Phytochemistry, 1965, Vol. 4, pp. 461 to 466. Pergamon Press Ltd. Printed in England

THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF SEVERAL ETHYL 1-ACYLINDOLE-3-ACETATES*

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(*Received* 23 *September* 1964)

Abstract—Several ethyl 1-acylindole-3-acetates have been synthesized and their biological activities in three plant systems ascertained. The 1-dichloroacetyl derivative was as active as the parent ester in the tomato ovary growth assay. The 2-chloropropionyl, 3-chloropropionyl, 4-nitrobenzoyl, and 4-aminobenzoyl derivatives were least active. The acetyl and chloroacetyl derivatives were of intermediate activity. The dichloroacetyl radical increased the activity of the parent ester as measured by delay in abscission of debladed bean petioles. The acetyl, chloroacetyl, and nitrobenzoyl compounds were less active, but more active than the parent compound. The 2-chloropropionyl, 3-chloropropionyl, and 4-aminobenzoyl derivatives were no more active than the parent ester and acid. All of the 1-acyl derivatives, except the acetyl, were as active in the *Avena* straight growth assay as both indole-3-acetic acid and ethyl indole-3-acetate over the range of concentrations used. Ethyl 1-acetylindole-3-acetate was of equivalent activity only at 1×10^{-5}M. The possible implications of these results in the concepts of structural requirements for auxin activity are discussed.

INTRODUCTION

SINCE the observation by Kögl[1] that IAA induced cell elongation, numerous derivatives of IAA have been assayed in an attempt to relate chemical structure to biological activity. Based on such studies, certain minimum structural requirements have been found to be necessary for activity as an auxin.

The aromatic ring and a carboxyl group with definite spatial character have been recognized[2–4] as requirements for auxin activity, especially in the straight growth assays. The absence of substitution immediately adjacent to the side chain has been related to activity in the phenoxy acid series and the indoleacetic acid series.[5–8] Thimann[9] has suggested that the structural requirements for activity are not adequately described by any of the earlier concepts

* Published with the approval of the Director of Michigan Agricultural Experiment Station as Journal Article No. 3415. This work was supported by a research grant from the National Science Foundation (GB-284).

† Portions were taken from a thesis submitted in partial fulfilment of the requirements for the degree of M.S., Michigan State University, 1961, by Roger W. Ritzert.

Abbreviations used: indole-3-acetic acid, IAA; ethyl indole-3-acetate, EIA; and ethyl 1-acylindole-3-acetates named as derivatives of EIA using the acyl radical and EIA, e.g. ethyl 1-acetylindole-3-acetate, acetyl-EIA.

[1] F. KÖGEL, A. J. HAAGEN-SMIT and H. ERXLEBEN, *Z. Physiol. Chem.* **228**, 90 (1934).
[2] J. B. KOEPFLI, K. V. THIMANN and F. W. WENT, *J. Biol. Chem.* **122**, 763 (1938).
[3] H. VELDSTRA, *Enzymologia*, **11**, 97 (1942).
[4] H. VELDSTRA and H. L. BOOIJ, *Biochim. Biophys. Acta*, **3**, 278 (1949).
[5] R. M. MUIR, C. H. HANSCH and A. H. GALLUP, *Plant Physiol.* **24**, 359 (1949).
[6] C. HANSCH and R. M. MUIR, *Plant Physiol.* **25**, 389 (1950).
[7] C. HANSCH, R. M. MUIR and R. L. METZENBERG, JR., *Plant Physiol.* **26**, 812 (1951).
[8] R. M. MUIR and C. HANSCH, *Plant Physiol.* **28**, 218 (1953).
[9] K. V. THIMANN, *Plant Physiol.* **33**, 311 (1958).

and further emphasized the importance of applying these requirements only to the specific assay involved.

Recently the spatial distance between the anionic charge of the carboxyl group (or any anionic charge) and a fractional positive charge on the nucleus, located on the nitrogen atom in the case of indole, has been shown to be essential for biological activity.[10] This has been supported[11] through the assay of several analogs differing in the distance separating the two charges and in their intensity. These results indicate that the degree of positive charge on the nitrogen should be reflected in the growth activity of the compound.

