

Short Communication

Gas chromatographic separation of pairs of isotopic molecules

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ABSTRACT

Nine pairs of isotopic (hydrogen/deuterium) molecules have been completely separated and quantitatively determined by gas chromatograph using a DB-5 column. All pairs exhibited an inverse isotope effect. The differences of enthalpy, entropy and free energy changes have been calculated for the chromatographic process. The data show that deuterium on the aliphatic part of a molecule makes more of a contribution to the inverse isotope effect than those on aromatic rings.

INTRODUCTION

The gas chromatographic (GC) separation of isotopically labeled molecules has been investigated during recent years [1–12]. Because this technique affords a convenient method to study isotope effects in solution and scale up might afford economically feasible separations of large amounts of material, studies on GC separation of isotopic molecules continue to receive widespread attention. A considerable amount of the literature studies on isotopic separations were conducted at relative low temperatures so that long times (sometimes more than 1 h) were required for the separations (see, *e.g.*, refs. 13–15). This has limited the utilization of this technique. Fortunately, the introduction of high-efficiency GC columns in recent years opens new opportunities for the analysis of isotopic labeled

molecules [16]. We report here the separation of several pairs of isotopic molecules. Quantitative determination of each component was possible, and the analysis was accomplished in a few minutes.

EXPERIMENTAL

The DB-5 column, purchased from J & W Scientific, is a 60 m × 0.32 mm fused silica column. The liquid phase was 5% diphenyl, 95% dimethylsilicone. A Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector was utilized; this gas chromatograph interfaced with an HP5971A mass-selective detector that was operated under the control of a Vectra 05/165 computer using HPG1034B software. Helium was used as the carrier gas.

The deuterated compounds used in this work were purchased from Aldrich except for

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bibenzyl- $d_{7\text{-benzyl}}$ which was provided by Professor R.D. Guthrie (Department of Chemistry, University of Kentucky).

Experiments were performed to estimate the response of the flame ionization detector to these compounds. The ratio of the GC peak areas is the same as the ratio of the weights of the compounds within the range of the experiment error (1–2%).

Retention volumes have been corrected using the retention time of methane to determine the dead volume.

RESULTS AND DISCUSSION

The data in Table I show the time and the temperature needed for complete separation of pairs of isotopic molecules. At 25°C, benzene- d_6 and benzene- d_0 were completely separated in 7 min. Liberti *et al.* [14] reported a complete separation of benzene- d_6 and benzene- d_0 on both squalane and silicone oil columns; however, in these cases the times needed were 34 and 61 min, respectively. At 45°C, toluene- $d_{3\text{-methyl}}$, toluene- d_8 and toluene- d_0 can be separated completely and can be measured quantitatively. A very good separation of octane- d_{18} and octane- d_0 was obtained at 80°C in 5 min. The mixture of naphthalene- d_8 and naphthalene- d_0 was separated at 110°C in 12 min. At 160°C, bibenzyl- $d_{7\text{-benzyl}}$ and bibenzyl- d_0 were separated in 15 min, and could be determined quantitatively. At

60°C, ethylbenzene- d_{10} , ethylbenzene- $d_{5\text{-ethyl}}$ and ethylbenzene- d_0 were completely separated. Ethylbenzene- $d_{5\text{-ring}}$ labeled was separated from ethylbenzene- d_0 at 35°C within 20 min. Typical gas chromatograms for these compounds (Figs. 1–3) show that the heavier species always eluted first. This phenomena is an inverse isotope effect [2,10,13–17]. Intermolecular Van der Waals forces make the major contribution to the inverse isotope effect. These are operative in the condensed phase and result in a shift in the zero point energy (ZPE) when a molecule of interest is transferred from the gas to the condensed phase. Since the ZPE is isotope sensitive, this results in a thermodynamic isotope effect. The sign of the effect correlates with the fact that the Van der Waals' interaction causes a red shift in

