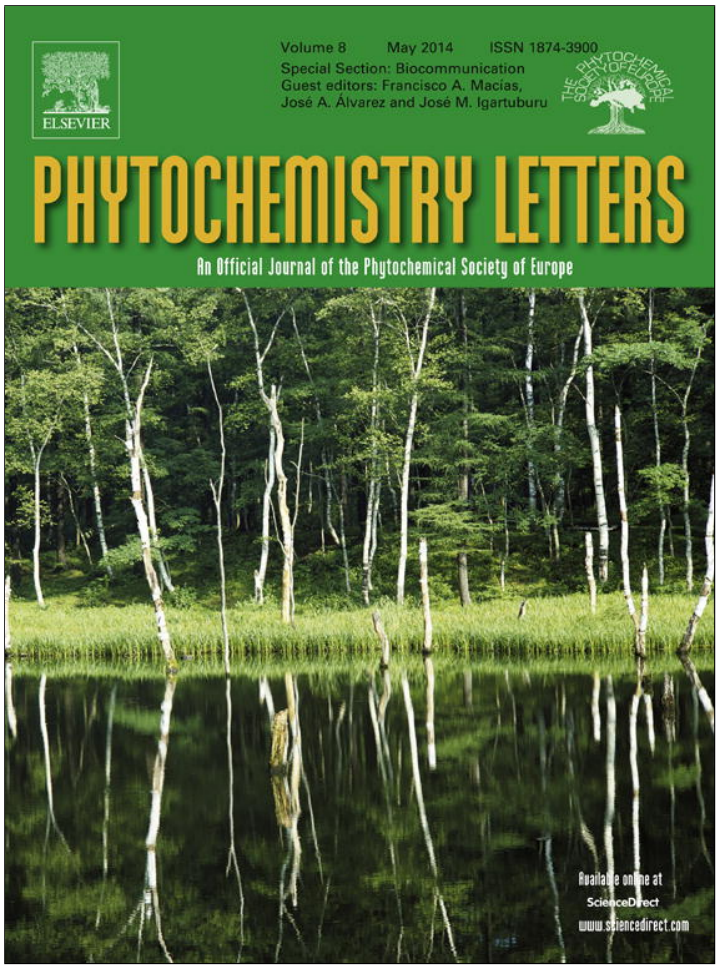

Phytochemistry Letters 8 (2014) 193–195

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Terpenes and steroids from leaves of *Oxandra sessiliflora* R. E. Fries

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ARTICLE INFO

Article history:
Received 15 September 2012
Received in revised form 22 September 2013
Accepted 26 September 2013
Available online 16 October 2013

Keywords:
Oxandra sessiliflora
Sesquiterpenes
4α,7β,10α-trihydroxyguai-5-ene

ABSTRACT

The EtOH extract from the leaves of *Oxandra sessiliflora* R. E. Fries (Annonaceae) was partitioned using hexane and CH_2Cl_2. After several chromatographic steps, caryophyllene oxide and spathulenol were isolated from hexane phase while, from CH_2Cl_2 phase, we isolated (*E*)-phytol, spathulenol, 4β,10α-dihydroxyaromadendrane, 1β,6α-dihydroxyeudesm-4(15)-ene, and 4α,7β,10α-trihydroxyguai-5-ene, the latter being a new sesquiterpene derivative. Additionally, a mixture of steroids (campesterol, sitosterol, and stigmasterol) was obtained from the CH_2Cl_2 phase. The isolated compounds were characterized by mass spectrometry and analysis of their ^1H and ^{13}C NMR spectroscopic data, including bidimensional analysis.

1. Introduction

The genus *Oxandra* (Annonaceae) is constituted by approximately 22 species (Lobão et al., 2005; Leboeuf et al., 1982) of which 14 occur in Brazilian territory, mainly in North and Northest regions (Forzza et al., 2010). Systematic phytochemical studies had previously been performed with stem bark and leaves of *O. xylopioides*, which resulted in the isolation of several alkaloids (including dimmeric derivatives), cycloartane triterpenoids, aromatic monoterpenoids, and steroids (El-Shanawany, 1985a; El-Shanawany et al., 1985b; Arango et al., 1987; Mérienne et al., 1987; Zhang et al., 1987; Guinaudeau et al., 1988; Rojano et al., 2007). Two other studies reported the isolation of numerous carvacrol derivative monoterpenes from stem bark of *O. espintana* (Hocquemiller et al., 1991) and that of alkaloids, sesquiterpenes and triterpenes from leaves of *O. asbeckii* (Tinto et al., 1992). In the present paper we report the first chemical study of *O. sessiliflora*, by means of which we were able to isolate and identify five sesquiterpenes – caryophyllene oxide (**1**), spathulenol (**2**), 4β,10α-dihydroxyaromadendrane (**3**), 1β,6α-dihydroxyeudesm-4(15)-ene (**5**), and a new derivative, 4α,7β,10α-trihydroxyguai-5-ene (**4**), the diterpene (*E*)-phytol (**6**) and a mixture of three steroids (campesterol, sitosterol and stigmasterol), from an EtOH extract obtained from leaves of this plant.

