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Oligomeric Flavanoids. Part 18[a]. Dimeric Prorobinetinidins from *Robinia pseudacacia*

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Abstract. The range of naturally occurring prorobinetinidins is extended by characterization of the novel robinetinidol-leucorobinetinidins **3**, **5**, **7**, and **9**, the robinetinidol-dihydrorobinetins **11**, **13**, and **15**, a robinetinidol-robinetin **17**, and a robinetinidol-flavone analogue **19**. The diversity regarding the oxidation level of the chain terminating moieties suggests that the biflavanoids in *Robinia pseudacacia* may be interrelated *via* oxidation/reduction of these units. The conspicuous absence of the effects of dynamic rotational isomerism about the interflavanyl bond in the ^{1}H NMR spectra of some of the derivatives is explained in terms of a preferred conformation of this bond rather than to 'free rotation'.

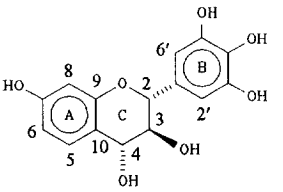
Prorobinetinidins with their 3′,4′,5′,7-tetrahydroxy phenolic functionality represent a relatively rare group of condensed tannins which nevertheless constitute the main components in the higher oligomeric fractions of the economically important black wattle ('Mimosa') bark extract[1-5]. In the durable heartwood of the locust tree (*Robinia pseudacacia* L.) the flavan-3,4-diol, leucorobinetinidin **1**, as incipient electrophile for prorobinetinidin biosynthesis, co-exists with a variety of monomeric flavonoids[6] as potential nucleophiles. These monomers, predominated by analogues with resorcinol A- and pyrogallol-type B-rings, however, invariably exhibit C-4 oxygenation which reduces the nucleophilicity of their A-rings compared to that of the corresponding functionality in the C-4 deoxy compounds, *e.g.* the flavan-3-ol, catechin[5]. The locust tree therefore represents a rare metabolic pool where oligomer formation occurs *via* the action of the very potent electrophile **1**[7] on chain terminating units apparently lacking the nucleophilicity that is associated with natural sources in which condensed tannin formation is paramount. We thus now disclose results relevant to a re-investigation of the polyphenols of *R. pseudacacia* with a view to expanding the scope of the chemistry of prorobinetinidin oligomers.

RESULTS AND DISCUSSION

The aqueous acetone (1:1) extract of the heartwood of *R. pseudacacia* was subjected to extensive enrichment procedures to effect a decrease in the concentration of the predominant metabolite leucorobinetinidin **1** and the dihydroflavonol, dihydrorobinetin **2** relative to that of the oligomeric analogues. Subsequent selection of the fractions most likely to contain the oligomers indeed afforded a series of novel dimeric compounds based exclusively upon 3′,4′,5′,7-tetraoxygenated monomeric precursors. These compounds comprised the robinetinidol-(4β,6) and (4α,2′)-leucorobinetinidins **3**, **5**, **7**, and **9**, the robinetinidol-(4α,2′), (4β,6), and (4α,8)-dihydrorobinetins **11**, **13**, and **15**, the robinetinidol-(4α,2′)-robinetin **17**, and the robinetinidol-(4β,2′)-tetrahydroxy-flavone **19**.

Owing to the high concentration of leucorobinetinidin **1** in a metabolic pool devoid of powerful nucleophilic species, participation of this flavan-3,4-diol as the chain extender unit in the formation of

a Part 17, W. Rossouw, A.F. Hundt, J.A. Steenkamp and D. Ferreira, *Tetrahedron*, 1994, in the press.



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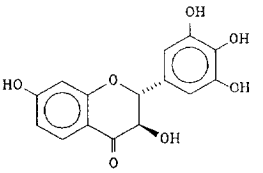

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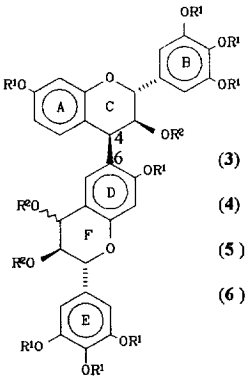
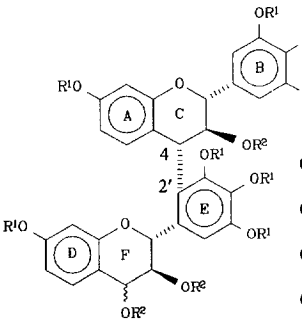
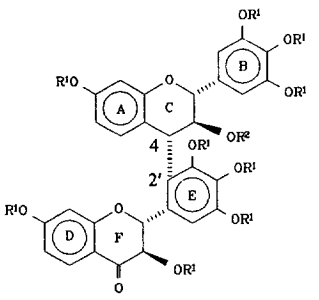


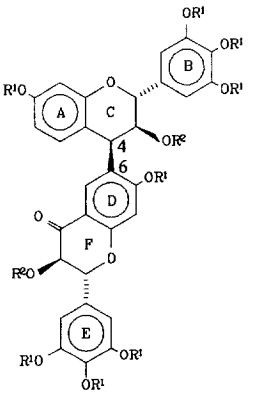
(3) ≡ , $R^1=R^2=H$
(4) ≡ , $R^1=Me, R^2=Ac$
(5) ≡ , $R^1=R^2=H$
(6) ≡ , $R^1=Me, R^2=Ac$



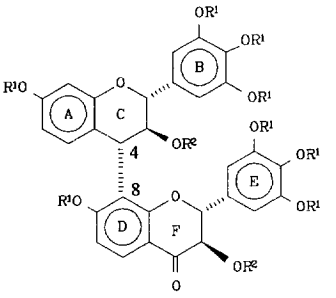
(7) ≡ , $R^1=R^2=H$
(8) ≡ , $R^1=Me, R^2=Ac$
(9) ≡ , $R^1=R^2=H$
(10) ≡ , $R^1=Me, R^2=Ac$



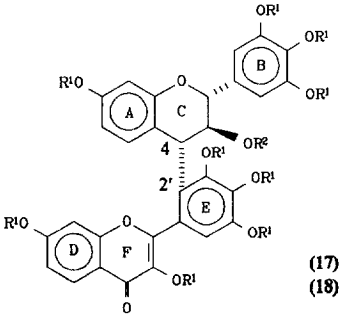
(11) $R^1=R^2=H$
(12) $R^1=Me, R^2=Ac$



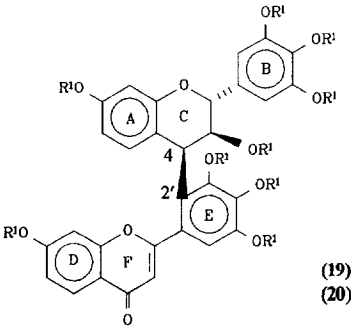
(13) $R^1=R^2=H$
(14) $R^1=Me, R^2=Ac$



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



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



(19) $R^1=H$
(20) $R^1=Me$

'unusual' oligoflavanoids in *R. pseudacacia* could be anticipated. The presence of the robinetinidol-type ABC-moiety in the methyl ether acetate derivatives of the aforementioned biflavanoids, *e.g.* **4**, was immediately evident from the ^{1}H NMR data (Tables 1 and 2) which exhibited the characteristic[3,5] two-proton singlet for the equivalent B-ring protons, an aromatic ABX-system for the A-ring, and an AMX-system for the protons of the heterocyclic C-ring. The spin systems of the remaining aromatic and heterocyclic protons were then reminiscent not only of the nature of the chain terminating DEF-unit but also of the mode of the interflavanyl linkage. Differentiation of the aromatic spin systems was effected *via* decoupling experiments using the heterocyclic 2-(for the B- and E-rings) and 4-protons (for the A- and D-rings), or *via* a 2D COSY experiment, a protocol that was applied throughout this paper.

