t_R (250 °C) 4.16 min; ^{1}H NMR (60 MHz) δ 1.05–2.08 (m, 9), 2.25 (br s, 1), 2.55 (br s, 1), 2.81 (m, 1), 2.91–3.28 (m, 2), 3.80 (s, 3), 6.55 (br s, 1.5), 6.70 (d, J = 2 Hz, 0.5), and 7.48 (d, J = 8 Hz, 1). Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 79.35; H, 8.01.

Epimerization of 10 to 11. The ketone **10** (130 mg, 0.5 mmol) was heated under reflux with a solution of NaOMe in MeOH (25 mL, 2%) for 3.5 h. After removing most of the MeOH, the residue was diluted with H_2O (10 mL) and extracted with Et_2O (3 × 15 mL). The Et_2O extract was washed with brine and dried. Removal of solvent followed by column chromatography of the residue afforded **11** (110 mg, 84%): IR 1660 (s), 1600 (s) cm^{-1}; t_R (250 °C) 1.7 min; ^{1}H NMR (60 MHz) δ 1.05–2.65 (m, 12), 3.05 (d, J = 4 Hz, 2), 3.73 (s, 3), 6.43 (m, 1.5), 6.60 (d, J = 2 Hz, 0.5), and 6.91 (d, J = 8 Hz, 1). Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 79.37; H, 7.93.

Epimerization of 12 to 13. Following the above procedure, the ketone **12** (130 mg, 0.5 mmol) was epimerized to give, after column chromatography, the *trans*-ketone **13** (100 mg, 77%): mp 85 °C; IR 1670 (s), 1600 (s) cm^{-1}; t_R (250 °C) 4.03 min; ^{1}H NMR (60 MHz) δ 1.13–2.23 (m, 11), 2.56–3.46 (m, 3), 3.85 (s, 3), 6.65 (m, 1.5), 6.85 (d, J = 2 Hz, 0.5), and 7.98 (d, J = 8 Hz, 1). Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 79.78; H, 8.19.

Cycloaddition of 6a with Maleic Anhydride to 14. A suspension of the dienone **6a** (50 mg, 0.3 mmol) and maleic anhydride (30 mg, 0.3 mmol) in xylene (2 mL) was refluxed for 22 h. In the beginning all of the solid was dissolved, but after 2–3 h refluxing, the adduct started crystallizing out. The crystals were collected by filtration and dried to afford **14** (70 mg, 87%): mp 188 °C; IR 1855, 1775 cm^{-1}; ^{1}H NMR (100 MHz) δ ($CDCl_3$) 3.72 (narrow t, J = 1 Hz, 2), 3.88 (s, 3), 4.16 (br t, J = 8 Hz, 2), 6.32 (t, J = 8 Hz, 1), 6.74 (t, J = 8 Hz, 1), 6.88 (m, 2), and 8.12 (d, J = 8 Hz, 1). Anal. Calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26. Found: C, 67.13; H, 4.27.

Acknowledgment. Financial support from DST, Govt. of India, is gratefully acknowledged. S.S. and G.S. wish to thank CSIR for research fellowships.

Metachromins D–H, New Cytotoxic Sesquiterpenoids from the Okinawan Marine Sponge *Hippospongia metachromia*

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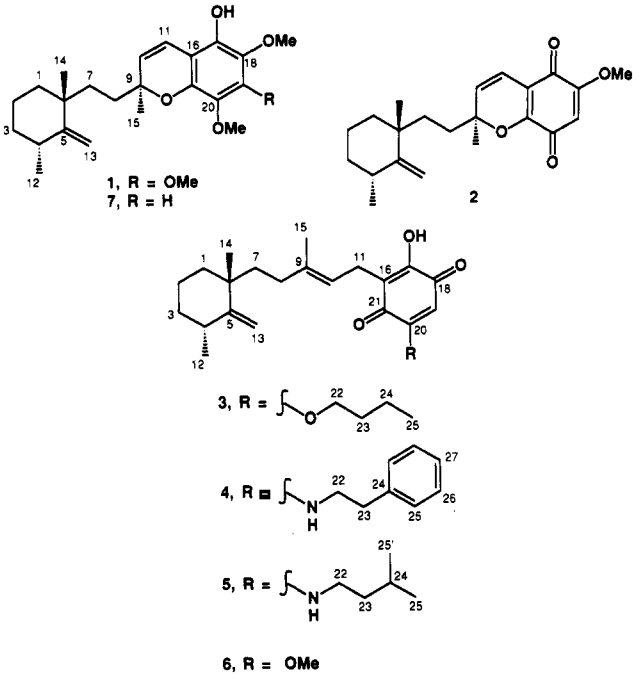
Received April 20, 1992

