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Note

Separation of saturated and unsaturated aldehyde and ketone 2,4-dinitrophenylhydrazone derivatives by reversed-phase high-performance liquid chromatography*

Gy. VIGH, Z. VARGA-PUCHONY and J. HLAVAY
Institute for Analytical Chemistry, University of Chemical Engineering, Veszprém (Hungary)
and
M. PETRÓ-TURCZA and I. SZÁRFÖLDI-SZALMA
Central Food Research Institute, Budapest (Hungary)
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Aldehydes and ketones are important, often characteristic constituents of various food aromas. Their concentration varies widely in fruit and vegetables.

The individual aldehydes and ketones can be determined only after separation of the components of the mixture. Such separations have been carried out by paper, thin-layer, classical column and gas chromatography. However, since these separations are beyond the scope of this paper they are not reviewed here.

High-performance liquid chromatography (HPLC) has proved suitable for the separation of certain carbonyl compounds. Thus Papa and Turner[1] used a partition system, while Carey and Persinger[2], Honda and Kakehi[3] and Heath *et al.*[4] used adsorption chromatography. Higher members of the aldehyde and ketone series could not be separated in either system. Kikta and Grushka[5], Piergiovanni and Volanterie[6] and Selim[7] used reversed-phase packings and acetonitrile–water as well as methanol–water eluents to separate some 2,4-dinitrophenylhydrazone (DNPH) derivatives of higher members of the *n*-aldehyde and 2-alkanone series. However, retention of the compounds as a function of the eluent composition was not studied in detail.

As we were interested in the carbonyl profile of fresh tomato and tomato products, and reversed-phase HPLC seemed a promising tool for the separation and tentative identification of the components in raw carbonyl aroma fractions preseparated by thin-layer chromatography (TLC)[8], a project was initiated to determine precise retention data (k' *vs.* eluent composition and carbon number) of DNPH derivatives of C_1–C_{12} saturated aldehydes, C_3–C_{11} saturated ketones, C_3–C_7 saturated symmetrical ketones and C_3–C_8 mono-unsaturated straight-chain aldehydes.

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Since the completion of this work and its submission for presentation at the 2nd Danube Symposium[8], Nakamura *et al.*[9] have reported the separation of DNPH derivatives of C_1–C_{10} aldehydes and ketones with methanol–water (4:1), as eluent on a μBondapak C_{18} column. They observed a linear relationship between the logarithm of the net retention volume and the solute carbon number, the line implied being the same for both the aldehyde and ketone DNPHs. Again, retention behaviour as a function of eluent composition was not studied.

EXPERIMENTAL

Chemicals

Saturated straight-chain aldehydes, C_1–C_{12}, *n*-alkan-2-ones, C_1–C_{11}, *n*-alk-2-ene aldehydes, C_3–C_6, diethyl ketone and di-*n*-propyl ketone were obtained from Fluka (Buchs, Switzerland). 2,4-Dinitrophenylhydrazine, methanol, ethanol and hydrochloric acid were analytical grade reagents purchased from Reanal (Budapest, Hungary).

Carbonyl DNPHs were prepared from the respective carbonyl compounds as described in ref. 10, and were purified by repeated recrystallization from ethanol.

Apparatus and procedure

A Waters liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.), 25 cm × 1/4 in. O.D. glass-lined (GLT) columns (SGE, Pty., Melbourne, Australia), a Type LC-55 variable-wavelength detector (Perkin-Elmer, Norwalk, CT, U.S.A. and a Type A25 dual-channel recorder (Varian Aerograph, Walnut Creek, CA, U.S.A.) were used. The GLT columns were slurry packed[11] with 10-μm Nucleosil C_{18} packing (Macherey, Nagel & Co., Düren, G.F.R.), jacketed[12] and thermostatted by a Type U10 circulating water-bath (MLW, Medingen, G.D.R.).

The actual water content of the eluents withdrawn from the inlet manifold of the pump was determined in triplicate by Karl Fisher titration with dead-stop end-point indication. Solutes were freshly dissolved daily in the eluent, since samples kept in transparent vials on the bench at ambient temperature for periods over 6 h displayed "twin" peaks.

The eluent flow-rate was 1.0 cm^3/min. Nine replicate measurements of each standard were made. Column dead volume was determined by injecting sufficient amounts of a 68% (v/v) methanol–water solution. The UV detector was set at 360 nm since most of the compounds studied had a strong and comparatively flat absorption peak in this region[13,14].

