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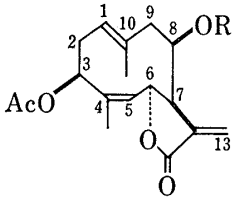
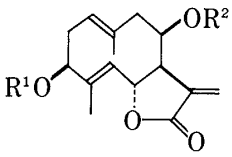
Hiyodorilactones D, E, and F, New Cytotoxic Sesquiterpene Lactones from *Eupatorium sachalinense* Makino

Three new cytotoxic germacranolides, hiyodorilactones D, E, and F were isolated from the leaves of *Eupatorium sachalinense* Makino, and their structures were determined to be **4**, **5**, and **6**, respectively, by spectral and chemical evidences.

Keywords——sesquiterpenes; hiyodorilactones D, E, and F; germacranolides; *Eupatorium sachalinense*; Compositae; HeLa test; Cope rearrangement; NMR at 270 MHz; nuclear Overhauser effect (NOE)

The isolation of two *in vivo* tumor inhibitory sesquiterpene lactones, hiyodorilactones A (**1**) and B (**2**), along with a cytotoxic sesquiterpene lactone, hiyodorilactone C (**3**), from





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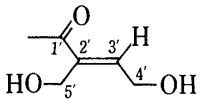
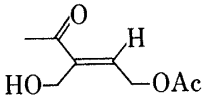
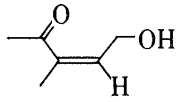
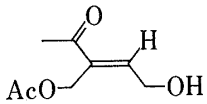
1 : 

4 : H 

2 : 

5 : H 

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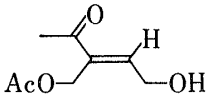
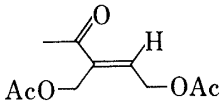
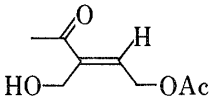
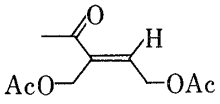
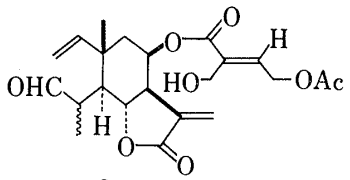
6 : 

7 : Ac 

11 : 

12 : 



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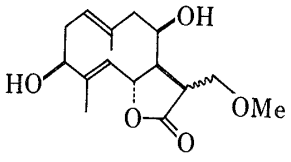

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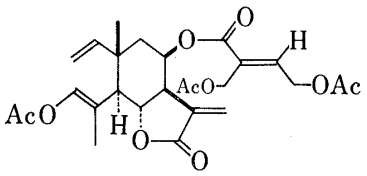

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Fig. 1

Eupatorium sachalinense Makino (Compositae) and the determination of their structures have been described.[1] Three additional new cytotoxic sesquiterpene lactones, hiyodorilactones D, E, and F, have now been isolated from the leaves of the same plant.[2] In this communication, we wish to report evidence leading to the structures **4**, **5**, and **6** for hiyodorilactones D, E, and F, respectively.

The molecular formula ($C_{22}H_{28}O_8$) of hiyodorilactone D (**4**), a pale yellow oil, $[\alpha]_D^{28}$ +81° (c=0.36, EtOH), was determined by the appearance of an $(M+1)^+$ peak at m/e 421 in the chemical ionization mass spectrum (CI-MS) using methane as reactant gas, and by the presence of a peak at m/e 360.1564 ($C_{20}H_{24}O_6$) due to $(M-AcOH)^+$ in the electron impact mass spectrum (EI-MS; MS); a molecular ion peak was absent. The infrared (IR) (ν_{max}^{neat} 3450, 1740, 1710, 1660, 1360, 890, and 870 cm^{-1}), the ultraviolet (UV) (end absorption at λ^{EtOH} 210 nm, ε 25700), and the proton magnetic resonance (NMR) spectra (Table I) suggested the presence of two olefinic methyls, an acetoxyl, hydroxyl(s), and an α-methylene-γ-lactone grouping.

Table I. NMR Spectral Data for **4**—**12** (δ values)

