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Polyoxygenated cyclohexane derivatives and other constituents from *Kaempferia rotunda* L.

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Abstract

Methanol extracts of *Kaempferia rotunda* L. rhizomes yielded seven compounds including six polyoxygenated cyclohexane derivatives identified as (−)-6-acetylzeylenol (**1**), four acylated derivatives of 1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol (**3**–**6**), a Diels–Alder adduct of 3-benzoyl-1-benzoyloxymethylcyclohexa-4,6-dien-2,3-diol (**7**), and a triacylated derivative of salicin (**9**). The cyclohexane diepoxide, crotepoxide (**8**), was also obtained. Spectroscopic methods were used for structure determination. The methanol extract of the rhizomes of *K. rotunda* and (−)-2-acetyl-4-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol (2-acetylrotepoxide B; **6**), had antifeedant activity against larvae of *Spodoptera littoralis*. (−)-Zeylenol (**2**) also showed antifeedant activity, whereas (−)-6-acetylzeylenol (**1**) was inactive.

Keywords: *Kaempferia rotunda*; Zingiberaceae; Cyclohexane epoxides; Zeylenol; Salicin; Diels–Alder adduct

1. Introduction

The Zingiberaceae is a family of perennial rhizomatous herbs that contains the economically important spices, ginger (*Zingiber officinale* L.), turmeric (*Curcuma longa* L.), galangal (*Kaempferia galanga* L.) and black cardamom (*Amomum subulatum* Roxb.). Not surprisingly, studies on the chemistry of this family have focused on flavour compounds (Pino et al., 2004; Naik et al., 2004; Chane-Ming et al., 2002). Some species also contain compounds of medicinal interest due to their antibacterial and antifungal activity (Wilson et al., 2005; bin Jantan et al., 2003) as well as antioxidant or platelet activating properties (Masuda et al., 2004). *Kaempferia rotunda* L. occurs in monsoon forests of India and Thailand and is also a popular ornamental. Although the essential oils of this species have been described (Sirat et al., 2005; Woerdenbag et al., 2004), other aspects of its phytochemistry are less well studied. It is, however, known to produce cyclohexane derivatives such as the diepoxide, crotepoxide (**8**) and the cyclohexene, (−)-zeylenol (**2**) (Marco-Contelles et al., 2004; Pancharoen et al., 1996; Pai et al., 1970). In our search for plant secondary metabolites with biological activity against insects we report the isolation, structural determination and antifeedant activity against larvae of *Spodoptera littoralis* of seven new compounds from *K. rotunda*, including six polyoxygenated cyclohexane derivatives and a triacylated derivative of salicin (Fig. 1).

2. Results and discussion

Analysis of a methanol extract of the rhizomes of *K. rotunda* by HPLC with diode-array detection revealed the presence of several apolar components (**1**, **3**–**9**) with similar UV spectra (λ_{max} 228 and 275 nm). These resembled the UV spectrum of senepoxide, a cyclohexene epoxide available as a standard in our natural product collection (source: leaves of *Friesodielsia obovata*, Annonaceae). Milligram quantities of **1** and **3**–**9** were obtained by repetitive isolation using either analytical or semi-preparative HPLC,

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and used for structure elucidation and insect bioassays. NMR spectra of **1**–**9** were acquired in CD_3OD, and their structures determined from 1D ^1H, 1D ^{13}C, 1D ROE, COSY, HSQC and HMBC datasets as appropriate. ^1H and ^{13}C NMR assignments are given in Tables 1–4.

Compound **1** was obtained as an off-white amorphous solid, $[\alpha]_D^{23}$ − 63.5° (*c* 0.6, MeOH), for which a molecular formula of $C_{23}H_{22}O_8$ was determined by HRESIMS. Its ^1H NMR spectrum was similar to that of (−)-zeylenol (**2**), an authentic sample of which was provided by Dr. Kou Hiroya (Hiroya and Ogasawara, 1999). However, on comparing the ^1H NMR spectra of **1** and **2**, the former displayed an additional resonance at δ_H 2.00 (3H, *s*), which together with two further resonances at δ_C 172.2 and 20.8 in the corresponding ^{13}C NMR spectrum indicated that it was an acetyl derivative of **2**. Sequential assignment of the ring protons of **1** from COSY data, and observation of a long-range connectivity from δ_H 5.53 (1H, *dd*, $J = 3.9$, 1.0 Hz, H-6) to the acetyl carbonyl confirmed that the acetyl group was located at C-6. The similarity between the multiplicities and coupling constants of the resonances in the ^1H NMR spectra of **1** and **2** indicated that these compounds had the same relative configuration. ROE correlations detected between the methylene protons of 1-CH_2OBz (δ 4.56 and 4.68) and H-2 (δ 4.22), and between one of the 1-CH_2OBz methylene protons (δ 4.68) and the acetyl methyl (δ 2.00), were consistent with this proposal. The CD spectrum of **1** was similar to that of **2** (Fig. 2), allowing the absolute configuration of **1** to be assigned. Thus, **1** was determined to be (−)-6-acetylzeylenol, a compound that has not been reported previously.