TABLE I

TIME AND TEMPERATURE NEEDED FOR COMPLETE SEPARATION OF PAIRS OF ISOTOPICALLY LABELED MOLECULES USING THE DB-5 COLUMN

Isotopic pair	Temperature (°C)	Time (min)
Benzene- d_6 / $-d_0$	25	7
Toluene- d_3 / $-d_0$	45	8
Toluene- d_8 / $-d_0$	50	7
Octane- d_{18} / $-d_0$	80	5
Naphthalene- d_8 / $-d_0$	110	12
Bibenzyl- d_7 / $-d_0$	160	15
Ethylbenzene- d_{10} / $-d_0$	60	10
Ethylbenzene- $d_{5\text{-ethyl}}$ / $-d_0$	60	10
Ethylbenzene- $d_{5\text{-ring}}$ / $-d_0$	35	21

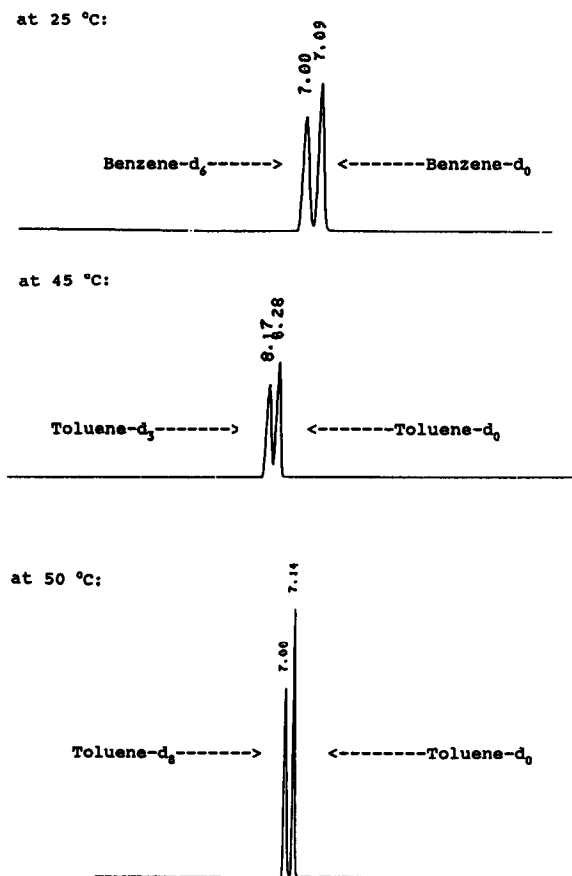


Fig. 1. Gas chromatograms of benzene- d_0 / $-d_6$, toluene- d_0 / $-d_3$ and toluene- d_0 / $-d_8$ on DB-5 capillary column. Values at peaks indicate retention times in min (also in Figs. 2 and 3).

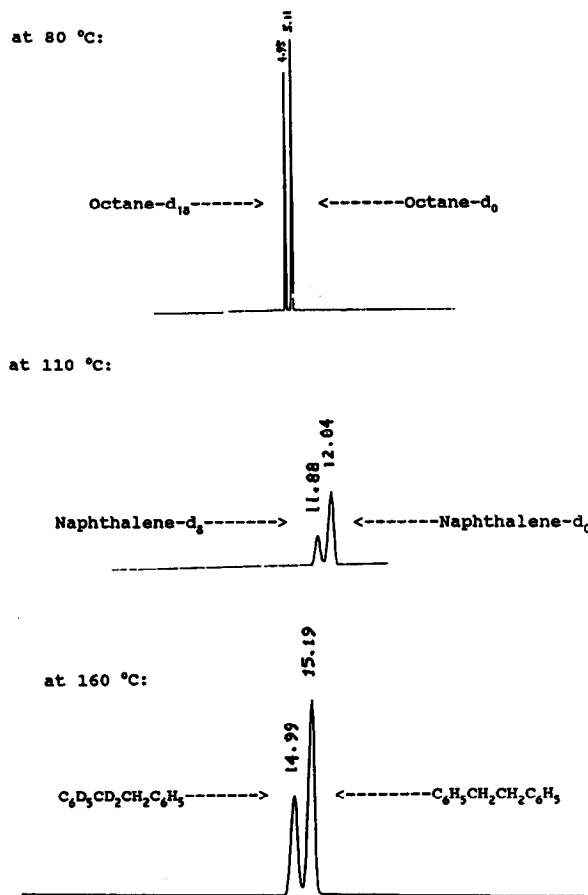


Fig. 2. Gas chromatograms of octane-d₀/-d₁₈, naphthalene-d₀/-d₈ and bibenzyl-d₀/-d₇ on DB-5 capillary column.

