may not occur under conditions in which ketene itself decomposes because of stabilization by resonance of the radical formed after loss of a methyl hydrogen from methylketene.

The ratios of products to ketone decomposed of Table I show distinctly different results for runs 4, 7 and 5 compared to those of the other three runs. In the first three, the ethylene and hydrogen yields are definitely higher but butane yields are lower. It is suspected that the use of different A-H6 lights for these sets of three runs is largely responsible for these differences although variations in contact time and temperature must also have a bearing on the matter, too. The weightings of the four competing reactions do not show the temperature dependence that one would expect had other variables such as light intensities and contact times been kept constant.

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CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH]

Synthetic and Degradative Investigations of the Structure of Folinic Acid-SF

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Mechanisms of synthesis of folinic acid-SF from folic acid and a mechanism of degradation of folinic acid-SF to folic acid have been investigated with regard to the structure of this synthetic member of the folinic acid group. Folinic acid-SF is readily degraded to folic acid under relatively mild conditions similar to those used in the isolation of folic acid from liver.

A group of factors, termed the folinic acid group, has been reported to be more effective than folic acid in preventing the toxicity of x-methylfolic acid for *Lactobacillus casei*[3] and in promoting the growth of *Leuconostoc citrovorum* 8081.[4ab] Several members of the folinic acid group have been prepared recently by formylation of members of the folic acid group, reduction of the formyl derivatives and heating the reduced products.[5] One member of this group of factors, folinic acid-SF which is synthesized by this procedure from folic acid, has been recently described.[6] The barium salt of a factor apparently identical with folinic acid-SF has been obtained from a reaction mixture resulting from reduction of either folic acid or formylfolic acid in formic acid.[7]

Paper chromatography and development of bioautographs of liver digest have indicated that the natural factor, folinic acid, and a slower moving factor not identical with folic acid constitute the major part of the principles of liver which promote the growth of *Lactobacillus casei* in place of folic acid.[8] By paper chromatography of mixtures of varying concentrations of folic acid with the digests of liver, it was shown that the supplemented folic acid could be detected in such small amounts that the original digests could not have contained appreciable amounts of folic acid relative to the concentration of these other factors.[8] Consequently, since folic acid has been isolated in relatively good yields from such liver digests,[9a,b] it appears likely that folinic acid and related factors may have been converted into folic acid during the isolation process.

In the present work, mechanisms of synthesis of folinic acid-SF from folic acid and reconversion of folinic acid-SF to folic acid have been studied in order to elucidate the structure of folinic acid-SF and in order to demonstrate that folinic acid-SF can be converted into folic acid by the relatively mild conditions employed in the isolation procedures whereby folic acid is obtained from liver.[9a,b]

The elementary analysis of folinic acid-SF corresponds closely to $C_{20}H_{23}N_7O_7$.[6] The factor is formed by heating the reduction product of N^{10}-formylfolic acid, and can be reconverted to N^{10}-formylfolic acid as subsequently demonstrated. Thus, folinic acid-SF appears to be a tetrahydrofolic acid combined with a formyl group. This is adequately demonstrated by the direct formation of folinic acid-SF by treatment of tetrahydrofolic acid in acetic acid solution with formic acid at room temperature. Tetrahydrofolic acid is prepared by hydrogenation of folic acid in acetic acid solution in the presence of a platinum catalyst.[10] Because of the ease of reduction of pyrazines in comparison to pyrimidines, it has been generally assumed that the structure of this reduction product is 5,6,7,8-tetrahydrofolic acid,[10] but no direct proof has been presented.

(1) Eli Lilly and Co. Post-doctoral fellow.

(2) A portion of this investigation is from a thesis presented by Frank Lynn Barger in partial fulfillment of the requirement for Master of Arts degree, University of Texas, Jan., 1951.

(3) T. J. Bond, T. J. Bardos, M. Sibley and W. Shive, THIS JOURNAL, **71**, 3852 (1949).

(4) (a) T. J. Bardos, T. J. Bond, J. Humphreys and W. Shive, *ibid.*, **71**, 3852 (1949); (b) H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

(5) W. Shive, T. J. Bardos, T. J. Bond and L. L. Rogers, THIS JOURNAL, **72**, 2817 (1950).

(6) E. H. Flynn, T. J. Bond, T. J. Bardos and W. Shive, *ibid.*, **73**, 1979 (1951).

(7) J. A. Brockman, B. Roth, H. P. Broquist, M. E. Hultquist, J. M. Smith, M. J. Fahrenbach, D. B. Cosulich, R. P. Parker, E. L. R. Stokstad and T. H. Jukes, *ibid.*, **72**, 4326 (1950).

(8) W. Shive, Paper presented at 117th Meeting, American Chemical Society, Houston, March, 1950.

(9) (a) J. J. Pfiffner, S. B. Binkley, E. S. Bloom and B. L. O'Dell, THIS JOURNAL, **69**, 1476 (1947); (b) E. L. R. Stokstad, B. L. Hutchings and Y. SubbaRow, *ibid.*, **70**, 3 (1948).

(10) B. L. O'Dell, J. M. Vandenbelt, E. S. Bloom and J. J. Pfiffner, *ibid.*, **69**, 250 (1947).

