Dysideasterols A – E, Five New Uncommon Polyhydroxylated Steroids from the South China Sea Sponge *Dysidea* sp.

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Five new uncommon polyhydroxylated steroids, dysideasterols A – E (**1** – **5**), were isolated from the South China Sea sponge *Dysidea* sp. Their structures were established by detailed analysis of spectral data and verified *via* single-crystal X-ray-diffraction analysis of compound **1**. The absolute configuration of **1** was determined by the modified *Mosher* method.

Introduction. – Sponges have yielded the most-varied and biogenetically unprecedented array of steroids found among the marine invertebrate phyla. Most of the 200 new monohydroxylated steroids found in marine organisms have been isolated from the sponges [1]. Recently, an increasing number of polyhydroxylated steroids have been isolated. Some authors have postulated that future studies would be likely to discover numerous examples of polar, polyhydroxylated steroids in marine organisms [2]. Sponges of the genus *Dysidea* are widely distributed in tropical and subtropical waters around the world [3]. It has been shown that highly functionalized steroids are widespread in this genus [2][4–9]. In particular, 9,11-dioxygenated steroids, such as (2α,3β,5α,6β,9α,11α)-cholesta-7,22-diene-2,3,5,6,9,11,19-heptol and its analogue [5] are the most common, whereas 9α,11α-epoxysteroids are infrequent. To the best of our knowledge, only one compound possessing 9α,11α-epoxy functionality has been reported so far [2].

As part of our ongoing research on the biologically active substances of Chinese marine invertebrates [10][11], we made a collection of the sponge *Dysidea* sp. off the Lingshui Bay, Hainan Province, China. On separation of the Et_2O-soluble fraction of an acetone extract of this sponge, five new uncommon polyhydroxylated steroids, named dysideasterols A – E (**1** – **5**, *Fig. 1*), were isolated. This paper describes the isolation and structure elucidation of those new compounds.

Results and Discussion. – Freshly collected animals from the South China Sea were immediately put at $-20°$ and kept frozen until used. Frozen material was extracted exhaustively with acetone. The acetone extract was then partitioned between Et_2O and H_2O. The organic extract was repeatedly subjected to column chromatography (silica gel) followed by HPLC purification to afford compounds **1** – **5**.

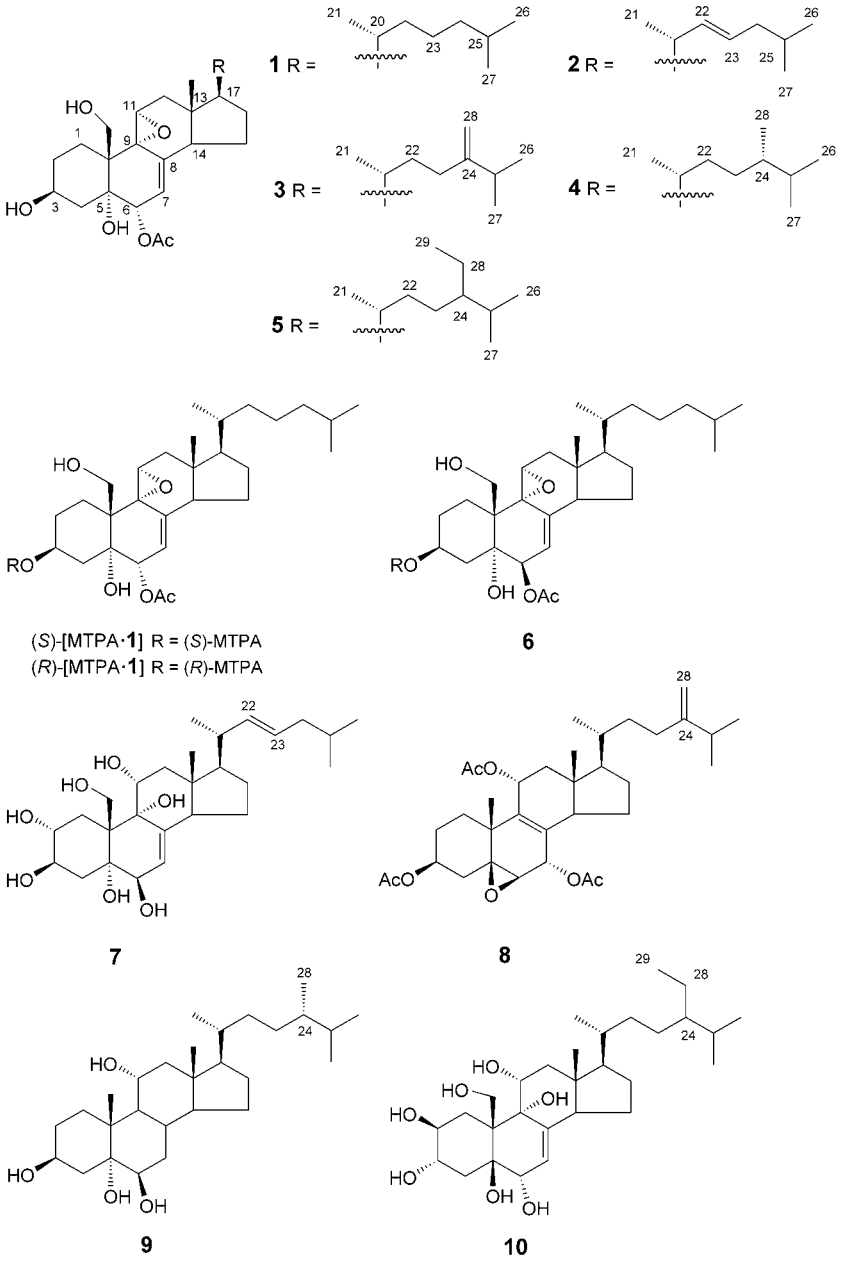

Fig. 1. *Chemical structures of compounds* **1**–**10**

All of those compounds showed very similar spectroscopic properties. Their IR spectra indicated the presence of OH groups (3498 cm^{-1}), an acetate function (1710, 1261 cm^{-1}), and a C=C bond (1639 cm^{-1}). Their ^{13}C-NMR spectra (*Table 1*) were also

