209. *Fungal Detoxication. Part VII.* Metabolism of 2,4-Dichlorophenoxyacetic and 4-Chloro-2-methylphenoxyacetic Acids by* Aspergillus niger.

By J. K. Faulkner and D. Woodcock.

In the metabolism of 2,4-dichlorophenoxyacetic acid by *Aspergillus niger* (replacement culture technique) the major product is 2,4-dichloro-5-hydroxyphenoxyacetic acid; a second metabolite has been identified as the hitherto unknown 2,5-dichloro-4-hydroxyphenoxyacetic acid. In a similar investigation with 4-chloro-2-methylphenoxyacetic acid, the previously unknown 4-chloro-5-hydroxy-2-methylphenoxyacetic acid has been identified as the major metabolite.

Interest in the fate of herbicides such as 2,4-dichlorophenoxyacetic acid (" 2,4-D ") in the soil, has stimulated investigations using soil bacteria and it seems likely that several species can bring about fission of the aromatic ring. Thus, Rogoff and Reid [1] reported an almost quantitative production of chloride ion from " 2,4-D " by a *Corynebacterium* sp. isolated from soil, whilst Fernley and Evans [2] using a soil *Pseudomonas* sp. obtained α-chloromuconic acid as a metabolite.

By contrast, non-specific nuclear hydroxylation without ring fission, has been the most notable feature of previous studies on the metabolism of aryloxyacetic acids by fungi,[3-5] considerable variation in the proportion of isomeric products being observed. The present Paper deals with the metabolism of " 2,4-D " and 4-chloro-2-methylphenoxyacetic acid (" MCPA ") using a replacement culture technique with *Aspergillus niger*.

The major metabolite obtained from " 2,4-D " has been identified as 2,4-dichloro-5-hydroxyphenoxyacetic acid, and it is interesting that this compound, together with its β-D-glucoside, has recently been shown [6] to be produced from " 2,4-D " in wheat coleoptiles. A second metabolite, present in much smaller amount, gave *p*-hydroxyphenoxyacetic acid when treated with nickel–aluminium alloy in boiling aqueous alkaline solution.[7] This compound has been identified as the hitherto unknown 2,5-dichloro-4-hydroxyphenoxyacetic acid, which was unambiguously prepared from 2,5-dichlorohydroquinone for comparison. The formation of this hydroxy-acid from " 2,4-D " necessitates hydroxyl–chlorine replacement, as was found in the metabolism of 2-chlorophenoxyacetic acid,[4] but it is novel

* Part VI, Evens and Woodcock, *J.*, 1963, 816.

[1] M. H. Rogoff and J. J. Reid, *J. Bacteriol.*, 1956, **71**, 303.
[2] H. N. Fernley and W. C. Evans, *Biochem. J.*, 1959, **73**, 22P.
[3] R. J. W. Byrde and D. Woodcock, *Biochem. J.*, 1957, **65**, 682.
[4] J. K. Faulkner and D. Woodcock, *J.*, 1961, 5397.
[5] D. R. Clifford and D. Woodcock, *Nature*, 1964, **203**, 763.
[6] H. D. Klämbt, personal communication.
[7] N. P. Buu-Hoï, N. D. Xuong, and N. van Bac, *Bull. Soc. chim. France*, 1963, 2442.

in that it also involves a chlorine shift. This migration recalls the production of homogentisic acid by *A. niger*, *P. notatum*, or *P. chrysogenum*, using tyrosine as sole nitrogen source.[8]

That this apparent reorientation of the chlorine atoms did not arise by hydroxylation of 2,5-dichlorophenoxyacetic acid present as impurity, was established by careful scrutiny of the 2,4-dichlorophenol used in the preparation of the original " 2,4-D." Moreover, unchanged " 2,4-D " recovered from a column separation, when re-used in the replacement culture process, resulted in further production of 2,5-dichloro-4-hydroxyphenoxyacetic acid. Final proof of the genesis of this acid was furnished by the use of ^{14}C-carboxy-labelled " 2,4-D " in the replacement culture technique with *A. niger*. Chromatographic separation of the metabolites on paper gave three radioactive spots, one being coincident with that corresponding to 2,5-dichloro-4-hydroxyphenoxyacetic acid.

