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Antioxidant Phenylpropanoid Esters of Triterpenes from *Dioclea lasiophylla*

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Abstract

The chloroform extract of steams of *Dioclea lasiophylla* furnished a new compound, namely, 3β-*E*-feruloyl oleanolic acid, in addition to 3β-*E*-caffeoyl oleanolic, betulinic and oleanolic acids, vanillin, methyl vanillate, pinoresinol and syringaresinol. The hexane extract contains sitosterol, stigmasterol, acetyl oleanolic acid, lupeol and lupenone. Antioxidant activities of isolates were measured using the 1,2-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay and the auto-oxidation of β-carotene in a linolenic acid suspension method. The 3β-*E*-caffeoyl-oleanolic acid, syringaresinol and the new compound, 3β-feruloyl oleanolic acid, exhibited antioxidant activities in both tests.

Introduction

Dioclea, belonging to the subfamily Papilionoideae of Leguminosae, is well represented in Brazil with *ca.* 150 species of climber, arborous and subarborous plants and rarely herbs. *Dioclea lasiophylla* Marth ex Benth. is a climber occurring in the Atlantic coastland and it is popularly known as "feijão-bravo" (Correa, 1984). A previous study performed with the ethyl acetate extract of leaves furnished *epi*gallocatechin-(2β→7,4β→8)-*epi*catechin, *epi*catechin, luteolin-3′β-D-glucopyranoside and chrysoeriol-7β-D-glucopyranoside (Barreiros et al., 2000). Now the presence of phytosterols and triterpenes in the hexane extract as well as the new compound named 3β-feruloyl oleanolic acid, as well as 3β-caffeoyl oleanolic acid, betulinic acid, oleanolic acid, vanillin, methyl vanillate, pinoresinol and syringaresinol from the chloroform extract, are described.

Material and methods

Plant material

The stem of *D. lasiophylla* was collected in the Campus of Universidade Estadual de Feira de Santana (BA), Brazil. A voucher (L.P. de Queiroz 4726) was deposited in the Herbarium of UEFS under number 24822.

Extraction and isolation

The powdered and dried material (1.8 kg) was subjected to extraction with methanol (4 L) and the methanol extract was partitioned between hexane/MeOH : H_2O (9 : 1), $CHCl_3$/MeOH : H_2O (6 : 4), EtOAc/H_2O and BuOH/H_2O (3 × 500 mL). The hexane extract was subjected to CC on SiO_2 60 using mixtures of hexane/EtOAc. The fractions obtained were grouped based on TLC analysis (Silica gel) using hexane/EtOAc (7 : 3) and observed by Liebermann-Bouchard and UV light 254 nm. The grouped fractions were then subjected to CC and/or recrystalization. These procedures permitted the isolation of lupenone (26.2 mg), lupeol (78.1 mg), 3β-acetyl-oleanolic acid (112.5 mg), β-sitosterol (126.2 mg) and stigmasterol (126.2 mg). The $CHCl_3$ extract was subjected to CC using $CHCl_3$ and mixtures of $CHCl_3$/EtOAc. The fraction eluted with $CHCl_3$ was purified by prep. TLC using $CHCl_3$/EtOAc (9 : 1), and furnished vanillin (3.0 mg) and methyl vanillate (3.3 mg). The fraction eluted with $CHCl_3$/EtOAc (9 : 1) afforded betulinic acid (6.8 mg) when eluted twice in prep. TLC on silica gel ($CHCl_3$/MeOH, 95 : 5). From the fraction eluted with $CHCl_3$/EtOAc (8 : 2), both oleanolic acid (8.4 mg) and pinoresinol (**1**, 5.8 mg) were obtained after prep. TLC which was also eluted twice with

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$CHCl_3$/MeOH (95 : 5). (+)-Syringaresinol (**2**, 7.2 mg) and 3β-caffeoyl oleanolic acid (**3**, 21.8 mg), in addition to the new compound 3β-feruloyl oleanolic acid (**4**, 13.7 mg), were isolated by prep. TLC using $CHCl_3$/MeOH (95 : 5) from the fraction eluted with $CHCl_3$/EtOAc (7 : 3) of main CC (Figure 1).

Antioxidant tests

Antioxidant activity was evaluated by the ability of the substance scavenging 1,2-diphenyl-2-picryl-hydrazyl (DPPH, Sigma) free radical, and was carried out according to an established protocol (Malterud et al., 1993). The antioxidant capacity was compared with those observed for standards BHT (Merck) and *n*-propyl gallate (Merck). The autoxidation of β-carotene (Merck)/linoleic acid (Aldrich) method was adapted from the literature (Hidalgo et al., 1994) and the activity was compared with commercial antioxidants such as propyl gallate, BHT, and α-tocopherol, previously isolated and characterized in our laboratory (David et al., 1996).

