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Studies on Marine Natural Products. VIII.[1] New Butenolides from the Gorgonian *Euplexaura flava* (Nutting)

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Four new butenolides (**1a**—**d**) were isolated from the Japanese gorgonian *Euplexaura flava* (Nutting). The structures of these compounds were elucidated on the basis of spectral data and chemical reactions.

Keywords——coelenterate; gorgonian; *Euplexaura flava* (Nutting); marine natural products; butenolide; structure elucidation

A variety of marine natural products have been isolated in recent years. Several butenolides[2,3] have also been isolated from marine sources, and some of these butenolides showed significant biological activities.[3] During the course of our investigations[4] on the chemical constituents of Japanese coelenterates, we have isolated four new butenolides (**1a**—**d**) from a gorgonian *Euplexaura flava* (Nutting). A part of this work has been described in a preliminary communication.[4c] The present paper is a full account of the isolation and structure elucidation of these butenolides on the basis of spectral properties and chemical reactions.

The isolation of the butenolides (**1a**—**d**) was carried out according to the procedure shown in Fig. 1. *Euplexaura flava* (Nutting) (wet weight 1.8 kg), collected on the coral reefs of Ishigaki Island (Okinawa, Japan) in 1978, was cut into pieces and extracted with methanol and then acetone. The combined methanol and acetone extracts were suspended in water and extracted with ethyl acetate. Repeated silica gel column chromatography of the ethyl acetate extract gave a mixture of four compounds (**1a**—**d**). High pressure liquid chromatography (HPLC) (Hitachi gel 3010) of the mixture using methanol as an eluant gave **1a** (2.38 g, $C_{21}H_{38}O_3$, mp 67°C) as colorless needles and an oily mixture of **1b**—**d** (2.7 g). Separation of **1b**—**d** was performed by silver nitrate-impregnated silica gel column chromatography. Elution with an *n*-hexane–acetone gradient (6: 1, 4: 1 and 3: 1) gave three oily compounds (**1b**) (63 mg, $C_{25}H_{42}O_3$), (**1c**) (340 mg, $C_{27}H_{44}O_3$) and (**1d**) (363 mg, $C_{27}H_{42}O_3$), respectively, in order of elution.

The physical data of **1a**—**d** are summarized in the Table. The presence of a hydroxyl group in each compound was suggested by the infrared (IR) absorptions at 3565—3560 cm^{-1} and was supported by the result of acetylation; treatment of **1a**—**d** with acetic anhydride in pyridine at room temperature for 3 d gave the corresponding monoacetates (**2a**—**d**). The ^1H nuclear magnetic resonance (^1H-NMR) spectra of **1a**—**d** show no signal due to a proton on carbon bearing an oxygen atom, indicating that the hydroxyl group is tertiary. The presence of an α,β-unsaturated γ-lactone (butenolide) moiety in **1a**—**d** was suggested by the IR (1765—1760 cm^{-1}) and ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectra [δ 171.4—172.4 (s, C=O), 146.6—147.3 (d, $\rangle C=\underline{C}H-$), 135.8—136.3 (s, $\rangle\underline{C}=CH-$)ppm]. The ^1H-NMR and ^{13}C-NMR spectra of **1a**—**d** also show the presence of a tertiary methyl group [^1H-NMR δ 1.68—1.69 (3H, s), ^{13}C-NMR δ 24.7—24.9 (q) ppm] and a saturated or unsaturated straight-chain hydrocarbon group; *n*-hexadecyl ($C_{16}H_{33}-$) for **1a**, *n*-eicosadienyl ($C_{20}H_{37}-$) for **1b**, *n*-docosatrienyl ($C_{22}H_{39}-$) for **1c** and *n*-docosatetraenyl ($C_{22}H_{37}-$) for **1d**. Regarding the unsaturated hydrocarbon moiety, the following partial structures were suggested. The ^1H-NMR signals at δ 2.76 (2H) of

