The Fungicidal Activity of Substituted Acetanilides and Related Compounds

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(Received 18 April 1953)

During a programme of research on the preparation and testing of possible non-metallic organic fungicides, consideration was given to halogenoacylamides, a class of compounds which has been reported in a patent (Maier-Bode, 1940) to possess the desired biological properties. Since few details are given in this publication, which records some of the specific compounds claimed to have fungicidal activity, a detailed examination of this type of compound was of interest and is described in the present paper.

The compounds tested are all *N*-arylamides, the type of compound which is quoted almost exclusively in the patent. An examination of the *N*-alkylamides was reserved for possible future study because three publications (Weaver & Whaley, 1947*a, b*; Swensen & Weaver, 1948) describing the preparation of groups of these compounds as potential fungicides suggested that their examination had already been undertaken by other workers. Indeed, this proved to be the case when a later publication (Leonard & Blackford, 1949) announced the relevant biological testing results. Amongst the compounds tested by Leonard & Blackford (1949) are eleven acetanilide derivatives, and the fungicidal activities which they report are compared below with those obtained by the present authors.

EXPERIMENTAL

Preparation of compounds

The compounds selected for study were, with four exceptions, acetanilide derivatives of the general formula

in which R was usually chlorine (though other monovalent groups were sometimes involved), R' was hydrogen or an *n*-alkyl radical, and in which the benzene ring also carried suitable substituents; the remaining compounds were closely related structurally. Those substances already recorded in the literature were prepared by the methods therein reported or by method A (below), whichever were deemed the more suitable. Most of the new acetanilide derivatives were prepared by one of the five general procedures indicated, and the relevant data concerning the yield, physical characteristics and analysis of individual compounds are summarized in Table 1. The preparation and

properties of other new substances are recorded separately. Some improved syntheses of known materials are also included. (All melting points are uncorrected.)

Method A. This process is essentially that of Jacobs & Heidelberger (1917). A solution or fine suspension of the amine (10 g. or 10 ml.) in a mixture of glacial acetic acid (50 ml.) and saturated aqueous sodium acetate (50 ml.) was stirred vigorously in a cold-water bath while the requisite acyl chloride (1.2 mol. prop.) was added during 30 min. Generally, the crude product separated directly from the reaction mixture either as an oil or as a crystalline solid. The mixture was diluted with water and any solid was collected, washed with water and recrystallized from ethanol or aqueous ethanol. When the product was an oil, the diluted reaction mixture was extracted with ether, the extract washed with aqueous Na₂CO₃ and water, and then fractionally distilled. *Trichloroacet-2-chloroanilide* (15) was isolated as a viscous oil which solidified after keeping at 0° for 3 weeks.

Method B. A solution of the amine (10 g.) in dry benzene (50–100 ml.) was carefully treated with chloroacetyl chloride (2–5 mol. prop.) in dry benzene (10–20 ml.) and the mixture refluxed for 1–2 hr. on a steam bath. The solvent and excess of chloroacetyl chloride were removed by distillation *in vacuo*, the solid residue was then triturated in very dilute HCl and, after washing with water, crystallized from ethanol or aqueous ethanol. In the case of *chloroacet-N-butylanilide* (25), 2 mol. prop. of the amine were used to 1 mol. prop. of acid chloride, and the product was isolated from the benzene layer after washing with 2.5*N*-HCl and water.

Method C. A mixture of the amine (10 g.) and chloroacetic anhydride (1.5–2.0 mol. prop.; Bischoff & Walden, 1894) was heated with conc. H₂SO₄ (1 drop) in an oil bath at 120–140° for 1 hr. While still warm, the reactants were poured into cold water and the precipitated oil triturated with a glass rod. When solidification took place, the resulting solid was collected, washed with water, and crystallized from ethanol or aqueous ethanol. When the crude product remained as an oil, it was isolated by ether extraction and purified by fractional distillation.

Method D. A solution of the chloroacetyl compound (1 mol. prop.) in the minimum of hot ethanol was mixed with a similar solution of KSCN (1.1 mol. prop.), and the mixture was refluxed on a steam bath for 1 hr. Most of the solvent was removed by distillation and the residue cooled and triturated several times in cold water. The pale-yellow plastic residues so obtained often gave considerable trouble in attempts to crystallize them, but purification was generally achieved by obtaining seed crystals from a small sample of the crude material (employing as solvent a mixture of ethanol and ethyl acetate, and using a cooling bath of ethanol: solid CO₂), and then using these seeds for a bulk crystallization with the same solvent and an ice:salt bath.

Method E. The chloroacetyl compound (1 mol. prop.) was added to a solution of NaI (4 mol. prop.) in dry acetone, and the mixture refluxed for 16–20 hr. on a steam bath. The solvent was removed by distillation, the residue triturated thoroughly in cold water, and the crude product finally purified with charcoal and crystallized from ethanol or aqueous ethanol.

Chloroacet-2:4-dichloroanilide (5). Chloroacetanilide (97 g.) and fused sodium acetate (105 g.) were dissolved in glacial acetic acid (750 ml.), and gaseous chlorine was passed in until the solution had absorbed the theoretical quantity for 2 mol. prop. (82 g.). During this process, the temperature of the liquid rose to 60–70°. The reactants were cooled, a stream of SO₂ was passed through the solution to decompose any *N*-chloro compounds, and the mixture poured into a large volume of cold water. The resulting solid was collected, washed with water and crystallized from ethanol (300 ml.); the product was obtained as stellate clusters of fine, colourless needles, m.p. 100° (94 g.; 69% yield). The pure *chloroacet-2:4-dichloroanilide* melted at 102°. (Found: C, 40.2; H, 2.8; N, 6.0. C₈H₆ONCl₂ requires C, 40.3; H, 2.5; N, 5.9%). This compound has also been made by method A from 2:4-dichloroaniline. Under the conditions described above, it was not possible to bring about further nuclear chlorination, and additional absorption of chlorine produces an *N*-chlorinated product.

Pentachloroaniline. Conc. HCl (45 ml.) and SnCl₂ · 2H₂O (30 g.) were added to a solution of pentachloronitrobenzene (10 g.) in glacial acetic acid (40 ml.) and the mixture was refluxed vigorously for 10 hr. The reactants were cooled, poured on ice and 5*N*-NaOH was added until the initial pasty precipitate of tin hydroxides had redissolved. The solid residue was collected, washed with 5*N*-NaOH, then with water and dried at 100° (8.6 g.; m.p. 226°). Very pale-brown needles were obtained on crystallization from a large volume of ethanol (5.1 g.; 46% yield, m.p. 229°). Willgerodt & Willeke (1910), who prepared the compound by chlorinating 2:4:6-trichloroaniline, reported a m.p. of 232°. It does not form a picrate.

Chloroacet-2:3:4:5:6-pentachloroanilide (7). Pentachloroaniline (5 g.) was dissolved in dry benzene (45 ml.), finely powdered anhydrous BaCO₃ (15 g.) was then added, followed by a solution of chloroacetyl chloride (5 ml.) in dry benzene (10 ml.). The mixture was refluxed for 4 hr., filtered hot, and the solid residue washed with benzene. The combined reaction liquor and washings were washed with water and aqueous NaHCO₃, and evaporated to a small volume. The resulting solid, after crystallization from a large volume of ethanol, formed colourless interlacing fine needles (1.6 g.; 25% yield; m.p. 216–217°). The pure *chloroacet-2:3:4:5:6-pentachloroanilide* melted at 217°. (Found: C, 28.4; H, 0.9. C₈H₃ONCl₅ requires C, 28.1; H, 0.9%).

Trichloroacet-*N*-methylanilide (22). This compound was prepared by method B in 45% yield and obtained pure from aqueous ethanol as stellate clusters of colourless prisms, m.p. 71.5–72°. (Found: C, 42.4; H, 3.3. Calc. for C₉H₈ONCl₃: C, 42.8; H, 3.2%). Spiegel & Spiegel (1907) gave m.p. 55°.

