greater than 10×10^{-4} cm^{-1}, far larger than experiment. Thus we reject these configurations.

(b) The next configuration in energy is illustrated in Figure 2, $(d_{x^2-y^2})^1(d_{xz} - O_2\pi_{gx})^1(d_{yz} - O_2\pi_{g*})^1$. Although this configuration corresponds to considerable unpaired spin density in the dioxygen π^* orbitals, it is straightforward to generate the appropriate analogy to eq 2-4. Assigning the ^{55}Mn hfs to the y,z molecular axes gives $P^{Mn} = 205 \times 10^{-4}$ cm^{-1} and to the x,z molecular axes gives $P^{Mn} = 192 \times 10^{-4}$ cm^{-1}. The former value is essentially $P_0^{Mn}(3+)$, while the latter is only slightly lower. Thus, the ^{55}Mn hfs calculated from the IEH wave functions are in agreement with experiment. The ^{17}O hfs tensors are calculated to have axial symmetry, $\mathbf{A}^O = -[10, -1, -1] \times 10^{-4}$ cm^{-1} and $-[12, 3, 3] \times 10^{-4}$ cm^{-1} for the near and far O atoms, respectively. Assignment of the ^{17}O hfs to the x,z axes is clearly inconsistent with experiment, for this would require the observed splittings to be $\sim$12 and $\sim$10 $\times 10^{-4}$ cm^{-1}, instead of the $\sim 2.5 \times 10^{-4}$ cm^{-1} found. However, a fortuitous correspondence of the ^{55}Mn hfs to the y,z molecular axes would give ^{17}O hfs that agree with the small observed values, in spite of the unpaired spin density on the dioxygen.

^{18}O-Isotope Effect in ^{13}C Nuclear Magnetic Resonance Spectroscopy. 2. The Effect of Structure

John M. Risley and Robert L. Van Etten*

Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907. *Received December 26, 1979*

Abstract: A series of ^{18}O-labeled organic compounds was synthesized in order to study further two aspects of the ^{18}O-induced shift of natural abundance ^{13}C NMR signals as compared to the respective ^{16}O compounds. First, the direct effect of the hybridization of the carbon atom was studied in ethers. The observed effect was a significant decrease in the ^{18}O-induced upfield shift when the carbon-atom hybridization was changed from sp^3 to sp^2. Second, the significance of the functional group on the ^{18}O-isotope effect was evaluated. The experimental data reveal that the functional group plays an important role in affecting the magnitude of the ^{18}O-induced shift. Large shifts are observed upon ^{18}O-substitution in aldehydes and ketones while alcohols and phenols exhibit smaller shifts. Carboxylic acids show the expected intermediate shifts. In addition, a small but experimentally significant substituent-group effect on the carbonyl group ^{18}O-isotope shift was observed upon changes in alkyl, aryl, or hydrogen substitution. The isotope-induced shift is discussed from a semitheoretical standpoint on the basis of known experimental results and theoretical predictions. In accord with these expectations, the ^{18}O shift on the ^{13}C resonance position is dependent both upon the chemical nature and the number of oxygens covalently bonded to the carbon atom.

Introduction

We recently reported the existence of an ^{18}O-isotope effect on ^{13}C NMR spectra.[1] Related examples of the effects of isotopic substitution on nuclear magnetic resonance signals have been known for a number of years. Ramsey and Purcell[2] predicted an isotope effect on nuclear magnetic resonance signals and Wimett[3] first observed the difference in the molecular shielding factors for $^2H_2$, $^2H^1H$, and $^1H_2$. However, it was not until the ^{2}H-isotope effects on ^{19}F NMR[4] and ^{1}H NMR[5] were reported that additional studies were begun on the isotope effect. The primary areas of interest were the ^{2}H-isotope and ^{13}C-isotope effects on ^{1}H and ^{19}F NMR. Batiz-Hernandez and Bernheim[6] reviewed the isotope effect on nuclear magnetic resonance signals through 1966.

Since then isotopic shifts involving other nuclei have been described. The primary ^{2}H-isotope effect on ^{19}F NMR was observed in gaseous ^{2}HF.[7] Supporting evidence for the ^{34}S-isotope effect, as well as the ^{13}C-isotope effect, in ^{19}F NMR was obtained with fluorine-labeled thiophene.[8] Deuterium-isotope effects were observed in ^{11}B NMR,[9] ^{13}C NMR,[10-12] ^{14}N NMR,[9] ^{15}N NMR,[9,13] ^{17}O NMR,[14] and ^{31}P NMR.[15] An additional ^{13}C-isotope effect[16] and a ^{34}S-isotope effect[17] in ^{13}C NMR were observed. ^{34}S was shown also to exert an isotope effect on the ^{95}Mo NMR signal.[18] ^{18}O was observed to exert an isotope effect on the ^{1}H NMR of [^{18}O]water which averaged $\sim$0.3 ppm upfield.[19] ^{18}O also was observed to exert an isotope effect on ^{31}P NMR,[20] ^{55}Mn NMR,[21,22] and ^{95}Mo NMR.[21]

The magnitudes of such isotope effects are found to be dependent on the chemical-shift range of the nucleus being observed, as well as upon the type of compound.[6] The experimental data also show that heavy-atom isotopic substitution generally causes an upfield shift in the resonance signal of the nucleus being observed. However, Kanazawa et al.[23] and Fraenkel et al.[24] reported

