THE BIOLOGICAL ACTIVITY OF 3-INDAZOLEACETIC ACID AND SOME OTHER INDAZOLE DERIVATIVES

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THE BIOLOGICAL ACTIVITY OF 3-INDAZOLEACETIC ACID AND SOME OTHER INDAZOLE DERIVATIVES

By

KENNETH PAUL HELLMAN

AN ABSTRACT

Submitted to the College of Science and Arts
Michigan State University of Agriculture and
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ABSTRACT

Since the designation of 3-indoleacetic acid (IAA) as a major plant growth hormone and the development of biological methods of assay for substances which effect plant growth, many compounds have been tested as plant growth regulators. The results of these investigations have led to the postulation of rules relating chemical structure and growth regulating properties.

The purpose of the present work was to study the effect of a change in the chemical structure of the indole nucleus of IAA (i.e., the substitution of a nitrogen atom for the carbon atom in the 2-position of the indole ring) on its growth regulating properties.

Five indazole compounds were synthesized and assayed: 3-indazoleacetic acid (IZAA), 3-indazolecarboxylic acid and its methyl and ethyl esters, and 3-dimethylaminomethylindazole. The effect of these compounds in three different biological assays was observed.

Results showed that at all concentrations used, IZAA was at least as active as the control IAA, but the other indazole derivatives showed little or no activity. From these results it was concluded that the rules relating activity and chemical structure were also applicable to the indazoles, especially regarding the interchangeability of nitrogen and carbon in the indole nucleus.

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INTRODUCTION

INTRODUCTION

In the years since the isolation of 3-indoleacetic acid (IAA) from urine by Kögl, Haagen-Smit and Erxleben (12), and its identification as a growth hormone in corn by Haagen-Smit and co-workers (9), there have been many attempts to isolate from plants other substances which have growth promoting properties. Some such compounds have been isolated, but few have growth promoting activity as great as that of 3-indoleacetic acid. Thus, 3-indoleacetic acid has established itself as the major naturally occurring plant growth hormone.

A large number of organic compounds, not known to occur naturally, have been synthesized and assayed in an attempt to discover which ones are capable of modifying plant growth. These plant growth regulators have been defined by Leopold (14) as "... organic compounds other than nutrients, small amounts of which are capable of modifying growth." This definition included substances which either stimulate, inhibit, or otherwise alter growth.

The applications of these plant growth regulators in agriculture are numerous. Various compounds have been found which result in stimulation of root formation on cuttings, earlier fruit set, production of seedless fruit, prolongation of dormancy, delay in blossoming of fruit trees, hastening of fruit maturity and coloring, and destruction of weeds. Because

of these and other applications, the development and manufacture of chemicals having growth-regulating properties has grown into a large and lucrative industry.

Methods of Bioassay

Several biological tests using plants have been devised to determine whether or not a compound has growth regulating properties. Most of these tests are based on a comparison of the effect on plant growth of the experimental compound with a known growth regulator such as 3-indoleacetic acid. The effect on plant growth usually observed is either stimulation or inhibition of cell elongation.

The three methods of assay used in this study were selected for various reasons: (1) they are well-known, simple and reliable; (2) they represent three different effects of growth regulators; (3) facilities for these tests were readily available.

The first is the Avena straight-growth test, the physiological basis for which is the simple stimultaion of cell elongation by organic compounds. In this test there is no transport limitation and no dependence upon differential growth to produce curvature. Sections of oat seedlings are cultured under controlled conditions in a solution containing a test compound, and the increase in length of the section is a measure of the activity of the compound.

The second test is the tomato ovary test. It is based

on the ability of some substances to set parthenocarpic fruit in the tomato. In this assay, lanolin solutions of the test substance are applied to the ovary of tomato flowers from which the stamens have been removed, and the diameter of the ovary is measured after a certain period of time. The growth of the ovary is a measure of the activity of the test substance.

The third assay is the cucumber root inhibition test. The physiological basis for this assay is the inhibition of growth of roots by low concentrations of auxins, compounds which promote growth in the manner of IAA. Most roots have an extremely low auxin requirement for optimal growth and consequently will respond to higher concentrations by growth inhibition. Thus, the activity of a compound can be determined by measuring the root growth of germinated cucumber seeds after they have been allowed to grow in a solution containing the compound for a controlled period of time.

Detailed procedures for these assays and their significance are presented later in this study.

