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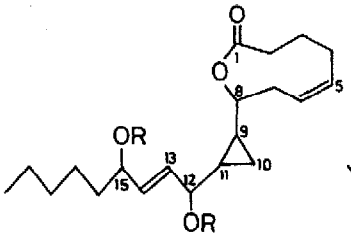
HALICHOLACTONE AND NEOHALICHOLACTONE, TWO NOVEL FATTY ACID METABOLITES FROM THE MARINE SPONGE HALICHONDRIA OKADAI KADOTA

Haruki Niwa, Kazumasa Wakamatsu, and Kiyoyuki Yamada*
Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

Summary: Halicholactone (**1**) and neohalicholactone (**2**), unusual fatty acid metabolites having a cyclopropane ring and a nine-membered lactone were isolated from the marine sponge Halichondria okadai Kadota. The planar structures of the new metabolites were elucidated on the basis of spectral and chemical means.

Marine sponges have yielded a variety of architecturally novel and pharmacologically interesting compounds.[1] We have examined the constituents of the sponge Halichondria okadai Kadota collected at Daiōzaki, Mie Prefecture, Japan and isolated two new fatty acid metabolites, halicholactone (**1**) and neohalicholactone (**2**). We wish to report herein the structural elucidation of these metabolites on the basis of spectral data coupled with chemical evidence.

The EtOAc-soluble material from the MeOH extract of the sponge was subjected to chromatography on silica gel (benzene-EtOAc → EtOAc → MeOH) followed by reversed-phase HPLC [ODS, MeOH-H_2O (7:3)] to give halicholactone (**1**)[2] (colorless oil; 2 x 10^{-3}% dry weight) and neohalicholactone (**2**)[3] [mp 69-70 °C (pentane-ether); 6 x 10^{-4}% dry weight].



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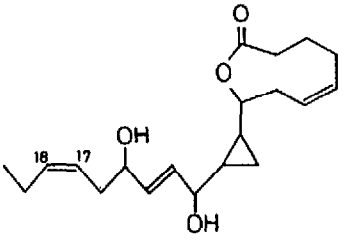
3 R = Ac



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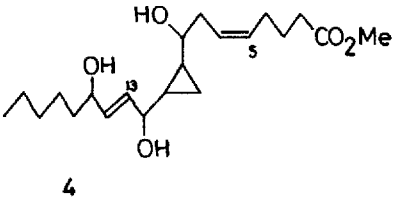

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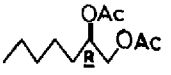

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The ^1H and ^{13}C NMR spectral data of **1** and **2** are summarized in Table 1. The IR spectrum of **1** indicated the presence of ester (or lactone) and hydroxyl functions. Acetylation of **1** (Ac_2O, Py) provided diacetate **3**,[4] (^1H NMR, Table 2), while methyl ester **4**[5,6] (^1H NMR, Table 2) was obtained upon methanolysis of **1** (NaOMe, MeOH). These chemical findings suggested **1** to be a diol lactone. The ^{13}C NMR spectrum of 1 showed the presence of a carbonyl carbon [δ 174.0 (s)] and three oxymethine carbons [δ 76.1 (d), 74.2 (d), and 72.3 (d)], supporting the above inference. The molecular formula of **1**, $C_{20}H_{32}O_4$, implying

