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SESQUITERPENES FROM *LIGULARIA INTERMEDIA*

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Key Word Index—*Ligularia intermedia*; Compositae; rhizomes; sesquiterpenes.

Abstract—Four new eremophilanolides were isolated from the rhizomes of *Ligularia intermedia*. Their structures were determined by spectroscopic methods and 2D-NMR techniques, to be 6α,9-dihydroxy-14β-carboxyfuranoeremophil-9(10)-ene, 7α, 8α-epoxyeremophilan-12β, 8β(14β,6α)-diolide, eremophil-7(11),8(9)-dien-12,8(14β,6α)-diolide and eremophil-7(8)-en-12,8(14β,6α)-diolide.

INTRODUCTION

In previous papers, we reported on the isolation and structural determination of sesquiterpenes and a diterpene from *Ligularia virgaurea* [1] and *Ligularia sagitta* [2], which grow in northwestern China. This paper reports on the isolation and characterization of four new sesquiterpenes from the rhizomes of *Ligularia intermedia* [3] as 6α,9-dihydroxy-14β-carboxyfuranoeremophil-9(10)-ene (**3**), 7α,8α-epoxy-eremophilan-12β,8β(14β,6α)-diolide (**4**), eremophil-7(11),8(9)-dien-12,8(14β,6α)-diolide (**5**) and eremophil-7(8)-en-12,8(14β,6α)-diolide (**6**), as well as two known compounds, bakkenolide A(**1**) [2, 4] and furanoeremophilan-4β,6α-olide (**2**) [5, 6].

RESULTS AND DISCUSSION

The petrol–ether extract of the dried rhizomes of *L. intermedia* was repeatedly separated by silica gel column chromatography and prep TLC to give six sesquiterpenes (**1**–**6**).

Compound **3** was assigned the molecular formula by HREIMS analysis (*m/z* 278.1156 $[M]^+$ Δ 0.3 mmu). IR absorption at 3235 and 1721 cm^{-1} implied that **3** possessed hydroxy groups and a carbonyl group. The ^1H NMR spectrum showed signals of two tertiary methyls (δ 1.19, *s*, CH_3-15; and δ 1.86, *s*, CH_3-13) and an active hydrogen (δ 12.39, *s*, 15-COOH). The ^{13}C NMR and DEPT spectra indicated that compound **3** possessed $2\times CH_3$ $3\times CH_2$, $3\times CH$ and seven quaternary carbons, one carboxylic acid group (δ 174.73) and three double bonds, and was, therefore, a tricyclic sesquiterpene. By comparison of the spectroscopic data with those reported for related compounds [5–8], **3** was identified as an eremophilane sesquiterpene. In the ^1H and ^{13}C NMR spectra the characteristic signals of a furan ring [δ 7.12, *s*, 12-H; δ 151.65 (C), 138.80 (CH), 127.09 (C), 104.28 (C) and 8.77(α-CH_3)] showed it to be a furanosesquiterpene. The hydroxy group had to be connected directly with the other double bond (δ 127.56, C; δ 165.81, C). Its ^1H NMR spectrum showed that the location of the double bond was at C-9/C-10. Due to the absence of the proton signal of CH_3-15 the carboxylic group had to be at C-15. The relative configuration of H-6 was determined by NOE; irradiation of CH_3-15 (δ 1.12) enhanced H-6 (δ 5.70, 6.2%). The structure of **3** was thus 6α,9-dihydroxy-4β-carboxyfurano-eremophil-9(10)-ene.

Compound **4** was assigned the molecular formula $C_{15}H_{18}O_5$ by HREIMS analysis (*m/z* 278.1156 $[M]^+$ Δ 0.2 mmu). IR absorptions at 1801 and 1768 cm^{-1} implied that **4** possessed two carbonyl groups. The ^1H NMR spectrum showed signals of a secondary methyl (δ 1.40, *d*, $J = 7.5$ Hz, CH_3-13), and a tertiary methyl (δ 1.19, *s*, CH_3-15). The ^{13}C NMR and DEPT spectra indicated that compound **4** possessed $2\times CH_3$, $4\times CH_2$, $4\times CH$ and five quaternary carbons (two ester carbonyl groups, δ 174.88 and 174.94), and was a pentacyclic eremophilane sesquiterpene. The ^{13}C NMR and DEPT spectra also showed that there were three saturated carbons connected to oxygen atoms directly (δ 87.11, C; 77.65, CH; 61.51, C). Because there was no hydroxyl absorption peak in the IR spectrum, **4** probably contained an epoxy group. There were three possible positions for such a group, i.e. (1)

