AN ATTEMPTED SYNTHESIS OF INDOLE-2-PROPIONIC ACID

and

THE OXIDATION OF m-XYLYL BROMIDE TO m-TOLUIC ACID

A Thesis submitted

by

Malcolm C. Stewart, B. Sc.,

to the

Post-Graduate Studies Committee

of the University of Manitoba

in partial fulfillment of the

requirements for the Degree

of

Master of Science

- 1939 -

The writer wishes to acknowledge his indebtedness to Dr. E. H. Charlesworth for the suggestion of these problems and for his very kind assistance throughout the course of the investigations.

C O N T E N T S

P R E F A C E

During recent years there has been a great deal of interest taken in the action of indole derivatives as plant hormones. Since, however, no intensive investigation has been made of derivatives substituted in the 2-position, the synthesis of indole-2-β-propionic acid, with which part I of this thesis deals, was undertaken with the view to having the compound tested for its possible action as a phytohormone.

Unfortunately, after some time, it was found that, due to the poor yields obtained in several of the preparations, supplies of the necessary chemicals would be sufficient for only one run. In view of the probably indefinite results to be obtained, work was begun on a second problem.

Part II of this presentation is an account of attempts to prepare m-toluic acid by the oxidation of m-xylyl bromide.

With the exception of photochemical oxidation, all previous attempts to prepare this acid by the oxidation of m-xylene have been unsuccessful, since both methyl groups attached to the benzene nucleus are attacked by oxidizing agents, resulting in the formation of isophthalic acid. However, from a con-

sideration of similar cases, it is conceivable, theoretically at least, that were a halogen such as bromine, substituted for hydrogen in one of the methyl groups in m-xylene, this group should be attacked by oxidizing agents in preference to the unsubstituted one. Thus by choice of the proper reagent and temperature conditions, it might be possible to obtain m-toluic acid. Since it involves the use of relatively inexpensive chemicals, this method, if successful, might well be of some commercial value in the manufacture of what is now a rather expensive compound.

PART I

I N T R O D U C T I O N

(a) A Survey of Indole Derivatives as Plant Hormones

Attempts to explain the various growth phenomena of plants (correlations, tropisms, bud-inhibition, etc.) have culminated in the isolation, within the last decade, of definite chemical substances, known as auxins or growth substances, which have been shown to be the cause of the afore-mentioned responses. In addition to these naturally-occuring substances, many synthetic compounds (including various indole derivatives) have also been found to be effective as phytohormones. Since the synthesis of the acid named in the title of this thesis was undertaken with the view to having its growth-promoting activity tested, a brief review of the subject of plant hormones will be given, with particular reference to derivatives of indole. A more detailed treatment, especially from the botanical point of view will be found in excellent books written by P. Boysen Jensen,[1] and by F. W. Went and K. V. Thimann[2] and also in review papers by these same authors.[3,4]

Since the early evidence for the existence and role of auxins came through the study of tropisms, it might be of interest, before continuing with a review

of the subject, to note the application of auxins to these phenomena.

It is a well-known fact that plants, when unilaterally illuminated, will grow towards the light. Many elaborate theories were evolved to account for this but the first simple one was proposed by Blaauw, whose experiments led him to state in 1918[5] that "whenever light causes a growth reaction, unequal distribution of the light will cause unequal growth, which we call phototropism" and hence that tropisms were simply a phenomenon of differential growth. The later work of Boysen Jensen and Paal further advanced this theory, giving rise to two possible explanations; first, that phototropism might be due to increased transmission of growth-promoting substance on the dark side of the plant, or second, that it might arise from decreased transmission of growth-promoting substance on the light side. That both of these occur simultaneously was finally shown by Cholodny in 1927[6] and Went in 1928.[7] From the experiments of both of these investigators was evolved the so-called Cholodny-Went theory which is now generally accepted. It may be stated as follows: "Growth curvatures whether induced by internal or by external factors, are due to an unequal distribution of auxin between the two sides of the curving organ."[2] (p.157)

Thus unilateral illumination causes an increased concentration of auxin on the dark side of the stem with a consequent increased growth-rate of this side resulting in a curvature toward the light. Experimental proof of this principle was advanced by Went[7] who arranged coleoptiles* of a species of grass (Avena sativa) on agar blocks in such a manner that, when illuminated from one side, the auxin from the light and dark sides diffused into separate blocks. It was found that the block from the shaded side contained the greater concentration of auxin. Similarly the concentration of auxin by gravity in the under side of the plant stem accounts for its upward growth.

In the case of roots, it has been found, first by Nielsen in 1930[8] and confirmed later by others, that the action of the phytohormones is to inhibit growth. Thus the concentration of auxin on the underside of the root under the influence of gravity, causes the upper portion to develop at a relatively greater rate with a consequent downward curvature of the root.

Although the actual isolation of growth-hormones is a recent development, the idea that the phenomenon of correlation is brought about by substances

---

* The coleoptile is a leaf-sheath which envelopes the growing point and first foliage leaf of the plant.

or "saps" was advanced as early as 1758 when Duhamel du Monceau[9] suggested the theory that correlation was brought about by two saps, one moving downward, the other upward, in the plant. The former was elaborated in the leaves and, after passing downward through the cortex, was used for the nutrition of the roots. If, however, this downward stream were intercepted by ringing or other means, it caused swellings, callus and root formation above the wound.

Sachs in 1880,[10] 1882,[11] and 1893[12] brought out a complete theory of such phenomena, his ideas differing, however, from the modern view by the assumption of the existence of many "specific substances" such as root-forming, flower-forming and other substances, which move in different directions through the plant. Light and gravity were assumed to affect the distribution of these special substances. It was, however, through the study of tropisms -- to which attention was directed about this time -- rather than correlations that auxin was finally isolated.

The first important work on this type of phenomena was that of C. Darwin, who showed in 1880[13] that the effects of light and gravity are perceived by the tip of the plant and that the stimulus is transmitted to the lower regions, which then react. This led him

to state that "we must, therefore, conclude that when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper to the lower part, causing the latter to bend." In regard to geotropism of roots, he concluded "that it is the tip alone which is acted on, and that this part transmits some influence to the adjoining parts, causing them to curve downwards".

Although these views met with some opposition, they were completely confirmed by the work of later investigators, among the latter being P. Boysen Jensen. In addition, Boysen Jensen[14,15,16] showed that the stimulus could be transmitted across a wound gap. By cutting off the tips of Avena coleoptiles, sticking them on again with gelatin and illuminating the tip only, he found that curvature appeared not only in the tip but also in the base. From this he concluded that "the transmission of the irritation is of a material nature produced by concentration changes in the coleoptile tip". He did not, however, postulate the presence of a special growth-promoting substance.

The experiments of Boysen Jensen were repeated and extended by Paal in 1914[17] and 1919,[18] who, after excluding the possibilities of the base being influenced by scattered light, by contact stimulus, or by the

assymmetrical weight of the bending tip, confirmed Boysen Jensen's results. In addition he showed that the stimulus could be transmitted by a layer of gelatin but could not cross mica or platinum foil and also that, even without light, curvatures could be induced in the base by cutting off the tip and replacing it on one side of the stump. From these results he concluded[18] that "the tip is the seat of a growth-regulating center. In it a substance (or a mixture) is formed and internally secreted, and this substance, equally distributed over all sides, moves downwards through the living tissue. In the growing zone it causes symmetrical growth. If the movement of this correlation carrier is disturbed on one side, a growth decrease on that side results, giving rise to a curvature of the organ".

Soding, in 1923[19] and again in 1925,[20] further confirmed the theory that the growth of the coleoptile is controlled by the tip through the agency of a diffusible substance. Success in isolating this substance was not, however, achieved for several years, although Seubert in 1925[21] showed that agar containing saliva, diastase, and malt extract caused a promotion of growth.

Isolation of auxin was finally obtained by F. W. Went in 1928[22] by placing coleoptile tips on agar blocks when it was found that the active substance

diffused into the agar. Such blocks when applied to one side of decapitated coleoptile tips caused a curvature of the stem away from the agar, the degree of curvature being proportional, within limits, to the concentration of auxin. This test, later known as the "Went Avena test" has since been used in the assay of the activity of other substances. In addition, by means of this test, certain of the properties of the substance were determined. Thus it was shown to be thermo- as well as photo-stable, and readily diffusible. By allowing the substance to diffuse from agar into a series of blocks of fresh agar and then assaying the activity of each block, the diffusion coefficient could be calculated and hence the molecular weight. The value so determined was 376 -- a figure which was later found to be in fairly good agreement with that calculated for auxin A.

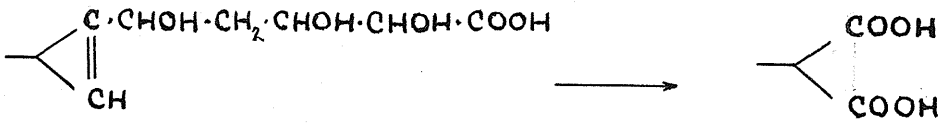
As mentioned previously, Seubert in 1925[21] had shown that growth-promoting substances may occur outside the plant. Extending these experiments, a systematic examination of animal excretions and tissues was made by Kogl and Haagen Smit in 1931,[23] and by Kogl, Haagen Smit and Erxleben in 1933.[24,25] Investigation showed that human urine was an extremely rich source of growth-substance. The bicarbonate-soluble

fraction of the ether extract of urine was extracted with petroleum ether and purified by partition between benzene and aqueous alcohol. It was then precipitated with lead acetate from weakly alkaline seventy per cent alcohol, treated with calcium hydroxide to precipitate a colored impurity, and finally heated with acid methyl alcohol. The product isolated proved, however, to be a lactone instead of the expected ester. It was then distilled in vacuo when the bulk of the active substance distilled at 125° to 130° under 0.1 millimeter pressure, yielding crystals of an acid with the molecular formula $C_{18}H_{32}O_5$, for which the name "auxin A" was proposed.

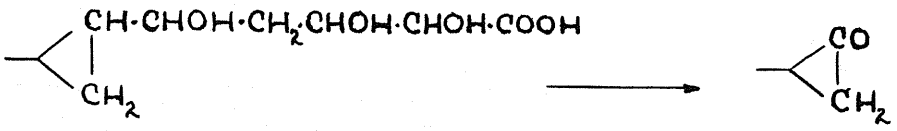
By a very similar method of purification, another active substance, auxin B, was subsequently isolated from malt and from corn germ oil by Kogl, Erxleben and Haagen Smit in 1934.[26] It proved on analysis to have the formula $C_{18}H_{30}O_4$, being isomeric with the lactone of auxin A. Its reactions showed it to be acidic in nature with an activity as a growth-promoter equal to that of the other hormone.

