

The isolation, structural determination and bioactivity of 1*E*,3*R*,4*S*,5*E*,7*Z*-1-bromo 3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene from a Namibian *Plocamium* species

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Abstract

A known compound namely 1*E*,3*R*,4*S*,5*E*,7*Z*-1-bromo-3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene was isolated from a Namibian *Plocamium* species for the first time and characterized by means of one and two dimensional Nuclear Magnetic Resonance (NMR) spectroscopic data and Mass Spectrometry (MS) analysis. The compound exhibited minimal inhibition against HIV-1 reverse transcriptase, with a 50% inhibitory concentration of > 1000µM. However, literature reviews indicate that this compound has good cytotoxic *in vitro* effects.

Keywords: halogenated monoterpene, *Plocamium*, cytotoxic

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1 Introduction

Marine algae (or seaweed) can be roughly divided as being either red, green or brown. Red algae (Rhodophyta) of the family Plocamiaceae and Rhizophyllidaceae produce a number of different biologically active linear and cyclic polyhalogenated monoterpenes (Kladi et al., 2004). Of the 47 different species of *Plocamium* that occur around the world, at least 7 species occur on Namibia's coastline (Bolton, 2014). Various polyhalogenated monoterpenes from around the world exhibit a wide range of biological activities including antifeedant effects on reef herbivores, antimicrobial, insecticidal, antitubercular and anticancer activities (Knott et al., 2005).

In the continuing investigation of biologically active metabolites from southern African *Plocamium* species (Afolayan et al., 2009; Knott et al., 2005; Mann et al., 2007) the natural product chemistry of a Namibian *Plocamium* species was investigated. In this work, a description of the isolation, structural elucidation and biological activity of the major metabolite obtained from a Namibian *Plocamium* species of marine alga is reported.

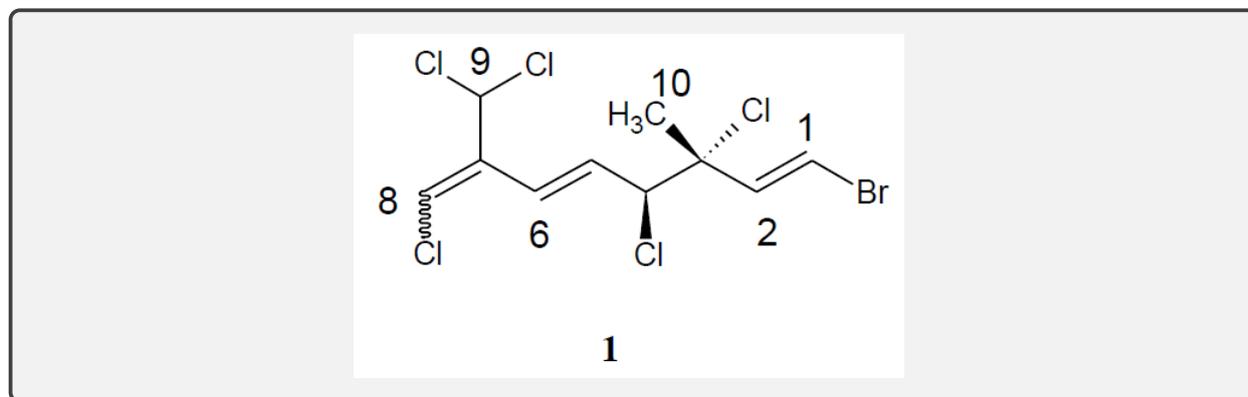
It appears that the major metabolites isolated from selected South African *Plocamium*, *Portieria* and *Laurencia* species are unique to each species (Knott, 2012) within the South African context. However, some degree of overlap occurs with regards to some of the minor secondary metabolites isolated in each species of a specific genus, within the South African context. This is most likely due to common biogenic pathways of origin. For example, metabolites from the genus *Plocamium* follow the ocimene biogenesis pathway (Naylor et al., 1983). However, this does not necessarily mean that the chemistry of various *Plocamium* species from Namibia will be the same as that of various *Plocamium* species from South Africa. Variations in geographical location, climate and seasonal changes are able to change the chemical profiles of certain marine algae.

