STUDIES ON ORGANIC FLUORINE COMPOUNDS. VIII.*—*N*-Substituted Fluoroacetamides as Insecticides and Rodenticides

By ERNST D. BERGMANN, P. MOSES and M. NEEMAN

N-Mono- and *NN*-disubstituted fluoroacetamides have been tested as potential larvicides, contact insecticides and rodenticides.

All monosubstituted amides were toxic to larvae of *Musca vicina* Macq. at a concentration of 100 p.p.m. Disubstituted amides were inactive or only slightly active. Only the amides monosubstituted by relatively small aryl radicals (phenyl, *p*-tolyl, *p*-fluorophenyl and *p*-chlorophenyl) showed contact toxicity to adult *Musca vicina* Macq. of a strain highly resistant to DDT.

All compounds were toxic to laboratory rats. The approximate LD_{50} values were lowest (4 to 8 mg./kg.) for *N*-mono-arylamides with small aryl radicals. Those with larger aryl substituents (α- and β-naphthyl, *p*-diphenylyl and *p*-bromophenyl) had somewhat higher LD_{50} values. The *molar* LD_{50} values of the *N*-arylamides fall within a narrow range.

The possible mode of action of the toxicants is discussed.

Introduction

Gitter, Blank & Bergmann[1] have reported the surprising observation that fluoroacetamide is —on a molar basis—much less toxic to mammals than is fluoroacetic acid or its esters and is, indeed, largely excreted unchanged in the urine.† This property of the amide has since been exploited by introducing it as a rodenticide[2, 3] instead of sodium fluoroacetate. Fluoroacetonitrile has also been reported to be non-toxic.[4, 5]

It has further been stated[6] that *N*-methyl-, *N*-(β-hydroxyethyl)- and *N*-(β-chloroethyl)-fluoroacetamide are convulsant poisons for mice, but show a markedly delayed action, and that fluoroacetanilide is a contact and systemic insecticide, but is not a good rodenticide, because of its low solubility in water.[3]

It appeared, therefore, worth while to study more systematically the influence of *N*-substitution on the larvicidal, insecticidal and rodenticidal properties of fluoroacetamide.

Experimental

Materials

The compounds reported in Tables I and II have been prepared by the condensation of fluoroacetyl chloride[7] with the appropriate amine in the presence of excess pyridine. A solution of fluoroacetyl chloride (0·055 mole) in two volumes of chloroform is added, dropwise and with stirring, to a solution of the amine (0·05 mole) in pyridine (0·1 mole) at 0°. The reaction mixture is kept at room temperature for 1–2 hours, and the chloroform distilled off.

The *monosubstituted* amides (Table I) were isolated by dissolving the residue in water and cooling the solution for 12 hours, whereupon the products crystallized out. They were recrystallized from aqueous methanol, with the exception of *N*-(*p*-diphenylyl)- and *N*-(*p*-methoxyphenyl)-fluoroacetamide, which were recrystallized from glacial acetic acid and a benzene–ligroin mixture, respectively.

The NN-*disubstituted fluoroacetamides* (Table II) and *N*-(fluoroacetyl)-piperidine and -morpholine, which were water-soluble liquids, were isolated by ether extraction of the chloroform residue and purified by fractionation under reduced pressure.

Methods of biological tests

Larvicidal activity (house flies).—The tests were carried out on third-stage larvae of *Musca vicina* Macq. (laboratory strain exhibiting slight resistance to DDT).

