Oligomeric Flavanoids. Part 2.† The first Profisetinidins with Dihydroflavonol Constituent Units

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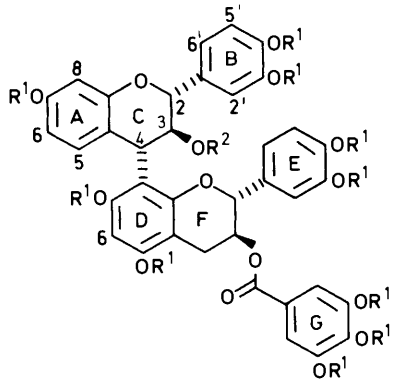
The range of naturally occurring profisetinidins is extended by characterization of the first (−)-fisetinidol-(−)-robinetinidol dimer and of a novel analogue based on the 3-*O*-galloyl ester of (+)-catechin. These metabolites are accompanied by (−)-fisetinidol-(4α,2′)-robinetin, (−)-fisetinidol-(4α,6)-(+)-taxifolin, and (−)-fisetinidol-(4α,6)- and (4β,6)-(+)-ampelopsins. The latter three compounds are representative of the first natural profisetinidins with dihydroflavonols as constituent units.

The biomimetic pool from which oligoflavanoids with $C_4(sp^3)$-$C_{6/8}(sp^2)$ interflavanyl linkages originate, presumably contains a variety of potential nucleophilic units. Despite this, the majority of these metabolites are constituted of a flavan-3-ol as repeating unit, originating most likely from an electrophilic flavan-3,4-diol entity, and a terminal 'lower' unit comprising a nucleophilic C-4 deoxy flavan-3-ol with phloroglucinol A-ring. Most prominent amongst these are the procyanidins[1–3] with their (+)-catechin and (−)-epicatechin constituent units, the profisetinidins[4–6] based on (−)-fisetinidol and (+)-catechin, the prorobinetinidins[7,8] composed of (−)-robinetinidol, (+)-catechin, and (+)-gallocatechin entities, and several other groups[9–12] with limited distribution, but also possessing a 'lower' flavan-3-ol unit with C-4 deoxy heterocycle. Examples where this entity is hydroxylated at C-4 are restricted to four dimeric and a single trimeric profisetinidin from the heartwood of *Acacia mearnsii*.[13–15] Such a limited natural distribution has been ascribed to a decrease in nucleophilicity of the A-ring functionality by the adjacent oxygenated methylene function.[15] Our continued search for novel prototypes of oligomeric flavanoids amongst the flora of Southern Africa has now revealed the presence of novel dimeric profisetinidins in which the 'lower' flavanyl units comprised of dihydroflavonol entities.

Results and Discussion

The methanol extractives of the heartwood of *Burkea africana*,[16] the red syringa, afforded the known flavonols, robinetin (3′,4′,5′,7-tetrahydroxyflavonol)[17] and myrecitin (3′,4′,5,5′,7-pentahydroxyflavonol),[18] the dihydroflavonol (+)-ampelopsin [(2*R*,3*R*)-2,3-*trans*-3′,4′,5,5′7-pentahydroxydihydroflavonol],[19] and the flavan-3-ol, (−)-robinetinidol [(2*R*,3*S*)-2,3-*trans*-3′,4′5′,7-tetrahydroxyflavan-3-ol][20,21] These compounds are accompanied by a variety of novel biflavanoids in which, amongst others, 2,3-*trans*-dihydroflavonols appear as 'terminating' flavanyl units.

The 3-*O*-galloyl ester (F-ring) of the (−)-fisetinidol-(4α,8)-(+)-catechin (**1**) was identified as the decamethyl ether acetate (**2**). Its 'conventional' nature was apparent from the ^{1}H n.m.r. data (Table) which revealed the characteristic AMX and AMXY spin patterns of the respective heterocyclic rings.[22] The relative 2,3-*trans*-3,4-*trans* (C-ring) and 2,3-*trans* (F-ring) configurations were evident from coupling constants [$J_{2,3(C)} = J_{3,4(C)}$ 10.0 Hz; $J_{2,3(F)}$ 10.0 Hz] compatible with such stereochemistry. The presence of three ABX systems and a one-proton singlet confirmed the aromatic substitution pattern, while the chemical



