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Note

Gas chromatographic determination of carbonyl compounds as their phenylhydrazones

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Volatile carbonyl compounds are important flavour components of foodstuffs. Because of their low concentration and relatively high volatility, carbonyl compounds are usually isolated from food products and pre-concentrated by distillation under normal or reduced pressure. They can then be separated and determined quantitatively by instrumental methods.

The most suitable method of determination seems to be the gas–liquid chromatographic (GLC) analysis of the free carbonyl compounds[1–5] or their 2,4-dinitrophenylhydrazone[6–7] or oxime[8] derivatives. Fedeli and Cirimele[9] indicated the possibility of separating some aldehyde phenylhydrazones by GLC.

The aim of our work was the investigation of the gas chromatographic determination of aldehydes and ketones as their phenylhydrazones.

EXPERIMENTAL

Apparatus

The gas chromatograph used was a JGC-810 (Jeol, Japan) equipped with a flame ionization detector. The recorder was a Model SRG (Sargent-Welch) equipped with a disc integrator. Stainless-steel columns, 3 m long and 3 mm I.D., were filled with 4% (w/w) SE-30 on Chromosorb W (A.W., H.P. DMCS-treated, 100–120 mesh). The columns were conditioned overnight at 300°, while nitrogen was passed through each column at a flow-rate of 10 ml/min. The other conditions of the operation were: temperature of the injection block, 280°; temperature of the flame ionization detector, 290°; initial column temperature, 110–170°; final column temperature, 280°; temperature programming rate, 10°/min; nitrogen flow-rate, 50 ml/min.

Reagents

Diethyl ether, ethyl acetate and water were refluxed for 2–3 h with 2 g of 2,4-dinitrophenylhydrazine and 5 ml of concentrated sulphuric acid per litre, then distilled in all-glass distillation apparatus and stored in all-glass containers protected from atmospheric carbonyl compounds with tubes filled with 2,4-dinitrophenylhydrazine. Hexane (BDH spectroscopic grade) was used without any purification. Phenylhydrazine hydrochloride, 0.5 *M* in aqueous 0.25 *M* sodium orthophosphate solution, was stored in a brown-glass bottle at room temperature for not longer than 2 weeks.

Before use, this reagent was shaken with activated carbon for half an hour, then filtered and extracted three times with diethyl ether and kept under hexane.

Procedure

Phenylhydrazones of the studied carbonyl compounds, which are listed in Table I, were prepared as follows. A 10-ml volume of 0.5 *M* phenylhydrazine hydrochloride in 0.25 *M* aqueous sodium orthophosphate solution, freshly extracted with diethyl ether, was placed in a 100-ml calibrated flask, then 10 ml of methanol were

TABLE I

EFFECT OF THE INITIAL COLUMN TEMPERATURE ON THE RELATIVE RETENTION TIMES OF PHENYLHYDRAZONES OF CARBONYL COMPOUNDS

The retention time of 2-hexanone phenylhydrazone was taken as unity. Temperature programming rate, 10°/min.

