Conformational Analysis of Leukotriene B_4 in Solution Based on High-Field Nuclear Magnetic Resonance Measurements†

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Abstract: The preferred conformations of leukotriene B_4 in solution were evaluated on the basis of the analysis of the proton–proton vicinal coupling constants, nuclear Overhauser effect data, and proton selective and nonselective relaxation times. The C5–C12 carbon atoms are planar, and the populations of the rotamers about C4–C5, C5–C6, C11–C12, C12–C13, C13–C14, and C15–C16 are calculated. The nuclear Overhauser effect data combined with proton selective and nonselective relaxation times, as well as conformational information from model compounds, are all found to be necessary to a thorough understanding of the conformations of the LTB_4 molecule because of its flexibility.

The metabolism of arachidonic acid involves its oxygenation via two enzymic systems. Cyclooxygenase transforms arachidonic acid to the prostaglandins, the thromboxanes, and prostacyclin, and lipoxygenases give rise to a new class of eicosanoids, the leukotrienes. In this metabolic process, 5-lipoxygenase oxidizes arachidonic acid to form 5(*S*)-hydroperoxy-6(*E*),8,11,14(*Z*)-eicosatetraenoic acid (5-HPETE), which is then converted to (5*S*)-5,6-epoxy-7(*Z*),9(*Z*),11(*E*),14(*E*)-eicosatetraenoic acid, leukotriene A_4 (LTA_4).[1,2] Leukotriene A_4 may be further metabolized by two enzymic pathways or may be chemically hydrolyzed by a nonenzymic pathway.

The first enzymic pathway involves conjugation with glutathione to produce leukotrienes C_4, D_4, and E_4. These compounds collectively account for the biological activity known as slow-reacting substance of anaphylaxis (SRS-A). These leukotrienes possess potent biological properties and can contract certain smooth-muscle preparations and constrict coronary and cerebral arteries and may induce changes in vascular permeability and mucus production. They may therefore be important mediators of human diseases such as bronchial asthma. The second enzymic pathway[2,3] involves insertion of H_2O to produce a 5,12-dihydroxyeicosatetraenoic acid, leukotriene B_4, in which the configuration of the hydroxyl groups is 5*S*,12*R* and the triene structure is cis,trans,trans (Figure 1). Two other 5,12-diHETEs can also be produced by the nonenzymic hydrolysis of leukotriene A_4. In this case, the double bonds in the triene structure are all trans and the hydroxyl groups have the configuration 5*S*,12*S* or 5*S*,12*R*. A fourth 5,12-dihydroxyeicosatetraenoic acid may be produced by a dual lipoxygenase attack and in this case the triene structure has the configuration trans,cis,trans and the hydroxyl groups are 5*S*,12*S*. The research on the leukotrienes has now been well reviewed.[4–7]

Leukotriene B_4 is a potent stimulator of the movement of leucocytes in vitro and can also induce vascular permeability changes.[7] A specific receptor has been shown to exist[8] for LTB_4 on human polymorphonuclear leukocytes, and LTB_4 causes a rapid elevation in the concentration of intracellular free calcium in both rabbit and human neutrophils, both from release from internal stores and from a net uptake from the extracellular medium.[9]

The geometrical assignments of the double bonds of the different leukotrienes as well as the stereochemical assignments have been made possible by total synthesis and subsequent comparison of synthetic and natural products.[10,11]

In the present study, our objective is to obtain the conformation of LTB_4 in aqueous solution. The determination of such a conformation is very important as it could be used in the design of analogues of LTB_4. It could also lead to a better understanding of the structure–activity relationships[12,13] and of the interaction of LTB_4 with its specific receptor. Some NMR studies have been published on derivatives of leukotrienes[14–17] but none on the leukotrienes in an aqueous system. The small amount of leukotrienes that are available and their instability are responsible for the scarcity of these important studies, and the nature of these derivatives makes it nearly impossible to obtain crystals suitable for X-ray analysis.

