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ICM0301s, New Angiogenesis Inhibitors from *Aspergillus* sp. F-1491

II. Physico-chemical Properties and Structure Elucidation

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ICM0301A (**1**), B (**2**) and their congeners (**3**~**8**) were isolated from a culture broth of *Aspergillus* sp. F-1491 as new angiogenesis inhibitors. Their structures were elucidated by spectroscopic analyses. ICM0301A and B have a substituted decalin skeleton containing two oxirane rings.

In the course of our screening for new angiogenesis inhibitors[1~3] from microbial products, we have isolated eight new structurally related compounds from a culture broth of *Aspergillus* sp. F-1491. The taxonomy of the producing strain and fermentation, isolation, and biological activities of ICM0301s are reported in the preceding paper[4]. In this paper, we describe the physico-chemical properties and structure elucidation of ICM0301s.

Results and Discussion

Physico-chemical Properties of ICM0301s

ICM0301s were isolated as white powders. Among them, ICM0301A (**1**) and B (**2**) were obtained as major products. The physico-chemical properties of **1** and **2** are summarized in Table 1. The UV spectra of all ICM0301s in MeOH exhibited essentially the same absorption maxima at 280 nm. Their IR spectra showed a $\nu_{\max}$ at around 1660 cm^{-1}. These observations suggested the presence of a conjugated dienone system.

Structure Elucidation of ICM0301 A (1)

The molecular formula of ICM0301A (**1**) was determined to be $C_{24}H_{34}O_3$ by HRFAB-MS, HRESI-MS and ^{13}C NMR analyses. The degree of unsaturation was calculated to be eight by its molecular formula. Since the ^{1}H and ^{13}C NMR spectra of **1** in CD_3OD at room temperature displayed broad signals, NMR experiments were carried out at 40°C. The ^{1}H NMR and HSQC spectra of **1** revealed 34 protons indicating the absence of hydroxyl group in the molecule.

The connectivity of two side chains (C-1~C-7 and C-18~C-20) was established by ^{1}H-^{1}H COSY with an aid of HMBC as shown in Fig. 2a. A substituted decalin moiety was elucidated as follow. The presence of two oxymethine carbons C-11 (δ_C 61.3), C-15 (δ_C 64.5) and two oxygenated quaternary carbons C-12 (δ_C 60.2), C-16 (δ_C 62.5) was suggested by the chemical shifts. This implies that each two of these carbons must form two ether moieties. Furthermore, large C-H coupling constants for *sp*^{3} oxymethine carbons at C-11 ($^{1}J_{C\text{-}H}$=175 Hz) and C-15 ($^{1}J_{C\text{-}H}$=172 Hz) indicated the presence of two oxirane rings[5]. In the remaining part (tentatively C-8~C-17), no more double bond exists in the molecule based on the ^{13}C NMR spectrum. Taking the degree of unsaturation into consideration, two ring systems besides two oxirane rings should be formed by the remainder. In the ^{1}H-^{1}H COSY spectrum of **1**, sequential proton networks were observed within H-11, 10, 9, 8, 17 and H-14, 13 through H-9. In the HMBC spectrum, cross peaks were observed from methyl

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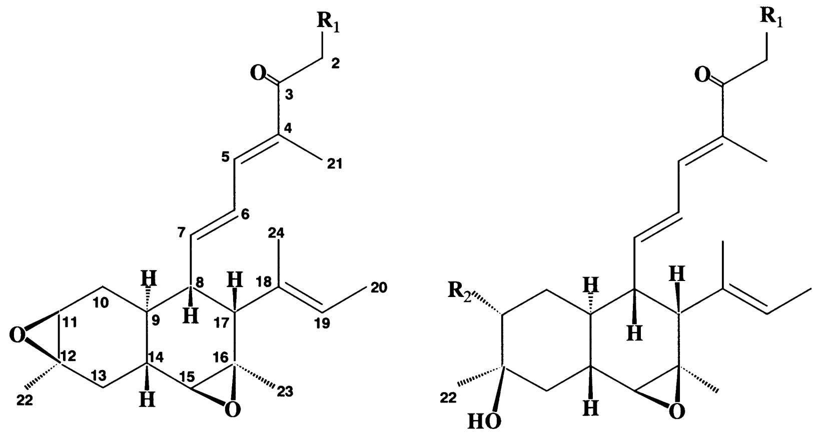
Fig. 1. Relative structures of ICM0301s.



