399. *Synthesis and Properties of Some Hydroxycinnamoyl Esters of Quinic Acid.*

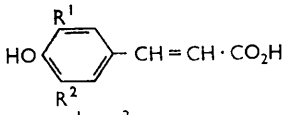
By E. Haslam, G. K. Makinson, M. O. Naumann, and (in part) Jill Cunningham.

The synthesis and characterisation of 1-, 4-, and 5-*O*-*p*-coumaroylquinic acid is described. On heating in sodium hydrogen carbonate solution migration of the *p*-coumaroyl group from position 1 ⟶ 3 and from 3 ⟷ 4 ⟷ 5 on the quinic acid core has been shown to occur, and a mechanism for these rearrangements is discussed. The structure of neochlorogenic acid has been confirmed as 5-*O*-caffeoylquinic acid (II; $R^1 = R^2 = R^3 = R^4 = H$, R^5 = 3,4-dihydroxycinnamoyl); the formation of this compound by isomerisation of chlorogenic acid, and its synthesis, are reported.

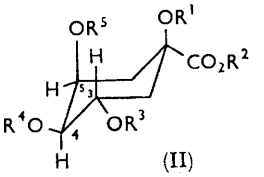
Esters and glycosides of the hydroxycinnamic acids (Ia—d) are the principal phenolic metabolites of many higher plants.[1] As esters, the acids are normally associated with glucose [1] or quinic acid [2] (II; all R's = H), but recent communications [3] report the occurrence in Nature of esters with shikimic acid (III), and several claims [4] exist for the isolation of esters with anthocyanins in which it is believed the ester linkage is in the sugar residue. Of the esters with quinic acid, the widely distributed 3-*O*-caffeoyl (3,4-dihydroxycinnamoyl) derivative, chlorogenic acid (II; $R^1 = R^2 = R^4 = R^5 = H$, R^3 = 3,4-dihydroxycinnamoyl), has been most extensively studied [2] but since 1950 the isolation of at least four other caffeoyl esters of quinic acid has been claimed. The structure of cynarin from the artichoke has been shown [5,6] to be 1,4-di-*O*-caffeoylquinic acid (II; $R^1 = R^4$ = 3,4-dihydroxycinnamoyl, $R^2 = R^3 = R^5 = H$), and that of isochlorogenic acid from coffee has been suggested [7] as 5-*O*-caffeoylquinic acid (II; $R^1 = R^2 = R^3 = R^4 = H$,

[1] Harborne and Corner, *Biochem. J.*, 1961, **81**, 242.
[2] Bate-Smith, *Chem. and Ind.*, 1954, 1457; Hermann, *Pharmazie*, 1956, **11**, 433.
[3] Maier and Metzler, American Chemical Society, 144th Meeting, Los Angeles, 1963.
[4] Harborne, *Fortschr. Chem. org. Naturstoffe*, 1962, **20**, 165.
[5] Panizzi and Scarpati, *Gazzetta*, 1954, **84**, 792.
[6] Panizzi, Scarpati, and Scarpati, *Gazzetta*, 1954, **84**, 806.
[7] Barnes, Feldman, and White, *J. Amer. Chem. Soc.* 1950, **72**, 4178.

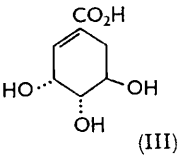
R^5 = 3,4-dihydroxycinnamoyl), but recently [8] the same structure was put forward for neochlorogenic acid isolated from peaches [9] and coffee.[8] No structure has been advanced for a fourth substance, pseudochlorogenic acid, obtained [10] from black-rot infected sweet potato. The proposed [11] route of biosynthesis of the hydroxycinnamic acids (Ia ⟶ Ib ⟶ Ic ⟶ Id) suggests that the occurrence in Nature of the *p*-coumaroyl (4-hydroxycinnamoyl) (Ia), feruloyl (Ic), and sinapoyl (Id) analogues of the various caffeoylquinic



(Ia: $R^1 = R^2 = H$)
(Ib: $R^1 = OH, R^2 = H$)
(Ic: $R^1 = OMe, R^2 = H$)
(Id: $R^1 = R^2 = OMe$)



(II)

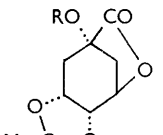


(III)

acids is to be expected, and recent reports [12,13] of the isolation and characterisation of the 3-*O*-feruloyl and 3-*O*-*p*-coumaroyl derivatives from coffee and cider apples, respectively, confirm these expectations. In addition, paper chromatography has revealed,[14] in a variety of plants, the *p*-coumaroyl counterpart of neochlorogenic acid, and as an ancillary study to structural work on isochlorogenic and neochlorogenic acid the four isomeric mono-*O*-*p*-coumaroylquinic acids have been synthesised and their properties studied.

A synthesis of 3-*O*-*p*-coumaroylquinic acid has been reported [15] and methods for the preparation of the 1-, 4-, and 5-isomers have been developed in this work along lines similar to those used for the corresponding galloylquinic acids.[16] Condensation of 4-acetoxycinnamoyl (*O*-acetyl-*p*-coumaroyl) chloride with 4,5-*O*-isopropylidenequinide (IV; R = H) gave the 1-ester (IV; R = *O*-acetyl-*p*-coumaroyl) from which the protecting acetyl and isopropylidene groups were removed and the lactone ring severed by hydrolysis with dilute acid.[17] Purification of the product by countercurrent distribution gave the amorphous 1-*O*-*p*-coumaroylquinic acid (II; R^1 = *p*-coumaroyl, $R^2 = R^3 = R^4 = R^5$ = H) which readily afforded the characteristic isopropylidene lactone (IV; R = *p*-coumaroyl), thus confirming its structure. The most satisfactory procedure for the preparation of 4- and 5-*O*-*p*-coumaroylquinic acid was by the condensation of *O*-acetyl *p*-coumaroyl chloride with an equimolar proportion of 1-*O*-ethoxycarbonylquinide (V; $R^4 = R^5$ = H, R^1 = ethoxycarbonyl). The intractable mixture of esters which resulted was treated directly with acetic acid to hydrolyse the lactone and ethoxycarbonyl groups,[15] and then with ammonia at 0° to remove the acetyl group. Countercurrent distribution of the products gave the crystalline 4- and 5-*O*-*p*-coumaroylquinic acids which were differentiated by titration with periodic acid, only the latter showing the presence of an α-glycol grouping. Alternatively, brief mineral-acid hydrolysis of the mixed ester condensation product gave a product identified by titration with periodic acid as 5-*O*-*p*-coumaroyl-1-*O*-ethoxycarbonylquinic acid (II; R^1 = ethoxycarbonyl, $R^2 = R^3 = R^4$ = H, R^5 = *p*-coumaroyl), and which on hydrolysis with water or acetic acid gave 5-*O*-*p*-coumaroylquinic acid. Selective hydrolysis of the di-ester (V; R^1 = ethoxycarbonyl, $R^4 = R^5$ = *p*-coumaroyl) gave poor yields of both 4- and 5-*O*-*p*-coumaroylquinic acid.

