quantities of heterologous protein being adsorbed. With the third antigen, *Pasteurella pestis* toxin, non-specific adsorption is substantial.

5. With the anthrax preparation, the antigen, when adsorbed on the polymer, retains its biological competence as judged by its ability to stimulate antibody production and to produce immunity to challenge in animals. Assays of the latter, Dr A. L. MacLennan for anti-plague serum and Dr H. Smith for anti-ovalbumin serum and also Sir Harry Melville, F.R.S., Dr H. T. Hookway and Mr D. K. Hale of the Department of Scientific and Industrial Research for helpful discussions.

We wish to thank our colleagues, Mr F. C. Belton, for supplies of anthrax antiserum and anthrax antigen and for assays of the latter, Dr A. L. MacLennan for anti-plague serum and *Pasteurella pestis* toxin and for mouse-toxicity tests, Dr H. Smith and Dr B. T. Tozer for anti-ovalbumin serum and also Sir Harry Melville, F.R.S., Dr H. T. Hookway and Mr D. K. Hale of the Department of Scientific and Industrial Research for helpful discussions.

REFERENCES


The Chemical Nature of the Toxic Compounds Containing Fluorine in the Seeds of Dichapetalum toxicarium

**BY R. A. PETERS, R. J. HALL AND P. F. V. WARD**

*A.R.C. Institute of Animal Physiology (Biochemistry Department), Babraham, Cambridge*

**AND N. SHEPPARD**

**The University Chemical Laboratory, Cambridge**

(Received 23 February 1960)

A fluoro-octadecenoic acid has been isolated in small amounts from the seeds of *Dichapetalum toxicarium* (Peters & Hall, 1959). It was obtained by fractionation of the oil soluble in acetone at −20° on silane-treated Hyflo Supercel columns with liquid paraffin and aqueous acetone as the stationary and mobile phases (Howard & Martin, 1950; Crombie, Comber & Boatman, 1955). In this Laboratory, the fraction from the columns eluted by aqueous 55 % (v/v) acetone was purified by precipitation as the calcium salt. However, analysis showed that, with one exception, all our small samples were contaminated with 5–10 % of an impurity. Reversed-phase paper chromatography showed that this fraction contained up to three components and it was clear that the structure of the fluoro-octadecenoic acid could not be determined finally by this method. The present paper describes an alternative method of preparing gram quantities of the pure fluoro fatty acid, and the determination of its structure as fluoro-oleic acid (ω-fluoro-cis-Δ⁹-octadecenoic acid). During the final stages of the purification another fluorine-containing component was found in small amounts. Unlike fluoro-oleic acid this is a solid at room temperature and its chemical structure has not yet been determined.

MATERIALS AND METHODS

*Special chemicals.* The activated charcoal was from Hopkin and Williams Ltd. and was previously washed with light petroleum (b.p. 40–60°). Linoleic acid, linoleic acid and oleic acid were from Nutritional Chemicals Ltd. and
elaidic acid was from K and K Laboratories Ltd. Embacel was from May and Baker Ltd. and Reoplex 400 from The Geigy Co. Ltd. Oil Red 4B was from George T. Gurr Ltd. The cellulose powder was Whatman Ashless Standard Grade. Samples of \( \omega \)-fluoro-octanoic acid, \( \omega \)-fluoronanoic acid and \( \omega \)-fluorodecanoic acid were kindly presented by Dr F. L. M. Pattison. Other chemicals were of A.R. quality where possible.

**Analytical methods**

**Double-bond estimations.** These were made essentially by Trappe’s (1938) method, except that the bromination was carried out in bromine–methanol without CHCl₃, and \( \chi \)-HCl (1 ml.) together with the 2% \((w/v)\) KI (1 ml.) was added after the bromination was complete.

