CHROM. 3630

Gas chromatography of isomeric fatty acid methyl esters

As an adjunct to a recent study[1] of the carboxylic acids present in Latakia tobacco leaf, separation of over twenty standard samples of methyl esters of isomeric saturated monocarboxylic acids containing 1–7 carbon atoms was achieved on various types of capillary column.

It would appear that no systematic study of the complete range of these lower isomeric compounds has been made on capillary columns. SCHOMBURG[2] has studied the retention characteristics of methyl esters of a large number of branched-chain acids of five or more carbon atoms on a wide range of stationary phases coated on capillary columns. VASIL'EV *et al.*[3] have reported the separation of the methyl esters of many lower isomeric acids, using a packed column of silicone grease.

Our aim in this paper is to show that separation of these closely related compounds can be achieved on capillary columns and that, by suitable choice of stationary phase, tentative identifications can be made on the basis of retention time.

The compounds investigated include the methyl esters of all saturated monocarboxylic acids containing 1–6 carbon atoms and as many isomers of 7 carbon atoms as were available at the time of the study.

Experimental

Preparation of compounds. Many of the compounds used were commercially available as methyl esters or as free carboxylic acids.

3-Methylpentanoic acid, 3-methylhexanoic acid, 4-methylhexanoic acid and 5-methylhexanoic acid were synthesised by a general method[4] using sodium diethylmalonate and the appropriate alkyl halide.

2-Methylbutyric acid, 2,2-dimethylbutyric acid and 2-methylhexanoic acid were prepared by a general method[5] employing the carboxylation of the corresponding Grignard reagent.

2-Ethylbutyric acid and 2,3-dimethylbutyric acid were prepared by an extension of a method of FISCHER *et al.*[6,7] using sodium ethyl cyanoacetate.

Conversion of acids to methyl esters was effected, in most cases, by refluxing with methanol–conc. sulphuric acid reagent. However, more sterically hindered acids, *e.g.* trimethylacetic acid, gave acceptable yields only when treated with methanol–boron trifluoride reagent.[8]

Gas chromatography. A Perkin-Elmer F.11 instrument, equipped with a flame-ionisation detector, was employed throughout.

The columns and conditions used for the separation were as follows:

(i) A stainless-steel open-tubular capillary column, 30 m × 0.25 mm I.D., coated with silicone-gum rubber SE-30 (SE30). Nitrogen carrier gas inlet pressure: 5 p.s.i.g. Operating temperature: linearly programmed from 35° at 4°/min.

(ii) A stainless-steel open-tubular capillary column, 50 m × 0.25 mm I.D., coated with trixylenyl phosphate (TXP). Nitrogen carrier gas inlet pressure: 5 p.s.i.g. Operating temperature: linearly programmed from 35° at 3°/min.

(iii) A column, similar to (ii) but coated with poly(phenyl ether), OS124 (PPE). Nitrogen carrier gas inlet pressure: 5 p.s.i.g. Operating temperature: linearly programmed from 35° at 2°/min.

TABLE I

RELATIVE RETENTION TIMES OF FATTY ACID METHYL ESTERS

Methyl ester of	*Boiling point**	*Relative retention time*			
		SE30	*TXP*	*PPE*	*DEGS*
Formic acid	32	1.51	2.73	—	0.86
Acetic acid	57	2.19	3.29	2.39	1.20
Propionic acid	80	3.35	3.76	3.06	1.64
iso-Butyric acid	92	4.12	4.10	3.42	1.86
n-Butyric acid	102	4.67	4.67	4.03	2.44
Tri-methylacetic acid	101	4.72	4.24	3.59	1.77
(±)-2-Methylbutyric acid	116	5.50	5.15	4.54	2.80
3-Methylbutyric acid	117	5.50	5.21	4.67	3.01
3,3-Dimethylbutyric acid	126	6.07	5.62	4.95	3.52
n-Pentanoic acid	127	6.08	6.08	5.52	3.88
2,2-Dimethylbutyric acid	127	6.24	5.62	4.95	3.20
(±)-2,3-Dimethylbutyric acid	136	6.55	6.20	5.53	3.88
2-Ethylbutyric acid	136	6.70	6.39	5.66	3.88
(±)-2-Methylpentanoic acid	138	6.86	6.39	5.94	4.16
(±)-3-Methylpentanoic acid	142	6.95	6.74	6.23	5.01
4-Methylpentanoic acid	144	7.09	6.90	6.42	5.20
n-Hexanoic acid	150	7.48	7.40	7.03	5.88
(±)-2-Methylhexanoic acid	157	8.06	7.66	7.32	6.18
(±)-3-Methylhexanoic acid	163	8.12	7.93	7.56	6.75
(±)-4-Methylhexanoic acid	161	8.55	8.41	8.25	7.32
5-Methylhexanoic acid	165	8.36	8.20	8.03	7.15
n-Heptanoic acid	173	8.78	8.75	8.60	7.92
n-Octanoic acid	193	10.00	10.00	10.00	10.00

* Approximate boiling point in °C at atmospheric pressure.

(iv) A support-coated open-tubular capillary column, 15 m × 0.5 mm I.D. coated with diethylene glycol succinate polyester (DEGS). Nitrogen carrier gas inlet pressure. 1 p.s.i.g. Operating temperature: linearly programmed from 35° at 1.5°/min.

The SE30 column was prepared in this laboratory according to a recently published method[9] and was somewhat shorter than the other open-tubular columns which were standard lengths supplied and coated by Perkin-Elmer Ltd.; the support-coated column was also supplied by Perkin-Elmer.

Results and discussion

Retention data for linearly temperature programmed analyses on four different stationary phases are detailed in Table I. All retention times were measured from sample injection and are presented as retention times relative to methyl *n*-octoate = 10.00.

The relative retention of times *n*-butyric ester, iso-butyric ester, *n*-pentanoic ester, *n*-hexanoic ester and *n*-heptanoic ester were almost identical on SE30 and TXP although the order of elution was not the same.

The order of elution on SE30 was largely determined by the boiling point of the esters but on the more polar stationary phases, especially DEGS, the order in which the compounds appeared was much influenced by their structure. On all stationary phases investigated, it was noted that 5-methylhexanoic ester was eluted before 4-

methylhexanoic ester; this appears to be anomalous on the basis of both the structures and (reported) boiling points of the two compounds.

The mixture was not wholly resolved on all the columns used. 2-Methylbutyric ester and 3-methylbutyric ester were coincident on SE30, as were 3,3-dimethylbutyric ester and *n*-pentanoic ester; both pairs of esters were resolved on the other stationary phases.

3,3-Dimethylbutyric ester and 2,2-dimethylbutyric ester were coincident on both TXP and PPE, but were separated on SE30 or DEGS.

2-Ethylbutyric ester and 2-methylpentanoic ester appeared together on TXP only.

n-Pentanoic ester and 2,3-dimethylbutyric ester were coincident on PPE and both were inseparable from 2-ethylbutyric ester on DEGS; all three compounds were resolved on TXP or SE30.

The retention characteristics of the DEGS support-coated column differ from those of the conventional open-tubular column with a similar number of theoretical plates (the latter being approximately three times the length of the former). The retention times of esters on the DEGS column were much shorter than on any of the conventional capillary columns, regardless of polarity.

However, this in no way detracted from the usefulness of this column when used in conjunction with other standard capillary columns for the identification of the compounds above.

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