396. *Toxic Fluorine Compounds containing the* C–F *link. Part IX.* Preparation of Carbon-labelled Sodium Fluoroacetate on a Micro-scale.*

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Pure sodium fluoroacetate labelled with ^{14}C in the methylene group has been prepared on a 100-mg. scale. A description is given of a specially designed micro-autoclave suitable for microfluorination. A convenient ampoule breaker has been devised to meet the special needs of this kind of work. Radioactive sodium fluoroacetate is to be used to test theories of fluoroacetate poisoning.

SINCE 1941 we have carried out extensive work on compounds containing the CH_2F group and possessing pronounced physiological properties (McCombie and Saunders, *Nature*, 1946, **158**, 382; Saunders and Stacey, *J.*, 1948, 1773). Thus, during a ten-minutes exposure to a concentration of *ca.* 0·1 mg. of methyl fluoroacetate per l., small animals exhibited no symptoms, but after 30—60 minutes convulsions occurred, followed by death. Many compounds related to this ester were synthesised and their activity examined. We deduced that for high toxicity the compound must be capable of conversion *in vivo* by

* Part VIII, *J.*, 1949, 2774.

oxidation and/or hydrolysis into fluoroacetic acid (Saunders, *J.*, 1949, 916). We also showed that in the series of ω-fluorocarboxylic acids, $F\cdot[CH_2]_n\cdot CO_2H$, when *n* is odd the compound is toxic and when *n* is even it is non-toxic (Saunders, *Nature*, 1947, **159**, 491; Buckle, Pattison, and Saunders, *J.*, 1949, 1471). Further, fixation of the α- and β-carbon atoms of the toxic methyl γ-fluorobutyrate in a ring system resulted in a compound of negligible toxicity (Pattison and Saunders, *J.*, 1949, 2745). The effects of methyl fluoroacetate and of sodium fluoroacetate *in vivo* are similar. Even when the dose is many times the lethal, a latent period precedes the onset of convulsions and subsequent death. Marais (*Onderstepoort J. Vet. Sci. Animal Ind.*, 1944, **20**, 67) isolated potassium fluoroacetate from the S. African plant "Gifblaar," *Dichapetalum Cymosum*, which is a well-known hazard to cattle. Sodium fluoroacetate acts as a systemic and contact insecticide (David, *Nature*, 1950, **165**, 493).

In 1948, Peters reported (*Proc. Roy. Soc. Med.*, 1948, **41**, 781): "There is not yet known any case in which fluoroacetate inhibits a single isolated enzyme." This is in contradistinction to the powerful anti-choline esterase activity of the phosphorofluoridates (fluorophosphonates; cf. McCombie and Saunders, *Nature*, 1946, **157**, 776).

Kalnitsky and Barron (*Arch. Biochem.*, 1949, **3**, 215), using kidney homogenate, found that fluoroacetate and γ-fluorobutyrate inhibited the oxidation of acetate, butyrate, etc., and that, *in vitro*, citrate accumulated in the presence of fluoroacetate. Liébecq and Peters (*J. Physiol.*, 1949, **108**, 215; *Biochim. Biophys. Acta*, 1949, **3**, 215) suggested that fluoroacetate entered the tricarboxylic acid cycle *via* the synthesis of a fluorotricarboxylic acid and by some means "jammed" the oxidation of citrate. Lotspeich, Peters, and Wilson (*J. Physiol.*, 1951, **115**, 25P; *Biochem. J.*, 1952, **51**, 20) also investigated the effect of "inhibitor fractions" isolated from tissues poisoned by fluoroacetate and found that the fluorocarboxylic acid fractions inhibited the reactions of aconitase.

The preparation of labelled sodium fluoroacetate will enable the formation of the fluorotricarboxylic acid to be further investigated. Thus the isolation, from tissues treated with radioactive sodium fluoroacetate, of radioactive material containing fluorine and chromatographically similar to the "tricarboxylic" acids would be strong evidence for the hypothesis that citrate accumulation is due to the formation of a fluorocitrate or related compound. Further the use of the radioactive "fluorotricarboxylic acid" fraction in a system containing aconitase should permit of the isolation of a radioactive enzyme–inhibitor complex.

The starting material for our preparation of radioactive sodium fluoroacetate was $^{14}CH_2Br\cdot CO_2H$ obtained from the Radiochemical Centre, Amersham. The acid had a total activity of 1·02 mc in 136 mg., and was supplied in a sealed ampoule. Because of the high cost and deliquescence of the material and the danger of ingestion, it was necessary to design a special ampoule breaker which could easily crack the ampoule in an evacuated system so that the radioactive acid could be transferred quantitatively and collected in a cooled U-tube by continuous pumping to high vacuum.

