Dilemmaones A−C, Unusual Indole Alkaloids from a Mixed Collection of South African Sponges

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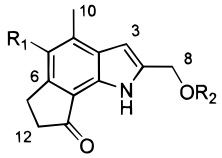
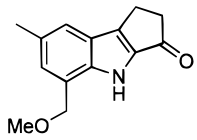
Three indole alkaloids, dilemmaones A−C (**1**−**3**), were isolated from a mixture of sponges that was collected near Cape Town, South Africa. The structures of dilemmaones A−C (**1**−**3**) were elucidated by interpretation of spectroscopic data. The dilemma caused by faulty differentiation of similar specimens in the field is discussed in detail.

As part of a program to explore the biomedical potential of Southern African marine invertebrates,[1] a variety of specimens was collected in the vicinity of Cape Town, and the specimens were screened for cytotoxicity. On the basis of preliminary screening, an extract of a mixed collection of sponges was selected for investigation. Bioassay-directed fractionation of the crude extract using a brine shrimp lethality assay led to the isolation of a sphingolipid as the active metabolite. However, examination of the inactive fractions by ^{1}H NMR spectroscopy revealed the presence of some interesting aromatic compounds, which were subsequently isolated as minor metabolites. In this paper, we report the isolation and structural elucidation of dilemmaones A (**1**), B (**2**), and C (**3**), which are unusual indole derivatives. Although the name of these new compounds, the dilemmaones, originally arose from the dilemma surrounding their sponge source, a process of elimination has suggested that one of the sponges in the mixed collection, *Ectyonanchora flabellata*, is the sponge producing these compounds.



1 R_1 = H, R_2 = Me
2 R_1 = R_2 = H
3 R_1 = OH, R_2 = Me



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A mixture of orange sponges, combined on the basis of their similar color and general appearance, was collected by hand using scuba off Hout Bay, South Africa, and a second collection of a single component of the mixture was collected the next day. Both samples were frozen immediately after collection. The error in sorting similar specimens in the field was not noted until the specimens were examined by experts (see below) after the chemical studies had been completed.

Table 1. ^{1}H and ^{13}C NMR Data for Dilemmaone A (**1**)

C no.	δ_C	δ_H	mult, no., *J* (Hz)	HMBC	NOESY
2	135.4				
3	100.8	6.51	s, 1 H	C-7a, C-3a	H-8, H-10
3a	127.6				
4	139.7				
5	118.2	6.98	s, 1 H	C-3a, C-7, C-10	H-10, H-11
6	152.9				
7	120.0				
7a	131.1				
8	67.7	4.60	s, 2 H	C-2, C-3, C-9	H-9
9	58.2	3.43	s, 3 H	C-8	H-8
10	19.9	2.61	s, 3 H	C-3a, C-4, C-5	H-3, H-5
11	29.5	3.22	t, 2 H, 5.5	C-6, C-7, C-12, C-13	H-5, H-12
12	36.7	2.73	t, 2 H, 5.5	C-6, C-11, C-13	H-11
13	206.5				
NH		9.53	br s, 1 H		H-8

The mixed sample of sponges was freeze-dried and sequentially extracted with hexanes, ethyl acetate, 1:1 ethyl acetate−methanol, and methanol. The 1:1 ethyl acetate−methanol-soluble material from the crude extract was chromatographed sequentially on a diol Sep Pak cartridge, a silica gel column, and silica HPLC to obtain dilemmaones A (**1**, 0.005% dry wt), B (**2**, 0.0012% dry wt), and C (**3**, 0.0035% dry wt), all of which were slightly contaminated with an orange pigment.

Dilemmaone A (**1**) was isolated as an optically inactive off-white solid, mp 146−148 °C. The molecular formula, $C_{14}H_{15}NO_2$, which was established by high-resolution mass measurement, requires eight degrees of unsaturation. Since the ^{13}C NMR spectrum (Table 1) contains a carbonyl signal at δ 206.5 and eight signals in the aromatic region, dilemmaone A (**1**) must be tricyclic. The eight aromatic carbon signals can be accommodated by an indole ring system, which gives rise to the NH signal at δ 9.53 in the ^{1}H NMR spectrum and the band at 3340 cm^{-1} in the infrared spectrum. The ^{1}H NMR spectrum (Table 1) also contained signals that could be assigned to an aromatic methyl group (δ 2.61), two aromatic protons (δ 6.51 and 6.98), a methoxy group (δ 3.43), a downfield methylene group (δ 4.60), and a $-CH_2CH_2-$ moiety (δ 3.22 and 2.73). The corresponding ^{13}C NMR signals were assigned from HMQC data. The HMBC correlation from the methoxy signal at δ 3.43 to the methylene carbon at δ 67.7 established the presence of

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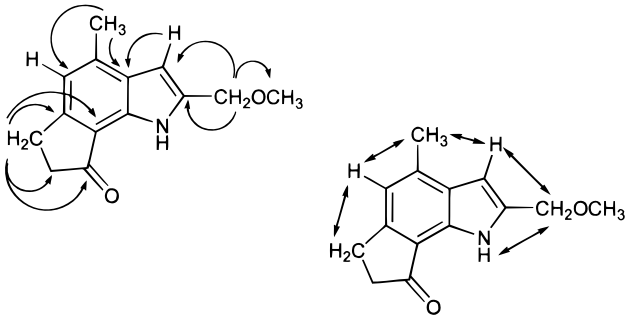

Figure 1. Key HMBC (left) and NOESY (right) correlations for dilemmaone A (**1**).

