1164, 1130, 1113; UV λ_{max}^{MeOH} nm: (log ε), 281.5 (4.19), 237 (4.58); MS *m/z*: 374 $[M]^+$, 356, 327, 222, 205, 204, 196, 193, 192, 191, 178, 175, 166, 163, 161, 153, 151.03651 ($C_8H_7O_3$, 100%), 137, 131, 124, 115, 109, 103, 93, 81, 77, 70, 65, 55.

4-*Acetoxy*-2,6-*di*(4'-*acetoxy*-3'-*methoxy*)*phenyl*-3,7-*dioxabicyclo*(3.3.0)*octane* (**2**). A mixture of **1** (15 mg), Ac_2O (0.5 ml) and pyridine (1.5 ml) was left overnight at room temp. Usual work-up yielded a viscous mass (12 mg); MS *m/z*: 500 $[M]^+$, 458, 416, 398, 369, 356, 328, 205, 191, 175, 163, 151, 137, 131, 97.

4-*Hydroxy*-2,6-*di*-(3',4'-*dimethoxy*)*phenyl*-3,7-*dioxabicyclo*-(3.3.0) *octane* (**3**). Compound **1** (10 mg) in dry MeOH (1 ml) was methylated with CH_2N_2 in ether to yield **3** (8 mg), mp 146–147° (hexane–CH_2Cl_2); MS *m/z*: 402 $[M]^+$, 384, 355, 341, 325, 281, 264, 236, 219, 210, 205, 192, 189, 177, 167, 165, 151, 139, 131, 119, 77, 69.

4-*Methoxy*-2,6-*di*-(3',4'-*dimethoxy*)*phenyl*-3,7-*dioxabicyclo*-(3.3.0)*octane* (**4**). A soln of **1** (10 mg) and MeI (0.2 ml) in dry acetone, 10 ml) was refluxed in presence of K_2CO_3 for 4 hr. The reaction mixture was filtered, the solvent removed and the residue purified by prep TLC to yield **4** (2 mg), mp 123.5° (hexane–CH_2Cl_2); MS *m/z*: 416 $[M]^+$, 383, 354, 218, 206, 191, 176, 166, 164, 151, 84, 71, 69, 57, 55.

2,6-*Di*-(4'-*hydroxy*-3'-*methoxy*)*phenyl*-3,7-*dioxabicyclo*(3.3.0) *octane* (**5**). A soln of **1** (10 mg) and diborane–DMSO complex in THF (1 ml) was heated at 50° for 4 hr. Excess of the reagent was decomposed by adding a satd aq. NH_4Cl soln to the reaction mixture. The THF was removed *in vacuo* and the resulting residue was extracted with $CHCl_3$ (3 × 20 ml). The organic layer was washed with H_2O, dried (Na_2SO_4) and the solvent removed to give a viscous mass which was purified by prep TLC to afford **5** (2.5 mg) identical in all respects with (+)-*pinoresinol*.

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3-*O*-FERULOYL-4-*O*-CAFFEOYLQUINIC ACID FROM COFFEE BEANS

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Key Word Index—*Coffea canephora*; Rubiaceae; robusta coffee bean; feruloyl-caffeoyl quinic acid; chlorogenic acids.

Abstract—A new phenolic ester was isolated from unroasted robusta coffee beans (*Coffea canephora*) by HPLC. The isolated compound was identified as an ester of caffeic acid and ferulic acid with quinic acid (3-*O*-feruloyl-4-*O*-caffeoylquinic acid) using ^1H NMR and mass spectroscopy.

INTRODUCTION

Quinic acid esters of hydroxycinnamic acid are of interest in plant physiology and food chemistry because of their ubiquitous occurrence in plants. Considerable amounts of them are contained in unroasted coffee beans [1–4]. The most common compounds are esters of caffeic acid, that is, chlorogenic acid and its derivatives [5]. They are often found together with ferulic acid esters [6].