[8] Scarpati and Esposito, *Tetrahedron Letters*, 1963, 1147.
[9] Corse, *Nature*, 1953, **172**, 771.
[10] Uritani and Miyano, *Nature*, 1955, **175**, 812.
[11] Neish and McCalla, *Canad. J. Biochem. Physiol.*, 1959, **37**, 537.
[12] Corse, Sondheimer, and Lundin, *Tetrahedron*, 1962, **18**, 1207.
[13] Williams, *Chem. and Ind.*, 1958, 1200.
[14] Cartwright, Flood, Roberts, and Williams, *Chem. and Ind.*, 1955, 1062.
[15] Haslam, Haworth, and Makinson, *J.*, 1961, 5153.
[16] Haslam, Haworth, and Lawton, *J.*, 1963, 2173.
[17] Josephson, *Annalen*, 1928, **467**, 292.



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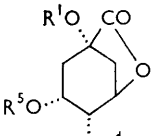

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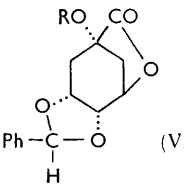
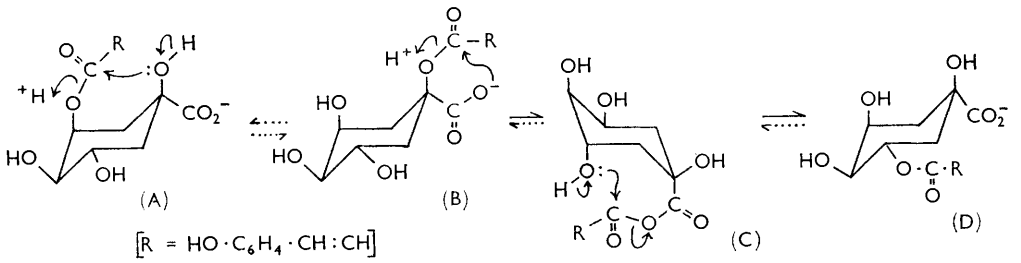


(VI)

The individual *p*-coumaroylquinic acids were characterised by analysis, optical rotation, paper chromatographic pattern, distribution coefficient (K) between ethyl acetate and water, and titration with periodic acid. Confirmation of their structures was obtained from proton resonance spectra; the position of the absorption due to the hydrogen atom at position 4 in quinic acid was diagnostic in differentiating the 3- and the 5-isomers from the 4-isomer, and 1-*O*-*p*-coumaroylquinic acid was distinguished by the absence of absorption (τ 4·6) due to a proton with an ester grouping in the α-position. Previous work [18] showed the preferred conformation of quinic acid to be (II) in which the 5-hydroxyl group is axial, since dehydrogenation over a platinum catalyst gave exclusively 5-dehydroquinic acid. The hydrogen at position 4 is thus in an axial position and flanked by two hydrogen atoms, one axially (position 3) and the other equatorially disposed (position 5), and Lemieux *et al.*[19] showed that such systems give rise to a double doublet in the proton resonance spectrum. In the case of the 3- and the 5-isomer this absorption (J_1 10, J_2 2 c./sec.) occurred at (τ 6·4) but in 4-*O*-*p*-coumaroylquinic acid it was moved to lower field (τ 4·85) owing to the deshielding effect of the ester group which is α to the proton at position 4.

Earlier attempts to prepare 1-*O*-*p*-coumaroylquinic acid led to the discovery of a novel series of rearrangements of acyl derivatives of quinic acid. Treatment of the ester (VI; R = *O*-acetyl-*p*-coumaroyl) with acid and alkali gave, in addition to the expected 1-*O*-*p*-coumaroylquinic acid, substantial amounts of the 3-*O*-*p*-coumaroyl isomer, and model experiments showed that 1-*O*-*p*-coumaroylquinic acid was isomerised to 3-*O*-*p*-coumaroylquinic acid by heating in sodium hydrogen carbonate solution. The previously observed [15] migration of a galloyl group in similar circumstances presumably follows an identical pathway, and a suggested mechanism for this rearrangement is shown (B ⟶ D). Formation of the anhydride intermediate (C) is analogous to mechanisms

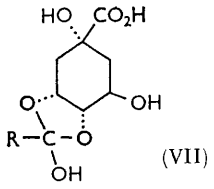


put forward to explain the rates of hydrolysis of acetylsalicylic acid [20] and ethyl hydrogen phthalate [21] in the pH range 5—8 in which it has been suggested that the ionised carboxyl group acts as a nucleophile. The presence of intermediates of an anhydride nature in the reaction was readily demonstrated by conducting the reaction in methanolic solution, when methyl *p*-coumarate was isolated. Similar rationalisations explain the production of

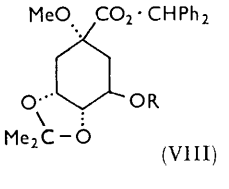
[18] Heyns and Gottschalk, *Chem. Ber.*, 1961, **94**, 343; Haslam, Haworth, and Knowles, *J.*, 1961, 1854.
[19] Lemieux, Kullnig, Bernstein, and Schneider, *J. Amer. Chem. Soc.*, 1958, **80**, 6098.
[20] Garrett, *J. Amer. Chem. Soc.*, 1957, **79**, 3401.
[21] Bender, Chloupek, and Neven, *J. Amer. Chem. Soc.*, 1958, **80**, 5384.

considerable quantities of ethyl gallate [16] when the lactone ring of (V; $R^4 = R^5 = H$, R^1 = 3,4,5-trihydroxybenzoyl) was severed in ethanolic barium hydroxide.