Many terpenoid quinones and phenols from marine sponges have been shown to exhibit interesting biological activities.[2] We have also reported the isolation and structural elucidation of three novel antineoplastic sesquiterpenoids, metachromins A, B, and C, from the Okinawan marine sponge *Hippospongia metachromia*.[3] In our continuing studies on bioactive metabolites from marine organisms,[4] we further examined extracts of the same sponge *H. metachromia* to obtain five new cytotoxic sesquiterpenoids, metachromins D–H (**1**–**5**), together with known related compounds metachromins A (**6**) and B (**7**). In this paper we describe the isolation and structural elucidation of **1**–**5**.



The purple-colored sponge *H. metachromia* was collected at Unten Harbour, Okinawa, and kept frozen until required. The methanolic extract of the sponge was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was subjected to silica gel column chromatography followed by silica gel TLC to yield metachromins D (**1**, 0.0037%, wet weight), E (**2**, 0.0076%), F (**3**, 0.0026%), G (**4**, 0.0010%), and H (**5**, 0.0013%) together with metachromins A (**6**, 0.17%) and B (**7**, 0.014%).

Metachromin D (**1**), a colorless oil, was shown to have the molecular formula $C_{24}H_{34}O_5$ by HREIMS (m/z 402.2408, M$^+$, Δ +0.2 mmu). The IR spectrum (ν_{max} 3400 cm^{-1}) suggested that **1** possessed hydroxyl group, and the UV absorptions (λ_{max} 226, 280, and 320 nm) were indicative of the presence of a phenol moiety. The ^{1}H NMR spectrum of **1** in $CDCl_3$ showed signals due to a secondary methyl (δ 1.03), two tertiary methyls (δ 1.01 and 1.41), three methoxy groups (δ 3.82, 3.85, and 3.94), an exomethylene (δ 4.69 and 4.76), one singlet deuterium-exchangeable proton (δ 5.58), and two doublet olefinic protons (δ 5.52 and 6.62, J = 10.3 Hz; cis-oriented). These data were reminiscent of a chromenol ring system.[5] The ^{1}H and ^{13}C NMR (Table I) data of **1** were similar to those of metachromin B (**7**), while three methoxy signals (δ_H 3.82, 3.85, and 3.94; δ_C 61.3, 61.4, and 61.5) in place of two methoxy signals (δ_H 3.82 and 3.83; δ_C 56.8 and 58.4) and an aromatic proton signal (δ_H 6.47) in **7** were observed for **1**. Irradiation of a hydroxyl proton (δ 5.53) caused 2.0 and 2.8% NOE of the olefinic proton at δ 6.62 (H-11) and the methoxy protons at δ 3.85 (CH_3O-22), respectively, since the hydroxyl group was attached to C-17. This result was coincident with the HMBC correlations for OH-17/C-16, OH-17/C-18, and H-11/C-17. Thus the structure of metachromin D was concluded to be **1**. Configurations of C-4, C-6, and C-9 in **1** were tentatively assigned as *R*, *R*, and

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Table I. ^{13}C NMR Chemical Shifts of Metachromins A (6), B (7), and D–H (1–5)

	1		2		3		4		5		6		7	
position	δ[a]	m[b]	δ	m	δ	m	δ	m	δ	m	δ	m	δ	m
1	38.72	t	38.70	t	38.70	t	38.67	t	38.76	t	38.68	t	38.68	t
2	21.89	t	22.68	t	21.90	t	21.87	t	21.95	t	21.88	t	21.76	t
3	37.20	t	37.07	t	37.26	t	37.24	t	37.32	t	37.24	t	37.10	t
4	33.98	d	33.94	d	33.97	d	33.94	d	34.02	d	33.94	d	33.94	d
5	159.42	s	158.95	s	159.75	s	159.76	s	159.81	s	159.70	s	159.37	s
6	39.05	s	39.05	s	39.25	s	39.23	s	39.32	s	39.22	s	38.97	s
7	35.35	t	35.95	t	39.99	t	40.03	t	40.11	t	39.98	t	34.82	t
8	35.40	t	34.91	t	34.04	t	34.01	t	34.01	t	34.02	t	35.17	t
9	78.90	s	84.31	s	138.37	s	137.37	s	137.85	s	138.47	s	78.24	s
10	127.27	d	128.04	d	118.92	d	119.41	d	119.75	d	118.72	d	129.58	d
11	116.93	d	115.19	d	21.90	t	21.87	t	21.95	t	21.87	t	117.17	d
12	19.61	q	19.61	q	19.62	q	19.59	q	19.68	q	19.61	q	19.48	q
13	103.71	t	103.95	t	103.47	t	103.42	t	103.52	t	103.46	t	103.59	t
14	24.98	q	24.95	q	24.66	q	24.63	q	24.72	q	24.65	q	24.81	q
15	26.13	q	27.54	q	16.40	q	16.33	q	16.39	q	16.40	q	25.80	q
16	104.99	s	115.07	s	118.12	s	119.41	s	119.75	s	118.25	s	110.20	s
17	140.42	s	178.60	s	151.11	s	154.00	s	156.00	s	151.23	s	140.81	s
18	132.94	s	158.89	s	183.16	s	184.55	s	182.31	s	183.00	s	135.96	s
19	135.09	s	105.39	d	102.39	d	91.83	d	91.53	d	102.20	d	101.01	d
20	146.21	s	181.45	s	160.58	s	149.55	s	150.01	s	161.10	s	139.35	s
21	142.83	s	151.86	s	181.62	s	182.25	s	178.90	s	181.54	s	137.36	s
22	61.28	q	56.47	q	69.85	t	43.93	t	41.08	t	56.77	q	56.83	q
23	61.41	q			29.23	t	34.30	t	37.32	t			58.41	q
24	61.52	q			19.09	t	137.37	s	25.95	d				
25					13.66	q	128.56	d	22.39	q				
26							128.96	d						
27							127.09	d						

