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FIVE NEW MONOTERPENES FROM THE MARINE RED ALGA *PORTIERIA HORNEMANNII*

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ABSTRACT-From the lipophilic extract of the marine red alga *Portieria hornemannii* (Lyngbye) P C Silva fifteen monoterpenes were isolated Of the fifteen, five were new natural products (2*Z*)-6-bromo-3-chloromethyl-1,7-dichloro-7-methylocta-2-ene (**1**), (2*Z*,6*E*)-3-chloromethyl-1-chloroocta-2,6-dien-8-al (**2**), 3-methoxymethyl-6-methoxyl-7-methylocta-1,7(10)-dien-3-ol (**3**), (2*Z*,6*S*)-3-chloromethyl-1-methoxylocta-2,7(10)-dien-6-ol (**4**) and (2*Z*,6*S*)-3-chloromethyl-6-methoxylocta-2,7(10)-dien-1-ol (**5**) The structures of all isolates were assigned on the basis of their spectroscopic data, ^{1}H and ^{13}C nmr, ir, uv and ms

INTRODUCTION

Marine algae of the families Plocamiaceae and Rhizophyllidaceae have been shown to be rich sources of polyhalogenated monoterpenes [1,2] Plants from the genus *Portieria* of the family Rhizophyllidaceae elaborate both acyclic and cyclic monoterpenes [1,2] The same plant species also elaborate different proportions of similar compounds depending on the location of collection Five previous investigations of *P hornemannii* [2,3,4] clearly illustrated that plants from different geographic locations did contain unique secondary metabolites In the current study we investigated the secondary metabolite content of a sample of *P hornemannii* from a further geographic location to obtain fifteen monoterpenes of which five were new natural products

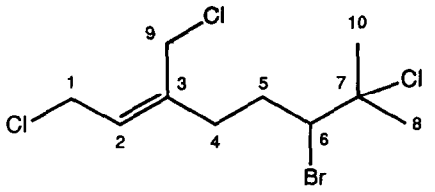
DISCUSSION

The fresh sample of *P hornemannii* (Rhizophyllidaceae) from Nelly Bay, Magnetic Island, Queensland, Australia, was extracted with a 1·1 mixture of methanol and dichloromethane, to afford 2 44 g (0 59%) of dichloromethane soluble material These solubles were then

chromatographed over silica to afford 11 fractions Hplc separation of selected fractions yielded fifteen monoterpenes Of these isolates five were as yet undescribed in the scientific literature

Compound **1** had the molecular formula of $C_{10}H_{16}Cl_3Br$, from mass spectrometry The presence of two sp^2 carbon resonances (127 0 (d), 139 6 (s) ppm) in the ^{13}C nmr spectrum of **1** indicated it to be an acyclic molecule Further, from the ^1H and ^{13}C nmr data the presence of two vinyl chloromethyl functions (38 9 (t), 4 15 (br d *J* 8 0 Hz), 40 4 (t), 4 09 (d, *J* 11 8 Hz), 4 17 (d, *J* 11 8 Hz) ppm), two methylene groups (32 1 (t), 1 93 (m), 2 52 (m), 33 5 (t), 2 34 (m), 2 68 (br ddd, *J* 4 5, 8 5 13 6 Hz) ppm), a bromine bearing methine function (64 3 (d), 4 01 (dd, *J* 1 7, 11 2 Hz) ppm), a quaternary carbon bearing chlorine (71 9 (s) ppm), two tertiary methyl groups (33 2 (q), 1 80 (s), 27 0 (q), 1 68 (s) ppm) and a single olefinic proton for the one carbon-carbon double bond (δ 5 76 (br t, *J* 8 0 Hz), 127 0 (d), 139 6 (s)) were discerned These data together with the results of short-range ^1H-^{13}C correlation (*J* 136 Hz) and ^1H-^1H COSY experiments enabled three major molecular fragments to be established, $ClCH_2$-CH=C(CH_2Cl)-, -CH_2-CH_2-CHBr-, -$(CH_3)_2$CCl These fragments can only combine in one way which is consistent with all of the spectroscopic data The resultant molecular framework, **1**, contained a single carbon-carbon double bond and one chiral centre that required stereochemical assignment The Δ^2 double bond was assigned as *Z* on the basis of the observed nOe interaction between the C2 vinyl proton (δ 5 76 (br t, *J* 8 0 Hz) and the protons of the C4 methylene group ((δ 2 34 (m), 2 68 (br ddd, *J* 4 5, 8 5, 13 6 Hz)) The stereochemistry at C6 cannot be deduced in a relative sense, however the rather deshielded nature of both the protons and carbon at C10 (33 2 (q), 1 80 (s) ppm) would tend to suggest that this group and the bromine function at C6 are eclipsed Compound **1** is (2*Z*)-6-bromo-3-chloromethyl-1,7-dichloro-7-methylocta-2-ene

