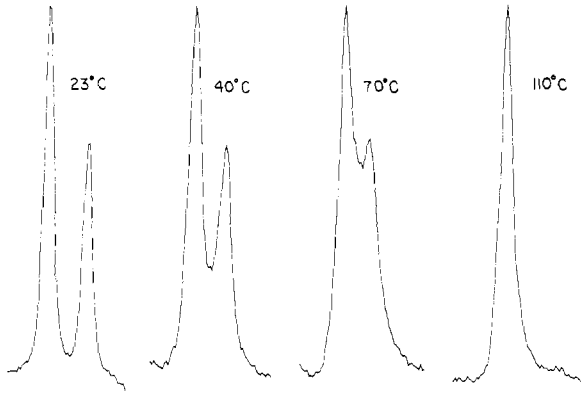

Figure 6. Effect of increasing temperature on the γ-methyl region of the β-decoupled 300-MHz ^{1}H NMR spectrum of parabactin in Me_2SO-d_6.

thermore, when the sample is cooled to −13 °C, the five lines of the α-methine become six lines, three sets of doublets (Figure 5), with identical coupling constants (J = 6.5 Hz). The γ methyls are not nearly as sensitive to cooling. When the β methine is decoupled, the five α-methine lines collapse to three lines just as the six γ-methyl lines collapse to three lines. Furthermore, and as expected under these decoupling conditions when the sample is observed at higher temperatures, e.g., 110 °C in Me_2SO-d_6, the three lines of the α methine and the two lines of the γ methyls appear as single lines (Figure 6). Determination of the exact coalescence temperature and Arrhenius energy is under study. These results are, of course, in complete accord with the idea of different conformers.[11,23] Because of the complexity of the β-methine signals, temperature experiments were not very revealing, although decoupling experiments clearly demonstrated its coupling to the α-methine and γ-methyl protons. We do not understand yet precisely how the temperature-dependent ^{1}H NMR spectrum of parabactin relates to its various conformers; however, this is currently under investigation.

The remainder of the spectrum, when taken in 10:1 $CDCl_3$/Me_2SO-d_6, is as expected. The six internal methylene protons of the spermidine backbone are in a δ 2.03–1.48 envelope while the eight amide methylene protons are under a δ 3.78–3.11 envelope. The β-proton signal is a complex envelope extending from δ 5.45 to 5.28. We were unable to run a two-dimensional ^{1}H spectrum, and therefore, further splitting information was not available. The aromatic proton signals consist of five well-separated envelopes: δ 6.52–6.67 (2 H), 6.78–6.92 (4 H), 7.06–7.15 (2 H), 7.27–7.35 (1 H), 7.51–7.71 (1 H). Finally, the NH and OH protons are as described by Neilands with the NH and non-hydrogen-bonded OH protons at δ 7.89–8.17 and the hydrogen-bonded protons δ 11.56–12.82.

The scheme described above represents the first synthetic route for accessing parabactin. This facile sequence offers the siderophore in high yield and can also be applied to generation of the nor- and homospermidine homologues with equal success. We are currently employing ^{1}H nuclear Overhauser effects to determine which conformations of parabactin belong to which set of oxazoline methine doublets. This will make it possible to pinpoint changes required for metal complexation and allow for a detailed examination of the siderophores' solution conformations.

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Registry No. 1, 78217-75-1; **2**, 82247-45-8; **3**, 82265-49-4; **4**, 82247-46-9; 2-hydroxybenzimino ethyl ether, 82247-47-0; parabactin, 74149-70-5; *N*-carbobenzoxy-L-threonine, 19728-63-3.

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NMR Determination of Site-Specific Deuterium Isotope Effects

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Nuclear magnetic resonance spectroscopists have long anticipated the day when very high-field magnets would be commerically available for obtaining high-resolution deuterium spectra.[1] Aside from a small isotope effect, ^{2}H chemical shifts correspond to ^{1}H shifts. Moreover, the relaxation mechanism of ^{2}H is reasonably well understood. But, more importantly, the relatively low natural occurrence (~0.015%) of ^{2}H precludes scalar coupling between two ^{2}H nuclei from ever being significant in the ^{2}H spectra of nonenriched compounds. Thus, as with other relatively rare isotopes, proton-decoupled ^{2}H spectra of most organic compounds consist of a single resonance for each chemically different nucleus, presuming of course that the magnetic field is large enough that chemically similar resonances can be resolved. Because spin–spin splittings are eliminated in these resonances, there is no problem with the second-order splittings which lead to banding of proton resonances in coupled, closely positioned proton spectra.