(1) Part 1: Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 252-253.
(2) Ramsey, N. F.; Purcell, E. M. *Phys. Rev.* **1952**, *85*, 143-144.
(3) Wimett, T. F. *Phys. Rev.* **1953**, *91*, 476.
(4) Tiers, G. V. D. *J. Am. Chem. Soc.* **1957**, *79*, 5585.
(5) Tiers, G. V. D. *J. Chem. Phys.* **1958**, *29*, 963-964.
(6) Batiz-Hernandez, H.; Bernheim, R. A. *Prog. Nucl. Magn. Reson. Spectrosc.* **1967**, *3*, 63-85.
(7) Hindermann, D. K.; Cornwell, C. D. *J. Chem. Phys.* **1968**, *48*, 2017-2025.
(8) Rodmar, S.; Rodmar, B.; Sharma, M. K.; Gronowitz, S.; Christiansen, H.; Rosen, U. *Acta Chem. Scand.* **1968**, *22*, 907-920.
(9) Shporer, M.; Loewenstein, A. *Mol. Phys.* **1968**, *15*, 9-15.
(10) Maciel, G. E.; Ellis, P. D.; Hafer, D. C. *J. Phys. Chem.* **1967**, *71*, 2160-2164.
(11) Lebel, G. L.; Laposa, J. D.; Sayer, G. G.; Bell, R. A. *Anal. Chem.* **1971**, *43*, 1500-1501.
(12) Grishin, Y. K.; Sergeyev, N. M.; Ustynynk, Y. A. *Mol. Phys.* **1971**, *22*, 711-714.
(13) Litchman, W. M.; Alei, M., Jr.; Florin, A. E. *J. Chem. Phys.* **1969**, *50*, 1897-1898.
(14) Lutz, O.; Oehler, H. *Z. Naturforsch. A* **1977**, *32*, 131-133.
(15) Borisenko, A. A.; Sergeyev, N. M.; Ustynyuk, Y. A. *Mol. Phys.* **1971**, *22*, 715-719. Stec, W. J.; Goddard, N.; Van Wazer, J. R. *J. Phys. Chem.* **1971**, *75*, 3547-3549.
(16) Weigert, F. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1972**, *94*, 6021-6025.
(17) Linde, S. A.; Jakobsen, H. J. *J. Magn. Reson.* **1975**, *17*, 411-412.
(18) Lutz, O.; Nolle, A.; Kroneck, P. *Z. Phys. A* **1977**, *282*, 157-158.
(19) Pinchas, S.; Meshulan, E. *J. Chem. Soc. D* **1970**, 1147-1148.
(20) Cohn, M.; Hu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 200-203. Lutz, O.; Nolle, A.; Staschewski, D. *Z. Naturforsch. A* **1978**, *33*, 380-382.
(21) Buckler, K. U.; Hasse, A. R.; Lutz, O.; Müller, M.; Nolle, A. *Z. Naturforsch. A* **1977**, *32*, 126-130.
(22) Haase, A. R.; Lutz, O.; Müller, M.; Nolle, A. *Z. Naturforsch. A* **1976**, *31*, 1427-1428.
(23) Kanazawa, Y.; Baldeschwieler, J. D.; Craig, N. C. *J. Mol. Spectrosc.* **1965**, *16*, 325-348.
(24) Fraenkel, G.; Asahi, Y.; Batiz-Hernandez, H.; Bernheim, R. A. *J. Chem. Phys.* **1966**, *44*, 4647-4649.

0002-7863/80/1502-4609$01.00/0

a downfield shift in the ^1H NMR signals of *cis*-1,2-difluoroethane and ammonium ion, respectively, when ^2H substitution was effected. Deuterium substitution also caused a downfield shift of the ^{13}C NMR signals of (1) the ring carbon meta to the cyano group and para to the deuterium substituent in *o*-deuteriobenzonitrile[11] and (2) the carbonyl carbon in [$^2H_6$]acetone.[10] Breitmaier et al.[25] confirmed the latter observation, and reported the downfield shift of the C-1 carbon of [$^2H_3$]acetonitrile. A downfield shift of the γ carbon in methyl 2,2-dideuteriopalmitate was also observed.[26]

Stothers[27] briefly reviewed the experimental applicability of the deuterium-isotope effect on ^{13}C NMR. The β-^2H-isotope[28] and β-(O)-^2H-isotope[29] effects have been used to assign conclusively the ^{13}C NMR signals of sugars in carbohydrate chemistry. Deuterium-isotope effects on ^{13}C NMR in amides, amino acids, and peptides[30] are potentially valuable in protein chemistry. The ^{18}O-isotope effect on ^{31}P NMR signals has already been applied to a number of biologically interesting problems with much success.[31]

Although the first report of an ^{18}O-isotope effect on an NMR signal appeared in 1970,[19] 6 years passed before a second report was published.[22] The following year one additional ^{18}O-isotope effect was reported.[21] The ^{18}O-isotope effect on ^{31}P NMR[20] has spurred a number of papers applying the isotope effect as mentioned above. Brief reviews of the results obtained by using the latter technique have recently been published.[32]

Even though isotope effects on NMR signals were widely known and it had been observed that ^2H, ^{13}C, and ^{34}S produced upfield shifts in ^{13}C NMR signals, the potentially valuable ^{18}O-isotope effect was reported only recently by us.[1] The natural abundance ^{13}C resonance of the hydroxyl carbon of ^{18}O-labeled *tert*-butyl alcohol was shifted upfield 0.035 ppm with respect to the unlabeled (^{16}O) compound. We further demonstrated a practical application of the isotopic shift by following the acid-catalyzed exchange of [^{18}O]*tert*-butyl alcohol in water by using ^{13}C NMR spectroscopy. A pseudo-first-order rate constant was calculated which was in excellent agreement with the rate constant measured earlier by mass spectroscopy.

Because this isotope effect is a new addition to the repertoire of isotope effects on NMR signals, a variety of fundamental experiments are necessary in order to define the scope of the isotope effect. Among these experiments, we felt it was important to examine the effect of structure on the ^{18}O-induced shift. In the present study, we have synthesized a number of ^{18}O-labeled compounds in order to study the ^{18}O-induced shift on ^{13}C NMR signals. First, the direct effect of the hybridization of the carbon atom bearing the oxygen upon the magnitude of the isotope shift was examined in the ethers phenetole and phenyl vinyl ether. Second, the effect of the functional group and compound type on the isotope shift was studied for a number of oxygen-containing functional groups.

Experimental Section

[^{18}O]Water (99 atom % excess ^{18}O, Norsk Hydro, Oslo) was used in the synthesis of the ^{18}O-labeled compounds except as noted. All other reagents were either analytical or spectrometric grade. Unlabeled water was glass distilled. Mass spectral analyses were performed on a CEC-21-110B mass spectrometer.

Syntheses. The following syntheses of the ^{18}O-labeled compounds were developed so as to proceed smoothly, be economical in the use of ^{18}O-water, and result in high isotopic incorporation in the desired products.

[α-^{13}C,$^{18}O_2$]Benzoic Acid. [α-^{13}C]Benzoic acid (90 atom % excess carboxyl ^{13}C, Stohler) and thionyl chloride were heated until evolution of HCl ceased. Excess thionyl chloride was removed by distillation. Concentrated ammonium hydroxide was added dropwise to the residue with the formation of [α-^{13}C]benzamide. The amide and thionyl chloride were again warmed until the evolution of HCl ceased. Excess thionyl chloride was distilled. [^{18}O]Water (95 atom % ^{18}O, Merck) was added to the residue, and the mixture was refluxed to hydrolyze the [α-^{13}C]-benzonitrile. Dilute HCl was added and [α-^{13}C,$^{18}O_2$]benzoic acid precipitated. Mass spectral analysis showed 72% ^{18}O incorporation, specifically 51% $^{18}O_2$, 41% $^{18}O^{16}O$, and 8% $^{16}O_2$.

