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A-type proanthocyanidin antioxidant from *Dioclea lasiophylla*

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Abstract

The A-type proanthocyanidin, epigallocatechin-(2β→7,4β→8)-epicatechin, together with the known epicatechin, luteolin 3′β-D-glucopyranoside, chrysoeriol 7β-D-glucopyranoside and 2-methylpentan-2,4-diol, were isolated from leaves of *Dioclea lasiophylla*. The structures were established on the basis of their spectral data. Antioxidant activities of isolates were measured using the auto-oxidation of β-carotene in a linolenic acid suspension method. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Dioclea lasiophylla*; Leguminosae; Proanthocyanidin; Flavan; Flavones; Antioxidant activities

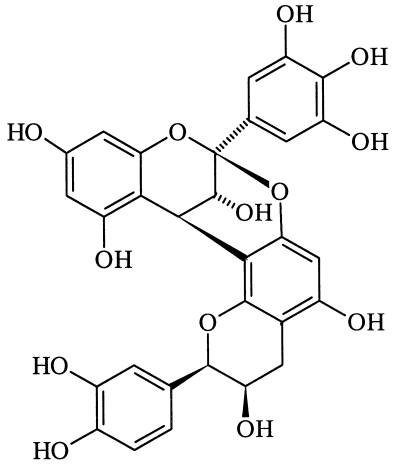
1. Introduction

Dioclea lasiophylla Mart. ex Benth. is a climber plant of the subfamily Papilionoideae (Leguminosae) occurring in Brazil on the northeastern Atlantic coast. In this country, *D. lasiophylla* and the other eleven species belonging to this genus embrace a set of plants called "feijão-bravo" and "mucunã". These plants are prescribed by local populations as an analgesic, as well as for the treatment of kidney stones and rheumatism (Correa, 1984). To date, there have been no previous phytochemical reports of *D. lasiophylla*. However it is noteworthy that from the roots of *D. grandiflora*, a plant also known as "mucunã", were isolated dioclein, a flavanone which possesses analgesic activity (Batista et al., 1995), and other flavonoids (Bhattacharyya et al., 1998).

This paper describes the isolation, structure elucidation, and the antioxidant activity of a new A2-type proanthocyanidin together with other flavonoids isolated from the EtOAc extract of leaves of *D. lasiophylla*.

2. Results and discussion

The EtOAc extract of leaves of *D. lasiophylla* was submitted to different fractionation procedures on Silica gel, Sephadex LH-20 and polyamide chromatographic columns. These procedures afforded the new proanthocyanidin **1**, as well as epicatechin, luteolin 3′β-D-glucopyranoside (**2a**), chrysoeriol 7β-D-glucopyranoside (**2b**) and 2-methylpentan-2,4-diol (**3**).



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The positive ion FABMS of **1** displayed a *quasi*-molecular ion peak $[M+H]^+$ at *m/z* 593, and the molecular formulae $C_{30}H_{24}O_{13}$ was confirmed by results of elemental analysis. The flavonoid nature of the constituent units of **1** was indicated through the NMR

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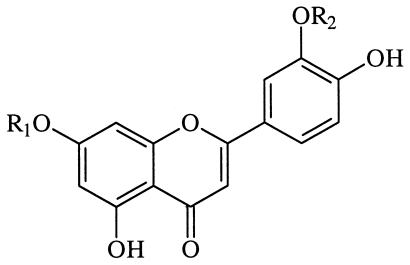
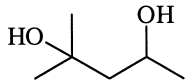


2a R_1=H R_2=Glu
2b R_1=Glu R_2=Me



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spectral data, which suggested that the lower unit was an epicatechin. The appearance of a broad singlet at δ 4.93 (H-2*l*) indicated a *cis* relationship between H-2 and H-3 of the lower unit. This statement was supported by the ^{13}C NMR chemical shifts of C-2*l* (δ 81.68) and C-3*l* (δ 66.90) which were consistent with the data found in literature (Agrawal et al., 1989) and for (−)-epicatechin also isolated from this plant. HETCOR analysis permitted correlation of all hydrogens bonded directly to corresponding carbons (Table 1). The doublet at δ 6.08 was attributed to H-8*u* by comparison with literature values (Bilia et al., 1996) and by NOE associations between H-8*u* to H-2′/6′*u* and H-6*u* to H-2*l*.

