Synthesis of Condensed Tannins. Part 7.† Angular [4,6:4,8]-Prorobinetinidin Triflavanoids from Black Wattle (' *Mimosa* ') Bark Extract

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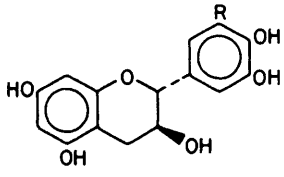
The triflavanoid fraction from the bark extract of the black wattle (*Acacia mearnsii*) comprises five angular prorobinetinidins with their constituent robinetinidol units [4,6:4,8]-linked to both (+)-catechin and (+)-gallocatechin. Synthetic proof of structure is provided for three bi-[(−)-robinetinidol]-(+)-catechin diastereoisomers. The complete dominance of prorobinetinidins in the higher oligomeric fractions correlates with the faster condensation rate of the parent (+)-leucorobinetinidin with the nucleophilic substrates compared with competing (+)-leucofisetinidin.‡

Black wattle (' Mimosa ') extract from the tannin-rich bark of *Acacia mearnsii* has been used universally as a tanning material for more than a century. However, in recent years the range of its industrial application has been progressively extended to the manufacture of depressants in ore flotation, flocculants in water treatment, various types of wood adhesives, and adhesives for packing materials.[1] These uses are all based on the 70% phenolic (' tannin ') content representing a molecular gradation over the mass range of 300 to 3 000 a.m.u., with a number-average mass of approximately 1 250 a.m.u.[2]

Amongst the simple flavonoids which collectively represent a relatively low percentage of the phenolic fraction, three flavan-3-ols, namely (+)-catechin (1), (+)-gallocatechin (2), and (−)-robinetinidol (4), predominate, while the flavan-3,4-diols (+)-leucofisetinidin (5) and (+)-leucorobinetinidin (6) occur at low concentrations.[3] The pair of phloroglucinol-type flavan-3-ols (+)-catechin and (+)-gallocatechin, as strongly nucleophilic substrates, and the aforementioned flavan-3,4-diols as potential electrophiles, represent likely participants in electrophilic substitution reactions as judged from biomimetic-type condensations.[4,5] The remaining flavonoids, mainly analogues of fisetinidin (7) and robinetinidin (8), all possess a 4-carbonyl function which not only precludes condensation at this point, but also deactivates their respective resorcinol A-rings towards electrophilic attack. Such selectivity in condensation is reflected by the isolation[4,5] of four [4,8]-linked biflavanoids, namely 2,3-*trans*-3,4-*trans*:- and 2,3-*trans*-3,4-*cis*:2′,3′-*trans*-(−)-fisetinidol-(+)-catechin (9) and (10) and 2,3-*trans*-3,4-*trans*:2′,3′-*trans*-(−)-robinetinidol-(+)-catechin (11) and 2,3-*trans*-3,4-*trans*:2′,3′-*trans*-(−)-robinetinidol-(+)-gallocatechin (12).§

The triflavanoid fraction, defined by two discrete areas designated as C and E on two-way paper chromatograms,[6,7] is readily separable from the remainder of the extract by preparative paper chromatography in 2% acetic acid, and hence into its ' homogeneous ' component units in butan-2-ol–water. The phenolic composition of the area of higher R_F in the latter system, component C, is demonstrated by its alkali fusion under anhydrous conditions[6] which gives resorcinol (also



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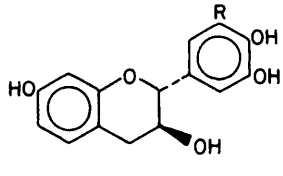
(2) R = OH



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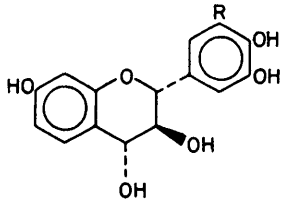

(5) R = H
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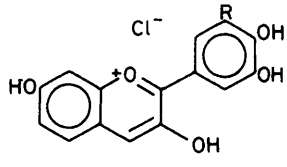
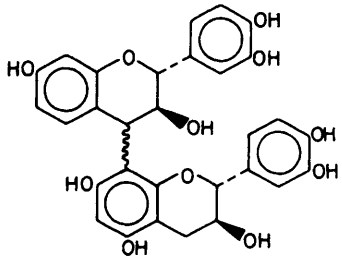
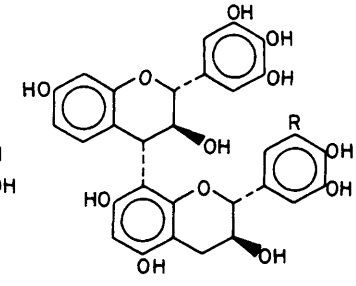


(7) R = H
(8) R = OH



(9) ⟨ = ⟩
(10) ⟨ = ▲



(11) R = H
(12) R = OH

† Part 6 is 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.

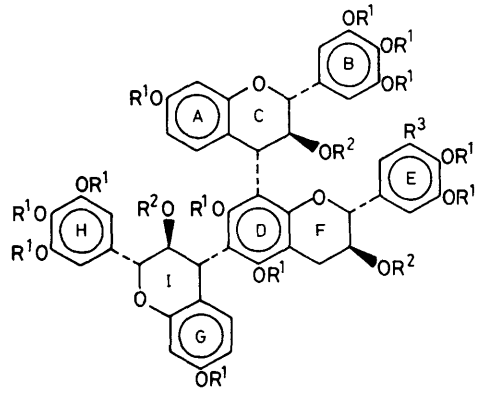
‡ In the following paper, (+)-leucofisetinidin (5) is called (+)-mollisacacidin.