Because of the structural similarity of chlorogenic acid derivatives, a highly effective separation method is required to resolve all compounds [7]. We have now developed a HPLC method which allows a clear separation of the possible positional isomers [8]. Seven phenolic compounds in unroasted coffee beans have been isolated by this method and the structure of the compounds have been confirmed by mass and ^1H NMR spectroscopy. In the course of separation of the phenolic compounds using reversed-phase HPLC we noticed an additional peak and isolated the compound. The isolated compound was identified as 3-*O*-feruloyl-4-*O*-caffeoylquinic acid by using mass and ^1H NMR spectroscopy and forms the subject of the present report.

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

Table 1. ^{1}H NMR chemical shifts of the peak 11 compound, quinic acid, caffeic acid and ferulic acid

Compound	Chemical shift (ppm)									
	C-4′–OH	C-3′–OH	C–H	C-2′–H	C-5′–H C-6′–H	C–H	C-3–H	C-4–H	C-5–H	OMe
Peak 11 compound	9.53	9.21	7.51 (*d*) 7.47 (*d*)	7.27 (*s*) 7.02 (*s*)	7.07 6.98 6.76 6.74	6.38 (*d*) 6.23 (*d*)	5.44 (*s*)	4.92 (*s*)	4.17 (*s*)	3.79 (*s*)
Quinic acid							3.75 (*s*)	3.24 (*s*)	3.88 (*s*)	
Caffeic acid	9.52 (*s*)	9.12 (*s*)	7.41 (*d*)	7.02 (*s*)	6.76 (*d*) 6.91 (*d*)	6.17 (*d*)				
Ferulic acid	9.54 (*s*)		7.49 (*d*)	7.28 (*s*)	6 78 (*d*) 7.08 (*d*)	6.36 (*d*)				3.81 (*s*)

isocratic and linear gradient elution 11 peaks were obtained. Seven of them were confirmed as chlorogenic acid derivatives already reported by ^{1}H NMR and mass spectra [8]. 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 and 3,4-*O*-dicaffeoylquinic acid, corresponding to peaks 1–3 and 5–8, respectively.

Isolated peak 11 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 11 compound is shown in Table 1. The peak positions and intensities clearly show that the compound consists of quinic acid (Q), caffeic acid (C) and ferulic acid (F) moieties in a molar ratio of 1:1:1. C_α–H(C):C_α–H(F):C_β–H(C + F) = 0.9:0.7:1.7. The coupling constants C_α–H(F):15.9, C_α–H(C):15.9, C_β–H(F or C):15.5 or 15.1 of C_α-protons and C_β-protons of caffeoyl and feruloyl moieties show the existence of *trans*-vinyl groups. The FD-mass spectrum of the peak 11 compound also indicates that this compound is feruloylcaffeoylquinic acid. The mass spectrum has an ion at m/z 531 $[M+H]^+$ and some additional peaks at m/z 535 $[M-H_2O+Na]^+$ and 553 $[M+Na]^+$.

The C-3, C-4 and C-5 protons of the quinic acid moiety were assigned on the basis of angular dependence of the spin–spin coupling constant [9]. The $J_{3,4}$ (8.4 Hz), spin–spin coupling constant between the C-3 proton (axial) and C-4 proton (axial) is larger than that of $J_{4,5}$ (1.7 Hz) (axial-equatorial). The chemical shifts of the C-3, C-4 and C-5 protons resemble those of 3,4-dicaffeoylquinic acid [8]. The C-3 and C-4 proton shift downfield by 1.56 and 1.67 ppm relative to those of free quinic acid. On the basis of the paramagnetic chemical shifts with acylation [6], it is possible to say that the ester bonds in the quinic acid moiety exist at C-3 and C-4–OH. However, we cannot determine which OH group of the C-3 and C-4 forms an ester bond with ferulic acid. To determine the position of the ester bond the chemical shifts of the C_α-protons of some chlorogenic acids were compared (Table 2). Except for 3,5-*O*-dicaffeoylquinic acid, the C_α-protons of the caffeoyl moieties substituted at C-3, C-4 and C-5–OH of quinic acid have individually intrinsic paramagnetic chemical shifts relative to that of free caffeic acid. The C_α-proton signals of the caffeoyl moieties esterified at C-3, C-4 and C-5–OH of quinic acid appear in the range 6.15–6.16 ppm, 6.23–6.27 ppm and 6.19–6.20 ppm, respectively. That is the case for the feruloyl moiety of 3-feruloylquinic acid (Table 2).

