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Supplementary Material Available: Tables III-VIII of rate constants for atropisomerization of (*S*)-(+)-BN in nematic solvents (2 pages). Ordering information is given on any current masthead page.

The ^{18}O Isotope Shift in ^{15}N NMR Spectroscopy. 3. Effects of Structure and Solvent

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Abstract: Various ^{15}N,^{18}O-labeled compounds were synthesized and the ^{18}O-induced isotope shifts on their ^{15}N NMR spectra were measured in order to examine the effects of structural changes on the magnitudes of the shifts. The measured shifts vary greatly, ranging from 0.027 ppm for a nitrile oxide to 0.159 ppm for an isoxazole. The large isotope shifts of isoxazoles compared to oximes and isoxazoline are attributed to the aromatic nature and shorter N-O bond lengths of the isoxazoles. The nature of substituents in the para position of some aromatic aldehyde and ketone oximes affects the magnitude of the ^{18}O-induced shift. Intramolecular hydrogen bonding significantly decreases the magnitude of the isotope shift in oximes. A study of the effect of solvents on the isotope shifts of three oximes is made. A significant decrease in the isotope shift is observed in solvents having an electron donor atom such as oxygen or nitrogen. This decrease in the magnitude of the isotope shift is ascribed to the formation of hydrogen bonds between the oxime hydroxyl proton and the oxygen or nitrogen of the solvent. Possible applications of the ^{18}O isotope shift in ^{15}N NMR are briefly discussed.

Effects of isotopic substitution on nuclear magnetic resonance signals have been known for some time. Ramsay and Purcell[1] first predicted an isotope effect on nuclear magnetic resonance signals, and this was soon followed by reports of a ^{2}H isotope induced shift on ^{1}H and ^{19}F NMR spectra.[2-4] Since then, isotope-induced shifts on NMR spectra of other nuclei have been increasingly studied and utilized. The advent of high-field Fourier transform NMR instruments with increased sensitivity and resolution has facilitated the measurement of many of the new isotope effects, including those due to oxygen. It was known that when oxygen-18 was substituted for an oxygen-16, an upfield shift occurred in the NMR signals of ^{1}H, ^{55}Mn, and ^{95}Mo.[5-7] Particularly following reports of ^{18}O isotope shifts on ^{31}P and ^{13}C NMR,[8,9] interest in the area has increased. Hansen has reviewed the isotope effects of all nuclei through 1981, and Forsyth has recently surveyed the isotope effects on ^{13}C NMR chemical shifts and coupling constants.[10,11] These isotope shifts are being extensively utilized to understand mechanisms of reactions and to elucidate biosynthetic pathways.[12-17]

The existence of the ^{18}O isotope shift on ^{15}N NMR was first demonstrated by Van Etten and Risley in studies with ^{15}N,^{18}O-labeled samples of sodium nitrite, silver nitrite, and sodium nitrate.[18] The ^{18}O shift on ^{15}N NMR was then employed to study the kinetics of the acid-catalyzed oxygen-exchange reaction between sodium nitrite and water. Subsequently it has been utilized to establish the source of oxygen in the oxidation of ammonium ion to nitrite ion by a *Nitrosomonas* species,[19,20] the conversion of nitrite ion to nitrate ion by *Nitrobacter agilis*,[21] and in the biosynthesis of 3-nitropropionic acid from L-aspartic acid by cultures of *Penicillium atrovenetum*.[22] This rapid utilization is due in part to the fact that NMR spectroscopy is a direct method with distinct experimental advantages over many conventional mass spectroscopic methods, particularly those that require a conversion to volatile compounds for analysis.

In the limited number of cases that have been studied so far, the magnitudes of the ^{18}O isotope induced shifts made it relatively easy to employ ^{15}N NMR spectroscopy as a tool to study the particular pathways. Because the ^{18}O isotope induced shift on ^{15}N NMR is a relatively new addition to the library of isotope effects on NMR signals, it is important to define the characteristics of the spectral shifts. Most fundamental among these would be an examination of the relationship between structure and magnitude of the shift. Recently we reported the synthesis of a key

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synthetic intermediate, [^{15}N,^{18}O]hydroxylamine hydrochloride.[23] In the present investigation, we describe the synthesis of a number of ^{15}N,^{18}O dual-labeled compounds and the measurement of the ^{18}O-induced shifts on the ^{15}N NMR spectra, in order to examine the effect of structure on the isotope shifts. Solvent effects on the isotope shifts of several oximes are also described.

Experimental Section

Sodium [^{15}N]nitrite (95 atom % ^{15}N, MSD Isotopes), sodium [^{15}N]-nitrate (99 atom % ^{15}N, MSD Isotopes), [^{18}O]water, (95+ atom % MSD Isotopes), borane methyl sulfide (2 M, Aldrich), deuteriochloroform (99.8 atom % ^{2}H containing 1% $Si(CH_3)_4$, Aldrich), deuteriodimethyl sulfoxide (99.5+ atom % ^{2}H, Baker), deuterioacetonitrile (99.8+ atom % ^{2}H, MSD Isotopes), and deuterium oxide (99.8 atom % ^{2}H, Aldrich) were used. All other reagents were of analytical grade. Glass-distilled water was used in all syntheses and other manipulations. The ^{18}O isotope enrichment in each compound was quantitated by ^{15}N NMR and by mass spectrometry using a Finnigan 4000 instrument equipped with a 9610 gas chromatograph and a Nova 4 Data System.

