New Phenolic Glucosides from the Leaves of Eurya Tigang

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NEW PHENOLIC GLUCOSIDES FROM THE LEAVES OF *EURYA TIGANG*

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ABSTRACT.—Three new compounds, 6′-*O*-coumaroyl-1′-*O*-[2-(4-hydroxyphenyl)ethyl]-β-D-glucopyranoside [**1**] (eutigoside A), 6′-*O*-coumaroyl-1′-*O*-[2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)ethyl]-β-D-glucopyranoside [**2**] (eutigoside B), and 6′-*O*-cinnamoyl-1′-*O*-[2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)ethyl]-β-D-glucopyranoside [**3**] (eutigoside C) have been isolated from the leaves of *Eurya tigang*, along with other known compounds (afzelin, quercitrin, *p*-coumaric acid, methyl-α-D-fructofuranoside, isorengyol, and euryanoside). Their structures were determined by chemical and spectroscopic methods (uv, ir, ms, ^{1}H-^{1}H COSY, and ^{1}H-^{13}C COSY).

Eurya tigang Sch. & Laut. (Theaceae) is a small tree growing in the highlands of Papua New Guinea. The leaves of *Eurya* are used in the traditional medicine of Papua New Guinea as covers for tropical ulcers and sores (1). In our continuing search for bioactive constituents from medicinal plants used in Papua New Guinea we have collected leaves of *E. tigang* for phytochemical and biological investigations (2). In an early investigation of this species, the presence of β-sitosterol, betulinic acid, and other triterpenes has been reported (3). This report deals with the isolation and structure elucidation of phenolic glucosides and their antiproliferatory activity against human bladder carcinoma T-24 cell and mouse keratinocyte MK cells.

RESULTS AND DISCUSSION

The air-dried leaves of *E. tigang* were extracted successively with petroleum ether, $CHCl_3$, and MeOH at room temperature. A combination of cc and dccc of the MeOH extract succeeded in the isolation of eutigosides A [**1**], B [**2**], and C [**3**].

Acid hydrolysis of compounds **1**, **2**, and **3** with 1 N HCl yielded glucose. Alkaline hydrolysis of compounds **1** and **2** resulted in coumaric acid, whereas that of **3** gave cinnamic acid. Desacyl moieties of compounds **2** and **3** were the same chromatographically, indicating that they differed due to their acyl moieties.

The fabms of compound **1** showed *m/z* 469 $[M + Na]^+$, 446 $[M]^+$, corresponding to the molecular formula $C_{23}H_{26}O_9$. The ^{1}H-nmr spectrum of compound **1** showed the presence of a trans olefinic system (δ 7.59 and 6.27 ppm, d, $J = 15.7$ Hz), aromatic protons of two AA′BB′ spin systems 6.64 (2H, d, $J = 8.4$ Hz, H-3, H-5), 6.78 (2H, d, $J = 8.4$, H-3″, H-5″), 7.03 (2H, d, $J = 8.4$, H-2, H-6), 7.39 (2H, d, $J = 8.4$ Hz, H-2″, H-6″), and two methylene groups typical of a phenylethyl alcohol moiety. The β-methylenic protons appeared as a multiplet at δ 2.83 ppm, whereas the α-methylenic protons resonated at 3.94 ppm and 3.66 ppm. An anomeric proton signal (4.32, d, $J = 7.8$ Hz) and further sugar proton signals (3.00–4.52, 6H) established the presence of a β-linked glucose moiety in the molecule (4).

The ^{13}C-nmr spectrum clearly showed that an acyloxy function was attached to the glucose moiety at C-6 due to a deshielding of the corresponding signal by 2.5 ppm. A short range ^{13}C-^{1}H 2D correlation experiment with the inverse mode permitted the as-

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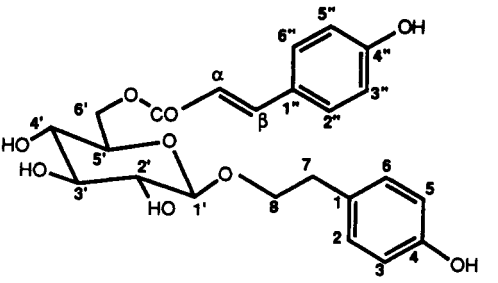

