

Note

***p*-Coumaroyl Malate Derivatives of the *Pandanus amaryllifolius* Leaf and Their Isomerization**

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A novel *p*-coumaroyl dimethyl malate (**1**) was isolated from the *Pandanus amaryllifolius* leaf in addition to three known analogs of *p*-coumaroyl dimethyl malate (**2–4**), and their structures were elucidated by analysis of the spectroscopic data. The *p*-coumaroyl malate derivatives were isolated as a mixture of *E* and *Z* isomers. To determine the cause of isomerization, the *p*-coumaroyl malate isolated in this study was synthesized. We concluded that the *Z* isomer might be an artifact generated from the *E* isomer through purification steps.

Key words *Pandanus amaryllifolius*; *p*-coumaroyl dimethyl malate; isomerization

Pandanus amaryllifolius (also known as the Pandan leaf) belongs to the Pandanus genus (Pandanaeae), and grows in Southeast Asia where it is traditionally used while cooking common nonaromatic rice to impart the aroma of cooked rice. 2-Acetyl-1-pyrroline was isolated from *P. amaryllifolius* and is the key aroma compound in cooked rice.^{1,2} Pandan leaves are also used medicinally in South East Asia for such purposes as refreshing the fatigued body and, reducing fever.³ In addition, their antioxidant activity⁴ and antihyperglycemic effects⁵ have been reported. Various alkaloids have also been isolated from *P. amaryllifolius*,^{6–9} and several studies have reported their isolation and structure.^{10–15}

Here, we report the isolation of a novel *p*-coumaroyl dimethyl malate (**1**) in addition to three known *p*-coumaroyl malate derivatives (**2–4**) (Fig. 1). We also describe the possible isomerization of **1** during the purification.

Results and Discussion

p-Coumaroyl malate derivatives (**1**, **2**, and **4**) and *p*-coumaric acid (**3**) were isolated from *P. amaryllifolius*. Raw *P. amaryllifolius* leaves purchased in the market were first treated by steam distillation to remove essential oil. After removal of essential oil, the leaves were dried and extracted with methanol (MeOH) under reflux, and organic solvent was removed by vacuum evaporation resulting in MeOH extracts. These extracts were separated by Diaion HP-20 column chromatography and successively eluted with water, 50% aqueous MeOH, MeOH and acetone. The 50% MeOH eluate was separated by gel permeation chromatography using TOSOH Toyo-peal HW-40 (F) column, yielding 12 fractions (fr. 2-1 to 2-12). Of them, fr. 2-6 was further subjected to Sephadex LH-20 column chromatography to afford six fractions (fr. 2-6-1 to 2-6-6). Fraction 2-6-2 was purified by reverse phase HPLC to isolate compound **1**. Compound **2** was isolated from fr. 2-7 by Sephadex LH-20 chromatography followed by HPLC purification using an octadecyl-silica (ODS) column. Then, fr. 2-9 was fractionated by reverse phase HPLC to give compounds **3** and **4**. Structure elucidation of known compounds **2**, **3**, and **4** was conducted to compare the spectral data with literature values or authentic samples and were identified as *p*-coumaroyl malate,¹⁶ *p*-coumaric acid, and *p*-coumaroyl malic acid

1-methyl ester,^{17,18} respectively.

Compound **1** was obtained as a pale yellow oil; its molecular formula was determined to be C₁₅H₁₆O₇ from high-resolution electron ionization mass spectrometry (EI-MS) and ¹H- and ¹³C-NMR spectral data. The ¹H-NMR spectrum of **1** had A₂B₂-type aromatic proton signals at δ 6.81 (d, *J*=8.6 Hz, 2H) and δ 7.48 (d, *J*=8.6 Hz, 2H), and doublet signals at δ 6.37 (d, *J*=15.9 Hz, 1H) and δ 7.66 (d, *J*=15.9 Hz, 1H), which

