Synthesis of Condensed Tannins. Part 5. The First Angular [4,6:4,8]-Triflavanoids and Their Natural Counterparts

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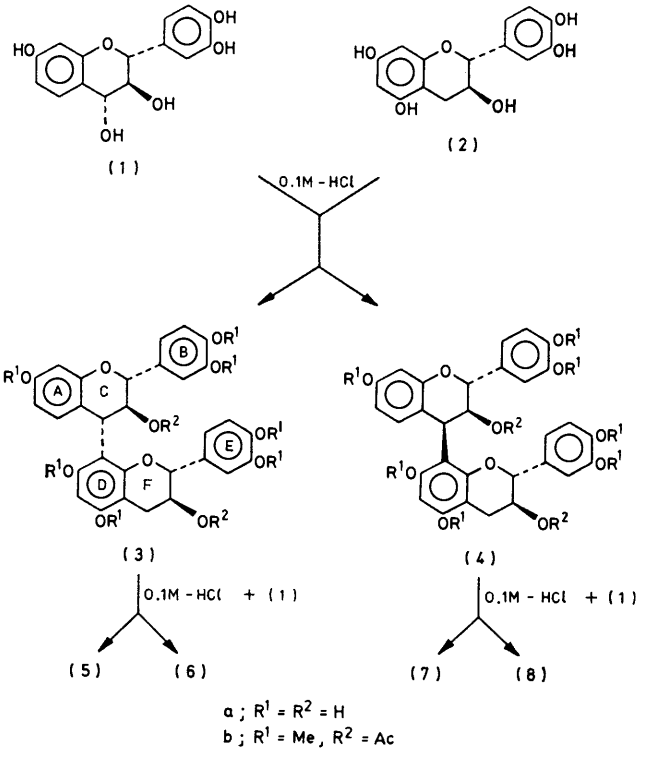
Angular triflavanoids comprising a group of four diastereoisomeric [4,6:4,8]-bi-[(−)-fisetinidol]-(+)-catechins with definable absolute configurations (2*R*,3*S*,4*S*- and 2*R*,3*S*,4*R*-2*R*,3*S*-2*R*,3*S*,4*S*; 2*R*,3*S*,4*S*- and 2*R*,3*S*,4*R*-2*R*,3*S*-2*R*,3*S*,4*R*) result from flavanyl-4-carbocation mediated condensation of (2*R*,3*S*,4*R*)-flavan-3,3',4,4',7-pentaol[(+)-mollisacacidin] at C-6 of the (+)-catechin units of all-*trans*-(2*R*,3*S*,4*S*-2*R*,3*S*)-[4,8]-(−)-fisetinidol-(+)-catechin and its 3,4-*cis*-(2*R*,3*S*,4*R*-2*R*,3*S*) diastereoisomer respectively. The natural coexistence of some of the foremention species of bi- and tri-flavanoids and their precursors indicate a comparable sequence of 4,8 preceding 4,6 condensation with (+)-catechin as common nucleophile.

THE biomimetic approach to condensed tannin synthesis based on the generation of flavanyl-4-carbocations from flavan-3,4-diols, and their condensation with nucleophilic flavan-3-ols to form [4,6]- and [4,8]-biflavanoids under mildly acidic conditions at ambient temperatures,[1,2] is presently extended to the first synthesis of 'angular' [4,6:4,8]-triflavanoids, thus expanding the applicability of the method. Such 'angular' triflavanoids occur naturally, and apparently represent key intermediates to more highly condensed tannins.[3]

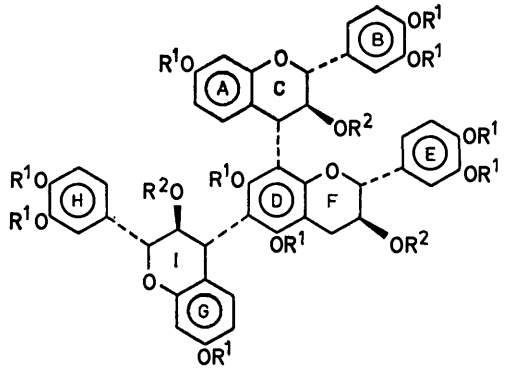
Condensation of the free phenolic (+)-mollisacacidin [(2*R*,3*S*,4*R*)-flavan-3,3',4,4',7-pentaol (1)], with the 4,8-coupled all-*trans*-biflavanoid, (2*R*,3*S*,4*S*-2*R*,3*S*)-(−)-fisetinidol-(+)-catechin [2] (3) under similar conditions gives, after successive methylation with diazomethane and acetylation, the decamethyl ether triacetates of two diastereoisomeric [4,6:4,8]-triflavanoids (5a) and (6a) in 7% overall yield. However, in contrast with the synthesis of biflavanoids,[1,2] the triflavanoids cannot readily be resolved as free phenolic forms. Their methyl ether acetates (5c), (6c) exhibit complex ^{1}H n.m.r. spectra at ambient temperatures due to line-broadening and duplication of resonances (*e.g.* acetyl protons), phenomena which are indicative of intermittent exchange [4] of rotational isomers.[5-7] Temperature elevation of the equally-populated rotamers in dimethyl sulphoxide [$(CD_3)_2SO$] to 177 and 167 °C respectively is necessary at 80 MHz to overcome the relatively high activation energy for 'fast' rotation about both interflavanoid bonds. Systematic temperature increase allows for the characteristic collapse, coalescence, and ultimate sharpening of all temperature-dependent resonances,[4] thus permitting differentiation between 3,4-*trans* and 3,4-*cis* configurations of coupled units and also definition of the point of coupling. Subsequent return to ambient temperatures effects reversion to the same line-broadened resonances of equally-populated rotamers undergoing 'intermittent' exchange. These phenomena are at variance with the speculative concept of left- and right-handed spiral arrangements proposed by Haslam *et al.*[8]

At elevated temperatures definition of the point of coupling is obtained by comparison at 360 MHz in $(CD_3)_2SO$ of the ^{1}H high-field aromatic resonances of the heptamethyl ether diacetate (3b) of the parent [4,8]-biflavanoid (3a) with those of the decamethyl ether



triacetates (5c), (6c) of the triflavanoids (5a), (6a) (*cf.* Figure 1). Thus, the high-field singlet [residual H-6 on the A-ring of the (+)-catechin unit] characteristic of the [4,8]-biflavanoid derivative (3b) [1,2] is replaced in the derivatives of both triflavanoids (5c), (6c) by a second † high-field ABC-system attributable to the introduced

† The ABC-system of the A-ring is already represented in the high-field aromatic region.



