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CONFORMATION ANALYSES OF D-(−)-QUINIC ACID AND SOME OF ITS DERIVATIVES BY NUCLEAR MAGNETIC RESONANCE

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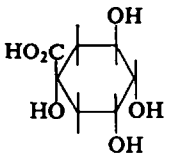
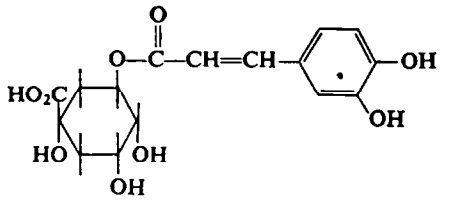
Abstract—D-(−)-Quinic acid, eight monoacyl esters, three diacyl esters, and 5-dehydroquinic acid have been examined by NMR spectroscopy and shown to exist in solution in a chair conformation with the carboxyl equatorial. The effects of this conformation on chemical and physical properties are discussed.

INTRODUCTION

D-(−)-QUINIC acid was shown to be a 3/145 tetrahydroxycyclohexane carboxylic acid by Fischer and Dangschat.[1] They later demonstrated, by conversion of D-(−)-quinic acid into shikimic acid and degradation of shikimic acid, that D-(−)-quinic acid had the configuration

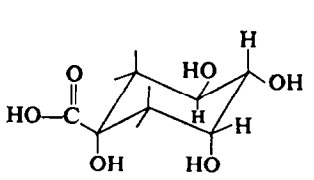


(I)



(II)

(I)‡ and not that of its mirror image.[3,4] In the course of their work chlorogenic acid, (II), was unequivocally determined to be 3-caffeoylquinic acid.[5]



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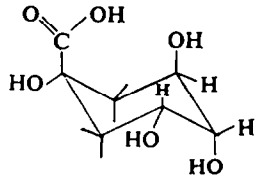

(IV)

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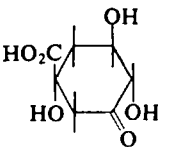
‡ This isomer may be named (1R:3R:4S:5R)-3/145-tetrahydroxycyclohexane-1-carboxylic acid by the Sequence Rule.[2]

[1] H. O. L. FISCHER and G. DANGSCHAT, *Ber. Deut. Chem. Ges.* **65B**, 1009 (1932).
[2] R. S. CAHN, C. K. INGOLD and V. PRELOG, *Experientia* **12**, 81 (1956).
[3] G. DANGSCHAT and H. O. L. FISCHER, *Naturwissenschaften* **26**, 562 (1938).
[4] K. R. HANSON, *J. Chem. Educ.* **39**, 419 (1962).
[5] H. O. L. FISCHER and G. DANGSCHAT, *Ber. Deut. Chem. Ges.* **65B**, 1037 (1932).

Quinic acid can exist in two chair conformations, (III) and (IV) (equatorial (e) and axial (a), CO_2H respectively), and a number of boat conformations. Boat conformations in general are less stable than the chair conformers and in quinic acid are much less favourable because of steric hindrance of the functional groups. Orloff[6] proposed the (a) CO_2H structure for quinic acid (IV), but the (e) CO_2H conformer (III) for chlorogenic acid. On the basis of the steric effect of the large carboxyl group, Heyns and Gottschalck[7] proposed the (e) CO_2H conformation of quinic acid also favoured by Hanson.[4] More recently, McCasland and coworkers[8] have shown that application of Brewster's calculation to the (e) CO_2H conformation yields excellent agreement between predicted and observed molecular rotations. Inoue *et al.*[9] on the basis of NMR spectra assigned the (e) CO_2H conformation, although the H_3 and H_5 proton positions were ambiguous in their spectrum. Oxidation of quinic acid by nitric acid,[10] air and platinum[7,11] or bacteria[12] gives 5-dehydroquinic acid (V). This result



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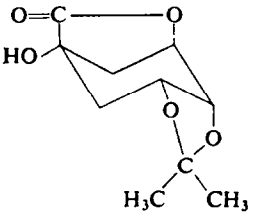

(VI)

supports the (e) CO_2H conformation, in which the 5-hydroxyl is axial, since equatorial hydroxyls are not oxidized under these conditions. However, the ready formation of 3-lactones, for example, acetonequinide, (VI), led Orloff[6] to favour the (a) CO_2H conformer. The present work, using high resolution NMR spectrometry both at 60 Mc/s and 100 Mc/s, was undertaken to clarify the conformation question on which the interpretations of the structures of the chlorogenic acids depended. It is shown without doubt that quinic acid and its acyl derivatives studied all exist in solution as a chair with the carboxyl group equatorial (III).

