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J. Nat. Prod., **1994**, 57 (12), 1626-1630• DOI: 10.1021/np50114a003 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 12, pp. 1626-1630, December 1994

SIX NEW EREMOPHILANE DERIVATIVES FROM TWO *LIGULARIA* SPECIES

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ABSTRACT.—Further phytochemical investigation of the roots of *Ligularia sagitta* and *L. veitchiana* afforded, in addition to several known compounds, six new eremophilane derivatives [**1–6**]. Their structures were elucidated by means of nmr spectroscopy.

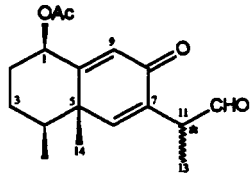
Some 27 *Ligularia* species have long been used as folk remedies due to their antibiotic, antiphlogistic, and antitumor activities (1). Eremophilane sesquiterpenes and pyrrolizidine alkaloids are the most widespread secondary metabolites in this genus (2,3). However, the isolation of a novel norditerpene with a previously unreported carbon skeleton from *Ligularia sagitta* (Maxim.) Mattf. ex Rehder & Kobuski (Compositae) (4) has increased interest in the phytochemistry of this species. In a reinvestigation on the roots of *Ligularia sagitta*, we have now isolated eight chemical components, of which four are novel (**1–4**). A further species, *Ligularia veitchiana* (Hemsl.) Greenm. (Compositae), from which several eremophilanolides have been isolated (5–7), has now yielded two additional previously unreported eremophilane derivatives (compounds **5** and **6**). We report herein the isolation and structural characterization of novel compounds **1–6**.

RESULTS AND DISCUSSION

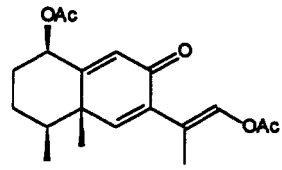
A petroleum ether-Et_2O-MeOH (1:1:1) extract of the powdered roots of *Ligularia sagitta* was subjected to cc on Si gel and then fractionated, as described in the Experimental section, to yield sagittolactone [**8**] (4), β-sitosterol, β-sitosterol β-D-glucopyranoside [**7**], petasin [**9**] (8), isopetasin [**10**] (9), and four new compounds, **1–4**. From a petroleum ether-Et_2O-Me_2CO (1:1:1) extract of *Ligularia veitchiana*, two minor eremophilane derivatives [**5** and **6**] were obtained by prep. tlc.

Compounds **1** and **2** were obtained as an epimeric mixture (ca. 1:1 by ^{1}H- and ^{13}C-nmr spectroscopy). The molecular formula, $C_{17}H_{22}O_4$, was indicated by its eims (*m/z* 290 $[M]^+$) and elemental analysis. In addition to acetoxy signals at δ 21.15 (CH_3) and 169.71 (*C*O), the ^{13}C-nmr spectrum of **1** and **2** showed fifteen signals. The ^{1}H-nmr spectra of **1** and **2** showed three methyl signals, the position and splitting patterns of which suggested these were eremophilane sesquiterpenes. Furthermore, two olefinic hydrogen singlets appeared at δ 6.37 (H-9) and 6.79 or 6.81 (H-6 of **1** and **2**). These signals, in conjunction with the ^{13}C-nmr resonances at δ 153.39/154.61 (CH), 135.38/135.24 (C), 184.84 (C), 127.94 (CH), and 160.46 (C), strongly suggested the presence of a 6(7),9(10)-dien-8-oxo moiety. This concept was supported by the α,β,α′,β′-unsaturated ketone absorption band at 1664 cm^{-1} in the ir spectrum. In addition to the molecular ion peak at *m/z* 290, the eims exhibited a significant $[M-1]^+$ peak at *m/z* 289. Thus, the ^{1}H-nmr signal at δ 9.66 (1H, s) and the ir absorption band at 1739 cm^{-1} were consistent with the presence of an aldehyde group. The location of this group at C-11 was deduced from the downfield methine proton at δ 3.72 and 3.67 attributed to H-11, as well as from the chemical shift of the methyl doublet (H-13) at δ 1.27 (Table 1).

