Identification of straight-chain unsaturated aldehydes by thin-layer partition chromatography

Aerial oxidation of oils and fats induces changes in flavour of these products, which are mainly caused by the formation of minute (1 mg/kg or less) amounts of aldehydes[1,2]. For the analysis of these generally strong-flavoured, oxidation products[3,4] various systems of thin-layer chromatography are eminently suited[5–9]. In these techniques the carbonyls, in the form of their 2,4-dinitrophenylhydrazones (DNPH), are separated.

BADINGS AND WASSINK[6] recently developed a method for the separation of C_2–C_{11} saturated aldehydes on Kieselguhr G chromatoplates impregnated with Carbowax 400. We have utilized this system for the study of the chromatographic

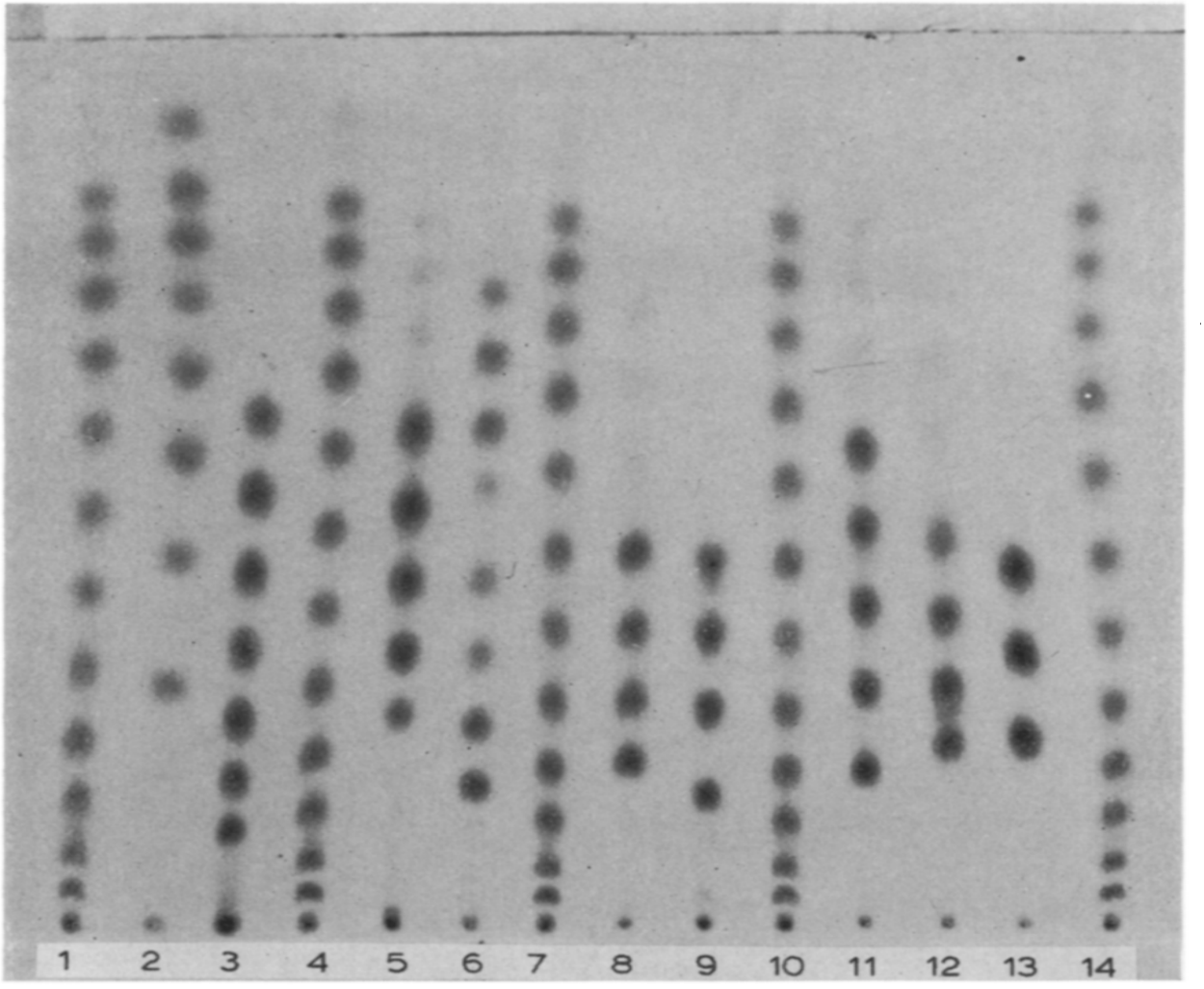

Fig. 1. Separation of aldehyde-DNPHs by thin-layer partition chromatography; $33^1/_3$% Carbowax 400 on Kieselguhr G, solvent: 100% light petroleum b.p. 100–120°. 