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Flavonoids from the root-bark of *Dioclea grandiflora*☆

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Abstract

The systematic investigation of the root-bark of *Dioclea grandiflora* permitted the isolation of three new flavanones: agrandol (5,7-dihydroxy-6-methoxy-8-prenylflavanone), paraibanol (3,5,7,2′,5′-pentahydroxy-6-methoxyflavanone), and diosalol (3,5,7,2′,5′-pentahydroxy-6-methoxy-8-prenylflavanone). Also identified were β-amyrin and 5,7,2′,5′-tetrahydroxy-6-methoxy-8-prenylflavanone. All structural assignments were based solely on spectral data.

Keywords: Dioclea grandiflora; Leguminosae; Flavanone; Flavanonol; 5; 7-dihydroxy-6-methoxy-8-prenylflavanone; Agrandol; 3; 5; 7; 2′; 5′-pentahydroxy-6-methoxy-8-prenylflavanone; Paraibanol; 3; 5; 7; 2′; 5′-pentahydroxy-6-methoxyflavanone; Diosalol

1. Introduction

Dioclea grandiflora Mart. (Leguminosae) which grows in the 'Caatinga' and 'Cerrado' regions of northeastern Brazil is used in popular medicine to treat prostate disorders and kidney stones (Lima, 1989). The $CHCl_3$ and aqueous extracts from the dried root-bark were shown to have significant CNS activity (Batista, Almeida & Bhattacharyya, 1995). In a previous study, a new dihydroflavonol, dioclenol, was isolated from the $CHCl_3$ soluble portion of the ethanolic root-bark extract (Bhattacharyya, Majetich, Spearing & Almeida, 1997), as were two new flavanones, dioclein (Bhattacharyya, Batista & Almeida, 1995) and dioflorin (Bhattacharyya, Majetich, Jenkins & Almeida, 1998). The major constituent, dioclein, was found to have significant analgesic activity (Batista et al., 1995). The promising biological activity of these compounds prompted us to identify the minor constituents of the $CHCl_3$ extract. Towards this end, a fresh extract was analysed and provided three new flavonoids (cf **1**, **2** and **3**), along with a previously isolated flavonoid (**4**) (Miyaiichi, Imoto, Tomimori & Namba, 1988) and β-amyrin (Manato & Kundu, 1994).

2. Results and discussion

The molecular formula of agrandol (**1**) ($C_{21}H_{22}O_5$), was deduced from the high resolution electron impact mass spectra (EIMS) and confirmed by its ^{13}C NMR spectral data (Table 2). It was clear from the ^{13}C NMR spectrum [signals at 42.2 ppm (C-3), 78.2 ppm (C-2) and 197.1 ppm (C-4)] that **1** was a flavanone (Agrawal, 1998). The C═O signal at 197.1 ppm implied the presence of an OH at C-5 since H-bonding with the C═O (C-4) causes this type of substituted flavanone to give a signal between 195.6 and 197.3 ppm, whereas the C═O signal for C-5 unsubstituted flavanones is usually found between 189.7 and 191.7 ppm (Agrawal, 1998). The ^1H NMR spectral data also justified our assignment of a flavanone skeleton for **1** (Table 1). In particular, the one proton doublet of doublets at δ 5.4 (J = 12.8, 3.3 Hz) for H-2 is

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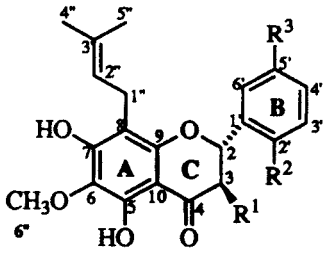
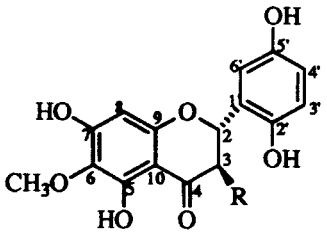


1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = R^3 = OH$



3 R = OH
4 R = H

distinctive for a flavanone (Mabry, Markham & Thomas, 1970), as is the splitting pattern observed for the C-3 methylene which produces a set of doublets of doublets at δ 3.03 for the C-3 axial proton (J = 17.2, 12.6 Hz), and another set of doublets of doublets at δ 2.85 for the C-3 equatorial proton (J = 17.2, 3.5 Hz).

The OH substitution pattern in the A-ring was established by UV analysis. Compound **1** in MeOH gave a major absorption band at 298 nm with a shoulder at 348 nm. Addition of NaOMe gave a 44 nm bathochromic shift with an increase in intensity. Bathochromic shifts were also noted for NaOAc

Table 1
^{1}H NMR (250 MHz) spectral data for flavonoids **1**–**3** in $CDCl_3$, whereas flavonoid **4** is in DMSO-d_6