The presence of an additional heterocyclic AMX-system comprising a conspicuously deshielded resonance indicative of the presence of a 4-acetoxy (F-ring) function[7,8], and only four remaining aromatic

Table 1. **^{1}H N.m.r. (300 MHz) peaks (p.p.m.) of prorobinetinidin derivatives 4, 6, 8, and 10 at 296K. Splitting patterns and J-values (Hz) are given in parentheses.**

Ring	H	**4**, $CDCl_3$	**6**, C_6D_6	**8**[a], $CDCl_3$	**8**[b], $CDCl_3$	**10**[a], $CDCl_3$	**10**[b], DMSO-d_6
A	5	6.73(d,8.5)	6.85(d,8.5)	6.90(d,8.5)	6.57(d,8.5)	6.82,6.74*(d,8.5)	
	6	6.47(dd,2.5,8.5)	6.50(dd,2.5,8.5)	6.48(dd,2.5,8.5)	6.41(dd,2.5,8.5)	6.46,6.53*(dd,2.5,8.5)	6.49-6.54
	8	6.59(d,2.5)	6.83(d,2.5)	6.55(d,2.5)	6.47(d,2.5)	6.55,6.52*(d,2.5)	
B	2/6	6.56(s)	6.79(s)	6.71(s)	6.54(s)	6.70,6.71*(s)	6.49(s)
C	2	5.20(d,6.0)	5.60(d,7.0)	4.83(d,9.5)	4.87(d,9.5)	4.78,4.93*(d,9.5)	4.95(d,9.5)
	3	5.53(dd,4.5,6.0)	6.04(dd,5.0,7.0)	6.05(t,9.5)	5.96(t,9.5)	5.98,5.84*(t,9.5)	5.57(t,9.5)
	4	4.70(d,4.5)	5.21(d,5.0)	4.68(d,9.5)	5.25(d,9.5)	4.55,4.71*(d,9.5)	5.13(d,9.5)
D	5	6.84(s)	7.21(s)	7.20(d,8.5)	7.06(d,8.5)	7.08,7.13*(d,8.5)	7.01(d,8.5)
	6	-	-	6.55(dd,2.5,8.5)	6.50(dd,2.5,8.5)	6.58,6.63*(dd,2.5,8.5)	6.61(dd,2.5,8.5)
	8	6.45(s)	6.42(s)	6.36(d,2.5)	6.62(d,2.5)	6.37,6.36*(d,2.5)	6.41(d,2.5)
E	2/6	6.64(s)	6.68(s)	-	-	-	-
	6	-	-	6.56(s)	6.78(s)	6.69,6.74*(s)	6.88(s)
F	2	5.15(d,10.5)	4.67(d,10.0)	5.73(d,10.5)	5.20(d,10.5)	5.54,5.72*(d,10.0)	4.86(d,10.0)
	3	5.45(dd,3.5,10.5)	5.95(dd,8.0,10.0)	5.92(dd,3.5,10.5)	5.69(dd,3.5,10.5)	5.95,5.85*(dd,7.5,10.0)	5.61(dd,7.5,10.0)
	4	6.04(d,3.5)	6.54(d,8.0)	6.28(d,3.5)	6.04(d,3.5)	6.31,6.26*(d,7.5)	5.48(d,7.5)
	OMe	3.71,3.78(x2), 3.80,3.82,3.84, 3.86(x2),each s	3.13,3.27,3.30 (x2),3.44(x2), 3.80,3.81,each s	3.23(3-E),3.69 (5-E),3.72(7-D), 3.74(7-A),3.82 (4-B),3.87(3/5-B),each s	3.67(7-D),3.68 (7-A),3.76(3/5-B),3.79(5-E), 3.80(4-B),3.94 (3-E),each s	3.23,3.73,3.74,3.77 (x2),3.82,3.87(x2), each s	
	OAc	1.82,1.83,2.09, each s	1.43,1.67,1.71, each s	1.70,1.92,2.09, each s	1.69,1.77,1.84, each s	1.71,1.86,2.08,each s	

[a] Peaks of the major rotamer
[b] Peaks of the minor rotamer
*** Peaks of compound 10 in DMSO-d_6**

Table 2 ^{1}H N.m.r. (300 MHz) peaks (p.p.m.)of prorobinetinidin **11** and derivatives **12**, **14**, **16**, **18**, and **20** at 296 K. Splitting patterns and J-values (Hz) are given in parentheses.

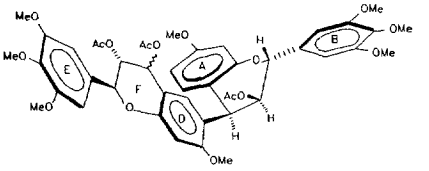
Ring	H	**11**, $(CD_3)_3CO$	**12**, $CDCl_3$	**14**, $CDCl_3$	**16**, $CDCl_3$	**18**, $CDCl_3$	**20**, $CDCl_3$
A	5	6.69(d,8.0)	6.78(d,8.5)	6.68(d,8.5)	6.62(d,8.5)	7.04,6.78[a](d,9.0)	6.77(dd,1.0,8.5)
	6	6.26(dd,2.5,8.0)	6.44(dd,2.5,8.5)	6.45(dd,2.5,8.5)	6.46(dd,2.5,8.5)	6.49(dd,2.5,9.0)	6.47(dd,2.5,8.5)
	8	6.33(d,2.5)	6.55(d,2.5)	6.56(d,2.5)	6.41(d,2.5)	6.51,5.70(d,2.5)	6.38(d,2.5)
B	2/6	6.53(s)	6.68(s)	6.56(s)	6.34(s)	6.62,6.19(s)	6.65(s)
C	2	4.45(d,9.5)	4.79(d,9.5)	5.12(d,7.5)	4.86(d,10.0)	4.65(d,9.5)	5.23(d,2.5)
	3	4.63(t,9.5)	6.02(t,9.5)	5.57(dd,5.0,7.5)	6.14(t,10.0)	5.95,6.10(t,9.5)	4.60(dd,2.5,3.5)
	4	4.46(d,9.5)	4.60(d,9.5)	4.77(d,5.0)	4.98(d,10.0)	4.09,5.04(d,9.5)	4.05(dd,1.0,3.5)
D	5	7.72(d,8.5)	7.86(d,8.5)	7.50(s)	7.90(d,9.0)	8.19,7.97(d,9.0)	7.65(d,8.5)
	6	6.60(dd,2.5,8.5)	6.63(dd,2.5,8.5)	-	6.76(d,9.0)	6.98,6.84(dd,2.5,9.0)	6.57(dd,2.5,8.5)
	8	6.33(d,2.5)	6.34(d,2.5)	6.50(s)	-	6.71,6.42(d,2.5)	5.44(d,2.5)
E	2/6	-	-	6.69(s)	6.35(s)	-	-
	6	6.88(s)	6.66(s)	-	-	6.67(s)	7.14(s)
F	2	4.65(d,11.5)	4.37(d,9.5)	5.36(d,12.5)	5.39(d,12.0)	-	-
	3	5.69(d,11.5)	5.74(d,9.5)	5.78(d,12.5)	5.46(d,12.0)	-	5.11(s)
	OMe		3.23(3-E),3.61 (3-F),3.74(7-A), 3.76(7-D),3.78 (4-E),3.81(4-B), 3.83(5-E),3.86 (3/5-B), each s	3.77,3.79(x2), 3.81,3.82,3.86, 3.87(x2),each s	3.58(3/5-B),3.70 (3/5-E),3.73 (7-A),3.75(4-E), 3.80(4-B),3.95 (7-D), each s	3.30(3-E),3.74(7-A), 3.79(4-B),3.82(7-D), 3.83(3/5-B),3.85(4-E), 3.86(5-E),3.87(3-F), each s	3.67,3.68,3.72 (x3),3.75,3.87, 3.92,3.94,each s
	OAc		1.66(s)	1.80,2.03,each s	1.54,2.01,each s	1.61(s)	-

[a] resonances of the minor rotamer

protons in their ^{1}H NMR spectra (Table 1), strongly indicated a leucorobinetinidin DEF-moiety in the octamethyl ether triacetates **4**, **6**, **8**, and **10**. Two one-proton singlets and a two-proton singlet in the aromatic region of the spectra of derivative **4** and **6** then signified a (4,6)-interflavanyl coupling for these compounds, while the (4,2′)-linkage for derivatives **8** and **10** was evident from the ABX-system and a single one-proton singlet in the aromatic region of their ^{1}H NMR spectra. The (4,6)-interflavanyl linkage in compounds **4** and **6** was unambiguously confirmed by a 2D COSY experiment which indicated $^4J_{HH}$ coupling of 5-H(D) to both 4-H(C) and 4-H(F). Allocation of the chemical shifts of 5- and 8-H(D) was additionaly corroborated by the strong NOE effect between 8-H(D) and 7-OMe(D) for both compounds **4** and **6**. NOE association of 3-OMe(E) with both 5-H(A) and 3-H(C) in derivatives **8** and **10** confirmed their (4,2′)-interflavanyl linkages.

