CHROM. 5898

CAPILLARY GAS CHROMATOGRAPHY OF FREE SATURATED C_2–C_6 FATTY ACIDS*

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(Received December 13th, 1971)

SUMMARY

Conditions were determined for the successful separation of free lower fatty acids by means of capillary gas chromatography. Base-line separation of all sixteen saturated C_2–C_6 fatty acids has been achieved on a steel capillary column of 45 m length and 0.25 mm I.D. with nitrogen as the carrier gas. The best stationary phase is Ucon LB-550-X with the addition of phosphoric acid, operated at 125°.

INTRODUCTION

It was just twenty years ago that the separation and analysis of C_1–C_{12} fatty acids by gas–liquid chromatography (GLC) on a column packed with Celite 545 coated with a mixture of silicone oil DC 550 and stearic acid (9:1) was published[1]. This work not only laid the basis for lipid chemistry, but also changed the direction and speed of the development of separation methods, thus opening an area of unprecedented research activity and fascinating possibilities that have moved forward the limits of human knowledge.

Since that time, the gas–liquid chromatography of fatty acids and glycerides has been used many times and there has, up to now, been nobody who has taken on the task of reviewing completely and critically the thousands of items of information available on the applications of GLC in the chemistry of fatty and other acids. Whereas a fairly reliable scheme[2] has been accepted for the separation of higher fatty acids in the form of their esters, the separation and analysis of the lower fatty acids still present certain difficulties. This fact is probably the reason why there have been very few papers[3–6] that attempt to solve the problem by capillary gas chromatography, the results being unsatisfactory.

* Dedicated to Dr. A. J. P. MARTIN and Dr. A. T. JAMES on the occasion of the 20th anniversary of the publication of their now classical paper on gas–liquid chromatography (of fatty acids).

TABLE I

BOILING POINTS, ADJUSTED RETENTION RATIOS, $R_{1,2}$ (RELATIVE TO *n*-PENTANOIC ACID), AND KOVÁTS RETENTION INDICES, I, OF FREE SATURATED C_2–C_6 FATTY ACIDS ON THREE STATIONARY PHASES

Acid	*Boiling point* (°C)	*Trimer acid*				*Tricresyl phosphate*				*Ucon LB-550-X*			
		$R_{1,2}$		I		$R_{1,2}$		I		$R_{1,2}$		I	
		120°	140°	120°	140°	110°	130°	110°	130°	125°	145°	125°	145°
Acetic	118	0.138	0.176	988	987	0.114	0.176	1098	1091	0.178	0.208	1076	1068
Propionic	141	0.276	0.317	1096	1094	0.274	0.314	1201	1190	0.314	0.347	1174	1166
Isobutyric	154	0.405	0.449	1162	1157	0.374	0.416	1251	1239	0.428	0.461	1228	1221
Butyric	162	0.498	0.542	1196	1192	0.501	0.534	1299	1283	0.542	0.572	1268	1262
Trimethylacetic	164	0.519	0.555	1203	1196	0.441	0.479	1278	1264	0.521	0.552	1261	1255
3-Methylbutyric	177	0.697	0.736	1251	1247	0.694	0.725	1351	1337	0.742	0.763	1323	1316
(±)-2-Methylbutyric	177	0.759	0.795	1265	1261	0.717	0.746	1357	1343	0.776	0.796	1330	1324
n-Pentanoic	186	1.000	1.000	1309	1302	1.000	1.000	1410	1396	1.000	1.000	1374	1368
2,2-Dimethylbutyric	186	1.080	1.079	1315	1313	0.933	0.943	1399	1385	1.040	1.039	1381	1376
3,3-Dimethylbutyric	190	1.215	1.212	1341	1338	1.605	1.539	1486	1474	1.542	1.489	1461	1445
(±)-2,3-Dimethylbutyric	190	1.236	1.229	1344	1341	1.154	1.148	1433	1421	1.215	1.200	1410	1403
2-Ethylbutyric	190	1.286	1.274	1351	1346	1.267	1.241	1448	1435	1.308	1.280	1426	1416
(±)-2-Methylpentanoic	193[a]	1.381	1.360	1362	1358	1.333	1.296	1456	1443	1.340	1.306	1431	1420
(±)-3-Methylpentanoic	198	1.431	1.426	1368	1366	1.449	1.407	1470	1458	1.427	1.386	1444	1431
4-Methylpentanoic	199[b]	1.524	1.490	1379	1374	1.540	1.480	1480	1467	1.485	1.436	1452	1438
n-Hexanoic	205	1.855	1.776	1411	1406	1.933	1.812	1516	1504	1.816	1.717	1495	1473