Bromoacetic acid could not be fluorinated directly. We were able to convert it quantitatively into methyl bromoacetate on a micro-scale. Fluorination of the ester at ordinary pressure gave poor yields and the adaptation of the method to small quantities of radioactive material proved difficult. It was, therefore, decided to use Saunders and Stacey's method (*loc. cit.*) in which fluorination is by potassium fluoride in a rotating autoclave. A micro-autoclave was designed for work with 100-mg. of volatile material. The volatile product contained only methyl bromoacetate and methyl fluoroacetate and the percentage composition was determined by refractive-index measurement and reference to a previously constructed calibration curve. The accuracy of the estimation was confirmed by fluorine analysis of known mixtures and in one instance by the fluorine analysis of the product.

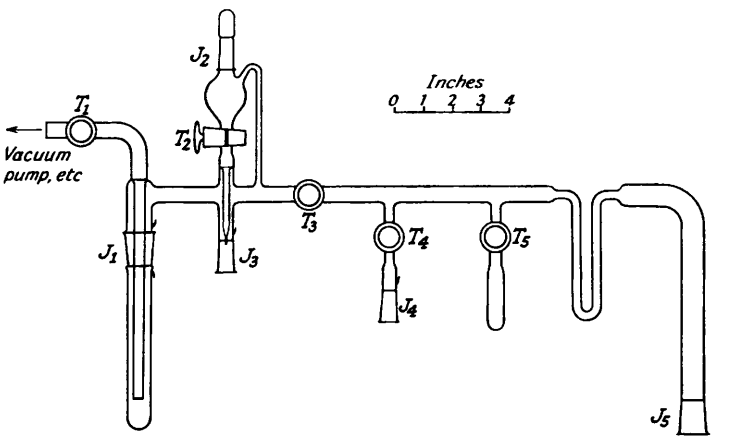
Fractional distillation of the product (*ca.* 50 mg.), by a special condensation unit (Fig. 5), gave methyl fluoroacetate of 94% purity. This was converted into sodium fluoroacetate by titration with sodium hydroxide solution in an apparatus containing a glass electrode and calomel half cell. The pure product was isolated by freeze-drying. Incidentally the value of the titre was a measure of the purity of the product as the equivalent weights of methyl bromoacetate and methyl fluoroacetate are sufficiently different.

Experimental

The vacuum apparatus is shown in Fig. 1. Apiezon " M " grease was used for all taps and joints, except J_1, J_2, J_3, T_2, and T_3 where silicone grease was employed.

Methyl Bromoacetate.—The radioactive bromoacetic acid was contained in a sealed-glass ampoule. In order to open this safely and conveniently a breaker (Fig. 2) was made from gun-metal alloy or stainless steel. The main component B had B24 cones about E and H, and was threaded on its inner surface. The ampoule was placed in HG, and held in place by the threaded cylinder A. The breaking-plug C, which had a rectangular-sectioned projection at its outer end, was screwed into E until its inner end reached F. After greasing, the knurled head D was placed over the end E, the rectangular hole in D fitting over the projection on C. The whole was then attached at J_5, which surrounded H. A tube, containing a small piece of soft iron sealed in glass to serve as a stirrer, was attached at J_3. In those experiments in which we diluted the radioactive bromoacetic acid, a break–seal ampoule containing the required amount of non-radioactive bromoacetic acid was attached at J_4 and a small magnetic breaker was placed next to the tip of the ampoule. After evacuation, T_3 was closed and D rotated slowly, thus propelling the breaking-plug C towards the ampoule. When it reached the ampoule an increased resistance to turning could be felt : when the ampoule broke a definite " kick " could be detected.

Fig. 1.



The U-tube was cooled in liquid oxygen, T_3 opened, and the whole apparatus evacuated continuously until all the radioactive bromoacetic acid had been transferred to the U-tube.

When dilution was necessary, T_3 was closed, the tip of the ampoule containing inactive bromoacetic acid was broken by oscillating a magnet about J_4, and the contents of this ampoule transferred to the U-tube. Dry air was then let into the apparatus *via* T_1 and the U-tube warmed to 55° and then cooled. This process was repeated twice. The mixed acid was transferred under high vacuum to tube J_3, where the process of mixing was repeated, this time with the help of the magnetic stirrer. When dilution was not required, the radioactive acid was transferred directly from the U-tube to J_3. In both cases a small sample of the homogeneous acid was transferred to the tube closed by T_5, and this could later be used for counting.