Table 1. *^{13}C-NMR Data* (125 MHz) *of Compounds* **1**–**5**. δ in ppm referenced to (D_5)pyridine (δ(C) 149.9).

	1	**2**	**3**	**4**	**5**
C(1)	20.0 (*t*)	19.9 (*t*)	19.9 (*t*)	19.9 (*t*)	19.9 (*t*)
C(2)	31.6 (*t*)	31.6 (*t*)	31.6 (*t*)	31.7 (*t*)	31.6 (*t*)
C(3)	66.8 (*d*)	66.7 (*d*)	66.7 (*d*)	66.7 (*d*)	66.7 (*d*)
C(4)	41.2 (*t*)	41.3 (*t*)	41.3 (*t*)	41.4 (*t*)	41.2 (*t*)
C(5)	74.4 (*s*)	74.4 (*s*)	74.3 (*s*)	74.4 (*s*)	74.4 (*s*)
C(6)	74.6 (*d*)	74.6 (*d*)	74.6 (*d*)	74.6 (*d*)	74.6 (*d*)
C(7)	122.7 (*d*)	122.7 (*d*)	122.6 (*d*)	122.6 (*d*)	122.6 (*d*)
C(8)	141.1 (*s*)	141.0 (*s*)	141.0 (*s*)	141.1 (*s*)	141.1 (*s*)
C(9)	61.1 (*s*)	61.0 (*s*)	61.0 (*s*)	61.0 (*s*)	61.0 (*s*)
C(10)	46.0 (*s*)	46.0 (*s*)	46.0 (*s*)	46.0 (*s*)	46.2 (*s*)
C(11)	54.7 (*d*)	54.6 (*d*)	54.6 (*d*)	54.6 (*d*)	54.6 (*d*)
C(12)	40.7 (*t*)	40.6 (*t*)	40.6 (*t*)	40.6 (*t*)	40.6 (*t*)
C(13)	44.4 (*s*)	44.2 (*s*)	44.4 (*s*)	44.4 (*s*)	44.4 (*s*)
C(14)	47.5 (*d*)	47.6 (*d*)	47.4 (*d*)	47.5 (*d*)	47.5 (*d*)
C(15)	22.7 (*t*)	22.6 (*t*)	22.6 (*t*)	22.6 (*t*)	22.6 (*t*)
C(16)	28.4 (*t*)	29.0 (*t*)	28.3 (*t*)	28.4 (*t*)	28.4 (*t*)
C(17)	56.7 (*d*)	56.5 (*d*)	56.6 (*d*)	56.8 (*d*)	56.8 (*d*)
C(18)	14.0 (*q*)	14.1 (*q*)	14.0 (*q*)	14.0 (*q*)	14.0 (*q*)
C(19)	61.2 (*t*)	61.2 (*t*)	61.1 (*t*)	61.1 (*t*)	61.1 (*t*)
C(20)	36.4 (*d*)	40.5 (*d*)	36.0 (*d*)	36.2 (*d*)	36.5 (*d*)
C(21)	18.7 (*q*)	20.8 (*q*)	18.6 (*q*)	18.5 (*q*)	18.7 (*q*)
C(22)	36.2 (*t*)	138.3 (*d*)	34.8 (*t*)	33.9 (*t*)	34.1 (*t*)
C(23)	24.3 (*t*)	126.9 (*d*)	31.4 (*t*)	30.7 (*t*)	26.6 (*t*)
C(24)	39.9 (*t*)	42.2 (*t*)	156.8 (*s*)	39.3 (*d*)	46.4 (*d*)
C(25)	28.4 (*d*)	28.8 (*d*)	34.2 (*d*)	31.8 (*d*)	29.6 (*d*)
C(26)	22.8 (*q*)[a])	22.4 (*q*)[a])	22.1 (*q*)[a])	17.8 (*q*)[a])	19.3 (*q*)[a])
C(27)	23.1 (*q*)[a])	22.5 (*q*)[a])	22.2 (*q*)[a])	20.5 (*q*)[a])	20.1 (*q*)[a])
C(28)	–	–	106.7 (*t*)	15.7 (*q*)	23.5 (*t*)
C(29)	–	–	–	–	12.3 (*q*)
*Me*COO	21.1 (*q*)	21.0 (*q*)	21.1 (*q*)	21.1 (*q*)	21.1 (*q*)
Me*C*OO	171.3 (*s*)	171.1 (*s*)	171.1 (*s*)	171.1 (*s*)	171.1 (*s*)

[a]) Signals may be interchanged.

very similar showing six C-signals between δ 55.0 and 75.0 indicating the presence of six sites of heteroatom functionality, along with a trisubstituted C=C (δ 122.7 (*d*), 141.1 (*s*)). Moreover, careful analysis of their ^{1}H,^{1}H-COSY, HMQC, and HMBC data revealed that compounds **1**–**5** all possessed an identical (3β,5α,6α,9α,11α)-9,11-epoxycholest-7-ene-3,5,6,19-tetrol steroid framework and varied only in the side chains. Therefore, it appeared that we had only to establish the substitution pattern and configuration of the most abundant steroid **1** to gain insight into the whole series.