RESULTS AND DISCUSSION

In the NMR spectrum of quinic acid (Fig. 1) three protons absorb in the region $\tau = 5{\cdot}0$–$6{\cdot}2$.* Since substitution of a hydroxyl group for a proton is known to shift the resonance of the remaining proton on the same carbon 2·4 ppm downfield, these absorption bands undoubtedly arise from the hydrogen atoms on carbons 3, 4, and 5. From the dependence of spin–spin coupling constants on dihedral angles in six-membered rings,[13] it is possible to predict the hyperfine structure of H_3, H_4, and H_5 for the two chair conformers of quinic acid (Fig. 2). The value of 9 c/s is assigned to the coupling of *trans*-diaxial protons that have a

* Measured in ppm from internal tetramethylsilane which is assigned the position of 10·00. Values increase with magnetic field.

[6] H. ORLOFF, *Chem. Rev.* **54**, 347 (1954).
[7] K. HEYNS and H. GOTTSCHALCK, *Chem. Ber.* **94**, 343 (1961).
[8] G. E. MCCASLAND, S. FURUTA, L. F. JOHNSON and J. N. SHOOLERY, *J. Org. Chem.* **29**, 2354 (1964).
[9] Y. INOUE, S. AOYAGI and K. NAKANISHI, *Chem. Pharm. Bull. (Tokyo)* **13**, 101 (1965).
[10] R. GREWE and J. P. JESCHKE, *Chem. Ber.* **89**, 2080 (1956).
[11] E. HASLAM, R. D. HAWORTH and P. F. KNOWLES, *J. Chem. Soc.* 1854 (1960).
[12] U. WEISS, B. D. DAVIS, and E. S. MINGIOLI, *J. Am. Chem. Soc.* **75**, 5572 (1953).
[13] R. U. LEMIEUX, R. K. KULLNIG, H. J. BERNSTEIN and W. G. SCHNEIDER, *J. Am. Chem. Soc.* **80**, 6098 (1959).

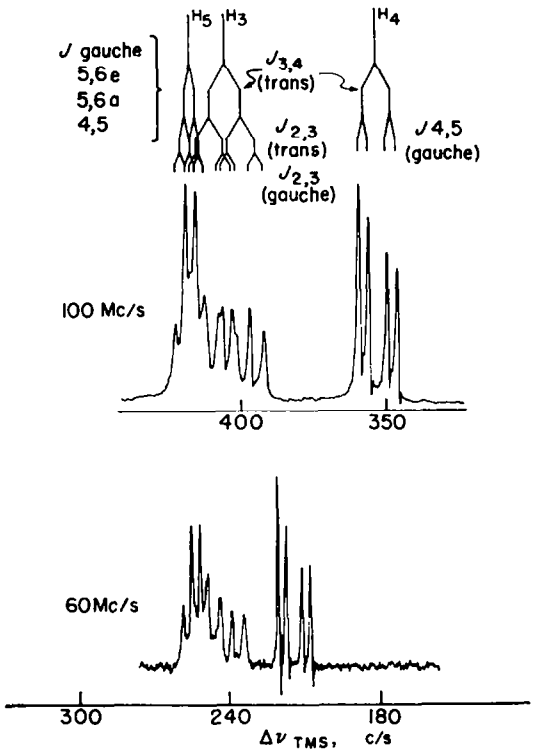

FIG. 1. NMR SPECTRA OF D-(−)-QUINIC ACID TAKEN IN D_2O AT 33°.

