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Isolation and Identification of Prenylated Coumarins from the Species *Flindersia brayleyana* F.Muell (Rutaceae)

Isolamento e Identificação de Cumarinas Preniladas da Espécie Flindersia brayleyana F. Muell (Rutaceae)

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The Rutaceae family is known for its representative genera in the society in which we live and also for being one of the most chemically versatile plant families. The genus *Flindersia* is part of this family and produces alkaloids and coumarins as the main secondary metabolites. Coumarins stand out for their many important biological activities and are identified as the predominant class in the species *Flindersia brayleyana*. Therefore, the main objective was the isolation and structural characterization of the secondary metabolites of *Flindersia brayleyana*. The structures of the isolated compounds were elucidated through the analysis of 1D and 2D spectra of ^{1}H NMR and ^{13}C NMR and GC/MS, involving comparison with data from the literature. The coumarins Seselin (1), Braylin (2), Cedrelopsin (3), *cis*-6-methoxykellactone (4), 6-methoxylomatin (5), and Brayleyanin (6) were identified.

Keywords: Rutaceae; *Flindersia brayleyana*; prenylated coumarins.

1. Introduction

Even before the advent of extensive studies, plant species were widely used by society to relieve pain and cure disease. Today it is known that plant species contain compounds of a chemical nature, called secondary metabolites, which are involved in the defense mechanism of plants and are the object of studies, in isolation or in extracts, to research their mechanism of action, and thus, to verify their activity in the organism of living beings.[1,2]

Therefore, the current work aimed to study the species *Flindersia brayleyana* (Rutaceae). The family Rutaceae, considered pantropical and with approximately 150 genera, is found abundantly in the tropics and subtropics, being one of the most chemically versatile plant families and known for its leaves, which have translucent spots through which essential oil is secreted from the glands, emitting strong aromas.[3-5]

The genus *Flindersia*, with 17 species, is differentiated from others of the Rutaceae family through fruit analysis: *Flindersia* has five carpels and the other genera only four.[6,7]

The bibliographic survey conducted with the genus *Flindersia* revealed alkaloids and coumarins as principal secondary metabolites in fixed components and monoterpenes and sesquiterpenes in essential oils, with coumarins being the dominant class in the species *Flindersia brayleyana*.[6,8,9]

Coumarins stand out for their medicinal potential and their very versatile bioactivities, represented by compounds that present anti-inflammatory, analgesic, antioxidant, anticoagulant, anti-HIV, and antimicrobial effects, among others.[10-12] This is due to the large number of structures present in this class of secondary metabolites. In addition, the majority of the recently identified pyranocoumarins are reported to belong to the Rutaceae family.[13] Given this, the objective of the current work is to isolate and identify secondary metabolites of the species *Flindersia brayleyana*.

2. Experimental

2.1. General experimental procedures

The following chromatographic techniques were applied: Column Chromatography (CC) was performed on silica gel 60 (0.063-0.200 mm, Merck); Preparative Thin Layer Chromatography

(PTLC) was performed on silica gel 60 PF_{254} containing gypsum (Merck); and Thin Layer Chromatography (TLC) was performed on aluminum chromatography sheets with silica gel 60 F_{254} (Merck). The following were used as mobile phase solvents, purchased from Synth (São Paulo, Brazil): Methanol (CH_3OH - 99.8%), dichloromethane (CH_2Cl_2 - 99.5%), *n*-hexane (98.5%), and acetone (99.5%). For the identification and elucidation of substances: 1D and 2D NMR experiments were performed on a 500 MHz Bruker Ascend 500 NMR spectrometer operating at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR. Deuterated chloroform ($CDCl_3$), tetradeuterated methanol (CD_3OD), and deuterated acetone ($(CD_3)_2CO$), containing TMS (tetramethylsilane) as an internal standard, were used. GC/EIMS were obtained on a spectrometer GC-MS 5975C Inert XL EI/CI/MS Agilent Technologies, coupled to a gas chromatography system 7890A, with the use of the positive ion mode of analysis.

2.2. Collection of material

The stem and wood bark of the species *Flindersia brayleyana*, were collected in November 2018 at Linhares, ES, Brazil (latitude 19°06'54" S, longitude 39°56'20" O). The voucher specimen (nº 8817) was deposited in the herbarium of the Universidade Federal de Viçosa (UFV).

