PERGAMON

Phytochemistry 59 (2002) 415–418

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

An isoflavanoid–neoflavonoid and an *O*-methylated isoflavone from the heartwood of *Dalbergia nitidula*

Madelyn Bekker, Elfranco Malan*, Jacobus A. Steenkamp, E. Vincent Brandt

Department of Chemistry, University of the Orange Free State, PO Box 339, Bloemfontein, 9300 South Africa

Received in revised form 4 October 2001

Abstract

An isoflavanoid (6→2) neoflavonoid dimer and a 4′,5′,7-trihydroxy-2′-methoxyisoflavone, both as the acetate derivatives were isolated from the heartwood of *Dalbergia nitidula*. Their structures were established by spectroscopic methods.

Keywords: *Dalbergia nitidula*; Leguminosae; Isoflavanoid–neoflavonoid; Absolute configuration; Methoxyisoflavone

1. Introduction

The relatively high natural abundance of flavonoid oligomers (Porter, 1994) is well documented in contrast to the limited occurrence of iso- and neo-flavonoid oligomers (Dewick, 1994; Donnelly and Boland, 1994). The first three pterocarpan-neoflavonoid dimers (daljanelins) and the isoflavanoid–neoflavonoid analogue **1** were identified from the heartwood of *Dalbergia nitidula* (Ferreira et al., 1995).

Arising from the re-investigation of the heartwood of *D. nitidula* with respect to the compounds present in low concentrations, we now report the isolation of a new isoflavanoid-neoflavonoid dimer **2** where the neoflavonoid unit is linked to the C-6 of the isoflavan moiety.

Although the 2′,4′,5′,7-substitution pattern is not unique amongst isoflavonoids (Agrawal, 1989; Dewick, 1994) the new *O*-methyl derivative 4′,5′,7-trihydroxy-2′-methoxyisoflavone **4** was isolated as the acetate derivative **5**.

2. Results and discussion

Excluding the 17 isoflavonoid compounds identified from the bark and heartwood before (Van Heerden et al., 1978, 1980; Bezuidenhoudt et al., 1984), the MeOH extract of the heartwood of *D. nitidula* yielded (3*S*)-6-(3-phenyl-5-hydroxy-6-methoxy benzo[b]furan-2-ylmethyl)vestitol (vestitol = 2′,7-dihydroxy-4′-methoxyisoflavan) **2** and the *O*-methylated isoflavone **4** as the acetate derivative **5**.

The C-8 linked neoflavanoid-isoflavan **1** was previously isolated from the heartwood of *D. nitidula* (Ferreira et al., 1995). The present compound **2** under discussion is an isomer of **1** with the neoflavonoid unit linked at C-6 of the vestitol moiety. The ^{1}H NMR data (Table 1) of compound **3** show an ABX-system, four singlets and the protons assigned to the unsubstituted B-ring, all of them in the aromatic region (δ 6.59–7.49). The isoflavan character is typical (Markham, 1982; Ferreira et al., 1995) from the H-2_{ax}, H-2_{eq} (δ 3.93 and δ 4.28), H-3 (δ 3.25–4.28) and the H-4_{ax}, H-4_{eq} (δ 2.97 and δ 2.87) resonance patterns of the C-ring in the heterocyclic region.

The EI–MS of compound **3** with molecular ion of [M^+] 650.2152 ($C_{38}H_{34}O_{10}$) along with the ^{1}H and ^{13}C spectra strongly suggested a dimeric structure.

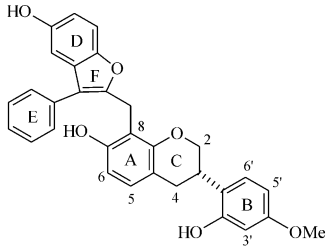
Using the isoflavan heterocyclic protons (C-ring) as reference the NOESY experiment (refer **3**) showed associations of 2-H_{eq} (C, δ 4.28) with 6′-H (B, δ 7.11) as well as 4-H_{eq} (C, δ 2.87) with 5-H (A, δ 6.96). The ABX-system of the B-ring was established from interactions between 6′-H(B) and 5′-H (B, δ 6.82) and between 3′-H (B, δ 6.64) with the 4′-OMe (δ 3.80).

In extension of the above assignments, the 5-H(A) showed associations with the isolated methylene group

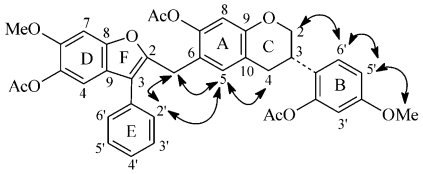
* Corresponding author. Tel.: +27-51-4013018; fax: +27-51-4307805.