ICM0301A (1) $R_1=CH_3$
ICM0301B (2) $R_1=H$

ICM0301C (3) $R_1=CH_3, R_2=Cl$
ICM0301D (4) $R_1=H, R_2=Cl$
ICM0301E (5) $R_1=CH_3, R_2=H$
ICM0301F (6) $R_1=H, R_2=H$
ICM0301G (7) $R_1=H, R_2=OCH_3$
ICM0301H (8) $R_1=H, R_2=OH$

Table 1. Physico-chemical properties of ICM0301A (**1**) and B (**2**).

	ICM 0301A(**1**)	ICM 0301B(**2**)
Appearance	White powder	White powder
Molecular formula	$C_{24}H_{34}O_3$	$C_{23}H_{32}O_3$
HRESI-MS(*m/z*)		
Observed	393.2406(M+Na)$^+$	379.2246(M+Na)$^+$
Calcd.	393.2406 for $C_{24}H_{34}O_3Na$	379.2249 for $C_{23}H_{32}O_3Na$
HRFAB-MS(*m/z*)		
Observed	371.2580(M+H)$^+$	357.2463(M+H)$^+$
Calcd.	371.2586 for $C_{24}H_{35}O_3$	357.2430 for $C_{23}H_{33}O_3$
$[\alpha]_D^{26}$	-194° (*c* 0.1, MeOH)	-222° (*c* 0.1, MeOH)
UV; λ(MeOH), nm(ε)	280 (26200)	280 (27500)
IR; ν cm^{-1}(KBr)	2970, 2915, 1660, 1630, 1440, 1375, 1260, 835	2965, 2925, 1655, 1630, 1435, 1375, 1255, 835

protons (H-22) to one quaternary carbon (C-12, δ_C 60.2), one methylene carbon (C-13, δ_C 36.8), one methine carbon (C-11, δ_C 61.3) and from H-23 to one quaternary carbon (C-16, δ_C 62.5), two methine carbons (C-15, δ_C 64.5 and C-17, δ_C 54.8), respectively. Cross peaks were also observed from H-15 to C-13 and C-14. These facts indicated the connectivity between C-11 and C-12, and between C-15 and C-16, respectively, resulting in the presence of substituted decalin ring. Cross peaks were observed from a methine proton (H-8) to C-7 and C-6 indicating that the long side chain should be attached at the C-8 position. Furthermore, cross peaks were observed from methyl protons of H-24 to C-17. Thus, the total planar structure of **1** was determined as shown in Fig. 2a.

The relative stereochemistry of **1** was elucidated by NOE difference and NOESY experiments (Fig. 2b). A large

Table 2. ^{1}H NMR data for **1**~**8**.

No	ICM0301A(1)$^{a)}$	ICM0301B(2)$^{a)}$	ICM0301C(3)$^{b)}$	ICM0301D(4)$^{c)}$
1	1.07 (3H, t, J=7.3)	-	1.06 (3H, t, J=7.4)	-
2	2.73 (2H, q, J=7.3)	2.31 (3H, s)	2.74 (2H, q, J=7.4)	2.30 (3H, s)
5	7.09 (1H, d, J=11.0)	7.12 (1H, d, J=11.0)	7.12 (1H, d, J=11.0)	6.94 (1H, d, J=11.0)
6	6.45 (1H, dd, J=15.0, 11.0)	6.46 (1H, dd, J=15.0, 11.0)	6.45 (1H, dd, J=15.0, 11.0)	6.33 (1H, dd, J=15.0, 11.0)
7	5.77 (1H, dd J=15.0, 10.0)	5.80 (1H, dd, J=15.0, 10.3)	5.78 (1H, dd, J=15.0, 10.5)	5.69 (1H, dd, J=15.0, 10.5)
8	2.29 (1H, ddd, J=11.0, 10.0, 5.0)	2.30 (1H, ddd, J=10.7, 10.3, 5.1)	2.40 (1H,ddd, J=11.0, 10.5, 5.0)	2.40 (1H, m)
9	1.38 (1H, m)	1.40 (1H, m)	2.05 (1H, m)	2.00 (1H,m)
10	1.13 (1H, dd, J=15.0, 12.0)	1.13 (1H, dd, J=15.0, 12.2)	1.62 (1H, m)	1.60 (1H, m)
	1.82 (1H, ddd, J=15.0, 5.6, 5.5)	1.82 (1H, ddd, J=15.0, 5.6, 5.5)	1.68 (1H, m)	1.68 (1H, m)
11	2.95 (1H, d, J=5.6)	2.98 (1H, d, J=5.6)	3.94 (1H, br)	3.90 (1H, br)
13	1.70 (1H, dd, J=13.0, 12.5)	1.70 (1H, dd, J=12.9, 12.2)	1.68 (1H, m)	1.55 (1H, m)
	2.15 (1H, dd, J=13.0, 3.0)	2.15 (1H, dd, J=12.9, 2.5)	1.80 (1H, m)	1.85 (1H, m)
14	1.64 (1H, ddd, J=13.0, 12.5, 3.0)	1.64 (1H, ddd, J=13.0, 12.2, 2.5)	2.00 (1H, m)	1.97 (1H, m)
15	2.78 (1H, s)	2.79 (1H, s)	2.78 (1H, s)	2.73 (1H, s)
17	2.57 (1H, d, J=5.0)	2.58 (1H, d, J=5.1)	2.64 (1H, d, J=5.0)	2.64 (1H, d, J=5.0)
19	5.29 (1H, q, J=7.0)	5.30 (1H, q, J=7.0)	5.34 (1H, q, J=7.0)	5.30 (1H, q, J=6.5)
20	1.66 (3H, d, J=7.0)	1.67 (3H, d, J=7.0)	1.66 (3H, d, J=7.0)	1.63 (3H, d, J=6.5)
21	1.85 (3H, s)	1.84 (3H, s)	1.85 (3H, s)	1.81 (3H, s)
22	1.33 (3H, s)	1.34 (3H, s)	1.34 (3H, s)	1.39 (3H, s)
23	1.21 (3H, s)	1.22 (3H, s)	1.22 (3H, s)	1.21 (3H, s)
24	1.69 (3H, s)	1.70 (3H, s)	1.73 (3H, s)	1.69 (3H, s)
11-OMe				