A third phenolic acid, present in trace amounts only in the fungal extract, differed chromatographically from 2,4-dichloro-3-hydroxy-, 2,4-dichloro-6-hydroxy-, 2-chloro-4-hydroxy-, and 4-chloro-2-hydroxy-phenoxyacetic acids, which might have been expected in the light of earlier results with *o*- and *p*-chlorophenoxyacetic acids;[4] it has not yet been identified.

In the metabolism of " MCPA " by *A. niger*, the previously unknown 4-chloro-5-hydroxy-2-methylphenoxyacetic acid has been identified as the major metabolite, an authentic specimen having been prepared by mononitration of " MCPA," esterification, reduction to the corresponding amine, diazotisation, and hydrolysis. The orientation of the nitro-group was established by conversion into the dichloro-2-methylphenoxyacetic acid, which was identical to the acid obtained by chlorination of 5-chloro-2-methylphenoxyacetic acid. Only trace amounts of other phenolic acids have been found in the fungal metabolic extract. The absence, in significant amount, of 5-chloro-4-hydroxy-2-methylphenoxyacetic acid in the " MCPA " metabolism, rather suggests that in the " 2,4-D " metabolism 2,5-dichloro-4- and 2,4-dichloro-5-hydroxyphenoxyacetic acids are not involved in a common mechanism of formation. In further replacement culture experiments, 2,5-dichloro-4-hydroxyphenoxyacetic acid was found to be the sole metabolite from 2,5-dichlorophenoxyacetic acid, and 2,4-dichloro-5-hydroxyphenoxyacetic acid did not undergo any further transformation when incubated with *A. niger*.

Experimental

Infrared absorption spectra were determined by using a Perkin-Elmer Infracord 137 spectrophotometer.

Replacement Culture Technique and Detection of Metabolites.—Cultures of *Aspergillus niger* van Tiegh. (Mulder strain), obtained from the Commonwealth Mycological Institute, were grown in penicillin flasks and the replacement culture technique carried out as previously described.[4] After incubation of the solution of the test acid (10^{-4}M) for 24 hr. under the fungal mat, the substrate (75 l.) was concentrated to 3 l. in a cyclone evaporator at 30°. It was then acidified to pH 2 and continuously extracted with ether for 16 hr., the extract washed with water, and the acidic material removed with sodium hydrogen carbonate solution. After acidification of the alkaline solution, the products were extracted with ether, the extract dried (Na_2SO_4), and the solvent removed.

Chromatography was on Whatman No. 1 paper by downward solvent flow with n-butanol–ethanol–3N-ammonia (4 : 1 : 5), and after drying at room temperature, chromatograms were examined under ultraviolet light (wavelength of maximum emission 2539 Å) and fluorescent spots marked. Phenolic compounds were detected by spraying with *p*-nitrobenzenediazonium fluoroborate (1% solution in 50% aqueous acetone) followed by a 20% solution of sodium acetate, and acids by spraying with Bromocresol Green (0·04% solution in 95% aqueous ethanol adjusted to pH 5).

Isolation of Metabolites.—Separation of metabolites was carried out by partition chromatography using silica gel as supporting medium for the stationary aqueous phase which was M-dipotassium hydrogen phosphate (adjusted to pH 10 with potassium hydroxide). The

[8] L. M. Utkin, *Biokhimiya*, 1950, **15**, 330.

mobile phase was n-butanol–chloroform (40 : 60) which had been half-saturated with the aqueous phase. Fractions were collected automatically.

