Article Type: Full Paper

Chemical composition and antimicrobial activity of *Ageratina deltoidea*

Amira Arciniegas,[a] Ana-L. Pérez-Castorena,*[a] Mariana Meléndez-Aguirre,[a] José Guillermo Ávila,[b] Ana María García-Bores,[b] José Luis Villaseñor,[c] and Alfonso Romo de Vivar[a]

[a]Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, Ciudad de México, México.

[b]Laboratorio de Fitoquímica, Unidad de Biología, Tecnología y Prototipos (UBIPRO), Universidad Nacional Autónoma de México, FES Iztacala, Tlanepantla 54090, México.

[c]Instituto de Biología, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, Ciudad de México, México.

e-mail: alperezc@unam.mx

The chemical study of *Ageratina deltoidea* afforded grandiflorenic acid (**1**), kaurenoic acid (**2**) and eight benzylbenzoates (**3** – **10**), two of them, 3',5'-dimethoxybenzyl 2,3,6-trimethoxybenzoate (**5**) and 4'-O-β-D-glucopyranosyloxy-3'-hydroxybenzyl 2,6-dimethoxybenzoate (**9**), described for the first time. In addition, the new sesquiterpene lactone deltoidin C (**13**), together with the known **11**, and **12**, the phenolic compounds: ayanin, 2,6-dimethoxybenzoic acid, methyl 3,4-dihydroxycinnamate, chlorogenic acid, and 3,5-dicaffeoylquinic acid were also isolated. The structures of these compounds were determined by spectroscopic methods and chemical reactions. The antibacterial and antifungal activities of compounds **1** – **12** were evaluated on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Deltoidin A (**11**) was the most active antibacterial agent (MIC 16.0 μg ml^{-1}) against *E.coli*, and the *ent*-kaurenoid derivatives (**1** – **2**) showed activity (MIC 31.0 μg ml^{-1}) against *S. aureus*.

Keywords: Sesquiterpene lactones; Benzylbenzoate derivatives; Antibacterial activity.

Introduction

The genus *Ageratina* (Asteraceae, Eupatoriae) comprises 196 perennial species segregated from the genus *Eupatorium*, located in tropical and subtropical regions from southern United States to South America.[1] This genus is characterized, mainly by the presence of terpenoids,[2][3] flavonoids,[4] and p-hydroxyacetophenone and thymol derivatives.[5] *Ageratina* species are used in Mexico to cure diarrhea, stomach pain and kidney problems.[6] In previous studies of *A. deltoidea*, bornyl p-coumarate was reported in roots,[7] methoxylated flavonoids were isolated from aerial parts,[4] and sesquiterpene lactones, diterpenes, and triterpenes were obtained from

leaves.[8] The aim of this paper was to contribute to the chemistry of the genus studying the aerial parts of *Ageratina deltoidea* (Jacquin) (former *Eupatorium deltoideum* Jacquin), and to seek for antibacterial agents. The antimicrobial activity of isolated compounds was evaluated against *Staphylococcus aureus, Escherichia coli* and *Candida albicans.*

Results and Discussion

The hexane, acetone, and MeOH extracts of aerial parts of *A. deltoidea* afforded two new benzylbenzoate derivatives (**5** and **9**), and a new sesquiterpene lactone (**13**) (*Fig. 1*). In addition, the known compounds: grandiflorenic acid (**1**), kaurenoic acid (**2**),[9] benzyl 2-hydroxy-6-methoxybenzoate (**3**),[10] 3'-methoxybenzyl 2,3,6-trimethoxybenzoate (**4**),[11] benzyl 2,5-dimethoxybenzoate (**6**),[12] benzyl 2,6-dimethoxybenzoate (**7**),[10] benzyl 2,3,6-trimethoxybenzoate (**8**),[13] deltoidin A (**11**),[8] 8β-angeloyloxyelemacronquistianthus acid (**12**),[14] verimol K (**10**),[15] ayanin,[16] 2,6-dimethoxybenzoic acid,[17] methyl 3,4-dihydroxycinnamate,[18] 3,5-dicaffeoylquinic acid, and chlorogenic acid[19] were also obtained. The structures of isolated compounds were determined by spectroscopic techniques and chemical reactions, and those of the known compounds by comparison of their physical and spectroscopic features with those reported in the literature. ^{13}C-NMR of compound **12** is reported since it was not available in the literature.

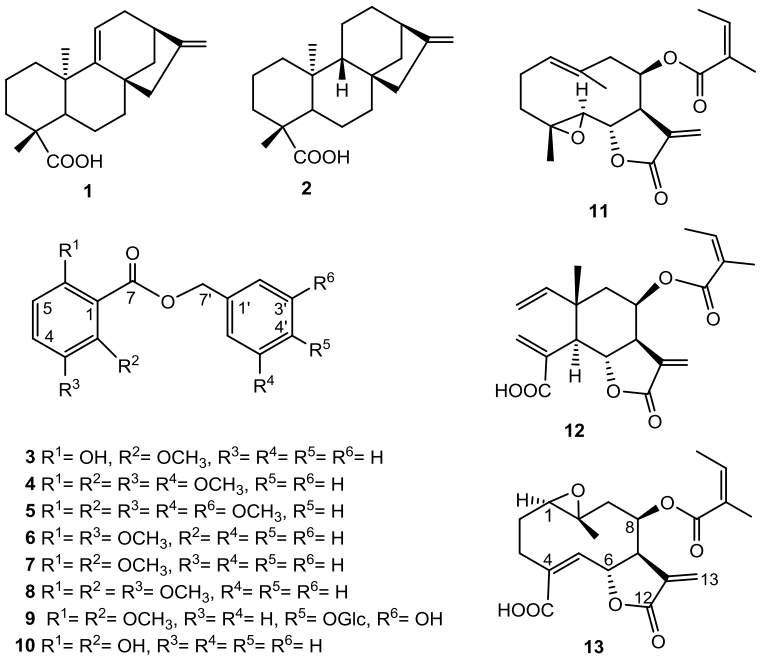

Figure 1. Structures of compounds **1** – **13**.

