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SESQUITERPENOIDS FROM *PETASITES FRAGRANS*

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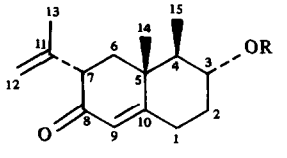
Key Word Index—*Petasites fragrans*; Compositae; Senecioneae; eremophilane; petasol; isopetasol; *S*-petasin; neo-*S*-petasin.

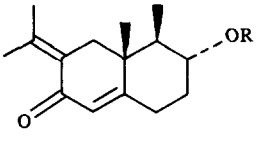
Abstract—Chemical analysis of *Petasites fragrans* yielded the eremophilane type of sesquiterpenes: petasol, isopetasol, *S*-petasin, neo-*S*-petasin, and a mixture of petasol derivatives, with caffeic acid methyl ester and a mixture of phytosterols. Their structures were elucidated by chemical and spectroscopic methods.

INTRODUCTION

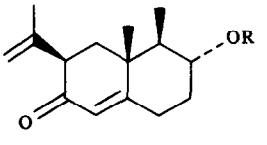
The components of several *Petasites* species have been investigated and shown to include petasin [1–3] and furanopetasin derivatives [4, 5] belonging to the eremophilane type of sesquiterpene, and bakkenolide derivatives [6, 7]. It has been reported that the petasin derivatives have spasmolytic properties [1–3, 8] and bakkenolide-A has cytotoxic [9] activity. We have now investigated *P. fragrans* and isolated petasol (**1**), isopetasol (**2**), *S*-petasin (**3**), neo-*S*-petasin (**4**), a mixture of petasin (**5**), isopetasin (**6**) and neopetasol angelate (**7**), in addition to a mixture of



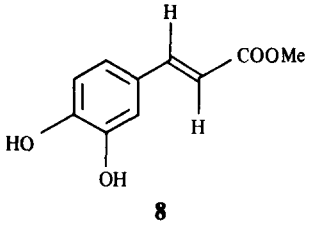
1 R = H
3 R = COCH═CH(SMe) *cis*–
5 R = COC(Me)═CH(Me) *cis*–



2 R = H
6 R = COC(Me)═CH(Me) *cis*



4 R = COCH═CH(SMe) *cis*–
7 R = COC(Me)═CH(Me) *cis*–



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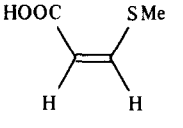

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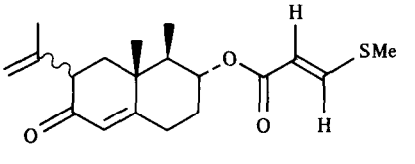

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Table 1. ^{1}H NMR chemical shifts of compounds **1**–**4** and **9** (δ in $CDCl_3$)

	1	2	3	4	9
H-3	3.63 *ddd* (11,10,4.4)	3.58 *ddd* (16,10,3.7)	5.00 *m*	5.00 *m*	—
H-6	—	2.92 *d* (13)	—	—	—
H-7	3.11 *dd* (13,5.8)	—	3.12 *dd* (13,6)	3.05 *dd* (9.8,6.6)	—
H-9	5.77 *br s*	5.76 *br s*	5.78 *br s*	5.83 *br s*	—
H-12 *trans**	4.83 *d* (1.0)	2.09 *s*	4.83 *br s*	4.73 *br s*	—
H-12 *cis*†	5.00 *qui* (1.7)		4.99 *br s*	4.93 *br s*	—
H-13	1.75 *s*	1.85 *s*	1.75 *s*	1.77 *s*	—
H-14	1.18 *s*	0.98 *s*	1.24 *s*	1.15 *s*	—
H-15	1.08 *d* (6.1)	1.14 *d* (6.3)	0.96 *d* (6.6)	0.99 *d* (6.6)	—
MeS–	—	—	2.41 *s*	2.41 *s*	2.42 *s*
α-CH=	—	—	5.84 *d* (10)	5.82 *d* (10)	5.87 *d* (10)
β-CH=	—	—	7.09 *d* (10)	7.10 *d* (10)	7.21 *d* (10)

**Trans*-relationship to the larger group.
†*Cis*-relationship to the larger group.
Values in parentheses are coupling constants (Hz).

phytosterols and caffeic acid methyl ester (**8**). The isolation of petasol (**1**) is the first report from a natural source.

RESULTS AND DISCUSSION

The dried and powdered whole plant of *P. fragrans* was extracted with methanol. After removal of the acidic and basic portions from the ethyl acetate soluble part of the methanol extract, the neutral portion was subjected to CC over Si gel to yield 11 compounds.