Chloroacet-*N*-pentylanilide (26). *N*-Pentylaniline (30 g.) dissolved in dry benzene (120 ml.) was mixed with a solution of chloroacetyl chloride (7 ml.) in dry benzene (25 ml.). The homogeneous liquid (kept dry with a CaCl₂ tube) was refluxed on a steam bath for 16 hr. and allowed to cool. The organic layer was then extracted thoroughly with 2.5*N*-HCl, washed with water, dried and evaporated. The residual oil, on distillation *in vacuo*, gave two fractions. The main

fraction, on redistillation, gave a pale-yellow oil, b.p. 139–141°/1 mm. (13 g.; 59% yield), which proved to be *chloroacet-*N*-pentylanilide*. (Found: N, 6.1. C₁₃H₁₈ONCl requires N, 5.8%). The subsidiary fraction, on redistillation, gave an oil, b.p. 117°/1 mm. (2.8 g.) which solidified on standing. Crystallization from a mixture of ethyl acetate and light petroleum (b.p. 80–100°) gave colourless plates, m.p. 124°. (Found: C, 66.1; H, 8.7. C₁₁H₁₇N.HCl requires C, 66.1; H, 9.0%). The identity of this material as *N-pentylaniline hydrochloride* was confirmed by a mixed m.p. with another sample prepared unambiguously from pentylaniline in dry ether and HCl.

***N*-*n*-Decylaniline.** A mixture of *n*-decyl bromide (66 g.; 1 mol. prop.) and aniline (138 g.; 5 mol. prop.) was refluxed for 4 hr., cooled, and made alkaline with 5*N*-NaOH. The organic layer was separated, dried over solid KOH, and fractionally distilled. The pure *N-n-decylaniline* was obtained as a colourless oil, b.p. 150–152°/1 mm. (60 g.; 86% yield). (Found: C, 82.6; H, 11.5. C₁₆H₂₇N requires C, 82.4; H, 11.6%). The *p*-toluenesulphonyl derivative separated from methanol as colourless monoclinic prisms, m.p. 44.5–46°. (Found: C, 71.6; H, 8.4. C₂₃H₃₃O₂NS requires C, 71.3; H, 8.5%). The *picrolonate* separated from ethanol as lemon-yellow rhombohedra, m.p. 127–128°. (Found: C, 62.9; H, 6.9. C₁₆H₂₇N · C₁₀H₈O₆N₄ requires C, 62.8; H, 7.0%).

***N*-Methyl-2:4-dinitroaniline.** This improved method of preparation is based on some observations of Valton (1925). Chloro-2:4-dinitrobenzene (10 g.) was dissolved in warm ethanol (100 ml.) and 20% aqueous methylamine added from a burette, while occasionally heating the reaction mixture on a steam bath, until an intense-red colour just persisted after 3 min. heating (about 10 ml. of amine solution were required). The red colour was cleared with a few drops of glacial acetic acid, and the mixture cooled. The product separated as bright-yellow needles (9.2 g.; 95% yield; m.p. 173–174°).

***N*-Methylpicramide.** The following description is more convenient than previous methods (cf. Romburgh, 1883). A solution of picryl chloride (23.3 g.; Boyer, Spencer & Wright, 1946) in warm ethanol (150 ml.) was periodically heated on a steam bath while 20% aqueous methylamine was added slowly from a burette until a deep-red colour just persisted in the solution after boiling for 2 min. The red colour was cleared with a few drops of glacial acetic acid. An oil, which had separated during the reaction, solidified on cooling the mixture. This solid was redissolved by heating and the addition of further ethanol to the reaction liquor. The product separated on cooling as a bright-yellow crystalline mass (21 g.; 93% yield; m.p. 111–112°).

Chloroacet-*N*-methyl-2:4-dinitroanilide (47). Chloroacet-*N*-methylanilide (18 g.) was added to fuming HNO₃ (100 ml.; sp.gr. 1.5) and the mixture heated on a steam bath under reflux for 30 min., cooled and poured on to ice. A yellow solid separated, which was collected, washed with water and crystallized from methanol. The product weighed 16 g. (60% yield; m.p. 77–79°). The pure *chloroacet-*N*-methyl-2:4-dinitroanilide* was obtained as bright-yellow prisms which melted at 80°, either alone or on admixture with the product (m.p. 80°), obtained on chloroacetylating 2:4-dinitroaniline by method C. This fact establishes the orientation of the two nitro groups entering the nucleus during the nitration. (Found: N, 15.3. C₉H₈O₆N₃Cl requires N, 15.4%).

Ethyl 4-chloroacetamidobenzoate (73). This compound was prepared by method A in 60% yield, but repeated crystal-

lization did not raise the m.p. above 110–111° (Einhorn (1899) reported m.p. 116°). (Found: N, 6.0. Calc. for $C_{11}H_{12}O_2NCl$: N, 5.8%.)

(*Phenylthio*)acet-*N*-methylaniide (75). Metallic sodium (2.9 g.) was dissolved in a mixture of thiophenol (13.8 g.) and anhydrous ethanol (25 ml.), and a solution of chloroacet-*N*-methylaniide (23.2 g.) in hot ethanol (100 ml.) added to the resulting solution. After refluxing for 2 hr., the reactants were cooled, poured into cold water and allowed to stand for a few moments, when the precipitated oil solidified. The solid was collected and crystallized from aqueous ethanol. The dry product weighed 27 g. (84% yield; m.p. 71°). A pure sample of (*phenylthio*)acet-*N*-methylaniide formed colourless triclinic prisms melting at the same temperature. (Found: N, 5.6. $C_{15}H_{16}ONS$ requires N, 5.4%.)

(*Phenylthio*)acet-*N*-benzylaniide (76). Metallic sodium (2.1 g.) was dissolved in a mixture of thiophenol (10 g.) and anhydrous ethanol (25 ml.), and a solution of chloroacet-*N*-benzylaniide (23.5 g.) in warm ethanol (100 ml.) added. After refluxing for 2 hr., the mixture was cooled and poured into water. The precipitated oil could not be induced to crystallize, and was therefore isolated by ether extraction and distillation *in vacuo*. The temperature rose rapidly to 240°/1 mm., when a steady fraction was obtained without decomposition. This gave a white crystalline powder (15.0 g.; 50% yield; m.p. 49°). The pure (*phenylthio*)acet-*N*-benzylaniide, crystallized from a mixture of benzene and light petroleum (b.p. 80–100°) as colourless rhombohedra, had the same m.p. (Found: C, 75.4; H, 5.3. $C_{21}H_{18}ONS$ requires C, 75.7; H, 5.7%.)

*Benzenesulphonyl*acet-*N*-methylaniide (77). (*Phenylthio*)acet-*N*-methylaniide (10 g.) was dissolved in glacial acetic acid (50 ml.), aqueous H_2O_2 (11.5 ml., 100 vol.) added slowly during 15 min., the solution allowed to stand for 15 min., and then heated on a steam bath for 2 hr. The cooled solution was poured into water and the precipitated oil scratched to solidify it. The crude product was collected and crystallized from aqueous ethanol, giving colourless monoclinic prisms (7.1 g.; 63% yield; m.p. 120–122°). The pure *benzenesulphonyl*acet-*N*-methylaniide melted at 122–122.5° (Found: N, 5.0. $C_{15}H_{16}O_3NS$ requires N, 4.8%.)

*Benzenesulphonyl*acet-*N*-benzylaniide (78). This material was obtained in a manner similar to that described in the preceding paragraph, using (*phenylthio*)acet-*N*-benzylaniide (5.1 g.) as the starting material. Crystallized from ethanol, the product formed colourless, tetragonal prisms (4.1 g.; 74% yield; m.p. 142°). The pure *benzenesulphonyl*acet-*N*-benzylaniide melted at 144°. (Found: N, 3.9. $C_{21}H_{18}O_3NS$ requires N, 3.8%.)