H	1	2	3	4
2	5.40 *dd* (1.28/3.3) 5.53[a] *dd* (10.0/3.3)	5.33 *d* (11.8) 5.38[a] (10.1)	5.29 *d* (11.7) 5.38[a] *d* (10.3)	5.50 *dd* (11.5/4.5)
3 (trans)	3.03 *dd* (17.2/12.6) 2.81[a] *m*[b]	4.47 *d* (11.8) 4.47[a] (10.2)	4.48 *d* (11.7) 4.61[a] *d* (10.3)	2.88 *m*[b]
3 (cis)	2.85 *dd* (17.3/3.5) 2.81[a] *m*[b]	–	–	2.77 *m*[b]
8	–	–	5.83 *s* 5.95[a] *s*	5.87 *s*
2′	7.40 *m*[b] 7.35–7.51[a] *m*[b]	–	–	–
3′	7.40 *m*[b] 7.35–7.51[a] *m*[b]	6.65 *d* (8.5) 6.57–6.71[a] *m*[b]	6.52 *d* (8.7) 6.57–6.77[a] *m*[b]	6.78 *m*[b]
4′	7.40 *m*[b] 7.35–7.51[a] *m*[b]	6.62 *dd* (8.8/2.8) 6.57–6.71[a] *m*[b]	6.46 *dd* (8.8/2.4) 6.57–6.77[a] *m*[b]	6.78 *m*[b]
5′	7.40 *m*[b] 7.35–7.51[a] *m*[b]	–	–	–
6′	7.40 *m*[b] 7.35–7.51[a] *m*[b]	6.90 *d* (2.6) 6.57–6.71[a] *m*[b]	6.67 *d* (2.5) 6.57–6.77[a] *m*[b]	7.68 *d* (2.0)
1″	3.29 *d* (7.2) 3.30[a] *t* (7.2)	3.10 *d* (7.3) 3.05[a] (7.0)	–	–
2″	5.21 *t* 5.10[a] *t*	5.04 *t* 4.59[a] *t*	–	–
4″	1.68 *s* 1.54[a] *s*	1.51 *s* 1.56[a] *s*	–	–
3″	1.64 *s* 1.58[a] *s*	1.49 *s* 1.50[a] *s*	–	–
6-OCH_3	3.96 *s* 3.67 *s*	3.74 *s* 3.69[a] *s*	3.64 *s* 3.65[a] *s*	3.65 *s*
5-OH	12.20 *s*	11.94 *s*	11.89 *s*	–
7-OH	10.30 *s*	10.25 *s*	9.02 *s*	–

[a] ^{1}H NMR (250 MHz) data for flavonoids **1**–**4** in $CDCl_3$ and CD_3OD.
[b] Signal overlaps.

Table 2
^{13}C NMR (62.9 MHz) spectral data for flavonoids **1**–**4** (DMSO-d_6)[a]

C	1	2	3	4*
2	78.2 (79.1)	77.9 (77.8)	77.9 (78.2)	(74.5)
3	42.2 (43.8)	70.7 (72.7)	70.5 (72.2)	(41.7)
4	197.1 (197.2)	198.7 (1983.3)	198.3 (197.2)	(197.3)
5	152.7 (152.4)	152.5 (151.9)	154.9 (154.7)	(154.8)
6	128.4 (128.3)	128.7 (128.6)	129.1 (129.3)	(128.8)
7	157.0 (155.9)	157.2 (157.1)	159.7 (159.6)	(158.9)
8	107.0 (107.4)	106.9 (108.4)	94.9 (95.5)	(95.0)
9	155.0 (152.4)	155.2 (155.6)	157.9 (158.6)	(158.9)
10	101.7 (103.0)	100.4 (100.4)	100.6 (100.8)	(102.3)
1′	139.0 (139.0)	123.3 (124.4)	123.1 (123.7)	(125.6)
2′	126.3 (126.1)	148.3 (147.5)	148.3 (148.1)	(148.4)
3′	130.4 (129.0)	116.1 (117.2)	116.3 (117.1)	(116.0)
4′	128.5 (128.8)	116.1 (116.6)	116.6 (116.5)	(115.6)
5′	130.4 (129.0)	149.6 (150.0)	149.7 (149.9)	(149.5)
6′	126.3 (126.1)	114.5 (113.8)	114.7 (114.1)	(112.7)
–OCH_3	60.3 (61.2)	60.4 (60.5)	60.1 (60.5)	(60.3)
1″	21.6 (22.1)	21.5 (21.4)	–	–
2″	122.4 (122.1)	122.3 (121.6)	–	–
3″	132.4 (132.2)	130.6 (131.6)	–	–
4″	25.5 (26.0)	25.6 (25.3)	–	–
5″	17.6 (18.0)	17.5 (17.1)	–	–

[a] The data shown in parentheses were recorded in $CDCl_3$ and CD_3OD.

(42 nm) and $AlCl_3$ (22 nm). These shifts are indicative of a 5,7-dihydroxyflavanone (Mabry et al., 1970) skeleton for agrandol (**1**).

The EIMS of **1** showed a molecular ion at m/z 354 (100%). Other significant ion fragments were observed at m/z 339 (M–CH_3), 311 (M–CH_3–CO), 299 (M-55), 251, 235, 207 and 195. The retro-Diels-Alder (RDA) fragmentation of the heterocyclic ring produced ions at m/z 251 $[A+1]^+$ and m/z 104 $[B]^{+\cdot}$ which led us to believe that the B-ring was devoid of substituents. The ^1H NMR spectral data confirmed the unsubstituted B-ring with a typical five proton multiplet centered at δ 7.4 ppm. The H-coupled ^{13}H NMR spectrum showed three doublets at 126.3 ppm (C-2′, C-6′), 130.4 ppm (C-3′, C-5′), and 128.5 (C-4′), along with a singlet at 139.0 ppm for C-1′. This led us to conclude that the B-ring was not substituted.

We interpreted the ion fragments at m/z 299 (M-55) and m/z 195 (250-55) to indicate that the A-ring bears a prenyl group; 55 amu corresponds to the loss of a C_4H_7 unit, a common fragmentation process whenever a prenyl sidechain undergoes allylic cleavage. Evidence for this was found in the ^1H NMR spectrum which showed two signals for the methyl groups at δ 1.64 and 1.68, a triplet for the vinylic proton at δ 5.21, and a doublet for the methylene protons at δ 3.29 (J = 7.2 Hz). The H-coupled ^{13}C NMR and DEPT spectra also confirmed the prenyl substituent with signals for C-1″ at 21.6 ppm, C-2″ at 122.4 ppm, C-3″ at 132.4 ppm, and the methyl groups at 25.5 and 17.6 ppm (Agrawal, 1998) (Table 2). The NMR spectral data and the ion fragments at m/z 330 [M–CH_3] and m/z 207 [A–CH_3] require that the A-ring also has an OCH_3 group. The ^1H NMR spectrum showed a three proton singlet at δ 3.96, while the H-coupled ^{13}C NMR spectrum gave a quartet at 60.3 ppm, consistent with an *ortho*-disubstituted Ar–OCH_3 group in contrast to Ar–OCH_3 groups having at least one unsubstituted *ortho*-position which generally appear between 55 and 57 ppm (Agrawal, 1998).

