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Antimalarial polyoxygenated cyclohexene derivatives from the roots of *Uvaria cherrevensis*

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ABSTRACT

Three new polyoxygenated cyclohexene derivatives named cherrevenisyls A and B (**1** and **2**), and ellipeiopsol E (**3**), along with fifteen known compounds, were isolated from the roots of *Uvaria cherrevensis*. Their structures were determined by spectroscopic methods including 2D NMR techniques and mass spectrometry. The absolute configurations of **1** and **2** were assigned. Compounds **1**, **2** and **5** showed antimalarial activity against *Plasmodium falciparum* with IC_{50} ranging from 3.34–7.34 μg/mL. Compounds **5**–**18** exhibited cytotoxicity against three cancer cell lines (KB, MCF-7 and NCI-H187) with IC_{50} values in ranging from 1.26–49.03 μg/mL.

1. Introduction

Uvaria cherrevensis (Pierre ex Finet & Gagnep.) L. L. Zhou, Y. C. F. S (Annonaceae) is a shrub that reaches up to 1.5 m in height and is found in deciduous forests throughout Thailand. Its synonym is *Ellipeiopsis cherrevensis* (Pierre ex Finet & Gagnep.) R. E. Fr and is known as "Nom maeo pa", "Phi phuan noi", and "Phi khao" in Thai [1]. A water decoction of its roots is used as traditional medicine to treat urinary disorders [2]. The genus *Uvaria* is known to be a rich source of polyoxygenated cyclohexene derivatives [3–8]. Previous investigations of the aerial parts of *E. Cherrevensis* (*U. cherrevensis*) led to the isolation of several polyoxygenated cyclohexene derivatives [9,10], as well as a cytotoxic C-benzoylated chalcone, flavonoids and alkaloids [10]. Recently, Auranwiwat et al. reported 2-phenylnaphthalenes and a cyclohexene from the stems and roots extracts of this plant [11]. In our continuing search for bioactive constituents from Thai plants, we noted that the roots extracts (EtOAc and MeOH) of *U. cherrevensis* showed cytotoxicity against KB cell lines with an IC_{50} value of 12.6 μg/mL. We report herein the isolation, the structural characterization, and bioactivities of three new polyoxygenated cyclohexene derivatives (**1**–**3**), together with fifteen known compounds (**4**–**18**). Among them, compounds **7**, **8** and **14** are reported for the first time from the genus *Uvaria*.

2. Experimental

2.1. General experimental procedures

Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter. IR spectra were taken on a Perkin-Elmer Spectrum One spectrophotometer. NMR spectra were recorded in $CDCl_3$ and CD_3OD on a Varian Mercury Plus 400 spectrometer, using residual $CHCl_3$ and CH_3OH as an internal standard. HRMS spectra were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of accurate masses. Column chromatography and preparative TLC were carried out on silica gel 60 (230–400 mesh) and PF_{254}, respectively.

2.2. Plant materials

The roots of *U. cherrevensis* were collected from Ban Na-khum Village, Ubonratana District, Khon Kaen Province, Thailand, in July 2011 and were identified by Prof. P. Chantaranothai, Department of Biology, Khon Kaen University, Thailand, where a voucher specimen S. Kanokmedhakul 19 was deposited.

2.3. Extraction and isolation

Air-dried roots of *U. cherrevensis* (1.1 kg) were ground to powder and then extracted successively with EtOAc (3 L × 3) and MeOH (3 L × 3). Removal of solvents from each extract under reduced pressure gave the crude EtOAc (57.5 g, 5.23%) and MeOH (65.9 g, 5.99%) extracts.

The EtOAc extract (55.0 g) was separated on silica gel column chromatography (CC), eluting with a gradient system of *n*-hexane-EtOAc (90:10 to 0:100 v/v) and then EtOAc-MeOH (100:0 to 0:100 v/v) to give six fractions, EF_1-EF_6. Evaporation of EF_1 gave compound **5** as a