In contrast to **1**, the ^1H and ^{13}C NMR spectra of **3**–**6** indicated that these compounds were polyoxygenated cyclohexane, rather than cyclohexene derivatives (Tables 1 and 2). However, spectral features common to **1** and **3**–**6** included resonances corresponding to a benzoyloxymethyl group and a benzoyl group. Further analysis of

Table 2
^{13}C NMR chemical shift assignments (δ) for **1** and **3**–**6** in CD_3OD at 30 °C

Atom	Assignment (δ in ppm)				
	1	**3**	**4**	**5**	**6**
1	75.5	62.7	63.4	61.9	61.6
2	71.6	67.9	70.6	69.7	72.2
3	75.0	77.7	71.8	74.6	69.3
4	130.7	70.5	76.8	71.8	76.8
5	127.0	70.4	67.8	70.1	67.4
6	72.0	63.7	62.8	62.5	62.5
1-CH_2OBz					
CH_2	67.9	65.9	65.9	65.5	65.9
2-OAc					
O*C*OCH$_3$				171.9	172.2
OCO*C*H$_3$				20.6	20.2
6-OAc					
O*C*OCH$_3$	172.2				
OCO*C*H$_3$	20.8				
-OBz (A)[a]					
CO	168.0	167.6	167.6	167.2	167.5
1	131.0	131.2	131.1	131.0	131.0
2,6	130.7	130.8	130.7	130.6	130.8
3,5	129.6	129.7	129.5	129.6	129.8
4	134.3	134.4	134.3	134.6	134.6
-OBz (B)					
CO	167.9	167.5	167.5	167.1	167.5
1	131.3	130.7	131.5	130.9	131.3
2,6	130.7	130.6	130.8	131.0	130.8
3,5	129.6	129.7	129.8	129.7	129.6
4	134.3	134.4	134.6	134.5	134.4

[a] For OBz entries, set (A) is that containing the most downfield shifted CO resonance.

Table 1
^1H NMR chemical shift assignments (δ) and coupling constant data for compounds **1** to **6** in CD_3OD at 30 °C

Atom	Assignment (δ in ppm, *J* in Hz)					
	1	**2**	**3**	**4**	**5**	**6**
2	4.22 *d* (7.1)	4.22 *d* (6.9)	4.38 *d* (4.6)	4.27 *d* (5.6)	5.87 *d* (6.3)	5.63 *d* (5.7)
3	5.74 *dddd* (7.1, 2.4, 1.6, 1.0)	5.70 *dm* (6.9)	5.19 *dd* (4.6, 2.0)	3.97 *dd* (5.6, 2.1)	5.29 *dd* (6.3, 2.1)	4.06 *dd* (5.7, 2.0)
4	5.95 *dd* (10.1, 2.5)	5.82 *ddd* (10.1, 2.5, 0.6)	3.98 *dd* (6.7, 2.0)	5.22 *dd* (5.6, 2.0)	3.97 *dd* (5.1, 2.0)	5.17 *dd* (5.7, 2.1)
5	5.88 *ddd* (10.1, 3.9, 1.6)	5.95 *ddd* (10.0, 4.1, 1.7)	4.22 *dd* (6.7, 2.9)	4.31 *dd* (5.7, 3.3)	4.20 *dd* (5.1, 3.6)	4.36 *dd* (5.7, 3.3)
6	5.53 *dd* (3.9, 1.0)	4.35 *d* (4.0)	3.66 *br d* (2.9)	3.63 *br d* (3.2)	3.65 *br d* (3.7)	3.64 *br d* (3.3)
1-CH_2	4.68 *d* (11.8)	4.64 *d* (11.6)	4.65 *d* (11.8)	4.69 *d* (11.8)	4.57 *d* (11.8)	4.50 *d* (11.8)
	4.56 *d* (11.8)	4.61 *d* (11.6)	4.21 *d* (11.8)	4.38 *d* (11.8)	4.26 *d* (11.8)	4.38 *d* (11.8)
2-OAc					2.04 *s*	2.15 *s*
6-OAc	2.00 *s*					
-OBz (A)[a]						
2,6	8.00 *m*	8.03 *m*	7.98 *m*	8.07 *m*	7.98 *m*	8.06 *m*
3,5	7.44 *m*	7.42 *m*	7.42 *m*	7.48 *m*	7.43 *m*	7.49 *m*
4	7.58 *m*	7.56 *m*	7.57 *m*	7.64 *m*	7.57 *m*	7.64 *m*
-OBz (B)						
2,6	8.00 *m*	8.00 *m*	7.85 *m*	7.99 *m*	7.93 *m*	8.01 *m*
3,5	7.44 *m*	7.42 *m*	7.26 *m*	7.33 *m*	7.36 *m*	7.34 *m*
4	7.58 *m*	7.56 *m*	7.49 *m*	7.55 *m*	7.54 *m*	7.56 *m*

[a] For OBz entries, set (A) is that containing the most downfield shifted H-2,6 resonance.

Table 3
^{1}H NMR and ^{13}C NMR chemical shift assignments (δ) for **7** in CD_3OD at 30 °C

Atom	Assignment (δ in ppm)		
	δ^{13}C	δ^1H (J in Hz)	HMBC[a]
Ring A			
1	138.5		
2	67.3	4.36 *dm* (8.8)	C-1, C-3
3	78.1	5.16 *dd* (8.7, 6.6)	C-2, C-4, CO (3-OBz)
4	33.7	3.33 *br m*	C-2, C-3, C-6, C-3′, C-4′, C-5′
5	41.1	3.16 *br d* (9.3)	C-4, C-5′
6	127.6	6.09 *dd* (3.4, 2.0)	1-CH_2OBz
Ring B			
1′	49.1		
2′	74.4	4.05 *br dd* (3.1, 2.2)	C-1′, C-3′, C-6′, C-5
3′	83.8	4.59 *t* (3.1)	C-2′, C-5′, C-4, CO (3′-OBz)
4′	37.0	3.13 *br dd* (6.9, 3.2)	C-2′, C-3′, C-5′, C-6′, C-5
5′	131.6	6.32 *dd* (8.2, 6.7)	C-1′, C-3′, C-4′
6′	134.4	5.84 *ddd* (8.2, 1.5, 1.0)	C-1′, C-3′, C-4′, 1′-CH_2OBz
1-CH_2OBz			
1	131.4		
2,6	130.6	8.02 *m*	C-4, CO (1-CH_2OBz)
3,5	129.7	7.46 *m*	
4	134.2	7.60 *m*	
CO	167.9		
CH_2	65.9	5.03 *dm* (12.8)	C-1, C-2, C-6, CO (1-CH_2OBz)
		4.83 *ddd* (12.8, 2.1, 1.1)	
3-OBz			
1	131.2		
2,6	130.6	7.87 *m*	CO (3-OBz)
3,5	129.5	7.38 *m*	
4	134.4	7.55 *m*	
CO	167.7		
1′-CH_2OBz			
1	131.3		
2,6	130.7	8.10 *m*	CO (1′-CH_2OBz)
3,5	129.8	7.50 *m*	
4	134.4	7.62 *m*	
CO	168.0		
CH_2	63.5	4.73 *d* (11.6)	C-1′, C-2′, C-5, C-6′,
		4.79 *d* (11.6)	CO (1′-CH_2OBz)
3′-OBz			
1	131.2		
2,6	130.6	7.89 *m*	CO (3′-OBz)
3,5	129.6	7.43 *m*	
4	134.3	7.59 *m*	
CO	167.9		

[a] Internal correlations in Bz rings not listed.

