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# Isotope effect in gas–liquid chromatography of labelled compounds

M. Matucha*

*Institute of Nuclear Biology and Radiochemistry, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague (Czechoslovakia)*

W. Jockisch

*Central Institute of Isotope and Radiation Research, Permoserstr. 15, O-0705 Leipzig (Germany)*

P. Verner

*Diagnostic Centre for Inherited Metabolic Disorders, Charles University, Karlovo nám. 32, 120 00 Prague 2 (Czechoslovakia)*

G. Anders

*Central Institute of Organic Chemistry, Permoserstr. 15, O-0705 Leipzig (Germany)*

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ABSTRACT

The isotope effect in the gas–liquid chromatography (GLC) of $^{14}C$- and $^{2}H$-labelled compounds was examined experimentally and theoretically. Capillary column gas chromatographic–mass spectrometric (GC–MS) data indicated that the isotope effect was very small for $^{14}C$-labelled higher fatty acids and large for perdeuterated *n*-alkanes. Measurement of the temperature dependence of the corrected retention volumes and separation factors of $C_{15}$–$C_{17}$ *n*-alkanes and their perdeuterated analogues yielded a relationship between the molar volume, enthalpy change and chromatographic retention: heavier isotopomers having lower molar volumes are eluted earlier when Van der Waals dispersion forces plays a dominant role in the solute–stationary phase interaction. The effect is proportional to the number of heavier atoms in the molecule and should be taken into account in the GLC and GC–MS of isotopically substituted compounds on efficient capillary columns.

## INTRODUCTION

Labelled (isotopically modified) compounds are often used in organic and analytical chemistry and in biochemistry and other life sciences as a powerful tool to trace the path of compounds of interest and/or to investigate their behaviour and transformations. One of important analytical methods used in applications of labelled compounds is gas chromatography: radio-gas chromatography (RGC) for radioactively labelled compounds and gas chromatography–mass spectrometry (GC–MS) for compounds substituted with stable isotopes. Relatively little attention has been paid to the theory of isotope effects in the gas–liquid chromatography (GLC) of labelled compounds, but based on earlier studies by others on chromatographic solute–stationary phase interactions [1,2] we have considered further the chromatographic behaviour of isotopic species.

In GC, the retention volumes of organic compounds labelled with deuterium or tritium have often been found to differ from those of isotopically unmodified substances [3–17]; the separation of

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$^{12}C$-, $^{13}C$- and $^{14}C$-isotopomers of methane by means of capillary gas–solid chromatography (GSC) was found to be low [16], whereas in GLC no isotope effect of $^{14}C$-labelled compounds has so far been reported. In the RGC of tritium-labelled compounds, "radioactivity was detected earlier than mass" [12,13]. Isotope effects in GC have been well studied in the past, but the data obtained were mostly evaluated according to the thermodynamics of the chromatographic process only and no clear theoretical conclusions were made [8–10].

A situation when lighter isotopomers are eluted earlier (in GSC at lower temperatures) is called the normal isotope effect. The "inverse" isotope effect was mostly found in GLC [3–17]; it was explained several years ago as being due to shorter internuclear carbon –hydrogen distances resulting in slightly lower molar volumes and boiling points of perdeuterated compounds [17]. The size and shape of the solute molecules, not mass differences as such, played a significant role [18].

In the GC–MS literature an inverse effect is often reported [19,20] and it is sometimes denoted a "chromatographic isotope effect" [20]. The introduction of several deuterium atoms into a small molecule usually results in a significantly decreased retention time in GC [21]. A recent report concerns the isotope effect in the "chromato-mass spectrometry" of deuterium-substituted amino acids [22] and also in the GC of low-boiling isotopic molecules on PLOT columns [23].

A very small isotope effect was observed in the GLC of methyl esters of [G-$^{14}C$]fatty acids obtained by biosynthesis, in contrast to that of perdeuterated *n*-alkanes of the same mass difference with respect to their isotopically unmodified analogues [18]. We confirmed the earlier hypothesis that the peak broadening of methyl esters of [G-$^{14}C$]fatty acids on highly efficient capillary columns is caused by overlapping of their isotopomers [24] using GC–MS. However, the method used did not permit us to determine the magnitude of the isotope effect.

