THE ANALYSIS OF OILS AND FATS BY GAS CHROMATOGRAPHY

III. SEPARATION FACTORS OF ACETATES, ALCOHOLS AND HYDROCARBONS

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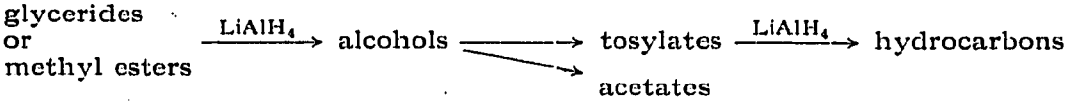
In recent years gas chromatography has found increasing use in the study of complex mixtures of methyl esters from oils and fats. The separation of long-chain alcohols and acetates by gas chromatography has not been investigated as thoroughly as the separation of methyl esters but with the increase in interest in the chemical communication aspect of molecular biology the separation and identification of alcohols and acetates by gas chromatography will become more important[1].

In a series of papers ACKMANN[2-5] has shown the usefulness of separation factors for the systematic identification of the methyl esters of unsaturated fatty acids obtained from complex lipids such as marine oils. It has been indicated[2] that unsaturated acid derivatives produced by modifying the carboxyl group may have the same Type II separation factors as the corresponding methyl esters when chromatographed under identical conditions, but only a limited number of results on dimethyl acetals[6,7] and acetates[7] were available to test this hypothesis. Separation factors obtained for short-chain monoalkenes have also been reported[8].

The purpose of the present work is to investigate if separation factors can be used for the systematic identification of long-chain unsaturated acetates, alcohols and hydrocarbons in a similar manner to their use for methyl ester identification. It might be expected that the relative retention times of long-chain unsaturated methyl esters, acetates and alcohols would follow the same pattern on polyester stationary liquids since LEFORT, PAQUOT AND POURCHEZ[9] observed only slight differences in retention times on a polyester substrate of methyl esters, acetates and alcohols with the same total carbon number. In the present investigation it was found that, with the polyester liquids used, the retention times increased in the series $R \cdot COOCH_3$, $R \cdot CH_2OOC \cdot CH_3$ and $R \cdot CH_2OH$.

LINK, HICKMANN AND MORRISSETTE[10-12] have shown that glycerides can be reduced quantitatively by aluminium lithium hydride to give mixtures of alcohols which were separated by gas chromatography of the acetates. In the present work the following reaction scheme was used for the preparation of the derivatives of long-chain fatty acids:



EXPERIMENTAL

Preparation of derivatives

Alcohols. A solution of 1 g of the oil or methyl ester in dry ether was added by means of a dropping funnel to a suspension of 0.1 g of aluminium lithium hydride in dry ether. The resulting mixture was refluxed gently for 15 min and then 2 ml of ethyl acetate was added to destroy the excess of the reducing agent. The mixture was acidified with diluted sulphuric acid and the alcohols were extracted with ether. The ether extracts were washed with water and dilute sodium hydrogen carbonate and dried over anhydrous sodium sulphate.

Acetates. 1 ml of acetyl chloride was added dropwise to 0.2 g of the alcohols in a dry test tube. The resulting solution was heated to boiling point and allowed to boil gently for 1 min. The excess of acetyl chloride was removed in a stream of dry nitrogen.

Tosylates[13]. 0.3 g of *p*-toluenesulphonyl chloride was added in small portions to a well-stirred solution of 0.2 g of the alcohols in 1 ml of pyridine. During the addition the temperature of the mixture was kept below 5°. After the addition of the *p*-toluenesulphonyl chloride was complete, the mixture was allowed to stand for 3 h below 5°. The resulting slurry was poured into dilute hydrochloric acid and the tosylates extracted with ether. The ether extracts were washed with water, dilute sodium hydrogen carbonate and dried over anhydrous sodium sulphate.

Hydrocarbons. The dried ether solution of the tosylates was added slowly, by means of a dropping funnel, to a suspension of 0.1 g aluminium lithium hydride in dry ether. The mixture was refluxed gently for 45 min and then the excess of reducing agent was destroyed as described above. The hydrocarbons were extracted with ether.

