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# Novel Iodinated Diterpenes from a Marine Cyanobacterium and Red Alga Assemblage

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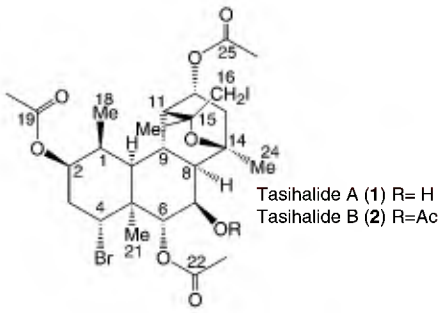
## ABSTRACT




**Tasihalides A and B have been isolated from an assemblage of a marine cyanobacterium, belonging to the genus *Symploca,* and an unidentified red alga. The gross structures and relative stereochemistries of these diterpenes were elucidated by spectroscopic means. In addition to possessing a novel cage structure, the tasihalides represent the only examples of iodinated diterpenes in nature.**

Halogenated natural products were once assumed to be either artifacts of the isolation procedure or freaks of nature.[1] Now it is known that organohalides are produced by a number of organisms for a variety of roles and often in significant quantities. For example, the 5 million tons of chloromethane emitted by terrestrial and marine biomass dwarfs the 26 000 tons produced annually by mankind.[2] The occurrence of these halogenated natural products though is dependent on the availability of the halide salts, so given the relatively high concentration of halides in seawater (0.5 M in $Cl^-$, 0.1 mM in $Br^-$, 1 $\mu$M in $I^-$, 73 $\mu$M in $F^-$),[2,3] marine natural products tend to incorporate these halogens more frequently than secondary metabolites from terrestrial sources. Somewhat surprisingly, though, the relative number of chlorinated, brominated, fluorinated, and iodinated metabolites parallels neither the concentration levels in the ocean nor the relative redox potentials of the halides. The incorporation of bromine or chlorine is by far the most common, with iodine rare and fluorine extremely rare.[3] For example, of the approximately 3800 halogenated natural products known, a number that excludes the iodine-containing thyroid hormones, less than 100 natural products contain iodine. Of these metabolites more than half are volatile compounds comprised of eight carbons or less, e.g., diiodomethane.[1,2] Examples of complex iodine-containing metabolites from a variety of terrestrial and marine sources include the depsipeptides geodiamolides[4] and doliculide (both iodotyrosines),[5] iodinated nucleosides,[6] and the structurally intriguing calicheamicin.[7]

Recently, we have been investigating the extracts of a

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(1) Gribble, G. W. *Acc. Chem. Res.* **1998**, *31*, 141–152.
(2) Gribble, G. W. *Prog. Chem. Org. Nat. Prod.* **1996**, *68*, 1–423.
(3) Butler, A.; Walker, J. V. *Chem. Rev.* **1993**, *93*, 1937–1944.
(4) Coleman, J. E.; Van Soest, R.; Andersen, R. J.; Kelsey, R. G. *J. Nat. Prod.* **1999**, *62*, 1137–1141.
(5) Hiroyuki, I.; Takayuki, N.; Makoto, O.; Kiyoyuki, Y. *J. Org. Chem.* **1994**, *59*, 4710–4711.
(6) Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Baird-Lambert, J. A.; Jamieson, D. D. *Aust. J. Chem.* **1983**, *36*, 165–170.

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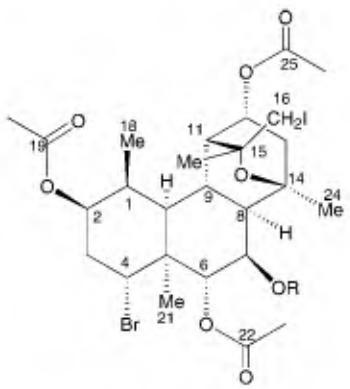
**Table 1.** NMR Spectral Data for **1** (500 MHz) Recorded in $CDCl_3$

