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COFFEE BEAN DICAFFEOYLQUINIC ACIDS

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Key Word Index—*Coffea* sp.; Rubiaceae; coffee; chlorogenic acids; cynarin; 1,3-dicaffeoylquinic acid.

Abstract—Recently the presence of 1,3-dicaffeoylquinic acid (IUPAC numbering) has been reported in green coffee beans. This paper presents ^{1}H NMR, polarimetric and chromatographic data which suggest that this report, based upon the interpretation of fast atom bombardment mass spectra, may be in error.

INTRODUCTION

A recent paper in this journal concerned with the fast atom bombardment mass spectrometry (FABMS) of chlorogenic acids referred to the identification of 1,5-dicaffeoylquinic acid (1,5-diCQA) in an extract prepared from coffee beans [1] and in support of this structural assignment appears to refer to its previous isolation from this source. The paper quoted [2] in fact recorded that it was not possible to recover any dicaffeoylquinic acids from roasted coffee, although there is now indisputable evidence that 3,4-diCQA, 3,5-diCQA and 4,5-diCQA do occur [3–5].

In order to prevent confusion it must be pointed out that refs [1], [2] and [5] use the obsolete pre-IUPAC numbering system. Refs [3] and [4] and this paper henceforward use the preferred IUPAC numbering sytem [6]. In this system the compound examined by FABMS becomes 1,3-diCQA.

The author has analysed over 200 samples of green coffee beans (including five species, several named cultivars and many commercial samples from most geographic origins) and various coffee products but has never detected chromatographically a compound corresponding to 1,3-diCQA.

This paper presents ^{1}H NMR and polarimetric data to confirm the identity of standard 1,3-diCQA (commercial cynarin) and typical chromatograms which show that 1,3-diCQA does not coincide with any component of the chlorogenic acid-rich extracts of typical green coffee beans.

RESULTS AND DISCUSSION

Cynarin gave a specific rotation $[\alpha]_D^{22}$ $-60°$ (*c* 2; MeOH) which is consistent with the previously published values [7, 8] which have recently been compiled in a review [4]. The ^{1}H NMR spectra are internally consistent and consistent with previously published data including recent high resolution 2D-NMR [5, 9–17]. The H-3, H-4 and H-5 protons of quinic acid were easily assigned at δ4.51, 3.88 and 4.71 respectively and the typical downfield chemical shift, upon acylation of the adjacent hydroxyl group, of δ1.34 for H-5 in 5-CQA and 1.52 for H-3 in cynarin, were clearly seen. Comparison of the ^{1}H NMR spectra of cynarin and 5-CQA clearly shows the presence of two *trans*-vinyl protons in the single caffeic acid residue in 5-CQA and four such in cynarin. Since there are only very small downfield chemical shifts of the protons H-4 (0.31 ppm) and H-5 (0.14 ppm) of cynarin the second caffeic acid residue is clearly not attached via either of the associated hydroxyl groups. Thus it must be attached at C-1 (where there is no associated proton).

Further evidence for esterification at C-1 is provided by the signals for the two protons at C-2. These are isochronous at δ2.47 in quinic acid and at 2.56 in 5-CQA. In cynarin they become distinct at 2.90 (H-2_{ax}) and 3.50 (H-2_{eq}). Haslam and Turner [13] have examined many quinic acid derivatives but noted such behaviour only with 1,3-diacyl quinic acids or related lactones, and attributed the behaviour to strong deshielding by the two electron withdrawing acyl residues on adjacent carbons.

The foregoing data are completely consistent with the conclusion that commercial cynarin is 1,3-dicaffeoylquinic acid. The chromatograms shown in Figs 1 and 2 clearly indicate that 1,3-diCQA does not occur in the extract of the Angola robusta, nor has the author ever observed a peak in any other coffee extract which might correspond. As would be expected from its greater polarity (two free equatorial hydroxy groups) it is well resolved from the diCQA (3,4; 3,5 and 4,5) normally accepted as present in coffee beans. In view of these observations some doubt must be shed upon the interpretation that Sakushima *et al.* [1] have placed on their FABMS data for chlorogenic acids, and upon the value of such spectra for discriminating individual chlorogenic acids.

EXPERIMENTAL

Material. Green robusta coffee beans from Angola, supplied by Sol Cafe, England were frozen in liquid N_2 and ground in a hammer mill to pass 0.7 mm. The ground bean (0.5 g) was extracted with boiling 70% MeOH (4 × 15 min, 22 ml) using a Tecator HT 1043 continuous extraction system, and the bulked extracts were diluted to 100 ml. The evaluation of this extraction procedure has been reported elsewhere [18].

