The Utilization of Chemical Shift and Spin-lattice (T_1) Relaxation Time Data for the Discrimination of Isomeric Indenoisoxazoles

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Treatment of 2-pivaloyl-1,3-indandione with hydroxylamine leads to the formation of a pair of isomeric indenoisoxazoles, the product formed dependent upon the cyclization conditions. Under acidic conditions, 8-*t*-butylindeno[1,2-*c*]isoxazol-7-one (**5**) is formed while under neutral or basic conditions, an oxime, **2**, is generated which may then be cyclized under acidic conditions to give 3-*t*-butylindeno[1,2-*c*]isoxazol-4-one (**4**). Although these isomeric indenoisoxazoles may be discriminated by chemical means, we were interested in developing an unequivocal method for distinguishing these and potentially other isomeric pairs by spectroscopic means. A ^{13}C-nmr based method for the discrimination of these isomers which is based on the utilization of chemical shift arguments and spin-lattice relaxation data is thus presented.

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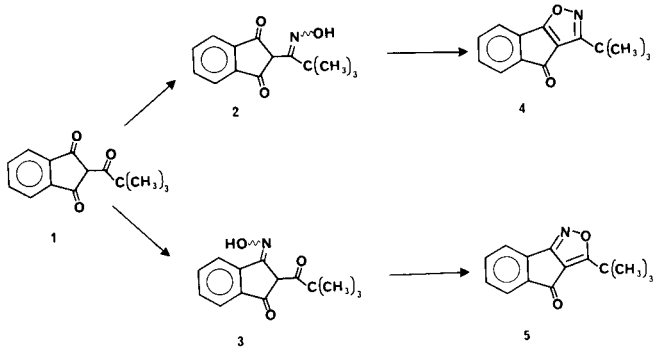
Through our continuing efforts directed toward the synthesis of novel tricyclic heterocycles which could serve as synthons for potential biologically active compounds, we have recently reported the synthesis of 3-*t*-butylindeno-[1,2-*c*]isoxazol-4-one (**4**) and 8-*t*-butylindeno[1,2-*c*]isoxazol-7-one (**5**) (2). Under acidic reaction conditions, isoxazole **5** is formed from the reaction of 2-pivaloyl-1,3-indandione (**1**) with hydroxylamine presumably through the intermediacy of oxime **3** (Scheme I). Under basic conditions hydroxylamine adds to the triketone, **1**, to give isolated oxime **2** which is then cyclized to isoxazole **4** with sulfuric acid in tetrahydrofuran. Previous workers have reported that when hydroxylamine adds to 2-acyl-1,3-indandiones that oxime formation occurs at the acyl carbonyl, but no specific evidence was given to support oximes of type **2** (3). Although both **4** and **5** have been identified by means of chemical degradative reactions and supported by mass spectral fragmentation patterns (2), we were interested in developing a method for distinguishing these closely related isomers spectroscopically. It is on this basis that we now report a means for the discrimination of **4** and **5** based on ^{13}C-nmr chemical shift arguments supported by spin-lattice (T_1) relaxation time considerations.

Scheme I



Upon comparison of the structures of **4** and **5**, the problem of distinguishing these compounds by spectroscopic means is clearly narrowed to developing a method of unequivocally assigning the three quaternary resonances, C3, C3a and C8b (4). This task is further complicated by the necessity of establishing the orientation of these positions relative to the isoxazole heteroatoms. Although ^{13}C-nmr assignments for a number of isoxazole derivatives have appeared (5,6), there are still no empirical guidelines amenable to the assignment of the spectra of the isomeric compounds generated in our previous study (2). Since all of the carbons crucial to the discrimination between the isomeric structures were quaternary, it seemed logical to attack this problem on the dual basis of chemical shift arguments and spin-lattice relaxation times. By using this approach, it would be possible to confirm the assignments made on the basis of the chemical shift arguments in much the same fashion that assignments of protonated resonances may be confirmed by the careful examination of heteronuclear spin-coupling constants (7). This approach also has the further advantage of providing a means for establishng the orientation of the isoxazole heteroatoms which chemical arguments alone could not provide.

