Syn-anti isomerization of 2,4-dinitrophenylhydrazones of volatile carbonyl compounds in capillary gas chromatographic-mass spectrometric analyses

Journal of Chromatography, 194 (1980) 135–144
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,726

SYN–ANTI ISOMERISATION OF 2,4-DINITROPHENYLHYDRAZONES OF VOLATILE CARBONYL COMPOUNDS IN CAPILLARY GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC ANALYSES

V. P. URALETS*
Institute of Organo-Element Compounds, Academy of Sciences, Moscow (U.S.S.R.)
and
J. A. RIJKS and P. A. LECLERCQ*
Laboratory of Instrumental Analysis, Eindhoven University of Technology, Eindhoven (The Netherlands)
(Received January 31st, 1980)

SUMMARY

Dinitrophenylhydrazone derivatives of volatile aldehydes and ketones were investigated by high-performance liquid chromatography (HPLC) and by gas chromatography–mass spectrometry (GC–MS). HPLC separations were slightly better than those obtained by existing procedures, but high-resolution GC is the method of choice.

The equilibrium of the *syn–anti* isomerization of the compounds is influenced by the nature of the solvent, the deactivation of the column and the injection and column temperatures. The GC profile can therefore be controlled by careful selection of the experimental conditions. It is shown that this phenomenon, in addition to the occurrence of characteristic values of the difference in the retention indices of *syn* and *anti* isomers for a given compound, can facilitate the identification.

Chemical ionization (methane) mass spectra of the compounds were found to contain more information than conventional electron impact spectra. Characteristic fragment ions are tabulated.

INTRODUCTION

Volatile carbonyl compounds are important flavour ingredients in many food products. For analytical purposes, these compounds are often converted into stable 2,4-dinitrophenylhydrazones (DNPHs), which can be selectively isolated from complex mixtures of flavour components.

After isolation of the DNPHs, the mixture can be separated chromatographically either as such or after regeneration of the free carbonyl compounds. Gas chromatographic (GC) studies on the liberated compounds[1–5] show that this method is not suitable for quantitative trace analyses. Moreover, reactions with the liberating agents may give artefacts[2,3].

* Present address: Institute of Nutrition, Academy of Medical Sciences, Ustinsky projezd 2/14, Moscow 109240, U.S.S.R.

0021-9673/80/0000–0000/$02.25

Many attempts to analyse DNPHs directly by GC have been reported[6–20]. In the gas chromatograms doubling of peaks has been observed in several instances[5,11,16,17], even when low-resolution packed columns were used. Obvious double peaks have been recorded with high-resolution capillary columns[18,19]. Purification of carbonyl compounds before derivatization to avoid peak doubling was not successful[18]. The doubling effects were attributed to either decomposition[10,16,20] or isomerization[17,18] of the compounds.

The occurrence of *syn* and *anti* isomers of DNPHs of aliphatic aldehydes was observed in 1961 by Van Duin[21], who studied these phenomena by conventional liquid chromatography. Published as a thesis, this work was apparently overlooked in the more recent GC studies cited above.

High-performance liquid chromatography (HPLC) has also been applied to the analysis of DNPHs[16,22,23]. This method is very attractive because the separation can be carried out at low temperature and the compounds can be detected with relatively high sensitivity. However, HPLC has a poor separation power in comparison with capillary GC.

Electron impact (EI) mass spectrometry (MS) has been applied to the identification of DNPHs[24–26] after separation by thin-layer or conventional column chromatography. EI mass spectral data are available for many DNPHs of saturated and unsaturated aldehydes and ketones[26–29]. Chemical ionization (CI) mass spectra of DNPHs were not found in the literature.

Although some attempts were made to optimize HPLC for the separation of DNPHs, the main purpose of this work was to study the GC behaviour of DNPHs on glass capillary columns. In particular, factors influencing isomerization phenomena, such as the nature of the solvent, deactivation of the column wall and the injection and column temperatures were investigated. CI mass spectra, using methane as reagent gas, are discussed.

EXPERIMENTAL

2,4-Dinitrophenylhydrazine was used as a reagent for preparing derivatives of aldehydes and ketones. The following procedures were applied for the preparation of DNPHs: (a) the conventional method and recrystallization from ethanol[30]; (b) derivatization on a column filled with neutral sorbent and the reagent[21]; (c) derivatization in pyridine solution[21].

