ACTA CHEMICA SCANDINAVICA 8 (1954) 119—126

Synthetic Plant Hormones

V *. Synthesis of α-3-Indole*iso*butyric Acid, an Antagonist of 3-Indoleacetic Acid, and of Some Other 1- and 3-Indole Acids

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In the preceding paper of this series the plant growth activity of some α-phenoxyacetic acids methylated in the side chain, and the working hypothesis which initiated our present line of research was discussed [1]. It was emphasized, that with one exception (phenoxyacetic acid itself, which is inactive at low concentrations but exhibits antiauxin activity at higher concentrations) all phenoxyacetic and α-phenoxypropionic acids discussed therein show strong auxin activity, whereas the α-phenoxy*iso*butyric acids are very potent auxin antagonists (antiauxins).

The present communication records an improved synthesis of α-3-indolepropionic acid (I) and the synthesis of α-3-indole*iso*butyric acid (II), compounds possessing special interest because of their close relation to 3-indoleacetic acid, one of the naturally occurring plant growth regulators.

Rather unexpectedly α-3-indole*iso*butyric acid (II) (m.p. 135°) could be obtained in a yield of about 30 % simply by the action of alkali, acetone and chloroform on indole, at room temperature. When the reaction was carried out at reflux temperature the yield of α-3-indole*iso*butyric acid decreased to about 10 %, but in addition a dibasic acid, $C_{16}H_{19}NO_4$ (IV) m.p. 228—230° (decomp.) was obtained.

From the known reactivity of the indole nucleus substitution could be expected to occur in position 3 or 1. Since the monobasic acid contains two "active" hydrogen atoms (Zerewitinoff), it must be a 3-indole derivative; it must possess an α- rather than a β-*iso*butyric acid side chain in view of the fact that it can also be obtained from acetonecyanhydrin and indole, a reaction completely analogous to the preparation of α-3-indolepropionic acid from indole and acetaldehydecyanhydrin (see below). Structures (III) and (IV) are possible for the dibasic acid, also containing two "active" hydrogen atoms. The acid (III) could, be prepared by addition of methacrylonitrile to ethyl α-indole-

* Part IV. Acta Chem. Scand. 7 (1953) 596.

*iso*butyrate, a method analogous to the preparation of β-1-cyanoethylindole from acrylonitrile and indole [2], and subsequent alkaline hydrolysis of the resulting nitrile-ester. The acid (III) had m.p. 163—164°, and is thus not identical with the above dibasic acid, m.p. 228—230°, which consequently must be α,α'-1,3-indoledi*iso*butyric acid (IV).

As acetonechloroform, 2,2,2-trichloro-*tert*butanol, could be considered a possible intermediate in the synthesis of α-3-indole*iso*butyric acid, and as indole is known to react with formaldehyde and hydrogen cyanide with formation of 3-indoleacetonitrile [3], attempts were made to condense indole with acetonecyanhydrin in ethanolic sodium hydroxide solution. A neutral product $C_{18}H_{16}N_2$, containing two "active" hydrogen atoms and surprisingly found to be identical with Oddo and Toffoli's [4] α,α-bis-(3-indole) ethane (V), α-3-indole-*iso*butyric acid and an acid having the formula $C_{11}H_{11}NO_2$, m.p. 111—112°, were obtained. Like α-3-indole*iso*butyric acid the latter was found to contain two "active" hydrogen atoms, and it was found to be identical with DL-α-3-indolepropionic acid previously prepared by Ellinger [6], further studied by Kögl and Kostermans [6] and resolved by Kögl and Verkaaik [7]. The melting point of the picrate, the specific rotation of the quinine salt of the (+)-acid prepared by Kögl's and Verkaaik's method as well as the melting point and specific rotation of the (+)-acid agreed with the figures given by the above authors.

