Journal of Natural Products
Vol. 52, No. 5, pp. 1173-1176, Sep-Oct 1989

METACHROMIN C, A NEW CYTOTOXIC SESQUITERPENOID FROM THE OKINAWAN MARINE SPONGE *HIPPOSPONGIA METACHROMIA*

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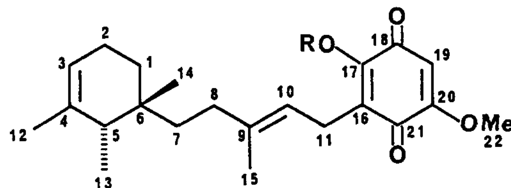
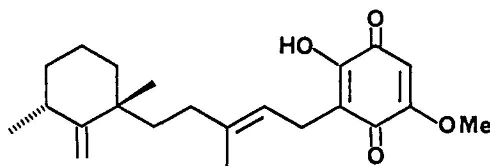
ABSTRACT.—A new sesquiterpenoid quinone, metachromin C [**1**], with potent cytotoxic activity has been isolated together with a known terpenoid metachromin A [**2**] from the Okinawan marine sponge *Hippospongia metachromia* and the structure determined by spectroscopic data, especially several types of 2D nmr spectra including ^{1}H-detected heteronuclear multiple-bond correlation (HMBC).

Interesting biological activities have been found for terpenoid quinones and phenols from marine sponges (1). In continuing studies on bioactive metabolites from marine organisms (2–6), we encountered cytotoxic extracts of the Okinawan marine sponge *Hippospongia metachromia* de Laubenfels (family Spongiidae, order Dictyoceratida). In this paper we describe the isolation and structure elucidation of metachromin C [**1**], a novel and biogenetically unusual sesquiterpenoid quinone with potent antileukemic activity.

The sponge *Hippospongia metachromia* collected at Okinawa Island was kept frozen until required. The MeOH extract of the sponge was partitioned between EtOAc and H_2O. The EtOAc solubles exhibiting antileukemic activity were subjected to a Si gel column [hexane-EtOAc (2:1)] and C_{18} hplc [MeCN-H_2O (7:3)] to yield metachromin C [**1**] (0.06% wet wt) and a known terpenoid, metachromin A [**2**] (0.10%) (1). Metachromin C [**1**] was obtained as a yellow solid from hexane, mp 90–91°; $[\alpha]^{26}D$ −29.7° (c = 0.2, $CHCl_3$). The ir (3350, 1640, and 1600 cm^{-1}) and uv [(MeOH) 208, 285, and 425 nm, (MeOH + OH^-) 206, 290, and 515 nm] absorptions suggested the presence



1 R=H
3 R=Ac



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of a hydroxy quinone moiety. The molecular formula $C_{22}H_{30}O_4$ for **1** was established by hreims (*m/z* 358.2118 [M]$^+$, Δ −2.6 mmu).

Comparison of the ^{1}H- and ^{13}C-nmr data of **1** (Table 1) and **2** (1) showed that **1** possessed the same 2-hydroxy-5-methoxybenzoquinone group as that in **2** and the remaining $C_{15}H_{25}$ part contained a sesquiterpene structure similar to **2**. The eims of **1** gave a peak at *m/z* 123, indicating the presence of a C_9H_{15} unit in the terminal part (1,7). The extensive analysis of ^{1}H-^{1}H COSY spectra of **1** and its acetate **3** (hreims *m/z* 400.2206 [M]$^+$, Δ −4.4 mmu for $C_{24}H_{32}O_5$) allowed assignment of all protons and established the following proton connectivities: H-1/H-2, H-2/H-3, H-3/H-12, H-5/H-13, H-7/H-8, H-8/H-10, H-10/H-11, and H-10/H-15. The only difference between **1** and **2** was the presence of a trisubstituted double bond (C-3 to C-4) bearing a methyl group (C-12, δ 22.7, *Z*) in **1** instead of an exomethylene (C-5 to C-13) as in **2**. Another trisubstituted double bond (C-9 to C-10) in **1** was assigned as *E* configuration (C-15, δ 16.2) (8), due to the observation of an nOe for the 15-methyl (6%) on irradiation of the C-11 methylene protons.

The assignment of all the protonated carbons in **1** (Table 1) was made on the basis of a ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC) experiment (9,10). The HMBC (11,12) spectra of **1** revealed cross peaks (Table 1) due to $^2J_{C\text{-}H}$ and $^3J_{C\text{-}H}$ to confirm the carbon-carbon connectivities. The con-

TABLE 1. ^{1}H- and ^{13}C-nmr Data for Metachromin C [**1**] and the Acetate **3**.

