From the 1090 g. of hulls, 1.188 g. of the pigment was obtained. This corresponds to a minimal yield of 0.109%.

Color Tests.—The isolated material gave the usual flavonol color tests.

The colors observed in ultraviolet light when the "chromogenic reagent" technique of Wender and Gage[10] was applied, agree with those obtained by these workers. An authentic sample of quercitrin gave identical results.

Absorption Spectrum.—A solution containing 1.4 mg. of the isolated pigment per one hundred ml. of 95% ethanol was used for the determination of the ultraviolet absorption spectrum. The pure solvent was used as a blank in the Beckman Model DU spectrophotometer. The ultraviolet absorption spectrum of authentic quercitrin of like concentration was also determined. Both gave absorption maxima at 255 and 352.5 mμ. Minima were observed at 235 and 280 mu.

Paper Chromatographic Analysis.—When the isolated substance was subjected to the paper chromatographic technique of Wender and Gage,[10] R_f values were observed which corresponded to those of quercitrin in the solvent systems used (phenol, ethyl acetate, *n*-butanol–acetic acid).

Hydrolysis Products.—The pigment was hydrolyzed by boiling a very small portion of it with 0.6% sulfuric acid for one and one-half hours.

L-Rhamnose was identified in the filtrate from the hydrolysis mixture by paper chromatography in *n*-butanol–acetic acid–water. The aglycone, quercetin, was likewise identified in the reaction mixture by this method. It was possible to distinguish quercetin from unhydrolyzed quercitrin by use of the chromogenic sprays.[10]

Acknowledgment.—This investigation was supported by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

Summary

Quercitrin has been found to be present in waste peanut hulls. A method for its isolation has been described.

(10) Wender and Gage, *Science*, **109**, 287 (1949).

NORMAN, OKLAHOMA RECEIVED MARCH 6, 1950

[CONTRIBUTION FROM THE CENTRAL LABORATORIES, GENERAL FOODS CORPORATION]

Isochlorogenic Acid. Isolation from Coffee and Structure Studies[1]

BY H. M. BARNES, J. R. FELDMAN AND W. V. WHITE

Early workers on the chemistry of coffee interpreted the green color formed upon the addition of ferric chloride to extracts of unroasted coffee as evidence for the presence of tannic acid. Prior to 1900, this fraction of coffee was known as "caffetannic acid" and was later called "chlorogenic acid" in view of the green color formed when solutions of the acid were made slightly alkaline and exposed to air. In 1907, Gorter[2] isolated the crystalline complex, potassium caffeine chlorogenate, from which he prepared the pure acid. The structure proposed by Gorter for the compound was later disproved by Freudenberg,[3] who proposed a structure that was subsequently established by Fischer and Dangschat[4] and is illustrated in Fig. 1.

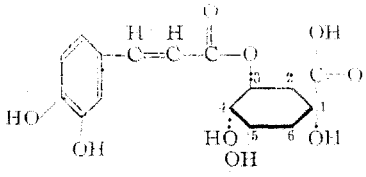


Fig. 1.—Chlorogenic acid.

The usual methods[5] of analysis indicated that the amount of this acid in coffee was about 6%, although neither Gorter[6] nor later workers could isolate more than two-thirds of this amount. Work in this laboratory has indicated that at least one-sixth of the material which shows up in the analyses as chlorogenic acid is markedly different in properties from that described by Gorter. This material has been isolated and purified, and the name "isochlorogenic acid" and the following structure are proposed for it

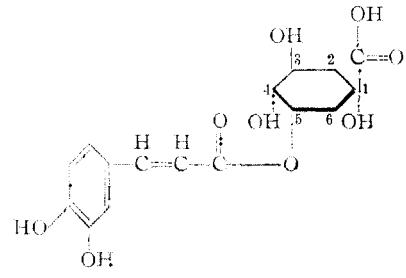


Fig. 2.—Isochlorogenic acid.

Isochlorogenic acid does not form a complex analogous to the crystalline potassium caffeine chlorogenate but is isolated from green coffee extracts by acidification with mineral acid and extraction with butyl acetate, a solvent in which chlorogenic acid is nearly insoluble. The crude acid is further purified by re-extraction of the acid from a solution buffered at *p*H 4.7.

