Bioactive Sesquiterpenes from A Taiwanese Marine Sponge *Parahigginsia* sp.

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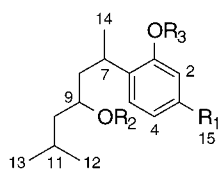
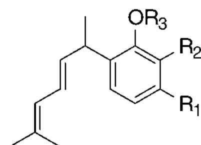
Five new sesquiterpenes, parahigginols A−D (**1**−**4**) and parahigginic acid (**5**), have been isolated from a Taiwanese marine sponge *Parahigginsia* sp. The structural assignments of the new compounds were based on their spectral data, including 1D and 2D NMR. Biological studies revealed that compounds **2**−**5** exhibited cytotoxicity against murine P-388 and human KB16, A549, and HT-29 tumor cells.

Sesquiterpene hydrocarbons such as curcuphenols have been found in the Caribbean gorgonian coral *Pseudopterogorgia rigida* and in a Japanese marine sponge *Epipolasis* sp.[1,2] These compounds showed interesting biological activities. For example, (+)-curcuphenol and dehydrocurcuphenol inhibit H, K-ATPase activity. (−)-Curcuphenol and related compounds showed antibacterial activity against *Staphylococcus aureus*. As part of our interest in the discovery of new and bioactive compounds from marine organisms,[3−6] we have investigated the Taiwanese marine sponge *Parahigginsia* sp. This species was collected from Green Island, Taiwan, at a depth of 10 m. Its skeleton appears to closely resemble that of *P. phakelioides* Dendy, which was found in 1924, but with two categories of oxeas. General silica gel column chromatography of the $(CH_3)_2CO$ extract of the sponge gave five new sesquiterpenes, parahigginols A−D (**1**−**4**) and parahigginic acid (**5**). We report here the isolation, structure elucidation, and cytotoxicity of these new sesquiterpene hydrocarbons.



1, $R_1 = CH_3$, $R_2 = H$, $R_3 = H$
2, $R_1 = CHO$, $R_2 = Ac$, $R_3 = H$
3, $R_1 = CH_3$, $R_2 = Ac$, $R_3 = H$
6, $R_1 = CH_3$, $R_2 = Ac$, $R_3 = Ac$



4, $R_1 = CHO$, $R_2 = OH$, $R_3 = H$
5, $R_1 = COOH$, $R_2 = H$, $R_3 = CH_3$

Results and Discussion

By combination of Si gel column and reversed-phase HPLC, the $(CH_3)_2CO$-soluble fraction from the $(CH_3)_2CO$ extract of the sponge *Parahigginsia* sp. yielded five new sesquiterpenes, which we have named parahigginols A−D (**1**−**4**) and parahigginic acid (**5**). The structures of these new compounds were determined by spectral methods including 2D NMR techniques such as COSY, HMQC, and HMBC.

Parahigginol A (**1**), $[\alpha]_D$ −4.8° ($CHCl_3$), was isolated as a viscous oil. Its molecular formula $C_{15}H_{24}O_2$ was established by high-resolution EIMS (*m/z* 236.1769) and was consistent with the data of ^{1}H, ^{13}C, and DEPT NMR. An IR band (3450 cm^{-1}) and UV absorption (276 nm) indicated the presence of a phenol moiety. The ^{1}H NMR spectrum (Table 1) of **1** was readily interpreted to support an aromatic methyl singlet (δ 2.28) and three aromatic signals for a 1,2,4-trisubstituted benzene ring (δ 6.71 s; 7.04 d, J = 7.8 Hz; 6.74 d, J = 7.8 Hz). Upon acetylation, compound **1** yielded a diacetate (**6**), which showed a molecular ion at *m/z* 320 in the EI mass spectrum. In addition, a methine proton was down-shifted from δ 3.35 in **1** to δ 4.76 in **6**. The COSY spectrum of **1** established the proton connectivities, in which a methyl doublet (δ 1.30, J = 7.5 Hz, H-14) was coupled to the methine multiplet at δ 3.33 (H-7). Another methine multiplet at δ 3.35 showed connection with two isolated methylenes (δ 1.70, 2H, m; δ 1.30, 1H, m; δ 1.50, 1H, m), respectively, indicating a hydroxyl group at the C-9 position. Finally, signals for an isopropyl group appeared at δ 0.80 (6H, d, J = 6.6 Hz) and δ 1.66 (1H, m). The assigned structure of parahigginol A was fully supported by ^{13}C NMR (Table 2) and DEPT spectra of **1**. Comparison of carbon data with those of curcuphenol revealed that they contain the same 2-hydroxy-4-methyl-1-alkylphenyl moiety (δ 153.9 s, 117.8 d, 128.7 s, 121.2 d, 126.2 d, 136.0 s). The remaining side chain consists of the other three methyl carbons (δ 21.0 q, 23.1 q, and δ 22.4 q), two methylene carbons (δ 46.8 t, 47.1 t), two methine carbons (δ 24.7 d, 27.1 d), and an oxygen-bearing carbon (δ 68.6 d).

