Determination of Multiple Equilibrium Isotopic Fractionation Factors at Natural Abundance in Liquid-Vapor Transitions of Organic Molecules

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This work presents a simple and easy steady-state distillation method for simultaneously determining the equilibrium isotopic fractionation factor α of different isotopomers of compounds at natural abundance in liquid-vapor phase transition. The measurement of α is precise (<1%) thanks to the use of natural abundance ^{2}H NMR for hydrogen isotope analysis, which provides the site-specific isotopic ratios of different hydrogen positions of a molecule. Moreover, for the heavy elements (C, O, and N), their site-specific isotopic ratios in different functional groups are measured precisely by isotope ratio mass spectrometry (IRMS). We studied the equilibrium isotopic fractionation factors of the major isotopomers of organic molecules containing typical functional groups. Of particular interest, the isotopic fractionation factors varied with the position in a molecule. These important results provide information on how the functional groups of a molecule contribute to the intermolecular forces in the condensed state.

Introduction

Isotopic fractionation takes place in all physical and chemical processes and has been the subject of numerous studies. In particular, the liquid-vapor transition has attracted much attention.[1,2] Isotopic fractionation in the phase transition is related to vapor pressure isotope effects (VPIE).[1a] In a reversible vaporization process, when the liquid and the vapor are in equilibrium, the isotopic composition in the two phases is constant at a given temperature. Equilibrium isotopic fractionation (EIF) reflects the difference of isotopic composition of an isotopomer (here isotopomer (isotopic isomer) is defined in agreement with most literature as "molecules that have the same constitution and same configuration but differ by isotopic substitution"; there are composition isotopomers and position isotopomers) mixture in the two phases in the reversible vaporization process when equilibrium is reached; it is characterized by the equilibrium isotopic fractionation factor or the separation factor, called α and defined as

$$\alpha = (N'/N)_{vap}/(N'/N)_{liq} \tag{1}$$

or

$$\alpha = (\Delta N'/\Delta N)_{liq}/[(N' - \Delta N')/(N - \Delta N)]_{liq}$$
$$= (dN'/dN)_{liq}/(N'/N)_{liq} \tag{1'}$$

where N' and N are mole fractions of the light and heavy isotopomers in the system. Integration of eq 1' leads to the following equation:

$$\ln(N'_{liq}/N'_{liq,0}) = \alpha \ln(N_{liq}/N_{liq,0}) \tag{2}$$

termed the Rayleigh equation:[1a,3]

$$\ln(R/R_0) = (\alpha - 1)\ln f \tag{3}$$

with $R = N_{liq}/N'_{liq}$, $R_0 = N_{liq,0}/N'_{liq,0}$ for the starting compound, and $f = N'_{liq}/N'_{liq,0}$. The term α can be determined by vapor pressure measurement or studied by distillation.[1a,3]

At natural abundance, the stable heavy isotope of hydrogen, deuterium, due to its low abundance is found essentially in monodeuterated isotopomers. When a molecule has several hydrogen sites, such as in most organic compounds, there are several monodeuterated isotopomers. The specific isotopic fractionation of site i of a deuterated isotopomer in the phase transition is

$$\alpha = (D/H)_{i,liq}/(D/H)_{i,vap} \tag{4}$$

and

$$\ln[(D/H)_{i,liq}/(D/H)_{i,liq,0}] = (\alpha - 1)\ln f \tag{5}$$

where $(D/H)_i$ is the specific isotopic ratio of site i:[7]

$$(D/H)_i = N_{D,i}/p_i N_H \tag{6}$$

in which $N_{D,i}$ is the number of site i monodeuterated isotopomers, N_H is the number of undeuterated molecules ($N_H \approx N_H + \Sigma N_{D,i}$), and p_i is the number of equivalent hydrogen atoms at site i. For example, for CH_3, $p_i = 3$.

For the measurement of α, the frequently used distillation method is Rayleigh distillation. In this case, a mixture of isotopomers is evaporated under equilibrium conditions until just enough residue for isotope analysis remains and α is calculated using eq 3. However, the Rayleigh distillation technique is rarely used nowadays, mainly because of the experimental difficulties connected with maintaining equilibrium conditions during the evaporation process.