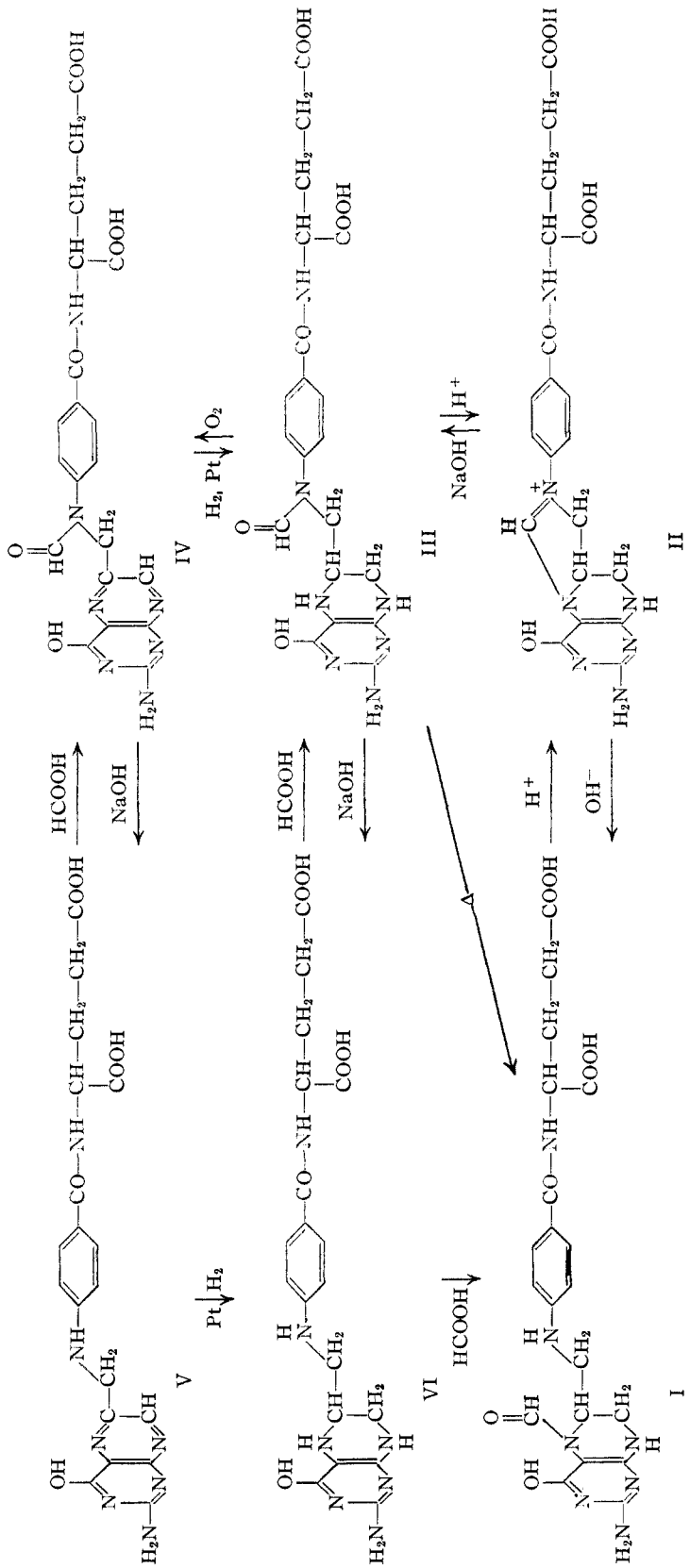


Fig. 1.—Interconversions of folic acid and folinic acid-SF.

Since titration data on folinic acid-SF indicate the presence of a weakly acidic group (pK_a 10.4),[6] it appears that the 4-hydroxy group of folic acid has remained in folinic acid-SF as a group capable of enolization. Consequently, considerable conjugation of double bonds in the pyrimidine portion of the molecule appears to have been retained in folinic acid-SF. Since dihydropyrimidines, *e.g.*, dihydrouracil or thymine glycol, have little if any absorption in the ultraviolet region,[11] it is difficult to account for the extremely high absorption of folinic acid-SF in the ultraviolet region without assuming that all three double bonds of the pyrimidine portion of folic acid remain intact in its conversion to folinic acid-SF. Since the benzene ring of the *p*-aminobenzoyl group of folic acid would not be reduced under the relatively mild condition whereby folinic acid-SF is formed, it appears that the pyrazine ring is the point at which the reduction occurs.

The point of attachment of the formyl group in folinic acid-SF does not appear to be simply at the N^{10}-position, since reduction of N^{10}-formylfolic acid to a tetrahydro derivative does not produce an appreciable yield of folinic acid-SF until the reaction mixture is heated. Thus, a rearrangement is indicated. If the rearrangement involves merely the migration of a formyl group to another position, the likely group to form such a stable formyl derivative is the N^5-group. By analogy, 2,4,5-triamino - 6 - hydroxypyrimidine, a similar compound, on formylation is converted into 5-formamido-2,4-diamino-6-hydroxypyrimidine.[12]

Direct evidence of the involvement of the N^5-position is obtained by destruction of folinic acid-SF in acid solution. This reaction appears to occur with the formation of an imidazoline ring (II, Fig. 1) with the single carbon unit bridging between the N^5- and N^{10}-positions. Destruction of the biological activity of folinic acid-SF occurs in acidic solution with the simultaneous formation of this product (II), which has an absorption maximum at 358 mμ as indicated in Fig. 2. The crystalline product[13] ob-

(11) L. F. Cavalieri and A. Bendich, THIS JOURNAL **72**, 2587 (1950).

(12) W. Traube and H. W. Dudley, *Ber.*, **46**, 3839 (1913); *cf.* W. Traube, *ibid.*, **33**, 1371, 3035 (1900); W. Wilson, *J. Chem. Soc.*, 1157 (1948).

(13) Crystalline acid degradation products from folinic acid-SF and from a crystalline barium salt which differed in its X-ray diffraction pattern from the barium salt of folinic acid-SF were obtained independently by Dr. E. H. Flynn of the Lilly Research Laboratories, Eli Lilly and Co., and by J. M. Ravel of the Biochemical Institute, respectively. Since the two acid degradation products differ to some extent in their X-ray diffraction patterns and since there is some doubt as to their identity, the present paper deals only with the product obtained from folinic acid-SF, and the authors are indebted to Dr. E. H. Flynn for furnishing the crystalline preparations mentioned in this paper.

tained from the reaction mixture does not differ appreciably in absorption spectrum from the crude product indicating essentially a total conversion to this acid degradation product (II). Titration data on the crude reaction mixture or the crystalline product indicate that a strongly basic group exists in the degradation product (II).

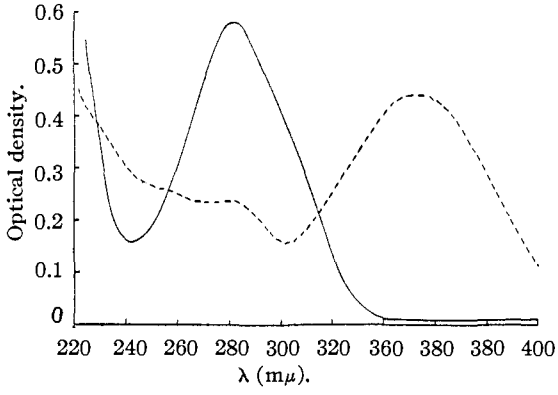


Fig. 2.—Acid degradation of folinic acid-SF; folinic acid-SF (10 γ per cc.): ——, in 0.1 *N* sodium hydroxide, - - - - - -, in 0.1 *N* hydrochloric acid after two hours.

The conversion of folinic acid-SF (I) to this acid degradation product may be represented by the equations indicated in Fig. 1. The structure of folinic acid-SF is tentatively assumed to be 5-formyl-5,6,7,8-tetrahydrofolic acid (I) in order to interrelate the various intermediates which are formed in the conversion of folic acid to folinic acid-SF by various methods and in the reconversion of folinic acid-SF to folic acid. The formation of the imidazoline structure of the product (II) is analogous to the formation of such derivatives from monoformyl derivatives of β-secondary diamines such as the N,N′-dimethyl derivative of *o*-phenylenediamine. For example, methylation of benzimidazole produces a compound identical with that obtained by acid treatment of the formyl derivative of the N,N′-dimethyl-*o*-phenylenediamine.[14,15] Since folinic acid-SF contains two aromatic groups attached to a 1,2-diamine, the monoformyl derivative of N,N′-diphenylethylenediamine (N-(2-anilinoethyl)-formanilide) serves better as a model for the study of the effect of acid on such compounds. The ultraviolet absorptions of this formyl derivative in acidic and basic solutions are indicated in Fig. 3. The absorption maximum at *p*H 2 is approximately 312 mμ while at *p*H 13 the maximum occurs at approximately 238 mμ. While this shift in the absorption maximum occurs rapidly in changing from strongly acid to strongly basic solutions, it occurs slowly at intermediate *p*H values. For example, at *p*H 4 the maximum at 238 mμ slowly declines with a corresponding increase in the absorption at 312 mμ showing that the shift of the maximum is not an immediate effect of *p*H but represents a more drastic change in the molecular structure. The change in the formyl derivative occurs very rapidly at *p*H values below 3. However, the reverse

(14) O. Fischer, *Ber.*, **34**, 936 (1901).

(15) C. W. Smith, R. S. Rasmussen and S. A. Ballard, THIS JOURNAL, **71**, 1082 (1949).