Expressions governing spin-lattice or T_1 relaxation (8) reveal that the relaxation of a given carbon resonance will be governed by dipolar relaxation processes which in the case of protonated resonances, are mediated by directly attached protons. In the case of non-protonated or quaternary carbon resonances, the efficiency of proton mediated relaxation falls off with increasing interatomic distance in an inverse sixth power relationship which is governed by the term r_{CH}^{-6} (8). Based on this observation, the assignment of numerous quaternary carbon resonances in complex molecules has been described in a recent chapter by Wehrli (9). Alternatively, in those cases where there are no protons located closer that 3.2 Å (three bonds), quater-

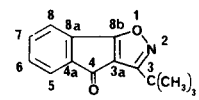
nary carbon relaxation may also be mediated by other heteroatoms with suitable properties such as ^{14}N (10,11) and ^{75}As (12).

From the relaxation considerations presented immediately above, we may develop a generalized case for the relaxation behavior of the quaternary carbons of the isoxazole portion of the molecule. In particular, we will develop the arguments for the C3a resonance first since it is expected to exhibit a reasonably constant ^{13}C-nmr chemical shift as well as spin-lattice relaxation behavior which would be predicted to be constant in both **4** and **5**. Pursuant to the ^{13}C-nmr chemical shift, the C3a resonance is expected to resonate in the range of δ 110-115 (5,6). The relaxation times for this resonance should also remain relatively constant since it is equidistant from both the ^{14}N and the *t*-butyl protons in both **4** and **5** which will provide its principle means of relaxation. Finally, because of its position in the molecule, C3a should also have the longest spin-lattice relaxation time of any carbon in the spectrum.

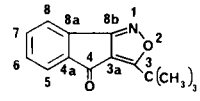
Chemical shift and relaxation prediction for C3 and C8b in **4** and **5** are somewhat more complex, but are still predictable. In the case of either molecule, the oxygen bearing quarternary carbon resonance is expected to resonate from 6-12 ppm downfield from its nitrogen bearing counterpart (5). In the case of **4**, the separation of these resonances in 8.6 ppm while in the case of **5** it is 13.6 ppm thus supporting this contention. A further consideration, however, is that of establishing the orientation of the heteroatoms of the isoxazole ring relative to the balance of the molecule. It is at this point that the development of relaxation time considerations becomes important to the overall discrimination between the isomers. Thus in the case of **4**, the relaxation of C3 resonance will be mediated by both the ^{14}N-^{13}C dipolar mechanism (10) by virture of the position of the nitrogen located at the 2-position, and by the nine *t*-butyl protons. In the case of **5**, the C3 resonance will, in contrast, be relaxed solely by the *t*-butyl protons since the 2-position is occupied by the oxygen atom in this isomer. The other quaternary carbon position, C8b, will be relaxed by the phenyl proton at C8 in the case of **4** and by both the phenyl proton and the ^{14}N-^{13}C dipolar mechanism (10) in the case of **5**. In summary, we would expect the oxygen bearing C8b quaternary carbon resonance of **4** to resonate downfield of its nitrogen bearing C3 counterpart and to further have a considerably longer relaxation time. Relaxation times for both C3 and C8b in **5**, in contrast, should be substantially the same; the relaxation time of the oxygen bearing resonance which would be downfield, shortened by its proximity to the *t*-butyl protons and that of the nitrogn bearing C8b resonance enhanced (relative to **4**) by the addition of the ^{14}N-^{13}C dipolar relaxation.

