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3-FERULOYLQUINIC ACID

A 3′-METHYL ETHER OF CHLOROGENIC ACID

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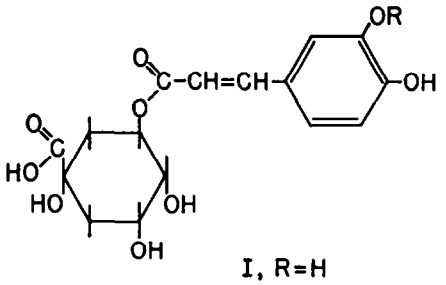
ALTHOUGH the chlorogenic acids are among the most widely occurring aromatic compounds in plants, the analogous feruloylquinic acids have been observed only on paper chromatograms.[2] This is surprising in view of the common occurrence of 4-hydroxy-3-methoxyphenylpropyl derivatives in lignins,[3] ketones, flavanoids and sugar esters[4] (not isolated) and a host of other natural products.

In the course of preparing isochlorogenic acid[5] by 200-tube countercurrent distribution for studies on purification and structure,[6] we were able to recover substantial amounts of a new ester which is one of the three feruloylquinic acids described by Pictet and Brandenberger. Like the chlorogenic acids, there should be four feruloylquinic acids possible. The correspondence between the several chlorogenic acids and their feruloyl analogs will remain in doubt for some while, since the chlorogenic acid isomer question itself is not clear. Chlorogenic acid[7] is known to be 3-caffeoylquinic acid, I.

The structures of the other reported isomers, neochlorogenic acid,[8] isochlorogenic acid,[6] pseudochlorogenic acid[9] and "Band 510"[10] are either not known or may be seriously questioned. In fact, there is some doubt as to one or more of them being



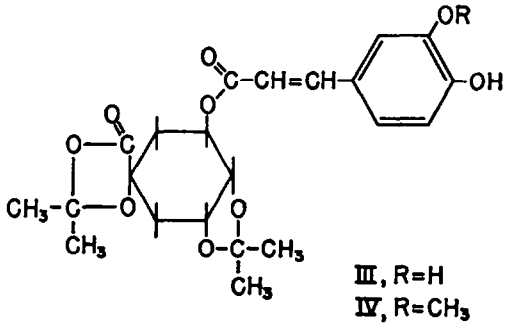
I, R=H
II, R=CH_3

[1] Syracuse University, New York.
[2] G. Pictet and H. Brandenberger, *J. Chromatog.* **4**, 396 (1960).
[3] K. Freudenberg, *Nature, Lond.* **183**, 1152 (1959).
[4] J. B. Harborne and J. J. Corner, *Arch. Biochem. Biophys.* **92**, 191 (1961); *Biochem. J.* **81**, 242 (1961).
[5] J. Corse and R. Bean, *Plant Phenolic Group Symposium on Chlorogenic Acids*, London, September 25 (1957).
[6] H. M. Barnes, J. R. Feldman and W. V. White, *J. Amer. Chem. Soc.* **72**, 4178 (1950).
[7] H. O. L. Fischer and G. Dangschat, *Ber. Dtsch. Chem. Ges.* **65**, 1037 (1932).
[8] J. Corse, *Nature, Lond.* **172**, 771 (1953).
[9] I. Uritani and M. Miyano, *Nature, Lond.* **175**, 812 (1955).
[10] E. Sondheimer, *Arch. Biochem. Biophys.* **74**, 131 (1958).

pure compounds. Certainly there cannot be five positional isomeric chlorogenic acids and only four hydroxyl groups on quinic acid.

Treatment of the feruloylquinic acid with acetone and zinc chloride[7] led to the formation of an amorphous diacetone compound. This derivative alone is almost sufficient evidence to assign structure IV to the compound, since of the four feruloylquinic esters possible, only the 3-feruloyl derivative, II, could be expected to form a diacetone compound under these conditions.[11] The 5-feruloyl ester would have to form a *trans*-isopropylidene linkage.

The proton magnetic resonance (PMR) spectrum (60·0 Mcs) of a solution of this diacetone compound in deuterochloroform containing 1%. tetramethylsilane (TMS) as an internal reference showed one aromatic methoxyl methyl ($\tau = 6{\cdot}10$)* and four ketal methyl groups ($\tau = 8{\cdot}37$ (2), 8·45, 8·63). The 3-ester is further supported by the PMR spectrum of a saturated deuterochloroform solution of the diacetone compound of chlorogenic acid, III. The positions and shapes of the cyclohexyl proton multiplets and the four ketal methyl peaks correspond almost identically to those obtained from the diacetone compound of feruloylquinic acid, IV. It seems very doubtful that such close agreement would exist if the ester linkage in II differed from the 3-linkage in chlorogenic acid I. However, a complete analysis of the cyclohexyl spin multiplets, which would definitely establish this point has not yet been carried out. The slight difference in shielding of two of the ketal methyls is almost certainly caused by the proximity of the ketal carbonyl group in the ketal ester rings of III and IV.