[a] δ in ppm. [b] Multiplicity in DEPT.

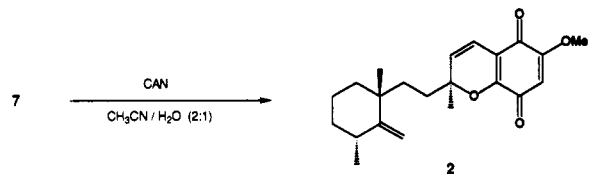
S, respectively, since **1** was considered to be generated through the same biosynthetic path as that of **7**.[3a]

Metachromin E (**2**) was obtained as an orange oil. The molecular formula of **2** was determined as $C_{22}H_{28}O_4$ by HREIMS (*m/z* 356.1996, M^+, Δ +0.9 mmu). The UV (λ_{max} 260, 290, 360, and 483 nm) and IR (ν_{max} 1670, 1610, and 1580 cm^{-1}) data of **2** indicated the presence of a benzoquinone moiety.[6] Comparison of the ^1H and ^{13}C NMR (Table I) data of **2** with those of **7** revealed that the structure of terminal cyclohexane ring (C-1–C-6) of **2** was identical with that of **7**, and the prominent differences were the presence of the benzoquinone moiety [δ_C 105.4 (d), 115.1 (s), 151.9 (s), 158.9 (s), 178.6 (s), and 181.5 (s)] for compound **2**. The connectivities around quaternary carbons, C-5, C-6, and C-9, of the sesquiterpene moiety were established by cross peaks of H_3-12/C-5, H-13/C-4, H-13/C-6, H_3-14/C-1, H_3-14/C-7, H-8/C-6, H_3-15/C-8, and H-11/C-9, while cross peaks of H-11/C-17 and H-11/C-21 allowed connection between the sesquiterpene moiety and the benzoquinone ring (C-11–C-16). The correlations of H-19/C-17, H-19/C-18, H-19/C-20, and H-19/C-21 observed in the HMBC spectrum of **2** established that a methoxy group (δ_H 3.82, MeO-22) was attached to C-18. Thus the structure of metachromin E was suggested to be **2**. This was confirmed by conversion of metachromin B (**7**) into metachromin E (**2**) through oxidation with cerium(III) ammonium nitrate (CAN)[7] (Scheme I). Treatment of metachromin B (**7**) with CAN in CH_3CN/H_2O (2:1) afforded a quinone compound, whose optical rotation and other spectral data were completely consistent with those of natural metachromin E (**2**). Thus the absolute configuration of **2** was determined to be 4*R*,6*R*,9*S*.