§ Formula (3) is that of (−)-fisetinidol.

β-resorcylic acid), phloroglucinol, and gallic and protocatechuic acids, and by its hydrolysis with 3M HCl–propan-2-ol under pressure [8] giving robinetinidin chloride (8) [9] together with a trace of fisetinidin chloride (7). The chromatographically homogeneous triflavanoid area of lower R_F, component E, gives only resorcinol (and β-resorcylic acid), phloroglucinol, and gallic acids on fusion (*i.e.* no protocatechuic acid), and only robinetinidin chloride from acid hydrolysis. These degradation products are in line with the finding that component C consists of a mixture of three angular triflavanoid diastereoisomers constituted of two (−)-robinetinidol units attached to (+)-catechin, while component E consists of two bi-[(−)-robinetinidol]-(+)-gallocatechin diastereoisomers.

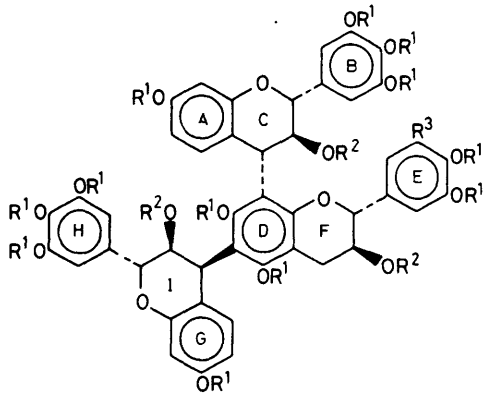
Purity of the triflavanoids was achieved by successive methylation (diazomethane) and acetylation, with preparative layer chromatography (p.l.c.) separation at each step. The resultant methyl ether triacetates [(13b)—(17b)] were differentiated by ^{1}H n.m.r spectroscopy at 165—170 °C, circular dichroism (c.d.), mass spectrometry, and finally by synthesis of all the diastereoisomeric prototypes represented in component C. Those from the homogeneous areas C (three compounds) and E (two) each exhibit molecular-mass values (M^+) of 1 160 and 1 190 respectively, indicating that they contain two and three methoxy-groups more than their [4,6 : 4,8]-bi-[(−)-fisetinidol]-(+)-catechin homologues (M^+ 1 100) previously synthesised, and also isolated from the heartwood of the same tree.[10,11] The predominant if not exclusive generation of robinetinidin chloride (8) from component C indicates that (−)-robinetinidol units replace (−)-fisetinidol units as [4,6 : 4,8]-substituents on (+)-catechin. The heterocyclic regions of the 80 MHz ^{1}H n.m.r. spectra of three dodecamethyl ether triacetates, $C_{63}H_{68}O_{21}$, isolated from area C in the proportions 3.2 : 3.6 : 1.0,* correspond exactly to those which characterize the [4,6 : 4,8]-all-*trans*-, 2,3-*trans*-3,4-*cis* : 2′,3′-*trans* : 2″,3″-*trans*-3″,4″-*trans*-, and 2,3-*trans*-3,4-*cis* : 2′,3′-*trans* : 2″,3″-*trans*-3″,4″-*cis*-bi-[(−)-leucofisetinidin]-(+)-catechins,[11] respectively, but the aromatic regions of the prorobinetinidins are distinguished in each instance by two singlets attributable to magnetically equivalent benzenoid protons of two pyrogallol moieties. Confirmation that these substituted pyrogallol groups are both associated with robinetinidol substituents (4) linked to (+)-catechin (1) was obtained by condensing (+)-leucorobinetinidin (6) from *Robinia pseudacacia* [12] with synthetic [4,8]-2,3-*trans*-3,4-*trans* : 2′,3′-*trans*-(−)-robinetinidol-(+)-catechin (11) [5] under mild, acidic conditions (0.1M HCl; 22 °C) to give [4,6 : 4,8]-all-*trans*-bi-[(−)-robinetinidol]-(+)-catechin (13a) and its [4,6]-3″,4″-*cis* isomer (14a). Similar condensation with the synthetic [4,8]-2,3-*trans*-3,4-*cis* : 2′,3′-*trans* isomer of the all-*trans*-biflavanoid (11) also provides the [4,6 : 4,8]-3,4-*cis*-3″,4″-*cis* triflavanoid analogue (17a). ^{1}H N.m.r. (80 MHz) spectra at 170 °C and c.d. spectra of their dodecamethyl ether triacetate derivatives [(13b), (14b), and (17b), respectively] were identical with those of their natural counterparts.