Relation of Chemical Structure to Growth Regulating Properties

The exhibition of growth regulating activity by compounds of diverse molecular structure led to the problem of the relationship between molecular structure and activity. In 1938, Koepfli *et al.* (11), studying a large number of active compounds, determined the minimum structural requirements for this acti-

vity to be the following: (1) a ring system as a nucleus, (2) a double bond in the ring, (3) a side chain, (4) a carboxyl group or a structure readily converted to a carboxyl group on the side chain at least one carbon atom removed from the ring, and (5) a particular space relationship between the ring and the carboxyl group. The exact nature of this spatial relationship was not defined by Koepfli.

As more compounds were added to the list of those having an effect on plant growth, these five requirements appeared insufficient to describe the essential features. Compounds were found which met the requirements but were inactive; others were active even though they did not meet Koepfli's structural requirements. This caused Veldstra in 1949 (22) to review the work up to that time, and he condensed the five requirements into two: (1) a basal ring system (nonpolar part) with high interface activity; and (2) a carboxyl group (polar part) in such a spatial position with respect to the ring system, that on absorption of the active molecule to a boundary, this functional group will be situated as peripherally as possible.

Even these rules, however, have been found not inclusive enough. Vander Kerk *et al.*, (21) found growth promoting activity in S-(carboxymethyl)-dimethyldithiocarbamic acid, a compound which has no ring system.

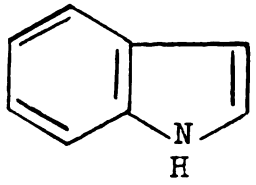
Thus we must be careful not to adhere too closely to any set of rules relating chemical structure with growth regulating properties. However, in the search for new compounds

which can effect growth in plants, the rules of Koepfli and Veldstra have served as a guide allowing a more organized line of investigation than was followed in previous research.

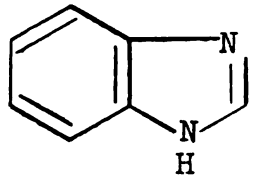
Statement of the Problem

When 3-indoleacetic acid, "heteroauxin," was determined to be a major naturally occurring plant growth hormone, workers in the field began synthesizing and testing numerous derivatives and analogs of IAA. The idea was to observe the differences in activity that accompanied the various changes in the structure of the IAA molecule. These investigations involved, in the main, substitutions in the rings, changes in the linear structure and functional groups of the side chain, moving the side chain to various positions on the indole nucleus, and substitution of the indole nitrogen with carbon, sulfur, and oxygen. Only a few workers investigated compounds with heterocyclic atoms in addition to the nitrogen in the 1-position of indole.

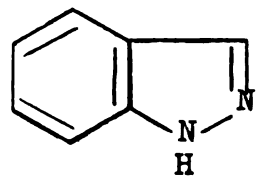
That indazole and some of its derivatives would be logical compounds to inspect for plant growth regulating activity was suggested by the work of Rebstock (15), who found that certain benzimidazole derivatives were active as inhibitors of the growth of plants.



indole



benzimidazole



indazole

Although no indazole compounds are known to occur in nature, it was of interest to investigate the plant growth regulating ability of indazole derivatives analogous to certain known active and inactive indole derivatives, especially the analog of IAA, 3-indazoleacetic acid (IZAA).

Additional interest in indazole was incited by Ainsworth (2) who reported that the indazole analogue of serotonin, a compound causing vasoconstriction in animals, showed pronounced physiological activity paralleling the actions of serotonin itself.

In the course of his work, Ainsworth (1,3) also investigated some of the problems of synthesis of indazoles. These compounds, synthesized by Emil Fischer in the 1880's (5,6,8), have been relatively untouched in the years since Fischer's work. Ainsworth, in addition to searching for physiologically active indazole compounds, also attempted to investigate some of Fischer's indazole syntheses, reevaluating dubious reactions, identifying questionable intermediates, and attempting new routes of synthesis in the light of some of the newer methods in synthetic organic chemistry.

The present work is resolved into two distinct problems: (1) the synthesis, by the simplest routes available, of indazole analogs of certain active indole compounds, and (2) the biological assay of these indazole derivatives, especially 3-indazoleacetic acid, to determine their effect on plant growth.