2. Results and discussion

The EtOH extract from the leaves of *O. sessiliflora* was sequentially partitioned using hexane and CH_2Cl_2. These phases were subjected to repeated chromatographic procedures on Sephadex LH-20 and on silica gel columns. Spectrometric analysis of purified fractions resulted in the identification of five sesquiterpenoids: caryophyllene oxide (**1**), spathulenol (**2**), 4β,10α-dihydroxyaromadendrane (**3**), 1β,6α-dihydroxyeudesm-4(15)-ene (**5**), and a new derivative that was characterized as 4α,7β,10α-trihydroxyguai-5-ene (**4**). Additionally, the diterpene (*E*)-phytol (**6**) and a mixture composed by three steroids (campesterol, sitosterol and stigmasterol) were also isolated. To our knowledge, this is the first report of **1**, **3–6** from the genus *Oxandra*.

The structures of terpenoids **1**–**3**, **5**, and **6** as well as those of the steroids were defined by analysis of their ^1H and ^{13}C NMR spectroscopic data and comparison with literature data (Iwabuchi et al., 1989; Rahman and Ahmad, 1992; Heymann et al., 1994; De-Eknamkul and Potduang, 2003; Moreira et al., 2003, 2007; Costa et al., 2008, 2009; Ibrahim et al., 2012). Moreover, the identification of campesterol (9.62%), sitosterol (75.67%) and stigmasterol (14.71%) was confirmed by LREIMS after separation by gas chromatography (GC).

Compound **4** (Fig. 1) was isolated as a colorless amorphous solid with a molecular formula $C_{15}H_{26}O_3$ as indicated by the HRESIMS *quasi*-molecular ion at *m/z* 253.0737 Da $[M-H]^-$. Its IR spectrum showed absorption bands at 3434 cm^{-1}, characteristic of hydroxyl

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http://dx.doi.org/10.1016/j.phytol.2013.09.014

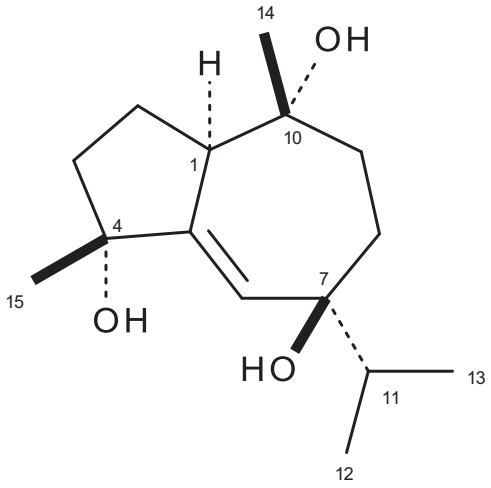

Fig. 1. Chemical structure of compound **4**.

group stretching and at 1647 cm^{-1}, assigned to olefin stretching. The ^{13}C, DEPT 135° and 90° NMR spectra displayed fifteen signals referring to four methyls, four methylenes, three methines, as well as four quaternary carbon atoms, three of these being oxygenated (δ 80.4, 90.0 and 75.4), which confirmed the occurrence of a trihydroxylated sesquiterpene. In addition, the signals at δ 127.1 (CH) and δ 149.9 (C) indicated the presence of an endocyclic double bond in the structure of **4**. These data, associated to the value of the insaturation equivalent (3), suggested the occurrence of a bicyclic sesquiterpene. Comparison of these data with those of the guaiane derivatives leptocladol A (Su et al., 2010) and guai-6-en-10β-ol (Lago et al., 2000) indicated that **4** has also a guaiane skeleton with a double bond at C-5 and two hydroxyl groups at C-4 and C-10.

