Some of the chemicals used in the manufacture of synthetic caffeine are urea, dimethylurea, and chloroacetic acid. For reasons of economy of manufacture, these chemicals are generally manufactured from petroleum and coal and their use from these sources would be the basis of the differentiation between synthetic and natural caffeine by the method used here. The application of C^{14} analyses to this problem was successfully done (results unpublished) in December 1956 in a series of experiments at the University of Michigan by Oliver Weinkauff of the Monsanto Chemical Co. and H. R. Crane of the University of Michigan. Subsequently, radiocarbon counting was done in other laboratories and recently known synthetic samples of caffeine from producers in America and Europe were analyzed by this method (*1*, *2*) based on oxidation of the sample to carbon dioxide, purification of carbon dioxide, and subsequent radiocarbon counting of carbon dioxide with a proportional counter (*3*). Results of analyses are listed in Table I.

Discussion

Caffeine sold in commerce has been successfully analyzed by radiocarbon analyses for identification as to its synthetic or natural origin. The two samples from German synthetic producers indicate that both sources used carbon which is radioactively extinct. In the United States, the two sources used one modern carbon atom in the synthetic process. In England, the producer would appear to have used two modern carbon atoms.

A knowledge of industrial organic chemistry and synthetic organic methods is necessary in judging results of a C^{14} analysis for caffeine differentiation. An example of the need of knowledge is the use of dimethyl sulfate as a methylating agent in making caffeine from natural theobromine. In this step, one carbon atom is added. Methanol used for manufacture of dimethyl sulfate was formerly made by the destructive distillation of wood, but now it is usually manufactured by oxidation of petroleum hydrocarbons, or from hydrogen and carbon monoxide and carbon dioxide, which are also petroleum products. Wood-derived methanol would contribute modern carbon radioactivity in a manner similar to that of natural carbon in the methyl groups acquired from the atmosphere in nature's synthesis of vegetative matter. On the other hand, petroleum-produced carbon dioxide would not possess any radiocarbon and the methylating agent made from it would contribute a dead carbon to the caffeine molecule. Such is the case with sample 304 in the table, where there is 90.1% modern carbon.

Widespread use of basic chemicals derived from petroleum and coal in the manufacture of synthetic caffeine makes possible differentiation between synthetic and natural caffeine by this method. Application of this method obviously is not restricted to caffeine, but to any natural synthetic pair in which the synthetic one is made from dead carbon.

Acknowledgment

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COCOA POLYPHENOLS

Changes in Cocoa Tannins during Processing

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Three polyphenols of chocolate were separated by chemical fractionation and paper chromatography after different steps in the manufacturing process. These polyphenols were characterized by color tests and by ultraviolet and infrared spectra. One was identified as (−)-epicatechin; the other two are apparently similar compounds. Roasting diminished the concentration of (−)-epicatechin, and alkalizing or conching caused stereochemical changes in its structure. The other two compounds also underwent stereochemical changes during roasting; no further change occurred with alkalization, but conching reversed the change caused by roasting.

THE MANUFACTURE of both cocoa and chocolate products from cocoa beans is similar for the first few steps in processing. The fresh beans are fermented, roasted, and then ground to form chocolate liquor. In making cocoa by the Dutch process this liquor is treated with alkali and pressed to remove fat. The pressed cake is then pulverized. There are several processes for making chocolate. In the one which we have followed flavorings are added to the roasted beans, which are then refined and conched. A full description of these processes is given by Chatt (*1*). The detailed chemical changes which occur during such processes are generally unknown.

