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Report

Kmeriol and Other Aromatic Constituents of *Kmeria Duperreana*

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The $CHCl_3$-soluble fraction of a crude extract of *Kmeria duperreana* exhibited cytotoxic activity when tested in both KB and P388 tumor-cell cultures. Bioassay-directed fractionation led to the isolation of a cytotoxic alkaloid, liriodenine (**1**). Other constituents obtained from the extract included scopoletin (**2**), (−)-3,4,5-trimethoxyphenyl β-D-glucopyranoside (**3**), (+)-syringaresinol β-D-glucopyranoside (**4**), and a new phenylpropanol, kmeriol (**5**), whose chemical structure was established through spectroscopic analysis.

KEY WORDS: *Kmeria duperreana;* Magnoliaceae; cytotoxicity test; isolation; kmeriol.

INTRODUCTION

As part of our continuing investigation of potential antitumor agents from plant sources, we have examined the stem bark of *Kmeria duperreana* (Pierre) Dandy (Magnoliaceae), a small tree growing in the tropical evergreen forests of Thailand. A MeOH extract of the dried plant material was fractionated by solvent extraction and found to be cytotoxic in both the KB nasopharyngeal carcinoma and the P388 murine leukemia cell lines (Table I). Chromatography of the $CHCl_3$-soluble portion of the MeOH extract afforded five aromatic compounds and several terpenoids. This paper is concerned with the cytotoxic activity of the plant and the isolation and characterization of aromatic constituents **1–5**, including a new phenylpropanol, kmeriol (**5**).

MATERIALS AND METHODS

General Experimental Procedures

Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Specific rotation was measured on a Perkin–Elmer 241 polarimeter. The UV spectra were obtained with a Beckman DU-7 spectrophotometer, and IR spectra were obtained on a Nicolet MX-1 FT-IR spectrometer. NMR spectra were recorded on a Nicolet MMC-360 instrument, operating at 360 MHz for proton resonance and 90.8 MHz for carbon resonance. Low-resolution MS was obtained with a Finnigan Model 4500 mass spectrometer, operating at 70 eV.

Plant Material

Stem bark of *K. duperreana* was collected in Thailand in 1986. Voucher specimens have been deposited at the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Solvent Extraction and Chromatographic Separation

The powdered, air-dried stem bark (7.7 kg) was exhaustively extracted with MeOH (20 liters × 4) at room temperature. The MeOH extract was concentrated *in vacuo* to a syrup, taken up in MeOH–H_2O (1:2), and successively partitioned with pentane, $CHCl_3$, and EtOAc.

The $CHCl_3$ fraction (170 g) was chromatographed on a silica gel column (1800 g), eluted with $CHCl_3$–petroleum ether, $CHCl_3$, and $CHCl_3$–MeOH mixtures of increasing polarity. A total of 100 fractions (1 liter each) was collected, monitored by thin-layer chromatography (TLC), and combined on the basis of similar TLC patterns.

Isolation of Kmeriol (5)

Combined fraction 29–39 (14.5 g) was separated on a column of silica gel (350 g) by flash chromatography, using a $CHCl_3$–EtOAc (1:1) mixture as eluting solvent. Subfractions 6–16 (2.6 g) were rechromatographed on a low-pressure column, eluted with $CHCl_3$–MeOH (95:5), to afford a fraction rich in **5**. Further purification by preparative TLC (hexane–propan-2-ol, 6:4) yielded an analytical sample of kmeriol (**5**; 45 mg; 0.0006% yield), mp 71–72°C, $[\alpha]_D$ 0° (MeOH); UV (EtOH) λ_{max} 210, 265 nm; IR (KBr) ν_{max} 3200–3300 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Table II; ms *m/z* (rel. int.) 242 (M^+, $C_{12}H_{18}O_5$, 62%), 224 (1%), 211 (M^+-CH_2OH, 6%), 195

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Table I. Results of Bioassays for Cytotoxicity Using KB and P388 Cell Lines

Sample	ED_{50} (μg/ml) KB	P388
Pentane fraction	20.2	14.8
$CHCl_3$ fraction	1.5	1.9
EtOAc fraction	7.7	9.2
MeOH–H_2O fraction	18.5	12.3
Liriodenine (1)	1.7	0.8
Scopoletin (2)	>50	>50
(−)-3,4,5-Trimethoxyphenyl β-D-glucopyranoside (3)	>50	>50
(+)-Syringaresinol β-D-glucopyranoside (4)	>50	>50
Kmeriol (5)	>50	>50

(1%), 181 (M^+-$C_2H_5O_2$, 100%), 167 (M^+-$C_3H_7O_2$, 24%), 151, 58, 43.

Isolation of Compounds 1–4

From fractions 40–49 of the first column, a semicrystalline deposit was obtained after standing at 4°C overnight. Repeated crystallization resulted in the isolation of scopoletin (2; 25 mg; 0.0003% yield).