E-mail address: malane@sci.uovs.ac.za (E. Malan).

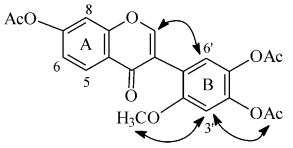
0031-9422/02/$ - see front matter
PII: S0031-9422(01)00462-9



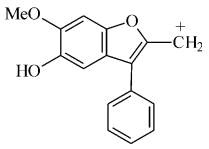
1



2 R=H
3 R=Ac (n.O.e. associations)



4 R=H
5 R=Ac (n.O.e. associations)



6 m/z 253

Table 1
^{1}H NMR (300 MHz, 296 K) data of isoflavanoid–neoflavonoid derivative **3** in $CDCl_3$. Splitting patterns and *J*-values are given in parentheses

Ring	H	**3**
A	5-H	6.96 (*s*)
	8-H	6.59 (*s*)
B	3′-H	6.64 (*d*, *J* = 3.0 Hz)
	5′-H	6.82 (*dd*, *J* = 3.0 and 10.0 Hz)
	6′-H	7.11 (*dd*, *J* = 1.0 and 10.0 Hz)
C	2_{eq}-H	4.24 (*ddd*, *J* = 2.0, 4.0 and 9.0 Hz)
	2_{ax}-H	3.93 (*dd*, *J* = 9.0 and 9.0 Hz)
	3-H	3.25 (*dddd*, *J* = 4.0, 5.5, 9 and 10.5 Hz)
	4_{ax}-H	2.97 (*ddd*, *J* = 1.0, 10.5 and 15.5 Hz)
	4_{eq}-H	2.87 (*ddd*, *J* = 2.0, 5.5 and 15.5 Hz)
D	4-H	7.25 (*s*)
	7-H	7.10 (*s*)
E	(2-6)-H	7.35–7.49 (*m*)
	–CH_2–	4.04 (*d*, *J* = 14.0 Hz)
	–CH_2–	4.00 (*d*, *J* = 14.0 Hz)
	OCH_3	3.88 (*s*)
	OCH_3	3.80 (*s*)
	$OCOCH_3$	2.33 (*s*)
	$OCOCH_3$	2.34 (*s*)
	$OCOCH_3$	2.17 (*s*)

at δ 3.99 and 4.05 (Ferreira et al., 1995) and the prominent 2′,6′-H at δ 7.47 of the E-ring. Interactions between the 2′,6′-H(E) with the isolated methylene group and the 4-H (D, δ 7.25) confirmed the interflavanyl bond comprising the methylene as linkage from C-6(A) $\rightarrow$ C-2(F) in **3**. The 7-H (D, δ 7.10) associated with 6-OMe(D). The above information suggested the structure of vestitol for the isoflavan moiety. COSY experiment confirmed all the above observations with couplings between 7-H (D, δ 7.10) to both 4-H (D, δ 7.25) and 6-OMe (D, δ 3.88). The correlations between 5-H (A, δ 6.96) and the isolated methylene group at δ 4.51 and δ 3.99, and between the 8-H (A, δ 6.59) established the A-ring. This information also confirmed the position of the methylene linkage between 6-C(A) to 2-C(F). Very important is the EIMS fragment *m*/*z* 253 (28.5%) **6** which confirmed the neoflavanoid moiety in accordance to the fragmentation as was proposed by Ferreira et al. (1995).

Carbon atom resonances were assigned on the basis of ^{1}H to ^{13}C connectivities e.g. HMQC and HMBC correlations (Table 2).

The D-ring of **3** was identified by coupling of 6-C (D, δ 159.55) with 6-OMe (δ 3.88, $^{3}J_{CH}$) and 7-H (D, δ 7.10, $^{2}J_{CH}$); 5-C (D, δ 149.54) correlated with 7-H (D, δ 7.10, $^{3}J_{CH}$) and 4-H (D, δ 7.25, $^{2}J_{CH}$), while 8-C (D, δ 153.86) showed coupling with 7-H (D, δ 7.10, $^{2}J_{CH}$) and 4-H (D, δ 7.25, $^{3}J_{CH}$) ; 9-C (D, δ 121.38) correlated with 7-H (D,

