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SHORT COMMUNICATION

CUAUTHEMONE SESQUITERPENES AND FLAVONES FROM *LAGGERA TOMENTOSA* ENDEMIC TO ETHIOPIA

Kibrom Gebreheiwot[1], Dibaba Amenu[2] and Nigist Asfaw[1*]

[1]Department of Chemistry, Addis Ababa University, P.O. Box 1176. Addis Ababa, Ethiopia
[2]Gilgel Beles College of Teachers Education, P.O. Box 36. Gilgel Beles, Ethiopia

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ABSTRACT. Three sesquiterpenes, 3-*O*-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)cuauthemone (**1**), 4-*O*-acetylcuauthemone-3-*O*-angelate (**2**), 4-*O*-acetylcuauthemone 3-*O*-(2'-hydroxy-2'-methyl-3'-acetoxybutyrate) (**3**) and two flavones, 3',4',5,7-tetrahydroxy-3,6-dimethoxyflavone (**4**), 3',5,6-trihydroxy-3,4',7-trimethoxyflavone (**5**) were isolated from the aerial parts of *Laggera tomentosa*. The compounds were characterized using spectroscopic techniques. Complete ^{1}H and ^{13}C NMR assignment of compound **1** was done for the first time. This is the first report on the occurrence of cuauthemone sesquiterpenes in the genus *Laggera.*

KEY WORDS: *Laggera tomentosa*, Asteraceae, Cuauthemone sesquiterpenes, 3-*O*-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)cuauthemone

INTRODUCTION

Laggera tomentosa (Sch. Bip. ex A. Rich) Oliv & Hiern (Asteraceae) known locally as "keskese", is a perennial fragment bushy herb (0.5-1.2 m high) endemic to Ethiopia. Traditionally, the juice of the crushed leaves is ingested as a treatment for stomachache, and is used against migraine. It is also used as a fumigant and for cleansing milk containers [1]. There are about 20 species in the genus *Laggera* and only few have been extensively investigated. Some *Laggera* species and their constituents exhibit anti-inflammatory, cytotoxicity and phytotoxicity [2]. A number of compounds have been isolated from the *Laggera* species, the most characteristic of which are the eudesmane sesquiterpenes and flavones [2-5]. Phytochemical studies on the essential oil of *L. tomentosa* have been reported before [6, 7]. However, there are no reports on the chemical investigation of the solvent extract of this species prior to this work.

RESULTS AND DISCUSSION

The petroleum ether and ethanol extract of the aerial part of *L. tomentosa* was subjected to exhaustive chromatographic separation which yielded three cuauthemone sesquiterpenes (**1-3**) and two flavones (**4** and **5**). These compounds are reported for the first time from *L. tomentosa*. The compounds, 4-*O*-acetylcuauthemone-3-*O*-angelate (**2**) [8], 4-*O*-acetylcuauthemone 3-*O*-(2'-hydroxy-2'-methyl-3'-acetoxybutyrate) (**3**) [9], 3',4',5,7-tetrahydroxy-3,6-dimethoxyflavone (**4**) [10, 11] and 3',5,6-trihydroxy-3,4',7-trimethoxyflavone (**5**) [12] (Figure 1) were identified by comparison of their spectroscopic data with reported values in the literature.

The structure of compound **1** was fully characterized in this work based on 1D and 2D-NMR data including DEPT-135, COSY, HSQC, and HMBC. Dominguez *et al.* [13] reported similar structure as **1** for a compound isolated from *Pluchea purpurescens* and the structure was elucidated from MS, IR and ^{1}H NMR data. The MS and IR data reported are similar with those of compound **1** isolated in this study. However, the ^{1}HNMR assignment by Dominguez *et al.* is

*Corresponding author. E-mail: nigista@chem.aau.edu.et; nigistasfaw@hotmail.com

different from some of the protons reported by us. This prompts us to do comprehensive NMR analysis of compound **1** (Table 1).