NMR Spectra

A Varian XL-200 NMR spectrometer (Sperry data system) fitted with a 10-mm broad band probe operating at 20.28 MHz and equilibrated at 25 °C was used for obtaining high-resolution ^{15}N NMR spectra. Quadrature phase detection with a sweep width of ±50 Hz (sometimes ±150 Hz), a 40–60° pulse angle, and an acquisition time of 10 s was used. The protons were efficiently decoupled with a custom-built Waltz broad band modulator and a ^{1}H power of 0.6 W, as previously described.[23] Modulator power was measured with a Bird 4430 RF wattmeter. The use of low-power decoupling minimized temperature gradients in the sample. Care was taken to exclude any paramagnetic impurity because most of the compounds reported here are excellent ligands for transition metals. The ^{15}N NMR spectra were recorded under conditions of optimum field homogeneity. As a result, high-resolution spectra with line widths at half-height as small as 60 mHz could be obtained, permitting the measurement of the ^{18}O-induced isotope shift with good precision. The FID, collected in 2–6K data blocks, were zero filled to 32K and Fourier transformed with a line broadening factor of 0.003 Hz. Some of the ^{15}N NMR spectra were recorded on a Varian XL-200 Advance NMR spectrometer fitted with a 5-mm broad band probe operating at 20.28 MHz. The ^{1}H NMR spectra were recorded on a Perkin-Elmer 90-MHz CW NMR spectrometer.

The isotope shifts of most of the compounds were measured in deuteriated chloroform solution. However, owing to the poor solubility of the oximes of 4-nitrobenzaldehyde, 2,6-dichlorobenzaldehyde, and 4-nitroacetophenone in $CDCl_3$, they were dissolved in a mixture of 1:4 (v/v) dimethyl sulfoxide–chloroform. Tetrahydrofuran was employed as the solvent for dimethylglyoxime, while acetone-d_6 in a coaxial 5-mm tube was used as an external lock. In the study of the effect of solvents upon the ^{18}O isotope shifts of three oximes, acetonitrile, chloroform, dimethyl sulfoxide, and pyridine were deuteriated, while benzene and tetrahydrofuran were nondeuteriated. When the latter two solvents were used, the isotope shifts were measured in a 10-mm probe. Acetone-d_6 in a coaxial 5-mm tube was inserted into the 10-mm tube to provide a lock signal.

Syntheses

Sodium [^{15}N,^{18}O]nitrite was prepared as reported earlier[23] from $Na^{15}NO_2$ and $H_2{}^{18}O$. The ^{15}N and ^{18}O enrichments were 95% and 30%, respectively. Sodium [^{15}N,^{18}O]nitrate employed for the synthesis of aromatic nitro compounds was prepared by a literature procedure.[24]

Silver [^{15}N,^{18}O]Nitrite. It was made by the metathetical reaction between $Na^{15}N^{18}O_2$ and $AgNO_3$. An aqueous solution of $AgNO_3$ was slowly added to an equimolar solution of $Na^{15}N^{18}O_2$ in water with vigorous stirring. After the mixture cooled, the pale-yellow precipitate of silver nitrite was filtered and washed sequentially with water, alcohol, and ether. It was dried in the dark and stored in an amber bottle. The yield was 95%.

[^{15}N,^{18}O]Hydroxylamine Hydrochloride. This key intermediate was made from $Na^{15}N^{18}O_2$.[23] Two samples of ^{15}N,^{18}O-hydroxylamine hydrochloride were made with respective ^{15}N,^{18}O enrichments of 95% and 30% in one preparation and 50% and 30% in the other. The latter was made by mixing equal amounts of $Na^{15}N^{18}O_2$ (^{15}N, 95%; ^{18}O, 30%) and $NaNO_2$ before reduction to $NH_2OH{\cdot}HCl$. The ^{15}N,^{18}O enrichments were checked by mass spectral analysis.

Synthesis of [^{15}N,^{18}O]Oximes. The oximes of aromatic aldehydes and ketones were made by reacting the respective carbonyl compound and [^{15}N,^{18}O]hydroxylamine hydrochloride. The general procedure was as follows.

In a small vial, sodium hydroxide (120 mg, 3 mmol) was dissolved in 0.5 mL of water and 1.5 mL of alcohol. The respective carbonyl compound (1.5 mmol) was added followed by 105 mg (1.5 mmol) of [^{15}N,^{18}O]hydroxylamine hydrochloride. The vial was immediately stoppered and shaken vigorously until the solution became almost homogeneous (about 10 min). It was allowed to stand for 5 min, and the total volume was adjusted to about 10 mL with water. A stream of carbon dioxide was passed through until the precipitation of the oxime was complete. The suspension was cooled, filtered, washed with water, and dried in a vacuum desiccator over KOH. The purity of the product was checked by its melting point and ^{1}H NMR spectrum. Thus, the oximes of *p*-methoxy-, *p*-(dimethylamino)-, *p*-chloro-, *p*-nitro-, *o*-hydroxy-, *o*-methoxy-, and 2,6-dichlorobenzaldehydes; acetophenone; *p*-methyl-, *p*-chloro-, and *p*-nitroacetophenones; and cyclopentanone were made. In the case of benzaldehyde oxime, a semisolid resulted on saturation with carbon dioxide because of the low melting point (mp 35 °C) of the oxime, so the isolation was slightly modified. The reaction mixture was saturated with NaCl, and the oxime was extracted with ether. The ether extract was dried with anhydrous $MgSO_4$, filtered, and evaporated. In the oxime preparations, the yields ranged from 75% to 95%.

