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BIOACTIVE MONOTERPENE GLYCOSIDES FROM *ERIGERON LINIFOLIUS*

CONSOLACION Y. RAGASA,* JOHN A. RIDEOUT,† JENNIFER O. SY, DOLORES ALCACHUPAS, VIC MARIE L. INTE and JOHN C. COLL†

Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila, 1004, Philippines; †Chemistry Department, Central Queensland University, Rockhampton, Queensland, 4702, Australia

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Key Word Index—*Erigeron linifolius*; Compositae; monoterpene glycosides; acetylated pinene glucosides; antimutagen; antimicrobial.

Abstract—The chloroform extract of *Erigeron linifolius* afforded two novel acetylated pinene glucosides. Their structures were elucidated by 1D and 2D NMR and FT-IR spectroscopy and mass spectrometry. The relative stereochemistry of the glucosides was established by a combination of coupling constant analyses and NOESY. The glucosides showed high antimutagenic and low antimicrobial activities. © 1997 Elsevier Science Ltd. All rights reserved

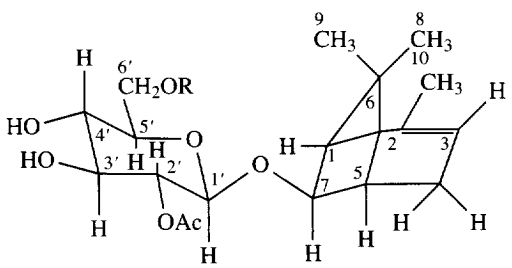
INTRODUCTION

Erigeron linifolius is widely distributed throughout the tropics. In the Philippines, the leaves are used for the treatment of rheumatism and as a prevention for too rapid conception. A cataplasm of the fresh plant is applied on wounds, contusions and dislocations [1]. Earlier studies on the genus *Erigeron* reported the isolation of sterols and triterpenes [2], monoterpenes and sesquiterpenes [3], flavanoid glycosides [4] and flavanoids [5–6]. We now report on the isolation, structure elucidation, antimutagenic and antimicrobial properties of two novel acetylated pinene glucosides (**1** and **2**) from *E. linifolius*. This is the first report on the isolation of pinene glucosides from the genus. However, a closely related monoterpene glucoside, (−)-cis-chrysanthenol *O*-β-D-glucopyranoside was isolated from *Dicoria canescens* [7] which belongs to the same family.

RESULTS AND DISCUSSION

The chloroform extract of the leaves of *E. linifolius* afforded two new monoterpene glycosides (**1** and **2**).

The structure of **1** was elucidated by NMR (^{1}H, ^{13}C, DEPT, COSY, NOESY, HMQC) and FT-IR spectroscopy, and mass spectrometry. The ^{1}H NMR spectrum indicated the presence of five methyl groups, two of which were from acetates (δ 2.08 and 2.10; IR



1 R = Ac
2 R = H

1742, 1238 cm^{-1}), an olefinic proton (δ 5.22) and eight protons attached to oxygenated carbons (Table 1). The COSY spectrum indicated two isolated spin systems as follows. The methylene protons on an oxygenated carbon at δ 4.25 (H-6a′, *dd*, *J* = 12, 1.7 Hz) and 4.42 (H-6b′, *dd*, *J* = 12, 4.1 Hz) were coupled to the hydrogen on an oxygenated carbon at δ 3.43 (H-5′, *m*), which was in turn coupled to the proton on an oxygenated carbon at δ 3.45 (H-4′, *dd*, *J* = 8, 9 Hz). The latter hydrogen was coupled to a proton on an oxygenated carbon at δ 3.60 (H-3′, *dd*, *J* = 9, 8 Hz), which was in turn coupled to a proton on an oxygenated carbon at δ 4.81 (H-2′, *dd*, *J* = 9, 8 Hz), which was further coupled to the hydrogen on an anomeric carbon at δ 4.46 (H-1′, *d*, *J* = 8 Hz). Based on chemical shift grounds, the acetates were attached to C-2′ and C-6′, while the hydroxyls (IR 3400, 1064, 1046 cm^{-1}) were attached to C-3′ and C-4′. Thus, **1** was a glycoside. Another isolated spin system was delinated

* Author to whom correspondence should be addressed.

