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Yun-Sen Li [a b], Zheng-Tao Wang [c], Shi-De Luo [d], Shao-Shun Li [b] & Da-Yuan Zhu [a]

[a] State Key Laboratory of Drug Research, Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, P.R. China

[b] Department of Natural Medicinal Chemistry, School of Pharmacy, Shanghai Jiaotong University, Shanghai 201203, P.R. China

[c] Department of Pharmacognosy, China Pharmaceutical University, Nangjing 210038, P.R. of China

[d] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P.R. of China

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Sensitized photooxidation of furanoeremophilane with singlet oxygen and their biogenetic relationship

YUN-SEN LI*†‡, ZHENG-TAO WANG§, SHI-DE LUO¶, SHAO-SHUN LI‡ and DA-YUAN ZHU†

†State Key Laboratory of Drug Research, Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, P.R. China
‡Department of Natural Medicinal Chemistry, School of Pharmacy, Shanghai Jiaotong University, Shanghai 201203, P.R. China
§Department of Pharmacognosy, China Pharmaceutical University, Nangjing 210038, P.R. of China
¶Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P.R. of China

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In an attempt to explore the biogenetic relationship of furanoeremophilane derivatives and eremophilan-8α,12-olides, produced in *Ligularia* and their structure-activity relationship, we studied the photosensitized oxidation of furanoeremophilane–type sesquiterpenes. Under the condition of several solvents solution Irradiation with a 200 W incandescent lamp of furanoeremophilan-14β,6α-olide isolated from *Ligularia vellerea*, in various solutions with methylene blue, rose bengal, toluidine blue and safranine T gave several products. The products were isolated by chromatographic procedure and their structures were elucidated as eremophilan-14β,6α,8α,12-diolide derivatives by NMR, IR and MS methods. A reaction mechanism has been proposed.

Keywords: Furanoeremophilane; Photosensitized oxidation; Biogenetic relationship; Structure–activity relationship

1. Introduction

Ligularia plants (Family Asteraceae) have been used as traditional Chinese herbs for dispelling phlegm and relieving cough [1,2]. Some interesting eremophilolides were isolated from *Ligularia* plants, which show various biological activities [2]. The rhizomes and roots of *Ligularia vellerea* (Franch.) Hand.-Mazz., distributed in the southwest of China, was used for cough and inflammation [1], and also for hepatitis in local areas. In order to find the hepatoprotective compounds, we previously investigated the chemical constituents of the roots and rhizomes of this plant and tested

*Corresponding author. Email: Sinodlsia@yahoo.com

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hepatoprotective activity of furanoeremophilan-14β,6α-olide (**a**), a main constituent of this herb [3]. Photooxidation of furans has been studied extensively [4]. Oxidative ring opening of furans leading to α,β-unsaturated γ-lactones has become a practical synthetic operation. We begin from furanoeremophilan-14β,6α-olide to obtain some eremophilolides to study the relationship of their hepatoprotective activities with their structures and deduce the biosynthesis pathway of the eremophilanolides in *Ligularia*.