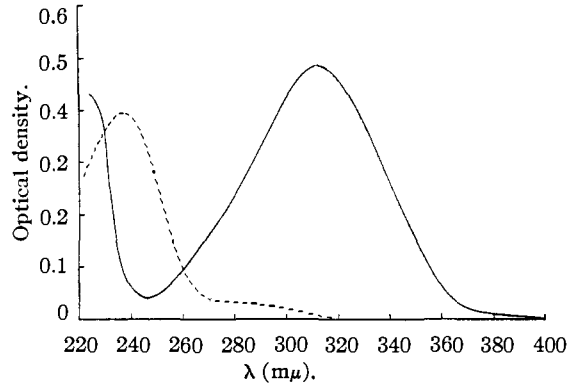
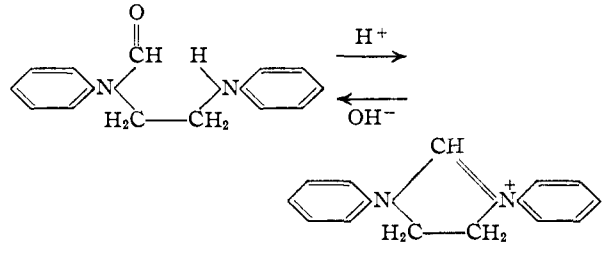


Fig. 3.—Conversion of N-(2-anilinoethyl)-formanilide to 1,3-diphenyl-2-imidazolinium chloride; N-(2-anilinoethyl)-formanilide (10 γ per cc.): - - - - - -, in 0.1 *N* sodium hydroxide; ——, in 0.1 *N* hydrochloric acid.

change does not occur rapidly except at *p*H values above 7. This shift in the point of maximum absorption suggests that a more highly conjugated system results. The following interrelationship appears to exist for this system.



The opening of the heterocyclic ring of the above compound in basic solution would result in the formation of the original formyl derivative since attachment of the formyl derivative to either nitrogen results in the formation of the same compound. However, the opening of the heterocyclic ring of the degradation product (II) of folinic acid-SF could proceed in two directions producing either an N^{10}- or N^5-formyl derivative. Treatment of the acid degradation product with dilute alkali results in some regeneration of folinic acid-SF (I); however, the major product appears to be the N^{10}-formyl derivative (III).

If solutions of the product (II) are adjusted to a *p*H above 9.5 aerobically and the basic solution is again acidified to *p*H 2, the characteristic absorption of the original product (II) does not return (Fig. 4). However, in such an alkaline solution one-half of a molecular equivalent of oxygen is rapidly consumed by the reaction mixture with the formation of a product which slowly oxidizes in acidic solution to N^{10}-formylfolic acid (IV). Apparently, the opening of the postulated heterocyclic ring in the degradation product appears to favor the formation of N^{10}-formyltetrahydrofolic acid (III) which is rapidly oxidized to the dihydro derivative.

However, N^{10}-formyltetrahydrofolic acid (III) would be expected to form the acid degradation product (II) in acidic solution. Since the N^{10}-formyltetrahydro derivative was extremely labile to oxidation by air, the absorption spectrum of the

product was determined *in vacuo* by adding the acid degradation product (II) to a buffer at *p*H 9.8, and the resulting product (III) *in vacuo* was treated at *p*H 2 with another buffer to obtain the absorption spectrum of the original compound. These results are indicated in Fig. 4.

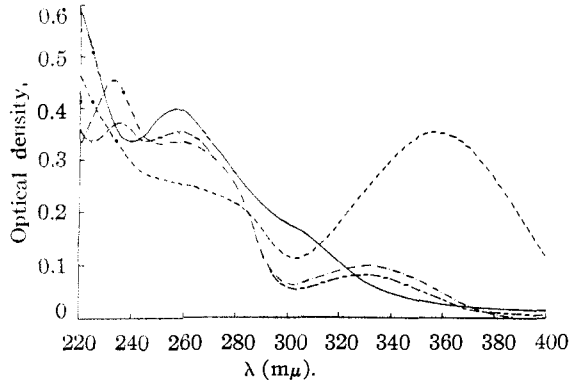


Fig. 4.—Interconversions of the acid degradation product of folinic acid-SF (II) and N^{10}-formyltetrahydrofolic acid (III) and the effect of oxygen on N^{10}-formyltetrahydrofolic acid: ——, acid degradation product (II) (10 γ per cc.) placed in buffer at *p*H 9.8 (anaerobic conditions, *in vacuo*); - - - - - -, solution at *p*H 9.8 (anaerobic conditions) adjusted to *p*H 2; -·-·-, acid product (II) (10 γ per cc.) placed in buffer at *p*H 9.5 (aerobic conditions); - —· — · - —, solution at *p*H 9.5 (aerobic conditions) adjusted to *p*H 2.

Ascorbic acid was found to be effective in preventing the oxidation of the N^{10}-formyltetrahydro derivative (III) even in strongly alkaline solution so that the absorption maximum of the acid degradation product (II) in the 350–360 mμ region reappears on acidification. In order to demonstrate that the product (II) is not merely the hydrochloride of N^{10}-formyltetrahydrofolic acid (III), the acid degradation product (II) protected by ascorbic acid was placed in a buffered solution at *p*H 6.9. At this *p*H, the absorption maximum at 350–360 mμ decreased slowly, requiring 10 to 20 minutes for the loss of the absorption in this region. When the solution was readjusted to *p*H 1.9, the 350–360 mμ maximum slowly reappeared and, after standing 10 minutes, approached the intensity of the acid degradation product (II). At intermediate *p*H values, the interconversions of these products occur at much slower rates. In this behavior the interrelationship of the acid degradation product (II) and N^{10}-formyltetrahydrofolic acid (III) is the same as that of the model compounds derived from N,N′-diphenylethylenediamine.

Folinic acid-SF can be destroyed in acidic solution, then the solution can be made alkaline in the presence of ascorbic acid to obtain N^{10}-formyltetrahydrofolic acid (III), and this product can be autoclaved in neutral solutions to obtain folinic acid-SF again in yields up to 40%. This series of transformations can also be carried out under anaerobic conditions in the absence of ascorbic acid. In addition, long standing in neutral or slightly alkaline solutions allows the conversion of the N^{10}-formyltetrahydro derivative (III) to folinic acid-SF in good yields. In 0.1 *N* sodium hydroxide, under anaerobic conditions the acid degradation product (II) is converted rapidly to mainly N^{10}-formyltetrahydrofolic acid which slowly hydrolyzes with the formation of tetrahydrofolic acid (Fig. 5). Small yields of folinic acid-SF, which is more stable to alkaline hydrolysis, are obtained simultaneously. Tetrahydrofolic acid[10] and folinic acid-SF have similar absorption maxima and minima.

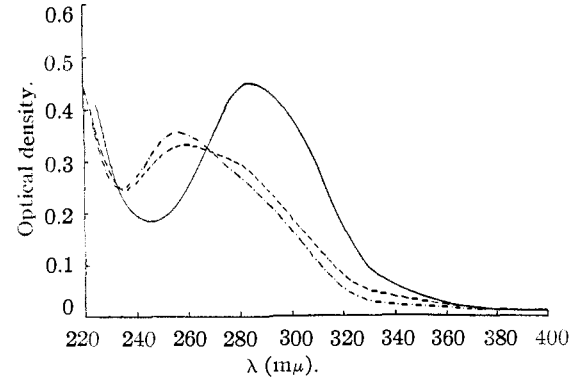


Fig. 5.—Effect of alkali on acid degradation product (II) of folinic acid-SF; acid product (II) (10 γ per cc.) in 0.1 *N* sodium hydroxide (anaerobic conditions, *in vacuo*): -·-·-, immediately; - - - - - -, after standing 2 hours; ——, after standing 23 hours.

