Metachromins R—T, New Sesquiterpenoids from Marine Sponge *Spongia* sp.

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Three new sesquiterpenoids, metachromins R—T (1—3), have been isolated from an Okinawan marine sponge *Spongia* sp. The structures and stereochemistry of 1—3 were elucidated on the basis of the spectroscopic data. Metachromins S (2) and T (3) showed modest cytotoxicity.

Key words metachromin R; metachromin S; metachromin T; sesquiterpenoid; sponge; *Spongia* sp.

Marine sponges contain a number of unique secondary metabolites with a diversity of biological activities,[1] and sponges of the genus *Spongia* are known to be a rich source of terpenoids[2—9] and polyketides.[10—12] In our continuing search for bioactive compounds from marine organisms, we previously isolated new sesquiterpenoid quinones, metachromins J—Q, from an Okinawan sponge *Spongia* sp. (SS-1037).[13—17] Futher investigation of extracts of this sponge resulted in the isolation of three new sesquiterpenoids, metachromins R—T (**1**—**3**). Here we describe the isolation and structure elucidation of **1**—**3** (Fig. 1).

Results and Discussion

The sponge *Spongia* sp. (SS-1037) collected off Gesashi, Okinawa, was extracted with MeOH. The extracts were partitioned between EtOAc and water. EtOAc-soluble materials were purified by a silica gel column (Hexane/Acetone) and C_{18} column ($MeOH/H_2O$) followed by reversed-phase HPLC (Cosmosil Cholester, $CH_3CN/H_2O/CF_3COOH$) to afford metachromins R (**1**, 0.0001%, wet weight), S (**2**, 0.0004%), and T (**3**, 0.0028%).

Metachromin R (**1**) was obtained as a purple oil and the molecular formula was established to be $C_{29}H_{37}NO_3$ by HR-EI-MS data [*m/z* 447.2773 $(M)^+$, Δ 0.0 mmu]. The IR spectrum indicated the presence of OH and/or NH (3510 cm^{-1}) and carbonyl (1730 cm^{-1}) functionalities. UV absorptions (338, 565 nm) suggested the presence of quinone chromophore. ^1H- and ^{13}C-NMR data of **1** were similar to those of metachromin G,[17] indicating that the difference between **1** and metachromin G was the presence of a trisubstituted double bond (C-3 to C-4) bearing a methyl group in **1** in place of an exomethylene (C-5 to C-13) in metachromin G. The ^1H–^1H COSY and TOCSY spectra of **1** revealed connectivities of five structural fragments, **a** (C-1 to C-4 and C-4 to C-12), **b** (C-7 to C-8), **c** (C-9 to C-11 and C-15), **d** (C-22 to N-20 and C-23), and **e** (C-25 to C-29) as shown in Fig. 2. The gross structure of sesquiterpene moiety (C-1 to C-15) in **1** was implied by HMBC cross-peaks for H_3-12 (δ_H 1.65) to C-5 (δ_C 42.7), H_3-13 (δ_H 0.89) to C-4 (δ_C 137.5) and C-6 (δ_C 34.0), H_3-14 (δ_H 0.83) to C-1 (δ_C 37.1), C-6 and C-7 (δ_C 29.7), and H_3-15 (δ_H 1.75) to C-8 (δ_C 33.4). HMBC cross-peaks for H_2-11 (δ_H 3.08) to C-16 (δ_C 119.7), C-17 (δ_C 156.0), and C-21 (δ_C 180.6), and H-19 (δ_H 5.39) to C-17, C-18 (δ_C 183.0), C-20 (δ_C 149.7), and C-21, H_2-23 (δ_H 2.95) and H-25/H-29 (δ_H 7.20) to C-24 (δ_C 138.1) suggested the existence of a quinone ring (C-16 to C-21) and a phenethylamine unit (C-22 to C-29 and N-20) in **1**. The connectivity of C-20 and C-22 through N-20 was deduced from the HMBC correlation for H_2-22 (δ_H 3.42) to C-20. Thus, the gross structure of metachromin R was elucidated as **1**.