Cynarin was supplied by Roth GmbH and Co KG Karlsruhe, West Germany; quinic acid by BDH Ltd, U.K. and 5-CQA by Sigma Chemical Co. Ltd., U.K. All other reagents were of the appropriate grade, obtained from normal sources.

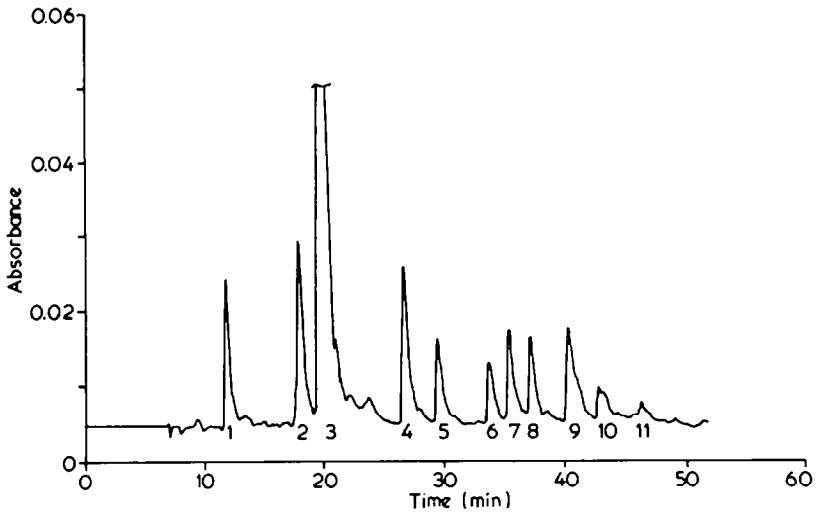

Fig. 1. Chromatogram of a 70% methanol extract of a green Angola robusta. Peak 1 = 3-CQA; 2 = 4-CQA; 3 = 5-CQA; 4 = 5-FQA; 5 and 6 = unknowns; 7 = 3,4-diCQA; 8 = 3,5-diCQA; 9 = 4,5-diCQA plus unresolved unknown; 10 and 11 = unknowns.

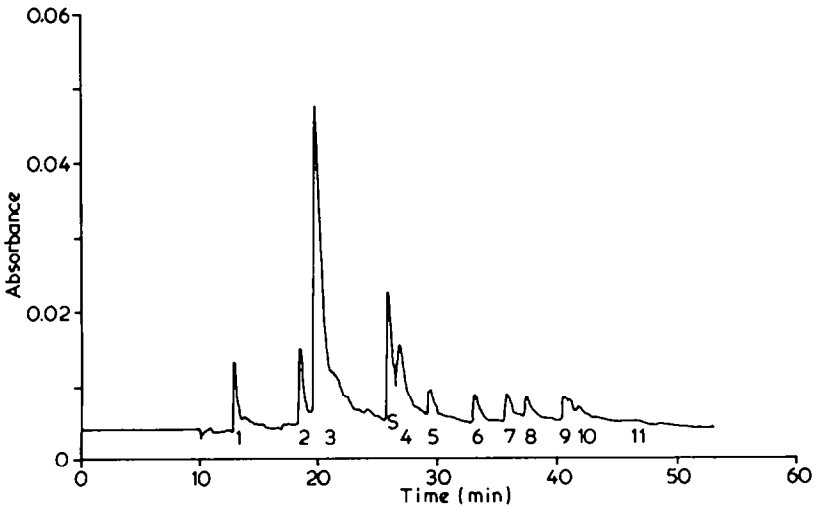

Fig. 2. Chromatogram of the green Angolan robusta extract spiked (1:1) with cynarin. S = Cynarin spike. Other peaks as Fig. 1.

Chromatography. A Waters Associates (Milford, Massachusetts, U.S.A.) liquid chromatograph consisting of two Model 6000A solvent delivering pumps, Model 660A solvent programmer, Model U6K injector and a Model 440 detector operating at 313 nm was used with a 25 cm × 5 mm stainless steel column, containing Spherisorb 5 ODS, and a linear gradient of 0.5% HCO_2H to 30% MeCN in 0.5% HCO_2H over 60 min.

^1H NMR spectra were obtained on a Bruker WH-90 Pulse Fourier Transform NMR Spectrometer operating at 90.02 MHz. The samples were maintained at 90° in 99.5% pyridine-d_5 (M.S.D. isotopes) in sealed tubes containing tetramethylsilane standard. 256 Transients were collected, in each case, into 8K data points.

