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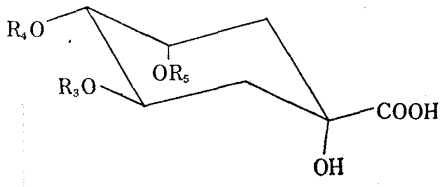
Isolation of 4,5-Di-O-caffeylquinic Acid from Coffee Beans*[1]

In 1950, Barnes, *et al.*[1] isolated "isochlorogenic acid" from coffee beans, but its 5-O-caffeylquinic acid structure[1] has remained in doubt.[2] On the other hand, 4-O-caffeylquinic acid and 5-O-caffeylquinic acid structures, respectively, have since been assigned[3] to neochlorogenic acid (III)[4] and "band 510" (IV)[4] isolated from artichoke leaves and coffee beans. It has further been shown that "isochlorogenic acid" is a mixture of three components.[5,6]



(I) quinic acid; $R_3=R_4=R_5=H$
(II) chlorogenic acid; R_3=caffeyl, $R_4=R_5=H$
(III) neochlorogenic acid; R_5=caffeyl, $R_3=R_4=H$
(IV) "band 510"; R_4=caffeyl, $R_3=R_5=H$
(V) 4,5-di-O-caffeylquinic acid; $R_4=R_5$=caffeyl, $R_3=H$

We have now isolated from unroasted Brazilian coffee beans a white powder to which a 4,5-di-O-caffeylquinic acid structure is assigned. The preliminary isolation and purification procedures were substantially the same as those described for "isochlorogenic acid."[1] The crude acid was further purified by three 50-plate counter-current-distributions between butyl acetate and phosphate buffer (one run at pH 5.7 followed by two runs at pH 4.8); this procedure removed a contaminant with $\lambda_{\max}^{\mathrm{MeOH}}$ 233 and 281 mμ as well as some chlorogenic acid (crystalline). In this way an acid, referred to as Compound I in this paper, was obtained*[2] with m.p. 140° (decomp.), $[\alpha]_D$ $-172°$ (c=1.0, MeOH); C, 56.31; H, 5.26 (calcd. for $C_{25}H_{24}O_{12}\cdot H_2O$: C, 56.17; H, 4.90); $\lambda_{\max}^{\mathrm{MeOH}}$ 330 mμ (log ε

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*[1] An outline of this work has been presented at the "Symposium on Recent Developments in Plant Polyphenolics," Delhi, October 1964. Dr. J. Corse and co-workers at Western Research Laboratory, Albany, California, have recently identified "isochlorogenic acid c" (cf. ref. 6) as 4,5-di-O-caffeylquinic acid (private communication).

*[2] The powder was dried at 80° for 20 hours *in vacuo*.

1) H.M. Barnes, J.R. Feldman, W.V. White : J. Am. Chem. Soc., **72**, 4178 (1950).
2) See for example : J. Corse, E. Sondheimer, R. Lundin : Tetrahedron, **18**, 1207 (1962).
3) M.L. Scarpati, P. Esposito : Tetrahedron Letters, No. 18, 1147 (1963).
4) J. Corse : Nature, **172**, 771 (1963).
5) M.L. Scarpati, M. Guiso : Ann. Chem. (Italy), **53**, 1315 (1963); also cf. footnote 1 in reference 3.
6) K.R. Hanson, M. Zucker : J. Biol. Chem., **238**, 1105 (1963).

4.53). Although Compound I could not be crystallized, it gave only a single spot on thin-layer chromatography (t.l.c.) under various conditions (Table I). Alkaline hydrolysis afforded caffeic acid and quinic acid.

Table I. Silica Gel Thin-layer Chromatography

Solvent	Rf-Value	
	Chlorogenic acid	Compound I
AcOH	0.54	0.59
AcOEt-AcOH-H_2O (9:2:2)	0.77	0.92
BuOH-EtOH-H_2O (4:1:5)	0.67	0.78
$CHCl_3$-H_2O-AcOH (2:1:1)[a]	0.9	0.84

[a] The upper phase was used.

Potentiometric titrations carried out on 33% ethanol solutions of quinic acid (I), chlorogenic acid (II) and Compound I (V) clearly showed that the neutralization equivalent of Compound I is larger than that of chlorogenic acid (found 363, calcd. 354) and is 536 (calcd. for monohydrate, 534).

