[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

ENZYME MODELS. I. THE PREPARATION OF SOME ARTIFICIAL CARBOXYLASES

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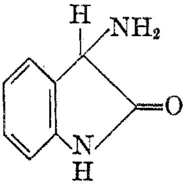
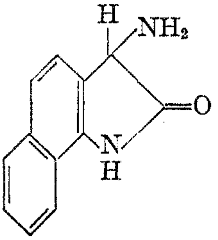
INTRODUCTION

The study of the decarboxylation of α-keto acids by means of enzymes is difficult, the sensitivity of the enzyme to reaction conditions interfering with sampling technique and also limiting the possible reaction conditions which can be employed. For these reasons the study of the reaction between simpler, less sensitive organic molecules upon α-keto acids affords a system whose kinetics may be followed more closely and over a wider range of reaction conditions. The ultimate value of such a study is the possible substitution of simple organic molecules for an enzyme and/or the possible elucidation of the mechanism of the decarboxylation of α-keto acids, a step in the oxidation of fatty acids.

Considerable work has already been done on the synthesis of carboxylase models by W. Langenbeck (1, 2). An excellent review of the subject has been published by M. G. J. Beets (3). It was early discovered (4) that primary amines have a marked catalytic effect upon the decarboxylation of α-keto acids. It has been shown that an amine group is more active when adjacent to a carboxyl group. Thus, β-aminobutyric acid is only about twice as active as methyl or ethylamine, while α-aminobutyric acid is about six times as active. Another advance was made with the discovery that certain amino-acid lactams are far more active than the aliphatic amino-acids. Thus, 3-aminoöxindole (I) is approximately 700 times as effective as methylamine. Other, even more active, amines are 3-amino-α-naphthoxindole (II) and 3-amino-6,7-benzo-α-naphthoxindole (III). III is the most active decarboxylation catalyst so far produced.



I



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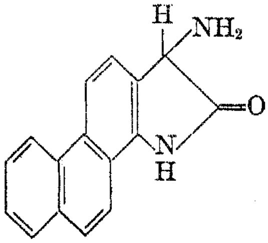

III

The present investigation is concerned with some aminoöxindoles and the benzal derivatives of certain ones of them. Subsequent papers will deal with kinetic studies and their interpretation.

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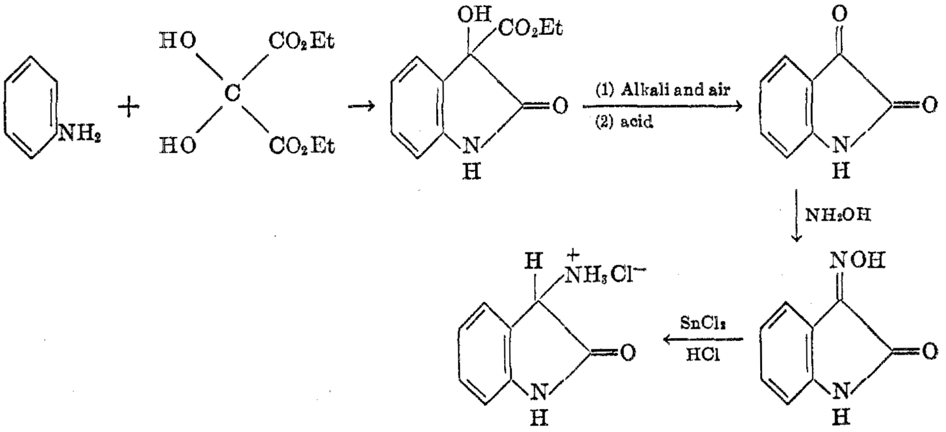
THE 3-AMINOÖXINDOLES

The general reactions for preparing the 3-aminoöxindole hydrochlorides may be formulated as follows:

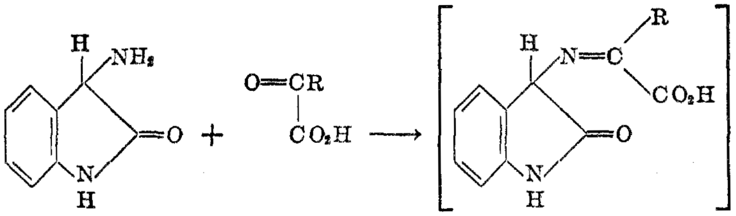


Using this reaction sequence I and II were prepared.

