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NEW SESQUITERPENE LACTONES FROM EUPATORIUM CHINENSE VAR. SIMPLICIFOLIUM (MAKINO) KITAM.

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Five new sesquiterpene lactones, eupachifolin-A, -B, -C, -D, and -E, were isolated from Eupatorium chinense var. simplicifolium (Makino) Kitam. and their structures were described. Eupachifolin-A belongs to the class of cis-$\Delta^{4,5}$,cis-$\Delta^{1,10}$-germacranolides, whereas eupachifolin-B, -C, -D, and -E are mutually related guaianolides. Structures and stereochemistries of the new sesquiterpene lactones were determined by chemical transformations and extensive use of ^{1}H and ^{13}C NMR spectrometry.

In connection with our search for sesquiterpene lactones with potential biological activity in the genus Eupatorium (Compositae)[1] we have examined the whole plants of Eupatorium chinense var. simplicifolium (Makino) Kitam..[2,3] In this communication we describe the isolation and structure determination of the new germacranolide sesquiterpene lactone, eupachifolin-A (1) and four mutually related guaianolide sesquiterpene lactones, eupachifolin-B (2), -C (3), -D (4), and -E (5). Structures and stereochemistries were established by chemical transformations and extensive application of ^{1}H and ^{13}C NMR spectrometry. The physical data of these five new sesquiterpene lactones are shown below.

Eupachifolin-A (1), $C_{20}H_{26}O_6$; colorless oil; $[\alpha]_D^{20}$ -66.2° (c=0.21, $CHCl_3$); UV (MeOH) 212 (ε=10,302), 312 nm (ε=33.7); CD curve $[\theta]_{211}$ -45513, $[\theta]_{225}$ 0, $[\theta]_{235}$ +17278, $[\theta]_{272}$ 0, $[\theta]_{320}$ +1685; IR ($CHCl_3$) 3400-3600 (OH), 1765 (γ-lactone), 1740 (ester), 1690 (α,β-unsaturated aldehyde), and 1640 cm^{-1} (C=C); m/e 362 (M^+), 344 ($M-H_2O$)$^+$, 333 (M-CHO)$^+$, and 260 ($M-C_2H_5CH(CH_3)COOH$)$^+$.

Eupachifolin-B (2), $C_{22}H_{26}O_8$; colorless oil; $[\alpha]_D^{18}$ -111.6° (c=0.19, MeOH); UV (MeOH) 213 (ε=15,608) nm; CD curve $[\theta]_{223}$ 0, $[\theta]_{230}$ +3451, $[\theta]_{252}$ 0, $[\theta]_{264}$ -992.2; IR ($CHCl_3$) 3450 (OH), 1770 (γ-lactone), 1740 (OAc), 1720 (α,β-unsaturated ester), and 1660 cm^{-1} (C=C); m/e 418 (M^+), 359 (M-OAc)$^+$, 302 ($M-CH(CH_2OH)=C(CH_3)COOH$)$^+$, 242 ($M-CH(CH_2OH)=C(CH_3)COOH-AcOH$)$^+$.

Eupachifolin-C (3), $C_{22}H_{26}O_7$; colorless oil; $[\alpha]_D^{20}$ -37.9° (c=0.21, $CHCl_3$); UV (MeOH) 217 (ε=11390)nm; CD curve $[\theta]_{240}$ 0, $[\theta]_{258}$ -1242, $[\theta]_{291}$ 0; IR ($CHCl_3$) 3500, 1765, 1730, 1715, and 1650 cm^{-1}; m/e 402 (M^+), 342, 303, 286, 261, 244, and 226.

