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Iridoid and *seco*-iridoid glucosides from *Chioccoca alba* (Rubiaceae)[1]

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Abstract

Phytochemical investigation of *Chioccoca alba* afforded three new iridoids, alboside I, alboside II and alboside III, and a new *seco*-iridoid alboside V. Alboside **IV** showed moderate activity towards the DNA repair-deficient mutant RS321 of *Saccharomyces cerevisiae*. The structural elucidation of the new compounds was performed by ES–MS and by 1D and 2D NMR spectroscopic methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Chioccoca alba; Rubiaceae; Iridoid glucosides; *Seco*-iridoid glucoside

1. Introduction

Chioccoca alba Hitch. (Rubiaceae) is a shrub endemic to America and is reported to be used in folk medicine as a tonic for ganglion inflammation, as well as being used as a diuretic. It is also used as an antiviral, antieodema and snakebite treatment and has aphrodisiac properties also (Costa, 1978; Dorvault, 1978). The pharmacological actions of the roots of *C. alba* have been described in the Pharmacopoeias of several European nations since the last century (Lewis & Elvin-Lewis, 1989; Mabberley, 1989) and in Brazil it is commercialized in the free markets as an adulterant of the Brazilian 'Ipecac' (Costa, 1978). Previous works on *C. alba* described the occurrence of lignans, coumarins (El-Hafiz, Weniger, Quirion, & Anton, 1991), triterpenes (Bhattacharyya & Cunha, 1992) and ketoalcohols (El-Hafiz et al., 1991; Ballini, Bosica, & Rafaini, 1995).

As part of our continuing interest in Brazilian medicinal plants, we now wish to report the occurrence of iridoids and a *seco*-iridoid, including the isolation and biological evaluation of the new compounds (**1**–**4**). The structures and relative stereochemistries were determined by application of 1D and 2D NMR spectroscopic techniques. The seco-iridoid **4** showed moderate activity against the mutant RS321 of *Saccharomyces cerevisiae*.

2. Results and discussion

The $CHCl_3$-soluble part of a CH_2Cl_2/MeOH (2:1 v/v) extract, prepared from the roots of *C. alba*, was fractionated by CC on silica gel and Sephadex LH-20 to afford compounds **1**–**4**. Compounds **1**–**3** showed

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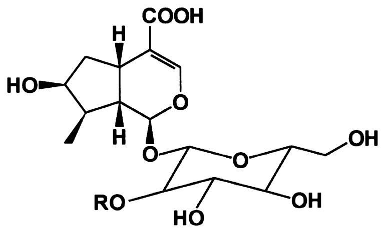

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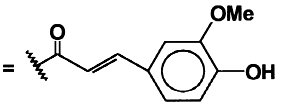

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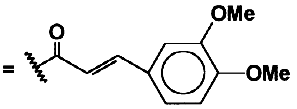

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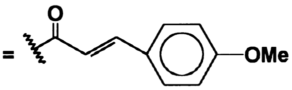

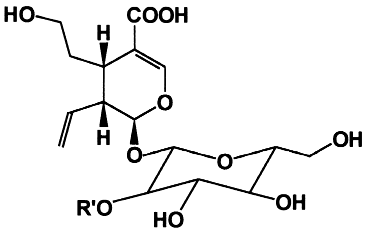

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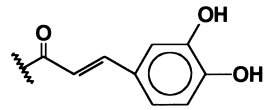

NMR signals typical of an iridoid skeleton (Garcia & Chulia, 1986; Mpondo, Garcia, & Lestani, 1989).