No	ICM0301E(5)$^{b)}$	ICM0301F(6)$^{b)}$	ICM0301G(7)$^{b)}$	ICM0301H(8)$^{c)}$
1	1.06 (3H, t, J=7.2)	-	-	-
2	2.74 (2H, q, J=7.2)	2.31 (3H, s)	2.33(3H, s)	2.32 (3H, s)
5	7.12(1H, d, J=11.0)	7.14 (1H, d, J=11.0)	7.16 (1H, d, J=11.0)	6.98 (1H, d, J=11.0)
6	6.46 (1H, dd, J=15.0, 11.0)	6.46 (1H, dd, J=15.0, 11.0)	6.47 (1H, dd, J=15.0, 11.0)	6.38 (1H, dd, J=15.0, 11.0)
7	5.82 (1H, dd J=15.0, 10.0)	5.85 (1H, dd, J=15.0, 11.0)	5.84 (1H, dd, J=15.0, 10.5)	5.73 (1H, dd, J=15.0, 10.0)
8	2.37 (1H, m)	2.37 (1H, m)	2.35 (1H, m)	2.40 (1H, ddd, J=11.0, 10.0, 5.0)
9	1.34 (1H, m)	1.35 (1H, m)	1.70 (1H, m)	1.82 (1H, m)
10	1.06 (1H, m)	1.05 (1H,m)	1.16 (1H, m)	1.41 (1H, m)
	1.32 (1H,m)	1.34 (1H, m)	1.62 (1H, m)	1.47 (1H, m)
11	1.30 (1H, m), 1.65 (1H, m)	1.30 (1H, m), 1.65 (1H, m)	3.00 (1H, br)	3.57 (1H, br)
13	1.40 (1H, t, J=13.0)	1.42 (1H, t, J=13.0))	1.55 (1H, m)	1.65 (1H, m)
	1.79 (1H, m)	1.80 (1H, m)	1.65 (1H, m)	1.75 (1H, m)
14	1.91 (1H, m)	1.92 (1H, m)	1.90 (1H, m)	1.96 (1H, m)
15	2.77 (1H, s)	2.77 (1H, s)	2.75 (1H, s)	2.75 (1H, s)
17	2.62 (1H, d, J=5.0)	2.61 (1H, d, J=5.0)	2.63 (1H, d, J=5.0)	2.67 (1H, d, J=5.0)
19	5.31 (1H, q, J=6.5)	5.30 (1H, q, J=7.0)	5.32 (1H, q, J=7.0)	5.31 (1H, q, J=7.0)
20	1.66 (3H, d, J=6.5)	1.65 (3H, d, J=7.0)	1.66 (3H, d, J=7.0)	1.66 (3H, d, J=7.0)
21	1.84 (3H, s)	1.84 (3H, s)	1.84 (3H, s)	1.85 (3H, s)
22	1.22 (3H, s)	1.23 (3H, s)	1.23 (3H, s)	1.32 (3H, s)
23	1.22 (3H, s)	1.23 (3H, s)	1.21 (3H, s)	1.25 (3H, s)
24	1.71 (3H, s)	1.71 (3H, s)	1.70 (3H, s)	1.71 (3H, s)
11-OMe			3.27 (3H, s)	

a) in CD_3OD at 40°C(500MHz)