Spectral data of isolates 3 and 4

3β-*E*-Caffeoyl-oleanolic acid or 3β-*E*-(3,4-dihydroxycinamoyl)-12-oleanen-28-oic acid (**3**). Amorphous powder, $C_{39}H_{54}O_6$, MM 618. $[\alpha]_D$ +68° (MeOH, *c* 0.034) ^{1}H NMR [300 MHz, *m*, *J* (Hz), CD_3OD]: 7.53 (*d*, 15.8, H-7′), 6.25 (*d*, 15.8, H-8′), 7.04 (*d*, 1.9, H-2′), 6.94 (*dd*, 1.9, 8.3, H-6′), 6.78 (*d*, 8.3, H-5′), 5.25 (*t*, 3.3, H-12), 4.56 (*dd*, 11.5, 4.9, H-3), 2.85 (*dd*, 11.5, H-2), 1.28 (*s*, H-23), 1.18 (*s*, H-27), 1.00 (*s*, H-25), 0.96 (*s*, H-26), 0.94 (*s*, H-29), 0.91 (*s*, H-24), 0.83 (*s*, H-30). ^{13}C NMR (75MHz, CD_3OD): 181.8 (C-28), 169.2 (C-9′), 149.5 (C-4′), 146.8 (C-3′), 146.7 (C-7′), 145.2 (C-13), 127.7 (C-1′), 123.5 (C-12), 122.9 (C-6′), 116.5 (C-5′), 115.6 (C-8′), 115.1 (C-2′), 82.3 (C-3), 56.8 (C-5), 49.0 (C-9), 47.6 (C-17), 47.3 (C-19), 42.9 (C-14), 42.7 (C-18), 40.6 (C-8), 39.4 (C-1), 39.0 (C-4), 38.2 (C-10), 34.9 (C-21), 33.9 (C-7), 33.8 (C-22), 33.6 (C-29), 31.6 (C-20), 28.8 (C-15), 28.7 (C-23), 26.5 (C-27), 24.7 (C-2), 24.5 (C-11), 24.0 (C-30), 24.1 (C-16), 19.4 (C-6), 17.7 (C-26), 17.4 (C-24), 16.8 (C-25).

3β-*E*-Feruloyl oleanolic acid or 3β-*E*-(3-methoxy-4-hydroxycinamoyl)-12-oleanen-28-oic acid (**4**). Amorphous powder, $C_{40}H_{56}O_6$. $[\alpha]_D$ +8,2° (MeOH, *c* 0.012) ^{1}H NMR [*m*, *J* (Hz), $(CD_3)_2CO$]: 7.54 (*d*, 15.8, H-7′), 6.30 (*d*, 15.8, H-8′), 7.17 (*d*, 2.0, H-2′), 7.03 (*dd*, 2.0, 8.2, H-6′), 6.87 (*d*, 8.2, H-5′), 5.25 (*t*, 3.3, H-12), 4.58 (*dd*, 10.7, 5.1, H-3), 3.91 (*s*, OCH_3), 1.20 (*s*, H-23), 1.00 (*s*, H-27), 0.96 (*s*, H-25), 0.94 (*s*, H-26), 0.92 (*s*, H-29), 0.90 (*s*, H-24), 0.83 (*s*, H-30). ^{13}C NMR [75 MHz, $(CD_3)_2CO$]: 178.9 (C-28), 167.2 (C-9′), 148.7 (C-4′), 146.3 (C-3′), 145.4 (C-7′), 144.9 (C-13), 127.5 (C-1′), 122.8 (C-12), 122.4 (C-6′), 116.2 (C-5′), 115.9 (C-8′), 114.9 (C-2′), 80.9 (C-3), 56.0 (OCH3), 55.9 (C-5), 48.3 (C-9), 46.8 (C-17), 46.7 (C-19), 42.5 (C-14), 42.1 (C-18), 40.1 (C-8), 38.8 (C-1), 38.5 (C-4), 37.7 (C-10), 34.4 (C-21), 33.5 (C-22), 33.4 (C-7), 33.3 (C-29), 31.2 (C-20), 30.3 (C-15), 28.4 (C-23), 26.2 (C-27), 24.3 (C-2), 24.0 (C-11), 23.8 (C-30), 23.6 (C-16), 18.9 (C-6), 17.5 (C-26), 17.2 (C-24), 15.7 (C-25). CIMS [M+H]+, *m/z* (rel. int.): 633 (10); 584 (5); 481 (5); 468 (5); 419 (10); 408 (15); 393 (20); 323 (20); 307 (30); 225 (30); 219 (20); 209 (20); 197 (100); 195 (50); 183 (41); 179 (43); 177 (42); 169 (41); 148 (23); 141 (6); 113 (10).