Metabolism of 2,4-*Dichlorophenoxyacetic Acid.*—Replacement culture fungal substrate (150 l.) yielded 3 g. of material soluble in sodium hydrogen carbonate. This was separated on a silica gel column (100 g.), and, following material of fungal origin (0·1 g.), the acids listed below were eluted in the order shown.

Metabolism of " 2,4-D " incubated with *A. niger*.

Phenoxyacetic acid	Wt. (g.)	R_F	Colour with diazo-reagent	Identification
2,4-Dichloro	1·9	0·69	—	Mixed m. p.
2,4-Dichloro-5-hydroxy...	0·41	0·23	Mauve-brown	Mixed m. p.; infrared
(Mixed fraction).....	0·12	—	—	—
2,5-Dichloro-4-hydroxy...	0·035	0·16	Light brown	Mixed m. p.; infrared
(Mixed fraction).....	0·058	—	—	—
Unknown	Trace	0·18	Blue	—

Methyl 2,4-*Dichloro*-5-*nitrophenoxyacetate.*—2,4-Dichloro-5-nitrophenoxyacetic acid [9] gave the methyl ester which crystallised from benzene–light petroleum (b. p. 60—80°) as pale yellow needles, m. p. 79—80° (Found: C, 38·3; H, 2·6; N, 4·9. $C_9H_7Cl_2NO_5$ requires C, 38·6; H, 2·5; N, 5·0%).

Methyl 5-*Amino*-2,4-*dichlorophenoxyacetate.*—The above nitro-ester (20 g.) was vigorously stirred with iron powder (20 g.) in boiling water (400 ml.), and glacial acetic acid (40 ml.) added dropwise during 2 hr. The mixture was cooled, extracted with ether, and, after washing with sodium hydrogen carbonate, the ethereal solution was dried (Na_2SO_4) and the solvent removed. The residue crystallised from benzene–light petroleum (b. p. 60—80°) as needles (8·2 g.), m. p. 103—104° (Found: C, 43·2; H, 3·6; N, 5·7. $C_9H_9Cl_2NO_3$ requires C, 43·2; H, 3·6; N, 5·6%).

2,4-*Dichloro*-5-*hydroxyphenoxyacetic Acid.*—(*a*) A suspension of the above amino-ester (8 g.) in sulphuric acid (*d* 1·84; 17 ml.) and water (17 ml.) was stirred at 0° during the dropwise addition of a solution of sodium nitrite (2·6 g.) in water (12 ml.). When diazotisation was completed, the excess of nitrous acid was destroyed by the addition of urea and the mixture was slowly added to a boiling solution from copper sulphate pentahydrate (200 g.) in water (200 ml.). The solution was cooled, extracted with ether, and phenolic material isolated by shaking the ethereal extract with 10% aqueous sodium hydroxide. The alkaline solution was refluxed for 1 hr., cooled, acidified, and extracted with ether. The ethereal extract was washed with sodium hydrogen carbonate solution, the washings acidified, and the product isolated with ether. Removal of the dried (Na_2SO_4) solvent, gave a residue which, after crystallisation from water (charcoal), yielded an acid hydrate, m. p. 170° (lit.,[10] 175·5°) (Found: C, 37·8; H, 3·2. Calc. for $C_8H_6Cl_2O_4,H_2O$: C, 37·6; H, 3·1%), $\nu_{max.}$ 3400, 3100, 1720, 1610, 1600, 1500, 1480, 1430, 1320, 1270, 1230, 1200, 1180, 1090, 870, 740, 705 cm.$^{-1}$. Sublimation *in vacuo* gave the anhydrous acid, m. p. 170° (Found: C, 40·2; H, 2·5; Cl, 29·8. Calc. for $C_8H_6Cl_2O_4$: C, 40·5; H, 2·5; Cl, 29·9%).

(*b*) The second compound to be eluted from the silica column crystallised from water as monoclinic prisms, m. p. 167—168° alone or mixed with the product from (*a*) above. Their infrared spectra were identical.

