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FLAVONOIDS AND TRITERPENE ESTER DERIVATIVES FROM *ERYTHROXYLUM LEAL COSTAE*

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Key Word Index—*Erythroxylum leal costae*; Erythroxylaceae; 8-hydroxyluteolin 8-rhamnoside; 6-hydroxyluteolin 6-rhamnoside; fatty acid esters of triterpenes.

Abstract—A methanolic leaf extract of *Erythroxylum leal costae* yielded quercetin 3-rhamnoside, epicatechin, and the new glycosides, 8-hydroxyluteolin 8-rhamnoside and 6-hydroxyluteolin 6-rhamnoside. Palmitic acid esterified with β-amyrin, lupeol and lupenyl acetate were also identified.

INTRODUCTION

Erythroxylum is the largest genus in the Erythroxylaceae and comprises approximately 97% of its species [1]. There are many wild species in Brazil, especially in the State of Bahia, where the greatest concentration occurs along the coast in Restinga and the Restinga forest on sandy soils [2]. These species are used locally in herbal medicines, although none of the chemical constituents have been fully characterized. However, previous chemical investigations of the genus have indicated the presence of alkaloids, terpenoids and phenolic compounds [1,3].

The present paper describes the identification of two new flavone glycosides **1** and **2**, three triterpenoids **5–7**, epicatechin (**3**) and quercetin 3-rhamnoside (**4**) from leaves of *E. leal costae* Plowman.

RESULTS AND DISCUSSION

The ^{13}C NMR spectra of the flavones **1** and **2** were very similar and both showed just one signal for an unsubstituted carbon in the A-ring at δ94.8 and 94.5, respectively. However, the two compounds could be distinguished by an analysis of their ^{1}H NMR spectra. Thus, in **1** H-6 appeared in higher field than H-8 in **2** (δ6.07 × 6.38) in accordance with literature data [4]. Thus, the aglycone moiety of **1** and **2** was assigned as 8-hydroxyluteolin and 6-hydroxyluteolin, respectively. The ^{13}C NMR data clearly showed that the sugar moiety was rhamnose. The position of rhamnose groups were determined by UV spectral analyses with the usual shift reagents [4], which established that both compounds had free 5-, 7-, 3′- and 4′-hydroxyl groups. Therefore, it is concluded that **1** and **2** are the new flavone glycosides, 8-hydroxyluteolin 8-rhamnoside and 6-hydroxyluteolin 6-rhamnoside. The known compounds epicatechin (**3**) and quercetin 3-rhamnoside (**4**) were also isolated from the ethyl acetate extract.

The ^{13}C NMR spectra (NOISE and DEPT) showed that compounds **5–7** were triterpenoids with oleanane (**5**) and lupane skeletons (**6** and **7**). The identification of the triterpenoid groups was established by the characteristic absorptions displayed for eight methyl groups and the values of unsaturated carbons at δ126.6 and 145.0 for compound **5**, and δ109.4 and 150.9 for **6**. When **5** and **6** were compared with acetyl derivatives of β-amyrin [5] and lupeol [6] it was observed that carbonyl groups were deshielded (Δδ*ca* 3.2). Furthermore, the spectra displayed new signals of methyl groups from palmitate moieties upfield (δ14.1) while no signals for the methyl groups from an acetoxyl group were observed. In addition, the C-3 signals of these compounds were displayed in a lower field (δ80.6) than those observed for β-amyrin (δ78.9) and lupeol (δ78.8) [5,6]. These data indicate that the compounds are fatty acid ester derivatives. Transesterification of **5** and **6** yielded β-amyrin, lupeol and methyl palmitate, which was characterized by GC–mass spectral analysis. Compound **5**, which was identified as β-amyril palmitate, has been found in *E. coca* [3] but neither **6** nor **7** (lupenyl palmitate and lupenyl acetate, respectively), have been isolated from the Erythroxylaceae.