**Carboxyl titration.** A blank titration, consisting of 2 ml. of 90% \((v/v)\) ethanol containing 20 μl. of 0.04% phenol red, was titrated with 5 ml \( \text{NaOH} \) in aqueous 90% \((v/v)\) ethanol until a bright-red colour persisted for at least 2 min. During this time a stream of CO₂-free air was passed through the solution. Up to 0.6 mg. of the fluoro acid in 0-2 ml. or less of carbon tetrachloride was then added to the blank, and the titration continued as before. The end point was sensitive to 2-3 μl. of 5 ml \( \text{NaOH} \) in a titration of 300-400 μl. Micropipettes and small-volume Ostwald–Van Slyke pipettes were used for the volume measurement of the samples. The titration was made with a Conway horizontal microburette with the tip below the surface of the solution.

**Fluorine estimation.** The fluorine from a long-chain \( \omega \)-fluoro fatty acid in our experience cannot be split off by heating with 7-5X-KOH, though we can confirm that 7-5X-NaOH or KOH will remove the fluorine from fluorooacetate (Saunders, 1957a; Mirosevic-Sorgo & Saunders, 1959). Some digestion in a Parr-type bomb is therefore necessary. Many attempts were made to obtain reproducible values for NaF when added to such a bomb and heated at 500°. Though new bombs gave reasonable recoveries at first, after a few analyses poor results were obtained. To overcome these difficulties the surface of the nickel bomb chamber was kept polished, 14-carat-gold washers were used instead of 9-carat and the surfaces were polished after each digestion, sodium peroxide was used instead of metallic sodium, and calcium nitrate was added to fix the fluorine during the digestion and to oxidize the residual carbon.

Samples were prepared for fluorine titration as follows. The purified oil (3-5 mg.), in carbon tetrachloride, was transferred to the nickel bomb chamber and the solvent evaporated away with a gentle stream of compressed air. To the oil was added 1 ml of 10% \((w/v)\) Ca(NO₃)₂ in acetone. The acetone was removed with compressed air. Sodium peroxide (100 mg.) was added and the bomb sealed and placed in a muffle furnace at 500–550° for 2 hr., and cooled in the muffle. The residue in the bomb, a white solid, was first extracted with 3-4 ml. of \( \text{HCl} \) perchloric acid. The bomb was then washed out with water, the volume of the digest solution was made to nearly 250 ml and the pH of the contents adjusted to approx. pH 6-0 with NaOH. The volume was made up to 250 ml and any insoluble material was removed by centrifuging. Fluoride was determined by thorium titration as described by Hall (1957) except that: 1 mN-thorium nitrate was used, the buffered alizarin sulphonate reagent was adjusted with 7-8 ml. of 1 mN-thorium nitrate for every 100 ml. of alizarin reagent (this step obviated the need for a blank thorium titration), and the titration was scaled down so that not more than 8 μg. of fluorine was titrated, with a reproducibility of ±0.01 ml. of 1 mN-thorium nitrate.

**Qualitative analysis by reversed-phase paper chromatography.** This was based upon the method of Schlenk, Gellerman, Tillotson & Mangold (1957) for the separation of \( \text{C}_{14}-\text{C}_{18} \) fatty acids. Instead of sheets of Whatman no. 1 filter paper treated with silicone, the fast hard paper Whatman no. 531 was used and liquid paraffin substituted for silicone. Sheets 46 cm. × 19 cm. were soaked in a 7% \((v/v)\) solution of liquid paraffin in ether and dried in air. Traces of impurities were then removed by washing with aqueous 85% \((v/v)\) acetic acid by descending chromatography for 48 hr. The papers were then dried at about 50°. Spots of 5 ml. of the fractions for analysis were put on the paper 3 cm. from one end. The chromatograms were developed at room temperature by ascending 85% \((v/v)\) acetic acid for 12-18 hr. and dried for 15 min. at 50°. The papers were suspended in iodine vapour, when the unsaturated fatty acids rapidly appeared as dark-brown spots (Brante, 1949). The method will detect a few micrograms of an unsaturated fatty acid.

