concentrations of the substrates were 0.010 or 0.020 M. In the conductimetric method 10–20 μL of a substrate in dioxane, THF, or hexane, all containing 0.025 M 2,6-lutidine, was injected into a thermally equilibrated and magnetically stirred solvent (20 mL) placed in a conductivity cell (cell constant 0.1005). The temperature of the thermostat (kerosene) was maintained within ±0.05 °C during the measurement. The conductivity changes followed a good first-order kinetics. The acetolysis and formolysis were conducted in the presence of 0.0250 M sodium acetate or sodium formate, respectively, and the rates followed by titrating the unreacted base with 0.01 M $HClO_4$–acetic acid by using crystal violet as an indicator. For quenching of an aliquot, carbon tetrachloride in acetolysis and acetic acid in formolysis were used. For titrimetric runs at 25.0 °C, a pipet-out method was used, whereas for those at 50.0, 75.0, and 100.0 °C, an ampule technique was employed.

Metachromins A and B, Novel Antineoplastic Sesquiterpenoids from the Okinawan Sponge *Hippospongia* cf. *metachromia*

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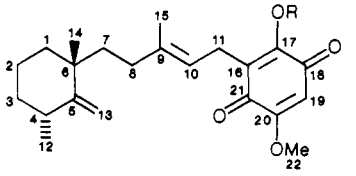
Received December 28, 1987

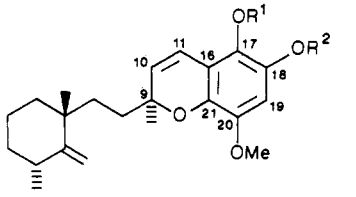
Many terpenoid quinones and phenols from marine sponges have exhibited interesting biological activities.[2] During our studies on bioactive metabolites from marine organisms,[3] we isolated a novel antineoplastic sesquiterpenoid quinone (1) and a chromenol (2), named metachromins A and B, respectively, from the Okinawan sponge *Hippospongia* cf. *metachromia*.[4] In this paper we describe the isolation and structure elucidation of 1 and 2.

The purple-colored sponge *Hippospongia* cf. *metachromia* was collected at Okinawa Island and kept frozen until required. The methanolic extracts were partitioned between ethyl acetate and water. The ethyl acetate soluble fraction exhibiting antineoplastic activity was subjected to chromatography on Sephadex LH-20 (methanol/chloroform, 1:1) followed by a silica gel column (hexane/ethyl acetate, 4:1) to afford metachromins A (1, 0.42% yield, wet weight) and B (2, 0.024% yield) together with a known quinone compound, isospongiaquinone[5] (0.18% yield).

Metachromin A (1) was obtained as orange crystals from hexane, mp 80–82 °C. The molecular formula of 1 was determined as $C_{22}H_{30}O_4$ by HREIMS (m/z 358.2154, Δ +1.0 mmu). The UV (MeOH, 286 and 435 nm; MeOH + KOH, 220, 289, and 520 nm) and IR (3340, 1630, and 1590 cm^{-1}) absorptions suggested the presence of a hydroxy

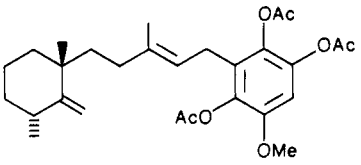


1, R = H
3, R = Ac
5, R = Me



2, R^1 = H; R^2 = Me
9, R^1 = R^2 = Me
10, R^1 = Ac; R^2 = Me
11, R^1 = Me; R^2 = H

quinone moiety.[5,6] This was supported by formation of an acetate (3) on acetylation with acetic anhydride and pyridine and a leuco-triacetate (4) on reaction with zinc dust and acetic anhydride in the presence of pyridine. Treatment of 1 with diazomethane afforded a methyl ether (5).



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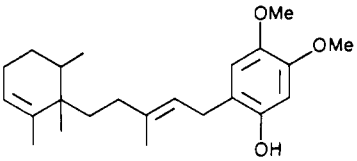
Comparison of the ^1H and ^{13}C NMR data of 1 with those of isospongiaquinone[5] or ilimaquinone[6] indicated that 1 possessed a 2-hydroxy-5-methoxybenzoquinone group. The ^1H NMR chemical shift for H-19 (δ 6.72) of the leuco-triacetate 4 implied that the substitution pattern of the benzoquinone group of 1 was the same as that of ilimaquinone.[6a] The ^1H and ^{13}C NMR spectra revealed that the remaining $C_{15}H_{25}$ part contained one tertiary and one secondary methyl groups and two olefins: an exomethylene and a trisubstituted double bond bearing a methyl group (δ_C 16.40 q; *E* configuration), from which the $C_{15}H_{25}$ part appeared to be a sesquiterpene having a structure similar to the sesquiterpene phenol 6 obtained from the sponge *Smenospongia echina*[7] or diterpenes such as agelasidine B or C[8] or ageline A[9,10] (7). The EIMS of 1 gave an intense