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

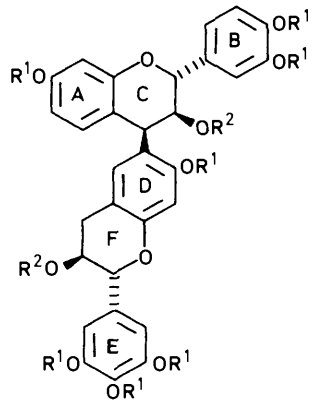
shift of the singlet (δ 6.46) is in accord with [4,8]-interflavanyl coupling.[23,24] Differentiation of the respective ABX systems was effected *via* spin decoupling experiments using the heterocyclic 2- (for the B- and E-rings) and 4-protons (for the A-ring) as reference signals a strategy which was applied throughout this paper. The sharp two-proton singlet in the low-field benzenoid region is indicative of the presence of a galloyl moiety, its location at the 3-*O* function of the F-ring being confirmed by the chemical shift of 3-H (F) (δ 4.97), by the large coupling constant [$J_{2,3(F)}$ 10.0 Hz] indicative of this bulky group dictating a predominant E-conformation for the F-ring,[25] and by the weak but selective n.O.e. association between the protons of the acetoxy group (δ 1.52) and the heterocyclic protons [δ 4.84 (0.2%), 6.10 (0.31%), 4.85 (0.27%)] of the C-ring. The C-ring coupling constants when taken in conjunction with a high amplitude negative Cotton effect (C.e.) in the c.d. spectrum at 222 nm indicate (2*R*,3*S*,4*S*) absolute configuration for the 'upper' unit.[26–28] Since the spectrum exhibited similar features at longer wavelengths than those of the (−)-fisetinidol-(4α,8)-(+)-catechin,[22] its lower unit may tentatively be assigned a (2*R*,3*S*)-absolute configuration.

The ^{1}H n.m.r. data (Table) of the heptamethyl ether diacetate (**4**) of the novel (−)-fisetinidol-(4β,6)-(−)-robinetinidol (**3**), representative of the first biflavanoid in which (−)-robinetinidol serves as a nucleophilic flavan-3-ol moiety in the biosynthetic pathway leading to this class of natural products, revealed an AMX spin pattern characteristic of the protons of a 2,3-*trans*-3,4-*cis* heterocyclic C-ring ($J_{2,3}$ 7.5; $J_{3,4}$ 5.5 Hz). The presence of two one-proton singlets in the aromatic region [δ 6.48, 8-H(D) (sharp); 6.56, 5-H(D) (broadened)] is typical of a resorcinol-type D-ring.[22] The two-proton aromatic singlet (δ 6.58) exhibiting benzylic coupling with 2-H(F), the A-portion of a heterocyclic

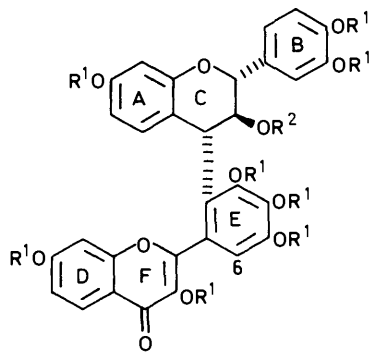
† Part 1, J. A. Steenkamp, J. C. S. Malan, D. G. Roux, and D. Ferreira, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1325.

Table. ^{1}H N.m.r. (300 MHz) peaks (p.p.m.) of biflavanoids (**2**), (**4**), (**6**), (**8**), (**10**), and (**12**). Splitting patterns and *J* values are given in parentheses

Ring	H	(**2**), $(CD_3)_2CO$,305 K	(**4**), $CDCl_3$, 298 K	(**6**), $CDCl_3$, 297 K	(**8**), $CDCl_3$, 297 K	(**10**), $CDCl_3$, 303 K	(**12**), $CDCl_3$, 298 K
A	5	6.68 (dd, 1.5, 8.5)	6.76 (d, 8.5)	7.04 (d, 8.0)	6.59 (dd, 1.0, 8.5)	6.58 (dd, 1.0, 8.5)	6.80 (d, 8.5)
	6	6.47 (dd, 2.5, 8.5)	6.46 (dd, 2.5, 8.5)	6.48 (dd, 2.5, 8.0)	6.37 (dd, 2.5, 8.5)	6.36 (dd, 2.5, 8.5)	6.42 (dd, 2.5, 8.5)
	8	6.36 (d, 2.5)	6.54 (d, 2.5)	6.49 (d, 2.5)	6.47 (d, 2.5)	6.48 (d, 2.5)	6.56 (d, 2.5)
B	2	6.73 (d, 2.0)	6.87 (d, 2.0)	6.90 (d, 2.0)	6.97 (d, 2.0)	6.98 (d, 2.0)	6.90 (d, 2.0)
	5	6.78 (d, 8.2)	6.80 (d, 8.2)	6.80 (d, 8.3)	6.86 (d, 8.2)	6.86 (d, 8.5)	6.80 (d, 8.5)
	6	6.63 (dd, 2.0, 8.2)	6.91 (dd, 2.0, 8.2)	6.99 (dd, 2.0, 8.3)	7.04 (dd, 2.0, 8.2)	7.04 (dd, 2.0, 8.5)	6.97 (dd, 2.0, 8.5)
C	2	4.84 (d, 10.0)	5.13 (d, 7.5)	4.66 (d, 9.5)	4.90 (d, 9.8)	4.91 (d, 9.5)	5.30 (d, 10.5)
	3	6.10 (t, 10.0)	5.53 (dd, 5.5, 7.5)	5.96 (t, 9.5)	5.99 (t, 9.8)	5.99 (t, 9.5)	5.55 (dd, 7.0, 10.5)
	4	4.85 (dd, 1.5, 10.0)	4.68 (d, 5.5)	4.08 (d, 9.5)	4.86 (dd, 1.5, 9.8)	4.87 (d, 9.5)	4.98 (d, 7.0)
D	5		6.56 (s)	8.18 (d, 9.0)			
	6	6.46 (s)		6.97 (dd, 2.2, 9.0)			
	8		6.48 (s)	6.70 (d, 2.2)	6.28 (s)	6.29 (s)	6.35 (s)
E	2/6	(Galloyl) 7.24 (s)	6.58 (s)			6.68 (s)	6.78 (s)
	2	6.63 (d, 2.0)			6.98 (d, 2.2)		
	5	6.78 (d, 8.2)			6.88 (d, 8.0)		
	6	6.74 (dd, 2.0, 8.2)		6.66 (s)	7.00 (dd, 2.2, 8.0)		
F	2	4.93 (d, 10.0)	4.97 (d, 7.8)		5.34 (d, 12.5)	5.34 (d, 12.1)	5.31 (d, 12.3)
	3	4.97 (m)	5.28 (m)		5.76 (d, 12.5)	5.74 (d, 12.1)	5.64 (d, 12.3)
	$4_{eq.}$	2.7 (m)	2.97 (dd, 5.5, 15.8)				
	$4_{ax.}$	3.21 (m)	2.74 (dd, 8.5, 15.8)				
OMe		3.52, 3.55, 3.73, 3.78 3.81, 3.84 (×3), 3.85, 3.87 (each s)	3.72, 3.78, 3.82 (×3), 3.84, 3.85 (each s)	3.29, 3.74, 3.83, 3.84, 3.85, 3.86 (×2), 3.87 (each s)	3.63, 3.72, 3.87, 3.88 (×2), 3.89, 3.90 (each s)	3.64, 3.72, 3.85 (×2), 3.86 (×2), 3.87, 3.89 (each s)	3.76, 3.82, 3.85, 3.86 (×2), 3.87 (×3) (each s)
OAc		1.52 (s)	1.79, 1.85 (each s)	1.59 (s)	1.60, 2.06 (each s)	1.65, 2.06 (each s)	1.64, 2.06 (each s)