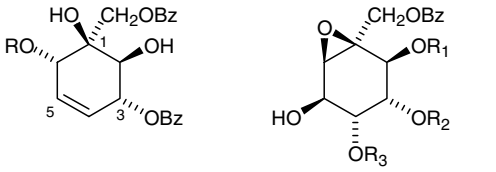
Table 4
^{1}H NMR and ^{13}C NMR chemical shift assignments (δ) for **9** in CD_3OD at 30 °C

Atom	Assignment (δ in ppm, J in Hz)	
	δ^{13}C	δ^1H
1	156.9	
2	127.7	
3	130.5	7.44 *dd* (7.5, 1.8)
4	124.1	7.09 *td* (7.5, 1.1)
5	130.8	7.33 *ddd* (8.3, 7.5, 1.8)
6	117.4	7.22 *dd* (8.3, 1.0)
β-Glc		
1′	102.9	5.030 *d* (7.8)
2′	73.3	3.63 *dd* (9.5, 7.8)
3′	78.8	5.033 '*t*' (9.4)
4′	69.9	3.53 *dd* (9.9, 9.3)
5′	75.5	3.72 *ddd* (9.9, 6.2, 2.4)
6′	64.4	4.39 *dd* (12.0, 2.4)
		4.24 *dd* (11.9, 6.2)
3′-OAc	172.6	
	21.1	2.13 *s*
6′-OAc	172.2	
	20.7	2.02 *s*
2-CH_2OBz		
CH_2	63.2	5.52 *d* (12.9)
		5.44 *d* (12.9)
CO	168.2	
1	131.6	
2,6	130.7	8.05 *m*
3,5	129.7	7.47 *m*
4	134.4	7.60 *m*

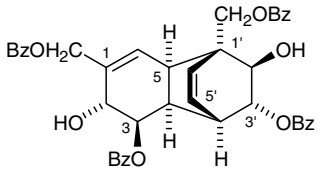
the NMR spectra of **3**–**6** indicated that these compounds were benzoylated derivatives of 1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol, and that **5** and **6** also contained an acetyl substituent (Fig. 1.). In each case, the ^{1}H NMR resonance assignments were readily confirmed using sequential correlations in COSY spectra from H-2 to H-6. The sites of substitution of the benzoyl, and in the case of **5** and **6**, the acetyl substituents, were determined using long-range correlations in HMBC spectra from ring methine protons to the corresponding carbonyls.

Compound **3**, an amorphous solid, had a molecular formula of $C_{21}H_{20}O_8$ by HRESIMS and $[\alpha]_D^{23} - 34.7°$ (c 0.5, MeOH). A long-range correlation in the HMBC spectrum from H-3 (δ_H 5.19) to a benzoyl carbonyl at δ_C 167.6 confirmed the site of acylation as C-3. Thus **3** was identified as (−)-3-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol, to which the trivial name (–)-rotepoxide A was assigned. An isomer, **4**, for which the molecular formula of $C_{21}H_{20}O_8$ was confirmed by HRESIMS, had $[\alpha]_D^{23} - 26.5°$ (c 0.5, MeOH). Although, its ^{1}H NMR spectrum resembled that of **3**, the resonance of H-4 was downfield shifted rather than that of H-3, suggesting that **4** was acylated at C-4. As expected, a long-range correlation was detected in the HMBC spectrum from H-4 (δ_H 5.22) to the benzoyl carbonyl at δ_C 167.6. Thus **4** was determined to be 4-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol or (−)-rotepoxide B.

Compounds **5** and **6**, the acetylated derivatives of **3** and **4**, gave protonated molecules at m/z 443 $[M+H]^+$ by APCI-MS, and their molecular formulae were established as $C_{23}H_{22}O_9$ by HRESIMS. Only **6** was obtained in pure form by HPLC (Section 3.3) and a 2:5 mixture of **5** and **6** was used for the spectroscopic analysis of **5**. The two sets of resonances were readily distinguished in ^{1}H and ^{13}C NMR spectra of the mixture, not only by their different relative intensities but also through correlations observed



3 $R_1 = R_3 = H$, $R_2 = Bz$
4 $R_1 = R_2 = H$, $R_3 = Bz$
5 $R_1 = Ac$, $R_2 = Bz$, $R_3 = H$
6 $R_1 = Ac$, $R_2 = H$, $R_3 = Bz$



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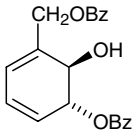
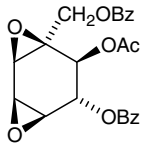
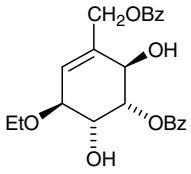


(–)-1,6-desoxypipoxide



8 crotepoxide



uvacalol C

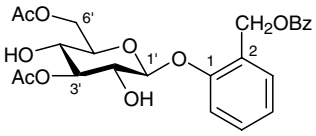


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Fig. 1. Constituents of *Kaempferia rotunda* rhizomes (**1**, **3**–**9**) and related compounds. Relative configurations are shown for **3**–**7**.