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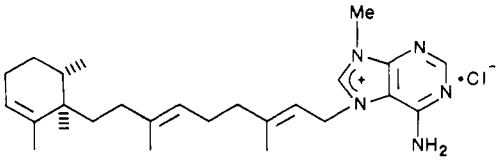

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(1) (a) Mitsubishi-Kasei Institute of Life Sciences. (b) Meijo University. (c) Kanazawa University.

(2) Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron* **1986**, *42*, 4197–4201, and references cited therein.

(3) (a) Ishibashi, M.; Ohizumi, Y.; Sasaki, T.; Nakamura, H.; Hirata, Y.; Kobayashi, J. *J. Org. Chem.* **1987**, *52*, 450–453. (b) Kobayashi, J.; Cheng, J.-F.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Lu, H.; Clardy, J. *Tetrahedron Lett.* **1987**, *28*, 4939–4942. (c) Ishibashi, M.; Ohizumi, Y.; Hamashima, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1127–1129. (d) Ohizumi, Y.; Nakamura, H.; Kobayashi, J.; Catterall, W. A. *J. Biol. Chem.* **1986**, *261*, 6149–6152.

(4) The sponge was identified as *Hippospongia* cf. *metachromia* by Dr. T. Hoshino, Mukaishima Marine Biological Station, Hiroshima University, Hiroshima, Japan.

(5) Kazlauskas, R.; Murphy, P. T.; Warren, R. G.; Wells, R. J.; Blount, J. F. *Aust. J. Chem.* **1978**, *31*, 2685–2697.

(6) (a) Luibrand, R. T.; Erdman, T. R.; Vollmer, J. J.; Scheuer, P. J.; Finer, J.; Clardy, J. *Tetrahedron* **1979**, *35*, 609–612. (b) Capon, R. J.; MacLeod, J. K. *J. Org. Chem.* **1987**, *52*, 5059–5060.

(7) Djura, P.; Stierle, D. B.; Sullivan, B.; Faulkner, D. J.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1980**, *45*, 1435–1441.

0022-3263/88/1953-2855$01.50/0

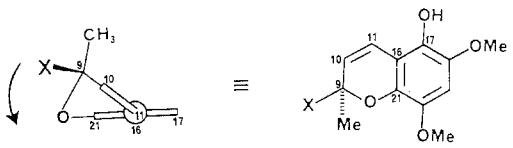
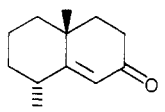


Figure 1. Perspective drawing of the chromenol moiety of metachromin B (**2**).

peak at m/z 123, indicating the presence of a C_9H_{15} unit in the terminal part.[7,11] Metachromin A (**1**) was treated with ozone, reduced with dimethyl sulfide, and cyclized in methanolic KOH to afford an octalone [**8**, m/z 178 (M^+)], which was identified as (8*R*,10*R*)-8,10-dimethyl-1-



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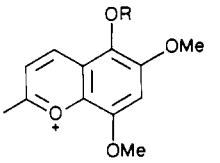
(9)-octal-2-one. Its 500-MHz ^1H NMR spectrum and EIMS as well as TLC behavior were identical with those of racemic **8** synthesized according to Still's procedure,[12] indicating a 1-alkyl-2-methylene-1,3-dimethylcyclohexane structure for the terminal part. The optical rotation of **8** prepared from **1** was negative ($[\alpha]^{24}_D$ −205° (*c* 0.3, $CHCl_3$)) and opposite to that of the known antipode of **8** with 8*S*,10*S* configuration ($[\alpha]^{20}_D$ +201.7° (*c* 2.0, $CHCl_3$)).[13] The structure of metachromin A was, therefore, established as **1** with the 4*R*,6*R* configuration.