Dysideasterol A (**1**) was obtained as optically active colorless crystalline solid. Its molecular formula was established as $C_{29}H_{46}O_6$ by HR-ESI-MS, which showed a quasi-molecular-ion peak at *m*/*z* 491.4258 ($[M+H]^+$). The comparison of the ^{1}H-NMR data (*Table 2*) of **1** and model compound **6**, a polyhydroxylated steroid isolated from an unidentified sponge *Dysidea* sp. [2], showed strong similarity of the data. To verify whether our structural assignment for compound **1** was correct, we subjected **1** to a single-crystal X-ray-diffraction analysis (see *Exper. Part*). A computer-generated perspective drawing of the final X-ray model is given in *Fig. 2*. Additional crystallo-

Table 2. *^{1}H-NMR Data for Compounds* **1** *and* **6**. δ in ppm, J in Hz.

	1		**6**[c])	
	C_5D_5N[a])	$CDCl_3$[b])	C_5D_5N	$CDCl_3$
H_α–C(1)	2.20 (*m*)			
H_β–C(1)	2.61 (*dd*, *J* = 14.4, 3.6)			
H_α–C(2)	2.36 (*m*)			
H_β–C(2)	2.20 (*m*)			
H_α–C(3)	4.81 (*m*)	4.02 (*m*)	4.75 (*m*)	4.06 (*m*)
H_α–C(4)	2.83 (*dd*, *J* = 12.7, 3.4)	2.04 (*dd*, *J* = 13.1, 4.8)	2.77 (*dd*, *J* = 14.2, 5.2)	2.07 (*dd*, *J* = 14.2, 5.2)
H_β–C(4)	2.19 (*t*-like, *J* = 12.7)			
H–C(6)	5.91 (*t*, *J* = 1.9)	5.26 (*t*, *J* = 1.9)	5.67 (br. *s*)	5.32 (*dd*, *J* = 3.5, 0.8)
H–C(7)	5.73 (br. *s*)	5.16 (*t*, *J* = 1.9)	5.86 (br. *s*)	5.22 (*dd*, *J* = 3.5, 1.0)
H_β–C(11)	3.97 (*d*, *J* = 5.5)	3.38 (*d*, *J* = 5.6)	3.92 (*d*, *J* = 4.7)	3.40 (*d*, *J* = 4.7)
H_α–C(12)	2.01 (*d*, *J* = 14.6)	1.81 (*d*, *J* = 15.0)	1.94 (*d*, *J* = 15.0)	
H_β–C(12)	2.36 (*dd*, *J* = 14.6, 5.5)	2.16 (*dd*, *J* = 15.0, 5.7)	2.29 (*dd*, *J* = 15.0, 4.7)	2.18 (*dd*, *J* = 15.0, 6.3)
H_α–C(14)	2.69 (*m*)	2.32 (*m*)	2.62 (*m*)	2.36 (*m*)
CH_2(15)	1.64 (*m*)			
CH_2(16)	1.24, 1.87 (2*m*)			
H_α–C(17)	1.26 (*m*)			
Me(18)	0.89 (*s*)	0.56 (*s*)	0.82 (*s*)	0.60 (*s*)
H_a–C(19)	4.27 (*d*, *J* = 11.5)	3.76 (*d*, *J* = 10.8)	4.21 (*dd*, *J* = 12.6, 5)	3.80 (*dd*, *J* = 12, 4.4)
H_b–C(19)	4.57 (*d*, *J* = 11.5)	3.95 (*d*, *J* = 10.8)	4.50 (*dd*, *J* = 12.6, 5)	
H–C(20)	1.03 (*m*)		1.50 (*m*)	
Me(21)	0.93 (*d*, *J* = 6.6)	0.85 (*d*, *J* = 6.1)	0.87 (*d*, *J* = 7)	0.92 (*d*, *J* = 7)
CH_2(22)	1.38 (*m*)			
CH_2(23)	1.16, 1.39 (2*m*)			
CH_2(24)	1.18 (*m*)			
H–C(25)	1.57 (*m*)		1.50 (*m*)	
Me(26)[d])	0.93 (*d*, *J* = 6.6)	0.82 (*d*, *J* = 6.5)	0.87 (*d*, *J* = 7)	0.86 (*d*, *J* = 7)
Me(27)[d])	0.93 (*d*, *J* = 6.6)	0.82 (*d*, *J* = 6.5)	0.87 (*d*, *J* = 7)	0.86 (*d*, *J* = 7)
AcO	2.10 (*s*)	2.12 (*s*)	2.01 (*s*)	2.15 (*s*)
OH–C(3)			6.21 (*d*, *J* = 1)	
OH–C(5)			5.82 (*s*)	
OH–C(19)	6.54 (br. *s*)		6.55 (*t*, *J* = 4)	