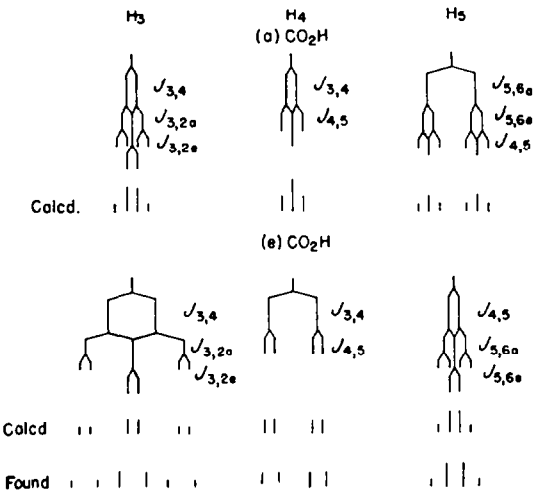

FIG. 2. PREDICTED FIRST-ORDER NMR SPECTRA FOR THE (a) AND (e) CO_2H CONFORMERS OF D-(−)-QUINIC ACID.

dihedral angle of 180° between them. A smaller coupling of 3–6 c/s arises from diequatorial or axial-equatorial gauche configurations. The signals from two protons are overlapped H_3 ($\tau = 5{\cdot}31$) and H_5 ($\tau = 5{\cdot}45$), but there is no doubt that the quartet centered at $\tau = 6{\cdot}09$ belongs to H_4 of the (e) CO_2H conformation. Although first-order theory predicts that H_4 should appear as a quartet of equal intensities, skewed intensities in spin–spin multiplets are often observed because first-order conditions are not satisfied.[14] The peaks are enhanced in the direction of the band(s) of the proton(s) producing the splitting. Large values of the ratio $J/\nu_0.\Delta\tau$ produce greater intensity variations. This variation, seen with quinic acid and chlorogenic acid (Fig. 3), is further illustrated in the spectra of 4-caffeoylquinic acid (Fig. 4)

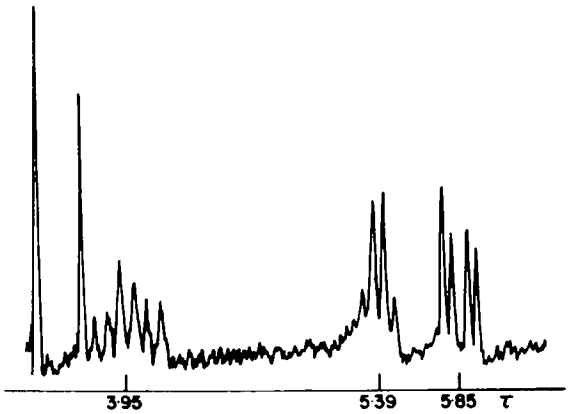

FIG. 3. 60 Mc/s NMR SPECTRUM OF CHLOROGENIC ACID TAKEN IN *d*-5-PYRIDINE AT 90°.

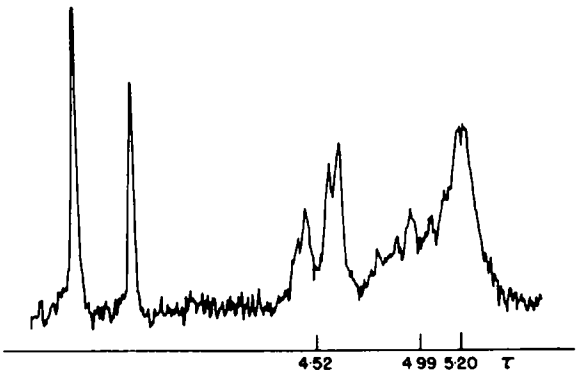

FIG. 4. 60 Mc/s NMR SPECTRUM OF 4-CAFFEOYLQUINIC ACID (BAND 510) TAKEN IN *d*-5-PYRIDINE AT 90°.

and 4,5-dicaffeoylquinic acid (Fig. 5). In the spectrum of 4-caffeoylquinic acid the H_4 proton bands are intensified in the direction of the overlapping H_3 and H_5 bands, upfield in this case. In 4,5-dicaffeoylquinic acid, the 9 c/s splitting of H_4 ($\tau = 4{\cdot}27$) by H_3 at $\tau = 5{\cdot}03$ gives rise to a doublet larger in the upfield direction. Each peak of this doublet is split in turn by H_5 (4 c/s) and the intensities increase downfield toward H_5 ($\tau = 3{\cdot}80$). The resulting quartet shows the expected variation in intensities.