Polarimetry. Cynarin (2% in MeOH) was examined at room temp. using a 2 dm tube and a sodium lamp.

Proton NMR data. ^1H NMR (90 MHz, pyridine-d_5): quinic acid. δ2.40 (1H, *dd*, $J_{6eq,6ax}$ = 13 Hz, $J_{5,6ax}$ = 10 Hz, H-6_{ax}), 2.47 (2H, *d*, $J_{2,3}$ = 4 Hz, H-2_{eq} and H-2_{ax}), 2.74 (1H, *dd*, $J_{6eq,6ax}$ = 13 Hz, $J_{5,6eq}$ = 4 Hz, H-6_{eq}), 3.88 (1H, *dd*, $J_{4,5}$ = 9 Hz, $J_{3,4}$ = 3 Hz, H-4), 4.51 (1H, *q*, $J_{2,3}$ = 4 Hz, $J_{3,4}$ = 4 Hz, H-3), 4.71 (1H, *td*, $J_{4,5}$ = 9 Hz, $J_{5,6eq}$ = 4 Hz, $J_{5,6ax}$ = 10 Hz, H-5). 5-Caffeoylquinic acid. δ2.49 (1H, *dd*, $J_{6eq,6ax}$ = 13 Hz, $J_{5,6ax}$ = 10 Hz, H-6_{ax}), 2.56 (2H, *d*, $J_{2,3}$ = 4 Hz, H-2_{eq} and H-2_{ax}), 2.81 (1H, *dd*, $J_{6eq,6ax}$ = 13 Hz, $J_{5,6eq}$ = 5 Hz, H-6_{eq}), 4.13 (1H, *dd*, $J_{4,5}$ = 9 Hz, $J_{3,4}$ = 3 Hz, H-4), 4.59 (1H, *q*, $J_{2,3}$ = 4 Hz, $J_{3,4}$ = 4 Hz, H-3), 6.05 (1H, *td*, $J_{4,5}$ = 9 Hz, $J_{5,6eq}$ = 4 Hz, $J_{5,6ax}$ = 10 Hz, H-

5 acyl), 6.45 (1H, *d*, $J_{\alpha,\beta}$ = 16 Hz, H-α), 6.95 (1H, *d*, $J_{5',6'}$ = 8 Hz, aromatic H-5'), 7.00 (1H, *d*, $J_{5',6'}$ = 8 Hz, aromatic H-6'), 7.15 (1H, *s*, H-2'), 7.85 (1H, *d*, $J_{\alpha,\beta}$ = 16 Hz, H-β). Cynarin. δ2.57 (1H, *dd*, $J_{6eq,6ax}$ = 14 Hz, $J_{5,6ax}$ = 10 Hz, H-6_{ax}), 2.90 (1H, *dd*, $J_{2eq,2ax}$ = 16 Hz, $J_{2ax,3}$ = 4 Hz, H-2_{ax}), 3.20 (1H, *dd*, $J_{6eq,6ax}$ = 14 Hz, $J_{5,6eq}$ = 4 Hz, H-6_{eq}), 3.50 (1H, *dd*, $J_{2eq,2ax}$ = 16 Hz, $J_{2eq,3}$ = 4 Hz, H-2_{eq}), 4.19 (1H, *dd*, $J_{4,5}$ = 8 Hz, $J_{3,4}$ = 3 Hz, H-4), 4.84 (1H, *td*, $J_{4,5}$ = 8 Hz, $J_{5,6ax}$ = 10 Hz, $J_{5,6eq}$ = 4 Hz, H-5), 6.03 (1H, *q*, $J_{2ax,3}$ = 4 Hz, $J_{2eq,3}$ = 4 Hz, $J_{3,4}$ = 3 Hz, H-3 acyl), 6.45 (1H, *d*, $J_{\alpha,\beta}$ = 16 Hz, H-α), 6.57 (1H, *d*, $J_{\alpha,\beta}$ = 16 Hz, H-α), 6.88 (1H, *d*, *J* = 8 Hz, aromatic), 6.90 (1H, *d*, *J* = 8 Hz, aromatic), 7.02 (1H, *d*, *J* = 8 Hz, aromatic), 7.06 (1H, *d*, *J* = 8 Hz, aromatic), 7.22 (1H, *s*, aromatic H-2'), 7.25 (1H, *s*, aromatic H-2'), 8.60 (1H, *d*, $J_{\alpha,\beta}$ = 16 Hz, H-β), 8.66 (1H, *d*, $J_{\alpha,\beta}$ = 16 Hz, H-β).

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