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Phenolic Compounds from the Leaves of *Eucalyptus microcorys* F. Muell.

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Abstract: A new acylated glycoside, 4-*O*-(4',6'-di-*O*-galloyl- -D-glucopyranosyl)-*trans*-*p*-coumaric acid, named microcoryn (**1**), together with sixteen known phenolic compounds, 5-*O*-(6'-*O*-galloyl- -D-glucopyranosyl)-gentisic acid (**2**), ellagic acid (**3**), gallic acid (**4**), kaempferol (**5**), quercetin (**6**), 3-*O*-galloyl- -D-glucose (**7**), 2,3,6-tri-*O*-galloyl- -D-glucose (**8**), 1,2,4,6-tetra-*O*-galloyl- -D-glucose (**9**), 1,2,3,4,6-penta-*O*-galloyl- -D-glucose (**10**), 4,6-hexahydroxydiphenoyl- -D-glucose (**11**), gemin D (**12**), tellimagrandin I (**13**), tellimagrandin II (**14**), isocoriariin F (**15**), oenothein C (**16**), and oenothein B (**17**) were isolated from the leaves of *Eucalyptus microcorys*. The structure of the new compound was elucidated by spectroscopic data, especially by 2D NMR techniques. This is the first phytochemical investigation of this plant's leaf extract.

Keywords: Acylated glycosides; ellagitannins; spectroscopic analyses.

1. Introduction

The *Eucalyptus* genus, which comprises more than 500 species, is highly diverse and displays significant adaptability and phenotypic plasticity [1]. Most of its species are native to Australia, but *Eucalyptus* trees are widely cultivated throughout the world and are the major fast-growing hardwood used in industrial pulpwood plantations across the globe [2]. Several *Eucalyptus* species are grown in Brazil, among which *E. microcorys*, mainly planted for its strong and durable timber, which is naturally resistant to decay [3-5]. Cycloeucalenol, cycloartenol, 24-methylencycloartanol and sitosterol were isolated from the heartwood [6,7], and sideroxylonals were quantified by HPLC in the leaf extract [8,9]. Furthermore, this species has potential medicinal use due to the high content of hydrolysable tannins in its leaves [10], as well as 1,8-cineole, which is the main component of its essential oil [10,11]. So far there have been no reports regarding the isolation and identification of this plant's phenolic compounds. Therefore, as part of our ongoing study on bioactive compounds from Mirtaceae species, we have isolated a new acylated glycoside, microcoryn (**1**), along with sixteen known phenolic compounds. In the present paper, we report on the isolation and structure elucidation of the new compound.

2. Materials and Methods

2.1. General

Column chromatography was run using Diaion HP-20 (Supelco) or Sephadex LH-20 (Sigma-Aldrich). Analytical TLC was carried out with Silica gel 60 F_{254} (Merck) plates, using formic acid-ethyl formiate-toluene (1:7:1) as the mobile phase. TLC spots were visualized by spraying plates with a 1% ethanolic solution of ferric

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chloride in HCl (0.1%) and UV light. IR spectra were recorded on a Perkin Elmer-440 infrared spectrophotometer with KBr pellets. UV spectra were measured with a Beckman DU-70 spectrophotometer. All NMR experiments were recorded on a Bruker Avance III 500 spectrometer operating at 500.13 MHz for ^{1}H and 125 MHz for ^{13}C, using TMS as internal reference. ESI-TOF MS spectra were recorded on a Bruker microTOF instrument.

2.2 Plant Material

Cultivated *E. microcorys* leaves were collected from ten trees at Ibama's National Forest (FLONA), located in Silvânia (S 16° 38´ 14´´; W 48° 39´ 06´´; 898 m), Goiás State, Brazil, in October 2011. Trees were 40 years old and originated from the seeds of the same progenies.

