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ARTICLE *in* ANALYTICAL CHEMISTRY · OCTOBER 1997

Impact Factor: 5.64 · DOI: 10.1021/ac970314d

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Stereochemical Analysis of Monodeuterated Isomers By GC/MS/MS

M. H. Taphanel,[†] J. P. Morizur,[†] D. Leblanc,[‡] D. Borchardt,[§] and T. H. Morton*[,‡,§]

COS, UMR CNRS 172, Université Paris VI, boîte 45, 4 place Jussieu, 75252 Paris Cedex 05, France, DCMR, URA CNRS 1307, Ecole Polytechnique, 91128 Palaiseau, France, and Department of Chemistry, University of California, Riverside, California 92521-0403

GC/MS/MS techniques to quantitate mixtures of acyclic stereoisomers are explored for a set of secondary *n*-alkyl phenyl ethers vicinally substituted with deuterium. Examination of a set of test compounds using a commercial quadrupole ion trap instrument (GC/QIT) reveals large differences between threo and erythro diastereomers when trapped molecular ions expel neutral alkene as a result of collisionally activated dissociation (CAD). All of the monodeuterated compounds give $PhOH^{\bullet+}$ (*m/z* 94) and $PhOD^{\bullet+}$ (*m/z* 95) as the principal fragments under GC/QIT/CAD conditions. The threo isomers give consistently larger values of the ion intensity ratio r = *m/z* 95/*m/z* 94 than do the corresponding erythro, with a ratio of ratios r_t/r_e as high as 3. A nonlinear calibration curve shows that the threo decomposes to a lesser extent than the erythro in mixtures of the two stereoisomers; hence, the precision of quantitation is sensitive to experimental error. Sustained off-resonance irradiation experiments in an FT-ICR give virtually complete dissociation, which results in a linear calibration curve. Ion−molecule reactions of the fragment ions indicate that alkene expulsion does not take place via a six-member cyclic transition state, while the stereochemical selectivity appears to rule out the intermediacy of ion−neutral complexes. Threo- and erythro-2-phenoxy-3-deuterio-*n*-octane (and their mixtures) are proposed as test compounds for evaluating the capability of GC/MS/MS instrumentation to perform stereochemical analyses on subnanomole samples of isotopically labeled molecules.

Spectrometric quantitation of acyclic stereoisomers at subnanomole levels remains a tantalizing goal. In many cases (e.g., isotopic substitution) chromatography cannot separate diastereomers, and mass spectrometric fragmentation offers the greatest promise for structural characterization. While mass spectrometry may not enjoy a reputation as the preferred technique for stereochemical analysis, the state of the art presented here offers a usable solution for functionalized *n*-alkanes that can be converted to their phenyl ethers. This paper describes an application of commercial gas chromatography/quadrupole ion trap (GC/QIT) instrumentation, which points the direction that further refinements must take in order for GC/MS/MS to provide a reliable method for assaying stereoisomeric purity. Such an approach ought to permit one to measure relative proportions of isomers that differ only by the configuration of hydrogen and deuterium attached to the same carbon atom in chemically nonequivalent positions. The GC interface allows an individual determination to be performed on each component in mixtures of homologues or structural isomers.

Mass spectrometry has long been known (though perhaps not widely recognized) to have the capacity to discern stereochemical differences between isomerically pure compounds. The pioneering work of Audier et al. showed that the ratio of two competing fragmentation pathways can be used to characterize acyclic diastereomers.[1] In that work, each example had two stereocenters, and the four possible isomers comprised two distinct pairs of nonsuperimposable mirror images (enantiomers). One set of enantiomers is often called the threo pair and the other the erythro pair. While two mirror image structures cannot manifest different chemical properties (in the absence of some additonal chiral agent), a threo compound may exhibit features that differentiate it from its erythro isomer.

In 1970−1971, Green and co-workers demonstrated that mass spectrometry can distinguish deuterated diastereomers by means of rearrangements involving five- and six-member cyclic transition states. In the fragmentation pattern of threo- and erythro-$CH_3CHClCH_2CH_2CHDCH_3$, for instance, they reported ion intensity ratios $[M - DCl]^{\bullet+}/[M - HCl]^{\bullet+}$ of 0.36 for the former and 0.26 for the latter.[2] They also measured a comparable level of stereoselectivity in acetic acid elimination from ionized threo- and erythro-$CH_3CH(OCOCH_3)CHDCH_3$ (d_1-*sec*-butyl acetate),[3] in which the stereocenters are adjacent, and subsequently examined the energetic dependence.[4] Ions ≤0.6 eV above threshold display stereoselectivity that decreases with energy, but in more highly excited ions the selectivity fluctuates (though the average does not vary from what is observed in the source mass spectra). In any event, other decompositions compete with the stereoselective pathways, which limits their utility for analytical purposes.

