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1-1-2019

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### Publication Details Citation

Auranwiwat, C., Maccarone, A. T., Carroll, A., Rattanajak, R., Kamchonwongpaisan, S., Blanksby, S. J., Pyne, S. G., & Limtharakul, T. (2019). Structure elucidation of cyclohexene (9Z)-octadec-9-enyl ethers isolated from the leaves of *Uvaria cherreensis* (Annonaceae). Faculty of Science, Medicine and Health - Papers: Part B. Retrieved from <https://ro.uow.edu.au/smhpapers1/603>

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## Structure elucidation of cyclohexene (9Z)-octadec-9-enyl ethers isolated from the leaves of *Uvaria cherreensis* (Annonaceae)

### Abstract

The phytochemical investigation of the leaf extract of *Uvaria cherreensis* (Annonaceae) yielded three new cyclohexene (9Z)-octadec-9-enyl ethers, cherrevenols M-O (1-3), and a known fatty ester derivative (4). The structures of the isolated compounds were elucidated by spectroscopic and computer-aided molecular modelling methods. Ozone Induced Dissociation (OzID) mass spectrometry was employed to determine the C-9 position of the side chain olefinic double bonds, while <sup>13</sup>C NMR spectroscopy indicated their (Z)-configurations. All isolated compounds were evaluated for their antimalarial and cytotoxic activities; all were inactive.

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Auranwiwat, C., Maccarone, A. T., Carroll, A. W., Rattanajak, R., Kamchonwongpaisan, S., Blanksby, S. J., Pyne, S. G. & Limtharakul, T. (2019). Structure elucidation of cyclohexene (9Z)-octadec-9-enyl ethers isolated from the leaves of *Uvaria cherreensis* (Annonaceae). *Tetrahedron Letters: the international journal for the rapid publication of all preliminary communications in organic chemistry*, 75 (15), 2336-2342.

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# Structure elucidation of cyclohexene (9Z)-octadec-9-enyl ethers isolated from the leaves of *Uvaria cherrevensis* (Annonaceae)

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## ABSTRACT

The phytochemical investigation of the leaf extract of *Uvaria cherrevensis* (*Annonaceae*) yielded three new cyclohexene (9*Z*)-octadec-9-enyl ethers, cherrevenols M-O (**1-3**), and a known fatty ester derivative (**4**). The structures of the isolated compounds were elucidated by spectroscopic and computer-aided molecular modeling methods. Ozone Induced Dissociation (OzID) mass spectrometry was employed to determine the C-9 position of the side chain olefinic double bonds, while <sup>13</sup>C NMR spectroscopy indicated their (*Z*)-configurations. All isolated compounds were evaluated for their antimalarial and cytotoxic activities; all were inactive.

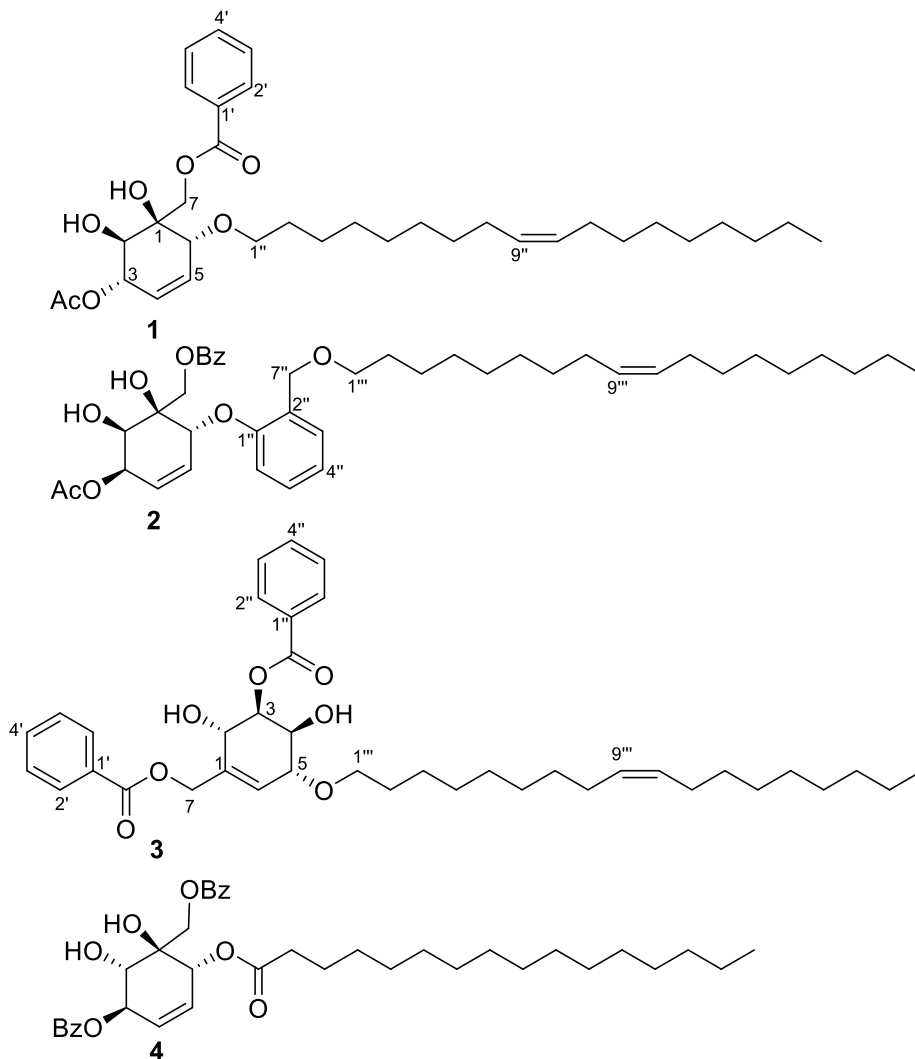
Keywords: *Uvaria cherrevensis*, cyclohexenes, Ozone Induced Dissociation (OzID)

