The Chemistry of Plant-growth Regulators. Part I. 2 : 4-Dichloro-6-hydroxyphenoxyacetic Acid and Related Compounds.

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Synthesis of 2 : 4-dichloro-6-hydroxyphenoxyacetic acid and of its methyl ether is described. The lack of growth-regulating properties of these compounds is briefly discussed in relation to some current theories of the mode of action of 2 : 4-dichlorophenoxyacetic acid (2 : 4-D). The direct nitration of 2 : 4-D is re-investigated.

Aryloxyalkanecarboxylic acids are important synthetic plant growth-regulators, particularly 2 : 4-di- and 2 : 4 : 5-tri-chlorophenoxyacetic acids. However, when chlorine or bromine is substituted in both the 2- and the 6-position, as in 2 : 6-di- and 2 : 4 : 6-tri-chlorophenoxyacetic acid, activity is lost. This apparent necessity for one free *ortho*-position has led to numerous hypotheses. Muir *et al.* (*Plant Physiol.*, 1949, **24**, 359; 1951, **26**, 369) suggested that 2 : 4-dichlorophenoxyacetic acid (2 : 4-D) reacts with a plant substrate in the *ortho*-position, Leaper and Bishop (*Bot. Gazz.*, 1951, **112**, 250) postulated oxidation to a *p*-quinone, and Holly, Boyle, and Hand (*Arch. Biochem.*, 1950, **27**, 143) believe nuclear hydroxylation (position unspecified) may occur during the metabolism of 2 : 4-D.

The synthesis of 2 : 4-dichloro-6-hydroxyphenoxyacetic acid is now reported and the preliminary testing of this compound discussed in relation to the above hypotheses.

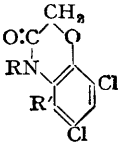
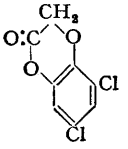
Although synthesis *via* 2 : 4-dichloro-6-nitrophenoxyacetic acid has not proved possible, it has led to an investigation of the nitration of 2 : 4-D. 2 : 4-Dichloro-6-nitrophenol could not be converted into the phenoxyacetic acid by known procedures (cf. Jacobs and Heidelberger, *J. Amer. Chem. Soc.*, 1917, **39**, 2191). Ultimately the sodium salt of the phenol was condensed with *n*-butyl chloroacetate in diethylene glycol, to give *n*-butyl 2 : 4-dichloro-6-nitrophenoxyacetate. Direct nitration of 2 : 4-D has been stated to give only 5-nitro-acid (Mahler, Speer, and Roberts, *Science*, 1949, **110**, 562; Wolfe, Wood, Klipp, Fontaine, and Mitchell, *J. Org. Chem.*, 1949, **14**, 900); our nitration with an excess of cold concentrated mixed acid gave largely the 5-nitro-acid, together with the 5 : 6-dinitro-acid and a small yield of the 6-nitro-acid.

The structure of the 6-nitro-compound was proved by reduction with ferrous hydroxide to the cyclic lactam (I; R = R′ = H) [cf. Jacobs and Heidelberger (*J. Amer. Chem. Soc.*, 1917, **39**, 1435) for reduction of *o*-nitrophenoxyacetic acid], which could not be hydrolysed to 6-amino-2 : 4-dichlorophenoxyacetic acid. Reduction of *n*-butyl 2 : 4-dichloro-6-nitrophenoxyacetate with hydrogen in the presence of Raney nickel also gave the lactam, butanol being eliminated. In contrast, hydrogenation with a palladium–charcoal catalyst gave a product, $C_8H_5O_3NCl_2$, containing one extra oxygen atom. This compound, soluble in concentrated sodium hydroxide solution (being immediately reprecipitated on addition of mineral acid) and insoluble in sodium hydrogen carbonate solution, reduces hot Fehling's solution but does not react with ammoniacal silver nitrate solution. It gives an intense wine red colour with alcoholic ferric chloride solution characteristic of hydroxamic acids and forms an apple-green copper derivative on treatment with cupric acetate in alcohol. Confirmation of the cyclic hydroxamic acid structure (I; R = OH; R′ = H) is afforded by reduction with zinc and hydrochloric acid to the lactam (I; R = R′ = H). Reduction with zinc in acetic acid gives both the lactam and the hydroxamic acid; in sulphuric acid it gives only the lactam.

