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# A Tetrachloro Polyketide Hexahydro-1*H*-isoindolone, Muironolide A, from the Marine Sponge *Phorbas* sp. Natural Products at the Nanomole Scale

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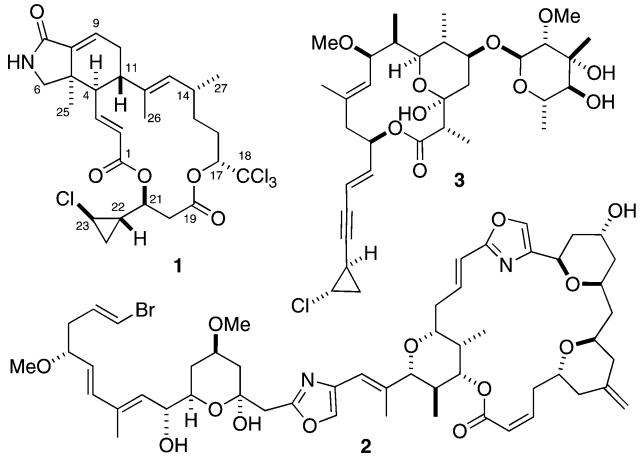
Modern marine sponges—the simplest metazoans—descend from ancestral lines at least 575 Mya that predate the Cambrian explosion.[1] Sponges are structured by associated complex microbial communities that often exceed in number the cells of their sponge hosts.[2] The extraordinary chemical diversity of sponge-derived natural products fuels speculation that many arise from heterogeneous microbial associations.[3]




We report here the structure of muironolide A (**1**), a new chemical entity from the same specimen of *Phorbas* that gave phorboxazoles A (**2**) and B,[4] and the phorbasides (e.g., phorbaside A, **3**).[5] The macrolide **1** is a new carbon skeleton bearing two unprecedented features: a hexahydro-1*H*-isoindolone-triketide ring and a trichlorocarbinol ester. The chlorocyclopropane in **1** is of *opposite* configuration to that of **3**, but the same as that of callipeltosides A−C.[6] The co-occurrence of **1** with **2** and **3** is demonstrable evidence of an extraordinarily diverse polyketide repertoire within this sponge which is likely the product of a heterogeneous sponge−microbe association. The elucidation of this exceedingly rare compound was achieved using the entire sample (90 $\mu$g, 152 nmole) by microcryoprobe NMR spectroscopy,[7] FTMS, circular dichroism (CD), and synthesis.

Muironolide A (**1**) was obtained by repeated HPLC (phenylhexyl column) of a fraction that also provided **3**. The very low abundance of **1** required special protocols for sample handling, structure elucidation, and bioassay. Analysis of the NMR data for **1**, obtained with a 1.7 mm NMR cryoprobe (600 MHz), was used to quantitate **1** prior to FTMS, CD, and chemical degradation−LCMS.

The formula of **1**, $C_{27}H_{33}Cl_4NO_5$, obtained from positive and negative ion ESI FTMS (*m/z* 592.11869, $[M + H]^+$, $\Delta$mmu = +0.41; 590.10339, $[M - H]^-$, $\Delta$mmu −0.1) revealed 10 double-bond equivalents of which six could be assigned to two trisubstituted and one vicinally disubstituted C=C double bonds, and three ester or amide C=O groups ($^{13}$C NMR; $\delta_c$ 169.7, 168.9, 164.4). The presence of four Cl atoms was confirmed by matching the isotope pattern of the pseudomolecular ion against a simulated MS spectrum. The carbon skeleton $^1$H and $^{13}$C NMR assignments of **1** were completed by interpetation of the gCOSY, gHSQC, and gHMBC spectra (see Table S1, Supporting Information) aided by strategic analysis of $^2J_{HH}$, $^3J_{HH}$, and $^1J_{CH}$ (*J*-coupled HSQC). HMBC data (see Supporting Information) showed correlations to the C=O groups that revealed the carbon chain of **1** comprises three ketolide segments as shown. A *trans*-chlorocyclopropane ring was identified by unusually large $^1J_{CH}$ values for the contiguous spin system C22 ($^1J_{CH}$ = 177 Hz), C23 ($^1J_{CH}$ = 200 Hz), and C24 ($^1J_{CH}$ = 173.4 Hz (×2) $CH_2$). The *trans*-2-chlorocyclopropyl ketide (CCK) element in **1** is united with a substituted 3-hydroxypropanoate unit, unlike **3** where it is conjugated to an ene-yne. The unusual geminal coupling of the isolated AB system $H_2$-6 ($^2J_{HH}$ = −8.8 Hz) was uniquely matched to a $\gamma$-lactam in a hexahydro-1*H*-isoindolone system.[8] This was supported by HMBC and NOESY correlations (Figure 1) from ring junction protons H4, H11 (Figure 1b) that secured the relative configurations of C4, C5, C11, and C14.