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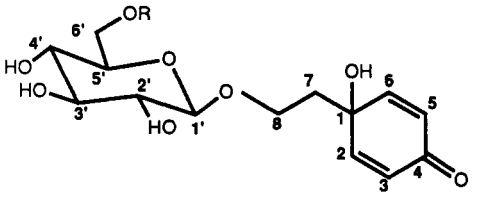

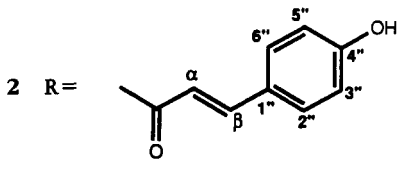

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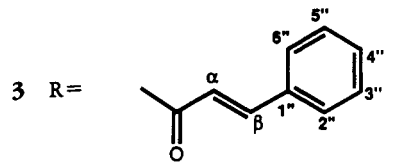

3 R=

sociation of all protons with the carbon atoms to which they were directly attached. The acyloxy function was identified as a coumaroyloxy group by comparison of ^{13}C-nmr (DEPT) data with earlier reported literature data (5–7). Thus, the structure of compound **1** was established at 6′-*O*-coumaroyl-1′-*O*-[2-(4-hydroxyphenyl)ethyl]-β-D-glucopyranoside.

Compound **2** had a mol wt of 462 and a molecular formula of $C_{23}H_{26}O_{10}$, and compound **3** had a mol wt of 446 and a molecular formula of $C_{23}H_{26}O_9$ derived from fabms and ^{13}C-nmr analysis.

The signals of the ^{1}H-nmr spectrum of compound **2** were in good accordance with the signals of the coumaroyl and 6′-substituted glucose moieties in compound **1**. The remaining ^{13}C-nmr signal at 187.8 ppm and signals at 69.2, 127.9, and 154.3 could be attributed to the 1-hydroxycyclohexa-2,5-dienone due to the upfield shift of the CH_2 protons at C-7 of compound **1** from 2.84 ppm to 2.04 ppm in the ^{1}H-nmr spectrum of **2**, which was also supported by ^{1}H-^{1}H and ^{13}C-^{1}H correlation experiments. By comparison of the ^{13}C-nmr spectra of compound **2** with those of structurally related known compounds (8,9), the structure of compound **2** was determined as 6′-*O*-coumaroyl-1′-*O*-[2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)ethyl]-β-D-glucopyranoside.

The uv and ir spectra of compounds **2** and **3** were nearly identical. The ^{1}H-nmr spectra of **2** and **3** presented analogous protons for 1-hydroxy-4-oxo-2,5-cyclohexadien-ethyl and glucose moieties. The residual resonances showed the lack of the

AA'BB' spin system of **1** and **2** and the presence of multiplets at 7.62 and 7.40 ppm, which could be assigned for a cinnamoyl moiety. Further evidence for the cinnamoyl moiety was provided by ^{13}C nmr (Table 1). The structure of compound **3** was elucidated as 6'-*O*-cinnamoyl-1'-*O*-[2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)ethyl]-β-D-glucopyranoside on the basis of comparison of spectroscopic data of known reported compounds (10–12).

Compounds **1**, **2**, and **3** showed moderate antiproliferative activity in the human bladder carcinoma T-24 cell line (13) and in the mouse keratinocyte MK cell line (14).

To our knowledge compounds **1**, **2**, and **3** are new compounds, and this is the first report of the occurrence of phenylpropanoid glycosides in the family Theaceae.

TABLE 1. ^{13}C-nmr Spectral Data of Eutigosides A [**1**], B [**2**], and C [**3**] (75 MHz, CD_3OD, ppm).