Diacetyl [^{15}N,^{18}O]Monoxime. An aqueous solution of $^{15}NH_2{}^{18}OH{\cdot}HCl$ (105 mg, 1.5 mmol in 1 mL of water) was added slowly to a stirred aqueous solution of diacetyl (0.13 mL, 1.5 mmol in 2 mL of water). A white precipitate formed in 2 min; stirring was continued for 30 min. The reaction mixture was saturated with NaCl and extracted with ether. The ether layer was dried with anhydrous sodium sulfate and filtered, and the solvent was evaporated: yield 110 mg (70%).

[^{15}N,^{18}O]Dimethylglyoxime. This was also made from diacetyl and hydroxylamine hydrochloride by reacting them in a 1:2 molar ratio. An aqueous solution of diacetyl (0.17 mL, 2 mmol in 1 mL of water) was added dropwise to a stirred solution of $^{15}NH_2{}^{18}OH{\cdot}HCl$ (300 mg, 4.3 mmol) in 1 mL of water. Stirring was continued for 3 h, and the precipitated dimethylglyoxime was filtered, washed with water, and dried in a vacuum desiccator over KOH: yield 195 mg (83%).

Pentane-2,3,4-trione-3-[^{15}N,^{18}O]oxime. Nitrosation of an active methylene compound[25] was utilized for synthesis. 2,4-Pentanedione (0.21 mL, 2 mmol) was dissolved in 0.4 mL of glacial acetic acid and cooled to 5 °C. A solution of $Na^{15}N^{18}O_2$ (145 mg, 2.1 mmol) in 0.3 mL of water was slowly added with vigorous stirring. After 30 min, it was diluted with water, saturated with NaCl, and extracted with ether. The ether extract was dried with Na_2SO_4 and evaporated. The yield was 225 mg (87%). The mass and ^{15}N NMR spectra showed that the percentage of ^{18}O in the product was lower than that in the starting $Na^{15}N^{18}O_2$, with approximately one-third of the ^{18}O label being lost during the synthesis.

Ethyl [^{15}N,^{18}O]Isonitrosoacetoacetate. By following the above procedure, the active methylene group of ethyl acetoacetate was nitrosated with $Na^{15}N^{18}O_2$ and acetic acid on a 2-mmol scale. Here again, the loss of oxygen-18 was about one-third: yield 290 mg (91%).

[^{15}N,^{18}O]-1-Nitrobutane. This was made by Victor Meyer reaction.[26] An ethereal solution of 1-bromobutane (0.32 mL, 3 mmol, in 20 mL of dry ether) was added slowly over a period of 1 h to a well-stirred suspension of silver [^{15}N,^{18}O]nitrite (480 mg, 3.1 mmol) in 25 mL of dry ether at 0 °C and in the dark. Stirring was continued for 24 h at 0 °C and another 24 h at room temperature. The precipitated silver bromide was removed by filtration and the residue washed with dry ether. The filtrate was fractionally distilled to remove ether, and then the temperature was raised to distill *n*-butyl nitrite. The fraction boiling at 75–100 °C was collected. Finally, 1-nitrobutane was distilled at reduced pressure and collected in a cold receiver: yield 250 mg (81%).

[^{15}N,^{18}O]Nitrobenzene.[27] Sodium [^{15}N,^{18}O]nitrate (175 mg, 2 mmol) was added to a stirred solution of benzene (0.18 mL, 2 mmol) in 10 mL of trifluoroacetic acid. After stirring for 24 h, ether was added and the acid was carefully neutralized with dilute sodium hydroxide. The ether layer was separated, and the aqueous layer was extracted once with ether. It was dried over anhydrous $MgSO_4$ and evaporated: yield 230 mg (93%). A high retention of the oxygen label was observed, with 35% ^{18}O in nitrobenzene compared to 39% in the sodium nitrate.

[^{15}N,^{18}O]Isoxazoles. A procedure involving a (3 + 2) cycloaddition starting from a 1,3-dicarbonyl compound and $NH_2OH{\cdot}HCl$ was followed.[28] Thus, benzoylacetone (324 mg, 2 mmol) in 5 mL of absolute

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ethanol was gently refluxed for 3 h with $^{15}NH_2{}^{18}OH{\cdot}HCl$ (145 mg, 2.1 mmol). The mixture was cooled and diluted with water until the precipitation of 3-methyl-5-phenylisoxazole was complete. The colorless crystals were collected on a Buchner funnel, washed with water, and dried in a vacuum desiccator: yield 300 mg (94%). 3,5-Diphenylisoxazole was made on a 2-mmol scale from dibenzoylmethane 448 mg) and $^{15}NH_2{}^{18}OH{\cdot}HCl$ (145 mg), following the same procedure employed for 3-methyl-5-phenylisoxazole. The yield of [^{15}N,^{18}O]-3,5-diphenylisoxazole was 430 mg (97%).

[^{15}N,^{18}O]-2,6-Dichlorobenzonitrile oxide was prepared by the oxidation of 2,6-dichlorobenzaldehyde oxime by *N*-bromosuccinimide (NBS).[29] The oxime (285 mg, 1.5 mmol) was dissolved in 1 mL of dimethylformamide (DMF) and cooled to below 10 °C. This solution was stirred while 81 mg of sodium methoxide was added, followed by the slow (10 min) addition of a solution of 267 mg of NBS (1.5 mmol) in 3 mL of DMF. Stirring was continued for an additional 30 min, and the reaction mixture was diluted with ice water. After standing at 0 °C for a few hours, the reaction mixture was filtered, and the product was washed thoroughly with water and dried in a vacuum desiccator over KOH. The purity of the product was checked by its ^1H and ^{13}C NMR spectra, and the ^{18}O shift was measured immediately because the compound decomposed upon storage.

