

CHROM. 7940

## GAS CHROMATOGRAPHY OF HOMOLOGOUS ESTERS

### VII\*. THE RETENTION BEHAVIOUR OF PYRUVATE ESTERS AND RELATED CARBONYL AND CARBOXYL COMPOUNDS

J. K. HAKEN\*\*, D. K. M. HO and CAROLYN E. VAUGHAN\*\*\*

Department of Polymer Science, The University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033 (Australia)

(Received August 17th, 1974)

---

#### SUMMARY

The retention behaviour of homologous pyruvate esters on a range of polysiloxane stationary phases is reported. The polysiloxane phases studied vary in character from essentially non-polar to those possessing donor and acceptor character within the concept of the Rohrschneider classification. The results are considered to-

gether with those of simple esters and ketones containing the  $\overset{\text{O}}{\parallel}\text{C}-\text{O}-$ ,  $\overset{\text{O}}{\parallel}\text{C}-$ ,  $\overset{\text{O}}{\parallel}\text{C}-\overset{\text{O}}{\parallel}\text{C}-$  and  $\overset{\text{O}}{\parallel}\text{C}-\text{CH}_2-\overset{\text{O}}{\parallel}\text{C}-$  structures

---

#### INTRODUCTION

Gas chromatography of pyruvic acid would appear to be limited to studies concerned with the separation of the various acids associated with the Krebs or tricarboxylic acid cycle where the methyl esters<sup>1-8</sup> have been the derivatives generally considered. The most extensive work relating to the pyruvate esters is that of Simmonds and co-workers<sup>8</sup>, who have shown that methylation with diazomethane and with methanol-hydrochloric acid each yields two products, namely the methyl ester plus methyl-2-methylglycidate and 2,2'-dimethoxypyruvate, respectively.

Lovelock<sup>9</sup> has reported that the electron affinity of a homologous series is determined principally by the affinity of the common functional group and is relatively little influenced by the hydrocarbon moiety. Pyruvates were shown<sup>10</sup> to possess un-

---

\* Part VI: J. R. Ashes and J. K. Haken, *J. Chromatogr.*, 101 (1974) 103.

\*\* To whom correspondence should be addressed.

\*\*\* Present address: Reckitts & Colman Pty. Ltd., 44 Wharf Road, West Ryde, 2114 N.S.W., Australia.

TABLE I  
RETENTION DATA FOR SOME SATURATED ESTERS AND KETONES ON NON-POLAR AND DONOR PHASES

Compound	Stationary phase											
	SE-30		OV-7		DC-710		100% Phenyl		DC-230		DC-530	
	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$
Methyl acetate	0.121	509	0.148	559	0.195	609	0.409	732	0.122	519	0.182	569
Ethyl acetate	0.190	592	0.242	648	0.306	690	0.593	803	0.189	599	0.265	642
Propyl acetate	0.332	695	0.438	755	0.552	796	0.962	895	0.339	704	0.453	747
Butyl acetate	0.570	794	0.764	855	0.960	895	1.591	991	0.606	810	0.756	846
Pentyl acetate	0.969	891	1.357	958	1.690	996	2.546	1081	1.077	914	1.235	942
Hexyl acetate	1.638	988	2.329	1056	2.879	1092	4.136	1174	1.856	1012	2.041	1040
2-Propanone	0.105	459	0.141	538	0.154	574	0.419	729	0.102	499	0.309	672
2-Butanone	0.184	569	0.259	652	0.295	690	0.677	825	0.189	608	0.506	770
2-Pentanone	0.311	673	0.430	746	0.507	786	0.935	889	0.327	706	0.790	858
2-Hexanone	0.530	778	0.738	848	0.921	892	1.645	1002	0.589	811	1.383	969
2-Heptanone	0.871	875	1.269	949	1.573	988	2.613	1094	1.047	913	2.284	1068
2-Octanone	1.462	977	2.154	1048	2.727	1086	4.226	1191	1.804	1010	3.790	1169
Methyl pyruvate	0.359	701	0.593	807	0.824	873	1.581	994	0.393	739	1.247	948
Ethyl pyruvate	0.521	774	0.889	882	1.211	941	2.161	1057	0.604	815	1.877	1029
Propyl pyruvate	0.845	870	1.426	971	1.969	1028	3.194	1135	1.004	906	2.840	1112
Butyl pyruvate	1.396	968	2.370	1066	3.308	1120	5.065	1227	1.738	1003	4.605	1207
Pentyl pyruvate	2.298	1067	3.957	1162	5.564	1212	8.032	1319	2.982	1100	7.556	1305
Hexyl pyruvate	3.772	1164	6.607	1258	9.308	1304	—	—	5.062	1194	—	—
2,3-Butanedione	0.171	555	0.246	642	0.295	690	0.742	843	0.167	586	0.444	744
2,3-Pentanedione	0.302	667	0.436	749	0.542	798	—	—	0.320	702	0.753	848
2,3-Hexanedione	0.495	764	0.705	839	0.885	885	1.323	959	0.538	795	1.198	941
2,3-Octanedione	1.392	968	2.075	1041	2.718	1085	3.968	1178	1.745	1004	3.309	1142
2,4-Pentanedione	0.534	779	0.849	874	1.141	931	2.290	1068	0.618	819	1.605	998
2,5-Hexanedione	1.015	906	2.030	1037	3.150	1110	6.806	1286	1.411	966	4.642	1201
3,5-Heptanedione	1.462	977	2.344	1064	3.176	1113	5.000	1224	1.855	1015	4.025	1181
4,6-Nonanedione	3.540	1152	5.649	1228	7.608	1268	10.742	1377	4.764	1183	9.062	1341

