Synthesis of Condensed Tannins. Part 15.† Structure of Natural 'Angular' Profisetinidin Tetraflavanoids: Asymmetric Induction During Oligomeric Synthesis

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Synthetic sequencing combined with the application of diagnostic chemical shifts of heterocyclic protons available from high-temperature n.m.r. spectroscopy permit the structural elucidation of the tridecamethyl ether tetra-acetates of four [4,6:4,8]-bi-(−)-fisetinidol-[4,6]-(−)-fisetinidol-(+)-catechin diastereoisomers derived from a fraction of the heartwood extract of *Acacia mearnsii*. In terms of the synthetic analogy these 'angular' profisetinidin tetraflavanoids arise from regiospecific 6-substitution on the 'upper' [4,8]-(−)-fisetinidol substituent on the (+)-catechin moiety of the triflavanoid analogue. Asymmetric induction by the nucleophilic substrate apparently directs the final step in each biomimetic condensation.

Occurrence of 'angular' [4,6:4,8]-triflavanoid profisetinidins and prorobinetinidins in the heartwoods and bark of the Leguminosae and Anacardiaceae has been demonstrated by us repeatedly, and proof of structure provided by direct synthesis in conjunction with ^{1}H n.m.r. spectroscopy.[1–4] Both the natural and synthetic substitution sequences on (+)-catechin [or (+)-gallocatechin] in these instances appear to be [4,8] followed by [4,6], regioselectivity being largely dictated by steric factors. Our continued interest in the 'angular' triflavanoids centres on the mode of the next step in the condensation sequence leading to higher oligomers. Our isolation of four tetraflavanoid profisetinidins from the heartwood of the black wattle tree (*Acacia mearnsii*) where they occur in association with four 'angular' triflavanoid analogues, therefore, presents an opportunity for examining this aspect. However, at ambient temperatures ^{1}H n.m.r. spectra of the tridecamethyl ether tetra-acetate of only one of the tetraflavanoids (**6**) gave sharply-defined resonances (*cf.* Figure 1) indicating arrested rotation about three interflavanoid bonds. Since the corresponding high resolution spectra of the remainder were line-broadened over a wide temperature range, it was obvious that the various bonding positions, the stereochemistry at C-4 of each flavanoid unit, the sequence of units and hence the locality of the biflavanoid moiety at either the 6 or 8 positions on the (+)-catechin unit could only be established by recourse to condensations of mono- and bi-flavanyl units of known stereochemistry. Among the attempted approaches, condensation of (+)-mollisacacidin trimethyl ether with [4,8]-(−)-fisetinidol-(+)-catechin biflavanoids[5] (Schemes 1 and 2) led directly to derivatives of the four natural tetraflavanoids obtained from the heartwood of the black wattle tree (*Acacia mearnsii*).

The problem of severely line-broadened ^{1}H n.m.r. spectra of the tetraflavanoid derivatives due to slow rotation (n.m.r. timescale) about the interflavanoid bonds as cited above was overcome by resorting to a combination of low frequency and high temperature (80 MHz; 170 °C) when the heterocyclic systems could be differentiated by spin-tickling experiments. Under these conditions high temperature shift parameters based on [4,6]- and [4,8]-bi- and 'angular' [4,6:4,8]-triflavanoid analogues[4] were valid, and in particular 2-H and 3-H shifts could be used to identify [4,8]-(2*R*)-3,4-*cis* substituents on (+)-catechin, and also to differentiate between 6-substitution of 3,4-*trans* units on (−)-fisetinidol and (+)-catechin moieties.[4,6] Coupling constants of 3,4-*trans*- and 3,4-*cis*-flavanyl units attached to (+)-catechin were consistent with those of the bi- and triflavanoid profisetinidins,[4] while 'terminal' 3,4-*cis*-(−)-fisetinidol units in bifisetinidol substituents in the tetraflavanoids show 'abnormal' coupling constants consistent with those in bi- and tri-flavan-3,4-diol profisetinidins.[6]