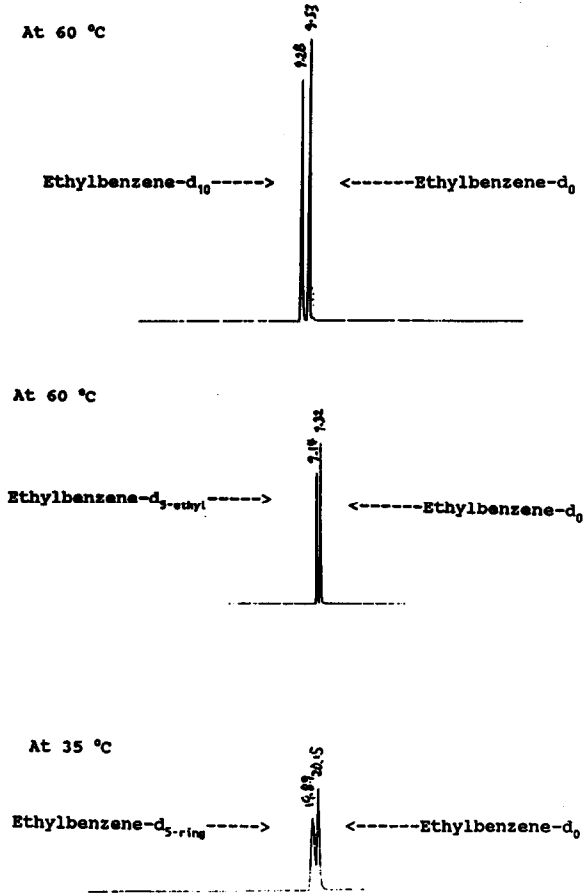


Fig. 3. Gas chromatograms of ethylbenzene-d₀/-d₁₀, -d_{5-ethyl} and -d_{5-ring} on DB-5 capillary column.

the carbon-hydrogen (or carbon-deuterium) vibrational modes [17,18].

To obtain the most favorable and the most convenient operating temperature, the separation factor has been measured over a fairly wide range (Fig. 4). For all of the systems studied except bibenzyl-d₇/-d₀, naphthalene-d₈/-d₀ and benzene-d₆/-d₀, the highest separation factors are observed around 300 K. The octane-d₁₈/-d₀ system has the highest separation factor and is very sensitive to temperature.

In Fig. 4, the logarithm of the ratio of the retention volumes obtained with a DB-5 column are plotted against the reciprocal of the absolute temperature. All of the isotopic mixtures show an inverse isotope effect and a relationship of the type $\log (V_R)_H / (V_R)_D = B/T + C$ describes the

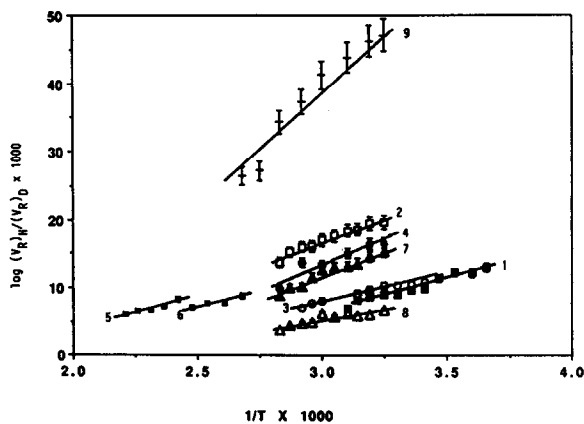


Fig. 4. Plot of the logarithms of the retention volumes versus $1/T$. 1 = C₆H₅CH₃/C₆H₅CD₃; 2 = ethylbenzene-d₀/-d₁₀; 3 = ethylbenzene-d₀/-d_{5-ring}; 4 = C₆H₅CH₃/C₆H₅CD₃; 5 = Bibenzyl-d₀/-d₇; 6 = C₈H₈/C₈D₈; 7 = ethylbenzene-d₀/-d_{5-ethyl}; 8 = C₆H₆/C₆D₆; 9 = octane-d₀/-d₁₈; D,d = deuterium.

variation of the separation factor with temperature [19]. The highest isotopic effect is shown by the pair octane-d₁₈/octane-d₀. The isotope effects of the pair of toluene-d₃/toluene-d₀ and toluene-d₈/toluene-d₀ are higher than that of benzene-d₆/benzene-d₀. However, the isotope effect for naphthalene-d₈/naphthalene-d₀ is about same as that of benzene-d₆/benzene-d₀. It can be concluded that the deuterium in the aliphatic part of the molecule plays a more important role in the GC separation than deuterium on the aromatic ring.

