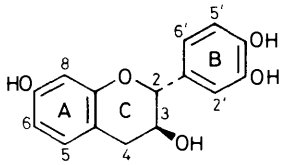
Oligomeric Flavanoids. Part 1. Novel Dimeric Profisetinidins from *Colophospermum mopane*

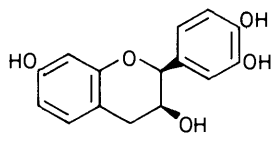
Jacobus A. Steenkamp, Johannes C. S. Malan, David G. Roux, and Daneel Ferreira *
Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa

The range of natural dimeric profisetinidins is extended by the recognition of a series of novel (4,6)-coupled analogues based not only on (−)-fisetinidol [(2*R*,3*S*)-2,3-*trans*-flavan-3,3′,4′,7-tetraol] but for the first time also on (+)-epifisetinidol [(2*S*,3*S*)-2,3-*cis*-flavan-3,3′,4′,7-tetraol] as constituent units, *e.g.* the (−)-fisetinidol-(4α,6)- and (4β,6)-(+)-epifisetinidols. They are accompanied by bis(4α,6′)-(−)-fisetinidol and (−)-fisetinidol-(4α,6′)-(+)-epifisetinidol, the first prototypes of naturally occurring B-ring coupled profisetinidins, and by the first dimeric peltogynoid analogue, (2*S*,3*S*,4*R*)-2,3-*cis*-3,4-*trans*-4[(2*R*,3*S*)-2,3-*trans*-3-hydroxy-3′,4′,7-trihydroxyflavan-6-yl]mopanane-3′,4′,7-triol.

Previous studies of the heartwood extractives of the mopane (*Colophospermum mopane* Kirk. *ex* J. Leonard) have revealed the presence of a variety of monomeric flavanoids based upon the 3′,4′,7-trioxygenated aromatic substitution pattern.[1–3] These included the mopanols, peltogynols, and fisetinidols, the flavan-3,4-diol, (+)-gleditsin, and indications of the presence of 'polymeric tannins'. Investigations of the latter group of compounds were, however, severely hampered by the exceptionally high concentration of (−)-fisetinidol (**1**) [(2*R*,3*S*)-2,3-*trans*-flavan 3,3′,4′,7-tetraol] and to a lesser extent of those of (+)-epifisetinidol (**2**) [(2*S*,3*S*)-2,3-*cis*-flavan-3,3′,4′,7-tetraol] and



(**1**)



(**2**)

the mopanols and peltogynols.[4–6] Following extensive fractionation and enrichment procedures we now report on novel biflavanoids dominated by those prototypes derived from 3′,4′,7-trihydroxylated precursors.

Results and Discussion

The methanol extract of mopane heartwood was chromatographed on cellulose and the polar fraction exhaustively extracted with anhydrous ether to remove a portion of the predominant metabolites (−)-fisetinidol (**1**) and (+)-epifisetinidol (**2**). Subsequent chromatographic fractionation of the residual mixture afforded a series of novel dimeric analogues based mainly upon 3′,4′,7-trioxygenated monomeric precursors, but also on monomers not previously encountered in this natural source. Known compounds amongst these dimeric analogues included the [4,6]-all-*trans*-bis-(−)-fisetinidol [(−)-fisetinidol-(4α,6)-(−)-fisetinidol*], the first[4,5] and, thus far, sole natural biflavanoid in which the A-rings of both flavanoid moieties are resorcinol units, (−)-fisetinidol-(4α,8)-(+)-catechin[6] [(+)-catechin is (2*R*,3*S*)-2,3-*trans*-flavan-3,3′,4′,5,7-pentaol], (−)-fisetinidol-(4β,8)-(+)-catechin,[6,†] and (+)-guibourtinidol-(4α,8)-(+)-catechin[7] [(+)-guibourtinidol is taken as the flavan-3-ol moiety derived from the (2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*-flavan-3,4,4′,7-tetraol, (+)-guibourtacacidin[8]]. These compounds are accompanied by a series of novel natural biflavanoids in which both (−)-fisetinidol (**1**) and (+)-epifisetinidol (**2**) function prominently as 'terminal' moieties, thus not only expanding the series of natural occurring bis-(−)-fisetinidols, but also introducing the first prototypes of condensed tannins in which (+)-epifisetinidol (**2**) serves as nucleophilic flavan-3-ol unit in the biosynthetic pathway leading to this class of natural products.