The relative stereochemistry of the cyclohexene ring in **1** was provided from NOESY correlations as shown in Fig. 3. NOESY cross-peaks for H-2β/H_3-14 and H-5/H_3-14 suggested the β configuration of C-14, while the α configuration of C-13 was implied from the NOESY correlation of H-1α/H_3-13.

Metachromin S (**2**) was obtained as a purple oil and the molecular formula was established to be $C_{26}H_{39}NO_3$ by HR-EI-MS data [*m/z* 413.2936 $(M)^+$, Δ −0.6 mmu]. IR (3280, 1730 cm^{-1}) and UV (337, 528 nm) absorptions of **2** suggested the presence of OH and/or NH and quinone functionalities. ^1H- and ^{13}C-NMR data of **2** were similar to those of metachromin R (**1**). Analysis of 2D NMR spectra (^1H–^1H COSY, TOCSY, HMQC, and HMBC) of **2**, revealed that the difference between **2** and **1** was the presence of an isobutylamino group (N-20 and C-22 to C-26) in **2** in place of a phenethylamino group (N-20 and C-22 to C-29) in **1**. Inspection of the NOESY spectrum of **2** revealed that the relative stereochemistry of **2** was same as that of **1**. Thus, the structure of matachromin S was elucidated as **2**.

Metachromin T (**3**) was obtained as a colorless oil and the molecular formula was established to be $C_{23}H_{32}O_4$ by HR-EI-

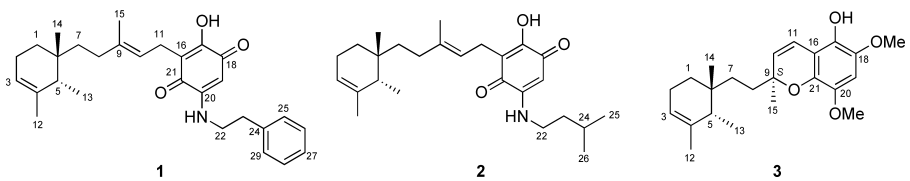

Fig. 1. Structures of Metachromins R—T (**1**—**3**)

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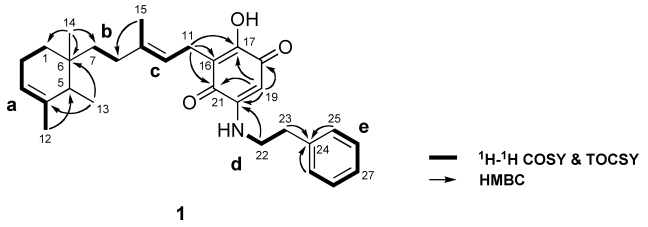

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Fig. 2. Selected 2D NMR Correlations of Metachromin R (**1**)

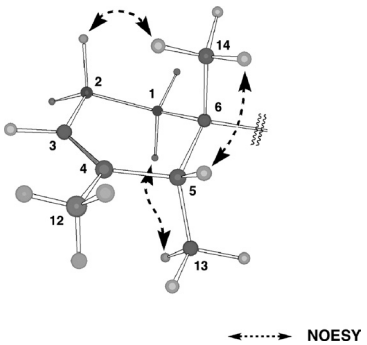

Fig. 3. Selected NOESY Correlations and Relative Stereochemistry of Cyclohexene Ring in Metachromin R (**1**)