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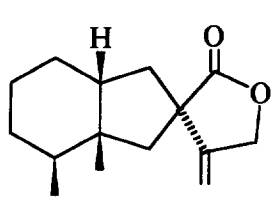
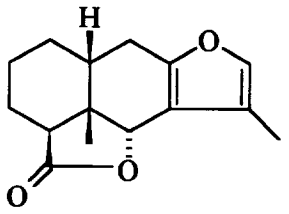


1



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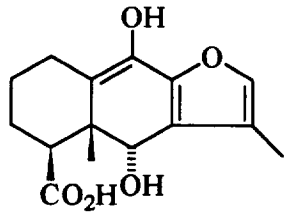

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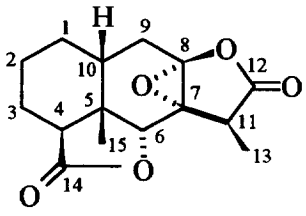

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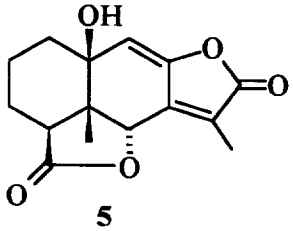

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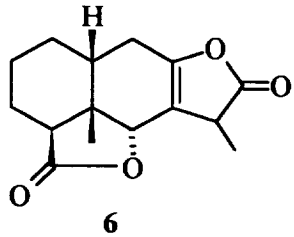

6

C-1—O—C-8 (2) C-8—O—C-7 and (3) C-7—O—C-6. Because the signal at δ 4.33 was a singlet, the connected form of epoxy group should be **2**. Unlike the ^{1}H NMR spectral data of compounds reported in literature (δ 2.68) [7, 8], the chemical shift of H-4 was upfield due to the shielding effect of the epoxy group. Thus, the configuration of the epoxy group was determined as α,α. In the ^{1}H–^{1}H NOESY spectrum, there were cross peaks between H-10 and CH_3-15, CH_3-13 and CH_3-15, H-6 and CH_3-15. Based on the assumption that CH_3-15 was in the β configuration, the relative configuration of H-10, H-6 and CH_3-13 should also be β. The ^{1}H and ^{13}C NMR data of **4** were assigned on the basis of ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, ^{1}H–^{1}H NOESY and ^{13}C–^{1}H COLOC LR spectra. Its structure was determined to be 7α,8α-epoxy-eremophilan-12β,8β(14β,6α)-diolide.

Compound **5** was assigned the molecular formula $C_{15}H_{16}O_5$ by HREIMS analysis (*m/z* 276.0984 $[M]^+$ Δ 1.3 mmu). The IR spectrum revealed the absorptions of a hydroxyl group (3446 cm^{-1}), two carbonyl groups (1792 and 1743 cm^{-1}) and double bonds (1660, 1445, 1394 cm^{-1}). The ^{1}H NMR spectrum contained signals for two methyl groups at δ 1.23 (*s*, CH_3-15) and 1.93 (*s*, CH_3-13). Three singlets at δ 5.72, 5.36 and 5.33 correspond to an olefinic proton, a proton of a hydroxy group and H-6. Assignments were made on the basis of NOE difference spectrum and D_2O exchange. In the NOE difference spectrum, irradiation of CH_3-15 (δ 1.13) caused enhancement of H-6 (δ 5.36, 16%). The signal at δ 5.33 (disappeared when D_2O was added), was the chemical shift of the proton of the hydroxy group. The signal at δ 5.72 must be due to the olefinic proton. The ^{13}C NMR and DEPT spectra indicated that **5** possessed 2 × CH_3, 3 × CH_2, 3 × CH and seven quaternary carbons (two ester carbonyl groups, δ 174.78 and 168.51; two double bonds, δ 148.04, C; 141.94, C; 126.69, C; and 111.05, CH), and was a tetracyclic sesquiterpene. The ^{13}C NMR and DEPT spectra also showed that **5** contained two carbon atoms connected to oxygen (δ 77.19, CH and 77.59, C), the hydroxy group should be at C-10. The structure of **5** was, therefore, identified as eremophil-7(11),8(9)-dien-12,8(14β,6α)-diolide.

