New Sesquiterpene Hydroquinones from a Taiwanese Marine Sponge, *Hippospongia metachromia*

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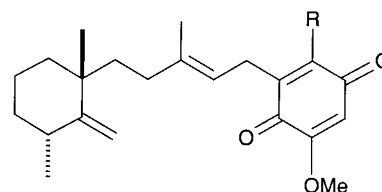
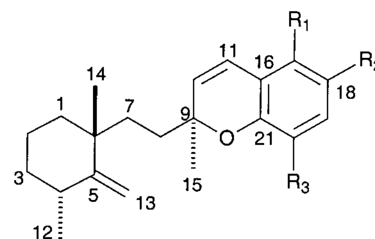
Received December 19, 2000

In addition to metachromins A (**1**), B (**2**), and E (**9**), two new sesquiterpene hydroquinones, hippochromins A and B (**3**, **4**), were isolated from the Taiwanese marine sponge *Hippospongia metachromia*. The structures of **4**−**6** were established by extensive 2D NMR analysis. Metachromins A (**1**) and B (**2**), hippochromin A diacetate (**5**), and metachromin B monoacetate (**8**) exhibited potent cytotoxicities against human colon (COLO-205) and nasopharyngeal (KB) tumor cells, while compounds **6**, **7**, and **9** were inactive.

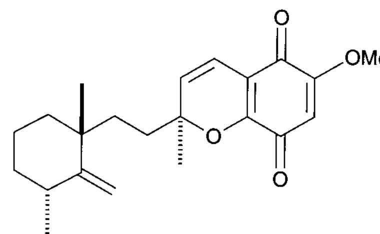
Marine sponges have proven to be a rich source for novel organic compounds with interesting biological activities. These natural products may play a role in warding off predators, and perhaps they also repel fouling organisms. Many terpenoid quinones and hydroquinones from marine sponges have been found to possess potent cytotoxicity against human tumor cells.[1−4] In our continued research on the isolation of bioactive substances from Taiwanese marine sponges,[5−8] we have undertaken a chemical investigation of *Hippospongia metachromia* de Laubenfels (family Spongiidae, order Dictyoceratida), a purple sponge collected along the seashore area of southern Taiwan. The acetone extract of the sponge was partitioned between $CHCl_3$ and H_2O to give a $CHCl_3$-soluble layer. The $CHCl_3$ solubles were subjected to a Si gel column, preparative TLC, HPLC chromatography, and chemical derivatization to yield nine compounds: metachromins A (**1**), B (**2**),[9] and E (**9**),[3] two new sesquiterpene hydroquinones, hippochromins A and B (**3**, **4**), and their derivatives (**5**−**8**). In this paper we wish to report the isolation and structure elucidation of the two new hippochromins A and B (**3**, **4**) and biological evaluation of these novel sesquiterpene hydroquinones. The structures of known sesquiterpene hydroquinones were identified by spectral comparison with the reported data. The structures of the new compounds **3**−**6** were determined by 2D NMR analysis as stated below.



1 R = OH
7 R = OMe



2 R_1 = OH, R_2 = R_3 = OMe
3 R_1 = R_2 = OH, R_3 = OMe
4 R_1 = R_3 = OH, R_2 = OMe
5 R_1 = R_2 = OAc, R_3 = OMe
6 R_1 = R_3 = OAc, R_2 = OMe
8 R_1 = OAc, R_2 = R_3 = OMe



