A Labdane Diterpene Glucoside from the Rhizomes of *Curcuma mangga*

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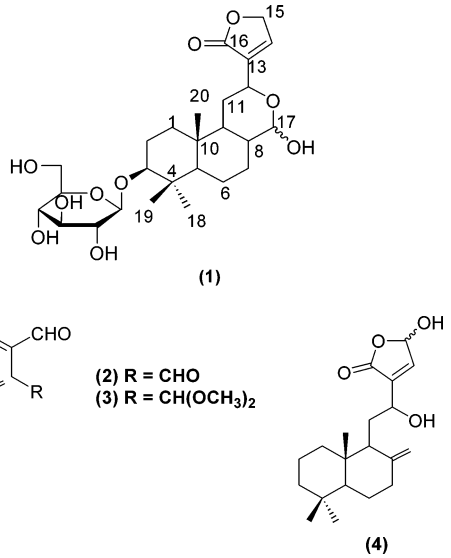
Received January 17, 2005

A new labdane diterpene glucoside, curcumanggoside (**1**), together with nine known compounds, including labda-8(17),12-diene-15,16-dial (**2**), calcaratarin A (**3**), zerumin B (**4**), scopoletin, demethoxycurcumin, bisdemethoxycurcumin, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, curcumin, and *p*-hydroxycinnamic acid, have been isolated from the rhizomes of *Curcuma mangga*. Their structures were determined using a combination of 1D (^{1}H NMR, ^{13}C NMR, DEPT) and 2D (COSY, HSQC, HMBC) NMR techniques. All diarylheptanoids and scopoletin showed significant antioxidant activity. Zerumin B, demethoxycurcumin, bisdemethoxycurcumin, and curcumin also exhibited cytotoxic activity against a panel of five human tumor cell lines.

Curcuma mangga is a member of the Zingiberaceae family commonly grown in Thailand, Peninsular Malaysia, and Java.[1] It is locally known as "manggo tumeric" because of its mango-like smell when the fresh rhizomes are cut. Due to this and its palatable taste, *C. mangga* is a popular vegetable, of which the tips of young rhizomes and shoots are consumed raw with rice. Medicinally, the rhizomes are used as a stomachic and for chest pains, fever, and general debility. It is also used in postpartum care, specifically to aid womb healing. Several species of *Curcuma* such as *C. domestica, C. zedoaria,* and *C. xanthorrhiza* have been extensively studied,[2−9] but there has not been any report on the phytochemical constituents of *C. mangga*. In this report, we describe the isolation and identification of a new labdane diterpene glucoside (**1**), together with nine known compounds identified as labda-8(17),12-diene-15,16-dial (**2**),[10,11] calcaratarin A (**3**),[12] zerumin B (**4**),[13] scopoletin,[14] demethoxycurcumin,[8,15] bisdemethoxycurcumin,[8] 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one,[16] curcumin,[7] and *p*-hydroxycinnamic acid, based on the analysis of their spectroscopic and spectrometric data (UV, MS, IR, ^{1}H NMR, and ^{13}C NMR) and comparison with literature values.



Compound **1** was obtained as a white amorphous solid, $[\alpha]_D$ +12.5°. The molecular formula was assigned as $C_{26}H_{40}O_{10}$ from the HREIMS (*m/z* 494.2514, $C_{26}H_{38}O_9$ [M − H_2O]$^+$) and EIMS (*m/z* 332, $C_{20}H_{28}O_4$ [M − H_2O − glucose]$^+$) for the aglycone. Its IR spectrum was consistent with the presence of an α,β-unsaturated γ-lactone (1752 cm^{-1})[13] and a hydroxyl group (3422 cm^{-1}).