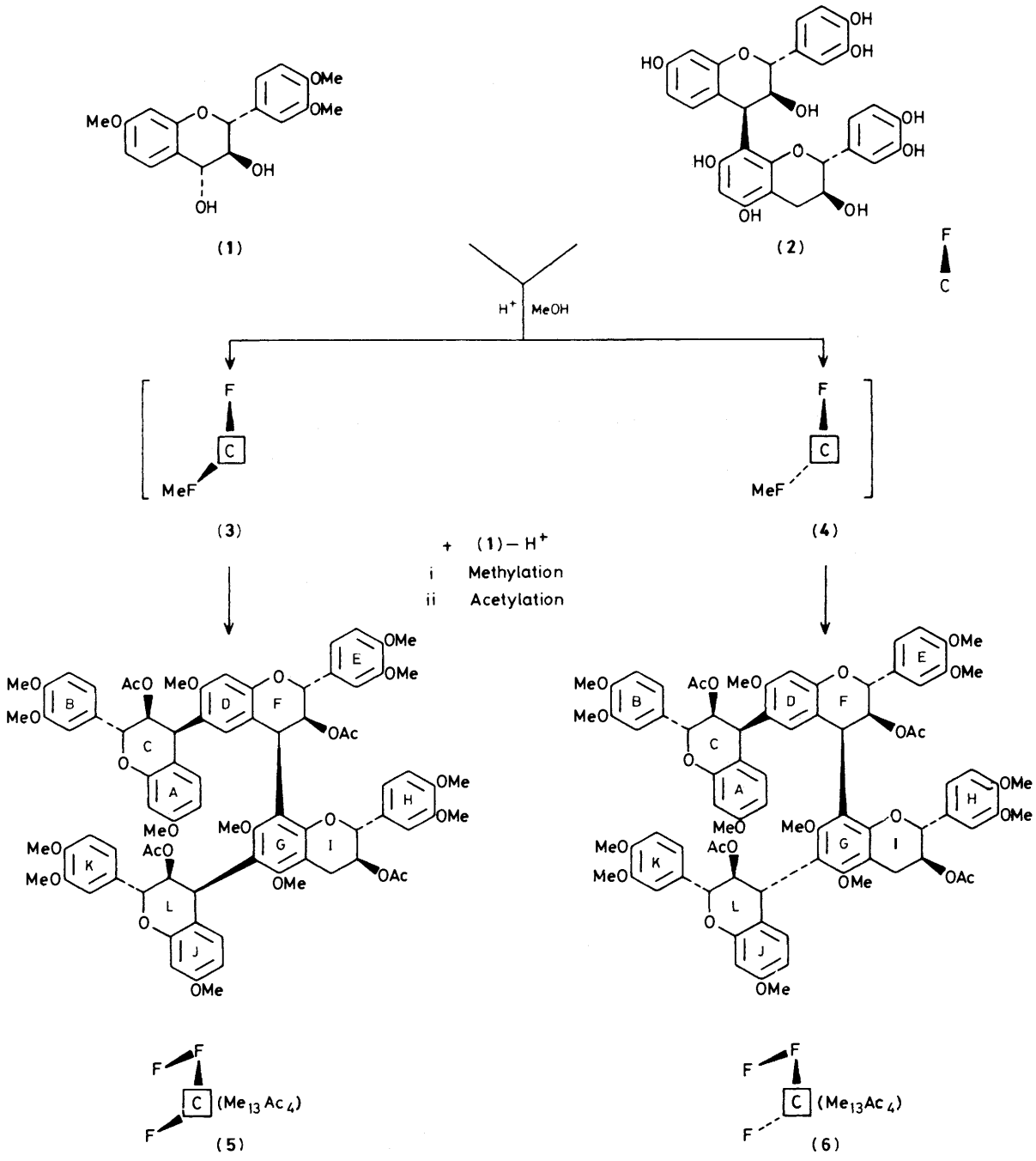
Condensation of (+)-*Mollisacacidin Trimethyl Ether* (**1**) *with* [4,8]-(−)-*Fisetinidol*-(+)-*catechin Biflavanoids* (**2**) *and* (**7**).— Condensation of the trimethyl ether of (+)-mollisacacidin (**1**) with [4,8]-3,4-*cis*-profisetinidin (**2**) gives two, (**5**) and (**6**), of the four theoretically possible profisetinidin tetraflavanoids. These products are identical to two of those obtained from the heartwood of *Acacia mearnsii* following methylation and acetylation of the tetraflavanoid fraction. Initially, preferential substitution at C-6 of the (+)-catechin moiety of the free-phenolic biflavanoid is likely[2,4] (*cf.* isolation of intermediates in the condensation represented in Scheme 2) to give the triflavanoid intermediates (**3**) and (**4**).‡ Since the 'nucleophilicity' at C-6 of the introduced (−)-fisetinidol trimethyl ether units is reduced as a consequence of etherification, further condensation is restricted to C-6 of the free-phenolic [4,8]-(−)-fisetinidol moieties to yield the tetraflavanoids (**5**) and (**6**). This approach introduces stereochemical variables at two points of interflavanoid bonding.

The high-temperature (197 °C) 500 MHz ^{1}H n.m.r. spectrum of the all-3,4-*cis*-tetraflavanoid derivative (**5**) exhibits an overlapping triplet [δ 5.504, $J_{2,3} = J_{3,4}$ = 8.0 Hz, 3-H(F)] and doublet of doublets [δ 5.496, $J_{2,3}$ 9.0, $J_{3,4}$ 7.0 Hz, 3-H(L)] at higher field and also a narrow doublet of doublets [δ 5.314, $J_{2,3}$ 6.0, $J_{3,4}$ 4.5 Hz, 3-H(C)]. These shifts and coupling constants correlate with two 2,3-*trans*-3,4-*cis*-profisetinidin units bonded directly to (+)-catechin[4] and a 'terminal' unit of similar configuration in the [4,8]-bi-(−)-fisetinidol moiety[6] respectively, and hence with an all-3,4-*cis* configuration of profisetinidin substituents.

By comparison the ^{1}H n.m.r. spectrum of the corresponding

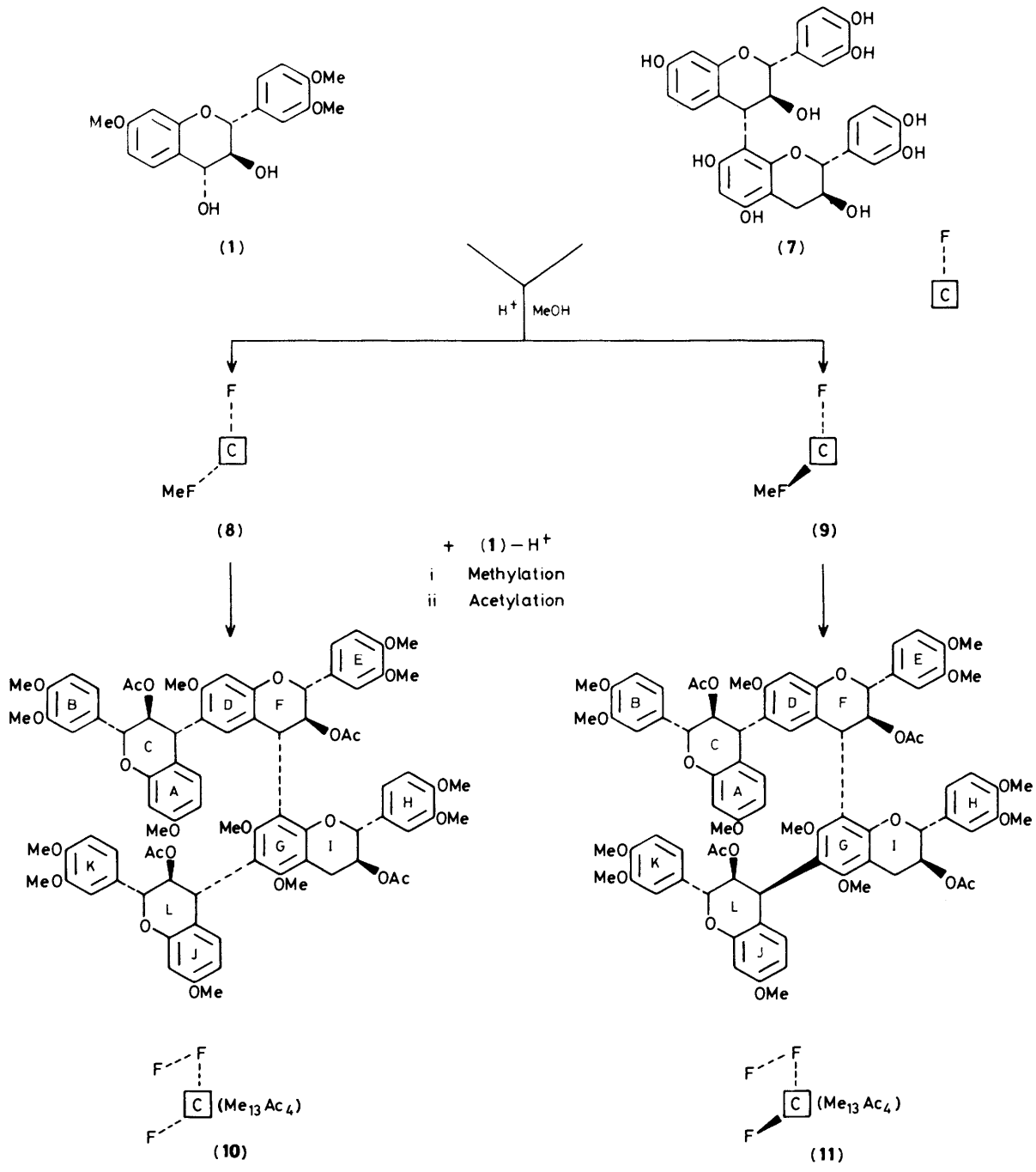
† Part 14 is the preceding paper.

