VOL. 53 NO. 6, JUNE 2000 THE JOURNAL OF ANTIBIOTICS pp. 579 — 590

Luminacins: A Family of Capillary Tube Formation Inhibitors from *Streptomyces* sp.

I. Taxonomy, Fermentation, Isolation, Physico-chemical Properties and Structure Elucidation

NOBUAKI NARUSE, RENA KAGEYAMA-KAWASE, YASUHIRO FUNAHASHI and TOSHIAKI WAKABAYASHI*

Tsukuba Research Laboratories, Eisai Co. Ltd.,
5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan

YOSHIO WATANABE, TOMOHIRO SAMESHIMA and KAZUYUKI DOBASHI

Central Research Laboratories, Mercian Corp.,
4-9-1 Johnan, Fujisawa, Kanagawa 251-0057, Japan

(Received for publication January 26, 2000)

A new family of capillary tube formation inhibitors, designated luminacins, has been discovered in the fermentation broth of a soil bacterium. The strain was identified as *Streptomyces* sp. Mer-VD1207 from taxonomic studies. By means of a series of chromatographic procedures, fourteen structurally related components, luminacins A_1, A_2, B_1, B_2, C_1, C_2, D, E_1, E_2, E_3, F, G_1, G_2, and H, were isolated, and their structures were elucidated on the basis of spectroscopic analyses.

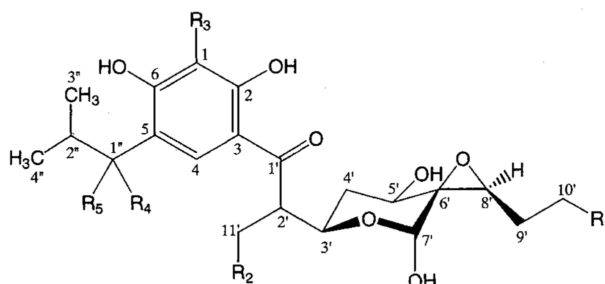
In the course of our screening studies to find novel angiogenesis inhibitors, an actinomycete strain Mer-VD1207 was discovered to produce a new family of inhibitors, designated luminacins[1]. The producing strain was identified as *Streptomyces* sp. by means of taxonomical studies and designated *Streptomyces* sp. Mer-VD1207. The inhibitor complex is lipophilic and was recovered from the fermentation broth by using nonfunctional porous polymer resin. It was separated by chromatography into fourteen components named luminacins A_1, A_2, B_1, B_2, C_1, C_2, D, E_1, E_2, E_3, F, G_1, G_2, and H. Spectral studies indicated that they all have similar structures (Fig. 1). Each component exhibited inhibitory activity against capillary vessel formation in the rat aorta matrix culture model[2]. The activity, including the mechanism of action, is described in an accompanying paper[3]. Here we present the taxonomy of the producing organism, together with details of the fermentation, isolation, physico-chemical properties, and structure elucidation, of this family of inhibitors.

Results and Discussion

Producing Organism

The producing organism, strain Mer-VD1207 was isolated from a soil sample collected in Kumamoto Prefecture, Japan. As shown in Fig. 2, the substrate and aerial hyphae of the cultured strain were well branched and the tops of the latter were straight. After growing, they divided to form a straight chain of spores. The mature spore chains comprised 10 to 50 spores each. The spores were cylindrical in shape, $0.6\sim0.8\times0.8\sim1.0\,\mu m$ in size, and had a smooth surface. Whirls, sclerotic granules, sporangia, and flagellated spores were not observed. The cultural characteristics in various agar media are shown in Table 1. In the tyrosine-agar (ISP-7) medium, the strain produced a melanoid pigment. The strain well utilized the following carbon sources: D-glucose, D-fructose, L-rhamnose, sucrose, inositol, D-mannose, L-arabinose, and D-xylose. L,L-

Fig. 1. Structures of luminacin components.



Luminacin Component	R_1	R_2	R_3	R_4, R_5
A_1	H	CH_2CH_3	H	=O
A_2	H	CH_2CH_3	H	=O
B_1	H	$CH(CH_3)_2$	H	=O
B_2	H	$CH(CH_3)_2$	H	=O
C_1	H	CH_2CH_3	CHO	OCH_3, H
C_2	H	CH_2CH_3	CHO	OCH_3, H
D	H	CH_2CH_3	CHO	H, H
E_1	H	$CH(CH_3)_2$	CHO	OCH_3, H
E_2	H	$CH(CH_3)_2$	CHO	OCH_3, H
E_3	H	$CH_2CH_2CH_3$	CHO	OCH_3, H
F	CH_3	CH_2CH_3	CHO	H, H
G_1	H	$CH(CH_3)_2$	CHO	H, H
G_2	H	$CH_2CH_2CH_3$	CHO	H, H
H	H	CH_2CH_3	$COCH_3$	H, H

Diaminopimelic acid was found in the cell wall, after hydrolysis, by means of cellulose TLC. Based on the taxonomic characteristics described above, Mer-VD1207 was classified as a member of the genus *Streptomyces* and a sample has been deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan with the accession number FERM P-15889.

Fermentation

A well-sporulated slant of *Streptomyces* sp. Mer-VD1207 was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of a medium composed of glycerol 2.0%, glucose 2.0%, soybean meal 2.0%, yeast extract 0.5%, sodium chloride 0.25%, calcium carbonate 0.32%, and 0.2% metal salt solution containing 0.25% copper sulfate, 0.25% manganese chloride, and 0.25% zinc sulfate, the pH being adjusted to pH 7.4 before sterilization. The flask was

Fig. 2. Scanning electron micrograph of aerial mycelium of strain Mer-VD1207.

Bar represents 1 μm.

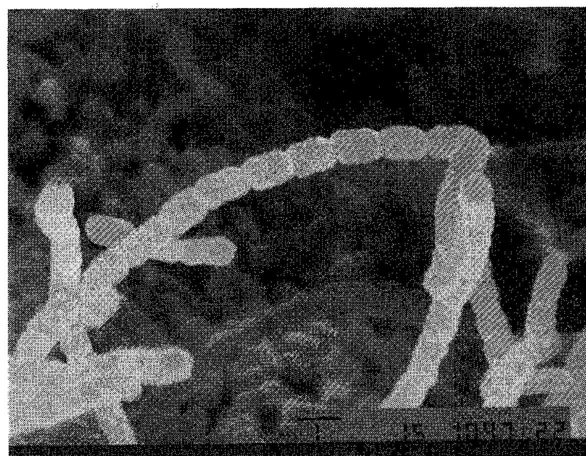


Table 1. Cultural characteristics of strain Mer-VD1207.