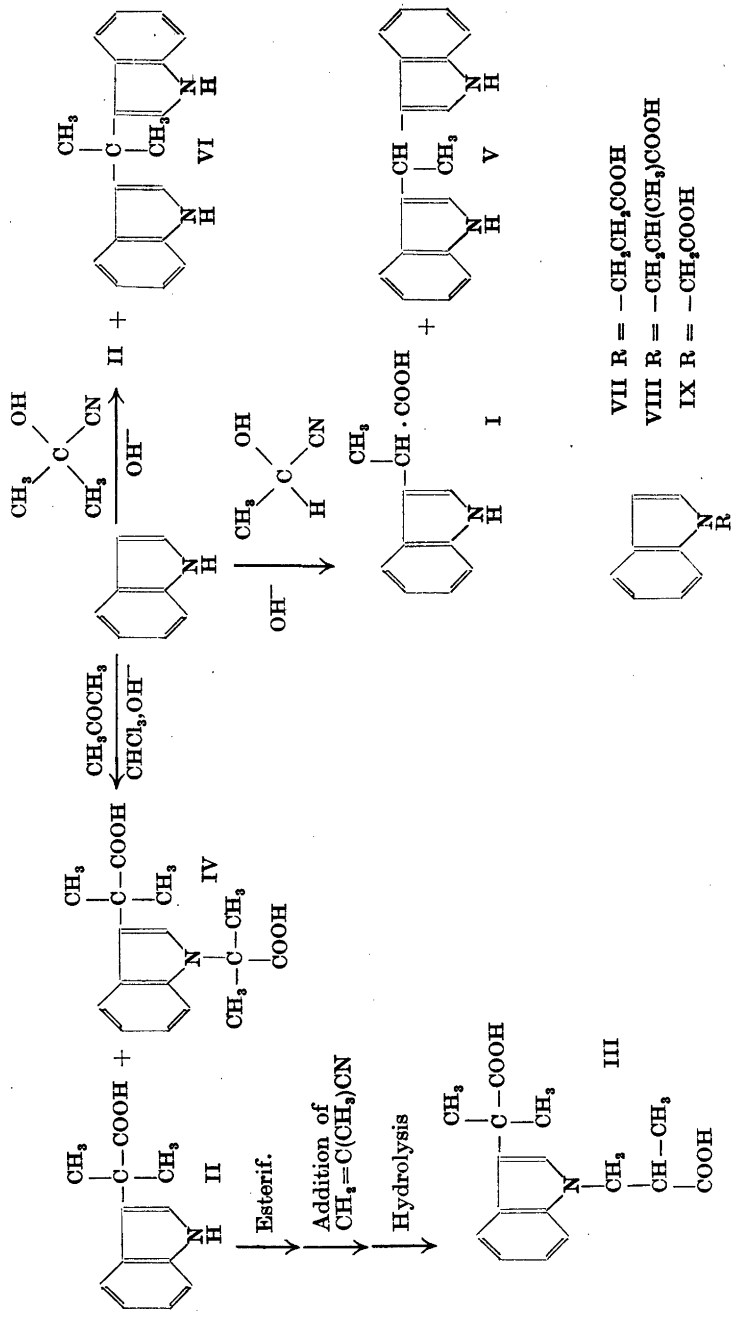
The production of these compounds is obviously due to a partial fission of the acetonecyanhydrin in the alkaline solution into acetone and cyanide ion, the acetone then undergoing a Meerwein-Ponndorf-Verley type of reaction with the ethanol present yielding *iso*propanol and acetaldehyde, the latter finally reacting with the indole.

This assumption was verified by carrying out the reaction in *iso*propanol instead of ethanol and α-3-indole*iso*butyric acid was now the only acid product, which could be isolated. In addition a neutral substance, $C_{19}H_{18}N_2$, m.p. 163°, identical with Scholtz's β,β-bis-(3-indole)propane [8] (VI) was obtained. When, on the other hand, the reaction was run with acetaldehydecyanhydrin in ethanol only α-3-indolepropionic acid (I) and α,α-bis-(3-indole)ethane were obtained.

For the preparation of α-3-indole*iso*butyric acid it was not necessary to use preformed acetonecyanhydrin. The same acid resulted, although in lower yield, when a mixture of acetone and potassium or sodium cyanide was used.