**Inhibition of citrate metabolism induced in guinea-pig kidney particles.** Guinea-pig kidney particles (Peters & Wakelin, 1957) were suspended in a mixture of 1% \((w/v)\) KCl solution and 0-1 m \( \text{KH}_{2} \text{PO}_{4} \) neutralized to pH 7.2 with NaOH. Citrate accumulation in the particles was induced by the fluoro acids as follows. A mixture of kidney-particle suspension (1-9 ml.), 0-8% \( \text{MgCl}_{2} \), 6H₂O solution (0-1 ml.), adenosine triphosphate (0-33 mg. of disodium salt in 0-1 ml.), triosidionate citrate (10 μmoles in 0-4 ml.), fluoro acid as the sodium salt in 0-2 ml. and 1% \((w/v)\) KCl solution (0-3 ml.) was incubated for 30 min. at 38°. After incubation aqueous 25% \((w/v)\) trichloroacetic acid solution (1 ml.) was added and solid material centrifuged off. Citrate was estimated in the supernatant solution by the method of Taylor (1953). A control experiment, with 1% \((w/v)\) KCl solution in place of the fluoro acid solution, served as a standard against which the citrate inhibition induced by the fluoro acids could be measured.

**Melting points.** A B.S.S. thermometer corrected for the emergent stem was used. The heating bath contained about 700 ml. of ice-cold water, the temperature of which was raised at a rate of 0-5°/min. on a hot plate containing a magnetic stirrer.

**Hydrogenation.** A small sample (10 mg.) of the fatty acid was dissolved in 1-5 ml. of ethanol. To this were added 1-5 ml. of water and 2 ml. of a Raney nickel suspension in ethanol (Covert & Adkins, 1932). The reaction mixture was kept at 65–85° for 4 hr. with a slow stream of hydrogen bubbling through it. The Raney nickel was separated, and the hydrogenated fatty acid solution was acidified with 0-5 ml. of 2X HCl and extracted with light petroleum (b.p. 40–60°).

**EXPERIMENTAL AND RESULTS**

The light-petroleum extract of the seeds of \( \text{D. toxicarium} \) was evaporated and saponified with methanolic KOH and partially purified with acetone at −20° (Peters & Hall, 1959). The resulting
oil, when analysed by reversed-phase paper chromatography, showed four major components. Two were oleic acid and linoleic acid \((R_f 0.27\text{ and }0.50\text{ respectively})\), one the fluoro fatty acids \((R_f 0.76)\) and the other a component running near the solvent front. The component containing the fluoro fatty acids was thought to be only fluoro-oleic acid, but in the final stages of the purification it proved to contain a small proportion of another fluoro acid which was a solid at room temperature.

The term ‘fluoro fatty acids’ has been used to describe this component, to distinguish it from the purified fluoro-oleic acid and the solid fluoro acid.

**Preparation of fluoro fatty acids by reversed-phase chromatography**

Attempts to utilize filter-paper sheets for the quantitative preparation of the fluoro fatty acids were unsuccessful. Large amounts of the seed oil soluble in acetone at \(-20^\circ\) when put on the paper did not separate even when thick paper was employed. Complete separation was achieved by the use of cellulose-powder columns treated with liquid paraffin.

**Location of the fluoro fatty acids.** The principle was to follow the acids with some coloured substance which would travel at approximately the same rate. Several oil-soluble dyes were tested, and the only one suitable was Oil Red 4B (xylenoazotoluene-azo-\(\beta\)-naphthol). An orange component found in three samples of this dye moved ahead of the main dye and travelled with the fluoro acids. The initial separations were made with small cylindrical columns, which are also necessary when there is little of the material. Later the same principle was used on a much larger scale in an apparatus constructed of glass and Tufnol plates (Hall, 1960). By this method approx. 2 g. of the pure fluoro-oleic acid has been prepared from about 150 g. of crude fat obtained from 1 kg. of seeds.

**Preparation of the cylindrical column.** Cellulose powder (500 g.) was suspended in 21. of aqueous 85\% \((v/v)\) acetic acid. About 200 ml. of the mixture at a time was filtered under reduced pressure in a 4 in. sintered-glass funnel. The pad of powder was washed through once with approx. 200 ml. of acetic acid. The cellulose was suspended in 21. of a 7\% \((v/v)\) solution of liquid paraffin in ether, and again filtered through the sintered funnel under reduced pressure. The cellulose was resuspended in 21. of 85\% acetic acid to remove excess of paraffin and filtered again. Traces of acetic acid left in the cellulose were removed either by spreading the powder on a dish and drying in air or in an oven at 50\°C.

