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Short Communication

Unusual deuterium isotope effect on the retention of formamides in gas–liquid chromatography

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ABSTRACT

The deuterium isotope effect on gas chromatographic retention of a series of N,N-dimethylformamide, N-methylformamide and formamide isotopomers was studied on methyl and phenyl methyl polysiloxane and polyethylene glycol stationary phases. The deuterium isotope effect was substantially affected by the position of deuterium in the molecule. Whereas methyl-deuterated $(C^2H_3)_2NCHO$ and C^2H_3NHCHO eluted, as expected, earlier than their non-labelled analogues, elution of formyl-deuterated $(CH_3)_2NC^2HO$, CH_3NHC^2HO and NH_2C^2HO was always delayed. The presence of chemically inequivalent deuterium atoms in $(C^2H_3)_2NC^2HO$ and $C^2H_3NHC^2HO$ resulted in final deuterium isotope effects to which the contributions of the individual groups were additive. The elution order of the individual isotopomers was not affected by the polarity of the stationary phase. Interpretation of the deuterium isotope effect observed is provided by the theory of vapour pressure isotope effects.

INTRODUCTION

Changes in the chromatographic retention of molecules with different isotopic composition have often been noted. Perhaps most reports deal with the effect of replacing ^1H by ^2H (deuterium) (for review, see ref. 1). The great majority of reported gas chromatographic (GC) separations result in earlier elution of heavier ^2H-labelled compounds, which is referred to as the inverse isotope effect [1]. Accordingly, in a series of isotopomers differing by a number of deuterium atoms within some structural unit, *e.g.* in an alkyl group [2,3] or on an aromatic ring [4], the compounds are eluted in the order of decreasing number of deuterium atoms. The normal isotope effect, *i.e.* later elution of heavier species, is observed mainly in absorption GC (GSC), for example on alkali-etched glass capillary columns [5,6]. Examples of normal isotope

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CH_3CH_3NHCHO	d_0-DMF	CH_3NHCHO	d_0-MF
$CH_3CH_3NHC^2HO$	d_1-DMF	CH_3NHC^2HO	d_1-MF
$CH_3C^2H_3NHCHO$	d_3-DMF	C^2H_3NHCHO	d_3-MF
$C^2H_3C^2H_3NHCHO$	d_6-DMF	$C^2H_3NHC^2HO$	d_4-MF
$C^2H_3C^2H_3NHC^2HO$	d_7-DMF		
		NH_2CHO	d_0-F
		NH_2C^2HO	d_1-F

Fig. 1. Structures of DMF, MF and F isotopomers.

effects in partition GC (GLC) are very scarce [3,5].

In this paper we report on an unusual observation, namely that the deuterium isotope effect on GC retention may be substantially affected by the position of deuterium atoms in the molecule. The observation is discussed in terms of the theory of vapour pressure isotope effects.

EXPERIMENTAL

Chemicals

Unlabelled N,N-dimethylformamide (d_0-DMF), N-methylformamide (d_0-MF) and formamide (d_0-F) were bought from Aldrich (Gillingham, UK), d_7-DMF was bought from Sigma (Poole, UK). Compounds d_6-DMF, d_3-DMF and d_3-MF were prepared from d_6-dimethylamine hydrochloride (Sigma), d_3-dimethylamine hydrochloride (a gift from Dr. R.P. Hanzlik, University of Kansas) and d_3-methylamine hydrochloride (Sigma), respectively, and ethyl formate [7]. By analogy, d_1-DMF, d_1-MF and d_1-F were prepared from the respective amine hydrochlorides and d_1-methyl formate (Aldrich). Compound d_4-MF was prepared from d_3-methylamine hydrochloride and d_1-methyl formate. N,N-Dimethylacetamide (DMA) was bought from Aldrich; propyl-N-methylcarbamate was prepared as described previously [8]. For structures of DMF, MF and F isotopomers see Fig. 1.

GC analysis

Retention times were measured on an HP-5890 A gas chromatograph with 3394 A integrator. The fused-silica capillary columns used were HP-20M (polyethylene glycol 20M), 25 m × 0.32 mm I.D., 0.3 μm film thickness, and HP-50+ (cross-linked 50% phenyl methyl silicone gum), 15 m × 0.53 mm I.D., 1.0 μm film thickness, both from Hewlett-Packard, and DB-1 (cross-linked 100% methyl silicone gum), 15 m × 0.53 mm I.D., 3.0 μm film thickness, from J & W Scientific. The injector was in a split mode with a 1:30 splitting ratio, and the nitrogen-selective detector was used. Helium was employed as carrier gas. Experimental conditions of the measurements and informative values of dead and retention times are shown in Table I.