Table 2. ^{1}H and ^{13}C NMR Data for Dilemmaones B (**2**) and C (**3**)

	2			**3**		
C no.	δ_C	δ_H	mult, no., *J* (Hz)	δ_C	δ_H	mult, no., *J* (Hz)
2	*a*			136.0		
3	99.6	6.50	s, 1 H	100.3	6.44	s, 1 H
3a	*a*			126.6		
4	*a*			139.3		
5	118.3	6.98	s, 1 H	147.8		
6	*a*			144.1		
7	*a*			120.0		
7a	*a*			123.1		
8	58.5	4.88	s, 2 H	67.6	4.62	s, 2 H
9	*a*			58.0	3.46	s, 3 H
10	19.9	2.61	s, 3 H	12.6	2.51	s, 3 H
11	26.9	3.22	t, 2 H, 5.5	23.4	3.15	t, 2 H, 5.5
12	36.7	2.61	t, 2 H, 5.5	36.4	2.77	t, 2 H, 5.5
13	*a*			206.1		
NH		9.68	br s, 1 H		9.37	br s, 1 H

[a] The signals due to quaternary carbons were not observed due to the small sample size.

a methoxymethylene group, while the correlations between the methylene signal at δ 3.22 and the adjacent methylene carbon at δ 36.7, the carbonyl carbon at δ 206.5, and two quarternary aromatic carbons at δ 120.0 and 152.9 indicated that the third ring was a cyclopentanone ring fused to the indole ring system. The location of the methyl and methoxymethylene groups and the location and orientation of the cyclopentane ring were unambiguously assigned by interpretation of the HMBC and NOESY experiments (Figure 1). In particular, the HMBC experiment eliminates the alternate structure **4**, which is compatible with the NOESY data and might be considered more attractive from a biosynthetic viewpoint.

Dilemmaone B (**2**) was isolated as an optically inactive buff solid, mp 168−170 °C. The molecular formula, $C_{13}H_{13}NO_2$, indicated that dilemmaone B (**2**) was a lower homologue of dilemmaone A (**1**). The ^{1}H NMR spectrum of **2** (Table 2) was almost identical to that of **1** except that it lacked a methoxyl signal and contained a methylene signal at δ 4.88 (rather than at δ 4.60), which is appropriate for a hydroxymethylene group. In the ^{13}C NMR spectrum, the C-8 signal was at δ 58.5 in **2**, rather than at δ 67.7 in **1**, which is close to the expected 10 ppm difference in methylene chemical shifts between primary alcohols and primary ethers. The remaining spectral data were all fully compatible with the proposed structure.

Dilemmaone C (**3**) was isolated as an optically inactive pale yellow solid, mp 187−190 °C. The molecular formula, $C_{14}H_{15}NO_3$, indicated that dilemmaone C (**3**) had one more oxygen atom than dilemmaone A (**1**). The IR spectrum contained a broad band at 3340 cm^{-1}, which was assigned to a hydroxyl group. The ^{1}H NMR spectrum of **3** (Table 2) was almost identical to that of **1** except that it lacked the H-5 aromatic proton signal at δ 6.98, suggesting that the hydroxyl group was at C-5. The ^{13}C NMR spectrum of **3** contained a signal at δ 147.8, which is appropriate for C-5, and the C-4 signal was shifted upfield from its position in **1**. All remaining spectral data were fully compatible with the proposed structure.

The structures of dilemmaones A−C (**1**−**3**), although simple, are quite unusual, particularly with respect to the methoxymethylene and hydroxymethylene substituents at C-2. We do not know if the methoxy group came from the methanol used for extraction, but this is certainly a possibility. The most similar structures in the marine natural products literature are the trikentrins from the sponge *Trikentrion flabelliforme*[2] and the herbindoles from an *Axinella* sp.[3] but these metabolites all lack a substituent at C-2.

