Gas chromatography of fluorinated fatty acids

II. Separation and identification of the methyl esters of 2-fluorofatty acids to 18 carbons

A rationale for our interest in the 2-fluorofatty acids has previously been reported[1]. In that paper, a gas chromatographic method was presented for the separation and identification of those free acids to 6 carbons and in combination with a similar series of unfluorinated aliphatic fatty acids. Since that time, GERSHON AND PARMEGIANI[2] have reported on the preparation and antifungal properties of additional members of the 2-fluorofatty acid series to 20 carbon atoms. Of further interest are the results of PATTISON, BUCHANAN AND DEAN[3], who reported the comparatively low mammalian toxicity of 2-fluorofatty acids, and that this was due to their inability to undergo β-oxidation.

The present report is concerned with a gas chromatographic study of the methyl esters of these acids alone and together with a similar series of methyl esters of unfluorinated fatty acids.

Experimental

Apparatus. All separations were carried out in an Aerograph Model 204 gas chromatograph, fitted with a flame ionization detector. The column employed was 5 ft. $\times$ $\frac{1}{8}$ in. O.D. stainless steel tube packed with 5 % diethyleneglycol succinate (DEGS) on acid washed Chromosorb W (80/100 mesh) with a flow rate of nitrogen of 25 ml/min. Retention data were obtained under isothermal conditions at two different temperatures. For the lower fatty acid esters, the column temperature was maintained at 85°, and the detector and injector temperatures were 100° and 140°, respectively. The higher fatty acid esters were chromatographed at a column temperature of 180° with the detector temperature at 205° and the injector temperature at 250°.

The mixture of the methyl esters of the 2-fluorofatty acids was separated using linear temperature programming at 5°/min from 100° to 200°, after which, isothermal conditions were maintained. The complete mixture of the methyl esters of the fluorinated and unfluorinated fatty acids was separated as above, except that the initial temperature was 75°. The detector temperature was kept at 210°, and the injector temperature was 220° in both cases.

Compounds. The methyl esters of the unfluorinated fatty acids were commercially available and the preparation of the 2-fluorofatty acids was according to the method of GERSHON AND PARMEGIANI[2]. Esterification of the 2-fluorofatty acids was performed by means of methanolic boron trifluoride[4]. All of the fluorinated fatty acid esters were purified by preparative gas chromatography in an Aerograph Autoprep Model A-700, and acetone solutions of the compounds were employed for injection into the chromatographs.

Results and discussion

Table I contains the analytical data characterizing the methyl esters of the 2-fluorofatty acids. A chromatogram of the separation of the methyl esters of the 2-fluorofatty acids can be seen in Fig. 1, and the gas chromatographic separation of the combined mixture of methyl esters of fatty acids and 2-fluorofatty acids is shown

TABLE I

ANALYTICAL DATA FOR METHYL ESTERS OF 2-FLUOROFATTY ACIDS

Methyl ester of	n_D^{25} *or m.p.*	$\nu_{max}^{C=O}$	*Formula*	*Calculated (%)*			*Found (%)*		
				C	*H*	*F*	*C*	*H*	*F*
Fluoroacetic acid[a]	1.3634[c]	1778, 1758							
2-Fluoropropionic acid[a,b]	1.3708[d]	1772, 1758							
2-Fluorobutyric acid[b]	1.3795[e]	1772, 1758	$C_5H_9FO_2$	49.93	7.55	15.82	49.83	7.45	15.98
2-Fluorovaleric acid[b]	1.3888[f]	1772, 1756	$C_6H_{11}FO_2$	53.72	8.27	14.16	53.91	8.44	14.01
2-Fluorohexanoic acid	1.3961	1778, 1754	$C_7H_{13}FO_2$	56.74	8.84	12.82	56.21	8.72	12.39
2-Fluoroheptanoic acid	1.4027	1775, 1754	$C_8H_{15}FO_2$	59.24	9.32	11.71	59.40	9.28	11.96
2-Fluorooctanoic acid	1.4091	1772, 1752	$C_9H_{17}FO_2$	61.29	9.72	10.78	61.44	9.73	10.54
2-Fluorononanoic acid	1.4131	1778, 1758	$C_{10}H_{19}FO_2$	63.13	10.07	9.99	63.16	10.04	9.81
2-Fluorodecanoic acid	1.4175	1778, 1755	$C_{11}H_{21}FO_2$	64.67	10.36	9.30	63.77	10.23	8.93
2-Fluoroundecanoic acid	1.4212	1778, 1752	$C_{12}H_{23}FO_2$	66.02	10.62	8.70	66.03	10.24	8.73
2-Fluorododecanoic acid	1.4240	1774, 1750	$C_{13}H_{25}FO_2$	67.20	10.85	8.18	67 56	10.54	7.93
2-Fluorotetradecanoic acid	1.4298	1772, 1758	$C_{15}H_{29}FO_2$	69.19	11.23	7.30	69.02	10.88	6.95
2-Fluorohexadecanoic acid	35–36°	1756	$C_{17}H_{33}FO_2$	70.79	11 53	6.59	71.23	11.35	6 46
2-Fluorooctadecanoic acid	37–38°	1755	$C_{19}H_{37}FO_2$	72.10	11.78	6.00	72.08	11.48	6.00