Combined fraction 50–60 (38 g) was chromatographed over a column of silica gel (1 kg), eluted with $CHCl_3$–MeOH mixtures. Subfractions 3–5 afforded liriodenine (1; 18 mg; 0.0002% yield). Subfraction 15 was further separated by flash chromatography to yield (+)-syringaresinol β-D-glucopyranoside (4; 130 mg; 0.002% yield), $[\alpha]_D$ −4.3° (MeOH), while subfraction 17 afforded (−)-3,4,5-trimethoxyphenyl β-D-glucopyranoside (3; 18 mg; 0.0002% yield), $[\alpha]_D$ −20.5° (MeOH), after repeated chromatography.

Each of compounds 1–3 was identical in all respects (co-TLC, $[\alpha]_D$, mmp, ^{1}H-NMR, ^{13}C-NMR, MS) to authentic samples.

Table II. ^{1}H and ^{13}C NMR Data for Kmeriol (5)

Position	Carbon chemical shift (δ, ppm)[a]	Proton chemical shift (δ, ppm)[b]
1	66.0	3.49, dd, J = 11.2, 6.8 Hz 3.66, dd, J = 11.2, 2.2 Hz
2	73.0	ca. 3.9, m
3	40.1	2.62–2.74, m
1′	136.4	—
2′	106.1	6.44, s
3′	153.1	—
4′	133.7	—
5′	153.1	—
6′	106.1	6.44, s
3′-OMe	56.0	3.83, s
4′-OMe	60.8	3.81, s
5′-OMe	56.0	3.83, s
OH	—	2.87, br

[a] 90.8 MHz, $CDCl_3$ as solvent, with TMS as internal standard. Assignments were aided by single-frequency off-resonance and attached-proton test experiments.

[b] 360 MHz, $CDCl_3$ as solvent, with TMS as internal standard. Assignments were aided by a COSY experiment.

Enzymatic Hydrolysis of (+)-Syringaresinol Di-*O*-β-D-glucopyranoside and Compound 4

A reference sample of (+)-syringaresinol di-*O*-β-D-glucopyranoside (liriodendrin; 6) (20 mg) was incubated with β-glucosidase (10 mg) in acetate buffer (0.1 *N* HOAc–0.1 *M* NaOAc, 1:2; pH 5.0) for 36 hr at 37°C. The reaction mixture was extracted with Et_2O (20 ml × 3) and the residue obtained from the organic phase was analyzed by TLC ($CHCl_3$–MeOH, 9:1). (+)-Syringaresinol was detected as the major product.

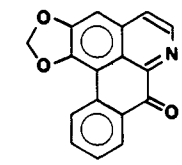
Compound 4 was hydrolyzed under similiar conditions to give an aglycone whose TLC behavior was identical to that of (+)-syringaresinol obtained from liriodendrin.

Bioassays for Cytotoxic Activity

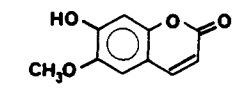
In vitro cytotoxic assays were performed using KB and P388 tumor cell lines as described in the literature (1–3).

RESULTS AND DISCUSSION

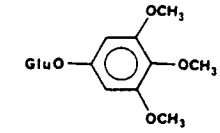
Fractionation of the MeOH extract of the stem bark was performed by extracting successively with pentane, $CHCl_3$, and EtOAc. Biological evaluation of these fractions for *in*



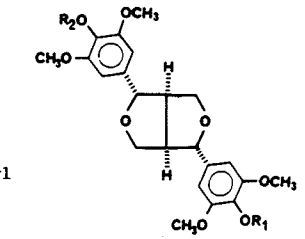
1 Liriodenine



2 Scopoletin

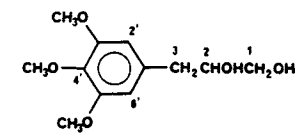


3 (-)-3,4,5-Trimethoxypehnyl β-D-glucopyranoside



4 (+)-Syringaresinol β-D-glucopyranoside: R_1 = Glucose, R_2 = H

6 Liriodendrin: R_1 = R_2 = Glucose



5 (±)-Kmeriol

Fig. 1. Chemical structures of compounds 1 to 6.

vitro cytotoxicity in both the KB and the P388 tumor cell lines indicated that the strongest activity was located in the $CHCl_3$-soluble fraction. Bioassay results are shown in Table I.

