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HIYODORILACTONES A AND B, NEW TUMOR INHIBITORY GERMACRADIENOLIDES FROM EUPATORIUM SACHALINENSE MAKINO

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Three new sesquiterpene lactones belonging to the class of [1(10)E, 4Z]-germacra-1(10),4-dienolide have been isolated from the leaves of Eupatorium sachalinense Makino, two of them (hiyodorilactones A and B) were found to show significant inhibitory activity in vivo against the Ehrlich ascites carcinoma. The structures of hiyodorilactones A, B, and C have been established to be 1, 2, and 3, respectively.

A number of cytotoxic germacranolides have been reported in these years,[1] however, few of them showed significant in vivo tumor inhibitory activity. We wish to report the isolation and the structure elucidation of hiyodorilactones A (1), B (2), and C (3), novel germacranolides, from the leaves of Eupatorium sachalinense Makino.[2] Hiyodorilactones A (1) and B (2) have significant tumor inhibitory properties[3] in vivo against the Ehrlich ascites carcinoma.

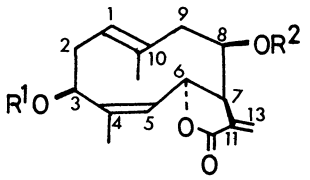
Fractionation of the methanolic extract was guided by the Hela assay. Successive solvent partitions and silica gel chromatography yielded three new cytotoxic lactones, hiyodorilactones A (1), B (2), and C (3).[4] Hiyodorilactone A (1) [pale yellow oil; $C_{22}H_{28}O_8$; $[\alpha]_D^{24}$ -121° (c 0.75, EtOH)] shows following spectral data. UV end absorption (EtOH) 210 nm (ε 14,800); IR (neat) 3400 (OH), 1760 (α,β-unsaturated γ-lactone), 1740 (ester), 1705 (α,β-unsaturated ester), and 1658 cm^{-1} (C=C); m/e 420.1793 (M^+), 289 $[M - CH(CH_2OH)=C(CH_2OH)COO]^+$ and 228 $[M - CH(CH_2OH)=C(CH_2OH)-COOH - AcOH]^+$; PMR: Table 1. These data are closely related to those of provincialin (4), a [1(10)E,4Z]-germacra-1(10),4-dienolide isolated from Liatris provincialis.[5] The remarkable difference between the PMR spectra of 1 and 4 was observed for the acyloxyl group at C-8 (cf. Table 1). Hiyodorilactone A (1) gave, on alkaline hydrolysis with K_2CO_3-aq. MeOH, two products 5 [$C_{18}H_{26}O_6$; colorless crystals; mp 141 - 142 °C; IR (KBr) 3510, 1765, 1720, and 1660 cm^{-1}; m/e 338 (M^+), 278 (M - AcOH)$^+$, 246 (M - AcOH - CH_3OH)$^+$; PMR: Table 1] and 6 [$C_{16}H_{24}O_5$; colorless oil; IR (neat) 3450, 1740, and 1663 cm^{-1}; m/e 296 (M^+), 278 (M - H_2O)$^+$, and 264 (M - CH_3OH)$^+$]. The compound (5) was shown to be identical with the product (5) obtained from 4 by the same treatment.[6] Therefore, hiyodorilactone A (1) should be [1(10)E,4Z]-3β-acetoxy-8β-acyloxy-6βH,7αH-germacra-1(10),4,11(13)-trien-12,6-olide.