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



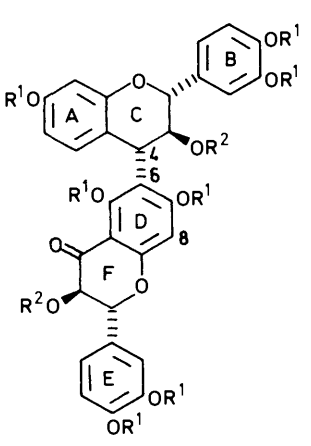
(**5**) $R^1 = R^2 = H$
(**6**) $R^1 = Me$, $R^2 = Ac$

AMXY system, similarly indicated a pyrogallol-type F-ring[8] with the remaining aromatic ABX systems hence attributable to the A- and B-rings of the (−)-fisetinidol entity. The absolute configuration, *i.e.* 2*R*,3*S*,4*R*:2*R*,3*S* which was deduced from heterocyclic coupling constants and c.d. data (high amplitude positive C.e. at 234 nm), was confirmed by synthesis *via* acid-mediated coupling[22,29] of (+)-mollisacacidin [(2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*-3′,4′,7-trihydroxyflavan-3,4-diol] and (−)-robinetinidol. The heptamethyl ether diacetate (**4**) of the expected (4β,6)-isomer (**3**) exhibited physical data (^{1}H n.m.r. and c.d.) identical with those of the corresponding derivative of the natural product. This compound is accompanied by the (4α,6)-isomer and a series of, amongst others, (4,2′), *i.e.* B-ring coupled analogues, details of which will be published elsewhere.

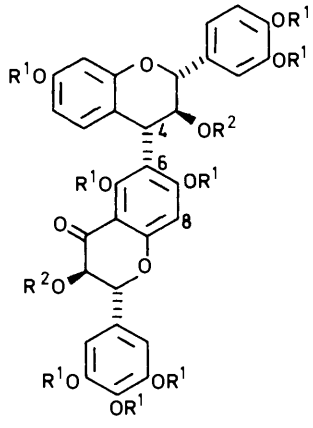
^{1}H N.m.r. data (Table) of the octamethyl ether diacetate (**6**) of the novel (−)-fisetinidol-(4α,2′)-robinetin (**5**), complementing the rare group of B-ring linked profisetinidins,[30] revealed a single heterocyclic AMX system, the coupling constants of which ($J_{2,3}$ 9.5 = $J_{3,4}$ 9.5 Hz) are consistent with a 2,3-*trans*-3,4-*trans* relative configuration. The aromatic region displayed three ABX systems, two of which could unequivocally be attributed to the A- (δ 6.48, dd, $J_{2,5}$ and 8.0 Hz, 6-H; 6.49, d, *J* 2.5 Hz, 8-H; 7.04, d, *J* 8.0 Hz, 5-H) and B-ring (δ 6.80, d, *J* 8.3 Hz, 5-H; 6.90, d, *J* 2.0 Hz, 2-H; 6.99, dd, *J* 2.0 and 8.3 Hz, 6-H) respectively *via* the appropriate spin decoupling experiments. The remaining ABX system (δ 6.70, d, *J* 2.2 Hz, 8-H; 6.97, dd, *J* 2.2 and 9.0 Hz, 6-H; 8.18, d, *J* 9.0 Hz, 5-H) with its deshielded *o*-doublet may, therefore, be ascribed to the D-ring of the robinetin moiety. These allocations for the A- and D-rings and thus proof that the latter ring could not be involved in interflavanyl coupling [*see* structure (**3**)] were unambiguously confirmed by observation of strong n.O.e. associations between 7-OMe