## 1. Introduction

Cyclohexene long-chain saturated fatty esters were isolated in earlier studies from two species of *Uvaria*, *U. dulcis* and *U. dac.*<sup>1,2</sup> These compounds exhibited cytotoxic activities.<sup>2</sup> Previous studies on *U. cherrevensis*, **Annonaceae family**, found various types of secondary metabolites, including alkaloids, cyclohexenes, flavonoids, naphthalene derivatives, and terpenoids.<sup>3-8</sup> We report here the first isolation of cyclohexene **(9Z)-octadec-9-enyl** ethers, cherrevenols M-O (**1-3**), from the leaf extracts of *U. cherrevensis*. The application of <sup>13</sup>C NMR and Ozone Induced Dissociation (OzID) mass spectrometry were used to determine the (Z) configuration and C-9 position of the olefinic moiety of the C-18 ether side chains, respectively. The latter would otherwise be difficult to determine because of the lack of 2D NMR correlations due to overlapping of the side chain <sup>1</sup>H and <sup>13</sup>C NMR resonances. The isolated compounds were evaluated for their antimalarial and cytotoxic activities.

## 2. Results and discussion

The leaf extracts of *U. cherrevensis* were subjected to chromatographic separation techniques which led to the isolation and characterization of three new **compounds**, cherrevenols M-O (**1-3**), and a known compound, dulcisene A (**4**) (Figure 1).<sup>2</sup>



**Figure 1.** The structures of isolated compounds from the leaves of *U. cherrevensis*.

Cherrevenol M (**1**) was obtained as a white amorphous solid. Its molecular formula,  $C_{34}H_{52}O_7$ , was deduced from its HRESIMS data, which showed an  $[M+Na]^+$  ion at  $m/z$  595.3615 (calcd. for  $C_{34}H_{52}O_7Na$ , 595.3611). The UV spectrum exhibited a maximum absorption band at 207, 229, and 278 nm while the IR spectrum showed bands for hydroxy and carbonyl groups at 3454 and 1722  $cm^{-1}$ , respectively. The  $^1H$  NMR spectroscopic data of **1** (Table 1) was very similar to that of ellipseiopsol A (Figure 2, see Supporting Information (Table S1) for details) previously isolated from this plant.<sup>3</sup> The main difference was the addition of resonances for an *O*-(*Z*)-octadec-

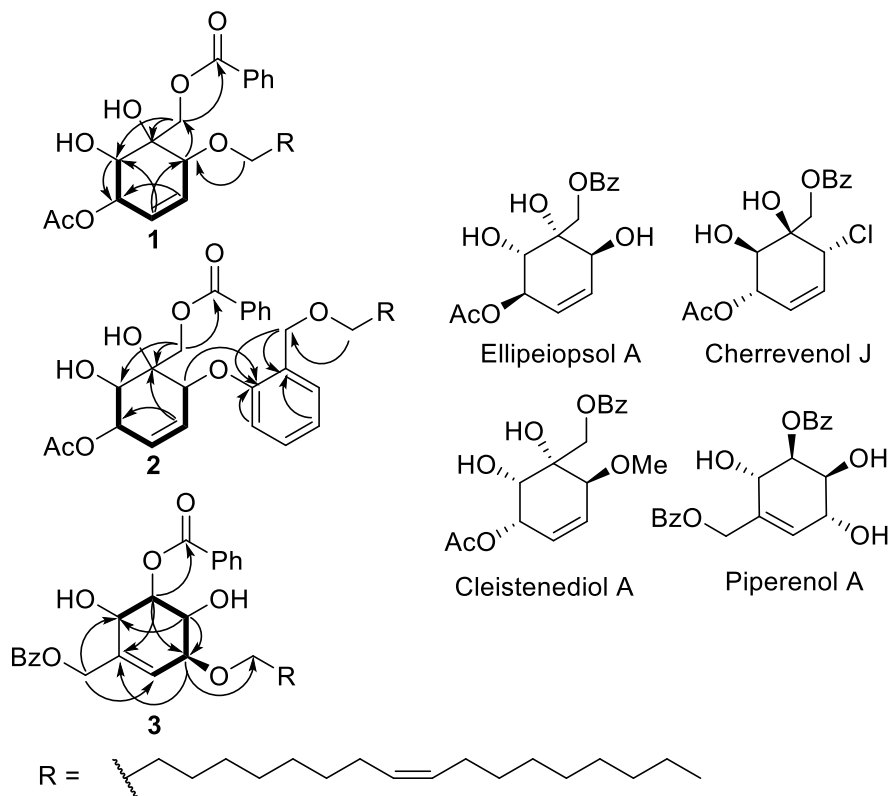
9-enyl moiety. The diastereotopic oxymethylene protons CH<sub>2</sub>-1'' ( $\delta_{\text{H}}$  3.65 and 3.47) showed **HMBC cross-peaks** to C-6 ( $\delta_{\text{C}}$  75.9) and thus the 6-*O*-(*Z*)-**octadec**-9-enyl moiety was attached to the C-6 oxygen substituent. The position of **the** double bond in the C-18 side chain was determined by OzID mass spectrometry.<sup>9,10</sup> In this experiment, the sodiated molecular ion ( $m/z$  595.3605) was isolated in an ion trap and exposed to ozone for 4 seconds. This produced the ozonide intermediate ( $m/z$  643.3470 [M+Na+O<sub>3</sub>]<sup>+</sup>) and its oxidative cleavage products corresponding to aldehyde ( $m/z$  485.2146) and Criegee ions ( $m/z$  501.2091) (Figures 4 and 5). These results allowed us to unequivocally assign the double bond at C-9''.<sup>11,12</sup> The <sup>13</sup>C NMR chemical shifts for C-8''/C-11'' ( $\delta_{\text{C}}$  27.37 and 27.35), C-9'' ( $\delta_{\text{C}}$  **130.0**) and C-10'' ( $\delta_{\text{C}}$  130.1) indicated that double bond had the (*Z*)-configuration.<sup>12</sup> The relative configuration of **1** at C-2 and C-3 was assigned from the  $J_{2,3}$  value of 6.3 Hz which indicated that H-2 and H-3 had a *trans* 1,2-diaxial-like relationship.<sup>8</sup> The NOESY spectrum showed a correlation between the diastereotopic CH<sub>2</sub>-7 ( $\delta_{\text{H}}$  4.79 and 4.60) resonances and the resonance for H-2 ( $\delta_{\text{H}}$  4.04) **and no correlations between H-6 ( $\delta_{\text{H}}$  3.94) and CH<sub>2</sub>-7 or between H-6 and H-2. This** indicated that **CH<sub>2</sub>-7 and H-2** were on the same face of the cyclohexene ring structure, **while H-6 was on the opposite face of the ring to H-2 and CH<sub>2</sub>-7.** When the proposed structure of **1** was generated computationally, [B3LYP/6-31G(d)] using the Gaussian 09 basis set. (Figure 3), the distance between H-2 and CH<sub>2</sub>-7 was calculated to be 2.58 Å, which correlated well with the experimental findings from the NOESY study. The specific rotation of **1** was of the same sign as that of cherrevenol J (Figure 2), which was also isolated from this plant,<sup>8</sup> { $[\alpha]_{\text{D}}^{24}$  -90 ( $c$  0.1, MeOH) and  $[\alpha]_{\text{D}}^{19}$  -84 ( $c$  0.2, MeOH), respectively}. In addition to this, the ECD spectra of these compounds exhibited similar negative Cotton effects at 207 and 203 nm, respectively. Therefore, **1** (cherrevenol M) was assigned as having the 1*R*,2*R*,3*S*,6*R* configuration.

