the fatty acids was found more satisfactory than the methods previously used. The removal of traces of silver from the diglycerides synthesized by Fischer's method has been simplified by the use of sodium iodide solution.

The synthesis and identification of the following compounds herein described have not been reported previously: α-monocaproin, α-mono-caproylo-acetoneglycerol, and the isomeric caproylo-distearins, capryllo-distearins and capro-distearins.
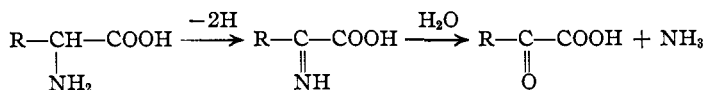
PITTSBURGH, PENNSYLVANIA

THE DEHYDROGENATION OF CERTAIN AMINES AND HYDRAZINES BY UNSATURATED COMPOUNDS (PRELIMINARY PAPER)

BY L. A. PINCK AND GUIDO E. HILBERT

RECEIVED SEPTEMBER 2, 1931   PUBLISHED FEBRUARY 5, 1932

The two theories of biological oxidation that probably have received most attention in recent years are those of Warburg[1] and of Wieland.[2] Warburg's theory is expressed as being a process in which the oxidation is catalyzed by iron. The active agent, "the respiration enzyme," is an iron complex of a compound related to hematin. Wieland's theory, which is definitely contradictory to that of Warburg, is pictured as being a transfer of hydrogen atoms from one molecule to the other. The oxidation is thus essentially a dehydrogenation. Wieland and Bergel[3] studied the oxidation of amino acids with animal charcoal and adduced evidence that the degradation takes place in the following manner

$$R-\underset{\underset{NH_2}{|}}{CH}-COOH \xrightarrow{-2H} R-\underset{\underset{NH}{\|}}{C}-COOH \xrightarrow{H_2O} R-\underset{\underset{O}{\|}}{C}-COOH + NH_3$$

in which the first step consists of a removal of hydrogen atoms. The oxidation of amines, which has been the subject of considerable work, has usually been carried out by such drastic reagents as the hypohalites[4] and potassium permanganate,[5] oxidizers which presumably do not exist in biological systems. Additional evidence has now been obtained in favor of Wieland's mechanism of biological oxidation. The dehydrogenation has

[1] Otto Warburg, "Über die katalytischen Wirkung der lebendigen Substanz," Berlin, 1928.

[2] Wieland, Ergebnisse Physiol., 20, 477 (1922).

[3] Wieland and Bergel, Ann., 439, 196 (1924).

[4] Hofmann, Ber., 14, 2725 (1881); Stieglitz, and co-workers, THIS JOURNAL, 36, 272 (1914); 38, 2046 (1916); Hellerman and Sanders, ibid., 49, 1742 (1927).

[5] Goldschmidt and Beuschel, Ann., 447, 197 (1926); Goldschmidt and Reichel, ibid., 456, 152 (1927).

been accomplished under mild conditions by the use of various unsaturated substances, containing active groups, which are known to occur in nature. The imide, which according to Wieland's theory is the initial product formed, has been isolated as such.

Such compounds as dibiphenyleneethylene, azobenzene, indigo and methylene blue, in which the linkages C=C, N=N, C=O and C=N, respectively, are known to be highly active, can dehydrogenate 9-fluorylamine. Dibiphenyleneethylene is also effective in dehydrogenating hydrazobenzene.

The reaction between 9-fluorylamine and dibiphenyleneethylene was found to take place very smoothly in liquid ammonia and is expressed by the equation
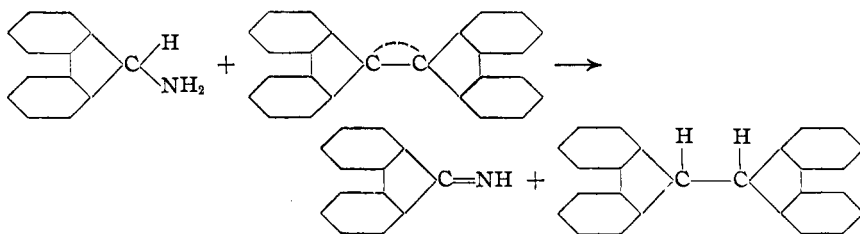
The reaction goes to completion at room temperature and is readily followed visually by the gradual disappearance of the red ethylene compound. Liquid ammonia appears to be the ideal solvent for carrying out these reactions since possible complications by interaction between fluorenone imide and 9-fluorylamine to form the Schiff base are eliminated. Attempts to carry out the reaction in other solvents such as alcohol benzene and pyridine have thus far been unsuccessful.

