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# New constituents from *Mikania laevigata* Shultz Bip. ex Baker

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ABSTRACT

A new phenylpropanoid and two new diterpenes were isolated from the leaves of the plant *Mikania laevigata* Shultz Bip. ex Baker. The structures of these compounds were established by 1D- and 2D-nuclear magnetic resonance spectroscopic techniques and mass spectrometry data. Taraxerol, lupeol, coumarin, syringaldehyde, *trans*-melilotoside, *cis*-melilotoside, adenosine, patuletin 3-*O*-β-D-glucopyranoside, kaempferol 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-glucopyranoside, methyl 3,5-di-*O*-caffeoyl quinate, and 3,3′,5-trihydroxy-4′,6,7-trimethoxyflavone were isolated too. In addition, the compounds dihydrocoumarin, spathulenol, caryophyllene oxide, kaurenoic acid, beyerenoic acid, and lupeol acetate were identified by GC–MS.



The genus *Mikania* (tribe Eupatorieae, subtribe Miikaniinae) is widespread in Brazil, and some species of this genus, known as 'guaco', are employed in popular medicine to treat a variety of diseases.[1] Approximately 35% of the species of this genus only occur in Brazil, including *Mikania laevigata* Shultz Bip. ex Baker,[2] a vine known as 'guaco-cheiroso' or 'guaco-do-mato'. The latter species is widely distributed in the southeastern and southern regions of Brazil and is registered in the Brazilian Pharmacopoeia.[3] Because of their bronchodilating properties, its leaves are widely used in the treatment of asthma and bronchitis.[4] In spite of the important medicinal uses of *M. laevigata*, there are few literature studies on its chemistry, but some correlations between its biological activities and some of its chemical constituents have been reported.[5–9] Among the main biological activities evaluated to date, *M. laevigata* has been shown to display anti-allergic and anti-inflammatory activities,[5,6] anti-ulcerogenic activity,[7] and antimicrobial activity against oral pathogens.[9] In previous phytochemical investigations, coumarin, cinnamoylgrandifloric acid, *ent*-kaur-15-en-19-oic acid, stigmast-22-en-ol, and syringaldehyde have been isolated,[10,11] and *trans*-*O*-coumaric acid and sesquiterpenes such as β-caryophyllene, germacrene D, and bicyclogermacrene have also been identified.[5,12] *M. laevigata* is often confused with *Mikania glomerata* Sprengel,[13] and it has been sold as the latter, because they both have related foliar morphology. *M. glomerata* is commonly known as 'guaco' or 'guaco verdadeiro' and is registered in the Brazilian Pharmacopoeia, too.[14] There are more literature studies on its chemical and biological properties, compared with *M. laevigata*.[15] *M. glomerata* is used as herbal medicine and is among the species with the highest number of herbal medicines registered with ANVISA (Agência Nacional de Vigilância Sanitária), which is a Brazilian agency responsible for the regulation and supervision of herbal products.[16] This plant also belongs to the list of plants which have a simplified registration in this regulator organ.[17] But there may be doubts about the certification that these herbal products actually come from *M. glomerata* and not from *M. laevigata*, due to confusion in botanical identification. A recent study has shown that while *M. laevigata* contains high concentrations of coumarin, *M. glomerata* has undetectable concentrations of this substance.[13] Coumarin is used as a chemical marker in the procedures for validation and standardization of extracts,[3] and one of the main substances responsible for the bronchodilating activity of *M. laevigata*. Furthermore, the wide distribution of the latter plant species in the southeastern and southern regions of Brazil can contribute to greater popular use of this plant. However, no herbal medicines from *M. laevigata* have been registered with ANVISA yet, but this plant, along with *M. glomerata*, is found in RENISUS (National List of Medicinal Plants of Interest to SUS), which integrates the current National Program for Medicinal Plants and Herbal Medicines from the Ministry of Health.[18,19] One of the goals of this program is the use of medicinal plants in basic pharmaceutical care in Brazil.

The present study deals with the isolation, identification, and structural elucidation of some constituents of *M. laevigata*.

