Journal of Chromatography, 178 (1979) 219–227

CHROM. 12,081

PREPARATION AND GAS CHROMATOGRAPHIC CHARACTERIZATION OF BENZYLOXIMES AND *p*-NITROBENZYLOXIMES OF SHORT-CHAIN (C_1–C_7) CARBONYLS

DONALD F. MAGIN
Philip Morris Research Center, P.O. Box 26583, Richmond, Va., 23261 (U.S.A.)
(Received May 9th, 1979)

SUMMARY

The formation and gas chromatography of benzyloximes and *p*-nitrobenzyloximes of some simple monocarbonyls (C_1–C_7) are described. Correlations are drawn between structure and retention behavior on a 12-m FFAP glass capillary column during linear temperature programming. Formation of these derivatives is simple and straightforward, requiring only about 1.5 h to prepare a sample for injection into the chromatograph. Additional sensitivity and selectivity is achieved because of the excellent response to the oximes by a nitrogen-selective detector.

INTRODUCTION

Relatively short-chain aldehydes and ketones are known to be important flavor components. Generally, their separation and analysis by gas chromatography (GC) presents little difficulty, since they are fairly volatile and heat stable. However, in complex systems such as food extracts, tobacco smoke, flavorings, air pollution samples and the like, these carbonyls may be present in only minor amounts, although they are still very important constituents. As a means of isolating these carbonyls, and sometimes for enhancing their detectability, derivatives have been made. The classical reagents used to prepare derivatives of chromatographic utility include hydroxylamines (formation of oximes), semicarbazides (formation of semicarbazones) and hydrazines (formation of hydrazones). Procedures for preparing these classical derivatives are found in many publications and textbooks (see for example, refs. 1 and 2).

In general, the main purposes of derivative formation in conjunction with chromatographic techniques are: to isolate the carbonyls from complex mixtures; to change the volatility and/or increase the differences between family members for easier chromatographic separation; and to improve the sensitivity of detection.

The formation of 2,4-dinitrophenylhydrazones has been the technique most commonly employed in conjunction with GC and high-performance liquid chromatography (HPLC), with detection both of the derivatives and of the carbonyls themselves after regeneration from the derivatives[3–16]. Other derivatives used specifically in the GC separation of carbonyls are oximes[17,18], methoximes[19] and phenylhydrazones[20,21].

Dns derivatives (1-dimethylaminonaphthalene-5-sulphonylhydrazones) have been made to provide a fluorescent tag for HPLC[22], but these derivatives are insufficiently volatile for separation by GC.

Oxime formation seems to have found most general applicability in the blocking of carbonyl functions of complex molecules (such as steroids) before silylation of hydroxyls, to avoid the possibility of enol formation; derivatives used for this purpose have been methoximes[23] and benzyloximes[24]. *p*-Nitrobenzyloximes and pentafluorobenzyloximes have recently been used to enhance detection sensitivity by electron-capture GC[25–28].

This paper reports on the use and characteristics of benzyloximes and *p*-nitrobenzyloximes of simple short-chain carbonyls in conjunction with glass-capillary GC techniques and nitrogen-selective detection.

EXPERIMENTAL

Derivative preparations

Aliquots of aqueous solutions of the aldehydes and ketones representing about 200–500 μg each were placed in 15-ml screw-capped vials fitted with Teflon®-lined caps. The carbonyls were of reagent grade, obtained mainly from Aldrich (Milwaukee, Wisc., U.S.A.). The volume was made to 10 ml with distilled water if necessary, and 50 mg of either O-benzylhydroxylamine hydrochloride (Aldrich) or *p*-nitrobenzyloxyamine hydrochloride (Regis, Morton Grove, Ill., U.S.A.) was added, followed by 10 drops of triethylamine (Eastman-Kodak, Rochester, N.Y., U.S.A.). The sealed vials were heated at 65–70° for 1 h, and allowed to cool. The solutions were transferred to 60-ml separatory funnels, acidified with 2 ml of 2 *N* hydrochloric acid, and extracted with three 3-ml portions of diethyl ether. The ether was evaporated to near dryness using dry nitrogen at room temperature, and the volume was reconstituted to 1 ml.