Table 1. 300 MHz ^{1}H NMR spectral data of compounds **1** and **2** in $CDCl_3$

^{1}H	**1**	**2**
1	2.09 (*s*, *br*)	2.09 (*s*, *br*)
3	5.22 (*s*, *br*)	5.21 (*s*, *br*)
4a, 4b	2.25 (*m*), 2.28 (*m*)	2.25 (*m*), 2.28 (*m*)
5	2.05 (*m*)	2.05 (*m*)
7	3.92 (*s*, *br*)	3.92 (*s*, *br*)
8	1.37 (3H, *s*)	1.35 (3H, *s*)
9	0.88 (3H, *s*)	0.87 (3H, *s*)
10	1.65 (3H, *d*, 1.7 Hz)	1.65 (3H, *s*, *br*)
1′	4.46 (*d*, 8 Hz)	4.46 (*d*, 8 Hz)
2′	4.81 (*dd*, 8, 9 Hz)	4.81 (*dd*, 8, 9 Hz)
3′	3.60 (*dd*, 8, 9 Hz)	3.61 (*dd*, 8, 9 Hz)
4′	3.45 (*dd*, 8, 9 Hz)	3.41 (*dd*, 8, 9 Hz)
5′	3.43 (*m*)	3.30 (*m*)
6a′, 6b′	4.25 (*dd*, 12, 1.7 Hz), 4.42 (*dd*, 12, 4.1 Hz)	3.84 (2H, *s*, *br*)
OAc	2.08 (*s*), 2.11 (*s*)	2.10 (*s*)

Table 2. 75 MHz ^{13}C NMR spectral data of compounds **1** and **2** in $CDCl_3$[a]

^{13}C	**1**	**2**
1	45.0	45.1
2	37.4	37.4
3	118.2	118.2
4	31.9	32.0
5	50.6	50.7
6	141.9	141.9
7	83.4	83.4
8	26.5	26.5
9	22.7	22.8
10	22.6	22.6
1′	99.0	99.0
2′	73.9	74.0
3′	75.4	75.6
4′	73.7	75.3
5′	70.7	70.6
6′	63.1	61.8
OAc	20.8, 21.0, 171.0, 171.8	21.1, 171.1

[a] Assignments are based on HMQC of **1**.

as follows. An olefinic hydrogen at δ 5.22 (H-3, *s*, *br*) was coupled to the allylic methyl at δ 1.65 (H-10, *d*, 1.7 Hz) and the allylic multiplets at δ 2.25 (H-4a) and 2.28 (H-4b). The latter hydrogen was coupled to the proton at δ 2.05 (H-5, *m*) which was coupled to the hydrogen on an oxygenated carbon at δ 3.92 (H-7, *s*, *br*), which was in turn coupled to the proton at δ 2.09 (*s*, *br*, H-1).

