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BIO-ACTIVE MONOTERPENES FROM RED SEAWEEDS

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Key Word Index—*Chondrococcus hornemanni*, Plocamiaceae, Rhodophyceae, red seaweed, monoterpenes, ochtodane, biotoxicity

Abstract—A new ochtodane from *Chondrococcus hornemanni* and the biotoxocity of other seaweed monoterpenes is reported

INTRODUCTION

Results accumulated to date show that red seaweeds from the families Plocamiaceae and Rhizophyllidaceae produce numerous halogenated monoterpenes which are divided into just four carbon skeletal types [1] Both crude extracts and individual compounds from such seaweeds are often of interest because of their varied biological activity This has ranged from ichthyotoxicity [2, 3], to fish antifeedant [3], antimicrobial [4, 5] and antifungal [6] activities We wish to describe further examples of pronounced bioactivity for these simple seaweed halogenated monoterpenes In addition we now report the isolation of a new ochtodane from *Chondrococcus hornemanni*

RESULTS AND DISCUSSION

The toxicity of Plocamiaceae crude extracts on goldfish originally prompted us to explore its chemistry [7] Interestingly, these crude extracts also show, at low concentrations, anti-insect activity, as summarized in Table 1 Purified metabolites including compounds **1–6**,‡ which are major components from *Plocamium cartilagineum* or *P violaceum*, also show significant anti-insect activity (Table 1) and are probably responsible for the biotoxicity of the crude extracts Moreover, the activity levels, especially inhibition of mosquito larvae emergence, are comparable to those of commercial products It should also be noted that **1–6** represent three of the known monoterpene skeleton types including 2,6-dimethyloctanes, 1-ethyl-1,3-dimethylcyclohexanes and 1-ethyl-2,4-dimethylcyclohexanes These same pure compounds (**1–6**) were next subjected to model pharmacological assays The results recorded in Table 2 show that compounds **2** and **6** exhibit the most significant activity levels

Compounds in the fourth skeletal family (e g ochtodanes or 1-ethyl-3,3-dimethylcyclohexanes) became available as a result of our recent field work in Tahiti A collection of *Chondrococcus hornemanni* yielded ochtodene (**7**) [3, 4], along with two new compounds 1,2-dichloro-ochtod-3,4-ene (**8**) and impure epi-1,2-dichloro-ochtod-3,4-ene (**9**) These were characterized by spectroscopic data Especially useful were ^{13}C NMR data, including APT and INEPT [8, 9, Myers, B L, Loo, J, Ball, D and Crews, P., unpublished] We completely assigned the ^{13}C NMR shifts in **7** [3] (see structures) based upon extensive SFORD Also, careful scrutiny of the ^{1}H NMR data for **7** showed a long range coupling $J_{4,8} = 2$ Hz and this, along with ^{13}C NMR methyl shifts at δ 20 4 and 28 9, were consistent with the assigned C-8 relative stereochemistry§ The equatorial C_6-Br in **8** ($C_{10}H_{14}BrCl_3$, $[M]^+$ at *m/z* 318, 320, 322, 324) was assigned by a ^{13}C NMR SFORD correlation of the proton at δ 4 56 (*dd*, J = 10 8, 7 2 Hz) to the carbon at δ 54 3 (*d*) The chemical shift of the latter vs models clearly indicates Br rather than Cl at C-6 An axial Cl at C-8 was also evident based upon the ^{13}C NMR methyl shifts vs models [10, 11] Compound **9** could not be completely purified, but it was isolated in HPLC fractions accompanied by **8** That these two were epimeric at C-2 was clearly evident by comparing their ^{13}C NMR chemical shifts With these data as a precedent, we believe that halogen regiochemistry in **10**, which appears to be uncertain [12–14] at C-6 and C-8, can now be confidently assigned as shown here

The new ochtodane **8** shows biotoxicity in the anticell division assay as summarized in Table 2 However, ochtodene **7** did not show activity in spite of its previously observed ichthyotoxicity [3]

EXPERIMENTAL

Our general analytical, chemical and chromatographic methods have been described previously [10] NMR spectra were recorded on a JEOL FX-100 PFT spectrometer operating at 25 0 MHz for ^{13}C and 100 MHz for ^{1}H Additional ^{13}C NMR spectra at 75 MHz were recorded at Syntex and ^{1}H NMR spectra at 360 MHz were recorded at the Stanford Magnetic Resonance Lab (SMRL) Mass spectra were obtained by direct inlet at 20 eV

‡Compounds **1–6** are well known and are not illustrated

§The following shifts (ppm) are predicted for a –CHX– in a –C(6)HX–C(Me)$_2$-C(8)HX array where X = Br C-6 = 55, C-8 = 56, X = Cl C-6 = 63, C-8 = 64–69 [11]

