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FOUR NEW GERMACRANOLIDES FROM EUPATORIUM LINDLEYANUM DC.

Kazuo ITO, Yoshihisa SAKAKIBARA, Mitsumasa HARUNA*, and Kuo-Hsiung LEE¶

Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya 468
¶Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, N.C. 27514, U.S.A.

Four new sesquiterpene lactones, eupalinin-A, -B, -C, and -D, the first of which showed significant inhibitory activity against KB cell culture, were isolated from Eupatorium lindleyanum DC. and their structures were determined by a combination of chemical and physical techniques.

Chemical examination of several Eupatorium species has produced a number of cytotoxic and antitumor germacranolides and guaianolides.[1] In the present communication, we wish to report the structure elucidation of four new germacranolides, eupalinin-A, -B, -C, and -D isolated from Eupatorium lindleyanum DC.,[2] the first showed significant inhibitory activity against KB cell culture.[3] The physical data of these four new germacranolides are shown below.

Eupalinin-A (1a), $C_{22}H_{28}O_8$; mp. 175-177°C; $[\alpha]_D^{20}$ -90° (c=0.2, MeOH); UV (MeOH) 220 nm (ε=14,628); CD curve $[\theta]_{209}$ -58536, $[\theta]_{223}$ 0, $[\theta]_{233}$ +12704; IR ($CHCl_3$) 3570 (OH), 1760 (α,β-unsat. γ-lactone), 1750 (OAc), 1720, 1705 (α,β-unsat. ester) and 1645 (C=C) cm^{-1}; m/e 420 (M^+), 321, 304 $[M-CH(CH_3)=C(CH_2OH)COOH]^+$, 279, 261, and 244 $[M-CH(CH_3)=C(CH_2OH)COOH-CH_3COOH]^+$; ^{1}H-NMR (cf. Table 1); ^{13}C-NMR (cf. Table 2).

Eupalinin-B (2), $C_{22}H_{28}O_8$; oil; $[\alpha]_D^{20}$ -68.6° (c=0.29, MeOH); UV (MeOH) 220 nm (ε=12,465); CD curve $[\theta]_{210}$ -68005, $[\theta]_{227}$ 0, $[\theta]_{233}$ +9506; IR ($CHCl_3$) 3470, 1760, 1750, 1720, and 1650 cm^{-1}; m/e 420 (M^+), 321, 304, 279, and 261.

Eupalinin-C (3a), $C_{22}H_{28}O_8$; oil; $[\alpha]_D^{20}$ -69.96° (c=0.23, MeOH); UV (MeOH) 215 nm (ε=12,446); CD curve $[\theta]_{209}$ -39689, $[\theta]_{223}$ 0, $[\theta]_{231}$ +9622; IR ($CHCl_3$) 3570, 1765, 1735, 1720, 1705, and 1650 cm^{-1}; m/e 420 (M^+), 321, 304, 279, and 261.

Eupalinin-D (4a), $C_{22}H_{28}O_8$; oil; $[\alpha]_D^{20}$ -38.8° (c=0.19, MeOH); UV (MeOH) 217 nm (ε=14,845); CD curve $[\theta]_{208}$ -53852, $[\theta]_{225}$ 0, $[\theta]_{232}$ +8803; IR ($CHCl_3$) 3470, 1760, 1735, 1720, and 1650 cm^{-1}; m/e 420 (M^+), 321, 304, 279, and 261.

Above physical data of eupalinin-A (1a) and -B (2) are closely related to those of acetylheliangine (1f), prepared by acetylation from heliangine (1e) which was isolated from Helianthus tuberosus L..[4] The remarkable difference of ^{1}H-NMR spectra between (1a), (2) and (1f) was observed for the ester groups. (cf. Table 1)

Partial hydrolysis of (1a) with 10% Na_2CO_3 in aqueous dioxane at room temperature afforded (1b): [$C_{17}H_{22}O_6$; oil; IR ($CHCl_3$) 3600, 3470, 1750, and 1670 cm^{-1}; m/e 322 (M^+), 279 (M-Ac)$^+$, 261 (M-Ac-H_2O)$^+$] and (1c): [$C_{20}H_{26}O_7$; oil; IR ($CHCl_3$) 3600, 1765, 1705, and 1650 cm^{-1}; m/e 378 (M^+), 360 (M-H_2O)$^+$, 261 (M-$CH(CH_3)=C(CH_2OH)CO-H_2O)^+$]. (1b) was also obtained by hydrolysis of (2) under the same condition with (1a). These results indicated that eupalinin-A (1a) and -B (2) have the same stereostructure except for the ester side chains.

