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New pentacyclic polyketide dimeric peroxides from a Taiwanese marine sponge *Petrosia elastica*

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Abstract—Two novel pentacyclic polyketide dimers, dihalenaquinolides A (**1**) and B (**2**), have been isolated from the marine sponge *Petrosia elastica* collected in Nan-wan, Taiwan. The structures **1** and **2** were established on the basis of extensive spectral analysis.

Tropical marine sponges are a fertile source of secondary metabolites with diverse and often novel molecular architecture.[1] In the course of our study on biologically active secondary metabolites from marine organisms,[2] we have undertaken the chemical examination of the Taiwanese marine sponge *Petrosia elastica* Keller.[3] The sponge material (1.85 kg) was extracted exhaustively with EtOH (3l × 3) to give a residue (27.3 g), which was partitioned between water (500 mL) and EtOAc (1 l × 3) furnishing the EtOAc residue (12.6 g). Chromatography of the residue over silica gel with solvents of increasing polarity from *n*-hexane/$CHCl_3$ through $CHCl_3$ to $CHCl_3$/MeOH gave six fractions (A–F). Fraction F on further chromatography over silica gel followed by preparative TLC ($CHCl_3$/MeOH, 95:5) furnished dihalenaquinolides A (**1**) and B (**2**).

Dihalenaquinolide A (**1**) has a formula $C_{42}H_{30}O_{10}$ as deduced from LRFAB and HRFAB-MS spectra. The FAB-MS showed a quasi-molecular ion peak at m/z 695 ($[M+H]^+$) together with a strong mass fragment at m/z 348 corresponding to the fragmentation of its constituent monomer ($C_{21}H_{16}O_5$), suggesting that compound **1** is a dimer. Its ^{1}H NMR spectrum showed the presence of four doublets at δ 6.65, 6.75. 6.85, and 6.95 each having a coupling constant 8 Hz, four singlets at δ 8.21, 8.26, 9.22 and 9.26, and a singlet for two methyls at δ 1.63, which is similar to the ^{1}H NMR spectrum of halenaquinol except for the presence of two sets of protons in compound **1** against one in halenaquinol.[4] Methylation of **1** with CH_2N_2 was not successful. This indicated the absence of aromatic hydroxyl group in its structure. Further its ^{1}H NMR spectrum showed the presence of a singlet for two methyls at δ 3.95 suggesting that the hydroxyl group in halenaquinol has been replaced by methoxyl group in compound **1**, which was further supported by the presence of methoxyl carbon at δ 55.7 in ^{13}C NMR spectrum. The presence of peroxide linkage was supported by the strong mass fragments at m/z 348 ($[M+H-C_{21}H_{15}O_5]^+$) and 333 ($[M-C_{21}H_{14}O_6]^+$) in the mass spectrum. The nature of the rings A, B, C, and D in the two monomers was observed symmetrical, which led to the doubling of signals or with a very small difference in its ^{13}C NMR values.[5] The similar nature of the two monomers was also supported by the presence of a singlet for two protons H-18/H-18′ at δ 8.21, another singlet for two protons H-1/H-1′ at δ 8.26, and the two singlets at δ 9.22 and 9.26 having a very small difference in their δ value can be assignable to H-11 or H-11′ in its ^{1}H NMR spectrum. The distortion to the symmetry was observed in ring E from C-13/C-13′ to C-16/C-16′. This inferred the only possibility for the unsymmetrical attachment of the two monomers at C-16 and C-13′ as shown in compound **1** and the positions of the methoxyl groups at C-13 and C-16′. NOESY spectrum showed the correlation between the methoxyl and the protons H-11 at δ 9.22 and H-18′ at δ 8.21, respectively, confirming further the unsymmetrical nature of the two monomers. A close comparison of the ^{13}C NMR values of halenaquinol and **1** revealed that the two carbons, C-13 and C-16′ in **1** are downfield by 1.3 and 1.7 ppm, respectively, which can be explainable by the

