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Dictyoceratin-A and -B, Novel Antimicrobial Terpenoids from the Okinawan Marine Sponge Hippospongia sp.[1]

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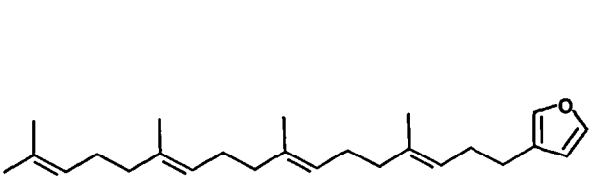
Abstract - Two new antimicrobial sesquiterpenoids, dictyoceratin-A (**3a**) and -B (**4a**) were isolated from the Okinawan marine sponge Hippospongia sp. along with known compounds, furospinulosin-1 (**1**) and ilimaquinone (**2**). The structures of dictyoceratin-A (**3a**) and -B (**4a**) were determined on the basis of spectral data and their absolute configurations were deduced to be antipodal to that of ilimaquinone (**2**) by comparing an ozonolysis product (**5**) with that (**6**) derived from ilimaquinone (**2**).

Marine sponges have yielded a variety of antimicrobial compounds having a hydroquinone moiety attached to a terpenoid skelton.[3] In the course of our study on physiologically active substances from marine organisms,[4,5] we found that crude extracts of the Okinawan marine sponge Hippospongia sp. (family Spongiidae, order Dictyoceratida), exhibited in vitro antimicrobial activity against Staphyrococcus aureus and Bacillus subtilis. From the sponge, we isolated two novel antimicrobial sesquiterpenoids containing a hydroquinone moiety, dictyoceratin-A (**3a**) and dictyoceratin-B (**4a**) together with known compounds, furospinulosin-1 (**1**)[6,7] and ilimaquinone (**2**).[7,8] In this paper, we describe the structural elucidation of dictyoceratin-A (**3a**) and -B (**4a**).

Specimens of the sponge were collected at Okinawa Islands in July, 1984, and were stored frozen until required. Ethanolic extracts were partitioned between hexane and 80% ethanol and the 80% ethanol portion was further divided into ethyl acetate soluble and water soluble materials. Chromatography of the hexane soluble part on a silica gel column using 95:5 benzene-acetone as eluant afforded furospinulosin-1 (**1**), ilimaquinone (**2**), a mixture of ilimaquinone (**2**) and dictyoceratin-B (**4a**) and crude dictyoceratin-A (**3a**). The mixture of **2** and **4a** was separated by a silica gel column (4:1 hexane-ethyl acetate) followed by a Sephadex LH-20 column (1:1 chloroform-methanol) to yield pure **4a**. Crude **3a** was subjected to silica gel and LH-20 column chromatography as same to **4a** to give pure **3a**. Additional amounts of **2**, **3a** and **4a** were obtained from the ethyl acetate soluble portion.

Dictyoceratin-A (**3a**) was obtained as colorless amorphous solid, mp 180-181.5 °C and showed UV absorption maxima at 218 (ε 24900), 268 (10000) and 300 nm (4350).



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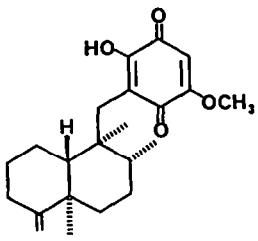

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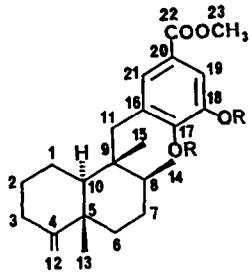
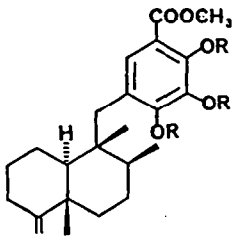


3a ; R=H
3b : R=Ac



4a : R=H
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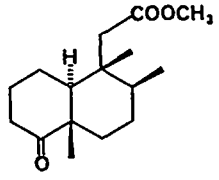

5
6 enantiomer

Table 1. ^{13}C NMR Data for Terpenoid Parts for Ilimaquinone (2), Dictyoceratin-A (3a), and Dictyoceratin-B (4a) in $CDCl_3$.[a]

Carbon	2	3a	4a	Carbon	2	3a	4a
C-1	23.2 t	23.1 t	23.1 t	C-9	43.4 s	42.1 s	41.8 s
C-2	28.0 t	27.6 t	27.8 t	C-10	50.3 d	48.1 d	48.1 s
C-3	36.8 t	36.9 t	36.7 t	C-11	32.5 t	32.9 t	33.1 t
C-4	153.5 s	150.7 s	149.1 s	C-12	102.6 t	103.0 t	102.7 t
C-5	40.5 s	40.1 s	40.2 s	C-13	20.5 q	20.5 q	20.6 q
C-6	36.3 t	36.5 t	36.3 t	C-14	17.9 q	17.4 q	17.5 q
C-7	27.9 t	27.8 t	27.9 t	C-15	17.3 q	17.4 q	17.5 q
C-8	38.2 d	36.3 d	36.3 d				

a: δ in ppm.

