NMR and MS Analysis of Decomposition Compounds Produced from *N*-Acetyl-L-glutamine at Low pH

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N-Acetyl-L-glutamine decomposition products glutamine, glutamic acid, pyroglutamic acid, *N*-acetylglutamic acid, and a novel compound, *N*-(2,6-dioxo-3-piperidinyl) acetamide, have been identified by NMR and MS techniques. *N*-Acetylglutamine, a modified amino acid, offers greater chemical stability than glutamine in conditions that are experienced during typical sterilization and shelf storage of liquid nutritionals. However, to support safety and stability studies, potential decomposition products of *N*-acetyl-L-glutamine needed to be identified. Therefore, atypically harsh conditions were used; an unbuffered (pH <3) 1 mg/mL water solution of *N*-acetyl-L-glutamine was heated to 100 °C for 3 h. One-dimensional proton and proton-decoupled carbon-13 NMR and electrospray LC-MS/MS techniques were employed to identify the molecular structures of the generated *N*-acetyl-L-glutamine decomposition products. Additionally, DEPT and two-dimensional NMR techniques TOCSY, GMQCOSY, GHSQC, and GHMBC were employed to derive the final structure of the acetamide.

Keywords: *Functional foods; NMR; LC-MS; HPLC; glutamine; N-acetylglutamine; decomposition products; stability*

INTRODUCTION

Rising health care costs, an aging population, and changing governmental regulations on food marketing and labeling have led to increased attention to functional foods, which are designed specifically to provide benefits beyond basic nutrition (Messina, 1995) and may actively and in a targeted fashion promote health. Many foods bought at the local grocery store such as cereal grains, fruits, and vegetables are now recognized for their health benefits (Jain et al., 1999). For instance, products with significant soy content have been found to cause a decline in low-density lipoprotein cholesterol (Carroll and Kurowska, 1995; Wong et al., 1998), leading to a reduced risk of heart disease. Functional foods are specifically formulated to deliver physiologically active ingredients or conditionally essential compounds that have been depleted due to events such as cancer, trauma, and sepsis. Glutamine is one such nutrient that is not produced in sufficient quantities during physiologically stressed conditions and can be supplemented through the use of functional foods to improve patient outcome.

Glutamine has many reported effects including maintenance of mucosal cell integrity and gut barrier function and reduction of sepsis in neonates (Klein and Jeejeebhoy, 1993; Roig et al., 1995; Lacey et al., 1996). Effects of glutamine supplementation for bone marrow transplant patients, including improved nitrogen balance, lower incidence of positive microbial cultures and clinical infection, and shorter recovery time, also have been reported (Ziegler et al., 1992). In HIV and AIDS patients, it is believed that tissue loss is related to glutamine depletion (Shabert and Wilmore, 1996), and glutamine is essential for wound healing (Caldwell, 1989). Glutamine may be considered a conditionally essential amino acid in certain catabolic disease states such as sepsis, trauma, major surgery, burns, and uncontrolled diabetes. During these events, the release of glutamine from skeletal muscle tissue is accelerated. However, circulating glutamine concentrations are reduced as the intake of glutamine by the gut is accelerated. Thus, the requirement for glutamine becomes greater than the ability of the body to produce it (Souba et al., 1985).

Use of a glutamine-fortified functional food could help meet the glutamine requirement of the body during physiological events that produce an exaggerated need. A problem exists, however, with the stability of free glutamine during sterilization and shelf storage of aqueous liquids. The stability of glutamine under model system conditions has been studied and found to be dependent on both pH and temperature. At room temperature, losses in glutamine were reported for extreme pH conditions after only a short period of time (15 days), converting to either pyroglutamic acid or glutamic acid. Significant losses were reported (minimally 63%) for all pH values after boiling for 60 min (Airaudo et al., 1987). One solution to the stability problem is to use glutamine that has been modified in a manner that improves stability without hindering bioavailability. If adequate stability (at least 90% of initial concentration through 12 months at room temperature) and bioavailability are established, *N*-acetyl-L-glutamine is one glutamine derivative that could be used as a source of glutamine.

