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CONSTITUENTS OF THE LEAVES OF *PETASITES FORMOSANUS* AND THEIR ANTIOXIDATIVE ACTIVITY

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Abstract — Three new naturally occurring phenylpropenoyl derivatives, sodium dupracine (**1**), (*E*)-dupracine methyl ester (**2**), (*Z*)-dupracine methyl ester (**3**), together with eight known compounds, were isolated from the leaves of *Petasites formosanus*. Their structures were established by spectral methods and chemical transformations. The isolated compounds showed significant antioxidative activity in DPPH radical scavenging assay.

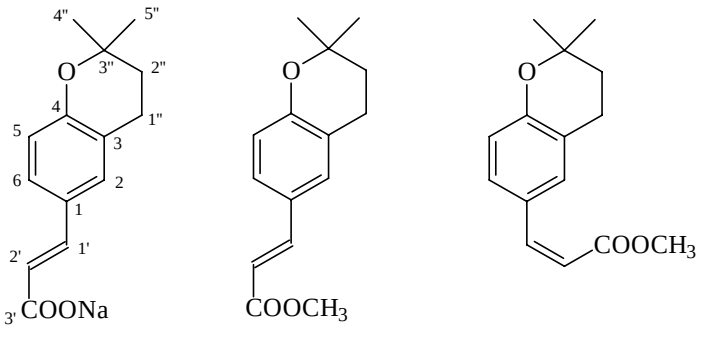
INTRODUCTION

Petasites formosanus Kitamura (Compositae) is a perennial herb and wildly distributed in Taiwan on the high altitude mountains. It has been used in folk medicine as an antidote, analgesic, expectorant, and for the treatment of hypertension and snakebite.[1] We have reported the isolation of several bakkenolides as cytotoxic and antiplatelet aggregation principles form the root of *P. formosanus*, recently. [2-4] In a continuation of our research on the bioactive compounds from natural sources, we have investigated the constituents of the leaves of *P. formosanus*, since the methanol extract showed the significant DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity. This paper deals with the isolation and

structural elucidation of three new phenylpropenoyl derivatives and eight known compounds from the leaves of *Petasites formosanus* and their antioxidative activity.

RESULTS AND DISCUSSION

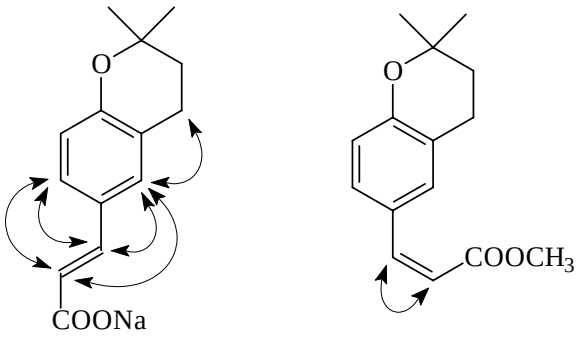
Sodium dupracine (**1**) was isolated as a white powder and HRFABMS spectrum established its molecular formula as $C_{14}H_{15}O_3Na$. The UV absorptions at 229 and 291 nm, together with the IR spectral absorption at 1556 cm^{-1} indicated the presence of a cinnamoyl moiety. In the ^1H NMR spectrum of **1**, ABX type protons at δ 7.25 (1H,dd, *J*=8.8, 2.3 Hz, H-6), 7.23 (1H,d, *J*=2.3 Hz, H-2), and 6.68 (1H, d, *J*=8.8 Hz, H-5) were assigned to H-6, H-2 and H-5 of 1,3,4-trisubstituted phenyl ring, respectively. A pair of trans coupling olefinic protons at δ7.33 and 6.34 (each 1H, d, *J*=16.0 Hz) were attributed to H-1' and H-2' of a conjugated carbonyl system, respectively. Besides, the signals at δ 2.80 (2H, t, *J*=6.4 Hz, H-1"), 1.82 (2H, t, *J*=6.4 Hz, H-2") and 1.32 (6H, s, Me×2) were typical of a 2*H*-2,2-dimethylpyran moiety. The location of the fusion of 2*H*-2,2-dimethylpyran moiety was determined to be C-3 and C-4 due to the NOE correlation between H-1" and H-2 in the NOESY experiment (Figure1). All these assignments were further supported by the NOE crosspeaks between H-1'and H-2, 6; H-2' and H-2, 6. The above results established the dupracine[5] structure. However, the carbonyl absorption in IR spectrum at 1556 cm^{-1} and the upfield shift of H-1' signal to δ 7.33 indicated that this carboxylic group should be in salt form. Acidification of **1** with 5 % HCl afforded sodium chloride, which was determined with an atomic absorption spectrometer. On the basis of the above data, **1** was assigned to be the sodium salt of dupracine.



