Two New Oleanane Saponins from Chiococca alba (L.) Hitch.

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Duas saponinas triterpénicas foram isoladas do extrato etanólico das raízes de Chiococca alba (L.) Hitch. (Rubiaceae). Suas estruturas foram elucidadas por RMN de 1H e 13C 1D e 2D e por espectrometria de massas de alta resolução com ionização por eletronebulização como 3-O-[β-D-glucopiranuril]-3-β-hidroxiolean-12,15-dien-28-oato de O-α-D-apiofuranosil (1→3)-[α-D-apiofuranosil (1→4)]-α-L-rhamnopyranosil (1→2)-α-L-arabinopyranosila (1) e 3-O-[β-D-glucopiranuril]-3-β-hidroxiolean-12,15-dien-28-oato de 28-O-α-D-apiofuranosil (1→3)-α-L-rhamnopyranosil (1→2)-α-L-arabinopyranosila (2).

Keywords: Chiococca alba, cainca, Rubiaceae, triterpene saponins

Introduction

Chiococca alba (L.) Hitch. (Rubiaceae), commonly known as “cainca” in Brazil, is a tropical and sub-tropical shrub spread all over the American continent. Its root bark is used in Brazilian traditional medicine for the treatment of several illnesses.1 Toxicological studies demonstrated absence of mutagenic activity in the ethanolic extract of the roots of C. alba and also low acute and sub acute toxicity by the oral route. Nevertheless, toxicity by parenteral routes was indeed pronounced.2

Previous works on C. alba reported the occurrence of lignans, coumarins, ketoalcohols,3 triterpenes,4 iridoids,5 flavonoids,6 two quinoline alkaloids7 and a nor-seco-pimarane.8

The present paper reports for the first time the isolation and structure elucidation of two triterpenoidal saponins from the ethanolic extract of the roots of C. alba.

Experimental

General experimental procedures

Optical rotations were measured on a Perkin Elmer model 243B polarimeter. Infrared spectra were recorded on a Nicolet Magna spectrometer. 1H NMR and 13C NMR spectra were recorded at 200 and 50 MHz (Varian Gemini 200), at 400 and 100 MHz (Bruker DRX 400) and at 600 and 150 MHz (Bruker DRX 600) in methanol-d4 and chloroform-d6 with TMS as internal standard.

The chemical shifts are reported in ppm. 1D and 2D experiments were made with the standard software provided. HRES/ESI/TOF MS analyses were made with a Bruker Daltonics microTOF instrument. Column chromatography: ODS silica gel (Aldrich Chemical Company, Inc); TLC: silica gel (0.25 mm precoated plates 60 F254, Merck). For preparative HPLC a 7.6 × 300 mm ODS column and UV detection at 210 nm were used. GC-MS analyses were recorded on a Shimadzu QP5000
GC/MS instrument (5% phenylmethyl silicone column, 30 m × 0.25 mm ID, 0.25 μm film thickness; programmed column temperature from 110 to 290 °C, 5 °C min⁻¹).

Plant material

Samples of *C. alba* were collected in Nova Friburgo, Rio de Janeiro, Brazil. The botanical identification was provided by Dr. Sebastião Neto, and a voucher specimen (RB395399) is deposited in the Herbarium of Rio de Janeiro Botanical Garden.

Extraction and isolation

Air-dried and powdered roots of *C. alba* (400 g) were extracted with ethanol. The extract was evaporated; the residue obtained (12 g) was suspended in water and successively partitioned with methylene chloride and butanol. The butanol fractions were combined, evaporated and the residue (4 g) was suspended in methanol and subjected to controlled precipitation with diethyl ether. The precipitate (2 g) was fractionated by column chromatography (octadecylsilane, 60 × 20 cm) using H₂O with increasing proportions of methanol (0% to 100%) to afford 10 fractions. TLC tests carried out with Liebermann-Bouchard and sulfuric orcinol reagents together with the observation the abundant foam formation, allowed the identification of the saponin enriched fractions. Further purification was carried out with reversed phase (octadecylsilane) preparative HPLC using methanol: 0.02% aqueous trifluoroacetic acid (60:40; v/v) to afford chiococcasaponin I (78 mg) and chiococcasaponin II (48 mg).

