2019

Uvarialuridols A-C, three new polyoxygenated cyclohexenes from the twig and leaf extracts of *Uvaria lurida*

Virayu Suthiphasilp
Mae Fah Luang University

Wisanu Maneerat
Mae Fah Luang University

Raymond J. Andersen
University of British Columbia

Brian O. Patrick
University of British Columbia

Piyaporn Phukhatmuen
Mae Fah Luang University

See next page for additional authors

Publication Details

Suthiphasilp, V., Maneerat, W., Andersen, R. J., Patrick, B. O., Phukhatmuen, P., Pyne, S. G. & Laphookhieo, S. (2019). Uvarialuridols A-C, three new polyoxygenated cyclohexenes from the twig and leaf extracts of *Uvaria lurida*. *Fitoterapia*, 138 104340-1-104340-6.

Uvarialuridols A-C, three new polyoxygenated cyclohexenes from the twig and leaf extracts of *Uvaria lurida*

Abstract

The first phytochemical investigation of *Uvaria lurida* resulted in the isolation and identification of three new polyoxygenated cyclohexenes, (+)-(1R,2S,3R,6S)-uvarialuridols A-C (1-3), together with 10 known compounds (4-13). All new structures were elucidated by spectroscopic methods and HRESIMS. The absolute configurations of compounds 1 and 5 were confirmed by X-ray diffraction analysis using Cu K α radiation. The absolute configurations of compounds 2-4 were identified from comparisons of their specific rotations and ECD spectra with those of known compounds. Compound 11 showed α -glucosidase inhibitory activity with an IC₅₀ value of 30 μ M which was better than the standard control, acarbose (74 μ M) whereas, compound 10 exhibited nitric oxide (NO) production inhibitory activity with an IC₅₀ value of 37 μ M.

Publication Details

Suthiphasilp, V., Maneerat, W., Andersen, R. J., Patrick, B. O., Phukhatmuen, P., Pyne, S. G. & Laphookhieo, S. (2019). Uvarialuridols A-C, three new polyoxygenated cyclohexenes from the twig and leaf extracts of *Uvaria lurida*. *Fitoterapia*, 138 104340-1-104340-6.

Authors

Virayu Suthiphasilp, Wisanu Maneerat, Raymond J. Andersen, Brian O. Patrick, Piyaporn Phukhatmuen, Stephen G. Pyne, and Surat Laphookhieo

Uvarialuridols A-C, three new polyoxygenated cyclohexenes from the twig and leave extracts of *Uvaria lurida*

Virayu Suthiphasilp^{a,b}, Wisanu Maneerat^{a,b}, Raymond J. Andersen^c, Brian O. Patrick^c, Piyaporn Phukhatmuen^{a,b}, Stephen G. Pyne^d and Surat Laphookhieo^{a,b,*}

^aCenter of Chemical Innovation for Sustainability (CIS) and ^bSchool of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

^cDepartment of Chemistry and Department of Earth, Ocean & Atmospheric Sciences, University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Z1

^dSchool of Chemistry and Molecular Biosciences, University of Wollongong, Wollongong, New South Wales 2522, Australia

*Phone: +66-5391-6238; fax: +66-5391-6776; e-mail: surat.lap@mfu.ac.th

Abstract

The first phytochemical investigation of *Uvaria lurida* resulted in the isolation and identification of three new polyoxygenated cyclohexenes, (+)-(1*R*,2*S*,3*R*,6*S*)-uvarialuridols A-C (**1-3**), together with 10 known compounds (**4-13**). All new structures were elucidated by spectroscopic methods and HRESIMS. The absolute configurations of compounds **1** and **5** were confirmed by X-ray diffraction analysis using Cu K α radiation. The absolute configurations of compounds **2-4** were identified from comparisons of their specific rotations and ECD spectra with those of known compounds. Compound **11** showed α -glucosidase inhibitory activity with an IC₅₀ value of 30 μ M which was better than the standard control, acarbose (74 μ M) whereas, compound **10** exhibited nitric oxide (NO) production inhibitory activity with an IC₅₀ value of 37 μ M.

Keywords: *Uvaria lurida*; Annonaceae; polyoxygenated cyclohexenes; Uvarialuridols A-C