The reduction of N^{10}-formylfolic acid to the tetrahydro derivative and acidification of the reaction mixture to *p*H 2 in the presence of ascorbic acid results in the formation of a mixture which has an absorption maximum at 358 mμ. Formylation of folic acid (V) to form N^{10}-formylfolic acid (IV), reduction to N^{10}-formyltetrahydrofolic acid (III) and rearrangement, upon heating, to the N^5-formyltetrahydrofolic acid (I) appear to be one mechanism of synthesis of folinic acid-SF (I).

Reduction of N^{10}-formylfolic acid (IV) appears to stop at the dihydro stage under certain conditions. Autoclaving of such a reaction mixture, particularly with ascorbic acid, results in material possessing folinic acid activity. Whether this is the result of a dismutation or merely reduction of part of the material to the tetrahydro stage while the remainder remains intact has not been determined. However, some experiments with the N^{10}-formyldihydrofolic acid obtained from the acid degradation product (II) have indicated that active material can be obtained by autoclaving this product with ascorbic acid in neutral solution.

The acid degradation product (II) slowly oxidizes at *p*H 2 with the consumption of approximately one molecular equivalent of oxygen within 24 hours. The product obtained on neutralization has an ultraviolet absorption corresponding to N^{10}-formylfolic acid (IV) (Figs. 6 and 7) and migrates on paper chromatograms at a rate corresponding to N^{10}-formylfolic acid. On standing in 0.1 *N* sodium hydroxide for 24 hours, the formylfolic acid thus obtained is hydrolyzed to a product which has the same ultraviolet absorption spectrum as folic acid (V) (Figs. 6 and 7), migrates on paper chromatograms at the same rate as folic acid, and, in crystalline form, has an X-ray diffraction pattern identical with that of folic acid. By this procedure, folinic acid-SF is converted by a series

of relatively mild changes in *p*H to folic acid. If natural folinic acid is likewise as easily transformed into folic acid, it appears probable that at least some of the folic acid isolated from liver is derived from folinic acid during the isolation process.

The formation of folinic acid-SF by reduction of folic acid to the tetrahydro derivative (VI) and formylation under a variety of mild conditions produces good yields of the active principle. Reduction of folic acid in formic acid solution and separation of the active principle as the barium salt without any heat treatment results in the isolation of a barium salt which is identical with the barium salt of folinic acid-SF. The optimal yield of folinic acid-SF in *formic acid* solution without heat treatment is best obtained under relatively mild conditions of temperature, etc., presumably because folinic acid-SF is destroyed by further formylation to form a diformyl derivative. In addition, simultaneous formation of the N^{10}-derivative occurs, since autoclaving the reaction product in neutral *aqueous* solution increases the yield of active material.

In this series of transformations, an intermediate hydroxymethylene bridge might be expected to result during the migration of the formyl group in either direction. Two stereoisomeric configurations are possible for such a bridge. The possibility that such hydroxymethylene bridges might represent the stable configuration of some such formyl derivatives cannot be excluded. Literature on more or less analogous pseudo bases formed by the action of alkali on certain cyclic quaternary ammonium salts has been very extensive.[15-18] Ring structures with hydroxymethylene bridges were early suggested for the formyl and other acyl derivatives of aromatic β-secondary diamines.[14,17,18] However, infrared absorption studies of some acyl compounds of similar structure indicate that these compounds exist, at least partially, in the open amide form rather than solely as ring structures with hydroxymethylene bridges.[15]

From certain of the synthetic reaction mixtures, a barium salt of a factor which has the same ultraviolet absorption spectrum and biological activity similar to folinic acid-SF has been isolated; however, the X-ray diffraction pattern of this factor is not identical with the pattern of the barium salt obtained from folinic acid-SF or the product from reduction of folic acid in formic acid solution. Since in the formation of folinic acid-SF, at the reductive stage, a new asymmetric center is created, two possible stereoisomers of the compound exist. This new crystalline form may be stereoisomeric with the barium salt of folinic acid-SF, may merely be a crystal modification, or may possibly have other structural differences.

Acknowledgments.—The authors are indebted to Margaret S. Lewis of The Biochemical Institute for much of the microbiological testing, to William Brown, H. L. Hunter and W. J. Schenck of the Lilly Research Laboratories, Eli Lilly and Co., for the analyses of the acid degradation product, to Dr. R. J. Herberg of the Lilly Research Laboratories for the potentiometric titration data, to Dr. E. H. Flynn for samples of folinic acid-SF as the crystalline free acid and for certain data on the acid degradation product, and to Dr. S. H. Simonsen of the Department of Chemistry and Dr. S. F. Kern of the Lilly Research Laboratories for some of the X-ray diffraction patterns.

(16) H. Decker, *Ber.*, **25**, 443 (1892).

(17) S. Niementowski, *ibid.*, **20**, 1874 (1887).

(18) O. Fischer and M. Rigaud, *ibid.*, **34**, 4202 (1901); **35**, 1258 (1902).

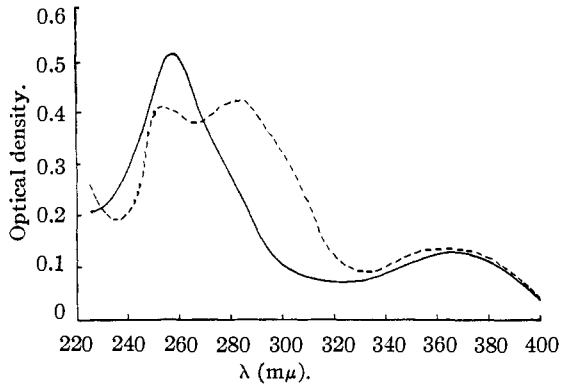


Fig. 6.—Conversion of folinic acid-SF to N^{10}-formylfolic acid and folic acid; acid degradation product (II) of folinic acid-SF after oxidation at *p*H 2 (10 γ per cc.) in 0.1 *N* sodium hydroxide: ——, immediately; - - - - - -, after standing 24 hours.

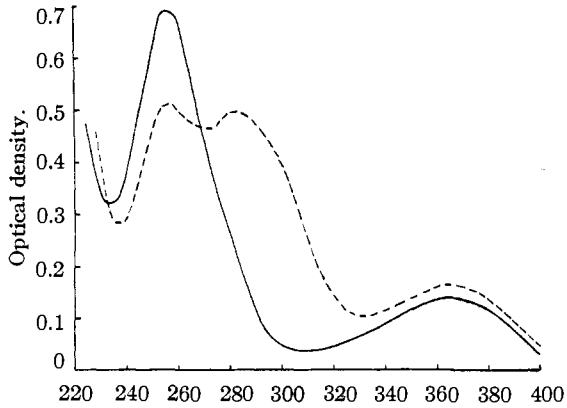


Fig. 7.—Hydrolysis of N^{10}-formylfolic acid to folic acid; N^{10}-formylfolic acid (10 γ per cc.) in 0.1 *N* sodium hydroxide: ——, immediately; - - - - - -, after standing 24 hours.