Similarly, the homogeneous area E may be resolved by preparative methods, after methylation and acetylation, into two compounds with molecular formulae $C_{64}H_{70}O_{22}$. The heterocyclic regions of their ^{1}H n.m.r. spectra again show precise identity with those of the all-*trans*- and [4,6]-3,4-*cis*-bi-[(−)-fisetinidol]-(+)-catechin [11] and [4,6]-3,4-*cis*-bi-[(−)-robinetinidol]-(+)-catechin analogues, while the aromatic region is characterized by three two-proton singlets (magnetically equivalent 2- and 6-protons of the 2-phenyl rings) as compared with two such singlets for the angular triflavanoid derivatives (13b), (14b), and (17b). Taken in conjunction with



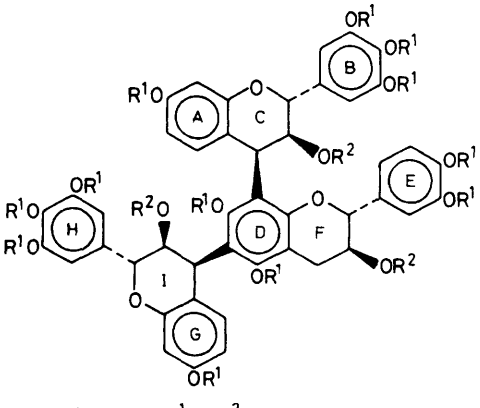
(13) a; $R^1 = R^2 = R^3 = H$
b; R^1 = Me, R^2 = Ac, $R^3 = H$

(15) a; $R^1 = R^2 = H$, R^3 = OH
b; R^1 = Me, R^2 = Ac, R^3 = OMe



(14) a; $R^1 = R^2 = R^3 = H$
b; R^1 = Me, R^2 = Ac, $R^3 = H$

(16) a; $R^1 = R^2 = H$, R^3 = OH
b; R^1 = Me, R^2 = Ac, R^3 = OMe



(17) a; $R^1 = R^2 = H$
b; R^1 = Me, R^2 = Ac

* The [4,6:4,8]-3,4-*cis*:3″,4″-*cis*-isomer (17b) of lowest concentration, as well as traces of the 3,4-*trans*:3″,4″-*cis*-isomer also isolated, may represent artefacts developed during chromatography of the free phenolic forms on silica.

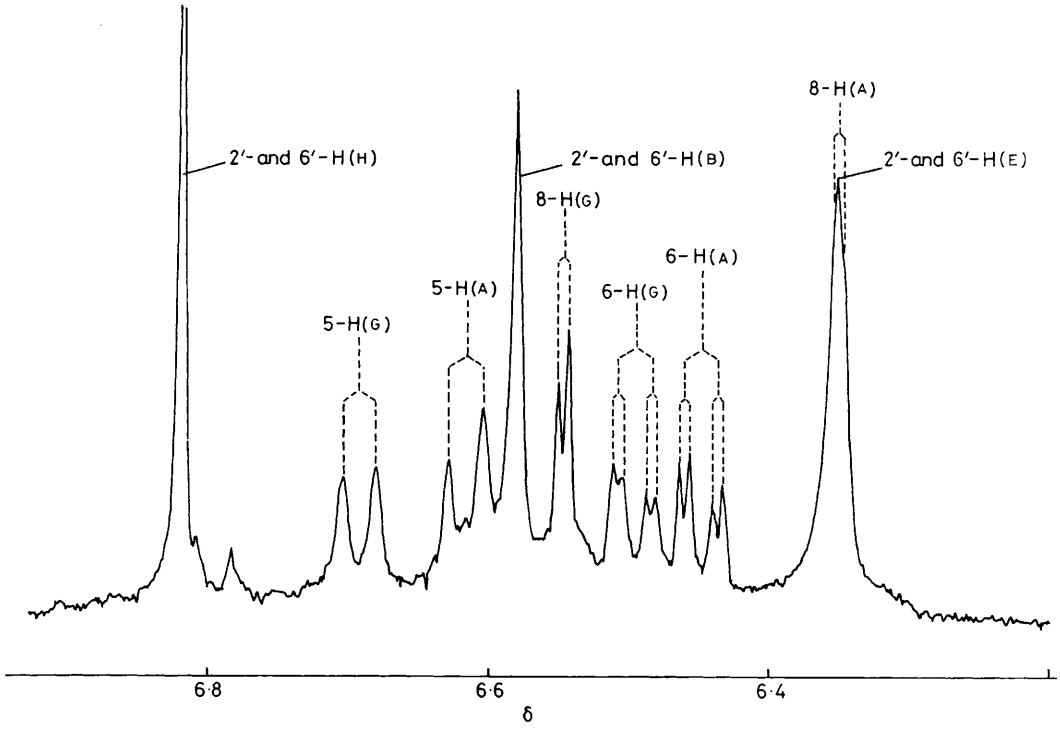


Figure 1. Aromatic region of the 360 MHz ^{1}H n.m.r. spectrum of the tridecamethyl ether triacetate of [4,6:4,8]-all-*trans*-bi-[(−)-robinetinidol]-(+)-gallocatechin, compound (15b)

the degradative and analytical evidence this indicates that the B-, E-, and H-rings are all represented by pyrogallol units and that the component E consists of [4,6 : 4,8]-all-*trans*-bi-[(−)-robinetinidol]-(+)-gallocatechin (15a) and its [4,6]-3″,4″-*cis* isomer (16a) in the ratio 1.8 : 1.