Whereas the relative 2,3-*trans*-3,4-*trans* configurations for the C-rings of compounds **8** and **10** were evident from ^{1}H NMR coupling constants of heterocyclic protons ($J_{2,3} = J_{3,4} = 9.5$ Hz), these values were less informative for derivatives **4** and **6** [Table 1: $J_{2,3} = 6.0$, 7.0; $J_{3,4} = 4.5$ and 5.0 Hz for **4** and **6** respectively]. Significant NOE association between 5-H(D) and 2-H(C) in both these compounds is reminiscent of 2,4-*trans*

orientated B- and D-rings and thus of 2,3-*trans*-3,4-*cis* relative configuration of their C-rings. Such deviations in coupling constants of heterocyclic protons have lately been ascribed to significant contribution of A-forms towards the C-ring conformational equilibrium[9,10]. However, conspicuous differences in the magnitude of the NOE associations between 2-H(C) and 5-H(D) (6.8 and 3.3% for **4** and **6** respectively), permitted only for the E-conformation **21**, and between 4-H(C) and 2-/6-H(B) (1.2 and 1.1% for **4** and **6**



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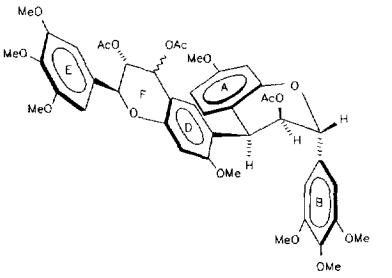

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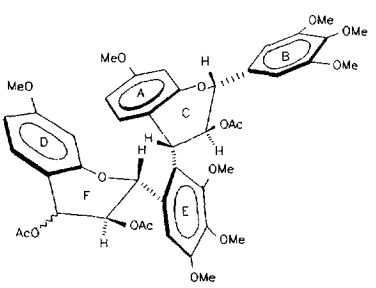
resp.), restricted to the A-conformation **22**, presumably indicates that the E-form **21** is the major contributor to the C-ring conformational equilibrium. The relative small coupling constants for the protons of these rings in both derivatives **4** and **6** may thus rather be attributed to a distorted ring, probably a sofa in stead of the half-chair, than to meaningful contributions of A- conformers. Coupling constants for the F-ring protons of derivatives **4**, **6**, **8**, and **10** permitted unambiguous assignment of the relative configurations of these rings *i.e.* 2,3- *trans*-3,4-*cis* for compounds **4** and **8** ($J_{2,3}$ = 10.5; $J_{3,4}$ = 3.5 Hz) and 2,3-*trans*-3,4-*trans* for analogues **6** and **10** ($J_{2,3}$ = 10.0, 10.0; $J_{3,4}$ = 8.0, 7.5 Hz for **6** and **10** resp.). High amplitude positive Cotton effects at *ca.* 240 nm. in the CD spectra of derivatives **4** and **6** indicated 4β-flavanyl substituents by application of the aromatic quadrant rule[11]. When taken in conjunction with coupling constants of C-ring protons, the absolute stereochemistry of this heterocycle may then be defined as 2*R*, 3*S*, 4*S* for both **4** and **6**. Negative Cotton effects at *ca.* 240 nm. in the CD spectra of derivatives **8** and **10** similarly facilitated definition of 2*R*, 3*S*, 4*R* absolute configuration of their C-rings. Comparison of the CD data of the prorobinetinidin derivatives **4** and **6** with those of analogous naturally occurring and synthetic profisetinidins[7] allowed designation of 2*R*, 3*S*, 4*S* and 2*R*, 3*S*, 4*R* configuration to the stereocenters of their F-rings respectively. The absolute configurations of the F-rings depicted in formulations **8** and **10** are, however, tentative and are based on the assumption that the DEF flavanyl units in the prorobinetinidins **4**, **6**, **8**, and **10** are biogenetically interrelated.

Whereas the ^{1}H NMR spectra of derivatives **8** and **10** each exhibit a duplicated set of signals, the spectra of compounds **4** and **6** are notably free of the effects of dynamic rotational isomerism about the interflavanyl bond[12,13] at ambient temperature. Such a single set of sharp resonances has previously invariably been ascribed to 'fast' rotation on the NMR time-scale[14]. However, prominent NOE associations of 5-H(D) with 2-H(C) and 5-H(A), and the conspicuous absence of associations between 7-OMe(D) and the latter protons are presumably reminiscent of the preponderance of a conformation **21** of the interflavanyl bond in which the 10-C(A), 4-C(C), 6-C(D), 7-C(D) dihedral angle approximates +90° to be consistent with molecular modeling work by Mattice[15,16]. A significant preference for this orientation may then explain the absence of signal duplication or broadened resonances in their ^{1}H NMR spectra. Information from a 2D-COSY-, an NOE-, and a decoupling experiment collectively facilitated allocation of the sets of signals of the constituent rotamers in the prorobinetinidin derivatives **8** and **10**. The NOE association of 3-OMe(E) with 3-H(C) in the dominant conformer **23** and of 3-OMe(E) with 4-H(C) in the minor rotamer **24** (rotamer



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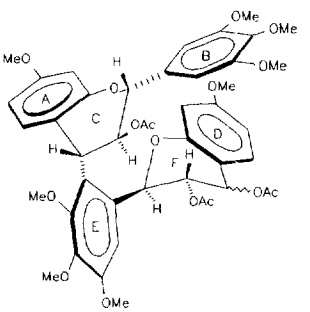

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populations *ca.* 70:30 and 80:20 for compounds **8** and **10** resp.) also permitted establishment of the preferred orientation about the interflavanyl bond such that the 10-C(A), 4-C(C), 2-C(E), 3-C(E) dihedral angle approaches $+90^{0}$.

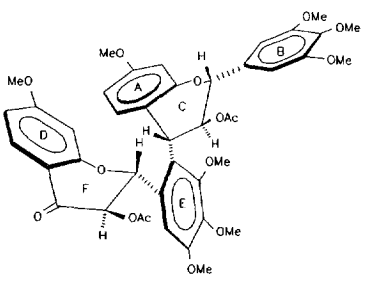
The mass spectral fragmentation patterns of derivatives **4**, **6**, **8**, and **10** are in agreement with those of analogous profisetinidin derivatives from *Acacia mearnsii*[7] and involve mainly loss of acetic acid, acetoxy radical, rupture of the interflavanyl bond, and RDA fragmentation of the C- and F-ring heterocycles. Compounds **3**, **5**, **7**, and **9** not only complement the rare series of oligoflavanoids with a flavan-3,4-diol chain terminating DEF unit[7] and of those with a C-E ring interflavanyl bond[17] (**7** and **9**), but represent the first entries into these classes that are exclusively based on leucorobinetinidin precursors of type **1**.

The next three prorobinetinidins **11**, **13**, and **15** are based on a 2,3-*trans* dihydroflavonol DEF-unit as was exemplified by the characteristic AB doublet (J = 9.5 - 12.5 Hz) for the heterocyclic protons in the ^{1}H NMR spectra (Table 2) of the free phenol **11** and of the derivatives **12**, **14**, and **16**. The four remaining aromatic protons are compatible with a dihydrorobinetin [*cf.* structure **2**] moiety coupled at various positions to the chain extender robinetinidol ABC-unit. Comparison of the 280-350 nm region of the CD spectra of derivatives **12**, **14**, and **16** with chiroptical data of dihydroflavonols[18] facilitated assignment of the 2*R*, 3*R* absolute configuration to the stereocenters of their F-rings and hence to definition of the DEF-units as a substituted (+)-dihydrorobinetin in the natural products **11**, **13**, and **15**. The (4,2′) coupling mode in compound **12** was evident from the presence of a one-proton singlet (δ 6.66) and an ABX-system with a conspicuously deshielded 5 proton (δ 7.86, J8.5 Hz), indicative of its *peri* position relative to the C-4 (F-ring) carbonyl, in the aromatic region of its ^{1}H NMR spectrum. A two-proton singlet (δ 6.69) and two one-proton singlets (δ 7.50, 6.50) in the same region of the spectrum of derivative **14** similarly indicated (4,6) counpling[3], while an aromatic two-proton singlet (δ 6.35) and an AB-system (δ 7.90, 6.76; J = 9.0 Hz) in the spectrum of derivating **16** were reminiscent of its (4,8) interflavanyl bond[19]. This rare coupling mode amongst proanthocyanidins with a 5-deoxy D-ring[19] was confirmed by the observed NOE association of 7-OMe(D) with both 6-H(D) and 4-H(C), the deshielded 5-H(D) doublet, and the chemical shift of 3-H(C) (δ 6.14, t, J = 10.0 Hz) which indicates a 4-linked flavanyl unit which is flanked at the point of attachment to aryl rings by two *ortho* oxygen substituents.