On acetylation with acetic anhydride and K_2CO_3 at room temperature, 1 yielded two acetates 7, $C_{24}H_{30}O_9$; colorless oil; IR (neat) 3480, 1760, 1745, 1720, and 1660 cm^{-1}; m/e 462 (M^+), 289 $[M - CH(CH_2OAc)=C(CH_2OH)COO]^+$, and 228 [M - AcOH -

Table 1. PMR spectra (60 MHz) of 1 - 11 (solvent $CDCl_3$, δ values)

Compd	C_3-H	C_6-H	C_8-H	C_4-Me	C_{10}-Me	C_{13}-H	3'-H	4'-H	5'-H	Ac	OMe
1	a	5.96dd (11;2)	a	1.80d (1)	1.84s	5.79d (2) 6.35d (2)	6.90t (5.5)	4.37d (5.5)	4.31s	2.12s	-
2	a	5.98dd (11.5;2)	a	1.80s	1.80s	5.79d (2) 6.36d (2)	6.81t (6)	4.31d (6)	1.80s	2.10s	-
3	a	5.86dd (11;2)	4.20m ($W_{\frac{1}{2}}$ 9)	1.80d (1.5)	1.90d (1.5)	5.70d (2) 6.38d (2)	-	-	-	2.07s	-
4	a	5.93dd (11.1;1.2)	a	1.83d (1.2)	1.77s	5.78d (1.9) 6.35d (2.1)				2.11s	-
5	a	5.73dd (11;4)	4.07m ($W_{\frac{1}{2}}$ 8)	1.80d (1)	1.81s	- -	-	-	-	2.06s	3.39s
6	4.42t (3.5)	6.12dd (11;4)	4.06m ($W_{\frac{1}{2}}$ 10)	1.72d (1)	1.82s	- -	-	-	-	-	3.41s
7	a	5.98dd (10;2.5)	a	1.81d (1)	1.86s	5.80d (2) 6.40d (2)	6.80t (6)	4.85d (6)	4.48s	2.08s 2.12s	-
8	a	5.90dd (10.5;2)	a	1.80d (1)	1.84s	5.78d (2) 6.37d (2)	6.94t (6)	4.87d (6)	4.80s	2.06s 2.06s 2.10s	-
9	a	5.82dd (10;3)	a	1.80brs	1.80brs	5.75d (2) 6.37d (2)	-	-	-	2.09s 2.00s	-
10	4.44t (3.5)	6.26dd (11;2.5)	4.14m ($W_{\frac{1}{2}}$ 8)	1.72d (1)	1.91s	5.68d (2) 6.40d (2)	-	-	-	-	-
12	a	5.96dd (2.5;10.5)	a	1.82d (1.3)	1.79brs	5.78d (2.1) 6.36d (2.1)	6.92t (6)	4.00d (6)	4.34s	2.12s	-

Coupling constants in parentheses are expressed in Hz. a) These signals could not be assigned because of overlapping with other signals. s; singlet, brs; broad singlet, d; doublet, t; triplet, dd; doublet of doublets



1 R^1 = Ac, R^2 = -CO-CH(3'-H)(HOH$_2$C-5')(CH$_2$OH-4')

2 R^1 = Ac, R^2 = -CO-CH(H$_3$C)(CH$_2$OH)

3 R^1 = Ac, R^2 = H

4 R^1 = Ac, R^2 = -CO-C(=CHCH$_2$OH)(CH$_2$OH)(CH$_2$O-CO-C=CHCH$_3$)

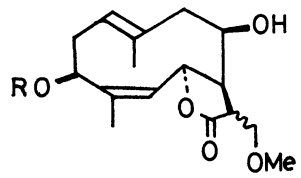
7 R^1 = Ac, R^2 = -CO-CH(HOH$_2$C)(CH$_2$OAc)