The angular nature of these compounds is confirmed by examination, for example, of the aromatic region of the tridecamethyl ether triacetate (15b) of the all-*trans* isomer by ^{1}H n.m.r. spectroscopy at 360 MHz (Figure 1). The spectrum is simplified in the sense that in addition to the three two-proton singlets at δ 6.36, 6.58, and 6.82, only two high-field ABC-systems attributable to the resorcinol-derived A- and G-rings of the 4,6- and 4,8-coupled (−)-robinetinidol units are discernible (*cf.* Figure 1 of reference 11). The c.d. spectra of the prorobinetinidin triflavanoids from wattle bark (Figure 2) and the above synthetic evidence indicates (*cf.* Figure 3 of reference 11) that the compounds have the 2″*R*,3″*S*,4″*S*-[(13a) (15a)] 2″*R*,3″*S*,4″*R*-2′*R*,3′*S*-2*R*,3*S*,4*S* [(14a) (16a)] and 2″*R*,3″*S*,4″*R*-2′*R*,3′*S*-2*R*,3*S*,4*R* (17a) absolute configurations, respectively. Qualitative evidence was also obtained of the presence of a low-R_F prorobinetinidin tannin fraction of higher mass than those of the triflavanoids.

The gradational sequence of oligomeric phenolic units of increasing mass, comprising more than 70% of black wattle bark extract, is populated exclusively at triflavanoid level by prorobinetinidins (13a)—(17a), whereas both profisetinidins [(9), (10)] and predominant prorobinetinidins [(11), (12)] constitute the associated biflavanoids.[5] The overall excess of prorobinetinidins in the extract apparently correlates with the more rapid rate of formation of bi- and tri-flavanoid prorobinetinidins than their profisetinidin analogues during biomimetic-type condensations.

These relative rates were confirmed by comparison of the specific rate constants for the condensation of each of the flavan-3,4-diols (+)-leucofisetinidin (5) and (+)-leucorobinetinidin (6) with the flavan-3-ol (+)-catechin (1) under pseudo-first-order conditions, using a ten-molar excess of the flavan-3-ol. The significant difference (*k ca.* 0.005 and *ca.* 0.01 min^{-1}, respectively) may be rationalized on the assumption that among the canonical forms representing those 4-carbocations which are generated under acid conditions, heterocyclic oxonium ions [(18), (19)] are stabilized to varying degrees by delocalization of the incipient benzylic charge over the B-ring, with a pyrogallol function, *e.g.* compound (19), being more effective than a catechol function as in compound (18), thus leading to higher condensation rates for (+)-leucorobinetinidin.

In the presence of excess of (+)-catechin (1), as above, condensation does not proceed significantly beyond the [4,8]-biflavanoid level, whereas with excess of the flavan-3,4-diols both [4,8]-bi- and [4,6 : 4,8]-tri-flavanoids result in sequence. Steric factors operating at the nucleophilic C-8 and -6 positions of (+)-catechin (1) obviously dominate the sequential nature of condensation up to triflavanoid level, the former position being sterically less hindered.[11]

Experimental

^{1}H N.m.r. spectra were recorded on Bruker WP-80 and WM-360 FT spectrometers in $(CD_3)_2SO$ at *ca.* 170 and *ca.* 190 °C, respectively, with Me_4Si as internal standard. Mass spectra were obtained with a Varian CH-5 instrument. Optical rotations were recorded on a Bendix NPL Automatic Polarimeter Type 143, and c.d. data in methanol on a Jasco J-20 spectropolarimeter. Analyses (C and H) were performed by Analytical Laboratories, Postfach 1249, D-5250 Engelskirchen, West Germany. Thin-layer chromatography (t.l.c.) was done on DC-Plastikfolin Kieselgel 60 PF_{254} (0.25 mm) and the plates were sprayed with H_2SO_4–HCHO (40 : 1) after development. Preparative plates [20 × 20 cm; Kieselgel PF_{254} (1.0 mm)] were air-dried and used without prior activation. Methylations were performed with an excess of

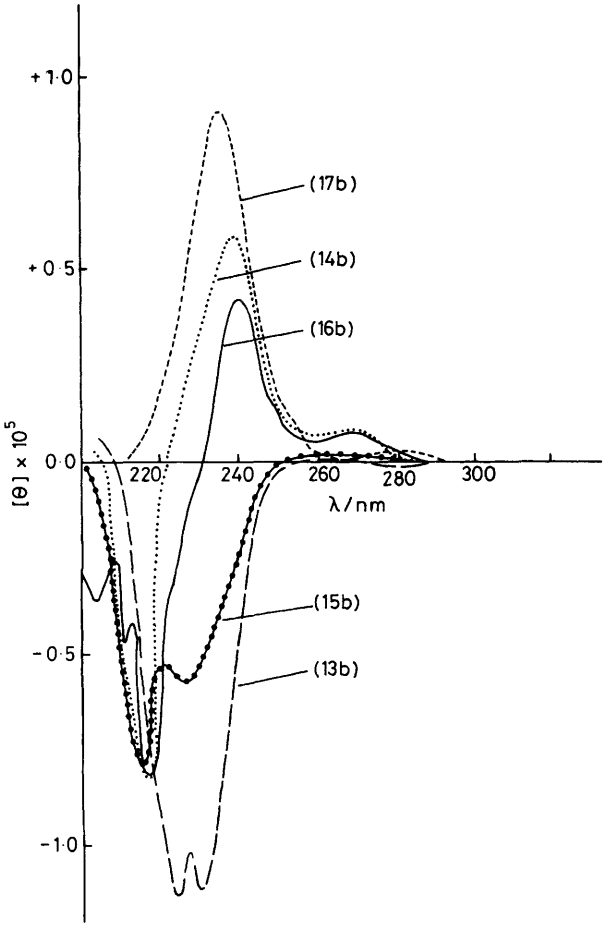
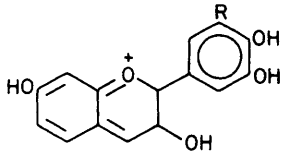


