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Note

Gas–liquid chromatographic analysis of 2,4-dinitrophenylhydrazones of monocarbonyl compounds in carrots using glass capillary columns

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Carbonyl compounds are known to be important constituents of the aromas of many raw and processed foods. These compounds are usually isolated from a very complicated mixture of food volatile constituents as their 2,4-dinitrophenylhydrazones (2,4-DNPH's). The derivatives are analytically separated and determined by column, paper or thin-layer chromatography (TLC)[1–4], and more recently by conventional gas–liquid chromatography (GLC) on packed columns[5,6]. However, although GLC is sensitive enough, it does not alone resolve all the numerous carbonyl compounds found in many foodstuffs in varying relative concentrations. Combined TLC–GLC has therefore been used by many investigators for analysing the carbonyl compounds as their 2,4-DNPH's in different materials, *e.g.* by Shibasaki and Iwabuchi[7] in "miso" aroma, Shimizu *et al.*[8] in roasted starch, Kallio and Linko[9] in Arctic bramble, Pyysalo[10] and Hiirsalmi *et al.*[11] in hybrids between raspberry and Arctic bramble and by Linko *et al.*[12] in carrot.

The present report deals with the analysis of carbonyl compounds as their 2,4-DNPH's using the glass-capillary GLC technique, and the adaptation of it to the analysis of the volatile constituents of carrots.

EXPERIMENTAL

Reference 2,4-DNPH derivatives of pure commercially available aldehydes and ketones, which are listed in Table I, were prepared according to Kallio *et al.*[5]. The 2,4-DNPH's were dissolved in ethyl acetate (guaranteed reagent; Merck, Darmstadt, G.F.R.) to give 0.2% solutions.

The carrots (*Daucus carota* L.) used in the study were of the Feonia Hunderup S-64 variety, and were obtained from Agricultural Research Centre Institute of Horticulture, Piikkiö, Finland. The handling of carrots was carried out according to Linko *et al.*[12].

The carbonyl compounds in the aroma distillate of carrots were isolated as their 2,4-DNPH's by a modification of the method[12] developed earlier by Kallio and Linko[9].

The GLC analyses of the derivatives were performed on a Varian Aerograph, Model 2100-40, equipped with a flame ionization detector. The capillary tubes were

TABLE I

RELATIVE RETENTION TIMES (r) OF THE 2,4-DNPH's OF CARBONYL COMPOUNDS
The retention times of n-hexadecane (internal standard) (a) and of the 2,4-DNPH of butan-2-one (b) were taken as unity.

No.	*Carbonyl compound*	r^a	r^b
1	Formaldehyde	1.71	0.67
2	Acetaldehyde	2.06 (2.01)*	0.82 (0.79)*
3	Prop-2-enal	2.29	0.90
4	Acetone	2.31	0.91
5	Propanal	2.33	0.92
6	2-Methylpropanal	2.45	0.96
7	Butan-2-one	2.54	1.00
8	n-Butanal	2.58	1.02
9	3-Hydroxybutan-2-one (acetoin)	2.63	1.03
10	3-Methylbutanal	2.72	1.07
11	Pentan-2-one	2.76	1.09
12	But-2-enal	2.78	1.10
13	n-Pentanal	2.86	1.12
14	n-Hexanal	3.18	1.25
15	n-Heptanal	3.58	1.41
16	6-Methylhept-5-en-2-one	3.87	1.52
17	5-Methylfurfural	3.96	1.56
18	n-Octanal	4.09	1.61

* Relative retention time of secondary peak.

prepared from Duran glass. The capillaries, 20 m long and 0.3 mm I.D., silylated with a 5:1 (v/v) mixture of hexamethyldisilazane (purum; Fluka, Buchs, Switzerland) and trimethylchlorosilane (puriss.; Fluka) were coated using a 0.6% (w/v) solution of OV-1 (Applied Science Labs., State College, Pa., U.S.A.) in chloroform (guaranteed reagent; Merck) by a static coating procedure of Grob[13]. After injection the column temperature was programmed to rise at a rate of 4°/min from 150° to 250°, after which the column was operated isothermally to the end of the analysis. The temperature of the injection block was 270° and that of the detector 320°. The split ratio between the column and the exit after the injection block was 1:20. The volume of sample injected varied from 0.1 to 0.3 μl. Nitrogen was used as the carrier gas and the make-up gas, at flow-rates of 1.5 and 30 ml/min, respectively.

RESULTS AND DISCUSSION

A typical gas chromatogram of the 2,4-DNPH's of n-alkanals from C_1 to C_3 and of acetone, obtained with the OV-1 capillary column, is shown in Fig. 1. As reported earlier, many of the 2,4-DNPH's give double peaks owing to the existence of isomers[5]. Pias and Gasco[6] could not distinguish the isomers because of the low efficiency of their GLC columns. In this study the resolving power of the column was sufficient to separate these double peaks (Fig. 1). The secondary peak was usually smaller than the main peak, with the exception of acetaldehyde derivative.