‡ Note the abbreviated [4,6]- and [4,8]-3,4-*cis* (**4**β) and 3,4-*trans* (**4**α) stereochemical notation in Scheme 1.



Scheme 1.

[4,6]-3,4-*trans*-diastereoisomer (**6**) is unique among the natural proanthocyanidins of high mass in that sharply-defined and unduplicated resonances are observed at ambient temperatures (*cf.* Figure 1). A strongly coupled and deshielded triplet [δ 6.14, $J_{2,3} = J_{3,4}$ = 9.75 Hz, 3-H(L)] indicates direct substitution of a 3,4-*trans*-profisetinidin unit on the (+)-catechin moiety, but further interpretation of the heterocyclic proton resonances from the 80 MHz (30 °C) spectrum presented difficulty due to their overlap, while progressive line-broadening occurs with temperature elevation. At high magnetic field strength (12.5 T; 500 MHz; 30 °C), however, the exceptionally sharp (Gaussian-enhanced) and well-resolved spectra (Figure 1) permit definition of the low-field heterocyclic 3-H(L) triplet (δ 6.14) and also two doublets of doublets [δ 5.49, $J_{2,3}$ 10.0, $J_{3,4}$ 6.8 Hz, 3-H(F) and δ 5.41, $J_{2,3}$ 4.75, $J_{3,4}$ 3.75 Hz, 3-H(C)], the latter pair representing 2,3-*trans*-3,4-*cis*-profisetinidin units, with the narrow couplings at δ 5.41 designating a terminal unit in the bi-(−)-fisetinidol moiety as before. The strongly shielded position of 2-H(I) (δ 4.13) and hence the large chemical shift difference ($\Delta\delta_{2\text{-H},3\text{-H}}$ 1.10) of I-ring protons correlates with the presence of a (2*R*)-2,3-*trans*-3,4-*cis*-profisetinidin unit at the 8-position of the (+)-catechin moiety.[4] Aromatic singlets at δ 5.66 [8-H(D)] and 6.55 [5-H(D)] confirm the [4,6]-junction betweeen profisetinidin units in the bifisetinidol moiety. Considerations of the synthetic methods, the above correlations, and spin-decoupling at 500 MHz permit allocation of all heterocyclic and aromatic protons as shown in Figure 1. The significance of the sharply defined ^{1}H n.m.r. spectra at ambient temperatures and the



Scheme 2.

stereochemical course of the condensations are discussed below.

Condensation of tri-*O*-methyl-(+)-mollisacacidin (**1**) with free-phenolic [4,8]-3,4-*trans*-(−)-fisetinidol-(+)-catechin (**7**) furnishes as in the previous instance, derivatives of two—(**10**) and (**11**)—out of a total of four possible tetraflavanoid diastereoisomers (*cf.* Scheme 2). The triflavanoid intermediates (**8**) and (**9**) were isolated from among the products after complete methylation and subsequent acetylation.

In the case of the tridecamethyl ether tetra-acetate of the all-*trans* diastereoisomer (**10**) the relative stereochemistry follows directly from the ^{1}H n.m.r. spectrum (80 MHz; 170 °C) in which three strongly coupled triplets [δ 5.92, 5.90, and 5.47, all with $J_{2,3} = J_{3,4} = 9.5$ Hz] are attributable to the 3-H resonances of the F-, L- and C-ring systems respectively. The two overlapping lowfield triplets (δ 5.92, 5.90) are similar to those of the all-*trans*-[4,6:4,8]-bi-(−)-fisetinidol-(+)-catechin 'angular' trimer[2] which constitutes the 'nucleus' of the tetramer. The remaining triplet (δ 5.47) possesses the same chemical shift as its counterpart in the [4,6]-bi-(−)-fisetinidol 'dimer'.[2,7]

Assignment of the remainder of the heterocyclic proton resonances was possible by means of spin tickling experiments. Mass spectrometry gave the $M^+ - 59$ peak [*m/z* 1 397 (0.5%)] as the ion of highest mass. The structure is defined by the above spectrometric evidence in conjunction with the method of synthesis.

The heterocyclic region of the ^{1}H n.m.r. spectrum (80 MHz; 170 °C) of the [4,6:4,8]-all-*trans*-bi-(−)-fisetinidol-[4,6]-3,4-