2.3 Extraction and Isolation

Air-dried and powdered leaves (670 g) were exhaustively extracted with 50% acetone at room temperature. After removing acetone under vacuum, the suspended aqueous extract was filtered to eliminate fats and chlorophylls and then partionated with ethyl acetate (6 x 100 mL). The gathered organic phase was concentrated to yield an ethyl acetate extract (6 g). The aqueous layer was lyophilized to yield a 32 g extract, which was dissolved in methanol (500 mL) to separate soluble (27 g) and insoluble (5 g) methanolic extracts. The ethyl acetate extract was subjected to column chromatography (200 g, Sephadex LH-20) and eluted with H_2O/EtOH/$(CH_3)_2CO$ (7:2:1) to afford ten combined fractions (*EA.1-EA.10*). *EA.7* (0.21 g) and *EA.9* (0.17 g) were separately subjected to Sephadex LH-20 CC eluting with increasing gradient of $CHCl_3$/EtOH and EtOH/MeOH to obtain compounds **1** (8 mg) and **8** (30 mg) from *EA.7* and **3** (4.4 mg), **5** (7.3 mg), **6** (3.3 mg), **9** (5.3 mg), **10** (15 mg), and **14** (22 mg) from *EA.9*.

The soluble methanolic extract (13.5 g × 2) was submitted to column chromatography over Diaion HP-20 (200 g) with MeOH/H_2O step-gradient elution (0-100%) to yield six fractions (*M1-M6*). *M2* (1.3 g) was further separated by CC over Sephadex LH-20 (eluted with increasing gradient of $CHCl_3$/EtOH, 1:1–1:9) and afforded compounds **4** (71 mg), **7** (6 mg), and **11** (22 mg). *M3* (6 g) was applied to Diaion HP-20 CC, eluted with 0-100% MeOH/H_2O to yield ten combined fractions (*M3.1-M3.10*). Fractions *M3.4* (0.2 g), *M3.5* (0.9 g), and *M3.*7 (2 g) were separately subjected to Sephadex LH-20 CC (eluting with gradients of $CHCl_3$/EtOH and EtOH/MeOH) to give compounds **12** (120 mg) and **15** (9 mg) from *M3.4*, **17** (350 mg) from *M3.5*, and **2** (14 mg) and **13** (110 mg) from *M3.*7. *M5* (6.2 g) was first submitted to Diaion HP-20 CC (0-100% MeOH/H_2O) and further purified by Sephadex LH-20 ($CHCl_3$/EtOH and EtOH/MeOH) to produce compound **16** (12 mg).

Microcoryn (**1**): white amorphous powder, $[\alpha]_D = -12$ (*c* 0.05, MeOH); UV (MeOH): $^{a}_{max}$ nm (log £): 216 (2.35), 276 (2.11); IR (KBr): ν_{max}: 3390, 2942, 1706, 1604, 1455, 1351, 1220, 1033 cm^{-1}. ESI-TOF MS: *m/z* 629.1136 $[M-H]^-$ (calc. for $C_{29}H_{25}O_{16}$, 629.1148). ^{1}H and ^{13}C NMR are listed in Table 1.

3. Results and Discussion

Ethyl acetate and methanol extracts obtained from the concentrated aqueous acetone homogenate of *E. microcorys* leaves were submitted to repeated column chromatography over Diaion HP 20 and Sephadex LH-20 to yield seventeen phenolic compounds (**1**-**17**). Known compounds 5-*O*-(6'-*O*-galloyl- -D-glucopyranosyl)-gentisic acid (**2**) [12], ellagic acid (**3**) [13], gallic acid (**4**), kaempferol (**5**) [14], quercetin (**6**), 3-*O*-galloyl- -D-glucose (**7**) [15], 2,3,6-tri-*O*-galloyl- -D-glucose (**8**)[16], 1,2,4,6-tetra-*O*-galloyl- -D-glucose (**9**) [17], 1,2,3,4,6-penta-*O*-galloyl- -D-glucose (**10**) [18], 4,6-hexahydroxydiphenoyl- -D-glucose (**11**) [19], gemin D (**12**) [19], tellimagrandin I (**13**) [20], tellimagrandin II (**14**) [21], isocoriariin F (**15**) [15], oenothein C (**16**) [15], and oenothein B (**17**) [22, 23] were identified by spectroscopic means and comparison with authentic samples or reported data. All compounds (Figure 1) were isolated from *E. microcorys* for the first time.

