Using a Pye Argon Chromatograph, a 4 ft. 1 % SE30 column at 225°, flash heater 270°, pressure 15 p.s.i. the reproducibility of the method was tested. Twelve samples of a pregnanediol diacetate solution, each 4 μg in 40 μl, were pipetted on to cylinders, evaporated, and the batch assayed using the automatic device described. Peak height and area by triangulation were determined and gave: height, mean 71 $\pm$ s.d. 3.0; area, 1330 $\pm$ s.d. 46 mm^2. The experiment was repeated the following day using the same column conditions but the evaporated samples in open glass cylinders were added and removed from the flash heater manually. This gave precision: height, mean 65 $\pm$ s.d. 3.9; area, mean 1250 $\pm$ s.d. 70 mm^2.

The apparatus has been used chiefly for overnight runs. Samples and standards are loaded in the evening and a separate time switch is set to stop the chart drive at the end of the run. The peaks are quantitated the following morning.

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Gas chromatography of fluorinated fatty acids

I. Separation and identification of lower 2-fluorofatty acids

There has been considerable interest, in recent years, in the biological activity of fluoro-organic compounds related to aliphatic fatty acids[1]. Fluoroacetic acid was found by MARAIS[2] to be the toxic principle of Gifblaar. Several ω-fluorofatty acids were identified in the seeds of *Dichapetalum toxicarium* by PETERS and coworkers[3,4]. In a study of the fate of fluoroacetic acid in nondividing yeast cells, ALDOUS[5] tentatively identified a fluorobutyric acid and a fluorohexanoic acid as metabolic products. Positive identification could not be made for lack of authentic samples.

As the result of our program on the synthesis of fluorinated molecules, a series of 2-fluorofatty acids became available. The separation and identification of this series of lower fluoroacids alone and in combination with a similar series of unfluorinated saturated aliphatic fatty acids by gas chromatography are the subject of this report.

Experimental

Apparatus. For the separation and identification of the 2-fluorofatty acids alone, a Perkin-Elmer Model 154D Vapor Fractometer fitted with a thermal conductivity

detector and a 100 cm × $^1/_4$ in. o.d. copper column was employed. The stationary phase was a modification of that employed by EMERY AND KOERNER[6] as follows: a mixture of 9 parts of Tween 80 and 1 part of 85 % phosphoric acid was coated on acid washed Chromosorb W (30/60 mesh) to make a 15 % liquid phase. The instrument was operated at a column temperature of 132° and a flow rate of 90 ml of helium per min. The column was conditioned at 140° overnight prior to use.

For the separation and identification of the lower 2-fluorofatty acids admixed with a similar series of unfluorinated saturated fatty acids, the Aerograph Hy-FI model 600-B fitted with a flame ionization detector was used. The column was a further modification of the one described above as follows: a copper tube 2.22 m × $^1/_8$ in. o.d. was packed with 100/120 mesh acid washed firebrick coated with 10 % (w/w) of a mixture of 9 parts of Tween 80 and 1 part of 85 % phosphoric acid. The column was conditioned overnight at 160° with a flow rate of nitrogen of 30 ml/min, and the operating temperature was 156° with the same flow rate of nitrogen.

Compounds. The fluoroacids to 2-fluorohexanoic acid were prepared by the method of GERSHON, SCHULMAN AND SPEVACK[7] by fluorination of C-alkyl ethyl cyanoacetates in toluene by perchloryl fluoride in the presence of sodium dispersion. The fluorinated esters were subsequently hydrolyzed to the fluoroacids. Fluoroacetic acid and the unfluorinated fatty acids to octanoic acid were commercially available. All compounds were purified by preparative gas chromatography in an Autoprep Model A-700 prior to use, and acetone solutions of the compounds were employed for injection into the chromatographs.

Results and discussion

The results of the separation of the lower 2-fluorofatty acids can be seen in Fig. 1 and retention data are summarized in Table I. The separation of the combined

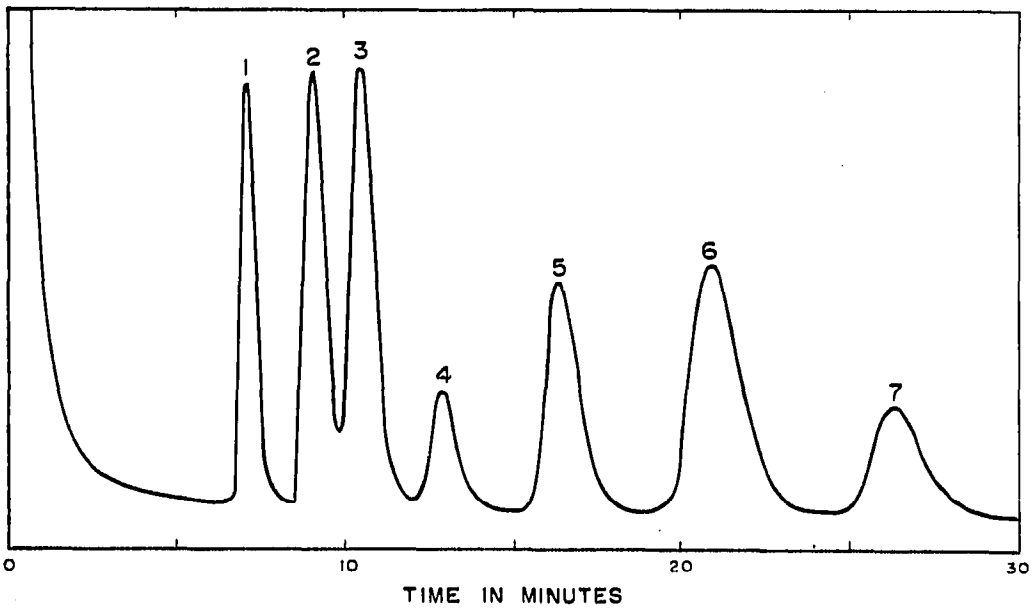

Fig. 1. Gas chromatogram of a mixture of 2-fluorofatty acids at 132°. The components are: (1) 2-fluoropropionic acid; (2) fluoroacetic acid; (3) 2-fluorobutyric acid; (4) 2-fluoro-3-methylbutyric acid; (5) 2-fluorovaleric acid; (6) 2-fluoro-3-methylvaleric acid + 2-fluoro-4-methylvaleric acid; (7) 2-fluorohexanoic acid.