| C/H no. | $\delta_H$[a] multiplicity (*J*, Hz) | $\delta_C$ [b,c] | COSY | HMBC[d,e] | NOESY |
|---|---|---|---|---|---|
| 1 | 2.40, qd (7.3, 3.6) | 33.1, d | 2, 18 | 3ax, 3eq, 10, 18 | |
| 2 | 5.04, br s | 73.9, d | 1, 3ax, 3eq | 3ax, 3eq, 18 | 1, 3ax, 3eq, 18 |
| 3ax | 2.53, ddd (−15.4, 11.5, 4.8) | 39.0, t | 2, 3eq, 4 | 4 | 21 |
| 3eq | 2.49, ddd (−15.4, 5.5, 1.7) | | 2, 3ax, 4 | | 4 |
| 4 | 5.34, dd (11.5, 5.5) | 54.5, d | 3ax, 3eq | 2, 3ax, 3eq, 6, 10, 21 | 3eq, 9 |
| 5 | | 45.3, s | | 3ax, 3eq, 4, 6, 9, 21 | |
| 6 | 5.50, d (3.1) | 76.6, d | 7 | 7, 7-OH, 8, 21 | 7-OH, 21 |
| 7 | 4.05, br s | 69.8, d | 6, 7-OH, 8 | 6, 7-OH | 7-OH, 8, 24 |
| 7-OH | 2.15, d (1.5) | | 7 | | |
| 8 | 1.71, d (11.1) | 47.2, d | 9, 13eq | 6, 7, 7-OH, 9, 10, 13eq, 13ax, 11, 24 | 7, 10, 13ax, 24 |
| 9 | 2.93, t (11.1) | 28.8, d | 8, 10 | 5, 7, 8, 10, 11, 12 | 4, 7-OH |
| 10 | 2.37, dd (11.1, 3.5) | 51.0, d | 9 | 1, 2, 6, 8, 9, 18, 21 | |
| 11 | 2.94, d (4.9) | 37.0, d | 12, 13eq | 9, 13eq, 16b, 17 | 12, 17, 18 |
| 12 | 5.34, t (4.9) | 68.8, d | 11, 13eq, 13ax | 9, 11, 13ax | 11, 13eq, 16a |
| 13eq | 2.36, dd (−15.6, 4.9) | 50.0, t | 8, 13b | 8, 11, 24 | 12, 13ax, 16a |
| 13ax | 1.47, d (−15.6) | | 13eq | | 12, 13eq, 24 |
| 14 | | 73.4, s | | 8, 12, 13eq, 13ax, 24 | |
| 15 | | 76.8, s | | 9, 11, 12, 16a, 16b, 17 | |
| 16a | 3.27, dd (−10.2, 1.1) | 15.3, t | 16b, 17 | 11, 17 | 12, 13eq, 16b |
| 16b | 3.10, d (−10.2) | | 16a | | 16a, 17 |
| 17 | 1.70, br s | 26.4, q | 16a | 11, 16a, 16b | 18 |
| 18 | 1.16, d (7.3) | 17.9, q | 1 | 1, 2 | 1, 2, 10, 11, 20 |
| 19 | | 171.1, s | | 2, 20 | |
| 20 | 2.21, s | 22.1, q | | | |
| 21 | 1.10, s | 19.2, q | | 4, 6, 10 | 1, 3ax, 6, 10 |
| 22 | | 169.5, s | | 6, 23 | |
| 23 | 2.08, s | 21.0, q | | | |
| 24 | 1.12, s | 21.2, q | | 13eq, 13ax | 7, 7-OH, 8, 13eq |
| 25 | | 169.9, s | | 12, 26 | |
| 26 | 2.13, s | 21.7, q | | | |

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Multiplicity deduced by HSQC. [d] Protons showing long-range correlation with indicated carbon. [e] Correlations were observed for $^nJ_{CH} = 7$ Hz.

collection of a marine cyanobacterium belonging to the genus *Symploca.* A recent reexamination of our voucher[8] though has revealed that the field-collected sample was not homogeneous and a small portion of this collection was a red alga. Unfortunately, too little material was present for a conclusive identification of the latter organism.

Already from this assemblage we have reported a series of peptides and depsipeptides and have named all of them with the prefix "tasi", meaning "ocean" in Chamorran.[9−11]




Tasihalide A (**1**) R= H
Tasihalide B (**2**) R=Ac

Further fractionation of the lipophilic and aqueous extracts has now led to the isolation and structure elucidation of the iodinated diterpenes, tasihalides A and B.