functions of both A- and D-rings and the respective 6- and 8-protons.* The sharp aromatic one-proton singlet (δ 6.66) exhibiting n.O.e. association with a single methoxy group only,* may then be ascribed to the 'residual' E-ring proton. Its shielding relative to the equivalent 2′-/6′-protons of hexa-*O*-methylmyrecitin (δ 7.36) is presumably a reflection of preference for a conformation (Dreiding models) in which the D/F-ring system is orthogonal relative to the E-ring, thus causing deviation of coplanarity of this ring with the α,β-unsaturated ketone moiety required for effective deshielding of 7-H(E). The (2*R*,3*S*,4*S*)-absolute configuration of the (−)-fisetinidol unit could again be deduced from the heterocyclic coupling constants and the negative C.e. at 233 nm of the c.d. spectrum.

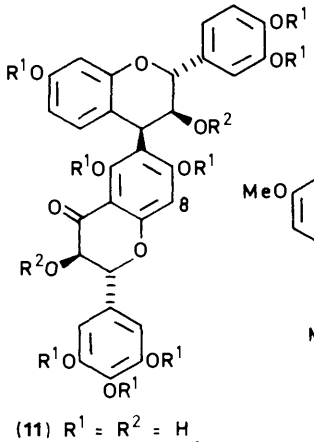
The series of naturally occurring profisetinidins with C-4 oxygenated heterocycles of their 'lower' flavanyl units[13–15,31] is extended by characterization of analogues where these units are based upon the dihydroflavonols, (+)-taxifolin [(2*R*,3*R*)-2,3-*trans*-3′,4′,5,7-tetrahydroxydihydroflavonol] and (+)-ampelopsin. This novel class of biflavanoids included (−)-fisetinidol-(4α,6)-(+)-taxifolin (**7**), (−)-fisetinidol-(4α,6)-(+)-ampelopsin (**9**), (−)-fisetinidol-(4β,6)-(+)-ampelopsin (**11**), and (4α,8)-(−)-



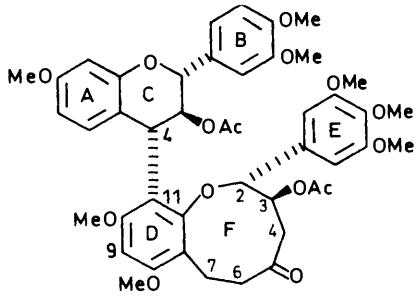
(**7**) R^1 = R = H
(**8**) R^1 = Me R^2 = Ac



(**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



(**13**)

fisetinidol-(+)-ampelopsin which was, however, subject to extensive methylene insertions during methylation with diazomethane and thus obtained as the benzoxonin-5-one (**13**) after acetylation. The first three compounds were similarly identified by the physical data of their phenolic methyl ether diacetates (**8**), (**10**), and (**12**). ^{1}H N.m.r. spectra of these derivatives are characterized by the presence of only five heterocyclic protons, *i.e.* an AMX system for the (−)-fisetinidol entity and an AB system for the dihydroflavonol unit with coupling constants (*J* 10.5—12.5 Hz) typical for 2,3-*trans* configuration of these structural types.[32,33]

The heterocyclic AMX system, indicative of 2,3-*trans*-3,4-*trans* configuration ($J_{2,3}$ = $J_{3,4}$ 9.8 Hz) for the C-ring of the (−)-fisetinidol-(4α,8)-(+)-taxifolin derivative (**8**), was correlated with the ABX systems of the resorcinol-type A-ring and the pyrocatechol B-ring and the AB system ($J_{2,3}$ 12.5 Hz) of ring F with the ABX system of the pyrocatechol E-ring by the appropriate spin decoupling experiments. The chemical shift of the residual D-ring proton singlet as parameter for distinguishing between C-6 and C-8 (+)-catechin derived biflavanoids has been established for C-4(F) deoxy analogues only.[23,24] Recourse was thus taken to an n.O.e. experiment which indicated strong association between the singlet (δ 6.28) and one of the D-ring methoxy groups [δ 3.63 (4.31%)] to establish coupling at C-6 of the (+)-taxifolin entity. Confirmation for the (−)-fisetinidol 'upper' unit followed from the heterocyclic coupling constants (C-ring) and the negative C.e. at 234 nm in the c.d. spectrum. Chiroptical properties very similar to those of (+)-fustin [(2*R*,3*R*)-2,3-*trans*-3′,4′,7-trihydroxydihydroflavonol][34] in the 280—350 nm region indicated identical absolute configurations at the C-2 and C-3 chiral centres of the dihydroflavanol entities. Collectively these data then define the absolute stereochemistry of the (−)-fisetinidol-(+)-taxifolin (**7**) as (2*R*,3*S*,4*S*:2*R*,3*R*).

