



Ent-labdane and beyerane diterpenes from *Erythroxylum betulaceum* Mart



Erika M. de O. Ribeiro^a, Juceni P. David^{b,*}, Jorge M. David^a, Maria L.S. Guedes^b,
Lucia M.X. Lopes^c, Zuzana Kršková^d, Jaroslav Dušek^d

^aInstituto de Química, Universidade Federal da Bahia, 40170-290, Salvador, BA, Brazil

^bFaculdade de Farmácia, Universidade Federal da Bahia, 40170-290, Salvador, BA, Brazil

^cInstituto de Química de Araraquara, Universidade Estadual Paulista, 14801-970 Araraquara, SP, Brazil

^dCharles University in Prague, Pharmaceutical Faculty in Hradec Králové, Heyrovského 1203, 50005 Hradec Králové, Czech Republic

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1. Subject and source

The Erythroxylaceae family comprises approximately 250 species distributed in four genera (Evans et al., 1981). *Erythroxylum* embraces ca 97% of all species of the family, which are distributed in the tropical and subtropical regions of the world with the main diversity occurring in Madagascar and South America, mainly in the Brazilian northeastern region (Hegnauer, 1981; Plowman and Hensold, 2004). The woods of *Erythroxylum betulaceum* Mart. was collected in the surroundings of Chapada Diamantina – BA (Brazil) and identified by M.L.S. Guedes, a voucher was deposited at the Herbarium Alexandre Leal Costa of Federal University of Bahia (UFBA) under number 69805.

2. Previous work

To date there are no previous phytochemical studies dealing with *E. betulaceum*. However, *Erythroxylum* is known by the presence of tropane alkaloids in leaves and fruits of various species (Oliveira et al., 2010). They are also a prolific source of assorted diterpenes (Connolly and Harding, 1972; Soman et al., 1983). The wood and fruits of *Erythroxylum* spp are known to contain *ent*-kaurane (McCrindle et al., 1968; Martin and Murray, 1968; Kapadi and Dev, 1964); *ent*-beyerane (Ansell et al., 1993a); *ent*-devadarane (Ansell et al., 1993a; Kapadi and Dev, 1964; dos Santos et al., 2006); ryanodane (Barreiros et al., 2007), *ent*-labdane (Ansell et al., 1993b) and *ent*-rosane (Ansell et al., 1993b, 1993c) diterpenes. Other common

* Corresponding author. Tel.: +55 7132836968; fax: +55 7132355166.

E-mail addresses: erikaoliveira1@yahoo.com.br (E.M.O. Ribeiro), juceni@ufba.br, jucenidavid@gmail.com (J.P. David), jmdavid@ufba.br (J.M. David), lopesxl@iq.unesp.br (Z. Lopes), zuzanaKrskova@seznam.cz (Z. Kršková).

compounds have also been reported in species of this genus such as flavonoids and oleanane triterpenes (Chavez et al., 1996; Johnson et al., 2003; Barreiros et al., 2002).

3. Present study

The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of the isolated compounds were acquired on Varian INOVA 500 (11.7 T) spectrometer, using standard pulse sequences for of homo- and heteronuclear correlation experiments. Optical rotations were measured using a Perkin–Elmer model 343 polarimeter. The melting points were measured on digital apparatus Microchemistry Ind. e Com. MQAPF-301. Conventional chromatographic methods were used for CC [silica gel 60 (Merck, 0.063–0.200 mm), (Acros organics, 0.035–0.070 mm)] and Sephadex LH-20 (Sigma). Silica gel TLC plates (Merck) were visualized with iodine fumes, Dragendorff and Liebermann Burchard reagents and viewed under UV light (254/366 nm).

The dried wood of *E. betulaceum* (964 g) was grounded and extracted twice with MeOH. The extract was suspended in H_2O and partitioned between CHCl_3 to obtain 4.0 g of CHCl_3 extract. This extract was submitted to Si gel CC employing mixtures of CHCl_3 :MeOH. The fractions eluted with CHCl_3 :MeOH (98:2) afforded vanillin (**1**, 4.4 mg) and, from the other fraction eluted with CHCl_3 :MeOH (95:5) diterpenes **2** (28.6 mg) and **3** (88.2 mg) (Fig. 1) were isolated. The fraction eluted with CHCl_3 :MeOH (93:7) was submitted to Sephadex LH-20 CC using CH_2Cl_2 :MeOH (1:1) as eluent and afforded *ent*-2 β ,19-dihydroxybeyer-15-en-1-one **4** (87.5 mg).