2.3. Extraction and isolation

The *Flindersia brayleyana* stem and wood bark (2 and 2.3 Kg, respectively) were dried and powdered, and the extraction was performed with methanol. Both of the methanolic extracts were partitioned with CH_2Cl_2, BuOH, EtOAc, and H_2O. The dichloromethane partition of the stem (47.3 g) was fractionated by silica gel CC, with a polar gradient of CH_2Cl_2:MeOH, obtaining 7 fractions (FCD1-FCD7). FCD1 (1.4 g) was chromatographed, with a polar gradient of CH_2Cl_2:MeOH, generating 7 fractions (FCD1.1 – FCD1.7). PTLC was performed with fraction FCD1.4 (20.0 mg), with 100% of CH_2Cl_2, obtaining seselin (**1**- 2.3 mg). The remainder of FCD1.4 (102.4 mg) was chromatographed, with a polar gradient of CH_2Cl_2:MeOH, generating 7 fractions (FCD1.4.1-FCD1.4.7). FCD1.4.5 (66.5 mg) was chromatographed, with a polar gradient of *n*-hexane:acetone, obtaining braylin (**2**- 30.5 mg) and cedrelopsin (**3**- 6.4 mg) of fractions FCD1.4.5.3 and FCD1.4.5.7, respectively. FCD5 (8.9 g) was chromatographed, with a polar gradient of *n*-hexane:acetone, generating 9 fractions (FCD5.1 – FCD5.9). Fraction FCD5.7 (54.7 mg) was chromatographed, with a polar gradient of *n*-hexane:acetone, obtaining *cis*-6-methoxykellactone (**4**- 34.6 mg) of fraction FCD5.7.4. Fraction FCD5.3 (669.2 mg) was chromatographed, with a polar gradient of *n*-hexane:acetone, obtaining 6-methoxylomatin (**5**- 35.2 mg) of fraction FCD5.3.6. The dichloromethane partition of wood bark (18.5 g) was fractionated by silica gel CC, with a polar gradient of CH_2Cl_2:MeOH, obtaining 13 fractions (FCMD1-FCMD13). FCMD6 (1,1 g) was chromatographed, with a polar gradient of *n*-hexane:acetone, generating 8 fractions (FCMD6.1 – FCMD6.8). Fraction FCMD6.4 (104.2 mg) was chromatographed, with a polar gradient of *n*-hexane:acetone, generating 7 fractions (FCMD6.4.1-FCMD6.4.7). FCMD6.4.5 (69.6 mg) was chromatographed, with a polar gradient of *n*-hexane:acetone, obtaining brayleyanin (**6** – 49.1 mg) of fraction FCMD6.4.5.3.

3. Results and Discussion

Six coumarins (Figure 1) were isolated and identified from the dichloromethane partitions of the stem and wood bark, which were identified as Seselin[14] (**1**), Braylin[15] (**2**), Cedrelopsin[16] (**3**), *cis*-6-methoxykellactone[17] (**4**), 6-methoxylomatin[18] (**5**), and Brayleyanin[9] (**6**). These compounds were characterized by their spectral data of ^1H and ^{13}C-NMR (1D and 2D) and GC/EIMS, involving comparison with data described in the literature (Supplementary Material).

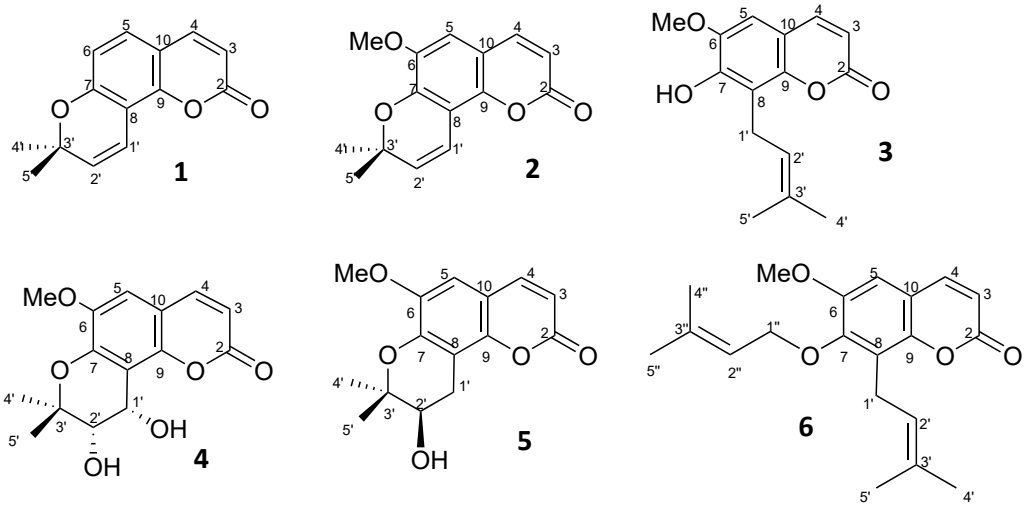


Figure 1. Prenylated coumarins isolated from *Flindersia brayleyana*

For the identification of these coumarins, the NMR spectra were analyzed (Table 1) considering some characteristic parameters of the basic coumarin skeleton $C_3(CH)_6O_2 = C_9H_6O_2$ (six sp^2 aromatic carbons with 2C and 4CH and one CH=CH-COO unit): a) the presence of two doublets (*J* around 9.0 Hz, Table 1) represented by hydrogen δ_H signals around 6.30 (H-3) and 7.60 (H-4, position receiving a mesomeric effect produced by conjugation with carbonyl group C-4 and also receiving anisotropic effect generated by the aromatic ring) in the ^{1}H NMR spectra, confirmed by the homonuclear interactions of H-3 and H-4 observed in the 2D ^{1}H-^{1}H-COSY spectra and correlated in the 2D HSQC ($^1J_{CH}$) spectra with the signals of atoms around δ_C 112.0 (CH-3) and 143.0 (CH-4), a position conjugated with the carbonyl carbon atom represented by the signal around δ_C 161 in the ^{13}C NMR spectra (Table 1); b) the availability of four positions in the aromatic ring (CH-4 to CH-8) for the location of substituents; and c) when there is oxygenated substitution in the C-5 carbon, H-4 hydrogen can be found with values above δ_H 8.00.[19]