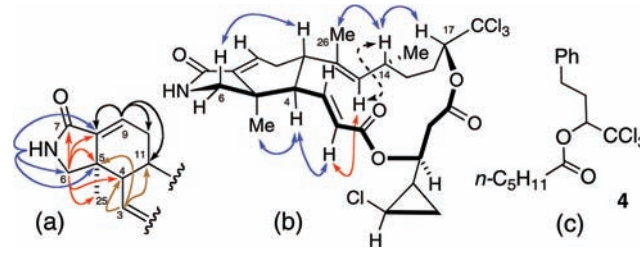



***Figure 1.*** Correlation analysis of **1**. (a) Partial HMBC data of the hexahydro-1*H*-isoindolone ring; (b) NOESY (mixing time $t_m$ = 400 ms) showing *syn*-facial (in blue) and transannular (in red) NOEs, and vicinal coupling (dashed) of H13−H14 ($^3J_{HH}$ = 9.1 Hz); (c) synthetic model **4**.[9]

The balance of the formula required placement of three chlorines and an additional ring. The former were assigned to trichloromethyl carbinol ester ($\delta_H$ 5.55, dd, H17, *J* = 10.8, 2.4 Hz; $\delta_C$ 80.3, C17; 99.3, Cq, C18) that was suppported by similar NMR data for synthetic **4** (Figure 1c).[9]

The absolute configurations at both C14 and C17 were based on NOESY data, *J*-based analysis (Figure 1) and CD (see below). Prominent NOEs observed from H17 to the methine H14 ($\delta$ 2.42, m) confirmed a *gauche*-turn element in the macrocycle.[10] Finally, a three-bond HMBC correlation from H17 to the ester C=O ($\delta$ 168.9, C19) completed the macrocyclic ring of **1**.

The absolute configuration of the macrolide ring system in **1** followed from interpretation of the strong negative bisignate Cotton effect observed in the CD spectrum of **1** [$\lambda$ 186 ($\Delta\varepsilon$ 58.5), 225 (−37.2)]. The latter is assigned to exciton coupling between the $\pi$−$\pi$* transitions of two $\alpha,\beta$-unsaturated carbonyl chromophores—the enamide C7−C9 and the enoate C1−C3. It follows from the rigid

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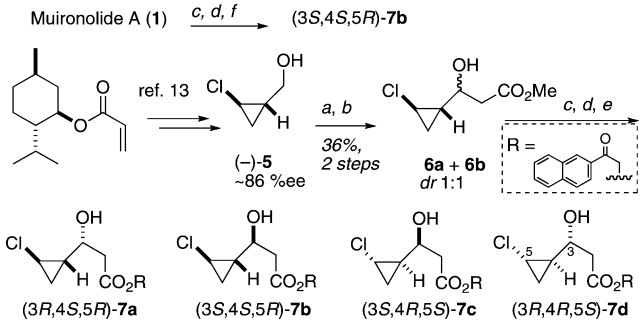
conformation of the isoindolone in **1** (Figure 1)[11] that the electronic transition dipole moments for the two $\pi-\pi^*$ transitions subtend an angle of $+116°$ or $-116°$ depending on chirality. Application of the Harada−Nakanishi nonempirical rule for ECCD[12] predicts the *negative* bisignate-Cotton effect observed for **1** is correlated with *negative* helicity of the two C=C−C=O structures in the depicted enantiomer. The chirality of **1**, therefore, corresponds to 4*R*,5*R*,11*S*,14*R*,17*R*.