A tube of Pyrex glass 45 cm. \(\times\) 10-12 mm. was used with a 100-120\(\mu\) sintered-glass disk 5 cm. from one end. Only sufficient of the paraffin-treated cellulose powder was introduced at a time to give a section approx. 1 cm. thick when compressed tightly with a glass rod which just fitted inside the column. To avoid irregular packing it was important that this rod should have an absolutely flat end at right angles to its length. The column was packed to within 5 cm. of its open end. At first the fatty acid mixture was applied in about 1 ml. of acetone, but the separation was unreliable. About 200 mg. of the crude fatty acids was evenly stained with 0.5 ml. of 0.1\% \((w/v)\) dye in ether and 1-5 g. of treated cellulose powder was added to this solution. The mixture was well stirred, transferred to the column and compressed with the glass rod so that a disk 3-5 mm. thick was formed. The packing of the column was completed to within 2 mm. of the open end and the packing finally covered with a circle of Whatman no. 531 filter paper. The column was clamped with the open end dipping into a beaker of solvent. Originally this was acetic acid-water \((17:3, v/v)\), but later formic acid-acetic acid-water \((2:2:1, v/v)\) was used. With 85\% acetic acid all the components separate but not widely; with formic acid the travel of oleic acid, linoleic acid and linolenic acid is much retarded and the degree of contamination of the fluoro fatty acids is decreased. The column was developed by ascending solvent for 18 hr. at 18-25\°C.

The fluoro fatty acids moved with the fast-running orange component of the marker dye. The parts of the column that were not wanted were removed to within 5 cm. of the beginning of the orange section. A layer of approx. 1 cm. of new cellulose loosely packed was inserted and the column eluted with the developing solvent. Small volumes of eluate \((0.2 \text{ ml.})\) were collected when the orange band started to come out, and these were continued until 2 ml. in all had been collected after the last of the dye had been eluted. The solvent was removed from each volume with a stream of air at room temperature or at about 35\°C. Each residue was examined by dissolving it in 0.5 ml. of carbon tetrachloride, and chromatographing 5 ml. portions on filter-paper sheets as described. Some of the earlier and later fractions contained small amounts of iodine-staining components other than the fluoro fatty acids. These were discarded. The fractions shown by paper chromatography to contain the fluoro fatty acids and no other components staining with iodine were pooled and the carbon tetrachloride was removed by evaporation.

**Purification of the fluoro fatty acids**

These fractions were contaminated with dye, liquid paraffin and non-acidic components of the seed oil of similar chromatographic mobility. To free the fluoro fatty acids from these contaminants they were precipitated as their calcium salts. The residues, in about 200 mg. quantities, after evaporation of the carbon tetrachloride were dissolved in 2 ml. of acetone and titrated with 0.25\(\text{N}\) NaOH to pH 8.0, any precipitate being removed by centrifuging. An equal volume of 10\% \((w/v)\) CaCl\(_2\) (anhydrous) in aqueous 50\% \((v/v)\) acetone, previously adjusted to pH 8.0, was added, and the contents of the tube were centrifuged at 4000 rev./min. The precipitate was suspended in 2 ml. of 1\% \((w/v)\) CaCl\(_2\) in 50\% \((v/v)\) acetone at pH 8.0 and centrifuged. The precipitate was washed twice with 5 ml. of light petroleum \((b.p. 40-80\°C)\), which removed most of the orange dye and the washings were discarded. After a further centrifuging, the precipitate was emulsified with 2 ml. of 2\(\text{N}\) HCl and extracted twice with 2 ml. of light petroleum \((b.p. 80-100\°C)\). After evaporation of the combined extracts to 1 ml., they were treated with 50 mg. of activated charcoal. The charcoal was separated by centrifuging and
washed with 1 ml of light petroleum (b.p. 80–100°). The washing combined with the supernatant light petroleum was washed once with 5 ml of water, made to 10 ml and left in the deep freeze overnight at −25°. Any precipitate at this stage was removed by centrifuging at −25°, the supernatant solution was separated and the light petroleum removed by evaporation. The clear oil obtained after evaporation of the light petroleum, when frozen and brought slowly to 20°, became an opaque gel. Upon centrifuging at 24 000 g for 10 min. at 15–20° the gel separated into a clear supernatant oil and a white solid, which was another fluoro acid. The solid was separated and washed three times with 0·2 ml of liquid paraffin, in which the fluoro-oleic acid is soluble. After the final washing the precipitate was dissolved with 2 ml of 90% (v/v) acetone to remove residual paraffin. The acetone solution of the solid phase was kept at −25°, when a crystalline precipitate formed which was centrifuged off at −25°. The precipitate was collected and dissolved in 1 ml of acetone. When converted into the sodium salt, it was insoluble in acetone but soluble in warm water. The sodium salt was treated with HCl and the free acid extracted with light petroleum (b.p. 80–100°). After evaporation of the solvent the white solid was dissolved in absolute methanol and centrifuged to remove the last traces of liquid paraffin. Evaporation of the methanol gave 14 mg of a white crystalline solid.