Medium	Growth	Aerial mass color	Reverse color	Diffusible pigment
Yeast extract-malt extract agar (ISP-2)	Good	Poor, grayish yellowish pink	Brown	Yellowish brown to brown
Oatmeal agar (ISP-3)	Poor	Poor, none	Brown	None
Inorganic salts-starch agar (ISP-4)	Good	Abundant, grayish yellowish pink [5dc]	Yellowish brown	None
Glycerol-asparagine agar (ISP-5)	Moderate	Poor, grayish yellowish pink	Brown	Yellowish brown to brown

incubated at 28°C for 48 hours on a rotary shaker. The seed culture (5 ml) was inoculated into a 3-liter Sakaguchi flask containing 500 ml of the same medium, and then fermented at 28°C for 48 hours on a reciprocal shaker. Then one-liter portions of the second seed culture were transferred into two 200-liter tank fermenters containing 100 liters of a production medium with the same composition as the seed medium. The fermentation was carried out at 28°C for 64 hours under agitation at 250 rpm with an aeration rate of 50 liters per minute.

Extraction and Purification

The harvested broth (200 liters) was separated with a filter press to afford a supernatant (170 liters) and mycelial cake. The broth supernatant was passed over Diaion HP-20 resin (20 liters) in a column, and the resin was washed with water and then 20% aqueous methanol (60 liters each). Subsequent elution was performed with 80% aqueous acetone (60 liters) and the eluate was concentrated to 10 liters *in vacuo*. The mycelial cake was extracted with methanol (100 liters), and the extract was concentrated to 24 liters. Both concentrates were mixed and extracted twice with ethyl acetate (17 liters). After concentration to dryness the organic layer gave 83 g of a dark brown oil (luminacin complex). One half of the crude complex was charged on a column of reverse phase silica gel (YMC-GEL ODS-AM 120-S50, 50 i. d.×950 mm) which had been equilibrated with acetonitrile-0.15% KH_2PO_4 buffer, pH 3.5 (1 : 1). Elution was carried out with the same solvent mixture in the ratio of 1 : 1 (4 liters), 11 : 9 (6.3 liters), 3 : 2 (7.8 liters), and finally 13 : 7 (5.7 liters). The eluate was collected in fractions (300 ml each) and each fraction was examined by HPLC on ODS (conditions are given in the Physico-chemical Properties section) and subjected to bioassay for angiogenesis inhibition. Luminacins A_1 and A_2 were eluted first, followed by luminacin components B_1 to H in alphabetical order. The relevant fractions were pooled and evaporated to remove acetonitrile, and the resultant aqueous solution was extracted with ethyl acetate. Repetition of the ODS chromatographic separation of the luminacin complex yielded crude solids mainly consisting of the following components: A_1 and A_2 mixture (789 mg), B_1 and B_2 mixture (120 mg), C_1 and C_2 mixture (756 mg), D and E_1 mixture (334 mg), E_2 and E_3 mixture (416 mg), F, G_1, and G_2 mixture (191 mg), and H (498 mg). Components C_1, C_2, and H were further purified by preparative HPLC using an ODS column (YMC J'sphere ODS-H80, 20 i. d.×250 mm, 4 μm, 80 Å) with the same acetonitrile-buffer system as above (C_1 and C_2, 3 : 2; H, 13 : 7; flow rate, 12 ml/minute), followed by preparative TLC on silica gel (Merck No. 5744; toluene-methanol, 9 : 1), providing C_1 (60.8 mg), C_2 (256.4 mg), and H (89.3 mg) as pale yellow powders. Components of A_1, A_2, B_1, and B_2 were effectively separated in the preparative silica gel TLC step after HPLC purification (acetonitrile-buffer, 3 : 2) to yield A_1 (57.0 mg), A_2 (131.8 mg), B_1 (5.8 mg), and B_2 (11.0 mg). Components D, E_1, E_2, E_3, and G_2 were isolated by

Table 2a. Physico-chemical properties of luminacin components A_1~D.

	Luminacin component			
	A_1	A_2	B_1	B_2
Appearance	White powder	White powder	White powder	White powder
Molecular formula	$C_{23}H_{32}O_8$	$C_{23}H_{32}O_8$	$C_{24}H_{34}O_8$	$C_{24}H_{34}O_8$
HRFAB-MS	Calcd for $C_{23}H_{33}O_8$: 437.2175 $(M+H)^+$ Found: 437.2175	Calcd for $C_{23}H_{33}O_8$: 437.2175 $(M+H)^+$ Found: 437.2153	Calcd for $C_{24}H_{35}O_8$: 451.2332 $(M+H)^+$ Found: 451.2330	Calcd for $C_{24}H_{35}O_8$: 451.2332 $(M+H)^+$ Found: 451.2354
$[\alpha]_D^{23}$ in $CHCl_3$	+2.5° (*c* 0.1)	-6.2° (*c* 0.1)	+1.9° (*c* 0.1)	-6.2° (*c* 0.1)
UV λ_{max} nm (ε)				
in 0.01 N HCl-MeOH	257 (44100), 280 (sh, 13900), 324 (6100)	257 (46000), 280 (sh, 13600), 324 (6000)	257 (41600), 280 (sh, 13400), 324 (5900)	257 (40800), 280 (sh, 13600), 325 (6000)
in 0.01 N NaOH-MeOH	233 (6200), 261 (18400), 316 (17000), 347 (16700)	233 (6300), 261 (18700), 316 (17300), 346 (16700)	233 (5700), 261 (16900), 317 (15900), 345 (15400)	233 (5400), 261 (16000), 317 (14700), 347 (16000)
HPLC retention time[a] (minutes)	3.7	3.7	4.8	4.8
TLC Rf[b]	0.20, 0.38	0.16, 0.35	0.23, 0.35	0.17, 0.35

	Luminacin component		
	C_1	C_2	D
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder
Molecular formula	$C_{25}H_{36}O_9$	$C_{25}H_{36}O_9$	$C_{24}H_{34}O_8$
HRFAB-MS	Calcd for $C_{25}H_{37}O_9$: 481.2438 $(M+H)^+$ Found: 481.2441	Calcd for $C_{25}H_{37}O_9$: 481.2438 $(M+H)^+$ Found: 481.2438	Calcd for $C_{24}H_{35}O_8$: 451.2332 $(M+H)^+$ Found: 451.2330
$[\alpha]_D^{23}$ in $CHCl_3$	-97.7° (*c* 0.1)	+50.2° (*c* 0.1)	-13.0° (*c* 0.1)
UV λ_{max} nm (ε)			
in 0.01 N HCl-MeOH	253 (27400), 276 (18000), 353 (7600)	253 (25400), 277 (18400), 352 (7600)	255 (27300), 276 (18000), 357 (7800)
in 0.01 N NaOH-MeOH	278 (18200), 332 (24600)	277 (17100), 334 (25200)	279 (18000), 338 (26400)
HPLC retention time[a] (minutes)	5.4	6.0	7.0
TLC Rf[b]	0.21, 0.40	0.21, 0.40	0.18, 0.23

[a] Column: YMC J'sphere ODS-H80, 4.6 i. d. × 75 mm, mobile phase: CH_3CN-0.15% KH_2PO_4 buffer (pH 3.5) = 3:2, flow rate: 1 ml/min, detection: UV at 254 nm.

