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Ophiuroidine, the first indolo[2,1-*b*]quinazoline alkaloid from the Caribbean brittle star *Ophiocoma riisei*

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Abstract—A new indoloquinazoline alkaloid, ophiuroidine **1**, having an indolo[2,1-*b*]quinazoline-6,12-dione skeleton, was isolated from the Caribbean ophiuroid, *Ophiocoma riisei*. The structure of **1** was determined as 4,8,9-trihydroxyindolo[2,1-*b*]quinazoline-6,12-dione from spectroscopic data. Ophiuroidine **1** is the first example of an indoloquinazoline alkaloid found in a marine invertebrate.

The phylum Echinodermata consists of asteroids, holothurians, crinoids, echinoids, and ophiuroids. Studies of the natural products of ophiuroids (brittle stars) are relatively rare compared to other echinoderms. The majority of secondary metabolites reported from brittle stars are polar steroids.[1] Furthermore, naphthaquinones,[2] carotenoids,[3] terpenes,[4] phenylpropanoids,[4] and cerebrosides[5] were isolated from brittle stars.

In the course of our search for antioxidants from marine organisms we have investigated an ethanol extract of the Caribbean brittle star *Ophiocoma riisei* Lütken, 1859 (order Ophiurida).[†] This extract showed a single spot on TLC plates after spraying with an ethanol solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Compound **1**, trivially named ophiuroidine, was isolated and showed moderate antiradical activity (IC_{50} 2.4×10^{-4} M) in a DPPH scavenging method comparable with that of the commonly used synthetic antioxidant BHT (IC_{50} 3.6×10^{-4} M). In this Letter, we describe the isolation and structural elucidation of novel compound **1**.

The EtOH extract of *O. riisei* (250 g, wet weight) was partitioned between Et_2O and H_2O. The aqueous layer was extracted with *n*-BuOH. The *n*-BuOH layer was concentrated and subsequently fractionated by silica gel (EtOAc–MeOH–H_2O, 100:16.5:13.5) and Sephadex LH-20 (MeOH) chromatography to give compound **1** (0.006% based on the dry weight of animals).

Compound **1** was obtained as an orange powder, mp >300 °C (decomp.); UV–vis (EtOH) λ_{max} (log ε) 224 (4.40), 254 (4.20), 284 (4.20), 312 (4.05), 320 (3.77), 399 (3.96) nm; UV–vis (EtOH–KOH) 240 (4.48), 320 (4.20), 500 (4.18) nm; IR (KBr) ν_{max} 3600–3200 (OH), 1711 (C=O), 1659 (C=O), 1644 (C=N), 1613, 1599, 1581 (C=C) cm^{-1}; EI-MS (70 eV) *m*/*z* (%) 296 (100) [M^+], 268 (96), 240 (12). Anal. Calcd for $C_{15}H_8N_2O_5$: C, 60.82; H, 2.72; N, 9.46. Found: C, 60.70; H, 2.79; N, 9.38.

Compound **1** has the molecular formula $C_{15}H_8N_2O_5$ as determined by microanalysis and EI-MS. Methylation of **1** with diazomethane yielded a trimethyl ether **2**, mp >300 °C ($CHCl_3$–MeOH), *m*/*z* 338 [M^+]. Compound **1** had limited solubility in common NMR solvents, therefore, trimethyl ether **2** was used for structure determination of the parent compound **1**.

The ^1H NMR spectrum of trimethyl ether **2** exhibited three-proton singlets for the three methoxyl groups and signals due to the five aromatic protons. The ^{13}C NMR spectrum of **2** revealed signals for 18 carbon atoms: 3 methyls, 5 methines, and 10 quaternary carbons (Table 1). Two of the quaternary carbons (δ 180.0 and 157.8) indicated the presence of two carbonyl

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† Collected near Havana, Cuba, at a depth of 3 m by hand using scuba.

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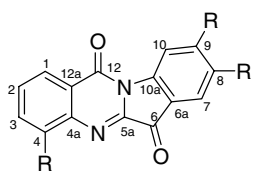
Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR data of **2**

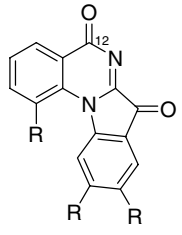
No.	$CDCl_3$		
	δC	δH (*J*)	HMBC
1	118.5 (CH)	7.97 dd (8; 1.2)	3, 4a, 12
2	130.8 (CH)	7.60 t (8)	3, 4, 12a
3	115.6 (CH)	7.29 dd (8; 1.2)	1, 4a
4	157.1 (C)		
4a	136.8 (C)		
5a	144.1 (C)		
6	180.0 (C)		
6a	114.7 (C)		
7	106.1 (CH)	7.33 s	6, 9, 10a
8	148.4 (C)		
9	157.3 (C)		
10	101.2 (CH)	8.21 s	6a, 8
10a	143.1 (C)		
12	157.8 (C)		
12a	124.9 (C)		
4-OMe	56.4 (CH_3)	4.05 s	4
8-OMe	56.3 (CH_3)	3.94 s	8
9-OMe	57.0 (CH_3)	4.10 s	9

groups in different chemical environments, the first signal possibly due to a ketone carbonyl group attached to an aromatic ring, the second signal possibly due to an amide carbonyl. This supposition was in agreement with the IR data of **2** (1718 and 1683 cm^{-1}).

