Toxic Fluorine Compounds

THE USE OF THE ω -FLUORINE ATOM IN THE STUDY OF THE METABOLISM OF FATTY ACIDS CONTAINING ETHYL, *n*-PROPYL AND ISOPROPYL BRANCHES

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1. Some ω -fluoroalkanoic acids containing a branched ethyl, *n*-propyl or isopropyl group in the α - or β -position have been prepared. 2. A study of the toxicity of these acids revealed that the ethyl group partially inhibited, and the *n*-propyl and isopropyl groups completely inhibited, the normal processes of oxidative degradation.

Pattison & Buchanan (1964) described the value of the ω -fluorine atom in elucidating the catabolism of fatty acids containing branched methyl groups; the rationale resided in the characteristic toxicological pattern of the homologous series of ω -fluoroalkanoic acids, $F \cdot [CH_2]_n \cdot CO_2H$. The method appears to be both general and simple, merely involving the preparation of a few compounds and the determination of their toxicity.

The ω -fluorine atom, in its role as a 'tag' (Pattison, 1959), offers several advantages over the more usual approach of isolating breakdown products after the administration of appropriate prototypes to animals. First, the determination of toxicity is simpler and less time-consuming than the isolation and estimation of individual catabolites. Secondly, the fluorine atom, being situated in the ω -position, eliminates the competitive biochemical route of ω -oxidation, allowing full attention to be directed to the structural features under study. Thirdly, the atomic dimensions of the fluorine atom, approximating those of a hydrogen atom, result in the ω -fluoro derivatives being structurally very similar to the parent compounds. The ω -fluorine atom is thus sterically less grotesque than the larger groups, e.g. phenyl, that were used in the early studies of β -oxidation (Knoop, 1904). Fourthly, small quantities of material may be administered, thus avoiding massive doses of prototypes, which, by flooding the system, can readily produce atypical results and from which individual catabolites are frequently isolated in such low percentage yield as to represent an insignificant fraction of the total products of metabolism.

Since previous results on methyl branching showed that *gem*-dimethyl groups in any position inhibited β -oxidation, and it has been well established that β -oxidation occurs up to the vicinity of the branch, no matter where the original branch

may be located (Weitzel, 1951; Pattison & Woolford, 1957), the problem reduces to the metabolism of the α - and β -monoalkyl derivatives.

The ω -fluoro acids were prepared by the general procedures previously used for some of the ω -fluoro branched-methyl derivatives (Pattison & Woolford, 1957; Pattison & Buchanan, 1964).

α -Alkyl- ω -fluoroalkanoic acids were obtained by malonate condensations:

β -Alkyl- ω -fluoroalkanoic acids were obtained from the corresponding α -alkyl- ω -fluoroalkanoic acids by Arndt-Eistert homologation:

RESULTS AND DISCUSSION

The toxicity determinations were carried out on male mice weighing 28–32 g. from a Wistar strain colony maintained at Suffield Experimental Station, Ralston, Alberta, Canada, since 1946. The compounds were administered by intraperitoneal injection in propylene glycol; the total volume never exceeded 0.1 ml. The LD₅₀ values and 95% fiducial limits were calculated by the method of

Table 1. *Toxicities of branched ω -fluoroalkanoic acids*

The 95% confidence limits are given in parentheses.

Compounds	LD ₅₀ found (mg./kg.)				
	R = H*	R = Me†	R = Et	R = Pr ⁿ	R = Pr ⁱ
8-Fluoro-octanoic acids					
F·[CH ₂] ₆ ·CHR·CO ₂ H (α -R)	0.64	1.1‡	5.5 (3.9-7.8)	> 75§	> 100
F·[CH ₂] ₅ ·CHR·CH ₂ ·CO ₂ H (β -R)	0.64	> 100	> 100	> 100	> 100
9-Fluorononanoic acids					
F·[CH ₂] ₇ ·CHR·CO ₂ H (α -R)	> 100	> 100	> 100	> 100	> 100
F·[CH ₂] ₆ ·CHR·CH ₂ ·CO ₂ H (β -R)	> 100	4.5 (1.80-11.2)	25.5 (17.0-38.3)	> 100	> 100

* Pattison, Hunt & Stothers (1956).