By a series of brilliant researches, the constitutional formulae of these two closely related compounds were determined by Kogl, Erxleben and Haagen Smit in 1933[27] and by Kogl and Erxleben in 1934[28] and 1935.[29]

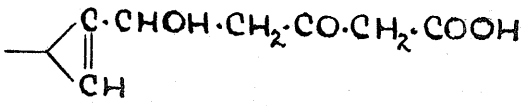
Their investigations have been summarized as follows, by Went and Thimann.[2] The acid and lactone were shown to have but one double bond, and the acid to have one carboxylic group. After addition of hydrogen at the double bond, the number of hydrogen atoms in the molecule was still two short of saturation, and hence there must be one ring in the molecule. In auxin A, the remaining three oxygen atoms were found to be in hydroxyl groups, while in auxin B, one hydroxyl and one keto-group could be identified. Oxidative degredation of both auxin A and B gave rise to a C_{13} dicarboxylic acid which contained no hydroxyl groups. Similar oxidation of the hydrogenated derivative, which is biologically inactive, yielded a neutral C_{13} ketone. The oxidation has therefore carried away all the hydroxyl groups, together with a chain of five carbon atoms. From the difference between the two oxidations it is also clear that the double bond was not in the side chain which was removed. Further reasoning indicated that this side chain contained the three hydroxyl groups and the carboxyl group, and established their relative positions. Hence the oxidations must be formulated as follows, substance I being auxin A, and II dihydro-auxin A:



I III

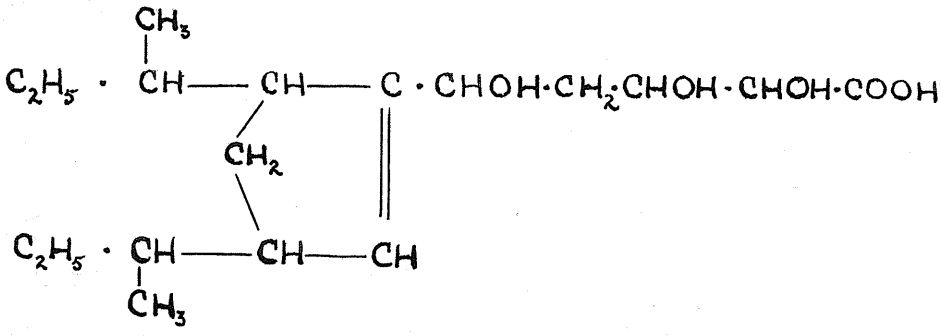


II IV

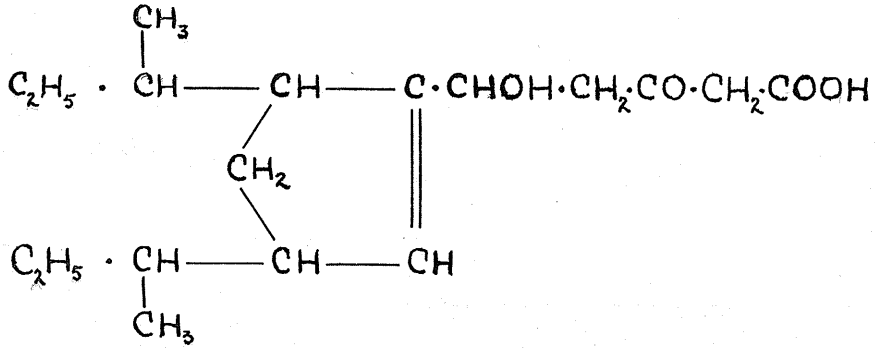


V

Since auxin B loses carbon dioxide to give a neutral ketone, it must have its keto-group in the β-position and therefore must be formulated as V. The structure of the C_{13} residue was worked out by degredation experiments, while finally the synthesis[29] of a dicarboxylic acid identical with the oxidation product III, (auxin-glutaric acid) confirmed the following formulae for auxin A and B:



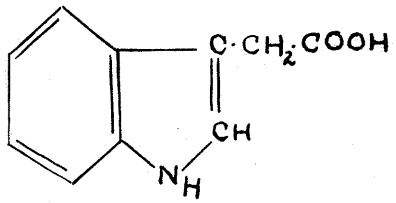
VI auxin A (auxentriolic acid)



VII auxin B (auxenolonic acid)

The development of a charcoal adsorption method for removing the active substance from urine led to the working up of larger volumes. Using this method, it was found[30] that, although two-thirds of the activity could be recovered by adsorption on charcoal and elution with 60% methyl acetate containing 5% concentrated ammonia, the subsequent steps in the usual isolation procedure failed to yield a crystalline auxin. The active substance was largely destroyed on attempting to lactonize but was finally obtained by

extraction with xylene and removal of impurities by precipitation with a mixture of barium acetate and potassium hydroxide. The filtrate yielded crystals which melted at 162° and showed high activity. This product, to which the name hetero-auxin was given, was subsequently shown to be identical with indole-3-acetic acid,* (VIII).

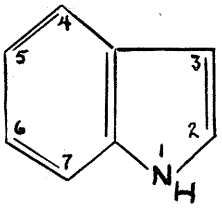


VIII

This identity was further supported by the fact that synthetic indole-3-acetic acid was found to have the same effect on plant growth as the naturally-occuring compound.

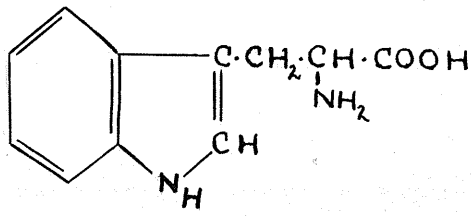
The discovery that a synthetic compound is active in influencing plant growth at once aroused interest in the possibility that other indole derivatives might also be active. Thus in the same paper

---

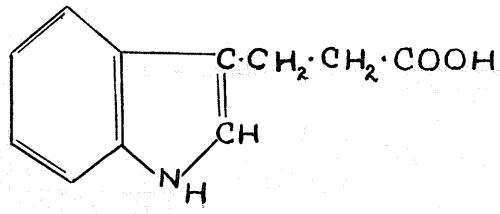


* The system of numbering the indole nucleus which will be followed in this presentation is shown at the left.

in which they recorded the isolation of hetero-auxin, Kogl, Haagen Smit and Erxleben also reported that tryptophane (indole-3-aminopropionic acid, IX), indole-3-β-propionic acid (X), indole-2-carboxylic acid and indole-3-carboxylic acid were all inert.

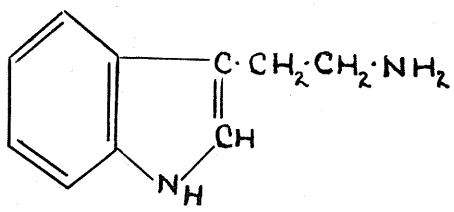


IX



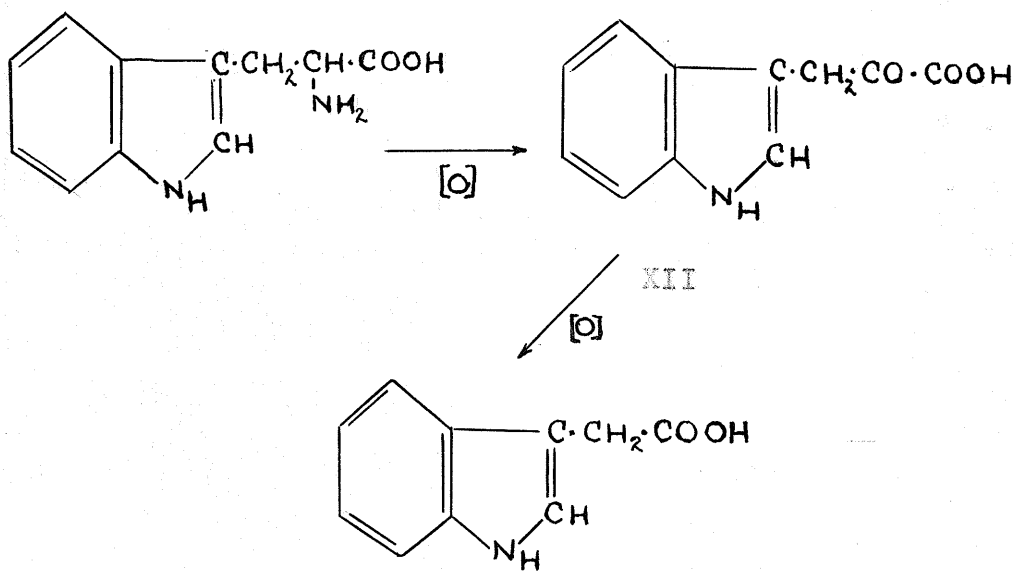
X

Since that time these derivatives have been retested and their small activities determined. It has been found[31] that if tryptophane is applied to "Avena", curvatures are produced after a lapse of two hours. F. Skoog[32] has reported that tryptamine (XI) also behaves in this manner.



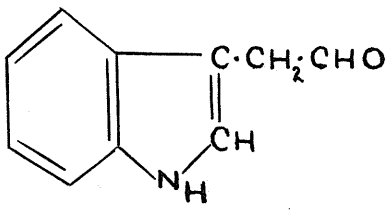
XI

Nielsen in 1928[33] had found that the medium on which Rhizopus suinus, a pathogenic fungi, had grown was rich in a substance active in producing plant curvatures. This was finally isolated by Thimann in 1935[34] and shown to be indole-3-acetic acid. It is almost certain that the mode of formation of this compound by the micro-organism is by a process of oxidative deamination of tryptophane present in the peptone used for the culture. It is presumed to take place in the following way through indole-3-pyruvic acid (XII):[2 (p.111)]



The same conversion by the plant is considered by Thimann and Went[31] to be extremely probable, thus accounting for the delayed activity. Although tryptamine (XI) does not contain a carboxylic group, it

could be oxidized to hetero-auxin (VIII) through indole-3-acetaldehyde (XIII).

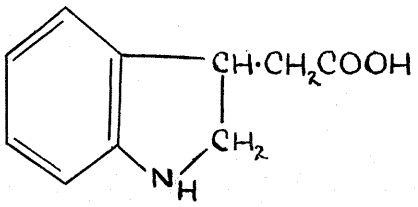


XIII

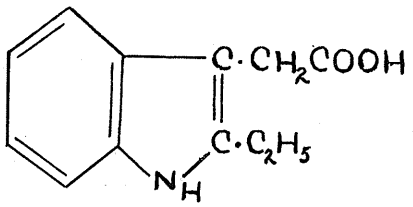
Indole-3-propionic acid has also been reported as being active by Zimmerman and Wilcoxon,[35] and by A. E. Hitchcock.[36] Its effectiveness in producing curvatures in the Went "Avena test" is, however, very much less than that of indole-3-acetic acid.

To determine the constitutional specificity of hetero-auxin, Kogl and Kostermans[37] prepared a large number of derivatives and tested them by the Avena curvature method. Among these derivatives were the methyl, ethyl, n-propyl and iso-propyl esters of indole-3-acetic acid. It was found that the methyl ester retained more than one-third of the activity of the free acid. With increasing size of the alkyl groups, the activity decreased at about the same rate, the iso-propyl ester being the least effective compound. A number of derivatives were prepared for the purpose of determining the effect of various substituents, or

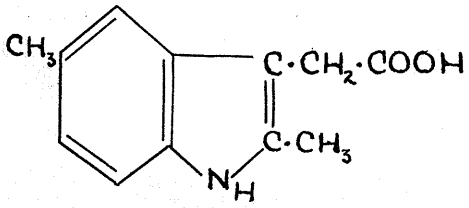
other modifications on the activity of hetero-auxin. Hydrogenation of the indole ring destroyed activity since both 2,3-dihydroindole-3-acetic acid (XIV) and its methyl ester were inactive.