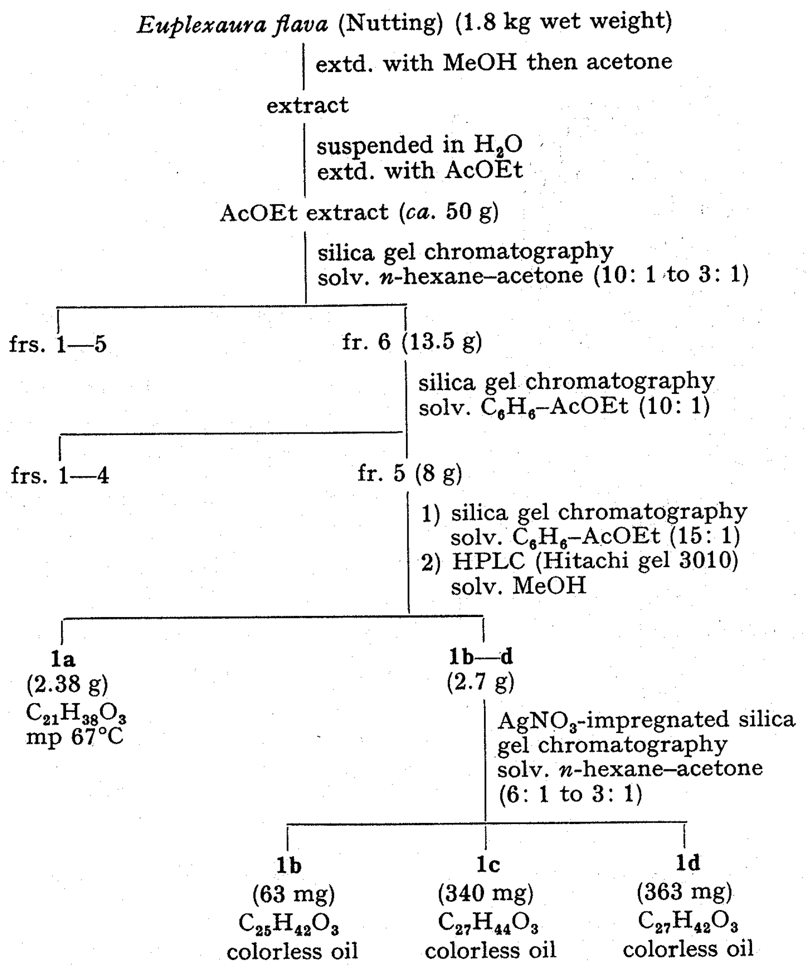

Fig. 1. Isolation of **1a**—**d** from *Euplexaura flava* (Nutting)

1b, 2.80 (4H) of **1c** and 2.80 (6H) of **1d**, which are assignable to methylene protons deshielded by two allylic double bonds, show the presence of $-CH=CH-CH_2-CH=CH-$ for **1b**, $-CH=CH-CH_2-CH=CH-CH_2-CH=CH-$ for **1c** and $(-CH=CH-CH_2-CH=CH-)_2CH_2$ for **1d**.

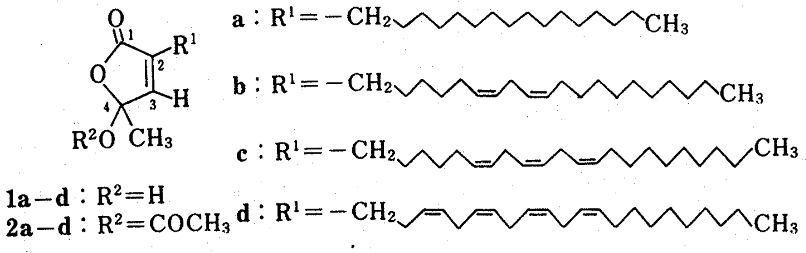

Fig. 2

These findings reveal that the compounds (**1a**—**d**) possess a butenolide moiety to which a tertiary hydroxyl group, a methyl group and a saturated or unsaturated long-chain hydrocarbon group are attached. The locations of these groups were determined on the basis of the spectral data and chemical reactions.

The ^{1}H-NMR signals at δ 6.76—6.78 ppm assignable to an olefinic proton at the β-position (C-3) of the α,β-unsaturated carbonyl system show that the above-mentioned groups are located at the C-2 and C-4 positions on the butenolide ring. The ^{13}C-NMR signals at δ 104.7—105.1 (s) ppm assignable to a quaternary carbon bearing two oxygen atoms indicate that the