2 Results and discussion

2.1 Identification of the major metabolite using NMR

Plocamium samples were collected from the intertidal zone at Swakopmund, Namibia and immediately frozen. A partially thawed sample was extracted with MeOH. This was partitioned with hexane using solvent-solvent separation to yield a surprisingly 'clean' compound **1**.

The major metabolite present in this *Plocamium* species was confirmed by NMR and MS



and identified based on a comparison of both experimental and literature values. This compound had previously been isolated from *Plocamium cartilagineum* (Mynderse and Faulkner, 1975), *Plocamium suhrrii* (Antunes et al., 2011) and *Plocamium rigidum* (Fakee, 2013). The molecular ion for this compound could not be observed in its electron ionisation mass spectrum. The mass spectrum exhibited an abundant ion at mass to charge ratio (m/z) 167, 169 (base peak), 171 (relative abundance: 3:4:1) which corresponds to an ion with a formula $C_4H_5BrCl^+$, based on the isotope pattern and MS data reported by Mynderse and Faulkner (1975). This fragment ion is formed by the homolytic cleavage of the 3,4-bond of compound **1**. The ^{13}C NMR data and 1H NMR data below for compound **1** are consistent with that reported by Fakee (2013) (Table 1). These correlations were also confirmed by means of a 1H - 1H Homonuclear Correlation Spectroscopy (COSY) experiment.

Table 1: ^{13}C NMR ($CDCl_3$, 100 MHz) data and 1H NMR ($CDCl_3$, 400 MHz) data for compound **1**. **Fakee (2013) NMR results included below.

Carbon no.	δ_C	δ_C^{**}	δ_H	δ_H^{**}
1	110.2	110.2	6.58, d, 16.0	6.57, d, 13.6
2	137.5	137.5	6.44, d, 16.0	6.45, d, 13.6
3	71.5	71.5		
4	68.2	68.2	4.53, d, 12.0	4.54, d, 6.8
5	127.4	127.4	6.33, m	6.33, dd, 15.5, 5.3
6	129.6	129.6	6.35, m	6.34, d, 15.6
7	138.5	138.5		
8	119.6	119.7	6.32, s	6.32, s
9	65.5	65.5	6.97, s	6.96, s
10	25.3	25.3	1.77, s	1.77, s

Of concern was the stereochemistry at position 4. However, the upfield shift of the methyl signal at position 10 from δ 1.82 to δ 1.77 suggests *S* stereochemistry at position 4 (Fakee, 2013; Mynderse and Faulkner, 1975).

Another point of contention was the nature of *E/Z* geometry at the $\Delta^{7,8}$ double bond. Literature values supported an *E* geometry, for example a proton shift of δ 6.33 at position 8 is characteristic of *E* geometry at the $\Delta^{7,8}$ double bond, while *Z* geometry at the $\Delta^{7,8}$ double bond has a proton shift of δ 6.28 (Mynderse and Faulkner, 1975). Fakee (2013) indicated that the carbon at position 8 has a shift of δ 119.7 for *E* geometry at the $\Delta^{7,8}$ double bond, and a shift at δ 119.3 for *Z* geometry at the $\Delta^{7,8}$ double bond. Based on both these literature values, an *E* geometry might have been assigned to the $\Delta^{7,8}$ double bond. However, a Nuclear Overhauser Enhancement Spectroscopy (NOESY) experiment showed strong correlations between H-8 and both H-4 and the methyl group at position 10 which strongly suggested *Z* geometry at the $\Delta^{7,8}$ double bond (Figure 1).