Sodium [$^{18}O_2$]Formate. The synthesis followed the outline for sodium [$^{18}O_2$]acetate given by Hutchinson and Mabuni.[33] Methyl orthoformate, $H_2{}^{18}O$, and *p*-toluenesulfonic acid were stirred together. Sodium methoxide (2.63 M) was added followed by more $H_2{}^{18}O$ in tetrahydrofuran. Crystals of sodium [$^{18}O_2$]formate were collected by filtration [70% yield, mp 255 °C]. Mass spectral analysis of the *p*-bromophenacyl ester (synthesized below) showed 91% isotopic purity.

***p*-Bromophenacyl Formate.** The following procedure was developed for the preparation of this ester. Absolute ethanol (2.0 mL), sodium formate (50 mg), and *p*-bromophenacyl bromide (α,*p*-dibromoacetophenone, 203 mg) were refluxed for 1.5 h. Water (0.5 mL) was added to dissolve the precipitated salt and the resulting solution was refrigerated overnight. Small brilliant plate-like crystals were deposited. The crystals were collected and dried in a desiccator: yield 99 mg (56%); mp 89 °C (lit.[34] mp 135 °C); ^1H NMR ($CDCl_3$/1% Me_4Si) δ 5.40 (2 H, s), 7.73 (2 H, s), 7.76 (2 H, s), 8.25 (1 H, s); ^{13}C NMR ($CDCl_3$/1% Me_4Si) δ 65.06, 129.08, 129.22, 132.28, 132.68, 159.87, 190.24; mass spectrum P_M = 242, P_{M+1} = 11%, P_{M+2} = 104%, P_{M+3} = 11%. Anal. Calcd for $C_9H_7O_3Br$: C, 44.47; H, 2.90; Br, 32.9. Found: C, 44.36; H, 3.05; Br, 33.0.

***p*-Bromophenacyl [$^{18}O_2$]Formate.** Absolute ethanol (2.0 mL), 100 mg of sodium [$^{18}O_2$]formate, and 408 mg of *p*-bromophenacyl bromide (α,*p*-dibromoacetophenone) were refluxed for 1.5 h. Water (0.5 mL) was added to dissolve the precipitated salt and the solution was refrigerated overnight. The small brilliant plate-like white crystals that were deposited were collected by filtration and dried in a desiccator: yield 182 mg (53%); mp 89 °C (lit.[35,36] mp 135 °C); ^1H NMR ($CDCl_3$/1% Me_4Si) δ 5.40 (2 H, s), 7.73 (2 H, s), 7.76 (2 H, s), 8.25 (1 H, s); ^{13}C NMR ($CDCl_3$/1% Me_4Si) δ 65.03, 129.08, 129.22, 132.28, 132.68, 159.82, 190.24. Mass spectral analysis showed 91% isotopic incorporation of ^{18}O into the carboxyl group, specifically 83% $^{18}O_2$, 16% $^{18}O^{16}O$ (14% carbonyl ^{18}O and 2% ether ^{18}O), and 1% $^{16}O_2$.

(25) Breitmaier, E.; Jung, G.; Voelter, W.; Pohl, L. *Tetrahedron* **1973**, *29*, 2485–2489.

(26) Tulloch, A. P.; Mazurek, M. *J. Chem. Soc., Chem. Commun.* **1973**, 692–693.

(27) Stothers, J. B. *Top. Carbon-13 NMR Spectrosc.* **1974**, *1*, 229–286.

(28) Gorin, P. A. J. *Can. J. Chem.* **1974**, *52*, 458–461. Gorin, P. A. J.; Mazurek, M. *Ibid.* **1975**, *53*, 1212–1223. Balza, F.; Cyr, N.; Hamer, G. K.; Perlin, A. S.; Koch, H. J.; Stuart, R. S. *Carbohydr. Res.* **1977**, *59*, C7–C11.

(29) Gagnaire, D.; Vincendon, M. *J. Chem. Soc., Chem. Commun.* **1977**, 509–510. Ho, S.-C.; Koch, H. J.; Stuart, R. S. *Carbohydr. Res.* **1978**, *64*, 251–256. Pfeffer, P. E.; Valentine, K. M.; Parrish, F. W. *J. Am. Chem. Soc.* **1979**, *101*, 1265–1274.

(30) Feeney, J.; Partington, P.; Roberts, G. C. K. *J. Magn. Reson.* **1974**, *13*, 268–274. Ladner, H. K.; Led, J. J.; Grant, D. M. *Ibid.* **1975**, *20*, 530–534. Newmark, R. A.; Hill, J. R. *Ibid.* **1976**, *21*, 1–7.

(31) Balakrishnan, M. S.; Sharp, T. R.; Villafranca, J. J. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 991–998. Bock, J. L.; Cohn, M. *J. Biol. Chem.* **1978**, *253*, 4082–4085. Cohn, M.; Hu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 200–203. Hackney, D. D.; Rosen, G.; Boyer, P. D. *Ibid.* **1979**, *76*, 3646–3650. Jarvest, R. L.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1979**, 364–366. Jordan, F.; Patrick, J. A.; Salamone, S., Jr. *J. Biol. Chem.* **1979**, *254*, 2384–2386. Lowe, G.; Sproat, B. S. *J. Chem. Soc., Chem. Commun.* **1978**, 565–566. Lowe, G.; Sproat, B. S. *Ibid.* 783–785. Lowe, G.; Sproat, B. S. *J. Chem. Soc., Perkin* Trans. *1* **1978**, 1622–1630. Lowe, G.; Potter, B. V. L.; Sproat, B. S.; Hull, W. E. *J. Chem. Soc., Chem. Commun.* **1979**, 733–735. Van Etten, R. L.; Risley, J. M. *Proc Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4784–4787. Webb, M. R.; McDonald, G. G.; Trentham, D. R. *J. Biol. Chem.* **1978**, *253*, 2908–2911.

(32) Cohn, M.; Rao, B. D. N. *Bull. Magn. Reson.* **1979**, *1*, 38–60. Rose, I. A. *Adv. Enzymol.* **1979** *50*, 361–395.

(33) Hutchinson, C. R.; Mabuni, C. T. *J. Labelled Compd. Radiopharm.* **1977**, *13*, 571–574.