Figure 2. C.d. spectra of do- and tri-decamethyl ether triacetates of [4,6:4,8]-bi-[(−)-robinetinidol]-(+)-catechins and [4,6:4,8]-bi-[(−)-robinetinidol]-(+)-gallocatechins [(13b)—(17b)]



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

diazomethane in methanol–diethyl ether over 48 h, and acetylations were performed in acetic anhydride–pyridine. Evaporations were done under reduced pressure at 50 °C in a rotary evaporator.

Isolation of Triflavanoid Prorobinetinidins from the Bark of Acacia mearnsii.—Freshly stripped black wattle bark (6.4 kg), cut across the grain into fine slivers with a stainless steel knife, was exhaustively extracted with ethyl acetate at ambient temperature to yield a solid extract (962 g) after evaporation. The extract was subjected to six successive partitions (each partition on a 160 g portion) between ethyl acetate and water (1 : 1 v/v) and the contents of the upper phases were grouped to give enriched fractions of components B, C, D, and E (*cf.* reference 6). The solids (246 g) obtained on removal of the organic solvent were fed into a Craig countercurrent machine (100 g in the first eight tubes), using a water–Bu^{s}OH–isohexane (5 : 4.5 : 0.5 v/v/v) system for development. After 150 transfers, every fifth tube was sampled and examined by two-way paper chromatography, using water-saturated Bu^{s}OH and then 2% HOAc as developer.

By using these chromatograms as reference, the tube contents were grouped as follows:

Tubes	Components [6]
1—29	low-R_F tannins
30—40	D and E
41—70	D, B, E, and C
71—80	E, B, C, and A
81—90	B, C, and A

The contents of tubes 30—85 were separated by preparative-paper chromatography (p.p.c.) on Whatman No. 3 paper in 2% HOAc at a loading of 250 mg per sheet. Bands were suitably cut to separate components B and D (R_F 0.44) and C and E (R_F 0.34); a toluene-*p*-sulphonic acid spray [13] gave an orange-red colour with the latter pair. Bands corresponding to components C + E were stripped with 70% EtOH. Solids from this fraction were separated in water-saturated Bu^{s}OH on pre-washed sheets of Whatman No. 3 paper at a loading of 60—70 mg/sheet. Bands at R_F 0.63 and 0.51 were treated as above to give C (R_F 0.63; 1.13 g) and E (R_F 0.51; 0.82 g) as homogeneous components.

Anthocyanidin pigments generated from these components at high dispersion and under pressure [8] were robinetinidin chloride (R_F 0.28), fisetinidin chloride (R_F 0.58; trace), and an orange pigment (R_F 0.61) from C, and robinetinidin chloride (R_F 0.27) and an orange pigment (R_F 0.59) from E (*cf.* reference 9). Formation of the orange pigment of unknown structure is characteristic of leuco- and pro-robinetinidins. No detectable amounts of either (+)-catechin or (+)-gallocatechin were generated under the above conditions.

Alkali fusion of components C and E with NaOH–KOH under anhydrous conditions [6] gave resorcinol (and β-resorcylic acid), phloroglucinol, and gallic and protocatechuic acids from C, whereas from E the same products were obtained but without protocatechuic acid. The main fusion products in each instance were resorcinol and gallic acid.

Paper ionophoresis [14] of component E in boric acid–borate buffer (pH 8.8) for 6 h at 5 mA on Whatman No. 3MM paper gave two main bands.

Resolution of the Methyl Ether Triacetate Derivatives of Prorobinetinidin Triflavanoids.—The contents (2 g) of a discrete band (R_F 0.34) corresponding to the homogeneous areas C and E, and isolated by p.p.c. on Whatman No. 3 paper in 2% HOAc, were methylated with diazomethane for 48 h. Separation by p.l.c. [twice; benzene–acetone (8 : 2 v/v) as developer] gave a single prominent band, R_F 0.20.

The components of the R_F 0.20 band (654 mg) was re-separated by p.l.c. [benzene–acetone (3 : 1 v/v) as developer] into three fractions at R_F 0.47 (band X, 100 mg), 0.43 (band Y, 124 mg), and 0.38 (band Z, 94 mg). Each of the components from these bands were then fully acetylated. The product of acetylation of the Z-component was separated by p.l.c. [twice; 1,2-dichloroethane–acetone (9 : 1 v/v) as developer] into two components, R_F 0.39 and 0.32.

