Sesquiterpenes and a Phenylpropanoid from *Cordia trichotoma*

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Two new secondary metabolites, the phenylpropanoid 3-(2′,4′,5′-trimethoxyphenyl)propanoic acid (**1**) and the sesquiterpene (+)-1β,4β,6α-trihydroxyeudesmane (**2**) were isolated from the heartwood of *Cordia trichotoma* Vell., along with the known sesquiterpenes (–)-1β,4β,7α-trihydroxyeudesmane (**3**) and (+)-1β,4β,11-trihydroxyoppositane (**4**). Their structures were elucidated by means of spectroscopic data interpretation, mainly 1D and 2D NMR and mass spectrometry.

Key words: Cordia trichotoma, Sesquiterpenes, Phenylpropanoid

Introduction

In our continuing efforts to discover bioactive metabolites from the Northeastern Brazil flora we have investigated the chemistry of some *Cordia* species. The genus *Cordia* (Boraginaceae), comprises about 250 species of trees, shrubs and subshrubs restricted to the New World. Various species of *Cordia* are common members of the Brazilian flora, with at least 65 species of this genus distributed in several Brazilian States (Taroda and Gibbs, 1986a, b). Some of them are of interest due to their use in folk medicine (Marston *et al.*, 1988; Sertié *et al.*, 1990; Ioset *et al.*, 2000a, b; Kuroyanagi *et al.*, 2001).

In a previous paper we have reported the isolation and characterization of steroids, sesquiterpenes, triterpenes and terpenoid quinones from *C. trichotoma* wood (Menezes *et al.*, 2001) whose timber is recognized for its durability in carpentry and construction (Lorenzi, 2000). In the present paper, results of an investigation of the heartwood of *C. trichotoma* are reported. The secondary metabolites, one phenylpropanoid (**1**) and three sesquiterpenes (**3**–**4**) were isolated and characterized by their spectral data.

Results and Discussion

Chromatography of a chloroform fraction of the heartwood of *C. trichotoma* resulted in the isolation of two novel compounds (Fig. 1), 3-(2′,4′,5′-trimethoxyphenyl)propanoic acid (**1**) and the (+)-1β,4β,6α-trihydroxyeudesmane (**2**), and two known sesquiterpenes, (–)-1β,4β,7α-trihydroxyeudesmane (**3**) and (+)-1β,4β,11-trihydroxyoppositane (**4**).

Compound **1** was isolated as colorless needles. The ^{1}H NMR spectrum showed singlet signals for three methoxyl groups at δ_H 3.80, 3.78 and 3.73, two triplets for methylene hydrogens in a CH_2–CH_2 moiety at δ_H 2.79 (2H, t, J = 7.9 Hz) and 2.49 (2H, t, J = 7.9 Hz) and two aromatic hydrogens as

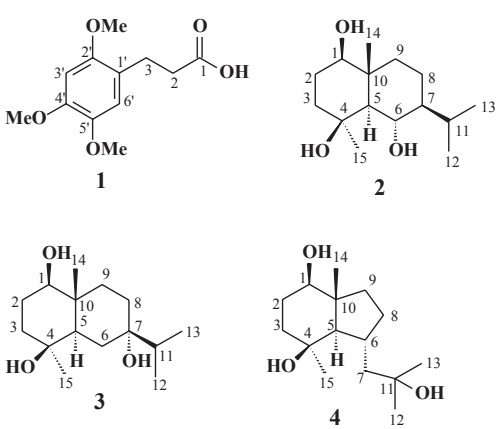

Fig. 1. Structures of 3(2′,4′,5′-triethoxyphenyl)propanoic acid (**1**), (+)-1β,4β,6α-trihydroxyeudesmane (**2**), (–)-1β,4β,7α-trihydroeudesmane (**3**) and (+)-1β,4β,11α-trihydroxyoppositane (**4**).

