Article

Trichotomol, a New Cadinenediol from Cordia trichotoma

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Um novo sesquiterpeno, nomeado trichotomol e compostos conhecidos como cordiacromo C, α -cadinol, ácido oleanólico, oncocalyxona A, β -sitosterol, glicosídeo do β -sitosterol, alantoína e sacarose foram isolados a partir do extrato etanólico do cerne de *Cordia trichotoma*. Suas estruturas foram determinadas por análises espectroscópicas e comparação com dados publicados para compostos estruturalmente relacionados.

A new sesquiterpene, named trichotomol, and known compounds cordiachrome C, α -cadinol, oleanolic acid, oncocalyxone A, β -sitosterol, β -sitosterol- β -D-glucoside, allantoin and sucrose were isolated from the heart wood ethanol extract of *Cordia trichotoma*. Their structures were assigned unambiguously by spectroscopic analyses and comparison with the published data for structurally related compounds.

Keywords: Cordia trichotoma, Boraginaceae, sesquiterpene, trichotomol, cordiachrome C

Introduction

Cordia trichotoma Vell. (Boraginaceae) is a tropical tree, popularly known as "frei jorge"1. According to a literature survey, several uses in traditional medicine such as cicatrizant, astringent, anti-inflammatory, antihelminthic, antimalarial, diuretic and to treat urinary infections, lung diseases and leprosy have been reported for several *Cordia* species²⁻⁴. No medicinal use has been reported for C. trichotoma, but its wood is recognized for its durability in carpentry and construction¹. Previous phytochemical investigations of plants from this genus have described several natural products structurally related to terpenoid quinone and hydroquino-nes⁵⁻ ⁷. In the last few years, several articles have been published on this kind of compounds, from Auxemma genus⁸⁻¹⁰, belonging to the same family and formerly considered synonimous of Cordia. To the best of our knowledge, except for a publication in which the presence of eudesmol isomers from C. trichotoma wood¹¹ has been recorded, there have been no other reports of any similar chemical investigation in the literature. In this paper we describe the isolation and structure elucidation of the known compounds: β -sitosterol, sitosterol- β -D-glucoside¹², oleanolic acid¹³, allantoin¹⁴, sucrose¹⁵, α -cadinol¹⁶, oncocalyxone A⁸, cordiachrome C⁵, and a new sesquiterpene, trichotomol (1). Although cordiachrome C (2) had been previously isolated from *C*. *millenii*, only the partial ¹H NMR data was provided but some doubt about its stereochemistry⁵ has remained. Here the complete ¹H and ¹³C spectral data and assignments for **2** are reported for the first time and used to corroborate the stereochemical aspects.

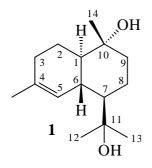
Results and Discussion

Compound **1** was obtained as colorless crystals, mp 159-160 °C and $[\alpha]_{589}$ = -17.1 (*c* 0.7, CHCl₃, 23 °C). Its IR spectrum revealed hydroxyl (3353 cm⁻¹ and 1116 cm⁻¹) and olefinic (1657 cm⁻¹) absorptions.

The molecular formula $C_{15}H_{26}O_2$, which indicates three double-bond equivalents, was deduced using EIMS, ¹³C NMR, and DEPT analyses. The ¹³C NMR (BB and DEPT) spectra displayed signals corresponding to four methyl, four methylene, four methine, and three nonhydrogenated carbons. Resonances due to two olefinic carbons at δ_C 134.3 (C) and 124.7 (CH) in the ¹³C NMR spectrum accounted for one double-bond equivalent,

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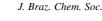
suggesting that **1** as a bicyclic compound. Two of the nonhydrogenated saturated carbons, $\delta_{\rm C}$ 74.2 and 72.1, were shifted to high frequency indicating they were attached to oxygen atoms. The EIMS spectrum did not present the molecular ion, but showed ions at m/z 220 (M - H₂O) and 202 (M - 2H₂O), in agreement with the presence of two hydroxyl groups for **1**. The ¹H NMR spectrum indicated resonances corresponding to four methyl groups, three of which were attached to carbons bearing hydroxyl groups: $\delta_{\rm H}$ 1.09 (s), 1.19 (s) and 1.20 (s), while the third one ($\delta_{\rm H}$ 1.64, s) due to the high frequency chemical shift seemed to be attached to a double bond. The presence of just one olefinic hydrogen $\delta_{\rm H}$ 6.14 (br s), suggested the presence of a trisubstituted double bond which is in accordance with ¹³C NMR data.