Eupachifolin-D (4), $C_{22}H_{27}ClO_8$; mp. 247-250°C colorless needles; $[\alpha]_D^{20}$ -92.6° (c=0.22, $CHCl_3$); UV (MeOH) 213.5 nm (ε=17,627); CD curve $[\theta]_{219}$ 0, $[\theta]_{226}$ +8831, $[\theta]_{241}$ 0, $[\theta]_{258}$ -2310; IR ($CHCl_3$) 3600, 1770, 1740, 1715, and 1650 cm^{-1}; m/e 454 and 456 (3:1, M^+), 418 ($M-HCl$)$^+$, 395 and 397 (3:1, M-OAc)$^+$, 278 and 280 (3:1, $M-C_5H_8O_2-AcOH$)$^+$, 242 ($M-C_5H_8O_2-AcOH-HCl$)$^+$.

Eupachifolin-E (5), $C_{20}H_{24}O_7$; mp. 262-264°C colorless needles; $[\alpha]_D^{20}$ -55.7° (c=0.2, pyridine); UV (MeOH) 213 nm (ε=18,114); CD curve $[\theta]_{227}$ 0, $[\theta]_{232}$ +2214, $[\theta]_{244}$ 0, $[\theta]_{260}$ -1439; IR (KBr) 3450, 1745, 1700, and 1650 cm^{-1}; m/e 376 (M^+), 358, 293, 276, and 258.

Eupachifolin-A (1), on acetylation with acetic anhydride and pyridine, gave a monoacetate (1a) [m/e 404 (M^+), 362 ($M-Ac+H$)$^+$, 303 ($M-C_5H_9O_2$)$^+$; IR ($CHCl_3$) 1765, 1740, and 1690 cm^{-1}]. Oxidation of (1) with pyridinium dichromate[4] in dichloromethane at room temperature afforded a keto-aldehyde

Table 1. ^{1}H NMR spectra of sesquiterpene lactones and derivatives

Compd.	H-1	H-2	H-3	H-5	H-6	H-7	H-8	H-9	H-13	H-14	H-15	H-2'	H-3'	H-4'	H-5'	OAc
(1)[a]	6.50t (8)	3.06dd (7;8)	4.58t (7)	5.18dd (1.5;10)	6.02dd (3.5;10)	2.58m	5.76m	2.85m	6.28;5.65d (2.5)(2)	9.45d (0.73)	1.79d (1.5)	2.34st (7)	1.54m	0.84t (7)	1.08d (7)	-
(1a)[a]	6.46dd (8;9)	3.09m	5.48t (7)	5.22dd (1.5;10)	5.66m[b]	2.58m	5.70m[b]	b	6.30;5.67d (2.5)(2)	9.42d (1)	1.80d (1.5)	2.31st (7)	1.47m	0.85t (7)	1.06d (7)	2.17s
(1b)[a]	6.72dd (8;9)	3.53;3.79dd (8;16)(9;16)	-	5.64m[b]	5.36dd (2.5;9)	2.67m	5.64m	2.50;2.96dd (9;14)(6.1;14)	6.30;5.68d (2.2)(1.9)	9.47d (1)	1.90d (1.5)	2.29st (7)	1.48m	0.85t (7)	1.04d (7)	-
(2)[a]	b	5.58m[b]	5.64sbr	2.84m[b]	4.64dd (8.5;10)	3.59m	5.53m[b]	2.24m[b]	6.29;5.52d (3.8)(3)	2.73s	2.04s	-	6.64m	4.29dbr (5.8)	1.78sbr	2.04s
(2a)[a]	b	5.64m[b]	5.75sbr	2.88m[b]	4.70m[b]	3.63m	5.65m[b]	2.30m[b]	6.43;5.62d (3.8)(3)	2.78s	2.05s	-	6.67m	4.75dbr (5.8)	1.83sbr	2.05s 2.13s
(2b)[a]	2.97dd (6;8)	5.22m	5.43sbr	3.16m	4.57dd (8;10)	3.42m	5.71m	1.61;2.36dd (9.5;15)(6;15)	6.31;5.53d (3.8)(3)	3.67;3.50d (10)(10)	1.91d (1.5)	-	-	-	-	1.98s
(2c)[a]	3.00dd (6;8)	5.52m[b]	5.42sbr	3.12m	5.48m[b]	3.32m	4.95m	1.77;2.46dd (12;14)(4;14)	6.24;5.63d (1.8)(2)	3.70dd(1;10) 3.34d(10)	1.66d (1.5)	-	-	-	-	2.01s
(2d)[a]	b	4.60dbr (4.5)	5.68m	b	4.70dd (11;8)	3.50m	5.53m	b	6.27;5.50d (3.5)(3)	2.73s	1.94sbr	-	5.93m	4.89m	1.83m	2.02s
(3)[a]	3.42dd (6;8)	5.58m[b]	5.64sbr	2.72m[b]	4.59dd (8.5;11)	3.18m	5.56m[b]	2.53;2.79dd (8;14)(6.5;14)	6.26;5.48d (3.8)(3)	5.03sbr 5.08sbr	1.99s	-	6.67m	4.32dbr (5.8)	1.78sbr	1.99s
(3a)[a]	1.94dd (5;8.5)	5.34dbr (5)	3.37s	2.59m	4.72m[b]	3.55m	5.56m[b]	b	6.33;5.53d (3.5)(3)	2.66s	1.69s	-	6.58m	4.68dbr (5.8)	1.82sbr	2.08s
(4)[a]	b	5.38m	5.80sbr	b	4.60dd (8;10)	3.96m	5.68m[b]	2.21;2.46dd (8;12)(4;12)	6.27;5.46d (3.8)(3)	3.50;3.60d (12)(12)	2.02d (1.5)	-	6.68m	4.32dbr (5.8)	1.76sbr	2.00s
(5)[c]	2.00dd (5;8)	4.60dbr (5)	3.60s	2.75m[b]	5.31dd (8;10.5)	3.88m	5.89dt (4;8)	2.42;3.43dd (8;14)(8;14)	6.36;5.54d (3.8)(3)	2.69s	1.77s	-	6.81m	1.52dbr (7)	1.72sbr	-
(5a)[a]	1.94dd (5;8.5)	5.36dbr (5)	3.38s	2.59m[b]	4.75dd (8.5;11.5)	3.55m	5.53m[b]	b	6.35;5.53d (3.8)(3)	2.68s	1.72s	-	6.73m	1.80dbr (7)	1.78sbr	2.10s

