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Dactyloquinones C, D and E novel sesquiterpenoid quinones, from the Okinawan marine sponge, *Dactylospongia elegans*

Hidemichi Mitome,[a] Takahiro Nagasawa,[a] Hiroaki Miyaoka,[a] Yasuji Yamada[a,*] and Rob W. M. van Soest[b]

[a]*School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan*
[b]*Institute for Systematics and Ecology, University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands*

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Abstract—Novel sesquiterpenoid quinones, dactyloquinone C (**5**), D (**6**) and E (**7**), each possessing a unique cyclic-ether moiety, were isolated from the Okinawan sponge *Dactylospongia elegans*. Structural determination in each case was made based on spectroscopic data.

Ilimaquinone (**1**), present in the Hawaiian sponge *Hippiospongia metachromia*,[1] has been reported for its unique, anti-HIV activities,[2] ability to protect cells from the toxic effects of ricin and diphtheria toxin,[3] and capacity to impede protein trafficking by reversibly breaking up the Golgi apparatus into small vesicles.[4] Thus, ilimaquinone should serve effectively as a probe for investigating important biological processes. This quinone belongs to the family of sesquiterpenoid quinones,[5–7] has a 4,9-friedodrimane sesquiterpene skeleton and contains 1,4-benzoquinone. The isolation from the Okinawan sponge *Dactylospongia elegans* and structural determination of dactyloquinones A (**2**) and B (**3**), all having a six-membered-ring made by ether linkages between C-10 and C-17 of ilimaquinone (**1**) and 5-*epi*-ilimaquinone (**4**),[8] respectively, were recently conducted by the authors.[9] These compounds are the first examples of dihydropyran-containing ilimaquinone-type sesquiterpenoid quinones. In search for other 4,9-friedodrimane-type sesquiterpenoid quinones from this sponge, the novel sesquiterpenoid quinones, dactyloquinone C (**5**), having a heretofore-unobserved seven-membered-ring formed via ether linkages between C-1 and C-17 of ilimaquinone (**1**), along with dactyloquinones D (**6**) and E (**7**), both possessing a six-membered-ring made from ether linkages between C-8 and C-17 of ilimaquinone (**1**) and 5-*epi*-ilimaquinone (**4**), respectively, were isolated for the first time in the present study. The isolation and structures of dactyloquinones C (**5**), D (**6**) and E (**7**) are presented in the following, as determined by spectroscopic analysis (Fig. 1).

Sponge specimens of *D. elegans*, obtained from the coral reef of Ishigaki Island, Okinawa, Japan, in November 2000, were initially extracted with MeOH and then acetone. The combined extracts were partitioned between H_2O and AcOEt. The AcOEt soluble portion was purified to give sesquiterpenoid quinones dactyloquinones C (**5**), D (**6**) and E (**7**), along with ilimaquinone (**1**),[1] dactyloquinones

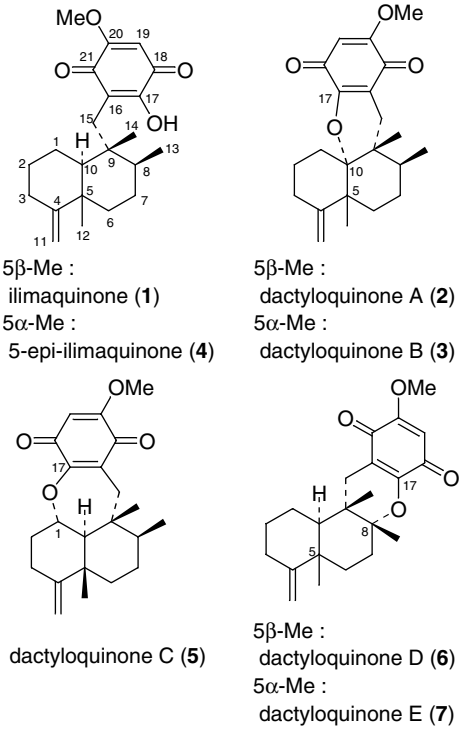

Figure 1.