Carbon	Compound		
	1	**2**	**3**
C-1	130.4	69.2	69.2
C-2	130.9	154.3	154.3
C-3	116.2	128.0	127.8
C-4	156.8	187.8	187.8
C-5	116.9	127.9	127.7
C-6	130.6	154.3	154.2
C-7	36.5	41.0	41.0
C-8	72.4	65.9	65.9
C-1'	104.9	104.9	104.4
C-2'	75.4	75.5	75.4
C-3'	78.0	77.9	77.9
C-4'	71.9	71.8	71.7
C-5'	75.1	75.0	75.0
C-6'	64.7	64.6	64.8
C-1''	127.1	127.0	135.7
C-2''	116.2	116.9	129.9
C-3''	130.9	131.2	129.2
C-4''	161.3	161.5	130.8
C-5''	130.6	131.2	129.2
C-6''	116.9	116.9	129.9
C-α	115.0	114.9	115.9
C-β	146.8	146.8	146.5
C=O	169.1	169.1	168.5

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Reversed-phase low pressure liquid chromatography (lplc) was carried out using a Büchi 681 and a Labomatic mplc column (71.3 cm × 1.85 cm i.d.). The column was packed with RP-18, particle size 40 μm (Bondesil, Analytichem International). The solvents used for lplc were hplc grade. Si gel 60 F_{254} plates from Merck (0.25 mm, Art. no. 5729) were used for tlc analysis.

Uv spectra were obtained on a Perkin-Elmer Lambda 3 spectrophotometer. Ir spectra were recorded on a Perkin-Elmer 781 spectrophotometer and fabms spectra on a ZAB 2-SEQ spectrometer. All nmr experiments were performed with a Bruker AMX-300 spectrometer operating at 300 (^1H) and 75.5 MHz (^{13}C). All samples prepared for nmr measurements were made as CD_3OD solutions with TMS (δ 0) as the internal standard.

PLANT MATERIAL, EXTRACTION, AND ISOLATION.—The plant material was collected on the road to Mt. Gahavisuka Park, North of Goroka, Eastern Highlands Province of Papua New Guinea in September

1988. The voucher specimens are deposited at the Herbarium (ZT, 11813) ETH, Zurich, Switzerland, UPNG Herbarium, Port Moresby, Papua New Guinea, and at the National Herbarium in Lae, Papua New Guinea. The air-dried leaves of *E. tigang* (1.7 kg) were percolated with petroleum ether, $CHCl_3$, and MeOH which gave 20 g, 35 g, and 46 g of crude extracts, respectively. The MeOH extract (10 g) was partitioned with *n*-BuOH–H_2O (4:6). The *n*-BuOH extract (0.9 g) was applied on dccc (Büchi, B-670) in ascending order using the organic phase of $CHCl_3$-MeOH-H_2O (7:13:8) as a stationary phase and eluting with the aqueous phase. Fractions 17–33 contained 60 mg of eutigoside A [**1**]. Eutigoside B [**2**] (40 mg) and eutigoside C [**3**] (30 mg) were isolated by Rp-18 lplc using MeOH-H_2O (8:2) as a solvent.

Eutigoside A [**1**].—Amorphous colorless powder: $[\alpha]^{20}D$ −26° (MeOH, $c = 0.80$); uv (MeOH) λ max nm 310, 295; ir (KBr) ν max cm^{-1} 3400, 1690, 1630, 1600, 1515; ^1H nmr (300 MHz, CD_3OD) coumaroyl moiety δ 6.27 (1H, d, $J = 15.7$ Hz, H-α), 7.59 (1H, d, $J = 15.7$ Hz, H-β), 7.39 (2H, d, $J = 8.4$ Hz, H-2″, H-6″), 6.78 (2H, d, $J = 8.4$ Hz, H-3″, H-5″); phenylethyl alcohol moiety δ 3.95 (1H, m, H_a-8), 3.66 (1H, m, H_b-8), 2.83 (2H, t-like, H-7), 7.03 (2H, d, $J = 8.4$, H-2, H-6), 6.64 (2H, d, $J = 8.4$ Hz, H-3, H-5); glucose moiety δ 4.32 (1H, d, $J = 7.8$ Hz, H-1′), 3.35–3.39 (2H, overlapping H-2′, H-3′), 3.22 (1H, t-like, H-4′), 3.53 (1H, m, H-5′), 4.50 (1H, dd, $J = 11.8$, 2.2 Hz, H_a-6′), 4.34 (1H, dd, $J = 11.8$, 2.2 Hz, H_b-6′); ^{13}C nmr see Table 1; fabms *m/z* (rel. int.) $[M + Na]^+$ 469 (40), $[M]^+$ 446 (15), consistent with $C_{23}H_{26}O_9$.

