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Oligomeric Flavanoids, Part 29‡: Structure and Synthesis of Novel Ether-linked [4-O-4] Bis-teracacinidins

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Abstract: The rare series of proteracacinidin-type oligoflavanoids is extended by identification of two novel [4-O-4] ether-linked analogues **7** and **10** from the heartwood of *Acacia galpinii*. Their structures and absolute configuration were established *via* spectroscopic methods and by stereoselective synthesis from the appropriate leucoteracacinidin flavan-3,4-diol precursors.

Proanthocyanidins possessing ether-type interflavanyl linkages are extremely rare except for the A-type oligomers which contain the conventional C_4 (C-ring)→C_6/C_8 (D-ring) bond as well as an additional ether linkage connecting C_2 (C-ring) and C_5/C_7 (D-ring).[1,2] Analogues which possess exclusive ether bonds are hitherto restricted to the 1,4-dioxane-type profisetinidins from *Acacia mearnsii*,[3,4] the recently reported (4→7:5→6) doubly-linked proteracacinidin from *A. caffra*,[5] two (C_4-O-C_3)-proteracacinidins from *A. galpinii*[6] as well as two (C_4-O-C_4)-promelacacinidins from *A. melanoxylon*.[7] No attempt was made to synthesise the latter compounds or to establish their absolute stereochemistry. We now describe the structure and synthesis of two novel (C_4-O-C_4)-linked proteracacinidins from *A. galpinii*, representing only the third and fourth members of the class of proanthocyanidins exhibiting this mode of interflavanyl coupling.

RESULTS AND DISCUSSION

In addition to the four leucoteracacinidins, oritin-4α-ol, epioritin-4α-ol **1**, epioritin-4β-ol and ent-oritin-4α-ol **4**, and the (C4-O-C3)-linked proteracacinidins, epioritin-(4β→3)-epioritin-4α-ol and epioritin-(4β→3)-oritin-4α-ol described in Part 28,[6] the methanol extract of the heartwood of *A. galpinii* also contains two novel (C_4-O-C_4)-coupled proteracacinidins, *ent*-oritin-(4α→4)-epioritin-4α-ol **7** and epioritin-(4β→4)-epioritin-4α-ol **10**. Owing to the complexity of the phenolic mixture the dimers **7** and **10** were identified as their hexamethyl ether diacetate derivatives **9** and **12**, the additional chromatographic steps offered by derivatization being a prerequisite for sample purity.

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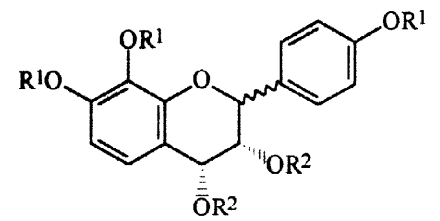
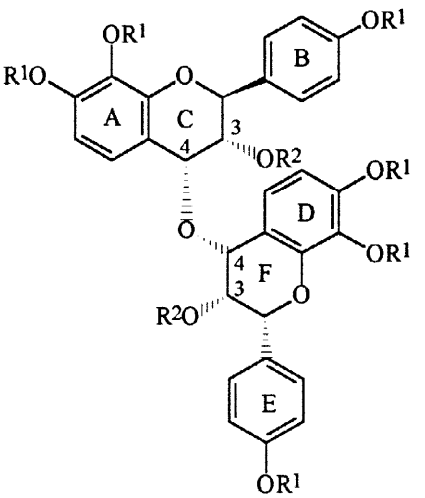
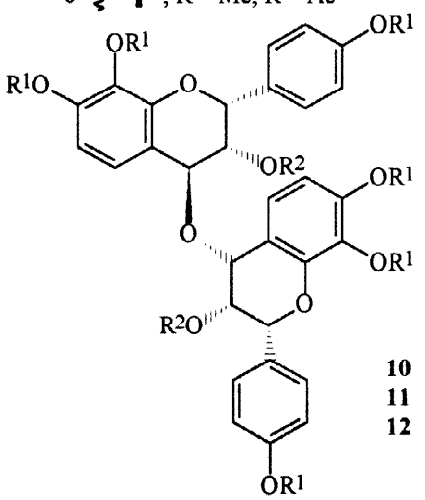


