New Eremophilenolides from *Ligulariopsis shichuana*

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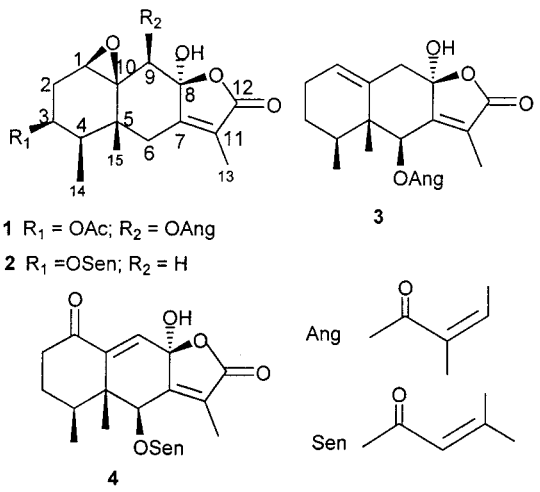
Four new eremophilenolides, **1**−**4**, have been isolated from the whole plant of *Ligulariopsis shichuana*. Their structures were elucidated by spectroscopic methods, including 2D NMR experiments. Structures of compounds **1** and **2** were unequivocally established by X-ray diffraction experiments. All of these sesquiterpenes have eremophilane skeletons with 7(11)-en-8(12) lactone units. Compounds **1** and **2** showed moderate antibacterial activities against *E. coli* and *B. subtilis*.

Ligulariopsis shichuana Y. L. Chen is the only species of *Ligulariopsis*, which is a new genus of Compositae (Asteraceae). Because *Ligulariopsis* has the apparent characteristics of both *Ligularia* and *Cacalia*,[1] which both belong to Compositae, it had been incorrectly assigned to *Cacalia longispsis*.[2] Many species of *Ligularia* in China have been studied by our research group, and a number of eremophilenolides have been isolated from them.[3,4] Many eremophilenolides derived from *Cacalia* have also been reported by Chinese researchers.[5] The eremophilenolides reported here provide phytochemical proof that *Ligulariopsis shichuana* is indeed closely related to *Ligularia* and *Cacalia*. In China, many species of *Ligularia* and *Cacalia* have long been used as traditional medicine[6] for antibacterial and antiinflammation purposes. We have screened *Ligulariopsis shichuana* by using antibacterial tests in order to find more medicinal proofs about close relationships of *Ligulariopsis*, *Ligularia*, and *Cacalia*.

Results and Discussion

The molecular formula of compound **1** was established as $C_{22}H_{28}O_8$ by HRESIMS (*m/z* 443.1671 $[M + Na]^+$) and ^{13}C NMR data (Table 2), thus indicating eight degrees of unsaturation in the molecule. In its ^1H NMR, there are signals for angeloyloxy and acetoxy groups (Table 1). Indeed, both *m/z* 320 $[M - AngOH]^+$ and *m/z* 360 $[M - AcOH]^+$ appeared in the EIMS spectrum. In addition to five carbons of the angloyloxy moiety and two acetoxy carbons, there are 15 carbon signals in the ^{13}C NMR spectrum, indicating a sesquiterpene skeleton (Table 2). The IR spectrum shows absorption at 1762 cm^{-1} for an α,β-unsaturated γ-lactone and at 3365 cm^{-1} for a hydroxy group. These data, taken together with three characteristic ^1H NMR methyl signals at δ 1.87 (d, J = 1.2 Hz, H-13), 0.98 (d, J = 5.4 Hz, H-14), and 1.17 (s, H-15) and a ^{13}C NMR signal for a quaternary carbon at δ_C 101.6, suggested an eremophil-7(11)en-8(12)-olide[3,4] structure for compound **1**, accounting for seven degrees of unsaturation. The remaining unsaturation could be attached to an epoxy, on the basis of signals in the ^{13}C NMR at 60.3 (CH) and δ_C 61.5 (C). Furthermore, the ^1H NMR spectrum supported a 1β,10β-epoxy (δ 3.37, d, J = 5.8 Hz, H-1α).[3] Compared with the data of eremophilenolides in the literature,[3,5] signals at δ 5.17(ddd, J = 4.4, 6.4, 10.8 Hz, H-3α) and 5.41(s, H-9) suggested that C-3 and C-9 are oxygenated with the angeloyloxy and acetoxy ester groups. Fortunately, a single crystal of **1** was obtained and its structure and relative stereochemistry were unequivocally established by X-ray diffraction analysis, as shown in Figure 1. Thus, compound **1** was identified as 3β-acetoxy-9β-angeloyloxy-1β,10β-epoxy-8α-hydroxyeremophil-7(11)-en-8β(12)-olide.



