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THE SYNTHESIS AND PHYSIOLOGICAL ACTIVITY OF SEVERAL α-ALKYLINDOLE-3-ACETIC ACIDS*

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Abstract—The effect of α-alkylation on the physiological activity of indole-3-acetic acid was determined by the synthesis of α-methyl-, α-ethyl-, α-propyl- and α-isopropyl-IAA and assessing their relative activity in the promotion of parthenocarpic tomato ovary growth, *Avena* coleoptile elongation, and buckwheat root elongation. Indole was condensed with the appropriate 2-hydroxyalkanoic acid in the presence of potassium hydroxide in a high pressure autoclave at 240–260° for 15–21 hr. Both α-methyl-IAA and α-ethyl-IAA were significantly more active in promoting parthenocarpic tomato ovary growth than IAA. α-Propyl-IAA was of intermediate activity and α-isopropyl-IAA had the same activity as IAA. In the *Avena* assay α-methyl-IAA and α-ethyl-IAA were equal in activity to IAA. Substitution of an α-hydrogen with a propyl group reduced the activity below that of IAA while substitution with the larger isopropyl group further significantly reduced biological activity. With the exception of α-isopropyl IAA, the activity in the buckwheat assay followed the same pattern as with the *Avena*. IAA, α-methyl-IAA, and α-ethyl-IAA all gave responses indicative of strong auxins while the response of α-propyl-IAA was typical of a weak auxin. α-Isopropyl-IAA showed neither inhibition nor stimulation of buckwheat root elongation.

INTRODUCTION

SPECIFIC molecular requirements of IAA have been recognized as essential for biological activity. Well established requirements are a nonpolar ring system and a side chain containing a group of anionic character, or one readily converted to an acid group.[1–3] The synthesis and bioassay of new compounds has led to the continued modification of these requirements. Most recently, Thimann and Porter[4] proposed that the critical property for auxin activity is an intramolecular distance of approximately 5·5 Å between the carbon of the carboxyl group and a fractional positive charge on the nucleus. Substitution of a methyl group at the one position of IAA, which would be expected to lower the fractional positive charge on the nitrogen, effectively reduced auxin activity. Likewise, substitution of electron withdrawing 1-acyl groups, which would increase the fractional positive charge on the nitrogen of ethyl indole-3-acetate, did not reduce the activity of the parent compound in the *Avena* straight growth assay. Further, 2-chloroindole-3-acetic acid was considerably more active than IAA in the pea curvature assay.[4] Methyl substitution at the two or seven position of IAA reduced biological activity, whereas, replacement of the carbon atom with a nitrogen atom at the two

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Abbreviations used: Indole-3-acetic acid, IAA; the α-alkyl-indole-3-acetic acids are named as derivatives of IAA using the alkyl radical and IAA, e.g. α-methylindole-3-acetic acid, α-methyl-IAA.

[1] J. B. KOEPFLI, K. V. THIMANN and F. W. WENT, *J. Biol. Chem.* **122**, 763 (1938).
[2] H. VELDSTRA, *Enzymologia* **11**, 97 (1944).
[3] H. VELDSTRA and H. L. BOOIJ, *Biochim. Biophys. Acta* **3**, 278 (1949).
[4] W. L. PORTER and K. V. THIMANN, *Plant Physiol.* **36**, Suppl., XXXIX (1961); *Phytochem.* **4**, 229 (1965).

or seven position had no effect, confirming that the nitrogen of the indole ring must be accessible.[5-8]

Specific structural requirements are necessary for the side-chain. The biological activity of indole-3-alkanoic acids varied with the length of the side-chain. Those acids with an even number of carbons were more active than those containing an odd number.[9] Wightman[10] established that the long chain indole-3-alkanoic acids were degraded by β-oxidation to IAA or indole-3-propionic acid, and that the biological activity observed was due to those degradation products.

