4].

of WF-1360 (400 MHz, $CDCl_3$).



methanol (150 : 1). Fraction (150 ml) contained active substance was collected as WF-1360F.

Each fraction was concentrated *in vacuo* to a small volume and *n*-hexane was added to give a purified powder (Fig. 1).

Physico-chemical Properties of WF-1360 and its Minor Homologs and Structures of WF-1360, WF-1360B, C, E and F

The physico-chemical properties of WF-1360, WF-1360A, B, C, D, E and F are summarized in

Fig. 3. IR spectrum of WF-1360 (KBr disk).

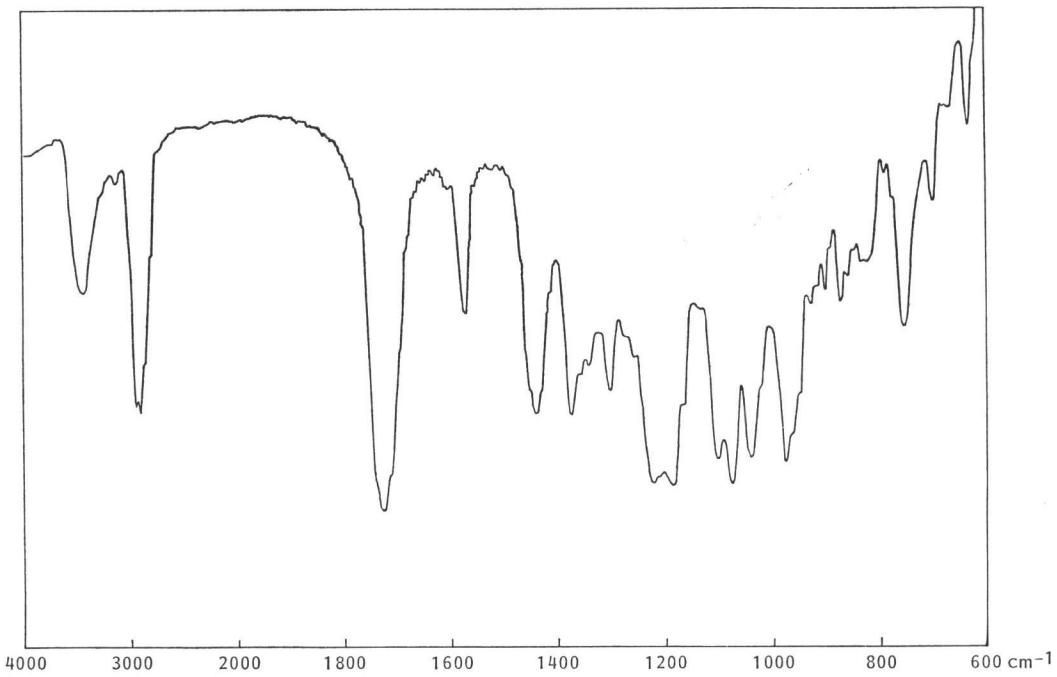


Fig. 4. UV spectrum of WF-1360 (MeOH).

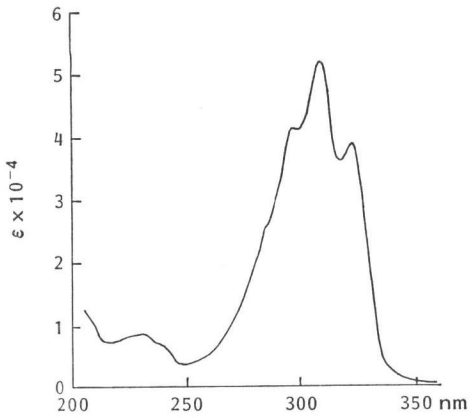


Table 1, and the ^{1}H NMR, IR and UV spectra of WF-1360 are shown in Figs. 2, 3 and 4. The major component, WF-1360, which is readily soluble in MeOH, EtOH, acetone, and EtOAc, soluble in $CHCl_3$ and benzene, slightly soluble in Et_2O and hexane, and insoluble in H_2O, gave a positive reaction to Dragendorff and iodine, though negative to Ninhydrin, Molisch, Ehrlich, ferric chloride reagents. During the structural study of WF-1360, we noticed that this product resembled rhizoxin (**1**), isolated by IWASAKI *et al.*[3] from the culture broth of *Rhizopus chinensis* Rh-2. Tables 2 and 4 show the ^{1}H and ^{13}C NMR spectral data of WF-1360, respectively, which were coincident with those of rhizoxin. The IR and UV data of WF-1360 were also corresponding to those of rhizoxin. These data showed that WF-1360 is identical with rhizoxin whose structure was already disclosed as **1** by IWASAKI *et al.*[3].