EIMS of compound **2** gave its molecular ion peak at *m/z* 362 $[M]^+$ and fragment ion peaks at *m/z* 262 $[M - SenOH]^+$and 244 $[M - SenOH - H_2O]^+$. Thus, its molecular formula was established as $C_{20}H_{26}O_6$ and was consistent with its ^{13}C NMR and DEPT data (Table 2). On the basis of comparison with the spectral data of **1**, compound **2** possesses the same skeleton and functionalities as **1**, except for the absence of an oxygenated functional group at C-9 and substitution of an acetoxy group by a senecioyloxy at C-3, which was supported by the ^1H NMR signals at δ 5.21 (ddd, J = 4.2, 6.8, 11.0 Hz, H-3). X-ray crystallography (Figure 2) provided unequivocal evidence of the structure and relative stereochemistry of **2** as 3β-senecioyloxy-1β,10β-epoxy-8α-hydroxyeremophil-7(11)-en-8β(12)-olide.

The molecular formula of compound **3** was determined by HRESIMS, *m/z* 369.1675 $[M + Na]^+$ as $C_{20}H_{26}O_5$. Its spectral data were very similar to those of **1** and **2** (Tables 1 and 2) and in agreement with a 8α-hydroxyeremophil-1(10),7(11)-dien-8β-olide structure.[7] The $^1H-^1H$ COSY spectrum confirmed a double bond between C-1 and C-10. An oxygenated proton δ 5.64 (q, J = 1.2 Hz) could be assigned to H-6. In the biogenetic consideration of eremophilane derivatives isolated from *Compositae* species,[8] methyls at C-4 and C-5 were both assigned the β-configuration. The configuration of 8(12) lactones was determined

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Table 1. ^{1}H NMR Data (δ value) of Compounds **1** and **2**[a] and **3** and **4**[b]

H	1	2	3	4
1	3.37(d, 5.8)	3.17(d, 5.4)	5.82(brt, 3.6)	
2α	2.16(dd, 12.0, 6.4)	2.20(dd, 11.8, 6.8)	2.11(dt, 11.0, 3.6)	2.32(dd, 14.6, 4.8)
2β	2.27(ddd, 12.0, 10.8, 5.8)	2.24(ddd, 11.8, 11.0, 5.4)	2.11(dt, 11.0, 3.6)	3.16(dd, 14.6, 8.0)
3α	5.17(ddd, 10.8, 6.4, 4.4)	5.21(ddd, 11.0, 6.8, 4.2)	1.57(m)	1.80(m)
3β			1.42(m)	1.65(m)
4α	1.75(qd, 5.4, 4.4)	1.77(qd, 5.6, 4.2)	1.85(qt, 6.8, 3.2)	2.87(qt, 6.8, 4.0)
6α	2.99(brd, 1.2, 14.0)	3.00(brd, 1.2, 14.2)	5.64(q, 1.2)	5.75(q, 1.2)
6β	2.40(d, 14.0)	2.40 (d, 14.2)		
9α	5.41(s)	2.31(d, 14.0)	2.78(d, 12.2)	6.21(s)
9β		1.75(d, 14.0)	2.51(brd, 12.2, 2.4)	
13	1.87(d, 1.2)	1.84(d, 1.2)	1.82(d, 1.2)	1.82(d, 1.2)
14	0.98(d, 5.4)	0.99(d, 5.6)	1.08(d, 6.8)	0.88(d, 6.8)
15	1.17(s)	1.05(s)	1.02(s)	0.80(s)
	OAng	OSen	OAng	OSen
	6.09(qq, 7.2, 1.5)	5.67(qq, 1.3)	6.29(qq, 7.6, 1.2)	6.58(qq, 1.2)
	1.95(dq, 7.2, 1.5)	2.16(d, 1.3)	2.08(dq, 7.6, 1.2)	2.25(d, 1.2)
	1.74(dq, 1.5, 1.5)	1.91(d, 1.3)	2.01(dq, 1.2, 1.2)	1.98(d, 1.2)
	OAc			
	2.07(s)			