The approach that was chosen is based on selective and nonselective proton longitudinal relaxation time measurements along with the nuclear Overhauser effect (NOE) experiments carried out at 400 MHz, since these data are extremely sensitive to the interproton distances.[18,19] For proton–proton intramolecular

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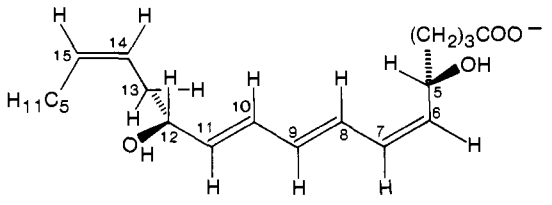

Figure 1. Structure of the potassium salt of LTB_4.

dipolar relaxation, the spin–lattice transition probabilities are W_0^{ij}, W_1^{ij}, and W_2^{ij}, respectively for zero-, single-, and double-quantum transitions involving the nuclear spins i and j. These are given as follows:

$$W_0^{ij} = (1/10)\gamma^4\hbar^2\tau_c/r_{ij}^6 \quad (1)$$

$$W_1^{ij} = (3/20)\gamma^4\hbar^2\tau_c/[(1 + \omega^2\tau_c^2)r_{ij}^6] \quad (2)$$

$$W_2^{ij} = (6/10)\gamma 4\hbar^2\tau_c/[(1 + 4\omega^2\tau_c^2)r_{ij}^6] \quad (3)$$

The relaxation rates are defined as

$$\sigma_{ij} = W_2^{ij} - W_0^{ij} \quad (4)$$

$$\rho_{ij} = W_0^{ij} + 2W_1^{ij} + W_2^{ij} \quad (5)$$

where σ_{ij} is the cross-relaxation term and ρ_{ij} is the direct relaxation term for a proton pair. The interproton distance is r_{ij}, ω is the Larmor frequency, and τ_c is the correlation time for molecular reorientation. The magnetogyric ratio is γ and $\hbar$ is Planck's constant h divided by 2π.

The nuclear Overhauser effect (NOE) is an excellent probe of molecular conformation because it depends on the interproton distance r^6_{ij}. It is defined as the fractional enhancement of nucleus d on saturating nucleus s ($f_d(s)$) and comprises a term (first) for *direct* interactions and one for *indirect* interactions (second):[18,20,21]

$$f_d(s) = (\sum_s N_s\sigma_{ds}/R_d) - \sum_{n\neq d,s} N_n\sigma_{dn}f_n(s)/R_d \quad (6)$$

where N_i is the number of equivalent nuclei i. The total direct relaxation rate is R_d:

$$R_d = \sum_n N_n\rho_{dn} \quad (7)$$

$$T_1^s = 1/R_d \quad (8)$$

The nonselective relaxation time is T_1^{NS} and is defined as:

$$T_1^{NS} = \frac{1}{R_d + S_d} \quad (9)$$

where $S_d = \sum_n N_n\sigma_{dn}$ equals the total cross-relaxation rate.

On the basis of these equations, a computer program was written that optimizes the NOE and the T_1^S and T_1^{NS} data for a given conformation. The optimum value of τ_c is thus obtained. Several possible conformations are used as input, and internuclear distances are estimated from Dreiding models. These conformations are obtained from a Karplus analysis of the coupling constants. This approach has been successfully applied to the study of prostacyclin and (6*S*)-prostaglandin I_1.[22]

Experimental Section

The potassium salt of LTB_4 was synthesized at Merck Frosst Canada according to Rokach et al.[23,24] The NMR measurements were carried out in D_2O ("100%", Aldrich) that was vacuum distilled. The leukotriene

