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300 MHZ ^{1}H NMR SPECTRA AND CONFORMATIONS OF BIOTIN AND RELATED HEXAHYDROTHIENOIMIDAZOLONE DERIVATIVES

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Abstract - ^{1}H NMR spectra of biotin and four related hexahydrothienoimidazolones in which the endo pentanoate side chain of biotin is replaced by another endo or exo substituent, and the urea nitrogen atoms are substituted with benzyl groups, have been obtained at 300 MHz. Vicinal coupling constants differentiate cis and trans proton pairs. The generalized Karplus equation was utilized to calculate dihedral angles from vicinal proton-proton coupling constants. The conformation of biotin in solution, calculated from coupling constants, is in good agreement with solid state X-ray crystallographic data.

The vitamin biotin, an essential nutrient and cofactor, is prepared commercially by total synthesis. In several syntheses of biotin, hexahydrothienoimidazolone derivatives of unknown configuration at the three adjacent asymmetric centers have been encountered.[1-11] Generally stereochemistry of these intermediates was assigned by chemical correlation. We felt that high-field ^{1}H NMR might provide a less tedious method for assignment of stereochemistry. Although ^{1}H NMR chemical shifts for biotin (1) and several of its derivatives have previously been reported,[12-31] and a complete set of spectral parameters for biotin in D_2O was reported recently,[32] the key coupling constants of biotin in a non-aqueous solvent were unreported.

The well-known phenomenon of pseudorotation[33-36] renders conventional coupling constant analysis fruitless for determination of stereochemistry in five-membered monocyclic compounds.[35-44] The Karplus equation[45] or modifications thereof,[46,47] generally cannot elucidate vicinal stereochemistry in these structures. In hexahydrothienoimidazolones, however, the cis ring fusion and planarity of the urea moiety considerably restrict pseudorotation. Calculations[48] performed on similar 5-membered rings[34,35] allow an estimation of 5-6 kcal/mole for the pseudorotational energy barrier in hexahydrothienoimidazolone 2. The preferred conformation of the tetrahydrothiophene ring is a C_s envelope with the sulfur endo.[49] Therefore coupling constant analysis can be used to determine stereochemistry.

Karplus,[45] in his pioneering work, fit the theoretical dependence of coupling constants upon vicinal proton dihedral angle approximately with a $\cos^2\phi$ function. Recently a generalized Karplus equation, (Equation 1)[40] a useful empirical extension of the original equation, has been developed which separates electronegativity effects (the last term) from orbital overlap considerations. Application of this empirical modified equation to hexahydrothienoimidazolone derivatives 1-5 greatly aids conformational analysis.

Equation 1. $$^3J = P_1\cos^2\phi + P_2\cos\phi + P_3 + \Sigma\Delta\chi_i\{P_4 + P_5\cos^2(\xi_i\phi + P_6|\Delta\chi_i|)\}$$

RESULTS AND DISCUSSION

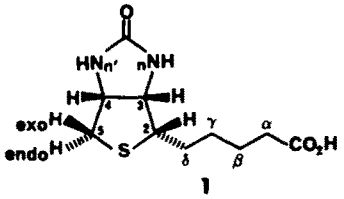
Chemical Shifts

The signals in the ^{1}H NMR spectrum of biotin (Table 1) were assigned on the basis of chemical shifts, coupling constants, and decoupling experiments, and are for the most part in accord with previous assignments.[8,12-32] Spectra of the remaining compounds were assigned in the same manner. We utilize tetrahydrothiophene numbering to designate the proton position, the endo- and exo- designations to describe orientation with respect to the cis-fused hexahydrothienoimidazolone ring, and α, β, γ, and δ to describe side chain proton position relative to the carboxylate of biotin, as shown in the structural formulas. The spectra of $CDCl_3$ solutions of 2 - 5, reported in Tables 1 and 2 were quite similar to DMSO-d_6 solutions. DMSO-d_6 was utilized as the solvent for the spectrum of biotin (1).