The molecular weight of alboside I (**1**) was indicated by the peak at *m*/*z* $[M+Na]^+$ 575 in the electrospray spectra ES–MS. Besides the molecular ion peak, the main information obtained from the ES–MS of **1** was given by the strong fragment at *m*/*z* 362 $[C_{16}H_{19}O_8+Na]^+$ corresponding to the loss of a feruloyl glucose moiety, which also was observed for the (*E*)-2′-feruloyl-loganin (Garcia & Chulia, 1986). The IR spectrum of **1** showed an hydroxyl absorption at 3500–3300 cm^{-1}, two-carbonyl absorptions at 1690, 1668 cm^{-1}, an aromatic ring at 1650, 1540 and 870 cm^{-1} and a UV λ max. 325, 235, 208 (MeOH). Three sets of signals were observed in the ^{1}H NMR spectrum (Table 1): hydrogens linked to the sp^2 carbons at δ 7.55 d ($J=16.0$ Hz), 7.35 d ($J=1.5$ Hz), 7.12 d ($J=1.5$ Hz), 6.95 d ($J=8.0$ Hz) 6.34 d ($J=16.0$ Hz) and 6.29 dd ($J=8.0$; 1.5 Hz); several signals from δ 5.14 to 3.18, a typical region for hydrogens in carbons linked to the hydroxyl functions; finally, signals at δ 1.74–2.99 m and 1.00 d ($J=7.0$ Hz). The ^{13}C NMR spectrum of **1** (Table 2) confirmed the presence of 26 carbons. According to the analysis of the DEPT spectrum these signals were attributed to two methyl, two methylene and 16 methine groups. These spectral features were very close to those observed for (*E*)-2′-feruloyl-loganin, an iridoid isolated from *Gentiana pedicellata* (Garcia & Chulia, 1986). Furthermore, the ^{1}H and ^{13}C NMR spectra of compound **1** did not show the presence of the carbomethoxy function ($\delta \approx 3.57$ s; ≈ 51.3 s) as in (*E*)-2′-feruloyl-loganin, a common feature in many iridoid metabolites. This fact, led us to suggest that compound **1** is an acid derivative, which already had been suspected by analysis of its IR spectra at 1680 cm^{-1}. The ^{1}H–^{1}H DQFCOSY relationships permitted the assignment of the feruloyl proton chemical shifts and also supported the substitution pattern. The location of the feruloyl unit at C-2′ was evidenced by this experiment, which indicated that the signal at δ 4.50 (d, $J=8.0$ Hz) attributed to H-1′ gave a cross peak with the H-2′ signal at δ 5.01 (t, $J=8.0$ Hz). In addition, the loganic acid unit was confirmed by ^{1}H–^{1}H DQFCOSY data in which the proton coupling correlations from H-1 $\leftrightarrow$ H-9; H-5 $\leftrightarrow$ (H-9, H-6$_a$, H-6$_b$, H-3); H-6$_a$, H6$_b$ $\leftrightarrow$ (H-5, H-7); H-8 $\leftrightarrow$ (H-7, H-9, H-10) and H-9 $\leftrightarrow$ (H-1, H-5, H-8) were observed. The ^{1}H–^{13}C one-bond correlation (HMQC) data were used to assign carbon resonances Table 2 to their attached protons, whereas the ^{1}H–^{13}C three-bond (HMBC) correlation were used to determine some conectivities in **1**. Especially important were the correlations from H-3 and H-5 proton resonances at δ 7.35 and 2.99 to the carbonyl resonance at δ 168.6 (C-11) and the correlation of the H-7″ and H-2′ at δ 7.55 and 5.01, respectively, to the resonance at δ 166.3 (feruloyl unity). The geometry of the unsaturated (C-7″, C-8″) in the feruloyl residue was established as (*E*) by judgement of the coupling constant between H-7″ and H-8″ ($J=16.0$ Hz) (Table 1).