Much attention has recently been paid to the relationship between structure and chromatographic retention [1,2]. One of the results is the linear relationship between the enthalpy change (heat of solution in the stationary phase) and the molar volume of the solute [2], which in our opinion can also be extended to isotopic species. The conclusions in recent papers on isotope effects in GC are that heavier isotopomers have smaller sizes or volumes [16,23]. Many years ago, GLC was suggested as a method for investigating isotope effects in solution to gain a fuller understanding of the intermolecular forces in solution [7], which is in accord with the new approaches [1,2]. However, also in older studies of isotope effects on thermodynamic properties of isotopic molecules in condensed systems the influence of isotopic substitution, molecular structure and geometry, molar volume and also polarizability were noticed [25,26]. Hence all factors of the chromatographic solute–stationary phase interaction should be considered.

## EXPERIMENTAL

### *Chemicals*

Methyl palmitate and *n*-hexane (analytical-reagent grade) were purchased from Merck (Darmstadt, Germany), [G-$^{14}C$]palmitic acid with a specific acitivity of 720 mCi/mmol (71,9% $^{14}C$) from the Institute for Research, Production and Application of Radioisotopes (Prague, Czechoslovakia), a mixture of $C_{15}$–$C_{17}$ *n*-alkanes, fully substituted with deuterium, from the Zentralinstitut für Isotopen- und Strahlungsforschung (Leipzig, Germany) and unsubstituted *n*-alkanes from Supelco (Bellefonte, PA, USA).

### *GC–MS analysis*

A Model 1020 B mass spectrometer (Finnigan, San Jose, CA, USA) coupled with a Sigma 3B gas chromatograph (Perkin-Elmer, Norwalk, CT, USA) was used under the following conditions: 12 m × 0.10 mm I.D. fused-silica open tubular (FSOT) column coated with BP-1 polydimethylsiloxane stationary phase (crosslinked and bonded, film thickness 0.1 μm) (SGE, Victoria, Australia), injector in the split mode, splitting ratio 1:100, operated at 230°C, oven temperature 165°C, helium as carrier gas, flow-rate 50 cm $s^{-1}$, GC–MS interface direct inlet at 260°C, manifold temperature 70°C and electron energy 70 eV.

GC of *n*-alkanes was performed on a Hewlett-Packard Model 5890 gas chromatograph on a 50 m × 0.25 mm I.D. FSOT column coated with PS-255 polydimethylsiloxane phase (cross-linked), film

thickness 0.2 $\mu$m, injector in the split mode, splitting ratio 1:100, carrier gas (hydrogen) linear velocity 40–50 cm $s^{-1}$, in the isothermal mode at temperatures between 120 and 200°C.

## RESULTS AND DISCUSSION

The peak broadening of methyl esters of [G-$^{14}$C]palmitic and oleic acid on highly efficient capillary column [24] and the knowledge of the mechanism of fatty acid biosynthesis [27–29] led us to investigate the causes of this effect by GC–MS; the putative cause was overlapping of the peaks of individual isotopomers contained in the [G-$^{14}$C]palmitic acid preparation. Because of the low intensity of molecular ions in the electron impact (EI) mass spectra of the methyl ester of [G-$^{14}$C]palmitic acid of high specific activity (the isotopic abundance of $^{14}$C was higher than 70%, *i.e.*, the preparation represented a rich mixture of isotopomers formed during biosynthesis), the isotope effect was not completely distinct [18]. The situation is clear from Fig. 1. A molecular ion of the non-radioactive methyl ester of palmitic acid having its origin in the non-radioactive inoculum is found at *m/z* 270, and the cluster of molecular ions of heavier radioactive isotopomers lies in the region *m/z* 280–302 with a maximum at *m/z* 298. The intensities were found to correspond to the specific acitivity determined by liquid scintillation counting and GC.

Mass chromatograms of molecular ions belonging to individual isotopomers were, however, of low intensity, showing only that the heavier isotopom-