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singlets at δ_H 6.77 and 6.60 consistent with a 1,2,4,5-tetrasubstituted aromatic ring. HBBD- and DEPT-^{13}C NMR spectra showed 12 resonance lines corresponding to three methoxyl, two methylene, six aromatic carbons (two methine and four non-hydrogenated) and one carbonyl group at δ_C 177.3 consistent with a carboxylic acid, in agreement with the IR absorption at ν_{max} 1705 cm^{-1}. These NMR data were consistent with the molecular formula $(C)_4(CH)_2(CH_2)_2(CO_2H)(OCH_3)_3 = C_{12}H_{16}O_5$, which was confirmed by the EIMS (*m/z* 240, $[M]^{\cdot+}$, 55.3%), with the base peak at *m/z* 181 correspondent to the 1,2,4-trimethoxytropilium ion. Based on these spectral data the structure of compound **1** was established as the 3-(2′,4′,5′-trimethoxyphenyl)propanoic acid. The HMQC and HMBC spectra were used to confirm structure **1** and to assign the ^{1}H and ^{13}C chemical shifts unambiguously (*vide* experimental). The methyl ester derivative, but not **1**, has been previously isolated from the root bark of *C. alliodora* (Ioset *et al.*, 2000b).

Compound **2** was isolated as colorless needles; $[\alpha]_D^{25}$ + 42° (*c* 0.05, MeOH). Its IR spectrum displayed strong absorptions at ν_{max} 3415 cm^{-1}, suggesting the presence of hydroxyl groups. The ^{1}H and ^{13}C NMR spectra of **2** (Table I), indicated clearly its polyhydroxylated sesquiterpene character. This conclusion was supported by the EIMS, which showed a molecular ion at *m/z* 256, consistent with a molecular formula $C_{15}H_{28}O_3$, and additional significant peaks at *m/z* 241 ($[M\text{-}CH_3]^+$), 223 ($[241\text{-}H_2O]^+$) and 205 ($[241-2H_2O]^+$), revealing the elimination of two H_2O molecules. Fifteen resonance lines of sp^3 carbon atoms were observed in the ^{13}C NMR spectrum of **2**, which were characterized by DEPT 135° experiment as corresponding to four methyl, four methylene, five methine and two non-hydrogenated carbon atoms. The two double-bond equivalent and the exclusively presence of ^{1}H and ^{13}C signals of sp^3 carbon atoms allowed to classify **2** as a bicyclic sesquiterpene. The ^{1}H NMR spectrum exhibited signals for two *gem*-dimethyl of an isopropyl moiety at δ_H 0.98 (d, *J* = 7.0 Hz, 3H-12) and 0.91 (d, *J* = 7.0 Hz, 3H-13), an angular methyl at δ_H 1.02 (s, 3H-14) and a methyl attached to an oxygenated carbon at δ_H 1.46 (s, 3H-15), five methine hydrogens at δ_H 3.90 (t, *J* = 10.3 Hz, H-6), 3.18 (dd, *J* = 10.7

Table I. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compounds **2**, **3** and **4**, in CD_3OD. Chemical shifts in δ (ppm) and coupling constants (*J*, in parentheses) in Hz*.