Compound **2** a monoterpene of the molecular formula $C_{10}H_{14}OCl_2$ contained five resonances (126 6 (d), 139 7 (s), 140 1 (s), 152 2 (d), 195 0 (d) ppm) in its ^{13}C nmr spectrum for sp^2 carbons, implying the molecule to be acyclic Other structural features obvious from both the ^{13}C and ^1H nmr data of **2** were the presence of two allylic chloromethyl groups (38 8 (t), 4 14 (d, *J* 7 9 Hz), 40 5 (t), 4 12 (s) ppm), the protons of one of these showing coupling to the vinyl proton with resonance at δ 5 69 (br t, *J* 7 9 Hz), two allylic methylene groups, with intercoupling protons (33 6 (t), 2 42 (m), 26 8 (t), 2 75 (m) ppm), one of which is adjacent to the carbon bearing the vinyl proton (δ 6 45 (br t *J* 7 1 Hz) of an α,β-unsaturated aldehyde moiety, a vinyl methyl group (9 4 (q), 1 77 (br s) ppm) and an α,β-unsaturated aldehyde moiety (195 0(d), 9 41 (s), 140 1 (s), 152 2 (d), 6 45 (br t, *J* 7 1 Hz)) The latter grouping being further confirmed by the uv maximum at 217 nm (ε 6900) With this information and ^{13}C nmr data comparison between **1** and **2** (Table 1), it was evident that **2** was an α,β-unsaturated aldehyde derivative of **1** Within the basic framework of **2** there is no chirality and as such the molecule is optically inactive as confirmed by its lack of an optical rotation, it does however contain two double bonds that required stereochemical assignment The results of a 2D-NOESY measurement made with **2** clearly evidenced nOe interactions between the protons at C6 (δ 6 45) and C8 (δ 9 41) and between the protons at C4 (δ



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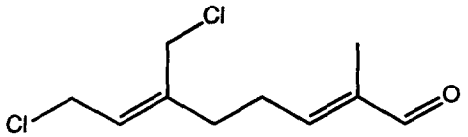

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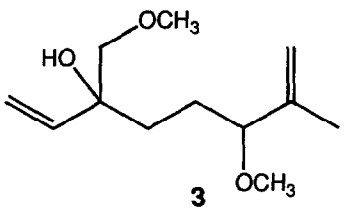

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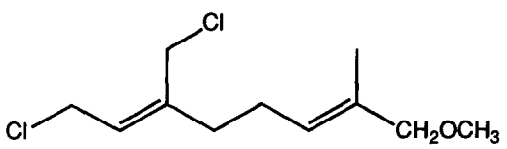

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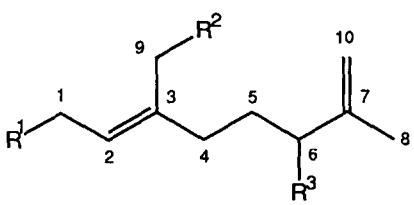
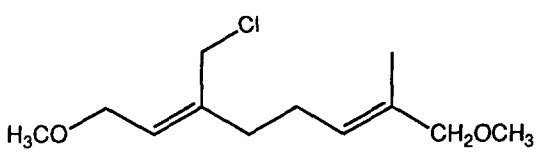


