Arch Pharm Res Vol 29, No 6, 497-502, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Cytotoxic C-Benzylated Chalcone and Other Constituents of *Ellipeiopsis cherrevensis*

Lalita Wirasathien, Thitima Pengsuparp, Masataka Moriyasu[1], Kazuko Kawanishi[1], and Rutt Suttisri

Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand and [1]Kobe Pharmaceutical University, 4-19-1 Motoyamakitamachi, Higashinada-ku, Kobe 658-8558, Japan

(Received January 27, 2006)

A new natural C-benzylated chalcone, 2',4'-dihydroxy-3'-(2-hydroxybenzyl)-6¢-methoxychalcone (**2**), along with two other flavonoids, tiliroside and kaempferol 3-*O*-rutinoside, and an oxoaporphine alkaloid, lanuginosine were isolated from the aerial parts of *Ellipeiopsis cherrevensis* (Annonaceae). Two known polyoxygenated cyclohexene derivatives, ferrudiol and zeylenol, and a new analog, ellipeiopsol D, were also isolated. The chalcone **2** exhibited cytotoxic activity against human small-cell lung-cancer (NCI-H187), epidermoid carcinoma (KB) and breast cancer (BC) cell lines with IC_{50} values of 1.40, 5.31 and 13.92 μg/mL, respectively. This compound also showed antimalarial activity against *Plasmodium falciparum* with an IC_{50} value of 7.1 μg/mL as well as antimicrobacterial activity against *Mycobacterium tuberculosis* with a MIC of 25 mg/mL.

Key words: *Ellipeiopsis cherrevensis*, Annonaceae, Flavonoids, Cyclohexene derivatives, Oxoaporphine alkaloid, Cytotoxicity

INTRODUCTION

Ellipeiopsis cherrevensis (Pierre ex Finet & Gagnep.) R. E. Fr. is a member of the very small genus *Ellipeiopsis* of the family Annonaceae. This plant can be found growing in deciduous forests throughout Thailand. Its root is used in traditional medicine as a treatment for urinary disorders (Mahidol University Foundation, 2001). An investigation of the aerial parts of *E. cherrevensis* revealed the presence of several polyoxygenated cyclohexene derivatives (Kijjoa *et al.*, 2002). However, the biological activity of the chemical constituents of *E. cherrevensis* has never been evaluated.

Preliminary testing of the ethanolic extract from the aerial parts of *E. cherrevensis* demonstrated significant cytotoxic activity against three cancer cell lines: NCI-H187 (human small-cell lung-cancer), KB (human epidermoid carcinoma) and BC (breast cancer), with IC_{50} values of 0.01, 0.15 and 3.54 μg/mL, respectively. Therefore, this study further investigated this plant phytochemically. Two known cyclohexene derivatives, ferrudiol (**1**) and zeylenol (**4**), and one new analog, ellipeiopsol D (**3**), were isolated along with three flavonoids, a new natural 2',4'-dihydroxy-3'-(2-hydroxybenzyl)-6'-methoxychalcone (**2**), tiliroside (**5**) and kaempferol 3-*O*-rutinoside (**6**), and an oxoaporphine alkaloid, lanuginosine (**7**). These compounds were tested for their cytotoxic activity against three cancer cell lines, the malarial parasite *Plasmodium falciparum,* and *Mycobacterium tuberculosis.*

Correspondence to: Rutt Suttisri, Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
Tel: 66-02-2188353, Fax: 66-02-2188354
E-mail: Rutt.S@Chula.ac.th

MATERIALS AND METHODS

Plant material

The aerial parts of *Ellipeiopsis cherrevensis* were collected in Nakhon Ratchasima, Thailand, in October, 2002, and were identified by one of the authors (R. Suttisri, Chulalongkorn University). A voucher specimen (No. RS02101) was deposited at the herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

General experimental procedures

The melting points were measured on a Fisher-Johns melting point apparatus and were uncorrected. The UV spectra were recorded using a Shimadzu UV-160A spec-

trometer in $CHCl_3$ or MeOH. The IR spectra were obtained on a Perkin Elmer FT-IR 1760X spectrophotometer. The ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Bruker Avance DPX-300 FT-NMR spectrometer. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained on either a JEOL JMN-A 500 or a Varian VXR-500 NMR spectrometer. TMS was used as the internal standard. The electrospray ionization-time of flight (ESI-TOF) mass spectra were recorded on a Micromass LCT mass spectrometer and the high resolution electrospray ionization (HR-ESI) mass spectra were recorded on a Hitachi M-4100 instrument. Column chromatography was performed on silica gel (Kieselgel 60, 230-400 mesh, Merck) and Sephadex LH-20 (Pharmacia). TLC was carried out on silica gel 60 F_{254} plates (0.25 mm, Merck), and the spots were detected under UV light and by spraying with a 10% (v/v) H_2SO_4 solution followed by heating at 110°C for 10 min.