**Table 1**<sup>1</sup>H (400 MHz) and <sup>13</sup>C (125 MHz) NMR spectroscopic data of cherrevenol M (**1**) in CDCl<sub>3</sub>

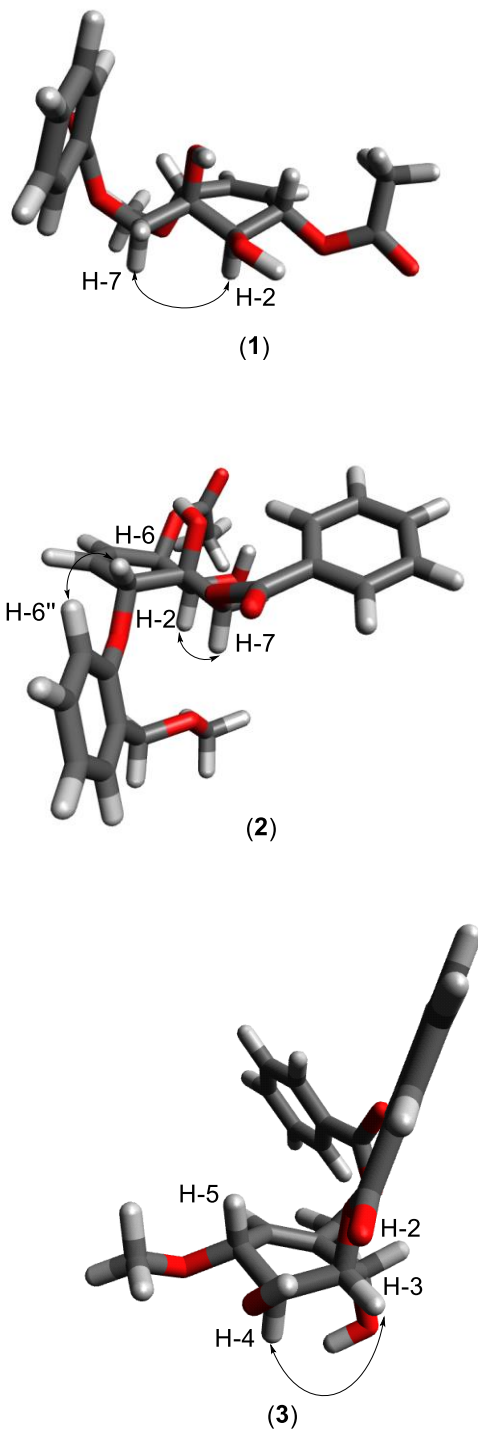
<b>1</b>							
Position	$\delta_{\text{H}}$ , ( <i>J</i> in Hz)	$\delta_{\text{C}}$ , type		Position	$\delta_{\text{H}}$ , ( <i>J</i> in Hz)	$\delta_{\text{C}}$ , type	
1		75.9 C		1''	3.65, dt (9.0, 6.4)	70.8	CH <sub>2</sub>
2	4.04, d (6.3)	71.5 CH			3.47, dt (9.0, 6.4)		
3	5.43, <b>m</b>	74.0 CH		2''	1.53, m	30.2	CH <sub>2</sub>
4	5.74, dd (10.2, 2.7)	126.9 CH		3''	1.26, m	26.3	CH <sub>2</sub>
5	6.03, ddd (10.2, 3.9, 1.8)	128.2 CH		4''-7'' and 12''-15''	1.26, m	29.92, 29.9, 29.85, 29.7, 29.6, 29.5, 29.47, 29.4	CH <sub>2</sub>
6	3.94, d (3.9)	75.9 CH		8''	2.00, <b>m</b>	27.37 <sup>a</sup>	CH <sub>2</sub>
7	4.79, d (12.0)	67.1 CH <sub>2</sub>		9''	5.34, m	<b>130.0</b>	CH
	4.60, d (12.0)			10''	5.34, m	130.1	CH
1'		129.9 C		11''	2.00, <b>m</b>	<b>27.35<sup>a</sup></b>	CH <sub>2</sub>
2',6'	8.05, dd (7.6, 1.5)	129.8 CH		16''	1.26, m	32.1	CH <sub>2</sub>
3',5'	7.46, t (7.6)	128.7 CH		17''	1.26, m	22.8	CH <sub>2</sub>
4'	7.59, tt (7.6, 1.5)	133.4 CH		18''	0.88, t (6.9)	14.3	CH <sub>3</sub>
<b>3-OC(O)Me</b>		171.9 C		<b>7-OC(O)</b>		167.3	C
<b>3-OC(O)Me</b>	<b>2.07, s</b>	21.3 CH <sub>3</sub>					

