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A COMPARATIVE STUDY OF THE PHENOLIC PRODUCTS IN THE HEARTWOOD OF *ACACIA KARROO* FROM TWO DIFFERENT LOCALITIES

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Key Word Index—*Acacia karroo*; Leguminosae; heartwood; (2′*S*,3′*R*)-3,10-dihydroxy-9-*O*-(6′-hydroxy-7′-*O*-methyl-2′-hydroxymethyldihydrobenzofuran-3-yl)dibenz-[*b*,*d*]-pyran-6-one; 8-*O*-methyl-epi-prosopin-4*β*-ol; 8-methoxyfustin; 7,8,3,4′-tetrahydroxy-3′-methoxyflavone; flavonoids.

Abstract—Known flavonoids were found in the heartwood of *Acacia karroo*. New natural products were isolated from the proposed subspecies *montana*. They are (2′*S*,3′*R*)-3,10-dihydroxy-9-*O*-(6′-hydroxy-7′-*O*-methyl-2′-hydroxymethyldihydrobenzo furan-3-yl)dibenz-[*b*,*d*]-pyran-6-one and its 10-*O*-methyl analogue; 8-*O*-methylepiprosopin-4*β*-ol; 8-methoxyfustin; and 7,8,3,4′-tetrahydroxy-3′-methoxyflavone. Apart from the difference in substitution patterns of the compounds isolated from the two plant sources, the flavonoids from subspecies *montana* are *O*-methylated as opposed to none occurring in *A. karroo*.

INTRODUCTION

According to Palgrave and von Breitenbach [1, 2] *Acacia karroo* is the most widespread acacia species in southern Africa but this very fact has resulted in confusion and differences of opinion amongst botanists in the past. Gerstner and Mey [3] and Robbertse [4] have proposed that *A. karroo* be subdivided into several subspecies but to date the Botanical Society of South Africa have resisted this proposal. This publication indicates a difference in chemical composition of the *A. karroo* found in the drier parts of the country on the western side of the Drakensberg and the same species found in the Loskopdam area in Eastern Transvaal on the hills surrounding the dam. Swartz and Robbertse proposed that the specimen from Loskopdam be classified as *Acacia karroo* subsp. *montana* [5].

The metabolic pool present in the heartwood of the two specimens (Table 1) was shown to differ considerably, the main differences were the presence of a greater variety of 7,8,3′,4′-substituted compounds and a large number of the *O*-methylated derivatives in the proposed subspecies *montana*. The two isomers 8-methoxyprosopin-4*β*-ol [6] and 8-methoxy prosopin-4α-ol[7] were isolated from *A. cultriformis* and *A. saxatilis* but 8-methoxy-epiprosopin-4*β*-ol isolated as the full acetate (**3**) from subspecies *montana* is new. The discovery of 7,3′,4′-trihydroxy-8-methoxydihydroflavonol (**4**) is reported for the first time.

Two partially *O*-methylated derivatives of 3,7,8,3′,4′-pentahydroxyflavone (melanoxetin) were recently reported to occur in the heartwood of *A. nigrescens* [8], viz. 3-*O*-methylated and 3,3′-*O*-methylated. We now report the presence of 3,7,8,4′-tetrahydroxy-3′-methoxyflavone (**5**) in subspecies *montana*.

RESULTS AND DISCUSSION

The two complex dibenzo-α-pyrones, 3,10-dihydroxy-9-*O*-(6′-hydroxy-7′-*O*-methyl-2′-hydroxy-methyldihydrobenzofuran-3-yl)dibenz-[*b*,*d*]-pyran-6-one and its 10-*O*-methyl analogue were isolated as acetates (**1**, **2**) from the heartwood of *montana* but could not be found in the heartwood of *A. karroo*. Similar heterodimeric compounds with different substitution patterns were previously discovered in the heartwoods of *Umtiza listerana* [9] and *A. mearnsii* [10].