Having determined that compound **1** was a 5,7-dihydroxyflavanone, the remaining substituents (OCH_3 and a prenyl sidechain) must be located at either C-6 or C-8. The data from an HMBC experiment permitted all of the A-ring substituents to be assigned (Fig. 1). The farthest downfield proton signal at δ 12.2 corresponded to the C-5-OH proton (Mabry et al., 1970). The strong crosspeak with the signal at 101.7 ppm (C-10) confirmed that the proton signal at δ 12.2 is due to the C-5-OH. This proton signal exhibited crosspeaks with the carbons at 128.4 ppm (OCH_3 ipso carbon) and 152.7 ppm. This allowed us to assign the carbon signal at 152.8 ppm to C-5. The relatively upfield shift of C-5 may be attributed to the presence of an *ortho* OCH_3 group. The coupling of the C-5-OH proton with the carbon at 128.4 ppm revealed that the OCH_3 must be at C-6. The OH proton at C-7 gave a signal at 10.3 ppm. There were crosspeaks with the carbons at 107.0 ppm (ipso prenyl carbon), 128.4 ppm (ipso OCH_3 carbon), and 157.0 ppm (C-7); this is only possible if the prenyl group is located at C-8 and the OCH_3 is at C-6. Moreover, the HMBC slice of the prenyl CH_2 protons at δ 3.1 gave crosspeaks with the carbons at 155.0 and 157.0 ppm, which requires that the C-9 signal is at 155.0 ppm, because in flavanones an oxygenated C-7 signal is always farther downfield than C-9 (Miyaiichi et al., 1988). The UV data were also consistent with a flavanone having a prenyl substituent at C-8. Compound **1** showed a 22 nm shift with added $AlCl_3$; however, flavanones with a C-6 prenyl group often do not show an $AlCl_3$-induced UV shift (Sherif, Gupta & Krishnamurti, 1980). In light of all these spectroscopic observations, agrandol (**1**) was deter-

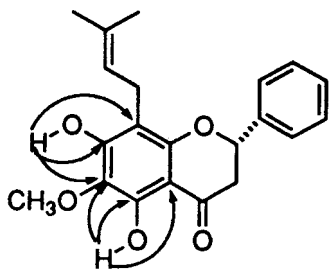


Fig. 1. Diagnostic CH-long-range correlations determined by the HMBC experiment of **1**.

mined to be 5,7-dihydroxy-6-methoxy-8-prenylflavanone.

Paraibanol (**2**), $C_{21}H_{22}O_8$ [(M^+ at m/z 402), mp 149–150°C] was isolated as yellow needles. A 3,5-dihydroxyflavanone was indicated by the ^{13}C NMR signals for C-2 and C-3 at 77.9 and 70.7 ppm, respectively, and a low field signal at 198.7 ppm for the C=O (C-4) (Agrawal, 1998) (Table 1). The dihydroflavanol skeleton of compound **2** was easily discerned from the ^1H NMR spectrum by the characteristic set of doublets at δ 5.33 (J = 11.8 Hz) for H-2 and at δ 4.47 (J = 11.8 Hz) for H-3 (Table 1). The large vicinal coupling constant indicated a *trans* relationship between these protons.

The UV spectrum of flavanonol **2** in MeOH showed an absorption maximum at 302 nm which changed to 342 nm and increased in intensity upon the addition of NaOMe. A 40 nm bathochromic shift with increased intensity is indicative of a 3,5,7-trihydroxyflavanone. Additional confirmation came from the 42 nm bathochromic shift induced by addition of NaOAc, and the 26 nm bathochromic shift caused by the addition of $AlCl_3$ (Mabry et al., 1970).

The EIMS of **2** showed, in addition to the molecular ion at m/z 402, significant fragments at 251, 235, 207, 195 (100%), 69 and 55. The ion fragments at m/z 251 $[A+1]^+$ and 152 $[B]^{+\cdot}$ resulted from the RDA fragmentation of the heterocyclic ring (Mabry & Markham, 1975). This fragmentation pathway also accounted for the ion at m/z 152, which required that there were two OH substituents on the B-ring. Similarly, the $[A+1]^+$ fragment at m/z 251, and the ion fragments at 235, 207, and 195, were also observed for compound **1** which implied that **2** possessed the same A-ring substitution pattern as **1**. Further analysis revealed that the ion fragments at m/z 195 (m/z 250-55), 69, and 55 also indicated a prenyl substituent in the A-ring. This was confirmed by the ^1H and ^{13}C NMR spectral data (Agrawal, 1998) as was the presence of an Ar–OCH_3 substituent.