<sup>a</sup>exchangeable carbons





**Figure 2.** Selected keys HMBC ( $^1\text{H} \rightarrow ^{13}\text{C}$ ) and COSY (bold line) correlations for compounds **1-3** and the structures of known natural products.



**Figure 3.** Experimental NOE correlations projected onto DFT minimized molecular models [B3LYP/6-31G(d)] of **1-3** (calculated on the corresponding Me ethers).

Cherrevenol N (**2**) was isolated as a colourless oil and showed an **HRESIMS**  $[M + Na]^+$  ion at  $m/z$  701.4019 (calcd for  $C_{41}H_{58}O_8Na$ , 701.4029). The UV spectrum showed absorption bands at 203, 230, and 275 nm and the IR spectrum showed bands for hydroxy and carbonyl functionalities at 3437 and 1723  $cm^{-1}$ , respectively. The  $^1H$  and  $^{13}C$  NMR spectroscopic data were similar to those of **1** except for additional resonances characteristic of a 2-hydroxyphenylmethanol moiety [ $\delta_H$  7.32 (1H, dd,  $J = 7.5, 1.8$  Hz), 7.26 (1H, td,  $J = 8.2, 1.8$  Hz), 6.99 (1H, td,  $J = 7.5, 1.8$  Hz), 6.96 (1H, d,  $J = 8.2$  Hz), 4.81, and 4.28 (each 1H, d,  $J = 11.9$  Hz)] in **2**. The oxymethine proton H-6 ( $\delta_H$  4.97) showed a **HMBC cross-peak** to C-1'' ( $\delta_C$  157.34) while  $CH_2$ -1''' ( $\delta_H$  3.49 and 3.46) correlated with C-7'' ( $\delta_C$  69.0) indicating that the 2-hydroxyphenylmethanol moiety was linked between C-6 of the cyclohexene ring structure and C-1''' of the (*Z*)-**octadec**-9-enyl substituent (Figure 2). The  $^{13}C$  NMR chemical shifts of C-8''', C-9''', C-10''' and C-11''' at  $\delta_C$  27.4, 130.0, 130.1, and 27.4, respectively were characteristic of a (*Z*)-olefinic configuration.<sup>12</sup> The OzID mass spectrum of **2** exhibited aldehyde and Criegee ions at  $m/z$  591.2566 and 607.2517, respectively that indicated the alkene was located at C-9''' as shown in Figures 4 and 5. The relative configuration of **2** was determined from the examination of the NOESY spectrum. **Key correlations were observed between H-2 ( $\delta_H$  4.29) and  $CH_2$ -7 ( $\delta_H$  4.87 and 4.72), and between H-6 ( $\delta_H$  4.97) and H-6'' ( $\delta_H$  6.96) which validated the optimized molecular model as the calculated distance between these protons were 2.52 Å and 2.00 Å, respectively. In contrast, H-6 showed no NOESY correlations to  $CH_2$ -7, indicating that these protons were on the opposite face of the ring structure.** In addition, the coupling constant value between H-2 and H-3 was 3.5 Hz which indicated their relative 1,2 equatorial-axial **like** relationship (Figure 3). The specific rotation of **2**  $\{[\alpha]_D^{25} -57$  ( $c$  0.1, MeOH) was opposite in sign to that of cleistenediol A  $\{[\alpha]_D^{20} +190$  ( $c$  0.3, MeOH) (Figure 2), isolated from *Cleistochlamys kirkii*,<sup>13</sup> and so suggests an

opposite configuration of the known compound. Therefore, the configuration of cherrevenol M (**2**) was assigned as 1*R*,2*R*,3*R*,6*R*.