(–)-3-Oxomanool (**2**): pale amorphous solid; $[\alpha]_D^{25} = -11^\circ$ (MeOH, $c = 0.01$); 5.84 (dd, $J = 10.5$ and 17.5 Hz, H-14), 5.14 (dd, $J = 1.5$ and 17.3 Hz, H-15a), 5.01 (dd, $J = 1.5$ and 10.5 Hz, H-15b), 4.82 (d, $J = 1.5$ Hz, H-17a), 4.51 (d, $J = 1.5$ Hz, H-17b), ^{13}C NMR (CDCl_3) δ ppm: 37.6 (C-1), 34.7 (C-2), 216.7 (C-3), 47.7 (C-4), 55.2 (C-5), 25.1 (C-6), 37.9 (C-7), 147.3 (C-8), 56.1 (C-9), 39.4 (C-10), 18.2 (C-11), 41.1 (C-12), 73.5 (C-13), 145.0 (C-14), 111.8 (C-15), 28.2 (C-16), 107.6 (C-17), 26.0 (C-18), 21.7 (C-19), 14.0 (C-20). The NMR data were similar with that of 3-oxo-manool previously isolated from *Erythroxyllum pictum* (Ansell et al., 1993a).

(–)-3 β -Hydroxymanool (**3**): yellow amorphous solid; $[\alpha]_D^{25} = -9^\circ$ (MeOH, $c = 0.01$); ^1H NMR (CDCl_3) δ ppm: 5.82 (dd, $J = 10.5$ and 17.5 Hz, H-14), 5.13 (dd, $J = 1.5$ and 17.5 Hz, H-15a), 4.98 (dd, $J = 1.5$ and 10.5 Hz, H-15b), 4.74 (d, $J = 1.5$ Hz, H-17a), 4.42 (d, $J = 1.5$ Hz, H-17b), 3.17 (dd, $J = 4.5$ and 12 Hz, H-3), 1.19 (H-16); 0.91 (H-18); 0.69 (H-19), 0.60 (H-20); ^{13}C NMR (CDCl_3) δ ppm: 37.0 (C-1), 27.8 (C-2), 78.8 (C-3), 39.1 (C-4), 54.6 (C-5), 23.9 (C-6), 38.1 (C-7), 148.1 (C-8), 56.9 (C-9), 39.5 (C-10), 17.8 (C-11), 41.2 (C-12), 73.6 (C-13), 145.0 (C-14), 111.6 (C-15), 28.0 (C-16), 106.7 (C-17), 28.2 (C-18), 15.3 (C-19), 14.4 (C-20). All data were similar with that of 3 β -hydroxymanool also previously isolated from *E. pictum* (Ansell et al., 1993a).

ent-2 β ,19-Dihydroxybeyer-15-en-1-one (**4**): white crystalline solid; $[\alpha]_D^{25} = +47^\circ$ (MeOH, $c = 0.019$); ^1H NMR (CD_3OD) δ ppm: 4.55 (dd, $J = 5.4$ and 15 Hz, H-2), 1.81 and 1.57 (H-3), 1.80 (H-5), 1.69 and 1.54 (H-6), 1.66 and 1.35 (H-7), 1.34 (H-9), 1.52 and 1.89 (H-11), 1.26 (H-12), 1.11 and 1.47 (H-14), 5.70 (d, $J = 5.6$ Hz, H-15), 5.51 (d, $J = 5.5$ Hz, H-16), 1.0 (s, H-17), 1.2 (s, H-18), 3.53 and 3.39 (d, $J = 10.95$ Hz, H-19), 1.1 (s, H-20); ^{13}C NMR (CD_3OD) δ ppm: 217.9 (C-1), 71.8 (C-2), 41.3 (C-3), 38.9 (C-4), 52.3 (C-5), 21.0* (C-6), 38.8 (C-7), 51.2 (C-8), 47.1 (C-9), 51.0 (C-10), 23.2* (C-11), 33.8 (C-12), 44.9 (C-13), 62.2 (C-14), 135.6 (C-15), 138.4 (C-16), 25.1 (C-17), 25.7 (C-18), 69.0 (C-19), 17.3 (C-20). All data were in accordance with *ent*-2 β ,19-dihydroxybeyer-15-en-1-one (Ansell et al., 1993b).

