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CAFFEOYLTYROSINE FROM GREEN ROBUSTA COFFEE BEANS

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Key Word Index—*Coffea canephora*; Rubiaceae; robusta coffee beans; *N*-caffeoyltyrosine.

Abstract—A component found in commercial green robusta coffee beans from many origins, but particularly characteristic of those from Angola, has been characterised as *N*-caffeoyltyrosine.

INTRODUCTION

From the results of paper chromatography [1] it has been apparent since 1960 that the chlorogenic acid (CGA)-rich fraction of commercial green robusta coffee beans from Angola might contain substances not present in the corresponding fraction of robustas from other origins. It was suggested [1] that *p*-coumaric acid was one of the components peculiar to Angolan beans. Studies in this laboratory have confirmed that commercial Angolan robustas are significantly ($p = 0.001$) different [2, 3], but the presence of *p*-coumaric acid could not be confirmed using reversed phase HPLC. This paper reports the isolation and characterisation of the main component contributing to the unique chromatographic profile of Angolan beans.

RESULTS AND DISCUSSION

A concentrated extract from a green Angolan robusta was subjected to reversed phase HPLC and the fraction containing component 7 repeatedly collected, bulked and concentrated under reduced pressure. Homogeneity, checked by analytical HPLC, was *ca* 98% as judged by peak areas at 276 and 313 nm.

Failure to isomerise and form a methyl cinnamate when treated with tetramethylammoniumhydroxide [4] indicated that this component was not a typical CGA. The UV spectrum in methanol showed maxima at 272 and 313 nm, the latter being 75% as intense. Positive reactions to ninhydrin (purple-grey) and molybdate (yellow) implied the presence respectively of $-NH_2$ or $>NH$ groups, and a 1,2-dihydroxy or 1,2,3-trihydroxyphenyl residue. A negative response to 4-dimethylaminocinnamaldehyde indicated that component 7 was not an indole derivative such as *N*-β-caffeoyltryptophan reported in robusta coffee beans by Morishita *et al.* [5].

Acid hydrolysis of component 7 yielded eight products, seven of which had stronger *A* at 313 than at 280 nm. The eighth component, which was detectable at 280 but not at 313 nm, co-chromatographed with authentic L-tyrosine in two analytical systems.

Proton NMR in D_2O at 300 MHz clearly showed component 7 to contain 12 unexchangeable protons. As listed in Table 1, these consisted of seven aromatic protons, two *trans* vinyl protons and three aliphatic protons. The two, two-proton doublets showing ortho coupling were assigned to a 1,4-disubstituted aromatic ring consistent with a tyrosine residue. The three aliphatic protons were assigned by their chemical shifts and coupling constants to the $-CH_2-CH<$ tyrosine side chain. The downfield shift (0.78 ppm) of the Hα proton relative to authentic L-tyrosine suggested that the associated amino function was acylated. The remaining aromatic protons, and the two *trans* vinyl protons, were assigned to a 1,3,4-trisubstituted aromatic ring. This part of the spectrum, after making allowances for the effects of acylation, was consistent with *trans*-caffeic acid (3,4-dihydroxycinnamic acid) which, unlike tyrosine, gives a yellow colour with the molybdate reagent.

By spiking the acid hydrolysate of component 7 it was possible to confirm that caffeic acid was one of the 313 nm-absorbing components present. However, after hydrolysis for only 1 hr the caffeic acid yield was considerably less relative to tyrosine than the 1:1 ratio indicated by NMR. Accordingly an equimolar mixture of caffeic acid and L-tyrosine was subjected to the hydrolysis procedure, and found to produce chromatograms identical to those given by the hydrolysate of component 7.

Taken collectively, these observations strongly suggest that component 7 is *N-trans*-caffeoyl-L-tyrosine which does not seem to have been reported previously. According to Meilgaard and Ravn [6] *N*-caffeoyltyramine occurs widely, *N*-caffeoyl DOPA occurs in Fabaceae, and as mentioned above, *N*-β-caffeoyl-L-tryptophan has been reported in robusta coffee beans.