[a]) At 400 MHz; δ referred to C_5D_5N (δ 7.31). [b]) At 400 MHz; δ referred to $CDCl_3$ (δ 7.22). [c]) Data from *Gunasekera* and *Schmitz* [2]. [d]) Signals may be interchanged.

graphic information is available[1]). The suggested absolute configuration of dyside-asterol A (**1**) is that of the natural sterols. However, to confirm this, **1** was esterified separately with (*S*)- and (*R*)-MTPA-Cl (=(αS)- and (αR)-α-methoxy-α-(trifluoro-methyl)benzeneacetyl chloride) and the corresponding MTPA esters (*R*)-[MTPA · **1**] and (*S*)-[MTPA · **1**], respectively, were analysed by a 2D-COSY experiment. This allowed us to assign the absolute configuration (*S*), *i.e.*, β, to the secondary-alcohol moiety at C(3), which, in turn, established the complete absolute configuration of **1** as (3β,5α,6α,9α,11α)-9,11-epoxycholest-7-ene-3,5,6,19-tetrol 6-acetate.

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[1]) Crystal data: $C_{29}H_{46}O_6$ *M*r 490.66; monoclinic, *P*2 (1); $a = 11.4638$ (12), $b = 6.2666$ (7), $c = 19.6140$ (2) Å; $V = 1402.5$ (3) Å^3; $Z = 2$; $D_{calc} = 1.162$ g cm^{-3}; λ 0.71073 Å. Atomic coordinates for this structure have been deposited at the *Cambridge Crystallographic Data Centre* (CCDC No. 251507); copy of the data can be obtained, free of charge, on application to *CCDC*, 12 Union Road, Cambridge CB21EZ, UK (fax: +44 (0) 1223-336033; or e-mail: deposit@ccdc.cam.ac.uk).

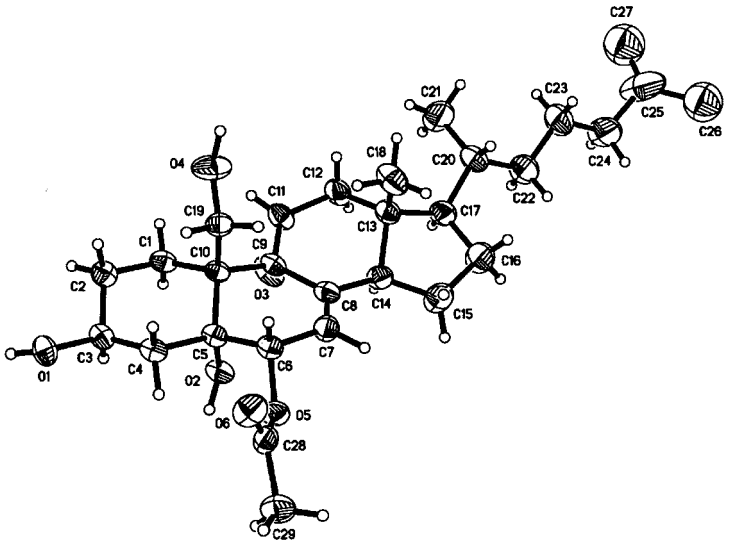

Fig. 2. *Computer-generated perspective drawing of compound* **1** *showing the relative configuration*

The ^{1}H-NMR spectrum of **1** (*Table 2*) exhibited signals for 4 of the 5 Me groups typical of a cholesterol: δ 0.56 (*s*, Me(18)), 0.816 (*d*, $J = 6.5$ Hz, Me(26)), 0.821 (*d*, $J = 6.5$ Hz, Me(27)), and 0.85 (*d*, $J = 6.1$ Hz, Me(21)). Although the *s* typical for Me(19) was absent, 2 *d* at δ 3.76 (*d*, $J = 10.8$ Hz, H_a–C(19)) and 3.95 (*d*, $J = 10.8$ Hz, H_b–C(19)) indicated the presence of an isolated CH_2OH group in place of Me(19). The presence of an acetate group was confirmed by the ^{1}H-NMR signal at δ 2.12 (*s*, AcO) and a ^{13}C-NMR signal in the ester carbonyl region (δ 171.3, *s*). The only substantial difference in the ^{1}H-NMR of **1** and **6** were the coupling constants between H–C(6) and H–C(7) (**1**: $J(6,7) = 1.9$ Hz; **6**: $J(6,7) = 3.5$ Hz) implying that **1** possessed the same (3β,5α,9α,11α)-9,11-epoxycholest-7-ene-3,5,19-triol steroid structure as **6** with a cholesterol-type side chain. The suggested configuration of **1** was further supported by the NOEs between H_a–C(19)/H_{ax}–C(6), H_{ax}–C(6)/H_{ax}–C(4), Me(18)/H_{ax}–C(11) and H_{eq}–C(12), and H_{ax}–C(14)/H_{ax}–C(12) and H_{eq}–C(17) (*Fig. 3*). The NOE between H_a–C(19) and H–C(6), together with the small coupling constant $J(6,7)$ (= 1.9 Hz; in accord with a dihedral angle $\Phi(6\beta,7) \approx 85°$), determined the configuration at C(6), which was not self-evident because of the twisted conformation of ring B.