The acylation of a hydroxyl group produces a paramagnetic (downfield) shift of about 1·5 ppm of the hydrogen(s) attached to the carbon bearing the acyloxy group. This shift is

[14] W. A. ANDERSON, *NMR and EPR Spectroscopy*, p. 158. Pergamon Press, Oxford (1960).

clearly seen in the NMR spectrum of chlorogenic acid (3-caffeoylquinic acid) (Fig. 3). As expected, the H_3 bands centre downfield at $\tau = 3{\cdot}95$ and the hyperfine structure of H_3, H_4, and H_5 can be readily distinguished. Table 1 lists the results of NMR spectral observations for a number of quinic acid derivatives.

Although quinic acid and its acylated derivatives are shown to be in the approximate (e) CO_2H conformation (III), we have not attempted to calculate the exact angles between the protons in each of the compounds by using one of the modified forms of the Karplus expression such as the one which has been applied to sugar conformational analysis[15] since Karplus has pointed out[16] that vicinal couplings will also be significantly effected by the electronegativity of substituents as well as by a number of other molecular properties. The dependence on electronegativity has been very well demonstrated by Williamson.[17]

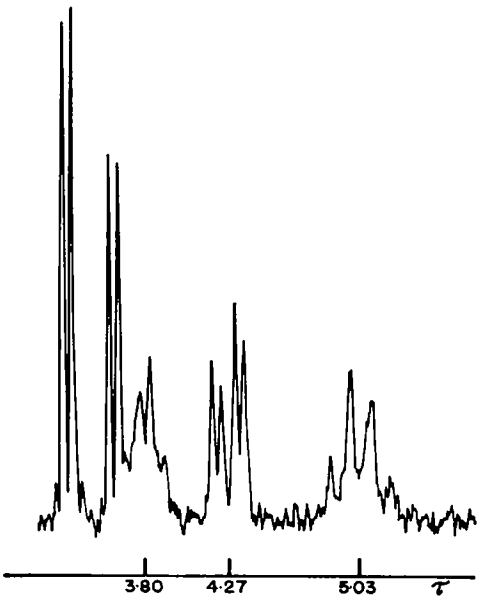

FIG. 5. 60 Mc/s NMR SPECTRUM OF 4,5-DICAFFEOYLQUINIC ACID TAKEN IN *d*-5-PYRIDINE AT 90°.

Williams and Bhacca recently reported a dependence of gauche coupling constants on the configuration of oxygen-bonded substituents in a number of steroid derivatives[18] which may become an important tool for the determination of configuration. When a hydroxyl or acetate group was equatorial, the coupling averaged $5{\cdot}5 \pm 1{\cdot}0$ c/s, whereas for axial substituents it was appreciably lower, varying from 2·5 to 3·2 c/s. All the gauche couplings in Table 1 are in accord with this observation, however, it must be pointed out that they are only accurate to $\pm 0{\cdot}5$ c/s.

Since chemical shifts are proportional to frequency while spin–spin couplings are independent of frequency, spectra taken at a higher resonance frequency will show fewer overlapping bands and the intensities and spacings will more closely approximate those

[15] R. W. LENZ and J. P. HEESCHEN, *J. Polymer Sci.* **51**, 247 (1961).
[16] M. KARPLUS, *J. Am. Chem. Soc.* **85**, 2870 (1963).
[17] K. L. WILLIAMSON, *J. Am. Chem. Soc.* **85**, 516 (1963).
[18] D. H. WILLIAMS and N. S. BHACCA, *J. Am. Chem. Soc.* **86**, 2742 (1964).

predicted by first-order theory. Figure 1 shows the 60 and 100 Mc/s spectra of quinic acid in deuterium oxide. The improved multiplet resolution available at 100 Mc/s is readily apparent. The bands representing H_3 and H_5 can be easily assigned at the higher frequency. The spectra obtained by Inoue, Aoyagi and Nakanishi[9] showed hyperfine structure only for H_4 of quinic acid and not for the other protons of quinic acid nor for the H_3, H_4 and H_5 protons on the two acylated derivatives considered.