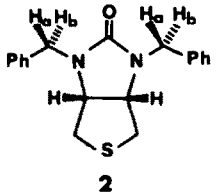
The chemical shift of H_2 is quite similar in 1, 3, 4, and 5, being relatively insensitive to the substituent at C_2. However H_2 is slightly more deshielded (0.008 ppm) in 4 where it is endo, than in 3 where it is exo due to the anisotropy of the phenyl group.[50] Similarly, the endo methyl of 3 is deshielded by 0.166 ppm more than the exo methyl of 4. The chemical shifts of H_3 and H_4 are also relatively insensitive to substitution at C_2.

In most previously reported spectra of biotin, H_{5endo} and H_{5exo} were not resolved or could not be unambiguously assigned. At 300 MHz, however, H_{5endo} and H_{5exo} are resolved and can easily be distinguished on the basis of coupling with H_4 (see discussion of coupling constants, below). The chemical shifts of H_{5endo} and H_{5exo} are also relatively insensitive to alkyl substitution at C_2. The chemical shift of H_{5exo} in 2-5 is nearly equal to the corresponding chemical shift in tetrahydrothiophene (2.73 ppm[43]), while H_{5endo} which is more deshielded by the benzyl groups resonates slightly downfield of H_{5exo}. In biotin (1), which lacks the benzyl groups, H_{5endo} resonates upfield of H_{5exo} and furthermore is upfield of H_{5endo} or H_{5exo} in 2-5. H_{5endo} and H_{5exo} were distinguished by coupling constant analysis and are consistent with a 2-D ^{1}H NMR experiment.[51]

The ^{1}H NMR spectrum of the pentanoate side chain of biotin is complex even at 300 MHz. Accordingly only one investigator previously interpreted this portion of the spectrum.[32] Interpretation was facilitated by decoupling, and assignments were verified by computer spectral simulation which allowed precise assignment of all chemical shifts and coupling constants. The H_α protons resonate at 2.18 ppm. The H_β and H_γ protons resonate at 1.51 and 1.34 ppm, respectively. The diastereotopic methylene protons H_δ and $H_{\delta'}$, which are adjacent to a chiral center resonate at 1.53 and 1.60 ppm. The carboxylic acid proton appears as a broad (26.6 Hz at half-height) singlet at 11.18 ppm which does not exchange appreciably with the urea protons. Previous investigators, with one exception,[12] were unable to locate this resonance.



1



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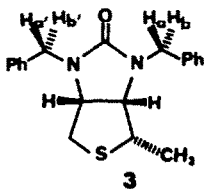

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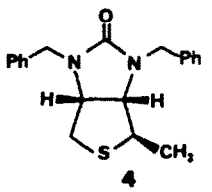

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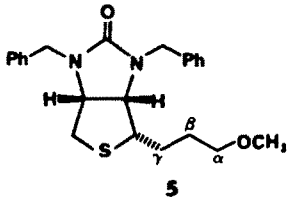

5

Table 1. ^{1}H NMR Chemical shifts (in ppm)[a]

	Compound				
	1	2	3	4	5
Proton					
2	3.145	(see H5)	3.280	3.288	3.104
3	4.183	4.005	3.833	4.025	3.849
4	4.352	4.005	3.973	4.072	3.976
5endo	2.574	2.749	2.800	2.860	2.738
5exo	2.810	2.688	2.739	2.740	2.654
a		4.200	4.174	4.214	4.124
a'		4.200	3.936	4.195	3.964
b		4.764	5.125	4.826	5.076
b'		4.764	4.735	4.792	4.731
n	6.472[b]				
n'	6.392[b]				
2-CH_3			1.338	1.172	
α	2.18				3.36
β	1.51				1.59
γ	1.34				1.78/1.80
δ	1.53				
δ'	1.60				
COOH	11.18[c]				
O-CH_3					3.325

[a] Aromatic protons resonated at 7.23 - 7.37 ppm in 2 - 4 and 7.22 - 7.31 ppm in 5. Assignments of a, a', b, and b' may be interchanged.
[b] Width at half-height = 7.50 Hz.
[c] Width at half height = 26.6 Hz.