2. Results and discussion

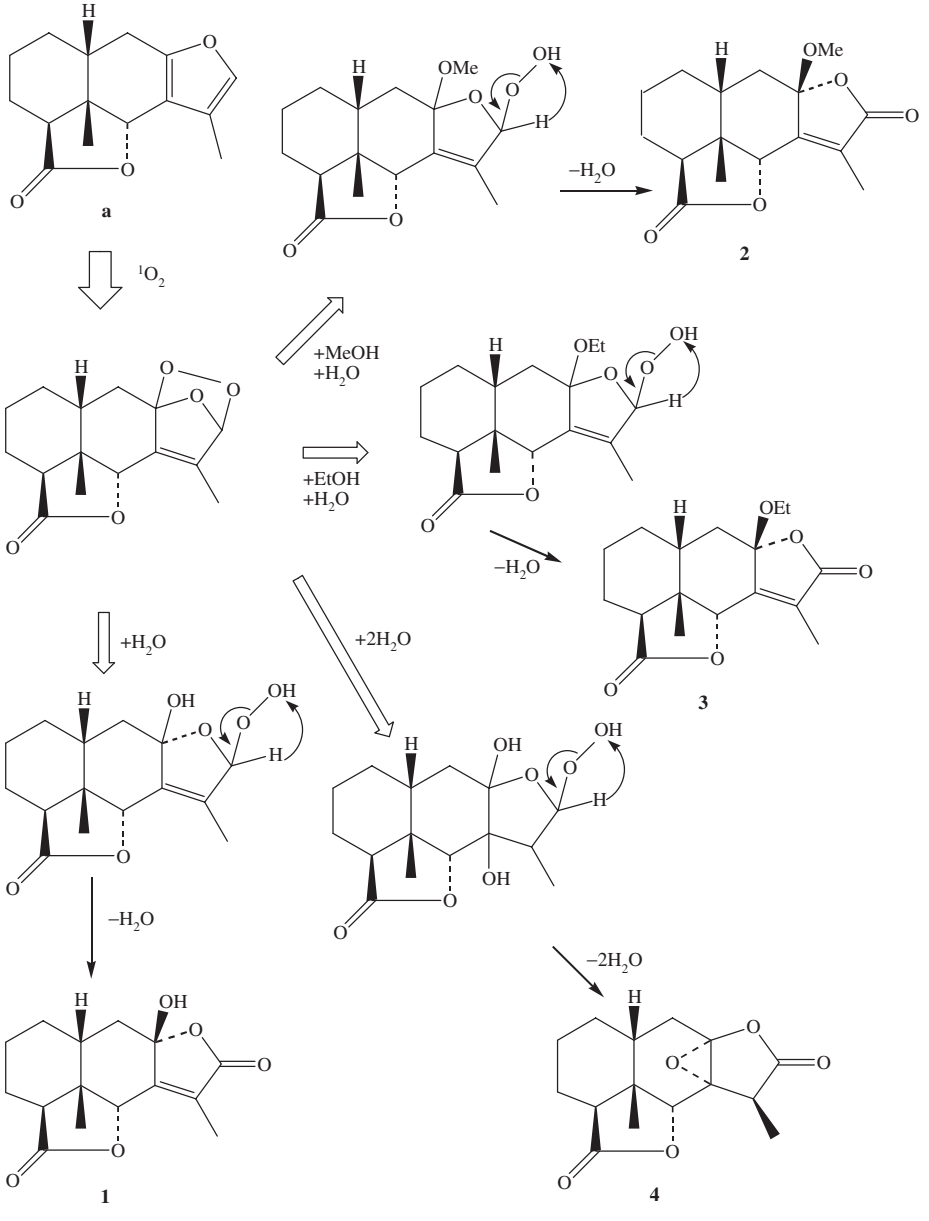
The oxidation of furans with singlet oxygen has widespread application in organic synthesis. The oxidation takes place through Diels–Alder type of cycloaddition to form an initial 1,4-endoperoxide. Many different types of products are formed depending on the reaction conditions. Thus, the intermediate peroxide may undergo solvolysis or rearrangement to yield 1,4-endiones, epoxy-1,4-diones, vinylic esters, bisepoxides or cyclic ketals [5]. In particular, the formation of enediones in alcoholic solvents appears to occur through the solvolysis of initially formed endoperoxides to yield alkoxy hydroperoxides that may be hydrolyzed to the corresponding enediones [6].

The compound furanoeremophilan-14β,6α-olide was oxidized with singlet oxygen in alcohol solvents (methanol or ethanol) and nonpolar solvents (acetone or acetonitrile) to provide four products (**1–4**, Scheme 1). The reaction mechanism and structure elucidation of these products are presented in Scheme 1.

Compounds **1** and **2** were obtained as colorless needles, with identical ^{1}H NMR, ^{13}C NMR, IR and MS data to those of the two known eremophilanodiolides, 8β-hydroxyeremophil-7(11)-en-12,8α(14β,6α)-diolide and 8β-methoxyeremophil-7(11)-en-12,8α(14β, 6α)-diolide, respectively, previously isolated by Zhao [7] from *Ligularia przewalskii*.

Compound **3** was the empirical formula of $C_{17}H_{22}O_5$ by EIMS ($[M]^+$ m/z 306) and elemental analysis (observed: C, 66.75%; H, 7.33%. Calcd.: C, 66.66%; H, 7.24%). The IR spectrum indicated two lactone groups (1788, 1761, 1078 cm^{-1}) and a double bond (1447 cm^{-1}). The ^{1}H NMR and ^{13}C NMR (DEPT) spectra (tables 1 and 2), as well as the IR spectrum, were very similar to those of 8β-hydroeremophil-7(11)-en-12,8α(14β,6α)-dioxide (**3a**) [5,6], except for the difference that was caused by an 8β-ethoxy group in **3**. The presence of the ethoxy group at C-8 was also supported by the C-8 signal (δ105.4, s), downfield 27.9 ppm from C-8 (δ 77.5, d) in **3a**. Furthermore, a carefully studied two-dimensional NMR spectra of **3** (COSY, HMQC, and HMBC) allowed the unequivocal assignments of all proton and carbon signals. **3** was identified as 8β-ethoxyeremophil-7(11)-en-12,8α(14β,6α)-diolide. Compound **4** was assigned the molecular formula $C_{15}H_{18}O_5$ by EIMS analysis (m/z 278), ^{13}C NMR and DEPT. Its ^{1}H NMR, ^{13}C NMR, IR and MS data were in accord with those of 7α,8α-epoxy-eremophilan-12β,8β(14β, 6α)-diolide previously isolated by Chen *et al*. from *Ligularia intermedia* [8].