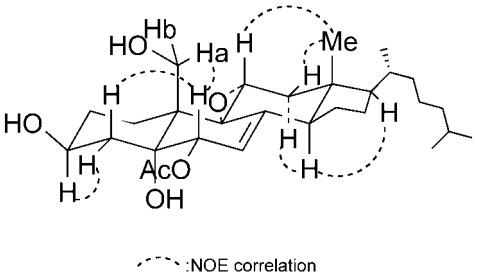

Fig. 3. *Selected NOESY correlations of* **1**

The $\Delta\delta$ ($= \delta_S - \delta_R$) values of the protons near the esterified C-atom (C-atom (C(3)) of (*S*)- and (*R*)-[MTPA·**1**] are summarized in *Fig. 4*. The negative $\Delta\delta$ values for H_α–C(2), H_a–C(19), H_b–C(19), H_β–C(11), H_α–C(12), H_β–C(12), and Me(18), and the positive $\Delta\delta$ values for H_α–C(4), H_β–C(6), H–C(7), H_α–C(14), and AcO were interpreted by the MTPA determination rule [12–17] and established (3*S*) configuration of **1**.

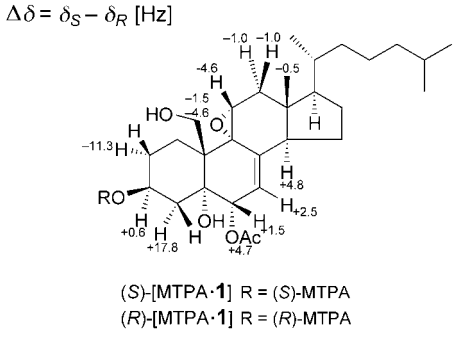

Fig. 4. *Δδ Values* [Hz] *obtained for the protons near C(3) of the* Mosher *esters (*S*)- and (*R*)-[MTPA·1]*

Dysideasterol B (**2**) was obtained as an optically active white amorphous powder. The molecular formula of **2** was inferred as $C_{29}H_{44}O_6$ from the HR-ESI-MS (*m/z* 511.3015 ($[M+Na]^+$)). The ^{1}H- and ^{13}C-NMR (*Table 1*) data of **2** were very similar to those of **1**. Comparison of the δ(C) assigned to the side-chain C-atoms of **2** with those of the model compound (2α,3β,5α,6β,9α,11α)-cholesta-7,22-diene-2,3,5,6,9,11,19-heptol (**7**), confirmed the proposed assignment [4].

The two additional olefinic signals in the ^{13}C-NMR spectrum of **2** (as compared to **1**), resonating at δ 138.3 and 126.9, as well as two additional downfield signals in the ^{1}H-NMR spectrum at δ 5.17 (*dd*, *J* = 8.6, 15.4 Hz, H–C(22) and 5.26 (*m*, H–C(23)), indicated the presence of a disubstituted C=C bond with (*E*)-configuration in the side chain of **2**.

Dysideasterol C (**3**) was obtained as an optically active white amorphous powder. The ESI-MS of **3** prominently showed a quasi-molecular-ion peak at *m/z* 1027.5 ($[2M+Na]^+$), and its molecular formula was inferred as $C_{30}H_{46}O_6$ from the HR-ESI-MS (*m/z* 525.3199 ($[M+Na]^+$)). It was immediately apparent from the ^{1}H- and ^{13}C-NMR spectra (*Table 1*) that **3** differed from **1** only in the side chain. The NMR data of the suggested methylene-substituted side chain of **2** agreed quite well with that of (3β,5β,6β,11α)-5,6-epoxyergost-8-ene-3,7,11-triol triacetate (**8**) [18], confirming the proposed structure of **3**.

The only substantial difference in the ^{1}H-NMR spectrum of **1** and **3** were the appearance of two additional downfield signals for **3** at δ 4.78 (br. *s*, H_a–C(28)) and 4.81 (br. *s*, H_b–C(28)). Similarly, the main differences in the ^{13}C-NMR spectra of **1** and **3** were the additional signals of an olefinic CH_2 group (δ 106.7) and a corresponding sp^2 quaternary C-atoms (δ 156.8) for **3** that could not be found in **1**.

Dysideasterol D (**4**) was obtained as an optically active white amorphous powder. The molecular formula of **4** was determined to be $C_{30}H_{48}O_6$ (HR-ESI-MS; *m/z* 527.3342 ($[M+Na]^+$), differing from the molecular formula of **3** by two additional H-atoms. The ^{13}C-NMR data of **4** revealed the presence of a Me group (δ 15.7 C(28)), located at C(24), instead of the CH_2= moiety of **3**. The configuration of C(24) was assigned as (*S*) since all ^{13}C-NMR data for the side chain of **4** were in good agreement with those of sarcoaldesterol B (**9**) [19].

Dysideasterol E (**5**) was obtained as an optically active white amorphous powder. The HR-ESI-MS of **5** established a molecular formula of $C_{31}H_{50}O_6$ (*m/z* 541.3492

($[M+Na]^+$). The ^{1}H- and ^{13}C-NMR spectral data were quite similar to those of **4**, indicating that they were side-chain homologues, **4** carrying a Me group at C(24) and **5** an Et group. A close agreement of the ^{13}C-NMR resonances assigned to the side-chain C-atoms of **5** with those of the model compound (2β,3α,5β,6α,9α,11α)-stigmast-7-ene-2,3,5,6,9,11,19-heptol (**10**) [5] confirmed the nature of the side chain. The absolute configuration at C(24) remains unidentified.