Carbon	Compound				
	1			**3**	
	δC[a]	δH[b] (*J*, Hz)	$^2J_{C\text{-}H}$, $^3J_{C\text{-}H}$[c]	δC[a]	δH[b] (*J*, Hz)
C-1	37.5 t	1.11 m, 1.42 m	H-14	37.5 t	1.11 m, 1.42 m
C-2	22.7 t	1.92 m		22.8 t	1.92 m
C-3	119.5 d	5.26 brs	H-12	119.7 d	5.26 brs
C-4	137.3 s		H-12	137.4 s	
C-5	42.7 d	1.65 m	H-12,H-13,H-14	42.9 d	1.59 m
C-6	34.0 s		H-13,H-14	34.1 s	
C-7	29.1 t	1.21 m, 1.29 m	H-14	29.2 t	1.18 m, 1.27 m
C-8	33.3 t	1.92 m	H-7,H-10,H-15	33.3 t	1.92 m
C-9	138.2 s		H-5,H-8,H-11	139.6 s	
C-10	118.7 d	5.14 brt (7.3)	H-8,H-11,H-15	117.5 d	5.01 brt (7.0)
C-11	21.7 t	3.1 brd (7.3)	H-10	20.3 t	3.14 brd (7.0)
C-12	22.7 q	1.65 s		22.8 q	1.65 s
C-13	14.6 q	0.89 d (7.0)		14.7 q	0.90 d (7.0)
C-14	22.5 q	0.84 s	H-1,H-7	22.7 q	0.84 s
C-15	16.2 q	1.75 s	H-10	16.4 q	1.73 s
C-16	118.1 s		H-11	116.9 s	
C-17	151.0 s	7.24 s (OH)	H-11,H-19	159.1 s	
C-18	181.3 s		H-19	179.9 s	
C-19	102.0 d	5.84 s		105.5 d	5.88 s
C-20	161.0 s		H-19,H-22	167.7 s	
C-21	182.8 s			181.4 s	
C-22	56.5 q	3.88 s	H-11	56.5 q	3.84 s
*C*OMe				171.5 s	
CO*Me*				22.8 q	2.35 s

[a]125 MHz, $CDCl_3$.
[b]500 MHz, $CDCl_3$.
[c]Observed in ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) experiments.

nectivities around quaternary carbons, C-4, C-6, and C-9, of the sesquiterpene moiety were established by cross peaks of C-1/H-14, C-3/H-12, C-4/H-13, C-5/H-12, C-6/H-13, C-7/H-14, C-8/H-15, and C-10/H-15, while cross peaks of C-17/H-11 and C-21/H-11 allowed connection between the sesquiterpene moiety and the quinone ring (C-11 to C-16). Thus the structure of metachromin C was concluded to be **1**. The vicinal methyl groups at C-5 and C-6 in **1** were assigned to be trans, since an nOe was observed for H-14 (3.5%) on irradiation of H-5. Furthermore, absolute configuration of C-6 in **1** was tentatively assigned as *R* while C-5 was assigned as *S*, because both **1** and **2** were considered to be generated through the same biosynthetic route and the absolute configuration at C-6 in **2** had been established to be *R* (1).

Metachromin C [**1**] contains the same biogenetically unusual carbon skeleton found in panicein A (13) or metachromin A [**2**] (1) isolated from the marine sponges *Halichondria panicea* and *Hippospongia* cf. *metachromia*, respectively. Metachromin C [**1**] exhibited potent cytotoxic activity against L1210 and L5178Y murine leukemia cells in vitro (14) with the IC_{50} values of 2.0 and 0.92 μg/ml, respectively. Metachromin C [**1**] as well as metachromins A [**2**] and B (1) showed potent coronary vasodilating activity, markedly inhibiting KCl (40 mM)-induced contraction of the rabbit isolated coronary artery with an IC_{50} value of 3×10^{-6} M.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Ir spectra were recorded on a Hitachi 260-50 ir spectrometer as KBr pellets and uv spectra on a JASCO 660 uv/VIS spectrophotometer for solutions in MeOH. Optical rotations were measured on a JASCO DIP-360 polarimeter. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-500 (500 MHz for ^{1}H nmr and 125 MHz for ^{13}C nmr) in $CDCl_3$ (δ_H 7.27 and δ_C 76.9 as standard) solution. Mass spectra (eims) were obtained with a Shimadzu GC-MX QP-1000A spectrometer at 70 eV. Wako C-300 Si gel was used for glass cc. Tlc was carried out on Merck Si gel GF_{254}.

COLLECTION, EXTRACTION, AND SEPARATION.—The sponge (1.4 kg, wet wt), collected at Unten Bay (70–80 m depth), Okinawa Island, in June 1987, was kept frozen until used; a voucher specimen is deposited in Mitsubishi Kasei Institute of Life Sciences. The MeOH (2×1500 ml) extract was suspended in 1 M aqueous NaCl (500 ml) and then extracted with EtOAc (3×500 ml). The EtOAc-soluble material (15.2 g) was partially subjected to a Si gel column (2.5×50 cm) eluted with hexane-EtOAc (2:1). A part (40 mg) of the fraction eluting from 350 ml to 500 ml was further separated by C_{18} reversed-phase hplc [YMC-Pack AM-324, Yamamura Chemical, 5μm, 10×300 mm; flow rate 2 ml/min; uv detection at 254 nm; eluent $MeCN-H_2O$ (7:3)] to afford metachromin C [**1**] (t_R 31.6 min, 7.3 mg) and metachromin A [**2**] (t_R 32.4 min, 10.3 mg) in 0.06 and 0.10% yield (wet wt of the sponge), respectively.

