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Two new butenolide derivatives from *Saussurea katochaete*

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Received May 2, 2005, accepted June 7, 2005

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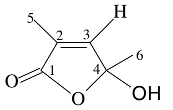
Pharmazie 62: 714–716 (2007) *doi: 10.1691/ph.2007.9.5597*

Two new butenolide derivatives, (4R,7R*/S*)-2,4-dimethyl-4-(1′-acetyl-3′-oxobutyl)-2- butenolide and its epimer, (4R,7S*/R*)-2,4-dimethyl-4-(1′-acetyl-3′-oxobutyl)-2-butenolide were isolated from the acetone extract of the whole plant of *Saussurea katochaete*. Their structures were established by various spectral methods including IR, 1D-, 2D-NMR and HR-ESIMS. Four known compounds were also isolated and identified as 2,4-dimethyl-4-hydroxy-2-butenolide, 3-hexene-2,5-dione, 3-hydroxyhexane-2,5-dione, 1-hydroxy-(*Z*)-3-hexene-2, 5-dione.

1. Introduction

The genus *Saussurea* (Compositae) consists of about 400 species distributed throughout the world, 264 species of them grown in China. Some of them have been used in traditional folk medicine (Shih et al. 1999). *Saussurea katochaete* is a perennial herbaceous plant growing in the North of China. In our phytochemical investigation of *Saussurea katochaete*, we found two new butenolide derivatives and four known compounds. These known compounds resemble a moiety of the new compounds. Butenolides occur widely in nature and possess an unusual range of biological activity (Caine et al. 1978; Larock et al. 1978). We describe herein the isolation and structural elucidation of the two new compounds.



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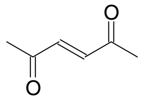

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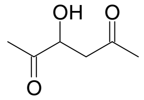

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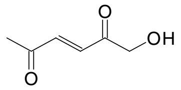

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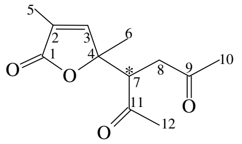

5 and 6

2. Investigations, results and discussion

The acetone extract of the whole plant of *Saussurea katochaete* was subjected to CC on silica gel and then fractioned, as described in the experimental section, to yield 2,4-dimethyl-4-hydroxy-2-butenolide (**1**) (Wendler et al. 1967), 3-hexene-2,5-dione (**2**) (Schepartz 1961), 3-hydroxyhexane-2,5-dione (**3**) (Thomas et al. 1978), 1-hydroxy-(*Z*)-3-hexene-2,5-dione (**4**) and two new butenolide derivatives, (4R,7R*/S*)-2,4-dimethyl-4-(1′-acetyl-3′-oxobutyl)-2-butenolide (**5**) and its epimer, (4R,7S*/R*)-2, 4-dimethyl-4-(1′-acetyl-3′-oxobutyl)-2-butenolide (**6**). The structures of compounds **1–3** were confirmed by comparing their corresponding spectroscopic properties (MS, IR) and melting points with the values reported in the literature. The spectral data (^{1}H- and ^{13}C NMR) of **1–4** are reported here for the first time.

Compound **5** was obtained as colorless gum, $[\alpha]^{D}_{26}$ −3.0° (C 1.0, CHCl$_3$). The HR-ESIMS gave a quasi-molecular ion peak, $[M+H]^+$ at $m/z = 225.1112$ (Calcd. 225.1121 for $C_{12}H_{17}O_4$), which corresponds to a molecular formula $C_{12}H_{16}O_4$. The results, 4 × CH$_3$, 1 × CH$_2$, 2 × CH and 5 × C from the ^{13}C NMR spectrum and DEPT experiment allowed also the establishment of the molecular formula $C_{12}H_{16}O_4$. The IR spectrum (KBr) indicated the presence of ketone groups (1712 cm^{-1}) and a α,β-unsaturated lactone (1664 and 1762 cm^{-1}). The ^{1}H- and ^{13}C NMR spectra (Table 2) of **5** displayed some characteristic signals for two acetyl groups and a α,β-unsaturated lactone. By comparing the spectral data of **5** with those of **1** (Table 1) and the data reported in the literature (Still et al. 1989), **5** appears to have a butenolide skeleton. The structure of **5** was further confirmed by 2D-NMR spectroscopic analysis. In the gHMBC spectrum, besides weak correlations of H-3 (δ 6.96) and H-5 (δ 1.90) with ester carbonyl carbon C-1 (δ 172.4), there were several stronger correlations between the proton signals CH$_3$-5 (δ 1.90) and H-3 (δ 6.96) with double bond carbon C-2 (δ 129.8); H-3 (δ 6.96) and H-6 (δ 1.42) with the quaternary carbon C-4 (δ 85.3). These correlations indicated that compound **5** has a substructure part **a** (Fig.), which was very similar to compound **1**. The ^{1}H$-$^{1}H COSY and gHMQC spectra suggesting the pre-