All the six minor components, WF-1360A, B, C, D, E and F, showed similar solubilities and gave similar color reactions to those of rhizoxin. Among these compounds, WF-1360B, C, D, E and F were proposed to have structures **2**, **3**, **4** and **5**, respectively, as follows:

The ^{1}H NMR data of these four compounds are summarized in Table 3 and the ^{13}C NMR data of these compounds are shown in Table 4 in comparison with those of WF-1360.

From its molecular formula, $C_{35}H_{47}O_8N$, WF-1360F was assumed to be a deoxy derivative of **1**.

Table 2. ^{1}H NMR data of WF-1360 in $CDCl_3$ (400 MHz).

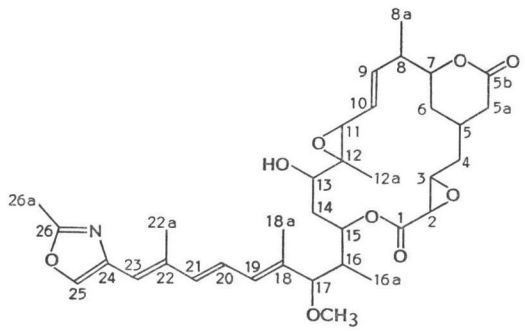
Chemical shift	Multiplicity	Coupling constant (Hz)	Assignment
7.54	s		H-25
6.57	dd	11, 15	H-20
6.38	d	15	H-21
6.27	s		H-23
6.07	d	11	H-19
5.63	dd	10, 15	H-9
5.36	dd	9.5, 15	H-10
4.61	dd	3, 9	H-15
3.88	ddd	3, 9.5, 12	H-7
3.28	ddd	1.6, 2, 10.5	H-3
3.23	d	9	H-17
3.20	d	9.5	H-11
3.15	s		17-OCH_3
3.02	dd	2.5, 11	H-13
2.96	d	1.6	H-2
2.73	m		H-5a
2.46	s		3H-26a
2.4~2.3	m		H-4, H-8, H-16
2.13	s		3H-22a
2.1~2.0	m		H-5, H-5a, H-14
1.97~1.85	m		H-6, H-14
1.82	s		3H-18a
1.44	s		3H-12a
1.22	d	6.3	3H-8a
1.01	d	6.7	3H-16a
0.93	m		H-6
0.80	m		H-4

In the ^{13}C NMR spectrum of WF-1360F, two olefinic carbon signals (C-2 and C-3) were newly observed at 124.9(d) and 146.1(d) ppm in place of the two epoxy-carbon signals (C-2, 54.2 and C-3, 56.0 ppm) in WF-1360, suggesting that WF-1360F is the 2,3-conjugated lactone derivative of **1**. This was corroborated by the ^{1}H NMR spectrum of WF-1360F, in which H-2 and H-3 appeared at δ 5.70 and 6.82, respectively. The coupling constant, $J_{2,3}$=16 Hz, showed the stereochemistry of the olefine to be *E* configuration. Thus, structure **5** was proposed for WF-1360F.

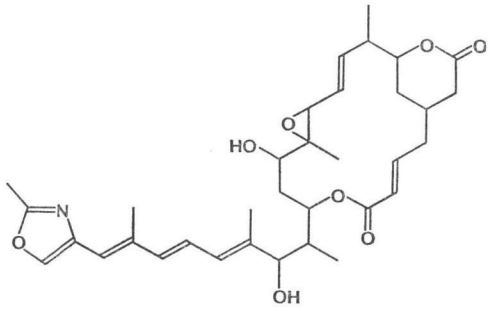
WF-1360B, $C_{34}H_{45}O_8N$, seemed to be a demethyl derivative of **5**. In the ^{13}C NMR spectrum of WF-1360B, no methoxy-carbon signal was observed, while the C-17 carbon signal was resonated at 78.9 ppm with an upfield shift (10.8 ppm) as compared with that of **5**. The ^{1}H NMR spectrum of WF-1360B also showed no methoxy-methyl protons and a downfield shift of H-17 (δ 3.27 in **5**→3.90). These data suggested that WF-1360B is a demethyl derivative of **5**, leading to structure **2** for WF-1360B.

