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Polyphenols from Honeybush Tea (*Cyclopia intermedia*)

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The fermented leaves and stems of *Cyclopia intermedia* are used to brew Honeybush tea, a herbal tea indigenous to South Africa. The plant is also used to manufacture a sweet herbal infusion used for restorative properties such as soothing coughs and alleviating bronchial complaints including tuberculosis, pneumonia, and catarrh. It is claimed to have a low tannin content and no caffeine and contains various antioxidants. Continued investigations into the phenolic content of the leaves and stems of *C. intermedia* yielded tyrosol and a methoxy analogue, 2-{4-[*O*-α-apiofuranosyl-(1″→6′)-β-D-glucopyranosyloxy]phenyl}ethanol, 4-[*O*-α-apiofuranosyl-(1″→2′)-β-D-glucopyranosyloxy]benzaldehyde, five glycosylated flavonols, two isoflavones, four flavanones, two isoflavones, and two flavones. Structure elucidation was done by NMR, CD, and MS methods. Because flavonoids are presumed to contribute significantly toward the scavenging effects of active oxygen species, our results indicate that the tentative claimed health-promoting properties may be attributed to the presence of these and other phenolics in *C. intermedia*.

KEYWORDS: ***Cyclopia intermedia*; Fabaceae; honeybush tea; polyphenols; flavonoids; isoflavonoids; glycosides; health beverage**

INTRODUCTION

Initial investigations (*1*, *2*) into the phenolic content of *Cyclopia intermedia* E. Mey (Fabaceae), a woody legume indigenous to South Africa from which a traditional herbal tea is brewed, have revealed the presence of phenolic metabolites purported to have significant pharmacological properties. The tea with a pleasant taste and characteristic honey flavor known as honeybush tea is brewed from the leaves and stems of several species mainly, *C. intermedia* and *Cyclopia subternata* Vogel. The presence of such metabolites in the tea extracts, in particular flavonoids with acknowledged antioxidant properties (*2*), together with the well-known application of the beverage by the people in the Cape fynbos (Cape macchia) region of South Africa as a medicinal concoction (*3*) prompted this continued investigation. On the basis of the chemical findings and belief that the tea contains, little, if any caffeine, the tea is fast gaining popularity as a health beverage.

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MATERIALS AND METHODS

Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 and 75 MHz, respectively, on a Bruker AVANCE DPX-300 spectrometer with TMS as the internal standard. MS spectra and mass estimations were obtained with a Kratos MS-80 mass spectrometer in the double focus EI mode. Although masses for fragment ions were recorded on the peracetate derivatives of compounds **5**, **6**, **9**, **15**, **17**, and **29**, no molecular ions were observed due to the instability of the glycosides, which require derivatization by permethylation of the free hydroxyls (*4*, *5*).

Chromatography and Derivatization. Qualitative thin-layer chromatography (TLC) was performed on 3 cm × 7 cm Kieselgel $60F_{254}$, 0.25 mm, aluminum plates (Merck). Following development, the TLC plates were sprayed with formaldehyde (40%)−sulfuric acid (2:98) or with anisaldehyde−sulfuric acid−ethanol (5:5:90) and heated to 120 °C. Preparative separations (PLC) were carried out by application of 10−25 mg mixture/plate on 20 cm × 20 cm glass plates coated with 1.0 mm Kieselgel PF_{254} (Merck), which were air-dried and used without prior activation. Small scale preparative separations were on 20 cm × 20 cm precoated silica gel PF_{254}, 0.25 mm glass plates (Merck). Compounds were eluted from the adsorbent with acetone after location by UV light 254 nm. Two-dimensional paper chromatography (PC) was done on 28.5 cm × 46 cm Whatman No. 1 paper in water-saturated *sec*-butanol and acetic acid−water (2:98), respectively, and the chromatograms were sprayed with bis-diazotized benzidine. Column chromatography (CC) was performed on Sephadex LH-20, and compounds were eluted at 0.5 mL/min, collecting fractions of 12 mL.

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All concentrations were done under reduced pressure at 40 °C in a rotary evaporator or by freeze-drying of aqueous solutions on a Virtis 12 SL freezemobile. Acetylations were performed with acetic anhydride/pyridine at 40 °C for 12 h.

Source of Plant Material. *C. intermedia* is one of the approximate 24 *Cyclopia* species endemic to the Cape fynbos region of South Africa. Samples of *C. intermedia* were collected in the Kouga Mountains of the Eastern Cape, and the dried, fermented shoots were supplied by Mr. Scheltema Nortje from the farm Nooitgedacht. A voucher specimen is being kept in the Chemistry Department of the University of the Free State.

Extraction and Fractionation. In order to remove chlorophyll, the pulverized shoots (6.0 kg) were extracted consecutively with chloroform (2 × 6.0 L, 24 h each) and acetone (2 × 5.0 L, 24 h each) at ~25 °C to yield dark green solids (155.5 and 86.8 g, respectively) on evaporation of solvents. Ensuing extractions with methanol (4 × 5.0 L, 24 h each, ~25 °C) and subsequently 70% acetone/water (5 × 5.0 L, 24 h each, ~25 °C) afforded brown solids following evaporation of the solvents. These were redissolved in water and freeze-dried to give 495.5 and 702.5 g of extract, respectively.

