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Abstract

A new scalemic 8,8a-dihydro caged xanthone (1) was isolated from the leaf extract of Garcinia propinqua. Five other known natural products, the three caged xanthones (2, 5 and 6) and the two neocaged xanthones, (3 and 4) were also isolated as scalemic mixtures. Their structures were characterized by spectroscopic methods. The enantiomeric ratios (er) of compounds 1-6 ranged from 1:0.7 to 1:0.9. These compounds were also resolved by semipreparative chiral HPLC. The absolute configurations of (+)-2 and (+)-3 were determined by single-crystal X-ray diffraction analysis using Cu Kα radiation while the absolute configurations of the other compounds were determined by comparisons of their ECD spectra. Compounds (-)-4, (+)-4, (-)-5, (+)-5, and (-)-6 showed potent cytotoxicities against a colon cancer cell line HCT116 with IC_{50} values of 2.60, 7.02, 1.47, 3.37, and 4.14μM, respectively, which were better than the standard control doxorubicin (IC_{50} 9.74μM).

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Resolution and identification of scalemic caged xanthones from the leaf extract of *Garcinia propinqua* having potent cytotoxicities against colon cancer cells

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Abstract: A new scalemic 8,8a-dihydro caged xanthone (**1**) was isolated from the leaf extract of *Garcinia propinqua*. Five other known natural products, the three caged xanthones (**2**, **5** and **6**) and the two neocaged xanthones, (**3** and **4**) were also isolated as scalemic mixtures. Their structures were characterized by spectroscopic methods. The enantiomeric ratios (er) of compounds **1**-**6** ranged from 1:0.7 to 1:0.9. These compounds were also resolved by semipreparative chiral HPLC. The absolute configurations of (+)-**2** and (+)-**3** were determined by single-crystal X-ray diffraction analysis using Cu Kα radiation while the absolute configurations of the other compounds were determined by comparisons of their ECD spectra. Compounds (−)-**4**, (+)-**4**, (−)-**5**, (+)-**5**, and (−)-**6** showed potent cytotoxicities against a colon cancer cell line HCT116 with IC_{50} values of 2.60, 7.02, 1.47, 3.37, and 4.14 μM, respectively, which were better than the standard control doxorubicin (IC_{50} 9.74 μM).

Keywords: *Garcinia propinqua*, Scalemic caged xanthones, Cytotoxicity, Electronic circular dichroism

1. Introduction

Caged xanthones are commonly found in *Garcinia* (Clusiaceae) [1-23] and many of them show interesting biological activities, especially cytotoxicity against several cancer cell lines [1-3,5,6,12,13,19]. One of the best-known caged xanthones is (–)-gambogic acid, a major component from the exuded resin of *G. hanburyi*, first isolated in 1949 by Land and Katz [14] which has been found to be cytotoxic for various cancer cell lines [21]. So far, over 100 caged xanthones have been isolated and identified from *Garcinia* [1-23]. Some of these have been isolated as scalemic mixtures or reported to have specific rotations of relatively small magnitudes [3,5,9,10,12,20]. In a previous study, we reported the isolation seven new scalemic caged xanthones from the stem bark extract of *G. propinqua*. Six of them were successfully resolved by chiral HPLC and their absolute configurations were determined [22]. In continuation of our study on the different parts of this plant (Fig. 1), we report herein the resolution and absolute configuration assignment of six scalemic caged xanthones, **1**-**6** (Fig. 2), isolated from the leaf extract of *G. propinqua*, collected from Doi Tung, Chiang Rai Province, Thailand. The cytotoxicities of the resolved caged xanthones against a colon cancer cell line are also reported.

2. Experimental

2.1. General

Melting points were measured on a Buchi melting point B-540 visual thermometer. The optical rotation values were measured with a Bellingham & Stanley APD440 polarimeter. The UV spectra were recorded with a Perkin-Elmer UV-vis or Varian Cary 5000 UV-Vis-NIR spectrophotometer. Infrared (IR) spectra were recorded on a PerkinElmer FTS FT-IR or PerkinElmer Frontier Optica FT-IR spectrophotometers. Electronic circular dichroism spectra were recorded on a JASCO J-815 spectrometer. The 1D and 2D NMR spectra data were obtained

on a 600 MHz Bruker AV-600 spectrometers in $CDCl_3$ with TMS as the internal standard. Chemical shifts are reported in parts per million (δ), and coupling constants (J) are expressed in hertz. Low- and high-resolution MS were recorded using ESI ionization and a TOF mass analyzer. Chiral HPLC was performed on a CHIRALPAK IA 15 column of 10 × 250 mm or CHIRALCEL OD-H column of 4.6 × 250 mm and attached to Waters 2487 Dual λ Absorbance detector. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (5-40 μm, SiliCycle Inc.) and silica gel 100 (63-200 μm, SiliCycle Inc.), respectively. Sephadex LH-20 was also used for CC. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

2.2 Plant material

The leaves of *G. propinqua* were collected from Doi Tung, Chiang Rai Province, Thailand in September 2011. The plant was identified by Mr. Matin Van de Bult (Doi Tung Development Project, Chiang Rai, Thailand), and the specimen (MFU-NPR0090) was deposited at the Natural Products Research Laboratory, School of Science, Mae Fah Luang University.

2.3. Extraction and isolation

Chopped and dried leaves of *G. propinqua* (2.5 kg) were extracted with MeOH (10 L) for three days at room temperature. Removal of the solvent under reduced pressure provided the crude extract (196 g), which was partitioned between water and CH_2Cl_2. The CH_2Cl_2 extract (96 g) was subjected to QCC over silica gel (100% hexanes to 100% EtOAc) to give 24 fractions (P1-P24). Fraction P3 (300 mg) was further fractionated by QCC (100% hexanes to 100% EtOAc) to afford seven subfractions (P3A-P3G). Subfraction P3D (100 mg) was further fractionated by CC (3:7 CH_2Cl_2/hexanes) to provide five subfractions (P3D1-P3D5). Compound **7** (2.3 mg) were obtained from subfraction P3D2 (10 mg) by PLC (1:19 EtOAc/hexanes).