The phytochemical study of dried leaves of *M. laevigata* enabled the identification of 21 compounds. Dried leaves of *M. laevigata* (660 g) were quickly rinsed (30 s at room temperature) in dichloromethane, which furnished the leaf rinse extract (1.5 g). The same leaves were then triturated and percolated with ethanol-95%, which gave 115.0 g of crude ethanolic extract after removal of the solvent under reduced pressure. The crude ethanolic extract

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was suspended in $CH_3OH/H_2O$ (9:1), filtered, and partitioned into hexane fraction (15.0 g). After that, the obtained hydroalcoholic fraction was concentrated, and then water was added until a $CH_3OH/H_2O$ ratio of 1:1 was achieved, followed by partitioning in dichloromethane fraction (10.3 g). Again, the resulting hydroalcoholic fraction was concentrated, and then water was added until a $CH_3OH/H_2O$ ratio of 3:7 was obtained, followed by partitioning in ethyl acetate fraction (12.7 g).

The dichloromethane fraction (3.0 g) was chromatographed on Sephadex LH-20 using methanol as eluent, which yielded 25 fractions. Fractions 13 and 14 were purified by preparative TLC on silica-gel using dichloromethane/methanol 95:5 as eluent and detection with KOH-5% in ethanol and UV 254 and 366 nm, which led to the isolation and identification of *ortho*-[(5′-hydroxy)-*cis*-cinnamoyl]-*trans*-cinnamic acid (**1**) (8 mg) (Fig. 1).

The ethyl acetate fraction (8.0 g) was submitted to chromatography on Sephadex LH-20 using methanol as eluent, which afforded 110 fractions. Fraction 17 gave a precipitate, which was washed with water and yielded a 19-*nor*kaur-16-ene-18-oic acid, namely de 19-*nor*kaur-16-ene-18-oic acid 2β-[[3-*O*-(3-hydroxy-1-oxo-3-phenypropyl)-2-(3-methyl-1-oxobutyl-4-*O*-(α-rhamnopyranosyl)-β-glucopyranosyl]oxy]-13,15α-hydroxy (**2**) (17 mg). Fractions 22 and 23 furnished a white precipitate, which was washed with methanol and yielded kaurane-3β,16β,17-triol 3-*O*-β-glucopyranosyl (**3**) (10 mg).

The compounds taraxerol (**4**), lupeol (**5**), coumarin (**6**), beyerenoic acid (**7**), kaurenoic acid (**8**), caryophyllene oxide (**9**), spathulenol (**10**), dihydrocoumarin (**11**), and lupeol acetate (**12**) were identified by GC–MS. The compounds lupeol (**5**),[20] taraxerol (**4**),[21] coumarin (**6**),[10] syringaldehyde (**13**),[22] *trans*-melilotoside (**14**),[23] *cis*-melilotoside (**15**),[24] methyl 3,5-di-*O*-caffeoyl quinate (**16**),[25] 3,3′,5-trihydroxy-4′,6,7-trimethoxyflavone (**17**),[26] patuletin 3-*O*-β-glucopyranoside (**18**),[27] quercetin 3-*O*-β-glucopyranoside (**19**),[28] kaempferol 3-*O*-β-glucopyranoside (**20**),[27] and adenosine (**21**)[28] were isolated and identified by the comparison of their spectral data with those published previously.

The $^1$H NMR spectrum of compound **1** (Table 1) displayed signals for olefinic protons of C-2′ at $\delta$ 6.50; 9.5 Hz and C-3′ at $\delta$ 8.08; 9.5 Hz as doublets, which is typical of the coumarin structure. In addition, the $^1$H NMR spectrum showed signals for olefinic protons of C-2 at $\delta$ 6.47; 15.1 Hz and C-3 at $\delta$ 7.76; 15.1 Hz as doublets, indicating *trans* configuration of the double bond, characteristic of cinnamic acid, as well as eight aromatic protons. The $^{13}$C spectrum

