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GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF 2,4-DINITROPHENYLHYDRAZONES OF CARBONYL COMPOUNDS

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SUMMARY

Fifteen carbonyl compounds that are known to be flavor components have been analyzed as their 2,4-dinitrophenylhydrazones by gas–liquid chromatography on a 2% SE-30 column both isothermally at 232° and with a linear temperature program at a rate of 4°/min from 200 to 270° and on a 12% F-60 column at 225°. The detectors employed were a hydrogen flame ionization detector and an electron capture detector. Mixtures of the 2,4-dinitrophenylhydrazones can be successfully resolved on both columns although derivatives of compounds such as propanal, propenal and acetone with equal numbers of carbon atoms overlap. Some of the 2,4-dinitrophenylhydrazones gave rise to double peaks in gas–liquid chromatography owing mainly to the influence of the solvent. Peaks which were only slightly asymmetric were obtained when ethyl acetate was the solvent. The sensitivity was of the order of nanograms when the flame ionization detector was employed and five-hundred times greater when the electron capture detector was employed.

INTRODUCTION

Volatile carbonyl compounds are important flavor components in foodstuffs. The pleasant odors of numerous natural products and products prepared from them, but also off-flavors are due to the presence of carbonyl compounds. The contents of the carbonyl compounds in these products are, however, very low in general and this renders their identification and analysis difficult.

Carbonyl compounds have usually been analyzed as their 2,4-dinitrophenylhydrazones using column, paper and thin-layer chromatography (TLC)[1–4]. The quantitative determination of individual carbonyl compounds by these methods is both laborious and time-consuming. Moreover, the accuracies and sensitivities of these methods are much lower than those of gas–liquid chromatographic (GLC) methods.

A number of 2,4-dinitrophenylhydrazones have been allowed to react with α-keto acids to liberate the carbonyl compounds which have then been analyzed by GLC[5–8].

Direct separation of 2,4-dinitrophenylhydrazones by GLC employing a hydrogen flame ionization detector has been used in only a few investigations[9,10]. BARRERA

et al.[11] employed this method to analyze the carbonyl compounds in apple, SHIBASAKI AND IWABUCHI[12] in combination with TLC to analyze the carbonyl compounds in 'miso' aroma and SHIMIZU *et al.*[13] to analyze the carbonyl compounds in roasted starch.

The work described below had as its aim the optimization of the resolution and sensitivity in the analysis of volatile carbonyl compounds as their 2,4-dinitrophenylhydrazones by GLC employing both a hydrogen flame ionization detector and an electron capture detector.

EXPERIMENTAL

The 2,4-dinitrophenylhydrazones of the studied carbonyl compounds, which are listed in Table I, were prepared by shaking 100 μl of each compound with 100 ml of a saturated solution of 2,4-dinitrophenylhydrazine (guaranteed reagent, E. Merck AG) in aqueous 2 *N* hydrochloric acid (guaranteed reagent, E. Merck AG) and allowing the mixture to stand at room temperature overnight. The formed precipitate was isolated by filtration, washed with 2 *N* hydrochloric acid and water, and dried over silica gel in a vacuum desiccator. After this treatment, the derivatives were sufficiently pure for GLC analysis.

TABLE I

RELATIVE RETENTION TIMES (r) OF 2,4-DINITROPHENYLHYDRAZONES OF CARBONYL COMPOUNDS

The retention time of the 2,4-dinitrophenylhydrazone of 2-butanone as determined with a 2% SE-30 column at 232° and with a linear temperature rise from 200 to 270° at a rate of 4°/min, and with a 12% F-60 column at 225° was taken as unity.

Carbonyl compound	*2% SE-30 column*			*12% F-60 column*
	200–270°[a]		*232°*[b]	*225°*[a]
	r	*Elution temperature* (°C)	r	r
Formaldehyde	0.52	219	0.49	0.38
Acetaldehyde	0.71	225	0.66	0.58
Propenal	0.84	230	0.81	0.76
Acetone	0.84	230	0.81	0.76
Propanal	0.86	231	0.81	0.78
2-Methylpropanal	0.94	233	0.92	0.88
2-Butanone	1.00	236	1.00	1.00
3-Methylbutanal (isovaleraldehyde)	1.13	240	1.40	1.23
2-Butenal	1.16	242	1.71	1.33
Hexanal	1.42	250	2.21	2.01
Furfural	1.62 (1.58)[c]	256	2.28 (2.10)[c]	2.59 (2.38)[c]
Heptanal	1.63	258	2.67	2.80
Octanal	1.83	265	3.10	3.90
Benzaldehyde	1.97	270	3.93	4.78
Nonanal	2.03	272	4.12	5.40

[a] Perkin-Elmer F-11 gas chromatograph equipped with a coiled glass column, 6 ft. long and 1/8 in. I.D.