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Hippochromin A (**3**) was an unstable natural compound and was isolated as its diacetate **5**, which had a molecular formula $C_{26}H_{34}O_6$, as determined by high-resolution FABMS, EIMS, and DEPT spectra. The ^1H NMR spectrum of **5** showed signals due to a secondary methyl (δ 1.03), two tertiary methyls (δ 1.02 and 1.34), an exomethylene (δ 4.69 and 4.74), and a pair of *cis*-oriented olefinic protons (δ 5.69 and 6.37, J = 10.0 Hz). These characteristic data were very close to those of metachromins, which contain a chromenol ring system.[9−11] Detailed comparison of ^1H and ^{13}C NMR data of **5** with those of metachromin B (**2**) revealed that **5** contained one methoxyl (δ 3.74) and two aromatic acetoxyl groups (δ 2.27, 2.32), rather than two methoxyl groups as in **2**. Although the ^{13}C NMR data in the chromenol ring system of **5** were different from those of **2**, the remaining signals corresponding to the left side of **5** were almost identical to those of metachromin B. The COSY spectrum of **5** established the connectivities (H-3/H-4, H-4/H-12, H-7/H-8, and H-10/H-11) and allowed assignment of most protons of **5**. The assignment of all protons and carbons was completed on the basis of HSQC and HMBC experiments. The connectivities between H-14/C-1, C-5, C-6 and Me-12/C-3, C-4, C-5 allowed the identification of 2,6-dimethylmethylenecyclohexane. The cross-peaks of H-15/C-8, C-9, C-10; H-11/C-9, C-16, C-17, C-21; and H-19/C-17, C-18, C-20, C-21 facilitated the elucidation of the skeleton of the chromenol derivative. The result of an HMBC spectrum confirmed not only the attachment of diacetate groups at C-17 and C-18 but also the methoxyl

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10.1021/np000610c CCC: $20.00 © 2001 American Chemical Society and American Society of Pharmacognosy
Published on Web 05/17/2001

Table 1. ^{1}H NMR Spectral Data ($CDCl_3$)[a,b] for Compounds **4**–**6**

H	**4**	**5**	**6**
1	1.27 m	1.24 m	1.24 m, 1.40 m
2	1.62 m	1.67 m	1.68 m
3	1.74 m	1.74 m	1.70 m
4	2.33 m	2.33 m	2.30 m
7	1.45 m	1.45 m	1.24 m, 1.40 m
8	1.70 m, 1.58 m	1.70 m	1.61 m, 1.68 m
10	5.65 (d, 10.2)	5.69 (d, 10.0)	5.67 (d, 10.2)
11	6.71 (d, 10.2)	6.37 (d, 10.0)	6.30 (d, 10.2)
12	1.03 (d, 6.5)	1.03 (d, 6.0)	1.01 (d, 6.5)
13a	4.69 s	4.69 s	4.68 s
13b	4.75 s	4.74 s	4.71 s
14	1.04 s	1.02 s	1.01 s
15	1.44 s	1.34 s	1.46 s
19	6.47 s	6.54 s	6.60 s
OMe	3.84 s	3.74 s	3.82 s
OAc		2.27 s	2.26 s
		2.32 s	2.30 s

[a] 300 MHz, δ in ppm (J in Hz). [b] Assignment obtained from HSQC and COSY.

group at C-20. The stereochemistry of **5** was assigned by observation of NOESY experiments and detailed comparison of the observed chemical shifts and coupling constants with those of metachromin B (**2**). The presence of cross-peaks of Me-12/H-13a/H-13b/H-8 and correlation of C17–OAc/H-11/H-10/Me-15 agreed with α-orientation of the methyl groups at C-4. The absence of NOESY between Me-14 and H-13b suggested a β-configuration of Me-14. Although we could not determine the stereochemistry at C-9, we observed correlations of H-10/H-8 and H-10/Me-15. Because compound **4** was oxidized to **9** and the fact that **5** and **6** showed the same NOESYs, the sterechemical assignment of **5** could be supported. The configurations of methyl groups at C-4, C-6, and C-9 were thus tentatively assigned as α, β, and α, respectively.

