be the *trans*, did not. This is the expected result if the above structure assignments are correct and if dehydrohalogenation is a *trans*-elimination, as is usually the case.[16] Since the physical properties reported by Komatsu and Kowamoto,[14] differ greatly from those of the above compound, some doubt is cast on the identity of their material.

Experimental

1,1-Dichlorocyclohexane.—Twenty moles (4,165 g.) of phosphorus pentachloride was placed in a 5-liter three-neck flask equipped with a dropping funnel, reflux condenser, mechanical stirrer and thermometer. Ten moles (1,030 ml.) of cyclohexanone was added dropwise at a rate of one to two drops per second. After about one-half the ketone was added the mixture became slushy and was stirred during the remainder of the addition, the temperature being kept below 65° throughout the process. The reaction mixture was then steam distilled by dropping it into boiling water contained in a three-neck flask properly equipped. The organic layer was separated, dried and fractionated to give first 536 g. (4.6 moles, 46% of theoretical) of 1-chlorocyclohexene, boiling range 141–143° and then 321 g. (2.1 moles, 21% of theoretical) of 1,1-dichlorocyclohexane, boiling range 169–173° which was purified by further fractionation.

Anal. Calcd. for $C_6H_{10}Cl_2$: Cl, 46.33; molecular refraction, 37.44. Found: Cl, 46.16; molecular refraction, 37.63.

The structure of this dichloride was proven by conversion to cyclohexanone. Ten grams of 1,1-dichlorocyclohexane, prepared as above, was stirred with 100 ml. of concd. sulfuric acid at 40° for one hour, at which time hydrogen chloride evolution had ceased and only one phase was present. The solution was poured onto ice and extracted several times with ether. Evaporation of the ether left about five grams of impure cyclohexanone which provided a 2,4-dinitrophenylhydrazone melting at 161–162° (literature,[17] 162°) after recrystallization.

(16) E. R. Alexander, "Ionic Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 118.

(17) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," 3rd. ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 262.

d,l-trans-**1,2-Dichlorocyclohexane.**—Three moles (294 g.) of cyclohexene was chlorinated at 30–40° in the absence of light by passing in chlorine gas with stirring until the gain in weight was 106 g. (50% of theoretical). The crude product was distilled, the dichloride being collected at 186–190°. Repeated fractionation gave a series of fractions having identical boiling points and indices.

Anal. Calcd. for $C_6H_{10}Cl_2$: Cl, 46.33; molecular refraction, 37.44. Found: Cl, 46.44; molecular refraction, 37.39.

cis-**1,2-Dichlorocyclohexane.**—*d,l-trans*-2-Chlorocyclohexanol was prepared by the method of Newman and VanderWerf.[18] The chlorohydrin (134 g., 1.4 moles, b.p. 84–85° (18 mm.), m.p. 29°, $n^{25}D$ 1.4832) was mixed with 233 g. (2.95 moles) of freshly distilled pyridine in a one-liter dropping funnel. This mixture was dropped slowly onto 338 g. (2.8 moles) of thionyl chloride (commercial grade) contained in a three-neck three-liter flask, fitted with a stirrer, thermometer and condenser, and maintained at 70–80° with a water-bath. Stirring and heating was continued for two hours after the addition was complete. Excess thionyl chloride was destroyed by dropwise addition of 500 ml. of ice-water and the organic layer separated and washed with 50-ml. portions of sodium carbonate solution. The product was then steam distilled, dried over calcium chloride and fractionated at reduced pressure. The yield of dichloride boiling at 114.4–114.5° (cor., 50 mm.) was 55 g. or 26%.

Anal. Calcd. for $C_6H_{10}Cl_2$: Cl, 46.33; molecular refraction, 37.44. Found: Cl, 46.49; molecular refraction, 37.24.

The above dichloride was dehydrochlorinated by refluxing 20 g. (0.13 mole) with 45 g. (0.35 mole) of freshly distilled quinoline. After the temperature of the vapor dropped to 155°, the mixture was distilled and material boiling up to 210° collected. Two distillations gave 4.0 g. of 1-chlorocyclohexene, b.p. 141–142°, $n^{25}D$ 1.4780 (literature[5] b.p. 141–142°, $n^{25}D$ 1.4772). A sample of *trans*-1,2-dichlorocyclohexane treated in a similar fashion produced no 1-chlorocyclohexene.

(18) M. S. Newman and C. A. VanderWerf, THIS JOURNAL, **67**, 233 (1945).

