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# A (4β → 5)-LINKED PROTERACACINIDIN DIMER FROM THE HEARTWOOD OF *ACACIA CAFFRA*

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**Key Word Index**—*Acacia caffra*; Leguminosae; heartwood: proteracacinidin dimer; teracacidin flavonoids.

**Abstract**—The proteracacinidin *ent*-oritin-(4β → 5) epioritin-4β-ol with 8-*O*-methylepioritin-4α-ol, 3-*O*-methyl-7,8,4′-trihydroxy-flavone and other teracacidin analogues were isolated from the heartwood of *Acacia caffra*.

## INTRODUCTION

*Acacia caffra* occurs in woodland and wooded grassland, frequently along rivers and streams. Its distribution is from Port Elizabeth in the south following the coastline up to Northern Natal from where it is spread inland across the greater part of the province of Transvaal. The sapwood is off-white and the heartwood heavy (air-dry 980 kg m$^{-3}$) and nearly black. It is cold resistant and one of the least thorny of the *Acacia* species in Southern Africa [1]. The substitution pattern of the flavonoids present in the heartwood is the same as the metabolic pool occurring in *Acacia galpinii*, viz. 7,8,4′-substituted [2, 3].

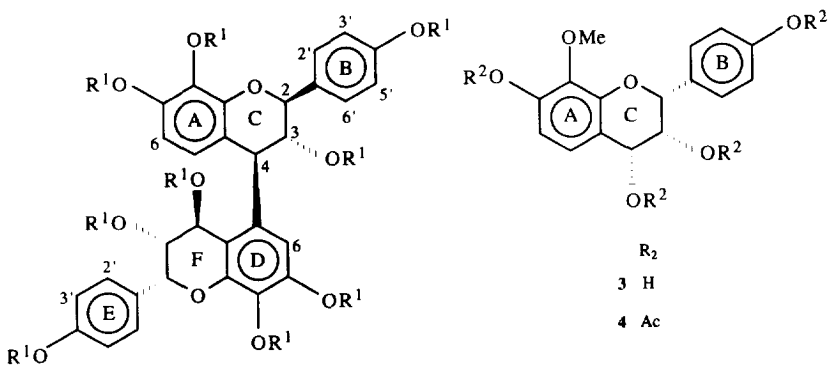
The 8-OMe-epioritin-4α-ol [3] represents an extension of the already known 8-*O*-methylated pyrogallol A-ring of the prosopin-4-ols isolated from *A. cultriformis* [4] *A. saxatilis* [5] and *A. montana* [6]. This is only the second *Acacia* species and plant in Southern Africa in which the teracacidin-type flavonoids were positively identified.

## RESULTS AND DISCUSSION

The newly discovered dimer **1** was accompanied by the two recently published dimers from *Acacia galpinii* [3], the monomeric analogues of dihydroflavonol, flavanone, flavonol, chalcone and flavan-3,4-diols. Epioritin-4α-ol, oritin-4α-ol and epioritin-4β-ol are present in a ratio of 4:2:1 with oritin-4β-ol available in trace quantities.

Compound **2** was shown to contain an AB and an AA′BB′ system for the A- and B-rings, respectively, resulting from the HOMODEC experiments. With H-4(C) (*d*, δ4.62, *J* = 10.0 Hz) of **2** as reference the doublet of doublets at δ5.64 (H-3, *C*) collapsed and the doublet at δ6.35 (*J* = 8.5 Hz) showed pronounced sharpening confirming it to be H-5(A). Irradiation of H-2(C) (*d*, δ5.04, *J* = 9.5 Hz) showed sharpening of the doublet at δ7.39 (*J* = 8.5 Hz) indicating it to be H-2′,6′(B). From H-2′6′(B) it was possible to determine the position of H-3′,5′(B) at δ6.97 (*d*, *J* = 8.5 Hz).

No HOMODEC coupling could be detected between H-4(F) (*d*, δ6.12, *J* = 3.0 Hz) of **2** and a singlet appearing



at $\delta$6.65 suggesting that it could be H-6(D). Using the same technique as described it was possible to assign the chemical shifts of the bottom unit protons for compound **2** (Table 1).

NOE experiments have shown an association of 6.7% between H-4(C) and H-2(C) of **2**, indicating them to be on the same side of the heterocyclic ring and also confirming the relative stereochemistry of the top unit to be 2,3-*trans*-3,4,-*trans* with *J* values the same as the all-*trans* monomer [3]. H-4(C) of **2** showed a strong association to H-4(F) of 15% which indicated that the coupling must be to C-5′ (D) of the bottom unit because no association would be possible when the coupling was at C-6 (D) (Dreiding model). The strong association of H-4(C) to H-4(F) also suggested a preferred conformation where the bottom unit is perpendicular to the plane of the top unit with the D-ring above the plane and the E- and F-rings on the same level as the top unit. No association could be detected between H-4(F) and H-5(A). This observation explained the broad appearance of all the heterocyclic protons at 296 K with almost no sharpening at an elevated temperature of 346 K.

NOE showed no association between H-4(F) and the singlet at $\delta$6.65 confirming the singlet to be H-6(D). Association between H-2(C) to H-2′,6′(B, 3.5%) and H-2(F) to H-2′,6′(E, 6.0%) confirmed their chemical shifts. The *J* values of the bottom unit (Table 1) is in accordance with the values of the 2,3-*cis*-3,4-*trans* monomer and no association could be detected between H-2(F) and H-4(F) supporting the above relative stereochemistry.