2:4-Dichlorophenoxyacet-4-chloroacetamidoaniide (80). 2:4-Dichlorophenoxyacetyl chloride was obtained essentially by the method of Freed (1946), but the crude residue, after removal of the excess of thionyl chloride, was crystallized from light petroleum (b.p. 60–80°) and not distilled *in vacuo*. By this means, the desired product was obtained as colourless crystals, m.p. 52.5–53.5°. (Found: C, 40.2; H, 2.4. Calc. for $C_8H_5O_2Cl_3$: C, 40.1; H, 2.1%.) Freed (1946) reported m.p. 44.5–45.5°.

2:4-Dichlorophenoxyacetyl chloride (20 g.) was mixed with a suspension of *p*-nitroaniline (25 g.) in dry benzene (450 ml.), and the mixture refluxed on a steam bath for 16 hr. and allowed to cool. The solid was collected, thoroughly shaken, first with 2.5*N*-HCl, then aqueous Na_2CO_3 , and finally water, dried at 100°, clarified with charcoal, and crystallized from a large volume of benzene. 2:4-Dichloro-

phenoxyacet-4-nitroaniide formed very pale-yellow prisms and plates (20.7 g.; 62% yield; m.p. 200–201°). (Found: C, 49.2; H, 2.8. $C_{14}H_{10}O_4N_2Cl_2$ requires C, 49.3; H, 2.9%.)

The above nitro compound (12.9 g.) was suspended in ethanol (300 ml.) and hydrogenated at ordinary pressure and temperature over Raney nickel. When this reaction was complete, the products were heated until the organic solid dissolved, the catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue was crystallized from ethanol (8.1 g.; 58% yield; m.p. 138°). Pure 2:4-dichlorophenoxyacet-4-aminoaniide formed colourless, interlacing needles, m.p. 140–141°. (Found: C, 54.2; H, 3.9. $C_{14}H_{12}O_2N_2Cl_2$ requires C, 54.0; H, 3.9%.) The *acetyl* derivative separated from ethanol as colourless, hair-like needles, m.p. 217–218°. (Found: C, 54.4; H, 4.0. $C_{16}H_{14}O_2N_2Cl_2$ requires C, 54.6; H, 4.0%.) The *picrate* separated from ethanol as yellow, interlacing needles, which darkened slowly from 170° and melted at 222° (decomp.). (Found: C, 44.7; H, 3.0. $C_{14}H_{12}O_2N_2Cl_2 \cdot C_6H_3O_7N_3$ requires C, 44.4; H, 2.8%.)

2:4-Dichlorophenoxyacet-4-aminoaniide (7.1 g.) was dissolved in hot, dry benzene (100 ml.), treated with chloroacetyl chloride (5.4 ml.) and the mixture refluxed for 2 hr. The benzene and excess of chloroacetyl chloride were removed by distillation *in vacuo*, the residue was triturated in water, and the resulting crude solid was crystallized from a large volume of glacial acetic acid.

2:4-Dichlorophenoxyacet-4-chloroacetamidoaniide was obtained as buff blades, m.p. 237–238°. (Found: C, 49.4; H, 3.3. $C_{16}H_{12}O_3N_2Cl_3$ requires C, 49.6; H, 3.4%.)

Oximinoacet-N-methylaniide (82). This compound was prepared according to the procedure given by Gilman & Blatt (1941) for the preparation of oximinoacetanilide, and was obtained as colourless prisms, m.p. 169–170°. (Found: C, 60.8; H, 5.6. Calc. for $C_9H_{10}O_2N_2$: C, 60.7; H, 5.6%.) Sandmeyer (1919) recorded m.p. 145°.

Bis(methylphenylcarbamylmethyl) sulphide (83). A hot solution of Na_2S , $9H_2O$ (6.6 g.) in water (10 ml.) was added to a solution of chloroacet-*N*-methylaniide (10 g.) in 50% (v/v) aqueous ethanol (100 ml.), and the mixture refluxed for 1 hr. The ethanol was evaporated and the product recrystallized from ethanol as stellate clusters of colourless prisms, m.p. 112–113° (7.1 g.; 78% yield). A pure sample of *bis(methylphenylcarbamylmethyl) sulphide* melted at 114°. (Found: N, 9.0. $C_{18}H_{20}O_2N_2S$ requires N, 8.6%.)

Bis(benzylphenylcarbamylmethyl) sulphide (84). This compound was prepared in 65% yield using the method described in the preceding paragraph; the starting material in this case was chloroacet-*N*-benzylaniide. The *bis(benzylphenylcarbamylmethyl) sulphide* separated from ethanol in the form of colourless, monoclinic prisms, m.p. 121–122°. (Found: N, 5.9. $C_{30}H_{28}O_2N_2S$ requires N, 5.9%.)

1:2-Bis(*N*-chloroacetylanilino)ethane (85). A mixture of 1:2-bisaniinoethane (14.7 g.), chloroacetyl chloride (20 ml.) and dry benzene (150 ml.) was refluxed for 2 hr., the solvent removed *in vacuo*, and the residue triturated in water. The resulting solid was collected, washed with water and crystallized from ethanol (19.5 g.; 77% yield; m.p. 152°). The compound, pure 1:2-bis(*N*-chloroacetylanilino)ethane, formed colourless, hexagonal prisms, m.p. 154–155°. (Found: C, 59.2; H, 4.9. $C_{18}H_{18}O_2N_2Cl_2$ requires C, 59.2; H, 4.9%.)

1:2-Bis(*N*-thiocyanoacetylanilino)ethane (86). 1:2-Bis(*N*-chloroacetylanilino)ethane (13.3 g.) was refluxed for 2 hr. with a solution of KSCN (7.8 g.) in ethanol (200 ml.). The

Table 1. *New compounds*

No.	Name	Method of preparation	Yield (%)	Physical properties	Analyses		
					Element	Found (%)	Required (%)
6	Chloroacet-2:4:6-trichloroanilide	B	72	Colourless interlacing needles, m.p. 180°	C	35.1	35.2
					H	2.05	1.8
					N	5.35	5.1
9	Dichloroacet-2-chloroanilide	A	53	Colourless matted needles, m.p. 109–110°	C	40.3	40.3
					H	2.75	2.5
10	Dichloroacet-3-chloroanilide	A	60	Colourless prismatic rods, m.p. 100°	C	40.0	40.3
					H	2.2	2.5
					N	6.1	5.9
11	Dichloroacet-4-chloroanilide	A	37	Colourless monoclinic prisms, m.p. 136–137°	C	40.3	40.3
					H	2.5	2.5
12	Dichloroacet-2:4-dichloroanilide	B	48	Colourless matted needles, m.p. 129°	C	35.2	35.2
					H	1.6	1.8
15	Trichloroacet-2-chloroanilide	A	47	Colourless oil, b.p. 136–138°/2.3 mm. Monoclinic prisms, m.p. 61–61.5°	C	35.3	35.2
					H	2.2	1.8
16	Trichloroacet-3-chloroanilide	A	47	Colourless glistening rectangular plates, m.p. 100°	C	35.0	35.2
					H	1.9	1.8
17	Trichloroacet-4-chloroanilide	A	41	Colourless plates and prisms, m.p. 125–126°	C	35.0	35.2
					H	1.9	1.8
18	Trichloroacet-2:4-dichloroanilide	B	43	Colourless monoclinic prisms, m.p. 74–75°	C	31.1	31.2
					H	1.6	1.3
19	Trichloroacet-2:4:6-trichloroanilide	B	63	Colourless monoclinic prisms, m.p. 119–120°	C	28.3	28.1
					H	0.9	0.9
24	Chloroacet- <i>N</i> -propylanilide	A	44	Pale straw-coloured oil, b.p. 160°/12 mm.	N	6.8	6.6
25	Chloroacet- <i>N</i> -butylanilide	B	40	Pale-yellow oil, b.p. 136–139°/3.5 mm.	N	6.1	6.2
27	Chloroacet- <i>N</i> -hexylanilide	C	52	Almost colourless oil, b.p. 147°/1.3 mm.	C	66.4	66.3
					H	8.1	7.9
28	Chloroacet- <i>N</i> -octylanilide	C	30	Almost colourless, oil, b.p. 171–173°/1.9 mm.	C	68.8	68.4
					H	8.6	8.5
29	Chloroacet- <i>N</i> -decylanilide	C	23	Pale-green oil, b.p. 180–182°/1.7 mm.	C	70.4	69.8
					H	9.3	9.1
30	Chloroacet- <i>N</i> -benzylanilide	A	65	Colourless plates, m.p. 75–76°	C	69.3	69.4
					H	5.45	5.4
38	Iodoacet- <i>N</i> -methylanilide	E	70	Colourless rectangular plates, m.p. 57–58°	C	38.9	39.3
					H	3.4	3.6
42	Chloroacet-2:4-dinitroanilide	C	77	Pale-yellow needles, m.p. 111–112°	N	16.2	16.2
43	Chloroacet-2:4:6-trinitroanilide	C	68	Thin, buff prisms, m.p. 208°	N	18.7	18.4
44	Chloroacet- <i>N</i> -methyl-2-nitroanilide	B	82	Colourless hexahedra, m.p. 78–79°	C	47.2	47.3
					H	4.0	3.9
45	Chloroacet- <i>N</i> -methyl-3-nitroanilide	B	81	Pale-cream irregular prisms, m.p. 107–108°	C	47.5	47.3
					H	3.9	3.9