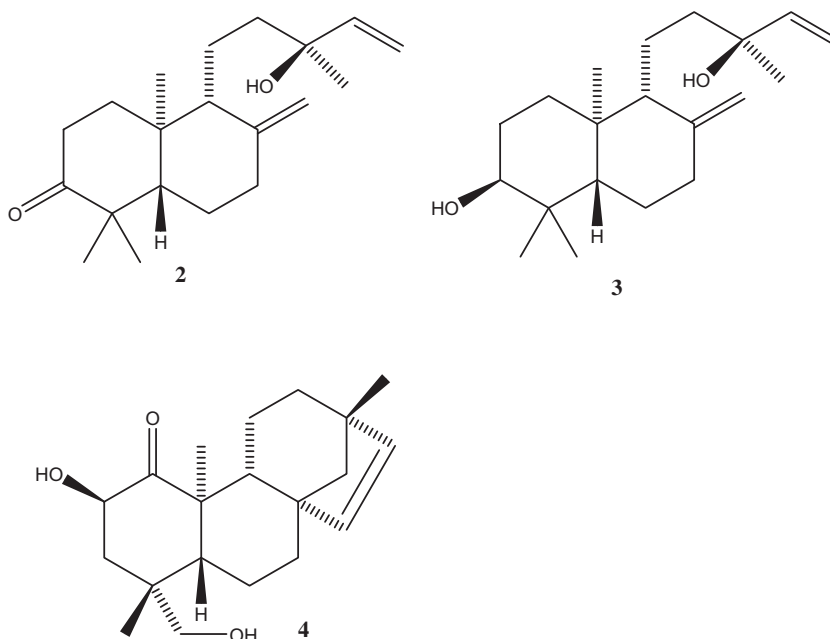


Fig. 1. Diterpenes from *Erythroxyllum betulaceum* Mart.

The identification of the isolated compounds were based on the spectra of ^1H and ^{13}C NMR including two dimensional techniques (gCOSY, gHSQC, gHMBC) and comparison with literature data. The labdane diterpenes were recognized as members of *ent*-series through their $[\alpha]_D$ values (David et al., 1998). The isolated compounds were identified as vanillin (**1**), 3-oxo (–)-manool (**2**), 3 β -hydroxy (–)-manool (**3**) and *ent*-2 β ,19-dihydroxybeyer-15-en-1-one (**4**).

4. Chemotaxonomic significance

Erythroxylum is the largest genus in the Erythroxylaceae family and comprises approximately 97% of its species, which are widely distributed in the tropics and subtropical regions showing extensive areas of diversity in South America (Plowman, 1987). In Bahia, the greatest concentration occurs along the coast in sandy soils of the “Restinga” and the Atlantic forest. This family is known by presence of three classes of compounds as chemical markers. Despite the occurrence of flavonoids in various families, flavonols and their 3-glycosides derivatives are considered markers of *Erythroxylum* (Barreiros et al., 2005). However this genus is best known as the source of tropane alkaloids, including cocaine. Tropane alkaloids are also chemotaxonomic markers of this family and this class of compound possesses restrictive occurrence in plant kingdom. A considerable number of these alkaloids are found in leaves and fruits of various species but, cocaine is present only in a few species (Oliveira et al., 2010). In addition, Erythroxylaceae is also a prolific source of different diterpenes, mainly labdanes and beyeranes of *ent*-series (Ansell et al., 1993c). In the present study, the vanillin (**1**) and three diterpenes (**2–4**) were purified from the timber of *E. betulaceum* (Fig. 1). Diterpenes **2**, **3** and **4** have been previously described in the family Erythroxylaceae; 3-oxo-manool (**2**) and 3 β -hydroxy-manool (**3**) has previously been reported only in timber *E. pictum* (Ansell et al., 1993a) and, to date, *ent*-2 β ,19-dihydroxybeyer-15-en-1-one (**4**) has been reported only in timber of *Erythroxylum australe* (Ansell et al., 1993b). Therefore, the relevance of this work lies in the fact of being the first phytochemical study realized with the species *E. betulaceum* and the isolated diterpenes are being described for the second time. So, the presence of diterpenes in *E. betulaceum* is in accordance with the chemical composition of the family. These results indicated that *E. betulaceum* possesses similar chemistry with other species of this family, with the presence of labdanes and beyerane diterpene derivatives. From a chemotaxonomical point of view, these diterpenes together tropane alkaloids may serve as potential chemotaxonomic markers for *Erythroxylum*.

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