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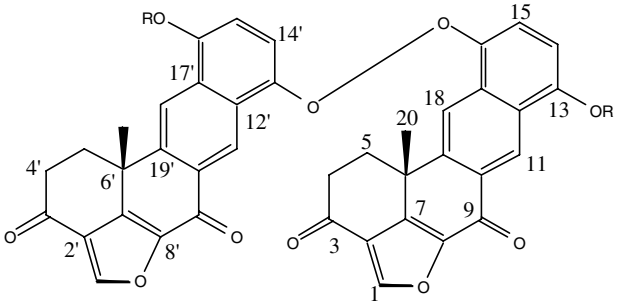
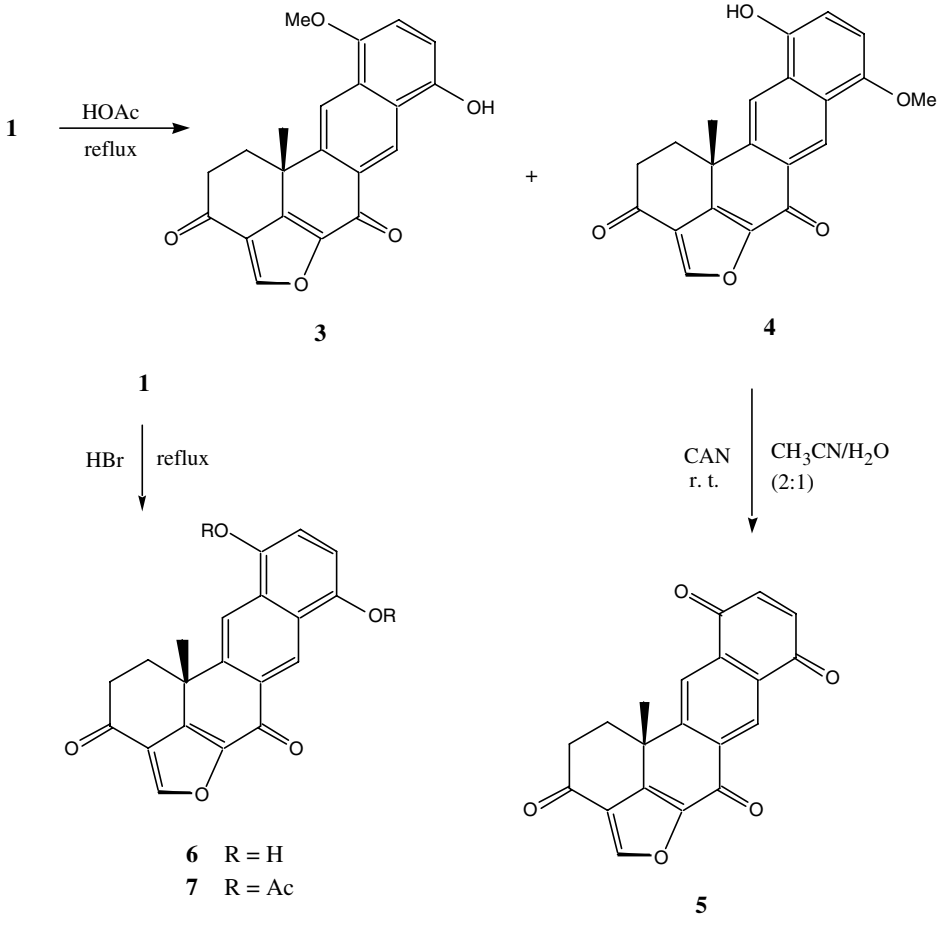


1 R = CH_3
2 R = CH_2CH_3



3 **4**

6 R = H
7 R = Ac

5

presence of methoxyl groups at those positions. The ^1H NMR data at δ 6.65 and 6.95 were assigned to H-14 and H-15, respectively, on the basis of COSY (H-14/H-15), HMBC (H-14/C-13, C-15, C-16; H-15/C-14, C-16), and NOESY spectral data. Similarly the other two protons at δ 6.85 and 6.75 were assigned to H-14′ and H-15′, respectively. Furthermore, the assignment of C-13, C-13′, C-16 and C-16′ at δ 148.6, 147.1, 145.5, and 147.2 was completed by HMBC too. Dihalenaquinolide A (**1**, 4 mg) was refluxed in acetic acid (4 mL) to give **3** and **4**, which were further reacted with CAN (ceric ammonium nitrate) in CH_3CN/H_2O (2:1) under room temperature to yield halenaquinone (**5**, 1.4 mg).[6] When compound **1** was refluxed with HBr (48%, 1 mL) in AcOH for 12 h, helenaquinol (**6**) was produced and was isolated as its diacetate form (**7**). The spectral data (^1H NMR, EI, and FAB-MS) of the known **5**–**7** are identical with those of authentic samples, respectively.[7] The new derivatives **3** and **4**, which exhibited more polar than **1**, were obtained as an inseparable diastereomeric mixture.[8]

