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# 3-*O*-CAFFEOYL-4-*O*-FERULOYLQUINIC ACID FROM GREEN ROBUSTA COFFEE BEANS

HIDEKO MORISHITA, HIDEO IWAHASHI* and RYO KIDO*

Faculty of Education, Wakayama University, Wakayama 640, Japan; *Department of Biochemistry, Wakayama Medical College, Wakayama 640, Japan

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**Key Word Index**—*Coffea canephora*; Rubiaceae; robusta coffee bean; 3-*O*-caffeoyl-4-*O*-feruloylquinic acid; chlorogenic acid.

**Abstract**—An additional compound, 3-*O*-caffeoyl-4-*O*-feruloylquinic acid, has been isolated from unroasted coffee beans (*Coffea canephora* var. *robusta*) and its structure determined by FD mass and $^1$H NMR spectroscopy.

## INTRODUCTION

The chlorogenic acids isolated from or detected in coffee beans to date include 3-*O*-caffeoylquinic acid [1], 4-*O*-caffeoylquinic acid [2], 5-*O*-caffeoylquinic acid [3], 3-*O*-feruloylquinic acid [4], 4-*O*-feruloylquinic acid [5, 6], 5-*O*-feruloylquinic acid [5], 3,4-*O*-dicaffeoylquinic acid [7], 3,5-*O*-dicaffeoylquinic acid [7], 4,5-*O*-dicaffeoylquinic acid [7] and 3-*O*-feruloyl-4-*O*-caffeoylquinic acid [8]. During a study of the chlorogenic acids in unroasted *Coffea canephora* var. *robusta* (robusta coffee) beans, we separated 11 components by HPLC [9]. The structures of nine of them have been elucidated [6, 8, 9]. In this paper we describe the isolation and identification of a new compound, 3-*O*-caffeoyl-4-*O*-feruloylquinic acid (compound **10**). IUPAC nomenclature is not used in this report.

## RESULTS AND DISCUSSION

Compound **10** was isolated from a 70% 2-propanol extract of unroasted *C. canephora* beans by HPLC, as described in our previous paper [8, 9]. The compound was eluted after 3,4-*O*-dicaffeoylquinic acid. The $^1$H NMR spectrum showed that **10** consisted of quinic acid (Q), caffeic acid (C) and ferulic acid (F) moieties in a molar ratio of 1:1:1, C3-H(Q):Cα-H(C):Cα-H(F) = 1.0:0.8:1.0. The protons in the C-3, C-4 and C-5 positions were assigned after decoupling experiments. Since the C-3 and C-4 protons of **10** showed paramagnetic chemical shifts of 1.66 and 1.69 ppm from the corresponding positions in free quinic acid, respectively, it was therefore a 3,4-disubstitution of quinic acid by cinnamic acids.

However, the position of the feruloyl group could not be distinguished from that of the caffeoyl group. In our previous paper we described a method to determine the position of ester bonds in caffeoylferuloylquinic acid isomers [8]. The Cα-proton of the caffeoyl moieties substituted at C-3, C-4 and C-5 OH of quinic acid had individually intrinsic chemical shifts relative to that of free caffeic acid. This was also the case for the feruloyl moiety. Accordingly, the constant chemical shifts of the Cα protons of caffeoylferuloylquinic acid permitted the determination of the position of the ester bonds. The chemical shifts of the Cα proton of **10** were compared with some chlorogenic acids. The Cα-proton peaks of these caffeoyl residues substituted at the C-3 OH of quinic acid showed small constant diamagnetic chemical shifts (0.01–0.02 ppm) compared with that of free caffeic acid. One of the Cα protons of **10** which resonated at 6.16 ppm was attributed to the caffeoyl group substituted at the C-3 OH of quinic acid. Since the Cα proton peak of 4-*O*-feruloylquinic acid shifted downfield by 0.14 ppm relative to that of free ferulic acid, another Cα proton which resonated at 6.47 ppm of **10** was attributed to the feruloyl group substituted at the C-4 OH of quinic acid. Thus the structure of **10** was shown conclusively to be 3-*O*-caffeoyl-4-*O*-feruloylquinic acid. On the other hand, the coupling constants of the Cα and Cβ protons (15.8 Hz, 16.0 Hz) showed the existence of two pairs of *trans*-vinyl protons in the caffeic acid and ferulic acid moieties of **10**. The FD mass spectrum of **10** showed peaks at 531 $[M + H]^+$, 535 $[M - H_2O + Na]^+$, 553 $[M + Na]^+$ and 569 $[M + K]^+$.