One-half ml. of a 1% acetone solution of each compound was added dropwise, with stirring, to 25 ml. of distilled water containing 0·3% of the fungistatic agent Nipagin M. Sieved wheat

* Part VII: *J. chem. Soc.*, 1956, p. 1524

† Dr. M. A. Phillips, private communication

Table I

N-*Monosubstituted fluoroacetamides*, $FCH_2{\cdot}CO{\cdot}NHR$

No.	R	Yield (%)	M.p., °C	Analysis N Calc.	Found
I	H	—	—	—	—
II	*iso*-butyl	75	35–36	10·5	10·3
III	*cyclo*-hexyl	38	93–94	8·8	8·9
IV	phenyl	78	71–72[a]	9·2	9·2
V	*p*-tolyl	95	124–126[b]	8·4	8·1
VI	*p*-methoxyphenyl	83	97–99	7·6	7·5
VII	*p*-fluorophenyl	60	114–115	8·2	8·2
VIII	*p*-chlorophenyl	96	126–127[c]	7·5	7·3
IX	*p*-bromophenyl	80	132	6·0	6·8*
X	*p*-diphenylyl	95	174–175[d]	6·1	6·2
XI	α-naphthyl	97	129–130	6·9	6·8
XII	β-naphthyl	97	105[e]	6·9	6·8
XIII	α-pyridyl	52	60–61	18·2	18·1

Notes : [a] Phillips :[3] m.p. 75–76°
[b] Price & Jackson :[8] m.p. 129–130°
[c] Phillips :[3] m.p. 131–132°
[d] Sawicki & Ray :[9] m.p. 179–180°
[e] Sawicki & Ray :[9] m.p. 106–107°
* Calc. : C, 41·4 ; H, 3·1. Found : C, 41·1 ; H, 3·5

bran (25 g.) was mixed with the resulting aqueous suspension, thus producing a toxicant concentration of 100 p.p.m. in the rearing medium. Control media were prepared similarly without toxicant. The test medium was distributed among 7 test tubes, each of which was inoculated with a 1-ml. portion from a culture of a standard bacterial flora.[10] Ten larvae, two days old,

Table II

NN-*Disubstituted fluoroacetamides*, $FCH_2{\cdot}CO{\cdot}NRR'$ [a]

No.	R	R′	Yield (%)	b.p., °c/mm.	n_D^{22}	d_4^{22}	Analysis, N Calc.	Found
XIV	*n*-propyl	*n*-propyl	75	57 0·025	1·4397	1·1019	8·7	8·2
XV	*n*-butyl	*n*-butyl	34	78 0·045	1·4423	0·998	7·4	7·2
XVI	*iso*-pentyl	*iso*-pentyl	60	100 0·03	1·4433	0·887	6·4	6·0
XVII	NRR′ = piperidino		20	78 0·025	1·4740	1·169	9·7	9·7
XVIII	NRR'_2 = morpholino		13	75 0·015	1·4768	1·296	9·5	9·2

[a] *NN*-diallyl compound described by Spegiale & Hamm[9a]

were introduced into each test tube and kept at 35 ± 1° and mortalities recorded after 48 hours. Duplicate tests were run on two different days.

Contact toxicity (house flies).—The test subjects were 2–3 days old females of *Musca vicina* Macq. of a strain highly resistant to DDT. A group of 40 flies was kept in contact for two hours at 27° with a one-day-old residual deposit of the toxicant on glass (1 g./sq. m.).[11] Knock-down counts were taken every 10 min. for the first 30 minutes, and thereafter every half hour.

Rodenticidal activity (rats).—The test subjects were male albino rats. The toxicants, in water or olive oil, were administered by stomach tube and/or by intraperitoneal injection. Symptoms of poisoning were observed, usually after 4–6 hours, and mortality counts were taken 24 hours to 6 days after the administration of the toxicant. Groups of four animals were used for each dose.

Results

The results are shown in Table III.

The observed proportional mortality values of larvae were analysed statistically by the comparison of individual means, as suggested by Tukey[12] and Hald.[13] This method permits the classification of means into groups which can be considered as belonging to a statistically homogeneous population of such means. The observed proportional mortality values of the larvae formed seven statistically homogeneous groups, as shown in Table IV.