These data were similar to those reported for α -cadinol¹⁶. Except for the observed differences, especially for the carbon atoms at δ_C 53.0 (C-7), 74.2 (C-11), 24.1 (C-12) and 32.1 (C-13), of **1** in respect to those of α -cadinol, what could be explained by the existence of an additional C-11 hydroxyl group in **1**. The slight difference between the chemical shift of methyls C-12 and C-13 (δ_C 24.1 and 32.1, respectively) revealed that there is no free rotation around the single bond C₇-C₁₁, as expected. From the NOESY data it was possible to assign unambiguously the chemical shift of both carbons through the dipolar interaction of H-6 (δ_H 1.93) with the slightly more protected H-12 (δ_H 1.19) and the equatorial H-8 (δ_H 1.75) with the other one H-13 (δ_H 1.20). HMQC data it was easy to assign both carbon chemical shifts.

The relative stereochemistry of **1** was determined by analysis of the NOESY spectrum. The observed nOes for H-1 α , H-2 α , H-9 α and H-7 α ; for H-6 β , H-2 β and 3H-14 β were consistent with a *trans* configuration of the A/B rings. These data also suggested that the configurations of HO-10 and HO(CH₃)₂C-7 groups were α and β , respectively (Figure 1). Based on these data, the structure of **1** was determined as the 10 α ,11-dihydroxy-4-cadinene, which is a new sesquiterpene.

Compound **2** was obtained as an orange oil, and its molecular formula, $C_{16}H_{18}O_2$, was suggested by ¹³C NMR,



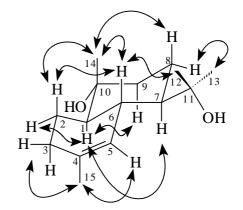


Figure 1. 1 H - 1 H dipolar correlations of 1 observed through NOESY experiment.

DEPT, and EIMS (m/z 242, [M]⁺). The IR spectrum of **2** showed the presence of carbonyl (1680 cm⁻¹) and olefinic (1656 cm⁻¹) groups.

Comparative analysis of BB and DEPT – ¹³C NMR spectra revealed six sp³ carbons (two methyls, two methylenes, one methine and one quaternary), two carbonyl groups and eight sp² carbons (three non-hydrogenated, three methines and two methylenes). The presence of a 1,4-benzoquinone moiety was revealed by the chemical shifts for H-2 $\delta_{\rm H}$ (6.70, d, J 9.2 Hz) and H-3 $\delta_{\rm H}$ (6.68, d, J 9.2 Hz), and for the carbons atoms C-1 ($\delta_{\rm C}$ 187.1) and C-4 ($\delta_{\rm C}$ 186.9).

The 500 MHz ¹H NMR spectrum presented information for all signals, including the homoallylic coupling of the methylene groups 2H-5 [$\delta_{\rm H}$ 2.66 (H-5 α), 2.24 (H-5 β)], and 2H-8 [$\delta_{\rm H}$ 2.60 (H-8 β), 2.44 (H-8 α)]. The signal at $\delta_{\rm H}$ 2.18 (dd, *J* 11.1 and 5.0 Hz) was attributed to H-10a, whose coupling constant values correspond to vicinal spin-spin interaction between hydrogens H-10 and H-10a.