The titrations also showed that whereas chlorogenic acid consumed only one mole of alkali in the pH range of 8 to 11 (where only one of the hydroxyl groups of the caffeic acid moiety is ionized), Compound I consumed two equivalents in the same pH range. This evidence suggests that Compound I is a dicaffeate. Furthermore, the pKa′

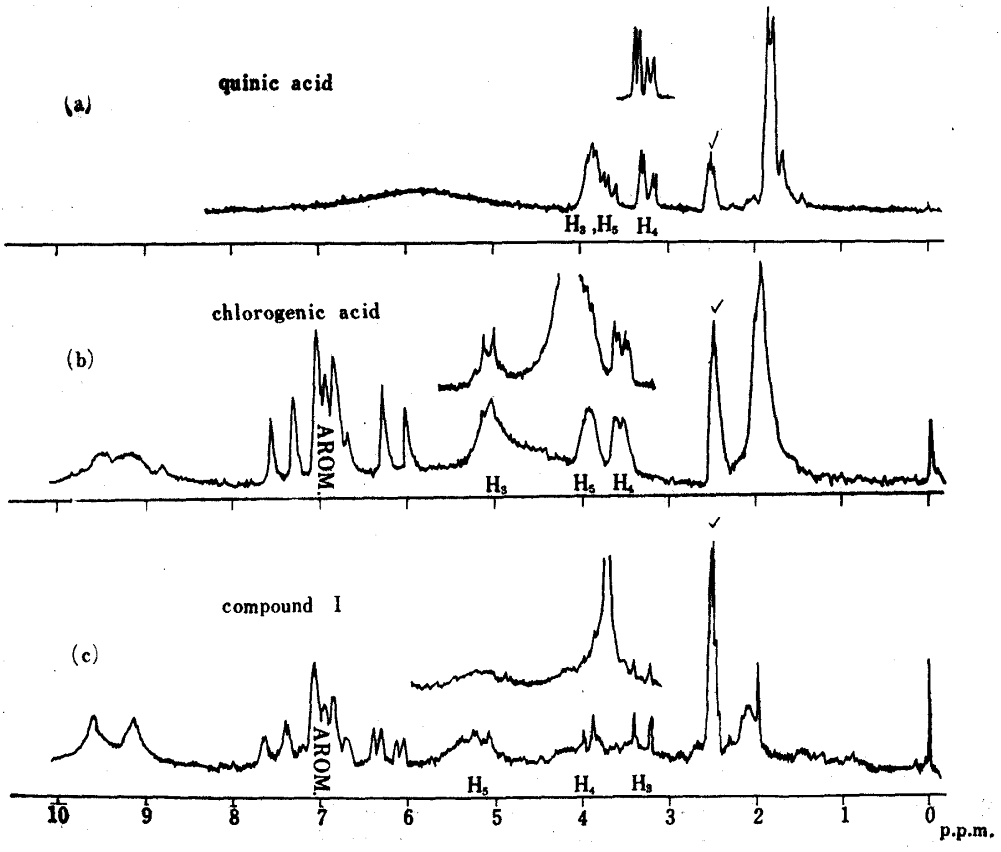


Fig. 1. Nuclear Magnetic Resonance spectra of (a) Quinic Acid, (b) Chlorogenic Acid and (c) Compound I in DMSO-d_6 and DMSO-d_6/D_2O

Only part of the spectra is shown for the latter solvent system. The peaks at ca. 2.5 p.p.m. are due to the solvent. Measured at 60 Mc. values in p.p.m. relative to TMS.

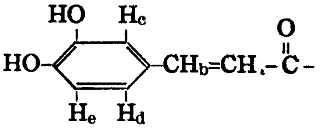
values of quinic acid, chlorogenic acid and Compound I are, respectively, 4.15, 4.25 and 4.25 (at 20°); the fact that the pKa′ of Compound I is identical with that of chlorogenic acid clearly shows that neither of the caffeyl groups is attached to the C_1-OH and also suggests that the conformation of the quinic acid moiety is the same in the two compounds, *i.e.*, C_1-COOH is equatorial.[7]

The hydroxyl group at C_3 also is not involved in the ester linkage since Compound I when dissolved in acetone containing 1% dry hydrogen chloride and left at room temperature for several days was gartly converted into a lactone; the product showed a distinct infrared band at 1787 cm^{-1} (γ-lactone, KBr), and on silica gel t.l.c. gave two spots, Rf 0.19 (starting material) and 0.53 in a solvent system of chloroform:ethanol (10:1). Lactonization is known to occur between the C_3-OH and C_1-COOH in quinic acid[8] and 5-O-caffeylquinic acid[9] and is accompanied by ring inversion.

It can therefore be concluded that Compound I is 4,5-di-O-caffeylquinic acid, and this is fully supported by the nuclear magnetic resonance data (Fig. 1 and Table II).

