Phytochemistry, Vol. 24, No. 10, pp. 2415–2422, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

BIFLAVANOID PROGUIBOURTINIDIN CARBOXYLIC ACIDS AND THEIR BIFLAVANOID HOMOLOGUES FROM *ACACIA LUEDERITZII*

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(*Received* 10 *December* 1984)

Key Word Index—*Acacia luederitzii*; Leguminosae; leucoguibourtinidins; proguibourtinidins; carboxylic acids; 3,4′,7-trihydroxyflavanyls; regio-isomerism; diastereoisomers; 6-carboxyl-(+)-catechin; biflavanoids.

Abstract—[4,6]- and [4,8]-Proguibourtinidin carboxylic acids (3,7,4′-trihydroxyl functionality) of 2,3-*trans*-3,4-*trans*: 2,3-*cis*- and 2,3-*trans*-3,4-*trans*: 2,3-*trans*-configuration based on (−)-epicatechin or (+)-catechin as constituent units, and their associated biflavanoid homologues, predominate in the heartwood of *Acacia luederitzii*. They are accompanied by stereochemical and functional analogues and by their putative flavan-3,4-diol and flavan-3-ol precursors.

INTRODUCTION

Pro- and leucoguibourtinidins (7,4′-dihydroxy phenolic functionality) represent a relatively rare group of compounds which, while occurring as minor components in the heartwoods of Australian *Acacia* spp. [1], predominate in the southern African species *Guibourtia coleosperma* [2, 3] (Rhodesian mahogany or large false mopane), *Julbernardia globiflora* (munondo) [4] and *Acacia luederitzii* (bastard umbrella thorn) [5]. The complex mixture of phenolic extractives from the last-mentioned source is of particular interest considering that it contains, among others, the first and hitherto only examples of natural biflavanoid carboxylic acids. Preliminary announcement [5] of the structures of some of these compounds made *ca* 15 years ago was without the advantage of our current detailed knowledge of ^{1}H NMR spectroscopy as regards diagnostic H-6, H-8, H-2, H-3 and OAc-3 chemical shift correlations [6–8], CD parameters [9, 10] and rotational isomerism [11]. The biflavanoid mixture in *A. luederitzii* Engl. was, accordingly, re-investigated in order to extend the previous study and also to provide unambiguous confirmation of our previous structural assignments.

RESULTS AND DISCUSSION

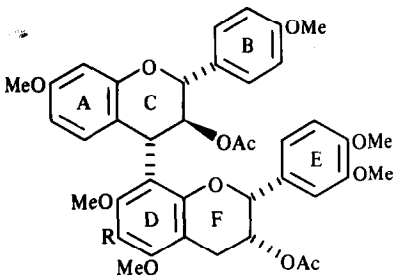
Due to the complexity of the mixture, the compounds were identified as their methyl ether acetates or methyl ester analogues **1**–**12** after preliminary separation. Alkali fusion under anhydrous conditions [12] of the free phenolic biflavanoids corresponding to their derivatives **1**–**10** gave the appropriate resorcinol, β-resorcylic acid, phloroglucinol, *p*-hydroxybenzoic and protocatechuic acid degradation products. With 3 M hydrochloric acid–propan-2-ol (1:4) at 100° under pressure [13] they yield the anthocyanidin guibourtinidin chloride (3,7,4′-trihydroxyflavylium chloride [14, 15]), establishing a common substitution pattern for their 'upper' units and, by a process of elimination, a phloroglucinol–catechol combination for the 'lower' units (except for the free phenolic equivalent of **10**).

The group of free phenolic biflavanyl carboxylic acids corresponding to the derivatives **1**, **3**, **5** and **7**, although chromatographically distinct, were readily separated from the remainder by the simple bicarbonate technique; evidence which correlates with the presence of aromatic ester groups (IR ν_{max} cm^{-1}: 1715) in their methylated derivatives. ^{1}H NMR spectroscopy of their methyl ether acetates recorded at elevated temperatures* indicates the presence of seven methoxyl and two acetoxyl proton resonances in each. Similarly, AA_1BB_1-systems of the B-rings and ABC proton coupling systems of the A-rings are evident in the benzenoid region. However, the aromatic singlet shift to high field in this region, which is characteristic of [4,8]- or [4,6]-biflavanoid units with a phloroglucinol-derived D-ring, is absent. The carboxyl function may, therefore, be allocated to the remaining position on the D-ring. Rationalization of the mass fragmentation spectra of the hexamethyl diacetyl methyl ester derivatives **1**, **3**, **5** and **7** (*m/z* 772, $[M]^+$) leads in each instance to the *m/z* 327 (**13**) fragment as an intense peak, following loss of a methoxyl radical from the carboxylic ester, and two reverse Diels–Alder fissions. Generation of this common fragment lends support to the above NMR evidence as regards the position of the carboxyl function.

The relative configurations of the 2,3-*trans*-3,4-*trans*: 2,3-*cis*-methyl ester derivatives **1** and **3** were evident from the ^{1}H NMR coupling constants of their heterocyclic protons ($J_{2,3}$ = 9.50, 9.75 Hz, $J_{3,4}$ = 9.75, 10.0 Hz: $J_{2,3}$ < 1 Hz) as from those of the 2,3-*trans*-3,4-*trans*: 2,3-*trans*-diastereoisomers **5** and **7** ($J_{2,3}$ = 9.25, 9.25 Hz, $J_{3,4}$ = 9.25, 9.50 Hz: $J_{2,3}$ = 7.0, 7.5 Hz).

'Conventional' proguibourtinidin analogues lacking absorption in the 1620–1800 cm^{-1} region and, thus, possessing no carboxyl function, were separated with considerable difficulty from a chromatographic overlay of higher oligomers. Their hexamethyl ether diacetates **2**, **4**, **6**

*Elevated temperatures were required to overcome spectral complexity contributed by rotational isomerism [11].



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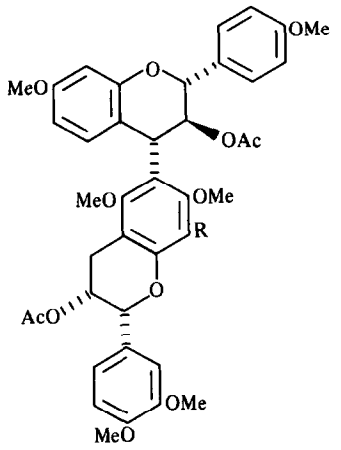

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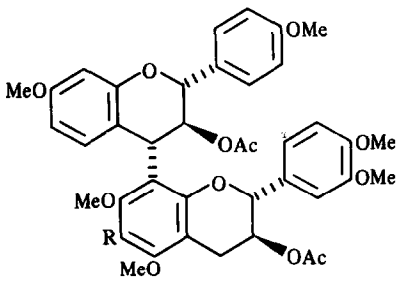

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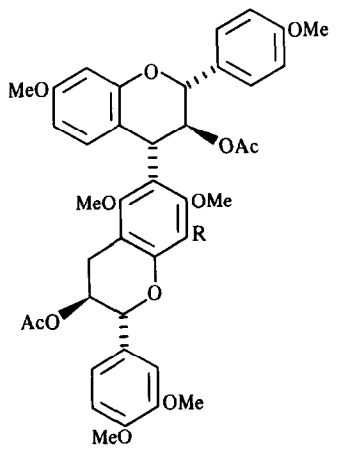

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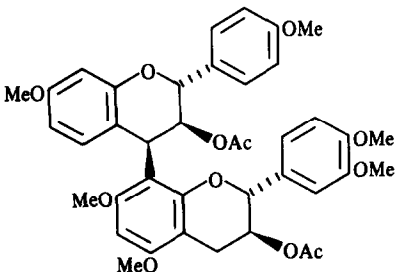

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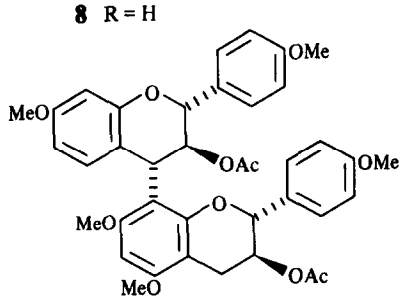

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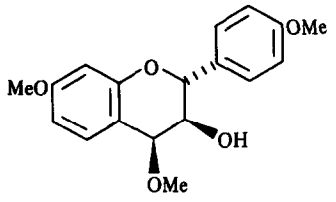