This reaction between indole and cyanhydrin is probably not restricted to formaldehyde-, acetaldehyde- and acetonecyanhydrin, but might be a rather general method for the preparation of side chain alkylated 3-indoleacetic acids.

β-1-Indolepropionic acid [9] (VII) was prepared *via* its nitrile by addition of acrylonitrile to indole by a method of I. G. Farbenindustrie [2]. The structure follows from the fact that it contains only one "active" hydrogen atom and no C—CH_3-group. When methacrylonitrile was used instead of acrylonitrile β-1-indole*iso*butyronitrile was obtained in high yield. Alkaline hydrolysis afforded the corresponding acid (VIII).


II
Esterif.
Addition of CH₂=C(CH₃)CN
Hydrolysis
III
IV
CH₃COCH₃
CHCl₃,OH⁻
II
OH⁻
VI
V
I
OH⁻
VII R = —CH₂CH₂COOH
VIII R = —CH₂CH(CH₃)COOH
IX R = —CH₂COOH

When the potassium salt of indole was heated with ethyl chloroacetate and the resulting mixture was hydrolysed with alkali 1-indoleacetic acid was obtained. This acid has recently been prepared by Smith and Moir [16] by a laborious route. Attempts to carry out this reaction with ethyl α-chloropropionate and ethyl α-bromo*iso*butyrate failed.

PHYSIOLOGICAL ACTIVITY

The plant growth activity of the substances has been tested by Prof.H. Burström *et al.*, Lund, employing their wheat root elongation technique [10,11] and is reported elsewhere by them. In accordance with the results of Kögl and Kostermans [6] D,L-α-3-indolepropionic acid was found to be a very potent auxin. The activity is, however, almost exclusively due to the dextrorotatory form of the acid (Ratio of the activity of the (+)-acid to that of the (±)-acid 2:1; Hansen [13]). This is in agreement with the results obtained by Kögl and Verkaaik [7,8] with the *Avena* curvature test, but not with the *Avena* straight growth test, in which both antipodes showed the same activity, and also agrees with the results obtained by Fredga, Matell and Åberg with optically active α-aryloxypropionic acids [14]. Smith and Moir's finding [15] that 1-indoleacetic acid possesses auxin activity was confirmed [13]. α-3-Indole*iso*butyric acid was found to be a very potent antiauxin [12]. β-1-Indolepropionic and β-1-indole*iso*butyric acid also possessed some antiauxin activity [13].

As 3-indoleacetic acid has been found to inhibit the growth of a number of microorganisms [16,17] it was of interest to study the effect of its branched homologues on various such organisms. Drs. N. Nielsen, A. B. Kabi, and G. Wallmark, Statens Bakteriologiska Laboratorium, have kindly carried out these tests. When tested in concentrations up to 0.1 mg per ml substrate α-3-indoleisobutyric acid had no significant effect on *Staph. aureus* and *albus*, *Enterococci*, *Ps. pyocyanea*, *Proteus vulg.*, *Salm. paratyphi*, *Pneumococci*, *α-Streptococci*, *β-Streptococci*, *M. tuberculosis*, some *Bacillus species*, *Escherichia coli*, *Candida albicans* and *Pullularia pullulans*. It showed an inhibitory effect on *Sarcina lutea* and *B. subtilis*.

D,L-α-3-Indolepropionic acid was tested in concentrations up to 1.0 mg per ml on *Sarcina lutea*, *Staph. aureus*, *B. subtilis*, *E. coli*, *Streptomyces griseus*, *Aspergillus niger*, *Penicillium chrysogenum* and *Pullularia pullulans*. In no case any significant effect was observed.