The structure of WF-1360E was disclosed to be **4** by comparison of its NMR data with those of **5**. New proton signals at δ 4.16 (2H, q, J=7 Hz) and 1.27 (3H, t, J=7 Hz) and new carbon signals at 60.6(t) and 14.2(q) demonstrated the presence of an ethyl ester group in WF-1360E. Observations of upfield shifts of H-7 (δ 3.75 in **5**→3.13) and C-7 (82.4 ppm in **5**→73.3) further indicated that the δ-lactone in **5** is changed, in WF-1360E, to a hydroxyethyl ester. These data, together with the molecular formula, $C_{37}H_{53}O_9N$, thus proposed that WF-1360E has structure **4**.

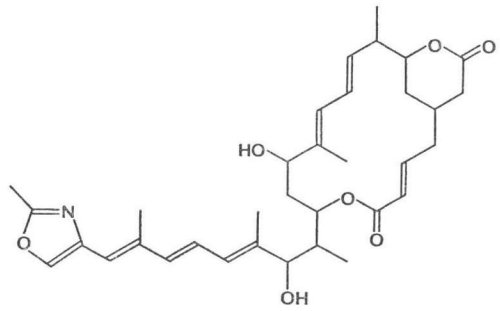
WF-1360C, $C_{34}H_{45}O_7N$, was likely a deoxy derivative of **2**. In the ^{1}H NMR spectrum, WF-1360C



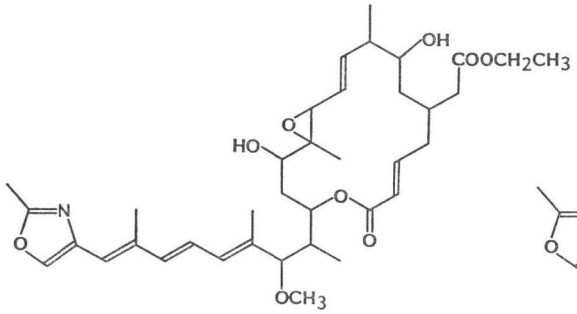
WF-1360 (**1**, Rhizoxin)



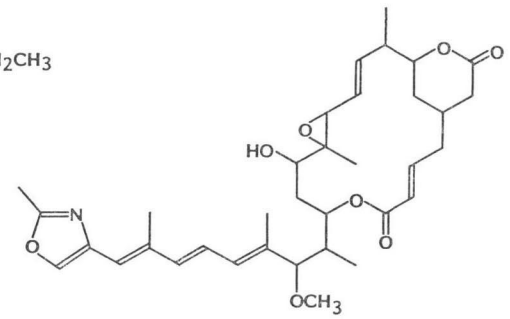
WF-1360B (**2**)



WF-1360C (**3**)



WF-1360E (**4**)



WF-1360F (**5**)

showed at δ 5.85 a 1 H doublet (J=11 Hz) which is attributed to H-11 (olefinic proton). The 12-Me and H-13 signals (allylic to the olefine) were consequently shifted downfield by 0.38 and 0.87 ppm, respectively, as compared with those of **2**. These data suggested that WF-1360C has an olefinic bond between C-11 and C-12 in place of the epoxide in **2**. This was corroborated by the ^{13}C NMR analysis of WF-1360C, in which new olefinic carbon signals attributable to C-11 and C-12 were observed at 129.8(d) and 139.3(s) ppm, respectively. WF-1360C was therefore proposed to have structure **3** without stereochemistry of the 11, 12-double bond.

The structures of WF-1360B, C, E and F were thus established, without stereochemistry, as being **2**, **3**, **4** and **5**, respectively. The structures of WF-1360A and D are the subject of future investigations.