1 **2** **3**

Scheme 1

(*E*)-Dupracine methyl ester (**2**) was obtained as colorless oil and HREIMS spectrum established its molecular formula as $C_{15}H_{18}O_3$. The cinnamoyl ester moiety absorptions of **2** were implied by the observation of UV spectrum at 234 and 316 nm. An IR spectral absorption at 1722 cm^{-1} indicated the presence of an ester carbonyl group in the molecule. The ^1H NMR spectrum of **2** showed the presence of an ABX aromatic protons at δ 7.40 (2H, m, H-2, H-6) and 6.74 (1H, d, *J*=8.8 Hz, H-5), a conjugated olefinic system at δ 7.58 and 6.35 (each 1H, d, *J*=16.0 Hz, H-1', 2'), and a 2*H*-2,2-dimethylpyran moiety at δ 2.81 (2H, t, *J*=6.8 Hz, H-1"), 1.83 (2H, t, *J*=6.8 Hz, H-2") and 1.32 (6H, s, Me×2), which indicated that the structure of **2** was similar to **1**. A methoxyl singlet at δ 3.72 in ^1H NMR spectrum together with the IR absorption at 1722 cm^{-1} inferred that **2** was a methyl ester of **1**. This compound has synthesized from 3-γ,γ-dimethylallyl-*p*-coumaric acid by Carrizo *et al.* in 1998.[6] This is the first report of **2** from natural source.



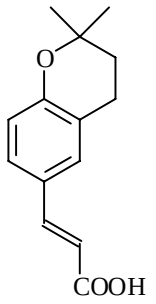
1 **3**

Figure 1 The NOE correlations of **1** and **3**

(*Z*)-Dupracine methyl ester (**3**) was isolated as colorless oil and its HREIMS spectrum gave identical molecular formula of **2**. The UV and IR spectral absorptions were also similar with those of **2**. The ^1H NMR spectrum revealed the presence of an ABX pattern signals at 7.53 (1H, d, *J*=2.2 Hz, H-2), 7.50 (1H, dd, *J*=8.8, 2.2 Hz, H-6) and 6.75 (1H, d, *J*=8.8 Hz, H-5), and a 2*H*-2,2-dimethylpyran moiety at δ 2.79 (2H, t, *J*=6.8 Hz, H-1"), 1.81 (2H, t, *J*=6.8 Hz, H-2") and 1.34 (6H, s, Me×2), a *cis* conjugated olefinic protons at δ 6.80 and 5.77 (each 1H, d, *J*=12.8 Hz, H-1', 2') and a methoxyl signal at δ 3.73 (3H, s, OMe). These data suggested that **3** was a *cis* isomer of **2**. The stereochemistry of **3** was also

inferred by the NOE cross-peaks between the two olefinic signals (Figure 1). On the basis of above data, the structure of **3** was determined as (*Z*)-dupracine methyl ester.

The known compounds, dupracine (**4**), (*E*)-3-[4-hydroxy-3-(3-methylbutenyl)phenyl]-2- propenoic acid (**5**), methyl-(*E*)-3-[4-hydroxy-3-(3-methylbutenyl)phenyl]-2-propenate (**6**), (*Z*)-3-[4-hydroxy-3-(3-methyl-butenyl)phenyl]-2-propenoic acid (**7**), (10*R*)-10-hydroxypheophorbide a methyl ester (**8**), (10*S*)-10-hydroxypheophorbide a methyl ester (**9**), uracil (**10**), bakkenolide-D (**11**), and methylparaben (**12**) were also isolated and identified from the leaves of *Petasites formosanus*. The structures of these known compounds were identified by comparison of their spectroscopic data (UV, IR, and MS spectroscopy) with literature values.