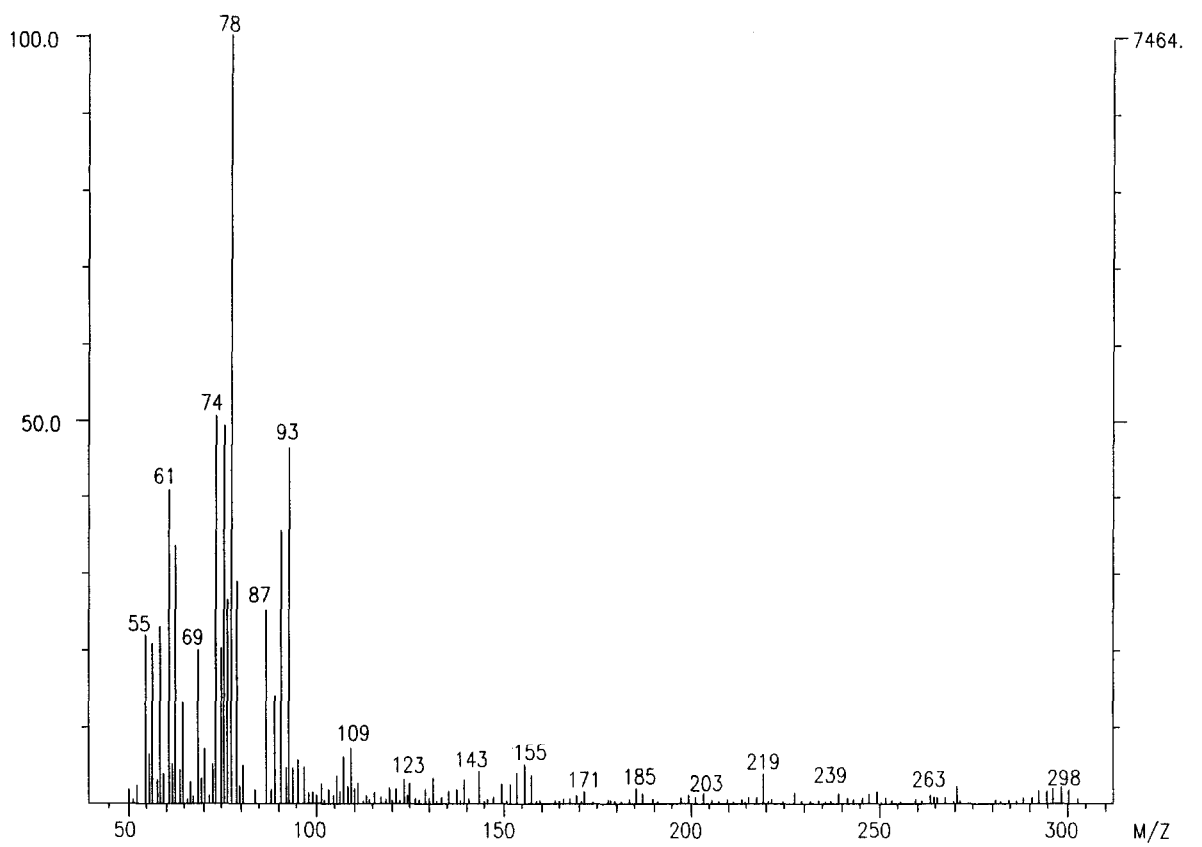


Fig. 1. EI mass spectrum of methyl ester of [G-$^{14}$C]palmitic acid at its peak maximum during GC–MS on a non-polar stationary phase. The molecular ions of *m/z* 270–302 confirm the presence of isotopomers; the mass peak at *m/z* 302 corresponds to palmitic acid containing only $^{14}$C atoms. The highest intensities belong to the $^{14}$C-fragments at *m/z* 78 and 93.

ers are eluted earlier [18]. The intensity of methylated $C_2$- and $C_3$-fragments of palmitic acid at *m/z* 74 and 87 is substantially higher than that of the other mass peaks in the spectrum. This can be also seen in Fig. 1, where the peaks at *m/z* 78 and 93 correspond to the $^{14}C$ fragments. The isotope effect in GLC on the capillary column used is then clear from the mass chromatograms of the fragments shown in Figs. 2 and 3, its magnitude being about 3 s. We must consider, however, that the fragments recorded in Figs. 2 and 3 do not originate from one isotopomer only, but that the position of the maxima agree relatively well with the position of the maxima of isotopomers of the highest content, *i.e.*, of [$^{12}C_{16}$]- and [$^{14}C_{14}$]palmitic acid methyl ester of molecular weights 270 and 298, respectively. At any rate, the isotope effect of larger molecules substituted with $^{14}C$ in GLC was confirmed; the heavier isotopomers are eluted earlier, which corresponds to their lower molar volumes [1,2] (and to the smaller size of the $^{14}C$ atom), as is apparent from the mass chromatograms in Figs. 2 and 3. Hence the advances of capillary GC are evident in the light of the older prediction about the impossibility of the GC separation of $^{14}C$-labelled species from the corresponding non-radioactive standards [30].