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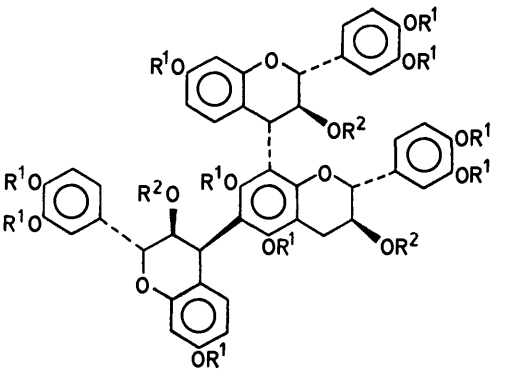
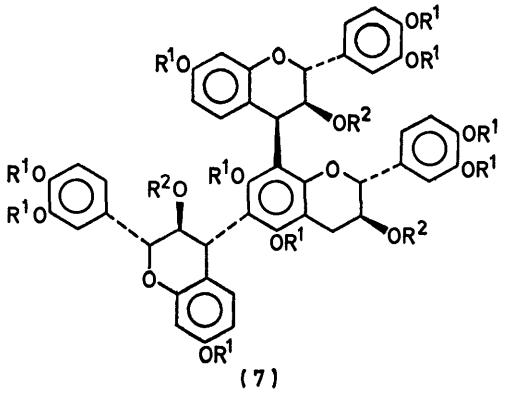


(6)

a; $R^1 = R^2 = H$
b; $R^1 = Me$, $R^2 = H$
c; $R^1 = Me$, $R^2 = Ac$



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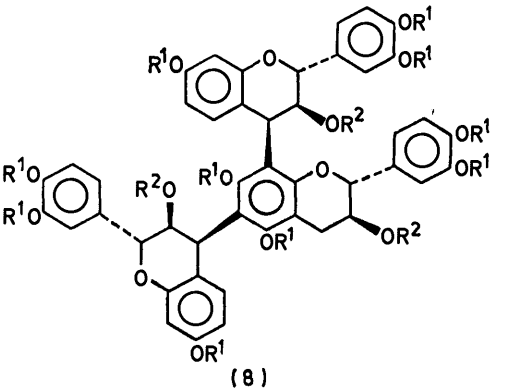


(8)

resorcinol-type G-ring. This establishes for the first time the *in vitro* formation of 'angular' triflavanoids in which the phloroglucinol A-ring of (+)-catechin (2) serves as common nucleophile in successive condensation reactions with the same flavan-3,4-diol (1), 4,8-preceding [1,2] 4,6-coupling.

Similarly, at elevated temperatures in $(CD_3)_2SO$ the *trans*-diaxial arrangements of the C- and I-ring heterocyclic protons of the triflavanoid decamethyl ether triacetate (5c) are characterized by closely overlapping H-3 triplet resonances (δ 5.77, 5.75), both indicative of large coupling constants ($J_{2,3} = J_{3,4} = 9.5$ Hz, *cf.* Table and Figure 2) to low field in the heterocyclic region). In contrast, the corresponding derivative of the diastereoisomer (6c) exhibits a single low-field triplet (δ 5.92) and a doublet of doublets to higher field (δ 5.38). The coupling constants ($J_{2,3}$ 8.0, $J_{3,4}$ 6.5 Hz) of the latter, as well as its characteristic upfield shift (Δδ − 0.38) (*cf.* Table), also indicates a second introduced unit but with a 3,4-*cis* configuration as expected.[1,2,9]

The remaining possible triflavanoid diastereoisomers of 2,3-*trans*-configuration (7a), (8a) were available from a similar condensation as cited above, but by using 3,4-*cis*-(2*R*,3*S*,4*R*-2*R*,3*S*)-[4,8]-(−)-fisetinidol-(+)-catechin (4a) as substrate. This intermediate is contaminated with a low percentage of the all-*trans*-(2*R*,3*S*,4*S*-2*R*,3*S*)-[4,6]-isomer from which it cannot be separated in the free phenolic form after direct synthesis from the parent compounds, (+)-mollisacacidin (1) and (+)-catechin (2).[1,9] However, apart from the resultant formation of a low proportion of the all-*trans*-triflavanoid (5a), its presence during the subsequent condensation was not disadvantageous. The decamethyl ether triacetates of the [4,6:4,8]-triflavanoids (7c), (8c) with the expected 3,4-*cis*-configuration of the 8-linked unit resulted in a 7% combined yield in the proportions of 1.6 : 1 respectively. They may readily be distinguished at 80 MHz [$(CD_3)_2SO$, 170 °C] * by narrowed triplets (ΣJ_s *ca.* 14 Hz) of the low-field H-3(C) heterocyclic resonances (δ 5.40, 5.44) assigned to the 3,4-*cis*-8-linked units, compared with those of their 3,4-*trans* counterparts (5c), (6c) (ΣJ_s 19.0 Hz; δ 5.75, 5.92) (*cf.* Table and Figure 2). Thus, while the all-*trans* (5c) and all-*cis* (8c) configurations of attached units may be readily distinguished by H-3 chemical shifts alone (δ 5.77, 5.75 and 5.44, 5.36 respectively, *cf.* Figure 2), those of 'mixed' 3,4-*trans*/3,4-*cis* configuration (6c),(7c) may be differentiated by the presence of two low-field triplets in the spectrum of the isomer of 6-3,4-*trans*:8-3,4-*cis* configuration (7c), as well as by pronounced chemical-shift differences between H-2

* Triflavanoids generally require examination at 500 MHz for accurate assignment of all protons (*cf.* Part 6).