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The term α can also be evaluated by isotopic analysis in the two phases in equilibrium. In this work, we used a very simple and easy method for its precise determination at natural abundance. In a chemical compound there are different natural isotopomers that will fractionate during a distillation. When a compound is heated in reflux conditions, an equilibrium between the liquid and its vapor is established at steady state. At that time the removal of a small quantity of its vapor through condensation will not alter the equilibrium isotopic composition in the two phases in the detection limit. The experiment thus consists of a steady-state distillation of a compound of which only a very small quantity ($\approx$1%) is removed as distillate. Then, the change of natural isotopic composition of the compound in the liquid phase during the distillation can be neglected while the isotopic composition of the distillate represents that of the vapor phase. Equation 1 can then be used to determine α of different isotopomers of a compound, and when a fractionating column is used, α can be calculated from the experimental value using the Fenske equation.[4,5]

$$\alpha_{exp} = \alpha^n \qquad (7)$$

with n equal to the number of theoretical plates.

The quality of the results of the study of fractionation of stable isotopes depends essentially on the precision of the isotopic analysis. When an enriched compound is used, many physical and chemical analytical techniques can be used. When working at natural abundance, only two main analytical methods can be useful for high precision analysis: isotope ratio mass spectrometry (IRMS) and NMR. The configuration of the isotope ratio mass spectrometer is different from a mass spectrometer for chemical analysis. The samples are converted to small molecules such as CO, CO_2, N_2, or H_2 before the analysis, and only overall isotopic ratios of all atoms of the element in a compound can be measured. For NMR, at present only ^{2}H NMR (SNIF NMR)[6,7] can be used in routine precise isotopic analysis. The most important merit of ^{2}H NMR is that the different natural monodeuterated isotopomers can be observed and that the site-specific isotopic ratios of a molecule can be measured simultaneously if their signals are separated. The ^{2}H NMR method has been used in the study of kinetic and equilibrium isotope effects of chemical reactions.[8-12] Its application in the study of liquid-vapor isotopic fractionation by Rayleigh distillation has also been reported.[13] Thanks to this technique, multiple deuterium isotope effects for compounds involved in a physicochemical process have been determined simultaneously at natural abundance in a one-pot experiment.

Taking advantage of the ^{2}H NMR and IRMS analytical techniques, multielement and multisite isotopic fractionation factors α of a compound can be determined with good accuracy. In this article, we will present some new and interesting results obtained for organic molecules by using the new experimental approach and the powerful isotopic analytical techniques.

Results and Discussion

The equilibrium isotopic fractionation factors of all the monodeuterated isotopomers and of some monosubstituted heavy element (C, N, and O) isotopomers of a variety of molecules have been studied: alcohol, amine, carboxylic acid, ester, cyanide, thiol, nitro compound, ketone, including cyclic, linear, and aromatic compounds. The site-specific $\alpha_{exp}(H/D_i)$ and the function-specific $\alpha_{exp}(^{13}C/^{12}C)$, $\alpha_{exp}(^{18}O/^{16}O)$, and $\alpha_{exp}(^{15}N/^{14}N)$ have been measured. Site-specific α_{exp} of the heavy elements was determined only when there is a single position

in the molecule containing that element. It cannot be measured if there are several sites containing the same heavy element. For example, if there are several carbon positions, only the average $\alpha_{exp}(^{13}C/^{12}C)$ for all these carbons can be obtained.

All results are summarized in Tables 1–13. In the tables, the α_{exp} calculated from $(D/H)_i$ or δX‰ (X = C, N, O) is the fractionation factor of site i monodeuterated isotopomer or X-labeled isotopomer relative to the unlabeled molecule. For example, the α_{exp} values correspond to $(D/H)_{OH}$ and $\delta^{18}O$‰-(OH) of methanol are $\alpha_{exp}(CH_3OH/CH_3OD)$ and $\alpha_{exp}(CH_3{}^{16}OH/CH_3{}^{18}OH)$, respectively. All the $(D/H)_i$ values are expressed in ppm. The standard deviations are in brackets.

$$\alpha_{exp} = \frac{(D/H)_{i,\text{average of starting and remaining}}}{(D/H)_{i,\text{distillate}}}$$

The precision of $(D/H)_i$ measured by ^{2}H NMR is very good. The uncertainty of $(D/H)_{OH}$ and $(D/H)_{COOH}$ is somewhat higher than $(D/H)_i$ of other sites because their ^{2}H NMR signals are wider and the signal/noise ratio is lower. The high reproducibility of the isotopic data measured with the starting compound and the liquid remaining confirms that the isotopic composition of the liquid phase does not change before and after the distillation in all experiments. The accuracy of the α_{exp} values calculated based on these data is excellent.