While this feature was being explored in a series of methylcyclohexanes, it was observed that the peak intensities of the ^{2}H singlets in most of the molecules corresponded reasonably well within experimental errors to the proton atomic ratios (e.g., see the ^{2}H spectrum of methylcyclohexane in Figure 1a and the quantitative data in Table I). However, the significant deviations from atomic ratios involved more peaks and were considerably larger in 1,1,3-trimethylcyclohexane (see the table and Figure 1b). Special attention is drawn to the low relative intensity of the 5-e peak. The data on peak intensities have been normalized to the number of protons in each molecule. Pending the availability of absolute intensity data, the use of normalized relative intensities focuses on the intensity deviations of each peak or site from the average molecular deuterium concentration. If the isotopic effect is either all depletion or all enhancement, then such a normalization procedure tends to minimize the extent of deviations from normal isotope ratios. A careful study of the T_1's and proton–deuterium NOE's for the two compounds given in Figure 1 indicates that there are no significant differences in relaxation parameters for any of the carbons in the two different molecules. No nuclear Overhauser enhancement of ^{2}H due to ^{1}H irradiation could be detected in either molecule. Therefore transient effects cannot be used to account for variations in the peak intensities from the intramolecular hydrogen ratios. Instead what is being observed are measurable variations in the ^{2}H to ^{1}H isotope ratio at different sites within 1,1,3-trimethylcyclohexane.

Marked isotope effects among the isotopes of hydrogen are of course legion, especially when hydrogen substitution, elimination, or transfer reactions are involved. Furthermore, enzymatic and other biologically significant reactions could be expected to be quite sensitive to such effects. Accordingly, ^{2}H spectra at natural

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Table I. Integrated Peak Intensities[a]

				Methycyclohexane						
position	3e, 5e	2e, 6e	4e	1a	3a, 5a	4a	2a, 6a	Me		
shift	1.66_3	1.63_8	1.61_4	1.30_2	1.21_8	1.11_3	0.87_5	0.85_9		
no. of H's	2	2	1	1	2	1	2	3		
peak intensity	2.06	1.84	0.73	0.98	2.14	1.15	1.80	3.30		
				1,1,3-Trimethylcyclohexane						
position	4e	3a, 5a	5e	2e, 6e	6a	Me(1e, 1a)	Me(3e)	2a	4a	
shift	1.64_1	1.49_9	1.41_5	1.32_9	1.03_4	0.88_2	0.83_0	0.75_2	0.71_4	
no. of H's	1	2	1	2	1	6	3	1	1	
peak intensity	0.83	1.09	0.60	1.95	1.11	7.25	3.35	1.07	0.75	
				d-Camphor (Naturally Occurring)						
position	3-exo	5-exo	4-	3-endo	5-endo	(6-exo,	6-endo)[b]	Me_c	Me_b	Me_a
shift	2.28_3	2.07_3	1.93_3	1.78_1	1.66_0	1.32_9		0.95_4	0.86_5	0.81_1
no. of H's	1	1	1	1	1	2		3	3	3
peak intensity	1.18	0.79	1.16	1.24	1.05	2.05		2.76	3.17	2.60
				d-Camphor (Synthetic)						
position	3-exo	5-exo	4-	3-endo	5-endo	(6-exo,	6-endo)[b]	Me_c	Me_b	Me_a
shift	2.27_1	2.07_0	1.93_3	1.76_8	1.65_5	$(1.34_7$	$1.32_6)$	0.95_2	0.85_9	0.80_6
no. of H's	1	1	1	1	1	(1	1)	3	3	3
peak intensity	1.47	0.92	1.11	1.52	0.86	(0.86	0.88)	2.61	3.27	2.49

[a] Integrated peak intensities have been normalized such that the sum of intensities equals the number of protons. [b] The close proximity of the 6-exo and 6-endo prevent an unequivocal assignment of the two peaks observed in the synthetic *d*-camphor. In the natural occurring *d*-camphor we were unable to resolve these two peaks.

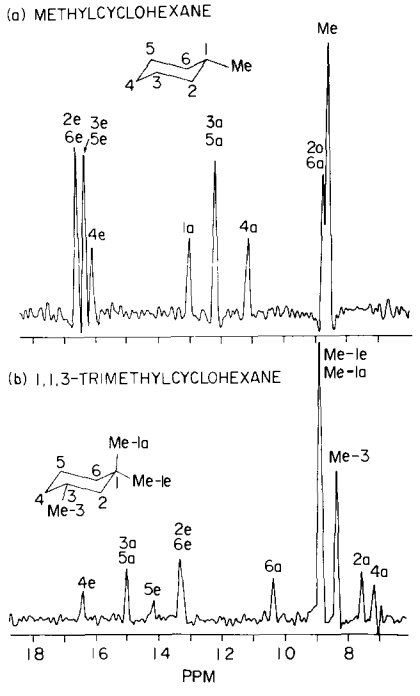

Figure 1. The 77-MHz deuterium magnetic resonance spectra of (a) methylcyclohexane and (b) 1,1,3-trimethylcyclohexane. The spectra obtained on the natural abundant ^2H isotope exhibit peak heights that correspond in methylcyclohexane and that do not correspond in 1,1,3-trimethylcyclohexane to the proton atomic ratios. Thus, site-specific isotope effects (enhancement and/or depletion) are observed in the latter compound.