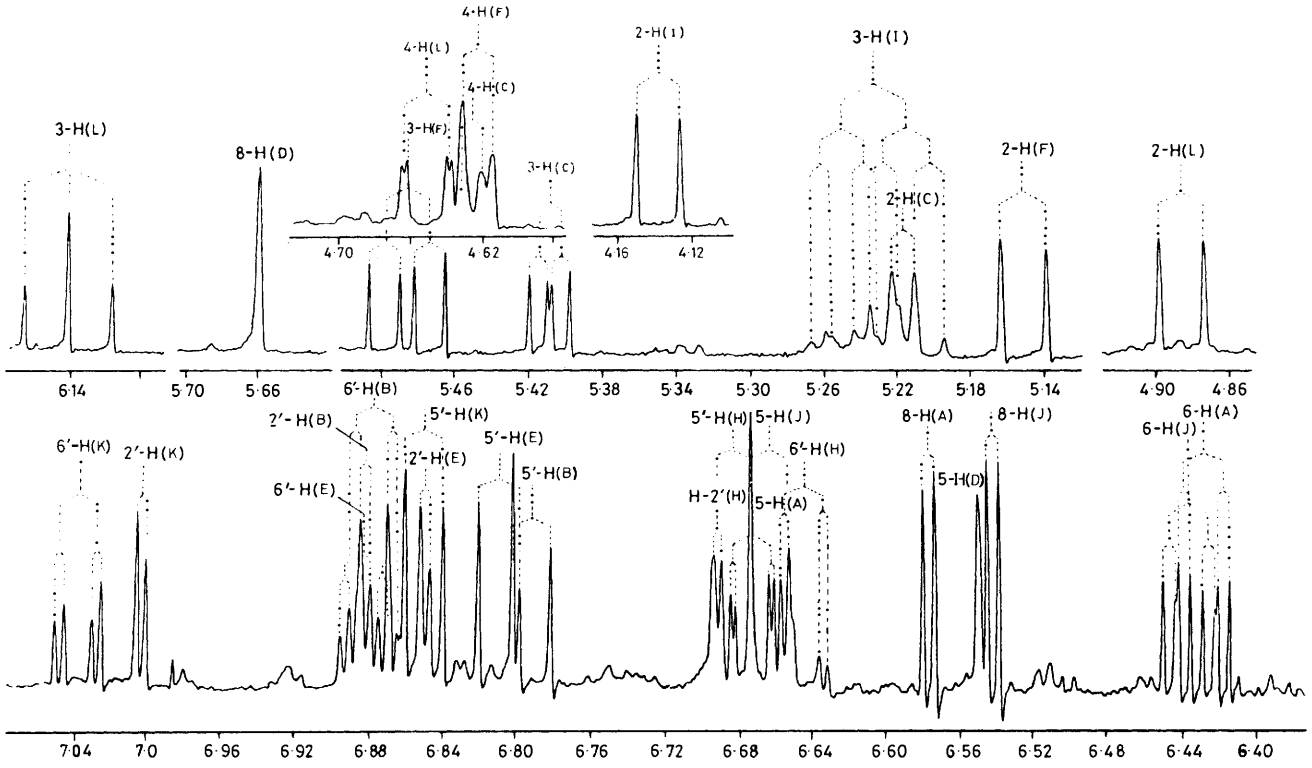
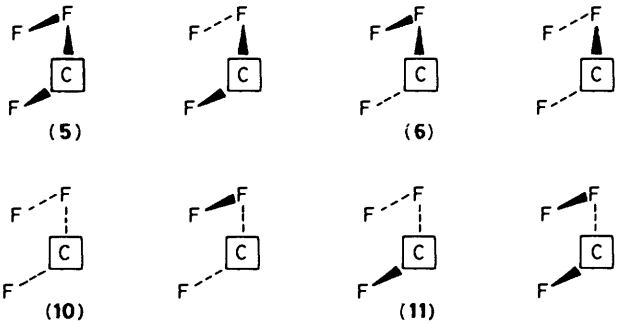


Figure 1. Heterocyclic and aromatic regions from the 500 MHz ^{1}H n.m.r. spectrum of [4,6:4,8]-bi-[2,3-*trans*-3,4-*cis*-(−)-fisetinidol]-[4,6]-2,3-*trans*-3,4-*trans*-(−)-fisetinidol-(+)-catechin tridecamethyl ether tetra-acetate (**6**) (31 °C; $CDCl_3$).

cis-(−)-fisetinidol-(+)-catechin diastereoisomer (**11**) exhibits a single lowfield triplet in the heterocyclic region [δ 6.06, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, 3-H(F)] and a similar triplet to higher field [δ 5.47, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 3-H(C)] which correlate with the presence of two units of 3,4-*trans*-configuration attached to the (+)-catechin moiety, and C-6 of a (−)-fisetinidol unit, and a doublet of doublets [δ 5.50, $J_{2,3}$ 8.0, $J_{3,4}$ 6.5 Hz, 3-H(K)] representative of a [4,6]-3,4-*cis*-(−)-fisetinidol substituent on (+)-catechin.[4] Absence of a shielded 2-H(I) resonance of the (+)-catechin moiety also places the aforementioned (2*R*)-3,4-*cis*-fisetinidol substituent at the 6-position.[4] Mass spectrometry again gives the $M^+ - 59$ ion (*m/z* 1 397, 0.5%) at highest mass.

The high-amplitude low wavelength Cotton effects in the c.d. spectra of the all-3,4-*cis* (**5**) and all-3,4-*trans* (**10**) tetraflavanoid derivatives (positive and negative respectively)[8–10] are almost mirror images presumably due to concerted (but opposing) contributions of chromophoric π–π* transitions around the C-4 chiral centres in each instance (*cf.* Figure 2). However, for those isomers of 'mixed' 3,4-stereochemistry the couplet (**6**) and high intensity doublet (**11**) at low wavelengths cannot be rationalized at present.

Synthetic and Biosynthetic Correlations for Profisetinidin Tetraflavanoids.—Of the eight possible (4,8)-biflavanoid-(4,6)-monoflavanoid-(+)-catechin profisetinidin 'tetramers' only four, (**5**), (**6**), (**10**), and (**11**), are formed preferentially by stepwise *in vitro* synthesis (Schemes 1 and 2), while those interposed in Scheme 3 (each pair differing at only one chiral centre) are either produced at low concentration or are absent from the reaction products. Thus in the final substitution step at C-6 of the 'upper' [4,8]-3,4-*cis*- and -3,4-*trans*-profisetinidin units of the 'angular' triflavanoid intermediates (**3**), (**4**), (**8**), and (**9**), the intromittent units adopt predominantly, if not exclusively, 3,4-*cis*- [(**5**), (**6**): Scheme 1] and 3,4-*trans*-configurations [(**10**), (**11**): Scheme 2]



( ) natural products

Scheme 3.

respectively. Such stereospecificity is the product of asymmetric induction effected in this instance* by the 'nucleophilic' triflavanoid substrate. Asymmetric induction is accordingly also invoked to rationalize the natural occurrence of only four tetraflavanoids in the heartwood of *Acacia mearnsii* in the presence of all four possible 'angular' triflavanoids [the free-phenolic equivalents of (**3**), (**4**), (**8**), and (**9**)] and the sustained excess of the potential electrophile, (+)-mollisacacidin.[2,6] The above conjecture, if valid, also implies regiospecificity during biogenesis of the tetraflavanoids, this aspect being controlled during *in vitro* biomimetic synthesis. Regioselectivity and asymmetric induction thus apparently combined to restrict the

* Asymmetric induction by an electrophile[11] is evident in the condensation of [4,6]-3,4-*cis*-(−)-fisetinidol-(+)-mollisacacidin hexamethyl ether triacetate with [4,6]- and [4,8]-(−)-fisetinidol-(+)-catechin synthons[5] to give 3,4-*trans* stereochemistry at the point of substitution.