TABLE 1. CHEMICAL SHIFTS AND FIRST-ORDER SPIN–SPIN COUPLING CONSTANTS OF QUINIC ACID AND DERIVATIVES AT 60 Mc/s

	H_3*	H_4	H_5	$\Delta\tau$†	$J_{3,4}$	$J_{4,5}$	$J_{3,2^a}$	$J_{3,2^e}$	$J_{5,6^a}$	$J_{5,6^e}$
D-(−)-Quinic acid	5·31	6·09	5·45	—	9	3	9	5	3	3
5-Dehydroquinic acid	6·01	5·64	—		9	—	9	6	—	—
1-Caffeoylquinic acid	5·22	5·96	5·37	—	8	3	8	5	3	3
1-Mesitoylquinic acid	5·39	5·97	5·39	—	9	3	9	5	4	4
Chlorogenic acid (3-Caffeoylquinic acid)	3·95	5·85	5·39	1·36	9	3	9	5	3	3
3-Feruloylquinic acid	3·92	5·85	5·39	1·39	9	3	9	5	3	3
3-Isoferuloylquinic acid	3·92	5·86	5·40	1·39	9	3	9	5	3	3
4-Caffeoylquinic acid	4·99	4·52	5·20	1·57	9	3	9	5	3	3
Neochlorogenic acid (5-Caffeoylquinic acid)	5·14	5·71	3·98	1·47	8	3	8	5	3	3
Dihydroneochlorogenic acid	5·29	5·85	4·15	1·30	8	4	8	5	4	4
3,4-Dicaffeoylquinic acid	3·83	4·39	5·16	1·48(H_3) 1·70(H_4)	9	3	‡	‡	3	3
3,5-Dicaffeoylquinic acid	3·91	5·50	3·96	1·40(H_3) 1·59(H_5)	9	3	‡	‡	‡	‡
4,5-Dicaffeoylquinic acid	5·03	4·27	3·80	1·40(H_4) 1·49(H_5)	7	4	7	7	4	4

The spectra were taken in deuteropyridine at 90° except for 5-dehydroquinic acid, wherein D_2O was the solvent (32°).

* Chemical shifts expressed in τ and first-order coupling constant (J) in c/s.

† $\Delta\tau$ = The difference between chemical shifts of proton attached to the carbon atom bearing the acyloxy group and its corresponding proton in quinic acid.

‡ No accurate measurements made because of overlapping multiplets.

All of the spin couplings that have so far been discussed involve protons separated by no more than three intermediary bonds. Long-range couplings over four or more bonds are frequently encountered when unsaturation is present in the coupling path. More rarely they are observed in saturated systems, presumably as a result of a very particular molecular geometry. Usually such couplings are small, of the order of 1 c/s or less, and care must be taken to avoid confusing them with second-order effects. In the series of compounds discussed here only the proton at C_4 of 5-dehydroquinic acid showed a discernible long-range coupling (Fig. 6). The assignment of the doublet-split doublet at $\delta' = 4{\cdot}36$ to H_4 (a) and of the doublet-split triplet at $\delta' = 3{\cdot}99$ to H_3 (a) is straightforward on the basis of their splitting patterns. The incompletely resolved 1·1 c/s doublet splitting of the H_4 multiplet superimposed on the *trans* coupling to H_3 undoubtedly results from coupling across the 5-carbonyl group to one of the protons on C_6. This latter resonance is not shown in the figure but is a typical "AB-type" methylene quartet with additional fine structure that has not been completely analyzed. Since a 60 Mc/s spectrum shows an identical H_4 splitting, it must result from a long-range coupling rather than from a second-order effect.

The practical effects of the (e) CO_2H conformation may be seen in two common instances: oxidation of quinic acid and separation of the chlorogenic acids. In cyclohexanol-type oxidations, the rate-determining step may be the proton transfer from the carbon that bears the reacting hydroxyl. Axial proton loss is a difficult process.[19] Thus, 5-dehydroquinic acid is the favored product by a number of reagents.[7,10–12] The separation of the isomeric chlorogenic and "isochlorogenic" acids has been done by counter-current distribution[20,21] or by partition chromatography.[22] Both processes depend on differential partition coefficients between pairs of solvents, one member of which has always been water. A solvation cage of water molecules can build up to a greater extent around equatorial hydroxyls than around the more sterically hindered axial hydroxyls. Thus, chlorogenic acid (II) and 4-caffeoylquinic acid each with one free equatorial and two axial hydroxyls, come very close together in both countercurrent distributions and liquid–liquid partition chromatography. Their distribution coefficients* are higher than those of neochlorogenic acid (5-caffeoylquinic acid) and 1-caffeoylquinic acid. Both of the latter isomers have two equatorial hydroxyls and one axial.