To the acid (*ca.* 100 mg.), dry ether (1 ml.) was added *via* J_2 and T_2. T_3 was closed and T_1 opened to the atmosphere, with suitable drying tubes. A solution of diazomethane (*ca.* 40 mg.) in ether (3 ml.) was then slowly added to the ice-cold solution of the acid. The two solutions soon formed separate layers which were gently mixed by means of the magnetic stirrer. The tube J_1 was cooled in ice–salt and the tube J_3 warmed to 55°, the ether and excess of diazomethane being collected in J_1. In preliminary experiments it was found essential to use a grease which was not very soluble in ether, otherwise the lubricant was washed down into tube J_3, making complete recovery of the methyl bromoacetate impossible. The tube J_3 was next cooled in liquid oxygen while the ether was removed under reduced pressure *via* T_1. By allowing J_3 to warm to room temperature for a short time, with the water-pump still running, the last traces of ether were removed. The methyl bromoacetate was then transferred to a tube at J_4 and T_4 was closed.

In a typical experiment 135·5 mg. of acid gave 144·3 mg. (97%) of ester, n_D^{15} 1·4582.

Fluorination of Methyl Bromoacetate.—The microautoclave *J* (Fig. 3) was designed so that losses could not occur on introduction or removal of material. Joints were vacuum- and pressure-tight, and volatile material was introduced and removed through a special attachment to the vacuum system. Thorough mixing was achieved by vibration in a vertical plane. The autoclave, made of stainless steel, had a socket (120°) into which fitted a stainless-steel cap *K* whose taper was slightly more acute, so that when the locking-nut *L* was screwed down firmly *K* and *J* came into contact along a circle. The attachment *M* was used for filling the autoclave. Its lower cone was similar to *K*, except for a central hole, which connected with a length of copper tubing and thence with a standard brass cone.

Potassium fluoride (*ca.* 65 mg.) and 12—14 stainless-steel balls (radius 3/32″) were placed in *J*, and *M* was then screwed down tightly, a trace of silicone grease being applied to the joint.

FIG. 2.

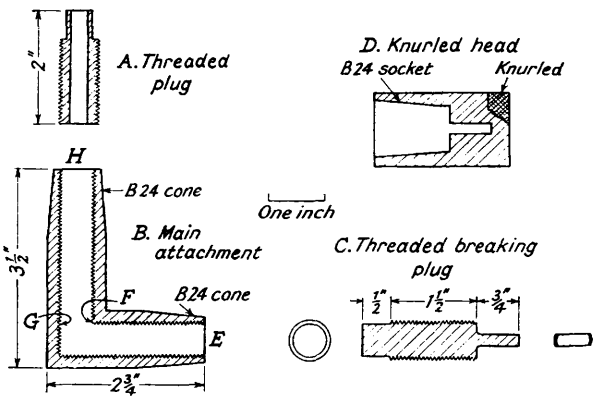


FIG. 3.

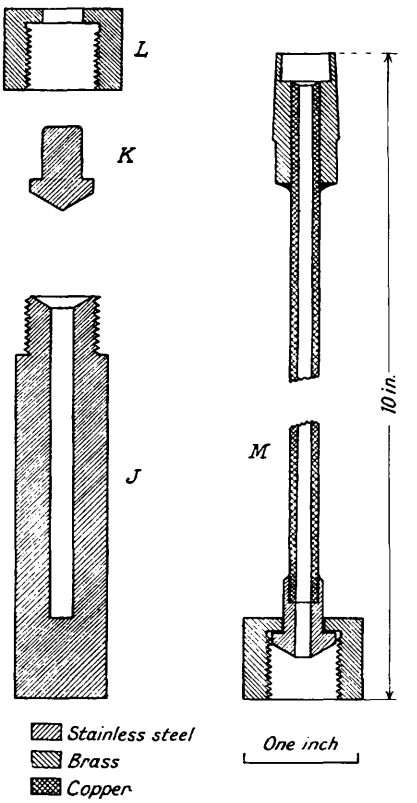
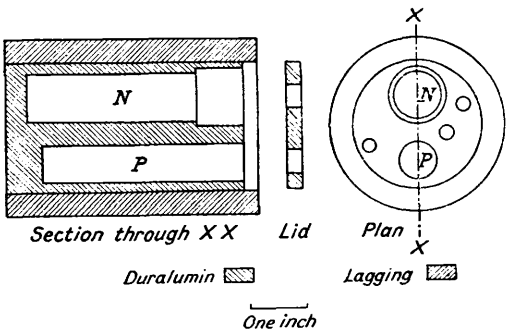


FIG. 4.



The whole was then attached at J_3 and pumped to high vacuum for 2 hr. to remove any water. In transference of methyl bromoacetate to the autoclave, T_1 was closed, T_4 opened, and *J* cooled in liquid oxygen, the copper tube and brass cone being kept at about room temperature. With *J* still cooled, tube J_1 was also cooled in liquid oxygen, while the system was pumped, the tube and cone being warmed to *ca.* 50°. Any methyl bromoacetate which had been retained in the tube was transferred either to *J* or to tube J_1; any ester which might have collected in J_1 was transferred to *J* and the process repeated until no more collected in J_1. Dry air was let in *via* T_1, *M* was detached, and the cap *K* and nut *L* were used to seal the autoclave.