Each of compounds **3**–**5** showed a characteristic EIMS fragment ion at *m/z* 123, which was attributed to a C_9H_{15} alkylated cyclohexane unit in the terminal part as observed in the EIMS of metachromin A (**6**).[3a] The molecular formula of metachromin F (**3**) was determined to be $C_{25}H_{36}O_4$ by HREIMS (*m/z* 400.2622, M^+, Δ +0.8 mmu). The IR and UV spectra of metachromin F (**3**) were indicative of the presence of a hydroxy quinone moiety (ν_{max} 3400, 1640, and 1600 cm^{-1}; λ_{max} 290 and 455 nm). The ^1H and ^{13}C (Table I) NMR data of **3** resembled those of metachromin A (**6**), while in place of a methoxy signal [δ_H 3.86 (s); δ_C 56.8 (q)] in **6**, signals due to a butoxy group [δ_H 3.94 (t), 1.85 (m), 1.44 (m), and 0.97 (t); δ_C 69.9 (t), 29.2 (t), 19.1 (t), and 13.7 (q)] were observed for **3**. The presence of a butoxy group in **3** was confirmed by EIMS fragment ions [400 (M^+), 385 ($M^+ - CH_3$), 372 ($M^+ - C_2H_4$), 358 ($M^+ - C_3H_6$), and 344 ($M^+ - C_4H_8$)]. The EIMS fragment ion at *m/z* 210 ($M^+ - C_{14}H_{23} + H$) supported the quinone moiety (C-11–C-25). The chemical shift for H-19 (δ_H 5.80) of **3** was similar to that (δ_H 5.84) of **6**, implying that the substitution pattern of the benzoquinone group was the same as that of **6**. This was confirmed by the HMBC correlations of H-19/C-17 and H-19/C-21. Thus the structure of metachromin F was concluded to be **3**.

Metachromin G (**4**) was obtained as a purple oil. The molecular formula of **4** was determined as $C_{29}H_{37}NO_3$ by HREIMS (*m/z* 447.2768, M^+, Δ −0.5 mmu). The IR and UV spectra suggested that **4** possessed a benzoquinone moiety (ν_{max} 3300, 1590, and 1510 cm^{-1}; λ_{max} 324 and 508 nm). The ^1H NMR spectrum of **4** was similar to that of metachromin A (**6**) except for the signals for two sp^3 methylenes [δ_H 3.42 (q) and 2.95 (t)], aromatic protons [δ_H 7.20 (2 H, d), 7.27 (1 H, t), and 7.34 (2 H, t)], and one

Scheme I



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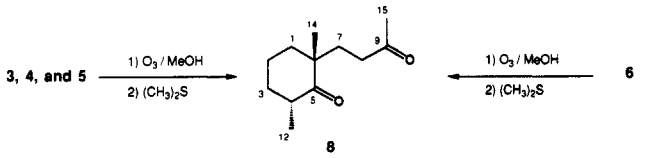
Scheme II



additional deuterium-exchangeable proton (δ_H 6.45). This suggested that 4 possessed a sesquitepene moiety (C-1–C-11), a benzoquinone ring (C-16–C-21), and a phenethylamine unit (C-22–C-27). The EIMS fragment ion at m/z 257 ($M^+ - C_{14}H_{23} + H$) supported the amino quinone moiety (C-11–C-27). The assignment of all the protonated carbons in 4 (Table I) was made by the HSQC experiment. The HMBC spectrum of 4 revealed the connectivities from C-20 to C-27 (cross peaks: H-22/C-20, H-22/C-24, H-23/C-25, H-25/C-24, H-26/C-24, and H-27/C-25). Thus the structure of metachromin G was concluded to be 4.

The molecular formula of metachromin H (5) was determined as $C_{26}H_{39}NO_3$ by HREIMS (m/z 413.2920, Δ -1.0 mmu). The IR (ν_{max} 3300, 1570, and 1520 cm^{-1}) and UV (λ_{max} 324 and 512 nm) of 5 suggested the presence of hydroxyl and/or amino groups and a benzoquinone ring. The ^{13}C signals due to C-1–C-21 of 5 resembled those of metachromin G (4). The EIMS fragment ion at m/z 223 ($M^+ - C_{14}H_{23} + H$) supported the amino quinone moiety (C-11–C-25). The extensive analysis of the ^1H–^1H COSY spectrum of 5 allowed assignment of all protons and established the following proton connectivities: H-1/H-2, H-2/H-3, H-3/H-4, H-4/H-12, H-7/H-8, H-10/H-11, H-22/H-23, H-23/H-24, and H-24/H-25,25′. The only structural difference between compounds 5 and 4 was the presence of an isobutylamino group [δ_H 0.94 (6 H, d, H_3-25,25′), 1.40 (1 H, m, H-24), 1.56 (2 H, m, H_2-23), 3.15 (2 H, m, H_2-22), and 6.40 (1 H, brs, NH)] in 5 in place of a phenethylamino group in 4. The structure of metachromin H was, therefore, assigned as 5.

The absolute configurations of metachromins F, G, and H (3, 4, and 5) were established as follows. Ozonolysis of metachromin A (6) followed by reduction with $(CH_3)_2S$ gave compound 8 ($[\alpha]^{19}_D$ -39°)[3a] (Scheme II). Each of metachromins F, G, and H (3, 4, and 5) was ozonized by the same method to afford compound 8, whose optical rotation and other spectral data were completely consistent with those of 8 derived from 6. Thus the absolute configurations of 3, 4, and 5 were determined to be 4R and 6R.