The ^{1}H NMR spectrum showed signals for three tertiary methyl groups (δ 0.89, 0.91, and 1.09) and an anomeric proton (δ 4.36) and resembled that of **4**, except for the presence of a glucopyranosyl moiety (δ 4.36, 3.25, 3.36, 3.31, 3.27, 3.87, 3.71), as well as an oxymethine (δ 3.25, H-3) and dioxymethine (δ 4.39, H-17) functionalities. The signals for the dioxymethine (δ 6.18, H-15) and *exo*-methylene protons in the spectrum of **4** were absent. The ^{13}C NMR spectrum gave 26 carbon signals including those of a glucopyranosyl group (δ 105.6, 74.5, 76.5, 70.5, 77.1, 61.6) whose glycosidic linkage was shown to be β based on the magnitude of the coupling constant of the anomeric proton (J = 8.0 Hz). Inspection of the HSQC and DEPT spectra of the aglycone moiety confirmed the presence of three methyl, six methylene, seven methine, and four quaternary carbons, one of which was for the carbonyl carbon belonging to the α,β-unsaturated γ-lactone moiety (δ 173.7/173.8, C-16) (Table 1).[13] Several of the ^{13}C signals were duplicated since the compound occurs as an epimeric mixture.[17] Three of the methine carbons were oxygenated (δ 70.3, 89.4/89.6, and 100.7), and on the basis of the ^3J correlations from the methyl protons at C-18 and C-19, observed in the HMBC spectrum, the oxymethine at δ 89.4/89.6 was assigned to C-3 of the diterpene skeleton (Table 1). Further correlation was observed with the anomeric proton of the pyranohexose unit, which established C-3 as the point of linkage to the aglycone.

Correlation of methylene protons at δ 4.89 (δ 71.3) with the carbonyl at δ 173.7/173.8 as well as the olefinic carbons at δ 134.2 (C-13) and 147.2/147.5 (C-14) was observed in the HMBC spectrum (Table 1). On the basis of this observation and comparison with literature data of the α,β-unsaturated γ-lactone moiety in neoandrographolide,[18] the methylene proton was assigned to H-15. The HMBC spectrum also revealed the correlation between the oxy-

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10.1021/np0500171 CCC: $30.25
Published on Web 06/28/2005

Table 1. ^{1}H and ^{13}C NMR Data of **1** and Short- (^1J) and Long-Range (^2J and ^3J) C−H Connectivity of **1** Established by HSQC and HMBC, Respectively

			HMBC correlations	
position	^{1}H NMR mult. (J in Hz)	^{13}C NMR[c]	^2J	^3J
1	1.16[a], 1.72[a]	36.9 (CH_2)		C-20
2	1.72[a], 2.02[a]	26.1(CH_2)		
3	3.25[a]	89.4/89.6[b] (CH)	C-2	C-18, C-19
4		39.1/39.3[b] (C)	C-3, C-5, C-18, C-19	
5	1.05[a]	54.5/54.6[b] (CH)	C-6	C-18, C-19
6	1.41[a], 1.72[a]	20.3/20.5[b] (CH_2)	C-5	
7	1.05[a], 2.02[a]	28.4/28.5[b] (CH_2)		C-17
8	1.41[a]	41.4 (CH)	C-7, C-17	C-11
9	1.16[a]	51.8 (CH)		C-7, C-20
10		35.6 (C)	C-1, C-5, C-20	
11	1.16[a], 1.94 (d, J = 11.0)	29.4/29.5[b] (CH_2)	C-9	
12	4.28 (br d, J = 8.0)	70.3 (CH)	C-11	C-14, C-15, C-17
13		134.2 (C)	C-12, C-14	C-11, C-15
14	7.61, s	147.2/147.5[b] (CH)	C-15	C-12
15	4.89, m	71.3 (CH_2)	C-14	
16		173.7/173.8[b] (C)		C-14, C-15
17	4.39, m	100.7 (CH)		C-7
18	1.09, s	27.3/27.4[b] (CH_3)		C-3, C-5, C-19
19	0.91, s	15.7/15.9[b] (CH_3)		C-3, C-5, C-18
20	0.89, s	13.4/13.5[b] (CH_3)		C-5, C-9
1′	4.36 (d, J = 8.0)	105.6 (CH)	C-2′	C-3
2′	3.25[a]	74.5 (CH)	C-1′, C-3′	
3′	3.36[a]	76.5 (CH)		C-5′
4′	3.31[a]	70.5 (CH)	C-3′, C-5′	C-6′
5′	3.27[a]	77.1 (CH)	C-6′	
6′	3.87 (dd, J = 5.0, 11.6)	61.6 (CH_2)	C-5′	
	3.71 (dd, J = 3.0, 11.6)			

[a] Multiplicity was not determined due to excessive overlapping signals. [b] Duplicated signals due to epimers. [c] Multiplicity of carbons was assigned by DEPT experiment.

