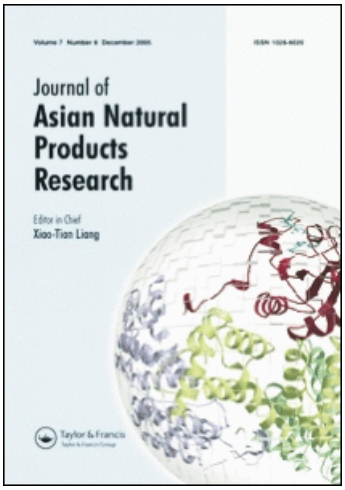

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NEW HURGHAMIDS FROM A RED SEA SPONGE OF THE GENUS *HIPPOSPONGIA*

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Three new N-acyl-2-methylene-β-alanine methyl esters, Hurghamides E–G (**5–7**), were isolated from a Red Sea sponge *Hippospongia* sp. Their structures were elucidated by extensive spectroscopic studies.

Keywords: Sponge; N-acyl-2-methylene-β-alanine methyl esters; Hurghamides E, F and G

INTRODUCTION

We previously reported the isolation and structure elucidation of four N-acyl-2-methylene-β-alanine methyl esters, Hurghamids A–D (**1**–**4**) (Fig. 1), from a Red Sea sponge *Hippospongia* sp. [1]. We have now separated three more new analogs, named hurghamides E–G (**5–7**) (Fig. 2). The isolation and structure elucidation of these new compounds are reported here.

RESULTS AND DISCUSSION

The sponge (500 g, wet weight) was collected by SCUBA off Hurghada in the Red Sea and stored frozen at −80°C until processed. The sponge was

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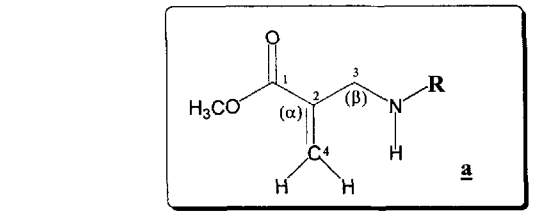

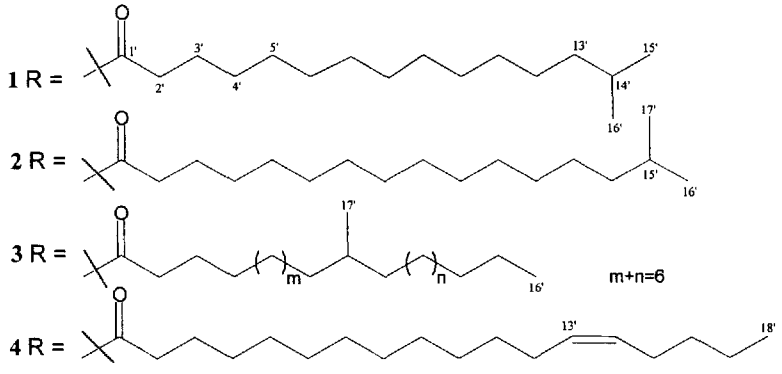

FIGURE 1

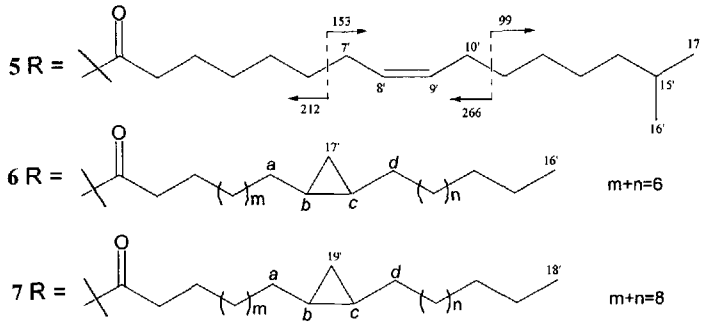

FIGURE 2

exhaustively extracted with Me_2CO, and the extract was partitioned between Et_2O and water. The organic phase after removing the solvent *in vacuo* was subjected to column chromatography on silica gel eluting with petroleum ether–Et_2O system. The major metabolite obtained by this procedure [1] was further separated by reverse-phase HPLC eluting with CH_3CN–H_2O (70:30) to give hurghamides E–G (**5**–**7**) together with known hurghamides reported previously [1].