[a] Obtained on a 200 MHz spectra recorder. [b] Obtained on a 400 MHz spectra recorder, chemical shifts in ppm from internal TMS, coupling constants in Hz.

Table 2. ^{13}C NMR Data of Compounds **1**−**4**[a]

C	1	2	3	4
1	60.3d	60.6d	130.2d	207.4s
2	24.4t	27.0t	24.3t	32.9t
3	68.8d	67. 5d	28.1t	27.8t
4	40.8d	42.6d	44.8d	36.1d
5	39.3s	40.3s	45.1s	50.9s
6	34.7t	34.9t	70.1d	70.5d
7	154.7s	157.4s	155.6s	153.5s
8	101.6s	102.5s	102.3s	103.9s
9	77.0d	39.3t	36.7t	118.2d
10	61.5s	61.5s	142.4s	159.1s
11	126.4s	124.9s	124.0s	125.3s
12	171.3s	172.1s	172.3s	172.8s
13	8.2q	8.2q	7.9q	8.7q
14	9.5q	9.6q	14.2q	9.4q
15	22.5q	25.2q	17.6q	16.9q
	OAng	OSen	OAng	OSen
	165.7s	166.3s	165.6s	166.8s
	126.6s	115.9d	126.4s	114.9d
	140.7d	157.8s	144.3d	160.9s
	20.3q	20.3q	20.5q	20.6q
	15.8q	20.3q	16.0q	20.6q
	OAc			
	171.8s			
	21.3q			

[a] Obtained on a 50 MHz spectra recorder.

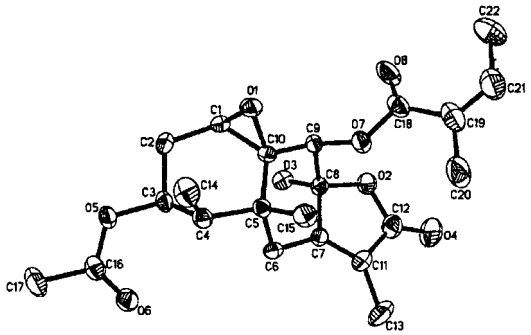

Figure 1. ORTEP diagram of the crystal structure of **1**.

using the rules reported by Naya.[9] For 8β-methoxyeremophil-7(11)-en-8α(12)-olide derivatives without oxygen functional groups at C-9, C-10, and C-1, the chemical shifts of the tertiary methyl group (CH_3-15) in the ^{1}H NMR are downfield from those of the secondary methyl group (CH_3-14), whereas this relationship is reversed in the 8α-methoxyeremophil-7(11)-8β(12)-olide. Therefore, the presence of signals at δ 1.02 (s, CH_3-15) and 1.08 (d, J = 6.8 Hz, CH_3-14) of **3** showed that it is an 8β(12)-olide. Naya also reported[10] the homoacyclic spin-coupling (J = 1.0−1.5 Hz) between H-6α and C-13 methyls observed in the 8β(12)-olides, where the angle between H-6α and CH_3-13 is 90°. This coupling is absent in 8α(12)-olides, where the angle between H-6α and CH_3-13 is only 20°. Thus, the coupling constant 1.2 Hz for H-6α and CH_3-13 in **3** indicated a β-angeloyloxy on C-6. From the above data, compound **3** was identified as 6β-angeloyloxy-8α-hydroxyeremophil-1(10),7(11)-dien-8β(12)-olide.