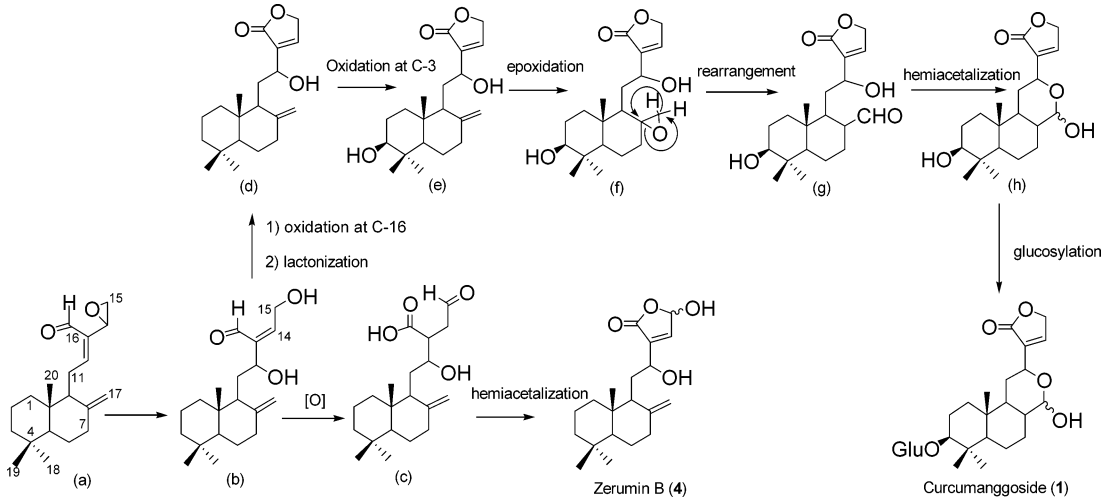

Figure 1. Presumed biogenesis of **1** and **4**.

methine proton at δ 4.28 and the carbons at δ 29.4/29.5 (C-11) and 147.2/147.5 (C-14), and thus, it was assigned to H-12. In addition, this resonance (δ 4.28) also correlated with the dioxymethine carbon at δ 100.7, which in turn correlated with the protons at δ 1.05 (H-7) and 1.41 (H-8). The proton of the dioxymethine unit (δ 4.39) exhibited cross-coupling with the carbons at δ 41.4 (C-8) and 70.3 (C-12). These observations revealed the linkage between the oxymethine and dioxymethine groups, and therefore, the ether bridge between C-12 and C17 was proposed (Table 1). On the basis of the above arguments the structure of compound **1** was established as curcumanggoside (12,17-epoxy-3,17-dihydroxylabda-13-en-16,15-olide3-*O*-β-glucopyranoside). All the protons and carbons could be fully assigned except for its epimeric identity. The relative stereochemistry at C-3 was established as 3β, by the NOESY correlation experiment. The signal at δ 3.25 (H-3) correlated with those at δ 4.36 (H-1′), 2.02 (H-2_{eq}), and 1.05 (H-5_{ax}). In addition, the chemical shifts of C-2, C-3, C-4, and C-1′ were consistent with those of viteoside A.[19] The configurations at C-8, C-12, and C-17 remain to be clarified.

Biogenetically curcumanggoside may presumably be derived alongside zerumin B (Figure 1) from the 12ξ,15-dihydroxylabda-8(17)-13-dien-16-al (b), a diterpene isolated from the aerial parts of *Alpinia chinensis*.[17] In the alternative pathway, oxidation at C-16 of the intermediate (b) to a carboxylic acid followed by lactonization (d) and hydroxylation at C-3 will give 3-hydroxylated labdane diterpene lactone (e). Epoxidation of the exocyclic alkene followed by

Table 2. Percentage Inhibition of Lipid Peroxidation by Compounds Isolated from *C. mangga* Using $Fe_3(CN)_6$ Method

compound[a]	% inhibition
curcumanggoside	0
labda-8(17),12-diene-15,16-dial	5.7 ± 0.1
calcaratarin A	11.5 ± 0.3
zerumin B	16.5 ± 0.6
scopoletin	94.9 ± 0.5
demethoxycurcumin	93.8 ± 0.1
bisdemethoxycurcumin	93.3 ± 02
1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	92.9 ± 0.1
curcumin	96.5 ± 0.1
α-tocopherol	45.2 ± 0.4
quercetin	51.3 ± 0.6
BHT	99.9 ± 0.3

[a] Concentration of 300 μM.