To obtain a relative measure of the impact of the aliphatic and the aromatic deuterium, the GC separations of ethylbenzene-d₁₀, ethylbenzene-d_{5-ethyl}, ethylbenzene-d_{5-ring} and ethylbenzene-d₀ were measured (Fig. 4). The separation factor for the pair of ethylbenzene-d₀/ethylbenzene-d_{5-ethyl} is much larger than that of ethylbenzene-d₀/ethylbenzene-d_{5-ring}. Also, the inverse isotope effect is additive. As can be seen from Fig. 5, the separation factor for the pair of ethylbenzene-d₀/ethylbenzene-d₁₀ is the sum of the separation factors for the pairs of ethylbenzene-d₀/ethylbenzene-d_{5-ethyl} and of ethylbenzene-d₀/ethylbenzene-d_{5-ring}.

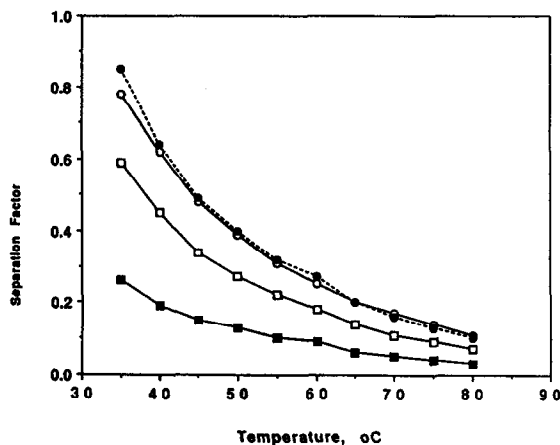


Fig. 5. Separation factors ($R_H - R_D$) for the pairs of ethylbenzene-d₀/ethylbenzene-d_{5-ring} (■), ethylbenzene-d₀/ethylbenzene-d_{5-ethyl} (□) and ethylbenzene-d₀/ethylbenzene-d₁₀ (○). ---●= represents the value calculated as the sum of the separation factor of ethylbenzene-d₀/ethylbenzene-d_{5-ring} and ethylbenzene-d₀/ethylbenzene-d_{5-ethyl}. R_H and R_D refer to retention times of the compounds containing hydrogen and deuterium, respectively.

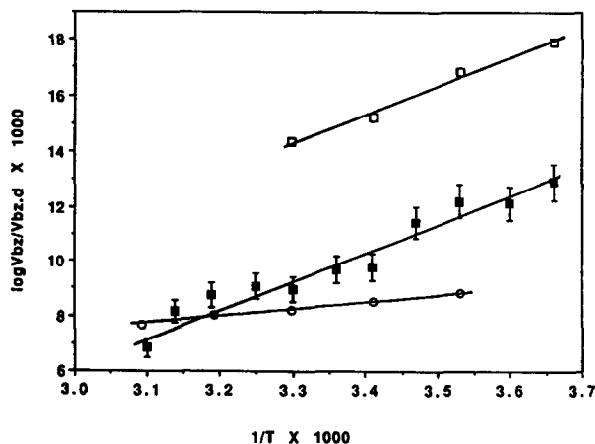


Fig. 6. Plot of the logarithms of the retention volumes versus $1/T$. ■ = DB-5 column; □ = squalane column; ○ = silicone oil 702. Vbz and Vbz-d are retention volumes of benzene-d₀ and benzene-d₆, respectively.

The logarithm of the ratio of retention volumes for the benzene-d₆/benzene-d₀ pair are compared for squalane, silicone oil and DB-5 columns (Fig. 6). The squalane and the silicone oil data are from ref. 14. The greatest isotope effect was obtained on a squalane column; the isotope effect on DB-5 column is greater than that of the silicone oil column.