The purity of the preparation is difficult to establish by classical criteria as neither the compound nor any derivative could be obtained in a crystalline form. The reactions tried included partial and exhaustive methylation, acetylation,

(1) Presented in part at the Atlantic City Meeting of the American Chemical Society in April, 1947.

(2) K. Gorter, *Bull. Dept. Agr. Indes Nierland*, **14**, 162 (1907); *Ann.*, **358**, 327–348; *ibid.*, **359**, 217–244 (1908).

(3) Karl Freudenberg, *Ber.*, **53B**, 232–239 (1920).

(4) H. O. L. Fischer and Gerda Dangschat, *Ber.*, **65B**, 1057–1040 (1932).

(5) Iodometric method, K. H. Slotta and K. Neisser, *ibid.*, **71B**, 1616 (1938); ultraviolet absorption method, R. G. Moores, D. L. McDermott and T. R. Wood, *Anal. Chem.*, **20**, 620 (1948).

(6) K. Gorter, *Ann.*, **379**, 110 (1911).

the formation of the isopropylidene derivatives, and salts with alkaloids and metals. To determine the degree of purity, the distribution techniques of Craig[7] were used. A marked solubility difference between the two isomers, a high degree of purity for a freshly prepared sample of isochlorogenic acid, and the existence of an equilibrium between an acid and neutral form were demonstrated. Optical rotation was used in the course of this work as a practical measure of purity for the several preparations.

Isochlorogenic acid like chlorogenic acid reduces silver and mercury salts, gives a catechol test with ferric chloride, and produces the same color[8] with nitrous acid in aqueous solution. Lead acetate precipitates both acids quantitatively. Both acids are soluble in alcohol and acetone but purified isochlorogenic acid, in contrast to the chlorogenic acid, is relatively insoluble in cold water and is very soluble in ethyl or butyl acetate.

Additional physical and chemical properties of the two compounds are shown in Table I.

TABLE I

PROPERTIES OF ISOCHLOROGENIC AND CHLOROGENIC ACIDS

		Isochlorogenic	Chlorogenic
Melting point, °C.		.....	206–210
Optical rotation, $[\alpha]^{20}D$		−230	−37
Molecular weight[a]		345	354
Molecular extinction (ϵ at 3225 Å.)		18,900	18,500
Neutralization equivalent		*ca.* 580	354–359
Saponification equivalent		163–185	176–183
Analyses,[b] %	Carbon	56.12[c]	54.3
	Hydrogen	5.18	5.1
Hydrolysis, % of theory	Caffeic acid	92	93
	Quinic acid	74	87

[a] Cryoscopic in dioxane. [b] Microanalyses by Mr. George Rupp of this laboratory. [c] Calculated for completely lactonized isochlorogenic acid, $C_{16}H_{16}O_8$: C, 57.1; H, 4.8, unlactonized $C_{16}H_{18}O_9$: C, 54.2; H, 5.1.

The concept that isochlorogenic acid is a position isomer of chlorogenic acid is based on the similarity of the molecular weights, ultimate composition, hydrolysis compounds, constant saponification equivalents, hydrogen consumption, and the relationships shown by the infrared and ultraviolet absorption spectra given in Figs. 3 and 4.

One of the significant differences between these isomers is the variability of the neutralization equivalent of the several preparations of isochlorogenic acid. This is explained on the basis of lactone formation between the hydroxyl at carbon 3 and the carboxyl at carbon 1 in the quinic acid portion of the molecule. Fischer[4] has stated in his proof of the structure of chlorogenic acid that no lactone should form with those hydroxyls at carbons 4 and 5 since they are *trans* to the carboxyl. Anhydrous chlorogenic acid can be heated *in vacuo* over phosphorus pentoxide at 140° without change in properties. Under these same conditions, however, the neutral equivalent of isochlorogenic acid shows a marked increase as given in Table II.

(7) O. B. Williamson and L. C. Craig, *J. Biol. Chem.*, **168**, 687–697 (1947); G.O. Rudkin and J. M. Nelson, THIS JOURNAL, **69**, 1470–1475 (1947).

(8) Hoepfner, *Chem. Ztg.*, **56**, 991 (1932).

