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Article

Fatty Acid Esters of Triterpenes from *Erythroxylum passerinum*

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O extrato hexânico das folhas de *Erythroxylum passerinum* além de sitosterol, β-amirina, lupeol e eritrodiol, forneceu palmitato de β-amirina, palmitato de 3β,28-diidroxi-olean-12-enila (eritrodiol); palmitato de 3β,11β-diidroxi-olean-12-enila; palmitato de 3β-hidroxi-11-oxo-olean-12-enila e palmitato de 3β-hidroxi-11,12-epoxi-friedoolean-14-enila. As estruturas das substâncias foram estabelecidas através da análise dos seus dados espectrométricos, bem como de seus derivados transesterificados, utilizando-se técnicas de RMN de ^{1}H e de ^{13}C, infravermelho e espectrometria de massas.

The hexane extract of leaves of *Erythroxylum passerinum* yielded besides sitosterol, β-amyrin, lupeol and erythrodiol, β-amyrin palmitate, 3β,28-dihydroxy-olean-12-enyl palmitate, 3β,11β-dihydroxy-olean-12-enyl palmitate, 3β-hydroxy-11-oxo-olean-12-enyl palmitate and 3β-hydroxy-11,12-epoxy-friedoolean-14-enyl palmitate. The structural elucidation of the isolates and of their derivatives were based on spectral data (^{1}H and ^{13}C NMR, IR and MS).

Keywords: *Erythroxylum passerinum*, Erythroxylaceae, fatty acid esters of triterpenes

Introduction

The genus *Erythroxylum* is the largest of the Erythroxylaceae family embracing 97% of its species, which are mainly spread through tropical and subtropical regions.[1,2] Many wild and endemic species of this genus, occur in the Bahia State, mostly along the coast in Restinga and the Restinga forest on sandy soils.[3] The occurrence of species in both Restinga and moist tropical forests soil is also common.[3,4]

The chemical composition of this genus is formed basically by alkaloids and terpenes, especially triterpenes which are found in leaves and fruits of its species.[2] The presence of fatty acids esterified with triterpenes in leaves has been a common characteristic of species that occur in the Brazilian Restinga.[4]

The present work describes the isolation of β-sitosterol, lupeol, β-amyrin, erythrodiol and of fatty acids esterified with triterpenes at the C-3 position (**1**-**5**). The following compounds were isolated and are described below: β-amyril palmitate (**1**), 3β-hydroxy-11-oxo-olean-12-enyl palmitate (**2a**), 3β,11β-dihydroxy-olean-12-enyl palmitate (**3**), 3β,28-dihydroxy-olean-12-enyl palmitate (**4**) and 3β-hydroxy-11,12-epoxy–friedoolean-14-enyl palmitate (**5**).

Results and Discussion

The structural elucidation of fatty acids esterified with triterpenes by NMR is only possible by analyses of ^{13}C data (PND and DEPT experiments). The ^{1}H NMR spectrum is not informative due to the predominance of the peak of methylene groups of the fatty acids. However, the triterpene moieties of these compounds can be preliminary checked by TLC employing the Lieberman-Burchard spray reagent as the developer. Otherwise the nature of the fatty acid contents can be identified by GC/MS analyses of their methyl esters produced through transesterification reactions.

The ^{13}C NMR spectra of compounds **1**-**4** showed methine and quartenary Csp^2 signals (δ 121.9 ± 0.3 and 144.6 ± 0.5) characteristic of oleanene triterpenes.[5] Detailed analyses of these spectra indicated that all triterpene moieties were esterified with fatty acids. This observation was supported by the presence in the spectra of all compounds of peaks of an additional methyl (δ 13.97 ± 0.06) and of acyl groups (*ca.* δ 173.4) whose carbonyls