The effect of α-alkyl substitution on the side-chain on biological activity was first studied by Kogl and Kostermans[11] who found α-methyl-IAA to be 80 per cent less active than IAA in the *Avena* curvature assay. Using the *Avena* straight growth assay[12] and pea curvature test,[13] where transport effects are minimized, the α-methyl derivative was equally as active as IAA. α-Methyl-IAA was equal to IAA in a wheat root assay[14] and more active than IAA in inhibiting oat root elongation.[15] Further, α-methyl-IAA was markedly more active than IAA in thickening of the fruit pedicel and promotion of parthenocarpic fruit growth in tomato.[16]

The synthesis and physiological activity of several α-alkyl derivatives of IAA in selected plant systems is the subject of this report.

RESULTS AND DISCUSSION

The synthesis of α-methyl-IAA, α-ethyl-IAA, α-propyl-IAA, and α-isopropyl-IAA was carried out by condensing indole with the appropriate 2-hydroxyalkanoic acid in the presence of potassium hydroxide in a high pressure autoclave at 240–260 for 15–21 hr. Their structure was confirmed by u.v. spectra, i.r. spectra, microanalysis, and melting points. From the known reactivity of the indole nucleus, condensation would be expected to occur at the one or three position. The presence of an NH stretching absorption around 2·90μ indicated that in each reaction, substitution occurred at the three position[17] (Table 1).

Both α-methyl-IAA and α-ethyl-IAA were significantly more active than the nonsubstituted acid in the promotion of parthenocarpic tomato fruit growth (Table 2). α-Propyl-IAA was of intermediate activity while α-isopropyl-IAA induced a response comparable to IAA. α-Ethyl-IAA was as active as α-methyl-IAA which we had previously reported[16] to be as effective as ethyl indole-3-acetate and other established nonindole fruit-setting chemicals (4-chlorophenoxyacetic acid and gibberellin A_3).

The enhanced activity of IAA substituted with the methyl, ethyl, or propyl radical on the α-carbon may be due to increased absorption and/or translocation as reported for α-methoxyindole-3-acetic acid.[18] However, no difference in stem curvature was noted with cucumber

[5] H. VELDSTRA, *Ann. Rev. Plant Physiol.* **4**, 151 (1953).
[6] K. V. THIMANN, *Plant Physiol.* **33**, 311 (1958).
[7] K. P. HELLMAN, H. M. SELL and S. H. WITTWER, *Phyton (Buenos Aires)* **17**, 11 (1961).
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[10] F. WIGHTMAN, *Can. J. Botany* **40**, 689 (1962).
[11] F. KOGL and D. KOSTERMANS, *Z. Physiol Chem.* **235**, 201 (1935).
[12] F. KOGL and B. VERKAAIK, *Z. Physiol. Chem.* **280**, 167 (1944).
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[14] B. A. M. HANSEN, *Botan. Notiser* **107**, 230 (1954).
[15] B. A. M. HANSEN, *Botan. Notiser* **107**, 318 (1954).
[16] M. J. BUKOVAC, K. K. SCHLENDER and H. M. SELL, *Nature* **202**, 617 (1964).
[17] F. MILLICH and E. I. BECKER, *J. Org. Chem.* **23**, 1096 (1958).
[18] J. W. MITCHELL and P. S. LINDER, *J. Agric. Fd. Chem.* **10**, 82 (1962).

TABLE 1. ABSORPTION CHARACTERISTICS OF THE α-ALKYLINDOLE-3-ACETIC ACIDS

Compound	Absorption maxima u.v.* mμ	NH† μ	C=O μ
IAA	281,290	2·96	5·90
α-Methyl-IAA	280,290	2·96	5·85
α-Ethyl-IAA	281,290	2·94	5·86
α-Propyl-IAA	280,290	2·92	5·90
α-Isopropyl-IAA	281,290	2·92	5·89

* Ethanol (95 per cent) was used as solvent.
† Spectra determined with KBr pellets.