The position of third hydroxyl group was determined based on the analysis of ^{1}H NMR data. This spectrum displayed singlets of two methyl groups linked to quaternary carbons at δ 1.16 (H-14) and 1.31 (H-15), as well as two doublets referring to methyl of an isopropyl group at δ 0.91 (3H, d, *J* 7.0 Hz, H-12) and at δ 0.94 (3H, d, *J* 7.0 Hz, H-13). Therefore, there were only two possible positions for the third hydroxyl group: C-7 or C-1. Since the signal of H-1 was observed at δ 2.88 (^{1}H, ddd, *J* 8.5, 6.0, 2.5 Hz), the hydroxyl group must be linked to C-7. Analysis of ^{1}H–^{1}H COSY spectrum, which showed correlations between the signal at δ 2.88 (H-1) and those at δ 1.96 (H-2) and δ 5.77 (H-6), as well as between the signal at δ 2.18 (H-11) and those at δ 0.91 (H-12) and δ 0.94 (H-13) confirmed such positioning.

Hydrogen-bearing carbon signals were assigned by analysis of the HMQC spectrum. Analysis of HMBC spectrum (Table 1) indicated ^{1}H–^{13}C long-range couplings between the signal at δ 80.4 (C-4) and those at δ 1.96 (H-2), 1.54/1.75 (H-3), 5.77 (H-6), and at δ 1.31 (H-15); between the peak at δ 34.1 (C-11) and those at δ 5.77 (H-6), 0.91 (H-12) and at δ 0.94 (H-13); as well as between the signal at δ 90.0 (C-7) and those at δ 2.18 (H-11), 0.91 (H-12), and at δ 0.94 (H-13). Finally, the presence of cross peaks between the signals at δ 51.5 (C-1) and those at δ 1.96 (H-2), 1.16 (H-14), 1.54/1.75 (H-3), 1.75/1.90 (H-9) and at δ 5.77 (H-6) confirmed a planar structure of **4** as 4,7,10-trihydroxyguai-5-ene, similar to another one previously reported in the literature (Yang and Shi, 2012).

Despite the similarities between the ^{1}H and ^{13}C NMR data of **4** and 4β,7β,10α,trihydroxi-guai-5-ene (Yang and Shi, 2012), some distinctive differences were detected in the NOESY spectrum. First of all, there were cross-peaks between H-1/H-11, establishing the isopropyl group in α position and, consequently, the hydroxyl group in β position at C-7. Moreover, correlations between H-14/H-15, but not between H-1/H-15 or H-1/H-14, were observed. Such correlations determined the relative configuration of C-1 and C-10 as previously depicted, but opposite to C-4. As a result, these data confirmed the structure of compound **4**, named 4α,7β,10α-trihydroxyguai-5-ene, as that presented in Fig. 1.

3. Experimental procedures

3.1. General procedures

Chromatographic separations were based on gel filtration over silica gel (70–230 mesh, Acros Organics), Sephadex LH-20 (Sigma), and pre-coated silica gel 60 PF_{254} TLC plates (Merck), the spots being visualized under UV lamp (254 and/or 366 nm) or by spraying $Ce(SO_4)_2$ solution followed by heating at 100 °C. IR spectra were recorded on a PerkinElmer Spectrum 100 spectrometer. ^{1}H (500 MHz), ^{13}C (125 MHz), ^{1}H–^{1}H COSY (500 MHz), NOESY

Table 1
^{13}C, ^{1}H and HMBC NMR data of compound **4** (500 MHz, δ/ppm, CD_3OD).

	^{13}C	^{1}H	HMBC (C → H)	
			$^{2}J_{CH}$	$^{3}J_{CH}$
C[a]				
4	80.4	–	H-15, H-3	H-6, H-2
5	149.9	–	H-1, H-6	H-15, H-3, H-2
7	90.0	–	H-11	H-12, H-13
10	75.4	–	H-14, H-9, H-1	H-2
CH				
1	51.5	2.88 (ddd, *J* = 8.5, 6.0, 2.5 Hz)	H-2	H-14, H-3, H-9, H-6
6	127.1	5.77 (d, *J* = 2.5 Hz)	H-11	H-1
11	34.1	2.18 (sept, *J* = 7.0 Hz)	H-12, H-13	–
CH_2				
2	24.6	1.96 (m)	H-3, H-1	–
3	41.1	1.54 and 1.75 (m)	H-2	H-15
8	27.0	1.30 and 1.96 (m)	H-9	H-11, H-6
9	40.1	1.75 and 1.90 (m)	–	H-14
CH_3				
12	17.1	0.91 (d, *J* = 7.0 Hz)	H-11	H-13
13	17.7	0.94 (d, *J* = 7.0 Hz)	H-11	H-12
14	23.3	1.16 (s)	–	H-1, H-9
15	28.1	1.31 (s)	–	H-3

[a] Multiplicity obtained from DEPT 90° and 135° spectra.