Compound **6** was assigned the molecular formula $C_{15}H_{18}O_4$ by HREIMS analysis (*m/z* 262.1208 $[M]^+$. IR absorptions at 1786 and 1760 cm^{-1} indicated that **6** possessed two carbonyl groups. The ^{1}H NMR spectrum showed signals of a secondary methyl (1.29, *d*, J = 7.2 Hz, CH_3-13, and a tertiary methyl (δ 1.20, *s*, CH_3-15. The ^{13}C NMR and DEPT spectra indicated that compound **6** possessed 2 × CH_3, 4 × CH_2, 4 × CH and five quaternary carbons (two ester carbonyl groups, δ 174.88 and 174.94; one double bond, δ 152.35 and 112.58) and was a tetracyclic sesquiterpene. By comparison of the spectroscopic data with those of compounds **3**–**5**, **6** was also identified as an eremophilane sesquiterpene. From the chemical shifts of carbon atoms of the double bond, it had to be connected directly to a lactone ring. The location of the double bond could be at (1) C-6/C-7 or (2) C-7/C-8. The ^{1}H NMR spectrum showed that the signal at δ 4.82 was a singlet, and indicated that the location of the double bond was at C-7—C-8 (1). The configuration of H-6 was determined by the NOE difference spectrum, irradiation of CH_3-15 (δ 1.20) caused enhancement of H-6 (δ 5.12, 9.3%). Thus, the structure of compound **6** was determined to be eremophil-7(8)-en-12,8(14β,6α)-diolide.

EXPERIMENTAL

Mps: uncorr.; ^{1}H and ^{13}C NMR: 500 (300) and 125 (75) MHz, respectively (int. standard: TMS); MS: 70 eV electron impact ion source..

Plant material. Ligularia intermedia was collected from Xinglong Mountain, Hebei Province in August, 1995. It was identified by Professor Hu-Biao Chen and Peng-Fei Tu, and a voucher specimen is deposited

in the Herbarium of the Department of Pharmacognosy, Beijing Medical University (Voucher no. 950828).

Extraction and isolation. The air-dried rhizomes of *L. intermedia* (2.5 kg) were pulverized and extracted at room temp. with petrol (60–90°)–Et_2O (2:1) (7 day × 2). The resultant extract was concd under vacuum to give a residue (66.4 g) which was subjected to CC on silica gel eluting with petrol containing gradually increased amounts of EtOAc. The crude CC frs obtained were further purified by repeated CC eluting with petrol (60–90°)–EtOAc and finally prep. TLC to afford **1** (30 mg, 1.2×10^{-3}%), **2** (12 g, 0.48%), **3** (125 mg, 5×10^{-3}%), **4** (150 mg, 6×10^{-3}%), **5** (30 mg, 1.2×10^{-3}%) and **6** (200 mg, 10^{-2}%).

6α,9-Dihydroxy-14β-carboxyfuranoeremophil-9(10)-ene (**3**) Platelets, mp 246–248°; $[\alpha]_D$ +140.1° (c = 0.23, Me_2CO); IR ν_{max}^{KBr} cm^{-1}: 3235, 1721, 1665, 1442, 1382, 1356, 1301, 1198, 1158, 1014; UV λ_{max}^{MeOH} nm: 220.5; ^1H NMR (DMSO-d_6, ppm): δ 1.12 (3H, *s*, H-15), 1.86 (3H, *s*, H-13), 1.20–2.50 (7H, *m*, H-1, 2, 3, 4); 5.70 (1H, *s*, H-6β), 7.12 (1H, *s*, H-12), 12.39 (1H, *s*, HOOC-14); ^{13}C NMR and DEPT: Table 1; HREIMS: 278.1151 $[M]^+$, calcd for $C_{15}H_{18}O_5$, 278.1154; EIMS *m/z* (rel. int.): 278(12), 261(15), 232(12), 214(14), 83(100), 55(56), 41(27).

7α,8α-Epoxy-eremophilan-12β,8β(14β,6α)-diolide (**4**). Needles mp 188–190°; $[\alpha]_D$ −23.1° (c = 0.45, Me_2CO); IR ν_{max}^{KBr} cm^{-1}: 1801, 1768, 1483, 1449, 1372, 1275, 1089, 1014, 988; UV λ_{max}^{MeOH} nm: 201.5, 213.0; ^1H NMR ($DCCl_3$,): δ 1.19 (3H, *s*, H-15), 1.40 (3H, *d*, J = 7.2 Hz, H-13), 1.40 (1H, *m*, H-1β), 1.45 (1H, *m*, H-2β), 1.46 (1H, *m*, H-3α), 1.76 (1H, *m*, H-2α), δ 1.83 (1H, *m*, H-1α), 1.90 (1H, *m*, H-3β), 1.96 (1H, *m*, H-10β), 2.20 (1H, *dd*, J = 3.2 and 11.8 Hz, H-4α), 2.29 (1H, *br d*, J = 4.3 Hz, H-9β), 2.40 (1H, *br d*, J = 6.6 Hz, H-9α), 3.02 (1H, *q*, J = 7.2 Hz, H-11α), 4.33 (1H, *s*, H-6β); ^{13}C NMR and DEPT: Table 1; HREIMS: 278.1156 $[M]^+$, calcd for $C_{15}H_{18}O_5$, 278.1154; EIMS *m/z* (rel. int.): 278(4), 250(4), 249(6), 207(23), 177(7), 135(100), 109(22), 95(43), 79(21), 67(12).