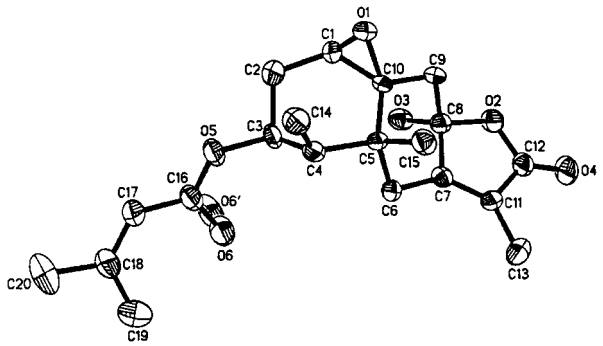

Figure 2. ORTEP diagram of the crystal structure of **2**.

The HRESIMS of compound **4** gave m/z 361.1651 [M + H]$^+$, establishing its molecular formula as $C_{20}H_{24}O_6$, consistent with its ^{13}C NMR and DEPT data (Table 2). The IR, ^{13}C NMR, and ^{1}H NMR (Tables 2 and 1) data all indicated this compound to be another eremophil-7(11)-en-8(12)-olide. The ^{13}C NMR data also showed a senecioyloxy group, a carbonyl, and a trisubstituted double bond on the skeleton. The ^{1}H NMR data showed signals at δ 6.21 (s, H-9) and 5.75(q, J = 1.2 Hz, H-6), allowing assignment of the double bond between C-9 and C-10 and placement of the senecioyloxy at C-6. Placement of a carbonyl group at C-1 was evident from the fragment of CH_3(14)CH(4)CH_2-(3)CH_2(2) in the ^{1}H−^{1}H COSY experiment. The upfield position of CH_3-15 (δ 0.80) and the downfield position of CH_3-14 (δ 0.88) in the ^{1}H NMR spectrum, as well as the appearance of homoacyclic coupling of 1.2 Hz between H-6α and CH_3-13 (Table 1), suggested the relative stereochemistry of a 8β(12)-olide. Thus, compound **4** was identified as 1-oxo-6β-senecioyloxy-8α-hydroxyeremophil-7(11),9(10)-dien-8β(12)-olide.

Table 3. Antibacterial Activities of **1** and **2**[a,b]

compound	*E. coli*	*B. subtilis*
1	++	++
2	++	+++
chloromycetin	+++	+++

[a] Zone diameter of growth inhibition: <10 mm (−), 10−12 mm (+), 13−15 mm (++), and 16−20 mm (+++). Diameter of the cup = 8 mm. [b] Concentrations of all compounds are at 100 μg/mL.

Compounds **1** and **2**, due to their abundance in the plant, were tested for antibacterial activity against *Escherichea coli* and *Bacillus subtilis*. The preliminary results (Table 3) indicated that compound **1** has moderate antibacterial activity against both bacteria, while compound **2** shows stronger antibacterial activity against *B. subtilis* than against *E. coli*.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer model 341 polarimeter. IR spectra were taken on a Nicolet AVATAR 360 FT-IR spectrometer. UV spectra were obtained on a UV−vis spectrophotometer Tu-1901. ^{1}H NMR (200 MHz, $CDCl_3$) and ^{13}C NMR (50 MHz, $CDCl_3$) spectra were recorded on an AD 200 FT-NMR. ^{1}H NMR (400 MHz, $CDCl_3$) and 2D NMR spectra (^{1}H−^{1}H COSY) were recorded on a Bruker AM 400 FT-NMR spectrometer with TMS as the internal standard. HRESIMS were obtained on a Bruker Daltonics APEX II 47e Specifications. EIMS data were obtained on a HP-5988 MS spectrometer. Silica gel (200−300 mesh) used for CC and silica GF_{254} for TLC were obtained from Qindao Marine Chemical Factory, Qindao, People's Republic of China. Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH. Melting points are uncorrected.