For the calculation of α from α_{exp}, the theoretical number of plates n of the column is needed. However, n depends not only on the properties of the column and the experimental conditions but also on the isotopomers. The theoretical number of plates also varies considerably according to the test compound. Kiss et al.[14] have determined the $\alpha(ROH/ROD)$ values of methanol (1.0283), ethanol (1.0215), 1-propanol (1.0190), and 1-butanol (1.0154) by vapor pressure measurements. Based on these values, n can be evaluated from the corresponding α_{exp} values measured in this work. For the four alcohols, $n = 13$, 12.4, 6.8, and 7.8 (the uncertainty of n, $\Delta n = \pm 1.1-2.3$, estimates based only on the error of α_{exp}) from methanol to 1-butanol, respectively.

Although it is difficult to evaluate n, it is essential to use a column, without which isotopic fractionation cannot be observed in the detection limit for most isotopic molecules, especially for those for which the VPIE is small, such as carbon-bound hydrogen-labeled isotopomers. Distillations successively with methanol, 1-butanol, and propionic acid were performed without the spinning band column, and their vapor at boiling point was directly condensed and collected. Under these conditions the theoretical number of plates may be minimized: $n \approx 1$. However, only slight deuterium isotopic fractionation for MeOD and $CH_3CH_2CO_2D$ was observed, while for the other deuterated isotopomers, unfortunately, no significant isotopic difference could be detected.

Although it is impossible to assume one precise n value for all isotopomers of all compounds and to determine its accurate value for each of them, a reasonably approximate n value for the different isotopomers of some compounds can be evaluated with certain known α values determined in previous works. For some compounds we evaluated the α values by using n obtained in this way. The term α_{exp} can also be directly used in the discussions for its interpretation based on two considerations. In the first place, the direction (normal or inverse) of isotope effects is independent of n. Furthermore, the order of relative magnitude of α_{exp} of the monodeuterated isotopomers of a compound may be independent of n because n is very close for its different isotopomers. Nevertheless, when comparing dif-

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TABLE 1: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of Methanol at 65 °C

	$(D/H)_{OH}$	$(D/H)_{CH_3}$	$\delta^{18}O‰(OH)$	$\delta^{12}C‰(CH_3)$
starting	148.7 (2.5)	129.3 (0.7)	14.9	−32.1
remaining	148.4 (2.8)	128.8 (0.6)	13.8	−32.3
distillate	103.1 (1.3)	132.6 (0.4)	−26.3	−27.7
α_{exp}	1.441 (0.044)	0.974 (0.008)	1.0424 (0.0005)	0.9950 (0.0005)
α^a	1.0285 (0.024)	0.9980 (0.0006)	1.0032 (0.0000)	0.9996 (0.0000)

^{a} $n = 13$ evaluated by using $\alpha = 1.0283$ for CH_3OH/CH_3OD.[14]

TABLE 2: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of Ethanol at 78 °C

	$(D/H)_{OH}$	$(D/H)_{CH_2}$	$(D/H)_{CH_3}$	$\delta^{18}O‰(OH)$
starting	129.5(2.3)	124.7(0.5)	92.8(0.3)	19.2
remaining	130.8(2.0)	124.7(0.3)	93.2(0.3)	19.4
distillate	99.9(2.2)	128.3(0.5)	96.9(0.3)	−10.2
α_{exp}	1.303(0.050)	0.972(0.007)	0.960(0.006)	1.0298(0.0005)
α^a	1.0216(0.0032)	0.9977(0.0006)	0.9967(0.0005)	1.0024(0.0000)

^{a} $n = 12.4$ evaluated by using $\alpha = 1.0215$ for C_2H_5OH/C_2H_5OD.[14]

TABLE 3: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of 1-Propanol at 97 °C

	$(D/H)_{OH}$	$(D/H)_{CH_2 OH}$	$(D/H)_{CH_2}$	$(D/H)_{CH_3}$	$\delta^{18}O‰(OH)$
starting	118.5 (1.7)	110.7 (0.7)	140.9 (0.4)	129.2 (0.5)	11.6
remaining	118.4 (2.2)	110.6 (0.5)	141.6 (0.7)	129.0 (0.2)	11.4
distillate	104.2 (2.1)	112.3 (0.6)	144.4 (0.4)	135.1 (0.2)	−11.9
α_{exp}	1.137 (0.042)	0.986 (0.011)	0.978 (0.007)	0.954 (0.004)	1.0237(0.0005)
α^a	1.0191(0.0055)	0.9979(0.0016)	0.9967(0.0010)	0.9931(0.0006)	1.0035(0.0001)