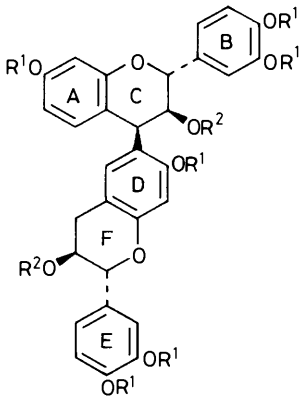
The bis(4α,6)-(−)-fisetinidol (**3**), representative of only the second natural biflavanoid with both A- and D-rings as resorcinol units, was characterised as the phenolic methyl ether acetate (**4**) by comparison of its spectroscopic data with those of a synthetic specimen.[5] Structural assessment of the novel pair of naturally occurring (−)-fisetinidol-(4α,6)-(**5**) and (4β,6)-(+)-epifisetinidol (**7**) was similarly effected by comparing the physical data of their hexamethyl ether diacetates (**6**) and (**8**) with those of their synthetic counterparts.[5]

Owing to the co-existence of the (−)-fisetinidol (**1**)/(+)-epifisetinidol (**2**) pair in high concentration and the (2*R*,3*S*,4*S*)-2,3-*trans*-3,4-*cis*-flavan-3,3′,4,4′,7-pentaol, (+)-gleditsin, recognition of the aforementioned novel bis-(−)-fisetinidol (**3**) and (−)-fisetinidol-(+)-epifisetinidols (**5**) and (**7**) could be anticipated. The diversity of the metabolic pool in *C. mopane* was, however, demonstrated by characterisation of two novel biflavanoids which are based on either flavan-3,4-diol or flavan-3-ol precursors unknown in this natural source. These compounds have been identified as (+)-guibourtinidol-(4α,6)-(−)-fisetinidol (**9**) and (−)-fisetinidol-(4α,8)-(+)-afzelechin (**11**) [(+)-afzelechin is (2*R*,3*S*)-2,3-*trans*-flavan-3,4′,5,7-tetraol[9]] by means of spectroscopic data of their phenolic methyl ether diacetates (**10**) and (**12**) respectively.

The relative configuration of (**10**) was evident from the ^{1}H n.m.r. coupling constants of their heterocyclic protons ($J_{2,3}$ = $J_{3,4}$ 9.0 Hz; $J_{2,3}$ 7.0 Hz). Correlation of the AA′BB′-system in the aromatic region (δ 7.32, 6.84, *J* 9.0 Hz) with the 2-H doublet (δ 4.99) of the heterocyclic AMX system and of 4-H (δ 4.54) of the latter system with 5-H (δ 6.63, d, *J* 8.5 Hz) of the highfield aromatic ABX system *via* spin–spin decoupling experiments defined the constitution of the (+)-guibourtinidol unit. The high-amplitude negative Cotton effect in the 220—240 nm

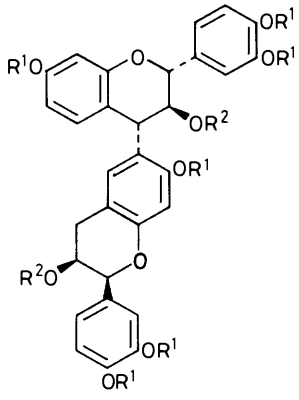
* Nomenclature based on the proposals by Hemingway *et al.* (R. W. Hemingway, L. Y. Foo, and L. J. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1209).

† Obtained from *C. mopane* for the first time.