Few derivatives of IAA in which the nitrogen atom is substituted have been evaluated, and these only in a few biological systems. In each case substitution on the nitrogen atom was reported to decrease activity of the parent compound. 1-Methylindole-3-acetic acid was 80 per cent less active in the *Avena* straight growth assay,[3] and 1-benzoylindole-3-acetic acid was less active in the tomato ovary assay than IAA.[12] Methyl 1-acetylindole-3-acetate applied to stems of bean plants caused less curvature than did methyl indole-3-acetate.[13]

We have synthesized several 1-acylindole-3-acetates and determined their biological activity in the *Avena* straight growth, tomato ovary growth, and petiole abscission assays. The synthesis of the acyl derivatives and effects of acyl substitution on the nitrogen atom of EIA on biological activity are herein described.

RESULTS AND DISCUSSION

Acetyl-EIA, chloroacetyl-EIA, dichloroacetyl-EIA, 2-chloropropionyl-EIA, 3-chloropropionyl-EIA, 4-nitrobenzoyl-EIA, and 4-aminobenzoyl-EIA were synthesized, and in addition to elemental analyses, the structures are supported by the absorption spectra in both the u.v. and i.r. regions (Table 1). In the i.r., the absence of NH absorption at 2·90 μ, and the addition of an amide carbonyl as shown by an absorption band between 5·86 μ and 6·02 μ show that the structures as postulated are correct.

EIA was more active than IAA in the tomato ovary growth assay (Table 2), and confirmed previously published results.[12, 14] Acetyl-EIA and chloroacetyl-EIA were less active than EIA and equal in activity to IAA; however, dichloroacetyl-EIA was more active than IAA and equal in activity to EIA. Replacement of the imino hydrogen on EIA with 2- and 3-chloropropionyl, or *p*-amino- and *p*-nitro-benzoyl groups resulted in a complete loss of biological activity.

It would appear that the acyl compounds are active without prior deacylation, unless such a reaction is highly specific. The observed activity of these substances may be dependent upon the nature of the substituent and its ability to substitute for hydrogen on the indole nitrogen.

The relative effectiveness of the EIA derivatives in delaying petiole abscission is also recorded in Table 2. EIA was more active than IAA, but dichloroacetyl-EIA was more active than any of the compounds synthesized. The acetyl, dichloroacetyl, and 4-nitrobenzoyl derivatives were less active, but more active than the parent compound, EIA. The two chloropropionyl derivatives and the 4-aminobenzoyl derivative were less active, but of the same order of activity as the parent acid and ester.

[10] W. L. PORTER and K. V. THIMANN, *Abstr. 9th Int. Botan. Congr., Montreal*, 305 (1959).
[11] W. L. PORTER and K. V. THIMANN, *Plant Physiol.* **36**, (Suppl.) XXXIX (1961).
[12] H. M. SELL, S. H WITTWER, T. L. REBSTOCK and C. T. REDEMANN, *Plant Physiol.* **28**, 481 (1953)
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TABLE 1. ABSORPTION SPECTRA OF THE ETHYL 1-ACYLINDOLE-3-ACETATES

Compound	Absorption maxima			
		I.R. (μ)‡		
	U.V. (mμ)*	NH	Ester C=O	Amide C=O
EIA	274, 280, 290	2·90	5·76	—
Acetyl-EIA	258, 277†	—	5·81	5·93
Chloroacetyl-EIA	245, 290, 300	—	5·78	5·86
Dichloroacetyl-EIA	250, 291, 304	—	5·79	5·88
2-Chloropropionyl-EIA	241, 292, 301	—	5·75	5·86
3-Chloropropionyl-EIA	248, 266, 292, 300	—	5·74	5·85
4-Nitrobenzoyl-EIA	260, 290†	—	5·82§	6·00§
4-Aminobenzoyl-EIA	251, 321	—	5·81§	6·02§

* In 95% ethanol.
† = shoulder.
‡ In toluene, corrected by employing a variable path cell.
§ Nujol mulls.

