Carbohydrate Research 343 (2008) 2699–2700

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

First record of L-quebrachitol in *Allophylus edulis* (Sapindaceae)

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ARTICLE INFO

Article history:
Received 12 June 2008
Received in revised form 17 July 2008
Accepted 22 July 2008
Available online 29 July 2008

Keywords:
Allophylus edulis
L-Quebrachitol
L-2-*O*-Methyl-*chiro*-inositol
Sapindaceae

ABSTRACT

Allophylus edulis, commonly called 'Chal chal', is a member of the Sapindaceae occurring in the Uruguayan and Brazilian native flora. During the phytochemical analysis of two Chal chal specimens from two well-differentiated geographical zones (Assis, São Paulo, Brazil, and Santa Lucía, Canelones, Uruguay), considerable amounts of L-quebrachitol were isolated from both samples. The isolation was carried out from the ethanolic twig extracts obtained by maceration of both vegetal samples. White easily distinguishable crystals were mechanically separated, washed, and characterized by 1D and 2D NMR experiments and by MS data. Such techniques confirmed that the crystals isolated from sources collected in both countries resulted in the same compound, L-quebrachitol, a natural product not previously reported for this species and one that has been investigated as a sugar substitute for diabetics. Worthy of note, the content of L-quebrachitol in *A. edulis* may be the chemical basis to explain its ethnobotanical uses, since infusions of this plant are used to treat diabetes in the practice of local traditional medicine.

Allophylus edulis (St. Hil.) Radlk (Sapindaceae), commonly called 'Chal chal', is a member of the Sapindaceae occurring in the Uruguayan and Brazilian native flora as well as in Bolivia, Argentina and the Guayanas.[1] Although this species has scarcely been studied, there are reports of its anti-hepatotoxic activity,[2] negative ionotropic activity,[3] and its inhibitory action of the angiotensin converting enzyme.[4]

During the course of a prospecting program of members of the native flora for anti-insectan products, members of Sapindaceae were studied.[5] Among them, *A. edulis* was biologically and chemically screened. This plant is commonly used as a source of tannins and essential oils.[6] Indeed a tisane (infusion) of the leaves is ethnically used as a throat anti-inflammatory agent and for intestinal disorders.[7]

L-Quebrachitol (**1**) is the 2-methyl ether of L-*chiro*-inositol. L-*chiro*-Inositol is a cyclitol whose isomers occur in various plant sources (i.e., Proteaceae,[8] Apocynaceae,[9] Elaeagnaceae,[10] Sapindaceae[11]). Quebrachitol was first described as a natural product in *Aspidosperma quebracho* (Apocynaceae).[12] Despite not being the most ubiquitous cyclitol, quebrachitol occurs in various species of Sapindaceae including *Acer pseudoplatanus* L., *Acer platanoides* L., *Cardiospermum halicacabum* L., *Alectryon excelsus* Gaertn., *Harpullia pendula* Planch., as well as members of the Aceraceae and Euphorbiaceae (within this family, *Hevea brasiliensis Muell. Arg.* is a good source of quebrachitol).[11] It has also been recently isolated from the leaves of *Allophylus cobbe* L. in Vietnam.[13] In nature, quebrachitol has been suggested to be a major contributor to osmotic pressure[14] and a cryoprotective agent for plants.[15] Besides, it has been shown to be a feeding stimulant for *Serrodes partita* (Lepidoptera: Noctuidae) larvae.[16] The present work reports the isolation of L-quebrachitol from specimens of *A. edulis* collected in two different locations. This natural product has not been previously reported in this species.

L-Quebrachitol was isolated as crystalline white powder; $[\alpha]_D^{25}$ −84 (0, 16% water);[17] ^{1}H NMR (D_2O, 400 MHz): δ 3.39 (dd, 1H, J_{2-1} 3.2, J_{2-3} 9.5 Hz, H-2); 3.44 (s, 3H, H–OMe); 3.60 (m, 2H, H-3, H-4); 3.73 (dd, 1H, J_{5-4} 9.6, J_{5-6} 3.2 Hz, H-5); 4.05 (dd, 1H, J_{6-1} 3.6, J_{6-5} 3.7 Hz, H-6); 4.25 (dd, 1H, J_{1-2} 3.5, J_{1-6} 3.6 Hz, H-1). ^{13}C NMR (100 MHz, D_2O): δ 57.333 (Me); 67.601 (C1); 70.877 (C5); 71.809 (C6); 72.369 (C4); 73.283 (C3); 80.602 (C2); HRESIMS: *m/z* 217. 0799 $[M+Na]^+$; Calcd for $C_7H_{14}O_6$, 194.0790. The recorded spectroscopic data were compared to the reported information for natural methyl inositols, and the compound was identified as L-quebrachitol.[18]

The ^{1}H NMR spectrum showed six signals between 3.3 and 4.3 ppm: one of them at δ 3.44 was assigned to three methoxyl protons, four (δ 3.39, 3.73, 4.05, and 4.25) were assigned to the methine protons, and a multiplet (δ 3.60) was attributed to two unresolved signals of the methine protons.