Extraction and isolation

The aerial parts of *E. cherrevensis* were separated into leaves and stems. The dried and powdered leaves (500 g) were macerated with hexane (4 × 3 L), $CHCl_3$ (4 × 3 L) and MeOH (4 × 3 L), respectively. Each extract was evaporated under reduced pressure at 45°C. The $CHCl_3$ extract (15 g) was chromatographed on a silica gel column (6 × 13 cm), using a hexane-EtOAc gradient (1:0 → 0:1) as the mobile phase, to give 13 combined fractions (LC01-LC13). Compound **1** (61.8 mg) was recrystallized from fraction LC04 in a mixture of hexane-EtOAc (2:1). Fraction LC06, which was subjected to two successive Sephadex LH-20 columns and eluted with $CHCl_3$-MeOH (2:1) and MeOH, respectively, yielded compound **2** (12.7 mg). Fractions LC08 and LC09, which was repeatedly chromatographed on a Sephadex LH-20 column using $CHCl_3$-MeOH (2:1) as the eluent, afforded compounds **3** (72.3 mg) and **4** (609.9 mg), respectively. The MeOH extract (20 g) was separated over a silica gel column (6 × 13 cm), and eluted with a $CHCl_3$-MeOH gradient (1:0 → 0:1), to give 14 pooled fractions (LM01-LM14). Recrystallization of fraction LM05 in a mixture of $CHCl_3$-MeOH (2:1) yielded compound **5** (12.3 mg). Fraction LM07 was subjected to silica gel column chromatography (3 × 26 cm) using $CHCl_3$-MeOH (2:1) as the mobile phase, which was followed by further Sephadex LH-20 column chromatography with MeOH as the eluent, to give compound **6** (18.6 mg).

The dried and milled stems of this plant (370 g) were macerated with hexane (3 × 2.5 L), $CHCl_3$ (3 × 2.5 L) and MeOH (3 × 2.5 L), respectively. The $CHCl_3$ extract (8 g) was separated over a silica gel column (5 × 21 cm) using a $CHCl_3$-MeOH gradient (9:1 → 0:1) as the eluent, into 13 fractions (SC01-SC13). Fraction SC06, which was subjected to a silica gel column and washed down with hexane-EtOAc (1:1), followed by a Sephadex LH-20 column using MeOH as the eluent to yield compound **7** (6.5 mg).

Ferrudiol (1)

Colorless needles; m.p. 182-183°C. UV λ_{max} ($CHCl_3$) nm (log ε): 243 (4.35), 276 (3.65). IR ν_{max} (KBr) cm^{-1}: 3449 (OH), 1716 (C=O), 1272, 1112, 712. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 4.29 (1H, d, *J* = 8.5 Hz, H-2), 4.74 (1H, d, *J* = 12.0 Hz, H-7a), 4.80 (1H, d, *J* = 12.0 Hz, H-7b), 5.80-5.84 (2H, m, H-3 and H-6), 5.86 (1H, dt, *J* = 10.3, 2.0 Hz, H-5), 5.98 (1H, dt, *J* = 10.3, 2.0 Hz, H-4), 7.30 (2H, tt, *J* = 8.0, 1.0 Hz, H-3' and H-5'''), 7.32 (2H, tt, *J* = 8.0, 1.5 Hz, H-3'' and H-5''), 7.44 (2H, tt, *J* = 7.8, 1.2 Hz, H-3' and H-5'), 7.47 (1H, tt, *J* = 8.0, 1.0 Hz, H-4'''), 7.49 (1H, tt, *J* = 8.0, 1.0 Hz, H-4''), 7.57 (1H, tt, *J* = 7.8, 1.2 Hz, H-4'), 7.90 (2H, dd, *J* = 8.0, 1.0 Hz, H-2''' and H-6'''), 7.97 (2H, dd, *J* = 7.8, 1.2 Hz, H-2' and H-6'), 8.07 (2H, dd, *J* = 8.0, 1.5 Hz, H-2'' and H-6''). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 62.7 (C-7), 72.9 (C-6), 75.5 (C-2), 76.1 (C-1), 76.7 (C-3), 127.9 (C-4), 128.3 (C-5, C-3''' and C-5'''), 128.4 (C-3'' and C-5ð), 128.5 (C-3' and C-5'), 129.0 (C-1'''), 129.4 (C-1''), 129.6 (C-1'), 129.7 (C-2''' and C-6'''), 129.9 (C-2' and C-6'), 130.0 (C-2ð and C-6''), 133.1 (C-4'''), 133.4 (C-4''), 133.6 (C-4'), 166.5 (C-7''), 166.6 (C-7'), 167.2 (C-7''').