Chromatographic separation

The gas chromatograph used was a Perkin-Elmer Model 3920, modified in this laboratory for use with glass capillary columns, and equipped with a nitrogen-selective detector. The Pyrex®-glass capillary was 12-m long and 0.4 mm I.D., pretreated by the method of Schieke *et al.*[29], and coated with FFAP (estimated liquid layer thickness 0.4 μm) by a dynamic coating procedure according to Schomburg *et al.*[30]. Helium was used as the carrier gas at an inlet pressure of 15 p.s.i., giving a flow velocity through the column of *ca.* 20 cm/sec (1.5 ml/min) at 100°. The chromatograph was fitted with a septum purge head with adjustable purge flow (Scientific Glass Engineering, Austin, Texas, U.S.A.), which was set to give a total pre-column carrier-gas split of *ca.* 20:1. The make-up gas was helium at 45 ml/min, and the detector air and hydrogen flows were 100 and 4 ml/min, respectively. The injection block temperature was 275°, and the detector was at 290°. Aliquots of 0.1–0.3 μl were injected .

The separation of the benzyloximes was carried out with a column oven temperature program of 100–180° at 2°/min, the temperature being kept at 180° to the end of the analysis. For the *p*-nitrobenzyloximes, the program was 170–200° at 2°/min, the final temperature again being held until the end of the analysis.

RESULTS

The adjusted retention times of the benzyloximes and *p*-nitrobenzyloximes of several low-molecular-weight carbonyl compounds (1–7 carbon atoms) are listed in Tables I and II. The adjusted retention times were calculated from distances on the

TABLE I

ADJUSTED RETENTION TIMES OF O-BENZYLOXIME DERIVATIVES

For chromatographic conditions, see text.

Aldehydes	*Retention time (min)*	*Ketones*	*Retention time (min)*
Formaldehyde	6.5	Acetone	12.6
Acetaldehyde	9.9	2-Butanone	14.2
Propanal	11.7	2-Pentanone	16.1
Butanal	17.6		
Pentanal	22.5	3-Pentanone	16.8
Hexanal	30.0	4-Heptanone	22.1
Heptanal	36.5		
Octanal	43.5	Cyclopentanone	34.5
		Cyclohexanone	38.4
Isobutanal	13.9	Cycloheptanone	45.0
Isopentanal	20.0		
		3-Methyl-2-butanone	14.4
Propenal	15.9	3-Methyl-2-pentanone	15.4
2-Butenal	27.9		
2-Hexenal	44.0	4-Methyl-2-pentanone	15.2
Methacrolein	17.9	5-Hexen-2-one	25.9
Benzaldehyde	67.7		

TABLE II

ADJUSTED RETENTION TIMES OF *O-p*-NITROBENZYLOXIME DERIVATIVES

For chromatographic conditions, see text.

Aldehydes	*Retention time (min)*	*Ketones*	*Retention time (min)*
Formaldehyde	6.6	Acetone	9.6
Acetaldehyde	9.0	2-Pentanone	12.1
Propanal	10.9		
Butanal	13.5	3-Pentanone	12.6
Pentanal	16.5	4-Heptanone	21.9
Hexanal	20.5		
Heptanal	24.6	Cyclopentanone	25.8
Octanal	31.7	Cyclohexanone	29.8
		Cycloheptanone	35.0
Isobutanal	11.2		
Isopentanal	14.5	3-Methyl-2-butanone	10.7
		3-Methyl-2-pentanone	13.6
Propenal	13.5		
2-Butenal	19.2	4-Methyl-2-pentanone	12.5
2-Hexenal	26.5		
		3-Buten-2-one	13.4
Methacrolein	14.0	3-Penten-2-one	15.0
Benzaldehyde	52.8	5-Hexen-2-one	17.6
		2-Methy-3-hexanone	15.9

chart paper and adjusted by subtraction of the dead volume of the system. Typical chromatograms of selected benzyloximes and *p*-nitrobenzyloximes can be seen in Figs. 1 and 2, respectively.