Hippochromin B (**4**) was an unstable natural compound also. Compound **4** had a molecular formula $C_{22}H_{30}O_4$ as determined by high-resolution EIMS. In $CDCl_3$ solution it was slowly oxidized to **9**.[12] The ^{1}H NMR spectrum of **4** showed signals similar to those of **5**, including a secondary methyl (δ 1.03), two tertiary methyls (δ 1.04 and 1.44), an exomethylene (δ 4.69 and 4.75), and a pair of *cis*-oriented olefinic protons (δ 5.65 and 6.71, J = 10.2 Hz). Upon acetylation, **4** yielded a diacetate (**6**), which showed a molecular formula $C_{26}H_{34}O_6$, the same as **5**, from measurement of high-resolution FABMS. The ^{1}H NMR data of **6** contained two aromatic acetoxyl groups (δ 2.26 and 2.30) and one methoxyl (δ 3.82), in addition to three methyl groups (δ 1.01, 1.01, and 1.46 s) and two exocyclic methylene protons (δ 4.68 and 4.71). The ^{13}C NMR spectrum of **6** exhibited almost identical values to those of **5** except those (C-15 to C-21) in the chromenol ring system (Table 2). The assignment of all protons and carbons was achieved on the basis of COSY, HSQC, and HMBC spectra. Connectivities of Me-14/C-1, C-5, C-6; Me-12/C-3, C-4, C-5; Me-15/C-8, C-9, C-10; and H-11/C-9, C-16, C-17, C-21 as well as H-19/C-17, C-18, C-20, C-21 in the HMBC of **6** were observed. The location of the methoxyl group at C-18 also agreed with the HMBC correlation of both **4** and **6**. The stereochemistry of the methyl groups at C-4, C-6, and C-9 was determined as α, β, and α on the basis of chemical correlation of **4** with **9**. During our NMR measurement, it was found that compound **4** was gradually oxidized to metachromin E (**9**). Because the stereochemistry of **9** has been established by Kobayashi et al.,[3] the structure of **4** was determined unequivocally. The NOESY correlation of **6** (Me-14/H-13a/H-13b/H-8 and C17-OAc/H-11/H-10/Me-15 and H-19/OMe) and the observation of similar chemical shifts and coupling constants between **5** and **6** also supported the stereochemical assignment.

Table 2. ^{13}C NMR Data[a,b] (75.4 MHz, $CDCl_3$) for Compounds **4**–**6** and **8**

C	**4**	**5**	**6**	**8**
1	39.0 t	38.9 t	39.0 t	38.7 t
2	21.7 t	21.7 t	21.8 t	21.9 t
3	37.0 t	37.1 t	37.1 t	35.2 t
4	33.8 d	33.9 d	33.9 d	33.9 d
5	158.9 s	159.1 s	159.3 s	159.5 s
6	34.8 s	34.0 s	34.2 s	34.0 s
7	38.9 t	38.4 t	38.5 t	37.2 t
8	35.1 t	35.2 t	35.1 t	34.8 s
9	78.4 s	79.0 s	79.3 s	78.7 s
10	131.3 d	132.3 d	131.2 d	131.9 d
11	117.2 d	116.4 d	116.5 d	116.9 d
12	19.5 q	19.5 q	19.6 q	19.6 q
13	103.7 t	103.6 t	103.6 t	103.7 t
14	24.9 q	24.5 q	24.8 q	24.9 q
15	25.8 q	25.7 q	23.2 t	26.0 t
16	110.3 s	116.5 s	115.5 s	116.0 s
17	129.7 s	132.3 s	131.1 s	132.0 s
18	140.7 s	136.0 s	146.1 s	146.1 s
19	100.5 d	106.6 d	106.8 d	99.8 s
20	139.3 s	144.5 s	134.8 s	144.5 s
21	137.1 s	138.3 s	140.3 s	136.8 s
OMe	56.8 q	56.4 q	56.6 q	56.8 q
				57.2 q
OAc		20.3 q	20.3 q	20.5 q
		168.5 s	168.7 s	169.1 s
		20.5 q	20.6 q	
		168.5 s	168.4 s	

[a] Mutiplicities were obtained from DEPT. [b] Assignment determined by HSQC and HMBC.

Table 3. Cytotoxicities of Compounds **1**, **2**, and **5**–**9** (IC_{50}, μg/mL)[a]

	compound	KB-16	COLO-250
1	metachromin A	1.80	0.10
2	metachromin B	0.68	0.26
5	hippochromin A diacetate	3.06	0.22
6	hippochromin B diacetate	>10	>10
7	metachromin A monomethylate	>10	>10
8	metachromin B monomethylate	1.32	0.53
9	metachromin E	>10	>10

[a] The concentration of compound that inhibited 50% (IC_{50}) of the growth of the human tumor cell lines KB-16 (nasopharyngeal carcinoma) or COLO-250 (colon carcinoma) according to the method described previously.[14]