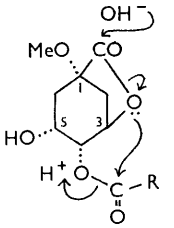
Further work has shown that on heating (90°) in sodium hydrogen carbonate solution facile migration of the *p*-coumaroyl group occurs in 3-, 4-, and 5-*O*-*p*-coumaroylquinic acid, although below 50° this was slow and in no case proceeded so readily as with 1-*O*-*p*-coumaroylquinic acid. In this way 3- and 5-*O*-*p*-coumaroylquinic acid were shown to be interconvertible, and both were obtained from 4-*O*-*p*-coumaroylquinic acid, which was isolated itself after brief treatment of 3-*O*-*p*-coumaroylquinic acid with sodium hydrogen carbonate. Rearrangement of the acyl group in these cases is thought therefore to proceed in a stepwise fashion (3 ⟷ 4 ⟷ 5) and probably by formation of the intermediate orthoesters such as (VII; R = 4-hydroxystyryl).



(VII)



(VIII)



(IX)

Although it was not possible to isolate 1-*O*-*p*-coumaroylquinic acid after treatment of 3-*O*-*p*-coumaroylquinic acid with sodium hydrogen carbonate, an alternative mechanism for the migration of the *p*-coumaroyl group between the 3- and the 5-position in which this acid acts as intermediate is shown in the above scheme (A ⟷ D). However, experiments with the 1-*O*-methyl derivatives of the *p*-coumaroylquinic acids favour the orthoester mechanism for the acyl migration, but as these have been only partly successful further work is in progress. Thus, although the 1-*O*-methyl derivatives of both 3- and 4-*p*-coumaroylquinic acid were synthesised satisfactorily and were shown to be interconvertible in sodium hydrogen carbonate solution, attempts to prepare 5-*O*-*p*-coumaroyl-1-*O*-methylquinic acid either by synthesis or following rearrangement of 4-*O*-*p*-coumaroyl-1-*O*-methylquinic acid were unsuccessful. Preparation of 3-*O*-*p*-coumaroyl-1-*O*-methylquinic acid (II; R^1 = Me, $R^2 = R^4 = R^5 = H$, R^3 = *p*-coumaroyl) was effected by an analogous method to that used [15] for the synthesis of 3-*O*-*p*-coumaroylquinic acid. Condensation of *O*-ethoxycarbonyl-*p*-coumaroyl chloride with diphenylmethyl 4,5-*O*-isopropylidene-1-*O*-methylquinate (VIII; R = H) yielded the ester (VIII; R = *O*-ethoxycarbonyl-*p*-coumaroyl) which with one equivalent of alkali gave the *p*-coumaroyl derivative (VIII; R = *p*-coumaroyl), and acid hydrolysis of the latter produced the crystalline 3-*O*-*p*-coumaroyl-1-*O*-methylquinic acid, characterised by titration with periodic acid, and from its proton resonance spectrum. Condensation of 1-*O*-methylquinide (V; R^1 = Me, $R^4 = R^5 = H$) with an equimolar proportion of *O*-ethoxycarbonyl-*p*-coumaroyl chloride gave a crystalline ester ($\varepsilon_{max.}$ 1775, 1745, and 1715 $cm.^{-1}$) which, surprisingly, on hydrolysis with alkali gave almost quantitative yields of 3-*O*-*p*-coumaroyl-1-*O*-methylquinic acid. The original ester has therefore been formulated as (V; R^1 = Me, R^4 = *O*-ethoxycarbonyl-*p*-coumaroyl, $R^5 = H$), and the hydrolysis with alkali as a concerted reaction in which the acyl group migrates (4 ⟶ 3) as the lactone ring is severed, as shown in (IX; R = 4-hydroxystyryl). Hydrolysis of the ester (V; R^1 = Me, R^4 = *O*-ethoxycarbonyl-*p*-coumaroyl, $R^5 = H$) with aqueous acetic acid followed by countercurrect distribution of the products gave 4-*O*-*p*-coumaroyl-1-*O*-methylquinic acid (II; R^1 = Me, $R^2 = R^3 = R^5 = H$, R^4 = *p*-coumaroyl) which was characterised by formation of the lactone (V; R^1 = Me, R^4 = *p*-coumaroyl), titration with periodic acid, and its proton resonance spectrum.

Work on the structure of neochlorogenic acid has confirmed the 5-*O*-caffeoylquinic acid [II; $R^1 = R^2 = R^3 = R^4 = H$, R^5 = caffeoyl (3,4-dihydroxycinnamoyl)] structure.[8] Neochlorogenic acid was first isolated by Corse [9] by countercurrent distribution of peach

purée; later workers, using paper chromatography [14] and partition chromatography on silica-gel,[22] extended Corse's observations, and the acid is known to occur (albeit often in very small amount) in the leaves and fruit of other plants. For this work it was isolated by countercurrent distribution of the acidic fraction from prunes, and in poor yield from hops and the leaves of *Betula verrucosa*. The acid crystallised from water as the hemihydrate, and, as with the related 4- and 5-*O*-*p*-coumaroylquinic acid, prolonged heating at an elevated temperature caused lactonisation. The compound from prunes (provided by Dr. J. Corse) was identical with neochlorogenic acid (provided by Dr. J. Corse), and the spectral characteristics and analysis supported its formulation as a monocaffeoylquinic acid. The *p*-coumaroylquinic acids were clearly divisible into two groups on the basis of the comparison of their partition coefficients (*K*) between ethyl acetate and water; the 1- and the 5-isomer (*K* 0·38) had higher water solubility than the 3- and the 4-isomer (*K* 1·75 and 1·1, respectively). A similar comparison between neochlorogenic acid (*K* 0·22) and chlorogenic acid (*K* 1·0) indicated that the former was either 1- or 5-*O*-caffeoylquinic acid, and proton resonance measurements, which showed the characteristic double doublet (τ 6·3; J_1 10, J_2 2 c./sec) and an unresolved band (τ 4·6) whose origin is described above in the case of the *p*-coumaroylquinic acids, were in accord with structure (II; $R^1 = R^2 = R^3 = R^4 = H$, R^5 = caffeoyl) but not with the other possibilities.