The purpose of the first part of the problem was to obtain any information which would confirm or add to Ainsworth's reinvestigation of the Fischer indazole synthesis. The purpose of the second part was twofold: (a) to discover an indazole compound which is active, either as a growth promoter or inhibitor, with the hope that it could be used in the field of agriculture and/or in the study of growth mechanisms in plants; and (b) to correlate the results of this work with the knowledge we now have concerning the relationship between molecular structure and growth regulating properties.

EXPERIMENTAL

EXPERIMENTAL

Synthesis of Compounds

3-Indazoleacetic acid was prepared from o-nitrocinnamic acid by a method analogous to Ainsworth's synthesis of 5-benzyloxy-3-indazoleacetic acid (2), which, in turn, is a modification of the indazole synthesis reported by Fischer and Tafel (8).

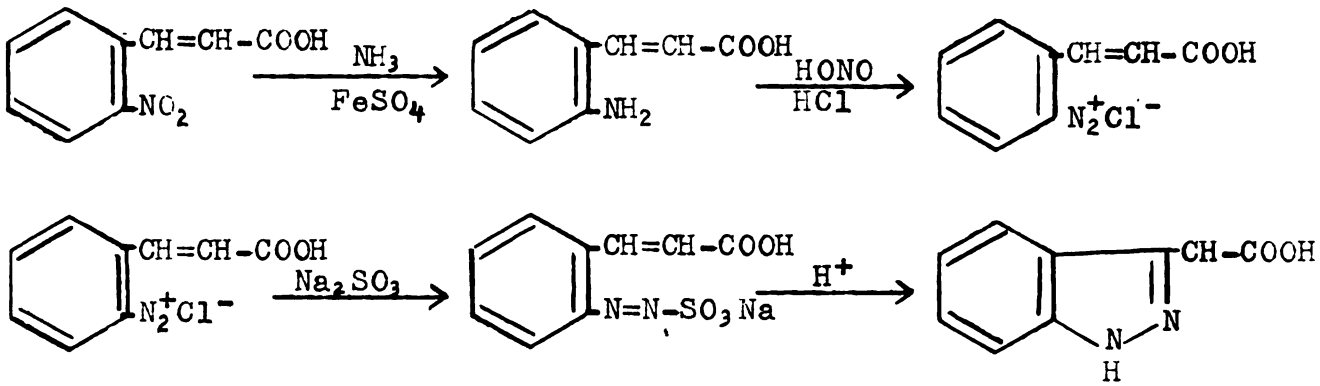
3-Indazolecarboxylic acid was prepared from isatin by the method described by Snyder, Thompson, and Hinman (17).

Preparation of the methyl and ethyl esters of 3-indazolecarboxylic acid was accomplished from the free acid by condensation with the corresponding anhydrous alcohol in the presence of concentrated sulfuric acid, using the method of Auwers (4) for the methyl ester, and that of Fischer and Speier (7) for the ethyl ester.

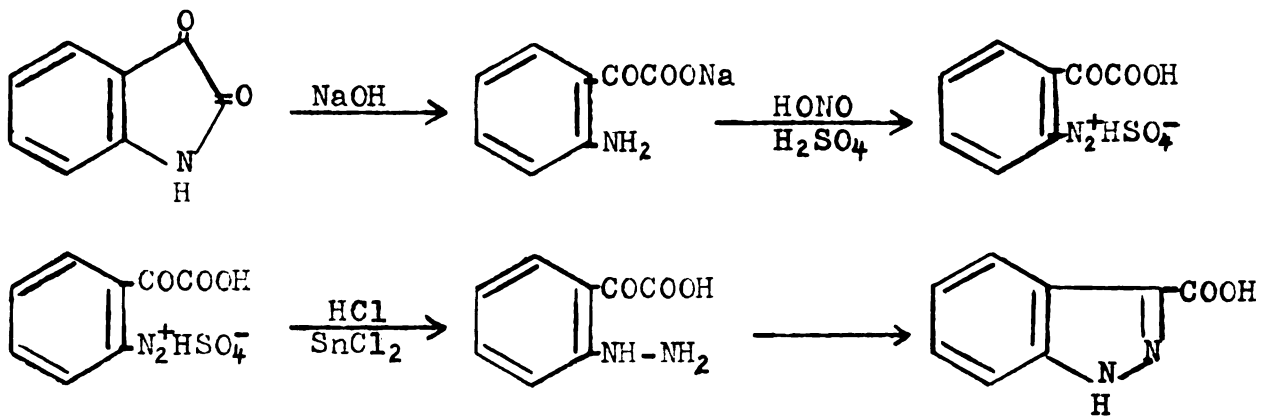
The indazole analogue of gramine, 3-dimethylaminomethylindazole, was synthesized from 3-indazolecarboxylic acid by the method of Snyder, *et al.* (17).