Compound **5** showed in the IR spectrum evidence of aromatic ester functionality (1734, 1597 and 1490 cm^{-1}). The molecular formula $C_{19}H_{22}O_7$ was deduced by HR-DART-MS and ^{13}C-NMR spectroscopy. In the DART-MS spectrum the base peak at *m/z* 195 indicated the presence of a trimethoxy benzoyl cation. The ^{1}H-NMR spectrum showed the resonances of a tetrasubstituted aromatic ring with two *ortho* protons at δ(H) 6.80 (*d*, *J* = 9.2 Hz) and δ(H) 6.59 (*d*, *J* = 9.2 Hz). These signals were assigned to H-C(4) and H-C(5), respectively, by means of the HMBC correlations of H-C(4) with C(2) and C(6) and of H-C(5) with C(1), C(3) and C(7), suggesting a 2,3,6-trimethoxybenzoate, as described for compounds **4**[11] and **8**.[13] A second aromatic ring was evident by the resonances of three protons with *meta* coupling at δ(H) 6.40 (*t*, *J* = 2.4 Hz) for H-C(4') and δ(H) 6.60 (2H, *t*, *J* = 2.4 Hz) for H-C(2') and H-C(6'). Correlation of these last

two protons with C(7') (δ(C) 66.9), and of H_2-C(7') (δ(H) 5.33, *s*) with the benzoyl carbonyl (δ(C) 165.8) in the HMBC spectrum, established the 3',5'-dimethoxybezyloxy moiety. In agreement with the above, the ^{13}C-NMR spectrum displayed 19 signals, including those of a carboxyl group, twelve aromatic carbons, a methylene and five methoxy groups, thus, compound **5** is 3',5'-dimethoxybenzyl 2,3,6-trimethoxybenzoate.

Compound **9** had the molecular formula $C_{22}H_{26}O_{11}$ determined by HR-FAB-MS and ^{13}C-NMR spectroscopy. The NMR data of compound **9** were similar to those of compound **5,** being evident the resonances of a methoxy benzoic acid benzyl ester and of a monosaccharide moiety. The 2,6-dimethoxybenzoate was deduced from the presence, in the ^{1}H-NMR spectrum, of resonances at δ(H) 7.35 (*t*, *J* = 8.4 Hz) and 6.70 (2H, *d*, *J* = 8.4 Hz), assigned to H-C(4) and to H-C(3) and H-C(5), respectively, by the HMBC correlations of H-C(4) with C(2) and C(6), and of H-C(3) and H-C(5) with C(1) and C(7). The benzyl group with three aromatic protons was defined by the resonances at δ(H) 6.86 (*d*, *J* = 2.0 Hz), 6.76 (*dd*, *J* = 8.4, 2.0 Hz), and 7.09 (*d*, *J* = 8.4 Hz). The first two were assigned to H-C(2') and H-C(6'), respectively, by the long range interactions with C(7') and C(4'), and the third one to H-C(5') by the cross peaks, observed in the HMBC experiment, between this proton and C(1') and C(3'). On acid hydrolysis, compound **9** afforded D-glucose ($[\alpha]^{25}_D$ = + 51.4, *c* 0.18, H_2O), identified by GC of its silylated derivative. Additionally, the H-C(1'') coupling constant (δ(H) 4.66, *d*, *J* = 8.0 Hz) allowed to identify the sugar moiety as β-D glucopyranose. Its attachment to C(4') was established by the correlation observed in the HMBC experiment between H-C(1'') and C(4') and by the NOESY interaction between H-C(1'') and H-C(5'). Finally, the presence of hydroxyl group at C(3') was deduced by the cross peak between H-C(2') and the hydroxyl group hydrogen at δ(H) 8.61 observed in the NOESY spectrum, and by the molecular formula ($C_{22}H_{26}O_{11}$) determined in the HR-FABMS, therefore, compound **9** was identified as 4'-O-β-Dglucopyranosyloxy-3'-hydroxybenzyl 2,6-dimethoxybenzoate.

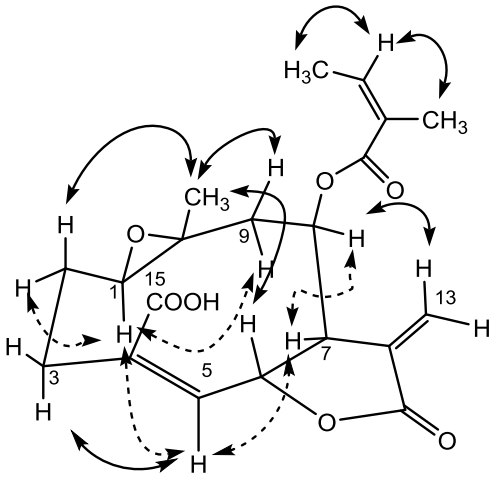

Figure 2. NOESY correlations of compound **13**.