	2		**3**		**4**	
C	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	80.4	3.18 (dd, 10.7, 4.1)	80.7	3.21 (dd, 11.9, 3.9)	80.4	3.32 (dd, 10.7, 4.3)
2	27.9	1.97 (dt, 10.7, 10.7, 3.1), H-β 1.56 (m), H-α	27.8	1.92, H-β 1.55, H-α	29.3	1.87 (13.4, 4.5), H-β 1.55 (m), H-α
3	42.7	1.68 (m), H-β 1.58 (m), H-α	40.8	1.69, H-β 1.50, H-α	42.7	1.64 (m), H-β 1.47 (dt, 13.4, 4.8), H-α
4	73.1	–	72.3	–	73.3	–
5	58.1	1.07 (d, 10.3)	46.3	1.46	60.9	0.94 (d, 10.9)
6	70.3	3.90 (t, 10.3)	29.5	1.58, H-β 1.43, H-α	33.7	2.27 (dq, 10.9, 3.8)
7	53.3	1.33 (m)	75.0	–	52.8	2.11 (d, 14.1) 1.36 (dd, 14.1, 10.9)
8	19.7	1.52 (m), H-β 1.37 (m), H-α	30.2	1.62, H-β 1.58, H-α	34.1	2.10 (m), H-β 1.41 (m), H-α
9	39.5	1.91 (td, 12.9, 3.1, 3.1), H-β 1.04 (m), H-α	35.9	1.65, H-β 1.42, H-α	40.9	1.59 (dd, 11.9, 7.9), H-β 1.22 (m), H-α
10	42.4	–	40.2	–	48.8	–
11	27.3	2.35 (m)	40.7	1.60	73.1	–
12	21.9	0.98 (d, 7.1)	17.7	0.95 (d, 6.9)	30.9	1.24 (s)
13	16.4	0.91 (d, 6.9)	17.5	0.96 (d, 6.9)	30.6	1.25 (s)
14	14.2	1.02 (s)	12.3	0.97 (s)	15.7	1.03 (s)
15	34.8	1.46 (s)	30.0	1.10 (s)	32.6	1.29 (s)

* Number of hydrogens bound to carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (*J*) obtained from 1D ^{1}H NMR spectra. Homonuclear 2D ^{1}H–^{1}H-COSY and heteronuclear 2D HMQC and HMBC spectra were also used in these assignments.

and 4.1, H-1), 2.35 (m, H-11), 1.33 (m, H-7) and 1.07 (d, J = 10.3, H-5), along with those due to two methylene groups, which appeared in the region at δ_H 1.97 – 1.37 ppm. These spectral data were used to postulate an eudesmane skeleton for **2**. The ^{13}C NMR spectra (HBBD and DEPT) showed the presence of three hydroxylated carbon atoms, two methine at δ_C 80.4 (CH-1) and 70.3 (CH-6) and one non-hydrogenated at δ_C 73.1 (C-4). The location of the hydroxyl group at C-1, C-4 and C-6 was established by means of the HMBC experiment, which was facilitated by comparison with the data described in the literature for an analogue sesquiterpene (Zhao *et al.*, 1997). A hydroxyl group attached to C-1 was evident by the long-range correlation between the angular methyl hydrogens at δ_H 1.02 (3H-14) with the carbon signals at δ_C 80.4 (CH-1, $^3J_{CH}$), 42.4 (C-10, $^2J_{CH}$) and 58.1 (CH-5, $^3J_{CH}$). The second hydroxyl group was located at C-4, based on observed correlations between the methyl hydrogens at δ_H 1.46 (3H-15, $^2J_{CH}$) with the carbon signals at δ_C 73.1 (C-4, $^2J_{CH}$) and 58.1 (CH-5, $^3J_{CH}$). The remaining hydroxyl group was linked to C-6 (δ_C 70.3) by the long-range correlation of the methine hydrogen at δ_H 1.07 (H-5, $^2J_{CH}$) with the carbon signal. The stereochemistry of **2** was solved by a combination of hydrogen coupling constants (J) of the chiral carbon atoms CH-1, CH-5, CH-6 and CH-7 and from the NOE effects revealed by ^1H-^1H-NOESY experiment (Fig. 2). The values corresponding to *vicinal* coupling of hydrogens H-1 (dd, J = 10.7 and 4.1) with H-2 (dt, J = 10.7, 10.7 and 3.1); H-5 (d, J = 10.3) with H-6 (t, J = 10.3) and this one with H-7 are consistent with the relative configuration shown in **2**, Fig. 1. Consistent with these observations, the NOESY spectrum of **2** (Fig. 2) also showed cross-peaks assigned to dipolar interaction of 3H-14 (δ_H 1.02) with H-2β (δ_H 1.97), H-6 (δ_H 3.90) and H-8β (δ_H 1.37); H-5α (δ_H 1.07) with H-1α (δ_H 3.18), H-3α (δ_H 1.58), 3H-15 (δ_H 1.46), H-7α (δ_H 1.33) and H-9α (δ_H 1.04). Thus, the structure of the new sesquiterpene isolated from *C. trichotoma* was determined as the (+)-1β,4β,6α-trihydroxyeudesmane (**2**). Table I shows all ^1H and ^{13}C NMR data assignments for **2**.

