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TWO NEW PHTHALATE DERIVATIVES FROM THE MARINE SPONGE *Haliclona* sp.

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Two new phthalate derivatives, N^1*-(2-aminoethyl)-*N^2*-isopentylphthalamide (**1**) and* N^1*-isobuty-*N^2*-tridecylphthalamide (**2**), were isolated from the marine sponge* Haliclona *sp. The structures of the new isolates were elucidated on the basis of extensive spectroscopic analysis and by comparison of the data with those of related secondary metabolites.*

Keywords: marine sponge *Haliclona* sp., phthalate derivatives.

Previous chemical investigation of the genus *Haliclona* (order Haplosclerida, family Haliclonidea) has displayed the presence of many bioactive secondary metabolites [1]. In our study of bioactive compounds from a *Haliclona* sp. collected from South China Sea, some secondary metabolites of this specimen have been reported [1–3]. Further search for bioactive metabolites from this specimen has led to the isolation of two new phthalate derivatives, N^1-(2-aminoethyl)-N^2-isopentylphthalamide (**1**) and N^1-isobuty-N^2-tridecylphthalamide (**2**) (Fig. 1). Herein we describe the isolation and structure elucidation of the new isolates.

Compound **1** was obtained as a white powder and had the molecular formula $C_{15}H_{23}N_3O_2$ as deduced from the HR-ESI-MS mass spectrum (found $[M + H]^+$ at *m*/*z* 278.1865, calcd $[M + H]^+$, 278.1869). The NMR data of **1** revealed the presence of two Me groups, four sp^3 CH_2 groups, one sp^3 CH group, four sp^2 CH groups, and four quaternary sp^2 C-atoms. The aromatic region of the NMR spectrum of **1** displayed the presence of a 1,2-disubstitued aromatic ring at δ 7.36 (1H, d, J = 8.5 Hz), 7.32 (1H, dd, J = 8.5, 6.8 Hz), 7.30 (1H, dd, J = 8.0, 6.8 Hz), and 7.28 (1H, d, J = 8.0 Hz), together with two carbonyl resonances at δ_C 170.1 and 169.2, confirming the phthalate characteristic of the molecule [4, 5].

The gross structure was determined by the aid of COSY and HMBC experiments (Fig. 1). The ^{1}H-^{1}H COSY spectrum displayed three fragments of H-2/H-3/H-4/H-5, H-3′/H-4′, and H-3″/H-4″/H-5″/H-6″ or H-7″. These data, together with the key HMBC correlations from H-2 to C-1′, H-3′ to C-1′, H-5 to C-1″, and H-3″ to C-1″, established the whole structure as N^1-(2-aminoethyl)-N^2-isopentylphthalamide (**1**).

Compound **2**, a white powder, was established as $C_{25}H_{42}N_2O_2$ based on the NMR and HR-ESI-MS data (found $[M + H]^+$ at *m*/*z* 403.3320, calcd $[M + H]^+$, 403.3325). The NMR data of **2** revealed the presence of three Me groups, 13 sp^3 CH_2 groups, one sp^3 CH group, four sp^2 CH groups, and four quaternary sp^2 C-atoms. The presence of a 1,2-disubstitued aromatic ring in **2** was revealed by four proton signals at δ_H 7.30 (1H, d, J = 8.7 Hz), 7.29 (1H, dd, J = 8.7, 6.7 Hz), 7.23 (1H, dd, J = 8.3, 6.7 Hz), and 7.20 (1H, d, J = 8.3 Hz). These proton resonances, together with two carbonyl resonances at δ_C 171.0 and 169.4, indicated that **2** possessed a phthalate moiety [4, 5]. In addition, the NMR and the signal for fragmentation in the ESI-MS/MS data of *m*/*z* 183 $[M - C_{12}H_{15}N_2O_2]^+$ indicated the presence of 13 aliphatic carbon chain ($(CH_2)_{12}CH_3$) in the structure.

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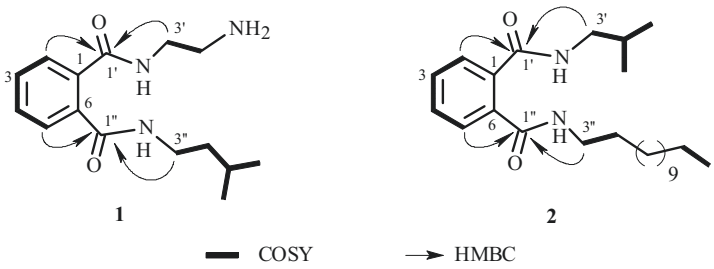

Fig. 1. Structures of compounds **1** and **2** and their key ^{1}H–^{1}H COSY and HMBC correlations.

The molecular framework was established by ^{1}H–^{1}H COSY and HMBC correlations (Fig. 1). The comprehensive analysis of the ^{1}H–^{1}H COSY correlations of **2** established the spin systems of H-2/H-3/H-4/H-5, H-3′/H-4′/H-5′ or H-6′, H-3″/H-4″, and H-12″/H-13″. These data, together with the key HMBC correlations from H-2 to C-1′, H-3′ to C-1′, H-5 to C-1″, and H-3″ to C-1″, established the whole structure as N^1-isobuty-N^2-tridecylphthalamide (**2**).