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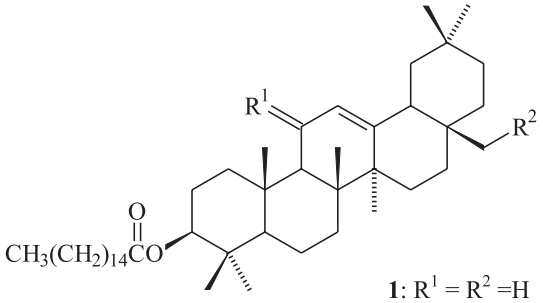
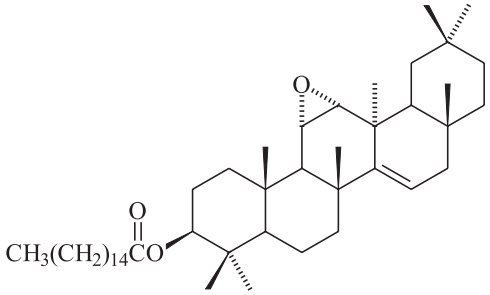


1: $R^1 = R^2 = H$
2: $R^1 = O$; $R^2 = H$
3: $R^1 = \beta$-OH; $R^2 = H$
4: $R^1 = H$; $R^2 = OH$



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were downshielded ($\Delta\delta$ *ca.* 3.2) relative to 3β-acetyl-amyrin. Comparison of the chemical shifts observed for the carbonyl carbons in the fatty acid moiety in these compounds with those found for 3β-acetyl-amyrin showed *ca.* 3 ppm downshield. The C-2 of the triterpene moieties in these compounds were shielded ($\Delta\delta$ 4 ppm), while the oxy methine carbons showed downshielding effect in comparison to C-3 of β-amyrin.[5] In addition, the C-3 signals of these triterpenes appeared at higher frequency (δ 80.5 ± 0.05 ppm) than that observed for β-amyrin (δ 78.9). The molecular ions recorded on the MS at *m/z* 678 (**2**), *m/z* 662 (**3**) and *m/z* 662 (**4**) were conclusive of the esterified nature of the triterpenes.

The identification of the triterpene moiety of **1** as being β-amyrin was possible by direct comparison of the NMR data of **1** with the data reported in the literature.[5] Transesterification of this compound yielded β-amyrin and methyl palmitate (*m/z* 270) which were characterized by GC analysis.

The ^{13}C NMR spectra (PND and DEPT) of compound **2** displayed characteristic signals for a α,β-unsaturated ketone group at δ 200.2, 128.1 and 170.6 . The presence of this group was corroborated by the ^{1}H NMR spectrum which displayed a singlet at δ 5.59 attributed to the α-carbonyl hydrogen (H-12). In addition, this assignment was confirmed by the presence of a signal at δ 2.36 due to H-9 in agreement with the methine signal at δ 61.7 displayed for C-9 in the ^{13}C NMR spectrum. In the ^{1}H NMR spectrum it was also possible to observe a multiplet signal at δ 4.50 due to H-3 bonded to the esterified carbon.

The mass spectrum of the transesterification product of **2** made it possible to recognize the palmitic acid esterified at the C-3 position of the triterpene through the methyl palmitate (*m/z* 270). Besides, comparison of the ^{13}C NMR data (Table) with those found in the literature for 3β,28-dihydroxy-11-oxo-olean-12-ene[6] allowed the identification of the structure of the triterpene moiety as being 3β-hydroxy-11-oxo-olean-12-ene.