[^{15}N,^{18}O]-3,5-Diphenylisoxazoline. Benzylideneacetophenone was prepared by Vogel's procedure.[30] The reported procedure for the synthesis of 3-(*p*-methoxyphenyl)-5-(*m*-nitrophenyl)isoxazoline was modified for making 3,5-diphenylisoxazoline from hydroxylamine hydrochloride and benzylideneacetophenone.[31] The ketone (1.04 g, 5 mmol), $^{15}NH_2{}^{18}OH{\cdot}HCl$ (0.35 g, 5 mmol), and ethanol (30 mL) were placed in a 100-mL flask equipped with a reflux condenser and an addition funnel. After stirring for 5 min under a nitrogen atmosphere, 20 mL of boiling distilled water was added. (The water was boiled in order to remove dissolved oxygen which would otherwise sensitize the decomposition of free base hydroxylamine.) A hot solution of potassium hydroxide (1 g in 10 mL of boiled water) was quickly run into the flask from the addition funnel. It was refluxed for 3 h and allowed to stand overnight at −15 °C. The crystallized material was filtered, washed thoroughly with water, and dried in a desiccator. The yield of pale-yellow crystals melting at 72 °C (reported[31] 74–5 °C) was 250 mg (22%).

Results and Discussion

All the compounds were dual labeled with ^{15}N and ^{18}O: the enrichment of ^{15}N was either 50% or 95% and of ^{18}O was 30%. In analogous studies of the ^{18}O isotope shift on ^{13}C NMR, most of the compounds had oxygens that could be exchanged with water oxygens in the presence of an acid or enzymes or at elevated temperatures. Here, on the contrary, such an exchange of oxygen between water and N–O bonds is seldom possible. Fry and Lusser found no detectable oxygen exchange between water and the oxygens of aliphatic and aromatic nitro compounds under acidic and alkaline conditions and at elevated temperatures.[32] We observed no exchange of oxygen between [^{18}O]water and hydroxylamine hydrochloride under a similar range of conditions. The facile exchange of oxygen is limited to inorganic nitrite, nitrate, and some oxides of nitrogen. With most other compounds, the N–O bonds do not reversibly break and reform but instead result in rearrangements.[33-37] Consequently, the compounds described here were all synthesized from suitable ^{15}N,^{18}O dual-labeled precursors.

As a nucleus of spin $^1/_2$, ^{15}N has no quadrupole moment. In addition, the longer spin–lattice relaxation times (T_1) predict very narrow natural line widths for the ^{15}N resonance frequencies when there is no interaction with a quadrupolar or paramagnetic nucleus and when there is no chemical exchange taking place at intermediate rates on the NMR time scale. This makes it possible to measure the ^{18}O isotope induced shift on ^{15}N NMR with relative ease. Representative high-resolution spectra showing the effect on the ^{15}N NMR signal of *p*-chlorobenzaldehyde oxime when ^{16}O is replaced by ^{18}O are shown in Figure 1. By convention, the signal due to the ^{15}N–^{16}O compound is assigned to be 0.0 ppm. In each of the compounds studied, an upfield shift is observed upon ^{18}O substitution. The ^{18}O isotope shifts of other compounds measured in the present study are given in Table I.

The magnitudes of such isotope shifts are a function of the shielding range of the resonant nucleus,[10] and hence the one-bond ^{18}O isotope shifts on ^{15}N NMR are expected to be significantly larger (per ^{18}O) than the one-bond ^{18}O isotope shifts on ^{13}C or ^{31}P NMR. Thus, the observed ^{18}O shifts on ^{15}N NMR range from 0.027 ppm in a nitrile oxide to 0.159 ppm in an isoxazole (Table I), while the ^{18}O isotope shifts on ^{13}C NMR range from 0.015 ppm (per ^{18}O) for the carbonate carbon of an orthocarbonate to

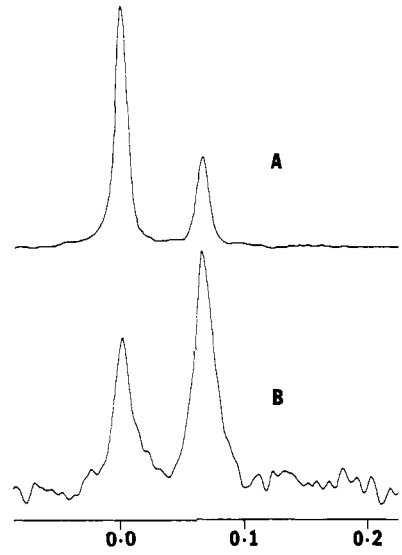

Figure 1. ^{15}N NMR spectra of ^{15}N-enriched *p*-chlorobenzaldehyde oxime containing different percentages of oxygen-18. An upfield shift of 0.063 ppm is observed upon replacement of oxygen-16 by oxygen-18: A, 27% ^{18}O; B, 60% ^{18}O.