Eremophil-7(11),8(9)-dien-12,8(14β,6α)-diolide (**5**). Platelets, decompd 200°; $[\alpha]_D$ +39.0° (c = 0.50, Me_2CO); IR ν_{max}^{KBr} cm^{-1}: 3446, 1792, 1743, 1660, 1445, 1394, 1370, 1061, 1035, 962; UV λ_{max}^{MeOH} nm: 210.0, 278.0; ^1H NMR (DMSO-d_6, ppm): δ 1.13 (3H, *s*, H-15), 1.93 (3H, *s*, H-13), 2.62 (1H, *dd*, J = 3.0 and 10.5 Hz, H-4α), 5.33 (1H, *s*, HO-10), 5.36 (1H, *s*, H-6β), 5.72 (1H, *s*, H-9); ^{13}C NMR and DEPT: Table 1; HREIMS: 276.0984 $[M]^+$, calcd for $C_{15}H_{18}O_5$, 276.0997; EIMS *m/z* (rel. int.): 276(4), 258(5), 219(13), 177(100), 100(25), 77(12).

Eremophil-7(8)-en-12,8(14β,6α)-diolide (**6**). Platelets, mp 164–165°; $[\alpha]_D$ −94.5° (c = 0.33, Me_2CO); IR ν_{max}^{KBr} cm^{-1}: 1786, 1760, 1693, 1468, 1446, 1385, 1091, 1006, 932; UV λ_{max}^{MeOH} nm: 201.5, 224.0; ^1H NMR (Me_2CO-d_6, ppm): δ 1.20 (3H, *s*, H-15), 1.30 (3H, *d*, J = 7.2 Hz, H-13), 2.60–1.20 (10H, *m*, H-1, 2, 3, 4, 9, 10), 3.45 (1H, *m*, H-12), 4.85 (1H, *d*, J = 1.2 Hz, H-6β); ^{13}C NMR and DEPT: Table 1; HREIMS: 262.1208 $[M]^+$, calcd for $C_{15}H_{18}O_4$ 262.1160; EIMS *m/z* (rel. int.): 262(16), 234(7), 218(22), 190(30), 175(13), 135(21), 109(22), 95(35), 81(33), 69(54), 55(68), 41(100).

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Table 1. The ^{13}C NMR data of compounds **2–6** (δ ppm)*

Carbon	2†	3‡	4†	5‡	6§
1	20.67, CH_2	36.26, CH_2	24.05, CH_2	22.80, CH_2	22.76, CH_2
2	18.93, CH_2	24.81, CH_2	19.83, CH_2	18.38, CH_2	21.00, CH_2
3	23.33, CH_2	18.36, CH_2	18.90, CH_2	34.89, CH_2	19.75, CH_2
4	41.43, CH	40.60, CH	39.54, CH	45.02, CH	40.48, CH
5	41.58, C	40.81, C	39.77, C	46.50, C	41.58, C
6	81.74, CH	70.71, CH	77.65, CH	77.20, CH	82.60, CH
7	120.10, C	127.09, C	61.51, C	141.94, C	112.58, C
8	150.75, C	151.65, C	87.11, C	148.04, C	152.35, C
9	25.41, CH_2	165.81, C	22.12, CH_2	111.05, CH	25.43, CH_2
10	37.11, CH	127.56, C	32.68, CH	71.60, C	36.70, CH
11	114.69, C	104.28, C	38.46, CH	126.69, C	41.64, CH
12	138.58, CH	138.80, CH	174.88, C	169.51, C	175.87, C
13	8.46, CH_3	8.79, CH_3	10.22, CH_3	9.23, CH_3	15.74, CH_3
14	176.63, C	174.73, C	174.94, C	174.78, C	178.92, C
15	20.22, CH_3	20.36, CH_3	20.32, CH_3	14.33, CH_3	19.55, CH_3

* The assignment of a few signals in the Table may be interchangeable.
† Recorded in $DCCl_3$; ‡, in DMSO-d_6; §, in Me_2CO-d_6.

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