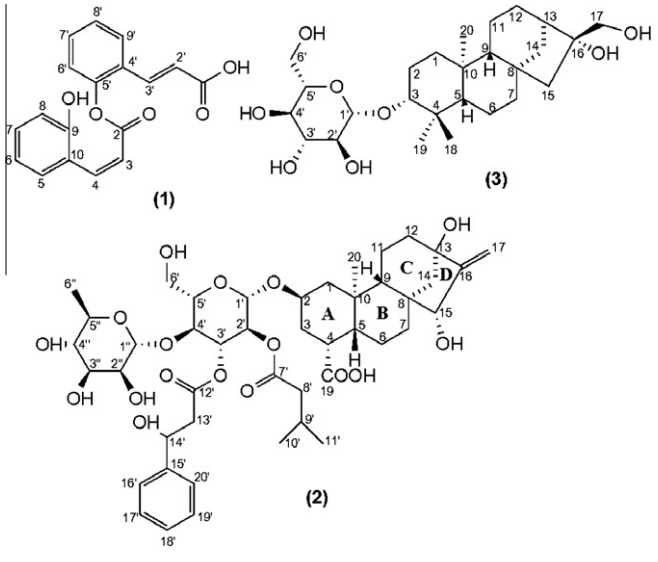


**Figure 1.** Structures **1**, **2** and **3**.

**Table 1**
$^1$H and $^{13}$C NMR data, and HMBC correlations for **1**

| Position | $\delta_H$ | $\delta_C$ | HMBC (H→C) |
|---|---|---|---|
| 1 | | 169.2 | |
| 2 | 6.49; *d*; 15.1 | 120.6 | |
| 3 | 7.74; *d*; 15.1 | 138.1 | C-9 |
| 4 | | 121.6 | |
| 5 | | 156.8 | |
| 6 | 6.91; *d*; 8.1 | 116.5[b] | C-8; C-4; C-5 |
| 7 | 7.18; *t*[a]; 7.5 | 131.1 | C-6; C-9; C-5 |
| 8 | 6.80; *t*[a]; 7.3 | 119.4 | C-6 |
| 9 | 7.52; *d*; 7.5 | 128.7 | C-7; C-3; C-5 |
| 1′ | | 160.2 | |
| 2′ | 6.50; *d*; 9.5 | 116.3[b] | C-4′; C-1′ |
| 3′ | 8.08; *d*; 9.5 | 144.5 | C-2′; C-4′; C-9′; C-5′; C-1′ |
| 4′ | | 119.0 | |
| 5′ | | 153.7 | |
| 6′ | 7.41; *d*; 8.3 | 116.5[b] | C-4′; C-5′, C-8′; C-7′ |
| 7′; | 7.63; *t*[a]; 7.9 | 132.2 | C-6′; C-8′; C-9′; C-5′ |
| 8′ | 7.36; *t*[a]; 7.5 | 124.8 | C-6′; C-9′ |
| 9′ | 7.73; *d*; 7.9 | 128.5 | C-7′; C-3′; C-5′ |

[a] Apparent triplet.
[b] Assignments may be interchanged.

exhibited 18 carbon signals corresponding to the molecular formula composed of a coumarin and a *trans*-*O*-coumaric acid. The assignments were greatly supported by comparison with the $^{13}$C NMR data reported previously for both coumarin[29] and *trans*-*O*-coumaric acid.[30] The molecular formula of this compound was assigned as $C_{18}H_{14}O_5$ on the basis of ESI-MS, which indicated the presence of these two moieties. The position of the connection between these two moieties was supported by HMBC correlations of C-1′ ($\delta$ 160.6) to both H-2′ ($\delta$ 6.50; *d*; 9.5 Hz) and H-3′ ($\delta$ 8.08; *d*; 9.5 Hz), which gave evidence that the ester group has adjacent olefinic protons with *cis* configuration. This is a phenolic compound that has not been reported to date. HRESIMS (negative mode) *m*/*z* $[M-H]^-$ 309.0785 (calcd for $C_{18}H_{13}O_5$, 309.0777).