MS data [*m/z* 372.2300 $(M)^+$, Δ −0.0 mmu]. IR and UV data of **3** were similar to those of metachromin B.[15] Comparison of ^{1}H- and ^{13}C-NMR data of **3** with those of metachromin B suggested that **3** possessed a 6,8-dimethoxy-2-methyl-2*H*-chromen-5-ol moiety. The connectivity of C-10 to C-11 revealed from ^{1}H–^{1}H COSY and HMBC cross-peaks for H_3-15 (δ_H 1.42) to C-8 (δ_C 33.8) and C-10 (δ_C 129.6), H-10 (δ_H 5.63) to C-8 and C-16 (δ_C 110.2), H-19 (δ_H 6.45) to C-17 (δ_C 140.8), C-18 (δ_C 135.8), C-20 (δ_C 139.4), and C-21 (δ_C 137.3), 18-OCH_3 (δ_H 3.80) to C-18, and 20-OCH_3 (δ_H 3.80) to C-20 also indicated the existence of the chromen moiety (Fig. 4). Inspection of the ^{1}H–^{1}H COSY and TOCSY spectra of **3** implied three structural fragments, C-4 to C-1 and C-12, C-5 to C-13, and C-7 to C-8. Analysis of HMBC spectrum of **3** implied the connectivities of H_3-12 (δ_H 1.63) to C-5 (δ_C 42.4), H_3-13 (δ_H 0.85) to C-4 (δ_C 137.0) and C-6 (δ_C 33.8), and H_3-14 (δ_H 0.81) to C-1 (δ_C 29.3), C-6 and C-7 (δ_C 32.6). These correlations revealed the connectivities of these three fragments and that **3** possessed a cyclohexene ring bearing three methyl groups like metachromins R (**1**) and S (**2**). Thus, the gross structure of metachromin T was elucidated as **3**.

The NOESY spectrum of **3** indicated that the relative stereochemistry of a cyclohexene moiety in **3** was the same as those of **1** and **2**. The absolute configuration at C-9 of metachromin T (**3**) was deduced from the CD spectrum as follows.[18,19] The CD spectrum of metachromin T (**3**) showed a positive Cotton effect around the 275 nm region (MeOH, $[\theta]_{275}$ +10000) due to the styrene chromophore, indicating that the chromenol ring had a negative chirality (a left-handed helix) as shown in Fig. 5, in which the bulky isoprenoid side chain of **3** preferentially adopted the pseudoequatorial conformation. Thus, the absolute configuration at C-9 of **3** was elucidated as *S*.

The absolute configurations at C-5 and C-6 in **1**—**3** were tentatively assigned as *S* and *R*, respectively, since **1**—**3** were

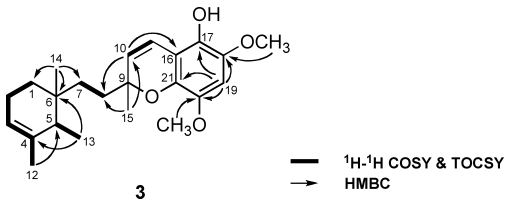

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Fig. 4. Selected 2D NMR Correlations of Metachromin T (**3**)

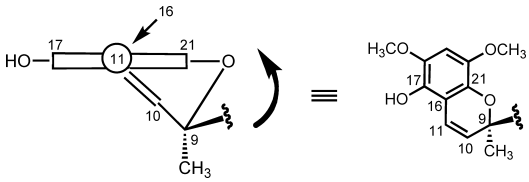

Fig. 5. Perspective Drawing of the Chromenol Moiety of Metachromin T (**3**)

considered to be generated through the same biosynthetic path as that of metachromin A in which the absolute configuration at C-6 had been established to be *R*.[15]

Metachromins S (**2**) and T (**3**) showed cytotoxicity against L1210 murine leukemia (IC_{50}, 5.2 and 3.0 μg/ml, respectively) and KB human epidermoid carcinoma cells (IC_{50}, >10 and 5.6 μg/ml, respectively) *in vitro*, while metachromin R (**1**) did not show such activity (IC_{50} >10 μg/ml).

Experimental

IR and UV spectra were recorded on JASCO FT/IR-5300 and Shimadzu UV-1600PC spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JMN-EX 400 and Bruker AMX-600, spectrometers. EI-MS spectra were recorded on a JEOL JMS-FABmate.