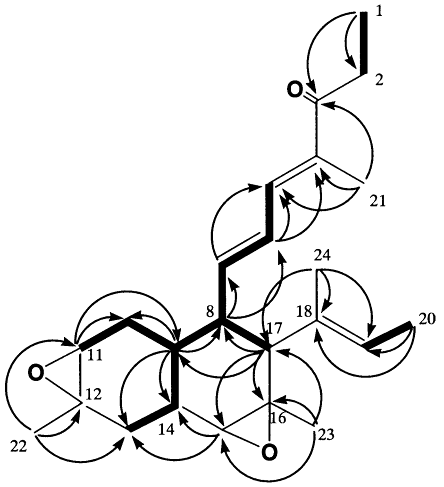
b) in CD_3OD at 24°C(400MHz)

c) in $CDCl_3$ at 24°C(400MHz)

Table 3. ^{13}C NMR data for **1**~**8**.

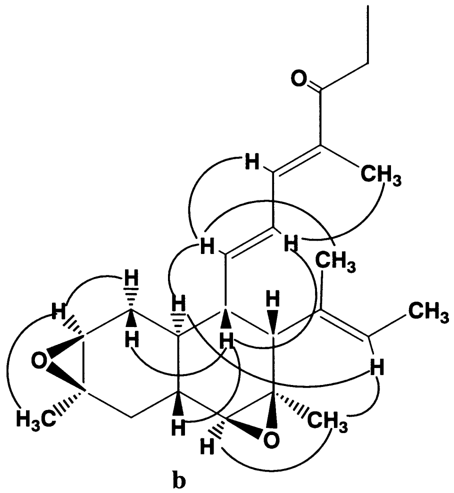
carbon number	ICM0301A(1)	ICM0301B(2)	ICM0301C(3)	ICM0301D(4)	ICM0301E(5)	ICM0301F(6)	ICM0301G(7)	ICM0301H(8)
1	9.2 q	-	9.3 q	-	9.3 q	-	-	-
2	30.8 t	25.6 q	31.3 t	25.6 q	31.3 t	25.6 q	25.6 q	25.6 q
3	205.2 s	202.4 s	205.1 s	200.0 s	205.1 s	202.5 s	202.5 s	199.9 s
4	135.3 s	136.0 s	135.2 s	135.1 s	134.9 s	135.6 s	135.7 s	135.0 s
5	139.9 d	141.1 d	139.9 d	139.0 d	140.1 d	141.6 s	141.5 d	139.1 d
6	128.6 d	128.6 d	128.7 d	127.5 d	128.3 d	128.3 d	128.6 d	127.3 d
7	146.5 d	146.7 d	146.6 d	144.9 d	147.6 d	148.0 d	147.6 d	145.6 d
8	45.4 d	45.4 d	44.7 d	43.7 d	46.1 d	46.1 d	45.7 d	44.2 d
9	34.3 d	34.1 d	31.5 d	30.2 d	38.5 d	38.5 d	31.3 d	30.1 d
10	31.6 t	31.6 t	36.0 t	34.9 t	28.0 t	28.0 t	29.8 t	34.4 t
11	61.3 d	61.4 d	66.7 d	65.3 d	40.0 t	40.0 t	84.4 d	74.0 d
12	60.2 s	60.1 s	73.2 s	72.9 s	70.7 s	70.7 s	72.7 s	72.2 s
13	36.8 t	36.9 t	39.1 t	38.2 t	44.8 t	44.8 t	40.5 t	39.2 t
14	35.2 d	35.3 d	37.9 d	36.7 d	38.5 d	38.5 d	38.1 d	37.0 d
15	64.5 d	64.5 d	65.5 d	63.9 d	66.0 d	66.0 d	65.9 d	64.1 d
16	62.5 s	62.5 s	62.6 s	61.3 s	62.7 s	62.7 s	62.6 s	61.5 s
17	54.8 d	54.6 d	55.1 d	53.5 d	55.0 d	55.0 d	55.2 d	53.1 d
18	134.2 s	134.1 s	134.2 s	132.5 s	134.6 s	134.5 s	134.4 s	132.8 s
19	126.9 d	127.0 d	127.3 d	126.2 d	128.0 d	126.9 d	127.1 d	125.6 d
20	13.6 q	13.6 q	13.6 q	13.6 q	13.6 q	13.6 q	13.6 q	13.7 q
21	11.7 q	11.5 q	11.7 q	11.4 q	11.7 q	11.4 q	11.4 q	11.5 q
22	23.1 q	23.0 q	29.0 q	29.2 q	31.6 q	31.6 q	27.7 q	27.7 q
23	22.1 q	22.1 q	22.4 q	22.0 q	22.4 q	22.4 q	22.4 q	22.0 q
24	19.5 q	19.5 q	18.9 q	19.0 q	19.0 q	19.0 q	19.5 q	19.5 q
11-OMe							57.2 q	