Also, because of very small differences in the chemical and chromatographic behaviour of $^{14}C$-labelled compounds and their unlabelled analogues, these compounds can be well recommended for chemical and biological research.

The isotope effect was also studied in more detail on perdeuterated *n*-alkanes in GLC on a capillary column with an efficiency higher than 200 000 plates for every species. An example of the separation of the isotopic species is shown in Fig. 4. All the data obtained on a capillary with a cross-linked polydimethylsiloxane stationary phase are given in

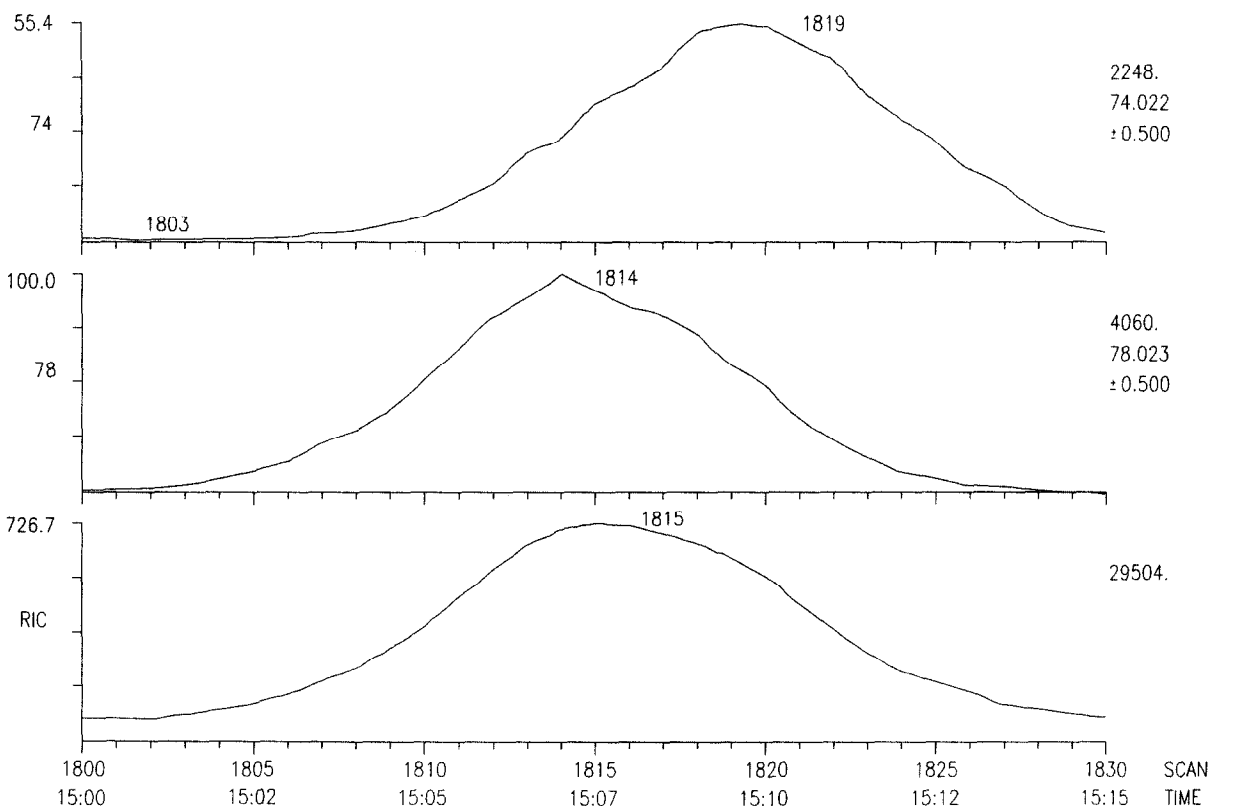


Fig. 2. Total ion and mass chromatograms at *m/z* 74 and 78 of isotopically unmodified and $^{14}C$-fragments $CH_2 = C(OH)OCH_3^+$ of the methyl ester of [G-$^{14}C$]palmitic acid. Time in min:s.