Liquid chromatography

The HPLC equipment used is described elsewhere[31]. A variable-wavelength Model PM 2D LC UV detector (Carl Zeiss, Jena, G.D.R.) was applied. Absorbances were measured at 358 nm. The stationary and mobile phase systems were similar to those described earlier for the separation of DNPHs[22]. Adsorption chromatography was performed on LiChrosorb SI-60 with ethyl acetate–isooctane (1:49) or isooctane–methylene chloride (3:2) as solvents. Reversed-phase chromatography was carried out on LiChrosorb RP-8 with acetonitrile–water (3:2) or dimethylformamide–water (3:1). Before use the solvents were degassed in an ultrasonic bath.

Gas chromatography

An Intersmat IGC 120 FB gas chromatograph (Intersmat Instruments, Pavillons sous Bois, France) equipped with a flame-ionization detector and an all-glass "moving needle" injector[32] was used at injection block temperatures of 200–400 °C (mainly 225 °C). Four Duran 50 glass capillary columns (40 m × 0.4 mm I.D.), rinsed with dry methylene chloride and dried with a gentle stream of dry nitrogen, were coated by a static procedure with SE-30 stationary phase using a 0.4% (w/w) solution in *n*-hexane[33]. Different treatments of the glass surface were applied before coating, in order to deactivate the column wall, with the exception of column 1, which was coated without prior treatment. Column 2 was deactivated with a 1% solution of benzyltriphenylphosphonium chloride (BTPPC) in methylene chloride, as described by Rutten and Luyten[34]. Column 3 was deactivated with a 5% solution of Carbowax 20M in methylene chloride according to Blomberg[35]. Column 4 was deactivated by Carbowax 20M vapour[36,37]. In all experiments the injector was deactivated essentially according to the same procedure as the column. The columns were operated isothermally between 200 and 260 °C, but mainly at 225 °C. The carrier gas was nitrogen at a flow-velocity of 10–20 cm/sec, the detector temperature was 280 °C and the sample size was $5 \cdot 10^{-7}$ to $5 \cdot 10^{-10}$ g per component in 1 μl of an appropriate solvent.

Mass spectrometry

A Finnigan Model 4000 quadrupole mass spectrometer (Finnigan, Sunnyvale, CA, U.S.A.) was used in the CI mode. The mass spectral data presented were acquired under the following conditions: ionizing electron energy, 79 eV; electron current, 0.20 mA; scan time, 1 sec per scan; and source temperature, 250 °C. Methane reactant gas was introduced via the make-up gas line. The ion source pressure was maintained at 0.15 Torr gauge reading. Samples were analysed by GC–MS using column No. 2 and helium as carrier gas. The column was coupled directly to the ion source via a platinum–iridium capillary (60 cm × 0.1 mm I.D.). The column was operated isothermally at 230 °C and the platinum–iridium interface was maintained at 250 °C. Samples were injected on to the column as described above at 250 °C.

RESULTS AND DISCUSSION

Fig. 1 shows part of a representative gas chromatogram of DNPHs derived from pentanal and heptanone-3 on capillary column No. 1. Two distinct peaks correspond to each compound. The baseline in between the pairs is elevated and has a slope. The composition of these complicated chromatographic zones was studied by GC–MS in the EI and CI (methane, isobutane) mode for DNPHs of different aldehydes and ketones. The mass spectra recorded at different points of one pair of peaks and in between were identical with and similar to those obtained by direct insertion of the sample into the mass spectrometer. Therefore, the peak doubling cannot be attributed to decomposition[10,16,20].

Preparative separation of the isomers of acetaldehyde DNPH was achieved by HPLC on LiChrosorb SI-60, using isooctane–ethyl acetate (49:1) as solvent. The identity of the isomers was established by proton NMR. The isomer eluting first in both GC and HPLC appeared to be the *syn* isomer:

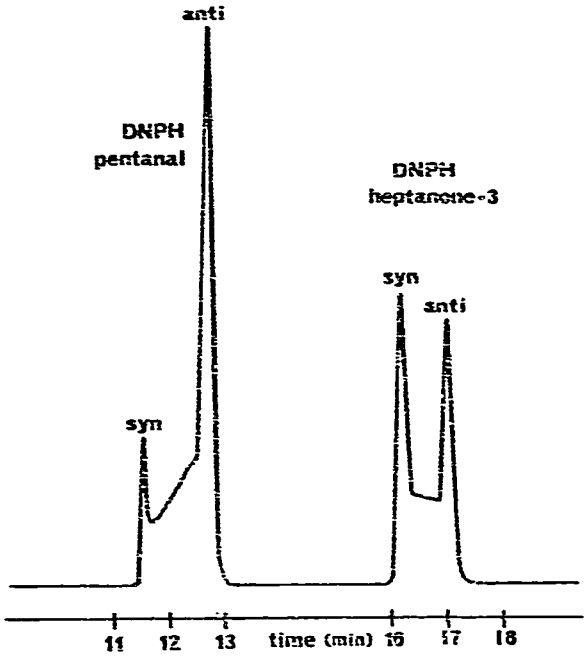
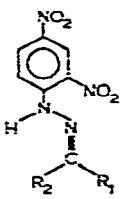
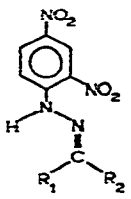


Fig. 1. Separation of *syn* and *anti* isomers of 2,4-dinitrophenylhydrazones of pentanal and heptanone-3 on a glass capillary column coated with SE-30 (column No. 1). Column and injector temperature, 225 °C; linear carrier gas (nitrogen) velocity, 200 mm/sec.

$R_1 = H$ (aldehydes)
$R_1 < R_2$ (ketones)



SYN



ANTI

The occurrence of a single peak for DNPHs of formaldehyde, acetone, diethyl ketone and other symmetrical carbonyl compounds will be easily understood.

The raised baseline between two DNPH isomers is evidently due to isomerization in the column during chromatography. This results in intermediate retention for components passing one part of the column as a *syn* and the other as an *anti* isomer.

Attempts have been made to suppress isomerization in the chromatographic column. Fig. 1 shows significant on-column isomerization (elevated baseline between DNPH isomers) on column No. 1, which was not treated with deactivating agents. Less isomerization was observed with column No. 2, which was treated with BTPPC as described above. Fig. 2 represents the separation of a DNPH mixture on this column. The column efficiency for heptanone-2 DNPH (capacity ratio, $k' = 6.5$) was 1500 theoretical plates per metre (2300 for n-$C_{24}H_{50}$, $k' = 5.4$). In contrast, Carbowax treatment (columns No. 3 and 4) decreased the column performance with respect to

the separation of DNPHs. Rapid isomerization was observed, resulting in enormous peak broadening, although separations of other compounds (hydrocarbons, pesticides, steroids) were successful. Column No. 2 was used in further experiments.