Parahigginols B (**2**), $[\alpha]_D$ −11.4° ($CHCl_3$), and C (**3**), $[\alpha]_D$ −9.5° ($CHCl_3$), were obtained as optically active oils that showed UV absorptions for the phenolic function similar to those of **1**. The IR spectrum of **2** further illustrated the existence of hydroxyl (3430 cm^{-1}), benzaldehyde (1698, 2700 cm^{-1}), and acetyl (1734 cm^{-1}) functionalities. The high-resolution EI mass spectra of **2** and **3** established their molecular formulas as $C_{17}H_{24}O_4$ (*m/z* 292.1660) and $C_{17}H_{26}O_3$ (*m/z* 278.1883), respectively. Moreover, the fragment ion at *m/z* 232 in **2** and ion at *m/z* 218 in **3** in the EI mass spectra indicated the loss of a molecule of acetic acid from each molecular ion. Close comparison of the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) of **2** and **3** with those of **1** revealed that compounds **2** and **3** are analogues of **1**. The presence of an acetyl group in **2** and **3** was also clear from observation of an acetyl singlet at δ 2.05 and 2.08 in each ^{1}H NMR spectrum. The methine proton of C-9 was shifted downfield from δ 3.35 in **1** to δ 4.75 in **2** and δ 4.76 in **3**, suggesting the acetoxy at the C-9 position for compounds **2** and **3**. Acetylation of **3** provided a product

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Table 1. ^{1}H NMR Data ($CDCl_3$) for Compounds **1**–**5**[a,b]

	1	**2**	**3**	**4**	**5**
2	6.71 (s)	7.19 (d, 1.4)	6.48 (s)		7.49 (s)
4	7.04 (d, 7.8)	7.39 (dd, 7.8, 1.4)	7.00 (d, 7.8)	7.09 (d, 8.2)	7.58 (d, 7.8)
5	6.74 (d,7.8)	7.29 (d, 7.8)	6.68 (d, 7.8)	6.86 (d, 8.2)	7.22 (d, 7.8)
7	3.33 (m)	3.40 (m)	3.31 (m)	4.02 (m)	3.81 (t, 6.6)
8	1.50 (m)	1.88 (m)	1.82 (t, 6.6)	5.75 (dd, 6.9, 15.2)	5.73 (dd, 6.6, 15.3)
	1.30 (m)				
9	3.35 (m)	4.75 (m)	4.76 (m)	6.35 (dd, 11.1, 15.2)	6.38 (dd, 11.1, 15.3)
10	1.70 (m)	1.52 (m)	1.51 (m)	5.83 (d, 11.1)	5.83 (d, 11.1)
		1.29 (m)	1.29 (m)		
11	1.66 (m)	1.52 (m)	1.29 (m)		
12	0.80 (d, 6.6)	0.79 (d, 6.5)	0.79 (d, 6.3)	1.76 (s)	1.74 (s)
13	0.80 (d, 6.6)	0.83 (d, 6.5)	0.85 (d, 6.3)	1.77 (s)	1.77 (s)
14	1.30 (d, 7.5)	1.26 (d, 6.9)	1.25 (d, 6.9)	1.38 (d, 6.6)	1.41 (d, 7.2)
15	2.28 (s)	9.75 (s)	2.11 (s)	9.81 (s)	5.40 (br s)
9-OAc		2.05 (s)	2.08 (s)		
OMe					3.90 (s)

[a] δ in ppm, J in Hz. [b] Assignments determined by COSY.