It is frequently seen that plants grown in high altitudes suffer damage induced by free radicals. Antioxidants are produced in plants as a result of stress. High quantity of **a** in *L. vellerea* and its potential protective ability against the free radical intoxication showed that this compound was a plant antioxidant. It was concluded that compound



Scheme 1. Oxidation of furanoeremophilan-14β,6α-olide with singlet oxygen.

a was oxidized by free radicals to create eremophilan-8,12-olides, which was validated by a photosensitized autooxidation of furanoeremophilan-14β,6α-olide (**a**).

A screen of hepatoprotective activity of compounds (**1–4** and **a**) with liver cells of mice and MTT bioassay as experimental methods was carried out. Compound **a** showed strong bioactivity on liver cells of mice. Compounds **1–4** showed no obvious bioactivity on liver cells of mice. Bioactivity to liver cells of mice of eremophilane derivatives was correlated with 7,8-furan ring on their structures.

Table 1. ^{1}H NMR data of compounds **1–4** (solvents: $CdCl_3$)[a].

Proton	Compound **1**	Compound **2**	Compound **3**	Compound **4**
1	1.89m	1.90m	1.89m	1.84m
1′	1.76m	1.76m	1.76m	1.41m
2	1.43m	1.44m	1.43m	1.78m
2′	1.38m	1.35m	1.34m	1.45m
3	1.42m	1.44m	1.43m	1.47m
3′	1.84m	1.84m	1.83m	1.90m
4α	2.27dd (12.2, 3.2)	2.25dd (12.0, 3.2)	2.25dd (12.1, 3.1)	2.23dd (11.8, 3.2)
6	5.00q (2.0)	4.90q (2.0)	4.91q (2.0)	4.33s
8β	–	–	–	–
9	1.77dd (13.2, 13.2)	1.77dd (13.4, 13.4)	1.76dd (13.0, 13.0)	2.41 br.d (6.6)
9′	2.28dd (13.2, 5.0)	2.28dd (13.4, 5.1)	2.28dd (13.0, 5.0)	2.29 br.d (4.3)
10β	2.18m	2.18m	2.20m	1.97m
11	–	–	–	3.02q (7.2)
13	2.03d (2.0)	2.01d (2.0)	2.00d (2.0)	1.41d (7.2)
14	1.30s	1.26s	1.28s	1.19s
OCH_3	–	3.22s	–	–
OCH_2CH_3			1.20t 3.51q 3.32q (6.9)	

[a] Chemical shifts (ppm), multiplicity, and coupling constant (Hz in parentheses).

Table 2. ^{13}C NMR data (DEPT) of compounds **1–4**.

Carbon no.	1 DEPT	2 DEPT	3 DEPT	4 DEPT
1	19.8CH_2	19.2CH_2	19.1CH_2	24.0CH_2
2	21.0CH_2	20.8CH_2	20.8CH_2	19.7CH_2
3	24.5CH_2	24.1CH_2	24.1CH_2	18.8CH_2
4	40.5CH	40.8CH	40.7CH	39.4CH
5	45.0C	44.6C	44.6C	39.7C
6	83.1CH	82.5CH	82.6CH	77.6CH
7	154.4C	151.1C	151.5C	61.5C
8	104.3C	105.6C	105.4C	87.1C
9	37.0CH_2	35.2CH_2	35.5CH_2	22.0CH_2
10	36.0CH	34.5CH	34.6CH	32.6CH
11	126.4C	129.2C	128.8C	38.4C
12	171.6C	170.3C	170.3C	174.9C
13	9.0CH_3	9.0CH_3	8.9CH_3	10.1CH_3
14	19.8CH_3	20.0CH_3	20.1CH_3	20.2CH_3
15	175.7C	174.9C	174.8C	174.9C
OCH_3		50.5		
OCH_2CH_3			58.2CH_2 15.0CH_3	