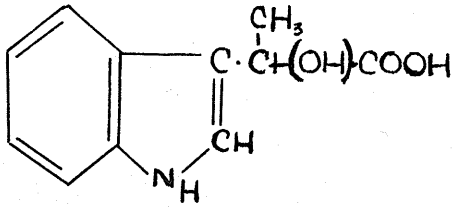
XIV



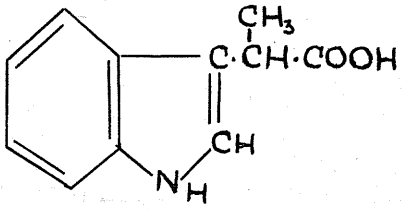
XV



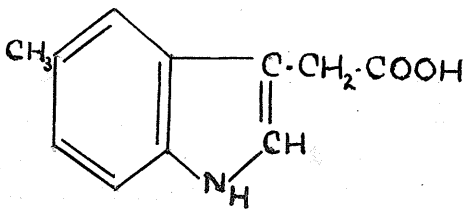
XVI



XVII



XVIII



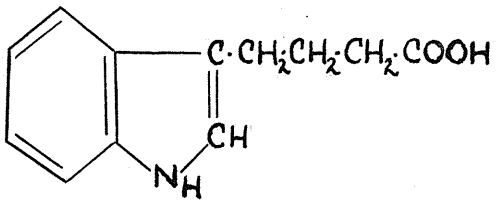
XIX

Other derivatives tested were 2-ethylindole-3-acetic acid (XV), 2,5-dimethylindole-3-acetic acid (XVI), indole-3-α-lactic acid (XVII), indole-3-pyruvic acid (XII), indole-3-α-propionic acid (XVIII), and 5-methyl-

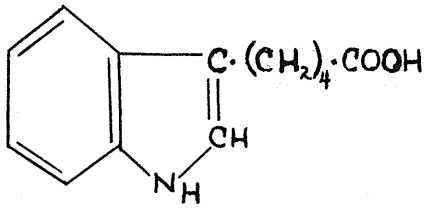
indole-3-acetic acid (XIX) and its methyl ester. Of these only the last three were found to be active, although Haagen Smit and Went[38] have reported that 2,5-dimethylindole-3-acetic acid (XVI) is 0.002 as effective as indole-3-acetic acid.

A further list of indole derivatives was published by F. Kogl.[39] Compounds not previously reported were N-methylindole-3-acetic acid and its ethyl ester, and 2-methylindole-3-acetic acid and its methyl ester. Both of the acids were found to be effective but the esters were inactive.

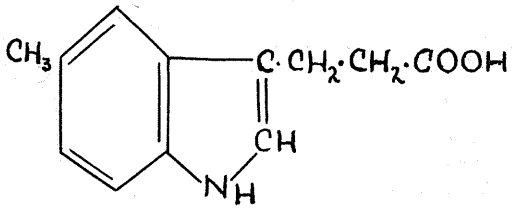
A great many compounds have been tested by Zimmerman, Wilcoxon and Hitchcock of the Boyce Thompson Institute, aromatic acids and esters other than indole derivatives being found effective as plant hormones for various responsee.[35,40] In addition, a number have been reported by Manske and Leitch.[41] The new indole derivatives shown to be active are as follows: indole-3-butyric acid (XX)(now being produced commercially for use as a growth stimulant), indole-3-valeric acid (XXI), 5-methylindole-3-propionic acid (XXII), and indylene-1:3-diacetic acid (XXIII). 2-Carboxyindole-3-butyric acid (XXIV) was also tested by Zimmerman and Wilcoxon[40] but was found to be ineffective.



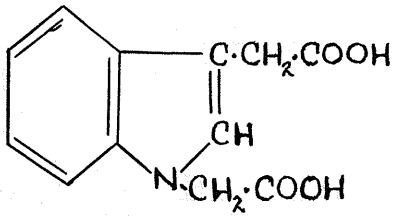
XX



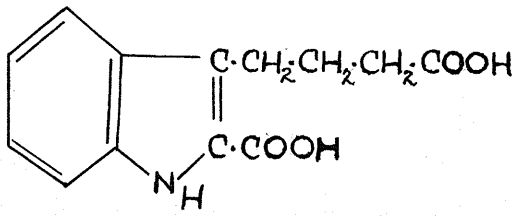
XXI



XXII

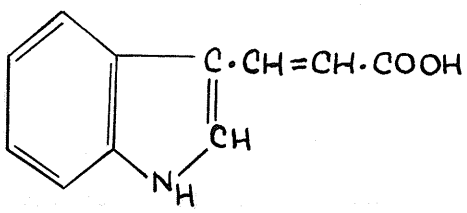


XXIII

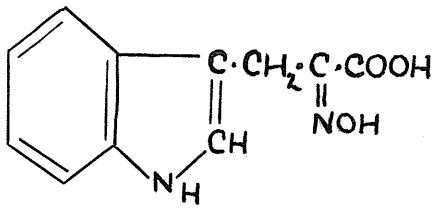


XXIV

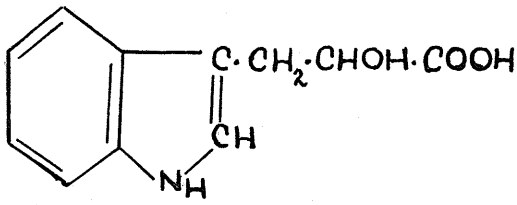
Bauguess[42] has also tested several indole derivatives, with resulting root initiation, stem bending, and bud inhibition in tomatoes, marigolds and stocks. Ones not previously mentioned in this thesis are indole-3-acrylic acid (XXV), indole-3-α-oximinopropionic acid (XXVI) and dl-indole-3-β-lactic acid (XXVII).



XXV



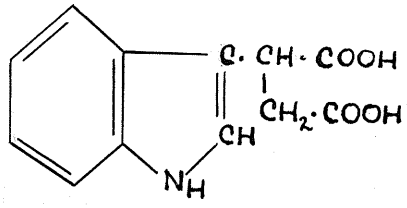
XXVI



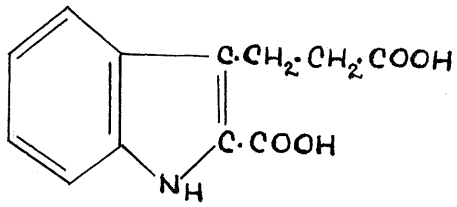
XXVII

More recently, an attempt has been made by Koepfli, Thimann and Went[43] to determine the relation between chemical structure and physiological activity and to present the minimum structural requirements for growth activity. For this purpose, a large number of compounds, among them many indole derivatives, were prepared and tested. A number of these had previously been reported by other investigators, but were tested again, since failure to promote curvatures in the "Avena" test does not necessarily imply complete inactivity in causing other plant responses. Of the derivatives examined, only the following have not been mentioned in preceding sections: indole-3-succinic

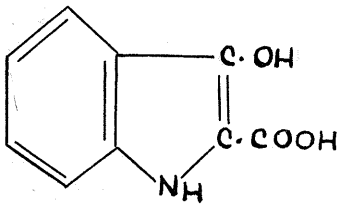
acid (XXVIII), 2-carboxyindole-3-propionic acid (XXIX), indoxylic acid (XXX), N-acetyl-3-hydroxyindole (XXXI), isatin (XXXII), 5-methoxyindole-3-propionic acid (XXXIII) and 6- and 7-methoxyindole-3-propionic acid.



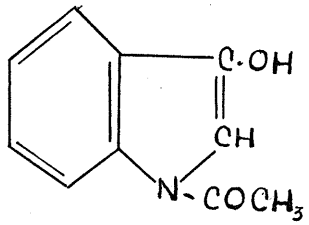
XXVIII



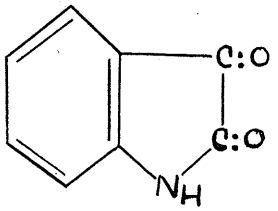
XXIX



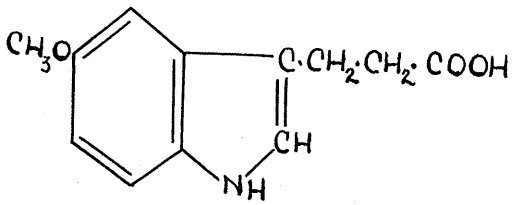
XXX



XXXI



XXXII



XXXIII

Skatole (3-methylindole) was also reported as inactive although Davies, Atkins and Hudson have found it effective in stimulating root development in willow branches.[44]

All indole derivatives which have been tested, together with the literature references, are tabulated below:

Table I.

Indole Derivatives reported Active

Derivative	Lit. Ref.
Indole-3-acetic acid	30,40,44,45
methyl ester	37,40,45
ethyl ester	37
n-propyl ester	37
isopropyl ester	37
Indole-3-propionic acid and methyl ester	36,40,41,44
Indole-3-butyric acid and methyl ester	35,40,41,45
Indole-3-valeric acid	35,40,41,43
Indole-3-pyruvic acid	37,39
Indole-3-acrylic acid	42
Indole-3-isopropionic acid	37,43
N-methylindole-3-acetic acid	39
2-Methylindole-3-acetic acid	39
5-Methylindole-3-acetic acid	37,39
Tryptophane	30,31
Tryptamine	32
5-Methylindole-3-propionic acid	35,41
Indylene-1:3-diacetic acid	35,41
Indole-3-α-oximinopropionic acid	42

Table II.

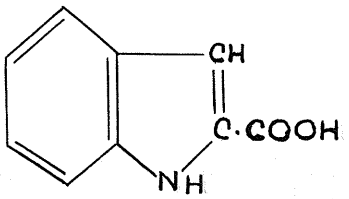
Indole Derivatives reported Inactive

Derivative	Lit. Ref.
Skatole (3-methylindole)	43
Indoxyl (3-hydroxyindole)	43
Indole-3-succinic acid	43
dl-Indole-3-β-lactic acid	42
Indole-3-α-lactic acid	37
2-Carboxyindole-3-butyric acid	35,43
Indole-2-carboxylic acid	30,39
Indole-3-carboxylic acid	30,39,43
2-Carboxyindole-3-propionic acid	43
Indoxylic acid	43
5-Methoxyindole-3-propionic acid	43
6-Methoxyindole-3-propionic acid	43
7-Methoxyindole-3-propionic acid	43
N-acetyl-3-hydroxyindole	43
Isatin	43
2,3-Dihydroindole-3-acetic acid	37,39
2-Ethylindole-3-acetic acid	37
2,5-Dimethylindole-3-acetic acid	37,39
ω-Skatolylmalonic acid	44
Ethyl N-methylindole-3-acetate	39
Methyl 2-methylindole-3-acetate	39

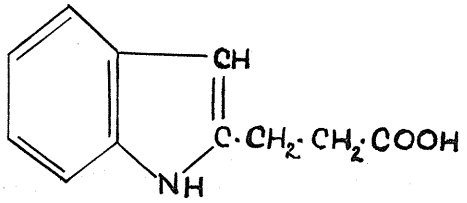
Koepfli, Thimann and Went recently undertook an intensive investigation of a large number of compounds with a view to determining the minimum structural requirements for activity as a phytohormone. From experimental evidence they conclude[43] that a compound in order to be active, must contain (1) a five- or six-membered homocyclic or heterocyclic nucleus, (2) a double bond in this ring, (3) a side chain, (4) a carboxyl group (or a group readily reduced to carboxyl) at least one carbon atom removed from the nucleus, and (5) a particular space relationship between the ring and the carboxyl group. It appeared that this question of space relationship was one of the most important since in a number of cases it was found that, while the cis-isomer was active, the trans-form was not. The most obvious difference between the cis- and trans-isomers is the distance between the carboxyl group and the nucleus, and this suggests that the growth activity of the cis-isomers is occasioned by the close proximity of the carboxyl group to the nucleus.

From a consideration of tables I and II, in which are listed all indole derivatives so far tested, it will be seen that, although derivatives with side-chains substituted in the 3-position of the indole nucleus have been thoroughly investigated, the only

mono-substituted compound tested with the substituent group in the 2-position is indole-2-carboxylic acid, (XXXIV).



XXXIV



XXXV

Activity of the latter would be excluded by number 4 of Koepfli, Thimann and Went's requirements, since the carbon atom of the carboxyl group is directly connected with the nucleus. Indole-3-β-propionic acid (XXXV), on the other hand, should fulfill all the requirements except possibly the last (5). It would therefore be of great interest to discover whether or not this substance is active as a plant hormone. It is regrettable that lack of chemicals forced the abandonment of this problem.