The molecular formula of compound **2** was deduced as $C_{45}H_{64}O_{17}$ from HRESIMS (negative mode) *m*/*z* $[M-H]^-$ 875.4059 (calcd for $C_{45}H_{63}O_{17}$, 875.4059), in agreement with the proposed structure. Spectral data are presented in Table 2. The carbons of *nor*kaurene aglycone showed typical signals of the C-17 exocyclic methylene ($\delta$ 108.8), the C-19 carboxylic acid ($\delta$ 178.7), as well as the characteristic $^{13}$C chemical shift of the hydroxylated carbons C-15 ($\delta$ 82.4) and C-13 ($\delta$ 79.7).[31] The glycosidic moiety was identified by TOCSY, HMBC, and HMQC experiments. The deshielding of C-2 ($\delta$ 74.2) and the correlation between this carbon and an anomeric proton ($\delta$ 4.78, *m*) in the HMBC spectrum indicated the position of this glycosylation. Analyses of the TOCSY 1D and $^{13}$C spectra allowed identification of a glucose and a rhamnose moiety (Table 2). Sugar protons H-2′ ($\delta$ 4.75; *m*) and H-3′ ($\delta$ 5.22; *m*) were shifted because of the 3-methyl-1-oxobutyl and 3,3-phenylhydroxypropanoyl substituents at C-2′ and C-3′, respectively. The position of attachment of the rhamnosyl moiety (α1-4) was established by comparison of the $^{13}$C chemical shift values with the literature data and was confirmed by the correlation between H-1″ ($\delta$ 4.8; *m*) and C-4′ ($\delta$ 76.0) observed in the HMBC spectrum.[31]

The stereochemistry of ring A substituents was determined by a detailed analysis of the $^1$H NMR spectrum. The *axial* proton on C-1 was detected as a broadened triplet ($\delta$ 0.76, *t*, 11.8 Hz), indicating coupling between H-$1_{ax}$–H-$1_{eq}$ and H-$1_{ax}$–H-$2_{ax}$, whose coupling constant and multiplicity establish that the moiety *O*-glycosyl in C-2 must be *equatorial*. The carboxylic acid must be *axial* because of the coupling constant and multiplicity of H-$3_{axial}$ ($\delta$ 1.2; *dt*; 5.2 Hz, 11.8 Hz), which evidence coupling between H-$3_{ax}$–H-$4_{eq}$ ($J$ = 5.2 Hz), H-$3_{ax}$–H-$3_{eq}$ ($J$ = 11.8 Hz), and H-$3_{axial}$–H-$2_{ax}$ ($J$ = 11.8 Hz).