In later preparations, after the washing with liquid paraffin, the solid was dissolved in methanol (2 ml.), when most of the paraffin separated. After removal of the methanol by evaporation, the solid was redissolved in methanol (1 ml) to separate the remaining paraffin and crystallized from light petroleum (b.p. 40–60°) at −25°.

Properties of fluoro-oleic acid

Table 1 gives the equivalent weight, the fluorine content and the number of double bonds.

Melting point. Some specimens melted at 12·5°; others softened at 16° and were not completely melted until 21°; the presence of quite small amounts of the solid fluoro fatty acid has a large influence. Our purest specimen of fluoro-oleic acid softened at 12·4° and melted at 13·5°. Another crystalline specimen melted at 18–22°. In this connexion, we have found that oleic acid containing 5% (w/v) of stearic acid melts at 30° instead of 13·5°, whereas with 1% it melted at 12° and with 2% at 16–20°.

Position of the double bond and fluorine atom. The position of the double bond was determined by ozonization and identification of the degradation products by gas chromatography. The purified oil (12 mg.) was dissolved in 2 ml of chloroform-acetic acid (2:1, v/v) and ozone was passed through the solution at 18–25°; losses by evaporation were made good. [The silver treatment described by Cason & Tavs (1959) was unsuitable.] After 16 hr. the solvents were removed in a current of air. The mixture was esterified with methanol and thionyl chloride (Carlson & Wadström, 1958) and examined by gas chromatography with a modified Martin.

Fig. 1. Separation of methyl esters of fatty acids by gas chromatography at 197° on a 4 ft. 3 in. column with Reoplex 400 (40%, w/w) as stationary phase and 60–80 mesh Embacel as support. N₂ pressure: 23 cm. Hg; N₂ flow: 120 ml/min. (a) Methyl esters of products of ozonolysis of fluoro-octadecenoic acid from D. toxicaireum. Peaks in order of appearance: (1) ether, (2) methanol, (3) and (4) methyl esters of acids produced by ozonolysis. (b) Methyl esters of products of ozonolysis of fluoro-octadecenoic acid from D. toxicaireum mixed with methyl ω-fluorononanoate and methyl azelate: (1) ether, (2) methanol, (3) and (4) methyl ester of acid produced by ozonolysis + methyl ω-fluorononanoate, (3) methyl ester of acid produced by ozonolysis + methyl azelate. (c) Mixture of samples of methyl esters: (1) ether, (2) methanol, (3) methyl ω-fluoro-octanoate, (4) methyl ω-fluorononanoate, (5) methyl ω-fluorodecanoate, (6) methyl suberate, (7) methyl azelate.
FLUORO-OLEIC ACID FROM D. TOXICARIA

Table 1. Analysis of fluoro-oleic acid

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Fluorine (%)</th>
<th>No. of double bonds</th>
<th>Equiv. wt.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.30±0.1</td>
<td>0.99±0.01</td>
<td>302±3</td>
</tr>
<tr>
<td>B</td>
<td>6.28±0.1</td>
<td>0.92±0.01</td>
<td>304±3</td>
</tr>
<tr>
<td>C8 fluoro-enoic acid requires</td>
<td>6.33</td>
<td>1.00</td>
<td>300</td>
</tr>
</tbody>
</table>

* Calculated from titration of CO2H groups.