(34) The *p*-bromophenacyl formate ester could not be synthesized in the presence of water since only the starting compound was recovered. The *p*-bromophenacyl ester of sodium formate was finally synthesized in absolute ethanol, although the yield was only 50%. This ester was a very interesting derivative since the melting point of the white plate-like crystals was 89 °C, much lower than the value (133 to 135 °C) reported by Moses and Reid[35] and Erickson et al.[36] These two groups had problems with yellowing of the crystalline mass due to decomposition and a consequent lowering of the melting point. In the present case, these crystals were stored in a dark bottle and it was found that after 10 months only slight decomposition had taken place as was evidenced by a melting point of 87 °C with a tinge of yellow. An elemental analysis of the ^{16}O-labeled crystalline material (mp 89 °C) was performed and the results agreed with the calculated percentages of C, H, and Br. The ^1H and ^{13}C NMR spectra, as well as mass spectral analysis, provided further confirmatory evidence that the ester had been synthesized. The conclusion reached was that the *p*-bromophenacyl formate ester had been synthesized and the melting point is 89 °C. The melting point is in more general agreement with the melting points of the other *p*-bromophenacyl normal alkanoic acid esters.[35]

(35) Moses, C. G.; Reid, E. E. *J. Am. Chem. Soc.* **1932**, *54*, 2101–2103.

(36) Erickson, J. L. E.; Dechary, J. M.; Kesling, M. R. *J. Am. Chem. Soc.* **1951**, *73*, 5301–5302.

[^{18}O]**Benzaldehyde.** The procedure given by Strain[37] for the synthesis of benzaldimine was followed. Benzaldehyde and ammonium hydroxide (d = 0.90) were sealed in a beaker for 1 week. The crystals of hydrobenzamide were collected by filtration, washed with ammonium hydroxide, and dried in a desiccator. The crude crystalline mass (mp 99 °C, lit.[37] mp 101 °C) was again dissolved in ammonium hydroxide, ammonium chloride was added as a catalyst, and the flask was sealed. After 5 days, the crystals of benzaldimine were collected and dried in a desiccator. [^{18}O]Water was acidified with H_2SO_4 and warmed to 95 °C in an oil bath; benzaldimine was added in small quantities with stirring and NH_3 was evolved. The product was extracted with ether and distilled. Mass spectral analysis showed 83% isotopic incorporation of ^{18}O.

[^{18}O]**Benzyl Alcohol.** [^{18}O]Benzaldehyde with some [^{18}O,^{16}O]benzaldehyde was reduced with sodium borohydride as described by Vogel.[38] A basic solution of sodium borohydride was added dropwise to a methanolic solution of [^{18}O]benzaldehyde such that the reaction temperature was between 18 and 25 °C. The methanol was removed by distillation and the residue was diluted with water. The product was extracted with ether, dried over anhydrous magnesium sulfate, and distilled under vacuum. The isotopic incorporation was the same as [^{18}O]benzaldehyde, 83%.

[^{18}O]**Phenol.** The procedure of Pinchas et al.[39] was followed with slight modification. Aniline hydrochloride and alcoholic H_2SO_4 were mixed with isoamyl nitrite at 10 °C until a clear solution was obtained. Ether was added and the precipitated diazonium salt was transferred to a stirred mixture of $H_2{}^{18}O$ in ether. The solution was warmed to 50 °C with stirring until N_2 evolution ceased. The product was vacuum distilled. Mass spectral analysis revealed 27% isotopic enrichment.

[^{18}O]**Phenetole.** The procedure described by Vogel[38] was used to prepare this compound. [^{18}O]Phenol and sodium hydroxide were stirred at 10 °C. Diethyl sulfate was added and the solution was refluxed for 2 h. The solution was diluted with water, the layers were separated, and the aqueous layer was extracted with ether. The combined ethereal extracts were washed with water, dilute sulfuric acid, and water and dried over anhydrous calcium chloride. The product was vacuum distilled. Mass spectral analysis showed 27% isotopic incorporation.

[^{18}O]β-**Bromophenetole.** The procedure of Marvel and Tanenbaum[40] was used. [^{18}O]Phenol, 1,2-dibromoethane, and water were mixed together and heated to reflux. A solution of 50% NaOH was then added over 1 h and the solution was refluxed an additional 10 h. The solution was extracted with ether and the ethereal extracts were dried over anhydrous sodium sulfate. The product was distilled under vacuum. Mass spectral analysis showed an isotopic enrichment of 27%.

[^{18}O]**Phenyl Vinyl Ether.** The procedure given by Wahl and Berthold[41] was followed. [^{18}O]β-Bromophenetole and powdered potassium hydroxide were mixed thoroughly. The mixture was heated in vacuo until the product distilled as a clear liquid. Unreacted [^{18}O]β-bromophenetole was recovered by dissolving the salt in water, extracting the solution with ether, and distilling the ethereal extract. The isotopic enrichment was 26%.

[^{18}O]**Acetone.** A catalytic amount of p-toluenesulfonic acid was added to a stirred solution of [^{18}O]water in excess 2,2-dimethoxypropane. The resulting endothermic reaction, evidence by condensation on the outside of the flask and a rapid decrease in the solution temperature, was immediate. The solution was allowed to warm to room temperature and then the product was distilled at atmospheric pressure. The yield was nearly quantitative. Mass spectral analysis revealed 94% isotopic incorporation.

[^{18}O]**Isopropyl Alcohol.** [^{18}O]Acetone was reduced with sodium borohydride by the general procedure of Vogel.[38] A solution of [^{18}O]-acetone and methanol was cooled to 18 °C. Sodium borohydride in 0.2 M potassium hydroxide was added dropwise such that the temperature did not exceed 25 °C. The endpoint of the reduction was determined by the presence of hydrogen evolution when a portion of the solution was dropped into dilute sulfuric acid. The product was liberated with dilute sulfuric acid and distilled at atmospheric pressure. The isotopic incorporation was the same as [^{18}O]acetone, 94%.

[^{18}O]**Butyraldehyde.** Butyraldehyde dibutyl acetal was synthesized from n-butyraldehyde, n-butanol, and p-toluenesulfonic acid (catalyst) according to Van Rissegham.[42] Water was removed as an azeotrope with benzene, using a Dean-Stark trap. After 7 h, the solution was vacuum distilled and the yield of the acetal was 95%. [^{18}O]Butyraldehyde was synthesized according to the method of Stasiuk et al.[43] The acetal and $H_2{}^{18}O$ plus p-toluenesulfonic acid were stirred in a closed flask at 63–65 °C for 2 h. The reaction was cooled and the product was distilled. The yield of [^{18}O]butyraldehyde was 90%. The isotopic enrichment was 70% by mass spectral analysis.

[^{18}O]**Benzophenone.** The procedure of Halmann and Pinchas[44] was followed. Dichlorodiphenylmethane and $H_2{}^{18}O$ were refluxed for 3 h under a calcium chloride drying tube. Excess $H_2{}^{18}O$ was removed to give the product, mp 47.5 °C (lit.[44] mp 48.1 °C). Mass spectral analysis indicated 89% isotopic incorporation.

