In Situ ^{15}N Nuclear Magnetic Resonance Observation of the Fischer Indolization Reaction. ^{15}N NMR Characterization of Amide–Imine Intermediates

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Abstract: The first application of natural-abundance ^{15}N NMR spectroscopy to the in situ observation of an ongoing chemical reaction is reported. Amide–imine intermediates in Fischer indolization reactions have been characterized by ^{15}N NMR. It is projected that "state-of-the-art" ^{15}N NMR instrumentation would allow natural-abundance studies of ongoing reactions where completion times are 2–3 h.

Introduction

Natural-abundance ^{15}N NMR studies are becoming customary for stable chemical species and systems,[1-11] but their use for in situ characterization of transient reaction intermediates apparently has not yet been reported. Label incorporation involving ^{15}N is, however, well-known in mechanistic investigations.[12-14] In the following, characterization of a stable amide–imine intermediate in the Fischer indole reaction by in situ, natural-abundance ^{15}N NMR is described.

A Fischer indole reaction leading to indomethacin (**6**) has been followed by in situ ^{13}C NMR spectroscopy where the first experimental characterization of an amide–imine type intermediate (**4**, Scheme I) was reported.[15] The same reaction has now been allowed to proceed at a lower temperature and followed by natural-abundance ^{15}N NMR. Further evidence confirming the structural assignment of **4** has been obtained.

