New Polyunsaturated Amino Ketones from a Guangxi Sponge *Haliclona* sp.

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Two new uncommon polyunsaturated amino ketones, (6*Z*,9*Z*,12*Z*,15*Z*)-1-[(2-phenylethyl)amino]octadeca-6,9,12,15-tetraen-3-one (**1**) and (6*Z*,9*Z*,12*Z*,15*Z*)-1-(diethylamino)octadeca-6,9,12,15-tetraen-3-one (**2**), were isolated from the Gangxi sponge *Haliclona* sp. The structures of new compounds **1** and **2** were determined by detailed analysis of their 1D- and 2D-NMR spectra, and high-resolution mass spectrometry.

Introduction. – Marine sponges, particularly sponges of the order *Haplosclerida*, have been established as a rich source of straight-chain polyunsaturated compounds with different chain length and oxygenation pattern [1]. Many of the reported long alkyl-chain compounds showed significant biological activities ranging from antifungal, antiplasmodial, to enzyme inhibitory activity [1–3]. Recent resource investigation of marine organisms carried out off Weizhou Island, Guangxi Autonomous Region, China, revealed that this region possesses a great deal of diversity of sponge species. Although the natural-product chemistry of marine organisms in waters off Weizhou Island remains largely unexplored, some efforts are being directed to the chemistry and biology of those organisms [4–7]. As part of these efforts, an orange-red sponge, identified as *Haliclona* sp., was collected off Weizhou Island at a depth of *ca.* 10 m. Chemical investigation of the Et_2O-soluble fraction of an acetone extract of this sponge led to the isolation of two new uncommon linear polyunsaturated amino ketones, (6*Z*,9*Z*,12*Z*,15*Z*)-1-[(2-phenylethyl)amino]octadeca-6,9,12,15-tetraen-3-one (**1**) and (6*Z*,9*Z*,12*Z*,15*Z*)-1-(diethylamino)octadeca-6,9,12,15-tetraen-3-one (**2**; *Fig. 1*). This report deals with the isolation and structure elucidation of these two new compounds.

Results and Discussion. – Freshly collected animals were immediately put at $-20°$ and kept frozen until used. The sponge material was extracted exhaustively with acetone. The acetone extract was then partitioned between Et_2O and H_2O. The Et_2O-soluble extract was subjected to repeated silica-gel and *Sephadex LH-20* column chromatography to afford two new compounds **1** (6.0 mg) and **2** (20.0 mg).

Compound **1** was isolated as a colorless oil. Its molecular formula was determined as $C_{26}H_{37}NO$ on the basis of HR-ESI-MS pseudomolecular-ion peak at *m*/*z* 380.2956