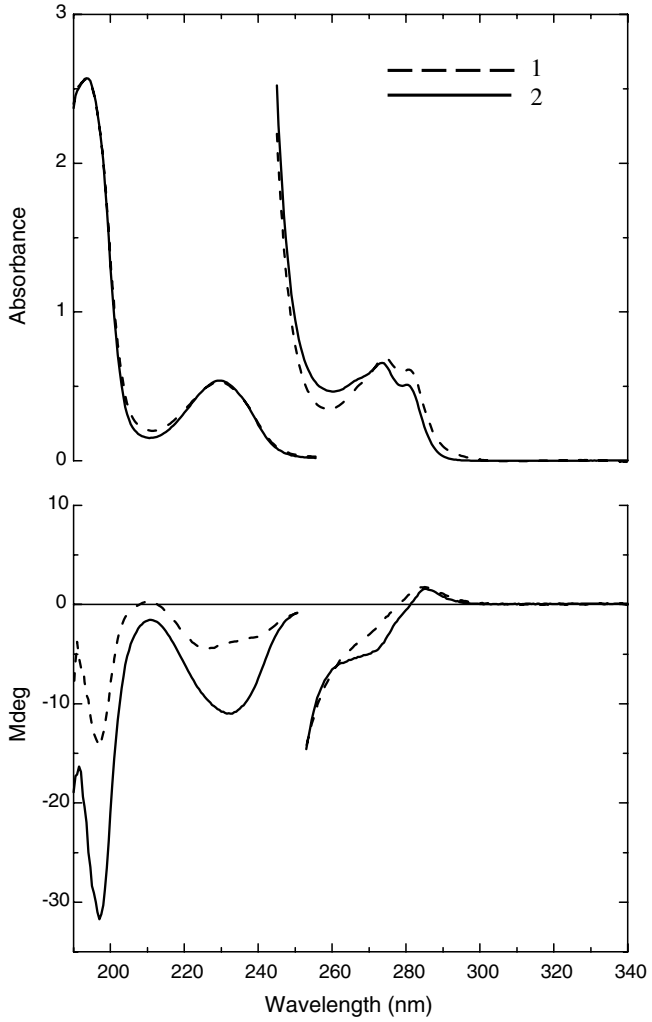

Fig. 2. CD spectra of (−)-6-acetylzeylenol (**1**) and (−)-zeylenol (**2**) (MeOH).

in 2D NMR. Comparison of the NMR spectral assignments for H-2 of **5** and **6** with those for **3** and **4** revealed downfield shifts of +1.49 and +1.36 ppm, respectively, suggesting that C-2 was the site of acetylation in these derivatives. As expected, long range correlations in the HMBC spectrum were observed from H-2 of **5** (δ 5.87) and H-2 of **6** (δ 5.63) to the acetyl carbonyls at δ_C 171.9 and 172.2, respectively. Similarly, long-range correlations from H-3 (δ 5.29) of **5** and H-4 of **6** (δ 5.17) to carbonyl carbons at δ_C 167.1 and 167.5, respectively, confirmed the sites of benzoylation. Thus **5** and **6** were determined to be 2-acetyl-3-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol (2-acetylrotepoxide A) and 2-acetyl-4-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol (2-acetylrotepoxide B), respectively (see Fig. 1).

The similar multiplicities and coupling constants for the ring proton resonances of **3**–**6** noted in their ^{1}H NMR spectra (Table 1) indicated that these compounds had the same relative configuration. Although relatively little has been published on the conformational analysis of cyclohexane epoxides, Naumov and Bezzubov (1967) reported that the half-chair conformation was preferred for these compounds and for cyclohexenes. The corresponding ring substituents are thus categorised as either quasi-axial or quasi-equatorial (Grossel, 1997). Zhou et al. (1999) used conformational analysis, Mosher's method and CD spectroscopy to determine the absolute configurations of a series of polyoxygenated cyclohexenes from *Uvaria calamistrata* roots, one of which, uvacalol C (3-benzoyl-1-benzoyloxymethyl-5-ethylcyclohex-6-en-2,3,4,5-tetrol) is similarly substituted to **3** (3-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol) (Fig. 1). The multiplicities and coupling constants for the resonances of H-2 to H-6 in **3** and uvacalol C are also similar, suggesting that the configurational relationships between neighbouring ring protons are the same. For example, the $^3J_{H\text{-}4,H\text{-}5}$

coupling constants of 6.7 and 6.3 Hz in **3** and uvacalol C, respectively, indicate *trans* quasi-axial relationships between H-4 and H-5. The configurational relationship between the 1,6-epoxide group of **3** and the neighbouring stereocentre at C-5 was established by analogy with data on the cyclohexane epoxide carba-sugars, cyclophellitol and 1,6-*epi*-cyclophellitol (Marco-Contelles, 2001). In these compounds, a ^3J coupling constant of approximately 2 Hz is encountered when an epoxide proton and a neighbouring ring proton are on the same face of the molecule (i.e. α,α or β,β), whereas when the protons are on opposite faces, the resulting coupling constant is less than 0.2 Hz (corresponding to a dihedral angle of close to 90°) (Tatsuta et al., 1991; Atsumi et al., 1990). In **3**, the coupling constant for $^3J_{\text{H-5,H-6}}$ was measured as 2.9 Hz (Table 1), thus if H-5 is α, as in uvacalol C, then H-6 must also be α (Fig. 1). Compound **3** was therefore assigned as rel-(1*S*,2*S*,3*R*,4*R*,5*R*,6*R*)-3-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol. A ROE correlation detected between the methylene protons of 1-CH_2OBz and H-2, and the absence of similar correlations between H-2 and H-4 and between H-3 and H-5, were consistent with this proposal. Similarly, **4**–**6** were assigned as rel-(1*S*,2*S*,3*S*,4*S*,5*R*,6*R*)-4-benzoyl-1-benzoyloxymethyl-1,6- epoxycyclohexan-2,3,4,5-tetrol, rel-(1*R*,2*S*,3*R*,4*R*,5*R*,6*R*)-2-acetyl-3-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol and rel-(1*R*, 2*S*, 3*S*,4*S*, 5*R*,6*R*)-2-acetyl-4-benzoyl-1- benzoyloxymethyl-1,6-epoxycyclohexan-2,3, 4,5-tetrol, respectively. Although **3**–**6** have not been reported previously, a related derivative, 2,3-diacetyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3, 4,5-tetrol, was obtained from *Piper wightii* (Piperaceae) by Boll et al. (1996). These authors did not comment on the stereochemistry of this compound, but it appears to have the same relative configuration as **3**–**6** based on a comparison of the corresponding ^{1}H-^{1}H coupling constants.