Chiococcasaponin I (1)

Amorphous white solid, UV transparent, [α]₂⁰° = −58 (MeOH; c 1). IR (KBr) νₘₐₓ/cm⁻¹: 3428, 2944, 1732, 1040, 986. ¹H NMR (methanol-d₄, 400 MHz) and ¹³C NMR (methanol-d₄, 100 MHz) (Table 1). HRES/ESI/TOF MS (negative mode) calculated for C₅₇H₈₈O₂₅, [M-H]⁻: m/z 1171.5536; found: 1171.5546. HRES/ESI/TOF MS (positive mode), calculated for C₅₇H₈₈O₂₅Na, [M+Na]⁺: m/z 1195.5512; found: 1195.5513.

Chiococcasaponin II (2)

Amorphous white solid, UV transparent, [α]₂⁰° = −46 (MeOH; c 1). IR (KBr) νₘₐₓ/cm⁻¹: 3426, 2946, 1736, 1137, 1077, 1053, 1034, 980. ¹H NMR (methanol-d₄, 400 MHz) and ¹³C NMR (methanol-d₄, 100MHz) (Table 1). HRES/ESI/TOF MS (negative mode) calculated for C₅₂H₈₀O₂₁Na, [M-H]⁻: m/z 1039.5114; found: 1039.5122. HRES/ESI/TOF MS (positive mode), calculated for C₅₂H₈₀O₂₁Na, [M+Na]⁺: m/z 1063.5089 found:1063.5084.

Acid hydrolysis

A solution of each saponin (5 mg per 2 mL of 0.5 mol L⁻¹ aqueous TFA) was poured in a Kontes heavy wall pressure resistant vessel and heated for 2 min in a microwave oven at full power. The reaction mixture was cooled, diluted with water and extracted with diethyl ether. The organic layer was concentrated under vacuum and the residue was methylated with diazomethane. 3β-hydroxyolean-12, 15-dien-28-oate methyl ester was identified by MS and NMR as the aglycone for both saponins.

MS (EI, 70eV) m/z 468 (2%); 260 (42%); 245 (14%); 231; 207; 201 (100%); 187 (14%); 173; 157; 145 (46%); 119; 105; 95; 81; 69; 55. ¹³C NMR (chloroform-d₁, 100 MHz): 16.1(C-25); 17.0 (C-23); 18.3 (C-26); 19.6 (C-6); 23.6 (C-30); 24.4 (C-11); 25.1 (C-27); 27.1 (C-2); 28.5 (C-24); 31.3 (C-20); 33.8(C-22); 33.9 (C-29); 33.9 (C-17); 34.1 (C-7); 35.7 (C-21); 38.2 (C-10); 39.8 (C-1); 40.3 (C-4); 41.3 (C-8); 43.5 (C-18); 44.6 (C-19); 45.6 (C-14); 48.7 (C-9); 57.2 (C-5); 91.2 (C-3); 124.7 (C-12); 128.6 (C-15); 137.8 (C-16); 141.5 (C-13); 176.1 (C-28).
Table 1. Carbon and proton NMR data for Chiococcasaponins I and II (100 and 400 MHz, CD,OD, internal TMS)

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<td>3.17 (dd, J 11.1 / 3.5 Hz)</td>
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**Rha at C-28**

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**Api**

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**Api**

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**GlcUA at C-3**

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<td>75.4</td>
</tr>
<tr>
<td>6 OH=O</td>
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resulting alditols were acetylated (acetic anhydride/pyridine, 1 h). The alditol acetates of arabinose, rhamnose and apiose were identified by direct comparison with authentic samples using GC/MS. The monosaccharides were also characterized by TLC on silica gel (butanol/acetic acid/water, 2:1:1). The spots on TLC plates were visualized by spraying with sulphuric-orcinol solution followed by heating. Glucuronic acid, arabinose and rhamnose were identified by comparison with authentic samples.