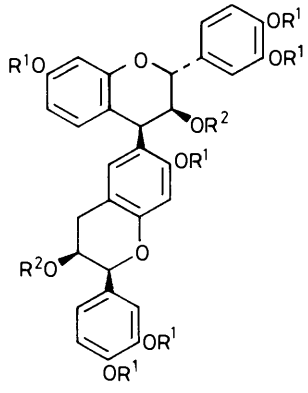
(**3**) $R^1 = R^2 = H$

(**4**) $R^1 = Me$, $R^2 = Ac$



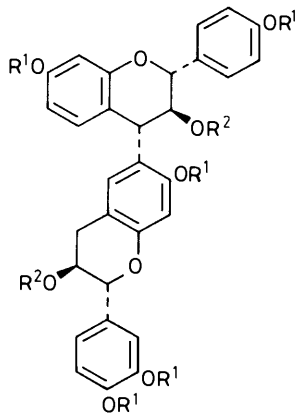
(**5**) $R^1 = R^2 = H$

(**6**) $R^1 = Me$, $R^2 = Ac$



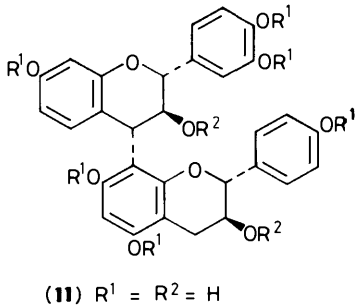
(**7**) $R^1 = R^2 = H$

(**8**) $R^1 = Me$, $R^2 = Ac$



(**9**) $R^1 = R^2 = H$

(**10**) $R^1 = Me$, $R^2 = Ac$



(**11**) $R^1 = R^2 = H$

(**12**) $R^1 = Me$, $R^2 = Ac$

region of the c.d. spectrum, in conjunction with the above coupling constants of the AMX-system, defined the absolute configuration of the proguibourtinidin moiety of this novel biflavanoid as 2*R*,3*S*,4*R* (*cf.* ref. 5). Confirmation of the terminal unit as a (−)-fisetinidol moiety with (2*R*,3*S*)-absolute configuration was obtained *via* biomimetic synthesis of (**10**) from (2*R*,3*S*,4*S*)-2,3-*trans*-3,4-*cis*-4′,7-dimethoxyflavan-3,4-diol [8,10] and (−)-fisetinidol (**1**) under mild acidic conditions (*cf.* ref. 5) followed by methylation and acetylation. Comparison of the physical data of the natural product derivative (**10**) with those of the synthetic analogue provided unambiguous proof for their identity.

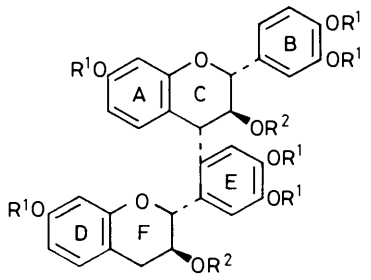
Proguibourtinidins with their 4′,7-dihydroxy phenolic functionality represent a relatively rare group of compounds which, while occurring as minor components in the heartwoods of Australian *Acacia* spp.,[11] predominate in the southern African species *Guibourtia coleosperma*.[8,10] *Julbernardia globiflora*,[12] and *Acacia luederitzii*.[13] The [4α,6]-(+)-guibourtinidol-(−)-fisetinidol (**9**) thus not only complements the above series of natural condensed tannins, but also represents the first proguibourtinidin which is based on (−)-fisetinidol (**1**) as nucleophilic flavan-3-ol moiety.

Comparison of the 300 MHz ^{1}H n.m.r. spectrum of the methyl ether diacetate (**12**) of the novel (−)-fisetinidol-(4α,8)-(+)-afzelechin (**11**), representative of only the second biflavanoid based upon a (+)-afzelechin terminal unit, with that of the (+)-guibourtinidol (4α,8)-(+)-catechin (*cf.* ref. 7) revealed their close structural resemblance. Notable differences included conspicuous shielding (Δδ 0.25) of the AA′ portion [δ 6.75, d, *J* 9.0 Hz, 2- and 6-H(E)] of the AA′BB′ spin system in the (+)-afzelechin analogue (**12**). The all-*trans* configuration was indicated by the heterocyclic proton coupling constants ($J_{2,3} = J_{3,4}$ 10.0 Hz; $J_{2,3}$ 8.0 Hz). Owing to the exact overlap of the AA′ and B portions of the respective B ring AA′BB′ and ABX spin systems in a variety of solvents at 300 MHz, confirmation for placement of these rings as part of the 'upper' or 'lower' units could not be obtained by the normal strategy of decoupling experiments using the respective heterocyclic 2-H resonance as reference signals. The 2-H of ring F, *i.e.* part of the heterocyclic AMXY system, however, exhibited a prominent n.O.e. correlation* with the AA′ doublet of the AA′BB′ system thus unequivocally proving the *p*-hydroxy substitution of ring B in the 'lower' flavan unit. Such an allocation is substantiated by the prominent *m/z* fragment 462 (62%) resulting from preferential RDA fragmentation [14] of the (+)-afzelechin moiety following initial loss of acetic acid from the 'upper' unit. The (2*R*,3*S*,4*S*)-absolute configuration of the fisetinidol unit was again deduced by combination of the ^{1}H n.m.r. coupling constants of its heterocyclic AMX system and the high-amplitude negative Cotton effect in the 220—240 nm region of its c.d. spectrum. Since unavailability of (+)-afzelechin excluded structural confirmation *via* synthesis, the absolute configuration of this unit was taken as 2*R*,3*S* by comparison of the c.d. data of the methyl ether acetate (**12**) with those of the corresponding derivative of the closely related (+)-guibourtinidol-(4α,8)-(+)-afzelechin from *Acacia luederitzii*.[7]

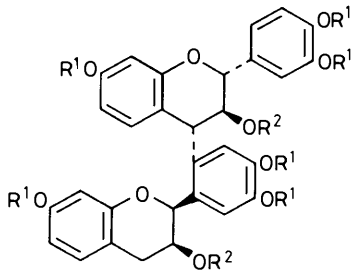
The 'conventional' [4,8 or 6]-biflavanoids are accompanied by two novel bis-fisetinidols (**13**) and (**15**), the first representatives of naturally occurring B ring coupled profisetinidins. The ^{1}H n.m.r. spectrum of the methyl ether acetate (**14**) of the (4α,6′)-bis-(−)-fisetinidol (**13**)† exhibited in the heterocyclic region an AMX ($J_{2,3} = J_{3,4}$ 10.0 Hz) and AMXY system ($J_{2,3}$ 6.2 Hz) characteristic of the all-*trans* configuration of both flavanyl units. Besides the expected three ABX systems, the aromatic region of the spectrum displayed two singlets (δ 6.84, 6.66) which did not sharpen significantly on irradiation of the 2-H(F) (δ 5.45) and 4-H(C) (δ 4.83) doublets. The latter two protons, however, exhibited prominent mutual n.O.e. association (19 and 15%) and also with respectively 2-H(E) (1.4%) and 5-H(E) (1.6%). This observation, when taken in conjunction

* Signal overlap hampered quantification.