***syn,anti*-7-[^{18}O]Carboxynorbornene (*syn,anti*-7-[^{18}O]Carboxybicyclo-[2.2.1]heptene).** This compound was prepared by hydrolysis of the methyl ester with Na^{18}OH and was a gift from Dr. Robert Peoples and Professor John Grutzner. The isotopic enrichment indicated it to be 83% of the mono-^{18}O compound.

NMR Spectra. The ^{18}O-isotope effects on ^{13}C NMR signals were measured at the natural abundance of carbon in all instances except one. The primary instrument used to measure the effect was a Varian CFT-20 spectrometer. Additional spectra were obtained on a Nicolet 150-MHz or 360-MHz spectrometer for some of the ^{18}O-labeled compounds. ^{1}H NMR spectra were recorded on a Varian A-60A spectrometer. The solvent was either deuterium oxide (99.7 atom % D, Merck) or deuteriochloroform (99.8 atom % D containing 1% Me_4Si, Aldrich).

The Varian CFT-20 instrument was fitted with an 8-mm probe. The sweep width was generally 300 Hz; however, 125, 200, and 400 Hz were occasionally used. A line-broadening factor was applied to the accumulated FID such that the resolution of the hydroxyl carbon in phenol was 0.24 Hz. Protons were broadband decoupled. A 45° pulse angle was applied and an 8K data block was used. A 8-mm tube was used throughout with the exception of *syn,anti*-7-[^{18}O]carboxynorbornene, where a 5-mm insert was used.

The ^{18}O-isotope effect was generally measured by the following procedure. The ^{13}C NMR spectrum of the ^{18}O-labeled compound was obtained. A measured quantity of unlabeled (^{16}O) compound was added to the ^{18}O sample and the spectrum was obtained for the labeled–unlabeled mixture. An additional measured quantity of unlabeled compound was added and the spectrum was again obtained. Thus triplicate spectra were obtained for the compound in most cases. In a few instances a Du Pont 310 curve resolver set for Lorentzian curves was used to simulate the spectra and to verify the isotopic shifts. The error in the measured isotope effect was ±0.002 ppm.

The instrumental probes used with the Nicolet 150-MHz and 360-MHz were 12 mm and 10 mm, respectively. A line-broadening factor of 0.10 to 0.20 s was applied. Protons were noise decoupled. Either 45° or 90° pulse angles were applied, 16K or 32K data blocks were used, and the spectra were generally used to confirm data obtained on the CFT-20 instrument.

Results

Figures 1–5 illustrate the shift in the ^{13}C NMR signal upon isotopic substitution with ^{18}O. The unlabeled (^{16}O) species has arbitrarily been assigned the value of 0.000 ppm in each figure. Spectra in Figures 1–3 and 5 were recorded on a Varian CFT-20 spectrometer while Figure 4 was recorded with a Nicolet 360-MHz spectrometer. Figure 1 exemplifies the ^{18}O-induced shifts observed for the ethers. The magnitudes of the shifts of the aldehyde (Figure 2) and ketone (Figure 3) are larger than those of phenol or of phenyl ethers (Figure 1). Figures 4 and 5 depict the shifts upon multiple or single isotopic substitution in carboxylic acids. The ^{18}O-induced upfield shifts of the ^{13}C NMR signals for each compound are given in Table I. The compounds are arranged according to functional group, the specific compound, and the ^{18}O-isotope effect per heavy atom.

There is a considerable range of isotope-induced shifts between different types of compounds. However, within a given group, such as alcohols, the range is not great. The unhindered sp^3-hybridized ethereal/hydroxyl carbon resonance signal is shifted around 0.025 ppm: benzyl alcohol (a primary alcohol) 0.023 ppm, isopropyl alcohol (a secondary alcohol) 0.023 ppm, p-bromophenacyl formate 0.025 ppm, phenetole 0.025 ppm, and β-bromophenetole 0.025 ppm. The sp^3-hybridized hydroxyl carbon in *tert*-butyl alcohol shows a markedly larger upfield shift (0.035

(37) Strain, H. H. *J. Am. Chem. Soc.* **1927**, *49*, 1558–1571.
(38) Vogel, A. "Textbook of Practical Organic Chemistry"; Longman: London, 1978.
(39) Pinchas, S.; Sadeh, D.; Samuel, D. *J. Phys. Chem.* **1965**, *69*, 2259–2264.
(40) Marvel, C. S.; Tanenbaum, A. L. "Organic Syntheses"; Wiley and Sons: New York, 1941; Collect. Vol. 1, pp 435–436.
(41) Wahl, A.; Berthold, E. *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 2175–2185.
(42) Van Risseghem, H. *Bull. Soc. Chim. Belg.* **1919**, *28*, 335–339.
(43) Stasiuk, F.; Sheppard, W. A.; Bourns, A. N. *Can. J. Chem.* **1956**, *34*, 123–127.
(44) Halmann, M.; Pinchas, S. *J. Chem. Soc.* **1958**, 1703–1705.

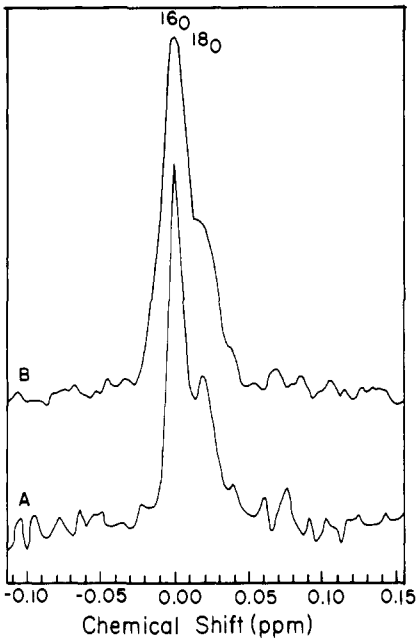

Figure 1. [^{18}O]Phenyl vinyl ether. Natural abundance ^{13}C NMR signals of the vinylic and phenyl ethereal carbons are shifted upfield by 0.018 ppm upon ^{18}O-isotopic substitution. (A) Phenyl carbon. (B) Vinylic carbon (26 atom % ^{18}O in $CDCl_3$/1% Me_4Si).

