formylation gave **4b** and **5b** in 15 and 61% isolated yields, respectively, and Wolff-Kishner reduction of **5b** produced guaiazulene (**6b**, 51%) as a dark purple oil.

Dienamine-fulvene cycloadditions (Scheme II) provided even more facile routes to 4-methyl-7-alkylazulenes, since the cycloadditions are completely regiospecific. The required pyrrolidine dienamines were prepared by reaction of the lithium salts of the *N*-cyclohexylimines of butyraldehyde or isovaleraldehyde (LDA, ether, 0 °C) with acetaldehyde at −70 °C,[14] followed by hydrolysis with oxalic acid and steam distillation, to give 2-ethylcrotonaldehyde (bp 130-131 °C)[15] and 2-isopropylcrotonaldehyde (bp 142-144 °C) in 48 and 52% yield, respectively. Refluxing these aldehydes with pyrrolidine and K_2CO_3 in toluene gave the corresponding pyrrolidine dienamines (**7a**, bp 65-70 °C (2 mm) and **7b**, bp 72-75 °C (4 mm)) as mixtures of geometric isomers.

The cycloadditions of the pyrrolidine dienamines **7a** and **7b** to methylfulvene, prepared by the method of Hafner and Sturm,[16] were carried out at room temperature in ether. Workup as described previously[7] gave the dihydroazulenes **8a** and **8b** in 52 and 50% yield, respectively. These dihydroazulenes were converted into the corresponding azulenes (**2a** and **2b**) in 17 and 35% yield, respectively, by refluxing with 5% Pd/C at 170 °C in triglyme. The azulenes prepared in this way are identical with the major isomers obtained from the thiophene dioxide cycloadditions, and could be converted as described above into chamazulene and guaiazulene.

Adaptations of these routes to the synthesis of hydroazulene sesquiterpenes are under investigation.

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References and Notes

(1) Plattner, Pl. A.; Lemay, L. *Helv. Chim. Acta.* **1940**, *23*, 897.
(2) Meisels, A.; Weizmann, A. *J. Am. Chem. Soc.* **1953**, *75*, 3865. Sõrm, F.; Novák, J.; Herout, V. *Collect. Czech. Chem. Commun.* **1953**, 18, 527.
(3) de Mayo, P. "Mono- and Sesquiterpenoids", Interscience: New York, 1959.
(4) In spite of the mixtures expected in three of the steps involved, the Hafner-Ziegler method has been used for the preparation of chamazulene: Hafner, K. unpublished results reported in Hafner, K. *Angew. Chem.* **1958**, *70*, 419. The Nozoe (Nozoe, T.; Itô, S. *Fortschr. Chem. Org. Naturst.* **1961**, *19*, 32, and references therein) and the Scott (Scott, L. T. *J. Chem. Soc., Chem. Commun.*, **1973**, 882) syntheses are not expected to exhibit sufficient regioselectivity to be adapted to syntheses of "natural" azulenes.
(5) Guaiazulene: Sõrm, F.; Gut, J.; Hlavnička, J.; Kučera, J.; Šedivý, L. *Collect. Czech. Chem. Commun.* **1951**, 16, 169. Chamazulene: Novák, J.; Sorm, F.; Sicher, J. *ibid.* **1954**, *19*, 1264. Guaiazulene and chamazulene: Mangini, L.; Gandiera, G. *Gazz. Chim. Ital.* **1960**, *90*, 947.
(6) Reiter, S. E.; Dunn, L. C.; Houk, K. N. *J. Am. Chem. Soc.* **1977**, *99*, 4199. See also Copland, D.; Leaver, D.; Menzies, W. B. *Tetrahedron Lett.* **1977**, 639.
(7) Dunn, L. C.; Chang, Y.-M.; Houk, K. N. *J. Am. Chem. Soc.*, **1976**, *98*, 7095.
(8) Dunn, L. C.; Houk, K. N. *Tetrahedron Lett.* **1978**, 3411.
(9) Hartough, H.; Conley, L. *J. Am. Chem. Soc.* **1947**, *69*, 3096.
(10) Melles, J. L.; Backer, H. J. *Recl. Trav. Chim. Pays-Bas.* **1953**, *72*, 314.
(11) The workup procedure consists only of evaporation and column chromatography to remove polar byproducts. Pyridine solvent more than doubles yields reported earlier[6] with other alkylthiophene dioxides.
(12) Meuche, D.; Molloy, B. B.; Reid, D. H.; Heilbronner, E. *Helv. Chem Acta.* **1963**, *46*, 2483.
(13) NMR resonances due to the ring protons of chamazulene in several solvents are essentially identical with those reported for guaiazulene.[12] Chamazulene, **6a**, in $CDCl_3$: δ 2.62 (Me_1), 7.56 (H_2), 7.15 (H_3), 2.75 (Me_4), 6.83 (H_5), 7.25 (H_6), 2.75 (CH_2), 1.29 (Me_7), 8.08 (H_8) (J_{23} = 4.0, J_{56} = 10.5, J_{68} = 1.5 Hz).
(14) Wittig, G.; Hesse, A. *Org. Synth.* **1970**, *50*, 66.
(15) This compound can also be obtained by direct condensation of acetaldehyde with butryaldehyde or from Chemical Samples Co.
(16) Sturm, D. Dissertation, Munich, 1964.
(17) On leave from the Indian Association for the Cultivation of Science, Jadavpur, India, 1976-1978.