Metabolites from the Methanol Extract. The methanol extract (4 × 95.0 g) was subjected to Craig (20 tubes) countercurrent distribution with water/*n*-butanol/hexane (5:4:1) as the dual phase system (200 mL of organic mobile phase and 200 mL of aqueous stationary phase per tube). Following paper chromatographic analysis, four fractions, A (tubes 1−3, 103.5 g), B (tubes 4−8, 42.0 g), C (tubes 9−14, 27.9 g), and D (tubes 15−20, 53.0 g), were selected.

Fraction B (21.0 g) was further separated by Sephadex LH-20 column chromatography (5 cm × 160 cm, flow rate of 1 mL/min, 32.0 min fractions) with ethanol as the eluant. The collected fractions were combined into 10 main fractions following analysis by TLC in benzene−acetone−methanol (5:4:1): B_1 (tubes 0−57, 1.25 g), B_2 (tubes 58−93, 0.20 g), B_3 (tubes 94−135, 2.98 g), B_4 (tubes 136−162, 1.78 g), B_5 (tubes 163−190, 2.90 g), B_6 (tubes 191−201, 1.75 g), B_7 (tubes 202−269, 1.25 g), B_8 (tubes 270−313, 2.92 g), B_9 (tubes 314−330, 2.55 g), and B_{10} (tubes 331−418, 1.04 g). Fractions B_1−B_3 and B_{10} did not contain compounds of interest pertaining to this investigation and were not further investigated.

Fraction B_4 (100 mg) was acetylated and separated by PLC (benzene−acetone−methanol, 90:8:2) to give three bands at R_f 0.84 (3.1 mg), 0.79 (4.6 mg), and 0.74 (3.5 mg). Purification of the R_f 0.84 band by PLC in the same solvent gave 4,*β*-di-*O*-acetyl-3-methoxytyrosol (**4**; R_f 0.84, 2.8 mg). ^{1}H NMR ($CDCl_3$) (δ_H): 6.98 (1H, d, J = 8.5 Hz, H-5), 6.84 (1H, d, J = 2.5 Hz, H-2), 6.81 (1H, dd, J = 2.5, 8.5 Hz, H-6), 4.30 (2H, t, J = 7.0 Hz, *β*-CH_2), 3.84 (3H, s, O*Me*), 2.95 (2H, t, J = 7.0 Hz, α-CH_2), 2.33 (3H, s, O*Ac*), 2.07 (3H, s, O*Ac*). Purification of the R_f 0.79 band by PLC in the same solvent afforded 4,*β*-di-*O*-acetyltyrosol (**2**; R_f 0.79, 4.2 mg) consistent with literature data (*6*).

The R_f 0.74 band represented the peracetate derivative (**8**; 3.1 mg) of 4-[*O*-α-apiofuranosyl-(1″→2′)-*β*-D-glucopyranosyloxy]benzaldehyde (**7**). ^{1}H NMR ($CDCl_3$) (δ_H): 9.95 (1H, s, *CH*O), 7.88 (2H, d, J = 8.5 Hz, H-2, -6), 7.15 (2H, d, J = 8.5 Hz, H-3, -5); glucose: 5.32 (1H, t, J = 10.0 Hz, H-3′), 5.14 (1H, d, J = 7.5 Hz, H-1′), 5.08 (1H, t, J = 10.0 Hz, H-4′), 4.23 (1H, d, J = 12.0 Hz, H-6′), 4.16 (1H, dd, J = 2.5, 12.0 Hz, H-6′), 4.01 (1H, dd, J = 7.5, 10.0 Hz, H-2′), 3.91 (1H, m, H-5′); apiofuranose: 5.21 (1H, d, J = 1.0 Hz, H-1″), 5.18 (1H, d, J = 1.0 Hz, H-2″), 4.60 (1H, d, J = 12.0 Hz, H-4″), 4.50 (1H, d, J = 12.0 Hz, H-4″), 4.30 (1H, d, J = 10.0 Hz, 5″-CH_2), 4.15 (1H, d, J = 10.0 Hz, 5″-CH_2); aliphatic O*Ac*: 2.14 (3H, s), 2.11 (3H, s), 2.07 (6H, s), 2.04 (3H, s). MS *m*/*z* (%): 688 (M^+, 0), 597 (30), 259 (10), 154 (57), 137 (60), 123 (59), 109 (100).

Acetylation of fraction B_5 followed by PLC in benzene−acetone−methanol (90:8:2) afforded an impure compound (R_f 0.62, 6.7 mg). This was subjected to further PLC purification in benzene−acetone−methanol (90:8:2 × 2) to give the peracetate (**6**; R_f 0.62, 6.7 mg) of 2-{4-[*O*-α-apiofuranosyl-(1″→6′)-*β*-D-glucopyranosyloxy]phenyl}-ethanol (**5**). ^{1}H NMR ($CDCl_3$) (δ_H): 7.15 (2H, d, J = 8.5 Hz, H-2, -6), 6.93 (2H, d, J = 8.5 Hz, H-3, -5); glucose: 5.28 (1H, t, J = 10.0 Hz, H-3′), 5.23 (1H, dd, J = 8.0, 10.0 Hz, H-2′), 5.05 (1H, d, J = 8.0 Hz, H-1′), 5.03 (1H, t, J = 10.0 Hz, H-4′), 3.73−3.86 (2H, m, H-5′, -6′), 3.61 (1H, dd, J = 7.0, 12.0 Hz, H-6′); apiofuranose: 5.37 (1H, d, J = 1.0 Hz, H-2″), 5.00 (1H, d, J = 1.0 Hz, H-1″), 4.79 (1H, d, J = 12.0 Hz, H-4″), 4.52 (1H, d, J = 12.0 Hz, H-4″), 4.21 (1H, d, J = 10.0 Hz, 5″-CH_2), 4.13 (1H, d, J = 10.0 Hz, 5″-CH_2), 4.26 (2H, t, J = 7.0 Hz, *β*-CH_2), 2.89 (2H, t, J = 7.0 Hz, *β*-CH_2); aliphatic O*Ac*: 2.12 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.04 (6H, s), 2.03 (3H, s), 2.02 (3H, s). MS *m*/*z* (%): 726 (M^+, 0), 599 (22), 259 (95), 139 (100), 129 (22), 109 (15).