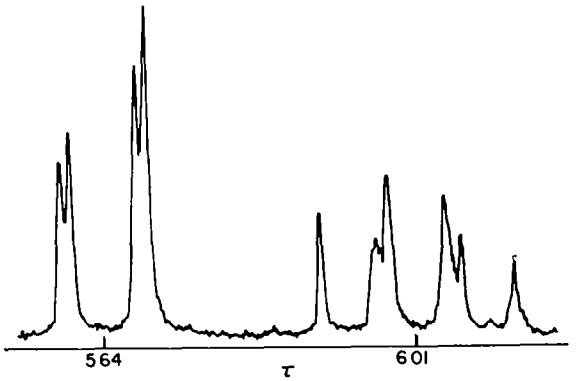

FIG. 6. 100 Mc/s NMR SPECTRUM OF 5-DEHYDROQUINIC ACID TAKEN IN D_2O AT 32°.

The facile migration[23–26] of the 1-acyl group to the other hydroxyls made it advisable to prepare the 1-mesitoyl ester. Addition to this highly hindered carbonyl function is not possible and therefore the mesitoyl cannot wander to other positions. No change in conformation was observed, however, although molecular models showed crowding between the bulky 1-mesitoyl moiety (axial) and the 5-hydroxyl (axial). The only chemical derivatives in which we have observed conformational change to the (a) CO_2H (aside from the quinic acid lactones, of course) are the trimethylsilyl ether esters of quinic acid and the chlorogenic acids.[27] The steric hindrance between the trimethylsilyl groups on the 1,5-axial hydroxyls

* Defined as the ratio of concentrations in upper to lower phase, usually an ethyl acetate–water (or buffered) solvent pair in these studies.

[19] T. STEWART, *Oxidation Mechanisms*, p. 45. Benjamin, New York (1964).
[20] J. W. CORSE, *Nature* **172**, 771 (1953).
[21] J. W. CORSE, R. E. LUNDIN and A. C. WAISS, JR., *Phytochem.* **4**, 527 (1965).
[22] E. SONDHEIMER, C. D. SZYMANSKI and J. W. CORSE, *J. Agr. Food Chem.* **9**, 146 (1961).
[23] P. BADIN, V. DEULOFEU and O. L. GALMARINI, *Chem. & Ind. (London)* 257 (1962).
[24] M. L. SCARPATI and P. ESPOSITO, *Tetrahedron Letters* 1147 (1963).
[25] K. R. HANSON, *Chem. & Ind. (London)* 1691 (1963).
[26] E. HASLAM, G. K. MAKINSON, M. O. NAUMANN and J. CUNNINGHAM, *J. Chem. Soc.* 2137 (1964).
[27] A. C. WAISS, JR. and J. CORSE. Unpublished observations.

((e) CO_2H) apparently is greater than that between the two trimethylsilyl groups on the diaxial 1-carboxyl and 3-hydroxyl ((a) CO_2H). The migration of acyl groups follows from the (e) CO_2H conformation, and it may be expected that anchimeric assistance will allow the migrations $1 \leftrightarrow 5 \leftrightarrow 4 \leftrightarrow 3$, only, and not $1 \leftrightarrow 3 \leftrightarrow 4 \leftrightarrow 5$ as proposed by Haslam *et al.*[26] which requires the unlikely intervention of an anhydride intermediate in the $1 \leftrightarrow 3$ step.

EXPERIMENTAL

Chlorogenic and neochlorogenic acids were isolated as described earlier.[20,22] The isolation of 4-caffeoylquinic acid ("Band 510") has been described but its unequivocal demonstration as a monocaffeoylquinic acid has not, and sufficient physical and hydrolytic data are included for this purpose. 1-Caffeoylquinic acid was prepared by hydrolysis of 1-(3',4'-dicarbomethoxycaffeoyl)-quinic acid,[28] and subsequent purification by countercurrent distribution. The three dicaffeoylquinic acids have been recently described.[21] 5-Dehydroquinic acid was prepared by nitric acid oxidation of quinic acid.[10] 3-Feruloylquinic acid was isolated from coffee by countercurrent distribution.[29]

The NMR spectra were taken on Varian A-60 and HR-100 spectrometers. Quinic acid and the acylated quinic acids were examined at 90° in *d*-5-pyridine; 5-dehydroquinic acid at 32° in D_2O. The quinic acid spectra shown in Fig. 1 were taken in D_2O at 33°.