COLUMBIA, S. C. RECEIVED OCTOBER 27, 1950

[CONTRIBUTION FROM THE MERCK INSTITUTE FOR THERAPEUTIC RESEARCH AND THE RESEARCH LABORATORIES, MERCK & CO., INC.]

Vitamin Activity of Lyxoflavin

BY GLADYS A. EMERSON AND KARL FOLKERS

Synthetic lyxoflavin has been found to be devoid of riboflavin activity when tested in conventional assays using rats and *L. casei*. A rat assay for unidentified vitamins has been devised in which the diet contains the known vitamins including vitamins B_2 and B_{12}. The protein is supplied by soybean meal, and thyroid powder is added to enhance development of a deficiency state. The addition of liver and fish-meal components stimulated growth indicating the presence of new unidentified vitamins. Lyxoflavin has shown growth-promoting or vitamin activity in this rat assay.

Data from tests with synthetic lyxoflavin for vitamin activity have been described, and it was concluded that lyxoflavin showed growth-promoting or vitamin-like activity.[1] We now wish to describe further information and details about this investigation of lyxoflavin, and to give additional data from later vitamin tests.

In 1947, L-lyxose was reported[2] as being isolated from human myocardium by steps including hydrolysis with cobra venom. The manner in which the lyxose is combined with other chemical groups was not apparent to these investigators, and the object of a later paper by Sodi and Garza[3] was to describe the isolation of L-lyxoflavin from human myocardium, and its synthesis. Sodi and Garza concluded that lyxoflavin is the form in which lyxose is found in the human myocardium. A 5-mg. specimen of the flavin was recorded as the result of fractionation of 10 kg. of human heart muscle. This flavin and its tetraacetate were compared in composition and melting point behavior with synthetic L-lyxoflavin and its tetraacetate, and it was concluded that these properties are identical. Although this comparison of natural and synthetic materials is meager for the identification of flavins, the veracity of the conclusion is the significant point. It seems likely that additional evidence for or against the occurrence of lyxoflavin in nature will be forthcoming and, until then, a favorable interpretation seems to be propitious.

(1) Emerson and Folkers, THIS JOURNAL, **73**, 2398 (1951).

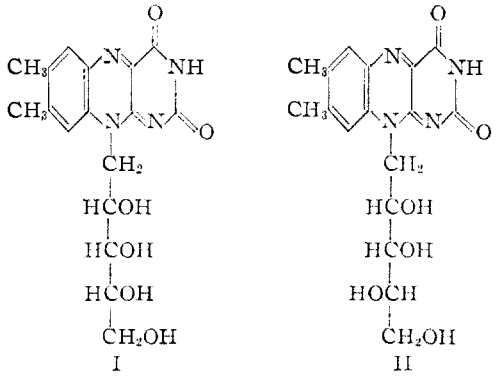
(2) Sodi, Velez and Carvallo, *Arch. inst. cardiol. Mex.*, **17**, 575 (1947); *C. A.*, **41**, 7480 (1947).

(3) Sodi and Garza, *Arch. Biochem.*, **22**, 63 (1949).

The existence of lyxoflavin seemed to Sodi and Garza[3] to account for lyxose, and it was believed that the lyxose is formed in the organism from mannose or galactose. The significance of the presence of lyxoflavin itself in human heart muscle was not discussed,[3] but a corresponding publication[4] is entitled "Stereoisomerism of vitamin B_2 in the Human Myocardium." The stereochemical relationship between vitamin B_2 and lyxoflavin may have no biological meaning.

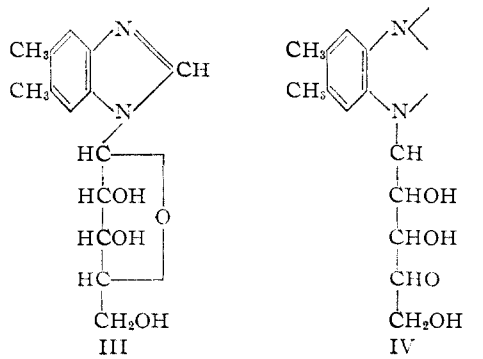
It is the question of the significance of the reported existence of lyxoflavin in nature which attracted our attention. A compelling idea was apparent—lyxoflavin might be a new member of the vitamin B complex. The basis of this idea was as follows.