Table 2. Proton-proton vicinal and geminal coupling constants (in Hz)

	Compound				
	1	2	3	4	5[d]
Vicinal:					
2,3	4.85[c]	(see 4,5)	5.61[c]	3.04[t]	5.49[c]
3,4	7.45[c]	-	9.59[c]	9.12[c]	9.47[c]
4,5endo	1.66[t]	2.50[t]	4.56[t]	4.45[t]	4.07[t]
4,5exo	4.67[c]	4.64[c]	6.08[c]	6.00[c]	6.06[c]
2,CH3	-	-	7.03	6.91	-
2,δ	6.3[b]				
2,δ'	9.0[b]				
CH2,CH2[a]	7.5[b]				7.5[b]
Geminal:					
5endo,5exo	-12.45	-12.16	-12.41	-12.36	-12.46
a,b (a',b')	-	-15.0	-15.2	-15.3	-15.3
δ,δ'	-13.5				

All coupling constants are ±0.05 Hz unless otherwise noted.
[a] Remaining methylene groups in side chain.
[b] +0.1 Hz.
[c] Vicinal protons are cis
[d] $J_{2,\gamma}$ = 6.4; $J_{2,\gamma'}$ = 9.1; $J_{\gamma,\gamma'}$ = 13.4 ± 0.1 Hz.
[t] Vicinal protons are trans

Benzyl groups have frequently been utilized to protect the urea functionality during biotin syntheses.[8-11] The benzylic protons H_a and H_b (or $H_{a'}$ and $H_{b'}$) are nonequivalent due to the inherent asymmetry of the hexahydrothienoimidazolone and preferred conformations of the benzyl groups.[51-53] The assignments in Table 1 are consistent with the interpretation that the differences in chemical shifts reflect proximity to the source of asymmetry at C_2. Thus in 2, which is symmetrical, H_a and $H_{a'}$ (or H_b and $H_{b'}$) are equivalent. In 4 which has a 2-exo substituent, the difference in chemical shift between H_a and $H_{a'}$ (or H_b and $H_{b'}$) is small. However in 3 and 5 in which the 2-substituent is endo, the difference in chemical shift between H_a and $H_{a'}$ (or H_b and $H_{b'}$) is larger. In 3 and 5 the chemical shift difference between H_b and $H_{b'}$ is greater than that between H_a and $H_{a'}$ because the magnetic dissimilarity between H_b and $H_{b'}$ is enhanced by the proximity of H_b to the 2-endo substituent.

The chemical shifts of H_n and $H_{n'}$ in biotin (1), which have been unambiguously assigned,[31] compare quite well with those previously reported,[12,16,26,29-31] where the downfield resonance is assigned to H_n which is closer to the pentanoate side chain. Although the precise chemical shifts of these protons depend upon concentration and impurities, the difference in chemical shift between H_n and $H_{n'}$ is reasonably constant. Protons H_n and $H_{n'}$ in biotin resonate separately with relatively sharp line widths of 7.5 Hz, implying that the proton exchange rate is equal and slow. Significant interaction of H_n with the carboxylate would be expected to differentiate the exchange rates of H_n and $H_{n'}$, and therefore their line widths. Irradiation establishes that there is no observable coupling between H_n and H_3 or between $H_{n'}$ and H_4. The apparent paradox between slow exchange and no observable coupling has occasionally been observed with other cyclic ureas and is not satisfactorily explained.[54-58]

Coupling Constants

Table 2 summarizes the coupling constant data determined in this investigation. Only geminal and vicinal coupling was observed; no significant long-range coupling was detected. Dihedral angles (accurate to ±5 °) were calculated from coupling constants by computer iteration from the generalized Karplus equation (Equation 1).[40] Coupling constants for biotin in DMSO-d_6 and for 2 - 5 have not previously been reported.