1. Introduction

The *Uvaria* genus belongs to the Annonaceae family and is widely distributed throughout tropical countries especially in South and Southeast Asia, Australia, and Papua New Guinea [1-3]. Its fruits are aggregates which are colored deep orange, yellow or a rich red and, for some species, are edible [4]. Many species of *Uvaria* have been used as traditional medicines. For example, the decoction of the roots of *U. cherrevensis* has been used for the treatment of urinary disorders, and the blood system and as a kidney tonic [5-8] whereas, the roots of *U. chamae* have been used as an antidiabetic and to treat infections [9] The wood and roots of *U. rufa* have been used to treat fever [10] The root and leaves of *U. narum* have been used to treat fevers, jaundice, rheumatic affections, and skin diseases [2,11-13]. Previous phytochemical investigations of the *Uvaria* genus resulted in the identification of cyclohexenes, [5,6,8,14-17] and flavonoids [6,10,14-16] as major phytochemical components with some minor compounds also isolated, including a naphthalene derivative [5], alkaloids [15], chalcones [6,9,16], benzoate esters [13,16], and a β -triketone derivative [18]. These isolated compounds displayed a wide range of biological activities, including antimalarial [5,9,16], cytotoxic [7,8,15,17], antifungal [13], α -glucosidase inhibitory [14], antitubercular [17], antitrypanosomal and antileishmanial activities [18]. *Uvaria lurida* (synonym: *Uvaria concava* Teijsm. & Binn.) [1] is a large woody scandent shrub which is distributed in the East of Thailand. To the best of our knowledge no phytochemical investigations or biological evaluations of this plant, as well as traditional medicine uses, have been reported. We report herein the first isolation and structure elucidation of three new polyoxygenated cyclohexenes (**1-3**) together with 10 known compounds (**4-13**) from the twig and leaf extracts of *U. lurida*, which were collected from Mae Fah Luang

University, Chiang Rai Province, Thailand. Some isolated compounds were tested for their α -glucosidase inhibitory and nitric oxide (NO) production inhibitory activities.

2. Experimental

2.1 General experimental procedures

The general information on instruments and chromatographic materials were the same as in previous reports [19-23] (General Experimental Procedures, Supplementary material).

2.2 Plant material

The twigs and leaves of *U. lurida* were collected in June 2017 at Health Park, Mae Fah Luang University, Chiang Rai Province, Thailand. The voucher specimen (MFU-NPR0164) was deposited at the Natural Products Research Laboratory of Mae Fah Luang University.

2.3 Extraction and isolation

The twigs of *U. lurida* (564.4 g) were chopped, air-dried and extracted with EtOAc (10 L) at room temperature and concentrated under reduced pressure to provide the EtOAc extract (36 g). The EtOAc extract was separated by quick column chromatography (QCC) over silica gel (100% hexanes to 100% EtOAc) to give eight fractions (Fr.U1- Fr.U8). Fr.U3 (86.8 mg) was fractionated by RP C₁₈ HPLC (2:8 MeOH: H₂O) to provide five fractions (Fr.U3A- Fr.U3E). Compound **10** (4.1 mg, t_R = 53.9 min) was obtained from Fr.U3C (27.3 mg) by RP C₁₈ HPLC (1.2:0.8 CH₃CN in H₂O, 2.0 mL/min) while compound **11** (4.1 mg) was isolated from Fr.U6 (3.65 g) by Sephadex LH-20 (100% MeOH) followed by PTLC (1:9 EtOAc: CH₂Cl₂).

Chopped and air-dried of *U. lurida* leaves (405.1 g) were extracted with EtOAc (10 L) at room temperature and concentrated under reduced pressure to yield the EtOAc extract (90 g).

The EtOAc extract was subjected to quick column chromatography (QCC) over silica gel (100% hexanes to 100% EtOAc) to give 10 fractions (Fr.L1-Fr.L10). Fr.L2 (2 g) was separated by CC (2:8 EtOAc: hexanes) to give compound **12** (15.1 mg). Fr.L4 (589.7 mg) was further separated by Sephadex LH-20 (100% MeOH) to provide six fractions (Fr.L4A- Fr.L4F). Fr.L4D (241.5 mg) was further purified by CC (3:7 CH₂Cl₂: hexanes) to give a mixture of compounds **1** and **4** (100.7 mg). Further purification of these two compounds by RP C₁₈ HPLC (1.2:0.8 CH₃CN in H₂O, 2.0 mL/min) gave compounds **1** (67.5 mg, *t_R* = 19.1 min) and **4** (30.5 mg, *t_R* = 21.8 min). Fr.L9 (1.7 mg) was purified by RP C₁₈ HPLC (9:1 MeOH: H₂O) to give 16 fractions (HPLC L9A- HPLC L9P). Compounds **7** (1.8 mg) and **8** (2.1 mg) were isolated by CC (100% CH₂Cl₂) from Fr.L9M (732.6 g). Fr.L9K (80.6 mg) was separated by RP C₁₈ HPLC (8:2 MeOH: H₂O) to give compound **13** (4.6 mg) and Fr.L9K1 (70.6 mg). Fr.L9K1 (70.6 mg) was further separated by CC (1:9 EtOAc: CH₂Cl₂) yielding compounds **2** (2.5 mg) and **3** (3.5 mg). Fr.L7 (380.4 mg) was separated by Sephadex LH-20 (100 % MeOH) to give seven (Fr.L7A-Fr.L7G). Fr.L7D (94.5 mg) was further purified by RP C₁₈ HPLC (9:1 MeOH: H₂O) to give six fractions (Fr.L7D1-Fr.L7D6). Fr.L7D2 (25.2 mg) was further separated by CC (1:9 EtOAc: CH₂Cl₂) to give compound **9** (15.7 mg). Compounds **5** (20.5 mg) and **6** (3.5 mg) were obtained from Fr.L7D3 (58.7 mg) by CC (2:8 EtOAc: CH₂Cl₂).