The ^{1}H NMR spectrum of **3** showed the H-12 signal as a doublet at δ 5.24 (d, *J* 3.7 Hz) whose multiplicity and position indicated that C-11 was a methine carbon. Thus the signal at δ 4.18 (dd, *J* 3.7 and 7.9 Hz) was due to the hidroxy methine H-11. The ^{13}C NMR spectra (PND and DEPT 135°) corroborated the previous statements through the peaks displayed at δ 67.7, 125.4 and 149.4. The observation of the additional oxy methine carbon peak at δ 67.7 and comparison of the ^{1}H and ^{13}C NMR data of this compound with the data described for 3β,11α-dihydroxy-olean-12-ene[5,7] (Table 1) allowed to establish the β relative stereochemistry for the hydroxy group at C-11 in compound **3**. The H-11 peak of 3β,11α-dihydroxy-olean-12-ene appeared at δ 4.50 (dd, *J* 3.6 and 9.0 Hz)[5] while in **3** it appeared at δ 4.18 (dd, *J* 3.7 and 7.9 Hz). Similarly, the chemical shift observed for the C-11 of 3β,11α-dihydroxy-olean-12-ene (δ 81.7) appears at higher frequency than for the 11β-derivative (δ 67.7). The deshielding effects of C-12 (δ 125.4) and C-9 (δ 56.3) of **3** when compared with the values observed for 3β,11α-dihydroxy-olean-12-ene (δ 121.1 and 49.7, respectively) could be explained by the different distances between H/C-9 and the *pseudo*-axial OH group in C-11. In compound **3** this group is closer to C-9 and C-12 than in the 11-α derivative. This statement is in agreement with the chemical shift of C-9 (δ 51.6) of the 11α-methoxy derivative.[8] The mass spectrum recorded for the transesterification product of **3** made it possible to recognize palmitic acid esterified at the C-3 position of the triterpene through the molecular ion at *m/z* 270 (methyl palmitate).

The triterpene moiety of compound **4** was also characterized by ^{13}C NMR spectral data of fatty acid ester. By comparison with the ^{13}C NMR data of β-amyrin and erythrodiol[5] the absence of a methyl peak (C-28) at δ 28.4 and the presence of an oxy methylene carbon at δ 69.7 were noted. These considerations made it possible to place this additional hydroxyl group at C-28. The mass spectrum of

Table 1. ^{13}C NMR spectral data for the fatty acid esters of triterpenes **2, 3** and **5** [75MHz, $CDCl_3$, δ (ppm)]

C	**2**	3β,28-dihydroxy-11-oxo-olean-12-ene[6]	**3**	3β,11α-dihydroxy-olean-12-ene[7]	3β-hydroxy-11α-methoxy-olean-12-ene[8]	**5**	11,12-epoxy-friedoolean-14-ene[9]
1	38.8	39.2	40.1	39.5	39.26	37.9	37.92
2	23.6	27.3	23.7	27.4	27.30	23.2	23.27
3	80.3	78.8	80.4	78.7	78.61	80.7	80.30
4	38.1	39.2	38.7	39.0	39.00	37.6	37.69
5	55.0	55.0	55.3	55.1	55.02	54.6	54.59
6	17.4	17.5	18.3	18.4	18.24	18.2	18.74
7	32.7	32.7	33.1	32.9	33.08	33.2	33.18
8	43.4	45.4	43.4	43.3	43.05	39.0	38.94
9	61.7	61.8	56.3	49.7	51.56	51.8	51.83
10	37.0	37.0	38.0	37.9	38.56	37.5	37.53
11	200.2	200.1	67.7	81.7	75.85	53.5	53.49
12	128.1	128.3	125.4	121.2	121.51	58.2	58.17
13	170.6	169.4	149.4	153.2	149.67	36.6	36.55
14	45.4	43.4	41.8	41.8	41.70	157.1	157.13
15	26.4	25.8	25.2	26.4	26.13	118.9	118.90
16	26.5	21.6	26.7	27.4	26.60	35.2	35.25
17	32.4	37.1	33.2	32.3	32.30	35.4	35.37
18	47.6	42.7	46.5	46.9	46.76	48.1	48.15
19	45.2	44.9	46.5	46.9	46.36	40.3	40.29
20	31.1	31.1	31.1	31.2	31.07	28.7	30.16
21	34.9	32.9	34.9	34.7	34.51	36.6	36.54
22	36.5	30.1	37.0	37.0	36.84	38.2	38.12
23	28.1	28.1	28.1	28.0	28.03	27.9	27.92
24	16.4	15.6	16.7	15.5	15.44	17.0	16.99
25	16.8	16.4	18.1	18.3	18.04	16.6	16.66
26	18.7	18.6	16.9	16.8	16.76	27.0	27.02
27	23.4	23.4	26.2	24.7	25.07	30.2	30.23
28	28.8	69.7	28.5	28.5	28.35	29.8	29.92
29	33.1	32.9	33.2	33.3	33.11	33.7	33.62
30	23.5	23.4	23.6	23.7	23.52	19.5	19.52
-COO-	173.7	-	173.6	-		174.5	-
H_3CCO-	14.1	-	14.1	-		14.1	
OCH_3					53.67		