The ^{13}C NMR spectrum displayed seven carbon resonances with chemical shifts within the region of heteroatom-linked carbon

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0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.07.014

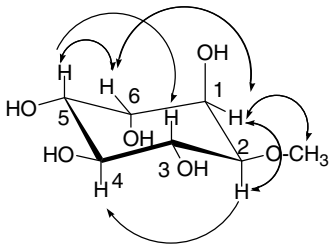

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Figure 1. Most significant NOEs.

atoms. The assignment of the protons was made by their spin-pattern analysis, COSY, HMQC, and HMBC experiments.

In spite of revealing the same R_f on TLC as D-3-*O*-methyl-*chiro*-inositol (D-pinitol), NMR experiments showed the cyclitol obtained from *A. edulis* was a different isomer, since the ^{13}C and ^{1}H chemical shifts from both samples were clearly different.

For the specific assignment as the isomer L-2-*O*-methyl-*chiro*-inositol (**1**), the values of the coupling constants (*J*) on the ^{1}H spectrum and the intensities of the signals from the NOEs spectra were taken into consideration. Each of the protons H-2 and H-5 couple with two different neighboring protons with one large (>9 Hz) and one small (<3.5 Hz) coupling constant. This coupling pattern indicates that both H-2 and H-5 have one axial and one equatorial proton neighbors. The signals corresponding to protons H-1 and H-6 show small coupling constants (<4 Hz), indicating either equatorial–equatorial or axial–equatorial couplings. While the H-3 and H-4 signals could not be differentiated on the basis of the ^{1}H spectra, analysis of NOEs showed an enhancement of the multiplet at δ 3.60 upon irradiation of H-5 or H-2, supporting the assignment of this multiplet to H-3 and H-4 (Fig. 1). The other results in NOE experiments supported the assigned stereochemistry for the remaining protons: irradiation of H-2, H-6 and the methyl group enhanced H-1; and they were in turn enhanced upon irradiation of H-1. H-6 was enhanced by irradiation of H-5, and irradiation of H-6 enhanced the H-5 signal.

This is the first report of L-quebrachitol in *A. edulis*, a plant that is used locally as an infusion to treat diabetes.[19] Interestingly, the potential of L-quebrachitol as a sugar substitute for diabetics has been investigated.[20] Even though the study reported that this methylcyclitol seems neither to prevent hypoglycemia nor to raise blood sugar content, a later work showed that L-quebrachitol has hypoglycemic effects in hyperglycemic rats.[21] It would be interesting to further investigate whether the local ethnobotanical use of *A. edulis* is related to its L-quebrachitol content.

1. Experimental

Extraction of plant material: *A. edulis* twigs were collected in the fall of 2005, at a riverbank nearby Montevideo city (Uruguayan sample) and at Assis, SP, Brazil (Brazilian sample). The Uruguayan species was identified by the Cátedra de Botánica, at the Chemistry School (Voucher number 4316 MVFQ), and the Brazilian species was identified by Inês Cordeiro (Voucher number Brumati 104). Plant material was air-dried, and ground (Kinematica AG, Polymix grinder) before extraction. Ethanolic extracts were produced under stirring (Labotec 23-2 stirrer, 50 rpm, 16 h) at room temperature (114 g of starting plant material treated with 1200 mL of ethanol). The procedure was repeated twice (re-extraction with 1200 mL, 88 h), and then the combined extracts were concentrated under vacuum to give a dark solid residue (Yields: Uruguayan sample, 9.4%; Brazilian sample, 8.1%). Acetone was added to this residue, and the solution was kept at 4 °C for 24 h to promote crystallization. The precipitate thus obtained was successively washed with hexane, chloroform, dichloromethane and ethyl acetate, yielding 51 mg of pure crystals for elucidation purposes. Although more material precipitated during the following days, no further purification was carried out. Methanol (HPLC grade) and deionized water (Milli-Q) were used throughout the whole study.

NMR spectra were recorded using a Bruker DPX-400 Avance Spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C) for solutions in D_2O (Cambridge Isotope Laboratories, Inc.).

ESI mass spectra were acquired in both positive- and negative-ion mode and recorded on a Quattro-LC spectrometer (Micromass, Manchester, UK) that held a quadrupole, hexapole quadrupole configuration (probe electrospray tip voltage 3 kV; cone voltage 25 V; nitrogen was used for both bath and nebulizing gas at 345 L/h and 27 L/h, respectively). Solutions were infused into the ESI source (5 μL/min) using a Harvard Apparatus model 1746 (Holliston, MA) pump. Accurate-mass measurements were performed on a quadrupole-time of flight instrument (UltrOTOF-Q, Bruker Daltonics, Billerica, MA). The optical rotation was determined in H_2O using a Zuzi automatic polarimeter, Model 412 with a sodium lamp operating at 589.3 nm at room temperature.

Acknowledgments

The authors thank OPCW (Grant L/ICA/ICB/111817/06), TWAS (Grant RGA 05-318), PEDECIBA (Grant PNUD- URU/06/004), and Programa ESCALA-AUGM (MD fellowship).

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