EXPERIMENTAL

Materials. Commercial green robusta coffee beans from Angola were kindly supplied by the International Coffee Organisation, London. The beans were frozen, ground and extracted with 70% MeOH as previously described [2]. The bulked extracts were treated with Carrez Reagent (1 ml A plus 1 ml B) to ppt. colloidal material, filtered and evapd to dryness at red. pres. The residue was redissolved in a minimal vol. of MeOH, refiltered and used for prep. HPLC. Caffeic acid and L-tyrosine were obtained from Sigma. All other reagents were normal commercial items of good quality.

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Table 1. ^{1}H NMR spectral data for component 7, L-tyrosine and caffeic acid

H	Component 7	L-Tyrosine	Caffeic acid
H_2 and H_6	6.79, 2H, *d*, $J = 8$ Hz	6.84, 2H, *d*, $J = 8$ Hz	
H_3 and H_5	7.12, 2H, *d*, $J = 8$ Hz	7.18, 2H, *d*, $J = 8$ Hz	
Hα	4.60, 1H, *t*, $J = 3$ and 9 Hz	3.82, 1H, *t*, $J = 3$ and 9 Hz	
Hβ, ax	2.94, 1H, *dd*, $J = 9$ and 15 Hz	3.01, 1H, *dd*, $J = 9$ and 15 Hz	
Hβ, eq	3.15, 1H, *dd*, $J = 15$ and 5 Hz	3.21, 1H, *dd*, $J = 15$ and 5 Hz	
Hα′	6.35, 1H, *d*, $J = 16$ Hz		6.21, 1H, *d*, $J = 16$ Hz
Hβ′	7.27, 1H, *d*, $J = 16$ Hz		7.56, 1H, *d*, $J = 16$ Hz
H_2′	7.05, 1H, *s*		7.02, 1H, *s*
H_5′	6.97, 1H, *d*, $J = 8$ Hz		6.93, 1H, *d*, $J = 8$ Hz
H_6′	6.86, 1H, *d*, $J = 8$ Hz		6.78, 1H, *d*, $J = 8$ Hz

General. Analytical HPLC of CGA-like components was performed as previously described [3] using a 3 μm reversed phase non-end capped C_{18} packing and an acidic (pH 2.5) acetonitrile gradient. The eluate was monitored sequentially at 280 and 313 nm. Prep. HPLC was performed in a similar manner but using a 25 cm × 8 mm column containing the equivalent 5 μm packing and a non-linear gradient profile [4]. Analytical amino acid analysis was performed on a Waters Pico Tag™ chromatographic system using a reversed phase column marketed for the analysis of protein hydrolysates and free amino acids after PITC derivatisation.

^{1}H NMR spectra were obtained at 300 MHz. Samples, dissolved in D_2O or D_2O–CD_3OD mixtures as appropriate, were examined at room temp. against a TMS standard.

A UV spectrum was obtained in 70% MeOH, against a 70% MeOH blank.

Acid hydrolysis. An aliquot (100 μl) of component 7 was refluxed with 6 M HCl (3 ml) and the hydrolysate sampled at 1, 4 and 7 hr. The hydrolysate was used directly for phenols analysis, and after derivatisation with PITC for amino acid analysis.

Transesterification. An aliquot (100 μl) of component 7 was treated with 10 μl tetramethylammoniumhydroxide (20% in EtOH) for 5 min at room temp. The reaction was stopped by adding 20 μl 2.5 M HOAc, and the reaction mixture analysed for phenols by direct injection.

Spot tests. Aliquots (10 μl) of component 7 were spotted on Whatman No 1 filter papers and treated individually with the ninhydrin reagent, the 4-dimethylaminocinnamaldehyde reagent [7] or the molybdate reagent [8]. The ninhydrin treated specimen was heated at 100° for 10 min before examination. Reagent blanks and positive controls (tyrosine, tryptophan and caffeic acid) were performed simultaneously.

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