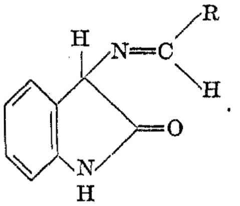
Specific directions for these syntheses are not in the literature and thus they are incorporated in the Experimental. Some difficulty was encountered in the analysis of II until it was noted that it had been obtained as a monohydrate. Its picrate likewise analyzes for the monohydrate. Langenbeck reports II only as the anhydrous compound (see Experimental).

THE SCHIFF BASES

A possible intermediate in the decarboxylation of the α-ketocarboxylic acids might be the Schiff base formed by condensation of the 3-amino group with the keto group.

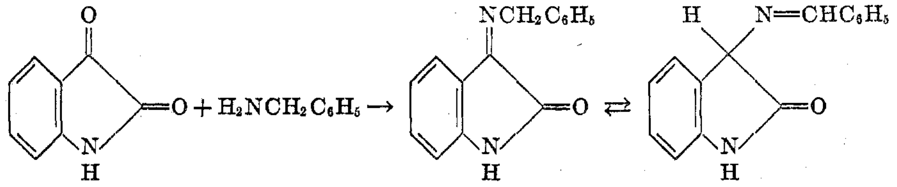


Following decarboxylation, the structure of the intermediate would be



Schönberg, *et al.* postulated such intermediates in the Strecker degradation, but did not isolate any (5, 6). Therefore, experiments were devised to obtain compounds of the latter type in order to test their efficacy as decarboxylation catalysts and so establish their function as intermediates.

Unfortunately, the 3-aminoöxindoles were found not to condense with either benzaldehyde or *p*-nitrobenzaldehyde without extensive decomposition. It proved possible, however, successfully to condense the isatins with benzylamine to form compounds which would be tautomeric with the desired ones. In this way the benzal derivatives of 3-aminoöxindole (IA), 3-amino-5-bromoöxindole (IVA), 3-amino-α-naphthoxindole (IIA), and 3-amino-6-hydroxy-α-naphthoxindole (VA), were synthesized. These compounds are tautomeric with the ones which might be produced during decarboxylation and so they were used without rigorous proof of the position of the double bond (Ia ⇄ Ib). As a possible clue to the position of the double bond, the colors of the compounds may be mentioned.[2]



Ia Ib

It is well-known that the *ortho*-quinoid structure, as in Ia, is invariably accompanied by color, while compounds not having this structure, as in Ib, are colorless, or absorb only in the ultraviolet region. Therefore, it is reasonable that the benzal derivatives IA and IVA which are colorless have structures analogous to Ib, while the benzal derivatives IIA and VA being colored correspond to Ia. The naming employed here assumes Ib for all. This has been done for simplification and should not be construed as tantamount to unequivocal assignment of structure.

EXPERIMENTAL

All temperatures are uncorrected.

Starting materials. Ethyl oxomalonate was prepared according to *Organic Syntheses* (7). *Isatin* was recrystallized to m.p. 200°. *5-Bromoisatin*, m.p. 252° (dec.), was prepared by bromination of isatin in acetic acid according to Borsche and Jacobs (8) [reported m.p. 247–248° (8), 255° (9)]. *5-Amino-1-naphthol* was purified by dissolving in water and treating repeatedly with charcoal until filtration gave a clear pale grey solution. Addition of a slight excess of a saturated solution of sodium bicarbonate gave an almost colorless precipitate which was filtered, washed with water, and dried under a vacuum at 35°. The product darkened rapidly and had to be used within 24 hours after purification, m.p. 185° (dec.). The dibenzoyl derivative melted at 272–273° [reported (10) 276°].

