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CYCLOHEXANE DIEPOXIDES FROM *KAEMPFERIA ROTUNDA*

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Key Word Index—*Kaempferia rotunda*; Zingiberaceae; rhizomes; cyclohexane diepoxides.

Abstract—Three new cyclohexane diepoxides (−)-(1*R*,2*R*,4*R*,5*S*,6*R*,7*R*)-4-benzoyloxymethyl-3,8-dioxatricyclo[5.1.0.0^{2,4}] octane-5,6-diol 6-acetate, (+)-(1*R*,2*R*,4*R*,5*S*,6*R*,7*R*)-4-benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0^{2,4}]octane-5,6-diol 5-acetate and (−)-(1*R*,2*R*,4*R*,5*S*,6*R*,7*R*)-4-benzoyloxymethyl-3,8-dioxatricyclo[5.1.0.0^{2,4}]
octane-5,6-diol 6-benzoate together with crotepoxide and (−)-zeylenol, were isolated from the rhizomes of *Kaempferia rotunda*. Their structures were characterized on the basis of spectral analyses and chemical correlations. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Kaempferia rotunda is a plant indigenous to Southeast Asia. Previous chemical investigation of this species led to the isolation of crotepoxide (**1**) [1]. As part of our study on the chemistry of some species of *Kaempferia* in Thailand, a number of cyclohexane oxide derivatives and diterpenes have also been characterized [2–4]. Further examination of *K. rotunda* has now yielded three new cyclohexane diepoxides (**3**–**5**), as well as (−)-zeylenol (**6**). Systematic number is shown in **1**; to maintain consistency with the literature, the conventional cyclohexane numbering shown in **2** is used hereafter.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of **3** resembled that of crotepoxide (**1**), except for the presence of one acetate signal less than that in the spectrum of **1**. The IR spectrum of **3** showed the presence of hydroxyl at 3600 cm^{-1} and carbonyl groups at 1740 cm^{-1} (ester). The ^{1}H NMR spectrum of **3** (Table 1) indicated the presence of one acetate (δ 1.90), one benzoate (δ 7.46–8.05) and one hydroxyl (δ 2.79) The signals for the protons on the epoxide rings appeared at δ 3.31 (*ddd*, J = 1.0, 2.5 and 3.7 Hz, H-4), 3.54 (*dd*, J = 2.7 and 3.7 Hz, H-5) and 3.70 (*d*, J = 2.7 Hz, H-6), respectively. The most informative absorption in the NMR spectrum of **3** was the resonance of H-2 at δ 4.13 (*dd*, J = 4.8 and 9.2 Hz) which was changed to a doublet (J = 4.8 Hz) upon addition of D_2O. The H-3 signal at lower field (δ 5.17, *d*, J = 2.5 and 4.8 Hz) suggested that C-3 carried the acetate ester.

The vicinal coupling constant value ($J_{2,3}$ = 4.8 Hz) reflected the different conformation of the cyclohexane ring of **3** compared with that of crotepoxide (**1**) ($J_{2,3}$ = 9.0 Hz). The evidence implied that the C-2 and C-3 substituents in **3** would not be in equatorial orientations. Presumably, the structure of **3** favoured the conformation with hydrogen bonding between the C-7 benzoyl and C-2 hydroxyl. However, acetylation of **3** gave crotepoide (**1**), which proved the stereochemistry unambiguously as well as the position of the hydroxyl and acetate groups in **3**.

The structural similarities between compounds **3** and **4** were apparent from their spectroscopic data. Their mass spectra (CI) were very similar in pattern. The ^{1}H NMR spectra of the two compounds (Table 1) suggested that they had the same structural features; however, some differences could be observed. The hydroxyl group of **4** was assigned to C-3, since the resonance of H-3 appeared at δ 3.97 (*ddd*, J = 1.7, 5.4 and 8.7 Hz) and the signal collapsed (*dd*, J = 1.7 and 8.7 Hz) upon addition of D_2O. The proton at C-2 was seen at low field (δ 5.46, *d*, J = 8.7 Hz), whose coupling constant indicated equatorial orientations of the C-2 and C-3 substituents. Likewise, acetylation of **4** yielded crotepoxide (**1**), which confirmed the structure of **4**.