¹H- and ¹³C-NMR spectra (DEPT and HMQC) also showed signals related to the methylene of a vinyl group – CH=CH₂ $\delta_{\rm H}$ [5.87 (dd, *J* 10.9 and 17.5 Hz, H-14), 4.98 (d, *J* 10.9 Hz, H-15a), 4.87 (d, *J* 17.7 Hz, H-15b)] and the methylene of an isopropenyl group –C(CH₃)=CH₂ $\delta_{\rm H}$ [4.88 (s, H-12a), 4.74 (s, H-12b), 1.73 (s, 3H-13)]. The heteronuclear long-range interaction between the methyl carbon CH₃-13 [$\delta_{\rm C}$ 23.2; $\delta_{\rm H}$ 1.73 (s)] and hydrogens 2H-12 $\delta_{\rm H}$ [4.88 (s) and 4.74 (s)] and H-7 ($\delta_{\rm H}$ 2.18, dd, *J* 11.1 and 5.0 Hz) observed in the HMBC spectrum, was also used to locate that methyl at carbon C-5 ($\delta_{\rm C}$ 145.1).

The *cis* relative configuration for the double bond moieties was supported from the chemical shift at δ 1.11 corresponding to the angular methyl (CH₃-16)⁵. The proposed stereochemistry was also supported by the NOESY experiment (Figure 2), that showed correlation between H-5 β , 3H-16 and H-7. Thus, **2** was identified as 6-ethenyl-5,6,7,8-tetrahydro-6-methyl-7-(1-methyl-ethenyl)-1,4-naphthalenedione.

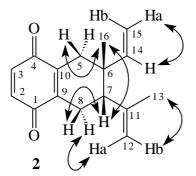


Figure 2. 1 H - 1 H dipolar correlations of 2 observed through NOESY experiments.

Experimental

General experimental procedures

Melting points were determined using a melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 1000 FT-IR instrument. EIMS data were obtained using a VG-Auto Spec mass spectrometer. Optical rotations were measured in a Perkin-Elmer 341 digital polarimeter. The NMR spectra were recorded in a Bruker DRX 500 [500 MHz (1 H) and 125 MHz (13 C)] spectrometer. Chemical shifts were recorded in δ (ppm) from TMS relative to the solvent absorption relative to TMS, $\text{CDCl}_3 \delta$ (7.24 and 77.0 ppm), and DMSO-d₆ (2.49 and 39.5 ppm). Column chromatography (CC) was performed using silica gel 60 (Merck). TLC analysis were performed on precoated silica gel UV₂₅₄ plates (Aldrich). Visualization of TLC plates was performed using a mixture of vanillin-perchloric acid-EtOH as a spray reagent. Spots were visualized by spraying the plates and then heating them at 100 °C for 1-3 min in a oven.

Plant material

Cordia trichotoma was collected in March 1998, at the Meruoca mountain, State of Ceará, Brazil, and identified by A. S. Nogueira de Castro and E. P. Nunes, botanists of the Universidade Federal do Ceará, where a voucher specimen is deposited (Herbarium Prisco Bezerra, N^o. 25.165).

Extraction and Isolation

The air-dried and pulverized heartwood (2.0 kg) was exhaustively extracted with EtOH at room temperature and then concentrated under vacuum to yield 124.0 g of a brown residue. The ethanol extract was first fractioned by CC with hexane, CHCl₃, EtOAc and MeOH. The hexane fraction was subjected to CC and eluted with mixtures of hexane and EtOAc of increasing polarities to give β -sitosterol (176.0 mg, mp 162-164 °C), oleanolic acid (25.0 mg, mp >300 °C), α-cadinol (58.0 mg, mp 73-74 °C) and the new compound **1** (186.0 mg, mp 158-160 °C). Similarly, CC of the CHCl₃ fraction, eluting with an hexane-EtOAc gradient, yielded oncocalyxone A (73.0 mg, mp 208-209 °C) and cordiachrome C (**2**, 32.6 mg). The EtOAc fraction gave βsitosterol-β-D-glucoside (287.0 mg, mp 289-292 °C), after repeated CC, using EtOAc-MeOH as eluent. From the MeOH fraction a precipitate was collected and was identified as sucrose (2.96 g, mp 185-186 °C). The residue from the supernanant MeOH fraction, after evaporation, was submitted to CC. Elution with increasing polarity with CHCl₃/EtOAc gave allantoin (630.0 mg, mp 230-232 °C).