The β-configuration of the acetoxy group at C-1 was deduced from the splitting pattern of H-1 (t, J=3.0 Hz). Based on the aforementioned information, **1** and **2** were deduced to have the basic structure, 1β-acetoxy-8-oxo-eremophila-6,9-dien-12-al. The small difference in the ^{13}C-nmr signals of C-4, C-6, C-7, C-11, C-13, and C-14 (Table



1 11**S*
2 11**R*



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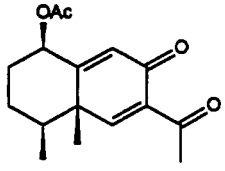

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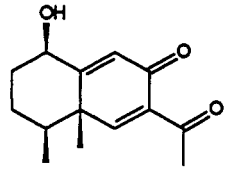

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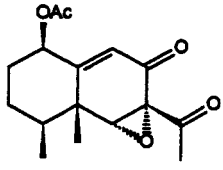

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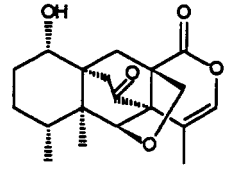

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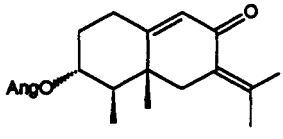

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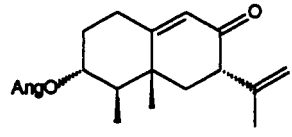

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2), and in the ^{1}H-nmr signal of H-6 indicated that the compounds were epimeric at C-11, an active center prone to facile enolization.

The ^{1}H- and ^{13}C-nmr data of **3** showed close similarities with those of **1** and **2** (Tables 1 and 2). However, the aldehyde group of **1** and **2** was absent in **3**, as judged from its ^{1}H-nmr, ^{13}C-nmr, and ir data (Tables 1, 2 and Experimental). The quartet seen for H-11 in **1** and **2** was also absent in the ^{1}H-nmr spectrum of **3**, while additional signals of a trisubstituted double bond were visible at δ 118.83 (C), 135.80 (CH) (Table 2), and δ 7.41 (1H) (Table 1). In view of the appearance of an olefinic hydrogen at such a low-field position and the presence of two acetoxy groups (^{1}H and ^{13}C-nmr data), an 11(12)-double bond and a 12-acetoxy substituent were most likely in **3**. The downfield location of H-12 was probably due to the 12-acetoxy group, and therefore suggested an *E*-configuration of the 11(12) double bond. The eims of **3** exhibited a molecular ion peak at *m/z* 332, together with two significant fragments at *m/z* 272 and *m/z* 212 due to the successive loss of two acetoxy groups. All of the above data were consistent with the structure proposed for this isolate [**3**].

Compound **4** exhibited ^{1}H- and ^{13}C-nmr spectra similar to those of **1–3**, except that **4** had only 14 skeletal carbon atoms (Table 2). In comparison with **1–3**, the H-13 signal appeared at noticeably lower field (δ 2.56, s), while H-6 was also shifted approximately 0.8 ppm downfield (Table 1). In the same way, the C-6 signal was shifted from δ 153.39/

TABLE 1. ^{1}H-Nmr Data of Compounds **1–6** ($CDCl_3$, 400 MHz).[a]

Proton	Compound					
	1[b]	**2**[b]	**3**	**4**	**5**	**6**
1	5.49 t (3.0)	5.49 t (3.0)	5.48 t (3.0)	5.50 t (3.0)	4.56 t (2.8)	5.44 t (3.0)
2	2.12 dddd (14.0,4.0, 4.0,3.0)	2.12 dddd (14.0,4.0, 4.0,3.0)	2.13 m	2.13 m	2.09 dddd (13.5,4.0, 4.0,3.2)	2.03 dddd (13.5,4.0, 4.0,3.2)
2'	1.85 dddd (14.0,14.0, 14.0,3.0)	1.85 dddd (14.0,14.0, 14.0,3.0)	1.86 dddd (14.0,14.0, 14.0,3.0)	1.86 dddd (14.0,14.0, 14.0,3.0)	2.02 dddd (13.5,13.5, 13.5,3.2)	2.03 dddd (13.5,13.5, 13.5,3.2)
3	1.46 m	1.46 m	1.48 m	1.49 m	1.52 m	1.56 m
3'	1.58 m	1.58 m	1.60 m	1.62 m	1.61 m	1.73 m
4	1.71 m	1.71 m	1.70 m	1.71 m	1.68 m	1.96 m
6	6.79 s	6.81 s	6.88 s	7.67 s	7.68 s	3.52 s
9	6.37 s	6.37 s	6.36 s	6.31 s	6.18 s	6.06 s
11	3.72 q (6.0)	3.67 q (6.0)	—	—	—	—
12	9.66 s	9.66 s	7.41 s	—	—	—
13	1.27 d (6.0)	1.27 d (6.0)	1.92 d (1.1)	2.56 s	2.56 s	2.35 s
14	1.26 s	1.26 s	1.26 s	1.29 s	1.38 s	1.30 s
15	1.16 d (6.4)	1.16 d (6.4)	1.13 d (6.6)	1.15 d (6.6)	1.15 d (6.6)	1.15 d (6.7)
OAc ...	2.06 s	2.06 s	2.06 s 2.18 s	2.06 s	—	2.06 s