The data in Figs. 5 and 6 show that there is a reasonably linear relationship between the re-

TABLE II

DIFFERENCE IN ENTHALPY OF FOR THE PAIRS OF ISOTOPIC MOLECULES RELATED TO THE CHROMATOGRAPHIC PROCESS ON DB-5 COLUMN

1 cal = 4.14 J.

Pairs of isotopic molecules	$\Delta H_H - \Delta H_D$ (cal)
Benzene-d ₆ /-d ₀	-45.8
Toluene-d ₃ -methyl/-d ₀	-32.1
Toluene-d ₈ /-d ₀	-67.6
Ethylbenzene-d ₁₀ /-d ₀	-62.5
Ethylbenzene-d _{5-ethyl} /-d ₀	-65.9
Ethylbenzene-d _{5-ring} /-d ₀	-27.6
Naphthalene-d ₈ /-d ₀	-34.9
Bibenzyl-d ₇ ^a /-d ₀	-42.2
Octane-d ₁₈ /-d ₀	-175.8

^a The structure of this compound is C₆D₅-CD₂-CH₂-C₆H₅.

tention volumes and the reciprocal of the temperature which can be expressed by eqn. 1:

$$\log(V_R)_H/(V_R)_D = -(\Delta H_H - \Delta H_D)/2.3RT + C \quad (1)$$

From the slope of each system on the graph, the enthalpy difference for the chromatographic process can be calculated; these results are given in Table II. The difference of entropy can be calculated by eqn. 2 and the difference in free energy by eqn. 3.

$$\Delta G_H - \Delta G_D = \Delta H_H - \Delta H_D - T(\Delta S_H - \Delta S_D) \quad (2)$$

$$\Delta G_H - \Delta G_D = -2.3RT \log(V_R)_H/(V_R)_D \quad (3)$$

The values of the difference in free energy and entropy are presented in Tables III and IV, respectively.

These fundamental thermodynamic data can be used to define the chromatographic process as well as the properties of compounds and the columns. The GC process should be considered

TABLE III

DIFFERENCE IN FREE ENERGY CHANGES $[-(\Delta G_H - \Delta G_D), \text{cal}]$ OBTAINED FROM RETENTION VOLUMES FOR PAIRS OF ISOTOPIC MOLECULES

Temperature (K)	Benzene ^a	Tol-CD ₃ ^b	Toluene-d ₈ ^c	EB-d ₁₀ ^d	EB-d ₅ -ethyl ^e	EB-d ₅ -ring ^f	Octane-d ₁₈ ^g	NP-d ₈ ^h	BB-d ₇ ⁱ
273	16.1	-	-	-	-	-	-	-	-
278	15.4	-	-	-	-	-	-	-	-
283	15.8	-	-	-	-	-	-	-	-
288	15.1	-	-	-	-	-	-	-	-
293	13.1	14.4	-	-	-	-	-	-	-
298	13.2	14.4	-	-	-	-	-	-	-
303	12.4	14.2	-	-	-	-	-	-	-
308	12.8	14.5	23.3	27.7	21.4	9.4	66.3	-	-
313	12.5	13.9	23.5	27.8	20.8	8.7	66.1	-	-
318	11.9	13.3	-	27.0	19.7	8.6	-	-	-
323	10.1	14.6	22.1	27.2	19.3	9.2	64.7	-	-
328	-	13.9	-	26.5	19.3	8.7	-	-	-
333	-	12.3	20.2	25.8	19.1	9.5	62.8	-	-
338	-	11.9	-	24.8	17.8	7.6	-	-	-
343	-	11.0	21.3	25.0	15.8	7.5	58.6	-	-
348	-	-	-	24.2	16.0	7.1	-	-	-
353	-	-	15.8	22.1	14.4	6.1	55.5	-	-
363	-	-	-	-	-	-	45.0	-	-
373	-	-	-	-	-	-	44.9	14.9	-
383	-	-	-	-	-	-	-	13.6	-
393	-	-	-	-	-	-	-	13.8	-
403	-	-	-	-	-	-	-	13.0	-
413	-	-	-	-	-	-	-	-	15.5
423	-	-	-	-	-	-	-	-	13.9
433	-	-	-	-	-	-	-	-	13.3
443	-	-	-	-	-	-	-	-	13.3
453	-	-	-	-	-	-	-	-	12.6

^a Benzene-d₆/-d₀.