The placement of the two OH groups on the B-ring was deduced from the ^1H and the ^{13}C NMR spectra. The ^1H NMR spectrum showed an ABM splitting system with two doublets at δ 6.65 (1H, J = 8.5 Hz) and δ 6.90 (1H, J = 2.6 Hz), along with a doublet of doublets at 6.62 ppm (1H, J = 8.8, 2.8 Hz). The *ortho* coupled signal at δ 6.65 corresponded to H-3′, while the *meta* coupled signal at δ 6.90 corresponded to H-6′, whereas the signal which showed both *ortho* and *meta* coupling at δ 6.62 was assigned to H-4′. Flavanonols having 2′,5′-dihydroxy substituted B-rings have similar ^1H NMR spectra (Bhattacharyya et al., 1995; Wollenweber, Mann, Munekazu, Tanaka & Mizuno, 1989). In the ^{13}C NMR spectrum of flavanoids, any substituent at C-2′ causes a small but significant upfield shift (3–4 ppm) of the C-2 signal,

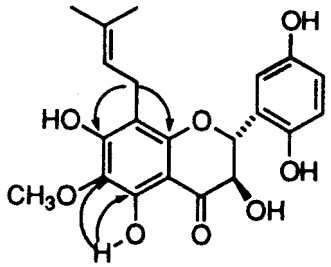


Fig. 2. Diagnostic CH-long-range correlations determined by the HMBC experiment of **2**.

which usually appears between 8.24–85.6 ppm (Agrawal, 1998). The C-2 signal for **2** appeared at 77.9 ppm, thereby indicating the presence of an hydroxyl substituent at C-2′. The ^{13}C NMR spectrum also showed aromatic B-ring signals, consistent with a 2′,5′-dihydroxy substitution pattern (Bhattacharyya et al., 1995).

Based on a partial structure of a 3,5,7,2′,5′-pentahydroxyflavanone for **2**, the OCH_3 and prenyl groups must be located at C-6 and C-8. The HMBC spectrum gave crosspeaks between the ^1H NMR signal at δ 11.90 (C-5-OH), which is H-bonded to the C-4 C=O (Mabry et al., 1970) and coupled with the carbon signal at 152.0 ppm (C-5), and the carbon signal at 128.7 ppm (Fig. 2). The relatively high field shift (152.0 ppm) indicated that C-5 was *ortho* to the OCH_3 and not to the prenyl group. The crosspeak of the C-5-OH with the ipso OCH_3 carbon (128.7 ppm) required that the OCH_3 was located at C-6, which is consistent with the literature data of similarly substituted flavanones (Agrawal, 1998). Thus, the prenyl substituent must be located at C-8. The prenyl methylene protons at δ 3.10 displayed crosspeaks with carbons at 157.2 ppm (C-7) and 155.2 ppm. This means that the signal at 155.2 ppm must be C-9. The well-resolved singlets in the proton spectrum for the prenyl methyl groups (δ 1.50 and 1.49) are consistent with a prenyl substituent at C-8 (Bohlmann, Zdero, Robinson & King, 1981). We concluded that paraibanol (**2**) was 3,5,7,2′,5′-pentahydroxy-6-methoxy-8-prenylflavanone.

The molecular formula of diosalol (**3**) ($C_{16}H_{14}O_8$), was deduced from the EIMS and the ^{13}C NMR spectra (Table 2). The ^{13}C NMR signals at 70.5 ppm (C-3), 77.9 ppm (C-2) and 198.3 ppm (C-4) were indicative of a 7-OH dihydroflavanol (Agrawal, 1998). A flavanol skeleton could also be deduced from analysis of the ^1H NMR spectral data (Table 1). In particular, the H-2 and H-3 protons gave two sets of doublets at δ 5.29 (J = 11.7 Hz) and δ 4.48 (J = 11.7 Hz). The 11.7 Hz vicinal coupling constant indicated a *trans* orientation between these protons (Mabry et al., 1970).

The UV spectrum in MeOH showed a major absorption band at 298 nm with a long wavelength shoulder. Upon addition of NaOMe, a 32 nm batho-

chromic shift, and increased intensity resulted. Addition of NaOAc gave a 36 nm bathochromic shift, which was reversed on treatment with H_3BO_3. Addition of $AlCl_3$ gave a 20 nm bathochromic shift that did not change with the addition of HCl. None of the shift reagents caused decomposition. On the basis of this information we concluded that the A-ring has a 5,7-dihydroxy substitution pattern (Mabry & Markham, 1975).

The EIMS of **3** showed, in addition to the molecular ion at *m/z* 334, significant peaks at 316 $[M–H_2)]^+$, 301 $[M–H_2O–CH_3]^+$, 183 (100%), 182, 167, 156, 123, 69, and 44. The base peak at *m/z* 183 and the ion fragment at *m/z* 152 were attributed to a retro-Diels-Alder fragmentation process, which necessitates that the B-ring fragment at *m/z* 152 contains two hydroxyl groups. Furthermore, the $[A+1]^+$ peak at *m/z* 183 and *m/z* 167 $[182\text{-}CH_3]^+$ required that the A-ring also contains an OCH_3 moiety. That **3** contains an Ar–OCH_3 substituent is confirmed by the signal at 60.1 (*q*) ppm in the ^{13}C NMR DEPT spectrum and a three proton singlet at δ 3.64 in the ^1H NMR spectrum. Moreover, the relatively lowfield of this signal demonstrates that both the positions *ortho* to the OCH_3 group are substituted (Agrawal, 1998).

The B-ring substitution pattern of the two OH groups was found to be 2′,5′-dihydroxy, as in flavanone **2**. The ^{13}C NMR spectral data confirmed the presence of a 2′-OH group by the unusually high field signal of C-2 at 77.9 ppm (Agrawal, 1998), while the ^1H NMR spectrum showed an ABM splitting pattern with a set of doublets at δ 6.76 (J = 2.5 Hz) and δ 6.52 (J = 8.7 Hz), and a second set of doublets at δ 6.46 (J = 8.8, 2.4 Hz). These signals displayed *ortho* and *meta* coupling constants and were assigned as follows: δ 6.76 (H-6′), δ 6.52 (H-3′), and δ 6.46 (H-4′). These coupling constants are comparable to those from compound **2** and known flavanones having 2′,5′-dihydroxyl substituents in the A-ring (Bhattacharyya et al., 1995; Wollenweber et al., 1989). The ^{13}C NMR spectrum also showed aromatic resonances consistent with this assignment.