1,2-hydrogen migration will lead to an aldehyde (g),[20] and glucosylation at position 3 will then yield curcumanggoside (**1**).

The presence of sesquiterpenoids and diarylheptanoids is very common in Zingiberaceae.[21,22] Diarylheptanoids have also been isolated from other genera of Zingiberaceous plants such as *Alpinia* and *Zingiber*. Labdanes are the only type of diterpene known to occur in the Zingiberaceous plants, and recently a number of related compounds have been isolated from the genera *Alpinia* (tribe Alpinieae)[12] and *Hedychium* (tribe Hedychieae).[23] The occurrence of diterpenoids in *Curcuma* is less common. The isolation of zerumin B, curcumanggoside, labda-8(17),12-diene-15,16-dial, and calcaratarin A from *C. mangga* created a chemotaxonomic interest in view of the taxonomical relation of *C. mangga* to other species in the taxa. The only other *Curcuma* species reported to contain a labdane-type diterpene is *C. heyneana*,[10] which yielded labda-8(17),12-diene-15,16-dial (**2**), which incidentally was also isolated from *C. mangga*. This report represents the first isolation and the occurrence of a labdane diterpene glycoside from the genus *Curcuma*.

Demethoxycurcumin, bisdemethoxycurcumin, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, curcumin, and scopoletin showed antioxidant activity with percentage inhibition of more than 90% (Table 2). Zerumin B (**4**), demethoxycurcumin, bisdemethoxycurcumin, and curcumin showed high cytotoxic activity against a panel of human tumor cell lines (Table 3). Compound **4** was found to be the most active toward MCF-7 cells with an IC_{50} value of 0.59 μM and showed moderate activity toward HL-60, HepG2, and DU-145 cells with IC_{50} values of 7.21, 25.33, and 11.21 μM, respectively.

Experimental Section

General Experimental Procedures. Melting points were recorded on a Kofler hot-stage apparatus and were uncorrected. The UV and IR spectra were recorded on Varian UV−vis 50 and Perkin-Elmer 1650 FTIR spectrophotometers, respectively. The ^1H and ^{13}C NMR were recorded at 500 and 125 MHz, respectively, in either $CDCl_3$, acetone-d_6, or CD_3OD using a Varian Unity 500 MHz spectrometer. EIMS and HREIMS were determined using a Finnigan MAT95XL-T mass spectrometer. The chemical shifts (δ) were determined from residual solvent peaks. Column and gel permeation chromatography were performed using silica gel (Merck 7734, Merck 9385) and Sephadex LH-20, respectively. TLC analyses were carried out on Merck silica gel DC-Plastikfolien 60 F_{254} plastic sheets with detection accomplished by spraying with 10% H_2SO_4 followed by heating at 100 °C, or by visualizing with a UV lamp at 254 and 366 nm.

Plant Material. The fresh rhizomes of *C. mangga* (25 kg) were collected in Johor, Malaysia, in October 2000. They were cleaned, chopped into smaller pieces (3−5 mm thickness), and dried in the shade. A voucher specimen (No. SK 149/02) was identified by Mr. Shamsul (a Resident Botanist) and deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, as well as the Forest Research Institute, Malaysia.