To assess stability in aqueous solutions, the behavior of *N*-acetyl-L-glutamine was studied at several pH

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values during storage and under different conditions of heating (M. Snowden, J. Baxter, M. Bergana, I. Reyzer, and V. Pound, unpublished results, 2000). Similar work has been reported for glutamine and pyroglutamic acid (Airaudo et al., 1987). To appropriately evaluate the toxicology of *N*-acetyl-L-glutamine, we needed to identify any potential degradation products. To produce enough sample mass for identification, we applied unusually harsh treatment (beyond the conditions typically encountered during the processing of foods) in an accelerated degradation study. Spectroscopic characterization and identification of the decomposition products produced during these conditions was performed using mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR). The results of these studies are reported.

MATERIALS AND METHODS

Materials. The following compounds were obtained from Sigma-Aldrich Co. (St. Louis, MO): *N*-acetyl-L-glutamine, *N*-acetyl-DL-glutamic acid, and L-glutamine. Pyroglutamic acid was purchased from Fluka (Buchs, Switzerland). The ^{2}H-labeled solvents deuterium oxide (D_2O, >99.996 atom % D) and dimethyl sulfoxide (DMSO-d_6, >99.9 atom % D) were obtained from Isotec Inc. (Miamisburg, OH). In some experiments, 3-(trimethylsilyl)tetradeutero sodium propionate (TSP) purchased from Wilmad Glass Co. (Buena, NJ) was used as an external reference.

Accelerated Degradation Study. An unbuffered (pH <3) 2 mg/mL solution of *N*-acetyl-L-glutamine was boiled (100 °C) under reflux for 3 h and freeze-dried. From an aqueous solution of the freeze-dried powder, each of four degradation products was purified by reverse phase HPLC to a single peak. The purified products were freeze-dried, redissolved in water, and again freeze-dried for subsequent assessment of the degradation products.

Analytical Methods. *HPLC.* The HPLC method utilized an Inertsil C8, 5 μm, 4.6 × 250 mm column from Keystone Scientific, Inc. A water mobile phase (pH adjusted to 2.2 with HCl) at 1 mL/min was employed isocratically with UV detection at 220 nm. Although typically it is not suggested to use entirely aqueous mobile phases with bonded phase columns, this column packing showed remarkable longevity. Care was taken to return the column to a 50% methanol solution over 20 min with a linear gradient for storage. Return to the analysis conditions was done in the same manner.

NMR Spectroscopy. The samples for NMR spectroscopy were prepared in Wilmad 327-PP 3 mm NMR tubes using ~0.15 mL of D_2O or DMSO-d_6. Samples from HPLC fractionation obtained in extremely small quantities were prepared in Shigemi 3 mm NMR tubes using ~70 μL of D_2O as solvent. NMR spectra were collected on a Varian Unity 500 spectrometer, equipped with either a Varian 3 mm PFG-ID probe for two-dimensional homo- and heteronuclear correlation experiments and one-dimensional ^{1}H experiments or a Nalorac 3 mm MCP probe for carbon-13 experiments. For both probes, the frequencies of 499.922 MHz for ^{1}H and 125.717 MHz for ^{13}C were employed. Probe temperature in all experiments was 27 °C. All shifts were cited in parts per million (ppm) from either an external TSP reference at 0.0 ppm for data sets collected in D_2O or internally by DMSO-d_6 peak shifts at 2.50 and 39.51 ppm for proton and carbon-13, respectively.

Two-Dimensional NMR. Four two-dimensional NMR techniques were used for structure identification and unequivocal signal assignments for the unknown compound. All two-dimensional data for the acetamide were collected in DMSO-d_6 at a temperature of 27 °C. Linear prediction was utilized to extend the number of points in the second dimension in all two-dimensional data sets, typically by a factor of 4. Additionally, zero filling and apodization with Gaussian window functions were applied for spectral enhancement in both the first and second dimensions.