Cherrevenol O (**3**) was isolated as a colourless oil. Its molecular formula, C<sub>39</sub>H<sub>54</sub>O<sub>7</sub>, was deduced from its HRESIMS data, which showed an [M +Na]<sup>+</sup> ion at *m/z* 657.3770 (calcd for C<sub>39</sub>H<sub>54</sub>O<sub>7</sub>Na, 657.3767). The UV spectrum exhibited adsorption bands at 234 and 274 nm, and the IR spectrum showed bands at 3423 and 1722 cm<sup>-1</sup> that corresponded to hydroxy and carbonyl groups, respectively. The NMR spectroscopic data of **3** were similar to those of piperenol A (Figure 2), isolated from *Piper cubeb*,<sup>14</sup> except that **3** contained <sup>1</sup>H and <sup>13</sup>C NMR resonances characteristic of a (*Z*)-**octadec**-9-enyl moiety. The oxymethine proton H-5 ( $\delta_{\text{H}}$  4.13/ $\delta_{\text{C}}$  76.8) and the diastereotopic oxymethylene protons CH<sub>2</sub>-1''' of the **octadec**-9-enyl moiety ( $\delta_{\text{H}}$  3.69 and 3.63/ $\delta_{\text{C}}$  70.4) showed **HMBC cross-peaks** to each other which indicated that the (*Z*)-**octadec**-9-enyl moiety was attached to the C-5 oxygen substituent of the cyclohexene ring (Figure 2). The <sup>13</sup>C NMR chemical shifts for C-8'''/C-11''' ( $\delta_{\text{C}}$  27.4), C-9 ( $\delta_{\text{C}}$  130.0), and C-10 ( $\delta_{\text{C}}$  130.1) corresponded to the (*Z*)-olefinic configuration.<sup>12</sup> The double bond position of the long chain unit was determined by the OzID method. More specifically, the OzID spectrum showed an ion at *m/z* 705.3600 [M+Na+O<sub>3</sub>]<sup>+</sup> for the corresponding ozonide. The corresponding aldehyde and Criegee ions were observed at *m/z* 547.2302 and 563.2248, respectively which indicated that the double bond was at C-9''' as shown in Figures 4 and 5. **The relative configuration of **3** was assigned on the basis of the *J*<sub>2,3</sub> value of 4.8 Hz and *J*<sub>4,5</sub> value of 6.5 Hz which indicated 1,2-diequatorial-like and 1,2-diaxial-like couplings, respectively.<sup>8</sup> The correlations between H-3 ( $\delta_{\text{H}}$  5.50) and H-4 ( $\delta_{\text{H}}$  4.31), and between H-3 and H-2 ( $\delta_{\text{H}}$  4.52) in the NOESY spectrum, were consistent with the calculated inter-proton distances of 2.52 Å and 2.54 Å, respectively from the optimized structure (Figure 3). These *J* values, NOESY correlations and molecular modelling results fully supported the proposed**

**structure of 3.** The ECD spectrum of **3** was similar to that of piperenol A<sup>14</sup> which exhibited negative Cotton effects at 234 and 235 nm, respectively. This in combination with the above data led to the assignment of the configuration of **3** (Cherrevenol O) as 2*S*,3*R*,4*S*,5*R*.

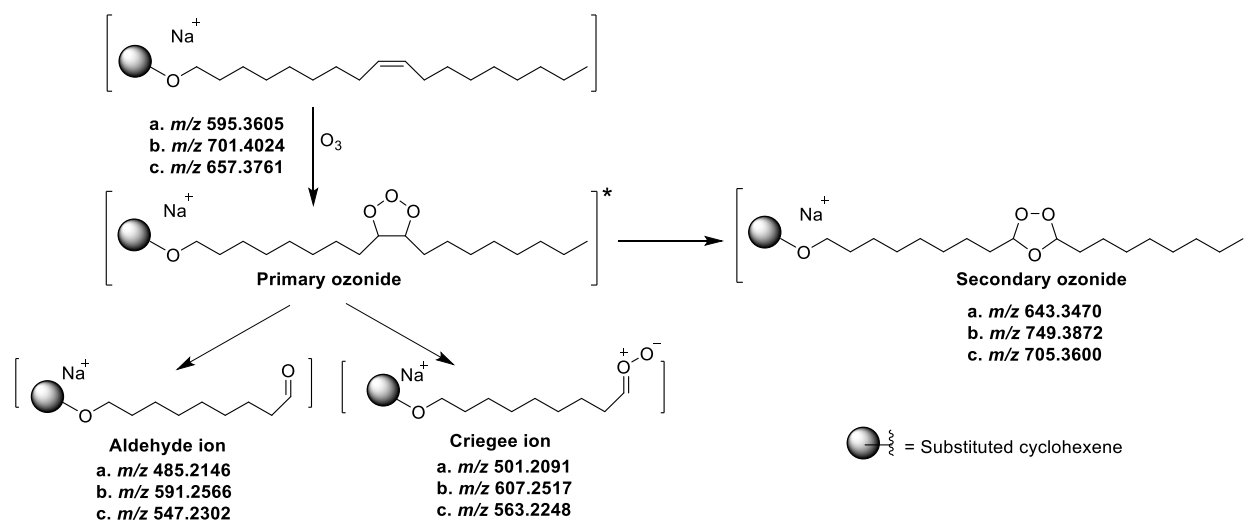
**Table 2**

<sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR data spectroscopic data of cherrevenols N-O (**2-3**) in CDCl<sub>3</sub>