A late eluting component (**7**) obtained as an amorphous solid by semi-prep. HPLC had a molecular formula of $C_{42}H_{36}O_{10}$ by HRESIMS and $[\alpha]_D^{23} + 27.9°$ (*c* 0.6, MeOH). The UV spectrum of this compound (λ_{max} 228 and 275 nm) was similar to those of **1** and **3**–**6**. Furthermore, the ^{1}H and ^{13}C NMR spectra of **7** indicated that it was highly benzoylated. A protonated molecule at *m*/*z* 701 $[M+H]^+$ and protonated fragments at *m*/*z* 579, 457, 335 and 213 in APCI-MS (four losses of 122 amu) provided evidence for the presence of 4 benzoyl moieties. The ^{1}H and ^{13}C NMR spectra of **7** further revealed resonances corresponding to a di-substituted double bond at δ 5.84 (1H, *ddd*, $J = 8.2$, 1.5, 1.0 Hz, δ_C 134.4) and 6.32 (1H, *dd*, $J = 8.2$, 6.7 Hz, δ_C 131.6), and a tri-substituted double bond at δ 6.09 (1H, *dd*, $J = 3.4$, 2.0 Hz, δ_C 127.6). Two sets of resonances representing the methylene protons of benzoyloxymethyl groups were also noted in the ^{1}H NMR spectrum. These features provided useful starting points for the elucidation of the full structure of **7** from 1D and 2D NMR data. The corresponding spectral assignments are listed in Table 3, together with H–^{13}C correlations from HMBC spectra. The fused ring structure of **7** can be rationalised as the result of Diels–Alder adduct formation from two molecules of 3-benzoyl-1-benzoyloxymethylcyclohexa-4,6-dien-2,3-diol (labelled 'A' and 'B' in Fig. 3). The relative configuration of the adduct was investigated using 1D site selective ROE experiments. Strong correlations were detected between H-2(A) and H-5′(B), H-5(A) and H-2′(B), and H-6′(B) and 1′-CH_2OBz(B). Weaker correlations were found between H-3(A) and H-5(A), and H-3′(B) and H-5′(B). A trans-diaxial relationship between H-2(A) and H-3(A) was indicated by the vicinal coupling constant ($^3J_{2,3}$) of 8.7 Hz. The corresponding value for $^3J_{2',3'}$ of 3.1 Hz, the ROE between H-3′(B) and H-5′(B), and the structural constraints of the bridged ring structure indicated that with H-2′ axial, H-3′ must be equatorial. The relative configuration of **7** deduced from these observations (Fig. 3) represents the Diels–Alder adduct that would be formed by *endo*-addition of two molecules of (2*S*,3*R*)-3-benzoyl-1-benzoyloxymethylcyclohexa-4,6-dien-2,3-diol (trivial name, (−)-1,6-desoxypipoxide, Fig. 1). Although *endo*-addition of the corresponding (2*R*,3*S*)-stereoisomer to produce the enantiomer of **7** is an equally valid configurational solution, it is interesting to note that the (2*S*,3*R*)-form was reported as a natural product by Schulte et al. (1982) from the roots of *Uvaria purpurea*. This was also proposed to be a metabolic precursor of polyoxygenated cyclohexane derivatives with the (2*S*,3*R*)-configuration such as (−)-zeylenol and crotepoxide. Given that

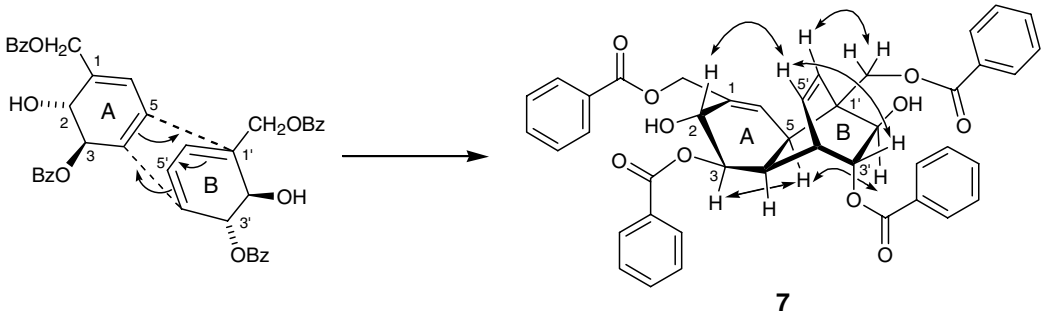

Fig. 3. Proposed mechanism of formation of **7** by Diels–Alder reaction. The relative configuration of **7** based on ROE connectivities (shown by double headed arrows) is illustrated.