The polyphenols of fresh and fermented beans have been studied extensively by Forsyth and coworkers (*2*, *3*, *4*). In the fresh bean they have identified two anthocyanins, three leucocyanidins, (−)-epicatechin, (+)-catechin, (+)-gallocatechin, and (−)-epigallocatechin. During fermentation the anthocyanins were apparently largely converted to complex leucoanthocyanins. Schubiger *et al.* (*9*) reported that in addition to the com-

Table I. Indicator Reagents Used to Reveal Polyphenols of Cocoa on Paper Chromatograms

Reagent	Specificity	Ref.
Ammoniacal silver nitrate, heat	Most organic compounds	(4)
Tetrazotized benzidine	Phenols in general, some other compounds	(6)
Toluene-*p*-sulfonic acid, heat	Catechins and leucoanthocyanins	(8)
Vanillin and hydrochloric acid	Catechins and leucoanthocyanins	(4)

Table II. R_f Values and Color Reactions on Paper Chromatograms of Acid Extracts and Ethyl Acetate Fractions of Fermented, Roasted, Alkalized, and Conched Samples

(Solvent: isopropyl alcohol–acetic acid–water, 3:2:1)

	R_f and Color	
Indicator	Acid extracts	Ethyl acetate fractions
Ammoniacal silver nitrate	0, brown	...
	0 → 0.50, brown	0 → 0.45, brown
	0.56, brown	0.52, brown (Compd. I)
	0.63, tan	0.59, tan (Compd. II)
	0.68, gray	0.67, gray (Compd. III)
	0.74, gray	0.74, gray (Compd. IV)
	0.81, gray	0.82, gray
Vanillin + HCl	0, pink	...
	0.025 → 0.50, pink	0.025 → 0.45, pink
	0.56, pink	0.52, pink (Compd. I)
	...	0.59, pink (Compd. II)

pounds found by Forsyth, five more phenolic substances are present. The only one of these compounds whose chemistry has been investigated after successive steps in the processing is (−)-epicatechin, and there is a difference of opinion regarding its fate. Forsyth (*2*) showed that during fermentation and drying about 80% of the epicatechin was lost from the cotyledons by exudation and oxidation. Lipscomb (*7*) found that the residual 20% of epicatechin was lost during roasting, but Schubiger *et al.* (*9*) deny that roasting destroys epicatechin. In the research reported here effects of roasting, alkalizing, and conching on cocoa polyphenols were investigated.

Materials and Methods

Points where samples were taken for analysis are lettered. Fermented Accra beans, *A*, were cleaned and roasted, *B*. The beans were cracked, shelled, and stored for several hours. They were then subjected to grinding and the raw chocolate liquor was produced. This was alkalized with potassium carbonate, *C*. Some of the roasted beans were mixed with sugar and cocoa butter (respective proportions, 44.5:50:5.5) and refined on a roll refiner. An additional 2% of alkalized cocoa butter was added and the mass was conched, *D*.

Thirteen-gram aliquots of the four samples were homogenized in a Waring Blendor with 100 ml. of 0.1*N* HCl for 3 minutes. The homogenate was allowed to stand at room temperature for 15 minutes and then filtered. In preliminary experiments this acid extract was spotted directly on paper for chromatography, but for most of the work reported here 50 ml. of the acid solution was extracted with 35 ml. of ethyl acetate by continuous shaking in a separatory funnel for 1 hour. The ethyl acetate was concentrated under reduced pressure at 35° C. to one third its initial volume. This solution was then used for characterization by chromatography and spectrophotometry.

For paper chromatography, the ascending method was used with Whatman No. 1 paper at room temperature. About 60 μl. of acid extract or 120 μl. of ethyl acetate extract were applied for each spot. About 25 different solvent systems were investigated, including those used previously by other investigators. Of all of these by far the best separation was achieved in a mixture of isopropyl alcohol–acetic acid–water in the ratio 3:2:1. This solvent was used routinely in the work described here. Nearly 20 different indicator reagents were tried at one time or another. Table I shows the most useful ones for the compounds investigated.

Individual components were isolated for spectrophotometry by applying the ethyl acetate extract in a horizontal band using 3 ml. per sheet of paper. After solvent development the separated bands were located by cutting a vertical strip and spraying with ammoniacal silver nitrate. Horizontal bands were then cut apart and eluted with flowing methanol. The methanol solutions were rechromatographed if necessary and used for ultraviolet spectrophotometry with the Beckman DU spectrophotometer. For infrared spectrophotometry the methanol solutions were evaporated to dryness under reduced pressure at 50° C. The residue was dissolved in 5 ml. of 5% aqueous potassium bromide (Harshaw infrared quality). This solution was lyophilized and the residue dried over phosphorus pentoxide. Pellets were pressed from the dry mixture of potassium bromide and sample, and spectra of the pellets recorded using Perkin-Elmer Model 21 and Baird-Atomic recording infrared spectrophotometers. All spectral data are expressed qualitatively in arbitrary units of absorbance, as the sample concentrations are unknown. For ultraviolet spectra the concentration of solution was adjusted to give an absorbance of about 1.0 at the wave length of maximum absorption. For infrared spectra the concentration of sample in potassium bromide was adjusted so that the major peaks fell in the range of 20 to 80% transmittance.