1-*Mesitoylacetone Quinide*

A solution of 13·3 g (0·073 M) of mesitoyl chloride[30] in 50 ml of chloroform was added dropwise with stirring to a cooled solution of 15·5 g (0·073 M) acetone quinide[31] in 50 ml of chloroform and 8 ml of triethylamine. After standing at room temperature overnight, the reaction mixture was poured into water. The chloroform layer was separated, washed with cold dil. HCl, water, $KHCO_3$ solution and dried over $MgSO_4$. The solution was filtered and the addition of light petrol caused the separation of 22·4 g (85 per cent) of fine white needles. These were recrystallized from ethyl acetate–light petrol, m.p. 115–116°; $[\alpha]_D^{27} -22·7°$ (0·44% in methanol). (Found: C, 66.3; H, 6.69. $C_{20}H_{24}O_6$ required: C, 66.65; H, 5.71%).

1-*Mesitoylquinic Acid*

1-Mesitoylacetone quinide (4 g) heated under reflux for 3 hr with 100 ml of 80% acetic acid to which 1 ml conc. HCl had been added. The reaction mixture was evaporated in dryness *in vacuo*, and the residue was refluxed with 25 ml N NaOH and 25 ml of ethanol for 1 hr. The alcohol was removed *in vacuo*, the cold aqueous solution was acidified with cold dil. HCl. The resulting crystals were collected; yield, essentially quantitative. The product was recrystallized from ethyl acetate–ether; m.p. about 110–115° (dec.) $[\alpha]_D^{27} -9·4°$ (0·32% in methanol). (Found: C, 60·4; H, 6·49. $C_{17}H_{22}O_7$ required: C, 60·34; H, 6·55%.) When the hydrolysis procedure was reversed, 1-*mesitoyl*-4,5-*isopropylidenequinic acid* could be isolated as the hemihydrate by basic hydrolysis of the lactone ring, m.p. 120–125° (dec.) (from ethyl acetate–light petrol); $[\alpha]_D^{27} -8·2°$ (0·73% in methanol); (Found: C, 61·8; H, 7·01. $C_{20}H_{26}O_7 . 0·5\ H_2O$ required: C, 61·95; H, 7·02%). Subsequent acid hydrolysis to remove the isopropylidene group resulted in extensive lactonization.

[28] Dr. R. R. JOHNSON. Private communication.
[29] J. W. CORSE, E. SONDHEIMER and R. E. LUNDIN, *Tetrahedron* **18**, 1207 (1962).
[30] R. P. BARNES, *Org. Syn.* **21**, 77 (1941).
[31] H. O. L. FISCHER, *Ber. Deut. Chem. Ges.* **54**, 775 (1921).

Dihydroneochlorogenic Acid

A solution of 0·5 g of neochlorogenic acid in 50 ml of ethanol was shaken with 200 mg of PtO_2 under hydrogen for 2 hr. The platinum was removed by filtration and the alcohol removed *in vacuo*. The residual crystals were recrystallized from ethanol; m.p. 165–167° $[\alpha]_D^{27}$ −27·6° (0·8% in methanol); (Found: C, 53·8; H, 5·85. $C_{16}H_{20}O_9$ required: C, 53·93; H, 5·66%).

4'-O-Methylchlorogenic Acid Diacetonide

A solution of 5·67 g of chlorogenic acid diacetonide[5] in 8 ml of methanol and 52 ml of dry ether was cooled in an ice bath and treated with an excess of ethereal diazomethane. After about 20 min the resultant crystals were collected and washed with ether; yield 3·03 g (52 per cent) m.p. 213–215°. Examination by thin-layer chromatography showed a small amount of impurity not removed by recrystallization. Purification was effected by chromatography on silicic acid using light petrol with increasing amounts of acetone (up to 6 per cent) as eluant. Recrystallization from ethyl acetate–light petrol afforded an analytical sample; m.p. (corr.) 222–223°. (Found: C, 61·4; H, 6·29. $C_{23}H_{28}O_9$ required: C, 61·59; H, 6·29%.)