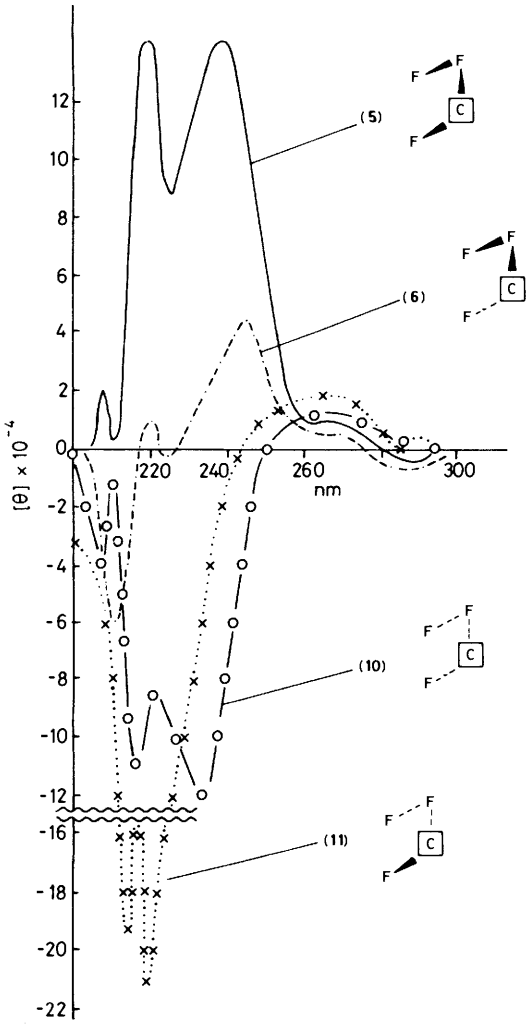


Figure 2. C.d. spectra of the tridecamethyl ether tetra-acetate derivatives (**5**), (**6**), (**10**), and (**11**) of the natural profisetinidin tetraflavanoids from *Acacia mearnsii* in methanol.

number of natural diastereoisomers formed, since a parallel phenomenon is observed in *Rhus lancea* (Anacardiaceae) for a (2*S*)-series of profisetinidin tetraflavanoids (*cf.* refs. 4, 12). The absolute stereochemistry of the four tetraflavanoids from *A. mearnsii* is as indicated in Schemes 1 and 2 from knowledge of the absolute stereochemistry of the participants during synthesis.

The sharply resolved ^{1}H n.m.r. spectrum provided by the [4,6:4,8]-3,4-*cis*-3,4-*cis*-bi-(−)-fisetinidol-[4,6]-3,4-*trans*-(−)-fisetinidol-(+)-catechin tridecamethyl ether tetra-acetate (**6**) at ambient temperatures (*cf.* Figure 1), and progressive line-broadening of resonance with temperature elevation indicates that this diastereoisomer requires high activation energy for rotation about its interflavanoid bonds ($\Delta G^{\ddagger}_{rot} > 20$ kcal mol^{-1}). This phenomenon which is unique among natural condensed tannins of this class represents the first instance in which considerations of helicity may in future be applied (*cf.* suggestions by Haslam and co-workers[13]); others being the unrelated biphenyl and *o*-terphenyl types of condensed tannins.[14]

Experimental

T.l.c. was performed on DC-Plastikfolin Kieselgel 60 PF_{254} (0.25 mm) and the plates sprayed with H_2SO_4–HCHO (40:1 v/v) after development. Preparative plates (p.l.c.) [Kieselgel PF_{254} (1.0 mm)] were air-dried and used without prior activation. Methylations were performed with an excess of diazomethane in methanol–diethyl ether at −15 °C for 48 h, while acetylations were carried out with acetic anhydride–pyridine. Evaporations were performed under reduced pressure at *ca.* 50 °C. ^{1}H N.m.r. spectra were recorded on Bruker WP-80, AM 300, and WH-500 FT instruments in $(CD_3)_2SO$ at 170 °C locked on the 'central' resonance (δ 2.49) of the protonated Me_2SO impurity, and in $CDCl_3$ at 19 and 31 °C with $SiMe_4$ as the internal standard, mass spectral data on a Varian CH-5 instrument, and circular dichroism (c.d.) on a JASCO J-20 spectropolarimeter. Analyses (C and H) were performed by Analytische Laboratorien, Fritz-Pregl-Strasse 24, 5270 Gummbersbach 1 Elbach, Germany.

Isolation of Tetraflavanoids as Derivatives from the Heartwood of Acacia mearnsii.—The wax-free extract[6] (2.14 kg) from the heartwood of the black wattle was separated in 20 g fractions on 5 × 125 cm cellulose columns (Solka-floc, Brown & Co., New Hampshire) with water as the eluant using 4 l per column. Fractions (100 ml) were collected from each column as follows and their contents combined. Fractions 1—5 [mainly (+)-mollisacacidin (170 g after crystallization)], fractions 6—13 [mainly bi- and tri-flavanoids (137 g)], and the subsequent 2.5 l eluant [mainly tri- and tetra-flavanoids (51.3 g)].

The tetraflavanoid fraction (48 g) was resolved into the following primary fractions (15 ml each) on two Sephadex LH-20 columns (140 × 4 cm) with ethanol–acetone (9:1 v/v) as the eluant to give the combined yields indicated: Fractions 18—40 [mainly triflavanoids (4.2 g)], 41—160 (11.7 g), 161—230 (4.7 g), and 231—280 (2.8 g).

