277. *Toxic Fluorine Compounds containing the* C–F *Link. Part V.* (a) *Fluorine-containing Ammonium Salts* (with I. G. E. Wilding). (b) *Relationship between Physiological Action and Chemical Constitution.*

By B. C. Saunders.

(*a*) An account is given of some hitherto-undescribed compounds containing both the fluoroethyl group and nitrogen. These compounds fall into two distinct groups: (i) toxic materials such as *ethyl fluoroacetamidoacetate* (I) and *2-fluoroethyl aminoacetate hydrochloride* (II); (ii) relatively non-toxic quaternary salts such as *trimethyl-2-fluoroethylammonium bromide* (IV).
(*b*) The toxic properties of the compounds containing the C–F link described in Parts I—V are reviewed, and certain well-defined conclusions are drawn regarding the relationship between physiological action and chemical constitution.

It is well known that the amino- and the acetamido-group and quaternary ammonium salt groups often render compounds physiologically active. Particular reference may be made to the work of Haworth and his collaborators on the toxic action associated with quaternary ammonium salts (*J.*, 1946, 176, 182).

In view of these facts and of the known toxic action of "fluoroacetates" it seemed worth while investigating compounds containing both fluorine and the above-mentioned groups. We prepared the first such compound in 1943 (Report No. 6 on Fluoroacetates to the Ministry of Supply, Sept. 30, 1943), namely, *ethyl fluoroacetamidoacetate*, $CH_2F{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO_2Et$ (I). It was a colourless crystalline solid which when injected into mice had an L.D. 50 of 20 mg./kg. The corresponding figure for methyl fluoroacetate is 6 mg./kg. The symptoms were similar in each case (delayed convulsant action).

2-Fluoroethyl aminoacetate hydrochloride (II) was readily prepared by esterifying glycine with fluoroethanol (FEA) according to the Fischer–Speier method:

$$NH_2{\cdot}CH_2{\cdot}CO_2H + CH_2F{\cdot}CH_2{\cdot}OH \xrightarrow{HCl} [NH_3{\cdot}CH_2{\cdot}CO_2CH_2{\cdot}CH_2F]^+\,Cl^- \quad (II.)$$

Using similar conditions with betaine and FEA none of the expected ester was obtained, the betaine remaining unchanged. The reaction between anhydrous trimethylamine and fluoroethyl chloroacetate, however, gave *2-fluoroethylbetaine hydrochloride* in excellent yield:

$$NMe_3 + CH_2Cl{\cdot}CO_2CH_2{\cdot}CH_2F \longrightarrow [NMe_3{\cdot}CH_2{\cdot}CO_2CH_2{\cdot}CH_2F]^+\,Cl^- \quad (III.)$$

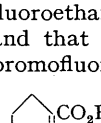
The hydrochloride (II) had an L.D. 50 of approx. 10 mg./kg. by subcutaneous injection into mice. The corresponding figure for 2-fluoroethylbetaine hydrochloride was 45 mg./kg. Both (II) and (III) produced fluoroacetate-like symptoms.

In preparing other compounds containing fluorine and quaternary ammonium groups, advantage was taken of the fact that of the two halogens in 1-bromo-2-fluoroethane, the bromine atom is the more reactive. When, *e.g.*, trimethylamine and bromofluoroethane were allowed to react at room temperature, addition took place and *trimethyl-2-fluoroethylammonium bromide* (IV) was produced. Triethylamine gave *2-fluorotetraethyl ammonium bromide* on being heated with bromofluoroethane.

$\bar{Br}\{\overset{+}{N}Me_3{\cdot}CH_2{\cdot}CH_2F$ (IV.) $C_5H_5\overset{+}{N}{\cdot}CH_2{\cdot}CH_2F\}\bar{Br}$ (V.) $[\overset{+}{N}HMe_2{\cdot}C_6H_4{\cdot}CH_2{\cdot}CH_2{\cdot}\overset{+}{N}HMe_2]\bar{Br}_2$ (VI.)

Pyridine gave *2-fluoroethylpyridinium bromide* (V) on being heated under reflux with bromofluoroethane.