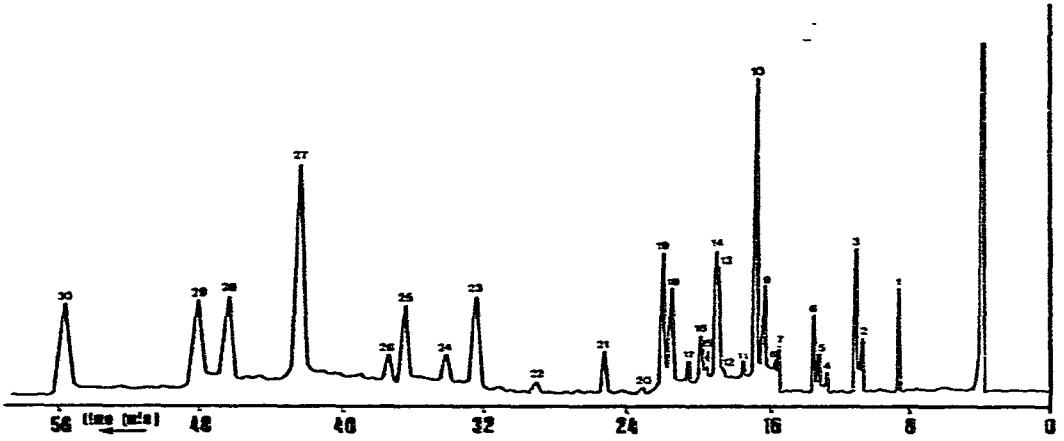


Fig. 2. Chromatogram of a mixture of 2,4-dinitrophenylhydrazones from a glass capillary column coated with SE-30 (column No. 2). Column and injector temperature, 225 °C; linear carrier gas (nitrogen) velocity, 160 mm/sec. Peaks: 1 = methanal; 2 = ethanal (*syn*); 3 = ethanal (*anti*); 4 = propanal (*syn*); 5 = acetone; 6 = propanal (*anti*); 7 = butanal (*syn*); 8 = butanone (*syn*); 9 = butanone (*anti*); 10 = butanal (*anti*) + 2-methylbutanal (*syn*); 11 = = 3-methylbutanal (*syn*); 12 = pentanone-2 (*syn*); 13 = 2-methylbutanal (*anti*); 14 = 3-methylbutanal (*anti*); 15 = pentanal (*syn*); 16 = pentanone-2 (*anti*); 17 = 4-methylpentanone-2 (*syn*); 18 = pentanal (*anti*); 19 = 4-methylpentanone-2 (*anti*); 20 = hexanone-2 (*syn*); 21 = hexanone-2 (*anti*); 22 = heptanone-2 (*syn*); 23 = heptanone-2 (*anti*) + heptanal (*syn*); 24 = hexenal-2; 25 = heptanal (*anti*); 26 = octanone-2 (*syn*); 27 = octanone-2 (*anti*); 28 = heptenal-2; 29 = octanal (*anti*) + nonanone-2 (*syn*); 30 = nonanone-2 (*anti*).

Within the column temperature range of 200–260 °C, increasing temperature promotes the isomerization rate. The slope between the pairs of isomer peaks increases significantly more than can be accounted for by decreasing retention times at higher temperatures. Temperatures of 220–235 °C are preferable, providing reasonable elution times and acceptable isomerization.

The injector temperature significantly influences the *syn/anti* ratio, as shown in Fig. 3 for derivatives of propanal, pentanal and heptanal. It should be noted that the injected sample contained mainly *anti* isomers, corresponding approximately to the ratios as in chromatogram A at a low injector temperature. Rapid isomerization was observed at 400 °C (Fig. 3B), which resulted in increased amounts of the *syn* isomers.

The initial ratio of isomers depends substantially on the derivatization procedure[21]. Conversions using a neutral adsorbent[21] yield high fractions of *syn* DNPHs (70%). Derivatives obtained from a pyridine solution[21] were mainly *anti* isomers (96%). The percentages given refer to butanal DNPH, as determined with GC column No. 2 at 225 °C and the injector also at 225 °C.

Kallio *et al.*[17] reported an influence of the solvent on the size of the secondary (*syn*) peak, although later this effect was not observed[18]. We examined several solvents to investigate this phenomenon. Isooctane and carbon disulphide did not change,

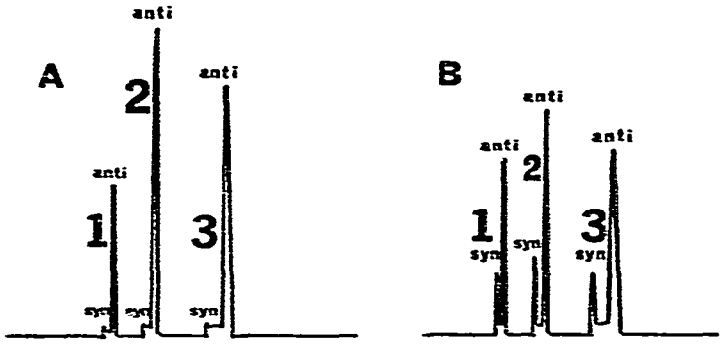


Fig. 3. Influence of injector temperature on the relative amounts of *syn*- and *anti*-DNPH isomers of propanal (1), pentanal (2) and heptanal (3). Glass capillary column coated with SE-30 (column No. 2) operated at 225 °C. Injector temperature: A, 235 °C; B, 400 °C.

over a period of 2 weeks, the *syn/anti* ratio of two samples of butanal DNPH, containing 70 and 4% of the *syn* isomer, respectively. In acetic acid, however, rapid isomerization was observed, resulting in an equilibrium corresponding to 25% of the *syn* and 75% of the *anti* isomer for both samples. Similar results were initially obtained with chloroform. After removal of hydrochloric acid, however, this solvent no longer promoted isomerization. Hence the isomerization in solutions is connected with the solvent acidity, as also follows from Van Duin's data[21]. It can be concluded that acid-catalysed interconversion leads to an equilibrium in the *syn–anti* composition. Thus, DNPH derivatives obtained in acidic medium show a constant *syn/anti* ratio in solutions[18].