1 ≡ , R^1=R^2=H
2 ≡ , R^1=Me, R^2=H
3 ≡ , R^1=Me, R^2=Ac
4 ≡ , R^1=R^2=H
5 ≡ , R^1=Me, R^2=H
6 ≡ , R^1=Me, R^2=Ac



7 R^1=R^2=H
8 R^1=Me, R^2=H
9 R^1=Me, R^2=Ac



10 R^1=R^2=H
11 R^1=Me, R^2=H
12 R^1=Me, R^2=Ac

The ^{1}H NMR data (Table) of derivatives **9** and **12** indicated the presence of two AB- and two AA'BB'-spin systems for aromatic protons as well as two AMX-systems for protons of the heterocyclic rings hence indicating the dimeric nature of both compounds. Differentiation of the spin systems and the connectivities between aromatic and heterocyclic protons were effected with 2D COSY experiments. The presence of six *O*-methyl and two *O*-acetyl resonances as well as FAB-MS data, indicating a molecular ion at m/z 730 reminiscent of a molecular formula $C_{40}H_{42}O_{13}$ for both compounds strongly suggested an ether-type interflavanyl linkage.

Application of the shielding phenomenon observed for 4-H(C) of the ABC chain extender unit of oligomeric proanthocyanidins relative to the chemical shifts of the same proton in the 3,4-di-*O*-acetyl derivative of the flavan-3,4-diol precursor,[7-10] indicated a C_4-O-C_4 ether bond [4-H(C), δ 4.91, 4.73 for **9** and **12**, respectively: 4-H(F), δ 5.13, 5.32 for **9** and **12**, resp.: 4-H(C), δ 6.33, 5.43 for diacetates **3** and **6**, resp., and δ 5.90 for the C-4 epimer of **3**].[6] The chemical shifts of the 3-H resonances of both the C- and F-rings of derivatives **9** and **12** (*cf.* Table) are reminiscent of methine hydrogens of an *O*-acetyl substituted carbon, hence supporting the ether linkage involving C-4(C) of both flavanyl constituent units.

Prominent $^4J_{HH}$ couplings, evident in the 2D COSY spectra of **9** and **12**, between 2-H(C) (δ 5.65, 5.50 for **9** and **12**, resp.) and 2′,6′-H(B) (δ 7.37, 7.46 for **9** and **12**, resp.), as well as between 2-H(F) (δ 5.29, 5.36 for **9** and **12**, resp.) and 2′,6′-H(E) (δ 7.43, 7.44 for **9** and **12**, resp.) differentiated the AA′B′B′ spin systems of the constituent flavanyl units. The A/C- and D/F-ring junctions were respectively connected *via* the observed benzylic coupling of 5-H(A) (δ 6.98, 7.10 for **9** and **12**, resp.) with 4-H(C) (δ 4.91, 4.73 for **9** and **12**, resp.) and of 5-H(D) (δ 6.84, 6.97 for **9** and **12**, resp.) with 4-H(F) (δ 5.13, 5.32 for **9** and **12**, resp.).

Table ^{1}H NMR peaks (δ_H) of proteracacinidin derivatives **9** and **12** and the 4-chloroflavan-3-ol derivative **13** in $CDCl_3$ at 300 MHz (296K). Splitting patterns and *J* values (Hz) are given in parentheses.