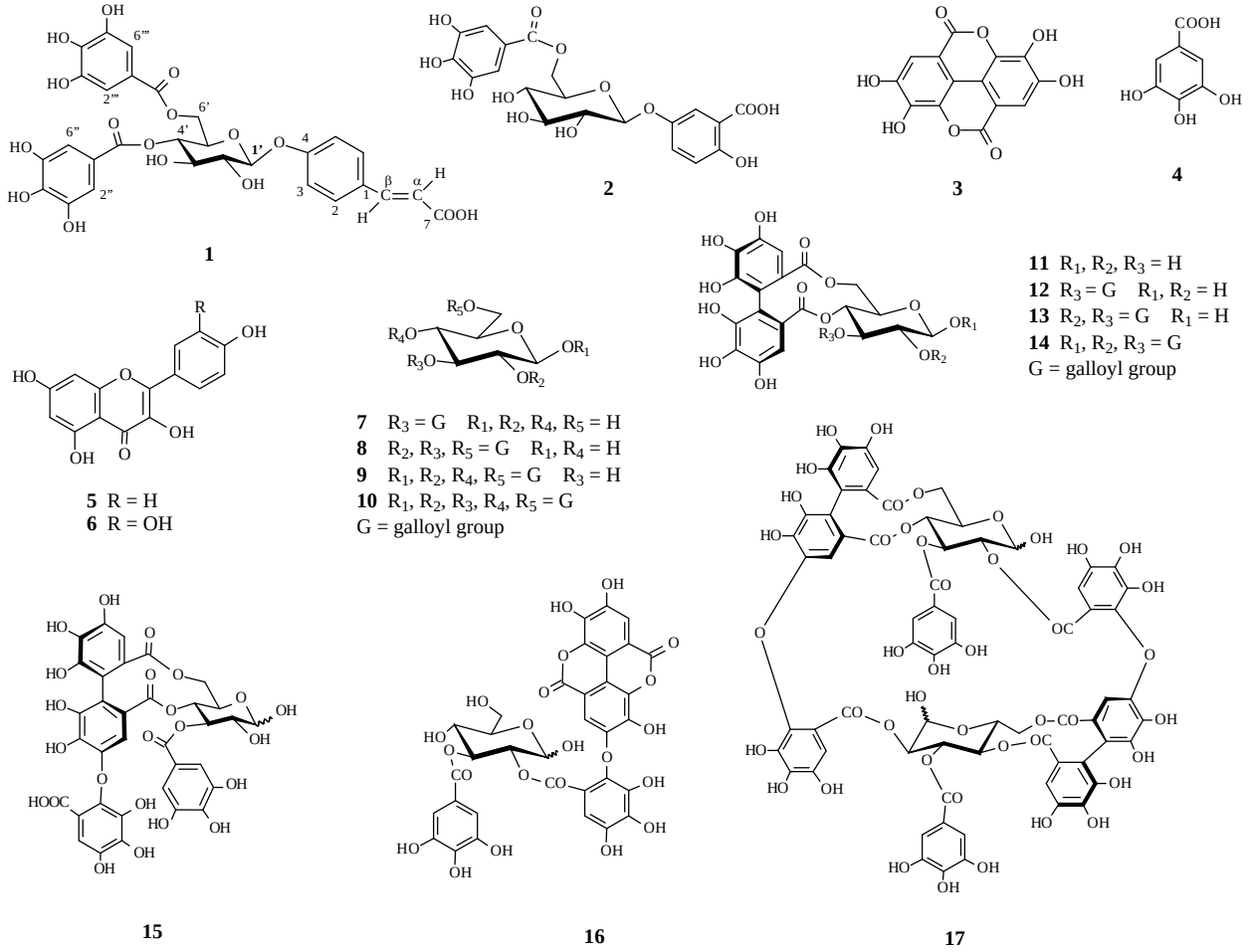

Figure 1. Structures of compounds **1-17**

Compound **1**, a white amorphous powder, $[\alpha]_D$ = –12 (MeOH), showed UV absorptions at λ 216 and 276 nm (log £ 2.35 and 2.11, respectively) and IR absorptions for OH groups (3390 cm^{-1}), conjugated C=O groups (1706 cm^{-1}), C=C (1629 cm^{-1}) and C–O (1220 cm^{-1}). The ESI-TOF mass spectrum exhibited the quasi molecular ion peak at *m/z* 629.1136 $[M-H]^{-1}$, corresponding to the molecular formula $C_{29}H_{26}O_{16}$. Analyses regarding the ^{1}H-NMR spectrum (Table 1) revealed the presence of two galloyl groups ($¢_H$ 7.18 and 7.20, each 2H, *s*) and a *trans-p*-coumaroyl group (*trans*-olefin signals at $¢_H$ 6.41 and 7.60, each 1H, *d*, *J* = 16.0 Hz as well as one 1,4-disubstituted benzene ring signals at $¢_H$ 7.18 and 7.63, each 2H, *d*, *J* = 8.6 Hz). In addition, the region $¢_H$ 3.70 – 5.25 showed a set of signals characteristic of a $^4C_1$-glucopyranosyl residue. The anomeric hydrogen signal appeared as a large doublet at $¢_H$ 5.24 (*J* = 7.6 Hz), which indicated the -configuration for the glucosidic linkage. The absence of an equilibrium mixture of ~ and anomers revealed the presence of an attached group at C-1', despite the low chemical shift of the anomeric hydrogen. The other glucose signals, which were assigned on the basis of ^{1}H-^{1}H COSY and ^{1}H-^{13}C-HSQC correlation maps, showed three deshielded signals, H-4' at $¢_H$ 5.15 and methylene hydrogens at $¢_H$ 4.22 and 4.47, suggesting that galloyl groups were attached to these