In 1988, Kondrat and Morton[5] described a similar level of stereoselectivity for $CH_3CH(OPh)CHDCH_3$, for which the ratio *r*

[†] Université Paris VI.
[‡] Ecole Polytechnique.
[§] University of California.

(1) Audier, H. E.; Felkin, H.; Fetizon, M.; Vetter, W. *Bull. Soc. Chim. Fr.* **1965**, 3236−3238. For reviews of this subject, see: Green, M. M. *Top. Stereochem.* **1976**, *9*, 35−110. Turecek, F. *Collect. Czech. Chem. Commun.* **1987**, *52*, 1928−1984. Turecek, F.; Splitter, J. S. *Applications of Mass Spectrometry to Organic Stereochemistry*; VCH Publishers; New York, 1994; Chapter 4.
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(5) Kondrat, R. W.; Morton, T. H.*Org. Mass Spectrom.* **1988**, *23*, 555−557.

S0003-2700(97)00314-4 CCC: $14.00

= $[PhOD]^{\bullet+}/[PhOH]^{\bullet+}$ has a value of 0.22 for threo and 0.16 for erythro. The ratio of intensity ratios from electron ionization, r_t/r_e in the range 1.3−1.4, remains virtually unchanged, regardless of whether molecular ion decomposition is examined in the source mass spectrum, by collisionally activated dissociation (CAD), or for metastable ions in the mass-resolved kinetic energy spectrum (MIKES, where ionized phenol constitutes the only fragments). In 1991, Audier and Morton[6] used CAD to analyze the stereospecificity of a unimolecular gas phase reaction that produces ionized $CH_3CH(OPh)CHDCH_3$.

For all of the examples above, hydrogen migration takes place prior to (or concomitant with) fragmentation. For isotopically labeled compounds H-transfer competes with D-transfer. To date, mass spectrometric assays of acyclic diastereomers have been limited to instances where a stereospecific reaction gives one stereoisomer from one starting material and the opposite stereoisomer from another starting material. Published analytical applications have looked at each starting material (and therefore each product) in independent experiments and have succeeded because the products turned out to be >90% diastereomerically pure.[6] Given the possibility that two stereoisomers in a mixture will not decompose to equal extents, the ratio of ratios r_t/r_e would have to increase substantially in order to quantitate a sample in which threo and erythro are mixed together. For linear alkyl phenyl ethers we find that enhancement of the ratio of ratios results simply from elongating the methylene chain in molecules having the general formula $CH_3(CH_2)_mCH(OPh)CHD(CH_2)_nCH_3$. Strictly speaking, the terms threo and erythro do not apply to these higher homolog as they do to the deuterated phenoxybutanes above. However, for the sake of consistency, threo and erythro will be used to designate all of the racemic diastereomers treated in this paper.

Since the analysis depends on intensity ratios of ions that are separated by 1 atomic mass unit, natural-abundance ^{13}C interferes with measurement of the higher mass peak in an ordinary mass spectrum. Therefore, MS/MS techniques, which isolate ions containing only ^{12}C, can give fragmentation patterns that are free from this interference. This paper compares results obtained using GC/MS/MS with those obtained using sustained off-resonance irradiation (SORI)[7] CAD in an FTICR. Advantages of the two techniques are compared.

EXPERIMENTAL SECTION

sec-Alkyl phenyl ethers were synthesized by established methods and purified by distillation from lithium aluminum hydride (or deuteride) at reduced pressure. Each of the vicinally deuterated phenoxyheptanes and octanes was prepared as a mixture of two positional isomers from the corresponding trans (to produce threo) or cis (to produce erythro) alkenes, by epoxidation with *m*-chloroperoxybenzoic acid in CH_2Cl_2, reaction in a sealed tube at 120−140 °C with trace sodium phenoxide in the presence of excess phenol, conversion of the resulting *vic*-phenoxy alcohol to the tosylate, and reduction with lithium aluminium deuteride. The major impurities (no more than a few percent) were corresponding phenoxyalkenes, which did not interfere with the present studies. Where appropriate, isomeric purity was assessed by 76.77 MHz ^2H NMR on a General Electric GN-500 instrument.