Gas–liquid chromatography

The mixtures of derivatives were separated, as described previously[14,15], using a Perkin Elmer 800 chromatograph with butanediol succinate (BDS) as the stationary liquid and also using a Pye Argon Chromatograph with operating conditions shown in Table I.

TABLE I

OPERATING CONDITIONS OF THE ARGON CHROMATOGRAPH

Stationary liquid	*% by weight*	*Support*	*Column temperature*	
			Acetates and alcohols	*Hydrocarbons*
EGA	10	Celite 545	185°	135°
EGSS-X	17	Gas-Chrom P	163°	125°
EGSS-Y	15.5	Gas-Chrom P	163°	125°

Mixtures were placed on the chromatographic columns as dilute solutions in ether. Retention times were measured to the peak maximum and were adjusted for column dead-volume. Equivalent chain lengths (ECL) were determined by the conventional graphic procedure[16].

RESULTS AND DISCUSSION

Linseed oil

Linseed oil is a source of the C_{18} fatty acids and is suitable for a preliminary study of derivatives of these acids. Retention times (BDS) of derivatives of the major component acids of linseed oil are shown in Table II. The methyl esters, acetates and alcohols were chromatographed at a column temperature of 200° and it was found that corresponding derivatives have similar relative retention times. The hydrocarbons were chromatographed at 130° and, as expected, larger separation factors were obtained. The relative retention times of all the derivatives were the same as those obtained from derivatives of pure acids.

TABLE II

RELATIVE RETENTION TIMES ON BDS STATIONARY PHASE

1 = Methyl esters, relative to methyl octadecanoate (= 1.00).
2 = Acetates, relative to octadecyl acetate (= 1.00).
3 = Alcohols, relative to octadecan-1-ol (= 1.00).
4 = Hydrocarbons, relative to octadecane (= 1.00).

	1	*2*	*3*	*4*
16:0	0.536	0.536	0.538	0.420
18:0	1.00	1.00	1.00	1.00
$18:1^{9}$	1.12	1.12	1.12	1.13
$18:2^{9,12}$	1.32	1.32	1.31	1.37
$18:3^{9,12,15}$	1.65	1.65	1.66	1.82

Sperm oil alcohols

The alcohols obtained by the hydrolysis of sperm oil were converted to (a) the corresponding acetates, and (b) the corresponding hydrocarbons. Retention data for these derivatives on four polyester stationary liquids are given in Table III. It is found that the acetates and the corresponding alcohols, when chromatographed at the same column temperature, have similar relative retention times. The hydrocarbons gave a similar retention pattern as the acetates and alcohols.

ECL values, calculated from the relative retention data, are given in Table IV. The ECL values for the hydrocarbons when multiplied by 100 give the corresponding KOVATS' retention indices. On each of the stationary liquids used it is found that the ECL values for the unsaturated hydrocarbons are, in general, lower than the ECL values for the corresponding alcohols and acetates. One consequence of this is that certain pairs of acetates which are not resolved can be separated when converted to the hydrocarbons, *e.g.* on EGSS-X, the $18:3^{9,12,15}$ acetate is not separated from the $20:1^{11}$ acetate, whereas the corresponding hydrocarbons are resolved. On EGA, the 16:2 acetate and alcohol are eluted after the 17:0 acetate and alcohol, whereas the 16:2 hydrocarbon is eluted before the 17:0 hydrocarbon.

Using the relative retention times of the C_{18} unsaturated derivatives the Type II separation factors were calculated (Table V). These separation factors arise from differences in volatility induced by the size of the end carbon chain. The magnitude of the Type II factors should be affected by the polarity of both the stationary phase

TABLE III

RELATIVE RETENTION TIMES OF DERIVATIVES OF SPERM OIL ALCOHOLS

Figures in parentheses are calculated values.
1 = Retention times of acetates relative to octadecyl acetate (= 1.00).
2 = Retention times of alcohols relative to octadecan-1-ol (= 1.00).
3 = Retention times of hydrocarbons relative to octadecane (= 1.00).