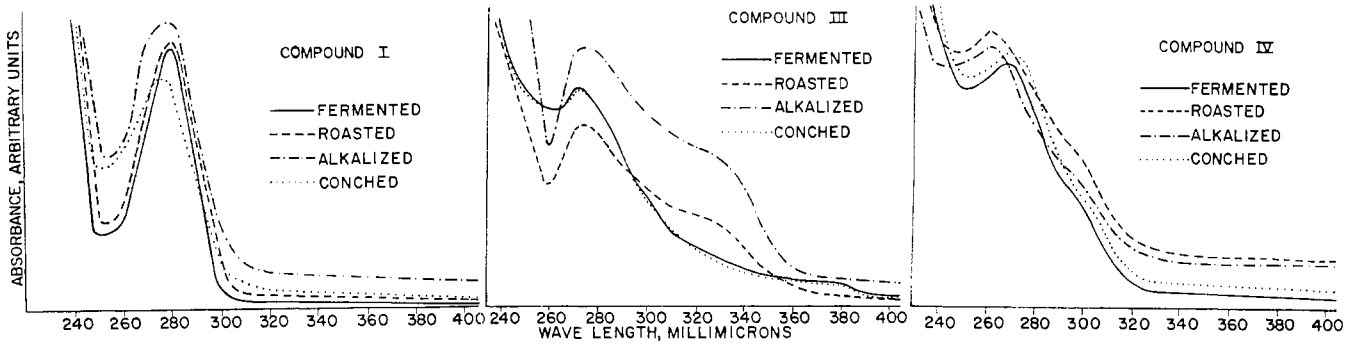


Figure 1. Ultraviolet absorption spectra of compounds I, III, IV at four different stages of processing

For comparison purposes known samples of some of the cocoa polyphenols were obtained.

Results

The fermented, roasted, and alkalized samples (*A*, *B*, and *C*) all contained about 12.6% of acid-extractable material on a dry weight basis. The ethyl acetate extracts contained only 0.61% of the weight of the original sample. Comparable figures for the conched sample were not obtained because of the large amount of sugar present.

Table II summarizes chromatographic results on the phenolic components of acid and ethyl acetate extracts of fermented beans. The spot at R_f = 0 in the acid extract is apparently a condensed catechin tannin. It was absent in the ethyl acetate extract, and the 0.56 spot was resolved into two fractions. The constituents in the ethyl acetate column to which numbers are assigned are those which were selected for further characterization. They all gave gray colors on spraying with 1% alcoholic ferric chloride; spectrophotometric results, to be presented below, confirmed that they were all phenolic compounds of a similar nature.

Qualitatively, all four of the processed samples (fermented, roasted, alkalized, conched) were identical with respect to these four constituents. The only change appeared to be a slight decrease in the amount of compound I and a concomitant increase in compound II as a result of roasting. The streak extending from R_f 0 → 0.50 corresponded to leucoanthocyanidins L_1 and L_2 of Forsyth (*2*). Of the four selected compound I was present in largest amount; when chromatographed in five different solvents, it gave R_f values identical with an authentic sample of (−)-epicatechin.

Compound II was present in insufficient amount to permit isolation for additional characterization. It might reasonably be an isomer of (−)-epicatechin formed during roasting. Compound III corresponds in R_f value to the (+)-catechin found by Forsyth (*3*). Compound IV has not been reported previously. Since it seems similar to the other catechins but moves faster in organic solvents, it might be afzelechin, which is less polar than (+)-catechin.

