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Preparation and Properties of 3-Indoleacetaldehyde[1]

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Introduction

The neutral plant growth substance, 3-indoleacetaldehyde (IAc), has been found to occur in several plant tissues by Larsen (1), Hemberg (2), and Gordon and Nieva (3). Its presence in the plant extracts was established indirectly by conversion to 3-indoleacetic acid (IAA) by aldehydrase systems and subsequent auxin assay. Gordon and Nieva (4) showed that IAc may be the immediate precursor of IAA in pineapple tissue.

Larsen (1) prepared microgram quantities of IAc from tryptophan by treatment with isatin or ninhydrin. The yields were less than 2% and the preparations were impure. The synthesis of 3-indoleacetaldehyde in quantities large enough to isolate in a pure form was first reported in 1952 by Brown *et al.* (5). The synthesis was carried out using indole as the starting material. The Grignard derivative of indole was prepared and treated with allyl bromide to give 3-allylindole. The 3-allylindole was treated with osmium tetroxide to produce 3,3-indolepropane-1,2-diol. This glycol was oxidized to 3-indoleacetaldehyde with periodate, and the aldehyde was purified by distillation at reduced pressure.

The preparation of 3-indoleacetaldehyde was achieved in this laboratory by a different method at about the same time in 1952, but interest in publishing the method was lost after the report by Brown *et al.* (5) appeared. Recently, several workers have indicated that they were unsuccessful in attempts to synthesize IAc by the method of Brown *et al.* (5) and that the compound was still unavailable for research studies in the plant hormone field. Therefore, the simple one-step method used successfully in this laboratory for the preparation of 3-indoleacetaldehyde is reported here. Results of tests that were run to establish the identity of the product and to determine its chemical and biological properties are also reported.

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The method of preparation consisted of oxidation of tryptophan with dilute sodium hypochlorite solution and was similar to the method used by Larsen (1) except that a different oxidizing agent was used and precautions were taken to prevent destruction of the aldehyde as it was being formed. The reaction is based on that studied by Langheld (6) who showed that when an α-amino acid is treated with sodium hypochlorite it is converted into an aldehyde containing one less carbon atom than the original amino acid. The oxidation of α-amino acids to aldehydes and nitriles has in fact been accomplished with a variety of reagents (7).

EXPERIMENTAL METHODS AND RESULTS

Preparation of 3-Indoleacetaldehyde from Tryptophan

To 100 ml. of distilled water were added 15 ml. of 10% NaOH solution and 3.0 g. of DL-tryptophan. After the tryptophan was completely dissolved, 4 *N* HCl was added until tryptophan crystals just started to separate at a pH near 8.5. The solution was diluted immediately with 1200 ml. of distilled water in a 4000-ml. Erlenmeyer flask. To this solution was added 700 ml. benzene followed by 200 ml. of 0.52% sodium hypochlorite solution made by mixing 1 part Clorox with 9 parts water. The flask and contents were shaken with a swirling motion immediately after the addition of the hypochlorite and during the entire duration of the experiment so that the aldehyde was taken up in the benzene as soon as it was produced. Care was taken to avoid an excess of hypochlorite which produced brown-colored products. This also happened if dilute solutions were not used. The flask was immersed in a hot water bath, and the temperature of the contents was raised to 50°C. over a period of 20 min. The temperature was held between 50 and 51°C. for 15 min. more. The benzene layer became yellow in color and later turned orange. The benzene was separated from the aqueous phase with a separatory funnel while still warm.

The benzene solution was concentrated to 65 ml. in a large evaporating dish placed in a swift current of air in a fume hood. Slight heat was supplied with a warm water bath at 40–50°C. The resulting benzene solution was shaken with 50 ml. of saturated sodium bisulfite solution in a closed wide-mouth bottle. Crystals of the IAc.$NaHSO_3$ formed immediately and were filtered almost dry by suction. The product was resuspended twice and shaken vigorously in 95% ethanol and filtered each time. The crystals were washed again with absolute alcohol and finally with ether. After drying at room temperature, the yield was 4.06 g. of white crystalline material. The yield was 90% of theoretical based on the amount of tryptophan used. Most of the IAc.$NaHSO_3$ was dissolved in a small amount of water making a saturated solution. The solution was filtered, and the IAc.$NaHSO_3$ was recrystallized by adding absolute ethanol to a final concentration of 90% ethanol, yielding 2.5 g. of recrystallized product.