Neither NOESY nor *J*-based methods could unequivocally relay the configuration of the CCK element to the other macrolide ring stereocenters. Consequently, we turned to microscale degradation of **1** and correlation of the products with standards of known configuration, which were prepared as follows (Scheme 1). Oxidation (PCC)

***Scheme 1***[a]




[a] Reagents: (a) PCC, Celite; (b) $BrCH_2CO_2Me$, Zn°, THF; (c) LiOH, THF−$H_2O$; (d) R-Br; (e) HPLC, Chiralpak AD, 3:7 *i*PrOH/hexane; (f) LCMS, Chiralpak AD-RH (1:9 $H_2O$−$CH_3CN$, 0.1% $HCO_2H$).

of optically enriched alcohol (−)-**5** (~86% ee)[13] to the corresponding volatile aldehyde,[14] followed by Reformatsky reaction[15] (Zn, $BrCH_2COOMe$, THF) gave an inseparable mixture (dr 1:1) of optically enriched methyl esters **6a** and **6b**. Saponification of the mixture (LiOH, THF-$H_2O$) and immediate derivatization with α-bromo-2-acetylnaphthalene (LiOH, $H_2O$, THF) gave a mixture of 2-naphthone esters, which were separated by chiral HPLC (Chiralpak AD, 3:7 *i*-PrOH/hexane) to give pure diastereomers, **7a** and **7b**, and their enantiomers **7c** and **7d**, respectively.[16]

Chiral LCMS (Chiralpak AD-RH, single ion monitoring, SIM, *m*/*z* 355.2, M + $Na^+$) separated all four stereoisomers with baseline resolution.[17] Treatment of a sample of **1** (30 μg) under the same conditions gave a peak $t_R$ = 11.13 min which coeluted with the standard **7b**. Therefore, the configuration the CCK group is 21*S*,22*S*,23*R* which completes the stereostructure of **1**.[18]

The novel skeleton of **1** represents the first report of a natural macrolactone of a 1,1,1-trichlorocarbinol[19,20] and one of only eight known chlorocyclopropanes. While hydro-1*H*-indol-2(3*H*)-ones, originating from tryptophan, are common in nature, hydro-1*H*-*iso*indolones are extremely rare. The remarkable finding of **1**, **2**, and **3**—three distinct classes of polyketide natural products—in *Phorbas*, deserves comment. Little resemblance is seen between the three compounds except for the presence of the 2-chlorocyclopropane ring in **1** and **3**, albeit with antipodal configurations. Phorbaside A (**3**) shares a similar macrolide ring with several cyanobacterial macrolide glycosides;[21] however, no other natural products described to date resemble **1** or **2**. The common link between **1** and **3** suggests both polyketides may be expressed by the same cyanobacterium living in association with *Phorbas*; however, the very low abundances of **1** (0.41 ppm/dry weight sponge) and **3** (11.6 ppm) compared to **2** (400 ppm) point to a different origin for phorboxazole A.

In conclusion, the structure of muironolide A (**1**), with unprecedented features, a macrocylic trichlorocarbinol ester embodying a hexahydro-1*H*-isoindolone together with a rare *trans*-chlorocyclopropane ring, was fully elucidated from a 90 μg sample. Highly mass-sensitive NMR spectroscopy enables discovery of natural products down to vanishingly small quantities and reveals diversity in macrolides from *Phorbas* that spans 3 orders of magnitude in abundance.[22]

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**Supporting Information Available:** Tabulated $^1H$, $^{13}C$ NMR data for **1**, CD, HSQC, HMBC, COSY, NOESY, preparation of **7a**,**7b**, degradation of **1**, and LCMS analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Five constraints on **1**—*anti*-orientation of H13 and H14 ($^3J_{HH}$ = 9.1 Hz), NOEs between H14−H17, $H_3$26−H14, the transannular NOE H2-H13, and $^3J_{H17\text{-}C15}$ = 2.5 Hz (HETLOC)—require the C14 methyl group be extended *exo* to the macrocycle and relays the configuration C5 to C14 and C17.
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(16) See Supporting Information for full characterization of all new compounds. The configurations of the major isomers are (3*R*,4*S*,5*R*)-**7a** and (3*S*,4*S*,5*R*)-**7b** were assigned from the modified Mosher's ester method and the known configuration (−)-**5** (ref. 13), and their enantiomers **7c** and **7d** were assigned by $^1H$ NMR and CD.
(17) Compounds **7a**−**d** eluted with retention times of $t_R$ = 13.84, 11.19, 9.05, and 9.90 min, respectively.
(18) Muironolide (**1**) showed weak cytotoxic activity against the HCT116 colon tumor cell line ($IC_{50}$ 96.5 μg/mL) and antifungal activity against *Cryptococcus neoformans* (MIC 16 μg/mL).
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