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METACHROMINS J AND K, NEW SESQUITERPENOIDS FROM MARINE SPONGE *SPONGIA* SPECIES

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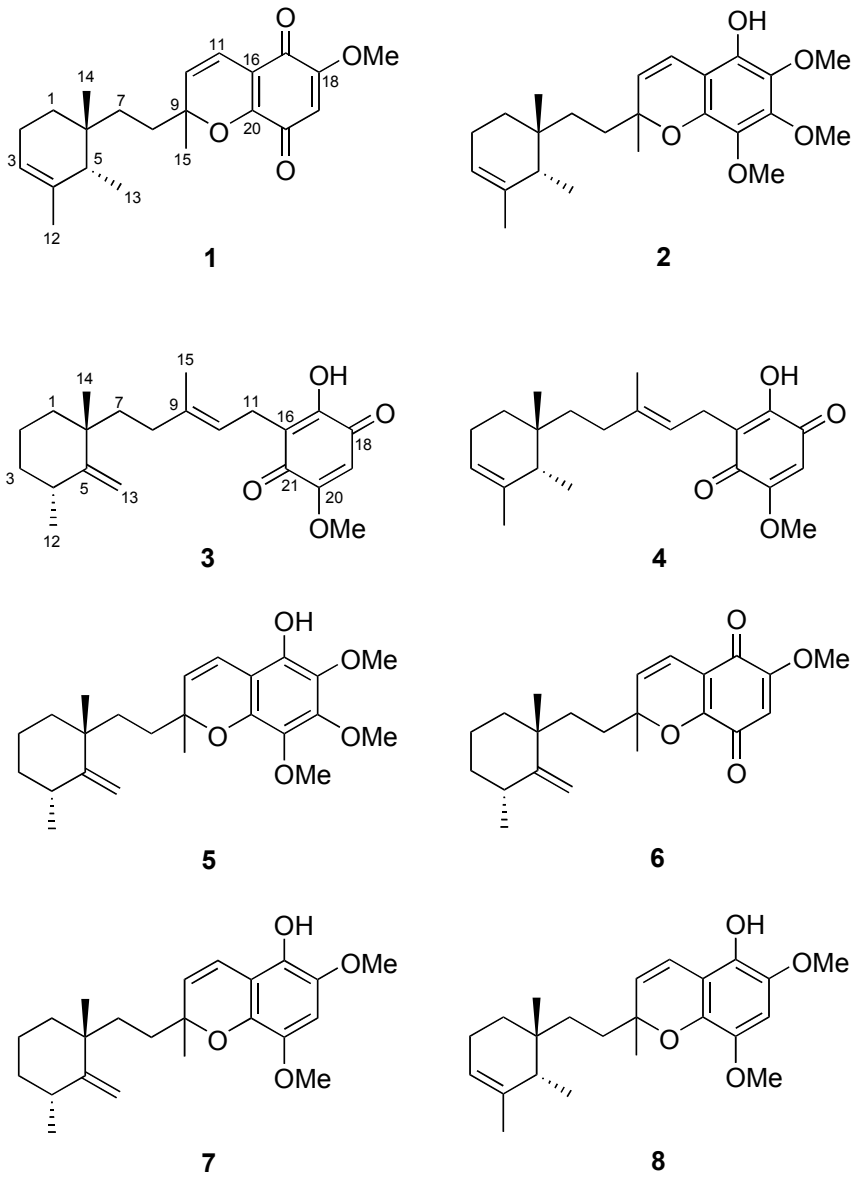
Abstract – Two new cytotoxic sesquiterpenoids, metachromins J (**1**) and K (**2**), have been isolated from an Okinawan marine sponge *Spongia* sp. (SS-1037). The structures of **1** and **2** were elucidated on the basis of the spectroscopic data.

INTRODUCTION

Marine sponges contain a number of unique secondary metabolites with a diversity of biological activities.[1] Marine sponges of the genus *Spongia* are a rich source of terpenoids[2] and polyketides.[3] Our recent study of extracts of an Okinawan sponge *Spongia* sp. (SS-1037) resulted in the isolation of two new sesquiterpenoids, metachromins J (**1**) and K (**2**), together with known sesquiterpenoids, metachromins A,[3] C,[4] D,[5] and E,[5] (**3** ~ **6**), previously isolated from the Okinawan marine sponge *Hippospongia metachromia*. Here we describe the isolation and structure elucidation of **1** and **2**.