Methyl 2,4-*Dichloro*-5-*methoxyphenoxyacetate.*—Obtained by methylation of acids from (*a*) or (*b*) above, this *ester* crystallised from ether and had m. p. 114—115° (Found: C, 45·4; H, 3·8. $C_{10}H_{10}Cl_2O_4$ requires C, 45·3; H, 3·8%). Infrared spectra were identical, $\nu_{max.}$ 1740, 1600, 1500, 1490, 1385, 1305, 1110, 1050, 1010, 890, 870, 830, 780, 735 cm.$^{-1}$. Alkaline hydrolysis gave 2,4-*dichloro*-5-*methoxyphenoxyacetic acid*, m. p. 168—169° (from aqueous acetone) (Found: C, 43·4; H, 3·25. $C_9H_8Cl_2O_4$ requires C, 43·0; H, 3·2%), $\nu_{max.}$ 3000, 1710, 1600, 1500, 1450, 1390, 1260, 1200, 1105, 1050, 875, 810, 750, 700 cm.$^{-1}$.

2,5-*Dichloro*-4-*hydroxyphenoxyacetic Acid.*—(*a*) 2,5-Dichlorohydroquinone [11] (1·8 g.) dissolved in a solution from sodium (0·23 g.) in ethyl alcohol (20 ml.) was refluxed during the dropwise addition of ethyl bromoacetate (1·7 g.) during 15 min. The mixture was cooled, diluted with water, adjusted to pH 10, and extracted with ether. The alkaline solution was then refluxed for 20 min., cooled, acidified, and extracted with ether. The *acid* was isolated by washing with sodium hydrogen carbonate solution, acidification, and re-extraction with ether. It crystallised

[9] G. W. K. Cavill and D. L. Ford, *J.*, 1954, 565.
[10] J. Moszew and J. Wojiechowski, *Roczniki Chem.*, 1954, **28**, 445.
[11] A. R. Ling, *J.*, 1892, 558.

from water (charcoal) and after sublimation *in vacuo* at 130° had m. p. 164° (Found: C, 38·6; H, 2·6; Cl, 28·8. $C_8H_6Cl_2O_4,\frac{1}{2}H_2O$ requires C, 39·0; H, 2·8; Cl, 28·9%). Methylation with diazomethane gave methyl 2,5-dichloro-4-methoxyphenoxyacetate, which crystallised from aqueous methanol and had m. p. 110—111°.

(*b*) The third acidic compound to be eluted from the silica column, crystallised from water as rectangular plates, m. p. 163—164° undepressed by admixture with the authentic specimen, m. p. 164°, described in (*a*) above (Found: C, 39·0; H, 2·9; Cl, 28·4; O (Unterzaucher), 29·6%; Equiv., 250, 251. $C_8H_6Cl_2O_4,\frac{1}{2}H_2O$ requires C, 39·0; H, 2·8; Cl, 28·9; O, 29·3%; Equiv., 246). Infrared spectra were identical. Treatment with an excess of diazomethane gave a methyl ester, m. p. 109—110°, identical (mixed m. p. and infrared spectra) with that described above (Found: C, 45·6; H, 3·8; OMe, 22·1. $C_{10}H_{10}Cl_2O_4$ requires C, 45·3; H, 3·8; OMe, 23·4%), $\nu_{max.}$ 1730, 1490, 1440, 1380, 1360, 1240, 1205, 1085, 1035, 980, 860, 780, 710 cm.$^{-1}$. Alkaline hydrolysis of this ester gave the *methoxy-acid*, m. p. 169° (Found: C, 43·6; H, 3·3; OMe, 12·1. $C_9H_8Cl_2O_4$ requires C, 43·0; H, 3·2; OMe, 12·3%), $\nu_{max.}$ 3000, 1760, 1500, 1440, 1375, 1215, 1095, 1035, 860, 815 cm.$^{-1}$.