To obtain the free aldehyde, 200 mg. of the IAc.$NaHSO_3$ was dissolved in 8 ml. water. A few drops of a saturated solution of Na_2CO_3 was added until the solution turned slightly turbid. After standing a few minutes, the free aldehyde was extracted by shaking the solution with three portions of peroxide-free ether. After evaporation of the ether and drying at reduced pressure, 82 mg. IAc was obtained as a colorless sirupy liquid.

Anal. Calcd. for $C_{10}H_9ON$: C, 75.4; H, 5.7%. Found: C, 74.9; H, 5.65%.

The procedure described above was used to prepare fresh quantities of the free aldehyde for the chemical and biological tests reported below.

Classification Tests and Derivatives

The freshly released 3-indoleacetaldehyde gave positive classification tests for aldehydes and the indole ring. A few milligrams of IAc in alcohol gave an intense violet color with Schiff's reagent. The compound with one less carbon atom, 3-indolealdehyde, did not give a positive test with Schiff's reagent. Cold potassium permanganate in aqueous or acetone solution was readily reduced by IAc. Ammoniacal silver nitrate was rapidly reduced producing black silver deposits. The IAc product gave a red color in the pine splinter test indicating the presence of an indole or pyrrole ring.

A few milligrams of the aldehyde when dissolved in 50% aqueous ethanol gave a yellow precipitate immediately after treatment with a few milliliters of saturated 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. The 2,4-dinitrophenylhydrazone was filtered off, recrystallized from hot benzene, and purified by chromatography on a column of silicic acid and Celite according to the method of Rosen *et al.* (8). Only one band appeared and it was eluted with benzene. On concentration of the benzene solution, crystals of the IAc 2,4-dinitrophenylhydrazone formed, which after drying melted at 200–201°C. (corr.).

Stability

Stability tests showed that once 3-indoleacetaldehyde was obtained in pure form it was much more stable than most reports indicated. A dilute solution of IAc in benzene when stored in a flask in the laboratory at room temperature for 4 months still gave a strong positive test for aldehyde with Schiff's reagent. After 14 months this solution gave only a weak test for aldehydes.

When a thin film of the freshly prepared IAc in a beaker was exposed to the air for 2 weeks, it still gave a positive test for aldehydes with Schiff's reagent. The thin film of IAc remained unchanged after several hours at room temperature, but it turned solid on standing 2 days. This probably was due to polymerization of some of the IAc. Evidently the aldehyde was not oxidized by air to the corresponding acid, as no IAA could be detected by paper chromatography of the product after the thin film of the IAc was exposed to the air for 2 weeks.

Indoleacetaldehyde was unstable in both acid and basic solutions. This may account for the many unsuccessful attempts to prepare this material in pure form. Ten milligrams of IAc was dissolved in 1 ml. alcohol, and this was mixed with 1 ml. of 1 *N* NaOH solution. The solution turned a bright red color in a few seconds, and after 15 min. the solution turned dark red and turbid. A reddish brown precipitate separated 15 min. later. After standing 1 hr. at room temperature, neither the precipitate nor an ether extract of the diluted supernatant gave a positive test for aldehydes with Schiff's reagent, indicating instability in dilute alkali. The development of a red color in alcoholic sodium hydroxide solution can be used as a color test for the characterization of IAc. Indolealdehyde and other indole compounds do not give a red color.

When 10 mg. of the aldehyde in 1 ml. ethanol was mixed with 1 ml. of 1 *N* HCl, the solution turned to a gray–brown color in 5 min., and in 10 min. a grayish brown gummy precipitate separated. Evidently IAc was unstable in 0.5 *N* acid solution as shown by the formation of a precipitate and the darkening effect, but the products gave a positive Schiff's test indicating that some of the aldehyde groups remained intact.

Identification by Oxidation to 3-Indoleacetic Acid

Proof of the identity of the 3-indoleacetaldehyde was obtained by oxidation to IAA by milk, xanthine oxidase (Schardinger enzyme), cold potassium permanganate solution, and other oxidizing agents for aldehydes. The IAA produced in each case was identified by filter paper chromatography using both R_f values and specific color tests for identification.