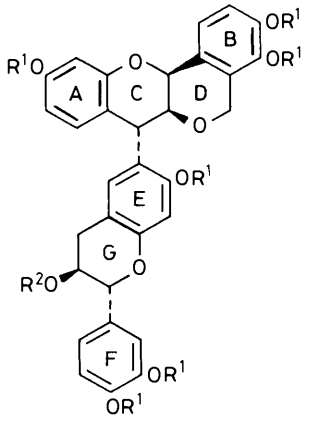
† The position of substitution is taken as C-6′ of ring B of the 'lower' flavan unit in order to retain trivial names for the respective constituent flavanyl moieties.



(**13**) $R^1 = R^2 = H$
(**14**) $R^1 = Me$, $R^2 = Ac$



(**15**) $R^1 = R^2 = H$
(**16**) $R^1 = Me$, $R^2 = Ac$



(**17**) $R^1 = R^2 = H$
(**18**) $R^1 = Me$, $R^2 = Ac$

with the observed n.O.e. association between the 2- and 5-H(E) singlets and a single methoxy resonance [δ 3.73 (3.1%); δ 3.68 (3.5%) resp.] in each instance, thus unambiguously defined coupling from C-4 (C-ring) to C-5 (B-ring) of the 'lower' (−)-fisetinidol unit.

The same strategy also facilitated structural elucidation of the (−)-fisetinidol-(4α,6′)-(+)-epifisetinidol (**15**) [$J_{2,3} = J_{3,4}$ 9.0 Hz: $J_{2,3}$ *ca.* 1.0 Hz for (**16**)] as its methyl ether diacetates (**16**).

The mass fragmentation spectra of the B ring coupled isomers (**14**) and (**16**) gave, in contrast to those of the more conventional A ring linked analogues [*e.g.* (**4**)], prominent fragment ions at *m/z* 518 (51 and 63% resp.) following loss of acetic acid from the C ring and RDA fragmentation of the heterocycle of the 'lower' flavan unit.

Although the c.d. spectra of both B ring coupled isomers (**14**) and (**16**) exhibited positive Cotton effects in the 220—240 nm region, thus confirming (2*R*,3*S*,4*R*)-absolute configuration for the 'upper' (−)-fisetinidol moiety, the stereochemistry of the 'lower' flavanyl units can only be deduced by comparison of spectroscopic data of the natural product derivatives with those of their synthetic counterparts with known absolute configuration at all chiral centres. We, therefore, embarked on a programme towards synthesis of this novel class of natural oligomeric flavanoids.

Since the catechol B ring of fisetinidols should exhibit reduced nucleophilicity when compared to that of the resorcinol-type A ring, a synthetic sequence, based on biomimetic principles, towards the B ring coupled isomers is expected to proceed under more drastic conditions than of those to 'conventional' [4,6]-linked analogues. Experimental conditions would, therefore, have to provide for either an increase of nucleophilicity of the catechol moiety or of the electrophilicity of the benzylic C-4 position of the flavan-3,4-diol unit. Furthermore the C-6 positions (A ring) as the more potent nucleophilic sites, would have to be either protected or deactivated in order to ensure C–C linkage from ring B and also to prevent the aforementioned positions to function prominently as nucleophiles once dimers of type (**13**) have been generated.

With the above in mind the following sequences were attempted. (i) Treatment of the 4-phenylthio ether of 3′,4′,-7-tri-*O*-methyl-(+)-mollisacacidin [(+)-mollisacacidin is (2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*-flavan-3,3′,4,4′,7-pentaol] with 6-bromo-(−)-fisetinidol * under nitrogen at pH 10 for 2 days at 50 °C. (ii) Similar conditions to the above, but in the presence of Hg^{II} salts in order to enhance the ability of the phenylthio leaving group. (iii) Reaction of 7-*O*-methyl-(−)-fisetinidol * with the 4-phenylthio ether of (+)-mollisacacidin at pH 10 for 2 days at 50 °C under nitrogen. These efforts towards effecting B ring coupling and thus unambiguous determination of the absolute stereochemistry of the 'lower' flavanyl units in (**13**) and (**15**) invariably led to intractable mixtures. In view of the predominance of (−)-fisetinidol (**1**) and (+)-epifisetinidol (**2**) in the heartwood of *C. mopane*, we favour a (2*R*,3*S*)-absolute configuration for the 'terminal' flavan-3-ol unit in (**13**) and (2*S*,3*S*) for the remaining isomer (**15**).