It is clear from our work and the reports of *Capon* and *Faulkner* [4] and *Gunasekera* and *Schmitz* [2] that highly functionalized steroids are widespread in the sponge genus *Dysidea*, but what role they play in the sponge remains undetermined. Their high level of functionality makes it unlikely that they contribute to the unique cell-membrane structure in the sponge; similar steroids do commonly not appear in other members of the Demospongiae. The similarities between these compounds and the crustecdysones could imply that they function as feeding deterrents to potential crustacean predators. An extension of this line of thought leads to the consideration that they could be kairomones which induce settling and metamorphosis in larvae of the dorid nudibranches known to be associated with the genus *Dysidea*.

Compound **1** was slightly cytotoxic against A-549, HL-60, and P-388 cell lines.

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Experimental Part

General. Reversed-phase HPLC: *Agilent-1100* liquid chromatograph, with a *VWD G1314A* detector set at 210 nm; purification with one semi-prep. *ODS HG-5* (5 µm) column (10 mm (i.d.) × 25 cm). CC = column chromatography. M.p.: *SGW X-4* hot-stage microscope; uncorrected. Optical rotations: *Perkin-Elmer 241-MC* polarimeter; in $CHCl_3$. X-Ray: *Bruker SMART-APEX-CCD* diffractometer. IR Spectra: *Nicolet Magna-FT-IR-750* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-500* spectrometer; $SiMe_4$ as internal standard; δ in ppm, *J* in Hz. MS: *Finnigan MAT-95* mass spectrometer.

Animal Material. Specimens of *Dysidea* sp., identified by Prof. *J.-H. Li* of the Institute of Oceanology, Chinese Academy of Sciences, were collected in June 2002, by scuba techniques at a depth of − 10 m off Sanya, Hainan Province, China, in the South China Sea. The sponge materials were frozen immediately and transferred to the Shanghai Institutes of Biological Sciences (SIBS), where it was kept at − 20° until extraction. The sponge is whitish in color and thinly encrusting to massive lobose in form. The surface of the sponge is densely conulose with blunt conules 1.0-mm high and 1.0–2.0-mm apart. The consistency is firm, crumbly, and sandy. The skeleton is dense, anisotropic, and consisting of sand-filled primary and secondary fibers, forming fascicles near the surface. The surface aspect and skeletal characters conform to the genus *Dysidea*, but there are no matching descriptions at the species level for this sponge. A voucher specimen is stored for inspection at the SIBS, registration No. 02SS-38.

Extraction and Isolation. The frozen animals (500 g of dry weight) were cut into small pieces and exhaustively extracted with acetone at r.t. The extract was concentrated, and the resulting residue was extracted with Et_2O and BuOH. The Et_2O-soluble portion was repeatedly subjected to CC (silica gel, MeOH/$CHCl_3$ gradient): mixture of **1**–**5**. This mixture was further purified by reversed-phase HPLC (*ODS-18* column (10 mm (i.d.) × 25 cm), MeOH/H_2O 85 : 15): pure **1** (24.1 mg), **2** (1.3 mg), **3** (1.4 mg), **4** (1.4 mg), and **5** (0.8 mg), all as amorphous white solids.

Dysideasterol A (= *3β,5α,6α,9α,11α)-9,11-Epoxycholest-7-ene-3,5,6,19-tetrol 6-Acetate*; **1**). Crystalline solid. M.p. 204–205°. $[\alpha]_D^{20} = +60$ ($c = 0.13$, $CHCl_3$). ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. EI-MS: 490 (M^+), 472 ($[M-H_2O]^+$), 412 ($[M-H_2O-AcOH]^+$). ESI-MS: 491.4 ($[M+H]^+$), 1003.4 ($[2M+Na]^+$).

Mosher *Esters of* **1**. (+)-(*R*)-MTPA acid was treated with oxalyl chloride in dry CH_2Cl_2 and a few drops of DMF for *ca*. 3 h under stirring at r.t.: (+)-(*S*)-MTPA-Cl. The (*R*)-[MTPA·**1**] was obtained by treating **1**

(5.0 mg) with freshly obtained (+)-(*S*)-MTPA-Cl in dry pyridine for *ca.* 16 h under stirring at r.t. The ester was purified by CC (silica gel): pure (*R*)-[MTPA · **1**] (0.8 mg).

Similarly, (*S*)-[MTPA · **1**] (1.1 mg) was prepared from (−)-(*S*)-MTPA acid.

*Data of (*R*)-[MTPA · **1**]:* ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.596 (*s*, Me(18)); 0.862 (*d*, *J* = 6.6, Me(26)); 0.867 (*d*, *J* = 6.6, Me(27)); 0.899 (*d*, *J* = 6.0, Me(21)); 1.862 (*d*, *J* = 14.9, H_α−C(12)); 2.154 (*s*, AcO); 2.198 (*dd*, *J* = 5.8, 14.9, H_β−C(12)); 2.355 (*t*, *J* = 9.8, H_α−C(14)); 3.396 (*d*, *J* = 5.5, H_β−C(11)); 3.818 (*d*, *J* = 10.6, H_a−C(19)); 3.965 (*d*, *J* = 10.6, H_b−C(19)); 5.162 (*d*, *J* = 2.1, H−C(7)); 5.297 (br. *s*, H_β−C(6)); 5.384 (*m*, H_β−C(3)).