^{a} $n = 6.8$ evaluated by using $\alpha = 1.0190$ for C_3H_7OH/C_3H_7OD.[14]

TABLE 4: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of 1-Butanol 118 °C

	$(D/H)_{OH}$	$(D/H)_{\alpha-CH_2}$	$(D/H)_{\beta-CH_2}$	$(D/H)_{\gamma-CH_2}$	$(D/H)_{CH_3}$	$\delta^{18}O‰(OH)$
starting	128.8 (2.2)	100.6 (0.6)	144.5 (0.6)	139.1 (0.3)	146.1 (0.4)	25.4
remaining	129.7 (2.5)	100.8 (0.4)	145.1 (0.3)	139.4 (0.7)	146.3 (0.7)	25.3
distillate	114.7 (1.8)	103.7 (0.8)	147.6 (0.4)	146.5 (0.7)	156.9 (0.5)	4.0
α_{exp}	1.127	0.971	0.981	0.951	0.932	1.0213
	(0.039)	(0.012)	(0.006)	(0.008)	(0.007)	(0.0005)
α^a	1.0154	0.9962	0.9975	0.9936	0.9910	1.0027
	(0.0045)	(0.0016)	(0.0008)	(0.0011)	(0.0010)	(0.0001)

^{a} $n = 7.8$ evaluated by using $\alpha = 1.0154$ for C_4H_9OH/C_4H_9OD.[14]

TABLE 5: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of 1-Propylamine at 48 °C

	$(D/H)_{NH_2}$	$(D/H)_{CH_2,\alpha}$	$(D/H)_{CH_2,\beta}$	$(D/H)_{CH_3}$	$\delta^{15}N‰(NH_2)$
starting					−3.2
remaining	143.4 (0.6)	116.1 (0.4)	129.3 (0.3)	127.0 (0.3)	−3.5
distillate	138.1 (0.8)	123.5 (0.6)	132.5 (0.5)	136.5 (0.3)	−5.2
α_{exp}	1.038 (0.007)	0.940 (0.006)	0.976 (0.005)	0.930 (0.003)	1.0019(0.0005)
α^a	1.0031(0.0008)	0.9949(0.0008)	0.9980(0.0006)	0.9940(0.0004)	1.0002(0.0001)

^{a} $n = 12$ evaluated by using $\alpha = 1.0031$ for $C_3H_7NH_2/C_3H_7NHD$.[15]

ferent compounds, the use of the elementary fractionation factor α is necessary.

For nearly all carbon-bound hydrogens, α_{exp} is inverse (i.e., the heavy isotopomer is more volatile), while all $\alpha_{exp}(ROH/ROD)$ and $\alpha_{exp}(RNH_2/RNHD)$ are normal (i.e., the heavy isotopomer is less volatile). For carboxylic acid, $\alpha_{exp}(RCOOH/RCOOD)$ is inverse. These results are in good accord with those obtained in previous studies.[1a,13] The results obtained for methanol also confirm the previous observation that the ^{18}O effect is normal and that the ^{13}C effect is inverse.[18]

Interpretation of vapor pressure isotope effects (VPIE) based on theoretical analysis has been well discussed.[1b,19,20] For the interpretation of the results, it is necessary to carry out detailed calculations in terms of the statistical mechanical theory of condensed-phase isotope effects since liquid is a complex dynamic system. Unfortunately, the necessary spectroscopic information is not available for most molecules studied in the work. As the observed isotope effect is the result of the interplay

between the normal isotope effect from hindered translations and rotations and the inverse isotope effect arising from the internal degrees of freedom, one should also consider properly the contributions to the fractionation factors from the hinder translational and rotational motions of the molecules in the liquid phase and from the internal vibration. At present, it is very difficult to apply the theoretical analysis to the interpretation of our results.

For all alcohols (Tables 1−4), both $\alpha(ROH/ROD)$ and $\alpha(R^{16}OH/R^{18}OH)$ are normal and big. A similar situation is observed for the amine (Table 5), for which both $\alpha(RNH_2/RNHD)$ and $\alpha(R^{14}NH_2/R^{15}NH_2)$ are normal and big too. Since in these compounds in liquid phase there are hydrogen bonds, big normal α values of these groups would be related to the formation of a hydrogen bond. For PrSH (Table 6), $\alpha_{exp}(PrSH/PrSD)$ is inverse. In this compound there is no hydrogen bond. As S−H is a poor proton donor, H-bonds can only be formed in the presence of a strong base.[21]

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TABLE 6: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of 1-Propanethiol at 67 °C