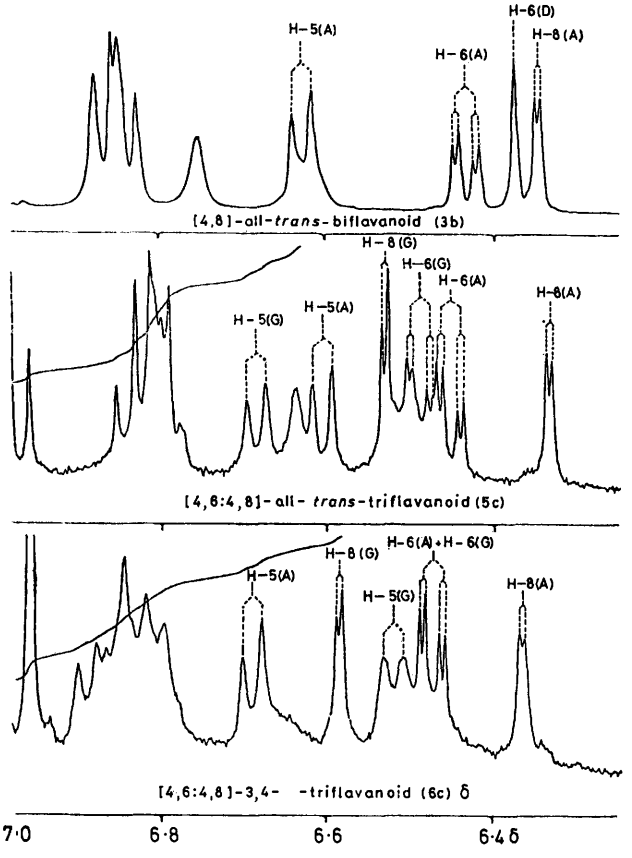


FIGURE 1 Expanded high-field aromatic resonances from ^1H 360 MHz spectra of methyl ether acetates of the all-*trans*-biflavanoid (3b) and the [4,6:4,8]-all-*trans*-(5c) and -2,3-*trans*-3,4-*cis*:2,3-*trans*:2,3-*trans*-3,4-*trans*-triflavanoids (6c)

(C- or I-rings) and highest up-field H-3 resonances ($\Delta\delta$ 0.19, 0.32 respectively). These aspects (Figure 2) have proved to be of diagnostic value at 80 MHz over a much wider range of 'angular' triflavanoids, since they represent the only feature of sharp differentiation at this frequency.

Although the absolute configurations of the four triflavanoids follow from the foregoing relative configurations, when taken in conjunction with their synthesis based on compounds of established absolute stereochemistry, further confirmation of the absolute configurations at C-4 of introduced units is available from circular dichroism based on the high-amplitude low-wavelength Cotton effects of those 4-arylflavan-3-ols [9-11] and biflavanoids [1,2] with 2,3-*trans*-stereochemistry. The all-*trans* decamethyl ether triacetate (5c) exhibits a high-amplitude negative doublet at *ca.* 220 and 235 nm (*cf.* Figure 3) tentatively attributed to those chiral centres which are attached to C-8 and C-6 respectively of the central (+)-catechin unit, and thus indicative of 4*S*-configurations in each instance. The 3,4-*trans* (C-ring) and 3,4-*cis* (I-ring) configurations of the isomeric derivative (6c) are in line with high-amplitude negative (*ca.* 220 nm) and positive (*ca.* 235) couplet (*cf.* Figure 3), which is apparently diagnostic of 4*S* and 4*R* absolute configurations at the same (C-8 and C-6) bonding points. Identical but opposite effects are exhibited as expected by derivatives of the remaining isomeric forms (7c), (8c) (*cf.* Figure 3). Notable is the enhanced magnitude of the low wavelength Cotton effects of the triflavanoid derivatives (5c)—(8c) compared with those of the parent biflavanoids (3b), (4b). This presumably reflects a degree of interaction of A-, D-, and G-benzenoid chromophores in the higher oligomers. From the foregoing collective evidence the absolute configurations of the synthetic [4,6:4,8]-triflavanoids (5a)—(8a) are established as 2*R*,3*S*,4*S*- and 2*R*,3*S*,4*R*-2*R*,3*S*-2*R*,3*S*,4*S*, and 2*R*,3*S*,4*S*- and 2*R*,3*S*,4*R*-2*R*,3*S*-2*R*,3*S*,4*R* respectively.

Mass fragmentation spectra are in agreement with the proposed triflavanoid structures (5c)—(8c), the molecular ions (M^+ 1 100) being substantiated by three successive losses of acetic acid. The mass spectra are also characterized by early loss of acetoxy- and methoxy-radicals (*e.g.* *m/e* 1 041 and *m/e* 1 010).

The prolonged reaction times (24 h) required for coupling of the flavan-3,4-diol to both [4,8]-biflavanoids compared with that for coupling to (+)-catechin (2 h), and also the lower yields of products (*ca.* 7% *vs.* 50% respectively) possibly reflects enhanced steric hindrance due to increased complexity of the nucleophilic substrate and its associated hydration sphere.

Chemical shifts and coupling constants of heterocyclic protons of 6- and 8-linked units in the decamethyl ether triacetates of angular triflavanoids *

Compound	3,4-Configuration of 2,3-*trans*-unit attached to (+)-catechin: 6-Linked (I-ring)	3,4-Configuration of 2,3-*trans*-unit attached to (+)-catechin: 8-Linked (C-ring)	Significant chemical shifts (δ) and coupling constants (Hz): 6-Linked (I-ring) H3	$J_{2,3}$	$J_{3,4}$	6-Linked (I-ring) H-2	8-Linked (C-ring) H-3	$J_{2,3}$	$J_{3,4}$	8-Linked (C-ring) H-2
(5c)	3,4-*trans*	3,4-*trans*	5.75 (t) ΣJ_e 19.0	9.5	9.5	4.77 (d)	5.77 (t) ΣJ_e 19.0	9.5	9.5	4.82 (d)
(6c)	3,4-*cis*	3,4-*trans*	5.38 (dd) ΣJ_e 14.5	8.0	6.5	5.19 (d)	5.92 (t) ΣJ_e 19.0	9.5	9.5	4.73 (d)
(7c)	3,4-*trans*	3,4-*cis*	5.83 (t) ΣJ_e 18.6	9.3	9.3	4.81 (d)	5,40 (t) ΣJ_e 14.0	7.0	7.0	5.08 (d)
(8c)	3,4-*cis*	3,4-*cis*	5.36 (dd) ΣJ_e 15.0	8.75	6.25	5.13 (d)	5.44 (t) ΣJ_e 14.1	7.0	7.0	5.06 (d)

d = doublet, dd = doublet of doublets, t = triplet.

* In $(CD_3)_2SO$ solution at 443 K (170 °C).