Experimental

Synthesis of Folinic Acid-SF from Folic and Formylfolic Acid.[19] Hydrogenation Experiments.—Either folic acid or formylfolic acid was dissolved in the solvent other than water indicated in Table I, 50 to 75 mg. of platinum oxide catalyst was added, and the mixture was stirred rapidly under hydrogen at atmospheric pressure until the indicated amount of hydrogen was absorbed. The reaction mixture was then evaporated to dryness *in vacuo*. The residue was dissolved in water containing enough sodium carbonate to make the solution slightly basic. Part of the reaction mixture was assayed microbiologically for folinic acid activity without further treatment; part was assayed after autoclaving at 120° for one hour; part was assayed after autoclaving with ascorbic acid (10 mg. per cc.) under the same conditions. The results are shown in Table I.

Reduction of folic acid or formylfolic acid in aqueous solution was carried out at approximately *p*H 7 obtained by the addition of sodium carbonate. The solution was stirred under a hydrogen atmosphere in the presence of 75 mg. of platinum oxide catalyst until the indicated amount of hy-

(19) From a paper presented at Gordon Research Conference, A. A. A. S., Vitamins and Metabolism Section, New London, August, 1950.

TABLE I

THE EFFECT OF VARIOUS CONDITIONS ON THE FORMATION OF FOLINIC ACID-SF FROM FOLIC ACID AND FORMYLFOLIC ACID

Reagent, mg.	Solvent, cc.	Temperature of hydrogenation, °C.	Molecular equivalents of hydrogen consumed	Yield,[a] % Unheated	Yield,[a] % Heated	Heated with ascorbic acid
Folic acid, 500	Water,[b] 50	31	1–2	<0.3	<0.3	0.3[c]
Folic acid, 500	Formic acid, 98%, 50	[d]	2	4–7	16	32
Folic acid, 500	Formic acid, 98%, 50	31	2	2	28	32
Formylfolic acid, 500	Water,[b] 50	31	1–1.5	<0.3	12–20	28–35
	Water,[b] 50	31	2	<0.3	10–18	20–30
Formylfolic acid, 500	Formic acid, 98%, 50	31	1	2	32	36
	Formic acid, 98%, 50	[d]	2	2	20	30
Formylfolic acid, 500	Acetic acid,[e] 40	[d]	2	2	18	32
	Formic acid, 98%, 10					
Formylfolic acid, 500	Acetic acid, 45	31	2	2	22	34
	Formic acid, 98%, 5					
Formylfolic acid, 500	Formic acid, 88%, 50	31	2	<0.3	12	18

[a] Yield in terms of folinic acid-SF as a standard; test organism, *Leuconostoc citrovorum*. [b] *p*H adjusted to approximately 7. [c] Ascorbic acid on autoclaving forms some oxalic acid which presumably furnishes the single carbon unit. [d] Temperature maintained just above freezing point of reaction mixture. [e] Methanol may also be used effectively in combination with formic acid as a solvent. Yields as high as 40% have been obtained after the reaction mixture was heated with ascorbic acid.

drogen was absorbed. Ascorbic acid in amounts four times the weight of folic acid or formylfolic acid was added prior to hydrogenation rather than after hydrogenation. The results are indicated in Table I.

Folinic Acid Preparation from Tetrahydrofolic Acid.—Folic acid (50 mg.) dissolved in 50 cc. of acetic acid was hydrogenated in the presence of platinum oxide as a catalyst in a manner similar to that described by O'Dell, *et al.*[10] The tetrahydrofolic acid thus formed was treated with varying concentrations of formic acid and allowed to stand for several hours anaerobically at room temperature. The maximum yield is usually obtained with approximately 10% by volume of formic acid (98%) in the acetic acid solution. Yields up to 20% based on microbiological assay using folinic acid-SF as a standard have been obtained in this manner. N-Methylformanilide can also be used as a formylating agent under similar conditions with comparable results.

Isolation of a Folinic Acid Preparation from an Unheated Reaction Mixture.—Folic acid (2.5 g.) was dissolved in formic acid at 10° and hydrogenated in the presence of platinum oxide (75 mg.) at as low a temperature as could be obtained without freezing the solution. After consumption of two molecular equivalents of hydrogen, the reaction mixture was dried *in vacuo* in the frozen state. The dried product was 7 to 9% as active as crystalline folinic acid-SF.

Florisil Column.—A column, 25 mm. in diameter, of 50 g. of florisil[19a] (60–100 mesh) was prepared in dilute alcohol (80% by volume of 95% alcohol). The dried product prepared by reduction of folic acid in formic acid solution was dissolved in 25 cc. of water containing sufficient sodium bicarbonate to neutralize the resulting solution to *p*H 7. The aqueous solution was diluted with 100 cc. of alcohol. Some precipitation occurred, and the insoluble material was removed by centrifugation. The solution was added to the column, the residue was dissolved in a small amount of water and diluted again with four volumes of alcohol, and the solution resulting after removing some precipitate was added to the column following the first 125 cc. The column was developed with alcohol (80% by volume of 95% alcohol), and 100-cc. fractions were collected. The first three 100-cc. fractions contained essentially no activity, and the next three 100-cc. fractions contained material sufficient to account for the initial activity. These fractions were evaporated *in vacuo* to dryness yielding 1.3 g. of material which was approximately 18% as active as folinic acid-SF.

Magnesol Column.—Magnesol[20] (50 g., regular industrial grade) was suspended in 5% barium chloride in sodium acetate-acetic acid buffer at *p*H 4.5. The magnesol as a slurry was poured into a column, 25 mm. in diameter, and washed with water. The active material from the florisil column was dissolved in 10 cc. of water and barium chloride (1.3 g.) was added. Some precipitation occurred, and the mixture was centrifuged. The solution was added to the column, and the residue was treated with water several times with each washing being added to the column after each preceding washing entered the column. The column was developed with water, and the first 450 cc. contained essentially none of the active material. The next 200 cc. contained the major portion of the active material. Evaporation of these active fractions gave 167 mg. of relatively pure barium salt, which was crystallized from water containing a small amount of barium chloride. The barium salt was recrystallized three times to obtain essentially colorless prisms.

Anal. Calcd. for $C_{20}H_{21}N_7O_7Ba \cdot 5H_2O$: C, 34.37; H, 4.47; Ba, 19.65; H_2O 12.89. Found: C, 34.65; H, 4.79; Ba, 19.49; H_2O, 11.9, 14.1.

Salts of Folinic Acid-SF

Barium Folinate-SF.—Crude crystalline folinic acid-SF (50 mg.) was treated with slightly more than an equivalent weight of barium hydroxide in 3 cc. of hot water. On cooling, considerable amorphous material precipitated but crystals slowly formed. The slow rate of formation of the crystals suggests the possibility of an impurity in the folinic acid-SF preparation which tends to inhibit the formation of the barium salt. However, on recrystallization of the crystalline material, a product was obtained which had an X-ray diffraction pattern similar to that of the crystals derived from the unheated preparation described above. These results are presented in Table II. Many dried samples of these barium salts do not give an X-ray diffraction pattern even though the materials have been recrystallized several times from water and possess well defined crystal patterns. However, the crystals in the mother liquor do give a pattern, although it is somewhat diffuse. In addition, the degree to which the sample is dried appears to determine to some extent the characteristics of the crystals. Thus, moist samples from the same preparation give patterns which differ as indicated in columns 3 and 4 of Table II. In order to compare the two barium salts obtained by different procedures, the crystalline samples, moistened with the mother liquor, were sealed in cellulose acetate tubes, and an exposure period of approximately six hours was employed. During this time, considerable drying of the samples had occurred. The patterns obtained in this manner from the two samples were essentially identical, and the differences, in almost all instances, can be accounted for in the combined lines obtained from the moist and dried samples of the same preparation.