The following is a summary of the reactions utilized in the synthesis of these compounds.

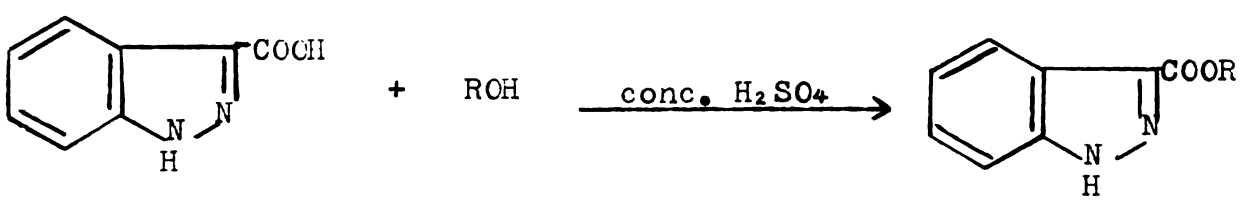
Preparation of 3-indazoleacetic acid



Preparation of 3-indazolecarboxylic acid

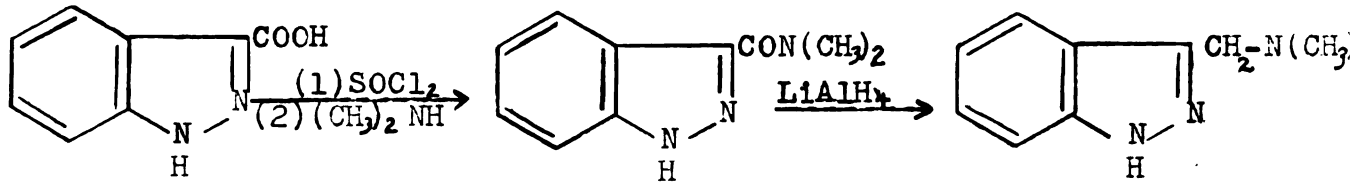


Preparation of methyl- and ethyl-3-indazolecarboxylate



R = CH_3 or C_2H_5

Preparation of 3-dimethylaminomethylindazole



Procedures

3-Indazoleacetic acid

o-Aminocinnamic acid (10). Six and two-tenths g. (0.03 mole) of o-nitrocinnamic acid[1] was dissolved in sufficient dilute ammonia to effect solution and was poured in a thin stream with vigorous shaking into a boiling solution of 48.7 g. of ferrous sulfate heptahydrate in 120 ml. of water. The mixture was immediately treated with small portions of concentrated ammonia, with shaking, until the boiling solution was alkaline to litmus. The solution was boiled for five minutes, then filtered hot with suction, adding ammonia if necessary to maintain alkalinity, and allowed to cool. Upon acidification with acetic acid, a mass of yellow crystals was deposited, and this was collected on a filter. After recrystallization from ethyl alcohol, 3.7 g. of yellow needles melting at

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[1]The o-nitrocinnamic acid was purchased from the Aldrich Chemical Company.

159-160°C.[1,2] was obtained.

3-Indazoleacetic acid. To a suspension of 3.7 g. of o-aminocinnamic acid in 45 ml. of water, sufficient concentrated hydrochloric acid was added to precipitate the hydrochloride. Then 1.8 g. of sodium nitrite was added at 20°C. After cooling the mixture to 0°C. in an ice-salt bath, a tan solid, presumably the diazonium salt, separated. To this cold diazonium salt mixture was added 6.5 g. of sodium sulfite. The temperature rose approximately 5°C., and an orange solution resulted, which was stirred for an additional fifteen minutes in the ice-bath. Seven ml. of 6 N hydrochloric acid was then added, the mixture heated to boiling and then allowed to cool. The resulting orange aqueous solution was then extracted with ether for 10 hours on a liquid-liquid extractor. The ether layer was concentrated in vacuo and a tan solid was obtained. This solid was recrystallized three times from water, treating with Norite each time. The yield was 1.5 g. of a white product which was crystalline in water, but which lost crystallinity when filtered. The product melted at 172°C.[3]

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[1]Tiemann and Oppermann (20) report the melting point as 158-159°C.