Compound **3**, $C_{19}H_{26}O_3S$, mp 123–125°, $[\alpha]_D$ +51.0°, was identified as *S*-petasin [10] on the basis of physical and spectral properties. The IR spectrum showed the presence of α,β-unsaturated carbonyl groups (1675 cm^{-1}), terminal methylenes (1650, 900 cm^{-1}), and a methyl thioether (1560 cm^{-1}). The ^{1}H NMR spectrum of **3** (Table 1) revealed an isopropenyl group as shown by the vinylic methyl singlet at δ 1.75 and two vinylic proton singlets at 4.83 and 4.99. A doublet at δ 0.96 (J = 6.6 Hz) and a singlet at 1.24 were assigned to methyl protons. A double doublet at δ 3.12 (J = 13 and 6 Hz) was assigned to the methine proton next to an isopropenyl group. A multiplet at δ 5.00 was assigned to the proton at the carbon atom bearing the ester group. A singlet at δ 5.78 was assigned to the vinylic proton. A singlet at δ 2.41 was assigned to the thio methyl protons and two doublets at 5.84 and 7.09 (AX-system, J = 10 Hz each) were assigned to the vinylic protons. The ^{13}C NMR spectrum of **3** (Table 2) confirmed the structure.

Alkaline hydrolysis of **3** with alcoholic potassium hydroxide gave **2** and β-methylthioacrylic acid (**9**) [7]. Compound **2**, mp 123.5–125.0°, $[\alpha]_D$ +113°, was analysed for $C_{15}H_{22}O_2$. The IR spectrum showed the presence of $\alpha,\beta;\alpha',\beta'$-unsaturated carbonyl (1650 cm^{-1}) and hydroxyl (3500 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** showed two vinylic methyl singlets at δ 0.99 and a methyl doublet at 1.11 (J = 5.9 Hz). The multiplet at δ 3.59 was assigned to the proton geminal to a hydroxyl group. The characteristic doublet at δ 2.92 (J = 13 Hz) was assigned to a proton attached to C-6. From these data **2** was deduced to be isopetasol (**2**).

Compound **4**, mp 83–84°, $[\alpha]_D$ −105°, displayed ^{1}H NMR signals that were nearly identical with those in the spectrum of **3** with the exception that a double doublet at δ 3.12 (J = 13 and 6 Hz) was replaced by a double doublet at 3.05 (J = 9.8 and 6.6 Hz) (Table 1). The ^{13}C NMR signals which are at δ 41.9 and 47.5 in the spectrum of **3** and are assignable to C-6 and C-7 were shifted to 37.5 and 43.3, respectively, in the spectrum of **4**. On the basis of spectral data (^{1}H NMR, ^{13}C NMR, UV, IR and mass spectra) and the opposite value of the optical rotation, **4** was deduced to be the 7β-epimer of **3**. The physical properties of **4** were similar to those of petasol ester C (**10**). Stoll [2] considered that the difference between petasol ester C (**10**) and **3** is in the *cis*, *trans*-isomerism of the ester moieties. However, the coupling constants of the vinylic protons were the same in each case (10 Hz). Neuenschwander *et al.* [11] gave the 7β-epimer of petasol

Table 2. ^{13}C NMR chemical shifts of compounds **1**–**4** (δ in $CDCl_3$)

C No.	1	2	3	4
1	30.8	30.6	30.8	30.5
2	35.1	35.5	31.9	33.7
3	70.9	75.8	73.1	73.4
4	50.2	49.2	50.5	50.2
5	39.7	42.2	40.2	39.9
6	41.6	41.4	41.9	37.5
7	50.2	127.2	47.5	43.3
8	198.2	191.6	198.2	198.5
9	124.1	126.4	124.5	123.4
10	167.3	166.1	166.1	165.9
11	143.1	143.1	143.3	143.0
12	114.1	22.3*	144.3	113.5
13	20.0	22.8*	19.5*	20.7*
14	17.2	17.5	17.4	19.2
15	10.4	11.0	10.6	11.6
MeS–	—	—	20.3*	21.0*
α-CH=	—	—	112.9	112.6
β-CH=	—	—	152.4	152.3
–COO–	—	—	166.6	167.8

*Indicates assignments which may be reversed within each column.

the name neopetasol, therefore, we have named compound **4** neo-*S*-petasin.