Table III

Toxicity of N-substituted α-fluoroacetamides

No.[a]		Toxicity to house flies		Toxicity to rats (LD_{50}, mg./kg.)[e]	
		Larvae, % mortality[b]	Adults, % mortality[d]	Oral	Intra-peritoneal
I		84	0		12 (0·16)
II		65	0	42 (0·32)	31 (0·23)
III		37			31 (0·20)
IV		70[c]	60		6 (0·04)
V		82[c]	57	7 (0·04)	4 (0·02)
VI		61		10 (0·06)	7 (0·04)
VII		83	95	2 (0·01)	5 (0·03)
VIII		75[c]	52		5 (0·03)
IX		72[c]	0		13 (0·06)
X		64	0	10 (0·04)	27 (0·12)
XI		51[c]	0		13 (0·06)
XII		59[c]	0		10 (0·05)
XIII		78	0	8 (0·05)	4 (0·03)
XIV		6	0	20 (0·12)	13 (0·08)
XV		2	0	37 (0·23)	24 (0·13)
XVI		1	20		90 (0·41)
XVII		26	0		150 (1·02)
XVIII		19	0		
XIX		74	0		2 (0·02)
Control	No toxicant	1	0	0	0

[a] The numbers correspond to those in Tables I and II. I is fluoroacetamide, XIX sodium fluoroacetate
[b] 48 hours' mortality at 100 p.p.m. of the toxicant in the larval rearing medium
[c] No significant mortality of larvae at 50 p.p.m.
[d] Mortality of female house flies after two hours' contact with a residual deposit of the toxicant (1 g./sq. m.)
[e] Values in parentheses are millimoles per kg.

Table IV

Group of toxicants (No. of compounds)	Group mean percentage mortality $\bar{p}$[a]	Confidence limits of $\bar{p}$[b] (P = 0·05)
A (I, II, IV, V, VII, VIII, IX, XIII, XIX)	76·9	72·6–82·4
B (VI, X, XII)	60·7	47·7–72·9
C (III, XI, XVII)	40·3	29·5–51·6
D (XVIII)	19·3	5·9–37·7
E (XIV, XV, XVI and control)	1·7	0·1–4·8

[a] The weighted group mean percentage mortality $\bar{p}$ was found by averaging the percentage mortality values of all test batches
[b] Estimated by the method of Hald[13]

Discussion

The most active larvicides were the *N*-arylamides IV, V, VII, VIII, IX, the *N*-(α-pyridyl) compound XIII, and the *N*-alkylamide II. These compounds showed activity of the same order as fluoroacetamide (I) and sodium fluoroacetate (XIX). Somewhat lower larvicidal activity was shown by the *N*-arylamides VI, X, XI and XII. The above findings indicate that *N*-aryl substituents of relatively large size decrease somewhat the larvicidal activity of the amides. Compound VI caused lower mortality than would be expected from this correlation.

The three *NN*-dialkyl-fluoroacetamides (XIV, XV and XVI) were inactive and fluoroacetyl-piperidine (XVII) and -morpholine (XVIII) showed only slight activity. It appears, therefore, that at least one unsubstituted amide hydrogen is required for appreciable larvicidal activity at 100 p.p.m. This correlation applies also to the aliphatic derivative *N-iso*butyl-fluoroacetamide (II). However, the presence of a free amide hydrogen does not necessarily ensure high larvicidal activity, as shown by the behaviour of *N-cyclo*hexyl-fluoroacetamide (III).

The only compounds which showed appreciable contact toxicity to adult flies were *N*-arylamides with relatively small substituents in the *para* position (IV, V, VII, VIII). Heavier *p*-substituents, such as those present in IX, X, XI and XII, reduce the activity, perhaps by

retarding penetration through the cuticula. The inactivity of XIII, as well as of fluoroacetamide (I) and of sodium fluoroacetate (XIX), may be due to their polar character which may likewise retard their cuticular penetration. The inactivity of the remaining compounds parallels in most cases their low larvicidal activity.