Preparation of Calcium Salt.—Folic acid was reduced in formic acid as described under the barium salt preparation. The reaction product after removal of formic acid was dissolved in water containing 10 g. of ascorbic acid at *p*H 7 and autoclaved for 1 hour. In order to remove the ascorbic acid, the reaction mixture diluted with an equal volume of

(19a) An adsorbent manufactured by The Floridin Co., Warren, Pennsylvania.

(20) An absorbent manufactured by Westvaco Chemical Division of Food Machinery and Chemical Corporation, South Charleston, West Virginia.

TABLE II

INTERPLANAR SPACINGS IN BARIUM FOLINATE-SF

Barium salt from crystalline folinic acid-SF Method 1,[a] Å.	Barium salt from unheated preparation Method 1,[a,b] Å.	Dried,[b] Å.	Wet,[b] Å.
		2.61	
		2.90	
3.05[c]	3.07[c]	3.04[c]	3.13
3.20	3.25		
3.36	3.49	3.49	3.41
3.76	3.78	3.77	
3.93	3.95	3.94	4.05
4.15		4.22	
	4.39		
4.57[c]	4.61	4.58[c]	
		4.81	4.77
	4.95		
5.11			5.10
5.67			
		5.85	
			6.08
6.28	6.25	6.21	
			6.54
6.77[c]	6.67[c]	6.68	
			7.00[c]
7.65	7.65		
			7.87
		8.02	
	8.73		
		9.84	
10.5[c]	10.5[c]	10.7	10.6[c]
12.1	12.4	12.8[c]	
13.7			

[a] Moist sample in sealed cellulose acetate tubing. Considerable loss of moisture occurred during exposure. [b] Same sample used for all three patterns. [c] Most intense lines.

alcohol was added to an alumina[21] column (120 g., pretreated with cyanide solution, washed with water and acetic acid, and activated at 180°) prepared in 50% alcohol. The active principle was eluted with 50% alcohol containing 5% by volume of concentrated ammonium hydroxide. The combined active fractions were evaporated to dryness *in vacuo*.

A magnesol column which was first used in the preparation of the calcium salt was prepared essentially as that described for the barium salt except that calcium chloride was employed in place of barium chloride. Active material from the alumina column, converted to the calcium salt, was chromatographed on the column. The active fractions from this magnesol column were evaporated, and the residue was fractionated by precipitation with dilute alcohol. The active precipitates were crystallized from water containing a small amount of calcium chloride and were recrystallized three times from water. The colorless prisms, after drying for two hours at 150°, were analyzed for calcium.

Anal. Calcd. for $C_{20}H_{21}O_7N_7Ca$: Ca, 7.84. Found: Ca, 7.80.

Degradation of Folinic Acid-SF

Acid Degradation Product (II) of Folinic Acid-SF.—The ultraviolet absorption spectrum of folinic acid-SF in 0.1 *N* sodium hydroxide is indicated in Fig. 2. If the solution is acidified anaerobically to *p*H 2 using a 0.2 *M* phosphate buffer and measurements made immediately, the extinction coefficient is essentially unchanged but the maximum shifts from 282 to 285 mμ. A slow reaction occurs at this *p*H with a gradual decrease in the maximum at 285 mμ and with the formation of a new maximum at 358 mμ. A maximal yield is obtained after approximately 18 hours. The reaction occurs more rapidly at *p*H 1, and the absorption spectrum of the final reaction mixture after two hours is depicted in Fig. 2. The rates at which the reaction occurs at *p*H 2 and *p*H 1 are indicated in Table III by the changes in ultraviolet absorption at 285 mμ and 358 mμ at various times.

TABLE III

EFFECT OF *p*H ON THE RATE OF DESTRUCTION OF FOLINIC ACID-SF

Time	Optical density[a] 285 mμ *p*H 1	285 mμ *p*H 2	358 mμ *p*H 1	358 mμ *p*H 2
8 min.	0.450	0.548	0.198	0.046
15 min.	.362	...	.304	...
30 min.	.288	...	.401	...
1 hr.	.246	0.340	.442	0.161
2 hr.	.246	.284	.448	.241
5 hr.	...	.239	...	.344
17 hr.	...	.205	...	.375

[a] Reaction mixture contains 10 γ per cc. of original folinic acid-SF.

If samples of folinic acid-SF are titrated to *p*H 2 or below and allowed to stand anaerobically until the destruction of the factor is complete, titration with alkali to *p*H 6 reveals that an uptake of almost an equivalent of acid has occurred. On standing in acidic solution at *p*H 2.8 and below, a crystalline product separates slowly from even relatively dilute solutions of folinic acid-SF. Samples of this acid degradation product were analyzed after drying *in vacuo* at 120° for two hours. *Anal.* Calcd. for $C_{20}H_{22}ClN_7O_6$: C, 48.83; H, 4.51; Cl, 7.21; N, 19.93; CHO, 5.90. Calcd. for $C_{20}H_{24}ClN_7O_7$: C, 47.11; H, 4.74; Cl, 6.95; N, 19.23; CHO, 5.69. Found: C, 48.01; 49.69, 49.21; 48.56; 48.45; H, 4.44; 5.14, 4.85; 5.47; 4.97; Cl, 6.90; N, 19.40; 19.60; 19.28; 19.66; 19.35; CHO, 6.08. The above analyses (different samples set off with semi-colons, duplicate analyses with commas) varied considerably because of the difficulty in obtaining a pure sample of this compound which is extremely labile and difficult to recrystallize. While the analyses do not clearly differentiate between the postulated cyclic imidazolinium salt and the hydrochloride of formyltetrahydrofolic acid, the carbon determination appears to exclude the latter structure. Although the hydrogen and nitrogen analyses correspond more closely to that of the hydrochloride of formyltetrahydrofolic acid, the values are not far removed from those of the postulated imidazolinium salt.

Potentiometric titration of the acid degradation product was carried out in the usual manner and showed the presence of four groups with pK_a values of 3.7, 4.8, 8.6 and 10.5. However, if the product after titration with alkali to *p*H 11.5 was titrated back to *p*H 2, only three groups with pK_a values of 3.8, 5.1 and 10.5 remained. Thus, during the process of titration the acid degradation product is destroyed with the formation of a new product which no longer possesses the basic group. If the acid degradation product is placed in a solution at *p*H 7 with 0.2 *M* phosphate buffer and immediately acidified to *p*H 2, the absorption maximum at 358 mμ corresponding to the acid degradation product is obtained; but if the acid degradation product is placed in a solution, buffered at *p*H 9.5 with 0.2 *M* phosphate, the absorption maximum at 358 mμ does not return in acidic solution. The absorption spectrum indicating this effect is shown in Fig. 4.

N^{10}-Formyldihydrofolic Acid.—A solution of 1 mg. of the acid degradation product of folinic acid-SF in 2.5 cc. of 0.1 *N* hydrochloric acid was placed in the main compartment of a Warburg vessel. Phosphate buffer (0.5 cc.) containing sufficient sodium hydroxide to neutralize the 2.5 cc. of 0.1 *N* acid to *p*H 9.6 was placed in the side arm, and 0.2 cc. of 10% potassium hydroxide was placed in the center well to absorb any traces of carbon dioxide which might be evolved. The vessel was attached to a Warburg manometer and placed in a 37° water-bath. The alkali containing buffer was tipped into the main compartment and the vessel was shaken. Four samples and a control without sample were treated similarly. The average oxygen uptake per micromole of acid degradation product was 0.52 micromole within a period of two minutes. Only a slow oxygen uptake occurs beyond that point. The ultraviolet absorption spectrum of this product has been indicated in Fig. 4; acidification of the solution does not allow the formation of the acid degradatiou

(21) Obtained from the Aluminum Company of America, Chemicals Division, Pittsburgh, Pennsylvania.

product as is also indicated in Figure 4. Since this product slowly oxidizes in the acid solution to a product which has the same ultraviolet absorption as N^{10}-formylfolic acid (Fig. 7), it appears that this product is N^{10}-formyldihydrofolic acid.