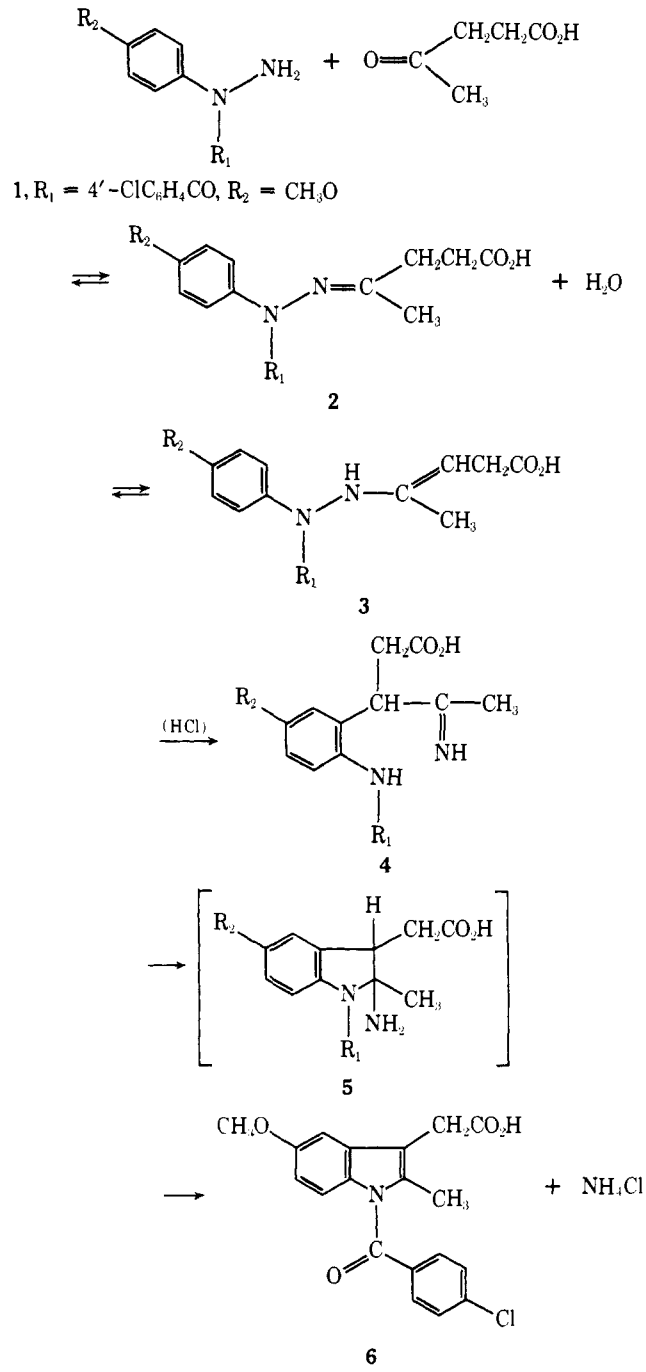
Experimental Section

Nitrogen-15 chemical shifts are reported relative to $^{15}NH_4^+$ as internal reference in the acidic reaction medium, with $^{15}NH_4Cl$ (Merck Sharp and Dohme, Canada) added to a separate reaction mixture preparation and the $-NH_3^+$ resonance of **1** otherwise used as a secondary reference at 53.8 ppm. Fourier transform acquisition times were 0.4 s and pulse nutation angle was 45°. Exponential weighting of free induction decays with a time constant of −0.2 s was performed before Fourier transformation. Spectra were obtained in the "Gyrocode Observe" mode of operation of a Varian XL-100 NMR spectrometer with full-time, broad-band proton decoupling. The reaction was followed in 12-mm o.d. tubes at 24–25 °C with field-frequency lock via the CD_3 group of the CD_3CO_2H solvent (Merck Sharp and Dohme, Canada). The reaction solution was prepared from 1.00 g of hydrazone **2** plus 2.0 mL of CD_3CO_2H saturated with HCl gas at about 0 °C (ice water bath) so that the sum of concentrations of **1**, **2**, and **4** was about 0.9 M. Total signal accumulation periods of 12 h each were made in sequence corresponding to 6, 18, and 30 h at 24–25 °C. The reference spectrum of **7** was obtained on a solution prepared from 500 mg of **1**, 180 mg of cyclohexanone, and 2.0 mL of CD_3CO_2H saturated at 15 °C with HCl. Approximately 1 mg of $^{15}NH_4Cl$ was added as internal reference. Compounds **1** and **2** have been described earlier.[15,16]

Results

Figure 1 presents a time-lapse view of the changes in the ^{15}N NMR spectrum during the reaction of **1**, **2**, and **4** in HCl–CD_3CO_2H medium with ^{15}N chemical shifts collected in Table I. It may be seen that certain stronger and weaker signals initially present (lower trace, Figure 1) disappear slowly. The strong, somewhat broadened signals are assigned to proton-bearing nitrogen atoms while the weaker, sharper lines must be due to nitrogen atoms not directly bonded to any hydrogen atoms. Signals at 53.8 and 187.6 ppm are assigned to proton-

Scheme I. The Fischer Indole Mechanism



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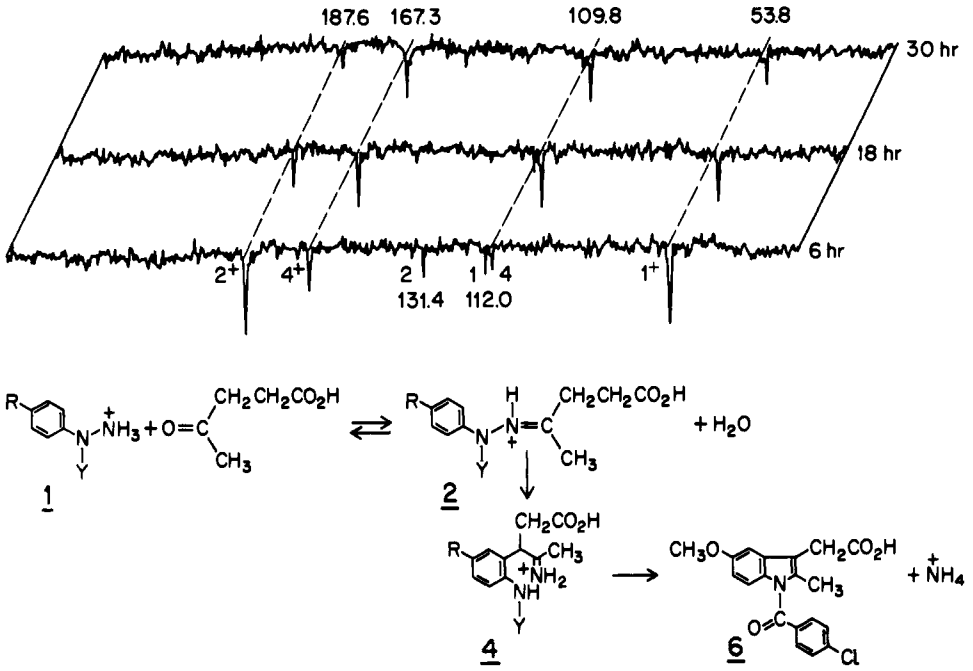

Figure 1. Time-lapse plot of ^{15}N NMR spectral changes in the Fischer indolization leading to indomethacin. Of particular interest is a pair of signals at 109.8 and 167.3 ppm which grow at first and then appear to decrease slightly. These are assigned respectively to amidic and iminium nitrogen atoms of the amide-imine intermediate **4**.