Ion−molecule reactions and SORI-CAD were studied using a Bruker Spectrospin CMS-47X FT-ICR spectrometer. For the reaction shown in eq 2, *m/z* 95 ions were produced by 14.5 eV electron ionization of $(CD_3)_2CHOPh$ in an external ion source and injected into an ICR cell containing 1×10^{-8} mbar of pyridine plus 1.5×10^{-7} mbar of argon, allowed to thermalize by collisions with the bath gas, isolated by a series of radio frequency ejection pulses to eliminate all other ions, and permitted to react in the ICR cell over a variable time period, following which the relative abundances of *m/z* 95, 81, and 80 ions were measured by ion cyclotron resonance.[7] For SORI-CAD of $CH_3CH_2CH(OPh)CHDCH_2CH_3$, the molecular ion (*m/z* 179) was produced by 13 ± 0.5 eV electron ionization in the external ion source and injected into an ICR cell containing 2×10^{-7} mbar of argon, isolated by a series of radio frequency ejection pulses to eliminate all other ions, allowed to thermalize for 2 s by collisions with the bath gas, and excited by a 70 μs pulse centered at 403 007 Hz (as compared to the cyclotron frequency of the parent ion, 402 869 Hz), and the fragments were analyzed by ion cyclotron resonance 0.2 s later. Between 90 and 95% of the parent ions dissociate under these conditions; *m/z* 94 and *m/z* 95 are the only fragment ions detected.

GC/MS experiments were performed on a Varian Saturn 3 QIT coupled to a Varian Star 3400CX gas chromatograph equipped with a 30 m DB5 fused-silica capillary column (0.25 mm i.d. and 0.25 μm film thickness). Helium pressure was adjusted to give a column head pressure of 10 psi. The GC injector was held at 180 °C, the GC/QIT transfer line at 260 °C, and the QIT manifold at 120 °C, while the GC was held 5 min at 70 °C and then ramped to 150° over a period of 32 min, during which the *sec*-phenoxy-*n*-alkanes eluted with the following retention times: 2-phenoxybutane, 11.4 min; 3-phenoxypentane, 15.6 min; 2-phenoxypentane, 16.2 min; 3-phenoxyhexane, 19.8 min; 2-phenoxyhexane, 20.8 min; 4-phenoxyheptane, 24.3 min; 3-phenoxyheptane, 25.0 min; 2-phenoxyheptane, 26.1 min; 4-phenoxyoctane, 28.8 min; 3-phenoxyoctane, 29.9 min; 2-phenoxyoctane, 31.3 min. For GC/QIT/CAD experiments 0.2−0.5 nmol samples in 1−2 μL of methanol were injected onto the GC (splitless mode), each component was ionized by 70 eV electron impact as the peaks eluted from the GC, and the $M^{\bullet+}$ ion was isolated in the QIT, stored for 5 ms (during which multiple collisions with helium thermalize the ion), and finally decomposed by resonant CAD for 20 ms with an amplitude setting of 0.2 V.[8] Two types of assessments are reported: of reproducibility, using one CAD mass spectrum recorded on each of ≥3 separate days (Table 1); and of precision, using at least two averaged CAD fragmentation patterns (each the mean of ≥3 individual CAD mass spectra) recorded on each of two separate days (Figure 1). Day-to-day variation is not significantly greater than the variation within one 24 h period.

RESULTS

This study examines effects of chain length and the position of functionality on mass spectrometric differentiation of the diastereomers of monodeuterated *sec*-alkyl phenyl ethers. Scheme