Compound **13** showed the molecular formula $C_{20}H_{24}O_7$ according to its HR-DART-MS and ^{13}C-NMR spectrum. The IR spectrum presented absorption bands at 1768, 1712, 1685, and 1640 cm^{-1} of conjugated γ-lactone, unsaturated acid, and ester groups. The NMR spectra showed the α-methylene-12,6-γ-lactone resonances at δ(H) 6.35 (*d*, *J* = 3.6 Hz, H-13a), 5.65 (*d*, *J* = 2.8 Hz, H-13b), 2.93 (*m*, H-7), and 6.06 (*dd*, *J* = 10.4, 8.8 Hz, H-6); and δ(C) 120.0 (*t*, C(13)), 168.7 (*s*, C(12)), 135.1 (*s*, C(11)), 53.5 (*d*, C(7)), and 73.1 (*d*, C(6)). A typical angeloyloxy chain was deduced from the resonance of a vinylic proton at δ(H) 6.15 (*qq*, *J* = 7.2, 1.6 Hz, H-C(3')) coupled to those of two methyl

groups at δ(H) 1.99 (*dq*, *J* = 7.2, 1.6 Hz, H_3-C(4')) and 1.84 (*quint*, *J* = 1.6 Hz, H_3-C(5')). The HMBC correlations of H-C(8) (δ(H) 5.78, br. *d* *J* = 6.0 Hz) and C(1') (δ(C) 166.0) permitted the location of this ester at C(8). A 1,10 epoxide function (δ(H) 2.76, *dd*, *J* = 11.6, 2.8 Hz, H-C(1); δ(C) 66.3, *d*, C(1) and 59.7, *s*, C(10)) was deduced from the cross peaks observed in the COSY and HMBC experiments. The relative configuration was proposed by the NOESY correlations (Fig. 2) of H-C(1) with Hα-C(2), Hα-C(9) and H-C(5); those of H-C(5) with H-C(7) and Hα-C(3); and of H_3-C(14) with Hβ-C(2), Hβ-C(9) and H-C(6), indicating a *trans*-1,10 epoxide function. In the same spectrum, the interaction of H-C(7) with H-C(8), and based on the biogenetic considerations regarding the α-orientation of H-C(7),[20] defined the β-orientation of the angeloyloxy moiety, and the correlations between H-C(3') and the methyl groups H_3-C(4') and H_3-C(5') stablished its Z configuration. In addition, the large coupling constant between H-C(6) and H-C(7) (8.8 Hz) suggested the β-orientation of H-C(6) and disclosed the *trans*-fused lactone ring of the new sesquiterpene lactone deltoidin C (**13**). The data of the sesquiterpene lactone moiety were in agreement with those reported for 1β,10α-epoxycronquistic acid,[21] which has a tigloyloxy group instead of an angeloyloxy residue.

Table 1. Antimicrobial activity of compounds **1** – **12**.[a] Minimal inhibitory concentrations (MIC, μg ml^{-1})

Compound	*S. aureus*	*E. coli*	*C. albicams*
1 – **2**[b]	31.0	NA	NA
11	NA	16.0	NA
12	125.0	125.0	NA
Chlorampenicol	12.5	12.5	NT
Nystatin	NT	NT	15.0

[a]Compounds **3**-**10** were not active. [b]Mixture 75:25 by HPLC of compounds **1** – **2** . NA: no activity. NT not tested

The antimicrobial activity of compounds **1** – **12** was evaluated against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* by the disk-diffusion method,[22] using chloramphenicol (MIC 12.5 μg ml^{-1}) and nystatin (MIC 15.0 μg ml^{-1}) as reference compounds for bacteria and fungus, respectively (Table 1). Results showed that benzylbenzoates (**3** – **10**) were not active in tested microorganisms, while the *ent*-kaurene derivatives mixture **1** – **2** (75% of **1** and 25% of **2** by HPLC) and sesquiterpene lactones exhibited substantial activities. The mixture of compounds **1** – **2** was active (MIC 31.0 μg ml^{-1}) against Gram-positive but inactive against Gram-negative bacteria, as expected from the published data for this type of diterpenoids.[23][24] This activity could be related with the capacity of insertion of these metabolites in the lipophilic region of the microorganism and the activity of the carboxylic acid as hydrogen donor group.[24] Among the sesquiterpene lactones, deltoidin A (**11**) showed the highest and selective activity against Gram-negative bacteria, inhibiting exclusively the growth of *E. coli* at a MIC of 16.0 μg ml^{-1}, while lactone **12** exhibited a weaker and non-selective effect against Gram-positive and Gram-negative bacteria (MIC of 125.0 μg ml^{-1} in both). It has been mentioned that, in sesquiterpene lactones the presence of an α-methylene-γ-lactone and of the 4,5 epoxide function, as in compound **11**, might be some of the structural requirements for antibacterial activity in sesquiterpene lactones,[25][26] however, the decrease of the activity in compound **12**, pointed out that probably the presence of the ten carbon macrocycle could have some influence. *Candida albicans* was not sensitive to any of the tested compounds. The activity of lactone **13,** obtained in small quantity, was not determined. Since the antimicrobial activity of ayanin,[27] 2,6-dimethoxybenzoic acid,[28] methyl 3,4-dihydroxycinnamate,[29] 3,5-dicaffeoylquinic acid,[30] and chlorogenic acid[31] has been extensively studied, they were not tested..