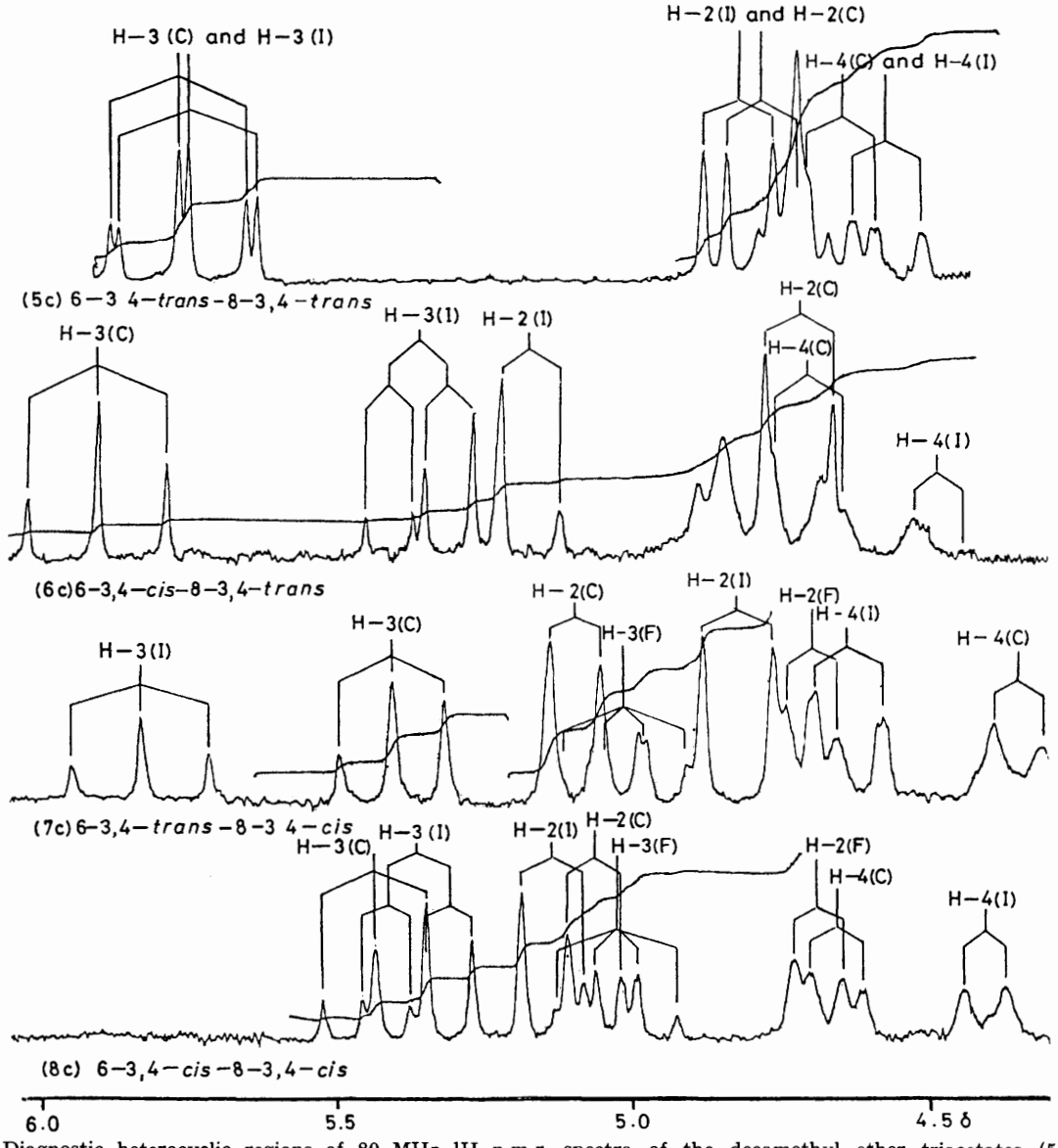


FIGURE 2 Diagnostic heterocyclic regions of 80 MHz ^{1}H n.m.r. spectra of the decamethyl ether triacetates (5c)—(8c) of diastereoisomeric [4,6:4,8]-triflavanoids

The natural co-occurrence of the four [4,6:4,8]-triflavanoids (5a)—(8a) is demonstrated by their isolation from a single source, the heartwood of the black wattle tree (*Acacia mearnsii*), and by the identity of the ^{1}H n.m.r. spectra (*ca.* 170 °C) and c.d. spectra (*cf.* Figure 3) of their decamethyl ether triacetates (5c)—(8c) with those of their synthetic counterparts. The pair of all-*trans* (5a) and 6-linked-3,4-*cis* (6a) isomers were also isolated in these laboratories some nine years ago by du Preez [12] from the heartwood of the mopane tree (*Colophospermum mopane*), where they are accompanied by their presumed initial [(+)-catechin, (+)-2,3-*trans*-3,4-*cis*-flavan-3,3′,4,4′,7-pentaol] and immediate {[4,8]-all-*trans*-(−)-fisetinidol-(+)-catechin (3)} precursors. Five angular isomers corresponding to (5a) and (6a) and their more highly oxygenated analogues have been isolated from black wattle bark (*A. mearnsii*), while other diastereoisomers occur in the heartwood of the karree tree (*Rhus lancea*), in all reflecting their ubiquitous distribution in nature.

The natural coexistence of [4,8]-bi- and 'angular' tri-flavanoids in the aforementioned species together with their precursors [13,14] indicates a comparable *in vivo* sequence (*cf.* synthetic scheme) of 4,8 preceding 4,6 condensation with (+)-catechin as common nucleophile. Furthermore, structural elucidation of natural angular tetraflavanoids by means of high magnetic field ^{1}H n.m.r. spectroscopy (*ca.* 10 T : 400 MHz and *ca.* 12.5 T : 500 MHz) from these sources suggests that these 'angular' triflavanoids may serve as intermediates for further structural elaboration to the more highly condensed tannins.

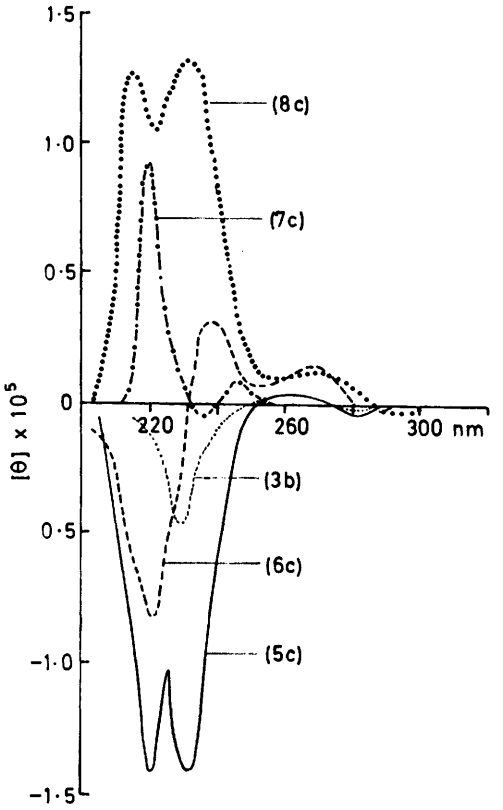


FIGURE 3 C.d. spectra of the methyl ether acetates of the all-*trans*-biflavanoid (3b) and the (4,6:4,8]-all-*trans*- (5c), 6-3,4-*cis*-8-3,4-*trans*- (6c), 6-3,4-*trans*-8-3,4-*cis*- (7c), and 6-3,4-*cis*-8-3,4-*cis*-triflavanoids (8c)