MS-FAB showed a molecular mass of 940 units thus supporting the structure of **2**. The strong positive Cotton effect $[\Phi]_{235.80\ nm} \times 3.156 \times 10^4$ in the CD spectrum of **2** confirmed the 4$\beta$-substitution at C-4 (C-ring) and the absolute stereochemistry and structure of **2** to be (2*S*, 3*R*, 4*R*-2*R*, 3*R*, 4*S*)-ent-oritin(4$\beta \rightarrow$ 5)-epioritin-4$\beta$-ol.

The $^1$H NMR of **4** displayed the expected AA′, AA′BB′ and AMX systems for the A-, B- and C-rings, respectively (Table 1). The *J* values are in accordance with that previously determined for epioritin-4$\alpha$-ol [3]. The large negative Cotton effect at $[\Phi]_{225.8\ nm}$ $4.973 \times 10^{-3}$ confirmed the stereochemistry to be 2*R*, 3*R*, 4*R*. NOE experiments could not detect any association of the methoxyl singlet at $\delta$3.87 to any of the protons suggesting the *O*-methoxyl group to be at the 8-position. The MS fragmentation of **4** confirmed the suggested structure with *m/z* 472 (34%) and the 8-position of the methoxyl with the RDA A-ring fragment of *m/z* 252 (8%). The structure of 3-*O*-methyl-7,8,4′-flavone was determined by $^1$H NMR and mass spectrometry and confirmed to be the same compound as was reported from *Acacia galpinii* [2].

## EXPERIMENTAL

The fatty components and compounds running higher than the flavan-3,4-diols on TLC were very effectively removed from the heavier fraction with a hexane/EtOAc (5:2) solution. The residue was separated on Sephadex LH-20 EtOH and CC (Merck Silica gel 7734), $C_6H_6$–$Me_2CO$ (2:1). Final purifications were done on Merck TLC Silica gel 5554 in $C_6H_6$–$Me_2CO$ (4:3) for the phenolic state and using 9:1 for the full acetates. From 6g of the residue separated on Sephadex, the isolated phenolic compounds were present in the combinations made from the fractions collected in the test tubes; 33–77/751 mg; 78–128/1519 mg; 129–160/444 mg; 161–236/473 mg and 237–280/171 mg. Acetates were prepared by $Ac_2O$–pyridine at 60° for 2 hr. CD spectra were determined in MeOH. Due to the complexity of the mixtures all the compounds were isolated and purified as the full acetates.

*Plant material. Acacia caffra* was collected at Loskopdam in the Eastern Transvaal and identified by Mrs P. Swartz of the National Botanical Institute at Pretoria. Milled heartwood (4.1 kg) was extracted with acetone and yielded 443 g of extract.

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

| Ring | H | 2 | 4 |
|---|---|---|---|
| A | 5 | 6.35 (*d*, 8.5) | 6.93 (*d*, 8.5, benzylic) |
| | 6 | 6.62 (*d*, 8.5) | 6.70 (*d*, 8.5) |
| B | 2′, 6′ | 7.39 (*d*, 8.5) | 7.48 (*d*, 8.5) |
| | 3′, 5′ | 7.17 (*d*, 8.5) | 7.11 (*d*, 8.5) |
| C | 2 | 5.04 (*d*, 9.5) | 5.39 (*br s*, 1.5) |
| | 3 | 5.64 (*dd*, 10.0, 9.5) | 5.65 (*dd*, 4.0, 1.5) |
| | 4 | 4.62 (*d*, 10.0) | 6.30 (*d*, 4.0) |
| D | 5 | — | |
| | 6 | 6.71 (*s*) | |
| E | 2′, 6′ | 7.40 (*d*, 8.5) | |
| | 3′, 5′ | 7.11 (*d*, 8.5) | |
| F | 2 | 5.34 (*br s*, 1.5) | |
| | 3 | 5.28 (*dd*, 3.0, 1.5) | |
| | 4 | 6.12 (*d*, 3.0) | |
| OAc | | 1.23, 1, 61, 1.87, 2.19, 2.23, 2.24, 2.56, 2.58, 2.59 (each *s*) | 1.90, 2.09, 2.29, 2.33 (each *s*) |
| OMe | | | 3.87 (*s*) |

*Ent-oritin-*(4$\beta$ → 5)*-epioritin-*4$\beta$*-ol* (*nonyl acetate*) (**2**). Isolated from phenolic fraction 129–160 ($R_f$ 0.17–0.29; B:A; 8:6 × 2). Full acetate has a $R_f$ 0.17 (B:A; 9:1 × 2). Non-crystalline, 7 mg. $^1$H NMR data (Table 1). (Found: $M^+$, 940.3352 $C_{48}H_{44}O_{20}$ requires 940.3356).

8-*OMe-epioritin-*4$\alpha$*-ol* (*tetraacetate*) (**4**). Isolated from phenolic fraction 33–77 ($R_f$ 0.44–0.52; B:A; 8:6 × 2). Full acetate has a $R_f$ 0.42 (B:A; 9:1 × 2). Non-crystalline, 4 mg. $^1$H NMR data (Table 1). (Found: $M^+$, 472.1850, $C_{24}H_{24}O_{10}$ requires 472.1852. EIMS (rel. int.) *m/z*: 472(34), 412(7), 370(46), 328(100), 252(8), 220(7).

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