Metachromins D–H (1–5) contain the same unusual carbon skeleton as that of metachromins A (6) or B (7).[3a] Amino quinones such as metachromins G (4) and H (5) are very rare from natural sources and are known only avarol derivatives[8] and smenospongine[9] from marine origin. Metachromins D–H (1–5) exhibited cytotoxicity against murine lymphoma L1210 cells with IC_{50} values of 3.0, 0.2, 0.6, 1.3, and 2.0 μg/mL, respectively, and human epidermoid carcinoma KB cells with IC_{50} values of 10, 0.4, 1.9, >10, and 6.4 μg/mL, respectively.

Experimental Section

General Methods. The 7.26 ppm resonance of residual $CHCl_3$ and 77.1 ppm of $CDCl_3$ were used as internal references for ^1H and ^{13}C NMR spectra, respectively. Wako C-300 silica gel was used for glass column chromatography. TLC was carried out on Merck silica gel GF_{254}.

Collection, Extraction, and Separation. The purple-colored sponge *H. metachromia* was collected by netting at Unten Harbour (-70 m), Okinawa Island, and kept frozen until used. The sponge (1.0 kg, wet weight) was extracted with methanol (1.5 L × 2). Evaporation of the extract afforded a residue (59.7 g), which was dissolved in a mixture of EtOAc (500 mL) and 1 M NaCl (500 mL). The aqueous layer was extracted with EtOAc (400 mL × 3). The EcOAc-soluble fraction was evaporated under reduced pressure to give a residue (8.1 g), part of which (1.0 g) was subjected to a silica gel column (2.4 × 39 cm) eluted with hexane/EtOAc [3:1 (600 mL) → 2:1 (200 mL) → 1:1 (150 mL) → 0:100 (100 mL)]. The fraction eluting from 130 to 210 mL was subjected to a silica gel column (1.4 × 24 cm) with $CHCl_3$, and the fraction (80–120 mL) was chromatographed on a silica gel column (0.5 × 11 cm) with toluene/MeOH (80:1) to give two fractions of a (4–10 mL) and b (10–30 mL). The fraction a was further separated on silica gel TLC with hexane/$CHCl_3$/acetone (3:1:1) to afforded metachromins G (4, 1.2 mg, 0.0010%, wet weight) and H (5, 1.6 mg, 0.0013%), and the fraction b was separated on silica gel TLC with toluene/EtOAc/CH_3CN (8:1:1) to give metachromins D (1, 4.7 mg, 0.0037%) and E (2, 9.5 mg, 0.0076%). The fraction (40–80 mL) of the second silica gel column (eluent, $CHCl_3$) was further purified by silica gel TLC with toluene/EtOAc (9:1) to afford metachromins F (3, 3.2 mg, 0.0026%) and B (7, 18.0 mg, 0.014%), and the fraction (220–300 mL) of the first column was evaporated under reduced pressure to afford a solid, which was crystallized from hexane to give metachromin A (6, 213 mg, 0.17%).

Metachromin D (1): a colorless oil; $[\alpha]^{22}_D$ +15° (*c* 0.7, $CHCl_3$); IR (neat) ν_{max} 3400, 1460, 1420, 1380, 1280, 1120, and 1040 cm^{-1}; UV (MeOH) λ_{max} 226 (ϵ 25000), 280 (10000), and 320 nm (3000); ^1H NMR ($CDCl_3$) δ 0.86 (1 H, m, H-2′), 0.97 (1 H, qd, J = 12.2 and 5.52 Hz, H-3′), 1.01 (3 H, s, H_3-14), 1.03 (3 H, d, J = 7.0 Hz, H_3-12), 1.25 (1 H, td, J = 12.7 and 4.9 Hz, H-1′), 1.41 (3 H, s, H_3-15), 1.46 (1 H, m, H-1), 1.59 (1 H, m, H-2), 1.63 (1 H, m, H-7′), 1.66 (2 H, m, H-8), 1.74 (1 H, m, H-3), 1.86 (1 H, ddd, J = 12.0, 10.3, and 5.9 Hz, H-7), 2.32 (1 H, m, H-4), 3.82 (3 H, s, MeO-23), 3.85 (3 H, s, MeO-22), 3.94 (3 H, s, MeO-24), 4.69 (1 H, s, H-13′), 4.76 (1 H, s, H-13), 5.52 (1 H, d, J = 10.3 Hz, H-10), 5.58 (1 H, s, OH-17), and 6.62 (1 H, d, J = 10.3 Hz, H-11); EIMS m/z (%) 402 (M^+, 20), 387 (2), 279 (15), 263 (5), 251 (100), 213 (10), and 149 (25); HREIMS m/z 402.2408 (M^+, calcd for $C_{24}H_{34}O_5$, 402.2406); HMBC (H/C) 1′/6, 1′/14, 7/8, 7/9, 7′/5, 7′/6, 7′/8, 7′/14, 8/7, 8/9, 10/8, 10/9, 10/16, 11/9, 11/17, 11/21, 12/4, 13/4, 13/6, 14/6, 14/7, 15/8, 15/9, 17/16, OH-17/17, and 17/18.