Results and Discussion

The structures of known phytosterols, triterpenes and lignans, were elucidated through spectroscopic analysis by direct comparison of data found in the literature (Ricca & Nicotra, 1978; Mahato & Kundu, 1994; Das et al., 1999). The TLC of **3** and **4** revealed an intense pink spot with Libermann-Burchard reagent and its triterpene nature was suggested by NMR spectral data. Structural elucidation of compound **3** was made through comparison with spectral data found in the literature for the caffeoyl oleanolic acid previously isolated from *Betula pubescens* Ehrh (Pan et al., 1994). The ^{1}H-^{13}C HETCOR spectra allowed unequivocal assignment of C-12 and C-6′, confirming other attributions in accordance with the literature.

Analyses of the ^{1}H NMR spectrum of compound **4** permitted identification of signals of *trans* double bond conjugated to C═O at δ 7.54 (*J* = 15.8 Hz) and δ 6.30 (*J* = 15.8 Hz) in addition to other characteristic signals of the triterpene skeletal. This spectrum also showed signals attributed to hydrogens in the AMX system of the aromatic ring. Also it was possible to observe a singlet of a methoxyl group at δ 3.91 and axial oxymethine at δ 4.56 (H-3) downshield when compared with the H-3 of oleanolic acid. These data suggested esterification of phenylpropanoid derivative at this position. In agreement, the ^{13}C NMR spectra displayed 40 signals of carbon and DEPT (135° and 90°) experiments helped to identify an oleanane moiety as part of the structure of this compound. The peak $[M+H]^+$ at *m/z* 633 displayed in the CIMS and the ^{13}C NMR data indicated the molecular formula $C_{40}H_{56}O_6$. The ^{13}C NMR also confirmed the oleanane skeletal through the sp^2 carbon and methyl peaks as well as the presence of a cinnamic acid derivative bearing a methoxyl group. The chemical shift values observed for this triterpene moiety were comparable with values found in literature for oleanolic acid (Mahato & Kundu, 1994). The signal (δ 80.9) downshielded (Δδ = 1.8 ppm) in comparison with C-3 (δ 79.1) of oleanolic acid and the presence of the ester carboxyl group confirmed the esterification at C-3 of oleanolic acid unity.

Comparison of ^{13}C NMR data of compound **4** with the **3** and isoferuloyl oleanolic acid found in the literature (Takahashi et al., 1999) allowed establishment of the location of the methoxyl group at C-3′ and recognized a ferulic acid esterified with the triterpene unit. There is no report of the occurrence of feruloyl oleanolic acid (**4**) in the literature.

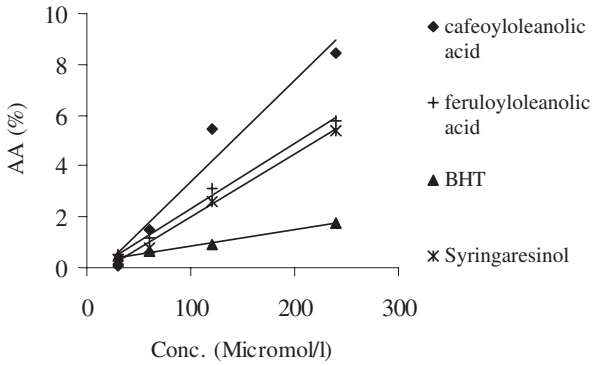

Figure 1. DPPH scavenging test of the isolates.

The IC_{50} values (mg/mL) for 50% of DPPH scavenging radicals were calculated by linear extrapolation of values obtained from curve of antioxidant activity versus concentration (Figure 1). This procedure was possible due to the linearity of response of activity for pirogallol through 60% of scavenging. The results show that compounds **2** (IC_{50} 3.25), **3** (IC_{50} 0.79) and **4** (IC_{50} 1.24) presented considerable antioxidant activity when compared with BHT (IC_{50} 0.45).

However, based on inhibition of autoxidation of β-carotene, the data with 95% of confidence indicated that compound **3** (AA = 61.95 ± 0.004; 116.6%) presented the highest antioxidant activity when compared to propyl gallate (AA = 53.14 ± 0.006; 100.0%) and BHT (AA = 60.04 ± 0.005; 113.1%). The 3β-feruloyl oleanolic acid (**4**) exhibited lower activity (AA = 47.17 ± 0.005; 88.8%) than propyl gallate and BHT, but superior to α-tocopherol (AA = 29.28 ± 0.004, 55.1%) and syringaresinol (**2**) which showed weak antioxidant activity (AA = 6.60 ± 0.007; 12.4%). These antioxidant activities were calculated relative to bleaching and further converted in AA percent taking into account propyl gallate as 100% of antioxidant activity.

The antioxidant ability of the triterpenes is mainly due to the presence of an aromatic ring bearing hydroxyl groups in these compounds and this effect depends on the number of these hydroxyl groups. So, that was the reason 3 showed higher antioxidant activity than **4**.

Although the presence of phytosterols and triterpenes are very common in plants, 3β-acetyl oleanolic acid and the 3β-caffeoyl oleanolic acid (**3**) are rare in nature. In spite of having already been previously reported in *Betula pubescens*, this is the first appearance of 3β-caffeoyl oleanolic acid in the Leguminosae.

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