Compounds I, III, and IV were separated by the band technique and methanol elution described above. After initial chromatography in isopropyl alcohol–acetic acid–water (3:2:1), compound I was eluted and rechromatographed twice, first in distilled water and then in the upper phase of butanol–acetic acid–water (4:1:5). Compounds III and IV were rechromatographed in 2% acetic acid. These rechromatography steps were necessary to achieve homogeneity of the compounds.

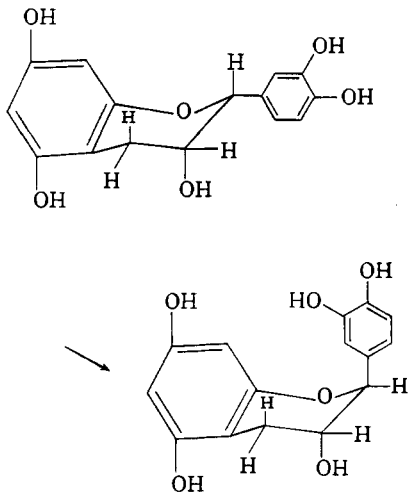
Despite the chromatographic evidence that each of these compounds remained unchanged during processing, differences were observed in the spectra of supposedly identical compounds isolated after certain steps in the processing. The ultraviolet spectrum of compound I at the four different stages is presented in Figure 1. Roasting produced no change in the spectrum, whereas both alkalizing and conching produced slight shifts of the absorption maximum to shorter wave lengths and a decrease in the height of the peak relative to the valley at about 260 mμ. Figure 2 covers the fingerprint region of the infrared spectrum for compound I after the four stages in processing. It shows, again, no change on roasting but changes on alkalizing or conching. The infrared spectrum of compound I isolated from fermented beans was identical to the spectrum of a known sample of (−)-epicatechin.

Corresponding curves are presented in Figures 1 and 2 for the ultraviolet and infrared spectra of compounds III and IV after the different steps in processing. The spectra for both of these compounds indicate a change on roasting which persisted when alkalized. However, if the roasted sample was conched, these compounds reverted back to their original forms.

Discussion

A warning must be inserted that the results reported here may apply only to the particular sample of cocoa beans used and to the way that these beans were processed. Sample variation has been noted by other workers (*9*), and different processing conditions are used throughout the industry. Roasting temperatures may vary as much as 50° and roasting time by as much as 2 hours. During alkalization different amounts and kinds of alkali may be used with different cooking times and temperatures. Conching temperature may vary from 40° to 90° C. and time from one to several days.

Consideration of chromatographic and spectral data shows that the qualitative changes during processing are very slight for the three compounds investigated. No modification of chromatographic mobility was found, and the slight spectral changes are suggestive of configurational differences rather than in any functional groups. The most likely site for such changes in (−)-epicatechin is the pyran ring. Epimerization of (−)-epicatechin to (+)-catechin involves inversion of configuration at C-2 and is promoted by heating (*5*) as diagrammed below:



This conversion represents a change of the cis form having an equatorial 2-aryl group to the trans form, with this group in the less stable axial conforma-

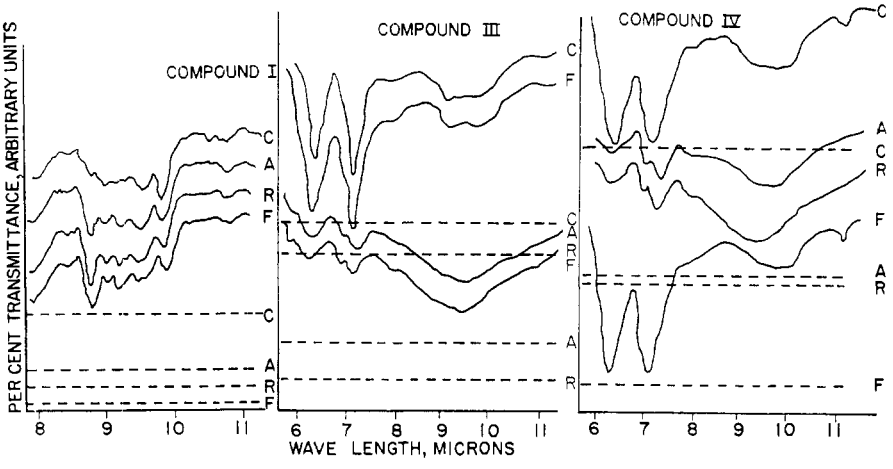


Figure 2. Infrared absorption spectra of compounds I, III, IV, respectively, at four different stages of processing

Stages:
F = fermentation
R = roasting
A = alkalization
C = conching
Dashed lines represent abscissas corresponding to each curve

tion. Some such change as this is what probably occurs during the alkalizing or conching processes. We have not isolated enough material to permit determining optical rotation. Such a measurement would be desirable.