1, 4, 7, 10, 14 = C_1–C_{12} alkanals; 2 = C_3–C_9 2-alkanones and 2-undecanone; 3 = C_6–C_{12} *trans*-2-, *trans*-4-alkadienals; 5 = *trans*-3-hexenal, *trans*-4-heptenal, *trans*-5-octenal, *trans*-6-nonenal and *trans*-7-decenal; 6 = C_5–C_{12} *trans*-2-alkenals; 8 = C_6–C_9 ω-alkenals; 9 = *trans*-2-, *trans*-4-heptadienal, *trans*-2-, *trans*-5-octadienal, *trans*-2-, *trans*-6-nonadienal and *trans*-2-, *trans*-7-decadienal; 11 = C_6-C_{10} *trans*-5-alkenals; 12 = *trans*-4-hexenal, *trans*-5-heptenal, *trans*-6-octenal and *trans*-7-nonenal; 13 = *trans*-2-, *trans*-7-octadienal, *trans*-2-, *trans*-7-nonadienal and *trans*-2-, *trans*-7-decadienal.

behaviour of a number of unsaturated aldehyde-DNPH's, special attention being paid to the effect of number and location of double bonds and to *cis–trans* isomerism.

Method and results

The chromatoplates (20 × 20 cm), consisting of Kieselguhr G (layer thickness 0.35 mm) impregnated with Carbowax 400 were prepared along the lines indicated by BADINGS AND WASSINK[6], except that 33.3 instead of 25 % Carbowax was used for impregnation. With this modification improved separation according to chain length