(+)-(1*R*,2*S*,3*R*,6*S*)-*Uvarialuridol A* (**1**): Colorless crystals; $[\alpha]_D^{23} +33$ (*c* 1, CHCl₃), mp 130-133 °C, UV (MeOH) λ_{max} (log ϵ) 275 (0.55), 231 (1.73), and 201 (1.75) nm, IR (neat) ν_{max} 3380, 1729, 1728, 1366, 1231, 717 cm⁻¹; ECD (5.31 × 10⁻³ M, MeOH) λ_{max} ($\Delta\epsilon$) 220 (+3.39), 244 (-3.05) nm. ¹H and ¹³C NMR, see Table 1; HRESITOFMS *m/z* 401.1214 [M + Na]⁺ (calcd for C₁₉H₂₂O₈Na, 401.1212).

(+)-(1*R*,2*S*,3*R*,6*S*)-*Uvarialuridol B* (**2**): Colorless viscous oil; $[\alpha]_{\text{D}}^{23} +26$ (c 1, MeOH), UV (MeOH) λ_{max} (log ϵ) 276 (0.46), 233 (1.59), and 201 (1.73) nm, IR (neat) ν_{max} 3481, 2932, 1718, 1269, 1249, 1097, and 711 cm^{-1} ; ECD (6.85×10^{-3} M, MeOH) λ_{max} ($\Delta\epsilon$) 203 (+4.36), 236 (+4.76), 277 (-3.22) nm. ^1H and ^{13}C NMR, see Table 1; HRESITOFMS m/z 463.1370 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{24}\text{H}_{24}\text{O}_8\text{Na}$, 463.1369).

(+)-(1*R*,2*S*,3*R*,6*S*)-*Uvarialuridol C* (**3**): Colorless viscous oil; $[\alpha]_{\text{D}}^{23} +38$ (c 1, CHCl_3), UV (MeOH) λ_{max} (log ϵ) 274 (0.57), 231 (1.63), and 203 (1.62) nm, IR (neat) ν_{max} 3428, 2933, 1724, 1723, 1282, 1270, 1097, and 711 cm^{-1} ; ECD (7.59×10^{-3} M, MeOH) λ_{max} ($\Delta\epsilon$) 209 (+3.32), 227 (+4.63), 252 (-4.48) nm. ^1H and ^{13}C NMR, see Table 1; HRESITOFMS m/z 359.1110 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{17}\text{H}_{20}\text{O}_7\text{Na}$, 359.1107).

2.4 Single crystal X-ray diffraction analysis of compounds **1** and **5**

Single colorless irregular-shaped crystals of **1** ($0.20 \times 0.18 \times 0.09 \text{ mm}^3$) and **5** ($0.35 \times 0.23 \times 0.16 \text{ mm}^3$) were mounted on a mylar loop in oil on a Bruker APEX-II CCD diffractometer. The crystal was kept at a steady $T = 90(2) \text{ K}$ during data collection. The structure was solved with the XT [24-25] structure solution program using the Intrinsic Phasing solution method and by using Olex2 [26] as the graphical interface. The model was refined with version 2018/3 of XL [24-25] using Least Squares minimization.

*X-ray crystallographic data for (+)-(1*R*,2*S*,3*R*,6*S*)-uvarialuridol A*: $\text{C}_{19}\text{H}_{22}\text{O}_8$, $M_r = 378.36$, tetragonal, $P4_1$ (No. 76), $a = 9.8944(2) \text{ \AA}$, $b = 9.8944(2) \text{ \AA}$, $c = 37.2068(8) \text{ \AA}$, $\alpha = \beta = \gamma = 90^\circ$, $V = 3642.51(17) \text{ \AA}^3$, $T = 90(2) \text{ K}$, $Z = 8$, $Z' = 2$, $\mu(\text{CuK}\alpha) = 0.912$, 6417 reflections measured, 6358 unique ($R_{\text{int}} = 0.064$) which were used in all calculations. The final wR_2 was 0.0668 (all data) and R_I was 0.0271 ($I > 2(I)$). The Flack parameter was refined to -0.03(9). The

crystallographic data of (+)-**1** have been deposited in the Cambridge Crystallographic Data Centre as CCDC 1941051 and data can be obtained free of charge from the via http://www.ccdc.cam.ac.uk/data_request/cif.