The presence of a dibenzo-α-pyrone moiety in **1** and **2** was revealed by ^{1}H NMR spectrometry and the appearance of the characteristically deshielded doublet [9, 11] of H-1 ($J = 9.0$ Hz, $\delta 8.93$ for **1** and $\delta 8.65$ for **2**). Proton decoupling experiments have shown an ABX-system ($J = 8.5$ and 2.5 Hz) for ring A using the H-1 doublet as reference and an AB-system ($J = 9.0$ Hz) for ring B using the H-7 doublet as reference in both **1** and **2**. The lactone carbonyl was confirmed by IR absorption at 1720 cm^{-1}. The 300 MHz ^{1}H NMR spectra also displayed an additional AB-system for both the acetates **1** and **2** which were allocated to the aromatic protons of the dihydrobenzofuran unit using the H-5′ doublet ($J = 8.5$ Hz) as reference. HOMODEC experiment exhibited a heterocyclic

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In memory of the late Professor D. G. Roux who died in June 1994.

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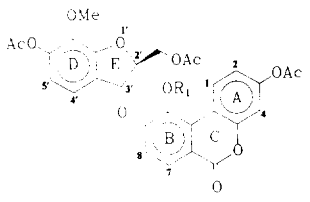
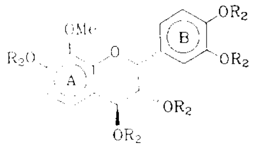
Table 1. Compounds present in the heartwoods of *A. karroo* and in subspecies *montana*

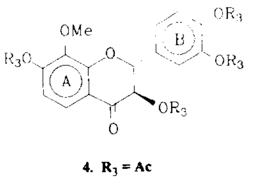
Compound	*A. karroo*	*montana*
3,10-Dihydroxy-9-*O*-(6′-hydroxy-7′-*O*-methyl-2′-hydroxymethyldihydrobenzofuran-3-yl) dibenzo[*b*,*d*]-pyran-6-one and its 10-*O*-methyl analogue	none	present
7,3′,4′-Trihydroxydihydroflavonol	present	present
7,3′,4′-Trihydroxyflavonol	none	present
7,3′,4′-Trihydroxyflavan-3-ol	present	present
7,8,3′,4′-Trihydroxydihydroflavonol	none	present
7,8,3′,4′-Tetrahydroxyflavonol	present	present
7,8,4′-Trihydroxy-3′-methoxyflavonol	none	present
7,8,4′-Trihydroxy-3,3′-dimethoxyflavone	none	present
5,7,3′,4′-Tetrahydroxyflavone	present	none
8-*O*-Methyl-7,3′,4′-trihydroxydihydroflavonol	none	present
8-*O*-Methylepiprosopin-4β-ol	none	present

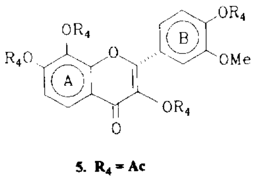


1. $R_1 = Ac$
2. $R_1 = Me$



3. $R_2 = Ac$





4. $R_3 = Ac$ **5.** $R_4 = Ac$

ABMX-system in both complex benzo-α-pyrones in which the methylene protons appeared as two doublet-of-doublets at δ4.61 and 4.57 (J = 3.0 and 12.5 Hz); δ4.15 and 4.13 (J = 5.5 and 12.5 Hz) for **1** and **2**, respectively. The non-equivalence of the methylene protons (different J values) is an indication of their proximity to the C-2′ stereogenic centre. The single acetoxyl groups at δ2.13 and 2.10 confirmed the aliphatic hydroxymethyl group in the two parent compounds. The benzylic relationship between H-4′ (δ7.03 and 7.0) and H-3′ (δ5.12 and 5.09, J = 8.0 Hz) was confirmed by decoupling experiments and during the same experiments showed the H-2′ (δ4.43 and 4.40, J = 8.0, 5.5 and 3.0 Hz) with non-equivalent coupling to the methylene protons (J = 3.0 and 5.5 Hz) to be on the neighbouring carbon, defining C-3′ to be the coupling site with the dibenzo-α-pyrone unit.

