Eremophilanes from *Petasites formosanus* KITAMURA

Yun-Lian LIN,* Jun-Chih OU, Chieh-Fu CHRN, and Yueh-Hsiung KUO[a]

National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C. Department of Chemistry, National Taiwan University,[a] Taipei, Taiwan, R.O.C. Received April 22, 1998; accepted July 23, 1998

Four new eremophilane-type sesquiterpenes, petasinones A (1), B (2), C (3), and D (4), together with petasin (5), isopetasin (6), petasol (7), isopetasol (8), S-petasin (9), and S-isopetasin (10) were isolated from the aerial parts of *Petasites formosanus*. The structures of new compounds were determined by chemical and spectroscopic methods.

Key words *Petasites formosanus*; Compositae; sesquiterpene; eremophilanes; petasinones A, B, C, D

Many eremophilane-type sesquiterpenes have been isolated from *Petasites*,[1-5] and they have shown antitumor properties[6] or inhibition of peptide-leukotriene biosynthesis[7,8] activities. The aerial parts of *Petasites* (*P.*) *formosanus* KITAMURA (Compositae) have long been used as folk medicine for the treatment of hypertension and tumors.[9,10] In order to isolate the pure components for bioassay test, we have now investigated and characterized its chemical principles.

The EtOAc-soluble fraction from an EtOH extract of the aerial parts of *P. formosanus* was subjected to column chromatography on activated charcoal to obtain fractions which were rich in sesquiterpenes. Further separation of these fractions by medium pressure liquid chromatography (MPLC) and HPLC on silica gel columns led to the isolation of four new eremophilanes, petasinones, A (**1**), B (**2**), C (**3**), and D (**4**), and six known compounds, petasin (**5**),[11,12] isopetasin (**6**),[11,12] petasol (**7**),[11,12] isopetasol (**8**),[11,12] *S*-petasin (**9**),[11,12] *S*-isopetasin (**10**),[11,12] petasones A and B,[13] *S*-petasitin,[13] and petasinol.[13] In this paper, we report the isolation and structural elucidation of these four new eremophilane sesquiterpenes.

Petasinone A (**1**) was obtained as a colorless oil and the molecular formula was determined to be $C_{19}H_{26}O_4$ by high resolution (HR-MS) and by the ^{13}C-NMR data. The IR spectrum showed the presence of an α,β-unsaturated carbonyl system with a β,β-disubstituted group (1665 cm^{-1}), an α,β-unsaturated ester (1705 and 1210 cm^{-1}), and a terminal methylene (1650, 890 cm^{-1}). Its UV spectrum also suggested the presence of an α,β-unsaturated ketone [λ_{max} 240 nm (log ε 4.46)]. The ^{1}H- and ^{13}C- NMR (Table 1) spectra indicated the presence of a singlet methyl group [δ_H 1.11 (s, H-14), δ_C 17.2 (C-14)], a doublet methyl group [δ_H 0.94 (d, J=6.6 Hz, H-15), δ_C 10.4 (C-15)], a trisubstituted double bond [δ_H 5.76 (d, J=1.5 Hz, H-9), δ_C 124.5 (C-9), 167.8 (C-10)], an isopropenyl group [δ_H 1.73 (s, H-13), δ_C 20.0 (C-13), δ_H 4.80 and 4.97 (br s, H-12), δ_C 143.0 (C-11), 114.4 (C-12)], a proton appearing as a triplet of doublets attached to the carbon atom bearing the ester group [δ_H 4.93 (td, J=11.0, 4.8 Hz, H-3), δ_C 72.5 (C-3)], a carbonyl group [δ_C 198.6 (C-8)], and an (*E*)-3-methoxyacryloyloxy group [δ_H 5.17 and 7.60 (each 1H, d, J=12.6 Hz, H-2′, H-3′), δ_C 95.9 (C-2′), 163.4 (C-3′); δ_H 3.68 (3H, s, OCH_3), δ_C 57.3 (OCH_3)] . This spectral data suggests that compound **1** is an eremophilene sesquiterpene derivative with an ester group at C-3. A methine proton [δ_H 3.09 (dd, J=14.4, 4.5 Hz] located between the carbonyl and isopropenyl groups was established by heteronuclear multiple bond correlation (HMBC) between H-13 and C-7, C-11; between H-12 and C-7, C-11; and between H-7 and C-8, C-6. The coupling pattern of H-3 at δ 4.93 (td, J=11.0, 4.8 Hz, $J_{3\beta,2\alpha}$=$J_{3\beta,4\alpha}$=11.0 Hz and $J_{3\beta,2\beta}$= 4.8 Hz) implies that the ester linked to C-3 is α-equatorial. The (*E*)-3-methoxyacrylate moiety was established by its NMR spectral data and by the HMBC technique. Comparing ^{1}H- and ^{13}C-NMR (Table 1) data of **1** with those of petasin (**5**),[11,12] the planar structure of compound **1** can be assigned as 3α-[(*E*)-3-methoxyacryloyloxy]-9,11-eremophiladien-8-one. Hydrolysis of **1** afforded isopetasol (**8**),[11,12] a result that also supported the assigned structure. The relative configurations of H-3, H_3-15 and H-7 were determined by nuclear Overhauser enhancement spectroscopy (NOESY) techniques and the whole structure is shown in Fig. 1.