The ^{13}C and DEPT NMR spectra of **1** indicated 20 carbon atoms with the following functionalities: two carbonyls of acetates at δ 170.0 and 171.8 (IR 1742, 1238 cm^{-1}) [1], seven oxygenated (one methylene and six methine, one of which has two oxygen functions at δ 98.9), one fully substituted olefin at δ 141.9 and one protonated olefin at δ 118.2, five methyl, one methylene, two methine and one quaternary carbon (Table 2). The ^{13}C and ^{1}H assignments for **1** were verified by HMQC. The DEPT spectrum revealed that **1** contained 28 protons attached to carbons. Of the 18 non-carbonyl carbons, seven were singly bonded to oxygen, including an anomeric carbon. Thus, **1** contained eight oxygen atoms. Since there are two acetates and an anomeric carbon which would account for six oxygen atoms, the remaining two oxygens probably belonged to two hydroxyl groups (IR 3400, 1081, 1064 cm^{-1}). Based on the foregoing analysis, the molecular formula of **1** is $C_{20}H_{30}O_8$. From the molecular formula, the index of hydrogen deficiency was six. With two carbonyls and an olefin in **1**, the rest of the deficiency could be accounted for by three carbocyclic systems. Since the glycoside contained two carbonyls and one ring system, the second fragment of **1** contained an olefin and a bicyclic system. Subtracting the glycoside from the molecular formula, the second fragment had 10 carbons and 15 hydrogens. This was deduced to be a monoterpene moiety since it is a multiple of isoprene. From the ^{13}C and DEPT spectra, only three carbons (one quaternary and two methyl singlets) were unaccounted for in the two fragments of the molecule deduced from the COSY spectrum. Attaching the quaternary carbon to C-1 and C-5 and the methyl carbons to the quaternary carbon indicated that the monoterpene moiety was α-pinene. The ^{1}H NMR spectrum of **1** minus the resonances for the glycoside was similar to that of α-pinene [8]. The α-pinene was attached to the anomeric carbon through C-7.

The identity of the glycoside remains to be determined. It was observed that the coupling constant between H-1′ and H-2′ (8 Hz), H-2′ and H-3′, (9 Hz), H-3′ and H-4′ (8 Hz) and H-4′ and H-5′ (9 Hz) fall within the range of axial–axial coupling (6–14 Hz). Thus, H-1′ to H-5′ were in the axial positions and the acetates, hydroxyls and α-pinene were in the equatorial positions. This would result in less steric effect and eliminate 1,3-diaxial interaction. Therefore, the glycoside is a β-glucoside. The relative stereochemistry of **1** was confirmed by NOESY. The correlation of ^{1}H NMR nuclei from NOESY is shown in Fig. 1. It was deduced from NOESY that the glucoside was attached to the α-pinene at the β position of C-7 since H-7 and H-1′ are close in space.

Structure **1** was verified by mass spectrum. It was

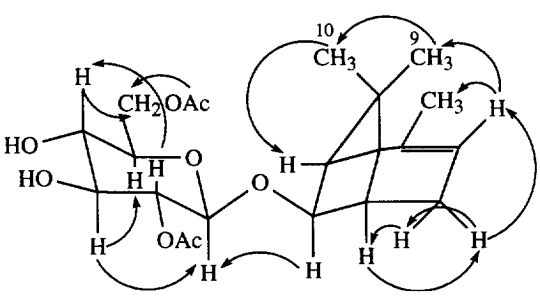


Fig. 1. Correlation of ^{1}H NMR nuclei from NOESY of **1**.

recognized that the spectrum did not show a $[M]^+$ peak. The highest *m/z* value was 247 which resulted from the loss of oxygenated α-pinene $[M-151]^+$. The proposed name of **1** is α-pinene-7β-*O*-β-D-2,6-diacetylglucopyranoside.

The structure of **2** was derived by analogy with **1**. The ^1H NMR spectrum of **2** (Table 1) indicated the absence of one of the acetates at δ 2.08 and the shielding of the hydrogens on the acetylated carbon at δ 4.42 (1H, *dd*, $J = 12$, 1.7 Hz) and 4.25 (1H, *dd*, $J = 12$, 4.1 Hz) for C-6′ of **1** changed to δ 3.84 (2H, *s br*) in **2**. This was supported by the ^{13}C NMR spectrum of **2** (Table 2) which revealed the absence of one of the acetates at δ 171.8 and 20.8. In addition, the acetylated carbon at δ 63.1 in **1** was shifted upfield to δ 61.8 in **2** when the acetate was replaced by a hydroxyl group. The structure was verified by mass spectroscopy with the highest *m/z* value at 205 which resulted from the loss of oxygenated α-pinene $[M-151]^+$. The proposed name of **2** is α-pinene-7β-*O*-β-D-2-acetylglucopyranoside.