RESULTS AND DISCUSSION

The sponge *Spongia* sp. (0.7 kg, wet weight) was extracted with MeOH, and the extracts were partitioned between EtOAc and water. The EtOAc-soluble materials were subjected to SiO_2 gel and C_{18} column chromatographies followed by repeated SiO_2 gel column chromatography and $AgNO_3$-SiO_2 TLC to afford metachromins J (**1**, 0.00013 %, wet weight) and K (**2**, 0.00011 %) together with known related sesquiterpenoids, metachromins A, C, D, and E (**3**-**6**).[4-6] Although the separation of metachromin B (**7**)[4] and its regioisomer (**8**) were tried with the preparative TLC method, they were oxidized by $AgNO_3$-SiO_2 TLC and changed into metachromin E[7] (**6**) and J (**1**), respectively.



Scheme 1. Structures of Metachromins J (**1**), K (**2**), A (**3**), C (**4**), D (**5**), E (**6**), B (**7**), and compound (**8**).

Metachromin J (**1**) showed the molecular ion peak at *m/z* 356 in the EIMS spectrum, and the molecular formula, $C_{22}H_{28}O_4$, was confirmed by HREIMS (*m/z* 356.1998 M^+, Δ +1.1 mmu) spectrum. The IR absorption at 1660 cm^{-1} of **1** was attributed to carbonyl group(s). The UV absorption at 303 nm indicated the presence of quinone moiety. ^1H and ^{13}C NMR data (Table 1) were similar to those of metachromin E (**6**)[6] [δ_H 1.50 (s, H-15), 5.79 (s, H-19), and 6.50 (d, *J* = 10.4 Hz, H-11); δ_C 84.3 (C-9), 178.6 (C-17), and 181.5 (C-20)], while proton signals for three singlet methyl (H-12, 14, and 15), a doublet methyl (H-13), and an olefin proton (H-3) lacking for **6** were observed. Furthermore, there were no exomethylene signals observed in the ^1H NMR spectrum of metachromin A[4] (**3**), suggesting that methachromin J (**1**) possessed a cyclohexene ring system like methachromin C[5] (**4**). Comparing ^1H and ^{13}C NMR spectral data of **1** with those of **4**, signals for the cyclohexene ring in **1** [δ_H 0.88 (d, *J* = 6.8 Hz,

H-13), 1.64 (s, H-12), and 5.26 (br s, H-3); δ_C 119.7 (C-3) and 137.2 (C-4)] were close to those of **4** [δ_H 0.88 (d, *J* = 6.8 Hz, H-13), 1.64 (s, H-12), and 5.26 (br s, H-3); δ_C 119.5 (C-3) and 137.2 (C-4)]. Thus, structure of metachromin J was elucidated to be **1**, possessing a hybrid structure of **4** and **6**.

Table 1 ^{1}H and ^{13}C NMR Data of Metachromins J (**1**) and K (**2**) in $CDCl_3$.

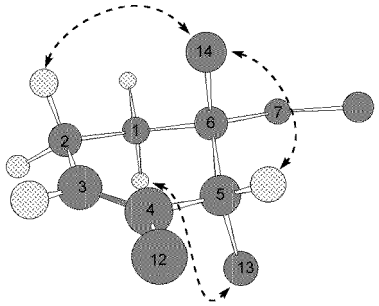
	1		**2**	
positn.	δ_H	δ_C	δ_H	δ_C
1	1.11 m 1.4 m	35.15 CH_2	1.11 m 1.43 m	34.64 CH_2
2	1.93[a] m	22.70 CH_2	1.94[a] m	22.90 CH_2
3	5.26 brs	119.67 CH	5.25 brs	119.69 CH
4		137.23 C		137.41 C
5	1.6 m	42.39 CH	1.6 m	42.62 CH
6		33.77 C	1.2 m 1.3 m	33.85 C
7	1.2 m 1.3 m	29.13 CH_2	1.80[a] m	29.31 CH_2
8	1.9[a] m	32.58 CH_2		32.86 CH_2
9		84.13 C		78.74 C
10	5.54 d, 10.4	128.00 CH	5.52 d, 10.0	127.20 CH
11	6.50 d, 10.4	115.06 CH	6.61 d, 10.0	116.92 CH
12	1.64[b] s	22.58 CH_3	1.64[b] s	22.82 CH_3
13	0.88[b] d, 6.8	14.63 CH_3	0.88[b] d, 7.2	14.73 CH_3
14	0.83[b] s	22.54 CH_3	0.84[b] s	22.75 CH_3
15	1.50[b] s	27.35 CH_3	1.40[b] s	26.05 CH_3
16		113.60 C		105.00 C
17		178.50 C	5.57 br s	140.41 C
18		158.80 C		128.44 C
19	5.79 s	105.30 CH		132.92 C
20		181.38 C		146.19 C
21		151.73 C		142.78 C
OH-17			5.58 br s	
OMe-18	3.82[b] s	56.40 CH_3	3.85[b] s	61.28 CH_3
OMe-19			3.81[b] s	61.41 CH_3
OMe-20			3.93[b] s	61.52 CH_3

[a]2H. [b]3H.