4	R^1=OCH_3	R^2=Cl	R^3=OH
5	R^1=OH	R^2=Cl	R^3=OCH_3
6	R^1=OCH_3	R^2=Cl	R^3=OCH_3
9	R^1=Cl	R^2=Cl	R^3=OH
11	R^1=Cl	R^2=Cl	R^3=Cl
12	R^1=Cl	R^2=Cl	R^3=OCH_3
14	R^1=OH	R^2=OH	R^3=OH



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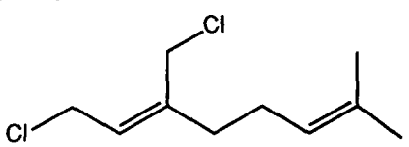

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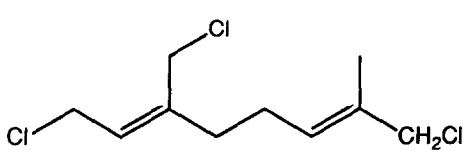

13

2 42) and the proton at C2 (δ 5 69) indicating the Δ^2 double bond to have the *Z* configuration and the Δ^6 double bond to have the *E* configuration Compound **2** is thus (2*Z*,6*E*)-3-chloromethyl-1-chloroocta-2,6-dien-8-al

Table 1 ^{13}C nmr (75 5 MHz, $CDCl_3$) data for compounds **1**, **2**, **3**, **4** and **5**

Carbon	1	2	3	4	5
1	38 9 t	38 8 t	117 5 t	68 2 t	58 7 t
2	127 0 d	126 6 d	138 4 d	127 0 d	129 2 d
3	139 6 s	139 7 s	78 4 s	139 2 s	138 8 s
4	33 5 t	33 6 t	29 7 t	31 1 t	31 6 t
5	32 1 t	26 8 t	26 8 t	32 8 t	31 5 t
6	64 3 d	152 2 d	85 7 d	75 3 d	85 0 d
7	71 9 s	140 1 s	144 0 s	147 2 s	144 1 s
8	27 0 q	195 0 d	16 2 q	17 6 q	16 3 q
9	40 4 t	40 5 t	47 0 t	41 7 t	41 5 t
10	33 2 q	9 4 q	114 0 t	111 3 t	113 9 t
11			56 0 q	58 2 q	56 0 q
12			50 1 q		

Compound **3** had the molecular formula $C_{12}H_{22}O_3$ by ^{13}C nmr spectroscopy and mass spectrometry The oxygen functionality within **3** was clearly present as two methoxyl functions (56 0 (q), 3 21 (s), 50 1 (q), 3 20 (s) ppm) and a tertiary alcohol (78 4 (s) ppm, 3300 cm^{-1}) From the ^{13}C nmr data of **3** (Table 1) four sp^2 carbon resonances could be discerned (114 0 (t), 117 5 (t), 138 4 (d), 144 0 (s) ppm) indicating **3** to be acyclic Also evident from the ^{13}C nmr data of **3** were the presence of resonances for a methine group bearing a methoxyl (85 7 (d), 3 48 (m) ppm), a methylene group bearing a methoxyl function (47 0 (t), 3 53 (d, *J* 11 8 Hz), 3 60 (d, *J* 11 8 Hz) ppm), an allylic methyl group (16 2 (q), 1 64 (s) ppm) and two methylene groups (29 7 (t), 1 74 (m), 1 57 (m), 26 8 (t), 1 57 (m), 1 48 (m) ppm) From these data and the results obtained from the ^1H-^1H COSY spectrum of **3** it was possible to delineate three major ^1H spin systems within **3** CH_2=CH-, -$CH_2(OCH_3)$-, -CH_2-CH_2-$CH(OCH_3)$-$C(=CH_2)$-CH_3 Clearly the three delineated ^1H spin systems must all connect to the tertiary carbon bearing the hydroxyl function and thus give rise to the framework **3** The two chiral centers (C3 and C6) within **3** have, to each other, no relative stereochemistry Compound **3** is 3-methoxymethyl-6-methoxyl-7-methylocta-1,7(10)-dien-3-ol