[b] Aerograph 2100-40 gas chromatograph equipped with a U-shaped glass column, 5 ft. long and 1/8 in. I.D.

[c] Relative retention times of secondary peaks.

The 2,4-dinitrophenylhydrazones were dissolved in ethyl acetate (guaranteed reagent, E. Merck AG) to give 0.2 % solutions, when the hydrogen flame ionization detector was employed and dissolved in benzene (for spectroscopy, E. Merck AG) to give 0.01 % solutions, when the electron capture detector was employed. Also hexane (guaranteed reagent, E. Merck AG), dioxane (extra pure, E. Merck AG) and dichloromethane (guaranteed reagent, E. Merck AG) were employed as solvents in some experiments. All the solvents were distilled before use. The volume of sample injected was in most cases 1–2 μl.

The gas chromatographs were a Varian Aerograph Model 2100-40 equipped with a U-shaped glass column 5 ft. long and 1/8 in. I.D. and a ^{63}Ni (8 mCi) electron capture detector and a Perkin-Elmer F-11 apparatus equipped with coiled glass columns 8 ft. long and 1/8 in. I.D. and two hydrogen flame ionization detectors. The column fillings were 2 % (w/w) SE-30 methyl silicone gum (for gas chromatography, Serva Entwicklungslabor, Heidelberg) and a 12 % (w/w) F-60 silicone fluid (D.C. 560, Varian Aerograph), both on Chromosorb W (60–80 mesh, acid-washed and trimethylchlorosilylated, Perkin-Elmer).

The columns were conditioned during the course of three days by heating them first to 150° and then gradually raising the temperature until it was finally 10° higher than the highest temperature at which they were employed in the analyses (see below). Nitrogen was passed through each column at a flow rate that was initially 5 ml/min, but was gradually increased to 32 ml/min. To increase the ability of the columns to resolve the dinitrophenylhydrazones, it proved advantageous to resilylate the heated columns before use. This was done by injecting into the column (detector removed) at 100° two 10-μl volumes of a 1:1 (v/v) mixture of hexamethyldisilazane (purum, Fluka AG) and trimethylchlorosilane (puriss., Fluka AG) while nitrogen was flowing through the column at a rate of 5 ml/min. Half an hour later the temperature of the SE-30 column was slowly raised to 280° and that of the F-60 column to 240°, at which temperature the column was held for ½ h while nitrogen flowed through the column at a rate of 32 ml/min.

When the Aerograph 2100-40 was used, the temperature of the 2 % SE-30 column was 232°, the detector temperature 325°, and the flow rate of nitrogen 45 ml/min. When the Perkin-Elmer F-11 was used, the temperature of the 2 % SE-30 column was raised from 200° to 270° at a rate of 4°/min and the temperature of the F-60 column was held at 225°, the flow rate of nitrogen through both columns being 32 ml/min. In all analyses the temperature of the injection block was 270°.

The relative amounts of the 2,4-dinitrophenylhydrazones in the injected samples were estimated by multiplying the height of the peak for each compound in the chromatogram by its width at half-height.

RESULTS AND DISCUSSION

Typical chromatograms of 2,4-dinitrophenylhydrazones of carbonyl compounds obtained with the 2 % SE-30 column are shown in Fig. 1. Chromatogram 1a was recorded in isothermal analysis with the electron capture detector and chromatogram 1b in programmed temperature analysis with a hydrogen flame ionization detector. It will be noted, as SOUKUP *et al.*[10] have found earlier, that carbonyl compounds can be analyzed as their 2,4-dinitrophenylhydrazones by GLC. The advantages of