(−)-6-acetylzeylenol (**1**), (−)-zeylenol (**2**) and crotepoxide (**8**) are all constituents of *Kaempferia rotunda*, it is tempting to speculate that these compounds, and by implication, the remaining polyoxygenated cyclohexane derivatives described in this paper, have a common origin in (2*S*,3*R*)-3-benzoyl-1-benzoyloxymethylcyclohexa-4,6-dien-2,3-diol. Compounds similar to **7** are uncommon in the literature, although Jolad et al. (1981) reported zeylena, an intramolecular Diels–Alder adduct of (2*S*,3*R*)-3-(*E*)-cinnamoyl-1-benzoyloxymethylcyclohexa-4,6-dien-2,3-diol, as a constituent of the roots of *Uvaria zeylanica*. The Diels-Alder adduct **7** was present in extracts of both fresh and freeze-dried *K. rotunda* rhizomes (Section 3.3). Furthermore, (−)-1,6-desoxypipoxide (its likely precursor) has been isolated and characterised with no report of dimer formation (Schulte et al., 1982). These observations suggest that **7** results from enzyme-catalysed, rather than artefactual, processes.

The ^{1}H and ^{13}C NMR spectra of **9** ($C_{24}H_{26}O_{10}$ by HRESIMS) contained resonances corresponding to an acylated sugar residue, two acetyl groups, an *o*-disubstituted phenyl and a benzoyloxymethyl group. Assignment of the sugar resonances from COSY, HSQC and HMBC data confirmed the presence of a β-glucopyranosyl residue (Table 4) (Duus et al., 2000). Both Glc H-3′ and CH_2-6′, which appeared at δ 5.033 (1H, '*t*', $J = 9.4$ Hz), and 4.39 (1H, *dd*, $J = 12.0$, 2.4 Hz) and 4.24 (1H, dd, $J = 11.9$, 6.2 Hz), respectively, were downfield shifted. Long-range connectivities between these proton resonances and the carbonyl carbons of acetyl groups were detected in the HMBC spectrum. Further connectivities from the anomeric proton at δ 5.030 (1H, *d*, $J = 7.8$ Hz) and the methylene protons of the benzoyloxymethyl group to the same oxygenated phenyl carbon at δ 156.9 indicated that the diacetylated *O*-β-glucopyranoside and benzoyloxymethyl groups were *o*-substituents of the phenyl ring. Thus **9** was determined to be 2-(benzoyloxymethyl)phenyl (3,6-di-*O*-acetyl)-β-glucopyranoside, a new acylated derivative of salicin (2-(hydroxymethyl)phenyl β-glucopyranoside). This type of compound is commonly found in *Populus* and *Salix* (Salicaceae), but does not appear to have been reported previously from the Zingiberaceae (Hegnauer, 1972; Hegnauer, 1990).

The methanol extract of the rhizomes of *K. rotunda* had significant antifeedant activity against the final stadium larvae of *Spodoptera littoralis* (Table 5). 2-Acetylrotepoxide B (**6**) was the only compound isolated from this extract to express antifeedant activity. (−)-Zeylenol (**2**), a polyoxygenated cyclohexane structurally related to **1**, and previously reported to occur in *K. rotunda* (Pancharoen et al., 1996) also had antifeedant activity.

Table 5
Effect of the methanol extract of *Kaempferia rotunda* and compounds isolated from this species on the feeding behaviour of larvae of *Spodoptera littoralis*

Compound	Feeding index[a]	FI_{50}[b]
1	16.5 (5.99)	>1000
2	50.8 (5.78)*	89
3	−11.0 (18.25)	ndr
4	12.6 (17.41)	>1000
6	42.8 (15.12)*	95
7	−12.7 (20.91)	ndr
9	18.7 (21.77)	>1000
Crotepoxide (**8**)	−15.7 (19.21)	ndr
Methanol extract	76.8 (11.67)**	56

[a] Feeding index $((C - T)/(C + T))\%$ at 100 ppm (mean (SEM)) $^{**}p < 0.01$, $^{*}p < 0.05$ Wilcoxon matched pairs test: $n = 8$–15.
[b] FI_{50} = Concentration (ppm) calculated to give a feeding index of 50% ndr = no dose-dependent response.

3. Experimental section

3.1. General experimental procedures

NMR data were acquired on either Bruker Avance 400 MHz or Varian 500 MHz instruments at 30 °C in CD_3OD. Chemical shifts were measured with respect to TMS as an internal standard at 0.00 ppm. Standard pulse sequences and parameters were used to obtain 1D ^{1}H, 1D selective ROE, 1D ^{13}C, COSY, HSQC and HMBC datasets. LC-MS was carried out on a Thermo-Finnigan LC/MS/MS system consisting of a 'Surveyor' autosampling LC system interfaced to a LCQ Classic quadrupole ion trap mass spectrometer. Chromatographic separation was performed on a 150 mm × 4.6 mm i.d. (5 μm particle size) Phenomenex Luna C18 column using a linear mobile phase gradient of 1 ml/min flow rate with water (A): MeOH (B): 5% HOAc in MeOH (C). Initial conditions were 80% A, 0% B and 20% C changing to 0% A, 80% B and 20% C at $t = 20$ min and maintained at these conditions to $t = 25$ min. Injection volumes were 10 μl and data analysis was performed using Xcalibur 1.2 software. The ion trap MS was fitted with an Atmospheric Pressure Chemical Ionisation (APCI) source operated under standard conditions; i.e. vaporiser temperature 450 °C, needle current 5 mA, heated capillary temperature 150 °C, sheath and auxiliary nitrogen gas pressure 80 and 20 psi, and the source voltages tuned for the optimal transmission of protonated rutin. The ion trap was set to monitor ions from *m*/*z* 125–1200 with collision energy of 45%. Optical rotation measurements (sodium D line, λ 589 nm, 23 °C) were made using a Perkin-Elmer 141 polarimeter with a 10 cm light path cylindrical cell of 1 ml volume. UV and CD spectra (23 °C, MeOH, 0.25 mg/ml) were acquired on an Applied Photophysics Ltd., Chirascan spectropolarimeter. 10 mm and 0.5 mm cell path lengths were employed in the wavelength ranges 450–240 nm and 300–180 nm, respectively, and all spectra were solvent baseline subtracted. UV spectra were also obtained online by HPLC coupled with diode array detection.

3.2. Plant material

Rhizomes of *Kaempferia rotunda* L. were collected from plants growing in the Princess of Wales Conservatory at the Royal Botanic Gardens, Kew, accession no. 1970-3567.