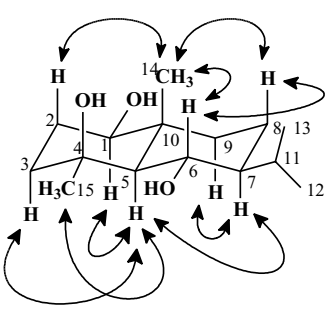

Fig. 2. Selected NOESY correlations for **2**.

Compound **3** and **4** were isolated as colorless prisms. They showed the same molecular formula, $C_{15}H_{28}O_3$, as **2**. Based on 1D and 2D (COSY, HMQC, HMBC, NOESY) NMR data and mass spectrometry the structures of the two compounds were established as (–)-1β,4β,7α-trihydroxyeudesmane and (+)-1β,4β,11-trihydroxyoppositane, respectively. Both compouds have been previously characterized from roots of *Homalomena aromatica* (Sung *et al.*, 1992). Despite its structure determination by spectroscopic data, complete ^1H and ^{13}C NMR data were assigned due to the differences reported for some carbon atoms.

Experimental

General

Melting points were measured on a digital Mettler Toledo FP90 apparatus and were uncorrected. The optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 1000 FT-IR spectrometer. Mass spectral data were acquired on a Shimadzu spectrometer. The NMR spectra were recorded in CD_3OD on a Bruker Avance DRX-500 (500 MHz for ^1H and 125 MHz for ^{13}C) spectrometer. Proton and carbon chemical shifts were referenced to residual MeOH (δ_H 4.87 and 3.31; δ_C 49.2). Silica gel 60 (Merck, 70–230 mesh) was used for column chromatography. Precoated silica gel plates (Merck, kieselgel 60 F_{254}, 0.20 mm) were used for analytical TLC. Chromatographic fractions were monitored by TLC visualized by spraying with vanillin/perchloric acid/EtOH followed by heating.

Plant material

The heartwood of *C. trichotoma* was collected in Acarape, State of Ceara, Brasil, in April, 2002. The plant was identified by Dr. Edson P. Nunes, and a voucher specimen (No. 25165) was deposited in the Herbarium Prisco Bezerra (EAC) of the Departamento de Biologia, Universidade Federal do Ceará.

Extraction and isolation

Air-dried and powdered heartwood (2.0 kg) of *C. trichotoma* was extracted with EtOH (2 × 8 l) at room temperature. The EtOH extract was taken to dryness under reduced pressure to yield 39 g of a dark brown gum. This extract was suspended in distilled H_2O/MeOH (7:3 v/v) and partitioned with $CHCl_3$ and *n*-BuOH. The $CHCl_3$ fraction (3.6 g) was chromatographed over silica gel and by elution with 0–100% EtOAc/*n*-hexane mixtures. The fractions were combined into 12 subfractions (F 01–12) based on TLC similarity. F 05 [850 mg, eluted with *n*-hexane/EtOAc (8:2 v/v)] and F 08 [150 mg, eluted with *n*-hexane/EtOAc (6:4 v/v)] yielded the two new compounds **1** (314 mg, 0.81%) and **2** (63 mg, 0.16%). While F 10 [170 mg, eluted with *n*-hexane/EtOAc (1:1 v/v)] yielded compound **4** (136 mg, 0.35%). F 09 [45 mg, eluted with *n*-hexane(EtOAc (6:4 v/v)] was rechromatographed over silica gel by elution with 20–100% EtOAc/*n*-hexane mixtures to afford **3** (7 mg, 0.02%).