Table 1: ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.62 MHz) data of 1–4 ($CDCl_3$, TMS, δ ppm)

No.	1		2		3		4	
	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)
1	172.5 s		27.8 q	2.38 s	30.7 q	2.27 s	66.6 t	4.12 d (17.2) 4.52 d (17.2)
2	131.1 s		198.4 s		209.1 s		194.6 s	
3	148.2 d	6.88 s	137.7 d	6.80 s	73.7 d	4.35 dd (6.4, 3.2)	126.5 d	6.08 d (10.0)
4	104.7 s		137.7 d	6.80 s	46.1 t	2.86 dd (17.2, 6.4) 3.00 dd (17.2, 3.2)	148.6 d	6.88 d (10.0)
5	10.1 q	1.88 s	198.4 s		207.0 s		194.5 s	
6	24.4 q	1.67 s	27.8 q	2.38 s	25.3 q	2.23 s	27.9 q	1.68 s

Table 2: ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.62 MHz) data of compound 5 and 6

No.	5		No.	6	
	δ_H (J Hz)	δ_C, DEPT		δ_H (J Hz)	δ_C, DEPT
1		172.4 s	1		172.5 s
2		129.8 s	2		130.0 s
3	6.96 s	150.8 d	3	7.06 s	150.8 d
4		85.3 s	4		84.6 s
5	1.90 s	10.6 q	5	1.94 s	10.5 q
6	1.42 s	22.4 q	6	1.43 s	23.2 q
7	3.36 dd (11.2, 2.8)	54.0 d	7	3.48 brd (11.2)	53.2 d
8	3.08 dd (18.4, 11.2)	42.8 t	8	2.78 dd (18.4, 11.2)	42.7 t
8′	2.74 dd (18.4, 2.8)		8′	Overlap with H-12	
9		205.7 s	9		205.9 s
10	2.15 s	29.6 q	10	2.10 s	29.5 q
11		207.9 s	11		209.0 s
12	2.32 s	32.1 q	12	2.45 s	33.0 q

Assignments were aided by spin splitting patterns, DEPT, ^{1}H−^{1}H COSY, gHMQC, gHMBC experiments, and chemical shift values (δ). The δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.00 ppm)

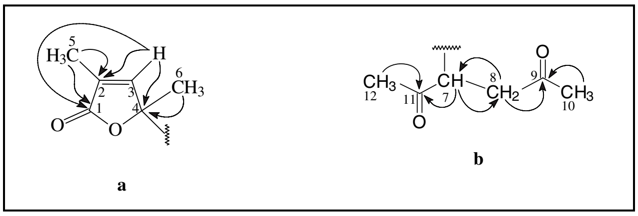

Fig.: Structure sets of compound **5** from 2D-NMR

sence of structure set, $-CHCH_2-$, together with the correlations between H-7 (δ 3.36), H-12 (δ 2.32) with the ketone carbonyl carbon C-11 (δ 207.9) and H-8 (δ 3.08), H-8′ (δ 2.75) and H-10 (δ 2.15) with another ketone carbonyl carbon C-9 (δ 205.7) in the gHMBC spectrum, indicated another substructure part **b** (Fig.), which was very similar to compounds **2** and **3**. Substructures **a** and **b** could be connected very easy by the correlation of H-7 (δ 3.36) with C-4 (δ 85.3) in the gHMBC spectrum. Thus, compound **5** was assigned to be 2,4-dimethyl-4-(1′-acetyl-3′-oxobutyl)-2-butenolide. Furthermore, **5** has two chiral carbons, C-4 and C-7. The absolute configuration of C-7 is difficult to determine, but the absolute configuration of C-4 can be deduced by the Cotton effect (CD spectrum). Since the CD curve for compound **5** has $\Delta\varepsilon_{max} = -0.05$ at 246 nm, the ring of butenolide has left-handed chirality in C=C−C=O (Beecham 1972). Through an established molecular model the absolute configuration of C-4 can be deduced as R. Consequently, compound **5** was identified as (4R,7R*/S*)-2,4-dimethyl-4-(1′-acetyl-3′-oxobutyl)-2-butenolide.