*No.**	*Parent carbonyl*	*Volume injected (nl)***	*Initial column temperature (°C)*						
			108	*120*	*131*	*139*	*150*	*160*	*170*
0	Phenylhydrazine	–	0.304	0.280	0.278	0.276	0.269	0.285	0.301
1	Formaldehyde	3.6	0.440	0.421	0.394	0.387	0.377	0.381	0.395
2	Acetaldehyde	9.0	0.567	0.539	0.515	0.503	0.479	0.478	0.481
3	Acetone	4.5	0.671	0.643	0.622	0.613	0.583	0.583	0.575
4	Propanal	9.0	0.674	0.647	0.626	0.617	0.589	0.586	0.577
5	2-Butanone	4.5	0.782	0.766	0.747	0.735	0.713	0.706	0.708
6	Butanal	–	0.800	0.787	0.768	0.755	0.731	0.732	0.726
7	Isopentan-2-one	6.0	0.846	0.833	0.818	0.812	0.796	0.789	0.789
8	2-Pentanone	6.0	0.885	0.869	0.863	0.855	0.848	0.837	0.840
9	Methyl *tert.*-butyl ketone	7.5	0.917	0.900	0.820	0.885	0.877	0.865	0.870
10	Pentanal***	–	0.926	0.927	0.928	0.913	0.911	0.911	0.921
11	Methyl isobutyl ketone	6.0	0.936	0.937	0.929	0.928	0.917	0.932	0.935
12	3-Hexanone	6.0	0.960	0.960	0.956	0.955	0.948	0.958	0.960
13	2-Hexanone (min)	6.0	9.92	8.70	7.80	7.13	6.18	5.27	4.45
14	Hexanal***	–	1.04	1.05	1.06	1.06	1.07	1.09	1.12
15	Cyclopentanone	4.5	1.06	1.08	1.09	1.09	1.10	1.11	1.13
16	2-Heptanone***	–	1.10	1.12	1.13	1.14	1.16	1.18	1.20
17	Heptanal	15.0	1.16	1.18	1.20	1.22	1.24	1.27	1.31
18	Cyclohexanone	–	1.18	1.20	1.22	1.24	1.26	1.29	1.33
19	2-Octanone***	–	1.21	1.23	1.25	1.27	1.31	1.35	1.40
20	5-Nonanone	7.5	1.25	1.28	1.30	1.32	1.35	1.39	1.43
21	Octanal	15.0	1.28	1.31	1.33	1.35	1.38	1.43	1.51
22	2-Nonanone***	–	1.31	1.35	1.38	1.41	1.47	1.52	1.60
23	Nonanal	15.0	1.40	1.43	1.46	1.49	1.55	1.62	1.70
24	2-Decanone***	–	1.41	1.47	1.50	1.55	1.62	1.69	1.80
25	Decanal	15.0	1.48	1.54	1.60	1.64	1.72	1.80	1.89
26	2-Undecanone	12.0	1.52	1.58	1.63	1.68	1.79	1.87	2.00
27	Undecanal	15.0	1.57	1.66	1.69	1.75	1.85	1.96	2.10

* Numbers of the carbonyl compounds are as in Fig. 4.

** Nanolitres of each carbonyl injected into the column are the same as those used to obtain the chromatogram in Fig. 4.

*** Positions of these phenylhydrazones were calculated by interpolation according to the equations given in Figs. 2 and 3.

added and the mixture diluted to volume with water. A 5–50-μl volume of each carbonyl compound was placed into the phenylhydrazine solution, which was then shaken vigorously and allowed to stand at room temperature overnight or heated for half an hour at 50° in a boiling water-bath. The phenylhydrazones formed were isolated by successive extractions with 10, 5 and 5 ml of hexane, diethyl ether or ethyl acetate. The solvent layers were combined in test-tubes with ether-tight stoppers and evaporated to dryness by placing the tubes in the water, sand or an oil-bath at 100° while directing a stream of nitrogen filtered through sodium hydrogen sulphite and silica gel (*ca.* 100 ml/min) into the heated tubes. The dried phenylhydrazones were dissolved in an exact volume (1, 5 or 10 ml) of hexane, diethyl ether or ethyl acetate, 50–100 mg of magnesium sulphate were added and the samples were stored in closed tubes with magnesium sulphate at 0–5°. The concentration of phenylhydrazones in the final solution was in the range of 0.3–3 μl/ml of each carbonyl containing substance. A 1–3-μl volume of this solution was injected into the gas chromatographic column and chromatographed under the conditions described above.

RESULTS AND DISCUSSION

A fairly linear correlation between the initial column temperature and the retention time of the phenylhydrazones was observed (Fig. 1). The differences between the retention times of the phenylhydrazones of formaldehyde, acetaldehyde, acetone and 2-butanone decreased when the initial column temperature was increased. For the other phenylhydrazones, the effect of the initial column temperature was much less. Satisfactory separation of the phenylhydrazones studied was obtained