Isatin oxime. The procedure is adapted from that of Borsche and Sander (11). A solution

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[2] In connection with catalytic studies, the Referee has pointed out that the compounds I, II, and III contain asymmetric carbon atoms. A study of the resolution and of possible differences in catalytic action of the enantiomorphs would be rewarding since the asymmetry persists in the intermediates.

of 48.6 g. (0.70 mole) of hydroxylamine hydrochloride in 100 ml. of water was added to 73.5 g. (0.50 mole) of isatin in 200 ml. of water contained in a three-necked flask equipped with a stirrer and reflux condenser. This was followed by 95.2 g. (0.70 mole) of sodium acetate trihydrate in 200 ml. of water. The mixture was warmed to 60° and maintained at this temperature for 45 minutes while being stirred vigorously. The color changed from deep red to light brown. Standing overnight at room temperature was followed by filtration, washing with water, and two recrystallizations from an alcohol-water mixture (carbon) to give 74.0 g. (0.46 mole, 91%) of bright yellow product, m.p. 223–225° (dec.) [reported (11) m.p. 221–222°].

Anal. Calc'd for $C_8H_6N_2O_2$: N, 17.28. Found: N, 17.16.

3-Aminoöxindole hydrochloride. The reduction of the isatin oxime was adapted from a method given in *Organic Preparations* (12).

A solution of 30 g. (0.185 mole) of isatin oxime was added over 30 minutes with stirring to 200 g. (1.05 mole) stannous chloride dissolved in 200 ml. of concentrated hydrochloric acid maintained throughout at 5°. After stirring for an additional 1.5 hours, the solution was allowed to warm to room temperature and stand overnight. Diluting to one liter was followed by passing in hydrogen sulfide and centrifuging to remove tin. This alternate precipitation and centrifugation was repeated several times. Finally, the solution was concentrated at reduced pressure under nitrogen to about 100 ml. to remove most of the hydrochloric acid, again diluted to one liter, saturated with hydrogen sulfide and filtered. The centrifuged and filtered solution was now concentrated to 75–100 ml. Filtering the cold solution gave colorless crystals which were washed with propanol-2, ether, and dried under a vacuum at 30°. The yield was 17 g. (0.092 mole; 50%).

Anal. Calc'd for $C_8H_9ClN_2O$: C, 52.04; H, 4.92.
Found: C, 52.5; H, 5.3.

The compound turns pink upon standing. This colored impurity could be removed with some loss by washing with ethanol.

The removal of tin with hydrogen sulfide is slow and accompanied by loss of product due to occlusion by tin sulfide. However, electrolytic deposition proved even slower and was accompanied by the danger of oxidation of the product by air or by the evolved chlorine gas. Ion exchange proved unfruitful because the ammonium ion was absorbed as well as the tin. Hence, the hydrogen sulfide method, while not ideal, was the best method tried.

6,7-Benzoisatin. This compound was prepared according to Martinet (13). Condensation of 1-naphthylamine with ethyl mesoxalate in glacial acetic acid gave 3-hydroxy-3-carbethoxy-α-naphthoxindole in 28% yield, m.p. 189° (reported 201°). This product was converted to 6,7-benzoisatin in 83% yield by air-oxidation in the presence of strong alkali followed by treatment with hydrochloric acid; on heating the product there was coloring at 203° and finally melting at 240° (dec.) [reported m.p. 255° (14)].

6,7-Benzoisatin oxime. This was prepared from 6,7-benzoisatin in a manner entirely analogous to that for isatin oxime, 8 g. (0.040 mole) gave 5.0 g. (0.024 mole; 59%) of orange crystals, m.p. 235–236° (dec.), from water-propanol-2. The reported melting point is "about 230° dec." (11).

3-Amino-α-naphthoxindole hydrochloride monohydrate. The procedure used, that of Langenbeck and coworkers (15), gave only the hydrated product and so the specific procedure employed here is given.

A solution of 10 g. of stannous chloride in 10 ml. of concentrated hydrochloric acid was added to a boiling solution of 1.0 g. (4.7 mmole) of 6,7-benzoisatin oxime in 200 ml. of ethanol. Reflux was continued for 4.5 hours, the mixture allowed to stand at room temperature for two days and filtered. The crude material was washed with ether, alcohol, again with ether, and finally air-dried to give 0.90 g. (3.8 mmole; 82%) of colorless product. The product was sensitive to heat and air.