46	Chloroacet- <i>N</i> -methyl-4-nitroanilide	<i>B</i>	69	Colourless triclinic prisms, m.p. 109.5–110°	C	47.0 4.2	47.3 3.9
48	Chloroacet- <i>N</i> -methyl-2:4:6-trinitroanilide	<i>C</i>	68	Pale-yellow prisms, m.p. 140°	N	17.6	17.6
49	Chloroacet- <i>N</i> -ethyl-2:4-dinitroanilide	<i>C</i>	42	Lemon-yellow prisms, m.p. 80–81°	C	41.8 3.9	41.8 3.5
50	Iodoacet- <i>N</i> -methyl-2-nitroanilide	<i>E</i>	62	Pale-yellow monoclinic prisms, m.p. 72.5–73.5°	N	14.5	14.6
51	Iodoacet- <i>N</i> -methyl-3-nitroanilide	<i>E</i>	76	Pale-yellow monoclinic prisms, m.p. 91–91.5°	C	33.7 3.0	33.8 2.8
52	Iodoacet- <i>N</i> -methyl-4-nitroanilide	<i>E</i>	86	Pale-yellow prisms and plates, m.p. 101–102°	H	34.1 2.9	33.8 2.8
53	Iodoacet- <i>N</i> -methyl-2:4-dinitroanilide	<i>E</i>	80	Bright-yellow prisms, m.p. 121–122°	C	34.0 2.8	33.8 2.8
54	Iodoacet- <i>N</i> -methyl-2:4:6-trinitroanilide	<i>E</i>	42	Lemon-yellow monoclinic prisms, m.p. 155–156°	C	29.5 2.2	29.6 2.2
56	Thiocyanoacet- <i>N</i> -benzylanilide	<i>D</i>	55	Colourless prisms, m.p. 59–60°	N	11.4	11.5
57	Thiocyanoacet- <i>N</i> -phenylanilide	<i>D</i>	93	Colourless tetragonal prisms, m.p. 87–88°	C	26.9 1.6	26.3 1.7
58	Thiocyanoacet- <i>N</i> -methyl-2-nitroanilide	<i>D</i>	62	Irregular buff prisms, m.p. 47.5–48.5°	H	13.8	13.7
59	Thiocyanoacet- <i>N</i> -methyl-3-nitroanilide	<i>D</i>	87	Irregular colourless plates, m.p. 84.5–85.5°	C	47.7 3.6	47.8 3.6
60	Thiocyanoacet- <i>N</i> -methyl-4-nitroanilide	<i>D</i>	71	Irregular colourless prisms, m.p. 113–114°	C	48.0 3.8	47.8 3.6
61	Thiocyanoacet- <i>N</i> -methyl-2:4-dinitroanilide	<i>D</i>	54	Pale-yellow prisms, m.p. 95°	N	18.7	18.9
62	Thiocyanoacet- <i>N</i> -methyl-2:4:6-trinitroanilide	<i>D</i>	64	Pale-yellow prisms, m.p. 131.5°	N	20.0	20.5
63	Thiocyanoacet- <i>N</i> -ethyl-2:4-dinitroanilide	<i>D</i>	72	Pale-yellow prisms, m.p. 85–85.5°	C	42.5 3.2	42.6 3.2
66	4-Chloroacetamidodiphenyl	<i>B</i>	52	Colourless, pearly plates, m.p. 178–179°	H	68.1 5.2	68.4 4.9
68	Chloroacet- <i>N</i> -methyl-4-hydroxyanilide	<i>A</i>	66	Colourless prisms, m.p. 165°	N	7.25	7.0
70	Chloroacet-4-methylthioanilide	<i>A</i>	59	Colourless hexagonal blocks, m.p. 134°	N	6.7	6.5
71	Chloroacet-4-thiocyanoanilide	<i>A</i>	40	Pale-yellow, rectangular prisms, m.p. 172°	N	12.7	12.4
74	<i>m</i> -Chloroacetamido- <i>xxx</i> -trifluorotoluene	<i>A</i>	54	Colourless prisms, m.p. 72°	N	6.2	5.9
79	Ethyl 4-chloroacetamidophenoxyacetate	<i>A</i>	46	Colourless prisms, m.p. 98.5–99.5°	C	53.4 5.0	53.0 5.2
90	β -Iodopropionanilide	<i>E</i>	90	Colourless rods, m.p. 123–124°	C	39.5 3.6	39.3 3.6
93	δ -Iodovaleranilide	<i>E</i>	52	Colourless needles, m.p. 79–80°	C	43.1 4.7	43.6 4.6

solvent was removed by distillation, cold water added to the residue, and the resulting solid collected and crystallized from ethanol (13.6 g.; 90% yield; m.p. 163–165°). The pure 1:2-bis(*N*-thiocyanocetylanylino)ethane formed long, colourless prisms, m.p. 164–166°. (Found: C, 58.6; H, 4.7. $C_{20}H_{18}O_2N_4S_2$ requires C, 58.5; H, 4.4%.)

***δ*-Chlorovaleranilide (91).** A mixture of *δ*-chlorovaleric acid (154 g.; Gaudry & Berlinguet, 1949) and thionyl chloride (410 ml.) was refluxed on a steam bath for 2 hr., the excess of thionyl chloride distilled at ordinary pressure, and the residue distilled *in vacuo*. *δ*-Chlorovaleryl chloride was obtained as a colourless liquid, b.p. 56–58°/2.5 mm. (144 g.; 83% yield). (Found: C, 38.6; H, 5.2. $C_6H_9OCl_2$ requires C, 38.7; H, 5.2%.)

A solution of aniline (85 ml., 2 mol. prop.) in dry ether (250 ml.) was added slowly to a solution of *δ*-chlorovaleryl chloride (71 g., 1 mol. prop.) in dry ether (250 ml.) and the mixture was refluxed for 1 hr. The ether was removed by distillation, the residue triturated in water (1 l.) containing conc. HCl (30 ml.), and then collected. The crude solid was crystallized from aqueous ethanol to give a yield of 81 g. (84%; m.p. 105–107°). The pure *δ*-chlorovaleranilide formed colourless, monoclinic prisms, m.p. 107–108°. (Found: C, 62.2; H, 6.8. $C_{11}H_{14}ONCl$ requires C, 62.4; H, 6.8%.)

***δ*-Chlorovaler-N-methylanilide (92).** This substance was prepared in a manner similar to that described above for *δ*-chlorovaleranilide, the crude product being isolated by ether extraction. The pure *δ*-chlorovaler-N-methylanilide was obtained as a colourless oil, b.p. 149–150°/1.5 mm. (51% yield). (Found: C, 64.3; H, 7.3. $C_{12}H_{16}ONCl$ requires C, 63.9; H, 7.1%.)