Conclusions

The isolation of benzyl benzoates, sesquiterpene lactones and kaurenic and quinic acids derivatives is in agreement with the chemistry of the genus. With exception of benzyl benzoate derivatives which were not active, the presence of *ent*-kaurene derivatives, sesquiterpene lactones, and quinic acid derivatives may be related to the curative effects attributed to some *Ageratina* species in popular medicine.

Experimental Section

General

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 343 polarimeter. UV and IR spectra were recorded on a Shimadzu UV 160U and a Bruker Tensor 27 spectrometer, respectively. 1D and 2D NMR spectra were obtained on a Bruker Avance III 400 MHz or a Varian-Unity Inova 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. For DART-MS a JEOL AccuTOF JMS-T100LC DART was used. FAB-MS were obtained on a JEOL MStation JMS-700 mass spectrometer. HPLC was carried out on an Agilent 1200 using a Luna A18(2) 100 × 2.0 mm, 3 μm column, eluted with a 0.2 ml min^{-1} gradient system of acetonitrile/H_2O 60:40 through 100:0 in 20 min. GC analysis was performed on an Agilent 6890 GC system with AT5 column (30 m × 0.25 mm, 0.1 μm film thickness) using a temperature gradient starting at 100 °C, which was raised to a final temperature of 240 °C in 10 min. Vacuum column chromatography (VCC) was carried out under vacuum on silica gel G 60 (Merck, Darmstadt, Germany). Flash column chromatography (FCC) was performed on silica gel 230-400 mesh (Macherey-Nagel, Germany). Analytical TLC was carried out on Si gel 60 GF_{254} or RP-18W/UV_{254} (10 – 40) μm, Macherey-Nagel, Germany) and preparative TLC on Si gel GF_{254} layer thickness 2.0 mm or RP-18W/UV_{254} layer thickness 1.0 mm, using 10 × 20 cm plates.

Plant Material

Ageratina deltoidea (Jacq.) R. M. King & H. Rob. was collected by the road to Españita, 2 km from the Calpulalpan-Apizaco road, Tlaxcala State, México, in October 2014, and authenticated by Dr. José Luis Villaseñor. A voucher specimen (MEXU 1398390) was deposited at the Herbario del Instituto de Biología, UNAM, México.

Extraction and Isolation

Dried and ground aerial parts (890 g) were extracted successively with hexane, acetone, and MeOH at room temperature. The hexane extract (23 g) was fractionated by VCC (hexane/EtOAc gradient system) to obtain fractions A-F. Fractions A afforded 390 mg of a mixture of grandiflorenic acid (**1**, 75%) and kaurenoic acid (**2**, 25%). This mixture (50 mg) by preparative HPLC (gradient system CH_3CN/H_2O 60:40 through CH_3CN) produced **1** (15 mg, amorphous powder, $[\alpha]^{25}_D = +35$, $c = 0.15$, EtOH) and **2** (5 mg, amorphous powder $[\alpha]^{25}_D = -95$, $c = 0.13$, EtOH). Fractions B by FCC (hexane/EtOAc 19:1) afforded compound **3** (30 mg, colorless oil). Fraction C by successive chromatographies yielded compounds **4** (45 mg, colorless oil) and **5** (6 mg, colorless oil). Fraction D was purified by FCC (hexane/EtOAc 4:1) to obtain compound **5** (5 mg) and mixture D-1. Mixture D-1 gave compound **11** (10 mg, colorless needles, M.p. 158 – 160 °C, $[\alpha]^{25}_D = -135$, $c = 0.20$, $CHCl_3$). Fraction E, by repeated chromatographies yielded compound **11** (650 mg) and ayanin (35 mg, yellow needles, M.p. 172 – 174 °C). Fraction F, by successive FCCs and a preparative TLC afforded compound **12** (25 mg, colorless needles, M.p. 132 – 135 °C, $[\alpha]^{25}_D = +18$, $c = 0.10$, $CHCl_3$). The acetone extract (35 g) was purified by VCC (hexane/acetone gradient system) to obtain

fractions G-K. Fraction G yielded a mixture of compounds **1** and **2** (20 mg), compound **3** (15 mg) and a mixture of β-sitosterol and stigmasterol (60 mg). Fraction H after several chromatographies afforded compounds **6** (5 mg, colorless oil), and **7** (7 mg, colorless oil). Fraction I gave compounds **11** (32 mg), **12** (10 mg), and **8** (7 mg, colorless oil). Fraction J by successive chromatographic purifications yielded mixtures J-1, J-2, J-3, these mixtures were treated with charcoal/ acetone to afford compounds **12** (30 mg) and **13** (6 mg). Fraction K by VCC (EtOAc/MeOH gradient system) yielded compound **9** (1.1 g). The MeOH extract (120 g) was fractionated by VCC with an EtOAc/MeOH gradient system to obtain fractions L-O. Fraction L was purified by several chromatographies to give compound **10** (2.5 mg, colorless oil), 2,6-dimethoxybenzoic acid (29 mg, white amorphous powder), and methyl 3,4-dihydroxycinnamate (20 mg, white amorphous powder). Fraction M by VCC (EtOAc/MeOH gradient system) produced compound **9** (1.8 g) and 3,5-dicaffeoylquinic acid (180 mg, white amorphous powder, $[\alpha]^{25}_D = -178$, $c = 0.10$, MeOH). Fraction N was purified by a Sephadex LH-20 column (MeOH) to yield compound **9** (850 mg) and 3,5-dicaffeoylquinic acid (180 mg). Fraction O was purified by a Sephadex LH-20 column (MeOH/H_2O 1:1) to afford chlorogenic acid (750 mg, white amorphous powder, $[\alpha]^{25}_D = -36$, $c = 0.10$, MeOH) .