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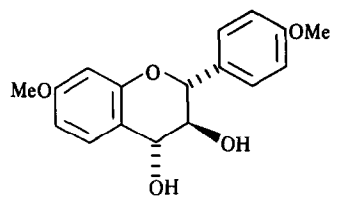

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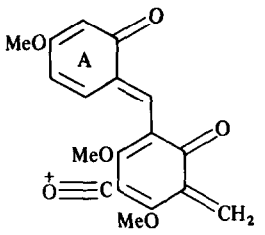
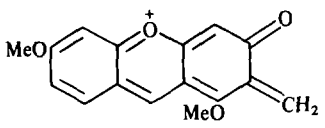


13 *m/z* 327 (76-88%)



14 *m/z* 269 (50-98%)

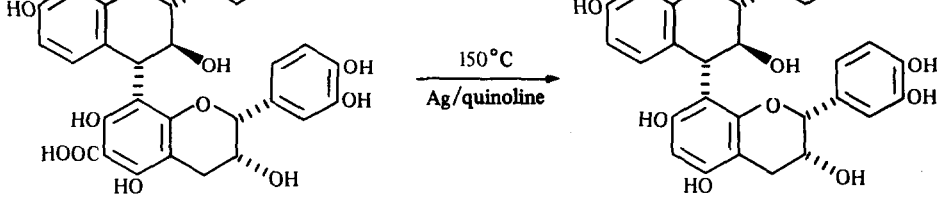
and **8** gave high temperature ^{1}H NMR spectra which were almost identical with those of the corresponding carboxyl methyl esters **1**, **3**, **5** and **7**, respectively, except for the additional presence of a high field aromatic singlet in each instance (δ6.17–6.19),† and the expected absence of a single methoxyl (methyl ester) resonance. Apart from the corresponding reduction (58 units) in the mass of their $[M]^+$ ions (*m/z* 714), the fragmentations differed from those of the methyl esters, the base peak at *m/z* 654 (100%) (cf. ref. [4]) representing acetic acid loss via a McLafferty rearrangement in each instance. Ions of consistently high intensity [**14**, *m/z* 269 (50–98%)] resulting from successive reverse Diels–Alder fragmentations, followed by methoxyl radical loss, as originally proposed by Pelter *et al.* [4], also distinguished the spectra of the hexamethyl ether diacetates, as well as those of their 'parent' hexamethyl ethers (93–100%).

The structural and stereochemical relationships between the [4,8]-2,3-*trans*-3,4-*trans*:2,3-*cis*-carboxylic ester **1** and its 'conventional' analogue **2** was provided by decarboxylation of the free phenolic form of the former **15** in quinoline with metallic silver as catalyst. The product, **16**, proved to be identical with [4,8]-proguibourtinidin-(−)-epicatechin by 2D chromatography, while the ^{1}H NMR spectrum of its hexamethyl ether diacetate was superimposable upon that of **2**. Similar relationships between the free phenolic equivalents of the pairs **3** and **4**, and **5** and **6** were established by chromatography.

Assessment of the aromatic bonding positions of the [4,8]- and [4,6]-2,3-*trans*-3,4-*trans*:2,3-*cis*-hexamethyl ether diacetates (**2** and **4**, respectively) and, hence, of their carboxylic methyl esters **1** and **3**, presented a problem since the chemical shift difference between their H-6 and H-8 (D-ring) resonances, respectively, in deuterochloroform at 100° (δ6.16 and 6.14 respectively) was negligible. In the face of this 'anomaly', the first encountered since our introduction of this method of differentiation [6], recourse was taken to comparison of the absolute values of 3-acetoxy proton shifts in both deuterochloroform and DMSO-d_6 with those of reference compounds of identical stereochemistry and bonding positions (cf. Table 1). Apart from a single divergence in the case of [4,6]-(−)-fisetinidol-(+)-catechin heptamethyl either diacetate (a reference compound), satisfactory agreements between the assignments for the proguibourtinidin derivatives **1**–**10** and suitable reference compounds provide the desired differentiation between the alternative bonding positions. Pelter *et al.*'s [4] globiflorins $3B_1$ and $3B_2$ from *Julbernardia globiflora* are apparently identical with the free phenolic forms of **4** and **8**, respectively, although no direct comparison has been attempted.

The ^{1}H NMR spectra of the [4,8]-2,3-*trans*-3,4-*trans*:2,3-*trans*-proguibourtinidin derivative **6** and of **10** [3'-demethoxy (E-ring) homologue of **6**] were superimposable except for one methoxyl resonance less and for an additional proton in the aromatic region of the latter. These findings are in agreement with a $[M]^+$ of 30 mass units less for **10** [*m/z* 684 (12%)] supported by the $[M-60]^+$ base peak [*m/z* 624 (100%)] and the *m/z* 269 (84.5%) ion (cf. **14**) characteristic of its homologues [4]. Similarly, the low relative abundance of those ions resulting from reverse Diels–Alder fragmentation which is normally attributable to a catechol-type E-ring [4] [namely *m/z* 222 (1.7%), 180 (6.6%) and 165 (6.2%)] compared with those generated from the [4,8]-proguibourtinidin-(+)-catechin methyl ether diacetate **6** [the known catechol-containing (E-ring) homologue] under identical conditions (13.9%, 72.8% and 76.8%, respectively) supports the presence of a phenol-type E-ring for **10**. Finally, evidence of a second AA_1BB_1 aromatic system in its ^{1}H NMR spectrum, the upfield position of the aromatic singlet [δ6.16, H-6(D)] [6], as

†In deuterochloroform or 1,2-dideuterotetrachloroethane.



15 **16**

Table 1. Chemical shifts of acetoxy ^{1}H NMR of [4,6]- and [4,8]-proguibourtinidin hexamethyl ether diacetates, their methyl carboxylates and homologues in $CDCl_3$

Bonding points and configurations of derivatives	Temperature (other solvents)	δ OAc-3 F-ring	δ OAc-3 C-ring	Δδ
[4,8]-2,3-*trans*-3,4-*trans*: 2,3-*cis*-				
Proguibourtinidin-(−)-epicatechin carboxylate (**1**)	100°	1.77	1.60	0.17
	170° (DMSO-d_6)	1.68	1.55	0.13
Proguibourtinidin-(−)-epicatechin (**2**)	100°	1.74	1.58	0.16
	170° (DMSO-d_6)	1.72	1.59	0.13
(+)-Catechin-(−)-epicatechin [16]	100°	1.72	1.60	0.12
[4,6]-2,3-*trans*-3,4-*trans*: 2,3-*cis*-				
Proguibourtinidin-(−)-epicatechin carboxylate (**3**)	170° (DMSO-d_6)	1.83	1.59	0.24
Proguibourtinidin-(−)-epicatechin (**4**)	100°	1.89	1.59	0.30
	170° (DMSO-d_6)	1.81	1.56	0.25
(+)-Catechin-(−)-epicatechin [16]	100°	1.84	1.64	0.20
[4,8]-2,3-*trans*-3,4-*trans*: 2,3-*trans*-				
Proguibourtinidin-(+)-catechin carboxylate (**5**)	100°	1.90	1.62	0.28
Proguibourtinidin-(+)-catechin (**6**)	100°	1.87	1.58	0.29
	170° (DMSO-d_6)	1.86	1.56	0.30
(−)-Fisetinidol-(+)-catechin [17, 18]	100°	1.84	1.56	0.28
(+)-Catechin-(+)-catechin [19]	100°	1.88	1.61	0.27
Proguibourtinidin-(+)-afzelechin (**10**)	100°	1.91	1.61	0.30
[4,8]-2,3-*trans*-3,4-*cis*: 2,3-*trans*-				
Proguibourtinidin-(+)-catechin (**9**)	100°	1.83	1.68	0.15
(−)-Fisetinidiol-(+)-catechin [17, 18]	100°	1.89	1.72	0.17
[4,6]-2,3-*trans*-3,4-*trans*: 2,3-*trans*-				
Proguibourtinidin-(+)-catechin (**8**)	180° (DMSO-d_6)	1.82	1.59	0.23
Proguibourtinidin-(+)-catechin carboxylate (**7**)	100°	1.85	1.67	0.18
(−)-Fisetinidol-(+)-catechin [17, 18]	100°	1.88	1.59	0.29
(+)-Catechin-(+)-catechin [19]	100°	1.91	1.67	0.23

well as the acetoxy shift correlation (cf. Table 1), indicate that the compound is a novel [4,8]-2,3-*trans*-3,4-*trans*: 2,3-*trans*-proguibourtinidin-(+)-afzelechin (cf. ref. [20]).