Keywords: marine metabolites; sponges; terpenes and terpenoids; quinones.
* Corresponding author. Tel.: +81-426-76-3046; fax: +81-426-76-3069; e-mail: yamaday@ps.toyaku.ac.jp

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Table 1. ^{13}C NMR data for **5**–**7** (125 MHz, $CDCl_3$)

No.	5	6	7
1	78.5 (CH)	22.0 (CH_2)	21.2 (CH_2)
2	35.5 (CH_2)	28.3 (CH_2)	24.2 (CH_2)
3	30.6 (CH_2)	32.6 (CH_2)	31.5 (CH_2)
4	157.2 (C)	158.6 (C)	152.9 (C)
5	40.2 (C)	39.4 (C)	38.5 (C)
6	37.4 (CH_2)	31.2 (CH_2)	31.0 (CH_2)
7	27.8 (CH_2)	30.4 (CH_2)	30.2 (CH_2)
8	41.8 (CH)	84.8 (C)	85.4 (C)
9	37.4 (C)	37.1 (C)	38.2 (C)
10	60.2 (CH)	44.8 (CH)	42.9 (CH)
11	104.1 (CH_2)	103.5 (CH_2)	106.3 (CH_2)
12	21.7 (CH_3)	20.9 (CH_3)	32.2 (CH_3)
13	16.4 (CH_3)	23.1 (CH_3)	23.1 (CH_3)
14	15.0 (CH_3)	21.1 (CH_3)	22.6 (CH_3)
15	34.8 (CH_2)	27.8 (CH_2)	28.0 (CH_2)
16	130.2 (C)	113.9 (C)	114.6 (C)
17	156.5 (C)	152.6 (C)	152.8 (C)
18	182.6 (C)	181.7 (C)	181.8 (C)
19	105.2 (CH)	104.8 (CH)	104.8 (CH)
20	159.0 (C)	159.4 (C)	159.5 (C)
21	182.6 (C)	181.0 (C)	181.2 (C)
20-OMe	56.4 (CH_3)	56.4 (CH_3)	56.4 (CH_3)

A (**2**) and B (**3**),[9] 5-*epi*-ilimaquinone (**4**),[8] smenospongine,[10] smenospondiol,[11] cyclospongiaquinone-1[12] and pelorol.[13]

The molecular formula of dactyloquinone C (**5**) was established as $C_{22}H_{28}O_4$ based on high-resolution mass measurement. There was indication of the presence of 1,4-benzoquinone chromophore from IR absorption at 1664 and 1648 cm^{-1} and UV absorption at λ_{max} 286 nm. ^{13}C NMR and DEPT spectra indicated 22 carbons comprised of the following: four methyls, five sp^3 methylenes, one sp^2 methylene, three sp^3 methines, one sp^2 methine, two sp^3 quaternary carbons and six sp^2 quaternary carbons (Table 1). ^1H and ^{13}C NMR correlations were evident from the HMQC spectrum (Tables 1 and 2). ^1H and ^{13}C NMR disclosed the presence of a dialkoxy-1,4-benzoquinone moiety (δ_H 3.81 (3H, s), 5.80 (1H, s); δ_C 56.4 (CH_3), 105.2 (CH), 130.2 (C), 156.5 (C), 159.0 (C), 182.6 (C), 182.6 (C)), oxygenated sp^3 methine (δ_C 78.5 (CH)) and *exo*-methylene (δ_H 4.62 (2H, s); δ_C 104.1 (CH_2), 157.2 (C)). COSY cross-peaks demonstrated the partial structures, C-3 to C-10 and C-6 to C-8, C-13.[14] The manner in which the above partial structures of dactyloquinone C are connected to each other was determined based on the following HMBC spectrum correlations: H-11/C-3, C-5; Me-12/C-4, C-5, C-6, C-10; Me-13/C-9; Me-14/C-8, C-9, C-10, C-15; H-15/C-8, C-9, C-10, C-14, C-16, C-17, C-21; H-19/C-17, C-18, C-20, C-21 and H-1/C-17. The 4,9-friedodrimane skeleton would thus appear connected to a dialkoxy-1,4-benzoquinone to produce a seven-membered-ring with ether linkages between C-1 and C-17. The planar structure of dactyloquinone C was thus established. The relative configuration of dactyloquinone C was found to be **5** from the following NOESY correlations: Me-12/H-1, H-7β, Me-14; Me-13/ H-7β, Me-14, H-15β; H-10/H-2α, H-6α, H-15α; H-8/H-6α, H-15α. The absolute configuration of dactyloquinone C may consequently be considered 1*S*,5*S*,8*S*,9*R*,10*S* assuming ilimaquinone (**1**) to be a precursor of dactyloquinone C (**5**).