3',5'-Dimethoxybenzyl 2,3,6-trimethoxybenzoate; **5**). Colorless oil. IR ($CHCl_3$): 1734, 1597, 1490. ^{1}H-NMR (400 MHz, $CDCl_3$): 6.80 (*d*, ^3J(H,H) = 9.2, H-C(4)); 6.60 (*t*, ^4J(H,H) = 2.4, H-C(2'), H-C(6')); 6.59 (*d*, ^3J(H,H) = 9.2, H-C(5)); 6.40 (*t*, ^4J(H,H) = 2.4, H-C(4')); 5.33 (*s*, H_2-C(7')); 3.821 (*s*, OMe-C(3)); 3.819 (*s*, OMe-C(2)); 3.79 (*s*, OMe-C(3'), OMe-C(5')); 3.78 (*s*, OMe-C(6)). ^{13}C-NMR (100 MHz, $CDCl_3$) 165.8 (*s*, C(7)); 160.8 (*s*, C(3'), C(5')); 150.7 (*s*, C(6)); 147.2 (*s*, C(2)); 146.9 (*s*, C(3)); 138.2 (*s*, C(1')); 119.2 (*s*, C(1)); 114.4 (*d*, C(4)); 106.3 (*d*, C(5)); 105.8 (*d*, C(2'), C(6')); 100.1 (*d*, C(4')); 66.9 (*t*, C(7')); 61.5 (*q*, OMe-C(3)); 56.6 (*q*, OMe-C(2)); 56.3 (*q*, OMe-C(6)); 55.3 (*q*, OMe-C(3'), OMe-C(5')). DART-MS: 363 $[M+H]^+$ (50), 195 $[C_{10}H_{11}O_4]^+$ (100). HR-DART-MS: 363.14440 ($[M + H]^+$, $C_{19}H_{23}O_7^+$; calc. 363.14438).

4'-O-β-D-Glucopyranosyloxy-3'-hydroxybenzyl 2,6-dimethoxybenzoate; **9**). White amorphous powder. $[\alpha]^{25}_D$ = - 40.5 (c = 0.2, MeOH). IR (KBr): 3323, 1724. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.61 (br. *s*, HO-C(3')); 7.35 (*t*, ^3J(H;H) = 8.4, H-C(4)); 7.09 (*d*, ^3J(H;H) = 8.4, H-C(5')), 6.86 (d, ^4J(H;H) = 2.0, H-C(2')); 6.76 (*dd*, ^3J(H;H) = 8.4, ^4J(H;H) = 2.0, H-C(6')); 6.70 (*d*, ^3J(H;H) = 8.4, H-C(3), H-C(5)); 5.46 (br. *s*, OH); 5.13 (*s*, H_2-C(7')); 5.05 (br. *d*, ^3J(H;H) = 3.6 Hz, OH); 5.01 (*d*, ^3J(H;H) = 4.8, OH); 4.46 (*t*, ^3J(H;H) = 6.0, HO-C(6'')); 3.75 (*s*, OMe-C(2), OMe-C(6)); Glucose: 4.66 (*d*, ^3J(H;H) = 8.0, H-C(1'')); 3.70 (*ddd*, ^2J(H;H) = 12.0, ^3J(H;H) = 6.0, ^3J(H;H) = 2.0, H-C(6"a)); 3.45 (*ddd*, ^2J(H;H) = 12.0, ^3J(H;H) = 6.0, ^3J(H;H) = 6.0 Hz, H-C(6"b)); 3.28 (*m*, H-C(2'')), 3.26 (*m*, H-C(3'')), 3.24 (*m*, H-C(5'')), 3.16 (*m*, H-C4'')). ^{13}C-NMR (100 MHz, (D_6)DMSO): 165.4 (*s*, C(7)); 156.6 (*s*, C(2), C(6)); 146.7 (*s*, C(3')); 145.0 (*s*, C-(4')); 131.2 (*d*, C(4)), 130.6 (*s*, C(1')), 118.9 (*d*, C(6')), 116.5 (*d*, C(5')), 115.6 (*d*, C(2')), 112.5 (*d*, C(1)), 104.2 (*d*, C(3), C(5)); 65.8 (*t*, C(7')); 55.8 (*q*, OMe-C(2), OMe-C(6)); Glucose: 102.3 (*s*, C(1'')); 77.2 (*d*, C(5'')); 75.9 (*d*, C(3'')); 73.3 (*d*, C(2'')); 69.8 (*d*, C(4'')); 60.7 (*t*, C(6'')). FAB-MS: 447 $[M+H]^+$, 165, 183; HR-FAB-MS: 467.1545 ($[M+H]^+$, $C_{22}H_{27}O_{11}^+$, calc. 467.1543).