To determine the possible medicinal applications of **1** and **2**, antimutagenicity and antimicrobial tests were conducted on the compounds. The Micronucleus test indicated that **1** at a dosage of 8 mg kg^{-1} mouse reduced the number of micronucleated polychromatic erythrocytes (MPCE) induced by mitomycin C [8.75 ± 2.18 MPCE/1000 PCE$\pm\sigma$ (15)] by 87.1%, while **2** reduced the MPCE by 84.8% at the same dosage. Statistical analysis using the *T*-test showed that there are significant reductions in MPCE at $\alpha = 0.01$. This implies that **1** and **2** are antimutagens.

Compounds **1** and **2** were also tested for antimicrobial activity by the disc diffusion method. Compound **1** at a concentration of 100 μg ml^{-1} produced partial inhibitory activity against *Escherichia coli*, *Staphylcococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhosa* and *Saccharomyces cerevisiae* but was inactive against *Candida albicans*. Compound **2** at the same concentration showed complete inhibitory activity against *E. coli* and *S. typhosa*, partial inhibitory activity against *S. aureus*, and *P. aeruginosa* but was inactive against *C. albicans* and *S. cerevisiae*. Thus, **2** has relatively higher antimicrobial activity than **1**. Although based on mean zone of inhibition (10 mm) which is also the disc diameter, **1** and **2** have low antimicrobial activity.

EXPERIMENTAL

General. NMR: 300 MHz (^1H) and 75 MHz (^{13}C) in $CDCl_3$; CC: Silica gel 60 (70–230 mesh); plastic backed plates coated with silica gel F_{254}. Plates were visualized by spraying with vanillin–H_2SO_4 and warming.

Erigeron linifolius was collected at the University of the Philippines, Diliman in April 1993. A voucher specimen is kept at the Chemistry Department of De La Salle University. The air-dried leaves (250 g) were extracted with $CHCl_3$ (2 l) to afford a crude extract (6 g). The extract was chromatographed on a column of silica gel (70–230 mesh) using increasing proportions of Me_2CO in $CHCl_3$ (10% increments) and MeOH in Me_2CO (10% increments) as eluents. The 30% Me_2CO in $CHCl_3$ fr. was rechromatographed in 30% Me_2CO in $CHCl_3$ to afford **1** [30.5 mg, colorless oil $[\alpha]_D = -38°$ (0.77, $CHCl_3$)]. The 50% Me_2CO in $CHCl_3$ fr. was rechromatographed in 40% acetone in $CHCl_3$ to afford **2** (16.2 mg, colorless oil).

Antimutagenicity test. The test compounds (8 mg kg^{-1} mouse) dissolved in DMSO (7.5 ml kg^{-1} mouse, solvent control) were administered simultaneously with mitomycin C (2.75 mg kg^{-1} mouse, positive control) to mice of the Swiss strain. For the control, only mitomycin C and DMSO were administered orally to the mice. Five mice were tested for each compound and control. The second administration was carried out after 24 hr. Six hr after the second administration, the mice were killed by dislocation of the neck. Blood from the bone marrow was smeared on slides (three per mouse). The slides were stained with May-Grunwald and Giemsa solns [8]. The numbers of MPCE/1000 PCE were counted by the use of a high power microscope.

Antimicrobial test. Test bacteria in nutrient broth were cultured and incubated at 37° overnight. Culture broth bacteria were incorporated in molten nutrient agar 40° to 42° at 2% concs of the test organisms to medium. Seeded medium were transferred in 10 ml portions to individual properly labeled petri dishes. Test plates were preincubated for 1 hr at 37° before conducting tests. Three separate discs were dipped into the test samples at a concn of 100 μg ml^{-1} and placed in proper distances upon the surface of the agar to allow development of inhibition zones. A fourth disc was dipped in the control (95% EtOH solvent) and placed upon the agar. All test plates were incubated upside down at 37° for 24 hr. The mean average diameter zones of inhibition produced by each test sample in three replication were measured in terms of milliliters.

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