EXPERIMENTAL *

α-3-Indole*iso*butyric acid (II) from indole, acetone, chloroform and alkali

a) *At room temperature.* Indole (117 g), dry acetone (1.0 l) and sodium hydroxide (220 g, pellets) were stirred in a three-necked flask (3 l capacity) equipped with a reflux condenser, dropping funnel and thermometer, and cooled in an ice bath. Chloroform (156 g) was added dropwise at such a rate, that the temperature of the mixture did not exceed 15° (about 2 hours). The ice bath was removed, and the mixture stirred for one hour followed by a further four hours with warming on a water bath. Excess acetone was removed by distillation, and the salt cake dissolved in water (1.5 l). The solution was extracted four times with ether (49 g of unreacted indole was recovered from the extracts) and acidified with dilute sulphuric acid (1 : 1). The oil which separated was taken up in ether, washed with water, dried and the ether removed. The product was dissolved in anhydrous ethanol (250 ml) containing 5 per cent sulphuric acid, heated under reflux for four hours and poured into water (1 l). The ester was extracted with ether, washed with water, with sodium bicarbonate solution, again with water, and dried. Removal of the ether yielded an oil, which distilled between 180 to 205°/3 mm Hg (55 g) which crystallised on standing for some hours. One crystallisation from benzene-petrol (1 : 1) gave

* All boiling and melting points uncorrected. Petrol refers to the fraction b.p. 40—60°.

pure *ethyl α-3-indoleisobutyrate*, m.p. 106—107°. The yield was 43 g (32 per cent calculated on indole actually consumed (Found OC_2H_5 19.4 %. $C_{14}H_{17}NO_2$ requires OC_2H_5 19.5 %).

When refluxed for four hours with excess 40 % aqueous sodium hydroxide and sufficient ethanol to make the mixture homogenous the ester was hydrolysed to *α-3-indoleisobutyric acid* in almost quantitative yield. The acid separated from a chloroform-petrol mixture in coarse needles, m.p. 135°. (Found C 70.9; H 6.40; N 6.90; equiv. weight 204; active H 1.7; C—CH_3 89 % of theory. $C_{12}H_{13}NO_2$ requires C 70.9; H 6.45; N 6.90; equiv. weight 203.2; active H 2.00.)

The *cyclohexylammonium salt* was prepared in ether solution, and crystallised from an ethanol-ether mixture in needles, m.p. 162—163° (decomp.). (Found N 9.2. $C_{18}H_{26}N_2O_2$ requires N 9.3.)

b) *At reflux temperature.* The above reaction was carried out at reflux temperature using a third of the previous quantities. The acid products were not esterified but their etheral solution was repeatedly extracted with sodium bicarbonate solution. The combined extracts were acidified to pH 5—6 with acetic acid and the solution was extracted four times with ether. *α-3-Indoleisobutyric acid* could be obtained in 5 to 10 % overall yield from this extract.

The aqueous raffinate was acidified to pH 1—2 with dilute sulphuric acid and repeatedly extracted with ether. The combined extracts were thoroughly washed with water, concentrated to a small volume and filtered through a short column of aluminium oxide. Evaporation of the ether yielded an oil which crystallised in a few days. The product was triturated with benzene-petrol (1 : 1) and the solid *α,α'-1,3-indolediisobutyric acid* (IV) crystallised from aqueous dioxane and from aqueous methanol in faintly pink needles, m.p. 228—230° (decomp.). Yield 5 to 10 %. (Found C 66.8; H 6.60; N 4.81; equiv. weight 145; active H 1.8. $C_{16}H_{19}NO_4$ requires C 66.4; H 6.62; N 4.84; equiv. weight 144.6; active H 2.00.)

α-3-Indole*iso*butyric (II) and α-3-indolepropionic acid (I) from indole and cyanhydrins

a) *Acetaldehydecyanhydrin.* A mixture of indole (12 g), acetaldehydecyanhydrin (8.8 g), sodium hydroxide (10 g, pellets) and absolute ethanol (50 ml) was heated with stirring at 135° C for 18 to 24 hours in a stainless steel autoclave with silver packings. (Lead packings must be avoided as even small amounts of lead favour undesirable side reactions leading to resinous materials.) The light yellow reaction mixtures from three such runs were combined and poured into water. Neutral products were extracted with ether.

