Sesquiterpenes from the Hainan Sponge *Dysidea septosa*

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Five new sesquiterpenes, named lingshuiolides A (**1**) and B (**2**), lingshuiperoxide (**6**), isodysetherin (**10**), and spirolingshuiolide (**12**), along with several known related analogues (**7**−**9**, **11**, and **13**−**15**), were isolated from the Hainan marine sponge *Dysidea septosa*. The structures of new compounds **1**, **2**, **6**, **10**, and **12** were determined by detailed analysis of 1D and 2D NMR spectra and by comparison with related model compounds. The absolute configuration of lingshuiolide B (**2**) was established by using the modified Mosher's method. Spirolingshuiolide (**12**) represents the first example of a sesquiterpene with a rearranged drimane skeleton. Compounds **7**−**9** and **15** exhibited significant inhibitory activity against human protein tyrosine phosphatase 1B (hPTP1B), an enzyme involved in the regulation of insulin signaling. In particular, **15** showed the most potent effect, with an IC_{50} value of 1.9 μg/mL.

Numerous investigations on the chemical composition of marine sponges of the genus *Dysidea* have been carried out, and many novel metabolites spanning a wide range of structure classes and biosynthetic origins were discovered. In particular, many *Dysidea* sponge metabolites exhibited various promising biological activities, ranging from ichthyotoxic, to cytotoxic, to anti-inflammatory, which attracted considerable attention from natural product chemists and pharmacologists.[1]

As part of our ongoing research on the biologically active substances from Chinese marine invertebrates,[2–5] we made a collection of the sponge *Dysidea septosa* off Lingshui Bay, Hainan Province, China. Separation of the Et_2O-soluble fraction of the acetone extract of this sponge resulted in the isolation of five new sesquiterpenes, namely, lingshuiolides A (**1**) and B (**2**), lingshuiperoxide (**6**), isodysetherin (**10**), and spirolingshuidolide (**12**), together with several known analogues (**7**−**9**, **11**, and **13**−**15**). This paper describes the isolation and structure elucidation of these new compounds.

Results and Discussion

The acetone extract of the sponge *D. septosa* was divided into Et_2O- and BuOH-soluble portions. Then, the Et_2O extract was repeatedly chromatographed on silica gel columns and reversed-phase HPLC to yield pure compounds **1**, **2**, **6**−**12**, and **13**−**15**, respectively.

The structures of known compounds were readily identified as hydroxybutenolide (**7**),[6] microcionin-4 (**8**),[7] dihydropallescensin-2 (**9**),[8] dysetherin (**11**),[9,10] furodysinin 3β,4β-epoxy lactone (**13**),[11] furodysinin lactone (**14**),[12] and nakafuran-8 (**15**),[13] respectively, by comparison of their NMR data with those reported in the literature.

Among the five new sesquiterpenes, compounds **1**, **2**, and **6** showed NMR data characteristic for a γ-hydroxyl-bearing butenolide moiety, like that of co-occurring **7**, while the NMR spectra of **10** and **12** were reminiscent of those of co-occurring **13** and **14**, displaying a common epoxy-bearing butanolactone segment. The structural elucidation of these new metabolites is described as follows.

Lingshuiolide A (**1**), a colorless oil, showed a molecular ion peak at *m/z* 266 (M^+) in the EIMS, and the molecular formula, $C_{15}H_{22}O_4$, was established by HREIMS (*m/z* 266.1532, calcd 266.1518, Δ = −1.4 mmu), indicating five degrees of unsaturation. The IR spectrum showed absorption bands at 3376 and 1762 cm^{-1} assignable to a hydroxyl group and an ester carbonyl group, respectively. The ^1H NMR spectrum displayed a broad one-proton singlet at δ_H 6.85 indicating an α,β-unsaturated γ-lactone, a broad one-proton singlet at δ_H 6.06 suggesting a hydrogen atom on the carbon bearing the lactone oxygen and hydroxyl oxygen atoms, one methyl singlet and a one-proton doublet at δ_H 1.66 and 5.85 (d, J = 4.6 Hz), respectively, ascribed to a Me-bearing trisubstituted double bond, and a one-proton broad singlet at δ_H 4.06 indicative of a hydrogen on a carbon bearing an allylic hydroxyl group. The ^{13}C NMR spectrum (Table 1) exhibited 15 signals ($3CH_3$, $3CH_2$, 5CH, 4C), whose chemical shift values and multiplicities (DEPT) confirmed the presence of an α,γ-disubstituted butenolide moiety [δ_C 97.2 (CH), 138.3 (qC), 143.7 (CH), and 172.3 (qC)], a Me-bearing trisubstituted double bond [δ_C 19.1 (CH_3), 145.1 (qC), 126.1