Confirmation of this structural assignment was obtained by a synthesis of 5-*O*-caffeoylquinic acid. Treatment of 1-*O*-ethoxycarbonylquinide with 3,4-diacetoxycinnamoyl chloride gave a mixture of esters which after treatment with acid gave 5-*O*-caffeoyl-1-*O*-ethoxycarbonylquinic acid (II; R^1 = ethoxycarbonyl, $R^2 = R^3 = R^4 = H$, R^5 = caffeoyl). Hydrolysis of the latter with water, and isolation of the products by countercurrent distribution, gave 5-*O*-caffeoylquinic acid, identical in all respects with neochlorogenic acid. Preparation of neochlorogenic acid was also readily achieved by isomerisation of chlorogenic acid in sodium hydrogen carbonate solution at 90°; the migration of the caffeoyl group from the 3- to the 5-position on the quinic acid core is presumably analogous to the previously described rearrangement of the *p*-coumaroyl esters. The reverse isomerisation (5 ⟶ 3) was also observed, and this ready interconversion of these esters suggested that neochlorogenic acid might arise in plant extracts as an artefact following migration of the caffeoyl group in chlorogenic acid during isolation. However, model experiments indicated that under similar conditions to those used in the extraction of neochlorogenic acid from prunes chlorogenic acid itself was unchanged. Surveys, by paper chromatographic analysis, of the occurrence of these two acids also support the conclusion that neochlorogenic acid is a naturally occurring isomer of chlorogenic acid; thus, the distribution of phenols in the leaves of the two common species of silver birch, *Betula alba* and *Betula verrucosa*, apart from the occurrence of neochlorogenic acid in the latter but not the former species, is qualitatively identical, and the acid was only isolated from leaves of *Betula verrucosa*.

Barnes, Feldman, and White [7] isolated the amorphous isochlorogenic acid from coffee, and, on the basis of analysis, titration with periodic acid, and the suggestion that the substance was readily lactonised, these authors put forward its structure as 5-*O*-caffeoylquinic acid. Isochlorogenic acid has been isolated from green coffee, and extensive countercurrent distribution between ethyl acetate and water indicated that it was a mixture of at least two closely related compounds, in agreement with the note of Scarpati and Esposito.[8] The major component, which is as yet amorphous, had the correct analysis for a di-*O*-caffeoylquinic acid, and had a partition coefficient between ethyl acetate and water which clearly indicated that it was not 5-*O*-caffeoylquinic acid. On acid hydrolysis it yielded approximately equivalent amounts of chlorogenic and neochlorogenic acid, which therefore favour its formulation as 3,5-di-*O*-caffeoylquinic acid (II; $R^1 = R^2 = R^4 = H$, $R^3 = R^5$ = caffeoyl). Further work is in progress on its constitution.

[22] Sondheimer, *Arch. Biochem. Biophys.*, 1958, **74**, 134.

Experimental

Paper chromatography was carried out in the systems: (A) 6% aqueous acetic acid, and (B) butan-2-ol–acetic acid–water (14 : 1 : 5), at 20 ± 2°. Caffeoyl esters were detected by their blue fluorescence under ultraviolet light, changing to green in the presence of ammonia, and *p*-coumaroyl esters by their blue fluorescence with ammonia in ultraviolet light. Countercurrent distribution was carried out between ethyl acetate and water, and the distribution analysed by measurement of the optical density, at 320 mμ, of aliquots (1 c.c.) (from the upper phase of each tube) diluted with ethanol (10 c.c.). Distribution coefficients were determined from the distribution curves, using the equation [23] $K = N/(n - N)r$, where n is the total number of transfers, N is the maxima on the abscissa, and r is the phase ratio ethyl acetate : water.

Proton resonance spectra were taken at 60 Mc./sec. using acetonitrile as internal standard. Samples of the caffeoylquinic acids were evaporated with deuterated water before solution in the same solvent. Samples of the *p*-coumaroylquinic acids were similarly evaporated, and dissolved in deuterated water containing one equivalent of sodium hydrogen carbonate which had been evaporated similarly with deuterated water.

Chlorogenic acid was isolated as described by Moores, McDermott, and Wood [24] from green coffee, and had R_F(A) 0·60, R_F(B) 0·70, and K (ethyl acetate : water) 1·0.

Periodic Acid Titrations.—Samples (0·2 g.) were dissolved in dioxan (10 c.c.) or water (50 c.c.), and 4% sodium metaperiodate solution (25 c.c.) and 2N-hydrochloric acid (5 c.c.) added. After standing at room temperature for 3 hr. potassium iodide and 5N-hydrochloric acid (5 c.c.) were added, and the liberated iodine estimated using N-sodium thiosulphate. Methyl *p*-coumarate was used as a blank to determine the uptake of periodate by the *p*-coumaroyl group, and under these conditions 1 mole consumed 0·54 mole of periodate.

1-O-(O-*Acetyl*-p-*coumaroyl*)-4,5-O-*isopropylidenequinide.*—A solution of *O*-acetyl-*p*-coumaroyl chloride (9·0 g.) and 4,5-*O*-isopropylidenequinide [25] (7·1 g.) in benzene (40 c.c.) containing pyridine (5 c.c.) was refluxed for 5 hr. washed with ice-cold 2N-hydrochloric acid (30 c.c.), saturated sodium hydrogen carbonate solution (3 × 20 c.c.), and water (20 c.c.), and the solvent removed at 30° after drying (Na_2SO_4). Crystallisation of the residue from ethyl acetate–light petroleum (b. p. 60—80°) gave needles (10·5 g.) of the *quinide*, m. p. 148° (Found: C, 62·5; H, 5·4. $C_{21}H_{22}O_8$ requires C, 62·7; H, 5·5%), $\nu_{max.}$ (Nujol) 1600, 1635, 1760, and 1800 cm.$^{-1}$.