The two novel (−)-fisetinidol-(+)-ampelopsin analogues, *i.e.* (−)-fisetinidol-(4α,6)(+)-ampelopsin (**9**) and (−)-fisetinidol-(4β,6)-(+)-ampelopsin (**11**) were similarly identified as their octamethyl ether diacetates (**10**) and (**12**). Their ^{1}H n.m.r. data (Table) exhibited striking resemblance to those of the (−)-fisetinidol-(+)-taxifolin derivative (**8**). Notable differences included replacement of one of the aromatic ABX spin systems with a broadened two-proton singlet [δ 6.68 for (**10**); 6.78 for (**12**)] characteristic of a pyrogallol-type B-ring. This unit was correlated with the (+)-ampelopsin moiety through observation of benzylic coupling between the above singlets and 2-H [δ 5.34, *J* 12.1 Hz for (**10**) 5.31, *J* 12.3 Hz for (**12**)] of the dihydroflavonol heterocycle. The 2,3-*trans*-3,4-*trans*- and 2,3-*trans*-3,4-*cis*-configurations for the (−)-fisetinidol units in (**10**) and (**12**) respectively were evident from the coupling constants of the heterocyclic AMX systems [$J_{2,3}$ = $J_{3,4}$ 9.5 Hz for (**10**); $J_{2,3}$ 10.5, $J_{3,4}$ 7.0 Hz for (**12**)]. Confirmation for substitution at C-6 of the (+)-ampelopsin entity in both (**10**) and (**12**) was obtained by observation of strong n.O.e. association of the residual D-ring proton [δ 6.29 for (**10**), 6.35 for (**12**)] with a single methoxy group [δ 3.64 (3.23%) for (**10**), 3.85 (3.1%) for (**12**)]. Finally, the absolute configurations, *i.e.* (2*R*,3*S*,4*S*:2*R*,3*R*) for (**10**) and (2*R*,3*S*,4*R*:2*R*,3*R*) for (**12**) were confirmed by the combined detail of the heterocyclic coupling constants (C-rings) and the high-amplitude C.e.'s [negative for (**10**) and positive for (**12**)] at low wavelengths in the c.d. spectra for the (−)-fisetinidol units and again by comparison of the 280—350 nm region with that of (+)-fustin.[34]

The (−)-fisetinidol-(4α,8)-(+)-ampelopsin analogue is apparently susceptible to extensive methylene insertion reactions during methylation with diazomethane (*cf.* ref. 31) and was thus obtained as the benzoxonin-5-one (**13**) after acetylation. The aromatic region of its ^{1}H n.m.r. spectrum exhibited features very similar to those of the (−)-fisetinidol-(+)-ampelopsin derivatives (**10**) and (**12**). Appropriate spin decoupling experiments again facilitated correlation of the aromatic ABX patterns with the respective heterocyles. Coupling constants reflected all-*trans* configuration for ring C ($J_{2,3}$ = $J_{3,4}$ 10.0 Hz) and 2,3-*trans* [δ 4.87, d, $J_{2,3(F)}$ 10.0 Hz, 2-H(F); 5.64, m, 3-H(F)] for the F-ring.

* Due to signal overlap, these associations could not be quantified.

N.O.e. difference spectroscopy confirmed coupling at C-8 of the (+)-ampelopsin moiety through association of the residual 9-H(D) singlet (δ 6.19) with both the methoxy groups [δ 3.46 (3.9), 3.79 (3.3%)] of the D-ring. Besides the AMX system of ring C and the signals due to 2- and 3-H (F-ring, see above), the heterocyclic region displayed three sets of signals which may be assigned to methylene groups. Their connectivities were elegantly demonstrated by spin decoupling experiments commencing with the 2-H(F) doublet. The strong negative C.e. at 221 nm in the c.d. spectrum when taken in conjunction with the heterocyclic coupling constants defined (2*R*,3*S*,4*S*) absolute configuration for the (−)-fisetinidol moiety. Due to lack of reference compounds the presumed (2*R*,3*R*) configuration of the lower unit could, however, not be verified experimentally.

Finally, possible synthetic routes towards the (−)-fisetinidol-(+)-ampelopsin dimers (**9**) and (**11**) were investigated. Owing to the expected reduced nucleophilicity of the A-ring of dihydroflavonols in comparison with those of the corresponding flavan-3-ol,[15] reaction of (+)-mollisacacidin, as synthon for the (−)-fisetinidol entity, and the dihydroflavonol, (+)-ampelopsin, under mild conditions, *i.e.* 0.1M HCl at ambient temperatures (see ref. 23) invariably failed. Similar failures were encountered under more drastic conditions (*see* Experimental section) and also when using 3′,4′,7-tri-*O*-methylmollisacacidin in order to enhance electrophilicity at its C-4 carbocationic centre.

Following the approach of Hemingway *et al.*[35,36] 4-phenylthio-(+)-mollisacacidin was treated with (+)-ampelopsin at pH 9 and room temperature for 90 h. Despite complete consumption of the thio ether, this method afforded the (−)-fisetinidol-(4α,6)-(+)-ampelopsin (**9**) in only 0.85% yield.