The ^{1}H NMR spectra of the heptamethyl ether diacetate of [4,8]-2,3-*trans*-3,4-*cis*: 2,3-*trans*-(−)-fisetinidol-(+)-catechin [17, 18] and the corresponding derivative of its [4,8]-proguibourtinidin-(+)-catechin homologue **9** {δ6.16 [$J_{2,3}$ = 8.5 Hz, $J_{3,4}$ = 6.5 Hz: $J_{2,3}$ = 7.6 Hz, *s*, H-6 (D)]} are almost superimposable and their acetoxy shift correlations (cf. Table 1) are in close agreement. The high amplitude positive Cotton effect at low wavelength in the CD spectrum of the hexamethyl ether diacetate **9** contrasts with the negative Cotton effects of all the remaining proguibourtinidin derivatives **1–8** and **10**, thus reaffirming its stereochemistry at C-4 (C-ring) and also those of its analogues [9, 10].

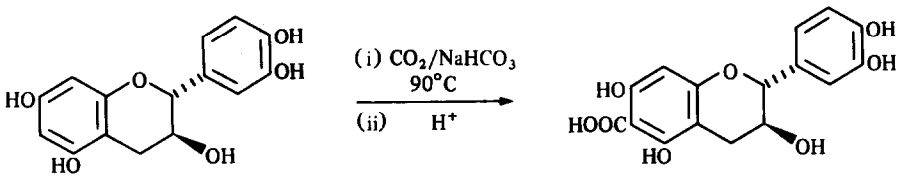
The above group of proguibourtinidin biflavanoids are associated with their putative precursors in *A. luederitzii* heartwoods as shown by the presence of 2,3-*trans*-3,4-*trans*- and 2,3-*trans*-3,4-*cis*-leucoguibourtinidins corresponding to their methyl ether derivatives **12** and **11**, respectively, and of (+)-catechin and (−)-epicatechin (chromatographic evidence) [5]. The co-occurrence of pairs of stereochemically and structurally identical [4,6]- and [4,8]-biflavanoids and their carboxylated homologues indicates that carboxylation occurs at both C-8 and C-6 of (+)-catechin and (−)-epicatechin moieties of the former pair, as a final step in the biogenetic sequence, since the 'extra' carbon atom of the carboxyl group cannot be accommodated in the generally accepted polyketide origin of the phloroglucinol ring.*

The feasibility of such conjecture in terms of a chemical analogy was examined by applying Von Kostanecki's bicarbonate technique [21] to (+)-catechin (**17**) when carboxylation occurred regiospecifically at the 6-position [6] rather than at the sterically less hindered 8-position. Considering that the C–Br bond in the 8-position is considerably less stable than its C–Br equivalent in the 6-position, from debromination experiments on 6,8-dibromo-(+)-catechin derivatives [6], the carboxylation of catechin seems to be under thermodynamic control. 6-Carboxyl-(+)-catechin (**18**) and the free phenolic proguibourtinidin carboxylic acids, corresponding to their derivatives **1**, **3**, **5** and **7**, were also subject to decarboxylation unless suitable precaution was taken.

Judging by the relative yields of methyl ether acetates **1–8** of the predominant proguibourtinidin biflavanoids in the approximate ratio of 1:9:2.5:3:1.5:10.5:1:7, compounds based on (+)-catechin (**5–8**) predominate over their (−)-epicatechin (**1–4**) analogues (ratio 4:3); biflavanoids over their carboxylated homologues (ratio 3:1); 6- over 8-carboxylated biflavanoids (ratio 7:5); and 8- over 6-linked biflavanoids (ratio 2:1). The last two comparisons imply that presumed enzymic 6- and 8-carboxylations occur with equal ease.

The present work confirms our previous assignments

*The alternative route via methylation and subsequent oxidation may be discarded due to absence of the necessary intermediates.



17 18

[5] of the major components from *A. luederitzii*. Our first proof of rotational isomerism among flavanoid oligomers [11] by ^{1}H NMR spectroscopy employing progressively increasing temperatures involved use of [4,6]-2,3-*trans*-3,4-*trans*: 2,3-*trans*-proguibourtinidin-(+)-catechin (**8**).

EXPERIMENTAL

^{1}H NMR spectra were recorded at 80 and 300 MHz in $CDCl_3$ (100°) and DMSO-d_6 (170°) with TMS as reference. CD spectra were determined in MeOH. Media used for the separation of components were: Whatman No. 3 for PC, DC-Plastikfolin Kieselgel 60 F_{254} 0.25 mm for TLC and Kieselgel PF_{254} (1 mm, 20 × 20 cm) for prep. TLC. TLC bands were located under UV and/or with H_2SO_4–HCHO (40:1) spray reagent. Methylations were performed with excess CH_2N_2 over 48 hr at −15° and acetylations in Ac_2O–pyridine. ^{1}H NMR spectra at high temp were essential for structural elucidation, for determining the purity of the various diastereoisomers, and for thus differentiating between them.

Extractions and preliminary separations. Drillings (1.5 kg) from the outer perimeter of the heartwood of *A. luederitzii* Engl. var. *luederitzii* were dewaxed by extraction with *n*-hexane (4 × 5 l.) at ambient temp, and, hence, extracted with Me_2CO–water (9:1, 4 × 5 l.) under similar conditions. The combined extract was evaporated under red. pres. to give a red-brown amorphous powder (330 g).

2D chromatography in H_2O satd butan-2-ol and 2% HOAc, respectively separated the mixture into the predominant areas at R_f 0.35/0.54 and 0.35/0.45 (biflavanoid carboxylic acids), 0.76/0.55 and 0.75/0.46 (biflavanoids), 0.80/1.0–4.0 (higher oligomers). The three groups of compounds all reduced ammoniacal $AgNO_3$ and gave orange-yellow, yellow and purple, respectively, with *p*-toluenesulphonic acid.

The crude extract (200 g) dissolved in EtOAc (1 l.) was treated with 7% aq. $NaHCO_3$ and the lower aq. phase extracted with the same solvent to ensure separation of the uncarboxylated phenols. The combined EtOAc extracts were dried over dry Na_2SO_4 and the solvent evaporated under red. pres. at 45° to give a red-brown amorphous phenolic powder (110 g). The carboxylic fraction was recovered by acidification with 3 M H_2SO_4 and extracted exhaustively with EtOAc, as above, to give a brown powder (75 g).

The phenolic fraction (30 g) in MeOH was applied to 150 46 × 57 cm sheets of Whatman No. 3 paper and the chromatograms developed by upward migration in 2% HOAc over 16 hr. Seven bands were differentiated with an ammonical $AgNO_3$ spray. These were stripped with 70% EtOH and the eluates concd to dryness, as above, to give fractions: R_f 0.79 (1.65 g), 0.68 (2.55 g), 0.55 (2.63 g), 0.43 (2.35 g), 0.31 (1.90 g), 0.18 (2.08 g), 0.10 (2.59 g).

Proguibourtinidin biflavanoids. [4,8]-2,3-trans-3,4-trans: 2,3-cis-*Proguibourtinidin*-(−)-*epicatechin hexamethyl ether diacetate* (**2**). The R_f 0.79 phenolic fraction, consisting of a single component, gave 3,7,4′-trihydroxyflavylium chloride [yellow (visible), yellow fluorescent (UV); R_f 0.64; UV λ_{max} nm: 485] [22] and its flavonol analogue [green (UV); R_f 0.25] from the anthocyanidin reaction [11]. Alkali fusion under dry conditions [10] gave *p*-hydroxybenzoic acid (R_f 0.51; red with diazotized *p*-nitroaniline), *β*-resorcylic acid (R_f 0.37), protocatechuic acid (R_f 0.18), resorcinol (R_f 0.80), and phloroglucinol (R_f 0.63) in *n*-BuOH–HOAc–H_2O (6:2:1). Similar reactions and degradation products resulted from the remaining biflavanoids listed below.

The 'dimeric' proguibourtinidin (700 mg) was methylated and purified by prep. TLC in C_6H_6–MeOH (9:1, twice) to give the hexamethyl ether as an amorphous powder (R_f 0.44, 161 mg). (Found: C, 67.9; H, 6.2. $C_{36}H_{38}O_{10}$ requires: C, 68.5; H, 6.1%.) MS *m/z* (rel. int.): 630 $[M]^+$ (14.3). (Found: $[M]^+$ 630.2446. $C_{36}H_{38}O_{10}$ requires: 630.2465.) $[\alpha]_D^{25}$ −115° (Me_2CO, *c* 0.7).