Oxidation with Milk. A suspension of 30 mg. IAc in 20 ml. water was mixed with 50 ml. of raw milk. The mixture was shaken and incubated 30 min. at 40°C. and then the mixture was incubated another 30 min. at 50°C. The casein was precipitated by adding 3 *N* HCl, and the filtrate was made alkaline to pH 10 and extracted with ether. A second ether extract was made after adjusting the pH to 2.5. After evaporation of the second ether extract, the residue was taken up in a few drops of ethanol and chromatographed along with pure IAA and IAc on Whatman No. 1 filter paper in *n*-butanol saturated with 2.8% aqueous ammonia. The paper was dried and lightly sprayed with Salkowski's reagent as prepared by Tang and Bonner (9). The product from the treatment of IAc with milk showed a large pink–red spot at R_f 0.29 in exactly the same position as pure IAA. On standing several hours after spraying the paper with Salkowski's reagent, another smaller brown-colored spot appeared just below the IAA spot. This spot was later identified as 3-indolecarboxylic acid. No IAA spot was detected in the original IAc solution or in an ether extract of untreated milk under the same conditions of extraction. The IAc showed up as a single gray–brown spot at R_f 0.93. It could also be located as an orange spot by spraying the paper with a solution of 2,4-dinitrophenylhydrazine in alcohol containing 1% hydrochloric acid.

The R_f values of various indole compounds tested in the same system were 0.21 for tryptophan, 0.23 for indolecarboxylic acid, 0.28 for indoleacetic acid, 0.34 for indolepropionic acid, 0.41 for indolebutyric acid, 0.84 for tryptamine, and 0.93 for indoleacetaldehyde.

Oxidation by Xanthine Oxidase (Schardinger Enzyme). Xanthine oxidase was prepared from raw milk as described by Ball (1) in his first step of purification. A few milligrams of IAc suspended in water were incubated with an equal volume of the enzyme contained in 0.1 *M* Na_2HPO_4 solution at 37°C. for 1 hr. The IAA was extracted and chromatographed as described above. IAA was produced in good yield as shown by a large pink–red spot at R_f 0.28.

In another experiment, a purified preparation of xanthine oxidase from Worthington Biochemical Sales Co. was used for the oxidation of IAc. An ether extract of the products of the reaction was made at pH 8.5, and another ether extract was made after acidification to pH 2.8. Paper chromatography of these extracts alongside pure IAA and the pure IAc used as the starting material showed that IAA was the principal product of the reaction (Fig. 1). Only unreacted IAc was present in the ether extract at pH 8.5. The acid fraction contained the red IAA spot at R_f 0.29 and a dark-brown spot near the solvent front as indicated on the paper chromatogram after spraying with Salkowski's reagent. This latter spot was colored light brown before spraying and was thought to be a mixture of colored impurities.

Oxidation of IAc to IAA by Potassium Permanganate and Other Oxidizing Agents. A sample of 130 mg. IAc was dissolved in 10 ml. acetone, and the solution was cooled in an ice bath. To the cold solution was added dropwise 4.35 ml. of 2% $KMnO_4$ solution. The permanganate was rapidly decolorized and after a few minutes, the solution was filtered off. After most of the acetone had evaporated from the solution, the

FIG. 1. Paper chromatogram showing the products from the oxidation of IAc with xanthine oxidase and the products from the reaction of IAc with cold $KMnO_4$ in acetone. Dots at the bottom show the origin where solutions were applied and the line at the top is the solvent front.

A: Ether extract at pH 8.5 of reaction products of xanthine oxidase and IAc. Only unreacted IAc is present. *B:* Ether extract at pH 2.8 of reaction products of xanthine oxidase and IAc, showing IAA spot as the principal product. *C:* Pure IAA as reference. *D:* Ether extract at pH 2.8 of reaction products of IAc and cold $KMnO_4$ in acetone, showing IAA as the principal product. *E:* Pure IAc used as starting material. *F:* Pure 3-indolecarboxylic acid as reference.

Materials were chromatographed by the ascending method for 7 hr. on Whatman No. 1 filter paper in *n*-butanol saturated with 2.8% aqueous ammonia. Spots appeared after spraying paper with Salkowski's reagent.

aqueous residue was made alkaline to pH 8.5 and extracted with ether. The aqueous phase was acidified to pH 2.8 and extracted with ether again. This acid fraction was chromatographed as described above. The red IAA spot at R_f 0.29 appeared immediately after spraying and a dark-brown spot of colored impurities appeared near the solvent front (Fig. 1). An intense blue-fluorescing spot in ultraviolet light was also noticed on the unsprayed paper just below the IAA spot and merging with it. This fluorescent spot was later shown to be anthranilic acid. Other spots could be detected by their fluorescence in ultraviolet light. But it was observed that IAA itself was oxidized by cold permanganate to anthranilic acid and other fluorescent products.

Treatment of IAc with cold silver oxide in alkaline solution produced some IAA as well as two or three other fluorescent products, but no anthranilic acid was produced.