Table 1. ^{1}H-NMR spectra (99.6 MHz) of (1a)-(4b)[a]

Compd.	H-3	H-6	H-8	C_{14}-Me	C_{15}-Me	H-13	H-3'	H-4'	H-5'	OAc
(1a)	5.23m	6.18dd (11;2)	5.20m	1.49s	1.92d (1.5)	5.77;6.36d (1.9;2)	6.96q (7.5)	1.90d (7.5)	4.32s	2.15s
(1b)	5.23m	6.08dd (11;2)	4.13m	1.60s	1.88d (1.5)	5.70;6.36d (1.9;2)	-	-	-	2.14s
(1c)	4.52m	6.68dd (11;2)	5.19m	1.48s	1.92d (1.5)	5.77;6.36d (1.9;2)	6.96q (7.5)	1.88d (7.5)	4.32s	-
(1d)[b]	4.45m	6.65dd (11;2)	4.06m	1.62s	1.83d (1.5)	5.74;6.30d (1.9;2)	-	-	-	-
(1e)	4.53m	6.72dd (11;2)	5.28m	1.48s	1.82d (1.5)	5.80;6.36d (1.9;2)	6.94m	1.80[c]	1.82[c]	-
(1f)	5.25m	6.19dd (11;2)	5.20m	1.48s	1.92d (1.5)	5.77;6.36d (1.9;2)	6.88m	1.80[c]	1.82[c]	2.14s
(2)	5.25m	6.18dd (11;2)	5.18m	1.48s	1.92d (1.5)	5.77;6.33d (1.9;2)	6.81m	4.34dbr (6.1)	1.82sbr	2.13s
(3a)	5.90dd (5.5;11.5)	5.68dd (11;2)	5.20m	1.58s	1.88d (1.5)	5.74;6.38d (1.9;2)	6.96q (7.5)	1.95d (7.5)	4.38s	2.10s
(3b)	5.82dd (5.5;11.5)	5.60dd (11;2)	4.13m	1.69s	1.84d (1.5)	5.69;6.38d (1.9;2)	-	-	-	2.06s
(3c)	4.96dd (5.5;11.5)	5.62dd (11;2)	5.20m	1.52s	1.88d (1.5)	5.79;6.36d (1.9;2)	6.96q (7.5)	1.93d (7.5)	4.32s	-
(3d)[b]	4.83dd (5.5;11.5)	5.60dd (11;2)	4.04m	1.62s	1.84d (1.5)	5.71;6.32d (1.9;2)	-	-	-	-
(4a)	5.84dd (5.5;11.5)	5.62dd (11;2)	5.18m	1.56s	1.86d (1.5)	5.77;6.32d (1.9;2)	6.79m	4.32dbr (6.1)	1.82sbr	2.05s
(4b)	4.97dd (5.5;11.5)	5.62dd (11;2)	5.20m	1.50s	1.88d (1.5)	5.78;6.36d (1.9;2)	6.78m	4.33dbr (6.1)	1.81sbr	-

a: Run in $CDCl_3$ on a JEOL FX-100 spectrometer with Me_4Si as internal standard. Values are parts per million; s, singlet; d, doublet; m, multiplet; dd, doublet of doublets; sbr, broad singlet; dbr, broad doublet; q, quartet. Figures in parentheses are coupling constants in Hertz.
b: Run in $CDCl_3/CD_3OD$. c: Signal partially obscured.

Table 2. ^{13}C-NMR spectra (25.05 MHz) of lactones[a]

Carbon atom	(1a)	(2)	(3a)	(4a)	(1e)	(5b)
1	60.0 d	60.0 d	58.9 d	59.0 d	60.5 d	59.6 d
2	30.5 t	30.4 t	31.6 t	31.7 t	32.5 t	31.7 t
3	72.8 d	72.7 d	68.6 d	68.6 d	72.0 d	68.7 d
4	138.4 s	138.3 s	137.6 s	137.6 s	141.4 s	137.8 s
5	125.7 d	125.4 d	125.3 d	125.3 d	126.0 d	125.2 d
6	74.5 d	74.5 d	72.9 d	72.9 d	74.1 d	73.1 d
7	48.3 d	48.3 d	48.5 d	48.6 d	48.4 d	48.5 d
8	76.7 d	76.3 d	76.4 d	76.3 d	76.1 d	76.7 d
9	43.3 t	43.3 t	43.3 t	43.3 t	43.5 t	43.3 t
10	58.1 s	58.1 s	57.0 s	56.9 s	58.6 s	58.6 s
11	136.7 s	136.5 s	136.6 s	136.4 s	137.1 s	136.5 s
12	169.0 s[b]	168.9 s[b]	168.7 s[b]	168.4 s[b]	169.1 s	169.1 s[b]
13	125.1 t	124.9 t	125.1 t	124.9 t	124.4 t	125.5 t
14	19.4 q	19.4 q	18.3 q	18.4 q	19.7 q	18.4 q
15	22.9 q	22.9 q	17.5 q	17.6 q	22.9 q	17.6 q
1'	165.8 s	165.4 s	165.9 s	165.8 s	166.3 s	165.6 s
2'	131.7 s	126.8 s	131.3 s	127.1 s	127.5 s	131.2 s
3'	142.1 d	142.4 d	142.5 d	142.1 d	138.6 d	145.3 d
4'	14.4 q	59.5 t	14.5 q	59.5 t	14.5 q	58.9 t
5'	56.3 t	12.5 q	56.5 t	12.7 q	11.9 q	57.1 t
$OCOCH_3$	169.2 s[b]	169.0 s[b]	169.4 s[b]	169.4 s[b]	-	169.9 s[b]

