Table V. Effect of PCA Content on Flavor Preference of Tomato Juice

(Triangulation procedure)

Controls, % (W/V.)			Treatments, % (W./V.)			Level of Significance, %	
Code	PCA added	Total PCA content	Code	PCA added	Total PCA content	Triangulation procedure	T-I value
A[a]	0	0.065	B[a]	0	0.090	1.0	0.10
A	0.075	0.140	B	0.050	0.140	N.S.[b]	N.S.
A	0	0.065	A	0.075	0.140	1.0	1.0
B	0	0.090	B	0.050	0.140	5.0	0.10
B	0	0.090	B	0.100	0.190	1.0	0.10
B	0.050	0.140	B	0.100	0.190	5.0	1.0

[a] A and B samples sterilized for 15 and 30 minutes, respectively, in boiling water.
[b] N.S. No significant difference.

produced a significant difference in flavor. Addition of greater amounts of PCA produced undesirable flavor and a greater level of significance between treated and untreated samples. The undesirable flavor was described by the taste panel as bitter, medicinal, chemical, and sour. This indicates that the PCA level has a great influence on the flavor of tomato juice. An increase in sterilization time will produce a greater amount of PCA, which in turn will adversely affect the flavor of the juice.

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COFFEE CONSTITUENTS

Isolation of Chlorogenic Acid and Its Isomers from Coffee

ERNEST SONDHEIMER and C. D. SZYMANSKI

Chemistry Department, State University College of Forestry at Syracuse University, Syracuse 10, N. Y.

J. W. CORSE

United States Department of Agriculture, Western Utilization Research and Development Division, Albany 10, Calif.

A method is presented for the sequential isolation of isochlorogenic, chlorogenic, and neochlorogenic acids, and another isomer designated "Band 510" from coffee beans. Isochlorogenic acid is removed first by extraction with butyl acetate, chlorogenic acid is then precipitated as the caffeine complex, and neochlorogenic acid and "Band 510" are separated by silicic acid column chromatography. The behavior of isochlorogenic acid during acid-base titrations was investigated.

ALTHOUGH CHLOROGENIC ACID was isolated from coffee in 1908 (*7*) and its structure firmly established in 1932 (*6*), interest in this compound is reviving. There is now good evidence for the existence of several isomers of chlorogenic acid and for their widespread occurrence (*12*). Possibly of greater interest is the fact that these compounds are closely related to shikimic acid, an intermediary in the biosynthesis of many aromatics, and that chlorogenic acid has been shown to inhibit the activity of indoleacetic acid oxidase (*10*) and potato phosphorylase (*11*). Also, further work is required to permit a complete structure assignment for the isomers of chlorogenic acid.

Methods for the individual isolation of these compounds have appeared in the literature. Chlorogenic acid, a major soluble constituent of green coffee beans, can be isolated as a crystalline solid from its potassium caffeine complex (*9*). Isochlorogenic acid has been obtained by Barnes, Feldman, and White (*1*) from coffee by extraction with *n*-butyl acetate as a noncrystalline solid. A preparation isolated by Uritani and Miyano (*13*) from sweet potatoes infected with black rot has been named pseudochlorogenic acid. This is a noncrystalline solid with properties that are very similar to isochlorogenic acid. Its existence as a bona fide isomer of chlorogenic acid has not been confirmed. Corse (*4*) has isolated crystalline neochlorogenic acid from peach purée by *n*-butyl alcohol extraction and countercurrent distribution. This isomer is widely distributed in plants, occurs in coffee beans, and seems to be the predominant isomer in members of the prunus family (*12*). A substance isolated from coffee beans by silicic acid column chromatography has been designated "Band 510" and appears to be a true isomer of chlorogenic acid (*12*). Combinations and modifications of the earlier isolation procedures have led to a method which permits the isolation of isochlorogenic acid, chlorogenic acid, Band 510, and neochlorogenic acid from coffee beans in one operation. The high chlorogenic acid content of coffee beans helps make this a convenient procedure. The present authors have also simplified

and improved the generation of chlorogenic acid from the caffeine complex.