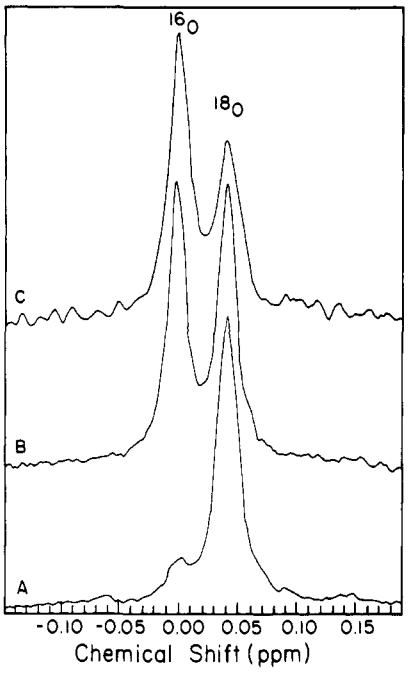

Figure 2. [^{18}O]Benzaldehyde. ^{18}O-Labeled natural abundance ^{13}C carbonyl NMR signal is shifted upfield by 0.043 ppm from its unlabeled analogue. (A) 0.15 mL of [^{18}O]benzaldehyde (83 atom % ^{18}O in $CDCl_3$/1% Me_4Si). (B) 0.10 mL of benzaldehyde added to A. (C) 0.20 mL of benzaldehyde added to A.

ppm) compared to the other ethereal/hydroxyl compounds.

The sp^2-hybridized carbon atoms exhibit both the smallest and largest ^{18}O-induced shifts. In aldehydes and ketones, the shift is the largest (per ^{18}O) observed: *n*-butyraldehyde 0.047 ppm, benzaldehyde 0.043 ppm (Figure 2), acetone 0.050 ppm, and benzophenone 0.045 ppm (Figure 3). In phenol, phenetole, and β-bromophenetole, the ethereal/hydroxyl carbon is shifted only 0.016 ppm upfield—the smallest shift observed. The phenyl and vinyl carbon atoms in phenyl vinyl ether (Figure 1) are shifted upfield 0.018 ppm.

The carboxyl carbon also shows some variation in its upfield shift. As the carboxylate ion in D_2O, the resonance signal of the

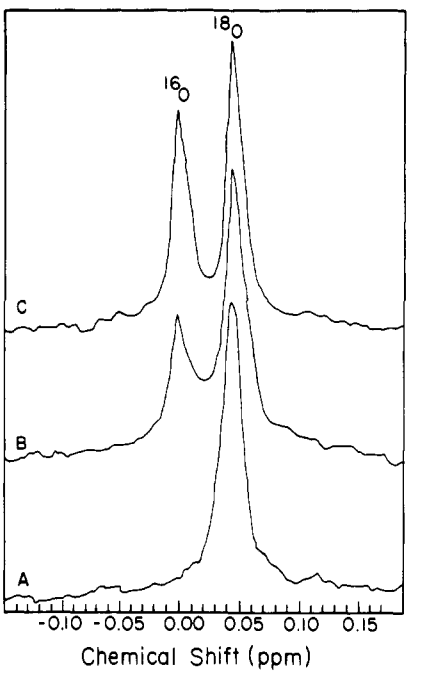

Figure 3. [^{18}O]Benzophenone. Isotopic substitution of ^{18}O into the carbonyl group shifted the natural abundance ^{13}C carbonyl NMR signal upfield by 0.045 ppm. (A) 0.5 g of [^{18}O]benzophenone (89 atom % ^{18}O in $CDCl_3$/1% Me_4Si). (B) 0.23 g of benzophenone added to A. (C) 0.43 g of benzophenone added to A.

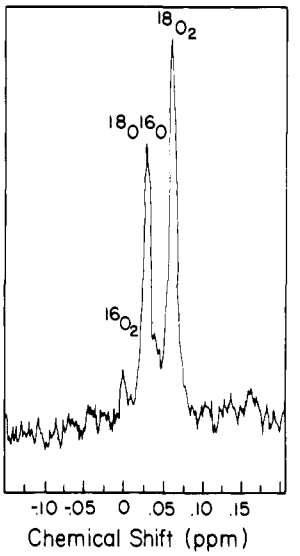

Figure 4. [α-^{13}C, $^{18}O_2$]Benzoic acid. The ^{13}C-enriched carboxyl carbon ^{13}C NMR signal is shifted upfield by 0.031 ppm per ^{18}O substituted into the carboxyl group (90 atom % ^{13}C; 72 atom % ^{18}O: 51% $^{18}O_2$, 41% $^{18}O^{16}O$, 8% $^{16}O_2$ in D_2O).

carboxyl carbon in sodium formate is shifted upfield a total of 0.050 ppm upon ^{18}O-substitution (0.025 ppm/^{18}O). The shift in the formate carboxyl carbon of the *p*-bromophenacyl ester is slightly larger, 0.054 ppm. Substitution of the phenyl group for the hydrogen atom of formic acid results in an even larger total upfield shift as is seen for the case of benzoic acid (0.062 ppm, Figure 4). However, the carboxyl carbon shift per ^{18}O did not change when norbornene replaced the hydrogen atom (Figure 5). The ^{18}O-isotope effect appears to be approximately additive as demonstrated by [α-^{13}C,$^{18}O_2$]benzoic acid (Figure 4) since the shift for the singly labeled species is 0.031-ppm upfield and the doubly labeled species is shifted upfield 0.062 ppm. Somewhat surprisingly, the isotope shifts appear to be relatively independent of solvent, since in the cases of acetone and benzoic acid, the isotope shifts do not change between deuteriochloroform and deuterium oxide solvents (Table I).

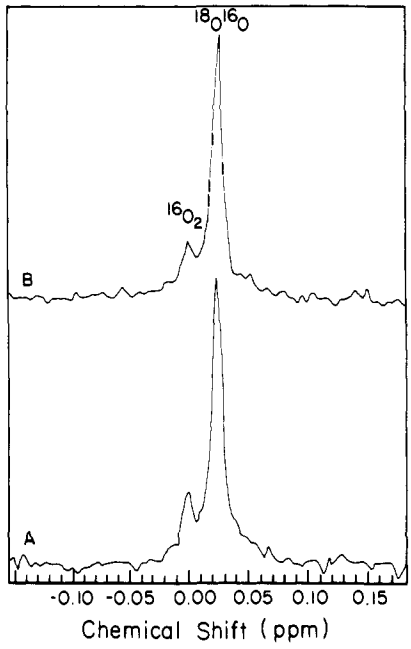

Figure 5. *syn,anti*-7-[^{18}O]Carboxynorbornene. The natural abundance ^{13}C NMR signal of the 7-carboxyl group is shifted upfield by 0.025 ppm in both the syn and anti epimers upon substitution of one ^{16}O by ^{18}O. (A) Anti. (B) Syn (83 atom % mono-^{18}O in $CDCl_3$/1% Me_4Si).