TABLE I

RETENTION DATA FOR 2-FLUOROFATTY ACIDS

Compound	*Relative time**
2-Fluoropropionic acid	0.43
Fluoroacetic acid	0.55
2-Fluorobutyric acid	0.64
2-Fluoro-3-methylbutyric acid	0.77
2-Fluorovaleric acid	1.00
2-Fluoro-4-methylvaleric acid	1.28
2-Fluoro-3-methylvaleric acid	1.29
2-Fluorohexanoic acid	1.60

* The values are retention times relative to 2-fluorovaleric acid. The observed value for this reference standard was 19.9 min at 132°.

mixtures of fatty acids and 2-fluorofatty acids is presented in Fig. 2 and the retention data are listed in Table II.

The chromatogram in Fig. 1 shows that this mixture of lower 2-fluorofatty acids can be resolved and identified by gas chromatography with the exception of 2-fluoro-3-methylvaleric acid and 2-fluoro-4-methylvaleric acid. If this pair of 2-fluorofatty acids is excluded from the mixture, a mixture of the six fluorofatty

TABLE II

RETENTION DATA FOR LOWER FATTY ACID MIXED WITH 2-FLUOROFATTY ACIDS

Compound	*Relative time**
Acetic acid	0.21
Propionic acid	0.29
Isobutyric acid	0.33
Butyric acid	0.41
3-Methylbutyric acid	0.50
Valeric acid	0.64
2-Fluoropropionic acid	0.88
Hexanoic acid	1.00
Fluoroacetic acid	1.07
2-Fluorobutyric acid	1.27
2-Fluoro-3-methylbutyric acid	1.55
2 Fluorovaleric acid	1.93
Octanoic acid	2.46
2-Fluorohexanoic acid	3.00

* The values are retention times relative to hexanoic acid. The observed value for this reference standard was 9.4 min at 56°.

acids together with eight unfluorinated fatty acids to octanoic acid can be separated and identified, as evidenced by the chromatogram shown in Fig. 2.

It is of interest to note that on both columns fluoroacetic acid is retained longer than 2-fluoropropionic acid, which is the reverse of that expected from their structures and boiling points[8]; whereas, in the case of acetic acid and propionic acid, the retention times are in the order of their structures and boiling points.

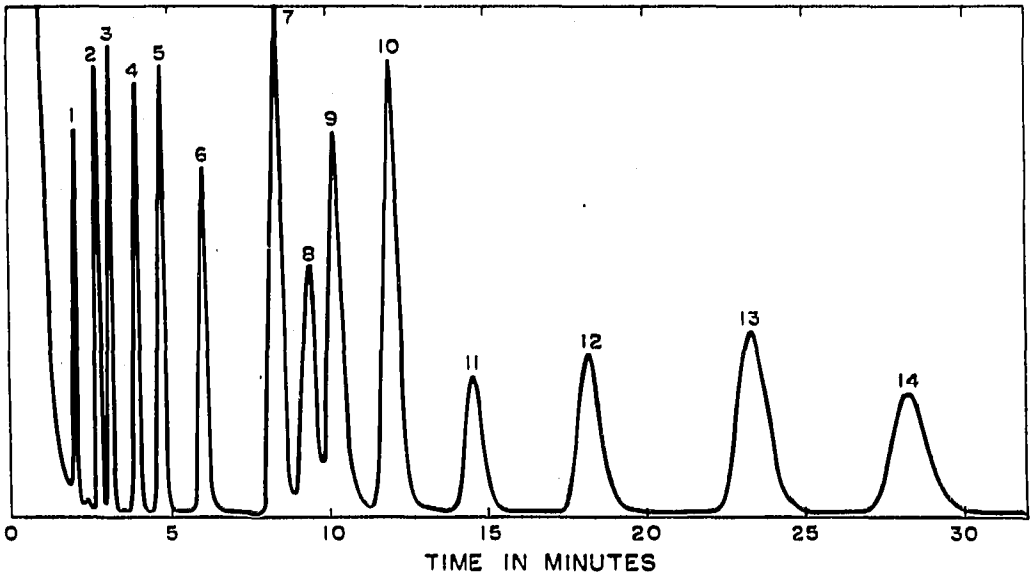

Fig. 2. Gas chromatogram of a mixture of fatty acids and 2-fluorofatty acids at 156°. The components are: (1) acetic acid; (2) propionic acid; (3) isobutyric acid; (4) butyric acid; (5) 3-methylbutyric acid; (6) valeric acid; (7) 2-fluoropropionic acid; (8) hexanoic acid; (9) fluoroacetic acid; (10) 2-fluorobutyric acid; (11) 2-fluoro-3-methylbutyric acid; (12) 2-fluorovaleric acid; (13) octanoic acid; (14) 2-fluorohexanoic acid.

In summary, a method for the gas chromatographic analysis of the lower 2-fluorofatty acids has been developed, and optimal conditions for the separation and identification of these acids in the presence of nonfluorinated fatty acids have been ascertained. These results should be applicable to the analysis of biological materials.

A study of the higher 2-fluorofatty acids to 20 carbons is in progress.

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