Vitamin B_2 has structure I, and lyxoflavin (II)



I II

differs from it only in the configuration of the groups about C_4 of the pentose side chain. Studies on the chemistry of vitamin B_{12} have shown that α-ribazole[5] (III) (1-α-D-ribofuranosido-5,6-dimethylbenzimidazole) is a unit of the molecule. The three compounds, vitamin B_2, vitamin B_{12} and lyxoflavin contain a 1,2-diamino-4,5-dimethylbenzene moiety which is linked through a nitrogen atom to a pentose (IV), and all three are concerned with the human body. Two of these three compounds are established vitamins for human nutrition; lyxoflavin, the third compound, might also be a vitamin. If so, lyxoflavin could be expected to have a biological role different from that of vitamins B_2, B_{12} and all other known vitamins. There is precedence (nicotinic acid, *p*-aminobenzoic acid and *meso*-inositol) for substances being known chemically before their vitamin-like role was elucidated. Szent-György's[6] broad concept of the definition of a vitamin is pertinent.



III IV

Research on the synthesis of L-lyxose and L-lyxoflavin was begun so that the latter compound could be made available in substantial quantities for biological and clinical tests for possible vitamin activity. Furthermore, lyxoflavin was desired for tests on the regression of lymphosarcoma transplants in mice, since it was found that 6,7-dichloro-9-(1′-D-sorbityl)-isoalloxazine[7] would enhance regression of the lymphosarcoma and yet was not an inhibitor of riboflavin; perhaps, lyxoflavin is the compound which participates *in vivo* in the inhibition reaction.

Before describing the results of biological tests with lyxoflavin, it is appropriate to consider other papers on flavins which appeared after this study of lyxoflavin had begun. *A priori*, it is probable that lyxoflavin might occur in nature in a bound form similar to the linkage of riboflavin in its flavin adenine dinucleotide. Lyxoflavin might exist in nature and laboratory concentrates from source materials as a phosphate and a purine dinucleotide and each would display different properties. Sanadi and Huennekens[8] have purified a new flavin dinucleotide of unknown relationship to lyxoflavin. Whitby[9] has enzymatically converted riboflavin into another product which appears to have an altered pentose moiety. Since the discovery of the "yellow ferment" of Warburg, at least ten flavin enzyme systems have been attributed to riboflavin. Clearly now, if lyxoflavin existed in any of these systems, physicochemical estimation of the flavin as "riboflavin" might not have detected a different flavin such as lyxoflavin. Paper chromatography[10] of flavin nucleotides would undoubtedly help clarify any question on flavin identity.

After lyxoflavin was synthesized by a modified and improved procedure,[11] it was possible to begin a series of biological tests for possible vitamin activity. The results of these first tests are as follows.

Testing of Lyxoflavin for Riboflavin Activity.—Lyxoflavin was tested for riboflavin activity by an established prophylactic method with weanling rats. The results are summarized in Table I, and it is evident that lyxoflavin is devoid of riboflavin activity in rats. This result is as it should be if lyxoflavin is a distinct vitamin-entity itself.

TABLE I

TEST OF LYXOFLAVIN FOR RIBOFLAVIN ACTIVITY

Groups of male rats (10 in each group)	Gain in weight 30 days, g.
Control (B_2 deficient)	11.5
Plus 10 μg. riboflavin	57.4
Plus 20 μg. riboflavin	109.2
Plus 100 μg. lyxoflavin	11.0

We are indebted to Dr. David Hendlin and Mrs. Jeanne Wahl for testing lyxoflavin for growth activity in the assay[12]

(4) Sodi and Garza, *Arch. inst. cardiol. Mex.*, **19**, 735 (1949); *C. A.*, **44**, 5368 (1950).

(5) Brink, Holly, Shunk, Peel, Cahill and Folkers, THIS JOURNAL, **72**, 1866 (1950).

(6) Szent-György, *Ann. Rev. Biochem.*, Vol. XI, Annual Reviews. Inc., Stanford University P. O., California, 1942, p. 309.

(7) Holly, Peel, Mozingo and Folkers, THIS JOURNAL, **73**, 332 (1951).

(8) Abstracts of papers, p. 60c; 117th Meeting, American Chemical Society; Detroit, Michigan, April, 1950.

(9) Whitby, *Nature*, **166**, 480 (1950).

(10) Crammer, *ibid.*, **161**, 349 (1948).

(11) Heyl, Cates, Koniuszy and Folkers, THIS JOURNAL, unpublished.