Dactyloquinone D (**6**) was shown to have the molecular formula, $C_{22}H_{28}O_4$, based on high-resolution mass measurement. 1,4-Benzoquinone chromophore appeared present based on IR absorption and UV absorption. All 22 carbons in ^{13}C NMR and DEPT spectra indicated the presence of four methyls, six sp^3 methylenes, one sp^2 methylene, one sp^3 methine, one sp^2 methine, three sp^3 quaternary carbons and six sp^2 quaternary carbons (Table 1). ^1H and ^{13}C NMR correlations were established from the HMQC spectrum (Tables 1 and 2). ^1H and ^{13}C NMR indicated a dialkoxy-1,4-benzoquinone moiety (δ_H 3.80 (3H, s), 5.76 (1H, s); δ_C 56.4 (CH_3), 104.8 (CH), 113.9 (C), 152.6 (C), 159.4 (C), 181.0 (C), 181.7 (C)) and oxygenated sp^3 quaternary carbon (δ_C 84.8 (C)) and *exo*-methylene (δ_H 4.48 (1H, s), 4.52 (1H, s); δ_C 103.5 (CH_2), 158.6 (C)). COSY cross-peaks demonstrated the partial structures, C-10 to C-3 and C-6 to C-7. All the partial structures indicated up to this point were connected to each other through quaternary carbons, as was evident from the following HMBC spectrum correlations: H-11/C-3, C-5; Me-12/C-4, C-6, C-10; Me-13/C-7,

Table 2. ^1H NMR data for **5**–**7** (500 MHz, $CDCl_3$)

No.	5	6	7
1α	–	1.56 (1H, m)	1.88 (1H, m)
1β	4.15 (1H, dt, *J*=5.2, 10.3 Hz)	1.48 (1H, m)	1.73 (1H, m)
2α	1.79 (1H,m)	1.16 (1H, m)	1.66 (1H, m)
2β	2.50 (1H,m)	1.85 (1H, m)	1.82 (1H, m)
3α	2.26 (1H,m)	2.06 (1H, m)	2.48 (1H, m)
3β	2.40 (1H, dt, *J*=4.7, 14.0 Hz)	2.25 (1H, dt, *J*=5.1, 13.7 Hz)	2.18 (1H, m)
6α	1.63 (1H, m)	2.02 (1H, m)	1.80 (1H, m)
6β	1.61 (1H, m)	1.48 (1H, m)	1.95 (1H, m)
7α	1.59 (1H, m)	2.04 (2H, m)	1.85 (1H, m)
7β	1.51 (1H, m)		2.07 (1H, td, *J*=13.6, 3.7 Hz)
8	1.38 (1H, m)	–	–
10	1.50 (1H, d, *J*=10.3 Hz)	1.02 (1H, dd, *J*=2.8, 12.1 Hz)	1.33 (1H, m)
11	4.62 (2H, s)	4.52 (1H, s), 4.48 (1H, s)	4.78 (1H, s), 4.79 (1H, s)
12	0.96 (3H, s)	1.11 (3H, s)	1.11 (3H, s)
13	1.03 (3H, d, *J*=6.6 Hz)	1.23 (3H, s)	1.20 (3H, s)
14	0.73 (3H, s)	1.06 (3H, s)	1.14 (3H, s)
15α	1.99 (1H, d, *J*=13.6 Hz)	2.83 (1H, d, *J*=19.0 Hz)	2.78 (1H, d, *J*=19.0 Hz)
15β	3.13 (1H, d, *J*=13.6 Hz)	1.90 (1H, d, *J*=19.0 Hz)	1.97 (1H, d, *J*=19.0 Hz)
19	5.80 (1H, s)	5.76 (1H, s)	5.78 (1H, s)
20-OMe	3.81 (3H, s)	3.80 (3H, s)	3.81 (3H, s)

C-9; Me-14/C-8, C-10, C-15; H-15/C-8, C-10, C-14, C-16, C-17, C-21; H-10/C-4; H-19/C-17, C-18, C-20, C-21 and 20-OMe/C-20. The 4,9-friedodrimane skeleton is thus shown to be connected to a dialkoxy-1,4-benzoquinone. The dihydropyran moiety was clearly shown present by the absence of absorption of hydroxyl groups in the IR spectrum and any affect from base addition, on the UV spectrum. The planar structure of dactyloquinone D was thus determined. The relative configuration of dactyloquinone D was indicated as **6** from the following NOESY correlations: Me-12/H-1β, H-6β; Me-14/H-1β, H-15β, Me-13; H-10/H-1α, H-6α; H-1α/H-15α. The Absolute configuration of dactyloquinone D was thus concluded to be 5*S*,8*R*,9*S*,10*R* with ilimaquinone (**1**) assumed to be a precursor of dactyloquinone D (**6**).

