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THE STRUCTURE AND SYNTHESIS OF THE FIRST DIMERIC PROTERACACINIDINS FROM *ACACIA GALPINII*

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Key Word Index—*Acacia galpinii*; Leguminosae; heartwood; epioritin-4α-ol; proteracacinidins; biomimetic synthesis.

Abstract—The first two proteracacinidin dimers were isolated from the heartwood of *Acacia galpinii*. Epioritin-4α-ol under mild acidic conditions yielded epioritin-(4β → 6)-epioritin-4α-ol and epioritin (4β → 6)-epioritin-4β-ol, confirming the structures of the two isolated dimers.

INTRODUCTION

In contrast to the many different proanthocyaninidins [1, 2] reported over recent years, the occurrence of condensed tannins with a pyrogallol A-ring is limited to the heartwoods of *Prosopis glandulosa* [3, 4] and *Acacia melanoxylon* [5]. A common feature of the above promelacacidins [3, 4] and the newly discovered proteracacidins is the C-4(C) to C-6(D) linkage of the top to the bottom unit of the dimer. Reinvestigation and improvements in separation techniques have led to the identification of epioritin-(4β→6)-epioritin-4α-ol (**9**) and epioritin-(4β→6)-epioritin-4β-ol (**11**) from the heartwood of *Acacia galpinii* [6]. The predominating epioritin-4α-ol, **1** in the heartwood was used as the sole starting material in the biomimetic synthesis under mild acidic conditions to synthesize the two dimers **9** and **11** in good yields (Table 1) compared to previous biflavonoid syntheses [4, 7].

RESULTS AND DISCUSSION

The proteracacinidins (**1**, **3**, **5**, **7**) are accompanied in the heartwood of *Acacia galpinii* with the usual pattern of dihydroflavonol, flavanone, flavonol, chalcone analogues [6] and the two newly discovered dimers, **9** and **11**. The epioritin-4α-ol (**1**) predominates with oritin-4α-ol (**5**) the second highest and the other two diastereoisomers (**3**, **7**) are present in low concentrations. The two dimers were isolated in the free phenolic state, but due to peak broadening no useful NMR data could be obtained and the two were converted to their full acetates **10** and **12**.

HOMODEC experiments on both **10** and **12** confirmed the substitution pattern of the top-units to be an AB and an AA′BB′ system for the A- and B-rings, respectively (Table 2). Irradiation of H-4(C) at δ4.45 of **10** has shown the collapse of the doublet of doublets at δ5.12 (H-3, C) and a pronounced sharpening of the doublet at δ6.94 ($J = 9.0$ Hz) proved to be H-5(A).

The position of the interflavonoid linkage of **10** from the C-4 position of the top-unit to the C-6 (D-ring) of the bottom unit was determined by long range proton decoupling experiments which showed coupling between H-4 (C, δ4.45) and the broad singlet at δ6.45 to be assigned as H-5 (D). This was consistent with the long range coupling between H-5 (D) to H-4F (δ6.16, *d*) in successive experiments. NOE difference spectroscopy confirmed an association between H-5 (D) to H-4 (F), 13.1%; H-4 (C), 11.2% and H-5 (A), 11.9%. HOMODEC also confirmed the chemical shifts of the bottom unit protons of **10** and **12**, and indicated an AA′BB′ system for the E-ring (Table 2).

The C-ring 2,3-*cis*-3,4-*trans* relative stereochemistry of **10** was determined by comparing the *J* values of H-2 to H-3 (1.5 Hz) and H-3 to H-4 (3.0 Hz), with that of the monomer, epioritin-4β-ol, **4**. The 2,3-*cis*-3,4-*cis* relative stereochemistry of the F-ring (**10**) was in accordance with the *J* values of the monomeric epioritin-4α-ol **2**, viz. H-2 to H-3 (1.5 Hz) and H-3 to H-4 (4.0 Hz).

The high amplitude positive Cotton effect $[\phi]_{235.5}$ 9.127×10^4 in the CD spectrum of the fully acetylated proanthocyanidin **10** confirmed the absolute stereochemistry at C-4 (C-ring) and of the top-unit to be 2*R*,3*R*,4*R* [8].