Table 2. ^{13}C NMR Data for Aromatic Parts of Dictyoceratin-A (3a) and Dictyoceratin-B (4a) in $CDCl_3$.

	3a			4a		
Carbon	δ	m[a]	J[b]	δ	m	J
C-16	125.7	s/t	/5 Hz(11-H)[c]	117.2	s/t	/5 Hz(11-H)[c]
C-17	160.8	s/m		160.2	s/m	
C-18	144.6	s/brs		130.4	s/brd	/3 Hz(19-OH)
C-19	114.0	d/d	164 Hz/7 Hz(21-H)[c]	147.1	s/d	/9 Hz(21-H)[c]
C-20	120.2	s/s		104.5	s/s	
C-21	127.0	d/q	160 Hz/6 Hz(11-H,19-H)[c]	124.7	d/t	161 Hz/6 Hz(11-H)c
C-22	168.9	s/sixtet	/4 Hz(19-H,21-H,OCH_3)[c]	170.7	s/quintet	/4 Hz(21-H,OCH_3)[c]
C-23	52.0	q/s	148 Hz/	52.0	q/s	148 Hz/

a: Multiplicity, direct coupling/long range coupling.

b: ^1H-^{13}C coupling constant, direct coupling/long range coupling.

c: Coupled proton assigned by low power proton selective decoupling.

Molecular formula $C_{23}H_{32}O_4$ for **3a** was obtained from its HREIMS (m/z 372.2308, Δ 0.7 mmu) and a remarkable fragment ion at m/z 191 suggested the presence of a rearranged driman skelton in **3a**. ^{1}H NMR spectrum of **3a** showed two singlet methyl, one broad methyl, one benzylic methylene and an exocyclic methylene signals at δ 0.88, 1.05, 1.00, 2.66 and 4.40, respectively, like that of ilimaquinone (δ 0.84, 1.06, 1.00, 2.50 and 4.43). These data and comparison of ^{13}C NMR data of **3a** with that of **2** (Table 1) revealed that the structure of terpenoid part of **3a** is identical with that of **2**. The remaining portion of **3a** was composed of $C_8H_7O_4$ and IR bands at 3500 and 1695 cm^{-1} indicated the presence of hydroxyl and ester functionalities, respectively. Acetylation of **3a** with acetic anhydride-pyridine yielded a diacetyl compound **3b** and meta-coupling was observed between aromatic proton signals at δ 7.38 and 7.50 (J=1.8 Hz), suggesting the presence of a 2,4,5,6-tetrasubstituted benzene ring. Substitution pattern of the benzene ring was deduced by analysis of ^{1}H-^{13}C long range couplings (Table 2)[9]: The ester carbonyl carbon coupled with two aromatic protons and methoxy protons, and the C-21 carbon coupled with the benzylic methylene protons. This was further supported by NOE experiment: NOE on 19-H (2%) and 21-H (2%) upon irradiation of the methoxy signal (δ 3.86), and NOE on 21-H (7%) upon irradiation of 11-H proton signal (δ 2.60).

Dictyoceratin-B (**4a**), colorless amorphous solid, mp 154.5-155.5 °C, showed a molecular ion peak at m/z 388.2258 ($C_{23}H_{32}O_5$, Δ 0.9 mmu) in the mass spectrum. The ^{1}H NMR spectrum of **4a** contained two singlet methyl, one broad methyl, one benzylic methylene and an exocyclic methylene signals at δ 0.84, 1.08, 1.00, 2.60 and 4.40, respectively, like that of **3a**. ^{13}C NMR signals for the terpenoid part of **4a** were superimposable to that of **3a**, suggesting that **4a** has the same terpenoid structure as **3a**. **4a** upon treatment with acetic anhydride-pyridine afforded a triacetyl compound **4b**. In the ^{1}H NMR, one aromatic proton, two hydroxyl proton and a hydrogen bonded hydroxyl proton signals were observed at δ 7.14, 5.48, 5.90 and 10.74, respectively, suggesting the presence of an additional hydroxyl group located at C-19 position in **4a**. The structure of aromatic part of **4a** was established by ^{13}C NMR data (Table 2), in which ^{1}H-^{13}C long range coupling between C-18 carbon and the hydrogen bonded hydroxyl proton (C-19OH) was observed.[10]

Ozonolyses of **3a** and **4a** followed by Jones oxidation and methylation with diazomethane gave an identical ketone **5** whereas ilimaquinone (**2**) afforded a ketone **6** by the same sequence of reactions.[7] The mass spectrum and the ^{1}H NMR spectrum of **5** were superimposable to those of **6**. However, CD spectrum of **5** was opposite in sign to that of **6**, suggesting that the structure of sesquiterpenoid parts of dictyoceratin-A and -B is antipodal to that of ilimaquinone (**2**).