X-ray crystallographic data for (-)-(1S,2S,3R)-zeylenone: C₂₁H₁₈O₇, $M_r = 382.35$, orthorhombic, $P2_12_12_1$ (No. 19), $a = 7.3739(2) \text{ \AA}$, $b = 12.5767(3) \text{ \AA}$, $c = 19.8326(5) \text{ \AA}$, $\alpha = \beta = \gamma = 90^\circ$, $V = 1839.26(8) \text{ \AA}^3$, $T = 90(2) \text{ K}$, $Z = 4$, $Z' = 1$, $\mu(\text{CuK}\alpha) = 0.876$, 16730 reflections measured, 3366 unique ($R_{\text{int}} = 0.0349$) which were used in all calculations. The final wR_2 was 0.0669 (all data) and R_1 was 0.0260 ($I > 2(I)$). The Flack parameter was refined to -0.01(7). The crystallographic data of (-)-**5** have been deposited in the Cambridge Crystallographic Data Centre as CCDC 1941052 and data can be obtained free of charge from the via http://www.ccdc.cam.ac.uk/data_request/cif.

2.5 α -Glucosidase inhibitory assay

A colorimetric α -glucosidase assay was performed using previously described method [14,23]. Acarbose was used as a positive control with an IC₅₀ value at 73.74 μM .

2.6 Nitric oxide production inhibitory assay and cell viability

The procedure for the nitric oxide (NO) production inhibitory assay used the Griess reagent reaction, which is slightly modified from previous studies [27]. In brief, RAW 264.7 cells were added in 96-well plate with 1×10^5 cells/well and incubated overnight at 37 °C and 5% CO₂. After that, cells were treated with various concentrations of sample or vehicle (DMSO) for 2 h, followed by LPS 10 $\mu\text{g/mL}$. Then 18 h incubation, NO production in the culture medium was determined using the Griess reagent for 10 min and the absorbance was measured at 550 nm.

Cell viability studies were carried out by the MTT assay, as previously described [27]. Indomethacin was used as a positive control with an IC₅₀ value of 150 μ M.

3. Results and discussion

The EtOAc extracts of the twigs and leaves of *U. lurida* were individually separated using various chromatographic techniques to provide three new polyoxygenated cyclohexenes (**1-3**) together with the ten known compounds; (+)-(1*R*,2*S*,3*R*,6*S*)-curcuminol F (**4**) [28], (-)-(1*S*,2*S*,3*R*)-zeylenone (**5**) [29], pinocembrin (**6**) [30], auriculatin (**7**) [31], auriculasin (**8**) [32], scandenone (**9**) [33], uvamalol F (**10**) [34], (2*E*)-3-(3,4-dihydroxyphenyl)-*N*-[2-(4-methoxyphenyl)ethyl]-2-propenamide (**11**) [35], goniopedalin (**12**) [36,37], and anabellamide (**13**) [38] identified by comparison of their spectroscopic and physical data with published values.

The structure of compound **1** was a polyoxygenated cyclohexene, which was confirmed by X-ray diffraction data analysis (Fig. 4, CCDC 1941051). Its molecular formula, C₁₉H₂₂O₈, was supported from the HRESITOFMS data ([M + Na]⁺ *m/z* 401.1214, calcd 401.1212). The NMR spectroscopic data of **1** (Table 1), which were in agreement with the X-ray structure, displayed resonances for two olefinic protons [δ_{H} 6.04 (dd, *J* = 10.3, 2.5 Hz, H-4)/ δ_{C} 130.2 and 5.88 (ddd, *J* = 10.3, 3.9, 2.5 Hz, H-5)/ δ_{C} 124.9], three oxymethine protons [δ_{H} 5.52 (d, *J* = 6.6 Hz, H-2)/ δ_{C} 71.5, 4.15 (d, *J* = 6.6 Hz, H-3)/ δ_{C} 76.5 and 5.49 (d, *J* = 3.9 Hz, H-6)/ δ_{C} 70.8], methyl protons of two acetate groups [δ_{H} 2.22 (s, COMe-2)/ δ_{C} 20.9 and 2.13 (s, COMe-6)/ δ_{C} 20.9], methoxy protons [δ_{H} 3.45 (s, OMe-3)/ δ_{C} 56.7], monosubstituted aromatic protons [δ_{H} 8.07 (m, H-2'/H-6')/ δ_{C} 129.7, 7.50 (m, H-3'/H-5')/ δ_{C} 133.4, and 7.63 (m, H-4')/ δ_{C} 128.5], one set of diastereomeric methylene protons [δ_{H} 4.64 (d, *J* = 11.8 Hz, H-1') and 4.47 (d, *J* = 11.8 Hz, H-

1')/ δ_C 65.9] and one hydroxy proton [δ_H 3.04 (s, OH-1)]. The NOSEY cross peaks (Supporting Information) of H-2 (δ_H 5.52) with OMe-3 (δ_H 3.43) and OH-1 (δ_H 3.04) with H-2 (δ_H 5.52) and H-6 (δ_H 5.49) indicated all of these protons were *syn*-orientation. The HMBC correlations displayed in Fig. 2 confirmed the locations of all substituents in the core skeleton. The (1*R*,2*S*,3*R*,6*S*) absolute configuration was established from the single-crystal X-ray diffraction analysis with Cu K α radiation (Fig. 4) which had the Flack parameter of -0.03(9). This compound displayed a positive Cotton effect at 220 nm and a negative Cotton effect at 245 nm (Fig. 3) and its specific rotation was $[\alpha]_D^{23} +33$ (*c* 1, CHCl₃). Therefore, compound **1** was assigned as (+)-(1*R*,2*S*,3*R*,6*S*)-uvarialuridol A.