The ^{1}H NMR spectrum of **5** (Table 1) showed a close resemblance to that of compound **3** with the notable difference being the presence of two benzoates. The H-2 signal resonated in the same region as that found for **3**. Moreover, a comparison of the H2-H3

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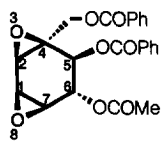

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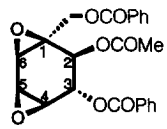

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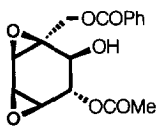

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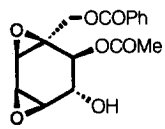

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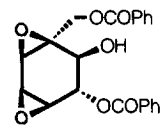

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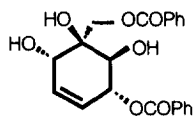

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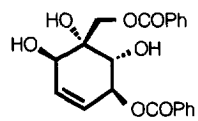

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coupling constant of **3** (4.8 Hz) with that of **5** (5.1 Hz) implied that the orientations of the C-2 and C-3 substituents were the same in these two compounds. The second benzoate was thus assigned to C-3. Acetylation of **5** provided further support for the the structure, which afforded boesenboxide (**2**). The ^{13}C NMR chemical shifts and assignments of compounds **3–5** are shown in Table 2.

Compound (**6**) possessed physical properties and spectral data (UV, IR and ^{1}H NMR) identical to those of zeylenol with the negative optical rotation ($[\alpha]_D^{22}$ −127°). The structure of **6** was thus identified as (−)-zeylenol [7].

This report provides further examples (**3–5**) of cyclohexane diepoxides, previously represented only by crotepoxide (**1**) and boesenboxide (**2**) [1, 2, 5, 6]. We reported previously the isolation of crotepoxide (**1**), boesenboxide (**2**), (+)-zeylenol (**7**) and (+)-zeylenol derivatives from the roots of *K. angustifolia* [2, 3]. We now report the isolation of (−)-zeylenol (**6**) which

Table 1. ^{1}H NMR chemical shifts and coupling constants (Hz) for cyclohexane diepoxides

H	1	2	3	4	5
H2	5.71 *dd*	5.92, *d*	4.13, *dd*	5.46, *d*	4.30, *dd*
	(9.0)	(9.5)	(4.8, 9.2)	(8.7)	(5.1, 10.0)
H3	4.99, *dd*	5.18, *dd*	5.17, *dd*	3.97, *ddd*	5.44, *dd*
	(1.6, 9.0)	(1.6, 9.5)	(2.5, 4.8)	(1.7, 5.4, 8.7)	(2.5, 5.1)
H4	(3.10, *ddd*	3.19, *ddd*	3.31, *ddd*	3.17, *ddd*	3.46, *ddd*
	(0.5, 1.6, 3.9)	(0.5, 1.6, 3.9)	(1.0, 2.5, 3.7)	(0.6, 1.7, 3.9)	(0.75, 2.5, 3.5)
H5	3.45, *dd*	3.44, *dd*	3.54, *dd*	3.43, *dd*	3.60, *dd*
	(2.7, 3.9)		(2.7, 3.7)	(2.7, 3.9)	(2.75, 3.5)
H6	3.67, *dd*	3.72, *dd*	3.70, *d*	3.62, *d*	3.74, *d*
	(0.5, 2.7)	(0.5, 2.7)	(2.7)	(2.7)	(2.75)
H7A, B	4.24, *d*	4.26, *d*	4.41, *d*	4.24, *d*	4.49, *d*
	4.57, *d*	4.60, *d*	4.54, *d*	4.60, *d*	4.61, *d*
	(12.1)	(12.0)	(12.0)	(12.0)	(12.0)
*m*ArH	7.47, *m*	7.42, *m*	7.47, *m*	7.46, *m*	7.38, *m*
*p*ArH	7.60, *m*	7.54, *m*	7.60, *m*	7.59, *m*	7.55, *m*
*o*ArH	8.03, *m*	8.02, 8.05, *m*	8.03, *m*	8.02, *m*	7.99, *m*
OAc	2.03, *s*	2.06, *s*	1.90, *s*	2.19, *s*	—
	2.12, *s*				
OH	—	—	2.70, *d*	2.63, *d*	2.85, *d*
			(9.2)	(5.4)	(10.2)