**Results and Discussion**

The HRES/ESI MS (positive mode) of chiococcasaponin I (1) exhibited a pseudomolecular ion peak [M+Na]+ at m/z 1195.5513 and the negative mode HRES/ESI-MS exhibited a pseudomolecular ion peak [M-H]- at m/z 1171.5546, both peaks being consistent with the molecular formula C43H54O38. Acid hydrolysis of I afforded the aglycone and the following monosaccharides: glucuronic acid, rhamnose, arabinose and apiose which were identified by GC/MS and TLC analysis. Due to the fact that apiose is a branched-chain sugar, incomplete acetylation is often observed for it when standard alditol acetylation conditions are utilized. This leads to the observation of two peaks for the partially acetylated apiose alditols in the GC/MS analyses. This behavior has already been described by Harris.9 The aglycone had its structure confirmed as 3β-hydroxyolean-12, 15-dien-28-oic acid by comparison of its 1H and 13C NMR spectra with literature data.4 This is the first report on the occurrence of a 15,16 unsaturated oleanolic acid saponin. 1H and 13C NMR data are shown in Table 1. 1D 1H and 13C NMR, DEPT and HSQC spectra confirmed the presence of five sugar residues corresponding to anomeric protons/carbons at 4.38/107.0, 5.12/101.4, 5.39/94.4, 5.19/112.5 and 5.29/112.2 ppm respectively. The anomeric proton at 4.38 ppm (d, J 7.3 Hz) displayed an array of correlations with signals at 3.24, 3.37, 3.51 and 3.76 ppm in the TOCSY spectrum. These 1H chemical shifts and the respective correlated carbons (HSQC) at: 75.3, 77.7, 73.3, 75.4 ppm together with the carboxyl group appearing at 176.0 ppm are consistent with the presence of a β-glucopyranuroyl residue. A further correlation, observed in the HMBC spectrum between the proton at 4.38 and the carbon at 91.1 ppm (C-3) confirmed the location of the β-glucopyranuroyl residue at C-3. Another correlation, between protons at 4.38 and 3.17 (aglycone H-3), observed in the T-ROESY spectrum reinforced the above deduction. The configurations at the anomeric carbons were assigned with the help of a NMR J-resolved heteronuclear experiment, on the basis of the measured J1H, C1H: 164 Hz for the signal at 94.4 ppm (C-1, Ara), 170.6 Hz for the signal at 30.6 ppm (C-2, Ara).
at 101.4 ppm (C-1, Rha), 157 Hz for the signal at 107.0 ppm (C-1, GlcUA), 169.8 Hz for the signal at 112.2 ppm (C-1, Api) and 169.8 Hz for the signal at 112.5 ppm (C-1, Api).

The individual spin systems for the other monosaccharide residues were assigned with the aid of the correlations between the anomeric protons (or methyl signal, for rhamnose) and the remaining protons in 2D TOCSY and COSY spectra. The chemical shifts of the apiofuranosyl residues were identified on the basis of C-3 being quaternary carbons (80.7, 80.4 ppm) and C-4 and C-5 being two methylenes. The assignments were double checked by comparison with literature chemical shift data and from HSQC and HMBC correlations. The sequence of the oligosaccharide chain was determined by a combination of DEPT, HSQC, HMQC, HMBC and T-ROESY experiments. Thus, the correlation observed in the HMBC spectrum between signals at 5.12 (H-1 rhamnose) and 75.4 ppm (C-1, GlcUA), 169.8 Hz for the signal at 112.2 ppm (C-1, Rha), 157 Hz for the signal at 107.0 ppm (C-1, Api) and 169.8 Hz for the signal at 112.5 ppm (C-1, Api). It is worth of mention the upfield shift (ca. –7 ppm) observed for C-3 in the rhamnose residue of 2 denoting absence of substitution at this site.

Acknowledgments

R.M. Borges acknowledges CNPq for the fellowship. The authors acknowledge Centro Nacional de Ressonância Magnética Nuclear de Macromoleculas Jiri Jonas, IBqM/UFRJ for 400 and 600 MHz NMR spectra.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

References

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Figure S1. $^{13}$C NMR of chiococasaponin I.

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Figure S2. $^1$H NMR of chiococcasaponin I.

Figure S3. Edited HSQC of chiococcasaponin I.
Figure S4. COSY of chiococcasaponin I.

Figure S5. TOCSY of chiococcasaponin I.
Two New Oleanane Saponins from Chiococca alba (L.) Hitch.

Figure S6. HMBC of chiococcasaponin I.

Figure S7. T-ROESY of chiococcasaponin I.
Figure S8. Mass spectrometry of Chiococcasaponin I (positive mode).
Figure S9. Mass spectrometry of Chiococcasaponin I (negative mode).
Figure S10. $^{13}$C NMR of chiococcasaponin II.
Two New Oleanane Saponins from *Chiococca alba* (L.) Hitch.

Figure S11. $^1$H NMR of chiococcasaponin II.

Figure S12. Edited HSQC of chiococcasaponin II.
Figure S13. COSY of chiococcasinon II.

Figure S14. TOCSY of chiococcasinon II.
Two New Oleanane Saponins from *Chiococca alba* (L.) Hitch.

Figure S15. HMBC of chiococcasaponin II.
Figure S16. Mass spectrometry of Chiococcasaponin II (positive mode).
Figure S17. Mass spectrometry of Chiococcasaponin II (negative mode).