# Ma'iliohydrin, a Cytotoxic Chamigrene Dibromohydrin from a Philippine *Laurencia* Species

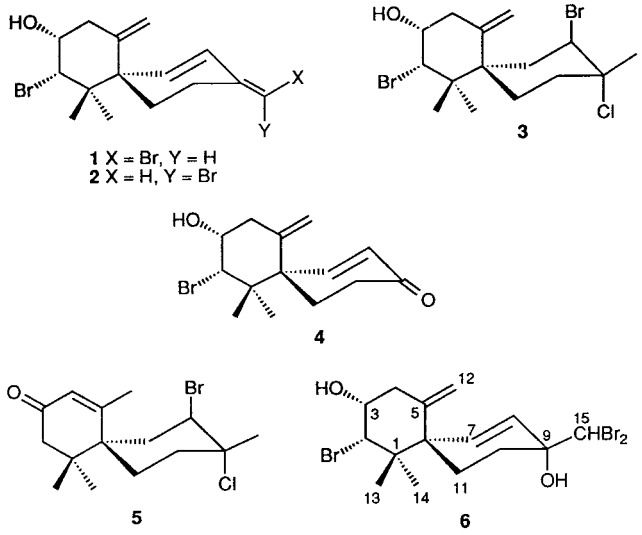
Ma. Elena Y. Francisco and Karen L. Erickson*

*Carlson School of Chemistry, Clark University, Worcester, Massachusetts 01610*

*Received October 27, 2000*

A new cytotoxic tribrominated chamigrene with a dibromohydrin functionality was isolated from an undetermined species of red alga, *Laurencia* sp. Its structure was determined by spectroscopic methods.

Red algae of the genus *Laurencia* are known to contain a wide variety of chamigrene sesquiterpenoid metabolites, the most common of which are halogenated.[1] We recently reported the isolation of vinyl bromides **1** and **2**, cartilagineol (**3**), ma'ilione (**4**), and enone **5** from *Laurencia* sp. collected off the coast of the island of Palawan in the Philippines.[2] Further investigation of the hexane and $CCl_4$ extracts of this species led to the isolation of a new cytotoxic tribrominated chamigrene, ma'iliohydrin. We report here its structure as **6**.




The algal organic extract was partitioned between hexanes and methanol−water (9:1), and then the methanol-soluble material was partitioned between $CCl_4$ and methanol−water (8:2). The hexanes and $CCl_4$ extracts were subjected to vacuum-liquid chromatography (VLC) on Si gel (20% EtOAc−$CH_2Cl_2$) to give **6** (1.3%) as a colorless oil.

Ma'iliohydrin's molecular formula was established as $C_{15}H_{21}O_2Br_3$ by high-resolution electron ionization mass spectroscopy (HREIMS), implying four degrees of unsaturation. The $^{13}C$ NMR and APT $^{13}C$ NMR spectra showed four quaternary carbons, five methines, four methylenes, and two methyls. A terminal double bond was identified from its $^{13}C$ NMR signals at $\delta$ 142.5 and 117.5 and its $^1H$ NMR signals at $\delta$ 4.84 and 5.09 (Table 1). A disubstituted double bond was also present ($^{13}C$ NMR $\delta$ 137.7 and 128.9, $^1H$ NMR $\delta$ 6.07 and 5.92). The five heteroatoms were located on a quaternary carbon ($\delta$ 72.2) and three methine carbons ($^{13}C$ NMR $\delta$ 71.9, 70.0, and 57.3; $^1H$ NMR $\delta$ 4.59, 4.14, and 5.57). The $^1H-^1H$ COSY spectrum established the proton sequence (Table 1).

**Table 1.** NMR Data for Compound **6**

| position | $^{13}C^a$ | $^1H^b$ (mult, $J$ = Hz) | $^1H-^1H$ COSY$^c$ |
|---|---|---|---|
| 1 | 42.7 | | |
| 2 | 70.0 | 4.59 (d, 2.9) | 3, 13, 14 (weak) |
| 3 | 71.9 | 4.14 (m) | 2, 4a, 4b |
| 4 | 37.9 | 2.56 (dd, 2.4, 15.1) | 3, 4b |
| | | 2.70 (dm, 15.0) | 3, 4a, 12a, 12b |
| 5 | 142.5 | | |
| 6 | 51.5 | | |
| 7 | 137.7 | 6.07 (d, 10.5) | 8, 11a |
| 8 | 128.9 | 5.92 (d, 10.6) | 7, 10a |
| 9 | 72.2 | | |
| 10 | 29.0 | 1.83 (m) | 8, 10b, 11a, 11b |
| | | 2.11 (m) | 10a, 11a, 11b |
| 11 | 22.6 | 1.90 (m) | 7, 10a, 10b, 11b |
| | | 2.04 (m) | 10a, 10b, 11a |
| 12 | 117.5 | 4.84 (br s) | 4b, 12b |
| | | 5.09 (br s) | 4b, 12a |
| 13 | 26.6 | 1.01 (s) | 2 (weak), 13 |
| 14 | 21.5 | 1.21 (s) | 2, 13 |
| 15 | 57.3 | 5.57 (s) | |
| 3-OH | | 2.23 (br s) | |
| 9-OH | | 2.60 (br s) | |

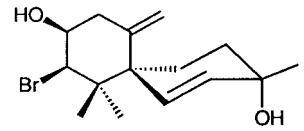
[a] Recorded at 50 MHz, $CDCl_3$ as internal standard ($\delta$ 77.0); multiplicity is based on the JMODXH spectra. [b] Recorded at 200 MHz. [c] With geminal protons, the smaller $\delta$-value is given the a designation, the larger $\delta$-value is given the b designation.