Ring	Proton	9	12	13
A	5	6.98(d,8.5)	7.10(d,8.5)	7.10(d,9.0)
	6	6.59(d,8.5)	6.67(d,8.5)	6.66(d,9.0)
B	2′,6′	7.37(d,9.0)	7.46(d,9.0)	7.51(d,9.0)
	3′,5′	6.89(d,9.0)	6.94(d,9.0)	7.01(d,9.0)
C	2	5.65(d,10.5)	5.50(br s,1.5)	5.56(br s,1.0)
	3	5.34(dd,10.5,2.5)	5.27(dd,1.5,3.0)	4.26(dd,1.0,2.5)
	4	4.91(d,2.5)	4.73(d,3.0)	5.12(d,2.5)
D	5	6.84(d,9.0)	6.97(d,9.0)	-
	6	6.53(d,9.0)	6.58(d,9.0)	-
E	2′,6′	7.43(d,9.0)	7.44(d,9.0)	-
	3′,5′	6.94(d,9.0)	6.92(d,9.0)	-
F	2	5.29(br s,1.0)	5.36(br s,1.0)	-
	3	5.76(dd,1.0,4.0)	5.85(dd,1.0,4.0)	-
	4	5.13(d,4.0)	5.32(d,4.0)	-
OMe		3.83,3.84,3.85,3.88 (x2),3.91 (each s)	3.83,3.84,3.87,3.90 , 3.93,3.95 (each s)	3.86,3.90,3.92, (each s)
OAc		1.86,1.97 (each s)	1.85,1.98 (each s)	-

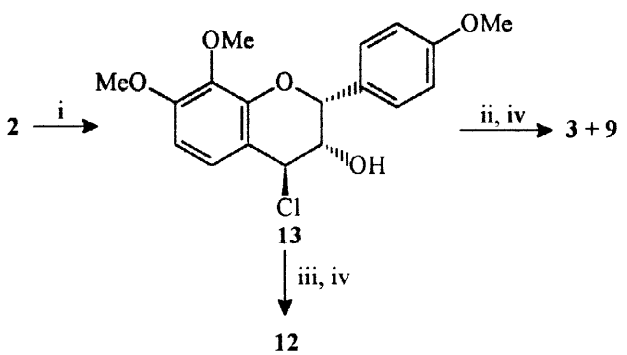
The coupling constants of the two heterocyclic ring systems [$J_{2,3(C)}$ = 10.5, 1.5; $J_{3,4(C)}$ = 2.5, 3.0 Hz for **9** and **12** resp.: $J_{2,3(F)}$ = 1.0; $J_{3,4}$ = 4.0 Hz for both **9** and **12**] indicated 2,3-*trans*-3,4-*cis*(C): 2,3-*cis*-3,4-*cis*(F) and 2,3-*cis*-3,4-*trans*(C): 2,3-*cis*-3,4-*cis*(F) relative configurations for the proteracacinidin derivatives **9** and **12**, resp.[5,10,11] In both compounds a strong NOE association was observed between 2- and 4-H(F) which confirmed the 2,4-*cis* relative configurations of the DEF constituent units. By the same token the conspicuous absence of NOE association between 2- and 4-H(C) in both derivatives was interpreted as confirmation of the 2,4-*trans* relative configuration of the ABC moieties.

A phase sensitive NOESY experiment of derivative **9** showed associations of 5-H(D) with 5-H(A), 2-H(C) and 4-H(C), of 5-H(A) with 3-H(F), 4-H(F) and 5-H(D) and of 4-H(C) with 4-H(F). The same experiment using derivative **12** indicated association of both 5-H(A) and 4-H(C) with 3-H(F),4-H(F) and

5-H(D), as well as of 5-H(D) with 2- and 3-H(C). Collectively these NOE effects are only reconcilable with C_4-O-C_4 interflavanyl linkages for both derivatives **9** and **12** of the novel proteracacinidin dimers **7** and **10**.

The CD spectra of the proteracacinidin derivatives **9** and **12** exhibited strong Cotton effects near 275 (positive and negative for **9** and **12**, resp.), 240 (- for both **9** and **12**), 225 (+ and - for **9** and **12**, resp.) and 220 nm (- and + for **9** and **12** resp.). The aromatic quadrant rule[12] is a powerful probe for establishing the absolute configuration at C-4 of 'conventional' $C_4 \rightarrow C_6/C_8$ coupled dimeric proanthocyanidins[8,13,14] but it cannot be used to the same effect for ether linked analogues. The CD data were only useful in a comparative capacity when derivatives 9 and 12 were also available via synthesis using flavan-3,4-diol precursors with established absolute configurations at C-2 and -3.