3.3. Extraction and isolation

Freeze dried material (25 g) of *K. rotunda* was ground to a powder and extracted with MeOH at room temperature for 24 h. The extract was filtered and solvent removed *in vacuo*. The residue was redissolved in 2 ml MeOH and subjected to analytical HPLC (Merck LiChrospher, 250 × 4.0 mm (i.d.), 5 μm particle size, 1 ml/min flow rate, two-solvent gradient system based on MeCN (A) and 0.04% TFA (B); A = 25% at $t = 0$ min, A = 70% at $t = 20$ min A = 70% at $t = 30$ min and A = 100% at $t = 40$ min). Compounds **1** and **3**–**9**, which represented major components of the MeOH extract, were detected at 230 nm and eluted at 22.6 (**1**), 15.2 (**3**), 15.6 (**4**), 19.4 (**5** and **6**), 33.9 (**7**), 20.7 (**8**) and 21.5 (**9**) min respectively (the same compounds were also present in a comparable analysis of fresh plant material). Scale-up to semi-preparative HPLC (Merck LiChrospher, 250 × 10.0 mm, 10 μm particle size, 4.5 ml/min flow rate) using the same gradient was achieved with no loss of resolution and yielded on repetitive manual collection; **1** (6.4 mg), **3** (7.2 mg), **4** (5.2 mg), **5** and **6** (19.3 mg), **7** (6.7 mg), **8** (2.9 mg) and **9** (3.5 mg). A small amount of pure **6** (5.4 mg) was obtained by repetitive reisolation using the analytical HPLC method above. All compounds were obtained as amorphous off-white solids.

3.4. (−)-6-Acetyl-1-benzoyloxymethylcyclohex-4-en-1,2,3,6-tetrol ((−)-6-acetylzeylenol) (*1*)

Off-white solid (MeCN); $[\alpha]_D^{23}$ − 63.5° (MeOH; *c* 0.6); UV (MeCN) λ_{max} nm: 228, 275; CD $\Delta\varepsilon_{235}$ −0.313, $\Delta\varepsilon_{280}$ +0.107 (MeOH; *c* 0.025). ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; APCI-MS (positive mode) *m/z*: 427 $[M+H]^+$; HRESIMS *m/z*: 444.1651 $[M+NH_4]^+$ (calc. for $C_{23}H_{26}O_8N$, 444.1653).

3.5. (−)-3-Benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol ((−)-rotepoxide A) (*3*)

Off-white solid (MeCN); $[\alpha]_D^{23}$ − 34.7° (MeOH; *c* 0.5); UV (MeOH) λ_{max} nm: 228, 275; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; APCI-MS (positive mode) *m/z*: 401 $[M+H]^+$; HRESIMS *m/z*: 401.1233 $[M+H]^+$ (calc. for $C_{21}H_{21}O_8$, 401.1231).

3.6. (−)-4-Benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol ((−)-rotepoxide B) (*4*)

Off-white solid (MeCN); $[\alpha]_D^{23}$ − 26.5° (MeOH; *c* 0.5); UV (MeOH) λ_{max} nm: 228, 275; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; APCI-MS (positive mode) *m/z*: 401 $[M+H]^+$; HRESIMS *m/z*: 401.1226 $[M+H]^+$ (calc. for $C_{21}H_{21}O_8$, 401.1231).

3.7. 2-Acetyl-3-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol (2-acetylrotepoxide A) (*5*)

Off-white solid (MeCN); UV (MeOH) λ_{max} nm: 228, 275; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; APCI-MS (positive mode) *m/z*: 443 $[M+H]^+$; HRESIMS *m/z*: 443.1337 $[M+H]^+$ (calc. for $C_{23}H_{23}O_9$, 443.1337).

3.8. (−)-2-Acetyl-4-benzoyl-1-benzoyloxymethyl-1,6-epoxycyclohexan-2,3,4,5-tetrol (2-acetylrotepoxide B) (*6*)

Off-white solid (MeCN); $[\alpha]_D^{23}$ − 3.1° (MeOH; *c* 0.5); UV (MeOH) λ_{max} nm: 228, 275; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; APCI-MS (positive mode) *m/z*: 443 $[M+H]^+$; HRESIMS *m/z*: 443.1337 $[M+H]^+$ (calc. for $C_{23}H_{23}O_9$, 443.1337).

3.9. Rotundol (*7*)

Off-white solid (MeCN); $[\alpha]_D^{23}$ + 27.9° (MeOH; *c* 0.6); UV (MeOH) λ_{max} nm: 228, 275; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 3; APCI-MS (positive mode) *m/z*: 701 $[M+H]^+$, 579 $[(M+H)-122]^+$, 457 $[(M+H)-(2\times122)]^+$, 335 $[(M+H)-(3\times122)]^+$, 213 $[(M+H)-(4\times122)]^+$; HRESIMS *m/z*: 701.2375 $[M+H]^+$ (calc. for $C_{42}H_{37}O_{10}$, 701.2381).

3.10. 2-(Benzoyloxymethyl)phenyl (3,6-di-O-acetyl)-β-glucopyranoside (*9*)

Off-white solid (MeCN); UV (MeOH) λ_{max} nm: 228, 275; ^{1}H NMR, see Table 4; ^{13}C NMR, see Table 4; APCI-MS (positive mode) *m/z*: 475 $[M+H]^+$; HRESIMS *m/z*: 475.1599 $[M+H]^+$ (calc. for $C_{24}H_{27}O_{10}$, 475.1599).