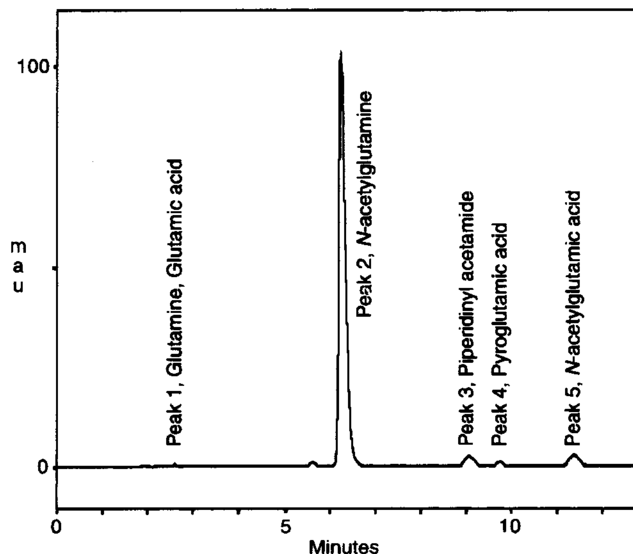

Figure 1. RP-HPLC chromatogram of thermally degraded *N*-acetyl-L-glutamine in an unbuffered (pH <3) 1 mg/mL aqueous solution. The HPLC method utilized an Inertsil C8, 5 μm, 4.6 × 250 mm column with an isocratic water mobile phase (pH adjusted to 2.2 with HCl) at 1 mL/min. The retention times for peaks 1−5 were 2.6, 6.3, 9.0, 9.7, and 11.4, respectively.

Table 1. Comparison of Proton NMR Data, Chemical Shift (ppm), and Multiplicity, Collected for *N*-Acetyl-L-glutamic Acid, *N*-Acetyl-L-glutamine, and the Piperidinyl Acetamide[a]

proton no.	*N*-acetyl-L-glutamic acid[b]		*N*-acetyl-L-glutamine[b]		*N*-(2,6-dioxo-3-piperidinyl) acetamide[c]	
1			7.27	S	10.77	S
1′			6.75	S		
3	4.18	M	4.13	DDD	4.53	M
4	1.93	M	1.93	M	1.91	M
4′	1.75	M	1.72	M	1.89	M
5	2.26	M	2.11	M	2.48	M
5′	2.26	M	2.11	M	2.71	M
8	8.09 (8.0)	D	8.10 (7.7)	D	8.21 (8.3)	D
12	1.84	S	1.84	S	1.87	S
COOH	~12.4[d]	S	12.50	S		

[a] All data were collected in DMSO-d_6. Some scalar couplings (Hz) are provided in parentheses. Multiplicity abbreviations used are as follows: multiplet (M), doublet (D), and singlet (S). [b] Data were collected using commercially available samples. Nuclei numbering for *N*-acetyl-L-glutamic acid and *N*-acetyl-L-glutamine use the corresponding numbers used for the piperidinyl acetamide. [c] Data were collected on HPLC fraction. [d] Very broad peak.

LC-MS/MS. The commercially available standards of *N*-acetyl-L-glutamine, pyroglutamic acid, and *N*-acetylglutamic acid were prepared individually and as a mixture at 1 nmol/μL in 0.1% trifluoroacetic acid (TFA) in H_2O. These standards along with the thermally degraded *N*-acetyl-L-glutamine solution were analyzed by electrospray mass spectrometry on a Finnigan LCQ-Classic. Samples were eluted isocratically at 0.2 mL/min from a 250 × 2 mm Inertsil C8 column with 0.1% TFA in H_2O. The mass spectrometer was operated in the positive ion, full-scan mode for both MS and MS/MS acquisitions. The electrospray ionization (ESI) probe was operated at 5.6 kV with sheath and auxiliary gases run at 75 and 3 (arbitrary units), respectively. The heated capillary temperature was maintained at 200 °C.