It was anticipated that the C-4 benzylic ether bonds in the proteracacinidins 7 and **10** would be highly susceptible to solvolysis in aqueous acidic medium. These conditions,[8] which have been applied universally for the formation of $C(sp^3) \rightarrow C(sp^2)$ interflavanyl linkages, would thus be less applicable to the formation of the ether linkage in compounds **7** and **10**. We thus opted to activate the eletrophilic attributes of one of the flavan-3,4-diols *via* formation of the 4-chloroflavan-3-ol derivative **13** in order to permit the formation of the crucial ether bond at near a neutral pH value.



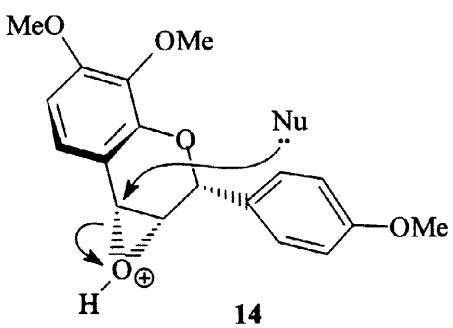
Scheme: Synthesis of the C_4-O-C_4 proteracacinidin derivatives **9** and **12**: *Reagents and conditions*: i, $SOCl_2$/THF; ii, flavan-3,4-diol derivative **5**, then K_2CO_3; iii, flavan-3,4-diol derivative **2**, then K_2CO_3; iv, Ac_2O/pyridine.

Synthesis of the proteracacinidin derivatives **9** and **12** (Scheme) hence involved the initial conversion of epioritin-4α-ol tri-*O*-methylether **2** into the 4β-chloroflavan-3-ol derivative **13** in quantitive yield using thionyl chloride in dry THF. Addition of a solution of a two molar excess of *ent*-oritin-4α-ol tri-*O*-methylether **5** in dry THF as the potential flavanyl-*O*-nucleophile followed by the immediate addition of anhydrous potassium carbonate then afforded a mixture comprising the epioritin-4α-ol and unchanged *ent*-oritin-4α-ol derivatives **3** and **6**, and the *ent*-oritin-(4α→4)-epioritin-4α-ol hexa-*O*-methylether diacetate **9** (14.9% yield) following separation by PLC and acetylation. A similar procedure using epioritin-4α-ol tri-*O*-methylether **2** as the nucleophile, gave epioritin-(4β→4)-epioritin-4α-ol hexa-*O*-methylether diacetate **12** (23.7% yield). The ^{1}H NMR, CD and MS data of derivatives **9** and **12** were identical to those of the same derivatives of the novel (C_4-O-C_4) coupled proteracacinidins **7** and **10** from *A. galpinii*. Unequivocal definition of

2*S*,3*R*,4*R*(C):2*R*,3*R*,4*R*(F) absolute configuration for **7** and 2*R*,3*R*,4*S*(C):2*R*,3*R*,4*R*(F) for **10** then followed from the known absolute stereochemistry of flavan-3,4-diol precursors **1** and **4** which had been meticulously established in Part 28.[6]

The stereoselective coupling of the 4β-chloroflavan-3-ol derivative **13** and epioritin-4α-ol tri-*O*-methylether **2** to give the (C_4-O-C_4)-bis-epioritin derivative **11** with retention of the C-4 configuration of the electrophilic precursor **13**, may be explained in terms of a neighbouring group mechanism involving intramolecular displacement of the *quasi*-axial C-4 chloro nucleofuge by the axial C-3 hydroxyl group.[15] The transient protonated epoxide **14** then permits preferential attack of the nucleophilic C-4 hydroxyl group of the epioritin-4α-ol derivative **2** from the less hindered β-face resulting in a highly stereoselective coupling step.