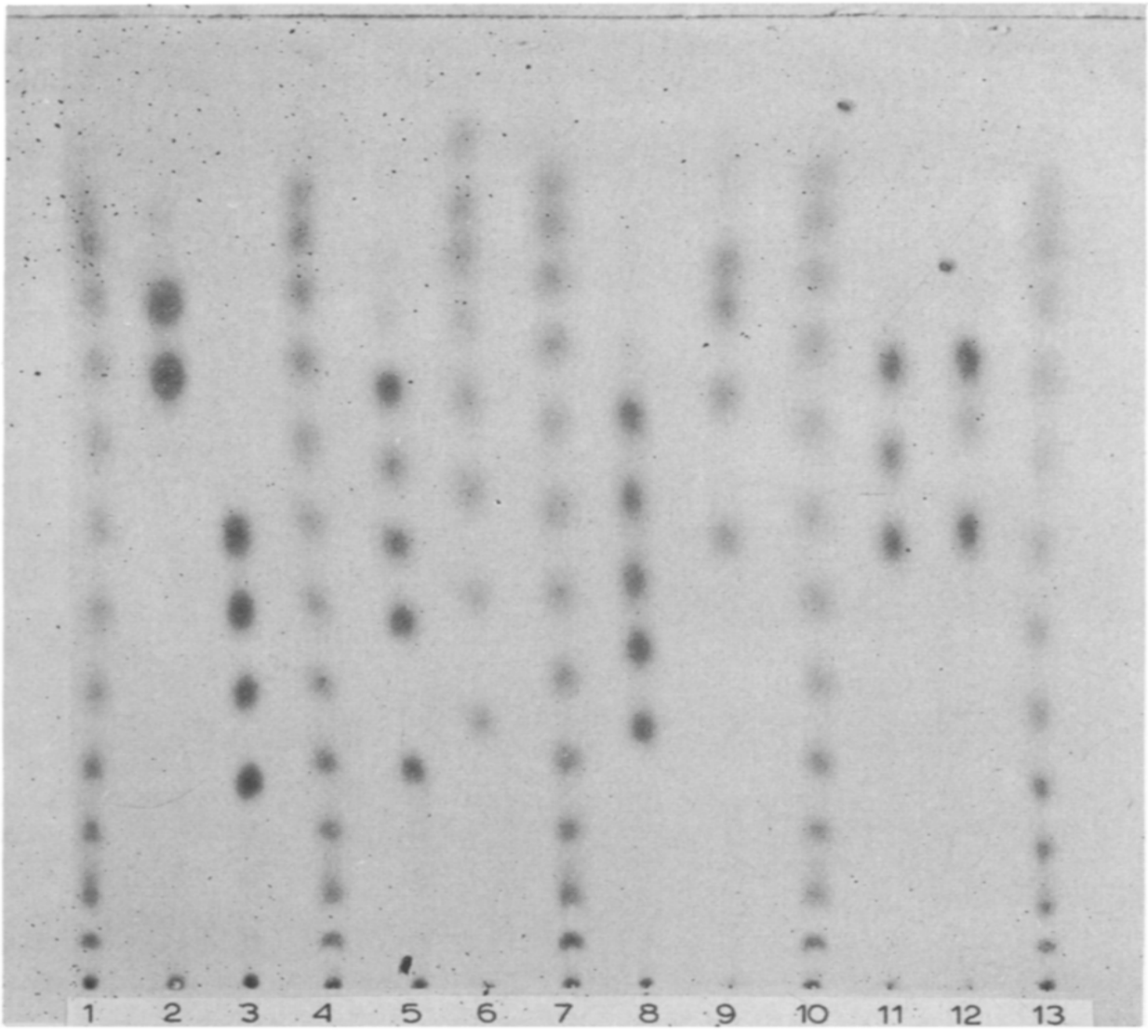

Fig. 2. Separation of aldehyde-DNPHs by thin-layer partition chromatography; $33^1/_3$% Carbowax 400 on Kieselguhr G; solvent: 100% light petroleum b.p. 100–120°. 1, 4, 7, 10, 13 = C_1–C_{12} alkanals; 2 = *trans*-2-, *cis*-5-, *cis*-8-tetradecatrienal and *trans*-2-, *cis*-6-, *cis*-9-pentadecatrienal; 3 = *trans*-2-, *cis*-4-heptadienal, *trans*-2-, *cis*-5-octadienal, *trans*-2-, *cis*-6-nonadienal and *trans*-2-, *cis*-7-decadienal; 5 = *trans*-2-, *cis*-4-heptadienal and C_9–C_{12} *trans*-2-, *cis*-4-alkadienals; 6 = C_3–C_9 2-alkanones and 2-undecanone; 8 = *cis*-3-hexenal, *cis*-4-heptenal, *cis*-5-octenal, *cis*-6-nonenal and *cis*-7-decenal; 9 = *cis*-4-, *cis*-7-decadienal, *cis*-6-, *cis*-9-dodecadienal, *cis*-4-, *cis*-7-tridecadienal and *cis*-5-, *cis*-8-tetradecadienal; 11 = *trans*-2-, *cis*-7-decadienal, *trans*-2-, *cis*-6-dodecadienal and *trans*-2-, *cis*-5-undecadienal; 12 = *trans*-2-, *trans*-7-decadienal, *trans*-2-, *trans*-6-dodecadienal and *cis*-6-, *cis*-9-dodecadienal.

was achieved for the DNPHs of saturated C_{10}–C_{12} aldehydes and C_8, C_9 and C_{11} methyl ketones.

Mixtures of 2 μg of the available DNPHs (methyl ketones, alkanals, monoenals, dienals and trienals) in 10 μl chloroform were spotted on to the plate, at distances of 1 cm, with a 10 μl micropipette. Subsequently, ascending chromatography was applied for 25–30 min at 20°, using light petroleum (b.p. 100–120°) as eluant.

From Figs. 1 and 2 it appears that the components of the various homologous series can be clearly separated by this technique. The accompanying, regularly distanced spots are not attributable to impurities, but most probably to *syn*-isomers. This phenomenon was not observed when the DNPHs had been stored dry.

