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NOTES

New Members of the Macrosphelides from *Microsphaeropsis* sp. FO-5050 IV

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In our previous study, we have reported novel anti-cell adherent compounds, macrosphelide A~D[1~3] from cultured broth of the fungus, *Microsphaeropsis* sp. FO-5050. The total synthesis and the absolute configuration of macrosphelides A and B have been also reported[4].

To clarify the structure-activity relationship of the macrosphelides and to apply the results to the design of more potent inhibitors of cell-cell adhesion, we have devoted our attention to the purification of other macrosphelide derivatives from the broth of strain FO-5050. This paper describes physico-chemical properties, structure determination and biological activities of two new members of the macrosphelides J (**1**), K (**2**) and another compound (**3**) which was isolated with the macrosphelide-related compounds because of similar HPLC profile and NMR spectrum.

The fermentation procedure employed with this strain was same as that reported previously[1], except for its 4 day duration. For the isolation of **1** and **2**, the EtOAc extract (14.5 g) of the cultured broth was chromatographed on silica gel ($CHCl_3$ - MeOH, 100 : 1 ~ 50 : 1 v/v) and ODS MPLC (C.I.G. column system, i.d. 22 × 100 mm; detection, UV at 210 nm; flow rate 9 ml/minute; sol. sys., CH_3CN - H_2O, 30 : 70 v/v). Compound **1** was finally purified by HPLC (Senshu Pak Pegasil-B ODS, i.d. 20 × 250 mm; detection, UV at 210 nm; flow rate, 7 ml/minute; sol. sys., MeOH - CH_3CN - H_2O, 25 : 20 : 55 v/v) and **2** was also purified by HPLC (Senshu Pak Pegasil-B ODS, i.d. 20 × 250 mm; detection, UV at 210 nm; flow rate, 7 ml/minute; sol. sys., CH_3CN - H_2O, 35 : 65 v/v) as colorless oil (91.6 and 7.0 mg respectively). Compound **3** was purified by silica gel column chromatography (eluted with $CHCl_3$ - MeOH, 50 : 1) and HPLC (Senshu Pak Pegasil-B ODS, i.d. 20 × 250 mm; detection, UV at 210 nm; flow rate, 7 ml/minute; sol. sys., CH_3CN - H_2O, 20 : 80 v/v) as a white powder (180 mg).

Physico-chemical properties are summarized in Table 1. The molecular formula of **1** was determined to be $C_{17}H_{24}O_9$ by HR-FAB-MS. The IR absorbance at 3442 cm^{-1} and 1730 cm^{-1} of **1** indicated the presence of hydroxyl group and ester carbonyl group, respectively. From its ^1H NMR spectrum (Table 2), **1** was assumed to be a derivative of macrosphelide B. The signals of δ 4.27 (dd, J = 18.9, 2.7 Hz, H-12), δ 2.66 ~ 2.81 (m, H-13)

Table 1. Physico-chemical data of **1** to **3**.

	1	**2**	**3**
Appearance	Colorless oil	Colorless oil	White powder
$[\alpha]_D^{20}$	−41° (*c* 0.20 MeOH)	−59° (*c* 0.20 MeOH)	−180° (*c* 0.50 MeOH)
Molecular weight	372	386	224
Molecular formula	$C_{17}H_{24}O_9$	$C_{18}H_{26}O_9$	$C_{11}H_{12}O_5$
Pos. FAB-MS (*m/z*)	373 $(M+H)^+$	387 $(M+H)^+$	225 $(M+H)^+$
HR Pos. FAB-MS (*m/z*)	Found 395.1320 ($C_{17}H_{24}O_9Na$) Calcd. 395.1318	Found 409.1488 ($C_{18}H_{26}O_9Na$) Calcd. 409.1475	Found 225.0772 ($C_{11}H_{13}O_5$) Calcd. 225.0763
UV λ_{max}^{MeOH} (log ε)	211 (4.02)	210 (3.98)	205 (3.87), 213 (3.82 sh)
IR ν_{max} (KBr)	3442, 1730, 1653, 1456	3440, 1747, 1732, 1625, 1454	3439, 3356, 1792, 1772, 1682, 1630
Melting point (°C)	—	—	141 ~ 143

Table 2. ^{1}H and ^{13}C NMR chemical shifts of macrospherlides J (**1**), K (**2**), and B.