Chloromethanesulphon-N-methylanilide (94). A solution of chloromethanesulphonyl chloride (20 g.; Johnson & Douglass, 1941) and methylaniline (27.2 g.) in dry benzene (200 ml.) was refluxed on a steam bath for 6 hr. The solvent was then evaporated, and the residual oil twice fractionally distilled. The pure chloromethanesulphon-N-methylanilide was thus obtained as a pale-yellow oil, b.p. 125–127°/1.0 mm. (10.8 g.; 37% yield). (Found: C, 43.5; H, 4.7; N, 6.7. $C_8H_{10}O_2NClS$ requires C, 43.8; H, 4.6; N, 6.4%.)

Chloromethanesulphon-N-ethylanilide (95). This compound was obtained as a pale-yellow oil, b.p. 130°/0.9 mm., by employing a method similar to that described in the preceding paragraph (38% yield). (Found: C, 46.7; H, 5.4; N, 6.1. Chloromethanesulphon-N-ethylanilide, $C_9H_{12}O_2NClS$, requires C, 46.3; H, 5.1; N, 6.0%.)

Biological methods

The methods described below were regarded as routine screening methods only and, whilst quantitative, the results obtained were used as a guide for further research.

The two methods were adopted to show the effects of the chemicals upon two different morphological features of fungi, viz. the mycelium and the spores. Only in the case of one test fungus were the tests carried out on both mycelium and spores. The fungi for the mycelial test were selected because of their regular, well-shaped colonies in culture and broad representation of pathogenic types. The organisms used for the spore germination test were chosen for their abundant spore production and general ease of management.

Formulation of materials. When the compound was a solid or a non-volatile liquid it was taken up in waterproofed limestone powder to give a dust containing 10% active

principle. In cases where that percentage was impossible lower ones had to be made. The dust base itself (containing about 1% lanoline and supplied by The Derbyshire Stone Co. Ltd., Matlock) has been found to be inactive, and it was assumed that the chemical under test underwent no change in form or activity. This is not to imply that formulation for practical use plays no part in activity, but for the laboratory tests, only the minimum amount of formulation was carried out to facilitate application of the compounds to the test organism. This dust formulation was used for one of the tests, while for the other the chemical was used in a pure state, as a solution in a suitable solvent, e.g. acetone, ethanol, $CHCl_3$, or water.

Mycelial test. This was based on a technique described by Forsberg (1949). In this method, pieces of sterilized button thread were plated out on malt-agar plates and these were inoculated with small pieces of the test fungus. These plates were then incubated at 23° for as long as was necessary for the surface of the agar to be covered with the fungal growth. During this period the mycelium probably grew in between the fibres of cotton in the thread and certainly over the outside, the outcome being that the pieces of thread were transformed into relatively manageable pieces of test organism. These lengths of thread were removed from the plates with sterile forceps and cut into pieces about $\frac{1}{4}$ in. long with sterilized scissors.

These small pieces were rolled in the dust containing the chemical under test in such a manner that the piece was completely covered with dust, the excess removed by tapping, and the pieces replated, five to each malt-agar plate. The pieces were so arranged that growth could take place from each piece, yielding reasonably large circular colonies. The incubation period, which varied from fungus to fungus, was governed by the time taken for the individual colonies to reach a size which just avoided intermingling of mycelium at the periphery of the colonies (see Pl. 4). At this stage measurements of colony diameters, in two directions at right angles, were made on all pieces, numbering ten per treatment, and the totals so obtained were compared one with the other.

Untreated controls were included in every set of plates, and a known fungicide was periodically included as a check on the technique. Two chemicals have been found satisfactory over the range of fungi, these being ferric dimethyl-dithiocarbamate (Fermate) and tetramethylthiuram disulphide (TMTD). These likewise were used as 10% dusts in the same base as the test chemicals.

In the assessment of the results of this test it was obvious that a chemical which allowed no growth in the test plates possessed fungicidal activity, whilst growth of the same order as the controls suggested complete lack of activity. The intermediate stages were arrived at by estimating the amount of inhibition, i.e. the difference between treated and untreated size, as a percentage of the untreated figure. The formula

$$\frac{C - T}{C} \times 100,$$

where C = untreated values, and T = treated values gave an estimate of the degree of control exerted by the chemical under test.

For a series of chemicals, therefore, a range of percentage inhibition was obtained and numbered according to the following schedule: 1 = 98–100%, 2 = 93–97%, 3 = 51–92%, 4 = 0–50%.

Fig. 1. Forsberg technique. Colony growths of test fungus (*Fusarium nivale*) from infected pieces of thread, showing the stage at which diameter measurements are taken.

Table 2 shows that a broad outline of the activity of any compound is easily obtained. This was sufficient for the purpose of studying the correlation between structure and activity.

The fungi used in this test were as follows: *Pythium debaryanum* Hesse, *Corticium solani* (Prill & Delacr.) Bourd. & Galz. = *Rhizoctonia solani* Kühn, *Botrytis cinerea* Fr., *Fusarium nivale* Auct., *F. caeruleum* (Lib.) Sacc., *Helminthosporium avenae* Eidam. Of these, the first-named is the most rapid growing and 2-3 days incubation sufficed, whilst the slower ones, *Fusarium nivale*, and *Helminthosporium* needed 7 or so days before the colonies reached an adequate size.

Spore germination test. This method was based on that laid down by the American Phytopathological Society (1943) and included some of the techniques used by Miller (1949) and Evans & Martin (1935). The method ensured the intimate association of test organism and chemical without the inclusion of any filler or inert base. It is more accurate and is capable of recording activity at a very low concentration of test chemical, but required more preparation than the first method.

The fundamental requirement of this method was absolute cleanliness of all the apparatus used. All the glassware used in this technique was cleaned out with chromic/nitric acid cleaning fluid, thoroughly washed with running water, rinsed several times with distilled water and finally washed with water, twice distilled in Pyrex glass. The test tubes and slides used were respectively filled with, and immersed in, doubly distilled water and autoclaved at 20 lb./sq.in. for 30 min. and then oven-dried. (At one time heating in 5% (w/v) KOH solution was included in the cleaning schedule, but it was discovered that the highly polished surface of the slides deteriorated fairly rapidly, giving rise to scratches and imperfections in which traces of chemicals from previous trials could remain and thus prejudice any further results. This KOH wash was therefore omitted.) The slides were then allowed to stand in pure CHCl_3 for a few minutes to remove traces of grease, removed and air-dried, dipped in ethanol and flamed, and placed under cover to await use. It was found that rigid adherence to this cleaning procedure was essential for consistent results.

The above method was used where the glass surface itself was the substrate upon which the test solutions were placed, but in certain cases, and also to control the spreading of liquid drops, it was necessary to coat the slides with a cellulose film. This was achieved by dipping the slides in a 2.5% (w/v) solution of cellulose nitrate in butyl acetate (Evans & Martin, 1935) and drying quickly in dry heat. When rapid drying was not carried out, the film adsorbed water vapour from the air and rapidly became opaque, thus impeding microscopic examination. These slides were again kept under cover until used.

The production of spores for this test followed the American recommendations in so far that the fungi were cultured on potato-dextrose agar slopes and incubated for 7 days at a temperature of 22°. These conditions appeared to be the optimum values for spore production (McCallan & Wilcoxon, 1939). The spores were removed from the slope by adding distilled water and rubbing the culture surface with a glass rod. This suspension was strained through muslin to remove fragments of mycelium and agar and the resulting suspension was centrifuged at 2000 rev./min. for about 5 min. The liquid was decanted and replaced with fresh distilled water, the spores resuspended and recentrifuged. This process was repeated three or four times in order

to ensure that all traces of nutrient solution from the agar medium were washed from the spores thereby avoiding the possibility of differential germinative capacity resulting from varying amounts of stimulant material included in the suspension. As germination was rather uncertain in distilled water, a synthetic stimulant was added in all cases to the spores for the actual test. This consisted of a solution containing 0.001% (w/v) potassium or sodium citrate, and 0.1% (w/v) sucrose (Miller, 1949). As this was the final dilution required a stock solution 100 times the recommended strength was made, autoclaved, and then stored in a refrigerator at about 3° and diluted as required for use.

