centration of flavin-adenine dinucleotide in the lantern than in the rest of the firefly's tissues. Also the concentration of this flavin coenzyme in the lantern, 36–70 γ per gram of dry weight, is one-fourth to one-half that found in liver, which is one of the tissues richest in flavin-adenine dinucleotide in the mammalian organism. It is, therefore, not unreasonable to suspect that flavin-adenine dinucleotide may play some role in the luminescent mechanisms of the firefly.

DEPARTMENT OF BIOLOGICAL CHEMISTRY
HARVARD UNIVERSITY MEDICAL SCHOOL
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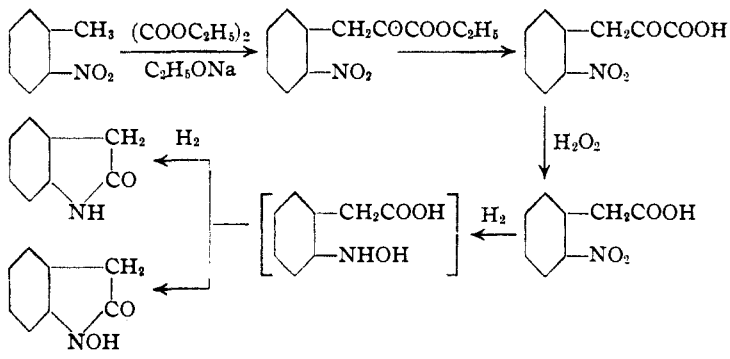
Synthesis of Oxindole

BY FREDERICK J. DI CARLO

The catalytic hydrogenation of *o*-nitrophenylacetic acid with Adams catalyst has been found to give oxindole in good yield. The starting acid was prepared by a modification of the method of Mayer and Balle.[1] It has been shown that ethyl *o*-nitrophenylpyruvate, which is formed by the condensation of ethyl oxalate with *o*-nitrotoluene, distills with steam. Consequently it was necessary to hydrolyze this ester completely before steam-distilling the excess *o*-nitrotoluene. This led to an increase of about 50% in the yield of *o*-nitrophenylpyruvic acid.

The oxidation of *o*-nitrophenylpyruvic acid to yield *o*-nitrophenylacetic acid has been reported.[1,2] Best results were obtained by the oxidation of a neutral solution with 3% hydrogen peroxide.



When the hydrogenation of *o*-nitrophenylacetic acid was carried out slowly in the presence of a small quantity of catalyst, an appreciable amount of 1,2-dioxindole was isolated. The hydrogenation of 1,2-dioxindole under similar conditions was ineffective. This suggests the formation of a hydroxylamine intermediate capable of concurrent slow ring closure to 1,2-dioxindole and rapid hydrogenation (followed by ring closure) to oxindole.

o-Nitrophenylpyruvic Acid.—A mixture of 43.8 g. (0.3 mole) of ethyl oxalate and 41.1 g. (0.3 mole) of *o*-nitrotoluene was poured into a cooled solution of 6.9 g. of sodium in 80 cc. of absolute alcohol. The mixture was refluxed for ten minutes. The volume was doubled by adding water and refluxing was continued for one and one-half hours in order to hydrolyze the ethyl *o*-nitrophenylpyruvate. Unreacted *o*-nitrotoluene was then recovered by steam distillation. The residue was cooled, acidified with hydrochloric acid and vigorously shaken in order to cause crystallization of the oil which separated. The *o*-nitrophenylpyruvic acid was filtered off, washed with water and dried; yield, 44 to 51 g. of crude product, m. p. *ca.* 115°. After treatment with charcoal and crystallization from water, the acid melted at 119–120°.

Oxindole.—A solution of 18.1 g. (0.1 mole) of *o*-nitrophenylacetic acid in 180 cc. of glacial acetic acid was subjected to hydrogenation at an initial pressure of 50 lb. per sq. in. in the presence of 0.2 g. of platinum oxide. When the reduction was complete (twenty minutes), the catalyst was removed by filtration and washed with a small portion of glacial acetic acid. After distillation of the solvent under diminished pressure, the residue was triturated with a solution of sodium carbonate, filtered and washed with water. The product was crystallized from water and 11.3 g. (88%) of oxindole was obtained as white needles, m. p. 127–129°.

Anal. Calcd. for C_8H_7NO: N, 10.52. Found: N, 10.60.