Petasinone B (**2**) was isolated as a colorless oil, and showed the molecular formula $C_{19}H_{26}O_4$, based on HR-MS. The UV absorption bands at 235 and 273 nm indicated two different conjugated carbonyl systems. The ^{1}H- and ^{13}C-NMR spectra (Table 1) of **2** were very similar to those of **1** except for the presence of an isopropylidene group [δ_H 1.82 (3H, s) and 2.06 (3H, d, J=1.5 Hz); δ_C 22.6 and 22.1] in place of an isopropenyl group, and AB system signals [δ_H 2.16 (1H, dq, J=13.6, 1.5 Hz, H_a-6), 2.89 (1H, d, J=13.6 Hz, H_b-6)] instead of ABX system signal. The protons of H-12 and H_a-6 showed homoallylic coupling (J=1.5 Hz), that indicates the isopropylidene group is conjugated with the carbonyl group. In the HMBC spectrum, cross peaks were observed between H_b-6 and C-5 (δ_C 42.2), H_b-6 and C-7 (δ_C 127.2), and H_b-6 and C-10 (δ_C 167.3), H_b-6 and C-11 (δ_C 143.3). Further evidence was obtained by hydrolysis, which afforded isopetasol (**8**). The relative configuration was determined by NOESY, in which nuclear Overhauser effect (NOE) was observed between H-3 and H_3-14.

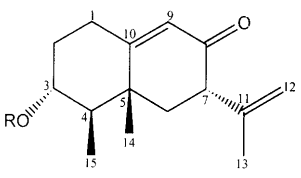
Petasinone C (**3**), was obtained as a colorless oil. HR-MS showed that **3** has the molecular formula $C_{19}H_{26}O_4$. The IR spectrum showed an α,β-unsaturated carbonyl group (1665 cm^{-1}). The ^{1}H- and ^{13}C-NMR (Table 1) spectral data revealed that compounds **3** and **2** are very similar, the only difference being the side chain ester group. Two olefinic proton signals [δ_H 4.82 and 6.43 (each 1H, d, J=7.0 Hz)][14] with a relatively small coupling constant and lower field shift for the methoxy group [δ_H 3.84 (3H, s)] suggested a (*Z*)-3-methoxyacryloyloxy moiety. Assignment of NMR signals was accomplished utilizing heteronuclear multiple quantum coherence (HMQC) and HMBC experiments. The relative stereochemistry was also established by NOESY experiment. Strong

* To whom correspondence should be addressed.

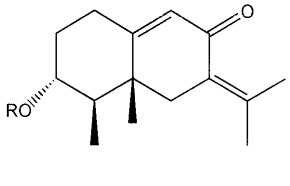
Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of **1**, **2**, **3** and **4** (300 and 75 MHz, in $CDCl_3$)