Acetylation of the product (75 mg) and purification in C_6H_6–Me_2CO (4:1) afforded the pure diacetate **2** as a colourless amorphous powder (R_f 0.58, 42.2 mg). (Found: C, 67.0; H, 5.9. $C_{40}H_{42}O_{12}$ requires: C, 67.2; H, 5.9%.) $[\alpha]_D^{25}$ −114° (Me_2CO; *c* 0.8); ^{1}H NMR ($CDCl_3$, 100°, 80 MHz): *δ* 7.43–6.31 (*m*, 11 × arom. H), 6.19 [*s*, H-6(D)], 6.03 [*t*, Σ*J* = 19.25 Hz, H-3(C)], 5.39 [*m*, H-3(F)], 5.02 [*br s*, *J* < 1 Hz, H-2(F)], 4.94 [*d*, *J* = 9.75 Hz, H-4(C)], 4.91 (*d*, *J* = 9.5 Hz, H-2(C)], 3.86, 3.82, 3.81, 3.80, 3.76, 3.75 (each *s*, 6 × OMe), 2.93 [*m*, CH_2-4(F)], 1.74 [*s*, OAc-3(F)], 1.58 [*s*, OAc-3(C)]; CD: $[\theta]_{290}$ 0, $[\theta]_{287}$ +920, $[\theta]_{284}$ +610, $[\theta]_{268}$ +6150, $[\theta]_{251}$ 0, $[\theta]_{224}$ −59010, $[\theta]_{220}$ −50400, $[\theta]_{217}$ −70690, $[\theta]_{201}$ 0.

[4,8]-2,3-trans-3,4-trans: 2,3-trans-*Proguibourtinidin*-(+)-*catechin hexamethyl ether diacetate* (**6**). The R_f 0.68 phenolic fraction (1.4 g) was repurified by prep. PC by upwards development in 2% HOAc as above. The band (R_f 0.40), after elution with 70% EtOH, gave a pale brown amorphous powder (420 mg). The product gave the same anthocyanidin and degradation products as cited above.

Methylation of the phenolic material (400 mg) and two successive purifications in C_6H_6–MeOH (9:1, twice) to rid the product of traces of impurity of similar R_f gave the hexamethyl ether as an amorphous solid (R_f 0.58, 62 mg), mp 104°, MS *m/z* (rel. int.) 630 $[M]^+$ (46). (Found: 630.2434. $C_{38}H_{38}O_{10}$ requires: 630.2465.) $[\alpha]_D^{25}$ −111.6° (Me_2CO; *c* 0.7).

After acetylation of the hexamethyl ether (37.5 mg) and prep. TLC in C_6H_6–Me_2CO (4:1), the pure diacetate **6** was obtained as an amorphous solid (R_f 0.62, 28 mg), mp 104°. (Found: C, 66.9; H, 6.0. $C_{40}H_{42}O_{12}$ requires: C, 67.2; H, 5.9%.) $[\alpha]_D^{25}$ −119.8° (Me_2CO *c* 0.9); ^{1}H NMR ($CDCl_3$, 100°, 80 MHz): *δ* 7.14 [*d*, *J* = 8.8 Hz, H-2′, H-6′(B)], 6.77 [*d*, *J* = 8.8 Hz, H-3′, H-5′(B)], 6.86–6.50 (*m*, 4 × arom. H), 6.39 [*dd*, *J* = 2.5, 8.5 Hz, H-6(A)], 6.31 [*d*, *J* = 2.5 Hz, H-8(A)], 6.17 [*s*, H-6(D)], 5.97 [*t*, Σ*J* = 19.5 Hz, H-3(C)], 5.09 [*m*, H-3(F)], 4.88 [*d*, *J* = 7.5 Hz, H-2(F)], 4.84 [*dd*, *J* = 1.0, 10.0 Hz, H-4(C)], 4.78 [*d*, *J* = 9.5 Hz, H-2(C)], 3.84, 3.81, 3.77 (× 3), 3.71 (each *s*, 6 × OMe), 3.06 [*dd*, *J* = 5.5, 16.0 Hz, H_{eq}-4(F)], 2.63 [*dd*, *J* = 7.5, 16.0 Hz, H_{ax}-4(F)], 1.87 [*s*, OAc-3(F)], 1.58 [*s*, OAc-3(C)]; CD: $[\theta]_{280}$ 0, $[\theta]_{260}$ +3980, $[\theta]_{251}$ 0, $[\theta]_{219}$ −95100, $[\theta]_{218}$ −87270, $[\theta]_{214}$ −105950, $[\theta]_{204}$ 0.

[4,6]-2,3-trans-3,4-trans: 2,3-trans-*Proguibourtinidin*-(+)-*catechin hexamethyl ether diacetate* (**8**). The same phenolic band,

R_f 0.68 (940 mg), from which the [4,8]-positional isomer **6** was derived, but obtained by identical means from the heartwood extractives of *A. luederitzii* Engl. var. *retinens*, was methylated. Separation of the methyl ethers by prep. TLC in C_6H_6–MeOH gave a product, R_f 0.63, which on subsequent separation in $CHCl_3$–MeCOEt (4:1) yielded two hexamethyl ethers at R_f 0.59 (70.8 mg), 0.51 (52.7 mg). The former, on acetylation, gave a diacetate with ^{1}H NMR and CD spectra identical with those of the [4,8]-positional isomer **6**.

The latter lower R_f (0.51) hexamethyl ether was isolated as a non-crystalline solid, mp 118°, MS *m/z* (rel. int.): 630 $[M]^+$ (34); $[\alpha]_D^{28}$ −88.5° (Me_2CO; *c* 0.6) which, on acetylation, gave the hexamethyl ether diacetate **8** as a non-crystalline compound. (Found: C, 66.8; H, 6.0. $C_{40}H_{42}O_{12}$ requires: C, 67.2, H, 5.9%.) MS *m/z* (rel. int.): 714 $[M]^+$ (15.1); $[\alpha]_D^{28}$ −87.3° (Me_2CO; *c* 0.9); ^{1}H NMR (DMSO-d_6, 180°, 300 MHz): δ7.21 [*d*, *J* = 8.5 Hz, H-2′, H-6′(B)], 6.91–6.79 [*m*, H-2′, H-5′, H-6′(E)], 6.84 [*d*, *J* = 8.5 Hz, H-3′, H-5′(B)], 6.54 [*dd*, *J* = 1.2, 8.5 Hz, H-5(A)], 6.33 [*dd*, *J* = 2.5, 8.5 Hz, H-6(A)], 6.31 [*s*, H-8(D)], 6.21 [*d*, *J* = 2.5 Hz, H-8(A)], 5.75 [*t*, Σ*J* = 19.2 Hz, H-3(C)], 5.13 [*m*, H-3(F)], 4.84 [*d*, *J* = 9.6 Hz, H-2(C)], 4.79 [*dd*, *J* = 1.2, 9.6 Hz, H-4(C)], 4.57 [*br d* (unresolved), H-2(F)], 3.80, 3.78, 3.76, 3.71, 3.70 (*br*), 3.60 (each *s*, 6 × OMe), 2.91 [*dd*, *J* = 5.5, 16.5 Hz, H_{eq}-4(F)], 2.64 [*dd*, *J* = 7.5, 16.5 Hz, H_{ax}-4(F)], 1.82 [*s*, OAc-3(F)], 1.59 [*s*, OAc(C)]; CD: $[\theta]_{290}$ 0, $[\theta]_{281}$ −580, $[\theta]_{277}$ 0, $[\theta]_{265}$ +2510, $[\theta]_{249}$ 0, $[\theta]_{221}$ −27420, $[\theta]_{216}$ −24500, $[\theta]_{213}$ −42000, $[\theta]_{208}$ −16330, $[\theta]_{205}$ −19830, $[\theta]_{200}$ −10500.