RESULTS AND DISCUSSION

The capacity factors of the *n*-aldehyde and 2-alkanone DNPHs determined at 23.8 °C and 26.3 °C, respectively, are shown in Tables I and II. Chromatograms of model mixtures of aldehyde and ketone DNPHs obtained with an eluent of 78.40% (v/v) methanol are shown in Figs. 1 and 2.

By analysing the data in Tables I and II it can be seen that log k' in both series can be described by a linear function of both the carbon number of the alkyl

TABLE I

k' VALUES OF SATURATED STRAIGHT-CHAIN ALDEHYDE DNPHs AT 23.8°C ON A NUCLEOSIL C_{18} COLUMN (NINE REPLICATES)

Errors are standard deviations.

C_n	*Eluent methanol concn. (% v/v)*				
	89.11 ± 0.43	*84.24 ± 0.1*	*78.40 ± 0.37*	*73.66 ± 0.31*	*68.23 ± 0.29*
C_1	0.38 ± 0.01	0.56 ± 0.01	0.90 ± 0.02	1.23 ± 0.03	1.94 ± 0.03
C_2	0.56 ± 0.01	0.82 ± 0.01	1.27 ± 0.03	1.74 ± 0.03	2.87 ± 0.05
C_3	0.70 ± 0.01	1.07 ± 0.01	1.81 ± 0.02	2.65 ± 0.02	4.65 ± 0.08
C_4	0.85 ± 0.01	1.35 ± 0.01	2.47 ± 0.04	3.80 ± 0.06	7.12 ± 0.10
C_5	1.06 ± 0.01	1.77 ± 0.02	3.48 ± 0.04	5.72 ± 0.11	11.41 ± 0.23
C_6	1.35 ± 0.02	2.36 ± 0.03	5.03 ± 0.08	8.69 ± 0.19	18.69 ± 0.38
C_7	1.73 ± 0.02	3.20 ± 0.04	7.32 ± 0.13	13.39 ± 0.28	—
C_8	2.25 ± 0.02	4.36 ± 0.08	10.85 ± 0.29	20.83 ± 0.39	—
C_9	2.93 ± 0.03	5.98 ± 0.12	16.03 ± 0.43	32.54 ± 0.65	—
C_{10}	3.82 ± 0.07	8.26 ± 0.18	23.82 ± 0.78	50.9 ± 2.2	—
C_{12}	6.58 ± 0.11	15.87 ± 0.47	52.9 ± 2.1	—	—

TABLE II

k' VALUES OF 2-*n*-ALKANONE DNPH STANDARDS AT 26.3°C

Details as in Table I.

C_n	*Eluent methanol concn. (% v/v)*				
	89.11 ± 0.43	*84.24 ± 0.1*	*78.40 ± 0.37*	*73.66 ± 0.31*	*68.23 ± 0.29*
C_3	0.73 ± 0.01	1.12 ± 0.01	1.87 ± 0.04	2.72 ± 0.02	4.26 ± 0.05
C_4	0.94 ± 0.01	1.52 ± 0.01	2.77 ± 0.05	3.85 ± 0.03	7.20 ± 0.08
C_5	1.13 ± 0.01	1.91 ± 0.01	3.75 ± 0.08	6.05 ± 0.04	10.82 ± 0.11
C_6	1.41 ± 0.01	2.53 ± 0.01	5.28 ± 0.09	9.11 ± 0.06	17.85 ± 0.17
C_7	1.79 ± 0.01	3.38 ± 0.02	7.63 ± 0.14	13.94 ± 0.09	—
C_8	2.30 ± 0.01	4.60 ± 0.02	11.14 ± 0.21	21.55 ± 0.14	—
C_{11}	5.11 ± 0.04	12.07 ± 0.05	36.19 ± 0.69	—	—

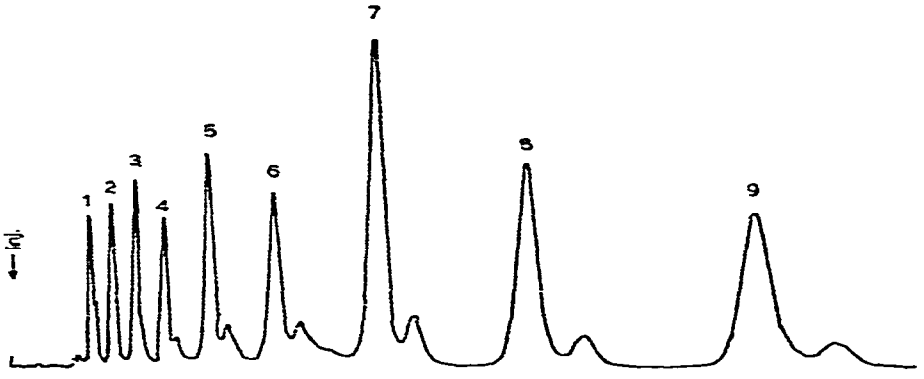

Fig. 1. Separation of a *n*-aldehyde DNPH model mixture with an eluent of 78.40% (v/v) methanol. Peaks: 1–9 = C_1–C_9 aldehyde DNPHs; small peaks eluted after the standards ("twins") are formed in the methanol solution upon standing.

