Abstract

From the rhizomes of *Etlingera pavieana* (Pierre ex Gagnep.) R.M. Sm., four phenylpropens, 

(E)-3-(4-methoxyphenyl)prop-2-en-1-amine (1), (E)-4-methoxycinamaldehyde (2), (E)-4-methoxycinamic acid (3) and (E)-1-methoxy-4-(3-methoxyprop-1-enyl)benzene (4), together with two other compounds, (E)-(E)-3-(4-methoxyphenyl)allyl)3-(4-hydroxyphenyl)acrylate (5) and 4-methoxybenzoic acid (6) were isolated. This is the first report on the presence of all compounds in *Etlingera*. Compounds 1 and 5 have been previously synthesised, but this is the first report of their isolation from a natural source. Compound 5 exhibited weak activity against *Mycobacterium tuberculosis* with MIC 50.00 µg/mL and cytotoxic activity against the KB, MCF7 and NCI–H187 cells with IC_{50} values of 25.11, 20.16 and 34.83 µg/mL, respectively.

**Keywords:** *Etlingera pavieana*; Zingiberaceae; (E)-3-(4-methoxyphenyl)prop-2-en-1-amine; (E)-(E)-3-(4-methoxyphenyl)allyl)3-(4-hydroxyphenyl)acrylate; cytotoxic activity.
Experimental

Plant material

The rhizomes of *E. pavieana* were collected from Chanthaburi province of Thailand. The voucher specimen was authenticated J. F. Maxwell, Taxonomist of the CMU Herbarium and deposited at the CMU Herbarium, Department of Biology, Faculty of Science, Chiang Mai University, Thailand with specimen number CMU01– Sirichan Tachai.

General experimental procedures

Melting points were determined on an Electrothermal apparatus and are uncorrected. IR spectra were obtained using a Tensor 27 FT-IR Spectrophotometer. $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer, operating at 400 and 100 MHz, respectively. Electron impact mass spectra were recorded with Agilent-HP 5973 mass spectrometer. TLC was carried out on precoated silica gel 60 F$_{254}$ (0.2 mm thick, Merck) plate. The TLC spots were visualized under UV light and by heating the plates after spraying with anisaldehyde-H$_2$SO$_4$ reagent. Silica gel column chromatography was performed on Merck silica gel 60 (finer than 0.063 mm).

Extraction and Isolation

The air dried powdered rhizomes of *E. pavieana* (1.35 kg) were successively extracted with *n*-hexane, CH$_2$Cl$_2$ and MeOH (3x3L) at room temperature. The extracts were concentrated under reduced pressure to obtain the *n*-hexane extract (brownish viscous oil, 9.66 g), the CH$_2$Cl$_2$ extract (brownish viscous oil, 10.76 g) and the methanol extract (dark brownish gummy, 75.30 g). The CH$_2$Cl$_2$ extract showed cytotoxic activities and were therefore investigated for active compounds. The CH$_2$Cl$_2$ extract (10.5 g) was chromatographed over silica gel and eluted with binary mixture of *n*-hexane–CH$_2$Cl$_2$ (95:5 to 0:100), CH$_2$Cl$_2$–EtOAc (95:5 to 0:100) and EtOAc–MeOH (95:5 to 50:50) which afforded 13 fractions (C$_1$–C$_{13}$). Precipitate from fraction C$_1$ (0.27g) was recrystallised in *n*-hexane–EtOAc to yield compound 1 (257.9 mg). Precipitate from fraction C$_2$ (0.31g) was recrystallised in *n*-hexane–EtOAc to yield compound 1 (310.0 mg). Filtrate of fractions C$_1$ and C$_2$ was combined and rechromatographed and eluted with CH$_2$Cl$_2$ to afford compound 2 (1.8 mg). Fraction C$_4$ (1.64 g) was rechromatographed using *n*-hexane–CH$_2$Cl$_2$ (50:50) to give 5 fractions (C$_{4a}$–C$_{4e}$). Fraction C$_{4b}$ was purified by was purified
by preparative layer chromatography using $n$-hexane-acetone (95:5) as eluent, to afford compound 3 (1.9 mg). Fraction $C_{4c}$ was rechromatographed and eluted with $n$-hexane–$CH_2Cl_2$–acetone (60:20:20) to give 6 fractions ($C_{4c1}$–$C_{4c6}$). Fraction $C_{4c6}$ was purified by silica gel column and eluted with $n$-hexane–$CH_2Cl_2$–MeOH (38:58:4) to afford compounds 4 (6.70 mg) and 5 (49.9 mg). Fraction $C_5$ was rechromatographed on silica gel and eluted with $CH_2Cl_2$–EtOAc (95:5 to 0:100) to give 9 fractions ($C_{5a}$–$C_{5i}$). Fraction $C_{5c}$ was rechromatographed and eluted with $CH_2Cl_2$–EtOAc (95:5) to yield compound 6 (3.6 mg). All compounds 1–6 were identified by interpretation of their spectral data including EIMS, $^1$H and $^{13}$C NMR (including DEPT135, COSY, HMQC and HMBC experiments), as well as by comparison of their spectral data with those reported in the literature. Placement of the methoxy group at the 4‴-OCH$_3$ of compound 5 was confirmed by HMBC correlation of the methoxy protons with C-3‴ (δ 114.03), C-4‴ (δ 159.65) and C-5‴ (δ 114.03) as shown in Figure S1 and Table S1.