[2]All melting points were determined with a Fischer-Johns assembly and are uncorrected.

[3]Ainsworth (2) reports the melting point as 168°C. (capillary). Fischer and Tafel (8) give 168-170°C. as the melting point.

3-Indazolecarboxylic acid

To a 500 ml., round bottomed, three neck flask equipped with a motor-driven stirrer was added 18.6 g. (0.18 mole) of concentrated sulfuric acid in 150 ml. of water. This solution was cooled to 0°C. by the addition of crushed ice. In a warm (50°C.) solution of 4.2 g. (0.105 mole) of sodium hydroxide in 65 ml. of water was dissolved 14.7 g. of isatin.[1] This dark solution was cooled to 0°C. and mixed with a solution (also at 0°C.) of 6.9 g. (0.1 mole) of sodium nitrite in 25 ml. of water. The combined solutions were then added to the rapidly stirred sulfuric acid solution from a dropping funnel, the tip of which extended below the surface of the acid solution. The rate of addition was rapid, but such that the temperature never rose above 4°C.; more crushed ice was added when needed. (To reduce the foaming which occurred as the solutions were mixed, a few ml. of ether was added when necessary. This procedure was continued throughout the period of stirring.) After the addition was complete, the brownish yellow solution was stirred for fifteen minutes. A cold (0°C.) solution of 54.1 g. (0.24 mole) of stannous chloride dihydrate in 85 ml. of concentrated hydrochloric acid was then added from a dropping funnel to the stirred solution. The mixture was stirred for another hour after addition was complete.

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[1]Isatin was purchased from Eastman Organic Chemicals.

The crude product, a yellow-brown paste, was collected on a Buchner funnel and recrystallized twice from large volumes of water using Norite to decolorize. The yield was 4.9 g. of yellow powder which melted at 267-268°C.[1]

Methyl-3-indazolecarboxylate

One and two-tenths g. (0.007 mole) of 3-indazolecarboxylic acid (prepared from isatin) was refluxed for three hours with 10.0 ml. of absolute methanol and 1.0 ml. of concentrated sulfuric acid. Two-thirds of the excess methanol was then removed by distillation. The residue was cooled and made slightly alkaline with dilute ammonium hydroxide. The yellow product was collected on a filter. Recrystallization from benzene and treatment with Norite gave 0.6 g. of yellow scales which melted at 168-169°C.[2]

Ethyl-3-indazolecarboxylate

Three g. of 3-indazolecarboxylic acid and 95 ml. of absolute ethanol (commercial absolute ethanol redistilled from ethyl succinate) were placed in a 300 ml. round bottomed flask, 9.5 ml. of concentrated sulfuric acid was added, and the mixture was refluxed for two hours. About two-thirds of the excess ethanol was then removed by distillation. The

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[1]Snyder, et al., (17) report a melting point of 268-268.5°C.

[2]Auwers (4) gives 168-169°C. as the melting point for methyl-3-indazolecarboxylate.

flask was cooled, and the contents were added to 70 ml. of ether in a separatory funnel and shaken. The ether was removed and the aqueous solution was extracted with two additional 50 ml. portions of ether. The combined ether extracts were washed with two 50 ml. portions of water followed by 50 ml. of 10% sodium bicarbonate solution. The etherial solution was dried over anhydrous potassium carbonate and then taken to dryness in a warm water bath. The yellow product which resulted was recrystallized from 50% ethanol, treating with Norite to decolorize. The yield was 1 g. of yellow needles melting at 135-137°C.[1]

3-Dimethylaminomethylindazole

N,N-Dimethyl-3-indazolecarboxylic acid amide. A slurry of 3.5 g. (0.022 mole) of 3-indazolecarboxylic acid (prepared from isatin) and 11.5 g. (0.09 mole) of purified thionyl chloride was heated under gentle reflux for two hours. The excess thionyl chloride was removed by distillation under reduced pressure. The flask containing the resulting red-orange solid was cooled in an ice-bath and to it was added a cold solution of 3.5 g. (0.07 mole) of dimethylamine in 50 ml. of dry benzene. After ten minutes of cooling and swirling, the slurry was filtered, the crude amide remaining on the filter. Concentration of the

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[1]Auwers and Dereser (4) report a melting point of 136-137°C.