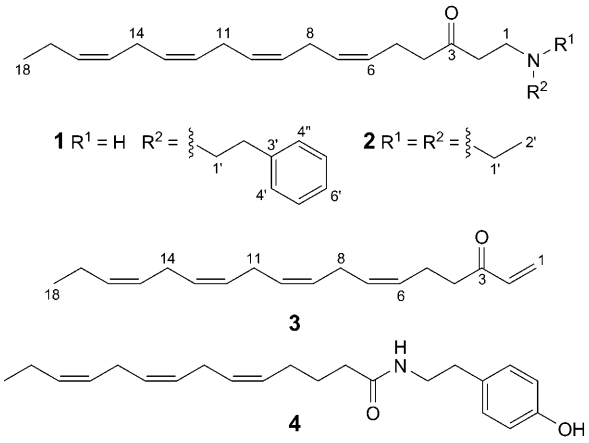


Fig. 1. *Structures of compounds* **1**–**4**

([$M+H$]$^+$; calc. for $C_{26}H_{38}NO$ 380.2953), indicating nine degrees of unsaturation. The IR spectrum indicated the presence of a secondary amine (3350 cm^{-1}), a ketone (1714 cm^{-1}), and a Ph moiety (1620, 770, and 700 cm^{-1}). The ^{13}C-NMR (*Table*), DEPT, and HMQC experiments revealed the presence of two quaternary C-atoms, 13 sp^2 CH and ten CH_2 groups, and one Me group. The low-field signals between 7.19 and 7.29 in the ^{1}H-NMR spectrum of **1** were indicative of a Ph moiety that was confirmed by ^{13}C-NMR resonances at δ(C) 139.6 (C(3′)), 128.7 (C(4′) and C(4″)), 128.5 (C(5′) and C(5″)), and 126.2 (C(6′)). The remaining five degrees of unsaturation were accounted for by a ketone CO group (δ(C) 209.8 (C(3))) and four disubstituted C=C bonds. The 2-phenylethylamine moiety of **1** was assigned by interpretation of COSY, HMQC, and HMBC data. In the COSY spectrum, the H-atoms resonating at δ(H) 2.90 (*t*, $J=6.6$, CH_2(1′)) showed a correlation with CH_2(2′) (δ(H) 2.81 (*t*, $J=6.6$)). Strong HMBCs between CH_2(1′) and C(1), C(2′), and C(3′), and between CH_2(2′), and C(3′), C(4′), and C(4″) confirmed the assignment of this moiety and its location at C(1) (δ(C) 44.0; *Fig. 2*).

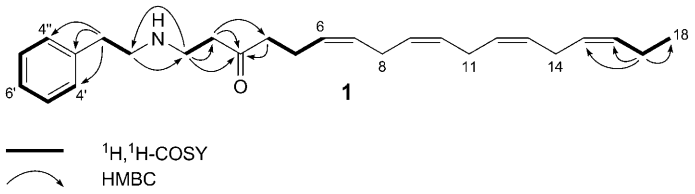


Fig. 2. *Selected ^{1}H,^{1}H-COSY and HMBC correlations for compound* **1**

The assignment of the polyunsaturated ketone part of **1** (C(1) to C(18)) was inferred from interpretation of the COSY, HMQC, and HMBC data. This assignment led to the assembly of C(1) to C(2), and C(4) to C(18) subunits (*Fig. 2*). The location of the C(3)=O moiety and the connectivities of subunits were confirmed by HMBCs.

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *for Compounds* **1** *and* **2**[a]). δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	2.90 (*t*, J = 6.6)	44.0 (*t*)	2.56 (*t*, J = 7.5)	47.3 (*t*)
CH_2(2)	2.65 (*t*, J = 6.3)	42.6 (*t*)	2.73 (*t*, J = 7.5)	40.5 (*t*)
C(3)	–	209.8 (*s*)	–	209.7 (*s*)
CH_2(4)	2.50 (*t*, J = 6.9)	42.7 (*t*)	2.46 (*t*, J = 6.6)	42.8 (*t*)
CH_2(5)	2.30–2.38 (*m*)	21.5 (*t*)	2.29–2.36 (*m*)	21.5 (*t*)
H–C(6)	5.30–5.40 (*m*)	129.0 (*d*)	5.20–5.30 (*m*)	129.0 (*d*)
H–C(7)	5.30–5.40 (*m*)	128.6 (*d*)	5.20–5.30 (*m*)	128.5 (*d*)
CH_2(8)	2.78–2.85 (*m*)	25.6 (*t*)	2.78–2.82 (*m*)	25.5 (*t*)
H–C(9)	5.30–5.40 (*m*)	128.3 (*d*)	5.20–5.30 (*m*)	128.3 (*d*)
H–C(10)	5.30–5.40 (*m*)	128.1 (*d*)	5.20–5.30 (*m*)	128.2 (*d*)
CH_2(11)	2.78–2.85 (*m*)	25.6 (*t*)	2.78–2.82 (*m*)	25.5 (*t*)
H–C(12)	5.30–5.40 (*m*)	128.0 (*d*)	5.20–5.30 (*m*)	128.0 (*d*)
H–C(13)	5.30–5.40 (*m*)	127.0 (*d*)	5.20–5.30 (*m*)	127.0 (*d*)
CH_2(14)	2.78–2.85 (*m*)	25.6 (*t*)	2.78–2.82 (*m*)	25.5 (*t*)
H–C(15)	5.30–5.40 (*m*)	127.8 (*d*)	5.20–5.30 (*m*)	127.8 (*d*)
H–C(16)	5.30–5.40 (*m*)	132.0 (*d*)	5.20–5.30 (*m*)	132.0 (*d*)
CH_2(17)	2.00–2.12 (*m*)	20.5 (*t*)	2.00–2.12 (*m*)	20.5 (*t*)
Me(18)	0.97 (*t*, J = 7.8)	14.3 (*q*)	0.94 (*t*, J = 7.2)	14.2 (*q*)
CH_2(1′)	2.90 (*t*, J = 6.6)	51.0 (*t*)	–	–
CH_2(2′)	2.81 (*t*, J = 6.6)	37.0 (*t*)	–	–
C(3′)	–	139.6 (*s*)	–	–
H–C(4′,4″)	7.19 (*d*, J = 7.8)	128.7 (*d*)	–	–
H–C(5′,5″)	7.29 (*t*, J = 8.1)	128.5 (*d*)	–	–
H–C(6′)	7.21 (*t*, J = 7.8)	126.2 (*d*)	–	–
2 CH_2(1′)	–	–	2.51 (*q*, J = 6.9)	46.8 (*t*)
2 Me(2′)	–	–	1.00 (*t*, J = 6.9)	11.6 (*q*)