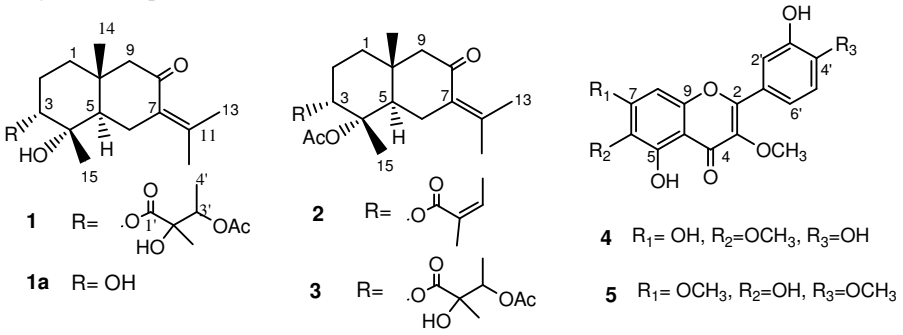

Figure 1. Structures of the compounds.

Table 1. ^{1}H, ^{13}C, and HMBC (H → C) spectral data of compound **1**[a] (in $CDCl_3$).

No.	δ_C (ppm)	δ_H (ppm)	HMBC	δ_H (ppm) [13]
1	33.4	1.45 (*m*), 1.28 (*m*)	$H^{1a}\rightarrow C^{10}$, $H^{1a}\rightarrow C^{14}$ $H^{1b}\rightarrow C^{10}$, $H^{1b}\rightarrow C^{14}$	No value
2	23.9	1.79 (*m*), 1.75 (*m*)	$H^{2}\rightarrow C^{10}$	No value
3	78.9	4.89 (*br t*)	$H^{3}\rightarrow C^{1}$, C^{4}, C^{5}, C^{15}, $C^{1'}$	4.91(ht, J = 3 Hz)
4	72.2	-		-
5	46.6	1.92 (*dd*, J = 4, 8 Hz)	$H^{5}\rightarrow C^{6}$, C^{7}, C^{9}, C^{14}, C^{15}	No value
6	25.5	2.91 (*dd*, J = 4, 16 Hz), 2.17 (*dd*, J = 12, 16 Hz)	$H^{6a}\rightarrow C^{5}$, C^{7}, C^{8}, C^{11} $H^{6b}\rightarrow C^{5}$, C^{7}, C^{8}, C^{11}	2.94 (ddbr, J = 4, 15 Hz), 2.09 (ddbr, J = 13, 15 Hz)
7	130.5	-		-
8	202.1	-		-
9	59.7	2.22 (*s*)	$H^{9}\rightarrow C^{1}$, C^{5}, C^{7}, C^{8}, C^{14}	1.93(dd, J = 13,5)
10	35.8	-		-
11	145.9	-		-
12	23.6	2.03 (*s*)	$H^{12}\rightarrow C^{7}$, C^{8}, C^{11}, C^{13}	2.23 (brs)
13	22.9	1.82 (*s*)	$H^{13}\rightarrow C^{7}$, C^{8}, C^{12}	1.83 (brs)
14	18.7	0.94 (*s*)	$H^{14}\rightarrow C^{1}$, C^{5}, C^{9}, C^{10}	0.93 (s)
15	21.5	1.26 (*s*)	$H^{15}\rightarrow C^{5}$	1.27 (s)
1'	174.7	-		-
2'	76.4	-		-
3'	74.4	5.12 (*q*, J = 4 Hz)	$H^{3'}\rightarrow C^{1'}$, $C^{4'}$, $C^{5'}$, $\underline{C}OCH_3$	5.13(q, J = 6.5 Hz)
4'	13.3	1.28 (*d*, J = 4 Hz)		1.30(d, J = 6.5 Hz)
5'	22.4	1.40 (*s*)	$H^{5'}\rightarrow C^{1'}$, $C^{3'}$	1.42(s)
-OAc	169.8 21.0	- 1.98 (*s*)	$CO\underline{C}H_3\rightarrow C^{3'}$, $\underline{C}OCH_3$	- 1.99(s)

[a] 400 and 100 MHz, respectively. The carbon assignments were made using the HSQC spectrum.