From the CD spectrum of **3**, it is presumed that the isopropenyl group attached to C-7 has the α-configuration because of the similarity of the CD curve to that of 2-oxo-5α-estr-1(10)-ene-3β,17β-diol diacetate in the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ region [12]. On the other hand, the CD spectrum of **4** seems to indicate that the isopropenyl group attached to C-7 has the β-configuration by comparison with the case of 2-oxo-5α-estr-1(10)-ene-3α,17β-diol diacetate [12].

Compound **1**, $[\alpha]_D$ +124°, was obtained as a viscous oil. High resolution mass spectrometry showed that **1** had the molecular formula $C_{15}H_{22}O_2$. The IR spectrum showed the presence of hydroxyl (3500–3300 cm^{-1}) and α,β-unsaturated carbonyl groups (1665 cm^{-1}) and a terminal methylene (1650, 900 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) demonstrated an isopropenyl group with the vinylic methyl singlet at δ 1.75 and two vinylic proton singlets at 4.83 and 5.00. A double double doublet at δ 3.63 (J = 11, 10 and 4.4 Hz) was assigned to the methine proton next to the isopropenyl group. The ^{13}C NMR spectrum revealed the presence of 1 × C, 3 × CH, 3 × CH_2, 3 × Me, 4 × sp^2C, and 1 × C=O. These data identified **1** as petasol. This is the first time that free petasol (**1**) has been isolated from a natural source although several petasol esters had been isolated so far.

A compound obtained from the fraction eluted with hexane–acetone (9:1) during the first CC was indicated to be an isomer of **1** (e.g. **2**) by its physical properties but loss of the sample prevented its further identification.

A mixture of compounds **5**–**7**, a yellow viscous oil, was inseparable by CC. Based on the spectral properties, the mixture consisted of mainly petasin (**5**) but containing some isopetasin (**6**) and neopetasol angelate (**7**). Alkaline hydrolysis of this mixture afforded **2**, which is known to be formed by isomerization during the hydrolysis of petasin esters [3].

Compound **8**, mp 148–152°, was identified as caffeic acid methyl ester by direct comparison with an authentic specimen. In recent years, it has been reported that hydroxycinnamic acids inhibited BP-induced neoplasma of the fore-stomach [13].

A mixture of sterols, melting at 130–136°, eluted with hexane–acetone (9:1) and consisted of sitosterol, stigmasterol and campesterol in the ratio 19:6:2.

Compounds **2**–**4** and the mixture of **5**–**7** are now being assayed for antitumor activity against solid type Ehrlich carcinoma using a slightly modified method of Egashira *et al.* [14]. Recently, Bohlmann and Otto [15] reported that isopetasol derivatives with an unsaturated acid ester moiety will selectively inhibit RNA synthesis in the ascites tumor of mouse.

EXPERIMENTAL

Mps were determined on a Kofler hot-stage apparatus and are uncorr. ^1H NMR spectra were measured at 100 MHz in $CDCl_3$ or CD_3OD soln and ^{13}C NMR spectra were at 25 MHz in $CDCl_3$ with TMS as a int. standard. Optical rotations were measured in $CHCl_3$ soln at room temp. CD were measured in MeOH soln.

The plant material was collected from the Experimental Station of Medicinal Plants, Faculty of Pharmaceutical Sciences, Hokkaido University, on 16 June 1981. The plant was transplanted from Takeda Kyoto Herbal Garden, Kyoto.

Extraction of P. fragrans. The whole plant of *P. fragrans* was dried (14 kg), powdered and extracted with MeOH (60 l.). The total MeOH extract was filtered and concd *in vacuo*. A part (500 g) of the extract (2 kg) was partitioned between EtOAc and H_2O. The EtOAc layer was extracted successively with satd $NaHCO_3$ and 2 N HCl.

The neutral layer (35.52 g) was chromatographed on 1 kg Si gel eluted with a hexane–Me_2CO gradient taking 1 l. fractions. Fractions 3–5 gave a mixture of sterols (69.2 mg). Fractions 4 and 5 contained a mixture of **5**–**7** which was not separated by further CC. Fractions 7 and 8 contained a mixture of **3** (739.5 mg) and **4** (130 mg), which were separated by CC eluting with CH_2Cl_2. Fractions 11–14 gave a mixture of **1** (7.4 mg) and **2** (6.3 mg), which were separated by CC on Si gel eluted with CH_2Cl_2. Fractions 20–32 contained 8.1 mg **8** which was purified by chromatography.

Identification of isolated compounds. A mixture of sitosterol, stigmasterol and campesterol in the ratio 19:6:2 was identified by GC/MS (1.5% SE-30 column at 250°) and comparison with authentic samples.