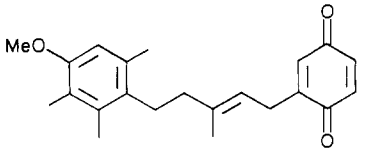
Metachromin B (**2**), a colorless oil, was shown to have a molecular formula, $C_{23}H_{32}O_4$, by HREIMS (m/z 372.2307, Δ +0.7 mmu). The IR spectrum suggested that **2** possessed no carbonyl groups and the UV absorption (MeOH, 223, 278, and 334 nm; MeOH + KOH, 221, 289, and 354 nm) was indicative of a phenol moiety. Comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1** revealed that the structure of the terminal cyclohexane ring of **2** was identical with that of **1**. For the remainder of the molecule, the ^1H NMR spectrum of **2** showed a tertiary methyl (δ 1.44), two methoxy groups (δ 3.82 and 3.83), one singlet deuterium-exchangeable proton (δ 5.34), one siglet (δ 6.47), and two doublet (δ 5.65 and 6.71, J = 10.0 Hz; cis-oriented) olefinic or aromatic protons. Irradiation of the tertiary methyl protons (δ 1.44, H_3-15) caused 12% NOE at the doublet (δ 5.65, H-10), implying that the tertiary methyl group was adjacent to the olefinic proton. These data were reminiscent of a chromenol ring system,[14,15] the cyclic derivative of a quinone. The ^{13}C NMR spectrum of **2** was consistent with the chromenol structure. Treatment of **2** with diazomethane in the presence of silica gel[16] afforded a trimethoxy derivative (**9**) and acetylation of **2** with acetic anhydride and pyridine yielded a monoacetate (**10**). The EIMS of **2**, **9**, and **10** gave prominent peaks due to the fragment ion A at m/z 221 (R = H), 235



A

(R = Me), and 263 (R = Ac), respectively, which were characteristic of chromenol derivatives.[17] The hydroxyl group was attached to C-17 as shown from an NOE experiment using acetate **10**. Irradiation of the acetate methyl protons (δ 2.33) caused an appreciable enhancement (4%) in the low field olefinic doublet at δ 6.36 (H-11). That the two methoxy groups of **2** were at C-18 and C-20 was shown by chemical conversion of metachromin A (**1**) to trimethoxychromenol **9** and its 9-epimer. Metachromin A (**1**) was heated in pyridine at 110 °C for 7 h, but in contrast to the literature[18] no cyclization occurred. However, the dimethoxy derivative **5**, on heating in pyridine at 110 °C for 7 h, was cyclized to dimethoxychromenol **11** and its 9-epimer in 49% yield. ^1H NMR spectrum and TLC analyses showed that neither **11** or its 9-epimer were not identical with **2**, thus supporting the conclusion that the hydroxyl group of **2** was not on C-18. Treatment of the mixture of **11** and 9-*epi*-**11** with diazomethane in the presence of silica gel[16] furnished the mixture of the trimethoxy derivative **9** and its 9-epimer. The 500-MHz ^1H NMR spectrum of the mixture (**9** and 9-*epi*-**9** from **1**) was identical with that of **9** from **2** except for the observation that the exomethylene protons of the former appeared to be split in a 1:1 ratio (**9**, δ 4.67 s and 4.73 s; 9-*epi*-**9**, δ 4.66 s and 4.72 s). Formation of this 1:1 diastereomeric mixture confirmed that metachromin B (**2**) was not an artifact of isolation.[19] The absolute configuration of the C-9 position of **2** was deduced on the basis of Kikuchi's method.[15] The CD spectrum of metachromin B (**2**) exhibited a positive Cotton effect around the 275-nm region (MeOH, $[\theta]_{275}$ +5200) due to the styrene chromophore, indicating that the chromenol ring has a negative chirality (a left-handed helix) as shown in Figure 1. The bulky isoprenoid side chain (X) of **2** preferentially adopts the pseudoequatorial conformation and thus the absolute configuration at C-9 is *S*. Hence the structure of metachromin B was **2**.

The carbon skeleton of **1** or **2** is unprecedented and appears biogenetically very unusual. Panicein A (**12**), a



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sesquiterpenoid quinone having an extensively rearranged skeleton, has been isolated earlier from the sponge *Halichondria panicea*,[19] but its biogenetic relationship to **1** or **2** remains unknown.[20]