**Spectroscopic data of isolated compounds**

(E)-3-(4-methoxyphenyl)prop-2-en-1-amine (1):
white crystal; m.p= 155.2-156.4 °C; EIMS: $m/z$ 147 (100), 132 (5), 121 (2), 115 (11), 91 (12), 77 (4); $^1$H NMR (400 MHz, CDCl$_3$): δ 7.28 (2H, d, $J$=8.7 Hz, H-2,6), 6.85 (2H, d, $J$=8.7 Hz, H-3,5), 6.42 (1H, d, $J$=15.6 Hz, H-3′), 6.04 (1H, dt, $J$=15.6, 7.7 Hz, H-2′), 3.81 (s, 3H, OCH$_3$), 3.48 (2H, d, $J$=7.7 Hz, H-1′); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 159.3 (C-4), 133.1 (C-3′), 129.5 (C-1), 127.6 (C-2,6), 122.30 (C-2′), 114.0 (C-3,5), 55.3 (OCH$_3$), 42.7 (C-1′); FTIR (neat): $\nu$ = 3427, 2916, 2848, 1603, 1574, 1173, 1510, 1244, 1024, 830, 804 cm$^{-1}$.

(E)-4-methoxycinnamaldehyde (2):
pale yellow amorphous solid; m.p= 57.0-59.2 °C; EIMS: $m/z$ 162 (100), 161 (80), 133 (22), 131 (61), 91 (37), 77 (19); $^1$H NMR (400 MHz, CDCl$_3$): δ 9.65 (1H, d, $J$=7.8 Hz, H-1′), 7.53 (2H, d, $J$=8.8 Hz, H-2,6), 7.45 (1H, d, $J$=15.8 Hz, H-3′), 6.95 (2H, d, $J$=8.8 Hz, H-3,5), 6.62 (1H, dd, $J$=15.8, 7.8 Hz, H-2′), 3.85 (s, 3H, OCH$_3$); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 193.7 (C-1′), 162.0 (C-4), 152.7 (C-3′), 130.3 (C-2,6), 126.6 (C-2′), 126.5 (C-1), 114.6 (C-3,5), 55.4 (OCH$_3$); FTIR (neat): $\nu$ = 2926, 2847, 1674, 1599, 1510, 1255, 969, 821 cm$^{-1}$. 
(E)-4-methoxycinnamic acid (3):
colourless crystals; m.p= 172.7-174.6 °C; EIMS: m/z 178 (100), 177 (13), 161 (32), 133 (20), 132 (15), 91 (7), 77 (19); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.74 (1H, d, \(J=15.9\) Hz, H-3'), 7.51 (2H, d, \(J=8.7\) Hz, H-2,6), 6.92 (2H, d, \(J=8.7\) Hz, H-3,5), 6.32 (1H, d, \(J=15.9\) Hz, H-2'), 3.85 (s, 3H, 4-OCH\(_3\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 171.3 (C-1'), 161.7 (C-4), 146.7 (C-3'), 130.1 (C-2,6), 126.8 (C-1), 115.0 (C-2'), 114.4 (C-3,5), 55.4 (OCH\(_3\)).; FTIR (neat): \(\nu\) = 3456, 2937, 1678, 1600, 1513, 1257, 825 cm\(^{-1}\).