EXPERIMENTAL

General. Mps uncorr. ^{1}H and ^{13}C NMR spectra of compounds **1–4** were recorded at 200 and 50 MHz,

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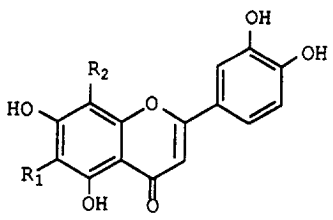
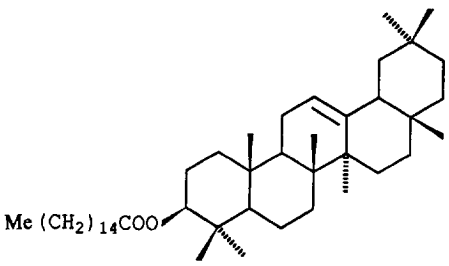
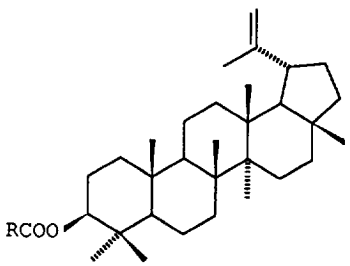


	R_1	R_2
1	H	ORha
2	ORha	H



5



	R
6	$-(CH_2)_{14}Me$
7	-Me

respectively; **5**–**7** presaturation at 300 and 75 MHz. ^{1}H NMR spectra using Me_2CO-d_6/D_2O were run utilizing water peak supression by a presaturation technique. GC-MS analyses were carried out in a GC chromatograph coupled with an ion trap mass detector, employing a DB5-MS column (30 m × 0.32 mm × 1 m).

Plant material was collected at Restinga of Parque Metropolitano do Abaeté, in the vicinity of Salvador, BA and were identified by Professor Maria Lenise S. Guedes. A voucher specimen has been deposited at Herbarium Alexandre Leal Costa, Biology Institute of Federal University of Bahia, under number 026391.

Isolation of the consitutents. The dried leaves of *E. leal costae* (1.410 kg) were extracted with EtOH. The crude extract was partitioned successively with hexane, $CHCl_3$, EtOAc and *n*-BuOH. The EtOAc fraction (1.67 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH (9:1 and 4:1) as eluent and yielded **3** (8.5 mg), **1** (20.9 mg), **2** (11.2 mg) and **4** (30.2 mg) after purification on a Sephadex LH-20 column eluted with $CHCl_3$–MeOH (1:4). The hexane fraction (13.9 g) was sepd by CC on silica gel using hexane–EtOAc (9:1) and (4:1) successively as solvents to yield pure **5** (1.98 g) and a mixture of **5**, **6** and **7**. The mixture yielded pure **6** after prep. TLC on silica gel using petrol–Et_2O (4:1) as solvent. The identification of **7** was carried out in a mixture with **5**.

Compound **1**. *8-Hydroxyluteolin 8-rhamnoside.* Yellow amorphous powder. Mp 179–181°. UV data λ_{max}^{MeOH} nm: 252, 264 (sh), 348; + $AlCl_3$ 272, 300 (sh), 432; + $AlCl_3$/HCl 270, 300 (sh), 366, 400; + NaOMe 266, 320, 392; + NaOAc 272, 320, 394; + H_3BO_3 262, 374. ^{1}H NMR (Me_2CO-d_6/D_2O): δ 6.24 (1H, *s*, H-3), 6.07 (1H, *s*, H-6), 7.22 (1H, *d*, *J* = indt., H-2′), 6.87 (1H, *d*, *J* = 6.8 Hz, H-5′), 7.13 (1H, *dd*, *J* = 6.8 and indt., H-6′), 5.13 (1H, *br s*, H-1″), 4.14 (1H, *br s*, H-2″), 3.13–3.69 (3H, *m*, H-3″, H-4″ and H-5″) and 0.74 (3H, *d*, *J* = 5.8 Hz, H-6″). ^{13}C NMR (Me_2CO-d_6/D_2O): δ165.0 (C-2), 104.8 (C-3), 178.2 (C-4), 158.6 (C-5), 94.8 (C-6), 157.2 (C-7), 134.8 (C-8), 161.0 (C-9), 102.1 (C-10), 122.6 (C-1′), 116.1 (C-2′), 144.7 (C-3′), 146.3 (C-4′), 116.5 (C-5′), 121.6 (C-6′), 99.4 (C-1″), 70.5 (C-2″), 70.6 (C-3″), 71.9 (C-4″), 71.2 (C-5″) and 16.8 (C-6″).