Table 2. ^{13}C NMR Data ($CDCl_3$) for Compounds **1**–**5**[a]

	1	**2**	**3**	**4**	**5**
1	153.0 (s)	154.5 (s)	153.5 (s)	141.7 (s)	153.5 (s)
2	117.8 (d)	115.6 (d)	116.8 (d)	148.1 (d)	117.0 (d)
3	128.7 (s)	135.4 (s)	129.3 (s)	118.7 (s)	129.3 (s)
4	121.2 (d)	123.1 (d)	121.4 (d)	123.9 (d)	122.2 (d)
5	126.2 (d)	127.5 (d)	126.5 (d)	118.7 (d)	126.9 (d)
6	136.0 (s)	140.5 (s)	136.6 (s)	140.4 (s)	136.8 (s)
7	27.0 (d)	28.6 (d)	27.7 (d)	35.9 (d)	27.0 (d)
8	46.8 (t)	42.5 (t)	43.1 (t)	133.7 (d)	133.5 (d)
9	68.6 (d)	72.1 (d)	72.4 (d)	126.2 (d)	127.9 (d)
10	47.1 (t)	44.0 (t)	44.0 (t)	124.8 (d)	124.4 (d)
11	24.7 (d)	24.7 (d)	24.7 (d)	134.3 (d)	135.4 (d)
12	21.0 (q)	22.5 (q)	20.7 (q)	18.3 (q)	18.4 (q)
13	23.1 (q)	22.7 (q)	22.6 (q)	25.9 (q)	26.0 (q)
14	22.4 (q)	21.9 (q)	22.1 (q)	19.7 (q)	19.5 (q)
15	21.6 (q)	192.0 (d)	22.6 (q)	196.2 (d)	166.9 (s)
OMe					52.1 (q)
9-OAc		21.4 (q)	21.5 (q)		
		172.1 (s)	172.1 (s)		

[a] Multiplicities determined by DEPT.

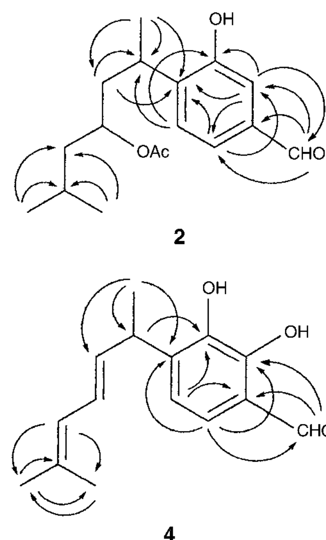

Figure 1. HMBC studies of compounds **2** and **4**.

identical with compound **6**, confirming the structure of **3**. The assignment of each proton and carbon signal in **2** and **3** was completed by COSY and DEPT experiments. Moreover, the aromatic methyl singlet (δ 2.28) in **1** was missing in **2**. Instead, an aldehyde function was inferred from a sharp singlet at δ 9.75 and a doublet at δ 192.0 in the ^{1}H and ^{13}C NMR spectra of **2**. To confirm the structure of **2**, HMQC and HMBC were performed, and the result of HMBC is shown in Figure 1. The HMBC study of **2** not only determined the structure of **2** but also supports the previous structure of **1**.