Compound **4** was a monomethoxylated monoterpene of the molecular formula $C_{11}H_{19}O_2Cl$ Both degrees of unsaturation, indicated by the molecular formula of **4**, were taken in two carbon-carbon double bonds (111 3 (t), 127 0 (d), 139 2 (s), 147 2 (s) ppm, the molecule was thus acyclic The functionalities within **4** were an allylic chloromethyl function (41 7 (t), 4 10 (s) ppm),

an allylic secondary alcohol (75 3 (d), 4 09 (dd, *J* 6 4, 6 8 Hz) ppm) and an allylic methoxymethyl group (68 2 (t), 4 02 (d, *J* 6 6 Hz) ppm) As for **3**, three individual proton spin systems could be discerned for **4** from the results of recording its ^{1}H-^{1}H COSY spectrum and ^{1}H-^{1}H double resonance studies, -C=CH-CH_2-OCH_3, -CH_2Cl, -CH_2-CH_2-CH(OH)-, -C(=CH_2)-CH_3 The three proposed molecular fragments combined to give **4** The one chiral centre within **4** (C6) was assigned as *S* on the basis of a direct comparison of all spectroscopic data for **4** with those of the known compound[4] **9** The $\Delta^{2,3}$ double bond was shown to have the *Z* configuration on the basis of the nOe observed between the proton at C2 (δ 5 60 (br t, *J* 6 6 Hz) and the C4 methylene group (δ 2 26 (m)) Compound **4** is thus (2*Z*,6*S*)-3-chloromethyl-1-methoxylocta-2,7(10)-dien-6-ol

Table 2 ^{1}H nmr (300 MHz, $CDCl_3$) data for compounds **1**, **2**, **3**, **4** and **5**

Carbon	1	2	3	4	5
1	4 15 (d, *J* 8 0 Hz)	4 14 (d, *J* 7 9 Hz)	5 23 (d, *J* 17 7 Hz), 5 34 (d, J 11 1 Hz)	4 02 (d, *J* 6 6 Hz)	4 25 (br d, *J* 6 8 Hz)
2	5 76 (br t, *J* 8 0 Hz)	5 69 (br t, *J* 7 9 Hz)	5 70 (dd, *J* 11 1, 17 7 Hz)	5 60 (br t, *J* 6 6 Hz)	5 64 (br t, *J* 6 8 Hz)
4	2 34 (m), 2 68 (br ddd, *J* 4 5, 8 5, 13 6 Hz)	2 42 (m)	1 74 (m), 1 57 (m)	2 26 (m)	2 21 (m)
5	1 93 (m), 2 52 (m)	2 75 (m)	1 57 (m), 1 48 (m)	1 72 (m)	1 76 (m)
6	4 01 (dd, *J* 1 7, 11 2 Hz)	6 45 (br t, *J* 7 1 Hz)	3 48 (m)	4 09 (dd, *J* 6 4, 6 8 Hz)	3 50 (dd, *J* 6 2, 7 3 Hz)
8	1 68 (s)	9 41 (s)	1 64 (s)	1 74 (s)	1 65 (br s)
9	4 09 (d, *J* 11 8 Hz), 4 17 (d, *J* 11 8 Hz)	4 12 (s)	3 53 (d, *J* 11 8 Hz), 3 60 (d, *J* 11 8 Hz)	4 10 (s)	4 10 (s)
10	1 80 (s)	1 77 (br s)	4 91 (br s), 4 95 (br s)	4 86 (br s), 4 96 (br s)	4 91 (br s), 4 96 (br s)
11			3 21 (s)	3 34 (s)	3 21 (s)
12			3 20 (s)		