(500 MHz), HMQC (500 and 125 MHz), and HMBC (500 and 125 MHz) NMR spectra were measured using CD_3OD as solvent on a Varian INOVA spectrometer. LREIMS (electronic impact – 70 eV) spectra were obtained on a Shimadzu QP-5050 spectrometer while HRESIMS (electron spray ionization – negative mode) spectrum was recorded on a Bruker Daltonics UltrOTOFq-ESI–TOF spectrometer. GC analyses were performed using a Shimadzu CG-17A with a mass selective detector QP5050A, equipped with a DB-5HT column (30 m × 0.25 mm internal diameter and 0.1 μm film thickness), using helium as carrier gas (1 mL/min). Temperature programming was performed as follows: 100–250 °C at 10 °C/min and 250–300 at 5 °C/min. The injector and interface temperatures were established as 260 °C and 300 °C, respectively.

3.2. Plant material

The leaves of *O. sessiliflora* were collected at Parque Ambiental de Teresina, Piaui State, Brazil in June 2009. The plant authentication was performed by Professor Roseli Farias Melo Barros and a voucher specimen (TEPB 27870) has been deposited at Herbario Graziela Barroso do Amaral (UFPI).

3.3. Extraction and isolation

The dried leaves (779 g) of *O. sessiliflora* were powdered and then exhaustively extracted with EtOH at room temperature. The combined EtOH solutions were concentrated under vacuum to afford 109 g (14%) of a green syrupy extract, which was subsequently dissolved in 1 L of MeOH–H_2O (2:1) and sequentially extracted using hexane and CH_2Cl_2. Part of the hexane fraction (10 g) was subjected to SiO_2 column chromatography using increasing amounts of EtOAc in hexane to yield 118 fractions (250 mL each), which were combined into 19 groups (A1–A19) after TLC analysis. Group A3 (1122 mg) was subjected to a flash SiO_2 column chromatography using a mixture of hexane:EtOAc 99:1 as eluent, to obtain **1** (109 mg). Group A6 (666 mg) was applied onto a Sephadex LH-20 column, eluted with hexane:CH_2Cl_2 (1:4), to yield **2** (34 mg). Part of the CH_2Cl_2 fraction (10 g) was subjected to SiO_2 column chromatography using increasing amounts of EtOAc in hexane producing 125 fractions (250 mL each), which were combined into 24 groups (B1–B24) after TLC analysis. The combined groups were individually subjected to Sephadex LH-20 column chromatography, with a hexane-CH_2Cl_2 (1:4) mixture as eluent. Groups B4 (81 mg) and B5 (33 mg) yielded **2** (13 mg) and **6** (8.5 mg), respectively. Group B9 (78 mg) generated a mixture of campesterol (**7**), sitosterol (**8**), and stigmasterol (**9**) (19 mg). Group B15 (214 mg) was divided into 20 hexane-CH_2Cl_2 (1:4)-elution fractions, which were pooled into three groups (B15/1–B15/3) after TLC analysis. Group B15/2 (74 mg) was further purified using flash SiO_2 column chromatography (hexane:EtOAc 8:2) to obtain **5** (13 mg). Group B18 (317 mg) afforded **3** (33 mg). Group B19 (208 mg) was also subdivided into 24 fractions, which we combined into five groups (B19/1–B19/5) after TLC analysis. Group B19/3 (73 mg) was further purified using flash SiO_2 column chromatography (hexane:$CHCl_3$:-MeOH 50:45:5) to beget **4** (14 mg).

3.4. 4α,7β,10α-Trihydroxyguai-6-ene (4)

Colorless amorphous solid; IR (KBr) λ_{max}/cm^1: 3434 (O–H); 2961/2925 (C–H); 1647 (C=C); 1102/1261 (C–O). HRESIMS: *m/z* 253.0737 $[M-H]^-$ (calculated: 253.1805, $C_{15}H_{25}O_3$). NMR spectroscopic data (500 MHz and 125 MHz, CD_3OD): see Table 1.

Acknowledgments

The authors would like to thank Prof. Roseli F. M. Barros (UFPI) for the botanical identification of the species, Dr. Ari J. S. Ferreira for reviewing the manuscript, and CNPq, CAPES, and FAPESP for the financial support.

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