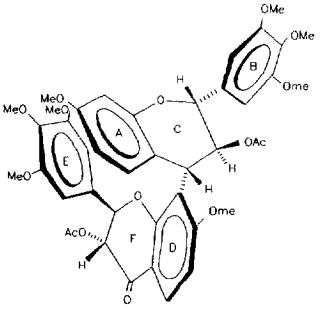
The relative 2,3-*trans*-3,4-*trans* configuration of the robinetinidol ABC-unit of phenol **11** and of the methyl ether acetate derivatives **12** and **16** was evident from $^{3}J_{HH}$ values (Table 2) of C-ring protons [$J_{2,3}$ = $J_{3,4}$ = 9.5 Hz for **11** and**12**; $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz for **16**]. An NOE association between 5-H(D) and 2-H(C) indicated 2,4-*trans* orientated B- and D-rings in derivative **14** hence confirming the 2,3-*trans*-3,4-*cis* C-ring configuration that could be inferred from the coupling constants ($J_{2,3}$ = 7.5; $J_{3,4}$ = 5.0 Hz) of the protons of this heterocycle. When taken in conjunction with high-amplitude negative Cotton effects at *ca.* 240 nm in the CD spectra of the prorobinetinidin derivatives **12** and **16**, indicating 4α-dihydrorobinetin units, and a positive Cotton effect in the same region in the spectrum of compound **14**, reminiscent of a 4β-flavanyl

moiety, these coupling constants confirmed 2*R*, 3*S*, 4*R* absolute configuration for derivatives **12** and **16**, and 2*R*, 3*S*, 4*S* for compound **14**. Owing to the identification of the free phenolic prorobinetinidin **11** in the natural product mixture, the 3-OMe(F) functionality in derivative **12**, confirmed by the chemical shifts of 3-H(C) (δ 6.02) and 3-H(F) (δ 5.74), represents an artefact of the methylation with diazomethane.

The ^{1}H NMR spectra of the robinetinidol-dihydrorobinetin **11** and of the derivatives **12**, **14**, and **16** are at ambient temperatures free of the effects of dynamic rotational isomerism about the interflavanyl bond which apparently indicates 'free' rotation about this bond on the NMR time-scale. However, the selective NOE association of 3-OMe(E) with 3-H(C) but not with 4-H(C) presumably again indicates preference for an interflavanyl conformation **25** in which the 10-C(A), 4-C(C), 2-C(E), 3-C(E) dihedral angle approximates +90^0 for derivative **12**. This conformation and the phenomenon of a predominant preference for a specific orientation are additionally supported by the observed NOE between 3-H(F) and 4-H(C) and the conspicuous absence of association between the former proton and 3-H(C) that could be anticipated should 'free rotation'



(25)



(26)

had occurred. The selective NOE between 2-H(F) and 6-H(E) presumably indicates a 1-C(E)-2-C(F) orientation in which repulsion of the bulky 3-OAc(C) and 3-OMe(F) is minimized. The significant shielding of 3-OMe(E) (δ 3.23) in the NMR spectrum of derivative **12** does not only support the orientation depicted in structure **25** but is presumably also reminiscent of an attractive π-alkyl interaction between the π-system of the A-ring and the methyl group. The NOE association of 5-H(D) with 2-H(C) and the marked absence of an NOE between the latter proton and 7-OMe(D) similarly points, as above, towards a preferred interflavanyl conformation with a 10-C(A), 4-C(C), 6-C(D), 7-C(D) dihedral angle approximating +90^0 for derivative **14**. In the (4,8)-prorobinetinidin derivative **16**, the NOE association of 7-OMe(D) with 4-H(C) but not with 3-H(C) and of both 7-OMe(A) and 8-H(A) with 2- and 6-H(E) exemplifies a conspicuous preference for the more crowded conformation **26.** Conformational analysis based on Dreiding models does, therefore, not permit predictions regarding preferred orientations about the interflavanyl bond. It's conformational itinerary is presumably controlled by more complex forces *i.e.* π-π- and π-alkyl interactions[10,20], and the tendency to minimize the surface area of the molecule, and hence solute-solvent contact[21]. The effect of adopting such a compressed conformation is precedented in the profisetinidin series of oligoflavonoids[22]. Whereas the ^{1}H NMR spectra of the methyl ether acetate of the (4α,8)-bis-fisetinidol is similarly 'free' of the effects of dynamic rotational isomerism under similar conditions[19], the spectrum of the same derivative of the fisetinidol-(4α,8)-catechin indicates the presence of two rotamers[22]. Considering the close resemblance of the three compounds in the vicinity of the interflavanyl bond it becomes evident that the factors controlling the conformations at the interflavanyl bond are at present largely obscured. Compounds **11**, **13**, and **15** represent the first natural prorobinetinidins with a dihydroflavonol constituent DEF unit.

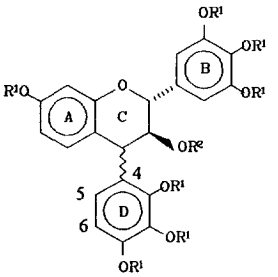
The structure of the novel robinetinidol-(4α,2′)-robinetin **17**, the first prorobinetinidin based on a flavonol DEF moiety, was established by comparison of the ^{1}H NMR-(Table 2) and CD-data of its

nonamethyl ether acetate **18** with those of the same derivative of fisetinidol-(4α,2′)-robinetin from *Burkea africana*[23]. In contrast to the ^{1}H NMR spectra of the aforementioned prorobinetinidins, *e.g.* **4**, the spectrum of derivative **18** showed the presence of two rotamers in a *ca.* 99:1 ratio. When more than one set of resonances are present in the ^{1}H NMR spectra of oligomeric proanthocyanidins and their derivatives it is often difficult to ascertain whether the additional signals result from impurities or from rotamer(s) especially where these signals constitute ≤ 1% of those of the main compound/rotamer. Unambiguous proof that the minor set of resonances in the spectrum of the prorobinetinidin derivative **18** is attributable to the protons of a second rotamer was obtained *via* a phase sensitive NOESY experiment[24] which indicated negative NOE effects between exchanging protons of the two rotamers *e.g.* 5-H(D) (δ 8.19, 7.97 for the major and minor rotamers respectively). An NMR experiment at progressively elevated temperature revealed that broadening of resonances sets in at *ca.* 35°C while coalescence occurs at 60-70°C with eventual line sharpening commencing at *ca.* 80°C. These distinct phases, *i.e.* sharp but duplicated resonances at ambient temperatures which coalesce at *ca.* 65°C and sharpen at *ca.* 80°C, then indicate slow, intermediate, and fast rotation, respectively, on the chemical shift time scale. The observed *ca.* 99:1 ratio of rotamers thus reflects a large difference in the relative energies of the two rotamers while the NMR results at elevated temperatures are reminiscent of a relatively low barrier to rotation of the conformers. The selective NOE association between 3-OMe(E) and 3-H(C) but not with 4-H(C) reflects the same preference for a conformation of type **25** that was advanced for the closely related prorobinetinidin derivative **12**.