[b] Silica gel 60 F_{254}, Merck, No. 5715 developed with the solvent systems: toluene-MeOH = 9:1, hexane-acetone = 7:3.

preparative HPLC on ODS (acetonitrile-buffer: D, E_1, E_2, E_3, 3:2; F, G_1, G_2, 13:7), and F and G_1 were finally separated by the next TLC step. Thus, components D (63.7 mg), E_1 (16.2 mg), E_2 (125.4 mg), E_3 (2.8 mg), F (9.2 mg), G_1 (10.4 mg), and G_2 (3.2 mg) were obtained as pale yellow powders.

Physico-chemical Properties

Luminacin components were obtained as white or pale yellow powders, soluble in methanol, chloroform, acetonitrile, acetone, ethyl acetate, and dimethyl sulfoxide, but insoluble in water. They gave positive responses to the iodine vapor, sulfuric acid, and ferric chloride tests. Their UV absorption spectra are pH-dependent. The physico-

Table 2b. Physico-chemical properties of luminacin components E_1~H.

	Luminacin component			
	E_1	E_2	E_3	F
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder	Pale yellow powder
Molecular formula	$C_{26}H_{38}O_9$	$C_{26}H_{38}O_9$	$C_{26}H_{38}O_9$	$C_{25}H_{36}O_8$
HRFAB-MS	Calcd for $C_{26}H_{39}O_9$: 495.2594 $(M+H)^+$ Found: 495.2598	Calcd for $C_{26}H_{39}O_9$: 495.2594 $(M+H)^+$ Found: 495.2600	Calcd for $C_{26}H_{39}O_9$: 495.2594 $(M+H)^+$ Found: 495.2600	Calcd for $C_{25}H_{37}O_8$: 465.2488 $(M+H)^+$ Found: 465.2459
$[\alpha]_D^{23}$ in $CHCl_3$	-82.6° (*c* 0.1)	+54.1° (*c* 0.1)	+4.4° (*c* 0.04)	-11.5° (*c* 0.1)
UV λ_{max} nm (ε)				
in 0.01 N HCl-MeOH	253 (27000), 276 (18600), 352 (7600)	254 (26300), 278 (18000), 351 (7500)	253 (27800), 278 (18900), 351 (7700)	255 (27700), 276 (18100), 356 (7800)
in 0.01 N NaOH-MeOH	277 (19400), 333 (26200)	277 (18600), 338 (26800)	276 (17600), 338 (26000)	279 (18600), 337 (25300)
HPLC retention time[a] (minutes)	7.4	8.3	8.3	9.4
TLC Rf[b]	0.18, 0.30	0.21, 0.30	0.18, 0.40	0.28, 0.42

	Luminacin component		
	G_1	G_2	H
Appearance	Pale yellow powder	Pale yellow powder	Pale yellow powder
Molecular formula	$C_{25}H_{36}O_8$	$C_{25}H_{36}O_8$	$C_{25}H_{36}O_8$
HRFAB-MS	Calcd for $C_{25}H_{37}O_8$: 465.2488 $(M+H)^+$ Found: 465.2475	Calcd for $C_{25}H_{37}O_8$: 465.2488 $(M+H)^+$ Found: 465.2482	Calcd for $C_{25}H_{37}O_8$: 465.2488 $(M+H)^+$ Found: 465.2487
$[\alpha]_D^{23}$ in $CHCl_3$	-11.2° (*c* 0.1)	+2.3° (*c* 0.04)	-1.6° (*c* 0.1)
UV λ_{max} nm (ε)			
in 0.01 N HCl-MeOH	255 (27300), 277 (18100), 356 (7700)	255 (27300), 277 (18400), 356 (7800)	255 (28100), 277 (18500), 356 (7900)
in 0.01 N NaOH-MeOH	278 (18700), 337 (24900)	279 (18800), 337 (25300)	278 (19200), 337 (25500)
HPLC retention time[a] (minutes)	9.4	9.7	10.1
TLC Rf[b]	0.23, 0.27	0.24, 0.29	0.25, 0.27

[a,b] See Table 2a.

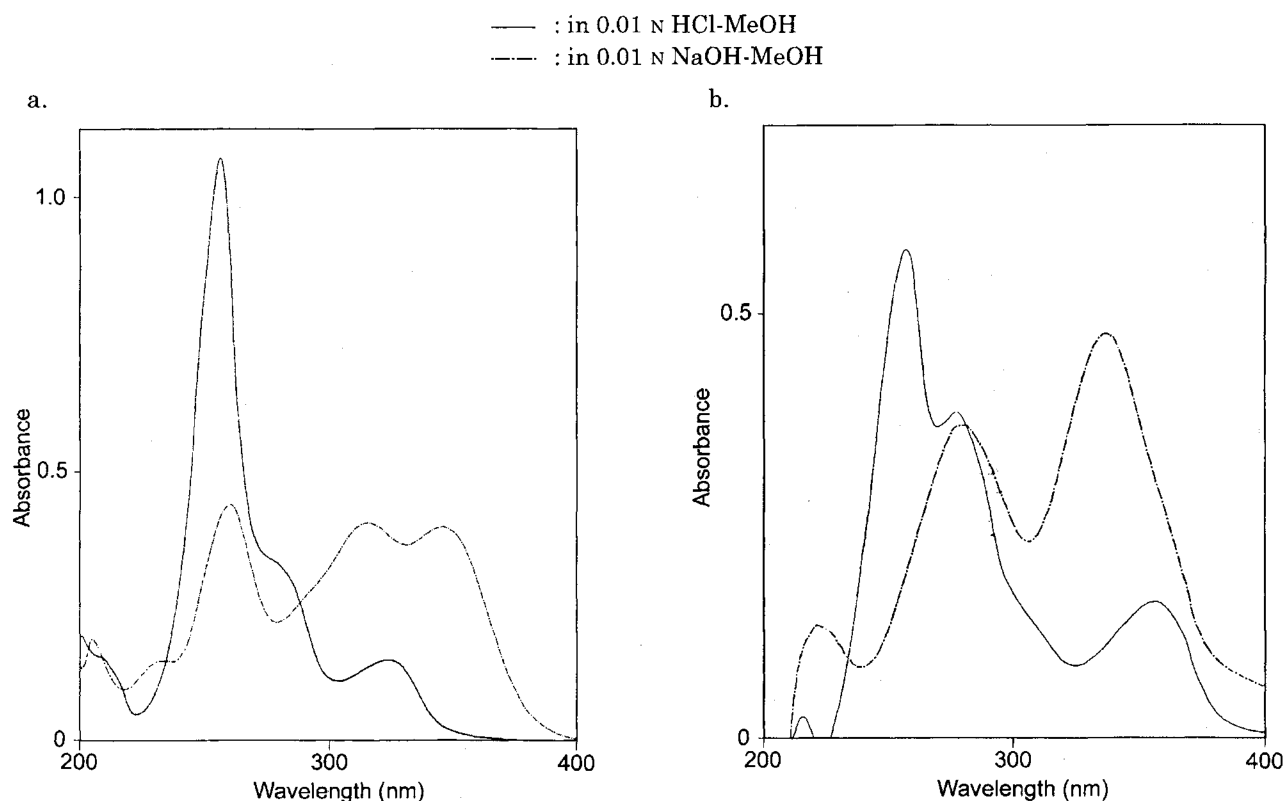
chemical properties of the luminacin components are summarized in Table 2. The UV and IR spectra of luminacins A_1 and C_2 as representative components are illustrated in Figs. 3 and 4.