The long-range correlations observed between the H-2*l* (δ 4.93) and C-1′*l* (δ 131.44), C-2′*l* (δ 115.90) and C-6′*l* (δ 120.36), displayed in the HETCOR spectra confirmed the catechol hydroxylation of the lower moiety. On the other hand, the H-2′*u*/H-6′*u* (δ 6.74) and C-2*u* (δ 100.13) exhibited a long range HETCOR cross-peak confirming the pyrogallol hydroxylation of aromatic ring at C-2*u*. Both the C-4*u* to C-8*l* and C-2*u* to O-C-7*l* interflavan linkages were determined by long range HETCOR, ^{1}H-^{1}H COSY correlations, NOE difference as well as through comparison with literature data (Baldé et al.,

Table 1
^{1}H NMR[a], ^{13}C NMR[b], HETCOR and long range ^{1}H-^{13}C spectroscopic correlation values for **1**

				LRHETCOR	
Position	δ-C	Multiplicity[c]	δ-H	J^2	J^3
2*u*	100.13	C	–	–	–
3*u*	68.02	CH	4.06 *d* (3.4)	–	104.25
4*u*	29.14	CH	4.41 *d* (3.4)	104.25; 107.14	100.13; 152.21; 152.06
5*u*	156.51	C	–	–	–
6*u*	98.26	CH	6.01 *d* (2.4)	–	–
7*u*	158.02	C	–	–	–
8*u*	96.60	CH	6.08 *d* (2.4)	–	–
9*u*	154.17	C	–	–	–
10*u*	104.25	C	–	–	–
1*u*	131.60	C	–	–	–
2*u*	107.48	CH	6.74 *s*	131.60; 146.26	134.58; 107.48; 100.13
3*u*	146.26	C	–	–	–
4*u*	134.58	C	–	–	–
5*u*	146.26	C	–	–	–
6*u*	107.48	CH	6.74 *s*	131.60; 146.26	134.58; 107.48; 100.13
2*l*	81.68	CH	4.93 br *s*	131.14	115.90; 120.36
3*l*	66.90	CH	4.24 *m*	–	102.39
4a*l*	29.85	CH_2	2.95 *dd* (17.2; 4.9)	102.39	152.06
4b*l*			2.76 *dd* (17.2; 1.9)	102.39	152.06
5*l*	156.90	C	–	–	–
6*l*	96.48	CH	6.11 *s*	152.21	107.14; 102.39
7*l*	152.21	C	–	–	–
8*l*	107.14	C	–	–	–
9*l*	152.06	C	–	–	–
10*l*	102.39	C	–	–	–
1*l*	131.14	C	–	–	–
2*l*	115.90	CH	7.16 *d* (2.0)	145.91	146.21; 120.36
3*l*	145.91	C	–	–	–
4*l*	146.21	C	–	–	–
5*l*	116.03	CH	6.82 *d* (8.2)	–	145.91; 131.14
6*l*	120.36	CH	6.98 *dd* (8.2, 2.0)	131.14	146.21

[a] 300 MHz, CD_3OD, δ (ppm), *J* (Hz).
[b] 75 MHz.
[c] Multiplicity of carbons obtained by DEPT experiments. *u* = upper unit (epigallocatechin), *l* = lower unit (epicatechin).

1991). Thus, H-4*u* (δ 4.41) showed correlations with C-8*l* (δ 107.14), C-7*l* (δ 152.21) and C-9*l* (δ 152.06). In addition, the ^{13}C NMR chemical shift of C-2*u* displayed at δ 100.13 clearly demonstrated that the linkage C–O–C should be located on this carbon. The NOE data supported the C-4*u* to C-8*l* interflavanyl linkage. Both correlations between H-2*l* and H-4*u* (12%) and H-6*u* (5%) indicated that **1** had a C-4*u* to C-8*l* linkage instead of to C-6*l*.