The above spectral data indicated that **3** was a 3,5,7,2′,5′-pentahydroxyflavanone, and that the remaining substituent, an *ortho*-disubstituted OCH_3, must be located at either C-6 or C-8. For corroboration of this we turned to the ^{13}C NMR spectrum. From this data we saw that the A-ring signals were similar to those of 5,7,2′,5′-tetrahydroxy-6-methoxyflavanone (**4**), which required that the OCH_3 substituent of **3** was at C-6 since the C-6 signals in isomeric compounds are normally upfield by 1 ppm (Miyaiichi et al., 1988). The ^{13}C NMR signal at 94.9 ppm was consistent with the assignment for an unsubstituted C-8 (Agrawal, 1998). The HMBC experiment proved that the C-5-OH proton (δ 11.90) gave crosspeaks with the carbons at 154.9 ppm (C-5), 129.1 ppm (OCH_3 ipso carbon), and 100.6 ppm (C-10) (Fig. 3). These assignments require that the OCH_3 substituent be at C-6. The H-8 aryl proton at δ 5.83 gave crosspeaks with the carbons at 100.6 ppm (C-10), 129.1 ppm (C-6), 157.8, and 159.7 ppm; thus, the signals at 157.8 and 159.7 ppm must be due to C-9 and C-7, respectively. Therefore, diosalol (**3**) must be 3,5,7,2′,5′-pentahydroxy-6-methoxyflavanone.

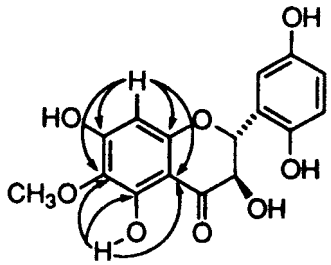


Fig. 3. Diagnostic CH-long-range correlations determined by the HMBC experiment of **3**.

Compound **4** [$C_{16}H_{14}O_7$ (M^+ at *m/z* 318), mp 120°C (dec)] was isolated as pale cream-colored needles and was found to be 5,7,2′,5′-tetrahydroxy-6-methylflavanone, a known natural product. The mp, EIMS, UV, and ^{13}C NMR spectral data were consistent with the data reported by Miyaiichi and co-workers (1988). Their structural assignment was substantiated using an HMBC experiment (Fig. 4) in which the C-5-OH proton at 12.1 ppm exhibited crosspeaks with carbons at 102.3 ppm (C-10), 128.8 ppm (OCH_3 ipso carbon), and 154.8 ppm (C-5). These correlations are only possible when the OCH_3 group is located at C-6.

The triterpene β-amyrin was isolated as white needles and characterized by comparison with published spectral data, along with the mp of the parent compound and its acetate (Manato & Kundu, 1994).

Examination of the seven flavonoids isolated thus far from *D. grandiflora* root-bark revealed three intriguing structural features: (1) all the compounds have a saturated heterocyclic ring; (2) the B-ring substitution

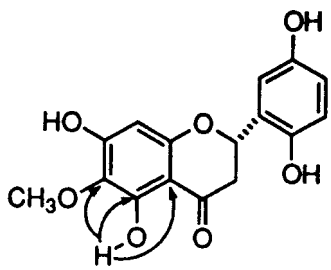


Fig. 4. Diagnostic CH-long-range correlations determined by the HMBC experiment of **4**.

pattern is somewhat related in that two of these compounds have no substituents, one has only a 2′-OH substituent and four others are oxygenated at both the 2′ and 5′ positions; and (3) none of these compounds have OCH_3 substituents on the B-ring. These characteristics suggest a common biogenetic precursor for these flavonoids. Flavanones are the well-accepted precursors of all tricyclic flavonoids, and are directly converted to flavanonols (Heller & Forkmann, 1988). The presence of only flavanones and flavanonols in the root-bark of *D. grandiflora* may have significant taxonomic and evolutionary implications.

3. Experimental

3.1. General procedures

Mps are uncorrected. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrophotometer operating at 250.13 MHz for ^1H NMR and 62.9 MHz for ^{13}C NMR. The HMBC spectra were recorded on a Bruker AMX 400 spectrophotometer operating at 400.13 MHz for ^1H NMR. ^1H–^{13}C coupling constants of 9 and 10 Hz were used. Quadrature detection in the indirectly observed dimension was obtained using the TPPI method (Marian & Wuthrich, 1983). Data were acquired with an acquisition time of 500 ms, 32 scans for each of 256 FID's. The HMBC data were processed with 0°-shifted sine-bell-squared functions in both dimensions and the data sets were filled to 2048×1024 real data points prior to the Fourier transformation. The HMBC spectra were presented in magnitude mode after the Fourier transform to gain maximum sensitivity of the spectrum. The NMR spectra were recorded in $CDCl_3$ unless sufficient CD_3OD was added to overcome any solubility problems. Chemical shifts are reported in ppm relative to the $CHCl_3$ signal present in trace amounts in $CDCl_3$ at δ 7.26 [^1H NMR] and 77.2 ppm [^{13}C NMR]. ^1H NMR spectral data are presented as follows; chemical shift (number of protons, multiplicity, coupling constants in Hertz), while ^{13}C NMR spectral data are presented as follows: chemical shift (multiplicity, carbon assignment). Carbon multiplicities were established using the DEPT experiment unless states otherwise.

