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6-Bromo-5-hydroxyindolyI-3-g!yoxylate from the Far Eastern Ascidian *Syncarpa oviformis*

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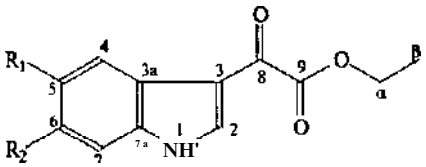
The ethanol extract of the marine ascidian *Syncarpa oviformis* (Kuril Islands, Russia) has been shown to contain three indole derivatives, ethyl indolyl-3-glyoxyIate (1), ethyl 6-bromoindolyl-3-glyoxylate (2), and ethyl 6-bromo-5-hydroxyindolyl-3-glyoxylate (3), along with /?-hydroxyphenylglyoxylate, 2,6-dimethylheptyl sulfate and (3Z)-3-decenyl sulfate. The structure of the novel compound 3 has been elucidated on the basis of NMR, MS, IR and UV analyses.

Keywords: ascidian, *Syncarpa oviformis,* indole alkaloids, 6-bromo-5-hydroxyindolyl-3-glyoxylate, sulfated alkanes/alkenes.

As part of our continuing study on new natural products from marine invertebrates, we investigated the ethanol extract of the Far Eastern ascidian *Syncarpa oviformis* collected near the Kuril Islands (Russia). Ascidians of the genus *Syncarpa* have not been chemically studied so far.

A new indole alkaloid, 6-bromo-5-hydroxyindolyl-3-glyoxylate (3), and the known indolyl-3-glyoxylate (1) [1], ethyl 6-bromoindolyl-3-glyoxylate (2) [2,3], p-hydroxyphenylglyoxylate, 2,6-dimethylheptyl sulfate [4,5] and (3Z)-3-decenyl sulfate [6,7] were isolated from the я-butanol-soluble materials of the extract using Sephadex LH-20 and RP HPLC chromatography. The ^{1}H and ^{13}C NMR spectra of 3 (Table 1) revealed signals of an ethyl glyoxylate moiety at 5_H 1.40 (3H, t, J = 7.2 Hz) and 4.39 (2H, q, J = 7.2 Hz), and 5_C 15.0 (CH_3), 63.7 (CH_2), 165.2 (C=0) and 180.7 (C=0). This was supported by two IR carbonyl bands at 1728 and 1651 cm'[1], and by EIMS peaks at *m/z* 240/238 (100%) [M-$COOCH_2CH_3$][4] and *m/z* 212/210 [M-side chain $COCOOCH_2CH_3$]$^+$. Also, the IR spectrum contained an absorption band of NH at 3455 cm"[1]. Accordingly,



1 R,=H, R_2=H
2 R,=H, R_2=Br
3 R,=OH, R_2=Br

Table I: 'H and ^{13}CNMR spectroscopic data for compound 3 inCDjOD.

	6_H(-/,Hz)	5_C	HMBC('Hto^{1j}C)
2	8.34 s	140.4 CH	3, 3a, 7a, 8
3		114.7 C	
3a		128.6 C	
4	7.82 s	108.8 CH	3, 5, 6, 7a
5		152.5 C	
6		109.4 C	
7	7.62 s	117.9 CH	3a, 5,6, 7a
7a		133.5 C	
8		180.7 C	
9		165.2 C	
a	4.39 q (7.2)	63.7 CH_2	**9,P**
0	1.40 t (7.2)	15.0CH_3	a

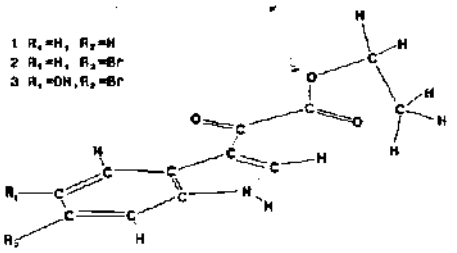


Figure 1: Quantum-chemically calculated conformation for compounds 1-3.

the NMR analysis suggested that **3** was a trisubstituted indole. Its ^{1}H NMR spectrum showed only three singlets of aromatic protons at δ_H 7.62, 7.82 and 8.34. The ^{13}C NMR spectrum of **3**, when compared with that of **2**, showed that **2** and **3** had an identical number of carbons, but **3** contained a tetrasubstituted carbon, with δ_C 152.5, instead of a trisubstituted one. The downfield shift of this signal, as well as the absorption band in the IR spectrum at 3531 cm^{-1}, supported the presence of a hydroxyl function in the aromatic ring. As a result of the inspection of these data, characteristic bromine isotopic patterns in the EI mass spectrum and the molecular formula $C_{12}H_{10}BrNO_4$ (HR EIMS), the OH and Br functions were concluded to be linked to a benzene ring in the indole nucleus. The positions of the substituents were established by HMBC experiments (Table 1) that confirmed the structure of ethyl 6-bromo-5-hydroxyindolyl-3-glyoxylate (**3**). The ^{13}C NMR spectrum for the indole part of **3** was similar to that of 6-bromo-5-hydroxy-3-indolecarboxyaldehyde isolated from the Caribbean sponge *Oceanapia bartschi* [8].