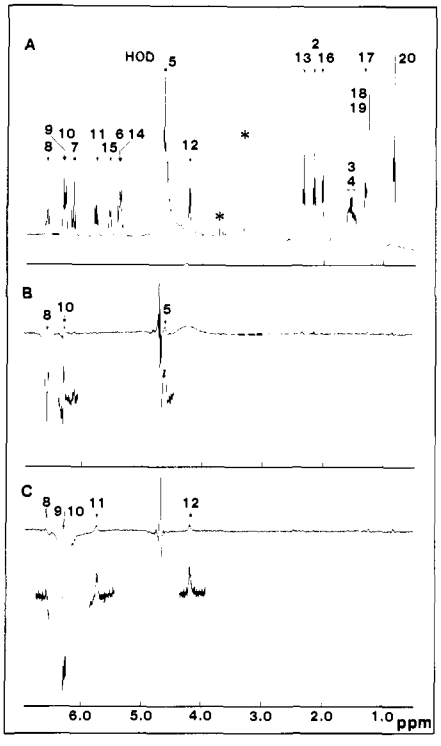

Figure 2. (A) Proton magnetic resonance spectrum (400 MHz) of LTB_4 in D_2O. The * indicates an impurity. (B) Steady-state NOE difference experiment carried out by saturating H8. (C) Steady-state NOE difference experiment carried out by saturating H9,10.

was received as a solution in methanol, and the solvent was removed under vacuum. The D_2O was then added and the samples were degassed and then pumped to dryness twice. Fresh 100% D_2O was then added (0.4 mL), and the solutions were degassed 4 times using the freeze–pump–thaw technique and then sealed under vacuum. All glassware was cleaned extensively, including a nitric acid wash, to eliminate paramagnetic impurities.

The proton magnetic resonance measurements were carried out in the Fourier transform mode using a Bruker WH-400/DS NMR spectrometer with an Aspect 2000 Data System, utilizing 16K of data memory for the NOE and the relaxation time measurements and up to 64 K of memory in evaluating the chemical shifts and coupling constants. The deuterium resonance of the solvent D_2O was used as the lock signal. The nonselective proton longitudinal relaxation time (T_1^{NS}) were obtained by using the inversion recovery (180°–τ–90°–t) two-pulse sequence. Usually about 10 τ values were included in the analysis of the data, based on the initial slope of a plot of ln ($M_\infty - M_\tau$) vs. τ. These M values were obtained from the measurement of the sum of the peak heights of a multiplet corresponding to a given proton. Additionally, two M_∞ values were measured for maximum accuracy. The selective longitudinal relaxation times (T_1^S) were measured by applying a selective 180° pulse to a given multiplet through the decoupler channel, at the specified frequency, and then detecting the magnetization after a time τ, using a nonselective 90° pulse. Before these measurements, the pulse widths were accurately calibrated. The relaxation times were obtained from a computer analysis of the data using a least-squares program.

The steady-state NOE difference experiments were carried out following procedures described previously.[25] Saturation power levels were determined by measuring the minimum power necessary to completely suppress a given multiplet. Each multiplet was irradiated for a period greater than $5T_1$. The reported NOE's are the average values for two or three experiments.

The chemical shifts for protons H5–H16,16′ and the corresponding coupling constants were obtained from a computer fit of the spectra by using the standard Bruker PANIC program. The chemical shifts for protons H2–H4 and H17–H20 are reported to first order. The computer fit to the data was carried out in several stages. First of all, decoupling proton H12 allows a computer fit for the five-spin case H7–H11. Decoupling of H8 allows a fit for H5–H7, and decoupling of H15 allows a fit for H11–H14. With this information, a spectrum was obtained with H5 decoupled and a seven-spin analysis was carried out for H6–H12. In

