fractive indices: α, 1.537; β, 1.565; γ, 1.571, all $\pm$ 0.002, agree well with values reported in the literature for natural sucrose.[20]

Rate of Hydrolysis of Synthetic Sucrose.—The hydrolysis of a 2% solution of the synthetic sucrose in 1 *N* hydrochloric acid was followed by observing the change of rotation at 23.5°. The rate of hydrolysis was compared with that of a similar solution of natural sucrose under the same conditions. The course of hydrolysis of both sugars is represented by a logarithmic curve, indicating a first order reaction, Fig. 2. The velocity constant *K*, under these conditions, is 0.0105. It is evident from Fig. 2 that the rate of hydrolysis of the synthetic product is identical with that of natural sucrose.

Sucrose Octaacetate.—A 0.5-g. sample of the synthetic sucrose was treated with 3.5 ml. of pyridine and 2.3 ml. of acetic anhydride at 0°. The mixture was kept at 3° for three days with frequent shaking until the sugar dissolved. The solution was then filtered and poured into 12.5 ml. of ice-water with stirring. The amorphous precipitate which separated out was removed by filtration and dissolved in chloroform. The chloroform solution was washed first with sodium bicarbonate solution and then with water. The chloroform phase was evaporated to a sirup, petroleum ether added, and the mixture stirred. Upon standing, the product crystallized out. The crystals were filtered and dried at 30° *in vacuo*. The yield of the acetylated product was 0.65 g.

Anal. Calcd. for $C_{12}H_{14}O_{11}(CH_3CO)_8$: CH_3CO, 50.7. Found: CH_3CO, 50.1. Specific rotation: $[\alpha]D$ +60° (in chloroform, *c* 2). Melting point, 69–70°. The specific rotation for sucrose octaacetate given in the literature is $[\alpha]D$ +59.6[21] and the melting point is 69°.[22] Irvine, *et al.*,[5] found the specific rotation for *iso*-sucrose octaacetate to be $[\alpha]D$ +20.3° and the melting point to be 131–132°.

The authors are indebted to Professor W. H. Dore for making the X-ray diffraction patterns of the synthetic and natural sucroses, and to Professor T. E. Rawlins for assistance in determining the optical properties of crystalline sucrose.

Summary

Synthetic crystalline sucrose has been obtained from glucose-1-phosphate and fructose through the action of sucrose phosphorylase from *Pseudomonas saccharophila*.

Data presented show that the chemical constitution of the synthetic product is identical with that of natural sucrose.

The de-phosphorolytic condensation of α-glucose-1-phosphate and fructose, resulting in the formation of sucrose, supports the conclusion that glucose exists in the sucrose molecule in the α-form.

(20) H. E. Merwin, "Int. Crit. Tables," **7**, 30 (1930).

(21) C. S. Hudson and J. M. Johnson, THIS JOURNAL, **37**, 2748 (1915).

(22) S. V. Shah and Y. M. Chakradeo, *Current Sci.*, **4**, 652 (1936).

BERKELEY, CALIFORNIA RECEIVED MAY 12, 1944

NOTES

The Flavin-Adenine Dinucleotide Content of Firefly Lanterns

BY ERIC G. BALL[1] AND PAULINE A. RAMSDELL

The recent note by Johnson and Eyring[2] suggesting that a flavoprotein plays a role in the process of luminescence by living organisms prompts us to publish the following data on the flavin-adenine dinucleotide content of firefly lanterns. The data were obtained by us during June, 1940, at which time the authors were working in the Department of Physiological Chemistry at the Johns Hopkins Medical School.

The lanterns of forty-five fireflies (species unidentified) caught the night before were severed from the insects' bodies and immediately dropped into 50 cc. of acetone and ground with a mortar and pestle. The suspension was centrifuged, the residue washed twice with two 10-cc. portions of acetone and then air dried. A total of 133 mg. of fine white powder was thus obtained which was stored over calcium chloride in the cold. The following day, 10 mg. of this powder was added in the dark to 1.0 cc. of water. Light was emitted immediately upon addition of the water to the powder and rapidly faded out. The suspension was then heated at 80° for ten minutes, cooled in running water, and centrifuged. An aliquot of the clear supernatant was then analyzed for flavin-adenine dinucleotide by means of its ability to restore oxygen consumption to a coenzyme-free *d*-amino acid oxidase system in the manner described by Warburg and Christian.[3] A flavin-adenine dinucleotide content of 70 γ per gram of dry material was found. A pure sample of flavin-adenine dinucleotide obtained from Professor Warburg served to standardize the enzyme preparation.

The lanterns were severed from another batch of ten fireflies. The total lantern material, which weighed 109 mg., was immediately ground with water in a mortar and then heated at 80° for ten minutes. The suspension was cooled and centrifuged. The remaining portions of the insects, which weighed 454 mg., were treated in a similar manner. Aliquots of both supernatants were then analyzed for their flavin-adenine dinucleotide content. The lantern portion was found to contain 9.1 γ of the coenzyme per gram of wet material. Since the lanterns contain about 75% water, this equals about 36 γ per gram of dry material. The flavin-adenine dinucleotide concentration in the rest of the insects' bodies was found to be not more than 15% of the lantern value.

There thus appears to be a much higher con-

(1) Present address: Department of Biological Chemistry, Harvard Medical School.

(2) Johnson and Eyring, THIS JOURNAL, **66**, 848 (1944).

(3) Warburg and Christian, *Biochem. Z.*, **298**, 150 (1938).

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.

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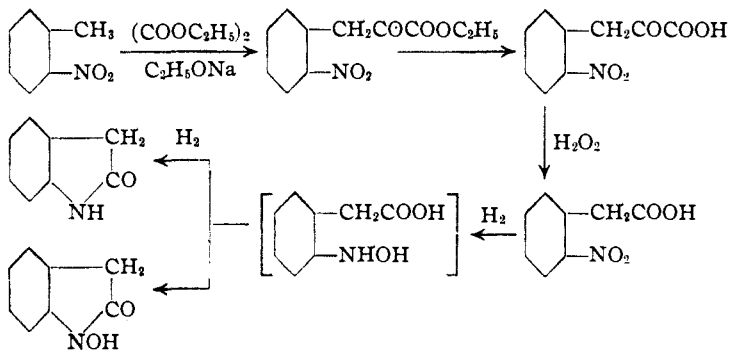
Synthesis of Oxindole

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.

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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).