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4-*O*-FERULOYLQUINIC ACID FROM GREEN COFFEE BEANS

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Key Word Index—*Coffea canephora*; Rubiaceae; robusta coffee bean; 4-*O*-feruloylquinic acid; chlorogenic acid.

Abstract—An additional quinyl ester of hydroxycinnamic acid was isolated from unroasted robusta coffee beans (*Coffea canephorra* var. *robusta*). The isolated compound was identified as 4-*O*-feruloylquinic acid using ^{1}H NMR and mass spectroscopy.

INTRODUCTION

During the course of an investigation of the chlorogenic acids from coffee beans we have clearly separated them into at least 11 components by HPLC [1]. Eight of them have been identified by mass and ^{1}H NMR spectroscopy [1, 2]. We have now isolated an unidentified compound in a pure state and identified it as 4-*O*-feruloylquinic acid by mass and ^{1}H NMR spectroscopy. The old and not the IUPAC nomenclature is used in this report [3].

RESULTS AND DISCUSSION

Crude green coffee bean extract was applied to a Fine Sil C_{18} semi-preparative column with 10 mM H_3PO_4 and methanol as eluents. By employing a combination of isocratic and linear gradient elution 11 peaks were obtained. Eight of them were identified as chlorogenic acid derivatives from ^{1}H NMR and mass spectra [1, 2]. They include 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, 3-*O*-feruloylquinic acid, 4,5-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, 3,4-*O*-dicaffeoylquinic acid and 3-*O*-feruloyl-4-*O*-caffeoylquinic acid, corresponding to peaks 1–3, 5–8 and 11, respectively. Peak 4 (20.6 min) was eluted after 3-*O*-caffeoylquinic acid (19.0 min) but before 3-*O*-feruloylquinic acid (22.0 min) from the ODS column.

The isolated peak 4 compound was examined by analytical HPLC and was homogeneous. The UV spectrum of this compound was almost identical to that of chlorogenic acid and showed λ_{max} at 325 nm. After rechromatography with water–methanol (1:1) as eluent, the eluant was lyophilized. The white amorphous powder obtained was used for measurements of ^{1}H NMR and mass spectra.

The ^{1}H NMR spectrum of the peak 4 compound was the sum of the spectra of ferulic acid and quinic acid, respectively. The peak position and intensities clearly show that the compound consists of quinic acid (Q) and ferulic acid (F) moieties in a molar ratio of 1:1, C_5-H(Q):C_2-H(F) = 1.0:1.1. The protons in positions C-3, C-4 and C-5 were assigned after decoupling experiments [2]. Since the C-4 proton of the peak 4 compound showed a paramagnetic chemical shift of 1.43 ppm from the corresponding position in free quinic acid the hydroxyl group at this position must be acylated [4]. Coupling constants (15.9 Hz) of the C_α proton of the feruloyl moiety showed the existence of *trans* vinyl groups.

In the EI mass spectrum of the TMSi derivative of the peak 4 compound, the $[M]^+$ was at *m/z* 727. The base peak at *m/z* 249, $[^{MeO}_{TMSO}>C_6H_3\text{–}CH{=}CH\text{–}COO]^+$ suggested a monoferuloylquinic acid structure.

From the spectral data described above, the structure for the peak 4 compound was concluded to be 4-*O*-feruloylquinic acid. Trugo *et al.* separated three feruloylquinic acid isomers in instant coffee as did Stegen *et al.* from green coffee beans using HPLC [5, 6]. In both reports the 4-isomer was tentatively assigned by comparing the retention times with that of the synthetic compound. It was reported that the 3-isomer was converted to the 4- and 5-isomers by alkaline treatment [7]. In order to prevent quinyl esters from interconversion, our extraction and isolation procedure has been carried out under neutral or acidic conditions at room temperature.

EXPERIMENTAL

Plant material. Robusta (*Coffea canephora* var. *robusta*) coffee beans from Java (fair-average quality) harvested in 1983 were obtained commercially. The green bean sample was ground in a rotating knife grinder and the ground material passed through a 500 μm sieve to remove coarse fragments.

Extraction. Finely ground unroasted coffee bean was extracted × 4 with 200 ml of 70% *iso*-PrOH for 30 min at room temp. The combined extracts were concd to *ca* 100 ml under red. pres. The resulting aq. soln was centrifuged for 30 min at 8000 *g* and the supernatant filtered through a Millipore filter (0.45 μm). The filtrate was frozen and stored at −20° in the dark until ready for use.

HPLC. An instrument fitted with a gradient programmer, column oven compartment and a variable wavelength UV detector was employed. The detector was set at 325 nm for prep. separation and was equipped with a 1 mm path length 8 μl cell. Sample was injected by a sample loop valve fitted with a 4 ml loop. The column was Fine Sil C_{18} (250 × 7.2 mm i.d.). The

elution solvents were 10 mM H_3PO_4 and MeOH. A combination of isocratic and linear gradient elution was used: 0–30 min, 5–50% MeOH in 10 mM H_3PO_4 (linear gradient); 30–50 min, 50% MeOH (isocratic); 50–55 min, 50–80% MeOH (linear gradient); 55–70 min, 80% MeOH (isocratic). For analytical separation, the UV detector was fitted with a 10 mm path length, 8 μl analytical cell and a variable loop injector was equipped with a 100 μl loop. A prepacked analytical column (250 × 4.6 mm i.d.) of Fine Sil C_{18-5} (5 μm) was employed with gradient elution with 10 mM H_3PO_4 and MeOH. The elution profile was: 0–15 min, 5–50% MeOH, 16–30 min, 50–70% MeOH. The column temp. was kept at 40°.

^{1}H *NMR*. The 360 MHz spectrum was recorded in DMSO with TMS as int. standard. Measurement was made at 25°. A 45° pulse width at a 1 sec pulse interval was used. Decoupling was performed using a homogated decoupling unit.

MS. Purified quinyl ester (*ca* 100 μg) was reacted with 100 μl of *N,O-bis*(trimethylsilyl)trifluoroacetamide(BSTFA) at 125° for 10 min. The derivatized sample was used for analysis. EIMS was obtained on 1 μg of the sample using a direct inlet probe. The probe temp. was varied from ambient to 200° and the spectrum was obtained at 116°. Ion source temp. and ion energy were 250° and 70 eV, respectively.

4-O-*Feruloylquinic acid*. White amorphous powder. UV $\lambda_{max}^{H_2O}$nm: 235, 245, 295 sh, 325. ^{1}H NMR: δ9.59 (1H, *s*, OH-4 ferulic acid), 7.57 (1H, *d*, J = 15.9 Hz, H_β ferulic acid), 7.32 (1*H*, *s*, H-2 ferulic acid), 7.11 (1H, *m*, H-5 ferulic acid), 6.80 (1H, *m*, H-6 ferulic acid), 6.50 (1H, *d*, J = 15.9 Hz, H_α ferulic acid), 4.67 (1H, *m*, H-4 quinic acid), 4.10 (1H, *s*, H-5 quinic acid), 4.01 (1H, *s*, H-3 quinic acid), 3.82 (3H, *s*, OMe ferulic acid). MS *m/z*: 727, penta-TMSi, $[M]^+$, 521 $[M-COOTMS]^+$, 431 $[M-OTMS-COOTMSi]^+$, 249 $[^{MeO}_{TMSO}>C_6H_3-CH=CH-COO]^+$.

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