Infrared (IR) spectra were recorded using KBr pellets on a Perkin-Elmer 1600 FT–IR. EIMS were recorded on a Finnigan 4000 spectrophotometer. UV data were collected on a Hewlett Packard 8452A diode array spectrophotometer. Shift reagents were prepared and used according to standard procedures (Mabry et al., 1970). Optical rotation data were acquired in MeOH using an Autopol IV automatic polarimeter.

3.2. Plant material

The plant material was collected in late January and early February of 1997, near João Pessoa, Brazil. The voucher specimen (Agra and Gois 1.1192, JPB 21.583) is deposited at JPB Herbarium, João Pessoa, Brazil.

3.3. Extraction, isolation and characterization

The plant material was extracted as described (Bhattacharyya et al., 1997). The $CHCl_3$ soluble part of the crude ethanolic extract was chromatographed on silica gel (E. Merck, 230–400 mesh) in 60 ml fr. using a gradient solvent (hexanes-$CHCl_3$–MeOH) with increasing polarity.

Compound **1** was isolated from frs 44–47 and was recrystallized from C_6H_6 to give dark yellow star-shaped crystals as clusters of needles: mp 156.5–157.5°C. $[\alpha]_D^{22.6}$ −41.2° (5.1×10^{-4} M). EIMS (probe) 70 eV, *m/z* (rel. int.): *m/z* 354 (M^+) (100%), 339, 311, 299, 281, 267, 235, 221, 207, 195, 179, 96, 69, 51. HRFABMS: *m/z* 355.1526 $[MH]^+$ ($C_{21}H_{22}O_5$ requires 355.1545). UV $\lambda^{CH_3OH}_{max}$ nm (log ϵ) 298 (4.18) and 348 sh (3.44) nm; 432 nm in NaOMe, 300 and 340 nm in NaOAc, 298 and 340 mn in $NaOAc/H_3BO_3$, 320 nm in $AlCl_3$ with and without HCl. ^1H NMR (250 MHz, $CDCl_3$) δ 7.40 (5H, *m*, H-1′–H-6′), 6.62 (1H, *s*, OH), 5.40 (1H, *dd, J* = 12.8, 3.3 Hz, H-2), 5.21 (1H, *t, J* = 7.2 Hz, H-2″), 3.96 (3H, *s*, OCH_3), 3.29 (2H, *d, J* = 7.2 Hz, H-1″), 3.03 (1H, *dd, J* = 17.2, 12.6 Hz, Ha-3), 2.85 (1H, *dd, J* = 17.3, 3.5 Hz, Hc-3), 1.68 (3H, *s*, H-5″), 1.65 (3H, *s*, H-4″); ^1H NMR (250 MHz, DMSO-d_6): δ 12.20 (1H, *s*, 5-OH), 10.30 (1H, *s*, 7-OH), 7.51–7.35 (5H, *m*, H-2′–H-6′), 5.53 (1H, *dd, J* = 10, 3.3 Hz, H-2), 5.10 (1H, *t, J* = 7.2 Hz, H-2″), 3.67 (3H, *s*, OCH_3), 3.30 (2H, *t, J* = 6.8 Hz, H-1″), 2.81 (2H, *m*, H-3), 1.58 (3H, *s*, H-5″), 1.54 (3H, *s*, H-4″). ^{13}C NMR (62.9 MHz, DMSO-d_6) 197.1 (*s*, C-4), 157.0 (*s*, C-7), 155.0 (*s*, C-9), 152.7 (*s*, C-5), 1390.0 (*s*, C-1′), 132.4 (*s*, C-3″), 130.4 (*d*, C-3′), 130.4 (*d*, C-5′), 128.5 (*d*, C-4′), 1287.4 (*s*, C-6), 126.3 (*d*, C-2′), 126.3 (*d*, C-6′), 122.4 (*d*, C-2″), 107.0 (*s*, C-8), 101.7 (*s*, C-10), 78.2 (*d*, C-2), 60.3 (*q*, C-6″), 42.2 (*t*, C-3), 25.5 (*q*, C-4″), 21.6 (*t*, C-1)″, 17.6 (*q*, C-5″) ppm; ^{13}C NMR (62.9 MHz, $CDCl_3$) 197.2 (*s*, C-4), 155.9 (*s*, C-7), 152.4 (*s*, C-5, C-9), 139.0 (*s*, C-1′), 132.2 (*s*, C-3″), 129.0 (*d*, C-3′, C-5′), 128.8 (*d*, C-4′), 128.3 (*s*, C-6), 126.1 (*d*, C-2′, C-6′), 122.1 (*d*, C-2″), 107.4 (*s*, C-8), 103.0 (*s*, C-10), 79.1 (*d*, C-2), 61.2 (*q*, C-6″), 43.8 (*t*, C-3), 26.0 (*q*, C-4″), 22.1 (*t*, C-1″), 18.0 (*q*, C-5″) ppm.

Compound **2** was isolated from frs 790–837. This material was further purified via chromatography, elution with 5% CH_3OH in $CHCl_3$, and then recrystallized from C_6H_6/CH_3OH to give bright yellow needles: mp 149.5–150°C. $[\alpha]_D^{23.0}$ +90.9° (2.4×10^{-5} M). EIMS (probe) 70 eV, *m/z* (rel. int.): 402 (M^+), 384, 369, 329,