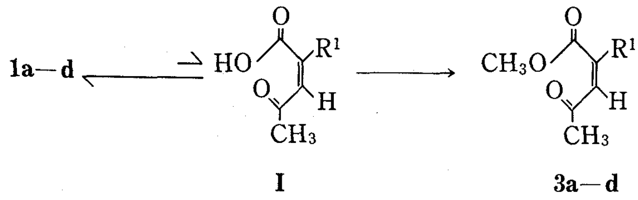

Fig. 3

hydroxyl group and either the methyl or long-chain hydrocarbon group are attached at the C-4 position. The positions of the methyl group at C-4 and the long-chain hydrocarbon group at C-2 were elucidated by chemical reaction as shown in Fig. 3. Treatment of **1a** with diazomethane in diethyl ether at room temperature for 1 h gave a methylketone (**3a**); mp 49—53°C; IR cm^{-1}: 1720, 1690, 1620; ^{1}H-NMR δ: 2.23 (3H, s), 3.79 (3H, s), 6.12 (1H, t, J=1.8 Hz) ppm; iodoform test (+). Similar reactions of **1b**—**d** with diazomethane gave the corresponding methylketones (**3b**—**d**). The formation of **3a**—**d** is explained by the presence of an equilibrium between the lactol (**1a**—**d**) and the corresponding keto carboxylic acid (I).[5)]

The mode of substitution on the butenolide ring was also supported by the observation of an acetylation shift[6)] in the ^{13}C-NMR spectra of the acetates (**2a**—**d**) when compared with the spectra of **1a**—**d**. On going from **1b** to **2b**, for example, the signals of both C-4 (104.7 ppm) and C-2 (136.3 ppm of **1b** shifted to lower field by +1.0 and +0.3 ppm, respectively, while the methyl signal at C-4 (24.9 ppm) and the olefinic carbon signal at C-3 (146.6 ppm) of **1b** appeared at higher field by −1.8 and −1.9 ppm, respectively, in **2b**.

TABLE. Physical Data for the Butenolides

	1a	**1b**	**1c**	**1d**
Molecular formula	$C_{21}H_{38}O_3$	$C_{25}H_{42}O_3$	$C_{27}H_{44}O_3$	$C_{27}H_{42}O_3$
Appearance	Colorless needles mp 67 °C	Colorless oil	Colorless oil	Colorless oil
IR $\nu_{\max}^{CHCl_3}$cm^{-1}	3560 1760 1655	3560 1760 1655	3565 1765 1655	3565 1765 1655
^{1}H-NMR($CHCl_3$) δ	0.88 (3H, t)	0.89 (3H, t)	0.86 (3H, t)	0.89 (3H, t)
	1.25(28H,br s)	1.28 (22H, br s)	1.30 (20H, br s)	1.30, 1.36 (14H)
	1.68 (3H, s)	1.68 (3H, s)	1.69 (3H, s)	1.69 (3H, s)
		2.05 (4H, br d)	2.05 (4H, br d)	2.05 (4H, br d)
	2.20 (2H, br t)	2.25 (2H, br t)	2.25 (2H, br t)	2.25 (2H, br t)
		2.76 (2H, t)	2.80 (4H, t)	2.80 (6H, br t)
		5.35 (4H, m)	5.35 (6H, m)	5.35 (8H, m)
	6.78 (1H, t, J=2 Hz)	6.77 (1H, t, J=2 Hz)	6.76 (1H, t, J=2 Hz)	6.78 (1H, t, J=2 Hz)
^{13}C-NMR($CDCl_3$) δ	14.1 (q) 24.7 (q)	14.1 (q) 24.9 (q)	14.1 (q) 24.9 (q)	14.1 (q) 24.9 (q)
	31.9 (t) 105.0 (s)	25.7 (t) 31.5 (t)	25.7 (t, 2C)	25.7 (t, 3C)
	135.8 (s) 147.3 (d)	104.7 (s)	31.5 (t)	31.5 (t)
	172.4 (s)	127.9 (d, 2C)	105.1 (s)	104.8 (s) 127.5 (d)
		130.1 (d, 2C)	127.7 (d, 2C)	127.8 (d) 127.9 (d)
		136.3 (s) 146.6 (d)	128.2 (d, 2C)	128.3 (d)
		171.4 (s)	130.3 (d, 2C)	128.5 (d, 2C)
			136.1 (s) 146.9 (d)	130.1 (d) 130.4 (d)
				136.1 (s) 146.8 (d)
			171.8 (s)	171.6 (s)

The positions of the carbon-carbon double bonds in the unsaturated hydrocarbon moiety of **1b**—**d** were determined by the following chemical degradation. Treatment of the methylketone (**3b**) with ozone at −78°C for 20 min followed by treatment with 30% hydrogen peroxide and then methylation with diazomethane gave a mixture of methyl esters. Gas chromatography–mass spectroscopy (GC–MS) of the products showed the formation of methyl *n*-undecanoate, which was identified by comparison of its retention time and mass fragmentation pattern with those of authentic methyl *n*-undecanoate. In similar experiments, *n*-caprate (*n*-decanoate) was obtained from both **3c** and **3d**. The (*Z*) geometries of all carbon-carbon double bonds in the hydrocarbon chains of **1b**—**d** were deduced from the ^{13}C-NMR spectra. The resonances at δ 25.7 ppm, integrating as one carbon atom for **1b**, two carbon atoms for **1c** and three carbon atoms for **1d** (Table), are characteristic of methylene carbons shielded by two allylic (*Z*)-double bonds.$^{7)}$

These results established the structure of **1a**—**d** as shown in Fig. 2. Compounds **1a**—**d** were each isolated in racemic form. This fact can be interpreted in terms of equilibration through I (Fig. 3).