The molecular formula of compound **2**, C₂₄H₂₄O₈, was deduced from its HRESITOFMS data, which showed an [M + Na]⁺ ion at *m/z* 463.1370 (calcd for C₂₄H₂₄O₈Na, 463.1369). The ¹H and ¹³C NMR spectroscopic data of **2** (Table 1) were similar to those of **1** except that the resonances for the acetoxy group (δ_H 2.13/ δ_C 20.9) at C-6 of **1** were replaced by resonances for a benzoyl unit [δ_H 8.05 (m, H-2''/H-6'')/ δ_C 129.7, 7.43 (m, H-3''/H-5'')/ δ_C 128.8, and 7.57 (m, H-4'')/ δ_C 133.9]. This was further supported by the HMBC correlations shown in Figure 1. The vicinal coupling constant between H-2 (δ_H 5.62) and H-3 (δ_H 4.16) of 6.5 Hz suggested H-2 and H-3 were in a 1,2-diaxial relationship [5]. The NOSEY cross peaks (Supplementary material) of OMe-3 (δ_H 3.43) with H-2 (δ_H 5.62) and OH-1 (δ_H 3.04) with H-2 (δ_H 5.62) and H-6 (δ_H 5.71) indicated the *syn*-orientation of H-2 with OMe-3, OH-1, and H-6. The ECD spectrum of **2** (Fig. 3) was similar to that of (+)-**1** and its specific rotation was also of the same sign $\{[\alpha]_D^{23} +26$ (*c* 1, MeOH) for (+)-**2** and $[\alpha]_D^{23} +33$ (*c* 1, CHCl₃) for (+)-**1**} indicating that the absolute configuration of (+)-**2** was the same as that of (+)-**1**. Therefore, compound **2** was identified as (+)-(1*R*,2*S*,3*R*,6*S*)-uvarialuridol B.

Compound **3** ($[\alpha]^{23}_{\text{D}} +38$, c 1, CHCl_3) was isolated as a colorless viscous oil. Its molecular formula, $\text{C}_{17}\text{H}_{20}\text{O}_7$, was established on the basis of HRESITOFMS data which showed ion $[\text{M} + \text{Na}]^+$ ion at m/z 359.1110 (calcd for 359.1107, $\text{C}_{17}\text{H}_{20}\text{O}_7\text{Na}$). The ^1H and ^{13}C NMR spectroscopic data of **3** (Table 1) were closely related to those of **2** except for the lack of resonances for the benzoyl unit at C-6 of **2**. This C-6 substituent was replaced by a hydroxy group in **3** which was indicated by the relative shielding of the oxymethine proton H-6 at δ_{H} 3.57 (m). Its specific rotation, ECD spectrum (Fig. 3), and NOSEY cross peaks (Supporting Information) were similar to those of **1** and **2**, suggesting that they had the same absolute configuration. Thus, compound **3** was assigned as (+)-(1*R*,2*S*,3*R*,6*S*)-uvarialuridol C.

Compound **4**, curcuminol F, was first reported in 2009 from *Curcuma wenyujin* (Zingiberaceae) by Ma and co-workers [39] and its relative configuration, (1*S*,2*R*,3*S*,6*S*), was determined from NOESY experiments. This compound had a specific rotation of -62.3° (c 0.1, MeOH). In 2015, Hongthong and co-workers [28] also isolated the enantiomeric compound from *Dasymaschalon sootepense* (Annonaceae), with a specific rotation $[\alpha]^{25}_{\text{D}} +35.6$, c 0.1, MeOH, *ent*-curcuminol F [28]. In this study, we isolated compound **4** which displayed NMR spectroscopic data similar to those compounds isolated from *C. wenyujin* and *D. sootepense*. Herein, the absolute configuration of compound **4** was established by the comparison of its ECD spectrum $\{\lambda_{\text{max}} (\Delta\epsilon)$ 207 (+3.24), 245 (-2.87) nm} to that of **1** $\{\lambda_{\text{max}} (\Delta\epsilon)$ 220 (+3.39), 244 (-3.05) nm} (Fig. 3). Therefore, compound **4** was identified as (+)-(1*R*,2*S*,3*R*,6*S*)-curcuminol F ($[\alpha]_{\text{D}}^{23} +43$, c 1, MeOH), corresponding to the same compound isolated by Hongthong.¹⁹

Compound **5**, zeylenone (lit. $[\alpha]^{28}_{\text{D}} -126.5$, c 0.747, CHCl_3) [29], was first isolated from *U. grandiflora* in 1997 by Liao and co-workers [29] but its absolute configuration was not identified. In this study, the (1*S*,2*S*,3*R*) absolute configuration of zeylenone was established from

X-ray crystallographic data analysis (Fig. 5, CCDC 1941052) and the Flack parameter was refined to -0.01(7) as well as its ECD spectrum which displayed a strong negative Cotton effect at 250 nm (Fig. 3). Therefore, compound **5** was identified as (-)-(1*S*,2*S*,3*R*)-zeylenone ($[\alpha]^{22}_{\text{D}}$ -33.7, *c* 1, CHCl₃).