Consequently, it is also apparent that glass capillary column and injector deactivation procedures have to eliminate the surface acidity (Duran 50 is slightly acidic) to suppress isomerization during chromatography.

The results obtained allow one to control the appearance of the chromatograms. To simplify the chromatograms, derivatization procedures should be applied that yield mainly *anti* isomers. Neutral solvents (isooctane or carbon disulphide) must be used for further treatment and low injector temperatures (approximately equal to the column temperature) have to be chosen to minimize the formation of *syn* isomers. On the other hand, the identification can be aided by the presence of *syn*-DNPH isomers in defined quantitative ratios and with appropriate retention data. This can easily be achieved by dissolving the DNPH mixture in acetic acid or by increasing the injector temperature. Thus, for analysis of an unknown mixture of DNPHs two chromatographic runs are advisable: one with suppressed *syn* isomer formation and another with both isomers present.

Retention indices of *syn*- and *anti*-DNPHs of 40 aldehydes and ketones are presented in Table I. *anti*-DNPH isomers of *n*-alkanals were used as reference compounds for the calculation of retention indices. Derivatives of the symmetrical carbonyl compounds, having only one isomer, and of methyl isopropyl ketone, methyl *tert.*-butyl ketone and α-unsaturated aldehydes producing negligible amounts of the *syn* isomer, are characterized by only one retention index value.

anti Isomers, having the (larger) alkyl group in the *anti* position with respect to the N–H group, are more exposed to stationary phase molecules than the corre-

TABLE I

RETENTION INDICES (I) OF 2,4-DINITROPHENYLHYDRAZONES OF CARBONYL COMPOUNDS ON SE-30 AT 225 °C

The *anti*-DNPH isomers of *n*-alkanals serve as reference compounds.

DNPHs of aldehydes	*I*		*ΔI**	*DNPHs of ketones*	*I*		*ΔI**
	syn	*anti*			*syn*	*anti*	
Methanal	100			Acetone	292		
Ethanal	188	200	12	Butanone	370	386	16
Propanal	272	300	28	Pentanone-2	442	468	26
Butanal	366	400	34	Hexanone-2	529	562	33
Pentanal	463	500	37	Heptanone-2	616	658	42
Hexanal	559	600	41	Octanone-2	709	755	46
Heptanal	657	700	43	Nonanone-2	805	851	46
Octanal	755	800	45	3-Methylbutanone-2		436	
Nonanal	854	900	46	Pentanone-3	451		
2-Methylpropanal	298	346	48	4-Methylpentanone-2	481	509	28
2-Methylbutanal	399	449	50	3,3-Dimethylbutanone-2		485	
3-Methylbutanal	418	451	33	3-Methylpentanone-2	493	517	24
2-Methylpentanal	476	533	57	2-Methylpentanone-3	488	503	15
3-Methylpentanal	523	561	38	2,4-Dimethylpentanone-3	529		
2-Ethylbutanal	469	526	57	2-Methylhexanone-3	547	574	27
Propenal		298		Heptanone-4	591		
Butenal-2		468		5-Methylhexanone-3	552	565	13
Hexanal-2		676		Heptanone-3	600	620	20
Heptenal-2		787		5-Methylhexanone-2	586	624	38
2,4-Hexadienal		749		6-Methylheptanone-3	649	666	17
				2,6-Dimethylheptanone-4	657		
				Nonanone-5	737		
				Nonanone-4	758	773	15
				Nonanone-3	776	806	30

* $\Delta I = I_{anti} - I_{syn}$.

sponding *syn* isomers. Therefore isomers with a higher retention index have the *anti* structure.

The elution sequence of DNPHs is similar to that observed for free carbonyl compounds on non-polar stationary phases[38]. However, hydrazones give twice as much retention data from one column as do free carbonyls, thus considerably increasing the possibilities of identification. The ΔI values in Table I are a measure of the distinction between the hydrocarbon groups attached to the (converted) carbonyl group. For compounds with the same number of carbon atoms, the ΔI value decreases in the order *n*-alkanals, methyl *n*-alkyl ketones, ethyl *n*-alkyl ketones and so on (*cf.*, 2-methylbutanal, pentanal, 3-methylbutanal). Within homologous series the ΔI value increases with increasing number of carbon atoms, and approaches a constant value. These regularities may facilitate the identification of unknown components.