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Table 1. Ion Intensity Ratios ($r = m/z$ 95/m/z 94) from CAD of Molecular Ions (Selected with a 3 amu Window) of Deuterated *sec*-Alkyl Phenyl Ethers

even ($n + m$)	$[PhOD^{\bullet+}]/[PhOH^{\bullet+}]$	odd ($n + m$)	$[PhOD^{\bullet+}]/[PhOH^{\bullet+}]$
phenoxybutanes		phenoxyheptanes	
threo-2-PhO-3-d_1	0.435 (0.015)	*threo*-2-PhO-3-d_1	0.55 (0.04)
erythro-2-PhO-3-d_1	0.36 (0.02)	*erythro*-2-PhO-3-d_1	0.21 (0.02)
2-PhO-1,1,1-d_3	0.13 (0.02)	2-PhO-1,1,1-d_3	0.12 (0.01)
		threo-3-PhO-2-d_1	0.37 (0.06)
phenoxyoctanes		*erythro*-3-PhO-2-d_1	0.21 (0.015)
threo-2-PhO-3-d_1	1.34 (0.21)	*threo*-3-PhO-4-d_1	0.355 (0.045)
erythro-2-PhO-3-d_1	0.47 (0.11)	*erythro*-3-PhO-4-d_1	0.14 (0.04)
threo-3-PhO-2-d_1	0.66 (0.33)	3-PhO-3-d_1	<0.05
erythro-3-PhO-2-d_1	0.39 (0.14)	*threo*-4-PhO-3-d_1	0.39 (0.03)
3-PhO-2,2,4,4-d_4	>20	*erythro*-4-PhO-3-d_1	0.14 (0.04)

[a]Standard deviations (in parentheses) represent averages of single spectra (each one corresponding to the m/z 95 ion intensity maximum) recorded on at least three separate days.

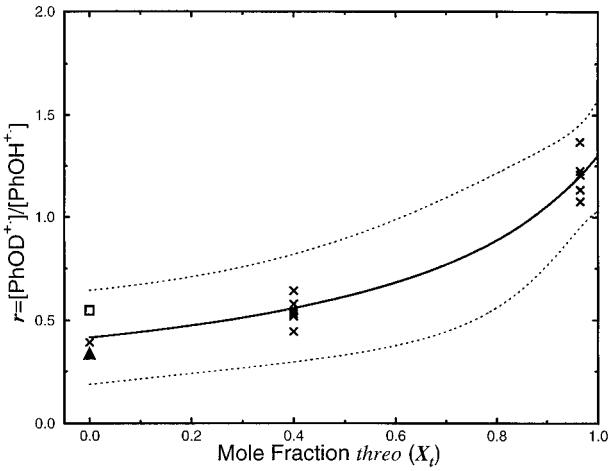
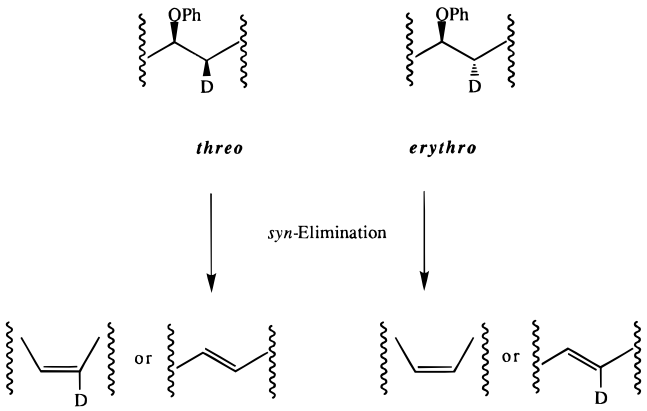


Figure 1. Ratio r vs mole fraction of *threo*-2-phenoxy-3-d_1-octane from GC/QIT/CAD experiments (2 amu window). Each point represents an average of at least three spectra averaged over an eluted peak; the filled triangle represents three points that coincide (within experimental uncertainty of 2%); the open square represents two points that coincide within experimental uncertainty; ×'s represent single points. The solid curve shows the best least-squares fit, and the dashed curves correspond to 95% confidence limits.[9]

Scheme 1



1 illustrates the consequences of elimination of alkene when the deuterium (or hydrogen) and oxygen come together in a cyclic transition state (syn elimination). Syn elimination from the threo isomer to yield an unlabeled double bond gives the trans geometry, while syn elimination from the erythro yields the unlabeled cis. If the expelled alkene retains the deuterium label, then threo forms a *cis*-d_1-alkene and erythro forms a *trans*-d_1-alkene.