5 degrees of unsaturation was inferred from the ^{13}C NMR spectrum (20 signals) and high-resolution mass measurement [m/z 318.2171 (M^+-H_2O) ($C_{20}H_{30}O_3$)] and was confirmed by the high-resolution mass spectrum of the diacetate **3** [m/z 420.2534 (M^+) ($C_{24}H_{36}O_6$)]. Detailed interpretation of the ^{13}C and ^1H NMR spectral data indicated the presence of the following groups in **1**: 1 x $-CO_2CH-$, 2 x $-CH=CH-$, 2 x $-CHOH-$, 1 x $CH_2\overset{-CH-}{\underset{-CH-}{\sim}}$, 8 x $-CH_2-$, 1 x $-CH_3$. The presence of a 1,2-disubstituted cyclopropane ring in **1** was deduced from the carbon signals [δ 19.5 (d; C-9), 8.2 (t; C-10), and 23.5 (d; C-11)] and the proton signals [δ 0.86 (1 H, m; H-9), 0.29 (1 H, ddd, J = 8, 5, 5 Hz; H-10), 0.47 (1 H, ddd, J = 8, 5, 5 Hz; H-10), and 1.03 (1 H, m; H-11)]. Each of the geminal protons at δ 0.29 and δ 0.47 (H-10) was coupled to the vicinal methine protons at δ 0.86 (H-9) and δ 1.03 (H-11) with the coupling constants of J_{cis} = 8 Hz and J_{trans} = 5 Hz, suggesting a trans ring substitution pattern.[7] Intensive studies of the $^1H-^1H$ COSY spectrum of **1** revealed the presence of partial structures **A-C** in **1**. These partial structures account for 18 among 20 carbons in **1**. Owing to the overlap of signals for the methylene protons appeared in the region of δ 1.1-1.4, the connectivity of these partial structures and the remaining two methylene carbons could not be clarified from the COSY spectrum. Further structural information for **1** was obtained from chemical degradation of **3**. Thus, reaction of **3** with OsO_4 (THF-Py) and subsequent $NaIO_4$ oxidation (EtOH-H_2O) provided a mixture of aldehydes, $NaBH_4$ reduction (MeOH) of which followed by acetylation (Ac_2O, Py) yielded (R)-diacetate **5**.[6,8] The structure of **5** including the absolute stereochemistry was established by comparison of spectral and chiroptical properties with those of the authentic **5**.[9] The formation of **5** from **3** indicated the presence of a partial structure **D** in **1**. On the basis of the partial structures **A-D**, the connectivity of all carbon atoms in **1** was established to give an extended partial structure **E**. The position of the lactone ring in **1** was deduced from the ^1H NMR spectral data of **1** and **3**: comparison of the ^1H NMR spectra of **1** and **3** (Table 1 and 2) revealed that the two signals for H-12 and H-15 in **1** underwent acetylation shift, while the chemical shift of the signal for H-8 was essentially unchanged in both **1** and **3**. These spectral findings indicated the C-1 carbonyl group and the C-8 oxygen atom to form a 9-membered lactone in **1**. The stereochemistry of the double bonds at C-5 and C-13 in **1** was assigned as Z and E, respectively from the coupling constants ($J_{5,6}$ = 11 Hz, $J_{13,14}$ = 16 Hz) in the ^1H NMR spectrum of **4**. In conclusion, the structure of halicholactone is established to be the formula **1**.

Neohalicholactone (**2**) has a molecular formula, $C_{20}H_{30}O_4$, which was determined by the ^{13}C NMR (Table 1) and high-resolution mass spectral data [m/z 316.2038 (M^+-H_2O) ($C_{20}H_{28}O_3$)]. Comparison of the spectral properties of **2** with those of **1** suggested that their structures were closely related and **2** was the dehydro compound of **1**. The methyl proton signal at δ 0.89 was coupled to the methylene proton signal at δ 1.97, which was in turn coupled to the olefinic proton at δ 5.51, indicating the presence of a partial structure **F** in **2**. The Z geometry of the double bond at C-17 in **2** was established by the chemical shift of the C-19 methylene carbon [δ 20.8 (t)] and the coupling constant (J = 11 Hz) between H-17 and H-18 in the NMR spectra of **2**. The intensive studies of $^1H-^1H$ COSY, ^{13}C and ^1H NMR spectra of **2**

Table 1. ^{13}C and ^{1}H NMR Spectral Data of Halicholactone (1) and Neohalicholactone (2)[a]