It should be noted that polyoxygenated cyclohexenes are well known as major compounds in the *Uvaria* genus [5,6,8,14-17,29,40]. The core cyclohexene structure contains one double bond and four chiral oxygenated carbons. The stereochemistry of these stereogenic carbons, as well as their substituent groups, is quite unique which is dependant on the enzymatic process in each plant species [5,6,8,14-17,29,40]. In this study, all cyclohexenes (**1-5**) contain a double bond at C-4/C-5 and the carbons containing the oxygenated substituent groups have the same stereochemistry: C-1 and C-2 are the same face of the molecules (β -orientation), whereas C-3 (α -orientation) is opposite to those of C-1 and C-2. Also, cyclohexenes **1-4** have the same stereochemistry at C-6 (α -orientation) whereas the OH-6 group of compound **5** was oxidized to a ketone group.

The biosynthetic pathway of polyoxygenated cyclohexenes from the *Uvaria* genus is known which comes from the shikimic acid pathway [5,8,40]. A putative biosynthetic pathway for **1-5** is shown in Scheme 1. Based on the results of our study, the key intermediate A is proposed as a precursor [8]. Epoxidation of the $\Delta^{1(6)}$ double bond followed by enzymatic acylation reactions, acetylation at C-2 or benzoxylation at C-3, would give the intermediates C or D, respectively. Epoxide ring opening (hydroxylation) of intermediate D leads to intermediate diol E, which could be further oxidation at OH-6 to give compound **5**. Compound **4** could be obtained from intermediates C and F via epoxide ring opening (hydroxylation) and acetylation at

C-3, respectively. On the other hand, selective methylation of intermediate F at C-3 could give **3**, which upon acetylation or benzylation at C-5 would give **1** or **2**, respectively.

Compounds **1**, **4-6**, **10**, and **11** were evaluated for their α -glucosidase inhibitory and NO production inhibitory activities. Compound **11** showed α -glucosidase inhibitory activity with an IC_{50} value of 30 μ M which was better than that of the standard control (IC_{50} value of acarbose = 74 μ M), whereas all remaining compounds were inactive at the concentration of 200 μ g/mL. In addition, compound **10** showed NO production inhibitory activity with an IC_{50} value of 37 μ M, while amide **11** showed weak NO production inhibitory with an IC_{50} value of 182 μ M. The standard control for NO production inhibitory is indomethacin with an IC_{50} value of 150 μ M.

Declaration of competing interests

The authors declare no competing financial interest.

Acknowledgments

This work was supported by Thailand Research Fund (BRG5980012 and PHD/0133/2559) and Mae Fah Luang University. Mae Fah Luang University and University of British Columbia are acknowledged for laboratory facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at

References

- [1] GBIF Global Biodiversity Information Facility, *Uvaria lurida* Hook.f. & Thomson.
<https://www.gbif.org/species/3154283/metrics>, 2018 (accessed 28 June 2019).

- [2] V.S. Parmar, O.D. Tyagi, A. Malhotra, S.K. Singh, S.K. Bisht, R. Jain, Novel Constituents of *Uvaria* Species, Nat. Prod. Rep. 11 (1994) 219-224.
- [3] C.V. Meade, A new species of *Uvaria* (Annonaceae) from Southeast Asia, Adansonia 27 (2015) 17-20.
- [4] C.K. Ruffo, A. Birnie, B. Tengnas, Edible Wild Plants of Tanzania, RELMA Technical Handbook Series 27, Nairobi, Kenya: Regional Land Management Unit (RELMA), Swedish International Development Cooperation Agency (Sida), 2002.
- [5] C. Auranwiwat, P. Wongsomboon, R. Rattanajak, S. Kamchonwongpaisan, A.C. Willis, S. Laphookhieo, S.G. Pyne, T. Limtharakul, Polyoxygenated Cyclohexenes and their chlorinated derivatives from the leaves of *Uvaria cherrevensis*, J. Nat. Prod. 82 (2019) 101-110.
- [6] C. Auranwiwat, P. Wongsomboon, T. Thaima, R. Rattanajak, S. Kamchonwongpaisan, A.C. Willis, W. Lie, S.G. Pyne, T. Limtharakul, 2-Phenylnaphthalenes and a polyoxygenated cyclohexene from the stem and root extracts of *Uvaria cherrevensis* (Annonaceae), Fitoterapia 120 (2017) 103-107.
- [7] L. Wirasathien, T. Pengsuparp, M. Moriyasu, K. Kawanishi, R. Suttisri, Cytotoxic C-benzylated chalcone and other constituents of *Ellipeiopsis cherrevensis*, Arch. Pharm. Res. 29 (2006) 497-502.
- [8] T. Jaipetch, S. Hongthong, C. Kuhakarn, P. Pailee, P. Piyachaturawat, K. Suksen, P. Kongsaree, S. Prabpai, N. Nuntasaen, V. Reutrakul, Cytotoxic polyoxygenated cyclohexene derivatives from the aerial parts of *Uvaria cherrevensis*, Fitoterapia 137 (2019) 104182.