filtrate by vacuum distillation to one-third its initial volume produced a little more of the crude amide which was combined with the main portion. Recrystallization from nitromethane, including a treatment with Norite, gave 2.3 g. of tan needles melting at 186-188°C.[1]

3-Dimethylaminomethylindazole. In the pot of a Soxhlet extractor was placed a slurry of 0.85 g. (0.025 mole) of lithium aluminum hydride in 50 ml. of tetrahydrofuran previously dried over sodium wire. In the thimble was placed 2.3 g. (0.012 mole) of N,N-dimethyl-3-indazolecarboxylic acid amide. The extractor was allowed to run for five hours, at the end of which time the thimble was empty and the solvent had assumed a yellow color. The excess lithium aluminum hydride was decomposed with a saturated solution of water in ether. The mixture was filtered immediately through a sintered glass funnel containing a Filter-Cel mat. The filtrate was dried over magnesium sulfate and then concentrated by distillation to a volume of about 10 ml. After standing overnight at 5°C., 1.5 g. of large white crystals were deposited from the solution. After treatment with Norite and recrystallization from nitromethane, the yield was 1.4 g. of white rhombic crystals melting at 126-127°C.[2]

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[1]Snyder, et al., (17) give a melting point of 187-188.5°C. for the amide.

[2]A melting point of 125-126°C. is reported by Snyder, et al. (17).

Biological Assay

Avena Straight-Growth Test (14)

The test solutions of the five indazole compounds to be tested, plus 3-indoleacetic acid to be run as a control, were made up in concentrations ranging from 2×10^{-9} M to 2×10^{-3} M.

Husked seeds of Victory oats were placed in water in a suction flask and evacuated. The seeds were soaked for two hours and then placed on glass plates covered with paper toweling, grooved side down, with the embryo projecting slightly over the edge. The glass was placed in a covered germinating dish in a darkroom and left for three days, adding water to keep the seeds moist but not wet. Three days after planting, the coleoptiles which were 20 to 30 mm. long were cut in uniform sections 3 to 5 mm. long. The apical 4 mm. of the coleoptile was discarded and the primary leaf removed. The sections were then floated in 10 ml. of the test solution in a Petri dish (10 sections per dish).

Growth was measured after twenty-four hours.

Tomato Ovary Test (16)

In this test, 1, 0.1, 0.01, and 0.001% solutions of the five indazole compounds and 3-indoleacetic acid in lanolin were made up as follows: a known quantity of the test compound was dissolved in anhydrous peroxide-free ether and diluted to

the desired concentration. An aliquot of this solution containing the required amount of compound was added to 2 g. of molten anhydrous lanolin and stirred until solution was complete. The ether was removed by immersing the tubes containing the solutions in a hot water bath. For higher dilutions, the same procedure was followed with smaller aliquots.

From 15 to 20 mg. of each lanolin solution was applied to the ovary of a tomato flower from which the stamens had been removed. Blossoms from the first flower clusters of tomato plants (variety Michigan State Forcing) of comparable physiological and nutritional status were employed.

The ovary diameters were measured after 6 days.

Cucumber Root Inhibition Test (15)

Test solutions of 5, 10, and 100 parts per million of the five indazole compounds, 3-indoleacetic acid, and 1-naphthaleneacetic acid were used.

A filter paper was placed in the bottom of a Petri dish, and ten cucumber seeds (variety Marketer) were spread evenly over the filter paper. Five ml. of the test solution was pipetted into each dish and the cover placed on the dish. The seeds were allowed to germinate at room temperature (approximately 25°C.) under laboratory conditions of alternating light and dark. After eight days the longest root radical was measured and the length of this radical used as a measure of the inhibitory power of the test compound as

compared to cucumber seeds treated with a solution of sodium acetate and acetic acid.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Synthesis

The synthesis of 3-indazoleacetic acid according to the method used by Ainsworth (2) was accomplished without much difficulty. However, in the case of the phenylazosulfonate intermediate and the 3-indazoleacetic acid itself, crystallization did not occur spontaneously as in Ainsworth's preparation. This is understandable, since Ainsworth was working with the 5-benzyloxy-derivatives of these compounds. The problem was handled in the case of the phenylazosulfonate by simply continuing the procedure on the solution of the intermediate since its isolation was not necessary. In the case of the IZAA, a crystalline product was obtained by extracting the compound with ether from its aqueous solution, evaporating the ether, and recrystalling the product from water.