3.11. Antifeedant bioassay

A binary choice bioassay using sucrose treated glass-fibre discs (Whatman 2.1 cm diameter) was used to investigate whether compounds influenced the feeding behaviour of final stadium larvae of *Spodoptera littoralis* (Lepidoptera) (Simmonds et al., 1990). Larvae were placed singly in a Petri dish with a control disc (*C*) and a disc treated with the test compound (*T*). The respective amounts eaten of each disc were used to calculate the feeding index $((C-T)/(C+T))\%$. Antifeedant activity is represented by positive values, whereas phagostimulant activity is represented by negative values. The compounds were each tested at 3–4 concentrations (50 ppm, 100 ppm, 250 ppm and 500 ppm). Each concentration was tested against between 8 and 15 different larvae. The Wilcoxon matched-pairs test was used to analyse the data. Regression analysis was used to calculate the concentration required to give a feeding index of 50% (FI_{50}).

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References

Atsumi, S., Umezawa, K., Iinuma, H., Nagagawa, H., Nakamura, H., Iitaka, Y., Takeuchi, T., 1990. Production isolation and structure determination of a novel β-glucosidase inhibitor, cyclophellitol from *Phellinus* sp. J. Antibiot. 43, 49–53.

bin Jantan, I., Yassin, M.S.M., Chin, C.B., Chen, L.L., Sim, N.L., 2003. Antifungal activity of the essential oils of nine Zingiberaceae species. Pharm. Biol. 41, 392–397.

Boll, P.M., Prasad, A.K., Tyagi, O.D., Wengel, J., Olsen, C.E., Kumar, N., Bisht, K.S., Parmar, V.S., 1996. Neolignans, cyclohexanes and alkaloids from *Piper wightii*. Recl. Trav. Chim. Pays-Bas 115, 9–12.

Chane-Ming, J., Vera, R., Chalchat, J.C., Cabassu, P., 2002. Chemical composition of essential oils from rhizomes, leaves and flowers of *Curcuma longa* L. from Reunion Island. J. Essent. Oil Res. 14, 249–251.

Duus, J.Ø., Gotfredsen, C.H., Bock, K., 2000. Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations. Chem. Rev. 100, 4589–4614.

Grossel, M.C., 1997. Alicyclic Chemistry. Oxford University Press, Oxford, pp. 28–29.

Hegnauer, R., 1972. Chemotaxonomie der Pflanzen, Bd 6. Birkhauser Verlag, Basel.

Hegnauer, R., 1990. Chemotaxonomie der Pflanzen, Bd 9. Birkhauser Verlag, Basel.

Hiroya, K., Ogasawara, K., 1999. The first enantiocontrolled synthesis of naturally occurring polyoxygenated cyclohexenylmethanol dibenzoates (−)-zeylenol, (−)-uvarigranol G, (−)-tonkinenin A and (+)-pipoxide. Chem. Commun., 2197–2198.

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

Marco-Contelles, J., 2001. Cyclohexane epoxides - Chemistry and biochemistry of (+)-cyclophellitol. Eur. J. Org. Chem. (9), 1607–1618.

Marco-Contelles, J., Molina, M.T., Anjum, S., 2004. Naturally occurring cyclohexane epoxides: Sources, biological activities and synthesis. Chem. Rev. 104, 2857–2899.

Masuda, Y., Kikuzaki, H., Hisamoto, M., Nakatani, N., 2004. Antioxidant properties of gingerol related compounds from ginger. Biofactors 21, 293–296.

Naik, J.P., Rao, L.J.M., Kumar, T.M.M., Sampathu, S.R., 2004. Chemical composition of the volatile oil from the pericarp (husk) of large cardamom (*Amomum subulatum* Roxb.). Flavour Frag. J. 19, 441–444.

Naumov, V.A., Bezzubov, V.M., 1967. Electron diffraction study of the structure of cyclohexene and cyclohexene oxide molecules. J. Struct. Chem. 8, 530–531, Engl. Trans. 466–7.

Pai, B.R., Rao, N.N., Wariyar, N.S., 1970. Occurrence of crotepoxide in *Kaempferia rotunda* L. Ind. J. Chem. 8, 468.

Pancharoen, O., Tuntiwachwuttikul, P., Taylor, W.C., 1996. Cyclohexane diepoxides from *Kaempferia rotunda*. Phytochemistry 43, 305–308.

Pino, J.A., Marbot, R., Rosado, A., Batista, A., 2004. Chemical composition of the essential oil of *Zingiber officinale* Roscoe L. from Cuba. J. Essent. Oil Res. 16, 186–188.

Schulte, G.R., Kopinid, M., Thebtaranonth, C., Thebtaranonth, Y., 1982. Studies on highly oxidized cyclohexanes. Constitution of a new key metabolic intermediate. Tetrahedron Lett. 23, 4303–4304.

Simmonds, M.S.J., Blaney, W.M., Fellows, L.E., 1990. Behavioural and electrophysiological study of antifeedant mechanisms associated with polyhydroxyalkaloids. J. Chem. Ecol. 16, 3167–3196.

Sirat, H.M., Jamil, S., Siew, L.W., 2005. The rhizome oil of *Kaempferia rotunda* Val. J. Essent. Oil Res. 17, 306–307.

Tatsuta, K., Niwata, Y., Umezawa, K., Toshima, K., Masay, N., 1991. Enantiospecific synthesis and biological evaluation of 1,6-epi-cyclophellitol. J. Antibiot. 44, 456–458.

Wilson, B., Abraham, G., Manju, V.S., Mathew, M., Vimala, B., Sundaresan, S., Nambisan, B., 2005. Antimicrobial activity of *Curcuma zedoaria* and *Curcuma malabarica* tubers. J. Ethnopharmacol. 99, 147–151.

Woerdenbag, H.J., Windono, T., Bos, R., Riswan, S., Quax, W.J., 2004. Composition of the essential oils of *Kaempferia rotunda* L. and *Kaempferia angustifolia* Roscoe rhizomes from Indonesia. Flavour Frag. J. 19, 145–148.

Zhou, G.X., Chen, R.Y., Yu, D.Q., 1999. New polyoxygenated cyclohexenes from *Uvaria calamistrata*. J. Asian Nat. Prod. Res. 1, 227–238.