Dictyoceratin-A and -B might be produced biogenetically via a pathway similar to ilimaquinone. It is estimated that the terminal polar functionalities of dictyoceratins and ilimaquinone are recognized when the enantiomeric bicyclic systems are formed. Such relationship is also found in that of agelasines and agelasidines.[5] Dictyoceratins and ilimaquinone showed antimicrobial activity against Staphyrococcus aureus and Bacillus subtilis and dictyoceratin-A exhibited the most potent activity among them. The minimum inhibitory concentrations against S. aureus and B. subtilis were 6.3 and 3.1 μg/ml, respectively.

EXPERIMENTAL

General. Melting points were taken on a Yanagimoto micro melting point apparatus. UV spectra were recorded on a Varian Cary-17 spectrophotometer. IR spectra were obtained on a Hitachi 260-50 spectrometer. CD spectra were taken on a JASCO DIP 360. Low resolution and high resolution EI mass spectra (HREIMS) were obtained with Shimazu GC-7000 and JEOL HX-100 instruments, respectively. ^{1}H (90 MHz) and ^{13}C (22.5

MHz) NMR spectra were obtained with a JEOL FX-90Q instrument. Chemical shifts were expressed in parts per million from internal tetramethylsilane (δ). Coupling constants are in hertz (Hz) and splitting pattern abbreviations are: s, singlet; d, doublet; q, quartet; br, broad.

Collection, Extraction and Isolation. Specimens of the marine sponge Hippospongia sp. were collected by SCUBA at Okinawa Islands (-10 - -20 m), freezed and stored at -20 °C until workup. The freezed specimens (510 g, wet weight) were cut into small pieces and extracted with ethanol (1l x 3). The ethanol soluble part of the extracts was partitioned between hexane and 80% ethanol. The hexane soluble portion was chromatographed on a silica gel column with 95:5 benzene-acetone as eluant to yield furospinulosin-1 (1, 1.14 g), ilimaquinone (2, 926 mg), a mixture of ilimaquinone and dyctyoceratin-B (4), and crude dyctyoceratin-A (3). The later two fractions were separated on a silica gel column (4:1 hexane-ethyl acetate) followed by an LH-20 column (1:1 chloroform-methanol) to afford pure compounds **4a** (27.6 mg) and **3a** (35.8 mg), respectively. The 80% ethanol soluble portion was suspended in water and extracted with ethyl acetate, and then with 1-butanol. Chromatography of ethyl acetate soluble material on LH-20 and silica gel columns as described above gave compounds **2** (721 mg), **3a** (30 mg) and **4a** (9.6 mg).

Furospinulosin-1 (1): A colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.60 (brs, 12H), 1.68 (brs, 3H), 2.0-2.6 (m, 16H), 5.09 (m, 4H), 6.25 (t, 1H, J=1 Hz), 7.19 (t, 1H, J=1 Hz), 7.30 (t, 1H, J=1 Hz); ^{13}C NMR ($CDCl_3$) δ 16.0 (q, 3C), 17.6 (q), 25.1 (t), 25.6 (q), 26.7 (t, 2C), 26.9 (t), 28.5 (t), 39.7 (t, 3C), 111.0 (d), 123.8 (d), 124.3 (d, 2C), 124.5 (d), 125.0 (s), 131.1 (s), 134.9 (s), 135.0 (s), 135.7 (s), 138.8 (d), 142.5 (d); EIMS m/z 354 (M^+).

Ilimaquinone (2): Golden color crystals; mp 111.5-113 °C; $[\alpha]_D^{25}$ -27.7° (c 1.12, $CHCl_3$); EIMS m/z 358 (M^+).