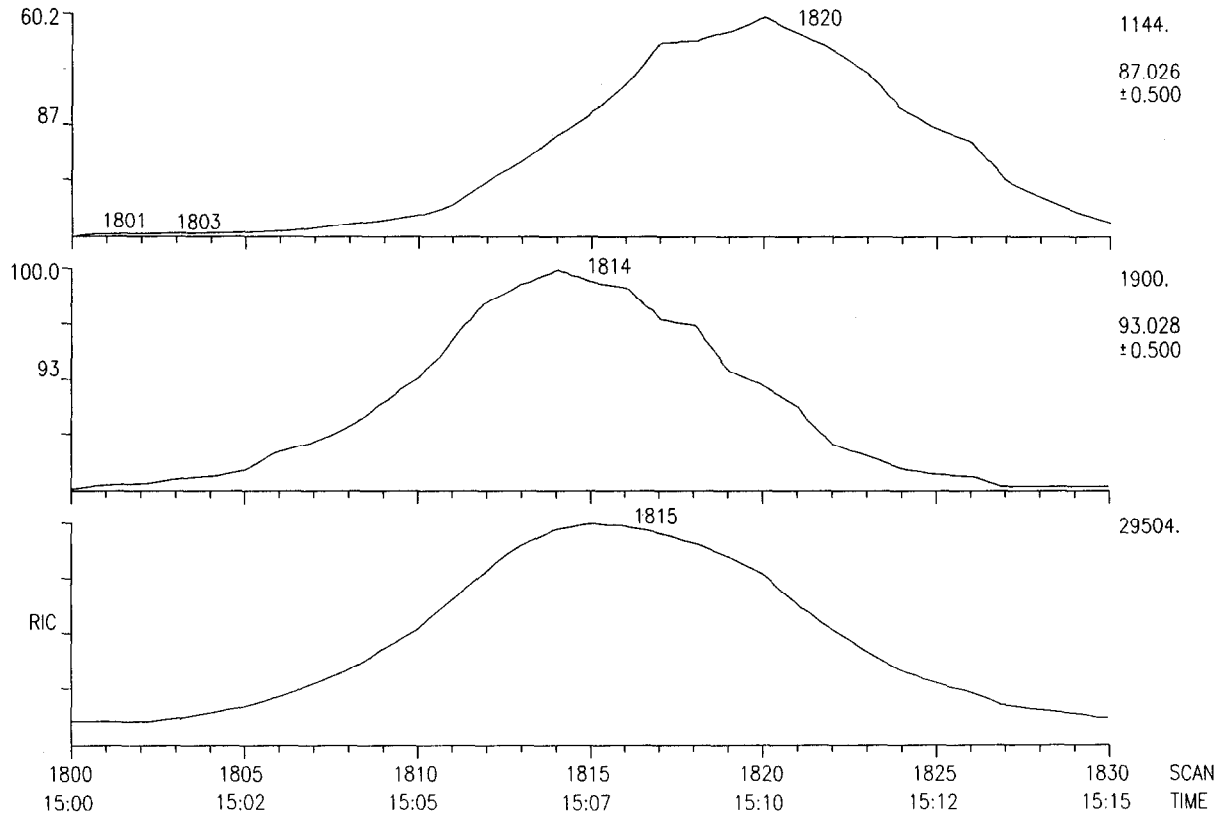


Fig. 3. Total ion and mass chromatograms at $m/z$ 87 and 93 of isotopically unmodified and $^{14}C$-fragments $CH_3OC(OH) = CHCH_2^+$ of the methyl ester of [G-$^{14}C$]palmitic acid. Time in min:s.

Table I. From these values one may calculate the differences in the enthalpy and entropy changes of the isotopomers (see Table II). The differences in enthalpy changes, $\Delta(\Delta H)$, are considerable and show a lower interaction of perdeuterated species with the stationary phase (owing to a larger number of deuterium atoms in $C_{15}$–$C_{17}$ *n*-alkane molecules) in comparison with the values of about $-60$ cal mol$^{-1}$ for molecules with a smaller number of deuterium atoms [8]. Also, the values of $(r_{12}-1)/N_D$ in Table I are interesting and confirm the earlier statements that the contribution to the value $r_{12}-1$ is approximately proportional to the number of $^2H$ atoms in the molecule [6,14]. In agreement with the results of Bermejo *et al.* [17], the separation factors depend on molar volumes, *i.e.*, on internuclear distances, as it was already claimed by Krumbiegel [15].