Subfraction P3C (10 mg) was further separated by PLC (1:19 EtOAc/hexanes) to give compounds **16** (2.3 mg) and **8** (2.9 mg). Compounds **5** (13.9 mg), **3** (8.7 mg), **6** (14.4 mg), and **11** (4.8 mg) were obtained from fraction P14 (62 mg), P15 (55 mg), P11 (50 mg), and P7 (30 mg), respectively, by washing with hexanes. Fraction P8 (500 mg) was separated CC (1:9 acetone/hexanes) to give three subfractions (P8A-P8C). Compound **12** (6.0 mg) was obtained from subfraction P8C (42 mg), whereas compound **9** (10.8 mg) was isolated from subfraction P8B (30 mg) by Sephadex LH-20 (100% MeOH). Compound **4** (9.5 mg) was obtained from subfraction P8A (50 mg) by CC (3:2 CH_2Cl_2/hexanes). Fraction P21 (500 mg) was further separated by Sephadex LH-20 (100% MeOH) to give compound **13** (13.0 mg). Fraction P9 (200 mg) was fractionated by Sephadex LH-20 (100% MeOH) to give three subfractions (P9A-P9C). Compound **2** (28.7 mg) was isolated from subfraction P9B (40 mg) by CC (1:9 acetone/hexanes). Fraction P18 (300 mg) was washed with hexanes to give compound **14** (4.5 mg) and the mother liquor of P18 was further separated by Sephadex LH-20 (100% MeOH) to give three subfractions (P18A-P18C). Compound **1** (16.2 mg) was isolated from subfraction P18A (50 mg). Finally, fraction P16 (200 mg) was separated by CC (1:9 acetone/hexanes) to give compounds **10** (4.3 mg), **17** (5.3 mg), and **15** (3.4 mg).

*2.4. Compound **1***

White solid; m.p. 221–223 °C; UV (MeOH) λ_{max} (log ε) 219 (3.81), 297 (3.65), and 337 (2.92) nm; IR (neat) ν_{max} 3436, 1740, 1638 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; ESITOFHRMS *m/z* 497.2547 $[M+H]^+$ (calcd for $C_{29}H_{37}O_7$, 497.2539).

*2.5 Resolution of compound **1** and ECD spectroscopic data of (–)-**1** and (+)-**1***

Resolution of the two enantiomers of **1** (15.0 mg) was performed by semi-preparative HPLC on a chiral column (CHIRALPACK IA 15 µL, 10 × 250 mm, flow rate 1 mL/min, *n*-

hexane/*i*PrOH, 99:1 v/v). Compound (–)-**1** (t_R 90 min) [(7.3 mg), $[\alpha]_D^{24}$ –66.6 (*c* 0.1, $CHCl_3$)]; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 224 (+0.85 × 10^2) and 306 (–0.88×10^2) nm] and (+)-**1** (t_R 95 min) [(4.7 mg), $[\alpha]_D^{24}$ +56.5 (*c* 0.1, $CHCl_3$)]; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 224 (-0.92 × 10^2) and 306 (+0.99 × 10^2) nm] were obtained.

2.6. Compound 2

Yellow solid; m.p. 180–184 °C; UV (MeOH) λ_{max} (log ε) 216 (3.16), 310 (2.64), 328 (2.83), and 355 (2.96) nm; IR (neat) ν_{max} 3462, 1739, 1635 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESITOFHRMS *m/z* 487.2094 $[M+Na]^+$ (calcd for $C_{28}H_{32}NaO_6$, 487.2097).

*2.7 Resolution of compound **2** and ECD spectroscopic data of (–)-**2** and (+)-**2***

Resolution of two enantiomers of **2** (10.0 mg) was performed by semi-preparative HPLC on a chiral column (CHIRALCEL OD-H, 4.6 × 250 mm, flow rate 1 mL/min, *n*-hexane/*i*PrOH, 98:2 v/v). Compound (–)-**2** (t_R 45 min) [(3.5 mg), $[\alpha]_D^{24}$ –24.2 (*c* 0.07, $CHCl_3$); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 209 (+0.32 × 10^2), 219 (–0.04 × 10^2), 236 (+0.09 × 10^2), 262 (+0.31 × 10^2), 280 (+0.42 × 10^2), 306 (+0.39 × 10^2), and 350 (–1.13 × 10^2) nm] and (+)-**2** (t_R 48 min) [(2.9 mg), $[\alpha]_D^{24}$ +38.2 (*c* 0.06, $CHCl_3$); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 208 (–0.64 × 10^2), 227 (+0.03 × 10^2), 239 (–0.12 × 10^2), 263 (–0.30 × 10^2), 280 (–0.40 × 10^2), 306 (–0.35 × 10^2), and 350 (+0.88 × 10^2) nm].

*2.8. Compound **3***

Yellow solid; m.p. 178–182 °C; UV (MeOH) λ_{max} (log ε) 213 (3.23), 252 (2.61), and 342 (3.02) nm; IR (neat) ν_{max} 3406, 1749, 1634 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESITOFHRMS *m/z* 501.2254 $[M+Na]^+$ (calcd for $C_{29}H_{34}NaO_6$, 501.2253).

*2.9. Resolution of compound **3** and ECD spectroscopic data of (–)-**3** and (+)-**3***

Resolution of the two enantiomers of **3** (8.4 mg) was performed by the method described for **1**, to give compounds (–)-**3** (t_R 70 min) [(4.0 mg), $[\alpha]_D^{24}$ −27.7 (*c* 0.08, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 232 (+0.82 × 10^2), 260 (+0.52 × 10^2), 310 (−0.53 × 10^2), 348 (+0.13 × 10^2), and 360 (+0.14 × 10^2) nm] and (+)-**3** (t_R 75 min) [(3.6 mg), $[\alpha]_D^{24}$ +34.4 (*c* 0.07, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 231 (−1.11 × 10^2), 258 (−0.67 × 10^2), 304 (+0.65 × 10^2), 316 (+0.72 × 10^2), 345 (−0.08 × 10^2) and 358 (−0.25 × 10^2) nm].

*2.10. Compound **4***

Yellow solid; m.p. 187–192 °C; UV (MeOH) λ_{max} (log ε) 211 (3.48), 257 (2.94), 306 (2.89), and 343 (3.23) nm; IR (neat) ν_{max} 3387, 1750, 1637 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESITOFHRMS *m/z* 463.2125 $[M–H]^-$ (calcd for $C_{28}H_{31}O_6$, 463.2121).

*2.11. Resolution of compound **4** and ECD spectroscopic data of (–)-**4** and (+)-**4***

Resolution of the two enantiomers of **4** (13.0 mg) was performed by the method described for **2**, to give compounds (–)-**4** (t_R 18 min) [(5.2 mg), $[\alpha]_D^{24}$ −28.3 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 238 (+0.61 × 10^2), 262 (+0.49 × 10^2), 301 (−0.34×10^2), and 341 (−0.22 × 10^2) nm] and (+)-**4** (t_R 21 min) [(4.8 mg), $[\alpha]_D^{24}$ +37.4 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 237 (−0.72 × 10^2), 262 (−0.57 × 10^2), 301 (+0.39 × 10^2), and 341 (+0.24 × 10^2) nm].

*2.12. Compound **5***

Yellow solid; m.p. 140–144 °C; UV (MeOH) λ_{max} (log ε) 218 (3.20), 297 (2.75), 326 (2.68), and 350 (2.75) nm; IR (neat) ν_{max} 3456, 1737, 1636 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESITOFHRMS *m/z* 501.2249 $[M+Na]^+$ (calcd for $C_{29}H_{34}NaO_6$, 501.2253).