EXPERIMENTAL

General Experimental Procedures. NMR spectra were recorded on a Bruker AV 500 MHz NMR spectrometer with TMS as internal standard (Bruker, Bremen, Germany). HR-ESI-MS data were obtained from a Bruker Maxis mass spectrometer (Bruker, Bremen, Germany). ESI-MS/MS data were obtained with a Thremo LCQ-DECA-XP LC-MS spectrometer. Semipreparative HPLC was performed on a Hitachi L-2400 HPLC system using a YMC ODS-H80 column (250 × 10 mm i.d., 4 μm) coupled to an Alltech ELSD 800 detector with the flow-splitter valve (Parker: NS) set at a split ratio of 20:1 (collector: detector). The silica gel GF_{254} used for TLC was supplied by the Qingdao Marine Chemical Factory, Qingdao, China. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in EtOH. All solvent ratios are measured v/v.

Animal Material. The sponge was collected in July 2005, off the coast of Hainan Island, China. The specimen was identified as *Haliclona* sp. by Dr. Kyung Jin Lee. A voucher specimen (0507003) was deposited at the Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, CAS.

Extraction and Isolation. The sponge *Haliclona* sp. (20 kg, wet) was extracted with ethanol (95%). Ethanol was evaporated *in vacuo* to afford a syrupy residue, which was suspended in distilled water and fractionated successively with $CHCl_3$ and *n*-butanol. The *n*-butanol soluble portion (7.21 g) was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 E, 230 mesh) using EtOH–H_2O (from 90:10 to 20:80) as eluent, giving 24 fractions (A–Z). Fraction B (6.2 g) was subjected to column chromatography using MeOH–H_2O (from 0 to 1:1) as eluent to afford 24 subfractions. Fraction B3-4 (155.0 mg) was subjected to column chromatography using MeOH–H_2O (from 0 to 6:94) as eluent to afford **2** (3.1 mg). Fraction B3-5 (640 mg) was subjected to column chromatography using $CHCl_3$–MeOH (from 0 to 7:3) as eluent to afford 15 fractions; then the B3-5-2 was subjected to column chromatography using $CHCl_3$–MeOH (from 0 to 95:5) as eluent to afford **1** (2.9 mg).

***N*^{1}-(2-Aminoethyl)-*N*^{2}-isopentylphthalamide (1)**. White powder. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.36 (1H, J = 8.5, H-2), 7.32 (1H, dd, J = 8.5, 6.8, H-3), 7.30 (1H, dd, J = 8.0, 6.8, H-4), 7.28 (1H, d, J = 8.0, H-5), 3.19 (2H, t, J = 8.2, H-3′), 2.98 (2H, t, J = 8.2, H-4′), 2.96 (2H, t, J = 8.1, H-3″), 1.70 (2H, m, H-5″), 1.56 (2H, m, H-4″), 0.90 (3H, d, J = 6.5, H-7″), 0.89 (3H, d, J = 6.5, H-6″). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 170.1 (C-1′), 169.2 (C-1″), 135.2 (C-1), 134.3 (C-6), 132.2 (C-3), 130.4 (C-4), 127.1 (C-2), 126.7 (C-5), 55.6 (C-4′), 42.1 (C-3′), 40.3 (C-3″), 34.3 (C-4″), 29.3 (C-5″), 22.9 (C-6″), 22.5 (C-7″). HR-ESI-MS *m/z* 278.1865 $[M + H]^+$, calcd 278.1869).

***N*^{1}-Isobuty-*N*^{2}-tridecylphthalamide (2)**. White powder. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.30 (1H, J = 8.7, H-2), 7.29 (1H, dd, J = 8.7, 6.7, H-3), 7.23 (1H, dd, J = 8.3, 6.7, H-4), 7.20 (1H, d, J = 8.3, H-5), 2.98 (2H, t, J = 8.1, H-3″), 2.96 (2H, t, J = 8.2, H-3′), 2.15 (2H, m, H-4′), 1.60 (2H, m, H-4″), 1.20–1.57 (16H, overlapping, H-5″–12″), 0.93 (3H, d, J = 6.7, H-5′), 0.91 (3H, d, J = 6.7, H-6′), 0.84 (3H, d, J = 6.3, H-13″). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 170.0 (C-1′), 169.4 (C-1″), 134.1 (C-1), 133.5 (C-6), 131.3 (C-3), 130.1 (C-4), 126.2 (C-2), 125.8 (C-5), 47.3 (C-3′), 39.3 (C-3″), 31.3 (C-4″), 30.6 (C-4′), 23.5 (C-5′), 22.9 (C-6′), 31.9–19.2 (8C, overlapping, C-5″–12″), 14.2 (C-13″). HR-ESI-MS *m/z* 403.3320 $[M + H]^+$, calcd 403.3325.

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