It is interesting to note the marked change in the reactivity of dibiphenyleneethylene when either one or both of the biphenylene groups are replaced by other substituents. Thus, when one of the biphenylene groups was replaced by hydrogen and a phenyl group, as in the case of benzalfluorene, the reaction was incomplete even on heating for eight days at 60°, as compared with a complete reaction of dibiphenyleneethylene within two days at 40°. Upon the introduction of two phenyl radicals in place of a biphenylene group, the compound did not react at all. The same was found to be true of tetraphenylethylene. This is not surprising since it is well known that the double bond of tetraphenylethylene is relatively inert and does not show any tendency to add either bromine[6] or nitrogen dioxide.[7] Thiele and co-workers,[8] in a study of the reduction of un-

[6] Biltz, *Ann.*, **296**, 231 (1897); Bauer, *Ber.*, **37**, 3321 (1904).

[7] Schlenk, *Ann.*, **394**, 213 (1912). The nitrogen dioxide enters the benzene rings instead of adding on the double bond.

[8] Thiele, *Ber.*, **33**, 3395 (1900); Thiele and Bühner, *Ann.*, **347**, 249 (1906); Thiele and Rüdiger, *ibid.*, **347**, 275 (1906); Thiele and Henle, *ibid.*, **347**, 290 (1906).

saturated hydrocarbons with aluminum amalgam, found that such compounds as tetraphenylethylene, dimethylfulvene, 1,4-diphenylbutadiene and both dihydrocinnamylidenefluorenes were not reduced. Since aluminum amalgam is without doubt a stronger reducing agent than 9-fluorylamine, this group of compounds will also probably not be affected by the latter. On the other hand, not only dibiphenyleneethylene and benzalfluorene but also benzalindene, cinnamylidenefluorene, etc., some of which probably will also dehydrogenate the amine, are reduced by aluminum amalgam.

A number of the dyes and azo compounds are also excellent dehydrogenators. Of this group three were studied, indigo (I), methylene blue (II) and azobenzene. The reactivity toward 9-fluorylamine was found

I   II
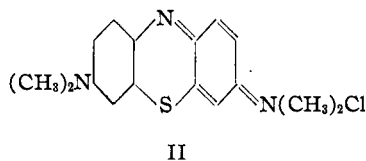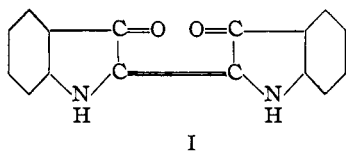
to increase in the order dibiphenyleneethylene, indigo, methylene blue and azobenzene. In the case of the dyes, the end-products are the leuco bases, whereas azobenzene is reduced to hydrazobenzene, which was found to be stable toward the amine. It is of interest to note that all the dehydrogenators used in this work absorb light in the visible region of the spectrum. As the color of a substance indicates low energy electronic transitions, it is possible that this may have some relation to chemical reactivity.

Other compounds can also take the place of 9-fluorylamine in the above reactions and. act as the source of hydrogen. Hydrazobenzene readily reacts with dibiphenyleneethylene; the end-products are dibiphenyleneethane and azobenzene. On allowing a solution of the amino acid tyrosine in liquid ammonia to interact with dibiphenyleneethylene, a slow reaction takes place, the results of which, however, will be published at a later date. On the other hand, benzhydrylamine, which is very similar in structure to 9-fluorylamine, is unaffected by any of the dehydrogenators. It appears that the ease of oxidation of 9-fluorylamine is a property conferred upon it by the five-membered ring, and depends upon its environment.

The fact that an active carbon–carbon double bond such as occurs in dibiphenyleneethylene can act as a dehydrogenator toward 9-fluorylamine and hydrazobenzene is new. There is no reason for believing that the reaction is necessarily limited to either an environment of liquid ammonia or to the fluorene type of compounds. It is probable that other very active carbon–carbon double bonds and free radicals will behave similarly, and that they will also be able to dehydrogenate compounds other than certain

amines and hydrazines.   Since 9-fluorylamine and benzhydrylamine are easily oxidized by oxygen, it is peculiar but also significant that they should show such a difference in behavior toward the above dehydrogenators in liquid ammonia.   It is hoped that further work will provide an explanation for this difference in behavior and also of the mechanism of the dehydrogenation.