2',4'-Dihydroxy-3'-(2-hydroxybenzyl)-6'-methoxychalcone (2)

Orange needles; m.p. 179-181°C. UV λ_{max} (MeOH) nm (log ε): 243 (3.92), 347 (4.31). IR ν_{max} (KBr) cm^{-1}: 3272, 1627 (C=O), 1449, 1338, 1235, 1115, 756, 568. HR-ESI-MS *m/z*: 376.1311 (calcd. for $C_{23}H_{20}O_5$: 376.1305). ^{1}H-NMR (500 MHz, acetone-d_6) δ: 3.90 (2H, s, H-7'), 3.95 (3H, s, 6'-OCH_3), 6.19 (1H, s, H-5'), 6.73 (1H, ddd, *J* = 7.6, 7.6, 1.2 Hz, H-5''), 6.82 (1H, dd, *J* = 7.6, 1.2 Hz, H-3''), 6.99 (1H, ddd, *J* = 7.6, 7.6, 1.2 Hz, H-4''), 7.25 (1H, dd, *J* = 7.6, 1.2 Hz, H-6''), 7.45 (3H, m, H-3 – H-5), 7.72 (2H, dd, *J* = 8.2, 1.8 Hz, H-2 and H-6), 7.83 (1H, d, *J* = 15.6 Hz, H-b), 8.04 (1H, d, *J* = 15.6 Hz, H-a), 15.04 (1H, s, 2'-OH). ^{13}C-NMR (125 MHz, acetone-d_6) δ: 22.9 (C-7'), 56.3 (6'-OCH_3), 92.4 (C-5'), 106.1 (C-3'), 108.1 (C-1'), 116.0 (C-3''), 120.6 (C-5''), 127.8 (C-1''), 127.9 (C-4''), 128.5 (C-a), 129.2 (C-3 and C-5), 129.8 (C-2 and C-6), 130.9 (C-4), 131.2 (C-6''), 136.5 (C-1), 142.7 (C-b), 155.2 (C-2''), 162.6 (C-6'), 164.2 (C-4'), 166.4 (C-2'), 193.2 (C=O).

Ellipeiopsol D (3)

Colorless crystals; m.p. 138-140°C. UV λ_{max} ($CHCl_3$) nm (log ε): 243 (3.96), 275 (3.31). IR ν_{max} (KBr) cm^{-1}: 3464 (OH), 1722 (C=O), 1275, 1116, 714. HR-ESI-MS *m/z*: 364.1159 (calcd. for $C_{18}H_{20}O_8$: 364.1152). ^{1}H-NMR (500

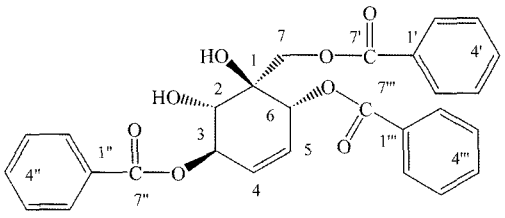
MHz, $CDCl_3$) δ: 2.03 (3H, s, 6-$OCOCH_3$), 2.05 (3H, s, 3-$OCOCH_3$), 4.04 (1H, d, *J* = 7.0 Hz, H-2), 4.46 (1H, d, *J* = 12.0 Hz, H-7a), 4.76 (1H, d, *J* = 12.0 Hz, H-7b), 5.44 (1H, d, *J* = 4.3 Hz, H-6), 5.49 (1H, m, H-3), 5.84 (1H, dd, *J* = 10.0, 2.0 Hz, H-4), 5.86 (1H, ddd, *J* = 10.0, 4.3, 2.0 Hz, H-5), 7.42 (2H, dd, *J* = 7.8, 1.3 Hz, H-3' and H-5'), 7.55 (1H, m, H-4'), 7.98 (2H, dd, *J* = 7.8, 1.3 Hz, H-2' and H-6'). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 21.1 (3-$OCOCH_3$ and 6-$OCOCH_3$), 66.5 (C-7), 70.2 (C-6), 71.3 (C-2), 73.1 (C-3), 74.7 (C-1), 125.9 (C-5), 128.5 (C-3' and C-5'), 128.8 (C-4), 129.7 (C-2' and C-6'), 129.8 (C-1'), 133.4 (C-4'), 167.0 (C-7'), 171.7 (3-$OCOCH_3$), 179.0 (6-$OCOCH_3$).