Chart 1

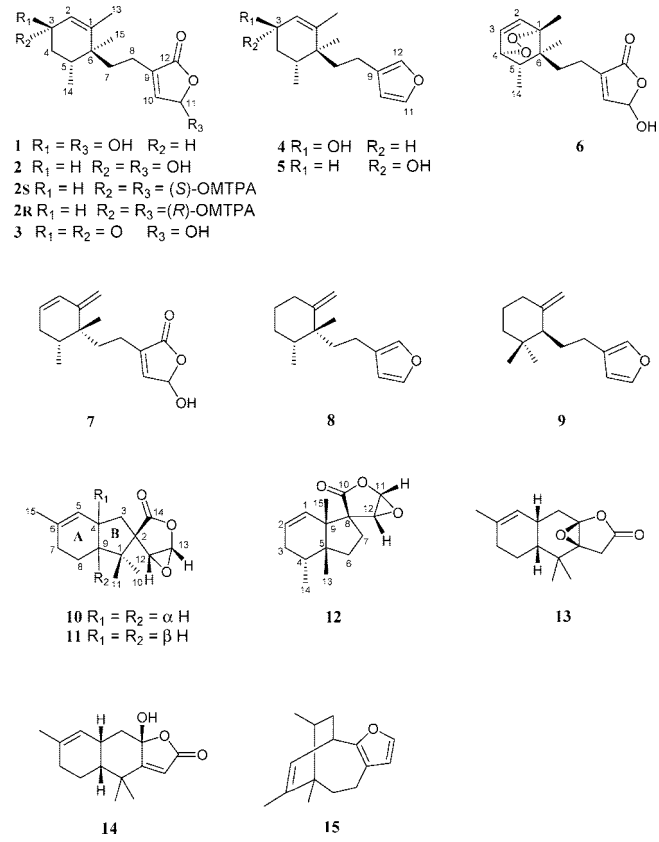

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Table 1. ^{13}C NMR Data of Compounds **1**, **2**, **6**, **10**, and **12**[a]

carbon	1	2	6	10	12
1	145.1, qC	141.6, qC	81.0, qC	48.4, qC	130.8, CH
2	126.1, CH	129.5, CH	137.9, CH	58.6, qC	127.9, CH
3	64.5, CH	67.8, CH	130.1, CH	41.9, CH_2	30.8, CH_2
4	35.7, CH_2	37.0, CH_2	76.3, CH	38.5, CH	31.2, CH
5	27.8, CH	31.6, CH_2	38.4, CH	121.8, CH	48.0, qC
6	41.0, qC	40.8, qC	40.7, qC	135.4, qC	34.5, CH_2
7	33.5, CH_2	33.1, CH_2	35.1, CH_2	30.4, CH_2	31.1, CH_2
8	20.4, CH_2	20.2, CH_2	20.9, CH_2	20.9, CH_2	58.4, qC
9	138.3, qC	138.5, qC	138.9, qC	51.0, CH	51.9, qC
10	143.7, CH	143.1, CH	143.0, CH	29.1, CH_3	177.7, qC
11	97.2, CH	96.8, CH	96.7, CH	22.4, CH_3	78.0, CH
12	172.3, qC	171.7, qC	171.4, qC	59.2, CH	59.4, CH
13	19.1, CH_3	18.7, CH_3	16.1, CH_3	77.9, CH	15.1, CH_3
14	15.6, CH_3	15.8, CH_3	15.5, CH_3	179.1, qC	15.8, CH_3
15	19.5, CH_3	20.5, CH_3	18.4, CH_3	23.6, CH_3	24.4, CH_3

[a] Bruker DRX-400 spectrometer. The chemical shift values are given in ppm and referenced to $CDCl_3$ (77.0 ppm).

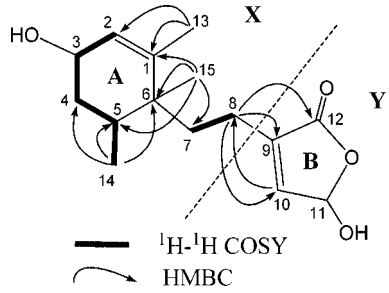

Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of compound **1**.