Fig. 2a. Structure of **1** elucidated by ^{1}H-^{1}H COSY (—) and HMBC (→) experiments.



a

Fig. 2b. NOE correlations of **1**.



b

coupling constant (J=13.0 Hz) due to the diaxial relationship between H-9 and H-14 indicated a *trans* junction for the decalin ring. The vicinal coupling constants between H-9 and H-8 (J=11.0 Hz) also indicated the diaxial relationship, whereas coupling constant between H-8 and H-17 (J=5.0 Hz) indicated an *axial-equatorial* relationship. The observation of an NOE between H-8 and the two protons H-10_{ax} and H-14 indicate the 1,3-diaxial relationship between H-8 and H-14 and the trans nature of the ring junction. An NOE was observed between H-11 and methyl protons (H-22), and between H-15 and methyl protons (H-23), respectively. On the other hand, no coupling was observed between H-11 and H-10_{ax} and between H-14 and H-15 perhaps due to the dihedral angles of near 90°. These results indicated that the two methyl protons (H-22 and H-23) exist on the α side of the decalin ring system. Accordingly, two epoxy moieties should exist on the β side of the decalin ring as shown in Fig. 2b. The geometry of the diene system was established to be 4*E*, and 6*E* based on the coupling constant ($J_{6,7}$=15.0 Hz) and the NOE observation between methyl protons (H-21) and H-6. The geometry of double bond at C-18 was elucidated as follow. NOE was observed between methyl protons (H-24) and an olefinic proton (H-7). On the other hand, NOE was observed between H-19 and H-23, H-9. Therefore, H-19 occupied near space to H-9 and methyl protons (H-23). Subsequently, two methyls (H-24 and H-20) should be located on the opposite side of H-19. These results are indicative of 18E configuration as shown in Fig. 2b. The structure of **1** was structurally related to fusarielin[6].

Structure of ICM0301 B (2)

The molecular formula of ICM0301B (**2**) was determined to be $C_{23}H_{32}O_3$ on the basis of the HRESI-MS and ^{13}C NMR information indicating the lack of one carbon and two hydrogen atoms compared with that of **1**. The UV and IR spectra were almost the same as **1**. The ^{13}C NMR spectra of **1** and **2** were also similar to each other except for the disappearance of signal due to one methylene carbon in **2**. In the ^{1}H NMR spectrum of **2**, acetyl protons were observed at δ_H 2.31 (s, 3H). These results indicated that a propionyl group of **1** was replaced by an acetyl group in **2**. Detailed NMR spectral analyses including ^{1}H-^{1}H COSY and HMBC experiments elucidated the planar structure of **2** as shown in Fig. 1. NOESY and NOE difference spectra of **2** showed that the relative stereochemistry of **2** was identical with that of **1**. Thus it was concluded that the structure of **2** was shown in Fig. 1.

The structures of other minor components were subsequently determined by comparing their spectral data with those of **1** and **2**.