A typical sample was a solution of a single isotopomer (1 m*M*) and an internal standard (1 m*M*) in acetone (1 μl). The internal standards were DMA for DMF, propyl-N-methylcarbamate (on HP-20M) or DMA (on HP-50+ and DB-1) for MF, and MF for F.

TABLE I

EXPERIMENTAL CONDITIONS OF GAS CHROMATOGRAPHIC MEASUREMENTS

	HP-20M				HP-50+				DB-1			
	Temp. (°C)	Flow-rate (ml/min)	t_M (min)	t_r[a] (min)	Temp. (°C)	Flow-rate (ml/min)	t_M (min)	t_r[a] (min)	Temp. (°C)	Flow-rate (ml/min)	t_M (min)	t_r[a] (min)
DMF	90	0.9	1.9	7.3	75	2.1	1.4	5.1	75	2.3	1.2	4.8
MF	130	0.7	2.0	7.7	75	2.1	1.4	5.0	75	2.3	1.2	4.3
F	130	0.7	2.0	12.1	75	2.1	1.4	3.5		Not measured[b]		

[a] t_R of the non-deuterated isotopomer.

[b] Not measured because of the strong tailing and dose dependence of the retention time.

Calculations

In this study, the deuterium isotope effect on chromatographic retention is expressed using relative retention, r_{12}, between a deuterated compound and its non-deuterated analogue: $r_{12} = t'_R(\text{deuterated})/t'_R(\text{non-deuterated})$. Here $t'_R = t_R - t_M$, where t_R is retention time, t'_R is corrected retention time and t_M is dead time. In the most straightforward way, r_{12} can be measured upon injection of both compounds together. This could not be applied here since in some cases peaks of isotopomers could not be resolved sufficiently. Thus, separate injection of each compound was necessary. The absolute values of retention time, however, may be affected by the drift or fluctuations in experimental conditions during measurement. On the other hand, accuracy of the retention parameters determined should be very high. Therefore, a retention of each isotopomer (i) including the non-deuterated compound was related to the retention of an internal standard (I.S.), which was added for this purpose to the sample. Auxiliary relative retentions r_{12aux} were calculated as $r_{12aux} = t'_R(\text{i})/t'_R(\text{I.S.})$ and, finally, the r_{12} value for each deuteromer was obtained:

$$r_{12} = r_{12aux}(\text{deuterated})/r_{12aux}(\text{non-deuterated}) .$$

RESULTS AND DISCUSSION

The deuterium isotope effect on the GC retention of a series of deuteromers of DMF, MF and F was studied on three fused-silica capillary columns of different polarity. As an index of the deuterium isotope effect, values of the relative retention r_{12} of a pair of deuterated/non-deuterated isotopomers were measured. The results (Table II) are consistent for DMF, MF and F, and can be summarized as follows.

(a) The presence of deuterium in the methyl position of formamides results in lower retention.

(b) The presence of deuterium in the formyl position of formamides results in higher retention. The formyl-related deuterium isotope effect is more pronounced than the methyl-related deuterium isotope effect: the corresponding values of an index $(r_{12} - 1)/N_D$ (where N_D is number of deuterium atoms in the molecule) were 0.009 to 0.024 and −0.001 to −0.008, respectively. The separation of MF isotopomers is shown in Fig. 2.

TABLE II

DEUTERIUM ISOTOPE EFFECT ON THE RETENTION OF N,N-DIMETHYLFORMAMIDE, N-METHYLFORMAMIDE, AND FORMAMIDE ISOTOPOMERS

Compound	r_{12} [a]		
	HP-20M	HP-50+	DB-1
d_0-DMF	1.000	1.000	1.000
d_1-DMF	1.023	1.013	1.009
d_3-DMF	0.998	0.989	0.990
d_6-DMF	0.995	0.979	0.982
d_7-DMF	1.017	0.991	0.989
d_0-MF	1.000	1.000	1.000
d_1-MF	1.017	1.014	1.010
d_3-MF	0.982	0.977	0.980
d_4-MF	1.000	–	–
d_0-F	1.000	1.000	–[b]
d_1-F	1.019	1.024	–[b]

[a] The values are mean of at least eight measurements; S.D. was less than 0.001 in most cases.

[b] Not measured because of the strong tailing and dose dependence of retention time.