If the N^{10}-formyldihydro derivative is formed by the placing of the acid degradation product into 0.1 *N* sodium hydroxide, the absorption spectrum changes slowly to one which resembles both dihydrofolic acid and folinic acid-SF in maxima and minima. The main product of the alkali treatment appears to be dihydrofolic acid.

N^{10}-Formyltetrahydrofolic Acid.—The absorption spectrum of the solution of the acid degradation product placed in buffered solutions at different *p*H values was determined in an absorption cell attached, by means of a ground glass joint, to an apparatus suitable for adding reagents *in vacuo*. By alternate freezing and evacuation, solutions of a desired *p*H could be prepared under anaerobic conditions. A solution of the degradation product adjusted to *p*H 9.8 gave the ultraviolet absorption spectrum shown in Fig. 4. On readjustment of the *p*H to a value of 2, the original absorption spectrum of the acid degradation product was obtained (Figure 4). Solutions protected with ascorbic acid gave similar results except that ascorbic acid interferes in the determination of ultraviolet absorption below 300 mμ.

To 4.5 cc. of phosphate buffer (*p*H 7) containing 500 γ per cc. of ascorbic acid, 0.5 cc. of 0.1 *N* hydrochloric acid containing 100 γ per cc. of the acid degradation product was added, and the solution was shaken vigorously. The ultraviolet absorption at 350 and 360 mμ was determined over a period of twenty minutes as indicated in Table IV. After twenty minutes, 0.2 cc. of 5 *N* hydrochloric acid was added to the 5 cc. of solution at *p*H 6.9 to bring the solution to *p*H 1.9. Again the solution was shaken vigorously, and the ultraviolet absorption determined in the same region.

TABLE IV

INTERCONVERSION OF THE ACID DEGRADATION PRODUCT AND N^{10}-FORMYLTETRAHYDROFOLIC ACID

	Optical density[a]			
	*p*H 6.9[b]		*p*H 1.9[c]	
Time, min.	350 mμ	360 mμ	350 mμ	360 mμ
1	0.365	0.355	0.169	...
1.5	...	...	.265	0.208
2	0.307	0.301	.333	.310
3	.270	.260	.345	...
4	.210	.203	...	...
5	.190	...	0.390	0.375
6	.166	0.150	.375	...
7	.132	.125	.365	.390
8	.126	...	...	.386
10	.095	0.090	...	.418
15	...	...	0.375	.418
20	0.054	0.051	...	...

[a] With 10 γ per cc. of compound. [b] Acid degradation product added to buffer at *p*H 6.9. [c] Product of *b* after 20 min. acidified to *p*H 1.9.

If a solution of the acid degradation product (100 γ per cc.) is supplemented with ascorbic acid (1.0 mg. per cc.), made alkaline to *p*H 10, allowed to stand for a few minutes, neutralized to *p*H 7 and autoclaved for one hour, as much as 40% yield based on folinic acid-SF is obtained. However, as much as a 3% yield of folinic acid-SF is obtained immediately merely by the treatment of the acid degradation product (II) with alkali. On treatment of the acid degradation product (II) with 0.1 *N* sodium hydroxide *in vacuo*, the initial absorption maximum (258 mμ) slowly changes to one corresponding to both folinic acid-SF and tetrahydrofolic acid[16] (Fig. 5). Since assay of the reaction mixture indicated only a 10–20% yield of folinic acid-SF, the main product appears to be tetrahydrofolic acid.

Degradation of Folinic Acid-SF to Formylfolic Acid and to Folic Acid.—A solution of 1 mg. of folinic acid-SF in 2.9 cc. of water containing just enough base to dissolve the factor was placed in the main compartment of a Warburg vessel; 0.5 cc. of 2 *M* phosphate buffer at *p*H 2.0 was placed in the side arm of the vessel; and 2–3 mg. of platinum oxide was placed in the center well of the vessel to absorb any traces of hydrogen which might be evolved. The vessel was attached to a Warburg manometer and placed in a 37° water-bath. The *p*H 2 buffer was tipped into the main compartment, the vessel was shaken continuously and manometer readings were taken at intervals to determine the oxygen uptake. Three controls and three similar samples were treated in this manner simultaneously.

AVERAGE OXYGEN UPTAKE

Hours	3	4.5	6.5	9	18	20
Moles O_2 per mole folinic acid-SF	0.45	0.71	0.82	0.92	0.99	0.97

Approximately 50 mg. of folinic acid-SF was dissolved in 220 cc. of 0.01 *N* hydrochloric acid. After removal of air by passage of nitrogen through the solution, the reaction mixture was allowed to stand anaerobically for 16 hours. The characteristic absorption spectrum for the acid degradation product (Fig. 2) was obtained at this stage, and the solution was then allowed to oxidize aerobically for a period of from one to two days. After the oxidation was complete, the solution was made alkaline in 0.1 *N* sodium hydroxide. The absorption spectrum for the product at this stage is indicated in Fig. 6 and is identical with that obtained with synthetic formylfolic acid (Fig. 7). After standing overnight, the absorption spectrum changed to that corresponding to folic acid as indicated in Figs. 6 and 7. The reaction mixture was neutralized and evaporated under reduced pressure to a volume of 20 cc. The *p*H was adjusted to 10 to 12, and the solution was treated with 80 mg. of charcoal. The filtrate was diluted to 120 cc. with boiling water containing enough acetic acid to bring the *p*H to 3–3.5. After this reaction mixture had stood in the refrigerator for several days, an amorphous precipitate appeared. The precipitate was dissolved in hot water, and the *p*H was adjusted to 3 with acetic acid. On standing at room temperature, crystals separated from the solution. The crystals were dried at room temperature *in vacuo*, and the X-ray diffraction pattern of the crystals was compared with that of folic acid. This comparison is indicated in Table V.

TABLE V

INTERPLANAR SPACINGS IN FOLIC ACID

Synthetic, Å.	Isolated,[d] Å.	Synthetic, Å.	Isolated,[d] Å.
1.61	..	2.87	2.84
..	1.64	3.00	3.02
1.67	..	3.09	...
1.73	..	3.22	3.22
1.76	1.75	3.34[a]	3.34[a]
1.83	..	3.65	3.62
1.94	..	3.89	3.88
1.99	2.01	4.05	...
2.03	..	4.33	4.30
2.08	2.09	4.62	4.61
2.17	..	4.92	...
..	2.21	5.31	5.24
2.25	..	5.50	...
2.33	2.32	...	5.60
2.46	2.45	6.00	...
2.59	2.62	6.80[b]	6.77[b]
2.66	..	8.23[c]	8.16[c]
2.74	..	16.5	16.6

[a] Highest intensity. [b] Second highest. [c] Third highest. [d] Isolated from reaction mixture derived from folinic acid-SF.