a: Run in $CDCl_3$ on a JEOL FX-100 spectrometer with Me_4Si as internal standard. s, singlet; d, doublet; t, triplet; q, quartet. Assignment established by single frequency off-resonance decoupling. b: Assignments may be interchanged.

The chemical shift (δ4.52) of the proton on the carbon bearing the newly generated hydroxyl group in (1c) is very similar to that (δ4.53) of H-3 in heliangine (1e). Furthermore, irradiation at the frequency of H-7 (δ2.92) in (1b) collapsed doublets of 13-Ha and -Hb into two singlets and also converted a doublet of doublets at δ6.08 (H-6) to doublet (J=11.0Hz), while a multiplet at δ4.13 (H-8) was simplified. Above results indicated that the ester side chain was attached to C-8 in (1b). Hence, the acetoxyl and ester side chain in eupalinin-A (1a) and -B (2) are also located at C-3 and C-8, respectively.

Hydrolysis of (1a) with 10% KOH in aqueous dioxane gave the corresponding diol (1d), mp. 232-235°C[5]; m/e 280 (M^+), which was identical in all respects with helianginol, prepared by hydrolysis from heliangine (1e).

Therefore, eupalinin-A (1a) and -B (2) have the trans-fused lactone, β-oriented acetoxyl and ester side chain attached at C-3 and C-8, respectively. And the 4,5-double bond is cis, whereas the 1,10-epoxy group is trans.

The structures of an α,β-unsaturated ester group at C-8 in (1a) and (2) were determined by the ^{1}H-NMR decoupling and NOE experiments.[6] Irradiation at the frequency of methyl protons (H-4') in (1a) enhanced 19.2 and 6.4% in the area intensity of H-3' and H-5' signals, respectively, but NOE between H-5' and H-3' was not observed. Hence, the structure of the ester group in (1a) was determined to be (2'E)-2'-hydroxymethyl-2'-butenoyloxyl.

On the other hand, the ^{1}H-NMR spectrum of the ester portion in (2) shows a broad doublet at δ4.34 instead of a singlet due to methylene protons (δ4.32) of hydroxymethyl group in (1a). Irradiation at the frequency of the vinyl methyl protons at C-5' produced a 15.1% increase in the area intensity of H-3' signal, but no observation of NOE between H-5' and H-4', whereas irradiation at H-4' produced a 15% increase in the area intensity of H-3' signal. These results indicated the structure of the ester group in (2) should be (2'Z)-4'-hydroxyl-2'-methyl-2'-butenoyloxyl.

From above experiments, the structures of eupalinin-A and -B were established to be (1a) and (2), respectively.

Eupalinin-C (3a) and -D (4a) are superimposable on the all spectral data of (1a) and (2), except for the ^{1}H-NMR signal due to C-3 proton of (1a) and (2), respectively.

On alkaline hydrolysis of (3a) and (4a) with 10% Na_2CO_3 in aqueous dioxane, (3a) yielded three compounds (3b):[mp. 248-251°C; IR ($CHCl_3$) 3400-3550, 1760, 1740, and 1658 cm^{-1}; m/e 322 (M^+), 279, 261], (3c):[mp. 185-188°C; IR ($CHCl_3$) 3600, 3480, 1765, 1710, and 1650 cm^{-1}; m/e 378 (M^+), 278, and 261], (3d):[mp. 259-261°C; m/e 280 (M^+)], whereas (4a) gave two compounds (3d) and (4b): [oil; IR ($CHCl_3$) 3400-3570, 1760, 1720, 1715, and 1655 cm^{-1}; m/e 378 (M^+)].