	1		**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		30.6		30.1		30.2		30.1
2		31.6		31.7		31.6		31.7
3	4.93 td (11.0, 4.8)[a]	72.5	4.87 td (11.0, 4.8)	72.8	4.83 td (11.1, 4.5)	72.5	4.91 td (11.0, 4.8)	73.3
4		47.4		46.3		46.2		46.5
5		41.7		42.2		42.2		42.2
6		40.0	2.89 d (13.6) 2.16 dq (13.6, 1.5)	41.1	2.87 d (13.5) 2.16 dq (13.5, 1.5)	41.1	2.90 d (12.6) 2.12 dq (12.6, 1.5)	41.1
7	3.09 dd (14.4, 4.5)	50.3		127.2		127.2		127.1
8		198.6		191.7		191.7		191.6
9	5.76 d (1.5)	124.5	5.75 d (1.5)	126.7	5.73 d (1.5)	126.5	5.76 d (1.5)	126.7
10		167.8		167.3		165.5		164.8
11		143.0		143.3		143.2		143.3
12	4.80 (br s) 4.97 (br s)	114.4	2.06 d (1.5)	22.1[b]	2.06 d (1.5)	22.1[b]	2.06 d (1.5)	22.1[b]
13	1.73 s	20.0	1.82 s	22.6[b]	1.85 s	22.5[b]	1.82 s	22.6[b]
14	1.11 s	17.2	1.01 s	17.2	1.00 s	17.1	1.02 s	17.1
15	0.94 d (6.6)	10.4	0.96 d (6.6)	10.7	0.94 d (6.6)	10.6	0.96 d (6.6)	10.7
1′		166.9		165.3		164.8		165.2
2′	5.17 d (12.6)	95.9	5.17 d (12.6)	95.9	4.82 d (7.0)	96.2	5.64 d (14.7)	113.1
3′	7. 60 d (12.6)	163.4	7.61d (12.6)	163.3	6.43 d (7.0)	160.5	7.74 d (14.7)	147.4
O-Me	3.68 s	57.3	3.68 s	57.3	3.84 s	62.5		
S-Me							2.32 s	14.3

a) Figures in the parentheses are coupling constants in Hz. *b*) Assignment may be interchanged.



1 R: $COCH^{1'}=CH^{2'}-^{E}CHO^{3'}Me$
5 R: $COCH=^{Z}CHMe$
7 R: H
9 R: $COCH=^{Z}CHSMe$



2 R: $COCH=^{E}CHOMe$
3 R: $COCH=^{Z}CHOMe$
4 R: $COCH=^{E}CHSMe$
6 R: $COCH=^{Z}CHMe$
8 R: H
10 R: $COCH=^{Z}CHSMe$

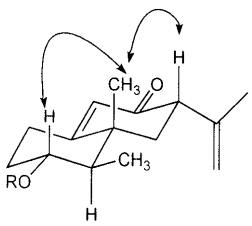


Fig. 1. NOESY Correlations Observed for the Related Eremophilanes in *P. formosanus*

NOE correlation between H_β-3 and H_3-14 indicated both rings A and B possessed a chair conformation, as shown in Fig. 1.

Petasinone D (**4**), isolated as colorless needles, mp 135—137, showed UV absorptions at 243 and 276 nm and IR absorption bands at 1690 and 1210 (ester), and at 1665 (α,β-unsaturated carbonyl) cm^{-1}. The ^{1}H- and ^{13}C-NMR data (Table 1) of **4** were similar to those of *S*-isopetasin (**10**),[11,12] except for (*E*)-3-methylthioacryloyloxy moiety [δ_H 5.64 and 7.74 (each 1H, d, J=14.7 Hz), δ_C 113.1 and 147.4] in place of the (*Z*)-3-methylthioacryloyloxy moiety in *S*-isopetasin (**10**). Strong NOE correlations between H_β-3 and H_3-14, revealed that the relative stereochemistry of rings A and B was the same as compound **1**.

Experimental

All NMR experiments were performed on Bruker AC-300 and DMX-300 instruments with $CDCl_3$ as solvent. MS were recorded in electron impact (EI) mode (70 eV) on a Finnigan TSQ-46C MS spectrometer. High resolution EI-MS were recorded on a JEOL SX-102A. IR spectra were run on a Perkin-Elmer 781 spectrophotometer. Optical rotation was obtained in EtOH on a JASCO DIP-370 polarimeter. UV spectra were measured on a Hitachi U-3200 spectrophotometer. MPLC were run using a BUCHI 688 chromatography pump (Kiesel gel 18—32 μm). Preparative HPLC was performed with a Hitachi L-6000 pump, using a Lichrosorb Si 60 (7 μm) 250×10 mm column with an ERC-7525 refractive index (RI) detector.