TABLE 2. EFFECT OF α-ALKYLINDOLE-3-ACETIC ACIDS ON THE PROMOTION OF PARTHENOCARPIC TOMATO OVARY GROWTH

Compound	Molar concentration 10^{-4}	10^{-3}	2×10^{-3}	10^{-2}
IAA	150[a]	196[a]	168[a]	179[a]
α-Methyl-IAA	361[a]	681[b]	734[b]	628[c]
α-Ethyl-IAA	175[a]	621[b]	686[b]	617[c]
α-Propyl-IAA	150[a]	168[a]	179[a]	407[b]
α-Isopropyl-IAA	145[a]	127[a]	124[a]	110[a]

α Diameter of ovary expressed as per cent of control. Means with different superscripts differ significantly at $P = 0{\cdot}05$.

seedlings when IAA or α-methyl-IAA was applied to one of the cotyledonary leaves, but seedlings treated with IAA recovered more rapidly than those treated with α-methyl-IAA.[19] Perhaps the difference in response of intact plants is based on a differential rate of degradation or inactivation of the compounds by the plant tissue. Therefore, the ease of inactivation as well as primary activity, absorption, and translocation may all contribute to the observed activity. Degradation and inactivation may be of special importance when using intact plants and observing responses which occur over a relatively long period of time as in the case of parthenocarpic fruit growth.

There was no marked difference in promotion of coleoptile elongation between α-methyl-IAA, α-ethyl-IAA, and IAA (Fig. 1). Substitution of an α-hydrogen with a propyl group significantly reduced activity, while substitution with an isopropyl radical further reduced the activity significantly below that of α-propyl-IAA.

In root elongation studies, the biological activity followed a pattern similar to that of the *Avena*; namely IAA, α-methyl-IAA, and α-ethyl-IAA all gave responses typical of strong auxins[20] (Fig. 2). At 10^{-8} M there was a slight stimulation of root elongation and at higher

[19] K. K. SCHLENDER, M.S. Thesis, Michigan State University (1963).
[20] B. ABERG, *Physiol. Plantarum* **5**, 305 (1952).

concentrations (10^{-7}–10^{-4} M) root growth was inhibited. The activity of α-propyl-IAA was typical of a weak auxin.[20] This response was very similar to that obtained with α,α-dimethyl-IAA (synthesized by the method of Erdtman and Jonsson[21]), which was reported to be a weak auxin in the *Avena* assay[6] and to promote root elongation and reverse the root growth inhibition of IAA in wheat.[22] With α-propyl-IAA, inhibition of root elongation occurred only at concentrations of 10^{-4} and 10^{-5} M, and at 10^{-4} M with α,α-dimethyl-IAA (Fig. 2).