Compound **1** appeared as a yellow solid. Its molecular formula was determined to be $C_{14}H_{12}O_3$ by the EIMS ($[M]^{\cdot+}$ at *m/z*=228).The ^{13}C-APT NMR data revealed the presence of fourteen carbon atoms, including six quaternary carbons (C_6, including one carbonyl O=C-O and two oxygenated, C-7 and C-9), six methines (including four olefinics), and two methyl groups. These data, which agree with the molecular formula $C_{14}H_{12}O_3$ indicating 9 degrees of unsaturation, four corresponding to the aromatic ring, three to α,β-unsaturated lactone including the CH=CHCOO unit, and two to the pyran ring sustaining two methyl groups, combined with the information provided by 1D and 2D spectral analysis were used to postulate the structure of **1** as a typical angular pyranocoumarin. The ^{1}H NMR spectrum shows the hydrogen signals of H-3 and H-4 as doublets (*J*=9.5 Hz, *cis*-interaction spin-spin) at δ_H 6.25 and 7.62, respectively, showing that C-5 is not replaced. The methyl groups 3H-4'and 3H-5' appear as a singlet signal at δ_H 1.50 (s, 6H), correlated in the HSQC with the carbon signal at δ_C 28.1 (Table 1). Analyzing the 2D ^{1}H-^{1}H-COSY spectrum, the interactions between the hydrogens H-3/H-4 (*J*=9.4 Hz), H-5/H-6 (*J*=8.4 Hz, *ortho*

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) NMR data of the prenylated coumarins **1**-**6**. Chemical shifts (δ, ppm) and coupling constants (*J,* in parenthesis) in Hz*

	1 ($CDCl_3$)		**2($CDCl_3$)**		**3 ($CDCl_3$)**		**4 ($(CD_3)_2CO$+CD_3OD)**		**5 ($CDCl_3$)**		**6 ($CDCl_3$)**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	161.2	-	161.1	-	161.6	-	161.2	-	161.8	-	161.2	-
3	112.6	6.25 (d, 9.5)	113.1	6.25 (d, 9.4)	113.1	6.29 (d, 9.4)	111.8	6.23 (d, 9.4)	112.9	6.24 (d, 9.4)	114.7	6.32 (d, 9.4)
4	143.9	7.62 (d, 9.5)	143.8	7.59 (d, 9.4)	143.7	7.60 (d, 9.4)	145.0	7.89 (d, 9.4)	144.0	7.61 (d, 9.4)	143.4	7.61 (d, 9.4)
5	127.8	7.23 (d, 8.4)	108.8	6.78 (s)	105.1	6.74 (s)	108.5	7.12 (s)	106.3	6.75 (s)	106.9	6.78 (s)
6	113.5	6.74 (d, 8.4)	145.6	-	143.7	-	146.2	-	146.2	-	150.0	-
7	143.4	-	145.9	-	147.5	-	146.8	-	147.9	-	149.8	-
8	109.3	-	110.2	-	114.3	-	112.3	-	108.7	-	124.9	-
9	150.2	-	144.8	-	148.3	-	149.0	-	148.6	-	147.6	-
10	112.8	-	111.4	-	111.2	-	111.1	-	111.1	-	114.4	-
1'	115.0	6.91 (d,10.1)	115.2	6.87 (d, 10.0)	22.2	3.59 (d, 7.2)	65.1	4.95 (d, 4.1)	26.0	3.15 (dd, 17.5, 4.9) 2.96 (dd, 17.5, 5.6)	23.0	3.58 (d, 7.2)
2'	130.8	5.75 (d, 10.1)	130.9	5.75 (d, 10.0)	120.7	5.31 (t, 7.2)	73.9	3.80 (d, 4.1)	68.2	3.91 (dd, 5.6, 4.9)	121.5	5.23 (qt, 7.2, 1.4)
3'	77.2	-	78.1	-	133.2	-	78.8	-	78.4	-	132.5	-
4'	28.1	1.50 (s)	28.3	1.52 (s)	25.8	1.70 (s)	24.7	1.47 (s)	24.8	1.41 (s)	25.7	1.68 (s)
5'	28.1	1.50 (s)	28.3	1.52 (s)	17.9	1.87 (s)	22.8	1.42 (s)	21.9	1.45 (s)	18.0	1.85 (s)
1''	-	-	-	-	-	-	-	-	-	-	70.0	4.60 (d, 7.2)
2''	-	-	-	-	-	-	-	-	-	-	120.3	5.55 (qt, 7.3, 1.3)
3''	-	-	-	-	-	-	-	-	-	-	138.4	-
4''	-	-	-	-	-	-	-	-	-	-	25.8	1.79 (s)
5''	-	-	-	-	-	-	-	-	-	-	17.9	1.71 (s)
MeO	-	-	56.5	3.89 (s)	56.3	3.95 (s)	55.5	3.84 (s)	56.35	3.88 (s)	56.1	3.90 (s)