Metachromin E (2): an orange oil; $[\alpha]^{22}_D$ -54° (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 1670, 1610, 1580, 1460, 1120, and 1040 cm^{-1}; UV (MeOH) λ_{max} 260 (ϵ 6000), 290 (8700), 360 (500), and 483 nm (300); ^1H NMR ($CDCl_3$) δ 0.86 (1 H, m, H-2′), 0.94 (1 H, m, H-3′), 1.01 (3 H, d, J = 6.8 Hz, H_3-12), 1.02 (3 H, s, H_3-14), 1.22 (1 H, m, H-1′), 1.43 (1 H, m, H-1), 1.50 (3 H, s, H_3-15), 1.54 (2 H, m, H_2-8), 1.56 (1 H, m, H-2), 1.60 (1 H, m, H-7′), 1.71 (1 H, m, H-3), 1.90 (1 H, m, H-7), 2.31 (1 H, m, H-4), 3.82 (3 H, s, MeO-22), 4.67 (1 H, s, H-13′), 4.69 (1 H, s, H-13), 5.57 (1 H, d, J = 10.3 Hz, H-10), 5.79 (1 H, s, H-19), and 6.51 (1 H, d, J = 10.3 Hz, H-11); EIMS m/z (%) 356 (M^+, 12), 341 (3), 237 (20), 205 (100), and 160 (10); HREIMS m/z (%) 356.1996 (M^+, calcd for $C_{22}H_{28}O_4$, 356.1987); HMBC (H/C) 1′/14, 7/14, 10/9, 10/11, 11/9, 11/21, 12/3, 12/4, 14/5, 13/4, 13/6, 14/1, 14/6, 14/7, 15/9, 15/10, 19/17, 19/18, and 19/21.

Metachromin F (3): a yellow oil; $[\alpha]^{22}_D$ -4° (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3400, 1640, 1600, 1370, 1300, and 1200 cm^{-1}; UV (MeOH) λ_{max} 290 (ϵ 16400) and 455 nm (250), ^1H NMR ($CDCl_3$) δ 0.83 (1 H, m, H-2′), 0.96 (1 H, m, H-3′), 0.97 (3 H, t, J = 6.6 Hz, H_3-25), 1.01 (3 H, s, H_3-14), 1.02 (3 H, d, J = 6.6 Hz, H_3-12), 1.21 (1 H, m, H-1′), 1.44 (2 H, m, H_2-24), 1.47 (1 H, m, H-1), 1.48 (1 H, m, H-7′), 1.57 (2 H, m, H-2, 7), 1.73 (1 H, m, H-3), 1.77 (3 H, s, H_3-15), 1.85 (2 H, m, H_2-23), 1.96 (2 H, m, H_2-8), 2.32 (1 H, m, H-4), 3.15 (1 H, d, J = 7.3 Hz, H_2-11), 3.94 (2 H, t, J = 6.6 Hz, H_2-22), 4.68 (1 H, s, H-13′), 4.70 (1 H, s, H-13), 5.16 (1 H, t, J = 7.3 Hz, H-10), and 5.80 (1 H, s, H-19); EIMS m/z (%) 400 (M^+, 40), 385 (3), 372 (3), 358 (3), 344 (3), 210 (65), 166 (30), 123 (55), 109 (100), and 57 (35); HREIMS m/z 400.2622 (M^+, calcd for $C_{25}H_{36}O_4$, 400.2614); HMBC (H/C) 1′/14, 8/9, 8/10, 10/8, 10/15, 11/9, 11/10, 11/17, 11/21, 12/3, 12/4, 14/1, 14/6, 14/7, 15/8, 15/9, 15/10, 19/17, 19/20, 19/21, 22/20, 22/23, 22/24, 23/22,

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23/24, 23/25, 24/22, 24/23, 24/25, 25/23, and 25/24.