The NMR spectra of **6** closely resembled that of vinyl bromides **1** and **2**, ma'ilione (**4**),[3] and rigidol (**7**).[4] The data clearly showed that ring A of these metabolites was unchanged in **6** and the internal double bond of ring B was retained as well. Remaining to be placed in ring B were two bromines and one oxygen, the latter as an OH group. This was accomplished by EIMS. The base peaks in the spectrum occurred at *m/z* 299/301, fragments resulting from the loss of $CHBr_2$ from the parent ions *m/z* 474/472, [M + 4, M + 2]. Further, the methine carbon at $\delta$ 57.3 with its corresponding proton singlet at $\delta$ 5.57 can be ascribed to this dibromomethine. The second hydroxyl group must then be attached to the quaternary carbon (C-9) at $\delta$ 72.2, completing the gross structural assignment of ma'iliohydrin as **6**. The stereochemistry of the various chiral centers is assumed to be that of its co-metabolites **1**−**5**, assigned previously.[2]

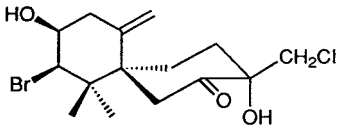
Only one other chamigrene halohydrin involving the C-15 methyl group has been reported. In 1978, working in the cold and under an anhydrous, inert atmosphere, **8** was isolated as an unstable component of *L. obtusa*.[5] Ma'iliohydrin (**6**), on the other hand, was obtained without taking any special precautions, and it could be stored in the cold without undergoing any serious decomposition.

* To whom correspondence should be addressed. Tel: 508-793-8866. Fax: 508-793-8861. E-mail: kerickson@clarku.edu.

10.1021/np0005053 CCC: $20.00 © 2001 American Chemical Society and American Society of Pharmacognosy
Published on Web 05/10/2001




7




8

The co-occurrence of **1**−**6** in the same organism suggests a biogenetic link among them. Addition of BrOH to **1** or **2**, which are found in a number of *Laurencia* species,[6−9] would generate **6**. Subsequent loss of $CH_2Br_2$ would give rise to the norchamigrene **4**.

Vinyl bromides **1** and **2**, cartilagineol (**3**), and ma'ilione (**4**) have all been reported as cytotoxic.[6,15] Ma'iliohydrin (**6**) also displayed cytotoxicity in the NCI 60-cell line human tumor screen[16] at a mean panel $GI_{50}$ concentration of $10^{-5}$ M. The greatest activity was seen in the NCI/ADR-RES breast cancer cell line, which displayed a $GI_{50}$ value of $10^{-8}$ M.

## Experimental Section

**General Experimental Procedures.** IR spectra were recorded on a Perkin-Elmer FT-IR spectrophotometer (PARAGON 500). The optical rotations were determined on a Rudolph Autopol II spectropolarimeter. $^1$H and $^{13}$C NMR spectra were recorded on Bruker AC 200 (200 MHz for $^1$H, 50 MHz for $^{13}$C) and Varian Unity 300 (300 MHz for $^1$H) spectrometers. $^1$H and $^{13}$C NMR spectra were determined in $CDCl_3$ as the solvent and reference (7.24 and 77.0 ppm, respectively). The HREIMS data were collected on a JEOL SX102 mass spectrometer operated at an accelerating voltage of 10 kV. Sephadex LH-20 (25−100 $\mu$, Sigma) was used for gel filtration, and Si gel type 60H (7736 EM Sciences) was used for vacuum liquid chromatography (VLC). Analytical and preparative TLC (PTLC) were carried out on precoated 60F254 plates (20 × 20 cm, 250 $\mu$, Sigma).

**Plant Material.** The red alga *Laurencia sp.* was collected at Taytay City, Palawan, Philippines, on February 29, 1992, by Dr. Hilconida Calumpong (Silliman University). Voucher samples were deposited at the Smithsonian by Emani Menez (0ALQ0348) and at the Marine Laboratory, Silliman University. Because they were sterile, the alga could not be speciated.