*2.13. Resolution of compound **5** and ECD spectroscopic data of (+)-**5** and (–)-**5***

158 Resolution of the two enantiomers of **5** (20.0 mg) was performed by the method
159 described for **2**, to give compounds (–)-**5** (t_R 5 min) [(4.6 mg) , $[\alpha]_D^{24}$ –74 (*c* 0.1, MeOH); ECD
160 (MeOH) λ_{max} ($\Delta\varepsilon$) 210 ($+0.44 \times 10^2$), 234 (-0.13×10^2), 260 ($+0.12 \times 10^2$), 285 ($+0.20 \times 10^2$),
161 313 ($+0.29 \times 10^2$), and 350 (-0.60×10^2) nm] and (+)-**5** (t_R 7 min) [(4.9 mg), $[\alpha]_D^{24}$ +58 (*c* 0.1,
162 MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 235 ($+0.04 \times 10^2$), 264 (-0.09×10^2), 283 (-0.10×10^2), 315
163 (-0.12×10^2), and 350 ($+0.34 \times 10^2$) nm].

164 *2.14. Resolution of compound **6** and ECD spectroscopic data of (+)-**6** and (–)-**6***

165 Resolution of the two enantiomers of **6** (20.0 mg) was performed by the method
166 described for **2**, to give compounds (–)-**6** (t_R 15 min) [(3.0 mg) , $[\alpha]_D^{24}$ –83 (*c* 0.06, MeOH); ECD
167 (MeOH) λ_{max} ($\Delta\varepsilon$) 211 ($+1.10 \times 10^2$), 229 (-0.42×10^2), 263 ($+0.47 \times 10^2$), 282 ($+0.67 \times 10^2$),
168 310 ($+0.69 \times 10^2$), and 352 (-1.65×10^2) nm] and (+)-**6** (t_R 17 min) [(3.5 mg), $[\alpha]_D^{24}$ +96 (*c* 0.07,
169 MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 209 (-0.87×10^2), 229 ($+0.29 \times 10^2$), 265 (-0.38×10^2), 282
170 (-0.51×10^2), 310 (-0.53×10^2), and 352 ($+1.30 \times 10^2$) nm].

171 *2.15. X-ray crystallographic analysis of compounds (+)-**2**, (+)-**3** and scalemic mixture **4***

172 Single-crystal X-ray diffraction analysis was collected on a Bruker APEX DUO
173 diffractometer with cross-coupled multilayer optics Cu-Kα radiation. Data were corrected for
174 absorption effects using the multi-scan technique (SADABS). The structure was solved by direct
175 methods.

176 Single-crystal X-ray data for (+)-**2**: Yellow tablet crystal of $C_{28}H_{32}O_6$, M = 464.53,
177 crystal system orthorhombic with a = 10.0612(7) Å, b = 11.6588(8) Å, c = 19.8385(13) Å, α =
178 90°, β = 90°, γ = 90°, v = 2327.1(3) Å, and chiral group $P2_12_12_1$, z = 4. The X-ray diffraction
179 analysis using Cu-K*a* radiation values were 7.50 cm^{-1}, 22624 reflections measured, 4027

independent reflections (R_{int} = 0.035). Final *R* indices: R_1 = 0.029 and wR_2 = 0.074. The standard deviation of an observation of unit weight was 1.03. The absolute configurations of (+)-**2** was assigned as *5S*, *7R, 10aR*, *22R* (Fig. 4) with a Flack x-parameter of 0.06(5).

Single crystal X-ray data for (+)-**3**: Yellow tablet crystal of $C_{29}H_{34}O_6$, *M* = 478.56, crystal system monoclinic with *a* = 11.7599(7) Å, *b* = 36.717(2) Å, *c* = 17.2160(9) Å, α = 90°, β = 90.767(3)°, γ = 90°, *v* = 7433.0(7) Å, and chiral group $P2_1$, *z* = 12. The x-ray diffraction analysis using Cu-K*a* radiation values were 7.19 cm^{-1}, 97789 reflections measured, 26173 independent reflections (R_{int} = 0.070). Final *R* indices: R_1 = 0.056 and wR_2 = 0.142. The standard deviation of an observation of unit weight was 1.04. The absolute configurations of compound (+)-**3** was assigned as *6S*, *7R, 10aS*, *22S* (Fig. 4) with a Flack x-parameter of -0.12(8).

Single-crystal X-ray data for scalemic mixture **4**: Yellow irregular crystal of $C_{28}H_{32}O_6$, *M* = 464.53, crystal system orthorhombic with *a* = 19.5660(8) Å, *b* = 8.0604(3) Å, *c* = 14.3543(6) Å, α = 90°, β = 90°, γ = 90°, *v* = 2263.81(6) Å, and chiral group $Pca2_1$, *z* = 4. The x-ray diffraction analysis using Cu-K*a* radiation values were 7.71 cm^{-1}, 12938 reflections measured, 3570 independent reflections (R_{int} = 0.039). Final *R* indices: R_1 [$I > 2.00\sigma(I)$] = 0.032 and wR_2 = 0.083, respectively. The standard deviation of an observation of unit weight was 1.06.

The X-ray Crystallographic data for compounds (+)-**2**, (+)-**3** and scalemic mixture **4** have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 1539175 for (+)-**2**, CCDC 1539176 for (+)-**3** and CCDC 1539178 for scalemic mixture **4**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

2.16. Cytotoxic Assay

Determination of the cytotoxicities of compounds on colon cancer cell (HCT116, American Type Culture Collection (ATCC), Manassas, VA, USA) was performed by the previous method [22]. Briefly, HCT116 cells (1×10^4 cells/well) were cultured in 96 well plate at 37 ºC for 24 h which were treated with compounds (10 µM in DMSO) in DMEM medium for 24 h. The medium was removed and fresh DMEM containing 0.5 mg/mL of MTT solution was added for 2 h. After removal of medium, the violet formazan crystals were dissolved in DMSO (100 µL) and the absorbance was measured at 570 nm using a microplate reader. Doxorubicin (IC_{50} 9.74 µM) was used as a positive control.