EXPERIMENTAL

M.p.s were determined with a Reichert hot-stage apparatus and are uncorrected. ^{1}H N.m.r. spectra were recorded on Bruker WP-80 and WM-360 FT spectrometers in $CDCl_3$ or $(CD_3)_2SO$ with Me_4Si as internal standard. Coupling constants were determined after suitable scale expansion. The free enthalpies of activation ($\Delta G^{\ddagger}_{rot.}$) were obtained by adopting the formulae $k_r = \pi\Delta\nu/\sqrt{2}$ and $\Delta G^{\ddagger}_{rot.} = 4.57\ T_c\ (10.32 + \log T_c/k_r)$ where $\Delta\nu$ represents the line separation without exchange and T_c the coalescence temperature (K) as outlined by Kessler.[4] Mass spectra were obtained with a Varian CH-5 instrument, and circular dichroism (c.d.) data in methanol on a Jasco J-20 spectropolarimeter. C and H Analyses were performed by Analytische Laboratorien, Fritz-Pregl-Strasse 24, 5 270 Gummersbach 1 Elbach, West Germany. Thin layer chromatography (t.l.c.) was done on DC-Plastikfolin Kieselgel 60 F_{254} (0.25 mm) and the plates sprayed with H_2SO_4–HCHO (40 : 1) after development. Preparative plates (p.l.c.) [20 × 20 cm, Kieselgel PF_{254} (1.0 mm)] were air-dried and used without prior activation. Methylations were performed with an excess diazomethane, while acetylations were in acetic anhydride–pyridine. Evaporations were done under reduced pressure at 50 °C in a rotary evaporator.

General Condensation and Work-up Procedures.—The (+)-3′,4′,7-trihydroxyflavan-3,4-diol [(+)-mollisacacidin (1)], and each of the synthetic [9] biflavanoids (3) or (4), were dissolved in 0.1M-HCl (50 ml) and stirred at room temperature for *ca.* 24 h. The solution was extracted with ethyl acetate (4 × 150 ml) and the combined extracts were dried (Na_2SO_4) after treatment with a minimum of hydrogen carbonate solution. Evaporation of the solvent to low volume (10 ml) followed by p.l.c. afforded the condensed product. Yields are based on the mass of biflavanoid offered.

Condensation of (2R,3S,4R)-2,3-trans-3,4-trans-*Flavan*-3,3′,4,4′,7-*pentaol* [(1), (+)-*Mollisacacidin*] *with* (2R,3S)-2,3-trans-8-[(2R,3S,4S)-2,3-trans-3,4-trans-3,3′,4′,7-*Tetrahydroxyflavan*-4-*yl*]*flavan*-3,3′,4′,5,7-*pentaol* {(3)a, [4,8]-3,4-trans-(−)-*Fisetinidol*-(+)-*catechin*}.—The 360 MHz ^{1}H n.m.r. spectrum of the heptamethyl ether diacetate of [4,8]-3,4-*trans*-(−)-fisetinidol-(+)-catechin (3) was recorded at the same temperature as those of the triflavanoid derivatives, thus enabling assignment through direct comparison, δ[$(CD_3)_2SO$, 200 °C] 6.93—6.79 (m), 6.76br (s, H-2′ (B or E)], 6.63 [d, *J* 8.3 Hz, H-5 (A)], 6.43 [dd, *J* 2.3 and 8.3 Hz, H-6 (A)], 6.38 [s, H-6 (D)], 6.36 [d, *J* 2.3 Hz, H-8 (A)], 5.89 [t, Σ*J* 19.5 Hz, H-3 (C)], 4.96 [m, H-3 (F)], 4.93 [d, *J* 7.5 Hz, H-2 (F)], 4.87 [d, *J* 9.7 Hz, H-2 + 4 (C)], 3.85, 3.80, 3.795, 3.79, 3.74, 3.73, 3.70 (s, 7 × OCH_3), 2.95 [dd, Σ*J* 21.7, $J_{3,4eq}$ 5.2, $J_{4ax.4eq}$ 16.5 Hz, H-4*eq* (F)], 2.70 [dd, Σ*J* 23.8, $J_{3,4ax}$ 7.5, $J_{4ax.4eq}$ 16.5 Hz, H-4*ax* (F)], 1.89 [s, 3-$COCH_3$ (F)], and 1.59 [s, 3-$COCH_3$ (C)].

(+)-Mollisacacidin (290 mg, 1 mmol) and [4,8]-3,4-*trans*-(−)-fisetinidol-(+)-catechin (590 mg, 1 mmol) were condensed in 0.1M-HCl (30 ml). The product was methylated and the methyl ethers after p.l.c. (1,2-dichloroethane–acetone, 7 : 3 v/v) gave two fractions, R_F 0.65 (14 mg) and 0.53 (385 mg). Acetylation of the former fraction and p.l.c. separation (benzene–acetone, 9 : 1 v/v) gave the heptamethyl ether diacetate, R_F 0.41, of the starting compound (2).[2] The latter fraction, comprising the methyl ethers (5b) and (6b) yielded two products, R_F 0.32 and 0.22, on acetylation followed by p.l.c. (benzene–acetone, 9 : 1 v/v).