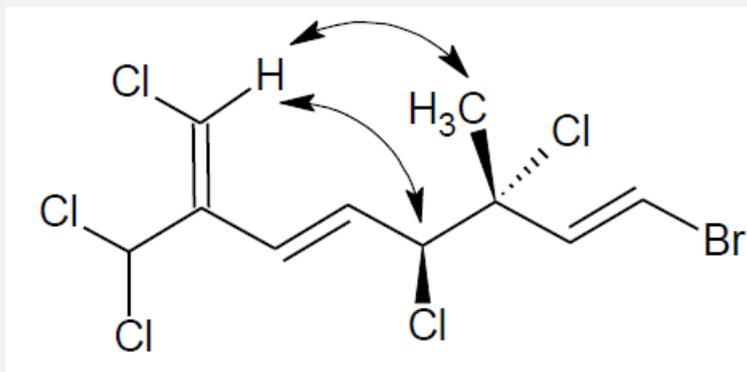
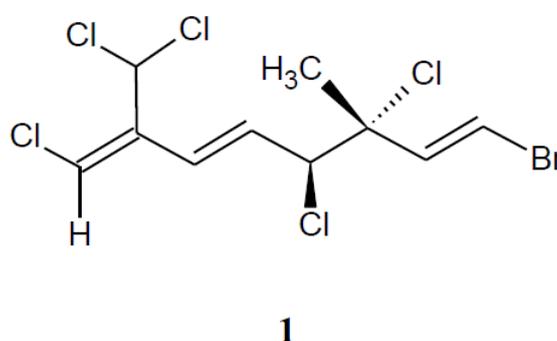


Figure 1: *Z* geometry at the $\Delta^{7,8}$ double bond based on NOESY correlations.

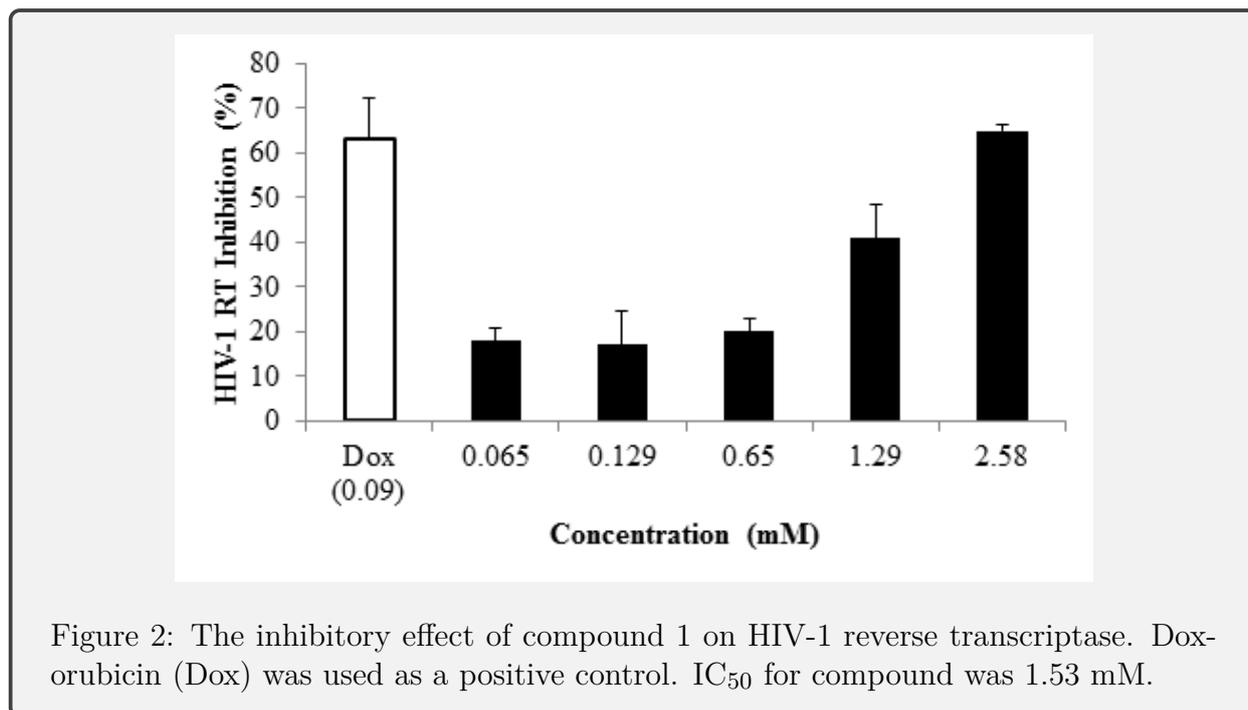
As a result, compound **1** was named, 1*E*,3*R*,4*S*,5*E*,7*Z*-1-bromo 3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene.