Zeylenol (4)

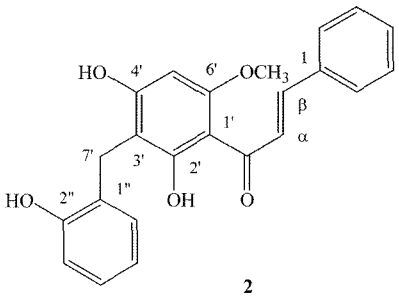
Colorless crystals; m.p. 104-105°C. UV λ_{max} ($CHCl_3$) nm (log ε): 243 (4.19), 276 (3.54). IR ν_{max} (KBr) cm^{-1}: 3462 (OH), 1679 (C=O), 1281, 1119, 712. ESI-TOFMS *m/z*: 384 $[M]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 4.22 (1H, d, *J* = 6.0 Hz, H-2), 4.32 (1H, d, *J* = 4.0 Hz, H-6), 4.72 (1H, d, *J* = 12.0 Hz, H-7a), 4.86 (1H, d, *J* = 12.0 Hz, H-7b), 5.69 (1H, m, H-3), 5.84 (1H, ddd, *J* = 10.3, 2.5, 0.5 Hz, H-4), 5.98 (1H, ddd, *J* = 10.3, 4.0, 2.0 Hz, H-5), 7.36 (4H, m, H-3', H-5', H-3" and H-5"), 7.51 (2H, m, H-4' and H-4"), 7.94 (2H, dd, *J* = 8.3, 1.5 Hz, H-2" and H-6"), 7.98 (2H, dd, *J* = 8.3, 1.5 Hz, H-2' and H-6'). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 66.7 (C-7), 68.6 (C-6), 70.8 (C-2), 74.2 (C-3), 75.9 (C-1), 126.8 (C-4), 128.4 (C-3', C-5', C-3" and C-5"), 129.2 (C-1'), 129.4 (C-1"), 129.7 (C-5), 129.8 (C-2', C-6', C-2" and C-6"), 133.4 (C-4'), 133.5 (C-4"), 167.1 (C-7"), 167.8 (C-7').

Tiliroside (5)

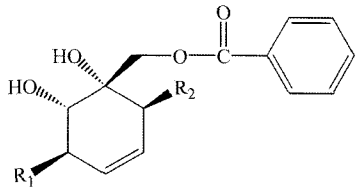
Pale yellow amorphous powder; m.p. 218-220°C. UV λ_{max} (MeOH) nm (log ε): 207 (4.87), 267 (4.67), 313 (4.78). IR ν_{max} (KBr) cm^{-1}: 3460 (OH), 1684 (C=O), 1608, 1502, 1356, 1183, 1068. ESI-TOFMS *m/z*: 594 $[M]^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.16-3.47 (4H, m, H-2" - H-5"), 4.08 (1H, dd, *J* = 11.1, 5.7 Hz, H-6"a), 4.27 (1H, d, *J* = 11.1 Hz, H-6"b), 5.44 (1H, d, *J* = 6.6 Hz, H-1"), 6.10 (1H, d, *J* = 16.9 Hz, H-8"'), 6.14 (1H, d, *J* = 1.8 Hz, H-8), 6.37 (1H, d, *J* = 1.8 Hz, H-6), 6.78 (2H, d, *J* = 7.8 Hz, H-2"' and H-6"'), 6.85 (2H, d, *J* = 8.7 Hz, H-2' and H-6'), 7.34 (1H, d, *J* = 16.9 Hz, H-7"'), 7.35 (2H, d, *J* = 7.8 Hz, H-3"' and H-5"'), 7.98 (2H, d, *J* = 8.7 Hz, H-3' and H-5'), 10.12 (1H, br s, 7-OH), 12.56 (1H, br s, 5-OH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 63.1 (C-6"), 70.1 (C-4"), 74.2 (C-2" and C-



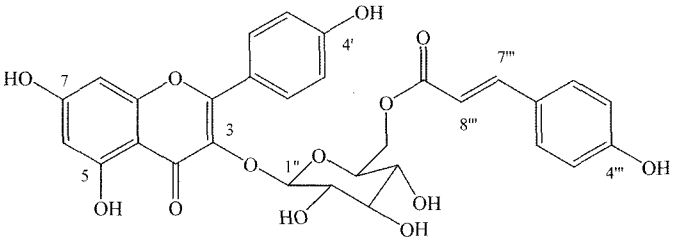
1



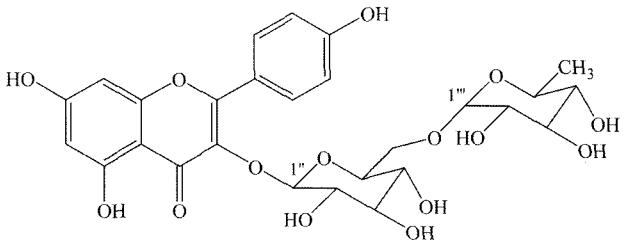
2



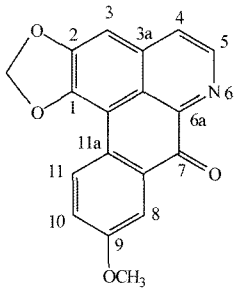
3 R_1 = OAc ; R_2 = OAc
4 R_1 = OBz ; R_2 = OH