Despite the fact that (+)-peltogynol as flavan-3,4-diol equivalent is known [15] since the mid-thirties, flavanoid oligomers based on these potentially electrophilic moieties have not yet been encountered in Nature. Prospectives of finding these compounds have been dampened by our recent observations of their formation in extremely low concentrations and under relatively drastic conditions during a biomimetic synthesis [16] from (+)-peltogynol and the flavan-3-ols (+)-catechin and (−)-fisetinidol. Our present approach of extensive fractionation and enrichment procedures of the complex flavanoid mixture from the mopane has, however, now revealed the presence of the first dimeric peltogynoid analogue, (2*S*,3*S*,4*R*)-2,3-*cis*-3,4-*trans*-4-(2*R*,3*S*)-2,3-*trans*-3-hydroxy-3′,4′,7-trihydroxyflavan-6-yl]mopanane-3′,4′,7-triol (**17**).

A diagnostic feature of the ^1H n.m.r. spectrum of the methyl ether acetate (**18**) is the presence of an isolated heterocyclic AB system (δ 5.26, 4.96, *J* 16.5 Hz), characteristic of the D ring methylene functionality of the peltogynoid skeleton.[3] Spin–spin decoupling of the remaining heterocyclic protons revealed the presence of an AMX system for the mopanoid unit [δ 5.11, 2-H (C); δ 4.21, 3-H(C); δ 5.08, 4-H(C)], the coupling constants ($J_{2,3}$ *ca.* 2.0 Hz; $J_{3,4}$ *ca.* 1.1 Hz) of which are consistent with 2,3-*cis*-3,4-*trans*-stereochemistry.[17] Irradiation of 2-H(C) led to sharpening of an aromatic *ortho*-doublet (δ 7.02) which was correlated with the doublet at δ 6.42 thus defining the B-ring as a C-2 substituted 3,4-dioxygenated unit and the 'upper' unit as a

* Details for their preparation will be published elsewhere.

Table. ^{1}H N.m.r. (300 MHz) peaks (p.p.m.) of the biflavanoids (**10**), (**12**), (**14**), and (**16**). Splitting patterns and *J* values (Hz) are given in parentheses

Ring	H	(**10**), $CDCl_3$, 365 K	(**12**), $CDCl_3$, 298 K	(**14**), $CDCl_3$, 365 K	(**16**), C_6D_6, 363 K
A	5	6.65 (d, 8.5)	6.67 (d, 9.0)	6.62 (d, 8.5)	7.22 (d, 8.5)
	6	6.42 (dd, 8.5, 2.5)	6.46 (dd, 9.0, 2.5)	6.43 (dd, 8.5, 3.0)	6.64 (dd, 8.5, 3.0)
	8	6.50 (d, 2.5)	6.42 (d, 2.5)	6.50 (d, 3.0)	6.74 (d, 3.0)
B	2	7.32 (d, 9.0)	6.58 (d, 2.0)	7.03 (d, 2.0)	7.18 (d, 2.0)
	3	6.84 (d, 9.0)			
	5	6.84 (d, 9.0)	6.69 (d, 7.0)	6.84 (d, 9.0)	6.67 (d, 8.0)
	6	7.32 (d, 9.0)	6.75 (dd, 7.0, 2.0)	7.01 (dd, 9.0, 2.0)	7.09 (dd, 8.0, 2.0)
C	2	4.99 (d, 9.0)	4.81 (d, 10.0)	5.07 (d, 10.0)	5.08 (d, 9.0)
	3	5.66 (dd, 9.0, 9.0)	6.00 (dd, 10.0, 10.0)	5.74 (dd, 10.0, 10.0)	5.96 (dd, 9.0, 9.0)
	4	4.54 (d, 9.0)	4.83 (d, 10.0)	4.83 (d, 10.0)	4.76 (d, 9.0)
D	5	6.63 (s)		6.92 (d, 9.0)	6.82 (d, 8.5)
	6		6.13 (s)	6.50 (dd, 9.0, 3.0)	6.53 (dd, 8.5, 2.0)
	8	6.46 (s)		6.50 (d, 3.0)	6.66 (d, 2.0)
E	2	6.90 (d, 2.0)	6.75 (d, 9.0)	6.84 (s)	7.48 (s)
	3		6.66 (d, 9.0)		
	5	6.83 (d, 7.0)	6.66 (d, 9.0)	6.66 (s)	6.81 (s)
	6	6.89 (dd, 7.0, 2.0)	6.75 (d, 9.0)		
F	2	4.95 (d, 7.0)	4.89 (d, 8.0)*	5.45 (d, 6.2)	5.86 (d, 1.0)
	3	5.27 (m)	4.78 (m)*	5.66 (m)	5.77 (m)
	$4_{eq.}$	2.92 (dd, 16.0, 5.0)	3.02 (dd, 16.0, 6.0)	3.02 (dd, 15.0, 5.0)	3.04 (dd, 18.0, 4.5)
	$4_{ax.}$	2.69 (dd, 16.0, 7.0)	2.58 (dd, 16.0, 9.0)	2.77 (dd, 15.0, 6.0)	
					2.82 (dd, 18.0, 3.0)
	OMe	3.74, 3.76, 3.79, 3.86, 3.88 (each s)	3.54, 3.75, 3.76, 3.81 (×2), 3.86 (each s)	3.68, 3.73, 3.74, 3.75, 3.85, 3.87 (each s)	3.33, 3.38 (×2), 3.45, 3.56, 3.59 (each s)
	OAc	1.88, 1.66 (each s)	1.87, 1.57 (each s)	1.62, 1.95 (each s)	1.50, 1.61 (each s)