III, R=H
IV, R=CH3

Fischer and Dangschat[7] methylated chlorogenic acid with diazomethane followed by silver oxide-methyl iodide treatment to yield a glassy pentamethyl ether of chlorogenic acid methyl ester. This was saponified to 1,4,5-trimethoxyquinic acid which formed the lactone, V, on distillation. Treatment of this lactone with ammonia gave the amide, VI. Methylation, saponification and ammonolysis of the feruloylquinic acid gave the identical amide as shown by mixed melting point and comparison of their PMR and IR spectra. Thus, the structure of the new ester has been shown conclusively to be 3-feruloylquinic acid, II.

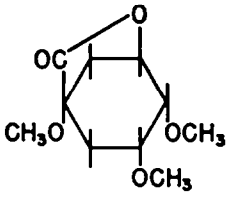
EXPERIMENTAL

M.p.s. were taken on a Kofler stage and are corrected.

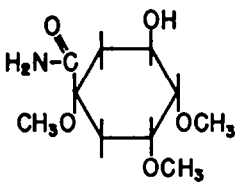
Isolation of 3-feruloylquinic acid. Finely ground, unroasted coffee (1000 g) was stirred for 4 hr

* Measured in p.p.m. from TMS which is assigned the value 10.00. Values increase with the magnetic field.

[11] W. R. Christian, C. J. Gogek and C. B. Purves, *Canad. J. Chem.* **29**, 911 (1951).



V



VI

with 6·1 of 70% isopropanol and filtered. The filtrate was concentrated under red. press. to a volume of 1200 ml and stored overnight at 1°. The resulting waxy precipitate was removed by filtration through a filteraid bed and the filtrate was again concentrated under vacuum, this time to a volume of 150 ml. The clear brown solution was acidified with 6 N HCl to pH 2·0 and made up to 300 ml with 10% sodium chloride solution. This solution was then divided into 100 ml portions and filled in the first 3 tubes of a 100-tube Craig countercurrent distribution apparatus[12] which holds 100 ml of each phase of solvent in every tube. Salt solution containing 5 ml conc HCl/l. (10%) equilibrated with ethyl acetate was used as the lower layer and the ethyl acetate thus treated was used as the upper phase. "Single withdrawal" technique[12] was used in this apparatus, and the fraction was collected between transfers 150–170 as 3-feruloylquinic acid. Isochlorogenic acid is in the first 10 tubes withdrawn; neochlorogenic acid is in tubes 20–45 and a mixture of chlorogenic acid and "Band 510" is in tubes 50–110. The combined feruloylquinic acid tubes were evaporated under red. press. to a volume of about 100 ml (considerable salt had precipitated) and a 4-fold volume of isopropanol added. The salt was removed by filtration and the filtrate again concentrated. The isopropanol precipitation was repeated until the sodium chloride was wholly removed. The light tan gum finally obtained was dissolved in ethyl acetate and pet ether added to turbidity. The yield of crystalline product varied with the source of the coffee. As indicated by Pictet and Brandenberger[2], "Robusta" variety was particularly rich, and yielded about 1 g per run; the Brazilian variety we had available for testing contained much smaller quantities. Recrystallization from ethyl acetate–pet ether gave colorless crystals, m.p. 196–197°; $[\alpha]_D^{25}$ −42·8° (ethanol); λ_{max} (ethanol) 325 mμ, E = 19, 200. (Found: C, 55·4; H, 5·59; equiv. wt. (titration), 375. Calc. for $C_{17}H_{20}O_9$:C, 55·4; H, 5·47%; equiv. wt., 368). The distribution ratio for 10% salt-ethyl acetate at pH 2·0 is 2·7; R_f on Whatman No. 1 paper, 2% acetic acid, 0·61; R_f, butanol–acetic acid–water (4:1:2·2), 0·74.

Saponification with potassium hydroxide gave an 80% yield of ferulic acid, m.p. 167–169°. The acidified aqueous residues contained a mixture of quinic acid and its lactone as shown by paper chromatography (two spots with butanol–acetic acid–water and a single spot with isopropanol–ammonia–water when sprayed with the modified Edward-Waldron spray).[13]

Crystalline quinic acid was separated by saponification of the mixture and keeping the reaction products cold during acidification, concentration and extraction with isopropanol; m.p. 162–163°; no lowering with an authentic specimen.

Diacetone compound (IV). In a similar manner to that described by Fischer and Dangschat[7], 0·5 g 3-feruloylquinic acid, 20 ml acetone and 3 g freshly fused zinc chloride was mixed and allowed to stand 3 days at room temp. The reaction mixture was poured into 100 ml ice water and 100 ml chloroform. A troublesome precipitate formed which could be removed by centrifugation. This did not occur in chlorogenic acid reactions. The chloroform extract was washed with water and dried Evaporation left 125 mg of amorphous product which did not crystallize. It was purified by precipitation from benzene solution by the addition of pet ether. (Found: C, 61·6; H, 6·71. Calc. for $C_{23}H_{30}O_9$:C, 61·60; H, 6·29).

