appreciation for computing time made available by DOE on the ER-Cray X-MP computer. We are grateful to Dr. W. R. Busing for making his WMIN program available to us and to Dr. D. Wolf for references.

Natural Abundance Deuterium NMR as a Novel Probe of Monoterpene Biosynthesis: Limonene

M. F. Leopold, William W. Epstein,* and David M. Grant*

Department of Chemistry, University of Utah
Salt Lake City, Utah 84112

Received June 8, 1987

Our interest in proton-decoupled, natural abundance deuterium nuclear magnetic resonance spectroscopy (^{2}H NMR) of monoterpenes has been sparked by the observation that at natural abundance, deuterium resonances of natural camphor-*d* have markedly different relative peak intensities compared to the proton spectrum and that these intensities are different from those observed for synthetic camphor (derived from α-pinene).[1] Although the first natural abundance ^{2}H NMR spectrum of *n*-butyl iodide was obtained in 1973,[2] experimental considerations have prevented its commonplace use (compared with ^{13}C NMR). However, the advent of modern high field NMR technology coupled with short T_1's (<7 s)[3] and negligible NOE's now allows measureable differences in deuterium peak intensities to be directly related to ^{2}H/^{1}H ratios at specific sites in a molecule. Thus, deuterium at natural abundance is the "perfect" isotopic tracer since no synthesis of labeled substrate is required and individual site-specific differences can be measured.

Martin et al. first noted that at natural abundance large variations in site-specific ^{2}H/^{1}H ratios occurred and that these integration values varied greatly from synthetic to naturally derived compounds. Both ethanol[5] and anethole[6,7] were found to have different identifiable ^{2}H/^{1}H site-specific ratios related to source of origin. More recently, primary KIE's of several well-understood reactions have been measured by using natural abundance ^{2}H NMR, and k_H/k_D values were comparable with literature values.[8] Analysis of α- and β-pinene suggested that isotopically sensitive partitioning within the pinene cyclase enzyme was occurring[8] and the measured KIE was similar to the theoretical value for such a partitioning.[9] A correlation between the optical purity and site-specific deuterium hydrogen ratios has been observed for α-pinene as well.[10]

Our study of monoterpene biosynthesis led us to select limonene (**4**), the simplest of the *p*-menthane monoterpenes, for analysis since the question of the genesis of the exocyclic double bond has not been satisfactorily answered experimentally. A shortened form of the proposed biosynthesis of limonene is shown in Scheme I.[11] Generation of this olefin required only proton loss from the α-terpinyl cation **3** postulated as a common intermediate in many bicyclic monoterpene biosyntheses. Although both C-7 and C-9 of **2** are derived from the methyl group of mevalonic acid (**1**)[12] and proton loss to form limonene is postulated to be regiospecific from C-9 of **3**, tracer studies of incorporation of 2-^{14}C mevalonic acid were far from conclusive.[13] Analysis of a more recent feeding study using [4,10-$^3H_4$,U-^{14}C]geraniol has suggested that proton abstraction occurs only from C-9, but incorporation was low and apparently not reproducible.[14]

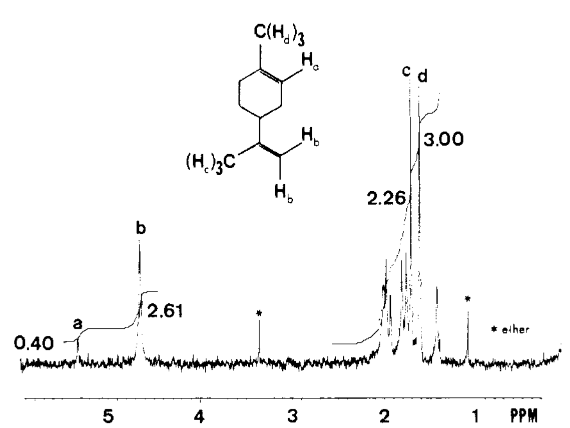
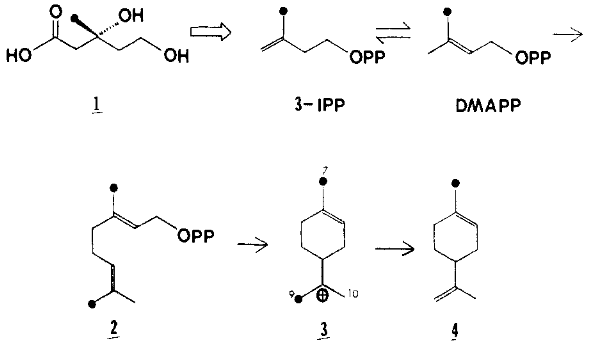