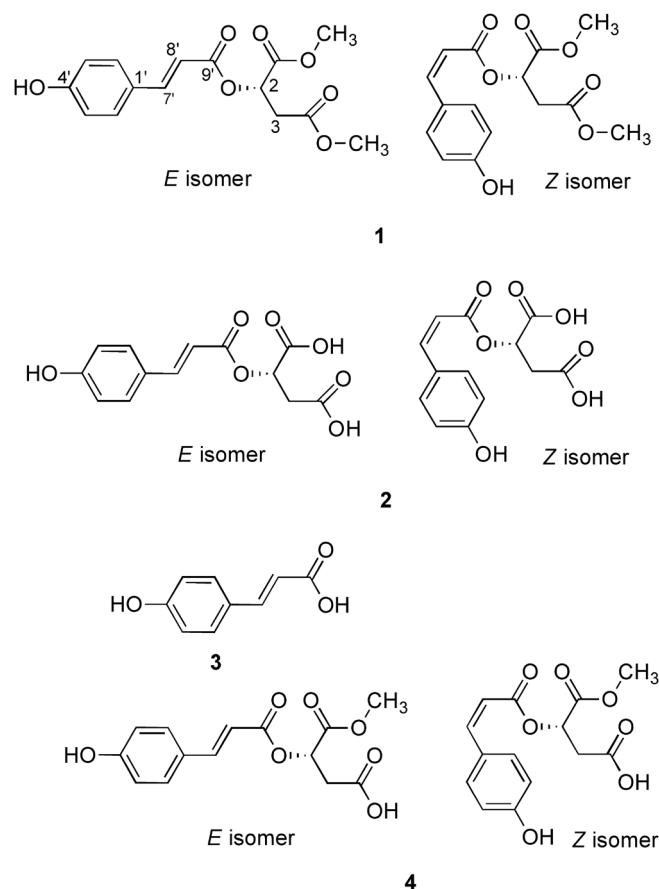


Fig. 1. Structures of Compounds Isolated from the Leaves of *Pandanus amaryllifolius*

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was attributed to olefinic protons. Furthermore, ^1H -NMR signals were observed for two methoxyl protons at δ 3.72 (s, 3H) and δ 3.77 (s, 3H), which correlated with signals at δ_{C} 52.8 and δ_{C} 53.3, respectively, in the heteronuclear single quantum correlation (HSQC) spectrum. Spin-spin coupling was observed between the methylene signal at δ 2.97 (m, 2H) and the methine signal at δ 5.52 (dd, $J=5.4$, 6.8 Hz, 1H) in the correlation spectroscopy (COSY) of the ^1H -NMR spectrum. The ^{13}C -NMR spectrum revealed 15 carbon signals, which arose from 2 methyl signals, 1 methylene, 5 methine and 5 quaternary carbons. In the heteronuclear multiple bond cor-

relation (HMBC) spectrum, long-range correlations of δ_{H} 5.52 (dd, $J=5.4$, 6.8 Hz, 1H) to δ_{C} 168.0 and δ_{C} 37.1 were observed. Long-range correlations were also observed from δ_{H} 6.37 (d, $J=15.9$, 1H) to δ_{C} 127.1 and δ_{C} 168.0, as shown in Fig. 2. These results indicate that **1** was *p*-coumaroyl dimethyl malate. The configuration of phenylpropanoide was confirmed as *trans* based on the J value of olefinic protons observed at δ_{H} 7.66 (d, $J=15.9$ Hz, 1H) and δ_{H} 6.37 (d, $J=15.9$ Hz, 1H). The *cis* form of **1** was also detected in the ^1H -NMR spectrum of **1**; thus, its structure elucidation was conducted in the same way. All of the ^1H - and ^{13}C -NMR spectral signals of the *trans* and *cis* isomers of **1** were assigned by two-dimensional NMR spectra and are listed in Tables 1, 2.

