Flavonoids and Terpenoids from *Luma gayana* (Barn.) Burret

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Z. Naturforsch. **54c,** 1140–1142 (1999); received July 15/August 6, 1999

Luma gayana (Barn.) Burret, Myrtaceae, Flavonoids, Terpenoids, Alkyl *p*-Coumarates

The flavonoids 5-hydroxy-7-methoxyflavanone, 6,8-dimethyl-5,7-dihydroxyflavanone and 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone, a mixture of alkyl esters of *p*-coumaric acid, the triterpenoids oleanolic acid and maslinic acid, the monoterpenoid 1α,2β,4β-trihydroxy-*p*-menthane, the sesquiterpenoid clovandiol and β-sitosterol were isolated from the aerial parts of *Luma gayana* (Barn.) Burret. This is the first report on the chemistry of this species.

Introduction

The genus *Luma* (Myrtaceae), native to Argentina and Chile, comprises only three species including *L. gayana* (Barn.) Burret, *L. apiculata* (DC) Burret and *L. chequen* (Mol.) A. Gray (Marticorena and Quezada, 1985). *L. gayana* is a shrub or small tree with thin hairy branches and ovate-oblong leaves with pointed tips and smooth margins. The leaves are hairy when young and do not exceed one centimeter in length. The solitary flowers which grow on peduncles longer than the leaves have four sepals, a corolla of four petals and numerous stamens. The fleshy edible fruit contains few seeds. This species occurs in Chile between Talca and Chiloe and is generally found along waterways or in swamps. It is used in folk medicine as a stimulant and astringent. Folk medicinal uses have also been mentioned for *L. chequen* and *L. apiculata* and extracts obtained from these two species have been evaluated for inhibitory activity against xanthine oxygenase (Theoduloz *et al.*, 1991). An extract of *L. chequen* has also been shown to inhibit the growth of a carcinoma cell line (Bhakuni *et al.*, 1976). A chemical investigation of *Myrceugenella chequen* (Mol.) Kaus, which is a synonym of *L. chequen*, showed the presence of quercetin, kaempferol, myricetin and myricetin glycosides (Hörhammer *et al.*, 1973). We report here the isolation of three flavonoids, a mixture of esters of *p*-coumaric acid, β-sitosterol, as well as one mono-, one sesqui- and two triterpenoids from *L. gayana*. This report is the first study of the chemistry of this species.

Material and Methods

General

Mps. were taken on an Electrothermal apparatus using open capillary tubes. NMR spectra were recorded on a Varian Unity 300 spectrometer (^{1}H: 300 MHz, ^{13}C: 75.4 MHz). EIMS (70 eV) were recorded on a Hewlett Packard 5988A spectrometer. APCIMS were recorded on a Finnegan TSQ 7000 spectrometer. Authentic samples of β-sitosterol and oleanolic acid were purchased from K& K Laboratories (Jamaica, N. Y.) and Aldrich (Milwaukee, W. I.), respectively. For column chromatography (CC) silica gel 60 was used (50–200 μm, Macherey & Nagel, Düren, Germany, for initial fractionation and 43–60 μm, Lagand, N. J., for subsequent CCs). For preparative thin layer chromatography (TLC) silica gel 60 G/F254 was used (Macherey & Nagel). Sephadex LH20 was purchased from Pharmacia (Upsala, Sweden).

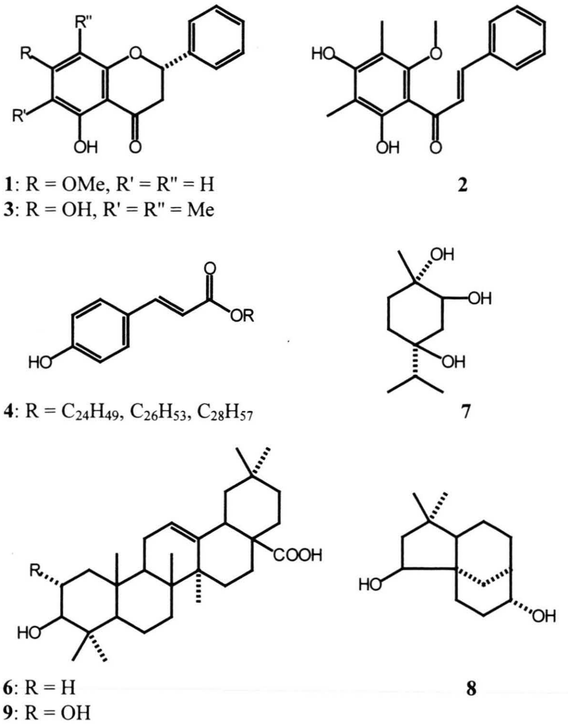
Plant material

L. gayana was collected by Luis González in May 1994 near Trehualemu in the Province Parral of the Chilean Region VII. The plant material was identified by Gloria Montenegro and a voucher specimen (no. 276) was deposited in the herbarium of the Pontificia Universidad Católica de Chile, Santiago, Chile. Intellectual Property Rights Agreements for plant collections and collaborative research have been fully executed between The University of Arizona and the collaborating institution in this study.