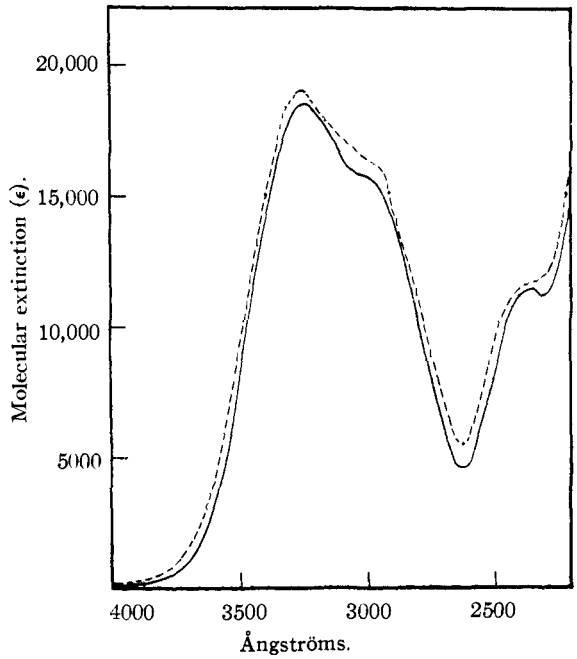


Fig. 3.

TABLE II

PERCENTAGE CHANGE IN CONSTANTS OF ISOCHLOROGENIC ACID UPON HEATING[a]

	8 Hours	16 Hours
Molecular extinction (ϵ at 3325 Å.), %	− 6	− 8
Optical rotation $[\alpha]^{20}D$	−13	−24
Neutralization equivalent	+19	+47

[a] Corresponding values at the end of a four-hour heating period are considered the initial point for the calculation of percentage change.

Since a hydroxyl at carbon 3 is *cis* to the carboxyl group, an incomplete conversion to a γ-lactone comparable to the lactone of quinic acid is indicated. This conclusion is supported by the small change in the extinction values relative to the change in neutralization equivalent shown in Table II. It is further supported by the saponification equivalents shown in Table I which were all close to the value for chlorogenic acid in spite of the fact that the neutralization equivalents of the initial preparations covered a wide range. Quinic acid, derived from isochlorogenic acid by acid hydrolysis of the hydrogenated product, is also partially lactonized. Chlorogenic acid under these conditions gives the free acid. No explanation is offered for any of the observed optical rotations.

Since the hydroxyl at carbon 3 is available for lactonization, the remaining points of attachment for the depside linkage are the hydroxyls at car-

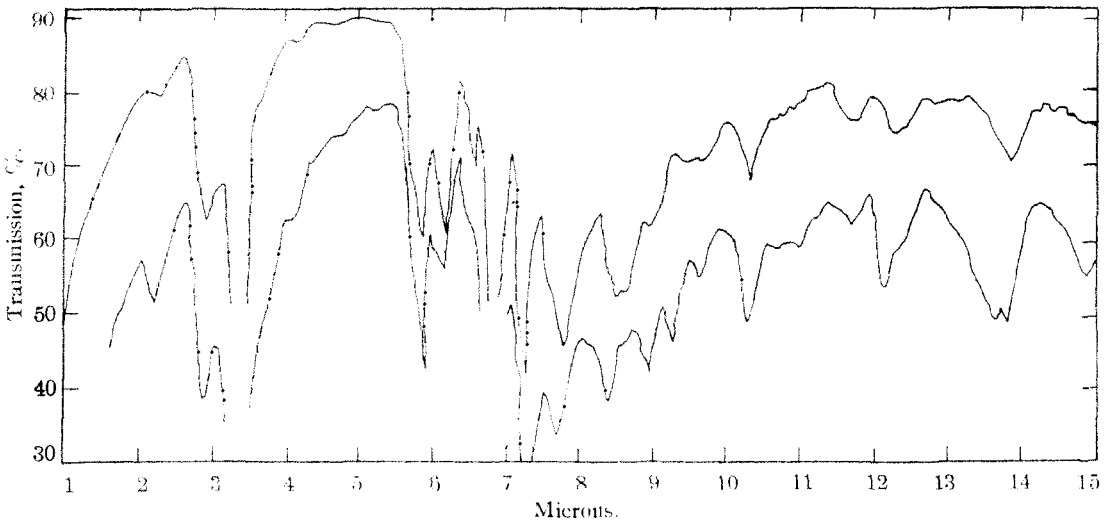


Fig. 4.—Upper curve, isochlorogenic acid; lower curve, chlorogenic acid.