a: Run in $CDCl_3$ at 99.6 MHz on a JEOL FX-100 spectrometer with Me_4Si as internal standard. Values are in parts per million; s, singlet; d, doublet; t, triplet; st, sixtet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; dbr, broad doublet. Figures in parentheses are coupling constants in Hertz.
b: Signal partially obscured or superimposed. c: Run in C_5D_5N.

Table 2. ^{13}C NMR spectra of sesquiterpene lactones and derivative

Carbon atom	(1)[a]	(2)[a]	(3)[a]	(4)[a]	(5)[b]	(5a)[a]
1	150.1 d	51.2 d	50.7 d	50.8 d	50.2 d	48.4 d
2	35.8 t	78.8 d	80.3 d	78.1 d	72.8 d	74.9 d
3	72.7 d	125.4 d	126.3 d	125.8 d	65.3 d	61.9 d
4	141.9 s[c]	150.6 s	148.2 s	151.9 s	65.8 s	65.9 s
5	127.8 d	55.5 d	56.0 d	54.8 d	50.8 d	50.2 d
6	73.0 d	80.4 d	80.0 d	81.3 d	77.3 d	75.9 d
7	47.0 d	46.6 d	48.0 d	47.2 d	48.8 d	48.2 d
8	73.0 d	67.0 d	68.0 d	66.9 d	67.8 d	66.7 d
9	28.2 t	35.8 t	39.0 t	36.3 t	36.7 t	36.1 t
10	137.8 s[c]	55.2 s	139.1 s	73.0 s	56.3 s	56.4 s
11	135.2 s[c]	133.7 s	134.0 s	134.3 s	135.2 s	133.4 s
12	175.1 s	169.4 s	169.2 s	169.6 s	168.9 s	168.6 s
13	123.9 t	123.1 t	122.4 t	122.2 t	121.6 t	122.8 t
14	194.4 d	56.2 t	120.1 t	54.6 t	56.2 t	56.4 t
15	24.5 q	17.5 q	17.2 q	18.1 q	18.8 q	18.1 q
1'	169.4 s	166.5 s	166.5 s	166.7 s	166.7 s	166.5 s
2'	41.0 d	127.5 s	127.8 s	127.6 s	128.2 s	127.7 s
3'	26.4 t	141.6 d	141.1 d	141.5 d	137.7 d	138.0 d
4'	11.5 q	59.5 t	59.6 t	59.5 t	14.1 q	14.4 q
5'	16.6 q	12.7 q	12.7 q	12.7 q	12.1 q	12.1 q
OAc	-	21.4 q	21.3 q	21.5 q	-	21.1 q