[4,6]-2,3-trans-3,4-trans : 2,3-cis-*Proquibourtinidin*-(−)-*epicatechin hexamethyl ether diacetate* (**4**). The R_f 0.55 phenolic fraction (400 mg) was methylated and the mixture of hexamethyl ethers was resolved by prep. TLC in C_6H_6–Me_2CO (4:1) into three fractions at R_f 0.40 (37.3 mg), 0.29 (39.1 mg), 0.25 (112 mg). Each methyl ether fraction was acetylated and the product purified or subjected to further separation. The hexamethyl ether diacetate from the R_f 0.40 methyl ether proved to be identical with **2** after purification in $(CH_2Cl)_2$–Me_2CO (19:1) (R_f 0.42, 19.2 mg). The diacetates resulting from the R_f 0.25 hexamethyl ethers on prep. TLC separation in C_6H_6–Me_2CO (9:1) gave two products at R_f 0.45 (24.4 mg), which proved to be identical with **6**, and at R_f 0.40 (47 mg). Purification of the latter in CH_2Cl_2–Me_2CO (49:1, × 6) gave a solid (R_f 0.62, 12.3 mg) of the hexamethyl ether diacetate **4**, MS *m/z* (rel. int.): 714 $[M]^+$ (1.3); ^{1}H NMR ($CDCl_3$, 100°, 80 MHz): δ7.38 [*d*, *J* = 8.8 Hz, H-2′, H-6′(B)], 6.89 [*d*, *J* = 8.8 Hz, H-3′, H-5′(B)], 7.06–6.23 (*m*, 7 × arom. H), 6.17 [*s*, H-8(D)], 6.06 [*t*, Σ*J* = 19.6 Hz, H-3(C)], 5.39 [*m*, H-3(F)], 4.97 [*dd*, *J* = 1.0, 9.75 Hz, H-4(C)], 4.88 [*d*, *J* = 10.0 Hz, H-2(C)], 4.69 [*br s*, *J* < 1 Hz, H-2(F)], 3.88, 3.81, 3.80 (twice), 3.76, 3.66 (each *s*, 6 × OMe), 2.94 [*m*, CH_2-4(F)], 1.89 [*s*, OAc-3(F)], 1.59 [*s*, OAc-3(C)]; CD: $[\theta]_{280}$ 0, $[\theta]_{267}$ +4250, $[\theta]_{252}$ 0, $[\theta]_{225}$ −26920, $[\theta]_{219}$ −16290, $[\theta]_{214}$ −26210, $[\theta]_{200}$ 0.

Significant ions in the mass fragmentations of the hexamethyl ether diacetates **2**, **4**, **6** and **8** are, respectively, MS *m/z* (rel. int.): 714 (6.5, 1.3, 11.0, 15.1), 654 (100, 100, 100, 100), 623 (21, 24, 28, 18.8), 594 (85, 77, 90, 59), 580 (5.3, 5.2, 5.5, —), 563 (18.8, 25, 14.8, 11.3), 492 (7.9, 4.9, 5.5, —), 491 (15.8, 16.8, 17.5, 13.2), 443 (74, 92, 66, —), 433 (17.6, 17.6, —, 14.8), 432 (53, 46, 55, 40), 431 (25, 28, 26, 16.9), 417 (23, 31, 24, —), 401 (21, 29, 22, 15.1), 387 (2.1, 2.4, 2.1, —), 327 (12.6, 9.9, 10.7, 9.4), 325 (9.7, 11.5, 18.6, 7.5), 300 (1.5, 1.8, 6.6, —), 297 (9.1, 8.8, 23, —), 285 (15.3, 16.9, 21, —), 284 (5.3, 2.9, 15.7, —), 269 (74, 98, 55, 50), 267 (11.8, 16.0, 12.4, 13.2), 222 (1.5, 2.4, 2.4, 7.5), 192 (1.2, 2.1, 2.4, 4.0), 180 (23, 44, 31, 30), 151 (47, 73, 62, 36).

The remaining fractions from the primary PC separation in 2% HOAc contained various admixtures of the above proguibourtinidin biflavanoids.

Proguibourtinidin biflavanoid carboxylic acids. The carboxylic fraction from the preliminary separation (14 g) was resolved by PC using upwards development in 2% HOAc. Two products at R_f 0.55 (1.28 g, R_f 0.35/0.45 in H_2O satd butan-2-ol and 2% HOAc, respectively) and 0.74 (1.12 g, R_f 0.35/0.54), both orange-pink with *p*-toluenesulphonic acid were obtained after elution of the bands with 20% Me_2CO and removal of the solvent. Both fractions gave the identical anthocyanidin and alkaline degradation products as their biflavanoid homologues (see above).

[4,8]-2,3-trans-3,4-trans : 2,3-cis-*Proguibourtinidin*-(−)-*epicatechin-6-carboxylic acid methyl ester hexamethyl ether diacetate* (**1**). The reseparated R_f 0.74 fraction (600 mg) was methylated and the product purified by prep. TLC in C_6H_6–Me_2CO (7:3). Since the methyl ether methyl esters at R_f 0.32 (145 mg) could not be resolved, they were acetylated and the diacetate esters separated in $(CH_2Cl)_2$–Me_2CO (47:3, × 3) giving two regioisomers at R_f 0.48 (40 mg), 0.52 (93 mg).

The R_f 0.48 isomer was isolated as an amorphous solid, mp 96°. (Found: C, 64.7; H, 5.6. $C_{42}H_{44}O_{14}$ requires: C, 65.3; H, 5.7%.) $[\alpha]_D^{25}$ −126.3° (Me_2CO; *c* 0.6), MS *m/z* (rel. int.): 772 $[M]^+$ (5.3). [Found for accurate mass determination on the *m/z* (rel. int.) 652 (38) ion: 652.2386. $C_{40}H_{40}O_{12}$ requires: 652.2308.] ^{1}H NMR ($CDCl_3$, 31°, 80 MHz): δ7.06 [*d*, *J* = 8.5 Hz, H-2′, H-6′(B)], 6.74 [*d*, *J* = 8.5 Hz, H-3′, H-5′(B)], 6.97–6.20 (*m*, 7 × arom. H), 6.10 [*t*, Σ*J* = 19.25 Hz, H-3(C)], 5.29 [*m*, H-3(F)], 5.04 [*s*, *J* < 1 Hz, H-2(F)], 4.92 [*d*, *J* = 9.5 Hz, H-2(C)], 4.76 [*dd*, *J* = 1.0, 9.75 Hz, H-4(C)], 3.96, 3.87 (twice), 3.81, 3.77, 3.75 (twice) (each *s*, 7 × OMe), 3.01 [*m*, CH_2-4(F)], 1.77 [*s*, OAc-3(F)], 1.60 [*s*, OAc-3(C)]; CD: $[\theta]_{290}$ 0, $[\theta]_{275}$ +3890, $[\theta]_{270}$ +3740, $[\theta]_{257}$ +4670, $[\theta]_{247}$ 0, $[\theta]_{227}$ −49300, $[\theta]_{218}$ −44530, $[\theta]_{215}$ −63030, $[\theta]_{200}$ −3110.

[4,6]-2,3-trans-3,4-trans : 2,3-cis-*Proguibourtinidin*-(−)-*epicatechin-8-carboxylic acid methyl ester hexamethyl ether diacetate* (**3**). The R_f 0.52 isomer was isolated as an amorphous solid, MS *m/z* (rel. int.): 772 $[M]^+$ (19.7); ^{1}H NMR (DMSO-d_6, 170°, 80 MHz): δ7.31 [*d*, *J* = 8.8 Hz, H-2′, H-6′(B)], 6.89 [*d*, *J* = 8.8 Hz, H-3′, H-5′(B)], 7.05–6.59 [*m*, 3 × arom. H(E)], 6.58 [*d*, *J* = 8.5 Hz, H-5(A)], 6.39 [*dd*, *J* = 2.5, 8.5 Hz, H-6(A)], 6.23 [*d*, *J* = 2.5 Hz, H-8(A)], 5.94 [*t*, Σ*J* = 19.25 Hz, H-3(C)], 5.32 [*m*, H-3(F)], 4.91 [*d*, *J* = 9.5 Hz, H-2(C)], 4.82 [*d*, *J* = 9.75 Hz, H-4(C)], 4.75 [*s*, *J* < 1 Hz, H-2(F)], 3.84, 3.81, 3.80, 3.75, 3.73, 3.69, 3.63 (each *s*, 7 × OMe), 3.03 [*m*, CH_2-4(F)], 1.83 [*s*, OAc-3(F)], 1.59 [*s*, OAc-3(C)]; CD: $[\theta]_{290}$ 0, $[\theta]_{275}$ +6180, $[\theta]_{260}$ 0, $[\theta]_{232}$ −32810, $[\theta]_{219}$ −8880, $[\theta]_{207}$ −68710, $[\theta]_{200}$ −15460.