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Table I. Chemical Shifts and Coupling Constants for LTB_4 in D_2O[a]

	chemical shift, ppm	coupling constant, Hz
H2	2.18	
H3,H4	1.4–1.7	$J_{4,5} = 6.3$
H5	4.65	$J_{5,6} = 9.2$
H6	5.43	$J_{6,7} = 11.0$
H7	6.19	$J_{7,8} = 11.3$
H8	6.62	$J_{8,9} = 14.6$
H9	6.35	$J_{9,10} = 10.7$
H10	6.32	$J_{10,11} = 14.7$
H11	5.81	$J_{11,12} = 7.2$
H12	4.25	$J_{12,13} = J_{12,13'} = 6.5$
H13,13′	2.35	$J_{13,13'} = -16$,[b] $J_{13,15} = J_{13',15} \sim 1.7$ $J_{13,14} = 7.3$, $J_{13',14} = 7.3$
H14	5.40	$J_{14,15} = 11.0$, $J_{14,16} \sim 1.5$
H15	5.58	$J_{15,16} = 7.5$
H16,16′	2.03	
H17	~1.32	
H18,19	~1.27	
H20	0.85	

[a] The chemical shifts are accurate to within ±0.01 ppm and the coupling constants to within ±0.2 Hz. The chemical shift reference is external DSS. [b] Assumed value in the computer analysis.

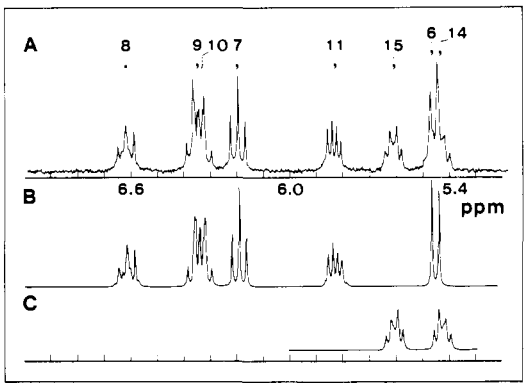

Figure 3. (A) Expansion of the spectrum for the low-field region of LTB_4 with couplings to H5 removed. (B) Computer simulation for protons H6–H12. Simulated spectrum for H12 is not shown. (C) Computer simulation for H14 and H15 which forms part of a six-spin system for H13–H16.

a second series of calculations, a fit to the H12–H15 data was carried out with H16 decoupled, decoupling of H13 allows a fit to H14–H16, and this was followed by a six-spin fit to the spectra for H13–H16.

Results

The assignment of the proton multiplets was carried out by following standard decoupling techniques and these are shown in Figure 2A. The chemical shifts and coupling constants are listed in Table I, and two examples of the computer fit to the data are shown in Figure 3. The proton assignments are in agreement with those reported for leukotriene B methyl ester diacetate in benzene-d_6.[15] The chemical shifts are solvent dependent but the coupling constants are in excellent agreement, implying similar conformations in both solvents. To check for aggregation, chemical shifts were recorded on a higher concentration sample (6.8×10^{-3} M) and dilutions were carried out down to $\sim 1.5 \times 10^{-4}$ M with no changes in chemical shift (within ~4 Hz). Hence the relaxation time data in Table II and the NOE data in Table III were obtained under conditions where intermolecular association is not present.

In the case of prostaglandin $F_{2\alpha}$,[26] hydrophobic interactions were not present at concentrations below 0.02 M, and this is also