	EGSS-X			*EGA*			*BDS*			*EGSS-Y*		
	1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
14:0	0.297	0.295	0.152	0.265	0.268	0.162	0.282	0.284	0.186	0.249	0.240	0.152
14:1	0.387	0.396	0.205	0.322	0.315	0.209	0.342	0.338	0.239	0.293	0.302	0.205
15:0	0.411	0.409	0.244	0.370	0.370	0.254	0.414	0.396	0.275	0.356	0.351	0.248
15:1	0.510	0.508	0.312	0.453	0.449	0.334	0.474	0.474	0.347	0.414	0.424	0.311
16:0	0.542	0.544	0.405	0.520	0.518	0.399	0.536	0.538	0.420	0.502	0.500	0.393
16:1	0.671	0.678	0.480	0.598	0.594	0.468	0.599	0.601	0.477	0.578	0.576	0.449
16:2	(0.86)	(0.86)	(0.70)	0.756	0.756	0.602	(0.73)	(0.73)	(0.61)	(0.70)	(0.70)	(0.64)
16:3	(1.22)	(1.22)	(0.93)	0.890	0.883	0.805	0.856	0.854	0.814	0.836	0.835	0.797
17:0	0.736	0.735	0.628	0.714	0.716	0.640	0.739	0.742	0.647	0.733	0.712	0.632
17:1	0.895	0.895	0.760	0.823	0.823	0.718	0.809	0.807	0.727	0.797	0.812	0.714
18:0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$18:1^{9}$	1.21	1.22	1.18	1.12	1.12	1.12	1.12	1.12	1.13	1.13	1.12	1.13
$18:2^{9,12}$	1.56	1.60	1.56	1.36	1.36	1.42	1.32	1.31	1.37	1.35	1.34	1.41
$18:3^{6,9,12}$	1.87	1.88	1.89	1.54	1.54	1.75	1.43	1.45	1.51	1.52	1.52	1.67
$18:3^{9,12,15}$	2.20	2.20	2.28	1.76	1.76	1.92	1.65	1.66	1.82	1.72	1.71	1.89
$20:1^{11}$	2.16	2.20	2.88	2.09	2.15	2.69	2.03	2.04	2.55	2.19	2.19	2.77

TABLE IV

ECL VALUES OF ACETATES AND HYDROCARBONS

1 = Acetates.
2 = Hydrocarbons.

	EGSS-X		*EGA*		*BDS*		*EGSS-Y*	
	1	*2*	*1*	*2*	*1*	*2*	*1*	*2*
14:0	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
14:1	14.85	14.60	14.60	14.53	14.48	14.62	14.43	14.60
15:0	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
15:1	15.80	15.50	15.60	15.60	15.55	15.51	15.45	15.48
16:0	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
16:1	16.70	16.42	16.44	16.34	16.33	16.27	16.40	16.29
16:2	(17.56)	(17.23)	17.15	16.85	(17.00)	16.80	(16.95)	(17.05)
16:3	(18.64)	(17.82)	17.65	17.51	17.48	17.52	17.45	17.48
17:0	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00
17:1	17.65	17.40	17.40	17.26	17.32	17.27	17.30	17.25
18:0	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
$18:1^{9}$	18.62	18.37	18.33	18.27	18.36	18.27	18.35	18.30
$18:2^{9,12}$	19.46	18.96	18.90	18.77	18.90	18.75	18.86	18.75
$18:3^{6,9,12}$	20.10	19.40	19.28	19.21	19.16	19.00	19.22	19.12
$18:3^{9,12,15}$	20.64	19.78	19.68	19.43	19.64	19.45	19.56	19.36
$20:1^{11}$	20.60	20.30	20.25	20.15	20.30	20.23	20.25	20.22

TABLE V

TYPE II SEPARATION FACTORS

1 = Acetates.
2 = Hydrocarbons.

	EGSS-X		*EGA*		*BDS*		*EGSS-Y*	
	1	*2*	*1*	*2*	*1*	*2*	*1*	*2*
3/6	1.41	1.46	1.29	1.35	1.25	1.33	1.27	1.34
6/9	1.29	1.32	1.21	1.27	1.18	1.21	1.19	1.25
3/9	1.82	1.93	1.57	1.71	1.47	1.61	1.52	1.68

and the support, the more polar the stationary phase, the greater the values of the separation factors. Changes in polarity should not necessarily have a large effect on the proportional differences in the separation factors, since these differences are due solely to the end carbon chain. This is illustrated by the fact that, when 3/6 and 6/9 separation factors for both acetates and hydrocarbons are plotted against the corresponding 3/9 values, an approximately linear relationship is obtained (Fig. 1). A similar relationship has been found for methyl esters[2]. One might expect, therefore, that derivatives of unsaturated acids, obtained by modifying the carboxyl group, would have the same Type II separation factors, if these derivatives were chromatographed under identical conditions. Type II separation factors for methyl esters, acetates, alcohols and hydrocarbons chromatographed on a BDS column at 180° are given in Table VI. These separation factors are independent of the nature of the group at the "carboxyl end" of the molecule.