The acetylated fraction B_6 (100 mg) was resolved into two bands, R_f 0.70 (3.6 mg) and 0.66 (8.8 mg), by PLC in benzene−acetone−methanol (90:8:2). Purification in benzene−acetone−methanol of the former band yielded 3′,4′,7-tri-*O*-acetylflavone (*7*) while the second band gave 3′,5,7-tri-*O*-acetyl-4′-*O*-methylluteolin (diosmetin) (*8*).

Fraction B_7 (100 mg) was acetylated and separated by PLC in benzene−acetone−methanol (90:8:2 × 2) into three bands, R_f 0.67 (3.0 mg), R_f 0.64 (14.5 mg), and R_f 0.41 (6.8 mg). These were further purified by PLC in benzene−acetone−methanol (90:8:2). The R_f 0.67 band afforded the peracetate of prunin (**20**; R_f 0.67, 2.8 mg) (*9*), while the R_f 0.64 (14.1 mg) and 0.41 (5.9 mg) bands gave the peracetates (**22** and **24**) of 7-*O*-*β*-D-glucopyranosyleriodictyol (**21**) and 5-*O*-*β*-D-glucopyranosyleriodictyol (**23**) (*10*), respectively.

Acetylation of fraction B_8 (100 mg) followed by PLC separation in benzene−acetone−methanol (90:8:2) gave four bands at R_f 0.64 (3.4 mg), 0.42 (4.2 mg), 0.38 (6.3 mg), and 0.33 (5.9 mg), which were subsequently further purified by PLC in the same solvent.

The R_f 0.64, 0.42, and 0.38 bands yielded the per-*O*-acetyl derivatives of known flavonols **13** (3.0 mg), **28** (3.8 mg), and **12** (6.1 mg) of 5-*O*-α-D-glucopyranosylkaempferol (**14**) (*11*), wistin (**27**) (*12*), and 6-*C*-*β*-D-glucopyranosyl kaempferol (**11**) (*13*, *14*), respectively.

The R_f 0.33 band gave the per-*O*-acetyl derivative (**10**; 4.6 mg) of 8-*C*-*β*-D-glucopyranosylkaempferol (**9**). ^{1}H NMR ($CDCl_3$) (δ_H): 6.54 (1H, s, H-6), 7.63 (2H, br d, J = 8.5 Hz, H-2′, -6′), 7.18 (2H, d, J = 8.5 Hz, H-3′, -5′); glucose: 5.40 (1H, br t, J = 10.0 Hz, H-2″), 5.27 (2H, br t, J = 9.0 Hz, H-3″, H-4″), 4.86 (1H, d, J = 10.0 Hz, H-1″), 4.35 (1H, dd, J = 2.5, 12.0 Hz, H-6″), 4.21 (1H, d, J = 12.0 Hz, H-6″), 3.90 (1H, m, H-5″); aromatic O*Ac*: 2.36 (3H, s), 2.17 (3H, s), 2.07 (3H, s); aliphatic O*Ac*: 2.01 (3H, s), 1.81 (3H, s), 1.59 (6H, s), 1.51 (3H, s). MS *m*/*z* (%): 784 (M^+, 0), 717 (40), 154 (88), 137 (95), 123 (68), 109 (100).

Fraction B_9 (100 mg) was acetylated and separated by PLC in benzene−acetone−methanol (90:8:2 × 2) to yield four bands at R_f 0.38 (3.4 mg), 0.30 (5.7 mg), 0.26 (4.2 mg), and 0.16 (5.1 mg), which were purified, respectively, by PLC in the same solvent. The R_f 0.38 band comprised the peracetate (**16**; 3.2 mg) of 3-hydroxy-6-[*O*-α-apiofuranosyl-(1‴→6″)-*β*-D-glucopyranosyloxy]-3′,4′-methylenedioxyflavonol (**15**). ^{1}H NMR ($CDCl_3$) (δ_H): 7.66 (1H, d, J = 2.5 Hz, H-5), 7.22 (1H, dd, J = 2.5, 8.5 Hz, H-7), 7.05 (1H, d, J = 8.5 Hz, H-8), 7.38−7.44 (3H, m, H-2′, -5′, -6′); glucose: 5.27 (2H, t, J = 8.5 Hz, H-2″, -3″), 5.07 (1H, d, J = 8.5 Hz, H-1″), 5.06 (1H, t, J = 8.5 Hz, H-4″), 3.76 (1H, dd, J = 2.5, 12.0 Hz, H-6″), 3.59 (1H, dd, J = 4.0, 12.0 Hz, H-6″), 3.83 (1H, m, H-5″); apiofuranose: 5.37 (1H, d, J = 1.0 Hz, H-2‴), 4.98 (1H, d, J = 1.0 Hz, H-1‴), 4.77 (1H, d, J = 12.0 Hz, H-4‴), 4.58 (1H, d, J = 12.0 Hz, H-4‴), 4.23 (1H, d, J = 10.0 Hz, H-5‴), 4.14 (1H, d, J = 10.0 Hz, H-5‴), 5.31 (2H, s, −OCH_2O−); aliphatic O*Ac*: 2.11 (3H, s), 2.08 (3H, s), 2.05 (12H, s), 2.03 (3H, s). MS *m*/*z* (%): 886 (M^+, 0), 759 (32), 259 (100), 154 (58), 121 (20), 109 (36).