Compd	$C_{(3)}$–H	$C_{(6)}$–H	$C_{(8)}$–H	$C_{(4)}$–Me	$C_{(10)}$–Me	$C_{(13)}$–H		$C_{(3')}$–H	$C_{(4')}$–H	$C_{(5')}$–H	Ac	OMe
4[a]	4.35m	5.22dd (8;10)	5.80m	1.78 s	1.50 s	5.63 d (3.2)	6.28 d (3.7)	6.73 t (6)	4.85d[e] (6)	4.35 s	2.07 s	—
5[b]	*d*)	5.25dd (9;10)	5.85m ($W_{1/2}$=10)	1.82 s	1.52 s	5.63 d (3.1)	6.33 d (3)	7.09 t (6)	4.51 d (6)	4.90 s	2.07 s	—
6[a]	*d*)	5.94dd (3;10)	*d*)	1.80 s	1.80 s	5.78 d (3)	6.35 d (3)	7.02 t (6)	4.42 d (6)	4.82 s	2.10 s 2.04 s	—
7[a]	*d*)	*d*)	5.85m ($W_{1/2}$=8)	1.79 (brs)	1.54 (brs)	5.64 d (3)	6.35 d (3.3)	6.99 t (6)	4.92 d (6)	4.83 s	2.12 s 2.10 s 2.00 s	—
8[a]	*d*)	5.24 t (9)	*d*)	1.74 (brs)	1.66 (brs)	—	—	—	—	—	—	3.37 s
9[a]	9.64 s	*d*)	*d*)	1.55 d (2)	1.28 s	5.78 d (3)	6.20 d (3)	6.74 t (6)	4.86 d (6)	4.40 s	2.10 s	—
10[c]	6.98 q (1)	4.64 t (12)	5.80m ($W_{1/2}$=13)	1.80 (brs)	1.20 s	5.50 d (3)	6.17 d (3)	6.96 t (6)	4.90 d (6)	4.80 (brs)	2.13 s 2.10 s 2.00 s	—
11[a]	*d*)	5.98dd (10;2.5)	*d*)	1.81 d (1)	1.86 s	5.80 d (2)	6.40 d (2)	6.80 t (6)	4.85 d (6)	4.48 s	2.08 s 2.12 s	—
12[a]	*d*)	5.90dd (10.5;2)	*d*)	1.80 d (1)	1.84 s	5.78 d (2)	6.37 d (2)	6.94 t (6)	4.87 d (6)	4.80 s	2.06 s 2.06 s 2.10 s	—

Coupling constants and $W_{1/2}$ values in parentheses are expressed in Hz.
a) Determined in $CDCl_3$ at 60 MHz.
b) Determined in acetone-d_6 at 60 MHz.
c) Determined in $CDCl_3$ at 100MHz.
d) These signals could not be assigned because of overlapping with other signals.
e) On irradiation of $C_{(3')}$–H, this doublet signal changed into a singlet.
s: singlet, brs: broad singlet, d: doublet, t: triplet, dd: double doublets, m: multiplet, q: quartet

Acetylation of **4** with acetic anhydride and potassium carbonate gave its diacetate (**7**), a colorless oil, NMR (Table I), MS m/e 504 (M^+), showing no IR absorption due to hydroxyl group. Therefore, **4** possesses two hydroxyls.

The proton magnetic double resonance (NMDR) experiments operated at 270 MHz (Tables IIa and IIb) revealed the presence of partial structures **A** and **B** (Fig. 2) for hiyodorilactone D (**4**).

1) T. Takahashi, H. Eto, T. Ichimura, and T. Murae, *Chem. Lett.*, **1978**, 1345.
2) Inhibitory effect (ID_{50}) against growth of HeLa cells was 2.5 μg/ml, 1—10 μg/ml, and 1—10 μg/ml for hiyodorilactones D, E, and F, respectively.

Table IIa. NMDR Expermental Data for the Protons in the Partial Structure (**A**) of Hiyodorilactone D (**4**)[a]

Observed protons		Signal change of observed proton(s) on irradiation of			
		$C_{(1)}$–H	$C_{(2\alpha)}$–H	$C_{(2\beta)}$–H	$C_{(3)}$–H
$C_{(1)}$–H	4.94dd (11;4)	—	brs[b]	d (11)	
$C_{(2\alpha)}$–H	2.33ddd (12;11;10)	dd (12;10)	—	dd (11;10)	dd (12;11)
$C_{(2\beta)}$–H	2.50ddd (12;6;4)	dd (12;6)	m ($W_{1/2}$=11)	—	dd (12;4)
$C_{(3)}$–H	4.35dd (10;6)		d (6)	d (10)	—
$C_{(10)}$–Me	1.52 s	Sharpened			

a) Measured in $CDCl_3$ at 270 MHz. Coupling constants and $W_{1/2}$ values in parentheses are expressed in Hz. s: singlet, brs: broad singlet, d: doublet, t: triplet, dd: double doublets, ddd: doublet of double doublets, m: multiplet.
b) Due to further coupling with $C_{(10)}$–Me other than with $C_{(2\beta)}$–H.