8 R^1 = Ac, R^2 = -CO-CH(AcOH$_2$C)(CH$_2$OAc)

9 R^1 = R^2 = Ac

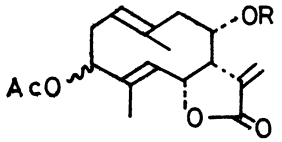
10 R^1 = R^2 = H

12 R^1 = Ac, R^2 = -COC(CH$_2$OH)=CHCH$_2$OH

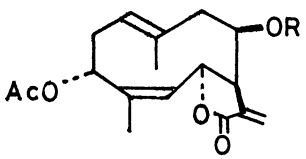


5 R = Ac

6 R = H



11 R = -CO-CH(HOH$_2$C)(CH$_2$OH)



13 R = -CO-CH(HOH$_2$C)(CH$_2$OH)

$CH(CH_2OAc)=C(CH_2OH)COOH]^+$; PMR: Table 1, and 8, $C_{26}H_{32}O_{10}$; colorless oil; IR (neat) 1760, 1740, 1720, and 1660 cm^{-1}; m/e 504 (M^+), 289 $[M - CH(CH_2OAc)=C(CH_2OAc)COO]^+$, and 228 $[M - CH(CH_2OAc)=C(CH_2OAc)COOH - AcOH]^+$; PMR: Table 1. These facts along with the results of the PMR decoupling and NOE experiments (Table 2)[7] on 7 suggest the presence of (2'E)-4'-hydroxy-2'-hydroxymethyl-2'-butenoyloxyl group at C-8 for 1. Thus the structure of hiyodorilactone A was established to be 1.

Hiyodorilactone B (2), $C_{22}H_{28}O_7$; pale yellow oil; $[\alpha]_D^{24}$ -140° (c 0.67, EtOH); UV end absorption (EtOH) 210 nm (ε 30,400); IR (neat) 3460, 1760, 1750, 1720, and 1660 cm^{-1}; m/e 404.1851 (M^+), 289 $[M - CH(CH_3)=C(CH_2OH)COO]^+$, and 228 $[M - AcOH - CH(CH_3)=C(CH_2OH)COOH]^+$; PMR: Table 1, shows the PMR spectrum closely related to that of hiyodorilactone A (1). In the PMR spectrum of hiyodorilactone B (2), the two proton singlet at δ 4.31 (CH_2OH at C-5') observed for 1 disappeared and an additional new olefinic methyl signal appeared at δ 1.80 as a slightly broadened singlet (due to a coupling with the proton at C-3'). The difference between the two spectra was considered to be due to a difference on the ester side chain at C-8. This was confirmed by the formation of 5 and 6 on alkaline hydrolysis of 2 under the same conditions as in the case of hiyodorilactone A (1).[6] The structure of the α,β-unsaturated ester grouping at C-8 was determined to be (2Z)-4-hydroxy-2-methyl-2-butenoyloxyl by the PMR decoupling and NOE experiments for 2 (cf. Table 3). Therefore, the structure of hiyodorilactone B should be 2.

Hiyodorilactone C (3) [$C_{17}H_{22}O_5$; colorless oil; $[\alpha]_D^{24}$ -109° (c 0.91, EtOH); UV end absorption (EtOH) 210 nm (ε 14,600); IR (neat) 3460, 1740, and 1660 cm^{-1}; m/e 306.1424 (M^+), 246 $(M - AcOH)^+$, and 228 $(M - AcOH - H_2O)^+$; PMR: Table 1] yielded, on acetylation with acetic anhydride and pyridine, an acetate (9) [$C_{19}H_{24}O_6$; colorless oil; m/e 348 (M^+) and 228 $(M - 2 \times AcOH)^+$; IR (neat) 1760, 1740, and 1655 cm^{-1}, no absorption due to hydroxyl group; PMR: Table 1]. On alkaline hydrolysis with 2 % aqueous KOH in dioxane, 3 gave a diol (10) [colorless crystals; $C_{15}H_{20}O_4$; mp 138 - 140.5 °C; m/e 264 (M^+), and 228 $(M - 2 \times H_2O)^+$; IR (Nujol) 3520, 3480, 1730, and 1660 cm^{-1}].[6] This diol (10) was shown to be identical with the diol obtained on alkaline hydrolysis of hiyodorilactone B (2) with 2 % aqueous KOH in dioxane. These results indicate that the structure of the diol is as shown in 10 and one of the hydroxyl group of 10 is acetylated in hiyodorilactone C (3). In the PMR spectrum of 3 the signal due to the proton at C-8 was observed at δ 4.20 (1H, m, $W_{\frac{1}{2}}$ = 9 Hz). The C-3 proton signal could not be assigned because of overlapping with the signals due to the protons at C-1 and C-5. In the spectrum of 10 the signals due to the protons at C-8 and C-3 were observed at δ 4.14 (1H, m, $W_{\frac{1}{2}}$ = 8 Hz) and δ 4.44 (1H, t, J=3.5 Hz), respectively. This observation is similar to that for 5 and 6 (cf. Table 1) and indicative of the presence of the acetoxyl group at C-3 in hiyodorilactone C (3). These results led to the structure (3) for hiyodorilactone C.