Table 2. ^{13}C NMR chemical shifts for cyclohexane diepoxides

C	3	4	5
1	56.0	59.8	56.7
2	66.4	73.3	70.4
3	69.7	69.1	66.8
4	50.9	53.7	53.3
5	47.9	48.1	48.1
6	53.6	54.7	51.2
7	64.6	62.6	64.6
ArC1′, ArC1″	129.3	129.1	129.2, 129.0
ArC2′, ArC2″	129.8	129.8	129.8, 129.8
ArC3′, ArC3″	128.5	128.6	128.5, 128.6
ArC4′, ArC4″	133.5	133.6	133.5, 133.7
ArC5′, ArC5″	128.5	128.6	128.5, 128.6
ArC6′, ArC6″	129.8	129.8	129.8, 129.8
2OCO	—	171.5	
3OCO	169.9	—	166.2
7OCO	166.0	165.9	165.6
Me	20.4	20.8	—

belongs to the same biosynthetic series as crotepoxide (**1**) but has never been reported from the genus *Kaempferia* before. (−)-Zeylenol (**6**) was first isolated from *Uvaria zeylanica* [7].

EXPERIMENTAL

Unless otherwise stated, analyses were carried out by Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok, Thailand. Mps are uncorr. UV were measured in EtOH solns. ^1H NMR of $CDCl_3$ solns were recorded at 400 MHz, ^{13}C NMR at 100 MHz. Optical rotations were measured for $CHCl_3$ solns.

Extraction. Crushed fresh rhizomes of *K. rotunda* L. (2.65 kg) were extracted with CH_2Cl_2 at room temp. Removal of solvent gave a dark brown oil (13 g). This extract was suspended in MeOH (25 ml) at room temp.; a solid (2.8 g) which formed was collected and identified as crotepoxide (**1**). Evapn of the filtrate gave a brown oil (7.2 g). A portion of the filtrate (4.13 g) was chromatographed over silica gel (400 g) and eluted with a hexane–EtOAc gradient. Successive frs obtained were combined on the basis of their behaviour on TLC and evapd to give 19 frs (0.20, 0.20, 0.14, 0.08, 0.16, 0.14, 0.39, 0.14, 0.08, 0.19, 0.10, 0.20, 0.46, 0.16, 0.18, 0.47, 0.26, 0.34 and 0.11 g, respectively).

Crotepoxide (**1**). Recrystallized from MeOH as needles, mp 152–154° (lit. [5] 152–153°). $[\alpha]_D^{22}$ +69° (lit. [5] +64°). IR and ^1H NMR identical with those of authentic sample; mmp underpressed.

(−)-(1R,2R,4R,5S,6R,7R)-4-*Benzoyloxymethyl*-3,8-*dioxatricyclo*[5.1.0.0^{2,4}]*octane*-5,6-*diol* 6-*acetate* (**3**). Frs 15 and 16 (0.65 g) were purified on a column of silica gel with CH_2Cl_2–Me_2CO (20:1, 15:1, 10:1 and 5:1) to give diepoxide **3** (0.13 g) and diepoxide **4** (0.19 g), as slightly yellow solids. Compound **3** was recrystallized from MeOH as needles, mp 141–142°. (Found: C, 60.1; H, 4.6. $C_{16}H_{16}O_7$ requires C, 60.0; H, 5.0%). $[\alpha]_D^{22}$ −58° (*c*, 0.10). IR ν_{max} $CHCl_3$ cm^{-1}: 3600, 1740, 1370, 1270, 1250, 1035, 720. UV λ_{max} EtOH nm (log ε): 280 (3.01), 270 (3.96). MS(CI) *m/z* (rel. int.): 321 $[M+H]^+$ (2), 319 (4), 280 (16), 279 (100), 139 (25), 105 (94), 83 (20).