The ready formation of insoluble lactams has been used to prove the structure of the 5 : 6-dinitro-acid. This acid is also formed on nitration of 2 : 4-dichloro-5-nitrophenoxyacetic acid, and as it is reduced with zinc and hydrochloric acid to a lactam (I; R = H, R′ = NH_2), the second nitro-group is in position 6.

An alternative route to 2 : 4-dichloro-6-hydroxyphenoxyacetic acid starting from 3 : 5-dichlorocatechol was next investigated. The 2-hydroxyl group of the dichlorocatechol

should be the more acidic, hence the catechol was converted into a monosodium salt and treated with ethyl chloroacetate. Only one monohydroxydichlorophenoxyacetate was isolated, together with 3 : 5-dichloro-1 : 2-di(ethoxycarbonylmethoxy)benzene and some unchanged dichlorocatechol. The dichlorohydroxyphenoxyacetic acid, obtained on hydrolysis of the ester, gives a light blue colour with alcoholic ferric chloride solution and couples

(I)  (II) 

with diazotised sulphanilic acid. It gives a positive Gibb's test (pale blue) indicating that the *para*-position to the hydroxyl group is free, whilst additional evidence of an unsubstituted *ortho*-position (relative to hydroxyl) is given by nitration and reduction, the product not forming a lactam. The hydroxy-derivative is thus the required 2 : 4-dichloro-6-hydroxyphenoxyacetic acid, and was characterised as the methyl ether and by cyclisation to the lactone (II).

Preliminary testing for plant-growth-regulating properties by spraying of a 0·1% solution of sodium 6-hydroxy-2 : 4-dichlorophenoxyacetate on mature tomato plants gave no detectable activity whilst a 0·1% solution of 2 : 4-D showed extreme epinasty, twisting, and eventually death. No significant reduction in elongation of the primary maize root was observed with the 6-hydroxy- or 6-methoxy-2 : 4-dichlorophenoxyacetic acids, even at ten times the strength effective for 2 : 4-D. On the contrary there was a small but highly significant increase in root elongation over that shown by distilled-water controls. These results suggest that hydroxylation in position 6 is not an essential step in the production of an active metabolite from 2 : 4-D; whilst not conclusive, such observations also render the quinone hypothesis less likely. Wain [" Plant Growth Substances," Roy. Inst. Chem. (London), 1953] briefly records that 2 : 4-dichloro-6-fluorophenoxyacetic acid possesses high activity in the Went pea test, and such a result suggests that a free *ortho*-position may not be essential for activity in the aryloxyalkanecarboxylic acids.

Experimental

Nitration of 2 : 4-*Dichlorophenoxyacetic Acid.*—2 : 4-Dichlorophenoxyacetic acid (110·5 g., 0·5 mole) was dissolved in concentrated sulphuric acid (300 ml.) at 0—5° and concentrated nitric acid (50 ml.) was added during 30 min. with vigorous stirring. The amber mixture was poured on ice and the yellow solid, so precipitated, was filtered off, well washed with water, and air-dried. Fractional crystallisation from benzene–light petroleum (b. p. 60—80°) gave the 2 : 4-dichloro-5-nitrophenoxyacetic acid (97 g.) as pale yellow prisms, m. p. 158·5—159° (Found : C, 36·3; H, 1·85; N, 5·2. Calc. for $C_8H_5O_5NCl_2$: C, 36·1; H, 1·9; N, 5·3%), and some 2 : 4-dichloro-6-nitrophenoxyacetic acid (3·0 g.), m. p. 128° alone or mixed with the acid (m. p. 128—128·5°) prepared from 2 : 4-dichloro-6-nitrophenol. The residues from this crystallisation were taken up in aqueous ethanol; some esterification occurred and an ethyl ester separated as colourless needles (1·0 g.), m. p. 84—85°. Hydrolysis gave 2 : 4-*dichloro*-5 : 6-*dinitrophenoxyacetic acid*, small yellow prisms, m. p. 185° (from ethanol) (Found : C, 30·8; H, 1·45; N, 8·8. $C_8H_4O_7N_2Cl_2$ requires C, 30·85; H, 1·3; N, 9·0%). The mother-liquors on further crystallisation yielded *ethyl* 2 : 4-*dichloro*-5-*nitrophenoxyacetate*, colourless plates, m. p. 95—97° (from ethanol) (Found : C, 40·5; H, 3·0; N, 4·8. $C_{10}H_9O_5NCl_2$ requires C, 40·8; H, 3·1; N, 4·8%). Hydrolysis gave the 5-nitro-acid, m. p. and mixed m. p. 159—160°.