The R_f 0.30 band yielded the peracetate (**26**; 5.0 mg) of 5-*O*-α-D-rutinosylnaringenin (**25**). ^{1}H NMR ($CDCl_3$) (δ_H): 6.57 (1H, d, J = 2.5 Hz, H-8), 6.45 (1H, d, J = 2.5 Hz, H-6), 7.48 (2H, m, H-2′, -6′), 7.17 (2H, d, J = 8.5 Hz, H-3′, -5′), 5.45 (1H, dd, J = 3.0, 13.0 Hz, H-2), 3.01 (1H, dd, J = 13.0, 16.0 Hz, H-3α), 2.78 (1H, dd, J = 3.0, 16.0 Hz, H-3*β*); glucose: 5.31 (1H, t, J = 7.0 Hz, H-3″), 5.19 (1H, m, H-4″), 5.15 (1H, d, J = 2.0 Hz, H-1″), 4.27 (1H, dd, J = 2.0, 12.0 Hz, H-6″), 4.18 (1H, dd, J = 3.0, 12.5 Hz, H-6″), 4.12 (1H, dd, J = 2.0, 7.0 Hz, H-2″), 3.86 (1H, m, H-5″); rhamnose: 5.31 (1H, t, J = 10.0 Hz, H-3‴), 5.19 (1H, m, H-2‴), 5.13 (1H, d, J = 3.0 Hz, H-1‴), 4.99 (1H, t, J = 10.0 Hz, H-4‴), 3.92 (1H, m, H-5‴), 0.93 (3H, d, J = 6.0 Hz, −CH_3); aromatic *OAc*: 2.34 (3H, s), 2.31 (3H, s); aliphatic O*Ac*: 2.18 (3H, s), 2.14 (3H, s); 2.07 (6H, s), 2.01 (3H, s), 1.97 (3H, s). Circular dichroism (CD) $[\theta]_{342,0}$ 6.946 × 10^4 and $[\theta]_{306,0}$ −4.425 × 10^3.

The R_f 0.16 band gave the per-*O*-acetyl derivative (**30**; 4.2 mg) of 7-[*O*-α-apiofuranosyl-(1″→6″)-*β*-D-glucopyranosyloxy]-4-methoxyisoflavone) (**29**). ^{1}H NMR ($CDCl_3$) (δ_H): 8.25 (1H, d, J = 8.5 Hz, H-5), 8.06 (1H, s, H-2), 7.12 (1H, d, J = 2.5 Hz, H-8), 7.05 (1H, dd, J = 2.5, 8.5 Hz, H-6), 7.55 (2H, d, J = 8.5 Hz, H-2′, -6′), 6.99 (2H, d, J = 8.5 Hz, H-3′, -5′); glucose: 5.34 (2H, m, H-2″, -3″), 5.23 (1H, d, J = 8.0 Hz, H-1″), 5.06 (1H, t, J = 10.0 Hz, H-4″), 3.80 (1H, dd, J = 2.5, 11.0 Hz, H-6″), 3.63 (1H, dd, J = 7.5, 11.0 Hz, H-6″), 3.93 (1H, m, H-5″); apiofuranose: 5.45 (1H, d, J = 1.0 Hz, H-2″), 5.02 (1H, d, J = 1.0 Hz, H-1″), 4.84 (1H, d, J = 12.0 Hz, H-4‴), 4.67 (1H, d, J = 12.0 Hz, H-4‴), 4.27 (1H, d, J = 10.0 Hz, H-5‴), 4.16 (1H, d, J = 10.0 Hz, H-5‴); O*Me*: 3.86 (3H, s); aliphatic O*Ac*: 2.12 (3H, s), 2.10 (6H, s), 2.06 (3H, s), 2.05 (3H, s), 2.03 (3H, s). MS *m*/*z* (%): 814 (M^+, 0), 788 (34), 269 (68), 259 (64), 154 (48), 139 (100), 109 (30).