METACHROMIN C [**1**].—A yellow solid, mp 90–91° (hexane); $[\alpha]^{26}D$ −29.7° (c = 0.2, $CHCl_3$); ν max (KBr) 3350, 2930, 1640, 1600, 1380, 1320, 1230, 1210 cm^{-1}; λ max (MeOH) 208 (ε 15000), 285 (17200), 425 nm (400); λ max (MeOH + NaOH, pH 10) 206 (ε 49500), 290 (11800), 515 nm (900); eims (rel. int.) *m/z* $[M]^+$ 358 (100), 343 (3), 207 (63), 189 (22), 168 (42), 123 (19); exact mass found *m/z* 358.2118, calcd for $C_{22}H_{30}O_4$ $[M]^+$ 258.2144.

METACHROMIN C MONOACETATE [**3**].—Metachromin C [**1**] (3.3 mg) was treated with Ac_2O (0.1 ml) and pyridine (0.2 ml) at room temperature for 14 h. Usual workup and purification by Si gel cc (0.7×5 cm), eluting with hexane-EtOAc (2:1), afforded monoacetate **3** (3.4 mg) as a yellow oil; $[\alpha]^{24}D$ − 16.3° (c = 0.2 in $CHCl_3$); λ max (MeOH) 206 (ε 9600), 270 nm (13500); ν max (film) 2930, 1780, 1660, 1610, 1450, 1370, 1330, 1240 cm^{-1}; eims (rel. int.) *m/z* $[M]^+$ 400 (36), 385 (2), 358 (83), 343 (2), 249 (100), 207 (82), 189 (20), 168 (38), 123 (24); exact mass found *m/z* 400.2206, calcd for $C_{24}H_{32}O_5$ $[M]^+$ 400.2250.

ACKNOWLEDGMENTS

We thank Dr. P. Bergquist, Department of Zoology, The University of Auckland, for identification of the sponge, Mr. Z. Nagahama for his assistance of collecting the marine sponge, and Ms. M. Hamashima for her technical assistance. This study was supported in part by Grant-in-Aid (63010043) for Cancer Research from Ministry of Education, Science, and Culture, Japan.

LITERATURE CITED

1. M. Ishibashi, Y. Ohizumi, J.-F. Cheng, H. Nakamura, Y. Hirata, T. Sasaki, and J. Kobayashi, *J. Org. Chem.*, **53**, 2855 (1988), and references cited therein.
2. J. Kobayashi, J.-F. Cheng, H. Nakamura, Y. Ohizumi, Y. Hirata, T. Sasaki, T. Ohta, and S. Nozoe, *Tetrahedron Lett.*, **29**, 1177 (1988).
3. J. Kobayashi, J.-F. Cheng, M.R. Wälchli, H. Nakamura, Y. Hirata, T. Sasaki, and Y. Ohizumi, *J. Org. Chem.*, **53**, 1800 (1988).
4. J.-F. Cheng, J. Kobayashi, Y. Ohizumi, H. Nakamura, Y. Hirata, and T. Sasaki, *J. Chem. Soc., Perkin Trans. 1*, 2403 (1988).
5. J.-F. Cheng, Y. Ohizumi, M.R. Wälchli, H. Nakamura, Y. Hirata, T. Sasaki, and J. Kobayashi, *J. Org. Chem.*, **53**, 4621 (1988).
6. J. Kobayashi, M. Ishibashi, M.R. Wälchli, H. Nakamura, Y. Hirata, T. Sasaki, and Y. Ohizumi, *J. Am. Chem. Soc.*, **110**, 490 (1988).
7. P. Djura, D.B. Stierle, B. Sullivan, D.J. Faulkner, E. Arnold, and J. Clardy, *J. Org. Chem.*, **45**, 1435 (1980).
8. J.J. Barchi Jr., R.E. Moore, and G.M.L. Patterson, *J. Am. Chem. Soc.*, **106**, 8193 (1984).
9. L. Muller, *J. Am. Chem. Soc.*, **101**, 4481 (1979).
10. M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1986).
11. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
12. A. Bax, A. Aszalos, Z. Dinya, and K. Sudo, *J. Am. Chem. Soc.*, **108**, 8056 (1986).
13. G. Cimino, S. De Stefano, and L. Minale, *Tetrahedron*, **29**, 2565 (1973).
14. M. Ishibashi, Y. Ohizumi, T. Sasaki, H. Nakamura, Y. Hirata, and J. Kobayashi, *J. Org. Chem.*, **52**, 450 (1987).

Received 20 March 1989