usually large cross sections for absorption of thermal electrons, a reaction with free electrons indicating formation of stable ions and acceptor character.

With certain of the early studies<sup>4-6</sup> the reported position of elution of methyl pyruvate varied in relation to methyl lactate and, with acid-catalysed esterification, the dimethoxy compound may have been inadvertently considered.

The identification of the esterification products<sup>8</sup> has allowed a study of the retention behaviour of homologous pyruvate esters on stationary phases of varying polar character and possessing some donor and acceptor properties. It has been shown with simple carbonyl compounds, *i.e.* esters and ketones, that retardation of elution of ketones occurs with phases such as QF-1 or OV-210, which possess acceptor character, to the extent that separation of mixtures is enhanced and it is of interest to consider more complex but comparable systems.

The present work considers the retention of homologous pyruvate esters on polysiloxane phases that vary in character from essentially non-polar dimethylpolysiloxane to those of accepted donor and acceptor character within the concept of the Rohrschneider classification. The generally expected enhancement of separation of keto compounds with acceptor phases is examined by a consideration of simple esters,

ketones and pyruvates containing the  $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{O}- \end{array}$ ,  $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}- \end{array}$ ,  $\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ -\text{C}-\text{C}-\text{O}- \end{array}$ ,  $\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ -\text{C}-\text{C}- \end{array}$  and  $\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ -\text{C}-\text{CH}_2-\text{C}- \end{array}$  structures.

## EXPERIMENTAL

The retention data used were obtained isothermally at 150° using 12 ft.  $\times$  1/4 in. O.D. aluminium columns with 10% stationary phase on 62-72 mesh acid-washed and silylated Celatom, as previously reported<sup>11</sup>.

The intercepts of the retention plots of the homologous series appropriate to the various functional groups were calculated from the line of best fit determined by regression analysis using a simple programmable calculator.

## RESULTS AND DISCUSSION

The retention data of the compounds examined are shown in Tables I and II as retentions relative to *n*-nonane ( $V_R$ ) and as Kováts indices while the characteristics and composition of the series of polysiloxane stationary phases used have been described previously<sup>11</sup>. Fig. 1a shows plots of the homologous series on SE-30. The simple esters and ketones with the same number of carbon atoms have substantially the same boiling points and their retentions are very similar, *i.e.* the esters have marginally higher boiling points and retention than the ketones. The retention and boiling points of the pyruvate esters are considerably higher than those of the simple esters and ketones. The conjugated diketones also have higher boiling points than the simpler series of the same chain length, but their retentions are lower although methylene-interrupted diketones, where the conjugation is lost owing to the presence of a methylene group, behave similarly to the pyruvate esters except that the slope of the plot is shallower.