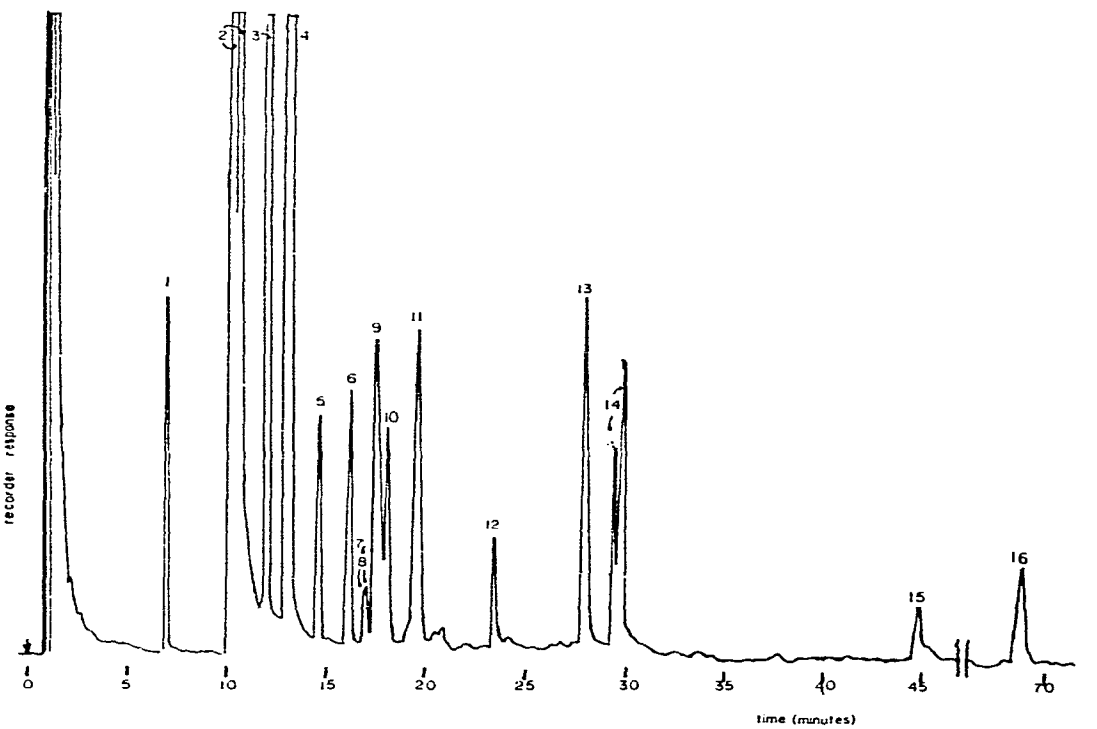

Fig. 1. Chromatogram of benzyloxime derivatives of some carbonyls (see text for chromatographic conditions). Peaks: 1 = formaldehyde; 2 = acetaldehyde (2 peaks); 3 = propanal; 4 = acetone; 5 = 2-butanone; 6 = propenal; 7 = 2-pentanone; 8 = 3-pentanone; 9 = butanal; 10 = methacrolein; 11 = isopentanal; 12 = pentanal; 13 = 2-butenal; 14 = hexanal (2 peaks); 15 = octanal; 16 = benzaldehyde.

Several of the benzyloximes gave double peaks when chromatographed, as can be seen readily in Fig. 1 for acetaldehyde and hexanal. In general, except for these two compounds, the major peak was much greater in size than the minor peak. This double peak formation has been noticed before for the 2,4-dinitrophenylhydrazones[11,16] of simple carbonyls, and the *p*-nitrobenzyloximes of prostaglandin esters[25], and is presumably due to the formation of *syn*- and *anti*-isomers of unsymmetrical carbonyls. For the purposes of the present work, the retention times cited for those compounds exhibiting this behavior are the times for the major peak.

To facilitate comparisons, it was desirable to present the retention data in graphical form. It has been well established that retention values can be plotted directly against carbon numbers for an homologous series during linear temperature programming to yield a straight-line relationship[31,32]; Figs. 3 and 4 show the data from Tables I and II in this format.