bons 1, 4 and 5. It is inferred from the following experiments that this ester grouping is at carbon 5. An increment in electrical conductivity in boric acid is shown by both chlorogenic and isochlorogenic acids (Table III). As adjacent hydroxyls must be present for the formation of cyclic boric acid ethers, the hydroxyl at carbon 4 must be free in both compounds.

TABLE III

MOLAR CONDUCTANCE (IN RECIPROCAL OHMS) AT *N*/1024 DILUTION IN *N*/8 BORIC ACID

	Sample	Sample with boric acid	Increment
Chlorogenic acid	125.1	188.7	63.6
Isochlorogenic acid	81.3	152.8	71.5
Hydrocaffeic acid	43.1	43.0	None

The choice between carbons 1 and 5 is made on the basis of the rates of oxidation with periodic acid of the vicinal hydroxyls of chlorogenic, isochlorogenic and quinic acids at 4° using the method of Pohle, Mehlenbacker and Cook.[9] In Fig. 5, the assumption is made that the values for ethyl caffeate may be subtracted from those of chlorogenic and isochlorogenic acid to eliminate the effect attributed to the catechol group. The hydroxyl at carbon 1 is eliminated as the point of attachment for the depside linkage as an oxidative curve corresponding to quinic acid rather than chlorogenic acid would have been obtained. The slower rate of oxidation of isochlorogenic acid is attributed to the limiting factor of the rate of opening of the lactone ring under the conditions of the experiment. Other factors which affect these rates are the steric relationship of the caffeic acid group and the *trans* arrangement of the hydroxyls, but the configuration is determined by the total oxygen consumed, which is approximately one mole each for chlorogenic and isochlorogenic acid. These results indicate vicinal hydroxyls in isochlorogenic acid. Coupled with the proof of lactonization, the most probable structure for isochlorogenic acid is that of 5-caffeylquinic acid.

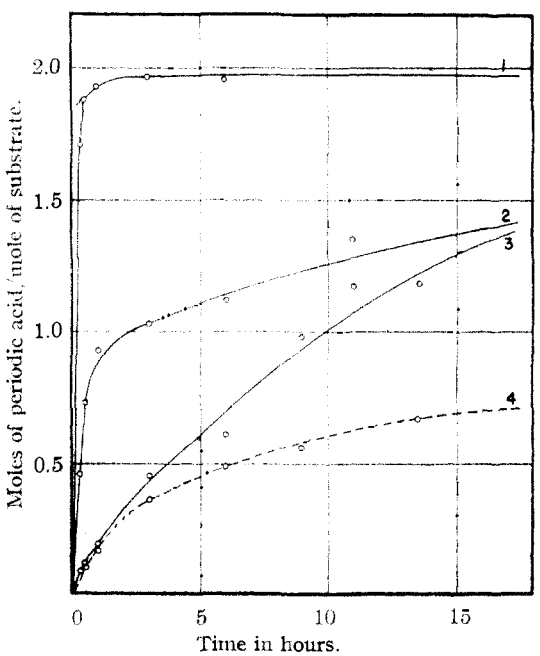


Fig. 5.—1, Quinic acid; 2, chlorogenic acid; 3, isochlorogenic acid; 4, ethyl caffeate.

Experimental

Isolation of Crude Isochlorogenic Acid.—One kilogram of flaked green coffee and 6 liters of 70% isopropyl alcohol are stirred for three hours at room temperature. The slurry is filtered through a large Buchner funnel, the filter cake stirred with another 6-liter portion of 70% isopropyl alcohol for fifteen minutes and filtered again. The filtrates are combined (total volume about 8 liters) and concentrated by distillation at reduced pressure to 2 liters. After standing overnight in the refrigerator, the concentrate is filtered with the aid of Celite to remove the precipitated impurities such as fats and waxes.

(9) Pohle, Mehlenbacker and Cook, *Oil and Soap*, **22**, 115 (1945).