The "inverse" isotope effect was also found during a GC–MS investigation of the catalytic exchange of hydrogen with deuterium in desipramine [31], a compound with methylaminopropyl group ($C_{18}H_{22}N_2$); five isotopomers ($^2H_0$, $^2H_1$, $^2H_2$, $^2H_3$ and $^2H_4$) were determined in the mixture, which formed also a broader peak than the isotopically unmodified standard. Similarly to [G-$^{14}C$]palmitic acid, the mass chromatograms in Fig. 5 show that the heavier species are again eluted earlier (the relatively large peak broadening was probably caused by the relatively low temperature of the ion source of the mass spectrometer).

A comparison of the isotope effect in the GLC of isotopically substituted compounds suggests a *ca.* 30-fold higher separation factors for perdeuterated $C_{15}$–$C_{17}$ *n*-alkanes as compared with [$^{14}C$]palmitic acid methyl ester with the same mass difference.

TABLE I

GLC DATA FOR PERDEUTERATED $C_{15}$–$C_{17}$ *n*-ALKANES WITH ISOTOPICALLY UNMODIFIED $C_{14}$–$C_{17}$ STANDARDS USING A 50 m × 0.25 mm I.D. FSOT COLUMN COATED WITH PS-255 CROSS-LINKED POLYDIMETHYLSILOXANE OF $d_f$ 0.2 μm

$t_r$ = Uncorrected retention time; $r_{12}$ = separation factor; $w_{1/2}$ = peak width at half-heigth; $R$ = resolution; $I$ = Kováts retention index; $N_D$ = number of deuterium atoms in molecule.

| Conditions | *n*-Alkane | $t_r$ | $w_{1/2}$ | $r_{12}$ | Log $r_{12}$ | $R$ | $I$ | $\frac{r_{12}-1}{N_D}$ |
|---|---|---|---|---|---|---|---|---|
| 120°C, $t_d$ = 1.354 min | $[^1H]C_{14}$ | 15.338 | 0.089 | | | | | |
| | $[^2H]C_{15}$ | 22.793 | 0.154 | 1.1256 | 0.0514 | 10.23 | 1478.3 | 0.00393 |
| | $[^1H]C_{15}$ | 25.486 | 0.156 | | | | | |
| | $[^2H]C_{16}$ | 38.326 | 0.291 | 1.1314 | 0.0536 | 10.14 | 1577.6 | 0.00386 |
| | $[^1H]C_{16}$ | 43.185 | 0.273 | | | | | |
| | $[^2H]C_{17}$ | 64.721 | 0.453 | 1.1443 | 0.0585 | 14.07 | 1680,2 | 0.00401 |
| | $[^1H]C_{17}$ | 73.865 | 0.312 | | | | | |
| 140°C, $t_d$ = 1.699 min | $[^1H]C_{14}$ | 8.134 | 0.044 | | | | | |
| | $[^2H]C_{15}$ | 11.104 | 0.068 | 1.1154 | 0.0474 | 9.25 | 1477.7 | 0.00361 |
| | $[^1H]C_{15}$ | 12.189 | 0.070 | | | | | |
| | $[^2H]C_{16}$ | 17.026 | 0.126 | 1.1203 | 0.0493 | 8.93 | 1576.9 | 0.00354 |
| | $[^1H]C_{16}$ | 18.870 | 0.117 | | | | | |
| | $[^2H]C_{17}$ | 26.509 | 0.181 | 1.1329 | 0.0542 | 10.49 | 1674.7 | 0.00369 |
| | $[^1H]C_{17}$ | 29.806 | 0.189 | | | | | |
| 160°C, $t_d$ = 1.877 min | $[^1H]C_{14}$ | 5.173 | 0.028 | | | | | |
| | $[^2H]C_{15}$ | 6.489 | 0.039 | 1.1045 | 0.0432 | 7.27 | 1477.2 | 0.00327 |
| | $[^1H]C_{15}$ | 6.971 | 0.039 | | | | | |
| | $[^2H]C_{16}$ | 9.009 | 0.059 | 1.1095 | 0.0451 | 7.99 | 1576.4 | 0.00322 |
| | $[^1H]C_{16}$ | 9.790 | 0.056 | | | | | |
| | $[^2H]C_{17}$ | 12.840 | 0.081 | 1.1212 | 0.0497 | 9.42 | 1674.0 | 0.00337 |
| | $[^1H]C_{17}$ | 14.169 | 0.085 | | | | | |
| 180°C, $t_d$ = 2.032 min | $[^1H]C_{14}$ | 3.838 | 0.020 | | | | | |
| | $[^2H]C_{15}$ | 4.472 | 0.024 | 1.0959 | 0.0398 | 5.74 | 1476.7 | 0.00300 |
| | $[^1H]C_{15}$ | 4.706 | 0.024 | | | | | |
| | $[^2H]C_{16}$ | 5.640 | 0.032 | 1.1012 | 0.0419 | 6.82 | 1575.7 | 0.00300 |
| | $[^1H]C_{16}$ | 6.005 | 0.031 | | | | | |
| | $[^2H]C_{17}$ | 7.350 | 0.042 | 1.1100 | 0.0453 | 8.20 | 1673.6 | 0.00310 |
| | $[^1H]C_{17}$ | 7.935 | 0.042 | | | | | |
| 200°C, $t_d$ = 2.124 min | $[^1H]C_{14}$ | 3.184 | 0.016 | | | | | |
| | $[^2H]C_{15}$ | 3.515 | 0.019 | 1.0897 | 0.0373 | 3.87 | 1476.0 | 0.00280 |
| | $[^1H]C_{15}$ | 3.640 | 0.019 | | | | | |
| | $[^2H]C_{16}$ | 4.110 | 0.023 | 1.0932 | 0.0387 | 4.73 | 1575.2 | 0.00274 |
| | $[^1H]C_{16}$ | 4.295 | 0.023 | | | | | |
| | $[^2H]C_{17}$ | 4.946 | 0.028 | 1.1017 | 0.0421 | 6.03 | 1673.0 | 0.00283 |
| | $[^1H]C_{17}$ | 5.233 | 0.028 | | | | | |