313, 285, 251, 235, 224, 207, 195 (100%), 180, 163, 149, 137, 123, 111, 95, 83, 69, 55, 44. HRFABMS: *m/z* 403.1411 $[MH]^+$ ($C_{21}H_{22}O_8$ requires 403.1392). IR ν_{max}^{film} cm^{-1}: 3652 (H bonded OH), 1652 (C═O), 1506, 1539, 1558 (Ar), 1456, 1102, 1026, 668. UV $\lambda^{CH_3OH}_{max}$ nm (log ϵ): 302 (4.18) and 344 (3.40) nm; 342 nm in NaOMe, 306 and 344 nm in NaOAc, 302 nm in $NaOAc/H_3BO_3$, 328 nm in $AlCl_3$ (with or without HCl). ^{1}H NMR (250 MHz, $CDCl_3$–CD_3OD): δ 6.9 (1H, *d, J* = 2.6 Hz, H-6′), 6.65 (1H, *d, J* = 8.5 Hz, H-3′), 6.62 (1H, *dd, J* = 8.8, 2.8 Hz, H-4′), 5.33 (1H, *d, J* = 11.8 Hz, H-2), 5.04 (1H, *t, J* = 7.2 Hz, H-2′), 4.47 (1H, *d, J* = 12.4 Hz, H-3), 3.74 (3H, *s*, Ar–OCH_3), 3.1 (2H, *d, J* = 7.3 Hz, H-1″), 1.51 (3H, *s*, H-4″), 1.49 (3H, *s*, H-5″); ^{1}H NMR (250 MHz, DMSO-d_6): δ 11.94 (1H, *s*, 5-OH), 10.25 (1H, *s*, 7-OH), 8.96 (1H, *s*, 3-OH), 6.71–6.57 (3H, *m*, H-3′, H-4′, H-6′), 5.38 (1H, *d, J* = 10.1 Hz, H-2), 4.59 (1H, *t, J* = 6.9 Hz, H-2″), 4.47 (1H, *d, J* = 10.2 Hz, H-3), 3.69 (3H, *s*, Ar–OCH_3), 3.05 (2H, *d, J* = 7.0 Hz, H-1″), 1.56 (3H, *s*, H-4″), 1.50 (3H, *s*, H-5″). ^{13}C NMR (62.9 MHz, $CDCl_3$–CD_3OD) 197.3 (*s*, C-4), 157.1 (*s*, C-7), 155.6 (*s*, C-9), 151.9 (*s*, C-5), 150.0 (*s*, C-5′), 147.5 (*s*, C-2′), 131.6 (*s*, C-3″), 128.6 (*s*, C-6), 124.4 (*s*, C-1′), 121.6 (*d*, C-2″), 117.2 (*d*, C-3′), 116.3 (*d*, C-4′), 113.8 (*d*, C-6′), 108.4 (*s*, C-8), 100.4 (*s*, C-10), 77.8 (*d*, C-2), 72.2 (*d*, C-3), 60.5 (*q*, C-6″), 25.3 (*q*, C-4″), 21.4 (*t*, C-1″), 17.1 (*q*, C-5″) ppm; ^{13}C NMR (62.9 MHz, DMSO-d_6) 198.7 (*s*, C-4), 157.2 (*s*, C-7), 155.2 (*s*, C-9), 152.5 (*s*, C-5), 149.6 (*s*, C-5′), 148.3 (*s*, C-2′), 130.6 (*s*, C-3″), 128.7 (*s*, C-6), 123.3 (*s*, C-1′), 122.3 (*d*, C-2″), 116.1 (*d*, C-3′), 116.1 (*d*, C-4′), 114.5 (*d*, C-6′), 106.9 (*s*, C-8), 100.4 (*s*, C-10), 77.9 (*d*, C-2), 70.7 (*d*, C-3), 60.4 (*q*, C-6″), 25.6 (*q*, C-4″), 21.5 (*t*, C-1″), 17.5 (*q*, C-5″) ppm.

Compound **3** was isolated from frs 919–1137. This material was purified further via chromatography, elution with 7% MeOH in $CHCl_3$, and then recrystallized from C_6H_6/Me_2CO to give pale yellow crystals: mp 142°C dec. $[\alpha]_D^{22.6}$ +30.1° (7.2×10^{-4} M). EIMS (probe) 70 eV, *m/z* (rel. int.): 334 (M^+), 316, 301, 289, 273, 251, 211, 195, 183 (100%), 167, 156, 141, 123, 113, 95, 83, 69, 55, 44. HRFABMS: *m/z* 335.0771 $[MH]^+$ ($C_{16}H_{14}O_8$ requires 335.0767). UV $\lambda^{CH_3OH}_{max}$ nm (log ϵ): 296 nm (4.18), 328 nm in NaOMe, 332 nm in NaOAc, 298 nm in $NaOAc/H_3BO_3$, 318 nm in $AlCl_3$ (with and without HCl). ^{1}H NMR (250 MHz, $CDCl_3$–CD_3OD): δ 6.67 (1H, *d, J* = 2.5 Hz, H-6′), 6.52 (1H, *d, J* = 8.7 Hz, H-3′), 6.46 (1H, *dd, J* = 8.8, 2.4 Hz, H-4′), 5.83 (1H, *s*, H-8), 5.29 (1H, *d, J* = 11.7 Hz, H-2), 4.48 (1H, *d, J* = 11.7 Hz, H-3), 3.64 (3H, *s*, Ar–OCH_3); ^{1}H NMR (250 MHz, DMSO-d_6): δ 11.89 (1H, *s*, 5-OH), 9.02 (1H, *s*, 7-OH), 8.81 (1H, *s*), 8.30 (1H, *s*), 6.77–6.57 (3H, *m*, H-6′, H-3′, H-4′), 5.95 (1H, *s*, H-8), 5.38 (1H, *d, J* = 10.3 Hz, H-2), 4.61 (1H, *d, J* = 10.3 Hz, H-3), 3.65 (3H, *s*, Ar–OCH_3). ^{13}C NMR (62.9 MHz, $CDCl_3$–CD_3OD) 197.2 (*s*, C-4), 159.6 (*s*, C-7), 158.6 (*s*, C-9), 154.7 (*s*, C-5), 149.9 (*s*, C-5′), 148.1 (*s*, C-2′), 129.3 (*s*, C-6), 123.7 (*s*, C-1′), 117.1 (*d*, C-3′), 116.5 (*d*, C-4′), 114.1 (*d*, C-6′), 100.8 (*s*, C-10), 95.5 (*d*, C-8), 78.2 (*d*, C-2), 72.2 (*d*, C-3), 60.5 (*q*, Ar–OCH_3) ppm; ^{13}C NMR (62.9 MHz, DMSO-d_6) 198.3 (*s*, C-4), 159.7 (*s*, C-7), 157.8 (*s*, C-9), 154.9 (*s*, C-5), 149.7 (*s*, C-5′), 148.3 (*s*, C-2′), 129.1 (*s*, C-6), 123.1 (*s*, C-1′), 116.3 (*d*, C-3′), 116.3 (*d*, C-3′), 114.7 (*d*, C-6′), 100.6 (*s*, C-10), 94.9 (*d*, C-8), 77.9 (*d*, C-2), 70.5 (*d*, C-3), 60.1 (*q*, Ar–OCH_3) ppm.