Table I. ^{18}O Isotope Induced Shifts on ^{15}N NMR Spectra of Various Compounds[a]

compd	$\Delta\delta$, ppm[b]
benzaldehyde oxime	0.064
p-methoxybenzaldehyde oxime	0.066
	0.051[c]
p-(dimethylamino)benzaldehyde oxime	0.066
p-chlorobenzaldehyde oxime	0.063
p-nitrobenzaldehyde oxime	0.048[c]
o-methoxybenzaldehyde oxime	0.059
o-hydroxybenzaldehyde oxime	0.051
2,6-dichlorobenzaldehyde oxime	0.048[c]
acetophenone oxime	0.068
p-methylacetophenone oxime	0.069
p-chloroacetophenone oxime	0.068
p-nitroacetophenone oxime	0.054[c]
cyclopentanone oxime	0.063
diacetyl monoxime	0.059
dimethylglyoxime	0.067[d]
pentane-2,3,4-trione-3-oxime	0.030
ethyl isonitrosoacetoacetate	0.035
1-nitrobutane	0.080[e]
nitrobenzene	0.075[e]
2,6-dichlorobenzonitrile oxide	0.027
3-methyl-5-phenylisoxazole	0.153
3,5-diphenylisoxazole	0.159
3,5-diphenylisoxazoline	0.074

[a] The solvent was $CDCl_3$ in all cases except those indicated. [b] All shifts are upfield, relative to the ^{16}O-substituted derivative. [c] The solvent was a Me_2SO–$CDCl_3$ mixture in a 1:4 v/v ratio. [d] THF was the solvent with deuterioacetone as an external lock. [e] Per ^{18}O.

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0.054 ppm for the carbonyl carbon on di-*tert*-butylketone.[38] The greater magnitude of the ^{18}O shifts on ^{15}N NMR makes their measurement and assignment somewhat easier, thus somewhat offsetting the disadvantage of the lower sensitivity of ^{15}N compared to ^{13}C NMR.

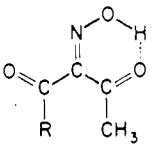
From Table I, it is also obvious that the isotope shifts vary significantly for different types of compounds. However, within a given class, such as oximes or isoxazoles, the variations are small. The isotope shifts of the oximes of aromatic aldehydes are slightly smaller than the shifts in the oximes of aromatic ketones. For example, the ^{18}O shift of benzaldehyde oxime is 0.064 ppm compared to 0.068 ppm in acetophenone oxime.

Unlike the parent carbonyl compounds, oximes exhibit geometrical isomerism. Depending on the nature and size of the substituents, either the *E* or *Z* forms or a mixture of both isomers may result. Here, only the thermodynamically more stable *E* isomers were formed in all the reactions. Even among the *E* isomers, steric interactions in acetophenone oxime would be larger than in benzaldehyde oxime. The larger ^{18}O isotope shifts in acetophenone oxime and other aromatic ketone oximes are attributed to changes in the electron density around nitrogen caused by steric interactions of the methyl and the hydroxyl groups. However, it should also be noted that the ^{18}O isotope shift for the carbonyl carbon of acetophenone is greater than that of benzaldehyde.[39,40]

The isotope shifts of the oximes of various para- and ortho-substituted benzaldehydes and para-substituted acetophenones are listed in Table I. Small electronic effects were observed. When there is a group capable of increasing the electron density in the benzene ring, the isotope shift becomes greater than that of an unsubstituted oxime. Thus, an isotope shift of 0.066 ppm was observed for both *p*-methoxy- and *p*-(dimethylamino)benzaldehyde oxime, as compared to 0.064 ppm in benzaldehyde oxime and 0.063 ppm in *p*-chlorobenzaldehyde oxime. A similar trend is also seen for the variation of isotope shifts with different para substituents of acetophenone oximes. Substituents induce changes in ^{15}N NMR chemical shifts by altering the electron density at nitrogen and the C–N bond order.[41] In turn, this causes changes in the paramagnetic shielding contribution to the overall screening of the nucleus. The isotope shift, being fundamentally vibrational in origin, is affected by the changes in the electron density and C–N bond order. Because the differences in isotope shifts of the oximes of para-substituted benzaldehydes and acetophenones are small, and the number of compounds studied so far is limited, no attempt is made to correlate the isotope shifts to Hammett constants.[40,42]

The isotope shifts of the two ortho-substituted benzaldehyde oximes are rather different from the rest of the oximes. First, *o*-methoxybenzaldehyde oxime exhibits a smaller isotope shift of 0.059 ppm as compared to 0.066 ppm of the *p*-methoxy analogue. The reason for this decrease in the isotope shift is not clear. When there is a hydroxyl group in the ortho position, as in salicylaldehyde oxime, the magnitude of the isotope shift is significantly decreased (to 0.051 ppm, compared to 0.064 ppm for benzaldehyde oxime). An X-ray crystallographic study and infrared spectral studies on *o*-hydroxybenzaldehyde oxime indicate that there is strong intramolecular hydrogen bonding between the *o*-hydroxy proton and the lone pair of electrons on the nitrogen.[43,44] The smaller isotope shift may thus be attributed to the deshielding of the nitrogen nucleus as a result of strong intramolecular hydrogen bonding.