Table II. Chemical Shifts and Coupling Constants of Quinic Acid (I), Chlorogenic Acid (II) and Compound I (V) (in DMSO-d_6)

	H_3	H_4	H_5	H_2, H_6	H_a[a]	H_b[a]	H_c[a]	H_d[a]	H_e[a]
(I)	3.7∼4.0	3.26(q)	3.7∼4.0	1.8∼1.9					
	J_{34} 8.3	J_{45} 3.0							
(II)	5.11	3.59(q)	3.95	1.9	6.18	7.47	7.10	7.04	6.83
	J_{34} 7.0	J_{45} 2.5			J_{ab} 16				
(V)	3.3	4.0(q)	5.2	2.1	6.32	7.54	7.06	7.04	6.79
	J_{34} 11.5	J_{45} small			J_{ab} 16				
					6.14	7.46			
					$J_{a'b'}$ 16				

a)


The quartet at 3.26 p.p.m. in Fig. 1a with a large (8.3 c.p.s., ax-ax) and a small splitting (3 c.p.s., ax-eq) is assignable to the C_4-H; the appearance of the C_4-H signal as a quartet in DMSO shows that the coupling between the C_4-H and the C_4-OH proton is unusually small (it as generally 4∼5 c.p.s.[10]). In chlorogenic acid (Fig. 1b) the signal of the C_3-H is shifted downfield by ca. 1.2 p.p.m. because of the ester linkage. The olefinic protons of the caffeyl moiety in chlorogenic acid appear as a clear AB quartet (16 c.p.s., J_{trans}) which straddles the aromatic proton pattern, and convincing evidence for the di-O-caffeyl structure in Compound I (Fig. 1c) is provided by the two sets of AB quartets both of which straddle the aromatic proton pattern.

Since the infrared spectrum of Compound I is very similar to that shown in the paper by Barnes, *et al.*,[1] it is conceivable that the major constituent of "isochlorogenic acid" is 4,5-di-O-caffeylquinic acid (Compound I).

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[7] It is highly improbable that ax- and eq-COOH groups on a cyclohexane ring would have the same pK value: cf. for example, P.F. Sommer, V.P. Arya, W. Simon: Tetrahedron Letters, No. 20, 18 (1960); P.F. Sommer, C. Pascual, V.P. Arya, W. Simon: Helv. Chim. Acta, **46**, 1734 (1963); C. Pascual, W. Simon: *Ibid.*, **47**, 683 (1964).

[8] H.O.L. Fischer: Ber., **54**, 775 (1921); H.O.L. Fischer, C. Taube: *Ibid.* **65**, 1009 (1932).

[9] E.A. Ruveda, V. Deulofeu, O.L. Galmarini: Chem. and Ind. (London), **1964**, 239.

[10] O.L. Chapman, R.W. Kind: J. Am. Chem. Soc., **86**, 1257 (1964).

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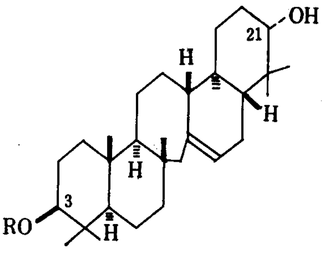
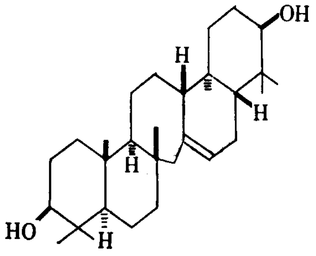
21-Episerratenediol, Isolation and its Structure

This communication is concerned with the constitution and stereochemistry of a triterpene-diol, 21-episerratenediol, which we have isolated from *Lycopodium serratum* THUNB. var. *Thunbergii* MAKINO (Lycopodiaceae) along with serratenediol, serratenediol-3-acetate and two new triterpenoids.

The structure of serratenediol and its 3-acetate, two major triterpenoid constituents, were already elucidated as formulated by I and II, respectively.[1,2]



I : R=H
II : R=$COCH_3$



III

Separation of minor triterpenoids was conveniently effected by repeated chromatography of derived acetates over silica gel and alumina. The physical constants of the three new triterpenoids and their acetates are listed in the Table. The nuclear magnetic resonance spectrum*1 of A-diacetate indicated the presence of a trisubstituted

		Alcohol		Acetate
(A)	21-Episerratenediol	$C_{30}H_{52}O_2$,	m.p. 289~290°	$C_{34}H_{56}O_4$, m.p. 225~229° $[\alpha]_D$ −29° (c=2.06, $CHCl_3$)
(B)	Serratriol	$C_{30}H_{50}O_3$,	m.p. 335~336°	$C_{36}H_{56}O_6$, m.p. 245~247° $[\alpha]_D$ +21° (c=1.9, $CHCl_3$)
(C)	Tohogenol	$C_{30}H_{52}O_3 \cdot \frac{1}{2}H_2O$,	m.p. 242~244°	$C_{34}H_{56}O_5$, m.p. 305~306° $[\alpha]_D$ +28° (c=1.6, $CHCl_3$)

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*1 NMR spectra were taken on Varian A-60 machine in $CDCl_3$ with Me_4Si as an internal standard by Mr. T. Shingu, Kyoto University, to whom we are indebted.

[1] Y. Inubushi, T. Sano, Y. Tsuda: Tetrahedron Letters, No. 21, 1303 (1964).

[2] Y. Tsuda, T. Sano, K. Kawaguchi, Y. Inubushi: *Ibid.*, No. 20, 1279 (1964).