Cod liver oil

This oil is a suitable source of unsaturated acids and the methyl esters of these acids have been used as secondary reference standards for comparison of relative retention times[17]. The retention times of the penta- and hexa-unsaturated methyl

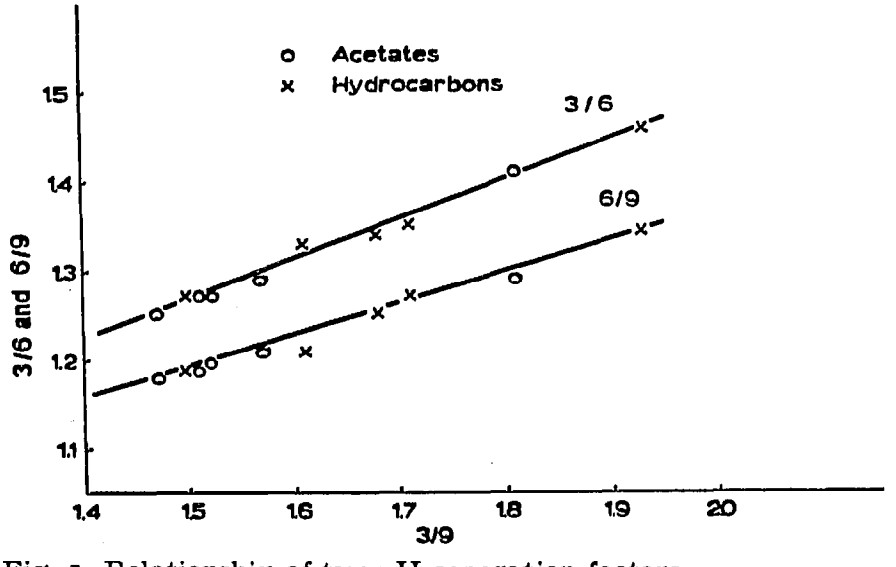


Fig. 1. Relationship of type II separation factors.

TABLE VI

TYPE II SEPARATION FACTORS, BDS AT 180°

	3/6	6/9	3/9
Methyl esters	1.26	1.19	1.51
Acetates	1.26	1.20	1.51
Alcohols	1.27	1.20	1.50
Hydrocarbons	1.27	1.19	1.50

esters from cod liver oil may also be used as starting points for the application of separation factors in these series of esters. It should then be possible to use, in a similar manner to methyl esters, acetates and hydrocarbons derived from the cod liver oil acids as secondary standards for the gas chromatographic examination of long chain unsaturated acetates and hydrocarbons.

Relative retention times (BDS) for C_{18}–C_{24} derivatives are given in Table VII. The methyl esters and acetates were separated at a column temperature of 200° and

TABLE VII

RELATIVE RETENTION DATA ON BDS OF COD LIVER OIL DERIVATIVES

	Methyl esters	*Acetates*	*Hydrocarbons*
18:0	1.00	1.00	1.00
$18:1^{9}$	1.12	1.12	1.12
$18:2^{9,12}$	1.32	1.31	1.32
$18:3^{6,9,12}$	1.43	1.43	1.53
$18:3^{9,12,15}$	1.65	1.63	1.70
$18:4^{6,9,12,15}$	1.84	1.82	1.98
$20:1^{11}$	2.04	2.03	2.43
$20:2^{11,14}$	2.42	2.41	2.86
$20:3^{8,11,14}$	2.63	2.61	3.35
$20:4^{5,8,11,14}$	2.94	2.90	3.84
$20:4^{8,11,14,17}$	3.31	3.30	4.28
$20:5^{5,8,11,14,17}$	3.61	3.60	5.05
$22:1^{13}$	3.66	3.63	5.33
$22:2^{13,16}$	—	—	6.32
$22:3^{10,13,16}$	—	—	7.51
$22:4^{7,10,13,16}$	5.30	5.25	8.42
$22:5^{4,7,10,13,16}$	5.80	5.74	9.60
$22:5^{7,10,13,16,19}$	6.61	6.49	10.8
$22:6^{4,7,10,13,16,19}$	7.34	7.14	13.7
$24:1^{15}$	6.70	6.50	11.6