Separations of DNPH mixtures using HPLC were similar to those reported by Selim[22]. A higher selectivity was obtained in the separation of DNPHs of alkanals and 2-alkanones with the same number of carbon atoms. This was accomplished by

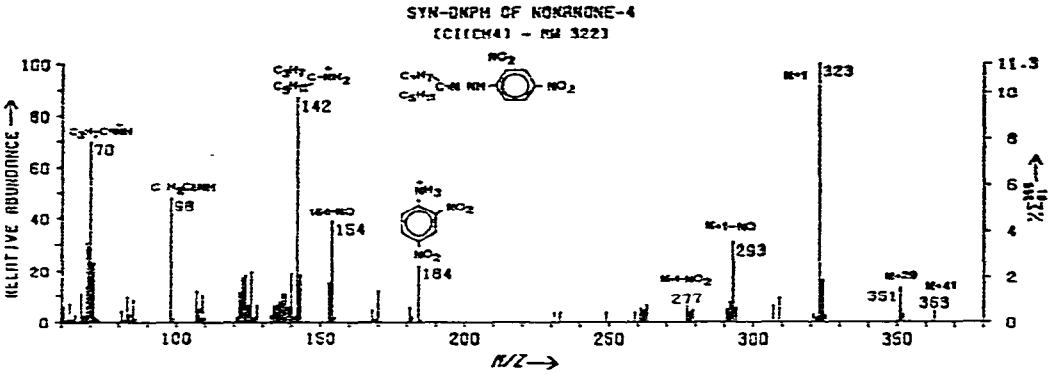


Fig. 4. Chemical ionization mass spectrum of the 2,4-dinitrophenylhydrazone of nonanone-4.

using a LiChrosorb RP-8 column with dimethylformamide–water (3:1) as solvent system. However, the relatively low separation power of this method, in comparison with capillary GC, restricts the use of HPLC for the analysis of DNPHs in complex mixtures.

CI (methane) mass spectra of many DNPHs were recorded. Fig. 4 shows, as an example, the CI spectrum of the nonanone-4 derivative. The spectra of the *syn* and *anti* isomers of the investigated compounds were found to be identical. The masses and relative abundances of characteristic ions from the CI spectra of DNPH derivatives are given in Tables II and III.

Protonated molecular ions, MH^+, appear to be the base peaks in most

TABLE II

SELECTED IONS FROM THE CI (METHANE) MASS SPECTRA OF 2,4-DINITROPHENYLHYDRAZONES OF ALDEHYDES

Ions with a mass below *m/z* 60 were not measured.

DNPH	*(m/z) relative abundance*						
	MH^+	$[MH - NO]^+$	$[MH - NO_2]^+$	*m/z* 184	*m/z* 154	$R_2HC{=}\overset{+}{N}H_2$	$R_2C{\equiv}\overset{+}{N}H$
Methanal	(211)100	(181) 5	(165)12	23	8		
Ethanal	(225)100	(195) 6	(179) 2	24	9		
Propanal	(239)100	(209)10	(193) 5	21	10		
Butanal	(253)100	(223)13	(207) 5	26	19	(72)29	(70) 64
2-Methylpropanal	(253)100	(223)15	(207) 3	45	28	(72)61	(70) 95
Hexanal	(281)100	(251) 5	(235) 2	25	4	(100)11	(98) 14
2-Methylpentanal	(281)100	(251) 3	(235) 1	27	1	(100) 6	(98) 8
2-Ethylbutanal	(281)100	(251) 4	(235) 1	20	2	(100) 8	(98) 9
Heptanal	(295)100	(265) 5	(249) 2	20	5	(114)15	(112) 14
Octanal	(309) 51	(279)19	(263) 5	28	65	(128)56	(126)100
Propenal	(237)100	(207) 7	(191) 3	13	4	(56)13	
Butenal-2	(251)100	(221) 3	(205) 1	5	1	(70)12	(68) 9
Hexenal-2	(279)100	(249) 3	(233) 1	4	1	(98) 8	(96) 6
Heptenal-2	(293)100	(263) 3	(247) 1	4	1	(112) 8	(110) 6
Hexadienal	(277) 48	(247)21	(231) 1	23	46	(96)92	(94)100

TABLE III

SELECTED IONS FROM THE CI (METHANE) MASS SPECTRA OF 2,4-DINITROPHENYLHYDRAZONES OF KETONES

Ions with a mass below *m/z* 60 where not measured. Alkyl group R_1 is smaller than or equal to R_2.