The nOe diff experiments also allowed assignment of the relative stereochemistry of **1**. The nOe associations observed between the H-2*l* to H-4*u* (12%) and to H-6*u* (5%), as well as effects of H-6*l* and H-3*u* (Fig. 1) were conclusive for understanding the mode of coupling of the two flavan-3-ol units. Thus, compound **1** and its peracetate derivative **1a** showed selective NOE effects of H-3*u* to H-6*l* and to H-2*l* (16%). These associations indicated 3,4 *trans* configuration of C-ring (Cronjé et al., 1990) and supported the relative configuration of C-3 and C-4 of the upper unit. The absolute configuration at C-4*u* of **1** was established by CD measurements in MeOH. The strong positive Cotton Effect at 227 nm

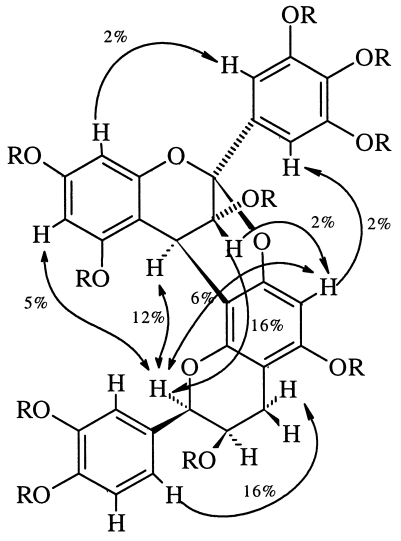

Fig. 1. Some nOe difference increments observed for **1** (R = H) and **1a** (R = Ac).

([ϕ]$_{227}$ + 119 573] indicated a 4β orientation and hence the 4*R* configuration (Barrett et al., 1979). On the basis of these observations, the structure of the proanthocyanidin was assigned as epigallocatechin-(2β→7,4β→8)-epicatechin.

The lipidol, 2-methylpentan-2,4-diol (**3**) was previously found in *Ligularia veitchiana* (Compositae) (Jia et al., 1992). This is the first report of isolation of the compound in Leguminosae.

The antioxidant activities of **1**, epicatechin, **2a** and **2b** were estimated by employing autooxidation of β-carotene in a linolenic acid suspension and comparing the results with those obtained from the commercial antioxidants, propyl gallate and α-tocopherol. The data collected (Fig. 2) indicated that epigallocatechin-(2β→7,4β→8)-epicatechin possesses considerable antioxidant activity (AA = 35) compared to propyl gallate (AA = 49) and α-tocopherol (AA = 27).

3. Experimental

^{1}H (300 MHz); ^{13}C NMR and DEPT experiments (75 MHz); ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY: Acetone-d_6 or methyl alcohol-d_4 as int. standard; MS (Finnigan MAT 90); UV and CD spectra in MeOH. Mps: uncorr. silica gel (70–230 Mesh-Merck); LH-20 Sephadex (Sigma), Polyamide 6 (J. B. Baker), TLC: precoated sheets of Si gel 60 F_{254} (Merck); *n*-propyl gallate (Merck).

3.1. Plant material

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

3.2. Isolation

Powdered dry leaves (404 g) were repeatedly partitioned between hexane/MeOH–H_2O (9:1) followed by $CHCl_3$/MeOH–H_2O (6:4), EtOAc/H_2O and BuOH/

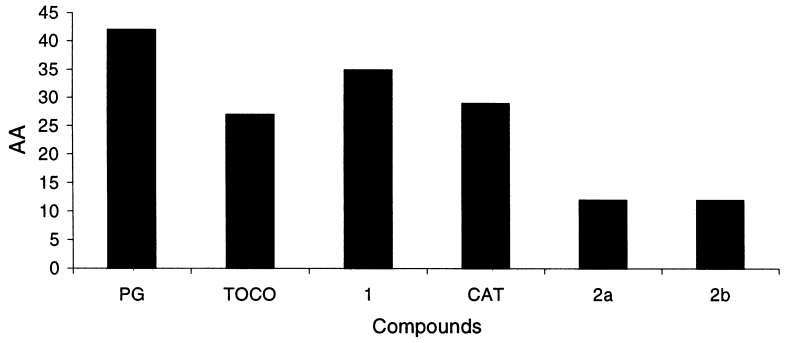

Fig. 2. Antioxidant activities (AA) of proanthocyanidin **1**, epicatechin (CAT) and the flavonoids **2a** and **2b** compared to *n*-propyl gallate (PG) and α-tocopherol (TOCO).