No. of carbon	1		2	
	δ_C (m)	δ_H (m, J in Hz)	δ_C (m)	δ_H (m, J in Hz)
1	174.0 (s)	-	174.1 (s)	-
2	33.6 (t)[b]	2.07 (2 H, m)	33.6 (t)[d]	2.08 (2 H, m)
3	25.3 (t)	1.55 (2 H, m)	25.3 (t)	1.55 (2 H, m)
4	26.5 (t)	1.77 (1 H, m) 2.37 (1 H, m)	26.5 (t)	1.76 (1 H, m) 2.37 (1 H, m)
5	131.7 (d)	5.35-5.45 (2 H, m)	131.8 (d)	5.35-5.45 (2H, m)
6	124.7 (d)		124.7 (d)	
7	33.9 (t)[b]	1.91 (1 H, ddd, 13, 7, 1.5) 2.34 (1 H, m)	33.9 (t)[d]	1.90 (1 H, ddd, 12, 7, 1.5) 2.34 (1 H, m)
8	76.1 (d)	4.32 (1 H, ddd, 12, 8, 1.5)	76.2 (d)	4.32 (1 H, ddd, 11, 9, 1.5)
9	19.5 (d)	0.86 (1 H, m)	19.5 (d)	0.85 (1 H, m)
10	8.2 (t)	0.29 (1 H, ddd, 8, 5, 5) 0.47 (1 H, ddd, 8, 5, 5)	8.2 (t)	0.27 (1 H, ddd, 8.5, 5, 5) 0.45 (1 H, ddd, 8.5, 5, 5)
11	23.5 (d)	1.03 (1 H, m)	23.4 (d)	1.02 (1 H, m)
12	74.2 (d)	3.53 (1 H, dd, 7, 4)	74.2 (d)	3.52 (1 H, m)
13	134.1 (d)[c]	5.65-5.75 (2 H, m)	134.7 (d)[e]	5.65-5.75 (2 H, m)
14	134.7 (d)[c]		135.3 (d)[e]	
15	72.3 (d)	3.92 (1 H, m)	71.5 (d)	3.98 (1 H, m)
16	37.3 (t)	1.50 (2 H, m)	35.2 (t)	2.23 (2 H, m)
17	25.1 (t)		133.3 (d)[e]	5.41 (1 H, m)
18	31.8 (t)	1.10-1.40 (6 H, m)	123.7 (d)	5.51 (1 H, dtt, 11, 8, 1)
19	22.6 (t)		20.8 (t)	1.97 (2 H, qdt, 8, 8, 1)
20	14.0 (q)	0.89 (3 H, t, 7)	14.2 (q)	0.89 (3 H, t, 8)

a) ^{13}C NMR spectra were taken in $CDCl_3$ at 125 MHz. ^{1}H NMR spectra were taken in C_6D_6 at 500 MHz. b), c), d), e) Assignments may be interchanged.

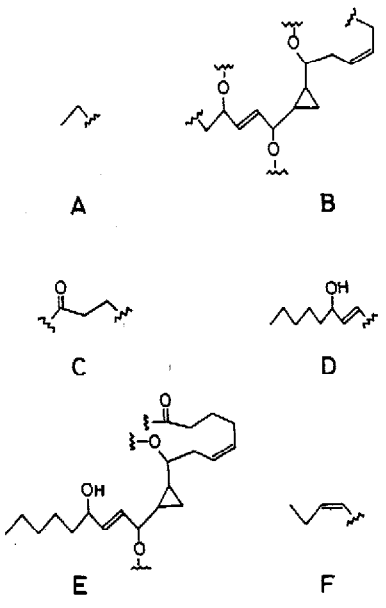
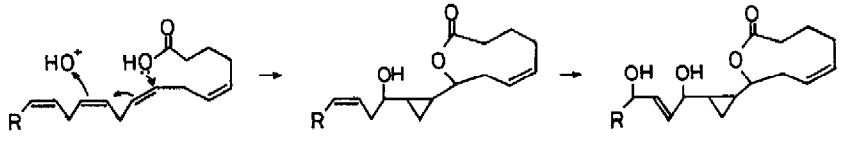


Table 2. ^{1}H NMR Spectral Data of the Derivatives 3 and 4[a]

No. of carbon	3 δ (m, J in Hz)	4 δ (m, J in Hz)
1	-	-
2	2.09 (2 H, m)	2.13 (2 H, t, 7)
3	1.60 (2 H, m)	1.61 (2 H, tt, 7, 7)
4	1.81 (1 H, m) 2.37 (1 H, m)	2.02 (2 H, dt, 7, 7)
5	5.30-5.40 (2 H, m)	5.41 (1 H, dt, 11, 7)
6		5.60 (1 H, dt, 11, 6)
7	1.77 (1 H, m) 2.33 (1 H, m)	2.35 (2 H, m)
8	4.31 (1 H, ddd, 8, 8, 1.5)	2.92 (1 H, ddd, 7, 7, 5)
9	0.77 (1 H, m)	0.93 (1 H, m)
10	0.35 (1 H, ddd, 8, 5, 5) 0.65 (1 H, ddd, 8, 5, 5)	0.37 (1 H, ddd, 8, 5, 5) 0.49 (1 H, ddd, 8, 5, 5)
11	0.92 (1 H, m)	1.10 (1 H, m)
12	4.94 (1 H, dd, 9, 4)	3.76 (1 H, dd 6, 5)
13	5.75-5.85 (2 H, m)	5.80 (1 H, dd, 16, 5)
14		5.76 (1 H, dd, 16, 5)
15	5.43 (1 H, dt, 7, 7)	4.09 (1 H, ddd, 7, 7, 5)
16	1.52 (2 H, m)	1.50 (2 H, m)
17		
18	1.10-1.40 (6 H, m)	1.20-1.42 (6 H, m)
19		
20	0.86 (3 H, t, 7)	0.91 (3 H, t, 7)
OAc	1.71 and 1.70	-
OMe	-	3.37