1,3-Diphenyl-2-imidazolinium Chloride.—N-(2-Anilinoethyl)-formanilide was prepared by the method of Zienty.[22] One gram of the compound was dissolved in 20 cc. of 6 *N* hydrochloric acid. After the reaction mixture had stood at room temperature for varying periods of time (usually 10 to 20 minutes but often much longer) a stable white gel was formed. Additional product continued to separate from the reaction product filtrate for several hours. The gel was broken up into small particles with a stirring rod and the liquid was removed in a suction funnel. The white powder which remained was dissolved in 20 cc. of hot water. Al-

(22) F. B. Zienty, THIS JOURNAL, **68**, 1388 (1946).

lowing the solution to cool resulted in the formation of a clear gel which was converted by the method above to an extremely bitter white powder which darkened above 200° melting with decomposition at 230–240°.

The salt was dried in a vacuum desiccator containing sulfuric acid for several days or *in vacuo* at 150° for one hour.

Anal. Calcd. for $C_{15}H_{15}ClN_2$: C, 69.63; H, 5.83; Cl, 13.71; N, 10.83. Found: C, 69.44; H, 5.95; Cl, 13.66; 13.70; N, 10.88.

This compound absorbed atmospheric moisture rapidly, taking up one molar equivalent of water.

Anal. Calcd. for $C_{15}H_{17}ClN_2O$: N, 10.12. Found: N, 10.05.

The absorption spectrum of N-(2-anilinoethyl)-formanilide in 0.1 *N* sodium hydroxide is indicated in Fig. 3. The transformation of this compound into 1,3-diphenyl-2-imidazolinium chloride is indicated by the change in the absorption spectrum in acidic solution as indicated in Fig. 3. This change in absorption spectrum is very rapid in strongly acidic solutions, and the reverse change takes place rapidly in strongly basic solutions; however, at intermediate *p*H ranges the rate of the transformations from one form to the other occurs more slowly. The rate of the transformation from the basic form to the acid form and the reverse is indicated in Table VI.

TABLE VI

EFFECT OF *p*H ON THE RATE OF INTERCONVERSIONS OF N-(2-ANILINOETHYL)-FORMANILIDE AND 1,3-DIPHENYL-2-IMIDAZOLINIUM CHLORIDE

	Optical density,[a] 312 mμ					
	N-(2-Anilinoethyl)-formanilide[b]			1,3-Diphenyl-2-imidazolinium chloride[c]		
Time	*p*H 3	*p*H 4	*p*H 4.5	*p*H 5	*p*H 6	*p*H 7
2 min.	0.109	...	0.013	...	0.555	0.539
5	.202	0.051	.023	0.541	.509	.459
10	.316	.088	.036	.537	.479	.356
20	.431	.120	.062	.518	.411	.181
1 hr.	.517	.308	.139	.507	.268	.049
2	.516	...	...	.501	.129	.011
20	.514	.451	.301	...	...	...

[a] At 10 γ per cc. of compound. [b] Compound (200 γ per cc.) in alcohol was added to 0.2 *M* phosphate buffer at indicated *p*H. [c] Compound (100 γ per cc.) in 0.1 *N* hydrochloric acid in 50% alcohol was added to 0.2 *M* phosphate buffer at indicated *p*H.

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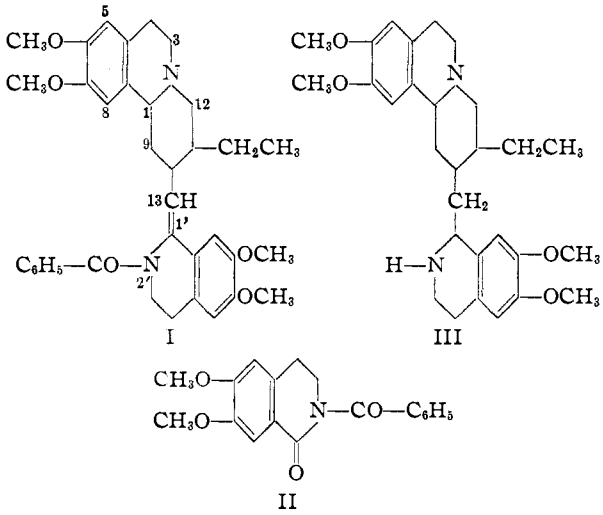
[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF KANSAS]

Studies on Emetine. II. Synthesis of N-Benzoylcorydaldine

BY MELVIN I. MOYER[1] AND WILLIAM E. MCEWEN

1-(β-3′,4′-Dimethoxyphenylethylamino)-6,7-dimethoxy-3,4-dihydroisoquinoline (V) was prepared by applying a Bischler–Napieralski reaction to *s*-bis-(β-3,4-dimethoxyphenylethyl)-urea (IV). A reaction of V with benzoyl chloride and aqueous sodium hydroxide solution afforded N-benzoylcorydaldine (II). The substituted urea (IV) was obtained by a reaction of ethyl acetonedicarboxylate with β-3,4-dimethoxyphenylethylamine. Some similar reactions involving β-phenylethylamine are also described.

Perphthalic acid oxidation or ozonolysis of N-benzoyl-O-methylpsychotrine (I) has been reported to yield a product, the elementary analysis of which is reasonably consistent with the formula of N-benzoylcorydaldine (II).[2] This result, in conjunction with the fact that reduction of O-methylpsychotrine affords both emetine (III) and a diastereoisomer, isoemetine,[2,3] has been advanced as evidence for the position of the non-aromatic double bond in O-methylpsychotrine.[4] In view of this, it seemed desirable to verify the isolation of II by synthesizing it independently and comparing its properties with those of the degradation product.



I III

II

Cyclization of *s*-bis-(β-3,4-dimethoxyphenylethyl)-urea (IV) according to the Bischler–Napieralski procedure afforded 1-(β-3′,4′-dimethoxyphenylethylamino) - 6,7 - dimethoxy - 3,4 - dihydroisoquinoline (V). Reaction of this with benzoyl chloride and aqueous sodium hydroxide solution gave N-benzoylcorydaldine (II). Its physical properties were in agreement with those reported for the product obtained by oxidative cleavage of N-benzoyl-O-methylpsychotrine (I).[2] Thus the position of the extra double bond in O-methylpsychotrine is probably confirmed.[5] In a similar reaction involving V, benzenesulfonyl chloride and aqueous alkali, a product was obtained, the elementary analysis and properties of which are consistent for N-benzenesulfonylcorydaldine.[6] Although Mohunta and Ray have reported that various 1-arylamino-6,7-dimethoxy-3,4-dihydroisoquinolines (VI) are resistant to hydrolytic reagents,[7] it is not surprising that the conditions employed

(1) U. S. Public Health Service fellow, predoctorate, National Heart Institute, June 5, 1950, to June 4, 1951.

(2) P. Karrer, C. H. Eugster and O. Ruttner, *Helv. Chim. Acta*, **31**, 1219 (1948).

(3) F. L. Pyman, *J. Chem. Soc.*, **111**, 419 (1917).

(4) In O-methylpsychotrine itself, the extra double bond may be located at C-13, C-1′, at C-1′, N-2′, or the substance may be an equilibrium mixture of the two isomers.

(5) The total synthesis of *d,l*-rubremetinium bromide has recently been reported. An intermediate product in the synthesis was either *d,l*-O-methylpsychotrine or one of its diastereoisomers. A. R. Battersby and H. T. Openshaw, *Experientia*, **VI**, 378 (1950).

(6) *Cf.* H. J. Barber, *J. Chem. Soc.*, 101 (1943).

(7) L. Mohunta and J. N. Ray, *ibid.*, 1263 (1934).