Table 2. Inhibition of citrate metabolism in guinea-pig-kidney particles induced by the fluoro acids

The inhibition of citrate metabolism induced by the fluoro acids in the guinea-pig-kidney-particle system described in the text was measured after incubation for 30 min. at 35°. In all the values a control containing no fluoro acid has been subtracted. Control values were of the order of 0.3 μmole.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Wt. (μg.)</th>
<th>Citrate remaining (μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium fluoroacetate</td>
<td>50.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Fluoro-oleic acid</td>
<td>2.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Unknown solid fluoro acid</td>
<td>2.5</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Chemical analysis by the Martin gas-density meter (Martin & James, 1956) constructed at Babraham. Figs. 1 (a) and (c) show that ozonization gave the methyl esters of azelaic acid and of ω-fluorononanoic acid. Hence the compound has a double bond between C8 and C10. The ω-position of the fluorine atom was proved by nuclear-magnetic-resonance determinations (see Appendix). With the evidence of the m.p. and the infrared spectrum the compound is ω-fluoro-oleic acid and not the trans form such as ω-fluoroelaidic acid. A further proof of the structure is given in Fig. 1 (b), where the products of ozonization were mixed with azelaic and ω-fluorononanoic acids. No additional chromatographic peaks separated.

Methyl ω-fluoro-oleate when chromatographed by the use of the Martin gas-density meter under the conditions used in Fig. 1 had a retention volume 2.7 times that for methyl olate, showing that the fluorine atom has a large retarding influence.

Hydrogenation. The acid was hydrogenated with Raney nickel in ethanol at 65–85° and a product was formed with m.p. 68–71°. This was consistent with a conversion into fluorostearic acid, but the matter was not pursued as the results were not always reproducible.

Infrared and ultraviolet spectra. The infrared spectrum of fluoro-oleic acid was compared (Fig. 2) with the spectra of oleic acid and of elaidic acid with a Perkin–Elmer Infra-ord. The main difference between 5% (w/v) solutions in carbon tetrachloride of oleic (cis) acid and elaidic (trans) acid was the peak for elaidic acid at 970 cm⁻¹ (10.3 μ). This peak was absent from fluoro-oleic acid, which confirms that it is the cis-compound, in agreement with previous deductions by Mr L. C. Thomas (personal communication). The CF band shows up at 1050 cm⁻¹. The ultraviolet spectrum of fluoro-oleic acid in ethanol was similar to that of oleic acid except for a small peak at 270–275 μμ.

Biological observations. A solution of fluoro-oleic acid in arachis oil (0.2 ml.) was injected intraperitoneally into rats. The lethal dose was 7–9 mg./kg. body wt. The amounts of fluoro-oleic acid producing citrate accumulation in guinea-pig-kidney particles were essentially the same as observed by Peters & Hall (1959). That is, 1.25 μg. of fluoro-oleic acid induced an accumulation of 2.6 μmole of citrate and 0.62 μg. an accumulation of 1.2 μmole. It was confirmed that the acid decreased citrate metabolism in the kidney particles much more than did fluoroacetate (Table 2).
Properties of the solid fluoro acid

This appeared to be not more than 5% of the toxic oil obtained after purification by precipitation as the calcium salt. The acid had m.p. 72-0-73-7° (corr.) with softening at 70-5°.

It has no double bond and the carboxyl-group titration gave a value for the equivalent weight of 286. The fluorine content was 6-8%. This could represent an acid with 17 carbon atoms but the acid inhibits aconitase in the guinea-pig-kidney-particle test (Table 2) and is toxic to rats; this indicates that it acts as an acid with an even number of carbon atoms (Peters, 1955) and is virtually as active as fluoro-oleic acid.