Dimethylaniline did not give the expected phenyldimethyl-2-fluoroethylammonium bromide, but gave the compound (VI) in small yield, the point of attack being the para-hydrogens of the dimethylaniline. This seems surprising in view of the unreactivity of the fluorine in bromofluoroethane. A possible explanation is that $[CH_2F{\cdot}CH_2{\cdot}C_6H_4{\cdot}NMe_2]^+Br^-$ (VII) is first formed and that in this compound the fluorine atom is much more reactive than in the original bromofluoroethane. The fluorine atom of (VII) would then react with the *p*-hydrogen atom of a second molecule of dimethylaniline.


(VIII.)

These fluoro-quaternary bromides proved to be not very toxic and had L.D. 50's of the order of 300 mg./kg.

In view of the biological importance of nicotinic acid, it was decided to prepare a quaternary salt from the acid or ester and bromofluoroethane. *3-Carbethoxy-N-2-fluoroethylpyridinium bromide* (VIII) was therefore prepared and examined. The L.D. 50 for subcutaneous injection into mice was 200 mg./kg., *i.e.*, it was relatively non-toxic compared with methyl fluoroacetate.

It seems possible to draw certain deductions from the above toxicities. It is to be noted that ethyl fluoroacetamidoacetate (I) would almost certainly be hydrolysable in the animal body to free fluoroacetic acid, and that (II) and (III) would similarly give 2-fluoroethanol (oxidisable *in vivo* to fluoroacetic acid). These three compounds do, in fact, show toxicities of the same order as that of methyl fluoroacetate (or of fluoroacetic acid); (III) is, however, rather less toxic than might be expected.

The inactivity of the quaternary bromides is probably due to the inability of the body to rupture the C–N link in the $CH_2F{\cdot}CH_2{\cdot}\overset{+}{N}<$ grouping. The toxicity figures nevertheless reveal an interesting feature. For example, a saline solution of 2-fluorotetraethylammonium bromide injected subcutaneously into mice gave the following results: 500 mg./kg. killed 1/1 in 10 minutes; 400 mg./kg. 4/4 within 90 minutes; 300 mg./kg. killed 2/4 within 2½ hours, and 200 and 100 mg./kg. killed 0/4 and 0/1, respectively. These figures shows a very *rapid* action at the higher concentrations, and the toxicity was therefore probably due to the quaternary ammonium ion rather than to the 2-fluoroethyl group.

Discussion.—A wide variety of compounds containing the C–F link has been described in this and in previous parts and it is now convenient to classify them according to their physiological activity. In general, two types of assessment of animal toxicity * have been made: (*a*) by inhalation (liquids) and given as L.C. 50 in mg./l.; (*b*) by injection (liquids and solids) and given as L.D. 50 in mg./kg. of body weight.

Methyl fluoroacetate (MFA) has an L.C. 50 of 0·1 mg./l. for rabbits, guinea-pigs, and rats. The figure for fluoroethanol (FEA) is similar. For intravenous injection into rabbits MFA, FEA, and free fluoroacetic acid (a solid) have L.D. 50's of the order of 0·25 mg./kg., and for subcutaneous injection into mice the figure is about 6 mg./kg. for each substance. Thus any one of these three substances can be conveniently taken as a reference standard. The symptoms are the same in each case, the action being that of a convulsant poison with delayed action. In these compounds the fluorine is very unreactive chemically; for example, the fluorine atom in fluoroacetic acid is unaffected by water or aqueous alkali, and boiling alcoholic potassium hydroxide solution removed only 50% of the fluorine in 20 hours.

For purposes of comparison the magnitude of the toxicity of fluoroacetic acid is represented as *B*; *A* indicates higher toxicity (up to a factor of 2) and *C* indicates a lower toxicity (down to about ¼ of that of fluoroacetic acid); *D* represents very low or negligible toxicity of the fluoroacetate type.

In class *B* are placed all simple esters $CH_2F{\cdot}CO_2R$ of fluoroacetic acid where R = Me, Et, Pr^n, Pr^i, Ph, etc. When substitution takes place in the α-hydrogen atoms, *e.g.* in methyl α-fluoropropionate or α-fluoro*iso*butyrate, then the compound is devoid of toxicity. This indicates the importance of the unsubstituted fluoromethyl group. In Part III (this vol., p. 912) it was shown that fluoroacetamide and a variety of substituted amides such as $CH_2F{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2Cl$ were, molecule for molecule, equally toxic with fluoroacetic acid and produced the same symptoms. The 2-chloroethyl group therefore contributed nothing appreciable to the toxicity of the molecule. The majority of the esters of fluoroethanol showed the toxicity of the parent alcohol; *e.g.*, 2-fluoroethyl chlorosulphonate, $CH_2F{\cdot}CH_2{\cdot}O{\cdot}SO_2Cl$, di-(2-fluoroethyl) sulphate and 2-fluoroethylglycine hydrochloride.

Fluoroacetaldehyde was as toxic as fluoroacetic acid.

All the toxic compounds mentioned above are either hydrolysable or oxidisable to fluoroacetic acid. In this connexion it should be noted that 1-chloro-2-fluoroethane was non-toxic. The chlorine atom in this compound was shown to be very unreactive chemically, hence hydrolysis to the toxic fluoroethanol in the animal body would be unlikely.

Compounds in which the fluorine atom is loosely bound are relatively non-toxic. Thus the COF group is not toxophoric as shown by the inactivity of acetyl fluoride, chloroacetyl fluoride, and ethyl fluoroformate. Also in the non-toxic class are the quaternary ammonium salts described in this paper, 2:2′-difluorodiethyl ethylene dithioglycol ether (Part IV, this vol., p. 916), was non-vesicant as well as non-toxic, whereas the corresponding chloro-compound is potent in both these respects. 2-Fluoroethyl sulphonyl chloride, $CH_2F{\cdot}CH_2{\cdot}SO_2Cl$, was also non-toxic by inhalation. These facts suggest that the body is unable to rupture the C–N and C–S bonds easily, and so the facile formation of fluoroethanol is prevented.

There is, however, another factor which must not be overlooked. In the highly toxic compounds the fluorine atom is firmly bound, and the toxic action may in some way be connected with this. In the compounds containing N or S in the 2-position to the F atom, the latter may

* See also Saunders and Stacey (*J.*, 1948, 697).

not be so firmly attached. There is some qualitative evidence to support this, but the matter requires further investigation.

The compounds in class *C* definitely show " fluoroacetate-like " activity, but are rather less potent than members of the standard class *B*.

2-Fluoroethyl fluoroacetate is a compound of outstanding toxicity (Part IV, *loc. cit.*). Its L.C. 50 for rabbits (inhalation) is 0·05 mg./l., *i.e.*, about half as great as for MFA. It is therefore placed in class *A*. Other factors apart from hydrolysis to fluoroethanol and fluoroacetic acid appear to be operative, and it seems that the molecule is toxic *per se*. The related fluoroacetylimino 2-fluoroethyl ether hydrochloride, $[CH_2F{\cdot}C(:NH_2){\cdot}O{\cdot}CH_2{\cdot}CH_2F]^+Cl^-$, was also placed in class *A*. This is understandable as it is readily hydrolysed by water to 2-fluoroethyl fluoroacetate. Other fluoroacetylimino ether hydrochlorides containing, however, only one fluorine atom fell into class *B*, as did also fluoroacetamidine hydrochloride itself.

Combination of " fluoroacetate " activity and certain other recognisable physiological effects have been successfully combined in fluoroaspirin (drugged sleep), triethyl-lead fluoroacetate (sternutation), difluoroethyl fluorophosphonate (miosis, but not powerful). In general, quaternary ammonium groups, and the $S{\cdot}CH_2{\cdot}CH_2Cl$ and $N{\cdot}CH_2{\cdot}CH_2Cl$ groups have not contributed anything to the potency of molecules containing them. Occasionally the $CH_2{\cdot}CH_2Cl$ group seems to have had some slight effect as in 2-chloroethyl fluoroacetate.

Apart from chemical considerations, purely physical phenomena, such as rate of diffusion through the cell membrane, must also play their part in determining the toxic action of a compound.

The following summarises the more important features of the above classification. The list is not exhaustive.

Class *A* : 2-Fluoroethyl fluoroacetate.

Class B^+ : 2-Chloroethyl fluoroacetate.

Class *B* : Fluoroacetic acids and salts, *e.g.*, sodium fluoroacetate, triethyl-lead fluoroacetate; all simple esters of fluoroacetic acid; fluoroacetamide and substituted amides; fluoroacetamidine hydrochloride; fluoroacetyl chloride and fluoride; fluoroethanol and its simple esters; fluoroacetaldehyde.

Class *C* : Fluoromethyl cyanide (but more toxic to rabbits); certain 2-fluoroethyl ethers; phenyl fluorothiolacetate, $CH_2F{\cdot}CO{\cdot}SEt$.

Class *D* : Esters of 1-alkylated fluoroacetic acids, $CHR'F{\cdot}CO_2R$ and $CR'R''F{\cdot}CO_2R$; acetyl and chloroacetyl fluoride; alkyl fluoroformates; quaternary ammonium salts, $CH_2F{\cdot}CH_2{\cdot}\overset{+}{N}R_3\overset{-}{X}$, chlorofluoroethane; sulphur-containing compounds, *e.g.*, $CH_2F{\cdot}CH_2{\cdot}SR$.

The synthesis will be described later of further highly toxic compounds containing the C–F link, which lend support to the views expressed above.

Experimental.

Ethyl Fluoroacetamidoacetate.—Glycine ethyl ester hydrochloride (2·8 g.) was dissolved in sodium carbonate solution (25 c.c. of 2N), fluoroacetyl chloride (1·94 g.) added, and the mixture shaken. After the evolution of carbon dioxide had ceased, the solution was carefully acidified with concentrated sulphuric acid (to avoid dilution). No precipitate was produced, so the mixture was extracted three times with ether (3 × 30 c.c.). The combined ethereal extracts were dried (Na_2SO_4), and the ether distilled off. The residue solidified on cooling in ice and salt and gave pale yellow crystals of the *ethyl* ester, m. p. 45—48°, which recrystallised from light petroleum (b. p. 40—60°) as white needle-shaped crystals (after seeding), m. p. 50—50·5°, soluble in water (Found : N, 8·62; F, 11·95. $C_6H_{10}O_3NF$ requires N, 8·94; F, 11·65%).

The condensation of glycine itself with fluoroacetyl chloride in the presence of alkali was attempted, but no derivative was isolated. Similarly fluoroacetic anhydride gave no derivative.

2-Fluoroethyl Aminoacetate Hydrochloride.—Glycine (7·5 g., 0·1 mol.) was added to fluoroethanol (37 g., 0·5 mol.), and the mixture heated under reflux while a stream of dry hydrogen chloride was passed through. After one hour all the glycine had disappeared, and after a further hour the product was cooled. The resultant mixture was extracted with alcohol–ether (1 : 2), leaving *2-fluoroethyl aminoacetate hydrochloride* as almost colourless crystals (12·9 g., 80%). After two recrystallisations from alcohol, the small colourless crystals had m. p. 150—150·5° (Found : N, 8·9; Cl, 22·8. $C_4H_9O_2NClF$ requires N, 8·86; Cl, 22·5%).

2-Fluoroethylbetaine Hydrochloride.—Anhydrous trimethylamine (12·2 g., 0·48 mol., 16 c.c.) and 2-fluoroethyl chloroacetate (29·5 g., 0·48 mol.) were mixed at the temperature of solid carbon dioxide–ether, and then allowed to warm to room temperature. Solid then began to separate and finally the mixture became quite warm. The solid was washed well with dry ether and filtered off. The *hydrochloride* was thus obtained as colourless hygroscopic crystals (36 g., 80%), m. p. 122° (Found : N, 6·8; Cl, 16·9. $C_7H_{15}O_2NClF$ requires N, 7·0; Cl, 17·7%).

Trimethyl-2-fluoroethylammonium Bromide.—Trimethylamine (11·8 g., 0·2 mol.) was mixed with bromofluoroethane (25·4 g., 0·2 mol.) at the temperature of a mixture of solid carbon dioxide and ether.

No reaction took place so the reagents were placed in a tube, sealed, and allowed to warm to room temperature. After 1½ hours' standing at room temperature much solid had separated. The tube was then opened, and the solid filtered off and washed with dry ether to remove pyridine and bromofluoroethane. The colourless crystalline *bromide* so obtained was pure, m. p. 244°; it contained fluorine and ionic bromine; yield 5·2 g. (14%) (this yield could probably have been increased on longer standing) (Found: N, 7·39; Br, 43·2. $C_5H_{13}NBrF$ requires N, 7·53; Br, 43·0%). The salt was readily soluble in water and fairly soluble in alcohol.

2-*Fluorotetraethylammonium Bromide*.—Triethylamine (10·1 g., 0·1 mol.) was heated under reflux with bromofluoroethane (12·7 g., 0·1 mol.) for 3 hours. The *bromide* which had separated was then filtered off and washed well with dry ether, being obtained as fine colourless cubes, m. p. 237° (4 g.) (Found: N, 5·92; Br, 35·1. $C_8H_{19}NBrF$ requires N, 6·14; Br, 35·1%.

2-*Fluoroethylpyridinium Bromide*.—Pyridine (7·9 g., 0·1 mol.) was heated under reflux with bromofluoroethane (12·7 g., 0·1 mol.) for 15 minutes; the product had then separated into two layers, and on cooling and scratching the lower layer crystallised. The crystals were filtered off, washed well with dry ether until free from pyridine and bromofluoroethane, and then dried in a vacuum. The *pyridinium bromide* (Found: N, 6·56; Br, 37·9. C_7H_9NBrF requires N, 6·79; Br, 38·9%) was obtained as colourless hygroscopic crystals, m. p. 180° (11 g., 53%). The compound contained fluorine.

Reaction between Bromofluoroethane and Dimethylaniline.—Anhydrous dimethylaniline (24·2 g., 0·2 mol.) was heated under reflux with anhydrous bromofluoroethane (25·4 g., 0·2 mol.). Solid began to separate after ¾ hour, and after 4¾ hours the solid present was filtered off and washed well with dry ether. The product thus obtained consisted of slightly hygroscopic, colourless, crystals (4 g.) which were recrystallised from aqueous alcohol. The compound (VI) was fluorine-free, contained ionic bromine, and had m. p. 199° (Hübner, Tölle, and Alhenstädt, *Annalen*, 1884, **224**, 346, give m. p. 200°) (Found: N, 6·8; Br, 37·6. Calc. for $C_{18}H_{26}N_2Br_2$: N, 6·5; Br, 37·2%).

3-*Carbethoxy*-N-2-*fluoroethylpyridinium Bromide*.—Ethyl nicotinate (2 g., 0·017 mol., prepared according to Engler, *Ber.*, 1894, **27**, 1787) was heated under reflux in an oil-bath with bromofluoroethane (2 g., 0·016 mol.) for 3 hours at 110°. The mixture had then become very viscous, but could not be crystallised from any organic solvent or water. On being left in a refrigerator, the viscous mass formed large cubic crystals.

The experiment was repeated with ethyl nicotinate (4 g., 0·032 mol.) and a large excess of bromofluoroethane (12 g., 0·1 mol.), the mixture being heated at 120° for 3 hours. The viscous reddish liquid which was formed was seeded with a few crystals from the preceding experiment and left at room temperature. After 2 days a reddish solid crystalline mass was formed. Some of the colour was removed by grinding the crystals (which were extremely hygroscopic) under acetone, and the *pyridinium bromide* was then dried in a vacuum desiccator over sulphuric acid; m. p. 86—88° (Found: Br, 28·9. $C_{10}H_{13}O_2NBrF$ requires Br, 28·8%). The *picrate* was prepared by adding a cold saturated solution of picric acid in alcohol to a concentrated alcoholic solution of the bromide. In a few minutes it began to crystallise in long yellow needles, which were filtered off, twice recrystallised from alcohol, and dried in a vacuum at 100° over phosphoric oxide; m. p. 128—129° (Found C, 44·7; H, 3·7; N, 13·46. $C_{16}H_{15}O_9N_4F$ requires C, 45·1; H, 3·52; N, 13·15%).

Attempted Condensation of Nicotinic Acid with Bromofluoroethane in Organic Solvents.—Nicotinic acid (1 g., 0·008 mol.) was dissolved in dioxan (5 c.c.), and bromofluoroethane (1 g., 0·008 mol.) added; the mixture was heated under reflux on an oil-bath for 3 hours, and the dioxan then removed on a water-pump, but the solid left in the reaction flask proved to be unchanged nicotinic acid. The procedure was repeated on the same scale with methanol and acetone as solvents, with similar results, and finally with bromofluoromethane in excess (10 g., 0·08 mol.) and without the addition of any solvent, but no condensation occurred under these conditions.

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