The structure of eucannabinolide isolated[8a] from *Eupatorium cannabium* has recently been revised from 11[8a] to 12,[8b] the latter of which is the same as 1 excepting the stereochemistry of the α,β-unsaturated acyloxyl group at C-8. In the first report on eucannabinolide[8a] the acyloxyl group was characterized as α,β-*cis*-bis-(hydroxymethyl)acryloyloxyl group on the basis of the PMR signals. However the chemical shift value (δ 4.00) for $C_{4'}$-H of eucannabinolide is different from that (δ 4.37) of hiyodorilactone A (1) (cf. Table 1). In the subsequent paper[8b] the stereochem-

istry of the acyloxyl group at C-8 remained undiscussed.[9]

Table 2. NOE of 7 in $CDCl_3$[a] (increases in signal heights, %)

Observed proton	Saturated proton	NOE
3'-H	4'-H	22
3'-H	5'-H	2
4'-H	5'-H	7
5'-H	4'-H	5

Table 3. NOE of 2 in $CDCl_3$[b] (increases in integrated signal intensities, %)

Observed proton	Saturated proton	NOE
3'-H	4'-H	28
3'-H	5'-H	18
4'-H	5'-H	nil

a) The NOE experiments were carried out using Brucker WH 270 spectrometer operating at 270 MHz in gated decoupling mode (PW 10.000 μsec, AQ 5.439 sec, ET=1 20.000 sec) for ca. 4% (w/v) degassed solution in $CDCl_3$. Accuracies are about ± 2% for NOE values. b) The NOE experiments were performed with JEOL PS-100 spectrometer operating at 100 MHz in the frequency-swept and internal TMS-locked mode, for ca. 8% (w/v) degassed solution in $CDCl_3$. Accuracies are about ± 5% for NOE values.

REFERENCES AND NOTES

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2) Collected at Nagano prefecture, Japan, August 1974 and 1976.

3) Life-prolonging effect of 1 and 2 for the mice suffering from Ehrlich ascites cancer was determined and expressed as the "life prolongation rate" (control being 100). 1: 253 (dose: 7.5 mg/kg/day) and 2: 175 (dose: 15 mg/kg/day). The authors wish to thank Dr. W. Tanaka, Dr. A. Matsuda, and Dr. Y. Nakayama, Nippon Kayaku Co., for the growth inhibition test against Ehrlich ascites carcinoma as well as the Hela cells, and for their valuable discussions.

4) Inhibitory effect (ID_{50}) against growth of Hela cells was 1.4 μg/ml, 0.6 μg/ml, and 2.0 μg/ml for 1, 2, and 3, respectively.

5) W. Herz and I. Wahlberg, J. Org. Chem., 38, 2485 (1973).

6) Since the PMR spectra of 1, 2, and 5 are similar to one another, it was suggested that the hydrolysis proceeded without skeketal or stereochemical transformations. The authors are thankful to Dr. I. Wahlberg, Swedish Tobacco Co., for a generous gift of an authentic sample of 5, together with its spectral data.

7) The authors wish to thank Prof. T. Miyazawa and Dr. Yokoyama, the University of Tokyo, for the measurements of NOE's at 270 MHz.

8) a) B. Drozdz, H. Grabarczyk, Z. Samek, M. Holub, V. Herout, and F. Šorm, Collect. Czech. Chem. Commun., 37, 1546 (1972). b) M. Holub and Z. Samek, ibid., 42, 1053 (1977).

9) No description on the anti-tumor activity of eucannabinolide was recorded in references 8a and 8b.

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