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NATURAL (−)-FISETINIDOL-(4,8)-(−)-EPICATECHIN PROFISETINIDINS

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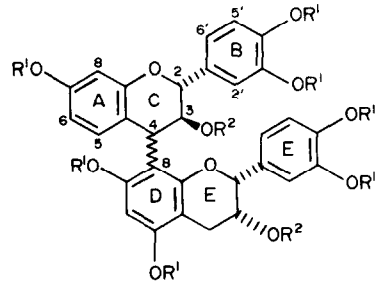
Key Word Index—*Guibourtia coleosperma*; *Colophospermum mopane*, *Baikiaea plurijuga*, Leguminosae; heartwood, profisetinidins, (−)-fisetinidol-(4α,8) and (4β,8)-(−)-epicatechins.

Abstract—The natural occurrence of the first profisetinidins associated with (−)-epicatechin is demonstrated in the heartwood of three legumes. ^{1}H NMR spectroscopy at 300 MHz permits assessment of the relative abundance of the predominant rotamers in each of the heptamethyl ether diacetates of the (−)-fisetinidol-(4α,8) and (4β,8)-(−)-epicatechins at ambient temperatures.

(−)-Epicatechin [(2*R*,3*R*)-2,3-*cis*-flavan-3,3′,4′,5,7-pentaol] figures prominently both as 'upper' and 'terminal' units in procyanidin- [1–3] and as 'terminal' units in proguibourtinidin- [4–6] condensed tannins. In contrast to the ubiquitous (−)-fisetinidol-(+)-catechin† profisetinidins- [7, 8], natural counterparts of the latter class of condensed tannins associated with (−)-epicatechin are hitherto unknown. Our search for tannin prototypes in the flora of southern Africa has now revealed the presence of (−)-fisetinidol-(4α,8) and (4β,8)-(−)-epicatechin **1** and **3** in the heartwoods of three species of the Caesalpinioideae, *Guibourtia coleosperma* (Rhodesian copalwood) [9], *Colophospermum mopane* (mopane) [9] and *Baikiaea plurijuga* (Rhodesian teak) [9].

The ethyl acetate extract of the heartwood of *G. coleosperma* afforded both the (4α,8)- and (4β,8)-(−)-fisetinidol-(−)-epicatechins **1** and **3**. Only the (4α,8)-isomer **1** was found in the heartwood extracts (methanol) of *C. mopane* and *B plurijuga*. Owing to the complexity of the phenolic mixtures of these sources, the novel metabolites **1** and **3** were identified as heptamethyl ether diacetates **2** and **4**. Their ^{1}H NMR spectra (300 MHz) (Table 1) at ambient temperatures exhibit duplicated signals characteristic of the effects of dynamic rotational isomerism [10].

At these temperatures the heterocyclic regions each display an AMX-($J_{2,3}=J_{3,4}=10.0$ Hz for **2**; $J_{2,3}=7.0$, $J_{3,4}=5.5$ Hz for **4**) and ABMX-system ($J_{2,3}=ca$ 1.0 Hz for both **2** and **4**) indicative of 2,3-*trans*-3,4-*trans*: 2,3-*cis*- and 2,3,-*trans*-3,4,-*cis*: 2,3,-*cis* relative configuration for **2** and **4** respectively. Spin decoupling experiments using the 2- (for ABX-systems of rings B and E) and 4-H (for ABX-system of ring A) resonances as reference signals, facilitated definition of the consitution of the 'upper' (−)-fisetinidol and 'lower' (−)-epicatechin moieties In addition to the aforementioned ABX-systems, the aromatic region of each spectrum exhibited a one-proton singlet



1 ⁄⁄⁄⁄ = ⁞⁞⁞⁞, R¹ = R² = H
2 ⁄⁄⁄⁄ = ⁞⁞⁞⁞, R¹ = Me, R² = Ac
3 ⁄⁄⁄⁄ = ▬, R¹ = R² = H
4 ⁄⁄⁄⁄ = ▬, R¹ = Me, R² = Ac

(δ6.18, 6.15 for **2** and **4** respectively). The chemical shifts of these were reminiscent of the proton of a C-8 substituted (−)-epicatechin unit [7, 11]. This was unequivocally confirmed by the strong NOE association of the 'residual' proton with both 5-(10.7, 10.8% for **2** and **4** respectively) and 7-OMe (10.1, 12.7% for **2** and **4** respectively) of the (−)-epicatechin 'lower' units. Confirmation for the structures of **2** and **4** and thus also the 2*R*,3*S*,4*S* (C-ring): 2*R*,3*R* (F-ring) and 2*R*,3*S*,4*R* 2*R*,3*R* absolute configuration of **1** and **3** respectively, was obtained by comparison of the ^{1}H NMR and CD data of **2** and **4** with those of the corresponding derivatives of their synthetic counterparts [7]. As ^{1}H NMR spectroscopy at 80 MHz only permitted partial assignment of signals for synthetic **2** and **4**, detailed analysis is included in this communication (Table 1).