The butenolides (**1a**—**d**) are regarded as derivatives of long chain fatty acids possessing a three carbon-branch at α-position. This type of long chain fatty acid derivatives$^{8)}$ is very rare in nature and **1a**—**d** are the first example isolated from marine sources. The compounds (**1a**—**d**) showed a significant anti-inflammatory effect at 100 μg/ml by the fertile egg test.$^{9)}$

Experimental

All melting points are uncorrected. IR spectra were taken with a Hitachi 215 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a JEOL PS-100 spectrometer (100 MHz) and a JEOL FX-100 spectrometer (25.0 MHz), respectively. Chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard. Mass spectra (MS) were measured with a Hitachi RMU-7L spectrometer and a Hitachi M-80 spectrometer. $AgNO_3$-impregnated silica gel was prepared as follows; silica gel (300 g, 40—63 μ, Merck) was suspended in 600 ml of 10% aqueous $AgNO_3$ solution, filtered with an aspirator and activated at 180°C for 3 h.

Isolation of 1a—d——The wet gorgonian *Euplexaura flava* (Nutting) (1.8 kg), collected on the coral reefs of Ishigaki Island (Okinawa, Japan) in 1978, was cut into pieces and extracted with MeOH (20 l) and then with acetone (20 l) at room temperature overnight. The combined extracts were suspended in H_2O and extracted twice with AcOEt (2 l). The AcOEt extract (*ca.* 50 g) was chromatographed on a silica gel column (700 g, 63—200 μ, Merck) with an *n*-hexane–acetone gradient (10: 1 to 3: 1). The fraction (13.5 g) eluted with *n*-hexane–acetone (4: 1) was further chromatographed repeatedly on a silica gel column; 1) silica gel 400 g, 63—200 μ, Merck, C_6H_6–AcOEt=15: 1, 2) silica gel 100 g, 40—63 μ, Merck, C_6H_6–AcOEt=10: 1. The fraction (5.5 g) containing **1a**—**d** was subjected to polystyrene gel column chromatography (Hitachi gel 3010, Hitachi Kasei) and elution with MeOH gave two fractions. The crystalline substance obtained from the first fraction was recrystallized from MeOH to give **1a** (2.38 g) as colorless needles. The oily substance obtained from the second fraction (2.7 g) was further chromatographed on a $AgNO_3$-impregnated silica gel column (300 g, 40—63 μ, Merck). Elution with *n*-hexane–acetone (6: 1, then 3: 1) gave **1b** (63 mg), **1c** (340 mg) and **1d** (363 mg) successively as colorless oils.

1a: mp 67°C. High resolution MS *m*/*z*: M$^+$ Calcd for $C_{21}H_{38}O_3$ 338.282. Found: 338.282.

1b: High resolution MS *m*/*z*: M$^+$ Calcd for $C_{25}H_{42}O_3$ 390.313. Found: 390.314.

1c: High resolution MS *m*/*z*: M$^+$ Calcd for $C_{27}H_{44}O_3$ 416.329. Found: 416.330.

1d: High resolution MS *m*/*z*: M$^+$ Calcd for $C_{27}H_{42}O_3$ 414.313. Found: 414.315.

The physical properties of **1a**—**d** are listed in the Table. All of these compounds gave an $[\alpha]_D$ value of 0°.

Acetylation of 1a—d——A mixture of **1a** (59 mg), acetic anhydride (0.5 ml) and pyridine (1 ml) was stirred at room temperature for 3 d. The reaction mixture was poured into ice-water and extracted with AcOEt. The AcOEt layer was washed successively with saturated aqueous $CuSO_4$ solution, H_2O and saturated aqueous NaCl solution, then dried over anhydrous Na_2SO_4. Removal of the solvent gave an oily substance, which was chromatographed on a silica gel column to give **2a** (24 mg) as colorless rods. Similar reactions of **1b**—**d** gave the corresponding monoacetates (**2b**—**d**).