Some researchers have reported that the *cis* isomers of phenylpropanoide might be artifacts commonly found after the isolation of *trans* cinnamic acid derivatives.¹⁶⁾ Therefore,

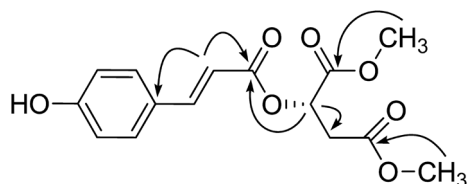


Fig. 2. Diagnostically Significant Long-Range Correlations of **1**

Table 1. ^1H -NMR Spectral Data of Compound **1**

Position	<i>E</i> Form	<i>Z</i> Form
2	5.52 (dd, $J=5.4$, 6.8 Hz, 1H)	5.47 (dd, $J=4.7$, 7.5 Hz, 1H)
3	2.97 (m, 2H)	2.92 (m, 2H)
2'6'	7.48 (d, $J=8.6$ Hz, 2H)	7.65 (d, $J=8.8$ Hz, 2H)
3'5'	6.81 (d, $J=8.6$ Hz, 2H)	6.75 (d, $J=8.8$ Hz, 2H)
7'	7.66 (d, $J=15.9$ Hz, 1H)	6.95 (d, $J=12.8$ Hz, 1H)
8'	6.37 (d, $J=15.9$ Hz, 1H)	5.78 (d, $J=12.8$ Hz, 1H)
2-COOMe	3.72* (s, 3H)	3.69** (s, 3H)
3-COOMe	3.77* (s, 3H)	3.77** (s, 3H)

Measured at 400 MHz in $\text{MeOH}-d_4$. ***: may be interchanged.

Table 2. ^{13}C -NMR Spectral Data of Compound **1**

Position	<i>E</i> Form	<i>Z</i> Form
1	171.7 (s) [†]	171.6 (s) [‡]
2	69.9 (d)	69.7 (d)
3	37.1 (t)	36.9 (t)
4	171.4 (s) [†]	171.3 (s) [‡]
1'	127.1 (s)	127.6 (s)
2',6'	131.6 (d)	134.1 (d)
3',5'	117.1 (d)	116.0 (d)
4'	161.8 (s)	160.6 (s)
7'	148.1 (d)	147.0 (d)
8'	114.1 (d)	115.3 (d)
9'	168.0 (s)	167.0 (s)
2-COOMe	52.8 (q)*	52.8 (q)**
3-COOMe	53.3 (q)*	53.3 (q)**

Measured at 100 MHz in $\text{MeOH}-d_4$. ***,[†],[‡]: may be interchanged.