	$(D/H)_{SH}$	$(D/H)_{CH_2-S}$	$(D/H)_{CH_2}$	$(D/H)_{CH_3}$
starting	91.1 (0.8)	87.0 (0.5)	130.6 (0.5)	128.5 (0.6)
remaining	92.3 (1.0)	87.4 (0.4)	130.7 (0.4)	128.8 (0.4)
distillate	98.7 (0.4)	91.7 (0.3)	134.9 (0.7)	134.8 (0.3)
α_{exp}	0.929 (0.010)	0.951 (0.006)	0.969 (0.006)	0.954 (0.004)

TABLE 7: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of Propionic Acid at 141 °C

	$(D/H)_{COOH}$	$(D/H)_{CH_2}$	$(D/H)_{CH_3}$	$\delta^{18}O\text{‰}(OH)$
starting	172.4 (1.8)	157.6 (0.6)	126.1 (0.3)	3.1
remaining	172.6 (1.5)	157.5 (0.5)	125.6 (0.5)	
distillate	217.8 (1.9)	159.7 (0.6)	126.5 (0.4)	1.4
α_{exp}	0.792 (0.010)	0.987 (0.005)	0.995 (0.004)	1.0017 (0.0005)

TABLE 8: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of Ethyl Propionate at 99 °C

	$(D/H)_{CH_2(ethyl)}$	$(D/H)_{CH_3(ethyl)}$	$(D/H)_{CH_2(propionate)}$	$(D/H)_{CH_3(propionate)}$
starting	119.2 (1.0)	109.2 (0.2)	141.1 (0.5)	133.7 (0.5)
remaining	119.1 (0.7)	108.3 (0.6)	140.8 (1.0)	133.8 (0.5)
distillate	121.1 (0.7)	110.0 (0.6)	142.9 (0.8)	139.0 (0.5)
α_{exp}	0.984 (0.009)	0.988 (0.007)	0.986 (0.007)	0.963 (0.005)

TABLE 9: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of Cyclohexanone at 155 °C

	$(D/H)_{(CH2)_2,\alpha}$	$(D/H)_{(CH2)_2,\beta}$	$(D/H)_{CH_2,\gamma}$	$\delta^{18}O\text{‰}(CO)$
starting	145.6 (0.3)	133.9 (0.4)	133.8 (0.6)	15.1
remaining	146.0 (0.3)	134.8 (0.3)	133.2 (0.9)	
distillate	152.1 (0.3)	137.8 (0.2)	134.3 (0.5)	17.8
α_{exp}	0.959 (0.003)	0.975 (0.003)	0.994 (0.007)	0.9974 (0.0005)

For methanol, we obtained the α values for all the three constitutive elements C, O, and H because this is a special case in which there is only one carbon atom in the molecule. Its $\alpha(^{12}C/^{13}C)$ of alkyl carbon is inverse. At the present time, reliable site-specific carbon-13 EIF values are difficult to obtain by NMR. Nevertheless, we have done an overall $^{13}C/^{12}C$ isotope ratio analysis for some compounds containing several carbon atoms in the equilibrated liquid/vapor phases by IRMS analysis. We have found the following $\delta^{13}C\text{‰}$ values: propylamine, -26.7 for the liquid and -22.4 for the vapor; acetonitrile, -29.8 for the liquid and -30.6 for the vapor. This means that the average ^{13}C EIF of the former is inverse and that of the latter is slightly normal. These results show that the site-specific VPIE is very complex and may involve many intermolecular interactions in the condensed phase.

For the alcohols, the significant normal EIFs $\alpha(ROH/ROD)$ and $\alpha(R^{16}OH/R^{18}OH)$ decrease as the alkyl chain length increases. The α values of all carbon-bound hydrogens are inverse. The methyl deuterated isotopomers have the most significant inverse EIF among all alkyl sites in the alcohol series, and this EIF becomes more and more significant from methanol to butanol.

For the carboxylic acid (Table 7), $\alpha_{exp}(RCO_2H/RCO_2D)$ is inverse but $\alpha_{exp}(RC^{16}O_2H/RC^{18}O_2H)$ is normal. Once again, this shows that the factors that influence α are very complex. The C=O and COH positions of carboxylic acids are averaged by rapid intermolecular proton transfer. This is confirmed by ^{17}O NMR, where the spectrum has only one signal for COOH.[22] Thus, the $\delta^{18}O$ value is that of the whole carboxylic group without distinguishing "CO" and "COH". For this compound, the α values of all carbon-bound hydrogens are inverse.