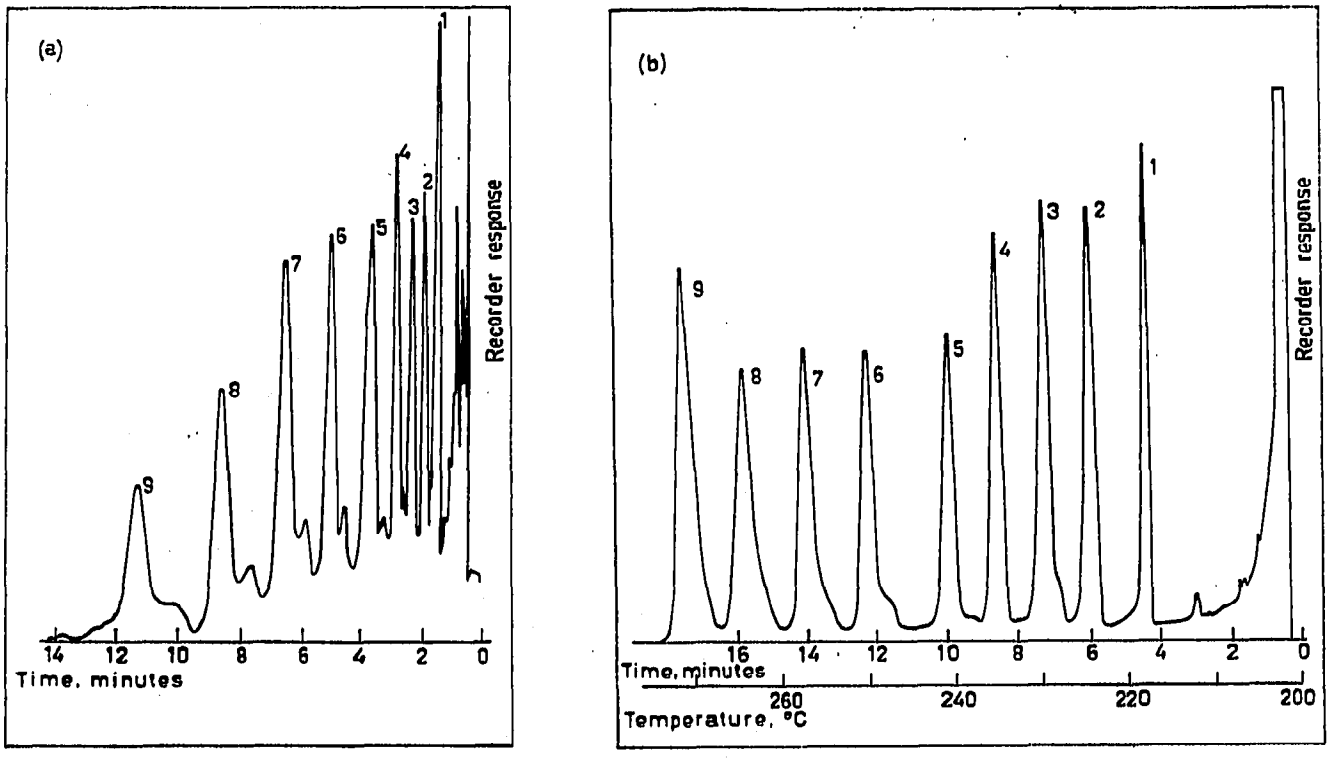

Fig. 1. Gas–liquid chromatogram of a mixture of 2,4-dinitrophenylhydrazones of carbonyl compounds resolved on a 2% SE-30 column. 1 = formaldehyde; 2 = acetaldehyde; 3 = acetone; 4 = 2-butanone; 5 = 2-butenal; 6 = hexanal; 7 = heptanal; 8 = octanal; 9 = nonanal. (a), Analysis at constant temperature (232°) on a Varian Aerograph 2100-40 equipped with a ^{63}Ni electron capture detector. Injected sample: 1.0 μl of hexane containing 100–200 pg amounts of each of the derivatives. Attenuation 2 × 10^{-10}A. (b). Programmed temperature analysis from 200 to 270° (4°/min) on a Perkin-Elmer F-11 chromatograph equipped with a hydrogen flame ionization detector. Injected sample: 1.0 μl of ethyl acetate containing about 1000 ng of each of the derivatives. Attenuation 128, range 1.

temperature programming are evident. The chromatograms show further that many of the dinitrophenylhydrazones give two peaks, of which one is larger in size than the other, which precedes the former. The occurrence of the double peak and the relative sizes of the two peaks depend on the compound, but especially on the solvent employed. Column temperature and retention time do not affect the formation of double peaks. Not all the compounds gave rise to double peaks, but the dinitrophenylhydrazone of furfural did irrespective of the solvent employed. The smaller of the two peaks (the secondary peak) was largest in size when the solvent was dioxane and dichloromethane, but decreased in size in the solvent order hexane, benzene and ethyl acetate. Only slightly asymmetric peaks were obtained with a few dinitrophenylhydrazones when ethyl acetate was the solvent (Fig. 1b), but clear double peaks were formed when hexane was the solvent (Fig. 1a). The double peaks are evidently due to the existence of isomeric 2,4-dinitrophenylhydrazones[14] as shown by the observation that the double peaks assume a constant form only some time after the derivatives are dissolved.