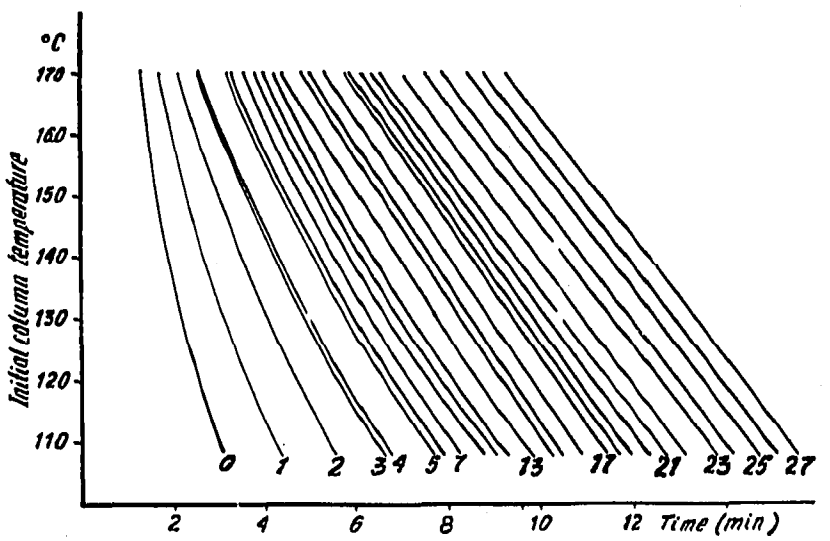

Fig. 1. Effect of the initial column temperature on the retention times of phenylhydrazones of different carbonyl compounds. Numbers of carbonyls are the same as in Table I. Conditions: column 3 m long, 3 mm I.D.; 4% SE-30 on Chromosorb W (A.W., DMCS, 100–120 mesh); temperature programming rate, 10 °C/min.

at initial column temperatures up to 170° (Fig. 1). Better resolution for some peaks was observed when the initial temperature was lower, but there was no need to use an initial column temperature of less than 140°.

When the initial column temperature was in the range 108–131°, a linear correlation between the number of carbon atoms in the carbonyl chain and the retention time was observed for both *n*-aldehydes and methyl ketones (Figs. 2 and 3). Fedeli and Cirimele[9] found a linear relationship between the length of the *n*-aldehyde carbon chain and the logarithm of the retention, but they did not state if a constant or programmed temperature was used.

Figs. 2 and 3 show that it is possible to calculate by interpolation the exact position of each peak on the chromatogram for *n*-aldehydes, 2-alkanones and

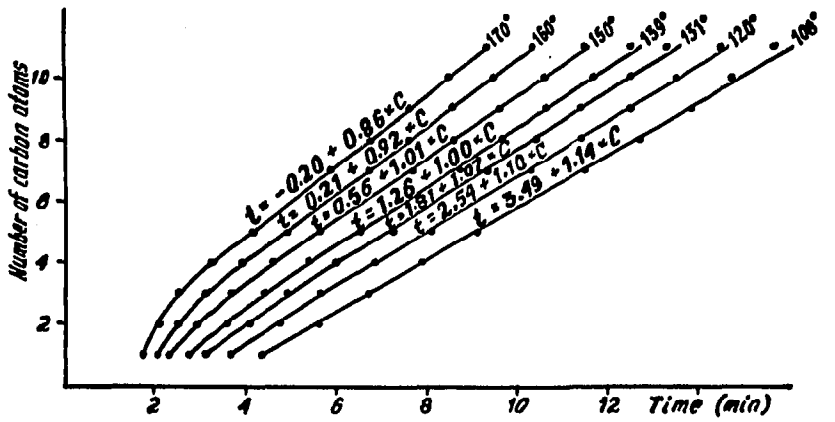

Fig. 2. Effect of the initial column temperature and the length of the carbonyl chain on the retention time of phenylhydrazones of *n*-alkanales. Conditions as in Fig. 1. The regression equations were calculated for *n*-aldehydes containing 4–11 carbon atoms in the chain; t = retention time (min) C= number of carbon atoms in the *n*-aldehyde chain.

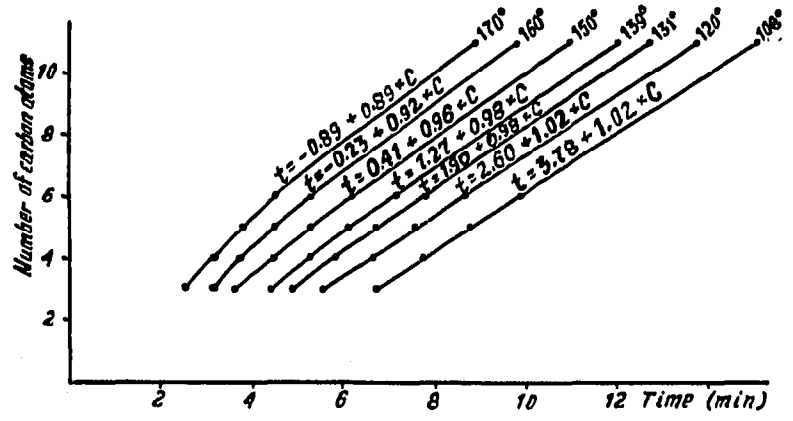

Fig. 3. Effect of the initial column temperature and the length of the carbonyl chain on the retention times of phenylhydrazones of 2-alkanones. Conditions as in Fig. 1. The regression equations were calculated for 2-alkanones containing 6–11 carbon atoms in the chain; t = retention time (min); C = number of carbon atoms in the 2-alkanone chain.

probably for other carbonyl compounds such as cyclic ketones, branched ketones and aldehydes. In general, 2-alkanones appear before *n*-alkanales and branched ketones before 2-alkanones. Cycloketones appear just after the peak of the *n*-aldehyde with one more carbon atom in the chain, *i.e.*, cyclopentanone appears just after *n*-hexanal and cyclohexanone after *n*-heptanal.