Table I. Shifts in ^{13}C Nuclear Magnetic Resonance Positions of Carbon Atoms Bound to Oxygen Upon Replacement of ^{16}O by ^{18}O

functional group	compd	^{18}O-isotope shift (ppm upfield from the corresponding ^{16}O compd)[a]
alcohol or phenol	phenol	0.016
	benzyl alcohol	0.023
	isopropyl alcohol	0.023[b]
	tert-butyl alcohol	0.035[b]
aldehyde	*n*-butyraldehyde	0.047
	benzaldehyde	0.043
carboxylic acid derivatives	sodium formate	0.025[b] (per ^{18}O)
	benzoic acid	0.031[c] (per ^{18}O)
	syn-7-carboxynorbornene	0.025 (per ^{18}O)
	anti-7-carboxynorbornene	0.025 (per ^{18}O)
ester	*p*-bromophenacyl formate	
	ether carbon	0.026
	carboxyl carbon	0.054 (per 2 ^{18}O's)
ether	phenetole	
	phenyl carbon	0.016
	ethyl carbon	0.025
	β-bromophenetole	
	phenyl carbon	0.016
	ethyl carbon	0.025
	phenyl vinyl ether	
	phenyl carbon	0.018
	vinyl carbon	0.018
ketone	acetone	0.050[c]
	benzophenone	0.045

[a] Solvent $CDCl_3$/1% Me_4Si unless otherwise noted. [b] Solvent D_2O. [c] Solvent $CDCl_3$ as well as D_2O.

Discussion

The isotopes ^{2}H, ^{13}C, and ^{34}S had been observed to affect the ^{13}C NMR signal by shifting the signal upfield[45] compared to the compound having the corresponding lighter isotope. Furthermore, ^{18}O had been shown to exert an isotope effect on other NMR signals as early as 1970. Qualitative data—Raman and infrared measurements, certain other heavy isotope effects on ^{13}C NMR, and the ^{18}O-isotope effect on other NMR signals—implied that ^{18}O should exert an isotope effect on ^{13}C NMR signals. We expected that this effect would usually result in an upfield shift in the resonance signal and that the magnitude of this shift would be dependent both on the type of compound and on the number of ^{18}O atoms bonded to the carbon.

The first published report was our description of the ^{18}O-isotope effect on the ^{13}C NMR signal of the hydroxyl carbon in *tert*-butyl alcohol.[1] Upon isotopic substitution, the natural abundance ^{13}C NMR signal was shifted upfield by 0.035 ppm. The variety of ^{18}O-labeled compounds investigated in the present report further illustrates the ^{18}O-isotope effect on ^{13}C NMR spectra. The ^{18}O-induced shifts are summarized in Table I and illustrated in Figures 1–5. The experimental data show that the heavy-oxygen isotope-induced shift is upfield and that the magnitude of the shift depends on the type of compound and the number of ^{18}O-substitutions.

The nature of the functional group plays a significant role in affecting the magnitude of the isotope-induced shift. Those functional groups which involve a single bond to oxygen—alcohols, ethers, and esters (ether carbon)—display relatively uniform isotopic shifts which depend on the hybridization of the carbon atom. (An apparent exception is *tert*-butyl alcohol which shows a markedly larger shift. The possible importance of steric effects will require further study.) The aldehyde and ketone functional groups exhibit significantly larger ^{18}O-induced shifts than do alcohols and ethers. In the case of the carboxyl functional group, the magnitude of the overall shift is consistent with contributions by one carbonyl oxygen plus one ether (or alcohol) oxygen. In the carboxylate ion and the rapidly tautomerizing carboxylic acids, the oxygen atoms are of course equivalent on the NMR time scale. The present data are too limited to analyze specifically the individual contributions to the shifts of the carboxyl functional group in esters.

The direct effect of a change in hybridization of the carbon atom upon the magnitude of the ^{18}O-isotope effect is illustrated by the two phenyl ethers phenetole and phenyl vinyl ether. The change from sp^3 to sp^2 hybridization significantly lowers the upfield ^{18}O-induced shift by 0.007 ppm. The isotopic shifts of the phenyl and vinyl carbon atoms in phenyl vinyl ether are the same (0.018 ppm). Although the total shift appears to be slightly larger than the phenyl carbon shift observed for phenol and phenetole (0.016 ppm), the difference is comparable in magnitude to the error in measurement (± 0.002 ppm). There is the possibility that the phenyl ring is contributing to the induced shift. The contribution may be quite small if the alcohols are used as a guide to compare to the ethers. Benzyl alcohol, isopropyl alcohol, and phenetole (ethyl carbon) show approximately the same sp^3-hybridization shift. Phenol and phenyl vinyl ether show approximately the same sp^2-hybridization shift. Because the isotope shift does not significantly change between the ethers and the alcohols, only a small effect, if any, results from the phenyl ring. However, further experimental evidence on this point would be desirable.

The shifts observed for the carboxyl carbons of singly and doubly ^{18}O-labeled carboxylic acids provides evidence for an ^{18}O-additivity effect, i.e., that the replacement of each equivalent ^{16}O by ^{18}O shifts the carbon resonance signal further upfield an equal amount. Examination of the data for the isotope-induced shift of the carbonyl group resonance reveals the existence of another effect which may be termed the substituent-group effect. This property may be illustrated as in Figure 6, where *n*-butyraldehyde is chosen as the central carbonyl component. Each arrow indicates the type of substituent change, i.e., hydrogen to alkyl, alkyl to aryl, hydrogen to hydroxyl, etc. The magnitude of the observed change in the isotopic shift is also given. For example, substitution of an alkyl group for a hydrogen (*n*-butyraldehyde to acetone) results in an increase in the total magnitude of the isotope shift of +0.003 ppm (upfield). Substitution of an aryl group for an alkyl (*n*-butyraldehyde to benzaldehyde) results in a decrease in the total upfield isotope shift by 0.004 ppm. Thus, there is a general substitution–isotope effect pattern exhibited by

(45) However, in addition to the examples cited in the introduction, other downfield heavy-atom isotope shifts have been found, including shifts of the ^{119}Cd and ^{199}Hg signals upon substitution by ^{13}C: Jokisaari, J.; Raisanen K.; Lajunen, L.; Passoja, A.; Pyykko, P. *J. Magn. Reson.* **1978**, *31*, 121–132. Jokisaari, J.; Raisanen, K. *Mol. Phys.* **1978**, *36*, 113.

