A New Isoquinoline Alkaloid from the Marine Sponge *Haliclona* Species

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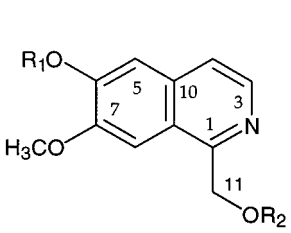
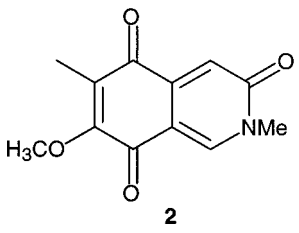
Two isoquinoline alkaloids, including the new compound **1**, were isolated from the cytotoxic fractions of an aqueous extract of the marine sponge *Haliclona* sp. The structures of these compounds were established as 1-hydroxymethyl-7-methoxyisoquinolin-6-ol (**1**) and mimosamycin (**2**) by conventional spectroscopic methods and by comparison with related compounds. Mimosamycin (**2**) was the principal cytotoxin with an IC_{50} of approximately 10 μg/mL against melanoma and ovarian human tumor cell lines.

Marine sponges belonging to the genus *Haliclona* have been the subject of extensive chemical studies. Recent investigations of *Haliclona* species have led to the isolation of alkaloids, macrolides, polyacetylenes, polyketides, steroids, and peptides.[2,3] Our analysis of the aqueous extract of a *Haliclona* sp. (order Haplosclerida, family Haliclonidae), collected from the Philippines, was initiated based on its cytotoxic activity in the U.S. National Cancer Institute (NCI)'s 60-cell antitumor screen.[4,5] Bioassay-guided fractionation of the extract provided two isoquinoline alkaloids, one of which was new.

The cytotoxic *Haliclona* extract was sequentially fractionated by wide-pore C_4 vacuum-liquid chromatography and gel permeation on Sephadex LH-20. All of the resulting chromatographic fractions were screened for cytotoxic activity. Final C_{18} HPLC purification of the active fractions provided a new isoquinoline alkaloid, which we established to be 1-hydroxymethyl-7-methoxyisoquinolin-6-ol (**1**), and the known compound mimosamycin (**2**), which was identified by comparison of its physical and spectral data with previously reported values.[6,7]



1 R_1 = H, R_2 = H
3 R_1 = Me, R_2 = H
4 R_1 = Ac, R_2 = Ac



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The molecular formula of compound **1** was established to be $C_{11}H_{11}NO_3$ by HRFABMS measurements. The ^{13}C NMR spectrum of **1** displayed 11 resonances, and DEPT experiments confirmed the presence of one methyl, one methylene, four methines, and five quaternary carbons. A broad absorption centered at 3400 cm^{-1} in the IR spectrum suggested the presence of hydroxyl functionalities in **1**, and this was supported by two deuterium-exchangeable ^1H NMR signals observed at δ 11.68 and 6.86 in DMSO-d_6. Acetylation of **1** afforded the diacetate derivative **4**, which confirmed the presence of two hydroxyl groups. Both the ^1H and ^{13}C NMR spectral data of **1** (Table 1) showed close correspondence with the NMR data reported for the synthetic compound 1-hydroxymethyl-6,7-dimethoxyisoquinoline (**3**).[8] However, the NMR data for **1** revealed the presence of only one methoxyl group (δ_H 4.00, δ_C 56.2), which suggested that compound **1** was an *O*-demethyl analogue of **3**. HSQC and HMBC experiments confirmed that compound **1** consisted of an isoquinoline ring system with a hydroxymethyl group at C-1 and two oxygenated substituents at C-6 and C-7. It was not possible to define the relative positions of the unassigned hydroxyl and methoxyl groups from the HMBC data, so selective 1D NOESY experiments were run. NOE interactions were observed between the methoxyl protons and H-8 (δ 7.58) and also between the hydroxymethyl protons (δ 5.34) and H-8. Thus, the methoxyl group was substituted at C-7, and the structure of the new alkaloid was assigned to be 1-hydroxymethyl-7-methoxyisoquinolin-6-ol (**1**). Although simple isoquinoline alkaloids have not previously been described from sponges in the genus *Haliclona*, oxidized and dimerized isoquinolinequinone derivatives have been reported.[9,10]

In a 2-day in vitro cytotoxicity assay,[11] compound **1** was inactive at a concentration of 50 μg/mL, while mimosamycin (**2**) exhibited an IC_{50} of approximately 10 μg/mL against the LOX (melanoma) and OVCAR-3 (ovarian) human tumor cell lines.