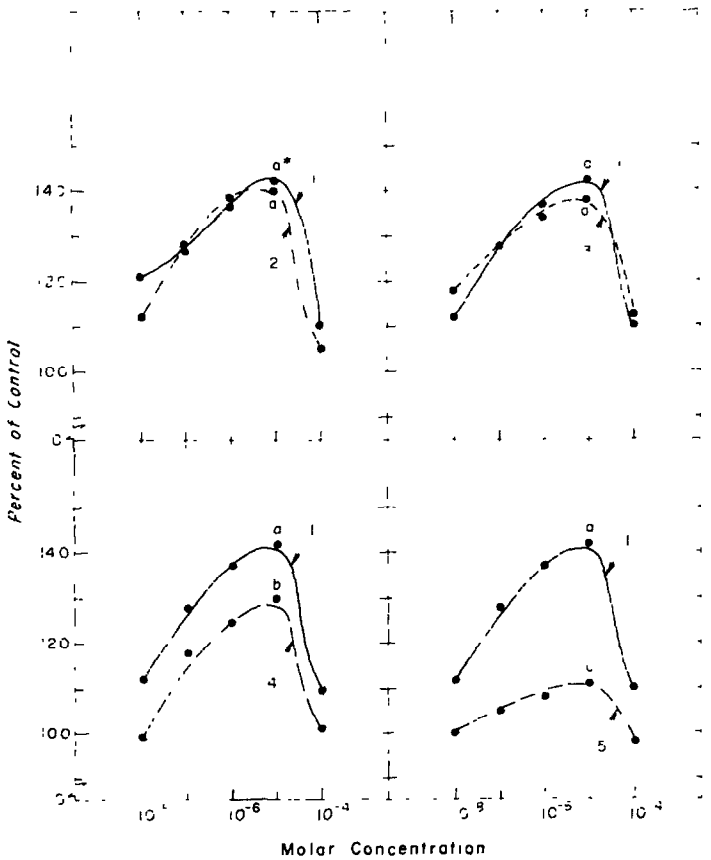

FIG. 1. EFFECT OF α-ALKYLINDOLE-3-ACETIC ACIDS ON THE ELONGATION OF *Avena* COLEOPTILES

Growth response curves are shown for the following treatments: (1) IAA, (2) α-Methyl-IAA, (3) α-Ethyl-IAA, (4) α-Propyl-IAA, (5) α-Isopropyl-IAA. * Points with different superscripts differ significantly at $P = 0{\cdot}05$.

At all lower concentrations, both compounds stimulated root elongation, reaching a maximum at the lowest concentration employed (10^{-8} M). Of special interest was the absence of either inhibition or stimulation of root growth at 10^{-8}–10^{-4} M with α-isopropyl-IAA.

Thimann[4] has emphasized the importance of a strong fractional positive charge on the nitrogen of the indole ring for auxin activity. The substitution of the various α-alkyl groups would be expected to have little effect on this charge. Millich and Becker[17] have noted that the NH stretching band of indole compounds shift to a higher frequency when electron

[21] H. ERDTMAN and A. JONSSON, *Acta Chem. Scand.* **8**, 119 (1954).
[22] H. BURSTROM, *Botan. Notiser* **108**, 400 (1955).

releasing substituents are located at the three position and to a lower frequency when the three position is substituted with electron attracting groups. Infrared data (Table 1) denotes no significant shift of the NH stretching band as the α-alkyl substituent was varied, indicating little if any change of the charge on the nitrogen.

Substitution with the α-alkyl groups may affect chemical and auxin activity through steric effects. A striking parallel was noted between the auxin activity of the α-alkyl derivatives and

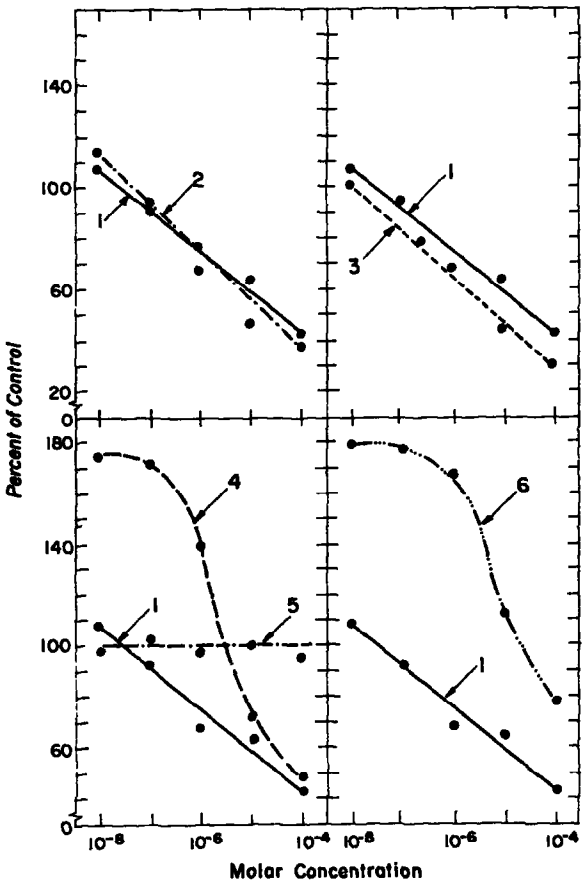

FIG. 2. EFFECT OF α-ALKYLINDOLE-3-ACETIC ACIDS ON THE ELONGATION OF BUCKWHEAT ROOTS.