$$\%\text{cell viability} = \frac{\text{absorbance of treated well}}{\text{absorbance of control well}} \times 100$$

$$\%\text{cytotoxicity} = 100 - \%\text{cell viability}$$

3. Results and discussion

Compound **1** was obtained as a white solid with m.p. 221–223 °C. Its molecular formula was determined as $C_{29}H_{36}O_7$, from its protonated molecular ion at *m/z* 497.2547 $[M+H]^+$ (calcd for 497.2539) in the ESITOFHRMS. The UV spectrum showed absorption bands at λ_{max} 219, 297, and 337 nm. The IR spectrum contained absorption bands at 3436, 1638, and 1740 cm^{-1} indicating the presence of a hydroxy group and conjugated carbonyl and unconjugated carbonyl groups. The ^1H and ^{13}C NMR spectra of compound **1** (Table 1), which were similar to those of doitunggarcinone F[22] isolated from the stem bark of *G. propinqua*, displayed resonances for: a H-bonded hydroxy proton [δ_H 12.09 (1H, s, OH-1)], an isolated aromatic proton [δ_H 6.08 (1H, s, H-2)/δ_C 99.5], a methoxy group [δ_H 3.29/δ_C 55.7], three methine protons [δ_H 4.35 (1H, dd, J = 4.4, 1.4 Hz, H-8)/δ_C 74.6, δ_H 2.88 (1H, dd, J = 4.5, 5.9 Hz, H-7)/δ_C 44.7, δ_H 3.30 (1H, d, J = 1.4 Hz, H-8a)/δ_C 47.2], a 1,1-dimethylallyl moiety [δ_H 6.24 (1H, dd, J = 10.5, 17.9 Hz, H-12)/δ_C

149.2, δ_H 5.35 (1H, d, J = 17.9 Hz, H-13a)/δ_C 113.4, δ_H 5.30 (1H, d, J = 10.5 Hz, H-13b)/δ_C 113.4, δ_H 1.66 (3H, s, H_3-15)/δ_C 27.3, and δ_H 1.62 (3H, s, H_3-14)/δ_C 30.3], a (–$OC(Me)_2$–$CHCH_2$–C–) unit [δ_H 2.54 (1H, d, J = 8.7 Hz, H-22)/δ_C 43.9, δ_H 1.97 (1H, dd, J = 6.3, 14.7 Hz, H-21a)/δ_C 20.2, δ_H 1.39 (3H, s, H_3-25)/δ_C 30.5, δ_H 1.38 (1H, dd, J = 8.7, 14.7 Hz, H-21b)/δ_C 20.2, and δ_H 1.12 (3H, s, H_3-24)/δ_C 27.4] and a prenyl group [δ_H 5.29 (1H, m, H-17)/δ_C 117.8, δ_H 2.86 (1H, dd, J = 14.6, 5.9 Hz, H-16a), δ_H 2.69 (1H, dd, J = 14.6, 9.1 Hz, H-16b)/δ_C 28.1, δ_H 1.66 (3H, s, H_3-20)/δ_C 26.0 and δ_H 1.62 (3H, s, H_3-19)/δ_C 18.0]. The only difference between the structures of compound **1** and doitunggarcinone F is that compound **1** had a C-3 hydroxy group (δ_H 6.96, s) while the latter compound had a C-3 methoxy group. In the HMBC spectrum, the C-3 hydroxy group showed cross-peaks with C-3 (δ_C 165.5) and C-4 (δ_C 111.4) (Fig. 3). Compound **1** was therefore identified as doitunggarcinone L.

Similar to doitunggarcinones F-K, previously isolated from the stem bark of this plant [22], doitunggarcinone L (**1**) could be resolved by semipreparative chiral HPLC to give compounds (**–**)**-1** (t_R 90 min), $[\alpha]_D^{24}$ –66.6 (*c* 0.1, $CHCl_3$) and (+)**-1** (t_R 95 min), $[\alpha]_D^{24}$ +56.5 (*c* 0.1, $CHCl_3$) in a ratio of ca 1:0.9 (Fig. S31, Supplementary material). The absolute configurations of (–)-**1** and (+)-**1** were identified by comparisons of their ECD spectra with those of (–)-doitunggarcinone F and (+)-doitunggarcinone F [22]. The ECD spectrum of (–)-**1** showed a positive Cotton effect around 224 and a negative effect around 306 nm, while (+)-**1** exhibited opposite signs of Cotton effects at those same wavelengths (Fig. 5), which were similar to those of (–)-doitunggarcinone F[22] and (+)-doitunggarcinone F [22], respectively. Thus, the absolute configuration of (–)-**1** was assigned as *5R*, *7S*, *8S*, *8aR*, *10aR*, *22S* and the configuration of (+)-**1** was assigned as *5S*, *7R*, *8R*, *8aS*, *10aR*, *22R*. Compounds (–)-**1** and (+)-**1** were assigned trivial names as (–)-doitunggarcinone L and (+)-doitunggarcinone L, respectively.

Compounds **2**-**5** were identified as isobractatin (**2**) [5], 3-*O*-methylneobractatin (**3**) [10], neobractatin (**4**) [10] and 3-*O*-methylbractatin (**5**) [10], by spectroscopic methods including NMR, ESITOFHRMS, UV, and IR as well as comparisons with literature data. The structure of compound **4** was also confirmed by single crystal X-ray diffraction analysis (Fig. 4). Compounds **2**-**5** were previously isolated from *G. bracteata*, with all having specific rotations of relatively small magnitudes. Isobractatin (**2**), $[\alpha]_D$ –3 (*c* 0.58, $CHCl_3$), was first isolated from the leaf extract of *G. bracteata* in 2000 by Thoison *et al*. [5] and was shown to be a scalemic mixture by analytical chiral HPLC analysis [5]. Compounds **3**-**5** were first isolated from the twig extract of *G. bracteata* by Na *et al*. in 2010 [10]. They had specific rotations of $[\alpha]_D^{26}$ +5.4 (*c* 0.17, MeOH) for **3** [10], $[\alpha]_D^{27}$ +9.4 (*c* 0.24, MeOH) for **4** [10], and $[\alpha]_D^{24}$ –1.3 (*c* 0. 44, MeOH) for **5** [10]. In this study, compounds **2**-**5** were resolved by semiprepatative chiral HPLC. All of them were scalemic mixtures with enantiomeric ratios (er) ranging from 1:0.7 to 1:0.9: Compound **2** (er = 1:0.8; (–)-**2**, t_R 45 min, $[\alpha]_D^{24}$ –24.2 (*c* 0.07, $CHCl_3$)/(+)-**2**, t_R 48 min, $[\alpha]_D^{24}$ +38.2 (*c* 0.06, $CHCl_3$)]), compound **3** (er = 1:0.9; (–)-**3**, t_R 70 min, $[\alpha]_D^{24}$ –27.7 (*c* 0.08, MeOH)/(+)-**3**, t_R 75 min, $[\alpha]_D^{24}$ +34.4 (*c* 0.07, MeOH), compound **4** (er = 1:0.7; (–)-**4**, t_R 18 min, $[\alpha]_D^{24}$ –28.3 (*c* 0.1, MeOH)/(+)-**4**, t_R 21 min, $[\alpha]_D^{24}$ +37.4 (*c* 0.1, MeOH) and compound **5** (er = 1:0.8; (–)-**5**, t_R 5 min, $[\alpha]_D^{24}$ –74 (*c* 0.1, MeOH)/(+)-**5**, t_R 7 min, $[\alpha]_D^{24}$ +58 (*c* 0.1, MeOH) (Fig. S31, Supplementary material). Each pair of enantiomers displayed identical ^1H and ^{13}C NMR spectra (Tables 1 and 2). Compounds (+)-**2** and (+)-**3** produced single crystals from CH_2Cl_2/MeOH (1:3 v/v) which were subjected to X-ray diffraction analysis using Cu Kα radiation to determine their absolute configurations.