Sponge 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 Separation The sponge (0.7 kg, wet weight) was extracted with MeOH, and the extract was partitioned between EtOAc and H_2O. A part (3.2 g) of EtOAc-soluble materials (10.5 g) were purified by a silica gel column (Hexane/Acetone and then MeOH) followed by a C_{18} column (MeOH/H_2O) to afford fractions Ia and Ib. Fraction Ia was purified by a silica gel column (Hexane/EtOAc) followed by reversed-phase HPLC (Cosmosil Cholester, Nacalai Tesque, Inc., 10×250 mm; eluent, CH_3CN/H_2O/CF_3COOH, 80 : 20 : 0.1; flow rate, 2.0 ml/min; UV detection at 330 nm) to give metachromin T (**3**, 5.9 mg, t_R 25.4 min). Fraction Ib was purified by reversed-phase HPLC (Cosmosil Cholester, Nacalai Tesque, Inc., 10×250 mm; eluent, $CH_3CN/H_2O/CF_3COOH$, 90 : 10 : 0.1; flow rate, 2.0 ml/min; UV detection at 320 nm) to afford metachromins R (**1**, 0.3 mg, t_R 32.2 min) and S (**2**, 0.9 mg, t_R 40.0 min).

Metachromin R (**1**): A purple oil; $[\alpha]_D^{22}$ −20.8° (c=0.2, $CHCl_3$); UV (MeOH) λ_{max} 206 (ε 43000), 338 (8000), 423 (420), and 565 (420) nm; IR (KBr) ν_{max} 3510 and 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.34 (2H, t, J=7.8 Hz, H-26 and 28), 7.27 (1H, t, J=7.8 Hz, H-27), 7.20 (2H, d, J=7.8 Hz, H-25 and 29), 6.43 (1H, m, NH), 5.39 (1H, s, H-19), 5.26 (1H, s, H-3), 5.13 (1H, t, J=6.6 Hz, H-10), 3.42 (2H, q, J=6.6 Hz, H_2-22), 2.95 (2H, t, J =7.2 Hz, H_2-23), 3.08 (2H, d, J=6.6 Hz, H_2-11), 1.93 (2H, m, H_2-2), 1.90 (2H, m, H_2-8), 1.75 (3H, s, H_3-15), 1.65 (3H, s, H_3-12), 1.61 (1H, m, H-5), 1.40 (1H, m, H-2a), 1.10 (1H, m, H-2b), 1.29 (1H, dt, J=13.2, 4.8 Hz, H-7a), 1.20 (1H, dt, J=13.2, 4.8 Hz, H-7b), 0.89 (3H, d, J=7.2 Hz, H_3-13), and 0.83 (3H, s, H_3-14); ^{13}C-NMR ($CDCl_3$) δ: 183.0 (s, C-18), 180.6 (s, C-21), 156.0 (s, C-17), 149.7 (s, C-20), 140.2 (s, C-9), 138.1 (d, C-24), 137.5 (d, C-4), 129.1 (2C, d, C-26 and 28), 129.0 (2C, d, C-25 and 29), 128.6 (d, C-27), 119.7 (s, C-16), 119.4 (d, C-10), 117.8 (d, C-3), 114.7 (d, C-19), 43.9 (t, C-22), 42.7 (d, C-5), 37.1 (t, C-1), 34.3 (t, C-23), 34.0 (s, C-6), 33.4 (t, C-8), 29.7 (t, C-7), 23.0 (q, C-14), 22.9 (q, C-12), 22.9 (t, C-2), 22.7 (t, C-11), 16.3 (q, C-15), and 14.8 (q, C-13); EI-MS *m/z* 447 $(M)^+$; HR-EI-MS *m/z* 447.2773 $(M)^+$ (Calcd for $C_{29}H_{37}NO_3$, 447.2773).