DNPH	*(m/z) relative abundance*							
	MH^+	$[MH-NO]^+$	$[MH-NO_2]^+$	*m/z* 184	*m/z* 154	$R_1 \backslash C=\overset{+}{N}H_2 / R_2$	$R_2C\equiv\overset{+}{N}H$	$R_1C\equiv\overset{+}{N}H$
Pentanone-2	(267)100	(237) 4	(221) 2	7	8	(86) 28	(70) 7	
Hexanone-2	(281)100	(251) 5	(235) 1	8	2	(100) 9	(84) 2	
3-Methyl-pentanone-2	(281) 86	(251)28	(235)10	12	21	(100)100	(84) 39	
4-Methyl-pentanone-2	(281)100	(251) 5	(235) 1	8	1	(100) 7	(84) 2	
3,3-Dimethyl-butanone-2	(281)100	(251) 4	(235) 2	9	2	(100) 4	(84) 1	
Heptanone-2	(295)100	(265) 4	(249) 1	5	0	(114) 4	(98) 5	
Heptanone-3	(295)100	(265) 4	(249) 2	6	1	(114) 4	(84) 5	
2,4-Dimethyl-pentanone-3	(295) 29	(265)13	(249) 3	4	8	(114) 31	(70)100	—*
Heptanone-4	(295)100	(265) 4	(249) 2	4	0	(114) 3	(70) 12	—*
Octanone-3	(309)100	(279)29	(263) 6	24	26	(128) 43	(98) 45	
Nonanone-4	(323)100	(293)31	(277) 6	22	39	(142) 87	(98) 48	(70)70
2,6-Dimethyl-heptanone-4	(323) 48	(293)14	(277) 4	13	22	(142) 84	(84)100	—*

* Ions R_1CNH^+ and R_2CNH^+ are coincident ($R_1 = R_2$).

instances. Exceptions include branched-chain and high-molecular-weight compounds. The high abundance of MH^+ ions is a great advantage over EI-MS, especially for the analysis of trace components by GC–MS. Adductions $[M + C_2H_5]^+$ and $[M+C_3H_5]^+$ (not included in Tables II and III) are always present, with abundances of 11–14% and 3–4% relative to MH^+, respectively. The DNPHs of methanal, ethanal and propanal, however, give significantly lower $[M + C_2H_5]^+$ peaks (5% of MH^+).

Common fragment ions are $[MH-NO]^+$, $[MH-NO_2]^+$, protonated dinitroaniline (*m/z* 184) and its product ion formed by loss of NO (*m/z* 154). Their peak heights are generally lower than 10% of the base peak with the following exceptions. Derivatives of saturated aldehydes yield more abundant *m/z* 184 ions (20–28%) than the DNPHs of alkenals and of lower ketones up to octanone (4–13%). DNPHs of 2-methylpropanal, octanal and hexadienal, as well as 3-methylpentanone-2, the branched heptanone, the octanone and both nonanones, show increased fragmentation. While most product ions are more abundant in these instances, the enhanced fragmentation apparently does not affect the formation of $[MH-NO_2]^+$ ions.

The occurrence of the ions R_1–$C(=\overset{+}{N}H_2)R_2$, R_1–$C\equiv\overset{+}{N}H$ and R_2–$C\equiv\overset{+}{N}H$ is of diagnostic value, enabling one to distinguish aldehydes from isomeric ketones.

ACKNOWLEDGEMENTS

This work was supported by the Scientific Exchange Agreement (S.E.A.). Thanks are due to Dr. J. H. Dhont, Dr. S. van Straten, Dr. H. Maarse (Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands) and Dr. H. T. Badings and Dr. H. van Duin (Netherlands Institute for Dairy Research NIZO, Ede, The Netherlands) for discussions on the analysis of carbonyl compounds. We also thank Dr. J. W. de Haan and Mr. L. J. M. van de Ven (Eindhoven University of Technology) for the NMR analyses.