Reduction of 2,5-*Dichloro*-4-*hydroxyphenoxyacetic Acid.*—This acid (10 mg.) was dissolved in a solution of sodium hydroxide (140 mg.) in water (1 ml.) and nickel–aluminium alloy (30 mg.) cautiously added. The mixture was refluxed for 1 hr., cooled, and the supernatant liquor decanted and acidified. Extraction with ether gave *p*-hydroxyphenoxyacetic acid (3·6 mg.), identified by mixed m. p. and infrared spectrum.

Ethyl 2,4-*Dichloro*-3-*nitrophenoxyacetate.*—2,4-Dichloro-3-nitrophenol [12] (15·5 g.) was dissolved in a solution from sodium (2 g.) in ethyl alcohol (150 ml.) and refluxed with ethyl bromoacetate (13·4 g.) for 2 hr. After distilling off most of the alcohol the residual liquid was diluted with water and extracted with ether. The extract was washed with 2% sodium hydroxide solution and water, and dried (Na_2SO_4). Removal of the solvent left the *ester* which crystallised from benzene–light petroleum (b. p. 60—80°) as needles, m. p. 85° (Found: C, 40·4; H, 3·2; N, 4·7. $C_{10}H_9Cl_2NO_5$ requires C, 40·8; H, 3·1; N, 4·8%).

Ethyl 3-*Amino*-2,4-*dichlorophenoxyacetate.*—The above nitro-ester (10 g.) was reduced with iron powder (10 g.) in aqueous acetic acid as described earlier. The oily *product* crystallised from light petroleum (b. p. 60—80°) as monoclinic prisms (5 g.) , m. p. 57—58° (Found: C, 45·8; H, 4·4; N, 5·65. $C_{10}H_{11}Cl_2NO_3$ requires C, 45·45; H, 4·2; N, 5·3%).

2,4-*Dichloro*-3-*hydroxyphenoxyacetic Acid.*—Ethyl 3-amino-2,4-dichlorophenoxyacetate (1 g.) was suspended in 66% (w/w) sulphuric acid (8·4 ml.) and vigorously stirred at 0° during the dropwise addition of a solution of sodium nitrite (0·5 g.) in 66% (w/w) sulphuric acid. When solution was complete, the excess of nitrous acid was destroyed by the addition of urea, and the diazonium solution slowly added to a boiling mixture of sulphuric acid (60 ml.) and water (40 ml.), boiling being continued until coupling no longer occurred with an alkaline solution of β-naphthol. The solution was cooled, diluted with water, and extracted with ether, and the extract washed with water and dried (Na_2SO_4). Removal of the solvent gave the *acid*, which crystallised from water (charcoal) and then had m. p. 171—172° (Found: C, 40·5; H, 2·6. $C_8H_6Cl_2O_4$ requires C, 40·5; H, 2·5%).