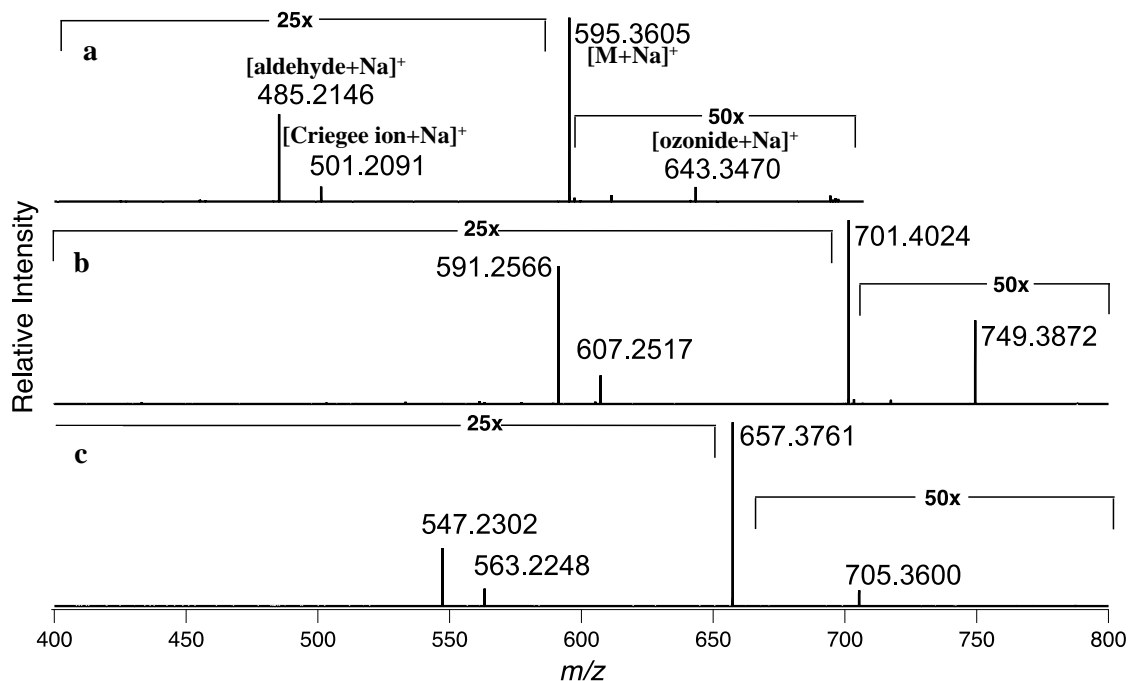
Position	<b>2<sup>a</sup></b>			<b>3<sup>b</sup></b>		
	$\delta_{\text{H}}$ , (J in Hz)	$\delta_{\text{C}}$ , type		$\delta_{\text{H}}$ , (J in Hz)	$\delta_{\text{C}}$ , type	
1		75.4	C		135.2	C
2	4.29, d (3.5)	71.2	CH	4.52, d (4.8)	67.7	CH
3	5.46, m	72.1	CH	5.50, dd (4.8, 2.4)	76.1	CH
4	5.84, dddd (10.2, 3.3, 1.5, 0.8)	127.1	CH	4.31, dd (6.5, 2.4)	69.2	CH
5	6.14, ddd (10.2, 2.9, 1.5)	128.9	CH	4.13, dd (6.5, 2.9)	76.8	CH
6	4.97, m	78.2	CH	6.09, d (2.9)	127.3	CH
7	4.87, d (12.3)	66.5	CH <sub>2</sub>	5.09, d (13.1)	65.2	CH <sub>2</sub>
	4.72, d (12.3)			4.88, d (13.1)		
1'		129.82	C		129.88	C
2',6'	7.93, dd (7.8, 1.4)	129.76	CH	8.04, dd (7.8, 1.4)	129.94	CH
3',5'	7.40, t (7.8)	128.6	CH	7.42, t (7.8)	128.61	CH
4'	7.55, tt (7.8, 1.4)	133.4	CH	7.59, tt (7.8, 1.4)	133.5	CH
1''		157.3	C		129.88	C
2''		127.8	C	8.02, dd (7.6, 1.2)	129.82	CH
3''	7.32, dd (7.5, 1.8)	130.5	CH	7.45, t (7.6)	128.58	CH
4''	6.99, td (7.5, 1.8)	122.0	CH	7.57, tt (7.6, 1.2)	133.4	CH
5''	7.26, td (8.2, 1.8)	129.6	CH	7.45, t (7.6)	128.58	CH
6''	6.96, d (8.2)	114.3	CH	8.02, dd (7.6, 1.2)	129.82	CH
7''	4.81, d (11.9)	69.0	CH <sub>2</sub>			
	4.28, d (11.9)					
1'''	3.49, dt (9.0, 7.0)	71.3	CH <sub>2</sub>	3.69, dt (9.3, 6.8)	70.4	CH <sub>2</sub>
	3.46, dt (9.0, 7.0)			3.63, dt (9.3, 6.8)		
2'''	1.56, dt (14.2, 6.9)	29.6	CH <sub>2</sub>	1.64, m	30.3	CH <sub>2</sub>
3'''	1.26, m	26.2	CH <sub>2</sub>	1.29, m	26.3	CH <sub>2</sub>
4'''-7''' and 12'''-15'''	1.26, m	29.92, 29.9, 29.7, 29.6, 29.57, 29.47, 29.47, 29.4	CH <sub>2</sub>	1.29, m	29.9, 29.9, 29.85, 29.7, 29.6, 29.5, 29.5, 29.45	CH <sub>2</sub>
8'''	1.99, m	27.4	CH <sub>2</sub>	2.03, q (6.5)	27.4	CH <sub>2</sub>
9'''	5.34, m	130.0	CH	5.36, m	130.0	CH
10'''	5.34, m	130.1	CH	5.36, m	130.1	CH

11'''	1.99, m	27.4	CH <sub>2</sub>	2.03, q (6.5)	27.4	CH <sub>2</sub>
16'''	1.26, m	32.1	CH <sub>2</sub>	1.29, m	32.1	CH <sub>2</sub>
17'''	1.26, m	22.8	CH <sub>2</sub>	1.29, m	22.8	CH <sub>2</sub>
18'''	0.88, t (6.9)	14.3	CH <sub>3</sub>	0.90, t (6.8)	14.3	CH <sub>3</sub>
3-OC(O)Me		170.1	C			
3-OC(O)Me	1.95, s	21.0	CH <sub>3</sub>			
3-OC(O)					166.64	C
7-OC(O)		167.1	C		166.60	C

<sup>a</sup> <sup>13</sup>C NMR recorded at 100 MHz, <sup>b</sup> <sup>13</sup>C NMR recorded at 125 MHz



**Figure 4.** Proposed mechanism for OzID fragmentation of 1-3 (a-c) [Criegee ion (representative structure shown but isomers of the carbonyl oxide cannot be excluded)]



**Figure 5.** The OzID spectra from sodium adducts of **1-3** (a-c).

These polyoxygenated long-chain cyclohexene compounds (**1-4**) were examined for their antimalarial activities against *P. falciparum* strains (TM4/8.2 and K1CB1) and for cytotoxicity against human mouth epidermal carcinoma (KB cells) and normal kidney epidermal cells of African green monkey (Vero cells). All compounds were inactive ( $IC_{50} > 50 \mu M$ ).