The absolute stereochemistry of the monomeric epioritin-4α-ol, **1**, precursor was determined to be 2*R*,3*R*, 4*R* and was also used in the biomimetic synthesis to synthesize the dimer **9** and on the grounds of the above facts we assign the same absolute stereochemistry to the bottom unit of **9** and its full acetate **10**. The heterocyclic regions of **10** and **12** showed an AMX-system for both

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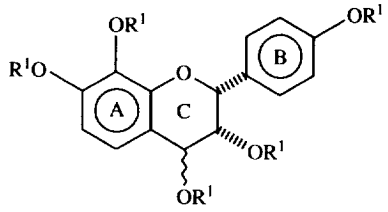

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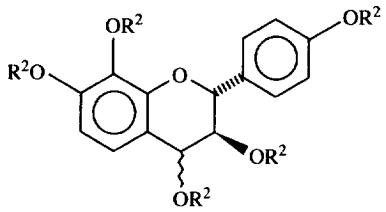

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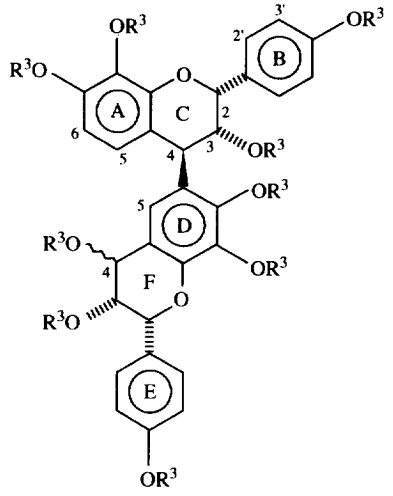

9 $R^3 = H$; { = ≡
10 $R^3 = Ac$; { = ≡
11 $R^3 = H$; { = ▲
12 $R^3 = Ac$; { = ▲

Table 1. Products obtained during synthesis (yield per cent)

Compounds	1	3	9	11
4 hr/40° average of four runs	23.8	14.3	11.1	6.3
10 min 40° (once only)	17	20.8	16	16

rings C and F. According to the J values obtained for the C- and F-rings ($J_{2,3} = 1.5$ and $J_{3,4} = 3.0$ Hz) they were both assigned a 2,3-*cis*-3,4-*trans* relative configuration and these values correlate with those of the monomer **4** (Table 2).

Spin decoupling experiments have shown a marked sharpening of the doublets at δ7.30 and 7.38 when H-2 (C, δ5.10) and H-2 (F, δ5.30) were irradiated, confirming the positions of H-2′, 6′ (B, δ7.30) and H-2′, 6′ (E, δ7.38). The C-4 (C) linkage to C-6 (D) was confirmed by NOE difference spectroscopy indicating an association from H-4 (C, δ4.42) to H-5 (A, *d*, δ6.90, 11.9%) and H-5 (D, *s*, δ6.82, 1.8%). With a similar experiment the position of H-5 (D, δ6.82) was determined and showed an association of 12.5% with H-4 (F, *d*, δ5.65). During the above NOE experiments an association of 3.8% was observed between H-2 (C, δ5.10) and H-5 (D) which is indicative of the bottom unit to have a preferred conformation relative to the C-ring and to be coupled in the axial position. A large positive Cotton effect $[\phi]_{232.8}$ 1.115×10^5 in the CD spectrum supported the previous observation and confirmed the absolute configuration of the C-ring to be 2*R*, 3*R*, 4*R* [7]. In the biomimetic synthesis pure epioritin-4α-ol **1** was stirred at 40° in a 0.1 M HCl aqueous solution under nitrogen [8]. It was observed that during the course of the experiment there was a gradual but definite epimerization of the epioritin-4α-ol, **1**, to epioritin-4β-ol, **3**, (Table 1). Initially, after 10 min of the reaction, only **3** and dimer **9** were formed in low concentrations, but after

Table 2. ^1H NMR (300 MHz), $CDCl_3$ data of **2**, **4**, **10** and **12**

Ring	H	2	4	10	12
A	5	7.13 (*d*, 9.0)	7.37 (*d*, 8.5)	6.95 (*d*, 9.0)	6.91 (*d*, 8.5)
	6	6.83 (*d*, 9.0)	6.79 (*d*, 8.5)	6.76 (*d*, 9.0)	6.79 (*d*, 8.5)
B	2′, 6′	7.42 (*d*, 9.0)	7.42 (*d*, 8.5)	7.28 (*d*, 9.0)	7.30 (*d*, 8.5)
	3′, 5′	7.11 (*d*, 9.0)	7.10 (*d*, 8.5)	7.03 (*d*, 9.0)	7.03 (*d*, 8.5)
C	2	5.41 (*br s*, 1.5)	5.32 (*br s*, 1.5)	5.09 (*br s*, 1.5)	5.10 (*br s*, 1.5)
	3	5.65 (*dd*, 1.5, 4.0)	5.20 (*dd*, 1.5, 3.0)	5.11 (*dd*, 1.5, 3.0)	5.09 (*dd*, 1.5, 3.0)
	4	6.31 (*d*, 4.0)	5.89 (*d*, 3.0)	4.50 (*d*, 3.0)	4.42 (*d*, 3.0)
D	5			6.45 (*br s*)	6.82 (*br s*)
E	2′, 6′			7.38 (*d*, 8.5)	7.38 (*d*, 8.5)
	3′, 5′			7.08 (*d*, 8.5)	7.09 (*d*, 8.5)
F	2			5.38 (*br s*, 1.5)	5.30 (*br s*, 1.5)
	3			5.57 (*dd*, 1.5, 4.0)	5.24 (*dd*, 1.5, 3.0)
	4			6.15 (*d*, 4.0)	5.65 (*d*, 3.0)
OAc		1.90, 2.10, 2.27, 2.31, 2.32 (each *s*)	1.85, 2.12, 2.26 (each *s*), 2.29 (*d*)	1.84, 1.86, 1.89, 2.40 (each *s*), 2.27 (3 × OAc), 2.29 (2 × OAc).	1.63, 1.64, 2.07 2.29, 2.30, 2.35 (each *s*), 2.27 (3 × OAc)