From the X-ray data analysis, the absolute configuration of (+)-**2** (Fig. 4) was established as *5S, 7R, 10aR*, *22R* with a Flack x-parameter of 0.06(5). The ECD spectrum of (+)-**2** (Fig. 5)

displayed a strong positive Cotton effect around 350 nm and negative Cotton effects around 208 and 280 nm. Thus, the absolute configuration of (–)-**2**, was assigned as *5R*, *7S*, *10aS*, *22S* because of its opposite signs of ECD Cotton effects (Fig. 5) as well as specific rotation, $[\alpha]_D^{24}$ –24.2 (*c* 0.07, $CHCl_3$), to that of (+)-**2**, $[\alpha]_D^{24}$ +38.2 (*c* 0.06, $CHCl_3$). The trivial names (+)-isobractatin and (–)-isobractatin were given for (+)-**2** and (–)-**2**, respectively.

The absolute configuration *6S, 7R, 10aS, 22S* of (+)-**3**, $[\alpha]_D^{24}$ +34.4 (*c* 0.07, MeOH), was established on the basis of single-crystal X-ray diffraction analysis (Fig. 4) with a Flack x-parameter of –0.12(8). Compound (–)-**3**, $[\alpha]_D^{24}$ –27.7 (*c* 0.08, MeOH), presented reverse absorption curves in the ECD spectrum (Fig. 5) indicating the absolute configuration of *6R, 7S, 10aR*, *22R*. Thus, (+)-3-*O*-methylneobractatin and (–)-3-*O*-methylneobractatin were assigned for compounds (+)-**3** and (–)-**3**, respectively.

The absolute configuration *6R*, *7S*, *10aR*, *22R* of (–)-**4** and *6S, 7R, 10aS*, *22S* of (+)-**4** were established by the comparisons of their ECD spectra (Fig. 5) and specific rotations ($[\alpha]_D^{24}$ –28.3 (*c* 0.1, MeOH) for (–)-**4** and ($[\alpha]_D^{24}$ +37.4 (*c* 0.1, MeOH) for (+)-**4**) to those of (–)-**3** and (+)-**3**. Therefore, compound (+)-**4** and (–)-**4** were assigned names as (+)-neobractatin and (–)-neobractatin, respectively.

Compound (–)-**5** displayed ECD Cotton effects (Fig. 5) and specific rotation, $[\alpha]_D^{24}$ –74 (*c* 0.1, MeOH), similar to those of (–)-cochinchinone C.[24] Thus, its absolute configuration was assigned as *5R*, *7S*, *10aS*, *22S*. The absolute configuration *5S*, *7R*, *10aR*, *22R* assigned to (+)-**5** was identified from the opposite signs of its ECD Cotton effects (Fig. 5) and specific rotation, $[\alpha]_D^{24}$ +58 (*c* 0.1, MeOH), with those of (–)-**5**. The names of (+)-**5** and (–)-**5** were assigned as (+)-3-*O*-methylbractatin and (–)-3-*O*-methylbractatin, respectively.

Bractatin (**6**) was previously resolved into its two enantiomers but their absolute configurations were not determined [5]. In this study, bractatin was resolved by semipreparative chiral HPLC which showed two peaks, t_R 15 min for (–)-**6** ($[\alpha]_D^{24}$ –83 (*c* 0.06, MeOH)) and 17 min for (+)-**6** ($[\alpha]_D^{24}$ +96 (*c* 0.07, MeOH)), (Fig. S31, Supplementary material) with an approximately enantiomeric ratio ca 1:0.7 ((–)-**6**/(+)-**6**). The ECD spectra of (+)-**6** and (–)-**6** (Fig. 5) were similar to those of (+)-**5** and (–)-**5**, respectively, indicating their absolute configurations were *5S*, *7R*, *10aR*, *22R* for (+)-**6** and *5R*, *7S*, *10aS*, *22S* for (–)-**6**.

Possible biosynthetic pathways for the generation of the caged xanthones **1**-**6** are shown in Scheme 1. The intermediate **13.1** could be obtained from xanthone **13** via double O-prenylation at C-5 and C-6 [22]. Claisen rearrangement of **13.1** could generate intermediate **13.2** (carbonyl group at C-6) or intermediate **13.3** (carbonyl group at C-5). These intermediates could then produce caged molecules having different bridge-head structures. Using the dextroisomer (+)-**6** for example, containing a caged bridge-head at C-5/C-7/C-10a, this molecule could be obtained from intermediate **13.2** via an intramolecular Diels-Alder reaction. The cyclization of the 3-OH onto the C-12 olefinic moiety of (+)-**6** would give compound (+)-**2**, whereas *O*-methylation at C-3 would produce compound (+)-**5**. In addition, the oxidation of (+)-**6** at C-8/C-8a and then *O*-methylation at C-8 would give compound (+)-**1**. While caged compound (+)-**3** with a different bridge-head, at C-6/C-7/C-10a, (called a neocaged xanthone) could obtain from intermediate **13.3** via an intramolecular Diels-Alder reaction. *O*-methylation at C-3 of compound (+)-**3** would then give compound (+)-**4**.

The remaining compounds were identified as doitunggarcinone A (**7**) [25, 26], doitunggarcinone B (**8**) [25, 26], morusignin J (**9**) [27], 1,3,5-trihydroxy-6´,6-dimethylpyrano (2´-2´,6,7)-4-(1,1-dimethylprop-2-enyl)-xanthone (**10**) [28], gartanin (**11**) [29], allanxanthone A

316 (**12**) [30], gerontoxanthone I (**13**) [31], 1,3,5,6-tetrahydroxy-4(1,1-dimethylprop-2-enyl)-7-(3-
317 methylbut-2-enyl)-xanthone (**14**) [28], 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**15**) [32],
318 blancoxanthone (**16**) [33], 10-*O*-methylmacluraxanthone (**17**) [34], (Fig. 2) by spectroscopic
319 methods as well as comparisons with the literature.