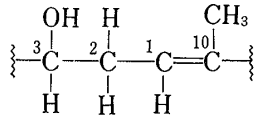
Table IIb. NMDR Experimental Data for the Protons in the Partial Structure (**B**) of Hiyodorilactone D (**4**)[a]

Observed protons		Signal change of observed proton(s) on irradiation of					
		$C_{(5)}$–H	$C_{(6)}$–H	$C_{(7)}$–H	$C_{(8)}$–H	$C_{(9\alpha)}$–H	$C_{(9\beta)}$–H
$C_{(5)}$–H	4.85 d (10)	—	brs[b]				
$C_{(6)}$–H	5.25dd (10;8)	d (8)	—	d (10)			
$C_{(7)}$–H	2.95m ($W_{1/2}$=17)		brs ($W_{1/2}$=8)	—	ddd (8;3.3;2.9)		
$C_{(8)}$–H	5.82m ($W_{1/2}$=12)			brs ($W_{1/2}$=9)	—	brs ($W_{1/2}$=6)	d (4.5)
$C_{(9\alpha)}$–H	2.84dd (15;4.5)				d (15)	—	d (4.5)
$C_{(9\beta)}$–H	2.34dd (15;3)				d (15)	d (3)	—
$C_{(13)}$–H	5.65 d (2.9)			s			
$C_{(13)}$–H	6.32 d (3.3)			s			
$C_{(4)}$–Me	1.80 s	Sharpened					

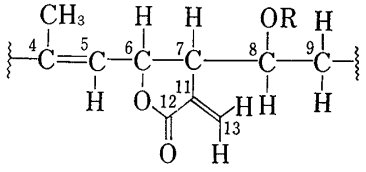
a) The same as the footnote a) in Table IIa.
b) The signal appears as a broadened singlet due to long-range coupling with $C_{(4)}$–Me.

The presence of an acyloxyl group in **B** was shown as follows. Treatment of **4** with potassium carbonate in aqueous methanol at room temperature gave an α-methoxymethyl-γ-lactone (**8**),[3] a colorless oil; IR ν_{max}^{neat} 3450, 1750, 1665, and 860 cm^{-1}; NMR (Table I): an appearance of a methoxyl signal and the absence of signals due to exo-methylene protons; MS *m/e* 296.1624 (M^+, $C_{16}H_{24}O_5$). This shows that **4** is an ester, $C_{15}H_{19}O_3$–O–CO–$C_6H_9O_3$ (**C**), and the acyloxyl group (–O–CO–$C_6H_9O_3$) was hydrolyzed with concomitant addition of methanol to the exo-methylene linkage[4] to yield **8**.

3) The whole structure (**8**) was shown for this lactone, after the determination of the structure of **4** as described below.
4) *E.g.*: W. Herz and I. Wahlberg, *J. Org. Chem.*, **28**, 2485 (1973).

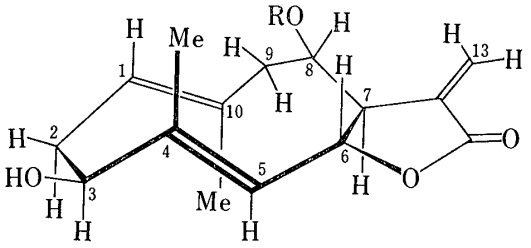


A



B

Fig. 2



D

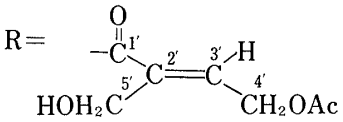


Fig. 3

Table III. NOE Data for Hiyodorilactone D (**4**)[a]

Observed protons	Saturated protons	NOE
$C_{(1)}$–H	$C_{(4)}$–Me	3
$C_{(2\alpha)}$–H	$C_{(3)}$–H	8
$C_{(3)}$–H	$C_{(10)}$–Me	4
$C_{(5)}$–H	$C_{(3)}$–H	4
$C_{(5)}$–H	$C_{(7)}$–H	3
$C_{(5)}$–H	$C_{(10)}$–Me	2
$C_{(6)}$–H	$C_{(4)}$–Me	9
$C_{(8)}$–H	$C_{(7)}$–H	5
$C_{(3')}$–H[b]	$C_{(4')}$–H	19
$C_{(3')}$–H	$C_{(5')}$–H	nil
$C_{(4')}$–H[b]	$C_{(5')}$–H	4
$C_{(5')}$–H[b]	$C_{(4')}$–H	9

a) The NOE experiments were carried out using a Brucker WH 270 spectrometer operating at 270 MHz in gated decoupling mode for *ca.* 4% degassed solution in $CDCl_3$. NOE values were estimated from increases in signal heights and expressed in %. Accuracies are about ±1% for NOE values.

b) The $C_{(3')}$–H, $C_{(4')}$–H, and $C_{(5')}$–H resonated at δ 6.75 (t, J=6.5 Hz), 4.88 (d, J=6.5 Hz), and 4.38 (s), respectively.