The molecular formula of iridoid **2** was established as $C_{27}H_{34}O_{13}$ by ES–MS (*m*/*z* 589 for $[M+Na]^+$), ^{13}C and ^{1}H NMR data. A further fragment at *m*/*z* 376 $[C_{17}H_{21}O_8+Na]^+$, attributable to feruloyl glucose, corroborated that feruloyl is linked to a glucose moiety. The IR and UV spectra of **2** were very similar to those of compound **1**. The ^{1}H NMR spectrum Table 1 of **2** possessed the same signals as **1** for the loganic acid residue. The ^{1}H NMR signals at δ 3.79 (3H, s) and 3.78 (3H, s), combined with DQFCOSY data, revealed that the difference between iridoid **2** and iridoid **1** is related to the aromatic residue, which was located at C-2′ in the glucose molecule. The HMQC spectrum correlated each carbon with its associated protons; expansions provide the following correlations

Table 1
^{1}H NMR spectral data for compounds **1**–**4** (δ in ppm, $CDCl_3$)[a]

H	**1**	**2**	**3**	**4**
1	5.14 d (6.0)	5.15 d (5.36)	5.37 d (5.6)	5.50 d (6.5)
3	7.35 d (1.5)	7.20 d (0.8)	7.28 d (1.5)	7.30 s
5	2.99 dddd (9.5; 8.5; 8.0; 1.7)	2.98 m	2.89–2.99 m	2.90 br dd (12.5; 6.5)
6a	2.22 ddd (13.5; 8.0; 1.7)	1.60–1.78 m	1.56 m	1.22 ddd (16.0, 12.5, 6.5)
6b	1.74 ddd (13.5; 8.5; 4.2)	2.08–2.15 m	2.16–2.26 m	1.66 dt (16.0; 6.5)
7	3.45 ddd (5.5; 4.5; 1.5)	3.68 m	3.50 m	3.70 m
8	2.08 dqd (9.2; 7.0; 4.5)	1.60–2.00 m	1.60 m	5.80 ddd (17.0; 10.0; 8.0)
9	1.95 ddd (9.2; 9.0; 6.0)	2.06–2.49 m	2.90–2.93 m	2.75 dd (8.0; 6.5)
10	1.00 d (7.0)	1.00 d (6.5)	1.01 d (7.0)	5.23 dd (10.0; 1.5), 5.50 dd (17.0; 1.5)
1′	4.50 d (8.0)	4.49 d (8.0)	4.49 d (8.0)	4.52 d (8.0)
2′	5.01 t (8.0)	5.08 t (8.0)	5.09 t (8.0)	4.99 dd (12.2; 3.7)
3′–6′	3.23–3.76 m	3.20–3.78 m	3.33–3.76 m	3.20–3.89 m
2″	7.12 d (1.5)	6.96 d (1.5)	7.46 dd (8.0, 2.0)	7.05 d (1.5)
3″	–	–	6.81 dd (8.0, 2.0)	–
5″	6.95 d (8.0)	7.35 d (8.0)	6.81 dd (8.0, 2.0)	6.75 d (8.0)
6″	6.29 dd (8.0; 1.5)	7.12 dd (8.0; 1.5)	7.46 dd (8.0, 2.0)	6.95 dd (8.0; 1.5)
7″	7.55 d (16.0)	7.65 d (16.0)	7.50 d (16.0)	7.43 d (15.8)
8″	6.34 d (16.0)	6.58 d (16.0)	6.50 d (16.0)	6.19 d (15.8)
OMe	3.81 s	3.79 s	3.78 s	–
OMe	–	3.78 s	–	–

[a] Coupling in Hz is given in parentheses.

Table 2
^{13}C NMR spectral data to compounds **1**–**4** (δ in ppm, $CDCl_3$)