The high-resolution EI mass spectrum of parahigginol D (**4**), $[\alpha]_D$ −73.5° ($CHCl_3$), showed the parent ion composition of $C_{15}H_{18}O_3$ at m/z 246.1254. The presence of a conjugated diene, aldehyde, and phenol functionalities was inferred from its UV (λ_{max} 232, 280 nm) and IR (3363, 1708, 1621, 1593, 1425 cm^{-1}) spectra. [7] The ^{1}H and ^{13}C NMR spectra of **4** and **5** were clearly analogous to those from dehydrocurcuphenol.[1] The conjugated diene system was also deduced from three strongly coupled olefinic protons (δ 5.75, 1H, dd, J = 6.9, 15.2 Hz; δ 6.35, 1H, dd, J = 11.1, 15.2 Hz; δ 5.83, 1H, d, J = 11.1 Hz). In addition to an aldehyde singlet at δ 9.81, in the aromatic region an AB quartet (δ 7.09, 1H; δ 6.86, 1H, J = 8.2 Hz) for a 1,2,3,6-tetrasubstituted phenolic ring moiety was observed. The COSY spectrum of **4** was in agreement with the structure of **4**. In confirmation, an HMBC spectrum of **4** exhibited correlations (CHO/C-2, C-3; Me-14/C-6, C-7, C-8; H-5, H-7/C-1) as illustrated in Figure 1. Parahigginic acid (**5**) ($[\alpha]_D$ −29.20) was determined to possess a 1,1-dimethyl-1,3-diene moiety (δ 1.74, 3H, s; δ 1.77, 3H, s; δ 5.83, 1H, d, J = 11.1 Hz; δ 6.38, 1H, dd, J = 11.1, 15.3 Hz; δ 5.73, 1H, dd, J = 6.6, 15.3 Hz) and a secondary methyl doublet (δ 1.41, J = 7.2 Hz) by interpretation of its ^{1}H NMR spectrum. Additionally, a methoxyl singlet (δ 3.90) and three aromatic signals (δ 7.49, 1H, s; δ 7.22, 1H, d, J = 7.8 Hz; δ 7.58, 1H, d, J = 7.8 Hz) for a 1,2,4-trisubstituted benzene ring were observed. These conclusions were supported by the COSY spectrum of **5**. While no aldehyde function was found, a benzoic acid function was deduced from its IR (3440 and 1700 cm^{-1}) and UV (λ_{max} 284 nm) spectra, and this was supported further by a carbon signal at δ 166.9 in the ^{13}C NMR spectrum of **5**. A DEPT spectrum of **5** was used to identify the remaining carbon signals for this sesqiterpene hydrocarbon as three methyl quartets (δ 18.4, 19.5, 26.0), a methine doublet (δ 37.0), three olefinic doublets (δ 133.5, 127.9, 124.4), an olefinic singlet (δ 135.4), three aromatic doublets (δ 117.0, 122.2, 126.9), and three aromatic singlets (δ 129.3, 136.8, 153.5).

Biological activity for these sesquiterpenes was measured using a cytotoxicity assay employing four tumor cell lines. As indicated in Table 3, compounds **2**–**5** exhibited mild to moderate activity against P-388 murine tumor cells and human mouth epidermoid (KB16), lung, and colon (HT-29) cancer cells. On the contrary, compound **1** was inactive as tested on these tumor cells. The related sesquiterpene (+) curcuphenol showed inhibitory activity against P-388

Table 3. Cytotoxicities (IC_{50}, μg/mL) of Compounds **1**−**5**[a]

compd, tumor cells	P-388	KB16	A549	HT-29
1, parahigginol A	>50	>50	>50	>50
2, parahigginol B	3.0	4.9	10	3.5
3, parahigginol C	2.5	3.9	2.8	2.6
4, parahigginol D	2.9	7.2	3.8	3.3
5, parahigginic acid	1.0	4.8	3.9	6.0
(+)-curcuphenol	0.1	8.3	NT[b]	8.1

[a] The concentration of compound inhibiting 50% (IC_{50}) of the growth of murine and human tumor cell lines, P-388 (murine leukemia), KB16 (human mouth epidermoid), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma) after 3, 3, 6, and 6 day drug exposure according to a published method.[7−9] [b] Not tested.