- [9] H. Koudokpon, N. Armstrong, T.V. Dougnon, L. Fah, E. Hounsa, H.S. Bankolé, F. Loko, E. Chabrière, J.M. Rolain, Antibacterial activity of chalcone and dihydrochalcone compounds from *Uvaria chamae* roots against multidrug-resistant bacteria, *Bio. Med. Res. Int.* (2018).
- [10] K. Deepalard, K. Kawanishi, M. Moriyasu, T. Pengsuparp, R. Suttisri, Flavonoid glycosides from the leaves of *Uvaria rufa* with advanced glycation end-products inhibitory activity, *Thai J. Pharm. Sci.* 33 (2009) 84-90.
- [11] P. Subrahmanya, M. Ashalatha, P.S. Prajna, Y. Ashutosh, B. Rama, evaluation of antibacterial and antioxidant properties of *Uvaria narum* (dunal) wall, *Int. Res. J. Pharm.* 5 (2011) 142-144.
- [12] S. Padyana, M. Ashalatha, R. Radhakrishma, S. Saraswathi, Pharmacognostic evaluation of an ethnomedicine - *Uvaria narum* (Dunal) Wall. *Int. J. Chem. Pharm. Anal.* 1 (2013) 5-8.
- [13] A.E. Varghese, B. Govindan, J. Madhavankutty, A.T. Valiyaveetil, M. Karadka, S. Baby, A new antifungal benzoic acid ester from *Uvaria narum*, *Nat. Prod. Res.* 32 (2018) 2657-2662.
- [14] P. Meesakul, S.G. Pyne, S. Laphookhieo, Potent α -glucosidase inhibitory activity of compounds isolated from the leaf extracts of *Uvaria hamiltonii*, *Nat. Prod. Res.* (2018) (in press) DOI: 10.1080/14786419.2018.1538996.
- [15] C. Auranwiwat, R. Rattanajak, S. Kamchonwongpaisan, S. Laphookhieo, S.G. Pyne, T. Limtharakul, Four new C-benzyl flavonoids from the fruit of *Uvaria cherreensis*, *Fitoterapia* 130 (2018) 198-202.

- [16] R. Lekphrom, K. Kanokmedhakul, F. Schevenels, S. Kanokmedhakul, Antimalarial polyoxygenated cyclohexene derivatives from the roots of *Uvaria cherrevensis*, *Fitoterapia* 127 (2018) 420-424.
- [17] A.P.G. Macabeo, A.G. Letada, S. Budde, C. Faderl, H.M. Dahse, S.G. Franzblau, G.J.D. Alejandro, G.K. Pierens, M.J. Garson, Antitubercular and cytotoxic chlorinated seco cyclohexenes from *Uvaria alba*, *J. Nat. Prod.* 80 (2017) 3319-3323.
- [18] T.A. Okpekon, J.M.E. Dade, M.V. Say, D.K. Yapo, P. Champy, B. Séon-Méniel, S.F. Yolou, C. Bories, Bingervone, an antiprotozoal β -triketone derivative from the roots of *Uvaria afzelii* (annonaceae), *Int. J. Pharm. Sci. Res.* 6 (2015) 4210-4215.
- [19] A. Raksat, W. Maneerat, R.J. Andersen, S.G. Pyne, S. Laphookhieo, Antibacterial Prenylated Isoflavonoids from the Stems of *Millettia extensa*, *J. Nat. Prod.* 81 (2018) 1835-1840.
- [20] W. Jaidee, R.J. Andersen, M.A.G. Chavez, Y.A. Wang, B.O. Patrick, S.G. Pyne, C. Muanprasat, S. Borwornpinyo, S. Laphookhieo, Amides and flavonoids from the fruit and leaf extracts of *Melodorum siamensis*, *J. Nat. Prod.* 82 (2019) 283-292.
- [21] T. Sriyatep, I. Siridachakorn, W. Maneerat, A. Pansanit, T. Ritthiwigrom, R.J. Andersen, S. Laphookhieo, Bioactive prenylated xanthenes from the young fruits and flowers of *Garcinia cowa*, *J. Nat. Prod.* 78 (2015) 265-271.
- [22] T. Sriyatep, R.J. Andersen, B.O. Patrick, S.G. Pyne, C. Muanprasat, S. Seemakhuan, S. Borwornpinyo, S. Laphookhieo, Scalemic caged xanthenes isolated from the stem bark extract of *Garcinia propinqua*, *J. Nat. Prod.* 80 (2017) 1658-1667.