the hydrocarbons at 150°. It is found that the corresponding methyl esters and acetates give similar relative retention times. The retention data and peak heights of the hydrocarbons show the same general pattern as the methyl esters and acetates. Two hydrocarbon peaks which have been tentatively identified as due to the $22:2^{13,16}$ and $22:3^{10,13,16}$ hydrocarbons, were found although the corresponding methyl esters and acetates were not detected.

It can be seen from the data in Table VIII, that for each series of derivatives a constant ratio of retention times is given by any pair of similarly unsaturated

TABLE VIII

RETENTION TIME RATIOS OF SIMILARLY UNSATURATED DERIVATIVES

	End carbon chain	*Methyl esters*	*Acetates*	*Hydrocarbons*
$18:1^{9}$	9			
$20:1^{11}$	9	1.82	1.81	2.17
$22:1^{13}$	9	1.79	1.79	2.19
$24:1^{15}$	9	1.83	1.79	2.18
$18:2^{9,12}$	6			
$20:2^{11,14}$	6	1.83	1.84	2.17
$22:2^{13,16}$	6	—	—	2.21
$18:3^{6,9,12}$	6			
$20:3^{8,11,14}$	6	1.84	1.83	2.19
$22:3^{10,13,16}$	6	—	—	2.24
$18:4^{6,9,12,15}$	3			
$20:4^{8,11,14,17}$	3	1.80	1.81	2.16
$20:4^{5,8,11,14}$	6			
$22:4^{7,10,13,16}$	6	1.80	1.81	2.19
$20:5^{5,8,11,14,17}$	3			
$22:5^{7,10,13,16,19}$	3	1.83	1.80	2.14
	Mean	1.82	1.81	2.18

derivatives. Once this ratio is found for any one group of similarly unsaturated derivatives, it can then be applied to any other group. The methyl esters and acetates, which were chromatographed at the same temparature give almost identical values for this ratio. The hydrocarbons, which were chromatographed at a lower temperature, give a greater value for this ratio.

ACKMANN[1] has suggested that it is probable that derivatives of unsaturated acids produced by modifying the carboxyl group will have the same Type II separation factors. Separation factors of Types I, II and III are given in Table IX. As expected, methyl esters and acetates gave similar separation factors, while the hydrocarbons gave higher values for the Type I and Type II separation factors.

CONCLUSIONS

Polyester stationary liquids can be used for the gas chromatographic separation of long-chain unsaturated acetates, alcohols and hydrocarbons. Separation factors which have been found useful for the systematic identification of unsaturated fatty acid esters can also be used for the systematic identification of the corresponding acetates, alcohols and hydrocarbons. It is found that, when chromatographed under identical conditions the separation factors are independent of the nature of the group at the "carboxyl end" of the molecule. The values of the separation factors are dependent on both the operating temperature and the polarity of the stationary liquid but the proportional differences in the Type II separation factors appear to be independent of these two variables.