Figure 1. Natural abundance, proton-decoupled ^{2}H NMR spectrum (61.4 MHz) of *R*-(+)-limonene. The impurity marked is ethyl ether used in extraction. The 2 M solution was prepared with C_6F_6 as the lock solvent and TMS as the chemical shift reference. The acquisition parameters used were as follows: acquisition time, 3 s; pulse width, 80°; number of transients, 10 000; probe temperature, 30 °C.

Scheme I



Natural *R*-(+)-limonene was isolated by steam distillation followed by ether extraction from Florida navel oranges collected exclusively from one tree. The purity of this sample was assessed by ^{1}H NMR and GC and found to be >98%; the measured optical rotation was $[\alpha]^{21}_D = 96.6°$ (neat). Selective ^{1}H-^{1}H decoupling experiments allowed assignment of the two methyls in the proton spectrum. This assignment is analogous in the natural abundance deuterium spectrum shown in Figure 1. The integrated intensity of the methyl hydrogens of C-7 is used as an internal standard for normalization since this methyl group remains unperturbed throughout the biosynthesis.[8] A relative depletion of 25% (2.26) is observed for the isopropenyl methyl hydrogens, H$_c$, relative to the hydrogens, H$_d$, of C-7. This depletion is clearly inconsistent with selective loss of a proton from C-10. If proton loss had occurred regiospecifically from C-10, then the integrated intensities of the methyl groups should be the same within experimental error.[15] This is not the case, and the relative depletion of deuterium noted in the isopropenyl methyl hydrogens can be directly traced to the 3-isopentylpyrophosphate (IPP) → dimethylallylpyrophosphate (DMAPP) isomerization.

The isopropenyl vinyl hydrogens, H$_b$, are enhanced to a relative value of 2.61 ± 0.13 (statistically this value should be 2 in the absence of any KIE). The enhancement of the isopropenyl vinyl hydrogens is expected since hydrogen abstraction is favored over deuterium abstraction, and k_H/k_D may be calculated from eq 1

---

(1) Grant, D. M.; Curtis, J.; Croasmun, W. R.; Dalling, D. K.; Wehrli, F. W.; Wehrli, S. *J. Am. Chem. Soc.* **1982**, *104*, 4492.

(2) Briggs, J. M.; Farnell, L. F.; Randall, E. W. *J. Chem. Soc., Chem. Commun.* **1973**, 70.

(3) Mantsch, H. H.; Saito, H.; Smith, I. C. P. *Prog. NMR Spectrosc.* **1977**, *11*, 211.

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

(5) Martin, G. J.; Martin, M. L.; Mabon, F.; Michon, M.-J. *J. Agric. Food Chem.* **1983**, *31*, 311.

(6) Martin, G. J.; Martin, M. L.; Malbon, F. *J. Am. Chem. Soc.* **1982**, *104*, 2658.

(7) Martin, G. J.; Javier, P.; Mabon, F. *Analusis* **1985**, *13*, 267.

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

(9) Jones, J. P.; Korzekwa, K. R.; Rettie, A. E.; Trager, W. F. *J. Am. Chem. Soc.* **1986**, *108*, 7074.

(10) Martin, G. J.; Janvier, P.; Akoka, S.; Mabon, F.; Jurczak, J. *Tetrahedron Lett.* **1986**, *27*, 2855.

(11) Croteau, R. B. *Chem. Rev.* **1987**, *87*, 929.

(12) Cornforth, J. W. *Tetrahedron* **1974**, *30*, 1515.

(13) Sandermann, W.; Bruns, K. *Planta Medica* **1965**, *13*, 364.