The spin systems of the robinetinidol-type ABC-moiety in the ^{1}H NMR spectrum (Table 2) of the remaining prorobinetinidin derivative **20** are accompanied by an aromatic ABX-system comprising a deshielded *o*-coupled resonance indicative of the presence of a carbonyl function in the **peri** position, a one-proton aromatic singlet (δ 7.14), and a one-proton singlet in the heterocyclic region (δ 5.11). Collectively this data is consistent with a 3′,4′,5′,7-tetramethoxyflavone DEF-unit substituted at C-2′ of its E-ring. Compound **19** thus represents the first proanthocyanidin with a flavone chain terminating moiety. ^{1}H NMR coupling constants of the C-ring protons ($J_{2,3}$ = 2.5; $J_{3,4}$ = 3.5 Hz) did not permit unequivocal assignment of relative configuration. A prominent NOE-association between 4-H(C) and 2/6-H(B) not only indicated a *trans* relationship between the B- and E-rings but is also reminiscent of a considerable contribution of the A-form towards the C-ring conformational equilibrium. Such an A-conformation would also permit anisotropic shielding of the vinylic 3-H(F) by the B-ring. Comparison of the coupling constants of C-ring protons with those of reference compounds[25] showed that these are best compatible with 2,3-*trans*-3,4-*cis* relative configuration. When taken in conjunction with a strong positive Cotton effect at 240 nm in the CD spectrum of derivative **20** indicative of a 4β-flavanyl group, the coupling constants thus permitted assignment of 2*R*, 3*S*, 4*S* absolute configuration for the novel prorobinetinidin **19**.

Finally, possible synthetic routes towards the robinetinidol-dihydrorobinetins **11**, **13**, and **15** were investigated with a view to unambiguously establish the absolute configuration of their DEF-unit and also to provide possible precursors to the robinetinidol- leucorobinitinidins **3**, **5**, **7**, and **9** *via* reduction of the carbonyl function. The alternative approach of utilizing the latter group of compounds as precursors to the dihydroflavonol analogues by oxidation of the benzylic 4-OH(F) would not be feasible due to the susceptibility of the pyrogallol and 4-C methine hydrogen functionalities to oxidation. In order to establish conditions for effecting coupling of C-4 of leuco- robinetinidin **1** at the pyrogallol-type B-ring of dihydrorobinetin **2**, the condensation reaction of the flavan- 3,4-diol **1** and pyrogallol was performed under conditions similar to those applied by Foo[26] in the coupling of melacacidin and pyrogallol.

Thus, treatment of leucorobinetinidin **1** and pyrogallol in 0.7% ethanolic HCl for 2 hours at room temperature followed by 4 hours at 50°C afforded a mixture comprising the 4-arylflavan-3-ols **27** and **29**, and the analogue **31** with rearranged pyran heterocycle[27]. Structural elucidation of this class of compounds is now firmly established[25,27] and has been performed by analysis of ^{1}H NMR- (Table 3) and CD-data of the methyl ether acetates **28**, **30**, and **32**. A notable feature of the above reaction is the formation of the product

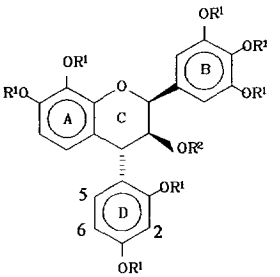


(27) ≡ , R^1=R^2=H

(28) ≡ , R^1=Me,R^2=Ac

(29) ≡ , R^1=R^2=H

(30) ≡ , R^1=Me,R^2=A



(31) R^1=R^2=H
(32) R^1=Me,R^2=Ac

Table 3. **^{1}H N.m.r. (300 MHz) peaks (p.p.m.) of the 4-arylflavan-3-ol derivatives 29, 31, and 33 in $CDCl_3$ at 296K. Splitting patterns and J-values (Hz) are given in parentheses.**

Ring	H	29	31	33
A	5	6.63(d,8.5)	6.77(d,8.5)	6.67(d,8.5)
	6	6.43(dd,2.5,8.5)	6.47(dd,2.5,8.5)	6.55(d,8.5)
	8	6.51(d,2.5)	6.56(d,2.5)	-
B	2/6	6.69(s)	6.57(s)	6.56(s)
C	2	4.96(d,9.5)	5.10(d,8.0)	5.01(d,1.0)
	3	5.67(t,9.5)	5.49(dd,5.5,8.0)	5.43(dd,1.0,2.0)
	4	4.55(d,9.5)	4.68(d,5.5)	4.46(d,2.0)
D	2	-	-	6.51(d,2.5)
	5	6.77(d,8.5)	6.62(d,8.5)	6.59(d,8.5)
	6	6.58(d,8.5)	6.58(d,8.5)	6.37(dd,2.5,8.5)
	OMe	3.73(7-A),3.81(1-D),3.82(2,3-D), 3.84 (4-B),3.86(3,5-B),each s	3.77(7-A),3.78(3-D),3.79(3,5-B), 3.81(4-B),3.83(2-D),3.87(1-D), each s	3.78(1-D),3.79(3,5-B),3.80(7-A), 3.87(3-D),3.88(8-A),3.97(4-B), each s
	OAc	1.65(s)	1.83(s)	1.89(s)

31 with rearranged C-ring from the 4β-arylflavan-3-ol **29** (see ref. **27** for the mechanism of this conversion). Although susceptible to facile pyran rearrangement under mild basic conditions, analogues with pyrocatechol B-rings undergo rearrangement under acidic conditions in extremely low yield[28]. The additional hydroxyl group in the pyrogallol B-ring of precursor **29** thus induces additional lability of the O-C_2 bond in comparison to analogues with a pyrocatechol B-ring.

Under similar conditions the condensation reaction of leucorobinetinidin **1** and dihydrorobinetin **2** afforded in *ca.* 3% yield a mixture of robinetinidol-(4α,2')-dihydro-robinetin **11** and the flavonol analogue **17**, the latter presumably representing an oxidation artefact of compound **11**. Comparison of ^{1}H NMR- and CD-data of the methyl ether acetates **12** and **18** with those of the corresponding derivatives of the natural

products confirmed their identity.

The chain terminating DEF-units in the aforementioned nine oligomers exhibit a remarkable diversity regarding the oxidation level of the heterocycle. This phenomenon suggest that the biflavanoids in *R. pseudacacia* may biogenetically be interrelated *via* oxidation/reduction of the terminal units.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer for solutions in $CDCl_3$, C_6D_6, $(CD_3)_2CO$, and DMSO-d_6 with Me_4Si as internal standard. J-values are given in Hz. Mass spectra and accurate mass estimations were obtained with a Varian CH-5 instrument with double focus and Field Desorption Ionization spectra on a Varian MAT-212 spectrometer. CD data was obtained in methanol on a JASCO J-710 spectropolarimeter. TLC was performed on pre-coated Merck plastic sheets (silica gel 60 PF_{254}, 0.25 mm) and the plates were sprayed with H_2SO_4-HCHO (40:1 v/v) after development. Preparative plates (PLC), 20x20 cm, Kieselgel PF_{254} (1.0 mm) were air-dried and used without prior activation. Separations on Sephadex LH-20 were on various column sizes and at differing flow rates in different solvent systems (to be specified in each instance). Methylations were performed with an excess of diazomethane in methanol-diethyl ether over a period of 48h at -15°C, while acetylations were in acetic anhydride pyridine at ambient temperature. Water-soluble phenolic were freeze-dried with a Virtis Freeze mobile 12 SL. Evaporations were done under reduced pressure at ~ 50°C in a rotary evaporator.

Phenolic Metabolites from the Heartwood of R. pseudacacia – Drillings (8.9 kg) of the heartwood of *R. pseudacadia* were extracted with aqueous acetone (1:1, v/v) for 7 days. The acetone was evaporated and the aqueous solution freeze-dried to give a yellow-brown powder (554 g). This (4x80 g) was partitioned between a butan-2-ol-water-hexane (5:4:1, v/v) mixture in a 20-tube, 100 cm^3 underphase, Craig countercurrent assembly. Following qualitative paper chromatographic analysis the fractions were combined as follows: 1 [tubes 1-2 (94.6 g)], 2 [3-5 (41.9 g)], 3 [6-9 (47.3 g], 4 [10-13 (47.6 g)], 5 [14-20 (47.2 g)]. Subsequent column chromatography of a portion (20 g) of fraction 3 on Sephadex LH-20 (5x170 cm column, flow rate of 20 cm^3/30 min) gave the following 12 fractions: A1 [tubes 1-29 (185 mg)], A2 [30-90 (2.28 g)], A3 [91-129 (119 mg)], A4 [130-200 (94 mg)], A5 [201-356 (439 mg)], A6 [357-434 (557 mg)], A7 [435-506 (482 mg)], A8 [507-552 (217 mg)], A9 [553-652 (807 mg)], A10 [653-748 (469 mg)], A11 [749-796 (395 mg)], and A12 [797-876 (326 mg)]. A portion (20 g) of fraction 2 was similarly resolved to afford the following 12 fractions: B1 [tubes 1-128 (241 mg)], B2 [129-161 (2.67 g)], B3 [162-267 (391 mg)], B4 [268-336 (319 mg)], B5 [337-372 (402 mg)], B6 [373-400 (327 mg)], B7 [401-466 (789 mg)], B8 [467-503 (427 mg)], B9 [504-528 (540 mg)], B10 [529-612 (1.67 g)], B11 [613-651 (483 mg)] and B12 [652-716 (872 mg)]. Fractions 1, 4, and 5 were not further investigated. The column fractions A1-A4 and B1-B4 consisted of known monomeric compound which were previously thoroughly investigated[6].