### 3. Conclusions

A phytochemical study of the leaf extracts of *U. cherrevensis* led to the isolation and characterization of three new cyclohexene (9*Z*)-**octadec-9-enyl** ethers (**1-3**), and a previously reported compound (**4**). While long chain ester derivatives are known (e.g. **4**), this is the first report of analogous ether derivatives. The structures of these compounds were characterized using spectroscopic techniques. Ozone Induced Dissociation (OzID) mass spectrometry proved critical in determining the double bond position of the **octadec-9-enyl** side chain. Importantly, ozonide formation was chemoselective for the less hindered side chain double bond. This method has been

successful in the determining the double bond position of natural fatty esters,<sup>15,16</sup> but to the best of our knowledge, this is the first example of the application of OzID to naturally occurring long chain ethers.

## 4. Experimental Section

### 4.1 General

Optical rotations were measured at the sodium D-line (590 nm) on a Jasco 2000 series polarimeter. Melting points were determined on a SANYO Gallenkamp melting point apparatus and are uncorrected. The UV-vis absorption spectra and circular dichroism (CD) were measured with a Jasco J-180 spectrophotometer. The infrared (IR) spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer. The NMR spectra were recorded using both a 400 MHz or a 500 MHz Bruker NMR spectrophotometer. Chemical shifts were recorded in parts per million ( $\delta$ ) in CDCl<sub>3</sub> ( $\delta_{\text{H}}$  7.26 and  $\delta_{\text{C}}$  77.0 ppm) with TMS as an internal reference. High Resolution Electrospray Ionization Mass Spectra (HRESIMS) were recorded on a Waters Xevo<sup>TM</sup> QToF mass spectrometer in positive ionization mode using the following conditions: 3 kV, 40 V, and 4 V applied to the ESI probe, sample, and extraction cone, respectively; cone and desolvation gas flows of 50 and 300 liters/hour, respectively. Spectra were corrected using leucine enkephalin reference peaks from ESI on a 200 pg/uL solution in 1:1 water:acetonitrile with 0.1% formic acid infused through the LockSpray<sup>TM</sup> source. Ozone-Induced Dissociation (OzID) mass spectrometry was performed on a modified Thermo Fisher Scientific Orbitrap Fusion mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel 60 GF<sub>254</sub> (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (62-400  $\mu\text{m}$ ) and type 60 (5-40  $\mu\text{m}$ ) for quick



column chromatography (QCC), and Sephadex LH-20 with MeOH elution. All solvents for extraction and chromatography were distilled or purchased in high purities prior to use.

#### 4.2 Plant Material

The leaves of *Uvaria cherrevensis* were collected from Doi Suthep National Park, Chiang Mai, Thailand in August 2015. This plant was identified by Dr. Tanawat Chaowasku, CMUB Herbarium, Chiang Mai University, Thailand, where a voucher specimen has been deposited (specimen no. T.Ritthiwigrom 5).

#### 4.3 Extraction and isolation

The leaves of *U. cherrevensis* (1.30 kg) were extracted with MeOH (3 X 3 L) and the extracts were evaporated. The residue was extracted with acetone (500 mL) and concentrated to give the crude acetone extract (126.8 g) as a dark brown gum. The extract was separated by Quick Column Chromatography (QCC) using hexanes to acetone to MeOH to give ten fractions (1-10). Fraction 3 (4.33 g) was subjected to Reversed Phase Column Chromatography (RPCC) by elution with MeOH to give three subfractions (3A-3C). Subfraction 3C (282.3 mg) was separated by CC over silica gel by elution with EtOAc/hexanes (1:4) to provide five subfractions (3C1-3C5). Compound **4** (39.2 mg) as a white amorphous solid, was obtained from subfraction 3C2 after evaporation. Subfraction 3C3 (31.0 mg) was purified after CC over silica gel by elution with MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:99) to provide compound **3** (24.8 mg) as a colourless oil. Subfraction 3C4 (61.1 mg) was isolated by Sephadex LH-20 CC after elution with MeOH to give three subfractions (3C4A-3C4C). Subfraction 3C4B (25.9 mg) was isolated by CC over silica gel by elution with MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1:99) to give compound **1** (7.2 mg) and **2** (10.7 mg) as a white amorphous solid and colourless oil, respectively.

4.3.1 *Cherrevenol M (1)*. White amorphous solid, mp. 70-71 °C;  $[\alpha]_D^{24} -90$  (*c* 0.1, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 207 (2.88), 229 (2.93), and 278 (2.55); ECD (*c* 3.22 mM, MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ); 207 (-1.19) and 229 (0.04); IR (neat)  $\nu_{\max}$  3454, 1722, 1276, and 1248  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz) data, see Table 1; HRESIMS  $m/z$  595.3615  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{34}\text{H}_{52}\text{O}_7\text{Na}$ , 595.3611).