[a]Data are provided in δ units, with multiplicities shown and *J* values indicated in parentheses.
[b]Isolated as an epimeric mixture.

154.61 to δ 160.73 when compared with **1** and **2**. Furthermore, two α,β-unsaturated ketone absorption bands were observable at 1664 and 1694 cm^{-1} in the ir spectrum of **4**. All of these observations supported the presence of an 11-ketone group. Biogeneti-

TABLE 2. ^{13}C-Nmr Data of Compounds **1–6**, **9**, and **10** ($CDCl_3$, 400 MHz, δ units).

Carbon	Compound							
	1[a]	**2**[a]	**3**	**4**	**5**	**6**	**9**[b]	**10**[c]
1	74.71	74.71	74.63	74.25	73.22	74.31	31.91	31.71
2	32.07	32.07	31.90	32.08	34.28	30.86	30.63	30.15
3	25.50	25.50	21.37	21.51	24.84	25.02	72.97	73.28
4	41.17	41.22	40.52	41.06	40.84	37.41	47.28	46.20
5	43.76	43.76	43.39	43.78	44.11	40.75	40.09	41.12
6	153.39	154.61	152.35	160.73	161.70	66.47	41.67	41.16
7	135.38	135.24	136.10	135.80	135.87	63.43	50.32	129.65
8	184.84'	184.84	183.78	185.31	184.33	190.90	198.51	191.07
9	127.94	127.94	129.30	128.79	126.75	125.78	124.58	126.72
10	160.46	160.46	159.21	159.40	165.20	157.74	159.78	155.67
11	45.39	45.48	118.83	198.50	198.60	200.30	143.29	150.63
12	200.89	200.89	135.80	—	—	—	114.44	22.61
13	12.92	12.98	13.67	30.93	30.87	28.08	20.56	21.13
14	18.00	18.05	17.74	18.13	18.59	18.46	10.52	10.83
15	16.06	16.06	15.93	16.08	15.95	15.66	29.66	29.69
OAc	169.71	169.71	169.68	169.65	—	169.34	—	—
	21.15	21.15	21.15	21.15	—	21.18	—	—
	—	—	167.82	—	—	—	—	—
	—	—	20.74	—	—	—	—	—

[a]Isolated as an epimeric mixture.
[b]Additional data for OAng: 166.62 (C), 127.88 (C), 138.08 (CH), 20.00 (CH_3), 17.16 (CH_3).
[c]Additional data for OAng: 165.18 (C), 127.28 (C), 138.10 (CH), 20.59 (CH_3), 17.14 (CH_3).

cally, this nor-eremophilane derivative should most likely originate via enzymatic oxidation of eremophilanes **1–3**. The molecular ion peak in the eims of **4** at *m/z* 276 and elemental analysis yielded a molecular formula of $C_{16}H_{20}O_4$, in agreement with the proposed structure **4**.

The structure of compound **5** followed from its ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2). Comparison with **4** clearly revealed that it was the deacetyl derivative of the latter compound. Acetylation of **5**, giving **4**, confirmed this assumption (Experimental).

The ^{1}H- and ^{13}C-nmr spectra of **6** indicated only slight differences from those of **4**. When comparing the ^{1}H-nmr data of **6** and **4**, the main difference was the absence of the olefinic H-6 signal and the appearance of a singlet at δ 3.51 (1H, s). This suggested the presence of an oxygenated function at C-6. Comparison of the ^{13}C-nmr data of **4** and **6** also supported the conclusion that the 6,7-double bond was replaced by a 6,7-epoxy functionality in **6** (Table 2). Furthermore, the required molecular formula of $C_{16}H_{20}O_5$ is in agreement with both the molecular ion peak at *m/z* 292 and elemental analysis. The α- configuration of the epoxide was deduced by nOe spectroscopy, where a clear nOe between H-6 and H-14 (6%) was observed.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were taken on a Perkin-Elmer 241 polarimeter. Uv spectra were measured on a Shimadzu UV-260 spectrometer, using MeOH as solvent. Ir spectra were measured on a 5DX-FTIR spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-400 Ft-nmr spectrometer using tetramethylsilane (TMS) as internal standard. Eims were obtained on a VG ZAB-HS spectrometer at 70 eV.