[4,6 : 4,8]-*all*-trans-*Bi*-[(−)-*robinetinidol*]-(+)-*catechin do-decamethyl ether triacetate* (13b), R_F 0.39, was isolated as a solid (16 mg), $[\alpha]_D^{23}$ −56.6° (*c*, 3.15) (Found: C, 65.0; H, 6.0. $C_{63}H_{68}O_{21}$ requires C, 65.2; H, 5.9%); *m/z*, 1 160 (M^+, 5.7%); δ (170 °C) 6.69 [s, 2- + 6-H (H-ring)], 6.41 [s, 2- + 6-H (B)],

5.84 [t, 3-H (C or I), ΣJ 18.6 Hz], 5.83 [t, 3-H (I or C), ΣJ 19.2 Hz], 4.88 [d, 2-H (C or I), J 9.6 Hz], 4.84 [d, 2-H (I or C), J 10.0 Hz], 4.71br [d, 4-H (C or I), J *ca.* 9.6 Hz], 4.63br [d, 4-H (I or C), J *ca.* 9.5 Hz], 2.94—2.69 [m, CH_2 (F)], 1.79 [s, 3-OAc (F)], 1.63 [s, 3-OAc (I)], and 1.62 [s, 3-OAc (C)]; c.d.: see Figure 2.

[4,6 : 4,8]-*all*-trans-*Bi*-[(−)-*robinetinidol*]-(+)-*gallocatechin tridecamethyl ether triacetate* (15b), R_F 0.32, was isolated as a solid (9 mg), $[\alpha]_D$ −47.2° (*c*, 3.12) (Found: C, 64.4; H, 6.0. $C_{64}H_{70}O_{22}$ requires C, 64.5; H, 5.9%); *m/z* 1 190 (M^+, 1.2%); δ (160 °C) 6.68 [s, 2- + 6-H (H)], 6.44 [s, 2- + 6-H (B)], 6.21 [s, 2- + 6-H (E)], 5.86 [t, 3-H (C or I), ΣJ 19.0 Hz] 5.84 [t, 3-H (I or C), ΣJ 19.0 Hz], 4.88 [2 × d, 2-H (C + I), J 9.5 Hz], 4.70br [d, 4-H (C or I), J *ca.* 9.5 Hz], 4.63br [d, 4-H (I or C), J *ca.* 9.5 Hz], 2.94—2.69 [m, CH_2 (F)], 1.80 [s, 3-OAc (F)], 1.63 [s, 3-OAc (I)], and 1.59 [s, 3-OAc (C)]; c.d.: see Figure 2.

The product of acetylation of the Y-component (R_F 0.43) gave a single product by p.l.c. [twice; benzene–acetone (9 : 1 v/v) as developer] (62 mg), R_F 0.26. However, subsequent separation by p.l.c. [thrice; 1,2-dichloroethane–acetone (9 : 1 v/v) as developer] gave two components at R_F 0.50 and 0.43.

[4,6 : 4,8]-2,3-trans-3,4-trans : 2″,3″-trans-3″,4″-cis-*Bi*-[(−)-*robinetinidol*]-(+)-*catechin dodecamethyl ether triacetate* (14b), R_F 0.50, was isolated as a solid (18 mg) (Found: C, 65.1; H, 5.8. $C_{63}H_{68}O_{21}$ requires C, 65.2; H, 5.9%); *m/z* 1 160 (M^+, 4.2%); δ (167 °C) 6.57 [s, 2- + 6-H (H)], 6.45 [s, 2- + 6-H (B)], 6.01 [t, 3-H (C), ΣJ 19.4 Hz], 5.45 [dd, 3-H (I), ΣJ 14.6 Hz], 5.23 [d, 2-H (I), $J_{2,3}$ 8.0 Hz], 4.81 [d, 2-H (C), $J_{2,3}$ 9.5 Hz], 4.78 [d, 4-H (I), $J_{3,4}$ *ca.* 6.5 Hz], 4.50br [d, 4-H (C), $J_{3,4}$ 9.5 Hz], 3.00—2.69 [m, CH_2 (F)], 1.83 [s, 3-OAc (F)], 1.61 [s, 3-OAc (I)], and 1.53 [s, 3-OAc (C)]; c.d.: see Figure 2.

[4,6 : 4,8]-2,3-trans-3,4-trans : 2″,3″-trans-3″,4″-cis-*Bi*-[(−)-*robinetinidol*]-(+)-*gallocatechin tridecamethyl ether triacetate* (16b), R_F 0.43, was isolated as a solid (5 mg) (Found: C, 64.3; H, 5.9. $C_{64}H_{70}O_{22}$ requires C, 64.5; H, 5.9%); *m/z* 1 190 (M^+, 1.7%); δ (167 °C) 6.59 [s, 2- + 6-H (H)], 6.48 [s, 2- + 6-H (B)], 6.27 [s, 2- + 6-H (E)], 6.03 [t, 3-H (C), ΣJ 19.2 Hz], 5.47 [dd, 3-H (I), ΣJ 14.9 Hz], 5.25 [d, 2-H (I), $J_{2,3}$ 8.0 Hz], 4.84 [d, 2-H (C), $J_{2,3}$ 9.5 Hz], 4.81br [d, 4-H (I), $J_{3,4}$ *ca.* 6.5 Hz], 4.50br [d, 4-H (C), $J_{3,4}$ *ca.* 9.5 Hz], 2.91—2.70 [m, CH_2 (F)], 1.85 [s, 3-OAC (F)], 1.65 [s, 3-OAc (I)], and 1.56 [s, 3-OAc (C)]; c.d.: see Figure 2.