In ethyl propionate (Table 8), the methyl of propionate has the most significant EIF while the effects of other alkyl sites are of the same order. The EIFs of the alkyl hydrogens of the

TABLE 10: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of Cyclohexane at 80 °C

	$(D/H)_{CH_2}$	$\delta^{13}C\text{‰}(CH_2)$
starting	130.9 (0.7)	-28.7
remaining	130.0 (0.5)	-28.2
distillate	135.5 (0.8)	-25.2
α_{exp}	0.963 (0.007)	0.9966 (0.0005)
$\alpha^{[a]}$	0.9942 (0.0011)	0.9995 (0.0001)

[a] $n = 6.5$ evaluated by using $\alpha = 0.994$ for $C_6H_{12}/C_6H_{11}D$.[16]

TABLE 11: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of Acetonitrile at 81 °C

	$(D/H)_{CH_3}$	$\delta^{15}N\text{‰}(CN)$
starting	94.9 (0.6)	-2.48
remaining	95.2 (0.5)	-2.34
distillate	102.2 (0.6)	-8.25
α_{exp}	0.9305 (0.008)	1.0059 (0.0005)
α^a	0.9919 (0.0011)	1.0007 (0.0001)

[a] $n = 8.8$ evaluated by using $\alpha = 0.9919$ for CH_3CN/CH_2DCN.[17]

amine are all inverse, and its methyl deuterium has the most significant EIF. A similar situation was observed for the thiol.

In cyclohexanone (Table 9), both $\alpha_{exp}(H/D)$ and $\alpha_{exp}(^{16}O/^{18}O)$ are inverse and the significance $\alpha_{exp}(H/D_i)$ decreases in the order of $CH_{2,\alpha} > CH_{2,\beta} > CH_{2,\gamma}$. In cyclohexane (Table 10), $\alpha(H/D)$ and $\alpha(^{12}C/^{13}C)$ are inverse. For acetonitrile (Table 11), both the inverse $\alpha_{exp}(H/D)$ and the normal $\alpha_{exp}(^{14}N/^{15}N)$ are significant. In the only aromatic compound studied in this work, *p*-xylene (Table 12), we observed an inverse EIF for the methyl group and a very weak normal effect for the ring hydrogen. This is the only normal α_{exp} value that we obtained for carbon-bound hydrogen.

Nitromethane (Table 13) is also a special molecule for which it is possible to determine the EIF of all monosubstituted

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TABLE 12: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of *p*-Xylene at 138 °C

	$(D/H)_{CH}$	$(D/H)_{CH_3}$
starting	63.2 (0.4)	87.4 (0.4)
remaining	63.2 (0.3)	87.0 (0.4)
distillate	61.9 (0.3)	90.4 (0.5)
α_{exp}	1.021 (0.008)	0.965 (0.007)

isotopomers. All the α_{exp}, (α_{exp}(H/D), α_{exp}($^{16}O/^{18}O$), α_{exp}($^{12}C/^{13}C$), and α_{exp}($^{15}N/^{14}N$)), are inverse but the EIFs are very weak.

By the present technique, a set of site-specific isotopic fractionation factors of a molecule can be observed. A molecule cannot be represented by a simple model such as a sphere or a dipolar rod. In intermolecular interactions, different functional groups of the molecule play different roles, especially in large organic molecules. Some groups contribute more and the others less, according to the nature of the intermolecular forces. Our results should provide information on the specific contribution of functional groups of a molecule to the intermolecular forces.

This work opens up new perspectives for the study of intermolecular forces through isotope effects. More compounds of diverse structures need to be studied, and the effects of physical factors, such as temperature, on the EIF need to be examined. Refined modeling and theoretical calculations should be conducted in order to explain not only the direction (normal or inverse) but also the relative size of the site-specific phase transition isotope effects of a compound. The theoretical analysis, coupled with experimental isotopic and spectroscopic data, will make possible a more profound understanding of specific intermolecular interactions in the liquid state.

Experimental Section

Determination of Experimental Equilibrium Fractionation Factor α_{exp} by Steady-State Distillation. The term α_{exp} was determined by distillation on a Cadiot spinning band column under atmospheric pressure. A 500 mL portion of compound was distilled at a reflux ratio of 1/20. When about 3−5 mL (the quantity necessary for NMR and IRMS analysis) of distillate was collected, the distillation was stopped: $\alpha_{exp} = (h/l)_{liq}/(h/l)_{vap} \approx (h/l)_{starting}/(h/l)_{distillate}$ or $\approx (h/l)_{remaining}/(h/l)_{distillate}$ and $\alpha = \alpha_{exp}^{1/n}$.