PLANT MATERIAL.—The roots of *L. sagitta* (voucher specimen No. ZR 25/888) and *L. veitchiana* (voucher specimen No. ZR 21/889) were collected in Zhang County, Gansu Province, People's Republic of China in August 1988 and August 1989, respectively. The plant material was identified by Prof. R.N. Zhao from the Department of Pharmacy, Lanzhou Medical College, where voucher specimens are deposited.

EXTRACTION AND ISOLATION.—The extraction and processing of the extract of *L. veitchiana* (50 g) have been described previously (7). Fractions 8 and 9 (86 mg) were combined and subjected to prep. tlc with $CHCl_3$-Me_2CO (10:1) as solvent to give 12 mg of **5** and 16 mg of **6**.

The air-dried roots of *L. sagitta* (5.5 kg) were powdered and extracted three times at room temperature with petroleum ether (60–90°)-Et_2O-MeOH (1:1:1) (each time for 4 days). A total of 420 g of extract was obtained after concentration *in vacuo*. One third of the obtained extract (ca. 140 g) was subjected to cc on Si gel (1 kg) with a petroleum ether-Me_2CO gradient (50:1→1:1). According to differences in composition indicated by tlc, 20 crude fractions were obtained. Fraction 13 (1.5 g) was further separated by cc on Si gel H (80 g), eluting with a $CHCl_3$-Me_2CO (20:1→1:1) gradient. Eluates 6–8 were combined and further purified by prep. tlc [petroleum ether-CH_2Cl_2-Et_2O (4:4:1), 3 developments] to afford a band containing 18 mg of a mixture of **1** and **2**. Eluates 10–14 were combined and purified by prep. tlc [petroleum ether-CH_2Cl_2-Et_2O (5:5:1), 6 developments] to afford 10 mg of **3** and 9 mg of **4**. Cc of fraction 7 (1.5 g) on Si gel (120 g) gave mainly fatty acids. Prep. tlc of eluates 3–5 with C_6H_{12}-Et_2O (14:1, 5 developments) afforded 7 mg of petasin [**9**] and 200 mg of isopetasin [**10**]. By cc and prep. tlc with C_6H_6/Me_2CO mixtures, fraction 11 yielded 60 mg of sagittolactone [**8**]. From fraction 17, 800 mg of β-sitosterol β-D-glucopyranoside [**7**] were obtained. Petasin and isopetasin were identified by comparison of their spectral data with literature values (8,9), and sagittolactone was directly compared with an authentic sample.

1β-Acetoxy-11 (R,S)*-8-oxoeremophil-6,9-dien-12-al* [*mixture of* **1** *and* **2**].—Colorless gum; $[\alpha]^{20}_D$ −25.6° (*c*=0.3, $CDCl_3$); uv λ max 249 nm (ε 12300); ir ν max (KBr) 1739, 1664, 1635, 1457, 1372, 1239, 1205, 1020, 914, 733 cm^{-1}; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* 290 $[M]^+$ (48), 289 $[M-1]^+$ (47), 261 $[M-CHO]^+$ (52), 247 $[289-Ac]^+$ (60), 230 $[M-AcOH]^+$ (70), 202 (100), 187 (35), 173 (32), 135 (36), 115 (48), 91 (74), 77 (54), 43 (90); *anal.* found C 70.32, H 7.56, calcd for $C_{17}H_{22}O_4$, C 70.34, H 7.59.

*1β,12-Diacetoxy-6,9,12*E*-trien-8-oxoeremophilane* [**3**].—Colorless gum; $[\alpha]^{20}_D$ −34.2° (*c*=0.5, $CDCl_3$); uv λ max 244 nm (ε 8270); ir ν max (KBr) 1740, 1664, 1633, 1455, 1373, 1236, 1212, 1020 cm^{-1}; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* 332 $[M]^+$ (5), 304 (4), 272 $[M-AcOH]^+$ (10), 230 (48), 212 $[M-2\times AcOH]^+$ (25), 173 (78), 115 (15), 91 (20), 43 (100); *anal.* found C 68.69, H 7.26, calcd for $C_{19}H_{24}O_5$, C 68.67, H 7.23.