[a] Previously prepared, lit. refs. 5 and 6.
[b] Previously prepared, lit. ref. 7.
[c] Lit. ref. 6, n_D^{20} 1.3679.
[d] Lit. ref. 7, n_D^{20} 1.3707.
[e] Lit. ref. 7, n_D^{20} 1.3809.
[f] Lit. ref. 7, n_D^{20} 1.3920.

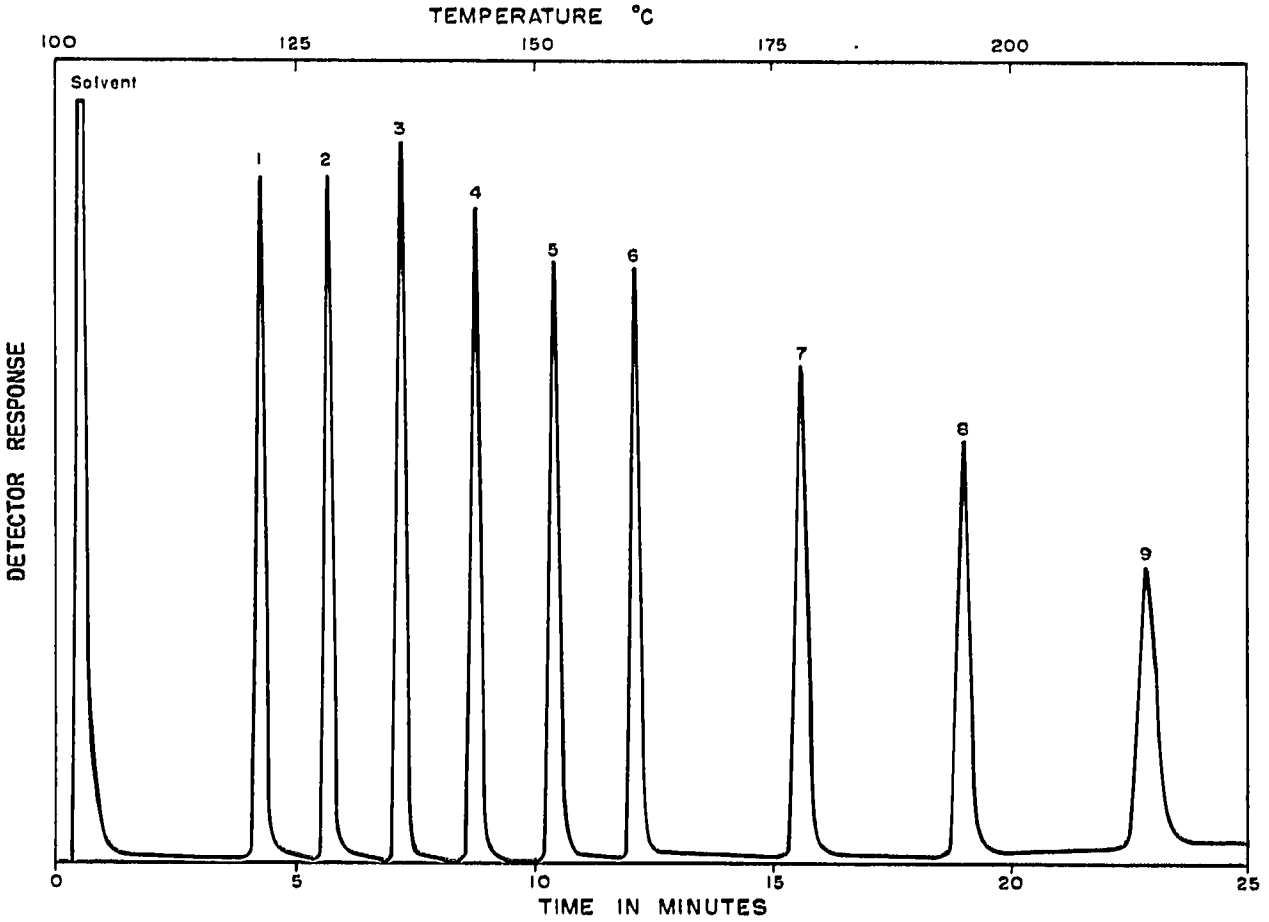

Fig. 1. Gas chromatogram of methyl esters of 2-fluorofatty acids resulting from linear temperature programming at 5°/min from 100° to 200°, after which isothermal conditions were maintained. The components are: 1 = methyl 2-fluoroheptanoate; 2 = methyl 2-fluorooctanoate; 3 = methyl 2-fluorononanoate; 4 = methyl 2-fluorodecanoate; 5 = methyl 2-fluoroundecanoate; 6 = methyl 2-fluorododecanoate; 7 = methyl 2-fluorotetradecanoate; 8 = methyl 2-fluorohexadecanoate; 9 = methyl 2-fluorooctadecanoate.