ated nitrogens of acylhydrazine **1** and hydrazone **2** based on their strength in the first run and their disappearance with time. The equilibrium between **1** and **2**, also involving levulinic acid and water, has already been noted.[15] Weaker signals at 112.0 and 131.4 ppm are assigned to the fully substituted, acylated nitrogen atoms of **1** and **2**, respectively. A separate measurement of the shift difference between the two nitrogen atoms of **1** in Me_2SO-d_6 solution containing excess concentrated HCl (in which **1** decomposes slowly) yielded a value of 59.3 ppm. Thus, the amidic nitrogen atom of **1** would be expected to appear near 113 ppm, given the assignment of the $-NH_3^+$ signal near 54 ppm. Protonation of hydrazone **2** at the unsaturated nitrogen atom is assumed as illustrated in Figure 1. The ^{15}N chemical shift of 187.6 ppm compares favorably with the 144 ppm reported for diphenylketimine hydrochloride but is quite distant from the 284-ppm shift of the free imine.[17] Large upfield shifts upon protonation have recently been reported for several *N*-(arylmethylidine)amines (Schiff bases), with typical iminium ^{15}N shifts near 175 ppm downfield from the ammonion ion.[18] Separate ^{13}C NMR observations of mixtures of **1** and **2** in acetic-levulinic acid medium with variable quantities of HCl admitted also demonstrate protonation of **2** upon HCl saturation. The ^{13}C signal due to the ketimine-type carbon in **2** moves from approximately 180 to 198 ppm for HCl concentrations of zero and ~1-2 M, respectively, accompanied by shift changes from about +1.5 to −3.5 ppm for several other carbon atoms.

Of particular interest here, however, is the appearance of a pair of slightly broad and therefore proton-bearing ^{15}N signals at 109.8 and 167.3 ppm. The intensities of these two peaks increase together at first and then decrease slightly with time, as seen in Figure 1. These signals behave in the manner expected for a chemical intermediate, some of which is being generated during the first signal accumulation interval. The experiment was not continued to the point of eventual disappearance of these signals; earlier ^{13}C studies[15] assure that almost total conversion to indomethacin would occur if enough time were allowed.

Discussion

Earlier ^{13}C NMR studies of indomethacin formation from

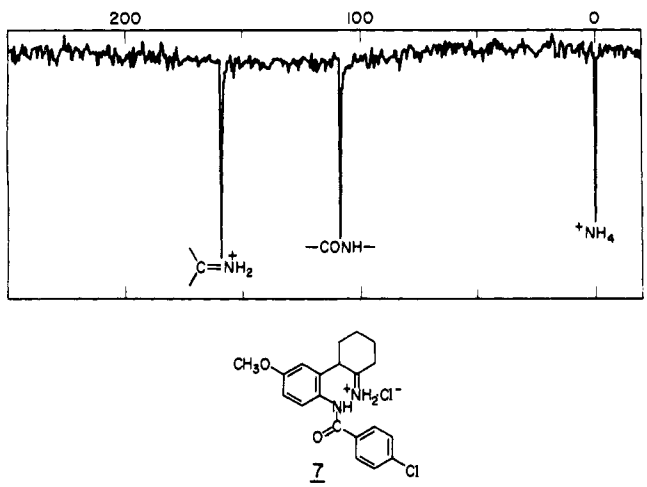

Figure 2. ^{15}N NMR spectrum of the cyclohexanone amide-imine Fischer indolization intermediate, **7**, recorded at 26-27 °C in CD_3CO_2H-HCl with $^{15}NH_4Cl$ reference at right. The amidic nitrogen of **7** is responsible for the signal at 108.2 ppm while the signal at 158.7 ppm is assigned to the iminium nitrogen nucleus.

1 or **2** had shown the presence of a single stable intermediate between **2** and **6** in Scheme I and assigned its structure as **4**.[15] This assignment depended in part upon a comparison of ^{13}C data for **4** with that of the corresponding amide-imine **7** (Figure 2) formed from **1** and cyclohexanone. Since the analogue was found to form somewhat more readily and proceed less rapidly to its Fischer indolization product, a ^{13}C reference spectrum of **7** was obtained free of interferences due to starting compounds or final products. The same was done here using ^{15}N observation, with the resultant spectrum presented in Figure 2. The product exhibits two signals, evidently due to proton-bearing nitrogen atoms by their widths, at 108.2 and 158.7 ppm. Based on literature shifts for *N*-benzoylaniline and diphenylketimine hydrochloride (Table I) assignments to amidic and imine-type nitrogen nuclei are as indicated on Figure 2. From the ^{15}N shifts for **7**, assignments for **4** of the amidic nitrogen at 109.8 ppm and the imine nitrogen at 167.3