A portion (160 mg) of fraction A5 was methylated and the mixture was resolved by PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) to give two main bands, A5.1 (R_F 0.33, 8 mg) and A5.2 (R_F 0.10, 11 mg).

Tri-O-*methyl*-3-O-*acetylrobinetinidol*-(4β,2′)-3′,4′,5,7-*tetra-methoxyflavone* **20**. – Band A5.1 was further purified by PLC in benzene-acetone (9:1, v/v) to give the title compound (6.1 mg, R_F 0.56) as a brown amorphous solid (Found M^+, 700.2518. $C_{39}H_{40}O_{12}$ requires M, 700.2520); δ_H (Table 2); CD $[\theta]_{350}$ $1.3x10^3$, $[\theta]_{331.1}$ $1.2x10^4$, $[\theta]_{319.3}$ $-5.6x10^1$, $[\theta]_{303.1}$ $-24x10^4$, $[\theta]_{280.1}$ $-4.2x10^1$, $[\theta]_{268.5}$ $1.6x10^4$, $[\theta]_{258.2}$ $1.3x10^4$, $[\theta]_{239.1}$ $6.5x10^4$, $[\theta]_{232.2}$ $7.2x10^4$, and $[\theta]_{224.2}$ $9.2x10^4$; m/z 700 (M^+, 8.1%), 699, 550, 476, 360, 340, 329, 326, 295, 224, 193, 181 (100), 151, 150, and 123.

Tetra-O-*methyl*-3-O-*acetylrobinetinidol*-(4β,6)-*tetra*-O-*methyl*-3-O-*acetylrobinetinidol*-4α-*acetate* **6**. – Band A5.2 was acetylated and purified by PLC in benzene-acetone (9:1, v/v) to give the title compound (9.5 mg, R_F 0.33) as a light brown amorphous solid (Found: M^+, 832,2945. $C_{44}H_{48}O_{16}$ requires M, 832.2942);

δ_H (Table 1); CD $[\theta]_{303.6}$ $-1.1x10^2$, $[\theta]_{290.7}$ $-7.4x10^3$, $[\theta]_{288.3}$ $-6.9x10^3$, $[\theta]_{280.9}$ $-1.1x10^4$, $[\theta]_{260.9}$ $1.6x10^{10}$, $[\theta]_{242.6}$ $5.0x10^4$, $[\theta]_{231.2}$ $2.0x10^4$, and $[\theta]_{219}$ $1.3x10^2$; m/z 832 (M^+, 1.1%), 772, 713, 653, 549, 507, 489, 445, 387, 327 (100), 252, 210, 193, and 181.

Tetra-O-*methyl*-3-O-*acetylrobinetinidol*-(4α,2′)-*penta*-O-*methylrobinetin* **18**. – Methylation of a portion (160 mg) of fraction A6 and PLC in benzene-acetone (9:1, v/v) gave a main band at R_F 0.26 (14 mg) which was further purified by PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) to afford a band at R_F 0.25 (12 mg). Acetylation and separation by PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) gave the title compound (R_F 0.23, 9.7 mg) as a yellowish amorphous solid (Found: M^+-HOAc; 698.2361. $C_{41}H_{42}O_{14}$ requires M-HOAc, 698.2364); δ_H (Table 2); CD $[\theta]_{350}$ $-2.8x10^1$, $[\theta]_{308.5}$ $-4.9x10^3$, $[\theta]_{291.3}$ 0, $[\theta]_{286.9}$ $1.4x10^3$, $[\theta]_{273}$ $3.6x10^2$, $[\theta]_{262.6}$ 0, $[\theta]_{239.4}$ $-3.7x10^4$, and $[\theta]_{227.6}$ $-5.9x10^2$; m/z 758 (M^+, 0%), 698 (M^+-HOAc, 5%), 667, 548, 517, 475, 387, 371, 340, 327, 252, 210, 181, 151, and 150.

Tetra-O-*methyl*-3-O-*acetylrobinetinidol*-(4α,2′)-*tetra*-O-*methyl*-3-O-*acetylrobinetinidol*-4α-*acetate* **10**. – A portion (160 mg) of fraction A7 was methylated and purified by PLC in hexane-benzene-acetone-methanol (40:40:13:7, v/v) to give two main bands at R_F 0.43 (20 mg) and 0.50 (11 mg). The R_F 0.43 fraction was further purified by PLC in benzene-acetone (8:2, v/v) to give a band at R_F 0.28 (16 mg) which was acetylated and resolved by PLC in benzene-acetone (9:1, v/v) to give derivative **10** (R_F 0.32, 12 mg) as a light brown amorphous solid (Found: M^+-HOAc, 772.2729. $C_{44}H_{48}O_{16}$ requires M-HOAc, 772.2731); δ_H (Table 1); CD $[\theta]_{301.1}$ $1.8x10^1$, $[\theta]_{289.9}$ $5.7x10^3$, $[\theta]_{281.9}$ 0, $[\theta]_{274.6}$ $-3.3x10^3$, $[\theta]_{261}$ $-1.2x10^3$, $[\theta]_{239.6}$ $-5.1x10^4$, and $[\theta]_{224.8}$; m/z 832 (M^+, 0%) 772 (M^+-HOAc, 8.2), 713, 653 (100), 638, 445, 387, 386, 327, 252, 210, 194, 181, and 135. The R_F 0.50 fraction was subjected to PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) to give a band at R_F 0.24 (10 mg) which was acetylated and separated by PLC in benzene-acetone (9:1, v/v) to afford an additional portion of the robinetinidol-(4α,2′)- robinetin derivative **18**.

Methylation of a portion (160 mg) of fraction A8 followed by PLC in hexane-benzene-acetone-methanol (8:8:3:3, v/v) afforded a single band at R_F 0.45 (10 mg) which was acetylated and purified by PLC in benzene-acetone (9:1, v/v) to give a further portion (R_F 0.32, 8 mg) of the robinetinidol-(4α,2′)-robinetinidol-4α-ol derivative **10**.

Tetra-O-*methyl*-3-O-*acetylrobinetinidol*-(4α,2′)-*tetra*-O-*methyl*-3-O-*acetylrobinetinidol*-4β-*acetate* **8** – A portion (160 mg) of fraction A9 was methylated and the mixture was resolved by PLC in hexane-benzene-acetone-methanol (40:40:13:7, v/v) to give three bands at R_F 0.48 (39 mg), 0.41 (20 mg), and 0.38 (14 mg). The R_F 0.41 band was further purified by PLC in benzene-acetone (9:1, v/v) to give a fraction at R_F 0.28 (16 mg) which was acetylated and separated by PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) to afford the title compound (R_F 0.31, 12 mg) as a light brown amorphous solid (Found: M^+ -HOAc, 772.2732. $C_{44}H_{48}O_{16}$ requires M-HOAc, 772.2731); δ_H (Table 1); CD $[\theta]_{299.6}$ $-2.4x10^1$, $[\theta]_{289}$ $1.2x10^4$, $[\theta]_{272.4}$ $1.1x10^3$, $[\theta]_{258.2}$ 0, $[\theta]_{241.9}$ $-3.8x10^4$, and $[\theta]_{235.9}$ $1.3x10^2$; m/z 832 (M^+, 0%), 772 (M^+-HOAc, 9.2%), 713, 653, 638, 445, 387, 386, 327 (100), 252, 210, 194, 181, and 135.