TABLE II

ISOTHERMAL RETENTION DATA FOR METHYL ESTERS OF FATTY ACIDS AND 2-FLUOROFATTY ACIDS TO EIGHT CARBON ATOMS

Methyl ester of	*Relative time**
Acetic acid	0.20
Propionic acid	0.25
Butyric acid	0.35
2-Fluoropropionic acid	0.51
2-Fluoroacetic acid	0.56
2-Fluorobutyric acid	0.77
Hexanoic acid	1.00
2-Fluorovaleric acid	1.29
2-Fluorohexanoic acid	2.25
Octanoic acid	3.35
2-Fluoroheptanoic acid	4.12
2-Fluorooctanoic acid	7.74

* The values are retention times relative to methyl hexanoate. The observed value for this reference standard was 3.1 min at 85°.

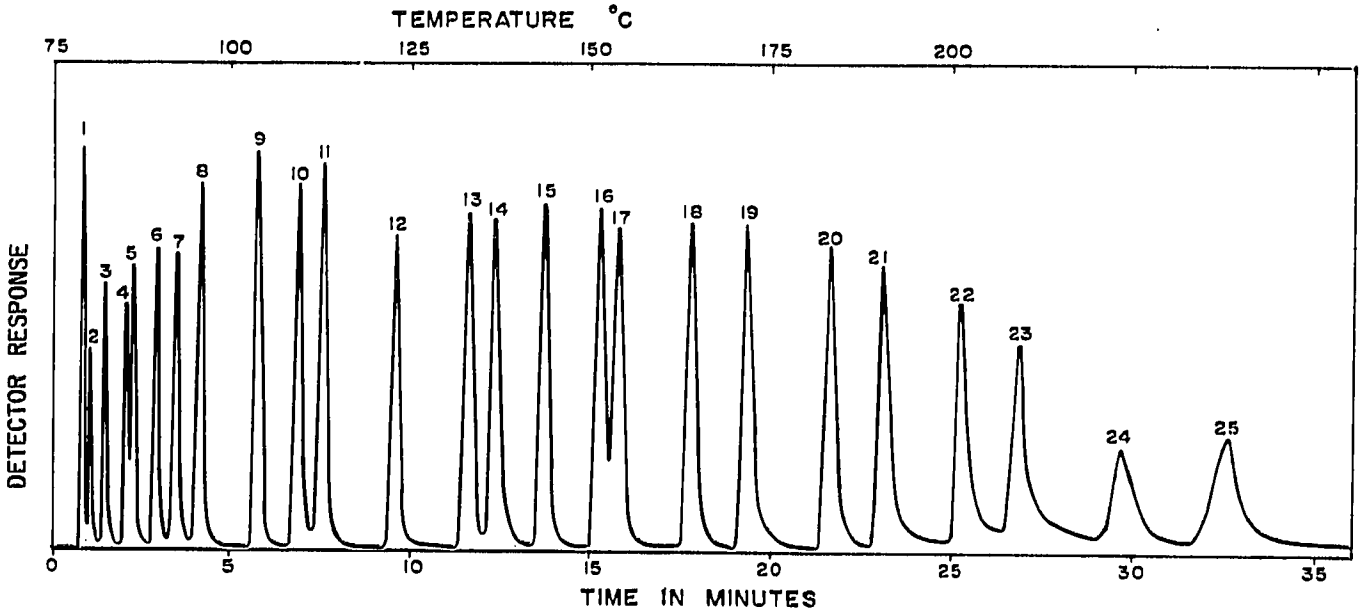

Fig. 2. Gas chromatogram of a mixture composed of methyl esters of fatty acids and methyl esters of 2-fluorofatty acids resulting from linear temperature programming at 5°/min from 75° to 200° after which isothermal conditions were maintained. The components are: 1 = methyl acetate; 2 = methyl propionate; 3 = methyl butyrate; 4 = methyl 2-fluoropropionate; 5 = methyl 2-fluoroacetate; 6 = methyl 2-fluorobutyrate; 7 = methyl hexanoate; 8 = methyl 2-fluorovalerate; 9 = methyl 2-fluorohexanoate; 10 = methyl octanoate; 11 = methyl 2-fluoroheptanoate; 12 = methyl 2-fluorooctanoate; 13 = methyl 2-fluorononanoate; 14 = methyl decanoate; 15 = methyl 2-fluorodecanoate; 16 = methyl dodecanoate; 17 = methyl 2-fluoroundecanoate; 18 = methyl 2-fluorododecanoate; 19 = methyl tetradecanoate; 20 = methyl 2-fluorotetradecanoate; 21 = methyl hexadecanoate; 22 = methyl 2-fluorohexadecanoate; 23 = methyl octadecanoate; 24 = methyl 2-fluorooctadecanoate; 25 = methyl eicosanoate.

TABLE III

ISOTHERMAL RETENTION DATA FOR METHYL ESTERS OF FATTY ACIDS TO TWENTY CARBON ATOMS AND 2-FLUOROFATTY ACIDS TO EIGHTEEN CARBON ATOMS

Methyl ester of	*Relative time**
2-Fluoroheptanoic acid	0.10
2-Fluorooctanoic acid	0.13
2-Fluorononanoic acid	0.15
Decanoic acid	0.17
2-Fluorodecanoic acid	0.21
Dodecanoic acid	0.26
2-Fluoroundecanoic acid	0.28
2-Fluorododecanoic acid	0.38
Tetradecanoic acid	0.49
2-Fluorotetradecanoic acid	0.76
Hexadecanoic acid	1.00
2-Fluorohexadecanoic acid	1.56
Octadecanoic acid	2.08
9-Octadecenoic acid	2.08
9,12-Octadecadienoic acid	2.30
9,12,15-Octadecatrienoic acid	2.69
2-Fluorooctadecanoic acid	3.26
Eicosanoic acid	4.43

* The values are retention times relative to methyl hexadecanoate. The observed value for this reference standard was 7.3 min at 180°.