(12) Snell and Stong, *Ind. Eng. Chem., Anal. Ed.*, **11**, 346 (1939).

with *L. casei* for riboflavin activity. Lyxoflavin had less than 0.2% of the activity of riboflavin in this microbial assay. This fact is useful to differentiate riboflavin and lyxoflavin or to conduct a differential assay with total pentose-isoalloxazine determined fluorometrically.

A Rat Assay for Unidentified Vitamins.—A test has been devised for an assay of new unidentified vitamins in various source materials. The assay employs rats maintained on a diet in which the protein is supplied by soybean meal. The dietary components are given in Table II. In addition, the diet contained 0.5% of thyroid powder which enhances the development of a deficiency state by increasing metabolism. The animals were placed at weaning on the diet and were maintained for 28 days at which time they were segregated into groups of like average weights. A number of source materials were tested for activity by addition to the basal ration. The source materials replaced a corresponding amount of the carbohydrate component.

TABLE II

BASAL DIET COMPOSITION

Components	G./100 g.
Soybean meal	60
Salt mixture #2	4
Dextrose	24
Crisco	10
Cod liver oil	2

Micronutrients in mg./100 g.: thiamine, 1; riboflavin, 2; pyridoxine, 1; calcium pantothenate, 10; nicotinamide, 10; inositol, 5; choline, 100; paraaminobenzoic acid, 30; biotin, 0.05; folic acid, 0.2; alpha tocopherol, 14.2; menadione, 14.2. The 0.01 mg./100 g. of vitamin B_{12} was added to the diet following 28-days depletion.

It was found that some of these materials must contain new unidentified vitamins, because their presence stimulated growth. The evidence for the presence of these unidentified nutrients is summarized in Table III.

TABLE III

RAT ASSAY OF NEW UNIDENTIFIED VITAMINS

Group of rats 9–11 males each	Average weight gain, g. in 15 days
Basal (with 0.5% thyroid powder)	64
Defatted liver power (10%), Viobin Corp.	77
Menhaden fish meal (10%)	79
Wilson's liver fraction L (10%)	70
Water insoluble liver solids (10%)	83

Ershoff[13] has reported that vitamin B_{12} failed to counteract the growth-depressing effect of massive doses of thyroid when this material was administered in conjunction with a diet containing casein. Retardation of growth was prevented by the administration of a water-insoluble liver fraction.

Testing of Lyxoflavin for Vitamin Activity in Rats.—Lyxoflavin was tested for vitamin activity in rats using a diet and test procedure described in the above section on a rat assay for unidentified vitamins. The results of Expts. 1 and 2 with lyxoflavin are summarized in Table IV. It may be seen that the average weight gain elicited by the daily oral administration of lyxoflavin was similar to that elicited by the addition of liver powder, fish meal and other liver source materials to the diet.

TABLE IV

TESTING OF LYXOFLAVIN FOR VITAMIN ACTIVITY IN RATS

Expt.	Groups of rats 9–11 males each	Average weight gain, g. in 15 days: Basal diet	Average weight gain, g. in 15 days: Plus 150 μg. lyxoflavin
1	(With 0.50% thyroid powder)	64	78
2	(With 0.50% thyroid powder)	64	88
3	(With 0.60% thyroid powder)	55	65
4	(With 0.75% thyroid powder)	58	67

(13) Ershoff, *Proc. Soc. Exp. Biol. Med.*, **73**, 459 (1950).

This test of lyxoflavin in rats has additional significance in that the supplementation of the lyxoflavin resulted in a weight gain equivalent to that given by natural source materials rather than a part of the weight gain elicited by natural source materials.

The effect of increasing the amount of thyroid powder which is added to the basal diet was investigated. In Expts. 3 and 4 (Table IV), the level of thyroid powder was increased to 0.60% and 0.75%, respectively. The administration of the same level (150 μg.) of lyxoflavin to the animals in these experiments resulted likewise in growth stimulation. It appeared that a level of 0.5–0.6% of thyroid powder is more satisfactory than a level of 0.75% since other groups of rats which received the high level showed excessive mortality.

Testing of Lyxoflavin for Tolerance in Mice.—Adult mice were given 10 mg. of lyxoflavin suspended in gum acacia daily *per os* for 30 days. They were indistinguishable from their undosed controls. The treated animals made the same weight gains as the controls; furthermore, all organs of the treated animals appeared normal at autopsy.

This intake of lyxoflavin represents *ca.* 2,000 times the daily requirement of the mouse for the closely related riboflavin.