1-O-p-*Coumaroylquinic Acid.*—1-*O*-(*O*-acetyl-*p*-coumaroyl)-4,5-*O*-isopropylidenequinide (4·2 g.), dissolved in acetone (30 c.c.) containing 3N-hydrochloric acid (13·3 c.c.), was heated under reflux for ½ hr., further 3N-hydrochloric acid (13·3 c.c.) was added, and the solution refluxed for 2 hr. After being cooled in ice, the mixture was extracted with ethyl acetate (3 × 30 c.c.) and the extract was washed with ice-cold saturated sodium hydrogen carbonate solution (4 × 20 c.c.). Acidification of the sodium hydrogen carbonate solution (2N-hydrochloric acid), extraction with ethyl acetate (3 × 15 c.c.), and removal of the solvent gave a gum which was subjected to countercurrent distribution between ethyl acetate and water (50 transfers; phase volume 40 c.c.). Concentration of the contents of tubes 2—23 gave a gum which was dissolved in ethyl acetate (10 c.c.), and 1-O-p-*coumaroylquinic acid* (0·9 g.) was precipitated, as an amorphous solid, with light petroleum (b. p. 60—80°) (Found: C, 57·0; H, 5·5. $C_{16}H_{18}O_8$ requires C, 56·8; H, 5·3%), $\nu_{max.}$ (KBr) 1600, 1630, and 1700 cm.$^{-1}$, $[\alpha]_D^{22}$ −5·0° (*c* 2·0 in methanol), R_F(A) 0·78, R_F(B) 0·75, K (ethylacetate : water) 0·38.

1-O-p-*Coumaroyl*-4,5-O-*isopropylidenequinide.*—A solution of 1-*O*-*p*-coumaroylquinic acid (1 g.) in acetone (25 c.c.) containing dry hydrogen chloride (1%) was slowly stirred at room temperature for 2 days, barium carbonate (10 g.) was added, and the stirring continued for a further 3 days. Filtration and removal of the solvent from the filtrate gave a gum which crystallised from aqueous ethanol as needles (0·3 g.) of the *quinide*, m. p. 179° (Found: C, 62·9; H, 5·5. $C_{19}H_{20}O_7$ requires C, 63·3; H, 5·6%), $\nu_{max.}$ (Nujol) 1640, 1710, and 1785 cm.$^{-1}$.

5-O-p-*Coumaroyl*-1-O-*ethoxycarbonylquinic Acid.*—A solution of *O*-acetyl-*p*-coumaroyl chloride (6·9 g.) and 1-*O*-ethoxycarbonylquinide [16] (6·9 g.) in benzene (60 c.c.) containing pyridine (15 c.c.) was refluxed for 3 hr., cooled, diluted with benzene (100 c.c.), washed with ice-cold 2N-hydrochloric acid (3 × 50 c.c.), saturated sodium hydrogen carbonate solution

[23] Craig and Craig, "Technique of Organic Chemistry," Vol. III, Interscience, New York, 1950, p. 207.
[24] Moores, McDermott, and Wood, *Analyt. Chem.*, 1948, **20**, 260.
[25] Fischer, *Ber.*, 1921, **54**, 775.

(3 × 50 c.c.), and water (50 c.c.), and dried ($MgSO_4$). Removal of the benzene gave a gum which was dissolved in acetone (75 c.c.); 4·5N-hydrochloric acid (22 c.c.) was added, and the mixture refluxed for ½ hr., when further 4·5N-hydrochloric acid (22 c.c.) was added and the solution refluxed for 2 hr. After being cooled to 0°, the solution was diluted with water (100 c.c.) and extracted with ethyl acetate (4 × 30 c.c.). Removal of the ethyl acetate gave a gum which was subjected to countercurrent distribution between ethyl acetate and water (40 transfers; phase volume 40 c.c.). Concentration of the contents of tubes 26—36 yielded a gum which crystallised from water to give the *quinic acid* as needles (2·0 g.), m. p. 202° (decomp.) (Found: C, 55·3; H, 5·6; OEt, 10·6. $C_{19}H_{22}O_{10}$ requires C, 55·6; H, 5·4; OEt, 11·0%), $\nu_{max.}$ (Nujol) 1745, 1710, and 1630 cm.$^{-1}$, $[\alpha]_D^{22}$ +36·8° (*c* 0·5 in ethanol), R_F(A) 0·87, R_F(B) 0·88. When titrated with periodate one mole of the compound consumed 1·51 moles of the reagent.

5-O-p-*Coumaroylquinic Acid.*—After being refluxed for 4 hr., a solution of 5-*O*-*p*-coumaroyl-1-*O*-ethoxycarbonylquinic acid (2·75 g.) in water (40 c.c.) was subjected to countercurrent distribution between ethyl acetate and water (40 transfers; phase volume 40 c.c.). Concentration of the contents of tubes 8—16, and crystallisation from water, gave 5-O-p-*coumaroylquinic* acid as prisms (0·9 g.), m. p. 194° (Found: C, 55·1; H, 5·5; OEt, 0·0. $C_{16}H_{18}O_8,\frac{1}{2}H_2O$ requires C, 55·3; H, 5·5; OEt, 0·0%), $\nu_{max.}$ (Nujol) 1715, 1690, and 1630 cm.$^{-1}$, $[\alpha]_D^{19}$ −5·6° (*c* 0·6 in methanol), R_F(A) 0·75, R_F(B) 0·72, *K* (ethyl acetate : water) 0·38.