Compound 1. C₁₅H₂₆O₂, 10α, 11-dihydroxy-4-cadinene. (185.6 mg, 1,49 %); colorless crystal, mp 159 - 160 °C (CHCl₃); [α]₅₈₉= - 17.1 (c 0.7, CHCl₃, 23 °C); IR ν_{max.}/ cm⁻¹ 3353, 2961, 2863, 1657, 1457, 1375, 1116 (KBr); ¹H NMR (CDCl₃, 500 MHz) δ 1.26 (m, H-1), 1.99 (m, H-2 α), 1.25 (m, H-2 β), 1.98 (m, H-3 β), 1.92 (m, H-3 α), 6.14 (s, H-5), 1.93 (m, H-6), 1.21 (m, H-7), 1.75 (m, H-8a), 1.03 (m, H-8 β), 1.46 (dq, J 3.4 and 12.5 Hz, H-9 α), 1.78 (dq, J 3.4 and 12.5 Hz, H-9β), 1.19 (s, 3H-12), 1.20 (s, 3H-13), 1.09 (s, 3H-14), 1.64 (s, 3H-15); ¹H NMR (DMSO-*d*₆, 500 MHz) δ 4.04 (s, HO-10), 4.11 (s, HO-11); ¹³C NMR (CDCl₃, 125 MHz,) δ 49.8 (CH-1), 22.7 (CH₂-2), 30.6 (CH₂-3), 134.3 (C-4), 124.7 (CH-5), 40.8 (CH-6), 53.0 (CH-7), 27.1 (CH₂-8), 42.3 (CH₂-9), 72.1 (C-10), 74.2 (C-11), 24.1 (CH₃-12), 32.1 (CH₃-13), 20.7 (CH₃-14), 24.1 (CH₃-15); EIMS (70 eV) m/z 220 (M – H₂O, 5), 202 (M – 2H₂O, 47), 43 (100).

Compound 2. C₁₆H₁₈O₂, 6-ethenyl-5,6,7,8-tetrahydro-6-methyl-7-(1-methylethenyl)-1,4-naphthalenedione. (32.6 mg, 0,026 %); orange oil; $[\alpha]_{589} = -1.11$ (c 0.27, CHCl₃, 23 °C); IR v_{max}/ cm⁻¹ 2920, 2851, 1680, 1656, 1464, 1376, 1278, 908, 725 (film); ¹H NMR (CDCl₃, 500 MHz) δ 6.69 (d, J 9.2 Hz, H-2), 6.67 (d, J 9.2 Hz, H-3), 2.66 (d, J 19.4 Hz, H-5 α), 2.24 (ddd, J 19.4, 4.1 and 2.4 Hz, H-5 β), 2.18 (dd, J 11.1 and 5.0 Hz, H-7), 2.60 (dd, J 19.9 and 2.6 Hz, H-8β), 2.44 (dddd, J 19.9, 11.1, 4.1 and 2.0 Hz, H-8α), 4.89 (s, H-12a), 4.74 (s, H-12b), 1.73 (s, Me-13), 5.87 (dd, J 17.5, and 10.9 Hz, H-14), 4.98 (d, J 10.9 Hz, H-15a), 4.87 (d, J 17.5 Hz, H-15b), 1.11 (s, Me-16); ¹³C NMR (CDCl₃, 125 MHz) δ 186.9 (C-1), 136.3 (CH-2), 136.3 (CH-3), 187.1 (C-4), 36.2 (CH₂-5), 37.7 (C-6), 49.4 (CH-7), 26.5 (CH₂-8), 141.6 (C-9), 140.7(C-10), 145.0 (C-11), 113.8 (CH₂-12), 23.1 (CH₃-13), 141.3 (CH-14), 113.4 (CH₂-15), 26.1 (CH₃-16); EIMS (70 eV) m/z 242 (M⁺, 15), 227 (M - CH₃,100), 199 (19).

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