Chromatography of the $CHCl_3$ fraction on silica gel led to the isolation of five aromatic compounds (Fig. 1). Four of these isolates were identified as liriodenine (1), scopoletin (2), (−)-3,4,5-trimethoxyphenyl β-D-glucopyranoside (3) (4), and (+)-syringaresinol β-D-glucopyranoside (4) (5). Compounds 1–3 were identified by co-TLC, and their physical and spectroscopic properties compared with those of authentic samples. Liriodenine (1) is the most widely distributed oxoaporphine in plants (6), and scopoletin (2) is also a common natural coumarin (7). The occurrence of (−)-3,4,5-trimethoxyphenyl β-D-glucopyranoside (3) was, however, reported only from *Parabenzoin praecox* recently (4). Compound 3 exhibited spectral data almost identical to those reported in the literature, with the exception that, in our hands, the C-4 carbon of the aglycone resonated at δ 134.2 ppm, instead of 145.8 ppm as observed previously (4). This chemical shift value is in agreement with that of a 3,4,5-trioxygenated phenyl moiety (5,8–10).

The fourth compound, (+)-syringaresinol β-D-glucopyranoside (4), was initially identified by interpretation of its spectroscopic data to be a lignan glucoside. It did not display a recognizable molecular ion in the electron-impact mass spectrum but exhibited a fragmentation pattern comparable to that of liriodendrin [(+)-syringaresinol di-*O*-β-D-glucopyranoside; 6] following the loss of the sugar portion (8). However, the two compounds showed different TLC behaviors in several solvent systems and therefore proved to be nonidentical. The ^{13}C-NMR spectrum of 4 was, on the other hand, almost identical to that of (+)-syringaresinol β-D-glucopyranoside, previously reported from *Eucommia ulmoides* (5), showing clearly the presence of only one glucose moiety in the molecule. The identity of the aglycone as syringaresinol was then confirmed by TLC comparison with liriodendrin following enzymatic hydrolysis. Workup of the aglycone portion of both hydrolysates gave the same product, (+)-syringaresinol. Compound 4 was thus determined to be (+)-syringaresinol β-D-glucopyranoside.

The fifth compound, kmeriol (5), was obtained as an amorphous powder, mp 71–72°C, $[\alpha]_D$ 0° (MeOH), displaying an $[M]^+$ at *m/z* 242 ($C_{12}H_{18}O_5$) and a UV spectrum [λ_{max} 210, 265 nm] characteristic of a benzenoid compound. IR absorption bands at 3300 cm^{-1} indicated the presence of hydroxyl groups, which were further shown to be nonphenolic since the UV spectrum did not alter on the addition of base. In the ^{1}H-NMR spectrum (Table II), prominent features included two methoxyl singlets at δ 3.81 and 3.83, the latter being integrated for six protons and therefore assignable to two magnetically equivalent methoxyl groups. The aromatic region of the spectrum was simple, displaying only a singlet at δ 6.44 integrated for two protons, attributable to two magnetically equivalent aromatic protons. Such NMR features are characteristic for a 3,4,5-trimethoxyphenyl moiety (4,5,8–10), which could be confirmed by the ^{13}C-NMR data (Table II). Thus, two methoxyl groups at C-3′ and C-5′ resonate at δ 56.0 ppm, while the 4′-OMe resonates at δ 60.8. In the downfield region of the carbon spectrum, only four signals were observed due to the symmetrical pattern of substitution on the benzene ring. Among them, the upfield signal could be assigned to two tertiary carbons (C-2′ and C-6′), and the most downfield one attested to C-3′ and C-5′.

The aliphatic part of the molecule was established through an examination of a COSY spectrum. Thus, a multiplet at δ 2.62–2.74 (benzylic methylene; 3-CH_2) was coupled to another multiplet (δ ca. 3.9) partially hidden under the methoxyl peaks. The latter could be assigned to a methine attached to oxygen (H-2). In addition, a hydroxymethylene group (1-CH_2) was revealed by two doublet of doublets resonating at δ 3.49 (J = 11.2, 6.8 Hz) and 3.66 (J = 11.2, 2.2 Hz), respectively. They were found not only coupled to each other, but also coupled to H-2. These data suggested a 3-substituted propan-1,2-diol structure and could be further supported by the mass fragmentation pattern and the ^{13}C-NMR spectrum (Table II). In the mass spectrum, sequential loss of C-1, C-2, and C-3 fragments was obvious. Finally, two hydroxyl protons were found in the ^{1}H-NMR spectrum at δ 2.87, which was exchanged on the addition of D_2O.

Compound 5 was thus established to be racemic (±)-3-(3′,4′,5′-trimethoxyphenyl)-1,2-propanediol and given the trivial name of kmeriol. To the best of our knowledge, this is the first report of the natural occurrence of this compound. A metabolite of the same general structure has been suggested by GC-MS evidence as a product of elemicin metabolism in rats (11).

All isolates were evaluated for cytotoxic activity in both the KB and the P388 systems, the results being included in Table I. Compounds 2–5 were devoid of any significant activity when tested in these bioassay systems. On the other hand, positive results were obtained for liriodenine (1), which is therefore considered to be an active principle of the plant extract. The cytotoxic activity of liriodenine in the KB system has been reported previously (12–14).

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