Structures of Luminacins C_1 and C_2

Since luminacin C_2 was available in the largest quantity, structural studies were first focused on this component. The structures of the other components were determined analogously.

The IR spectrum (Fig. 4, b) displayed absorptions at 3400, 1630, and 1090 cm^{-1} due to hydroxyl, carbonyl, and ether moieties, respectively. The molecular formula was established as $C_{25}H_{36}O_9$ by HRFAB-MS, which corresponds to the number of protons and carbons observed by NMR. Analysis of the ^1H NMR, ^{13}C NMR, DEPT, and HSQC spectra revealed the presence of seven quaternary carbons: one ketone carbonyl, five aromatic and one aliphatic (C–O) carbons; and the following proton-bearing carbons: one formyl, one aromatic CH, five CH–O, two CH–C, four CH_2–C, one methoxyl, and four methyls. The

Fig. 3. UV spectra of luminacins A_1 (a) and C_2 (b).

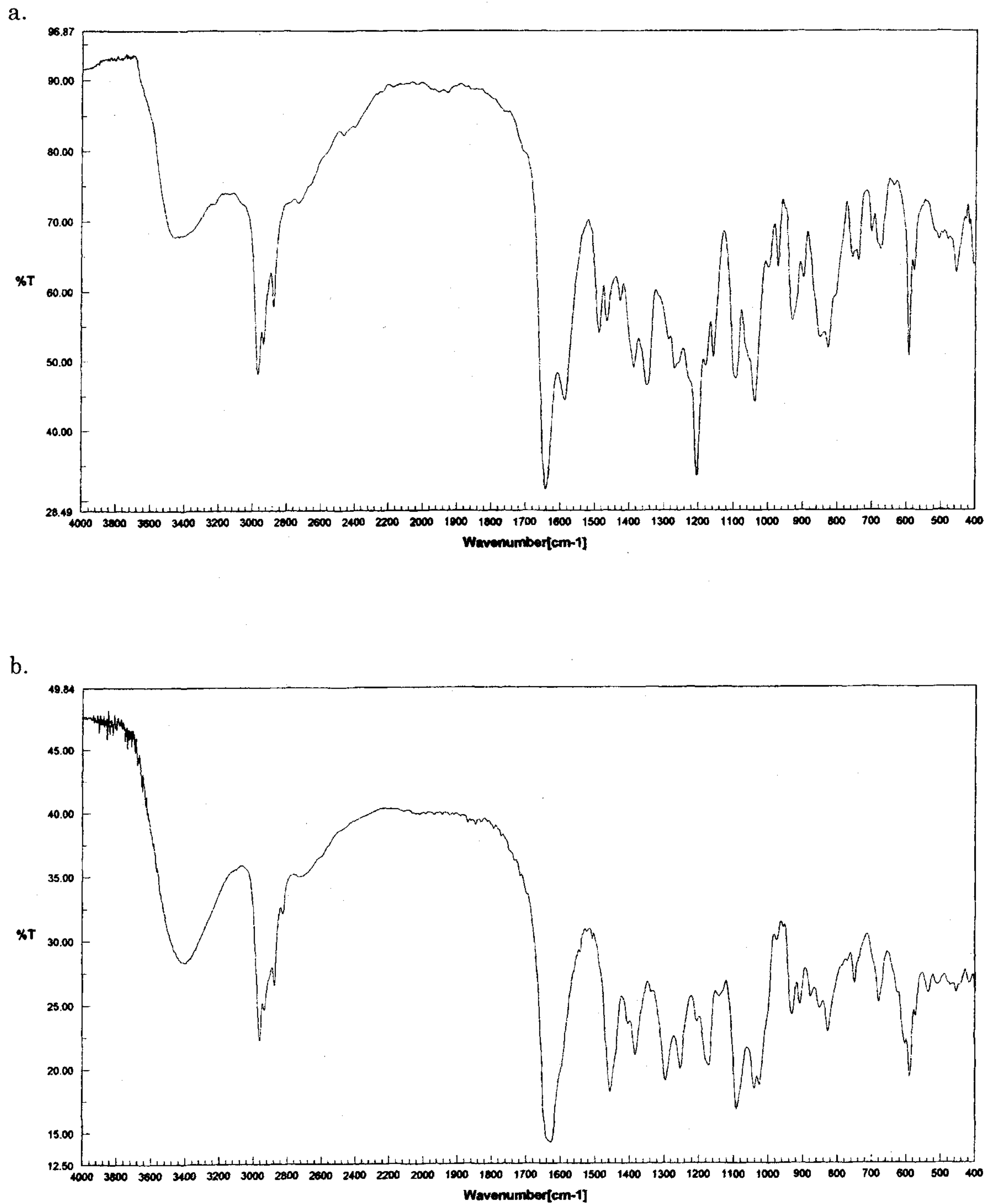


four D_2O-exchangeable proton signals were assigned to two phenolic (δ 14.21 and 12.99) and two aliphatic hydroxyl (δ 2.99 and 1.62) protons. The ^{1}H-^{1}H correlations observed in the ^{1}H-^{1}H COSY spectrum revealed three partial structures besides an alkyl hydroxyl group at C-7′ represented by solid lines in Fig. 5. Connection of these partial structures was made by means of HMBC experiments optimized for 8 Hz couplings, demonstrating a spiropyran skeleton with an epoxy function (Fig. 5). The ^2J and ^3J ^{1}H-^{13}C correlations in the HMBC spectrum also revealed a pentasubstituted benzene moiety. The connectivities between C-2′ and C-3 *via* the carbonyl group C-1′, and C-1″ and C-5 provided the complete planar structure of luminacin C_1. The positions of the substituents of the benzene chromophore were confirmed by NOE correlations detected in the NOESY spectrum (Fig. 6). The formyl proton correlated to two phenolic hydroxyl protons, indicating that the sequence of –OH, –CHO, and –OH was in this order. The positions of the two alkyl chains of the benzene ring were also confirmed by the NOE correlations with aromatic H-4, as shown in Fig. 6.

The relative stereochemistry of the spiropyran moiety, including the epoxy group, was determined to be as shown in Fig. 6 from vicinal coupling constants and NOE correlations from NOESY experiments. The large values of the coupling constants ($^3J_{H\text{-}3',H\text{-}4'(axial)}=11$ Hz, $^3J_{H\text{-}4'(axial),H\text{-}5'}=11$ Hz) unequivocally defined the axial orientations of the protons at the C-3′, C-4′ (proton at 1.44 ppm), and C-5′ positions. The NOEs between H-3′ and the 1,3-diaxially disposed H-5′ and OH-7′ supported the normal chair conformation for the pyran ring. The NOEs between H-5′ and H-8′, and H-7′ and H_2-9′ established the relative configuration of the C-8′-ethyl group and the epoxy ring.