Discussion of Results.—There is accepted evidence today for the existence of at least several new animal and microbial factors of vitamin-like activity. It is to be expected that one of these, in pure form, would show activity only in those few systems where an inadequate amount of the factor is present in the diet or medium. Since lyxoflavin shows growth activity in a rat assay, it is of interest now to test it in many other assays, and to attempt to directly correlate its activity with that of other known factors. Dr. Wayne Umbreit has kindly tested lyxoflavin for activity in assays for the glycerol factor[14] and for the pyruvate oxidation factor.[15] This latter factor is believed to be identical with the acetate factor and protogen.[16] Dr. Umbreit reported that lyxoflavin showed no activity in either assay.

Gardner, Wenis and Lee[17] have also synthesized lyxoflavin, but reported no vitamin tests. They treated it with Russell's viper venom and concluded that the likelihood of lyxose arising from lyxoflavin is doubtful. Sodi and Garza,[3] however, seemed to believe that lyxoflavin came from lyxose. It was also observed[17] that the natural occurrence of lyxoflavin is not too well established, that it has R_F values which are different from those of Whitby's flavin.[9] They[17] found that it is pharmacologically almost inert in heart and smooth muscle preparations. Although there are exceptions, most of the known vitamins are pharmacologically inert.

The syndrome characteristic of riboflavin deficiency in man[18,19] was exceedingly common prior to food enrichment. Because of possible similar biosynthesis of riboflavin and lyxoflavin, a clinical study of lyxoflavin in disease might reveal a new human deficiency and its therapy. Through the courtesy of Dr. Tom Spies such clinical tests have been initiated. Preliminary tests[20] on patients with hypertension revealed no objective evidence of change, but the individuals believed they were

(14) Van Demark, *J. Bact.*, **59**, 533 (1950).

(15) O'Kane and Gunsalus, *ibid.*, **56**, 499 (1948).

(16) Snell and Broquist, *Arch. Biochem.*, **23**, 326 (1949).

(17) Abstracts of Papers, p. 2c, 119th Meeting, American Chemical Society, Boston, Mass., April, 1951.

(18) Sebrell and Butler, *Pub. Health Rep.*, **53**, 2282 (1938).

(19) Spies, Bean, Vilter and Huff, *Am. J. Med. Sci.*, **200**, 697 (1940).

(20) Personal communication.

more relaxed. There was no effect on temperature, pulse, respiration, urine or blood; thus, administration is safe for trial in a number of diseases.

RAHWAY, N. J. RECEIVED MAY 23, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

The Synthesis, Resolution and Proof of Configuration of the Isomers of *m*-Tyrosine[1]

BY ROBERT R. SEALOCK, MERRILL E. SPEETER[2] AND RICHARD S. SCHWEET

m-Tyrosine has been obtained by hydriodic acid decomposition of 2-methyl-4-(3′-acetoxybenzal)-5-oxazolone in 60–70% yields. The oxazolone, in turn, was prepared by an Erlenmeyer condensation between acetylglycine and *m*-hydroxybenzaldehyde in yields of 67–75%. Resolution was accomplished by means of the brucine salt of the formyl derivative. Of the resulting amino acids, the levorotatory isomer was shown to possess the L-configuration by means of the D-amino acid oxidase and the Lutz-Jirgenson methods. Results with the D-isomer were in complete accord. Catalytic reduction of acetamido-*m*-hydroxycinnamic acid yielded acetylcyclohexylamine.

In a study of the role of ascorbic acid in phenylalanine and tyrosine metabolism, the individual optical isomers of *m*-tyrosine were required. The amino acid was therefore synthesized and resolved and the configuration of the respective isomers established.

By means of the classical Erlenmeyer-oxazolone procedure, the racemic compound has been previously prepared by Blum[3] and Abderhalden and Schairer.[4] Likewise, the synthesis has been achieved by the diketopiperazine method.[5,6] The methods, however, were unsatisfactory in view of the large amounts required for subsequent resolution and the extensive feeding and metabolic experiments to be conducted. As an alternative, Dakin's acetylglycine modification[7] of the Erlenmeyer scheme proved more useful. Condensation of acetylglycine and acetic anhydride with *m*-hydroxybenzaldehyde yielded 2-methyl-4-(3′-acetoxybenzal)-5-oxazolone which was readily converted to DL-*m*-tyrosine in one step by the hydriodic acid procedure of Lamb and Robson.[8]

Resolution was accomplished with the formyl derivative, prepared by the method of Clarke as described by du Vigneaud and Meyer.[9] The resolving agent was *l*-brucine with which an alcohol insoluble levorotatory and a water insoluble dextrorotatory salt were obtained. The resulting formyl derivatives proved to be levorotatory and dextrorotatory, respectively. Each in turn yielded a free amino acid exhibiting a rotation of opposite direction from that observed with the formyl compound. The one corresponding to the insoluble brucine salt proved to be dextrorotatory and the unnatural isomer. The other proved to be levorotatory and to be the amino acid possessing the natural configuration. Thus *m*-tyrosine affords a further illustration of amino acids exhibiting opposite direction of rotation when in the form of an acyl derivative.