Compound **6** was isolated as colorless gum, $[\alpha]_{26}^{D}$ −5.0° (C 0.5, $CHCl_3$). Its molecular formula was confirmed as $C_{12}H_{16}O_4$ according to the EI-MS (molecular ion peak at $m/z = 224$ $[M]^+$) and ^{13}C NMR spectrum and DEPT experiment. The ^{1}H NMR, ^{13}C NMR and IR spectral data of **6** were very similar to those of **5**, except for a very small difference in the ^{1}H NMR spectrum. In the ^{1}H NMR spectrum of **5**, the chemical shift of H-7 was at δ 3.36 (dd, J = 11.2, 2.8 Hz), H-8 was at δ 3.08 (dd, J = 18.4, 11.2 Hz) and H-8′ was at δ 2.74 (dd, J = 18.4, 2.8 Hz). In the ^{1}H NMR spectrum of **6**, the chemical shift of H-7 was at δ 3.48 (brd, J = 11.2 Hz), H-8 was at δ 2.78 (dd, J = 18.4, 11.2 Hz) and H-8′ was overlapped with H-12. The difference between **5** and **6** in their ^{1}H NMR spectra indicated that the two compounds **5** and **6** were epimeric at C-7. This could be confirmed by the CD spectrum: The CD curve for compound **6** has $\Delta\varepsilon_{max} = -0.03$ at 246 nm, the stereo-model of the butenolide has left-handed chirality in the C=C−C=O system like compound **5**, and the absolute configuration of C-4 is also R, the difference between **6** and **5** in the ^{1}H NMR spectrum was incurred by the chiral carbon of C-7. So, compound **6** was identified as (4R,7S*/R*)-2,4-dimethyl-4-(1′-acetyl-3′-oxobutyl)-2-butenolide.

3. Experimental

3.1. Equipment

Optical rotation: polarimeter 241 (Perkin Elmer), solvent $CHCl_3$. IR-spectra were recorded on a Nicolet 170SX FT-IR instrument. ^{1}H NMR (400.13 MHz), ^{13}C NMR (100.62 MHz) and 2D-NMR were recorded on a Varian INOVA-400 FT-NMR spectrometer in $CDCl_3$ with TMS as int. standard. EI-MS spectra were determined on a HP-5988 (A. E. I. Brunner) mass spectrometer and HR-ESIMS was recorded on a Bruker APEX II. Silica gel (200–300 mesh) was used for column chromatography and silica gel GF_{254} for TLC. Spots were detected on the TLC under UV light or by heating after spraying with 98% H_2SO_4 : EtOH (v : v) 5 : 95.

3.2. *Plant material*

The plant material, *Saussurea katochaete* (Compositae) was collected in Huzhu County, Qinghai province, P. R. China in August 2002 and was identified by adjunct Prof. Ji Ma, Faculty of Pharmacy, First Military Medical University of PLA, Guangzhou, P. R. China. A voucher specimen (NO. 2002003) has been deposited at Key Laboratory of Natural Medicine for Gansu province.

3.3. *Extraction and isolation*

The air-dried whole plant (5.0 kg) was extracted three times with acetone at room temperature, and the extracts were concentrated under reduced pressure. The extract (90 g) was subjected to column chromatography (CC) on a silica gel (1000 g) with petroleum ether -acetone (v : v, 40 : 1–1 : 1) as eluent to give ten fractions (Fr. 1-Fr. 10). Fr. 2 yielded **2** (225 mg) after CC on a silica gel eluting with petroleum ether-acetone (v : v, 15 : 1). Fr. 3 was chromatographied using petroleum ether-EtOAc (v : v, 10 : 1) as eluent to afford a mixture of **5** and **6**, which was further separated by CC using petroleum ether-acetone (v : v, 8 : 1) as eluent to give **5** (14 mg) and **6** (12 mg). Fr. 4 yielded **1** (13 mg) after CC on a silica gel eluting with petroleum ether-acetone (v : v, 8 : 1). Fr. 5 was subjected to CC on a silica gel using petroleum ether-EtOAc (v : v, 10 : 1) as eluent to give **3** (13 mg) and **4** (11 mg).

3.4. *(4R,7R*/S*)-2,4-Dimethyl-4-(1′-acetyl-3′-oxobutyl)-2-butenolide (compound 5)*

Colorless gum, $[\alpha]_{26}^{D}$ −3.0° (C 1.0, $CHCl_3$). IR (KBr, cm^{-1}): 2985, 2930, 1762 (ester carbonyl group), 1712 (ketone carbonyl groups), 1664 (C=C), 1361, 1184, 1161, 1057, 1001, 950, 879, 764. HR-ESIMS: m/z = 225.1112 $[M+1]^+$ (Calcd. 225.1121 for $C_{12}H_{17}O_4$). ^1H NMR and ^{13}C NMR see Table 2.

3.5. *(4R,7S*/R*)-2,4-Dimethyl-4-(1′-acetyl-3′-oxobutyl)-2-butenolide (compound 6)*

Colorless gum, $[\alpha]_{26}^{D}$ −5.0° (C 0.5, $CHCl_3$). IR (KBr, cm^{-1}): 2985, 2930, 1758 (ester carbonyl group), 1713 (ketone carbonyl groups), 1664 (C=C), 1361, 1159, 1059, 1000, 950, 882, 764. EI-MS: m/z = 224 $[M]^+$, 179, 125, 111 $[a]^+$ or $[M\text{-}b]^+$, 94, 43. ^1H NMR and ^{13}C NMR see Table 2.

Acknowlegement: This work was supported by the National Natural Science Foundation of China (NSFC 20021001, 20372029 and 20475057).

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