Nitration of 2 : 4-*Dichloro*-5-*nitrophenoxyacetic Acid.*—The 5-nitro-acid (2·0 g.) in concentrated sulphuric acid (10 ml.) was treated with concentrated nitric acid (2 ml.) at 30°. Isolation, as above, gave 2 : 4-dichloro-5 : 6-dinitrophenoxyacetic acid as pale yellow prisms (1·29 g.), m. p. and mixed m. p. 185° (from ethanol).

2 : 4-*Dichloro*-6-*nitrophenoxyacetic Acid.*—2 : 4-Dichloro-6-nitrophenol (52 g., 0·25 mole) in absolute alcohol (500 ml.), on addition of sodium ethoxide solution (5·75 g. of sodium in 500 ml. of alcohol) yielded the bright red salt. This mixture, after 6 hours' refluxing with *n*-butyl chloroacetate (38·8 g., 0·25 mole), contained no chloride ion. Diethylene glycol (200 ml.) was then added and the ethanol removed by slow distillation (to 150°), the red salt gradually dissolv-

ing and sodium chloride being precipitated. The crude butyl ester separated as a yellow oil when the mixture was poured into water. Ether-extraction and removal of unchanged phenol with sodium carbonate solution gave n-*butyl* 2 : 4-*dichloro*-6-*nitrophenoxyacetate* (40 g.) as a yellow viscous oil, b. p. 182—184°/2·0 mm. (Found : C, 44·8; H, 3·9. $C_{12}H_{13}O_5NCl_2$ requires C, 44·7; H, 4·1%). Hydrolysis gave the *acid*, pale yellow needles, m. p. 128—128·5° (from benzene) (Found : C, 35·9; H, 1·9; N, 5·1. $C_8H_5O_5NCl_2$ requires C, 36·1; H, 1·9; N, 5·3%).

6 : 8-*Dichloro*-3 : 4-*dihydro*-3-*oxobenzoxazine* (I; R = R′ = H; Ring Index numbering).—2 : 4-Dichloro-6-nitrophenoxyacetic acid (5·3 g.) in boiling water, (500 ml.) was treated with ferrous sulphate solution (98 g. of the heptahydrate in 500 ml. of water), and sodium hydroxide solution (28 g. in 100 ml. of water) slowly added according to Jacobs and Heidelberger's general procedure (*loc. cit.*). The *oxazine* was isolated as colourless needles (4·0 g.), m. p. 268° (from ethanol) (Found : C, 44·2; H, 2·25. $C_8H_5O_2NCl_2$ requires C, 44·1; H, 2·3%). The lactam is insoluble in 10N-hydrochloric acid but soluble in 2N-sodium hydroxide, being reprecipitated on acidification.

Catalytic Reduction of n-*Butyl* 2 : 4-*Dichloro*-6-*nitrophenoxyacetate*.—(*a*) The butyl ester (3·2 g.) in 95% ethanol (100 ml.) was shaken at room temperature and pressure with Raney nickel (pH of solution 7·5—8·0) until no further hydrogen was taken up (85% absorption). From the solution, which gave no colour with alcoholic ferric chloride solution, the insoluble lactam (I; R = R′ = H) was soon precipitated as colourless needles (1·8 g.), m. p. 268—270° (from ethanol). Butanol was detected in the filtrate.

(*b*) In an analogous reduction of the butyl ester (3·2 g.), with 10% palladium–" Norit " only 70% of the theoretical volume of hydrogen was absorbed. After removal of catalyst and solvent, 6 : 8-*dichloro*-3 : 4-*dihydro*-4-*hydroxy*-3-*oxobenzoxazine* (I; R = OH, R′ = H) crystallised as colourless needles (2·4 g.), m. p. 229—230° (from ethanol) (Found : C, 41·1; H, 2·1; N, 5·8. $C_8H_5O_3NCl_2$ requires C, 41·0; H, 2·15; N, 6·0%). It gives an intense wine-red colour with alcoholic ferric chloride, and is reduced quantitatively to the lactam (I; R = R′ = H), m. p. and mixed m. p. 269—270°, by zinc and hydrochloric acid.