RESULTS AND DISCUSSION

An aliquot of the *N*-acetyl-L-glutamine solution from the accelerated degradation study was separated by reverse phase HPLC to give five peaks. The resulting

Table 2. Comparison of LC-MS Data of *N*-Acetyl-L-glutamine, Pyroglutamic Acid, and *N*-Acetyl-L-glutamic Acid Standards to Corresponding Data of Thermally Degraded, Unbuffered *N*-Acetyl-L-glutamine Solution[a]

		m/z		
identification	RT[b] (min)	(MH^+)[c]	$(2MH^+)$[d]	principal ions in MS-MS[e]
N-acetyl-L-glutamine standard	6.36	189.1	376.9	**172.0**, 143.0, 130.1
peak 2	6.32	189.1	376.8	**172.0**, 142.9, 130.1
peak 3 (unknown)	8.75	171.0		142.9, **129.2**
pyroglutamic acid standard	9.42	130.1	258.9	115.8, **84.3**, 65.9
peak 4 (very weak)	8.99	130.2	258.9	**84.1**
N-acetyl-L-glutamic acid standard	10.58	190.1	378.8	**172.0**, 143.9, 130.1
peak 5	10.24	190.1	378.9	**172.0**, 143.9, 130.1

[a] LC-MS data were not collected for a glutamini standard, a glutamic acid standard, or peak 1. [b] Retention time. [c] Protonated molecular ion. [d] Protonated dimer. [e] Boldface type for an ion denotes base peak.