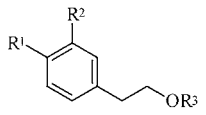
The R_f 0.16 band yielded the peracetate (**18**; 4.9 mg) of 3-*O*,6-*C*-di-*β*-D-glucopyranosylkaempferol (**17**). ^{1}H NMR ($CDCl_3$) (δ_H): 6.67 (1H, s, H-8), 8.08 (2H, d, J = 8.5 Hz, H-2′, -6′), 7.40 (2H, d, J = 8.5 Hz, H-3′, -5′); 6-*C*-glucose: 5.71 (1H, t, J = 10.0 Hz, H-2″), 5.32 (1H, t, J = 9.0 Hz, H-3″), 5.16 (1H, t, J = 9.0 Hz, H-4″), 4.82 (1H, d, J = 10.0 Hz, H-1″), 4.47 (1H, dd, J = 5.5, 12.5 Hz, H-6″), 4.30 (1H, dd, J = 4.0, 12.5 Hz, H-6″), 3.82 (1H, m, H-5″); 3-*O*-glucose: 5.95 (1H, dd, J = 2.0, 10.0 Hz, H-2‴), 5.50 (1H, t, J = 9.0 Hz, H-4‴), 5.43 (1H, t, J = 9.0 Hz, H-3‴), 4.59 (1H, d, J = 10.0 Hz, H-1‴), 4.50 (1H, dd, J = 2.5, 12.5 Hz, H-6‴), 4.20 (1H, dd, J = 4.0, 12.5 Hz, H-6‴), 3.77 (1H, m, H-5‴); aromatic O*Ac*: 2.56 (3H, s), 2.51 (3H, s), 2.37 (3H, s); aliphatic O*Ac*: 2.11 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.04 (3H, s), 2.00 (3H, s), 1.94 (3H, s), 1.89 (3H, s), 1.77 (3H, s). MS *m*/*z* (%): 1073 (M^+, 0), 599 (42), 137 (42), 123 (55), 109 (100).

RESULTS AND DISCUSSION

In addition to the initial findings of the occurrence of flavonoids (*1*, *2*), our ongoing investigation of the leaves and stems of *C. intermedia* further revealed the presence of phenols and their glycosides, glycosylated flavonols, flavanones, isoflavonoids, and flavones. The methanol extract of the fermented leaves and stems of *C. intermedia* afforded a complex mixture of phenolic compounds. This necessitated extensive enrichment and fractionation procedures using Craig countercurrent distribution, Sephadex LH-20 gel chromatography, and final purification by preparative TLC, following derivatization. Because of the number of steps required for purification, substantial losses were encountered, which precluded reliable quantification of the constituents. Several closely related glycosides were difficult to purify and tended to remain as mixtures, in varying proportions, often spread across a number of consecutive tubes of the Craig countercurrent extractor. The classes of phenolic compounds identified were as follows: $C_6{\cdot}C_1$ and $C_6{\cdot}C_2$ metabolites, both as the aglycones and their glycosides, and a variety of glycosylated $C_6{\cdot}C_3{\cdot}C_6$ metabolites including flavonols, flavanones, isoflavones, and flavone aglycones.

$C_6{\cdot}C_1$ and $C_6{\cdot}C_2$ Metabolites. Consecutive enrichment via Craig countercurrent distribution and column chromatography on Sephadex LH-20 in ethanol afforded a simple hydroxyphenylethanol, tyrosol (**1**) (*6*), and the 3-methoxy analogue (**3**) (**Figure 1**). These were accompanied in the same fraction by the new 4-*O*-glycosyl derivatives 2-{4-[*O*-α-apiofuranosyl-(1″→6′)-*β*-D-glucopyranosyloxy]phenyl}ethanol (**5**) and 4-[*O*-α-apiofuranosyl-(1″→2′)-*β*-D-glucopyranosyloxy]benzaldehyde (**7**). Acetylation of the four compounds yielded the peracetates **2**, **4**, **6**, and **8**, respectively (**Figures 2**, **3**, and **4**).

^{1}H NMR spectra of compounds **2**, **6**, and **8** all display a simple aromatic AA′BB′ spin system indicative of the 1,4-disubstituted phenyl ring. This is replaced for derivative **4** by an aromatic ABX system, in accordance with the presence of the methoxy substituent (δ 3.84) on the ring. For the peracetate derivatives **2**, **4**, and **6**, the protons of the substituted ethyl group resonate as two pairs of aliphatic methylene triplets (J = 7.0 Hz) at δ 2.95 and 4.30. The aldehyde **8** instead displays a characteristic formal singlet at δ 9.95. In addition, the ^{1}H NMR spectra of **6** and **8** show aliphatic protons reminiscent of similar disaccharide moieties with the two isolated methylene groups (4″- and 5″-CH_2), typical of the apiofuranosyl unit, resonating as two pairs of doublets (J = 12.0 and 10.0 Hz, respectively) for both compounds. A conspicuous shielding, however, of H-2′ (δ 4.01) in the glucopyranosyl unit of **8** as compared to that of compound **6** (δ 5.23) suggests that the apiofuranosyl moiety is most likely linked via C-2′ of the former as opposed to the more common linkage via C-6′ in the latter. Nuclear Overhauser effect (NOE) association of the anomeric proton of the apiofuranosyl unit with H-2′ of the glucopyranosyl unit in **8** and with 6′-CH_2 in **6** unambiguously confirms the respective C-1″−O−C-2′ and C-1″−O−C-6′ connectivity of the sugar units. The remaining glycosidic protons were verified by correlation spectroscopy (COSY) experiments and vicinal coupling constants, which also designated the α-configuration for the apiofuranosyl unit by the



1, R^1=R^2=R^3=H
2, R^1=R^3=Ac, R^2=H
3, R^1=R^3=H, R^2=OMe
4, R^1=R^3=Ac, R^2=OMe
5, R^2=R^3=H, R^1= apiofuranosyl-(1"→6')-β-*D*-glucopyranosyl
6. R^2=H, R^3=Ac, R^1=hexa-*O*-acetylapiofuranosyl-(1"→6')-β-*D*-glucopyranosyl,

Figure 1. Structures of tyrosol derivatives **1**–**6**.