After completing the structural studies, we decided to extract the second sample of the sponge to obtain more material for biomedical screening. The second sample, which was identical to the voucher specimen of the first collection, was devoid of aromatic compounds. When we examined each piece of sponge from the first collection, which had been dried before storage, we found that it consisted of 47% *E. flabellata* Levi, 41% *Crambe chelastra* Levi, and only 12% of the voucher specimen, which was identified as a member of the genus *Antho* (subgenus *Dirrhopalum*, order Poecilosclerida). Since the dilemmaones do not resemble the known compounds from *Crambe* spp.[4] and were not found in the second collection of *Antho* sp., we suspect that they are from *E. flabellata*, but this obviously needs to be confirmed. Our experience reinforces the need to cleanly separate different specimens in the field. If there is any slight difference in color, both interior or exterior, surface texture, or firmness to the touch, the samples should be kept separate until after the initial bioactivity screening or until they have been examined by an expert.

Experimental Section

General Experimental Procedures. IR and UV spectra were recorded on a Perkin-Elmer FT-IR and a Lambda 3B instrument, respectively. All NMR data were recorded in $CDCl_3$ using either a Varian Inova 300 MHz or a Gemini 400 MHz spectrometer. HRCIMS data were obtained from the UC Riverside Regional Mass Spectrometry Facility. All solvents were distilled before use.

Animal Material. Two collections of orange sponges were made by hand using scuba on two consecutive days off Hout Bay, South Africa, and both were immediately frozen. The first sample (collection no. SAF96015) was found to consist of 47% *E. flabellata* Levi, 1963, 41% *C. chelastra* Levi, 1960, and 12% *Antho* (*Dirrhopalum*) sp. (by dry weight). The second sample (collection no. SAF96015-2) was a homogeneous collection of *Antho* sp., a voucher specimen of which has been deposited at the Natural History Museum, London (BMNH 1997:3:21:1). In life, the sponge (*Antho* sp.) forms a

relatively thick, tough, slightly elastic, encrustation that is bulbous or mammilate in places. The surface is finely dimpled. The exterior color of the sponge is bright reddish orange and it is a brighter orange internally. The ectodermal skeleton consists of a thick crust of paratangential auxiliary subtylostyles with microspined heads. The choanosomal skeleton consists of a basal isotropic renieroid reticulation of acanthostrongyles, with a primary dendritic plumose skeleton of smooth or slightly spined primary subtylostyles, arising from the sponge base. Hastate acanthostyles echinate the primary tracts. Microscleres are palmate isochelae accompanied by at least three sizes of toxas. The sponge is an undescribed species of the genus *Antho*, subgenus *Dirrhopalum* (order Poecilosclerida, family Microcionidae). Dried specimens of *E. flabellata* and *C. chelastra* are available from the authors.

Isolation of Dilemmaones A−C. The frozen sponge material was lyophilized and extracted sequentially with hexanes, EtOAc, 1:1 EtOAc/MeOH, and MeOH. The EtOAc/MeOH extract was dried and the residue (3.14 g) partitioned between EtOAc and H_2O to obtain an organic fraction (1.34 g) that was fractionated on a diol Sep Pak (12 g, 35 mL) using CH_2Cl_2, 20% MeOH in CH_2Cl_2, and MeOH as eluants. The CH_2Cl_2 fraction (240 mg) was chromatographed on silica gel using a gradient from hexanes to EtOAc. Final purification was achieved by HPLC on silica using 7:3 EtOAc/hexanes as eluant to obtain dilemmaones A (**1**, 4.9 mg, 0.005% dry wt), B (**2**, 1.2 mg, 0.0012% dry wt), and C (**3**, 3.4 mg, 0.0035% dry wt).

Dilemmaone A (1): off-white solid; mp 146−148 °C; IR (AgCl) 3340, 1655, 1130 cm^{-1}; UV ($CHCl_3$) 310 nm (ϵ 4100), 250 nm (ϵ 8060); ^1H NMR (300 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; LRCIMS *m/z* (rel int), 230 $[M + H]^+$ (100), 200 (8), 198 (11); HRCIMS *m/z* 230.1177 $(M + H)^+$ (calcd for $C_{14}H_{16}NO_2$, 230.1181).

Dilemmaone B (2): buff solid; mp 168−170 °C; IR (AgCl) 3380, 1645, 1220, 1020, 770 cm^{-1}; UV ($CHCl_3$) 310 nm (ϵ 4230), 250 nm (ϵ 8420); ^1H NMR (300 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; LRCIMS *m/z* (rel int), 216 $[M + H]^+$ (100), 200 (22), 198 (8); HRCIMS *m/z* 216.1026 $(M + H)^+$ (calcd for $C_{13}H_{14}NO_2$, 216.1024).

Dilemmaone C (3): pale yellow solid; mp 187−190 °C; IR (AgCl) 3340, 1670, 1145 cm^{-1}; UV ($CHCl_3$) 310 nm (ϵ 12 330), 250 nm (ϵ 7950); ^1H NMR (300 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; LRCIMS *m/z* (rel int), 246 $[M + H]^+$ (100), 245 (15) 216 (17), 215 (9), 214 (23); HRCIMS *m/z* 246.1140 $(M + H)^+$ (calcd for $C_{14}H_{16}NO_3$, 246.1130).

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