* In [$^2H_6$]acetone.

mopanoid moiety. Inspection of the remaining proton systems and comparison with those of the bis-(−)-fisetinidols [*e.g.* (**10**)], in conjunction with the appropriate decoupling experiments, led to characterisation of the 'lower' unit as a C-6 substituted (−)-fisetinidol moiety. In view of the predominance of (−)-fisetinidol (**1**) in the heartwood of *C. mopane* and also of the C-2 epimeric relationship between (−)-fisetinidol and (+)-epifisetinidol (**2**), presumably reflecting a similar relationship between a (2*R*,3*S*)-and a (2*S*,3*S*)-mopanol [*cf.* ref. 17 for the natural existence of a (2*S*,3*S*)-2,3-*cis*-3,4-*cis*-peltogynol], the absolute configuration of the novel biflavanoid (**17**) may tentatively be assigned as (2*S*,3*S*,4*R*:2*R*,3*S*). Proof for such an assumption will, however, be possible only when 2,3-*cis*-mopanols become available from natural sources or *via* synthesis in order to facilitate the preparation of oligomers of type (**17**).

Experimental

^{1}H N.m.r. spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3$, C_6D_6 and $(CD_3)_2SO$ with Me_4Si as internal standard. Mass spectra were obtained with a Varian CH-5 instrument and c.d. data in methanol on a Jasco J-20 spectropolarimeter. T.l.c. was performed on precoated Merck plastic sheets (DC-Plastikfolien Kieselgel 60 F_{254}, 0.25 mm) and compounds were located by H_2SO_4–HCHO (40:1 v/v) spray reagent. Preparative plates (p.l.c.), 20 × 20 cm, Kieselgel PF_{254} (1.0 mm) were air-dried and used without prior activation. Separations on Sephadex LH-20 columns (5 × 100 cm) were in 10% methanol–ethanol at a flow rate of 20 cm^3 per 32 min. Methylations were performed with an excess of diazomethane in methanol–diethyl ether over 48 h at −15 °C, while acetylations were carried out in acetic anhydride–pyridine at ambient temperatures. Evaporations were done under reduced pressures at *ca.* 60 °C in a rotary evaporator. C and H analyses were performed by Analytische Laboratorien, Fritz-Pregl Strasse 24, 5270 Gummersbach 1 Elbach, West Germany. In those instances where available quantities were too low for elemental analysis, purity was assessed by ^{1}H n.m.r. spectroscopy in conjunction with accurate mass determination.

Extraction and Fractionation of the Heartwood of Colophospermum mopane.—Drillings (4 kg) of the heartwood were extracted with MeOH (10 L) in a Soxhlet apparatus. Evaporation of the solvent produced a dark brown resin (250 g).

The dewaxed methanol extract (246 g) was chromatographed on 5 × 125 cm cellulose ('Solka Floc', Brown Co., New Hampshire, U.S.A.) columns (20 g per column) with water as eluant. Only the first 2 l fraction, starting with introduction of the sample on the column, was collected. Extraction with ethyl acetate followed by drying and evaporation of the solvent produced a brown, amorphous residue (56 g) which was subsequently extracted with anhydrous ether in a Soxhlet apparatus. Evaporation of the solvent produced a light brown solid (10 g) which consisted mainly of monomeric (−)-fisetinidol and (+)-epifisetinidol. The residue (46 g) of the ether extraction was chromatographed by p.l.c. in benzene–acetone–methanol (6:3:1) to give three bands of *ca.* 4 cm width at R_F 0.61 (13.2 g), 0.44 (3.7 g), and 0.28 (8.7 g). The fraction at R_F 0.61 consisted mainly of (−)-fisetinidol and (+)-epifisetinidol.