Dactyloquinone E (**7**) had the molecular formula, $C_{22}H_{28}O_4$, as indicated by high-resolution mass measurement. A dialkoxy-1,4-benzoquinone chromophore was shown present by IR and UV absorptions, and the planer structure of which was found consistent with dactyloquinone D (**6**) based on ^{1}H and ^{13}C NMR spectra and two-dimensional NMR (COSY, HMQC and HMBC) data. ^{13}C NMR spectra of dactyloquinone E were quite similar to those of dactyloquinone D, except for the C-4 (Δ 5.7 ppm) and Me-12 (Δ 11.3 ppm). Dactyloquinone E would thus appear to be the stereoisomer of dactyloquinone D at the C-5 position. The relative configuration of dactyloquinone E was established as **7** based on the following NOESY correlations: Me-12/H-3α, H-10; H-6α/H-7α, H-10; H-15α/H-10; H-2α/H-3α; Me-14/H-2β, H-7β, H-15β; Me-13/H-7β, H-15β; H-2β/H-3β; H-7β/H-6β. The absolute configuration of dactyloquinone E may thus be concluded to be 5*R*,8*R*,9*S*,10*R* with 5-*epi*-ilimaquinone (**4**) assumed a precursor of dactyloquinone E (**7**).

Dactyloquinone C (**5**) was found here to possesses a heretofore-unobserved seven-membered ether ring, formed through linkages between C-1 and C-17 of ilimaquinone (**1**). Dactyloquinones D (**6**) and E (**7**) are the first examples of 4,9-friedodrimane-type sesquiterpenoid quinones, each possessing a six-membered-ring constructed of ether linkages between C-8 and C-17 of ilimaquinone (**1**) and 5-*epi*-ilimaquinone (**4**), respectively.

Oxidative cyclization from the α-face at C-1, C-8 and C-10 of ilimaquinone (**1**) may provide, respectively, dactyloquinones C (**5**), D (**6**) and A (**2**)[9] in the biosynthetic pathway. By this process, dactyloquinones E (**7**) and B (**3**)[9] may be produced from 5-*epi*-ilimaquinone (**4**) at C-8 and C-10, respectively. The biological activity of dactyloquinones C (**5**), D (**6**) and E (**7**) is presently being examined.

1. Experimental

1.1. General

Optical rotation was measured with a JASCO DIP-360 polarimeter. IR spectrum was recorded with a JASCO FT-IR/620 spectrometer, UV spectrum with a JASCO V-550 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-500 spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard (s, singlet; d, doublet; t, triplet; m, multiplet). EIMS spectrum was obtained with a Thermo Quest TSQ 700 spectrometer and a high resolution EIMS (HREIMS) spectrum was obtained using a VG Auto Spec E spectrometer. Flash column chromatography was carried out on Kanto Chemical Silica Gel 60N (spherical, neutral) 40–50 μm or ODS Wakogel® LP-40 C-18. HPLC separations were performed using a YMC-Pack R&D ODS (250×20 mm^2) column and a UV detector (254 nm).

1.2. Animal material

Sponge specimens (dark brown thin encrustations) were obtained from the coral reef of Ishigaki Island, Okinawa, Japan, at a depth of 5 m by hand using SCUBA, in November 2000.

The sponge specimen was *D. elegans* (Thiele, 1899), class Demospongiae, order Dictyoceratida, family Thorectidae. A voucher specimen has been deposited at University of Amsterdam (ZMA POR. 16688), and another is maintained at Tokyo University of Pharmacy and Life Science (S-00-7).

1.3. Extraction and isolation

Wet specimens (2.5 kg) were cut into small pieces and extracted with MeOH (12.5 L×3) and then acetone (7.0 L×2). The combined extracts were concentrated and partitioned between AcOEt (2.5 L×4) and water (2.0 L) to give an AcOEt-soluble portion (10.7 g).

