Phytochemistry, Vol. 21, No. 8, pp. 2139–2141, 1982.
Printed in Great Britain.

0031-9422/82/082139-03$03.00/0

DIASTEREOISOMERS OF CYCLOCYMOPOL AND CYCLOCYMOPOL MONOMETHYL ETHER FROM *CYMOPOLIA BARBATA*

OLIVER J. MCCONNELL, PATRICIA A. HUGHES and NANCY M. TARGETT

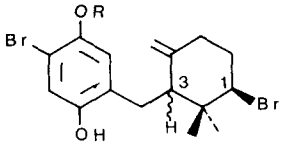
Skidaway Institute of Oceanography, P.O. Box 13687, Savannah, GA 31406, U.S.A.

(*Revised received* 23 *November* 1981)

Key Word Index—*Cymopolia barbata*; Dascycladaceae; marine natural products; terpene-quinols; cyclocymopol; cyclocymopol monomethyl ether.

Abstract—Optically active diastereoisomers of the brominated monoterpene quinols, cyclocymopol and cyclocymopol monomethyl ether, were isolated from the green marine alga *Cymopolia barbata* and characterized.

In 1965, Nadal *et al.* [1] isolated a broad-spectrum antibiotic complex which they named sarganin from three marine algae including *Cymopolia barbata* (L.) Lamouroux, collected near Puerto Rico. The diethyl ether extracts from these algae showed *in vitro* activity against bacteria and fungi, and inhibited KB tumour cells *in vitro*. Subsequently, several brominated monoterpene-quinols were isolated and characterized from *C. barbata* collected in Bermuda [2]. We have identified additional biological activities from the extract and components of *C. barbata* collected in the Florida Keys. The activities include avoidance by molluscs [3], inhibition of feeding by sea urchins [4] and inhibition of plant growth [O. J. McConnell, P. A. Hughes, N. M. Targett and S. C. Phatak, unpublished work]. Consequently, we have carefully re-examined the chemistry of *C. barbata*. We report here that *C. barbata* contains optically-active diastereoisomers of cyclocymopol (**1a**, **1b**) (1 - bromo - 3 - (4 - bromo - 2,5 - dihydroxybenzyl) - 2,2 - dimethyl - 4 - methylenecyclohexane) and cyclocymopol monomethyl ether (**2a**, **2b**). The presence of optically-active diastereoisomers in green marine algae is unprecedented, although a few examples of optically-active diastereoisomers from red [5–7] and brown [8] marine algae are known.

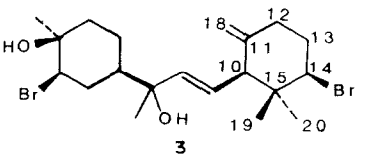


1a (R=H): **2a** (R=Me) : H (C-3)-equatorial

1b (R=H): **2b** (R=Me) : H (C-3)-axial

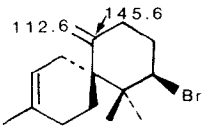
Silica gel chromatography of an ether-soluble extract (1.0% fr. wt) of *C. barbata* yielded diastereoisomers of cyclocymopol (**1a**, **1b**) (1:1 mixture of $\alpha:\beta$ epimers at C-3, 0.01% fr. wt) and cyclocymopol ether (**2a**, **2b**) (3:1 mixture of $\alpha:\beta$ epimers at C-3, 0.02% fr. wt). The previously identified compounds cymopol (0.07% fr. wt), cymopol monomethyl ether (0.01%), cymopolone (0.001%) and cymopochromenol (0.004%) were also isolated [2]. Isocymopolone was not detected [2]. The α-diastereoisomer of cyclocymopol (**1a**) was purified by HPLC on μ-Porasil and Partisil 10 (5% EtOAc in hexanes). Fractions containing various ratios of **1a** and **1b** were obtained, but none contained **1b** alone. From similar HPLC conditions (0–5% EtOAc in hexanes), a 5% enrichment from the original mixture of **2a** over **2b** was affected (3:1, **2a**/**2b** to 4:1, **2a**/**2b**).