5-*Amino*-6 : 8-*dichloro*-3 : 4-*dihydro*-3-*oxobenzoxazine* (I; R = H, R′ = NH_2).—2 : 4-Dichloro-5 : 6-dinitrophenoxyacetic acid (25 mg.), on reduction with zinc dust in warm 5N-hydrochloric acid solution, gave an immediate precipitate of the 5-*amino-lactam*, isolated as colourless needles (17·5 mg.), m. p. 325° (decomp.) (from ethanol) (Found : C, 41·2; H, 2·55; N, 11·9. $C_8H_6O_2N_2Cl_2$ requires C, 41·2; H, 2·6; N, 12·0%). Acetylation (sodium acetate–acetic anhydride) gave a *diacetyl* derivative which crystallised as almost colourless plates, m. p. 227—228°, from ethanol (Found : C, 45·6; H, 3·0; N, 8·8. $C_{12}H_{10}O_6N_2Cl_2$ requires C, 45·5; H, 3·15; N, 8·8%).

2 : 4-*Dichloro*-6-*hydroxyphenoxyacetic Acid*.—The 3 : 5-dichlorocatechol (Dakin, *Amer. Chem. J.*, 1909, **42**, 477) (8·0 g.; m. p. 85—86°) was added to a solution of sodium (1·15 g.) in butanol (60 ml.) under an atmosphere of hydrogen. Sodium chloride was precipitated from the pale yellow solution on addition of *n*-butyl chloroacetate (8·0 g.) during 2 hr. Direct alkaline hydrolysis of the reaction mixture, followed by steam-distillation to remove butanol, gave colourless crystals after acidification. Unchanged dichlorocatechol (1·5 g.) was recovered by repeated extraction of a sodium hydrogen carbonate solution of these phenoxyacetic acids with ether until the ethereal extracts no longer gave a positive ferric chloride test. The mixed acids (9·0 g.), obtained from the sodium hydrogen carbonate solution, were separated by repeated crystallisation from water. 2 : 4-*Dichloro*-6-*hydroxyphenoxyacetic acid* was isolated as colourless needles (3·1 g.), m. p. 132° (Found : C, 40·45; H, 2·5. $C_8H_6O_4Cl_2$ requires C, 40·5; H, 2·55%). The *methyl ester*, prepared in the normal manner, crystallised as colourless needles, m. p. 91°, from light petroleum (Found : C, 42·9; H, 3·15. $C_9H_8O_4Cl_2$ requires C, 43·1; H, 3·2%). Evaporation of the aqueous filtrate from the 6-hydroxy-acid gave 3 : 5-*dichloro*-1 : 2-*di*(*ethoxycarbonylmethoxy*)*benzene* (1·1 g.) which finally crystallised as rosettes of colourless needles, m. p. 189—191°, from benzene (Found : C, 40·6; H, 2·65. $C_{10}H_8O_6Cl_2$ requires C, 40·7; H, 2·7%).

2 : 4-*Dichloro*-6-*methoxyphenoxyacetic Acid*.—The 6-hydroxy-acid (20 mg.) was treated with an excess of methyl sulphate in 2N-sodium hydroxide, and the colourless oil formed immediately hydrolysed, to yield the 2 : 4-*dichloro*-6-*methoxyphenoxyacetic acid*, crystallising as colourless needles (20 mg.), m. p. 176°, from benzene (Found : C, 43·1; H, 3·2. $C_9H_8O_4Cl_2$ requires C, 43·1; H, 3·2%).

Cyclisation of 2 : 4-*Dichloro*-6-*hydroxyphenoxyacetic Acid*.—The 6-hydroxy-acid (50 mg.) was refluxed for 2 hr. with acetic anhydride (2 ml.). Excess of anhydride was removed under reduced pressure, to give the 5 : 7-*dichloro*-2-*oxobenzo*-1 : 4-*dioxan* (II) (40 mg.), isolated as

colourless prisms, m. p. 134—134·5°, from benzene–light petroleum (Found: C, 44·0; H, 1·95. $C_8H_4O_3Cl_2$ requires C, 43·9; H, 1·85%). This compound is insoluble in sodium hydrogen carbonate solution, but easily dissolves in warm 2N-sodium hydroxide.

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