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colourless oil (746.2 mg). Fraction EF_2 was purified by silica gel flash column chromatography (FCC), eluting with a gradient system of *n*-hexane-EtOAc (90:10 to 0:100 v/v) and then EtOAc-MeOH (100:0 to 0:100 v/v) to give ten subfractions, $EF_{2.1}$-$EF_{2.10}$. Subfraction $EF_{2.5}$ was purified on silica gel CC, eluting with a gradient system of *n*-hexane-EtOAc (80:20 to 0:100 v/v) to yield compound **6** as a colourless oil (269.8 mg). Subfraction $EF_{2.6}$ was separated by silica gel FCC, eluting with a gradient system of *n*-hexane-EtOAc to yield compound **7** as a colourless oil (190.0 mg) and an additional amount of **5** (36.3 mg). Subfraction $EF_{2.8}$ was chromatographed on silica gel FCC, eluting with a gradient system of *n*-hexane-EtOAc (80:20 to 0:100 v/v) to give compound **8** as a colourless solid (26.4 mg) and compound **9** as a colourless oil (17.3 mg). Subfraction $HF_{2.9}$ was purified on silica gel CC, gradually eluting with *n*-hexane-EtOAc (80:20 to 0:100 v/v) and EtOAc-MeOH (80:20 to 0:100 v/v) to give five subfractions, $EF_{2.9.1}$-$EF_{2.9.5}$. Subfraction $MF_{2.9.2}$ was subjected to silica gel CC, using the same solvent system as that of subfraction $EF_{2.8}$ above to give compound **10** as a colourless oil (55.6 mg). Subfraction $EF_{2.9.3}$ was purified by silica gel CC eluting with a gradient system of *n*-hexane-CH_2Cl_2 (80:30 to 0:100 v/v) to yield compounds **1** and **2** as white amorphous solids (49.0 and 25.0 mg). Subfraction $EF_{2.9.5}$ was further purified by silica gel FCC, using the same solvent system as that of subfraction $EF_{2.8}$ to give compounds **11** and **12** as two yellow oils (16.4 and 55.2 mg). Fraction EF_3 was chromatographed on silica gel CC and eluted with a gradient of *n*-hexane–EtOAc (90:10 to 0:100 v/v) to give nine subfractions, $EF_{3.1}$-$EF_{3.9}$. Subfraction $EF_{3.2}$ was subjected to silica gel CC, eluting with a gradient system of *n*-hexane-CH_2Cl_2 (70:30 to 0:100 v/v) to yield compound **13** as a white solid (11.5 mg). Subfraction $EF_{3.3}$ was separated by silica gel CC, using the same solvent system as that of subfraction $EF_{3.2}$ to yield compound **14** as a colourless amorphous powder (11.2 mg) and compound **15** as colourless crystals (37.1 mg). Subfraction $EF_{3.5}$ was separated by silica gel CC, eluting with a gradient system of CH_2Cl_2-EtOAc (100:0 to 0:100 v/v) to give compound **16** as orange needles (80.5 mg) and compound **17** as colourless needles (44.6 mg). Subfraction $EF_{3.9}$ was re-crystallized from *n*-hexane-EtOAc (30:70) to yield **3** as a pale yellow amorphous solid (40.2 mg). Fraction EF_4 was separated by silica gel CC, eluting with a gradient system of CH_2Cl_2-MeOH (100:0 to 0:100 v/v) to give compound **4** as a white amorphous solid (20.2 mg). Fraction EF_6 was separated by silica gel CC, eluting with a gradient system of CH_2Cl_2-MeOH (80:20 to 0:100 v/v) to give compound **18** as a yellow solid (120.5 mg).

The MeOH extract (60.0 g) was separated on silica gel CC, eluting with a gradient system of *n*-hexane-EtOAc (60:40 to 0:100 v/v) and then EtOAc-MeOH (80:20 to 0:100 v/v) to give seven fractions, MF_1–MF_7. Fraction MF_2 was separated by CC, eluting with a gradient system of *n*-hexane-EtOAc (60:40 to 0:100 v/v) to give four subfractions, $MF_{4.1}$-$MF_{4.4}$. Subfraction $MF_{4.1}$ afforded an additional amount of **14** (11.7 mg). Subfraction $MF_{4.2}$ was purified by preparative TLC, using *n*-hexane-CH_2Cl_2-EtOAc (20–10:70) as eluent to yield an additional amount of **11** (R_f = 0.42, 9.3 mg) and **12** (R_f = 0.40, 16.7 mg). Fraction MF_3 was further subjected to silica gel FCC, eluting with a gradient system of *n*-hexane-EtOAc (50:50 to 0:100 v/v) and then MeOH to give an additional amount of **14** (11.9 mg). Fraction EF_5 was separated by silica gel CC, eluting with a gradient system of CH_2Cl_2-MeOH (80:20 to 0:100 v/v) to give an additional amount of **17** (20.2 mg).