Table 2
^{13}C NMR data (ppm) of compound **3** at 296 K

Ring	C	ppm	Ring	C	ppm
A/C	2-C	70.86	D/F	2-C	151.66
	3-C	30.10		3-C	118.48
	4-C	27.22		4-C	113.40
	5-C	131.32		5-C	149.54
	6-C	121.07		6-C	159.55
	7-C	148.10		7-C	127.78
	8-C	110.99		8-C	153.86
	9-C	153.93		9-C	121.38
	10-C	120.55			
B	1′-C	112.97	E	1′-C	132.58
	2′-C	137.13		2′-C	129.33
	3′-C	108.84		3′-C	129.24
	4′-C	149.81		4′-C	128.25
	5′-C	125.19		5′-C	129.33
	6′-C	96.39		6′-C	129.24
				CH_2	27.22
				OCH_3	56.73
				OCH_3	55.87
				$OCOCH_3$	21.43
				$OCOCH_3$	21.06
				$OCOCH_3$	20.98
				$OCOCH_3$	169.97
				$OCOCH_3$	169.74
				$OCOCH_3$	169.74

$^3J_{CH}$) and 4-H (D, $^2J_{CH}$), all these connectivities confirmed the proposed structure of **3**.

The resonance signal of 1′-C (E, δ 132.58) showed coupling with the E-ring phenyl protons at δ 7.49. The F-ring was determined from correlations of 2-C (F, δ 151.66) to the isolated methylene group protons at δ 4.51 and 3.99 ($^2J_{CH}$), as well as couplings from 3-C (F, δ 118.48) to the E-ring protons, the 4-H (D, δ 7.25) and the isolated methylene protons (all $^3J_{CH}$).

The substitution pattern of the A-ring was evident from the 6-C (A, δ 121.97) and 10-C (A, δ 120.55) couplings with 8-H (A, δ 6.59, $^3J_{CH}$); the 6-C(A) also coupled with the isolated methylene protons ($^2J_{CH}$), disclosing the position of linkage to the A-ring. The 7-C (A, δ 148.10) correlated with 8-H (A, δ 6.59, $^2J_{CH}$) and 5-H (A, δ 6.96, $^3J_{CH}$), while 9-C (A, δ 153.93) coupled with both 8-H(A) and 5-H(A) to complete the identification of the A-ring. The 4′-C (δ 149.81) of the B-ring coupled with the 4′-OMe protons (δ 3.80, $^3J_{CH}$), 6′-H (δ 7.11, $^3J_{CH}$) and 3′-H (δ 6.64, $^2J_{CH}$). Further correlations from 2′-C (B, δ 137.13) to 6′-H (δ 7.11, $^3J_{CH}$) and between 1′-C (B, δ 112.97) and 6′-H (B, δ 7.11, $^2J_{CH}$) confirmed the substitution pattern of the B-ring.

The CD-data of **3** (see 3.2) are in agreement with that of (+)-vestitol and the compound, (3*S*)-8-(5-hydroxy-6-methoxy-3-phenylbenzo[b]furan-2-ylmethyl)vestitol, isolated by Ferreira et al., (1995) and could be unambiguously assigned to have the 3*S* absolute configuration. The newly discovered isoflavanoid (6→2) neoflavonoid **2** (as the full acetate **3**) is isomeric to the (8→2) linked compound.

Table 3
^{1}H (300 MHz, 296 K) and ^{13}C NMR assignments for compound **5** in $CDCl_3$. Splitting patterns and *J*-values are given in parentheses

Ring	H	**5**	C	ppm
A/C	2-H	7.93 (*s*)	C-2	154.72
A/C	5-H	8.32 (*d*, *J* = 9.0 Hz)	C-3	122.45
	6-H	7.19 (*dd*, *J* = 2.0 and 9.0 Hz)	C-4	175.07
	8-H	7.32 (*d*, *J* = 2.0 Hz)	C-5	128.15
			C-6	120.14
			C-7	154.95
			C-8	111.49
			C-9	157.08
			C-10	122.15
B	3′-H	6.87 (*s*)	1′-C	116.84
	6′-H	7.09 (*s*)	2′-C	152.22
			3′-C	108.19
	OMe	3.87 (*s*)	4′-C	147.68
	OAc	2.39 (*s*)	5′-C	137.69
	OAc	2.33 (*s*)	6′-C	125.42
	OAc	2.16 (*s*)		
			$O\underline{C}OCH_3$	169.34
				169.04
				169.04
			$O\underline{C}H_3$	56.61