Methylation of chlorogenic and feruloylquinic acids. A solution of 1·5 g feruloylquinic acid in 10 ml methanol was treated at 0° with an excess of ethereal diazomethane by allowing reaction to proceed for 20 min.[7] The excess diazomethane and solvents were removed *in vacuo* and 14 g silver oxide, 24 ml methyl iodide and 10 ml pure dioxane were added to the residue. After refluxing overnight, the silver salts were removed, washed well with methanol, and the combined filtrate and

[12] L. C. Craig and D. Craig, *Technique of Organic Chemistry*, (Edited by A Weissberger) Chap. IV; Vol. III. Interscience, New York (1950).

[13] R. A. Cartwright and E. A. Roberts, *Chem. & Ind.* 230 (1955).

washings again evaporated to dryness. The resulting tan gum was dissolved in 30 ml warm methyl iodide, 15 g silver oxide was added and the mixture was again refluxed for 18 hr. The silver salts were removed by filtration, washed with methanol and the combined filtrate and washings concentrated and distilled; b.p. 180–185° at 0·012 mm; yield 1·14 g of light yellow gum. Although Fischer and Dangschat obtained a satisfactory elementary analysis for this compound (prepared from chlorogenic acid), PMR spectra showed our compound to be impure, as was our chlorogenic acid-derived product. There was indication that the diazomethane treatment had caused some side reaction with the conjugated double bond. It has been further observed in similar reactions that silver oxide can cleave esters; in this case methyl 3,4-dimethoxycinnamate and methyl 0-tetramethylquinate would have been formed. The crude distillation could not be expected to fractionate them. These secondary products, however, were eliminated in the subsequent saponification and lactonization steps.

0,0,0,-1,4,5-*Trimethylquinic acid amide* (VI). A mixture of 0·75 g of the above methyl 0-pentamethylchlorogenate from the feruloylquinic acid in 5 ml methanol and 10 ml N NaOH was heated at 50° for 4 hr. The resulting solution was cooled and acidified with hydrochloric acid, which precipitated 0·31 g 3,4-dimethoxycinnamic acid (88%). The filtrate was extracted twice with equal volumes of ether (which were discarded) and then evaporated to dryness *in vacuo*. The residue was extracted with isopropanol and the salt filtered off. The filtrate was again evaporated and treated with isopropanol to remove the small amount of salt remaining. Vacuum concentration left an oil 1,4,5-trimethoxyquinide, V, which was distilled; b.p. 110–115°/0·016 mm (yield 0·20 g). The quinide was dissolved in 4 ml methanol, cooled to 0° and saturated with dry ammonia. After standing overnight at room temp the excess ammonia and solvent were evaporated. The residue was sublimed *in vacuo* and recrystallized from ethyl acetate–pet ether; yield 0·13 g of 0,0,0-1,4,5-trimethoxylquinic acid amide, VI; m.p. 125–126°.

Saponification of a sample of methyl 0-pentamethylchlorogenate prepared from chlorogenic acid, followed by distillation of the trimethoxyquinide and amination as above, gave the identical amide, m.p. 125–126°, no lowering on admixture of the two. The IR spectra of the two amide samples (in $CHCl_3$) was completely superimposable; characteristic maxima 5·98 μ, 9·20 μ, 9·73 μ.

PMR spectra of dil.deutero-chloroform solutions of the two amides were also superimposable. No attempt was made to completely analyze the spectrum, but all peaks appeared to be consistent with the assigned structure. Two broad (approximately 11 c.p.s.) peaks at low field ($\tau - 3{\cdot}20$) and 3·71) could be assigned to the amide protons both on the basis of position and shape. These two peaks arise from a difference in the magnetic environment of the two protons which occurs because of the absence of rotational averaging about the carbon-nitrogen bond due to its double-bond character.

An exceedingly broad hydroxyl peak occurs at 5·29 which was identified by the sensitivity of its position and shape to trace amounts of acid which were added to the sample. A series of incompletely resolved multiplets of low intensity in the region from 5·75 to 6·75 can be assigned to the protons on carbon atoms 3, 4 and 5 which also bear oxygen atoms. Three sharp methoxy proton resonances occur at 6·49, 6·60 and 6·71. A moderately strong band occurs in the 7·58 to 8·30 region, with its center at 7·95 which can be attributed to the two pairs of ring-methylene protons both on the basis of position and the band's general resemblance to two overlapping "AB" portions of ABX multiplets.

We wish to thank Miss Geraldine Secor and Mr. Lawrence White for the microanalyses and Mr. Dennis Patterson for his technical help.