Metachromin G (4): a purple oil; $[\alpha]^{20}_D$ -18° (c 0.2, C_6H_6); IR (neat) ν_{max} 3300, 1590, 1510, 1380, and 1210 cm^{-1}; UV (MeOH) λ_{max} 211 (ϵ 29700), 324 (14700), and 508 nm (800); ^1H NMR ($CDCl_3$) δ 0.83 (1 H, m, H-2'), 0.99 (1 H, m, H-3'), 1.01 (3 H, s, H_3-14), 1.02 (3 H, d, J = 6.6 Hz, H_3-12), 1.19 (1 H, m, H-1'), 1.46 (1 H, m, H-1), 1.50 (1 H, m, H-7'), 1.57 (1 H, m, H-7) 1.58 (1 H, m, H-2), 1.73 (1 H, m, H-3), 1.75 (3 H, s, H_3-15), 1.97 (2 H, m, H_2-8), 2.33 (1 H, m, H-4), 2.95 (2 H, t, J = 7.0 Hz, H_2-23), 3.09 (2 H, d, J = 7.0 Hz, H-11), 3.42 (2 H, q, J = 7.0 Hz, H_2-22), 4.68 (1 H, s, H-13'), 4.71 (1 H, s, H-13), 5.16 (1 H, t, J = 7.0 Hz, H-10), 5.39 (1 H, s, H-19), 6.45 (1 H, brt, NH), 7.20 (2 H, d, J = 8.0 Hz, H-25, 25'), 7.27 (1 H, t, J = 8.0 Hz, H-27), and 7.34 (2 H, t, J = 8.0 Hz, H-26, 26'); EIMS m/z (%) 447 (M^+, 90), 356 (60), 296 (20), 257 (60), 166 (90), 123 (30), 105 (100), and 91 (45); HREIMS m/z 447.2768 (M^+, calcd for $C_{29}H_{37}NO_3$, 447.2673); HMBC (H/C) 1/14, 1'/14, 7/6, 7/14, 8/9, 8/10, 10/8, 11/10, 11/16, 11/21, 12/3, 12/4, 13/4, 13/6, 14/1, 14/6, 14/7, 15/8, 15/9, 15/10, 19/17, 19/21, 22/20, 22/23, 22/24, 23/22, 23/24, 23/25, 25/26, 25/27, 26/24, 26/25, and 27/26.

Metachromin H (5): a purple oil; $[\alpha]^{19}_D$ -9° (c 0.2, C_6H_6); IR (neat) ν_{max} 3300, 3250, 1570, 1520, 1380, and 1200 cm^{-1}; UV (MeOH) λ_{max} 324 (ϵ 12800) and 512 nm (700); ^1H NMR ($CDCl_3$) δ 0.83 (1 H, m, H-2'), 0.94 (6 H, d, J = 6.6 Hz, H-25, 25') 0.98 (1 H, m, H-3') 1.01 (3 H, s, H_3-14), 1.02 (3 H, d, J = 6.6 Hz, H_3-12), 1.21 (1 H, m, H-1'), 1.40 (1 H, m, H-24), 1.46 (1 H, m, H-1), 1.52 (2 H, m, H_2-7), 1.56 (2 H, m, H_2-23), 1.58 (1 H, m, H-2), 1.73 (1 H, m, H-3), 1.76 (3 H, s, H_3-15), 2.31 (1 H, m, H-4), 3.10 (2 H, d, J = 7.0 Hz, H_2-11), 3.15 (2 H, m, H_2-22), 4.68 (1 H, s, H-13'), 4.71 (1 H, s, H-13), 5.17 (1 H, t, J = 7.0 Hz, H-10), 5.35 (1 H, s, H-19), 6.40 (1 H, brs, NH), and 8.20 (1 H, brs, OH); EIMS m/z (%) 413 (M^+, 65), 356 (8), 289 (15), 275 (12), 262 (30), 223 (100), 166 (30), 152 (30), and 123 (15); HREIMS m/z 413.2920 (M^+, calcd for $C_{26}H_{39}NO_3$, 413.2930).

Conversion of Metachromin B (7) into Metachromin E (2) by Oxidative Demethylation. Aqueous ceric ammonium nitrate (24 μL, 0.5 M, 12.0 μmol) was added to a solution of metachromin B (7, 1.5 mg) in CH_3CN/H_2O (2:1, 1 mL) at 25 °C. After 30 min the mixture was diluted with water (2 mL) and extracted with EtOAc (4 mL × 2). The combined organic extracts were washed brine and dried over magnesium sulfate, and the solvent was removed to give metachromin E (2, 1.1 mg, 74%).