Structure of ICM0301 C (3)

The molecular formula of ICM0301C (**3**) was determined to be $C_{24}H_{35}O_3Cl$ by HRESI-MS and ^{13}C NMR spectra together with the characteristic mass fragment patterns of chlorine containing molecules. The ^{1}H and ^{13}C NMR spectra of **3** showed a close similarity to those of **1** except for the H-11, C-10, C-11, C-12 and C-22 chemical shifts (Table 2). The ^{1}H-^{1}H COSY and HMBC spectra of **3** indicated the presence of the same carbon skeleton as that of **1**. Although one oxirane ring was preserved in **3** based on the large coupling constant ($^{1}J_{C\text{-}15,H\text{-}15}$=175 Hz), the other one might be cleaved based on the coupling constant ($^{1}J_{C\text{-}11,H\text{-}11}$=152 Hz). This value strongly supported the presence of chloromethine[7] in the molecule. In addition, the observation that the quaternary carbon (C-12, δ_C 60.2) in **1** was shifted to low field (δ_C 73.2) due to the presence of the hydroxymethine in **3** was consistent with this conclusion. The remaining parts of **3** showed a close similarity to **1** in the NMR spectroscopic properties including ^{1}H-^{1}H COSY, HMBC, NOESY spectra. Therefore, the structure of **3** was proposed as shown in Fig. 1.

Structure of ICM0301 D (4)

ICM0301D (**4**) was obtained as a white powder. Most of the spectroscopic properties of **4** were similar to those of **3**. The molecular formula of **4** was determined to be $C_{23}H_{33}O_3Cl$ on the basis of the HRESI-MS, suggesting that **4** was one methylene unit lower than that of **3**. The ^{1}H and ^{13}C NMR spectra of **3** and **4** were similar to each other except for the terminal parts of long side chains. Consequently, the structure of **4** was determined as shown in Fig. 1.

Structure of ICM0301 E (5)

The molecular formula of ICM0301E (**5**) was determined to be $C_{24}H_{36}O_3$. Compound **5** contained four methylene carbons based on the observation of DEPT experiments. Among them, three methylene carbons (C-2, C-10, C-13) in **5** were identical with those of **3** on the basis of NMR analyses. On the other hand, one more methylene (C-11), which was readily identified by the correlation between H-10 protons in ^{1}H-^{1}H COSY, was observed in the DEPT spectrum of **5**. The remaining parts of **5** were identical with those of **3**. These results indicated that the methylene (C-11) in **3** was replaced by chloromethine in **5**. Thus, the structure of **5** was determined as shown in Fig. 1.

Structure of ICM0301 F (**6**)

The molecular formula ($C_{23}H_{34}O_3$) of ICM0301F (**6**) and most of the spectroscopic properties were very similar to those of **5**. Just as is the case for compound **2** and **4**, the difference between **6** and **5** exists in the terminal of long side chains as shown in Fig. 1.

Structure of ICM0301 G (**7**)

The molecular formula of ICM0301G (**7**) was determined to be $C_{24}H_{36}O_4$ on the basis of HRESI-MS and the ^{13}C NMR. In the ^1H NMR spectrum of **7**, characteristic methoxy protons at δ_H 3.27 were observed, in distinction with signals seen in the spectra of compounds **1**~**6**. The methyl protons correlated to C-11 methine carbon (δ_C 84.4) in the HMBC spectrum indicating the presence of methoxymethine at C-11. The remaining parts of **7** were identical with those of **4** and **6**. Thus, the structure of **7** was determined as shown in Fig. 1.

Structure of ICM0301 H (**8**)

The molecular formula of ICM0301H (**8**) was determined to be $C_{23}H_{34}O_4$ on the basis of HRESI-MS, which is one carbon and two protons less than that of **7**. In the ^1H NMR spectrum of **8**, a methoxy group was not observed. In the ^{13}C NMR spectrum, a low-field methine carbon (C-11, δ_C 84.4) in **7** was shifted to high-field (δ_C 74.0) in **8**. These results indicated that a methoxy group in **7** was replaced by a hydroxyl group in **8** as shown in Fig. 1.

As described above, ICM0301s were isolated as new angiogenesis inhibitors. Among them, ICM0301A and B were produced as major products. On the other hand, minor compounds (**3**~**8**) were isolated from larger scale fermentations. Taking the chemical reactivity of epoxy functional group into consideration, some of minor components might be artifacts of the isolation procedures.

Materials and Methods

UV spectra were measured on a Hitachi 228A spectrometer. IR spectra were recorded on a Horiba FT-200 fourier transform infrared spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. HRESI-MS spectra were measured with a JEOL JMS-T100LC. HRFAB-MS spectra were measured with a VG AutoSpec mass spectrometer. The ^1H and ^{13}C NMR spectra of **1** and **2** were measured on a JEOL JNM-A500 spectrometer at 40°C using TMS as an internal reference. Compounds **3**~**8** were measured on a JEOL JNM-A400 spectrometer at 24°C.

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