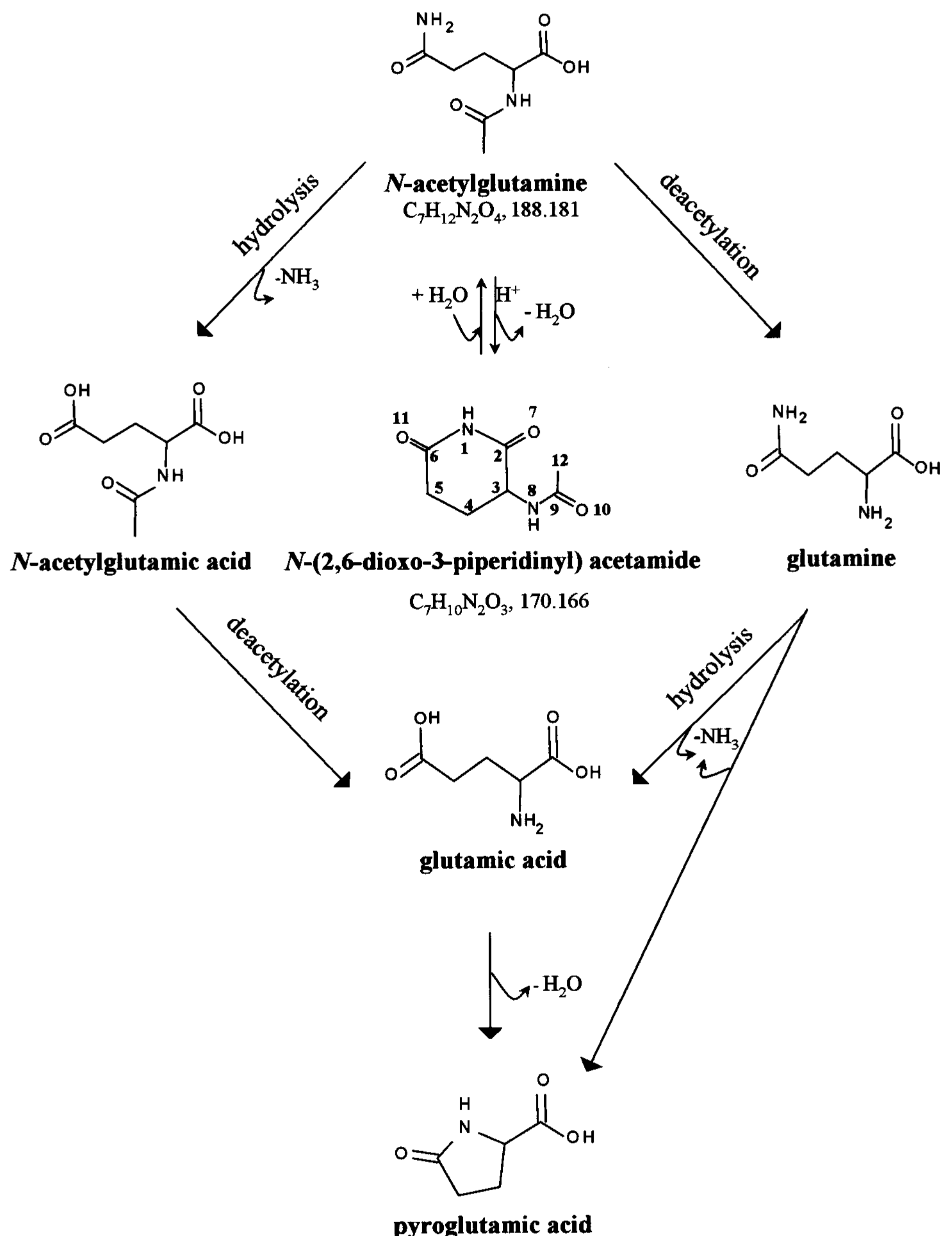

Figure 2. Decomposition scheme proposed for *N*-acetylglutamine with associated molecular structures. The numbering scheme used for NMR assignments is provided for *N*-(2,6-dioxo-3-piperidinyl) acetamide.

HPLC chromatogram is shown in Figure 1. Using this method, *N*-acetyl-L-glutamine was eluted at ~6.2 min and remained the largest peak in the chromatogram (83.6% of total peak area).

Commercially available samples of L-glutamine and known degradation products of glutamine (glutamic acid and pyroglutamic acid) were individually chromatographed. Their retention times, 2.6, 2.8, and 9.7 min, respectively, were found to be consistent with three HPLC peaks from the *N*-acetyl-L-glutamine boiled solution. Similarly, the peak with retention time of 11.39 min was found to be consistent with the *N*-acetyl (NAc) analogue of glutamic acid, leaving one unassigned HPLC peak in the chromatogram.