One of the primary purposes of this investigation was establishment of characteristic ^3J coupling constants for protons oriented cis and trans to each other on the hexahydrothienoimidazolone ring. In biotin (1), $^3J_{3,4}$ for the cis ring juncture protons is 7.45 Hz in DMSO-d_6. Previously reported $^3J_{3,4}$ values for biotin in D_2O range from 7.6 to 9.0 Hz. The dihedral angle between H_3 and H_4 may be calculated as 28° in biotin, based upon the generalized Karplus equation.[40] This compares with the dihedral angle of 12° which we calculate from the crystallographic data.[49] The $^3J_{3,4}$ coupling constants are 9.59, 9.12, and 9.47 Hz in 3, 4, and 5, respectively, corresponding to 2°, 11°, and 4°, dihedral angles between H_3 and H_4 indicating nearly eclipsed protons. The larger dihedral angle between H_3 and H_4 in 4 versus 3 or 5 indicates a distortion of the tetrahydrothiophene ring from the C_s symmetrical envelope conformation. The preferred conformation of 4 is a twist-envelope with C_2 out of the plane formed by C_3, C_4, and C_5 to mimimize eclipsing interactions between the 2-exo methyl substituent, H_3, and H_4.

Previously reported coupling constants of biotin in D_2O[12,23,32] are generally in agreement with our coupling constants in DMSO-d_6, however $^3J_{4,5endo}$ has previously been reported as 0 to 1 Hz in contrast to our value of 1.66 Hz. The $^3J_{2,3}$, $^3J_{4,5endo}$ and $^3J_{4,5exo}$ coupling constants reveal that in all cases cis coupling constants are at least 1.5 Hz larger than the corresponding trans coupling constants: The trans coupling constants for 1 - 5 range from 1.66 to 4.56 Hz, while the cis coupling constants (excluding $^3J_{3,4}$) range from 4.64 to 6.08 Hz. According to the generalized Karplus equation, the dihedral angle between trans protons is 100° to 120°, and the dihedral angle between cis protons (excluding the angle between H_3 and H_4) is 38° to 52°. In biotin the dihedral angles, calculated from the generalized Karplus equation, between the cis protons H_2-H_3 and H_4-H_{5exo} are both approximately 45°, while the dihedral angles calculated from literature X-ray crystallographic data[49] are 54° and 30°, respectively. The trans dihedral angle between H_4 and H_{5endo} is calculated as 101° from the coupling constant and 98° from the crystallographic data. The good agreement between the conformations in solution and solid state, determined by coupling constant analysis and crystallography, confirms that the hexahydrothienoimidazolone ring is relatively rigid, in contrast to five-membered monocycles which undergo facile

pseudorotation.[35-38,59] Therefore relative stereochemistry of substitution in hexahydrothienoimidazolones can easily be determined by application of the empirical generalized Karplus equation.

The ^{3}J coupling constants between the methylene groups of the pentanoate side chain in biotin in DMSO-d_6, determined by computer spectral simulation, are all 7.5 Hz as expected. The values for $^{3}J_{2,\delta}$ and $^{3}J_{2,\delta'}$ are 6.3 and 9.0 Hz, in good agreement with those previously reported,[32] and also in agreement with 5.

The value of $^{2}J_{5endo,5exo}$ ranges between -12.16 and -12.7 Hz for compounds 1 to 5. The corresponding coupling constant previously reported for biotin in D_2O is slightly higher. The value of $^{2}J_{a,b}$ remains essentially constant in 2 - 5.