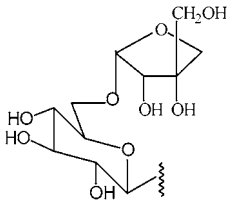
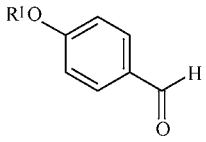


Figure 2. Structure of apiofuranosyl-(1″→6′)-*β*-D-glucopyranosyl sugar moiety.



7, R^1= apiofuranosyl-(1"→2')-β-*D*-glucopyranosyl
8, R^1= hexa-*O*-acetylapiofuranosyl-(1"→2')-β-*D*-glucopyranosyl

Figure 3. Structures of 4-[*O*-α-apiofuranosyl-(1″→2′)-*β*-D-glucopyranosyloxy]benzaldehyde **7** and its peracetate derivative **8**.

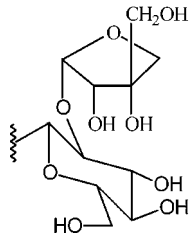
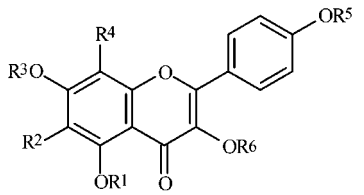


Figure 4. Structure of apiofuranosyl-(1″→2′)-*β*-D-glucopyranosyl.



9, $R^1=R^2=R^3=R^5=R^6=H$,
R^4=2-β-*D*-glucopyranosyl
10, $R^1=R^3=R^5=R^6$=Ac, $R^2=H$,
R^4=tetra-*O*-acetyl-2-β-*D*-glucopyranosyl
11, $R^1=R^3=R^4=R^5=R^6=H$,
R^2=2-β-*D*-glucopyranosyl
12, $R^1=R^3=R^5=R^6$=Ac, $R^4=H$,
R^2=tetra-*O*-acetyl-2-β-*D*-glucopyranosyl
13, $R^2=R^3=R^4=R^5=R^6=H$,
R^1=2-α-*D*-glucopyranosyl
14, $R^3=R^5=R^6$=Ac, $R^2=R^4=H$,
R^1=tetra-*O*-acetyl-2-α-*D*-glucopyranosyl
17, $R^1=R^3=R^4=R^5=H$,
$R^6=R^2$=2-β-*D*-glucopyranosyl
18, $R^1=R^3=R^5$=Ac, $R^4=H$,
$R^6=R^2$=tetra-*O*-acetyl-2-β-*D*-glucopyranosyl

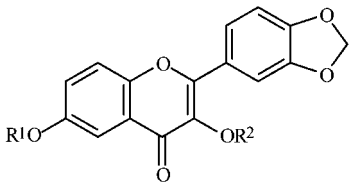
Figure 5. Structures of kaempferol derivatives **9**–**14**, **17**, and **18**.

extent of the coupling between H-1″ and H-2″ (J = 1.0 Hz for both compounds). Similarly, a β-glucopyranosyl unit was confirmed for **6** as well as **8** by $^3J_{HH}$ = 8.0 Hz between H-1′ and H-2′ and by $^3J_{HH}$ = 10.0 Hz between H-2′ and H-3′. The positions of the *O*-methyl (**4**) and *O*-glycosidic (**6** and **8**) linkages, relative to the aliphatic moieties on the ring, were derived from dipolar couplings (^{1}H NMR: two-dimensional nuclear Overhauser enhancement spectroscopy experiments) between appropriate protons of the substituents and the neighboring aromatic protons.

Flavonols. Five glycosylated flavonols all displaying the characteristic absence of heterocyclic protons of the flavonoid C ring were purified and identified as *O*-acetyl derivatives by ^{1}H NMR, COSY, and NOESY experiments. The known monoglucosylated kaempferols **11** (*13*) and **13** (*11*) were identified by comparison of the ^{1}H NMR data of their *O*-acetyl derivatives with literature data (**Figure 5**).

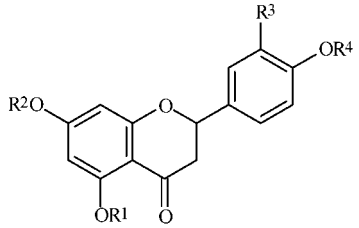
Identification of the new 8-C-β-D-linked glucopyranosyl flavonols **9** and **17** (**Figure 5**) was performed on their *O*-acetyl derivatives whose ^{1}H NMR spectra displayed two aromatic doublets of doublets integrating for two protons each consistent with a 4′-oxygenated B ring. A residual aromatic singlet displayed in the ^{1}H NMR spectra of **9** and **17** allocated to H-8 was based on the coupling via 6-position to the glucosyl unit and absence of H-6 (A ring) in their ^{1}H NMR spectra (*15*, *16*). Flavonol **17** has an additional C-3−O−C-1″ linked glucosyl moiety whose connectivity to the C ring was confirmed by the association between glucosyl protons H-2‴ and H-4‴ with H-2′,6′ of the B ring observed in the NOESY experiment.