A typical chromatogram of the phenylhydrazones of some carbonyl compounds is shown in Fig. 4. Under the conditions described above, it is possible to separate more than 20 carbonyl compounds having up to 11 carbon atoms in less than 15 min. It was possible to separate methyl *tert.*-butyl, methyl isobutyl, ethyl propyl and methyl *n*-butyl ketones, but is was not possible to separate acetone and propionaldehyde (Table I).

Double peaks of two isomers (*sym.* and *anti*) for some phenylhydrazones were observed, especially when hexane was used as the solvent. When more polar solvents such as diethyl ether or methyl or ethyl acetate were used, the first peak (*sym.*) was so small that it had no practical value. Only for cyclohexanone was the area of the first peak about 10% of that of the main peak.

Diethyl ether or ethyl acetate solutions of phenylhydrazones of carbonyl compounds were stable at 5–10° for several months when stored in closed tubes with magnesium sulphate. Hexane solutions were not stable and usually decomposed after a few days of storage.

The determination of carbonyl compounds as their phenylhydrazones seems to be easier than as their 2,4-dinitrophenylhydrazones[6,7], as the preparation of the derivatives is simpler and less time consuming and their separation from aqueous solution by extraction with diethyl ether is also very easy, rapid and quantitative. The diethyl ether can then easily be evaporated, thus giving a sufficiently concentrated solution of phenylhydrazones. The GLC analysis of the phenylhydrazones can be

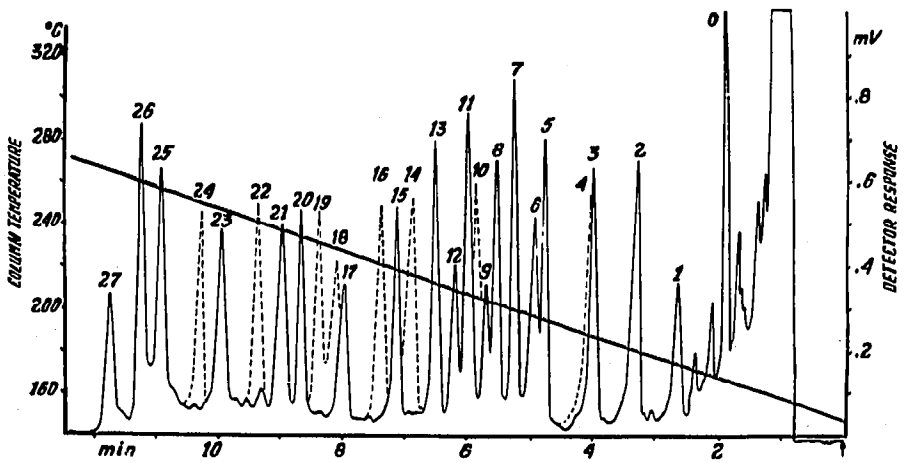

Fig. 4. Typical chromatogram of phenylhydrazones of carbonyl compounds. Conditions: initial column temperature 147°; attenuation 1.5×10^{-8} A (5×10^{3}); 3 μl of an ethyl acetate solution of phenylhydrazones contained the amounts of parent carbonyls given in Table I. The other conditions were the same as in Fig. 1. The positions of the peaks indicated by broken lines were calculated by interpolation according to the equations in Figs. 2 and 3. The straight line across the chromatogram indicates the column temperature.

done on stainless-steel columns instead of glass columns, which are needed for the 2,4-dinitrophenylhydrazones. The retention temperatures of the phenylhydrazones are about 50° lower than those for the 2,4-dinitrophenylhydrazone derivatives, which increases the column stability and decreases bleeding of the liquid phase. The high stability of the phenylhydrazones and the short time required for analysis permits the preparation and analysis of several samples per day and the storage of derivatives for long periods of time.

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