Fig. 2. Separation of a *n*-alkan-2-one DNPH model mixture with an eluent of 78.40% (v/v) methanol. Peaks: 1–6 = C_3–C_8 ketone DNPH standards.

chain and the methanol content of the eluent. In the range studied, a computerized least-squares fitting method gives for the *n*-aldehyde DNPHs

$$\log k' = 1.609 + 0.555\,C_n - (0.0239 + 0.00500\,C_n)\,[\mathrm{MeOH}]$$

and for the 2-alkanone DNPHs:

$$\log k' = 1.352 + 0.582\,C_n - (0.0203 + 0.00538\,C_n)\,[\mathrm{MeOH}]$$

where [MeOH] is the methanol content of the eluent in %(v/v).

Calculated and measured data agree well. The data and the results of this analysis contradict the implications of Fig. 5 in ref. 9, *i.e.*, ketone and aldehyde DNPHs have identical k' values. The equations obtained for the aldehyde and ketone DNPHs differ sufficiently indicating the possibility of at least a partial separation of the equal-carbon-number aldehyde and ketone DNPH pairs. This chromatogram is shown in Fig. 3. The separation selectivity, $\alpha_{\mathrm{ketone/aldehyde}}$, decreases with increasing carbon number.

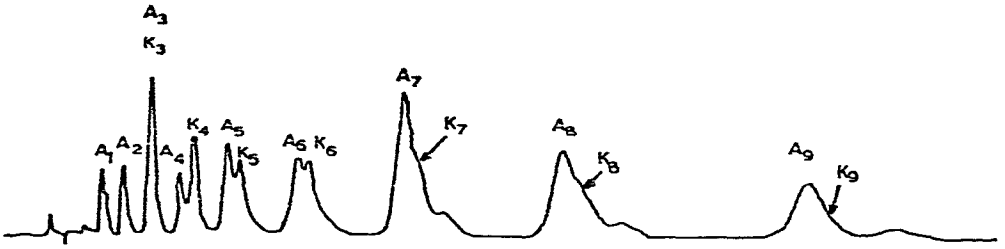

Fig. 3. Separation of *n*-aldehyde and ketone DNPH standards with an eluent of 78.40% (v/v) methanol at 25.0°C.

The comparative elution orders at 20.0 °C of *sym*-*n*-alkyl ketone, *n*-alkyl ketone, *n*-alkyl aldehyde and *n*-alk-2-ene aldehyde DNPH standards were also studied briefly. The k' values are shown in Table III. In the case of chains of equal carbon numbers the elution order is: unsaturated aldehyde < *n*-aldehyde < *n*-ketone < *sym*-ketone. Following the solvophobic retention theory of Horváth *et al.*[15], this order can be readily explained by assuming that the net decrease of the hydrophobic surface area brought about by the association of the aliphatic chain of the solute and the C_{18} moiety on the surface of the packing increases in the order *n*-aldehyde < *n*-ketone < *sym*-ketone DNPH, with the more hydrophilic dinitrophenylhydrazone part

TABLE III

k' VALUES OF DNPHs AT 20.0°C

Eluent, 78.40% (v/v) methanol; Nucleosil C_{18} column (nine replicates).

C_n	*sym-Ketone*	*Alkane-2-one*	*n-Aldehyde*	*n-Alk-2-ene aldehyde*
3	2.23 ± 0.01	2.23 ± 0.01	2.13 ± 0.02	1.90 ± 0.02
4	—	3.29 ± 0.01	2.91 ± 0.02	2.83 ± 0.02
5	4.79 ± 0.04	4.47 ± 0.01	4.16 ± 0.01	3.98 ± 0.02
6	—	6.40 ± 0.01	6.05 ± 0.02	5.61 ± 0.03
7	9.57 ± 0.09	9.38 ± 0.02	8.81 ± 0.03	8.18 ± 0.03
8	—	13.94 ± 0.04	13.32 ± 0.05	11.95 ± 0.06

"sticking out" toward the bulk of the eluent. The elution order of the unsaturated aldehyde–saturated aldehyde DNPH pairs also agrees with former general conclusions (*e.g.*, in ref. 16) that unsaturation in the chain decreases the retention volume.

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