Table I

^{13}C-NMR Chemical Shift Assignments and Spin-lattice Relaxation Times for 3-*t*-Butylindeno[1,2-*c*]isoxazol-4-one (**4**) and 8-*t*-Butylindeno[1,2-*c*]isoxazole-7-one (**5**) as 0.73 Molar Solutions in Deuteriochloroform at 33° and an Operating Frequency of 25.158 MHz



4



5

	4		**5**	
	δ ^{13}C (ppm)	T_1 (sec)	δ ^{13}C (ppm)	T_1 (sec)
C3	167.60	61.8 ± 4.3	178.45	80.4 ± 5.8
C3a	117.97	139.7 ± 5.6	115.12	147.8 ± 9.4
C4	189.55	103.7 ± 5.1	181.00	107.1 ± 8.6
C4a	131.13	73.3 ± 2.8	133.36	72.4 ± 3.5
C8a	140.56	71.7 ± 1.5	142.71	77.4 ± 1.4
C8b	181.17	110.9 ± 3.7	169.75	79.3 ± 4.5
C5	119.51		122.66	
C6	131.35		131.26	
C7	132.91		134.11	
C8	124.80		124.86	

Having established these basic premises concerning the chemical shift and spin-lattice relaxation behavior expected for **4** and **5**, the chemical shift and relaxation data for these compounds were acquired. Because of the long relaxation times anticipated for these carbons, the relaxation data was acquired using the progressive saturation method (13) which, although reported to be somewhat less accurate than other methods by Levy and Peat (14), is considerably more time efficient than inversion recovery and other available methods. The resonance assignments and spin-lattice relaxation times measured for the quaternary carbons of **4** and **5** are collected in Table I. As predicted above, the chemical shift of the C3a resonance remained relatively constant, resonating at δ 117.97 in **4** and δ 115.12 in **5**. The relaxation time for this resonance in the two molecules was likewise the longest observed, measured at 139.7 and 147.8 seconds in **4** and **5** respectively. From here, we may now turn our attention to the C3 and C8b quaternary carbon assignments. In the case of **5**, these resonances were assigned at δ 178.45 and δ 169.75 respectively. It will also be noted from the table that the relaxation times, as predicted, were essentially the same. The other isomer, **4**, in contrast, provides a somewhat different picture. The C3 resonance in **4**, which bears the isoxazole nitrogen was assigned to the signal observed at δ 167.60 which exhibited a relaxation time of 61.8 seconds. As predicted, this is somewhat shorter than the relaxation time of the corresponding C8b resonance in **5** which also bears the nitrogen atom. The oxygen bearing counterpart, C8b, was assigned to the resonance at 181.17 which exhibited a relaxation time of 110.9 seconds. This behavior is also con-

sistent with that predicted above.

In summary, we have shown that it is possible to assign the structues of 3-*t*-butylindeno[1,2-*c*]isoxazol-4-one and 8-*t*-butylindeno[1,2-*c*]isoxazol-7-one through careful development of chemical shift arguments with support of the assignments through the measurement of the spin-lattice relaxation times. With care, it should be possible to extend this development to include other ring systems in which the orientation of the heteroatoms may be in question.

EXPERIMENTAL

Samples of **4** and **5** were synthesized as reported in the previous paper (2). The samples for measurement of the spin-lattice relaxation times were prepared by dissolving exactly 250 mg of the compound in 3.0 ml of deuteriochloroform which had been previously degassed with a stream of zero grade argon for thirty minutes. After the sample was dissolved, it was further subjected to three freeze-pump-thaw cycles to ensure that all traces of disssolved oxygen had been removed. Reference spectra were obtained from these same samples, in which the reported chemical shifts were referenced to the center line of the deuteriochloroform multiplet which was taken as 76.9 ppm downfield of tetramethylsilane. Spectra were obtained on a Varian XL-100-15 spectrometer operating at 25.158 MHz in the Fourier transform mode and equipped with a Nicolet 1180 data system interfaced through a Model 293A' pulse programmer. The spectrometer was also equipped with a Model TT-760 decoupler which for these studies was set at δ 7.0 in the proton spectral window with sufficient power that $\gamma H_2/2\pi$ = 2.9 KHz. The progressive saturation data were obtained using a series of fifteen *tau* values which ranged from 2.0 to 512 seconds, the first ten acquisitions taken at each *tau* value discarded to ensure the establishment of magnetic equilibrium for that degree of saturation.

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(4) Compounds **4** and **5** are renumbered as shown in Table I for ease of discussion. This numbering does not refer to the chemical nomenclature.

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