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)

Lara P. S. Nascimento, Michel de S. Passos, Thalya S. R. Nogueira, Milena G. C. Vieira, Antônio Sérgio N. Moreira, Raimundo Braz-Filho, Ivo J. C. Vieira*

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 1H NMR and 13C 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. 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 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...
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NMR experiments were performed on a 500 MHz Bruker Ascend 500 NMR spectrometer operating at 500 MHz for $^1$H NMR and 125 MHz for $^{13}$C NMR. Deuterated chloroform (CDCl$_3$), tetradeuterated methanol (CD$_3$OD), and deuterated 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$_3$OH - 99.8%), dichloromethane (CH$_2$Cl$_2$ - 99.5%), n-hexane (98.5%), and acetone (99.5%). For the identification and elucidation of substances: 1D and 2D -hexane:acetone, obtaining 7 fractions (FCMD6.4.1-FCMD6.4.7). Fraction FCMD6.4 (69.6 mg) was chromatographed, with a polar gradient of n-hexane:acetone, obtaining braylinin ($\sim$ 2.3 mg). The remainder of FCMD1.4 (102.4 mg) was chromatographed, with a polar gradient of CH$_2$Cl$_2$: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, 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 the stem and wood bark (18.5 g) was fractionated by silica gel CC, with a polar gradient of CH$_2$Cl$_2$:MeOH, obtaining 13 fractions (FCMD1-FCMD13). FCM6 (669.2 mg) was chromatographed, with a polar gradient of n-hexane:acetone, generating 8 fractions (FCMD6.1 – FCMD6.8). Fraction FCD6.4 (104.2 mg) was chromatographed, with a polar gradient of n-hexane:acetone, generating 7 fractions (FCMD6.4.1-FCMD6.4.7). FCM6.4.5 (69.6 mg) was chromatographed, with a polar gradient of n-hexane:acetone, obtaining brayleyanin (6- 49.1 mg) of fraction FCD6.4.5.3.

### 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$_2$Cl$_2$, BuOH, EtOAc, and H$_2$O. The dichloromethane partition of the stem (47.3 g) was fractionated by silica gel CC, with a polar gradient of CH$_2$Cl$_2$:MeOH, obtaining 7 fractions (FCD1-FCD7). FCD1 (1.4 g) was chromatographed, with a polar gradient of CH$_2$Cl$_2$:MeOH, generating 7 fractions (FCD1.1 – FCD1.7). PTLC was performed with fraction FCD1.4 (20.0 mg), with 100% of CH$_2$Cl$_2$, obtaining seselin (1- 2.3 mg). The remainder of FCD1.4 (102.4 mg) was chromatographed, with a polar gradient of CH$_2$Cl$_2$: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 the stem and wood bark (18.5 g) was fractionated by silica gel CC, with a polar gradient of CH$_2$Cl$_2$:MeOH, obtaining 13 fractions (FCMD1-FCMD13). FCM6 (1,1 g) was chromatographed, with a polar gradient of n-hexane:acetone, generating 8 fractions (FCMD6.1 – FCMD6.8). Fraction FCD6.4 (104.2 mg) was chromatographed, with a polar gradient of n-hexane:acetone, generating 7 fractions (FCMD6.4.1-FCMD6.4.7). FCM6.4.5 (69.6 mg) was chromatographed, with a polar gradient of n-hexane:acetone, obtaining brayleyanin (6- 49.1 mg) of fraction FCD6.4.5.3.

### 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$^{14'}$ (6). These compounds were characterized by their spectral data of $^1$H 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*](image-url)
For the identification of these coumarins, the NMR spectra were analyzed (Table 1) considering some characteristic parameters of the basic coumarin skeleton \( \text{C}_3(\text{CH})_6\text{O}_2 = \text{C}_9\text{H}_6\text{O}_2 \) (six \( \text{sp}^2 \) aromatic carbons with 2C and 4CH and one CH=CH-COO unit): a) the presence of two doublets (\( J \approx 9.0 \text{ Hz} \), Table 1) represented by hydrogen \( \delta \text{H} \) 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 \( ^1\text{H} \) NMR spectra, confirmed by the homonuclear interactions of H-3 and H-4 observed in the 2D \( ^1\text{H}-^1\text{H}-\text{COSY} \) spectra and correlated in the 2D HSQC (\( ^1\text{J}_{\text{CH}} \)) spectra with the signals of atoms around \( \delta \text{C} \) 112.0 (CH-3) and 143.0 (CH-4), a position conjugated with the carbonyl carbon atom represented by the signal around \( \delta \text{C} \) 161 in the \( ^{13}\text{C} \) 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 \( \delta \text{H} \approx 8.00 \).