The R_F 0.48 band was further resolved by PLC in benzene-acetone (9:1, v/v) to give a fraction at R_F 0.41 (37 mg). Acetylation and subsequent separation by PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) afforded an additional portion (R_F 0.37, 34 mg) of the robinetinidol-(4α,2′)-robinetin derivative **18**. The R_F 0.38 band was also re-subjected to PLC in benzene-acetone (9:1, v/v) to give a fraction at R_F 0.57 (9 mg) which was acetylated and separated by PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) to give a further batch (R_F 0.38, 7 mg) of derivative **18**.

Methylation of a portion (100 mg) of fraction A10 and PLC in benzene-acetone (8:2, v/v) afforded two main bands at R_F 0.46 (7 mg) and 0.34 (20 mg). The R_F 0.46 band was acetylated and purified by PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) to give the robinetinidol-(4α,2′)-robinetin derivative **18** (R_F 0.38, 6 mg). The R_F 0.34 band was further purified by PLC in hexane-acetone-ethyl acetate (13:4:3, v/v) (R_F 0.30, 14 mg), acetylated, and finally resolved by PLC in the same solvent system to give a further portion (R_F 0.35, 11 mg) of the robinetinidol-(4α,2′)-robinetinidol-4α-ol derivative **10**.

Robinetinidol-(4α,2′)-*dihydrorobinetin* **11**. – A portion (100 mg) of fraction A11 was re-chromatographed on Sephadex LH-20 in ethanol-water (1:1, v/v) (2.5x75 cm column, flow rate of 20 cm^3/30 min) to give only one fraction [tubes 389-440 (20 mg)] worth further investigation. This material comprised of the title compound as a light brown amorphous solid (Found: M^+, 592.1221. $C_{30}H_{24}O_{13}$ requires M, 592.1217); δ_H (Table 2). A portion (10 mg) of compound **11** was methylated and separated by PLC in benzene-acetone (8:2, v/v) to give a band at R_F 0.40 (9 mg) which was acetylated and purified by PLC in benzene-acetone (9:1, v/v) to give *tetra*-O-*methyl*-3-O-*acetylrobinetinidol*-(4α,2′)-*penta*-O-*methyldihydro-robinetin* **12** (R_F 0.38, 9 mg) as a light-brown amorphous solid (Found: M^+-HOAc, 700.2518. $C_{41}H_{44}O_{14}$-HOAc requires M, 700.2520); δ_H (Table 2); CD $[\theta]_{350}$ $4.3x10^3$, $[\theta]_{333.4}$ $1.3x10^4$, $[\theta]_{321}$ $-7.5x10^1$, $[\theta]_{305.7}$ $-2.0x10^4$, $[\theta]_{292.1}$ $-5.6x10^1$, $[\theta]_{288}$ $6.0x10^3$, $[\theta]_{279}$ $4.2x10^3$, $[\theta]_{268.3}$ $6.9x10^3$, $[\theta]_{247.1}$ $22.5x10^1$, $[\theta]_{238.7}$ $-2.3x10^4$, $[\theta]_{233.4}$ $-1.1x10^2$, $[\theta]_{227.1}$ $4.2x10^4$, and $[\theta]_{224.8}$ $5.0x10^3$; m/z 760 (M^+, 0%), 700 (M^+-HOAc, 24), 669, 610, 550, 508, 387, 373, 342, 327, 252, 210, 181, 151 (100), and 150.

Methylation of a portion (160 mg) of fraction A12 gave a complex mixture without prominent compounds (TLC) which was not further investigated. Attention was thus next focussed on the fractions B5-B12 from the second column chromatographic separation.

A portion (160 mg) of fraction B5 was methylated and the mixture was separated by PLC in hexane-benzene-acetone-methanol (10:10:3:2, v/v) to give a main band at R_F 0.40 (10 mg) which was acetylated and resolved by PLC in benzene-acetone (9:1, v/v) to give an additional portion (R_F 0.32, 8 mg) of the robinetinidol-(4α,2′)-robinetinidol-4α-ol derivative **10**.

Tetra-O-*methyl*-3-O-*acetylrobinetinidol*-(4α,8)-*tetra*-O-*methyl*-3-O-*acetyldihydroro- binetin* **16.** – A portion (160 mg) of fraction B6 was methylated and purified by PLC in hexane-benzene-acetone-methanol (22:21:4:3, v/v) to give a main band at R_F 0.22 (36 mg). This was acetylated and the mixture was purified by PLC in benzene-acetone (9:1, v/v) to give the derivative **16** (R_F 0.26, 31 mg) as a light brown amorphous solid (Found: M^+-HOAc, 728.2468. $C_{42}H_{44}O_{15}$ require M-HOAc, 728.2469); δ_H (Table 2); CD $[\theta]_{350}$ $2.1x10^3$, $[\theta]_{334.6}$ $9.1x10^3$, $[\theta]_{324.3}$ $-3.7x10^1$, $[\theta]_{305.9}$ $-2.2x10^4$, $[\theta]_{289.3}$ $-5.0x10^3$, $[\theta]_{276}$ $-1.6x10^4$, $[\theta]_{258.1}$ $-7.0x10^3$, $[\theta]_{238}$ $-6.1x10^4$, and $[\theta]_{226.2}$ $1.4x10^2$; m/z 788 (M^+, 0%), 728 (M^+-HOAc, 65), 669 (100), 505, 445, 401, 387, 341, 327, 285, 253, 252, 210, 181, 149, 136, and 121.

Tetra-O-*methyl*-3-O-*acetylrobinetinidol*-(4β,6)-*tetra*-O-*methyl*-3-O-*acetylrobinetinidol*-4β-*acetate* **4**. – Methylation of a portion (100 mg) of fraction B7 and PLC in hexane- benzene-acetone-methanol (22:21:4:3, v/v) gave two main bands at R_F 0.17 (38 mg) and 0.23 (14 mg). The R_F 0.17 band was acetylated and separated by PLC in benzene-acetone [9:1, v/v (x2)] to give a fraction at R_F 0.39 (20 mg) which was further resolved by PLC in toluene-2-butanone into two bands at R_F 0.52 (10 mg) and 0.56 (8.2 mg). The former band comprised of the title compound as a brown amorphous solid (Found: M^+, 832.2944. $C_{44}H_{48}O_{16}$ requires M, 832.2942); δ_H (Table 1); CD $[\theta]_{306.1}$ 0, $[\theta]_{290.6}$ $-5.8x10^3$, $[\theta]_{287.5}$ $-5.3x10^3$, $[\theta]_{282}$ $-6.6x10^3$, $[\theta]_{264.4}$ 0, $[\theta]_{241.3}$ $6.3x10^4$, $[\theta]_{223}$ $2.5x10^4$, and $[\theta]_{214.7}$ 0; m/z 832 (M^+, 1.8%), 772, 713, 653, 549, 507, 489, 445, 387, 327 (100), 252, 210, 193, and 181. The R_F 0.56 band consisted of the robinetinidol-(4β,6)-robinetinidol-4α-ol derivative **6**.

The R_F 0.23 band (14 mg) was acetylated and resolved by PLC in hexane-acetone-ethyl acetate [12:4:3, v/v (x2)] into two fractions at R_F 0.35 (3 mg) and 0.46 (7 mg). The former fraction afforded *tetra*-O-*methyl*-3-O-*acetylrobinetinidol*-(4β,6)-*tetra*-O-*methyl*-3-O-*acetyl- dihydrorobinetin* **14** as a light brown amorphous solid (Found: M^+, 788.2679. $C_{42}H_{44}O_{15}$ requires M, 788.2680); δ_H (Table 2); CD $[\theta]_{350}$ $7.5x10^2$, $[\theta]_{329.2}$ $-1.0x10^1$, $[\theta]_{309.4}$ $-1.1x10^4$, $[\theta]_{277.30}$ 0, $[\theta]_{242.2}$ $5.2x10^4$, and $[\theta]_{221.1}$ $-7.0x10^1$; m/z 788 (M^+, 4.7%), 728, 668, 505, 445, 401, 387, 341, 3327, 284, 253, 252, 210 (100), and 181. The R_F 0.46 fraction gave an additional portion of the robinetinidol-(4α,2′)-robinetinidol-4β-ol derivative **8**.

The remaining B-fractions were similarly methylated/purified (PLC) and acetylated/- purified (PLC) to give further crops of the derivatives described above. Fraction B8 gave derivative **8** (9 mg), B9 gave derivatives **10** (11 mg) and **18** (31 mg), B10 afforded derivatives **8** (18 mg), **10** (19 mg), and **18** (8.6 mg),

B11 gave derivative **12** (6.8 mg), and B12 consisted of a complex mixture which did not merit further investigation.