(8) Nakamura, H.; Wu, H.; Kobayashi, J.; Kobayashi, M.; Ohizumi, Y.; Hirata, Y. *J. Org. Chem.* **1985**, *50*, 2494–2497.
(9) Capon, R. J.; Faulkner, D. J. *J. Am. Chem. Soc.* **1984**, *106*, 1819–1822.
(10) Wu, H.; Nakamura, H.; Kobayashi, J.; Kobayashi, M.; Ohizumi, Y.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2495–2504.
(11) Takeda, R.; Naoki, H.; Iwashita, T.; Mizukawa, K.; Hirose, Y.; Isida, T.; Inoue, M. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 1125–1132.
(12) Still, W. C.; VanMiddlesworth, F. L. *J. Org. Chem.* **1977**, *42*, 1258–1259.
(13) Maurer, B.; Francheboud, M.; Grieder, A.; Ohloff, G. *Helv. Chim. Acta* **1972**, *55*, 2371–2382.
(14) Dave, M.-N.; Kusumi, T.; Ishitsuka, M.; Iwashita, T.; Kakisawa, H. *Heterocycles* **1984**, *22*, 2301–2307.
(15) Kikuchi, T.; Mori, Y.; Yokoi, T.; Nakazawa, S.; Kuroda, H.; Masada, Y.; Kitamura, K.; Kuriyama, K. *Chem. Pharm. Bull.* **1983**, *31*, 106–113.
(16) Ohno, K.; Nishiyama, H.; Nagase, H. *Tetrahedron Lett.* **1979**, 4405–4406.
(17) Mayer, H.; Metzger, J.; Isler, O. *Helv. Chim. Acta* **1967**, *50*, 1376–1393.
(18) McHale, D.; Green, J. *Chem. Ind. (London)* **1962**, 1867.
(19) Cimino, G.; De Stefano, S.; Minale, L. *Tetrahedron* **1973**, *29*, 2565–2570.

Metachromins A (1) and B (2) exhibited potent antitumor activity against L1210 murine leukemia cells in vitro with the IC_{50} values of 2.40 and 1.62 μg/mL, respectively.[21] Both compounds also 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 each.

Experimental Section

General Methods. Melting points were obtained on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. UV and IR spectra were taken on a JASCO UVIDEC-660 and a Hitachi 260-50 spectrometer, respectively. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-500 or JEOL FX90Q spectrometer in $CDCl_3$. The 7.27 ppm resonance of residual $CHCl_3$ and 76.9 ppm resonance of $CDCl_3$ were used as internal references, respectively. Electron impact mass spectra (EIMS) were obtained on a JEOL HX-100 or Shimadzu GCMS-QP1000A spectrometer operating at 70 eV. CD spectra were recorded on a JASCO J-40A spectrometer.

Collection, Extraction, and Separation. The purple-colored sponge *Hippospongia* cf. *metachromia* was collected by netting at Unten Bay (−70 m), Okinawa Island, in March 1987, shipped via air to Tokyo, and kept frozen until used. The sponge (700 g, wet weight) was extracted twice with methanol (1.5 L and 1 L). Evaporation of the extract afforded 45 g of residue, which was dissolved in a mixture of ethyl acetate (500 mL) and 1 M NaCl (500 mL). The aqueous layer was extracted with ethyl acetate (500 mL × 3) and then with 1-butanol (500 mL × 3). The ethyl acetate soluble fraction was evaporated under reduced pressure to give a crude residue (16 g), part of which (910 mg) was subjected to gel filtration on Sephadex LH-20 (2.1 × 106 cm) eluted with chloroform/methanol (1:1). The fraction eluting from 160 mL to 190 mL (350 mg) was further separated by a silica gel column chromatography (Wako gel C-300, Wako Chemical, 2.3 × 42 cm) with hexane/ethyl acetate (4:1) as the eluant to give metachromin B (2, 9 mg) in the 120–170-mL fraction, known isospongiaquinone (70 mg) in the 230–360-mL fraction, and metachromin A (1, 160 mg) in the 400–560-mL fraction.