Tasihalide A (**1**) was isolated from the lipophilic extract of the assemblage designated NIH304. The residue from the repeated extraction of this assemblage with 4:1 $CH_3CN/CH_2Cl_2$ was separated by a combination of solvent partitioning, silica gel chromatography, and repeated reversed-phase HPLC. Final purification of **1** ($t_R$ 20 min) was achieved on an Ultracarb 30 ODS column (250 × 10 mm) with 70% aqueous $CH_3CN$ to afford 0.8 mg of an optically active oil [$[\alpha]^{23}_D$ −18 (*c* 0.6, MeOH)].[12]


(7) Lee, M. D.; Manning, J. K.; Williams, D. R.; Kuck, N. A.; Testa, G. O.; Borders, D. B. *J. Antibiot.* **1989**, *42*, 1070−1087.

(8) The sample was collected at Short Drop-off, Palau. It was identified by V. J. Paul, and a voucher is maintained in formalin at the Smithsonian Marine Station, Fort Pierce, FL.

(9) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J *J. Nat. Prod.* **2003**, *5*, 620−624.

(10) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *66*, 1006−1009.

(11) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 1336−1339.


(12) **Tasihalide A (1):** amorphous powder; $[\alpha]^{24}_D$ −18 (*c* 0.6, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 207 (4.3), 253 (1.2) nm; IR (film) $\nu_{max}$ 3501, 1735, 1372, 1236, 1026, 977 $cm^{-1}$; ESI *m/z* (relative intensity) $[M + Na]^+$ 707 and 709 (1:1); HR-ESI *m/z* $[M + NH_4]^+$ 702.1154 (calcd for $C_{26}H_{38}{}^{79}BrIO_8NH_4$ 702.1133, 2.1 mDa error).

Inspection of the richly detailed proton and carbon NMR spectra (Table 1), along with IR and UV/vis data, set the stage for the gross structure determination. The HSQC and the broadband-decoupled $^{13}C$ NMR spectra established that **1** possessed 6 quaternary, 10 methine, 3 methylene, and 7 methyl carbons, that is, a total of 37 hydrogens attached to 26 carbons, amounting to 349 mass units in the molecule. One more proton was inferred from the $^{1}H$ NMR and HSQC spectra which revealed a $^{1}H$ signal at $\delta_H$ 2.15 indicative of an exchangeable proton that was attributed to an alcohol proton on the basis of a 3501 $cm^{-1}$ band in the IR spectrum. This brought the total proton count to 38. The alcohol group also accounted for one of the six oxygenated $sp^3$-carbons ($\delta_C$ 76.8, 76.6, 73.4, 73.9, 69.8, and 68.8). Three more of these oxygenated carbons were ascribed to the acyloxy carbons of ester linkages based on the presence of signals for three acetate groups in the carbon and proton spectra (carbonyl carbon signals at $\delta_C$ 171.1, 169.9, 169.5, and methyl proton singlets at $\delta_H$ 2.21, 2.13, 2.08). Further analysis of the $^{1}H$ NMR spectrum revealed that one of the methylene proton signals comprised an AB system at $\delta_H$ 3.27 and 3.10, which showed a $^{1}J_{CH}$ of 150 Hz to a carbon that resonated at $\delta_C$ 15.3. This unusual combination of values was indicative of a heavy-atom effect and indicated that either bromine or iodine was attached to this carbon.[13] The presence of iodine was confirmed by the UV/vis spectrum, recorded in MeOH, which showed an n−$\sigma^*$ transition at 253 nm characteristic of this halide. Therefore, the preliminary data suggested that nine oxygens and one iodine were present in **1**.

Difficulties arose at first in confirming these conclusions through mass spectral analysis of **1**. FAB and MALDI-TOF mass spectrometry both failed to provide a clear-cut pseudomolecular ion peak under a variety of conditions. Eventually, $[M + Na]^+$ peaks at *m/z* 707 and 709 were obtained by ESIMS.[14] The isotope pattern of 1:1 for these two peaks indicated that bromine was present in **1**. The elemental composition of **1** was therefore $C_{26}H_{38}O_8BrI$ as confirmed by a high-resolution ESI measurement of a $[M + NH_4]^+$ peak at *m/z* 702.1154 (2.1 mDa error).