The existence of 2′,4′,5′,7-substituted isoflavones are well known (Burns et al., 1984; Rao et al., 1984; Agrawal, 1989; Dewick, 1994) but to our knowledge the isolation of a 2′-methoxy isoflavone **4** as the acetate derivative **5** is a first. The ^{1}H NMR data (Table 3) of **5** showed the characteristic 2-H (C, *s*, δ 7.93) at low field (Rao et al., 1984), together with an ABX-system and two singlets at δ 6.87 and δ 7.09 for the A- and B-rings resp. From 2D COSY correlations between 2-H(C) and 6′-H (B, *s*, δ 6.87), the 2′-OMe with 3′-H (B, *s*, δ 6.87), together with the n.O.e. association of the 3′-H with 4′-OAc and the associations indicated on **5** it was possible to identify the B-ring. The low field doublet at δ 8.32 of the 5-H (A, *J* = 9.0 Hz) is typical of the proton adjacent to the 4-C (δ 175.07) carbonyl (Burns et al., 1984). With the 5-H as reference it was possible to establish the ABX-system of the A-ring [Table 3 (**5**)]. With the assistance of HMQC and HMBC it was possible to assign the carbons (Table 3) and confirm the structure of **5**. EIMS showed a molecular ion *m*/*z* 426 (M^+, 60%) with RDA-fragments *m*/*z* 136 (A-ring, 100%) and *m*/*z* 164 (B-ring, 20%), which confirmed the suggested structure **5** with the OMe-group as a substituent on the B-ring.

3. Experimental

^{1}H NMR spectra were recorded on a Bruker AVANCE DPX 300 spectrometer for solns. as indicated, with Me_4Si as internal standard. Electron impact–mass spectroscopy (EI–MS) data were recorded on a VG-70E instrument when tuned to function in the EI–MS mode. CD data were collected in MeOH as solvent on a Hitachi 150-20 spectropolarimeter. TLC was performed on pre-coated Merck plastic sheets (silica gel 60 PF_{254}, 0.25 mm) and the plates were sprayed with H_2SO_4–HCHO (40:1, v/v) after development. Preparative plates (PLC) [20×22 cm, Kieselgel PF_{254} (1.0 mm)] were air dried and used without prior activation. Column chromatography was done on Sephadex LH-20 in 120×4 cm columns, at a flow rate of 30 cm^3/h using ethanol as eluent. Flash column chromatography (FCC) was carried out in a glass column (54×6.5 cm) charged with Merck Kieselgel 60 (230–400 mesh) using benzene-EtAc (9:1, v/v) as eluent at a flow rate of 60 cm^3/min. Acetylations were conducted in Ac_2O-pyridine at 50 °C for 24 h. Evaporations were done under reduced pressure at ambient temp. in a rotary evaporator, and freeze drying of aqueous solutions on a Virtis 12 SL freezemobile.

3.1. Metabolites from the methanol extract of the heartwood of D. nitidula

The ether extract (20 g) was subjected to column chromatography on Sephadex LH-20 (120×4 cm column at a flow rate of 30 cm^3/h) in ethanol as eluent.

Four fractions were collected and combined after TLC monitoring (benzene–Me_2CO–methanol, 6:3:1; v/v/v) viz. 1 (2.84 g), 2 (3.12 g), 3 (2.90 g) and 4 (4.96 g).

All four combinations were derivatized in Ac_2O-pyridine and separately subjected to FCC using benzene–EtAc (9:1, v/v) as eluent, fractions 1.1 (0.1 g), 1.2 (0.05 g), 1.3 (0.03 g) and 1.4 (0.55 g) were collected and subsequently separated on PLC with benzene–EtAc (9:1, v/v) as solvent system. Liquiritigenindiacetate (R_f 0.43, 35 mg) was obtained from fraction 1.1; vesticarpin-diacetate (R_f 0.27, 3 mg) was identified from fraction 1.2 and fraction 1.3 yielded 2′,7-diacetoxy-4′-methoxyisoflavone (R_f 0.24, 7.5 mg). The new 4′,5′,7-triacetoxy-2′-methoxyisoflavone **5** was isolated from fraction 1.4 (R_f 0.26, 5 mg).

Fractions 2, 3 and 4 yielded all the compounds as was reported in the literature (Letcher and Shirley, 1976; Van Heerden et al., 1980; Bezuidenhout et al., 1984; Ferreira et al., 1995).