At room temperature the ^{1}H NMR spectra of **2** and **4** indicated two predominant rotameric forms, i.e 41:9 and 39:11 for **2** and **4** respectively. The magnitude of the rotational barrier is such that in DMSO-d_6 at 453 K, the upper limit of the Bruker AM-300 system, the spectra of both **2** and **4** still displayed severe line-broadening in the aromatic and heterocyclic regions. This contrasts with the isomeric (−)-fisetinidol-(+)-catechin pair [7] where

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†(−)-Fisetinidol is (2*R*,3*S*)-2,3-*trans*-flavan-3,3′,4′,7-tetraol and (+)-catechin its 5-oxy analogue

Table 1 ^{1}H NMR (300 MHz) peaks (ppm) of (−)-fisetinidol-(4α 8) and (4β,8)-(−)-epicatechin heptamethyl ether diacetates **2** and **4** in $CDCl_3$ at 298 K

Ring	H	2*	4*
A	5	6 74 (*d*, 8 5)	6 60 (*d*, 8 5)
	6	6 43 (*dd*, 2 5, 8 5)	6 11 (*dd*, 2 5, 8 5)
	8	6 47 (*d*, 2 5)	5 90 (*d* 2 5)
B	2	6 57 (*d*, 2 0)	6 92 (*d*, 2 0)
	5	6 68 (*d*, 8 0)	6 78 (*d*, 8 0)
	6	6 75 (*dd*, 2 0, 8 0)	6 93 (*dd*, 2 0, 8 0)
C	2	4 90 (*d* 10 0)	5 47 (*d* 7 0)
	3	6 17 (*t*, 10 0)	5 62 (*dd*, 5 5, 7 0)
	4	4 88 (*d*, 10 0)	4 80 (*d*, 5 5)
D	6	6 18 (*s*)	6 15 (*s*)
E	2	6 54 (*d*, 2 0)	6 77 (*d*, 2 0)
	5	6 66 (*d* 8 0)	6 68 (*d*, 8 0)
	6	6 42 (*dd*, 2 0, 8 0)	6 50 (*dd*, 2 0, 8 0)
F	2	4 98 (*br s*, *ca* 1 0)	4 32 (*br s*, *ca* 1 0)
	3	5 25 (*m*)	5 29 (*m*)
	4_{ax}	2 81 (*dd*, 1 5, 18 5)	2 84 (*dd* 1 5, 18 5)
	4_{eq}	2 94 (*dd*, 4 5, 18 5)	2 95 (*dd*, 5 5, 18 5)
	OMe	3 41, 3 70, 3 74, 3 79, 3 82, 3 83 (5-D), 3 86 (7-D) (each *s*)	3 39, 3 74, 3 77 (7-D), 3 82, 3 83, 3 84 (5-D), 3 85 (each *s*)
	OAc	1 54, 1 72 (each *s*)	1 84, 1 87 (each *s*)

*Peaks of the predominant rotamer only,
Splitting patterns and *J*-values (Hz) are given in parentheses

only the (4β,8)-derivative exhibited comparable restriction to rotation. Such differences in activation energy to induce 'free rotation' about the interflavanyl bond in the (−)-fisetinidol-(−)-epicatechin- and (+)-catechin analogues are presumably attributable to the effect of the axial 3-OAc(F) in the former group The considerable higher energy requirements of the 3,4-*cis* analogues, e g **4**, may originate from significant contributions of A-conformers of the C-ring [12], these being evident from small $J_{2,3(C)}$ values [7.0 and 6 8 Hz for the (−)-epicatechin and (+)-catechin homologues respectively] ^{1}H NMR experiments aimed at defining the preferred conformation about the interflavanyl bond [13] invariably failed as a result of the 'slow exchange' of rotamers at ambient temperatures Such a phenomenon was evident from the prominent NOE association of 7-OMe(D) with H-4(C) in both **2** (1 1%) and **4** (1 6%)

Although efforts to identify the (4,6)-regio-isomers of **1** and **3** in the above sources have hitherto failed, we have indirect evidence of their natural occurrence from related tetrahydropyrano[2,3-*f* and *g*]chromenes [14] The extremely low proportions in which the (4,6)-analogues were formed *in vitro* [7] may reflect similar minor concentrations in Nature hence complicating their isolation

EXPERIMENTAL

^{1}H NMR spectra were recorded at 300 MHz in $CDCl_3$ (298 K) or DMSO-d_6 (453 K) with TMS as reference CD spectra were determined in MeOH. Media used for the separation of components were Sephadex LH-20/ethanol or Fractogel TSK HW-40(S)/EtOH for CC, DC-plastikfolin 60 F_{254} for TLC and Kieselgel PF_{254} (1 mm × 20 × 20 cm) for prep TLC TLC bands were located under UV and/or with H_2SO_4–HCHO (40 1) spray reagent and compounds recovered from the absorbent with Me_2CO Methylations were performed with excess CH_2N_2 over 48 hr at −15° and acetylations in Ac_2O-pyridine

(−)-Fisetinidol-(4α,8) and (4β,8)-(−)-epicatechins **1** *and* **3** *from G coleosperma* Heartwood drillings (6 kg) were extracted (EtOAc) and fractionated (Craig countercurrent assembly followed by CC on Sephadex LH-20 EtOH) according to the procedures previously described [14] Methylation of a portion (4 g) of fraction 2F (10 89 g) followed by prep TLC (C_6H_6–Me_2CO, 4 1, ×2) afforded six bands, $2F_1$ (R_f 0 62, 186 mg), $2E_2$ (R_f 0 58, 253 mg), $2E_3$ (R_f 0 54, 222 mg), $2E_4$ (R_f 0 48, 268 mg), $2E_5$ (R_f 0 45, 251 mg) and $2E_6$ (R_f 0 36, 678 mg) Fraction $2E_6$ was acetylated and resolved by prep TLC (C_6H_6–Me_2CO, 9 1) to give two bands at R_f 0 62 (120 mg) and 0 50 (90 mg) The former band afforded 3-*O*-acetyl-tri-*O*-methyl-(−)-fisetinidol-(4β,8)-3-*O*-acetyl-tetra-*O*-methyl-(−)-epicatechin **4** as a white solid [7], ^{1}H NMR data (Table 1) MS and CD data [7] The R_f 0 50 band gave the (4α,8)-isomer **2** as an amorphous white solid [7], ^{1}H NMR data (Table 1), MS and CD data [7]

(−)-Fisetinidol-(4α,8)-(−)-epicatechin **1** *from* B plurijuga Heartwood drillings (4 3 kg) were extracted (MeOH), enriched (Craig procedure) and fractionated (CC on Sephadex LH-20/EtOH) according to the procedures described earlier [14] A portion (1 5 g) of fraction 3H (2 64 g) was subjected to CC (Fractogel TSK HW-40(S) EtOH) to give three fractions $3H_1$ (172 mg), $3H_2$ (342 mg) and $3H_3$ (638 mg) in order of increasing R_f Methylation of fraction $3H_3$ and prep TLC (hexane–Me_2CO–EtOAc, 11 6 3, ×2) afforded a main band at R_f 0 46 (262 mg) This was acetylated and subsequently resolved by prep TLC ($CHCl_3$–EtOAc, 9 1) to two bands at R_f 0 51 (119 mg) and 0 33 (68 mg) The R_f 0 51 fraction consisted of the known [7] (−)-fisetinidol-(4α,8)-(+)-catechin heptamethyl ether diacetate and the R_f 0 33 band of the (−)-fisetinidol-(4α,8)-(−)-epicatechin heptamethyl ether diacetate **2**, with physical data identical to those of the compound from *G coleosperma*

(−)-Fisetinidol-(4α,8)-(−)-epicatechin **1** *from* C mopane The

fractionation procedure for the methanol extract of the heartwood leading to fraction 4 3 has previously been described [15, 16]. This fraction (417 mg) was methylated and resolved by prep TLC (C_6H_6–Me_2CO–MeOH, 90 9 1, × 3) to give two bands at R_F 0 17 (23 8 mg) and 0 09 (145 8 mg) The latter band was acetylated and the mixture resolved by prep TLC (hexane–Me_2CO–EtOAc, 7 2 1, × 9) to four fractions at R_F 0 33 (8 5 mg), 0 29 (51 1 mg), 0 25 (20 5 mg) and 0 09 (16 7 mg) The R_F 0.25 fraction afforded the (−)-fisetinidol- (4α,8)-(−)-epicatechin heptamethyl ether diacetate **2** with spectroscopic data identical to those from *G coleosperma* and *B plurijuga*

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