Eutigoside B [**2**].—Amorphous pale yellow powder: $[\alpha]^{20}D$ −23° (MeOH, $c = 0.50$); uv (MeOH) λ max nm 310, 295; ir (KBr) ν max cm^{-1} 3400, 1710, 1690, 1630, 1600, 1515; ^1H nmr (300 MHz, CD_3OD) coumaroyl moiety δ 6.35 (1H, d, $J = 16.2$ Hz, H-α), 7.64 (1H, d, $J = 16.2$ Hz, H-β), 7.75 (2H, d, $J = 8.7$ Hz, H-2″, H-6″), 6.98 (2H, d, $J = 8.7$ Hz, H-3″, H-5″); 1-hydroxy cyclo-2,5 dienone moiety δ 3.96 (1H, m, H_a-8), 3.66 (1H, m, H_b-8), 2.03 (2H, t-like, H-7), 7.10 (2H, d, $J = 10$, H-2, H-6), 6.07 (2H, d, $J = 10$ Hz, H-3, H-5); glucose moiety δ 4.32 (1H, d, $J = 7.8$ Hz, H-1′), 3.35–3.39 (2H, overlapping H-2′, H-3′), 3.29 (1H, t-like, H-4′), 3.52 (1H, m, H-5′), 4.48 (1H, dd, $J = 11.8$, 2.2 Hz, H_a-6′), 4.34 (1H, dd, $J = 11.8$, 2.2 Hz, H_b-6′); ^{13}C nmr see Table 1; fabms *m/z* (rel. int.) $[M + Na]^+$ 485 (20), $[M]^+$ 452 (25), consistent with $C_{23}H_{26}O_{10}$.

Eutigoside C [**3**].—Amorphous pale yellow powder: $[\alpha]^{20}D$ −20° (MeOH, $c = 0.70$); uv (MeOH) λ max nm 310, 286; ir (KBr) ν max cm^{-1} 3400, 1710, 1690, 1630, 1600, 1515; ^1H nmr (300 MHz, CD_3OD) cinnamoyl moiety δ 6.55 (1H, d, $J = 16.2$ Hz, H-α), 7.72 (1H, d, $J = 16.2$ Hz, H-β), 7.66 (2H, m, H-2″, H-6″), 7.40 (3H, m, H-3″, H-4″, H-5″); 1-hydroxy cyclohexadienone moiety δ 3.96 (1H, m, H_a-8), 3.66 (1H, m, H_b-8), 2.04 (2H, t-like, H-7), 6.99 (2H, d, $J = 9$, H-2, H-6), 6.05 (2H, d, $J = 9$ Hz, H-3, H-5); glucose moiety δ 4.33 (1H, d, $J = 7.8$ Hz, H-1′), 3.35–3.39 (2H, overlapping H-2′, H-3′), 3.22 (1H, t-like, H-4′), 3.52 (1H, m, H-5′), 4.53 (1H, dd, $J = 11.8$, 2.2 Hz, H_a-6′), 4.34 (1H, dd, $J = 11.8$, 2.2 Hz, H_b-6′); ^{13}C nmr see Table 1; fabms *m/z* (rel. int.) $[M + Na]^+$ 469 (30), $[M]^+$ 446 (25), consistent with $C_{23}H_{26}O_9$.

ACIDIC AND ALKALINE HYDROLYSIS.—Compounds **1–3** were applied on a tlc plate with reference sugars and hydrolyzed at 90° for 30 min with HCl fumes according to Kartnig and Wegschaider (15). After drying, the tlc plate ws eluted with the lower phase of $CHCl_3$-MeOH-H_2O (7:13:8) and detected with anisidine reagent (16). In the same way eutigosides were hydrolyzed with 1 N NaOH and detected with Na_2CO_3-$KMnO_4$ (1:1) spray reagent.

BIOASSAYS.—Compounds **1**, **2**, and **3** showed weak in-vitro growth inhibition (antitumor activity) in the human bladder carcinoma T-24 cells as well as the MK mouse keratinocytes. IC_{50} values were found to be 28.7, 31.9, and 29.3 μg/ml in human bladder carcinoma T-24 cells and 9.2, 9.4, and 12.6 μg/ml in MK mouse keratinocytes, respectively, for compounds **1**, **2**, and **3**.

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