Table 3. ^{1}H NMR chemical shifts of WF-1360B, C, E and F.

Proton	WF-1360B	WF-1360C	WF-1360E	WF-1360F
2	5.70	5.63	5.70	5.70
3	6.84	6.80	6.78	6.82
7	3.78	3.71	*ca.* 3.13	3.75
8a	1.20	1.22	1.05	1.18
9	5.57	5.18	5.52	5.55
10	5.35	6.23	5.15	5.34
11	3.27	5.85	3.24	3.25
12a	1.42	1.80	1.33	1.42
13	3.13	4.00	3.02	3.07
15	4.68	4.68	4.78	4.62
16a	1.00	0.98	1.00	1.00
17	3.90	3.90	*ca.* 3.18	3.27
17-OCH_3			3.15	3.17
18a	1.93	1.90	1.84	1.89
19	6.14	6.21	6.09	6.10
20	6.55	6.57	6.58	6.59
21	6.38	6.40	6.38	6.38
22a	2.12	2.14	2.14	2.14
23	6.23	6.26	6.25	6.26
25	7.52	7.53	7.53	7.54
26a	2.45	2.47	2.45	2.48
CH_2CH_3			4.16	
CH_2CH_3			1.27	
4				
5	2.79 (1H)	2.78 (1H)	2.6~2.2 (5H)	2.78 (1H)
5a	2.58 (1H)	2.55 (1H)	2.15~1.9 (4H)	2.56 (1H)
6	2.4~2.3 (2H)	2.4~2.25 (2H)	1.9~1.7 (3H)	2.45~2.3 (2H)
8	2.2~2.05 (2H)	2.2~2.05 (3H)	1.09 (1H)	2.15~1.9 (3H)
14	2.0~1.7 (6H)	2.0~1.65 (5H)		1.85~1.7 (4H)
16	0.74 (1H)	0.7 (1H)		0.73 (1H)
-OH				

^{1}H NMR data were measured in $CDCl_3$ at 400 MHz. Chemical shifts are in ppm downfield of TMS.

Biological Activities

Antimicrobial Activity

Antimicrobial activity of WF-1360 components (WF-1360, WF-1360A, B, C, D, E and F) was determined by a serial broth dilution method in bouillon media for bacteria and in Sabouraud media for fungi. Minimum inhibitory concentration (MIC) was expressed in terms of μg/ml after overnight incubation at 37°C for bacteria and 48~72 hours incubation at 28°C for fungi and yeast. The results are shown in Table 5.

WF-1360 components inhibited *Aureobasidium pullulans* No. WK-1, *Aspergillus niger* IFO 04417 and *Penicillium chrysogenum* ATCC 10002 at low concentrations. However, WF-1360 components have weak antimicrobial activities against *Escherichia coli* NIHJ JC-2, *Staphylococcus aureus* 209P, *Bacillus subtilis* ATTC 6633 and *Candida albicans*.

Antitumor Activity

Cytotoxic activity *in vitro* of WF-1360 components was determined as follows. Concentration of the compounds required for 50% inhibition of cell growth (IC_{50}; μg/ml) was examined by plotting the logarithms of the concentration *versus* the growth rate (percentage of control) of the treated cells. The

Table 4. ^{13}C NMR assignments of WF-1360, WF-1360B, C, E and F[a].