The IZAA obtained in this manner had properties-melting point, solubility, neutralization equivalent-identical to those of 3-indazoleacetic acid reported in various places in the literature, and, in addition, had an ultraviolet absorption spectrum identical to that reported by Ainsworth for 3-indazoleacetic acid (3).

An attempt was made to prepare the methyl and ethyl esters of IZAA from the free acid, the former by the use of diazomethane and the latter via the procedure for ethyl-3-indazolecarboxylate. In both cases, the lack of a sufficient amount

of starting material (IZAA) resulted in yields of these liquid esters that were so small and impure that purification for biological assay was not deemed practicable.

The method finally used for the preparation of 3-dimethylaminomethylindazole was uncovered after several unsuccessful attempts had been made to synthesize this compound from indazole by a Mannich-type reaction analogous to that used by Kühn and Stein (13) for a synthesis of gramine. Snyder *et al.* (17) encountered the same problem but they offered no explanation for the failure of the Mannich reaction in this case. Apparently, for some reason, the 3-position of indazole is less reactive than the 3-position of indole.

All of the methods of synthesis used in this work, with the exception of the few modifications already mentioned, were previously devised by other workers. All were found to be satisfactory procedures. Thus, the synthesis portion of the present study can be considered work of a confirmatory nature regarding the methods of synthesis employed.

Biological Assay

The results of two *Avena* straight growth tests run at different times show that at all concentrations assayed, 3-indazoleacetic acid was at least as active as the control 3-indoleacetic acid. The optimum activity for both was at a concentration of 2×10^{-4} M. Activity of IAA began to decrease as the concentration was decreased, whereas IZAA seemed to maintain its high activity at 2×10^{-5} M as well. At lower concentrations, IZAA activity also diminished.

Other indazole compounds tested showed little or no growth-promoting activity. 3-Indazolecarboxylic acid and 3-dimethylaminomethylindazole showed very slight activity at 2×10^{-4} M and 2×10^{-3} M, respectively, and 3-methylindazolecarboxylate seemed to show a slight inhibitory effect at 2×10^{-3} M, but these effects were so slight as to be regarded insignificant.

A summary of the results of the *Avena* straight-growth assay is shown in Table I.

The other two assays employed, the tomato ovary test and the cucumber root inhibition test, showed, in general, the same results; that is, in both cases 3-indazoleacetic acid demonstrated activity as great as that of the IAA control. In the fruit-set test, none of the other compounds tested were active, whereas in the cucumber test, slight inhibitory action was also demonstrated by 3-indazolecarboxylic acid. The results of these tests are summarized in Table II and Table III.

TABLE I

RELATIVE GROWTH PROMOTING EFFECT OF VARIOUS INDAZOLE COMPOUNDS IN THE AVENA STRAIGHT GROWTH ASSAY[1]

Compound	Molar concentration						
	$2x10^{-3}$	$2x10^{-4}$	$2x10^{-5}$	$2x10^{-6}$	$2x10^{-7}$	$2x10^{-8}$	$2x10^{-9}$
3-Indoleacetic acid (control)	+	++++	+++	++	+	-	-
3-Indazoleacetic acid	+	++++	++++	++	-	-	-
3-Indazolecarboxylic acid	-	+	-	-	-	-	-
Methyl-3-indazolecarboxylate	slight inhib.	-	-	-	-	-	-
Ethyl-3-indazolecarboxylate	-	-	-	-	-	-	-
3-Dimethylaminomethylindazole	+	-	-	-	-	-	-

[1]The number of plus signs indicates the relative activity; a dash (-) indicates inactivity.

TABLE II

RELATIVE ACTIVITY OF VARIOUS INDAZOLE COMPOUNDS IN THE TOMATO OVARY TEST[1]

Compound	Percent (by weight) concentration in lanolin			
	1.0	0.1	0.01	0.001
3-Indoleacetic acid (control)	++++	++	+	+
3-Indazoleacetic acid	+++	++++	+	-
3-Indazolecarboxylic acid	-	-	-	-
Methyl-3-indazolecarboxylate	-	-	-	-
Ethyl-3-indazolecarboxylate	-	-	-	-
3-Dimethylaminomethylindazole	-	-	-	-

[1]The number of plus signs indicates the relative activity; a dash (-) indicates inactivity.