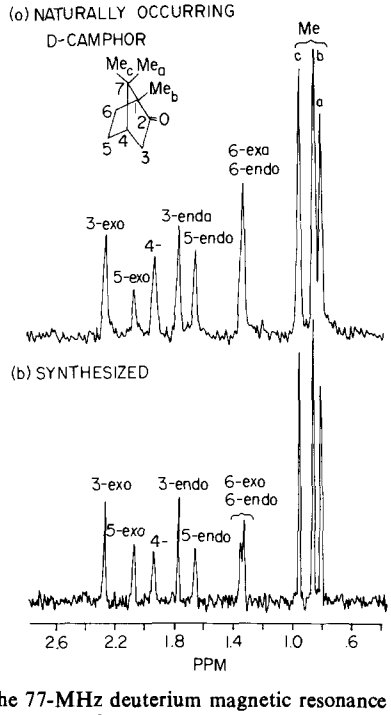

Figure 2. The 77-MHz deuterium magnetic resonance spectra of two samples of *d*-camphor of different origin. (a) *d*-Camphor, obtained from Sigma Chemical (no. 107C-0342), was secured from naturally occurring sources. (b) *d*-Camphor, obtained from Spectrum Chemical Manufacturing Corp. (no. 19048-C10), was synthesized from α-pinene and resolved into the relevant optical isomer. Attention is drawn to significant variations between the two spectra and also to deviations in peak heights from the proton atomic ratios. Both a site-specific isotope effect and a chemical-history effect are observed in these spectra.

abundance were obtained on a variety of natural products. The proton-decoupled ^2H NMR spectra of two different samples of *d*-camphor are shown in figure 2, wherein the $^2H/^1H$ isotope ratios as represented by the peak intensities vary from one peak to another by a factor of almost 2-fold. Furthermore, the spectra of the two different samples of camphor (one from nature (Figure 2a) and the other (Figure 2b) synthesized from α-pinene) also vary from each other in some peaks by more than experimental error. These results exhibit that either isotope depletion or enrichment not only is site specific but also depends upon the chemical history of the substance. Thus, isotope effects first noted for 1,1,3-trimethylcyclohexane have become the general rule for natural products such as *d*-camphor. Spectra obtained on other natural products also indicate significant variations in the ^2H peak intensities from expected hydrogen atomic ratios. It is anticipated that these variations provide important information on the origin and/or the mechanism of biosynthesis of such compounds. It is interesting to note that a *dl*-camphor synthesized from α-pinene has a spectrum very similar to that found in Figure 2b.

It is well recognized in the biosynthesis of organic compounds in plants that the products are depleted in ^2H comparison to the water used in the synthesis.[2,3] The common method of analysis

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of the $^2H/^1H$ ratios is by complete combustion of the organic matter to CO_2 and H_2O and reduction to H_2 in a uranium furnace and mass spectroscopic analysis of the isotropic ratio of the whole compound without any indication of isotopic ratios as a function of site within the molecule. Some effort has been made to be more selective in analysis of $^2H/^1H$ ratios. In particular, work has been done on the nonexchangable hydrogens of cellulose.[4,5] While these studies focused on one type of site by eliminating the hydroxyl hydrogens, the $^2H/^1H$ ratios were not site specific for the C-H hydrogens. Rauschenbach et al.[6] compared $^2H/^1H$ ratios for synthetic and fermented ethanols and also determined the isotopic ratio in the methyl group of the ethanol by oxidation to acetic acid and analysis of the corresponding acetate salt. Martin and Martin[7,8] recently have used ^2H NMR to show that the isotopic distribution of ^2H in ethyl and vinyl groups varies from molecule to molecule.