murine and human KB16 as well as human HT-29 carcinoma cells at 0.1, 8.3, and 8.1 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Horiba FT-720 and Hitachi T-2001 U-3210 spectrophotometers, respectively. High-resolution EI and EI mass spectra were taken on VG Quattro 5022 and JEOL JMS-SX 102 mass spectrometers. ^{1}H and ^{13}C NMR, COSY, DEPT, HMBC, and HMQC spectra were recorded on Varian FT-300 and Bruker 300-AC spectrometers. Chemical shifts are given in δ (ppm) and coupling constants in Hz. Silica gel 60 and RP-18 were used for CC and HPLC and precoated silica gel plates (Kieselgel 60 F_{254}, 1 mm) for preparative TLC.

Animal Materials. The sponge belongs to a novel, as yet undescribed species of the genus *Parahigginsia* (class Demospongiae, order Poecilosclerida, family unknown).[8] It was collected about 500 m off the Green Island, Taiwan, at a depth of 10 m scuba, in May 1995, and stored at −20 °C before extraction. *Parahigginsia* sp. showed a golden to brown mass measured 10 cm in height and 25 cm in breath with the lamella 8 mm in thickness. The surface was rough, and the consistency was firm. The interior was brown with spicules inside the tissue. The main skeleton is lax and irregular, composed of loose, wisp, and stout oxea, which run toward the surface, from which they may project in loose radiate tufts of usually more slender spicules. The spicules include slightly curved oxea with a size range of 1050−1120 × 4−35 μm, acanthoxea 320−420 × 4−17 μm, small oxea 56−175 × 3−5 μm, and an asterose microsclere 12−20 μm. According to a personal communication, this species will be named *P. sheni* sp. n. A voucher specimen (registration no. GSP-22) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

Extraction and Isolation. The frozen animals (0.8 kg) were ground and repeatedly extracted with acetone (5 L) at room temperature. The combined extracts were evaporated under vacuum to give a brown residue, which was partitioned between $CHCl_3$ and water. The $CHCl_3$ layer (4.2 g) was subjected to silica gel column chromatography (200 g) and eluted with a mixture of $CHCl_3$−MeOH (10:1) to provide three fractions. The major fraction A was rechromatographed on silica gel and eluted with *n*-hexanes−EtOAc by increasing polarity to provide five fractions, I−V. Fractions I, III, and IV were further purified by a reversed-phase HPLC (RP-18) column using 25% aqueous MeOH as solvent to give **3** (120 mg), **4** (70 mg), and **5** (3 mg), respectively. All products were yellowish oils. Fraction B was applied on a preparative TLC plate and developed with *n*-hexanes−EtOAc (3:1) to yield compound **1** (17 mg) and **2** (11 mg).

Parahigginol A (1): yellowish oil; $[\alpha]_D$ −4.8° (*c* 0.075, $CHCl_3$); IR (neat) ν_{max} 3450, 3307, 1539, 1506, 1456, 945, 862, 808 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 218 (3.4), 276 (3.0) nm; ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 1 and 2, respectively; EIMS *m/z* (rel int) 236 (24), 218 (12), 203 (14), 175 (4), 161 (30), 147 (35), 135 (100), 121 (26), 115 (22), 105 (14), 91 (37), 77 (13), 55 (14), 43 (31); HREIMS *m/z* 236.1769 (calcd for $C_{15}H_{24}O_2$ 236.1776).