The cytotoxicities of the sesquiterpene hydroquinones were evaluated in vitro against human tumor cell lines. Metachromins A (**1**) and B (**2**), hippochromin A diacetate (**5**), and metachromin B monoacetate (**8**) exhibited potent cytotoxicities against human colon (COLO-205) tumor cells at concentrations of 0.1, 0.26, 0.22, and 0.53 μg/mL, respectively. These four sesquiterpenoids also inhibited the growth of nasopharyngeal (KB) tumor cells with IC_{50} values of 1.8, 0.68, 3.06, and 1.32 μg/mL, respectively. However, compounds **6**, **7**, and **9** were inactive when tested on these tumor cells (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. Low-resolution EIMS and FABMS spectra were recorded on a VG Quattro 5022 mass spectrometer, and high-resolution MS spectra were measured on a JEOL HX 110 mass spectrometer. The ^{1}H, ^{13}C NMR, COSY, HSQC, HMBC, and NOESY spectra were recorded on a Bruker FT-300 or a Varian FT-500 spectrometer. The

chemical shifts are given in δ (ppm) and coupling constants in Hz. Si gel 60 (Merck) was used for CC, and precoated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC.

Animal Material. *Hippospongia metachromia* was collected from Nan-Wan, Taiwan, in May 1997. The sample was stored in a refrigerator at −20 °C before extraction. The purple sponge has an irregular shape with several branches. A voucher specimen (SPSF-6) has been deposited in the Department of Marine Resources, National Sun Yat-sen University.

Extraction and Isolation. The fresh sponge (300 g, wet wt) was crushed into small pieces and extracted with acetone (2 L × 3). The combined filtrate was concentrated under vacuum to give a suspension, which was adjusted to 500 mL by the addition of H_2O. The suspension was extracted exhaustively with $CHCl_3$ (500 mL). The $CHCl_3$ layer was concentrated to give a residue (4 g), which was applied to a Si gel (100 g) column eluted with *n*-hexane (0.5 L) and *n*-hexane/EtOAc of increasing polarity (2 L) to afford fractions A (0.16 g) and B (2.18 g, contained **3** and **4**). Part of fraction A (50 mg) was applied on a preparative TLC plate (Si gel) and developed with *n*-hexane/$CHCl_3$/MeOH (5:5:1) to yield fraction I (contained metachromin B, **2**, 15 mg) and metachromin E (**9**, 20 mg). Fraction I was acetylated (Ac_2O/pyridine, each 1 mL) to give a residue, which was chromatographyed by normal-phase HPLC (Si gel column, *n*-hexane/$CHCl_3$/MeOH, 5:5:1) to yield compound **8** (10 mg). Part of fraction B (400 mg) was acetylated to afford a mixture, which was applied on a preparative TLC plate (Si gel) and developed with *n*-hexane/$CHCl_3$/MeOH (5:5:1) to yield metachromin A (**1**, 94 mg) and fraction II (mixture of **5** and **6**). Fraction II was separated by reversed-phase HPLC (LiChrosorb RP-C_{18} column, 85% MeOH) to yield hippochromin A diacetate (**5**, 70 mg) and hippochromin B diacetate (**6**, 22 mg). Metachromin A (**1**, 20 mg) was methylated and followed by normal-phase HPLC (Si gel column, *n*-hexane/$CHCl_3$/MeOH, 30:10:1) to yield compound **7** (13 mg).

Compound 4: UV (MeOH) λ_{max} (log ϵ) 278 (3.67), 290 (3.70) nm; ^{1}H and ^{13}C NMR ($CDCl_3$), in Tables 1 and 2, respectively. HREIMS *m*/*z* 358.2142 (calcd for $C_{22}H_{30}O_4$, 358.2144); EIMS (70 eV) *m*/*z* (rel int) 358 (0.9), 356 (4.5), 341 (2), 249 (10), 235 (12), 221 (100), 205 (66), 191 (15), 177 (7), 123 (15), 91 (19), 81 (39), 69 (9), 55 (26).