This is caused by the substantially lower molar volumes of compounds with peripheral deuterium atoms relative to the molar volume of compounds with skeletal $^{14}C$ atoms; the latter compounds exhibit a smaller decrease in their size compared with the hydrogen isotopes.

The interactions encountered in GLC differ from those appearing in GSC; they include Van der

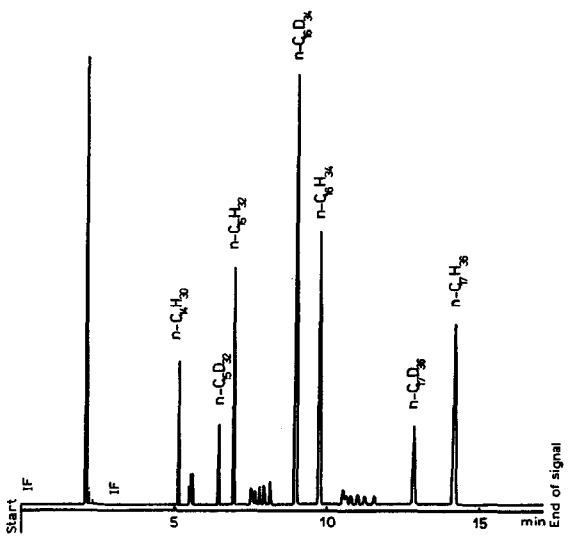


Fig. 4. Gas chromatogram of perdeuterated $C_{15}$–$C_{17}$ *n*-alkanes with isotopically unmodified $C_{14}$–$C_{17}$ standards on a FSOT column coated with PS-255 cross-linked polydimethylsiloxane (50 m × 0.25 mm I.D., $d_f$ 0.20 μm) at 160°C and carrier gas (hydrogen) linear flow-rate 44 cm $s^{-1}$.

Waals dispersion forces, dipole–dipole interactions, influence of hydrogen bonds and polarizability [1], and therefore also isotope effects have a different character. In accordance with the enthalpy–molar volume–chromatographic retention relationship [2], the heavier isotopomers were found to have lower retentions, especially when dispersion forces played a dominant role.

## CONCLUSIONS

The isotope effect in the GLC of $^{14}C$-labelled compounds has been confirmed; it is small but measurable on highly efficient capillary columns.

Deuterated compounds, in comparison with $^{14}C$-labelled species, exhibit much larger effects, which are caused by substantially lower molar volumes, and are proportional to the number of deuterium atoms in the molecules.