The molecular formula of luminacin C_1 was determined as $C_{25}H_{36}O_9$, which was identical with that of C_2. The spectral data of these components were quite similar, and their planar structures established by means of 2D NMR measurements were identical. The main differences were in the specific optical rotation and the chemical shifts in the ^{13}C NMR spectra at around the C-2′ position (C-2′, $\Delta\delta$=0.5 ppm; C-11′, $\Delta\delta$=0.3 ppm; C-3, C-3′, C-4′, $\Delta\delta$=0.2 ppm). Therefore, luminacin C_1 was deduced to be a

Fig. 4. IR spectra of luminacins A_1 (a) and C_2 (b) in KBr.



diastereoisomer of luminacin C_2 at the C-2′ position.

Structures of Luminacins E_1, E_2, and E_3

Luminacins E_1, E_2, and E_3 showed the same molecular composition, $C_{26}H_{38}O_9$, CH_2 more than the formula for C_1 and C_2. The NMR analysis indicated that this additional unit was located in the C-2′ alkyl side chain of each component. Luminacins E_1, E_2, and E_3 had the same planar structure as C_1 and C_2 with an *n*-butyl (E_1 and E_2) or isobutyl (E_3) group instead of the *n*-propyl group at the C-2′ position of C_1 and C_2. The difference between E_1 and E_2

Fig. 5. ^{1}H-^{1}H COSY and HMBC correlations of luminacin C_2.

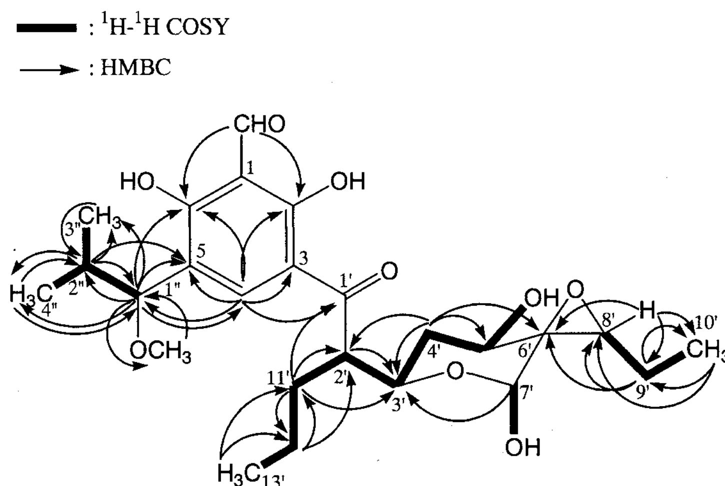
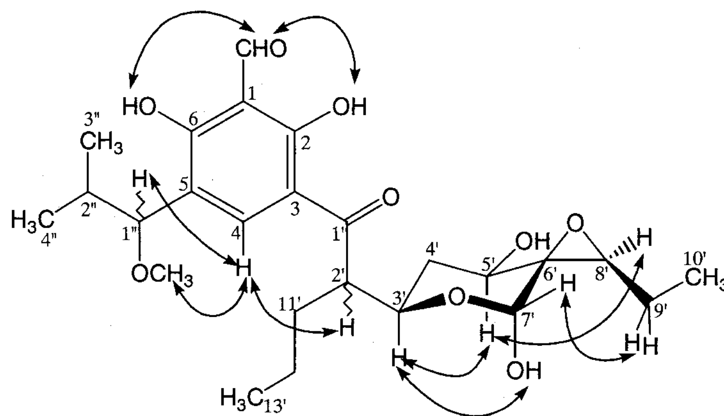


Fig. 6. NOESY correlations of luminacin C_2.



was ascribed to epimeric configurations at the C-2′ position, analogous to the situation described above.

Structures of Luminacins D, F, G_1, G_2, and H

This group of luminacin components lacks the C-1″ methoxyl group and thus has an isobutyl side chain in common at the C-5 position of the benzene ring.

The molecular formula of luminacin D was determined as $C_{24}H_{34}O_8$, which is OCH_2 less than those of C_1 and C_2. Detailed NMR analysis characterized it as a C-5 isobutyl analogue of C_1 or C_2. The ^{13}C chemical shifts at around the C-2′ position were more similar to those of C_2 than to those of C_1, indicating that D has the same stereochemistry as C_2.

The molecular compositions of luminacins F, G_1, G_2, and H were determined as $C_{25}H_{36}O_8$. The NMR analyses of G_1 and G_2, including 2D NMR experiments, revealed that they were the C-1″-demethoxyl analogues of E_1 (E_2) and E_3, respectively.

The ^{1}H and ^{13}C NMR spectra of luminacin F showed an additional CH_2 compared with luminacin D. The ^{1}H-^{1}H connectivities in the ^{1}H-^{1}H COSY spectrum clearly demonstrated that this methylene was a part of an *n*-propyl group located at the C-8′ position. Thus, luminacin F is the C-8′-*n*-propyl analogue of luminacin D.

In the NMR spectra of luminacin H, no formyl group

Table 3a. ^{1}H NMR data (399.65 MHz in $CDCl_3$) of luminacin components A_1~D.

Chemical shifts in ppm (multiplicity, coupling constants in Hz) relative to $CHCl_3$ (7.27 ppm).