P.l.c. separation of the main 41—160 fraction in benzene–acetone–methanol (6:3:1 v/v; × 2) gave two fractions at R_F 0.31 (2.33 g, triflavanoids) and 0.23 (2.31 g, tetraflavanoids). The latter fraction was methylated, purified by p.l.c. in benzene–acetone (7:3 v/v), R_F 0.28 (752 mg), and then acetylated. Separation of the acetate (400 mg) by similar means in benzene–acetone–methanol (90:9:1 v/v; × 2) gave two products at R_F 0.30 (98.5 mg) and 0.23 (123 mg).

(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,4R)-2,3-trans-3,4-cis-3-*acetoxy*-6-[(2R,3S,4S)-2,3-trans-3,4-cis-3-*acetoxy*-3′,4′,7-*trimethoxyflavan*-4-*yl*]-3′,4′,7-*trimethoxyflavan*-4-*yl*}-3′,4′,5,7-*tetramethoxyflavan* (**5**). The tridecamethyl ether tetra-acetate, R_F 0.30 (98.5 mg), of the all-3,4-*cis*-tetraflavanoid was isolated as a *colourless solid*, giving ^{1}H n.m.r., c.d., and mass spectra identical with those of its synthetic counterpart.

(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*-6-[(2R,3S,4S)-2,3-trans-3,4-cis-3-*acetoxy*-3′,4′,7-*trimethoxyflavan*-4-*yl*]-3′,4′,7-*trimethoxyflavan*-4-*yl*}-3′,4′,5,7-*tetramethoxyflavan* (**6**). The tridecamethyl ether tetra-acetate, R_F 0.23 (123 mg) of the [4,6]-3,4-*trans*-diastereoisomer of the all-3,4-*cis*-compound (**5**) was isolated as a *colourless solid* which gave ^{1}H n.m.r., c.d., and mass spectra identical with those of its synthetic counterpart.

Methylation of fractions 161—230 (2 g) from the Sephadex LH-20 column and p.l.c. separation in benzene–acetone–methanol (78:20:2 v/v; × 2) gave a single band, R_F 0.31 (376 mg). Acetylation and subsequent separation in benzene–acetone–methanol (90:8:2 v/v; × 5) gave two products, R_F 0.41 (69 mg) and 0.34 (44 mg).

(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*-6-[(2R,3S,4R)-2,3-trans-3,4-trans-3-*acetoxy*-3′,4′,7-*trimethoxyflavan*-4-*yl*]-3′,4′,7-*trimethoxyflavan*-4-*yl*}-3′,4′,5,7-*tetramethoxyflavan* (**11**). The tridecamethyl ether

tetra-acetate, R_F 0.41 (69 mg), was isolated as a *colourless solid* with ^{1}H n.m.r., c.d., and mass spectra identical with those of its synthetic counterpart.

(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,4S)-2,3-trans-3,4-trans-3-*acetoxy*-6-[(2R,3S,4R)-2,3-trans-3,4-trans-3-*acetoxy*3′,4′7-*trimethoxyflavan*-4-*yl*]-3′,4′,7-*trimethoxyflavan*-4-*yl*}-3′,4′,5,7-*tetramethoxyflavan* (**10**). The all-*trans* diastereoisomer, R_F 0.34 (44 mg), was isolated as a *colourless solid*, which gave ^{1}H n.m.r., c.d., and mass fragmentation spectra identical with those of its synthetic counterpart.

Synthesis of Derivatives of Natural Tetraflavanoid Profisetinidins

[4,8]-3,4-cis-(−)-*Fisetinidol*-(+)-*catechin* (**2**) *as the Substrate*.—The free-phenolic biflavanoid (562 mg, 1 mmol) and tri-*O*-methyl-(+)-mollisacacidin (**1**) (996 mg, 3 mmol) were dissolved in methanol (15 ml) and to this was added HCl (0.6 ml; 1M). The solution was kept at 50 °C for 5 days, concentrated to *ca.* 5 ml, and after addition of water (30 ml) the mixture was extracted with ethyl acetate (4 × 50 ml). The combined extracts were dried (Na_2SO_4), concentrated and then methylated with diazomethane. P.l.c. separation in benzene–acetone–methanol (78:20:2 v/v; × 2) gave a single product at R_F 0.28 (392 mg). Acetylation followed by p.l.c. separation in 1,2-dichloroethane–acetone (9:1 v/v; × 3) gave two products at R_F 0.45 (20.9 mg) and 0.40 (71.3 mg).

[4,6:4,8]-3,4-cis- 3,4-cis-*Bi*-(−)-*fisetinidol*-[4,6]-3,4-cis-(−)-*fisetinidol*-(+)-*catechin methyl ether acetate* (**5**). The tridecamethyl ether tetra-acetate, R_F 0.45 (20.9 mg) was isolated as a colourless solid (Found: C, 66.5; H, 5.9. $C_{81}H_{84}O_{25}$ requires C, 66.7; H, 5.8%); δ[$(CD_3)_2SO$; 500 MHz; 197 °C]* 7.08—6.33 (m, 20 × Ar H), 5.504 [t, 3-H(F), $J_{2,3} = J_{3,4} = 8.0$ Hz], 5.496 [dd, 3-H(L), $J_{2,3}$ 9.0, $J_{3,4}$ 7.0 Hz], 5.314 [dd, 3-H(C), $J_{2,3}$ 6.0, $J_{3,4}$ 4.5 Hz], *ca.* 5.3 [m, 3-H(I)], 5.280 [d, 2-H(L), $J_{2,3}$ 9.0 Hz], 5.276 [d, 2-H(C), $J_{2,3}$ 6.0 Hz], 5.270 [d, 2-H(F), $J_{2,3}$ 8.0 Hz], 5.134 [br d, 4-H(C), $J_{3,4}$ 4.5 Hz], 4.820 [d, 4-H(F), $J_{3,4}$ 8.0 Hz], 4.63 [br unresolved d,* 4-H(L)], 4.37 [br unresolved d,* 2-H(I)], 3.813, 3.798, 3.790, 3.780, 3.766 (× 2), 3.760(br), 3.752, 3.724, 3.716, 3.572(br), 3.194 (each s, 13 × OMe), 2.96 [br m, 4-H_{eq}(I)], 2.832 [br dd, 4-H_{ax}(I), *J* 7.5 and 15 Hz], and 1.855, 1.677, 1.630(br), 1.555(br) (each s, 4 × COMe); c.d. spectrum (*cf.* Figure 2).