5,7,2′,5′-Tetrahydroxy-6-methoxy-8-prenylflavanone (**4**) was isolated from frs 809–837. This material was purified further via chromatography, elution with 5% MeOH in $CHCl_3$, to give **4** as cream-colored crystals: mp 120°C (dec). $[\alpha]_D^{22.1}$ −214.3° (1.7×10^{-4} M). EIMS (probe) 70 eV *m/z* (rel. int.): 318 (M^+), 300, 285 (100%), 264, 257, 236, 222, 213, 183, 167, 152, 136, 129, 111, 97, 83, 69, 55. HRFABMS: *m/z* 318.0705 ($C_{16}H_{14}O_7$ requires 318.0740). IR ν_{max}^{film} cm^{-1}: 2356 (br OH), 1652 (conj. C═O), 1558, 1539, 1520, 1505, 1456, 1164, 1092, 1018, 667. UV $\lambda^{CH_3OH}_{max}$ nm (log ϵ): 294 nm (4.13) and 342 nm (sh), 328 nm in NaOMe, 328 nm in NaOAc, 296 nm in $NaOAc/H_3BO_3$, 314 nm in $AlCl_3$ (with and without HCl). ^{1}H NMR (250 MHz, $CDCl_3$ and CD_3OD): δ 7.68 (1H, *d, J* = 2.0 Hz, H-6′), 6.78 (2H, *m*, H-3′, H-4′), 5.87 (1H, *s*, H-8), 5.50 (1H, *dd, J* = 11.5, 4.5 Hz, H-2), 3.65 (3H, *s*, Ar–OCH_3), 2.84–2.71 (2H, *m*, H-3); ^{13}C NMR (62.9 MHz, $CDCl_3$ and CD_3OD) 197.3 (*s*, C-4), 158.9 (*s*, C-7), 158.8 (*s*, C-9), 154.8 (*s*, C-5), 149.5 (*s*, C-5′), 148.4 (*s*, C-2′), 128.8 (*s*, C-6), 125.6 (*s*, C-1′), 116.0 (*d*, C-3′), 115.6 (*d*, C-4′), 112.7 (*d*, C-6′), 102.3 (*s*, C-10), 94.0 (*d*, C-8), 74.5 (*d*, C-2), 60.3 (*q*, OCH_3), 41.7 (*t*, C-3) ppm.

β-Amyrin (**5**) was isolated from frs 44–47. Recrystallization from MeOH provided β-amyrin as white needles: EIMS (probe) 70 eV *m/z* (rel. int.): 426 (M^+), 399, 365, 339, 251, 218 (100%), 205, 189, 147, 119, 95, 69, 55. ^{13}C NMR (62.9 MHz, $CDCl_3$) 145.1 (*s*), 121.6 (*d*), 79.0 (*d*), 55.1 (*d*), 47.6 (*d*), 47.2 (*d*), 46.8 (*t*), 41.6 (*s*), 39.7 (*s*), 38.7 (*s*), 38.5 (*t*), 37.1 (*t*), 36.9 (*s*), 34.6 (*t*), 33.3 (*q*), 32.6 (*t*), 32.4 (*s*), 31.0 (*s*), 28.3 (*q*), 28.0 (*q*), 27.2 (*t*), 26.9 (*t*), 26.1 (*t*), 25.9 (*q*), 23.6 (*q*), 23.5 (*t*), 18.3 (*t*), 16.7 (*q*), 15.5 (*q*), 15.4 (*q*) ppm.

β-Amyrin acetate was prepared in standard fashion and was recrystallized from C_6H_6/MeOH to yield white needles: mp 250–250.25°C. EIMS (probe) 70 eV *m/z* (rel. int.): 468 (M^+), 408, 365, 339, 257, 218 (100%), 205, 189, 147, 119, 95, 69, 55. ^{13}C NMR (62.9 MHz, $CDCl_{34}$) 171.2 (*s*), 145.4 (*s*), 121.8 (*d*), 81.1 (*d*), 55.4 (*d*), 47.7 (*d*), 47.4 (*d*), 46.9 (*t*), 41.8 (*s*), 40.0 (*s*), 38.4 (*s*), 37.9 (*t*), 37.3 (*t*), 37.0 (*s*), 34.9 (*t*), 33.5 (*q*), 32.7 (*t*), 31.3 (*s*), 28.6 (*q*), 28.2 (*q*), 27.0 (*t*), 26.3 (*t*), 26.1 (*q*), 23.9 (*q*), 23.7 (*t*), 21.5 (*q*), 18.4 (*t*), 17.0 (*q*), 15.7 (*q*) ppm (Tables 1 and 2).

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