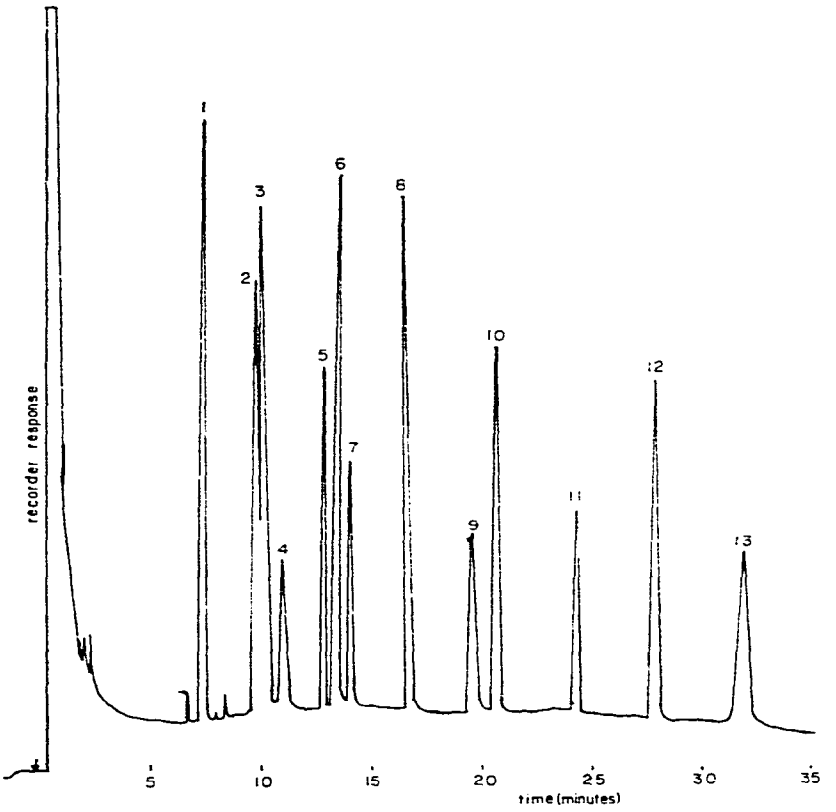

Fig. 2. Chromatogram of *p*-nitrobenzyloxime derivatives of some carbonyls (see text for chromatographic conditions). Peaks: 1 = formaldehyde; 2 = acetaldehyde; 3 = acetone; 4 = propanal; 5 = 3-pentanone; 6 = pentenal; 7 = butanal; 8 = pentanal; 9 = 2-butenal; 10 = hexanal; 11 = heptanal; 12 = 2-hexenal; 13 = octanal.

The break near the propanal datum point for the aldehyde for both the benzyloximes and *p*-nitrobenzyloximes is probably due to the initial column oven temperature being too high[33]. This type of behavior has been noticed by Dal Nogare and Juvet[31], who noted that a lower initial temperature or slower flow-rate would improve the linearity at low retention times.

Similar deviation from linearity can be observed from the data reported by Linko *et al.*[16] for the glass capillary column separation of 2,4-dinitrophenylhydrazones.

CONCLUSIONS

From the retention time *versus* carbon number relationship shown in Figs. 3 and 4 and the data in Tables I and II, the following conclusions can be drawn:

(1) With the exceptions as noted above, the benzyloximes and *p*-nitrobenzyloximes of *n*-aldehydes lie along a straight line;