*Number of hydrogens bonded to carbon atoms deduced by ^{13}C-APT-NMR spectra. Chemical shifts and coupling constants (*J*) corresponding to hydrogen signals were obtained from 1D ^{1}H NMR spectrum. 2D ^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, HSQC ($^1J_{CH}$) and HMBC ($^2J_{CH}$ and $^3J_{CH}$) spectra were also used in these structural elucidations

relation), and H-1'/H-2' (*J*=10.1 Hz, *cis*-configuration) were confirmed (Table 1). The dimethylated pyranic ring was also deduced by observing in the 2D HMBC spectrum the correlations of 3H-4'/3H-5' (δ_H 1.50, s) with both C-3' (δ_C 77.2, $^2J_{CH}$) and CH-2' (δ_C 130.8, $^3J_{CH}$).

Thus, these NMR spectral data and the EIMS, revealing only four principal peaks at *m/z*=228 ([M]$^{+\cdot}$, 15.0 %, 213 (M-15, 100.0 %), and 185 (M-15-28, 22,5 %) together with the proposed fragmentation mechanisms summarized in Scheme 1, enabled characterization of this prenylated coumarin as Seselin (**1**).

Compound **2** was isolated as yellowish crystals. The EIMS displayed a molecular ion peak at *m/z*=258, consistent with a molecular formula of $C_{15}H_{14}O_4$ and compatible with the presence of one methoxyl group (MeO) in comparison with compound **1** (Scheme 1). In fact, the 1D ^{1}H and ^{13}C-APT NMR spectral data in combination with the HMBC experiment indicated that **2** was very similar to Seselin (**1**), except for the presence of an additional methoxy group represented by signals at δ_H 3.89/δ_C 56.3, confirmed by the 2D HMBC through heteronuclear correlation of the carbon signal at δ_C 145.6 (C-6) with 3H (δ_H 3.89, $^3J_{CH}$) of the methoxyl group (MeOC-6). The location of a methoxyl group at C-6 was also confirmed by spatial dipolar interaction of H-5 (δ_H 6.78, s) with both H-4 (δ_H 7.59, d, *J*=9.4 Hz) and H_3CO (δ_H 3.89, s) revealed by 2D^{1}H-^{1}H-NOESY (Figure 2).

Compound **3** was isolated as a yellow oil and showed a molecular formula $C_{15}H_{16}O_4$ deduced by the [M]$^{+\cdot}$ ion peak at *m/z*=260. Comparative analysis of the ^{1}H and ^{13}C NMR spectral data of **2** and **3** revealed a significant difference only in the region involving a pyran ring in **2** and the presence of an isoprene unit ($CH_2CH=CMe_2$, δ_H/δ_C: 3.59 (d, *J*=7.2 Hz)/ 22.2 (CH_2-1'), 5.31/120.7 (CH-2'), -/133.2 (C-3'), 1.70 (s)/25.8 (CH_3-4'), and 1.87 (s)/17.9 (CH_3-5',γ effect of the CH_2-1') in **3** (Table 1). The methoxyl group linked to C-6 was confirmed by the HMBC correlation between δ_H 3.89 (OMe) and δ_C 143.7 (C-6, $^3J_{CH}$). The HMBC spectrum of **3** also showed heteronuclear correlations of two singlet signals 3H-4' (δ_H 1.70) and 3H-5' (δ_H 1.87) with both CH-2' (δ_C 120.7, $^3J_{CH}$) and C-3' (δ_C 133.2, $^2J_{CH}$), 3H-4' (δ_H 1.70) with CH_3-5' (δ_C 17.9, $^3J_{CH}$), and 3H-5' (δ_H 1.87) with CH_3-4' (δ_C 25.8, $^3J_{CH}$) confirming the presence of the two methyl groups 3H-4' and 3H-5' bonded to the same carbon atom C-3' (3'-CMe_2). As observed in **2**, the location of the

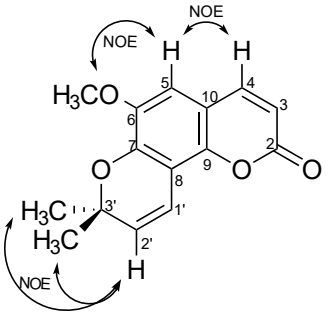


Figure 2. Correlation 2D ^{1}H-^{1}H-NOESY of compound **2**

methoxyl group in C-6 was also confirmed by spatial dipolar interaction of H-5 (δ_H 6.74, s) with both H-4 (δ_H 7.60, d, *J*=9.4 Hz) and H_3CO (δ_H 3.89, s), revealed by 2D ^{1}H-^{1}H-NOESY (Figure 3). The presence of the isoprenyl unit in the *ortho* position of the hydroxyl group can also can be used to justify the presence of the peak at *m/z*=204 ([M]$^{+\cdot}$ -$H_2C=CMe_2$) of the EIMS spectrum of **3** and formed by a fragmentation reaction below that postulated (Scheme 2).