Metachromin A (1): orange needles, mp 80–82 °C (hexane); $[\alpha]^{27}_D$ −11° (*c* 1, $CHCl_3$); IR (KBr) 3340, 2930, 1630, 1590, 1370, 1300, and 1200 cm^{-1}; UV (MeOH, pH 7) 206 (ε 15000), 286 (14000), and 435 nm (500); UV (MeOH + KOH, pH 10) 220 (ε 36000), 289 (12000), and 520 nm (1200); ^1H NMR ($CDCl_3$) δ 1.02 (3 H, d, *J* = 6.5 Hz, H_3-12), 1.02 (3 H, s, H_3-14), 1.77 (3 H, br s, H_3-15), 2.32 (1 H, m, H-4), 3.16 (2 H, d, *J* = 7.4 Hz, H_2-11), 3.86 (3 H, s, MeO), 4.68 (1 H, s, H-13), 4.70 (1 H, s, H′-13), 5.16 (1 H, t, *J* = 7.4 Hz, H-10), 5.84 (1 H, s, H-19), and 7.30 (1 H, br s, exchangeable, OH-17); ^{13}C NMR ($CDCl_3$) δ 16.40 q, 19.61 q, 21.88 t, 24.65 q, 28.62 d, 33.94 t, 34.02 t, 37.24 t, 38.68 t, 39.22 s, 39.98 t, 56.77 q, 102.20 d, 103.46 t, 118.25 s, 118.78 d, 138.47 s, 151.23 s, 159.70 s, 161.10 s, 181.54 s, and 183.00 s; EIMS, *m/z* (relative intensity) 358 (M^+, 90), 343 (4), 276 (15), 234 (27), 219 (32), 207 (86), 168 (71), and 123 (100); exact mass, found *m/z* 358.2154, calcd for $C_{22}H_{30}O_4$ (M) 358.2144.

Metachromin B (2): colorless oil $[\alpha]^{24}_D$ +8° (*c* 1, $CHCl_3$); IR (neat) 3400, 2900, 1480, 1440, 1245, 1190, 1060, and 1035 cm^{-1}; UV (MeOH, pH 7), 207 (ε 15000), 223 (15000), 278 (7000), and 334 nm (1800); UV (MeOH + KOH, pH 11) 221 (ε 31000), 289 (5800), and 354 nm (2700); CD (MeOH) $[\theta]_{275}$ +5200; ^1H NMR ($CDCl_3$) δ 1.01 (3 H, s, H_3-14), 1.02 (3 H, d, *J* = 7.0 Hz, H_3-12), 1.44 (3 H, s, H_3-15), 2.36 (1 H, m, H-4), 3.82 (3 H, s, MeO), 3.83 (3 H, s, MeO), 4.66 (1 H, s, H-13), 4.75 (1 H, s, H′-13), 5.34 (1 H, s, exchangeable, OH-17), 5.65 (1 H, d, *J* = 10.0 Hz, H-10), 6.47 (1 H, s, H-19), and 6.71 (1 H, d, *J* = 10.0 Hz, H-11); ^{13}C NMR ($CDCl_3$) δ 19.48 q, 21.76 t, 24.81 q, 25.80 q, 33.94 d, 34.82 t, 35.17 t, 37.10 t, 38.68 t, 38.97 s, 56.83 q, 58.41 q, 78.24 s, 101.01 d, 103.59 t, 110.20 s, 117.17 d, 129.58 d, 135.96 s, 137.36 s, 139.35 s, 140.81 s, and 159.37 s; EIMS, *m/z* (relative intensity) 372 (M^+, 33), 357 (1.5), 249 (15), and 221 (100); exact mass, found *m/z* 372.2307, calcd for $C_{23}H_{32}O_4$ (M) 372.2300.

Metachromin A Monoacetate (3). Metachromin A (1, 7.4 mg) was treated with acetic anhydride (0.2 mL) and pyridine (0.2 mL) at room temperature for 14 h. Usual workup and purification by silica gel column chromatography (0.7 × 7 cm), eluting with hexane/ethyl acetate (3:1), afforded monoacetate **3** (3.9 mg): ^1H NMR ($CDCl_3$) δ 1.02 (3 H, s), 1.03 (3 H, d, *J* = 6.2 Hz), 1.75 (3 H, br s), 2.35 (3 H, s), 3.16 (2 H, d, *J* = 7.3 Hz), 3.84 (3 H, s), 4.69 (2 H, s), 5.04 (1 H, t, *J* = 7.3 Hz), and 5.90 (1 H, s); EIMS, *m/z* 400 (M^+), 358, 249, 207, and 109.

Metachromin A Leuco-Triacetate (4). A mixture of metachromin A (1, 9.5 mg), acetic anhydride (0.5 mL), pyridine (0.5 mL), and zinc dust (30 mg) was stirred at room temperature for 18 h. After evaporation, the mixture was passed through a silica gel column (1.1 × 15 cm), eluting with hexane/ethyl acetate (3:1), to yield leuco-triacetate **4** (8.3 mg): ^1H NMR ($CDCl_3$) δ 1.02 (3 H, s), 1.03 (3 H, d, *J* = 6.6 Hz), 1.74 (3 H, br s), 2.27 (6 H, s), 2.30 (3 H, s), 3.20 (2 H, d, *J* = 6.6 Hz), 3.79 (3 H, s), 4.71 (2 H, br s), 5.03 (1 H, t, *J* = 6.6 Hz), and 6.72 (1 H, s); EIMS, *m/z* 486 (M^+), 444, 402, 360, 320, 278, 236, 207, 168, 147, and 123.