(E)-1-methoxy-4-(3-methoxypent-1-enyl)benzene (4):
yellow solid; m.p= 114.3-115.8 °C; EIMS: m/z 147 (100), 132 (9), 91 (20), and 71 (8); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.36 (2H, d, \(J=8.7\) Hz, H-2,6), 6.88 (2H, d, \(J=8.7\) Hz, H-3,5), 6.65 (1H, d, \(J=15.8\) Hz, H-1'), 6.13 (1H, dt, \(J=15.8\), 7.6 Hz, H-2'), 3.86 (1H, d, \(J=7.6\) Hz, H-3'), 3.82 (s, 3H, 4-OCH\(_3\)), 2.88 (s, 3H, 3'-OCH\(_3\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 160.3 (C-4), 138.7 (C-1'), 128.3 (C-1), 128.2 (C-2,6), 114.3 (C-3,5), 113.2 (C-2'), 59.4 (C-3'), 55.5 (C4-OCH\(_3\)), 39.2 (C1'-OCH\(_3\)); FTIR (neat): \(\nu\) = 2937, 1600, 1511, 1245, 1124, 975, 814 cm\(^{-1}\).

(E)-(E)-3-(4-methoxyphenyl)allyl)3-(4-hydroxyphenyl)acrylate (5):
pale yellow solid; m.p= 109.7-110.5 °C; EIMS: m/z 310 (6), 309 (22), 279 (10), 173 (24), 147 (100), 121 (14); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.67 (1H, d, \(J=16.0\) Hz, H-3'), 7.43 (2H, d, \(J=8.6\) Hz, H-2,6), 7.35 (2H, d, \(J=8.7\) Hz, 2''',6'''), 6.86 (4H, t, \(J=8.6\) Hz, H-3,5, 3''',5'''), 6.65 (1H, d, \(J=15.8\) Hz, H-3''), 6.34 (1H, d, \(J=16.0\) Hz, H-2'), 6.22 (1H, dt, \(J=15.8\), 6.6 Hz, H-2''), 5.37 (s, 1H, 4'-OH), 4.84 (1H, d, \(J=6.6\) Hz, H-1''), 3.81 (s, 3H, 4'''-OCH\(_3\)); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 167.1 (C-1'), 159.6 (C-4'''), 157.6 (C-4), 144.6 (C-3'), 133.9 (C-3''), 130.0 (C-2,6), 127.9 (C-2''',6'''), 128.6 (C-1''), 127.2 (C-1), 121.1 (C-2''), 115.8 (C-3,5), 115.5 (C-2'), 114.0 (C-3''',5'''), 65.3 (C-1''), 55.3 (OCH\(_3\)); FTIR (neat): \(\nu\) = 3338, 1680, 1600, 1511, 1250, 1168, 971, 832 cm\(^{-1}\).

4-methoxybenzoic acid (6):
white solid; m.p= 184.2-184.6 °C; EIMS: m/z 152 (M\(^+\), 88), 135 (100), 107 (11) and 77 (20); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.07 (2H, d, \(J=8.8\) Hz, H-2,6), 6.95 (2H, d, \(J=8.8\) Hz, H-3,5), 3.88
(s, 3H, 4-OCH₃); $^{13}$C NMR (100 MHz, CDCl₃): δ 171.3 (C-1’), 164.0 (C-4), 132.3 (C-2,6), 121.6 (C-1), 113.7 (C-3,5), 55.5 (OCH₃); FTIR (neat): ν = 3464, 1686, 1603, 1427, 1261, 845 cm⁻¹.