Treatment of IAc with dilute hydrogen peroxide produced some IAA, but the principal product was the blue-fluorescing material which was isolated in crystalline form. It melted at 146–147°C. while authentic samples of anthranilic acid melted at 149°C. The melting point of a mixture of the two materials was not depressed, showing that they were identical. Each oxidizing agent produced different products from the others, and the same agent produced different products depending on the conditions of the reaction. Under very mild conditions IAA was produced as the principal product with the oxidizing agents used. In most cases, a small amount of 3-indolecarboxylic acid was also detected as a brown-colored spot at R_f 0.23 which usually appeared the day after spraying. It was observed that the reaction of IAA with potassium permanganate also yielded some of this material.

Absorption Spectra

The infrared spectrum of freshly released 3-indoleacetaldehyde was run as a liquid film and found to be identical to that reported by Brown *et al.* (5). It showed strong absorption bands at 3400, 1710, and 747 $cm.^{-1}$, medium strong bands at 1465, 1430, 1350 (resolved into two bands at 1340 and 1365) and 1100 $cm.^{-1}$, medium bands at 2830, 1230, 1075, and 1015 $cm.^{-1}$, and small bands at 3030, 2700, 1630, 1560, 1500, 1390, 1260, 1185, 1155, 1130, 1050, and 925 $cm.^{-1}$.

The infrared spectrum of the crystalline sodium bisulfite addition product of IAc in Nujol did not show the carbonyl stretching band at 1710 $cm.^{-1}$. It showed strong to medium-strong absorption bands at 3400, 3200, 1460, 1245, 1210, 1170, 1050, 1005, 800, and 742 $cm.^{-1}$.

The ultraviolet absorption spectrum of the IAc in ethanol showed absorption maxima at wavelengths of 220, 273, 281, and 290 $m\mu$. The bisulfite addition product in water gave a similar ultraviolet absorption curve with maxima at wavelengths of 219, 278, and 287 $m\mu$.

Biological Properties of 3-Indoleacetaldehyde

Avena Test. Bentley and Housley (11) reported that IAc had one-tenth as much activity as IAA in the *Avena* straight-growth test. They found that during the assay the aldehyde was converted to an acidic substance which accounted for all of the activity. They concluded that IAc itself was either inactive or inhibitory.

The crystalline IAc.$NaHSO_3$ prepared in this laboratory was tested in the *Avena* curvature test and found, in confirmation of Bentley and Housley, to be about one-tenth as active as IAA. It produced the maximum curvature at a concentration of 2 mg./l. as shown in Table I, whereas IAA usually gave a maximum curvature with a solution about one-tenth as concentrated.

Slit Pea Test. In the slit pea curvature test, where red light was used to increase sensitivity (12), IAc and IAc.$NaHSO_3$ were compared with IAA on a molar basis (Table II). At the highest level (128×10^{-5} M) tested, IAc.$NaHSO_3$ was not toxic and it produced much more curvature than

TABLE I

Activity of 3-Indoleacetaldehyde Sodium Bisulfite Product in the Avena *Curvature Test*

Concn. of IAc.$NaHSO_3$	Curvature[a]
mg./l.	*deg.*
2000	0
200	13.1
20	16.4
2	28.2
1	18.1
0.5	12.8
0.25	3.8
0.2	2.7

[a] Average of ten plants.

TABLE II

Comparison of the Activity of 3-Indoleacetaldehyde and Its Sodium Bisulfite Addition Product with 3-Indoleacetic Acid in the Slit Pea Test

Concn.	Curvature[a]		
	IAc	IAA	IAc.$NaHSO_3$
$\times 10^{-5}$ *M*	*deg.*	*deg.*	*deg.*
128	195[b]	94	361
32	474	422	321
8	154	352	114
2	30	375	55
0.5	5	186	20

[a] Average of 30 stem halves of 15 slit pea sections.

[b] Toxic as indicated by browning and loss of turgor.

IAA or IAc. The free aldehyde was insoluble at the highest level and it was also toxic at this level as shown by the brown coloring and flaccid condition of the pea sections. At lower levels IAA was much more active than IAc and IAc.HSO_3, but at the higher levels IAc produced more curvature than IAA produced.

Rooting of Cuttings. Soaking Chinese hibiscus cuttings for 24 hr. in an aqueous solution containing 200 mg./l. IAc.$NaHSO_3$ initiated roots on 10 out of the 16 cuttings that were treated and placed in moist sand for 1 month. Only 4 our of 13 untreated cuttings developed roots, indicating that the IAc.$NaHSO_3$ had a favorable effect on root initiation.