The aqueous solution containing the acid reaction products was acidified with dilute (1 : 1) sulphuric acid. The oil, which separated was dissolved in ether, thoroughly washed with water and dried. Removal of the ether afforded a light brown oil (24 g), which crystallised when stored in a refrigerator over night. Crystallisation from a benzene-petrol mixture (2 : 1) yielded an acid (19 g), m.p. 106—108°, which by repeated crystallisation was raised to 111—112°. (Found N 7.38, 7.41; equiv. weight 189; active H 1.8; C—CH_3 92 % of theory. Calc. for $C_{11}H_{11}NO_2$ N 7.40; equiv. weight 189.2; active H 2.00.)

The acid gave a picrate, m.p. 145—146°. Picrate of D,L-α-3-indolepropionic acid, Kögl and Kostermans [7], m.p. 146—147°. Following the method of Kögl and Verkaaik [7] for the resolution of D,L-α-3-indolepropionic acid a quinine salt, having $[\alpha]_D^{20} = -105°$ (95 % ethanol, $c = 1$) was obtained. The (+)-acid, m.p. 137—138°, $[\alpha]_D^{20} = +73°$ (95 % ethanol, $c = 0.4$) was obtained from the quinine salt. The acid m.p. 111—112° must therefore be D,L-*α-3- indolepropionic acid.* (Literature [5,6] m.p. 111°; the *dextro*rotatory form m.p. 138—139°, $[\alpha]_D = +76 \pm 2°$ (ethanol, $c = 1$). The quinine salt of the latter $[\alpha]_D = -104 \pm 2°$ (ethanol, $c = 1$).

The *cyclohexylammoniumsalt* of D,L-α-3-indolepropionic acid was prepared in ether and crystallised from acetone in needles. M.p. 183—185° (decomp.). (Found C 71.1; H 8.5; $C_{17}H_{24}N_2O_2$ requires C 70.8; H 8.39.)

The ether solution containing the neutral reaction products was evaporated yielding a brown oil, from which indole (5 g) was recovered by steam distillation. The nonvolatile residue was dissolved in ethanol and set aside for crystallisation. A colourless product

(13.5 g) was obtained from ethanol as crystals, m.p. 162—163°, undepressed on admixture with *α,α-bis-(3-indole)ethane* (V) m.p. 158—160° prepared by the method of Oddo and Toffoli [4]. (Found C 82.8; H 6.24; N 10.78; active H 1.9; C—CH_3 93 % of theory; mol. weight (Rast) 258, 262. Calc. for $C_{18}H_{16}N_2$ C 83.0; H 6.19; N 10.76; active H 2.00; mol. weight 260.4.)

b) *Acetonecyanhydrin.* Indole (12 g), acetonecyanhydrin (10.6 g), sodium hydroxide (10 g pellets) and anhydrous *iso*propanol (50 ml) were treated exactly as described for acetaldehydecyanhydrin. The yield of α-3-indole*iso*butyric acid from three such runs was 15 g. The m.p. was 135° alone and in admixture with acid prepared by the acetonechloroform reaction. Of a neutral compound, crystallising in colourless prisms from aqueous ethanol and melting at 163°, was obtained 14 g in addition to the acid. (Found N 10.1; 10.2; active H 2.0; C—CH_3 93 % of theory. Calc. for $C_{13}H_{18}N_2$; N 10.2; active H 2.00.) This compound was identical (mixed m.p.) with *β,β-bis-(3-indole)propane* (VI), obtained by Scholtz [6] from indole and acetone in acetic acid solution.

A mixture of α-3-indole*iso*butyric and α-3-indolepropionic acid resulted when the reaction was carried out in ethanol instead of *iso*propanol. Only unchanged indole and α,α-bis-(3-indole)ethane could be isolated from the neutral fraction. The main product was an uncrystallisable oil, which was not further investigated. It was probably a mixture of α,α-bis-(3-indole)ethane and β,β-bis-(3-indole)propane.