Although enzymes would obviously be more effective in bringing about the desired couplings, the aforementioned failures clearly indicate that formation of biflavanoids derived from dihydroflavonols as 'lower' unit is less facile than of those based on a C-4 deoxy analogue. This may well indicate that selective oxidation of the C-4 methylene group of the F-ring of conventional (−)-fisetinidol-(+)-catechin or (+)-gallocatechin biflavanoids might represent a plausible alternative for direct coupling of flavan-3,4-diol and dihydroflavonol as means of biosynthesis of this novel group of oligomeric flavanoids. Efforts in this direction as well as alternative approaches towards their *in vitro* synthesis are presently being investigated.

Experimental

^{1}H N.m.r. spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3$ or $(CD_3)_2CO$ 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 pre-coated 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 were in ethanol at a flow rate of 22 cm^3 32 min^{-1}. 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. 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 Burkea africana.—Drillings (3.65 kg) of the dried heartwood were extracted with MeOH in a Soxhlet apparatus. The extract was dewaxed with hexane (5 × 300 cm^3) and evaporated to give a dark brown powder (569 g). This (5 × 40 g) was partitioned between a butan-2-ol–water–hexane (4:5:1, v/v) mixture in a 20-tube, 100 cm^3 underphase, Craig countercurrent assembly.

Following qualitative paper chromatographic analysis the fractions were combined as follows: 1 [tubes 1—4 (43.8 g)], 2 [5—8 (27.7 g)], 3 [9—12 (21.8 g)], 4 [13—16 (22.1 g), 5 [17—20 (23.1 g)]. Subsequent column chromatography of fraction 5 on Sephadex LH-20 (22 cm^3 eluant/tube) yielded the following nine fractions: 5A [tubes 1—169 (785 mg)], 5B [170—182 (300 mg)], 5C [183—212 (280 mg)], 5D [213—240 (500 mg)], 5E [241—290 (240 mg)], 5F [291—392 (550 mg)], 5G [396—429 (340 mg)], 5H [430—504 (480 mg)], 5I [505—556 (347 mg)].

Acetylation and subsequent purification by p.l.c. [1,2-dichloroethane–acetone (95:5, v/v, ×2)] of fraction 5F (100 mg) afforded hexa-*O*-acetyl-(+)-ampelopsin[19] (8.7 mg; R_F 0.69) and penta-*O*-acetylrobinetin[17] (15.6 mg; R_F 0.66), the physical data (^{1}H n.m.r., c.d., and m.s.) of which were identical with those of authentic samples. Owing to their complexity and partial overlap of components with those in fraction 4 of the countercurrent distribution, the remaining fractions were thus not further investigated.

Fraction 4 from the countercurrent distribution was subjected to column chromatography on Sephadex LH-20 (22 cm^3 eluant/tube) to afford ten fractions: 4A [tubes 184–221 (503 mg)], 4B [222—307 (300 mg)], 4C [308—350 (382 mg)], 4D [351—374 (214 mg)], 4E [375—404 (356 mg)], 4F [405—500 (532 mg)], 4G [502—553 (552 mg)], 4H [554—653 (604 mg)], 4I [654—694 (539 mg)], and 4J [695—991 (1.48 g)].

A portion of fraction 4A (100 mg) was methylated and the mixture resolved by p.l.c. [benzene–acetone (8:2)] into a single dominant band at R_F 0.55 (15 mg) which afforded, after acetylation and purification, the methyl ether acetate of (−)-robinetinidol[20,21] the physical data of which were identical with those of an authentic sample.

Methylation of fraction 4D (214 mg) followed by p.l.c. [benzene–acetone (8:2)] afforded hexa-*O*-methylmyrecitin[18] (75 mg, R_F 0.23), the physical data of which were identical with those of an authentic sample.

The chemical contents of fractions 4B—4E, and 4H were basically the same as those of the remaining fractions and were thus not further investigated.

Tri-O-*methyl*-3-O-*acetyl*-(−)-*fisetinidol*-(4β,6)-*tetra*-O-*methyl*-3-O-*acetyl*-(−)-*robinetinidol* (**4**).—Fraction 4F (532 mg) was methylated and a portion (360 mg) of the mixture resolved by p.l.c. [benzene–acetone (8:2)] into three bands at R_F 0.76 (28 mg), 0.54 (27 mg), and 0.20 (32 mg).

Acetylation of the R_F 0.20 fraction followed by p.l.c. [benzene–acetone (8:2)] afforded the heptamethyl ether diacetate (19 mg; R_F 0.59) as a *white amorphous solid* (Found: M^+, 744.2773. $C_{41}H_{44}O_{13}$ requires M^+, 744.2782); ^{1}H n.m.r. data (Table); c.d. $[\theta]_{295}$ 0, $[\theta]_{279}$ 1.08 × 10^4, $[\theta]_{365}$ 0, $[\theta]_{234}$ 4.65 × 10^5, and $[\theta]_{212}$ 0.

The R_F 0.76 and 0.54 fractions eventually afforded the methyl ethers of the 3-*O*-galloyl ester of (−)-robinetinidol and a related benzopyran-8-one, details of which will be published elsewhere.