TABLE IX

SEPARATION FACTORS OF TYPES I, II AND III

Carboxyl end chain ratio		*End carbon chain*	*Methyl esters*	*Acetates*	*Hydrocarbons*
Type I					
4/7	$22:6^{4,7,10,13,16,19}$	3	I.II	I.IO	I.I8
	$22:5^{7,10,13,16,19}$	3			
4/7	$22:5^{4,7,10,13,16}$	6	I.09	I.09	I.I4
	$22:4^{7,10,13,16}$	6			
5/8	$20:5^{5,8,11,14,17}$	3	I.09	I.09	I.I8
	$20:4^{8,11,14,17}$	3			
5/8	$20:4^{5,8,11,14}$	6	I.I2	I.II	I.I5
	$20:3^{8,11,14}$	6			
6/9	$18:4^{6,9,12,15}$	3	I.II	I.I2	I.I7
	$18:3^{9,12,15}$	3			
6/9	$18:3^{6,9,12}$	6	I.08	I.09	I.I6
	$18:2^{9,12}$	6			
8/II	$20:3^{8,11,14}$	6	I.09	I.08	I.I7
	$20:2^{11,14}$	6			
Type II					
	$22:6^{4,7,10,13,16,19}$	3	I.26	I.24	I.28
	$22:5^{4,7,10,13,16}$	6			
	$22:5^{7,10,13,16,19}$	3	I.25	I.23	I.24
	$22:4^{7,10,13,16}$	6			
	$20:5^{5,8,11,14,17}$	3	I.23	I.24	I.3I
	$20:4^{5,8,11,14}$	6			
	$20:4^{8,11,14,17}$	3	I.26	I.26	I.28
	$20:3^{8,11,14}$	6			
	$18:4^{6,9,12,15}$	3	I.29	I.27	I.29
	$18:3^{6,9,12}$	6			
	$18:3^{9,12,15}$	3	I.25	I.24	I.29
	$18:2^{9,12}$	6			
	$18:2^{9,12}$	6	I.I8	I.I7	I.I8
	$18:1^{9}$	9			
	$20:2^{11,14}$	6	I.I9	I.I9	I.I8
	$20:1^{11}$	9			
	$18:3^{9,12,15}$	3	I.47	I.45	I.52
	$18:1^{9}$	9			
Type III					
4/7	$22:5^{7,10,13,16,19}$	3	I.I5	I.I4	I.II
	$22:5^{4,7,10,13,16}$	6	Calc. (I.I4)	(I.I3)	(I.08)
5/8	$20:4^{8,11,14,17}$	3	I.I3	I.I4	I.II
	$20:4^{5,8,11,14}$	6	Calc. (I.I5)	(I.I3)	(I.09)
6/9	$18:3^{9,12,15}$	3	I.I5	I.I4	I.II
	$18:3^{6,9,12}$	6	Calc. (I.I4)	(I.I3)	(I.08)

SUMMARY

Separation factors, which have been calculated from gas chromatographic retention data can be used for the systematic identification of long-chain unsaturated acetates and hydrocarbons in a similar manner to that used for the identification of the corresponding methyl esters.

REFERENCES

1 J. MEINWALD AND Y. C. MEINWALD, *J. Am. Chem. Soc.*, 88 (1966) 1305.
2 R. G. ACKMANN AND R. D. BURGHER, *J. Chromatog.*, 11 (1963) 185.
3 R. G. ACKMANN, *J. Am. Oil Chemists' Soc.*, 40 (1963) 558.
4 R. G. ACKMANN, *J. Am. Oil Chemists' Soc.*, 40 (1963) 564.
5 R. G. ACKMANN AND P. M. JANGAARD, *J. Am. Oil Chemists' Soc.*, 40 (1963) 744.
6 G. M. GRAY, *J. Chromatog.*, 4 (1960) 52.
7 J. W. FARQUAR, *J. Lipid Res.*, 3 (1962) 21.
8 R. G. ACKMANN, *J. Chromatog.*, 12 (1963) 271.
9 D. LEFORT, C. PAQUOT AND A. POURCHEZ, *Oléagineux*, 16 (1961) 253.
10 W. E. LINK, H. M. HICKMANN AND R. A. MORRISSETTE, *J. Am. Oil Chemists' Soc.*, 36 (1959) 20.
11 W. E. LINK, H. M. HICKMANN AND R. A. MORRISSETTE, *J. Am. Oil Chemists' Soc.*, 36 (1959) 300.
12 W. E. LINK AND R. A. MORRISSETTE, *J. Am. Oil Chemists' Soc.*, 37 (1960) 668.
13 P. VEERAVAGU, R. T. ARNOLD AND E. W. EIGENMANN, *J. Am. Chem. Soc.*, 86 (1964) 3072.
14 G. R. JAMIESON AND E. H. REID, *J. Chromatog.*, 17 (1965) 230.
15 G. R. JAMIESON AND E. H. REID, *J. Chromatog.*, 20 (1965) 232.
16 E. P. WOODFORD AND C. M. VAN GENT, *J. Lipid Res.*, 1 (1960) 188.
17 R. G. ACKMANN AND R. D. BURGHER, *J. Am. Oil Chemists' Soc.*, 42 (1965) 38.