The proposed structures of **1** and **2** were confirmed by mass spectral analysis, yielding $[M]^+$ m/z 606 (11%) and m/z 578 (16.8%), respectively. Fission of the ether linkage [6] confirmed the substituents on the dihydrobenzofuran (rings D and E) by fragments m/z 294 (45 and 53%); m/z 252 (48 and 42%) and m/z 209 (100 and 22%) for **1** and **2**. The dibenzo-α-pyrone (rings A, B and C) were represented by fragments $[M]^+$ m/z $[606 - (294 + 42)] = m/z$ 270 (53%); m/z $[270 - 28] = m/z$ 242 (58%) for **1** and $[M]^+$ m/z $[578 - (294 + 42)] = m/z$ 242 (100%); m/z $[242 - 28] = m/z$ 214 (58%) for **2**. Repeated NOE experiments could only detect an association of the 10-*O*-methyl group (3.7%) with the H-1 proton of **2**, with this information and with the mass spectral fragments of m/z 294 (45 and 53%), m/z 270 (53%) and m/z 242 (100%) it was possible to assign the positions of the *O*-methyl groups to C-7′ for **1** and C-10 with C-7′ for **2**. CD measurement of **2** has shown a strong negative Cotton effect at $[\theta]_{205\ \mathrm{nm}}$ 1.345×10^{-4} and with the large H-2′ to H-3′ coupling constant (J = 8.0 Hz) typical of a 2′,3′-*trans*-configuration it suggested a 2′*S*,3′*R*-configuration for both **1** and **2** [9, 12].

^{1}H NMR spectral and decoupling experiments have shown that AB- and ABX-systems were present for the A- and B-rings in each of the three compounds **3**–**5**. In the case of both **3** and **4** no association between the two single *O*-methyl groups (δ3.80 and 3.84) and any protons could be detected, suggesting them to be at the 8-position. Mass spectral analyses have shown RDA fragments m/z 211 (4.2%), 169 (7.1%) and m/z 167 (100%), 166 (58.5%) for the A-rings of **3** and **4**, respectively, confirming the *O*-methyl group in both instances to be at the 8-position. Fragments m/z 152 (16%), 136 (100%) and m/z 152 (78.5%) confirmed the B-ring substitution for both compounds.

The ^{1}H NMR spectrum displayed an AMX-system for **3** in the heterocyclic region at δ5.29 (J = 1.0 Hz, H-2), δ5.31 (J = 3.0 and 1.0 Hz, H-3), and δ6.17 (J = 3.0 Hz, H-4). NOE difference spectroscopy could not detect any association between H-4 and H-2 which validated the assignment of the relative stereochemistry to be 2,3-*cis*-3,4-*trans* [13]. A large positive Cotton effect at $[\theta]_{228.5\ nm}$ 7.216×10^3 confirmed it to be a 4β-ol and the compound to be the full acetate of (2*R*,3*R*,4*S*)-8-*O*-methylepiprosopin-4β-ol (**3**).

A distinct *trans*-diaxial coupling between H-2 and H-3 (J = 12.0 Hz) [8] in the ^{1}H NMR spectrum of **4** along with the lowfield doublet δ7.66 (J = 9.5 Hz) are positive indicators for a dihydroflavonol. IR absorption at 1685 cm^{-1} confirmed the carbonyl at C-4. The CD curve of **4** was exactly the same as the curve for an authentic sample of (2*R*,3*R*)-(+)-fustin isolated from *Acacia mearnsii* [14], confirming the compound to be the full acetate of (2*R*,3*R*) 8-*O*-methyl-7,3′,4′-trihydroxydihydroflavonol (**4**).

NOE experiments exhibited an association of the 3′-*O*-methyl (δ4.0) with H-2′ (δ7.73, 3.5%) in **5**. Mass spectral analysis supported the suggested structure of **5** with a molecular ion of 484 (13%). With fragments *m/z* 152 (16%), 153 (100%) for the A-ring and *m/z* 151 (23%) for the B-ring the position of the 3′-*O*-methyl group was confirmed and the structure of 3,7,8,4′-tetraacetoxy-3′-methoxyflavone (**5**) was established.

On the basis of these chemical features in conjunction with the morphological differences reported, there is enough evidence to confirm the botanical separation of *Acacia karroo* from its subspecies *montana*.