NMR Analysis. Deuterium NMR spectra were recorded at 61.4 MHz under broadband proton decoupling using a Bruker DPX 400 spectrometer equipped with a ^{19}F lock device. Frequency window 1200 Hz, memory size 16 K, scan number 500 to 1400 according to the sample, and exponential multiplication corresponding to a line broadening of 0.5 or 1 Hz. Six spectra were recorded for each sample and an average $(D/H)_i$ value was calculated from the six measurements.

The $(D/H)_i$ values (in ppm) were determined using an external reference, TMU (tetramethylurea − Institute for Reference Materials and Measurements (IRMM), Retieseweg, B-2440, Belgium), of which the isotopic ratio $(D/H)_R$ was precisely

calibrated. $(D/H)_i$ was calculated from the following equation:

$$(D/H)_i = (D/H)_R P_R m_R M_S S_i/(P_i m_S M_R S_R) \qquad (8)$$

where P_i and P_R are the stoichiometric numbers of hydrogens in site i and in the reference; M_S, m_S and M_R, m_R are, respectively, the molecular weight and mass of the samples and of the reference; S_i and S_R are, respectively, the site i mono-deuterated molecule and the reference signal surface area of the ^2H NMR spectrum, obtained using a curve-fitting program (Interlis, Eurofins Scientific, Nantes, France).[23]

IRMS Analysis. The overall carbon-13 and nitrogen-15 contents of samples were measured by isotope ratio mass spectrometry using a Finnigan MAT Delta S spectrometer coupled with a Fisons NA 2100 elemental analyzer. The overall oxygen-18 content of sample was measured in the same way with a Micromass Optima coupled with a Carlo Erba NA 1500 elemental analyzer.

For all carbon and nitrogen analyses, after distillation, samples were accurately weighed into tin capsules that were folded over and loaded onto the sample carousel of the elemental analyzer. They were then dropped sequentially into the combustion region of the elemental analyzer filled with Chromic oxide (temperature: 1035 °C). Combustion in the presence of oxygen and (70 mL/min, 70 KPa) was followed by a copper reduction stage at approximately 650 °C, which ensured total conversion of nitrogen oxides to nitrogen. Water of combustion was removed by an online magnesium perchlorate water trap. The resultant gases (CO_2 and N_2) were then separated by a packed column (Porapak Q, 80 cm) at room temperature using a helium flow rate of 110 mL/min prior to transfer to the IRMS region.

For all oxygen analyses, after distillation, samples were dried on 5 Å molecular sieve. For analysis, accurately weighed samples were sealed in tin capsules and loaded onto the sample carousel of the elemental analyzer. They were then dropped sequentially into the pyrolysis region of the elemental analyzer filled with nickelized carbon (temperature: 1050 °C). The resultant gases (N_2 and CO) were then separated by a packed column (molecular sieve 5 Å, 1.5 m) prior to transfer to the IRMS region.

Samples were weighted to obtain an equivalent of 0.5 mg C for carbon analysis, an equivalent of 0.2 mg N for nitrogen analysis, and an equivalent of 2 mg O for oxygen analysis.

The isotope content of the sample (S) is expressed in per thousand (‰) on the relative δ-scale which refers the isotope ratio of the sample to that of an international reference (R) as follows:

$$\delta‰ = \frac{R_S - R_R}{R_R} x1000 \qquad (9)$$

with $R = {}^{13}C/{}^{12}C$, $^{15}N/^{14}N$, or $^{18}O/^{16}O$. The carbon-13 isotope content of compounds is expressed using Pee Dee Belemnite (PDB) as reference. The nitrogen-15 isotope content of compounds is expressed using atmospheric nitrogen as reference. The oxygen-18 is expressed using V−SMOW as reference. Values obtained are the mean of two or three measurements,

TABLE 13: Determination of the Equilibrium Isotopic Fractionation Factors in Liquid/Vapor Transition of Nitromethane at 101 °C

	$(D/H)_{CH_3}$	$\delta^{15}N‰(NO_2)$	$\delta^{13}C‰(CH_3)$	$\delta^{18}O‰(NO_2)$
starting	114.2 (0.2)	−5.8	−26.2	27.3
remaining	114.3 (0.2)	−5.5	−26.2	27.1
distillate	116.0 (0.3)	−3.3	−25.1	28.4
α_{exp}	0.985 (0.003)	0.9976 (0.0005)	0.989 (0.0005)	0.9988 (0.0005)

and the precision of the determination is usually better than 0.3 ‰.

$$\alpha_{exp} = \frac{\delta_{liq}/1000 + 1}{\delta_{vap}/1000 + 1} \qquad (10)$$

References and Notes

(1) (a) Jancsó, G.; Van Hook, W. A. *Chem. Rev.* **1974**, *74*, *4*(6), 689 and references therein. (b) Jancsó, G.; Rebelo, L. P. N.; Van Hook, W. A. *Chem. Soc. Rev.* **1994**, 257. (c) Jancsó, G.; Rebelo, L. P. N.; Van Hook, W. A. *Chem. Rev.* **1993**, *93*, 5.