(c) The presence of chemically inequivalent deuterium atoms results in the final deuterium isotope effect, to which the contributions of individual deuterium atoms are additive. Thus, r_{12} of d_7-DMF or d_4-MF is consistent with that

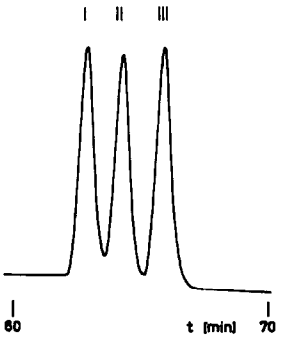

Fig. 2. Resolution of C^2H_3NHCHO (I), CH_3NHCHO (II) and CH_3NHC^2HO (III) on fused-silica capillary column HP-20M 25 m × 0.32 mm I.D., 0.3 μm film thickness; column temperature 70°C; carrier gas helium, 1.0 ml/min.

estimated from the individual r_{12} values of partially deuterated analogues d_6-DMF and d_1-DMF, or d_3-MF and d_1-MF, respectively.

(d) The observations described under (a) to (c) are similar on strongly polar (HP-20M), slightly polar (HP-50+), and low polar (DB-1) columns.

Numerous reports on GC of isotopomers are consistent in that the deuterium isotope effect related to deuterium in methyl or methylene groups is regularly the inverse effect [1,9,10]. This has been interpreted to be a consequence of shorter C–^{2}H internuclear distances as compared with C–H bonds, resulting in lower molar volumes and thus weaker dispersion forces. The effect on dispersion forces is thus responsible for the inverse deuterium isotope effect related to C^2H_3 groups in DMF and MF (even when the contribution of these forces to overall interaction does not have to be prevailing). Dispersion forces seem to predominate in the interaction of formamides with non-polar stationary phases such as DB-1, as judged from the elution in the order of F, MF and DMF, *i.e.* with increasing molar volume. In contrast, these compounds are eluted in the opposite order where polar interactions (dipole–dipole, dipole–induced dipole, etc.) prevail, as in the case of elution from polar HP-20M column. The increasing role of polar interactions in the order of DMF to F is consistent with the order of boiling points: DMF, 153°C; MF, 185°C; F, 195°C.

Unlike methyl-deuterated compounds, the normal DIE as observed with formyl-deuterated formamides seems to be surprising and difficult to account for by simple considerations. The key to understanding this phenomenon is provided by the theory of isotope effects, especially vapour pressure isotope effects, supported by numerous experimental data (for review, see ref. 11). All available reports show that deuterium at saturated carbon displays inverse vapour pressure isotope effect, whereas substitution in groups that form hydrogen bonds and associate often results in normal vapour pressure isotope effect. There is an intrinsic relationship between isotope effect and molecular motion; normal isotope effects correlate with blue and inverse isotope effects with red frequency shifts.

Good examples to study the vapour pressure isotope effect related to deuterium in different groups of the same compound are methylacetylenes and alkylamines [12–14]. Here, deuterium in the methyl group results in an inverse vapour pressure isotope effect, whereas CH_3CC^2H, $CH_3N^2H_2$ and $(CH_3)_2N^2H$ exhibit a normal vapour pressure isotope effect. The above effects were interpreted with the aid of Bigeleisen theory [15]. In a most simplified way, the vapour pressure isotope effect is calculated using the equation $\ln P'/P = A/T^2 - B/T$, where P' and P are the vapour pressures of the heavier and lighter isotopomers, respectively, A (lattice term) and B (zero-point energy term) represent external and internal frequency modes, respectively, and T is temperature. In this model, replacement of H by ^{2}H in the methyl group affects only the A term, which is always positive so that inverse vapour pressure isotope effects occur. On the other hand, the location of deuterium in methynic or amino groups affects both A and B terms, the latter being responsible for the normal vapour pressure isotope effect observed. Analysis of the shape of an experimental plot of $\ln P'/P$ *vs.* T for CH_3CC^2H, $CH_3N^2H_2$ and $(CH_3)_2N^2H$ revealed temperature-dependency of B, which is indicative of molecular association in condensed phases [12–14]. This view was further reinforced by finding that the vapour pressure isotope effect as measured for $CH_3N^2H_2$ or $(CH_3)_2N^2H$ in solution with hexane changed from a normal to inverse effect at high dilution [13,14], in which the association was negligible.

The data on formamides presented here are obviously in good agreement with those on methylacetylenes and amines. Thus, the normal isotope effect related to GC retention of formyl-deuterated formamides is likely to reflect stronger interaction of those compounds, via the formyl group (compared with their non-labelled counterparts), with each other as well as with other molecules such as GC stationary phases. Owing to the polar nature of those interactions one can expect that the magnitude of the deuterium isotope effect on GC retention of formyl-deuterated formamides will increase with polarity of the stationary phase. This was in fact the case, except for F (Table II). Another similarity was observed between the sets of

methylacetylene, methyl- and dimethylamine and formamide isotopomers, namely that the presence of chemically inequivalent deuterium atoms resulted in a final deuterium isotope effect to which contributions of individual deuterium atoms were additive.

In conclusion, the deuterium isotope effect on GC retention of formamides reported here, despite its unusual character, complies well with the general theory of isotope effects.

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