Growth response curves are shown for the following treatments: (1) IAA, (2) α-Methyl-IAA, (3) α-Ethyl-IAA, (4) α-Propyl-IAA, (5) α-Isopropyl-IAA, (6) α,α-Dimethyl-IAA.

Taft E_s values.[23] Taft E_s values are a near quantitative measure of the total steric effect which can be associated with a substituent relative to a standard, in this case a methyl group.[24] Generally, decreasing E_s values parallel increasing van der Waals radii,[24] and refer to effects on rates of chemical reactions or to equilibriums that involve a transition state. These values

[23] R. W. TAFT, Jr., *J. Am. Chem. Soc.* 74, 3120 (1952).

[24] R. W. TAFT, Jr., In *Steric Effects in Organic Chemistry* (Edited by N. S. NEWMAN), p. 556. John Wiley, New York (1956).

compared with biological activity may not be an ideal analogy, since auxin may not be covalently bound at the active site. However, in a qualitative sense, they give some idea of the change in forces operating within a molecule when substituents are varied. Qualitatively, the E_s values of Taft and the physiological activity were proportional to each other in coleoptile elongation. With the exception of α-isopropyl-IAA in the buckwheat assay, the relative E_s values and physiological activity where, $CH_3 = C_2H_5 > C_3H_7 > iso\text{-}C_3H_7$. Therefore, it is highly probable that the influence of α-alkyl substitution on physiological activity which accompanied the increase in size of the α-substitution was due to steric hindrance preventing the molecule from fitting the active site in a way which allowed for optimum activity.

EXPERIMENTAL

The compounds were synthesized by condensing the appropriate 2-hydroxy-alkanoic acid with indole, in the presence of KOH following a procedure similar to that employed by Johnson and Crosby[25] for the synthesis of IAA. The reaction was carried out in a 300 ml high pressure stainless steel autoclave (Autoclave Engineers, Erie, Pa.). Absorption spectra in the u.v. region were obtained in 95% EtOH with a Beckman DK-2 spectrophotometer using a quartz cell of 1 cm light path, and in the i.r. region on KBr pellets with a Beckman IR-5 spectrophotometer. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan. All melting points reported are uncorrected.

D,L-α-*Methylindole*-3-*Acetic Acid*

To a mixture of 27·0 g (0·41 mole) of KOH and 35·1 g (0·30 mole) of indole, were added slowly 35·3 g (0·30 mole) of 70% aq. lactic acid, allowing the heat of neutralization to melt the indole. The autoclave was sealed and the contents stirred at 240–260° for 16 hr. The resultant mixture was cooled to 40°, 50 ml of water added, and then heated for 1 hr at 100° to dissolve the potassium salt. After cooling to room temperature, the reaction mixture was extracted twice with ethyl ether to remove the unreacted indole. The aqueous phase was acidified with conc. HCl. The product separated from the aqueous phase as a crude oil and was extracted twice with ethyl ether. The latter ether extracts were combined, treated with Norite A, and dried over $MgSO_4$. The ether was removed *in vacuo* and the product crystallized from benzene–light petroleum, yield 52 g (91 per cent of theory). After several recrystallizations from benzene–light petroleum, a constant melting point of 113–114° (Ref. 22 reports 111–112°) was obtained.

D,L-α-*Ethylindole*-3-*Acetic Acid*

Twenty g (0·19 mole) of 2-hydroxybutyric acid was added to a mixture of 16 g (0·24 mole) of KOH and 20·6 g (0·175 mole) of indole contained in the autoclave, sealed, and the contents treated as described above. The resulting brown oil was crystallized from benzene–light petroleum, yielding 15·4 g (43 per cent of theory) of white crystals, m.p. 106°. (Calcd. for $C_{12}H_{13}NO_2$: C, 70·90; H, 6·45; N, 6·90. Found: C, 71·03; H, 6·43; N, 6·81%).