The concentrate is acidified to *p*H 2.5 with sulfuric acid and extracted with three one-liter portions of butyl acetate. The combined solvent, washed with three 250-ml. portions of water, is concentrated to the point where solids appear. The crude acid is precipitated at this point by the addition of 10-20 volumes of chloroform, filtered, and the precipitate dried overnight at 70° *in vacuo;* yield based on the original weight of green coffee is approximately 0.75% (n. e., 541; $[\alpha]^{20}D$ −185°).

Purification of Isochlorogenic Acid.—The crude acid appeared to be a mixture as it could not be obtained in a crystalline form. A 10-mg. sample was therefore distributed over an 8-transfer Craig separation with butyl acetate and aqueous 2 *M* phosphate, *p*H 5.2, as the two solvents. The fraction of the solute present in each plate was calculated from the optical density at 3250 A. Independently it was shown that all fractions thus obtained had the same absorption coefficients. Therefore, optical density was a valid measurement of the weight of material in each plate. The results are shown in Fig. 6.

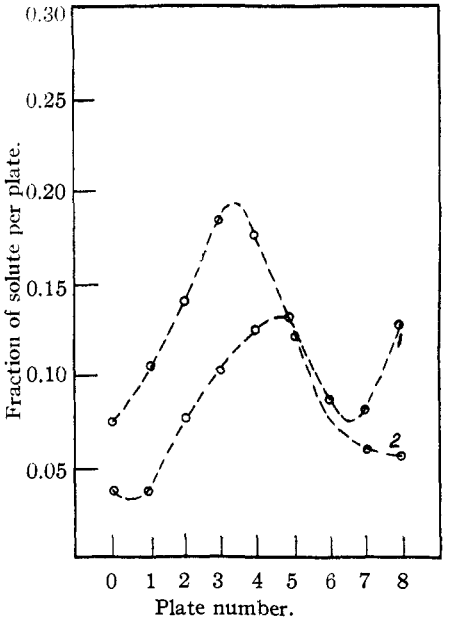


Fig. 6.—1, Crude acid, *p*H 5.2; 2, plates 0-6; Curve 1, redistributed at *p*H 5.2.

Plates 0-6 (Curve 1, Fig. 6) were again distributed at *p*H 5.2 over an 8-plate separation (Curve 2, Fig. 6). At *p*H 4.7, all of this material can be separated from the impurity (chlorogenic acid), plates 7 and 8, Curve 1, and is found in the first three plates. This behavior is somewhat similar to that of the chlorogenic-like materials isolated by Rudkin and Nelson[7] from the sweet potato. Unlike their sample, isochlorogenic acid could not be separated into several components over a 24-transfer distribution.

The following method was thereupon adapted to the purification of the crude acid. The acid from the green coffee extract is put into the first of three 1000-ml. separatory funnels, each containing 500 ml. of butyl acetate. Three liters of aqueous 2 *M* phosphate buffer, *p*H 4.7, are passed in 500-ml. portions consecutively through the three funnels. The butyl acetate fractions are combined and washed three times with 200 ml. of water. The solids are isolated as described previously in the isolation of the crude acid. Recoveries greater than 60% (overall yield, *ca.* 0.5%) are thus effected having these constants: $[\alpha]^{20}D$ −210 to 212°, n. e., 580.

Separation into Neutral and Acidic Fraction.—A separation of the neutral component from the acid was effected by extraction from *p*H 6.3 buffer. The neutral material in the butyl acetate was found to have an optical rotation of $[\alpha]^{20}D$ −230°. The product, however, was still amorphous and when titrated potentiometrically in 50% (v./v.) ethanol, the neutralization equivalent (586-598) indicated the restoration of the initial equilibrium of acid and lactone. The optical rotation of this mixture was still −230°.

As a consequence, it was necessary to investigate the possibility of a mobile equilibrium. About 10 mg. of sample, $[\alpha]^{20}D$ −217°, n. e., 580, was put into 10 ml. of *p*H 6.3 phosphate buffer and extracted twice with an equal volume of butyl acetate. The butyl acetate was concentrated to one-half its volume and the solute immediately distributed over an 8-plate system with *p*H 5.2 buffer. All of the material (4-5 mg.) was found in the first three plates.