It was shown that the actual density of the spore suspension influenced the germinating capacity of the organism and that a final concentration of 50000 spores/ml. was a satisfactory value (McCallan & Wilcoxon, 1939). In all tests, the suspension was adjusted to this figure by dilution from the original suspension calculated from counts made on a haemocytometer cell.

As pure chemicals only were involved in this test, the first procedure was to make a solution in a suitable solvent. For this particular series acetone was used throughout and a stock solution of chemical prepared. The usual test strengths were 50, 25 and 15 p.p.m., but in some cases where the strongest solution failed to reveal activity differences relative to chemical structure, tests were carried out using 200 p.p.m. solution. Thus, stock solutions of 200 p.p.m. chemicals were made up and used, as such, or diluted to give 50, 25 and 15 p.p.m. where necessary.

For these tests the cleaned, uncoated glass slides were used and on to each of the three slides used for each chemical, 0.25 ml. was pipetted. The low surface tension of acetone ensured that the liquid spread over the whole surface, and as the slides were levelled beforehand, the chemical was assumed to be distributed evenly. The acetone was allowed to evaporate, thus giving a deposit of chemical of 0.6-0.7 $\mu\text{g./sq.cm.}$ when a 50 p.p.m. solution was used. (The fungicidal standard in this test was copper, usually as the sulphate; it had been found (Byrde, 1952) that 0.2 $\mu\text{g./sq.cm.}$ was sufficient to prevent 50% of the spores germinating, and from previous experience it was obvious that 0.6 $\mu\text{g./sq.cm.}$ of copper would completely inhibit germination. It could thus be assumed that test chemicals showing high activity at the various concentrations compared well with copper under the conditions of test.) The spore suspensions, of correct density and made up in stimulant solutions of requisite concentration, were pipetted on to the treated surfaces, three drops to a slide, each drop being 0.05 ml. and spreading to about 1 cm. in diameter. The slides were then distributed at random on glass racks, placed in the incubation chamber which maintained a high relative humidity, the chamber was sealed with a vaselined lid and the whole incubated at 22° for a period of 17-24 hr. (The period of incubation does affect the germination to some extent but as large differences in activity were sought, the effect of an incubation period of even 48 hr. was relatively unimportant.)

After the incubation period the spores were examined microscopically using a $\frac{3}{4}$ in. objective and $\times 10$ eyepiece. The total number of spores in each field was counted, sufficient fields being examined to give a total of 150 per slide (i.e. about 450/treatment) and the spores divided into germinated and ungerminated groups. In all tests there was an untreated set of slides, to show the germination capacity of the spores themselves. If this figure was not in the region of 95-100%, the test was regarded as invalid.

In order to compare successive tests, the percentage of germinated spores was calculated for each treatment and the percentage of spores inhibited from germination was also calculated by expressing the difference between untreated and treated germination percentages as a fraction of the untreated percentage, thus

$$\frac{C - T}{C} \times 100,$$

where C = untreated germination percentage, and T = treated germination percentage. This had the effect of removing from the final figures the effect of the few spores in each batch which invariably failed to germinate and produced a result based on the assumption that all the spores were viable and that failure to germinate was occasioned by the treatment alone.

This procedure helped to minimize the effect of using different lots of spores on different days. The same categories were adopted as for the mycelial test.

Only one fungus found a use in both tests. This was *Botrytis cinerea* whilst the other two used in the test were *Sclerotinia fructigena* Aderh. & Ruhl. and *Alternaria brassicae* (Berk.) Sacc. All three fungi were easily cultured but the latter tended to be spasmodic in spore production and sometimes cultures consisted solely of white vegetative mycelium. *S. fructigena* proved to be most sensitive to fungicidal activity.

RESULTS

The antifungal activities of the ninety-five organic compounds tested are given in Table 2 (mycelial test) and Table 3 (spore germination test). The effects of nuclear chlorination on chloroacetanilide (1), which may be regarded as a reference compound, can be seen in the activities of compounds 2-7 (Table 2). The excellent control effected by compound 1 on all the six experimental fungi was maintained on introducing a chlorine atom either *ortho* or *meta* to the chloroacetamido group, but this activity was diminished with the substituent in the *para* position, as was the case when two chlorine atoms were present in the nucleus (5). Further halogenation completely destroyed the activity (6, 7). The same general tendency was observed with the dichloroacetanilides (8-13) and the trichloroacetanilides (14-19), though in these cases the demarcation was not so regular. This may be due to the fact that these two groups of compounds were considerably less active than the chloroacetanilides. Polychlorination in the acetyl group reduced the overall activity of these compounds, the maximum activity being exhibited by the monochloroacetyl derivatives (acetanilide itself is inactive). This effect is particularly well illustrated by the *N*-methylanilides (20-22).

The activities of a series of *N*-substituted homologues of chloroacetanilide have been determined (Table 3; 20, 23-29) as biological activity can increase considerably in such a series. In this particular instance, the general control of the fungi was poor at the concentration of 200 p.p.m., but a definite maximum of control of *S. fructigena* was

obtained with chloroacet-*N*-pentylanilide (26). This series was not continued beyond chloroacet-*N*-decylanilide. Two other compounds of the same type (30, 31, Table 2) also showed little activity.

The effect of different halogen atoms in the nucleus is illustrated with compounds 32, 4, 33 and 34 (Table 2), containing one such atom in the *para* position of the nucleus. The fluorine compound (32) had the same activity as the chlorine compound (4), but the substitution of a bromine atom (33) reduced this activity considerably, while it disappeared completely in the iodine compound (34).

A more important change in biological activity was shown on changing the halogen atom in the acetyl group. Here, the mycelial test (Table 2) demonstrated that the high activity of chloroacetanilide (1) is maintained in bromoacetanilide (35) and iodoacetanilide (36). Further, the activity of the last two compounds was only slightly diminished when their concentration was reduced to 50 p.p.m. in the spore germination test (Table 3), while the activity of chloroacetanilide (1) had disappeared. The same effects were noted with the *N*-methyl derivatives (20, 37, 38).

The effects of nuclear nitration upon fungicidal activity were well illustrated by compounds 1, 39-43 (Table 2), where a marked similarity with the effects of nuclear chlorination was noticeable. Nitro groups in the *ortho* and *meta* positions of the rings did not reduce the activity of the parent compound (1), but when the substituent was *para* to the chloroacetamido moiety, the activity was slightly reduced (41), while the introduction of further nitro groups ultimately destroyed the activity completely (42, 43). The corresponding group of *N*-methyl derivatives (20, 44-48) were submitted to the spore germination test. Here, the much lower concentration employed (200 p.p.m.) masked the activity of even the potentially active members of the series, which was uniformly inactive. However, a similar series of nitro derivatives (50-54) based on iodoacet-*N*-methylanilide (38), which had already been seen to be more active than chloroacet-*N*-methylanilide (20), did manifest considerable activity at 200 p.p.m. (Table 3). Here, again, increasing nuclear substitution tended to reduce the activity exhibited by the mono-substituted compounds.

The effects of various other nuclear substituents upon the activity of chloroacetanilide has been examined with compounds 67-73 (Table 2), and 74, 79, 80 (Table 3). None of these derivatives showed an enhanced activity, while most of them were much weaker fungicides. Replacement of the benzene nucleus by naphthalene (64, 65) and diphenyl (66) likewise resulted in no improved activity. The union of two chloroacetanilide molecules into one molecule by an ethylene bridge (85) also produced no marked activity.

Table 2. Results of mycelial test

(Materials applied as dusts containing 10% chemical in an inert base.)