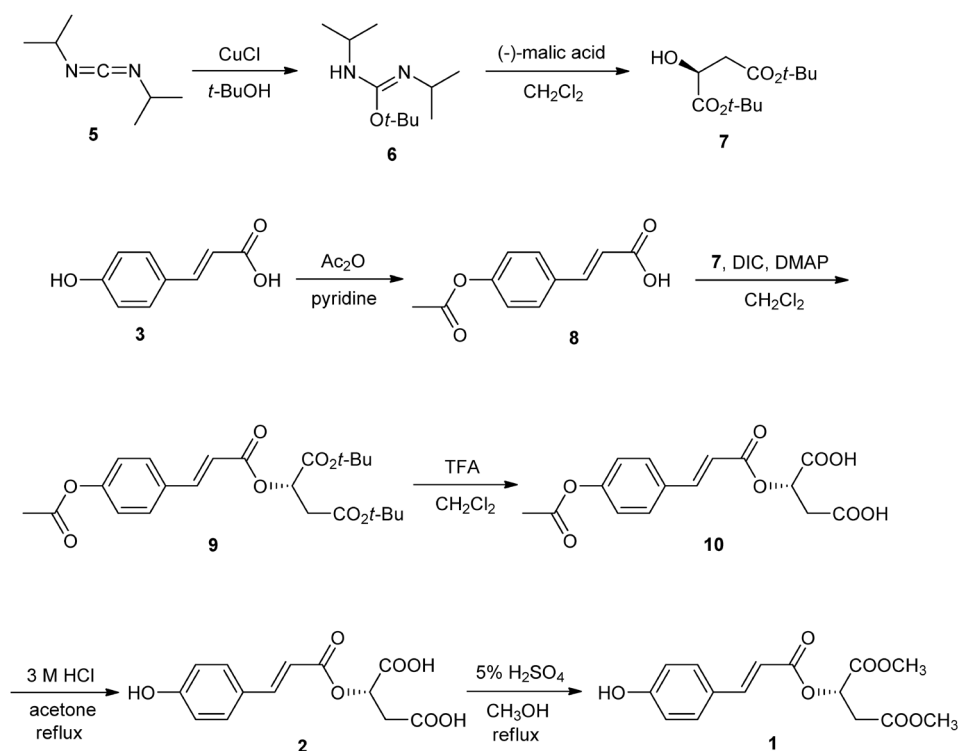


Chart 1. Synthesis of **1**

to confirm the possible isomerization of *p*-coumaroyl dimethyl malate (**1**) and *p*-coumaroyl malate (**2**), these isolated compounds were synthesized according to published methods¹⁹ as shown in the Chart 1. In brief, esterification of *tert*-butyl malate ester (**7**) and 4-*O*-acetyl coumaric acid (**8**) was conducted after which the protecting groups *tert*-butyl and acetyl were removed to yield *p*-coumaroyl malate (**2**). Finally methylation of *p*-coumaroyl malate (**2**) in MeOH containing a catalytic amount of sulfuric acid afforded *p*-coumaroyl dimethyl malate (**1**). Synthesized compound **1** was dissolved in deuterated MeOH (CD₃OD) and stored under a fluorescent light. After 24 h, the ¹H-NMR spectrum was measured to confirm isomerization of **1**. The results indicated that the ratio of *E* and *Z* isomers of **1** was 12:1. In contrast, synthesized **1** was subjected to HPLC equipped with a UV detector (wave-length was set to 254 nm) to give the corresponding peak. After drying, the ¹H-NMR spectrum of the isolated peak was measured and showed isomerization of **1** with an *E* and *Z* ratio of was 6:1. HPLC purification strongly induced isomerization of **1** compared to fluorescent light radiation. However, *p*-coumaric acid (**3**) and 4-*O*-acetyl coumaroyl malate (**10**), which was intermediate product of the synthesis process, were not isomerized through HPLC purification. For isomerization to occur, position 4 of the aromatic ring of 4-*O*-acetyl coumaroyl malate must be a hydroxyl group. These results indicated that *Z* isomer of **1**, **2** and **4** might be artifact generated from the *E* isomer through purification steps. On the other hand, absolute configuration at C-2 of **1** was determined as *R* based on comparison of the optical rotation of **1** with that synthesized from L-malate.

Experimental

General Experimental Procedures High resolution (HR) EI-MS was recorded on a JMS-700 (JEOL). ¹H, ¹³C, and two-dimensional NMR spectra were recorded at 400 MHz on an Agilent 400-MR NMR system at room temperature. ¹H chemical shifts were revealed in ppm relative to the quintet at δ 3.31 and ¹³C chemical shifts relative to the septet at δ 49.2 referring to solvent signal. Optical rotations were measured at P-2300 (JASCO).

Plant Material *P. amaryllifolius* was obtained from the market (QWACCHI Inc., Chiba, Japan). A voucher specimen (No. PNM-2013-1) was deposited in the Laboratory of Pharmacognosy and Natural medicines.

