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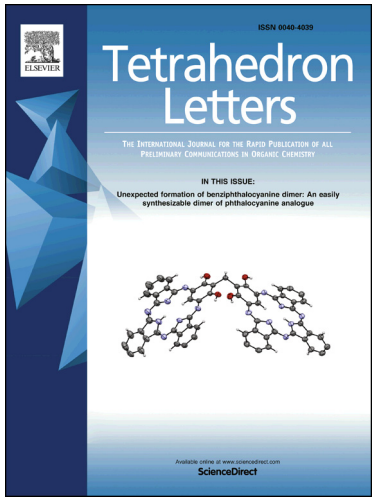

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Cytotoxic indoles alkaloid from *Pseudovibrio denitrificans* BBCC725

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Abstract:

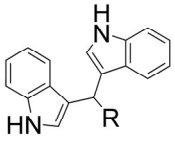
In this study, we investigated a culture supernatant of a *Pseudovibrio denitrificans* strain isolated from sea water. Five indole alkaloids were isolated from this culture. These include four known compounds, arundine (**1**), vibrindole A (**2**), 1,1,1-tris(indol-3-yl)methane (**3**), 7,7-bis(indol-3-yl)-*p*-cresol (**4**) and the new tetra(indol-3-yl)ethanone (**5**). Compound **5** showed interesting cytotoxic effect against L929 cells (EC_{50} = 7 µM) and A549 cells (EC_{50} = 8 µM).

Keywords:

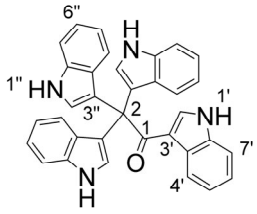
Pseudovibrio denitrificans; Marine natural products; Indole alkaloids; Cytotoxic activity.

The seas and oceans cover about 70% of the Earth's surface, are complex, ancient and relatively poorly explored biotopes[1,2] and thus, a source of unique natural products that may contain structural features not found in terrestrial sources. There are approximately 10^6 prokaryotic cells per ml of surface seawater throughout the world's oceans[3] and these microorganisms have developed unique metabolic and physiological capabilities that ensure their survival in a heterogeneous and fluid habitat (light, salinity, temperature nutrient gradients among others). These adaptations in many cases are dependent on specific bioactive compounds which in turn are a rich source for the discovery of new natural compounds.

For more than a decade our group has been building a collection of marine bacteria strains and a screening of different biological activities has been conducted using culture supernatants of these strains. Among these supernatants, those of a *Pseudovibrio denitrificans* BBCC725 culture showed interesting cytotoxic activities against a melanoma cell line, and was further investigated. Extraction of the secondary metabolites was conducted from centrifugation pellets (8500G during 10 minutes) of cells and sterile Amberlite XAD7 resin (160g of dry weight), that had been added 4 hours prior to a 10 L Marine Broth culture grown for 16 days at 25 degrees under static conditions. The pellet was macerated in methanol/acetone (1/1); the macerate was collected by filtration, evaporated, dissolved in water (1L) and extracted 3 times with 1L of ethyl acetate. Chromatographic separation coupled to activity-based screening against L929 mouse fibroblasts cells, allowed us to isolate four known compounds: arundine (**1**),[4] vibrindole A (**2**),[5] 1,1,1-tris(indol-3-yl)methane (**3**),[6] 7,7-bis(indol-3-yl)-*p*-cresol (**4**)[7] along with one novel compound (**5**) (Fig. 1). Compounds **1–4** were identified by comparison with spectral data (^{1}H and ^{13}C NMR, and MS) previously reported in the literature.[5–8]



1: R = H
2: R = CH_3
3: R = Indol-3-yl
4: R = 4-Hydroxyphenyl



Tetra(indol-3-yl)ethanone
5

Figure 1. Structures of compounds **1**-**5**.

Table 1

NMR spectroscopic data for tetra(indol-3-yl)ethanone (**5**) in CD_3OD

position	δ_C, type	δ_H (*J* in Hz)	COSY	HMBC[a]
1	198.4, C			
2	57.8, C			
2'	135.8, CH	7.23, s		1, 3', 7'a, 3'a
3'	117.5, C			
3'a	128.9, C			
4'	123.5, CH	8.43, brd (7.5)	5'	6', 7'a
5'	123.1, CH	7.17, td (7.2, 1.2)	4'	3'a, 7'
6'	123.9, CH	7.15, m	7'	4', 7'a
7'	112.6, CH	7.28, brd (7.9)	6'	3'a, 5'
7'a	137.0, C			
2"	125.8, CH	6.94, brs		3", 3"a, 7"a
3"	121.0, C			
3"a	129.4, C			
4"	122.9, CH	7.14, brdd (7.2, 1.2)	5"	
5"	119.4, CH	6.65, brt (7.5)	4", 6"	3"a, 7"
6"	122, CH	6.91, brt (7.5)	5", 7"	4", 7"a
7"	112.1, CH	7.26, d (8.0)	6"	3"a, 5"
7"a	138.5, C			

[a]HMBC correlations, optimized for 8 Hz, are from proton stated to the indicated carbon.