(CH)], and a hydroxyl-bearing carbon [δ_C 64.5 (CH)]. From these spectral data compound **1** was deduced to be a bicyclic structure with two olefins and one carbonyl group. Analysis of the 2D NMR spectra of **1** revealed that compound **1** was composed of two parts (Figure 1): partial structures **X** (from C-1 to C-8 including C-13 to C-15) and **Y** (from C-9 to C-12).

For the partial structure **X**, the presence of two spin−spin systems (H-2 to H-5 and H_3-14; H_2-7 to H_2-8) were evident by analysis of the $^1H-^1H$ COSY spectrum. Further, HMBC correlations from H_3-15 to C-1, C-5, C-6, and C-7, from H_3-14 to C-4, C-5, and C-6, and from H_3-13 to C-1, C-2, and C-6 suggested that the two above-mentioned spin systems were connected through the quaternary carbon C-6. Moreover, the ^1H and ^{13}C NMR chemical shifts of the moiety **X** were found almost identical to those of pelseneeriol-1 (**4**),[14] a furanosesquiterpene recently isolated from the Portuguese nudibranch *Doriopsilla pelseneeri*, securing the assignment for the partial structure **X**. The identification of partial structure **Y** was straightforward. Direct comparison of the ^1H and ^{13}C NMR data of C-9 to C-12 of **1** with those of 2-oxomicrocionin-2-lactone (**3**)[15] and co-occurring **7** readily identified an α,γ-disubstituted butenolide moiety. Finally, subunits **X** and **Y** were linked through the bond C-8 to C-9 by the observation of $^1H-^{13}C$ long-range correlations from H_2-8 to C-9, C-10, and C-12 and from H-10 to C-8 (Figure 1). Thus, the planar gross structure of **1** was determined.

There are three chiral centers (C-3, C-5, and C-6) around the cyclohexene ring of **1**. The *cis* relationship between H_3-14 and H_3-15 was determined by analogy with model compound **4**.[14]As described in ref 14, the ^{13}C NMR chemical shift of CH_3-14 has a similar value (15.7−16.0 ppm) in both *cis* and *trans* isomers, whereas the carbon value of CH_3-15 is smaller in the *cis* (20.9−21.1 ppm) than in the *trans* (26.3−26.6 ppm) isomer due to the greater γ-type interaction between the two methyl groups in *cis* compounds. The β-orientation of the hydroxyl group at C-3 was inferred by analysis of the splitting pattern of H-3, which was the same as the corresponding proton in model compound **4**. Moreover, no ROESY cross-peak between H-3 and H-5 gave further support to this assignment.

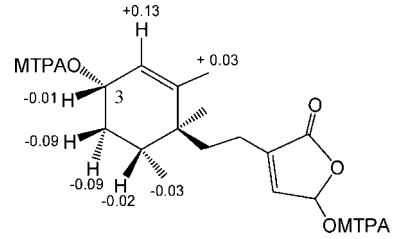

Figure 2. Δδ values ($\delta_S - \delta_R$) (ppm) for the protons near C-3 of (*S*)- and (*R*)-MTPA esters of **2**.

Lingshuiolide B (**2**) has a molecular formula $C_{15}H_{22}O_4$, as deduced from its HREIMS spectrum, the same as **1**. Detailed analysis of 1D and 2D NMR spectra revealed that the planar structure of **2** was identical to that of **1**. As shown in Table 1, the ^{13}C NMR data from C-6 to C-12 and C-13 to C-15 of **2** are almost the same as those of **1**, whereas the differences between them mainly happened at the cyclohexene ring. The multiplicity of carbinolic signal H-3 (δ_H 4.06, br s, in **1** and δ_H 4.20, m, in **2**) indicated that compound **2** differs from **1** only in the relative stereochemistry of the hydroxyl group at C-3. Since the configuration of 3-OH of **1** was already assigned as β, the hydroxyl group at C-3 of **2** was determined adopting the α-configuration. The diagnostic ROESY correlation between H-3 and H-5 further confirmed this assignment. The different chemical shift values from C-1 to C-5 between **1** and **2**, due to different orientations of the hydroxyl group, were in good agreement with what was observed in the model compounds **4** and **5**,[14] a pair of C-3 epimers. Thus, lingshuiolide B was determined as the C-3 epimer of **1**.