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An ^{18}O Isotope Shift upon ^{13}C NMR Spectra and Its Application to the Study of Oxygen Exchange Kinetics

Sir:

Numerous examples of the effects of isotopic substitution upon the NMR resonance positions of various nuclei are known. The ^2H-isotope effects on ^1H NMR spectra and ^2H- and ^{13}C-isotope effects on ^{19}F NMR spectra have been known for some time.[1,2] The ^{13}C- and ^{15}N-isotope effects on ^{59}Co NMR spectra and the ^{34}S-isotope effect on ^{13}C NMR spectra have been characterized;[3-5] the β-deuterium (*O*-deuterium) isotope effect on ^{13}C NMR spectra has been successfully employed in carbohydrate research.[6] Most recently ^{18}O-isotope effects have been reported for ^{55}Mn and ^{95}Mo NMR spectra[7] and for ^{31}P NMR spectra.[8] The latter observation has already proved useful[9-11] for studying the exchange of phosphate oxygen from phosphate ion, and it is clear that a comparable technique would be of significant utility in carbon chemistry. Jameson[12] has predicted an ^{18}O-isotope effect on ^{13}C NMR spectra, in particular an upfield shift in ^{18}O-labeled $^{13}CO_2$ which is dependent on the number of ^{18}O atoms in the molecule. We have now observed such an isotope shift in ^{13}C NMR spectra and it provides a very useful method for studying oxygen exchange kinetics.

We find an upfield shift in the natural abundance ^{13}C NMR spectrum of the hydroxyl carbon of *tert*-butyl alcohol when ^{18}O rather than ^{16}O is bonded to the carbon. [^{18}O]-*tert*-butyl alcohol was synthesized by passing dry HCl into 99 atom % excess [^{18}O]water (Norsk Hydro, Oslo), adding *tert*-butyl alcohol to the acidic [^{18}O]water, and allowing the exchange reaction to proceed at 55 °C for 60 h. The [^{18}O]-*tert*-butyl alcohol was then isolated by addition of salt, separated, dried, and distilled. Mass spectral analysis showed it to contain 83.7% ^{18}O. All natural abundance ^{13}C NMR spectra were recorded on a Varian CFT-20 spectrometer equipped with a 10-mm variable-temperature probe. A 5-mm internal diameter capillary of D_2O was used to provide an instrumental lock signal. A total of 50 scans, 200-Hz sweep width, 20.5-s acquisition