(2) The methyl ketone homologs also lie along a straight line, although the

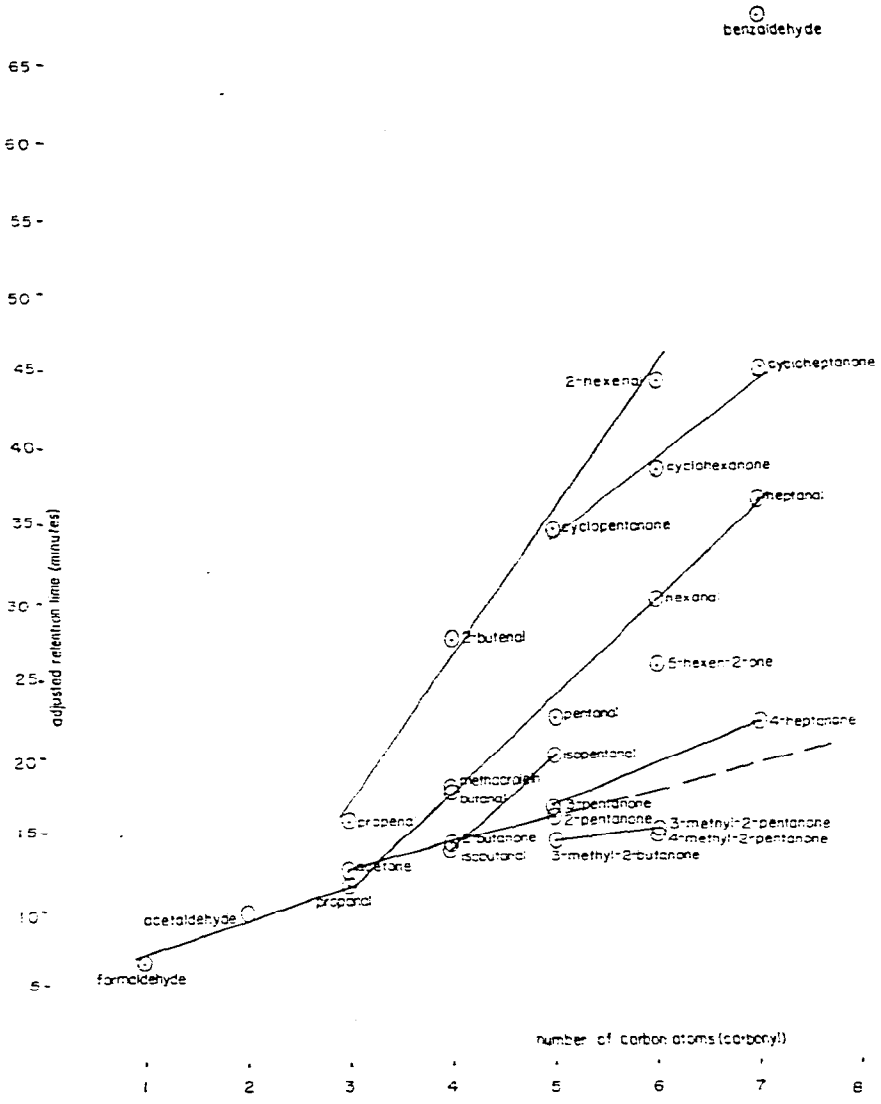

Fig. 3. Relationship between adjusted retention time and carbon number for benzyloximes of some carbonyls. See Table I and text for details.

slope is less than for the *n*-aldehydes. The lines for the aldehydes and methyl ketones approach each other for 3-carbon compounds (propanal and acetone), and the difference between them increases as chain length increases;

(3) Iso-branching in the alkyl chain of the aldehyes reduces the retention, although the slope of the line is similar to that for the *n*-aldehydes. Note that, for both derivatives, the isobutanal point lies very close to the 2-butanone point, but the increase in retention with increasing alkyl chain length for the isoaldehydes is greater than for the methyl ketones. Branching along the alkyl chain of ketones also seems to reduce the retention, with the effects of a branching methyl group perhaps