3-Isoferuloylquinic Acid

A solution of 3 g of the crude diacetonide above in 15 ml of water, 10 ml of ethanol and 2 ml of conc. HCl was heated on the steam bath for 1 hr, allowed to stand for 1 hr and evaporated to dryness *in vacuo*. The crude crystalline product was then purified by countercurrent distribution in a Craig apparatus which holds 10 ml of each solvent in 200 tubes. The sample was dissolved in the first two tubes; the solvent pair was acetate–10% NaCl, pH 2·0. A total of 192 transfers was made and the fraction in tubes 130–160 (N_{max} 146; $K = 3{\cdot}1$) was collected. The water and ethyl acetate were removed *in vacuo* at 50°. The residue was extracted with 200 ml of isopropanol and the sodium chloride removed by filtration. The filtrate was again evaporated and the treatment with isopropanol was repeated. Three recrystallizations from water yielded 0·91 g of 3-isoferuloylquinic acid; m.p. 201–202°; $[\alpha]_D^{25}$ −39·8° (0·5% in ethanol); λ_{max} 326 mμ; $\epsilon = 21{,}000$ (ethanol). (Found: C, 55·5; H, 5·38; neutral equivalent, 359. $C_{17}H_{20}O_9$ required: C, 55·43; H, 5·47%; neutral equivalent 368.)

The R_f on Whatman No. 1 paper in 2% acetic acid was 0·59 and in butanol–acetic acid–water (1:2:2) was 0·61. The spots fluoresced blue-white in u.v. light, turned faintly yellow when exposed to ammonia and then fluoresced pink, in common with other isoferulic acid esters.

Saponification of 25 mg of the depside with aqueous sodium hydroxide gave a near-quantitative yield of isoferulic acid on acidification, m.p. 228–229°; no lowering on admixture with an authentic specimen.

4-Caffeoylquinic Acid ("*Band* 510")

"Band 510" isolated by column chromatography was dried at 68° over P_2O_5 to constant weight; $\epsilon_{322\,m\mu}$ 15,400 (H_2O); $[\alpha]_D^{25}$ −53·6° (2% in H_2O) pK_a 3·4; (Found: C, 53·7; H, 5·61; neutral equiv. 376; mol. wt. (osometer in butanol, average of 4 determinations, 384 ± 18) $C_{16}H_{18}O_9$ required: C, 54·24; H, 5·12%; neutral equiv. and mol. wt. 354).

Saponification was effected by allowing 195 mg of "Band 510" in 2 ml 1 N sodium hydroxide to be stored 24 hr at room temperature under a nitrogen atmosphere. The addition of 0·6 ml glacial acetic acid caused the immediate precipitation of caffeic acid. The mixture was

extracted six times with ether, the ether evaporated, the residue weighed, and the quantity of caffeic acid estimated from the u.v. absorption spectrum of an aliquot in 95% ethanol. This indicated 85 per cent recovery based on a 1:1 caffeic acid–quinic acid ester. The caffeic acid moiety of "Band 510" has been fully characterized previously. The aqueous phase was placed on a 1 × 18 cm column of Dowex 2-X8, 100–200 mesh, and eluted with 0·8 N acetic acid in a modification of the method of Weinstein, Porter and Laurencot.[32]

Only one band was observed, and it had the same peak effluent volume as authentic D-quinic acid. Titration data indicated 76 per cent recovery of quinic acid based on a 1:1 caffeic acid–quinic acid ester.

The quinic acid containing fractions were combined and the solvent evaporated in vacuum below 40°. The remaining colourless syrup was crystallized from ethanol, m.p. 157–160° mixed m.p. with authentic D-quinic acid 157–161°. Optical rotation $[\alpha]_D^{25} = -44{\cdot}7\%$ (1% in H_2O).

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[32] L. H. WEINSTEIN, C. A. PORTER and H. J. LAURENCOT, Jr., *Contrib. Boyce Thompson Inst.* **21**, 439 (1962).