Experimental

9-Fluorylamine.—Wieland and Roseeu[9] in a cursory statement mentioned that the reduction of fluorenone ketazine on treatment with zinc dust in acetic acid yielded 9-fluorylamine.   Kuhn and Jacob[10] later described the method in greater detail.   As the procedure used by us differed somewhat from that of the previous investigators, its description is given here.   It has been our experience that this is the best method for the preparation of 9-fluorylamine.

Fluorenone ketazine was prepared in the manner described by Wieland and Roseeu. A yield of 93.5 g. was obtained from 100 g. of fluorenone.   A solution of 50 g. of fluorenone ketazine in 250 cc. of glacial acetic acid was treated with 80 g. of zinc dust and boiled for six hours.   After filtration of the hot solution, the zinc residue was digested with boiling acetic acid and filtered.   The combined filtrates were diluted with 3 liters of water and the small amount of precipitate matter filtered.   From this precipitate was isolated 2.4 g. of Kerp's 9-fluorylacetylamine (according to Kuhn and Jacob γ-9-fluorylacetylamine).   The filtrate was made strongly alkaline with aqueous ammonia.   The amine which separated was dried (m. p. 60–61°) and precipitated as the hydrochloride from a benzene solution.   The amine hydrochloride was recrystallized from dilute hydrochloric acid, from which it separated as long needles that melted with decomposition at 255°; yield 37 g.   The free amine was obtained in the usual manner and recrystallized twice from hexane; m. p. 64–65°.   Approximately 10 g. of 9-fluorylamine is soluble in 150 cc. of boiling hexane.

Dibiphenyleneethane.—This was prepared by heating a toluene solution of 9-chlorofluorene under reflux in the presence of copper powder.   The recrystallized product melted at 247°.

Reaction of 9-Fluorylamine with Dibiphenyleneethylene.—Several preliminary qualitative experiments were carried out in liquid ammonia in order to determine the optimum conditions of the reaction.   9-Fluorylamine is appreciably soluble in liquid ammonia at room temperature.   The solution, however, becomes pale green, probably due to some oxidation by a trace of oxygen.   Dibiphenyleneethylene is somewhat soluble in liquid ammonia, imparting to it an orange-yellow color.   The reaction between 9-fluorylamine and dibiphenyleneethylene is not photochemical and as one would expect is greatly accelerated by heat.   As 9-fluorylamine is a weak base, a solution of its hydrochloride in liquid ammonia is just as effective as the free amine. Wet and sodium-dried liquid ammonia are equally satisfactory in carrying out the reaction of the amine with the ethylene compound.

A sealed tube containing 0.500 g. of 9-fluorylamine hydrochloride, 0.758 g. of dibiphenyleneethylene[11] and about 15 cc. of sodium-dried liquid ammonia was heated at a temperature of 40°.   After heating for two hours an appreciable reaction had already taken place, as was shown by the appearance of long colorless prismatic needles.   The

[9] Wieland and Roseeu, *Ann.*, **381**, 232 (1911).

[10] Kuhn and Jacob, *Ber.*, **58**, 1432 (1925).

[11] Wagner and Schmidt, *ibid.*, **43**, 1796 (1910).   The hydrocarbon is best recrystallized from a solution of propyl and amyl alcohol.

reaction was practically completed in twenty-four hours. The progress of this reaction was followed by the gradual disappearance of the red ethylene compound.

After opening the tube, the ammonia was evaporated and the residue dried in a vacuum desiccator. The residue was then extracted with petroleum ether. The filtrate was allowed to evaporate in a desiccator containing paraffin. Pale yellow crystals separated that melted at 119°; mixed melting point with fluorenone imide[12] 122°. Further efforts to bring the imide to a higher state of purity were without success chiefly because of its sensitivity toward moisture. In order to confirm the fact that this substance was fluorenone imide, its ether solution was treated with dry hydrogen chloride. The orange-colored hydrochloride was precipitated; yield 0.45 g. It melted with decomposition at 265°; mixed decomposition point 268°. The hydrochloride was hydrolyzed by dissolving in water. The fluorenone was filtered, recrystallized from petroleum ether and melted at 83.5°; mixed m. p. with fluorenone unchanged.