With the donor phenyl columns the retention of all of the series is increased

TABLE II  
RETENTION DATA FOR SOME SATURATED ESTERS AND KETONES ON ACCEPTOR STATIONARY PHASES

Compound	Stationary phase													
	XE-60		F-400		F-500		OV-225		QF-1		SILAR SCP		XF-1150	
	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$	$V_R$	$I$
Methyl acetate	0.461	737	0.236	617	0.312	672	0.492	759	0.563	772	0.800	850	0.944	894
Ethyl acetate	0.667	815	0.336	687	0.446	742	0.712	835	0.812	855	1.136	923	1.213	927
Propyl acetate	1.088	920	0.586	796	0.754	845	1.144	932	1.283	960	1.750	1013	1.878	1040
Butyl acetate	1.760	1022	0.989	899	1.258	945	1.839	1030	2.025	1064	2.870	1116	2.944	1135
Pentyl acetate	2.814	1122	1.643	999	2.086	1045	3.025	1132	3.200	1169	4.538	1212	4.542	1227
Hexyl acetate	4.490	1221	2.721	1098	3.450	1143	4.839	1229	4.925	1267	7.025	1303	6.833	1313
2-Propanone	0.526	773	0.225	626	0.309	672	0.577	798	0.680	813	0.989	907	1.185	946
2-Butanone	0.887	883	0.385	727	0.506	770	0.942	897	1.000	903	1.463	994	1.769	1043
2-Pentanone	1.353	971	0.620	817	0.790	858	1.442	984	1.560	1006	2.130	1078	2.538	1131
2-Hexanone	2.271	1080	1.294	926	1.383	969	2.327	1081	2.493	1116	3.463	1187	3.923	1237
2-Heptanone	3.624	1178	2.011	1039	2.283	1068	3.788	1179	3.880	1219	5.426	1288	6.015	1341
2-Octanone	5.789	1276	3.209	1128	3.790	1169	6.019	1273	6.013	1321	8.463	1388	9.092	1441
Methyl pyruvate	2.812	1125	0.834	873	1.247	948	2.962	1129	2.787	1142	5.426	1288	6.508	1360
Ethyl pyruvate	3.684	1181	1.294	956	1.877	1029	3.846	1182	3.827	1216	6.870	1341	7.892	1407
Propyl pyruvate	5.504	1265	2.011	1039	2.840	1112	5.769	1265	5.693	1308	9.981	1425	11.185	1491
Butyl pyruvate	8.617	1359	3.273	1132	4.605	1207	9.135	1358	8.760	1409	15.352	1521	16.723	1589
Pentyl pyruvate	13.609	1455	5.455	1228	7.556	1305	14.500	1451	13.400	1508	23.574	1617	25.123	1688
Hexyl pyruvate	—	—	—	—	—	—	—	—	—	—	35.870	1711	37.308	1784
2,3-Butanedione	0.962	900	0.332	699	0.444	744	0.962	902	1.000	903	1.537	1005	1.954	1067
2,3-Pentanedione	1.504	993	0.567	800	0.753	848	1.577	1002	1.533	1002	2.537	1118	2.969	1169
2,3-Hexanedione	2.226	1076	0.909	889	1.198	941	2.269	1075	2.253	1092	3.574	1194	4.231	1255
2,3-Octanedione	5.774	1276	2.578	1086	3.309	1142	6.019	1273	5.587	1304	8.759	1395	9.585	1454
2,4-Pentanedione	3.774	1186	1.144	933	1.605	998	3.750	1177	3.053	1163	6.833	1340	8.169	1415
2,5-Hexanedione	8.902	1366	3.027	1117	4.642	1201	9.712	1370	10.733	1456	21.796	1600	20.723	1641
3,5-Heptanedione	11.684	1423	3.005	1115	4.025	1181	11.981	1413	6.467	1338	20.796	1589	25.800	1594
4,6-Nonanedione	17.534	1509	6.994	1275	9.062	1341	17.096	1485	12.573	1493	26.981	1647	28.662	1720

progressively but the relative position of each of the series remains unaltered. DC-530, containing aminoalkyl groups<sup>12</sup>, reacted chemically with all of the series containing the carbonyl functional group.