IAc.$NaHSO_3$ was also tested on African violet leaf cuttings to see if it would stimulate root formation. African violet leaves from the same clone with petioles 4–5 cm. in length were used. The basal end (2 cm.) of the petiole was moistened with water and dipped in a rooting powder. Rooting

TABLE III

The Effect of the Sodium Bisulfite Addition Product of 3-Indoleacetaldehyde on Root and Bud Formation on African Violet Leaf Cuttings

Concn. of IAc.$NaHSO_3$ in Pyrax	No. of leaves treated	No. of rooted leaves	Avg. No. of roots per leaf	No. of leaves with buds	Avg. No. of buds per leaf
%					
0	43	16	2	4	0.2
0.1	43	43	16	41	5.3
1.0	43	43	17	43	5.6

powders containing 0, 0.1, 1, and 10% IAc.$NaHSO_3$ in an inert powder (Pyrax) were used. After treatment, the petioles of the leaves were inserted in moist sand contained in wooden flats. After 48 days the number of roots and buds on each leaf cutting were counted. The results which are summarized in Table III show that a level of as little as 0.1% IAc.$NaHSO_3$ in Pyrax caused a very pronounced increase in root formation and bud formation. A concentration of 1% IAc.$NaHSO_3$ was equally effective. Later on, many more new plants developed from the IAc.$NaHSO_3$-treated leaves than from the control leaves treated with Pyrax alone. The highest level of 10% IAc.$NaHSO_3$ killed the treated petioles, but many roots and buds formed on the bases of the remaining leaf blades.

Fruit Setting. An aqueous solution containing 500 mg./l. IAc.$NaHSO_3$ was sprayed on the first and second flower clusters of 20 Rutgers tomato plants growing in the greenhouse during the winter. The flower clusters of 20 control plants were sprayed with distilled water. One spray was applied to each cluster on the day it was in full bloom. The plants were growing under unfavorable crowded conditions. A total of 41 tomato fruit developed on the clusters sprayed with IAc.$NaHSO_3$ while only 12 fruit developed on the clusters sprayed with water. Inspection showed that some of the indoleacetaldehyde-treated fruit were parthenocarpic. It appears that the aldehyde is effective in setting fruit on tomato plants. It has the advantage over certain other fruit-setting compounds of the auxin type in that it fails to cause epinastic and formative effects on the sprayed tomato plants.

Leaf Curling. Freshly prepared IAc and IAc.$NaHSO_3$ were compared with IAA in their ability to cause curling of bean leaves. The primary leaves of Pinto bean plants were sprayed on the lower surfaces with aqueous solutions of the compounds. IAA caused pronounced upward curling of the leaves at a concentration of 2×10^{-5} *M* whereas it took 128×10^{-5} *M* IAc to cause as much curling. The bisulfite addition product had about the same activity as the free aldehyde. On a molar basis, IAA was 64 times as active as IAc and IAc.$NaHSO_3$ in the leaf-curling test.

Summary

A simple method for the preparation of the neutral plant growth hormone, 3-indoleacetaldehyde (IAc), from tryptophan by treatment with sodium hypochlorite was found. The IAc was isolated as the pure crystalline sodium bisulfite addition compound (IAc.$NaHSO_3$) which was stable for many years. The free aldehyde was prepared fresh as needed for chemical and biological studies by treating a solution of IAc.$NaHSO_3$ with a few drops of sodium carbonate solution. The purified IAc product was a colorless sirupy liquid which was stable for several hours at room temperature and for several weeks in organic solvents, but it was unstable in dilute acidic and basic solutions.

The IAc product was identified by classification tests and oxidation to 3-indoleacetic acid (IAA) by milk, xanthine oxidase, cold potassium permanganate, and other oxidizing agents. The infrared absorption bands of IAc and IAc.$NaHSO_3$ and the ultraviolet absorption maxima for these two compounds are reported.

The IAc.$NaHSO_3$ caused maximum curvature of *Avena* coleoptiles at a concentration of 2 mg./l. and was about one-tenth as active as IAA. In the slit pea curvature test, IAc and IAc.$NaHSO_3$ at high levels caused more curvature than IAA, but at low levels they were much less active.

On a molar basis IAA was 64 times as active as IAc in causing upward curling of bean leaves. The IAc.$NaHSO_3$ product was effective in initiating root growth on Chinese hibiscus stem cuttings and African violet leaf cuttings and in setting fruit on greenhouse-grown tomato plants.

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