Position	Luminacin component						
	A_1	A_2	B_1	B_2	C_1	C_2	D
1	6.43 (s)	6.46 (s)	6.44 (s)	6.46 (s)	-	-	-
1-CHO	-	-	-	-	10.42 (s)	10.43 (s)	10.41 (s)
1-$COCH_3$	-	-	-	-	-	-	-
2-OH	13.21 (s)*	13.21 (s)*	13.22 (s)*	13.213 (s)*	14.17 (s)	14.21 (s)	14.15 (s)
4	8.39 (s)	8.42 (s)	8.43 (s)	8.46 (s)	8.08 (s)	8.05 (s)	7.74 (s)
6-OH	13.18 (s)*	13.17 (s)*	13.18 (s)*	13.205 (s)*	12.97 (s)	12.99 (s)	12.98 (s)
2'	3.51 (ddd, 10,8,3.5)	3.59 (ddd, 10,8,3.5)	3.58 (m)	3.69 (ddd, 10.5,7,3.5)	3.61 (m)	3.68 (ddd, 9.5,7.5,4.5)	3.56 (dt, 4,9)
3'	4.46 (ddd, 11,8,1.5)	4.41 (ddd, 11,8,1.5)	4.39 (ddd, 11,9.5,2)	4.34 (ddd, 11,7,1.5)	4.40 (ddd, 11,10,2)	4.41 (ddd, 11,7.5,1.5)	4.38 (ddd, 11,9,1.5)
4'	2.32 (ddd, 11,5,1.5) 1.54 (m)	2.05 (ddd, 11,5,1.5) 1.41 (q, 11)	2.34 (ddd, 11,5,2) 1.53 (m)	2.03 (ddd, 11,4.5,1.5) 1.45 (q, 11)	2.05 (ddd, 11,5,2) 1.44 (q, 11)	2.03 (ddd, 11,5,1.5) 1.48 (q, 11)	2.02 (ddd, 11,4.5,1.5) 1.42 (q, 11)
5'	4.27 (dt, 5,11)	4.22 (dt, 5,11)	4.27 (dt, 5,11)	4.21 (dt, 4.5,11)	4.19 (dt, 5,11)	4.19 (dt, 5,11)	4.20 (dt, 4.5,11)
5'-OH	1.68 (d, 11)	1.51 (d, 11)	1.63 (d, 11)	1.54 (d, 11)	1.56 (d, 11)	1.62 (d, 11)	1.55 (d, 11)
7'	4.81 (d, 2.5)	5.02 (d, 3)	4.82 (br s)	5.01 (d, 3)	4.97 (d, 3)	4.96 (d, 2.5)	4.99 (d, 3)
7'-OH	2.82 (d, 2.5)	2.59 (d, 3)	2.49 (br s)	2.59 (d, 3)	2.89 (br s)	2.99 (d, 2.5)	2.74 (d, 3)
8'	3.30 (t, 6.5)	3.31 (t, 6.5)	3.30 (dd, 7,6)	3.31 (t, 6.5)	3.28 (t, 6.5)	3.27 (t, 6.5)	3.29 (dd, 7,6)
9'	1.57 (m)	1.63 (m)	1.58 (m)	1.64 (m)	1.64 (dq, 6.5,7.5)	1.60 (m)	1.61 (m)
10'	1.06 (t, 7.5)	1.09 (t, 7.5)	1.06 (t, 7.5)	1.10 (t, 7.5)	1.08 (t, 7.5)	1.07 (t, 7.5)	1.08 (t, 7.5)
10'-CH_3	-	-	-	-	-	-	-
11'	1.75 (m) 1.61 (m)	1.85 (2H, m)	1.84 (ddd, 13.5,11,4) 1.33 (ddd, 13.5,10,3.5)	1.91 (ddd, 13.5,10.5,5) 1.66 (m)	1.80 (2H,m)	1.82 (2H,m)	1.83 (2H,m)
11'-$\underline{CH_2}CH_3$	1.25 (m)	1.25 (m)	-	-	1.28 (m)	1.24 (m)	1.25 (m)
11'-$CH_2\underline{CH_3}$	0.89 (t, 7.5)	0.90 (t, 7.5)	-	-	0.89 (t, 7.5)	0.88 (t, 7.5)	0.88 (t, 7.5)
11'-$\underline{CH}(CH_3)_2$	-	-	1.44 (m)	1.50 (m)	-	-	-
11'-$CH(\underline{CH_3})_2$	-	-	0.893 (d, 6.5) 0.887 (d, 6.5)	0.91 (6H,d, 6.5)	-	-	-
11'-$\underline{CH_2}CH_2CH_3$	-	-	-	-	-	-	-
11'-$CH_2\underline{CH_2}CH_3$	-	-	-	-	-	-	-
11'-$CH_2CH_2\underline{CH_3}$	-	-	-	-	-	-	-
1"	-	-	-	-	4.33 (d, 6)	4.34 (d, 6.5)	2.47 (dd, 13.5, 7) 2.42 (dd, 13.5, 7)
1"-OCH_3	-	-	-	-	3.23 (s)	3.22 (s)	-
2"	3.59 (sept, 7)	3.55 (sept, 7)	3.59 (sept, 7)	3.57 (sept, 7)	1.92 (m)	1.96 (m)	1.91 (m)
3", 4"	1.29 (d, 7) 1.28 (d, 7)	1.29 (d, 7) 1.28 (d, 7)	1.30 (d, 7) 1.28 (d, 7)	1.30 (d, 7) 1.29 (d, 7)	0.93 (d, 6.5) 0.90 (d, 6.5)	0.96 (d, 6.5) 0.88 (d, 6.5)	0.92 (d, 6.5) 0.91 (d, 6.5)

* Assignment could be interchanged in each column.

signal was observed, however extra carbonyl (δ_C 205.9) and methyl (δ_C 33.6, δ_H 2.99) signals were present. The $^2J_{H,C}$ correlation in the HMBC spectrum revealed direct connectivity between these signals, *i.e.*, a methylcarbonyl group. The location of this $COCH_3$ group at the C-1 position was confirmed by means of NOESY experiments, which showed NOE correlations between the singlet methyl proton and two phenolic protons (δ 15.11 and 14.73). Therefore, luminacin H corresponds to the C-1-methylcarbonyl analogue of luminacin D.

Structures of Luminacins A_1, A_2, B_1, and B_2

The UV and IR spectra of these components were significantly different from those of the components mentioned above (Figs. 3 and 4). The difference was assumed to be attributable to the benzene chromophore. Luminacins A_1 and A_2 had the same molecular formula,

Table 3b. ^{1}H NMR data (399.65 MHz in $CDCl_3$) of luminacin components E_1~H.