Ozonolysis of Metachromins A (6) and F-H (3-5). A solution of 6 (20.0 mg) in MeOH (4 mL) was saturated with ozone at -78 °C for 10 min. After excess ozone was removed by a nitrogen stream, dimethyl sulfide (0.04 mL) was added, and the mixture was stirred at 0 °C for 30 min and then at room temperature for 30 min. The solvent and excess reagent were evaporated under reduced pressure. The residue was purified by a silica gel column (1.0 × 20 cm), eluting with hexane/EtOAc (3:1) to afford compound 8 (6.7 mg, 61%): a colorless oil; $[\alpha]^{18}_D$ -39° (c 1.0, $CHCl_3$); IR (neat) ν_{max} 1700, 1450, 1350, and 990 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (3 H, d, J = 6.2 Hz, H_3-12), 1.17 (3 H, s, H_3-14), 1.30 (1 H, qd, J = 13.1 and 3.7 Hz, H-3'), 1.55 (1 H, m, H-1'), 1.61 (1 H, m, H-1), 1.65 (1 H, m, H-2'), 1.71 (2 H, t, J = 8.1 Hz, H_2-7), 1.88 (1 H, tt, J = 13.1 and 3.7 Hz, H-2), 2.04 (1 H, dddd, J = 12.1, 6.2, 5.5 and 2.9 Hz, H-3), 2.15 (3 H, s, H_3-15), 2.42 (1 H, dt, J = 16.8 and 8.1 Hz, H-8'), 2.55 (1 H, dt, J = 16.8 and 8.1 Hz, H-8) and 2.62 (1 H, qd, J = 6.2 and 5.5 Hz, H-4); ^{13}C NMR ($CDCl_3$) δ 14.87 (q, C-12), 21.21 (t, C-2), 23.32 (q, C-14), 29.83 (q, C-15), 32.28 (t, C-7), 36.53 (t, C-3), 39.09 (t, C-1), 39.22 (t, C-8), 41.08 (d, C-4), 47.56 (s, C-6), 209.26 (s, C-9), and 216.71 (s, C-5); EIMS m/z (%) 196 (M^+), 139 (3), 126 (10), 111 (5), 95 (15), and 43 (100); HREIMS m/z 196.1478 (M^+, calcd for $C_{12}H_{20}O_2$, 196.1463).

According to essentially the same procedure as described above, 3 (6.5 mg), 4 (3.0 mg), and 5 (7.4 mg) afforded 8 [2.7 mg (85%) from 3, $[\alpha]^{19}_D$ -32° (c 0.4, $CHCl_3$); 0.6 mg (46%) from 4, $[\alpha]^{17}_D$ -32° (c 0.05, $CHCl_3$); and 1.0 mg (29%) from 5, $[\alpha]^{20}_D$ -32° (c 0.1, $CHCl_3$)]. Spectral data (^1H NMR, IR, and EIMS) of the ozonized compounds were identical with those of compound 8 derived from 6.

Acknowledgment. We thank Dr. P. Bergquist, The Uniersity of Auckland, for identification of the sponge and Mr. Z. Nagahama for his help with the sponge collection. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

Supplementary Material Available: All spectra of metachromins D-H (1-5) and compound 8 (47 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Regioselective Alcoholysis of Flavonoid Acetates with Lipase in an Organic Solvent

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Received July 8, 1992

Flavonoids are natural compounds widely distributed in higher plants. Many of them possess biological activities potentially exploitable in the biomedical field as antiinflammatory,[1-3] antiinfluenza virus,[4] antiulcer,[3] capillary protecting,[2] antitumor,[5-7] and in radical scavanger activity.[8-10] Inhibition of protein-tyrosine kinase[11,12] and phosphatidyl-inositol kinase[13] by flavonoids has also been reported.

Chemical elaboration of the basic flavonoid structure to obtain either rare natural compounds, for instance *O*-methyl flavonoids, or semisynthetic products often requires protection/deprotection of specific hydroxyl function(s). In a previous work we have investigated the partial hydrolysis of peracetylated di- and trihydroxyflavonoids catalyzed by a lipase from *Pseudomonas cepacea* (referred to as *Pseudomonas* sp.[14]) and we have found that this reaction takes place with a high degree of regioselectivity. In the present paper we report the results obtained by the application of this method to peracetates of additional hydroxylated compounds of the same class, namely luteolin, kaempferol, kaempferide, and quercetin. The synthesis of an *O*-methyl flavonoid, ombuin, is also described.

A flavone ester, luteolin tetraacetate (1), and three flavonol esters, kaempferol tetraacetate (2), kaempferide triacetate (3), and quercetin pentaacetate (4), were subjected to alcoholysis catalyzed by *P. cepacea* lipase in THF.

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