6-Bromo-5-hydroxy-3-indolecarboxyaldehyde from the Caribbean sponge Oceanapia bartschi

Francesco Cafieri, Ernesto Fattorusso*, Yosef Mahajnah, and Alfonso Mangoni

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli “Federico II”.
Via D. Montesano 49, I-80131 Napoli, Italy

Z. Naturforsch. 48b, 1408–1410 (1993); received March 29, 1993
Oceanapia bartschi, Oceanapid, Sponges, Indole Derivatives, Bromoindoles

The organic extract from Oceanapia bartschi has been shown to contain the antibiotic di­
terpenes Ambiol A (1) and three indole derivatives, 3-bromoindole (2), 6-bromo-3-indole­
carboxyaldehyde (3), and 6-bromo-5-hydroxy-3-indolecarboxyaldehyde (4). The structure of
the novel compound 4 has been established from its spectroscopic (H and 13C NMR, UV, IR, and MS) data.

Introduction

Among the marine sponges of the family Oceanapid, only two species belonging to the genus Calyx (C. nicaensis and C. podatypa) have been chemically investigated, and they have been shown to elaborate a number of very interesting sterols [1–5]. Much less interest has been devoted to sponges of the genus Oceanapia, which, to our knowledge, have not been until now chemically in­vestigated. As a part of our continuing work on bio­active marine products, we have now studied an Oceanapia species (Oceanapia bartschi), which was collected along the coast of Little San Salvador Island (Bahamas) during Summer 1990. From this organism we have isolated the furanoditerpene 1, an antibiotic compound previously found in a Dysidea sponge [6]. The methanol extract of the sponge also contained three simple indole derivatives, 2–4.

Compounds 2 and 3 have already been isolated from Ptychodera flavalaysanica [7] and from an uniden­tified marine pseudomonad [8], respectively, while the bromohydroxyindolaldehyde 4 appears to be a novel compound. This paper describes the isolation of 1–3 and the structure determination of the novel compound 4.

Results and Discussion

Specimens of O. bartschi were cut in small pieces and extracted with methanol. The chloroform sol­ubles of the concentrated alcoholic extract were partitioned by Medium Pressure Liquid Chroma­tography (MPLC) on silica gel. Fractions eluted with petroleum ether-chloroform (1:1) contained 1–3. Final purification of the individual compounds was achieved by reversed phase HPLC (compounds 1 and 3) and preparative TLC (compound 2).

Comparison of spectral data of 1 with those re­ported in the literature, showed it to be a product previously isolated in large amounts from Dysidea ambilia, a marine sponge belonging to the order Dictyoceratida [6]. Compounds 2 and 3 were identified as 3-bromoindole and 6-bromo-3-indolecarboxy­
aldehyde, respectively, by comparison of their physical properties with those described in the literature [7–8].

Compound 4 was isolated from the n-butanol soluble fraction of the methanol extract of the sponge by chromatography on Sephadex LH20, followed by reversed phase HPLC. High resolution mass spectrometry showed a molecular formula C9H6BrN2O. The IR spectrum contained hydroxyl (3365 cm⁻¹) and carbonyl (1636 cm⁻¹) absorption bands. The aldehydic nature of the latter function was suggested by the 1H singlet at δ = 9.83 in the 1H NMR and by the resonance at δ = 187.3 in the 13C NMR spectrum. Additional signals were ob­served at δ = 8.15 (1H, s, H-2), δ = 8.01 (1H, s, H-7), and δ = 7.58 (1H, s, H-4) in the 1H NMR spect­rum, and at δ = 159.2 (C-5), δ = 139.8 (C-2), δ = 129.4, and δ = 127.7 (C-3a and C-7a), δ = 118.6 (C-3), δ = 118.2 (C-7), δ = 114.1 (C-6), and δ = 108.9 (C-4) in the 13C NMR spectrum.

As a result of the inspection of molecular formu­la and NMR spectra, the OH and CHO functions, together with a bromine atom, were concluded to be linked to an indole nucleus. This was supported from the UV absorption band at 255 nm.

The positioning of the substituents was accom­plished by NOE and 1H 13C decoupling experiments,
which allowed the measurement of diagnostic long-range \(^1\)H \(^{13}\)C coupling constants. The formyl group must be linked at C-3, as indicated by the large NOE enhancement of the sharp singlet resonating at \(\delta = 8.15\) (H-2) by irradiation at \(\delta = 9.83\) (aldehydic proton), together with a less intense enhancement of the broad singlet resonating at \(\delta = 7.58\) (H-4). The latter signal was shown to be long-range coupled with the broad singlet at \(\delta = 8.01\) (H-7) by spin-spin decoupling experiments. The small coupling constant (\(J \approx 1\) Hz) observed between the two protons was indicative of their para relationship. As a consequence, the bromine atom and the hydroxyl group must be linked at C-6 and C-5, respectively, or vice versa. The latter possibility was excluded on the basis of following argument. The \(^{13}\)C NMR spectra of 4 contained signals for five unprotonated carbon atoms (\(\delta = 159.2, \delta = 114.1, \delta = 118.6, \delta = 127.7,\) and \(\delta = 129.4\)). The singlet at \(\delta = 118.6\) was assigned to C-3, since it displayed a very large coupling constant (23 Hz) in the fully coupled \(^{13}\)C NMR spectrum, which could only be accounted for by a two-bond coupling with the formyl proton (\(\delta = 9.83\)). Of the remaining four carbon singlets, the one resonating at \(\delta = 114.1\) was assigned to the carbon linked to the bromine atom on the basis of its chemical shift, as well as the one resonating at \(\delta = 159.2\) was assigned to the carbon atom attached to the OH group.