With the acceptor columns, the retention of the ketones was preferentially enhanced. The retention plots on the acceptor column of lowest polar character, *i.e.* F-400, are shown in Fig. 1b where it is apparent that the plot of the simple ketones now has an intercept greater than that of the alkyl esters, while the plot of the conjugated diketones is essentially the same as that of the esters. As the acceptor nature of the

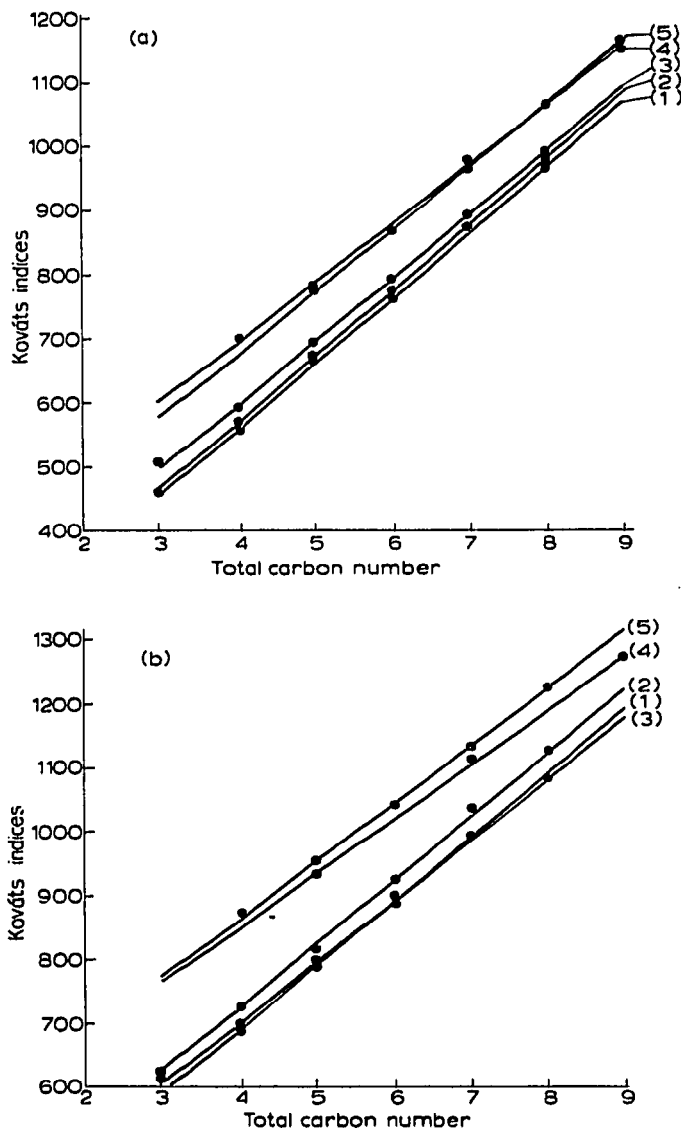


Fig. 1.

(Continued on p. 322)

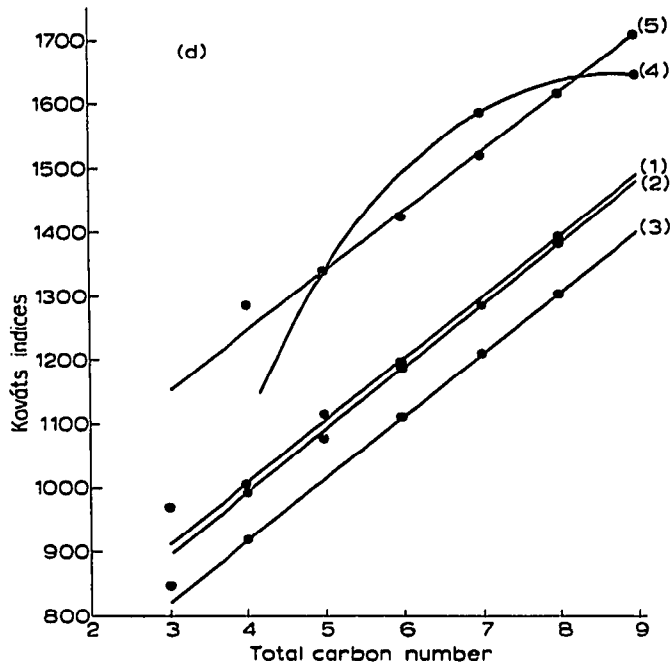
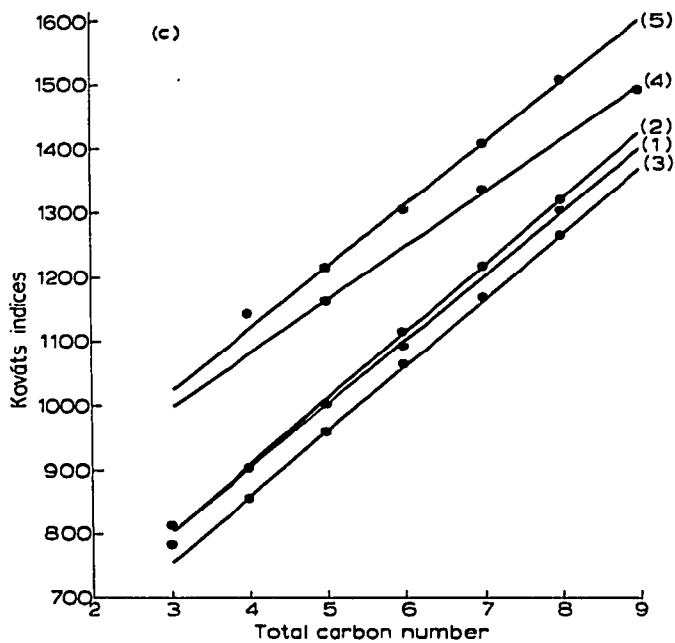