Biflavanoids from the R_F 0.44 *Fraction.*—The fraction of R_F 0.44 was subjected to column chromatography on Sephadex LH-20 using 10% methanol–ethanol as eluant to yield five

fractions with the following retention times (starting with introduction of the sample on the column): 1 [45—60 h (0.54 g)], 2 [61—73 h (0.24 g)], 3 [74—88 h (0.39 g)], 4 [89—105 h (0.58 g)], and 5 [106—130 h (0.29 g)]. Subsequent methylation of each fraction afforded mixtures of the methyl ethers.

3-O-*Acetyl-di*-O-*methyl*-(+)-*guibourtinidol*-(4α,6)-3-O-*acetyl-tri*-O-*methyl*-(−)-*fisetinidol* (**10**).—The methylated mixture from fraction 1 was resolved by p.l.c. [benzene–acetone (9:1)] to give two bands at R_F 0.33 (63 mg) and 0.17 (47 mg), the latter of which has not yet been identified. Acetylation of the R_F 0.33 fraction followed by p.l.c. [hexane–ethyl acetate–acetone (60:25:15, ×2)] afforded the pentamethyl ether diacetate (15 mg; R_F 0.72) as a *white amorphous solid* (Found: M^+, 684.2571. $C_{39}H_{40}O_{11}$ requires M^+, 684.2571); ^{1}H N.m.r. data (Table); c.d. $[\theta]_{233}$ 0, $[\theta]_{219}$ −79 000, $[\theta]_{200}$ 0.

Fraction 2 was methylated and the mixture resolved by p.l.c. in benzene–acetone (9:1, ×2), into two bands at R_F 0.25 (72 mg) and 0.17 (31 mg).

Acetylation and subsequent purification by p.l.c. [hexane–ethyl acetate–acetone (60:25:15, ×2)] of the bands at R_F 0.25 and 0.17 afforded the hexamethyl ether diacetates (58 and 20 mg) of (−)-fisetinidol-(4β,6)-(−)-fisetinidol and (−)-fisetinidol-(4β,6)-(+)-epifisetinidol respectively, the physical data (^{1}H n.m.r., c.d., and m.s.) of which were identical with those of authentic samples.[5]

Bis(4α,6′)-[3-O-*acetyl-tri*-O-*methyl*-(−)-*fisetinidol*] (**14**).—The methylated mixture (fraction 3) was resolved by p.l.c. [benzene–acetone (9:1, ×2)] to give one band at R_F 0.31 (94 mg). Acetylation of the R_F 0.31 fraction followed by p.l.c. [hexane–ethyl acetate–acetone (60:25:15, ×2)] afforded the hexamethyl ether diacetate (34 mg; R_F 0.42) as a *white amorphous solid* (Found: C, 67.0; H, 6.0. $C_{40}H_{42}O_{12}$ requires C, 67.2; H, 5.9%); ^{1}H n.m.r. data (Table); c.d. spectrum $[\theta]_{276}$ 0, $[\theta]_{245}$ −500, $[\theta]_{225}$ −21 000, $[\theta]_{214}$ 0, $[\theta]_{210}$ 6 000, $[\theta]_{200}$ 0.

3-O-*Acetyl-tri*-O-*methyl*-(−)-*fisetinidol*-(4α,6′)-3-O-*acetyl-tri*-O-*methyl*-(+)-*epifisetinidol* (**16**).—Fraction 4 was methylated and the mixture resolved by p.l.c. [benzene–acetone (8:2, ×2)] into two bands at R_F 0.56 (36 mg) and 0.42 (232 mg). Acetylation of the R_F 0.56 fraction followed by p.l.c. [hexane–ethyl acetate–acetone (60:25:15, ×2)] gave a prominent band at R_F 0.62 which was subjected to a further fractionation by p.l.c. [benzene–hexane–acetone (6:3:1, ×2)] to afford the hexamethyl ether diacetate (17 mg; R_F 0.50) as a *white solid* (Found: C, 67.0; H, 5.9. $C_{40}H_{42}O_{12}$ requires C, 67.2; H, 5.9%); ^{1}H n.m.r. data (Table): c.d. spectrum $[\theta]_{302}$ 0, $[\theta]_{291}$ 11 000, $[\theta]_{260}$ 1 000, $[\theta]_{245}$ 18 100, $[\theta]_{236}$ 0, $[\theta]_{230}$ −13 000, $[\theta]_{223}$ 0.