2.2 Bioactivity

IC₅₀ values of 1*E*,3*R*,4*S*,5*E*,7*E*-1-bromo 3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene against the MCF-7 breast cancer cell line is an impressive 13.7 μ M. Compounds bearing the *gem*-dichloro moiety are known to exhibit moderate to good cytotoxic activity against this breast cancer cell line (Fakee, 2013). In addition, compound **1** was also very bioactive towards oesophageal cancer cells with an IC₅₀ (μ M) of 6.6 (Antunes et al., 2011). For the oesophageal cancer cell line test, the known anticancer drug cisplatin has an IC₅₀ value of 13 μ M.

Interestingly, compound **1** showed minimal inhibitory activity towards HIV-1 reverse transcriptase (Figure 2) and the IC₅₀ of the compound was > 1000 μ M. The anti-HIV results are indicative that compound **1** might demonstrate good inhibitory activity if tested at higher concentrations.



From a chemotaxonomic perspective, it is interesting to observe that South African *Plocamium* species can be classified on the basis of their major metabolites which are unique to each species. Compound **1** is the major metabolite found in the South African species of *Plocamium suhrii*. Although the chemistry of the major secondary metabolite isolated and characterised from this Namibian *Plocamium* sample is characteristic of *Plocamium suhrii*, further taxonomical investigations still need to confirm the full identity of this Namibian *Plocamium* sample. Bolton (2015) states, "The taxonomy of *Plocamium* in southern Africa

is not properly sorted out". Until the taxonomy of *Plocamium* in southern Africa is well established, tentative identification of *Plocamium* species in Namibia will remain an uncertain element of this research.

3 Conclusion

Compound **1** is easy to extract and is available in relatively large quantities off the Namibian coast where *Plocamium* species are commonly found. Literature reviews reveal that compound **1** exhibits good *in vitro* cytotoxic activity. Although compound **1** demonstrated minimal inhibitory activity towards HIV-1 reverse transcriptase, further *in vitro* testing still needs to be conducted on other HIV-enzymes before we can fully exclude compound **1** as a potentially important anti-retroviral lead compound.

4 Materials and Methods

4.1 Plant material

Plocamium species were collected by hand at the intertidal zone at Swakopmund in May 2014 and kept frozen until extraction. The voucher specimen has been stowed away at the School of Pharmacy, University of Namibia.

4.2 Extraction

Plocamium species (1.598 g wet mass) was steeped in 100 ml MeOH overnight. The partially concentrated methanolic extract was filtered through cotton wool and partitioned three times with hexane (3 × 30 ml). The hexane extracts were concentrated and weighted 0.271 g (dry weight 16.96%).

4.3 General experimental procedures

The ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were all recorded on a Bruker 400 NMR spectrometer using standard pulse sequences. Spectra were referenced to residual

protonated solvent resonances (CHCl_3 δ_H 7.25, δ_C 77.0). Chemical shifts were reported in parts per million (ppm), while coupling constants were reported in Hertz (Hz).

Gas Chromatography-Mass Spectrometry (GC-MS) analyses were performed on a Thermo Scientific Focus GC coupled to an ITQ 700 MS using Xcalibur Software, version 2.1, for data acquisition. A SGE BP5MS capillary GC column (30 m \times 0.25 mm i.d., film thickness of 0.25 μm) was used with helium as carrier gas at a flow rate of 1.0 mL/min (constant flow) and a split ratio of 10. The GC injector was maintained at a temperature of 220 $^\circ\text{C}$. Samples were injected in the split mode using a split ratio of 1:10. The oven temperature was programmed at 5 $^\circ\text{C}/\text{min}$ from 40 $^\circ\text{C}$ to 300 $^\circ\text{C}$. Electron ionization-Mass Spectrometry (EI-MS) data were acquired at 70 eV and a mass range of m/z 25 to 625 was scanned. Ion source and interface temperatures of 200 and 250 $^\circ\text{C}$, respectively, were used for the analysis.

Compound 1: (1*E*,3*R*,4*S*,5*E*,7*Z*)-1-bromo-3,4,8-trichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene (**1**): colourless oil; NMR data available in Table 1. As previously reported by Mynderse and Faulkner (1975).