Since the other two compounds studied have unknown structures, it is not possible to ascribe particular structural changes to them. Their properties generally indicate that they are similar to the catechins and that therefore similar conformational changes probably occur with them during processing. However, the order of change is just the reverse of the order observed with (−)-epicatechin. Apparently the high temperature of roasting has no effect on this compound which is already in the stable cis, aryl-equatorial conformation. On alkalizing or conching the more unstable form appears. By analogy, compounds III and IV may start out in an unstable configuration which changes to a stable configuration during roasting and then back again to the unstable form during conching, but not during alkalizing.

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FRUIT JUICE CONSTITUENTS

Determination of Linaloöl and α-Terpineol in Florida Orange Products

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The principal volatile constituents of orange peel juice that contribute to its bitter flavor have been identified as linaloöl and α-terpineol. A gas-liquid chromatographic method has been developed for their determination in juices and peel oils and examples illustrating possible applications are given. Taste levels of these substances were determined in orange juices.

In recent years, there has been a substantial increase in the yield of juice from a given amount of oranges. Much of it is due to the increased efficiency of mechanical extractors, less good juice being discarded with peel and pulp. There is always some possibility, however, that when extraction pressures are carried to extremes, some juice from the peel may find its way into the final product. This possibility has been noted by Pobjecky (*3*).

Swift and Veldhuis (*5*) reported on a comparison of a number of components and properties in commercial juices, segment juices, and peel juices. Included were soluble solids, acidity, pH, Brix-acid ratio, reducing sugars, sucrose, total sugars, soluble pectic substances, ascorbic acid, flavonoids, and diacetyl as well as viscosity, odor, and fluorescence. This study showed that the general composition of these three types of juice differs considerably, but it was not intended to show which specific compounds might contribute to off-flavors. It was observed that peel juice was bitter and was detectable, when added to the extent of 3 to 5%, to good orange juice.

The present investigation was undertaken for the purpose of identifying certain peel juice constituents capable of contributing bitterness or other off-flavors and of devising means for their determinations. Only relatively soluble, steam-volatile substances are considered in this paper. The principal flavor-influencing constituents found were linaloöl and α-terpineol.

Linaloöl and α-terpineol have both been identified in single-strength orange and grapefruit juices by Kirchner and Miller (*1, 2*). Linaloöl has been found to be a constituent of domestic orange peel oils by Poore (*4*). The present work verifies these findings by different means and describes a method for simultaneous determination of these substances. Some examples of the application of the method are given and certain implications are presented.

Experimental

Identification of Linaloöl and α-Terpineol. Peel juice used in this study was obtained from a commercial plant where peel from juice extractors was being treated for the recovery of peel oil. Juice was squeezed from the peel between fluted rolls and centrifuged to recover oil. This peel juice was taken to the laboratory and clarified with a continuous precoat filter using Hyflo-Supercel filter aid. The filtrate obtained was a bright yellow, apparently homogeneous solution. When a portion of this was distilled, the distillate extracted with ether, and the solvent evaporated from the extract, an oil was obtained in small yield. A portion of this was introduced into an Aerograph gas-liquid chromatographic (GLC) apparatus and it gave two prominent peaks in a region where a normal peel oil showed only very small ones. The areas of the large hydrocarbon peaks of normal peel oils were greatly reduced for the extract. Several runs of the extract were made on the GLC apparatus and the effluent gases corresponding to each of these two large peaks, linaloöl and α-terpineol, respectively, which eluted after the peel oil hydrocarbons were passed through a condensing system and the fractions were accumulated. These were examined as