Metachromin A Methyl Ether (5). To metachromin A (1, 12 mg) in tetrahydrofuran (0.5 mL) was added an excess of a solution of diazomethane in ether (1 mL). After 15 h of standing at room temperature, the solution was evaporated to afford methyl ether **5**: ^1H NMR ($CDCl_3$) δ 1.02 (3 H, s), 1.02 (3 H, d, *J* = 6.4 Hz), 1.76 (3 H, br s), 3.16 (2 H, d, *J* = 7.4 Hz), 3.81 (3 H, s), 4.06 (3 H, s), 4.68 (1 H, s), 4.69 (1 H, s), 5.08 (1 H, t, *J* = 7.4 Hz), and 5.73 (1 H, s); EIMS, *m/z* 372 (M^+), 357, 233, 147, and 109.

(8*R*,10*R*)-8,10-Dimethyl-1(9)-octal-2-one (8). A solution of metachromin A (1, 11 mg) in methanol (2 mL) was saturated with ozone at −78 °C for 15 min. After excess ozone was removed by a nitrogen stream, dimethyl sulfide (0.02 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. To the residue were added 5% KOH aqueous solution (0.4 mL) and methanol (4 mL), and the solution was heated under reflux for 2 h. After addition of brine (10 mL) to the cooled solution, extraction with chloroform (10 mL × 3) and purification by silica gel column chromatography (1.1 × 16 cm), eluting with hexane/ethyl acetate (85:15), afforded octalone **8** (2 mg): colorless oil, $[\alpha]^{24}_D$ −205° (*c* 0.3, $CHCl_3$); IR (neat) 1665, 1605, 1445, 1370, 1260, and 1235 cm^{-1}; UV (MeOH) 238 nm (ε 12000); CD (MeOH) $[\theta]_{308}$ +700 and $[\theta]_{235}$ −7800; ^1H NMR ($CDCl_3$) δ 1.03 (3 H, d, *J* = 6.4 Hz), 1.25 (3 H, s), and 5.80 (1 H, d, *J* = 2.0 Hz); EIMS, *m/z* 178 (M^+), 163, 150, 136, 121, 107, 93, and 79.

Metachromin B Methyl Ether (9). Metachromin B (2, 5 mg) was treated with an excess of diazomethane in ether solution (10 mL) in the presence of silica gel (Wako gel C-300, 4 mg) at room temperature for 19 h. Evaporation of the solvent and separation by silica gel column chromatography (1.1 × 17 cm), eluting with hexane/ethyl acetate (4:1), yielded methyl ether **9** (2 mg): ^1H NMR ($CDCl_3$) δ 1.01 (3 H, s), 1.02 (3 H, d, *J* = 6.6 Hz), 1.45 (3 H, s), 2.32 (1 H, m), 3.79 (3 H, s), 3.83 (3 H, s), 3.84 (3 H, s), 4.67 (1 H, s), 4.73 (1 H, s), 5.68 (1 H, d, *J* = 10.1 Hz), 6.47 (1 H, s), and 6.67 (1 H, d, *J* = 10.1 Hz); EIMS, *m/z* 386 (M^+), 263, 249, 235, 220, 205, 147, and 123.

Metachromin B Monoacetate (10). Treatment of metachromin B (2, 3 mg) with acetic anhydride (0.2 mL) and pyridine (0.2 mL) at room temperature overnight followed by usual workup furnished monoacetate **10**: ^1H NMR ($CDCl_3$) δ 1.00 (3 H, s), 1.01 (3 H, d, *J* = 6.6 Hz), 1.44 (3 H, s), 2.33 (3 H, s), 3.79 (3 H, s), 3.86 (3 H, s), 4.68 (1 H, br s), 4.73 (1 H, br s), 5.71 (1 H, d, *J* = 10.1 Hz), 6.36 (1 H, d, *J* = 10.1 Hz), and 6.50 (1 H, s); EIMS, *m/z* 414 (M^+), 372, 263, 221, 191, 147, and 123.