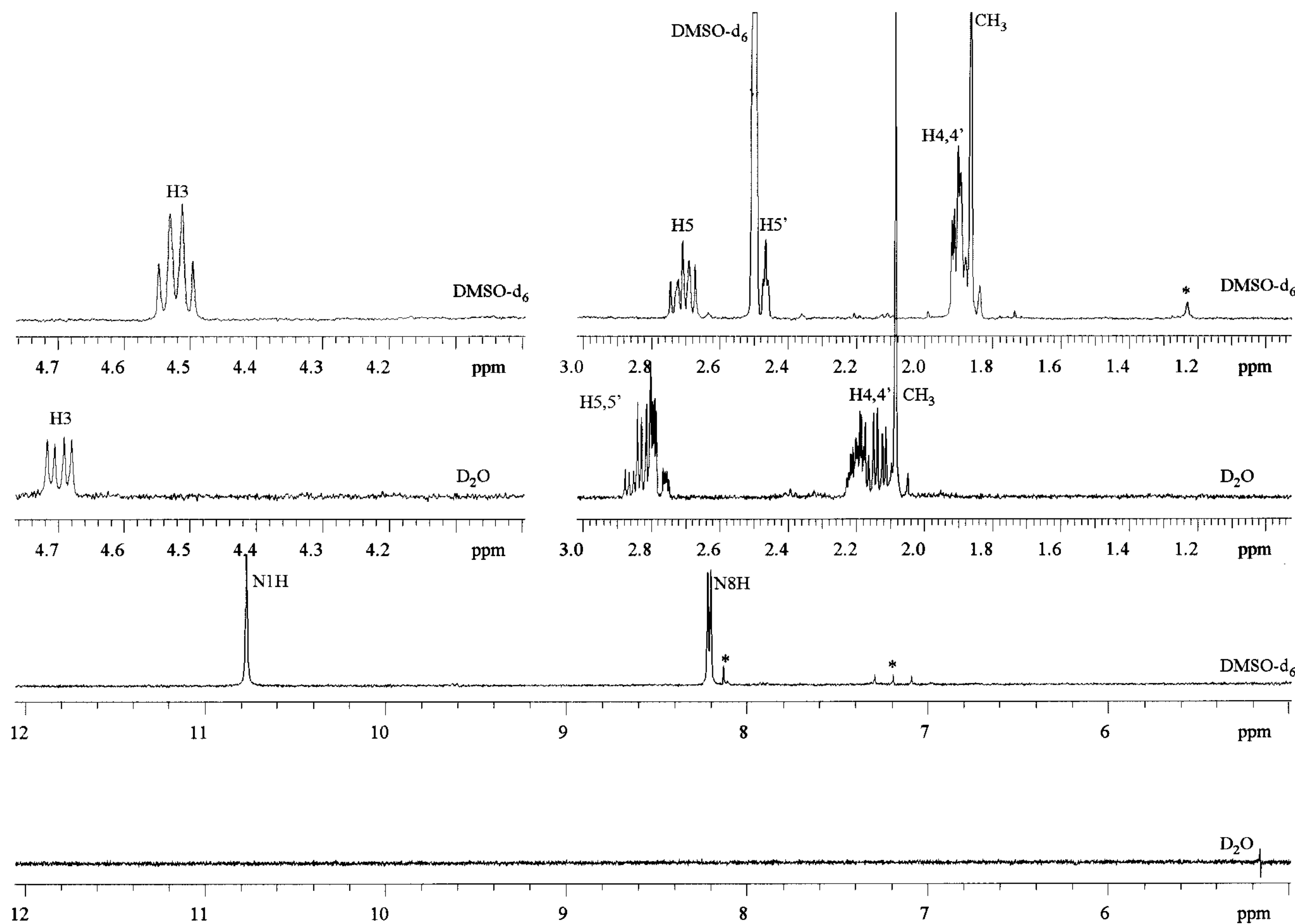

Figure 3. Comparison of the ^{1}H NMR spectra of *N*-(2,6-dioxo-3-piperidinyl) acetamide collected in DMSO-d_6 and D_2O solvents. Certain regions have been omitted due to the intensities of the peaks from water. Resonances have been labeled according to the numbering scheme shown in Figure 2, and impurities have been labeled with asterisks. See text for further details.

NMR and MS studies were performed to confirm the tentative HPLC peak assignments and for identification of the unknown HPLC peak. For NMR analyses, the HPLC peaks were isolated and purified utilizing the HPLC method without modification. Electrospray MS analyses were performed on-line using the same HPLC method with modifications as described under LC-MS/MS. Proton NMR and MS data, along with the HPLC data, confirmed the identification of the four peaks assigned to the decomposition products glutamine, glutamic acid, pyroglutamic acid, and *N*-acetylglutamic acid. Proton NMR data for these four products are provided in Table 1, and the MS data are shown in Table 2. The structures of the identified decomposition products are provided in Figure 2.