REFERENCES

1 M. Keeney, *Anal. Chem.*, 29 (1957) 1485.
2 J. W. Ralls, *Anal. Chem.*, 32 (1960) 332.
3 L. A. Jones and R. J. Monroe, *Anal. Chem.*, 37 (1965) 935.
4 R. V. Golovnya and V. P. Uralets, *Nahrung*, 16 (1972) 497.
5 H. Halvarson, *J. Chromatogr.*, 57 (1971) 406.
6 R. J. Soukup, R. J. Scarpellino and E. Danielczik, *Anal. Chem.*, 36 (1964) 2255.
7 E. Fedeli and M. Cirimele, *J. Chromatogr.*, 15 (1964) 435.
8 R. E. Leonard and J. E. Kiefer, *J. Gas Chromatogr.*, 4 (1966) 142.
9 W. G. Galetto, R. E. Kepner and A. D. Webb, *Anal. Chem.*, 38 (1966) 34.
10 R. Barrera, L. Gasco and F. de la Crus, *An. Quim.*, 64 (1968) 517.
11 K. Shibasaki and S. Iwabuchi, *Nippon Shokuhin Kogyo Gakkai-Shi (J. Food Sci. Technol., Tokyo)*, 17 (1970) 193.
12 Y. Shimizu, S. Matsuto, Y. Mizunuma and J. Okada, *Nippon Shokuhin Kogyo Gakkai-Shi (J. Food Sci. Technol., Tokyo)*, 17 (1970) 385.
13 M. F. Fracchita, F. J. Schuette and P. K. Mueller, *Environ. Sci. Technol.*, 1 (1967) 915.
14 M. M. E. Metwalley, C. H. Amundson and T. Richardson, *J. Amer. Oil Chem. Soc.*, 48 (1971) 149.
15 M. Deki and M. Yoshimura, *Chem. Pharm. Bull.*, 23 (1975) 1374.
16 L. J. Papa and L. P. Turner, *J. Chromatogr. Sci.*, 10 (1972) 744.
17 H. Kallio, R. R. Linko and J. Kaitaranta, *J. Chromatogr.*, 65 (1972) 355.
18 Y. Hoshika and Y. Takata, *J. Chromatogr.*, 120 (1976) 379.
19 R. R. Linko, H. Kallio and K. Rainio, *J. Chromatogr.*, 155 (1978) 191.
20 J. B. Pias and L. Casco, *Chromatographia*, 8 (1975) 270.
21 H. van Duin, *Ph.D. Thesis*, Free University of Amsterdam, Amsterdam, 1961.
22 S. Selim, *J. Chromatogr.*, 136 (1977) 271.
23 M. A. Carey and A. F. Persinger, *J. Chromatogr. Sci.*, 10 (1972) 537.
24 D. P. Schwartz and A. I. Wirtanen, *Acta Chim. Scand.*, 22 (1968) 1717.
25 D. F. Brown, V. J. Senn, F. G. Dollear and L. A. Goldblatt, *J. Amer. Oil Chem. Soc.*, 50 (1973) 16.
26 H. T. Badings, *Ph.D. Thesis*, Agricultural University of Wageningen, Wageningen, 1970.
27 J. B. Stanley, D. F. Brown, V. J. Senn and F. J. Dollear, *J. Food Sci.*, 40 (1975) 1134.
28 R. J. C. Kleipool and J. T. Heins, *Nature* (London), 203 (1964) 1280.
29 S. R. Heller and G. W. A. Milne, *EPA/NIH Mass Spectral Data Base*, Vols. I–IV, U.S. Department of Commerce, Washington, D.C., 1978.
30 R. J. Shriner, R. C. Fuson and D. Y. Curtin, *The Systematic Identification of Organic Compounds. A Laboratory Manual*, Wiley, New York, 4th ed., 1956, p. 219.
31 R. S. Deelder and P. J. Hendricks, *J. Chromatogr.*, 83 (1973) 343.
32 P. M. J. van den Berg and Th. Cox, *Chromatographia*, 5 (1972) 301.
33 G. A. F. M. Rutten and J. A. Rijks, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 279.
34 G. A. F. M. Rutten and J. A. Luyten, *J. Chromatogr.*, 74 (1972) 177.
35 L. Blomberg, *J. Chromatogr.*, 115 (1975) 365.
36 J. J. Franken, R. C. M. de Nijs and F. L. Schulting, *J. Chromatogr.*, 144 (1977) 253.
37 R. C. M. de Nijs, J. J. Franken, R. P. M. Dooper, J. A. Rijks, H. J. J. M. de Ruwe and F. L. Schulting, *J. Chromatogr.*, 167 (1978) 231.
38 V. P. Uralets and R. V. Golovnya, *Zh. Anal. Khim.*, 33 (1978) 782.