The isotope effects in the GLC of labelled compounds should be taken into account in RGC and in GC–MS; heavier isotopomers are eluted earlier,

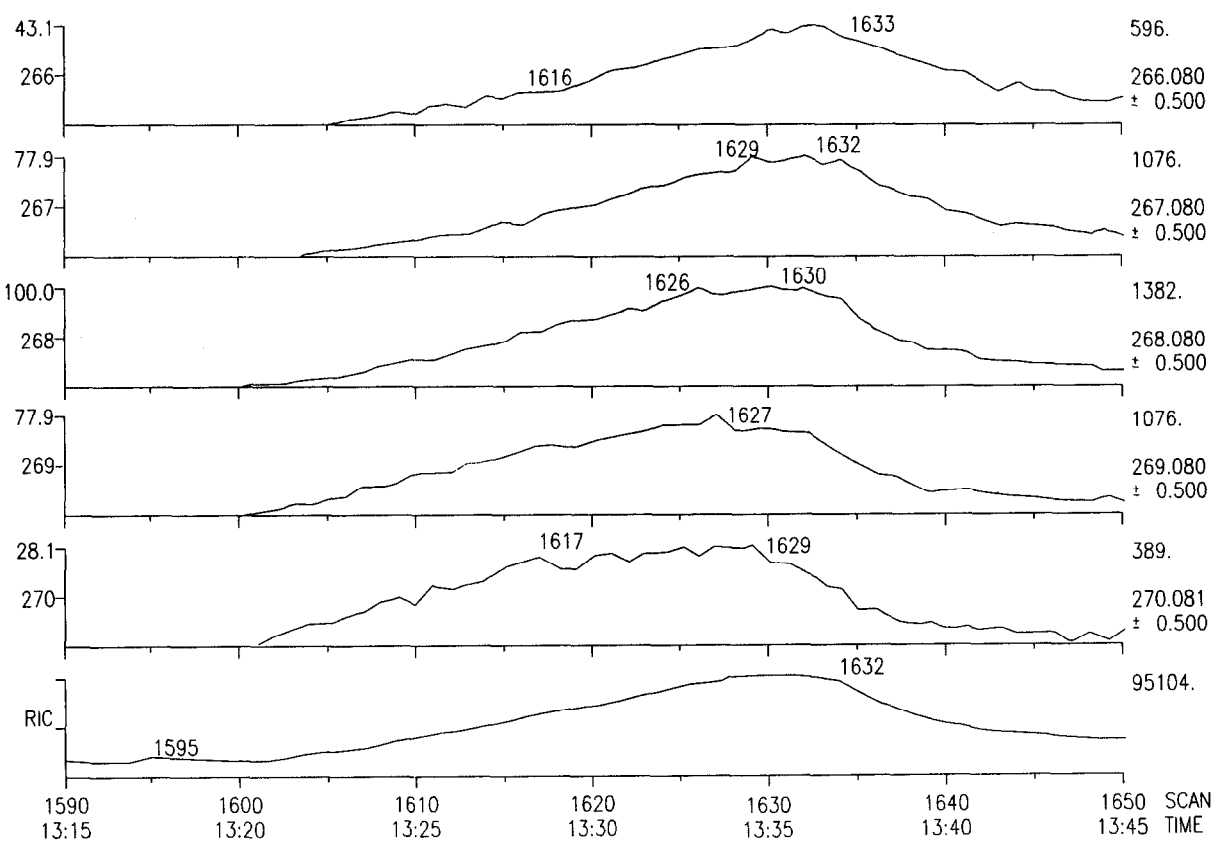


Fig. 5. Total and molecular ion chromatograms of catalytically deuterated desipramine, *i.e.*, of its $^2H_0$, $^2H_1$, $^2H_2$, $^2H_3$ and $^2H_4$ isotopomers. Time in min:s.

TABLE II

DIFFERENCES IN THE ENTHALPY AND ENTROPY CHANGES BETWEEN *n*-ALKANES AND THEIR PERDEUTERATED ANALOGUES CORRESPONDING TO THE CHROMATOGRAPHIC INTERACTION WITH POLYDIMETHYLSILOXANE PS-255

$R$ = Correlation coefficient.

| *n*-Alkane pair | $\Delta(\Delta H)$ (cal mol$^{-1}$) | $\Delta(\Delta S)$ (cal K$^{-1}$ mol$^{-1}$) | $R$ |
|---|---|---|---|
| *n*-$C_{15}$ | −152.6 | −0.1534 | 0.9974 |
| *n*-$C_{16}$ | −158.5 | −0.1583 | 0.9992 |
| *n*-$C_{17}$ | −177.4 | −0.1827 | 0.9984 |

predominantly when the solute–stationary phase interaction is due to dispersion forces.

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