Since lingshuiolide B contains a secondary hydroxyl group at C-3, the absolute configuration of **2** was determined by applying the modified Mosher's method.[16] Compound **2** was esterified separately with (*R*)- and (*S*)-MTPA chloride in dry pyridine at room temperature to yield the corresponding MTPA esters **2S** and **2R**, respectively. Assignment of the ^1H NMR signals of the esters was achieved by carefully analyzing the $^1H-^1H$ COSY spectra. The Δδ ($\delta_{S\text{-ester}} - \delta_{R\text{-ester}}$) values of the protons near the chiral carbon (C-3) were summarized as shown in Figure 2. Negative Δδ values were recorded for the protons of H_2-4, H-5, and Me-14 and positive Δδ values were observed for the protons of H-2 and Me-13. According to the Mosher's determination rule, the absolute stereochemistry of the hydroxyl group at C-3 was established as *S*. Consequently, the absolute stereochemistry at C-5 and C-6 was determined as both *R*, respectively. The configuration of C-11 remains undefined. Although the hydroxyl group on C-11 could also be esterified by (*R*)- and (*S*)-MTPA chloride, the Δδ ($\delta_S - \delta_R$) values of H-10 and H-11 in both 11-MTPA esters were nearly zero. It should be pointed out that these results were in good agreement with the phenomenon observed when treating cacospongionolide F,[17] which contains a similar γ-hydroxybutenolide moiety, with (*R*)- and (*S*)-MTPA chloride.

Lingshuiperoxide (**6**) was obtained as an optically active, white, amorphous powder. The EIMS of **6** showed a molecular ion peak at *m/z* 280, and its molecular formula was inferred as $C_{15}H_{20}O_5$ from the HREIMS (*m/z* 280.1307, calcd 280.1311). It was immediately apparent from the ^1H and ^{13}C NMR data that **6** differs from **1** and **2** only in the ring A, where the methyl-bearing trisubstituted double bond in **1** and **2** was replaced by a disubstituted double bond [δ_H 6.39 (1H, br d, J = 8.2 Hz), 6.59 (1H, dd, J = 8.2 and 6.1 Hz); δ_C 130.1 (CH), 137.9 (CH)]. In addition, careful analysis of $^1H-^1H$ COSY and HMQC spectra revealed the clear proton connectivities from H-2 to H-5 and H_3-14. Thus, starting from H-4 (δ_H 4.33, δ_C 76.3), a series of distinct correlations between H-4 and H-3 (δ_H 6.59), between H-3 and H-2 (δ_H 6.39), between

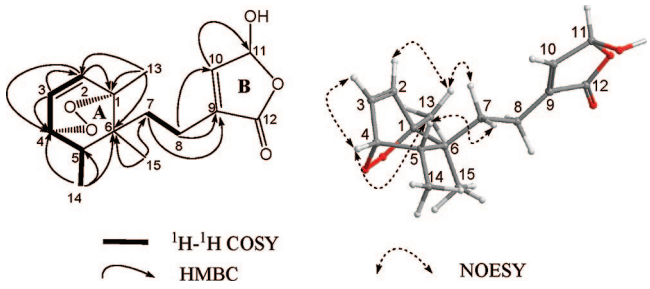

Figure 3. Key 2D NMR correlations of compound **6**.

H-4 and H-5 (δ_H 2.29), and between H-5 and H_3-14 (δ_H 0.82) were observed (Figure 3). One butenolide moiety (ring B) and one cyclohexene ring accounted for the five sites of unsaturation. Consequently, the last two oxygen atoms unassigned had to be ascribed to a peroxide bridge that linked at C-1 and C-4, respectively, to complete the required unsaturation degrees of **6**. Finally, by analogy to compounds **1** and **2**, the relative stereochemistry of H_3-14 and H_3-15 was assigned as *cis* (both α), whereas the configuration for the peroxide bridge was established by analysis of the ROESY spectrum. The ROESY correlations between H-4 and H-5, between H-3 and H-4, between CH_3-13 and H-7a, between CH_3-13 and H-2, and between H-5 and H-7b indicated that the peroxide bridge is also α-oriented. The *cis* nature of the double bond at $\Delta^{2(3)}$ was assigned on the basis of the coupling constant between H-2 and H-3 (J = 8.2 Hz).