† Pattison & Buchanan (1964).

‡ Estimated value.

§ Mortality figures: at 100 mg./kg., 3/5; at 75 mg./kg., 0/5.

Litchfield & Wilcoxon (1949). The results are shown in Table 1.

Some α -branched ω -fluoroheptanoic acids (F·[CH₂]₅·CHR·CO₂H; R = Et, Prⁿ and Prⁱ) were prepared as intermediates in the synthesis of the β -alkyl- ω -fluoro-octanoic acids. They were non-toxic (LD₅₀ > 100 mg./kg. in all cases); the unbranched 7-fluoroheptanoic acid was also non-toxic (LD₅₀ > 80 mg./kg.).

The toxicity results shown in Table 1 include the previously reported values for the parent unbranched ω -fluoroalkanoic acids (Pattison, Hunt & Stothers, 1956) and the corresponding acids containing a branched methyl group (Pattison & Buchanan, 1964).

For the 8-fluoro-octanoic acids containing an α -alkyl branch, it is clear that the α -methyl group offers no hindrance to the normal β -oxidation process. When the branched group is increased in size from methyl to ethyl, the same result is apparent qualitatively, but quantitatively the acid is fivefold less active than the branched methyl analogue. This is probably a steric effect, whereby the larger groups increasingly inhibit the action of the enzyme.

The 7-fluoroheptanoic acids and 9-fluorononanoic acids containing an α -alkyl branch are all non-toxic for one of two reasons: first, the acids containing a methyl or an ethyl branch allow normal β -oxidation to occur but give rise to a non-toxic end product; secondly, β -oxidation of the acids containing a larger branch is inhibited on steric grounds.

A similar pattern can be seen with the 9-fluorononanoic acids containing a β -alkyl branch. The β -methyl group causes complete reversal of toxicity, as explained by Pattison & Buchanan (1964). The β -ethyl group mimics this to a 5.5-fold smaller extent. A steric effect again provides the most likely explanation. As before, a further increase in

size of the branch to n -propyl and isopropyl results in the expected loss of toxic properties.

The 8-fluoro-octanoic acids containing a β -alkyl branch are all non-toxic, as expected, either because a non-toxic catabolite is formed (R = Me or Et) or because degradation is inhibited (R = Prⁿ or Prⁱ). The fact that 8-fluoro-2- n -propyloctanoic acid shows some slight toxicity suggests two possibilities: that some ω -oxidation of the n -propyl branch may be occurring, which by subsequent β -oxidation and decarboxylation might give rise to traces of the toxic 8-fluoro-octanoic acid; or that the n -propyl group may be a less effective steric inhibitor (relative to the isopropyl group) in the formation of the enzyme-substrate complex.

In summary, a branched ethyl group, being larger, gives results that are qualitatively similar to those obtained with the branched methyl group, whereas still larger groups, such as n -propyl and isopropyl, in any position cause complete inhibition of β -oxidation. It is perhaps significant that the 'inhibiting effect' of the ethyl group relative to the methyl group, in both the α - and β -positions, is approximately the same; this consistency provides additional support for the explanation of steric inhibition. Groups still larger than propyl may also inhibit β -oxidation unless some competitive reaction, such as ω -oxidation of the branch, can occur.

Williams (1959) has surveyed much of the earlier work relating to branched acids. In general, acids containing branches larger than methyl in the α - or β -position are metabolized only by ω -oxidation; in no instance has β -oxidation removed the branched group (Weitzel, 1951; Keil, 1947). Because ω -oxidation is ruled out in the present work by the presence of the ω -fluorine atom, it might be supposed that the tendency for β -oxidation to occur would be correspondingly enhanced. Hence the observed lack of toxicity of