The AcOEt-soluble portion was chromatographed on Si gel using a hexane–AcOEt (3:1) to AcOEt gradient and MeOH as eluent to produce fractions 1 (1.3 g), 2 (2.5 g) and 3 (5.9 g). Fraction 2 was subjected to flash Si gel column chromatography (elution with hexane–AcOEt (3:1–5:3)) to give fractions 2-1, 2-2 and 2-3. Fraction 2-1 was subjected to repeated flash Si gel and ODS column chromatography to provide dactyloquinone C (**5**) (1.9 mg). From fraction 2-2, ilimaquinone (**1**) (369.7 mg) was obtained.[9] On fraction 2-3, repeated flash Si gel column chromatography (elution with hexane–AcOEt (5:3)) was conducted to give fractions 2-3-1, 2-3-2 and 2-3-3. Fraction 2-3-1 was subjected to repeated flash ODS column chromatography (elution with MeOH–acetone (3:1) and acetone–water (4:1)) and ODS-HPLC (elution with MeOH–water (6:1 to 4:1)) to give dactyloquinone A (**2**) (9.6 mg), 5-*epi*-ilimaquinone (**4**) (15.8 mg), cyclospongiaquinone-1 (0.7 mg), dactyloquinone B (**3**) (1.0 mg), dactyloquinone D (**6**) (3.5 mg) and E (**7**) (1.4 mg), respectively. Fraction 2-3-2 was subjected to repeated flash Si gel column chromatography (elution with $CHCl_3$–MeOH (19:1) and hexane–AcOEt (2:1)) to produce smenospondiol (11.0 mg), pelorol (106.0 mg) and smenospongine (11.8 mg).[8]

1.3.1. Dactyloquinone C (5). Pale yellow amorphous; $[\alpha]_D^{27}=-22.7°$ (*c* 0.22, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 286 (3.7) nm; IR (KBr) ν_{max} 1664, 1648 cm^{-1}; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively; HMBC correlation (H/C) H-11/C-3, C-4, C-5; Me-12/C-4, C-5, C-6, C-10; Me-13/C-7, C-8, C-9; Me-14/C-8, C-9, C-10, C-15; H-15/C-8, C-9, C-10, C-14, C-16, C-17, C-21; H-10/C-1, C-2, C-5,

C-6, C-12, C-14, C-15; H-1/C-2, C-17; H-19/C-17, C-18, C-20, C-21; 20-OMe/C-20; EIMS *m/z* 356 [M^+] (62), 341 (12), 189 (79), 188 (49), 187 (100), 168 (61); HREIMS *m/z* 356.2018 (calcd for $C_{22}H_{28}O_4$, 356.1988).

1.3.2. Dactyloquinone D (6). Pale yellow amorphous; $[\alpha]_D^{27}=-26.7°$ (*c* 0.15, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 291 (4.1) nm; IR (KBr) ν_{max} 1664, 1642, 1603 cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2, respectively; HMBC correlation (H/C) H-11/C-3, C-4, C-5; Me-12/C-4, C-5, C-6, C-10; Me-13/ C-7, C-8, C-9; Me-14/C-8, C-9, C-10, C-15; H-15/C-8, C-9, C-10, C-14, C-16, C-17, C-21; H-10/C-1, C-4, C-5, C-12, C-14, C-15; H-19/C-17, C-18, C-20, C-21; 20-OMe/C-20; EIMS *m/z* 356 [M^+] (100), 189 (43), 175 (71); HREIMS *m/z* 356.1992 (calcd for $C_{22}H_{28}O_4$, 356.1988).

1.3.3. Dactyloquinone E (7). Pale yellow amorphous; $[\alpha]_D^{27}=+33.3°$ (*c* 0.09, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 290 (4.2) nm; IR (KBr) ν_{max} 1663, 1641, 1602 cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2, respectively; HMBC correlation (H/C) H-11/C-3, C-4, C-5; Me-12/C-4, C-5, C-6; Me-13/C-7, C-8, C-9; Me-14/C-8, C-9, C-10, C-15; H-15/C-8, C-9, C-10, C-14, C-16, C-17, C-21; H-10/C-1, C-4, C-5, C-12, C-14, C-15; H-19/C-17, C-18, C-20, C-21; 20-OMe/C-20; EIMS *m/z* 356 [M^+] (52), 341 (8), 189 (63), 188, (45), 187 (73), 161 (90), 147 (100); HREIMS *m/z* 356.1974 (calcd for $C_{22}H_{28}O_4$, 356.1988).

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14. This numbering is in accordance with that for ilimaquinone.