Compound **5**, of the molecular formula $C_{11}H_{19}O_2Cl$, was spectroscopically extremely similar to compound **4** The major differences between the two molecules being the position of the hydroxyl and methoxyl functions All of the spectroscopic data for **5** were in total accord with the methoxyl being at C6 and the hydroxyl at C1 Similar stereochemical assignments within **5** were required

as for **4** The double bond stereochemistry was once again assigned as *Z* on the basis of the nOe observed between the C2 proton (δ 5 64 (br t, *J* 6 8 Hz)) and the protons of the C4 methylene group (δ 2 21 (m)) The single chiral centre C6 was assigned as *S* from direct comparison of all of its spectroscopic data with those for the previously reported compound[4] **6** Compound **5** is (2*Z*,6*S*)-3-chloromethyl-6-methoxylocta-2,7(10)-dien-1-ol

Together with the afore mentioned metabolites we also isolated and identified from this sample of *P hornemannii* the acyclic compounds **6-14** and the cyclic monoterpene octodene[5] This was by no means every monoterpene to be found within this sample We have some spectroscopic evidence for trace amounts (<1 mg) of at least ten other metabolites of this type The results of the current investigation of *P hornemannii* plants provide further evidence of the secondary metabolite variation within plants of the same species depending on where the sample was collected It also serves as another example of the co-occurrence of cyclic and acyclic monoterpenes within the same algal sample

EXPERIMENTAL

GENERAL PROCEDURES

As per reference 6

PLANT MATERIAL

All plant materials were collected by divers using self contained underwater breathing apparatus (SCUBA) Voucher specimens were deposited with the James Cook University's Botany Department Herbarium (JCT A7929)

EXTRACTION AND ISOLATION

Fresh plant material (411 8 g) was extracted with MeOH/DCM (1/1) to yield 2 44 g of DCM soluble material Separation of this material by vacuum liquid chromatography (VLC) over silica with hexane containing increasing proportions of ethyl acetate, followed by methanol, afforded 11 fractions, each of approximately 80 ml HPLC separation (Knauer 250 mm x 8 mm HPLC column packed with LiChrosorb Si60 5μm and eluant DCM/hexane 1/9) of fraction 2 yielded five pure compounds

(2*Z*)-6-bromo-3-chloromethyl-1,7-dichloro-7-methylocta-2-ene (**1**) (2 3 mg, 0 09 %), a clear oil $[\alpha]_D^{25}$ +47 0° (c, 0 12, $CHCl_3$), IR (film) ν_{max} 2910, 1450, 1370, 1380, 1250, 1100, cm^{-1}, ^1H nmr (see Table 2), ^{13}C nmr (see Table 1), HREIMS, obsd , *m/z* 319 944, $C_{10}H_{16}Cl_3Br$ requires *m/z* 319 950, EIMS, *m/z* (% rel int), 320 (M^+ [$^{35}Cl_3{}^{79}Br$], 1), 249 ([$^{35}Cl^{79}Br$], 5), 205 ([$^{35}Cl_2$], 65), 169 ([^{35}Cl], 23), 133 (17), 103 (37), 91 (35), 79 (45), 69 (100)

Compound **10** (13 mg, 0 53 %) with identical physical and chemical properties to those of the known metabolite[3] **10**

Compound **11** (40 mg, 1 64 %) with identical physical and chemical properties to those of the known metabolite[3] **11**

Compound **12** (45 mg, 1 85 %) with identical physical and chemical properties to those of the known metabolite[4] **12**

Compound **13** (2 mg, 0 08 %) with identical physical and chemical properties to those of the known metabolite[3] **13**

HPLC separation (Knauer 250 mm x 8 mm HPLC column packed with LiChrosorb Si60 5μm and eluant ethyl acetate/hexane 1/19) of fraction 4 yield four pure compounds

3-methoxymethyl-6-methoxyl-7-methylocta-1,7(10)-dien-3-ol (**3**) (5 1 mg, 0 21 %), a clear oil $[\alpha]_D^{25}$ +4 7° (c, 0 17, $CHCl_3$), IR (film) ν_{max} 3350, 2910, 1500, 1090 cm^{-1}, ^1H nmr (see Table 2), ^{13}C nmr (see Table 1), HREIMS, obsd , *m/z* 197 158, $C_{12}H_{21}O_2$ requires *m/z* 197 154, EIMS, *m/z* (% rel int), 320 (M^+ -OH, 3), 183 (8), 165 (7), 151 (31), 119 (59), 98 (52), 85 (100), 55 (54)