5



6



7

5"), 76.3 (C-3"), 93.7 (C-6), 98.8 (C-8), 101.0 (C-1"), 103.8 (C-10), 113.6 (C-8'''), 115.1 (C-2' and C-6'), 115.7 (C-2''' and C-6'''), 120.7 (C-1'), 124.9 (C-1'''), 130.1 (C-3''' and C-5'''), 130.7 (C-3' and C-5'), 133.0 (C-3), 144.5 (C-7'''), 156.2 (C-2 and C-5), 159.6 (C-4'''), 159.8 (C-4'), 161.0 (C-9), 164.2 (C-7), 166.0 (C-9'''), 177.2 (C-4).

Kaempferol 3-*O*-rutinoside (6)

Pale yellow amorphous powder; m.p. 182-184°C. UV λ_{max} (MeOH) nm (log ε): 208 (4.51), 267 (4.33), 348 (4.25). IR ν_{max} (KBr) cm^{-1}: 3422 (OH), 1659 (C=O), 1608, 1510, 1364, 1182, 1064. ESI-TOFMS *m/z*: 594 $[M]^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.97 (3H, d, *J* = 6.0 Hz, H-6'''), 3.03-3.69 (10H, m, H-2" - H-6" and H-2''' - H-5'''), 5.09 (1H, d, *J* = 8.0 Hz, H-1'''), 5.29 (1H, d, *J* = 7.2 Hz, H-1"), 6.18 (1H, br s, H-6), 6.39 (1H, br s, H-8), 6.86 (2H, d, *J* = 8.4 Hz, H-3' and H-5'), 7.97 (2H, d, *J* = 8.4 Hz, H-2' and H-6'), 12.54 (1H, br s, 5-OH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 18.0 (C-6'''), 67.0 (C-6"), 68.4 (C-5'''), 70.1 (C-2'''), 70.5 (C-3'''), 70.7 (C-4"), 71.9 (C-4'''), 74.3 (C-2"), 75.9 (C-5"), 76.5 (C-3"), 93.9 (C-8), 98.9 (C-6), 100.8 (C-1"), 101.4 (C-1'''), 103.9 (C-10), 115.1 (C-3' and C-5'), 120.9 (C-1'), 130.8 (C-2' and C-6'), 133.2 (C-3), 156.4 (C-2), 156.7 (C-9), 159.8 (C-4'), 161.1 (C-5), 164.3 (C-7), 177.2 (C-4).

Lanuginosine (7)

Orange amorphous powder; m.p. >300°C. UV λ_{max} (MeOH) nm (log ε): 246 (3.06), 263 (2.92), 273 (2.95), 438 (2.30). IR ν_{max} (KBr) cm^{-1}: 2920, 1712 (C=O), 1604, 1462, 1378, 1341, 1304, 1265, 1229, 1035, 962. ESI-TOFMS *m/z*: 306 $[M+1]^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.97 (3H, s, 9-OCH_3), 6.33 (2H, s, -OCH_2O-), 7.12 (1H, s, H-3), 7.28 (1H, d, *J* = 9.3 Hz, H-10), 7.75 (1H, d, *J* = 5.1 Hz, H-4), 7.98 (1H, br s, H-8), 8.54 (1H, d, *J* = 9.3 Hz, H-11), 8.86 (1H, d, *J* = 5.1 Hz, H-5). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 55.8 (9-OCH_3), 102.3 (-OCH_2O-), 102.4 (C-3), 108.2 (C-1a), 110.3 (C-8), 122.6 (C-10), 122.7 (C-1b), 124.3 (C-4 and C-7a), 129.1 (C-11), 132.9 (C-11a), 135.9 (C-6a), 144.8 (C-5), 145.3 (C-3a), 148.0 (C-1), 151.8 (C-2), 159.8 (C-9), 182.3 (C-7).

Cytotoxicity assay

The colorimetric method reported by Skehan *et al.* (1990) was used to examine the cytotoxicity of the isolated compounds against three human cancer cell lines; small-cell lung-cancer (NCI-H187), epidermoid carcinoma (KB) and breast cancer (BC). Ellipticine was used as the reference material. IC_{50} values < 5 μg/mL, 5-10 μg/mL, 10-20 μg/mL and > 20 μg/mL were considered to be strongly active, moderately active, weakly active and inactive, respectively.

Antimalarial assay

The antimalarial activity against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain) was determined using the procedure previously described in the literature (Trager and Jensen, 1976). A quantitative assessment of the activity was performed in duplicate using the micro-culture radioisotope technique (Desjardins *et al.*, 1979). The 50% inhibitory concentration (IC_{50}) represents the concentration of the compound causing a 50% reduction in the growth of *P. falciparum*, as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by the parasite. Dihydroartemisinin was used as the positive control. IC_{50} values < 10 mg/mL were considered active.