*Data of (*S*)-[MTPA · **1**]:* ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.595 (*s*, Me(18)); 0.862 (*d*, *J* = 6.6, Me(26)); 0.866 (*d*, *J* = 6.6, Me(27)); 0.897 (*d*, *J* = 6.1, Me(21)); 1.860 (*d*, *J* = 14.8, H_α−C(12)); 2.165 (*s*, AcO); 2.195 (*dd*, *J* = 5.7, 14.8, H_β−C(12)); 2.372 (*t*, *J* = 9.8, H_α−C(14)); 3.387 (*d*, *J* = 5.5, H_β−C(11)); 3.814 (*d*, *J* = 10.5, H_a−C(19)); 3.956 (*d*, *J* = 10.5, H_b−C(19)); 5.193 (*d*, *J* = 2.0, H−C(7)); 5.301 (br. *s*, H_β−C(6)); 5.385 (*m*, H_β−C(3)).

X-Ray Diffraction of **1**[1]). Colorless block crystals of **1** were obtained by recrystallization (MeOH). The crystal (0.375 × 0.186 × 0.179 mm) belongs to the monoclinic system, space group *P*2 (1), with *a* = 11.4638 (12), *b* = 6.2666 (7), *c* = 19.6140 (2) Å; *V* = 1402.5 (3) Å^3, *Z* = 2, D_{calc} = 1.162 g cm^{-3}; λ 0.71073 Å. Intensity data were measured with a *Bruker SMART-APE × CCD* diffractometer. A total of 8637 reflections were collected in the range $1.78^\circ < 2\theta < 28.34^\circ$ by using the ω-2θ scan technique at 293(2) K. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. SADABs were applied to the absorption data corrections. All non-H-atoms were given anisotropic thermal parameters. The refinement converged to the final *R* = 0.0628, R_w = 0.1461 for 6061 observed reflections with $I > 2\sigma(I)$ and 536 variable parameters.

Dysideasterol B (*=(3β,5α,6α,9α,11α)-9,11-Epoxycholesta-7,22-diene-3,5,6,19-tetrol 6-Acetate*; **2**). White powder. $[\alpha]_D^{20} = +42$ (*c* = 0.11, $CHCl_3$). ^{1}H-NMR (C_5D_5N, 600 MHz): 0.82 (*s*, Me(18)); 0.84 (*d*, *J* = 6.7, Me(26), Me(27)); 0.94 (*d*, *J* = 6.7, Me(21)); 1.96 (*s*, AcO); 2.23 (*dd*, *J* = 5.5, 14.6, H_β−C(12)); 2.54 (*dd*, *J* = 3.6, 14.6, H_β−C(1)); 2.60 (*m*, H_α−C(14)); 2.72 (*dd*, *J* = 3.8, 12.6, H_α−C(4)); 3.88 (*d*, *J* = 5.6, H_β−C(11)); 4.18 (*dd*, *J* = 4.8, 10.7, H_a−C(19)); 4.48 (*dd*, *J* = 3.8, 10.7, H_b−C(19)); 4.71 (*m*, H_β−C(3)); 5.17 (*dd*, *J* = 8.6, 15.4, H−C(22)); 5.26 (*m*, H−C(23)); 5.64 (br. *s*, H−C(7)); 5.76 (*s*, 1 OH); 5.83 (br. *s*, H−C(6)); 6.14 (br. *s*, 1 OH); 6.49 (br. *s*, 1 OH). ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 489.4 ($[M+H]^+$), 999.1 ($[2M+Na]^+$). HR-ESI-MS: 511.3015 ($[M+Na]^+$, $C_{29}H_{44}O_6^+$; calc. 511.3036).

Dysideasterol C (*=(3β,5α,6α,9α,11α)-9,11-Epoxyergosta-7,24(28)-diene-3,5,6,19-tetrol 6-Acetate*; **3**). White powder. $[\alpha]_D^{20} = +42$ (*c* = 0.12, $CHCl_3$). ^{1}H-NMR (C_5D_5N, 600 MHz): 0.78 (*s*, Me(18)); 0.85 (*d*, *J* = 6.4, Me(21)); 1.01 (*d*, *J* = 6.7, Me(26)); 1.02 (*d*, *J* = 6.7, Me(27)); 1.98 (*s*, AcO); 2.54 (*dd*, *J* = 3.5, 14.0, H_β−C(1)); 2.58 (*m*, H_α−C(14)); 2.73 (*dd*, *J* = 3.6, 13.5, H_α−C(4)); 3.87 (*d*, *J* = 5.3, H_β−C(11)); 4.18 (*dd*, *J* = 4.5, 10.5, H_a−C(19)); 4.48 (*dd*, *J* = 3.8, 10.5, H_b−C(19)); 4.71 (*m*, H_β−C(3)); 4.78 (br. *s*, H_a−C(28)); 4.81 (br. *s*, H_b−C(28)); 5.63 (br. *s*, H−C(7)); 5.74 (*s*, 1 OH); 5.83 (*s*, H−C(6)); 6.14 (br. *s*, 1 OH); 6.49 (br. *s*, 1 OH). ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 1027.5 ($[2M+Na]^+$). HR-ESI-MS: 525.3199 ($[M+Na]^+$, $C_{30}H_{46}O_6^+$; calc. 525.3192).