Extraction and Isolation Steam distillation was employed to remove essential oil from *P. amaryllifolius*. Briefly, steam was passed over the wet leaf of *P. amaryllifolius* (1.88 kg). Then, the leaves were dried and extracted with MeOH in reflux to yield a MeOH extract (72 g). The MeOH extract was subjected to Diaion HP-20 chromatography and eluted successively with water→50% aqueous MeOH→MeOH→acetone to afford the corresponding soluble fractions. Of them, the 50% aqueous MeOH soluble fraction (fr. 2, 5.6 g) was subjected to chromatography on a Toyopearl HW-40F column eluted with 50% aqueous MeOH to give twelve fractions (fr. 2-1 to fr. 2-12). Fraction 2-6 (130 mg) was separated on a Sephadex LH-20 column eluted with chloroform–MeOH (1:1)→chloroform to afford six fractions (fr. 2-6-1 to fr. 2-6-6). Fraction 2-6-2 (19.6 mg) was purified with HPLC on an ODS column eluted with 50% aqueous acetonitrile containing formic acid at 0.1% to give compound **1** (3.7 mg). Fraction 2-7 (110 mg)

was subjected to Sephadex LH-20 chromatography eluted with chloroform–MeOH (2:1)→(1:1) to give six fractions (fr. 2-7-1 to fr. 2-7-6). Compound **2** (10.8 mg) was purified by HPLC with an ODS column eluted with MeCN–H₂O (19:81) from fr. 2-7-3 (29.4 mg). Fraction 2-9 (81 mg) was purified by HPLC equipped with an ODS column eluted with 50% aqueous acetonitrile containing formic acid at 0.1% to give compound **3** (3.0 mg) and **4** (4.9 mg).

***p*-Coumaroyl Dimethyl Malate (**1**)** Pale yellow oil; [α]_D: –16.3° (*c*=0.1, MeOH); UV (MeOH) λ_{max} 314 nm given from photodiode array detector equipped with HPLC system, ¹H-NMR (400 MHz, MeOH-*d*₄), see Table 1; ¹³C-NMR (100 MHz, MeOH-*d*₄), see Table 2; HR-EI-MS *m/z* 308.0897 [M]⁺ (calcd for C₁₅H₁₆O₇, 308.0896).

Synthesis of *p*-Coumaroyl Malate Derivatives The isolated compounds **1** and **2** were synthesized according to a modified procedure.¹⁹ In brief, *N,N'*-diisopropyl-*O*-*tert*-butylisourea (**6**) was obtained from the reaction of diisopropylcarbodiimide (DIC) (**5**) (40.8 g, 323 mmol) and *tert*-butyl alcohol (27.8 g, 375 mmol) with a catalytic amount of copper (I) chloride for 4 d (yield: 78.1%). Malate **7** was thereafter obtained by adding excess **6** (38.8 g, 194 mmol) to a solution of (–) malic acid (3.6 g, 26.9 mmol) in dichloromethane (300 mL) at room temperature (yield: 14.0%). Acetylation of commercially available *p*-coumaric acid (**3**) (1 g, 6.0 mmol) by treatment with acetic anhydride (1.74 mL, 18.4 mmol) in pyridine (10 mL) gave 4-*O*-acetyl coumaric acid (**8**) (yield: 78%). The coupling reaction of the obtained coumaric acid derivative (**8**) (1.0 g, 5.05 mmol) and malate **7** (1.2 g, 5.05 mmol) in the presence of DIC (0.65 mL) and *N,N*-dimethyl-4-aminopyridine (DMAP) (255.6 mg) afforded the condensation product (**9**) (yield: 18.3%). Hydrolysis of compound **9** by treatment with trifluoroacetic acid (TFA) followed by removal of the acetyl group under acidic conditions (3 M HCl–acetone, reflux) gave *p*-coumaroyl malate (**2**) (total yield: 11.0%). Methylation of **2** was achieved by treatment of MeOH involving 5% sulfuric acid (H₂SO₄) in reflux to obtain compound **1** (total yield: 4.6%).

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Conflict of Interest The authors declare no conflict of interest.

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