Position	Luminacin component						
	E_1	E_2	E_3	F	G_1	G_2	H
1	-	-	-	-	-	-	-
1-CHO	10.42 (s)	10.42 (s)	10.43 (s)	10.42 (s)	10.42 (s)	10.42 (s)	-
1-$COCH_3$	-	-	-	-	-	-	2.79 (s)
2-OH	14.21 (s)	14.21 (s)	14.20 (s)	14.15 (s)	14.20 (s)	14.16 (s)	15.11 (s)*
4	8.13 (s)	8.08 (s)	8.03 (s)	7.74 (s)	7.78 (s)	7.73 (s)	7.71 (s)
6-OH	12.98 (s)	12.98 (s)	12.99 (s)	12.99 (s)	13.00 (s)	12.99 (s)	14.73 (s)*
2'	3.72 (ddd, 10,7,3.5)	3.75 (ddd, 10.5,7,3.5)	3.61 (dt, 5,8)	3.56 (dt, 4.5,8.5)	3.65 (ddd, 10.5,7.5,3.5)	3.54 (dt, 5.5,8)	3.58 (dt, 4.5,8)
3'	4.33 (ddd, 11,7,1.5)	4.30 (m)	4.40 (ddd, 11.5,8,1.5)	4.38 (ddd, 11,8.5,2)	4.30 (ddd, 11,7.5,2)	4.38 (ddd, 11,8,1.5)	4.38 (ddd, 11,8,1.5)
4'	2.02 (ddd, 11,4.5,1.5) 1.47 (q, 11)	2.02 (ddd, 11,4.5,2) 1.46 (q, 11)	2.04 (m) 1.43 (q, 11.5)	2.03 (ddd, 11,4.5,2) 1.42 (q, 11)	1.99 (ddd, 11,4.5,2) 1.45 (q, 11)	2.03 (ddd, 11,5,1.5) 1.42 (q, 11)	2.02 (ddd, 11,4.5,1.5) 1.42 (q, 11)
5'	4.17 (dt, 4.5,11)	4.17 (dt, 4.5,11)	4.19 (dt, 5,11.5)	4.20 (dt, 4.5,11)	4.18 (dt, 4.5,11)	4.20 (dt, 5,11)	4.20 (dt, 4.5,11)
5'-OH	1.53 (d, 11)	1.59 (d, 11)	1.53 (d, br s)	1.50 (d, 11)	1.50 (d, 11)	1.50 (d, 11)	1.54 (d, 11)
7'	4.95 (d, 3)	4.94 (d, 2.5)	4.98 (d, 2.5)	5.01 (d, 3.5)	4.99 (d, 3)	5.00 (d, 2.5)	5.01 (d, 2.5)
7'-OH	2.69 (br s)	2.95 (d, 2.5)	2.69 (d, 2.5)	2.52 (d, 3.5)	2.50 (d, 3)	2.69 (d, 2.5)	2.73 (d, 2.5)
8'	3.28 (dd, 7,6)	3.27 (dd, 7,6)	3.28 (dd, 7,6)	3.34 (dd, 7,6)	3.29 (dd, 7,6)	3.30 (dd, 7,6)	3.30 (dd, 7,6)
9'	1.61 (2H, m)	1.59 (2H, m)	1.60 (2H, m)	1.66 (m) 1.55 (m)	1.60 (2H, m)	1.63 (2H, m)	1.64 (2H, m)
10'	1.08 (t, 7.5)	1.07 (t, 7.5)	1.08 (t, 7.5)	1.55 (m)	1.09 (t, 7.5)	1.09 (t, 7.5)	1.09 (t, 8)
10'-CH_3	-	-	-	0.99 (d, 7.5)	-	-	-
11'	1.89 (ddd, 13.5,10,4.5) 1.58 (m)	1.90 (ddd, 13,10.5,4.5) 1.59 (m)	1.84 (2H, m)	1.83 (2H, m)	1.90 (ddd, 13.5,10.5,4.5) 1.63 (m)	1.84 (2H, m)	1.83 (2H,m)
11'-$\underline{CH_2}CH_3$	-	-	-	1.24 (m)	-	-	1.25 (m)
11'-$CH_2\underline{CH_3}$	-	-	-	0.89 (t, 7.5)	-	-	0.88 (t, 7.5)
11'-$\underline{CH}(CH_3)_2$	1.46 (m)	1.46 (m)	-	-	1.45 (m)	-	-
11'-$CH(\underline{CH_3})_2$	0.88 (d, 6.5) 0.87 (d, 6.5)	0.88 (d, 6.5) 0.86 (d, 6.5)	-	-	0.88 (d, 6.5) 0.87 (d, 6.5)	-	-
11'-$\underline{CH_2}CH_2CH_3$	-	-	1.19 (m)	-	-	1.20 (m)	-
11'-$CH_2\underline{CH_2}CH_3$	-	-	1.28 (m)	-	-	1.28 (m)	-
11'-$CH_2CH_2\underline{CH_3}$	-	-	0.84 (t, 7)	-	-	0.85 (t, 7)	-
1"	4.35 (d, 6.5)	4.34 (d, 6)	4.34 (d, 6)	2.47 (dd, 13.5,7) 2.42 (dd, 13.5,7)	2.50 (dd, 14,7) 2.41 (dd, 14,7)	2.48 (dd, 13.5,7) 2.42 (dd, 13.5,7)	2.47 (dd, 13.5,7) 2.41 (dd, 13.5,7)
1"-OCH_3	3.23 (s)	3.22 (s)	3.23 (s)	-	-	-	-
2"	1.92 (m)	1.90 (m)	1.92 (m)	1.91 (m)	1.92 (m)	1.91 (m)	1.92 (m)
3", 4"	0.92 (d, 7) 0.91 (d, 7)	0.94 (d, 7) 0.90 (d, 7)	0.94 (d, 7) 0.90 (d, 7)	0.93 (d, 7) 1.29 (d, 7)	0.93 (d, 6.5) 0.92 (d, 6.5)	0.93 (d, 6.5) 0.92 (d, 6.5)	0.92 (d, 6.5) 0.91 (d, 6.5)

* Assignment could be interchanged in each column.

$C_{23}H_{32}O_8$, and B_1 and B_2 had the same molecular formula, $C_{24}H_{34}O_8$, which corresponds to CH_2 more than the former. The ^{1}H and ^{13}C NMR spectra of this group of the components showed in common the presence of an additional sp^2 methine and a carbonyl, while the signals of the formyl function seen in other components were absent. By means of detailed 2D NMR analyses of these components including ^{1}H-^{1}H COSY, HSQC, and HMBC experiments, the 2,3,5,6-tetrasubstituted benzene chromophore with an isopropylcarbonyl side chain at the C-5 position was clearly demonstrated. The C-1-deformyl structure was also confirmed by the NOE correlations of the C-1 aromatic proton to the adjacent phenolic protons observed in the NOESY spectrum.

The planar structures of A_1 and A_2 based on the NMR data were identical. The difference between these components was deduced to be due to the stereochemistry at the C-2′ position, in analogy with the case of C_1 and C_2.

The structures of luminacins B_1 and B_2 were elucidated as the C-2′-isobutyl analogues of A_1 and A_2. The chiralities at their C-2′ positions were deduced to correspond to those of A_1 and A_2, respectively, on the basis of the ^{13}C chemical

Table 4. ^{13}C NMR data (100.4 MHz in $CDCl_3$).

Chemical shifts in ppm relative to $CDCl_3$ (77.00 ppm).