Acetylation of Parahigginol A (1). Acetylation (Ac_2O−pyridine 1:1; room temperature) of **1** (5 mg) gave, after workup, parahigginol A diacetate (**6**, 4 mg): $[\alpha]_D$ −13.5° (*c* 0.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.81 (1H, s, H-2), 7.01 (1H, d, J = 8.0 Hz, H-4), 7.12 (1H, d, J = 8.0 Hz, H-5), 2.91 (1H, m, H-7), 4.76 (1H, m, H-9), 1.20−1.85 (5H, m, H-8, 10, 11), 0.84 (3H, d, J = 6.5 Hz, H-12), 0.86 (3H, d, J = 6.5 Hz, H-13), 1.20 (3H, d, J = 6.9 Hz, H-14), 2.31 (6H, s, H-15, Ac), 1.96 (3H, Ac); EIMS *m/z* (rel int) 320 (0.3), 278 (2), 260 (26), 218 (41), 217 (26), 203 (18), 161 (17), 148 (21), 147 (20), 135 (100), 121 (32), 115 (25), 105 (24), 91 (51), 77 (15), 55 (13), 43 (27).

Parahigginol B (2): yellowish oil; $[\alpha]_D$ −11.4° (*c* 0.575, $CHCl_3$); IR (neat) ν_{max} 3369, 2850, 1734, 1699, 1541, 1509, 1455, 1024, 820 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 224 (4.0), 265 (3.8), 316 (3.4) nm; ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 1 and 2, respectively; EIMS *m/z* (rel int) 292 (2), 249 (0.5), 232 (62), 217 (4), 175 (26), 162 (73), 149 (100), 135 (35), 121 (36), 115 (16), 103 (39), 91 (56), 77 (52), 55 (50), 43 (94); HREIMS *m/z* 292.1660 (calcd for $C_{17}H_{24}O_4$ 292.1675).

Parahigginol C (3): yellowish oil; $[\alpha]_D$ −9.5° (*c* 2.1, $CHCl_3$); IR (neat) ν_{max} 3361, 1708, 1621, 1592, 1454, 1423, 1024, 809 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 219 (3.8), 278 (3.4), 284 (3.4) nm; ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 1 and 2, respectively; EIMS *m/z* (rel int) 278 (1), 260 (0.1), 245 (0.1), 218 (13), 203 (7), 175 (3), 161 (24), 147 (28), 135 (100), 121 (12), 115 (13), 105 (8), 91 (20), 77 (8), 55 (9), 43 (99); HREIMS *m/z* 278.1883 (calcd for $C_{17}H_{26}O_3$ 278.1884).

Acetylation of Parahigginol C (3). Acetylation (Ac_2O−pyridine 1:1; room temperature) of **3** (25 mg) gave, after workup, a solid (20 mg) that showed spectral data (^{1}H NMR, EIMS, [α]) identical with those of **6**.

Parahigginol D (4): yellowish oil; $[\alpha]_D$ −73.5° (*c* 0.225, $CHCl_3$); IR (neat) ν_{max} 3363, 1708, 1621, 1593, 1425, 1024, 946, 810 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 232 (4.2), 280 (4.0) nm; ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 1 and 2, respectively; EIMS *m/z* (rel int) 246 (51), 231 (20), 203 (17), 177 (51), 157 (20), 149 (28), 135 (9), 121 (17), 115 (25), 103 (13), 91 (56), 77 (72), 55 (50), 41 (100); HREIMS *m/z* 246.1254 (calcd for $C_{15}H_{18}O_3$ 246.1256).

Parahigginic acid (5): yellowish oil; $[\alpha]_D$ −29.2° (*c* 0.15, $CHCl_3$); IR (neat) ν_{max} 3400, 1718, 1700, 1685, 1437, 1026, 989 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 216 (4.0), 249 (3.6), 257 (3.6), 264 (3.5), 299 (3.2) nm; ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data, see Tables 1 and 2, respectively; EIMS *m/z* (rel int) 260 (39), 245 (35), 229 (16), 213 (19), 203 (39), 185 (18), 179 (42), 159 (35), 131 (29), 115 (48), 105 (21), 91 (57), 77 (43), 55 (47).

Cytotoxicity Test. The cytotoxic activities of compounds against P-388, KB16, A549, and HT-29 cells were assayed by the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay with some modifications.[9] Detailed procedures were reported previously.[5,6] The IC_{50} was defined as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cells in the MTT assay. Results are given in Table 3.

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