1,2-Dioxindole.—The hydrogenation of a solution of 18.1 g. of *o*-nitrophenylacetic acid in 180 cc. of glacial acetic acid in the presence of 0.05 g. of platinum oxide required several hours and a poorer yield of oxindole was obtained (75%). Acidification of the sodium carbonate washings with hydrochloric acid caused the precipitation of a mixture of *o*-nitrophenylacetic acid and 1,2-dioxindole. The former was removed with dilute sodium bicarbonate solution; 0.9 g. (m. p. 143.5°) separated upon addition of hydrochloric acid. The 1,2-dioxindole was treated with charcoal and twice recrystallized from water; 1.0 g. was obtained in the form of glistening plates, m. p. 198–199° A mixed melting point with the product prepared by the method of Reissert[2] showed no depression. 1,2-Dioxindole reduced Fehling solution on heating.

Anal. Calcd. for $C_8H_7O_2N$: N, 9.39. Found: N, 9.30.

When 0.1 g. of platinum oxide was employed, the hydrogenation required forty-five minutes. The yield of oxindole was 85% and 0.2 g. of pure dioxindole was isolated. Use of 0.02 g. of platinum oxide caused but little reduction within twenty-four hours and 80% of the *o*-nitrophenylacetic acid was recovered.

Brucine Salt of 1,2-Dioxindole.—1.8 g. of brucine was added to a warm solution of 0.75 g. of 1,2-dioxindole in methyl alcohol. The salt separated and was crystallized from ethyl alcohol; cubic crystals, m. p. 223°. Its aqueous solution became intensely blue upon the addition of a drop of ferric chloride solution.

Anal. Calcd. for $C_{31}H_{33}O_6N_3$: N, 7.73. Found: N, 7.77.

A solution of 2.5 g. of 1,2-dioxindole in 250 cc. of glacial acetic acid was subjected to hydrogenation for six hours in the presence of 0.05 g. of platinum oxide at an initial pressure of 50 lb. per sq. in. The solvent was distilled under reduced pressure and the residue was dissolved in a warm solution of sodium carbonate. Acidification of the carbonate solution resulted in the separation of 2.3 g. of pure 1,2-dioxindole.

DEPARTMENT OF CHEMISTRY
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(1) Mayer and Balle, *Ann.*, **403**, 188–189 (1914).
(2) Reissert, *Ber.*, **30**, 1043 (1897); **41**, 3924 (1908).

Esterification of Fatty and Amino Acids with 1,2-Epoxides in Aqueous Solution

BY HEINZ FRAENKEL-CONRAT AND HAROLD S. OLCOTT

It has recently been shown[1] that 1,2-epoxides

(1) Fraenkel-Conrat, *J. Biol. Chem.*, **154**, 227 (1944).

react readily with proteins in neutral aqueous solution at room temperature. Esterification of the carboxyl groups appeared to be the predominating reaction. Since little attention had previously been given to the use of epoxides as esterifying agents,[2,3] model experiments were performed in which fatty acids and amino acids were treated with ethylene oxide, 1,2-propylene oxide, or epichlorohydrin in aqueous solution or suspension at room temperature.

In agreement with Brönsted,[2] dissociation of the acids was found to favor the reaction. Thus in four days acetic acid alone (0.06 *M*) was only 3% esterified by a 30-fold excess of propylene oxide, while, in the presence of a small amount of alkali metal ions, esterification of the acid approached completion. This catalytic action was produced not only by hydroxides directly but also by neutral salts, most readily by halides, which yield hydroxyl ions with the excess reagent according to the equation

$$CH_3—\underset{\diagdown O \diagup}{CH}—CHR + X^- + H_2O \longrightarrow CH_3CHOH—CHRX + OH^-$$

Possibly because simple synthetic methods have not been available, many monoesters of lower fatty acids with 1,2-diglycols are not known. As examples of the possible preparative use of the reaction of epoxides with fatty acids, ethylene glycol monovalerate and propylene glycol-1-monobutyrate were synthesized as follows:

The fatty acid (0.1) mole was treated in water solution or suspension with 0.01 mole NaOH and 1 to 2 moles of the epoxide. After standing four to six days at room temperature (with occasional shaking if the system was biphasic), the solution had become neutral through esterification of the free acid. The ester was then extracted with ether, washed with potassium carbonate, dried and distilled.