Compound 5: $[\alpha]^{26}_D$ −3.4° (*c* 0.6, $CHCl_3$); IR (neat) ν_{max} 2931, 1770, 1481, 1371, 1192, 1036, 931, 897 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), in Tables 1 and 2, respectively; COSY data H-3/H-4, H-4/H-12, H-7/H-8, H-10/H-11; HMBC data H-10/C-9, C-15, C-16; H-11/C-9, C-16, C-17, C-21; H-19/C-17, C-18, C-20, C-21; Me-12/C-3, C-4, C-5; Me-14/C-1, C-5, C-6; Me-15/C-8, C-10; OMe/C-20; NOESY data H-10/H-11, H-10/Me-15, H-11/OAc-17, H-13a/H-13b, H-13a/Me-12, H-13b/H-8, H-19/OMe-20; FABMS *m*/*z* 442 [M]$^+$, 465 [M + Na]$^+$; HRFABMS *m*/*z* 442.2362 (calcd for $C_{26}H_{34}O_6$, 442.2355); EIMS (70 eV) *m*/*z* (rel int) 442 (7), 400 (2), 358 (4), 291 (100), 249 (66), 207 (34), 189 (7), 123 (10), 107 (7.6), 95 (13), 91 (9), 81 (23), 67 (14), 55 (11).

Compound 6: $[\alpha]^{26}_D$ −12.8° (*c* 0.6, $CHCl_3$); IR (neat) ν_{max} 2925, 2856, 1772, 1483, 1448, 1373, 1205, 1020, 968, 887 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 227 (4.50), 270 (3.94), 280 (3.87) nm; ^{1}H and ^{13}C NMR ($CDCl_3$), in Tables 1 and 2, respectively; COSY data H-2/H-3, H-3/H-4, H-4/H-12, H-7/H-8, H-10/H-11; HMBC data H-1/C-2, C-14; H-2/C-1; H-7/C-8; H-10/C-9, C-15, C-16; H-11/C-9, C-16, C-17, C-21; H-13/C-4, C-5, C-6; H-19/C-17, C-18, C-20, C-21; Me-12/C-3, C-4, C-5; Me-14/C-1, C-5, C-6; Me-15/C-8, C-9, C-10; OMe/C-18; NOESY data H-10/H-11, H-10/Me-15, H-11/OAc-17, H-13a/H-13b, H-13a/Me-12, H-13b/H-8, H-19/OMe-18; FABMS *m*/*z* 442 [M]$^+$, 465 [M + Na]$^+$; HRFABMS *m*/*z* 442.2364 (calcd for $C_{26}H_{34}O_6$, 442.2355); EIMS (70 eV) *m*/*z* (rel int) 442 (4), 400 (2), 358 (3), 291 (100), 249 (86), 207 (58), 191 (15), 123 (7), 107 (6), 95 (9), 91 (7), 81 (16), 69 (14), 55 (12).

Cytotoxicity Assays. The in vitro cytotoxicity assay against KB (nasal pharyngeal carcinoma) and COLO-205 (colon carcinoma) tumor cells by the methylene blue dying method was based on reported procedures.[13−15] The cells for bioassay were cultured in RPMI-1640 medium supplemented with a 5% CO_2 incubator at 37 °C. In summary, the assay depends on binding the methylene blue to the fixed monolayer at pH 8.5 and, after washing the monolayer, releasing dye by lowering the pH. Entries and control standard agents were prepared at concentrations of 1, 10, 40, and 100 μg/mL. The detailed procedures of this experiment are in a previous report.[14] Finally, the 96-well tray was dipped into a 0.01 M borated-buffer solution four times to remove the dye. Then, 100 μL/well ethanol/0.1 M HCl (1:1 v/v) was measured on a microtiter plate reader (Dynatech, MR 7000) at a wavelength of 650 nm. The IC_{50} value was defined by comparison of untreated cells at the concentration of the test sample resulting in 50% reduction of absorbance. Mitomycin C was employed as the standard compound, which exhibited an IC_{50} value of 0.05 μg/mL under the above conditions.

Acknowledgment. We thank Ms. Chao-lein Ho and Shiu-ching Yu of the NSC Southern NMR and MS Instrument Center in National Sun Yat-sen University, for measurements of some NMR and MS spectral data. Dr. G. H. Lee, Institute of Oceanology, Academia Sinica, is gratefully acknowledged for his identification of the sponge *Hippospongia metachromia*. The authors especially thank Prof. J. Kobayashi, Faculty of Pharmaceutical Sciences, Hokkaido University, for his help in providing NMR spectra of metachromin A. This research was supported by a grant from the National Science Council, Republic of China (NSC 89-2320-B-110-009).

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NP000610C