A new flavonol glycoside (**15**) was purified and identified as its per-*O*-acetyl derivative, 3-acetyl-6-[*O*-apiofuranosyl-(1‴→6″)-β-D-glucopyranosyloxy]-3′,4′-methylenedioxyflavonol (**16**) (**Figure 6**). In the ^{1}H NMR spectrum of **16**, resonances between δ 3.5 and 5.5, reminiscent of the glucosyl moiety identical to that of compound **5**, and a two proton singlet at δ 6.00, characteristic of the protons of the methylenedioxy functionality, were displayed. NOE of the $-O-CH_2-O-$ to the 2′-H and 5′-H established the B ring protons. The position



15, R^1=apiofuranosyl-(1‴→6″)-β-*D*-glucopyranosyl, R^2=H
16, R^1=hexa-*O*-acetylapiofuranosyl-(1‴→6″)-β-*D*-glucopyranosyl, R^2=Ac

Figure 6. Structures of 3-hydroxy-6-[*O*-α-apiofuranosyl-(1‴→6″)-β-D-glucopyranosyloxy]-3′,4′-methylenedioxyflavonol **15** and its peracetate derivative **16**.



19, $R^1=R^4=H$, $R^3=H$,
R^2=2-β-*D*-glucopyranosyl
20, $R^1=R^4$=Ac, $R^3=H$,
R^2=tetra-*O*-acetyl-2-β-*D*-glucopyranosyl
21, $R^1=R^4=H$, R^3=OH,
R^2=2-β-*D*-glucopyranosyl
22, $R^1=R^3$=Ac, R^3=OAc,
R^2=tetra-*O*-acetyl-2-β-*D*-glucopyranosyl
23, $R^2=R^4=H$, R^3=OH,
R^1=2-β-*D*-glucopyranosyl
24, $R^2=R^3$=Ac, R^3=OAc,
R^1=tetra-*O*-acetyl-2-β-*D*-glucopyranosyl
25, $R^2=R^3=R^4=H$, R^1=rutinosyl
26, $R^2=R^4$=Ac, $R^3=H$,
R^1=hexa-*O*-acetylrutinosyl

Figure 7. Structures of derivatives with a flavanone aglycone **19**–**26**.

of the disaccharide moiety was confirmed by the appropriate NOESY and COSY experiments. Because of its peri-position to the carbonyl, a conspicuously deshielded meta doublet for H-5 (δ 7.67) suggests 6-oxygenation of the A ring. This was unambiguously confirmed by a NOESY association of the anomeric proton H-1″ of the glucosyl unit with the anticipated five and seven A ring protons. The C-6″−*O*−C-1‴ connectivity of the glucosyl and the apiofuranosyl moieties was confirmed as above by the association between the glucosyl H-6″ and the apiofuranosyl proton H-1‴ in the NOESY experiment.

Besides possessing physiological activities such as antioxidant (*17*), anticancer (*18*), antitumor, antiallergic, antiviral, antitoxic, and antihistamine activities (*19*), flavonols inhibit the production of lipid peroxidation and oxyradical production, which are implicated in pathological conditions such as aging, atherosclerosis, antiinflammatory, hepatotoxicity, and iron toxicity (*20*, *21*). Flavonols also have the ability to inhibit platelet aggregation, adhesion, and secretions (*19*).

Flavanones. Flavanones (**19**, **21**, **23**, and **25**) (**Figures 7** and **8**) characterized by the presence of the 3-CH_2 [two doublet of doublets (~δ 2.75−3.0)] and the 2-H [doublet of doublets (δ 5.5)] (*22*) resonances in their ^{1}H NMR spectra were purified and identified as their *O*-acetyl derivatives (**20**, **22**, **24**, and **26**).

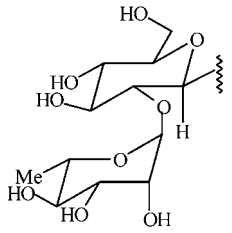


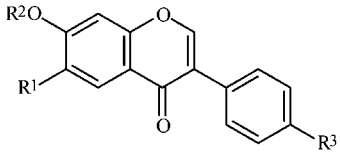
Figure 8. Structure of a rutinosyl sugar moiety.

In the ^{1}H NMR spectra, each of the four flavanones displays two meta doublets integrating for one proton each in the aromatic region, reminiscent of 5,7-dioxygenated A rings. The AA′BB′ systems exhibited by **19** and **25** indicate a para-substituted B ring, which was confirmed by association of 2-H(C) with 2′,6′-H(B) in the NOESY experiments. Similar association of 2′,6′-H(B) with 2-H(C) confirms an ABX system in flavanones **21** and **23**. The presence of the β-D-glucopyranosyl unit on ring A with a C-7−*O*−C-1″ glycosidic linkage in **19** and **21** was apparent from NOE association of both 5-H and 8-H with the anomeric 1″-H. A notable difference was prominent in the C-5−O−C-1″ glycosyl connectivity to the aglycone of flavanones **23** and **25**, where **23** possesses a β-configuration as opposed to **25** with an α-configuration. This was confirmed by a larger coupling constant of 1″-H (J = 7.5 Hz) and a prominent association of the anomeric hydrogen to those of the glucosyl moiety (3′-H and 5′-H) bearing a 1,3-diaxial relationship eminent in **23** and a smaller coupling constant (J = 3.0 Hz) of the anomeric proton in **25**. The C-2″−O−C-1‴ linkage of the glucosyl to the rhamnosyl unit in **25** was evident in the shielded 2″-H, which appeared at δ 4.12 in the ^{1}H NMR spectrum. Confirmation is via association of this resonance (δ 4.12, dd, J = 2.0, 7.0 Hz) to 1‴-H in the NOESY experiments.