Carbon	WF-1360	WF-1360B	WF-1360C	WF-1360E	WF-1360F
1	168.0 s	165.6 s	166.2 s	165.1 s	165.3 s
2	54.2 d	124.8 d	124.5 d	124.9 d	124.9 d
3	56.0 d	146.4 d	146.7 d	146.4 d	146.1 d
4	35.9 t	38.0 t	38.3 t	37.9*t	38.0 t
5	29.2 d	29.6 d	29.7 d	32.0 d	29.7 d
5a	36.4 t	36.8 t	36.9 t	40.7 t	36.9 t
5b	169.3 s	170.0 s	170.3 s	173.1 s	169.9 s
6	34.2 t	33.7 t	34.6 t	37.5*t	33.9 t
7	82.3 d	82.3 d	83.2 d	73.3 d	82.4 d
8	45.3 d	44.9 d	45.3 d	45.6 d	45.0 d
8a	16.9 q	16.6 q	16.6 q	17.1 q	16.6 q
9	139.5 d	140.1 d	134.6 d	141.5 d	140.1 d
10	126.4 d	126.5 d	126.1*d	125.5 d	126.5 d
11	63.8 d	64.8 d	129.8*d	63.9 d	64.9 d
12	65.1 s	65.7 s	139.3 s	65.6 s	65.7 s
12a	11.7 q	12.4**q	12.8 q	11.7 q	12.2 q
13	77.4 d	76.9*d	77.9 d	78.3 d	77.0 d
14	31.9 t	32.5 t	33.5 t	31.8 t	31.8 t
15	76.8 d	75.4*d	74.9 d	74.1 d	74.8 d
16	38.0 d	39.3 d	40.0 d	39.4 d	38.3 d
16a	9.7 q	9.4 q	9.6 q	10.2 q	9.9 q
17	89.3 d	78.9 d	78.3 d	89.3 d	89.7 d
17-OCH_3	56.1 q			56.3 q	56.2 q
18	136.2 s	138.7 s	138.7 s	136.2 s	136.5 s
18a	11.4 q	12.3**q	11.0 q	11.1 q	11.5 q
19	129.3 d	127.0 d	126.7*d	129.2 d	129.4 d
20	123.8 d	124.3 d	124.2 d	124.1 d	124.0 d
21	137.7 d	137.6 d	137.3 d	137.6 d	137.7 d
22	136.6 s	136.9 s	137.1 s	136.9 s	136.8 s
22a	14.3 q	14.4 q	14.4 q	14.4 q	14.3 q
23	120.8 d	120.6 d	120.4 d	120.7 d	120.7 d
24	138.5 s	138.8 s	138.7 s	138.7 s	138.8 s
25	136.0 d	135.9 d	135.8 d	135.9 d	136.0 d
26	160.8 s	161.0 s	161.0 s	160.9 s	160.9 s
26a	13.8 q	13.8 q	13.8 q	13.8 q	13.8 q
CH_2CH_3				60.6 t	
CH_2CH_3				14.2 q	

[a] ^{13}C NMR data were measured in $CDCl_3$ at 67.5 MHz. Chemical shifts are in ppm downfield of TMS.
*,** Interchangeable assignments.

results are shown in Table 6. The WF-1360 components are highly active against mouse leukemia P388 cells.

Additionally, the antitumor activity *in vivo* of WF-1360 was determined in mice. Lymphoid leukemia L1210 and melanotic melanoma B16 were implanted intraperitoneally in BDF_1 mice (female, 8 weeks old) at an inoculum size of 1×10^5 and 1×10^6 per mouse, respectively. Twenty-four hours after the implantation of tumor cells, graded doses of WF-1360 were administered to mice intraperitoneally. Treatments were on day 1, 2, 3 and 4 (qd 1→4). WF-1360 was solubilized in methanol, concentrated *in vacuo* and then suspended in the sterilized water. Control animals received intraperitoneal doses of physiological saline solution. Five mice were used for each experimental group. Mitomycin C (Kyowa) was used as a reference compound. Antitumor activity was evaluated by the mean survival

Table 5. Antimicrobial spectrum of WF-1360, WF-1360A, B, C, D, E and F.

Microorganism	Minimum inhibitory concentration (μg/ml)						
	WF-1360	A	B	C	D	E	F
Escherichia coli NIHJ JC-2	>100	>100	>100	>100	>100	>100	>100
Staphylococcus aureus 209P	>100	>100	>100	>100	>100	>100	>100
Bacillus subtilis ATCC 6633	>100	>100	>100	>100	>100	>100	100
Candida albicans	>100	>100	>100	>100	>100	>100	>100
Aureobasidium pullulans No. WK-1	0.003	1.5	0.025	0.2	0.8	0.4	0.006
Aspergillus niger IFO 04417	0.15	>10	0.6	>10	>10	>10	0.15
Penicillium chrysogenum ATCC 10002	0.0125	>10	0.15	1.25	>10	0.6	0.02

A: WF-1360A, B: WF-1360B, C: WF-1360C, D: WF-1360D, E: WF-1360E, F: WF-1360F.