the product from the transesterification of **4** confirmed the structure of the triterpene moiety as 3β-28-dihydroxy-olean-12-ene (*m/z* 442).

Detailed analyses of the ^{13}C NMR data of **5** pointed out that it also was esterified with a fatty acid. The MS exhibited a molecular ion at *m/z* 678 corroborating with this statement. Analyses of the ^{13}C NMR spectra (PND and DEPT 135°) of compound **5** made it possible to recognize signals at δ 118.9 and 157.1 and signals for methyl groups which were very close to the values found for a taraxarene skeleton (or friedoolean-14-ene). However the presence of signals due to two methyne carbons, at δ 53.5 and 58.2, both deshielded in comparison with the methyne groups of taraxerol[5], and the absence of two methylene groups suggested the presence of an epoxy group at the C-11 and C-12 positions. Besides, the downshielded methyne group at δ 51.8 (C-9) and the upshielded quaternary carbons at δ 36.6 (C-13) and δ 48.2 (C-18) in comparison with C-9 (δ 48.9) and C-13 (δ 37.9) of taraxerol confirmed the presence of an oxirane group in that position. These data were confirmed by the ^1H NMR spectrum that exhibited absorptions at δ 2.80 (d, *J* 4.8 Hz, H-12), 3.11 (t, *J* 4.8 Hz, H-11), 5.55 (dd, *J* 3.3 and 7.9 Hz, H-15) and at δ 4.52 (dd, *J* 4.8 and 11.0 Hz) observed for the oxy methyne C-3. The latter signal was characteristic of esterification at this position. Comparison of these data with those found in the literature for taraxerol and for the acetyl derivative of 11α,12 α-epoxy-taraxerol[9] allowed to identify the triterpene moiety as being 3β-hydroxy-11α,12 α-epoxy-friedoolean-14-ene (Table 1). The mass spectrum of the product from the transesterification of pure **5** also made it possible to recognize palmitic acid esterified at the C-3 of the triterpene.

This is the first time that fatty acid esters of the triterpenes **2**, **3** and **5** are reported in the literature. Although the triterpene moiety 3β, 11β-dihydroxy-olean-12-ene (**3**) has been obtained through synthesis[10,11] this is the first report of its isolation from a natural source. A significant

number of plant species in different families contain fatty acids esterified with triterpenes or steroids, usually as a mixture of fatty acids,[12,13] however, previous studies of *Erythroxylum* also showed only palmitates.[4]

Experimental

GC/MS analyses were carried out in a GC chromatograph Varian mod. Saturn II coupled to an ion trap mass detector, employing a DB1-MS column (30 m x 0.32 mm x 1 μm). Mass spectra by direct injection (70 eV) of the esterified triterpenes were obtained on a HP mass selective detector equipment Mod. 5973. NMR spectra were recorded on a Varian Gemini 300 equipment using $CDCl_3$ as solvent and assigning the residual solvent signal as internal reference. Infrared spectra were recorded on a JASCO mod. Valor III spectrophotometer.

Botanical material was collected at the restinga of Reserva do Parque da Lagoa do Abaeté, in the vicinity of Salvador- BA (Brazil). Identification of the plant species was carried out by Prof. Maria Lenise S. Guedes. A voucher is deposited at Herbarium Alexandre Leal Costa of Biology Institute of Federal University of Bahia, under number 24280.