[a]) δ Values referenced to $CDCl_3$ (δ(H) 7.26, δ(C) 77.0) as internal standard. Assignments deduced from the analysis of mononuclear and heteronuclear spectra.

HMBCs between CH_2(1) and C(1′), C(2), and C(3), and between CH_2(2) and C(1), C(3), and C(4) secured the position of the ketone moiety as well as the assignment of fragment C(1)–C(2). Similarly, the structure assignment of the terminal Et group was supported by HMBCs between CH_2(17) and C(15), C(16), and C(18). Furthermore, COSY correlations from CH_2(16) to CH_2(17) and from CH_2(17) to CH_2(18) supported the assignment.

Finally, the (*Z*)-geometries of the four C=C bonds of polyunsaturated ketone part were assigned by ^{13}C-NMR, since the resonances observed for the olefinic C-atoms (C(6) (δ(C) 129.0), C(7) (128.6), C(9) (128.3), C(10) (128.1), C(12) (128.0), C(13) (127.0), C(15) (127.8), and C(16) (132.0)), as well as those of the allylic CH_2 groups (C(8) (δ(C) 25.6), C(11) (25.6), and C(14) (25.6)) showed the typical δ(C) values for a CH_2-interrupted (*Z*)-C=C bond system [8–10]. The (*Z*)-geometries of these C=C bonds were further confirmed by comparing the ^{13}C chemical shifts of C(3)–C(18) of **1** with those of the corresponding part of the model compound **3** (*Fig. 1*), which is a metabolite of a marine sponge of the genus *Callyspongia* [11]. On the basis of above

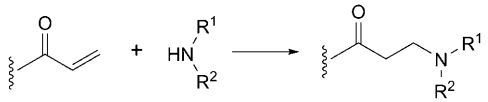
evidences, the structure of **1** was determined as (6*Z*,9*Z*,12*Z*,15*Z*)-1-[(2-phenylethyl)-amino]octadeca-6,9,12,15-tetraen-3-one.

The HR-ESI-MS (positive ion-mode) of compound **2** showed a $[M+H]^+$ peak at *m*/*z* 332.2951 for a molecular formula of $C_{22}H_{37}NO$, equivalent to five degrees of unsaturation. ^{1}H- and ^{13}C-NMR data of compound **2** had some similarities whith those of **1**, indicating that **2** was also a polyunsaturated amino ketone. After a detailed examination of 1D- and 2D-NMR data of **2**, we observed that the only difference between **2** and **1** was the substituents at the N-atom, where the H-atom and 2-phenylethyl group in **1** were replaced by two Et groups in **2**. The presence of a Et_2N moiety in **2** was supported by ^{13}C-NMR (δ(C) 46.8 (C(1′)) and 11.6 (C(2′))) and ^{1}H-NMR data (δ(H) 2.51 (*q*, *J* = 6.9, CH_2(1′)) and 1.00 (*t*, *J* = 6.9, Me(2′))). Detailed anlysis of the 2D-NMR spectra allowed the unambiguous elucidation of the structure of **2**. Thus, the structure of compound **2** was determined as (6*Z*,9*Z*,12*Z*,15*Z*)-1-(diethylamino)octadeca-6,9,12,15-tetraen-3-one.