[4,8]-2,3-trans-3,4-trans : 2,3-trans-*Proguibourtinidin*-(+)-*catechin-6-carboxylic acid methyl ester hexamethyl ether diacetate* (**5**). The R_f 0.55 carboxylic fraction (800 mg) was methylated and the mixture separated by prep. TLC in C_6H_6–Me_2CO (7:3), R_f 0.34 (268 mg). Due to incomplete resolution the product was acetylated and the diacetates first purified in C_6H_6–Me_2CO (9:1), R_f 0.45 (125 mg) and, finally, resolved in $(CH_2Cl)_2$–Me_2CO (39:1, × 4) to give two pure products at R_f 0.42 (71 mg), 0.37 (35.5 mg).

The R_f 0.42 diacetate methyl ester **5** was isolated as an amorphous solid, mp 103.5°, $[\alpha]_D^{25}$ −113.8° (Me_2CO; *c* 0.8), MS *m/z* (rel. int.): 772 (6.9). [Found for accurate mass determination on the *m/z* (rel. int.) 652 (67) ion: 652.2299. $C_{40}H_{40}O_{12}$ requires: 652.2308.] ^{1}H NMR ($CDCl_3$, 31°, 80 MHz): δ7.07 [*d*, *J* = 8.8 Hz, H-2′, H-6′(B)], 6.74 [*d*, *J* = 8.5 Hz, H-3′, H-5′(B)], 6.92–6.28 (*m*, 6 × arom. H), 5.70 [*t*, Σ*J* = 19.25 Hz, H-3(C)], 5.09 [*m*, H-3(F)], 4.92 [*d*, *J* = 7.5 Hz, H-2(F)], 4.83 [*d*, *J* = 9.25 Hz, H-2(C)], 4.73 [*dd*, *J* = 1.0, 9.25 Hz, H-4(C)], 3.95, 3.87 (twice), 3.78, 3.77, 3.75, 3.71 (each *s*, 7 × OMe), 3.07 [*dd*, *J* = 5.0, 16.0 Hz, H_{eq}-4(F)], 2.70 [*dd*, *J* = 7.5, 16.0 Hz, H_{ax}-4 (F)], 1.90 [*s*, OAc-3(F)], 1.62 [*s*, OAc-3(C)]; CD: $[\theta]_{285}$ 0, $[\theta]_{270}$ +6180, $[\theta]_{250}$ 0, $[\theta]_{230}$ −72570, $[\theta]_{225}$ −77970, $[\theta]_{215}$ −96500, $[\theta]_{200}$ −10810.

[4,6]-2,3-trans-3,4-trans : 2,3-trans-*Proguibourtinidin*-(+)-*catechin-8-carboxylic acid methyl ester hexamethyl ether diacetate*

(7). The R_f 0.37 diacetate methyl ester was obtained as an amorphous solid, mp 102.8°, $[\alpha]_D^{25}$ −114.1° (Me_2CO: *c* 0.8), MS *m/z* (rel. int.): 772 $[M]^+$ (20). [Found for accurate mass determination on the *m/z* (rel. int.) 652 (60) ion: 652.2298. $C_{40}H_{40}O_{12}$ requires: 652.2308.] ^1H NMR ($CDCl_3$, 31°, 80 MHz): δ7.17 [*d*, *J* = 8.5 Hz, H-2′, H-6′(B)], 6.84 [*d*, *J* = 8.5 Hz, H-3′, H-5′(B)], 6.96–6.75 (*m*, 3 × arom. H), 6.60 [*d*, *J* = 8.5 Hz, H-5(A)], 6.35 [*dd*, *J* = 2.5, 8.5 Hz, H-6(A)], 6.25 [*d*, *J* = 2.5 Hz, H-8(A)], 6.02 [*t*, Σ*J* = 19.0 Hz, H-3(C)], 5.25 [*m*, H-3(F)], 4.80 [*d*, *J* = 9.25 Hz, H-2(C)], 4.69 [*dd*, *J* = 1.0, 9.5 Hz, H-4(C)], 4.43 [*d*, *J* = 7.0 Hz, H-2(F)], 3.95, 3.90, 3.89, 3.84, 3.79 (twice), 3.68 (each *s*, 7 × OMe), 3.02 [*dd*, *J* = 5.0, 16.0 Hz, H_{eq}-4(F)], 2.67 [*dd*, *J* = 7.5, 16.0 Hz, H_{ax}-4(F)], 1.85 (*s*, OAc-3(F)], 1.67 [*s*, OAc-3(C)]; CD: $[\theta]_{285}$ 0, $[\theta]_{275}$ +6180, $[\theta]_{260}$ 0, $[\theta]_{230}$ −44 780, $[\theta]_{219}$ −40 920, $[\theta]_{214}$ −51 720, $[\theta]_{200}$ −11 580.

Significant ions in the mass spectral fragmentations of methyl ester hexamethyl ether diacetates **1**, **3**, **5** and **7** are, respectively, MS *m/z* (rel. int.): 772 $[M]^+$ (6.5, 8.8, 3.2, 20), 741 (8.7, 10.1, 15.9, 41), 726 (5.4, 8.2, 7.1, 29), 715 (6.0, 8.4, 7.0, —), 712 (89, 82, 90, 82), 697 (10.7, 14.8, 19.2, 47), 681 (24, 26, 41, 53), 670 (10.3, 14.3, —, —), 652 (38, 35, 67, 60), 637 (14.6, 19.9, 27, 49), 621 (14.4, 19.3, 24, 51), 593 (16.9, —, 22, 49), 549 (19, —, 29, 51), 501 (65, 67, 82, 67), 477 (10.1, 13.4, —, —), 475 (31, 37, 45, 55), 490 (30, 29, 35, —), 459 (24, 30, 35, 52), 445 (6.5, 8.5, 8.2, 26), 443 (11.0, 12.3, 16.1, 33), 431 (8.5, 10.8, 9.8, 24), 385 (13.2, 16.3, 11.4, 34), 371 (67, 67, 78, 65), 327 (83, 82, 88, 76), 325 (8.1, 8.4, 9.4, 25), 300 (4.6, 2.7, 3.0, 7.4), 294 (2.8, 3.3, 3.1, 7.4), 269 (10.5, 10.4, 6.7, 22), 267 (21, 30, 32, 52), 222 (7.3, 8.1, 6.8, 22), 180 (79, 83, 84, 66), 151 (90, 86, 89, 76).

Isolation of minor phenolic components: leuco- and proguibourtinidins. The carboxyl-free fraction (20 g) was separated on a Sephadex LH-20 column (200 × 5 cm) with 10% Me_2CO–EtOH, after suitable pre-treatment of the substrate with the same solvent. Successive fractions (15 ml each) were collected and, after examination by 2D chromatography in H_2O satd butan-2-ol and 2% HOAc, they were suitably grouped in the following elution sequence.

(+)-2,3-trans-3,4-cis-4,4′,7-*Trimethoxyflavan*-3-*ol* (**11**). The fraction R_f 0.85/0.55 was methylated and the product (27 mg) purified by prep. TLC in $(CH_2Cl)_2$–Me_2CO (97:3) to give a colourless solid, R_f 0.48 (8.8 mg), ^1H NMR ($CDCl_3$, 30°, 80 MHz): δ7.39 [*d*, *J* = 8.8 Hz, H-2′, H-6′(B)], 7.15 [*d*, *J* = 8.5 Hz, H-5(A)], 6.94 [*d*, *J* = 8.8 Hz, H-3′, H-5′(B)], 6.53 [*dd*, *J* = 2.5, 8.5 Hz, H-6(A)], 6.47 [*d*, *J* = 2.5 Hz, H-8(A)], 5.05 [*d*, *J* = 10.0 Hz, H-2(C)], 4.27 [*d*, *J* = 4.0 Hz, H-4(C)], 4.06 [*m*, H-3(C)], 3.82, 3.77 (each *s*, 2 × OMe), 3.50 [*s*, OMe-4(C)], 2.53 [*d*, *J* = 8.7 Hz, OH-3(C)] (cf. ref. [24] and synthetic racemate, ref [2]).

Acetylation of the methyl ether gave the 3-*O*-acetyl derivative, MS *m/z* (rel. int.): 358 $[M]^+$ (21), 298 (14), 284 (19), 267 (54), 256 (22), 192 (59), 191 (12.7), 181 (17.6), 167 (58), 166 (21), 165 (19.3), 161 (10.7), 150 (100); CD: $[\theta]_{280}$ 0, $[\theta]_{290}$ +145, $[\theta]_{247}$ +116, $[\theta]_{234}$ +4840, $[\theta]_{232}$ +4165, $[\theta]_{231}$ +5330, $[\theta]_{227}$ 0, $[\theta]_{225}$ −1890, $[\theta]_{223}$ 0, $[\theta]_{217}$ −3490, $[\theta]_{212}$ −678.