Table 2. *Physical constants and analytical results of ω -fluoroalkanoic acids*

Compound	Boiling point		n_D^{25}	Analytical results			
	(°c)	(mm. Hg)		C (%)		H (%)	
				Calc.	Found	Calc.	Found
Alkyl- ω -fluoroalkylmalonic acid esters							
F·[CH ₂] ₅ ·CEt(CO ₂ Et) ₂	106	0.25	1.4296	60.9	61.1	9.1	9.4
F·[CH ₂] ₆ ·CEt(CO ₂ Et) ₂	135	2.00	1.4320	62.0	62.2	9.4	9.4
F·[CH ₂] ₇ ·CEt(CO ₂ Et) ₂	119	0.35	1.4322	63.1	63.0	9.6	9.6
F·[CH ₂] ₅ ·CPr ^m (CO ₂ Et) ₂	107	0.80	1.4317	62.0	62.0	9.4	9.2
F·[CH ₂] ₆ ·CPr ^m (CO ₂ Et) ₂	106	0.20	1.4330	63.1	63.4	9.6	9.4
F·[CH ₂] ₇ ·CPr ^m (CO ₂ Et) ₂	126	0.30	1.4351	64.1	64.4	9.8	9.9
F·[CH ₂] ₅ ·CPr ⁱ (CO ₂ Et) ₂	100	0.20	1.4352	62.0	62.2	9.4	9.2
F·[CH ₂] ₆ ·CPr ⁱ (CO ₂ Et) ₂	108	0.25	1.4366	63.1	63.4	9.6	9.8
F·[CH ₂] ₇ ·CPr ⁱ (CO ₂ Me) ₂	122	0.55	1.4405	62.0	62.2	9.4	9.5
α -Alkyl- ω -fluoroalkanoic acids							
F·[CH ₂] ₅ ·CHEt·CO ₂ H	120	1.50	1.4271	61.3	61.5	9.7	9.6
F·[CH ₂] ₆ ·CHEt·CO ₂ H	135	0.30	1.4297	63.1	63.0	10.1	9.9
F·[CH ₂] ₇ ·CHEt·CO ₂ H	126	0.20	1.4326	64.7	64.6	10.4	10.3
F·[CH ₂] ₅ ·CHPr ^m ·CO ₂ H	108	0.30	1.4301	63.1	63.3	10.1	10.1
F·[CH ₂] ₆ ·CHPr ^m ·CO ₂ H	126	0.55	1.4334	64.7	65.0	10.4	10.4
F·[CH ₂] ₇ ·CHPr ^m ·CO ₂ H	132	0.55	1.4353	66.0	66.2	10.6	10.7
F·[CH ₂] ₅ ·CHPr ⁱ ·CO ₂ H	107	0.30	1.4358	63.1	63.2	10.1	9.9
F·[CH ₂] ₆ ·CHPr ⁱ ·CO ₂ H	122	0.50	1.4353	64.7	65.0	10.4	10.5
F·[CH ₂] ₇ ·CHPr ⁱ ·CO ₂ H	121	0.40	1.4357	66.0	66.3	10.6	10.4
β -Alkyl- ω -fluoroalkanoic acids							
F·[CH ₂] ₅ ·CHEt·CH ₂ ·CO ₂ H	118	0.80	1.4359	63.1	63.3	10.1	10.1
F·[CH ₂] ₆ ·CHEt·CH ₂ ·CO ₂ H	125	0.50	1.4372	64.7	64.5	10.4	10.2
F·[CH ₂] ₅ ·CHPr ^m ·CH ₂ ·CO ₂ H	108	0.20	1.4360	64.7	64.8	10.4	10.4
F·[CH ₂] ₆ ·CHPr ^m ·CH ₂ ·CO ₂ H	127	0.33	1.4381	66.0	66.2	10.6	10.6
F·[CH ₂] ₅ ·CHPr ⁱ ·CH ₂ ·CO ₂ H	118	0.30	1.4414	64.7	64.9	10.4	10.4
F·[CH ₂] ₆ ·CHPr ⁱ ·CH ₂ ·CO ₂ H	120	0.30	1.4401	66.0	66.0	10.6	10.7

the *n*-propyl and isopropyl derivatives clearly shows that β -oxidation is effectively blocked by such branched groups.

EXPERIMENTAL

Refractive indices were measured on a thermostatically controlled Bausch and Lomb refractometer. Analyses were performed by the Schwarzkopf Microanalytical Laboratory, Woodside, 77, New York, N.Y., U.S.A. Fractional distillation was carried out on a 23-plate platinum-plated spinning-band column (18 in.). Physical constants and analytical results are listed in Table 2.

α -Alkyl- ω -fluoroalkanoic acids

Alkyl- ω -fluoroalkylmalonic acid esters. *Diethyl 5-fluoropentyl-n-propylmalonate* was prepared as follows. Diethyl *n*-propylmalonate (41.7 g., 0.207 mole) was added over a period of 5 min. to a stirred solution of sodium (7.2 g., 0.313 g. atom) in ethanol (120 ml.). The solution was stirred and heated under reflux for a further 30 min., and then 5-fluoropentyl bromide (Pattison & Howell, 1956) (35.0 g., 0.207 mole) was run in over 10 min. The resultant mixture was refluxed in an oil bath for 4 hr.; the reflux condenser was then set for distillation, and the bulk of the solvent

was distilled off. Ice-water (250 ml.) was added, and the ester layer was separated and added to two ether extracts of the aqueous layer. The ethereal solution was dried over CaCl₂ and distilled. The yield of product, after fractionation, was 41.6 g. (77%).

The following were obtained in the same way: *diethyl ethyl-5-fluoropentylmalonate* (72%), *diethyl ethyl-6-fluoroheptylmalonate* (66%), *diethyl ethyl-7-fluoroheptylmalonate* (56%), *diethyl 6-fluoroheptyl-n-propylmalonate* (62%) and *diethyl 7-fluoroheptyl-n-propylmalonate* (59%).

Diethyl 5-fluoropentylisopropylmalonate was prepared as follows. A mixture of NaH (6.3 g. of a 56.5% dispersion in mineral oil, 0.149 mole) and dry dimethylformamide (110 ml.) was placed in a 250 ml. flask fitted with a stirrer, a reflux condenser protected by a drying tube, and a dropping funnel. Diethyl isopropylmalonate (30.0 g., 0.149 mole) was added over a period of 15 min. and the solution was stirred until the evolution of hydrogen had ceased (about 1 hr.). 5-Fluoropentyl bromide (Pattison & Howell, 1956) (25.1 g., 0.149 mole) was added slowly and the mixture was heated to 85° in an oil bath and kept at that temperature until the pH had become steady at approx. 6.5 (about 43 hr.). The solution was poured into ice-water (400 ml.). The resultant mixture was extracted with ether and the extracts were washed with water and dried over CaSO₄. Distillation and fractionation gave 35.8 g. (83%) of the pure product.

The following were obtained in the same way: *diethyl 6-fluorohexylisopropylmalonate* (80%) and *dimethyl 7-fluoroheptylisopropylmalonate* (87%).

α -Alkyl- ω -fluoroalkanoic acids. *2-Ethyl-8-fluoro-octanoic acid* was prepared as follows. Diethyl ethyl-6-fluorohexylmalonate (31.8g., 0.11 mole), ethanol (50ml.) and 10% (w/v) NaOH solution (250ml.) were gently heated under reflux until a homogeneous solution was obtained (3–4 hr.). The ethanol was removed by distillation and the residue was cooled to room temperature, acidified with a mixture (1:1 v/v) of conc. HCl and ice, and extracted with ether (4 $\times$ 150ml.). The ether solution was washed with cold water (50ml.) and dried over CaSO₄. The solvent was removed and the residue, consisting of crude ethyl-6-fluorohexylmalonic acid, was decarboxylated by heating in an oil bath at 140–150° for 1.5 hr. Distillation and fractionation of the product gave 19.2g. (92%) of the pure acid.

The following were obtained in the same way: *2-ethyl-7-fluoroheptanoic acid* (82%), *2-ethyl-9-fluorononanoic acid* (85%), *7-fluoro-2-n-propylheptanoic acid* (83%), *8-fluoro-2-n-propyloctanoic acid* (89%), *9-fluoro-2-n-propylnonanoic acid* (73%), *7-fluoro-2-isopropylheptanoic acid* (70%), *8-fluoro-2-isopropyloctanoic acid* (70%) and *9-fluoro-2-isopropylnonanoic acid* (78%).

β -Alkyl- ω -fluoroalkanoic acids

α -Alkyl- ω -fluoroalkanoic acid chlorides. *2-Ethyl-8-fluoro-octanoic acid* (25.0g., 0.132 mole) was added dropwise to gently refluxing thionyl chloride (21.5g., 0.180 mole) at such a rate that the reaction remained under control (about 30 min.). The solution was then heated under reflux for a further 2.5 hr. and distilled without further treatment. The yield of the acid chloride, b.p. 120°/14 mm. Hg, was 25.6g. (94%).

The following were obtained in the same way (yield and b.p. are shown in parentheses): *2-ethyl-7-fluoroheptanoyl chloride* (85%; 105°/12 mm. Hg), *7-fluoro-2-n-propylheptanoyl chloride* (82%; 113°/14 mm. Hg), *8-fluoro-2-n-propyloctanoyl chloride* (87%; 129°/14 mm. Hg), *7-fluoro-2-isopropylheptanoyl chloride* (88%; 126°/14 mm. Hg) and *8-fluoro-2-isopropyloctanoyl chloride* (69%; 130°/16 mm. Hg).

β -Alkyl- ω -fluoroalkanoic acids. *3-Ethyl-8-fluoro-octanoic acid* was prepared as follows. A solution of 2-ethyl-7-fluoroheptanoyl chloride (14.0g., 0.072 mole) in anhydrous ether (80ml.) was added over 15–20 min. to a stirred solution of diazomethane (6.0g., 0.143 mole) in ether (450ml.) at 0–5°. The solution was stirred for a further 2 hr. at that temperature, and was left overnight at room temperature. The solvent was then removed in a rotary evaporator at 20–25°. The yellow residue of crude diazoketone was dissolved in dioxan (250ml.) and was added dropwise to a stirred mixture of freshly precipitated Ag₂O (4g.), Na₂CO₃ (10g.), Na₂S₂O₃ (6g.) and water (400ml.) at 70°. This mixture was stirred for 2 hr. at 65–70° with intermittent addition of further quantities of Ag₂O to maintain a slight excess at all

times. The mixture was heated for 10 hr. at 90°, filtered, acidified with dilute HNO₃ and extracted with ether. The ether solution was separated into neutral and acidic fractions with NaHCO₃ solution and the acidic fraction (in ether) was dried over MgSO₄ and distilled to give the crude acid. Fractionation gave 6.0g. (44%) of the pure product.

3-Ethyl-9-fluorononanoic acid (36%) was obtained in the same way.

9-Fluoro-3-n-propylnonanoic acid was prepared as follows. *8-Fluoro-2-n-propyloctanoyl chloride* (19.0g., 0.085 mole) in anhydrous ether (100ml.) and diazomethane (8.0g., 0.19 mole) in anhydrous ether (600ml.) gave the crude diazoketone as in the previous reaction. This was dissolved in dioxan (137ml.) and added to a solution of AgNO₃ (1.0g.) in water (15ml.) and aq. NH₃ (sp.gr. 0.88) (29ml.). The resultant liquid was refluxed gently on a water bath for 12 hr. The black residue was filtered off; the solution was then diluted with twice its volume of ice-water and extracted with ether (4 $\times$ 100ml.). After removal of the solvent, the resultant crude *9-fluoro-3-n-propylnonanoic acid* was hydrolysed by being heated with a solution of NaOH (40g.) in water (200ml.) and ethanol (50ml.) on a steam bath until a clear solution was obtained and no more NH₃ was evolved. The solution was cooled, acidified with a mixture (1:1, v/v) of conc. HCl and ice, and extracted with ether (4 $\times$ 100ml.). The ether solution was dried over MgSO₄, distilled and fractionated to give 10.5g. (57%) of the pure acid.

The following were prepared in the same way: *8-fluoro-3-n-propyloctanoic acid* (37%), *8-fluoro-3-isopropyloctanoic acid* (37%) and *9-fluoro-3-isopropylnonanoic acid* (40%).

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