The toxicity to rats of the *N*-aryl-fluoroacetamides (IV to XII) and of the *N*-(α-pyridyl) amide (XIII) showed fairly uniform LD_{50} values when calculated *on a molar basis*. All but two of the molar LD_{50} values ranged from 0·02 to 0·06 mmole/kg., with those of X (intraperitoneal, 0·12 mmole/kg.) and VII (oral, 0·01 mmole/kg.) being outside this range. The LD_{50} values of these two compounds, obtained by the alternative method of administration, however, fell within the expected range (X, oral, 0·04 mmole/kg.; VII, intraperitoneal, 0·03 mmole/kg.). Sodium fluoroacetate had a molar LD_{50} value near the lower limit of this range (0·02 mmole/kg.), while the molar LD_{50} of fluoroacetamide, in accord with previous results of Gitter *et al.*,[1] was found to be considerably higher (0·16 mmole/kg.).

The fifteen observed molar LD_{25}, LD_{50} and LD_{75} values of the *N*-aryl-amides (IV to XII) and the *N*-(α-pyridyl)amide (XIII) ranged from 0·015 mmole/kg. to 0·109 mmole/kg. Assuming that these values, as the random variable, have a normal distribution, it is possible to test the null hypothesis that the differences between these values are not significant (as P = 0·05). The estimated standard deviation for this distribution was 2·37. The standardized range of these fifteen values was therefore $W_{15} = \frac{10{\cdot}9 - 1{\cdot}5}{2{\cdot}37} = 3{\cdot}96$, which is smaller than $W_{15,0{\cdot}95} = 4{\cdot}80$.

Hence the two extreme molar dose values (in the mortality range 25–75%) did not deviate significantly from the remaining 13 values.

These findings can be explained by the hypothesis that the monosubstituted amides (IV–XIII) exert their toxic action after conversion into fluoroacetate. The relative rates of penetration, hydrolysis and excretion should thus determine the overall biological response to each of these compounds.

The hypothesis appears to be supported by the constancy of the *molar* rat toxicities, although the statistical weight of the data obtained is relatively low in view of the small numbers of test animals used. Furthermore, no case seems to have been reported in which a *NN*-substituted amide is hydrolysed by an amidase,[14] and even the hydrolysis of acetamide by organ extracts is very slow.[15, 16] It has also recently been reported[17] that salicylamide is excreted after conjugation, but without hydrolysis.

Acknowledgments

The biological tests have been carried out by Dr. H. Ederi, Israel Institute for Biological Research, Ness-Zionah, and Messrs. K. R. S. Ascher and Z. Levinson, Research Laboratories, Medical Corps, Israel Defence Army. The statistical tests have been carried out by Mr. S. Zacks of the Israel Research Council.

Department of Organic Chemistry
Hebrew University
Jerusalem
and
Laboratory of Applied Organic Chemistry
Israel Research Council
Jerusalem

Received 10 October, 1956

References

[1] Gitter, S., Blank, I., & Bergmann, E. D., *K. Ned. Akad. Wetensch.*, 1953, [C] **56**, 4. Cf. Gitter, S., *Biochem. J.*, 1956, **63**, 182
[2] Chapman, C., & Phillips, M. A., *J. Sci. Fd Agric.*, 1955, **6**, 231
[3] Phillips, M. A., *World Crops*, 1955, **7**, No. 12
[4] Gryszkiewicz-Trochimowski, E., Sporzynski, A., & Wnuk, J., *Rec. Trav. chim. Pays-Bas*, 1947, **66**, 419
[5] Buckle, F. J., Heap, R., & Saunders, B. C., *J. chem. Soc.*, 1949, p. 912
[6] Saunders, B. C., 'The Chemistry and Toxicology of Organic Fluorine and Phosphorus Compounds', 1953, Roy. Inst. Chem., Lect. Monogr. & Rep., No. 1, p. 10
[7] Truce, W. E., & Sack, B. H., *J. Amer. chem. Soc.*, 1948, **70**, 3959

[8] Price, C. C., & Jackson, W. G., *J. Amer. chem. Soc.*, 1947, **69**, 1065
[9] Sawicki, E., & Ray, F. E., *J. Amer. chem. Soc.*, 1953, **75**, 4346
[9a] Speziale, A. J., & Hamm, P. C., *J. Amer. chem. Soc.*, 1956, **78**, 5580
[10] Silverman, P. H., & Levinson, F. H., *Biochem. J.*, 1954, **58**, 291
[11] Kocher, C., Roth, W., & Treboux, J., *Anz. Schädlingsk.*, 1953, **26**, 65
[12] Tukey, J. W., *Biometrics*, 1949, **5**, 99
[13] Hald, A., 'Statistical Theory with Engineering Applications', 1952, p. 705 (New York: Wiley & Sons)
[14] See Zittle, Ch. A., in 'The Enzymes', 1951, Vol. I, p. 936 ff. (New York: Academic Press)
[15] Bray, H. G., James, S. P., Ryman, B. E., & Thorpe, W. V., *Biochem. J.*, 1948, **42**, 274
[16] Bray, H. G., James, S. P., Thorpe, W. V., Wasdell, M. R., & Wood, P. P., *Biochem. J.*, 1949, **45**, 467
[17] Hoffmann, W., *Arzneim. Forsch.*, 1955, **5**, 723 (*Chem. Abstr.*, 1956, **50**, 5171)

FOOD PERISHABILITY: THE DETERMINATION OF THE VULNERABILITY OF FOOD SURFACES TO BACTERIAL INFECTION

By D. H. F. CLAYSON and RUTH M. BLOOD

A technique has been devised whereby the limiting conditions of equilibrium relative humidity, and consequently of osmotic pressure, for bacterial growth can be demonstrated, using bacteria in thin films. With *Salmonella typhimurium* and *Escherichia coli* Type I, growth is prevented at and below an e.r.h. of 92% and with *Staphylococcus aureus*, at or below 85%. From the results, it may be deduced that the surface drying that occurs during baking or on exposure of moist foods to normal atmospheres does afford protection against the increase of surface bacterial contamination. The possibility of the production of variants resisting high osmotic pressure cannot be excluded, but no clear evidence of their production was obtained during the present studies.

By a modification of the technique, a heterogeneous article of food can be dissected and the vulnerability of the different parts of the growth of bacterial infection can be determined.

In an earlier paper[1] attention was drawn to the probability that bacterial growth on certain moist foods could be prevented by the formation at their surfaces of layers of high osmotic pressure by evaporation either during cooking or on exposure to atmospheres of moderate or low relative humidity. If the food has been sterilized, or rendered free from all but innocuous heat-resistant organisms, and is subject to bacterial contamination only at its surface, one might deduce that, so long as the osmotic pressure of the surface layer is too high to allow the growth of the contaminants, they will not become established in the food.

Before accepting this deduction it is necessary to establish that growth of various bacterial species does not occur above certain osmotic pressures, otherwise it is possible that contaminating bacteria may grow slowly through the surface layer of high osmotic pressure and thrive when it has been penetrated. The present paper is concerned with the development and application of an apparatus for the study of bacterial growth in nutrient films in equilibrium with atmospheres of known relative humidities, and, therefore, of osmotic pressures corresponding to those relative humidities. This technique is similar in principle to that used by various workers to establish the limiting equilibrium relative humidities (e.r.h.) for various moulds. It is considered preferable to that used by Scott[2] (who studied bacterial growth in media of which the e.r.h. were determined initially by an isopiestic method but were not controlled during the periods of growth) for the following reasons:

(*a*) Hydrolytic changes produced by bacterial growth would have some effect on the osmotic pressure and hence the e.r.h. of a given medium, although these changes may be slight at the stage at which growth can first be detected. In Scott's media, the predominating constituents