in Fig. 2. Isothermal retention data for the methyl esters of the lower fatty acids and fluorinated fatty acids are included in Table II. Table III contains the corresponding data on the methyl esters of the higher fatty acids and 2-fluorofatty acids.

The chromatograms of Figs. 1 and 2 show that the mixtures of the methyl esters of 2-fluorofatty acids alone and admixed with unfluorinated fatty acids can be separated. For biological studies, it is generally more desirable to employ isothermal conditions, and consequently the retention data of Tables II and III were obtained. Since odd numbered fatty acids are uncommon in biological systems, they were excluded from our study, with the exception of propionic acid. This resulted in less overlapping and more easily interpretable results. Methyl octadecanoate and methyl 9-octadecenoate were not separated under the conditions reported. These esters have been separated on a column containing a higher percentage of liquid phase, but in such a column, overlapping occurs between some of the fluorinated and unfluorinated esters.

It should be noted that methyl fluoroacetate and methyl 2-fluoropropionate came off the column in reversed order as compared with the unfluorinated esters. The same observation was previously reported[1] for the corresponding fluorinated acids. On chromatographing methyl chloroacetate and methyl 2-chloropropionate under conditions similar to those employed for the corresponding fluoroesters, methyl 2-chloropropionate came off the column before methyl chloroacetate. Thus, it appears that this effect may not be peculiar to the fluorinated acids, but may be explained on the basis that the halogenoacetic acids and methyl esters are more polar than the halogenopropionic acids and methyl esters, and that on a polar column the polar effect exceeds the effect due to the boiling point.

Boyce Thompson Institute for Plant Research, Inc.,
Yonkers, N.Y. 10701 (U.S.A.)

HERMAN GERSHON
J. A. A. RENWICK

1 H. GERSHON AND J. A. A. RENWICK, *J. Chromatog.*, 20 (1965) 134.
2 H. GERSHON AND R. PARMEGIANI, *J. Med. Chem.*, 10 (1967) 186.
3 F. L. M. PATTISON, R. L. BUCHANAN AND F. H. DEAN, *Can. J. Chem.*, 43 (1965) 1700.
4 L. D. METCALFE AND A. A. SCHMITZ, *Anal. Chem.*, 33 (1961) 363.
5 E. GRYSZKIEWICZ-TROCHIMOWSKI, A. SPORZYNSKI AND J. WNUK, *Rec. Trav. Chim.*, 66 (1947) 413.
6 B. C. SAUNDERS AND G. J. STACEY, *J. Chem. Soc.*, (1948) 1773.
7 A. YA. YAKUBOVICH AND P. GITEL, *Zh. Obshch. Khim.*, 36 (1966) 874; *C.A.*, 65 (1966) 10493.

Received December 1st, 1966