Although nitrogenous long-chain compounds, such as long-chain amides (exemplified by compound **4** (*Fig. 1*) [12]) and alkylamino alcohols, are frequently encountered in marine invertebrates [1–3], linear amino ketone compounds are rarely reported. To the best of our knowledge, this is the first report on the isolation of polyunsaturated amino ketones from a nature source.

The biogenetic pathway of **1** and **2** might be somewhat different from that of amides (exemplified by compound **4**) and alkylamino alcohols [12–14]. As outlined in the *Scheme*, compound **3** should be their common precursor that reacts with 2-phenylethylamine and Et_2NH, will generate compounds **1** and **2**, respectively [15]. It raises a question about the real origin of **2**, since Et_2NH was used in the isolation process. To clarify this fact, the original extract of the sponge was analyzed by the TLC, and no trace of **2** was found there, suggesting that compound **2** is probably an artifact.

Scheme. *Possible Biogenetic Pathway of Compounds* **1** *and* **2**



3

	R^1	R^2
1	H	$PhCH_2CH_2$
2	Et	Et

Compounds **1** and **2** have been evaluated for cytotoxity against tumor cell lines A-549, HL-60, and P-388, but they were all inactive at a concentration of 10 μg/ml. Other bioassay studies such as anti-inflammatory and antimicrobial activities are currently underway.

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Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2; *Qingdao Haiyang Chemical Group Co.*; 200–300 mesh) and *Sephadex LH-20* (*Amersham Biosciences*). TLC: Precoated silica-gel plates (*Yantai Zi Fu Chemical Group Co.*; *G60*, *F-254*). Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *NicoletMagna FT-IR 750* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Varian Mercury 400* (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer; chemical shifts δ in ppm, with residual $CDCl_3$ (δ(H) 7.26, δ(C) 77.0) as internal standard, coupling constant *J* in Hz. ^1H- and ^{13}C-NMR assignments were supported by ^1H,^1H-COSY, HMQC, and HMBC experiments. ESI-MS and HR-ESI-MS: *Q-TOF-Micro-LC-MS-MS* mass spectrometer; in *m*/*z*.

Biological Material. Specimens of *Haliclona* sp. were collected at Weizhou Island, Guangxi Autonomous Region, P. R. China, in 2007, at a depth of 10 m, and were frozen immediately after collection. A voucher specimen (No. WZ-9) is available for inspection at the Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The frozen animals (310 g dry weight) were cut into small pieces and exhaustively extracted with acetone (3×1.5 l) at r.t. The extract was concentrated, and the resulting residue was partitioned between Et_2O and H_2O. The Et_2O-soluble extract was chromatographed on a SiO_2 column using light petroleum ether (PE) with increasing amount of AcOEt as eluent. The fraction eluted with AcOEt/PE 5:5 was further purified by CC (SiO_2; $CHCl_3$/MeOH/Et_2NH 95:5:1) to afford compounds **1** (6.0 mg) and **2** (20.0 mg).

(6Z,9Z,12Z,15Z)-1-[(2-Phenylethyl)amino]octadeca-6,9,12,15-tetraen-3-one (**1**). Colorless oil. IR (KBr): 3350, 3012, 2925, 2852, 1714, 1620, 1454, 1373, 1263, 1122, 770, 700. ^1H- and ^{13}C-NMR: see *Table.* HR-ESI-MS: 380.2956 ($[M+H]^+$, $C_{26}H_{38}NO^+$; calc. 380.2953).

(6Z,9Z,12Z,15Z)-1-(Diethylamino)octadeca-6,9,12,15-tetraen-3-one (**2**). Colorless oil. IR (KBr): 3012, 2925, 2850, 1716, 1374, 1265, 1120. ^1H- and ^{13}C-NMR: see *Table*. HR-ESI-MS: 332.2951 ($[M+H]^+$,$C_{22}H_{38}NO^+$; calc. 332.2953).

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