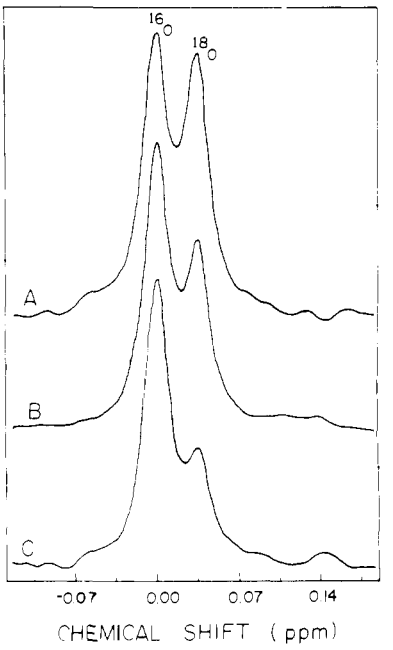

Figure 1. Natural abundance ^{13}C spectra of C-1 of *tert*-butyl alcohol made from known concentrations of [^{18}O]- and [^{16}O]-*tert*-butyl alcohol in water-deuterium oxide. An upfield shift occurs owing to ^{18}O isotopic substitution at the hydroxyl carbon. The unlabeled hydroxyl carbon is arbitrarily assigned the value 0.0 ppm. The spectra were recorded on a Varian CFT 20 spectrometer. Curve A, 48% [^{18}O]-*tert*-butyl alcohol; curve B, 40% [^{18}O]-*tert*-butyl alcohol; curve C, 31% [^{18}O]-*tert*-butyl alcohol.

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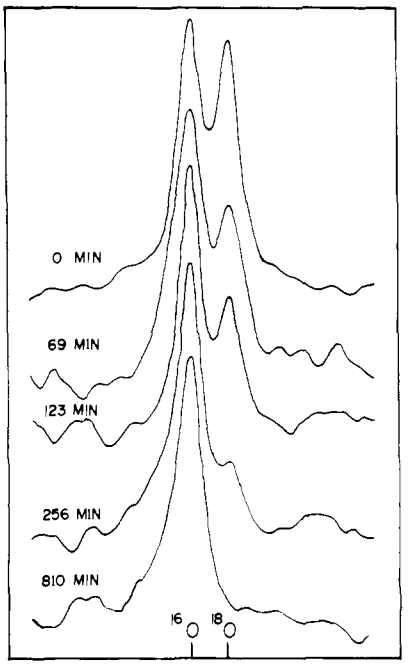

Figure 2. The acid-catalyzed exchange of the hydroxyl oxygen of *tert*-butyl alcohol as measured by ^{13}C isotope shift NMR. ^{18}O-Labeled *tert*-butyl alcohol was dissolved in 0.16 N HCl solution made up using normal [^{16}O]water. The loss of ^{18}O from the C-1 position of the alcohol was measured as a function of time. By 810 min no further change was measureable. The ^{13}C spectra were recorded on a Varian CFT-20 instrument at 65 ± 2 °C.

time, 45° pulse angle, and 8K data block were used to record the spectra in the Fourier transform mode. The exponential weighting for the free induction decay was 1.0 Hz and the resolution was 0.50 Hz. The shift separation was verified on Nicolet 150-MHz and Nicolet 360-MHz NMR instruments. Figure 1 illustrates the ^{18}O-isotope effect on the hydroxyl carbon resonance position. It shows ^{18}O-labeled *tert*-butyl alcohol diluted with known amounts of [^{16}O]-*tert*-butyl alcohol. The upfield shift is 0.035 ppm in water. The separation of the ^{13}C resonance positions was 0.7 Hz at 20 MHz, 1.32 Hz at 37.7 MHz, and 3.17 Hz at 90 MHz.

The utility of the ^{18}O-isotope shift effect on carbon was demonstrated by following the kinetics of the loss of label from a sample of [^{18}O]-*tert*-butyl alcohol. The reaction mixture for the exchange experiment contained 0.16 N HCl and 1.88 M [^{18}O]-*tert*-butyl alcohol in 3.3-mL total volume. The variable-temperature probe on the CFT-20 was equilibrated at 65 ± 2 °C for 1 h, while [^{18}O]-*tert*-butyl alcohol and glass-distilled water made acidic with HCl were equilibrated separately in a water bath at 65 °C for 30 min. The reaction was begun by addition of the [^{18}O]-*tert*-butyl alcohol to the acid solution. Figure 2 shows the ^{13}C NMR spectra of the hydroxyl carbon at five times during the course of the reaction. The disappearance of the ^{18}O-labeled signal is readily detectable and quantitative changes are easily measured.

^{13}C NMR spectra such as those shown in Figure 2 were resolved using a Du Pont 310 curve resolver set for Lorentzian curves. A kinetic plot of quantitative data for the exchange experiment gave a pseudo-first-order rate constant of 5.18×10^{-5} s^{-1}, in excellent agreement with the literature value[13] of 5.33×10^{-5} s^{-1} obtained by conventional mass spectrometric methods.