^{13}C NMR and DEPT-135 indicated that **1** has 22 carbon atoms: 8 quaternary, 3 methine, 4 methylene and 7 methyl carbons. The spectra also showed one ketone carbonyl at δ 202.1, two ester carbonyls at δ 196.8 and 174.7, and two olefinic carbons at δ 130.5 and 145.9. The H, H-COSY spectrum showed strong correlation between the two protons on C-6 indicating that methylene protons on C-6 are diasteriotopic and C-5 is a stereogenic center. In HMBC spectrum

methyl protons appearing at δ 0.94 showed correlations with C-10, C-9, C-5 and C-1 indicating CH_3-14 is attached to C-10. Moreover, protons on CH_3-12 and CH_3-13 correlated with the two olefinic carbons (C-7 and C-11) and carbonyl carbon C-8 (Table 1). This observation suggests the presence of an α,β-unsaturated carbonyl group which is supported by the UV spectrum. Correlation of H-3 with carbonyl carbon C-1' indicated the position of the side chain to be C-3. This was also confirmed by hydrolysis experiment which yielded cuauthemone (**1a**) [14]. The relative configuration of the stereocenters (C-3, 4, 5, and 10) has been defined by comparison with related compound in the literature [14].

Among the 20 *Laggera* species, *L. pterodonta*, *L.alata*, *L. crispata* and *L. decurrens* have been extensively investigated and 51 eudesmanes sesquiterpenes and five flavonoids have been reported from these species [2]. The cuauthemone eudesmanes and flavonoids isolated from *L. tomentosa* have not been reported from the above mentioned species. The cuauthemone eudesmanes are rather characteristic of the genus *Pluchea* [14], which belong to the same tribe, *Plucheeae*, as the genus *Laggera.* This study indicates that *L. tomentosa* is chemically related to some *Pluchea* species, due to the co-occurrence of cuauthemone sesquiterpenes, rather than to the *Laggera* species studied so far. Further studies need to be carried out to establish the chemotaxonomic relationship of the species within the genus *Laggera*, and between *L. tomentosa* and *Pluchea* species.

EXPERIMENTAL

General. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker Avance 400MHz spectrometer with TMS as internal standard. The ultraviolet and visible (UV-Vis) spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer in the range 200-1000 cm^{-1}. Infrared (IR) spectra were obtained on Perkin-Elmer BX Infrared spectrometer using KBr in the range 400-4000 cm^{-1}. Positive ion HR-MS spectrum was recorded on Bruker Micro TOF spectrometer at School of Chemistry, University of Nottingham, UK. Melting points were recorded using Thomas HOOVER Capillary melting point apparatus. TLC analyses were carried out on TLC plates 0.2 mm thick layer of Merck silica gel 60 F_{254} coated on aluminum foil. Compounds on TLC were detected using UV lamp and spraying with 1% vanillin in sulfuric acid for sesquiterpenes and 5% methanolic KOH solution for flavones.

Plant material. *Laggera tomentosa* was collected from Daletti, Western Shoa of Ethiopia (26 km far from Addis Ababa, near Alemgena) in November 2005. A voucher specimen (SD 6487) is deposited at the National Herbarium (ETH), Department of Biology, Addis Ababa University.