C[a,b]	**1**	**2**	**3**	**4**
1	98.9 (CH)	98.8 (CH)	98.8 (CH)	98.7 (CH)
3	150.2 (CH)	150.5 (CH)	150.5 (CH)	149.7 (CH)
4	112.4 (C)	111.9 (C)	111.9 (C)	112.2 (C)
5	31.6 (CH)	31.5 (CH)	315 (CH)	30.3 (CH)
6	39.1 (CH_2)	39.2 (CH_2)	39.2 (CH_2)	29.1 (CH_2)
7	77.0 (CH)	76.4 (CH)	76.4 (CH)	61.1 (CH_2)
8	39.3 (CH)	39.5 (CH)	39.5 (CH)	135.1 (CH)
9	45.5 (CH)	45.3 (CH)	45.3 (CH)	43.5 (CH)
10	13.7 (CH_3)	13.6 (CH_3)	13.6 (CH_3)	118.6 (CH_2)
11	168.6 (C)	168.2 (C)	168.2 (C)	166.5 (C)
1′	96.0 (CH)	95.9 (CH)	95.9 (CH)	95.4 (CH)
2′	73.4 (CH)	73.2 (CH)	73.2 (CH)	73.1 (CH)
3′	76.5 (CH)	76.8 (CH)	76.8 (CH)	76.8 (CH)
4′	70.3 (CH)	70.2 (CH)	70.2 (CH)	70.1 (CH)
5′	77.4 (CH)	77.3 (CH)	77.3 (CH)	77.3 (CH)
6′	61.4 (CH_2)	61.3 (CH_2)	61.3 (CH_2)	62.7 (CH_2)
1″	127.1 (C)	126.7 (C)	126.9 (C)	125.3 (C)
2″	115.5 (CH)	110.3 (CH)	130.2 (CH)	113.8 (CH)
3″	148.1 (C)	151.0 (C)	114.4 (CH)	145.8 (C)
4″	146.9 (C)	149.0 (C)	161.2 (C)	148.8 (C)
5″	121.3 (CH)	111.5 (CH)	114.4 (CH)	116.0 (CH)
6″	112.2 (CH)	123.1 (CH)	130.2 (CH)	121.4 (CH)
7″	114.5 (CH)	115.8 (CH)	115.6 (CH)	114.9 (CH)
8″	144.8 (CH)	144.8 (CH)	144.3 (CH)	145.1 (CH)
9″	166.3 (C)	166.3 (C)	166.2 (C)	166.5 (C)
OMe	55.9 (CH_3)	55.3 (CH_3)	55.7 (CH_3)	–
OMe	–	55.6 (CH_3)	–	–

[a] Measured at 100.57 MHz.
[b] Multiplicities of carbons (in parentheses) determined by a DEPT experiment.

in iridoid **2**. The doublet at δ_H 5.15 (H-1) correlated to the carbon peak at δ 98.8, the doublet at δ_H 4.49 (H-1′) correlated to the carbon peak at δ 95.9, the multiplet at δ_H 3.68 (H-7) correlated to the carbon at δ 76.4 and the triplet at δ_H 5.08 (H-2′) correlated to the carbon at δ 73.2. In addition, the presence of two methoxy groups at δ 55.3 and 55.6 also were confirmed, which showed correlations to the protons resonating at δ 3.78 and 3.79, respectively. The HMBC spectrum of **2** provided long-range interactions supporting these assignments. Of particular interest were the correlations between H-2′ (glucosyl unity) at δ 5.08 and C-9″ (carbonyl of the dimethoxycaffeoyl) at δ 166.3. The methoxy protons at δ 3.78 and 3.79, correlated to the corresponding ring carbons to which they were attached, C-3″ at δ 151.01 and C-4″ at δ 149.0, respectively. Additionally, the sp^2 methine proton at δ 7.20 correlated to the carboxylic function at C-11 δ 168.2, C-4 at δ 111.9, C-5 at δ 31.5 and C-1 at δ 98.8, while the C-10 methyl protons at δ 1.00 correlated to C-7 at δ 76.4, C-8 at δ 39.5 and C-9 at δ 45.3

The minor compound isolated, iridoid **3**, also had the same structural characteristics as evidenced by similar spectral features. Detailed analysis of all ^{1}H and ^{13}C NMR spectral data, including 1D and 2D (Tables 1 and 2) demonstrated that the main difference observed for **3** is due to the aromatic residue, that in this iridoid, the glucose of the loganic acid structure is substituted in the C-2′ position by a *p*-methoxycoumaroyl moiety. The molecular formula of alboside III