TABLE 2. EFFECT OF THE 1-ACYLINDOLE-3-ACETATES ON TOMATO OVARY GROWTH AND BEAN PETIOLE ABSCISSION

Compound, 1×10^{-3} M in lanolin	Tomato ovary growth*	Bean petiole abscission†
Control	3·3 f	100
IAA	5·3 de	123
EIA	7·7 ab	138
Acetyl-EIA	5·7 cd	162
Chloroacetyl-EIA	5·3 de	169
Dichloroacetyl-EIA	7·2 bc	200
2-Chloropropionyl-EIA	3·8 ef	123
3-Chloropropionyl-EIA	3·7 ef	115
4-Nitrobenzoyl-EIA	3·7 ef	154
4-Aminobenzoyl-EIA	3·3 f	123

* Diameter of ovary (in millimeters) was measured 4 days after treatment. The mean values were compared. Those followed by the same letter or letters are not significantly different at the 5% level.
† Activity, expressed as per cent of control, is time required for abscission of the debladed petiole after treatment.

All of the derivatives were active in the *Avena* straight growth assay (Fig. 1) and, with the exception of acetyl-EIA, were approximately equal to both IAA and EIA. Acetyl-EIA was of comparable activity only at 1×10^{-5} M. We did not observe the enhanced activity of EIA over IAA reported by Nitsch and Nitsch.[15] The two chloroacetyl derivatives showed optimum activity at 1×10^{-6} M, while the other derivatives were most active at the highest concentration used, 1×10^{-5}.

[15] J. P. NITSCH and C. NITSCH, *Plant Physiol.* 31, 94 (1956).

Porter and Thimann[10, 11] have suggested that a strong fractional cationic charge of the heterocyclic nitrogen atom combined with an anionic charge which is of a desirable distance from it (5·5 Å) may be a criterion for activity in the pea curvature test for the indolic auxins. This does not necessarily suggest that the heterocyclic nitrogen must be non-substituted, but that the integrity of the cationic nature of the nitrogen must be maintained. It has been observed[16] that 1-methylindole-3-acetic acid has a growth response curve similar to that reported here for acetyl-EIA. The inductive effect of the methyl radical and methyl group of

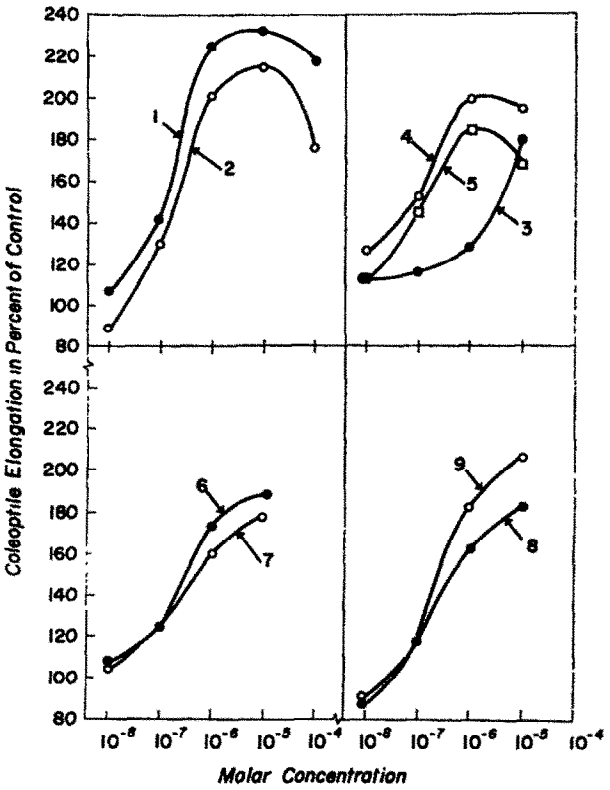

FIG. 1. EFFECT OF THE ETHYL 1-ACYLINDOLE-3-ACETATES ON THE GROWTH OF *Avena* COLEOPTILES. Growth response curves are shown for the following treatments: (1) IAA, (2) EIA, (3) Acetyl-EIA, (4) chloroacetyl-EIA, (5) dichloroacetyl-EIA, (6) 2-chloropropionyl-EIA, (7) 3-chloropriopionyl-EIA, (8) 4-nitrobenzoyl-EIA, (9) 4-aminobenzoyl-EIA. Assay methods were as described in the text.

the acetyl radical thus appear to be comparable in their effect on the observed activity. The remaining acyl substituents are all electron withdrawing and comparable to hydrogen in maintaining the necessary charge. The observed activity for acetyl-EIA suggests that the amides are not hydrolyzed during the course of the assay.