After spraying the plate with dilute alcoholic alkali, the DNPHs undergo their characteristic colour change, as described by LAPPIN AND CLARK[10], which could serve as an aid in the analysis and identification of carbonyl compounds.

Discussion

The relative migration (R_H) of the DNPHs with respect to hexanal-DNPH has been established. The relevant data, which show little scattering, are collected in Table I.

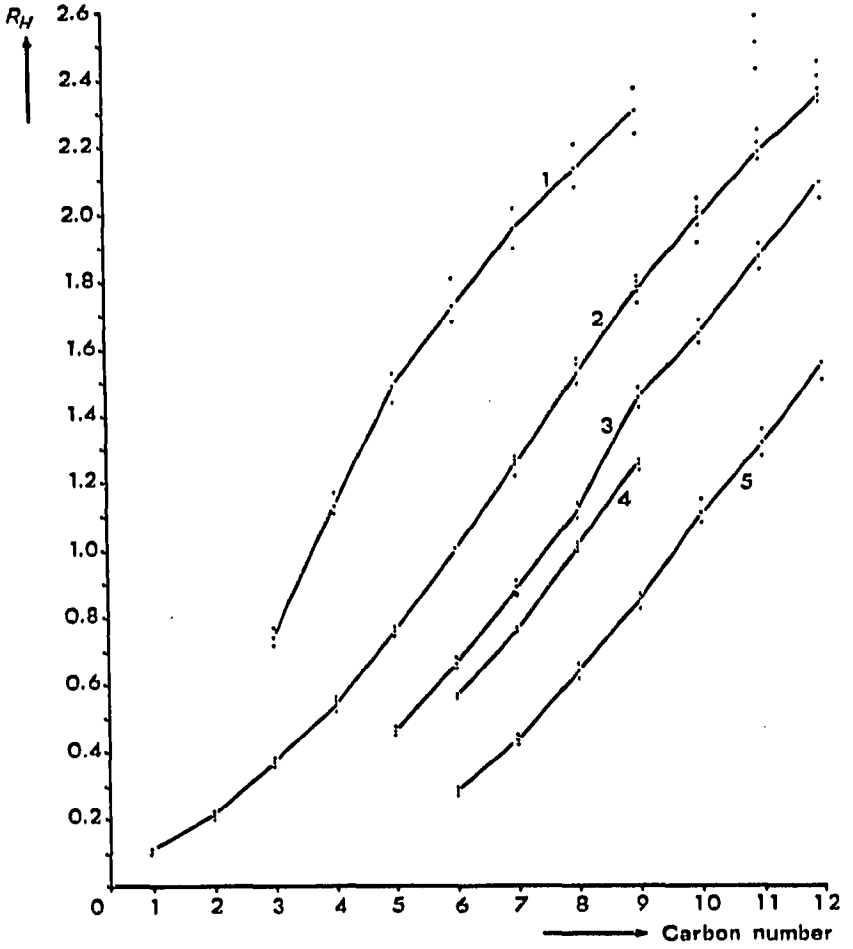

Fig. 3. Migration of aldehyde-DNPHs, R_H = distance moved by hydrazone/distance moved by hexanal-DNPH. 1 = 2-Alkanones; 2 = alkanals; 3 = *trans*-2-alkenals; 4 = ω-alkenals; 5 = *trans*-2-, *trans*-4-alkadienals.

