581. *Toxic Fluorine Compounds Containing the* C–F *Link. Part VII. Evidence for the β-Oxidation of ω-Fluoro-carboxylic Acids* in vivo.

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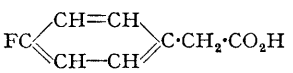
The highly toxic γ-fluorobutyric acid has been "blocked" in the β-position as in *ethyl γ-fluoro-ββ-dimethylbutyrate* (I) and in α- and β-positions as in *methyl 2-fluoromethyl-3* : 6-endo-*methylene-Δ⁴-tetrahydrobenzoate* (IV) and in the related *compounds* (II, III, and V). The non-toxicity of these new fluoro-compounds affords additional support for β-oxidation of ω-fluoro-carboxylic acids *in vivo* (cf. Part VI).

In Part VI (this vol., p. 1461) we showed that there was a striking alternation in the physiological properties of ω-fluoro-carboxylic esters of the general formula $F\cdot[CH_2]_n\cdot CO_2R$. Thus when n was an odd number the compound was highly toxic to animals, whereas when n was even the compound was non-toxic. All the toxic compounds were powerful convulsant poisons and showed symptoms of the "fluoroacetate" type (Part I, *J.*, 1948, 1773). These results were discussed from the point of view of degradation in the animal body, and the conclusion drawn was that, while several factors were involved, the major chemical change concerned was undoubtedly β-oxidation.

We then sought an independent method of proving the β-oxidation theory. This consisted in synthesising ω-fluoro-compounds which contained the "skeleton" of the toxic members, but which could not undergo β-oxidation in the body. If these new compounds were toxic, the β-oxidation theory would have to be abandoned, whereas if they were non-toxic compared with the "parent-acid", then excellent support, of a kind hitherto not considered, would be obtained.

Structurally, the prevention of β-oxidation was achieved by two means: (*a*) side-chain inhibition and (*b*) ring inhibition. (*a*) The β-oxidation of the highly toxic ethyl γ-fluorobutyrate could presumably be stopped by effectively "blocking" the β-position in the chain. A simple compound satisfying this condition was *ethyl γ-fluoro-ββ-dimethylbutyrate*, $CH_2F\cdot CMe_2\cdot CH_2\cdot CO_2Et$ (I), which was found to be entirely devoid of toxic properties. (*b*) The α- and β-carbon atoms of methyl γ-fluorobutyrate were "fixed" by making them part of a ring system. It was considered most unlikely that the animal body could degrade such compounds by a process of β-oxidation. The following compounds in this class were synthesised and found to be non-toxic: *methyl 2-fluoromethyl-4* : 5-*dimethyl-Δ⁴-tetrahydrobenzoate* (II), *methyl 2-fluoromethyl-4* : 5-*dimethylhexahydrobenzoate* (III), *methyl 2-fluoromethyl-3* : 6-endo*methylene-Δ⁴-tetrahydrobenzoate* (IV), and *methyl 2-fluoromethyl-3* : 6-endo*methylene hexahydrobenzoate* (V).

One further compound should be mentioned in this connexion, namely *p*-fluorophenylacetic acid (VI), which has the carbon "skeleton" of the highly toxic 5-fluoropentanecarboxylic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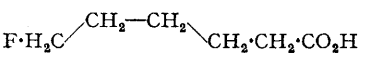
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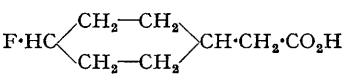
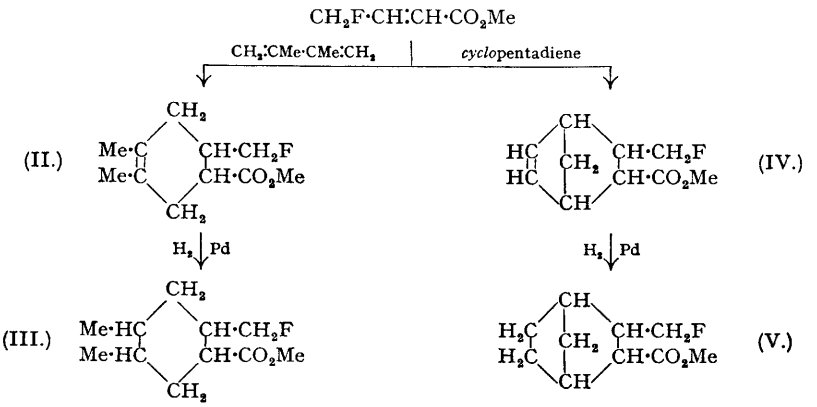

(VIII.)

acid (VII). It seemed unlikely that (VI) could be broken down *in vivo* to fluoroacetic acid, and as expected it was non-toxic. It should be mentioned, however, that aromatic compounds are capable of certain types of oxidative breakdown in the animal body. Jaffé, for example (*Z. physiol. Chem.*, 1909, **62**, 58), isolated small quantities of muconic acid from the urine of dogs and rabbits which had received considerable quantities of benzene.

Synthetic Methods.—ββ-Dimethylglutaric acid (prepared from mesityl oxide) was converted into the disilver salt, which, by an improvement of the method of Windaus and Klänhardt

(*Ber.*, 1921, **54**, *B*, 581), was converted into ββ-dimethyl-γ-butyrolactone. The latter on treatment with constant-boiling hydrobromic and sulphuric acid gave *γ-bromo-ββ-dimethylbutyric acid* which readily gave its *ethyl* ester. The pure *fluoro*-ester was obtained from this by heating with silver fluoride, although the yield was low.

The synthesis of (II), (III), (IV), and (V) from methyl γ-fluorocrotonate was accomplished according to the annexed scheme. Methyl γ-fluorocrotonate had previously been prepared, and was reported by Kharasch and his co-workers (private communication) to be a highly toxic compound. It possesses the carbon structure of methyl γ-fluorobutyrate, and the double bond in the αβ-position would undoubtedly facilitate oxidation. The American workers



prepared methyl γ-fluorocrotonate by an ingenious five-stage process from epifluorohydrin. For the above synthesis we prepared it by the fluorination of methyl γ-bromocrotonate using silver fluoride. The bromo-ester was obtained (1) from methyl crotonate by means of *N*-bromosuccinimide (Zeigler, *Annalen*, 1942, **551**, 103) and (2) by the addition of bromine to methyl vinylacetate and the subsequent removal of hydrogen bromide with sodium ethoxide (Glattfeld and Rietz, *J. Amer. Chem. Soc.*, 1940, **62**, 976).

The Diels–Alder additions of methyl γ-fluorocrotonate to 2 : 3-dimethylbuta-1 : 3-diene and *cyclo*pentadiene were effected by heating the reactants in sealed tubes at 110—120° for about 3 hours. The reduction of the unsaturated products (II and IV) was carried out at room temperature, with palladium as catalyst. In both cases the theoretical quantity of hydrogen was absorbed, although the hydrogenation of the dimethyl derivative was much slower than that of the *endo*methylene compound.

p-Fluorophenylacetic acid had been obtained by Dippy and Williams (*J.*, 1934, 1466) by a rather laborious process. We have now prepared it by an alternative and much improved method from *p*-fluorotoluene. This was treated with sulphuryl chloride and a trace of peroxide giving *p*-fluorobenzyl chloride, thus providing a further example of the free-radical chlorination process first described by Kharasch and Brown (*J. Amer. Chem. Soc.*, 1939, **61**, 2142). The chloride was then converted into the cyanide and thence into the free acid. *p*-Fluorophenylacetic acid was hydrogenated, in the presence of Raney nickel, under varying conditions in alcohol, in a high-pressure hydrogenator, in an attempt to prepare 4-fluoro*cyclo*hexylacetic acid (VIII). This compound bears an even closer resemblance to 5-fluoropentanecarboxylic acid than does *p*-fluorophenylacetic acid. The reduction of the aromatic nucleus could not, however, be effected without the loss of the fluorine atom. Either *p*-fluorophenylacetic acid was recovered unchanged or *cyclo*hexylacetic acid was formed. Experiment 3 (Experimental) is of particular interest: the conditions were clearly the most favourable for producing the required fluoro-compound, since a mixture of equal parts of the unchanged aromatic compound and fully reduced defluorinated product was obtained, yet no trace of ethyl 4-fluoro*cyclo*hexyl acetate was detected.

Experimental.

Silver ββ-Dimethylglutarate.—ββ-Dimethylglutaric acid (120 g., 0·75 mol.) was added to water (300 c.c.), and the mixture was made just alkaline to phenolphthalein using 30% sodium hydroxide solution and then just acid with dilute nitric acid. A slight excess of aqueous ammonia was added and the mixture was then boiled until the steam no longer turned red litmus blue. The neutral glutarate solution was diluted to 2 l., and silver nitrate solution (300 g., 1·8 mols.; in 500 c.c. of water) was slowly

added with constant shaking. The dense, colourless precipitate was filtered, washed several times with water, and drained for 3—4 hours. It was dried by heating in an evaporating-basin for 12 hours on a boiling water-bath, and then by storage over phosphoric oxide *in vacuo* in the dark for 24 hours. It was thus obtained as a faintly-brown crystalline mass (270 g., 96%; overall yield from mesityl oxide, 75%) (Found: C, 23·0; H, 3·0. Calc. for $C_7H_{10}O_4Ag_2$: C, 22·5; H, 2·7%).

ββ-Dimethyl-γ-butyrolactone.—Silver ββ-dimethylglutarate (70 g., 0·19 mol.) and fine sand (240 g.) were intimately ground in a mortar, and then iodine (56 g., 0·44 atom) was added and thoroughly mixed in. The mixture was then slowly heated in a flask immersed in an oil-bath, with constant shaking. At 100° a reaction started with a steady evolution of iodine vapour. When this reaction had subsided, the flask was heated at 150° for 1½ hours. After cooling, the mixture was thoroughly extracted with ether until the extracting liquid was free from iodine. The extract was then washed twice with a solution of potassium carbonate (200 g.), sodium sulphite (50 g.), and potassium hydroxide (5 g.) in water (250 c.c.). The colourless anhydrous layer was finally washed with water and dried (K_2CO_3). After removal of the ether, the residue was usually still contaminated with iodine. Further quantities of silver ββ-dimethylglutarate (making a total of 270 g.) were treated in a similar manner. The several residues were then dissolved in a small quantity of ether and washed twice with aqueous sodium hydrogen sulphite and finally water before drying (K_2CO_3). On distillation under reduced pressure, a colourless liquid was collected which solidified to a resinous mass. B. p. 79·3°/10 mm. M. p. 57·5°. Yield, 35 g. (42·5% based on the silver salt used) (Found: C, 62·7; H, 8·9. Calc. for $C_6H_{10}O_2$: C, 63·1; H, 8·8%).

Recovery of ββ-dimethylglutaric acid from the above degradation. The alkaline washings were neutralised with concentrated hydrochloric acid, evaporated to about half bulk, cooled, and exhaustively extracted with ether. The extract was washed with water, dried, and distilled. The residue, on cooling, solidified. It was slightly brown and was therefore recrystallised twice from benzene (charcoal). Colourless crystals were obtained, having m. p. 101°. M. p. of equal mixture of original acid and recovered acid, 100°. Yield, 39 g. (34% based on silver ββ-dimethylglutarate used) (Found: C, 52·5; H, 7·48. Calc. for $C_7H_{12}O_4$: C, 52·5; H, 7·5%).

The degradation of silver ββ-dimethylglutarate thus yields ββ-dimethyl-γ-butyrolactone in 42·5% yield, while 34% of the silver salt is converted into the parent acid, which can be used again.

γ-Bromo-ββ-dimethylbutyric Acid.—To a cooled mixture of constant-boiling hydrobromic acid (600 g., 3·6 mols.) and concentrated sulphuric acid (140 c.c.) was added ββ-dimethyl-γ-butyrolactone (35 g., 0·31 mol.). This was set aside overnight and was then heated on a boiling water-bath for 10½ hours. The product was cooled and diluted to about 4 l., whereupon the bromo-acid separated as a brown oil. This was extracted 3 times with ether, the extract dried (Na_2SO_4), and the ether removed. The residue was redissolved in a small quantity of ether and shaken twice with sodium thiosulphate solution and then water before drying again. After removal of the ether, the residue was fractionated. Some unchanged lactone came over first (b. p. 80—82°/11 mm.; 3·5 g.), and *γ-bromo-ββ-dimethylbutyric acid* distilled as a colourless liquid, b. p. 127°/12 mm. (10 g., 18·5%) [Found: C, 36·92; H, 5·9; Br, 41·4 (Stepanov). $C_6H_{11}O_2Br$ requires C, 37·0; H, 5·7; Br, 41·0%]. It was essential to isolate the bromo-acid in a pure condition before esterification, because the b. p. of the ethyl ester (95°/12 mm.) is close to that of the unchanged lactone, making subsequent purification difficult.

Ethyl γ-Bromo-ββ-dimethylbutyrate.—A mixture of the bromo-acid (23 g., 0·12 mol.), ethyl alcohol (40 c.c.), and concentrated sulphuric acid (4 c.c.) was heated under reflux in an oil-bath at 120° for 1·3 hours. On cooling, the mixture was diluted and thoroughly extracted with ether. After drying (Na_2SO_4) and removal of the ether, *ethyl γ-bromo-ββ-dimethylbutyrate* (20·3 g., 77%) distilled at 95—97°/12 mm. (Found: Br, 35·6. $C_8H_{15}O_2Br$ requires Br, 35·9%). It possessed an odour almost identical with that of its isomer, ethyl 5-bromopentanecarboxylate.

Ethyl γ-Fluoro-ββ-dimethylbutyrate.—Four portions of ethyl γ-bromo-ββ-dimethylbutyrate (5 g., 0·03 mol.) were fluorinated at 75—80° with silver fluoride (10 g., 0·08 mol.) for 15 minutes. The product was extracted with ether, filtered, washed with water, and dried. After removal of the ether, the residue was fractionated and the liquid of b. p. <80°/13 mm. was collected as the crude fluoro-ester. In every case, some bromo-ester was recovered and this was refluorinated. The yield of crude fluoro-ester was low (1·1 g., 7·5%). Purification by standard macro-methods was therefore impossible, and the micro-method of distillation described in Part VI of this series (this vol., p. 1461) was employed. The crude fluoro-ester was distilled at 13 mm. By slowly heating the oil-bath, in which was immersed the bulb of the flask, a fraction was collected at the oil-bath temperature of 85—95°. This fraction was redistilled, yielding samples at oil-bath temperatures of (*a*) 85—90° and (*b*) 90—95°. The latter sample was found to be pure *ethyl γ-fluoro-ββ-dimethylbutyrate*, a colourless liquid, b. p. *ca.* 80°/13 mm. (by deduction from oil-bath temperature), 193°/762 mm. (by the method of Emich, *Monatsh.*, 1917, **38**, 219) (Found: C, 59·7; H, 9·03. $C_8H_{15}O_2F$ requires C, 59·3; H, 9·3%), having a smell almost identical with that of its isomer, ethyl 5-fluoropentanecarboxylate.

Methyl γ-Fluorocrotonate.—Methyl γ-bromocrotonate (10 g., 0·056 mol.) and silver fluoride (10 g., 0·08 mol.) were mixed thoroughly and then heated gently under reflux until a vigorous reaction occurred with evolution of hydrogen fluoride. The product was cooled and extracted with ether, and the ethereal extracts were washed with water and dried (Na_2SO_4). After removal of the ether, the residue yielded a small fraction of crude methyl γ-fluorocrotonate, b. p. 47°/16 mm., and then unchanged bromo-ester. This fluorination was repeated several times, the recovered bromo-ester being used in each case. Yield: 2·5 g. of crude fluoro-ester from 18·5 g. of pure methyl γ-bromocrotonate. The fluoro-ester was redistilled, and the colourless, pleasant-smelling liquid of b. p. 46°/16 mm. was collected, (2·1 g., 17%) (Found: C, 50·5; H, 6·3; F, 16·6. Calc. for $C_5H_7O_2F$: C, 50·8; H, 5·9; F, 16·2%). Care is needed in handling this material on account of its very high toxicity.

Methyl 2-Fluoromethyl-3: 6-endomethylene-Δ⁴-tetrahydrobenzoate.—Dicyclopentadiene was monomerised by slow distillation through a long column. It was then redistilled immediately before use. *cyclo*Pentadiene (3·5 g., 0·053 mol.) and methyl γ-fluorocrotonate (5 g., 0·043 mol.) were carefully sealed in a thick-walled Carius tube, and then heated in an oil-bath at 110° for 4 hours. After cooling, the

tube was opened and the contents were directly distilled. A small quantity of unchanged methyl γ-fluorocrotonate distilled, and then the remainder of the liquid at 103—104°/13 mm. as a viscous liquid with a pleasant odour. After refractionation, the *tetrahydrobenzoate* distilled at 103°/13 mm. (5 g., 54% based on *cyclo*pentadiene, 64% based on methyl γ-fluorocrotonate) (Found: C, 64·9; H, 7·2; F, 10·4. $C_{10}H_{13}O_2F$ requires C, 65·2; H, 7·1; F, 10·3%).

Methyl 2-Fluoromethyl-3 : 6-endomethylenehexahydrobenzoate.—Methyl 2-fluoromethyl-3 : 6-*endo*-methylene-Δ^4-tetrahydrobenzoate was hydrogenated at room temperature and atmospheric pressure with palladium as catalyst. Palladium oxide catalyst (300 mg.), suspended in absolute alcohol (20 c.c.), was reduced in the hydrogenation apparatus. When the uptake of hydrogen had ceased, the tetrahydrobenzoate (2·18 g.), dissolved in absolute alcohol (50 c.c.), was introduced into the flask and the rate of absorption of hydrogen was noted. The addition was complete in 30 minutes:

Time, mins.	0	1·5	2·5	5·5	7·5	13	20	30
H_2 uptake, c.c.	0	84	134	274	279	284	287	288

2·18 G. of the tetrahydrobenzoate require 288·5 c.c. at 24°/760 mm. The product was filtered, the ethyl alcohol removed, and the residue fractionated. The *hexahydrobenzoate* was obtained as a colourless, pleasant-smelling liquid of b. p. 107°/15 mm. (1·5 g., 69%) (Found: C, 64·8; H, 8·2. $C_{10}H_{15}O_2F$ requires C, 64·5; H, 8·1%). Bromine-water and potassium permanganate were not decolorised. The compound contained fluorine, but a quantitative determination was not carried out, since the theoretical figure was very similar to that of the tetrahydrobenzoate.

Methyl 2-Fluoromethyl-4 : 5-dimethyl-Δ^4-tetrahydrobenzoate.—Methyl γ-fluorocrotonate (5 g., 0·043 mol.) and 2 : 3-dimethylbuta-1 : 3-diene (3·5 g., 0·043 mol.) were sealed in a thick-walled Carius tube and heated in an oil-bath at 110—120° for 7 hours. The product was distilled: the dimethylbutadiene was removed as a gas under reduced pressure, a small amount of methyl fluorocrotonate was recovered, and finally *methyl 2-fluoromethyl-4 : 5-dimethyl-Δ^4-tetrahydrobenzoate*, b. p. 122°/15 mm., was collected as a colourless, pleasant-smelling liquid. This redistilled at 121°/13 mm. (2 g., 24%). The compound contained fluorine (Found: C, 65·6; H, 8·7. $C_{11}H_{17}O_2F$ requires C, 66·0; H, 8·5%).

Methyl 2-Fluoromethyl-4 : 5-dimethylhexahydrobenzoate.—The foregoing compound was hydrogenated at room temperature and atmospheric pressure with palladium as catalyst. The reaction was carried out as described for the 3 : 6-*endo*methylene compound, but 1 g. of unsaturated ester was used, with a correspondingly reduced amount of palladium oxide. The uptake of hydrogen was very much slower, possibly owing to steric effects. The 4 : 5-*dimethylhexahydrobenzoate* was isolated as before, and fractionation yielded a clear, colourless, viscous liquid, b. p. 124°/15 mm. (0·6 g., 60%). The compound contained fluorine (Found: C, 65·6; H, 9·7. $C_{11}H_{19}O_2F$ requires C, 65·4; H, 9·5%).

p-*Fluorobenzyl Chloride.*—Benzoyl peroxide was dried, and recrystallised by dissolving it in a minimum quantity of chloroform and precipitating it with an excess of methanol. It was then filtered off, drained and dried.

Sulphuryl chloride (18 g., 0·13 mol.) was mixed with *p*-fluorotoluene (31 g., 0·28 mol.) in a flask fitted with a reflux condenser and calcium chloride tube, and benzoyl peroxide (0·18 g., 0·0007 mol.) was added. The mixture was then heated in the dark until it started to boil. Evolution of hydrogen chloride and sulphur dioxide began at once, and the reaction proceeded spontaneously for the first 5 minutes, no heat being required. The mixture was then gently heated under reflux for 30 minutes in the dark, after which no more sulphur dioxide or hydrogen chloride was evolved. After being allowed to cool overnight, the mixture was directly distilled through a column under reduced pressure. *p*-Fluorotoluene (13 g.) was recovered and *p*-fluorobenzyl chloride was collected as a colourless liquid, b. p. 70—71°/17·5 mm. (12·2 g., 85% relative to sulphuryl chloride used).

p-*Fluorobenzyl Cyanide.*—*p*-Fluorobenzyl chloride (10 g., 0·07 mol.) in alcohol (10 c.c.) was warmed under reflux on a water-bath, with sodium cyanide (5 g., 0·1 mol.) in water (5 c.c.), for 2 hours. The sodium chloride was removed by filtration and washed with a small quantity of alcohol. As much as possible of the alcohol was removed by heating the mixture on a water-bath, and the *p*-fluorobenzyl cyanide was separated from the aqueous layer. It was dried ($CaCl_2$) for $\geqslant$20 minutes and distilled under reduced pressure, as a colourless liquid, b. p. 115—116°/16 mm. (6·7 g., 72%), smelling strongly of hydrogen cyanide.

p-*Fluorophenylacetic Acid.*—*p*-Fluorobenzyl cyanide (6·0 g., 0·045 mol.) was boiled under gentle reflux with 70% sulphuric acid (40 c.c.) for 20 minutes. The mixture was cooled and an equal volume of water was added. The solid was filtered off, washed with water, and recrystallised from water (charcoal). Beautiful, colourless, diamond-shaped leaves were obtained, having m. p. 85° (4·1 g., 60%). The equivalent weight, determined by titration of a hot solution with sodium hydroxide (phenolphthalein), was 156 (Found: C, 62·2; H, 4·4. Calc. for $C_8H_7O_2F$: C, 62·3; H, 4·5%; equiv., 154).

Attempted Preparation of 4-Fluorocyclohexylacetic Acid.—*p*-Fluorophenylacetic acid (3 g., 0·02 mol.) was dissolved in ethyl alcohol (200 c.c.) in a high-pressure hydrogenator, and Raney nickel (1·5 g.) was added. The mixture was then hydrogenated under varying conditions with stirring. The product was filtered from the nickel, and the filtrate distilled. Results are shown in the table. Esterification of the acid occurred in every instance.

Experiment number.	Time, hours.	Temp.	Pressure, atms.	Product.
1	½	165°	195	Ethyl *p*-fluorophenylacetate.
2	2	200	165	,, ,,
3	½	245	170	1 : 1 Mixture of ethyl *p*-fluorophenylacetate and ethyl *cyclo*hexylacetate.
4	2¼	200	180	Ethyl *cyclo*hexylacetate.
5	3½	200	160	,, ,,
6	5	180	180	,, ,,

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