The mixture of petasin (**5**), isopetasin (**6**) and neopetasol angelate (**7**) was a viscous oil which was not separated. IR ν_{max}^{neat} cm^{-1}: 1710 (C=CCOOR), 1675 (C=CCO), 1650 sh, 890 (>C=CH_2), 1630 (C=C). MS *m/z*: 316 $[M]^+$ 216 $[M$ − angelic acid$]^+$, 148 $[216$ − isoprene$]^+$ (100), 83 $[C_4H_7CO]^+$. ^1H NMR ($CDCl_3$): δ 0.98 (3H, *d*, J = 6.6 Hz, Me-15), 1.26 (3H, *s*, Me-14), 1.74 (3H, *s*, Me-13), 1.89 (3H, *s*, $=C\langle^{COO}_{Me}$), 2.02 (3H, *d*, J = 7.1 Hz, $=CH\underline{Me}$), 2.93 (1H, *d*, J = 11 Hz, H-6), 3.07 (1H, *dd*, J = 9, 5 Hz, H-7), 3.14 (1H, *dd*, J = 13, 5 Hz, H-7), 4.84 (1H, *br s*, H-12 *trans*), 4.99 (1H, *br s*, H-12 *cis*), 5.78 (1H, *br s*, H-9), 6.08 (1H, *dq*, J = 1.5, 7 Hz, $=C\langle^{Me}_{H}$).

Alkaline hydrolysis of a mixture of compounds **5**–**7**. A mixture of **5**–**7** was dissolved in 5% KOH–MeOH (7 ml) and the mixture was refluxed for 70 min. After cooling, the mixture was diluted with H_2O and extracted with Et_2O (50 ml × 3). The combined Et_2O fractions were washed with 2 N HCl, 5% $NaHCO_3$ and satd NaCl soln, and dried over Na_2SO_4. The Et_2O fractions were evaporated to give a product (205.5 mg) from which CC afforded colorless needles, identified as isopetasol (**2**), mp 125–126.5°, $[\alpha]_D$ +115° ($CHCl_3$; *c* 1.02). (Found: C, 76.68; H, 9.27; $C_{15}H_{22}O_2$ requires: C, 76.88; H, 9.27%.) IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1650 (C=CCOC=C), 1610 (C=C). UV λ_{max}^{EtOH} nm (ε): 247 (9000), 280 (6300). MS *m/z*: 234 $[M]^+$, 216 $[M - H_2O]^+$, 201 $[216 - Me]^+$, 161 (100).

S-Petasin (**3**). Mp 123–125°, $[\alpha]_D$ +51.0° ($CHCl_3$; *c* 1.03). (Found: C, 68.14; H, 7.91; S, 9.70. $C_{19}H_{26}O_3S$ requires C, 68.23; H, 7.84; S, 9.59%.) IR ν_{max}^{Nujol} cm^{-1}: 1675 (C=CCO), 1650, 890 (C=CH_2), 1630 (C=C), 1560 (Me S–), 1220. UV λ_{max}^{EtOH} nm (ε): 237 (12 500), 290 (12 400). MS *m/z*: 334 $[M]^+$, 319 $[M - Me]^+$, 216 $[M$ − β-methylthioacrylic acid$]^+$, 201 $[216 - Me]^+$, 148 $[216$ − isoprene$]^+$ (100), 105, 101. CD (MeOH; *c* 4.8 × 10^{-5}): $[\theta]_{340}$ −111, $[\theta]_{310}$ −4660 (negative maximum), $[\theta]_{255}$ 0, $[\theta]_{222}$ +29 600 (positive maximum).

Alkaline hydrolysis of **3**. *S*-Petasin (**3**) (101.1 mg) was dissolved in 5% KOH–MeOH (7 ml), and then the mixture was stirred overnight at room temp. After removal of the solvent *in vacuo*, the residue was acidified with 2 N HCl and extracted with Et_2O (8 ml × 3). The extract was washed with 5% $NaHCO_3$ soln and satd NaCl soln and dried over Na_2SO_4. The subsequent evaporation of the solvent followed by further CC and recrystallization from hexane–Et_2O gave a product (35.5 mg), mp 123.5–125°, $[\alpha]_D$ +113° ($CHCl_3$; *c* 0.9). This material was identical (mp, IR, UV, ^1H NMR, MS, $[\alpha]_D$) with the isopetasol (**2**) obtained from alkaline hydrolysis of **5**–**7**.