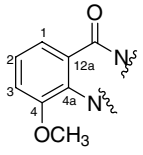
The presence of a 1,2,3-trisubstituted aromatic ring in **2** was evident from the coupling constants of three protons at δ 7.29, 7.60, and 7.97 (Table 1). The substitution pattern in this ring was substantiated by COSY, NOESY, and HMBC experiments. The ^{1}H–^{1}H-COSY spectrum of **2** revealed proton correlations only in the 1,2,3-trisubstituted aromatic ring. In the NOESY spectrum of **2** an NOE between the protons of the methoxyl group at C-4 (δ 4.05) and the proton at δ 7.29 (H-3) showed their adjacency. In the HMBC spectrum, the proton at δ 7.29 (H-3) and the proton at δ 7.97 (H-1) correlated with the carbon at δ 136.8 indicating it to be C-4a. The downfield shift of C-4a signal indicated that it was connected to a nitrogen atom. The proton at δ 7.60 (H-2) correlated with the carbon at δ 124.9 indicating it to be C-12a, and with the oxygenated carbon at δ 157.1 (C-4) bearing the methoxyl group (δ 4.05). The HMBC correlation between the proton at δ 7.97 (H-1) and the carbon at δ 157.8 showed the presence of an amide carbonyl C-12. These data suggested the partial structure A.



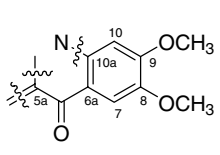
1 R = OH
2 R = OMe
3 R = H



2a



A

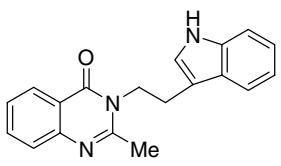


B

The ^{1}H NMR spectrum of **2** exhibited two one-proton singlets at δ 7.33 and 8.21 (Table 1). HMBC, NOESY, and COSY data indicated that both protons belong to the same aromatic ring, and this ring is 1,2,4,5-tetrasubstituted. Indeed, the NOESY spectrum of **2** revealed two pairs of NOE's, the first between the protons of the methoxyl group at C-8 (δ 3.94) and the proton at δ 7.33 (H-7), and the second between the protons of the methoxyl group at C-9 (δ 4.10) and the proton at δ 8.21 (H-10). In the HMBC spectrum, the proton at δ 7.33 (H-7) in turn, correlated with the carbon at δ 157.3 (C-9) and with the carbon at δ 143.1 (C-10a). The downfield shift of C-10a signal indicated that it was connected to a nitrogen atom. The proton at δ 8.21 (H-10), in turn, correlated with the carbon at δ 148.4 (C-8) and with the carbon at δ 114.7 indicating it to be C-6a. The ^{1}H–^{1}H-COSY spectrum showed the absence of correlations for these aromatic protons, therefore the two protons in this ring were *para*-situated with respect to one another, and the second ring was determined as a 1,2,4,5-tetrasubstituted. The proton at δ 7.33 also showed a three-bond correlation to the ketone carbonyl carbon at δ 180.0, and indicated the *ortho*-position of the carbonyl relative to this proton. Of all the carbons of **2**, the quaternary carbon at δ 144.1 (C-5a) did not show HMBC correlations with any protons, therefore this carbon was located next to ketone carbonyl C-6. Thus, the second partial structure B was constructed.

The ^{1}H and ^{13}C NMR spectra and the unsaturation requirements of the molecular formula indicated **2** to be tetracyclic. Two ways of joining partial structures A and B are possible. These combinations lead to two skeletons, indolo[2,1-*b*]quinazoline-6,12-dione (**2**) and indolo[1,2-*a*]quinazoline-5,7-dione (**2a**), as the possible tetracyclic backbone of ophiuroidine. The indolo[2,1-*b*]quinazoline-6,12-dione skeleton is present in the known alkaloid tryptanthrine **3**.[6–14] To determine the correct structure for **2**, we compared ^{13}C NMR data for a carbonyl group of a 4-quinazolinone moiety in compounds having the N_3-substituted 4(3*H*)-quinazolinone moiety [**3** (δ 158.1),[6] **4** (δ 161.3),[15] and **5**(δ 160.6)[16] and the N_1-substituted 4(1*H*)-quinazolinone moiety [**6** (δ 168.8)[17] and **7** (δ 169.8)[18]].

This comparison showed that the ^{13}C chemical shifts of the carbonyl group in N_1-substituted 4(1*H*)-quinazolinones have higher values than those in N_3-substituted 4(3*H*)-quinazolinones. The ^{13}C chemical shift of the carbonyl group at δ 157.8 for **2** clearly indicates the presence of an N_3-substituted 4(3*H*)-quinazolinone moiety in **2**. Comparison of the ^{13}C NMR data for **2** with that for **3**[6] confirmed that **2** and **3** have the same skeleton. Thus the trimethyl ether of ophiuroidine had the structure as shown in **2** and hence the structure of **1** was determined to be 4,8,9-trihydroxyindolo[2,1-*b*]quinazoline-6,12-dione.



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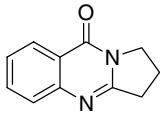

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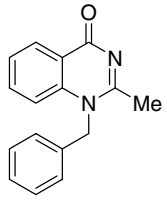

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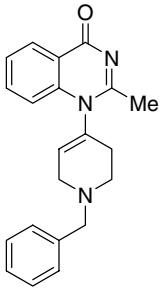

7

Tryptanthrine was reported earlier in the literature as the active component of indigo plants, such as *Isatis tinctoria*,[7] *Polygonum tinctorium*,[8] *Strobilanthes cusia*,[9] *Wrightia tinctoria*,[10] and in the cannon ball tree *Couroupita guaianensis*.[11] It was isolated from *Candida lipolytica* grown under conditions where large amounts of tryptophan were added to the culture solution.[12] Recently, tryptanthrine was isolated from the North Sea bacterium *Cytophaga* sp.[6,13] Its synthesis was described 50 years prior to it being discovered as a natural product.[14] To our knowledge, ophiuroidine **1** is the first example of an indolo[2,1-*b*]quinazoline alkaloid isolated from a marine invertebrate.

Acknowledgments

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