The lagged heating-block (Fig. 4) was made of duralumin. The microautoclave was placed in the hole *N* and a 50-w heater in *P*. The whole was then mechanically shaken in a vertical plane for 3½ hr. at 220°, the axis of the autoclave being vertical. After cooling, *L* and *K* were removed from *J*, and replaced by *M*. This was attached at J_3, and the product transferred to a small tube attached by means of an adaptor at J_4.

Some decomposition of the methyl bromoacetate occurred during the reaction. [After

heating of 158·2 mg. of methyl bromoacetate without potassium fluoride at 220° in the autoclave for 3½ hr., 140·1 mg. (89%) of the ester were recovered unchanged.]

The percentage of fluoroacetate in the product was estimated by comparing the values of the refractive index with those found for known mixtures of methyl bromoacetate and methyl fluoroacetate. Results are tabulated. The yield quoted makes no allowance for the methyl bromoacetate recovered.

Methyl bromoacetate (mg.)	Product mg.	n	$CH_2F{\cdot}CO_2Me$ (%) in product	Yield (%)
102·6	36·7	1·3897	72	43
113·1	63·8	1·4022	56	53
93·0	36·2	1·3965	63	41
123·6	43·0	1·3962	63	37
114·0	65·2	1·4096	47	45

Removal of Residual Methyl Bromoacetate.—The fractionation unit (Fig. 5) was attached by J_6 at J_3, the tube containing the fluorinated ester being attached at J_7. The U-tube S was

FIG. 5.

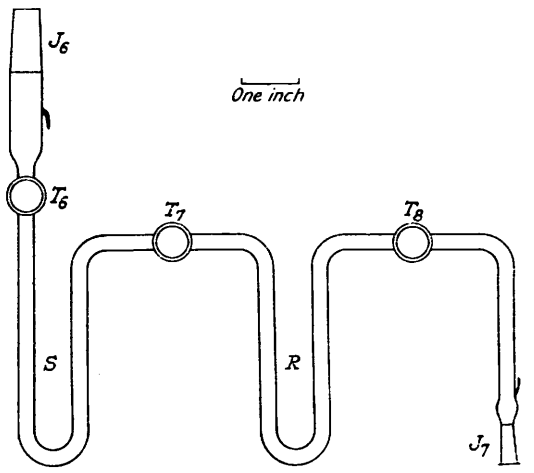
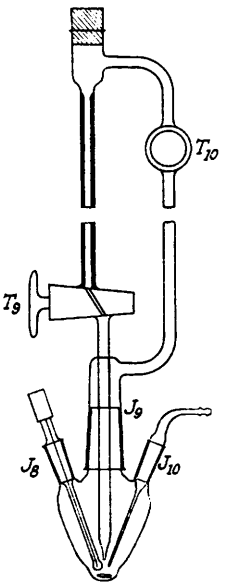


FIG. 6.



cooled in liquid oxygen, R in a bath of melting ethyl malonate (−54°), and the tube at J_7 in a bath of melting carbon tetrachloride (−25°). The system was continuously evacuated, T_6, T_7, and T_8 being open. When all the mixture had been transferred from the tube J_7 to S and R, T_1 and T_7 were closed. The material from S was transferred to a small weighed tube at J_4, while that from R was transferred to the tube J_7.

Preliminary experiments were carried out on the micro-fractionation of synthetic mixtures of non-radioactive methyl bromoacetate and methyl fluoroacetate of known composition. For example, with mixtures containing 47—49% of methyl fluoroacetate, the lower-boiling fraction (collected in S) contained 94—100% of fluoroacetate. Moreover, 77—57% of the fluoroacetate from the original mixture was now in this lower-boiling fraction. When material from the micro-autoclave was used the percentage of fluoroacetate in the lower-boiling fraction was within the range 94—100%, as shown by refractive-index measurement. Furthermore, infra-red spectroscopy proved that this material contained at least 90% of methyl fluoroacetate.

Sodium Fluoroacetate.—The titration cell (Fig. 6, shown attached to a pressure-equalised burette), containing a small iron strip sealed in glass to serve as a stirrer, and with stoppers at J_8 and J_{13}, was attached by J_9 at J_3. The methyl fluoroacetate (42 mg.) was then transferred to this cell under high vacuum. After removal of the cell, water (1 ml.) was immediately added to avoid loss by volatilisation. A glass electrode was placed at J_8 and a tube connected to a calomel half-cell at J_{10}. The cell was attached to the burette, with T_{10} open. The mixture

was stirred magnetically and slowly titrated with N-sodium hydroxide (0·43 ml.) to pH 11 (42 mg. of methyl fluoroacetate requires 0·45 ml. of N-NaOH).

The electrodes and burette were removed and washed. The solution was removed and the pure sodium fluoroacetate (45 mg.) isolated by freeze-drying.

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