Fig. 1. Retention plots of (1) conjugated diketones, (2) *n*-alkyl ketones, (3) *n*-alkylesters, (4) methylene-interrupted diketones and (5) pyruvates on (a) SE-30, (b) F-400, (c) QF-1 and (d) SILAR 5CP.

phase is increased, the sequence of the separations alters. Retention plots on QF-1 (Fig. 1c) show the two ketone series to be separated but with substantially increased retention compared with the alkyl esters, while with a further increase in polarity, *i.e.* on XE-60, the retention of the conjugated diketones has increased relative to the simple ketones such that the plots are almost superimposed.

Retention plots of the most polar of the columns, *i.e.* XF-1150 and SILAR 5CP (Fig. 1d), show that the conjugated diketones now have significantly greater retentions than the simple alkyl ketones. While the conjugated diketones, owing to their boiling points, might have exhibited somewhat higher retention on low-polarity phases, the behaviour observed with phases of increasing acceptor character is to be expected. Retardation of the acceptor carbonyl group with modest acceptor phases<sup>13</sup> is readily apparent together with the more significant interaction of the conjugated carbonyls with the more polar acceptor phases. The retention of the pyruvate esters on the acceptor columns is enhanced with respect to the methylene-interrupted diketones.

The retention increments due to the carboxyl group of the *n*-alkyl esters on various phases have been reported previously<sup>11</sup> and were found as the intercept of the linear retention relationship calculated from the line of best fit determined by regression analysis where the abscissa was considered as the total number of carbon atoms due to the alkyl groups. With the esters previously reported, the incremental value for the carboxyl group was observed to increase as the polar character of the stationary phase was increased. Reasonable reproducibility of those values is indicated by two reports using SE-30 where the value of 324 (ref. 11), as shown in Table III and obtained by considering *n*-alkyl esters at 150°, is higher, as expected, than the value of 290 reported by Zulaica and Guiochon<sup>14</sup> for dibasic esters at 220°. Table III shows values of increments calculated for the carbonyl group in simple alkyl and in diketones

and the  $\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ -\text{C}-\text{C}-\text{O}- \end{array}$  structure of the pyruvate esters.

TABLE III  
RETENTION INCREMENTS FOR CARBONYL FUNCTIONAL GROUPS

Stationary phase	$\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{O}- \end{array}$	$\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}- \end{array}$	$\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ -\text{C}-\text{C}- \end{array}$	$\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ -\text{C}-\text{C}-\text{O}- \end{array}$	$\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ -\text{C}-\text{CH}_2-\text{C}- \end{array}$
SE-30	324	265	354 (177)	480	596 (298)
DC-230	320	304	384 (192)	528	642 (321)
DC-530	377	391	—	—	—
OV-7	363	351	446 (223)	596	701 (351)
DC-710	417	391	497 (249)	666	767 (384)
OV-25	507	—	—	—	—
100% Phenyl	548	532	658 (329)	789	914 (457)
F-400	417	421	507 (254)	680	766 (383)
F-500	473	463	547 (274)	748	830 (415)
XE-60	539	581	710 (355)	903	1050 (525)
OV-225	558	609	719 (360)	900	1050 (525)
QF-1	571	589	700 (350)	921	1001 (500)
SILAR 5CP	663	688	818 (409)	1057	1218 (609)
XF-1150	718	736	876 (439)	1116	1305 (653)