Compound **5** was obtained as an intense red powder. The HRESI-MS analysis of **5** indicated a molecular formula of $C_{34}H_{24}N_4O$, from an exact monoisotopic experimental mass at *m/z* 527.1844 Da for $[M+Na]^+$ (calcd. 527.1848), corresponding to a compound with 25 degrees of unsaturation. The first impression based on one-proton NMR signal integrations was that we had obtained a 3:1

mixture of two simple aromatic compounds. Eventually, careful analysis demonstrated that **5** was in fact a pure compound with three equivalent indoles.

On one side of the molecule, the ^{1}H NMR spectrum was very similar to the spectra recorded for indole moieties in compounds **1**-**4**, with one singlet at δ 6.93 (H2"), and an *ortho*-disubstituted benzene ring 4-spin system at δ 7.13 (H4"), 6.64 (H5"), 6.90 (H6") and 7.24 (H7"). HMBC correlations between the singlet proton at δ 6.93 and 3 quaternary carbons at δ 138.5 (C7"a), 129.4 (C3"a) and 121.0 (C3") ppm confirmed the presence of a 3-substituted indole moiety.

On the other side, a second *ortho*-disubstituted benzene ring was identified based on the four proton signals at δ 8.42 (H4'), 7.14 (H5'), 7.16 (H6'), and 7.27 (H7') along with their COSY and HMBC correlations. Another singlet proton was present at δ 7.22 (H2'). This proton was coupled to quaternary carbons at δ 117.5 (C3'), 128.9 (C3'a) and 137.0 (C7'a), these last two carbons being also coupled to H5' and H7', and to H4' and H6', respectively. This pattern indicated that this portion of the molecule was also an indole moiety, although some protons and carbons (H2'/C2', H4'/C4') were significantly shifted downfield indicating that an electron withdrawing group was linked to C3'. Indeed, H2' (δ 7.22 ppm) was correlated to a carbonyl group at δ 198.4 ppm (C1), although the correlation was very weak.

Based on HRESIMS, one quaternary carbon at δ 57.8 ppm had to be added to the structure. The free valences and the 3:1 relative stoichiometry between the indole moieties indicated that this signal should correspond to C2, on which three indole groups were attached. To test this hypothesis HRMS fragmentation experiments at 30 eV were conducted on the $[M+Na]^+$ adduct of **5**. The main fragments had *m/z* 383.1390 and *m/z* 360.1494, corresponding to the $(Indole)_3CNa^{+\bullet}$ radical cation (calcd. for $C_{25}H_{18}N_3Na^{+\bullet}$ 383.1393) and the tri(1H-indol-3-yl)methylium ion (calcd. for $C_{25}H_{18}N_3^+$ 360.1495), respectively. Both daughter ions originate from the breaking of the C1-C2 single bond,

therefore confirming the proposed structure for compound **5** which was named tetra(indol-3-yl)ethanone based on IUPAC convention.

The cytotoxicity of compounds **1-5** was measured using the ATP-lite test (Perkin Elmer) on L929 mouse fibroblasts and on A549 human adenocarcinoma cell lines with 72 hours of contact. Each test was conducted three times in triplicate. The results are reported in Table 2. The new compound **5** was significantly cytotoxic, with half maximal effective concentration (EC50) between 7 and 8 µM.

Table 2

Cytotoxicity of compounds **1**-**5** against L929 and A549 cells

	EC_{50} (µM)	
	L929	A549
Arundine (**1**)	> 100	>100
Vibrindole A (**2**)	> 100	>100
1,1,1-Tris(indol-3-yl)methane (**3**)	> 100	>100
7,7-Bis(indol-3-yl)-*p*-cresol (**4**)	27.4	28.0
Tetra(indol-3-yl)ethanone (**5**)	7.0	8.0
Doxorubicin	0.25	0.5

This report is the first description of tetra(indol-3-yl)ethanone (**5**) that has been isolated from *Pseudovibrio denitrificans* BBCC725 along with known analogous indole alkaloids. Although the cytotoxicity of 3,3'-diindolylmethane derivatives has been previously described,[9] it is interesting to point out that tetra(indol-3-yl)ethanone (**5**) was the most active of all five compounds (**1-5**) tested by our assays.

Acknowledgments

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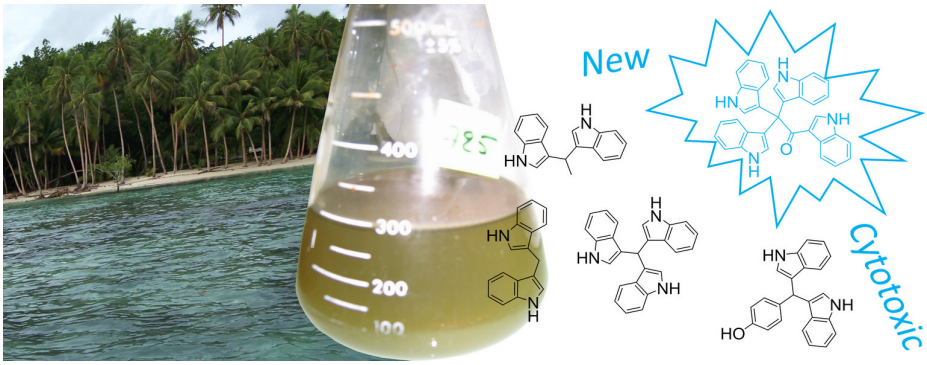

- Five indole alkaloids were isolated from *Pseudovibrio denitrificans*
- Full description of the new alkaloid tetra(indol-3-yl)ethanone is provided
- Tetra(indol-3-yl)ethanone is cytotoxic