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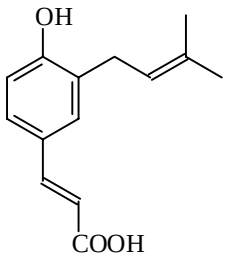

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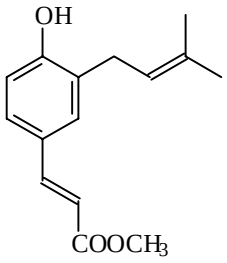

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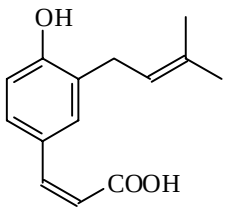

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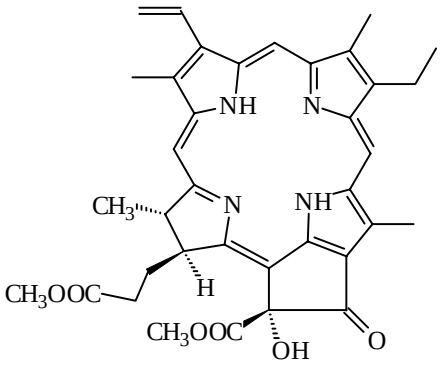

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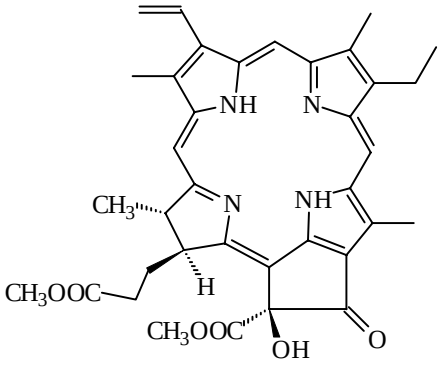

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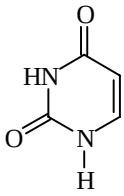

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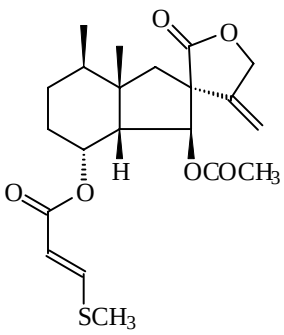

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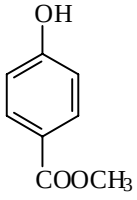

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The isolated compounds were subjected to evaluate their free radical-scavenging activity. The results were compared with α-tocopherol (α-Toc), which was commonly used in the food industry as antioxidant (IC_{50} 0.15 mg/mL, ~0.35 mM).[7] Among them, (*E*)-3-[4-hydroxy-3-(3-methylbutenyl)-phenyl]-2-propenoic acid (**5**), methyl-(*E*)-3-[4-hydroxy-3-(3-methylbutenyl)phenyl] -2-propenate (**6**) and (*Z*)-3-[4-hydroxy-3-(3-methyl-butenyl)phenyl]-2-propenoic acid (**7**) showed strong DPPH radical-scavenging effect activity with an IC_{50} values, 0.50, 0.53 and 0.46 mM, respectively (Table 1). These results implied that *P. formosanus* may be able to afford protection against oxidative damage.

Table 1. The DPPH radical-scavenging activity of the constituents isolated from the leaves of *Petasites formosanus* and α-Toc.

Sample	Conc. (mM)	Inhibition (%)	IC_{50}
1	1.0	-	
4	1.0	-	
5	1.0	96.6	
	0.5	52.2	0.50
	0.25	24.1	
	0.125	14.3	
6	1.0	86.3	
	0.5	48.1	0.53
	0.25	27.7	
	0.125	17.3	
7	1.0	80.1	
	0.5	55.3	0.46
	0.25	38.5	
	0.125	28.9	
11	1.0	-	
12	1.0	-	
α-Toc	1.0	79.4	
	0.5	77.9	0.20
	0.25	62.6	
	0.0625	10.6	

EXPERIMENTAL

General Experimental Procedures. Melting points were measured on a Yanagimoto MP-S3 micro melting point apparatus and are uncorrected. The UV spectra were recorded on a Hitachi UV-3210 spectrophotometer in MeOH solution. The IR spectra were measured on a Shimadzu FTIR-8501 spectrophotometer as KBr disks. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Varian-400 Unity Plus 400 spectrometer. Chemical shifts are shown in δ values with tetramethylsilane as an internal reference. The MS spectra were performed in the EI or FAB (matrix: glycerol) mode on a VG70-250S mass spectrometer. Optical rotations were obtained on a Jasco DIP-370 polarimeter.