The $NaHCO_3$ soln was concd *in vacuo*, acidified with 2 N HCl

and extracted with Et_2O (8 ml × 3). The extract was washed with satd NaCl soln and dried over Na_2SO_4. After removal of the solvent, the residue (21.6 mg) was crystallized from hexane–Me_2CO to give **9** as colorless needles of β-methylthioacrylic acid, mp 106–120.5°. (Found: C, 40.89; H, 5.21; S, 27.03. $C_4H_6O_2S$ requires C, 40.66; H, 5.12; S, 27.14%.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000 (OH), 1670 (C=CCOOH), 1570 (MeS–). UV λ_{max}^{EtOH} nm (ε): 284 (11 200). MS *m/z*: 118 $[M]^+$, 103 $[M-Me]^+$ (100), 101 $[M-OH]^+$, 100 $[M-H_2O]^+$, 73 $[M-COOH]^+$, 45 $[COOH]^+$.

Neo-S-petasin (**4**). Mp 83–84°, $[\alpha]_D$ −105° ($CHCl_3$; *c* 0.94). (Found: C, 68.25; H, 7.87; S, 9.37. $C_{19}H_{26}O_3S$ requires C, 68.23; H, 7.84; S, 9.59%.) IR ν_{max}^{Nujol} cm^{-1}: 1690 (C=CCOOR), 1660 (C=CCO), 1640, 900 (>C=CH_2), 1620 (C=C), 1560 (MeS–). UV λ_{max}^{EtOH} nm (ε): 253 (13 200), 290 (13 200). MS *m/z*: 334 $[M]^+$, 319 $[M-Me]^+$, 216 $[M-\beta$-methylthioacrylic acid$]^+$, 201 $[216-Me]^+$, 148 $[216-$isoprene$]^+$ (100), 105, 101. CD (MeOH; *c* 4.0×10^{-5}): $[\theta]_{327}$ 0, $[\theta]_{316}$ +334 (positive maximum), $[\theta]_{216}$ 0, $[\theta]_{226}$ −15 100 (negative maximum).

Petasol (**1**). Viscous yellow oil, $[\alpha]_D$ +124° ($CHCl_3$; *c* 0.74). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–3300 (OH) 1665 (C=CCO), 1650 sh, 900 (>C=CH_2), 1625 (C=C), 1030 (C–O). UV λ_{max}^{EtOH} nm (ε): 236 (9300). EIMS *m/z*: 234 $[M]^+$, 219 $[M-Me]^+$, 216 $[M-H_2O]^+$, 201 $[216-Me]^+$, 166 $[M-$isoprene$]^+$ (100), 122, 94, 79. HRMS *m/z*: Calcd for $C_{15}H_{22}O_2$ 234.1618. Found 234.1616.

Isopetasol (**2**). This was identified as the product of the hydrolysis of **3**, $[\alpha]_D$ +68.3° ($CHCl_3$; *c* 1.38). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH), 1655 (C=CCOC=C), 1615 (C=C). UV λ_{max}^{EtOH} nm (ε): 243 (7700), 278 (3200). EIMS *m/z*: 234 $[M]^+$, 216 $[M-H_2O]^+$, 201 $[216-Me]^+$, 161 (100), 147, 91. HRMS *m/z*: Calcd for $C_{15}H_{22}O_2$ 234.1619. Found 234.1609.

Caffeic acid methyl ester (**8**). Mp 148–152°, IR ν_{max}^{Nujol} cm^{-1}: 3200 (OH), 1680 (C=CCOOR), 1625 (C=C), 1600, 1540 (aromatic). UV λ_{max}^{EtOH} nm (ε): 219 (9200), 237 (6800), 245 (7100), 301sh (9100), 332 (12 700). EIMS *m/z*: 194 $[M]^+$, 163 $[M-OMe]^+$ (100), 145 $[163-H_2O]$, 135 $[M-COOMe]^+$, 117 $[135-H_2O]^+$, 89. HRMS *m/z*: Calcd for $C_{10}H_{10}O_4$ 194.0579. Found 194.0569. ^1H NMR (CD_3OD): δ 3.75 (3H, *s*, COOMe), 6.24 (1H, *d*, *J* = 16 Hz, α-CH=), 6.76 (1H, *d*, *J* = 8.1 Hz, ar. H-5), 6.94 (1H, *dd*, *J* = 8.3, 2.0 Hz, ar. H-6), 7.03 (1H, *d*, *J* = 2.0 Hz, ar. H-1), 7.54 (1H, *d*, *J* = 16 Hz, β-CH=).

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