The infrared spectrum in carbon tetrachloride was similar to that of fluoro-oleic acid, except that the band at 1460 cm⁻¹ was much more pronounced. The absence of the band at 970 cm⁻¹ indicates that it is not a trans-isomer. The ultraviolet spectrum resembled that of oleic acid. The small peak at 270-275 mμ was absent.

DISCUSSION

The first experiments on the toxic fluoro oil from D. toxicarium (Peters, Wakelin, Birks, Martin & Webb, 1959) indicated that it was a long-chain fatty acid. The constitution of the main component is now proved, so far as this can be done without synthesis. That of the solid fluoro acid is not yet clear; its fluorine content, carboxyl-group titration and absence of CH—CH suggest a saturated acid with possibly 17 carbon atoms. The biochemical tests indicate that it behaves to the enzymes concerned as an acid with an even number of carbon atoms. This suggests either a branched-chain fluoro-octadecenoic acid with an even number of carbon atoms in the fluorine-containing chain or an ω-fluoro hydroxy acid with 16 carbon atoms. Hansen, Shorland & Cooke (1953, 1954) have shown that some branched-chain fatty acids found in mutton fat all have low optical rotations. With the small amount of the solid fluoro acid available, the optical rotation in propan-1-ol was not measurable, from which we concluded that the compound was optically inactive or had [α]D < 2-0. This observation did not confirm the presence of a branched-chain structure or a hydroxyl group but the subject is being investigated.

The fluorine content of the soil in which plants that contain organic fluorine compounds grow raises some points requiring investigation. Dichapetalum cymosum, the leaves of which contain fluoroacetate, is stated to grow in a soil rich in fluorine (Saunders, 1957b). On the other hand, in a personal communication, Dr Dagmar Wilson said that she saw no evidence of fluorosis in Sierra Leone, where Dichapetalum toxicarium grows, from which she concluded that the soil content in this part was low in fluorine. If this is so, Dichapetalum toxicarium must concentrate fluorine compounds.

We now know the main compound responsible for the condition of 'broke back' (Peters, 1955) in Sierra Leone and also for the circumstance that causes the seeds of Dichapetalum toxicarium to be called 'ratsbane'.

APPENDIX

Notes on the Nuclear-Magnetic-Resonance Spectrum of ω-Fluorodecanoic Acid and of a Fluoro-Octadecenoic Acid of Unknown Structure

By N. SHEPPARD

The hydrogen (proton-) resonance spectra of the two compounds were obtained in chloroform solution with a Varian V-4300B spectrometer at 40 Mcyc./sec. Fig. 3 shows those parts of the spectra where resonances are expected to occur for CH bonds adjacent to the electronegative fluorine atom, as in CH₂F⁻ or CHF⁻. The remainder of the spectra were typical of long-chain carboxylic acids.

This part of the spectrum of ω-fluorodecanoic acid is essentially as expected for the [F]⁻—CH₃—[CH₂]₆ group. It consists of a widely spaced pair of bands (separation 48 cyc./sec., centre of gravity 0-95) each with triplet contours (fine structure spacing ~6 cyc./sec.), one being sharp and the other blurred. The separation of 48 cyc./sec. is of the size expected for spin–spin splitting of the methylene resonance by the magnetic F nucleus (spin 1/2); the smaller triplet spacing is of the magnitude and type to be anticipated for spin–spin interaction with the second CH₃ group along the chain. The only unusual and unexplained feature of the spectrum is the blurred nature of the triplet at higher field.

The spectrum of the unknown fluoro-octadecenoic acid shows two bands (a triplet at v 14 cyc./sec. with spacing 6-1 cyc./sec. and a blurred triplet at 61 cyc./sec.) of the same position and appearance as those shown in the first spectrum, with an additional stronger triplet centred at v +1 cyc./sec. and with a spacing of 3-6 cyc./sec. The first two bands clearly show the presence of the F·CH₂·CH₃— group in the molecule. The third