Plant Material. The leaves of *Petasites formosanus* was collected from Al-Li mountains in Taiwan in August 1992 and verified by Prof. C. S. Kuoh. A voucher specimen was deposited in the Herbarium of Cheng Kung University, Tainan, Taiwan.

Extraction and Separation. The leaves of *Petasites formosanus* (4.3 kg) was extracted with MeOH (5 L × 5) at rt for 8 h, and the extract was concentrated to give a deep brown syrup (760 g). The residue was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer (85 g) was directly chromatographed on a silica gel column by elution with a gradient of $CHCl_3$-Me_2CO to afford nine fractions. Fr. 4 underwent column chromatography over silica gel using *n*-C_6H_{14}-Me_2CO (6:1) as an eluent to yield crystal (**9**) (1.1 mg, mp 212-213°C) and oil (**12**) (4.3 mg). Fr. 5 was rechromatographed on a silica gel column and eluted with C_6H_6-EtOAc (6:1) to give crystal (**8**) (2.3 mg, mp 232-233°C). The H_2O soluble layer was partitioned between H_2O and n-BuOH. A crystalline solid obtained from n-BuOH layer was chromatographed over sephadex G-10 to give crystal (**5**) (2 mg, mp 146-147°C). Then, the filtrate was chromatographed on Diaion HP-20 and eluted with a gradient of H_2O and MeOH to give 26 fractions. Fr. 1 was chromatographed on sephadex G-10 to afford crystal (**11**) (5.6 mg, mp 207-208°C) and crystal (**10**) (0.8 mg, mp >300°C), successively. Fr. 2 was chromatographed on RP-C18 to afford crystal (**10**) (0.4 mg, mp >300°C). Fr. 11 underwent column chromatography over silica gel using $CHCl_3$-Me_2CO-MeOH (5:1:1) as an eluent to yield crystal (**4**) (12 mg, mp 188-189°C),

crystal (**5**) (1.3 mg, mp 146-147°C) and crystal (**7**) (0.4 mg, mp122-123°C), successively. Fr. 13 was chromatographed on silica gel and eluted with $CHCl_3$-Me_2CO-MeOH (7:2:2) to afford crystal (**6**) (0.5 mg, mp 86-87°C), crystal (**7**) (0.2 mg, mp122-123°C), oil (**3**) (0.4 mg), oil (**2**) (4.3 mg), and crystal (**5**) (0.5mg, mp 146-147°C), respectively. Fr. 24 was chromatographed on silica gel and eluted with $CHCl_3$ and Me_2CO (4:1) to yield oil (**3**) (0.2 mg) and crystal (**4**) (1.2 mg, mp188-189°C).

Sodium dupracine (1) was obtained as colorless powder (mp >300°C); HRFABMS *m/z*: 255.0999 $[M+1]^+$ (Calcd for $C_{14}H_{16}O_3Na$: 255.1000); UV λ_{max}nm: 229, 291; IR $\nu_{max}cm^{-1}$: 2929, 1556, 1494, 1409, 1122; FABMS (rel. int.) *m/z*: 232 ($[M-Na+H]^+$, 83), 177 (100), 149 (20); ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.33 (1H, d, *J*=16.0 Hz, H-1'), 7.25 (1H, dd, *J*=8.8, 2.3 Hz, H-6), 7.23 (1H, d, *J*=2.3 Hz, H-2), 6.68 (1H, d, *J*=8.8 Hz, H-5), 6.34(1H, d, *J*=16.0 Hz, H-2'), 2.80 (2H, t, *J*=6.4 Hz, H-1"), 1.82 (2H, t, *J*=6.4 Hz, H-2"), 1.32 (6H, s, 3"-Me×2).

Acidification of 1. **1** (0.5 mg) was dissolved in 1 mL of 5 % HCl. The solution was eluted on a Sephadex LH-20 column with H_2O to afford NaCl (0.5 mg), which was determined with an atomic absorption spectrometer. It was then eluted with MeOH to give crystal dupracine (**4**).