Experimental Section

General Experimental Procedures. General experimental procedures have been described previously.[12]

Animal Material. Samples of the sponge *Haliclona* sp. were collected from a depth of −10 m at Jessie Beazley Reef, Sulu Sea, Philippines, in 1995 by the Coral Reef Research Foundation. A voucher specimen (0CDN3179) for this collection is maintained at the Smithsonian Institution, Washington, D.C.

Extraction and Isolation. The frozen sponge samples (1275 g) were ground into a coarse powder and extracted with H_2O. Solid materials were removed by centrifugation, and the resulting aqueous solution was freeze-dried to provide 57.3 g of aqueous extract. A 10.0 g aliquot of the crude extract was subjected to C_4 vacuum-liquid chromatography eluted with increasing amounts of MeOH in H_2O. The cytotoxic fraction (134 mg) was separated on a column of Sephadex LH-20 eluted with MeOH−H_2O (9:1) to afford two active fractions. Purification of this material (15 mg total) by C_{18} HPLC using a linear

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Table 1. NMR Data for 1-Hydroxymethyl-7-methoxyisoquinolin-6-ol (**1**)[a]

position	^{13}C mult[b,c]	^{1}H mult *J* (Hz)[c]	HMBC	^{13}C mult[b,d]	^{1}H mult *J* (Hz)[d]
1	154.6 s			156.2 s	
3	129.3 d	8.19 d (6)	C-1, C-4, C-5	129.2 d	8.15 d (6)
4	120.9 d	8.01 d (6)	C-3, C-5, C-9	122.8 d	7.99 d (6)
5	109.2 d	7.47 s	C-4, C-6, C-7, C-9	110.8 d	7.45 s
6	156.0 s			158.2 s	
6-OH		11.68 bs			
7	151.8 s			153.7 s	
7-OMe	56.2 q	4.00 s	C-7	57.2 s	4.11 s
8	104.7 d	7.58 s	C-1, C-6, C-7, C-10	104.7 d	7.54 s
9	119.0 s			120.7 s	
10	135.8 s			138.1 s	
11	58.6 t	5.34 s	C-1, C-9	59.6 t	5.45 s
11-OH		6.86 bs			

[a] ^{1}H and ^{13}C spectra acquired at 500 and 125 MHz, respectively. [b] Multiplicity inferred from the DEPT pulse sequence. [c] DMSO-d_6. [d] CD_3OD.

gradient from 20 to 100% MeCN in H_2O (0.1% TFA) over 30 min yielded, in order of elution, compounds **1** (3.1 mg) and **2** (2.0 mg).

1-Hydroxymethyl-7-methoxyisoquinolin-6-ol (1): white gum; UV (MeOH) λ_{max} (log ϵ) 239 (4.35), 253 sh (3.87), 258 (3.73), 279 (3.56), 325 (3.32), 363 (3.31) nm; IR (film) ν_{max} 3400, 1682, 1505, 1435, 1298, 1174, 844, 721 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 206.0820 [M + H]$^{+}$ (calcd for $C_{11}H_{12}NO_3$ 206.0817).

Acetylation of 1. A 1.0 mg aliquot of **1** was dissolved in 0.5 mL of pyridine, and 1.0 mL of acetic anhydride was added to it. The reaction mixture was left in the dark for 48 h at room temperature. Evaporation of the mixture under a stream of N_2 afforded virtually pure diacetate derivative **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (1H, d, J = 6 Hz), 8.03 (1H, d, J = 6 Hz), 7.79 (1H, s), 7.62 (1H, s), 6.01 (2H, s), 4.05 (3H, s), 2.41 (3H, s), 2.30 (3H, s); FABMS [M + H]$^{+}$ *m/z* 290 (appropriate for $C_{15}H_{16}NO_5$).

Cytotoxicity Evaluations. DMSO solutions of chromatography fractions and aliquots of the purified compounds were assayed for cytotoxic properties in a 2-day in vitro assay, experimental details of which have been reported previously.[11]

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