Deltoidin C; **13**). Colorless oil. $[\alpha]^{25}_D = +13$ (c = 0.1, $CHCl_3$). IR ($CHCl_3$): 2932 (large), 1768, 1712, 1685, 1640. ^{1}H-NMR (400 MHz, $CDCl_3$): 6.35 (*d*, ^4J(H;H) = 3.6, H-C(13a)); 6.18 (*d*, ^3J(H;H) = 10.4, H-C(5)); 6.15 (*qq*, ^3J(H;H) = 7.2, ^4J(H;H) = 1.6, H-C(3')); 6.06 (*dd*, ^3J(H;H) = 10.4, ^3J(H;H) = 8.8, H-C(6)), 5,78 (br. *d*, ^3J(H;H) = 6.0, H-C(8)); 5.65 (d, ^4J(H;H) = 2.8, H-C(13b)); 2.96 (*m*, Hβ-C(3)); 2.93 (*m*, H_2-C(7)); 2.86 (*dd*, ^2J(H;H) = 15.6, ^3J(H;H) = 6.0, Hβ-C(9)); 2.76 (*dd*, ^3J(H;H) = 11.6, ^3J(H;H) = 2.8, H-C(1)); 2.42 (*ddd*, ^2J(H;H) = 12.8, ^3J(H;H) = 12.8, ^3J(H;H) = 5.6, Hα-C(3)), 2.24 (br. *d*, ^2J(H;H) = 14.0, Hα-C(2)); 1.99 (*dq*, ^3J(H;H) = 7.2, ^5J(H;H) = 1.6, H_3-C(4')); 1.84 (*quint*, ^4J(H;H) = ^5J(H;H) = 1.6, H_3-C(5')); 1.63 (*dddd*, ^2J(H;H) = 14.0, ^3J(H;H) = 12.8, ^3J(H;H) = 11.6, ^3J(H;H) = 4.8, Hβ-C(2)); 1.32 (*dd* ^2J(H;H) = 15.6, ^3J(H;H) = 3.0, Hα-C(9)); 1.12 (*s*, H_3-C(14)). ^{13}C-NMR (100 MHz, $CDCl_3$): 169.1 (*s*, C(15)); 168.7 (*s*, C(12)); 166.0 (*s*, C(1')); 142.4 (*d*, C(5)); 141.3 (*d*, C(3')); 136.2 (*s*, C(4)); 135.1 (*s*, C(11)); 126.3 (*s*, C(2')); 122.0 (*t*, C(13)); 73.1 (*d*, C(6)); 66.3 (*d*, C(1)); 66.1 (*d*, C(8)); 59.7 (*s*, C(10)); 53.5 (*d*, C(7)); 42.7 (*t*, C(9)); 31.4 (*t*, C(3)); 25.7 (*t*, C(2)); 20.3 (*q*, C(5')); 18.6 (*q*, C(14)); 15.9 (*q*, C(4')). DART-MS *m/z* 394 $[M+H_2O]^+$ (50), 377 $[M+H]^+$ (100), 277 (45); HR-DART-MS: 377.16017 ($[M+H]^+$, $C_{20}H_{25}O_7^+$, calc. 377.16003).

8β-Angeloyloxyelemacronquistianthus acid; **12**). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.9 (*s*, C(15)); 169.3 (*s*, C(12)); 166.8 (*s*, C(1')); 145.8 (*d*, C(1)); 139.7 (*d*, C(3')); 135.0 (*s*, C(4)); 134.7 (*s*, C(11)); 130.0 (*t*, C(3)); 127.0 (*s*, C(2')); 120.1 (*t*, C(13)); 113.2 (*t*, C(2)); 75.7 (*d*, C(6)); 65.7 (*d*, C(8)); 52.0 (*d*, C(7)); 49.4 (*d*, C(5)); 42.6 (*t*, C(9)); 42.3 (*s*, C(10)); 20.6 (*q*, C(5')); 18.8 (*q*, C(14)); 15.9 (*q*, C(4')).

Hydrolysis of compound **9**

Compound **9** (100 mg) was refluxed for 1 h with MeOH (3 ml) and 2 N HCl (3 ml). The reaction mixture was evaporated and purified by VCC eluting with EtOAc-MeOH gradient system to obtain fractions A and B. Fraction A by FCC (hexane-EtOAc 3:2) yielded 2,6-dimethoxybenzoic acid (10 mg), and fraction B by FCC (EtOAc-MeOH 7:3) produced D-glucose (7 mg) ($[\alpha]^{25}_D$ = + 51.4, *c* = 0.18, H_2O) identified by GC of the silylated derivative.

Antibacterial Activity

The antibacterial activity was measured by the disk-diffusion method.[22] *Staphylococcus aureus* ATCC 12398, *Escherichia coli* ATCC 25922 were used. Microorganisms were grown overnight at 37 °C in 10 ml of Mueller-Hinton broth (Bioxon). The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 0.5 standard (1.0×10^8 CFU ml^{-1}).[32] Petri dishes containing Mueller Hinton agar (Bioxon) were inoculated with these microbial suspensions. Solutions of pure compounds were prepared. For polar compounds sterile distilled water and for non-polar compounds dimethyl sulfoxide were used. Disks of filter paper (Whatman no. 5) of 5 mm diameter were impregnated with 10 μl of each solution (final doses per disk: 400 μg) and placed on the agar surface. Disks impregnated with dimethyl sulfoxide or distilled water were used as negative controls. Disks with chloramphenicol were used as positive controls. Each experiment was repeated three times.