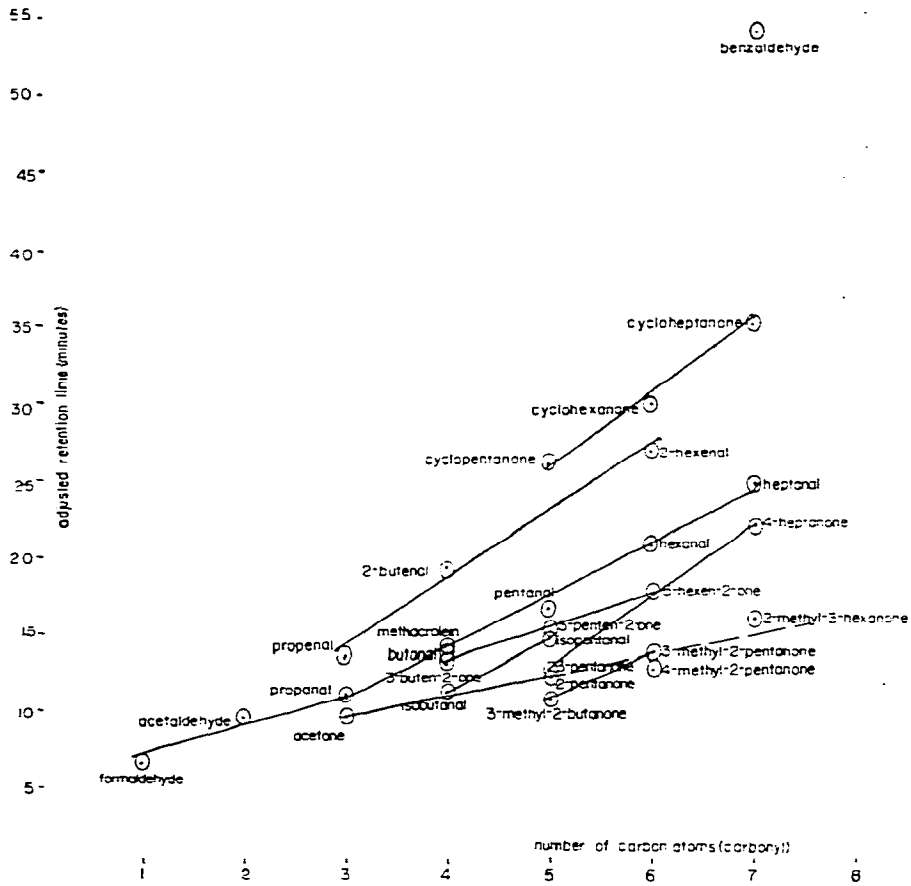

Fig. 4. Relationship between adjusted retention time and carbon number for *p*-nitrobenzyloximes of some carbonyls. See Table II and text for details.

being slightly greater as it is moved along the chain away from the keto group (as in 3-methyl-2-pentanone compared to 4-methyl-2-pentanone);

(4) Unsaturation along the alkyl chain increases retention markedly for both aldehydes and methyl ketones. This effect becomes larger as the chain length increases, as can be seen for the aldehydes by comparing the propenal–2-butenal–2-hexenal line to the lines for the *n*-aldehydes for both derivatives. It is noticeable for the ketones by comparing the 3-buten-2-one–3-penten-2-one–5-hexen-2-one line for the *p*-nitrobenzyloximes to the corresponding methyl ketones, and for the benzyloximes by comparing the 5-hexen-2-one point to the extrapolation of the methyl ketone line. Also, the fact that the 5-hexen-2-one *p*-nitrobenzyloxine point lies on the line drawn between the 3-buten-2-one and 3-penten-2-one points indicates that moving the unsaturation site farther away from the keto group has little additional effect on the retention.

(5) Moving the keto group inward toward the centre of the alkyl chain appears to increase retention for both the benzyloximes and *p*-nitrobenzyloximes, with the effect increasing from 3-pentanone (which is only slightly more retained than 2-pentanone) to 4-heptanone (which is distinctly above the methyl ketone line). This

divergence appears greater for the *p*-nitrobenzyloximes than for the benzyloxime derivatives.

(6) The presence of an aromatic system in the molecule greatly increases the retention of both derivatives, as can be seen from the points for the benzaldehyde derivatives.

(7) Ring closure of the alkyl side chain of the ketones to form cycloketones markedly increases the retention of both derivatives, and the effect increases with increasing carbon length for cyclopentanone to cycloheptanone.

(8) The effects of these changes in the alkyl portions of the molecule seem to be at least qualitatively additive. For example, the methacrolein derivatives elute with about the same retention time as butanal. The structure of methacrolein incorporates a methyl-group side chain and an unsaturation site. As noted before, branching decreases retention (as for isobutanal), and the presence of an unsaturation site increases retention (as for 2-butenal). These effects seem nearly to cancel each other when combined in the methacrolein molecule. Another example of this additive property can be seen from the *p*-nitrobenzyloxime of 2-methyl-3-hexanone. Again, branching has been shown to decrease retention, and the migration of the keto group inward toward the center of the chain has been shown to increase retention. Yet 2-methyl-3-hexanone falls only slightly above the line drawn for the methyl ketones (-2-ones) and substantially below the point for 4-heptanone.

Similar conclusions were drawn by Pias and Gasco[12] about several of the relationships noted when the 2,4-dinitrophenylhydrazones of some carbonyl compounds were investigated. They found the expected straight-line relationships for the *n*-aldehydes and methyl ketones, and found similar qualitative deviations from these lines for branching and for double-bonds within the alkyl chain (except for propanal–propenal on OV-3 at 205°, which exhibited a negative increment in retention index). They also noticed the surprisingly high index of the benzaldehyde derivative compared to the retention of the heptanal.

In the present study, the effect of moving the keto group toward the middle of the alkyl chain appears to increase the retention of the benzyloximes and *p*-nitrobenzyloximes, although there is little difference between 3-pentanone and 2-pentanone. Clearly, though, the points for 4-heptanone lie well above the expected value from an extrapolation of the methyl ketone line. Pias and Gasco noticed a decrease in retention for the 2,4-dinitrophenylhydrazones going from 2-pentanone to 3-pentanone, although the retention indices differ by only about 1%.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the assistance of Beverly Wood, who performed much of the analytical work. Thanks also go to David Douglas for providing the chromatography column, and to Duane Watson for his helpful discussions.