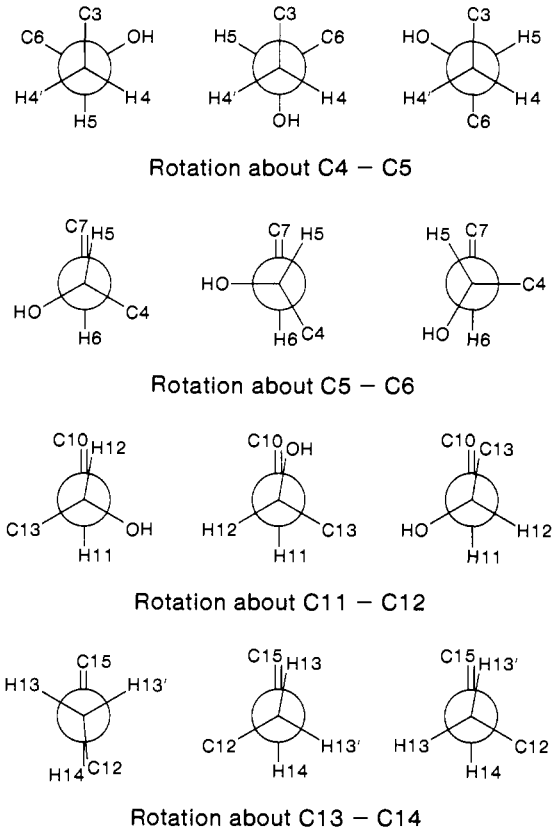

Figure 4. The possible conformations about C4–C5, C5–C6, C11–C12, and C13–C14 as discussed in the text.

apparent with prostacyclin and its analogues.[22] Consequently, the present studies are carried out at a concentration where aggregation is minimal.

The molecular modeling was carried out in the following manner. Carbon atoms C1–C5 are trans, as expected in analogy with butane.[27] As well, C15–C20 are chosen to be trans. The triene portion of the molecule is trans,[15,16] and the present study supports this conclusion, since the observed coupling constants between olefinic protons across a connecting single bond average 11.0 Hz (J(H7–H8) = 11.3, J(H9–H10) = 10.7 Hz).

To calculate the rotamer populations about C4–C5, C5–C6, C11–C12, C12–C13, C13–C14, and C15–C16, the observed coupling constants were analyzed by using the Karplus equation in the form[28]

$$^3J = (7 - (\cos \phi) + (5 \cos 2\phi))(1 - \Delta\chi)$$

where $\Delta\chi$ = substituent electronegativity (0 for carbon, 0.2 for oxygen). For rotation about C4–C5, J(H4–H5) = J(H4′–H5) = 6.3 Hz. Three rotamers are possible, but the Karplus analysis indicates that the population of the rotamer where H5 bisects H4 and H4′ is small (Figure 4). $p_1 J_{60°} + [(1 - p_1)(J_{60°} + J_{180°})/2] = 6.3$, $p_1 = 0.14$ $p_2 = p_3 = 0.43$. The two remaining conformers are predominant and are equal in population. This is also true for rotation about C12–C13, where the observed coupling is 6.5 Hz.

For rotational isomerism about sp^2–sp^3 carbon–carbon single bonds, the preferred conformation is the one where the C—H bond at the sp^3 center eclipses the C=C double bond.[29–31] As the Karplus curve for allylic proton–proton coupling constants does

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Table II. Selective and Nonselective Proton Relaxation Times for the Leukotriene B_4 Protons at 400 MHz[a]

sample		H2	H6	H7	H8	H9	H10	H11	H12	H13	H15	H16
3.4×10^{-3} M LTB_4	$T_1^{NS}{}_{obsd}$	0.71		0.95	0.69			1.15	0.98	0.44	1.52	0.77
1.6×10^{-3} M LTB_4	$T_1^{NS}{}_{obsd}$	0.67		0.93	0.72			1.06	0.88	0.42	1.45	0.75
1.6×10^{-3} M LTB_4 plus 0.8×10^{-4} M EDTA	$T_1^{NS}{}_{obsd}$	0.76		0.95	0.71			1.16	0.99	0.43	1.45	0.75
	$T_1^{NS}{}_{av}$	0.71	1.10	0.94	0.71	0.96	0.96	1.12	0.95	0.43	1.47	0.76
	$T_1^{NS}{}_{calcd}$		0.99	0.94	0.65 (0.51)	0.96	1.15	0.95	0.85			
	$T_1^{S}{}_{obsd}$ (av)			1.08	0.83			1.19				
	$T_1^{S}{}_{calcd}$			1.10	0.77 (0.60)			1.11				

[a] T_1^{NS} data for H6, H9, and H10 are estimated for second-order and overlapping multiplets from the null points. The data in brackets are for the rotamer with H5 trans to H6. $\tau_c = 0.30$ ns.