J. Chromatog., 24 (1966) 427–431

TABLE I

R_H VALUES OF ALDEHYDE-DNPHs

Type of aldehyde	*Double bond at*	*Chain length*														
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Alkanals		0.11	0.21	0.38	0.54	0.76	1.00	1.25	1.53	1.79	2.05	2.27	2.47			
trans-Alkenals	2					0.43	0.61	0.85	1.09	1.41	1.63	1.89	2.16			
	3						0.67	0.93								
	4						0.62	0.80								
	5						0.55	0.82	1.09	1.40	1.70					
	6							0.75	1.05	1.36						
	7								1.00	1.31	1.65					
	8									1.26						
cis-Alkenals	3						0.60	0.83								
	4						0.57	0.85								
	5							0.80	1.04							
	6									1.28						
	7										1.56					
trans,trans-Alkadienals	2, 4						0.27	0.43	0.62	0.81	1.05	1.32	1.56			
	2, 5								0.70							
	2, 6									0.97			1.76			
	2, 7								0.62	0.92	1.22					
trans,cis-Alkadienals	2, 4							0.45		0.85	1.10	1.34	1.55			
	2, 5								0.68			1.35				
	2, 6									0.93			1.66			
	2, 7									0.88	1.15					
cis,cis-Alkadienals	4, 7										1.07			1.87		
	5, 8														2.08	
	6, 9												1.57			
trans,cis,cis-Alkatrienals	2, 5, 8														1.66	
	2, 6, 9															1.92

On plotting the R_H values as a function of the chain length for the components of various homologous series, smooth curves are generally obtained. An exception is the curve for *trans*-2-monoenals, which shows a distinct bend at C_9 (Fig. 3). A similar phenomenon was observed by NONAKA, PIPPEN AND BAILEY[11] for the series of saturated aldehydes.

With the exception of the monoenals having a terminal double bond approximately equal R_H values were established for the homologues ($> C_3$) of C_{n-3}-methyl ketones, C_{n+1}-monoenals, C_{n+3}-dienals, and C_{n+5}-trienals (see Table I). These R_H values only differ from those of the homologues of the saturated aldehydes in that they are invariably in between two successive members (C_n — C_{n-1}) of that series. However, the R_H values of the members belonging to the series of saturated aldehydes C_n equal those of the ω-alkenals having a chain length C_{n+2}. Similar rules were formulated in the past from the results of column and paper chromatographic methods of analysis[11–14].

Consequently, now that it has appeared that the behaviour of unsaturated aldehyde-DNPHs on chromatoplates is less dependent on their stereochemical configuration (see Table I), but rather on the number and location of their double bonds, the analysis of carbonyls from oils and fats can be largely simplified. On applying the above-mentioned technique to a complex DNPH mixture an orienting identification can be obtained chromatographically in about 30 min, after which a detailed analysis can be carried out more rapidly. Work on this aspect is in hand and will be published in the near future.

Acknowledgements

The author thanks Dr. J. P. WARD for submitting some unsaturated aldehyde-DNPHs and Mr. G. A. JONGENOTTER for carrying out the experiments.

Unilever Research Laboratory, Mercatorweg 2, Vlaardingen (The Netherlands) P. W. MEIJBOOM

1 P. HAVERKAMP BEGEMANN AND J. C. KOSTER, *Nature*, 202 (1964) 552.
2 J. G. KEPPLER, J. A. SCHOLS, W. H. FEENSTRA AND P. W. MEIJBOOM, *J. Am. Oil Chemists' Soc.*, 42 (1965) 246.
3 C. H. LEA AND P. A. T. SWOBODA, *Chem. Ind. (London)*, (1958) 1289.
4 P. W. MEIJBOOM, *J. Am. Oil Chemists' Soc.*, 41 (1964) 326.
5 K. DE JONG, K. MOSTERT AND D. SLOOT, *Rec. Trav. Chim.*, 82 (1963) 837.
6 H. T. BADINGS AND J. G. WASSINK, *Neth. Milk Dairy J.*, 17 (1963) 132.
7 G. URBACH, *J. Chromatog.*, 12 (1963) 196.
8 P. W. MEIJBOOM AND G. JURRIENS, *J. Chromatog.*, 18 (1965) 424.
9 D. P. SCHWARTZ AND O. W. PARKS, *Microchem. J.*, 7 (1963) 403.
10 G. R. LAPPIN AND L. C. CLARK, *Anal. Chem.*, 23 (1951) 541.
11 M. NONAKA, E. L. PIPPEN AND G. F. BAILEY, *Anal. Chem.*, 31 (1959) 875.
12 A. M. GADDIS AND R. ELLIS, *Anal. Chem.*, 31 (1959) 870.
13 H. VAN DUIN, *Neth. Milk Dairy J.*, 12 (1958) 74.
14 D. A. FORSS AND E. H. RAMSHAW, *J. Chromatog.*, 10 (1963) 268.

Received March 25th, 1966