Antifungal Activity

Candida albicans ATCC 14065 was assayed by the method described for bacteria, using Petri dishes containing CzapekDox Agar (Bioxon), Nystatin (30 μg/disc) was used as positive control and dimethyl sulfoxide as negative control. Each experiment was repeated three times.[22]

Quantitative assays

For quantitative assays, seven doses of pure compound (0.0155, 0.031, 0.0625, 0.125, 0.250, 0.5, 1.0 mg ml^{-1}) dissolved in sterile dimethyl sulfoxide was added to rapidly mixing in Muller- Hinton or Czapek Dox agar for bacteria and yeast respectively (5 ml) and poured into 6 cm petri dishes. When the agar reached the room temperature, test bacteria or yeast were inoculated at the concentration of 10^5 CFU ml^{-1}. MIC values were taken as the lowest concentration that prevents visible bacterial or yeast growth after 24 h of incubation at 37 °C or 28 °C. Chloramphenicol and Nystatin were used as reference antibiotics and dimethyl sulfoxide as negative control. Each experiment was repeated three times, and results are expressed as mean ± standard deviation.[33]

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

Acknowledgements

We are indebted to Rubén Gaviño, Isabel Chaves, Héctor Ríos, Ángeles Peña, Elizabeth Huerta, Rocío Patiño, Beatriz Quiroz, Luis Velasco, Javier Pérez, Carmen García, Carmen Márquez, Eréndira García, and Lucero Ríos for technical assistance.

Author Contribution Statement

ARV, ALPC, and AA designed the experiments and prepared the manuscript. AA and MMA isolated and characterized the compounds. JGA and AMGB performed the biological experiments. JLV collected and identified the plant. All the authors approved the final manuscript, and declare no conflict of interest associated with this work.

References

[1] R. M. King, H. Robinson, 'Studied in the Eupatorieae (Compositae). XIX. New combinations in *Ageratina*', *Phytologia* **1970**, *19*, 208 - 229.

[2] N. Fang, S. Yu, T. J. Mabry, K. A. Abboud, S. H. Simonsen, 'Terpenoids from *Ageratina saltillensis*', *Phytochemistry* **1988**, *27*, 3187 - 3196.

[3] G. Tamayo-Castillo, J. Jakupovic, F. Bohlmann, V. Castro, R. M. King, '*Ent*-clerodane derivatives and other constituents from representatives of the subgenus *Ageratina*', *Phytochemistry* **1989**, *28*, 139 - 141.

[4] S.-L. Yang, R. A. King, M. F. Roberts, 'The flavonoids of *Ageratina deltoidea*', *Biochem. Syst. Ecol.* **1990**, *18*, 485 - 486.

[5] F. Bohlmann, S. Banerjee, C. Wolfrum, J. Jakupovic, R. M. King, H. Robinson, 'Sesquiterpene lactones, geranylnerol and tremetone derivatives from *Ageratina* species', *Phytochemistry* **1985**, *24*, 1319 - 1321.

[6] A. Aguilar, J. R. Camacho, S. Chin, P. Jácquez, M. E. López, 'Herbario Medicinal del Instituto Mexicano del Seguro Social. Información Etnobotánica', Instituto Mexicano del Seguro Social, México, 1994, pp. 47 - 48.

[7] V. M. Hernández, R. Miranda, M. Martínez, P. Joseph-Nathan, 'Presence of bornyl *p*-coumarate in the roots of *Eupatorium deltoideum*', *J. Nat. Prod.* **1986**, *49*, 1173 - 1174.

[8] L. Quijano, J. S. Calderón, F. Gómez G., J. T. Garduño, T. Ríos C., 'Deltoin A and B, two new germacrolides from *Eupatorium deltoideum*', *Phytochemistry* **1980**, *19*, 1975 - 1977.

[9] R. G. Enriquez, J. Barajas, B. Ortiz, A. J. Lough, W. F. Reynolds, M. Yu, I. Leon, A. Gnecco, 'Comparison of crystal and solution structures and ^{1}H and ^{13}C chemical shifts for grandiflorenic acid, kaurenoic acid, and monoginoic acid', *Can. J. Chem.* **1997**, *75*, 342 - 347.

[10] S. Z. Choi, S. U. Choi, S. Y. Bae, S. N. Pyo, K. R. Lee, 'Immunobiological activity of a new benzyl benzoate from the aerial parts of *Solidago virga-aurea* var. *gigantea*', *Arch. Pharm. Res.* **2005**, *28*, 49 - 54.

[11] X. A. Dominguez, H. Sanchez, E. del Río, G. Vazquez, M. Abdel Aal, 'Desoxypetiolaside and other constituents from *Fleischmania pycnocephala* and *Ageratina virburnoides*', *Rev. Latinoamer. Quím.* **1988**, *19*, 43 - 44

[12] M. Kodpinid, C. Sadavongvivad, C. Thebtaranonth, Y. Thebtaranonth, 'Benzyl benzoates from the root of *Uvaria purpurea*', *Phytochemistry* **1984**, *23*, 199 - 200.

[13] F. Bohlmann, J. Jakupovic, M. Lonitz, 'Über Inhaltsstoffe der *Eupatorium*-group', *Chem. Ber.* **1977**, *110*, 301 - 314.