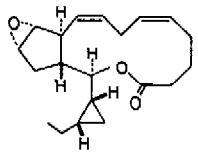
a) Spectra were taken in C_6D_6 at 500 MHz.

Scheme 1. Biogenesis of Halicholactone (1) and Neohalicholactone (2)



defined the assignments of all protons and the connectivity of all carbons, elucidating the structure of neohalicholactone to be depicted as the formula **2**.

A number of cyclopropane-containing fatty acid metabolites, the cyclopropane ring of which is formed by transfer of a methylene group from S-adenosyl methionine to a double bond have been isolated from various bacteria and plant species.[10] In contrast, the isolation of cyclopropane-containing fatty acid metabolites from marine sources is quite rare; hybridalactone (**6**)[7] and a compound **7**[11] have been isolated recently. Halicholactone (**1**) and neohalicholactone (**2**) may be biosynthesized from arachidonic acid and eicosapentaenoic acid, respectively via a cyclization mechanism as shown in Scheme 1. It is noteworthy that performic oxidation of linoleic acid provided a cyclopropane-containing acid **8**,[12] supporting the biogenesis of **1** and **2**. Halicholactone (**1**) exhibited weak inhibitory activity (IC_{50} = 630 μM) against 5-lipoxygenase of guinea pig polymorphonuclear leukocytes.



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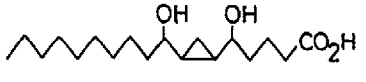

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References and Notes

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2. **1**: $[\alpha]_D^{23}$ -85.4° (c 1.16, $CHCl_3$); IR ($CHCl_3$) 3640, 3460 (broad), 1730 cm^{-1}; EIMS m/z 318 (M^+ - 18), 265, 247, 238, 209.
3. **2**: $[\alpha]_D^{16}$ -54.2° (c 0.73, $CHCl_3$); IR ($CHCl_3$) 3600, 3400 (broad), 1730 cm^{-1}; EIMS m/z 316 (M^+ - 18), 265, 247, 229.
4. **3**: colorless oil; $[\alpha]_D^{17}$ -64.4° (c 0.68, $CHCl_3$); IR ($CHCl_3$) 1735 cm^{-1}; EIMS m/z 420 (M^+), 360, 349, 318, 300, 251, 209, 191.
5. **4**: colorless oil; $[\alpha]_D^{17}$ +1.7° (c 0.23, $CHCl_3$); IR ($CHCl_3$) 3600, 3380 (broad), 1730 cm^{-1}; EIMS m/z 350 (M^+ - 18), 332, 319, 314, 301, 279, 261, 209.
6. Satisfactory high-resolution mass spectral data were obtained.
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8. **5**: colorless oil; $[\alpha]_D^{17}$ +5.2° (c 0.23, $CHCl_3$); ^1H NMR ($CDCl_3$, 90 MHz) δ 0.89 (3 H, br t, J = 7 Hz), 1.10-1.76 (8 H, m), 2.06 (6 H, s), 4.03 (1 H, dd, J = 12, 6 Hz), 4.24 (1 H, dd, J = 12, 4 Hz), 5.07 (1 H, m); IR ($CHCl_3$) 1730 cm^{-1}; EIMS m/z 216 (M^+), 173, 157, 143, 97.
9. The authentic **5** ($[\alpha]_D^{15}$ +4.1° (c 0.58, $CHCl_3$) was prepared by acetylation of (R)-1,2-heptanediol[13] ($[\alpha]_D^{14}$ +18.8° (c 0.47, EtOH); Lit.[13] $[\alpha]_D^{22}$ +16.8° (c 11.8, EtOH)].
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