Irradiation at the frequency of H-7 (δ2.95) in (3c) collapsed the signals of 13-Ha and -Hb into two singlets and converted a doublet of doublets (H-6, δ5.62) into a clear doublet (J=11.0Hz) and a multiplet at δ5.20 (H-8, unchanged peak by hydrolysis) was also simplified. On the other hand, irradiation at H-7 (δ2.89) in (4b) produced the same result as (3c).

These experiments showed that the acetoxyl and the ester group in eupalinin-C (3a) and -D (4a) are also located at C-3 and C-8, respectively.

The diol (3d) was prepared by hydrolysis from epoxyeupaformosanin (5b) which was obtained from eupaformosanin (5a)[7] on epoxidation with m-chloroperbenzoic acid.

The structures of the ester group at C-8 in (3a) and (4a) were determined to be represented by formula (A) and (B) (Fig. 1) by the ^{1}H- and ^{13}C-NMR spectra. (cf. Table 1 and 2)

From the above data, eupalinin-C (3a) and -D (4a) are the α-epimers of the acetoxyl group at C-3 of eupalinin-A (1a) and -B (2), respectively.

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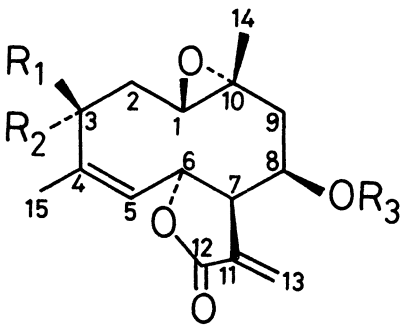
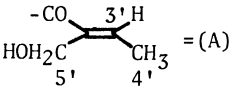
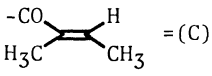


(1a): R_1=OAc, R_2=H, R_3=  =(A)

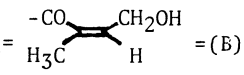
(1b): R_1=OAc, R_2=R_3=H

(1c): R_1=OH, R_2=H, R_3= (A)

(1d): R_1=OH, R_2=R_3=H

(1e): R_1=OH, R_2=H, R_3=  =(C)

(1f): R_1=OAc, R_2=H, R_3= (C)

(2) : R_1=OAc, R_2=H, R_3=  =(B)

(3a): R_1=H, R_2=OAc, R_3= (A)

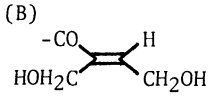
(3b): R_1=R_3=H, R_2=OAc

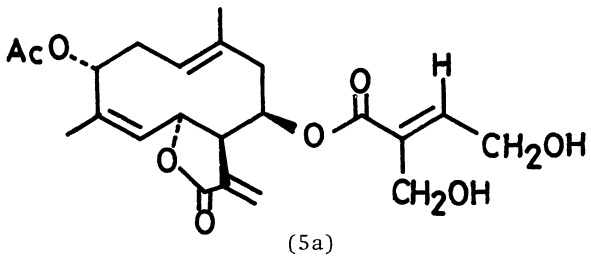
(3c): R_1=H, R_2=OH, R_3= (A)

(3d): R_1=R_3=H, R_2=OH

(4a): R_1=H, R_2=OAc, R_3= (B)

(4b): R_1=H, R_2=OH, R_3= (B)

(5b): R_1=H, R_2=OAc, R_3= 



(5a)

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1) e.g.: W. Herz and I. Wahlberg, J. Org. Chem., 38, 2485 (1973); T. Takahashi, E. Eto, T. Ichimura, and T. Murae, Chem. Lett., 1345 (1978), and the references cited therein.

2) Collected at Gifu prefecture, Japan, August 1977.

3) Eupalinin-A (1a) showed cytotoxity (ED_{50}) against KB (human epidermoid carcinoma of mouth) cell culture at 4-10 μg/ml. This activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, by a literature method.[8]

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5) The specimen of helianginol (1d) had a reported melting point of 191-192°C[4] and 214-218°C[10], while melting point of our material (1d) from heliangine (1e) as well as from eupalinin-A (1a) was 232-235°C.

6) The NOE experiments were carried out using a JEOL FX-100 spectrometer operating at 99.6 MHz in gated decoupling mode (PW 12.000μsec, AQ 4.096 sec, PI 30.000sec) for ca. 8% (w/v) degassed solution in $CDCl_3$.

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8) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schmacher, and B. J. Abbott, Cancer Chemother. Reports (Part 3), 3, 17 (1972).

9) The geometry of the 4,5-double bond in heliangine (1e) was determined to be cis by observation of 14.5% NOE between C_{15} methyl protons and H-5.

10) S. J. Torrance, T. A. Geissman, and M. R. Chedekel, Phytochemistry, 8, 2381 (1969)

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