An isopropyl alcohol extract of coffee beans was concentrated under reduced pressure, acidified, and extracted with *n*-butyl acetate. The organic phase contained the isochlorogenic acid. Further concentration of the aqueous portion and neutralization yielded the crystalline caffeine complex of potassium chlorogenate. After removal of this material by filtration, the filtrate was extracted with *n*-butyl alcohol, the butyl alcohol was concentrated, and a mixture of solid Band 510 and neochlorogenic acid was precipitated by the addition of chloroform to the butyl alcohol concentrate. These two substances were separated by silicic acid column chromatography. One kilogram of coffee beans yielded 15.5 grams of crude isochlorogenic acid, 12.4 grams of crystalline chlorogenic acid, 1.15 grams of Band 510, and 1.03 grams of crystalline neochlorogenic acid.

Experimental

Isolation of Isochlorogenic Acid. This portion of the procedure is essentially unchanged from that used by Barnes, Feldman, and White (*1*). A mixture of 1 kg. of ground, unroasted Santos coffee beans and 4 liters of 70% isopropyl alcohol was stirred at room temperature for 4 hours, the mixture filtered, and the residue re-extracted with another 4-liter portion of solvent. The combined extract was concentrated under reduced pressure to 1230 ml., stored overnight at 0° C., and filtered through Celite. The filtrate, which had a pH of 5.3, was acidified to pH 2.6 with concentrated sulfuric acid and extracted three times with 1-liter portions of butyl acetate. The combined butyl acetate extracts, which contained the isochlorogenic acid, were washed twice with 250-ml. portions of water. The wash water was discarded. Concentration of the washed butyl acetate extract under reduced pressure was continued until precipitate formation was discernable. Addition of 1 liter of chloroform yielded 15.5 grams of crude isochlorogenic acid. This material can be purified by the procedure of Barnes *et al.* (*1*).

Isolation of Chlorogenic Acid. After butyl acetate extraction, the coffee extract contained chlorogenic acid, Band 510, and neochlorogenic acid. This solution was treated with small portions of 10% aqueous potassium hydroxide until a pH of 5.0 was obtained. The solution was then concentrated in vacuum to 250 ml. and stored overnight at 0° C. The resulting precipitate, which seemed to be mainly potassium sulfate, was removed by filtration and discarded. To the filtrate, 250 ml. of 95% ethyl alcohol was added, the mixture filtered immediately, and the precipitate again discarded. Fifteen grams of caffeine and 5 grams of potassium acetate were then dissolved in the aqueous ethyl alcohol solution. After storage at 0° C. for 48 hours the mixture was filtered, yielding 35 grams of crude chlorogenate-caffeine complex. The filtrate, which contained the Band 510 and neochlorogenic acid, was saved.

Recrystallization of the chlorogenate complex from 150 ml. of 33% ethyl alcohol yielded 26 grams. Conversion to chlorogenic acid was achieved by dissolving the recrystallized complex in 45 ml. of 1*N* hydrochloric acid and extracting six times with 100-ml. portions of chloroform. The chloroform extracts, which contained the caffeine, were discarded. Overnight storage of the aqueous phase at 0° C. yielded a white precipitate which was filtered off, washed with water, and dried. The yield of chlorogenic acid was 12.4 grams, 81% of the theoretical, $E_{1\text{ cm.}}^{1\%}$ at 322 mμ = 510. This material can be recrystallized from water in 80 to 90% yield.

Isolation of Band 510 and Neochlorogenic Acid. The 500 ml. of aqueous ethyl alcohol filtrate remaining from the chlorogenate complex isolation was concentrated in vacuum to about one third of its volume when crystals again precipitated. These were filtered off and discarded since they were almost pure caffeine. The filtrate was extracted three times with 100-ml. portions of chloroform to remove the rest of the caffeine and the extracts were discarded.