(2R,3R)-2,3-trans-3-*Acetoxy*-6,8-*bi*-[(2R,3S,4S)-2,3-trans-3,4-trans-3-*acetoxy*-3′,4′,7-*trimethoxyflavan*-4-*yl*]-3′,4′,5,7-*tetramethoxyflavan* (5c).—The R_F 0.22 fraction afforded the all-*trans*-decamethyl ether triacetate as a *colourless solid* (92 mg, 4.5%) (Found: C, 66.6; H, 5.8. $C_{61}H_{64}O_{19}$ requires C, 66.5; H, 5.9%); δ[360 MHz, $(CD_3)_2SO$, 210 °C] * 7.11 [d, *J* 2.0 Hz, H-2′ (H)], 7.07 [dd, *J* 2.0 and 8.0 Hz, H-5 (H)], 6.98 [d, *J* 8.0 Hz, H-6′ (H)], 6.90—6.75 [m, 5 × H (B and E)], 6.69 [d, *J* 8.5 Hz, H-5 (G)], 6.64br s, H-2′ (B or E)], 6.61 [d, *J* 8.5 Hz, H-5 (A)], 6.53 [d, *J* 2.3 Hz, H-8 (G)], 6.49 [dd, *J* 2.3 and 8.5 Hz, H-6 (G)], 6.46 [dd, *J* 2.5 and 8.5 Hz, H-6 (A)], 6.34 [d, *J* 2.3 Hz, H-8 (A)], 5.95 [t, Σ*J* 19.5 Hz, H-3 (C or I)], 5.94 [t, Σ*J* 19.5 Hz, H-3 (I or C)], 5.00 [d, *J* 9.7 Hz, H-2 (C or I)], 4.96 [d, *J* 9.7 Hz, H-2 (C or I)], 4.90br [m, H-2 + 3 (F)], 4.83 [d, *J* 9.7 Hz, H-4 (C)], 4.73br [d, *J ca.* 9.5 Hz, H-4 (I)], 3.84, 3.835, 3.81, 3.79, 3.76, 3.74, 3.71, 3.66, 3.39br, 2.95br (s, 10 × OCH_3), 3.03 [dd, *J ca.* 5.0 and 16.0 Hz, H-4*eq* (F)], 1.85 [s, 3-$COCH_3$ (F)], 1.67 [s, 3-$COCH_3$ (I), and 1.63 [s, 3-$COCH_3$ (C)]; δ[360 MHz, $(CD_3)_2SO$, 24 °C] 3.57, 3.50 (s, OCH_3), 3.40, 3.36 (s, OCH_3), 2.98, 2.93 (s, OCH_3), 1.85, 1.81 (s, $COCH_3$), 1.70, 1.66 (s, $COCH_3$), and 1.53, 1.43 (s, $COCH_3$); T_c [$(CD_3)_2SO$, 80 MHz] 100 °C, $\Delta G^{\ddagger}_{rot.}$ [$(CD_3)_2SO$, 80 MHz] 19.3 kcal mol^{-1}.

(2R,3S)-2,3-trans-3-*Acetoxy*-6-[(2R,3S,4R)-2,3-trans-3,4-cis-3-*acetoxy*-3′,4′,7-*trimethoxyflavan*-4-*yl*]-8-[(2R,3S,4S)-2,3-trans-3,4-trans-3-*acetoxy*-3′,4′,7-*trimethoxyflavan*-4-*yl*]-

* Small differences in the temperature-dependent chemical shifts of resonances between 360 and 80 MHz (*cf.* Table) are also due to relative differences in overcoming activation energies for ' fast ' rotation at different magnetic field strengths.

3',4',5,7-*tetramethoxyflavan* (6c).—The R_F 0.32 fraction gave the 3,4-*cis*-decamethyl ether triacetate as a *colourless solid* (51 mg, 2.44%) (Found: C, 66.9; H, 6.1. $C_{61}H_{64}O_{19}$ requires C, 66.5; H, 5.9%); δ[360 MHz, $(CD_3)_2SO$, 210 °C] 7.05—6.72 [m, 9 × H (B, E and H)], 6.69 [d, *J* 8.0 Hz, H-5 (A)], 6.58 [d, *J* 2.6 Hz, H-8 (G)], 6.51 [dd, Σ*J* *ca.* 11 Hz, H-6 (G)], 6.47 [dd, *J* 2.6 Hz, H-6 (A)], 6.36 [d, *J* 2.3 Hz, H-8 (A)], 6.11 [t, Σ*J* 19.5 Hz, H-3 (C)], 5.53 [dd, Σ*J* 16.5, $J_{2,3}$ 9.5, $J_{3,4}$ 7.0 Hz, H-3 (I)], 5.38 [d, *J* 9.5 Hz, H-2 (I)], 5.02br m, H-2 + 3 (F)], 4.91 [d, *J* 9.5 Hz, H-4 (C)], 4.90 [d, *J* 10.0 Hz, H-2 (C)], 4.74br [m, H-4 (I)], 3.80, 3.79, 3.78, 3.75, 3.72, 3.70, 3.67, 3.43br, 2.81br (s, 10 × OCH_3), 2.93 [dd, *J* 5.0 and 16.0 Hz, H-4*eq* (F)], 2.84 [dd, *J* 8.0 and 16.0 Hz, H-4*ax* (F)], 1.89 [s, 3-$COCH_3$ (F)], 1.65 [s, 3-$COCH_3$ (I)], and 1.58 [s, 3-$COCH_3$ (C)]; δ[360 MHz, $(CD_3)_2SO$, 37 °C] 3.65, 3.63 (s, OCH_3), 3.55, 3.39 (s, OCH_3), 2.93, 2.92 (s, OCH_3), 1.89, 1.88 (s, $COCH_3$), 1.60, 1.57 (s, $COCH_3$), 1.56, 1.51 (s, $COCH_3$); T_c [$(CD_3)_2SO$, 80 MHz] 63 °C, $\Delta G^{\ddagger}_{rot.}$ $(CD_3)_2SO$, 80 MHz] 18.3 kcal mol^{-1}.

Condensation of (2R,3S,4R)-2,3-trans-3,4-trans-*Flavan*-3,3',4,4',7-*pentaol* [(1), (+)-*Mollisacacidin*] *with* (2R,3S)-2,3-trans-8-[(2R,3S,4R)-2,3-trans-3,4-cis-3,3',4',7-*Tetrahydroxyflavan*-4-*yl*]*flavan*-3,3',4',5,7-*pentaol* {(4a), [4,8]-3,4-cis-(−)-*Fisetinidol*-(+)-*catechin*}.—(+)-Mollisacacidin (870 mg, 3 mmol) and [4,8]-3,4-*cis*-(−)-fisetinidol-(+)-catechin * (1.57 g, 8 mmol) were condensed in 0.1M-HCl (70 ml). Extraction of the solution with ethyl acetate (8 × 100 ml) gave the phenolic product (2.22 g) which was separated by p.l.c. (benzene–acetone–methanol, 6 : 3 : 1 v/v × 3) into three fractions, R_F 0.33 (117 mg), 0.26 (832 mg), and 0.19 (380 mg). Methylation of fraction R_F 0.26, 832 mg, for 24 h with diazomethane gave after p.l.c. separation in benzene–acetone (7 : 3 v/v) two products, R_F 0.37 (253 mg) and 0.30 (74 mg). Acetylation of the R_F 0.37 (253 mg) methyl ether fraction followed by p.l.c. separation in 1,2-dichloroethane–acetone (19 : 1 v/v, × 2) gave two methyl ether acetate fractions of R_F 0.34 (74 mg) and 0.26 (118 mg).