4- *and* 5-O-p-*Coumaroylquinic Acid.*—To a solution of 1-*O*-ethoxycarbonylquinide [15] (2·5 g.) in chloroform (100 c.c.) containing pyridine (15 c.c.) a solution of *O*-acetyl-*p*-coumaroyl chloride (2·5 g.) in chloroform (25 c.c.) was added during 1 hr. The mixture was left at room temperature for 15 hr., diluted with chloroform (200 c.c.), and extracted (organic layer) with ice-cold 2·5N-hydrochloric acid (2 × 100 c.c.), saturated sodium hydrogen carbonate solution (2 × 100 c.c.), and water (100 c.c.). After drying ($MgSO_4$) and removal of the chloroform, the resultant gum was dissolved in 80% acetic acid (75 c.c.) and the solution refluxed for 24 hr. Removal of the solvent gave a further gum, which was dissolved in acetone (100 c.c.); 2N-ammonium hydroxide (50 c.c.) was added and the solution left at 0° for 48 hr., when paper chromatographic analysis showed it to contain 5-*O*-*p*-coumaroylquinic [R_F(A) 0·75, R_F(B) 0·72], 4-*O*-*p*-coumaroylquinic [R_F(A) 0·68, R_F(B) 0·76], and *p*-coumaric acid [R_F(A) 0·53, R_F(B), 0·92]. The solution was concentrated to 50 c.c. at 25°, acidified with N-sulphuric acid, and extracted with ethyl acetate (6 × 100 c.c.). The product remaining on removal of the ethyl acetate was subject to countercurrent distribution (50 transfers; phase volume 40 c.c.). Concentration of tubes 8—15, followed by crystallisation from water, gave 5-*O*-*p*-coumaroylquinic acid (0·50 g.), m. p. and mixed m. p. 193—194°. Concentration of tubes 20—30 and crystallisation from water gave, as plates (0·32 g.), 4-*O*-*p*-coumaroylquinic acid, m. p. 192—193° (decomp.) (Found: C, 55·6, 55·5; H, 5·7, 5·4. $C_{16}H_{18}O_8,\frac{1}{2}H_2O$ requires C, 55·3; H, 5·5%), $\nu_{max.}$ (Nujol) 1710, 1690, and 1630 cm.$^{-1}$, $[\alpha]_D^{20}$ −47·3° (*c* 1·4 in methanol), R_F(A) 0·68, R_F(B) 0·75, *K* (ethyl acetate : water) 1·1.

4,5-O-*Isopropylidene*-1-O-*methylquinic Acid.*—N-Sodium hydroxide (52·8 c.c.) was added to a solution of 4,5-*O*-isopropylidene-1-*O*-methylquinide (12 g.) in acetone (50 c.c.) and the mixture maintained at 50° for 1 hr., cooled to 0°, and N-sulphuric acid (52·0 c.c.) added. The aqueous solution was extracted with ethyl acetate (4 × 50 c.c.) and the extract dried ($MgSO_4$). Removal of the solvent and crystallisation of the residue from ethyl acetate–light petroleum gave the *acid* as needles (9 g.), m. p. 115° (Found: C, 53·6; H, 7·3. $C_{11}H_{18}O_6$ requires C, 53·7; H, 7·3%), $\nu_{max.}$ (Nujol) 1700 cm.$^{-1}$.

Diphenylmethyl 4,5-O-*Isopropylidene*-1-O-*methylquinate.*—Diphenyldiazomethane (9·6 g.) and 4,5-*O*-isopropylidene-1-*O*-methylquinic acid (9·0 g.) were refluxed in chloroform (300 c.c.) for 6 hr.; removal of the solvent and crystallisation of the residue from light petroleum (b. p. 60—80°) gave the *diphenylmethyl ester* as needles (11 g.), m. p. 102° (Found: C, 69·4; H, 6·8. $C_{24}H_{28}O_6$ requires C, 69·9; H, 6·8%), $\nu_{max.}$ (Nujol) 1725 cm.$^{-1}$.

Diphenylmethyl 3-O-(O-*Ethoxycarbonyl*-p-*coumaroyl*)-4,5-O-*isopropylidene*-1-O-*methylquinate.*—To the above ester (10 g.) in benzene (100 c.c.) containing pyridine (10 c.c.) was added *O*-ethoxycarbonyl-*p*-coumaroyl chloride (6·8 g.) and the mixture refluxed for 6 hr. After being cooled in ice the benzene solution was washed with 2N-hydrochloric acid (3 × 20 c.c.), sodium hydrogen carbonate solution (2 × 20 c.c.), and water (20 c.c.), and dried ($MgSO_4$). The oil remaining after removal of the solvent was crystallised from ethyl acetate–light petroleum (b. p. 60—80°), to yield the *quinate* as needles (12·4 g.), m. p. 87° (Found: C, 68·2; H, 6·3. $C_{36}H_{38}O_{10}$ requires C, 68·6; H, 6·1%), $\nu_{max.}$ (Nujol) 1630, 1710, 1725, and 1750 cm.$^{-1}$.

Diphenylmethyl 3-O-p-*Coumaroyl*-4,5-O-*isopropylidene*-1-O-*methylquinate*.—N-Sodium hydroxide (2·2 c.c.) was added dropwise to a solution of the foregoing quinate (1·26 g.) in ethanol (10 c.c.) at 40°. After ½ hr., ice–water (30 c.c.) and N-sulphuric acid (2·2 c.c.) were added, and the precipitate collected, washed with water until free from acid, and crystallised from ethyl acetate–light petroleum (b. p. 60—80°). The *quinate* formed needles (1·0 g.) m. p. 202° (decomp.) (Found: C, 70·9; H, 6·4. $C_{33}H_{54}O_8$ requires C, 71·0; H, 6·2%), $\nu_{max.}$ 1730, 1707, and 1633 cm.$^{-1}$.

3-O-p-*Coumaroyl*-1-O-*methylquinic Acid*.—A solution of the above ester (3·5 g.) in 80% aqueous acetic acid (35 c.c.) was refluxed for 6 hr.; the solvent was removed at 30°, and the solid residue extracted with ice-cold sodium hydrogen carbonate solution (50 c.c.). Acidification of the sodium hydrogen carbonate solution (N-hydrochloric acid), collection of the precipitate, and recrystallisation from water gave the *quinic acid* as needles (1·2 g.), m. p. 248° (Found: C, 57·5; H, 5·8. $C_{17}H_{20}O_8$ requires C, 58·0; H, 5·7%), $\nu_{max.}$ (Nujol) 1630, 1690, and 1720 cm.$^{-1}$, $[\alpha]_D^{22}$ −47·5° (*c* 0·5 in ethanol), *K* (ethyl acetate : water), 3·4.

4-O-(O-*Ethoxycarbonyl*-p-*coumaroyl*)-1-O-*methylquinide*.—A solution of *O*-ethoxycarbonyl-*p*-coumaroyl chloride (3·45 g.) in chloroform (30 c.c.) was added during 1 hr. to a solution of 1-*O*-methylquinide (2·55 g.) in the same solvent (20 c.c.) containing pyridine, and the mixture set aside at room temperature for 18 hr., and washed with ice-cold 2N-hydrochloric acid (20 c.c.), saturated sodium hydrogen carbonate solution (20 c.c.), and water. After drying (Na_2SO_4), removal of the chloroform gave a gum which crystallised from benzene–light petroleum (b. p. 60—80°), to give the *ester* as prisms (2·88 g.), m. p. 127° (Found: C, 59·0; H, 5·6. $C_{20}H_{22}O_9$ C, 59·1; H, 5·5%), $\nu_{max.}$ (Nujol) 1775, 1745, and 1715 cm.$^{-1}$, $[\alpha]_D^{20}$ +21·8° (*c* 0·8 in ethanol).