(**3**) $C_{26}H_{32}O_{12}$ was indicated by the peak at *m/z* 559 $[M+Na]^+$ in the ES–MS together with ^{13}C and ^{1}H NMR spectral data. Analysis of the fragments in the ES–MS spectrum of **3** showed a diagnostic peak at *m/z* 346 $[C_{16}H_{19}O_7+Na]^+$, which confirmed the placement of the *p*-methoxycoumaroyl group at the glucose moiety. The IR spectrum showed a hydroxyl absorption at 3465 cm^{-1} and strong aromatic C–H stretching bands at 3055 and 3020 cm^{-1}. The UV data are also in agreement with the structure proposed. The difference in the aromatic residue present in this molecule compared with **1** and **2** was clearly evidenced by ^{1}H NMR (2H, δ 7.46 dd, $J=8.0$, 2.0 Hz; 1H, 7.50 d, $J=16.0$ Hz; 2H, 6.81 dd, $J=8.0$, 2.0 Hz; 1H, 6.50 d, $J=16.0$ Hz and 3H, s, 3.78), which was confirmed by ^{13}C NMR data Table 2 as the *p*-methoxycoumaroyl. The HMBC spectrum of **3** showed a correlation between H-2′ and C-9″ (δ 166.2, carbonyl of *p*-methoxycoumaroyl) corroborating the position of the aromatic residue in C-2′ of the loganic acid derivative **3**, which to our knowledge is new.

Seco-iridoid **4** was obtained as a gum with the molecular formula $C_{25}H_{30}O_{13}$ (ES–MS *m/z* 561 $[M+Na]^+$). Its UV spectrum suggested the presence of an aromatic acyl moiety. The ^{1}H NMR Table 1 and ^{13}C NMR Table 2 spectral data were assigned by interpretation of ^{1}H–^{1}H DQFCOSY, DEPT 135, HMQC and HMBC spectra. The ^{1}H NMR spectrum of **4**, analyzed with the aid of DQFCOSY, showed resonances for aromatic protons at δ 7.43 (1H, d, $J=15.8$ Hz), 7.05 (1H, d, $J=1.5$ Hz), 6.95 (1H, dd, $J=1.5$; 8.0), 6.75 (1H, d, $J=8.0$ Hz) and 6.19 (1H, d, $J=15.8$ Hz). These chemical shift values and the absence of methoxy protons were indicative of a caffeoyl unit in molecule **4**. In fact, the ^{13}C NMR spectral data Table 2 were consistent with the caffeoyl residue. The site of esterification was determined to be the 2′-position of the glucopyranosyl moiety in **4**. The ^{1}H and ^{13}C NMR spectral data for the *seco*-iridoid skeleton were very close to those related to secologanol (Mpondo et al., 1989); the main difference observed in **4** was the absence of a methoxy function at δ_H 3.68 and δ_C 51.7, attributed to carbomethoxy at C-11, in the structure of secologanoal. The presence of a carbonyl function at δ 166.5 led us to attribute to compound **4** a carboxyl group rather than a carbomethoxyl function. The other spectral characteristics were in agreement with the structure proposed. In the ^{1}H–^{1}H DQFCOSY MNR spectrum, the H-7 (δ 3.70) was coupled with the H-6 resonances (δ 1.22 and 1.66), which in turn were coupled with the H-5 signal (δ 2.90). HMQC data supported the above observation with a correlation displayed between H-7 (δ 3.70) and C-5 (δ 30.3). In addition, the following selected ^{13}C–^{1}H long range correlation corroborated the structure proposed: the H-3 signal (δ 7.30) with the C-1 (δ 98.7), C-5 (δ 30.3) and C-11 (δ 166.5); the H-1 signal (δ 5.50) with the C-3 (δ 149.7), C-8 (δ 135.1) and C-1′ (δ 95.4) and the H-2′ signal (δ 4.99) with the C-1′ (δ 95.4), C-3′ (δ 76.8) and C-9′ (δ 166.5). These data are in agreement with those reported for secologanoal (Mpondo et al., 1989).