[14] F. Bohlmann, C. Zdero, R. M. King, 'Neue germacranolide, heliangolide und elemanolide aus *Cronquistianthus chachapoyensis*', *Liebigs Ann. Chem.* **1984**, 240-249.

[15] L.-K. Sy, G. D. Brown, 'Novel phenylpropanoid lignans from *Illicium verum*', *J. Nat. Prod.* **1998**, *61*, 987 - 992.

[16] E. Malan, D. G. Roux, 'Flavonoids from *Distemonnanthus nbenthamianus* Baillon. Mathoxylated flavones and inter-relationship of benthamianin, a [2]benzopyrano[4,3-*b*][1]benzopyran', *J. Chem. Soc. Perkin Trans. 1* **1979**, 2696 - 2703.

[17] L. Di, K.-J. Wang, C.-C. Zhu, N. Li, 'Chemical constituents from rhizomes of *Curculigo capitulate*', *Bull. Korean Chem. Soc.* **2010**, *31*, 2999 - 3002.

[18] C.-N. Xia, H.-B. Li, F. Liu, W.-X. Hu, 'Synthesis of *trans*-caffeate analogues and their bioactivities against HIV-1 interase and cancer cell lines', *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6533 - 6557.

[19] M. Znati, H. B. Jannet, S. Cazaux, J. P. Souchard, F. H. Skhiri, J. Bouajila, 'Antioxidant, 5-lipoxygenase inhibitory and cytotoxic activities of compounds isolated from *Ferula lutea* flowers', *Molecules* **2014**, *19*, 16959 - 16975.

[20] J. Richards, J. Hendrickson, 'Biosynthesis of Terpenes, Steroids and Acetogenins' W. A. Benjamin Inc., New York, 1964, pp 225-237.

[21] M. Miski, D. A. Gage, T. J. Mabry, 'Terpenoids of *Piptothrix sinaloae*', *Phytochemistry* **1987**, 26, 2753 – 2757.

[22] J. C. Marín-Loaiza, J. G. Avila, M. Canales, T. Hernández, C. L. Céspedes, 'Antifungal and antibacterial activities of *Pittocaulin* spp.', *Pharm. Biol.* **2008**, *46*, 66 - 71.

[23] S. R. Gutiérrez, O. Fuentes, A. N. Téllez, R. Torrenegra, 'Principios activos antibacterianos de *Espeletia barclayana*', *Rev. Latinoamer. Quim.* **1998**, *26*, 71 - 74.

[24] A. Urzúa, M. C. Rezende, C. Mascayano, L. Vazquez, 'A structure-activity study of antibacterial diterpenoids', *Molecules* **2008**, 13, 882 -891.

[25] E. Forville de Andrade, D. Carpiné, J. L. A. Dagostin, A. Barison, A. L. Rüdiger, G. I. Bolzón de Muñiz, M. L. Masson, 'Identification and antimicrobial activity of the sesquiterpene lactone mixture extracted from *Smallanthus sonchifolius* dried leaves', *Eur. Food Res. Technol.* **2017**, DOI 10.1007/s00217-017-2918-y.

[26] E. Cartagena, S. Montanaro, A. Bardón, 'Improvement of the antibacterial activity of sesquiterpene lactones', *Rev. Latinoamer. Quim.* **2008**, *36*, 43 - 51.

[27] A. Urzua, J. Echeverria, J. Espinoza, M. Wilkens, 'Lipophilicity and antibacterial activity of resinous exudates of *Haplopappus litoralis*, *H. chrysantemifolius* and *H. scrobiculatus*', *BLACPMA* **2012**, *11*, 369 - 376.

[28] M. Friedman, P. R. Hanika, R. E. Mandrell, 'Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica*', *J. Food Prod.* **2003**, *66*, 1811 - 1821.

[29] J. Kyselka, D. Rabiej, M. Dragoun, F. Kreps, Z. Burčová, I. Němečková, J. Smolová, M. Bjelková, A. Szydłwska-Czerniak, Š. Schmidt, L. Šarman, 'Antioxidant and antimicrobial activity of linseed lignans and phenolic acids', *Eur. Food Res. Technol.* **2017**, *243*, 1633 - 1644.

[30] J. Han, Q.-Y. LV, S.-Y. Jin, T.-T. Zhang, S.-X. Jin, X.-Y. Li, H.-L. Yuan, 'Comparison of anti-bacterial activity of three types of di-*O*-caffeoylquinic acids in *Lonicera japónica* flowers base on microcalorimetry', *Chin. J. Nat. Med.* **2014**, *12*, 108 - 113.

[31] F. Kabir, S. Katayama, N. Tanji, S. Nakamura, 'Antimicrobial effects of chlorogenic acid and related compounds', *J. Korean Soc. Appl. Biol. Chem.* **2014**, *57*, 359 - 365.

[32] H. E. Lennette, A. Balows, J. W. Hausler, H. J. Shadomy, '*Manual de microbiología clínica*', Fourth ed. Médica Panamericana, Argentina, 1987 pp. 336-352, 358-382, 1192, 1200 - 1245.

[33] R. Serrano, T. Hernández, M. Canales, A. M. García-Bores, A. Romo de Vivar, C. L. Céspedes, J. G. Avila, 'Ent-labadane type diterpene with antifungal activity from *Gymnosperma glutinosum* (Spreng.) Less. (Asteraceae)', *BLACPMA* **2009**, *8*, 412 - 418.

Entry for the Table of Contents ((required for publication))