Thus, we have demonstrated the ^{18}O-isotope shift upon ^{13}C NMR spectra and provided an example of its use. The technique should be widely applicable. The present study employed natural abundance spectra and a Fourier transform instrument operating at 20 MHz for ^{13}C, so that instruments of very high magnetic field strength are not necessary for kinetic studies providing that curve resolution or electronic integration are employed. However, since the quantitative data obtained from the resolved spectra closely approximated those obtainable by peak-height measurements, it seems probable that the greater separation available with instruments of higher field strength should permit even more convenient quantitation. Further gains in sensitivity (thus providing in effect the possibility of following more rapid exchange reactions) can be achieved by measurements with ^{13}C-enriched compounds. Typical among the uses to which these procedures should be applicable may be mentioned the measurement of oxygen exchange accompanying both enzymatic and nonenzyme-catalyzed reactions of carbonyl and carboxyl group derivatives.

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References and Notes

(1) H. Batiz-Hernandez and R. A. Bernheim, *Prog. Nucl. Magn. Reson. Spectrosc.*, **3**, 63–85 (1967).
(2) J. B. Stothers, *Top. Carbon-13 NMR Specfrosc.*, **1**, 229–286 (1974).
(3) P. C. Lauterbur, *J. Chem. Phys.*, **42**, 799–800 (1965).
(4) A. Loewenstein and M. Shporer, *Mol. Phys.*, **9**, 293–294 (1965).
(5) S. A. Linde and H. J. Jakobsen, *J. Magn. Reson.*, **17**, 411–412 (1975).
(6) S.-C. Ho, H. J. Koch, and R. S. Stuart, *Carbohydr. Res.*, **64**, 251–256 (1978).
(7) K. U. Buckler, A. R. Haase, O. Lutz, M. Muller, and A. Nolle, *Z. Naturforsch. A*, **32**, 126–130 (1977).
(8) M. Cohn and A. Hu, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 200–203 (1978).
(9) D. R. Webb, G. G. McDonald, and D. R. Trentham, *J. Biol. Chem.*, **253**, 2908–2911 (1978).
(10) J. Bock and M. Cohn, *J. Biol. Chem.*, **253**, 4082–4085 (1978).
(11) R. L. Van Etten and J. M. Risley, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 4784–4787 (1978).
(12) C. J. Jameson, *J. Chem. Phys.*, **66**, 4983–4988 (1977).
(13) I. Dostrovsky and F. S. Klein, *J. Chem. Soc.*, 791–796 (1955).

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Synthesis of Thymosin α_1

Sir:

Thymosin α_1, an acetyl octacosapeptide, isolated from calf thymus gland by Goldstein et al.[1] was reported to exhibit biological activities that are important for the development of thymus-dependent lymphocytes (T cells) in man and in animals.[2] Its amino acid sequence was determined to be[3] Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn. In this communication, a solution synthesis[4] of thymosin α_1 is described. The synthetic product was found to be identical with the natural material, confirming the proposed structure of the polypeptide hormone.

As outlined in Scheme I, Boc-Ile-Thr(Bzl)-Thr(Bzl)-Lys(Z)-OH (**3**) was treated with 4 N HCl in THF to remove the Boc group and the ensuing tetrapeptide hydrochloride salt was coupled with Boc-Val-Asp(OBzl)-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Glu(OBzl)-HNNH$_2$ (**2**) by the azide method[5] to give the protected decapeptide Boc-Val-Asp(OBzl)-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Glu(OBzl)-Ile-Thr(Bzl)-Thr(Bzl)-Lys-(Z)-OH (**7**); 76.2%; mp 268–271 °C. Anal. $C_{106}H_{133}N_{11}O_{24}$, C, H, N. Amino Acid Anal. $Asp_{1.05}$, $Ser_{1.88}$, $Glu_{1.09}$, $Thr_{2.93}$, $Val_{0.98}$, $Ile_{1.01}$, $Lys_{1.06}$. Azide coupling between Ac-Ser(Bzl)-Asp(OBzl)-Ala-Ala-HNNH$_2$ (**1**) and **7** after removal of Boc group from **7** with TFA provided the protected N-terminal acetyl tetradecapeptide Ac-Ser(Bzl)-Asp(OBzl)-Ala-Ala-Val-Asp(OBzl)-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Glu(OBzl)-

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