Treatment of the above ester (4·0 g.) in aqueous acetone solution (1 : 1; 50 c.c.) with N-sodium hydroxide (40 c.c.) during ½ hr., followed by acidification (H_2SO_4) and extraction with ethyl acetate (2 × 50 c.c.), gave a gum which crystallised from water as needles (3·0 g.), m. p. 248° undepressed on admixture with 3-*O*-*p*-coumaroyl-1-*O*-methylquinic acid.

4-O-p-*Coumaroyl*-1-O-*methylquinic Acid*.—A solution of 4-*O*-(*O*-ethoxycarbonyl-*p*-coumaroyl)-1-*O*-methylquinide (2·5 g.) was refluxed in aqueous acetic acid (33%; 100 c.c.) for 24 hr.; removal of the solvent gave a gum which was subjected to countercurrent distribution between ethyl acetate and water (50 transfers; phase volume (40 c.c.). Concentration of the contents of tubes 31—37 gave a gum which crystallised from water, to give the *quinic acid* (0·95 g.) as plates, m. p. 193—194° (Found: C, 58·1; H, 5·9. $C_{17}H_{20}O_8$ requires C, 58·0; H, 5·7%), $\nu_{max.}$ (Nujol) 1635 and 1700 cm.$^{-1}$, $[\alpha]_D^{21}$ −44·1° (*c* 1·0 in ethanol), *K* (ethyl acetate : water) 2·3.

4-O-p-*Coumaroyl*-1-O-*methylquinide*.—A solution of the above acid (0·5 g.) in acetic acid (1·0 c.c.) was heated at 90° for 3 hr. Crystallisation of the residue from acetone–water gave the *lactone* as glistening plates (0·25 g.), m. p. 209—210° (Found: C, 60·9; H, 5·7. $C_{17}H_{18}O_7$ requires C, 61·1; H, 6·0%), $\nu_{max.}$ (Nujol) 1785, 1710, and 1635 cm.$^{-1}$.

4,5-*Di*-O-(O-*acetyl*-p-*coumaroyl*)-1-O-*ethoxycarbonylquinide*.—A mixture of 1-*O*-ethoxycarbonylquinide (4·9 g.) and *O*-acetyl-*p*-coumaroyl chloride (11·2 g.) dissolved in benzene (100 c.c.) containing pyridine (12 c.c.) was refluxed for 24 hr., diluted with benzene (500 c.c.), and the organic layer washed separately with 2N-hydrochloric acid (3 × 50 c.c.), sodium hydrogen carbonate solution (3 × 50 c.c.), and water (50 c.c.), and dried ($MgSO_4$). Removal of the solvent gave the *ester* as prisms (10·5 g.), m. p. 172° (Found: C, 61·7; H, 5·0. $C_{32}H_{30}O_{13}$ requires C, 61·7; H, 4·8%), $\nu_{max.}$ (KBr) 1790, 1750, 1710, and 1630 cm.$^{-1}$

4,5-O-*Benzylidenequinide*.—Quinide (16·2 g.), benzaldehyde (40 c.c.), and powdered zinc chloride (13·3 g.) were stirred together at 25° for 48 hr. before dilution with ethyl acetate (250 c.c.). The solution was washed successively with 10% sodium hydrogen sulphite solution (4 × 100 c.c.), sodium hydrogen carbonate (50 c.c.), and water (3 × 30 c.c.), and dried ($MgSO_4$). Removal of the solvent, and crystallisation of the residue from benzene–light petroleum, gave the *quinide* as prisms (16·8 g.), m. p. 94° (Found: C, 63·9; H, 5·3. $C_{14}H_{14}O_5$ requires C, 64·1; H, 5·4%).

1-O-(O-*Acetyl*-p-*coumaroyl*)-4,5-O-*benzylidenequinide*.—A solution of *O*-acetyl-*p*-coumaroyl chloride (5·7 g.) and 4,5-*O*-benzylidenequinide (5 g.) in benzene (50 c.c.) containing pyridine (10 c.c.) was refluxed for 16 hr., washed with 2N-hydrochloric acid (3 × 50 c.c.), saturated sodium hydrogen carbonate solution (3 × 50 c.c.), and water (50 c.c.), and dried ($MgSO_4$). Crystallisation of the residue, after removal of the benzene, from methanol gave needles (5·4 g.) of the *ester*, m. p. 146° (Found: C, 66·2; H, 4·7. $C_{25}H_{22}O_8$ requires C, 66·6; H, 4·9%), $\nu_{max.}$ 1633, 1710, 1766, and 1810 cm.$^{-1}$.

Treatment of the above ester (8·7 g.) with 2N-hydrochloric acid (32 c.c.) in acetone (60 c.c.) at 60° for 1 hr., followed by removal of the solvents, gave a gum which was heated at 45° for 2 hr. with saturated sodium hydrogen carbonate solution (30 c.c.). Acidification of the solution and extraction with ethyl acetate (3 × 25 c.c.) gave a gum which was subjected to countercurrent distribution between ethyl acetate and water (40 transfers; phase volume 40 c.c.). Tubes 8—20 gave the amorphous 1-*O*-*p*-coumaroylquinic acid (0·3 g.), characterised as the isopropylidene lactone, m. p. and mixed m. p. 177°, as described above. Tubes 26—32 gave 3-*O*-*p*-coumaroylquinic acid (0·5 g.), m. p. and mixed m. p. 246—247° (from water).