Dictyoceratin-A (3a): Amorphous solid; mp 180-181.5 °C; $[\alpha]_D^{25}$ +5.80° (c 0.965, $CHCl_3$); UV (EtOH) λmax 218 (ε 24900), 268 (10000), 300 nm (4350); IR (KBr) νmax 3500, 3330, 1695, 1605, 1440, 1300 cm^{-1}; EIMS (%) m/z 372 (M^+, 3), 357 (2), 341 (4), 302 (2), 191 (100), 182 (54), 165 (25), 135 (31), 121 (33), 109 (14), 95 (100), 81 (7); ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, s, 15-CH_3), 1.00 (3H, br, 14-CH_3), 1.05 (3H, s, 13-CH_3), 1.0-2.4 (m, 12H), 2.66 (2H, s, 11-CH_2), 3.86 (3H, s, OCH_3), 4.40 (2H, brs, $=CH_2$), 6.12 (2H, brs, OH), 7.38 (1H, d, J=1.8 Hz, 19-H), 7.50 (1H, d, J=1.8 Hz, 21-H); HREIMS m/z 372.2308 (M^+, $C_{23}H_{32}O_4$ requires M, 372.2301).

Dictyoceratin-B (4a): Amorphous solid; mp 154.5-155 °C, $[\alpha]_D^{25}$ -1.22° (c 1.12, $CHCl_3$); UV (EtOH) λmax 221 (ε 33800), 275 nm (12600); IR (KBr) νmax 3550, 3450, 1670, 1440, 1300 cm^{-1}; EIMS (%) m/z 388 (M^+, 1.5), 373 (1), 357 (1.5), 301 (2), 198 (100), 175 (39), 166 (31), 135 (21), 121 (22), 109 (10), 95 (50), 91 (10); ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, s, 15-CH_3), 1.00 (3H, br, 14-CH_3), 1.08 (3H, s, 13-CH_3), 1.0-2.4 (12H, m), 2.60 (2H, s, 11-CH_2), 3.90 (3H, s, OCH_3), 4.40 (2H, brs, $=CH_2$), 5.48 (1H, brs, OH), 5.90 (1H, brs, OH), 7.14 (1H, s, 21-H), 10.74 (1H, brs, OH); HREIMS m/z 388.2258 (M^+, $C_{23}H_{32}O_5$ requires M, 388.2249).

Acetylation of Dictyoceratin-A (3a) and -B (4a). **3a** (2 mg) and **4a** (2 mg) were treated with a mixture of acetic anhydride (0.05 ml) and pyridine (0.05 ml) at room temperature for 1 h and evaporated to dryness to give the diacetate **3b** and the triacetate **4b**, respectively. **3a**: EIMS m/z 456 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, s), 1.00 (3H, br), 1.08 (3H, s), 2.28 (3H, s), 2.33 (3H, s), 2.58 (2H, s), 3.89 (3H, s), 4.38 (1H, brs), 4.44 (1H, brs), 7.75 (2H, s). **4b**: EIMS m/z 514 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, s), 0.98 (3H, br), 1.08 (3H, s), 2.27 (3H, s), 2.31 (6H, s), 2.55 (2H, s), 3.84 (3H, s), 4.45 (2H, brs), 7.73 (1H, s).

Ozonolyses of Dictyoceratin-A (3a) and -B (4a) and Ilimaquinone (2). A solution of **3a** (5 mg) in dry acetone (2.5 ml) was saturated with ozone at -78°C for 1.5

h. After removing excess ozone using a nitrogen stream Jones reagent (6 N, 0.23 ml) was added at 0 °C and the mixture was stirred for 1 h. The resulting mixture was diluted with water and then extracted with diethyl ether (5 ml x 4). The combined ether extract was washed with water and extracted with 5% sodium hydrogen carbonate (5 ml x 4). The extract after acidified with 1 N HCl was extracted with diethyl ether (5 ml x 4). The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to give a mixture of carboxylic acids, which was treated with diazomethane at room temperature overnight. The resulting mixture was purified by HPLC (Develosil 60-3, 10 x 250 mm, hexane-diethyl ether 4:1, flow rate 3.1 ml/min) to give **5** (1.5 mg, t_R=15.4 min). By the same sequence of reactions, **5** and **6** were obtained from **4a** and **2**, respectively. **5**: CD (EtOH) $[\theta]_{296}$ -16 (from **3a**), -19 (from **4a**); EIMS (%) m/z 266 (M^+, 2), 251 (8), 248 (6), 193 (34), 192 (24), 174 (100); ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, s), 0.88 (3H, d, J=5.1 Hz), 1.10 (3H, s), 2.34 (2H, d, J=2 Hz), 3.58 (3H, s). **6**: CD (EtOH) $[\theta]_{296}$ +60; ^{13}C NMR ($CDCl_3$) δ 16.1 (q), 17.3 (q), 18.9 (q), 21.2 (t), 25.6 (t), 26.4 (t), 32.7 (t), 37.3 (t), 37.5 (t), 41.3 (s), 42.8 (t), 48.9 (s), 49.2 (d), 51.1 (q), 171.7 (s), 215.3 (s); HREIMS m/z 266.1900 (M^+, $C_{16}H_{26}O_3$ requires M, 266.1882).

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