The photosensitized autooxidation of **a** gave four products, 8β-hydroxyeremophil-7(11)-en-12,8α(14β,6α)-diolide (**1**), 8β-methoxyeremophil-7(11)-en-12,8α (14β,6α)-diolide (**2**), 8β-ethoxyeremophil-7(11)-en-12,8α(14β,6α)-diolide (**3**) and 7α,8α-epoxy-eremophilan-12β,8β(14β,6α)-diolide (**4**), identical with compounds isolated from *L. vellerea* respectively. The result revealed that **a** might be one precursor of **1–4**. A proposed biosynthesis pathway of **1–4** was given as follows: the furan ring of **1** was oxidized with singlet oxygen catalyzed by enzymes produced in the plant itself,

to form an initial 8,12-endoperoxide, then the intermediate peroxide ring opened and passed through a Diels–Alder cycloaddition to create the α,β-unsaturated-lactone ring of eremophilan-8,12-olides.

3. Experimental

3.1. *General*

Column chromatography (CC): silica gel, 200–300 mesh. TLC: Precoated silica GF_{254} plates, detection at 254 nm, and by ceric sulfate reagent. Optical rotations: JASCO DEP-370 polarimeter. All melting points were obtained on a Koffler apparatus and are uncorrected. The IR spectra were obtained on a Bio-Rad FTS-135 spectrometer with KBr pellets. The NMR spectra were recorded on a Bruker AM-400 or DRX-500 instrument with TMS as an internal standard and $CdCl_3$ as a solvent. ^{1}H NMR, ^{1}H-^{1}H COSY spectra were measured at 400.13 or 500.13 MHz; ^{13}C NMR and DEPT spectra were recorded at 100.6 MHz; HMBC spectrum was obtained at 500.13 MHz/125.8 MHz. ^{13}C NMR assignments were determined by ^{13}C-^{1}H COSY and HMQC spectra. The EIMS were carried out on a VG Auto Spec-3000 spectrometer at 70 eV.

3.2. *Extraction and isolation*

Dried and powdered roots and rhizomes of *L. vellerea* (10.0 kg) were extracted with EtOH (25 L × 3) at room temperature. After removal of the solvent in *vacuo*, an extract of 580.0 g was afforded. The extract was suspended in H_2O and partitioned with EtOAc. 100.0 g of the evaporated EtOAc part (total 196.0 g) were subjected to repeated chromatography on a silica gel column (200–300 mesh, 1.0 kg), the column was eluted with $CHCl_3$ and a gradient of Acetone in $CHCl_3$. Six crude fractions were obtained after combining the eluates by TLC monitoring. The first fraction (12 g) was chromatographed on a silica gel column (200–300 mesh, 120 g), eluted with petroleum ether/EtOAc (15 : 1, elution volume: 100 mL) and further purified by crystallizing in EtOAc to afford colourless needles (5.0 g). The ^{1}H NMR, ^{13}C NMR, IR and MS data were in accordance with those of furanoeremophilan-14β,6α-olide previously isolated by Chen *et al*. from *L. intermedia* [7].

3.3. *Typical synthesis procedure*

Methylene blue (100 mg) was added to a solution of furanoeremophilan-14β,6α-olide (50 mg) in methanol (200 mL), and the resulting solution was irradiated with a 200 W incandescent lamp under an oxygen atmosphere at 25°C for 4 h. The residue obtained after removal of the methanol under reduced presure, was chromatographed on a column of silica gel (50 g). Elution with petroleum ether–EtOAc (10 : 1) gave two principal fractions: the less polar fraction (19 mg) and the more polar fraction (25 mg). From the more polar fraction, a crystalline product (15 mg) was obtained after recrystallization from petro-ether, which was found to be identical with 8β-methoxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (m.p., TLC, IR, MS and NMR spectra). The less polar fraction was repeatedly chromatographed on a column of silica gel (60 g).

Elution with petro-ether (20 : 1, 15 mL) gave 7α,8α-epoxy-eremophilan-12β,8β(14β,6α)-diolide.

The same procedure was carried out for the other nine photooxidative reactions.

Fifty milligrams furanoeremophilan-14β,6α-olide in 400 mL Me_2CO containing 100 mg menthylene blue for 24 h, 7α,8α-epoxy-eremophilan-12β,8β(14β,6α)-diolide (5 mg) and 8β-hydroxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (10 mg) were obtained.

Fifty milligrams furanoeremophilan-14β,6α-olide in 400 mL acetonitrile containing 100 mg methylene blue for 4 h, 7α,8α-epoxy-eremophilan-12β,8β(14β,6α)-diolide (14 mg) and 8β-hydroxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (9 mg) were obtained.