The material insoluble in petroleum ether was treated with 10 cc. of hot toluene and filtered from the ammonium chloride. The toluene filtrate was concentrated to half of its original volume, diluted with an equal volume of ethyl alcohol, cooled and filtered; yield 0.54 g. Upon recrystallization it separated in beautiful white silky needles melting at 245°; mixed m. p. with dibiphenyleneethane, 245.5°.

No reaction was observed to take place when dibiphenyleneethylene and 9-fluorylamine were heated in such solvents as toluene, alcohol and pyridine.

The Reaction of 9-Fluorylamine with Benzalfluorene.—Upon heating the theoretical quantities of 9-fluorylamine and benzalfluorene[13] in liquid ammonia at 40° for one week, the reaction was found to be incomplete. For this reason the experiment was carried out at a higher temperature, using an excess of 9-fluorylamine.

A bomb tube containing 0.75 g. of 9-fluorylamine hydrochloride and 0.50 g. of benzalfluorene in liquid ammonia was heated at 60° for eight days. The tube was opened, the ammonia evaporated and the residue, a yellow oil, was dissolved in ether. The ether solution was treated with dry hydrogen chloride, which precipitated the hydrochlorides of fluorenone imide and 9-fluorylamine. The hydrochlorides, after filtering, were treated with water. The amine hydrochloride went into solution and the imide was hydrolyzed to fluorenone. The fluorenone was removed by filtration, recrystallized and identified; yield 0.055 g. Various attempts to separate benzylfluorene from benzalfluorene in the ether-soluble residue were unsuccessful.

The Dehydrogenation of 9-Fluorylamine with Indigo.—Since the completion of this reaction can be determined by the disappearance of the blue color of the solution, an excess of the amine was used. To a bomb tube containing wet liquid ammonia, was added 0.500 g. of 9-fluorylamine hydrochloride and 0.250 g. of indigo. The indigo was found to be insoluble at a temperature of −75°. However, it was sufficiently soluble at room temperature to give to the solvent a deep blue color. The reaction proceeded quite rapidly as in a few minutes the blue color of the solution had become greenish-yellow with the exception of a narrow band of blue at the surface of the liquid with a layer of a dark green color directly below. Upon shaking the tube more indigo went into solution, imparting to it a deep blue color, which again faded on standing, with the formation of the colored rings. This procedure was repeated several times, after which the rings at the surface were no longer formed. This phenomenon was without doubt due to the oxidation of the indigo white formed in the reaction by a slight amount of oxygen in the gaseous space of the tube. Upon shaking the tube in the latter stages of the experiment, the solution became dark green instead of blue,

---

[12] The preparation of fluorenone imide by treating fluorenone with liquid ammonia will be described in a later publication.

[13] Thiele, *Ber.*, **33**, 852 (1900).

indicating the presence of a considerable quantity of the yellow fluorenone imide. The bomb tube, after standing at room temperature for twenty-four hours, still contained a small amount of undissolved indigo. The reaction was then brought to completion by heating for fifteen minutes at 40°. The tube was opened and the indigo white oxidized to indigo by atmospheric oxygen. The ammonia was evaporated and the residue triturated with petroleum ether. The petroleum ether extract was treated with dry hydrogen chloride, which precipitated the hydrochlorides of fluorenone imide and 9-fluorylamine. The hydrochlorides were dissolved in water, which hydrolyzed the imide. The fluorenone was separated, purified and identified. The petroleum ether filtrate from the hydrochlorides was evaporated and yielded from 10–20 mg. of fluorene, the presence of which cannot be accounted for.

Another experiment was performed in which an excess of indigo was used. Here the fluorenone imide hydrochloride was obtained free from the amine hydrochloride and identified. The petroleum ether filtrate in this case did not contain any fluorene.

The Reaction of 9-Fluorylamine with Methylene Blue.—In one experiment 0.3 g. of 9-fluorylamine hydrochloride and 0.2 g. of methylene blue were treated with wet liquid ammonia. The solution turned a cherry red at room temperature and behaved similarly to the indigo experiment with regard to the fading of the dye in solution and the formation of the colored rings at the surface of the liquid. After one and one-half hours the reaction was complete and the solution was of a yellow color. The tube was opened and the leuco base oxidized to methylene blue. The imide and excess amine were precipitated as hydrochlorides and hydrolyzed; yield of fluorenone, 0.090 g. (theoretical, 0.113 g.).