D,L-α-*Propylindole*-3-*Acetic Acid*

A solution of 4 g (0·034 mole) of indole, 5 g (0·093 mole) of KOH in 25 ml of tetralin, and 4 g (0·033 mole) of 2-hydroxyvaleric acid was heated (230–250°) in a sealed autoclave for 18 hr and worked up as before to yield a crude oil. Crystallization from benzene–light petroleum yielded 4·5 g (63 per cent of theory) of white crystals, m.p. 88°. (Calcd. for $C_{13}H_{15}NO_2$: C, 71·89; H, 6·91; N, 6·45. Found: C, 71·78; H, 6·48; N, 6·35%).

[25] H. E. JOHNSON and D. G. CROSBY, *J. Org. Chem.* **28**, 1246 (1963).

D,L-α-*Isopropylindole*-3-*Acetic Acid*

Four g (0·034 mole) of indole, 5 g (0·093 mole) of KOH and 25 ml of tetralin were placed in the autoclave with 4 g (0·033 mole) of 2-hydroxyisovaleric acid (prepared by the basic hydrolysis of 2-bromoisovaleric acid, the crude 2-hydroxyisovaleric acid, was crystallized from cold chloroform, m.p. 83–85°; Ref. 26 reports 85°), and treated in a manner similar to that above. In this case, the product crystallized upon acidification with conc. HCl, and was recrystallized from benzene, giving 6·4 g (88 per cent of theory), m.p. 138°. (Calcd. for $C_{13}H_{15}NO_2$: C, 71·89; H, 6·91; N, 6·45. Found: C, 71·96; H, 6·89; N, 6·46%).

Biological Assays

Comparative activity in inducing parthenocarpy was evaluated by applying 10 μl of test solution (0, 10^{-4}, 10^{-3}, 2×10^{-3}, 10^{-2} M) to the ovaries of emasculated tomato flowers (*Lycopersicon esculentum* L. cv. Michigan-Ohio Hybrid). Three flowers of the first cluster of each of three plants were used for each treatment. Each plant represented a replication. Ovary diameter was measured after nine days. Analysis of variance was performed on the data and the means for chemicals at each concentration were compared for significance using Duncan's multiple range test.[27]

Effects on cell elongation were established with the coleoptile straight growth assay,[28] using *Avena sativa* (Cv. Torch). The compounds were dissolved for assay in citrate phosphate buffer solution (pH 5·0) containing 2% sucrose and 0·1% Tween 80. Ten coleoptiles, 4·5 mm in length, were placed in each test tube with 2 ml of the designated solution and incubated for 24 hr on a revolving drum (1 rev/min). Five replications were used for each treatment and the mean values were expressed as per cent of the length of coleoptiles incubated in buffer, sucrose, and Tween 80 solution. Statistical comparison among treatments were delineated at 10^{-5} M.[27]

For the buckwheat root assay, (*Fagopyrum esculentum* Cv. Japanese) seeds were sown, on porcelain plates covered with paper toweling, in culture dishes. After 24 hr in the dark at 31°, ten uniform seeds with a radicle approximately 5 mm in length were selected, and transferred to a filter paper in a Petri dish containing 4·5 ml of the desired test solution. Radical length was measured after 48 hr in the dark at 31°. Three replications were utilized for each treatment and the mean values were expressed as per cent of the control growth in distilled water.

[26] H. J. Backer and D. van der Veen, *Rec. Trav. Chim.* **55**, 885 (1936).
[27] D. B. Duncan, *Biometrics* **11**, 1 (1955).
[28] J. P. Nitsch and C. Nitsch, *Plant Physiol.* **31**, 94 (1956).