0939–5075/99/1200–1140 $ 06.00

Extraction and isolation

Air dried and ground aerial parts of *L. gayana* (520 g) were extracted at room temperature with CH_2Cl_2 (4 × 4 l). Removal of the solvent yielded the crude extract (21 g) which was subjected to CC on silica gel with mixtures of hexane-EtOAc (0–100% EtOAc) followed by MeOH. Thirty eight fractions were collected of which fraction 38 (8.3 g) eluted with MeOH. Fractions 17–20 were combined (2.7 g) and further separated by CC on silica gel (hexane-EtOAc, 8:2) to yield **1** (36.9 mg) and a fraction which after further purification by CC on Sephadex LH20 with MeOH-CH_2Cl_2 (1:1) gave a mixture of flavonoids (1.2 g) which consisted almost exclusively of **2**. Treatment of 570 mg of this material with cold methanol gave **3** (8.2 mg) as an insoluble residue. The soluble portion was recrystallized from hexane to give pure **2** (385 mg). From fraction 21 (563 mg), a white solid precipitated which was collected and purified by CC on silica gel (hexane-EtOAc, 4:1) and Sephadex LH20 (MeOH-CH_2Cl_2, 1:1) to give **4** (150 mg). Fraction 23 (0.3 g) was subjected to CC on silica gel with hexane-EtOAc (4:1) to yield **5** (51.7 mg). Fractions 33–35 were combined (4.1 g) and chromatographed on silica gel with mixtures of hexane and EtOAc. The fraction eluting with 20% ethyl actetate (740 mg) was further separated by preparative TLC with hexane-EtOAc (1:3) to give two fractions. The fraction with the higher R_f value was further purified by CC on Sephadex LH20 (MeOH-CH_2Cl_2, 1:1) and gave **6** (29.5 mg). From the fraction with the lower R_f value compounds **7** (58.9 mg) and **8** (13.8 mg) were obtained by CC (silica gel, hexane-EtOAc, 1:1). Fractions 36 and 37 were combined (1.0 g) and subjected to CC (silica gel, hexane-EtOAc, 1:4). The resulting fractions were evaluated by TLC. Compound **9** (5.6 mg) was isolated by reversed phase HPLC (econosil C18, 10 μm, CH_3CN-MeOH-H_2O, 14:1:5) from a selected fraction (15.7 mg). The ratio of solvents is based on v/v throughout.



1: R = OMe, R' = R'' = H
3: R = OH, R' = R'' = Me

2

4: R = $C_{24}H_{49}$, $C_{26}H_{53}$, $C_{28}H_{57}$

7

6: R = H
9: R = OH

8

Fig. 1. Structures of compounds **1–4** and **6–9**.

Results and Discussion

The isolated compounds **1-9** (Fig. 1) were identified by comparison of their melting points, ^{13}C and ^1H NMR data with those reported in the literature. Compounds **1-3** were identified as the flavonoids 5-hydroxy-7-methoxyflavanone (**1**, González *et al.*, 1989), 6,8-dimethyl-5,7-dihydroxyflavanone (**3**, Tanriseven *et al.*, 1987) and 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**, Wollenweber *et al.*, 1985). NMR and mass spectrometry showed that **4** was a mixture of esters of *p*-coumaric acid and tetracosanol, hexacosanol and octacosanol (ratios determined by APCIMS were 1:10:3) (Chatterjee *et al.*, 1980). Compounds **5** and **6** were identified as β-sitosterol and oleanolic acid by comparison of their ^1H NMR spectra with those of authentic samples. Compound **9** was identified as the triterpenoid maslinic acid (Tchivounda *et al.*, 1991). Compounds **7** and **8** were identified as 1α,2β,4β-trihydroxy-*p*-menthane (**7**, Thappa *et al.*, 1976) and clovandiol (**8**, Delgado *et al.*, 1984).

Acknowledgement

The authors thank Mr. Luis González for the collection of the plant material. This study was supported by the ICBG "Bioactive Agents from Dryland Biodiversity of Latin America" grant 2 UO1 TW 00316 from the National Institutes of Health (NIH), National Science Foundation

(NSF) and US Department of Agriculture (USDA) (to BNT), by a grant from the National Institute of Environmental Health Sciences (NIEHS) (Environmental Health Service Core Center Grant P30ES06694 to the Southwest Environmental Health Sciences Center) and grant FONDECYT 1980967 (to GM). AW gratefully acknowledges funding provided by a scholarship from the Royal Thai Government. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the granting agencies.

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