EXPERIMENTAL

Plant material. Acacia karroo was collected from the farm of the late Mr Andries van Rensburg (Plessisdraai near Hoopstad in the Orange Free State) and identified by Mr R. Ward (Department of Botany at the University of Durban-Westville). Mrs P. Swartz (NBI in Pretoria) collected and identified specimens of *Acacia karroo* subs. *montana* on the hills at Loskopdam in the Eastern Transvaal.

Extraction and isolation. Drillings from the heartwood of both *A. karroo* (4.1 kg) and *montana* (3.6 kg) were twice extracted with Me_2CO over periods of 2 days at 30°. After removal of the solvent the powdered extract was defatted with hexane to yield amorphous powders of 65 g and 76 g, respectively.

After separation (40 g) by counter-current chromatography in a water–butan-2-ol–n-hexane (5:4:1) using 6 separating funnels with an aq. lower phase of 200 ml and an equivalent vol. of organic upper phase, the contents (3.4 g) of the first three funnels (upper phase) were combined. CC (Merck 7734, C_6H_6–Me_2CO, 2:1) resulted in frs where the isolated compound was present in bands with R_f 0.45–0.57 (**1** and **2**), R_f 0.37–0.42 (**4** and **5**) and R_f 0.22 (**3**) when analysed on Merck TLC 5554 in C_6H_6–Me_2CO (2:1). All R_f-values for the full acetates on TLC in a mixture of C_6H_6–Me_2CO (9:1) will be reflected along with the other details of the physical data. Acetylations were in Ac_2O–pyridine at 30°. All ^{1}H NMR spectra were run in $CDCl_3$.

(2′S,3′R)-3,10-*Diacetoxy*-9-O-(6′-*acetoxy*-7′-O-*methyl*-2′-*acetoxy methyldihydrobenzofuran*-3-*yl*)*dibenz*-[b,d]-*pyran*-6-*one* (**1**). Non-crystalline, 3 mg, R_f 0.50. MS: *m/z* (rel. int.): 606 (11) $[M]^+$, 420 (34), 294 (45), 284 (56), 270 (52.6), 269 (88), 252 (48.5), 242 (57.5), 209 (100%). ^{1}H NMR: δ8.93 (*d*, J = 9.0 Hz, H-1), 8.09 (*d*, J = 8.5 Hz, H-7), 720 (*d*, J = 8.5 Hz, H-5′), 7.15 (*d*, J = 8.5 Hz, H-8), 7.09 (*dd*, J = 8.5 and 2.5 Hz, H-2), 7.03 (*d*, J = 8.5 Hz, H-4), 7.03 (*d*, J = 2.5 Hz, H-4), 5.12 (*d*, J = 8.0 Hz, H-3′), 4.61 (*dd*, J = 12.5 and 3.0 Hz, $-CH_2-$), 4.43 (*m*, J = 12.5, 8.0 and 5.5 Hz, H-2′), 4.15 (*dd*, J = 12.5 and 5.5 Hz, $-CH_2-$), 3.89 (*s*, OMe), 2.39, 2.36, 2.35, 2.13 (*s*, 4 × OAc).

(2′S,3′R)-3-*Acetoxy*-10-O-*methyl*-9-O-(6′-*acetoxy*-7′-O-*methyl*-2′-*acetoxy methyldihydrobenzofuran*-3-*yl*)*dibenz*-[b,d]-*pyran*-6-*one* (**2**). Non-crystalline, 5 mg, R_f 0.48. MS: $[M]^+$ *m/z* 578.1426, $C_{30}H_{26}O_{12}$ requires: 578.1422, *m/z* (rel. int.): 578 (16.8), 392 (33), 294 (53), 256 (56), 252 (42), 242 (100), 241 (45.5), 214 (58), 209 (22%). ^{1}H NMR: δ8.65 (*d*, J = 9.0 Hz, H-1), 8.07 (*d*, J = 8.5 Hz, H-7), 719 (*d*, J = 8.5 Hz, H-5′), 7.12 (*d*, J = 8.5 Hz, H-8), 7.01 (*dd*, J = 2.5 Hz, H-4), 7.0 (*d*, J = 8.5 Hz, H-4′), 7.0 (*dd*, J = 9.0 and 2.5 Hz, H-2), 5.09 (*d*, J = 8.0 Hz, H-3′), 4.57 (*dd*, J = 12.5 and 3.0 Hz, $-CH_2-$), 4.40 (*m*, J = 12.5, 8.0 and 5.5 Hz, H-2′), 4.13 (*dd*, J = 12.5 and 5.5 Hz, $-CH_2-$), 3.87, 4.01 (*s*, × OMe), 2.37, 2.33, 2.10, (*s*, 3 × OAc).