**Antimycobacterial Assay**

The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H₃⁷Ra using the green fluorescent protein microplate assay (GFPMA) (Changsen et al., 2003). The lowest drug concentration effecting and inhibition of ≥ 90% was considered the MIC. The standard drugs, rifampicine, streptomycin, isoniazid, oflaxacin and ethambutol showed MIC values of 0.00312-0.025, 0.156-0.313, 0.0234-0.0468, 0.391-0.781 and 0.234-0.469 µg/mL, respectively.

**Cytotoxic activity**

The cytotoxicity against human oral cavity cancer (KB), human breast cancer (MCF7) and human small cell lung cancer (NCI-H187) cells was determined by resazurin microplate assay (REMA) which was a modified method of the use of a fluorescent dye for mammalian cell cytotoxicity (O’Brien et al., 2000). Briefly, cells at a logarithmic growth phase were harvested and diluted to $9 \times 10^4$ cells/ml in fresh medium. Successively, 5 µl of test sample, diluted in 10% DMSO and 45 µl of cells suspension were added to 384-well plates. Plates were incubated at 37 °C in 5% CO₂ incubator for 5 days. After that, 12.5 µl of 62.5 µg/ml resazurin solution was added to each well and the plates were then incubated at 37 °C for 4 h. Fluorescence signal was measured using SpectraMax M5 multidetection microplate reader at dual wavelengths of 530 and 590 nm. IC₅₀ values were derived from dose-response curves, using six concentrations of 2-fold serially diluted samples, by the SOFTMax Pro software (Molecular device). The standard drugs ellipticine exhibited IC₅₀ values against KB and NCI-H187 at 1.10, 0.220 and 1.21 µg/mL and tamoxifen exhibited IC₅₀ values against MCF7 at 5.83 µg/mL.

**References**

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Table S1. $^1$H-, $^{13}$C-NMR and HMBC data of compound 5

| Positions | $\delta^1$H (Mult., $J$ in Hz) | $\delta^{13}$C | HMBC |
|-----------|---------------------------------|---------------|------|
|           | Compound 5                      | Ref. (Hu et al., 2005) | Compound 5 | |
| 1'        | 4.84 ($d$, 6.6)                 | 4.87 ($d$, 6.4) | 167.13 | C-1', 2'', 3'' |
| 1''       | 6.34 ($d$, 16.0)                | 6.37 ($d$, 16.0) | 65.32 | C-1', 3' |
| 2'        | 6.23 ($dt$, 15.8, 6.6)          | 6.36 ($dt$, 16.0, 6.4) | 115.57 | C-1'', 1''' |
| 2''       | 7.67 ($d$, 16.0)                | 7.67 ($d$, 16.0) | 121.10 | C-1', 2, 3, 5, 6 |
| 3'        | 6.65 ($d$, 15.8)                | 6.71 ($d$, 16.0) | 144.62 | C-1'', 2'', 6'' |
| 1         | 127.27                          | -              |      | |
| 1'''      | 128.59                          | -              |      | |
| 2, 6      | 7.43 ($d$, 8.6)                 | 6.94-7.43 (m)  | 130.00 | C-3', 4 |
| 2''', 6'''| 7.35 ($d$, 8.7)                 | 6.94-7.43 (m)  | 127.90 | C-3'', 4''' |
| 3, 5      | 6.85 ($t$, 8.5)                 | 6.94-7.43 (m)  | 115.84 | C-1, 4 |
| 3''', 5'''| 6.87 ($t$, 8.6)                 | 6.94-7.43 (m)  | 114.03 | C-1''', 4''' |
| 4         | 157.59                          | -              |      | |
| 4''''     | 159.65                          | -              |      | |
| 4-OH      | 3.81 (s)                        | 3.93 (s)       | 115.84 | C-3'''', 4''''' |
| 4''''-OCH$_3$ | 3.31 (s)               | 3.93 (s)       | 55.30 |      |

Figure S1. Selected HMBC correlations of compound 5