320 Caged xanthones **1**-**6** were evaluated for their cytotoxicities against the colon cancer cell
321 line (HCT116). Compounds (−)-**4**, (+)-**4**, (−)-**5**, (+)-**5**, and (−)-**6**, showed potent inhibitory
322 cytotoxicities with IC_{50} values of 2.60, 7.02, 1.47, 3.37, and 4.14 μM, respectively, better than
323 that of the positive control doxorubicin (IC_{50} 9.74 μM). Compound (−)-**2** had weak cytotoxicity
324 (IC_{50} > 50 μM) while all remaining resolved caged xanthones ((−)-**1**, (+)-**1**, (+)-**2**, (−)-**3**, (+)-**3**,
325 and (+)-**6**) were found to be inactive. It is interesting to note that the levorotatory compounds
326 showed cytotoxicity greater than their dextrorotatory counterparts. The structural difference
327 between compounds **3** and **4** is only at C-3. Compound **3** contained a C-3 methoxy group
328 whereas compound **4** had a C-3 hydroxy group which played an important role in the
329 cytotoxicity. Also, the double bond at C-8/C-8a of compounds **5** and **6** might be a crucial
330 structural element for cytotoxicity as indicated by the different potencies of the compounds **1** and
331 **2** [22].

332 **4. Conclusions**

333 Previous phytochemical studies of the stem bark [22], root [23] and twig [25] of *G.*
334 *propinqua*, have resulted in the isolation of benzophenones, xanthones, and caged xanthones. In
335 our studies, the latter two types of compounds were the major compounds of this plant. All caged
336 xanthones previously isolated from this plant were scalemic mixtures. In the present study, we
337 isolated an additional six scalemic caged xanthones (**1**-**6**) with enantiomeric ratios ranging from
338 1:0.7 to 1:0.9. Interestingly, none of these compounds were isolated from the stem bark [22] and

twig [25] of this plant. These compounds were resolved by chiral HPLC and their absolute configurations were identified using X-ray crystallography and ECD spectroscopy. Five enantiomeric caged xanthones (–)-**4**, (+)-**4**, (–)-**5**, (+)-**5**, and (−)-**6** showed potent cytotoxicities against a colon cancer cell line which may have potential for further development as anticancer lead compounds.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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Fig. 1. *Garcinia propinqua* (These photos were taken by Surat Laphookhieo).

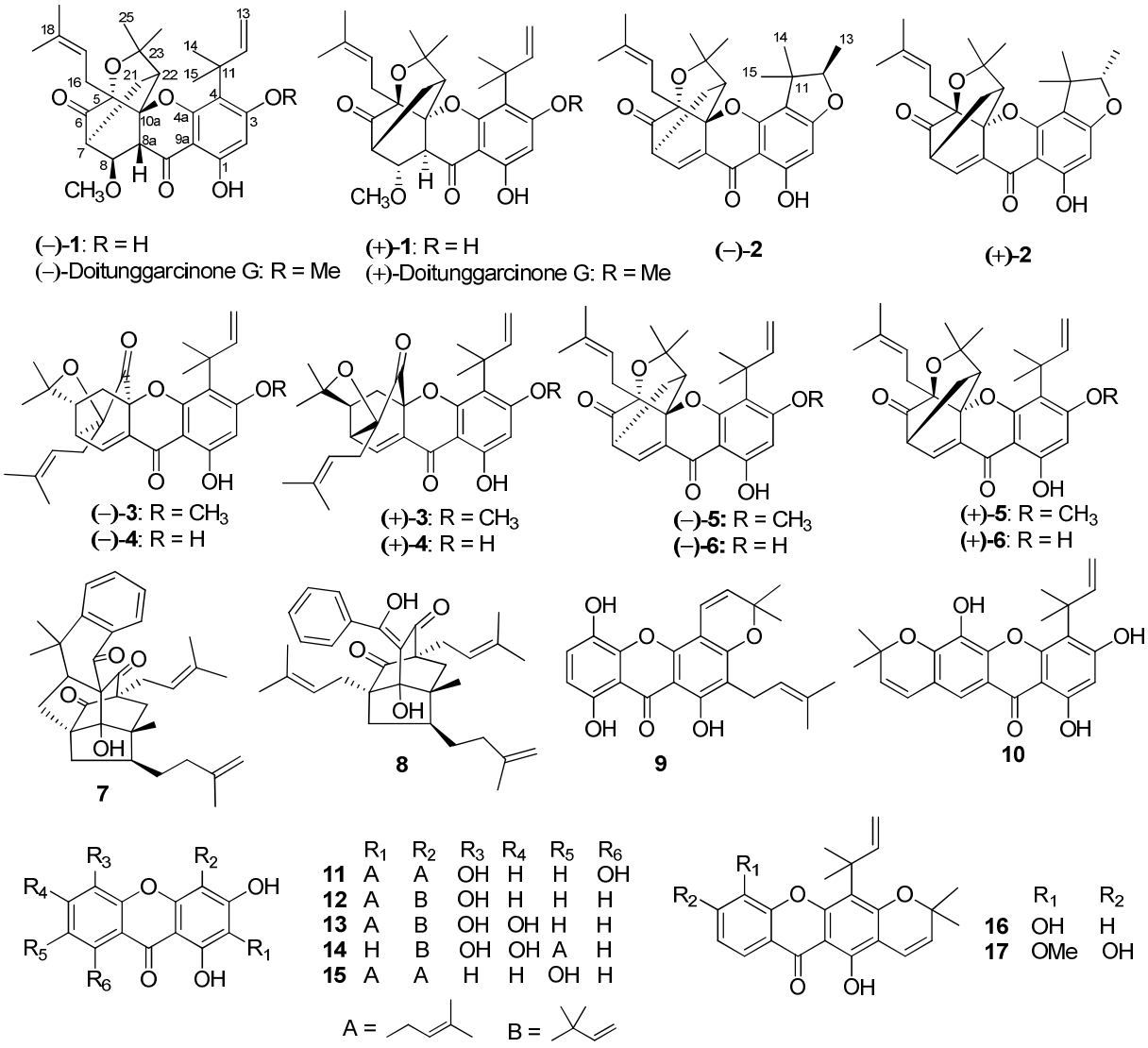


Fig. 2. Compounds isolated from the leaves of *G. propinqua*.