(2R,3S)-2,3-trans-3-*Acetoxy*-6-[(2R,3S,4S(-2,3-trans-3,4-trans-3-*acetoxy*-3',4',7-*trimethoxyflavan*-4-*yl*]-8-[(2R,3S,4R-2,3-trans-3,4-cis-3-*acetoxy*-3',4',7-*trimethoxyflavan*-4-*yl*]-3',4',5,7-*tetramethoxyflavan* (7c).—The R_F 0.26 fraction gave the decamethyl ether triacetate as a *colourless solid* (136 mg, 4.45%) (Found: C, 66.8; H, 6.1. $C_{61}H_{64}O_{19}$ requires C, 66.5; H, 5.9%; δ[80 MHz, $(CD_3)_2SO$, 170 °C] 6.97—6.01 (m, 15 × arom. H), 5.83 [t, ΣJ_s 18.5 Hz, H-3 (I)], 5.40 [t, ΣJ_s 14.0 Hz, H-3 (C)], 5.08 [d, $J_{2,3}$ 7.0 Hz, H-2 (C)], 5.00 [m, H-3 (F)], 4.81 [d, $J_{2,3}$ 9.25 Hz, H-2 (I)], 4.69br [d, $J_{3,4}$ 7.0 Hz, H-2 (F)], 4.61 [split d, $J_{3,4}$ 9.5 Hz, $J_{benzylic}$ 1.0 Hz, H-4 (I)], 4.33br [d, $J_{2,3}$ *ca.* 6.7 Hz, H-4 (C)], 3.68, 3.65, 3.63, 3.62, 3.59, 3.58, 3.53, 3.36br, 3.19br, 2.85br (s, 10 × OCH_3), 3.03—2.66 (m, CH_2), 1.77 [s, 3-$COCH_3$ (F)], 1.61 [s, 3-$COCH_3$ (I)], and 1.56 [s, 3-$COCH_3$ (C)].

(2R,3S)-2,3-trans-3-*Acetoxy*-6,8-*bi*-[(2R,3S,4R)-2,3-trans-3,4-cis-3-*acetoxy*-3',4',7-*trimethoxyflavan*-4-*yl*]-3',4',5,7-*tetramethoxyflavan* (8c).—The R_F 0.34 fraction gave the decamethyl ether triacetate as a *colourless solid* (85 mg, 2.76%) (Found: C, 66.2; H, 6.0. $C_{61}H_{64}O_{19}$ requires C, 66.5; H, 5.9%); δ[80 MHz, $(CD_3)_2SO$, 170 °C] 6.97—6.06 (m, 15 × arom. H), 5.44 [t, ΣJ_s 14.1 Hz, H-3 (C)], 5.36 [dd, ΣJ_s 15.0 Hz, H-3 (I)], 5.13 [d, $J_{2,3}$ 8.75 Hz, H-2 (I)], 5.06 [d, $J_{2,3}$ 7.0 Hz, H-2 (C)], 5.03 [m, ΣJ_s 16.5 Hz, H-3 (F)], 4.69 [d, $J_{2,3}$ 6.25 Hz, H-2 (F)], 4.65br [d, $J_{3,4}$ 7.0 Hz, H-4 (C)], 4.41br [d, $J_{2,3}$ *ca.* 6.0 Hz, H-4 (I)], 3.66, 3.65, 3.63, 3.625, 3.622 (× 2), 3.58, 3.55, 3.23br, 3.08br (s, 10 × OCH_3), 2.82 (d, CH_2), 1.80 [s, 3-$COCH_3$ (F)], 1.65 [s, 3-$COCH_3$ (I)], and 1.59 [s, 3-$COCH_3$ (C)].

Acetylation of the R_F 0.30 (74 mg) methyl ether fraction followed by p.l.c. separation (× 2) in benzene–acetone (9 : 1 v/v) gave a single product, R_F 0.23, as a *colourless solid* (11 mg, 0.4%). ^1H N.m.r. spectroscopy showed it to be the all-*trans*-[4,8]-decamethyl ether triacetate (5c). This compound presumably has its origin in the low percentage of [4,6]-all-*trans*-(−)-fisetinidol-(+)-catechin associated with the parent [4,8]-3,4-*cis*-(−)-fisetinidol-(+)-catechin.[9]

Significant ions in the mass fragmentation spectra of the decamethyl ether triacetates (5c)—(8c) and their respective relative abundance are *m/e* 1 100 (M^+, 3.6, 7.7, 2.0, 1.3%), 1 041 (71, 68, 5.0, 9.2), 1 040 (78, 77, 8.3, 8.2), 1 010 (71, 60, 13.0, 11.5), 1 009 (73, 77, 22, 12.4), 982 (48, 29, 6.5, 4.3), 981 (72, 59, 7.2, 5.5), 980 (75, 67, 7.2, 4.8), 921 (64, 32, 4.8, 2.3), 920 (39, 20, 3.6, 1.8), 878 (1.7, 2.3, —, —), 847 (9.7, 23, 10.4, 7.7), 743 (29, 76, 21, 8.6), 387 (10.4, 5.6, 2.3, 1.2), 357 (10.0, 22, 5.6, 4.3), 297 (70, 76, 40, 19.4), 222 (7.0, 19.8, 3.5, 4.7), 180 (70, 79, 48, 35), 165 (45, 55, 32, 23), 151 (100, 100, 100, 100), and 137 (47, 32, 19.2, 16.6).

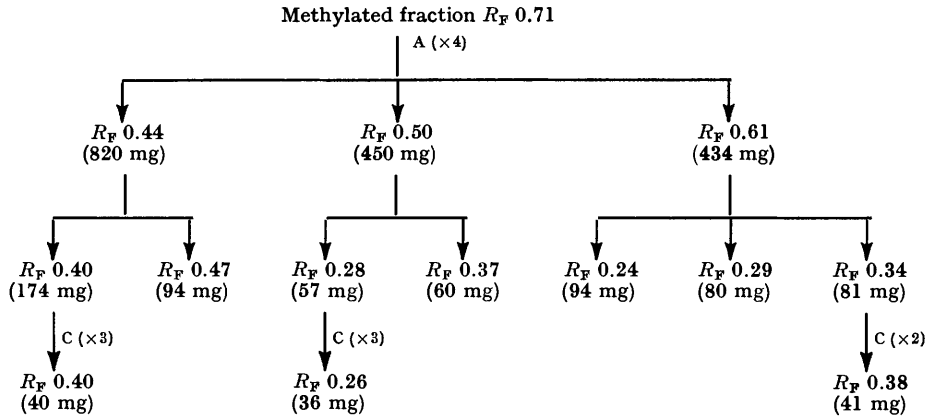
Isolation of Bi- and Tri-flavanoid Profisetinidins from the Heartwood of Colophospermum mopane.—Two-way paper chromatography of the methanol-soluble heart-wood extracts of *C. mopane* in water-saturated butan-2-ol and 2% acetic acid (upward migrations) show the dominant components as (in decreasing order of mobility in the second system): (+)-2,3-*trans*-3,4-*cis*-flavan-3,3',4,4',7-pentaol [14] (R_F 0.67, 0.68), (−)-fisetinidol [14] (0.76, 0.58), (+)-epifisetinidol [14] (0.72, 0.46), a biflavanoid (0.55, 0.41), bi- and triflavanoids (0.68, 0.30), and mopanols-peltogynols (0.55—0.68, 0.10). The relative concentrations of bi- and triflavanoids increase from the sapwood-heartwood interface to the heartwood centre.