The aqueous phase was acidified with concentrated sulfuric acid to pH 2.5 and extracted three times with 150-ml. portions of *n*-butyl alcohol. The combined butyl alcohol extracts, which contained the Band 510 and neochlorogenic acid, were concentrated in vacuum to 225 ml. and stored overnight at 0° C. A small amount of precipitate had settled out; this was removed by filtration and discarded. The filtrate was concentrated further under vacuum and 900 ml. of chloroform was added to the remaining syrup. After overnight storage at 0° C. the mixture was filtered, the precipitate washed with chloroform and dried in vacuum, yielding 10.7 grams of hygroscopic solids with an $E_{1\text{ cm.}}^{1\%}$ at 320 mμ of 335. The estimated chlorogenic acid content of this mixture is approximately 65%, based on the assumption that all the ultraviolet absorption is due to chlorogenic acids. An exploratory silicic acid chromatogram of this material showed the relative proportions of caffeic and isochlorogenic acids, chlorogenic acid, Band 510, and neochlorogenic acid to be 1:3,5:7:5, respectively. From this mixture, Band 510 and neochlorogenic acid were isolated by silicic acid chromatography.

The silicic acid column technique used here is an adaptation of the method described previously (*12*). The column consists of 160 grams of silicic acid plus 110 ml. of 0.5*N* sulfuric acid and the first development solvent. A band of 2.5 grams of the chlorogenic acids mixture in 5 ml. of 0.5*N* sulfuric acid plus 10 grams of silicic acid was applied to the top of the column. Development was carried out with *n*-butyl alcohol–chloroform mixtures that had been equilibrated with 0.5*N* sulfuric acid, 3500 ml. of 15% by volume *n*-butyl alcohol, then 1000 ml. of 25% *n*-butyl alcohol, and finally 1500 ml. of 35% *n*-butyl alcohol. Fractions of 100 ml. were collected. The chlorogenic acids were detected by their ultraviolet absorption at 330 mμ in a Beckman

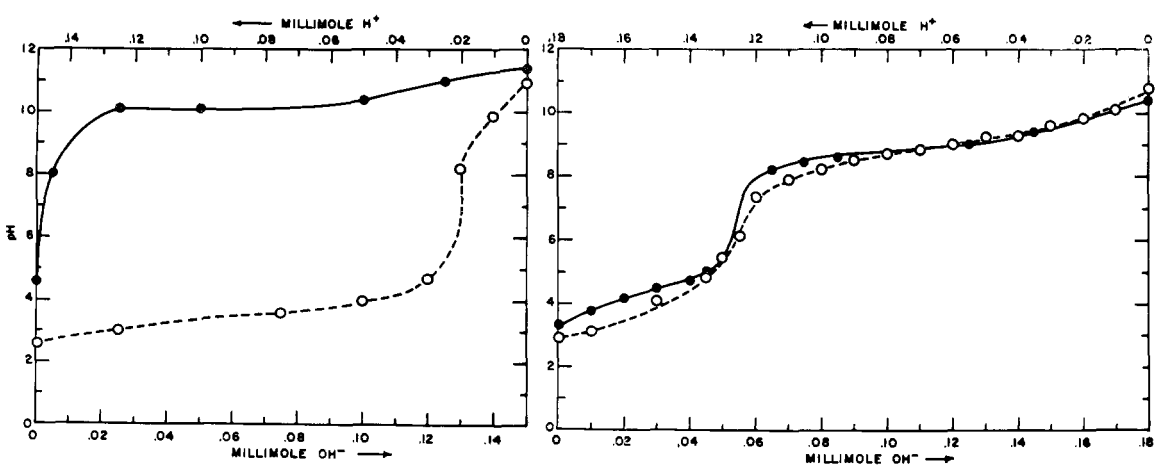


Figure 1. Titration curves for 23.5 mg. of quinic acid-3-lactone (left) and 33.9 mg. of isochlorogenic acid (right)

———Base titration – – – – Back-titration with acid

Model DU spectrophotometer. Band 510 was eluted with the 25% butyl alcohol mixture, peak effluent volume 3800 ml., and neochlorogenic acid with the 35% butyl alcohol solvent, peak effluent volume 5300 ml.