(14) Akhila, A.; Banthorpe, D. V.; Rowan, M. G. *Phytochem.* **1980**, *19*, 1433.

(15) Analytical studies in this laboratory have determined the experimental error for deuterium integration is 5%.

0002-7863/88/1510-0616$01.50/0

$$\frac{D_{\mathrm{CH_2}}}{D_{\mathrm{CH_3}}} = \frac{(n-1)k_{\mathrm{H}}/k_{\mathrm{D}}}{(n-1)k_{\mathrm{H}}/k_{\mathrm{D}} + 1} \qquad (1)$$

where D equals the normalized integration value and n equals the number of protons available for abstraction. Thus k_H/k_D is found to be 3.35.[16] All of the data are consistent with the loss of a proton from C-9 of **3** and with the intermediacy of the α-terpinyl cation in limonene biosynthesis. The use of this spectral technique provides great simplicity in determining the regiospecificity of proton abstraction as well as in calculating the KIE without the use of labeled substrates or enzyme isolation.

Acknowledgment. This research was supported by NIH Grant GM 08521-27. Grateful thanks are owed to Dr. Cheves T. Walling for his useful discussions on KIE's. Our thanks also go to David Bar-Zvi of Flamingo Gardens, Ft. Lauderdale, FL, for his generous donation of the navel oranges.

---

(16) Melander, L.; Saunders, W. H. *Reaction Rates of Isotopic Molecules*; John Wiley & Sons: New York, 1980; pp 91–111.

Acyclic Stereocontrol via an Electron-Transfer Process. Remarkable Stereochemical Difference between One- and Two-Electron Events

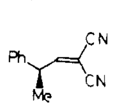
Yoshinori Yamamoto,*[†] Shinji Nishii,[†] and Toshiro Ibuka[‡]

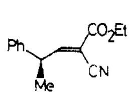
Department of Chemistry, Faculty of Science
Tohoku University, Sendai 980, Japan
Faculty of Pharmaceutical Sciences
Kyoto University, Kyoto 606, Japan

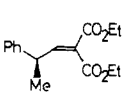
Received September 8, 1987

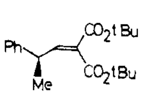
Acyclic stereocontrol is of current importance in practical and theoretical organic chemistry.[1] Stereochemistries of nucleophilic and electrophilic reactions have been widely investigated,[1] and their theoretical studies have also been carried out recently.[2] However, stereochemical study of a radical process in acyclic systems is very rare, although the theoretical prediction has been made.[2] Furthermore, the acyclic stereochemistry via an electron-transfer process has not yet been investigated systematically.[3] We report, for the first time, a surprising stereochemical difference between a nucleophilic process and an electron-transfer process in an acyclic system.

The nucleophilic addition with organometallic compounds, the free-radical addition, and the allylation via photoinduced electron transfer were investigated by using the Michael acceptors (**1–4**) bearing a chiral center at the γ-position.[4] The diastereoselectivities are summarized in Table I. Organocopper addition to **1** gave the syn isomer **5a** predominantly regardless of the reagent (entries 1–4). On the other hand, other organometallic reagents such as the allyltin and the aluminum ate complex afforded the anti isomer **6a** predominantly (entries 5 and 6). The stereochemistries of **5** and **6** were determined unambiguously by comparison with an authentic material (Supplementary Material).[5]

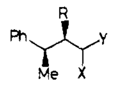

1 (–1.52 V)


2 (–1.73 V)

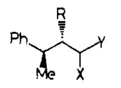

3 (<–2.0 V)


4

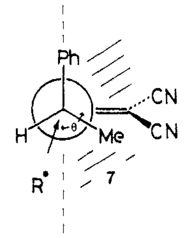
(E_{red} values in volts vs SCE measured in MeCN)


5 (syn)

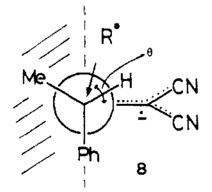
a, X=Y=CN
b, X=CN, Y=CO_2Et
c, X=Y=CO_2Et
d, X=Y=CO_2tBu


6 (anti)

a, X=Y=CN
b, X=CN, Y=CO_2Et
c, X=Y=CO_2Et
d, X=Y=CO_2tBu



7



8

The cuprate addition to **2** again gave the syn adduct **5b** predominantly (entries 10 and 12). Quite interestingly, however, the alkylcopper addition to **2** produced the anti adduct **6b** predominantly (entries 9 and 11). The same tendency was observed for the organocopper addition to **3** and **4** (entries 13–20).