Compound **2**. *6-Hydroxyluteolin 6-rhamnoside.* Crystal. Mp 182–183°. UV data λ_{max}^{MeOH} nm: 256 (sh), 270, 350; + $AlCl_3$ 278, 302, 430; + $AlCl_3$/HCl 278, 302, 362, 390 (sh); + NaOMe 272, 324, 402; + NaOAc 284, 324, 344, 396; + H_3BO_3 284, 324, 364. ^{1}H NMR (Me_2CO-d_6/D_2O): δ6.18 (1H, *s*, H-3), 6.38 (1H, *s*, H-8), 7.35 (1H, *d*, *J* = indt., H-2′), 6.94 (1H, *d*, *J* = 7.6 Hz, H-5′), 7.26 (1H, *dd*, *J* = 7.6 and indt, H-6′), 5.25 (1H, *br s*, H-1″), 4.19 (1H, *br s*, H-2″), 3.25–3.75 (3H, *m*, H-3″, H-4″ and H-5″) and 0.80 (3H, *d*, *J* = 5.49 Hz, H-6″). ^{13}C NMR (Me_2CO-d_6/D_2O): δ165.1 (C-2), 104.8 (C-3), 178.5 (C-4), 161.7 (C-5), 135.1 (C-6), 157.5 (C-7), 94.5 (C-8), 158.5 (C-9), 102.4 (C-10), 122.3 (C-1′), 116.0 (C-2′), 145.2 (C-3′), 148.7 (C-4′), 116.4 (C-5′), 121.8 (C-6′), 99.4 (C-1″), 70.7 (C-2″), 70.9 (C-3″), 71.9 (C-4″), 71.2 (C-5″) and 17.1 (C-6″).

Compound **3**. *(epicatechin) and compound* **4** *(quercetin 3-rhamnoside)* were identified by comparison of physical and NMR data with literature values [7].

Compound **5**. *β-Amyril palmitate.* White wax. ^{13}C NMR ($CDCl_3$): δ38.2 (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 (CO_2), 14.1 (CH_3), 29.6 (*n*CH_2).

Compound **6**. *Lupenyl palmitate.* White wax. ^{13}C NMR ($CDCl_3$): δ38.3 (C-1), 23.7 (C-2), 80.6 (C-3), 37.8 (C-4), 55.3 (C-5), 18.2 (C-6), 34.2 (C-7), 40.8 (C-8), 50.3 (C-9), 37.0 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.5 (C-16), 42.9 (C-17), 48.2 (C-18), 48.0 (C-19), 150.9 (C-20), 29.7 (C-21), 39.9 (C-22), 27.9 (C-23), 16.6 (C-24), 16.1 (C-25), 15.9 (C-26), 14.5 (C-27), 17.9 (C-28), 109.4 (C-29), 19.3 (C-30), 173.4 (CO_2), 14.1 (CH_3) and 29.5 (*n*CH_2).

Transesterification procedure. Compounds **5** and **6** (150 mg each) were refluxed in dry MeOH (20 ml) with sodium methoxide (20 mg) for 1 hr. The reaction mixture was extracted successively with H_2O and $CHCl_3$. The organic phase was sepd, dried over Na_2SO_4 and evapd to give methyl palmitate **5a** (44.8 mg). Addition of HCl (1%) to the remaining water phase following extraction with $CHCl_3$ yielded β-amyrin (76.6 mg) and lupeol (79.5 mg).

Compound **5a**. *Methyl palmitate.* Oil. GC-MS. R_t 15: 54. 70 eV, *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).

Compound **7**. *Lupenyl acetate.* The identification was performed by comparison of NMR data with literature data [6].

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