The fractions considered to contain the material of highest purity were combined and stored overnight at 0° C. This cold storage caused the precipitation of a small quantity of aqueous phase which contained a high proportion of the sulfuric acid but only insignificant amounts of the chlorogenic acid. Filtration, therefore, afforded a very effective method of removal of sulfuric acid prior to solvent removal. The eluates from four columns with comparable peak effluent volumes were combined, most of the solvent was removed under reduced pressure, and the chlorogenic acids were precipitated by the addition of chloroform. Overnight storage at 0° C., filtration, and drying of the precipitate in a vacuum desiccator over phosphorus pentoxide yielded 1.15 grams of noncrystalline Band 510, $E^{1\%}_{1\ cm.}$ at 320 mμ = 417, from the fractions with peak effluent volume of 3800 and 0.92 gram of neochlorogenic acid, $E^{1\%}_{1\ cm.}$ at 320 mμ = 391, m.p. 171–6° C., from the fractions with peak effluent volume of 5300. A second crop of neochlorogenic acid was obtained by concentration of the filtrate from the first crop and reprecipitation with chloroform; yield, 0.11 gram; $E^{1\%}_{1\ cm.}$ at 320 mμ = 460; m.p. 175–9° C.

For analysis, Band 510 was dried at 100° C. over phosphorus pentoxide for 45 minutes. This raised the $E^{1\%}_{1\ cm.}$ at 320 mμ to 435.

Calculated for $C_{16}H_{18}O_9$: C, 54.24; H, 5.12; neutral equivalent, 354; ash, 0.0. Found: C, 55.06; H, 5.47; neutral equivalent, 350; ash, 0.55.

Hydrolysis of Band 510 with sodium hydroxide, followed by acidification, extraction with ether to remove caffeic acid, and paper chromatography of the aqueous phase by the procedure of Cartwright and Roberts (*3*) gave one spot which had the same R_f value as quinic acid.

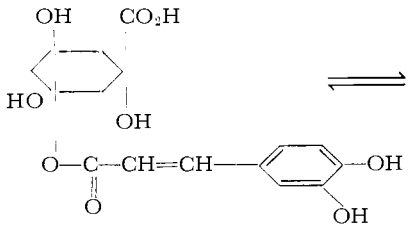
The first crop of neochlorogenic acid, 900 mg., was recrystallized from 95% ethyl alcohol, yielding 460 mg., $E^{1\%}_{1\ cm.}$ at 322 mμ = 490.

Titrations of Isochlorogenic Acid. The isochlorogenic acid had been purified by the procedure of Barnes, Feldman, and White (*1*). The material, 33.9 mg., was dissolved in a minimum of water containing a small amount of methanol, and titrated electrometrically with 0.02*N* sodium hydroxide. The solution started turning yellow, because of ionization of the phenolic groups, near pH 7. Alkali addition was continued until a pH above 10 was reached and the solution was then held at room temperature for 30 minutes. It was back-titrated with 0.02*N* hydrochloric acid. The results are shown in Figure 1. A neutral equivalent of 615 was obtained; Barnes *et al.* (*1*) reported a value of approximately 580; the calculated neutral equivalent for an anhydrous chlorogenic acid isomer is 354.

The quinic acid-3-lactone was prepared from the isopropylidene derivative by mild acid hydrolysis according to the procedure of Fischer (*5*). Titration of 23.5 mg. was carried out under the same conditions as used for the isochlorogenic acid (Figure 1). The saponification equivalent was 184; calculated value, 174. 4,5-Isopropylidene quinic acid-3-lactone was similarly titrated; saponification equivalent, 220; calculated value, 214.