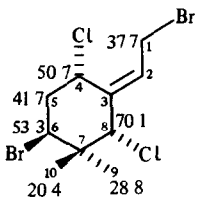
Table 1 Anti-insect activity against insect-sensitive synchronized instars*

	Manduca tobacco hornworm larva-III (μ g/larva) LD-50	*Aedes* mosquito larvae (ppm) EC-50	*Musca* fly larvae (μ g/prepupa) ED-50	*Heliothis* tobacco budworm larva-III (μ g/larva) ED-50
Crude extracts				
P cartilagineum	> 100	0 0054	—	—
P violaceum	2 8	> 1 0	92	50
P oregonum	> 100	> 1 0	> 100	—
Natural products†				
Cartilagineal (**1**)	> 100	> 0 1	> 100	> 100
7-Bromochloromethyl-3,4,8-trichloro-3-methyl-1,5,7-octa-triene (**2**)	> 100	—	30	> 100
Violacene (**3**)	2 4	> 1 0	65	32
Plocamene B (**4**)	> 100	0 05	—	—
Plocamene D (**5**)	> 100	> 1 0	> 100	> 100
Plocamene E (**6**)	> 100	> 0 1	> 100	—
Standards				
Benzene hexachloride	> 100	0 14	0 03	4 3
Methoprene	> 100	0 0002	0 004	> 100
Padan	22 0	0 41	1 0	> 10
Permethrin	0 029	—	0 35	0 018
Testing threshold‡	100	1 0	100	100

*ED-50 or EC-50 values are effective dose or concentration required reduce metamorphosis to 50%, but in the case of methoprene ID-50 and IC-50 values are cited

†For chemical structures see **1**, ref [7], **2**, ref [10], **3–6**, ref [2], methoprene, ref [15]

‡Values greater than the testing threshold indicate little or no activity



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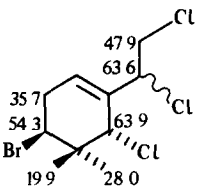

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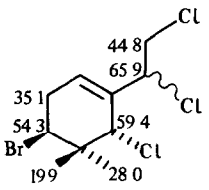

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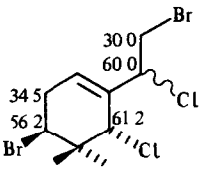

10 [14]

with temperature programming, 100° for 1 min followed by 3°/min to 200° Rotations were measured on a Perkin Elmer polarimeter with a quartz microcell (0 1 ml) Bioassays were carried out according to past procedures [15–17]

The alga (60 14 g, dry wt), *C hornemanni*, from Bora Bora, Tahiti (June 1982), extracted with CH_2Cl_2 immediately after collection yielded a crude oil (0 848 g) Partitioning between hexane–MeOH (1 1) yielded semi-pure fractions 0 230 g (hexane), 0 618 g (MeOH) ^{1}H NMR (360 MHz, benzene-d_6) of the MeOH fraction revealed characteristic peaks for compounds **7–9** The MeOH fraction was subjected to flash chromatography (solvent gradient of hexane to EtOAc) A total of five fractions were collected, and Fr 1 (hexane) and Fr 2 (hexane–EtOAc, 4 1) were combined (0 398 g) then subjected to HPLC

Ochtodene (**7**) HPLC fractions 15–20 gave **7** as a clear oil (74 mg) with spectral properties and rotation $[\alpha_D] = +162°$ (*c* 0 065, $CHCl_3$) matching those in the literature [3, 4] An SFORD ^{1}H NMR at δ1 15 gave a sharp ^{13}C peak at δ28 8

1,2-*Dichloro-ochtod*-3,4-*enes* (**8** *and* **9**) HPLC fractions 8 and 9 (14 mg) and 10–13 (82 mg) contained respectively pure **8** and a mixture of **8** and **9** (ratio of 5 1) Specific physical properties for **8** were $[\alpha_D] = +50°$ (*c* 0 045, $CHCl_3$), ^{1}H NMR (360 MHz, $CDCl_3$) δ5 88 (*dd*, *J* = 5 4, 3 6 Hz, H-4), 4 60 (*t*, *J* = 7 2 Hz, H-2), 4 56 (*dd*, *J* = 10 8, 7 2 Hz, H-6a), 4 52 (*s*, H-8e), 3 95 (*dd*, *J* = 10 8, 7 2 Hz, H-1), 3 85 (*dd*, *J* = 10 8, 7 2 Hz, H-1), 2 94 (*ddd*, *J* = 19 8, 7 2, 5 4 Hz, H-5e), 2 69 (*ddd*, *J* = 19 8, 10 8, 3 6 Hz, H-5a), 1 30 (*s*, Me-10), 1 10 (*s*, Me-9) Spin decoupling at δ5 88 (H-4) revealed a clear ABX pattern at δ2 69 (*J* = 3 6 Hz eliminated), 2 94 (*J* = 5 4 Hz eliminated) and 4 56 ^{13}C NMR (benzene-d_6, 25 and 75 MHz) 134 4 (*s*, C-3), 131 0 (*d*, C-4), 63 9 (*d*, C-8), 63 6 (*d*, C-2), 54 3 (*d*, C-6), 47 9 (*t*, C-1), 39 9 (*s*, C-7), 35 7 (*t*, C-5), 27 9 (*q*, C-10), 19 7 (*q*, C-9) SFORD at 75 MHz was used to assign C-6, mass spectrum, *m/z* 318, 320, 322, 324 $[M]^+$, 283, 285, 287 $[M-Cl]^+$, 203, 205, 207 $[M-Cl, -HBr]^+$, 169, 167 $[M-Cl, -HBr,$