a: Run in $CDCl_3$ at 25.05 MHz 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: Run in C_5D_5N.
c: Assignment may be interchanged.

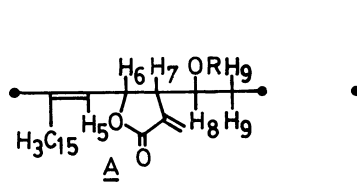
derivative (1b)[mp. 138-141°C; UV (MeOH) 216 (ε=14,284), 309 nm (ε=212); IR ($CHCl_3$) 1775, 1742, 1698, and 1645 cm^{-1}; m/e 360 (M^+), 276 ($M-C_5H_9O+H)^+$, 258 ($M-C_5H_{10}O_2)^+$].

From above experiments and physical data, eupachifolin-A (1) has a secondary hydroxyl, α-methylene γ-butyrolactone, α,β-unsaturated aldehyde, and 2-methylbutyroyl ester group. Therefore, the presence of three moieties [A, B, and C] in (1) can be elucidated by exhaustive analysis of the above physical data, particularly ^1H NMR spectrum (cf. Table 1) with aid of spin decoupling experiments together with ^{13}C NMR spectrum (Table 2) of (1). Irradiation at the frequency of H-7 (δ2.58) collapsed 13-Ha and Hb into singlets and also changed a multiplet (H-8, δ5.76) into a doublet of doublets (J=4,9Hz) and doublet of doublets (H-6, δ6.02) into a clear doublet (J=10Hz), whereas on irradiation at δ5.76 (H-8) the multiplet at δ2.85 (H-9) became simplified. Irradiation at the frequency of H-6 converted H-7 into broad singlet and also changed a doublet of doublets at δ5.18 (H-5, J=1.5,10Hz) into a quartet (J=1.5Hz), whereas on irradiation at δ5.18 the doublet at δ1.79 (15-CH_3, J=1.5) became singlet. The presence of [B] and [C] can also be based on the IR and UV spectra combined with ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum exhibit a one-proton triplet at δ6.50 (J=8Hz), presumably due to the proton β to the aldehyde function. The identity of this signal was confirmed by single-frequency off resonance decoupling in the ^{13}C NMR spectrum which resulted in collapse of the doublet at δ150.1 to a singlet. Irradiation at δ3.06 (H-2) caused collapse of two triplets at δ6.50 and δ4.58 to singlets, respectively. These data were in good agreement with the ^{13}C NMR spectral data (cf. Table 2).
On the other hand, the ester portion was a 2-methylbutyroyloxyl group as evidenced by the presence of a sixtet (δ2.34) coupled to a methyl doublet (δ1.08) and a multiplet (δ1.54) which was also coupled to a methyl triplet (δ0.84). In accordance with these deductions the mass spectrum exhibited characteristic peaks at m/e 278, 260, and the base peak at m/e 85 (C_5H_9O). From the above data, the structure of eupachifolin-A, which incorporates [A], [B], and [C] and satisfies all requirements, should be (1), exclusive of its stereochemistry. The geometries of the 1,10- and 4,5-double bonds in (1) were determined to be cis by observation of 14.8% NOE between H-1 and aldehyde proton (H-14) as well as of 12.3% NOE between H-5 and 15-CH_3 protons. Irradiation at the 15-CH_3 protons also produced a 9.2% enhancement in the area intensity of H-3 signal, suggesting that H-3 and H-5 spatially close to 15-CH_3 group. Irradiation at H-9 and H-2 produced 14.8 and 13.5% enhancements, respectively, in the area intensity of H-6 signal. These results indicate that H-6, H-9, and H-2 are also quite close one another.
From above experiments, the conformation of eupachifolin-A in chloroform solution should be (D).
The stereochemistry of H-8 was determined to be α-orientation by the small half band width of H-7 ($W_{\frac{1}{2}}$ 3Hz) and a doublet (J=3Hz) of H-8, which were obtained on irradiation at H-6 and H-9, respectively, in (1). This configuration (α) was also supported by the appearance of two doublet of doublets at δ2.50 (9-Ha, J=9,14Hz) and δ2.96 (9-Hb, J=6.1,14Hz) in (1b)[5] Hence, the structure of eupachifolin-A should be represented by (1) except for the stereochemistry of C-2'.