2.3.1. Cherrevenisyl A (1)

White amorphous solid; $[\alpha]_D^{26}$ + 94.4 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 268 (4.25) 331 (4.23); IR (KBr) ν_{max} 2927, 1717, 1601, 1491, 707 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 745.2269 $[M + Na]^+$ (calcd for $C_{41}H_{38}O_{12}Na$ 745.2261).

2.3.2. Cherrevenisyl B (2)

White amorphous solid; $[\alpha]_D^{26}$ + 9.2 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 235 (4.5); ECD (*c* 0.09 mM, MeOH) λ_{max} ($\Delta\varepsilon$): 206 (+ 80.35), 235 (− 26.49); IR (KBr) ν_{max} 2925, 1715, 1601, 1561, 712 cm^{-1}; ^1H and ^{13}C NMR see Table 1; HRESIMS *m/z* 683.2109 $[M + Na]^+$ (calcd for $C_{36}H_{36}O_{12}Na$ 683.2104).

Table 1
^1H and ^{13}C NMR spectral data (δ, ppm) for compounds **1** and **2** ($CDCl_3$).

Position	1		2	
	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type
Ring A				
1		134.3, C		134.3, C
2	5.79 d (9.2)	67.6, CH	5.56 d (9.2)	67.6, CH
3	5.27 dd (9.2,7.6)	74.0, CH	5.06 dd (9.2, 7.2)	73.2, CH
4	3.34 t (7.6)	31.9, CH	3.30 br m	31.6, CH
5	3.17 brm	39.3, CH	3.10 m	39.1, CH
6	6.11 brs	128.0, C	6.07 brs	128.2, C
1-CH_2OBz				
1		129.8, C		129.6, C
2,6	8.05 d (7.2)	129.7, C	8.01 d (7.2)	129.5, CH
3,5	7.48 d (7.2)	128.5, CH	7.45 m	128.6, CH
4	7.5 m	133.1, CH	7.58 m	133.4, CH
CO		166.0, C		166.4, C
CH_2	4.79 ABq (12.8)	64.3, CH_2	4.73 *br s*	64.4, CH_2
2-OAc	1.97 *s*	170.2, C; 20.1, CH_3	2.01 s	170.7, C; 20.5, CH_3
3-OAc	1.92 *s*	170.3, C; 20.7, CH_3	2.01 s	170.6, C; 20.5, CH_3
Ring B				
1′		46.5, C		46.4, C
2′	5.17 d (3.2)	74.4, CH	5.10 d (2.8)	74.6, CH
3′	4.53 t (3.2)	78.0, CH	4.51 t (2.8)	78.2, CH
4′	3.02 dd (6.8, 3.2)	35.2, CH	3.01 dd (6.6, 2.8)	35.0, CH
5′	6.47 dd (8.0, 6.8)	129.8, CH	6.40 dd (8.0,6.6)	130.7, CH
6′	5.86 d (8.0)	132.6, CH	5.83 d (8.0)	132.6, CH
1′-CH_2OBz				
1		129.5, C		129.3, C
2,6	8.00 d (7.2)	129.6, CH	7.95 d (7.2)	129.5, CH
3,5	7.49 (m)	128.6, CH	7.42 m	128.5, CH
4	7.60 (m)	133.5, CH	7.58 m	133.3, CH
CO		166.1, C		166.3, C
CH_2	4.69 d (11.6) 4.56 d (11.6)	61.2, CH_2	4.64 d (12.0) 4.54 d (12.0)	61.2, CH_2
2′-OAc	2.03 s	169.7, C; 20.8, CH_3	2.03 s	170.4, C; 20.7, CH_3
3′-OBz				
1		129.3, C		
2,6	7.98 d (7.2)	129.6, CH		
3,5	7.46 m	128.4, CH		
4	7.57 m	133.4, CH		
CO		165.2, C		
3′-OAc			2.06 s	170.9, C; 20.4, CH_3

2.3.3. Ellipeiopsol E (3)

Pale yellow amorphous solid; $[\alpha]_D^{26}$ − 165.0 (*c* 0.1, CH_3OH; IR (KBr) ν_{max} 3372, 2971, 2938, 1719, 1601, 1583, 714 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 387.1062 $[M + Na]^+$ (calcd for $C_{18}H_{20}O_8Na$ 387.1056).

2.4. Antimalarial assay

Antiplasmodial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen [23]. Quantitative assessment of antimalarial activity *in vitro* was determined by means of the microculture radioisotope technique, based upon the method described by Desjardins et al. [24]. The inhibitory concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* incorporation of [^{3}H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin.