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OLIGOMERIC FLAVANOIDS. PART 14[a]. PROGUIBOURTINIDINS BASED ON (-)-FISETINIDOL AND (+)-EPIFISETINIDOL UNITS.

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Abstract —— The rare series of proguibourtinidins (3,4′,7-trihydroxy functionality) based on the fisetinidol (3,3′,4′,7-tetrahydroxy functionality) 5-deoxy flavan-3-ol unit is extended by identification of (+)-guibourtinidol-(4β,6)-(-)-fisetinidol and the (+)-guibourtinidol-(4α,6) and (4β,6)-(+)-epifisetinidols. These metabolites are formed in low yields *via* acid-catalyzed coupling of (+)-guibourtacacidin and (-)-fisetinidol, the high activation energy of generating a 4-C benzylic carbocation being attributable to the poor electron donating properties of the phenol B-ring of the flavan-3,4-diol. Stereo-electronic principles operating in the *in vitro* process presumably also control their formation in Nature.

Pro- and leucoguibourtinidins with their 4′,7-dihydroxy phenolic functionality represent a relatively rare group of condensed tannins which, while occurring as minor components in Australian *Acacia* spp.[1], predominate in the Southern African species *Guibourtia coleosperma* (large false mopane)[2,3], *Julbernardia globiflora* (munondo)[4], and *Acacia luederitzii* (bastard umbrella thorn)[5,6]. Known analogues from the last three sources are invariably based on 5-oxygenated 'lower' flavan-3-ol units, *i.e.* (+)-catechin, (-)-epicatechin, and (+)-afzelechin. Our recent demonstration of the occurrence of (+)-guibourtinidol-(-)-fisetinidols in the heartwood of *Colophospermum mopane* (mopane)[7,8], reputed for its exceptionally high concentrations[7] of the 5-deoxy flavan-3-ols (-)-fisetinidol[b] and (+)-epifisetinidol[b], indicated that these 5-deoxy analogues may feasibly act as nucleophiles in the biosynthetic pathway leading to this class of proguibourtinidins. A systematic approach towards the chemistry and extension of this rare series of oligoflavanoids thus became objectives in our continuing investigation of the diverse metabolic pool of the mopane.

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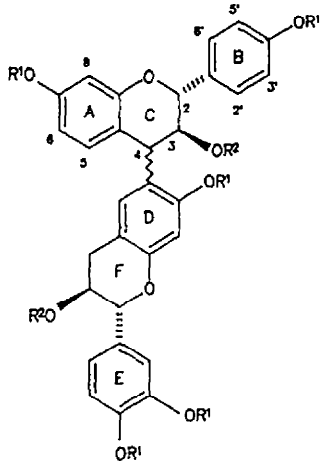
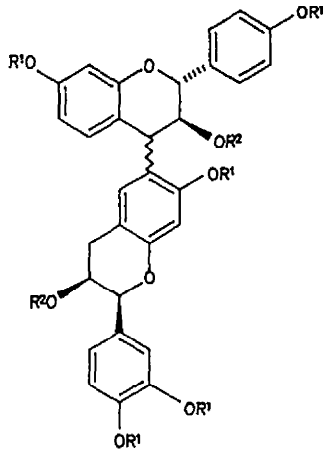
[a]Part 13. J.C.S. Malan, J.A. Steenkamp, D.A. Young, and D. Ferreira, *Tetrahedron*, 1989, paper AM 9295.

[b](-)-Fisetinidol is (2*R*,3*S*)-2,3-*trans*-flavan-3,3′,4′,7-tetraol and (+)-epifisetinidol the (2*S*,3*S*)-2,3-*cis* C-2 epimer.

RESULTS AND DISCUSSION

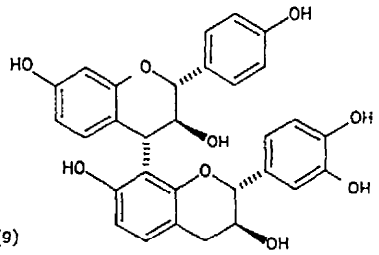
The (+)-guibourtinidol-(4α,6)-(-)-fisetinidol 1[7], (+)-guibourtinidol-(4α,8)-(-)-fisetinidol 9[8] and (+)-guibourtinidol-(4α,6′)-(-)-fisetinidol 10[8] are accompanied in the heartwood of *C. mopane* by the (+)-guibourtinidol-(4β,6)-(-)-fisetinidol 3 and the (+)-guibourtinidol-(4α,6) and (4β,6)-(+)-epifisetinidols 5 and 7. Owing to the complexity of the phenolic mixture these novel metabolites were identified as pentamethyl ether diacetates, *e.g.* 4, the additional chromatographic stages offered by such an approach being a prerequisite for compound purity.





(1) ≡ 1, R^1 = R^2 = H
(2) ≡ 2, R^1 = Me, R^2 = Ac
(3) ≡ 3, R^1 = R^2 = H
(4) ≡ 4, R^1 = Me, R^2 = Ac

(5) ≡ 5, R^1 = R^2 = H
(6) ≡ 6, R^1 = Me, R^2 = Ac
(7) ≡ 7, R^1 = R^2 = H
(8) ≡ 8, R^1 = Me, R^2 = Ac



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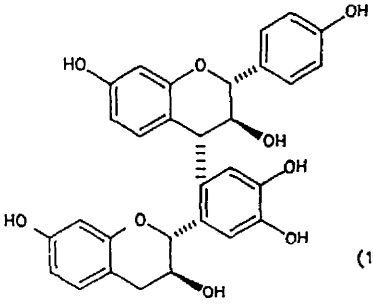


(10)

Comparison of the ^{1}H NMR data (Table) of the pentamethyl ether diacetate 4 of the (+)-guibourtinidol-(4β,6)-(-)-fisetinidol 3 with those of the (4α,6)-analogue 2 reveals their close structural resemblance. The relative 2,3-*trans*-3,4-*cis* (C-ring): 2,3-*trans* (F) configuration is evident from the coupling constants of heterocyclic protons [$J_{2,3}$ 7.5, $J_{3,4}$

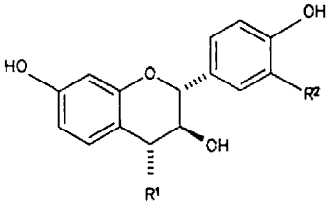
5.0 Hz; $J_{2,3}$(F) 7.5 Hz]. Correlation of the AA'BB' system in the aromatic region (δ7.27, 6.85, J9.0 Hz) with the 2-H doublet (δ5.14) of the heterocyclic AMX system and of 4-H (δ4.79) of the latter system with 5-H(A) (δ6.77, d, J8.5 Hz) of the highfield aromatic ABX system *via* decoupling experiments, defines the constitution of the (+)-guibourtinidol unit. Substitution at 6-C of the 'lower' 2,3-*trans* fisetinidol moiety is confirmed by the characteristic appearance of the D-ring protons as singlets[7] [δ6.55 (br.), 6.46; 5- and 8-H(D) respectively]. NOE experiments not only confirmed these allocations but also facilitated assignment of all methoxy resonances. The same protocol of decoupling experiments using the 2-(C- and F) and 4-H(C) resonances as reference signals, supported by appropriate NOE data, also enables structural definition of the pentamethyl ether diacetates 6 and 8 of the novel (+)-guibourtinidol-(4α,6) and (4β,6)-(+)-epifisetinidols 5 and 7 [$J_{2,3}$(F) *ca.* 1.0 Hz for the 2,3-*cis* DEF moieties of both 6 and 8] (Table).

Table ^{1}H NMR peaks (p.p.m.) of (+)-guibourtinidol-fisetinidol derivatives 2, 4, 6, and 8 at 300 MHz in $CDCl_3$. Splitting patterns and J-values (Hz) are given in parentheses.

Ring	H	2, 365 K	4, 296 K	6, 345 K	8, 296 K
A	5	6.65(d,8.5)	6.77(d,8.5)	6.67(d,8.5)	6.80(d,8.5)
	6	6.42(dd,2.5,8.5)	6.46(dd,2.5,8.5)	6.43(dd,2.5,8.5)	6.47(dd,2.5,8.5)
	8	6.50(d,2.5)	6.55(d,2.5)	6.51(d,2.5)	6.55(d,2.5)
B	2/6	7.32(d,9.0)	7.27(d,9.0)	7.32(d,9.0)	7.26(d,9.0)
	3/5	6.84(d,9.0)	6.85(d,9.0)	6.84(d,9.0)	6.85(d,9.0)
C	2	4.99(d,9.0)	5.14(d,7.5)	5.01(d,9.0)	5.16(d,7.5)
	3	5.66(dd,9.0,9.0)	5.49(dd,5.0,7.5)	5.68(t,9.0)	5.50(dd,5.0,7.5)
	4	4.54(d,9.0)	4.79(d,5.0)	5.47(d,9.0)	4.77(d,5.0)
D	5	6.63(br.s)	6.55(br.s)	6.63(br.s)	6.59(br.s)
	8	6.46(s)	6.46(s)	6.49(s)	6.51(s)
E	2	6.90(d,2.0)	6.88(d,2.0)	7.01(d,2.0)	7.01(d,2.1)
	5	6.83(d,7.0)	6.82(d,8.5)	6.84(d,8.5)	6.85(d,8.5)
	6	6.89(dd,2.0,7.0)	6.91(dd,2.0,8.5)	6.94(dd,2.0,8.5)	6.94(dd,2.1,8.5)
F	2	4.95(d,7.0)	4.97(d,7.5)	5.03(br.s,*ca* 1.0)	5.03(br.s,*ca* 1.0)
	3	5.27(m)	5.28(m)	5.37(m)	5.38(m)
	4_{ax}	2.92(dd,5.0,16.0)	2.74(dd,8.0,16.0)	2.74(dd,5.0,17.5)	2.77(dd,2.2,17.5)
	4_{eq}	2.69(dd,7.0,16.0)	2.95(dd,5.5,16.0)	3.10(dd,4.5,17.5)	3.15(dd,4.5,17.5)
	OMe	3.74(7-D),3.76(7-A),3.79(4-B),3.86(3-E),3.88(4-E) (each s)	3.71(7-D),3.77(7-A),3.78(4-B),3.84(3-E),3.86(4-E) (each s)	3.76(7-A/7-D),3.79(4-B),3.86(4-E),3.87(3-E)(each s)	3.74(7-D),3.78(4-B),3.79(7-A),3.88(4-E),3.90(3-E)(each s)
	OAc	1.66,1.88(each s)	1.76,1.90(each s)	1.66,1.88(each s)	1.76,1.94(each s)

The absolute configurations, *i.e.* 2*R*,3*S*,4*S* for 3 and 7, and 2*R*,3*S*,4*R* for 5, of the proguibourtinidin moieties are evident[9] from the sign of the high-amplitude Cotton effects (positive for 4 and 8, negative for 6) in the 225-240 nm region of the CD spectra of their derivatives, and the known relative configurations as defined by coupling constants of the heterocyclic AMX protons. Confirmation of the (2*R*,3*S*)-(-)-fisetinidol (for 3) and (2*S*,3*S*)-(+)-epifisetinidol (for 5 and 7) 'terminal' units was obtained *via* their biomimetic synthesis.

Thus, condensation[10] of (+)-guibourtacacidin 11 [(2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*-flavan-3,4,4′,7-tetraol][3] and (-)-fisetinidol 12 in 0.1M HCl for 140h at 45°C affords a mixture com-



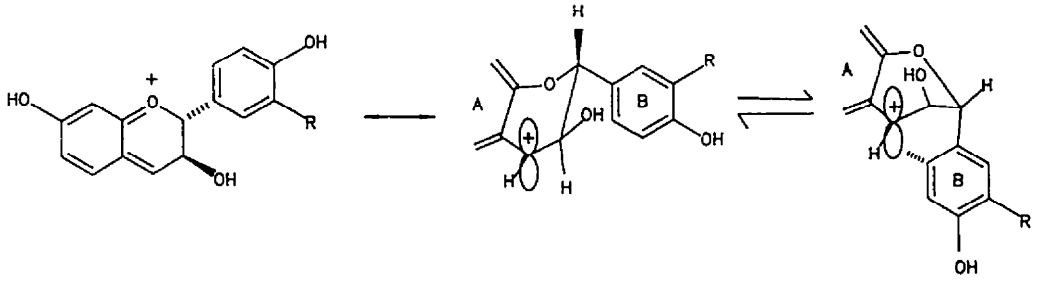
(11) R¹= OH, R²= H
(12) R¹= H, R²= OH
(13) R¹= R²= OH

prising considerable quantities of (-)-fisetinidol and (+)-epifisetinidol (*ca* 4:1), and the proguibourtinidins 1, 3, 5, and 7 in 18.8% overall yield[c] . Their pentamethyl ether diacetates are identical to those of the natural products by comparison of ^{1}H NMR (Table) and CD data.

Notable in the above conversion is the prolonged reaction time, conspicuously low yields, more severe conditions compared to those for the condensation of (+)-mollisacacidin 13 [3′-oxy analogue of (+)-guibourtacacidin 11] with (+)-catechin[10] (20°C, 2h, 45% yield) and (-)-fisetinidol[11] 12 (40°C, 24h, 36% yield), and the predominant formation of the (4*β*,6)-analogues 3 and 7 relative to that of the (4*α*,6)-isomers 1 and 5 (*ca* 4:1). Since the prevailing conditions do no permit interconversion of the (4*β*,6)- and (4*α*,6)-analogues, the observed product ratio presumably reflects kinetic control[12] of coupling of the flavan-3-ol moiety to the carbocationic intermediates 16 and 18 (compare, however, ref. 13). Whereas the enhanced rate of the reaction of (+)-mollisacacidin 13 with (+)-catechin

[c]Tri- and tetra-flavanoid analogues (*cf.* ref. 11) are formed in proportions which did not merit further investigation.

compared to that with (-)-fisetinidol 12 is attributable to the superior nucleophilicity of the phloroglucinol-type A-ring of (+)-catechin *vs* the resorcinol-type A-ring of (-)-fisetinidol, differences in the relative rates of condensation of (+)-mollisacacidin 13 and (+)-guibourtacacidin 11 with (-)-fisetinidol 12 are to be sought in the rates of formation of the 4-C carbocationic intermediates 16 and 17 derived from the flavan-3,4-diols. The significant difference may be rationalized on the assumption that the A-ring delocalized 4-carbocations which are generated under acidic conditions, are stabilized to varying degrees by delocalization of the benzylic charge over the B-ring. Although heterocyclic oxonium ions of types 14 and 15 were invoked[14] to explain the higher rate of condensation of (+)-leucorobinetinidin[(2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*-flavan-3,3′,4,4′,5′,7-hexaol] with (+)-catechin compared to that of (+)-mollisacacidin 13, recent demonstration of the conformational mobility of the pyran heterocycle[15-17] indicates that the benzylic carbocations 16 and 17 may be additionally stabilized by charge donation from the B-ring *via* A-conformations 18 and 19 (Scheme). The more electron-rich pyrocatechol function in 19 is more effective than the mono-oxygenated moiety in 18 thus leading to higher condensation rates for (+)-mollisacacidin 13.

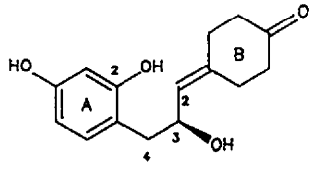


(14) R = H
(15) R = OH

(16) R = H
(17) R = OH

(18) R = H
(19) R = OH

Scheme



(20)

The relative drastic reaction conditions required for inducing formation of the 4-C carbocation 16 also leads to protonation of the (-)-fisetinidol heterocyclic oxygen hence initiating its conversion to the 2-C epimer, (+)-epifisetinidol, by recyclization *via*

2-OH(A) and the *Si*-face at 2-C in a presumed quinone-methide intermediate 20. Replacement of (-)-fisetinidol by (+)-epifisetinidol as nucleophile in stereoselective coupling with the benzylic carbocation 16 may then explain the genesis of the (+)-guibourtinidol-(4α,6) and (4β,6)-(+)-epifisetinidols 5 and 7. Such epimerization at 2-C of the (-)-fisetinidol moiety may, however, also occur at the 'dimeric' level, *e.g.* 1 → 5 and 3 → 7, by a similar mechanism.

A notable feature regarding the acid-catalyzed epimerizations is the apparent absence of a similar phenomenon at 2-C of the 'upper' guibourtinidol moiety. Such an observation may indicate an increased susceptibility to quinone-methide formation at the pyrocatechol E-ring under acid-catalysis compared to the phenol B-ring in proguibourtinidins 1, 3, 5 and 7.

The sparse group of fisetinidol based proguibourtinidins 1, 9, and 10, their natural occurrence hitherto being restricted to the heartwood of *C. mopane*, is thus complemented by identification of the three additional analogues 3, 5, and 7. Stereo- and electronic factors governing their *in vitro* formation presumably also control the *in vivo* process.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3$ with TMS as internal standard. Accurate mass estimations were obtained with a Kratos MS80 instrument and CD data in methanol on a Jasco J-20 spectropolarimeter. TLC was performed on precoated Merck Plastic sheets (silica gel 60 PF_{254}, 0.25 mm) and the plates sprayed with H_2SO_4-HCHO (40:1) after development. Preparative plates (PLC), 20x20 cm, Kieselgel PF_{254} (1.0 mm) were air-dried and used without prior activation. CC was on Sephadex LH-20 with EtOH as eluant. Methylations were performed with an excess of CH_2N_2 in MeOH-diethyl ether over 48h at -15°C, while acetylations were in Ac_2O-pyridine at ambient temperatures. Evaporations were performed under reduced pressure at *ca* 60°C.

Proguibourtinidins 3, 5, *and* 7 *from the heartwood of C. mopane* — The extraction (MeOH), fractionation, and derivatization/purification procedures leading to fraction 1.1.1.2 were fully described in Part 9[18] and those leading to fractions 1.1.2.2 and 2.2.4 in Part 8[8].

Fraction 1.1.1.2 (6.4 mg) afforded the (+)-*guibourtinidol*-(4β,6)-(-)-*fisetinidol pentamethyl ether diacetate* 4 as an amorphous white solid (Found: M^+, 684.2569. $C_{39}H_{40}O_{11}$ requires M, 684.2571); ^{1}H NMR (Table); CD $[\Theta]_{293}$ 0, $[\Theta]_{280}$ -0.3×10^4, $[\Theta]_{252}$ 0, $[\Theta]_{236}$ 7.5×10^4, $[\Theta]_{232}$ 9.2×10^4, and $[\Theta]_{223}$ 0.

Fraction 1.1.2.2 (2.1 mg) gave the (+)-*guibourtinidol*-(4β,6)-(+)-*epifisetinidol pentamethyl ether diacetate* 8 as a white solid (Found: M^+, 684.2560. $C_{39}H_{40}O_{11}$ requires M, 684.2571); ^{1}H NMR data (Table); CD $[\Theta]_{296}$ 0, $[\Theta]_{287}$ -0.6×10^4, $[\Theta]_{282}$ -0.4×10^4, $[\Theta]_{269}$ -1.7×10^4, $[\Theta]_{253}$ 0, $[\Theta]_{237}$ 17.8×10^4, and $[\Theta]_{226}$ 0.

The methyl ether fraction 2.2.4 (75.3 mg) was acetylated and separated by PLC in C_6H_6-hexane-Me_2CO (70:25:5, x4) to give a main band at Rf 0.35 (23.8 mg). De-acetylation with a 1% solution of KOH in methanol for 1h at 45°C followed by PLC in C_6H_6-Me_2CO (85:15)

gave two bands at Rf 0.45 (3.0 mg) and 0.31 (18.9 mg). The latter band was re-subjected to PLC in hexane-Me_2CO-EtOAc (7:2:1, x12) to give two bands at Rf 0.51 (4.2 mg) and 0.44 (7.6 mg). Acetylation of the latter gave the *(+)-guibourtinidol-(4α,6)-(+)-epifisetinidol pentamethyl ether diacetate* 6 as a white solid (8.0 mg) (Found: M^+, 684.2556. $C_{39}H_{40}O_{11}$ requires M, 684.2571); ^1H NMR data (Table); CD $[\Theta]_{300}$ 0, $[\Theta]_{289}$ $1.0x10^4$, $[\Theta]_{279}$ 0, $[\Theta]_{268}$ $-0.7x10^4$, $[\Theta]_{255}$ 0, $[\Theta]_{245}$ $2.0x10^4$, $[\Theta]_{240}$ 0, $[\Theta]_{238}$ $-15.7x10^4$, $[\Theta]_{223}$ $-18.8x10^4$, and $[\Theta]_{208}$ 0. Acetylation of the Rf 0.45 band (3.0 mg) band gave the known[7] (+)-guibourtinidol-(4α,8)-(+)-catechin hexamethyl ether diacetate. The Rf 0.51 band (4.2 mg) gave an additional sample of the same compound.

Synthesis of proguibourtinidins 1, 3, 5, and 7. — (-)-Fisetinidol (250 mg) was dissolved in 0.1M HCl (100 ml), a solution of (+)-guibourtacacidin (100 mg) in 0.1M HCl (20 ml) added, and the mixture stirred for 140h at 45°C. The mixture was chilled with ice, extracted with EtOAc (5x100 ml), and the combined extract was dried with Na_2SO_4. Evaporation of the solvent afforded a tan powder (235 mg) which was subjected to CC (2.7x90 cm column, flow rate - 1 ml/min, 16.0 ml fractions, first 600 ml of eluant discarded) to give three fractions[d], 1 (tubes 6-16, 191.6 mg), 2 (32-42, 23.6 mg), and 3 (46-54, 12.8 mg). Fraction 1 comprised of a mixture (4:1) of (-)-fisetinidol and (+)-epifisetinidol.

Methylation of fraction 2 followed by PLC in C_6H_6-Me_2CO (9:1, x2) gave two bands at Rf 0.19 (8.6 mg) and 0.15 (4.5 mg). Acetylation of the Rf 0.19 band afforded the (+)-guibourtinidol-(4β,6)-(-)-fisetinidol pentamethyl ether diacetate 4. Similar treatment of the Rf 0.15 band gave the (+)-guibourtinidol-(4β,6)-(+)-epifisetinidol pentamethyl ether diacetate 8. The physical data of 4 and 8 were identical to those of the corresponding derivatives of the natural products.

Fraction 3 (12.8 mg) was methylated and purified by PLC in C_6H_6-Me_2CO (8:2) to give a main band at Rf 0.39 (8.9 mg). Acetylation and subsequent PLC in C_6H_6-Me_2CO-MeOH (97:2:1, x2) gave two bands at Rf 0.33 (3 mg) and 0.29 (1.6 mg). The Rf 0.33 band gave the (+)-guibourtinidol-(4α,6)-(-)-fisetinidol pentamethyl ether diacetate 2, and the Rf 0.29 band the (+)-guibourtinidol-(4α,6)-(+)-epifisetinidol pentamethyl ether diacetate 6. These compounds were identical to the same derivatives of the natural products by comparison of their ^1H NMR and CD data. Although the ^1H NMR data of 2 was previously published[7], it is included in the table for comparative purposes.

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[d]Followed by a fraction (*ca* 2.0 mg) comprising tri- and tetra-flavanoid analogues which was not further investigated.

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