**Table 2**
$^{1}H$ and $^{13}C$ NMR data, and HMBC correlations for **2** and **3**

| Position | Compound **2** | | | | Compound **3** | | |
|---|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | HMBC (H→C) | TOCSY | $\delta_H$ | $\delta_C$ | HMBC (H→C) |
| 1a | 0.76; t[a], 11.8 | 48.5 | C-3; C-10; C-5; C-9; C-2 | | 0.72, m | 39.3 | C-3 |
| 1b | 2.3, m | | | | 1.7, m | | |
| 2a | 4.2, m | 74.2 | C-1′ | | 1.7, m | 23.9 | |
| 2b | — | | | | 2.0, m | | |
| 3a | 1.2; td; 5.2; 11.8 | 35.6 | C-4; C-1; C-5; C-19; C-2 | | 3.50, dd, 4.2; 11.6 | 85.2 | C-4; C-18; C-19; C-1′ |
| 3b | 2.39; d; 11.8 | | | | — | | |
| 4 | 2.6; m | 44.6 | C-3; C-10; C-5; C-19; C-2 | | — | 37.9 | |
| 5 | 1.4; m | 50.3 | | | 0.63, d. 11.5 | 55.8 | C-3; C-4; C-6; C-7; C-18; C-19 |
| 6a | 1.6; m | 26.5 | | | 1.3, m | 20.7 | C-7; C-18 |
| 6b | 2.6; m | | | | 1.4, m | | |
| 7a | 1.6; m | 35.9 | | | 1.6, m | 42.6 | |
| 7b | 1.4; m | | | | 1.4, m | | |
| 8 | — | 46.9 | | | — | 44.9 | |
| 9 | 0.9; m | 53.2 | C-20; C-11; C-12; C-14; C-8; C-15 | | 0.92, m | 57.0 | C-8 |
| 10 | — | 41.6 | | | — | 38.7 | |
| 11a | 1.4; m | 20.9 | | | 1.6, m | 18.9 | |
| 11b | 1.7; m | | | | 1.5, m | | |
| 12a | 1.4; m | 40.1 | | | 1.8, m | 27.0 | |
| 12b | 1.7; m | | | | 1.6, m | | |
| 13 | — | 79.7 | | | 2.48, sl | 46.1 | |
| 14a | 1.5; m | 44.0 | C-13; C-15 | | 1.9, m | 38.7 | C-13 |
| 14b | 1.9; m | | | | 2.0, m | | |
| 15a | 3.76; br s | 82.4 | C-14; C-9; C-16; C-17 | | 1.8, m | 54.0 | C-16 |
| 15b | — | | | | 1.7, m | | |
| 16 | — | 160.8 | | | — | 81.9 | |
| 17a | 5.23; br s | 108.8 | C-12; C-16; C-13; C-15 | | 4.09, d, 10.9 | 66.7 | C-13; C-15; C-16 |
| 17b | 5.20; br s | | | | 4.15, d, 10.9 | | |
| 18 | — | — | | | 1.20, s | 28.9 | C-3; C-4; C-19 |
| 19 | — | 178.7 | | | 0.89, s | 17.1 | C-3; C-4; C-18 |
| 20 | 0.98; s | 17.1 | C-10; C-1; C-5; C-9 | | 0.94, s | 18.1 | C-10 |
| 1′ | 4.78; m | 100.0 | C-2 | Correlated with H-2′ | 4.93, d, 7.8 | 102.5 | C-3; C-5′ |
| 2′ | 4.75; m | 73.4 | C-1′; C-7′; C-4′ | Irradiated signal | 4.05, t, 8.4 | 75.4 | C-1′; C-4′ |
| 3′ | 5.22; m | 75.7 | C-12′; C-2′; C-4′ | Correlated with H-2′ | 4.33, t, 9.0 | 78.8 | C-2′; C-4′ |
| 4′ | 3.8; m | 76.0 | C-6′ | Correlated with H-2′ | 4.23, t, 9.2 | 72.2 | C-2′; C-6′ |
| 5′ | 3.50; dl, 9.6 | 76.6 | C-2′ | Correlated with H-2′ | 4.01, m | 78.5 | C-4′; C-6′ |
| 6′a | 3.8; m | 61.7 | | Correlated with H-2′ | 4.61, dd, 1.7; 11.5 | 63.3 | C-4′; C-5′ |
| 6′b | 3.71; dd; 2.8; 12.2 | | | Correlated with H-2′ | 4.39, dd, 5.6; 11.5 | | |
| 7′ | — | 173.7 | | | | | |
| 8′ | 1.9; m | 44.1 | C-9′; C-7′ | | | | |
| 9′ | 1.9; m | 26.6 | C-8′; C-7′ | | | | |
| 10′ | 0.91; dl; 4.9 | 22.9 | C-7′; C-8′; C-9′ | | | | |
| 11′ | 0.91; dl; 4.9 | 22.9 | | | | | |
| 12′ | — | 171.7 | | | | | |
| 13′a | 2.79; dd; 8.0; 16.0 | 45.3 | C-15′; C-12′; C-14′ | | | | |
| 13′b | 2.72; dd; 5.3; 16.0 | | | | | | |
| 14′ | 5.03; dd; 5.3; 8.0 | 71.3 | C-16′; C-20′; C-15′; C-12′; C-13′ | | | | |
| 15′ | — | 145.2 | | | | | |
| 16′; 20′ | 7.3; m | 127.2 | | | | | |
| 17′; 19′ | 7.3; m | 129.5 | C-15′ | | | | |
| 18′ | 7.2; m | 128.8 | | | | | |
| 1″ | 4.8; m | 102 | C-5″; C-2″; C-4′ | Correlated with H-6″ | | | |
| 2″ | 3.59; dd; 2.9; 9.3 | 71.9 | C-4″ | Correlated with H-6″ | | | |
| 3″ | 3.80; m | 72.4 | C-4″ | Correlated with H-6″ | | | |
| 4″ | 3.34; m | 73.9 | C-6″; C-5″; C-2″ | Correlated with H-6″ | | | |
| 5″ | 3.65; dd; 6.2; 9.0 | 70.8 | C-4″ | Correlated with H-6″ | | | |
| 6″ | 1.25; d; 6.2 | 18.1 | C-5″; C-4″ | Signal irradiated | | | |