The experiments investigated decomposition of the molecular ions ($M^{\bullet+}$) of ethers having the general formula $CH_3(CH_2)_mCH(OPh)CHD(CH_2)_nCH_3$. Table 1 summarizes the values of the m/z 95 to m/z 94 ion intensity ratio $r = [PhOD]^{\bullet+}/[PhOH]^{\bullet+}$ for the cases $n = m = 0$, all the isomers for $n + m = 3$, and a comparison of $n = 0$, $m = 4$ with $n = 1$, $m = 3$. The m/z 95/m/z 94 ion intensity ratio r corresponds to the relative proportions of expulsion of unlabeled vs labeled alkene. Under the experimental conditions, alkene loss to yield $C_6H_5OH^{\bullet+}$ contributes the most intense fragmentation. Apart from the phenoxybutanes (which can have only one *sec*-alkyl structure), each of the alkyl phenyl ethers was prepared from the corresponding alkene as a mixture of positional isomers, which were separated by GC. For instance, *trans*-2-octene yielded a mixture of *threo*-2-phenoxy-3-d_1-octane and *threo*-3-phenoxy-2-d_1-octane (whose ^2H NMR chemical shifts are separated by 0.08 ppm) in the proportion 53:47 (based on NMR integration).

Ion intensity ratio r reflects two types of competition: transfer from the labeled methylene vs the unlabeled group on the other side of the oxygen-bearing carbon, and H- vs D-transfer from the deuterated methylene group. The phenoxyheptanes were chosen for particular scrutiny because they represent the shortest chain length for which competition can be systematically examined from three aspects: (i) 2-phenoxy-3-d_1-heptanes reflect competition between a deuterated methylene and a methyl (just as do the 2-phenoxybutanes); (ii) 4-phenoxy-3-d_1-heptanes exemplify competition between deuterated and undeuterated methylenes that are positionally equivalent; and (iii) comparison between nonequivalent methylenes is provided by 3-phenoxy-2-d_1- and 3-phenoxy-4-d_1-heptane.

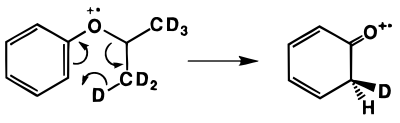
The mechanistic basis for diastereomeric differences remains to be elucidated. At this point our data warrant the following inferences:

(a) Even- and odd-membered chains of comparable length show comparable values of the ratio of ratios r_t/r_e, even though individual values of r may differ substantially. The value of r_t/r_e does not alter dramatically in going from 2-phenoxyheptanes to the 2-phenoxyoctanes;

(b) Intervention of ion−neutral complexes must play a negligible role. Only deuterium transfer is observed in d_4-3-phenoxy-

octane (Table 1), while deuterium geminal to phenoxy (cf. 3-phenoxy-3-d_1-heptane in Table 1) does not transfer.

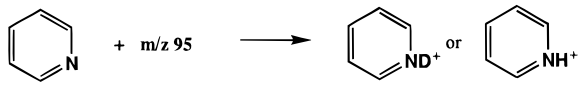
(c) The decomposition operates via some kind of four-membered cyclic transition state, as shown by FT-ICR experiments for ionized isopropyl phenyl ether (*i*PrOPh$^{\bullet+}$) described below (which accord with previously published results[9]). Decomposition via six-member cyclic transition states[3] (such as eq 1 portrays) must represent a very minor pathway, at most.



(1)

m/z 95

In order to gauge the extent of eq 1 in the simplest 2-phenoxyalkane, we have investigated the *m*/*z* 95 ion from ionized $(CD_3)_2CHOPh$. The ion formed by expulsion of neutral alkene reacts with a gaseous base such as pyridine as illustrated in eq 2.



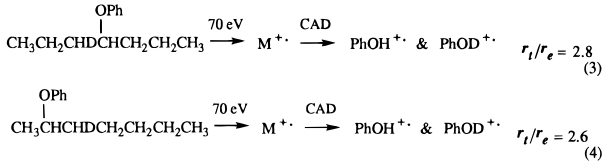
m/z 81 m/z 80 (2)

Had this fragment the ionized cyclohexadienone structure drawn in eq 1, it would transfer H^+ and D^+ in nearly equal proportions. By contrast, the ionized phenol structure PhOD$^{\bullet+}$ should transfer only D^+ to the gaseous base.