The structure of **2b**, cyclocymopol monomethyl ether ($C_{17}H_{22}Br_2O_2$), was deduced from spectral data and confirmed by an X-ray crystallographic study of the corresponding acetate [2]. MS, IR and UV data of the 4:1 mixture of **2a** and **2b** were virtually identical with reported values of **2b** [2]. Detailed analysis of ^1H NMR data at 400 MHz and ^{13}C NMR data (25 MHz, $CDCl_3$, TMS as internal standard) allowed structure assignment of **2a**. In **2a**, the α-Br proton at C-1 absorbs at δ 4.44 (*dd*, $J = 4.4$, 11.8 Hz) whereas in **2b**, the 1,3-diaxial deshielding effect of the benzyl moiety on the α-Br proton is absent and this proton resonates upfield at δ 4.18. The allylic methine proton at C-3 in **2a** is equatorial and appears at δ 2.44 (*dd*, $J = 3.4$, 12 Hz). In **2b**, this proton is axial and absorbs upfield at δ 2.31. Based on the model compounds obtusadiol (**3**) [9], which has a β-side chain at C-10 (C-3 in **2**), and 10-bromo-β-chamigrene (**4**) [7] (Fig. 1), ^{13}C NMR values for the exomethylene carbons in **2a** correspond to δ 112.3 and 144.9. A resonance at δ 63.0 in **2a** was assigned to the bromine-bearing carbon at C-1. In obtusadiol (**3**), this absorption occurs downfield at δ 66.4 because the shielding (steric) effects from the C-3 axial substituent found in **2a** is absent [9]. ^{13}C NMR chemical shifts of gem-dimethyls



3

Carbon no.	δ (ppm)
10	56.5
11	147.2
12	37.5
13	22.0
14	66.4
15	41.1
18	110.3
19	16.1
20	26.1



4

Fig. 1. ^{13}C NMR data for obtusadiol (**3**) and 10-bromo-β-chamigrene (**4**).

in conformationally rigid cyclohexanes can be used to confirm the nature and stereochemistry of substituents on adjacent carbons [10]. In 1-bromo-2,2,3-trimethylcyclohexane, the chemical shifts of the gem-dimethyls are δ 16 and 29 when the bromine and remaining methyl are equatorial. They are δ 23 and 29 when the bromine is equatorial and the methyl axial. The latter values correspond most closely to δ 23.6 and 27.6, which were observed for **2a**.

Högberg *et al.* [2] assigned the structure of **1b**, the β-epimer of cyclocymopol at C-3, by comparing ^1H NMR and MS data with **2b** and by chemical conversion of **2b** to **1b**. The close spectral similarities of **1a** with **1b**, **2a** and **2b** allowed structure assignment. MS analysis of **1a** verified the molecular formula of $C_{16}H_{20}Br_2O_2$. The IR spectrum exhibited hydroxyl (3450 cm^{-1}), gem-dimethyl (1395 and 1375 cm^{-1}) and olefin (900 cm^{-1}) absorptions. The UV spectrum revealed absorptions characteristic of a bromoquinol moiety (λ 220 sh (log ε 4.34) and λ_{max} 303 nm (log ε 3.95)). **1a** exhibited a ^1H NMR absorption at δ 4.42 (*dd*, $J = 4.4$, 11.2 Hz) corresponding to the axial α-Br proton at C-1 which is coupled to two protons at δ 2.05–2.25. The equatorial allylic methine proton at δ 2.45 (*dd*, $J = 3.4$, 12 Hz) is coupled to two benzylic protons at δ 2.57 (*dd*, $J = 12$, 13 Hz) and 2.88 (*dd*, $J = 3.4$, 13 Hz). ^{13}C NMR absorptions corresponding to the axial methyls at C-2 of **1a** and **1b** were observed at δ 17.7 and 23.8, respectively. In **1a**, C-1 and C-3 absorb at δ 62.9 and 55.0, respectively, and in **1b**, at δ 66.4 and 52.1, respectively.

The biogenesis presumes an enzymatic conversion of cymopol (2 - bromo - 5 - (3,7 - dimethyl - octa - 2(*E*),6(*E*) - dienyl)quinol) to the 2(*Z*) - olefinic isomer followed by bromonium-ion induced cyclization [2, 11] to yield diastereoisomers of **1** and **2**. We did not detect either the *Z*-isomer of cymopol or the *Z*-isomer of cymopolone, isocymopolone [2].