Metabolism of 4-*Chloro*-2-*methylphenoxyacetic Acid.*—Sodium hydrogen carbonate-soluble material (2·53 g.) obtained from fungal replacement culture (170 l.) was separated on a silica column (100 g.). Unchanged 4-chloro-2-methylphenoxyacetic acid (1·3 g.; m. p. 116—120°) was eluted first, followed by the major product (0·71 g.) which gave a tan-coloured spot with the diazo-reagent. Crystallisation from water gave 4-*chloro*-5-*hydroxy*-2-*methylphenoxyacetic acid*, m. p. 139—140°, identical (mixed m. p. and infrared spectra) with the authentic specimen prepared as described below (Found: C, 46·1; H, 4·8%; Equiv., 238. $C_9H_9ClO_4,H_2O$ requires C, 46·1; H, 4·7%; Equiv., 234·5), $\nu_{max.}$ 3300, 1750, 1620, 1590, 1500, 1410, 1330, 1290, 1230, 1200, 1170, 1085, 1030, 880, 830, 680 cm.$^{-1}$. Methylation in ethereal solution with diazomethane gave *methyl* 4-*chloro*-5-*methoxy*-2-*methylphenoxyacetate*, m. p. 103° (Found: C, 53·9; H, 5·3. $C_{11}H_{13}ClO_4$ requires C, 54·1; H, 5·3%), $\nu_{max.}$ 1730, 1610, 1510, 1440, 1390, 1320, 1280, 1210, 1170, 1060, 1015, 895, 830 cm.$^{-1}$. Hydrolysis of this ester by refluxing with 10% aqueous sodium hydroxide gave 4-*chloro*-5-*methoxy*-2-*methylphenoxyacetic acid*, m. p. 145° (Found: C, 51·8; H, 4·9. $C_{10}H_{11}ClO_4$ requires C, 52·1; H, 4·8%), $\nu_{max.}$ 1720, 1620, 1510, 1450, 1280, 1270, 1210, 1175, 1060, 895, 875, 815, 730, 700 cm.$^{-1}$.

4-*Chloro*-2-*methyl*-5-*nitrophenoxyacetic Acid.*—A solution of " MCPA " (25 g.) in sulphuric

[12] L. G. Groves, E. E. Turner, and G. I. Sharpe, *J.*, 1929, 512.

acid (*d* 1·84; 125 ml.) was stirred at 7—10° during the gradual addition (40 min.) of a mixture of nitric acid (*d* 1·42; 12·5 ml.) and sulphuric acid (*d* 1·84; 125 ml.). The solution was poured on to ice and the product collected, washed with water, and crystallised from methanol. Purified by acid hydrolysis of the ethyl ester and recrystallised from benzene it had m. p. 152—153° (Found: C, 44·0; H, 3·4; N, 5·7. $C_9H_8ClNO_5$ requires C, 44·0; H, 3·3; N, 5·7%). The *ethyl ester*, crystallised from light petroleum (b. p. 40—60°), had m. p. 55° (Found: C, 48·2; H, 4·3; N, 5·35. $C_{11}H_{12}ClNO_5$ requires C, 48·3; H, 4·4; N, 5·1%). A *dinitro-acid* (probably 5,6-), obtained from the mother-liquors, had m. p. 193·5—194·5° (Found: C, 37·0; H, 2·8; N, 9·5. $C_9H_7ClN_2O_7$ requires C, 37·2; H, 2·4; N, 9·6%).

Ethyl 5-Amino-4-chloro-2-methylphenoxyacetate.—The above nitro-ethyl ester (5 g.) was reduced in aqueous acetic acid solution with iron powder (5 g.) at 60° as previously described. The *amino-ester* (4·1 g.), crystallised from light petroleum (b. p. 60—80°), had m. p. 79—80° (Found: C, 54·5; H, 5·9; N, 6·0. $C_{11}H_{14}ClNO_3$ requires C, 54·2; H, 5·75; N, 5·75%). The *benzoate*, crystallised from ethanol, had m. p. 130—131° (Found: C, 62·2; H, 5·1; N, 4·4. $C_{18}H_{18}ClNO_4$ requires C, 62·2; H, 5·2; N, 4·0%).

4-Chloro-5-hydroxy-2-methylphenoxyacetic Acid.—A solution of the above amino-ester (1 g.) in a mixture of sulphuric acid (*d* 1·84; 2 ml.) and water (9 ml.) was diazotised and the diazonium solution decomposed by pouring into a boiling solution of copper sulphate (100 g.) in water (100 ml.). The solution was then diluted, cooled, and the *hydroxy-acid* (0·54 g.) isolated as previously described for the corresponding dichloro-compound. Crystallised from water (charcoal), it had m. p. 140° (Found: C, 46·1; H, 4·5. $C_8H_9ClO_4,H_2O$ requires C, 46·1; H, 4·7%). The *methyl methoxy-ester*, prepared using diazomethane, had m. p. 103° (Found: C, 53·7; H, 5·3. $C_{11}H_{13}ClO_4$ requires C, 54·0; H, 5·3%). Alkaline hydrolysis of this ester gave *4-chloro-5-methoxy-2-methylphenoxyacetic acid*, m. p. 145° (Found: C, 51·8; H, 4·9. $C_{10}H_{11}ClO_4$ requires C, 52·1; H, 4·8%).