Anal. Calc'd for $C_{12}H_{11}ClN_2O$: N, 11.9.
Calc'd for $C_{12}H_{11}ClN_2O \cdot H_2O$: N, 11.1. Found: N, 11.2.

3-Amino-α-naphthoxindole picrate monohydrate. A solution of 0.5 g. of the amine hydrochloride in 10 ml. of methanol was boiled with 10 ml. of a saturated solution of picric acid in methanol. Filtration gave 0.8 g. (80%) of the granular, yellow picrate, decomposing without melting at 130–135°.

Anal. Calc'd for $C_{18}H_{13}N_5O_8$: C, 50.59; H, 3.07; N, 16.39.

Calc'd for $C_{18}H_{13}N_5O_8 \cdot H_2O$: C, 48.54; H, 3.40; N, 15.73.

Found: C, 48.47; H, 3.47; N, 15.91.

3,6-Dihydroxy-3-carbethoxy-α-naphthoxindole. The literature procedure (13) for this compound did not give the desired product easily. After a number of experiments the procedure adopted here gave better results.

A solution of 21.1 g. (0.11 mole) of ethyl mesoxalate [made by mixing 19.1 g. (0.11 mole) of ethyl oxomalonate with 2.0 g. of water] in 10 ml. of acetic acid was added to 15.9 g. (0.10 mole) of 5-amino-1-naphthol in 30 ml. of acetic acid. Acetic acid (10 ml.) was used to wash in residual ethyl mesoxalate. After heating 30 minutes on a steam-bath and cooling to room temperature, 150 ml. of a saturated solution of sodium sulfate was added and the mixture allowed to stand for 15–20 minutes. The precipitate was filtered, washed with water, and recrystallized twice from water-ethanol (charcoal) to give 5.5 g. (0.019 mole; 17%) of a light red-brown powder, m.p. 202° (dec.) [reported (16) 200–201° (dec.)].

6-Hydroxy-benz[g]isatin. The isatin was obtained from the above compound as reported in the literature (16).

SCHIFF BASES

Benzal of 3-aminoöxindole. A. *Hot condensation.* A stream of nitrogen was bubbled through a solution of 7.4 g. (0.050 mole) of isatin (m.p. 200°) and 6.42 g. (0.060 mole) of benzylamine in 150 ml. of ethanol for about 5 minutes to sweep out air. Five drops of acetic acid were added and the solution refluxed for 30 minutes. No crystals deposited upon cooling overnight, but water caused the separation of a gummy material. Recrystallization from benzene-petroleum ether (b.p. 60–70°), ethanol-water (charcoal), and again from benzene-petroleum ether gave 4.1 g. (0.017 mole: 35%) of an almost colorless product with an orange tinge, m.p. 228° (dec.).

Anal. Calc'd for $C_{15}H_{12}N_2O$: C, 76.25; H, 5.12; N, 11.86.

Found: C, 75.91; H, 5.40; N, 11.80.

It was interesting that the platelets formed exhibited, when dry, static electrical properties to a remarkable degree. The particles could not be piled up in a heap on the bottom of the container but would align themselves along the bottom and walls of the vessel so that each particle maintained a maximum distance from its neighbor. For example, it was not found possible to add more than 3 or 4 mg. of the substance to a standard micro-porcelain boat, as once the walls and bottom of the boat were coated, additional compound when added would fly out of the boat. None of the other benzal compounds possessed this unusual property. No experiments were carried out in an effort to discharge the static electricity. A similar effect was recently reported for C^{14}-containing barium carbonate (17).