(+)-2,3-trans-3,4-trans-7,4′-*Dimethoxyflavan*-3,4-*diol* (**12**). The fraction R_f 0.90/0.55 was methylated and the methyl ether (32 mg) purified by prep. TLC in $(CH_2Cl)_2$–Me_2CO (97:3). The dimethyl ether, R_f 0.50 (7.9 mg) was isolated as needles from EtOH–H_2O, mp 75°, ^1H NMR ($CDCl_3$, 30°, 80 MHz) δ7.42 [*d*, *J* = 8.8 Hz, H-2′, H-6′(B)], 7.31 [*d*, *J* = 8.5 Hz, H-5(A)], 6.97 [*d*, *J* = 8.8 Hz, H-3′, H-5′(B)], 6.61 [*dd*, *J* = 2.5, 8.5 Hz, H-6(A)], 6.42 [*d*, *J* = 2.5 Hz, H-8(A)], 4.85 [*d*, *J* = 8.0 Hz, H-4(C)], 4.84 [*d*, *J* = 10.0 Hz, H-2(C)], 3.89 [*dd*, Σ*J* = 18.0 Hz, H-3(C)], 3.84, 3.77 (each *s*, 2 × OMe), 1.56 (*br s*, 2 × OH) (cf. ref. [21] and racemate, ref. [2]).

Acetylation of the dimethyl ether afforded the diacetate as a solid (6.5 mg), MS *m/z* (rel. int.): 386 $[M]^+$ (7.8), 327 (35), 326 (60), 285 (41), 284 (64), 283 (60), 267 (100), 257 (29), 256 (60), 255 (28), 241 (28), 239 (10.8), 227 (37), 192 (31), 177 (50), 176 (36), 165 (48), 156 (64), 150 (93); CD: $[\theta]_{293}$ 0, $[\theta]_{268}$ −1215, $[\theta]_{247}$ +347, $[\theta]_{226}$ −14 060, $[\theta]_{221}$ −12 150, $[\theta]_{218}$ −13 880, $[\theta]_{214}$ −8680, $[\theta]_{212}$ −11 630, $[\theta]_{205}$ −3820. The CD spectrum is in complete agreement with that of (+)-2,3-*trans*-3,4-*trans*-leucofisetinidin trimethyl ether diacetate (cf. ref. [23]) and leucoguibourtinidin from *A. luederitzii* accordingly belongs to the (+)-series of leucoanthocyanidins with 2*R*,3*S*,4*R*-absolute configuration (cf. ref. [24]).

[4,8]-2,3-trans-3,4-cis: 2,3-trans-*Proguibourtinidin*-(+)-*catechin hexamethyl ether diacetate* (**9**). The fraction R_f 0.75/0.55 was methylated and the product (80 mg) subjected to preliminary purification by prep. TLC in C_6H_6–Me_2CO (4:1). The resultant methyl ether, R_f 0.44 (32 mg), was acetylated and the product purified in $(CH_2Cl)_2$–Me_2CO (97:3) to give a colourless solid, R_f 0.22 (5.2 mg), ^1H NMR ($CDCl_3$, 100°, 80 MHz): δ7.22 [*d*, *J* = 8.8 Hz, H-2′, H-6′(B)], 6.77 [*d*, *J* = 8.5 Hz, H-3′, H-5′(B)], 6.92–6.18 (*m*, 6 × arom. H), 6.16 [*s*, H-6(D)], 5.53 [*dd*, *J* = 6.5, 8.5 Hz, H-3(C)], 5.28 [*d*, *J* = 8.5 Hz, H-2(C)], 5.12 [*m*, H-3(F)], 4.93 [*d*, *J* = 6.5 Hz, H-4(C)], 4.75 [*d*, *J* = 7.6 Hz, H-2(F)], 3.80 (twice), 3.73, 3.72, 3.68, 3.60 (each *s*, 6 × OMe), 3.07 [*dd*, *J* = 5.5, 16.0 Hz, H_{eq}-4(F)], 2.65 [*dd*, *J* = 7.5, 16.0 Hz, H_{ax}-4(F)], 1.83 [*s*, OAc-3(F)], 1.68 [*s*, OAc-3(C)]; MS *m/z* (rel. int.): 714 $[M]^+$ (81), 654 (28), 624 (8.8), 594 (81), 579 (13), 563 (18.3), 491 (87), 443 (76), 433 (35), 432 (70), 417 (32), 401 (17.3), 387 (5), 327 (16.7), 325 (15.4), 301 (19), 297 (29), 285 (82), 284 (46), 269 (100), 267 (40), 222 (6), 192 (4.4), 180 (80), 151 (83); CD: $[\theta]_{350}$ 0, $[\theta]_{295}$ −1980, $[\theta]_{283}$ 0, $[\theta]_{267}$ −6430, $[\theta]_{252}$ 0, $[\theta]_{232}$ +74 260, $[\theta]_{200}$ +2140.

[4,8]-2,3-trans-3,4-trans: 2,3-trans-*Proguibourtinidin*-(+)-*afzelechin pentamethyl ether diacetate* (**10**). The fraction R_f 0.90–0.75/0.50–0.55 was methylated and the mixture of methyl ethers (15 mg) was resolved in C_6H_6–Me_2CO (4:1) by prep. TLC to give three fractions at R_f 0.55 (55 mg), 0.48 (47 mg), 0.42 (61 mg). Each fraction was acetylated and the products separated by prep. TLC in $(CH_2Cl_2$–Me_2CO (97:3).

The diacetates from the methyl ether fraction, R_f 0.55, separated into two products at R_f 0.28 (4.7 mg), 0.34 (2.3 mg). The former was identical with the hexamethyl ether diacetate of [4,8]-2,3-*trans*-3,4-*trans*: 2,3-*cis*-proguibourtinidin-(−)-epicatechin (**2**). The latter was isolated as a solid, ^1H NMR ($CDCl_3$, 100°, 80 MHz): δ7.16 [*d*, *J* = 8.8 Hz, H-2′, H-6′(B)], 6.78 [*d*, *J* = 8.8 Hz, H-3′, H-5′(B)], 6.69 [*br d*, *J* ~ 8 Hz, H-2′, H-6′(E)], 6.56 [*br d*, *J* ~ 8 Hz, H-3′, H-5′(E)], 6.42 [*dd*, *J* = 2.5, 8.5 Hz, H-6(A)], 6.43 [*d*, *J* = 2.5 Hz, H-8(A)], 6.16 [*s*, H-6(D)], 5.96 [*t*, Σ*J* = 19.5 Hz, H-3(C)], 5.08 [*m*, H-3(F)], 4.94 [*d*, *J* = 7.5 Hz, H-2(F)], 4.88 [*dd*, *J* = 1.0, 10.0 Hz, H-4(C)], 4.79 [*d*, *J* = 9.5 Hz, H-2(C)], 3.81, 3.78 (twice), 3.77, 3.75 (each *s*, 5 × OMe), 3.03 [*dd*, *J* = 5.5, 16.0 Hz, H_{eq}-4(F)], 3.66 [*dd*, *J* = 7.5, 16.0 Hz, H_{ax}-4(F)], 1.91 [*s*, OAc-3(F)], 1.61 [*s*, OAc-3(C)]; MS *m/z* (rel. int.): 684 $[M]^+$ (3.6), 624 (84), 593 (41), 563 (76), 549 (19), 533 (24), 461 (28), 449 (16.6), 443 (76), 432 (75), 419 (17.4), 417 (45), 401 (57), 327 (10.6), 325 (22), 313 (75), 299 (10.1), 297 (21), 285 (26), 269 (83), 267 (30), 241 (25), 226 (15.3), 216 (32), 192 (4.5), 180 (6.6), 179 (17.2), 165 (6.2), 151 (23), 150 (81), 149 (24); CD: $[\theta]_{283}$ 0, $[\theta]_{265}$ +9210, $[\theta]_{248}$ 0, $[\theta]_{225}$ −48 180, $[\theta]_{200}$ −10 860.

The diacetate from the methyl ether fraction, R_f 0.48, yielded the [4,8]-2,3-*trans*-3,4-*cis*: 2,3-*trans*-isomer, **9**, R_f 0.22 (15.1 mg), while the diacetate from the R_f 0.42 fraction corresponded to the [4,8]-2,3-*trans*-3,4-*trans*: 2,3-*trans*-isomer, **6**, R_f 0.21 (8.2 mg).