The retention of configuration at C-4 of the 4β-chloroflavan-3-ol electrophile **13** during formation of the epioritin-(4β→4)-epioritin-4α-ol derivative **11** is in agreement with the stereochemical course of the coupling of **13** and oritin-4α-ol tri-*O*-methylether *via* participation of C_3-OH (Part 28).[6] We anticipated that coupling of **13** and the *ent*-oritin-4α-ol derivative **5** would also proceed *via* the neighbouring group mechanism. The rather unexpected formation of the 4α-ether bond (F-ring) in **8**, *i.e.* with inversion of configuration at C-4 of the 4β-chloroflavan-3-ol **13**, presumably reflects reaction conditions incapable of triggering the neighbouring group mechanism hence resulting in an S_N2-type mechanism which requires the approaching hydroxyl nucleophile to force out the nucleofugal chloride. The requisite alignment for such a concerted process may be facilitated by mutual hydrogen bonding of the C-3 hydroxyl groups of both **5** and **13** which is effectively permitted by the *axial* C-4 hydroxyl group of flavan-3,4-diol **5** compared to the equatorial orientation of the same functionality in nucleophile **2.** In addition, unfavourable 1,3-diaxial interaction between H-2 *ax* of a putative oxirane of type **14** and the approaching nucleophile **5** would not favour a neighbouring group



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mechanism. The stereochemical course of the coupling steps in the formation of the dimeric proteracacinidins **8** and **11** (Scheme) must, incidentally, closely resemble the biosynthetic process leading to **7** and **10** where the same flavan-3,4-diols **4** and **1** with their respective *axial* and *equatorial* C-4 hydroxyl groups presumably serve as the nucleophilic flavanyl units. The formation of the all-*cis*-flavan-3,4-diol derivative **2** in the

coupling reaction of the 4β-chloro-flavan-3-ol **13** and the *ent*-oritin-4α-ol derivative **5** is explicable in terms of solvolysis of the former compound during workup and chromatography. Inversion of configuration is effected by intermolecular hydrogen bonding between the *axial* C-3 hydroxyl group and water hence permitting S_N2 displacement of chloride.

The co-occurrence of the ether-linked proteracacinidins **7** and **10** in the heart-wood of *A. galpinii* in addition to some carbon-carbon bonded analogues, is a further manifestation of the much reduced nucleophilicity of the pyrogallol A-ring which permits other centres to participate in the formation of interflavanyl bonds.

EXPERIMENTAL

^{1}H NMR Spectra were recorded on a Bruker AM-300 spectrometer for solutions as indicated, with Me_4Si as internal standard. FAB Mass spectra were recorded on a VG 70-70E instrument with a VG 11-250J data system and an iontech saddlefield FAB gun. TLC was performed on precoated 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. Methylations were performed with an excess of diazomethane in MeOH-diethyl ether over a period of 48h at -15 °C, while acetylations were conducted in acetic anhydride-pyridine at ambient temperature. Evaporations were done under reduced pressure at ambient temperature in a rotary evaporator.

The extraction of the heartwood of A. galpinii and fractionation using a Craig coutercurrent distribution to give the three fractions A (59.3 g), B (70.8 g) and C (27.3 g), and the separation of a portion (25 g) of fraction B using Sephadex LH-20 in EtOH, affording fractions A-Z were described in Part 28[6] and need not be repeated.

***Ent*-oritin-(4α→4)-epioritin-4α-ol hexa-*O*-methylether diacetate 9.** A portion (80 mg) of fraction K was methylated and the mixture separated by PLC in hexane-C_6H_6-Me_2CO-MeOH (40:40:13:7, v/v) to give four bands at R_F 0.53 (28 mg), 0.70 (12 mg), 0.76 (6 mg) and 0.86 (3 mg). The quantities and complexity of the R_F 0.70, 0.76 and 0.86 did not merit further investigation. Acetylation of the R_F 0.53 band followed by PLC in C_6H_6-Me_2CO (97:3, v/v, x4) afforded a band at R_F 0.44 (12 mg). Further purification by PLC in C_6H_6-Me_2CO-MeOH (47:46:5:2, v/v) gave the title compound **9** (R_F 0.46, 5 mg) as *white amorphous solid.* (Found: M^+, 730.2705. $C_{40}H_{42}O_{13}$ requires M, 730.2704); δ_H (Table); CD $[\theta]_{280.5}$ 1830, $[\theta]_{245}$ -2838, $[\theta]_{235.8}$ 3429, $[\theta]_{225.4}$ 17970, $[\theta]_{218.2}$ -8651 and $[\theta]_{211.7}$ 8434.