Reaction of 9-Fluorylamine with Azobenzene.—Five-tenths of a gram of 9-fluorylamine hydrochloride and 0.42 g. of azobenzene dissolved completely in wet liquid ammonia at room temperature, producing a deep orange color. The reaction was carried out at a temperature of 40°. Within fifteen minutes an appreciable reaction had taken place, as was indicated by the decrease in intensity of the color of the solution. In thirty minutes the reaction was apparently complete, since on cooling the solution to about −75°, no azobenzene was precipitated. The tube was opened, the ammonia evaporated and the residue leached with ether. The ether filtrate was treated with dry hydrogen chloride and the precipitated hydrochlorides hydrolyzed. The fluorenone was separated (yield 0.360 g.), and after recrystallization melted at 82°; mixed melting point with an authentic specimen was unchanged.

The identification of hydrazobenzene was effected in another experiment in which the same quantities of reagents were used. The contents of the tube were transferred to a Dewar flask and the ammonia evaporated in a stream of nitrogen in order to prevent oxidation. The hydrazobenzene was rearranged to benzidine in the usual manner, filtered from fluorenone, precipitated first as the sulfate and then as the free base, which was identified; yield 0.16 g.

Reaction of Hydrazobenzene with Dibiphenyleneethylene.—A liquid ammonia solution containing 0.500 g. of dibiphenyleneethylene and 0.290 g. of hydrazobenzene was heated at 60° for a week. At the end of this time the reaction was complete. The progress of the reaction was easily followed by observing the gradual disappearance of the red ethylene compound, and the simultaneous increase in the colorless ethane. The tube was opened, the ammonia removed, the residue extracted with ether and filtered. The ether was removed from the filtrate and the red residue recrystallized from petroleum ether. The red needles (0.26 g.) which separated were shown to be azobenzene. The residue (0.28 g.) that was insoluble in ether was recrystallized twice from a solution of toluene and alcohol and melted at 247°; mixed m. p. with dibiphenyleneethane unchanged.

Systems that Did Not Interact.—The following products did not react under the conditions specified and in every case were recovered unchanged unless otherwise noted. In every case the solvent used was liquid ammonia.

(1)   Tetraphenylethylene[14] and 9-fluorylamine were heated at 60° for twenty hours.

(2)   Diphenylbiphenyleneethylene[15] and 9-fluorylamine hydrochloride heated at 40° for six days and then maintained at room temperature for one month.

(3)   Stilbene and 9-fluorylamine hydrochloride, allowed to stand at room temperature for four months.

(4)   Hydrazobenzene and 9-fluorylamine hydrochloride, heated at 60° for six days and kept at room temperature for three weeks.

(5)   Benzhydrylamine and methylene blue, heated at 60° for three days and kept at room temperature for four days, benzophenone was not detected upon hydrolysis of the products.

(6)   Dibiphenyleneethylene and benzhydrylamine, allowed to stand at room temperature for one month.

(7)   Benzhydrylamine hydrochloride and indigo, heated at 60° for twelve days, the indigo was decomposed; however, no benzophene was obtained on hydrolysis of the products.

Summary

1.   Evidence has been obtained in favor of Wieland's theory of dehydrogenation as being one of the mechanisms of biological oxidation.

2.   The dehydrogenation of 9-fluorylamine to fluorenone imide in liquid ammonia has been effected by dibiphenyleneethylene, benzalfluorene, azobenzene, indigo and methylene blue.

3.   Dibiphenyleneethylene has been found to dehydrogenate hydrazobenzene to azobenzene.

[14] Schlenk and Bergmann, *Ann.*, **463**, 15 (1928).

[15] Kaufmann, *Ber.*, **29**, 75 (1896).

Washington, D. C.

THE ISOLATION OF CAROTENE

By Harry N. Holmes and Henry M. Leicester

Since von Euler,[1] von Euler and Karrer and T. Moore[2] showed that carotene can replace vitamin A in the diet of a rat suffering from a deficiency of this substance, the problem of obtaining the pigment with greater ease than formerly has become important.   It seems certain that animals convert the carotene of plant food into vitamin A, hence the occurrence of the vitamin in butter, egg yolk and cod liver oil.

The methods used in the past[3] for the isolation of carotene have, in

[1] Von Euler, *Helv. Chim. Acta*, **12**, 278 (1929).

[2] Moore, *J. Soc. Chem. Ind.*, **49**, 238 (1930); *Biochem. J.*, **24**, 692(1930).

[3] For a summary of such methods, see Palmer, "Carotinoids and Related Pigments," The Chemical Catalog Co . New York, **1922**, Chap. 8.