Fraction 4G was methylated and the mixture resolved by p.l.c. [benzene–acetone (8:2)] into bands 4G1 (R_F 0.15, 48 mg) and 4G2 (R_F 0.07; 94 mg).

Tri-O-*methyl*-3-O-*acetyl*-(−)-*fisetinidol*-(4α,6)-*tetra*-O-*methyl*-3-O-*acetyl*-(+)-*taxifolin* (**8**).—Acetylation of 4G1 followed by p.l.c. [1,2-dichloroethane–acetone (99:1)] afforded the heptamethyl ether diacetate (4.1 mg; R_F 0.65) as a *light brown solid* (Found: M^+, 758.2569. $C_{41}H_{42}O_{14}$ requires M^+, 758.2574); ^{1}H n.m.r. data (Table); c.d. $[\theta]_{360}$ 0, $[\theta]_{335}$ 1.18 × 10^5, $[\theta]_{320}$ 0, $[\theta]_{301}$ −1.45 × 10^5, $[\theta]_{279}$ 0, $[\theta]_{268}$ 8.99 × 10^4, $[\theta]_{248}$ 0, and $[\theta]_{234}$ −3.39 × 10^5.

Tri-O-methyl-3-O-acetyl-(−)-fisetinidol-(4α,6)-penta-O-methyl-3-O-acetyl-(+)-ampelopsin (**10**).—Acetylation of 4G2 and p.l.c. [1,2-dichloroethane–acetone (85:15)] gave the octamethyl ether diacetate (3.5 mg; R_F 0.69) as a *light brown amorphous solid* (Found: $M^+ - HOAc$, 728.2463. $C_{42}H_{44}O_{15} - HOAc$ requires M^+, 728.2469); ^1H n.m.r. data (Table); c.d. $[\theta]_{326}$ 0, $[\theta]_{303}$ -1.15×10^5, $[\theta]_{279}$ 0, $[\theta]_{264}$ 4.95×10^4, $[\theta]_{248}$ 0, $[\theta]_{234}$ -4.0×10^5, and $[\theta]_{205}$ 0.

Fraction 4I was methylated and the mixture resolved by p.l.c. [benzene–acetone (8:2)] into 4I1 (R_F 0.12; 138 mg) and 4I2 (R_F 0.08; 61 mg).

Acetylation of 4I1 followed by p.l.c. [benzene–acetone (9:1, ×2)] gave two bands at R_F 0.53 (42 mg) and 0.49 (13 mg).

Tri-O-methyl-3-O-acetyl-(−)-fisetinidol-(4β,6)-penta-O-methyl-3-O-acetyl-(+)-ampelopsin (**12**).—Subsequent purification of the R_F 0.53 fraction by p.l.c. [benzene–acetone (9:1)] afforded the octamethyl ether diacetate (4.2 mg) as a *light brown solid* (Found: $M^+ - HOAc$, 728.2472. $C_{42}H_{44}O_{15} - HOAc$ requires M^+, 728.2469); ^1H n.m.r. data (Table); c.d. $[\theta]_{320}$ 0, $[\theta]_{308}$ 1.19×10^4, $[\theta]_{288}$ 0, $[\theta]_{266}$ -1.03×10^5, $[\theta]_{251}$ 0, $[\theta]_{232}$ 6.67×10^5, $[\theta]_{219}$ 0, $[\theta]_{217}$ -5.56×10^4, and $[\theta]_{213}$ 0.

Tri-O-methyl-3-O-acetyl-(−)-fisetinidol-(4α,2′)-penta-O-methylrobinetin (**6**).—The fraction at R_F 0.49 afforded the octamethyl ether acetate as a *light brown solid* (Found: M^+, 728.2476. $C_{40}H_{40}O_{13}$ requires M^+, 728.2469); ^1H n.m.r. data (Table); c.d. $[\theta]_{335}$ 0, $[\theta]_{300}$ -6.40×10^4, $[\theta]_{265}$ 0, $[\theta]_{233}$ -3.65×10^5, and $[\theta]_{217}$ 0.

Tri-O-methyl-3-O-acetyl-(−)-fisetinidol-(4α,11)-[2-(3′,4′,5′-trimethoxyphenyl)-3-acetoxy-8,10-dimethoxy]-benzoxonin-5-one (**13**).—Acetylation of 4I2 followed by p.l.c. [benzene–acetone (9:1)] afforded the octamethyl ether diacetate (11.2 mg; R_F 0.47) as a *light brown solid* (Found: M^+, 830.3128. $C_{45}H_{50}O_{15}$ requires M^+, 830.3149); $\delta_H(CDCl_3$; 300 MHz; 25 °C) 6.95 [dd, *J* 8.5 and 2.0 Hz, 6-H(B)], 6.88 [d, *J* 2.0 Hz, 2-H(B)], 6.82 [d, *J* 8.5 Hz, 5-H(B)], 6.55 [s, 2-H and 6-H(E)], 6.27 [d, *J* 2.5 Hz, 8-H(A)], 6.19 [s, 9-H(D)], 6.07 [dd, *J* 8.5 and 2.5 Hz, 6-H(A)], 5.85 [dd, *J* 8.5 and 1.1 Hz, 5-H(A)], 5.64 [m, 3-H(F)], 5.48 [t, *J* 10.0 Hz, 3-H(C)], 4.87 [d, *J* 10.0 Hz, 2-H(F)], 4.51 [br d, *J* 10.0 Hz, 2-H(C)], 4.36 [dd, *J* 10.0 and 1.1 Hz, 4-H(C)], 3.25 [dd, *J* 12.0 and 10.2 Hz, 4-H(F)], 3.14 [m, 6-H(F)], 2.83 [dd, *J* 11.5 and 5.5 Hz, 4-H(F)], 2.62 [m, 7-H(F)], 2.25 [m, 7-H(F)], 3.55 [m, 6-H(F)], 3.88, 3.86, 3.79, 3.75 (×2), 3.64, 3.46, 3.45 (each s, 8 × OMe), and 1.72 and 1.71 (each s, 2 × OAc); c.d. $[\theta]_{260}$ 0, $[\theta]_{235}$ -4.57×10^5, $[\theta]_{227}$ -2.73×10^5, $[\theta]_{221}$ -5.91×10^5, and $[\theta]_{200}$ 0.