TABLE III
RELATIVE INHIBITION OF CUCUMBER ROOT FORMATION BY VARIOUS INDAZOLE COMPOUNDS[1]

Compound	Concentration (parts per million) 100	10	5
1-Naphthaleneacetic acid (control)	++++	++++	++++
3-Indoleacetic acid (control)	+++	++	-
3-Indazoleacetic acid	+++	++	++
3-Indazolecarboxylic acid	++	+	+
Methyl-3-indazolecarboxylate	+	-	-
Ethyl-3-indazolecarboxylate	+	-	-
3-Dimethylaminomethylindazole	inactive	-	-

[1]The number of plus signs indicates the relative activity; a dask (-) indicates inactivity.

1-Naphthaleneacetic acid was used as an additional control in the cucumber root inhibition test. Since it is one of the compounds most active in this assay, it is normally used as a standard. Neither the 3-indoleacetic acid or the 3-indazoleacetic acid proved as active as the naphthaleneacetic acid, but here it must be noted again that the IAA and IZAA showed equal inhibitory effects. In fact, at a concentration of 5p.p.m., IZAA had much greater activity than IAA, and it is these latter two compounds which are to be compared.

The results, which show 3-indazoleacetic acid to be equally as effective as a plant growth regulator as the naturally occurring 3-indoleacetic acid, and which show the other indazole compounds assayed to be relatively inactive, tend to bear out both Koepfli's and Veldstra's structural requirements for plant growth activity. This is somewhat as expected since the substitution of a nitrogen for a carbon in the 2-position of indole would not be expected to alter either the stereochemistry of the planar ring system or the resonance forms to any great extent. Thus 3-indazoleacetic acid should obey Koepfli's and Veldstra's rules for a plant growth regulator.

There was reason to suspect that 3-indazoleacetic acid might possibly act as an auxin antagonist or inhibitor. This, however, was not the case since assays show that it acts as a powerful auxin on Avena. Thimann (19) observed a similar high activity of 7-aza-3-indoleacetic acid, in which the carbon in the 7-position of the indole nucleus is substituted by a nitro-

gen atom. This observation caused Thimann to conclude that the concept of "anti-auxin" or auxin antagonist needs considerable modification, and that these terms should be defined by the specific bioassay in which they function.

Thus, the present work adds support to the theory of the interchangeability of carbon and nitrogen in the ring, exemplified previously by the high activity of 3-indeneacetic acid (18) and confirmed by Thimann's work with 7-aza-3-indoleacetic acid.

Applying this theory, it is not surprising that 3-indazolecarboxylic acid and its esters are inactive since, as in the case of their inactive indole analogues, the carboxyl group is not one carbon removed from the ring as demanded by Koepfli, and a particular space relationship is not maintained, as required by both Koepfli and Veldstra.

The case of the 3-dimethlaminomethylindazole is not so clear, however, since some activity would be expected in the light of the activity of the indole analogue, gramine. The reason for the inactivity of this indazole derivative, assuming it is not attributable to any stereochemical differences between the indole and indazole analogs, is not clear and bears further examination.

The general conclusions which might be drawn as a result of this work have already been stated by Thimann (19) regarding indoles. These conclusions, which can also be applied to indazoles are (1) that the empirical rules relating structure

of aromatic compounds to activity also seem to hold good in the indazole series, but (2) that the requirements for activity, though evidently stringent, are not too well described by any of the recently proposed theories.

SUMMARY

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A study was made of the growth-regulating properties of various indazole compounds, 3-indazoleacetic acid in particular, using three biological assays: (1) the Avena straight growth test, (2) the tomato ovary test, and (3) the cucumber root inhibition test. A comparison was made with the activity of 3-indoleacetic acid in the same tests to determine the effect of an additional nitrogen atom in the ring.

Five indazole compounds were synthesized and assayed: 3-indazoleacetic acid, 3-indazolecarboxylic acid and its methyl and ethyl esters, and 3-dimethylaminomethylindazole. The methods of preparation used were confirmed as satisfactory routes of synthesis.

Results showed that at all concentrations used, IZAA was at least as active in all three assays as the control IAA. In each case the other indazole compounds showed little or no activity.

It was concluded from these observations that the existing rules correlating activity with molecular structure, especially regarding the interchangeability of nitrogen for carbon in the ring, seemed to be valid in the indazole series, but that these rules may not be entirely definitive.

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