Results and Discussion

Isochlorogenic Acid. The properties described for isochlorogenic acid by Barnes, Feldman, and White (*1*) are consistent with their suggestion that this preparation is an isomer of chlorogenic acid. However, their evidence for the structure assignment of isochlorogenic acid as caffeyl-5-quinic acid (*1*) has been questioned by Bean and Corse (*2*). Barnes *et al.* (*1*) also observed a curious discrepancy between the molecular weight determinations, which appear to be normal for a monocaffeyl quinic acid, and the neutral equivalent which is abnormally high. They attribute this result to a mobile equilibrium between the acid (I) and lactone forms (II) of isochlorogenic acid. On re-examination, the present authors confirmed the high neutral equivalent of Barnes *et al.* for isochlorogenic acid. However, evidence for the presence of a lactone is lacking. It could be shown that under conditions that caused opening of the lactone ring of quinic acid-3-lactone (Figure 1), no comparable change occurred with isochlorogenic acid. Had the high neutral equivalent of isochlorogenic acid been due to the presence of a lactone, saponification would have produced a 40% increase in the free acid content.



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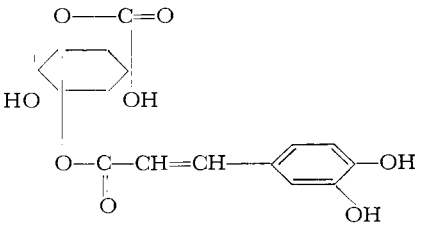

II

The infrared spectrum of isochlorogenic also indicates the absence of lactone groups. In potassium bromide pellets, quinic acid has an absorption band at 5.96 microns which is shifted to 5.58 microns on lactonization. The corresponding band in isochlorogenic acid and chlorogenic acid is at 5.90 microns. Since chlorogenic acid cannot lactonize and lactone formation in quinic acid caused a 0.4-micron shift, even a small percentage of isochlorogenic acid lactone should be detectable.

Although the data presented here make it highly improbable that a mobile equilibrium exists between an acid and a lactone form of isochlorogenic acid, they do not require a revision of the structure assignment. All that can be concluded from these observations is that the difference in the molecular weight determinations and the neutral equivalent of isochlorogenic acid is not due to an equilibrium mixture of acid and lactone.

Chlorogenic Acid. The customary procedure for the conversion of the potassium chlorogenate–caffeine complex to chlorogenic acid with tartaric acid and chloroform (*9*) results in low yields. A simplified procedure, using hydrochloric acid instead of tartaric acid, which at the same time improves the yields is described in the experimental section.

Band 510. It has been shown previously that this substance resembles the other chlorogenic acids with respect to its ultraviolet and infrared spectra, its color reactions with sodium hydroxide and ferric chloride, and its levorotation in aqueous solutions. Alkaline hydrolysis yielded caffeic acid in 80% yield, based on the assumption that Band 510 is a monocaffeyl quinic acid.

Further evidence that Band 510 is a chlorogenic acid isomer is now available. If noncrystalline Band 510 is dried at 100° C. in vacuum, the carbon and hydrogen analyses are much closer to the calculated values for a monocaffeylquinic acid than those previously reported. Also, the water-soluble alkaline hydrolysis product of Band 510 has been tentatively identified as quinic acid by paper chromatography. The neutral equivalent, obtained electrometrically, is also very close to the value calculated for a monocaffeylquinic acid.

Attempts to obtain Band 510 in crystalline form have been only partially successful. Only once, when the substance was stored at −15° C. over *n*-butyl alcohol and chloroform for several weeks, were crystals obtained. The crystalline structure was verified by x-ray photographs. This preparation, although chromatographically identical with the noncrystalline substance, did not induce crystallization of the amorphous material. Possibly this is due to very stringent requirements for a specific water concentration of the solvent. On the melting point stage, the crystalline Band 510 changes to a glass at temperatures slightly above 100° C., indicating loss of crystalline structure under conditions that would lead to loss of water of crystallization.