Conversion of 5 into 9 and Its 9-Epimer. Metachromin A methyl ether (5, 21 mg) in pyridine (10 mL) was heated at 110

(20) For other sesquiterpenes possessing rearranged skeletons, see: (a) Fenical, W. *Science (Washington, D.C.)* **1982**, *215*, 923–928. (b) Bittner, M. L.; Silva, M.; Paul, V. J.; Fenical, W. *Phytochemistry* **1985**, *24*, 987–989. (c) Fukuzawa, A.; Shea, C. M.; Masamune, T.; Furusaki, A.; Katayama, C.; Matsumoto, T. *Tetrahedron Lett.* **1981**, *22*, 4087–4088. (d) Fukuzawa, A.; Matsue, H.; Masamune, T.; Furusaki, A.; Katayama, C.; Matsumoto, T. *Chem. Lett.* **1984**, 1349–1350. (e) Corbett, R. E.; Chee, T. L. *J. Chem. Soc., Perkin Trans. 1* **1976**, 850–857. (f) Kubo, I.; Miura, I.; Pettei, M. J.; Lee, Y.-W.; Pilkiewicz, F.; Nakanishi, K. *Tetrahedron Lett.* **1977**, 4553–4556. (g) El-Feraly, F. S.; McPhail, A. T.; Onan, K. D. *J. Chem. Soc., Chem. Commun.* **1978**, 75–76.

(21) The IC_{50} of isospongiaquinone against L1210 was 0.88 μg/mL. Isospongiaquinone also showed a coronary vasodilating activity.

°C for 7 h. After evaporation of the solvent under reduced pressure, the residue was purified by a silica gel column chromatography (1.1 × 15 cm), eluting with hexane/ethyl acetate (3:1), to give a mixture (10.3 mg) of the chromenol derivative 11 and its 9-epimer: ^{1}H NMR ($CDCl_3$) δ 1.00 (3 H, d, J = 7 Hz), 1.01 (3 H, s), 1.46 (3 H, s), 2.31 (1 H, m), 3.77 (3 H, s), 3.80 (3 H, s), 4.65 (0.5 H, s), 4.66 (0.5 H, s), 4.70 (0.5 H, s), 4.72 (0.5 H, s), 5.19 (1 H, s), 5.72 (1 H, d, J = 9.9 Hz), 6.47 (1 H, s), and 6.56 (1 H, d, J = 9.9 Hz); EIMS, m/z 372 (M^+), 221, 207, and 191. The mixture (8.3 mg) of 11 and 9-*epi*-11 was treated with an excess of diazomethane in an ether solution (15 mL) in the presence of silica gel (Wako gel C-300, 4 mg) at room temperature for 19 h. Evaporation of the solvent and purification by a silica gel column chromatography (1.1 × 17 cm), eluting with hexane/ethyl acetate (4:1), afforded a mixture (1.8 mg) of trimethoxychromenol derivative 9 and its 9-epimer: ^{1}H NMR ($CDCl_3$) δ 1.01 (3 H, s), 1.02 (3 H, d, J = 6.4 Hz), 1.45 (3 H, s), 3.79 (3 H, s), 3.83 (3 H, s), 3.84 (3 H, s), 4.66 (0.5 H, s), 4.67 (0.5 H, s), 4.72 (0.5 H, s), 4.73 (0.5 H, s), 5.68 (1 H, d, J = 10.1 Hz), 6.47 (1 H, s), and 6.67 (1 H, d, J = 10.1 Hz); EIMS, m/z 386 (M^+), 263, 235, 220, 205, 147, and 123.

Bioassay Methods. Rabbits (2–3 kg) were sacrificed by cervical dislocation and the hearts were excised in cold Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl, 120; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 25.2; and glucose, 5.8; pH 7.4. The coronary artery was excised and cut into helical strips, ~1 mm in width and 10 mm in length. The strip was tied at each end by surgical silk. One end was connected to a force-displacement transducer and the other end was secured to a glass tissue holder. The 20-mL organ bath containing the solution was gassed with 95% O_2:5% CO_2 and the temperature was maintained at 36 °C. A resting tension of 800 mg was applied to each strip. Isometric force was monitored continuously by a polygraph.

The assay method of antitumor activity in vitro has been previously reported.[3a]

Acknowledgment. We thank Mr. Z. Nagahama for help with collections, Mr. T. Hayase of Central Research Laboratories, Mitsubishi Chemical Industries Ltd., for help with NMR measurements, and Ms. M. Hamashima and A. Muroyama of this institute for technical assistance.