Extraction and isolation. Eight kilograms of the dried powdered rhizomes was extracted three times with acetone to give 900 g of crude extract (11.25% yield). The acetone extract (250 g) was partitioned into a hexane/H_2O mixture to furnish 150 g of hexane-soluble fraction. The aqueous phase was further extracted with EtOAc to give 45 g of EtOAc-soluble and 25 g of H_2O-soluble portions. The EtOAc fraction (40 g) was adsorbed on 40 g of silica gel. This was then introduced onto the top of a glass column (9.5 cm × 90 cm) packed with 1 kg of silica gel. The column was eluted in ascending polarity manner with hexane/EtOAc, followed by EtOAc/MeOH mixtures to afford 13 fractions (A−M). Fraction C (2 g) was subjected to column chromatography on silica gel eluted in ascending polarity with hexane/EtOAc mixtures to give labda-8(17),12-diene-15,16-dial (**2**, colorless oil, 5 mg), calcaratarin A (**3**, colorless oil, 5 mg), zerumin B (**4**, white crystal, $CHCl_3$/hexane, 25 mg), and *p*-hydroxycinnamic acid (white amorphous solid, 10 mg). Repeated column chromatography of the combined fractions D, E, F, and G (5 g) on silica gel using CH_2Cl_2/MeOH mixtures and further purification by gel permeation chromatography on Sephadex LH-20 (MeOH) afforded scopoletin (yellowish needles, EtOAc/hexane, 5 mg), demethoxycurcumin (orange powder, 250 mg), bisdemethoxycurcumin (orange powder, 20 mg), 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (orange powder, 5 mg), and curcumin (orange powder, 150 mg). Further column chromatography of the combined fractions L and M (2 g) on silica gel using CH_2Cl_2/MeOH (gradient) afforded compound **1** (15 mg, white amorphous solid). All known compounds were identified by comparison of spectroscopic data with the literature.

Curcumanggoside (1): white amorphous solid; $[\alpha]_D$ +12.5° (MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (5.72) nm; IR ν_{max} (KBr) 3422 (OH), 2940 (C−H), 1752 (α,β-unsaturated γ-lactone), 1168, 1024 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz); ^{13}C NMR (CD_3OD, 125 MHz), see Table 1; EIMS *m/z* 332 $[M - H_2O - glucose]^+$ (15), 315 (100), 297 (34), 271 (28), 133 (35), 121 (39), 69 (92), 41(96); HREIMS *m/z* 494.2514 $[M - H_2O]^+$ (calcd for $C_{26}H_{40}O_{10}$).

Bioassay Evaluation. All compounds except *p*-hydroxycinnamic acid were evaluated for antioxidant activity using $Fe_3(CN)_6$ according to the established protocol.[24] Quercetin, α-tocopherol, and butylated hydroxytoluene (BHT) were used as standards. The percentage of lipid peroxidation inhibition was evaluated on the basis of the absorbance on the last day

Table 3. Cytotoxic Activity of Zerumin B, Demethoxycurcumin, Bisdemethoxycurcumin, and Curcumin[a−d]

compound	HL-60	HepG2	MCF-7	DU-145	NCI-H460
zerumin B	7.21 ± 2.6	25.33 ± 3.3	0.59 ± 1.8	11.21 ± 3.1	nd
demethoxycurcumin	24.21 ± 2.3	>50	18.51 ± 8.4	21.7 ± 4.2	25.7 ± 6.1
bisdemethoxycurcumin	25.12 ± 4.1	>50	33.78 ± 1.3	30.1 ± 2.4	88.1 ± 6.8
curcumin	34.22 ± 4.8	35.53 ± 4.1	10.44 ± 1.3	20.6 ± 4.9	27.6 ± 1.1

[a] Results are expressed as IC_{50} values (μM) ± SD of three experiments performed in triplicate. [b] Compounds **1**, **2**, **3**, and 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one could not be evaluated because of their insufficient amount. [c] Scopoletin is inactive against all cell lines tested (IC_{50} > 50 μM). [d] Key to cell lines used: HL-60 = human leukemia, HepG2 = liver cancer, MCF-7 = breast cancer, DU-145 = human prostate cancer, NCI-H460 = lung cancer cells.

of the experiment (usually on the 7th day, at which time the absorbance of control reached a maximum and begins to decrease) and was calculated as

$$\% \text{ inhibition} = (ABS_{Control} - ABS_{Sample})/ABS_{Control} \times 100\%$$

The in vitro cytotoxicity of zerumin B, scopoletin, demethoxycurcumin, bisdemethoxycurcumin, and curcumin was measured against a panel of five human cancer cell lines as previously described.[25]

Acknowledgment. The authors wish to thank the Ministry of Science, Technology and Innovation, Malaysia, for the grant provided under the Intensified Research in Priority Areas Research Grant (No. 09-02-04-0067).

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NP0500171