(+)-(1R,2R,4R,5S,6R,7R)-4-*Benzoyloxymethyl*-3,8-*dioxatricyclo*[5.1.0.0^{2,4}]*octane*-5,6-*diol* 5-*acetate* (**4**). Compound **4** was recrystallized from MeOH as needles, mp 135–136°. (Found: C, 59.8.; H, 4.7. $C_{16}H_{16}O_7$ requires C, 60.0; H, 5.0%). $[\alpha]_D^{22}$ +36° (*c*, 0.10). IR ν_{max} $CHCl_3$ cm^{-1}: 3500, 1730, 1378, 1120, 1030, 720. UV λ_{max} EtOH nm (log ε): 280 (3.03), 267 (3.98). MS (CI) *m/z* (rel. int.): 321 $[M+H]^+$ (0.7), 319 (4), 279 (100), 242 (31), 186 (6), 139 (39), 105 (86), 83 (39).

Acetylation of **3**. To a soln of **3** (23 mg) in dry pyridine (0.5 ml), Ac_2O (0.25 ml) was added and the mixt. stirred at room temp. overnight. After usual work-up, the crude product was purified on a silica gel column using CH_2Cl_2–MeOH (40:1) to yield a colourless solid (20 mg). Recrystallization from MeOH gave colourless needles, mp 148–150°. $[\alpha]_D^{22}$ +67.9° (*c*, 0.05). ^1H NMR, IR and UV identical to those of crotepoxide (**1**) [5].

Acetylation of **4**. Compound **4** (35 mg) was acetylated in the usual manner to give a crude product (41 mg). Purification by chromatography and recrystallization from MeOH gave crotepoxide (**1**) as colourless needles, mp 150–152°, $[\alpha]_D^{22}$ +74.6° (*c*, 0.10). IR, ^1H NMR and UV identical to those of crotepoxide (**1**) [5].

(−)-(1R,2R,4R,5S,6R,7R)-4-*Benzoyloxymethyl*-3,8-*dioxatricyclo*[5,1,0,0^{2,4}]-*octane*-5,6-*diol* 6-*benzoate* (**5**). Fr. 12 (0.20 g) was purified on a column of silica gel with CH_2Cl–Me_2CO (20:1, 15:1 and 10:1) to give diepoxide **5** (95 mg) as a slightly yellow solid. Recrystallization from MeOH yielded needles, mp 177–178°. (Found: C, 66.3; H, 4.4. $C_{21}H_{18}O_7$ requires C, 66.0; H, 4.7%). $[\alpha]_D^{22}$ −76° (*c*, 0.16). IR ν_{max} $CHCl_3$ cm^{-1}: 3600, 1720, 1370, 1270, 1020, 720. UV λ_{max} EtOH nm (log ε): 281 (3.31), 274 (3.37). MS (CI) *m/z* (rel. int.): 383 $[M+H]^+$ (100), 289 (1), 261 (14), 243 (8), 193 (2), 151 (8), 139 (9), 123 (85).

Acetylation of **5**. Compound **5** (15 mg) was acetylated in the usual way. After work-up, the crude product (20 mg) was recrystallized from MeOH as needles, mp 173–174°. $[\alpha]_D^{22}$ +31° (*c*, 0.12). ^1H NMR, IR and UV identical to those of (+)-boesenboxide (**2**) [2].

(−)-*Zeylenol* (**6**). Frs 15 and 16 (0.65 g) were sepd on a column of silica gel with CH_2Cl_2–Me_2CO (20:1, 15:1, 10:1 and 5:1) to give a yellow oil (0.19 g). A portion of the oil (41 mg) was purified by prep. TLC using CH_2Cl_2–MeOH (20:1) to yield a solid (18 mg). Recrystallization from EtOAc gave needles, mp 132–133° (lit. [7] 144–145°), $[\alpha]_D^{22}$ −127° (*c*, 0.20, $CHCl_3$) (lit. [7] −116.3°). ^1H NMR, IR and UV identical to those of (−)-zeylenol (**6**) [7].

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