Tri-O-methyl-3-O-acetyl-(−)-fisetinidol-(4α,8)-tetra-O-methyl-3-O-galloyl-(+)-catechin (**2**).—Fraction 4J was methylated and the mixture resolved by p.l.c. [benzene–acetone (8:2)] into two bands at R_F 0.23 (84 mg) and 0.16 (81 mg).

Acetylation of the R_F 0.23 fraction followed by p.l.c. [benzene–acetone (9:1)] afforded the decamethyl ether acetate (4 mg; R_F 0.37) as a *light brown amorphous solid* (Found: $M^+ - HOAc$, 836.3052. $C_{49}H_{52}O_{16} - HOAc$ requires M^+, 836.3044); ^1H n.m.r. data (Table); c.d. $[\theta]_{320}$ 0, $[\theta]_{270}$ 1.62×10^5, $[\theta]_{244}$ 0, $[\theta]_{222}$ -5.24×10^5, and $[\theta]_{203}$ 0.

The R_F 0.16 fraction is presently being investigated, details of which will be published elsewhere.

Attempted Acid-catalysed Condensation of (+)-Mollisacacidin and 3′,4′,7-Tri-O-Methyl-(+)-mollisacacidin with (+)-Ampelopsin.—All attempts to couple (+)-mollisacacidin and the methyl ether of (+)-mollisacacidin with (+)-ampelopsin under varying conditions of temperature (20—60 °C), solvent (aqueous MeOH and aqueous dioxane) and reaction times (24—160 h) in a 0.1M HCl solution invariably failed.

Base-catalysed Condensation of 4-Phenylthio-(+)-mollisacacidin with (+)-Ampelopsin.—(+)-Ampelopsin (640 mg, 2 mmol) and 4-phenylthio-(+)-mollisacacidin (520 mg, 1.37 mmol) were dissolved in alkaline buffer at pH 9 [0.025M Na_2CO_3; 0.025M $NaHCO_3$; 1:1 (v/v); 58 cm^3] and the reaction mixture stirred at ambient temperature for 90 h under a nitrogen blanket. The reaction was then terminated by the addition of 4% acetic acid solution (reaction mixture acidified to pH 6). The mixture was extracted with ethyl acetate (3 × 100 cm^3) and the combined extracts dried (Na_2SO_4). After evaporation of the solvent the recovered solids were separated by column chromatography. Methylation of the fraction (retention time: 60—80 h) followed by p.l.c. [benzene–acetone (8:2)] afforded a fraction at R_F 0.28 (33 mg). Acetylation of this fraction and p.l.c. [benzene–acetone (9:1)] gave tri-*O*-methyl-3-*O*-acetyl-(−)-fisetinidol-(4α,6)-penta-*O*-methyl-3-*O*-acetyl-(+)-ampelopsin (13.4 mg; 0.85%), the physical data of which were identical with those of the natural counterpart (**10**).

Acid-catalysed Condensation of (+)-Mollisacacidin and (−)-Robinetinidol.—(+)-Mollisacacidin (182 mg, 0.6 mmol) and (−)-robinetinidol (200 mg, 0.69 mmol) was dissolved in 0.1M HCl (20 cm^3) and the mixture kept at 50 °C for 24 h. The reaction was terminated by the addition of $NaHCO_3$ solution and the mixture extracted with ethyl acetate (3 × 200 cm^3). After drying (Na_2SO_4) and evaporation of the solvent the recovered solids were separated by column chromatography on Sephadex LH-20. Methylation of the fraction with retention time 80—88 h followed by p.l.c. [benzene–acetone (85:15) ×2] afforded a fraction at R_F 0.33 (22.1 mg). Acetylation of this fraction gave tri-*O*-methyl-3-*O*-acetyl-(−)-fisetinidol-(4β,6)-tetra-*O*-methyl-3-*O*-acetyl-(−)-robinetinidol (**4**) as an amorphous white solid (20.6 mg), with physical data identical with those of the natural product. Details of the remaining compounds from this condensation will be published elsewhere.

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