Table 2 Bio-assay activity with animal model preparations

Compound*	Guinea pig heart auricle contraction rate and force	Frog heart ventricle contraction force	Sea urchin cell division inhibition	Guinea pig ileum agonist, antagonist, or potentiator
Cartilagineal (1)	No effect	—	No effect	Weak histamine antagonism
7-Bromochloromethyl-3,4,8-trichloro-3-methyl-1,5-7-octatriene (2)	No effect	No effect	76%	45% Histamine antagonism 25% Acetylcholine antagonism
Violacene (3)	Force + 50%	Mild effect	No effect	Agonist
Plocamene B (4)	No effect	Mild effect	No effect	Acetylcholine potentiation Histamine potentiation
Plocamene D (5)	No effect	No effect	No effect	No effect
Plocamene E (6)	Force + 30%	−21%	No effect	Acetylcholine antagonism Histamine antagonism
Ochtodene (7)	—	Mild effect	No effect	—
Dichloro-ochtodene (8)	No effect	Mild effect	18%	—

*All dose concentrations = 16 μg/ml

$-HCl]^+$ Specific physical properties for **9** were ^1H NMR (360 MHz, $CDCl_3$) δ 5.78 (*dd*, J = 5.4, 3.6, H-4), 4.65 (*t*, J = 7.2 Hz, H-2), 4.51 (*dd*, J = 10.8, 7.2 Hz, H-6a), 4.45 (*s*, H-8e), 3.89 (*dd*, J = 10.8, 7.2 Hz, H-1), 3.75 (*dd*, J = 10.8, 7.2 Hz, H-1), 2.94 (*ddd*, J = 19.8, 7.2, 5.4 Hz, H-5a), 1.25 and 1.11 (*s*, *s*, Me-9 and 10), ^{13}C NMR (25 and 75 MHz, benzene-d_6) δ 134.6 (*s*, C-3), 127.4 (*d*, C-4), 65.9 (*d*, C-2), 59.4 (*d*, C-8), 54.3 (*d*, C-6), 44.8 (*t*, C-1), 39.9 (*s*, C-7), 35.1 (*t*, C-5), 28.0 (*q*, C-10), 19.9 (*q*, C-9)

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REFERENCES

1 Naylor, S, Hanke, F J, Manes, L V and Crews, P (1983) *Prog Chem Org Nat Prod* **44**, 189
2 Crews, P, Kho-Wiseman, E and Montana, P (1978) *J Org Chem* **43**, 116
3 Paul, V J, McConnell, O J and Fenical, W (1980) *J Org Chem.* **45**, 3401
4 McConnell, O J and Fenical, W (1978) *J Org Chem* **43**, 4238
5 Stallard, M O and Faulkner, D J (1974) *Comp Biochem Physiol* **49B**, 25
6 Stierle, D B, Wing, R M and Sims, J J (1979) *Tetrahedron* **35**, 2855
7 Crews, P and Kho, E (1974) *J Org Chem* **39**, 3303
8 Patt, S L and Shoolery, J N (1982) *J Magn Reson* **46**, 535
9 Freeman, R and Morris, G A (1979) *J Am Chem Soc* **101**, 760
10 Crews, P, Naylor, S, Hanke, F J, Hogue, E R, Kho, E and Braslau, R J (1984) *J Org Chem* **49** (in press)
11 Crews, P and Kho-Wiseman, E (1978) *Tetrahedron Letters* 2483
12 Burreson, B J, Wollard, F X and Moore, R E (1975) *Chem Letters* 1111
13 Burreson, B J, Wollard, F X and Moore, R E (1975) *Tetrahedron Letters* 2155
14 Sims, J J, Rose, A F and Izac, R R (1978) in *Marine Natural Products Chemistry* (Scheuer, P J, ed) Vol 2, p 179 Academic Press, New York
15 Henrick, C A, Wiley, W E and Staal, G B (1976) *J Agric Food Chem* **24**, 207
16 Quistad, G B, Cerf, D C, Schooley, D A and Staal, G B (1981) *Nature* **289**, 176
17 Jacobs, R S, White, S and Wilson, L S (1981) *Fed Proc* **40**, 26