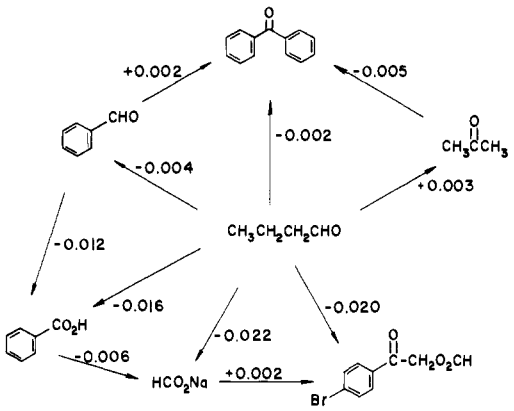

Figure 6. Substituent-group effects on the carbonyl group ^{18}O-isotopic shift. *n*-Butyraldehyde is chosen as the central component. The arrows show the direction of substituent changes (i.e., alkyl to aryl, etc.) and the magnitude of the experimentally observed change in the ^{18}O-induced upfield shift accompanying the indicated substitution. Note that two major types of changes are illustrated: changes of substituents on carbonyl compounds (upper half of figure) and functional group changes (lower part of figure).

the carbonyl group. The substitution of an aryl group for a hydrogen increases the isotope shift. The alkyl to aryl group substitution decreases the isotope shift, and the substitution of a hydroxyl or alkoxyl group for an aryl, alkyl, or hydrogen greatly decreases the isotope shift (per ^{18}O).

The experimental data show that there is no simple correlation between the isotope effect and the carbon–oxygen bond length. However, within a given functional group or among closely related functional groups, there is an approximate correlation between the carbon–oxygen bond length and the isotope shift. The isotope shifts for those functional groups which form single bonds to oxygen decrease with shorter bond lengths. A similar relationship exists for conjugated and isolated carbon–oxygen double bonds in the aldehydes and ketones. Conjugated carbon–oxygen derivatives exhibit smaller isotopic shifts than the nonconjugated systems which have longer bonds. However, among the different functional groups no general relationship such as an increase in the isotope shift with an increase (or decrease) in the carbon–oxygen bond length is evident.

It is rather surprising that experimental evidence establishing the ^{18}O-isotope effect on ^{13}C NMR signals had not been presented prior to our report.[1] In addition to the theoretical calculations of Jameson,[46] there were various lines of evidence which qualitatively indicated that such an ^{18}O-isotope effect should exist. The vibrational energies of an isotopically substituted molecule (compared to its unlabeled analogue) have been important considerations in explanations of the isotope effect on NMR chemical shifts. This emphasis particularly applies for isotopically substituted hydrogen,[6] but vibrational energies have also been invoked to successfully explain other isotopically induced shifts.[47] In the latter instance, a lower Larmor frequency (corresponding to an upfield shift) is predicted if the vibrational energies are decreased in the isotopically substituted molecule compared to the unlabeled molecule. This relationship has been shown to hold in those cases where it was investigated. The latest example is the ^{18}O-isotope effect on ^{31}P NMR in [^{18}O]inorganic phosphate.[20]

In theory, the vibrational frequencies of isotopically substituted compounds are inversely proportional only to the reduced mass and therefore are independent of the force constant. Thus a decrease in the vibrational frequencies would be observed upon heavy isotopic incorporation. Pinchas and colleagues have studied the effects of ^{18}O-substitution on the Raman and IR spectra of numerous organic compounds.[48] In accord with theoretical expectations, they found that the vibrational frequencies were decreased and the magnitude of the changes in the vibrational frequencies depended on the type of compound and on the number of ^{18}O atoms present.

In the case of NMR, some theoretical aspects of the isotope shift have been considered. For polyatomic molecules, the major contribution to isotopic shifts is considered to arise from the anharmonic vibration term of the chemical shielding.[46] If this term is negative, then the heavy-atom isotope shift will be positive; that is, there will be an upfield shift of the isotopically substituted molecule. This will usually be the case. Moreover, the magnitude of the isotope shift will be approximately proportional to the number of isotopically substituted atoms. An example would be the values calculated for the ^{18}O-isotope effect on the ^{13}C NMR signal of carbon dioxide, estimated to be 0.1017 and 0.1844 ppm upfield for mono- and di-^{18}O substitution, respectively, relative to the unlabeled molecule.[46]

In the present study, we have provided detailed experimental evidence for the existence of an ^{18}O-isotope effect on ^{13}C NMR spectra. Both qualitative expectations and the theoretical predictions have been verified. ^{18}O-isotopic substitution into simple organic compounds results in an upfield shift in the ^{13}C NMR signal, the magnitude of which is dependent on the type of compound and the number of ^{18}O atoms covalently bonded to the carbon atom. Our study shows that there are at least three factors which in varying degrees account for the experimentally observed shifts: (1) the structure of the oxygen-containing group, (2) the hybridization of the oxygen-bearing carbon atom, and (3) the structure of the molecule bonded to the oxygen-bearing carbon atom. Because the largest isotopic shifts are observed for the sp^2 hybridized carbon in carbonyl compounds, the structure of the oxygen-containing group and to a lesser extent the structure of the balance of the molecule covalently bonded to the oxygen-bearing carbon atom are the overriding factors in affecting the ^{18}O-isotope shifts. When an alkyl group, aryl group, or hydrogen is interchanged, the substituent-group effect on the carbonyl group ^{18}O-isotope shift is experimentally small but significant. However, when an alkyl group, aryl group, or hydrogen is replaced with a hydroxyl or alkoxyl group, a large substituent-group effect is seen on the carbonyl group ^{18}O-isotope shift. For those compounds which involve a single bond to oxygen the overriding factor is apparently the hybridization of the carbon atom; the structure of the oxygen-containing group and of the rest of the molecule are, in general, of minor importance.

There are many aspects of the ^{18}O-isotope effect on ^{13}C NMR signals still to be investigated and the present work begins to lay the foundation for such additional investigations. Most significantly, oxygen-exchange reactions occurring at carbon can be readily studied by using NMR techniques. Indeed, after our initial publication, Darensbourg[49] has already provided several interesting applications of the technique for studying metal–carbonyl interactions in organometallic chemistry. On the basis of the present results, we anticipate that a considerable variety of practical applications using this technique will be described.[50]

Acknowledgments. This investigation was supported by research Grant GM 27003-10 from the National Cancer Institute and by N.I.H. Grant RR 01077 from the Division of Research Resources.

(46) Jameson, C. J. *J. Chem. Phys.* **1977**, *66*, 4977–4982. Jameson, C. J. *Ibid.* 4983–4988.

(47) Lauterbur, P. C. *J. Chem. Phys.* **1965**, *42*, 799–800.

(48) Pinchas, S.; Laulicht, I. "Infrared Spectra of Labelled Compounds"; Academic Press: London, 1971; pp 238–280.

(49) Darensbourg, D. J. *J. Organomet. Chem.* **1979**, *174*, C70–C76. Darensbourg, D. J.; Baldwin, B. J. *J. Am. Chem. Soc.* **1979**, *101*, 6447–6449.

(50) After submission of the present work, further examples of the ^{18}O effect on ^{13}C NMR spectroscopy have been described: Vederas, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 374–376. Where the compounds are comparable, his results are consistent with ours and he also finds that the magnitudes of the shifts are apparently independent of solvent. That communication also provides the important result that an ^{18}O-induced isotope shift occurs with amides.