A Simple Direct Procedure for the Regiospecific Preparation of Chloro Aromatic Compounds

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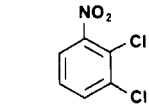
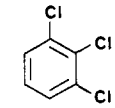
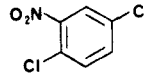
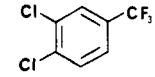
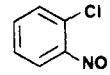
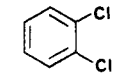
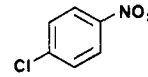
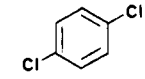
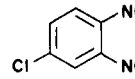
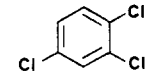
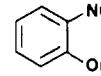
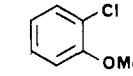
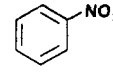
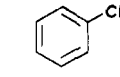
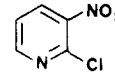
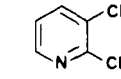
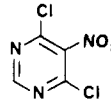
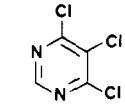
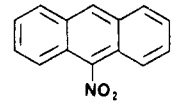
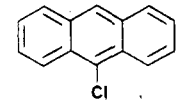
Received December 18, 1987

A common problem facing organic chemists is the regiospecific chlorination of aromatic rings. One practical solution to this problem is the substitution of aromatic nitro groups by chlorine. The Sandmeyer reaction[1] is normally used to accomplish this conversion. The nitro group is reduced to an amine, diazotized, and reacted with copper chloride to give the corresponding chloro aromatic. A number of variations on this basic reaction are also known.[2] Other methods of taking aromatic nitro to chloro functions include irradiation in chloroform/hydrogen chloride solution,[3] alkylative reduction by Grignard reactions quenched with sodium hypochlorite,[4] and treatment with chlorine or thionyl chloride in the vapor phase.[5] Here we report the successful application of two readily available organophosphorus reagents to accomplish the same transformation. Thus, phosphorus pentachloride and phenyltetrachlorophosphorane[6] (PTCP) have each been used to convert nitro aromatics directly to the analogous chloro aromatic materials. Prior to our work, PTCP was almost unknown as a reagent for organic synthesis. Timokhin et al.[7] reported the reaction of cyclohexene with PTCP to give *trans*-1,2-dichlorocyclohexane and 3-chlorocyclohexene. Mitrasov et al.[8] found that treatment of aliphatic aldehydes and ketones with PTCP produced geminal dichlorides. PTCP has also been used to form tetrazines from hydrazides.[9] Most recently, we have used PTCP in phenylphosphonic dichloride at 170 °C to convert nitro aromatics to chloro aromatics. The reaction is straight-forward and high-yielding and offers a simple, inexpensive alternative to the less desirable processes mentioned previously. We have tested our procedure on a variety of substrates and our results are recorded in

Table I. Chloro Aromatics from Nitro Aromatics Using PPTC/BPOD

starting material	product	% yield
		94
		67
		78
		73
		86
		66
		93
		81
		82
		90

(1) Kornblum, N. *Org. React. (N.Y.)* **1944**, *2*, 262.
(2) (a) Kim, Y. H. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1065. (b) Kim, Y. H.; Shinhama, K.; Oae, S. *Tetrahedron Lett.* **1978**, 4519. (c) Doyle, M. P.; Siegfried B.; Dellaria, J. F., Jr. *J. Org. Chem.* **1977**, *42*, 2426.
(3) Frater, G.; Havinga, E. *Tetrahedron Lett.* **1969**, 4603.
(4) Bartoli, G.; Bosco, M. *Synthesis* **1980**, 616.
(5) (a) Meyer, H. *Monatsh.* **1915**, *36*, 723. (b) Tang, D. E. P. 159388, 1985.
(6) Phenylphosphorus tetrachloride can be prepared easily by treating phenylphosphorus dichloride in a suitable solvent with chlorine gas.
(7) Timokhin, B. V.; Dmitriev, V. K.; Dmitriev, A. I. *Zh. Obshch. Khim.* **1984**, *54*, 1290.
(8) Mitrasov, Y. N.; Vladyko, E. D.; Kormachev, V. V. USSR SU 1051097, 1051096, 1983.
(9) (a) Yagupol'skii, L. M.; Matyshecheva, G. I.; Mikhailov, V. S.; Bulygina, L. A. USSR SU 498300, 1976. (b) Matyushecheva, G. I.; Mikhailov, U. S.; Yagupol'skii, L. M. *Zh. Org. Khim.* **1974**, *10*, 124.

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0022-3263/88/1953-2858$01.50/0