The structure of **1** was elucidated from the 2D NMR data (Table 1). Analysis of the COSY and HMBC data, recorded in $CDCl_3$ at 500 MHz, established three fragments (Figure 1). The proton and carbon chemical shifts of fragments A−C

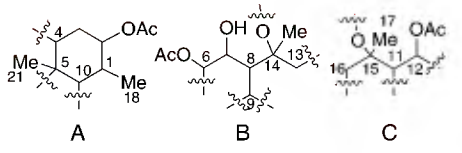



**Figure 1.** Partial structures from 2D NMR.

suggested that the halides were attached to C-4 and C-16. The iodine was connected to C-16 ($\delta_C$ 15.3) and the bromine to C-4 ($\delta_C$ 54.5) based on the carbon chemical shifts in accordance with literature values.[13] An HMBC correlation from H-21 to C-6 and a COSY cross-peak from H-9 to H-10 established the C-5/C-6 and C-9/C-10 junctions, respectively. Fragment C was linked to this unit by a COSY correlation from H-12 to H-13 and a HMBC cross-peak to C-10 from H-9. The two downfield carbons (C-14 and C-15) were connected via an ether linkage to account for the remaining oxygen atom and the final degree of unsaturation required by the molecular formula.

The relative stereochemistry of the ring system in **1** was established by analysis of proton−proton coupling constants and NOESY correlations (Figure 2). A large proton−proton

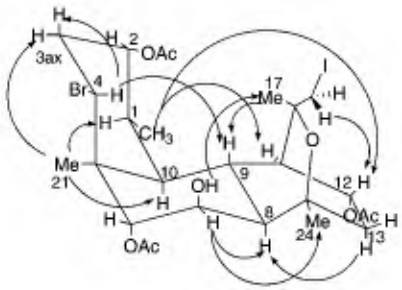


**Figure 2.** Key NOESY correlations observed in **1**.

coupling between H-$3_{ax}$ and H-4 ($^{3}J_{H\text{-}3ax/H\text{-}4}$ = 11.5) established the axial orientation of both these protons. Likewise a small proton−proton coupling between H-$3_{ax}$ and H-2 ($J_{H\text{-}2/H\text{-}3ax}$ = 4.5) indicated that this latter proton was in an equatorial position. Three other protons H-8, H-9, and H-10 were also assigned axial configurations based on large proton−proton couplings. NOESY correlations from H-4 to H-9 and from H-21 to H-1 indicated that the A/B ring junction was *cis*, while the axial position of H-8 and H-9, determined from coupling constants, indicated that the B/C ring junction was *trans*. The configuration of the B-ring with respect to the oxabicyclic systems was elucidated from NOESY cross-peaks from H-8 and H-16 to H-13.

A related compound, tasihalide B (**2**, 0.8 mg),[15] was isolated from the 30% aqueous EtOH extract of the assemblage. The proton NMR spectrum of **2** was nearly identical to **1** except for the downfield shift of H-7 and the presence of an extra methyl singlet at $\delta_H$ 2.17. HR-ESI established a molecular formula of $C_{28}H_{44}NO_9BrI$ based on a $[M + NH_4]^+$ of 744.1246 (0.7 mDa error) for the peracetylated analogue **2**.[16] NMR analysis (see the Supporting

(13) Silverstein, R. M.; Bassler, C. G.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*; Wiley & Sons: New York, 1981; p 269.

(14) The fragmentation of the 709-ion peak was followed in a series of MS/MS experiments, which provided ions at *m/z* 627, 499, 439, and 383 from loss of $H^{81}Br$, HI, and two successive McLafferty rearrangements that resulted in the elimination of two molecules of acetic acid (−60).

(15) **Tasihalide B (2):** amorphous powder; $[\alpha]^{23}_D$ −13 (*c* 0.4, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 202 (3.1), 253 (1.3) nm; IR (film) $\nu_{max}$ 1735, 1372, 1236, 1026, 977 $cm^{-1}$; ESI *m/z* (relative intensity) $[M + NH_4]^+$ 744 and 746 (1:1); HR-ESI *m/z* $[M + NH_4]^+$ 744.1246 (calcd for $C_{28}H_{40}$-$^{79}BrIO_9NH_4$ 744.1239, 0.7 mDa error).

Information) confirmed the gross structure and relative stereochemistry to be otherwise identical to **1**.