Carboxylation of (+)-*Catechin.* 6-*Carboxymethyl*-3-*O*-*acetyl*-5,7,3′,4′-*tetra*-*O*-*methyl*-(+)-*catechin.* (+)-Catechin (4 g) dissolved in satd $NaHCO_3$ (150 ml) was heated on a boiling water bath for 2.5 hr while CO_2 was bubbled through. After cooling, unreacted (+)-catechin was extracted with EtOAc (5 × 50 ml). The bicarbonate soln was acidified with 3 M H_2SO_4 and the carboxylic acid extracted with EtOAc. After drying (dry Na_2SO_4)

and removal of the solvent, the pale brown solid (1.2 g), R_f 0.34 (H_2O satd butan-2-ol) and 0.52 (2% HOAc) was methylated. The product was purified by prep. TLC in C_6H_6–Me_2CO (17:3), R_f 0.32 (488 mg), and acetylated. Prep. TLC of the product in Et_2O–hexane (3:1) gave 3-*O*-acetyl-5,7,3',4'-tetra-*O*-methyl-(+)-catechin, R_f 0.66, probably as a product of decarboxylation and its 6-carboxymethyl derivative as a colourless solid, R_f 0.43 (cf. ref. [6]), MS *m/z* 494 $[M]^+$; ^1H NMR ($CDCl_3$, 25°, 60 MHz): δ6.89 [*s*, H-2', H-4', H-5'(B)], 6.36 [*s*, H-8(A)], 5.35 [*m*, H-3(C)], 5.10 [*d*, *J* = 6.0 Hz, H-2(C)], 3.92, 3.88 (twice), 3.82, 3.78 (each *s*, 5 × OMe), 2.85 [*d*, CH_2-4(C)], 1.95 [*s*, OAc-3(C)].

Reduction of the 6-carboxymethyl derivative. $LiAlH_4$ (100 mg) in dry tetrahydrofuran (25 ml) was added to a soln of the methyl ester (200 mg) in dry tetrahydrofuran (20 ml) and the mixture stirred for 2.5 hr under reflux at 65°. After cooling, the excess $LiAlH_4$ was destroyed with H_2O satd EtOAc and the complex decomposed with 3 M H_2SO_4. After extraction with EtOAc (3 × 50 ml), the extract was washed with H_2O (3 × 100 ml), dried and taken to dryness under red. pres. The product, a mixture of 6-hydroxymethyl- and 6-methoxymethyl-5,7,3',4'-tetra-*O*-methyl-(+)-catechin, was purified by prep. TLC in C_6H_6–Me_2CO (7:3), R_f 0.29 (63 mg). The mixture was separated after acetylation in C_6H_6–Me_2CO (9:1).

6-Acetoxymethyl-3-O-acetyl-5,7,3',4'-tetra-O-methyl-(+)-catechin. The compound, R_f 0.45 (26.6 mg), was isolated as a pale yellow oil, MS *m/z* (rel. int.): 460 $[M]^+$ (25.5). (Found: *m/z* 460.1724. $C_{24}H_{28}O_9$ requires: *m/z* 460.1734.) ^1H NMR ($CDCl_3$, 25°, 60 MHz): δ6.90 [*br s*, H-2', H-4', H-6'(B)], 6.36 [*s*, H-8(A)], 5.39 [*m*, H-3(C)], 5.16 (*br s*, CH_2), 5.10 [*d*, *J* = 6.0 Hz, H-2(C)], 3.88 (twice), 3.79, 3.74 (each *s*, 4 × OMe), 3.03 [*dd*, *J* = 5.5, 16.0 Hz, H_{eq}-4(C)], 2.73 [*dd*, *J* = 8.0, 16.0 Hz, H_{ax}-4(C)], 2.07 (*s*, CH_2OAc-6), 1.94 [*s*, OAc-3(C)].

3-O-Acetyl-6-methoxymethyl-3',4',5,7-tetra-O-methyl-(+)-catechin. The compound, R_f 0.34 (28 mg), was isolated as a pale yellow oil, MS *m/z* (rel. int.): 432 (1); ^1H NMR ($CDCl_3$, 25°, 60 MHz): δ6.90 [*br s*, H-2', H-4', H-5'(B)], 6.35 [*s*, H-8(A)], 5.37 [*m*, H-3(C)], 5.08 [*d*, *J* = 6.0 Hz, H-2(C)], 4.60 (*s*, CH_2), 3.85 (× 3), 3.79, 3.75 (each *s*, 5 × OMe), 2.95 [*dd*, *J* = 5.5, 16.0 Hz, H_{eq}-4(C)], 2.71 [*dd*, *J* = 8.0, 16.0 Hz, H_{ax}-4(C)], 1.94 [*s*, OAc-3(C)].

The chemical shifts of the residual A-ring protons of the above 6-functionalized derivatives (δ6.36, 6.35) confirm (cf. ref. [6]) the position of substitution and, hence, that of carboxylation of (+)-catechin.

Acknowledgements—Support by the Sentrale Navorsingsfonds of this university and by the Foundation for Research Development, Council for Scientific and Industrial Research, Pretoria, South Africa is gratefully acknowledged. Wood samples of *A. luederitzii* var. *luederitzii* and var. *retinens* were supplied, respectively, by the Director, South West Africa Herbarium, Windhoek and by the Director, Botanical Research Institute, Pretoria, South Africa.

REFERENCES

1. Tindale, M. D. and Roux, D. G. (1974) *Phytochemistry* **13**, 829.
2. Saayman, H. M. and Roux, D. G. (1965) *Biochem. J.* **96**, 36.
3. Steynberg, J. P., Ferreira, D. and Roux, D. G. (1983) *Tetrahedron Letters* 4147.
4. Pelter, A., Amenechi, P. I., Warren, R. and Harper, S. H. (1969) *J. Chem. Soc. C* 2572.
5. du Preez, I. C., Rowan, A. C. and Roux, D. G. (1970) *J. Chem. Soc., Chem. Commun.* 492.
6. Hundt, H. K. L. and Roux, D. G. (1981) *J. Chem. Soc., Perkin Trans.* 1, 1227.
7. Viviers, P. M., Kolodziej, H., Young, D. A., Ferreira, D. and Roux, D. G. (1983) *J. Chem. Soc., Perkin Trans.* 1, 2555.
8. Kolodziej, H., Ferreira, D. and Roux, D. G. (1984) *J. Chem. Soc., Perkin Trans.* 1, 343.
9. Botha, J. J., Young, D. A., Ferreira, D. and Roux, D. G. (1981) *J. Chem. Soc., Perkin Trans.* 1, 1213.
10. van der Westhuizen, J. H., Ferreira, D. and Roux, D. G. (1981) *J. Chem. Soc., Perkin Trans.* 1, 1220.
11. du Preez, I. C., Rowan, A. C., Roux, D. G. and Feeney, J. (1971) *J. Chem. Soc., Chem. Commun.* 315.
12. Roux, D. G. (1958) *J. Am. Leather Chem. Assoc.* **53**, 384.
13. Pigman, W., Anderson, E., Fisher, R., Buchanan, M. A. and Browning, B. L. (1953) *Tappi* **36**, 4.
14. Freudenberg, K., Karimullah and Steinbrunn, G. (1935) *Justus Liebig's Ann. Chem.* **518**, 37;
15. Roux, D. G. and de Bruyn, G. C. (1963) *Biochem. J.* **87**, 439.
16. Delcour, J. A., Serneels, E. J., Ferreira, D. and Roux, D. G. (1985) *J. Chem. Soc., Perkin Trans.* 1 (in press).
17. Young, D. A., Cronjé, A., Botes, A. L., Ferreira, D. and Roux, D. G. (1985) *J. Chem. Soc., Perkin Trans.* 1 (in press).
18. Botes, A. L. (1983) M.Sc. Thesis, University of the Orange Free State, South Africa.
19. Delcour, J. A., Ferreira, D. and Roux, D. G. (1983) *J. Chem. Soc., Perkin Trans.* 1, 1711.
20. Hillis, W. E. and Carle, A. (1960) *Austr. J. Chem.* **13**, 390.
21. Von Kostanecki, S. (1885) *Chem. Ber.* **18**, 3205.
22. Roux, D. G. (1957) *Nature* **179**, 305.
23. Viviers, P. M., Young, D. A., Botha, J. J., Ferreira, D. and Roux, D. G. (1982) *J. Chem. Soc., Perkin Trans.* 1, 535.
24. du Preez, I. C. and Roux, D. G. (1970) *J. Chem. Soc. C* 1800.