Isolation of the constituents

The dried leaves of *E. passerinum* (2.6 kg) were extracted with MeOH. The crude extract (93.4 g) was filtered over SiO_2 using hexane, hexane/EtOAc (8:2) and EtOAc as solvent systems. These procedures furnished residues A (1.1 g), B (1.21 g) and C (2.06 g), respectively. Residue B was submitted to CC on SiO_2 eluted with hexane/EtOAc (98:2). The fractions were grouped and sequentially submitted to purification through CC on SiO_2 using ether/petrol mixtures. The fraction eluted with ether/petrol 95:5 gave compound **2** (5.6 mg) while from the fractions eluted with a 98:2, 9:1 and 8:2 mixtures, β-amyril palmitate (**1**), compound **5** (13.5 mg) and **3** (17.8 mg) were obtained, respectively. Residue C, after purification through CC on SiO_2 eluted with hexane/EtOAc 95:5, followed by preparative TLC (hexane/EtOAc 9:1), furnished lupeol, β-amyrin, erythrodiol and erythrodiolyl palmitate or 3β, 28-dihydroxy-olean-12-enyl palmitate (**4**) besides sitosterol.

Transesterification of fatty acid esters of triterpenes. The esters of **1** - **5** were respectively refluxed in dry MeOH (20 mL) with sodium methoxide (20 mg) for 1 h. The reaction product was extracted with H_2O and CH_2Cl_2. The organic phase was separated, dried over Na_2SO_4 and evapored. From the esters of compounds **1** – **5** the respective methyl esters of palmitic acid were obtained (43.8 mg, 2.3 mg, 8.3 mg, 3.2 mg and 7.6 mg). Addition of HCl (1%) to the remaining aqueous phase, followed by extraction with CH_2Cl_2 yielded the triterpene moieties β-amyrin (**1**); 3β-hydroxy-11-oxo-olean-12-ene (**2**); 3β, 11β-dihydroxy-olean-12-ene (**3**); erythrodiol (**4**) and 3β-hydroxy-11,12-epoxy-friedoolean-14-ene. (**5**).

β-Amyrin palmitate (**1**). White wax. $[\alpha]_D^{25}$ +26.3° (*c* 4.0 hexane); ^{13}C NMR ($CDCl_3$): δ 38.2 (C-1), 22.7 (C-2), 80.6 (C-3), 37.7 (C-4), 55.2 (C-5), 18.2 (C-6), 32.8 (C-7), 39.7 (C-8), 47.1 (C-9), 36.9 (C-10), 23.5 (C-11), 121.6 (C-12), 145.0 (C-13), 41.6 (C-14), 26.8 (C-15), 26.0 (C-16), 32.3 (C-17), 47.8 (C-18), 47.4 (C-19), 31.1 (C-20), 35.6 (C-21), 37.1 (C-22), 28.4 (C-23), 16.6 (C-24), 15.6 (C-25), 16.8 (C-26), 25.9 (C-27), 28.0 (C-28), 33.4 (C-29), 23.6 (C-30), 173.6 (COO), 14.1 (CH_3), 29.6 (CH_2)$_n$.

3β-Hydroxy-11-oxo-olean-12-enyl palmitate (**2**). White wax. $[\alpha]_D^{25}$ +28.0° (*c* 3.0 $CHCl_3$); MS (*m/z*): 678 (8, $C_{46}H_{78}O_2$), 423 (16), 273 (100), 232 (87); ^{13}C NMR ($CDCl_3$): Table. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.59 (s, H-12); 4.50 (dd, H-3); 1.35 (s, CH_3); 1.16 (s, CH_3); 1.13 (s, CH_3), 0.90 (s, 4 x CH_3) and 0.83 (s, CH_3). IR ν_{max} /cm^{-1}: 2926, 2855, 1735 (COO), 1665 (C=O) (film).