Table III. Observed and Calculated NOE's for LTB_4 in D_2O (~0.003 M in D_2O) at 400 MHz[a]

satd protons					
	obsd proton		7	12	15
6, 14	NOE calcd		8.2 (8.1)	3.2	5.6
	NOE obsd		7.2	3.9	7.8
	obsd proton	6			
7	NOE calcd	8.6 (8.3)			
	NOE obsd	8.8			
	obsd proton	5	7	10	
8	NOE calcd	7.6 (9.4)	2.1 (1.9)	7.7	
	NOE obsd	9.1	2.5	5.6	
	obsd proton	8	11	12	
9, 10	NOE calcd	5.9 (4.5)	8.6	3.3	
	NOE obsd	3.9[b]	7.5	6.3	
	obsd proton	9	12		
11	NOE calcd	6.5	3.5		
	NOE obsd	5.9	3.1		
	obsd proton	11	12	14	15
13	NOE calcd	4.5	6.9	6.1	0.9
	NOE obsd	4.7	8.3	6.7	1.9
	obsd proton	15			
16	NOE calcd	5.3			
	NOE obsd	5.0			

[a] Data in brackets are for the rotamer with H5 trans to H6. Optimized correlation time $\tau_c = 0.30 \pm 0.05$ ns. [b] The observed signal is very close to the saturated signal and hence the observed NOE may be slightly smaller than expected.

not differ very much from that for vicinal coupling constants between 60° and 180°, the above equation was used throughout.[32] For rotation about C5–C6, J(H5–H6) = 9.2 Hz, and an eclipsed conformation with a population of about 85% is calculated in which H5 and H6 are trans (Figure 4). This leads to a steric interaction between H5 and H8 because of the extra cis double-bond substitution. As well, this results in a calculated T_1^{NS} of 0.51 s for H8 where the observed is 0.71 s, and the T_1^{S} is calculated to be 0.60 s, whereas the observed is 0.83 s. The calculated T_1 data are too short. However, for a dihedral angle of 155° the vicinal coupling for J(H5–H6) is predicted to be 9 Hz. In our analysis, two preferred rotamers are used, as shown in Figure 4 with dihedral angles of H5–H6 $\cong \pm 150°$. In this way, the stabilization due to the eclipsed conformation is still present but is decreased by the H5–H8 interaction. This gives a much better fit to the T_1 data. A comparison of the T_1 and NOE data for both possibilities is presented in Tables II and III.

For rotation about C11–C12, the eclipsed conformation is preferred with H11 trans to H12[29-31] (Figure 4). In addition, from an examination of the models, the OH can be eclipsed with C10, and C13 can also be eclipsed with C10. Applying the Karplus relationship, the ratio of populations is 60:20:20 for J(H11–H12) = 7.2 Hz for the three conformations. Finally, rotation about C13–C14 can be represented as shown in Figure 4 with H13 eclipsing C15 and H13′ eclipsing C15. The case where C12 eclipses C15 was not considered because of steric interactions associated with the C14–C15 cis double bond. The third rotamer has C12 trans to C15. The three rotamers will have equal populations since J(H13–H14) = 7.3 Hz. In addition, rotation about C15–C16 can be represented in the same manner.