Antimicrobacterial assay

An assay of the antituberculosis activity of the isolated compounds against *Mycobacterium tuberculosis* H_{37}Ra was performed in duplicate using the microplate Alamar blue method (Collins and Franzblau, 1997). Isoniazid and kanamycin sulfate were used as the reference compounds. Minimum inhibitory concentrations (MICs) < 200 μg/mL were considered active.

RESULTS AND DISCUSSION

Polyoxygenated cyclohexene derivatives are rare natural compounds that are found in only a few plant genera e.g. *Uvaria* of the Annonaceae (Kodpinid *et al.*, 1983; Takeuchi *et al.*, 2002; Xu *et al.*, 2005) and *Piper* of the family Piperaceae (Ruangrungsi *et al.*, 1992; Koul *et al.*, 1996). The genus *Ellipeiopsis* is closely related to *Uvaria* and the occurrence of these compounds in *E. cherrevensis* has been reported (Kijjoa *et al.*, 2002). This study isolated two known cyclohexene derivatives (compounds **1** and **4**), a new cyclohexene derivative (**3**) and a new natural C-benzylated chalcone (**2**) from the $CHCl_3$ extract from the leaves of *E. cherrevensis*. Compounds **1** and **4** were identified as ferrudiol (Schulte *et al.*, 1982; Kijjoa *et al.*, 2002) and zeylenol (Jolad *et al.*, 1981; Pan and Yu, 1995), respectively, by a comparison of their spectral data with that reported in the literature.

The high resolution MS and NMR data showed the orange compound **2** to have a molecular formula of $C_{23}H_{20}O_5$. The IR spectrum showed absorption peaks for a hydroxyl (3272 cm^{-1}) and conjugated carbonyl (1627 cm^{-1}) groups. The UV absorption maxima of compound **2** at 243 and 347 nm indicated this compound to be a chalcone (Markham, 1982). A chelated hydroxyl proton resonance in the ^{1}H-NMR spectrum at δ 15.04 and a carbon signal at δ 193.2 were consistent with a 2'-hydroxychalcone skeleton. The proton spectrum also showed characteristic *trans* double bond signals at δ 8.04 and 7.83 (both 1H, d, *J* = 15.6 Hz, H-a and H-b, respectively). The C-benzylated nature could be determined from the

number of aromatic proton and carbon signals as well as by a singlet resonance of the benzylic methylene protons appearing at δ 3.90 (H-7'). A comparison of the NMR spectral data for compound **2** with those previously reported for other C-benzylated chalcones revealed its ring substitution patterns to closely resemble those of 2',4'-dihydroxy-3'-(2,6-dihydroxybenzyl)-6'-methoxychalcone (Rahman *et al.*, 2003), which was isolated from *Desmos chinensis*. Detailed analysis of the data indicated that the difference in compound **2** was its benzyl moiety, which was substituted by one hydroxyl group at position 2" only. Therefore, compound **2** was identified as 2',4'-dihydroxy-3'-(2-hydroxybenzyl)-6'-methoxychalcone, which was previously reported to be an intermediate product in the chemical synthesis of uvaretin, an antitumour and antimicrobial dihydrochalcone found in several *Uvaria* species (Malterud *et al.*, 1985). However, to the best of our knowledge, this is the first report of its occurrence in nature.

The molecular formula of compound **3**, $C_{18}H_{20}O_8$, was determined by high resolution ESI mass spectrometry. Its IR spectrum suggested the presence of a hydroxyl group (3464 cm^{-1}), an ester carbonyl group (1722 cm^{-1}) and a monosubstituted phenyl ring (1602, 1453 and 712 cm^{-1}). The UV absorption maxima at 243 and 275 nm indicated the presence of one or two benzoyl group(s). The ^{1}H-NMR spectrum of compound **3** showed the signals of the five protons of a monosubstituted phenyl ring (at δ 7.42, 7.55 and 7.98) and two sharp singlets at δ 2.03 and 2.05, each integrating for three protons, which were assigned to two acetoxy methyl groups. The two olefinic proton signals at δ 5.84 (1H, dd, J = 10.0, 2.0 Hz, H-4) and 5.86 (1H, ddd, J = 10.0, 4.3, 2.0 Hz, H-5) with HMQC correlations with ^{13}C-NMR signals at δ 128.8 (C-4) and 129.5 (C-5), respectively, represent the double bond between positions 4 and 5 within the cyclohexene ring. A methine proton bearing a hydroxy group appeared as a doublet at δ 4.04 (J = 7.0 Hz, H-2), whereas the two acetoxyl groups could be established at C-3 and C-6 based on the downfield shifts of H-3 at δ 5.49 and H-6 at δ 5.44. Long-range HMBC correlations between both H-7 protons (at δ 4.04 and 4.76) and the carbonyl carbon of the benzoate (at δ 167.0), and a comparison with previously reported data, confirmed that the benzoate was at the usual C-7 position. The relative stereochemistry of compound **3** was established from its large $J_{2,3}$ value (7.0 Hz), indicating a *trans* pseudo-diaxial arrangement of H-2 and H-3, while the large $J_{5,6}$ value (4.3 Hz) indicated H-6 as being pseudoequatorial and the substituents at C-3 and C-6 as *cis* (Jolad *et al.*, 1981). Therefore, compound **3** was determined to be the new 6-acetate analog of ellipeiopsol A (Kijjoa *et al.*, 2002) and was named ellipeiopsol D.