3β,11β-dihydroxy-olean-12-enyl palmitate (**3**). White wax. $[\alpha]_D^{25}$ +45.0° (*c* 4.3 $CHCl_3$); MS (*m/z*): 662 (38, $C_{46}H_{80}O_2$ – H_2O), 407 (19), 255 (45), 234 (35), 218 (58), 203 (49), 189 (35); ^{13}C NMR ($CDCl_3$): Table; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.24 (d, *J* 3.7 Hz, H-12); 4.50 (m, H-3), 4.18 (dd, *J* 7.9 and 3.7 Hz);); 1.22 (s, CH_3); 1.09 (s, CH_3); 1.01 (s, CH_3), 0.88 (s, 4 CH_3) and 0.84 (s, CH_3). IR ν_{max} /cm^{-1}: 3437 (HO), 2926, 2855, 1732 (COO) (film).

Erythrodiol or *3β,28-dihydroxy-olean-12-enyl palmitate* (**4**). White wax; MS (*m/z*): 662 (4, $C_{46}H_{80}O_2 - H_2O$), 425 (3), 218 (13), 203 (100), 189 (12). ^{13}C NMR ($CDCl_3$): δ 38.1 (C-1), 23.4 (C-2), 80.4 (C-3), 37.6 (C-4), 55.1 (C-5), 18.1 (C-6), 32.4 (C-7), 39.7 (C-8), 47.3 (C-9), 36.8 (C-10), 23.4 (C-11), 122.1 (C-12), 144.1 (C-13), 41.6 (C-14), 25.4 (C-15), 25.8 (C-16), 31.8 (C-17), 42.2 (C-18), 46.3 (C-19), 34.0 (C-20), 30.9 (C-21), 38.1 (C-22), 27.9 (C-23), 16.6 (C-24),15.4 (C-25), 16.6 (C-26), 25.8 (C-27), 69.7 (C-28), 33.1 (C-29), 23.5 (C-30), 173.6 (-COO-), 14.0 (CH_3), 29.7 (CH_2)$_n$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.18 (t, *J* indt., H-12); 4.50 (m, H-3), 3.55 (d, *J* 11 Hz, H-28); 3.21 (d, *J* 11 Hz, H-28); 1.30 (s, CH_3); 1.17 (s, CH_3); 0.96 (s, CH_3), 0.94 (s, CH_3); 0.90 (s, CH_3); 0.87 (s, CH_3); and 0.86 (s, CH_3). IR ν_{max} /cm^{-1}: 3445, 2926, 2855, 1733, 1464, 1379, 1250, 1179, 1095, 1046 (film).

3β-hydroxy-11,12-epoxy-friedoolean-14-enyl palmitate (**5**). White wax; MS *m/z*: 678 (8, $C_{46}H_{80}O_3$), 423 (25), 301 (15), 286 (29), 255 (31). ^{13}C NMR ($CDCl_3$): Table; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.55 (dd, *J* 7.9 and 3.3 Hz, H-15); 4.52 (dd, *J* 11.0 and 4.8 Hz, H-3); 3.11 (t, *J* 4.8 Hz, H-11), 2.80 (d, *J* 4.8 Hz, H-12); 1.10 (s, CH_3); 1.08 (s, 2

CH_3); 1.00 (s, CH_3), 0.97 (s, CH_3); 0.87 (s, CH_3); 0.83 (s, CH_3). IR ν_{max} /cm^{-1}: 2920, 2852, 1731, 1249, 987, 720 (film).

Methyl palmitate; Oil; GC-MS, EIMS *m/z* (rel. int.): 270 $[M]^+$ (22), 214 (5), 227 (18), 213 (5), 199 (10), 185 (10), 171(10), 157 (5), 143 (21), 129 (8), 115 (5), 101 (8), 87 (69), 74 (100), 55 (45).

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