Table I. Nitrogen-15 Chemical Shifts of Indomethacin Precursors and Related Compounds[a]

compd	solvent	$\delta^{15}N$, amide	$\delta^{15}N$, imine	ref
1	CD_3CO_2H-HCl	112.0	53.8[b,c]	this work
2	CD_3CO_2H-HCl	131.4	187.6[c]	this work
4	CD_3CO_2H-HCl	109.8	167.3[c]	this work
7	CD_3CO_2H-HCl	108.2	158.7[c]	this work
PhCONHPh	$CDCl_3$	102.[d]		19
$Ph_2C{=}NH_2^+$, Cl^-	SO_2		144.[c,e]	17

[a] In parts per million, relative to internal NH_4Cl except as noted. [b] N_2 of the acylhydrazine **1** is listed in the imine column for convenience; it has not yet reacted to form an imine in **1** itself. [c] Nitrogen is protonated to the appropriate "inium" form. [d] Measured for ^{14}N by ^1H-{^{14}N} double resonance; shift related to NH_4^+ by analogous measurement on 4.5 M NH_4NO_3 in 3 N aqueous HCl. [e] Shift referenced to ammonium chloride (aqueous solution) based on the shift of the latter reported in ref 17.

ppm are straightforward, and the amide-imine nature of the Fischer indolization intermediate is even more clearly established than before.[15] The correspondence for amidic and ketimine nitrogen atoms for **4** and **7** with the literature values for similar structures in Table I reinforces the structural assignments for both reaction intermediates.

Conclusions

^{15}N NMR offers a sensitivity at natural abundance of almost 50-fold less than ^{13}C, but, in very special circumstances such as described in this work, ^{15}N NMR is capable of contributing to the structural characterization of stable reaction intermediates which contain one or more nitrogen atoms, preferably proton bearing. The reaction to be studied must necessarily be quite slow and performed at a high concentration. When nuclear Overhauser effect (NOE) enhancements are taken into consideration, the sensitivity question with ^{15}N becomes more complicated than for ^{13}C owing to the negative magnetogyric ratio of the former. Just as ^{13}C NOE enhancements other than the maximum may be observed, ^{15}N-{^1H} NOE may produce intensities between +1 and −4, possibly even leading to zero intensity. Since ^{13}C spectra of the reaction of **1** or **2** to produce **6** in media containing an excess of levulinic acid had been found to exhibit intermediate NOE enhancements at 18-20 °C,[15] presumably owing to the high viscosity of the solutions examined, the present ^{15}N studies were conducted with no excess of levulinic acid and at a slightly higher observation temperature. The estimated single correlation time of 2.7 ns reported in ref 15 for hydrazone **2** would have led to a NOE-enhanced ^{15}N resultant intensity of −1.3 for fully dipolar relaxation, which would have made ^{15}N spectroscopy a more than 65-fold less sensitive monitor than ^{13}C observation. No ^{15}N NOE measurements were attempted here but omission of levulinic acid from the reaction medium presumably provided greater sensitivity in the ^{15}N reaction study than would have been obtained had concentrations and observation temperature been identical with those of ref 15. Indeed, an attempt to obtain a reference spectrum of **7** at about double the concentration used for Figure 2 almost failed. Broad, weakly negative-going signals could barely be detected in a 20-h experiment, where the signal-to-noise ratio was about five times worse than in Figure 2.

Finally, it may be suggested that the use of larger volume NMR probes, but perhaps without the employment of greater H_0 field strengths (due to complications involving negative NOE enhancement), constitutes the best approach to improvement of the sensitivity of natural-abundance ^{15}N NMR for the study of evolving chemistry at moderate to high concentrations. Both increased field strength and large sample volume will be desirable for dilute reactions involving small molecules where NOE enhancement may be expected to be at its full strength for proton-bearing nitrogen atoms. Based on the illustration in ref 1, obtained with considerably greater sample volume and at almost twice the applied field strength as in the present study, it may be projected that signal-to-noise ratios comparable to or better than those obtained for the amide-imine intermediate visualized here could be obtained using 0.5-h accumulation periods, in the absence of NOE complications. For proton-bearing nitrogen atoms, the recently reported J-cross-polarization (JCP) technique[20] should circumvent possibly diminished NOE enhancements and provide even greater sensitivity than the maximum possible in ordinary proton-decoupled observations. Thus, the most modern of ^{15}N NMR equipment and techniques will allow several samplings of a reaction lasting only 2-3 h. The mechanistic insight which in situ observations of ongoing chemistry can provide strongly recommends that natural-abundance ^{15}N NMR be employed frequently where applicable.

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