Position	Luminacin component													
	A_1	A_2	B_1	B_2	C_1	C_2	D	E_1	E_2	E_3	F	G_1	G_2	H
1	105.1	105.4	105.1	105.4	109.2	109.1	109.3	109.2	109.2	109.1	109.4	109.4	109.4	109.5
1-CHO	-	-	-	-	194.4	194.4	194.3	194.4	194.4	194.3	194.3	194.3	194.3	-
1-$\underline{CO}CH_3$	-	-	-	-	-	-	-	-	-	-	-	-	-	205.9
1-$CO\underline{CH_3}$	-	-	-	-	-	-	-	-	-	-	-	-	-	33.6
2	169.7	169.9	169.7	169.9	167.9	167.9	168.0	167.9	167.9	167.8	168.0	168.0	168.0	170.0
3	111.9*	112.0*	111.9*	112.0*	113.0	113.2	112.5	113.1	113.3	113.1	112.5	112.7	112.6	112.1
4	135.6	135.5	135.7	135.5	137.6	137.5	139.4	137.6	137.6	137.4	139.4	139.4	139.5	137.8
5	114.9*	114.5*	115.1*	114.6*	120.3	120.3	121.0	120.3	120.2	120.3	121.0	121.0	121.0	121.5
6	168.7	169.2	168.7	169.2	167.3	167.4	167.5	167.3	167.3	167.3	167.5	167.5	167.5	167.7
1'	207.5	206.5	207.8	206.6	207.2	207.3	206.7	207.2	207.2	207.2	206.7	206.7	206.7	206.6
2'	50.0	49.9	48.3	47.7	49.8	49.3	49.4	47.6	47.2	49.5	49.4	47.4	49.6	49.3
3'	70.2	69.8	71.0	70.6	69.6	69.8	69.8	70.3	70.5	69.7	69.8	70.6	69.8	69.9
4'	37.3	37.3	37.5	37.1	37.1	36.9	37.3	36.9	36.6	36.9	37.9	37.1	37.3	37.4
5'	62.5	62.4	62.5	62.5	62.6	62.6	62.4	62.6	62.7	62.5	62.4	62.5	62.4	62.5
6'	61.9	61.8	61.8	61.8	61.8	61.8	61.8	61.7	61.7	61.7	61.5	61.8	61.8	61.8
7'	94.0	94.5	94.1	94.5	94.5	94.5	94.5	94.5	94.5	94.5	94.5	94.5	94.5	94.5
8'	59.8	59.9	59.8	59.8	59.7	59.7	59.8	59.7	59.7	59.7	58.6	59.8	59.8	59.8
9'	20.6	20.6	20.6	20.6	20.6	20.7	20.6	20.6	20.6	20.6	31.2	20.6	20.6	20.6
10'	10.4	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	28.3	10.5	10.5	10.5
10'-CH_3	-	-	-	-	-	-	-	-	-	-	14.2	-	-	-
11'	31.2	32.3	38.4	39.2	31.8	32.1	32.1	38.7	38.7	29.6	32.1	39.0	29.7	32.2
11'-$\underline{CH_2}CH_3$	20.6	20.6	-	-	20.6	20.6	20.6	-	-	-	22.3	-	-	20.5
11'-$CH_2\underline{CH_3}$	14.2	14.2	-	-	14.1	14.2	14.2	-	-	-	13.9	-	-	14.2
11'-$\underline{CH}(CH_3)_2$	-	-	26.0	26.1	-	-	-	26.1	26.2	-	-	26.2	-	-
11'-$CH(\underline{CH_3})_2$	-	-	23.8 21.9	23.7 22.0	-	-	-	23.6 21.8	23.6 21.9	-	-	23.7 22.0	-	-
11'-$\underline{CH_2}CH_2CH_3$	-	-	-	-	-	-	-	-	-	29.5	-	-	29.5	-
11'-$CH_2\underline{CH_2}CH_3$	-	-	-	-	-	-	-	-	-	22.8	-	-	22.8	-
11'-$CH_2CH_2\underline{CH_3}$	-	-	-	-	-	-	-	-	-	13.8	-	-	13.8	-
1''	209.1	208.9	209.1	208.9	80.6	80.5	37.9	80.6	80.7	80.5	37.3	38.0	37.9	38.6
1''-OCH_3	-	-	-	-	57.2	57.2	-	57.2	57.2	57.2	-	-	-	-
2''	34.9	34.8	34.9	34.8	33.8	33.7	28.3	33.8	33.9	33.8	29.3	28.2	28.3	28.1
3'', 4''	19.7 19.1	19.6 19.3	19.7 19.1	19.6 19.2	18.7 17.6	18.7 17.9	22.3 22.2	18.7 17.5	18.7 17.7	18.6 17.6	20.6 19.9	22.4 22.2	22.4 22.2	22.4 22.3

* Assignments could be interchanged in each column.

shifts adjacent to this position, and a comparison of their specific optical rotation values.

Conclusion

Luminacin components were isolated as a family of novel angiogenesis inhibitors from the extract of *Streptomyces* sp. Mer-VD1207. The structures of the components were established by means of spectroscopic analysis, mainly NMR experiments. These components can be classified into three groups according to the C-1 substituent of the benzene chromophore: C-1-formyl (luminacins C_1, C_2, D, E_1, E_2, E_3, F, G_1, and G_2), C-1-

methylcarbonyl (H), and C-1-nor (A_1, A_2, B_1, and B_2) groups. It is interesting to note that the C-1 substituent considerably affected the angiogenesis inhibitory activity in the rat aorta matrix culture model (C-1-formyl, relatively strong; C-1-methylcarbonyl, medium; and C-1-nor, weak), while the other structural differences mentioned above (*i.e.*, the stereochemistry at C-2′ and the alkyl side chains at the C-5, C-2′, and C-8′ positions) had little effect[3].

A search of the literature revealed two molecules closely related or identical to luminacin components. One of these was SI-4228A, which has the same planar structure as luminacins C_1 and C_2, and was claimed to have anti-microbial, immunosuppressive, and antitumor activity[4]. Four side chain analogues were also mentioned. The second related compound, claimed to be a promoter of low density lipoprotein (LDL) uptake[5], has a planar structure corresponding to the C-1″ hydroxyl analogue of luminacins C_1 and C_2.

Experimental

General

The optical rotation was measured on a JASCO DIP-1000 digital spectropolarimeter. The IR spectra were determined on a JASCO FT/IR-300E spectrometer and the UV spectra on a Shimadzu UV-2400PC spectrometer. MS were run with a JEOL JMS-SX102A spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-α400 NMR spectrometer.

Taxonomic Studies

Methods and media described by the International Streptomyces Project (ISP)[6] were used to determine most of the cultural and physiological characteristics. The morphological observations were made with a scanning electron microscope (JEOL JSM-T300) on cultures grown on inorganic salts - starch agar (ISP-4) at 28°C for 2 weeks. The colors of the substrate and aerial mass were assigned according to the Color Harmony Manual[7]. Carbohydrate utilization was investigated according to the procedure of PRIDHAM and GOTTLIEB[8].

References

1) Luminacins A_1, A_2, B_1, B_2, C_1, C_2, D, E_1, E_2, E_3, F, G_1, G_2, and H were formerly designated as VD1207 U1, U2, A1, A2, B, C, D, E, F, F′, G1, G2, G′, and H, respectively.
2) WAKABAYASHI, T.; R. KAGEYAMA, N. NARUSE, N. TSUKAHARA, Y. FUNAHASHI, K. KITOH & Y. WATANABE: Borrelidin is an angiogenesis inhibitor; disruption of angiogenic capillary vessels in a rat aorta matrix culture model. J. Antibiotics 50: 671~676, 1997
3) WAKABAYASHI, T.; R. KAGEYAMA-KAWASE, N. NARUSE, Y. FUNAHASHI & K. YOSHIMATSU: Luminacins: a family of capillary tube formation inhibitors from *Streptomyces* sp. II. Biological activities J. Antibiotics 53: 591~596, 2000
4) SUZUKI, M.; I. KOBAYASHI & K. MITSUTAKE (Idemitsu Kosan Co.): A new antibiotic SI-4228, method for the production and agricultural microbicide containing the same as an active constituent. Jpn. Kokai Tokkyo Koho 116,686, 1983
5) HAMAGUCHI, T.; T. AKAMA & Y. KAWAMURA (Taisho Pharmaceutical Co.): A benzaldehyde compound. Jpn. Kokai Tokkyo Koho 228,144, 1994
6) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
7) TAYLOR, H. D.; L. KNOCHE & W. C. GRANVILLE (*Ed.*): Color Harmony Manual, Container Corporation of America, Chicago, 1948
8) PRIDHAM, T. G. & D. GOTTLIEB: The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bacteriol. 56: 107~114, 1948