Extraction and isolation. The dried and milled aerial parts of *L. tomentosa* (500 g) were extracted by maceration with petroleum ether (54-93 °C) at room temperature for 24 hours, and then evaporated *in vacuo* to yield 22.8 g crude extract. The residue was then soaked with ethanol twice at room temperature for up to 24 hours each and then evaporated *in vacuo* to yield 54 g solid material. The dried petroleum ether extract (22.8 g) was chromatographed over a column packed with 200 g silica gel using petroleum ether containing increasing amounts of EtOAc as solvents and 40 fractions were collected. The fractions were monitored by TLC and combined to 17 fractions. Fraction 8 (pet-ether/EtOAc, 7:3) after cleaning up on Sephadex LH-20 column [eluent, $CHCl_3$/MeOH (2:1)] was further purified on a silica gel column [eluent, pet-ether/EtOAc (7:3)] to yield compound **2** (28 mg). Fraction 17, after cleanup on Sephadex LH-20, [$CHCl_3$/MeOH (2:1)] and further fractionation on a silica gel column as above gave compound **3** (32 mg). About 20 g of the ethanol extract was applied to a silica gel CC and eluted with $CHCl_3$/MeOH in increasing polarity. A total of 29 fractions were collected and combined in to 12 fractions on the bases of similar TLC profile.

Fraction 4, after cleanup on a Sephadex LH-20 column [$CHCl_3$/MeOH (2:1)] and further fractionation on a silica gel column [$CHCl_3$/EtOAc (9:1)] gave a fraction which on further purification using chromatotron ($CHCl_3$/MeOH; 40:1) afforded compound **1** (163 mg). Fraction 5 was passed through Sephadex LH-20 [$CHCl_3$/MeOH (2:1)] and upon further purification on a silica gel column (using $CHCl_3$/MeOH; 20:1) and chromatotron ($CHCl_3$/MeOH; 20:1) to afford **4** (43 mg) and **5** (38 mg).

*3-O-(3'-Acetoxy-2'-hydroxy-2'-methylbutyryl)cuauthemone (**1**)*. Colorless solid, m.p. 137-138 ^oC; IR: (KBr) ν_{max}: 3510, 2954, 2942, 2884, 1732, 1667, 1584, 1449, 1380, 1267, 1204, 1148, 1105, 1080, 1065, 1019, 966 cm^{-1}. UV spectrum λ_{max} ($CHCl_3$) 256 nm. HR-MS *m/z*: 411.2377 $[M+H]^+$ (calc. for $C_{22}H_{35}O_7$: 411.2383), 393.2272$[M-OH]^+$ (calc. for $C_{22}H_{33}O_6$: 393.2277), 217.1587 (calc. for $C_{15}H_{21}O$: 217.1593). ^1H NMR: see Table 1. ^{13}C NMR: see Table 1.

*4-O-Acetylcuauthemone-3-O-angelate (**2**)*. White gummy substance; IR (NaCl) ν_{max} (cm^{-1}) 2931, 1456, 1673, 1732, 1367, 1244, 1162, UV λ_{max} (in $CHCl_3$) 250 nm; ^1H NMR: see [8]; ^{13}C NMR: see [8].

*4-O-Acetylcuauthemone 3-O-(2'-hydroxy-2'-methyl-3'-acetoxybutyrate) (**3**)*. Brown gummy substance; IR (KBr) ν_{max} (cm^{-1}) 3467, 2936, 14571671, 1738, 1371, 1245.99, 1144.90, UV λ_{max} ($CHCl_3$) 250 nm; ^1H NMR: see [9]; ^{13}C NMR: see [15].

*3',4',5,7-Tetrahydroxy-3,6-dimethoxyflavone (**4**)*. Yellow solid, m.p. 201-203 ^oC; IR (KBr) ν_{max}: 3403, 3152, 2935, 2369, 1654, 1600, 1555, 1466, 1220, 1216, 1172, 990 cm^{-1}; UV λ_{max} (MeOH) 352, 256, 214 nm; ^1H NMR: see [10]; ^{13}C NMR: see [10].

*3',5,6-Trihydroxy-3,4',7-trimethoxyflavone (**5**)*. Yellow solid with melting point of 207-209 ^oC; IR**:** (KBr) ν_{max} 3493, 3323, 2942, 1677, 1603, 1577, 1514, 1485, 1458, 1355, 1275, 1252, 1213, 1166, 1136, 1091, 1061, 1032, 988, 819 cm^{-1}; UV λ_{max} (MeOH) 349, 280, 214nm; ^1H NMR: see [12] ; ^{13}C NMR: see [12].

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