2a: mp 48—53°C. High resolution MS *m*/*z*: M$-$AcOH Calcd for $C_{21}H_{36}O_2$ 320.271. Found: 320.270. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1765, 1730. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, t), 1.80 (3H, s), 2.05 (3H, s), 2.28 (2H, br t), 7.15 (1H, t, *J*=2 Hz).

2b: Colorless oil. High resolution MS *m*/*z*: M$-$AcOH Calcd for $C_{25}H_{40}O_2$ 372.303. Found: 372.301. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1770, 1730. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t), 1.80 (3H, s), 2.03 (3H, s), 2.75 (2H, t, *J*=6 Hz), 5.34 (4H, m), 7.14 (1H, t, *J*=2 Hz).

2c: Colorless oil. High resolution MS *m*/*z*: M$^+$ Calcd for $C_{29}H_{46}O_4$ 458.340. Found: 458.341. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1775, 1735. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t), 1.79 (3H, s), 2.02 (3H, s), 2.79 (4H, t, *J*=6 Hz), 5.35 (6H, m), 7.15 (1H, t, *J*=2 Hz).

2d: Colorless oil. High resolution MS *m/z*: M$^+$ Calcd for $C_{29}H_{44}O_4$ 456.324. Found: 456.326. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1770, 1730. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t), 1.79 (3H, s), 2.05 (3H, s), 2.82 (6H, br t), 5.36 (8H, m), 7.15 (1H, t, $J=2$ Hz).

Reaction of 1a—d with Diazomethane——Excess diazomethane in Et_2O was added to a solution of **1a** (100 mg) in 10 ml of Et_2O. The mixture was stirred at room temperature for 1 h and was concentrated under reduced pressure to give a colorless oil. Silica gel column chromatography of the oil gave a crystalline substance which was recrystallized from *n*-hexane to give **3a** (35 mg) as colorless rods. Similar reactions of **1b**—**d** gave the corresponding methylketones (**3b**—**d**).

3a: mp 49—53°C. High resolution MS *m/z*: M$^+$ Calcd for $C_{22}H_{40}O_3$ 352.298. Found: 352.300. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1720, 1690, 1620. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, t), 2.23 (3H, s), 2.32 (2H, br t), 3.79 (3H, s), 6.12 (1H, t, $J=1.8$ Hz).

3b: Colorless oil. High resolution MS *m/z*: M$^+$ Calcd for $C_{26}H_{44}O_3$ 404.329. Found: 404.328. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1720, 1685, 1610. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t), 2.21 (3H, s), 2.76 (2H, t, $J=6$ Hz), 3.75 (3H, s), 5.29 (4H, m), 6.09 (1H, t, $J=1.5$ Hz).

3c: Colorless oil. High resolution MS *m/z*: M$^+$ Calcd for $C_{28}H_{46}O_3$ 430.345. Found: 430.344. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1720, 1685, 1610. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, t), 2.22 (3H, s), 2.81 (4H, t, $J=6$ Hz), 3.78 (3H, s), 5.36 (6H, m), 6.11 (1H, t, $J=2$ Hz).

3d: Colorless oil. High resolution MS *m/z*: M$^+$ Calcd for $C_{28}H_{44}O_3$ 428.329. Found: 428.329. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1720, 1685, 1610. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, t), 2.22 (3H, s), 2.81 (6H, br t), 3.78 (3H, s), 5.37 (8H, m), 6.11 (1H, t, $J=1.5$ Hz).

Ozonolysis of 3b—d——Ozone was passed through a solution of the methyl ketone (**3b**) (19 mg) in 2 ml of a mixture of CH_2Cl_2–MeOH (1: 1) at -16—$-14°$ until the pale blue color persisted for more than 2 min. Excess ozone was removed by bubbling O_2 through the solution at this temperature. Then 30% H_2O_2 (0.3 ml) was added and the mixture was allowed to warm to room temperature. After 1 h, excess diazomethane in Et_2O was added and the mixture was stirred for 30 min. Excess Et_2O was added to the reaction mixture and the mixture was washed successively with 10% aqueous $Na_2S_2O_3$ solution, saturated aqueous $NaHCO_3$ solution, H_2O, and saturated aqueous NaCl solution, then dried over anhydrous Na_2SO_4. Removal of the solvent gave a mixture of methyl esters. GC–MS (3% SE-30, He, 70°C) of the mixture showed a main peak whose retention time and mass fragementation pattern were in good accordance with those of methyl *n*-undecanoate. Similar ozonolysis of both **3c** and **3d** yielded methyl *n*-caprate.

References and Notes

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