Because of an appearance of each $C_{(3)}$–H, $C_{(9\alpha)}$–H, and $C_{(9\beta)}$–H signal as double doublets (Tables IIa and IIb), the partial structures [**A**(C_5H_8O) and **B** ($C_{10}H_{11}O_2$–OR)] can be combined between $C_{(3)}$ and $C_{(4)}$ and between $C_{(9)}$ and $C_{(10)}$ to form a germacranolide ($C_{15}H_{19}O_3$–OR; R=–CO–$C_6H_9O_3$) in accordance with the observation (**C**) mentioned above.

Based on this germacranolide structure, the coupling constant values listed in Tables IIa and IIb and observed data of the nuclear Overhauser effect (NOE) (Table III) can be compatible with a unique stereostructure (**D**; Fig. 3) having a [1(10)*E*,4*E*]-6βH,7αH-germacra-1(10),4,11(13)-trien-12,6-olide skeleton for **4**. The structure of the acyloxyl group was determined to be (*E*)-4-acetoxy-2-hydroxymethyl-2-butenoyloxyl[5] by the NMR (Table I) and the NOE (Table III) experiments.

The [1(10)*E*,4*E*]-germacra-1(10),4-diene structure was confirmed for **4** by the following Cope rearrangement.[6] When **4** was heated at 190° for 7 min, an aldehyde (**9**), a pale yellow oil; IR ν_{max}^{neat} 3450, 2750, 1760, 1745, 1720, 1660, and 875 cm^{-1}; NMR (Table I); MS *m/e* 360 $(M-AcOH)^+$, was obtained. The same treatment of **7** yielded an enol acetate (**10**), a color-

5) Fragment ion peak at *m/e* 247 due to $[M-OCOC(CH_2OH)=CH(CH_2OAc)]^+$ was observed in the MS spectrum of **4**.

less oil, IR ν_{max}^{neat} 1780, 1750, 1725, 1680, 1665, 1640, and 865 cm^{-1}; NMR (Table I); MS *m/e* 504 (M^+).

Hiyodorilactone D is represented by [1(10)*E*,4*E*]-3β-hydroxy-8β-[(*E*)-4-acetoxy-2-hydroxymethyl-2-butenoyloxy]-6βH,7αH-germacra-1(10),4,11(13)-trien-12,6-olide (**4**; **D**).

Hiyodorilactone E (**5**), colorless needles, mp 149° (dec.), $[\alpha]_D^{18}$ +119° (*c*=0.22, EtOH); IR ν_{max}^{KBr} 3400, 1745, 1740, 1718, 1660 (sh), 1655, and 885 cm^{-1}; UV: end absorption at λ^{EtOH} 210 nm (ε 19000), has the same molecular formula, $C_{22}H_{28}O_8$,[7] as that of **4**. Treatment of **5** with acetic anhydride in the presence of potassium carbonate afforded a diacetylated product which was found to be identical with **7** derived from hiyodorilactone D (**4**). The MS spectrum of **5** showed a fragment ion peak at *m/e* 247 due to a loss of the acyloxyl group (–O–CO–$C_6H_9O_3$). These results together with the NMR spectral data (Table I) led to the presence of an (*E*)-2-acetoxymethyl-4-hydroxy-2-butenoyloxyl grouping in the side chain.

The structure **5** is given for hiyodorilactone E.

Hiyodorilactone F (**6**), a pale yellow oil, $[\alpha]_D^{30}$ −141° (*c*=0.21, EtOH); IR ν_{max}^{neat} 3450, 1760, 1740, 1720, 1660, 1370, and 885 cm^{-1}; UV: end absorption at λ^{MeOH} 210 nm (ε 25000); NMR (Table I); MS *m/e* 462.1915 (M^+, $C_{24}H_{30}O_9$), showed spectral data closely related to those of a monoacetylated product (**11**)[1] derived from hiyodorilactone A (**1**).[1] Acetylation of **6** with acetic anhydride and potassium carbonate yielded a monoacetylated product (**12**), which proved to be identical with **12**[1] obtained from **11** by the same treatment. These two compounds (**6** and **11**) have the same molecular formula ($C_{24}H_{30}O_9$). The fragment ion peak due to an elimination of the acyloxyl group (–O–CO–$C_6H_9O_3$) in the side chain was observed at *m/e* 289 for both compounds (**6** and **11**).

Hiyodorilactone F should be represented by [1(10)*E*,4*Z*]-3β-acetoxy-8β-[(*E*)-2-acetoxymethyl-4-hydroxy-2-butenoyloxy]-6βH,7αH-germacra-1(10),4,11(13)-trien-12,6-olide (**6**).

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Department of Chemistry,
Faculty of Science,
The University of Tokyo,
Bunkyo-ku, Tokyo

Takeyoshi Takahashi
Tomoko Ichimura
Tatsushi Murae

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7) Determined by the elemental analysis.