We anticipated that the marked stereochemical contrast between the organocoppers and other organometallic reagents in the addition to **1** and between the cuprates and alkylcoppers in the addition to **2–4** would be a reflection of the electron-transfer process.[6] Actually, the photoinduced electron-transfer allylation[7] of **1** with allyltin gave **5a** predominantly (entry 7), although the desired adduct was obtained in very low yield. The radical addition[8] of BuI to **1** produced **6a** preferentially (entry 8), and the similar radical allylation[8] of **1** with allyl iodide also gave the anti isomer predominantly.

The addition of ordinary organometallics such as allyltin–$TiCl_4$ and allylsilane–$TiCl_4$ to **3** gives the anti isomer **6c** predominantly,[1] and this stereoselectivity is in good agreement with the selectivity predicted by a modified Felkin or Cram rule. Therefore, the diastereoselection exhibited in the alkylcopper addition to **2–4** as well as the stereoselectivity in entries 5 and 6 fall under the category of the normal selectivity predictable from nucleophilic additions.

The diastereoselectivities of the organocopper addition in the presence of *p*-dinitrobenzene (pDNB),[9] which possesses a strong electron acceptor ability, are summarized in Table II. *The syn selectivity was changed to the anti selectivity.* pDNB presumably accepts an electron from RCu[10] and thus prevents formation of the radical anion of **1** (entries 1 and 3). In these cases, both the anti preference and low conversion were observed. The R_2CuLi–pDNB system also exhibited the anti preference, while the *conversion was enhanced* (entries 2, 5–7). The reason of this enhancement is not clear,[11] but it seems that pDNB again prevents

---

[†] Tohoku University.
[‡] Kyoto University.

(1) For example, see: Yamamoto, Y. *Acc. Chem. Res.* **1987**, *20*, 243 and references cited therein.

(2) Houk, K. N.; Paddon-Row, M. N.; Rondan, N. G.; Wu, Y. D.; Brown, F. K.; Spellmeyer, D. C.; Metz, J. T.; Li, Y.; Loncharich, R. J. *Science (Washington, D.C.)* **1986**, *231*, 1108.

(3) Yamamoto, Y.; Maruyama, K. *J. Am. Chem. Soc.* **1985**, *107*, 6411.

(4) The asymmetric induction in **1** was studied previously. Kruger, D.; Sopchik, A. E.; Kingsbury, C. A. *J. Org. Chem.* **1984**, *49*, 778.

(5) The stereochemistry determined by us was in agreement with the previous assignment[4] and made by ^1H NMR. However, the isomer ratio in the addition of Me_2CuLi did not agree (entry 2 vs Table I of ref 4).

(6) (a) House, H. O. *Acc. Chem. Res.* **1976**, *9*, 59. (b) Posner, G. H. *An Introduction to Synthesis using Organocopper Reagents*; John Wiley: 1980. See, also: (c) Corey, E. J.; Boaz, N. W. *Tetrahedron Lett.* **1985**, *26*, 6015.

(7) For example, Mizuno, K.; Toda, S.; Otsuji, Y. *Chem. Lett.* **1987**, 203.

(8) Giese, B.; G-Gomez, J. A.; Witzel, T. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 69.

(9) Yang, D.; Tanner, D. D. *J. Org. Chem.* **1986**, *51*, 2267.

(10) When pDNB was added to a colorless ether solution of Me_2CuLi, the color immediately changed to dark brown.

(11) This interesting observation may be rationalized as follows. $(R_2CuLi)^{\bullet+}$ produced by an electron transfer may react more rapidly and more cleanly with the Michael acceptors than R_2CuLi itself. $(RCu)^{\bullet+}$ might easily decompose to $R^{\bullet}$ and thus lose capability of the conjugate addition. Further aspects are now under active investigation.