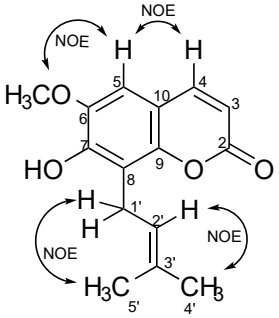
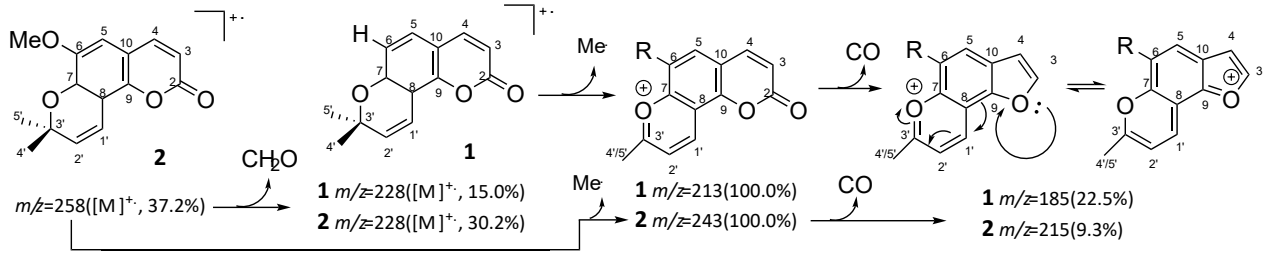


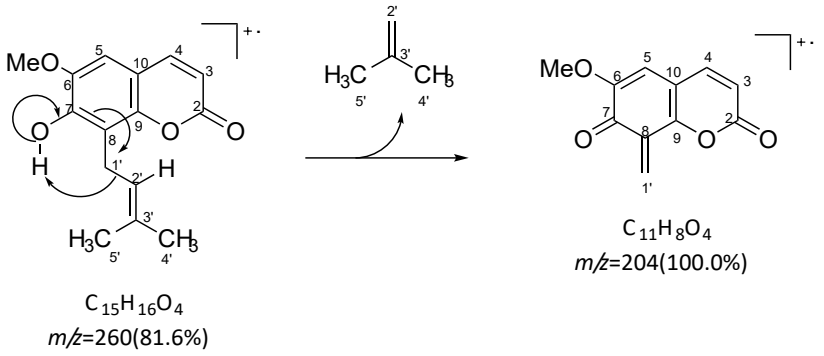
Figure 3. Correlation 2D ^{1}H-^{1}H-NOESY of compound **3**

Thus, all these data confirmed the presence of the isoprenyl unit in **3**, enabling its identification as Cedrelopsin (**3**).

The molecular formula $C_{15}H_{16}O_6$ of **4** (yellow solid) was established by EIMS (*m/z*=292, [M]$^{+\cdot}$). The ^{1}H and ^{13}C NMR spectral data (Table 1) of **4** were very similar to those of **2**, differing only in the sp^2 CH-1' and CH-2' carbons of the pyran ring of **2,** now in **4** as methine carbons sp^3 sustaining hydroxyl groups: δ_H/δ_C 4.91 (d, *J*=4.0 Hz)/65.1 (HOCH-1', γ effect of the methyl groups CH_3-4' and CH_3-5') and 3.80 (d, *J*=4.0 Hz)/73.9 (HOCH-2')**.** The value of *J*=4.1 Hz observed



Scheme 1. Proposed fragmentation mechanisms of **1** and **2** (only peaks classified as principals)



Scheme 2. Proposed fragmentation mechanisms of **3** (only peaks classified as principals)

in the hydrogen signals of H-1' and H-2' suggest an axial/equatorial or equatorial/equatorial interaction.

The presence of the peak at *m/z*=220 (peak basic) in the EIMS spectrum of **4**, formed by a fragmentation reaction below that postulated (Scheme 3), supported these conclusions.

