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SHORT COMMUNICATION

IDENTIFICATION OF SEVERAL COMPONENTS OF ISOCHLOROGENIC ACID

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Abstract—"Isochlorogenic acid" has been shown to be a complex mixture of closely related compounds. Three of the major fractions have been purified and shown by NMR spectroscopy to be 4,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid. A fourth major fraction appears to be a mixture of the 3'-methyl ethers of 3,5-dicaffeoylquinic acid.

INTRODUCTION

ISOCHLOROGENIC acid, originally isolated from coffee,[1] was proposed to be 5-caffeoylquinic acid. The chemical evidence for this assignment has been questioned[2] and the resolution of isochlorogenic acid into three or more components[3-6] has reopened the whole question. Scarpati and Guiso[5] reported three dicaffeoylquinic acids as components of isochlorogenic acid and Hanson and Zucker[3] likewise showed at least three components: *a*, *b*, *c* by partition chromatography. In addition, neochlorogenic acid has been shown to be 5-caffeoylquinic acid.[4,6]

We have recently demonstrated that acylation of the hydroxyl groups in quinic acid produces paramagnetic shifts of 1·4–1·6 ppm in the NMR peaks of the protons on the same carbon atom.[6] These shifts have been used to confirm the structures of the chlorogenic acids.

EXPERIMENTAL

When isochlorogenic acid is subjected to 200-transfer counter-current distribution (CCD) (ethyl acetate—1M phosphate buffer, pH 5·5), three major fractions separate (Fig. 1), corresponding to the Hanson and Zucker[3] nomenclature. The partition coefficients and Rcg[3] values are: *a*, K = 2·8, Rcg = 0·54, 0·67; *b*, K = 0·53, 0·94, Rcg = 0·87, 0·94; *c*, K = 0·18, Rcg, 1·15.

Isochlorogenic acid *c*, was obtained by acidification of the aqueous layer from the CCD band and extraction with ethyl acetate. The *a* and *b* fractions were separated by 500-transfer CCD using ethyl acetate-1 M phosphate, pH 5·7. All three fractions were purified by precipitation of ethyl acetate solutions by chloroform. Isochlorogenic acid *b* was recrystallized from

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[1] H. M. BARNES, J. R. FELDMAN and W. V. WHITE, *J. Am. Chem. Soc.* **72**, 4178 (1950).
[2] R. BEAN and J. CORSE, Paper presented at the Plant Phenolics Group Symposium on Chlorogenic Acids, University of London, Sept. 25, 1957.
[3] K. R. HANSON and M. ZUCKER, *J. Biol. Chem.* **238**, 1105 (1963).
[4] M. L. SCARPATI and P. ESPOSITO, *Tetrahedron Letts* 1147 (1963).
[5] M. L. SCARPATI and M. GUISO, *Ann. Chim. (Rome)* **53**, 1315 (1963).
[6] A. C. WAISS, R. E. LUNDIN and J. CORSE, *Chem. & Ind. (London)*, 1984 (1964).

ethyl acetate, m.p. 170–172° (dec.). The fraction peaking at tube 165 was separated by 500-transfer CCD using ethyl acetate-1 M phosphate pH 6·2, and the resulting main fraction purified as before. Analytical chromatography[3] however, showed it still to be a mixture.

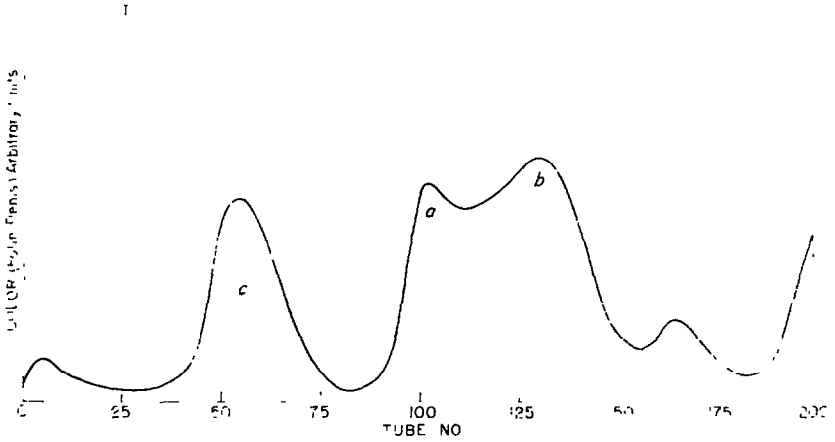

FIG. 1. COUNTERCURRENT DISTRIBUTION PATTERN OF "ISOCHLOROGENIC ACID"
200-transfer run; ethyl acetate: 1 M phosphate buffer, pH 5·5.