Octodene (5 mg, 0.21 %) with identical physical and chemical properties to those of the known metabolite [5]

Compound **6** (20 mg, 0 82 %) with identical physical and chemical properties to those of the known metabolite[4] **6**

Compound **7** (65 mg, 2 67 %) with identical physical and chemical properties to those of the known metabolite[4] **7**

Two monoterpenes were isolated from fraction 5, which was chromatographed in an identical manner to fraction 4

(2*Z*,6*E*)-3-chloromethyl-1-chloroocta-2,6-dien-8-al (**2**) (3 1 mg, 0 13 %), a clear oil $[\alpha]_D^{25}$ 0 0° (c, 0 10, $CHCl_3$), IR (film) ν_{max} 2910, 1715, 1450, 1250 cm^{-1}, UV λ_{max} (EtOH) 217 nm (ε 6900), ^1H nmr (see Table 2), ^{13}C nmr (see Table 1), HREIMS, obsd , *m/z* 185 073, $C_{10}H_{14}OCl$ requires *m/z* 185 073, EIMS, *m/z* (% rel int), 320 (M^+ -$CH_3[^{35}Cl_2]$, <1), 185 ([^{35}Cl], 1), 169 (2), 149 (6), 143 (9), 131 (35), 69 (100)

Compound **8** (17 mg, 0 70 %) with identical physical and chemical properties to those of the known metabolite[4] **8**

HPLC separation (Knauer 250 mm x 8 mm HPLC column packed with LiChrosorb Si60 5μm and eluant ethyl acetate/hexane 3/17) of fraction 9 yielded a single pure compound

Compound **9** (22 mg, 0 90 %) with identical physical and chemical properties to those of the known metabolite[4]**9**

HPLC separation (Knauer 250 mm x 8 mm HPLC column packed with LiChrosorb Si60 5μm and eluant chloroform) of fraction 7 yielded three monoterpenes

(2*Z*,6*S*)-3-chloromethyl-1-methoxylocta-2,7(10)-dien-6-ol (**4**) (5 7 mg, 0 21 %), a clear oil $[\alpha]_D^{25}$ -6 3° (c, 0 19, $CHCl_3$), IR (film) ν_{max} 3400, 2910, 1450, 1100, 900 cm^{-1}, ^1H nmr (see Table 2), ^{13}C nmr (see Table 1), HREIMS, obsd , *m/z* 203 086, $C_{10}H_{16}O_2Cl$ requires *m/z* 203 084, EIMS, *m/z* (% rel int), 203 (M^+ -$CH_3[^{35}Cl]$, 1), 186 (4), 183 (3), 171 (6), 151 (14), 137 (30), 133 (19), 132 (27), 119 (33), 79 (58), 71 (81), 41 (100)

(2*Z*,6*S*)-3-chloromethyl-6-methoxylocta-2,7(10)-dien-1-ol (**5**) (2 3 mg, 0 10%), a clear oil $[\alpha]_D^{25}$ -18 3° (c, 0 12, $CHCl_3$), IR (film) ν_{max} 3400, 2910, 1450, 1100, 900 cm^{-1}, ^1H nmr (see Table 2), ^{13}C

nmr (see Table 1); HREIMS, obsd , *m/z* 201 104, $C_{11}H_{18}OCl$ requires *m/z* 201 104, EIMS, *m/z* (% rel int), 218 (M^+ [^{35}Cl], <1), 201 (M^+ -OH[^{35}Cl], 1), 183 (1), 169 (2), 151 (4), 119 (6), 107 (5), 85 (100)

Compound **14** (3 mg, 0 12 %) with identical physical and chemical properties to those of the known metabolite[4] **14**

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