Double peaks of this kind have previously been observed to be produced by the 2,4-dinitrophenylhydrazone of furfural by LEONARD AND KIEFER[15]. SHIBASAKI AND IWABUCHI[12], however, obtained the same retention times for the red and yellow isomers of furfural 2,4-dinitrophenylhydrazone. This observation and the absence

of double peaks in the chromatograms of SOUKUP and co-workers[10] may have been due to insufficient resolving power of the columns they used. GALETTO *et al.*[16] did not find that the 2,4-dinitrophenylhydrazone of isovaleraldehyde decomposes on passage through a GC column when they studied the compounds that emerged from the column by TLC. LEONARD AND KIEFER[15] reported that the 2,4-dinitrophenylhydrazone of formaldehyde is not decomposed by heat in preparative GLC. It thus seems odd that BARRERA *et al.*[11] claimed that dinitrophenylhydrazones undergo catalytic decomposition in the injection block and in the support in GLC.

When they analyzed amide and thioester derivatives of pyruvic acid 2,6-dinitrophenylhydrazone by GLC, BASETTE *et al.*[17] also observed that some of the derivatives gave double peaks when the resolution was increased. To avoid this, they reduced the resolving power of the column by increasing its temperature. In the present work, the formation of double peaks was avoided by choosing a suitable solvent, which was found to be ethyl acetate when the hydrogen flame ionization detector was employed and benzene when the electron capture detector was used.

The results of the GLC analyses of the 2,4-dinitrophenylhydrazones of the studied carbonyl compounds show that the use of an electron capture detector leads to essentially greater sensitivity and selectivity. Thus, whereas amounts of the order of nanograms can be analyzed with a hydrogen flame ionization detector, amounts about five hundred times smaller can still be analyzed with an electron capture detector. The linear response range is very broad, 10–10000 ng, when a hydrogen flame ionization detector is used, but as shown by the results of experiments with 2-butanone, the range is very narrow, 20–500 pg, when an electron capture detector is employed. The non-linearity of the response is a limitation to precise quantitative application of the electron capture detector, but its high sensitivity and selectivity makes it extremely well suited to trace analytical problems. SOUKUP and co-workers[10] found that the detectability of 2,4-dinitrophenylhydrazones with the hydrogen flame ionization detector, limited by the column background, is in the range of 10^{-3}–10^{-5} mg, which is the range of detection values obtained in this study with the hydrogen flame ionization detector.

The relative retention times on SE-30 and F-60 columns of 2,4-dinitrophenylhydrazones of fifteen carbonyl compounds with the retention time of the derivative of 2-butanone as unity are collected in Table I. It will be noted that the derivatives of *n*-alkanals are clearly separated as FEDELI AND CIRIMELE[9] found earlier. Sufficient numbers of members of other homologous series were not available for a similar analysis. It is seen, however, that the peaks for the dinitrophenylhydrazones of propanal, propenal and acetone with three carbon atoms overlap. According to SOUKUP *et al.*[10], *n*-butyraldehyde, 2-butanone and isobutyraldehyde, on one hand, and *n*-valeraldehyde, 2-pentanone and isovaleraldehyde, on the other, are resolved, although poorly, on a similar silicone SF-96 column. It is possible to separate such derivatives into classes by absorption chromatography prior to GLC to avoid overlapping[18].

When the results obtained with the non-polar SE-30 and the intermediately polar F-60 column are compared, it is seen that the resolving powers are similar. The SE-30 column was, however, thermally more stable than F-60 column, which rapidly began to bleed. Weak bleeding occurred also with the SE-30 column which led to a gradual shortening of the retention times and limited the useful lifetime of the

column to about three weeks. The other tested stationary phases GE XE-60 (nitrile gum) and Carbowax 20-M were not found suitable for the resolution of the 2,4-dinitrophenylhydrazones.

The above results reveal that many carbonyl compounds that are important components of natural flavors can be analyzed even in nano–subnanogram amounts by GLC of their 2,4-dinitrophenylhydrazones using a hydrogen flame ionization detector and, especially, an electron capture detector. The possibility of separating and analyzing such groups of compounds containing a common functional group is important for progress in the investigation of natural and artificial flavoring substances.

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