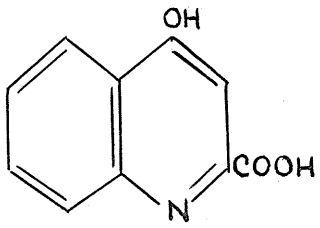
(b) A Review of Indole Derivatives in Physiological Experiments

Considerable interest has also been taken in indole derivatives in connection with experiments concerning the possibility of replacing tryptophane (indole-3-α-aminopropionic acid, IX) in the animal diet by various synthetic compounds closely related structurally to the natural substance. Such investigations are important in that they indicate the possible path of metabolism of the amino acid in the body and in addition the types of chemical reactions which the animal organism is capable of accomplishing.

The general method followed in such experiments is to feed to white rats diets deficient in the amino acid under consideration. A decreased growth rate results. The diet is then supplemented by the synthetic compound. Resumption of growth, as checked by control animals on an unsupplemented diet, indicates that the compound is being utilized and hence that either it is being converted into the amino acid or is being used in its place, i.e., is a possible intermediary product in metabolism.

When tryptophane is administered to normal rabbits, it is partially eliminated as γ-hydroxy-

quinoline-4-carboxylic (kynurenic) acid,[46] (XXXVI).



XXXVI

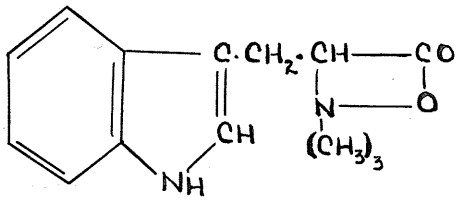
Production of this acid has also been used as a criterion in estimating the extent of utilization of indole derivatives, although it has been stated that "this substance is not a link in the chain of normal tryptophane oxidation in the animal body but rather is an end-product of a set of side reactions brought into play especially when tryptophane is administered in excess of ordinary metabolic requirements".[47]

Since the discovery of the amino acids, many synthetic compounds have been tested for their ability to replace the natural substance in the diet. The first of such experiments with tryptophane was performed by Sure in 1925,[48] who, after carrying out experiments to ascertain whether indole and alanine would condense to form tryptophane, came to the conclusion that they do not. Two years later, Jackson undertook similar, more intensive, investigations with various indole derivatives.[49] Indole-3-aldehyde

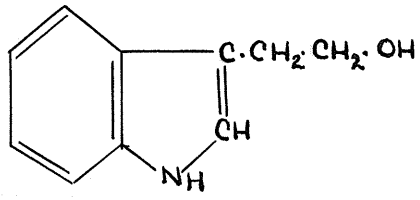
and l-indole-3-β-lactic acid were tested. The first was chosen since it had been reported that furfuraldehyde condenses with acetic acid in rabbits and dogs to give furfuracrylic acid. A similar reaction in the case of indole-3-aldehyde followed by addition of ammonia in the proper fashion would give tryptophane. The indole-lactic acid was of importance since it had been found[50] possible to replace histidine with dl-β-4-imidazole lactic acid. In neither case, however, was the growth of white rats appreciably influenced by the addition of these compounds. The active lactic acid was then subjected to the probable racemizing action of long boiling in barium hydroxide solution and tested again, when it was found that it still had no effect. Both Ichihara and Iwakura[51] and Bauguess and Berg[52] have since obtained positive results with the racemic form of indole-3-lactic acid. The latter investigators attribute Jackson's failure to incomplete racemization of the acid.

In 1929,[53] Jackson reported experiments with nine additional indole derivatives, with position 3 side-chains as follows: the betaine of tryptophane (XXXVII), indole-3-ethyl alcohol (XXXVIII), indole-3-α-benzoylaminoacrylic acid (XXXIX), methylene tryptophane (XL), indole-3-butyric acid (XX), indole-3-α-

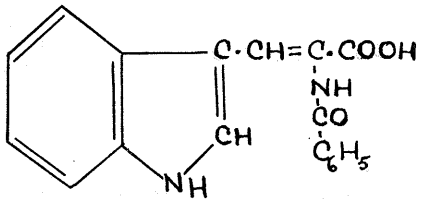
uraminopropionic acid (XLI), indole-3-propionic acid (X), indole-3-pyruvic acid (XII) and indole-3-ethylamine, (tryptamine, XI).



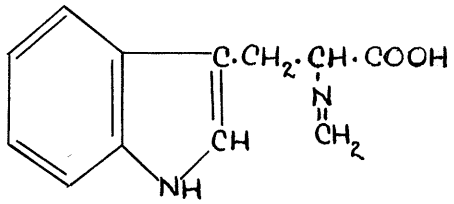
XXXVII



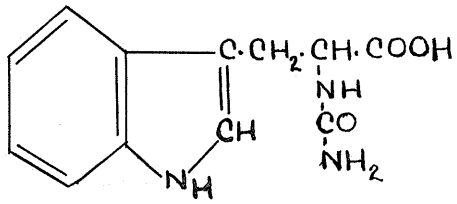
XXXVIII



XXXIX



XL



XLI

With the exception of indole-3-pyruvic acid, the derivatives employed had no appreciable influence on growth. Further brief experiments were carried out with indole-3-acetic acid, indole-3-acetonitrile and isatin but gave wholly negative results. This shows

how very specific the animal organism is in its requirements for compounds of a particular structure since it was unable to bring about the rather superficial alterations necessary to convert them to tryptophane. The fact that indole-3-pyruvic acid replaced tryptophane in the diet is in agreement with the many demonstrations in the literature of the close physiological relations between amino acids and the corresponding pyruvic acids. In this connection, it may be noted that Harrow and Sherwin[54] had previously found that imidazole pyruvic acid would to a certain extent replace histidine in the diet. In addition, Ellinger and Matsuoka[55] in 1920 reported that indole pyruvic acid gave rise to an increased kynurenic acid output.

The inactivity of indole-3-propionic acid and the activity of indole-3-pyruvic acid was confirmed by Berg, Rose, and Marvel in 1927.[56] Hence this latter acid may be regarded as a normal metabolite of tryptophane.

Indole-3-β-acrylic acid (XXV) and indole-3-β-(α-oximino)-propionic acid (XXVI) were studied by Bauguess and Berg.[52,57] Although indole-3-acrylic acid would require only the directive addition of ammonia to the double-bonded carbons to be converted into tryptophane, while the oximino compound needed only reduction

to tryptophane or hydrolysis to indole-pyruvic acid, neither derivative showed any capacity to replace tryptophane for purposes of growth.

These same derivatives in addition to l- and dl-indole-3-lactic acid and indole-3-pyruvic acid were examined by Bauguess and Berg from the standpoint of kynurenic acid production in the rabbit.[50] It was found that indole-3-oximinopropionic acid, indole-3-acrylic acid and l-indole-3-lactic acid yielded no appreciable quantity of kynurenic acid. dl-Indole-3-lactic acid and indole-3-pyruvic acid, on the other hand, were converted into kynurenic acid, but much less completely than was l-tryptophane. The d-indole-3-lactic acid was recovered in greater amounts than indole-3-pyruvic acid and hence, though not conclusive evidence, is at least favorable to the view that the latter is the more likely intermediate in the oxidation of tryptophane in the animal organism. The wide divergence between the amount of kynurenic acid excreted after the l-tryptophane administration and after the administration of either of the two non-amino acids is taken as indicating that neither of the latter is a normal intermediate in the production of kynurenic acid from l-tryptophane. In addition the data obtained suggest that the mechanisms for the oxidation of l-tryptophane and for the produc-

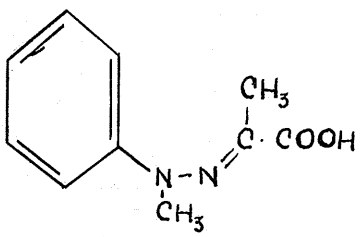
tion of kynurenic acid from that amino acid are independent of each other. In the former process, indole-3-pyruvic acid may be a normal metabolite; in the latter it probably has no role. Its partial conversion (or that of d-indole-3-lactic acid) into kynurenic acid may be explained by its previous partial conversion into l-tryptophane which then undergoes metabolism by a different path to produce kynurenic acid.

Many derivatives of tryptophane itself have been tested both for growth and for kynurenic acid production. Since, however, these are not strictly the type of indole derivatives with which this thesis is concerned, no mention will be made of them.

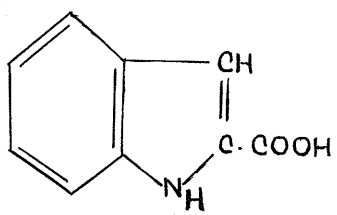
D I S C U S S I O N

A great deal of interest has been taken in recent years in the action of indole derivatives as plant hormones. While the indole-3 series of acids has been intensively investigated, few derivatives with the substituent group in the 2-position have been tested. The synthesis of indole-2-propionic acid was therefore undertaken for the purpose of ascertaining its activity as a phytohormone. As indicated by the equations on a later page, it was hoped to bring about the formation of this acid through ring closure of the phenylhydrazone of methyl ethyl β-ketoadipate by means of Nef's modification of the Fischer indole synthesis.

The first example of this important method for the preparation of indole derivatives was discovered by E. Fischer[59] who boiled the methylphenylhydrazone of pyruvic acid (XLII) with alcoholic hydrochloric acid. The product, $C_{10}H_9O_2N$, proved on investigation to be an indole derivative (1-methylindole-2-carboxylic acid, XLIII).

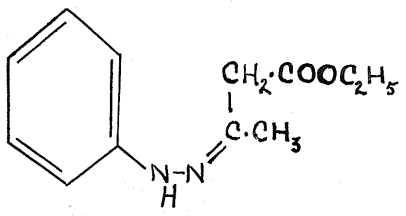


XLII

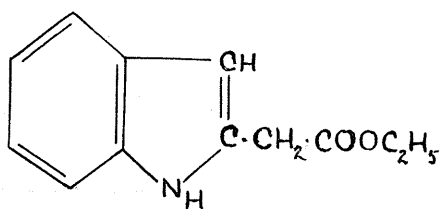


XLIII

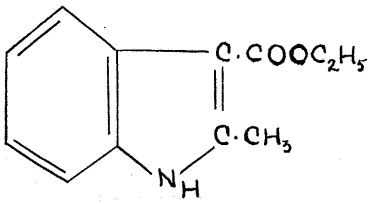
It was later found that this reaction became very general if zinc chloride was substituted for alcoholic hydrochloric acid as the condensing agent,[60] and that it would proceed in the presence of one percent of zinc chloride, cuprous chloride, cuprous bromide or platinous chloride.[61] Certain other reagents will also bring about the reaction. Thus Nef[62] found that when the phenylhydrazone of acetoacetic ester was dissolved in concentrated sulphuric acid and the reaction mixture then poured into a large volume of water, a product separated which crystallized from alcohol in compact needles with a melting point of 134°. Analysis of the substance agreed with the formula $C_{12}H_{13}O_2N$, indicating that one mole of ammonia must have been split from a mole of the hydrazide, $C_{12}H_{16}O_2N_2$, (XLIV). Of the two products which might have been formed, indole-2-acetic ester (XLV) or 2-methyl-indole-3-carboxylic ester (XLVI), Nef decided that the latter was the one obtained, a supposition which was later confirmed by Walker.[63]



XLIV

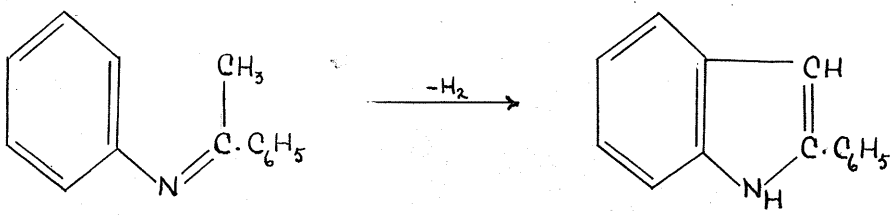


XLV



XLVI

It is interesting to note that no entirely satisfactory explanation appears to have yet been offered for the course of the Fischer indole synthesis. Hollins[64] considers Reddelien's hypothesis to be the most acceptable. The latter discovered[65] that acetophenone anil is oxidized by phenylhydrazine to 2-phenylindole, the phenylhydrazine being reduced in the process to ammonia and aniline:



Phenylhydrazones were found to have the same oxidizing power, being themselves reduced to the ketone-imide and aniline. For the Fischer indole synthesis, Reddelien therefore suggested the following stages:

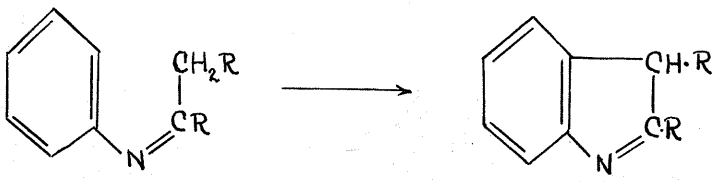
1) Reduction of the phenylhydrazone (during oxidation in (3) to aniline and ketone imide --

$$C_6H_5NH.N{:}CR.CH_2R + H_2 \longrightarrow C_6H_5NH_2 + NH{:}CR.CH_2R$$

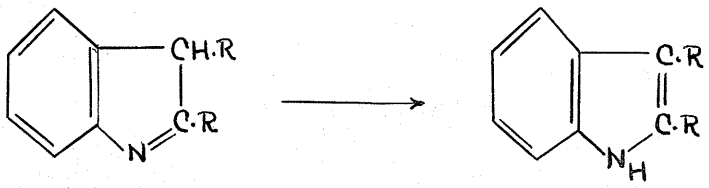
2) Elimination of ammonia between these two products--

$$C_6H_5NH_2 + NH{:}CR.CH_2R \longrightarrow C_6H_5N{:}CR.CH_2R + NH_3$$

3) Ring-closure by oxidation by the phenylhydrazone--



4) Rearrangement of the resulting indolenine--



Robinson and Robinson[66] have advanced several arguments against such a formulation. It requires fission of the hydrazone and recombination of the parts and fails to take cognisance of the extreme sensitiveness of ketoneimines and anils to hydrolysis by acids. In addition, it involves compensating oxidation and reduction with intermediate condensation, which is in practice somewhat difficult to effect, and

it offers no explanation of the fact that the necessary reagent is an acidic substance. Furthermore, they have found that indole syntheses proceed normally in the presence of foreign aromatic amines. Thus pure tetrahydrocarbazole is obtained from cyclohexanonephenylhydrazone in the presence of p-toluidine, methylaniline, and p-nitroaniline in glacial acetic acid solution; similarly the yield of nitrotetrahydrocarbazole from cyclohexanone-p-nitrophenylhydrazone is unaffected by the addition of an equivalent of aniline sulphate. In this case, the Reddelien hypothesis would require that the liberated p-nitroaniline is so much more reactive than aniline that the anil is exclusively formed from the nitro derivative, whereas experience shows that the amino group of aniline is far more prone to take part in condensations than that of p-nitroaniline.

Robinson and Robinson[67] have themselves advanced an explanation of the mechanism of the reaction which appears to be more satisfactory than any so far put forward, although little direct experimental proof is available. According to their hypothesis, the reaction occurs in three stages:

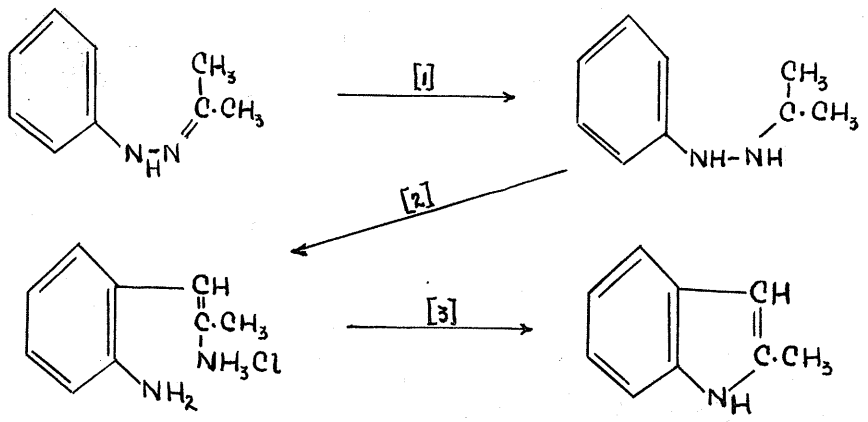
1) Transformation of the hydrazone into an unsaturated hydrazine, which is the isomeric change of an enimic into an enamic modification. This is assumed to occur

by the addition of the acid reagent and decomposition of the additive product.

2) The benzidine-type rearrangement of the resulting hydrazine.

3) Ring-formation by elimination of ammonium salt from the product, analogous to the formation of piperidine from the hydrochloride of pentamethylene-diamine.

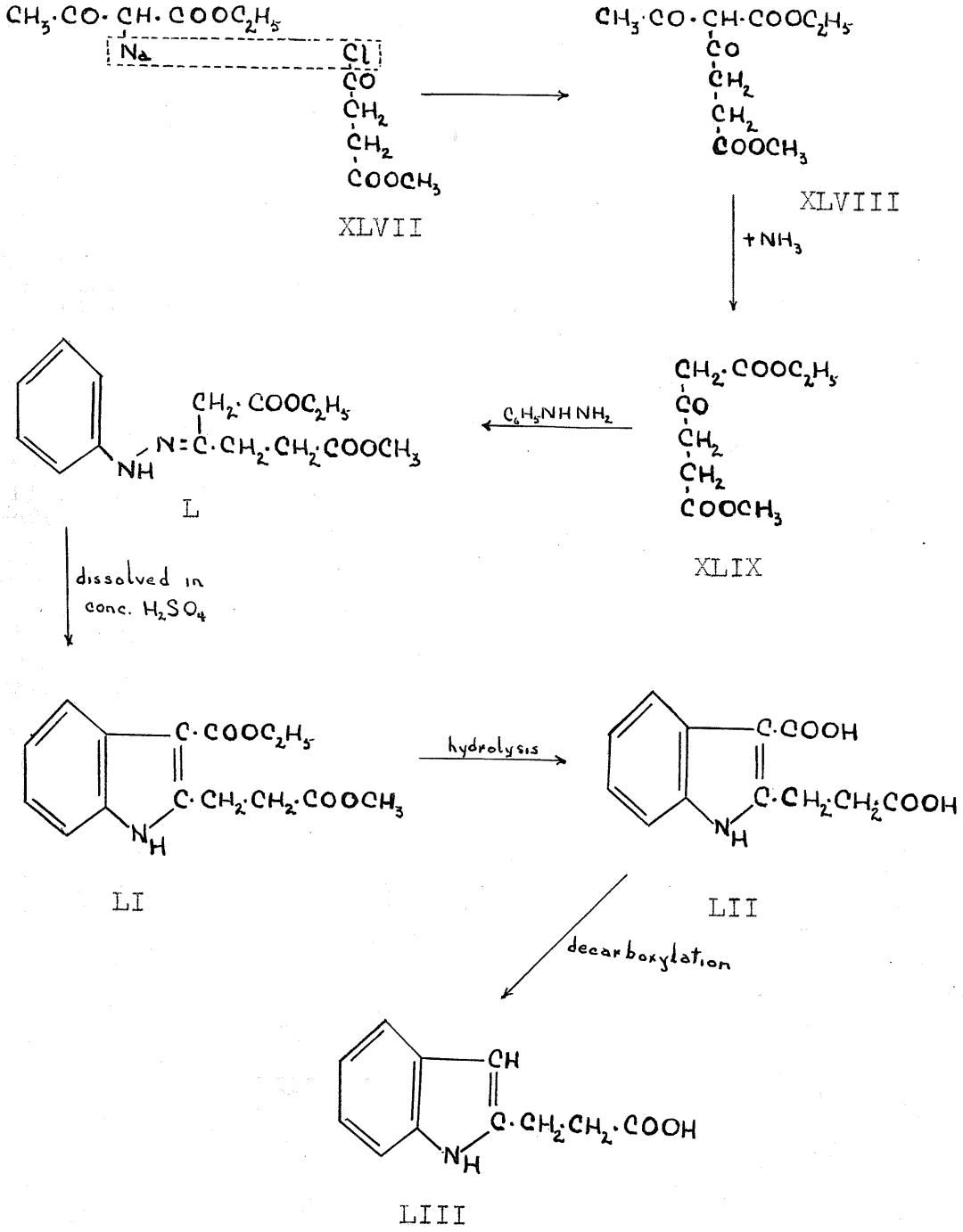
These changes are represented below in the case of the synthesis of 2-methylindole from the phenyl-hydrazone of acetone:



Possibly the only criticism against such a hypothesis is the lack of direct experimental evidence.

As stated previously, the method employed by Nef for the preparation of 2-methylindole-3-carboxylic ester was followed in an attempt to synthesize indole-2-

propionic acid. The reactions are outlined in the equations given below:



The mixed ketoadipic ester (XLIX) was prepared in five stages from succinic acid. Succinic acid was first converted into the anhydride through the action of acetyl chloride followed by distillation under atmospheric pressure. Refluxing with methyl alcohol resulted in the formation of the half methyl ester from which the half chloride (3-carbomethoxy-propionyl chloride, XLVII) was obtained by treatment with thionyl chloride. Yields in all three preparations were excellent.

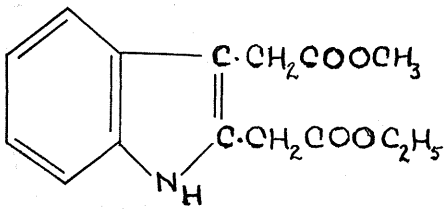
Two methods were employed in effecting the condensation between acetoacetic ester and the half ester half chloride of succinic acid (XLVII). The first of these -- that of J. C. Bardhan[69] -- using the sodium derivative of ethyl acetoacetate was found long and tedious and subsequently use was made of Spassow's procedure[70] employing magnesium in benzene as the condensing agent.

Through treatment of the product of the above reaction (methyl ethyl α-acetyl-β-ketoadipate, XLVIII) with ammonia, an acetyl group was split off as acetamide with the formation of methyl ethyl β-ketoadipate (XLIX).

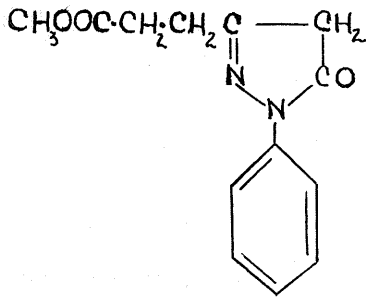
The phenylhydrazone (L) of the ketoadipic ester was next prepared and subjected to ring closure with

concentrated sulphuric acid following the method employed by Nef[62] in the synthesis of 2-methylindole-3-carboxylic ester. It was considered that the ring would close in the manner indicated yielding the dibasic ester (LI) which after hydrolysis and decarboxylation should yield indole-2-propionic acid (LIII).

Ring closure might take place in another way, however, yielding indole-2,3-diacetic ester (LIV), and there also exists the possibility of the formation of a pyrazalone (LV).



LIV



LV

The reaction mixture, after dilution with water, was extracted with ether, washed with 5% KOH solution, dried and the solvent removed. This neutral fraction (A) represented the bulk of the product and gave a positive, though faint, test for indole derivatives with Ehrlich's reagent.[71] As it should be the dibasic ester (LI), it was hydrolyzed with alcoholic potash yielding a yellowish-orange crystalline product which gave a strong positive Ehrlich's reaction. The

dibasic acid (LII) was then decarboxylated and after standing several days in the cold, partially solidified yielding small clusters of colorless, needle-like crystals dispersed in a dark red oil. This product also gave a strong Ehrlich's test. Due to the small amount of substance obtained, attempts at recrystallization were unsuccessful.