Early in the investigation of the unknown compound (Figure 1, peak 3), insight into its structure was obtained by observation of decomposition or equilibrium structures that developed in D_2O during lengthy NMR experiments. After purification, the fraction was lyophilized and reconstituted in D_2O for NMR study. Within a short period, typically overnight at 27 °C, new resonances appeared in the proton NMR data. It was found that the new peaks were consistent with peaks collected for *N*-acetyl-L-glutamine and *N*-acetyl-L-glutamic acid; the *N*-acetyl-L-glutamine peaks were the larger of the two sets of new peaks. These data are very important, demonstrating that the *N*-acetyl-L-glutamine structure was regenerated upon hydration, and indicate that the nitrogen nuclei of *N*-acetyl-L-glutamine were retained in the unknown structure (unlike other degradation products such as pyroglutamic acid and *N*-acetyl-L-glutamic acid). The data collected in D_2O helped to characterize the nitrogen nuclei by NMR where direct detection by nitrogen NMR would have required isotopic labeling or a much larger sample amount than available. Table 2 shows that the protonated molecular ion generated in the MS analysis of the unknown (m/z 171.0) was 18 Da less than that of *N*-acetyl-L-glutamine (m/z 189.1). Both the D_2O proton NMR and MS data support the identification of the unknown structure as a dehydration product of *N*-acetyl-L-glutamine.

Utilizing DMSO-d_6 as an NMR solvent provided good solution stability for the unknown compound, and the exchangeable protons could be observed under these conditions. Two of the peaks observed at approximately 8.2 and 10.8 ppm in DMSO-d_6 proton NMR data were not observed in the corresponding data collected in D_2O. A comparison of the two proton NMR data sets collected in different solvents is shown in Figure 3. Supported by proton peak area integration, this observation is consistent with the retention of two of the original four exchangeable protons in the *N*-acetyl-L-glutamine parent structure. One of the exchangeable protons observed in DMSO-d_6, the 8.3 Hz doublet at 8.2 ppm, is assigned to the nitrogen proton (N8H) of the NAc group on the basis of scalar coupling features as well as chemical shift (see Figure 2 for nuclei numbering used for NMR peak assignment). Similar chemical shift and coupling constant values of 8.10 ppm and 7.7 Hz, respectively, were

Table 3. Proton-Decoupled Carbon-13, DEPT, and Two-Dimensional NMR Data of *N*-(2,6-Dioxo-3-piperidinyl) Acetamide in DMSO-d_6

heteronucleus no.	^{13}C chemical shift (δ)	carbonyl identification[a] and multiplicity[b]	proton no.	GMQCOSY correlations (from diagonal)	GHMBC correlations
N1	–	–	H1	–	–
C2	172.28	C=O	–	–	NH8, H3, H4
C3	49.03	CH	–	3→8, 3→4,4′	**NH1**,[c] NH8, H5, H5′, H4
C4	24.36	CH_2	–	4→3, 4→5, 4→5′	H3, H5, H5′
C5	30.90	CH_2	H5	5→5′, 5→4,4′	NH1, H3, H4
	–	–	H5′	5′→5, 5′→4,4′	–
C6	172.97	C=O	–	–	H1, H5, H5′, H4
N8	–	–	H8	8→3	–
C9	169.26	C=O	–	–	H3, N8H, H12
C12	22.52	CH_3	–	–	–

[a] Determined by proton-decoupled carbon-13. [b] Determined by carbon-13 DEPT. [c] Boldface type denotes correlation found to support ring formation.