Dihalenaquinolide B (**2**) has been deduced as $C_{44}H_{34}O_{10}$ from HRFAB-MS and DEPT spectrum. Its ^1H NMR spectrum showed the presence of four doublets at δ 6.64,

Table 1. Cytotoxicity of polycyclic hydroquinones and quinones against human tumor cells (growth inhibition, %)[a]

Compound (10 μg/mL)	PC-3[b]	Hep3B[c]
Dihalenaquinolide A (**1**)	56	5
Dihalenaquinolide B (**2**)	35	5
Halenaquinone (**5**)	60	47
Halenaquinol (**6**)	98	20
Halenaquinol diacetate (**7**)	43	31
Xestoquinone	100	2
Xestoquinolide	50	5
Taxol (0.1 μg/mL)	80	85

[a] Positive compound: % of control >50%.
[b] PC-3: human prostate cancer cells.
[c] Hep3B: human hepatoma cancer cells.

6.77, 6.86, 6.94 corresponds to H-14, H-15, H-15′, H-14′, respectively, three singlets at δ 8.21 (H-18/H-18′), 8.26 (H-1/H-1′) and 9.24 (H-11/H-11′), and a singlet for two methyls at δ 1.64 similar to that of **1** except the presence of a quartet for two methylene groups at δ 4.14 ($J = 6.9$ Hz) and a triplet for two methyl groups at δ 1.52 ($J = 6.6$ Hz) in place of the singlet for methoxyl group at δ 3.95 in **1**. From its EIMS it was observed that **2** is 28 mass units greater than **1** suggests that compound **2** might be having ethoxyl group at C-13 instead of methoxyl group of **1**. This was further supported the presence of the two ^{13}C NMR signals at δ 64.2 and 14.9 for OCH_2CH_3. The ^{13}C NMR values were assigned for each carbon and were in good agreement with the structure.[9]

The cytotoxic activity of the isolated pentacyclic hydroquinones and quinones were tested in vitro against human prostate (PC-3) and hepatoma (Hep3B) tumor cell lines. As shown in Table 1, compounds **1**, **5**, **6**, and xestoquinone selectively inhibited the growth of PC-3 tumor cells at 10 μg/mL, while compounds **2**, **7**, and xestoquinolide were inactive. None of the compounds showed growth inhibition toward human Hep3B tumor cells.

Cytotoxicity Assay. The bioassay used against PC-3 (human androgen-independent prostate carcinoma) and Hep3B (human hepatocellular carcinoma) tumor cells was based on a sulforhodamine B (SRB) assay method.[10] The procedure of assay was carried out as previously described.[11] Taxol was used as a standard compound.