Plant Material. The whole plant of *L. shichuana* was collected in August 2000, in Mount Taibai in Qinglin Mountains, Shanxi Province, People's Republic of China, and was identified by Prof. Y. J. Zhang, Department of Biology, Lanzhou University. A voucher specimen (No.2000823) is deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The dried whole plant of *L. shichuana* (1.0 kg) was pulverized and then percolated with acetone (5.0 L three times; 5 days/time) at room temperature. The solvent was removed under reduced pressure to give a residue (36.0 g). The residue was subjected to column chromatography on silica gel (500 g) with a gradient of petroleum ether−acetone as eluent. After solvent removal, fraction A (0.5 g), fraction B (0.6 g), and fraction C (3.0 g) of petroleum ether−acetone (10:1, 8:1, and 5:1, respectively) were obtained. Fraction A was then separated by column chromatography on silica gel (7.5 g) with a gradient of petroleum ether−EtOAc (10:1, 8:1, 5:1) as eluent. The eluate of petrolum ether−EtOAc (5:1) (10 mg) was purified on preparative TLC, developed with $CHCl_3$−acetone (25:1), to give **3** (2.2 mg) and **4** (2.0 mg). Fraction B was purified by column chromatography on silica gel (10.0 g) and eluted repeatedly with petroleum ether−acetone (6:1). Crude **2** (20 mg) was further purified by preparative TLC developed with petroleum ether−acetone (3:1; ×3) to yield pure **2** (12 mg), which was crystallized from methanol. Fraction C was purified by column chromatography with petrolum ether−acetone (5:1) as eluent. Crystals of **1** deposited from the eluent were recrystallized from methanol to yield pure **1** (30 mg).

3β-Acetoxy-9β-angeloyloxy-1β,10β-epoxy-8α-hydroxyeremophil-7(11)-en-8β(12)-olide (1): white columns (MeOH); mp 212−214 °C; $[\alpha]^{25}_D$ −71° (*c* 0.41, acetone); UV ($CHCl_3$) λ_{max} (log ϵ) 241 (1.980) nm; IR (KBr) ν_{max} 3365, 1762, 1725, 1707, 1647 cm^{-1}; EIMS *m/z* 420 $[M]^+$ (1.5), 360 (1.1), 320 (1.7), 260 (100), 83 (60), 43(27); HRESIMS *m/z* 443.1671 $[M + Na]^+$ (calcd for $C_{22}H_{28}O_8Na$, 443.1676); ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2.

3β-Senecioyloxy-1β,10β-epoxy-8α-hydroxyeremophil-7(11)-en-8β(12)-olide (2): white needles (MeOH); mp 182−184 °C; $[\alpha]^{25}_D$ −123° (*c* 0.75, acetone); UV ($CHCl_3$) λ_{max} (log ϵ) 242 (1.899) nm; IR (KBr) ν_{max} 3392, 1774, 1718, 1652 cm^{-1}; EIMS *m/z* 362 $[M]^+$ (2.1), 279 (78), 262 (59), 244 (84), 83 (100); ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2.

6β-Angeloyloxy-8α-hydroxyeremophil-1(10),7(11)-dien-8β(12)-olide (3): colorless gum; $[\alpha]^{25}_D$ −107° (*c* 0.18, acetone); UV ($CHCl_3$) λ_{max} (log ϵ) 245 (2.829) nm; IR (KBr) ν_{max} 3413, 1789, 1730, 1642 cm^{-1}; EIMS *m/z* 328 $[M - C_5H_6O]^+$ (2.9), 246 (100), 228 (20), 83 (32); HRESIMS *m/z* 369.1675 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_5Na$, 369.1672); ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2.

1-Oxo-6β-senecioyloxy-8α-hydroxyeremophil-7(11),9(10)-dien-8β(12)-olide (4): colorless gum; $[\alpha]^{25}_D$ −120° (*c* 0.10, acetone); UV ($CHCl_3$) λ_{max} (log ϵ) 275 (0.750), 239 (0.505) nm; IR (KBr) ν_{max} 3407, 1796, 1730, 1643 cm^{-1}; EIMS *m/z* 260 $[M - C_5H_8O_2]^+$ (30), 232 (38), 204 (17), 83 (100); HRESIMS *m/z* 361.1651 $[M + H]^+$ (calcd for $C_{20}H_{25}O_6$, 361.1646); ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2.