	1			2			Macrosphelide B		
	δ_C (mult.)	δ_H	(mult., J [Hz])	δ_C (mult.)	δ_H	(mult., J [Hz])	δ_C (mult.)	δ_H	(mult., J [Hz])
1	169.75 (s)			169.73 (s)			170.4 (s)		
2a	41.05 (t)	2.66~2.81	(2H, m)	41.03 (t)	2.66~2.81	(2H, m)	40.6 (t)	2.83	(1H, dd, 16.2, 10.9)
2b								2.64	(1H, dd, 16.2, 3.0)
3	68.47 (d)	5.43~5.47	(1H, m)	68.44 (d)	5.43~5.46	(1H, m)	67.7 (d)	5.45	(1H, m)
5	164.21 (s)			164.24 (s)			164.3 (s)		
6	123.30 (d)	6.12	(1H, dd, 15.6, 1.8)	123.28 (d)	6.13	(1H, dd, 15.9, 1.5)	122.4 (d)	6.08	(1H,dd, 15.8, 3.0)
7	144.32 (d)	6.81	(1H, dd, 15.9, 3.9)	144.30 (d)	6.81	(1H, dd, 15.9, 3.9)	144.5 (d)	6.94	(1H, dd, 15.8, 3.6)
8	74.70 (d)	4.23	(1H, br s)	74.71 (d)	4.23	(1H, br s)	74.6 (d)	4.32	(1H, br s)
9	76.55 (d)	4.96	(1H, dq, 4.2, 6.6)	76.52 (d)	4.95	(1H, dq, 6.6, 4.2)	75.7 (d)	5.08	(1H, m)
11	172.81 (s)			173.24 (s)			165.2 (s)		
12	74.70 (d)	4.27	(1H, dd, 18.9, 2.7)	73.05 (d)	4.36	(1H, dd, 9.6, 3.0)	132.1 (d)	7.03	(1H, d, 15.8)
13	42.01 (t)	2.66~2.81	(2H, m)	42.70 (t)	2.66~2.81	(2H, m)	132.6 (d)	6.73	(1H, d, 15.8)
14	202.73 (s)			202.73 (s)			196.2 (s)		
15	75.38 (d)	5.22	(1H, q, 6.8)	75.36 (d)	5.23	(1H, q, 6.6)	76.5 (d)	5.08	(1H, m)
3Me	19.95 (q)	1.37	(3H, d, 6.6)	19.93 (q)	1.37	(3H, d, 6.6)	19.7 (q)	1.36	(3H, d, 6.6)
9Me	18.21 (q)	1.45	(3H, d, 6.6)	18.22 (q)	1.45	(3H, d, 7.2)	17.8 (q)	1.49	(3H, d, 6.6)
12-CH_2				66.85 (t)	3.65	(1H, dq, 8.9, 7.2)			
					3.47	(1H, dq, 8.6, 7.2)			
12-Me	58.79 (q)	3.40	(3H, s)	15.01 (q)	1.18	(3H, t, 6.9)			
15Me	15.05 (q)	1.41	(3H, d, 7.2)	15.01 (q)	1.41	(3H, d, 7.2)	16.0 (q)	1.43	(3H, d, 6.9)

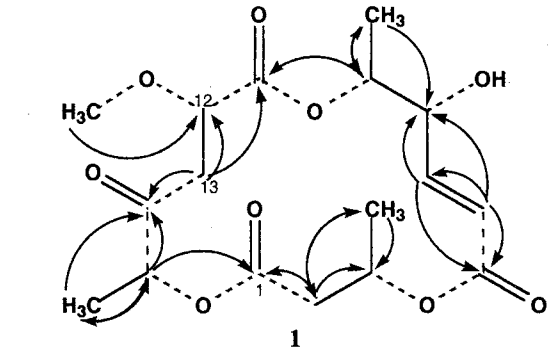
δ from TMS in $CDCl_3$.

and δ 3.40 (s, 12-OMe) were newly observed when compared with those of macrosphelide B. In addition, two olefinic protons of δ 6.73 and δ 7.03 which had been detected in macrosphelide B were absent. In the ^{13}C NMR spectrum of **1** (Table 2), one methine (δ 74.70, d, C-12), one methylene (δ 42.01, t, C-13) and one methyl (δ 58.79, q, 12-OMe) were detected, but the two olefinic carbons (C-12 and C-13 in macrosphelide B) were absent. The low-field methyl signal showed the presence of a methoxy group. The partial structures of 1 were proposed by PFG (pulse field gradient)-^{1}H-^{1}H COSY and PFG-HMQC. By the PFG-HMBC experiment, these components were connected as shown in Fig. 1. Thus, **1** was elucidated to be a 12,13-hydro-12-methoxy-macrosphelide B.