positions. The total structure was achieved via the ^{1}H-^{13}C long-range correlation map from the HMBC NMR experiment (Figure 2). The attachments of the two galloyl groups at C-4' and C-6' positions of the glucopyranosyl core were confirmed by long-range correlations between carbonyl carbons at $¢_C$ 166.6 and respective aromatic and glucose hydrogens (Figure 2). The strong HMBC correlation observed from H-1' of the glucose to the C-4 of the *trans-p*-coumaric acid at $¢_C$ 160.6 defined the acetal linkage between C-1' and O-C-4. The structure was therefore established to be 4-*O*-(4',6'-di-*O*-galloyl- -D-glucopyranosyl)-*trans-p*-coumaric acid and was named microcoryn.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** (acetone-d_6 + D_2O, ¢ in ppm, *J* in Hz).

Position	¢$_H$	¢$_C$
1		130.9
2 and 6	7.63 (2H, *d*, *J* = 8.6)	129.6
3 and 5	7.18 (2H, *d*, *J* = 8.6)	116.6
4		160.5
α	7.60 (1H, *d*, *J* = 16.0)	144.1
β	6.41 (1H, *d*, *J* = 16.0)	116.4
C=O		168.2
1’	5.24 (1H, *d*, *J* = 7.6)	100.4
2’	3.73 (1H, *br t*)	73.9
3’	4.00 (1H, *br t*)	74.2
4’	5.15 (1H, *t*, *J* = 9.6)	71.1
5’	4.28 (1H, *m*)	72.4
6’	4.47 (1H, *dd*, *J* = 1.8, 12.0)	62.8
6’	4.22 (1H, *dd*, *J* = 7.8, 12.0)	
1’’		121.9
2’’ and 6’’	7.18 (2H, *s*)	109.1
3’’ and 5’’		146.3
4’’		139.4
C=O		166.6
1’’’		121.9
2’’’ and 6’’’	7.20 (2H, *s*)	109.1
3’’’ and 5’’’		146.3
4’’’		139.4
C=O		166.6

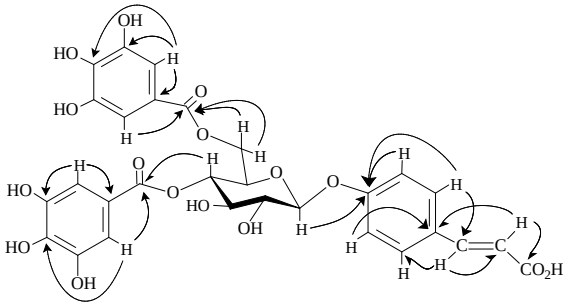

Figure 2. Key HMBC (H ➔ C) correlations of compound **1**.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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