EXPERIMENTAL

High-field ^{1}H NMR were recorded at 300 MHz on a Nicolet NT-300NB superconducting NMR with a 1280 data system and a 293C pulse programmer. A pulse duration of 5 usec (70° flip angle), a delay time of 500 usec, and an aqusition time of 4.28 sec was utilized. Homonuclear decoupling experiments were performed by single frequency irradiation. The Nicolet program NMCSIM was utilized for spectral simulation. Typical 32 K proton data sets were transformed after 64 transients had been accumulated. The sample was rotated at 20 - 30 rps at 22 °C in a 5 mm ^{1}H or ^{13}C probe. Manual and computer shimming were performed in order to obtain a line width of less than 0.5 Hz for TMS which was included (0.25 %) as an internal reference. Sample concentrations ranged from 10 to 40 mg / mL. No concentration dependence was observed for 2-5 in $CDCl_3$. Slight broading of the N-H protons of 1 was observed with increasing concentration. All spin systems were completely analyzed; thus each coupling was measured twice, and in all cases agreement was better than ±0.1 Hz. Crucial ABX and AMX systems were simplified by decoupling before calculation of coupling constants by standard analysis.[55-57]

The generalized Karplus equation with appropriate values for P_i[40] was used to calculate proton-proton dihedral angles from coupling constants. Huggins electronegativity values[60] were used to calculate X_i with exclusion of beta effects. A BASIC program for a Hewlett-Packard 2647A graphics terminal was written to plot J as a function of ϕ and to compute both values of ϕ for a given J by iteration on both sides of a calculated minimum. A related program has been described in detail.[36] Dihedral angles were calculated from the X-ray crystallographic data[49] with the program PLANE of the Enraf-Nonius structure determination package.

(3aα,4β,6aα)-Hexahydro-2-oxo-1H-thieno[3,4-d]imidazole-4-pentanoic acid (1, biotin). d-Biotin was purchased from Mann Research Laboratories. ^{13}C NMR data has been previously reported.[27,32]

(3aα,6aα)-1,3-Dibenzylhexahydro-1H-thieno[3,4-d]imidazol-2(3H)-one (2). This compound was synthesized essentially as previously described.[8] ^{13}C NMR ($CDCl_3$) δ159.5, 137.35, 128.8, 128.3, 127.7, 61.43, 37.5, 46.48.

(3aα,4β,6aα)-1,3-Dibenzylhexahydro-4-methyl-1H-thieno[3,4-d]imidazol-2(3H)-one (3). Oxidation of 2 with $NaIO_4$ in aqueous acetonitrile afforded a 9:1 mixture of exo and endo sulfoxides.[8] The exo sulfoxide in THF was treated with methyllithium (260 mol %) at -78 °C for 15 min followed by iodomethane (500 mol %) at -78 to -30 °C for 4 h. This alkylated sulfoxide was reduced with triphenylphosphine (175 mol %) in carbon tetrachloride to afford (2) in 40 % yield. Mp 98 - 100 °C. ^{13}C NMR ($CDCl_3$) δ 160.2, 137.0, 128.7, 128.3, 127.7, 62.6, 61.6, 47.66, 47.65, 46.7, 35.3, 15.78.

(3aα,4α,6aα)-1,3-Dibenzylhexahydro-4-methyl-1H-thieno[3,4-d]imidazol-2(3H)-one (4). Oxidation of 2 with iodobenzene dichloride in aqueous pyridine afforded a 1:1 mixture of endo and exo sulfoxides.[8] The endo sulfoxide was alkylated as described above in 11 % yield. Reduction of this sulfoxide with triphenylphosphine afforded 3 in 50 % yield. Mp 97 - 99 °C. ^{13}C NMR ($CDCl_3$) δ 160.0, 137.3, 128.3, 128.1, 127.8, 68.0, 61.8, 48.1, 46.8, 46.6, 36.2, 20.1.

(3aα,4β,6aα)-1,3-Dibenzylhexahydro-4-(3-methoxypropyl)-1H-thieno[3,4-d]imidazol-2(3H)-one (5). Preparation of 5 has been described previously.[10] Mp 212 - 214 °C. ^{13}C NMR ($CDCl_3$) δ 160.8, 136.7, 136.5, 128.4, 127.9, 127.3, 71.84, 62.9, 58.4, 47.6, 46.3, 34.54, 28.9, 25.3.

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