Quantum-chemical study of the geometrical and electronic structures of the glyoxylates **1-3** suggested that global minimums on the potential energy surfaces (PES) for these compounds corresponded to the conformation shown in Figure 1. The geometry implied the proximity of H-2 and the carbonyl at position 9 with, presumably, an intramolecular hydrogen bond, which explained the significant downfield shift of the proton signal in the NMR spectrum.

The compounds **1-3** had ethyl glyoxylate residues, and ethanol was used at almost every step of the isolation. We observed that these compounds lacked EtO-, but attached CD_3O- during the recording of ^{1}H and ^{13}C NMR spectra at room temperature (decreased intensities of signals of EtO- in **1-3** and increased intensities of signals of free EtOH). This was confirmed by EIMS , which showed the glyoxylates with attached deuterated methoxyl. Thus, the ethoxy group in the glyoxylates may be of artificial derivation. It should be noted that homofascaplysin B (bis-indole with an indolyl-3-glyoxylate fragment) from the sponge *Fascaplysinopsis reticulata* has a methyl glyoxylate moiety (MeOH was used for isolation) [9], 3-bromohomofascaplysin B from the ascidian *Didemnum* sp. also contains a methyl glyoxylate moiety, and homofascaplysin B-1 and 3-bromohomofascaplysin B-1 have ethyl glyoxylate (EtOH and MeOH were used for isolation) [10].

Indoles [11-13 and references cited therein] appear to be common metabolites of marine ascidians. However, according to the above-mentioned studies, sponges were recognized in the first place as a rich source of indoles (bromoindoles). The simple related bromotryptophan derivatives have been isolated from a variety of sponges from Southern China, Bahamas, UK, Western Australia and the North Atlantic [3,8,14-16], from a marine Californian Pseudomonad bacterium [17], from the Caribbean ascidian *Stomozoa murrayi* and the bacterium *Acinetobacter* sp. associated with this ascidian [18]. It is of special interest that a marine Pseudomonad produced indole-3-carboxaldehyde, 6-bromoindole-3-carboxaldehyde, and the antibiotic *p*-hydroxybenzaldehyde [17], and the extract of the ascidian *S. oviformis* contained the related compounds **1**, **2** and *p*-hydroxyphenyl-glyoxylate with an ethyl glyoxylate moiety instead of an aldehyde in the same position. So, for this structural similarity, these glyoxylates may have originated from some associated microorganisms or microbial diet. It seems to be an inviting prospect to isolate a microbial producer of either indolyl-3-glyoxylic acid or its derivatives, since substituted indolyl-3-glyoxylic acid derivatives have generated considerable interest as anticancer agents [19].

The bromoindole **2**, 2,6-dimethylheptyl sulfate and (3*Z*)-3-decenyl sulfate showed moderate cytotoxicity against Ehrlich carcinoma cells *in vitro* [20], with EC_{50} values of 61 μg/mL, 35 μg/mL and 97 μg/mL, respectively. The alkaloid **3** at a concentration of 100 μg/mL inhibited non-specific esterase activity in mouse lymphocytes up to 44.2% compared with control cells.

Experimental

General experimental procedures: UV spectra were obtained in methanol using a CECIL CE 7200 spectrophotometer; IR spectra were recorded on a Bruker Vector 22 FTIR spectrophotometer in $CDCl_3$ for **1-3**. EI and LSI (negative mode) mass spectra