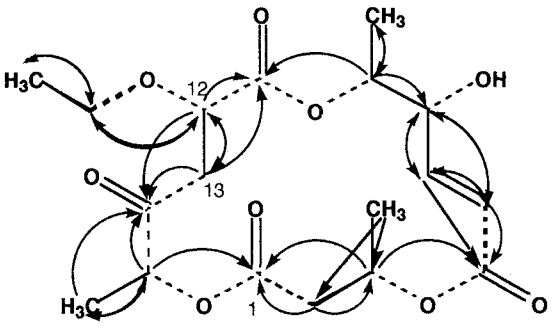
The molecular formula ($C_{18}H_{26}O_9$) of **2** was assigned based on the HR-FAB-MS. The IR absorptions at 3442 cm^{-1} and 1730 cm^{-1} most likely correspond to a hydroxyl and an ester group. In the ^{1}H and ^{13}C NMR spectra (Table 2), an additional methylene (δ_H 3.65, 3.47, δ_C 66.85, t, 12-CH_2) was observed compared with those of **1**. In addition, the low-field methyl proton of **1** at δ 3.40 (s, 12-Me) was shifted to high-field (δ 1.18) in **2**. From ^{1}H-^{1}H COSY, the direct connection between 12-CH_2 and 12-Me was observed. By the PFG-HMBC experiment, the structure of **2** was determined as shown in Fig. 1.

The molecular formula ($C_{11}H_{12}O_5$) of **3** was determined by the HR-FAB-MS. The IR spectrum indicated the presence of hydroxyl (3439 cm^{-1} and 3356 cm^{-1}, br), carbonyl and γ-lactone (1792 cm^{-1} and 1772 cm^{-1}). The ^{13}C NMR including DEPT method displayed eleven signals related to four quaternary carbons (δ 196.81, 172.27, 158.79, and 59.69), five methines (δ 150.74, 125.60, 71.89, 67.91, and 32.43), one methylene (δ 87.41), and one methyl (δ 15.94). In the ^{1}H NMR (Table 3) spectrum, the addition of D_2O caused the loss of two proton signals at δ 5.96 and 6.54, and simplified the signals at δ 4.23 and 5.16, respectively. These results indicated the presence of two hydroxyl groups and their direct correlations to δ 4.23 and 5.16. ^{1}H-^{1}H COSY suggested a partial structure of CH_3–CH(6)–CH(5)=CH(4) chain including two *cis* form protons (δ 6.60 and δ 5.98, J=10.2 Hz), and HMBC correlation of H-4/C-3 and H-5/C-3 confirmed that the structure of C-3–C-4=C-5 is adjacent to a ketone. Also

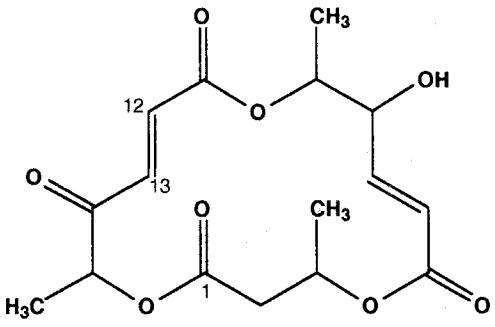
Fig. 1. Structures of **1**, **2** and macrosphelide B.



1



2



Macrosphelide B

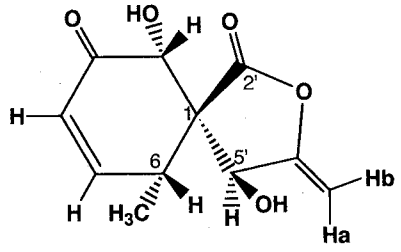
Arrows show ^{1}H-^{13}C long range couplings detected by HMBC experiments ($J=6.0$ Hz). Solid lines show the correlation detected by ^{1}H-^{1}H COSY.

Table 3. ^{1}H and ^{13}C NMR chemical shifts of **3**.