Values for the individual carbonyl group in the two series are shown in brackets in Table III where it is apparent that the increments observed with the conjugated diketones are lower than for the simple alkyl ketones. With the methylene-interrupted diketones the situation is dependent on the type of stationary phase considered such that with the lower polarity phases, *i.e.* SE-30, DC-230, the increments are somewhat higher than for an isolated carbonyl while with the more polar phases the increments are all increased as compared with the conjugated diketones but are still somewhat less than for an isolated carbonyl. A diketone where the carbonyl groups are separated by two methylene groups exhibits greater retention than a comparable methylene-interrupted diketone and the incremental value on acceptor phases would be closer to the value for the isolated carbonyl group. The reduction of interaction on separation of the functional groups is, of course, expected, but for conjugated carbonyl groups a much greater value might have been expected. The retention behaviour of conjugated compounds has not been extensively studied.

Hofstetter and co-workers<sup>15</sup>, with a variety of unsaturated methyl esters, observed a uniform increase in retention with the presence of non-conjugated unsaturation and on the basis of limited data an accentuated effect with conjugation of double bonds, either with the ester carbonyl group or with themselves, was apparent.

More detailed studies of the methyl octadecenoates<sup>16</sup>, octadecadienoates<sup>17</sup> and some acetylenic esters<sup>16</sup> show that retention is increased when unsaturation occurs near the chain ends but that a double bond at the  $\Delta 13$  position frequently shows greater retention than at the  $\Delta 12$  position where conjugation with the carbonyl group occurs. From studies of the isomeric butenoic esters<sup>18</sup>, it is evident that the shape of the molecule under the resulting steric hindrance is of considerable importance in the retention observed, such that screening of an effective functional group in the solute minimizes its interaction with similar types of groups in the solvent present.

#### ACKNOWLEDGEMENT

The sample of SILAR 5CP was kindly provided by Dr. R. S. Henly of Applied Science Laboratories Inc., State College, Pa., U.S.A.

#### REFERENCES

- 1 C. Kowala, Z. Kranz and K. Murray, *Aust. J. Chem.*, 15 (1962) 832.
- 2 H. P. Burchfield and E. E. Storrs, *Biochemical Application of Gas Chromatography*, Academic Press, New York, 1962, pp. 588-592.
- 3 S. F. Spencer, *Facts Methods*, 3, No. 3 (1962-1963) 7.
- 4 T. S. Rumsey, C. H. Noller, J. C. Burns, D. Kalb, C. L. Rhykerd and D. L. Hill, *J. Dairy Sci.*, 47 (1964) 1418.
- 5 N. W. Alcock, *Anal. Biochem.*, 11 (1965) 335.
- 6 M. Gee, *Anal. Chem.*, 37 (1965) 926.
- 7 M. G. Horning, E. A. Boucher, A. M. Moss and E. C. Horning, *Anal. Lett.*, 11 (1968) 713.
- 8 P. G. Simmonds, B. C. Pettitt and A. Zlatkis, *Anal. Chem.*, 39 (1967) 163.
- 9 J. E. Lovelock and S. R. Lipsky, *J. Amer. Chem. Soc.*, 82 (1960) 431.
- 10 J. E. Lovelock, *Nature (London)*, 189 (1961) 729.
- 11 J. R. Ashes and J. K. Haken, *J. Chromatogr.*, 101 (1974) 103.
- 12 J. R. Ashes and J. K. Haken, *Anal. Chem.*, 45 (1973) 1131.



- 13 W. J. A. VandenHeuvel, E. O. A. Haaihte and E. C. Horning, *J. Amer. Chem. Soc.*, 83 (1961) 1513.
- 14 F. T. Zulaica and G. Guiochon, *Bull. Soc. Chim. Fr.*, 1 (1963) 1242.
- 15 H. H. Hofstetter, N. Sen and R. T. Holman, *J. Amer. Oil Chem. Soc.*, 42 (1965) 537.
- 16 F. D. Gunstone, I. A. Ismail and M. L. K. Jie, *Chem. Phys. Lipids*, 1 (1967) 376.
- 17 W. W. Christie, *J. Chromatogr.*, 37 (1968) 27.
- 18 J. R. Ashes and J. K. Haken, *J. Chromatogr.*, 69 (1971) 39.