^b Toluene-d₃-methyl/-d₀.

^c Toluene-d₈/-d₀.

^d Ethylbenzene-d₁₀/-d₀.

^e Ethylbenzene-d₅-ethyl/-d₀.

^f Ethylbenzene-d₅-ring/-d₀.

^g Octane-d₁₈/-d₀.

^h Naphthalene-d₈/-d₀.

ⁱ C₆D₅-CD₂CH₂-C₆H₅/-d₀.

TABLE IV

DIFFERENCE IN ENTROPY $[-(\Delta S_H - \Delta S_D) \times 10^2, \text{ cal}]$ OBTAINED FROM RETENTION VOLUMES FOR PAIRS OF ISOTOPIC MOLECULES

Temperature (K)	Benzene ^a	C ₆ H ₅ -CD ₃ ^b	Toluene-d ₈ ^c	EB-d ₁₀ ^d	EB-d ₅ -ethyl ^e	EB-d ₅ -ring ^f	Octane-d ₁₈ ^g	NP-d ₈ ^h	BB-d ₇ ⁱ
273	10.9	—	—	—	—	—	—	—	—
278	10.9	—	—	—	—	—	—	—	—
283	10.6	—	—	—	—	—	—	—	—
288	10.7	—	—	—	—	—	—	—	—
293	11.2	6.0	—	—	—	—	—	—	—
298	10.9	5.9	—	—	—	—	—	—	—
303	11.0	5.9	—	—	—	—	—	—	—
308	10.7	5.7	14.4	11.3	14.4	5.9	35.6	—	—
313	10.6	5.8	14.1	11.1	14.4	6.0	35.1	—	—
318	10.7	5.9	—	11.2	14.5	6.0	—	—	—
323	11.1	5.4	14.1	10.9	14.4	5.7	34.4	—	—
328	—	5.6	—	11.0	14.2	5.8	—	—	—
333	—	6.0	14.2	11.0	14.0	5.4	33.9	—	—
338	—	6.0	—	11.1	14.2	5.9	—	—	—
343	—	6.2	13.5	10.9	14.6	5.9	34.2	—	—
348	—	—	—	11.0	14.3	5.9	—	—	—
353	—	—	14.7	11.5	14.6	6.1	34.1	—	—
363	—	—	—	—	—	—	37.1	—	—
373	—	—	—	—	—	—	36.2	5.4	—
383	—	—	—	—	—	—	—	5.6	—
393	—	—	—	—	—	—	—	5.4	—
403	—	—	—	—	—	—	—	5.5	—
413	—	—	—	—	—	—	—	—	6.5
423	—	—	—	—	—	—	—	—	6.7
433	—	—	—	—	—	—	—	—	6.7
443	—	—	—	—	—	—	—	—	6.5
453	—	—	—	—	—	—	—	—	6.5

^a Benzene-d₆/-d₀.^b Toluene-d_{3-methyl}/-d₀.^c Toluene-d₈/-d₀.^d Ethylbenzene-d₁₀/-d₀.^e Ethylbenzene-d_{5-ethyl}/-d₀.^f Ethylbenzene-d_{5-ring}/-d₀.^g Octane-d₁₈/-d₀.^h Naphthalene-d₈/-d₀.ⁱ C₆D₅-CD₂CH₂-C₆H₅/-d₀.

to consist of two processes; one is due to the condensation evaporation process for each eluted component and the other is due to their mixing with the liquid partitioning material [7]. Of the two processes, the mixing process is assumed to be relatively isotope insensitive. This leaves the condensation-evaporation process as the important one [18].

CONCLUSIONS

Pairs of isotopic molecules have been completely separated and quantitatively determined

by GC using a DB-5 column. An inverse isotope effect was obtained for all pairs of the isotopically labelled molecules. Very good separations were obtained at low temperatures with a short elution time. The differences of ΔG , ΔH and ΔS for a DB-5 column have been calculated. The data clearly show that deuterium on the aliphatic part of a molecule plays a more important role in determining the inverse isotope effect than those on the aromatic ring; this is shown by the fact that the vapor pressure isotope effect per D for the aromatics is less than that per D for aliphatics [10].

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