TABLE I ^{13}C-NMR data[a, c] of hurghamides E–G (**5**–**7**)

Carbon	**5**	**6**	**7**
1	166.5 s	166.5 s	166.5 s
2	136.4 s	136.4 s	136.4 s
3	40.5 t	40.3 t	40.5 t
4	127.4 t	127.1 t	127.4 t
OCH_3	172.9 s	172.9 s	172.9 s
1′	36.8 t	36.8 t	36.8 t
2′	25.7 t	25.7 t	25.7 t
3′	25.7 t	25.7 t	25.7 t
a	27.2 t	30.2 t	30.2 t
b	129.8 d	15.7 d	15.7 d
c	129.9 d	15.9 d	15.9 d
d	27.1 t	30.2 t	30.2 t
14′	39.0 t	31.9 t	
15′	28.0 d	22.5 t	
16′	22.6 q	13.0 q	31.8 t
17′	22.6 q	10.9 t	22.5 t
18′			13.7 q
19′			10.8 t

[a] Bruker AM-500; δ values are reported in ppm referenced to $CHCl_3$ (δ 77.0); assignments were deduced from the analysis of mono- and hetero-nuclear spectra.
[b] The multiplicity was determined by DEPT technique.
[c] The methylenes not reported contributed to a large signal centered at δ 29.8.

All of these compounds showed very similar spectroscopic properties. Their IR spectra indicated the presence of a secondary amide (3400 sh, 3420, 1665, 1520 and 1260 cm^{-1}), an ester (1720 cm^{-1}), and a terminal methylene (950 cm^{-1}). Careful analysis of their ^{1}H–^{1}H COSY, HMQC, TOCSY and HMBC led to the characteristic of a common N-acyl-2-methylene-β-alanine methyl ester moiety (partial structure **a**) which was confirmed by the comparison of the ^{1}H- and ^{13}C-NMR data (Table I) with those of co-occurring hurghamides [1]. In fact, the difference among them happened only in the nature of the acyl part (R).

Hurghamide-E (**5**) had a molecular formula $C_{22}H_{39}NO_3$ as deduced from EIMS spectrum (*m/z* 365, M^+) and ^{13}C-NMR data. The ^{1}H-NMR spectrum of **5** is very similar to hurghamide-B (**2**) showing also the presence of a terminal isopropyl group (δ 0.87, d, $J = 7$ Hz, 6H) in the molecule. In fact, **5** differs from **1** only by the presence of an isolated double bond (δ 5.35, 2H) in its acyl part. The *cis*-stereochemistry of this double bond was deduced from the ^{13}C-NMR chemical shift of the allylic methylene carbons (δ 27.2, 27.1) [2] while the position of this double bond was tentatively assigned at $\Delta^{8'}$ on the basis of EIMS data. In fact, the EIMS spectrum of **5** displayed, apart from the intense fragments reported previously [1], diagnostic fragments at *m/z* 153 and 99 indicating the position of the double bond.

Hurghamide-F (**6**), with also the molecule formula $C_{22}H_{39}NO_3$, is an isomer of **5**. The acyl part of **6** had to be unbranched as only a 3H triplet at δ 0.88 was present in the methyl region of the ^{1}H-NMR spectrum. In addition, the alkyl chain (R) contained a cyclopropane ring, instead of a double bond, as shown by the three upfield resonances at δ −0.34 (1H, ddd, J=5, 5, 4Hz, H-17′a), 0.56 (ddd, J=8, 8, 4Hz, H-17′b), and δ 0.65 (2H, m, H-b and H-c). Detailed analysis of ^{1}H−^{1}H COSY, HMQC, TOCSY and HMBC spectra permitted assignment of the carbons of cyclopropane ring and the two flanking methylene groups as reported in Table I. The large difference between the ^{1}H-NMR chemical shifts of the ring geminal methylene protons is a clear indication of the *cis*-stereochemistry of the ring substituents. Comparison of NMR data with those reported for model compound (plakoside A) [3] confirmed this assignment. To locate the cyclopropane ring chemical degradation was necessary. Unfortunately, scarcity of the material prevented from determination the position of the cyclopropane ring.

Hurghamide-G (**7**) ($C_{24}H_{43}NO_3$, two CH_2 more than that of **6**) showed ^{1}H-NMR spectrum almost identical to that of **6** containing also a *cis*-substituted cyclopropane ring (Table I) and a terminal methyl (H_3-18′, δ 0.87). Clearly, **7** is a superior homologue of **6**. The position of cyclopropane ring remains to be determined.

This is the first report of 2-methylene-β-alanine methyl esters, N-acylated by unbranched aliphatic fatty acids containing cyclopropane rings, isolated from a marine source. Interestingly, hurghamides (**1**–**7**) show structural similarity with a sleep-inducing lipid, *cis*-octadecenamide (oleamide) [4]. Further study should be conducted to test if hurghamides possess also sleep-inducing activity and to understand what is the biological role of these metabolites during the life cycle of the animal, as well as their biogenetic origin.

EXPERIMENTAL SECTION

General Experimental Procedures

The IR spectra were recorded on a Bio-Rad FTS 7 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer. Chemical shifts are reported in ppm referenced to $CHCl_3$ as internal standard (δ 7.26 for proton and δ 77.0 for carbon). ^{1}H- and ^{13}C-NMR assignments were supported by ^{1}H−^{1}H COSY, HMQC and HMBC experiments. EIMS spectra were recorded on a CARLO

ERBA VG TRIO 2000 instrument. Reverse-phase HPLC purification was performed on a Waters liquid chromatograph using a Uvidec-100-III detector. Both analytical [5u, 4.6 mm (I.D.) × 25 cm] and semi-preparative [5u, 10 mm (I.D.) × 25 cm] columns were SPHERISORB-S5 ODS2. Commercial Merck Si gel plates were used for TLC. The chromatograms were sprayed with 0.1% $Ce(SO_4)_2$ in 2 N H_2SO_4 and heated at 80°C for 5 min to detect the spots.