Isodysetherin (**10**)[18] was isolated as an optically active, colorless oil with $[\alpha]_D^{20}$ +81 (*c* 0.26, $CHCl_3$). The molecular formula of **10** was determined to be $C_{15}H_{20}O_3$ by HREIMS {*m/z* 248.1415 $[M]^+$, Δ = +1.1 mmu}. The carbon framework of **10** was readily recognized to be the same as dysetherin (**11**)[9,10] by comparing their ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. In fact, the significant difference between the ^{1}H NMR spectra of **10** and **11** was the signals assigned to the methine proton pairs (H-4 δ 2.85 in **10** and 2.96 in **11**; H-9 δ 1.66 in **10** and 2.32 in **11**) and the germinal methyl groups (Me-10 δ 1.38 in **10** and 1.18 in **11**; Me-11 δ 1.08 in **10** and 1.14 in **11**). Furthermore, in their ^{13}C NMR spectra, it was noticed that the resonances for C-4, C-9, Me-10, and Me-11 appeared at δ 38.5, 51.0, 29.1, and 22.4 in **10**, while the corresponding carbons in **11** resonated at δ 36.3, 45.1, 22.5, and 26.2, respectively. These differences suggested that **10** is a diastereomer of **11** with opposite A/B ring junction. A significant NOE correlation between H-4 and H-9 in the NOESY spectrum of **10** confirmed the *cisoid* rings' linkage. The other NOE correlations between H-4/H-3α, H-9/Me-10, H-12/Me-10, and H-3α/H-12 supported that the orientations of H-4 and H-9 in **10** were α instead of β as in **11**, and the configuration of C-2 was the same as that of **11**. On the basis of the above observations, the structure of compound **10** was determined as a 4,9-epimer of dysetherin, named isodysetherin.

Spirolingshuiolide (**12**)[18] was isolated as an optically active, colorless oil with $[\alpha]_D^{20}$ −31 (*c* 0.16, $CHCl_3$). Its molecular formula was deduced as $C_{15}H_{20}O_3$ from the HREIMS data {*m/z* 248.1401 $[M]^+$, Δ = +1.1 mmu}, the same as **10**. Nevertheless, the spectroscopic properties of **12** were somewhat different from those of **10**. The structural determination of spirolingshuiolide was aided by the previous experience acquired during the structural elucidation of isodysetherin. The ^{13}C NMR data of **12** displayed three sp^3 methines, two sp^2 methines, three sp^3 methylenes, three methyls, and four quaternary carbons. All the protons were connected to carbons by HMQC experiment. The presence of one disubstituted double bond [δ_H 5.32 (br d, J = 10.0 Hz), 5.80 (ddd, J = 10.0, 4.8, 2.4 Hz); δ_C 130.8 (CH), 127.9 (CH)], one epoxy group [δ_H 3.86 (d, J = 2.2 Hz), 5.48 (d, J = 2.2 Hz); δ_C 59.4 (CH), 78.0 (CH)], and an ester carbonyl [δ_C 177.0 (q C)] were easily recognized by characteristic ^{1}H and ^{13}C NMR resonances. These data accounted

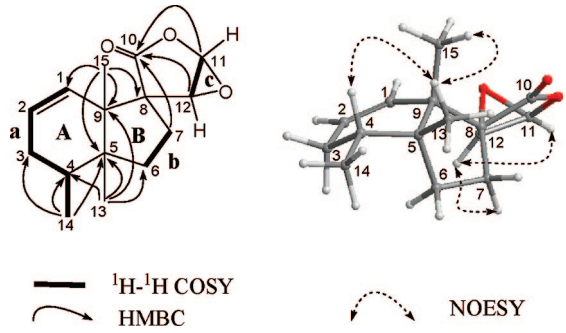

Figure 4. Key 2D NMR correlations of compound **12**.

for three of the required six sites of unsaturation. Consequently, spirolingshuiolide must possess three rings. Analysis of the ^{1}H NMR spectrum of **12**, aided by ^{1}H−^{1}H COSY, HMQC, and TOCSY, revealed the proton connectivities for three partial structures, **a**−**c** (Figure 4).

For the partial structure **a**, the olefinic proton (δ_H 5.32, H-1) exhibited clear correlation with the adjacent olefinic proton (δ_H 5.80, H-2), which, in turn, was further correlated with the methylene proton at δ 2.02 (H-3b); on the other hand, the sharp doublet signal at δ 0.95 (H_3-14) was linked to the methine at δ 1.94 (H-4), which, in turn, was also coupled with H_2-3. For the partial structure **b**, the correlations between H_2-6 (δ 1.76, 1.96) and H_2-7 (δ 1.98, 2.18) were observed. The clear cross-peaks between H-11 (δ 5.48) and H-12 (δ 3.86) indicated the presence of the partial structure **c**. All the subunits, bearing in mind two unassigned methyls [δ_H 0.79 (s), 1.03 (s); δ_C 15.1 (CH_3), 24.4 (CH_3)] and three quaternary carbons (C-5, δ 48.0; C-8, δ 58.4, C-9, δ 51.9), were connected by extensive interpretation of well-resolved HMBC spectra. Significant ^{1}H−^{13}C long-range correlations, as shown in Figure 4, connected H_3-13 to C-5 and H_3-15 to C-9 and located the spirocarbon at C-8. Thus, the planar structure of **12** was unambiguously assigned.