The anticipated synchronous Cotton effects (negative for the $\pi\rightarrow\pi^*$ transition at ca. 300 nm and positive for the $n\rightarrow\pi^*$ transition at ca. 340 nm) that were compatible with flavanones possessing 2S absolute configuration (*23*) were evident in the CD of the derivatives of flavanones **19**, **21**, **23**, and **25**.

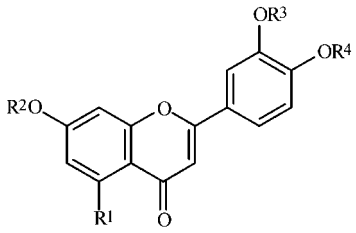
Many reports of physiological activity including antimicrobial, antiviral, and antiinflammatory (*19*), as well as the vitamin-like activity of citrin, a mixture of eriodictyol and hesperidin (*19, 24*), have been attributed to flavanones.

Isoflavones. A known isoflavone wistin (**27**) and a new diglycosylated isoflavone (**29**) were isolated, and their structures were confirmed as *O*-acetyl derivatives **28** and **30**, respectively (**Figures 2** and **9**). The isoflavone character was immediately apparent from the typical one proton singlet at δ 7.95−8.07 characteristic of the vinylic H-2 resonance of isoflavones (*25*). The presence of protons in the δ 3.5−5.5 region in their ^{1}H NMR spectra in conjunction with the acetoxy groups indicated an apiofuranosylglucosyl sugar moiety. Two doublets of doublets integrating for two protons in their ^{1}H NMR spectra were indicative of a para-substituted B ring. NOE association of the 4′-methoxy protons with 3′,5′-H and of 2-H with 2′,6′-H confirmed the substitution. The ABX pattern attributed to the 7-*O* substitution on ring A in isoflavone **30** was unequivocally confirmed by an NOE association of the 1″-H with 6-H and 8-H. Similar association of the anomeric 1″-H and 8-H and of 6-OMe with the anisotropically deshielded 5-H in isoflavone **28** unequivocally confirmed the substitution on the A ring. The C-1‴−O−C-6″ connectivity of the apiofuranosyl moiety to the glucosyl unit was unambiguously confirmed by association of 1‴-H with the C-6″ methylene protons of the glucosyl moiety.



27, R^1=R^3=OMe, R^2=2-β-*D*-glucopyranosyl
28, R^1=R^3=OMe,
R^2=tetra-*O*-acetyl-2-β-*D*-glucopyranosyl
29, R^1=H, R^3=OMe,
R^2= apiofuranosyl-(1‴→6″)-
β-*D*- glucopyranosyl
30, R^1=H, R^3=OMe,
R^2= hexa-*O*-acetylapiofuranosyl-
(1‴→6″)-β-*D*- glucopyranosyl

Figure 9. Structures of derivatives with an isoflavone aglycone **27**–**30**.



31, R^1=R^2=R^3=R^4=H
32, R^1=H, R^2=R^3=R^4=Ac
33, R^1=OH, R^2=R^3=H, R^4=Me
34, R^1=OAc, R^2=R^3=Ac, R^4=Me

Figure 10. Structures of derivatives with a flavone aglycone **31**–**34**.

In addition to their characteristic feature of acting as phytoalexins in the defense against fungal infection in plants (*26*), isoflavones have physiological effects in humans that include anticancer (*18*), estrogenic (*27*), and antimicrobial activities (*28*).

Flavones. Isolated as their *O*-acetyl derivatives **32** and **34**, flavone **31** was accompanied by the new analogue **33** (**Figure 10**). The characteristic sharp singlet of the vinylic 3-H near δ 6.7 ppm in the ^{1}H NMR spectra (*29*) indicated the flavone character. Two sets of ABX systems in the ^{1}H NMR spectrum of **31** attributable to the 7-acetoxy and 3′,4′-diacetoxy A and B ring, respectively, were distinguished by association between 3-H and 2′-H and 6′-H in the NOESY experiments. Two aromatic meta-coupled doublets in isoflavone **33** are reminiscent of 6-H and 8-H. The 4′-*O*-methyl substituent on the B ring was simply confirmed by NOE association with 5′-H in the COSY experiment. Association of 3-H with 2′-H and 6′-H further confirmed the ABX pattern on ring B of isoflavone **31**. The antioxidant, diuretic (*19*), and antispasmodic (*30*) properties of flavones are well-established.

Our continued investigations of a series of enriched fractions of the methanol extract from the fermented leaves and shoots of *C. intermedia* have thus revealed a considerable number and variety of additional flavonoids as compared to those previously reported (*2*). Honeybush tea that is brewed from *C. intermedia*, therefore, contains a significant number and amount of polyphenols, which are associated with diverse physiological properties. The latter would probably also account partially for its traditional application as a medicinal concoction, but it would presently probably find greater acclaim for health-promoting properties such as its antioxidant activity and low caffeine content.

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