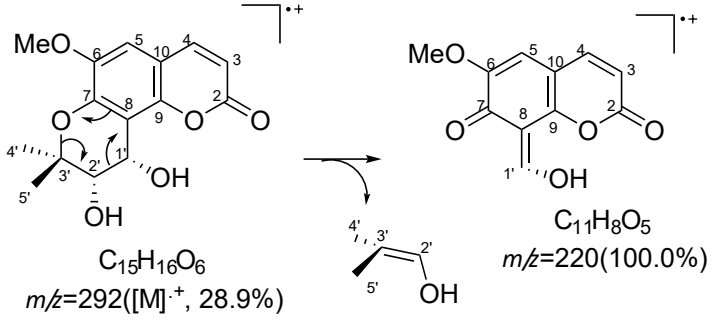
Structure **4a** (Figure 4) reveals results obtained by 2D ^{1}H-^{1}H-NOESY spectrum of **4**, including a postulated relative configuration.

Compound **5** (*m/z*=276) appeared as a dark yellow solid. The EIMS displayed a molecular ion peak at *m/z*=276 ($[M]^{\cdot+}$), consistent with a molecular formula of $C_{15}H_{16}O_5$. Comparative analysis of the ^{1}H and ^{13}C NMR (1D and 2D) spectral data of **5** and **4** revealed results that were similar, except for the absence of signals corresponding HOCH-1' (δ_H 4.95 (d, *J*=4.1 Hz, H-1')/δ_C 65.1, CH-1') in the spectra of **4,** and the presence of signals attributed to methylene group H_2C-1' [δ_H 3.15 (dd, *J*=17.5, 4.9 Hz, H-1'a) and 2.96 (dd, *J*=17.5, 5.6 Hz, H-1'b)/δ_C 26.0, CH_2-1'] in the spectra of **5**. The signal of the H-2' in the ^{1}H NMR spectrum of **5** was, as anticipated, observed as a double-doublet at δ_H 3.91 (dd, *J*=4.9, 5.6 Hz)/δ_C 68.2 revealing vicinal spin-spin couplings with the two hydrogen atoms 2H-1' [δ_H 3.15 (dd, *J*=17.5, 4.9 Hz, H-1'a) and 2.96 (dd, *J*=17.5, 5.6 Hz, H-1'b) /δ_C 26.0. The values of the vicinal coupling constants *J*=4.9 Hz and *J*=5.6 Hz were used to postulate the axial position (Figure 5) for a hydroxyl group on carbon CH-2' (equatorial position for hydrogen H-2'), because when involving an equatorial-axial interaction the *J* ranges from 0-5 Hz and axial-axial from 6-14 Hz.[20]

The presence of the peak at *m/z*=206 (peak basic) in the EIMS spectrum of **5**, formed by a fragmentation reaction below that postulated (Scheme 4), supported these conclusions.

Figure 5 presents important spatial correlations obtained by 2D ^{1}H-^{1}H-NOESY spectrum of **5**, prenylated coumarin isolated from *Flindersia brayleyana* and known as 6-methoxylomatin (**5**).

Compound **6** (*m/z*=328) was isolated as a yellow solid. Comparison of the EIMS of this compound (**6**, *m/z* 328 $[M]^{\cdot+}$,



Scheme 3. Proposed fragmentation mechanisms of **4** (only peaks classified as principals)

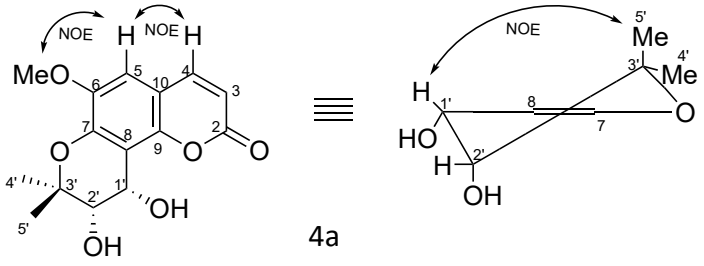


Figure 4. Correlation 2D ^{1}H-^{1}H-NOESY of compound **4**

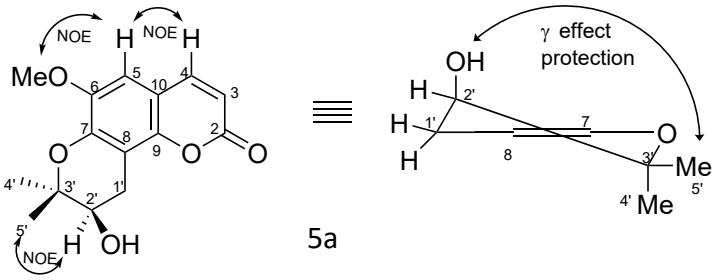
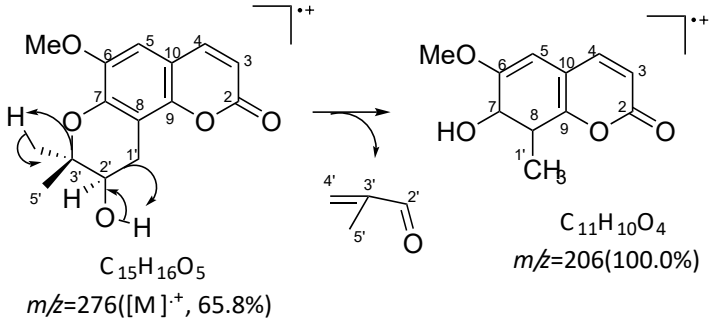