[a] At 748 torr.
[b] At 752 torr.

With this short report concerning the above problem, we wish, above all, to pay honour to the inventors of GLC with whom to live in the same age and to meet with their ideas in everyday work is, in itself, a fortune.

EXPERIMENTAL

Apparatus

A Fractovap GI (Carlo Erba, Milan, Italy) gas chromatograph equipped with a flame-ionization detector was used for these studies. The recorder was a Leeds and Northrup Speedomax G with a full-scale deflection of 1 mV. Nitrogen was used as the carrier gas.

Chromatographic columns

The columns, fabricated from stainless-steel tubing, were 45 m long and had an I.D. of 0.25 mm. The plug method[3] was used for coating the columns. A solution (0.4–0.6 ml) of the liquid phase (90 mg) and orthophosphoric acid (10 mg, 85 % w/w) in acetone (1.0 ml) was forced through the tubing with the aid of nitrogen at a velocity of 0.8–1.2 cm/sec. The columns were conditioned for 6 h at approx. 20° below the maximum acceptable temperature for each stationary phase.

Chemicals

Trimer acid was a product of Emery Industries (Cincinnati, Ohio, U.S.A.), and tricresyl phosphate and Ucon LB-550-X were supplied by Carlo Erba. The majority of the model fatty acids were commercial products of 97–98 % purity (measured by GLC). 3-Methylpentanoic, 2-methylbutyric, 2,2-dimethylbutyric, 2-ethylbutyric and 2,3-dimethylbutyric acids were prepared by methods described elsewhere[6].

RESULTS AND DISCUSSION

The acids studied and their boiling points, retention ratios and Kováts retention indices (measured with a precision of ±2 index units on three stationary phases at two temperatures) are given in Table I.

A mixture of 16 compounds has to be dealt with, of which many have boiling points very close to each other, such as 3-methylbutyric and (±)-2-methylbutyric acids; (±)-2,3-dimethylbutyric, 3,3-dimethylbutyric and 2-ethylbutyric acids; and (±)-2-methylpentanoic, (±)-3-methylpentanoic and 4-methylpentanoic acids.

Owing to the very similar physico-chemical properties of some isomers, the application of capillary columns is justifiable, although it was necessary[6] to use several columns of different polarities to attain a complete separation of some isomers of lower fatty acids in the form of their methyl esters. We tried out the previously used stationary phases trimer acid[4] and tricresyl phosphate[5], but, instead of using dimethylnaphthalenesulphonic acid as an additive, orthophosphoric acid[3] was employed, which proved to be especially suitable for the separation of free fatty acids.

For some pairs of acids, capillary chromatography on trimer acid does not give good separations. Thus, for instance, the minimum plate number ($n_{req.}$) necessary for a base-line separation of the pairs butyric acid–trimethylacetic acid and 3,3-dimethylbutyric acid–(±)-2,3-dimethylbutyric acid is 152,000 and 123,000, respectively.

The retention order of the acids corresponds mainly with the sequence of their boiling points. On the tricresyl phosphate column, the elution of the acids is similar, except for trimethylacetic, 2,2-dimethylbutyric and 3,3-dimethylbutyric acids. The rise in the relative volatility is due to the steric hindrance of the COOH group, which brings about a weakening of the hydrogen bond with the stationary phase. The shift of the retention of 3,3-dimethylbutyric acid up to the end of the group of branched-chain acids is caused by the contribution of the induction due to the three methyl groups at the end of the chain.