Acetylation of the R_F 0.42 fraction followed by p.l.c. [hexane–ethyl acetate–acetone (60:25:15, ×2)] gave two bands at R_F 0.47 (59 mg) and 0.42 (43 mg) which afforded the hexamethyl ether acetates of (−)-fisetinidol-(4α,6)-(−)-fisetinidol and (−)-fisetinidol-(4α,6)-(+)-epifisetinidol respectively, the physical data (^{1}H n.m.r., c.d. and m.s.) of which were identical with those of authentic samples.[5]

(2S,3S,4R)-2,3-cis-3,4-trans-4-[(2R,3S)-3-*Acetoxy*-3′,4′,7-*trimethoxyflavan*-6-*yl*]-3′,4′,7-*trimethoxymopanane* (**18**).—Fraction 5 was methylated and the mixture resolved by p.l.c. [benzene–acetone (8:2, ×2)] to give two bands at R_F 0.56 (123 mg) and 0.39 (35 mg). Acetylation of the R_F 0.56 fraction followed by p.l.c. [hexane–ethyl acetate–acetone (60:25:15, ×2)] gave a prominent band which was subjected to a further fractionation by p.l.c. [benzene–hexane–acetone (6:3:1, ×2)] to afford the hexamethyl ether acetate (3 mg; R_F 0.50) as a *white solid* (Found: M^+, 684.2571. $C_{39}H_{40}O_{11}$ requires M^+, 684.2571); δ_H(C_6D_6, 300 MHz, 25 °C) 7.02 [d, *J* 8.5 Hz, 6-H(B)], 6.98 [br dd, *J* 9.0 and 2.0 Hz, 6-H(F)], 6.95 [d, *J* 8.0 Hz, 5-H(A)], 6.94 [d, *J* 2.5 Hz, 2-H(F)], 6.85 [br s, 5-H(E)], 6.80 [d, *J* 2.5 Hz, 8-H(A)], 6.63 [s, 8-H(E)], 6.58 [dd, *J* 8.0 and 2.5 Hz, 6-H(A)], 6.51 [d, *J* 8.0 Hz, 5-H(F)], 6.42 [d, *J* 8.5 Hz, 5-H(B)], 5.46 [m, 3-H(G)], 5.26 [d, *J* 16.0 Hz, $H_{eq.}$(D)], 5.11 [br d, $J_{2,3}$ 8.0 Hz, 2-H(G)], 5.10 [d, $J_{2,3}$ 2.0 Hz, 2-H(C)], 5.09 [d, $J_{3,4}$ 1.0 Hz, 4-H(C)], 4.96 [d, *J* 16.0 Hz, $H_{ax.}$(D)], 4.21 [dd, *J* 2.0 and 1.0 Hz, 3-H(C)], 3.47, 3.37, 3.33, 3.32, 3.23 (each s, 6 × OMe), and 1.51 (s, OAc); c.d. spectrum $[\theta]_{280}$ 0, $[\theta]_{237}$ 4 500, $[\theta]_{227}$ 0, $[\theta]_{215}$ −7 800, $[\theta]_{204}$ 0.

Biflavanoids from the R_F 0.28 *Fraction*.—The R_F 0.28 fraction (8.7 g) from the crude preparative t.l.c. separation was subjected to column chromatography on Sephadex LH-20 using 10% methanol–ethanol (v/v) as eluant to give nine fractions with the following retention times: 1 [35—52 h (0.52 g)], 2 [53—70 h (1.13 g)], 3 [71—88 h (1.08 g)], 4 [89—107 h (1.12 g)], 5 [108—128 h (0.92 g)], 6 [129—148 h (0.80 g)], 7 [149—210 h (0.92 g)], 8 [211—246 h (0.48 g)], and 9 [247—300 h (0.70 g)] which were subsequently subjected to methylation.

3-O-*Acetyl-di*-O-*methyl*-(+)-*guibourtinidol*-(4α,8)-3-O-*acetyltetra*-O-*methyl*-(+)-*catechin*.—The methylated mixture (fraction 1) was resolved by p.l.c. [benzene–acetone (8:2, ×3)] to give two bands at R_F 0.44 (24 mg), and 0.39 (27 mg). Acetylation of the R_F 0.44 fraction followed by p.l.c. [hexane–ethyl acetate–acetone (60:25:15, ×2)] afforded the hexamethyl ether diacetate (5 mg; R_F 0.54) as a white solid, the physical data (^{1}H n.m.r., c.d. and m.s.) of which were identical with those of an authentic sample.[7]

3-O-*acetyl-tri*-O-*methyl*-(−)-*fisetinidol*-(4α,8)-3-O-*acetyl-tri*-O-*methyl*-(−)-*afzelechin* (**12**).—Acetylation of the R_F 0.39 fraction followed by p.l.c. [hexane–ethyl acetate–acetone (60:25:15, ×3)] afforded the hexamethyl ether diacetate (2 mg; R_F 0.65) as a *white amorphous solid* (Found: M^+, 714.2665. $C_{40}H_{42}O_{12}$ requires M^+, 714.2676; ^{1}H n.m.r. data (Table); c.d. $[\theta]_{250}$ 0, $[\theta]_{240}$ −11 000, $[\theta]_{230}$ −33 000, $[\theta]_{200}$ 0.

The remainder of the above nine column fractions afforded, amongst others, the novel class of ring-isomerised condensed tannins (phlobatannins) which was the subject of a previous communication.[18]

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