The buffer phase was acidified to *p*H 5.2 with phosphoric acid and immediately distributed over an 8-plate system. As shown in Fig. 7, a purity greater than 92% calculated from the theoretical curve[7] was achieved. However, if allowed to stand in buffer solution for 38 hours after acidification, the purity dropped to 79% (Fig. 6).

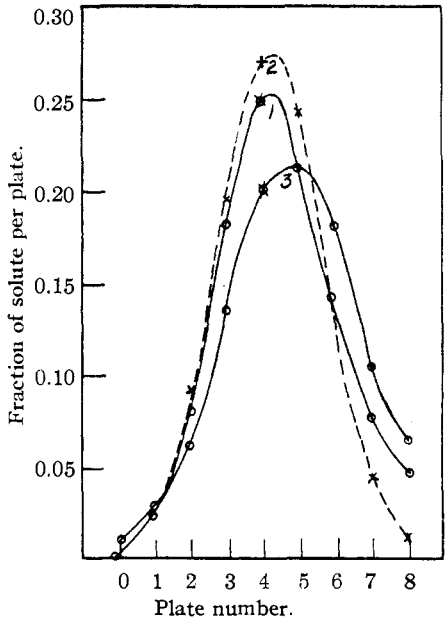


Fig. 7.—1, Experimental 2, theoretical 3, experimental after 38 hours.

It is indicated from this information that the acid and lactone forms are in an equilibrium determined by conditions of extraction and isolation. Failure to obtain a crystalline derivative is probably due to the presence in any preparation of these two forms.

Optical Rotation.—All optical rotations were measured at 2% concentration in 50% (v./v.) 95% ethanol-water using a 2-decimeter tube.

Absorption Measurements.—All ultraviolet measurements were made at a concentration of approximately 20 mg. of sample and 5 cc. of 95% ethanol per liter of water using a Model DU Beckman Quartz Spectrophotometer. Infrared measurements were made on a Model IR-2 Beckman Spectrophotometer, using a Nujol mull of the sample.

Saponification Techniques.—The semi-micro saponification method described by Huntress and Mulliken[10] modified by the use of potentiometric titration was employed to determine saponification equivalents.

(10) Huntress and Mulliken, "Identification of Pure Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 21.

To isolate caffeic acid, 5 g. of sample is dissolved in 50 ml. of *N* sodium hydroxide under an atmosphere of nitrogen and allowed to stand overnight at room temperature before acidification with an equivalent quantity of sulfuric acid. Caffeic acid is isolated from the saponification mixture by continuous extraction with ether, the ether removed by distillation, and the residue recrystallized from water, m. p. 218–220°.

After removal of the caffeic acid, quinic acid may be isolated by acetone extraction of the dry saponification residue or by formation of the copper acetate complex. Better yields are obtained by acid hydrolysis[11] of the hydrogenated chlorogenic or isochlorogenic acid, and the quinic acid is further identified by preparation of the 1,4,5-triacetylquinlactone.[12] The quinic acid derived from isochlorogenic acid is partially lactonized, but may be saponified to the correct titration value.

(11) A. Watanabe, *J. Pharm. Soc. Japan*, **56**, 71 (Abstracts in German) 13, (1936); *Chem. Zentr.*, **107**, I, 4901 (1936); *cf. C. A.* **31**, 2062 (1936).

(12) Erwig and Koenig, *Ber.*, **22**, 1157 (1889).

Hydrogenation.—Chlorogenic and isochlorogenic acids were hydrogenated in absolute ethanol using 5% palladium on charcoal as a catalyst. The suspension after hydrogenation was filtered through Celite, the filtrate taken to dryness, and the residue taken up in water and lyophilized. The theoretical amount of hydrogen was absorbed by each compound without significant change in neutralization equivalent. However, the optical rotation of hydrogenated isochlorogenic acid $[\alpha]^{20}D$ −34 to −39°, was approximately that of chlorogenic or hydrochlorogenic acid, $[\alpha]^{20}D$ −37°.

Summary

A new compound, "isochlorogenic acid," has been isolated from green coffee. Evidence is presented for the proposed structure, 5-caffeylquinic acid, a position isomer of chlorogenic acid.