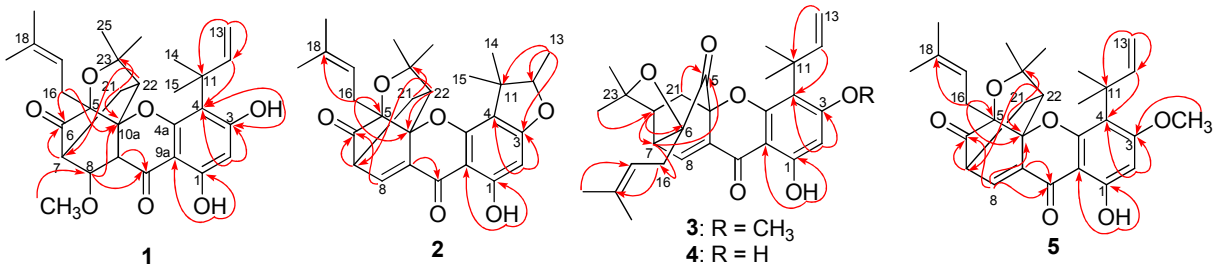
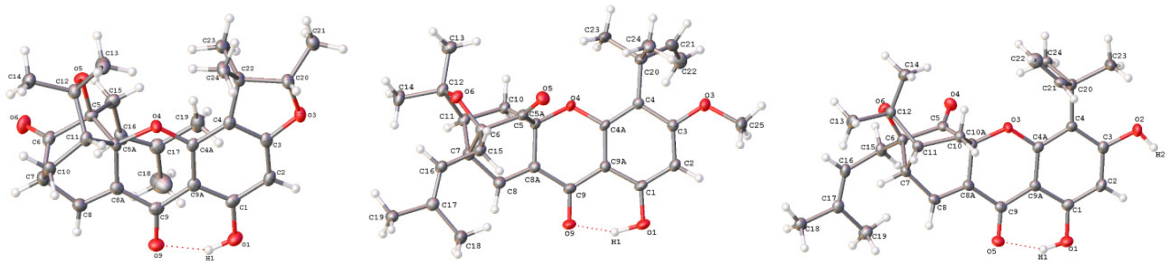
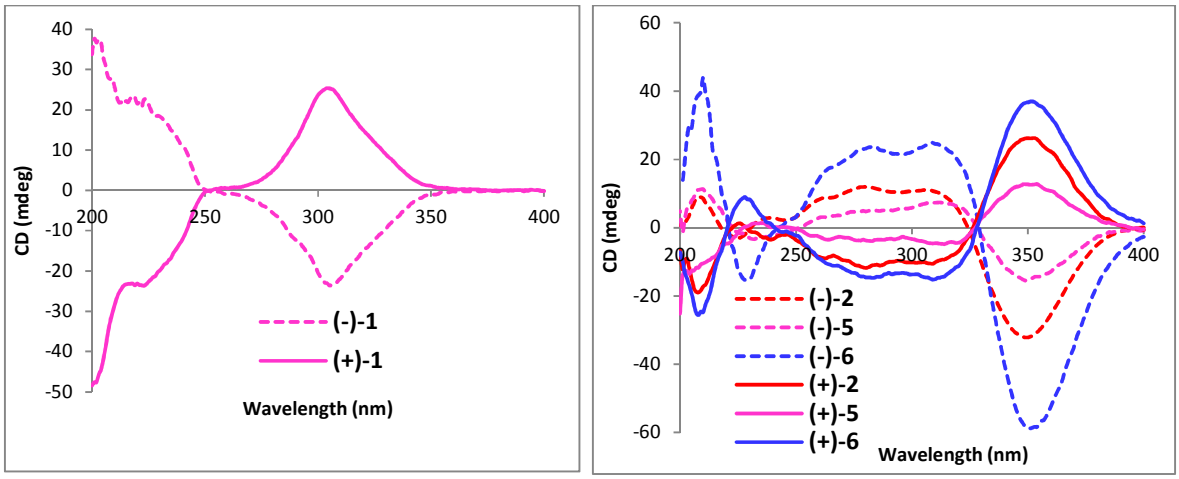


Fig. 3. Selected HMBC correlations of compounds **1**-**5**.



(+)-**2** (+)-**3** (±)-**4**

Fig. 4. X-ray ORTEP diagrams of compounds (+)-**2**, (+)-**3** and (±)-**4**.



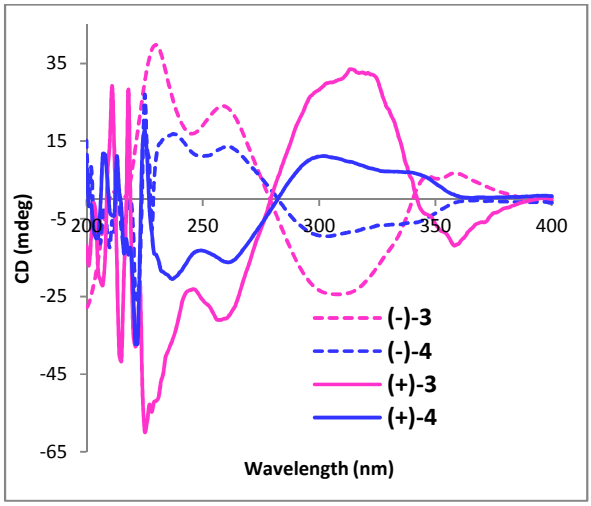


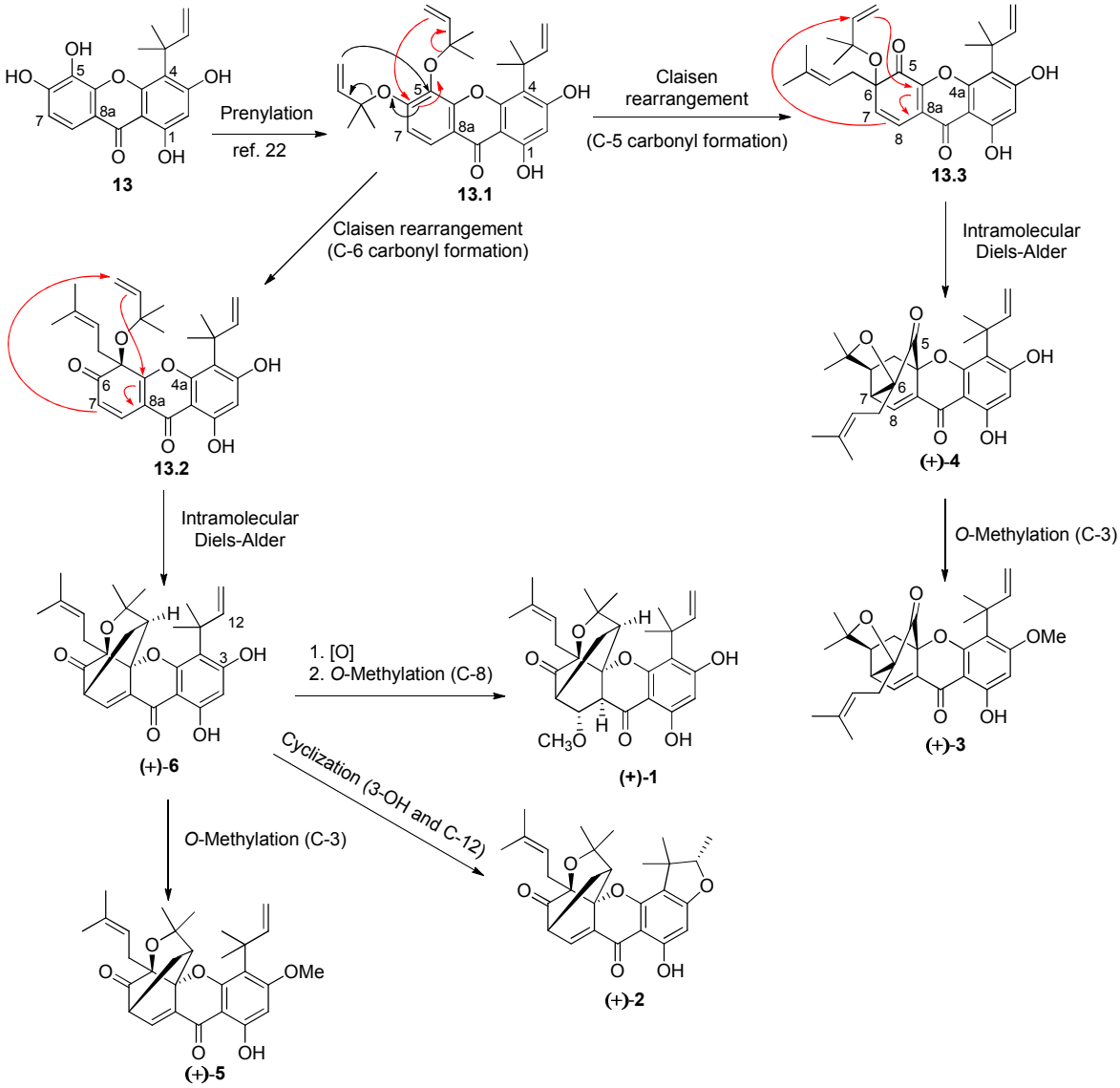
Fig. 5. ECD spectra of resolved compounds **1-6** in MeOH.