The configuration of the two amino acid isomers was determined by the application of the specific D-amino acid oxidase method originally suggested by Krebs.[10] The levorotatory isomer was not oxidized by the enzyme, whereas the dextrorotatory isomer was readily oxidized to the alpha-keto acid. Confirmation of the configuration was obtained by the physical-chemical method of Lutz and Jirgenson[11,12] which depends upon the natural isomer exhibiting a positive shift in rotation with increasing concentrations of acid.

In the process of arriving at the final synthesis, catalytic reduction of acetamido-*m*-hydroxycinnamic acid was attempted. The reduced product proved to be acetyl-β-cyclohexylalanine instead of the desired acylamino acid. The proof of the structure of the compound was accomplished by means of appropriate derivatives particularly those described by Herbst and Shemin.[13] The latter authors obtained the same compound from the catalytic reduction of acetamidocinnamic acid.

Experimental

m-Hydroxybenzaldehyde was prepared from commercial *m*-nitrobenzaldehyde[14] and acetylglycine was likewise prepared by a method already in the literature.[15]

2-Methyl-4-(3′-acetoxybenzal)-5-oxazolone.—In a liter flask were thoroughly mixed 61 g. (0.5 mole) of *m*-hydroxybenzaldehyde, 58.6 g. (0.5 mole) of acetylglycine, 41 g. (0.5 mole) of anhydrous sodium acetate and 143.5 ml. (1.5 moles) of acetic anhydride. The mixture was placed in a boiling water-bath for six hours with a reflux condenser attached. It was allowed to cool to room temperature with the resultant formation of a solid mass of crystalline material, at which point 300 ml. of cold water were gradually worked into the mixture. After storage in the cold overnight, the product was separated by filtration and thoroughly washed with several 100-ml. portions of ice-cold water. The air-dried material, which was canary yellow in color, melted at 116–118°. In different preparations 82–92 g. corresponding to 67–75% yields were obtained. The crude material could be recrystallized from hot ethyl acetate through careful addition of Skellysolve B, in which case the melting point was 118–120°. Ordinarily the crude material was not recrystallized but was used directly in the next reaction.

Anal. Calcd. for $C_{13}H_{11}O_4N$: N, 5.71. Found: N, 5.73, 5.68, 5.68.

(1) Presented before the Division of Biological Chemistry at the 110th Meeting of the American Chemical Society in Chicago, Illinois, September, 1946.

(2) The Upjohn Company, Kalamazoo, Michigan.

(3) L. Blum, *Arch. Exp. Path. Pharmakol.*, **59**, 269 (1908).

(4) E. Abderhalden and W. Schairer, *Fermentforschung*, **12**, 295 (1931).

(5) H. Ueda, *J. Biochem. (Japan)*, **8**, 397 (1928).

(6) H. Ueda, *Ber.*, **61**, 146 (1928).

(7) H. D. Dakin, *J. Biol. Chem.*, **82**, 439 (1929).

(8) J. Lamb and W. Robson, *Biochem. J.*, **25**, 1231 (1931).

(9) V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **98**, 295 (1932).

(10) H. A. Krebs, *Biochem. J.*, **29**, 1620 (1935).

(11) O. Lutz and B. Jirgenson, *Ber.*, **64**, 1221 (1931).

(12) O. Lutz and B. Jirgenson, *ibid.*, **63**, 448 (1930).

(13) (a) R. M. Herbst and D. Shemin, "Org. Syntheses," 2nd edition, Coll. Vol. 2, 491 (1946); (b) D. Shemin and R. M. Herbst, THIS JOURNAL, **61**, 2471 (1939).

(14) R. B. Woodward, *Org. Syntheses*, **25**, 55 (1944).

(15) R. M. Herbst and D. Shemin, "Org. Syntheses," 2nd edition, Coll. Vol. 2, 11 (1946).