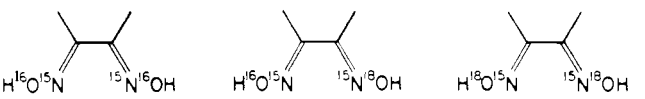
Cyclopentanone oxime, the only alicyclic ketone oxime studied here, exhibits an isotope shift of 0.063 ppm. This value is quite close to the isotope shift of acetone oxime reported earlier.[23] Surprisingly, the isotope shift in pentane-2,3,4-trione-3-oxime (structure **1**, R = CH_3) is very low, 0.030 ppm as compared to



1 R = CH_3, OC_2H_5

0.061 ppm for acetone oxime. Thus, replacement of the two methyl groups by two acetyl groups causes the isotope shift to decrease by about one-half. Such a difference could be due either to the electron-withdrawing nature of the acetyl group or to intramolecular hydrogen bonding (structure **1**) or to both. To determine why, we compared the ^{18}O shifts of some related compounds. Diacetyl monoxime was synthesized and confirmed to be the *E* isomer from its melting point and ^1H and ^{13}C NMR spectra. The oxime hydroxyl and the carbonyl groups are trans to each other in this isomer. Consequently, there is no intramolecular hydrogen bonding of the oxime hydroxyl group to the carbonyl oxygen. The isotope shift is found to be 0.059 ppm, very close to the shift of acetone oxime. Thus, it seems likely that it is the strong intramolecular hydrogen bonding and not the inductive (–I) effect of the acetyl group that induces a large decrease in the isotope shift observed in petane-2,3,4-trione-3-oxime. The infrared spectral studies show that a large shift in the IR stretching frequency of the O–H bond occurs when it is involved in a strong intramolecular hydrogen bond such as the stable six-membered-ring arrangement in pentane-2,3,4-trione-3-oxime.[45] Strong intramolecular hydrogen bonding lengthens the O–H bond and alters the electron density around the nitrogen as well as the C–N bond order, thereby causing changes in the overall screening of the nitrogen nucleus. On the basis of IR and ^1H NMR spectral studies of some ^{18}O-labeled compounds, it is also reported that the strength of an ^{18}O–H···X hydrogen bond is different from its value for the corresponding ^{16}O–H···X bonds.[46,47] Therefore, it appears that the combined effects of strong intramolecular hydrogen bonding involving a six-membered ring, and possibly small differences in the strength of hydrogen bonds involving $^{16}O/^{18}O$ isotopes, are responsible for the relatively small isotope shift of pentane-2,3,4-trione-3-oxime. Support for this conclusion also comes from the 0.035 ppm isotope shift observed for a structurally similar compound, namely, ethyl isonitrosoacetoacetate (Structure **1**, R = OC_2H_5). This compound also forms a strong intramolecular hydrogen bond involving a six-membered ring similar to that in structure **1**.

An interesting ^{15}N NMR spectrum was observed in the case of the labeled dimethylglyoxime, which exhibited four peaks (Figure 2). In dimethylglyoxime, the ^{15}N- and ^{18}O-enriched isotopes were 95% and 27%, respectively. The various isotopomers are shown in **2**–**4**. (The $^{14}N,^{14}N$ and $^{14}N,^{15}N$ isotopomers are



2 **3** **4**

neglected since they are each less than 1% of the total.) The chemical and magnetic environments of both nitrogens are identical in structure **2** and also in structure **4**. Therefore, they appear as single peaks at 0.0 and 0.067 ppm for the $(^{15}N^{16}O)_2$

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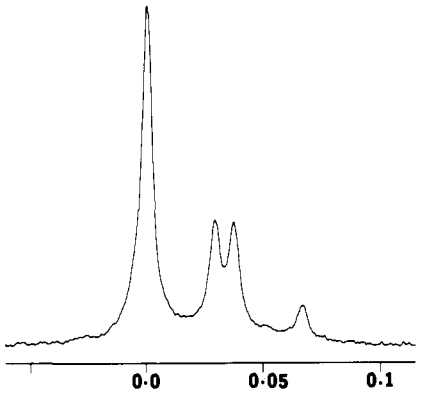
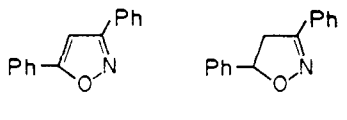


Figure 2. ^{15}N NMR spectrum of ^{15}N,^{18}O-enriched dimethylglyoxime (95% ^{15}N, 27% ^{18}O). An upfield shift of 0.067 ppm is seen for the $(^{15}N,^{18}O_2)_2$ isotopomer, relative to the $(^{15}N,^{16}O_2)_2$ isotopomer. The two symmetric peaks centered at 0.0335 ppm are part of an AB quartet arising from the mixed isotopomer, [$^{15}N^{16}O$,$^{15}N^{18}O$]dimethylglyoxime. The outer lines of this AB quartet are not observable because their intensities are about 1.4% of the inner lines. The three-bond ^{15}N,^{15}N coupling constant is calculated to be 5.7 Hz.

and $(^{15}N^{18}O)_2$ isotopomers, respectively. However, in structure **3**, one of the nitrogens is bonded to ^{16}O while the other is bonded to ^{18}O. Consequently, they are nonidentical, and the difference in their chemical shifts is 0.067 ppm (1.36 Hz on a spectrometer operating at 20.3 MHz). Because their chemical shift difference is less than the coupling constant $^3J_{NN}$, structure **3** forms an AB spin system consisting of four lines with a symmetric weak, strong, strong, and weak intensity distribution. In the present spectrum only the inner two strong lines are observed with a separation of 0.16 Hz (l_2–l_3). With the knowledge of ν_A–ν_B as 1.36 Hz and l_2–l_3 as 0.16 Hz, we can calculate the three-bond ^{15}N–^{15}N coupling constant and the ratio of the intensities (I) of the inner to the outer lines.[48] From the calculations, we obtain a three-bond ^{15}N–^{15}N coupling constant of 5.7 Hz, while the ratio of I(inner):I(outer) is 72. The outer lines (l_1 and l_4) of the AB spectrum could not be observed because of their low intensity, 1.4% of the inner lines (l_2 and l_3).