In the FTICR *m*/*z* 95 reacts with neutral pyridine to give (relative to the intensity of unreacted *m*/*z* 95 = 100) *m*/*z* 81/*m*/*z* 80 ratios of 60:1 after 4 s reaction time and 1400:50 after 20 s reaction time. Because these data show very little H-transfer and no evidence for a slowly reacting minor component of *m*/*z* 95, eq 1 must constitute no more than 7% of the pathway by which *i*PrOPh$^{\bullet+}$ expels propene. If this conclusion can be generalized to all 2-phenoxy-*n*-alkanes, stereochemical discrimination must reflect constraints of a four-member cyclic transition state.

When ionized 2-phenoxyalkanes dissociate, some of the the $C_6H_5OH^{\bullet+}$ fragments carry hydrogen that originated from the methyl. Audier and co-workers demonstrated this in 1987 for 2-phenoxybutane and 2-phenoxypentane,[9] and our data for CD_3-$CHOPh(CH_2)_4CH_3$ corroborate that result for 2-phenoxyheptane. Chain length appears to have a negligible effect, in terms of competition between methyl and methylene, and the value of *r* reported for 2-phenoxy-1,1,1-d_3-pentane[10] is quite close to the values for the d_3-compounds in Table 1. If we assume the same isotope effect as for decomposition of ionized $CD_3CH(OPh)CH_3$ to $C_6H_5OH^{\bullet+}$, k_H/k_D = 1.4,[11] approximately one-seventh of the ionized linear 2-phenoxyalkanes decompose via hydrogen transfer from the methyl.

One might therefore anticipate that the 2-phenoxy-3-d_1-alkanes should display the highest values of r_t/r_e. Despite this expectation, competition between equivalent chain positions (where only one is monodeuterated) shows a comparable level of selectivity. 4-Phenoxyheptane has a plane of symmetry, which is abolished by deuterating one of the methylenes. In the near-symmetric d_1 analog depicted in eq 3 (4-phenoxy-3-d_1-heptane) the stereoselectivity r_t/r_e is no less than for its 2-phenoxy isomer, drawn in eq 4.



Nonequivalent methylenes manifest unequal degrees of selectivity. The values of r_t/r_e are not the same for the two different vicinally deuterated 3-phenoxyheptanes: r_t/r_e = 1.8 for 2-d_1 and 2.5 for 4-d_1. It turns out that *erythro*-3-phenoxy-4-d_1-heptane exhibits a significantly smaller value of *r* than does *erythro*-3-phenoxy-2-d_1-heptane, while the two *threo*-3-phenoxy-d_1 positional isomers give the same values of *r*.

The origin of the chain length effects requires further exploration, but our lack of complete understanding does not impede the use of stereoselective mass spectrometry as an analytical tool. The example of 2-phenoxy-3-d_1-octane serves to illustrate the capabilities (and current limitations) of GC/QIT/CAD. Sets of averaged spectra give the data summarized in Figure 1 for threo, erythro, and a 40:60 mixture of the two. The value of *r* for the mixture indicates that the relative extents of CAD are not the same for the two stereoisomers: threo contributes proportionately less than its mole fraction X_t. A weighting factor, *W*, must therefore be applied, and the three-point calibration curve in Figure 1 is based on a fit using eq 5.

$$r = \frac{r_e(1 - X_t)}{WX_t + (1 - X_t)} + \frac{r_t W X_t}{WX_t + (1 - X_t)} \qquad (5)$$

The purity of individual diastereomers was assessed by ^{2}H NMR, in which the threo and erythro have chemical shifts separated by 0.2 ppm: the product from *trans*-2-octene contains 96.5% threo and 3.5% erythro, while the product from *cis*-2-octene is >99% erythro. A nonlinear least-squares fit of the data gives a weighting factor W = 0.29, r_e = 0.42 (SD = 0.12), and r_t = 1.31 (SD = 0.11). The reliability of the three-point calibration can be best gauged by the 95% confidence intervals (dotted lines) for the least-squares curve.[12]

In our QIT experiments, only a fraction of the molecular ions dissociate. The weighting factor *W* should become unity if all the ions undergo decomposition. In order to compare QIT CAD with other techniques for collisionally activated dissociation, we prepared pure *threo*- and *erythro*-3-phenoxy-4-d_1-*n*-hexane ($m = n = 1$), which contains no positional isomers and therefore does not require the GC interface. The ^{2}H NMR spectra, reproduced in Figure 2, demonstrate their diastereomeric purity. These compounds (as well as a 40:60 mixture) were studied both by GC/QIT and by SORI-CAD in the FT-ICR. The GC/QIT calibration gives a curve much like that in Figure 1. The SORI-CAD, in which >90% of the *m*/*z* 179 ions dissociate, gives a straight line plot. Figure 3 compares results of the two experiments. The