REFERENCES

1 R. H. Brandenberger and H. Brandenberger, in K. Blau and C. S. King (Editors), *Handbook of Derivatives for Chromatography*, Heyden & Son, London, 1977, Ch. 6.

2 R. L. Shriner, R. C. Fuson and D. Y. Curtin, *The Systematic Identification of Organic Compounds*, Wiley, New York, 1965.
3 J. W. Ralls, *Anal. Chem.*, 32 (1960) 332.
4 R. I. Stephens and A. P. Tezler, *Anal. Chem.*, 32 (1960) 1047.
5 J. W. Ralls, *Anal. Chem.*, 36 (1964) 946.
6 L. A. Jones and R. J. Monroe, *Anal. Chem.*, 37 (1965) 935.
7 H. Halvarson, *J. Chromatomffl.*, 57 1971) 406.
8 R. J. Soukup, R. J. Scarpellino and E. Danielczik, *Anal. Chem.*, 36 (1964) 2255.
9 W. G. Galetto, R. E. Kepner and A. D. Webb, *Anal. Chem.*, 38 (1966) 34.
10 R. E. Leonard and J. E. Kiefer, *J. Gas Chromatogr.*, 4 (1966) 142.
11 H. Kallio, R. R. Linko, and J. Kaitaranta, *J. Chromatogr.*, 65 (1972) 355.
12 J. B. Pias and L. Gasco, *Chromatographia*, 8 (1975) 287.
13 C. Bachmann, R. Baumgartner, H. Wick and J. P. Colombo, *Clin. Chim. Acta*, 66 (1976) 287.
14 S. Selim, *J. Chromatogr.*, 136 (1977) 271.
15 C. T. Mansfield, B. T. Hodge, R. B. Hege and W. C. Hamlin, *J. Chromatogr. Sci.*, 15 (1977) 301.
16 R. R. Linko, H. Kallio and K. Rainio, *J. Chromatogr.*, 155 (1978) 191.
17 J. W. Vogh, *Anal. Chem.*, 43 (1971) 1618.
18 M. Bieganowska and T. Wawrzynowicz, *Chromatographia*, 8 (1975) 617.
19 H. M. Fales and T. Luakkainen, *Anal. Chem.*, 37 (1965) 955.
20 E. Fedeli and M. Cirimele, *J. Chromatogr.*, 15 (1964) 435.
21 J. Korolczuk, M. Daniewski and Z. Mielnicznk, *J. Chromatogr.*, 88 (9173) 177.
22 R. W. Frei and J. F. Lawrence, *J. Chromatogr.*, 83 (1973) 321.
23 M. G. Horning, A. M. Moss and E. C. Horning, *Anal. Biochem.*, 22 (1968) 284.
24 P. G. Devaux, M. G. Horning, R. M. Hill and E. C. Horning, *Anal. Biochem.*, 41 (1971) 70.
25 F. A. Fitzpatrick, M. A. Wynalder and D. G. Kaiser, *Anal. Chem.*, 49 (1977) 1032.
26 K. T. Koshy, D. G. Kaiser and A. L. Vanderslik, *J. Chromatogr. Sci.*, 13 (1975) 97.
27 T. Hambaro, K. Kigasawa, T. Iwata and M. Ibuki, *J. Chromatogr.*, 114 (1975) 81.
28 T. H. Jupille, *Amer. Lab.*, May (1976).
29 J. D. Schieke, N. R. Comins and V. Pretorius, *J. Chromatogr.*, 112 (1975) 97.
30 G. Schomburg, H. Husmann and F. Weeke, *J. Chromatogr.*, 99 (1974) 63.
31 S. Dal Nogare and R. S. Juvet, Jr., *Gas Chromatography/Theory and Practice*, Interscience, New York, 1962, p. 337.
32 L. S. Ettre and A. Zlatkis, *The Practice of Gas Chromatography*, Interscience, New York, 1967, p. 387.
33 W. Jennings, *Gas Chromatography with Glass Capillary Columns*, Academic Press, New York, 1978, p. 84.