The separation of the two acids was effected after esterification with ethanol containing 5 % sulphuric acid. The esters distilled together at 150—210°/5 mm Hg giving a semisolid distillate, which when dissolved in hot petrol and kept over night in a refrigerator yielded ethyl α-3-indole-*iso*butyrate in an almost pure state. (M.p. 106—107° after one crystallisation.) The solvent was evaporated from the original mother liquor and the residual oil hydrolysed with excess 20 per cent aqueous sodium hydroxide. Acidification gave D,L-α-3-indolepropionic acid as an oil which crystallised on standing. Further crystallisation from benzene-petrol gave pure acid m.p. 111—112°. The yield of each acid was 10 to 15 %.

D,L-β-1-Indole*iso*butyronitrile

Indole (30 g), methacrylonitrile (35 g), ethanolic sodium ethoxide solution from sodium (0.5 g) and absolute ethanol (50 ml), and cupric acetate (0.2 g) were heated in a stainless steel autoclave at 120—130° for four hours. The reaction mixture was dissolved in ether, washed thoroughly with water, dried, the solvent removed and the residual oil distilled. The yield of D,L-*β-1-indoleisobutyronitrile* b.p. 160—164°/3 mm Hg was 35 g (74 %). (Found N 15.1; $C_{12}H_{12}N_2$ requires N 15.2.)

D,L-β-1-Indole*iso*butyric acid (VIII)

The above nitrile was refluxed for three hours with excess 40 per cent aqueous sodium hydroxide and sufficient ethanol to give a homogenous mixture. The liquid was poured into water and acidified; the resultant oil was taken up in ether, washed, dried and the solvent evaporated. The residual oil soon solidified. Crystallisation from benzene-petrol gave D,L-*β-1-indoleisobutyric acid*, m.p. 74°. Yield 70 %. (Found N 7.0; equiv. weight 203; active H 0.9; C—CH_3 76 % of theory. $C_{12}H_{13}NO_2$ requires N 6.9; Equiv.weight 203.2; active H 1.00.)

D,L-β-3-(1-α-Carboxypropyl)indole*iso*butyric acid (III)

Ethyl α-3-indole*iso*butyrate (8.0 g), methacrylonitrile (4.0 g) ethanolic sodium ethoxide from sodium (0.1 g) and absolute ethanol (25 ml), and cupric acetate (0.05 g) were mixed and heated as described for β-1-indole*iso*butyronitrile. The reaction mixture was heated under reflux for five hours with a solution of potassium hydroxide (20 g) in water (25 ml) and ethanol (100 ml), poured into water (500 ml), filtered and acidified. The acid products were extracted with ether, washed and dried. Removal of the ether gave a noncrystallisable oil (8.3 g) which was dissolved in ethanol (30 ml) and excess *cyclo*hexylamine added. After four hours the precipitated salt was collected and thoroughly extracted with hot ethanol. The yield of the insoluble *cyclohexylammonium salt of* D,L-α-3-

(1-α-carboxypropyl)indoleisobutyric acid m.p. 184—188° (decomp.) was 7.0 g. (Found N 8.65; $C_{28}H_{45}N_3O_4$ requires N 8.62.) The combined ethanol mother liquors were concentrated and α-3-indole*iso*butyric acid *cyclo*hexylammoniumsalt (4 g) was precipitated with acetone.

The D,L-*α-3-(1-α-carboxypropyl)indoleisobutyric* acid was obtained from its *cyclo*hexylammonium salt by treatment with dilute sulphuric acid. Crystallisation from chloroform-petrol gave colourless needles, m.p. 163—164°. (Found N 4.80; equiv.weight 145; active H 1.7; C—CH_3 78 % of theory. $C_{16}H_{19}NO_4$ requires N 4.84; equiv.weight 144.6; active H 2.00.)