[4,6:4,8]-3,4-cis-3,4-cis-*Bi*-(−)-*fisetinidol*-[4,6]-3,4-trans-(−)-*fisetinidol*-(+)-*catechin methyl ether acetate* (**6**). The tridecamethyl ether tetra-acetate, R_F 0.40 (71.3 mg), was isolated as a *colourless solid* (Found: C, 66.5; H, 5.9. $C_{81}H_{84}O_{25}$ requires C, 66.7; H, 5.8%); δ ($CDCl_3$; 500 MHz; 31 °C) 7.039 [dd, 6-H(K), *J* 2.0 and 8.0 Hz], 7.003 [d, 2-H(K), *J* 2.0 Hz], 6.882 [dd, 6-H(E), *J* 2.0 and 8.0 Hz], 6.880 [d, 2-H(B), *J* 2.0 Hz], 6.874 [dd, 6-H(B), *J* 2.0 and 8.0 Hz], 6.850 [d, 5-H(K), *J* 8.0 Hz], 6.849 [d, 2-H(E), *J* 2.0 Hz], 6.810 [d, 5-H(E), *J* 8.0 Hz], 6.790 [d, 5-H(B), *J* 8.0 Hz], 6.691 [d, 2-H(H), *J* 2.0 Hz], 6.685 [d, 5-H(H), *J* 8.0 Hz], 6.674 [dd, 5-H(A), *J* 0.9 and 8.1 Hz], 6.661 [dd, 5-H(J), *J* 1.0 and 8.2 Hz], 6.645 [dd, 6-H(H), *J* 1.8 and 8.0 Hz], 6.577 [d, 8-H(A), *J* 2.5 Hz], 6.550 [s, 5-H(D)], 6.542 [d, 8-H(J), *J* 2.3 Hz], 6.435 [dd, 6-H(J), *J* 2.5 and 8.5 Hz], 6.425 [dd, 6-H(A), *J* 2.5 and 8.5 Hz], 6.142 [t, 3-H(L), Σ*J* 19.5 Hz], 5.660 [br s, 8-H(D)], 5.485 [dd, 3-H(F), *J* 6.75 and 9.75 Hz], 5.410 [dd, 3-H(C), *J* 3.75 and 4.75 Hz], 5.235 [m, 3-H(I)], 5.215 [d, 2-H(C), *J* 4.75 Hz], 5.146 [d, 2-H(F), *J* 9.75 Hz], 4.885 [d, 2-H(L), *J* 9.6 Hz], 4.653 [dd, 4-H(L), *J* 1.0 and 9.75 Hz], 4.624 [d, 4-H(C), *J* 3.75 Hz], 4.623 [d, 4-H(F), *J* 6.75 Hz], 4.130 [d, 2-H(I), *J* 9.5 Hz], 3.886, 3.843, 3.831, 3.826, 3.808, 3.801, 3.793, 3.727, 3.706, 3.673, 3.643, 3.351, 2.931 (each s, 13 × OMe), 3.313 [dd, 4-H_{eq}(I), *J* 6.25 and 16.2 Hz], 2.601 [dd, 4-H_{ax}(I), *J* 9.5 and 16.2 Hz], and 1.769, 1,709, 1.627, 1.559 (each s, 4 × COMe); c.d. (*cf.* Figure 2).

[4,8]-3,4-trans-(−)-*Fisetinidol*-(+)-*catechin* (**7**) *as the Substrate*.—The free-phenolic biflavanoid (1.124 g, 2 mmol) and tri-*O*-methyl-(+)-mollisacacidin (**1**) (1.992 g, 6 mmol) were dissolved in methanol (30 ml) and the solution after treatment with HCl (1.25 ml; 1M) kept at 50 °C for 5 days. The reaction mixture was concentrated thereafter to *ca.* 10 ml, water (40 ml) added, and the solution was then extracted with ethyl acetate (4 × 80 ml). The combined extract was dried (Na_2SO_4), the solution evaporated and the products methylated with diazomethane.

P.l.c. separation of the methyl ethers eluting with 1,2-dichloroethane–acetone (8:2 v/v; × 2) gave two fractions at R_F 0.30 (220 mg) and 0.22 (320 mg), representing tri- and tetraflavanoid products respectively.

Acetylation of the former (R_F 0.30) fraction, p.l.c. separation eluting with hexane–acetone–ethyl acetate (50:35:15 v/v; × 3) of the product, R_F 0.59 (63.8 mg), and re-separation in benzene–acetone (9:1 v/v) gave two triflavanoid decamethyl ether triacetates at R_F 0.31 (14.6 mg) and 0.36 (21.3 mg). These compounds were identical with the corresponding derivatives of [4,6:4,8]-all-*trans*-bi-(−)-fisetinidol-(+)-catechin and its [4,6]-3,4-*cis*-isomer respectively[2,6] and hence indicative of the formation of the intermediates (**8**) and (**9**) (Scheme 2).

Acetylation of the latter (R_F 0.22) fraction, and successive p.l.c. separations with hexane–acetone–ethyl acetate (50:35:15 v/v; × 2) (R_F 0.37; 128 mg) and benzene–acetone (9:1 v/v; × 4) gave two fractions at R_F 0.35 (50.3 mg) and 0.29 (27.9 mg).

[4,6:4,8]-3,4-trans-3,4-trans-*Bi*-(−)-*fisetinidol*-[4,6]-3,4-cis-(−)-*fisetinidol*-(+)-*catechin methyl ether acetate* (**11**). The tridecamethyl ether tetra-acetate, R_F 0.35 (50.3 mg) was isolated as a *colourless solid* (Found: C, 66.6; H, 5.8. $C_{81}H_{84}O_{25}$ requires C, 66.7; H, 5.8%); δ [$(CD_3)_2SO$; 80 MHz; 180 °C] 6.97—6.34 (m, 20 × Ar H), 6.06 [t, 3-H(F), Σ*J* 19.0 Hz], 5.50 [dd, 3-H(L), *J* 6.75 and 8.0 Hz], 5.47 [t, 3-H(C), Σ*J* 20.0 Hz], 5.29 [d, 2-H(L), *J* 8.0 Hz], 5.03 [m, 3-H(I)], 4.84 [d, 2-H(C), *J* 10.0 Hz], 4.84 [d, 2-H(F), *J* 9.5 Hz], 4.81 [d, 2-H(I), *J* 7.0 Hz], 4.62 [d, 4-H(F), *J* 9.5 Hz], 4.37 [d, 4-H(C), *J* 10.0 Hz], 4.22 [d, 4-H(L), *J* 6.75 Hz], 3.75, 3.74, 3.73, 3.72, 3.71, 3.69, 3.68, 3.64 (× 2), 3.63, 3.55, 3.51 (× 2) (each s, 13 × OMe), 3.10 [dd, 4-H_{eq} (I), *J* 6.25 and 16.0 Hz], 2.74 [dd, 4-H_{ax} (I), *J* 8.75 and 16.0 Hz], and 1.83, 1.55, 1.52, 1.51 (each s, 4 × COMe); c.d. spectrum (*cf.* Figure 2).