Relative stereochemistry of methyl groups at C-13 and C-14 in **1** and the conformation of the cyclohexene ring were assigned as trans and pseudochair form, respectively, which were the same as those of **4**, since NOESY correlations H-1/H_3-13, H-2/H_3-14, and H-5/H_3-14 (Figure 1) were observed for **1**.

HREIMS spectral data [*m/z* 402.2412 M^+, Δ +0.6 mmu] of metachromin K (**2**) suggested the molecular formula, $C_{24}H_{34}O_5$. The IR absorption at 3430 cm^{-1} was attributed to hydroxyl group(s). The UV absorption at 282 nm indicated the presence of aromatic ring system. ^{1}H and ^{13}C NMR spectral data (Table 1) of **2** suggested that **2** possessed a cyclohexene ring like metachromins C (**4**) and J (**1**). On the other hand, the residual proton and carbon resonances were similar to those of a chromenol moiety for

metachromin D[6] (**5**) [δ_H 1.41 (s, H-15), 3.82, 3.85, 3.94 (s, three methoxy groups), and 6.62 (s, H-11); δ_C 78.9 (C-9), 140.4 (C-17), and 146.2 (C-20)]. Thus, the structure of metachromin K was assigned as **2**. Relative configurations of the cyclohexene ring in **2** elucidated to be the same as those of **4** from NOESY correlations.



1 ←- - → NOESY

Figure 1. Selected NOESY correlations and relative stereochemistry of cyclohexene ring in metachromin J (**1**)

Metachromins J (**1**) and K (**2**) exhibited weak cytotoxicity against murine lymphoma L1210 cells (IC_{50}, 1.0 and 12.6 μg/mL, respectively) and human epidermoid carcinoma KB cells (IC_{50}, 9.9 and >20 μg/mL) *in vitro*.

EXPERIMENTAL

General Experimental Procedures. The IR and UV spectra were recorded on JASCO FT/IR-5300 and Shimadzu UV-1600PC spectrophotometer, respectively. ^1H and ^{13}C NMR spectra were recorded on JEOL JMN-EX 400, Bruker ARX-500 and AMX-600 spectrometers. EIMS spectra were recorded on a JEOL FABmate spectrometer at 70 eV.

Animal Material. The sponge *Spongia* sp. (order Dictyoceratida, family Spongiidae) was collected off Gesashi, Okinawa, and kept frozen until used. The voucher specimen (SS-1037) was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (0.7 kg, wet weight) was extracted with MeOH (1.8 and 1.9 L) at rt, and the extract (30 g) was partitioned between EtOAc (500 mL x 3) and H_2O (500 mL). Parts (1.16 g) of the EtOAc soluble materials (10.54 g) were subjected to SiO_2 gel (hexane/acetone, 80:20) and C_{18} (MeOH/H_2O, 95:5 and 90:10) column chromatographies to give two fractions (**a**) and (**b**), respectively. The fraction (**a**) was purified by a SiO_2 gel column (hexane/EtOAc, 95:5) followed by $AgNO_3$-SiO_2 TLC (hexane/EtOAc, 3:1) to afford metachromins J (**1**, 0.00013 %, wet weight, R_f 0.30) and E (**6**, 0.00010 %, R_f 0.17). The fraction (**b**) was subjected to $AgNO_3$-SiO_2 TLC (hexane/EtOAc, 2:1) to give metachromins K (**2**, 0.00011 %, R_f 0.58) and D (**5**, 0.00011 %, R_f 0.50). The fraction eluted with

hexane/acetone (80:20) in the first SiO_2 gel column was subjected to $AgNO_3$-SiO_2 TLC (hexane/acetone, 1:1) to yield metachromins A (**3**, 0.0089 %, R_f 0.55) and C (**4**, 0.0013 %, R_f 0.42).

Metachromin J (1): red oil; $[\alpha]^{24}_D$ –38° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} 303 (ε 8300), 258 (7000), and 203 (18000) nm; IR (neat) ν_{max} 1660, 1610, and 1580 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* (%) 356 ($[M]^+$, 15) and 205 (100); HREIMS *m/z* 356.1998 $[M]^+$ (calcd for $C_{22}H_{28}O_4$, 356.1987).

Metachromin K (2): colorless oil; $[\alpha]^{24}_D$ +13° (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} 282 (ε 11000), 224 (26000), and 204 (30000) nm; IR (neat) ν_{max} 3430, 1650, 1620, and 1460 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* (%) 402 ($[M]^+$, 10), 279 (5) and 252 (100); HREIMS *m/z* 402.2412 $[M]^+$ (calcd for $C_{24}H_{34}O_5$, 402.2406).

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7. This will be a possible transformation, since it had been reported that **7** was changed to **6** by oxidation. Although metachromins E (**6**) and J (**1**) occurred naturally, they were easily generated through oxidation from metachromin B (**7**) and its regioisomer (**8**).