In analyzing the T_1 and NOE data, several models were tried, and because of the large number of possible rotamers, it was impossible to determine the allowed conformations. However, with the aid of the Karplus analysis of the coupling constants, the T_1 and NOE data can be used to confirm the possible conformations and to select the best conformation about C5–C6. A fit to the T_1 and NOE data was obtained for the above mentioned conformations. The reorientational correlation time τ_c for the C–H vectors for the planar C5–C12 portion of the molecule is taken to be isotropic in agreement with the data for prostacyclin[22] and prostaglandin $F_{2\alpha}$.[26] The observed and calculated NOE data are compared in Table III and the optimized fit to the data is achieved with $\tau_c = 0.30 \pm 0.05$ ns (95% confidence limits).[33] This value of the correlation time was used to obtain the best fit to the T_1^{S} and T_1^{NS} data, and the results are shown in Table II. This correlation time is confirmed from the T_1^{S}/T_1^{NS} ratio as these relaxation times have a different frequency dependence.[34] An average value of 0.34 ns is calculated for H7, H8, and H11, which is within the error limits.

Discussion

The LTB_4 molecule in solution can adopt a wide range of conformations. To determine the populations of the various rotamers, a complete analysis of the observed coupling constants is required, and the preferred conformers are determined from a comparison of LTB_4 with model compounds. The analysis of the T_1^{S}, T_1^{NS}, and NOE data was then used to confirm the conformations predicted from coupling constants.

It has previously been shown[15,16] that the triene units of LTB_4 and leukotriene D_4 are planar, and the olefinic protons about interconnecting single bonds are in a transoid arrangement. In the present study, it is shown that C5–C12 is planar, and for the preferred conformation (60%) about C11–C12, H12 is in the same plane as C5–C12 and is trans to H11. Proton H5 is only slightly offset from this plane because of the steric interaction between H5 and H8 (Figure 1). The remainder of the molecule is flexible. Consequently, the C1–C5 atoms are taken to be all trans, and the coupling constants suggest that there are two predominant, equally populated rotamers about C4–C5. This is also true for rotation about C12–C13. Rotation about C13–C14 and C15–C16 can be represented by three equally populated rotamers, and finally C15–C20 are taken to be trans.

The observed NOE data and the selective and nonselective relaxation times were fit to the proposed conformations. A comparison of the observed and calculated NOE data is given in Table II, both for the conformation with H5 trans to H6 (in

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brackets) and with equal populations of two rotamers with the H5–H6 dihedral angle equal to ~±150°. The overall fit to the data is good. The correlation time used in the analysis was verified by measuring the T_1^S/T_1^{NS} ratio. The NOE and T_1^S and T_1^{NS} data (Table II) support the conformations with the H5–H6 dihedral angle equal to ~±150°. The latter situation is preferred as it is consistent with the H5–H6 coupling constant and steric considerations.

Because the LTB_4 molecule is flexible, an analysis of all of the available parameters must be undertaken, together with conformational studies of model compounds, in order to evaluate the preferred conformations for LTB_4. This includes an analysis of the coupling constants, the T_1^S, T_1^{NS} and NOE data. By use of this combined approach, the conformational analysis of a complex molecule such as LTB_4 is possible.

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Structure of *syn*-Vinyl Alcohol Determined by Microwave Spectroscopy

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Abstract: The microwave spectra of *syn*-vinyl alcohol and nine isotopically substituted species have been measured and analyzed between 12 and 40 GHz. The compounds have been prepared by very low pressure pyrolysis of appropriately isotopically substituted species of cyclobutanol. The pyrolysis products have been pumped directly through the microwave Stark cell. Rotational constants and all quartic centrifugal distortion constants have been fitted to the measured transition frequencies. The complete substitution structure of *syn*-vinyl alcohol has been determined from the moments of inertia of all isotopic species.

Vinyl alcohol ($H_2C{=}CHOH$, ethenol), the simplest enol compound, is an unstable tautomer of acetaldehyde. Its existence as a reaction intermediate was proposed over 100 years ago.[1] In 1973, Blank and Fischer[2] observed its NMR spectrum by using the CIDNP technique during photolysis of acetaldehyde.

In 1976, Saito[3] reported the first identification in the gas phase. He produced vinyl alcohol by very low pressure pyrolysis of ethylene glycol and measured its microwave spectrum. Vinyl alcohol can exist in two planar conformations depending on the orientation of the hydroxyl group. Saito[3] showed from the rotational constants of the parent and the OD isotopic species that the syn conformer has been observed.