Compound 1 appeared as a yellow solid. Its molecular formula was determined to be \( \text{C}_{14}\text{H}_{12}\text{O}_3 \) by the EIMS ([M]+ at \( m/z = 228 \)). The \( ^{13}\text{C}-\text{APT} \) NMR data revealed the presence of fourteen carbon atoms, including six quaternary carbons (\( \text{C}_9 \), including one carbonyl O=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 \( \text{C}_{14}\text{H}_{12}\text{O}_3 \) indicating 9 degrees of unsaturation, four corresponding to the aromatic ring, three to \( \alpha \),\( \beta \)-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 \( ^1\text{H} \) NMR spectrum shows the hydrogen signals of H-3 and H-4 as doublets (\( J = 9.5 \text{ Hz} \), cis-interaction spin-spin) at \( \delta \text{H} \approx 6.25 \) 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 \( \delta \text{H} \approx 1.50 \) (s, 6H), correlated in the HSQC with the carbon signal at \( \delta \text{C} \approx 28.1 \) (Table 1). Analyzing the 2D \( ^1\text{H}-^1\text{H}-\text{COSY} \) spectrum, the interactions between the hydrogens H-3/H-4 (\( J = 9.4 \text{ Hz} \)), H-5/H-6 (\( J = 8.4 \text{ Hz} \), ortho)

| 1 (CDCl₃) | 2(CDCl₃) | 3 (CDCl₃) | 4(CD₃₂CO+CD₂D) | 5 (CDCl₃) | 6 (CDCl₃) |
|-----------|----------|-----------|----------------|-----------|----------|
| \( \delta \text{C} \) | \( \delta \text{H} \) | \( \delta \text{C} \) | \( \delta \text{H} \) | \( \delta \text{C} \) | \( \delta \text{H} \) | \( \delta \text{C} \) | \( \delta \text{H} \) | \( \delta \text{C} \) | \( \delta \text{H} \) |
| 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) | 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 | - | 114.1 | - | 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) |
| 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, 6.1) | 68.2 | 3.91 (dd, 5.6, 4.9) | 121.5 (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'' | - | - | - | - | - | - | - | - | 7.00 | 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 \( ^{13}\text{C}-\text{APT-NMR} \) spectra. Chemical shifts and coupling constants (\( J \)) corresponding to hydrogen signals were obtained from 1D \( ^1\text{H} \) NMR spectrum. 2D \( ^1\text{H}-^1\text{H}-\text{COSY} \), \( ^1\text{H}-^1\text{H}-\text{NOESY} \), HSQC (\( ^1\text{J}_{\text{CH}} \)) and HMBC (\( ^2\text{J}_{\text{CH}} \) and \( ^3\text{J}_{\text{CH}} \)) spectra were also used in these structural elucidations.
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The 1D 1H and 13C NMR data for 3 revealed a significant difference from compounds 1 and 2, with signals at δH/δC 3.89 (OMe) and 56.3 (MeOCH₂) confirming the presence of the two methoxyl groups in C-6. The HMBC spectrum of 3 also showed heteronuclear correlations of two singlet signals 3H-4' (δH 1.70) and 3H-5' (δH 1.87) with both CH-2' (δC 120.7, δCH 292), and CH-1' (δC 123.2, δCH 292), 3H-4' (δH 1.70) with CH₂-5' (δC 143.7, δCH 45.6), and 3H-5' (δH 1.87) with CH₂-4' (δC 25.8, δCH 45.6) confirming the presence of the two methyl groups 3H-4' and 3H-5' bonded to the same carbon atom C-3' (3'-CMe₂). As observed in compound 2, the location of the methoxyl group in C-6 was also confirmed by spatial dipolar interaction of H-5 (δH 6.74, s) with both H-4 (δH 7.60, d, J=9.4 Hz) and H₂CO (δH 3.89, s), revealed by 2D 1H-1H-NOESY (Figure 2). The presence of the isoprenyl unit in the ortho position of the hydroxyl group can also be used to justify the presence of the peak at m/z=204 ([M]+ -H₂=C=CMe₂) of the EIMS spectrum of 3 and formed by a fragmentation reaction below that postulated (Scheme 2).