The retention times on an OV-1 capillary column of 2,4-DNPH's of 18 carbonyl compounds, relative to the retention time of the derivative of butan-2-one, are presented in Table I. The Table shows that the 2,4-DNPH's of n-alkanals are well

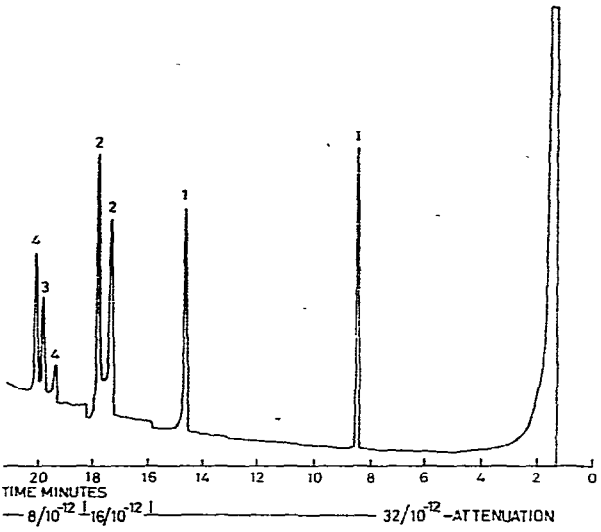

Fig. 1. Gas–liquid chromatogram of a mixture of the 2,4-DNPH's of carbonyl compounds resolved on an OV-1 glass capillary column. The temperature of the column was programmed to rise at a rate of 4°/min from 150° to 250°, after which the column was operated isothermally. 1 = Formaldehyde; 2 = acetaldehyde; 3 = acetone; 4 = propanal; I = hexadecane, internal standard.

separated. Although the number of members of different homologous series was small, it can be seen that the retention times of the 2,4-DNPH's of saturated carbonyl compounds of equal chain length increase in the following order: iso-branched aldehydes < methyl ketones < straight-chain aldehydes. Thus within the series mentioned above,

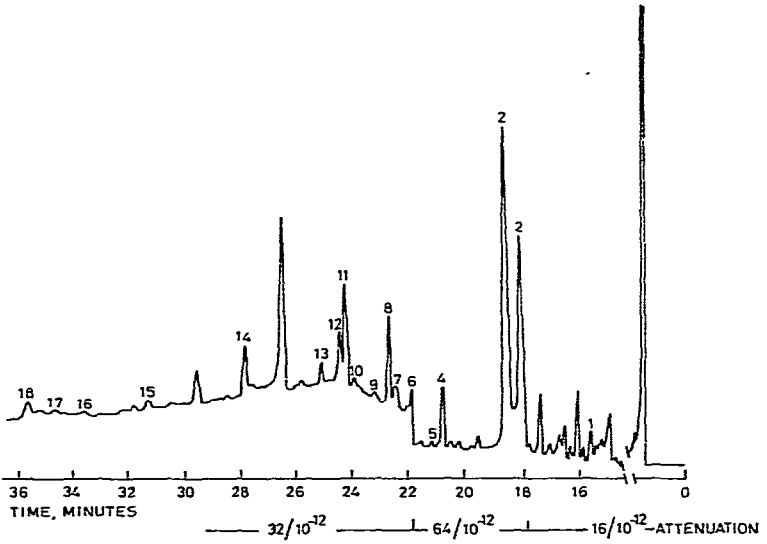

Fig. 2. Gas–liquid chromatogram of the 2,4-DNPH's of carbonyl compounds isolated from the aroma distillate of carrot, carried out under the conditions described in Fig. 1. See Table I for the meaning of the peak numbers.

for example, the members having four and five carbon atoms are resolved clearly on the OV-1 capillary column. Pias and Gasco[6] found a similar order of elution for the 2,4-DNPH's of carbonyl compounds in conventional GLC analyses on packed OV-3 and OV-7 columns. On the other hand, 3-hydroxybutan-2-one (acetoin) was eluted much quicker in our analyses than in those of Pias and Gasco. This is probably due to the difference in the stationary phases. A distinct advantage of GLC analyses on a capillary column is the resolution of the 2,4-DNPH's of prop-2-enal, acetone and propanal, which usually more or less overlap in conventional GLC analyses on packed columns[5,6].

Fig. 2 illustrates the successful application of the capillary column GLC technique to the analysis of 2,4-DNPH derivatives of the volatile carbonyl compounds of carrots. The peaks in the chromatogram are numbered according to the reference compounds in Table I. These compounds had been identified earlier in the volatile constituents of carrots as their 2,4-DNPH's by means of TLC–GLC–MS data[12]. In that study the major component between the peaks 13 and 14 was shown to be methylbutenal. However, most of the derivative peaks overlapped seriously in conventional GLC analysis with packed columns. The only way to analyse them gas-chromatographically was to perform preliminary TLC separation of the 2,4-DNPH's (*cf.* also Kallio and Linko[9]). This is laborious and time-consuming, and according to the results presented here it can easily be avoided by analysing the 2,4-DNPH's of carbonyl compounds on capillary columns.

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