An indirect experimental estimate[4] of the difference between the heats of formation of vinyl alcohol and acetaldehyde in the gas phase at 25 °C gave 13.2 kcal/mol. Although vinyl alcohol is thermodynamically substantially less stable than its keto tautomer it has sufficient kinetic stability to be observed. A half-life of about 30 min at room temperature was reported[5] if vinyl alcohol was stored in a Pyrex flask.

In the most recent ab initio calculation Bouma and Radom[6] predicted the structure of vinyl alcohol. They showed that the syn conformer should be lower in energy than the anti form by 2.2 kcal/mol.

In this paper we report the measurements of the rotational spectra of *syn*-vinyl alcohol, all its singly substituted D, ^{13}C, and ^{18}O species, and two multiply substituted species. The vinyl alcohols have been produced by very low pressure pyrolysis of isotopic species of cyclobutanol. We have found recently that cyclobutanol or 3-thietanol are far better starting compounds than ethylene glycol for the preparation of vinyl alcohol.[7] The complete substitution structure of *syn*-vinyl alcohol has been deduced from the moments of inertia of the parent and all isotopic species.

Experimental Section

Pyrolysis. Very low pressure pyrolysis (thermolysis) allows cleavage of saturated four-membered rings at opposite bonds.[8] Thus, pyrolysis of cyclobutanol produced vinyl alcohol and ethylene with negligible side reactions. Temperatures above 800 °C were necessary in order to reach completion of the reaction. At these temperatures, however, it could not be avoided that a substantial fraction of vinyl alcohol isomerizes to acetaldehyde.

Cyclobutanol vapor was pyrolyzed in a quartz tube of 8-mm inner diameter heated over a length of 16 cm with an electric oven. The maximal yield of vinyl alcohol was obtained with the oven temperature around 800–900 °C. The emerging gases from the heated zone containing vinyl alcohol were pumped immediately through the Stark cell at pressures of 10 to 30 mtorr. The pressure and flow rate were adjusted with two valves, a needle valve between the sample reservoir and the oven and another valve between the Stark cell and the pump. The lifetime of vinyl alcohol in the Stark cell was 15–30 s.

Synthesis of Isotopic Cyclobutanols. Samples containing pure isotopic species or mixtures of isotopic species of cyclobutanol were prepared following known procedures.

$\overline{CH_2CH_2CH_2CHOD}$: Direct exchange of cyclobutanol with D_2O produced cyclobutanol-*O-d*. The procedure was repeated in order to increase the deuterium content.

$\overline{CHDCH_2CH_2CHOH}$, $\overline{CHDCH_2CHDCHOH}$, $\overline{CD_2CH_2CH_2CHOH}$, $\overline{CD_2CH_2CHDCHOH}$, **and** $\overline{CD_2CH_2CD_2CHOH}$: Acid-catalyzed exchange of cyclobutanone with a 1:1 mixture of D_2O:H_2O gave a mixture of singly, doubly, triply, and quadruply deuterium substituted cyclobutanones in the α position. The mixture of cyclobutanones was reduced with $LiAlH_4$ to the corresponding cyclobutanols.

$\overline{CH_2CH_2CH_2CDOH}$: Cyclobutanone was reduced with $LiAlD_4$ in an ethereal solution. The solution was treated with H_2O and gave cyclobutanol-*1-d*.

$\overline{CD_2CH_2CD_2CDOH}$: $\overline{CD_2CH_2CD_2CO}$ produced by acid-catalyzed exchange of cyclobutanone was reduced with $LiAlD_4$ as above.

$\overline{CH_2CH_2CH_2{}^{13}CHOH}$: $CH_3{}^{13}COOH$ (0.5 g) was pyrolyzed in a quartz tube packed with quartz pieces and heated over a length of 35 cm

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