EXPERIMENTAL

Isolation of diastereomers of cyclocymopol (**1a**, **1b**) *and cyclocymopol monomethyl ether* (**2a**, **2b**). Freshly frozen *C. barbata* (L.) Lamouroux (identified by J. Norris; a voucher specimen has been deposited at the National Museum of Natural History, Smithsonian Institution, Washington, D.C.), collected in shallow waters (−1 m) in the Florida Keys (January 1980), was homogenized and repeatedly extracted with *iso*-PrOH–CH_2Cl_2 (1:1). The vol. of the filtrate was reduced *in vacuo* and the residue partitioned between Et_2O and H_2O. The Et_2O-soluble extract (16 g) was obtained from 1.6 kg of fresh algae (1.0% extract, fr. wt). Gravity flow gradient Si gel chromatography (Grace Chemical, grade 62) with hexanes, CH_2Cl_2, EtOAc and MeOH yielded mixtures of **1a**, **1b**, **2a**, **2b**, cymopol, cymopol monomethyl ether, cymopolone and cymopochromenol [2]. Repeated low pres. isocratic Si gel chromatography [12] using 5%, 10% and 15% EtOAc–hexanes yielded diastereoisomers of cyclocymopol (**1a**, **1b**) (1:1 mixture of α:β epimers at C-3, 0.01% fr. wt) and cyclocymopol monomethyl ether (**2a**, **2b**) (3:1 mixture of α:β epimers at C-3, 0.02% fr. wt).

Cyclocymopol (**1a**, **1b**). HPLC of the diastereoisomeric mixture on μ-Porasil (2 × 30 cm, 66–80 kg/cm^2) and Partisil 10 (50 cm, 13–16 kg/cm^2) with 5% EtOAc–hexanes yielded pure **1a** and mixtures of **1a** and **1b**. **1a** provided the following data: $[\alpha]_D^{24}$ −3.1° (*c* 0.16, $CHCl_3$); high resolution MS (probe) 70 eV, *m/z*: M^+ = 401.986, $C_{16}H_{20}{}^{79}Br_2O_2$ requires 401.983; ^1H NMR (400 MHz, $CDCl_3$, TMS as int. standard): δ 1.08 (3H, *s*), 1.21 (3H, *s*), 2.05–2.12 (1H, *m*), 2.18–2.25 (2H, *m*), 2.36 (1H, *m*), 2.45 (1H, *dd*, $J = 3.4$, 12 Hz) 2.59 (1H, *dd*, $J = 12$, 13 Hz), 2.88 (1H, *dd*, $J = 3.4$, 13 Hz), 4.33 (1H, *br s*) 4.42 (1H, *dd*, $J = 4.4$, 11.2 Hz), 4.48 (1H, *s*, OH), 4.65 (1H, *br s*) 5.02 (1H, *s*, OH), 6.67 (1H, *s*), 6.83 (1H, *s*); ^{13}C NMR of (1:1) mixture of **1a** and **1b** (25 MHz, $CDCl_3$ TMS as int. standard): δ 17.7, 23.8, 26.7, 27.6, 28.5, 31.8, 34.6, 35.5, 36.2, 52.1, 55.0, 62.9, 66.4, 110.2, 112.2, 116.7, 117.4, 117.9, 129.0, 144.9, 145.1, 146.0 and 147.2; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450, 1395, 1375 and 900; UV λ_{max}^{MeOH} nm: 220(sh) (4.34), 303 (3.95).

From a least squares fit of optical rotation data of mixtures of **1a** and **1b** that were obtained at the same temp. and concn, $[\alpha]_D^{24}$ of **1b** was determined to be −16° (*c* 0.6, $CHCl_3$) [2].

Cyclocymopol monomethyl ether (**2a**, **2b**). Isocratic HPLC of the diastereoisomeric mixture of cyclocymopol monomethyl ether on μ-Porasil (2 × 30 cm, 66–80 kg/cm^2) and Partisil 10 (50 cm, 13–16 kg/cm^2) with 0–5% EtOAc–hexanes yielded a 4:1 mixture of **2a** and **2b**. MS (probe) 70 eV, *m/z*: 416/418/420 (1:3:1) $[M]^+$; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3615, 3430, 1395, 1375, 906; UV λ_{max}^{MeOH} nm: (log ε) 219(sh) (3.81), 297 (3.77); ^1H NMR data assigned to **2a** (400 MHz, $CDCl_3$, TMS as int. standard): δ 1.08 (3H, *s*), 1.23 (3H, *s*), 2.05–2.10 (1H, *m*), 2.18–2.25 (2H, *m*), 2.39 (1H, *m*), 2.44 (1H, *dd*, $J = 3.4$, 12 Hz), 2.62 (1H, *dd*, $J = 12$, 13 Hz), 2.91 (1H, *dd*, $J = 3.4$, 13 Hz), 3.80 (3H, *s*), 4.31 (1H, *br s*), 4.44 (1H, *dd*, $J = 4.4$, 11.2 Hz), 4.63 (1H, *s*, OH), 4.62 (1H, *br s*), 6.53 (1H, *s*), 6.92 (1H, s); ^{13}C NMR data assigned to **2a** (25 MHz, $CDCl_3$, TMS as int. standard): δ 23.6 (*q*), 27.6 (*q*), 27.8 (*t*), 31.8 (*t*), 34.6 (*t*), 39.9 (*s*), 55.1 (*d*), 57.0 (*q*), 63.0 (*d*), 108.4

(s), 112.3 (t), 115.0 (d), 119.8 (d), 127.7 (s), 144.9 (s), 147.6 (s), 149.6 (s).