Figure 5. Correlation 2D ^{1}H-^{1}H-NOESY of compound **5**



Scheme 4. Proposed fragmentation mechanisms of 5 (only peaks classified as principals)

relative abundance 100.0%, molecular formula $C_{20}H_{24}O_4$, Scheme 5) with compound **3** (*m/z* 260 $[M]^{+}$, relative abundance 81.6%, molecular formula $C_{15}H_{16}O_4$) revealed a difference of C_5H_8 compatible with one additional isoprenyl unit (Me_2C=CH-CH_2- substituting one hydrogen atom in the molecule of **3**) bonded to an oxygen atom, justifying the significant difference in the chemical shifts of the ^{1}H/^{13}C (δ_H 4.60 (d, *J*=7.2 Hz)/δ_C 70.0) in **6**.

In fact, in the^{1}H NMR spectrum of **6,** four singlet signals were observed corresponding to four methyl groups, with values of δ_H 1.85, 1.79, 1.71, and 1.68, chemical shifts suggesting a bond in four sp^2 carbon atoms, also in accordance with the presence of two isoprenyl units.

Comparative analysis of the ^{1}H and ^{13}C NMR spectral data of **3** and **6** revealed significant differences only in the region involving the substituents sustained by aromatic carbon atoms C-6, C-7, and C-8, compatible with the etherification of the hydroxyl group bonded to carbon atom 7 with one isoprenyl unit (Table 2 and Scheme 5).

Two doublets of each compound are attributed as the characteristic signals of the hydrogens H-3 [**3/6**: δ_H 6.29 (d, 9.4 Hz)/6.32 (d, 9.4 Hz)] and H-4 [**3/6**: δ_H 7.60 (d, 9.4 Hz)/7.61 (d, 9.4 Hz)], correlated in the 2D HSQC with carbon signals of the CH-3 (**3/6**: δ_C 113.1/114.7) and CH-4 (**3/6**: δ_C 143.7/143.4). The signals with a value of δ_C 23.0 and δ_H 3.58 (d, 7.2 Hz) were assigned to CH2-1'. The signals at δ_C 70.0/δ_H 4.60 (d, 7.2 Hz) were attributed to methylene oxygenated CH2-1'' (1''-CH_2-O) bonded to C-7 carbon. This location was unequivocally confirmed by the heteronuclear spin-spin interaction between carbon C-7 (δ_C 149.8) and hydrogens 2H-1' [δ_H 3.58 (d, *J*=7.2 Hz), $^{3}J_{CH}$], 2H-1'' [δ_H 4.60 (d, *J*=7.2 Hz), $^{3}J_{CH}$), and H-5 (δ_H 6.78 (s), $^{3}J_{CH}$] revealed by cross-peaks observed in the HMBC spectrum of **6** (Table 2). The analog result was observed in the HMBC spectrum of **3** revealing interactions only of C-7 (δ_C 147.5) with 2H-1' [δ_H 3.59 (d, *J*=7.2 Hz), $^{3}J_{CH}$ and H-5 (δ_H 6.74 (s), $^{3}J_{CH}$] (Table 2).

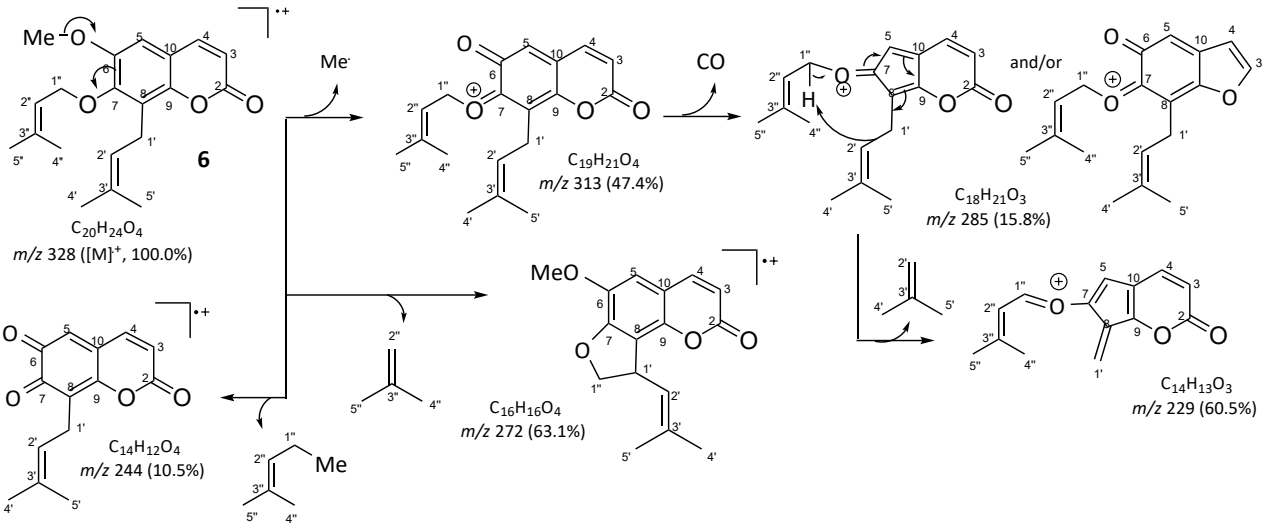
For further clarification, Table 1 shows only the values of the chemical shifts of ^{1}H (H and coupling constants in Hz) and ^{13}C (δ_C, number of hydrogens bonded to carbon atoms deduced by ^{13}C-APT) of **1** to **6**. The results of the extensive application of 1D and 2D NMR spectral techniques were also used to confirm the structure and establish the ^{1}H and ^{13}C resonance assignments of **1-6** (**3** and **6** in Table 2 to exemplify, with the additional results of heteronuclear long-range couplings also used to prepare Table 1).