Eupachifolin-B (2), on acetylation with acetic anhydride and pyridine, gave a diacetate (2a) [IR ($CHCl_3$) 1765, 1735, 1720, and 1665 cm^{-1}; m/e 460 (M^+), 446, 418, 358, 303, and 242]. These data of (2a) showed that the structure of (2) was similar to that of deoxygraminiliatrin (2d) isolated from Liatris graminifolia,[6] except for the position of an acetoxyl group. On alkaline hydrolysis with 10% KOH in dioxane followed by acetylation, (2) yielded a monoacetate (2b)[7][mp. 176-178°C; UV (MeOH) 205 nm (ε=8,309); CD curve $[\theta]_{260}$ -1174; IR ($CHCl_3$) 3400, 1770, 1735, and 1665 cm^{-1}; m/e 320 (M^+), 305, 302, 290, 260, and 242] and (2c)[mp. 213-215°C; UV (MeOH) 205 nm (ε=8,242); CD curve $[\theta]_{214}$ +31613, $[\theta]_{236}$ 0, $[\theta]_{252}$ -5268; IR ($CHCl_3$) 3450, 1770, 1750, and 1665 cm^{-1}; m/e 302 ($M-H_2O)^+$, 276, 260, 242, and 230]. From above data, ^1H NMR spectrum (Table 1) with aid of spin decoupling experiments together with ^{13}C NMR spectrum (Table 2) of (2), eupachifolin-B (2) has a 2β-acetoxyl, 8β-ester group, 1α-H, 5α-H, 10α-hydroxyl,[8] and 6β-H,7α-H-trans-fused lactone ring.

The structure of the ester group at C-8 in (2) was determined by the ^1H NMR decoupling and NOE experiments: irradiation at the frequency of the vinyl methyl protons at C-5' produced a 13.3% enhancement in the area intensity of H-3' signal, but no observation of NOE between H-5' and H-4'. From above data, the structure of eupachifolin-B should be (2).