Acknowledgements

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5. Dihalenaquinolide A: $[\alpha]_D^{25}$ +45° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 229.4, 300.6 nm; IR ($CHCl_3$) ν_{max} 3104, 3035, 1727, 1685, 1523, 1427, 1018, 995, 767, and 667 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (s, 2H, H-1/H-1′), 2.8–3.0 (m, 4H, H-4/H-4′), 2.28 and 2.76 (m, 4H, H-5/H-5′), 9.22 (s, ^{1}H, H-11), 9.26 (^{1}H, H-11′), 6.65 (d, $J = 8$ Hz, ^{1}H, H-14), 6.85 (d, $J = 8$ Hz, ^{1}H, H-14′), 6.95 (d, $J = 8$ Hz, ^{1}H, H-15), 6.75 (d, $J = 8$ Hz, ^{1}H, H-15′), 8.21 (2H, H-18/H-18′), 1.63 (s, 6H, H-20/H-20′), and 3.95 (s, 6H, 2 × OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$): δ 150.9 (C-1/C-1′), 122.4 (C-2/C-2′), 192.3, 192.4 (C-3, C-3′), 36.7 (C-4/C-4′), 34.0 (C-5/C-5′), 35.7 (C-6/C-6′), 147.9 (C-7/C-7′), 144.7 (C-8/C-8′), 172.8, 172.9 (C-9, C-9′), 130.0 (C-10/C-10′), 123.8 (C-11/C-11′), 122.4 (C-12), 123.8 (C-12′), 148.6 (C-13), 147.1 (C-13′), 104.1 (C-14), 109.0 (C-14′), 111.4 (C-15), 107.1 (C-15′), 145.5 (C-16), 147.2 (C-16′) 124.6 (C-17), 124.8 (C-17′), 118.5, 118.7 (C-18, C-18′), 147.5 (C-19/C-19′), 31.9 (C-20/C-20′), and 55.7 (OCH_3); FAB-MS *m*/*z*: 695 ($[M+H]^+$, 0.7), 694 (M^+, 0.5), 349 ($[M-C_{21}H_{13}O_5]^+$, 23.6), 348 ($[M-_{21}H_{14}O_5]^+$, 17.4), 334 ($[M-C_{21}H_{13}O_6]^+$, 6.2), 333 ($[M-C_{21}H_{14}O_6]^+$, 6.1), 307 (4.93), 289 (4.85), 154 (88.6), and 136 (88.5); HRFAB-MS *m*/*z*: $[M+H]^+$ 695.1912 (calcd for $C_{42}H_{31}O_{10}$, 695.1917); TLC R_f: 0.70/CH_2Cl_2–MeOH (49:1), 0.53/CH_2Cl_2–MeOH (99:1).
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8. Compounds **3** and **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.24, 9.22 (s, 2H, H-1), 8.30, 8.27 (s, 2H, H-18), 8.23 (s, 2H, H-11), 6.66, 6.87 (d, $J = 8.1$ Hz, 2H, H-14), 6.79, 6.91 (d, $J = 8.1$ Hz, 2H, H-15), 3.98 (s, 6H, OCH_3), 2.85 (m, 6H), 2.32 (m, 2H), 1.67 (s, 6H, H-20); EIMS (70 eV) *m*/*z* 348 (M^+, 77), 333 ($[M-Me]^+$, 100); FAB-MS *m*/*z*: 349 ($[M+H]^+$, 3.2); TLC R_f: 0.63/CH_2Cl_2–MeOH (49:1).
9. Dihalenaquinolide B: $[\alpha]_D^{25}$ +70.8° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 227.8, 299.8 nm; IR ($CHCl_3$) ν_{max} 3135, 3058, 2904, 1677, 1608, 1106, 1025, 917, 767, and 671 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (2H, H-1/H-1′), 2.80–2.98 (m, 4H, H-4/H-4′), 2.29 and 2.90 (m, 4H, H-5/H-5′), 9.24 (2H, H-11/H-11′), 6.64 (d, $J = 8$ Hz, 1H, H-14), 6.86 (d, $J = 8$ Hz, 1H, H-14′), 6.94 (d, $J = 8$ Hz, 1H, H-15), 6.77 (d, $J = 8$ Hz, 1H, H-15′), 8.26 (2H, H-18/H-18′), 1.64 (6H, H-20/H-20′), 4.14 (q, $J = 6.9$ Hz, 4H, OCH_2CH_3), and 1.52 (t, $J = 6.6$ Hz, 6H, OCH_2CH_3); ^{13}C NMR (300 MHz, $CDCl_3$): δ 150.1 (C-1/C-1′), 122.4 (C-2/C-2′), 192.4, 192.5 (C-3/C-3′), 36.7 (C-4/C-4′), 34.0 (C-5/C-5′), 35.7 (C-6/C-6′), 144.0 (C-7/C-7′), 144.7 (C-8/C-8′), 172.8, 172.9 (C-9/C-9′), 129.9 (C-10/C-10′), 123.8 (C-11/C-11′), 122.4 (C-12/C-12′), 148.6 (C-13), 147.1 (C-13′), 105.1 (C-14), 109.1 (C-14′), 111.5 (C-15), 108.3 (C-15′), 145.5 (C-16), 147.2 (C-16′), 124.6 (C-17/C-17′), 118.4, 118.6 (C-18, C-18′), 147.5 (C-19/C-19′), 31.7 (C-20/C-20′), 64.1 (OCH_2CH_3), and 14.8 (OCH_2CH_3); FAB-MS *m*/*z*: 723

([M+H]$^+$, 0.7), 722 (M$^+$, 0.4), 363 ([M−$C_{22}H_{16}O_5$]$^+$, 49), 362 ([M−$C_{22}H_{17}O_5$]$^+$, 37), 348 (M−$C_{22}H_{15}O_6$+H]$^+$, 7.6), 347 ([M−$C_{22}H_{16}O_6$]$^+$, 6), 307 (8.3), 289 (6.7), 154 (90.6), and 136 (100); HRFAB-MS m/z: [M]$^+$ 722.2165 (calcd for $C_{44}H_{34}O_{10}$, 722.2152); [M+H]$^+$ 723.2231 (calcd for $C_{44}H_{35}O_{10}$, 723.2230); TLC R_f: 0.58/CH_2Cl_2–MeOH (99:1).

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