In a \(^{13}\)C NMR spectrum recorded on selective irradiation of H-4 the quaternary carbon atom at \(\delta = 159.2\) resonated as a doublet (\(J = 7.5\) Hz), the proton responsible for the splitting being evidently H-7. Since in a six-membered aromatic system such a large constant can only be due to a three-bond carbon-proton coupling [9], this carbon atom, and therefore the hydroxyl group, must be located at position 5 of the indole nucleus, while the bromine atom is at position 6. Compound 4 was therefore established as 6-bromo-5-hydroxy-3-indolecarboxaldehyde.

**Experimental**

**General**

Electron impact high resolution mass spectra were obtained at 70 eV on a Kratos MS 50 mass spectrometer. Fourier transform IR spectra were recorded on a Bruker IFS-48 spectrophotometer. \(^1\)H and \(^{13}\)C NMR experiments (solvents CDCl\(_3\) and CD\(_3\)OD) were performed with a Bruker AMX-500 spectrometer. Proton chemical shifts are relative to the residual chloroform and methanol proton singlets (\(\delta = 7.26\) and 3.34, respectively). \(^{13}\)C NMR spectra were referenced to the center peak of CDCl\(_3\) at 77.0 ppm.

**Extraction and isolation**

*Oceanapia bartschi* was collected by SCUBA in the lagoon of Little San Salvador Island (Bahamas), during Summer 1990. A reference specimen is on file at our laboratories. Freshly collected specimens (250 g, dry weight after extraction) were frozen when still alive and dispatched to our laboratory. The animals were extracted exhaustively with MeOH, and the extract partitioned between water and CHCl\(_3\). The organic layer was evaporated to dryness, affording 1.2 g of a crude mixture, that was chromatographed on a silica gel column.

**Ambliol-A (1)**

Fractions 5–8 eluted with n-hexane/CHCl\(_3\) (1:1), after removal of the solvent in vacuo afforded a product that was chromatographed by reversed-phase HPLC on an RP-18 column using CH\(_3\)OH/H\(_2\)O (95:5) as eluent, to give pure compound 1 (8.4 mg), whose \(^1\)H NMR and mass spectra were identical to those of Ambliol-A isolated by Walker and Faulkner [6].

**3-Bromoindole (2)**

Fractions 12–13 eluted with CHCl\(_3\) and rechromatographed by TLC, using as eluent n-hexane/Et\(_2\)O (8:2) afforded compound 2 (5.2 mg), whose chemical and spectral properties were identical to those reported in the literature [7].

**6-Bromo-3-indolecarboxylic acid (3)**

Fractions 20–21 eluted with CHCl\(_3\)/CH\(_3\)OH (9:1) were purified by HPLC on an RP-18 column using CH\(_3\)OH as eluent to give 3 (5.6 mg), whose
chemical and spectral properties were identical to those reported in the literature [8].

6-Bromo-5-hydroxy-3-indolecarboxyaldehyde (4)

The aqueous layer from the partitioning of the methanol extract was extracted with n-butanol four times. After removal of the solvent in vacuo, the residue was fractionated on a Sephadex LH-20 column using methanol as eluent. Fractions 12–13 were collected (19.6 mg) and rechromatographed by HPLC on an RP-18 column using CH$_3$OH–H$_2$O (7:3) as eluent, to give pure 4 (4.1 mg). HRMS: $m/z = 240.9568$ (M$^+$, C$_9$H$_6$81BrN$_2$O$_2$, calcd. 240.9560) and 238.9595 (M$^+$, C$_9$H$_6$79BrN$_2$O$_2$, calcd. 238.9581); IR $\nu_{\text{max}} = 3365$ and 1636 cm$^{-1}$; UV $\lambda_{\text{max}} = 255$ nm ($\varepsilon = 5800$); $^1$H and $^{13}$C NMR data are reported in Table I.

This work is a result of a research supported by M.U.R.S.T, Rome, Italy (40% and 60%). We wish to thank Prof. W. Fenical for giving us the opportunity to participate in an expedition to the Caribbean Sea, during which the sponge O. bartschi was collected, and Dr. M. Pansini (University of Genova, Italy) for identifying the sponge. Mass spectra were provided by “Servizio di spettrometria di massa del CNR e dell’Università di Napoli”. The assistance of the staff is gratefully acknowledged.

<table>
<thead>
<tr>
<th>Pos.</th>
<th>$\delta_{1H}$ (mult.)</th>
<th>$\delta_{13C}$ (mult.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8.15 (s)</td>
<td>139.8 (CH)</td>
</tr>
<tr>
<td>3</td>
<td>118.6 (C)</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>127.7 (C)*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.58 (s)</td>
<td>108.9 (CH)</td>
</tr>
<tr>
<td>5</td>
<td>159.2 (C)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>114.1 (C)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8.01 (s)</td>
<td>118.2 (CH)</td>
</tr>
<tr>
<td>7a</td>
<td>129.4 (C)*</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>9.83 (s)</td>
<td>187.3 (CH)</td>
</tr>
</tbody>
</table>

* These resonances may be reversed.

References: