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Toxic Fluorine Compounds

20. THE USE OF THE ω -FLUORINE ATOM IN THE STUDY OF THE METABOLISM OF BRANCHED-CHAIN FATTY ACIDS*

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Branched-chain fatty acids occur in the lipid fractions of certain bacteria and micro-organisms, and in many types of synthetic fats; hence a study of their mode of breakdown is of some medical importance. In the past, a common approach to this type of problem has been to isolate individual catabolites after administering acid prototypes to animals, and hence to deduce a likely metabolic pathway (see, e.g., Williams, 1959). The present paper describes a 'chemical' approach to this type of biochemical problem, based on the unique toxicological properties of the ω -fluorine atom (Pattison, 1959*b*). The rationale centres around the fact that compounds that ultimately can give rise to ω -fluoroalkanoic acids, $F\cdot[CH_2]_n\cdot CO_2H$, containing an even number of carbon atoms are toxic whereas those that give ω -fluoroalkanoic acids with an odd number of carbon atoms are non-toxic

(Buckle, Pattison & Saunders, 1949; Pattison, Hunt & Stothers, 1956*a*).

For fatty acids with a single branched methyl group, $CH_3\cdot[CH_2]_n\cdot CHMe\cdot[CH_2]_m\cdot CO_2H$, two routes of degradation, depending on the value of m , are possible (Scheme 1). The problem thus reduces to the metabolism of the α - and β -methyl derivatives. Theoretically there are two ways in which each of these can break down. However, work by Pattison & Woolford (1957*b*) indicated that, in the overall β -oxidation process, the α -methyl group is eliminated as part of a C_3 fragment, whereas the β -methyl group is eliminated as part of a C_4 fragment, i.e. route (a) in each case. *gem*-Dimethyl groups appeared to inhibit β -oxidation, as expected.

To confirm these conclusions, it was decided to prepare all possible variations of methyl substitution in two adjacent ω -fluoroalkanoic acids, and to determine the toxicities (see Table 1).

* Part 19: Pattison & Dear (1963).

One other acid, 10-fluoro-2-methyldecanoic acid, $F \cdot [CH_2]_8 \cdot CHMe \cdot CO_2H$, was prepared for testing for antitubercular activity. ω -Fluorotuberculo-stearic acid, $F \cdot [CH_2]_{18} \cdot CHMe \cdot [CH_2]_8 \cdot CO_2H$ (Pattison & Woolford, 1957*a*), is active *in vitro* against virulent *Mycobacterium tuberculosis* but inactive *in vivo*; it was thought that high lipophilicity might account for this discrepancy. 10-Fluoro-2-methyl-decanoic acid retains most of the original characteristic features of the longer-chain member, but contains eight carbon atoms less. (These, it might be supposed, would be lost *in vivo* by β -oxidation.) Thus the two compounds might be considered to be basically the same, but the decreased lipophilicity of the shorter-chain member might confer more favourable properties.

RESULTS

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, since 1946. The compounds were usually administered by intraperitoneal injection in propylene glycol; the total volume never exceeded 0.1 ml. The LD_{50} values and 95% fiducial limits were calculated by the method of Litchfield & Wilcoxon (1949). The results are shown in Table 1.

The toxicities of two of the malonate intermediates were determined for comparison: under the same conditions of testing, diethyl 6-fluoro-hexylmethylmalonate, $F \cdot [CH_2]_6 \cdot CMe(CO_2Et)_2$, had LD_{50} (to mice) 18.1 (15.3–27.1) mg./kg., and the next higher homologue, diethyl 7-fluoroheptylmethylmalonate, $F \cdot [CH_2]_7 \cdot CMe(CO_2Et)_2$, had LD_{50} greater than 94 mg./kg.

The antitubercular testing showed that 10-fluoro-2-methyldecanoic acid gave only partial inhibition of growth of the virulent human tubercle bacillus *Mycobacterium tuberculosis* var. *hominis* (H37 RV) at the highest concentration tested (20 μ g./ml.). Its precursor, diethyl 8-fluoro-octylmethylmalonate, $F \cdot [CH_2]_8 \cdot CMe(CO_2Et)_2$, gave no inhibition.

Scheme 1. Possible routes of degradation of fatty acids with a single branched methyl group, $CH_3 \cdot [CH_2]_n \cdot CHMe \cdot [CH_2]_m \cdot CO_2H$, when m is odd (eqn. 1) or even (eqn. 2).

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

Predictions are based on the conclusions outlined in the introduction; 'toxic' and 'non-toxic' are relative terms, and are arbitrarily assigned as LD_{50} values of less than 25 mg./kg. and more than 75 mg./kg. respectively.

Compounds	Prediction	LD_{50} found (mg./kg.)
8-Fluoro-octanoic acids		
$F \cdot [CH_2]_7 \cdot CO_2H^*$	Toxic	0.64
$F \cdot [CH_2]_6 \cdot CHMe \cdot CO_2H$ (α -Me)	Toxic	1.1†
$F \cdot [CH_2]_5 \cdot CHMe \cdot CH_2 \cdot CO_2H$ † (β -Me)	Non-toxic	118
$F \cdot [CH_2]_6 \cdot CMe_2 \cdot CO_2H$ (α -Me ₂)	Non-toxic	> 100
$F \cdot [CH_2]_5 \cdot CMe_2 \cdot CH_2 \cdot CO_2H$ † (β -Me ₂)	Non-toxic	121
9-Fluorononanoic acids		
$F \cdot [CH_2]_8 \cdot CO_2H^*$	Non-toxic	> 100
$F \cdot [CH_2]_7 \cdot CHMe \cdot CO_2H$ (α -Me)	Non-toxic	> 100
$F \cdot [CH_2]_6 \cdot CHMe \cdot CH_2 \cdot CO_2H$ (β -Me)	Toxic	4.5 (1.80–11.2§)
$F \cdot [CH_2]_7 \cdot CMe_2 \cdot CO_2H$ (α -Me ₂)	Non-toxic	> 100
$F \cdot [CH_2]_6 \cdot CMe_2 \cdot CH_2 \cdot CO_2H$ (β -Me ₂)	Non-toxic	185
10-Fluorodecanoic acids		
$F \cdot [CH_2]_9 \cdot CO_2H^*$	Toxic	1.5
$F \cdot [CH_2]_8 \cdot CHMe \cdot CO_2H$ (α -Me)	Toxic	1.7–2.0†
$F \cdot [CH_2]_7 \cdot CHMe \cdot CH_2 \cdot CO_2H$ † (β -Me)	Non-toxic	65

* Pattison *et al.* (1956*a*).

† Estimated values.

‡ Pattison & Woolford (1957*b*).

§ 95% confidence limits.

DISCUSSION

Three different reactions were involved in the preparation of the various branched acids:

(a) Nucleophilic substitution by the methylmalonate anion gave rise to the three α -methyl derivatives:

(b) Unsymmetrical anodic coupling formed the β -methyl and the $\beta\beta$ -dimethyl esters, which in turn could be hydrolysed to the free acids:

(c) The Barbier-Wieland degradation was employed for obtaining the $\alpha\alpha$ -dimethyl derivatives:

This is the first successful application of the Barbier-Wieland degradation to monofluoroalkanoic acids. In a previous attempt (Buckle & Saunders, 1949) the fluorine was preferentially replaced by bromine during the reaction; this was substantiated in our first attempts in this work, with methyl 10-fluorodecanoate as a model. However, by forming the carbinol in the cold and rapidly quenching the reaction mixture with acid, the halogen exchange was entirely avoided; the required products were thus obtained in satisfactory yield.

The complete success of the predictions, as shown in Table 1, provides a novel type of proof for the breakdown of the branched-chain fatty acids. The striking difference in the metabolism of the two types of monomethyl branches (α - and β -) is well exemplified in the two isomeric fluorinated methyl-octanoic acids: the mere shift of the methyl group to the adjacent carbon atom results in one acid being over 100 times as toxic as the other. It is also of interest to consider the progressive substitution of methyl groups in the β -position of 9-fluorononanoic acid. The unsubstituted acid is of course non-toxic; the substitution of one methyl group results in a remarkable increase in toxicity; whereas the substitution of a second methyl group causes the acid to be non-toxic again.

In short, the presence of an α -methyl group in

these fluoro acids has apparently no effect on the β -oxidation process, whereas the β -methyl group causes complete reversal of the toxicological pattern. These results, when applied to non-fluorinated fatty acids, substantiate the statement of the overall oxidation process shown by routes (1a) and (2a) in Scheme 1.

Such a conclusion is consistent with other lines of biochemical investigation. Published work on the metabolic fate of α -methylalkanoic acids has suggested that intermediate steps involve malonate formation or simply elimination of propionyl-CoA by the standard β -oxidation process (see, e.g., Williams, 1959; Green & Wakil, 1960); but, whatever the mechanism, the overall loss of a C_3 fragment has been inferred or confirmed by several workers (Kay & Raper, 1924; Carter, 1941; Atchley, 1948; Coon & Abrahamsen, 1952; Robinson, Bachhawat & Coon, 1956).

The metabolic fate of β -methylalkanoic acids has been studied less extensively. Intermediates that have been proposed include β -unsaturated acids, formed by dehydrogenation (Pattison & Woolford, 1957b), and methyl ketones, formed by the loss of two carbon atoms (Bloch, 1944; Zabin & Bloch, 1949; White, Handler, Smith & Stetten, 1959). Though the evidence thus favours the overall loss of a C_4 unit by some as yet unconfirmed mechanism, the work described in the present paper gives unequivocal support for such a conclusion.

In the *gem*-dimethyl derivatives the quaternary carbon atom at the α - or β -position must inevitably block β -oxidation by inhibiting dehydrogenation of the CoA thiol ester. For example, 3,3-dimethylbutanoic acid has been recovered unchanged in the rabbit urine as its ester glucuronide (Dziewiatkowski & Lewis, 1945), 4-fluoro-3,3-dimethylbutanoic acid is non-toxic (Pattison & Saunders, 1949), and 2,2-dimethyloctadecanoic acid and 2,2-dimethylnonadecanoic acid are metabolized only by ω -oxidation (Bergström, Borgström, Tryding & Westöö, 1954; Tryding & Westöö, 1956). ω -Oxidation occurs only to a slight extent with α -methylalkanoic acids (Tryding & Westöö, 1957), but this must be completely inhibited by the presence of an ω -fluorine atom. Indeed, this elimination of a competitive reaction route from the system is one of the attractive features of the technique involving the ω -fluorine atom, allowing full attention to be directed to the key portion of the molecule.

The use of the ω -fluorine atom has been used for the elucidation of the catabolism of many different types of compounds (Pattison, 1959a, b). Work is in progress in applying this technique to fatty acids containing other kinds of branching, and to a variety of other functional groups.

EXPERIMENTAL

Melting points (uncorrected) were determined on a Kofler hot stage, and densities with a Fisher-Davidson gravitometer. Infrared-absorption spectra were obtained with Beckman IR-7 and IR-5 infrared spectrophotometers. Refractive indices were measured on a thermostatically-controlled Bausch and Lomb refractometer. Analyses were performed by the Schwarzkopf Micro-analytical Laboratory, Woodside 77, New York, N.Y., U.S.A. Most of the reactions leading to acids were followed by vapour-phase chromatography of the corresponding methyl esters, and by infrared spectroscopy. Fractional distillation was carried out on a 23-plate platinum-plated spinning-band column (18 in.).

 ω -Fluoro- α -methylalkanoic esters and acids

(a) ω -Fluoroalkylmethylmalonic acid diethyl esters. Diethyl 7-fluoroheptylmethylmalonate was prepared as follows. Anhydrous dimethylformamide (50 ml.) and sodium hydride dispersion (56.5% in mineral oil) (1.73 g., 40.6 m-moles) were placed in a 100 ml. three-necked flask fitted with a stirrer, a reflux condenser protected by a drying tube, and a dropping funnel. Diethyl methylmalonate (7.07 g., 40.6 m-moles) was added dropwise with stirring over 20 min. After the initial vigorous evolution of hydrogen had ceased, the mixture was heated at 70–90° for 4.5 hr. to complete the formation of the enolate. The mixture was allowed to cool, and 7-fluoroheptyl bromide (Pattison & Howell, 1956) (8.0 g., 40.6 m-moles) was added rapidly with stirring. The mixture was then stirred for 18 hr. at 80–90°, when the pH of the solution had levelled off at approx. 7.5. The product was isolated by dilution with water, ether extraction, drying of the extract (over MgSO_4), removal of the ether and fractional distillation. Some unchanged 7-fluoroheptyl bromide (0.72 g.) was recovered. The yield was 8.05 g. (74.9% based on bromide utilized). The diethyl 7-fluoroheptylmethylmalonate had b.p. 93.5–96.5°/0.075 mm. Hg, n_D^{25} 1.4298 and d_4^{20} 1.012 (Found: C, 62.1; H, 9.4. $\text{C}_{15}\text{H}_{27}\text{FO}_4$ requires C, 62.0; H, 9.4%).

Diethyl 6-fluorohexylmethylmalonate, obtained similarly in 60% yield from 9.0 g. of 6-fluorohexyl bromide (Pattison & Howell, 1956), had b.p. 89–92°/0.075 mm. Hg, n_D^{25} 1.4284 and d_4^{20} 1.012 (Found: C, 61.0; H, 8.9. $\text{C}_{14}\text{H}_{25}\text{FO}_4$ requires C, 60.9; H, 9.1%).

Diethyl 8-fluoro-octylmethylmalonate, obtained similarly in 55.3% yield from 8-fluoro-octyl chloride (6.0 g.) and sodium iodide (6.0 g.), had b.p. 102–103°/0.07 mm. Hg and n_D^{25} 1.4320 (Found: C, 63.3; H, 9.5. $\text{C}_{16}\text{H}_{29}\text{FO}_4$ requires C, 63.1; H, 9.6%). The heating period in this preparation was 52 hr. The 8-fluoro-octyl chloride was prepared from 1,8-dichloro-octane by the partial fluorination technique (Pattison & Howell, 1956) in 53.2% yield, b.p. 84.5–87.5°/9 mm. Hg and n_D^{25} 1.4273.

(b) ω -Fluoro- α -methylalkanoic acids. 9-Fluoro-2-methylnonanoic acid was prepared as follows. A mixture of diethyl 7-fluoroheptylmethylmalonate (7.67 g., 26.4 m-moles), 6N-hydrochloric acid (60 ml.) and dioxan (150 ml.) was heated under reflux for 41 hr. Isolation of the products [dilution with water, ether extraction, washing well with water, drying (over MgSO_4) and removal of the solvent] gave a mixture of 7-fluoroheptylmethylmalonic acid (m.p. 118–121°) and the required nonanoic acid. The former

was decarboxylated by heating at 155° under slight vacuum. Fractional distillation of the combined samples gave the pure acid. The yield was 4.03 g. (80.5%). The 9-fluoro-2-methylnonanoic acid had b.p. 88–89°/0.07 mm. Hg and n_D^{25} 1.4308 (Found: C, 63.0; H, 10.0. $\text{C}_{10}\text{H}_{19}\text{FO}_2$ requires: C, 63.1; H, 10.1%).

8-Fluoro-2-methyloctanoic acid, obtained similarly in 88.8% yield, had b.p. 102–106°/0.2–0.35 mm. Hg and n_D^{25} 1.4274 (Found: C, 61.6; H, 9.7. $\text{C}_9\text{H}_{17}\text{FO}_2$ requires: C, 61.3; H, 9.7%). No intermediate malonic acid derivative was observed.

10-Fluoro-2-methyldecanoic acid, obtained similarly in 56.8% yield, had b.p. 92–93°/0.15 mm. Hg and n_D^{25} 1.4356 (Found: C, 64.9; H, 10.3. $\text{C}_{11}\text{H}_{21}\text{FO}_2$ requires C, 64.7; H, 10.4%). After the acid treatment, most of the product was in the form of 8-fluoro-octylmethylmalonic acid [recrystallized from light petroleum (b.p. 60–80°)], m.p. 99–101.5° (Found: C, 57.8; H, 8.4. $\text{C}_{12}\text{H}_{21}\text{FO}_4$ requires C, 58.0; H, 8.5%). Decarboxylation was accomplished by heating for 1.5 hr. at 150° under 9 mm. Hg pressure.

 ω -Fluoro- $\beta\beta$ -dimethylalkanoic esters and acids and ω -fluoro- β -methylalkanoic acids

The reactions were carried out in a Kolbe electrolysis cell (Pattison, Stothers & Woolford, 1956b; Pattison & Woolford, 1957a, b).

(a) ω -Fluoro- $\beta\beta$ -dimethylalkanoic esters and acids. Methyl 9-fluoro-3,3-dimethylnonanoate was prepared as follows. Methyl hydrogen $\beta\beta$ -dimethylglutarate (Cason, Sumrell & Mitchell, 1950) (5.65 g., 31.7 m-moles) and sodium (0.21 g., 9.5 mg.atoms) were dissolved in methanol (20 ml.). A current of 1.6 A was applied, and, as the current dropped, a solution of 6-fluorohexanoic acid (Pattison *et al.* 1956b) (8.5 g., 63.4 m-moles) and methyl hydrogen $\beta\beta$ -dimethylglutarate (16.95 g., 95.1 m-moles) in methanol (50 ml.) was added dropwise to maintain the current at 1.5–1.6 A. This current was continued until the solution had become slightly alkaline (3.5 hr.). The direction of the current was reversed periodically throughout the reaction. The mixture was diluted with water, acidified with acetic acid and extracted three times with ether. The ether extracts were washed twice with 5% (w/w) NaHCO_3 and dried over anhydrous MgSO_4 . After removal of the ether, the residue was fractionally distilled. Three products were obtained: 1,10-difluorodecane (yield 1.92 g., 17%), b.p. 88–98°/9 mm. Hg and n_D^{25} 1.4114; dimethyl $\beta\beta\beta\beta$ -tetramethylsuberate (yield 9.13 g., 27.8%), b.p. 80–82°/0.1 mm. Hg and n_D^{25} 1.4421; and the required methyl 9-fluoro-3,3-dimethylnonanoate (yield 6.99 g., 50.4%), b.p. 57.5–59°/0.1 mm. Hg, n_D^{25} 1.4265 and d_4^{20} 0.949 (Found: C, 65.9; H, 10.5. $\text{C}_{12}\text{H}_{23}\text{FO}_2$ requires C, 66.0; H, 10.6%).

The free 9-fluoro-3,3-dimethylnonanoic acid was obtained by heating the crude ester under reflux for 3 hr. with 10% (w/w) NaOH. The mixture was diluted with water and extracted with ether. The solution was acidified (with conc. HCl) and extracted with ether. Drying of the extracts (over MgSO_4), removal of the ether and fractional distillation gave the pure product. The 9-fluoro-3,3-dimethylnonanoic acid had b.p. 97–98°/0.06 mm. Hg and n_D^{25} 1.4369 (Found: C, 64.6; H, 10.6. $\text{C}_{11}\text{H}_{21}\text{FO}_2$ requires C, 64.7; H, 10.4%).

Methyl 10-fluoro-3,3-dimethyldecanoate, obtained similarly in 31.4% yield from 7-fluoroheptanoic acid (Pattison *et al.* 1956b), had b.p. 135–135.5°/8 mm. Hg, n_D^{25} 1.4302 and

d_{40}^{20} 0.938 (Found: C, 67.4; H, 11.0. $C_{13}H_{22}FO_2$ requires: C, 67.2; H, 10.9%). Separation of the ester from the accompanying 1,12-difluorododecane required very careful fractionation on the spinning-band column, with a reflux ratio of 20:1.

(b) ω -Fluoro- β -methylalkanoic acids. 9-Fluoro-3-methylnonanoic acid was obtained by the same procedure from 6-fluorohexanoic acid; in this case the appropriate half ester was methyl hydrogen β -methylglutarate (Linstead, Lunt & Weedon, 1950). The intermediate ester was hydrolysed, as described above, without purification, giving the acid in 21.4% overall yield. The 9-fluoro-3-methylnonanoic acid had b.p. 92–93°/0.06 mm. Hg and n_D^{25} 1.4319 (Found: C, 63.0; H, 10.3. $C_{10}H_{18}FO_2$ requires C, 63.1; H, 10.1%).

8-Fluoro-3-methyloctanoic acid, 10-fluoro-3-methyldecanoic acid and 8-fluoro-3,3-dimethyloctanoic acid have been described by Pattison & Woolford (1957b).

ω -Fluoro- α -dimethylalkanoic acids

Trial experiments involving the Barbier–Wieland degradation were carried out with methyl 10-fluorodecanoate as a model. Phenylmagnesium chloride in tetrahydrofuran (Ramsden, Balint, Whitford, Walburn & Cserr, 1957) was first examined as the Grignard reagent least likely to promote halogen exchange, but reaction was very sluggish even under reflux conditions. The use of phenylmagnesium bromide, which in the past had caused halogen exchange (Buckle & Saunders, 1949), resulted in the formation only of 9-bromononanoic acid [recrystallized from light petroleum (b.p. 30–60°)], m.p. 35–36° (Found: C, 45.7; H, 7.3. Calc. for $C_9H_{17}BrO_2$: C, 45.6; H, 7.2%). Halogen exchange and the required degradation had thus occurred. When this last reaction was performed in the cold and the mixture quenched with acid as soon as the solid complex was precipitated, satisfactory yields of the required 9-fluorononanoic acid were obtained, b.p. 83–84°/0.05 mm. Hg and n_D^{25} 1.4321 (Found: C, 61.5; H, 9.6. Calc. for $C_9H_{17}FO_2$: C, 61.3; H, 9.7%).

Methyl 10-fluorodecanoate. This ester was obtained in 87% yield by the method of Clinton & Laskowski (1948), b.p. 63–64.5°/0.1 mm. Hg and n_D^{25} 1.4232 (Found: C, 65.0; H, 10.2. $C_{11}H_{21}FO_2$ requires C, 64.7; H, 10.4%).

8-Fluoro-2,2-dimethyloctanoic acid. Phenylmagnesium bromide was prepared from dry magnesium turnings (1.4 g., 57.5 mg.atoms) and bromobenzene (8.64 g., 55 m-moles) in anhydrous ether. The Grignard reagent was cooled in ice, and methyl 9-fluoro-3,3-dimethylnonanoate (5.8 g., 26.5 m-moles) in anhydrous ether was added dropwise over 10 min. with stirring. Cooling was continued for a further 10 min. and the mixture was then allowed to stand at room temperature for 45 min., when precipitation of the Grignard reagent–ester complex started. The mixture was immediately treated with excess of cold 6N-hydrochloric acid, sufficient to give a clear solution. The crude diphenylcarbinol was isolated by separation and drying of the ether layer, and removal of the ether and lower-boiling substances by distillation. It was then converted into the crude diphenylethylene derivative by refluxing with excess of acetic anhydride (100 ml.) for 1.5 hr. (Lane & Wallis, 1941). The product was isolated by addition of water, evaporation to small volume, addition of a further quantity of water, extraction with ether, washing the extracts with 5% $NaHCO_3$, drying (over $MgSO_4$) and removal of the ether. The crude material was then cleaved oxidatively

(Fierz-David & Kuster, 1939). It was dissolved in acetic acid (50 ml.) contained in a 250 ml. flask equipped with a stirrer, a reflux condenser and a dropping funnel. A solution of chromium trioxide (9.42 g., 94.2 m-moles) in water (8 ml.) and acetic acid (20 ml.) was added dropwise with stirring and occasional cooling over 30 min. It was then stirred at room temperature for 15 min. and at 70–100° for 30 min.; finally it was allowed to stand at room temperature for 6 hr. The mixture was cooled in an ice bath and the excess of chromium trioxide was decomposed by the dropwise addition of an excess of methanol (100 ml.). Most of the solvent was removed by evaporation and sufficient water was added to dissolve the precipitated salts. The aqueous solution was then extracted with ether; the extracts were washed with water and extracted with 1% NaOH solution to dissolve the acid. Acidification, extraction with ether, drying (over $MgSO_4$), removal of the ether and fractionation gave 8-fluoro-2,2-dimethyloctanoic acid (yield 2.58 g., 51.1%), b.p. 96–96.5°/0.16 mm. Hg and n_D^{25} 1.4314 (Found: C, 63.0; H, 10.1. $C_{10}H_{18}FO_2$ requires C, 63.1; H, 10.1%).

9-Fluoro-2,2-dimethylnonanoic acid. This acid, obtained similarly in 53.1% yield from methyl 10-fluoro-3,3-dimethyldecanoate, had b.p. 96–98°/0.16 mm. Hg and n_D^{25} 1.4355 (Found: C, 64.8; H, 10.2. $C_{11}H_{21}FO_2$ requires C, 64.7; H, 10.4%).

SUMMARY

1. The ω -fluorine atom has been used as a tracer for studying metabolic breakdown processes.
2. Its application to fatty acids containing a single branched methyl group showed that an α -methyl group is eliminated as part of a C_3 fragment, whereas a β -methyl group is eliminated as part of a C_4 fragment (possibly in two stages).
3. Its application to fatty acids containing α - or β -gem-dimethyl groups showed that β -oxidation is blocked.
4. 10-Fluoro-2-methyldecanoic acid showed only slight activity against *Mycobacterium tuberculosis* var. *hominis*.
5. The Barbier–Wieland degradation has been applied successfully for the first time to mono-fluoroalkanoic acids.

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Nature of Endogenous Reserve Material in a Strain of *Pseudomonas fluorescens-putida* Intermediate

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The nature of endogenous reserve materials in various pseudomonads is still incompletely known. Initially Norris, Campbell & Ney (1949) showed that the endogenous respiration of *Pseudomonas aeruginosa* is unaffected by aeration or by the addition of common uncouplers like dinitrophenol or azide, and that it proceeds undiminished in the presence of an exogenous substrate. In subsequent experiments, Warren, Ells & Campbell (1960) showed that the endogenous metabolism of both *P. aeruginosa* and *Pseudomonas fluorescens* involved liberation of ammonia, thus suggesting that the endogenous reserve material is nitrogenous in nature, possibly the cellular protein or the acids of the free amino acid pool. This ammonia could be re-incorporated rapidly into the cell as soon as any oxidizable substrate like glucose was added, suggesting the synthesis of an oxidatively assimilable material (Gronlund & Campbell, 1961). But whether these oxidatively assimilable materials are valid reserves for endogenous respiration is not

clear (Gibbs & Wood, 1952), and as such there is no clear-cut demonstration of any cellular nitrogenous constituent that may be considered to act as an endogenous reserve material in the pseudomonads. The present study was undertaken to investigate the nature of endogenous respiration in an intermediate strain of the *Pseudomonas fluorescens* group and to find out whether any particular cellular component is likely to be the substrate for endogenous respiration in that organism.

EXPERIMENTAL

Materials. Uniformly labelled L-[^{14}C]phenylalanine was purchased from The Radiochemical Centre, Amersham, Bucks. Azaserine was kindly given by Dr John R. Dice of Parke, Davis and Co., Mich., U.S.A., and actinomycin by Dr Junichi Kawamata of Osaka University, Japan.

Growth conditions. Cells of the *Pseudomonas fluorescens-putida* intermediate strain (Ganguly, 1955) were grown on a rotary shaker at room temperature (approx. 30°) in a synthetic medium which contained (g./l.): $(\text{NH}_4)_2\text{HPO}_4$,