Action of Sodium Hydrogen Carbonate Solution on the Individual p-*Coumaroylquinic Acids.*—The acid (0·5 g.) and saturated sodium hydrogen carbonate solution (5 c.c.) were heated at 90° for 30 min., and the solution was acidified (2N-sulphuric acid) and extracted with ethyl acetate (6 × 30 c.c.). After removal of the solvent the product was subjected to countercurrent distribution between ethyl acetate and water (120 transfers; ethyl acetate phase 10 c.c., aqueous phase 15 c.c.). After analysis of the distribution, and from a knowledge of the distribution coefficients (K) of the isomeric acids, the appropriate tubes were concentrated and the products isolated, crystallised, and identified by m. p. and mixed m. p. The results are discussed on p. 2140.

Isolation of Neochlorogenic Acid.—Californian prunes (1500 g.), after removal of the stones, were crushed with 70% propan-2-ol (10 l.) in a Kenwood mixer, and the extract filtered through fine muslin and then through cellulose in a Buchner funnel, to give a clear pale brown solution. Removal of the solvent at 30° gave a brown gum which was dissolved in saturated sodium hydrogen carbonate solution (20 c.c.) and extracted with ethyl acetate (10 × 20 c.c.). The sodium hydrogen carbonate solution was then acidified (N-H_2SO_4) and extracted with ethyl acetate (15 × 50 c.c.). Removal of the solvent gave a brown gum (3·0 g.) which paper chromatography showed to contain predominantly chlorogenic acid, R_F(A) 0·60, R_F(B) 0·70, and neochlorogenic acid, R_F(A) 0·65, R_F(B) 0·62. The gum was subjected to countercurrent distribution between ethyl acetate and water (60 transfers; phase volume 40 c.c.) and the contents of the tubes analysed in solvent system B. Tubes 5—12 gave neochlorogenic acid as needles (0·25 g.), m. p. 210° (from water) (lit.,[8] 204—206°) (Found, on drying for 24 hr. at 100°/0·2 mm.: C, 52·9; H, 5·2. Calc. for $C_{16}H_{18}O_9,\frac{1}{2}H_2O$: C, 52·9; H, 5·0%). Found, on drying for 30 hr. at 125°/0·2 mm.: C, 54·3; H, 5·2. Calc. for $C_{16}H_{18}O_9$: C, 54·2; H, 5·1%), $\nu_{max.}$ (Nujol) 1630, 1690, and 1705 cm.$^{-1}$, $[\alpha]_D^{21}$ +1·8° (*c* 0·6 in MeOH), R_F(A) 0·65, R_F(B) 0·62. Tubes 25—33 gave chlorogenic acid (0·35 g.), m. p. and mixed m. p. 208° (from water) (lit.,[24] 208°) (Found, on drying for 24 hr. at 100°/0·2 mm.: C, 53·3; H, 5·1. Calc. for $C_{16}H_{18}O_9,\frac{1}{2}H_2O$: C, 52·9; H, 5·0%). Found, on drying for 30 hr. at 135°/0·2 mm.: C, 54·0; H, 5·1. Calc. for $C_{16}H_{18}O_9$: C, 54·2; H, 5·2%, $\nu_{max.}$ (Nujol) 1630 and 1700 cm.$^{-1}$, $[\alpha]_D^{20}$ −29·1° (*c* 1·2 in MeOH), R_F(A) 0·60, R_F(B) 0·70.

Interconversion of Chlorogenic and Neochlorogenic Acids.—The acid (0·3 g.) was heated at 90° in saturated sodium hydrogen carbonate solution (5 c.c.) for 30 min. On cooling, the mixture was acidified (N-H_2SO_4) and extracted with ethyl acetate (8 × 25 c.c.). The extract was separated by countercurrent distribution (75 transfers; phase volume ethyl acetate 10 c.c., water 15 c.c.). Tubes 2—10 and 23—35 gave neochlorogenic acid and chlorogenic acid, respectively, undepressed in m. p. (from water) on admixture with authentic samples.

5-O-*Caffeoyl*-1-O-*ethoxycarbonylquinic Acid.*—3,4-Di-*O*-acetylcaffeoyl chloride (5·1 g.) was added in portions during ¼ hr. to a refluxing solution of 1-*O*-ethoxycarbonylquinide (4·0 g.) in benzene (50 c.c.) containing pyridine (7 c.c.), and the heating continued for 3 hr. After it had cooled, the mixture was washed with ice-cold 2N-hydrochloric acid (3 × 20 c.c.), sodium hydrogen carbonate solution (3 × 20 c.c.), and water (20 c.c.), and dried ($MgSO_4$). On removal of the solvent the residual gum (7·2 g.) was dissolved in acetone (50 c.c.), 4N-hydrochloric acid (17 c.c.) was added, and the mixture refluxed for 2 hr., diluted with water (75 c.c.), and extracted with ethyl acetate (5 × 50 c.c.). The extract was separated into acidic and neutral portions with sodium hydrogen carbonate solution, and the former fraction subjected to countercurrent distribution between ethyl acetate and water (40 transfers; phase volume 40 c.c.). Tubes 20—30 gave a gum which was freeze-dried from water to give the *quinic acid* as a white amorphous powder (1·25 g.) (Found: C, 53·3; H, 5·4; OEt, 10·8. $C_{19}H_{22}O_{11}$ requires C, 53·5; H, 5·2; OEt, 10·6%), $\nu_{max.}$ (Nujol) 1740, 1710, and 1630 cm.$^{-1}$, $[\alpha]_D^{22}$ +27·5° (*c* 0·7 in ethanol).

5-O-*Caffeoylquinic Acid.*—A solution of 5-*O*-caffeoyl-1-*O*-ethoxycarbonylquinic acid (0·6 g.) in water (30 c.c.) was refluxed for 3 hr. and then subjected to countercurrent distribution

between ethyl acetate and water (40 transfers; phase volume 40 c.c.). Tubes 2—12 gave 5-*O*-caffeoylquinic acid as prisms (0·12 g.), m. p. (from water) and mixed m. p. with neochlorogenic acid 210° (Found: C, 53·1; H, 5·2; OEt, 0·0. Calc. for $C_{16}H_{18}O_9,\frac{1}{2}H_2O$: C, 52·9; H, 5·0%), $\nu_{max.}$ (Nujol) 1630, 1690, and 1705 cm.$^{-1}$, $[\alpha]_D^{20}$ +2·0° (*c* 0·9 in MeOH), *K* (ethyl acetate : water) 0·22, R_F(A) 0·63, R_F(B) 0·62.

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