X-ray Crystal Structure of Compound (1). Crystal data: $C_{22}H_{28}O_8$, formula wt 420.44; crystal dimensions 0.52 × 0.52 × 0.38 mm, orthorhombic, space group $P2_12_12_1$, a = 7.440(1) Å, b = 12.589(2) Å, c = 22.981(5) Å, V = 2152.5(5) Å^3, Z = 4, D_c = 1.297 g/cm^3, $F(000)$ = 896, Mo Kα (λ = 0.71073 Å). The reflection data were collected on a Siemens P4, using graphite-monochromated radiation. A total of 3016 reflections were collected in the range 1.77° ≤ θ ≤ 27.25°, of which 2865 unique reflections with $I > 2\sigma(I)$ were used for refinement. The final R and R_w were 0.044 and 0.111, respectively. The structure was solved by the direct method using the program SHELXS-97. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were included at calculated positions and not refined.

X-ray Crystal Structure of Compound (2). Crystal data: $C_{20}H_{26}O_6$, formula wt 360.40, crystal dimensions 0.58 × 0.20 × 0.08 mm, monoclinic, space group $P2_1$, a = 7.062(2) Å, b = 6.340(2) Å, c = 21.066(5) Å, V = 930.9(5) Å^3, Z = 2, D_c = 1.289 g/cm^3, $F(000)$ = 386, Mo Kα (λ = 0.71073 Å). The reflection data were collected on a Siemens P4, using graphite-monochromated radiation. A total of 2611 reflections were collected in the range 1.96° ≤ θ ≤ 27.50°, of which 2338 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. The final R and R_w were 0.050 and 0.105, respectively. The structure was solved as described for compound **1**.

Antibacterial Assays. Evaluation of antibacterial activity was carried out in the Department of Biology, Lanzhou University, employing the cup-plate method.[11] Chloromycetin was used as a positive control. Two strains of bacteria, *E. coli* and *B. subtilis*, were cultured in beef broth and incubated at 37 °C for 24 h. After dilution of beef broth, the two bacteria were cultured in agar medium dishes respectively, six cups (8 × 10 mm) were put onto the dishes, and each tested compound (0.2 mL of 100ug/mL) was respectively added into the cups under aseptic conditions. Then the dishes were cultured at 37 °C for 24 h. The zone of inhibition of the growth of bacteria, produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity. Each test was performed twice.

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Supporting Information Available: X-ray crystallographic data of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Chen, Y.-L. *Acta Phytotaxonomica Sinica* **1996**, *34*, 631−634.
(2) Institute of Botanic Boreali-Occidentali, Academica Sinica. *Flora Tsinlingensis*; Science Press: Beijing, 1985; Vol. 1, Chapter 5, p 298.

(3) Jia, Z.-J.; Zhao, Y.; Tan, R.-X.; Yang, L. *Phytochemistry* **1992**, *31*, 2785−2787.
(4) Wang, W.-S.; Gao, K.; Yang, L.; Jia, Z.-J. *Plant. Med.* **2000**, *66*, 189−191.
(5) Zhang, S.-M.; Zhao, G.-L.; Li, R.; Lin, G.-Q. *Phytochemistry* **1998**, *48*, 519−524.
(6) Jiangsu College of New Medicine. *A Dictionary of the Traditional Chinese Medicines*; Shanghai Science and Technology Press: Shanghai, 1977; pp 22, 2303, 2348, 1806.
(7) Wiemer, D. F.; Wolfe, L. K.; Fenical, W. *Tetrahedron Lett.* **1990**, *31*, 1973−1976.
(8) Zhao, Y.; Parsons, S.; Smart, B. A.; Tan, R.-X.; Jia, Z.-J.; Sun, H.-D.; Rankin, D. W. H. *Tetrahedron* **1997**, *53*, 6195−6208.
(9) Naya, K.; Kanazawa, R.; Sawada, M. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 3220−3225.
(10) Naya, K.; Nooi, N.; Makiyama, Y.; Takashina, H.; Imagawa, T. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3002−3006.
(11) Xu, S.-Y.; Bian, Q.-L.; Chen, X.-Y. *Pharmacology Experimental Methods*; People's Health Press: Beijing, 1982; p 1346.

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