Eupachifolin-C (3), -D (4), and -E (5) are mutually related guaianolide sesquiterpene lactones. Acetylation of (3) followed by epoxidation with m-chloroperbenzoic acid gave (3a)[IR ($CHCl_3$) 1765, 1745, 1720, and 1660 cm^{-1}; m/e 476 (M^+), 417, 356, 319, 275, and 259], which was completely identical with (3a), prepared from eupachifolin-B (2) on epoxidation with m-chloroperbenzoic acid followed by acetylation. Eupachifolin-D (4) was an HCl adduct of (2). Treatment of (4) with neutral alumina (MERCK) caused quantitative conversion to (2). From above experiments, the structures of eupachifolin-C and -D would be represented by (3) and (4), respectively. On the other hand, eupachifolin-E (5), on acetylation with acetic anhydride and pyridine, gave a monoacetate (5a)[IR ($CHCl_3$) 1770, 1750, 1715, and 1650 cm^{-1}; m/e 418 (M^+), 359, 276, 258, and 83], the ^1H NMR spectrum of which was almost superimposable on that of (3a) except for the ester group. The structure of the ester group at C-8 in (5) was determined by NOE experiments: irradiation at δ1.52 (4'-CH_3) produced a 22.3% enhancement in the area intensity of H-3' signal but NOE between 5'-CH_3 and H-3' was not observed. From above results and physical data, the structure of eupachifolin-E should be (5).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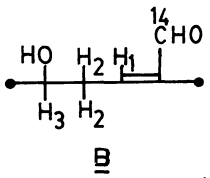
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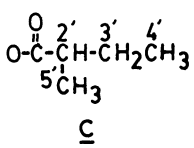
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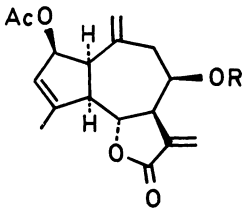
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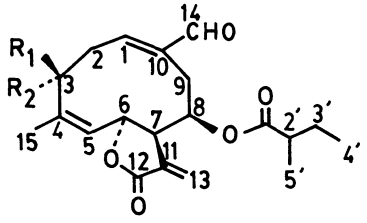
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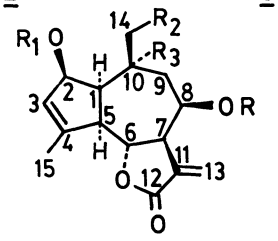
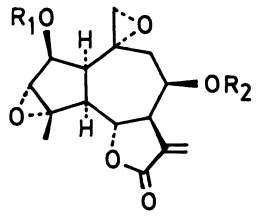

(2): R_1=Ac, R_2+R_3=-O-, R=(A), R_4=H
(2a): R_1=Ac, R_2+R_3=-O-, R=(A), R_4=Ac
(2d): R_1=H, R_2+R_3=-O-, R=(A), R_4=Ac
(4): R_1=Ac, R_2=Cl, R_3=OH, R=(A), R_4=H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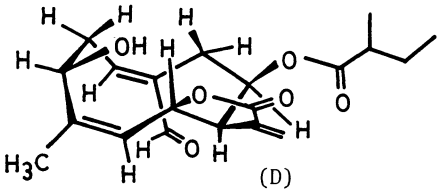
(5): R_1=H, R_2=Tigroyl
(5a): R_1=Ac, R_2=Tigroyl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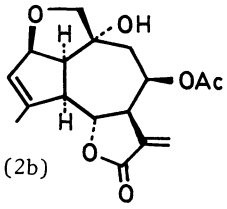
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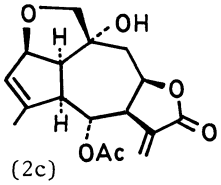
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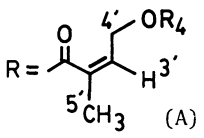
(A)

REFERENCES AND NOTES

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2) Collected at Aichi prefecture, Japan, August 1977.
3) S. Nakajima and K. Kawazu, Heterocycles, 10, 117 (1978)
4) E. J. Corey and G. Schmidt, Tetrahedron Lett., 399 (1979)
5) The conformation of (1b) in solution was similar to that of (1) by observation of 9.5% NOE between H-6 and 9-Ha (δ2.50) as well as of 12% NOE between H-6 and H-2.
6) W. Herz, J. Poplawski, and R. P. Sharma, J. Org. Chem., 40, 199 (1975)
7) The stereochemistry of the hydroxyl group at C-10 was determined to be α-orientation by the no observation of low-field shift at H-6 of (2b).
8) The observed low-field shift of H-7 (δ3.59) in (4), compared with H-7 (δ3.96) in (2), is explainable only if the hydroxyl group attached to C-10 is α-oriented. (see Table 1).

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