HIV-1 reverse transcriptase assay

A non-radioactive reverse transcriptase colorimetric enzyme-linked immunosorbent assay (ELISA) kit from Roche Diagnostics (Mannheim, Germany) was used to test compound inhibition to reverse transcriptase (RT). The protocol out-lined in the kit was followed using 4ng of enzyme per well (Fonteh et al., 2009; Kapewangolo et al., 2013). The compound was tested at five different concentrations 0.065, 0.129, 0.65, 1.29 and 2.58 mM. Doxorubicin (Sigma-Aldrich, St Louis, MO, USA) was used as a positive control.

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References

- [1] Afolayan, A.F.; Mann, M.G.A.; Lategan, C.A.; Smith, P.A.; Bolton, J.J.; Beukes, D.R. Antiplasmodial halogenated monoterpenes from the marine red alga, *Plocamium cornutum*. *Phytochemistry*, 70, 597-600 (2009).

- [2] Antunes, E.M., Afolayan, A.F., Chiwakata, M.T., Fakee, J., Knott, M.G., Whibley, C.E., Hendricks, D.T., Bolton, J.J., & Beukes, D.R. Identification and in vitro anti-esophageal cancer activity of a series of halogenated monoterpenes isolated from the South African seaweeds *Plocamium suhrii* and *Plocamium cornutum*. *Phytochemistry*, 72, 769-772 (2011).
- [3] Bolton, J. Pers. Comm. University of Cape Town, Cape Town, South Africa (2014)
- [4] Bolton, J. Pers. Comm. University of Cape Town, Cape Town, South Africa (2015)
- [5] Fakee, J. The Isolation and Characterisation of Secondary Metabolites from Selected South African Marine Red Algae (Rhodophyta). MSc thesis, Rhodes University, Grahamstown, South Africa, 27 (2013).
- [6] Fonteh, P.N., Keter, F.K., Meyer, D., Guzei, I.A., Darkwa, J. Tetra-chloro-(bis-(3,5-dimethylpyrazolyl)methane)gold(III) chloride: an HIV-1 reverse transcriptase and protease inhibitor. *Journal of Inorganic Biochemistry*, 103, 190-194 (2009).
- [7] Kapewangolo, P., Hussein, A.A., Meyer, D. Inhibition of HIV-1 enzymes, antioxidant and anti-inflammatory activities of *Plectranthus barbatus*. *Journal of Ethnopharmacology*, 149, 184-190 (2013).
- [8] Kladi, M.; Vagias, C.; Roussis, V. Volatile halogenated metabolites from marine red algae. *Phytochem. Rev.*, 3, 337-366 (2004).
- [9] Knott, M.G. The natural product chemistry of South African *Plocamium* species. MSc thesis, Rhodes University, Grahamstown, South Africa, 68 (2003).
- [10] Knott, M.G.; Mkwanzani, H.; Arendse, C.E.; Hendricks, D.T.; Bolton, J.J.; Beukes, D.R. Plocoralides A-C, polyhalogenated monoterpenes from the marine alga, *Plocamium corallorhiza*. *Phytochemistry*, 66, 1108-1112 (2005).
- [11] Knott, M.G. Isolation, structural characterisation and evaluation of cytotoxic activity of natural products from selected South African marine red algae. PhD thesis, Rhodes University, Grahamstown, South Africa (2012)
- [12] Mann, M.G.A.; Mkwanzani, H.; Antunes, E.M.; Whibley, C.E.; Hendricks, D.T.; Bolton, J.J.; Beukes, D.R. Halogenated monoterpene aldehydes from the South African marine Alga *Plocamium corallorhiza*. *J. Nat. Prod.*, 70, 596-599 (2007).
- [13] Mynderse, J.S., & Faulkner, D.J. Polyhalogenated monoterpenes from the red alga *Plocamium cartilagineum*. *Tetrahedron*, 31, 1963-1967 (1975).
- [14] Naylor, S., Hanke, F.J., Manes, L.V., & Crews, P. Chemical and biological aspects of marine monoterpenes. *Prog. Chem. Nat. Prod.*, 44, 211 (1983).

Appendix