**Extraction and Isolation.** The frozen algal mass was ground with dry ice, extracted with $H_2O$ at 3 °C for 4 h, filtered, and freeze-dried. The dried marc was extracted with MeOH−$CH_2Cl_2$ (1:1), then MeOH, at 25 °C for 16 h. The filtered extracts were then combined and concentrated in vacuo to give a greenish-black sludge. A sample (1.29 g) of the crude extract was dissolved in MeOH−$H_2O$ (9:1) and extracted with hexanes (3 × 200 mL). After separation of the layers, the hexane was evaporated to give 803 mg of a black-brown oil. The MeOH layer was diluted with water to give a ratio of 8:2 and extracted with $CCl_4$ (3 × 200 mL). The $CCl_4$ layer was dried and concentrated, yielding 165 mg of a dark brown oil. Each extract was permeated through Sephadex LH-20 with hexanes−$CH_2Cl_2$−MeOH (2:5:1) to give eight fractions. The fourth and fifth fractions, after repeated PTLC with hexanes−$^i$PrOH (45:1), afforded a total of 15 mg of **6**.

**Compound 6:** colorless oil; $[\alpha]_D$ −9.6° (*c* 0.26, $CHCl_3$); IR (film) 3447, 3076, 2925, 1457, 1371, 1075, 1032, 750, 698 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; EIMS *m/z* 474 [M + 4], 472 [M + 2], 470 [M$^+$], 302 (16), 301/299 (98, 100), 202 (21), 201 (15), 121 (12), 107 (22), 91 (41, $C_6H_9^+$), 77 (30), 55 (67, $C_4H_7^+$); HREIMS *m/z* 471.9095 (calcd for $C_{15}H_{21}O_2{}^{79}Br_2{}^{81}Br$, 471.9072), *m/z* 473.9064 (calcd for $C_{15}H_{21}O_2{}^{79}Br^{81}Br_2$, 473.9052).

**Cytotoxicity Studies.** The cytotoxicity profile of ma'iliohydrin (NSC 719212-L) was determined in the NCI 60-cell line human tumor screen, detailed descriptions of which have been published.[16]

**Acknowledgment.** We thank K. Snader and D. Newman, NPB/DPT, National Cancer Institute, for coordinating the collection; H. Calumpong, Silliman University, for the collection, T. McCloud, NPB/DPT, National Cancer Institute, for initial extractions, and L. Pannell, LBC/NIDDK, National Institutes of Health, for the mass spectra.

## References and Notes

(1) (a) Gribble, G. W. Naturally Occurring Organohalogen Compounds. A Comprehensive Survey. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Gamm, Ch., Eds.; Springer: New York, Vol. 68, pp 50−57. (b) Erickson, K. L. Constituents of *Laurencia*. In *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1983; Vol. 5, pp 164−171, 229, 242−242.

(2) Francisco, M. E. Y.; Turnbull, M. M.; Erickson, K. L. *Tetrahedron Lett.* **1998**, *39*, 5289−5292.

(3) There is an error in the numbering system given in structure **3** of ref 2. C-13 should be *trans* to the bromine and C-14 *cis* to the bromine.

(4) Konig, G. M.; Wright, A. D. *J. Nat. Prod.* **1997**, *60*, 967−970. In this reference the $\delta$ values for the *gem*-dimethyl protons should be interchanged (Wright, A. D. Personal communication).

(5) Gonzalez, A. G.; Martin, J. D.; Martin, V. S.; Norte, M. *Tetrahedron Lett.* **1978**, 2035−2036.

(6) Rashid, M. A.; Gustafson, K. R.; Cardellina, J. H.; Boyd, M. R. *Nat. Prod. Lett.* **1995**, *6*, 255−259.

(7) Coll, J. C.; Wright, A. D. *Aust. J. Chem.* **1989**, *42*, 1591−1603.

(8) Schmitz, F. J.; Michaud, D. P.; Schmidt, P. G. *J. Am. Chem. Soc.* **1982**, *104*, 6415−6423.

(9) (a) Suzuki, M.; Kurosawa, E. *Tetrahedron Lett.* **1978**, 4805−4808. (b) Suzuki, M.; Furusaki, A.; Hashiba, N.; Kurosawa, E. *Tetrahedron Lett.* **1979**, 879−882.

(10) Suzuki, M.; Kurosawa, E.; Kurata, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3795−3796.

(11) Suzuki, M.; Kurosawa, E. *Tetrahedron Lett.* **1978**, 4805−4808.

(12) Gonzalez, A. G.; Martin, J. D.; Martin, V. S.; Norte, M. *Tetrahedron Lett.* **1979**, 2719−2722.

(13) Brennan, M. R.; Erickson, K. L.; Minott, D. A.; Pascoe, K. O. *Phytochemistry* **1987**, *26*, 1053−1057.

(14) Guella, G.; Mancini, I.; Chiasera, G.; Pietra, F. *Helv. Chim. Acta* **1990**, *73*, 1612−1620.

(15) Juagdan, E. G.; Kalidindi, R.; Scheuer, P. *Tetrahedron* **1997**, *53*, 521−528.

(16) (a) Boyd, M. R. *Princ. Pract. Oncol. Updates* **1989**, *3*, 1−12. (b) Boyd, M. R. In *Cancer Drug Discovery and Development; Vol. 2, Drug Developments: Preclinical Screening, Clinical Trial and Approval*; Teicher, B. A., Ed.; Humana: Totowa, NJ, 1997; pp 23−42.

NP0005053