The product of acetylation of the X-component (R_F 0.47) gave a single component on p.l.c. [twice; benzene–acetone (9 : 1 v/v) as developer] (38 mg), R_F 0.28, which was purified [p.l.c., thrice; 1,2-dichloroethane–acetone (9 : 1 v/v) as developer] to give the pure component, R_F 0.52 [4,6 : 4,8]-2,3-trans-3,4-cis : 2″,3″-trans-3″,4″-cis-*Bi*-[(−)-*robinetinidol*]-(+)-*catechin dodecamethyl ether triacetate* (17b) which was isolated as a solid (5 mg) (Found: C, 65.2; H, 6.0. $C_{63}H_{68}O_{21}$ requires C, 65.2; H, 5.9%); *m/z* 1 160 (M^+, 10.8%); δ (167 °C) 6.60 [s, 2- + 6-H (H)], 6.45 [s, 2- + 6-H (B)], 5.53 [t, 3-H (C), ΣJ 13.5 Hz], 5.45 [dd, 3-H (I), ΣJ 14.5 Hz], 5.19 [d, 2-H (I), $J_{2,3}$ 8.5 Hz], 5.14 [d, 2-H (C), $J_{2,3}$ 7.0 Hz], 4.72br [d, 4-H (C), $J_{3,4}$ *ca.* 6.5 Hz], 4.50br [d, 4-H (I), $J_{3,4}$ *ca.* 6.5 Hz], 3.0—2.75 [m, CH_2 (F)], 1.83 [s, 3-OAc (F)], 1.69 [s, 3-OAc (I)], and 1.64 [s, 3-OAc (C)]; c.d.: see Figure 2.

Significant ions in the mass-fragmentation spectra of the dodecamethyl ether triacetates (13b), (14b), and (17b) and their respective relative abundances are *m/z* 1 160 (M^+) (5.7, 4.2, 10.8%), 1 100 (45, 46, 73), 1 040 (55, 63, 64), 980 (19.0, 16.3, 32), 908 (4.1, 3.6, 6.8), 877 (19.1, 26, 73), 848 (19.8, 11.8, 29), 817 (19.9, 66, 75), 788 (12.8, 21, 30), 773 (11.7, 86, 71), 757 (17.1, 25, 1.7), 713 (100, 99, 91), 656 (10.5, 10.7, 16.5), 653 (52, 37, 33), 625 (2.8, 16.2, 22), 595 (16.5, 12.2, 22), 565 (23, 25, 39), 534 (3.9, 12.6, 9.4), 388 (5.7, 15.6, 8.4), 387 (7.8 19.8, 22), 328 (49, 43, 44), 327 (86, 88, 91), 252 (6.4, 9.5, 10.5) 222 (11.8, 8.0, 11.1), 210 (79, 78, 86), 181 (93, 100, 100), 180 (18.2, 37, 30), and 151 (83, 86, 89). Similarly the significant ions of the tridecamethyl ether triacetates (15b) and (16b) are *m/z* 1 190 (M^+) (1.2, 1.7%), 1 130 (64, 37), 1 070 (49, 54), 1 010 (16.5, 15.7), 938 (2.6, 3.0), 907 (11.4, 4.4), 878 (5.4, 6.6), 847 (14.8, 63), 818 (9.9, 24), 803 (10.5, 85), 787 (18.1, 29), 743 (100, 96), 686 (5.7, 4.1), 683 (73, 48), 656 (10.3, 11.3), 655 (38, 42), 626 (9.0, 8.1), 595 (29, 35), 565 (12.1, 10.1), 418 (11.9 9.5), 417 (10.7, 22), 328 (5.7, 41), 327 (82, 88), 252 (12.4, 22), 210 (68, 85), and 181 (93, 100).

Synthesis of Diastereoisomeric Bi-[(−)-*robinetinidol*]-(+)-*catechins.*—A solution of (+)-leucorobinetinidin (6) (1.22 g) and (+)-catechin (1) (2.25 g) in 0.1M HCl (100 ml) was stirred for *ca.* 1 h at ambient temperature (*ca.* 25 °C) until the reaction as monitored by t.l.c. [benzene–acetone–methanol (5 : 4 : 1 v/v/v) as developer], was nearly complete. The solution was kept at 0 °C for a further 15 h, and the combined solutions from six such condensations were extracted with ethyl acetate (8 × 400 ml). The combined extracts were dried (anhydrous Na_2SO_4). P.l.c. separation of the solid product (19.69 g) in the same system gave two fractions at R_F 0.30 (1.22 g) and 0.38 (1.52 g).

The R_F 0.30 fraction, composed exclusively of the [4,8]-all-*trans*-(−)-robinetinidol-(+)-catechin (11),[5] and (+)-leucorobinetinidin (6) (1.2 g) were dissolved in 0.1M HCl (100 ml) and the solution was stirred for 21 h at ambient temperature (15—25 °C). The solution was extracted with ethyl acetate (5 × 400 ml) and, after being dried (Na_2SO_4) the combined extracts gave a phenolic mixture (2.28 g). P.l.c. using benzene–acetone–methanol (5 : 4 : 1 v/v/v) as developer gave a triflavanoid fraction at R_F 0.33 (311 mg). Methylation with diazomethane and separation [p.l.c., twice; benzene–acetone (8 : 2 v/v) as developer] gave the pure dodecamethyl ethers at R_F 0.21 (65 mg). Acetylation and separation [p.l.c., twice; benzene–acetone (9 : 1 v/v) as developer] gave the dodecamethyl ether triacetate of [4,6 : 4,8]-all-*trans*-bi-[(−)-robinetinidol]-(+)-catechin at R_F 0.29 (20 mg) and its [4,6]-3″,4″-*cis* isomer at R_F 0.35 (28 mg). These compounds, (13b) and (14b), respectively, were shown to be identical with their natural counterparts by ^1H n.m.r. [$(CD_3)_2SO$; 170 °C], mass spectrometry, and c.d.