were recorded on an AMD-604S mass spectrometer. The ^1H and ^{13}C NMR spectra were obtained in CD_3OD on a Bruker DRX-500 spectrometer at 500 and 125 MHz, respectively, with TMS as internal standard. The gas-phase conformational analyses utilized the Gaussian-03 package of quantum-chemical programs [21] on the basis of the density functional theory (DFT) and three-parameter hybrid functional B3LYP in the 6-311G(d,p) basis set. Column chromatography was performed using Sephadex LH-20 (25-100 μ, Pharmacia, Sweden) and silica gel (L 40/100 μm, Chemapol, Czechoslovakia). TLC analyses were carried out on aluminum plates precoated with silica gel (5-17 μ, Sorbfil, Russia). HPLC was performed on a Du Pont Series 8800 Instrument with a RIDK-102 refractometer using either an Agilent ZORBAX Eclipse XDB-C8 (4 x 150 mm) column for **1-3** or Supelco Discovery®C8 (4.6 x 250 mm) column for sulfated hydrocarbons in mixtures of MeOH or EtOH and H_2O. All solvents used were distilled from glass prior to use. In the enzyme bioassay, fluorescence was detected using a fluorescence plate reader (Fluoroscan Ascent, Thermo Labsystems, Helsinki, Finland).

Animal material: The colonial ascidian *Syncarpa oviformis* (class Ascidiacea, order Stolidobranchia, family Styelidae, subfamily Polyzoinae) was collected by scuba at 15 m depth near Shikotan island (Kuril Islands, 43°52,5 N, 146°47,0 E) during a cruise of the r/v "Academik Oparin" in July 2005. The species was identified by K.E. Sanamyan (Kamchatka Branch of the Pacific Institute of Geography FEB RAS, Petropavlovsk-Kamchatsky, Russian Federation). A voucher specimen (031-079) is on deposit in the collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia.

Extraction and isolation: The collected ascidian (approximately 8 kg) was frozen, stored at -15°C and then extracted with ethanol at room temperature. The extract was evaporated *in vacuo* and partitioned between *n*-hexane and H_2O, then the H_2O-fraction was extracted with *n*-BuOH. *N*-Hexane-soluble materials were subjected to column chromatography on Sephadex LH-20 in the system $CHCl_3$: EtOH (1:1), to give the fraction of indoles, and after crystallization 6-bromoindolyl-3-glyoxylate (**2**; 3.6 mg) was obtained. The mother solution was separated by reversed-phase HPLC in 40% MeOH to yield 0.5 mg of indolyl-3-glyoxylate (**1**) and 0.4 mg of *p*-hydroxyphenylglyoxylate. The *n*-butanolic extract, after evaporation *in vacuo*, was subjected to column chromatography over Sephadex LH-20 to give two fractions of low molecular weight compounds. One of the fractions was separated by reversed-phase HPLC in 25% EtOH to yield 6-bromo-5-hydroxyindolyl-3-glyoxylate (**3**; 1.2 mg). Another fraction was purified by chromatography over silica gel to give a subfraction of sulfated hydrocarbons using the system $CHCl_3$:EtOH (2:1). The reversed-phase HPLC of the subfraction in 45% MeOH afforded 2,6-dimethylheptyl sulfate (7.5 mg) and (3*Z*)-3-decenyl sulfate (3.5 mg).

Ethyl 6-bromo-5-hydroxyindoly-3-glyoxylate (3)

Yellowish amorphous powder.
IR (KBr): 3531, 3455, 2976, 2929, 2871, 2856, 1728, 1651, 1518, 1427, 1344, 1098 cm^{-1}.
UV (EtOH) λ_{max}: 207, 267, 292, 331 nm.
^1H NMR: Table 1.
^{13}C NMR: Table 1.
MS (EI, 70 eV) m/z (%): 313/311 $[M]^+$ (13), 240/238 $[M–COOEt]^+$ (100), 212/210 $[M–COCOOEt]^+$ (11), 185/183 (6), 159 (31), 131 (13), 103 (14), 75 (14).
HR EI MS: *m/z* $[M]^+$ calcd for $C_{12}H_{10}BrNO_4$: 310.9793; found: 310.9772.

Bioassay (esterase activity): Mouse lymphocytes (splenocytes) were obtained from mouse spleen. For this purpose, a spleen was isolated and cut, using scissors, into small-sized slices in PBS (pH 7.4), and then pressed through nylon gauze (280 mesh). The obtained suspension was washed twice in PBS by centrifugation (2000 rpm, 10 min). Then 200 μL of the cell suspension [final cell concentration (2-5) × 10^6 cells/mL] was placed into wells of a 96-well microplate containing 20 μL solutions of the test compounds. The incubation was conducted within 1 h at 37°C. Then, 10 μL of fluorescein diacetate solution in DMSO (final concentration 50 μg/mL) was added to each well, and the microplate was incubated additionally for 15 min at 37°C. The intensity of fluorescence was measured at λ_{ex} = 485 nm, λ_{em} = 518 nm.

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