The tasihalides are exceptional in two respects. While brominated and chlorinated terpenoids have been reported before, there are no other examples of iodinated diterpenes that have been found in nature.[17,18] The structural core of the tasihalide is also unprecedented in a natural product. It is the combination of the oxabicyclic system in the C-ring, the *cis*-decalin system, and the methylation pattern that makes the carbon skeletons of **1** and **2** unique. Thus, the tasihalides represent the first examples of a new structural class of diterpenes. The closest structural relatives to **1** and **2** are tricyclic synthetic compounds that have been prepared from cembrane diterpenes treated with electrophiles.[19] This makes it tempting to speculate that **1** and **2** arise from a halogenation-initiated cyclization of an oxygenated cembrane diterpene. Such haloperoxidase-mediated electrophilic cyclizations have recently been demonstrated in vitro using bromoperoxidases cloned from red algae (Rhodophyta).[20,21] In the case of the oxabicyclic ring, nucleophilc attack on C-15 by the tertiary alcohol[22] attached to C-14 during the halogenation of an exocyclic methylene would generate the carbon skeleton depicted below.

In general, marine cyanobacteria are well-known sources of biologically active molecules. With few exceptions, most of these metabolites are formed by the union of polyketide and nonribosomal peptide biosynthetic machinery which results in a variety of modified amino acid structural motifs. In fact, this merger is so common that pure peptides[23] or acetogenins[24] are rarely isolated from marine cyanobacterial extracts. Likewise, reports of compounds from marine cyanobacteria derived from terpenoid biosynthesis are almost nonexistent in the literature.[25,26] By comparison, halogenated terpenoids are ubiquitous in red algae, especially the genus *Laurencia*.[18] On the basis of these biosynthetic considerations, it seems more likely that the trace amount of red algae in the sample is responsible for the tasihalides rather than the cyanobacterium.[27]

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**Supporting Information Available:** The isolation procedure, tabulated NMR data for **2**, and NMR spectra for **1** and **2** in $CDCl_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) It is unlikely that **2** is an artifact, given that readily apparent acylating agents, such as ethyl acetate or ammonium acetate, were not used during the isolation procedure.

(17) We thank G. W. Gribble for checking on this matter.

(18) Two iodinated sesqiterpenes have been reported from a red alga belonging to the genus *Laurencia*. Izak, R. R.; Sims, J. J. *J. Am. Chem. Soc.* **1979**, *101*, 6136−6137.

(19) Shpatov, A. V.; Shakirov, M. M.; Raldugin, V. A. *Russ. J. Org. Chem.* **2000**, *36*, 1127−1138.

(20) Carter-Franklin, J. N.; Parrish, J. D.; Tschirret-Guth, R. A.; Little, R. D.; Butler, A. *J. Am. Chem. Soc.* **2003**, *125*, 3688−3689.

(21) Ishihara, J.; Shimada, Y.; Kanoh, N.; Takasugi, Y.; Fukuzawa, A.; Murai, A. *Tetrahedron* **1997**, *53*, 8371−8382.

(22) Analysis of models suggests the tertiary alcohol on C-14 and the isopropenyl side chain (C-15, C-16, C-17) in this biosynthetic precursor would both be axial and in close proximity due to a twist-boat conformation in the C-ring. This conformation would be thermodynamically favorable since it would alleviate the steric interaction between C-18 (ring A) and the isopropenyl side chain (C-15, C-16, C-17).

(23) For examples of pure peptides from marine cyanobacteria, see: (a) Nogle, L. M.; Marquez, B. L.; Gerwick, W. H. *Org. Lett.* **2003**, *5*, 3−6. (b) Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 1336−1339.

(24) For an example of a pure ketide, see: MacMillan, J. B.; Molinski, T. F. *Org. Lett.* **2002**, *4*, 1535−1538.

(25) The indole alkaloid lyngbyatoxin A, from a Hawaiian strain of the marine cyanobacterium *Lyngbya majuscula*, is likely formed by a mixed terpene−peptide biosynthesis. Structure: Cardellina, J. H., III; Marner, F. J.; Moore, R. E.; *Science* **1979**, *204*, 193−195. Biosynthesis of a related analogue: Irie, K.; Kajiyama, S.-I.; Funaki, A.; Koshimizu, K. *Tetrahedron* **1990**, *46*, 2773−2788.

(26) Terrestrial cyanobacteria are known to produce terpenoids; see: (a) Jaki, B.; Orjala, J.; Heilmann, J.; Linden, A.; Vogler, B.; Sticher, O. *J. Nat. Prod.* **2000**, *63*, 339−343. (b) Prinsep, M.; Thomson, R. A.; West, M. L.; Wylie, B. L. *J. Nat. Prod.* **1996**, *59*, 786−788 and references therein.

(27) The average yield of the peptides isolated from this collection based on the dry extract was 0.16%, while the yields of **1** and **2** were both 0.04%.