The biological activity of the compounds isolated, **1**–**4**, were tested using the bioassay with genetically engineered strains of the yeast *S. cerevisiae* for mechanism-based anticancer activity (Johnson et al., 1986; Gunatilaka, Samaranayake, Kingston, Hofmann, & Johnson, 1992; Gunatilaka, Kingston, & Johnson, 1994). The new iridoids **1**–**3** were inactive and the *seco*-iridoid **4** showed moderate activity only in the mutant strain RS321, with an IC_{12} value of 120 µg/ml.

3. Experimental

3.1. Instrumentation and chromatography materials

Silica gel (Merck, 230–400 and 70–230 Mesh) were used for all column chromatography unless otherwise stated and solvents were distilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity spectrometer at 400 and 100.57 MHz, respectively with TMS as a internal standard. IR spectra were obtained on a Nicolet spectrometer. ES–MS: at 70 EV VG Platform II. Optical activities were measured on a Polamat A, Carl Zeiss Jena.

3.2. Plant material

C. alba root was collected at Mogi Guaçu, São Paulo and classified by Dr. Lúcia Rossi. The voucher specimen is deposited in the Herbarium of the Botanical Garden “Maria Eneida P.K. Fidalgo”.

3.3. Biological testing

The experimental methods utilized in the screening procedure have been described elsewhere (Johnson et al., 1986; Gunatilaka et al., 1992; Gunatilaka et al., 1994).

3.4. Extraction and isolation of constituents

Dried and powdered roots of *C. alba* (261.6 g) were extracted with CH_2Cl_2/MeOH (2:1). The resulting CH_2Cl_2/MeOH (2:1) extract was filtered and concd in vacuum to afford a brown gum (24.5 g). The $CHCl_3$ soluble part (9.75 g) of the CH_2Cl_2/MeOH extract was applied to a flash Si gel column (100 g) and eluted with $CHCl_3$ containing increasing amounts of MeOH to give 14 fractions. The fraction 8 (1.44 g) was applied to a Si gel column (50 g) 230–400 mesh and eluted with $CHCl_3$/MeOH/H_2O (65:30:5 to 50:50:10) and MeOH to give 31 fractions. Fractions 6 (356.0 mg), 11 (48.0 mg) and 16 (94.0 mg) were applied to a Sephadex LH-20 CC, eluted with MeOH to give **1** (12.0 mg), **2** (6.0 mg), **3** (5.0 mg) and **4** (10 mg), respectively.

3.5. *Alboside I (**1**)*

Amorphous solid. $[\alpha]_D$ −46.7° (H_2O; $c=2.4$); UV λ_{max}^{MeOH} nm: 325, 235, 208; IR_{max}^{film} KBr cm^{-1}: 3453, 1695, 1630, 1520, 1077, 520. ES–MS *m/z*: 575 $[M+Na]^+$, 557, 362, 221, 200, 202; ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively).

3.6. *Alboside II (**2**)*

Amorphous solid. $[\alpha]_D$ −30.7° (MeOH; $c=0.9$); UV λ_{max}^{MeOH} nm: 330, 238, 208; IR_{max}^{film} KBr cm^{-1}: 3465, 1693, 1630, 1515, 1455, 1262, 1169, 1068, 1020. ES–MS *m/z*: 589 $[M+Na]^+$, 571, 376, 214, 202; ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively).

3.7. *Alboside III (**3**)*

Amorphous solid. $[\alpha]_D$ −30.7° (MeOH; $c=0.9$); UV λ_{max}^{MeOH} nm: 338, 235, 208; IR_{max}^{film} KBr cm^{-1}: 3465, 1693, 1630, 1515, 1455, 1262, 1169, 1068, 1020. ES–MS *m/z*: 559 $[M+Na]^+$, 541, 346, 202, 184; ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively)

3.8. *Alboside IV (**4**)*

Gum. $[\alpha]_D$ −51.5° (H_2O; $c=0.5$); UV λ_{max}^{MeOH} nm: 325, 238, 210; IR_{max}^{film} KBr cm^{-1}: 3464, 1693, 1626, 1519, 1387, 1276, 1187, 1072, 1030. ES–MS *m/z*: 561 $[M+Na]^+$, 543, 347, 186; ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively).

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