479 **Table 1.** ^{1}H NMR spectroscopic data of compounds **1**–**5** (600 MHz in $CDCl_3$).

Position	**1**	**2**	**3**	**4**	**5**
1	-	-	-	-	-
2	6.08, s	6.02, s	6.08, s	6.02, s	6.12, s
3	-	-	-	-	-
4	-	-	-	-	-
4a	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	2.88, dd (4.5, 5.9)	3.50, dd (4.9, 6.8)	3.73, m	3.76, dd (4.8, 6.7)	3.49, dd (5.1, 7.0)
8	4.35, dd (4.4, 1,4)	7.47, d (6.8)	7.16, d (6.1)	7.19, d (6.7)	7.48, d (7.0)
8a	3.30, d (1.4)	-	-	-	-
9	-	-	-	-	-
9a	-	-	-	-	-
10a	-	-	-	-	-
11	-	-	-	-	-
12	6.24, dd (10.5,17.9)	4.54, q (6.7)	6.32, dd (11.1,17.1)	6.47, dd (10.4,17.8)	6.14, dd (10.6,17.3)
13	5.35, d (17.9)	1.30, d (6.7)	4.86, d (17.1)	5.47, d (17.8)	4.76, d (17.3)
	5.30, d (10.5)		4.75, d (11.1)	5.36, d (10.4)	4.69, d (10.6)
14	1.62, s	1.42, s	1.60, s	1.72, s	1.66, s
15	1.66, s	1.51, s	1.57, s	1.61, s	1.63, s
16	2.86, dd (5.9, 14.6)	2.64, m	2.50, m	2.49, m	2.63, dd (7.7)
	2.69, dd (9.1, 14.6)		2.07, dd (8.5, 14.4)	2.09, dd (8.1, 14.7)	
17	5.29, m	4.35, m	5.02, t (7.6)	5.02, t (7.6)	4.38, t (7.7)
18	-	-	-	-	-
19	1.62, s	1.38, s	1.72, s	1.73, s	1.40, s
20	1.66, s	1.07, s	1.59, s	1.59, s	1.09, s
21	1.97, dd (6.3, 14.7)	2.34, dd (4.9, 13.5)	2.50, m	2.49, m	2.33, dd (4.9, 13.4)
	1.38, dd (8.7,14.7)	1.39, m	1.79, m	1.86, dd (9.9, 13.2)	1.31, dd (9.1, 13.4
22	2.54, d (8.7)	2.55, dd (10.8, 14.3)	2.16, m	2.19, dd (4.8, 9.9)	2.49, d (9.1)
23	-	-	-	-	-
24	1.12, s	1.27, s	1.34, s	1.35, s	1.23, s
25	1.39, s	1.74, s	1.35, s	1.36, s	1.69, s
OH-1	12.09, s	13.15, s	12.77, s	12.54, s	13.27, s
OH-3	6.96, s	-	-	7.52, s	-
OMe-3	-	-	3.79, s	-	3.77, s
OMe-8	3.29, s	-	-	-	-

Table 2. ^{13}C NMR spectroscopic data of compounds **1**–**5** (150 MHz in $CDCl_3$).

Position	**1**	**2**	**3**	**4**	**5**
1	162.5	166.3	163.8	163.3	164.2
2	99.5	92.6	94.1	99.2	94.0
3	165.5	168.2	168.3	165.7	168.3
4	111.4	112.4	114.4	111.5	113.6
4a	158.0	156.5	158.3	159.8	159.3
5	86.7	84.6	199.9	199.9	85.1
6	209.1	203.9	79.4	79.2	204.3
7	44.7	46.7	44.9	44.9	47.5
8	74.6	134.4	134.2	134.2	134.2
8a	47.2	135.0	135.0	134.3	133.5
9	194.6	178.9	178.8	178.6	180.2
9a	103.1	101.3	102.0	102.4	101.7
10a	88.7	90.8	83.7	83.9	91.5
11	41.4	43.7	40.9	41.4	41.6
12	149.2	91.5	151.6	149.9	150.9
13	113.4	15.9	106.1	113.3	106.7
14	30.3	25.1	29.5	28.3	29.0
15	27.3	19.6	29.0	27.6	28.0
16	28.1	28.5	30.4	30.4	29.4
17	117.8	117.5	117.6	117.4	118.2
18	133.9	133.7	136.4	136.5	135.3
19	18.0	25.5	18.3	18.3	25.7
20	26.0	16.3	25.9	26.1	17.1
21	20.2	27.8	32.8	32.9	26.6
22	43.9	48.9	42.5	42.4	49.5
23	81.1	83.0	83.6	83.9	83.3
24	27.4	28.6	26.9	26.9	31.0
25	30.5	30.5	29.7	29.7	31.4
OMe-3	-	-	55.6	-	55.5
OMe-8	55.7	-	-	-	-



Scheme 1. Plausible biosynthetic pathway of compounds **1**–**6**.