The isotope shifts of 1-nitrobutane and nitrobenzene are 0.080 and 0.075 ppm per oxygen-18, respectively, and nicely illustrate the additivity of the shift per ^{18}O (Figure 3). The aryl nitro compound exhibits a smaller isotope shift than does the alkyl derivative. Substitution of a phenyl group for an alkyl group would be expected to deshield nitrogen through anisotropic ring current and resonance effects. However, the carboxyl group, although isoelectronic with the nitro group, shows an increased ^{18}O isotopic shift of about the same magnitude upon replacement of the alkyl group by a phenyl group.[12,39]

It was interesting to find that the isoxazoles exhibit much larger upfield isotope shifts than the other compounds. The shift is 0.159 ppm in 3,5-diphenylisoxazole (5, Figure 4A) and 0.153 ppm in 3-methyl-5-phenylisoxazole. From crystal structure studies of



5 6

3,5-diphenylisoxazole[50] and 4-(dimethylamino)benzaldehyde oxime,[51] the N–O bond lengths are 1.407 and 1.420 Å, respectively. The shorter N–O bond length of the isoxazole is consistent with a larger ^{18}O-induced isotope shift. In addition, theoretical esti-

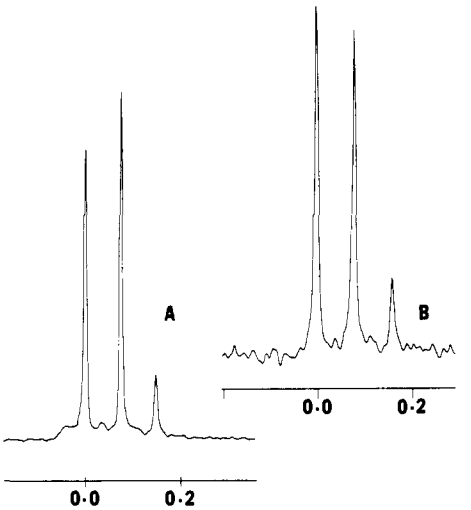


Figure 3. ^{15}N NMR spectra of nitro compounds illustrating the additivity of the ^{18}O isotope shift. A, [^{15}N,^{18}O]nitrobenzene. An upfield shift of 0.075 ppm per oxygen-18 substitution is observed. B, [^{15}N,^{18}O]-1-nitrobutane. The shift is 0.080 ppm per oxygen-18.

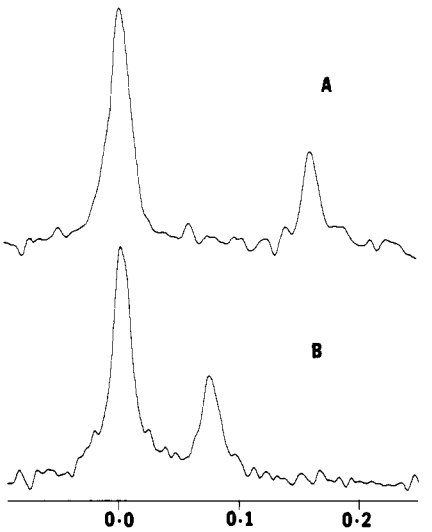


Figure 4. Comparison of ^{18}O isotope shifts in two five-membered heterocyclic compounds. A relatively large shift of 0.159 ppm is observed for [^{15}N,^{18}O]-3,5-diphenylisoxazole (A), whereas it is 0.074 ppm in [^{15}N,^{18}O]-3,5-diphenylisoxazoline (B). The N–O bond order is larger and the bond length is shorter in the aromatic isoxazole.

mates of aromaticity indicate that isoxazole is aromatic, although with less resonance stabilization energy than furan and pyrrole.[49] In the isoxazole ring, the four π bonding electrons and a pair of nonbonding electrons make up the six electrons of the aromatic system. For reasons of orbital symmetry, two nonbonding electrons of oxygen (not nitrogen, in spite of its lesser electronegativity) mix with the other four π bonding electrons. The change in hybridization of oxygen from sp^3 in an oxime to sp^2 in isoxazole with subsequent overlap of its nonbonding electrons alters the electron density, bond order, and bond length of the C–N and the N–O linkages. Consistent with this, the ^{18}O isotope shift on the ^{13}C NMR spectrum of the C-5 carbon of 3,5-diphenylisoxazole was 0.040 ppm,[52] compared with a value of 0.018 ppm for the

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(52) The high-resolution ^{13}C NMR spectrum of ^{15}N,^{18}O-labeled 3,5-diphenylisoxazole (^{15}N, 95 atom %; ^{18}O, 27 atom %) affords additional information on the ^{13}C–^{15}N coupling constant and the ^{18}O-induced secondary isotope shift on the ^{13}C–^{15}N coupling constants. From the ^{13}C NMR spectrum of the C-5 carbon, the C–N coupling constant (the sum of $^2J_{C-N}$ through oxygen and $^3J_{C-N}$ through the C-4 and C-3 carbons) is 1.46 Hz for the [^{15}N,^{16}O]isotopomer and 1.39 Hz for the [^{15}N,^{18}O]isotopomer. The difference of 0.07 Hz between the coupling constants of the two isotopomers is the ^{18}O-induced secondary isotope shift on the ^{13}C–^{15}N coupling constant. The ^{13}C NMR spectrum of the C-3 carbon shows that the one-bond C–N coupling constant is 2.88 Hz and the ^{18}O-induced secondary isotope shift on $^1J_{C-N}$ is 0.06 Hz.