H_2O. The EtOAc phase (6.9 g) was applied to a Si gel column eluted with mixtures of EtOAc/MeOH. Fractions (20 ml) were collected and combined in batches of five fractions on basis of TLC. The second fraction (556 mg dry wt) was submitted to gel permeation Sephadex LH-20 chromatography, using $CHCl_3$–MeOH (3:7) as eluent to furnish compound **1** (163 mg), epicatechin (59.2 mg) and 2-methyl-pentane-2,4-diol (35 mg). The fourth fraction (549 mg dry wt) was subjected to Polyamide 6 column chromatography, using as an eluent, mixtures of H_2O/MeOH in order of increasing amounts of MeOH. From the fraction eluted with 70% of MeOH was obtained the compounds **2a** (128.3 mg) and **2b** (22.7 mg).

3.3. Epigallocatechin-(2β→7,4β→8)-epicatechin (*1*)

Brown crystals; mp 223–225°C. $C_{30}H_{24}O_{13}$ (Found: C, 54.1; H, 5.0%. $C_{30}H_{24}O_{13}.4H_2O$ requires: C, 54.2; H, 4.8%). CD: $[\phi]211\ -79573$, $[\phi]_{227} + 119573$. UV λ^{MeOH} max. nm (log ε): 210 (5.16), 225 (5.10), 277 (3.98); NMR data: Table; POS FABMS $[M+H]^+$, *m/z* (rel. int.): 593 (5); 441 (4); 303 (10); 287 (8); 277 (8); 207 (31); 149 (9); 115 (100).

3.4. Epigallocatechin-(2β→7,4β→8)-epicatechin peracetate (*1a*)

Amorphous powder. ^{1}H NMR [300 MHz, *m*, J (Hz), CD_3OD]:4.63 (*d*, 4.3, H-3*u*), 5.30 (*d*, 4.3, H-4*u*), 6.87 (*d*, 2.1, H-6*u*), 6.55 87 (*d*, 2.1, H-8*u*), 7.47 (*s*, H-2′/H-6′*u*), 5.43 (br*s*, H-2*l*), 5.33 (*m*, H-3*l*), 6.54 (*s*, H-6*l*), 7.25 (*d*, 7.9, H-2′*l*), 2.99 (*dd*, 2.6, 17.8, H-4*l*), 2.75 (*dd*, 4.1, 17.8, H-4*l*), 2.30–1.90 (CH_3CO). ^{13}C NMR (75 MHz, CD_3OD): 104.60 (C-2*u*), 68.80 (C-3*u*), 28.76 (C-4*u*), 151.08 and 151.11 (C-5*u*/C-7*u*), 110.87 (C-6*u*), 107.54 (C-8*u*), 153.51 (C-9*u*), 99.33 (C-10*u*), 136.61 (C-1′*u*), 121.09 (C-2′/C-6′*u*), 143.40 (C-3′/C-5′*u*), 137.19 (C-4′*u*), 78.95 (C-2*l*), 67.88 (C-3*l*), 26.25 (C-4*l*), 146.48 (C-5*l*), 104.72 (C-6*l*), 155.13 (C-7*l*), 115.08 (C-8*l*), 151.67 (C-9*l*), 109.87 (C-10*l*), 137.13 (C-1′*l*), 125.65 (C-2′*l*), 144.59 (C-3′*l*), 144.07 (C-4′*l*), 124.14 (C-5′*l*), 127.24 (C-6′*l*), 171.89–168.56 ($C\ OCH_3$), 20.93–19.99 ($COC\ H_3$).

3.5. Antioxidant activities (AA)

The AA of the isolates were measured using the method of autoxidation of β-carotene in a suspension of linoleic acid (Hidalgo et al., 1994). The results were compared to those from the commercial antioxidant propyl gallate and α-tocopherol isolated from natural sources. Thus, the values observed for the compounds were: **1**, AA = 35 (0.17 μM); epicatechin, AA = 29 (0.34 μM); **2a**, AA = 12 (0.22 μM); **2b**, AA = 12 (0.22 μM); *n*-propyl gallate, AA = 43 (0.47 μM) and α-tocopherol, AA = 27 (0.23 μM).

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