Notes

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Gas chromatographic separation of esters of fluoro analogs of citric acid cycle intermediates

As an approach to the study of the rate limiting role of citric acid cycle enzymes in tissue metabolism, specific fluoro carboxylic acid enzyme inhibitors were synthesized[1], and their effect on isolated enzymes and multienzyme systems investigated[2]. Application of fluoro carboxylic acid enzyme inhibitors as metabolic probes in organ systems and in the whole animal requires analytical methods capable of detecting these fluoro acids in various tissues, which contain also the physiological nonfluorinated carboxylic acid enzyme substrates. In view of the recent progress in the gas chromatographic analysis of the citric acid cycle esters[3], the results presented in this paper show that gas chromatography may offer a feasible solution to this analytical problem.

Materials

Ethyl 3-fluorolactate[4,5], diethyl fluorosuccinate, 3-fluoro-2-oxoglutarate, diethyl 3-fluoro-2-hydroxyglutarate, triethyl 2-fluoroisocitrate[4], diethyl 2-fluoroglutarate, triethyl fluoropropane-1,1,3-tricarboxylate[6] and fluorocitrate[7] were synthesized according to previously developed methods. The syntheses of fluoromalonate[8] diethyl fluorooxalacetate[9] and diethyl 3-fluoromalate[10] were carried out by procedures published by other workers.

Diethyl 2-oxoglutarate, triethyl citrate and triethyl isocitrate were prepared by the addition of ethanol to a mixture of the carboxylic acids in trifluoroacetic anhydride[11]. Other esters (see Table I) were obtained commercially. Before analysis on the DEGS column, the ester samples were first purified by preparative gas chromatography on a 20-ft. SE-30 column.

Apparatus. The Varian Aerograph Model 700 was used, with thermal conductivity detection. A column (12 ft. × 1/8 in. O.D.) containing diethylene glycol succinate (DEGS), 12% on Chromosorb W, was operated at a helium flow rate of 67 ml/min (50 p.s.i.). A column (5 ft. × 1/4 in. O.D.) containing silicone polymer (SE-30) 20% on Chromosorb W, was operated at a flow rate of 150 ml/min (20 p.s.i.) Individual esters were preparatively purified on a column (20 ft. × 3/8 in. O.D.) of SE-30, 30% on Chromosorb W. The input temperature was 225°. Injected samples contained 0.1 to 0.5 μl of each ester.

RESULTS AND DISCUSSION

Succesful separation of esters of carboxylic acids and their fluoro homologs was obtained with a DEGS column. Retention times were measured for each ester at three column temperatures 25° apart in the range 100–225°. Nearly straight lines were obtained by plotting the logarithm of the retention time against the reciprocal of the temperature. The retention times were related to diethyl succinate as summarized

TABLE I

RELATIVE RETENTION TIMES OF ETHYL ESTERS OF CARBOXYLIC ACIDS AND CORRESPONDING FLUORO ANALOGS ON DEGS COLUMNS AT 125°, 150°, 175° AND 200°

Ethyl ester	*Temp. (°C)*	*Relative retention time*[a]	*Fluoro analog*	*Relative retention time*	*Ratio*[b]
Lactic	125	0.28	3-fluoro	0.66	2.3
	150	0.36		0.77	2.1
	175	0.50		0.66	1.3
Malonic	125	0.65	fluoro	1.0	1.5
	150	0.66		1.0	1.5
	175	0.77		0.87	1.1
Succinic	125	1.0	fluoro	1.5	1.5
	150	1.0		1.5	1.5
	175	1.0		1.2	1.2
Glutaric	125	1.4	2-fluoro	2.6	1.9
	150	1.4		2.3	1.6
	175	1.3		1.8	1.4
	200	1.3		1.6	1.2
Oxalacetic	150	2.5	fluoro	3.0	1.2
	175	dec.		2.3	
	200	dec.		2.1	
Malic	150	3.9	3-fluoro	3.0	0.77
	175	3.1		2.4	0.77
	200	2.7		2.1	0.78
2-Oxoglutaric	150	3.9	3-fluoro	3.2	0.78
	175	3.7		3.2	0.86
	200	3.0		2.7	0.90
3-Hydroxyglutaric	150	3.2	2-fluoro	3.7	1.2
	175	4.4		4.8	1.2
	200	3.5		4.6	1.3
Propane tricarboxylic	150	4.5	fluoro	4.6	1.0
	175	5.9		6.7	1.2
	200	4.2		5.0	1.2
Citric	175	13.0	2-fluoro	18.0	1.4
	200	8.7		11.4	1.3
Isocitric	175	dec.	2-fluoro	18.0	
	200	dec.		19.4	

[a] Diethyl succinate = 1.00.
[b] The ratio of fluoro to nonfluoro compound retention times.

in Table I. For mixtures of each ester with its fluoro analog, the ratio of retention times is summarized in the last column.

In most cases the nonfluoro ester emerged from the column before the corresponding fluoro ester. The fluoro ester emerged first in the cases of diethyl malate and diethyl oxoglutarate.

Extraneous peaks were observed at 125° for all esters of keto acids. At 175°, triethyl isocitrate decomposed completely and fluoroisocitrate was partially decomposed. In general, the fluoro analogs appeared to be more thermally stable than the nonfluoro esters.

Trimethylsilyl[12] and trimethylsilyl oximino derivatives[13] were prepared from the hydroxy and keto esters, but gas chromatographic separation of these derivatives of fluoro analogs from the corresponding nonfluoro ester was not successful.

Esters of hydroxy and keto carboxylic acids and their fluoro homologs were

successfully separated by isothermal gas chromatography on a DEGS column, as the basis of an analytical procedure for the study of tissue distribution and *in vivo* metabolic effects of fluoro inhibitors.

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