(2) (a) Rozen, A. M. *Fractionation of isotopes in distillation columns*; Atomizdat: Moscow, 1960. (b) Robinovich I. B. *Influence of isotopy on the physicochemical properties of liquids*; Consultants Bureau: New York, 1970.

(3) Baertschi, P.; Thürkauf, M. *Helv. Chim. Acta* **1960**, *43*, 80.

(4) Fenske, *Ind. Eng. Chem.* **1932**, *24*, 482.

(5) Rose, A.; Rose, E. In *Techniques of Organic Chemistry*, *Vol. IV, Distillation*; Perry, E. S., Weisberger, A., Eds.; Interscience Publishers: New York, 1965; p 1.

(6) Martin, G. J.; Martin, M. L. *Tetrahedron Lett.* **1981**, *22*(36), 3525.

(7) Martin, M. L.; Martin, G. J. In *NMR Basic Principles and Progress*; Diehl, P., Fluck, E., Günther, H., Kosfeld, R., Seelig, J., Eds.; Springer-Verlag: Berlin, 1990; Vol. 23, p 1.

(8) Pascal, R. A., Jr.; Baum, M. W.; Wagner, C. K.; Rodgers, L. R.; Huang, D. S. *J. Am. Chem. Soc.* **1986**, *108*, 6477.

(9) (a) Zhang, B. L.; Wu, W. X.; Gao, Z. H.; Sun, X. Y. *Acta Chimica Sinica* **1986**, *44*, 437. (b) Zhang, B. L. *Magn. Reson. Chem.* **1988**, *26*, 955.

(10) Zhang, B. L.; Martin, M. L. *J. Am. Chem. Soc.* **1992**, *114*, 7089.

(11) Zhang, B. L.; Mabon, F.; Martin, M. L. *J. Phys. Org. Chem.* **1993**, *6*, 367.

(12) Zhang, B. L.; Pionnier, S. *J Phys. Org. Chem.* **2001**, *14*(4), 239.

(13) Moussa, I.; Naulet, N.; Martin, M. L.; Martin, G. J. *J. Phys. Chem.* **1990**, *94*, 8303.

(14) (a) Kiss, I.; Jákli, Gy.; Jancsó, G.; Illy, H. *J. Chem. Phys.* **1967**, *47*(11), 4851. (b) Kiss, I.; Jákli, Gy.; Jancsó, G.; Illy, H. *Acta Chim. Acad. Sci. Hung.* **1968**, *56*(3), 271.

(15) Kiss, I.; Jákli, Gy.; Illy, H.; G. Jancsó *Report KFKI-71-68*, **1971**.

(16) Jákli, G.; Tzias, P.; Van Hook, W. A. *J. Chem. Phys.* **1978**, *68*(7), 3177.

(17) Jákli, Gy.; Koritsánszky, T.; Jancsó, G. *Ber. Bunsen-Ges. Phys. Chem.* **1981**, *85*, 773.

(18) Borowitz, J. L.; Klein, F. S. *J. Phys. Chem.* **1971**, *75*(12), 1815.

(19) Bigeleisen, J. *J. Chem. Phys.* **1961**, *34*, 1485.

(20) (a) Wolfsberg, M. *J. Chim. Phys. Physicochim. Biol.* **1963**, *60*(1/2), 15. (b) Stern M. J.; Van Hook W. A.; Wolfsberg M. *J. Chem. Phys.* **1963**, *39*, 3179.

(21) Pimentel, G. C.; McClellan, A. L. *The hydrogen bond*; W. H. Freeman & Co.: San Francisco, 1960; p 201.

(22) Kintzinger, J. P. In *NMR Basic Principles and Progres*; Diehl, P., Fluck, E., Kosfeld, R., Eds.; Springer-Verlag: Berlin, 1981; Vol.17, p 1.

(23) Martin, Y. L. *J. Magn. Reson. Ser. A* **1994**, *111*, 1.