Epioritin-(4β→4)-epioritin-4α-ol hexa-*O*-methylether diacetate 12. A portion (100 mg) of fraction M was methylated and subjected to the same procedure of separation and purification as for compound **9**. The first separation gave band M-3 (R_F 0.31, 21 mg) which was acetylated and purified as above to give the title compound **12** (R_F 0.42, 9 mg) as a *white amorphous solid.* (Found: M+, 730.2706. $C_{40}H_{42}O_{13}$ requires M,

730.2704); δH (Table); CD $[\theta]_{274.2}$ -3976, $[\theta]_{241}$ -14370, $[\theta]_{228.6}$ -4458, $[\theta]_{221.9}$ 13560, $[\theta]_{213.1}$ 6262 and $[\theta]_{206}$ 20300.

(2R,3S,4S)-2,3-cis-3,4-trans-4-chloro-3-hydroxy-7,8,4'-trimethoxyflavan 13. Epi-oritin-4α-ol tri-*O*-methylether **2** (50 mg) was dissolved in dry THF (10 ml) and treated with 1.2 equivalents of $SOCl_2$ (0.013 ml) under an N_2-atmosphere at room temperature for 10 min. The solvent was removed under vacuum to give the 4-chloroflavan-3-ol derivative **13** in quantative yield; δ_H (Table).

Synthesis of the C_4-O-C_4 proteracacinidin derivatives 9 and 12.

***Ent*-Oritin-(4α→4)-epioritin-4α-ol hexa-*O*-methylether 8**. *Ent*-oritin-4α-ol tri-*O*-methylether **5** (100 mg) was dissolved in dry THF (10 ml) and added to the chlorinated product **13**. Anhydrous K_2CO_3 (250 mg) was added immediately. The mixture was stirred under nitrogen for 12h at 22°C and a further 2h at 40°C. The K_2CO_3 was removed and the reaction volume was reduced under vacuum. PLC separation in C_6H_6-Me_2CO (4:1, v/v) gave three main bands at R_F 0.33 (12 mg), 0.31 (53 mg) and 0.27 (9.6 mg). Bands R_F 0.31 and 0.27 yielded *ent*-oritin-4α-ol tri-*O*-methylether **5** and epioritin-4α-ol tri-*O*-methylether **2** respectively.

***Ent*-oritin-(4α→4)-epioritin-4α-ol hexa-*O*-methylether diacetate 9**. Acetylation of the R_F 0.33 band followed by purification by PLC in C_6H_6-Me_2CO (9:1, v/v) yielded the title compound (R_F 0.46, 10 mg) as an amorphous solid; δ_H ($CDCl_3$) (Table); M^+, 730.2704, $C_{40}H_{42}O_{13}$ requires M^+, 730.2704; CD data were in accordance with those of the same derivative of the natural product.

Epioritin-(4β→4)-epioritin-4α-ol hexa-*O*-methylether **11**. The procedure and the quantities used were the same as for compound **8** but for epioritin-4α-ol tri-*O*-methylether 2 was used as nucleophile. PLC separation of the reaction mixture in C_6H_6-Me_2CO (4:1, v/v) gave two bands at R_F 0.43 (20 mg) and 0.27 (56 mg). The band at R_F 0.27 was identified as epioritin-4α-ol tri-*O*-methylether **2**.

Epioritin-(4β→4)-epioritin-4α-ol hexa-*O*-methylether diacetate **6**. Acetylation of the band at R_F 0.43 and subsequent PLC separation in C_6H_6-Me_2CO (9:1, v/v) yielded the title compound (18 mg, R_F 0.51) as an *amorphous solid*. δ_H ($CDCl_3$) (Table), M^+, 730.2704, $C_{40}H_{42}O_{13}$ requires M^+, 730.2704; CD data were in accordance with those of the same derivative of the natural product.

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