3-(2′,4′,5′-trimethoxyphenyl)propionic acid (**1**): Colorless needles, m.p. 95–96° C. – IR (KBr): ν_{max} = 3514, 3438, 1705, 1645, 1524, 1452, 1206, 1034 cm^{-1}. – EIMS: *m/z* (rel. int.) = 240 ($[M]^+$, 55.3), 225 (13.4), 197 (9.8), 181 (100), 151 (33.0). – ^1H NMR (CD_3OD): δ = 6.77 (1H, s, H-6′), 6.60 (1H, s, H-3′), 3.80 (3H, s, 4′-OCH_3), 3.78 (3H, s, 2′-OCH_3), 3.73 (3H, s, 5′-OCH_3), 2.79 (2H, t, *J* = 7.92 Hz, H-3), 2.49 (2H, t, *J* = 7.92 Hz, H-2). – ^{13}C NMR (CD_3OD): δ = 177.3 (C-1), 153.5 (C-2′), 149.9 (C-4′), 144.2 (C-5′), 122.0 (C-1′), 116.6 (C-6′), 99.4 (C-3′), 57.7 (5′-OCH_3), 56.9 (4′-OCH_3), 56.7 (2′-OCH_3), 35.6 (C-2), 26.7 (C-3).

(+)-1β,4β,6α-trihydroxyeudesmane (**2**): Colorless needles, m.p. 220–221° C. – $[\alpha]_D^{25}$ + 42° (*c* 0.05, MeOH). – IR (KBr): ν_{max} = 3415, 2935, 2870, 1461, 1376, 1074, 1026 cm^{-1}. – EIMS: *m/z* (rel. int.) = 256 ($[M]^+$, < 1), 241 ($[M\text{-}CH_3]^+$, 3.5), 223 ($[241\text{-}H_2O]^+$, 3.8), 205 ($[241-2H_2O]^+$, 2.1), 123 (7.9), 101 (46.9), 81 (23.0), 55 (28.3), 43 (100). – ^1H and ^{13}C NMR spectral data: see Table I.

(–)-1β,4β,7α-trihydroxyeudesmane (**3**): Colorless prisms, m.p. 138–141° C (Lit. m.p. 135–141° C). – $[\alpha]_D^{25}$ – 1.1° (*c* 0.05, MeOH). – IR (KBr): ν_{max} = 3435, 2933, 2859, 1466, 1376, 1271, 1027 cm^{-1}. – EIMS: *m/z* (rel. int.) = 256 ($[M]^{\cdot+}$, < 1), 213 ($[M\text{-}CH(CH_3)_2]^+$, 17.8), 195 ($[213\text{-}H_2O]^+$, 71.4), 177 ($[213-2H_2O]^+$, 32.1), 43 (100). – ^1H and ^{13}C NMR spectral data: see Table I.

(+)-1β,4β,11-trihydroxy-8(7→6)-abeoeudesmane (**4**): Colorless prisms, m.p. 179–180° C (Lit. m.p. 174–175° C). – $[\alpha]_D^{25}$ + 12° (*c* 0.05, MeOH). – IR (KBr): ν_{max} = 3353, 2971, 2863, 1465, 1373, 1266, 1185, 1024 cm^{-1}. – EIMS: *m/z* (rel. int.) = 256 ($[M]^{\cdot+}$, < 1), 241 ($[M\text{-}CH_3]^+$, 1.6), 223 ($[241\text{-}H_2O]^+$, 9.7), 205 ($[241-2H_2O]^+$, 2.1), 179 (23.0), 147 (14.1), 123 (48.7), 59 (30.9), 43 (100). – ^1H and ^{13}C NMR spectral data: see Table I.

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