Collection of the Biological Material

The specimens were collected by Dr. E. Mollo off Hurghada on the Red Sea using SCUBA-diving at a depth of −10 m. They were immediately frozen and transferred to Naples and kept at −80°C until extraction. Voucher specimens are available for inspection at ICMIB of Naples and at Istituto di Zoologia of Genova.

Extraction and Isolation

A frozen sponge (20 g, dry weight after extraction) was extracted with Me_2CO (2.5 l). After concentration, the aqueous residues were extracted with Et_2O (3 × 150 ml). The combined ether extracts were taken to dryness, yielding oily residues (1.6 g) which was chromatographed on silica gel column using eluants of increasing polarity from light petroleum ether to Et_2O. The fraction eluting with petroleum ether–Et_2O (3:7) yielded crude hurghamides that was again chromatographed on a silica gel column eluting with the same eluants mentioned above. This procedure yielded formally pure hurghamides (0.26 g, 1.3% dry weight) as a white amorphous wax. Prep. HPLC isolation of Hurghamides E–G (**5**–**7**) was carried out by isocratic elution with CH_3CN–H_2O (70:30). This yielded, in order of increasing polarity, **6** (1.6 mg), **5** (0.9 mg), **7** (1.0 mg) together with other known hurghamides [1]. The flow rate for the separation was 2 ml/min.

Spectral Data of **5** A white amorphous wax. IR (liquid film) ν_{max} 3260, 2880, 2810, 1715, 1640, 1550, 1460, 1380, 940, 720 cm^{-1}; UV (MeOH) λ_{max} 205 nm (ε 14700); ^{1}H-NMR δ 4.08 (2H, br d, J = 6 Hz, H_2-3), 6.25, 5.83 (each 1H, br s, H_2-4), 5.93 (1H, m, N–H), 5.34 (2H, m, olefinic protons), 3.78 (3H, s, OCH_3), 2.17(2H, t, J = 7, 7 Hz, H_2-2′), 2.02 (4H, m, methylenes adjacent to the double bond), 1.62 (2H, m, H_2-3′), 1.16 (2H, m, H_2-13′), 1.52 (1H, m, H-14′), 0.87 (6H, d, J = 7 Hz, H_3-16′ and H_3-17′); ^{13}C-NMR: see Table I; EIMS m/z (%): 365 (M^+, 12%), 334 (14%), 306 (15%), 170 (80%), 153 (32%), 142 (42%), 116 (90%), 114 (100%), 99 (25%).

Spectral Data of **6** A white amorphous wax. IR and UV are same as those of **5**; ^{1}H-NMR δ 4.09 (2H, br d, $J=6$ Hz, H_2-3), 6.25, 5.83 (each 1H, br s, H_2-4), 5.92 (1H, m, N–H), 3.78 (3H, s, OCH_3), 2.17 (2H, t, $J=7$, 7 Hz, H_2-2′), 1.62(2H, m, H_2-3′), 1.37, 1.12 (each 2H, m, H_2-*a* and H_2-*d*), 0.89 (3H, t, $J=7$, 7 Hz, H_3-16′), 0.65 (2H, m, H-*b* and H-*c*), 0.56 (1H, ddd, $J=8$, 8, 4 Hz, H-17′b), −0.34 (1H, ddd, $J=5$, 5, 4 Hz, H-17′a); ^{13}C-NMR: see Table I; EIMS *m/z* (%): 365 (M^+, 10%), 334 (14%), 170 (50%), 142 (32%), 116 (90%), 114 (100%).

Spectral Data of **7** A white amorphous wax. IR and UV are same as those of **5**; ^{1}H-NMR δ 4.09 (2H, br d, $J=7$ Hz, H_2-3), 6.25, 5.83 (each 1H, br s, H_2-4), 5.92 (1H, m, N-H), 3.78 (3H, s, OCH_3), 2.17 (2H, t, $J=7$, 7 Hz, H_2-2′), 1.63 (2H, m, H_2-3′), 1.37, 1.12 (each 2H, m, H_2-*a* and H_2-*d*), 0.89 (3H, t, $J=7$, 7 Hz, H_3-18′), 0.65 (2H, m, H-*b* and H-*c*), 0.56 (1H, ddd, $J=8$, 8, 4 Hz, H-19′b), −0.34 (1H, ddd, $J=5$, 5, 4 Hz, H-19′a); ^{13}C-NMR: see Table I; EIMS *m/z* (%): 393 (M^+, 13%), 362 (14%), 170 (50%), 142 (37%), 116 (92%), 114 (100%).

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