EXPERIMENTAL

Ultraviolet spectra were obtained from ethanol solutions of the compounds using a Beckman Model DK-2 spectrophotometer. Infrared spectra were determined on either toluene

[16] R. W. RITZERT, Unpublished results.

solutions or Nujol mulls of the compounds with a Beckman Model IR-5 spectrophotometer. The absorption spectra are summarized in Table 1. Microanalytical analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan. All melting points are uncorrected. Ethyl indole-3-acetate was prepared according to the methods of Nametkin *et al.*[17] or Jackson.[18]

Ethyl 1-*Acetylindole*-3-*acetate*

To a solution of 15·9 g (0·1 mole) of 1-acetylindole in 30 ml of dry benzene under reflux was added a small quantity of cuprous chloride followed by the dropwise addition of 11·4 g (0·1 mole) of ethyl diazoacetate[19] in 10 ml of benzene at a rate necessary for a constant evolution of nitrogen gas. After 3 hr, the mixture was cooled, filtered, and the benzene removed *in vacuo*. The residual oil was distilled at 164–167° and 0·6 mm. The distillate upon redistillation gave 2·7 g of yellow oil, b.p. 142–147° and 0·3 mm. The product was dissolved in ethyl ether-hexane, cooled, and the supernatant separated from the resulting yellow oil. The supernatant liquor upon cooling at 0° gave 1 g of colorless crystals, m.p. 90°. (Found: C, 68·50; H, 6·12; N, 5·73. Calc. for $C_{14}H_{15}NO_3$: C, 68·55; H, 6·16; N, 5·71%.)

Ethyl 1-*Chloroacetylindole*-3-*acetate*

To a hot solution of 4·06 g (0·02 mole) of EIA in 15 ml of dry benzene, a solution of 2·3 g (0·02 mole) of chloroacetyl chloride in 10 ml of benzene was added dropwise. The mixture was then refluxed for 24 hr, cooled, and the benzene removed *in vacuo*. The residue was dissolved in ethanol-ethyl ether and crystallized upon cooling. Recrystallization from ethanol gave 2·15 g of colorless needles, m.p. 118–119°. (Found: C, 59·66; H, 5·19; N, 4·87. Calc. for $C_{14}H_{15}ClNO_3$: C, 60·11; H, 5·04; N, 5·00%.)

Ethyl 1-*Dichloroacetylindole*-3-*acetate*

A solution of 4·06 g (0·02 mole) of EIA was dissolved in 25 ml of dry toluene and the solution brought to reflux. The dropwise addition of 2·95 g (0·02 mole) of dichloroacetyl chloride was begun and the mixture refluxed for 30 hr. The mixture was cooled and the toluene removed under vacuum. The residue was dissolved in ethanol, treated with Norite A, and filtered. The product was precipitated by the addition of water and cooling. Recrystallization from ethanol gave 2·65 g of colorless needles, m.p. 74°. (Found: C, 53·91; H, 4·28; N, 4·32. Calc. for $C_{14}H_{13}Cl_2NO_3$: C, 53·32; H, 4·17; N, 4·45%.)

Ethyl 1-(2-*Chloropropionyl*)*indole*-3-*acetate*

Similar treatment of 4·06 g (0·02 mole) of EIA with 2·6 g (0·02 mole) of 2-chloropropionyl chloride in boiling dry toluene for 48 hr gave a residue which was dissolved in hexane-ethanol, and the solution placed in the cold to obtain the product. Recrystallization from ethanol yielded 1·04 g of light yellow platelets, m.p. 63°. (Found: C, 61·21; H, 5·42; N, 4·77. Calc. for $C_{15}H_{16}ClNO_3$: C, 61·33; H, 5·49; N, 4·76%.)