4.3.2 *Cherrevenol N (2)*. Colourless oil;  $[\alpha]_D^{25} -57$  (*c* 0.1, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 203 (4.17), 230 (3.96), and 275 (3.59); ECD (*c* 0.33 mM, MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ); 203 (-72.1) and 230 (-41.3); IR (neat)  $\nu_{\max}$  3437, 1723, 1274, and 1233  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) data, see Table 2; HRESIMS  $m/z$  701.4019  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{41}\text{H}_{58}\text{O}_8\text{Na}$ , 701.4029).

4.3.3 *Cherrevenol O (3)*. Colourless oil;  $[\alpha]_D^{24} -44$  (*c* 0.1,  $\text{CHCl}_3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 234 (3.61) and 274 (3.34); ECD (*c* 0.53 mM, MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ); 234 (-2.62); IR (neat)  $\nu_{\max}$  3423, 1722, and 1272  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz) data, see Table 2; HRESIMS  $m/z$  657.3770  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{39}\text{H}_{54}\text{O}_7\text{Na}$ , 657.3767).

#### 4.4 Ozone Induced Dissociation (OzID) Mass Spectrometry

OzID was performed using a modified Thermo Fisher Scientific Orbitrap Fusion mass spectrometer. Compounds **1-3** were separately dissolved in MeOH to 100  $\mu\text{M}$  and then diluted to produce 10  $\mu\text{M}$  analyte in MeOH with 90  $\mu\text{M}$  sodium acetate. Infusion via syringe pump at 5  $\mu\text{L}/\text{min}$  through an EASY-Max NG source under the following conditions produced an adequate sodium adduct signal: +4.5 kV spray voltage, values of 4, 5, and 1 for sheath, auxiliary, and sweep gas (nitrogen), respectively, 300 and 30 °C ion transfer tube and vaporizer, respectively. The RF lens was set to 60 % to transmit ions through the front of the instrument. This mass spectrometer

has recently been modified in a manner similar to that reported recently by Paine *et al.*<sup>17</sup> Oxygen with 0.5% nitrogen (Coregas, New South Wales, Australia) was supplied at 20 psig and 0.2 Standard liters per through a Titan 30 ozone generator (Absolute Ozone, Alberta, Canada) producing 225 g/Nm<sup>3</sup> ozone (10.5% by volume in oxygen) as measured in a MiniHICON analyzer (Teledyne API, California, USA). Gas flow was directed through a destruct catalyst (Teledyne API) before expulsion from the laboratory however, prior to destruction, a portion was split-off through a 25  $\mu\text{m}$  ID/10 cm length PEEKsil restriction (Trajan Scientific Australia, Victoria, Australia) into a flow of helium determined by a regulator upstream of a 50  $\mu\text{m}$  ID/10 cm length of PEEKsil (Trajan). Under normal operating conditions e.g. in the absence of ozone, the regulator is set such that the ion gauge reads *ca.*  $6 \times 10^{-5}$  Torr near the ion trap region. The mixture of ozone, oxygen and helium traveled to the ion trap through a 1.4 m length of grey PEEK tubing (381  $\mu\text{m}$  ID) which bypasses the helium open split inlet. In an MS<sup>3</sup> experiment sodiated precursor ions were isolated through the quadrupole with a 0.8 Da window and allowed to pass into the HCD cell (zero setting on the collision energy) followed by reisolation in the ion trap (2.0 Da window) for 4 seconds in the presence of ozone. Mass analysis was then performed in the orbitrap analyzer at a 50,000 resolution setting (specified at  $m/z$  200) and a 10000 AGC target. Spectra reported here represent the average over a 1 min acquisition time. The fluoranthene internal calibrant option was used to improve mass accuracy. We note the ozone generator is interlocked to a model 106-L ambient ozone monitor (2B Technologies, Colorado, USA) for safety such that ozone production ceases at or above 60 ppbv laboratory ozone concentration.

#### 4.5 Computational Methods

The 3D structures generated were prepared with ChemDraw 17.0 and Avogadro: an open-source molecular builder and visualization tool, Version 1.2.0.61.<sup>18</sup> The Density Functional

Theory (DFT) used to minimize the three-dimensional structures was calculated with B3LYP/6-31G(d)<sup>19-21</sup> using the Gaussian 09 basis set.<sup>22</sup>

#### 4.6 Bioactivity assays

Antimalarial activity against *Plasmodium falciparum* TM4/8.2 (a wild type sensitive strain) and K1CB1 (multidrug resistant strain) was carried out as described in a previous report.<sup>6</sup> The cytotoxicity against normal Vero cells (kidney epithelial cells of African green monkey, *Cercopithecus aethiops*) and KB cells (human mouth epidermal carcinoma cells) were also carried out as previously described.<sup>6</sup>

#### **Acknowledgements**

We thank the Thailand Research Fund and Office of the Higher Education Commission (Grant No. MRG6180098) for supporting this project and the Center of Excellence for Innovation in Chemistry (PERCH-CIC) and Chiang Mai University, and the University of Wollongong for partial support. C.A. acknowledge the Graduate School of Chiang Mai University and the Science Achievement Scholarship of Thailand (SAST). A.T.M. thanks Drs. B.L.J. Poed and D.L. Marshall in the Central Analytical Research Facility at Queensland University of Technology for assistance with OzID implementation on the Orbitrap Fusion mass spectrometer in Wollongong and Assoc. Prof. T.W. Mitchell in the UOW School of Medicine for securing funding to purchase the Orbitrap Fusion mass spectrometer and associated OzID hardware. A.C. wishes to acknowledge that this research was undertaken with assistance of resources from the National Computational Infrastructure (NCI), which is supported by Australian Government. S.K. thanks NSTDA's Cluster Program Management.

#### **Appendix A. Supplementary data**

Supplementary data to this article can be found online at <http://>

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