Behavior of Chlorogenic Acids in Strongly Acidic Solution. Jean and Reid (*8*) have reported that treatment of chlorogenic acid with 5*N* sulfuric acid for 15 minutes at 20° C. produced two isomers, one of which they believe to be identical with neochlorogenic acid. They detected these isomers by paper chromatography. Since the present authors have been separating the chlorogenic acids on silicic acid columns containing 0.5*N* sulfuric acid, it was considered necessary to determine whether any isomerization occurred under these isolation conditions. Chlorogenic acid isolated from coffee as the caffeine complex and recrystallized from water was therefore chromatogramed on a silicic acid column with 0.5*N* sulfuric acid as the stationary phase. No isomers were detected on scanning at 330 mμ, under conditions known to permit detection of 2 to 3% of transformation products when 1 mg. or more of an isomer is applied to the column. (Since the chlorogenic acid isomers have high absorption coefficients, the $E_{1\ cm.}^{1\%}$ at 330 mμ in butyl alcohol–chloroform being above 450, they can be detected at these levels by ultraviolet scanning.) Even when the chlorogenic acid in 5*N* sulfuric acid was held for 15 minutes at 20° C., the conditions used by Jean and Reid, and then chromatogramed on the silicic acid column, no significant changes in the elution pattern were observed. Similar results were obtained with neochlorogenic acid. Thus, the claim for acid-catalyzed isomerization of chlorogenic acid has not been confirmed. Possibly the discrepancy between the results reported in this article and those of Jean and Reid is due to differences in the purity of the chlorogenic acid samples.

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BEVERAGE PRESERVATION

Pasteurization of Palm Sap (Neera)

B. P. BALIGA and A. C. IVY

Department of Clinical Science, University of Illinois College of Medicine, Chicago, Ill.

Palm sap (Neera) tapped from coconut palm or date palm can be pasteurized and bottled at 170° to 175° F. without affecting its natural flavor. Pasteurization above 185° or 190° F. for 4 to 5 minutes imparts a "cooked" flavor. Yeasts are killed by holding pasteurization for 25 to 30 minutes. The presence of air increases the rate of fermentation. Addition of sodium benzoate makes pasteurization more effective. The effectiveness of benzoate is increased by a small amount of citric or malic acid, but these acids alone (pH 3.75) have no significant effect. The addition of malic acid gives the sap an off-flavor resembling that of apple juice.

THE PRESERVATION OF PALM SAP (Neera) has attracted considerable attention in India. Neera has a flavor which appeals to the palate of many Indian people, and, probably because of its vitamin content, a legend has developed regarding its value as a nutritious drink. As it contains considerable sugar, the natives have used it as the source of a fermented beverage, a use prohibited in certain states in India. The governments of these states have agencies which collect the sap and distribute it in large containers to selected stores in the cities, where it is consumed on the premises. Certain governmental officials have been interested in ascertaining whether the unfermented sap could be bottled and distributed as is done with soft drinks.

Various methods have been suggested for the preservation of Neera in bottles, but none has been found practical from either a pilot-plant or commercial point of view (*1*, *4*). Pasteurization has often been mentioned, but its practical application has not been systematically studied.

Pasteurization, more particularly flash pasteurization (*5*), has been widely used in the preservation of fruit juices in the United States. Pasteurization should be preferable for Neera, as prolonged heating may cause off-flavors.

The present studies were conducted to find the optimum conditions under which Neera could be pasteurized with and without the addition of chemical preservatives, the sap being deaerated to check oxidative deterioration.

Methods

Palm sap used in these studies was packed in dry ice and shipped by air to Chicago from Bombay, India. The sample was received within 48 hours and was fresh and clear. No fermentation had taken place. During experimentation palm sap was stored at 3° C.

To ascertain the approximate temperature and rate at which the microorganisms would be killed, a number of samples of palm sap were pasteurized by "holding" pasteurization. Temperatures were taken and plate counts were made on special agar as described by Pederson, Beavens, and Goersline (*2*). The hot pasteurized sap was introduced into sterile soft-drink bottles, which were immediately crowned. Later the