(*E*)-Dupracine methyl ester (2) was obtained as colorless oil; HREIMS *m/z*: 246.1259 $[M]^+$ (Calcd for $C_{15}H_{18}O_3$: 246.1256); UV λ_{max}nm: 234, 316; IR $\nu_{max}cm^{-1}$: 2925, 2852, 1722, 1633, 1494, 1153; EIMS (rel. int.) *m/z*: 246 (M^+, 100), 191 (92), 148 (61), 131 (21), 76 (21); ^{1}H-NMR (Acetone-d_6, 400 MHz): δ 7.58 (1H, d, *J*=16.0 Hz, H-1'), 7.40 (2H, m, H-2, H-6), 6.74 (1H, d, *J*=8.8 Hz, H-5), 6.35 (1H, d, *J*=16.0 Hz, H-2'), 3.72 (3H, s, OMe), 2.81 (2H, t, *J*=6.8 Hz, H-1"), 1.83 (2H, t, *J*=6.8 Hz, H-2"), 1.32 (6H, s, 3"-Me×2); ^{13}C-NMR (Acetone-d_6, 100 MHz): δ 167.2 (C-3'), 156.6 (C-4), 144.8 (C-1'), 130.3 (C-6), 127.6 (C-2), 126.3 (C-3), 121.7 (C-1), 117.8 (C-2'), 114.7 (C-5), 75.1 (C-3"), 50.8 (OMe), 32.4 (C-2"), 26.4 (3"-Me×2), 22.2 (C-1").

(*Z*)-Dupracine methyl ester (3) was obtained as colorless oil; HREIMS *m/z*: 246.1253 $[M]^+$ (Calcd for $C_{15}H_{18}O_3$: 246.1256); UV λ_{max}nm: 231, 316; IR $\nu_{max}cm^{-1}$: 2926, 2855, 1722, 1495, 1265, 1153, 1122; EIMS (rel. int.) *m/z*: 246 (M^+, 100), 191 (74), 149 (22), 131 (22); ^{1}H-NMR (Acetone-d_6, 400 MHz): δ 7.53 (1H, d, *J*=2.2Hz, H-2), 7.50 (1H, dd, *J*=8.8, 2.2 Hz, H-6), 6.80 (1H, d, *J*=12.8 Hz, H-1'), 6.75

(1H, d, *J*=8.8 Hz, H-5), 5.77 (1H, d, *J*=12.8 Hz, H-2'), 3.73 (3H, s, OMe), 2.79 (2H, t, *J*=6.8 Hz, H-1"), 1.81 (2H, t, *J*=6.8 Hz, H-2"), 1.34 (6H, s, 3"-Me×2).

Free Radical-Scavenging Activity Assay. The effect of isolated compounds on the DPPH radical was estimated according to the method of Yamaguchi *et al*.[8] with minor modifications. A sample was dissolved in 0.1 mL DMSO and then added to 0.1 mL of 0.1 mM DPPH in ethanol. The mixture was shaken vigorously and allowed to stand for 30 min at rt in the dark. The absorbance at 517 nm by DPPH was measured by a μ Quant universal microplate spectrophotometer. α-Toc (Sigma Chemical Co.) was used as a standard agent. The capability to scavenge the DPPH radical was calculated using the following equation.[11]

Scavenging effect (%) = [1-(absorbance of sample at 517 nm / absorbance of control at 517 nm)]×100

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REFERENCES

1. C. M. Hsu and N. Y. Chiu, 'The Illustrated Wild plants of Taiwan', SMC Publishing Inc., Taipei, 1986, pp. 262-264.

2. C. H. Liao, F. N. Ko, T. S. Wu, and C. M. Teng, *J. Pharm. Pharmacol.*, 1997, **49**, 1248.

3. T. S. Wu, M. S. Kao, P. L. Wu, F. W. Lin, and L. S. Shi, *Phytochemistry*, 1999, **52**, 901.

4. T. S. Wu, M. S. Kao, P. L. Wu, F. W. Lin, L. S. Shi, M. J. Liou, and C. Y. Li, *Chem. Pharm. Bull.*, 1999, **47**, 375.

5. L. A. Golovina and G. K. Nikonov, *Khimiya Prirodnykh Soedinenii*, 1973, **6**, 700.

6. R. F. Carrizo, M. E. Sosa, L. S. Favier, F. Penna, E. Guerreiro, O. S. Giordano, and C. E. Tonn, *J. Nat. Prod.*, 1998, **61**, 1209.

7. L. S. Lai, S. T. Chou, and W. W. Chao, *J. Agric. Food Chem.*, 2001, **49**, 963.

8. T. Yamaguchi, H. Takamura, T. Motoba, and J. Terao, *Biosci. Biotechnol. Biochem*., 1998, **62**, 1201.