Metachromin S (**2**): A purple oil; $[\alpha]_D^{22}$ +45.1° (c=0.2, $CHCl_3$); UV (MeOH) λ_{max} 203 (ε 33000), 212 (26000), 243 (8100), 337 (26000), and 528 (590) nm; IR (KBr) ν_{max} 3280 and 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 6.38 (1H, s, NH), 5.35 (1H, s, H-19), 5.26 (1H, br s, H-3), 5.14 (1H, m, H-10), 3.16 (2H, m, H_2-22), 3.09 (2H, br d, J=6.0 Hz, H_2-11), 1.93 (2H, m, H_2-2), 1.93 (2H, m, H_2-8), 1.74 (3H, s, H_3-15), 1.65 (3H, s, H_3-12), 1.61 (1H, m, H-5), 1.55 (2H, m, H_2-23), 1.40 (1H, m, H-7a), 1.30 (1H, m, H-7b), 1.40 (1H, m, H-24), 1.11 (1H, m, H-1), 0.94 (6H, d, J=6.6 Hz, H_3-25 and 26), 0.90 (3H, d, J=7.2 Hz, H_3-13), and 0.83 (3H, s, H_3-14); ^{13}C-NMR ($CDCl_3$) δ: 182.4 (s, C-18), 178.8 (s, C-21), 154.9 (s, C-17), 149.7 (s, C-20), 137.9 (s, C-9), 137.5 (d, C-4), 119.7 (s, C-16), 119.7 (d, C-3), 119.4 (d, C-10), 91.5 (d, C-19), 42.8 (d, C-5), 41.1 (t, C-22), 37.7 (t, C-1), 36.9 (t, C-23), 34.1 (s, C-6), 33.4 (t, C-8), 29.2 (t, C-7), 25.9 (d, C-24), 22.9 (q, C-12), 22.9 (t, C-2), 22.7 (q, C-14), 22.3 (2C, q, C-25 and 26), 21.8 (t, C-11), 16.3 (q, C-15), and 14.8 (q, C-13); EI-MS m/z 413 $(M)^+$; HR-EI-MS m/z 413.2936 $(M)^+$ (Calcd for $C_{26}H_{39}NO_3$, 413.2942).

Metachromin T (**3**): A colorless oil; $[\alpha]_D^{22}$ −41.8 (c=0.5, $CHCl_3$); UV (MeOH) λ_{max} 203 (ε 18000), 223 (15000), 267 (5800), 277 (7200), 288 (5700), and 334 (1800) nm; IR (KBr) ν_{max} 3540 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 6.70 (1H, d, J=10.2 Hz, H-11), 6.45 (1H, s, J=10.2 Hz, H-19), 5.63 (1H, d, J=10.2 Hz, H-10), 5.43 (1H, br s, OH), 5.24 (1H, s, H-3), 3.80 (3H, s, H_3-23), 3.80 (3H, s, H_3-22), 1.93 (2H, br s, H_2-2), 1.76 td 1H (1H, dt, J=13.2,4.8 Hz, H-8a), 1.66 (1H, dt, J=13.2, 3.6 Hz, H-8b), 1.63 (3H, s, H_3-12), 1.59 (1H, m, H-5), 1.43 (1H, m, H-1a), 1.42 (3H, s, H_3-15), 1.09 (1H, m, H-1b), 1.39 (1H, m, H-7a), 1.28 (1H, dt, J=13.2, 4.8 Hz, H-7b), 0.85 (3H, d, J=7.2 Hz, H_3-13), and 0.81 (3H, s, H_3-14); ^{13}C-NMR ($CDCl_3$) δ: 140.8 (s, C-17), 139.4 (s, C-20), 137.3 (s, C-21), 137.0 (d, C-4), 135.8 (s, C-18), 129.6 (d, C-10), 119.6 (d, C-3), 117.1 (t, C-11), 110.2 (s, C-16), 100.4 (d, C-19), 78.1 (s, C-9), 58.1 (t, C-23), 56.8 (t, C-22), 42.4 (d, C-5), 33.8 (s, C-6), 33.8 (t, C-8), 32.6 (t, C-7), 29.3 (t, C-1), 25.5 (q, C-15), 22.8 (q, C-14), 22.8 (q, C-12), 22.6 (t, C-2), and 14.6 (q, C-13); EI-MS m/z 372 $(M)^+$; HR-EI-MS m/z 372.2300 $(M)^+$ (Calcd for $C_{23}H_{32}O_4$, 372.2300).

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