[a] Apparent triplet.

The stereochemistry of the 15-OH substituent was deduced to be alpha by comparison of its $^{13}C$ NMR spectrum data with those for *ent*-15α-hydroxylated and *ent*-15β-hydroxylated derivatives. Although the difference in chemical shift at the position of hydroxylation (C-15) between epimers is small, C-9 is shielded in the *ent*-15α-hydroxylated derivatives by approximately 9 ppm.[32]

The spectral data revealed similarity with the compound wedeloside, the major toxic constituent of *Wedelia asperrima* (Asteraceae).[33] The optical activity of compound **2** is $\alpha_D^{25} = -103.1$ (*c* 0.017, $CH_3OH$).

Compound **3** was determined to be $C_{26}H_{44}O_8$ by HRESIMS—negative mode (*m*/*z* $[M-H]^-$ 483.2961 (calcd for $C_{26}H_{43}O_8$, 483.2961). The $^{13}C$ NMR spectrum, together with $^{1}H$ NMR, DEPT, and two-dimensional spectra (Table 2) showed the presence of six signals assignable to β-glucopyranose, and the remaining 20 signals were presumed to be due to a diterpenoid with a kaurane skeleton because of its occurrence in *Mikania* sp. The quaternary carbon at $\delta$ 81.9, the methylene at $\delta$ 66.7, and their correlations observed in the HMBC spectrum suggested hydroxyl substituents in the carbons C-16 and C-17, respectively. The position of the glucose moiety was established by HMQC and HMBC spectra. In the HMBC spectrum, the methyl proton signals C-18 and C-19 ($\delta$ 0.89 and 1.20) correlated with an oxygenated carbon ($\delta$ 85.2), and this carbon correlated with the anomeric proton ($\delta$ 4.93; 7.8 Hz). These

results indicate that the position of the *O*-glycosyl linkage is at C-3. Since the proton on C-3 appeared as a double doublet at $\delta$ 3.50 ($J$ = 11.6, 4.2 Hz), it can be inferred that it is in an $\alpha$-orientation. The stereochemistry at C-16 was determined to be in $\alpha$-orientation with respect to the hydroxyl group by comparison of the chemical shift values obtained for C-16 and C-17 with those reported in the literature.[34]

This compound has not been previously published. The optical activity of compound **3** is $\alpha_D^{25} = -90.4$ ($c$ 0.01, $CH_3OH$).

Because *M. laevigata* is a plant of such important popular use and is described in the Brazilian Pharmacopeia, knowledge about its chemistry is essential. In this way, this research provided for a wider view of the chemistry of this species, since fractions of low polarity and more polar ones were identified.

In conclusion, this phytochemical study allowed identification of 21 compounds, including sesquiterpenes, kaurane diterpenes, flavonoids, and coumarin. A phenylpropanoid derivative, a kaurane, and *nor*kaurane diterpenes are being reported for the first time. Many of these constituents or compounds with similar chemical structures had already been identified in previous studies of *Mikania glomerata*, for which identification of beyerenoic acid, kaurenoic acid, coumarin, lupeol, lupeol acetate, syringaldehyde, and *trans*-*O*-coumaric acid were reported. This is important because both species have popular use for the same therapeutic purposes.

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## Supplementary data

Supplementary data (HRESIMS, $^1$H and 2D NMR spectra for the compounds **1**, **2** and **3**, general experimental details, and spectroscopic data of the other isolated constituents) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.10.102.

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