Plant Material The aerial parts of *P. formosanus* were collected from Ali mountain, in the central part of Taiwan, and identified by comparison with a voucher specimen which had been deposited at the Herbarium of the Department of Botany of National Taiwan University (No: TAI 197973, collected on March 21, 1985).

Extraction and Isolation The dried aerial parts of *P. formosanus* (5 kg) were extracted with 95% EtOH (×3). The EtOH extract was evaporated *in vacuo* to yield a black residue (295 g), which was taken up in H_2O and partitioned successively with EtOAc and *n*-BuOH. The EtOAc fraction (146 g) was chromatographed on activated charcoal column, and eluted from 10% EtOAc/hexane to EtOAc gradient. with Fractions rich in sesquiterpenes (20—70% EtOAc/hexane) were further separated by MPLC and HPLC on a silica gel column, and led to the isolation of ten eremophilanes–petasinone A (**1**) (15 mg), petasinone B (**2**) (32 mg), petasinone C (**3**) (38 mg), petasinone D (**4**) (35 mg), petasin (**5**) (1.3 g), isopetasin (**6**) (250 mg), petasol (**7**) (36 mg), isopetasol (**8**) (21 mg), *S*-petasin (**9**) (3.4 g), and *S*-isopetasin (**10**) (1.2 g).

Petasinone A (**1**): Colorless oil. $[\alpha]_D^{25}$ +11° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 240 (4.46), 276 (3.58). IR (film) cm^{-1}: 3020, 1705, 1665, 1650, 1625, 1210, 1150, 1000, 890, 800. ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): Table 1. EI-MS (70 eV) (rel. int.) *m/z*: 318 $[M]^+$ (8), 216 (92), 161 (90), 148 (60), 85 (100). HR-MS *m/z*: 318.1826 (M^+, Calcd for $C_{19}H_{26}O_4$: 318.1832).

Petasinone B (**2**): Colorless oil. $[\alpha]_D^{25}$ +18° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 235 (4.45), 273 (3.76). IR (film) cm^{-1}: 3020, 1700, 1665, 1620, 1210, 1120, 1015. ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): Table 1. EI-MS (70 eV) (rel. int.) *m/z*: 318 $[M]^+$ (15), 278 (15), 216 (100), 201 (60), 161 (100), 148 (38), 85 (64). HR-MS *m/z*: 318.1826 (M^+, Calcd for $C_{19}H_{26}O_4$: 318.1830).

Petasinone C (**3**): Colorless oil. $[\alpha]_D^{25}$ +36° (c=0.7, MeOH). UV λ_{max}^{MeOH} nm (log ε): 234 (4.82), 275 (3.95). IR (film) cm^{-1}: 3020, 1700, 1660, 1615, 1200, 1150, 1100, 1020, 980, 880. ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): Table 1. EI-MS (70 eV) (rel. int.) *m/z*: 318 $[M]^+$ (12), 216 (70), 201 (52), 161 (88), 148 (100), 105 (44), 85 (88). HR-MS *m/z*: 318.1828 (M^+, Calcd for $C_{19}H_{26}O_4$: 318.1832).

Petasinone D (**4**): Colorless needles (from EtOH), mp 135—137 °C. $[\alpha]_D^{25}$ +42° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 243 (4.10), 276 (4.40). IR (Kbr) cm^{-1}: 3040, 3020, 1690, 1665, 1620, 1570, 1210, 1150, 1000, 860, 690. ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): Table 1. EI-MS (70 eV) (rel. int.) *m/z*: 334 $[M]^+$ (16), 218 (100), 161 (90), 148 (25). HR-MS *m/z*: 334.1597 (M^+, Calcd for $C_{19}H_{26}O_3S$: 334.1604).

Alkaline Hydrolysis of Petasinone A (1) Compound **1** (8 mg) was dissolved in 5% methanolic sodium hydroxide (1 ml) and refluxed for 1 h. After removal of the solvent, the residue was acidified with 1 N HCl, and extracted with EtOAc (10 ml×3). The extract was washed with 5% $NaHCO_3$ solution and H_2O, successively, and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded isopetasol (**8**) (4 mg).

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