The existence of caffeoylferuloylquinic acid in coffee beans was predicted by Corse *et al.* [7]. In 1980, Van der Stegen and Van Duijn reported the isolation of a derivative of chlorogenic acid, which yielded a small amount of a mixture of caffeic acid, ferulic acid and monocaffeoylmonoferuloylquinic acid on hydrolysis [10]. However, they could not identify their compound because of the small amount of compound obtained. Previously we described the identification of 3-*O*-feruloyl-4-*O*-caffeoyl quinic acid from robusta coffee beans [8]. Compound **10** is the reversed positional isomer, i.e. 3-*O*-caffeoyl-4-*O*-feruloylquinic acid.

## EXPERIMENTAL

*Plant material. C. canephora* var. *robusta* (robusta coffee) beans from Java (fair average quality), harvested in 1983, were obtained commercially.

*Extraction and isolation.* The green bean sample was ground in a rotating knife grinder and the ground material passed through a 32 mesh (500 μm) sieve to remove coarse fragments. Finely ground unroasted coffee beans (10 g) were extracted × 4 with 70%

2-propanol at room temp. with continuous stirring. The pooled extracts were concentrated under red. press. The resulting aq. soln was filtered through a Millipore filter (pore size 0.45 μm). The filtrate was frozen and stored at −20° in the dark. The extract was applied to a Finepak SIL $C_{18}$ semi-preparative column (250 × 7.2 mm i.d.). Eleven peaks were obtained by employing a combination of isocratic and linear gradient elution: 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). Nine of the peaks were confirmed as chlorogenic acid derivatives already identified by MS and $^1$H NMR. They included 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, 4-*O*-feruloylquinic 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 1–8 and 11 [8, 9] (Table 1). In this investigation, we isolated an unidentified compound corresponding to peak 10. The peak compound was collected and examined for homogeneity by analytical HPLC. After re-chromatography with $H_2O$–MeOH (1:1), the eluant was lyophilized. The white amorphous powder obtained was used for measurements of $^1$H NMR and FDMS.

*$^1$H NMR.* The 360 MHz spectrum was recorded in DMSO with TMS as internal standard. Measurements were made at 25 and 80°. A 45° pulse width at 1 sec pulse intervals was employed. Decoupling was performed using a homogated decoupling unit.

*3-O-Caffeoyl-4-O-feruloylquinic acid.* White amorphous powder; visible $\lambda_{max}$ ($H_2O$) 325 nm; MS *m/z* 531 $[M+H]^+$, 535 $[M-H_2O+Na]^+$, 553 $[M+Na]^+$ and 569 $[M+K]^+$; $^1$H NMR: δ7.53 (1H, *d*, *J* = 16 Hz, H-β ferulic acid), 7.41 (1H, *d*, *J* = 16 Hz, H-β caffeic acid), 7.29 (1H, *s*, H-2′ ferulic acid), 7.06 (1H, *m*, H-5′ ferulic acid), 7.03 (1H, *s*, H-2′ caffeic acid), 6.97 (1H, *m*, H-5′ caffeic acid), 6.75 (1H, *m*, H-6′ ferulic acid), 6.71 (1H, *m*, H-6′ caffeic acid), 6.47 (1H, *d*, *J* = 15.8 Hz, H-α ferulic acid), 6.16 (1H, *d*, *J* = 15.8 Hz, H-α caffeic acid), 5.41 (1H, *m*, H-3 quinic acid), 4.93 (1H, *m*, H-4 quinic acid), 4.17 (1H, *s*, H-5 quinic acid), 3.79 (3H, *s*, OMe ferulic acid).

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Table 1. Retention times of chlorogenic acids in green robusta coffee beans on Fine SIL/$C_{18-5}$ using a gradient elution

| Peak No. | Compound | $R_t$ (min) | Ref. |
|---|---|---|---|
| 1 | 5-*O*-Caffeoylquinic acid | 16.1 | [10] |
| 2 | 4-*O*-Caffeoylquinic acid | 18.1 | [10] |
| 3 | 3-*O*-Caffeoylquinic acid | 19.0 | [10] |
| 4 | 4-*O*-Feruloylquinic acid | 20.6 | [6] |
| 5 | 3-*O*-Feruloylquinic acid | 22.0 | [10] |
| 6 | 4,5-*O*-Dicaffeoylquinic acid | 22.5 | [10] |
| 7 | 3,5-*O*-Dicaffeoylquinic acid | 22.8 | [10] |
| 8 | 3,4-*O*-Dicaffeoylquinic acid | 24.6 | [10] |
| 9 | Caffeoyltryptophan | 25.5 | [11] |
| 10 | 3-*O*-Caffeoyl-4-*O*-feruloylquinic acid | 26.4 | * |
| 11 | 3-*O*-Feruloyl-4-*O*-caffeoylquinic acid | 27.0 | [8] |

Conditions: column, Finepak SIL $C_{18-5}$, 25 × 0.46 cm i.d.; flow rate = 1.0 ml/min; gradient elution (solvent A, 10 mM $H_3PO_4$; solvent B, MeOH), from 5% B to 50% B in 15 min and then 70% B in 15 min. Average of three runs.

*Present investigation.

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