The aqueous alkaline washings from the above ethereal solution gave an acidic fraction (B) on acidification and extraction with ether. It was decarboxylated but did not solidify on cooling. Repeated attempts to crystallize the product from solvents were also unsuccessful. This substance, however, did give a positive test with Ehrlich's reagent.

Nothing definite, therefore, can be stated concerning the identity of these products, although the results of the Ehrlich's tests would indicate that an indole derivative was formed. A pyrazalone such as (LV) would not give a positive Ehrlich's reaction, while the fact that the acid decarboxylated on heating (as shown by the evolution of carbon dioxide) precludes the possibility that the product of the reaction was indole-2,3-diacetic acid (LIV). It therefore appears extremely likely that indole-2-propionic acid was formed although possibly in very small amounts.

E X P E R I M E N T A L

Preparation of Ethyl 2-methylindole-3-carboxylate (XLVI)

Before attempting the synthesis of indole-3-propionic acid, it was decided to repeat Nef's preparation of the above compound in order to obtain proficiency in the method.

The phenylhydrazone of acetoacetic ester was prepared as follows: 11 grams (1 mole) of freshly distilled phenylhydrazine were added slowly with cooling to a solution of 13 grams (1 mole) of acetoacetic ester in 40 ccs. of dry ether containing a small amount of calcium chloride. After standing for two hours, more ether was added and the solution washed with 10% sodium hydroxide, 10% sulphuric acid and water to remove unchanged acetoacetic ester and phenylhydrazine. After drying over potassium carbonate, the ether was removed in vacuo over sulphuric acid. The hydrazone separated in yellowish-orange needles and was allowed to stand for twelve hours, after which time it was washed with hexane to remove the small amount of oil which remained. The hydrazone was obtained in the form of very light yellow, needle-like crystals. Yield, 6.5 grams.

To convert this into the indole derivative, the

above product was slowly added to 15 ccs. of concentrated sulphuric acid cooled in a freezing mixture. At the beginning of the addition, a reddish solution was obtained which soon became brown in color. Following the addition, the reaction mixture was allowed to stand at room temperature for 15 - 20 minutes and was then poured into ice-water. A white, gummy material separated which, through treatment with hot ligroin in which it is sparingly soluble, was obtained as a white crystalline solid with a melting point of 133°- 134°. Nef recorded a melting point of 134° for this product.

A second run failed to yield the same substance. Instead, a red, crystalline compound was obtained which, after recrystallization from ligroin, melted at 170°. Although this product was not investigated further, it appeared to be the free acid (2-methylindole-3-carboxylic acid) for which Ciamician and Magnanini have reported[72] a melting point of 170°- 172°. (Walker,[63] 176°)

Apparently then the ring closure is sometimes accompanied by de-esterification and acidic fractions as well as neutral fractions must be investigated in reactions of this kind.

Preparation of Succinic Anhydride

Following the method of Bone, Sprankling and Sudborough,[68] two moles (236 grams) of succinic acid were refluxed on the water bath for about two hours with an excess (336 grams) of acetyl chloride. After cooling, the anhydride was filtered off, transferred to a distillation flask and distilled under atmospheric pressure, the portion coming over between 240° and 260° being collected. The distillate slowly solidified to a white mass with a melting point of 118°-120°. Yields were about 59% of theoretical.

It was noticed that following the treatment with acetyl chloride, a small quantity of succinic acid appeared to be unchanged. The subsequent distillation, however, converted this into the anhydride.

Preparation of Methyl Hydrogen Succinate

The method of Bone, Sprankling and Sudborough[68] was employed. One mole (100 grams) of succinic anhydride were refluxed for 45 - 50 minutes with two moles (64 grams) of methyl alcohol which had previously been dehydrated by refluxing over calcium oxide for eight hours followed by distillation. The excess alcohol was then removed by distillation, and the residue recryst-

allized from hot carbon disulphide. The succinate was obtained in the form of glistening white plates, melting point 58°- 59°. (Bone, Sprankling and Sudborough, 58°)

It was later found unnecessary to recrystallize this product since yields in the next preparation were not greatly reduced by the use of the crude material.

Preparation of 3-Carbomethoxy-propionyl chloride (XLVII)

One mole (132 grams) of methyl hydrogen succinate was heated with one mole plus 15% (140 grams) of thionyl chloride on the water bath at the boiling point (78°C) of the thionyl chloride until the evolution of hydrochloric acid gas ceased. The product was then distilled under reduced pressure, the fraction coming over between 90° and 100° at 12 - 18 mms. pressure being collected. 3-Carbomethoxy-propionyl chloride is a colorless liquid with a pungent odor and boils at 93° at 18 mms. Yields were 80% of the theoretical.

Preparation of Methyl ethyl β-keto-α-acetyl adipate (XLVIII)

The method first employed in this preparation was that of J. C. Bardhan.[69]

Finely-divided sodium was first prepared as

follows: 23 grams of sodium were placed in a 3 liter flask and covered with xylene. It was then heated under a reflux condenser over a free flame until the sodium was melted. The flame was removed and the flask shaken vigorously until the sodium had solidified in small particles. After cooling, the xylene was poured off, the sodium washed three times by decantation with small quantities of dry ether and ether (1000 ccs.) added. The flask was then cooled in a freezing mixture and a solution of ethyl acetoacetate (130 grams) in dry ether (300 ccs.) added. Following the addition, the mixture was allowed to stand for 2 hours. It was then refluxed gently on the steam bath for 1 1/2 hours and allowed to stand overnight, the mixture being protected from moisture by the insertion of a calcium chloride tube in the end of the condenser. At the end of this time, 150 grams of succinic half-ester half-chloride in 400 ccs. of dry ether were gradually introduced with cooling and after standing overnight, the mixture was refluxed for one hour to complete the reaction. Ice and dilute HCl were then added and the separated aqueous layer was extracted four times with ether. The combined ethereal solutions were washed with a little water, dried over calcium chloride and the ether distilled off. The residual oil, consisting of a mixture of C-acyl and O-acyl esters was treated

with an ice-cold concentrated solution of sodium carbonate and the undissolved oil collected in ether, the extraction being repeated several times until no more oil dissolved in the sodium carbonate solution. The combined alkaline solution (about 6 liters) was extracted once more with ether to remove any oily matter and cautiously acidified with ice-cold dilute HCl, and the oil which separated collected in ether, dried over calcium chloride and the ether removed. The residual oil was then distilled under reduced pressure, the portion boiling between 165° and 180° at 19 mms. being collected.

Methyl ethyl β-keto-α-acetyl adipate is a colorless liquid boiling at 172°- 174° at about 18 mms. and gives a deep red coloration with ferric chloride in alcoholic solution. The yields obtained varied from 16% to 21%.

Analysis: Found - C = 53.4% : H = 6.64%
Calculated for $C_{11}H_{16}O_6$ -- C = 54.1% : H = 6.56%

The simpler and shorter method of A. Spassow[70] was later employed.

To a solution of ethyl acetoacetate (66.7 grams) in about 200 grams of dry benzene were added 8 grams of magnesium turnings and a slight excess (150 grams)

of half-ester half-chloride. The mixture was heated under a reflux condenser on an oil bath at 80°- 90° for 2 1/2 to 3 hours, during which time gases were evolved. The slightly dark product was cooled, the liquid decanted and the unchanged magnesium washed with ether. The residue in the flask, cooled in a freezing mixture, was decomposed with ice-water and the aqueous solution, after filtering off from the magnesium, was added to the benzene-ether solution. The mixture was extracted with ether and the ethereal solution dried over sodium sulphate. After driving off the ether, the residual oil was distilled under reduced pressure as before.

An average yield of 20% was obtained.

Preparation of Methyl ethyl-β-keto adipate (XLIX)

The diketo ester (60 grams) was dissolved in dry ether (120 ccs.), cooled in a freezing mixture and saturated with dry ammonia gas. The flask was then loosely corked and allowed to stand overnight. After this time, the solution was washed twice with water, and with cold dilute HCl, dried over sodium sulphate and distilled under reduced pressure. The fraction distilling between 154° and 160° at 14 mms. being collected.

Preparation of Phenylhydrazone of Methyl ethyl β-keto-adipate (L)

The method of preparation was the same as that described for the phenylhydrazone of acetoacetic ester. In this case however, crystallization of the product was much slower requiring two to three days standing in the cold. The phenylhydrazone was then washed with hexane to remove a small amount of oil and was obtained in the form of light yellow, needle-like crystals with a melting point of 70°. The yield from 25 grams of the ketoadipic ester and 13.7 grams of phenylhydrazine was only 5 grams.

Preparation of Indole-2-propionic acid (LIII)

The 5 grams of the phenylhydrazone of methyl ethyl β-ketoadipate were dissolved in 15 ccs. of concentrated sulphuric acid cooled in a freezing mixture, the temperature being kept at 15° - 20° during the addition. The solution was allowed to stand at room temperature for 15 - 20 minutes and was then poured into ice-water. Since no solid or oil separated, the mixture was extracted four times with ether and the ethereal solution washed four times with 100 cc. portions of 5% potassium hydroxide and with water. The ethereal solution was dried over calcium chloride and

the ether distilled giving 2.5 grams of a dark oil, fraction A.

Acidification of the alkaline washings with dilute sulphuric acid followed by extraction with ether and removal of the solvent after drying over calcium chloride, yielded an acidic fraction (B), consisting of 1 gram of a dark red, viscous oil.

Both products were tested as follows for indole derivatives:[71] A small amount of p-dimethylaminobenzaldehyde (Ehrlich's reagent) was dissolved in about 5 ccs. of 95% ethyl alcohol in a test tube to give an approximately 5% solution. Three or four drops of concentrated hydrochloric acid were then added, followed by the addition of a very small amount of the unknown substance. Development of a color either in the cold or on warming gently indicates the presence of an indole derivative.

Fraction A gave a faint reddish coloration while fraction B gave a stronger reaction (orange-red color).

The neutral fraction A was hydrolyzed by refluxing for three hours with 10 ccs. of 95% ethyl alcohol, 4 ccs. of water and 3 grams of potassium hydroxide. More water was added and the alcohol removed. On acidification, a brownish oil separated which was

taken up with ether and dried over calcium chloride. On evaporating the ether, an oil was obtained which, on cooling, solidified completely giving 1.1 grams of yellowish-orange, needle-like crystals. This product gave a permanganate coloration with Ehrlich's reagent.

This substance -- presumably the dicarboxylic acid, (LII) -- was then heated in an oil bath at 160° to 170° when decarboxylation occured in the course of a few minutes. The product was a brownish oil which, on standing in the cold for two days, partially solidified yielding a very small amount of white crystals dispersed in a dark red oil. The yield was only 0.7 grams of both tar and crystalline material. The crystals gave a positive (dark red) Ehrlich's reaction.

Although this product appeared to recrystallize from either 50% acetic acid or a benzene-petroleum ether mixture, the small amount obtained made filtration impossible.

The acid fraction (B) was decarboxylated in the same manner as described above. A dark red oil was obtained which gave a positive Ehrlich's test but repeated attempts to crystallize this product were unsuccessful.

Owing to lack of chemicals and the time necessary for the many preparations involved, the problem had to be abandoned at this point.

S U M M A R Y

1. A review of indole derivatives in connection with plant hormones and physiological experiments has been presented.

2. All indole derivatives which have been tested for activity as phytohormones have been tabulated.

3. The method by which an attempt was made to synthesize indole-2-propionic acid has been described.

4. Although nothing definite can be stated concerning the identity of the product isolated, results of the Ehrlich's tests indicate that the indole derivative was formed.