The C_α-proton peak of 3,5-*O*-dicaffeoylquinic acid shifts downfield to a great extent compared to those of other 5-substituted ones. Conformational change of the molecule and/or the influence of trace amount of counter ion may explain the discrepancy. The C_α-proton peaks of the caffeoyl residue substituted at C-3–OH of quinic acid show almost the same frequency as those of free caffeic acid. A similar result was observed for ferulic acid (6.36 ppm) and 3-*O*-feruloylquinic acid (6.38 ppm), although these peaks shift downfield as whole. In spite of the exception observed for 3,5-*O*-dicaffeoylquinic acid, the constant paramagnetic chemical shifts of the C_α-protons of the chlorogenic acids allows the determination of the position of ester bonds in the peak 11 compound. The existence of the C_α-proton peaks resonating at 6.38 and 6.23 ppm shows that the peak 11 compound is 3-*O*-feruloyl-4-*O*-caffeoylquinic acid. Similar results were observed for the peak 9 compound, 3-*O*-caffeoyl-4-*O*-feruloylquinic acid (Table 2).

The method described here to determine the position of the ester bond of caffeoylferuloylquinic acid is consistent for chlorogenic acid derivatives so far isolated, 3-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid and 3-*O*-feruloylquinic acid. To confirm the validity of the method other quinyl esters of cinnamic acid derivatives will be isolated and ^{1}H NMR spectra of the compound will be measured. Work along this line is in progress.

EXPERIMENTAL

Extraction. Finely ground unroasted coffee bean was extracted × 4 with 200 ml of 70% 2-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 was filtered through a Millipore filter (pore size 0.45 μm). The filtrate was frozen and stored at −20° in the dark until ready for use.

A HPLC instrument with a gradient programmer, column oven compartment and a variable wavelength UV detector was

Table 2. ^{1}H NMR chemical shifts of C protons of some chlorogenic acid derivatives (ppm)*

Compound	Substituted position			
	3	4	5	Free
CA				6.17
5-*O*-CQA			6.20 (0.03)	
4-*O*-CQA		6.27 (0.10)		
3-*O*-CQA	6.15 (−0.02)			
4,5-*O*-DCQA		6.24 (0.07)	6.19 (0.02)	
3,5-*O*-DCQA	6.16 (−0.01)		6.25 (0.08)	
3,4-*O*-DCQA	6.15 (−0.02)	6.24 (0.07)		
FA				6.36
3-*O*-FQA	6.38 (0.02)			
3-*O*-F-4-*O*-CQA	6.38 (0.02)	6.23 (0.06)		
3-*O*-C-4-*O*-FQA	6.16 (−0.01)	6.47 (0.11)		

*Chemical shifts from free caffeic and ferulic acid are presented in parentheses.

Abbreviations: CA, caffeic acid; CQA, caffeoylquinic acid; DCQA, dicaffeoylquinic acid; FQA, feruloylquinic acid.

employed. The UV detector was set at 325 nm, for prep. separation and was equipped with a 1 mm path length 8 μl prep. cell. Sample was injected by a sample loop valve fitted with a 4 ml loop. The column used was a Fine Sil C_{18} semi-prep. column (250 × 7.2 mm id). 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 sepn, 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 Finepak C_{18} prepacked analytical column (250 × 4.6 mm id) of Fine Sil C_{18}-5 (5 μm) was employed. Gradient elution with 10 mM H_3PO_4 and MeOH was used. 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° and 80°. A 45° pulse width at 1.0 sec pulse interval was employed. Decoupling was performed using a homo-gated decoupling unit.

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