4,5-Dichloro-2-methylphenoxyacetic Acid.—(*a*) Ethyl 5-amino-4-chloro-2-methylphenoxyacetate (1 g.) was diazotised as described above and added to a boiling solution of cuprous chloride (1 g.) in hydrochloric acid (*d* 1·18; 10 ml.). The product was extracted with ether, the ether distilled off, and the residue refluxed with 10% aqueous sodium hydroxide until all had dissolved. The solution was cooled, acidified, and the solid collected and washed with water. The *acid* (0·4 g.), crystallised from aqueous methanol, had m. p. 163—164° (Found: C, 46·1; H, 3·5. $C_9H_8Cl_2O_3$ requires C, 45·95; H, 3·4%).

(*b*) 5-Chloro-2-methylphenoxyacetic acid (1 g.), prepared from 5-chloro-*o*-toluidine, was dissolved in glacial acetic acid (5 ml.) and chlorinated at room temperature to give a gain in weight of 0·35 g. The solution was diluted with water, the product collected, and crystallised from aqueous methanol. It had m. p. 164—165°, undepressed by admixture with product from (*a*) above. Eckstein, Dyszer, and Niedzwialowska [13] give m. p. 161—162°, but no microanalyses.

Ethyl 4-Chloro-2-methyl-6-nitrophenoxyacetate.—Prepared from 4-chloro-2-methyl-6-nitrophenol (1·87 g.) and ethyl bromoacetate (1·8 g.), the ester crystallised from light petroleum (b. p. 60—80°) and had m. p. 70° (Found: C, 48·7; H, 4·4; N, 5·3. $C_{11}H_{12}ClNO_5$ requires C, 48·3; H, 4·4; N, 5·1%). Alkaline hydrolysis gave the corresponding *acid*, m. p. 177—178° (from benzene) (Found: C, 44·1; H, 3·5; N, 5·9. $C_9H_8ClNO_5$ requires C, 44·0; H, 3·3; N, 5·7%).

2,6-Dichloro-4-nitrophenoxyacetic Acid.—2,6-Dichloro-4-nitrophenol (10·4 g.) dissolved in a solution of sodium (1·2 g.) in ethanol (100 ml.) was refluxed with ethyl bromoacetate (8·4 g.) for 6 hr. After distilling off most of the solvent, the mixture was cooled, diluted with water, made alkaline, and extracted with ether. After washing the extract with water and drying (Na_2SO_4), the solvent was removed and the residual oil distilled (7·1 g., b. p. 184—186°/0·1 mm.). Hydrolysis by refluxing with 30% (w/w) sulphuric acid for 2 hr. gave the *acidic product*, m. p. 185—186° (from benzene–ether) (Found: C, 36·25; H, 1·9. $C_8H_5Cl_2NO_5$ requires C, 36·1; H, 1·9%).

The authors thank Miss C. S. Brown for valuable technical assistance, Miss D. M. Fieldgate and Mr. D. R. Clifford for the microanalyses, Dr. F. W. Beech for certain large-scale facilities, and Mr. C. P. Lloyd-Jones for the autoradiograph.

Department of Agriculture and Horticulture, University of Bristol, Research Station, Long Ashton, Bristol. [*Received, July 10th*, 1964.]

[13] Z. Eckstein, E. Dyszer, and T. Niedzwialowska, *Roczniki Chem.*, 1964, **38**, 51.