30 min monomer **3** and dimer **11** were also present. Four hr of reaction time under nitrogen seemed to be the optimum period reflected in Table 1, after which there was a decrease in yield of the two dimers to 3–4% of each after 24 hr. Only once were we able to get an astonishing yield after 10 min (Table 1) when the nitrogen cylinder ran empty half-way through the experiment and we were not able to repeat the performance.

Epioritin-4β-ol, **3**, was separated and purified from the reaction mixture to establish its relative (Table 2) and absolute stereochemistry as 2*R*,3*R*,4*S* from the positive Cotton effect ($\phi_{226.9}$ 1.24×10^4). The absolute stereochemistry of dimer **11** could now be assigned as (2*R*,3*R*,4*R*–2*R*,3*R*,4*S*)-epioritin-epioritin-4β-ol, because the monomeric **3** is the logical precursor.

EXPERIMENTAL

CD spectra were determined in MeOH. Acetates were prepd by Ac_2O–pyridine at 60° for 2 hr. Compounds from the acetone extract were first concd by counter-current technique and then sepd on Sephadex LH-20 EtOH and CC (Merck 7734), C_6H_6–Me_2CO, 2:1. Final purification was done on Merck TLC 5554 in C_6H_6–Me_2CO, 4:3 for the phenolic state and using 9:1 for the full acetates. From 6 g of semi-crude material sepd on Sephadex, the isolated phenolic compounds were present in the bands with R_f 0.33 (**1**, 782 mg), 0.29 (**5**, 236 mg), 0.28 (**3**, 31 mg), 0.17 (**9**, 51 mg) and 0.10 (**11**, 23 mg) when C_6H_6–Me_2CO, 4:3 (× 3) was used as eluant.

A 0.1 M HCl aq. soln (80 ml) in N_2 was stirred at 40°, **1** (100 mg) was added and the reaction quenched with excess ice after the required time had elapsed. This was followed by immediate extraction of the reaction mixt. with EtOAc, evapd under red. pres. and the residue analysed.

Plant material. Acacia galpinii was collected at Steelpoort Valley in the Eastern Transvaal and identified by Mrs P. Swartz of the National Botanical Institute at Pretoria. Heartwood drillings (3.6 kg) were extracted with acetone and yielded 270 g of extract.

Epioritin-(4β→6)*-epioritin-*4β-ol (*nonyl acetate*) **10**. Non-crystalline, 75 mg. R_f 0.14. MS-FAB:$[M]^+$ *m/z* 940. Found:C, 61.4; H, 4.4. $C_{48}H_{44}O_{20}$ requires C, 61.3; H, 4.7%.

Epioritin-(4β→6)*-epioritin-*4β-ol (*nonyl acetate*) **12**. Non-crystalline, 31 mg. R_f 0.12. MS-FAB:$[M]^+$ *m/z* 940. Found:C, 61.5; H, 4.5. $C_{48}H_{44}O_{20}$ requires C, 61.3; H, 4.7%.

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REFERENCES

1. Harborne, J. B. (ed.) (1988) in *The Flavonoids: Advances in Research since* 1980. pp. 21–62. Chapman and Hall, London.
2. Harborne, J. B. (ed.) (1994) in *The Flavonoids, Advances in Research since* 1986. pp. 23–55. Chapman and Hall, London.
3. Jacobs, E., Ferreira, D. and Roux, D. G. (1983) *Tetrahedron Letters* **24**, 4627.
4. Young, E., Brandt, E. V., Young, D. A., Ferreira, D. and Roux, D. G. (1986) *J. Chem. Soc. Perkin Trans. I* 1737.
5. Foo, L. Y. (1986a) *J. Chem. Soc., Chem. Commun.* 236.
6. Malan, E. and Roux, D. G. (1975) *Phytochemistry* **14**, 1835.
7. Steynberg, J. P., Burger, J. F. W., Cronje, A., Malan, J. C. S., Young, D. A. and Ferreira, D. (1990) *Phytochemistry* **29**, 275.
8. Botha, J. J., Ferreira, D. and Roux, D. G. (1981) *J. Chem. Soc., Perkin Trans. I* 1235.