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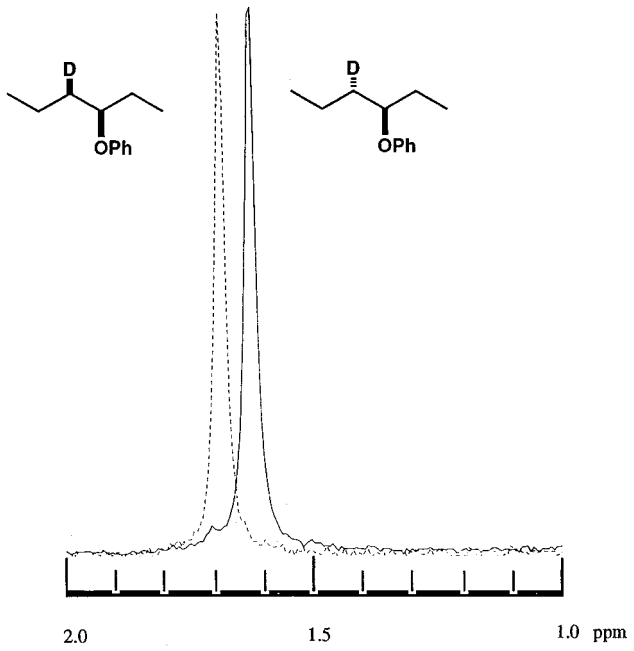

Figure 2. ^{2}H NMR spectra of erythro (dashed line) and threo (solid line) 3-phenoxy-4-d_1-*n*-hexane in chloroform solution at room temperature.

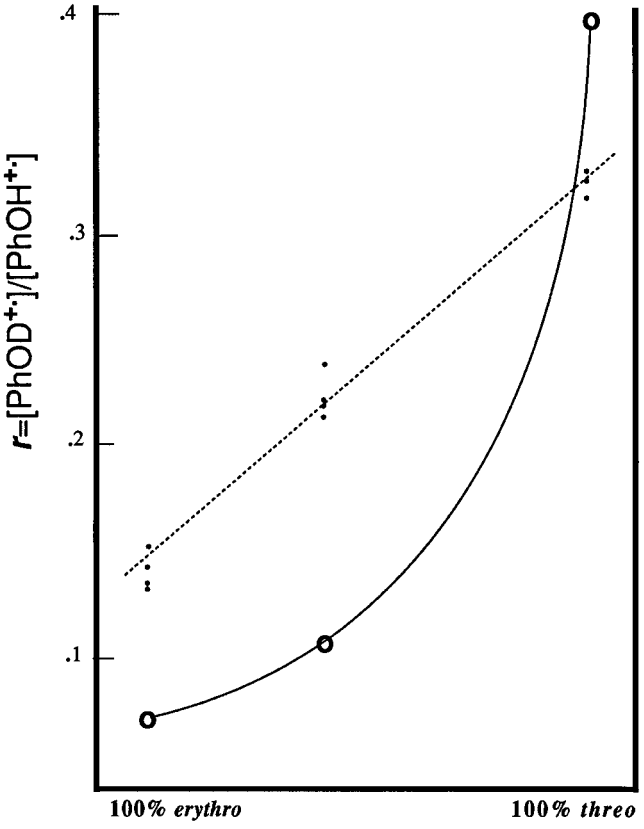

Figure 3. Comparison of CAD spectra of 3-phenoxy-4-d_1-*n*-hexanes (pure diastereomers and a 40:60 mixture) using two different instruments and techniques: GC/QIT (solid curve with open circles representing average values of *r*; error bars omitted for clarity) and FT-ICR SORI (dashed line, with individual data points).

Discussion below briefly outlines the relative merits of the two CAD methods.