The best separation was obtained on a polypropylene glycol, Ucon LB-550-X. The $n_{req.}$ values necessary for a base-line separation of all the critical pairs of acids at 125° were less than 100,000 theoretical plates. It can be seen from the data in Table I that a further increase in the temperature leads to poorer separations with most of the acids studied.

Fig. 1 shows a chromatogram of a mixture of all the free saturated C_2–C_6 fatty acids, obtained on a column with Ucon LB-550-X at 125°.

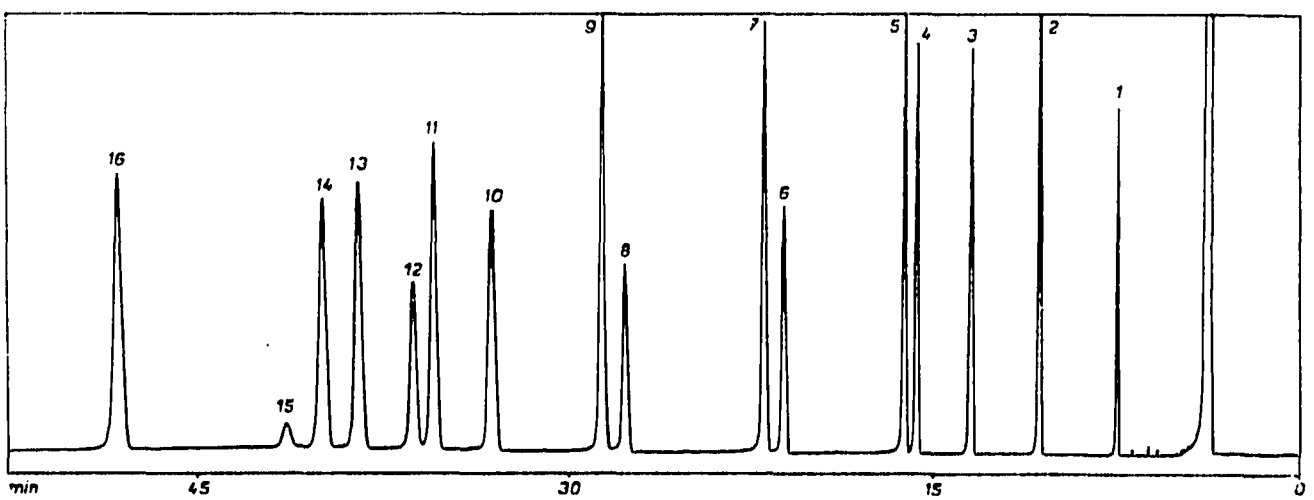

Fig. 1. Capillary gas chromatogram of free saturated C_2–C_6 fatty acids. Steel capillary column, 45 m × 0.25 mm I.D.; Ucon LB-550-X + phosphoric acid, 125°. 1 = Acetic acid; 2 = propionic acid; 3 = isobutyric acid; 4 = trimethylacetic acid; 5 = butyric acid; 6 = 3-methylbutyric acid; 7 = (±)-2-methylbutyric acid; 8 = *n*-pentanoic acid; 9 = 2,2-dimethylbutyric acid; 10 = (±)-2,3-dimethylbutyric acid; 11 = 2-ethylbutyric acid; 12 = (±)-2-methylpentanoic acid; 13 = (±)-3-methylpentanoic acid; 14 = 4-methylpentanoic acid; 15 = 3,3-dimethylbutyric acid; and 16 = *n*-hexanoic acid.

The occurrence of symmetrical peaks and the high efficiency (70,000 theoretical plates) makes it possible to analyze free fatty acids even at very different mutual ratios of the latter. The method was applied to the analysis of lower fatty acids in biological materials and in foodstuffs.

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