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CHEMICAL CONSTITUENTS OF MARINE SPONGE *Callyspongia* sp. FROM THE SOUTH CHINA SEA

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Callyspongia sp. belonges to the order Haplosclerida, family Callyspongiidae [1]. In order to discover marine natural products with new structures and bioactive activities, we have investigated the marine sponge *Callyspongia* sp., collected from the South China Sea [2]. Nine known compounds were isolated and identified as ethyl 2-(1*H*-indol-3-yl)acetate (**1**), 1*H*-indole-3-carbaldehyde (**2**), phenylalanine (**3**), 2-phenylacetamide (**4**), hydroxydihydrobovolide (**5**), (–)-loliolide (**6**), 4-hydroxyphenylacetic acid (**7**), *p*-methoxyphenylacetic acid (**8**), and (*E*)-4-(4-hydroxyphenyl)-3-buten-2-one (**9**). All compounds were isolated from the marine sponge *Callyspongia* sp. for the first time.

The sponge was collected by hand in July 2005, off the coast of Hainan Island, China. The sponge was extracted with 95% EtOH. The extract was concentrated under reduced pressure and partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was partitioned between 85% EtOH and petroleum ether. The H_2O layer was partitioned between *n*-BuOH and H_2O. The 85% EtOH layer was initially subjected to column chromatography over silica gel to provide five fractions. Fraction 1 was separated by Sephadex LH-20, reversed-phase flash column chromatography, and silica gel column chromatography to afford **2** (7.8 mg), **3** (5.5 mg), **4** (7.1 mg), and **9** (4.6 mg). Fraction 2 was separated by silica gel column chromatography, Sephadex LH-20, and HPLC to afford compounds **1** (7.1 mg), **5** (3.3 mg), and **6** (4.0 mg). The *n*-BuOH fraction was subjected to reversed-phase flash column chromatography, eluting with MeOH–H_2O gradient to afford seven fractions. Fraction 4 was further isolated by Sephadex LH-20 and reversed-phase flash column chromatography to give compounds **7** (3.5 mg), and **8** (6.1 mg).

Compound 1. Colorless oil. ESI-MS *m/z* 204 $[M + H]^+$. It was characterized as ethyl 2-(1*H*-indol-3-yl) acetate by comparison of the physicochemical properties and ^1H and ^{13}C NMR spectral data with those reported in the literature [3].

Compound 2. White powder (MeOH); mp 194–196°C. It was characterized as 1*H*-indole-3-carbaldehyde by comparison of the physicochemical properties and ^1H and ^{13}C NMR spectral data with those reported in the literature [4].

Compound 3. White powder (MeOH); mp 282–284°C; ESI-MS *m/z* 163 $[M – H]^-$. It was characterized as phenylalanine by comparison of the ^1H and ^{13}C NMR spectral data with those reported in the literature [5].

Compound 4. White powder (DMSO); mp 155–156°C; ESI-MS *m/z* 134 $[M – H]^-$. It was characterized as 2-phenylacetamide by comparison of the physicochemical properties and ^1H and ^{13}C NMR spectral data with those reported in the literature [6].

Compound 5. White solid ($CHCl_3$); ESI-MS *m/z* 197 $[M – H]^-$. It was characterized as hydroxydihydrobovolide by comparison of the ^1H and ^{13}C NMR spectral data with those reported in the literature [7, 8].

Compound 6. White powder (MeOH); mp 122–122.5°C; ESI-MS *m/z* 197 $[M + H]^+$. It was characterized as (–)-loliolide by comparison of the ^1H and ^{13}C NMR spectral data with those reported in the literature [9, 10].

Compound 7. Colorless oil. It was characterized as 4-hydroxyphenylacetic acid by comparison of the ^1H and ^{13}C NMR spectral data with those reported in the literature [11].

Compound 8. Colorless oil. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.13 (2H, d, J = 8.5, H-7), 6.77 (2H, d, J = 8.5, H-7), 3.69 (3H, s), 3.54 (2H, s). The ^1H NMR spectra of **8** showed a close similarity to those of **7**. Compound **8** was characterized as *p*-methoxyphenylacetic acid by comparison of its behavior on TLC with an authentic sample.

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Compound 9. Light yellow solid. ESI-MS *m/z* 163 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.44 (2H, d, J = 8.5), 6.88 (2H, d, J = 8.5), 7.48 (1H, d, J = 16), 6.60 (2H, d, J = 16), 2.38 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 27.3 (C-1), 199.5 (C-2), 124.6 (C-3), 144.2 (C-4), 126.8 (C-5), 130.4 (C-6, C-10), 116.2 (C-7, C-9), 158.7 (C-8). According to the spectral data, compound **9** was determined to be (*E*)-4-(4-hydroxyphenyl)-3-buten-2-one. It was further identified by comparison of its behavior on TLC with an authentic sample.

Indole alkaloids have been isolated from a variety of marine invertebrates, including sponges, coelenterates, tunicates, and bryozoans. Some of these metabolites showed a wide spectrum of pharmacological activities such as antiviral, cytotoxic, anti-inflammatory, and antifungal activities [12]. In our investigation, two indole alkaloids **1**, **2** were isolated from the marine sponge *Callyspongia* sp.

Hydroxydihydrobovolide (**5**) was previously reported from butter fat and fermented tobacco leaves as an aroma constituent, from timothy chokes as a fungitoxic substance, from liverwort cell culture as an allelochemical, from cress seedlings as a plant growth inhibitor, and from the leaves and twigs of *Litsea verticillata* as an anti-HIV substance [8].

(–)-Loliolide (**6**) was isolated from *Fumaria officinalis* L. as early as 1938, but its structure was clarified by Hodges and Porter only in 1964 [9]. Since that time, (–)-loliolide has been detected in many higher plant species and also in marine mollusks. (–)-Loliolide was found to be a germination inhibitor and recently reported as a repellent for ants *Atta cephalotes* [10].

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