No.	δ_C (mult.)	δ_H (mult., J [Hz])
1	59.69 (s)	
2	71.89 (d)	4.23 (1H, d, 2.4)
2-OH		5.96 (1H, d, 4.2)
3	196.81 (s)	
4	125.60 (d)	5.98 (1H, dd, 10.2, 2.4)
5	150.74 (d)	6.60 (1H, d, 10.2)
6	32.43 (d)	3.24 (1H, m)
6-Me	15.94 (q)	1.08 (3H, d, 7.8)
2′	172.27 (s)	
4′	158.79 (s)	
4′-CH_2a	87.41 (t)	4.76 (1H, t-like, 2.4)
4′-CH_2b		4.58 (1H, t-like, 2.1)
5′	67.91 (d)	5.16 (1H, br s)
5′-OH		6.54 (1H, d, 4.8)

δ from TMS in DMSO-d_6.

Fig. 2. Structure of 6-epi-5′-hydroxy-mycosporulone (**3**).



consistent with this conclusion was the observation that the respective high and low field shifts of C-4 (δ 125.60) and C-5 (δ 150.74). Because HMBC cross peaks of 5′-OH/C-4′,4′-CH_2/C-4′,4′-CH_2/C-5′ were detected, a partial structure of CH(OH)(5′)-C(4′)-CH_2 can be constructed. Judged by the very low shift value (δ 158.79), C-4′ was very likely to be adjacent to an electrophilic group. Further, this electrophilic group also caused the upfield shift of the oxo-methylene (C-4′) group to δ 87.14 due to the mesomeric effect. The presence of HMBC cross peaks of 5′-OH/C-1, 2-OH/C-1 and 6-CH_3/C-1 suggested that C-1 is located in the center of C-2, C-6 and C-5′. The presence of cross peaks of H-6/C-2′, H-5′/C-2′ and H-2/C-2′ suggested that C-2′ was next to C-1. Based on the above arguments, a spiro-lactone structure of compound **3** can be constructed as shown in Fig. 2.

The stereo-structure was determined by differential NOE experiments. The NOE between H-2 (δ 4.23) and H-6 (δ 3.24), between H-2 and H-5′ (δ 5.16), and 6-CH_3 (δ 1.08) and 5′-OH (δ 6.54) were observed. When 2-OH was irradiated, the positive NOE for H-5′ and negative NOE for 5′-OH were observed. In the meantime, when H-5′ was irradiated, the positive and negative NOE for geminal protons of 4′-CH_2a (δ 4.76) and 4′-CH_2b (δ 4.58)

were observed, respectively. From this result, 4′-CH_2a was concluded to be in the same side and 4′-CH_2b in the opposite side for H-5′, respectively. Therefore, the relative stereochemistry of **3** was determined as shown in Fig. 2. Although **3** is closely related to mycosporulone, previously isolated from *Coniothyrium sporulosum*[5], the compounds differ by oxidation state and the stereochemistry of 6-Me. Therefore, **3** was determined to be 6-epi-5′-hydroxymycosporulone.

Biological activities of new macrosphelides and 6-epi-5′-hydroxymycosporulone were examined according to the previous methods[1]. These compounds were evaluated in an adhesion assay system using human leukemia cells (HL-60 cells) and HUVECs (human umbilical vein endothelial cells). The IC_{50} values of all compounds tested were greater than 100 μg/ml and did not indicate any effects on the cell growth of HL-60 and B16/BL6 melanoma when tested at 50 μg/ml.

We have previously isolated the anti-cell adhesion molecule, macrosphelide A, from the fermentation broth of *Microsphaeropsis* sp. FO-5050. Since the inhibitor of cell adhesion could suppress inflammation and cancer metastasis[6], we have made efforts to find more potent inhibitors among the derivatives of macrosphelide. In this paper, we described the isolation of two new derivatives of macrosphelide, but neither showed inhibitory activity. The primary structural difference between the macrosphelides J and K and macrosphelide B was a double bond at C-12. On the other hand, macrosphelides J and K were not artifacts of macrosphelide B, because of its stablility in MeOH or EtOH solution. These results show the double bond at C-12 to be important for the inhibitory activity. The analysis of the structure-activity relationships between the macrosphelides may become the basis for the development of an inhibitor of the cell-adhesion molecule.

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