The full significance of observing site-specific isotopic effects upon the $^2H/^1H$ ratio is emphasized when it is realized that such information is not readily available from the traditional mass spectrometric methods of measuring isotope ratios. Although mass spectral methods are much more sensitive than NMR techniques and have a much larger dynamic range, such methods can only measure the average molecule $^2H/^1H$ isotope ratio because of the facile scrambling of the molecular hydrogens (both ^1H and ^2H) in the typical mass spectrometric ion. Futhermore, these hydrogen rearrangements can be isotopic selectivities as large as 10^5 in some instances.[9] Such large isotope effects would completely mask the significant but still smaller intramolecular ratios that we have been recently observing. Thus, high-field deuterium magnetic resonance, ^2H NMR, techniques provide a unique method for determining this potentially valuable information.

Site-specific isotopic ratios have the potential for more complete characterization of the rate-controlling processes in rather complex chemical and biosynthesis processes. Detailed information on mechanistic steps should be available from variations in the $^2H/^1H$ ratio as a function of molecular site. This technique, applicable at natural abundance, allows analysis without destruction of the sample and removes the possibility of artifical inhibition of a metabolic system by excess deuterium present in labeled compounds. Finally, as found in our two camphors, one may anticipate the opportunity of observing the chemical history of two identical molecules derived from different precursors from variations in the intramolecular $^2H/^1H$ ratios stampled into the molecule by alternative synthetic pathways. With improved sensitivity and spectral dispersion at very high fields, proton-decoupled ^2H NMR can now realize its full potential.[1] The significant isotope effects observed herein further enhance the importance of ^2H NMR techniques.

Experimental Section. The 76.77-MHz ^2H spectra were recorded on a Bruker WM500 spectrometer operating in Fourier transform mode. Methylcyclohexane and 1,1,3-trimethylcyclohexane were run as neat liquids, and *d*-camphor was prepared as a nearly saturated solution in chloroform. Each sample contained 10% tetramethylsilane as a chemical shift reference. Spectra requiring about 400 scans were acquired at 8-s intervals by utilizing a 90° pulse and a 2-s data aquisition of 2048 points. Broad-band decoupling at 5 W of power was used with the power reduced to 0.4 W during the interval between accumulations to allow the nuclear magnetization of the sample to recover under reduced dielectric heating. Free induction decays were filtered by using a Lorentzian to Gaussian line-shape transformation and zero filled to 8192 points before Fourier transformation. Assignment of resonance lines to specific molecular sites was accomplished from analysis of the corresponding 500-MHz proton spectra or from associating the protons with a specific carbon peak by using selective proton decoupling.

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Registry No. Methylcyclohexane, 108-87-2; 1,1,3-trimethylcyclohexane, 3073-66-3; *d*-camphor, 464-49-3.

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Tricyclo[5.1.0.0^{2,8}]octa-3,5-diene (Octavalene)

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The unique properties of the $(CH)_8$ hydrocarbons[1] have attracted the attention of many organic chemists. As a member of this series the title compound (**8**) enjoys special interest. The butadiene bridge should substantially widen the dihedral angle of the bicyclo[1.1.0]butane system from the normal value of about 122°,[2] resulting in an increased strain energy. This, as well as the electronic interaction of the π system and the strained σ system, leads one to anticipate that **8** will exhibit unusual chemical and spectroscopic properties. Furthermore, the Woodward-Hoffmann rules allow a thermally induced [1,5] carbon shift. Repetition of this degenerate rearrangement would finally equilibrate all CH groups. Thus, **8** could be te bullvalene equivalent of the $(CH)_8$ family.

Interested in such prospects, several research groups have undertaken to synthesize **8**. When 8,8-dibromobicyclo[5.1.0]octa-2,4-diene was treated with methyllithium, the resulting carbene rearranged to a dihydropentalene[3,4] instead of inserting into the syn-6-CH bond as intended. Likewise, the tricarbonyl iron complex of the dibromide with methyllithium failed to give the desired insertion product.[5] In analogy to the preparation of benzvalene via cyclopentadienylcarbene[6] the intramolecular [2 + 1] or [6 + 1] cycloaddition of cycloheptatrienylcarbene to give **8** has been attempted. However, only cyclooctatetraene, heptafulvene, benzene, and acetylene could be identified as reaction products.[7] Also, the disrotatory opening of the cyclobutene portion in tetracyclo[4.2.0.0^{2,4}.0^{3,5}]oct-7-ene, which should produce **8**, could not be achieved without rearrangement of the bicyclo[1.1.0]butane system.[8] In addition, a straightforward route to prepare 4-bromooctavalene by cyclopropane ring enlargement of the dibromocarbene adduct of homobenzvalene and subsequent hydrogen bromide elimination failed because under all conditions tried a *trans*-bis(homobenzene) derivative was formed in the first step,[9,10] most probably in an acid-catalyzed process.[10] Here, we

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