Table 6. Cytotoxicity of WF-1360, WF-1360A, B, C, D, E and F against leukemia P388.

	IC_{50} (μg/ml)						
	WF-1360	A	B	C	D	E	F
Cytotoxicity against leukemia P388	0.0001	0.003	0.0008	0.0015	0.12	0.0016	0.0002

Table 7. Antitumor activity of WF-1360 against leukemia L1210.

Drug	Dose (mg/kg/day)	Mean survival time (days)	T/C (%)
WF-1360	1.0	13.1	151
	0.5	11.3	131
	0.25	10.4	121
	0.125	9.3	108
	0.062	8.6	100
Mitomycin C	1.0	12.8	149
Control		8.6	100

Table 8. Antitumor activity of WF-1360 against melanoma B16.

Drug	Dose (mg/kg/day)	Mean survival time (days)	T/C (%)
WF-1360	1.0		Toxic
	0.5	29.8	153
	0.25	27.7	142
	0.125	24.6	126
	0.162	21.3	109
Mitomycin C	1.0	27.8	143
Control		19.5	100

time of group of mice and also expressed as T/C% value (mean survival time of treated group/mean survival time of non-treated group (control), $\times 100$).

The results are shown in Tables 7 and 8. WF-1360 was quite active against leukemia L1210 and melanoma B16. Doses between 0.25~1.0 mg/kg against L1210 and 0.125~0.5 mg/kg against B16 on the qd$\times$4 schedule resulted in a significant increase in the life span.

Acute Toxicity

The acute toxicity of WF-1360 was determined in *dd*Y mice (female, 5 weeks old) by a single intraperitoneal injection of graded doses of the compound into five mice. The LD_{50} was 2.5 mg/kg.

Discussion

A complex of the antitumor antibiotics (WF-1360, WF-1360A, B, C, D, E and F), which exhibited *in vitro* and *in vivo* antitumor activity, was isolated from *Rhizopus* sp. No. F-1360.

Recently it was reported by IWASAKI *et al.* that *Rhizopus chinensis* produced an antibiotic designated as rhizoxin which exhibited potent antifungal activity[3]. Rhizoxin is a 16-membered-ring lactones having an oxazole ring[3,4]. Comparison of physico-chemical properties of WF-1360 with those of rhizoxin showed that the compounds are identical. The structures of WF-1360B, C, E and F ware proposed to be **2**, **3**, **4** and **5**, respectively, on the basis of spectroscopic evidence. The structural study on WF-1360A and D is the subject of future work.

An *in vivo* evaluation of the antitumor activities of WF-1360 is now in progress in the Division of Cancer Treatment, National Cancer Institute (U.S.A.).

Addendum in Proof

WF-1360 is identical to compound FR-900216.

Acknowledgment

The authors wish to express their sincere thanks to the members of Nagoya Pilot Plant of Fujisawa Pharmaceutical Co., Ltd. for preparation of fermentation products.

References

1) OKUHARA, M.; H. TANAKA, E. KINO, S. KIYOTO, M. KOHSAKA & H. AOKI (Fujisawa): FR-900216 substance and its preparation. Japan Kokai 60-27,390, Feb. 12, 1985
2) KIYOTO, S.; M. OKUHARA, E. KINO, H. TANAKA, H. AOKI, M. KOHSAKA & H. IMANAKA (Fujisawa): FR-900216 substance obtainable by cultivating ATCC 20577 for use as an active therapeutic substance and pharmaceutical antitumor composition comprising FR-900216 substance. Eur. Pat. Appl. 132,772, Feb. 13, 1985
3) IWASAKI, S.; H. KOBAYASHI, J. FURUKAWA, M. NAMIKOSHI, S. OKUDA, Z. SATO, I. MATSUDA & T. NODA: Studies on macrocyclic lactone antibiotics. VII. Structure of a phytotoxin "rhizoxin" produced by *Rhizopus chinensis*. J. Antibiotics 37: 354~362, 1984
4) IWASAKI, S.: Structures and activities of rhizoxin and analogues. Abstract of papers, the 29th Meeting of Kanto Branch. Pharmaceutical Society of Japan. pp. 37~40, Tokyo, Nov. 9, 1985