The relative stereochemistry of **12** was established by NOESY experiment. The NOE correlations of Me-13/Me-15 and Me-13/H-4 showed that the rings A/B were *cis* fused and the configuration of H-4 was β. The NOESY correlation between the oxygen-bearing proton signals (δ 3.86, H-12 and 5.48, H-11) revealed the H-11 and H-12 were *cis* oriented to each other. The relative configuration of the spirocarbon (C-8) was tentatively determined as *S** by a NOESY cross-peak observed between H-12 and H-7b (Figure 4).

In conclusion, 12 sesquiterpenes belonging to six different structural classes were isolated and structurally characterized from the Hainan sponge *D. septosa*. It is interesting to note that dihydropallescensin-2 (**9**)[8] had been previously isolated from the nudibranch *Cadlina luteomarginata* collected near San Diego, California, and furodysinin 3β,4β-epoxy lactone (**13**)[11] had been previously isolated from the nudibranch *Chromodoris funereal* collected in Iwayama Bay, Palau. In particular, interestingly, pelseneeriol-1 (**4**) and pelseneeriol-2 (**5**),[14] two furanosesquiterpenes structurally closely related to compounds **1**, **2**, and **6**−**8**, had been recently reported from the Portuguese nudibranch *Doriopsilla pelseneeri*, and they were hypothetically suggested to originate from its dietary sponge of genus *Dysidea* even though no direct experimental observation supported their hypothesis. This study provides the indirect experimental evidence that the sponge *D. septosa* might be the potential prey of the above-mentioned nudibranchs. Further study should be conducted to collect these nudibranchs in the same region of South China Sea, where the title sponge was found, and to investigate these nudibranchs chemically so as to unambiguously confirm their prey−predator relationship.

The discovery of these new sesquiterpenes, particularly spirolingshuiolide (**12**), has added to an extremely diverse and complex assay of sponge sesquiterpenes, which is rapidly expanding. Now, there is a strong interest in performing further studies aimed at

experimentally proving the true biogenetic origin and the effective biological and ecological role that spirolingshuiolide (**12**) and related sesquiterpenes play in the life cycle of the sponge and finally at confirming their structural peculiarities by synthesis.

It has been discovered that protein tyrosine phosphatase 1B (PTP1B) is involved both physiologically and pathologically in regulating the signaling of the insulin receptor. Knockout studies in mice have shown that PTP1B-deficient mice displayed enhanced insulin sensitivity and resistance to diet-induced obesity. PTP1B thus becomes a promising target in the treatment of type-II diabetes and obesity.[19] For this reason, the development of small-molecule inhibitors of PTP1B has attracted considerable attention in both academic research and pharmaceutical investigation. In fact, a number of reports describing natural products as PTP1B inhibitors have appeared in the past decade.[20] In light of this observation, all the new and known compounds isolated in this study were evaluated for their inhibitory activity against PTP1B. The bioassay results showed that compounds **7**−**9** had moderate inhibitory activities, with IC_{50} values of 8.8, 11.6, and 6.8 μg/mL, respectively, while compound **15** had the strongest PTP1B inhibitory effect, with an IC_{50} value of 1.9 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. UV spectra were recorded on a Varian Cary 300 Bio spectrophotometer. IR spectra were recorded on a Nicolet-Magna FT-IR 750 spectrometer. NMR spectra were measured on a Bruker DRX-400 spectrometer with the residual $CHCl_3$ (δ_H 7.26 ppm, δ_C 77.0 ppm) as an internal standard. EIMS and HREIMS data were obtained on a Finnigan-MAT-95 mass spectrometer. ESIMS and HRESIMS spectra were recorded on a Q-TOF Micro LC-MS-MS mass spectrometer. Reversed-phase HPLC (Agilent 1100 series liquid chromatography using a VWD G1314A detector at 210 nm and a semipreparative ODS-HG-5 [5 μm, 10 mm (i.d.) × 25 cm] column) was also employed. Commercial Si gel (Qing Dao Hai Yang Chemical Group Co., 200−300 and 400−600 mesh) was used for CC, and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC.