The R_F 0.38 (1.52 g) fraction from the primary condensation, consisting of an inseparable mixture of the predominant [4,8]-2,3-*trans*-3,4-*cis* : 2′,3′-*trans*-(−)-robinetinidol-(+)-catechin (10) and low proportions of its [4,6]-all-*trans*-isomer, was treated with (+)-leucorobinetinidin (6) (1.5 g) under identical conditions to give a phenolic material (3.25 g). Separation by p.l.c. gave a triflavanoid fraction, R_F 0.34 (451 mg) as before, and after methylation [R_F 0.14; benzene–acetone (8 : 2 v/v) as developer] (69 mg) and acetylation the dodecamethyl ether triacetate (17b) of the isomeric [4,6 : 4,8]-3,4-*cis*-3″,4″-*cis*-bi-[(−)-robinetinidol]-(+)-catechin was obtained [R_F 0.26 in 1,2-dichloroethane–acetone (19 : 1 v/v)] (33 mg) and shown identical to the natural product from black wattle bark extract.

Relative Reaction Rates of (+)-*Leucofisetinidin and* (+)-*Leucorobinetinidin with* (+)-*Catechin.*—Confirmation was sought of the apparently higher condensation rate of (+)-leucorobinetinidin (6) with (+)-catechin (1) compared with that of (+)-leucofisetinidin (5) as observed under conditions of synthesis.

(+)-Leucofisetinidin (5) (29 mg, 0.1 mmol) and (+)-leucorobinetinidin (6) (30.6 mg, 0.1 mmol) were treated individually under identical conditions (25 °C; 20 ml 0.1M HCl) with a tenfold excess of (+)-catechin (1) (290 mg, 1 mmol) to ensure pseudo-first-order conditions. The reaction mixtures were sampled at regular intervals by withdrawal of 1 ml aliquots,

and addition to each of these of saturated aqueous $NaHCO_3$ (0.2 ml) followed immediately by glacial acetic acid (0.15 ml). The presence of a dilute organic acid was essential in order to obviate oxidation of the pyrogallol function of (+)-leucorobinetinidin (6) at the pH of the hydrogencarbonate solution. A suitable quantity (0.08 ml) of each treated aliquot sample was applied to a two-way paper chromatogram, which was developed in turn in water-saturated Bu^{s}OH (A) and then in 2% acetic acid (B).

Calibration of similar chromatograms [15] by the application of varying volumes (0.08—0.01 ml range) of (+)-leucorobinetinidin and (+)-leucofisetinidin solutions prepared under identical conditions, but for the absence of (+)-catechin, permitted assessment of the progressive reduction in the concentrations of these compounds as a function of time during condensations, and hence approximations of the specific rates from the first-order rate expression $\ln c_0 - \ln c = kt$.

The rate constant (k 0.0145 min^{-1}) for (+)-leucorobinetinidin after 30 min is approximately twice that for (+)-leucofisetinidin (0.0065 min^{-1}), but decreases with time (k 0.0124, 0.0059 min^{-1}, respectively, after 60 min), indicating that the mechanism is complex, possibly due to an intermediate solvolysis step.

During the above condensations [10 molar excess of (+)-catechin] and even with 1.5 molar excess of (+)-catechin the appropriate 3,4-*trans*- and 3,4-*cis*-biflavanoids develop without evidence of triflavanoid formation [the following R_F values refer to paper chromatography with developer systems A and B, respectively (see above)]; (+)-catechin (R_F 0.52, 0.40) combines with (+)-leucofisetinidin (0.53, 0.52) to give the [4,8]-all-*trans*- (0.33, 0.35) and -3,4-*cis*-(−)-fisetinidol-(+)-catechin (0.44, 0.37), and with (+)-leucorobinetinidin (0.37, 0.51) to give the [4,8]-all-*trans*- (0.22, 0.33) and -3,4-*cis*-(−)-robinetinidol-(+)-catechin (0.36, 0.30). Appropriate triflavanoid formation is evident when a five molar excess of (+)-leucorobinetinidin relative to (+)-catechin is used.

Acknowledgement

Fresh bark of *A. mearnsii* was kindly collected by Mr. D. F. C. Garbutt, Wattle Research Institute, Pietermaritzburg.

Support by the Sentrale Navorsingsfonds of this University, by the Council of Scientific and Industrial Research, Pretoria, including tenure of a Research Assistantship by P. M. V., by the Wattle Bark Industry of South Africa Marketing Committee, Pietermaritzburg, and by the Leather Industries Research Institute, Grahamstown, is acknowledged. Mass spectra were recorded by Dr. J. M. Steyn, Department of Pharmacology of this University, and 360 MHz ^1H n.m.r. spectra by Dr. W. E. Hull, Bruker Analytische Messtechnik GmbH, Silberstreifen, Rheinstetten-Forchheim, West Germany.

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Received 25*th March* 1982; *Paper* 2/519