DISCUSSION

This work illustrates scope and limitations of mass spectrometric analysis of acyclic compounds that exhibit diastereomerism solely as a consequence of isotopic substitution. If the goal is to tell which of two pure stereoisomers is present in a sample, GC/QIT/CAD solves the problem in many cases. Most of the examples in Table 1 show significant differences between threo and erythro that can serve to identify a pure sample. Analytical problems of this sort are often encountered in enzymology, where elucidation of the mechanism of a highly stereoselective reaction hinges on determining the placement of an isotopic label. On the other hand, not all enzymic reactions exhibit complete stereochemical fidelity.[13] In such instances, the analytical task becomes more challenging not only to identify the major isomer but also to measure its proportion relative to the minor isomer. Here a large difference between isomers does not suffice to guarantee the success of a spectrometric approach, for one may contribute more strongly to the signal than the other.

Mass spectrometry, in particular, can exhibit differential response characteristics when quantitation requires the measurement of fragment ion intensity ratios. In the example of the 2-phenoxy-3-deuterio-*n*-octanes, the stereochemical sensitivity r_t/r_e is ~3. At the same time, however, the erythro isomer tends to overwhelm the threo in the GC/QIT CAD of mixtures, with a relative weighting factor for the latter of $W = 0.3$, when the isolated molecular ion does not completely dissociate. The curve in Figure 1 displays the consequences of the tradeoff between r_t/r_e and W. The dashed lines represent 95% confidence limits and give an idea of the precision of mixture analysis using GC/QIT/CAD on a commercial instrument operating under routine conditions. Quantitation of a mixture becomes possible for $X_t > 0.5$, with an uncertainty that decreases with increasing mole fraction of threo. Hence, if the majority diastereomer happens to be threo, then GC/QIT is a method of choice, since the technique is especially sensitive in this domain.

In more general situations (or when the analyte contains majority erythro), a linear relationship between mole fraction and ion abundance ratio is to be preferred. Such is the case when virtually all of the ions undergo collisionally activated decomposition, such as in the SORI-CAD experiments summarized in Figure 3. At present, however, SORI-CAD conditions have yet to be optimized for use with a GC interface.

The two diastereomers of most of the vicinally deuterated *sec*-phenoxy-*n*-alkanes exhibit substantial differences in the CAD mass spectra of their molecular ions. Ionized phenol (or its deuterated analog) is virtually the only fragment ion produced, which removes complications arising from competing dissociation pathways. The major limitation of MS/MS for mixture quantitation comes from scatter in the values of the m/z 95/m/z 94 intensity ratio r. The variation in r from day to day (cf. Table 1) turns out not to be much greater than the variance among measurements made on the same day (even when r is averaged over several spectra in a given GC/MS trace). Further refinement therefore relies upon improvements in the precision with which r can be determined, and efforts are underway to attain this goal.

In order to guide subsequent developments we suggest the 2-phenoxy-3-deuterio-*n*-octane diastereomers as test compounds. In part, this choice derives from the large ratio of ratios, r_t/r_e observed here. In addition, the pertinence of this problem to assessing the metabolism of xenobiotics lends these compounds particular relevance. Hydroxylation of octane by the enzyme

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cytochrome P-450 oxidase has been extensively studied and shows a decided preference for the 2-position.[14] A number of isozymes are known,[15] but a practical stereochemical probe of the competition of retention vs inversion of configuration has yet to be devised, so that the effects of protein structure and reaction conditions can be systematically investigated. Stereospecific conversion of secondary alcohols to their phenyl ethers represents a straightforward derivatization, following which the mass spectrometric strategy outlined here is appropriate. Since enzymic hydroxylation of octane also produces 1-octanol and 3-octanol, chromatography will also have to play a role in the analysis, as well.

In the long run, GC/MS/MS shows promise for toxicological and pharmacokinetic studies that address the stereochemistry of an isotopic label. The recognized advantages of mass spectrometry—rapidity, well-developed interfaces with data systems, high sensitivity, and small sample size—are now augmented by a demonstration of its potential for analyzing stereoisomeric mixtures.

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ACKNOWLEDGMENT

The advice of Prof. Subir Ghosh regarding statistical analysis is greatly appreciated. This work was supported by the CNRS and by NSF Grant CHE 9522604. Portions of this work were presented in the session on Analytical Chemistry and Nanosciences at the 36th IUPAC Congress, Geneva, Switzerland, 21 August 1997.

Received for review March 21, 1997. Accepted August 4, 1997.[⊗]

AC970314D

[⊗] Abstract published in *Advance ACS Abstracts*, September 15, 1997.