Biological Material. The specimens of *D. septosa* identified by Professor J.-H. Li of Institute of Oceanology, Chinese Academy of Sciences, were collected by scuba at Lingshui Bay, Hainan Province, China, in November 2001, and were frozen immediately after collection. A voucher sample (SS-38) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The frozen sample (500 g dry weight) was lyophilized and exhaustively extracted with acetone. The extract was concentrated *in vacuo*, and the resulting residue was extracted with Et_2O and *n*-BuOH, respectively. The Et_2O-soluble portion was repeatedly subjected to silica gel column chromatographies (using increasing concentrations of EtOAc in petroleum ether as the eluent) to give **1** (50.0 mg), **2** (55.0 mg), **6** (4.8 mg), **7** (18.7 mg), **8** (18.7 mg), **9** (18.7 mg), **12** (0.8 mg), **13** (10.9 mg), **14** (10.7 mg), and **15** (41.7 mg), respectively. The portion eluted with EtOAc/petroleum ether (95:5) was further purified by semipreparative RP-HPLC (MeOH/H_2O, 70:30; 2.5 mL/min; UV 210 nm) to give **10** (1.5 mg, t_R = 37.7 min) and **11** (3.2 mg, t_R 38.8 min).

Lingshuiolide A (1): colorless oil; $[\alpha]_D^{20}$ +85.4 (*c* 0.37, $CHCl_3$); IR (KBr) ν_{max} 3376, 2968, 1762, 1658, 1444, 1010 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (1H, br s, H-10), 6.06 (1H, br s, H-11), 5.85 (1H, br s, H-2), 4.06 (1H, m, H-3), 2.22 (1H, m, H-8b), 1.89 (1H, m, H-8a), 1.82 (1H, m, H-4α), 1.77 (1H, m, H-5), 1.66 (3H, s, Me-13), 1.61 (2H, m, H_2-7), 1.39 (1H, m, H-4β), 0.92 (3H, s, Me-15), 0.90 (3H, d, J = 6.5 Hz, Me-14); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m*/*z* 266 [M]$^+$ (5), 248 (10), 203 (10), 128 (36), 121 (100); HREIMS *m*/*z* 266.1532 (calcd for $C_{15}H_{22}O_4$, 266.1518).

Lingshuiolide B (2): colorless oil; $[\alpha]_D^{20}$ −2.0 (*c* 0.33, $CHCl_3$); IR (KBr) ν_{max} 3355, 2964, 1751, 1658, 1446, 1010 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.82 (1H, br s, H-10), 6.07 (1H, br s, H-11), 5.47 (1H, br s, H-2), 4.20 (1H, m, H-3), 2.22 (1H, m, H-8b), 1.89 (1H, m, H-8a), 1.82 (1H, m, H-4α), 1.77 (1H, m, H-5), 1.66 (3H, s, Me-13), 1.61 (2H, m, H_2-7), 1.39 (1H, m, H-4β), 0.92 (3H, s, Me-15), 0.90 (3H, d, J = 6.5 Hz, Me-14); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m*/*z* 266 [M]$^+$ (5), 248 (45), 230 (35), 204 (55), 121 (80), 84 (100); HREIMS *m*/*z* 266.1510 (calcd for $C_{15}H_{22}O_4$, 266.1518).

Lingshuiperoxide (6): colorless oil; $[\alpha]_D^{20}$ −7.3 (*c* 0.41, $CHCl_3$); IR (KBr) ν_{max} 2921, 1745, 1461, 1376 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92 (1H, br s, H-10), 6.59 (1H, dd, J = 6.1, 8.2 Hz, H-3), 6.39 (1H, br d, J = 8.2 Hz, H-2), 6.11 (1H, br s, H-11), 4.33 (1H, m, H-4), 4.11 (1H, br s, OH), 2.38 (1H, m, H-8b), 2.33 (1H, m, H-8a), 2.29 (1H, m, H-5), 1.99 (1H, m, H-7b), 1.66 (1H, m, H-7a), 1.30 (3H, s, Me-13), 0.83 (3H, s, Me-15), 0.82 (3H, d, J = 6.6 Hz, Me-14); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; ESIMS *m*/*z* 303 [M + Na]$^+$; HRESIMS *m*/*z* 303.1209 (calcd for $C_{15}H_{22}O_5Na$, 303.1208).