[4,6:4,8]-3,4-trans-3,4-trans-*Bi*-(−)-*fisetinidol*-[4,6]-3,4-trans-(−)-*fisetinidol*-(+)-*catechin methyl ether acetate* (**10**). The tridecamethyl ether tetra-acetate, R_F 0.29 (27.9 mg), was isolated as a *colourless solid* (Found: C, 66.5; H, 5.9. $C_{81}H_{84}O_{25}$ requires C, 66.7; H, 5.8%); δ [$(CD_3)SO$; 80 MHz; 170 °C) 7.03—6.29 (m, 20 × Ar H), 5.92 [t, 3-H(F), Σ*J* 19.0 Hz], 5.90 [t, 3-H(L), Σ*J* 19.0 Hz], 5.47 [t, 3-H(C), Σ*J* 19.0 Hz], 5.04 [m, 3-H(I)], 4.91 [d, 2-H(L), *J* 9.5 Hz], 4.87 [d, 2-H(F), *J* 9.5 Hz], 4.82 [d, 2-H(C), *J* 9.5 Hz], 4.72 [d, 2-H(I), *J* 8.0 Hz], 4.72 [d, 4-H(L), *J* 9.5 Hz], 4.56 [d, 4-H(F), *J* 9.5 Hz], 4.34 [d, 4-H(C), *J* 9.5 Hz], 3.76 (× 2), 3.73 (× 3), 3.71 (× 2), 3.68, 3.66, 3.63, 3.63, 3.54, 3.48 (each s, 13 × OMe), 3.12 [dd, 4-H_{eq}(I), *J* 5.5 and 16.0 Hz], 2.70 [dd, 4-H_{ax}(I), *J* 8.75 and 16.0 Hz], and 1.80, 1.56, 1.55, 1.45 (each s, 4 × COMe); c.d. spectrum (*cf.* Figure 2).

Mass fragmentation spectra (relative abundances) of the tridecamethyl ether tetra-acetates of the tetraflavanoid profisetinidins (**5**), (**6**), (**10**), and (**11**) are respectively as follows: 1 456 (M^+ 0, 0, 0, 0%), 1 397 (0, 0, 0, 0.3), 1 396 (0, 0.5, 0.3, 0), 1 337 (0.3, 0.3, 0.7, 0), 1 175 (0.4, 0, 0, 0), 1 174 (0.2, 0, 0, 0), 743 (0.3, 0, 0, 0), 684 (0, 1.5, 0.5, 0), 683 (0, 1.5, 0.5, 0), 653 (0.4, 0, 0.1, 0), 623 (0.8, 0.7, 0, 0), 593 (1.3, 0, 0.9, 0.6), 537 (0, 0, 0.4, 0), 386 (0, 0, 0.2, 0), 357 (0.4, 0, 0.7, 0.4), 356 (0, 0, 0.2, 0), 327 (0.6, 0, 0.3, 0.2),

* ^{1}H n.m.r. spectra were also recorded at 80 MHz (170 °C) and 300 MHz (170 °C).

298 (3.5, 2.6, 1.6, 3.4), 297 (11.5, 9.3, 9.1, 6.5), 222 (10.1, 6.8, 11.7, 8.5), 180 (76.8, 64.6, 67.6, 56.0), and 151 (77.1, 62.5, 62.0, 56.4).

Acknowledgements

Support by the South African Council of Scientific and Industrial Research, Pretoria, the Marketing Committee of the South African Wattle Industry, and the Sentrale Navorsingsfonds of the University of the Orange Free State is acknowledged. Sections of the wood of *Acacia mearnsii* were kindly supplied by Mr. D. F. C. Garbutt, Chemistry Section of the Wattle Research Institute, Pietermaritzburg; 300 MHz ^{1}H n.m.r. spectra were recorded by Dr. E. V. Brandt of this Department and mass spectra by Dr. J. M. Steyn, Department of Pharmacology of this University.

References

1 J. J. Botha, D. Ferreira, D. G. Roux, and W. E. Hull, *J. Chem. Soc., Chem. Commun.*, 1979, 510.
2 J. J. Botha, P. M. Viviers, D. A. Young, I. C. du Preez, D. Ferreira, D. G. Roux, and W. E. Hull, *J. Chem. Soc., Perkin Trans. 1*, 1982, 527.
3 P. M. Viviers, J. J. Botha, D. Ferreira, D. G. Roux, and H. M. Saayman, *J. Chem. Soc., Perkin Trans. 1*, 1983, 17.
4 P. M. Viviers, H. Kolodziej, D. A. Young, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2555.
5 D. A. Young, A. Cronjé, A. L. Botes, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, preceding paper.
6 P. M. Viviers, D. A. Young, J. J. Botha, D. Ferreira, D. G. Roux, and W. E. Hull, *J. Chem. Soc., Perkin Trans. 1*, 1982, 535.
7 J. A. Steenkamp, D. Ferreira, D. G. Roux, and W. E. Hull, *J. Chem. Soc., Perkin Trans. 1*, 1983, 23.
8 J. J. Botha, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Chem. Commun.*, 1978, 698.
9 J. J. Botha, D. A. Young, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1213.
10 J. H. van der Westhuizen, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1220.
11 D. A. Young, D. Ferreira, and D. G. Roux, *J. Polym. Sci., Part* A-1. *Polymer Chem.*, in the press.
12 D. A. Young, H. Kolodziej, D. Ferreira, and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, following paper.
13 A. C. Fletcher, L. J. Porter, E. Haslam, and R. J. Gupta, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1628.
14 E. Jacobs, D. Ferreira, and D. G. Roux, *Tetrahedron Lett.*, 1983, 4627.

Received 22*nd October* 1984; *Paper* 4/1795