Fraction 4 yielded amongst the other known compounds the new 6-C-(3-phenyl-5-acetoxy-6-methoxybenzo[b]furan-2-ylmethyl)vestitol-triacetate **3** (R_f 0.30, 4 mg, TLC hexane–Me_2CO–EtAc 7:1:2, v/v/v) after the initial FCC separation using hexane–Me_2CO–EtAc, 11:6:3, v/v/v as eluent.

*3.2. (3S)-6-(3-Phenyl-5-acetoxy-6-methoxy benzo[b]furan-2-ylmethyl)-vestitol-triacetate **3** as a light brown solid*

(Found: M^+, 650.2152. $C_{38}H_{34}O_{10}$ requires M, 650.2150.) ^1H NMR: Table 1. ^{13}C NMR: Table 2. CD $[\theta]_{238}$ 104, $[\theta]_{245}$ 780, $[\theta]_{252}$ 281, $[\theta]_{260}$ 473, $[\theta]_{270}$ 11, $[\theta]_{288}$ −1510, $[\theta]_{294}$ −969 and $[\theta]_{300}$ −45. EI–MS, *m/z* 650 (M^+, 62%), 374 (8), 359 (10), 282 (10), 240 (10), 150 (30), 137 (100), 134 (20).

*3.3. 4′,5′,7-Triacetoxy-2′-methoxyisoflavone **5** as a yellow-brown solid*

(Found: M^+ 426.0951. $C_{22}H_{18}O_9$ requires M, 426.0950.) ^1H NMR: Table 3. ^{13}C NMR: Table 3. EI–MS, *m/z* 426 (M^+, 60%), 164 (15), 136 (100).

Acknowledgements

We gratefully thank the National Research Foundation, Pretoria and the 'Sentrale Navorsingsfonds' of the UOFS for the financial support. Our thanks are due to Dr. R Bekker (NMR) and Dr. L Fourie (MS) for their assistance.

References

Agrawal, P.K., 1989. Carbon—13 NMR of Flavonoids. Elsevier, Amsterdam, pp. 192–207.

Bezuidenhoudt, B.C.B., Brandt, E.V., Roux, D.G., 1984. Synthesis of isoflavanoid oligomers using a pterocarpan as inceptive electrophile. Journal of the Chemical Society, Perkin Transactions 1, 2767–2778.

Burns, D.T., Dalgarno, B.G., Gargan, P.E., Grimshaw, J., 1984. An isoflavone and a coumestan from *Eysenhardtia Polystacha*—Robert Boyle's flourescent acid- base indicator. Phytochemistry 23, 1. 167–169.

Dewick, P.M., 1994. In: Harborne, J.B. (Ed.), The Flavonoids. Advances in Research since 1986. Chapman & Hall, London, pp. 117–238.

Donnelly, D.M.X., Boland, G., 1994. In: Harborne, J.B. (Ed.), The Flavonoids. Advances in Research since 1986. Chapman & Hall, London, pp. 239–258.

Ferreira, J.A., Nel, J.W., Brandt, E.V., Bezuidenhoudt, B.C.B., Ferreira, D., 1995. Oligomeric isoflavonoids. Part 3. Daljanelins A–D, the first pterocarpan- and isoflavanoids–neoflavonoid analogues. Journal of the Chemical Society, Perkin Transactions 1, 1049–1056.

Letcher, R.M., Shirley, I.M., 1976. Phenolic compounds from the heartwood of *Dalbergia nitidula*. Phytochemistry 15, 353–354.

Markham, K.P., 1982. The Flavonoids. Advances in Research. Chapman & Hall, London, pp. 19–37.

Porter, L.J., 1994. In: Harborne, J.B. (Ed.), The Flavonoids. Advances in Research since 1986. Chapman and Hall, London, pp. 23–115.

Van Heerden, F.R., Brandt, E.V., Roux, D.G., 1978. Structure and synthesis of some complex pyranoisoflavonoids from the bark of *Dalbegia nitidula* Welw. ex Bak. Journal of Chemical Society, Perkin Transactions 1, 137–145.

Van Heerden, F.R., Brandt, E.V., Roux, D.G., 1980. Synthesis of the pyranoisoflavonoid, heminitidulan. Isoflavanoid and rotenoid glycosides from the bark of *Dalbergia nitidula* Welw. ex Bak. Journal of the Chemical Society, Perkin Transactions 1, 2463–2469.

Rao, E.V., Murthy, M.S.R., Ward, R.S., 1984. Nine isoflavones from *Tephrosia Maxima*. Phytochemistry 23 (7), 1493–1501.