Drillings (5.5 kg) from the central heartwood, extracted over a period of 5 days with daily renewal of the solvent, gave a brown solid (326 g). The complex mixture was partitioned in a counter-current system (ethyl acetate–benzene–water, 9 : 1 : 10 by vol.; 10 transfers) and the contents grouped as follows: tubes 2—6 (upper and lower phases) and 8—10 (upper phases only). The solids (40.5 and 19.0 g respectively) recovered from the enriched fractions were purified by preparative paper chromatography (Whatman No. 3 : 18½ × 22¼ in) using the same solvent systems but in reverse sequence, and applying 200 mg per sheet for 2% acetic acid (upward migration) and 50 mg for water-saturated butan-2-ol (downwards). The appropriate bands were cut and eluted with 70% ethanol in each instance; the 40.5 g fraction gave 8.4 g, R_F 0.50; while the 19.0 g fraction gave 10.5 g, R_F 0.40 in the former (2% acetic acid) system, and subsequently the products, 8.4 g (0.50) gave 2.2 g, R_F 0.60, and 10.5 g (0.40) gave 6.3 g, R_F 0.71, in water-saturated butan-2-ol.

Alkali fusion of the R_F 0.60 and 0.71 phenolic fractions under anhydrous conditions with KOH [15] each gave β-resorcylic acid, protocatechuic acid, resorcinol, and phloroglucinol.

Derivatives of (2R,3S,4S:2R,3S)-*All*-trans-[4,8]-(−)-*fisetinidol*-(+)-*catechin*.—The phenolic fraction R_F 0.60 (2.2 g) was methylated with an excess of diazomethane, and the products subjected to p.l.c. (benzene–acetone, 7 : 3 v/v). The fraction of highest concentration (R_F 0.25, purple with formaldehyde–sulphuric acid spray) gave a non-crystalline colourless solid (70 mg), m.p. 110 °C, $[\alpha]_D^{28}$ −12.8° (*c*, 0.7 in

* Containing 1—5% of the [4,6]-all-*trans*-(−)-fisetinidol-(+)-catechin as contaminant.[2]



acetone) (Found: C, 67.3; H, 6.1. $C_{37}H_{40}O_{11}$ requires C, 67.3; H, 6.0%), *m/e* 660 (M^+).

The heptamethyl ether (40 mg) was acetylated and the resultant diacetate subjected to p.l.c. (benzene–acetone, 8 : 1 v/v). The product (R_F 0.42, 28 mg) was a non-crystalline solid, m.p. 103 °C, $[\alpha]_D^{28}$ −136.5° (*c*, 0.4 in acetone), M^+ 774, with ^{1}H n.m.r. and c.d. spectra identical with those of the synthetic products.[2]

Methylation of the phenolic fraction R_F 0.71 (6.0 g) gave a complex mixture of methyl ether derivatives which required a series of multiple development p.l.c. separations in the solvent systems: A, ethyl methyl ketone–toluene (6 : 4 v/v); B, benzene–acetone (8 : 3 v/v); and C, benzene–methanol (9 : 1 v/v) in order to obtain their desired purity (*cf.* Scheme).

Derivatives of (2R,3S,4R:2R,3S)-*all*-trans-[4,6]-*bi*-[(−)-*fisetinidol*].—Fraction R_F 0.38 (*cf.* Scheme), the hexamethyl ether, was isolated as a non-crystalline colourless solid, m.p. 109 °C, $[\alpha]_D^{28}$ −55.0° (*c*, 0.4 in acetone) (Found: C, 68.7; H, 6.2. $C_{36}H_{38}O_{10}$ requires C, 68.6; H, 6.0%).

Acetylation gave the hexamethyl ether diacetate as a non-crystalline colourless solid, m.p. 105 °C, $[\alpha]_D^{28}$ −41.5° (*c*, 0.7 in acetone) (Found: C, 68.8; H, 6.1. $C_{40}H_{42}O_{12}$ requires C, 67.3; H, 5.9%), M^+ 714; ^{1}H n.m.r. and c.d. spectra were identical with those of the synthetic derivative.[2]

(2R,3S,4S:2R,3S:2R,3S,4S)-[4,6:4,8]-*All*-trans-*bi*-[(−)-*fisetinidol*]-(+)-*catechin* (5a).—The fraction R_F 0.26 (*cf.* Scheme), the decamethyl ether (5b), was obtained as a non-crystalline *colourless solid*, m.p. 163 °C, $[\alpha]_D^{28}$ −134.0° (*c*, 0.3 in acetone) (Found: C, 67.6; H, 6.1. $C_{55}H_{58}O_{16}$ requires C, 67.8; H, 5.9%), M^+ 974.

Acetylation gave the decamethyl ether triacetate (5c) as a non-crystalline colourless solid, m.p. 135 °C, M^+ 1 100; ^{1}H n.m.r. and c.d. spectra were identical with those of the corresponding all-*trans* derivative of the synthetic triflavanoid (5a).

(2R,3S,4R:2R,3S:2R,3S,4S)-[4,6:4,8]-2,3-trans-3,4-cis:2,3-trans:2,3-trans-3,4-trans-*Bi*-[(−)-*fisetinidol*]-(+)-*catechin* (6a).—The fraction R_F 0.40 (*cf.* Scheme), representing the decamethyl ether (6b), was a non-crystalline *colourless solid*, m.p. 143 °C, M^+ 974.

Acetylation gave the 3,4-*cis*-decamethyl ether triacetate (6c) as a non-crystalline colourless solid, m.p. 136 °C, $[\alpha]_D^{28}$ +5.6° (*c*, 0.4 in acetone) (Found: C, 66.9; H, 6.1. $C_{61}H_{64}O_9$ requires C, 66.6; H, 5.8%), M^+ 1 100; ^{1}H n.m.r. and c.d. spectra were identical with those of the corresponding derivative of the isomeric synthetic 3,4-*cis*-triflavanoid (6c).

Isolation of the complete series of triflavanoid diastereoisomers (5a)—(8a) from the heartwood of *Acacia mearnsii* is detailed in Part 6.[16]

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