No.	Name	1 = 98-100% Inhibition A = Good general activity		2 = 93-97% Inhibition B = Fair general activity		3 = 51-92% Inhibition C = Some activity		4 = 0-50% Inhibition D = Little or no activity		Overall symbol	
		<i>Pythium debaryanum</i>	<i>Corticium solani</i>	<i>Botrytis cinerea</i>	<i>Fusarium nivale</i>	<i>Fusarium caeruleum</i>	<i>Helmholtz sporium avenae</i>				
1	Chloroacetanilide	1	2	1	1	1	2			A	
2	Chloroacet-2-chloroanilide	1	2	2	2	1	2			A	
3	Chloroacet-3-chloroanilide	1	2	1	1	1	1			A	
4	Chloroacet-4-chloroanilide	3	4	2	3	1	1			B	
5	Chloroacet-2:4-dichloroanilide	3	3	1	2	2	3			B	
6	Chloroacet-2:4:6-trichloroanilide	4	4	4	4	4	4			D	
7	Chloroacet-2:3:4:5:6-pentachloroanilide	4	4	4	4	4	4			D	
8	Dichloroacetanilide	1	1	3	4	4	1			C	
9	Dichloroacet-2-chloroanilide	4	3	4	4	4	3			D	
10	Dichloroacet-3-chloroanilide	1	1	3	1	3	1			B	
11	Dichloroacet-4-chloroanilide	3	2	3	3	4	3			C	
12	Dichloroacet-2:4-dichloroanilide	4	3	4	4	4	3			D	
13	Dichloroacet-2:4:6-trichloroanilide	4	4	4	4	4	3			D	
14	Trichloroacetanilide	1	2	3	1	4	1			B	
15	Trichloroacet-2-chloroanilide	3	1	3	4	4	3			C	
16	Trichloroacet-3-chloroanilide	3	2	4	3	4	2			C	
17	Trichloroacet-4-chloroanilide	3	3	4	4	4	3			D	
18	Trichloroacet-2:4-dichloroanilide	3	1	4	4	4	3			D	
19	Trichloroacet-2:4:6-trichloroanilide	3	1	3	3	4	1			C	
20	Chloroacet- <i>N</i> -methylanilide	1	1	1	1	1	1			A	
21	Dichloroacet- <i>N</i> -methylanilide	2	2	3	4	4	3			C	
22	Trichloroacet- <i>N</i> -methylanilide	3	2	3	4	4	3			D	
30	Chloroacet- <i>N</i> -benzylanilide	4	3	4	4	4	4			D	
31	Chloroacet- <i>N</i> -phenylanilide	3	2	4	4	3	4			D	
32	Chloroacet-4-fluoroanilide	.	1	4	4	4	1			B	
33	Chloroacet-4-bromoanilide	.	2	4	4	4	4			C	
34	Chloroacet-4-iodoanilide	.	4	4	4	4	4			D	
35	Bromoacetanilide	.	1	1	1	1	1			A	
36	Iodoacetanilide	.	1	1	1	1	1			A	
37	Bromoacet- <i>N</i> -methylanilide	.	1	1	1	1	1			A	
38	Iodoacet- <i>N</i> -methylanilide	.	1	1	1	1	1			A	
39	Chloroacet-2-nitroanilide	1	1	3	1	1	2			A	
40	Chloroacet-3-nitroanilide	1	1	3	1	1	1			A	
41	Chloroacet-4-nitroanilide	1	1	4	3	3	1			B	
42	Chloroacet-2:4-dinitroanilide	.	3	3	3	3	1			C	
43	Chloroacet-2:4:6-trinitroanilide	.	4	4	4	4	3			D	
55	Thioxyanoacet- <i>N</i> -methylanilide	1	1	2	3	2	4			A	
56	Thioxyanoacet- <i>N</i> -benzylanilide	3	4	4	4	4	4			D	
67	Chloroacet-4-hydroxyanilide	.	2	4	4	4	1			C	
68	Chloroacet-4-hydroxy- <i>N</i> -methylanilide	.	4	4	4	4	3			D	
69	Chloroacet-4-methoxyanilide	3	2	3	3	4	1			C	
70	Chloroacet-4-methylthioanilide	4	1	4	3	4	3			C	
71	Chloroacet-4-thioxyanoanilide	3	3	4	3	4	3			C	
72	4-Chloroacetamido- <i>N</i> -dimethylaniline	3	1	4	3	4	2			B	
73	Ethyl 4-chloroacetamidobenzoate	.	1	4	4	3	1			D	
83	Bis(methylphenylcarbamylmethyl) sulphide	.	4	4	4	4	4			D	
84	Bis(benzylphenylcarbamylmethyl) sulphide	4	4	4	4	4	4			D	

Table 3. Results of spore germination test

No.	Name	Concentration of chemical (p.p.m.)												Overall symbol				
		200				50				25					15			
		BC	SF	AB		BC	SF	AB		BC	SF	AB			BC	SF	AB	
1	Chloroacetanilide	.	.	.		4	4	4		.	.	.	.	.	.	.	D	
20	Chloroacet- <i>N</i> -methylanilide	4	3	4		4	4	3	4	3	3	4	4	4	4	4	D	
23	Chloroacet- <i>N</i> -ethylanilide	4	4	4		4	4	4	4	.	.	.	.	.	.	.	D	
24	Chloroacet- <i>N</i> -propylanilide	4	4	4		4	4	4	4	.	.	.	.	.	.	.	D	
25	Chloroacet- <i>N</i> -butylanilide	4	3	4		4	4	4	4	.	.	.	.	.	.	.	D	
26	Chloroacet- <i>N</i> -pentylanilide	4	2	4		4	4	4	4	.	.	.	.	.	.	.	D	
27	Chloroacet- <i>N</i> -hexylanilide	4	4	4		.	.	.	.	.	.	.	.	.	.	.	D	
28	Chloroacet- <i>N</i> -octylanilide	4	4	4		.	.	.	.	.	.	.	.	.	.	.	D	
29	Chloroacet- <i>N</i> -decylanilide	4	4	4		.	.	.	.	.	.	.	.	.	.	.	D	
32	Chloroacet-4-fluoroanilide	.	.	.		4	4	4	4	.	.	.	.	.	.	.	D	
33	Chloroacet-4-bromoanilide	.	.	.		4	4	4	4	.	.	.	.	.	.	.	D	
34	Chloroacet-4-iodoanilide	.	.	.		4	4	4	4	.	.	.	.	.	.	.	D	
35	Bromoacetanilide	.	.	.		1	1	3	1	1	1	3	2	1	4	4	B	
36	Iodoacetanilide	.	.	.		1	1	3	1	1	1	3	2	1	4	4	B	
37	Bromoacet- <i>N</i> -methylanilide	.	.	.		1	2	4	4	4	3	4	4	4	4	4	C	
38	Iodoacet- <i>N</i> -methylanilide	.	.	.		1	1	4	4	4	3	4	4	4	3	4	C	
43	Chloroacet-2:4:6-trinitroanilide	.	.	.		4	4	3	4	4	4	4	.	.	.	.	D	
44	Chloroacet- <i>N</i> -methyl-2-nitroanilide	4	4	4		.	.	.	.	.	.	.	.	.	.	.	D	
45	Chloroacet- <i>N</i> -methyl-3-nitroanilide	4	4	4		.	.	.	.	.	.	.	.	.	.	.	D	
46	Chloroacet- <i>N</i> -methyl-4-nitroanilide	4	4	4		.	.	.	.	.	.	.	.	.	.	.	D	
47	Chloroacet- <i>N</i> -methyl-2:4-dinitroanilide	4	4	4		.	.	.	.	.	.	.	.	.	.	.	D	
48	Chloroacet- <i>N</i> -methyl-2:4:6-trinitroanilide	.	.	.		4	3	4	4	4	4	4	.	.	.	.	D	
49	Chloroacet- <i>N</i> -ethyl-2:4-dinitroanilide	4	4	4		.	.	.	.	.	.	.	.	.	.	.	D	
50	Iodoacet- <i>N</i> -methyl-2-nitroanilide	1	1	3		.	.	.	.	.	.	.	.	.	.	.	B	
51	Iodoacet- <i>N</i> -methyl-3-nitroanilide	1	1	1		.	.	.	.	.	.	.	.	.	.	.	A	

Changes have been made in the monovalent substituent in the acetyl group. The most interesting of these was the replacement of the halogen atom by the thiocyno group. This change did not, apparently, affect the fungicidal activity associated with the parent chloroacet-*N*-methylanilide (20), for thiocynoacet-*N*-methylanilide (55) exhibited good general activity in the mycelial test (Table 2), and only poor activity at 200 p.p.m. in the spore germination test (Table 3). No enhancement of activity has been detected with other thiocynoacetyl compounds, namely 56 (Table 2), 57 and 86 (Table 3), and the series of nitro derivatives 58–63 (Table 3). A number of other changes in the acetyl substituent did not produce any interesting increases of activity (75–78, 81, 82, Table 3; 83, 84, Table 2).