Dysideasterol D (*=(3β,5α,6α,9α,11α)-9,11-Epoxyergost-7-ene-3,5,6,19-tetrol 6-Acetate*; **4**). White powder. $[\alpha]_D^{20} = +53$ (*c* = 0.12, $CHCl_3$). ^{1}H-NMR (C_5D_5N, 600 MHz): 0.76–0.85 (*m*, Me(18), Me(21), Me(26), Me(27), Me(28)); 1.92 (*d*, *J* = 14.4, H_α−C(12)); 1.98 (*s*, AcO); 2.54 (*dd*, *J* = 3.8, 14.0, H_β−C(1)); 2.59 (*m*, H_α−C(14)); 2.72 (*dd*, *J* = 3.9, 13.6, H_α−C(4)); 3.88 (*d*, *J* = 5.6, 1 H_β−C(11)); 4.18 (*dd*, *J* = 4.4, 10.5, H_a−C(19)); 4.48 (br. *d*, *J* = 10.5, H_b−C(19)); 4.71 (*m*, H_β−C(3)); 5.64 (br. *s*, H−C(7)); 5.74 (*s*, 1 OH); 5.83 (br. *s*, H−C(6)); 6.14 (br. *s*, 1 OH); 6.48 (br. *s*, 1 OH). ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 505.0 ($[M+H]^+$), 1031.7 ($[2M+Na]^+$). HR-ESI-MS: 527.3342 ($[M+Na]^+$, $C_{30}H_{48}O_6^+$; calc. 527.3349).

Dysideasterol E (*=(3β,5α,6α,9α,11α)-9,11-Epoxystigmast-7-ene-3,5,6,19-tetrol 6-Acetate*; **5**). White powder. $[\alpha]_D^{20} = +30$ (*c* = 0.16, $CHCl_3$). ^{1}H-NMR (C_5D_5N, 600 MHz): 0.81–0.87 (*m*, Me(18), Me(21), Me(26), Me(27), Me(29)); 1.92 (*d*, *J* = 14.4, H_α−C(12)); 1.98 (*s*, AcO); 2.58 (*m*, H_α−C(14)); 2.72 (*dd*, *J* = 3.2, 13.3, 1 H_α−C(4)); 3.88 (*d*, *J* = 5.6, H_β−C(11)); 4.18 (*dd*, *J* = 4.1, 10.3, H_a−C(19)); 4.48 (br. *d*, *J* = 10.3, H_b−C(19)); 4.72 (*m*, H_β−C(3)); 5.64 (br. *s*, H−C(7)); 5.74 (*s*, 1 OH); 5.83 (br. *s*, H−C(6)); 6.14 (br. *s*, 1 OH); 6.48 (br. *s*, 1 OH). ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 519.4 ($[M+H]^+$, 1059.5 ($[2M+Na]^+$). HR-ESI-MS: 541.3492 ($[M+Na]^+$, $C_{31}H_{50}O_6^+$; calc. 541.3505).

REFERENCES

[1] V. M. D'Auria, L. Minale, R. Riccio, *Chem. Rev.* **1993**, *93*, 1839.
[2] S. P. Gunasekera, F. J. Schmitz, *J. Org. Chem.* **1983**, *48*, 885.
[3] S. H. Gorde, J. H. Cardellina II, *J. Nat. Prod.* **1984**, *47*, 76.

[4] R. J. Capon, D. J. Faulkner, *J. Org. Chem*. **1985**, *50*, 4771.
[5] R. R. West, J. H. Cardellina, *J. Org. Chem*. **1988**, *53*, 2782.
[6] R. R. West, J. H. Cardellina, *J. Org. Chem*. **1989**, *54*, 3234.
[7] S. Isaacs, R. Berman, Y. Kashman, *J. Nat. Prod*. **1991**, *54*, 83.
[8] T. S. Milkova, B. P. Mikhova, N. M. Nikolov, S. S. Popov, *J. Nat. Prod*. **1992**, *55*, 974.
[9] A. Casapullo, L. Minale, F. Zollo, *Tetrahedron Lett*. **1995**, *36*, 2669.
[10] X.-C. Huang, D. Zhao, Y.-W. Guo, H.-M. Wu, Z.-H. Wang, Y.-S. Lin, *Bioorg. Med. Chem. Lett*. **2004**, *14*, 3117.
[11] Z.-Y. Shao, J.-N. Cai, Q.-Z. Ye, Y.-W. Guo, *J. Asian Nat. Prod. Res.* **2002**, *4*, 205.
[12] J. A. Dale, H. S. Mosher, *J. Am. Chem. Soc*. **1973**, *95*, 512.
[13] G. R. Sullivan, J. A. Dale, H. S. Mosher, *J. Org. Chem*. **1973**, *38*, 2143.
[14] T. Kusumi, I. Ohtani, Y. Inouye, H. Kakisawa, *Tetrahedron Lett*. **1988**, *29*, 4731.
[15] I. Ohtani, T. Kusumi, O. M. Ishtsuka, H. Kakisawa, *Tetrahedron Lett*. **1989**, *30*, 3147.
[16] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Org. Chem.* **1991**, *56*, 1296.
[17] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc*. **1991**, *113*, 4092.
[18] S. Isaacs, R. Berman, Y. Kashiman, *J. Nat. Prod*. **1991**, *54*, 83.
[19] A. Umeyama, N. Shoji, M. Ozeki, S. Arihara, *J. Nat. Prod*. **1996**, *59*, 894.

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