Hydrolysis of *a*, *b* and *c* gave only caffeic and quinic acids, in each case. The main fraction of the band 165 gave ferulic acid in addition. The NMR spectra showed the purity of *a*, *b* and *c* to be greater than 95% per cent and that they were free of contamination by other quinic acid esters. Their physical constants are given in Table 1. It is to be emphasized that in this

TABLE 1. PHYSICAL CONSTANTS OF ISOCHLOROGENIC ACID FRACTIONS

Fraction	%C	%H	Neutral equiv.	Log ε 329 mμ	Rcg	Rcg (Lit.)[7]	$[\alpha]_D^{26}$
a	56·5	4·73	526	4·51	0·87	0·86	−288°
b	57·6	4·74	549	4·55	0·94	0·94	−197
c	56·4	4·80	534	4·51	1·15	1·10	−166
4,5-Dicaffeoylquinic acid[7]					1·14		(−173°)[7]
$C_{25}H_{26}O_{13}$	58·14	4·68	522				
$C_{25}H_{26}O_{13}\frac{1}{2}H_2O$	58·02	4·87	531				
$C_{25}H_{26}O_{13}H_2O$	56·17	4·90	540				

series of compounds, the NMR spectra and optical rotations are more sensitive indicators of purity than elementary analyses. The chemical shifts of the protons on C_3, C_4 and C_5 (H_3, H_4 and H_5) of quinic acid from the NMR spectra of the three isomers, taken in deuteropyridine at 90° are given in Table 2.

[7] M. L. SCARPATI, G. ORIENTE and L. PANIZZI, *Ann. Chim. (Rome)* **48**, 997 (1958).

TABLE 2. CHEMICAL SHIFTS (τ) OF ESTERS OF QUINIC ACID

	H_3	H_4	H_5	$\Delta\tau$*
Quinic acid	5·31	6·09	5·45	—
Isochlorogenic acid *c*	5·03	4·27	3·80	1·82 H_4 1·65 H_5
Isochlorogenic acid *a*	3·83	4·39	5·16	1·48 H_3 1·70 H_4
Isochlorogenic acid *b*	3·91	5·50	3·96	1·40 H_3 1·49 H_5

* $\Delta\tau$ is the difference in chemical shifts of the protons attached to carbon atoms bearing acyloxy groups and its corresponding proton in quinic acid.

DISCUSSION

In addition to the unequivocal identification of protons H_3, H_4 and H_5 by their chemical shifts and expected spin–spin coupling of the quinic acid protons, the NMR spectra clearly show the presence of four *trans* vinyl protons ($J = 16$ cps) from the caffeic acid moieties in each of the dicaffeoyl quinic acids. This confirms the disubstituted nature of the compounds. It is apparent from Table 2 that isochlorogenic acid *a* is 3,4-dicaffeoylquinic acid, *b* is the 3,5-diester and *c* is the 4,5-isomer. The optical rotation of two of the isomers, *b* and *c*, show their probable identity with fractions *C* and *A* of Scarpati and Guiso.[5] The correspondence of *a* with *B* is uncertain because of the large difference in rotation: $[\alpha]_D^{26}$ $a = -288°$; $[\alpha]_D$ $B = -225°$.

"Fraction 165" showed chemical shifts and vinyl protons corresponding to 3,5-disubstitution of quinic acid by *trans*-cinnamic acids. Further, O-methyl protons (6·25 τ) appeared in the spectrum, but at about one-half the intensity of the expected diferuloyl derivative. The finding of the monoferuloyl-monocaffeoylquinic acid, band 165, is to be expected; since 3-feruloylquinic acid is known[8] and the 4- and 5-isomers appear to be present in coffee.[9] The position of the feruloyl group cannot be distinguished from that of the caffeoyl by NMR spectroscopy.

Note added in revision. Recently K. Nakanishi (Symposium on Recent Advances in Plant Phenolics, New Delhi, India, Oct. 4–6, 1964) reported the isolation of 4,5-dicaffeoylquinic acid from coffee and its identification by NMR spectroscopy. M. L. Scarpati and M. Guiso *Tetrahedron Letters* No. 39, 2851 (1964) identified their isomers *A*, *B* and *C* by chemical means; $A = c$; $B = a$; $C = b$. The discrepancy in rotation of *B* and *a* (60°) remains.

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[8] J. CORSE, E. SONDHEIMER and R. LUNDIN, *Tetrahedron* **18**, 1207 (1962).
[9] J. CORSE, unpublished work.