The two other flavonoids, compounds **5** and **6**, were isolated from the MeOH extract of the *E. cherrevensis* leaves and identified as tiliroside [kaempferol 3-*O*-β-D-(6"-*p*-coumaroyl) glucopyranoside] (Nikaido *et al.*, 1987) and kaempferol 3-*O*-rutinoside (Ho *et al.*, 2002), respectively. An investigation of the $CHCl_3$ extract of its stems yielded compound **7**, which was identified as the oxoaporphine alkaloid, lanuginosine (Wijeratne *et al.*, 1996; Zhang *et al.*, 2002). These compounds were identified by an analysis of their spectral data and a comparison with the literature. This is the first report of these three compounds being isolated from this plant.

C-benzylated flavonoids are known to have cytotoxic activity (Cole *et al.*, 1976; Lasswell Jr. and Hufford, 1977). In this study, 2',4'-dihydroxy-3'-(2-hydroxybenzyl)-6'-methoxychalcone (**2**) showed strong cytotoxicity against human small cell lung cancer cell line (NCI-H187) with an IC_{50} value of 1.40 μg/mL (IC_{50} of ellipticine as the positive control was 0.35 μg/mL). It was moderately cytotoxic against human epidermoid carcinoma (KB) (IC_{50} = 5.31 μg/mL) and weakly cytotoxic against the breast cancer (BC) cell line (IC_{50} = 13.92 μg/mL). This C-benzylated chalcone also showed antimalarial activity against *Plasmodium falciparum* with an IC_{50} value of 7.1 μg/mL and antimicrobacterial activity against *Mycobacterium tuberculosis* H_{37}Ra with a MIC of 25 μg/mL. C-benzylated flavonoids are rarely found and have only been reported from *Uvaria* species of the Annonaceae. The existence of compound **2** in *Ellipeiopsis* confirms the close relationship between these two genera.

No other constituents of *E. cherrevensis* isolated in this study showed antimalarial or cytotoxic activity against the three cancer cell lines tested, even though there have been reports of the antitumor activity of cyclohexene derivatives (Pan *et al.*, 1998; Xu *et al.*, 2005) and tiliroside (**5**) (Esteves-Souza *et al.*, 2002). Ellipeiopsol D (**3**), zeylenol (**4**) and lanuginosine (**7**) were active against the microbe *Mycobacterium tuberculosis* with MIC values of 200, 100 and 100 mg/mL, respectively. Lanuginosine was previously reported to have antimicrobial (Ferdous *et al.*, 1992) and antiplatelet activity (Pyo *et al.*, 2003).

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Bioassay Research Facility of BIOTEC, NSTDA, Thailand for evaluating biological activities. This work was supported by grants from the Biodiversity Research and Training Program (BRT) and Thailand Research Fund.

REFERENCES

Cole, J. R., Torrance, S. J., and Wiedhopf, R. M., Uvaretin, a new antitumor agent from *Uvaria acuminata* (Annonaceae). *J. Org. Chem.*, 41, 1852-1855 (1976).

Collins, L. and Franzblau, S. G., Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.*, 41, 1004-1009 (1997).

Desjardins, R. E., Canfield, C. J., Haynes, J. D., and Chulay, J. D., Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.*, 16, 710-718 (1979).

Esteves-Souza, A., Sarmento da Silva, T. M., Fernandes Alves, C. C., de Carvalho, M. G., Braz-Filho, R., and Echevarria, A., Cytotoxic activities against Ehrlich carcinoma and human K562 leukaemia of alkaloids and flavonoids from two *Solanum* species. *J. Braz. Chem. Soc.*, 13, 838-842 (2002).

Ferdous, A. J., Islam, M. O., Hasan, C. M., and Islam, S. N., *In vitro* antimicrobial activity of lanuginosine and oxostephanine. *Fitoterapia*, 63, 549-550 (1992).