HOBOKEN, N. J. RECEIVED JANUARY 14, 1950

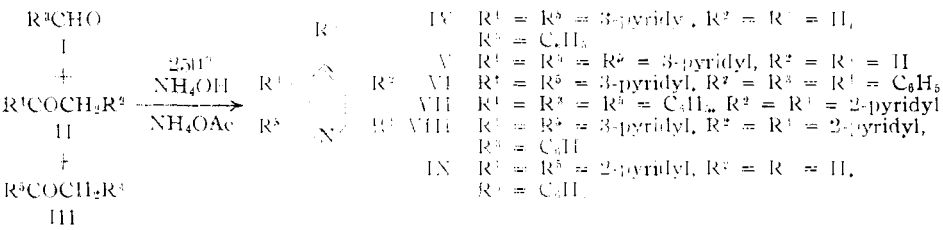
(CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS)

Pyridines. VI. Polypyridyls by the Chichibabin Synthesis[1]

BY ROBERT L. FRANK AND EDWARD F. RIENER

A recent investigation[2] of the Chichibabin synthesis of pyridines has demonstrated its particular usefulness for the preparation of symmetrically substituted aryl pyridines. The reaction has now been applied to the synthesis of pyridyl-substituted pyridines, or polypyridyls, some of which were desired for pharmacological studies.

Yields of the polypyridyls (IV–IX) were for the most part in the range 23–32%, comparable with those of the corresponding aryl pyridines[2] and satisfactory considering the simplicity of the procedure.



IV $R^1 = R^5 = 3$-pyridyl, $R^2 = R^4 = H$, $R^3 = C_6H_5$

V $R^1 = R^3 = R^5 = 3$-pyridyl, $R^2 = R^4 = H$

VI $R^1 = R^5 = 3$-pyridyl, $R^2 = R^3 = R^4 = C_6H_5$

VII $R^1 = R^3 = C_6H_5$, $R^2 = R^4 = 2$-pyridyl

VIII $R^1 = R^5 = 3$-pyridyl, $R^2 = R^4 = 2$-pyridyl, $R^3 = C_6H$

IX $R^1 = R^5 = 2$-pyridyl, $R^2 = R^4 = H$, $R^3 = C_6H_5$

It has been shown that condensations of this type may occur[2,3] in two ways, either with the aldehyde (I) reacting so as to introduce the group R^3 at the *gamma* position of the pyridine, as in the above equation, or with the aldehyde (I) reacting at the *alpha* position to give products represented by Structures IV-IX in which R^1 (or R^5) and R^3 are interchanged. Thus the problem of assignment of structure arises in those examples in which R^1 (or R^5) is not identical with R^3, namely, IV, VI, VIII and IX.

In two previous examples,[2] as shown by unequivocal syntheses of aryl-substituted pyridines, the group introduced by the aldehyde (I) has been found to appear at the *gamma* position of the pyridine formed. A similar unequivocal synthesis, the reaction of benzaldi-(2-acetylpyridine) with hydroxylamine to form 2,5-di-(2-pyridyl)-4-phenylpyridine (IX), has now provided evidence for the correctness of Structure IX for the identical compound prepared by the Chichibabin reaction. Attempts to prepare the 1,5-diketones which would react with hydroxylamine to form structures IV, VI and VIII have been unsuccessful, so we are forced to assign these structures only tentatively, although with rather good assurance of their correctness.

Further information on the scope and limitations of the Chichibabin synthesis has been provided by this study. For example, the starting materials II and III must be identical or the products are complex mixtures, as evidenced by a number of reactions described in the Experimental Part. An additional limitation is that when formaldehyde is used as Compound I (R^3 = H), only tars are obtained. Compound I must be an aromatic aldehyde.[2]

The preparation of two polypyridyls (VII and VIII) gave rise to a side reaction not encountered in our previous study.[2] These compounds were formed in yields of only 5.4%. The explanation lies in the structures of the starting materials, 2-

(1) For the previous communication on pyridine chemistry see Frank and Phillips, THIS JOURNAL, **71**, 2804 (1949).

(2) Frank and Seven, *ibid.*, **71**, 2629 (1949).

(3) Chichibabin and co-workers, *J. prakt. Chem.*, **107**, 109, 122, 129, 132, 138, 145, 151 (1924).