Table II. Effect of Solvent on the ^{18}O Isotope Induced Shifts in ^{15}N NMR Spectra of Oximes[a]

	solvent					
compd	C_6H_6	$CHCl_3$	CH_3CN	C_5H_5N	THF	Me_2SO–$CHCl_3$ (1:4 v/v)
p-chlorobenzaldehyde oxime	0.065	0.063	0.053	0.049	0.050	0.050
p-chloroacetophenone oxime	0.069	0.068	0.059	0.057	0.058	0.057
o-methoxybenzaldehyde oxime	0.059	0.058	0.055	0.052	0.054	0.053

[a] Except for benzene and THF, the solvents were deuteriated. Acetone-d_6 in a 5-mm tube was used as the lock when benzene and THF were used as the solvent. The ^{18}O isotope induced shifts are accurate to ±0.001 ppm.

isotope shift of phenyl vinyl ether.[40]

Because they are present in a cyclic structure, the C–N and N–O bonds of an isoxazole would exhibit different vibrational characteristics than in an oxime.[53] The isotope shift is fundamentally vibrational in origin, arising from the anharmonic vibration, centrifugal distortion, and a small contribution from harmonic vibration.[54,55] In order to measure the isotope shift in a cyclic but nonaromatic heterocyclic compound, we synthesized 3,5-diphenylisoxazoline (**6**) and measured the isotope shift (Figure 4B). The measured value of 0.074 ppm is close to the isotope shifts of oximes, so it seems likely that the large isotope shifts observed in isoxazoles are due to π electron delocalization, with consequent effects on N–O bond order and bond length in the aromatic compound.

Of the compounds studied here, the smallest isotope shift (0.027 ppm) was observed with a benzonitrile oxide. In contrast to the other compounds, the nitrogen in this compound is linked to oxygen through a formal coordinate–covalent bond involving sp hybridization, with virtually linear $-C{\equiv}N{\rightarrow}O$ bonds.[56] In nitrile oxide there is no lone pair on nitrogen because it becomes the bonding pair of electrons in the $N{\rightarrow}O$ coordinate–covalent bond. Therefore, the smaller isotope shift in 2,6-dichlorobenzonitrile oxide may be attributed to the low bond order and the absence of lone-pair electrons on nitrogen.[57]

Solvent Effects on the Isotope Shifts. Nitrogen is subject to structural and electronic influences similar to those experienced by carbon so the chemical shift behavior of nitrogen often closely parallels that of carbon. However, the features that distinguish nitrogen from carbon often result from the presence of an unshared electron pair. Hydrogen bonding, protonation, molecular association, and ionic interactions involving the lone pair and solvent may cause differences in shielding of nitrogen. Thus, hydrogen bonding solvents cause a downfield shift of up to 30 ppm, while protonating solvents cause even larger downfield shifts (100–150 ppm).[58,59] Therefore, we were interested to examine the effect of solvents on the isotope-induced shifts. Six solvents and three oximes were chosen in order to study the effect of solvents on the ^{18}O isotope shifts on ^{15}N NMR. The results are presented in Table II.

Solvent changes cause significant changes in the magnitudes of the isotope shifts. Although the isotope shifts in benzene and chloroform are almost the same, the magnitude of the isotope shift is found to decrease by as much as 0.016 ppm on changing the solvent from benzene to pyridine. Specific solvent–solute interactions are expected to be minimal in benzene and chloroform. However, in a solvent like pyridine, hydrogen bonding can occur involving the lone-pair electrons of the solvent and the O–H proton of the oximes. Such interactions should deshield the nitrogen nucleus, as indicated by the downfield shifts of a few parts per million in the resonance position. It appears that such deshielding effects also cause decreases in the magnitudes of the isotope shift. Solvents such as acetonitrile, tetrahydrofuran, and dimethyl sulfoxide–chloroform mixtures also cause decreases in the magnitudes of the isotope shift for each of the oximes, to about the same extent as that observed in pyridine. Although the effect of solvent on the isotope shift of *o*-methoxybenzaldehyde oxime is not as pronounced as with *p*-chlorobenzaldehyde oxime, the general trend remains the same for the aldehyde and ketone oximes that were studied. No correlation was apparent between the dielectric constant of the solvent and the magnitude of the decrease in the isotope shift in the respective solvents. Rather, the similar values of the isotope shifts for each of the oximes in pyridine, THF, Me_2SO–$CDCl_3$, and acetonitrile may be attributed to the presence in the solvents of donor atoms with lone electron pairs. An attempt to measure the ^{18}O shift in the protonating solvent trifluoroacetic acid (TFA) was not successful due to broadening of the ^{15}N signal.

In summary, the ^{18}O isotope induced shifts on ^{15}N NMR are shown to exhibit many of the characteristics recognized in analogous systems. The shifts are all upfield compared to those of the ^{16}O compounds, the magnitudes are relatively large (as would be anticipated from the chemical shift range of ^{15}N), the shifts are additive, and their magnitude depends on the structure and hybridization of the compound. In most cases the magnitudes of the shifts may be predicted reasonably well from appropriate models, but these isotope shifts are noticeably more sensitive to solvent effects than are the corresponding ^{18}O shifts on ^{13}C spectra. The ^{18}O isotope shift on ^{15}N NMR should be useful in studying a variety of kinetic and mechanistic problems such as rearrangement reactions of organic nitrogen compounds and biosynthetic problems such as the formation of the isoxazole ring in the antitumor antibiotic AT-125 produced by *Streptomyces sviceus*.[60] The present study should facilitate many such investigations.

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