Isodysetherin (10): colorless oil; $[\alpha]_D^{20}$ +81 (*c* 0.26, $CHCl_3$); IR (KBr) ν_{max} 2966, 1776, 1461, 1070 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.47 (1H, d, J = 2.3 Hz, H-13), 5.34 (1H, br s, H-5), 3.60 (1H, d, J = 2.3 Hz, H-12), 2.85 (1H, m, H-4), 2.09 (1H, dd, J = 8.2, 14.0 Hz, H-3α), 1.93 (1H, m, H-3β), 1.89 (2H, m, H_2-7), 1.64 (2H, m, H_2-8), 1.66 (1H, m, H-9), 1.64 (3H, s, Me-15), 1.38 (3H, s, Me-10), 1.08 (3H, s, Me-11); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m*/*z* 248 [M]$^+$ (50), 233 (35), 203 (50), 135 (45), 119 (100); HREIMS *m*/*z* 248.1415 (calcd for $C_{15}H_{20}O_3$, 248.1412).

Spirolingshuiolide (12): colorless oil; $[\alpha]_D^{20}$ −31 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 2962, 1772, 1460, 1068 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.80 (1H, ddd, J = 2.4, 4.8, 10.0 Hz, H-2), 5.48 (1H, d, J = 2.2 Hz, H-11), 5.32 (1H, br d, J = 10.0 Hz, H-1), 3.86 (1H, d, J = 2.2 Hz, H-12), 2.18 (1H, m, H-7a), 2.02 (1H, m, H-3b), 1.98 (1H, m, H-7b), 1.96 (1H, m, H-6b), 1.94 (1H, m, H-4), 1.82 (1H, m, H-3a), 1.76 (1H, m, H-6a), 1.03 (3H, s, Me-15), 0.95 (3H, d,J = 6.6 Hz, Me-14), 0.79 (3H, s, Me-13); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m*/*z* 248 [M]$^+$ (5), 219 (20), 203 (25), 121 (100); HREIMS *m*/*z* 248.1401 (calcd for $C_{15}H_{20}O_3$, 248.1405).

Preparation of (*S*)- and (*R*)-MTPA Ester. The **2S** derivative was obtained by treating **2** (1.5 mg) with (*R*)-MTPA-Cl in dry pyridine for ca. 16 h under stirring at RT. The reaction mixture was purified by CC (silica gel) to afford pure **2S** (1.2 mg). In a similar manner, **2R** (1.3 mg) was prepared from (*S*)-MTPA-Cl.

2S: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, br s, H-11), 6.81 (1H, br s, H-10), 5.46 (1H, br s, H-2), 5.53 (1H, m, H-3), 2.26 (1H, m, H-8b), 1.98 (1H, m, H-8a), 1.85 (1H, m, H-4α), 1.83 (1H, m, H-5), 1.67 (3H, s, Me-13), 1.63 (2H, m, H_2-7), 1.51 (1H, m, H-4β), 0.91 (3H, s, Me-15), 0.89 (3H, d, J = 6.5 Hz, Me-14).

2R: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, br s, H-11), 6.81 (1H, br s, H-10), 5.33 (1H, br s, H-2), 5.54 (1H, m, H-3), 2.26 (1H, m, H-8b), 1.98 (1H, m, H-8a), 1.94 (1H, m, H-4α), 1.85 (1H, m, H-5), 1.64 (3H, s, Me-13), 1.62 (2H, m, H_2-7), 1.60 (1H, m, H-4β), 0.91 (3H, s, Me-15), 0.92 (3H, d, J = 6.5 Hz, Me-14).

Biological Assay. Recombinant PTP1B catalytic domain was expressed and purified according to a previous report.[21] The enzymatic activities of the PTP1B catalytic domain were determined at 30 °C by monitoring the hydrolysis of pNPP. Dephosphorylation of pNPP generates product pNP, which was monitored at an absorbance of 405 nm by the EnVision multilabel plate reader (PerkinElmer Life Sciences, Boston, MA). In a typical 100 μL assay mixture containing 50 mmol/L 3-[*N*-morpholino] propanesulfonic acid (MOPs), pH 6.5, 2 mmol/L pNPP, and 30 nmol/L recombinant PTP1B, activities were continuously monitored and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve. The IC_{50} was calculated with Prism 4 software (Graphpad, San Diego, CA) from the nonlinear curve fitting of the percentage of inhibition (% inhibition) versus the inhibitor concentration [I] by using the following equation: % Inhibition = $100/(1 + [IC_{50}/[I]]^k)$, where k is the Hill coefficient.

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Supporting Information Available: 1D and 2D NMR and HREIMS spectra of compound **12**. This information is available free of charge via the Internet at http://pubs.acs.org.

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