4. Conclusions

Six prenylated coumarins, **1**-**6** were isolated from *Flindersia brayleyana*, confirming the major presence of this class of secondary metabolites in this species. These secondary metabolites have been previously identified from the wood bark of the same species, in addition, these coumarins are present in several genera of the Rutaceae family.

The structures of these prenylated coumarins (**1-6**) were elucidated using 1D and 2D ^{1}H NMR and ^{13}C NMR spectral data, based on characteristic peaks and correlations observed in the experiments, and also, low-resolution mass spectra, confirming the proposed structure to from the mass obtained.

It was possible to notice that **1** and **2** have similarities between their structures, being different only by a methoxy



Scheme 5. Proposed fragmentation mechanisms of 6 (only peaks classified as principals)

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) NMR data of the prenylated coumarins **3** and **6**, including direct ($^{1}J_{CH}$), observed in the HSQC, and long-range couplings of hydrogen and carbon atoms in the HMBC ($^{2}J_{CH}$ and $^{3}J_{CH}$) spectra, in $CDCl_3$ as solvent. Chemical shifts (δ, ppm) and coupling constants (*J*, Hz, in parenthesis)*

	3 ($CDCl_3$)				**6 ($CDCl_3$)**			
	HSQC		**HMBC**		HSQC		HMBC	
	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$
2	161.6	-	H-3	H-4	161.2	-	H-3	H-4
3	113.1	6.29 (d, 9.4)	H-4		114.7	6.32 (d, 9.4)		
4	143.7	7.60 (d, 9.4)	H-3		143.4	7.61 (d, 9.4)	H-5	
5	105.1	6.74 (s)		H-4	106.9	6.78 (s)		H-4
6	143.7	-	H-5	MeO-6	150.0	-	H-5	MeO-6
7	147.5	-		H-5; 2H-1’	149.8	-		H-5; 2H-1’; 2H-1”
8	114.3	-	2H-1’	H-2’	124.9	-	2H-1’	
9	148.3	-		H-4; 2H-1’	147.6	-		H-4; H-5; 2H-1’
10	111.2	-	H-5	H-3	114.4	-	H-4	H-3
1’	22.2	3.59 (d, 7.2)	H-2’		23.0	3.58 (d, 7.2)		
2’	120.7	5.31 (t, 7.2)	2H-1’	3H-4’; 3H-5’	121.5	5.23 (qt, 7.2, 1.4)	2H-1’	3H-4’; 3H-5’
3’	133.2	-	3H-4’; 3H-5’	2H-1’	132.5	-	3H-4’; 3H-5’	2H-1’
4’	25.8	1.70 (s)		H-2’; 3H-5’	25.7	1.68 (s)		H-2’; 3H-5’
5’	17.9	1.87 (s)		H-2’; 3H-4’	18.0	1.85 (s)		H-2’; 3H-4’
1’’	-	-	-	-	70.0	4.60 (d, 7.2)		
2’’	-	-	-	-	120.3	5.55 (qt, 7.2, 1.3)	2H-1’’	3H-4’’; 3H-5’’
3’’	-	-	-	-	138.4	-	3H-4’’; 3H-5’’	2H-1’’
4’’	-	-	-	-	25.8	1.79 (s)		H-2’’; 3H-5’’
5’’	-	-	-	-	17.9	1.71 (s)		H-2’’; 3H-4’’
MeO	56.3	3.95 (s)			56.1	3.90 (s)		

*Number of hydrogens bonded to carbon atoms deduced by -^{13}C-APT-NMR spectra. Chemical shifts and coupling constants (*J*) corresponding to hydrogen signals were obtained from 1D ^{1}H NMR spectrum. 2D ^{1}H-^{1}H-COSY and ^{1}H-^{1}H-NOESY spectra were also used in these structural elucidations

group in C-6; **3** and **6** are similar to have isoprenyl groups and, therefore, do not form the pyranic ring; and **4** and **5** are similar due to the presence of hydroxyl groups in the pyranic ring.

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