Fifty milligrams furanoeremophilan-14β,6α-olide in 200 mL EtOH containing 100 mg methylene blue for 4h, 8β-hydroxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (18 mg) and 8β-ethoxyeremophil-7(11)-en-12,8α(14β,6α)-diolide (12 mg) were obtained.

Forty milligrams furanoeremophilan-14β,6α-olide in 150 mL methanol containing 50 mg Rose Bengal for 8 h, 8β-methoxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (28 mg) was obtained.

Fifty milligrams furanoeremophilan-14β,6α-olide in 200 mL methanol containing 50 mg Toluidine blue for 8 h, 8β-methoxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (32 mg) was obtained.

Fifty milligrams furanoeremophilan-14β,6α-olide in 200 mL Me_2CO containing 50 mg Toluidine blue for 4 h, 7α,8α-epoxy-eremophilan-12β,8β(14β,6α)-diolide (35 mg) was obtained.

Fifty milligrams furanoeremophilan-14β,6α-olide in 200 mL EtOH containing 50 mg Toluidine blue for 6 h, 8β-hydroxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (15 mg) and 8β-ethoxyeremophil-7(11)-en-12,8α(14β,6α)-diolide (10 mg) were obtained.

Fifty milligrams furanoeremophilan-14β,6α-olide in 200 mL acetonitrile containing 50 mg Toluidine blue for 12 h, 8β-hydroxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (25 mg) was obtained.

Fifty milligrams furanoeremophilan-14β,6α-olide in 200 mL methanol containing 50 mg Safranine T for 12 h, 8β-methoxyeremophil-7(11)-ene-12,8α,14β,6α-diolide (23 mg) was obtained.

3.3.1. Compound 1. Colourless needles. EIMS: *m*/*z* 278 (M^+, 70), 263 (4), 250 (4), 232 (17), 217 (6), 205 (33), 191 (13), 177 (24), 169 (17), 159 (26), 149 (12), 142 (17), 135 (30), 124 (29), 109 (76), 95 (59), 79 (50), 67 (100), 55 (65); ^{1}H NMR data (table 1); ^{13}C NMR data (table 2).

3.3.2. Compound 2. White needles. m.p. 182–183°C; $[\alpha]_D^{25}$: +43.0° ($CHCl_3$, c 1.0); IR (KBr): 1789, 1760, 1700, 1444, 1305, 1190, 989 cm^{-1}; EIMS: *m*/*z* 292 (M^+, 69), 277 (7), 261 (22), 232 (24), 218 (12), 205 (66), 191 (32), 177 (14), 159 (46), 147 (16), 140 (37), 119 (18), 109 (69), 95 (58), 79 (47), 67 (100), 55 (47); ^{1}H NMR data (table 1); ^{13}C NMR data (table 2).

3.3.3. Compound 3. White needles. TLC: R_f 0.49 (petroleum ether–EtOAc, 5 : 1); $[\alpha]_D^{25}$: +69.0° ($CHCl_3$, c 0.50); IR (KBr): 2950, 2885, 1796, 1742, 1686, 1448, 1364, 1321, 1292,

1251, 1217, 1182, 1147, 1098, 1049, 973 cm^{-1}; EIMS: *m/z* 306(M^+, 25), 291 (M^+-CH_3, 2), 278 (3), 261 (10), 232 (5), 217 (4), 205 (15), 197 (2), 187 (5), 175 (5), 159 (15), 145 (8), 133 (10), 119 (10), 109 (19), 95 (53), 79 (45), 67 (100), 55 (56). ^{1}H NMR data (table 1); ^{13}C NMR data (table 2).

3.3.4. Compound 4. Colourless needles. m.p. 189–191°C; $[\alpha]_D^{25}$: −18.6° (Me_2CO, c 0.59); IR (KBr): 2985, 2946, 2874, 1804, 1779, 1490, 1451, 1373, 1332, 1305, 1277, 1173, 1130, 1089, 1017, 987, 964, 949 cm^{-1}; EIMS: *m/z* 278 (M^+, 45), 250 (25), 222 (51), 207 (62), 177 (14), 135 (82), 122 (34), 107 (31), 95 (81), 79 (69), 67 (53); ^{1}H NMR data (table 1); ^{13}C NMR data (table 2).

3.4. *Biological assay*

3.4.1. Screening of hepatoprotective activity of compounds (1–4 and a). The hepatoprotective bioassay was accomplished according to the procedures outlined earlier. (methylthiazoltetorzolium, MTT bioassay) Compound **a** showed strong hepatoprotective activity on liver cells of mice at 10 μM/mL. Compounds **1–4** had no obvious hepatoprotective activity on liver cells of mice.

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