A small group of compounds (87–93) was prepared for the purpose of observing the change of fungicidal properties on moving the halogen atom in the halogenoacetanilides away from the carbonyl group. Table 3 shows that the considerable activity exhibited by bromoacetanilide (35) completely vanished in β -bromopropionanilide (89), and the same was equally true for the analogous iodo compounds (36, 90); nor did the activity reappear on moving the iodine atom even further away from the carbonyl group, as in δ -iodovaleranilide (93).

Two compounds (94, 95) were prepared in which the carbonyl group of chloroacetanilide had been replaced by the sulphonyl group. This change was accompanied by no increase in fungicidal properties.

DISCUSSION

The biological activities recorded in Table 2 confirm the claims of Maier-Bode (1940) that, in general, the halogenoacetanilides do possess fungicidal activity when formulated as 10 % dusts in inert solid diluents. This activity, however, was only maintained in a few cases when the concentration of the active constituent was reduced to 200 p.p.m. or smaller values. It has also been confirmed that chloroacetanilide is less active than chloroacet-*N*-methylanilide, but *N*-alkylation did not always result in increased activity, e.g. trichloroacetanilide was more active than trichloroacet-*N*-methylanilide.

Leonard & Blackford (1949) have shown that chloroacetanilide inhibited the growth of *Aspergillus niger* more efficiently than did dichloroacetanilide which, in turn, was more efficient than trichloroacetanilide. These findings have been confirmed for other fungi in the present work, which has demonstrated that nuclear chlorination also reduces the activity. In addition, it has confirmed the superior activity of bromoacetanilide, which was equalled by that of iodoacetanilide.

The molecular size of the test chemical naturally affects its biological activity since, if the active halogenoacetamido group becomes masked by a

large nucleus, the compound will be inactive. Thus, no significant activity was found in compounds with a high molecular weight. The effect of increasing the molecular weight by substitution is well illustrated in two series of compounds, where the continued introduction of chlorine atoms (1–7) and nitro groups (39–43) gradually reduced a significant activity to zero.

Most of the chemicals described in this work have undesirable physiological properties. It was expected that the halogenoacetyl derivatives would be inherent lachrymators and skin irritants, but they occasioned no marked unpleasantness during synthesis because of their low volatility. In the form of dusts and sprays, however, the skin irritancy became very noticeable, a fact which would have precluded their practical application. (The same type of problem has been encountered by McCallan, 1950.) When the halogen atom in the acyl radical is not adjacent to the carbonyl group, as in β -iodopropionanilide, the compound did not exhibit irritancy, but unfortunately it was also no longer a fungicide.

The unpleasant physiological properties seem to be greatly diminished or eliminated completely in the nitro derivatives of the halogenoacetanilides, where appreciable fungicidal activity was maintained by the mononitro compounds. Skin irritation was also removed by replacing the halogen atom in the acetyl radical by the thiocyno group. The resulting thiocynoacet-*N*-methylanilide had the same order of activity as chloroacet-*N*-methylanilide but, like the latter, this activity faded on dilution to 200 p.p.m.

SUMMARY

1. The fungicidal activities of ninety-five non-metallic organic compounds derived structurally from acetanilide have been tested; sixty-two of these compounds are new, and their preparation and physical characteristics are described.

2. Two biological methods have been employed: (a) the mycelial test, in which the chemical was used as a 10 % dust in an inert solid diluent, and (b) the spore germination test, in which the chemical was used as an acetone solution at concentrations of 200 p.p.m. or less.

3. This group of compounds exhibits general fungicidal activity at the higher concentration of 10 %, but few of the chemicals retain their activity at 200 p.p.m.

4. Most of the compounds, particularly in the form of dusts and sprays, possess unpleasant physiological properties which precludes any practical application.

The authors wish to thank Miss A. P. Wilson and Dr H. A. Stevenson for their encouragement and interest in the work, and Miss J. Keefe and Mr B. E. Leggetter for technical assistance.

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The Deamidation of Nicotinamide by Bacteria

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(Received 21 May 1953)

In a previous paper from this laboratory it was shown that nicotinamide was rapidly broken down to nicotinic acid by washed suspensions of *Lactobacillus arabinosus* 17-5 (Hughes & Williamson, 1952a). In the present paper it is shown that the breakdown of nicotinamide in this micro-organism is hydrolytic, nicotinic acid and ammonia being formed stoichiometrically. Cell-free extracts which deamidate nicotinamide have been prepared and some properties of the enzyme investigated. A number of other species of lactobacilli were tested and all found to deamidate nicotinamide. In contrast to this, none of eleven other species of bacteria, with the single exception of a strain of *Staphylococcus albus*, were active.

EXPERIMENTAL

Organisms and growth media. *Lactobacillus arabinosus* 17-5, *Lb. bulgaricus*, National Collection of Type Cultures, strain 76, *Lb. delbrueckii*, NCTC, strain 5125, *Lb. casei*, NCTC, strain 8001, and *Lb. helveticus*, NCTC, strain 8103, two locally isolated strains of *Staphylococcus aureus* and NCTC, strain 6775, several locally isolated strains of *Staph. albus* and haemolytic streptococci were grown at 37° on an enzymic casein hydrolysate medium (Nossal, 1951) adjusted to pH 7.6. In some experiments *Lb. arabinosus* was grown on the semi-synthetic medium used for the microbiological assay of nicotinic acid (McIlwain & Hughes,

1948). Twelve strains of lactobacilli isolated from beer by Dr T. K. Walker, Manchester College of Technology, were grown at 30° on the casein hydrolysate medium adjusted to pH 5.0. *Acetobacter aceti*, *Aceto. acetosum*, *Aceto. capsulatum*, *Aceto. suboxydans* and *Aceto. turbidens* (supplied by Dr T. K. Walker) were grown at 30° on the yeast extract-glucose medium described by Walker & Tosic (1945). *Proteus morgani*, NCTC, strain 2812, and a local strain of *P. vulgaris* and *Escherichia coli* were grown on a medium consisting of acid-hydrolysed casein (McIlwain & Hughes, 1944) and 2% yeast autolysate. A mutant strain of *Aerobacter aerogenes*, exacting for nicotinic acid, was grown on the inorganic salt medium described by Devi & Higginbottom (1949). Three locally isolated strains of *Pseudomonas fluorescens* were grown on the medium of Koser & Baird (1944).

The organisms were harvested by centrifugation, washed twice and suspended in 0.9% (w/v) NaCl to give a suspension containing from 5 to 40 mg. dry wt./ml.

Estimation of enzyme activity. The rate of deamidation of nicotinamide was estimated by measuring ammonia formation: 0.01M nicotinamide (0.5 ml.), and 0.2M phosphate buffer, pH 7.0 (1.0 ml.) were placed in one side of a double side-arm test tube (Hughes & Williamson, 1952b) and the enzyme solution (0.5 ml. previously adjusted to pH 7) in the other. After 5 min. incubation in a water bath at 40° the contents of the side arms were mixed. The reaction was stopped after varying periods of incubation by the addition of 1.0 ml. of 2N-H₂SO₄ and ice-cooling. The ammonia was distilled in a modified Parnas apparatus, the distillate nesslerized, and the colours read in a Beckman spectrophotometer at 625 mμ. Ammonia was also estimated in the