1-Indoleacetic acid (IX)

Molten indole (22 g) was stirred with finely powdered potassium hydroxide (17 g) in a fractionating flask (200 ml capacity) inserted deeply in a salt bath kept at 200—225°, until no more water distilled (6 to 8 hours). The flask was removed from the bath and allowed to cool a little. Dry ethyl chloroacetate (36 g) was added and the flask was heated at 140—150° for four hours with occasional shaking, and cooled. A solution of potassium hydroxide (25 g) in water (75 ml) was added, and the mixture heated on a water bath for three hours and poured into water (250 ml). After some hours unreacted indole (7 g) was collected, and the filtrate was acidified. The crystalline product which separated, was collected, washed, dried, dissolved in ether, and the solution filtered through a short column of aluminium oxide and then evaporated to dryness. The crude *1-indoleacetic acid* (12 g) was dissolved in hot ethanol, and decolourised with animal charcoal. On cooling the acid was obtained as light brown needles of m.p. 168—170°, raised on crystallisation to 174—175° (decomp.). Smith and Moir report m.p. 178.4—179.4°. The acid is difficult to obtain in a colourless state, and rapidly deteriorates on storing. (Found C 68.4; H 5.19; equiv. weight 176; active H 0.8. Calc. for $C_{10}H_9NO_2$; C 68.6 H 5.18; equiv. weight 175.2; active H 1.00.)

Colour reactions

Fischer's fir chip test and the *p*-dimethylaminobenzaldehyde test are not very satisfactory in this series of indole compounds. In the fir chip test all give reddish colours, but compounds with free 3-position give an initial blueish coloration.

SUMMARY

The convenient synthesis of α-3-indole*iso*butyric acid (II) and D,L-α-3-indolepropionic acid (I) by novel methods, the condensation of indole with acetone and chloroform and condensation with cyanhydrins in the presence of alkali are described. D,L-β-1-indole*iso*butyric acid (VIII) has been prepared and an improved method for the synthesis of 1-indoleacetic acid (IX) is reported. The root growth activity of the acids is discussed as well as the activity of the first two on a number of microorganisms.

The authors wish to acknowledge their indebtedness to *Bönnelyche & Thuröe AB* and to *Cancernämnden* for financial support, to *Statens Tekniska Forskningsråd* for a Fellowship to one of them (Å. Jönsson) and to Miss E. Duintjer for skilful assistance.

REFERENCES

1. Jönsson, Å. *Acta Chem. Scand.* **7** (1953) 596.
2. I. G. Farbenindustrie A. G. *Brit.pat.* 457.621.
3. *U. S. pat.* 2.222.344 (1940), *Chem. Abstracts* **35** (1941) 1807; *Jap. pat.* 161.544 (1944), *Chem. Abstracts* **43** (1949) 2236.
4. Oddo, B., and Toffoli, C. *Gazz. chim. ital.* **64** (1934) 359, cited from *Chem. Zentr.* **1934 : II** 3253.

5. Ellinger, A. *Ber.* **38** (1905) 2887.
6. Kögl, F., and Kostermans, D. G. F. R. *Hoppe-Seylers Z. physiol. Chem.* **235** (1935) 201.
7. Kögl, F., and Verkaaik, B. *Hoppe-Seylers Z. physiol. Chem.* **280** (1944) 167.
8. Scholtz, M. *Ber.* **46** (1913) 1088.
9. I. G. Farbenindustrie A. G. *French pat.* 48.570 (1938).
10. Burström, H. *Physiol. Plantarum* **3** (1950) 175.
11. Wilske, C., and Burström, H. *Physiol. Plantarum* **3** (1950) 58.
12. Burström, H. *Physiol. Plantarum* **4** (1951) 470.
13. Hansen, B. *Private communication.*
14. For a summary of their results see Matell, M. *Stereochemical studies on Plant Growth Substances*, p. 30, Almqvist & Wiksells Boktryckeri AB, Uppsala 1953, and Veldstra, H. *Ann. Rev. Plant Physiol.* **4** (1953) 151.
15. Smith, W. S., and Moir, R. Y. *Can. J. Chem.* **30** (1952) 411.
16. Dubos, R. J. *Proc. Soc. Exptl. Biol. Med.* **63** (1946) 317.
17. Handler, Ph., and Kamin, H. *Proc. Soc. Exptl. Biol. Med.* **66** (1947) 251.

Received October 28, 1953.