Acknowledgements—This research was partially supported by the Georgia Sea Grant Program (NOAA), Merck, Sharp and Dohme and CIBA-GEIGY. We thank J. Norris (Smithsonian Institution) for taxonomic identification of *C. barbata*, Prof. Pelletier (University of Georgia) for optical rotation data, C. Pape (University of Georgia) for ^{13}C NMR data, and L. Abbey (Georgia Institute of Technology) for MS data. High resolution ^{1}H NMR data were obtained at the University of South Carolina NMR facility which is supported by a grant from the National Science Foundation (CHE78-18723).

REFERENCES

1. Nadal, N. G. M., Rodriguez, L. V. and Casillas, C. (1965) *Antimicrob. Ag. Chemo.* 131.
2. Högberg, H.-E., Thompson, R. H. and King, T. (1976) *J. Chem. Soc. Perkin Trans.* 1, 1696.
3. Targett, N. M. and McConnell, O. J. (1982) *J. Chem. Ecol.* **8**, 115.
4. McConnell, O. J., Hughes, P. A., Targett, N. M. and Daley, J. (1981) *J. Chem. Ecol.* (in press).
5. Crews, P. (1977) *J. Org. Chem.* **42**, 2634.
6. Faulkner, D. J. (1977) *Tetrahedron* **33**, 1421.
7. Sims, J. J., Rose, A. F. and Izac, R. R. (1978) in *Marine Natural Products* (Scheuer, P., ed.) Vol. 2, pp. 297–378. Academic Press, New York.
8. Gerwick, W. H. and Fenical, W. (1981) *J. Org. Chem.* **46**, 22.
9. Howard, B. M. and Fenical, W. (1978) *Tetrahedron Letters* 2453.
10. Crews, P. and Kho-Wiseman, E. (1978) *Tetrahedron Letters* 2483.
11. Fenical, W. (1978) in *Marine Natural Products* (Scheuer, P., ed.) Vol. 2, pp. 173–245, Academic Press, New York.
12. Meyers, A. I., Slade, J., Smith, R. K., Mihelich, E. D., Hershenon, F. M. and Liang, C. D. (1979) *J. Org. Chem.* **44**, 2247.

Phytochemistry, Vol. 21, No. 8, pp. 2141–2142, 1982.
Printed in Great Britain.

0031-9422/82/082141-02$03.00/0

HALOGENATED PHLOROGLUCINOLS FROM *RHABDONIA VERTICILLATA*

ADRIAN J. BLACKMAN and DAVID J. MATTHEWS

Chemistry Department, University of Tasmania, Hobart, Tasmania, Australia 7000

(*Received* 2 *December* 1981)

Key Word Index—*Rhabdonia verticillata*; Rhabdoniaceae; halogenated phenols; bromo- and chlorophloroglucinols.

Abstract—Six bromo- and/or chloro- derivatives of phloroglucinol have been obtained from the red alga *Rhabdonia verticillata*.

INTRODUCTION

A variety of bromophenols have been isolated from marine organisms, particularly Rhodophyta (red seaweeds) [1, 2]. These compounds have been found to occur primarily in the Rhodomelaceae (Ceramiales). We now report, for the first time, the presence of previously unknown halophenols in a member of the Rhabdoniaceae (Gigartinales).

RESULTS AND DISCUSSION

The major compound (0.2%, dry wt) obtained from *Rhabdonia verticillata* was 2,4-dibromo-1,3,5-trihydroxybenzene (dibromophloroglucinol), **1**. Its identity was established by spectroscopic analysis and conversion into 1,3,5-trimethoxybenzene, **3**, via **2** and confirmed by comparison of **1**, **2** and **3** with synthetic materials. Dibromophloroglucinol has not been previously isolated as such from natural sources although the corresponding trimethyl ether, **2**, has been obtained after treatment of the ethanolic extract of *Rytiphlea tinctoria* (Rhodomelaceae) with diazomethane [3].

Since *Rhabdonia verticillata* contained smaller amounts of other halogenated phenols which could not be separated from **1** by Si gel chromatography, the crude extract was subjected to GC/MS after forming trimethylsilyl and trimethoxy derivatives. The following halogenated phloroglucinols, in order