Ethyl 1-(3-*Chloropropionyl*)*indole*-3-*acetate*

In the same way 4·06 g (0·02 mole) of EIA and 2·6 g (0·02 mole) of 3-chloropropionyl chloride in boiling toluene for 48 hr., gave a residue which was crystallized from ethanol–

[17] S. S. NAMETKIN, N. N. MEL'NIKOV and K. S. BOKAREV, *Zhur. Pricklad. Khim.* **29**, 459 (1956).
[18] R. W. JACKSON, *J. Biol. Chem.* **88**, 659 (1930).
[19] L. B. LAFORGE, N. A. GERSDORFF, N. GREEN and M. S. SCHECHTER, *J. Org. Chem.* **17**, 381 (1952).

water at 0°. The product upon recrystallization from ethanol gave 1·6 g of colorless needles, m.p. 75 . (Found: C, 61·89; H, 5·28; N, 4·97. Calc. for $C_{15}H_{16}ClNO_3$: C, 61·33; H, 5·49; N, 4·76%.)

Ethyl 1-(4-*Nitrobenzoyl*)*indole*-3-*acetate*

In this case 10 g of EIA was acylated with 9·2 g of 4-nitrobenzoyl chloride in toluene for 48 hr under reflux. The solvent was removed *in vacuo* and the residue cooled at 0 . The resulting solid material was taken up in absolute ethanol leaving a mass of yellow crystals (6·5 g) which were filtered, dried, and recrystallized from absolute ethanol, m.p. 114–115 . (Found: C, 64·56; H, 4·41; N, 8·04. Calc. for $C_{19}H_{16}O_5N_2$: C, 64·77; H, 4·58; N, 7·95%.)

Ethyl 1-(4-*Aminobenzoyl*)*indole*-3-*acetate*

A suspension of 300 mg of palladium on charcoal (5%) in 40 ml of 85% ethanol in a vessel fitted with a gas dispersion tube, cold finger, and outlet was activated by the addition of hydrogen gas for 15 min at 70 . Hydrogen was then passed through the mixture for 1 hr after the addition of 1 g of ethyl 1-(4-nitrobenzoyl)indole-3-acetate. The mixture was filtered and the filter cake washed with 40 ml of hot ethanol. The combined washings and filtrate yielded white crystals. Additional product was obtained upon concentration of the mother liquor. The combined crystals were recrystallized from absolute ethanol, yielding 410 mg, m.p. 170·2–172 . (Found: C, 70·89; H, 5·53; N, 8·88. Calc. from $C_{19}H_{18}O_3N_2$: C, 70·79; H, 5·63; N, 8·69%.)

Biological Assays

Comparative parthenocarpic activity was determined by applying quantities of a 1×10^{-3} M lanolin suspension of each chemical to ovaries from emasculated tomato flowers. Three flowers of the first cluster of three plants were used for each chemical. Ovary diameter was measured after 4 days. The data were subjected to an analysis of variance and comparison between treatment means was performed as suggested by Duncan.[20]

The abscission assay was performed by applying equal quantities of lanolin solutions (1×10^{-3} M) of the appropriate chemical to the petiolar stub of one of the primary leaves of recently debladed bean (cv. Contender) plants. Two plants were used for each treatment and each treatment was replicated four times. Time from treatment to abscission of the petiole stub was recorded and expressed as percent of control.

The coleoptile cylinder test described by Nitsch and Nitsch[15] was employed as the straight growth assay, using *Avena* (var. Torch) seeds. The initial length of the coleoptiles was 4·5 mm. Solubility of the compounds was facilitated by using 0·1% Tween 80 in phosphate-citrate buffer solution (pH 5·0). The mean of three replicates of ten sections each were used to determine the elongation.

Acknowledgement—The authors wish to express appreciation to Mrs. Carolyn Titus for her technical assistance during the course of this work.

[20] D. B. DUNCAN, *Biometrics* **11**, 1 (1955).