5. From a repetition of Nef's synthesis of ethyl 2-methylindole-3-carboxylate, it appears that ring closure through the action of concentrated sulphuric acid is sometimes accompanied by de-esterification and hence acidic fractions as well as neutral fractions must be investigated in reactions of this kind.

A D D E N D A

Some time after the attempted recrystallization of indole-2-propionic acid from a benzene-petroleum ether mixture, it was noticed that the evaporation of the solvent had left several well-developed, almost colorless crystals. These were separated mechanically from the dark red impurities and crystallized once more from a benzene-petroleum ether mixture. They were found to have a melting point of 90° with sintering from 88° and did not give a positive Ehrlich's reaction. This latter fact indicates that these crystals were probably the pyrazolone derivative (LV) which has previously been mentioned as a possible product of this synthesis.

Since the mother liquors from the first recrystallization gave a positive test with Ehrlich's reagent, it is evident that the indole-2-propionic acid must be present in the dark red oil from which the above crystals were separated.

B I B L I O G R A P H Y

1. Growth Hormones in Plants - P. Boysen Jensen, trans. by G. S. Avery, Jr., and P. R. Burkholder.
2. Phytohormones - F. W. Went and K. V. Thimann
3. K. V. Thimann - Ann. Rev. Biochem., IV, 545 (1935)
4. P. Boysen Jensen - Ann. Rev. Biochem., VII, (1938)
5. A. H. Blaauw - Med. Landbouwhoogeschool, 15, 89 (1918)
6. N. Cholodny - Bio. Central., 47, 604 (1927)
7. F. W. Went - Rec. trav. bot. neerl., 25, 1 (1928)
8. N. Nielsen - Jahrb. wiss. Bot., 73, 135 (1930)
9. Duhamel du Monceau - La physique des Arbres, Paris (1758)
10. Julius Sachs - Arb. Bot. Inst. Wurzburg, 2, 452 (1880)
11. J. Sachs - Arb. Bot. Inst. Wurzburg, 2, 609 (1882)
12. J. Sachs - Physiologische Notizen - VI, Marburg (1893)
13. C. Darwin - The Power of Movement in Plants, London (1880)
14. P. Boysen Jensen - Ber. d. bot. Ges., 28, 118 (1910)
15. P. Boysen Jensen - Bull. Acad. Roy. Danmark, 1911, no., 1,3.
16. P. Boysen Jensen - Ber. d. bot. Ges., 31, 559 (1913)
17. A. Paal - Ber. d. bot. Ges., 32, 499 (1914)
18. A. Paal - Jahrb. wiss. Bot., 58, 406 (1919)
19. H. Soding - Ber. d. bot. Ges., 41, 396 (1923)

20. H. Soding - Jahrb. wiss. Bot., 64, 587 (1925)

21. E. Seubert - Z. f. Bot., 17, 49 (1925)

22. F. W. Went - Rec. trav. bot. neerl., 25, 1 (1928)

23. F. Kogl and A. J. Haagen Smit - Proc. Kon. Akad. Wetensch., 34, 1411 (1931)

24. F. Kogl, A. Haagen Smit and H. Erxleben - Z. physiol. Chem., 214, 241 (1933)

25. F. Kogl, A. Haagen Smit and H. Erxleben - Z. physiol. Chem., 220, 137 (1933)

26. F. Kogl, H. Erxleben and A. Haagen Smit - Z. physiol. Chem., 225, 215 (1934)

27. F. Kogl, H. Erxleben and A. Haagen Smit - Z. physiol. Chem., 216, 31 (1933)

28. F. Kogl and H. Erxleben - Z. physiol. Chem., 227, 51 (1934)

29. F. Kogl and H. Erxleben - Z. physiol. Chem., 235, 181 (1935)

30. F. Kogl, A. Haagen Smit and H. Erxleben - Z. physiol. Chem., 228, 90 (1934)

31. K. V. Thimann and F. W. Went - Phytohormones, p. 115

32. F. Skoog - J. Gen. Physiol., 20, 311 (1937)

33. N. Nielsen - Planta, 6, 376 (1928)

34. K. V. Thimann - J. Biol. Chem., 109, 279 (1935)

35. P. W. Zimmerman and F. Wilcoxon - Cont. Boyce Thomp. Inst., 7, 209 (1935)

36. A. E. Hitchcock - Cont. Boyce Thomp. Inst., 7, 87 (1935)

37. F. Kogl and D. G. Kostermans - Z. physiol. Chem., 235, 201 (1935)

38. A. Haagen Smit and F. W. Went - Proc. Kon. Akad. Wetensch. Amsterdam, 38, 835 (1935)

39. F. Kogl - Ber., 68A, 16 (1935)

40. P. W. Zimmerman and A. E. Hitchcock - Cont. Boyce Thomp. Inst., 7, 439 (1935)

P. W. Zimmerman and A. E. Hitchcock - Cont. Boyce Thomp. Inst., 8, 63 (1936)

P. W. Zimmerman, A. E. Hitchcock and F. Wilcoxon - Cont. Boyce Thomp. Inst., 8, 105 (1936)

41. R. H. F. Manske and L. C. Leitch - Can. J. Res., 14, 1 (1936)

42. L. C. Bauguess - Am. J. Bot., 22, 910 (1935)

43. J. Koepfli, K. V. Thimann and F. W. Went - J. Biol. Chem., 122, 763 (1938)

44. W. Davies, G. A. Atkins and P. C. Hudson - Ann. Botany, 1, 389 (1937)

45. G. S. Avery, P. R. Burkholder and H. B. Creighton - Am. J. Bot., 24, 226 (1937)

46. A. Ellinger - Ber., 37, 1801 (1904)

47. R. W. Jackson and W. T. Jackson - J. Biol. Chem., 96, 697 (1932)

48. A. Sure - Am. J. Physiol., 72, 260 (1925)

49. R. W. Jackson - J. Biol. Chem., 73, 523 (1927)

50. G. Cox and W. C. Rose - J. Biol. Chem., 68, 781 (1926)

51. K. Ichihara and N. Iwakura - Z. physiol. Chem., 195, 202 (1931)

52. L. C. Bauguess and C. P. Berg - J. Biol. Chem., 104, 675 (1934)

53. R. W. Jackson - J. Biol. Chem., 84, 1 (1929)

54. B. Harrow and C. P. Sherwin - J. Biol. Chem., 70, 683 (1926)

55. A. Ellinger and Z. Matsuoka - Z. physiol. Chem., 109, 259 (1920)

56. C. Berg, W. Rose and C. S. Marvel - J. Biol. Chem., 85, 219 (1929)

57. L. C. Bauguess and C. P. Berg - Proc. Iowa Acad. Sci., 40, 110 (1933)

58. L. C. Bauguess and C. P. Berg - Proc. Iowa Acad. Sci., 41, 174 (1934); J. Biol. Chem., 104, 691 (1934)

59. E. Fischer - Ber., 16, 2245 (1883)

60. E. Fischer - Ber., 19, 1567 (1886)

61. Arbusov and Tichvinski - J. Russ., 45, 69,70,694 (1923)

62. Nef - Ann., 266, 72 (1891)

63. Walker - Am. Chem. J., 15, 576 (1893); 16, 435 (1894)

64. C. Hollins - The Synthesis of Nitrogen-ring Compounds (1924)

65. G. Reddelien - Ann., 388, 179 (1912)

66. G. M. Robinson and R. Robinson - J. Chem. Soc., 125, 827 (1924)

67. G. M. Robinson and R. Robinson - J. Chem. Soc., 113, 639 (1918)

68. W. Bone, C. Sprankling and J. Sudborough - J. Chem. Soc., 85, 530 (1904)

69. J. C. Bardhan - J. Chem. Soc., 1948 (1936)

70. A. Spassow - Ber., 70, 2381 (1937)

71. Hawk and Bergeim - Prac. Physiol. Chemistry, p. 303

72. G. Ciamician and Magnanini - Ber., 21, 1926

PART II

I N T R O D U C T I O N

The need for large supplies of m-toluic acid and the cost of such quantities, made it desirable to see if a cheap method of preparing this chemical was available.

A search through the literature revealed little information concerning such methods. The general method for preparing organic acids by the hydrolysis of the corresponding nitriles is, of course, applicable in the case of m-toluic acid and constitutes one of the earliest methods for the preparation of this acid. m-Tolunitrile may be obtained by means of the Sandmeyer reaction from m-toluidine and may then be hydrolyzed by boiling with 75 percent sulphuric acid as described by Buchka and Schachtebeck.[1]

With only three exceptions, the remaining procedures described in the literature involve the oxidation of m-xylene.

L. Szperl has reported[2] that m-xylyl alcohol when heated with sulphur in an atmosphere of carbon dioxide, is partially converted into m-toluic acid but no mention is made of the yield. A. A. Morton and J. R. Stevens[3] have prepared this acid through the action of carbon dioxide on m-chlorotoluene in the

presence of sodium. A 58 percent yield of m-toluic acid was obtained by heating a solution of m-chlorotoluene in benzene in the presence of sodium with carbon dioxide under a pressure of thirty pounds. In a later paper,[4] Morton has reported similar experiments in which m-chlorotoluene, when dissolved in benzene and heated with sodium and carbon dioxide under a pressure of fifty pounds was converted into m-toluic acid in 98 percent yields. A third method, patented by A. Verley,[5] involves the introduction of a methyl group into the benzene nucleus. The preparation is carried out by agitating methyl alcohol and benzoic acid with a 25 percent pyrosulphate mixture composed of an alkyl sulphate, pure sulphuric acid (monohydrate) and sodium pyrosulphate for three to four hours at a temperature of 70°. m-Toluic acid and its methyl ester are obtained, the yields being unreported.

As mentioned in the preceding paragraph, the other methods recorded in the literature involve the oxidation of m-xylene.

The earliest of these was reported by Reuter[6] who prepared m-toluic acid by boiling m-xylene with dilute nitric acid (two volumes of acid of density 1.4, and three volumes of water). It has also been found[7] that this acid is formed in very small amounts by the electrochemical oxidation of m-xylene, an emulsion of

xylene and dilute sulphuric acid being used.

It was first noticed by Weger in 1903,[8] that certain aromatic hydrocarbons are converted into compounds with acidic properties under the influence of light and air. Nine years later, Ciamician and Silber[9] confirmed the formation of acids from such hydrocarbons as toluene, the xylenes and cymene by sunlight in the presence of air and water. Twenty grams of xylene and 200 cubic centimeters of water placed in large flasks of 13 liters capacity and left exposed to light for about six months gave a 31 percent yield of m-toluic acid.

About this same time, Benrath and Meyer,[10] when studying the action of aromatic hydrocarbons on quinones in sunlight, found that phenanthraquinone was completely oxidized to diphenic acid and the hydrocarbon partially to the corresponding monobasic acid. This fact was made use of by A. Eckert,[11] who patented in 1922 a method by which the oxidation of any aromatic compound is brought about by the action of free oxygen under exposure to light in the presence of anthraquinone or its substitution products such as chloranthraquinone. According to Sudborough,[12] Eckert in his patent specification states that he can obtain a 40 percent yield of benzoic acid by exposing toluene under the above conditions for six weeks, but no mention was made of the yields from the three

xylenes. In these reactions, the anthraquinone acts as a catalyst and remains unaltered at the end of the oxidation. To explain this action, it is assumed by Eckert[13] that the anthraquinone derivative gives up its oxygen to the hydrocarbon and thereby is reduced to the corresponding anthrahydroquinone. The latter is an extremely unstable substance and oxidizes very easily in contact with air back to anthraquinone and the reaction can thus repeat itself.