Ho, H. M., Chen, R. Y., Leung, L. K., Chan, F. L., Huang, Y., and Chen, Z. Y., Difference in flavonoid and isoflavone profile between soybean and soy leaf. *Biomed. Pharmacother.*, 56, 289-295 (2002).

Jolad, S. D., Hoffmann, J. J., Schram, K. H., and Cole, J. R., Structures of zeylenol and zeylena, constituents of *Uvaria zeylanica* (Annonaceae). *J. Org. Chem.*, 46, 4267-4272 (1981).

Kijjoa, A., Bessa, J., Pinto, M. M. M., Anatachoke, C., Silva, A. M. S., Eaton, G., and Herz, W., Polyoxygenated cyclohexene derivatives from *Ellipeiopsis cherrevensis*. *Phytochemistry*, 59, 543-549 (2002).

Kodpinid, M., Sadavongvivad, C., Thebtaranonth, C., and Thebtaranonth, Y., Structures of b-senepoxide, tingtanoxide, and their diene precursors. Constituents of *Uvaria ferruginea*. *Tetrahedron Lett.*, 24, 2019-2022 (1983).

Koul, J. L., Koul, S. K., Taneja, S. C., and Dhar, K. L., Oxygenated cyclohexanes from *Piper cubeb*. *Phytochemistry*, 41, 1097-1099 (1996).

Lasswell, Jr., W. L. and Hufford, C. D., Cytotoxic C-benzylated flavonoids from *Uvaria chamae*. *J. Org. Chem.*, 42, 1295-1302 (1977).

Mahidol University Foundation. Kok Ya E-San, Amarin Printing, Bangkok, p. 103 (2001).

Malterud, K. E., Undheim, J., and Erdal, J. E., Synthesis of uvaretin, an antitumor and antimicrobial flavonoid. *Tetrahedron Lett.*, 26, 4807-4810 (1985).

Markham, K. R., Techniques in flavonoid identification, Academic Press, London, pp. 36-51 (1982).

Nikaido, T., Ohmoto, T., and Sankawa, U., Inhibitors of adenosine 3',5'-cyclic monophosphate phosphodiesterase in *Daphne genkwa* Sieb. *et* Zucc. *Chem. Pharm. Bull.*, 35, 675-681 (1987).

Pan, X. P. and Yu, D. Q., Two polyoxygenated cyclohexenes from *Uvaria grandiflora*. *Phytochemistry*, 40, 1709-1711 (1995).

Pan, X., Qin, Y., Chen, R., and Yu, D., Study on the polyoxygenated cyclohexenes from *Uvaria boniana*. *Yaoxue Xuebao*, 33, 275-281 (1998).

Pyo, M. K., Yun-Choi, H. S., and Hong, Y. J., Antiplatelet activities of aporphine alkaloids isolated from leaves of *Magnolia obovata*. *Planta Med.*, 69, 267-269 (2003).

Rahman, M. M., Qais, N., and Rashid, M. A., A new C-benzylated chalcone from *Desmos chinensis*. *Fitoterapia*, 74, 511-514 (2003).

Ruangrungsi, N., Prathanturarug, S., Lange, G. L., and Organ, M. G., An *N*-methyl aristolactam and an oxygenated cyclohexane derivative from *Piper ribesoides*. *Phytochemistry*, 31, 2397-2400 (1992).

Schulte, G. R., Ganem, B., Chantrapromma, K., Kodpinid, M., and Kobkull, S., The structure of ferrudiol. A highly oxidized constituent of *Uvaria ferruginea*. *Tetrahedron Lett.*, 23, 289-292 (1982).

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, 82, 1107-1111 (1990).

Takeuchi, Y., Shi, Q. W., Sugiyama, T., and Oritani, T., Polyoxygenated cyclohexenes from the Chinese tree, *Uvaria purpurea*. *Biosci. Biotechnol. Biochem.*, 66, 537-542 (2002).

Trager, W. and Jensen, J. B., Human malaria parasites in continuous culture. *Science*, 193, 673-675 (1976).

Wijeratne, E. M. K., Hatanaka, Y., Kikuchi, T., Tezuka, Y., and Gunatilaka, A. A. L., A dioxoaporphine and other alkaloids of two annonaceous plants of Sri Lanka. *Phytochemistry*, 42, 1703-1706 (1996).

Xu, Q. M., Zou, Z. M., Xu, L. Z., and Yang, S. L., New polyoxygenated cyclohexenes from *Uvaria kweichowensis* and their antitumour activities. *Chem. Pharm. Bull.*, 53, 826-828 (2005).

Zhang, Z., ElSohly, H. N., Jacob, M. R., Pasco, D. S., Walker, L. A., and Clark, A. M., New sesquiterpenoids from the roots of *Guatteria multivenia*. *J. Nat. Prod.*, 65, 856-859 (2002).