Acid-catalysed reaction of leucorobinetinidin **1** *and pyrogallol.* – A solution of leucorobinetinidin (100 mg) and pyrogallol (300 mg) in 0.7% ethanolic HCl (25 cm^3) was stirred at room temperature for 2h and at 50°C for 4h. Water (200 ml) was added and the mixture was extracted with ethyl acetate (5x100 cm^3). The combined organic layers was dried (Na_2SO_4) and the solvent was evaporated to give a dark brown powder (370 mg). This was separated on Sephadex LH-20 in ethanol-water (1:1, v/v) (5x90 cm column, flow rate of 20 cm^3/30 min) to give three fractions: 1 [tubes 61-101 (222 mg), pyrogallol], 2 [121-143 (38 mg)], and 3 [150-173 (10 mg)].

Methylation of fraction 2 and PLC in benzene-acetone (9:1, v/v) gave two bands at R_F 0.13 (5 mg) and 0.23 (22 mg). The R_F 0.13 band was acetylated and purified by PLC in benzene-acetone (9:1, v/v) to give (2R,3S,4R)-2,3-cis-3,4-trans-3-*acetoxy*-3′,4′,5′,7,8- *pentamethoxy*-4α,4-(*di*-O-*methylresorcyl*)*flavan* **32** as a light brown amorphous solid (R_F 0.52, 5 mg) (Found: M^+, 554.2154. $C_{30}H_{34}O_{10}$ requires M, 554.2152; δ_H (Table 3); CD $[\theta]_{304.1}$ -1.0×10^2, $[\theta]_{290.4}$ -7.3×10^2, $[\theta]_{287.2}$ 0, $[\theta]_{271.9}$ 6.3×10^3, $[\theta]_{254.2}$ 1.8×10^3, $[\theta]_{244.6}$ 6.1×10^3, and $[\theta]_{240.2}$ 1.2×10^3. Acetylation of the R_F 0.23 band and PLC in benzene-acetone [9:1, v/v (x2)] gave *tetra*-O-*methyl*-3-O-*acetylrobinetinidol*-(4β,4)-*tri*-O- *methylpyrogallol* **30** as a light brown amorphous solid (R_F 0.56, 22 mg) (Found: M^+ 554.2151. $C_{30}H_{34}O_{10}$ requires M, 554.2152); δ_H (Table 3); CD $[\theta]_{301.8}$ 0, $[\theta]_{283.6}$ -2.8×10^0, $[\theta]_{270.1}$ 0, and $[\theta]_{239.2}$ 1.2×10^1.

Fraction 3 was methylated and separated by PLC in benzene-acetone (9:1, v/v) to give a single band at R_F 0.16 (8 mg). This was acetylated and purified by PLC in benzene- acetone [9:1, v/v (x2)] to give *tetra*-O-*methyl*-3-O-*acetylrobinetinidol*-(4α,4)-*tri*-O- *methylpirogallol* **28** as a pale yellow amorphous solid (R_F 0.57, 7 mg) (Found: M^+, 554.2148. $C_{30}H_{34}O_{10}$ requires M, 554.2151); δ_H (Table 3); CD$[\theta]_{293.8}$ 0, $[\theta]_{287.3}$ 2.7×10^3, $[\theta]_{277.8}$ 1.9×10^1, $[\theta]_{270.2}$ -1.4×10^3, $[\theta]_{258.7}$ -5.4×10^2, $[\theta]_{238}$ -2.6×10^4, and $[\theta]_{226}$ 1.4×10^2.

Acid-catalyzed reaction of leucorobinetinidin **1** *and dihydrorobinetin* **2**. – A solution of leucorobinetinidin (300 mg) and dihydrorobinetin (600 mg) in 0.7% ethanolic HCl (100 cm^3) was stirred under argon for 15h at 50°C. The mixture was chilled with ice and extracted with ethyl acetate (6x150 cm^3). The combined organic layers was dried (Na_2SO_4) and the solvent evaporated to give a red brown powder (684 mg). A portion (200 mg) of this mixture was methylated and resolved by PLC in hexane-benzene-acetone-methanol (21:21:5:3, v/v) to give two bands at R_F 0.40 (7 mg) and 0.46 (6 mg) in the 'dimeric region'. These bands were separately acetylated and purfied by PLC in benzene-acetone (9:1, v/v) to give respectively the robinetinidol-(4α,2′)- dihydrorobinetin derivative **12** (R_F 0.38, 6 mg) and the robinetinidol-(4α,2′)-robinetin derivative **18** (R_F 0.44, 4 mg) which were identical to the same derivatives of the natural products **11** and **17** by comparison of their ^1H NMR- and CD-data.

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REFERENCES

1. Roux, D.G. *Nature* (London), 1957, 180, 793.

2. Roux , D.G.; Evelyn, S.R. *Biochem, J.*, 1958, 69, 530.

3. Botha, J.J.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1981, 1235.

4. Viviers, P.M.; Botha, J.J.; Ferreira, D.; Roux, D.G.; Saayman, H.M. *J. Chem. Soc., Perkin Trans. 1,* 1983, 17.
5. Cronjé, A.; Steynberg, J.P.; Brandt, E.V.; Young, D.A.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1,* 1993, 2467.
6. Roux, D.G.; Paulus, E. B*iochem. J.*, 1962, 82, 324, and references cited therein.
7. Viviers, P.M.; Young, D.A.; Botha, J.J.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1,* 1982, 535.
8. Van Heerden, F.R.; Brandt, E.V.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1,* 1981, 2483.
9. Porter, L.J.; Wong, R.Y.; Benson, M.; Chan, B.G.; Vishwanadhan, V.N.; Gandour, R.D.; Mattice, W.L. *J. Chem. Res.,* 1986, (S)86; (M)830.
10. Steynberg, J.P.; Brandt, E.V.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 2,* 1991, 1569.
11. DeAngelis, G.G.; Wildman, E.C. *Tetrahedron,* 1969, 25, 5099.
12. Weinges, K.; Marx, H.D.; Göritz, K. *Chem. Ber.*, 1970, 103, 2336.
13. Du Preez, I.C.; Rowan, A.C.; Roux, D.G. *Chem. Commun.*, 1971, 315.
14. Brandt, E.V.; Young, D.A.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1,* 1987, 2353.
15. Vishwanadhan, V.N.; Bergman, W.R.; Mattice, W.L. *Macromolecules,* 1987, 20, 1539.
16. Vishwanadhan, V.N.; Mattice, W.L. *J. Comput. Chem.*, 1986, 7, 711.
17. Steenkamp, J.A.; Malan, J.C.S.; Roux, D.G.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1,* 1988, 1325.
18. Van der Westhuizen, J.H.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1,* 1980, 1003.
19. Malan, J.C.S.; Steenkamp, J.A.; Steynberg, J.P.; Young, D.A.; Brandt, E.V.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1,* 1990, 209.
20. Hunter, C.A.; Saunders, J.K.M. *J. Am. Chem. Soc.*, 1990, 112. 5525.
21. Foo, L.Y.; Porter, L.J. *J. Chem. Soc., Perkin Trans. 1,* 1983, 1535.
22. Steynberg, J.P.; Brandt, E.V.; Ferreira, D.; Helfer, C.A.; Mattice, W.L.; Gornik, D.; Hemingway, R.W. *Magn. Reson. in Chem.*, 1994, in the press.
23. Malan, J.C.S.; Young, D.A.; Steenkamp, J.A.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1,* 1988, 2567.
24. Bodenhausen, G.; Kogler, H.; Ernst, R.R. *J. Magn. Res.*, 1984, 58, 370.
25. Van Zyl, P.W.; Steynberg, J.P.; Brandt, E.V.; Ferreira, D. *Magn. Reson. in Chem.*, 1993, 31, 1057.
26. Foo, L.Y. *J. Chem. Soc., Chem. Commun.*, 1985, 1273.
27. Steynberg, J.P.S.; Burger, J.F.W.; Young, D.A.; Brandt, E.V.; Ferreira, D. *Heterocycles,* 1989, 28, 923.
28. Young, D.A.; Cronjé, A.; Botes, A.L.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1,* 1985, 2521.

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