By a very similar method to that employed by Ciamician and Silber, Stephens[14] has reported the preparation of m-toluic acid from m-xylene through the action of gaseous oxygen. In these experiments, the dry hydrocarbons were used and oxygen was bubbled through them at elevated temperatures in dim, diffused light. Yields of the acid were very poor however; ninety grams of m-xylene when treated with oxygen for 30 days at 100° giving only 0.98 grams of toluic acid and 1.9 grams of toluic aldehyde. The method therefore appears to be of little commercial importance.

Another similar method of oxidation is through the action of water and oxygen in the presence of catalyzers such as the oxides of heavy metals, for example, uranium oxide, vanadium pentoxide, copper oxide, chromium oxide and others.[15] The hydrocarbon is heated

with water and the metal oxide with oxygen under pressure to a temperature of over 150°, yielding a mixture of aldehyde and acid.

From the foregoing summary it is apparent that no success has been met with in preparing m-toluic acid from m-xylene through the action of the more common oxidizing agents such as permanganate, dichromate, peroxide, etc. It is to be expected, of course, that the oxidizing agent would attack both of the methyl groups simultaneously with the subsequent formation of isophthalic acid. It has been demonstrated many times that the presence of a halogen atom in a methyl group renders that group more susceptible to attack by oxidizing agents. With this idea in mind, it was decided to attempt the oxidation of an ω-halogen-substituted xylene in the hope that the resulting yields might justify the commercial use of this method in the manufacture of m-toluic acid.

Accordingly, m-xylyl bromide was prepared by Atkinson and Thorpe's method and submitted to the oxidizing action of such common oxidizing agents as hydrogen peroxide, potassium dichromate, chromic acid, nitric acid and potassium permanganate. The oxidations with chromic acid were carried out in acetic acid solution and in the presence of sulphuric acid, while potassium permanganate was used in acidic, basic and

neutral (acetone) solution. In most cases also the experiments were carried out at various temperatures in an attempt to find the conditions leading to the best yields of m-toluic acid. The reaction product from each oxidation was treated with hot carbon tetrachloride since it was found that, of the two possible products of the oxidation of m-xylyl bromide -- i.e., isophthalic acid and m-toluic acid -- only the latter was soluble in this solvent. After recrystallization from hot water, identification was then made by means of melting point determinations, isophthalic acid melting at 330° and m-toluic at 108.5°

m-Xylyl bromide was found to be more difficult to oxidize than was expected, and a mild oxidizing agent such as hydrogen peroxide appeared to have no effect. The other reagents employed did oxidize the compound but in most cases the reaction proceeded beyond the desired point and resulted in the formation of isophthalic acid. Only in the case of alkaline potassium permanganate was any appreciable quantity of m-toluic acid obtained, the yields being so poor however, that the method is unlikely to have any commercial importance.

E X P E R I M E N T A L

Preparation of m-Xylyl bromide

150 grams of m-xylene were placed in a two liter flask fitted with a reflux condenser, thermometer and a dropping funnel. The flask was heated to 125°- 130° in an oil bath and 302 grams of bromine were run in very slowly below the surface of the xylene. After the addition of the bromine, the product was poured into an evaporating dish and placed in a vacuum dessicator over potassium hydroxide until free of hydrogen bromide. The m-xylyl bromide was then separated by decantation from a white crystalline solid which had separated out, and distilled at atmospheric pressure, the distillate being collected between 210° and 225°. Yields were about 35% of the theoretical.

m-Xylyl bromide was an exceedingly unpleasant compound with which to work due to its pronounced lachrymatory properties.

Oxidation with Nitric Acid

Five grams of m-xylyl bromide were refluxed for 1 1/2 hours with 25 ccs. of dilute nitric acid (2 vols. of concentrated acid to 3 vols. of water). The white crystalline solid which separated was then filtered and

washed with cold water. The melting point of the crude product was 290°. Treatment with carbon tetrachloride in which m-toluic acid is soluble, failed to yield any appreciable quantity of this acid. From its high melting point, the product was isophthalic acid.

Oxidation with Potassium Dichromate

A mixture of 5 grams of m-xylyl bromide and 100 ccs. of water was heated in an oil bath to 80°- 85° and a solution of 5.3 grams of the dichromate in 7.1 grams of concentrated sulphuric acid and 25 ccs. of water was added with constant stirring. Heating and stirring was continued for one hour when the mixture was cooled and poured into a large volume of water. Since no solid separated, the mixture was extracted with ether and the ethereal solution washed with water and 10% KOH. After drying over $CaCl_2$, the ether was distilled off yielding 5 grams of unchanged m-xylyl bromide. Acidification of the KOH washings failed to yield any solid product.

The above oxidation was repeated twice. In the first case the mixture was heated to 100°- 105° with constant stirring for 1 1/2 hours, and in the second, was refluxed for the same length of time. The only identifiable product, aside from unchanged m-xylyl bromide, was isophthalic acid.

Oxidation with Chromic Acid

5 grams of m-xylyl bromide dissolved in 25 ccs. of glacial acetic acid were heated in an oil bath to 105°- 110° and a solution of 3.6 grams of chromic acid in 250 ccs. of hot glacial acetic acid slowly added with constant stirring. After 2 1/2 hours, the solution was cooled, diluted with water and extracted with ether. The ethereal solution was washed with 10% KOH, dried over calcium chloride and the solvent removed. 3.5 grams of unchanged m-xylyl bromide were obtained. Acidification of the KOH washings yielded 1.0 gram of a white solid which was identified by its melting point as being isophthalic acid.

The above oxidation was repeated, the xylyl bromide and chromic acid being refluxed in 75 ccs. of glacial acetic acid for 1 1/2 hours. The only solid product obtained was isophthalic acid.

The oxidation was again repeated, a mixture of 5 grams of m-xylyl bromide, 3.6 grams of chromic acid and 8 ccs. of concentrated sulphuric acid in 50 ccs of water being refluxed for 1 1/2 hours. The mixture was then diluted with water and extracted with ether. The ethereal solution was washed with water, 10% KOH, dried over calcium chloride and the solvent removed yielding

3 grams of unchanged m-xylyl bromide. Acidification of the KOH washings gave 1.5 grams of a brownish solid which, after recrystallization from hot water, was obtained as a white solid melting above 300° and identical with isophthalic acid. No m-toluic acid could be obtained through treatment of this product with carbon tetrachloride.

Oxidation with Hydrogen Peroxide

Five grams of m-xylyl bromide were dissolved in 20 ccs. of glacial acetic acid and heated in an oil bath to 90°- 100°. Forty grams of 30% hydrogen peroxide were then added with constant stirring. After 1 1/2 hours, the mixture was cooled and extracted with ether. The ethereal solution was washed with water and 10% KOH and dried over calcium chloride. Removal of the solvent yielded 4 grams of unchanged m-xylyl bromide. Acidification of the alkaline washings gave 0.1 grams of a white solid which was identified through its melting point as being isophthalic acid. No m-toluic acid appeared to have been formed.

Oxidation with Potassium Permanganate

(1) In acetone:

20 grams of m-xylyl bromide

dissolved in acetone were refluxed with 33 grams of finely-ground potassium permanganate for 30 - 35 hours. Although the solution was not entirely colorless at the end of this time, it was poured into 400 ccs. of water and manganese dioxide removed by passing sulphur dioxide through the mixture. After standing for a day, it was found that a white solid had separated. This product was filtered off and identified by its melting point (329°) as being isophthalic acid.

(2) In acidic solution:

A solution of 3.5 grams of potassium permanganate in 130 ccs. of water and 50 ccs. of 10% sulphuric acid was slowly added to 5 grams of m-xylyl bromide contained in a flask heated on the water bath. When the addition was complete, the mixture was refluxed gently for 2 hours, when the colorless solution was made alkaline with sodium hydroxide and filtered to remove manganese dioxide. The filtrate was then acidified and again filtered. A small amount of a grayish solid was obtained which melted at 303° and from which no m-toluic acid could be separated. Unchanged m-xylyl bromide was also present in the reaction mixture indicating that the oxidation was incomplete, but since there appeared to be no m-toluic acid present, no further oxidations by this method were attempted.

(3) In alkaline solution:

A mixture of 10 grams of m-xylyl bromide, 10 ccs. of 10% KOH and 100 ccs. of water were refluxed for three hours, a solution of 11.4 grams of potassium permanganate in 1200 ccs. of water being added slowly from a dropping-funnel. At the end of this time, the solution was cooled and made strongly alkaline by the addition of a further quantity of KOH. The manganese dioxide was then filtered off, the filtrate being extracted with ether to remove any unchanged m-xylyl bromide and then concentrated to small bulk. On acidification with dilute sulphuric acid, a white solid separated which was filtered off and washed with cold water. A yield of 5.3 grams was obtained, the product sintering at 99° but not completely melting even at 200°.

The crude material was then treated with hot carbon tetrachloride in which m-toluic acid is soluble. Evaporation of the solvent yielded 3.1 grams of a white solid sintering at 85° and melting at 104°. After three recrystallizations from hot water, 1.0 gram of a product with a melting point of 108°- 109° was obtained, an analysis of which agreed with m-toluic acid.

Analysis; Found - C = 70.2% ; H = 5.94%

Calculated for $C_8H_8O_2$ - C = 70.6% ; H = 5.88%

The yield of pure m-toluic acid was 13.6% of the theoretical when calculated on the basis of m-xylyl bromide, or 4.7% of the theoretical, calculated on the basis of m-xylene.

Although this oxidation was repeated at a temperature of 70°- 80°, the lower temperature resulted in greatly decreased yields of the desired m-toluic acid. It therefore appears that the method outlined above is the most satisfactory of any of the attempted oxidations for the preparation of m-toluic acid from m-xylyl bromide.

S U M M A R Y

1. A review of other methods for the preparation of m-toluic acid has been given.

2. The oxidation of m-xylyl bromide to m-toluic acid by means of many of the more common oxidizing agents has been attempted.

3. Of the reagents employed, alkaline potassium permanganate gave the best yields of m-toluic acid, but even in this case, yields were less than 14 percent of the theoretical, making it unlikely that this method will be of any commercial value.

4. The method of Morton et al., who obtained m-toluic acid from m-chlorotoluene in a reported 88 percent yield through the action of carbon dioxide under pressure in the presence of sodium, seems feasible for any laboratory possessing the necessary apparatus and would appear to be worthy of further investigation.

B I B L I O G R A P H Y

1. Buchka and Schachtebeck - Ber., 22, 841

2. L. Szperl - Roczniki Chemji, 6, 728 (1926)

3. A. A. Morton and J. R. Stevens - J. Am. Chem. Soc., 53, 4028 (1931)

4. A. A. Morton, W. J. LePevre and I. Hechenbleikner - J. Am. Chem. Soc., 58, 754 (1936)

5. A. Verley - F.P., 632124 (1927)

6. Reuter - Ber., 17, 2028

7. Fr. Fichter and J. Meyer - Helv. chim. Acta, 8, 74 (1925)

8. Weger - Ber., 36, 309 (1903)

9. G. Ciamician and P. Silber - Ber., 45, 38 (1912)

10. Benrath and Meyer - Ber., 45, 2707 (1912)

11. A. Eckert - E.P., 182487 (1922)

12. J. Sudborough, H. Watson and B. Narayanan - J. Ind. Inst. Sci., 8A, 1 (1925)

13. A. Eckert - Ber., 58, 313 (1925)

14. H. N. Stephens - J. Am. Chem. Soc., 48, 1824 (1926)

15. I. G. Farbenindustrie Akt.-Ges. - F.P., 676826 (1929)

16. E. F. Atkinson and J. F. Thorpe - J. Chem. Soc., 91, 1696 (1907)