- [23] P. Meesakul, C. Richardson, S.G. Pyne, S. Laphookhieo, α -Glucosidase inhibitory flavonoids and oxepinones from the leaf and twig extracts of *Desmos cochinchinensis*, J. Nat. Prod. 82 (2019) 741-747.
- [24] G.M. Sheldrick, *SHELXT* – Integrated space-group and crystal-structure determination, Acta. Cryst. A71 (2015) 3-8.
- [25] G.M. Sheldrick, Crystal structure refinement with *SHELXL*, Acta. Cryst. C71 (2015) 3-8.
- [26] O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard, H. Puschmann, *OLEX2*: a complete structure solution, refinement and analysis program, J. Appl. Cryst. 42 (2009) 339-341.
- [27] J.P. Dzoyem, A.M. Lannang, C.D. Mbazoa, A.E. Nkengfack, N. Sewald, J.N. Eloff, Anti-inflammatory activity of benzophenone and xanthone derivatives isolated from *Garcinia* (Clusiaceae) species, Phytochem. Lett. 14 (2015) 153-158.
- [28] S. Hongthong, C. Kuhakarn, T. Jaipetch, S. Prabpai, P. Kongsaree, P. Piyachaturawat, S. Jariyawat, K. Suksen, J. Limthongkul, A. Panthong, N. Nuntasaen, V. Reutrakul, Polyoxygenated cyclohexene derivatives isolated from *Dasymaschalon sootepense* and their biological activities, Fitoterapia 106 (2015) 158-166.
- [29] Y.H. Liao, L.Z. Xu, S.L. Yang, J. Dai, Y.S. Zhen, M. Zhu, N.J. Sun, Three cyclohexene oxides from *Uvaria grandiflora*, Phytochemistry 45 (1997) 729-732.
- [30] M. Kuroyangi, T. Noro, S. Fukushima, R. Aiyama, A. Ikuta, H. Ttokawa, M. Morita, Studies on the constituents of the seeds of *Alpinia katsumadai* Hayata, Chem. Pharm. Bull. 31 (1983) 1544-1550.

- [31] M. Shabbir, A. Zaman, Structure of auriculatin, extractive of *Milletia auriculata*, J. Chem. Soc. (C) (1968) 1899-1901.
- [32] A.E. Nkengfack, D.R. Sanson, Z.T. Fomum, M.S. Tempesta, 8-prenylluteone, a prenylated isoflavone from *Erythrina eriotriocha*, Phytochemistry 28 (1989) 2522-2526.
- [33] N. Minhaj, H. Khan, S.K. Kapoor, A. Zaman, Extractives of *Milletia auriculata*-III, Tetrahedron 32 (1976) 749-751.
- [34] S. Wang, R.Y. Chen, S.S. Yu, D.Q. Yu, Uvamalols D–G: novel polyoxygenated seco-cyclohexenes from the roots of *Uvaria macrophylla*, J. Asian Nat. Prod. Res. 5 (2003) 17-23.
- [35] E. Anis, I. Ains, S. Ahmed, G. Mustafa, A. Malik, N. Afza, S.M.A. Hai, S. Shahzad-Ul-Hussan, M.I. Choudhary, α -Glucosidase inhibitory constituents from *Cuscuta reflexa*, Chem. Pharm. Bull. 50 (2002) 112-114.
- [36] X.N. Yang, Y.S. Jin, P. Zhu, H.S. Chen, Amides from *Uvaria microcarpa*, Chem. Nat. Compd. 46 (2010) 324-326.
- [37] S.K. Talapatra, D. Basu, P. Chattopadhyay, B. Talapatra, Aristololactams of *Goniothalamus sesquipedalis* wall. Revised structures of the 2-oxygenated aristololactams, Phytochemistry 27 (1988) 903-906.
- [38] N.J. Nwodo, F.B.C. Okoye, D. Lai, A. Debbab, R. Brun, P. Proksch, Two trypanocidal dipeptides from the roots of *Zapoteca portoricensis* (Fabaceae), Molecules 19 (2014) 5470-5477.

- [39] Z.J. Ma, Z.K. Meng, P. Zhang, Chemical constituents from the radix of *Curcuma wenyujin*, *Fitoterapia* 80 (2009) 374-376.
- [40] Y.M. Hsu, T.Y. Wu, Y.C. Du, M. El-Shazly, L. Beerhues, T.D. Thang, H.V. Luu, T.L. Hwang, F.R. Chang, Y.C. Wu, 3-Methyl-4,5-dihydro-oxepine, polyoxygenated seco-cyclohexenes and cyclohexenes from *Uvaria flexuosa* and their anti-inflammatory activity, *Phytochemistry* 122 (2016) 184–192.