1β-Acetoxy-6,9-dien-8-oxoeremophil-11-nor-11-ketone [**4**].—Colorless gum; $[\alpha]^{20}D$ −34.6° (*c*=0.5, $CDCl_3$); uv λ max 247 nm (ε 9900); ir ν max (KBr) 1741, 1695, 1664, 1633, 1455, 1373, 1235, 1208, 1020 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* 276 $[M]^+$ (10), 261 (3), 234 $[M-Ac]^+$ (20), 216 (52), 201 (49), 173 (100), 145 (54), 103 (22), 91 (25), 77 (28); *anal.* found C 69.58, H 7.27, calcd for $C_{16}H_{20}O_4$, C 69.57, H 7.25.

1β-Hydroxy-6,9-dien-8-oxoeremophi-11-nor-11-ketone [**5**].—Colorless gum; $[\alpha]^{20}D$ −36.0° (*c*=0.6, $CHCl_3$); uv λ max 248 (ε 10100); ir ν max (KBr) 3482, 1696, 1657, 1624, 1450, 1391, 1281, 1202, 1025, 959 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* 234 $[M]^+$ (26), 219 (16), 216 $[M-H_2O]^+$ (44), 201 (55), 173 (100), 145 (78), 103 (25), 77 (52); *anal.* found C 71.81, H 7.72, calcd for $C_{14}H_{18}O_3$, C 71.79, H 7.69. Compound **5** (10 mg) was treated with 2 ml Ac_2O-pyridine (1:1, v/v) overnight. After vacuum concentration, the residue (16 mg) was subjected to prep. tlc with petroleum ether (60–90°)-Et_2O (3:1) and yielded 11 mg **4** (identified by comparing their $[\alpha]^{20}D$ value, and ir, uv, ^1H- and ^{13}C-nmr spectra).

1β-Acetoxy-6α,7α-epoxy-9-en-8-oxoeremophil-11-nor-11-ketone [**6**].—Colorless gum; $[\alpha]^{20}D$ −11.6° (*c*=0.4, $CHCl_3$); uv λ max 236 nm (ε 8960); ir ν max (KBr) 1745, 1715, 1710, 1394, 1265, 1237, 1208, 960, 590 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* 292 $[M]^+$ (4), 250 $[M-Ac]^+$ (70), 232 (18), 207 (43), 189 (22), 179 (33), 164 (30), 151 (48), 133 (20), 105 (20), 91 (30), 43 (100); *anal.* found C 65.77, H 6.83, calcd for $C_{16}H_{20}O_5$, C 65.75, H 6.85.

ACKNOWLEDGMENTS

The authors express their gratitude to the National Natural Science Foundation of China and the doctoral program of the State Education Commission of China for financial support.

LITERATURE CITED

1. Jiangsu College of New Medicine, People's Hygiene Publisher, Beijing, "A Dictionary of the Traditional Chinese Medicines," 1977, pp. 7, 154, 549, 1152, 2349.
2. Y. Asada and T. Furuya, *Chem. Pharm. Bull.*, **32**, 475 (1984).
3. T. Sato, Y. Moriyama, H. Nagano, Y. Tanahashi, and T. Takahashi, *Bull. Chem. Soc. Jpn.*, **48**, 112 (1975).
4. H.M. Chen, Z.J. Jia, and L. Yang, *Phytochemistry*, **31**, 2146 (1992).
5. Y. Zhao, Z.J. Jia, R.X. Tan, and L. Yang, *Phytochemistry*, **31**, 2785 (1992).
6. Z.J. Jia, Y. Zhao, and R.X. Tan, *Planta Med.*, **58**, 365 (1992).
7. Z.J. Jia, Y. Zhao, and R.X. Tan, *J. Nat. Prod.*, **56**, 494 (1993).
8. D. Herbert and C. Djerassi, *J. Am. Chem. Soc.*, **82**, 4337 (1960).
9. A. Stoll, R. Morf, A. Rheiner, and J. Renz, *Experientia*, **12**, 360 (1956).

Received 26 April 1994