B. *Cold condensation.* This procedure gave a less gummy product in slightly improved yield. A stream of nitrogen was bubbled through 7.4 g. (0.050 mole) of isatin, 6.42 g. (0.060 mole) of benzylamine, and 3 ml. of acetic acid in 150 ml. of ethanol for 15 hours. An amorphous brown precipitate (5.3 g.) was collected, triturated in a mortar with water, and dried. The dried material was dissolved in toluene and petroleum ether (b.p. 60–70°) was added to incipient crystallization. The solution was heated to boiling to yield a voluminous, colorless precipitate which was filtered hot and washed with cold toluene. The product (4.5 g., 0.019 mole; 38%) did not depress the melting point of the previously prepared material.

Benzal of 3-amino-5-bromoöxindole. A solution of 11.3 g. (0.050 mole) of 5-bromoisatin, 6.42 g. (0.060 mole) of benzylamine, and 3 ml. of acetic acid in 150 ml. of ethanol was agitated for 15 hours by a stream of nitrogen. A rose-colored precipitate was collected; the addition of 3 volumes of water precipitated an additional quantity of solid. The combined precipitates were dried, ground with toluene, filtered, and the solution heated just to boiling. Hot filtration gave 1.0 g. (0.0036 moles; 6.4%) of a white powder, m.p. 223–224° (dec.). The compound slowly became red as the temperature rose, and was quite brilliant just under the melting point.

Anal. Calc'd for $C_{15}H_{11}BrN_2O$: N, 8.89. Found: N, 8.99.

Benzal of 3-amino-α-naphthoxindole. A mixture of 3.0 g. (15 mmole) of 6,7-benzoisatin, 2.1 g. (20 mmole) of benzylamine, and 1 ml. of acetic acid in 115 ml. of ethanol was agitated overnight by a stream of nitrogen. A small quantity of insoluble material remaining was filtered and discarded. Addition of 50 ml. of water gave a precipitate, and further addition of 150 ml. of water (charcoal) gave a second crop. The combined materials were dissolved in acetone, water was added to incipient crystallization (charcoal), the solution was filtered and the product precipitated with additional water.

The product appeared to be a labile substance. When a toluene solution was passed through a packed alumina column, about 90% was adsorbed as a brown zone while the remainder was recovered from the percolate as an orange zone substance. Within a few minutes the orange solution became tan and the original compound could be recovered from the toluene solution.

The over-all yield was 2.6 g. (9.1 mmole; 60%).

Anal. Calc'd for $C_{19}H_{14}N_2O$: N, 9.79. Found: N, 9.29.

Benzal of 3-amino-6-hydroxy-α-naphthoxindole dihydrate. A solution of 1.6 g. (7.5 mmole) of 6-hydroxy-6,7-benzoisatin, 0.91 g. (8.5 mmole) of benzylamine, and 0.3 ml. of acetic acid in 70 ml. of ethanol was agitated with a stream of nitrogen for 15 hours. The addition of 200 ml. of water and cooling in an ice-bath gave a brick red product which was dried at 35° at reduced pressure. Upon standing the material decomposed with the odor of benzaldehyde. The compound was washed with ether immediately before analysis and also before an enzymatic run.

Anal. Calc'd for $C_{19}H_{14}N_2O_2$: N, 9.27.
Calc'd for $C_{19}H_{18}N_2O_4$: N, 8.28. Found: N, 8.37.

The *dialcoholate* was prepared by dissolving 0.1 g. of the dihydrate in ethanol, heating to boiling, filtering, and evaporating to dryness in a current of nitrogen. It was analyzed after drying for two hours at reduced pressure at 67°.

Anal. Calc'd for $C_{23}H_{26}N_2O_4$: N, 7.10. Found: N, 7.31.

SUMMARY

1. The syntheses of 3-aminoöxindole hydrochloride, 3-amino-α-naphthoxindole hydrochloride, and 3,6-dihydroxy-3-carbethoxy-α-naphthoxindole have been improved. The picrate of 3-amino-α-naphthoxindole has been prepared.

2. The benzal derivatives of 3-aminoöxindole, 3-amino-5-bromoöxindole, 3-amino-α-naphthoxindole, and 3-amino-6-hydroxy-α-naphthoxindole have been prepared. The latter compound has been obtained as the dihydrate and also as the dialcoholate.

3. A tentative interpretation of the color of the benzal derivatives is offered.

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