(2R,3R,4S)-8-O-*methyl*-3,4,7,3′,4′-*pentaacetoxy*-*epi*-*prosopin*-4β-*ol* (**3**). Non-crystalline, 11 mg, R_f 0.44. MS: $[M]^+$ *m/z* 530.1426, $C_{26}H_{26}O_{12}$ requires: 530.1422, *m/z* (rel. int.): 530 (8), 470 (13.5), 428 (8), 386 (3), 344 (2), 194 (5), 211 (4.2), 210 (3.4), 169 (7.1), 168 (8.8), 152 (16), 150 (8.5), 136 (100%). ^{1}H NMR: δ7.38 (*dd*, J = 8.5 and 2.5 Hz, H-6′), 7.28 (*d*, J = 2.5 Hz, H-2′), 7.21 (*d*, J = 8.5 Hz, H-5′), 7.01 (*d*, J = 8.5 Hz, H-5), 6.17 (*d*, J = 8.5 Hz, H-6), 6.17 (*d*, J = 3.0 Hz, H-4), 5.31 (*dd*, J = 3.0 and < 1.0 Hz, H-3), 5.29 (*d*, J < 1.0 Hz, H-2), 3.80 (*s*, OMe), 2.31, 2.28, 2.27, 2.13 1.88 (*s*, 5 × OAc).

(2R,3R)-8-O-*methyl*-3,7,3′,4′-*tetraacetoxydihydroflavone* (**4**). Non-crystalline, 15 mg, R_f 0.37. MS: $[M]^+$ *m/z* 486.1163, $C_{24}H_{22}O_{11}$ requires: 486.1160, *m/z* (rel. int.): 486 (60), 444 (60), 402 (30), 360 (17), 290 (9.5), 289 (31.5), 258 (11.5), 167 (100), 166 (58.5), 152 (78.5%). ^{1}H NMR: δ7.66 (*d*, J = 8.5 Hz, H-5), 7.43 (*dd*, J = 8.5 and 2.5 Hz, H-6′), 7.31 (*d*, J = 2.5 Hz, H-2′), 7.26 (*d*, J = 8.5 Hz, H-5′), 6.82 (*d*, J = 8.5 Hz, H-6), 5.70 (*d*, J = 12.0 Hz, H-2), 5.45 (*d*, J = 12.0 Hz, H-3), 3.84 (*s*, OMe), 2.33, 2.30, 2.29, 2.06 (*s*, 4 × OAc). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1685.

3,7,8,4′-*Tetraacetoxy*-3′-*methoxyflavone* (**5**). Non-crystalline, 8 mg, UV λ_{max}^{MeOH} nm: 239, 252, 274, 304 (log ε 4.26, 4.32, 4.07, 4.20). MS: $[M]^+$ *m/z* 484.1003, $C_{24}H_{20}O_{11}$ requires: 484.1005, *m/z* (rel. int.): 484 (13), 442 (13), 400 (7.5), 358 (6), 316 (6), 287 (4), 153 (100), 152 (16), 151 (23%). ^{1}H NMR: δ7.96 (*d*, J = 9.0 Hz, H-5), 7.77 (*dd*, J = 9.0 and 2.5 Hz, H-6′), 7.73 (*d*, J = 9.0 Hz, H-2′), 7.35 (*d*, J = 9.0 Hz, H-5′), 7.13 (*d*, J = 9.0 Hz, H-6), 4.00 (*s*, OMe), 2.38, 2.36, 2.33, 2.32 (*s*, 4 × OAc). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1768.

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