Both esters boiled at 56 to 57° (0.5 to 1 mm.). They were isolated in 58 to 63% yield. The refractive index at 25° was 1.4300 for ethylene glycol monovalerate and 1.4246 for propylene glycol-1-monobutyrate. *Anal.* Calcd. for $C_7H_{14}O_3$: C, 57.5; H, 9.6; saponification number, 384. Found for ethylene glycol monovalerate: C, 57.2; H, 9.6; saponification number, 387. Found for propylene glycol 1-monobutyrate: C, 57.0; H, 9.7; saponification number, 386.

In order to demonstrate that the addition of acids to unsymmetrical epoxides, *e. g.*, propylene oxide, occurred on carbon atom 1 in aqueous solution as it is known to do in anhydrous media, propylene glycol 1-monobutyrate was also prepared by refluxing an alcoholic solution of 1-chloro-2-propanol and sodium butyrate. The product, obtained in poor yield, boiled at 57° (1 mm.) and showed a refractive index of 1.4245.

Titrations and *p*H measurements demonstrated that amino acids or acylated amino acids were also readily esterified by epoxides. Thus, the interaction of propylene oxide with benzoyl-*d,l*-alanine, in the presence of one-tenth of the equivalent amount of sodium hydroxide, led to the disappearance of the undissolved material within one day. After three days the *p*H of the mixture had risen from 3.5 to 7.

In contrast to the carboxyl groups, the amino groups, determined by the Van Slyke manometric method,[4] appeared to react much more readily in the uncharged state, *i. e.*, in alkaline solution or only after all acids originally present had been "neutralized" by combination with the epoxide. Complete disappearance of the primary amino groups of 0.08 *M* solutions of monosodium glutamate or of alanine in the presence of sodium acetate (0.08 *M*) occurred within two days of treatment with excess propylene oxide (3 *M*). Surprisingly, the dipolar ion did not react as readily in the absence as in the presence of other electrolytes.

Attempts to isolate the epoxide derivatives of amino acids in pure form were unsuccessful since neither they nor a number of their derivatives could be made to crystallize. The products were very soluble in water and alcohol and could not be distilled without decomposition or molecular rearrangement.

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(2) Brönsted, Kilpatrick and Kilpatrick, THIS JOURNAL, **51**, 428 (1929).

(3) Bauer and Mauthe, U. S. Patent 1,979,601 (1934).

(4) Van Slyke, *J. Biol. Chem.*, **83**, 425 (1929).

A Method of Synthesis for Aromatic Aminoaldehydes and Aminoketones

BY WU HAO-TSING

It has been found that the reaction of hydrogen cyanide or nitriles, hydrogen chloride and phenols,[1] which has always been considered to be specific for aromatic hydroxy compounds, can be extended to the aromatic amine, aniline. Preliminary studies have demonstrated that *p*-aminobenzaldehyde and *p*-aminoacetophenone can be prepared by this method, and it seems likely that the reaction can be extended. Further work on the improvement of the yield and on the synthesis of related compounds is now in progress.

It is believed that an addition compound (I) is first produced and this on heating rearranges to the carbon substitution product (II) which on hydrolysis gives the carbonyl derivative (III).

$$RCN^a + C_6H_5NH_2 \xrightarrow[\text{Dry ether}]{HCl} \underset{\text{I}}{R—C(=NH)—N(H)—C_6H_5} \xrightarrow{\text{heat}} \underset{\text{II}}{R—C(=NH)—C_6H_4NH_2} \xrightarrow{H_2O} \underset{\text{III}}{R—C(=O)—C_6H_4NH_2}$$

[a] In the two experiments reported R has been hydrogen and a methyl group. It is thought other alkyl groups or possibly aryl groups can be used.

Experimental

(1) **Preparation of *p*-Aminobenzaldehyde.**—Ten grams of dry hydrogen cyanide and 9.3 g. of aniline were added to 70 cc. of ether which had been previously saturated with dry hydrogen chloride. When the mixture thus obtained was enclosed in a bottle and gently heated for several hours, an oily liquid, brown in color, was precipitated. This oily liquid was then transferred into a sealed tube, and heated at 250–300° for one hour. The contents of the sealed tube were afterward put into a solution of potassium hydroxide, boiled for a few minutes, extracted with ether and recrystallized from water in the form of leaflets. These melted at 70–72° (percentage of nitrogen determined, 11.60; calculated, 11.57).

(2) **Preparation of *p*-Aminoacetophenone.**—*p*-Aminoacetophenone was prepared by the reaction of aniline

(1) Hoesch, *Ber.*, **48**, 1122 (1915).