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

The molecular formula C₁₅H₁₆O₆ of 4 (yellow solid) was established by EIMS (m/z=292, [M]+). The 1H and 13C NMR spectral data (Table 1) of 4 were very similar to those of 2, differing only in the sp² CH-1' and CH-2' carbons of the pyran ring of 2, now in 4 as methine carbons sp² sustaining hydroxyl groups: δH/δC 4.91 (d, J=4.0 Hz)/65.1 (HO-CH-1'), γ effect of the methyl groups CH₂-4' and CH₂-5' and 3.80 (d, J=4.0 Hz)/73.9 (HO-CH-2'). The value of J=4.1 Hz observed...
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 ¹H-¹H-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]+), consistent with a molecular formula of C₁₅H₁₆O₅. Comparative analysis of the ¹H and ¹³C NMR (1D and 2D) spectral data of 5 and 4 revealed results that were similar, except for the absence of signals corresponding HOCH-1’ (δH 4.95 (d, J=4.1 Hz, H-1’)/δC 65.1, CH-1’) in the spectra of 4, and the presence of signals attributed to methylene group H₂C-1’ [δH 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)/δC 26.0, CH₂-1’] in the spectra of 5. The signal of the H-2’ in the ¹H NMR spectrum of 5 was, as anticipated, observed as a double-doublet at δH 3.91 (dd, J=4.9, 5.6 Hz)/δC 68.2 revealing vicinal spin-spin couplings with the two hydrogen atoms 2H-1’ [δH 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)/δC 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 ¹H-¹H-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]+, 

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\text{Scheme 2. Proposed fragmentation mechanisms of 3 (only peaks classified as principals)}
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\text{Scheme 3. Proposed fragmentation mechanisms of 4 (only peaks classified as principals)}
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\text{Figure 4. Correlation 2D ¹H-¹H-NOESY of compound 4}
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relative abundance 100.0%, molecular formula C_{20}H_{24}O_{4}, relative abundance 81.6%, molecular formula C_{15}H_{16}O_{4}) revealed a significant difference in the chemical shifts of the 1H/13C family. For further clarification, Table 1 shows only the values of characteristic signals of the hydrogens H-3 ([δ_H 3.59 (d, J=7.2 Hz), J_CH 70.0) in compound 5, with compound 3 (m/z 250 [M]⁺, relative abundance 100.0%, molecular formula C_{20}H_{25}O_{4}, Scheme 5) with compound 3 (m/z 260 [M]⁺, relative abundance 81.6%, molecular formula C_{20}H_{25}O_{4}) revealed a difference of C_{5}H_{8} compatible with one additional isoprenyl unit (Me=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 1H/13C family.

In fact, in the 1H 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 sp² carbon atoms, also in accordance with the presence of two isoprenyl units.

Comparative analysis of the 1H and 13C 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 ([δ_H 6.29 (d, 9.4 Hz)/6.32 (d, 9.4 Hz), J_CH 70.0) in the 2D HSQC with carbon signals of the CH-3 (δ_C 113.1/114.7) and CH-4 (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), J_CH 6.67 (s), J_CH 6.74 (s), J_CH 7.60 (d, J=7.2 Hz), J_CH 70.0) 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), J_CH 6.67 (s), J_CH 6.74 (s), J_CH 7.60 (d, J=7.2 Hz), J_CH 70.0) in the HMBC spectrum of 5 revealing cross-peaks observed in the HMBC spectrum of 6 (Table 2). For further clarification, Table 1 shows only the values of the chemical shifts of 1H (H and coupling constants in Hz) and 13C (δ_C, number of hydrogens bonded to carbon atoms deduced by 13C-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 1H and 13C 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 1H NMR and 13C 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 group.
Table 2. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) NMR data of the prenylated coumarins 3 and 6, including direct (JCH), observed in the HSQC, and long-range couplings of hydrogen and carbon atoms in the HMBC (JCH and JCH) spectra, in CDCl₃ as solvent. Chemical shifts (δ, ppm) and coupling constants (J, Hz, in parenthesis)*

|       | HSQC | HMBC | 3 (CDCl₃) | HSQC | HMBC | 6 (CDCl₃) |
|-------|------|------|-----------|------|------|-----------|
| 2     | 161.6| -    | H-3       | 161.2| -    | H-3       |
| 3     | 113.1| 6.29 (d, 9.4) | H-4 | 114.7| 6.32 (d, 9.4) | H-5 |
| 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       | 150.0| -    | MeO-6     |
| 7     | 147.5| -    | H-5; 2H-1' | 149.8| -    | H-5; 2H-1'; 2H-1''|
| 8     | 114.3| -    | 2H-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       | 114.4| -    | H-4       |
| 1'    | 22.2 | 3.59 (d, 7.2) | H-2' | 23.0 | 3.58 (d, 7.2) | H-3 |
| 2'    | 120.7| 5.31 (t, 7.2) | 2H-1' | 3H-4'; 3H-5' | 121.5| 5.23 (q, 7.2, 1.4) | 2H-1' | 3H-4'; 3H-5' |
| 3'    | 133.2| -    | 3H-4'; 3H-5' | 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 (q, 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 ¹³C-APT-NMR spectra. Chemical shifts and coupling constants (J) corresponding to hydrogen signals were obtained from 1D ¹H NMR spectrum. 2D ¹H-¹H-COSY and ¹H-¹H-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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