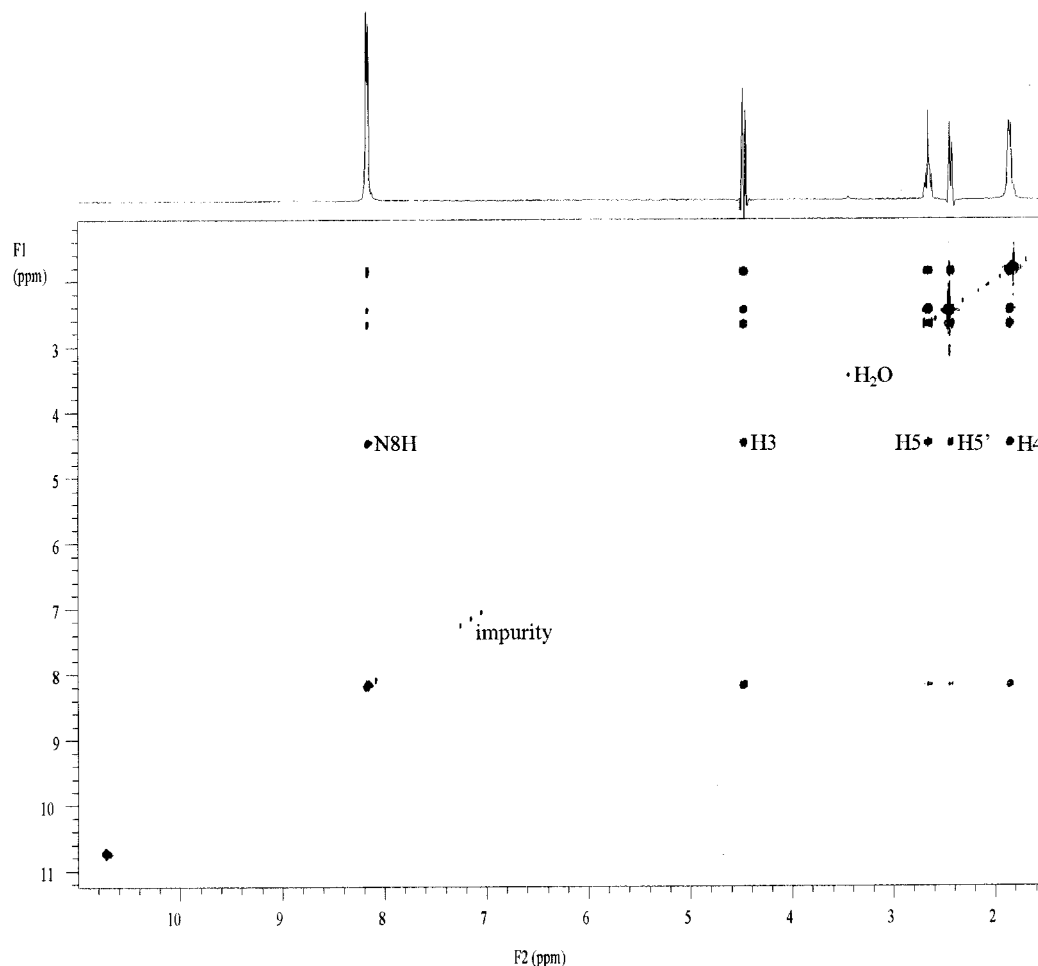

Figure 4. TOCSY spectrum of *N*-(2,6-dioxo-3-piperidinyl) acetamide in DMSO-d_6. The trace represents the diagonal peak at 4.5 ppm. Resonances have been labeled according to the numbering scheme shown in Figure 2. The resonances from DMSO-d_6 and the CH_3 peaks non-degenerate with H4 and H5 resonances are observed as intense resonances along the diagonal.

observed for the corresponding resonance in *N*-acetyl-L-glutamine (see Table 1). Comparison of MS data for the unknown and *N*-acetyl-L-glutamine showed a difference of 18 amu consistent with a loss of H_2O. Therefore, if a hydroxyl group is lost from the carboxyl group as a result of the dehydration process, the remaining exchangeable proton is likely positioned on the second nitrogen (N1). Characterization of these nitrogen protons and their assignments are further substantiated in two-dimensional NMR data discussed below.

The proton-decoupled carbon-13 NMR data of the unknown structure in DMSO-d_6 yielded seven carbon nuclei for the unknown, three of which resonated in the carbonyl region. Carbon-13 DEPT spectra, which classify the protonated carbon-13 nuclei (methine, methylene, or methyl), indicated that the unknown structure has one methine, two methylenes, and one methyl, in addition to the three carbonyl resonances observed in the proton-decoupled carbon-13 NMR spectrum. Therefore, the total number of carbon nuclei and the multiplicity of the carbon nuclei remain unchanged from those of the parent compound, *N*-acetyl-L-glutamine. These data are compiled in Table 3.

A GMQCOSY pulse sequence (MQ=2) was utilized to determine proton−proton scalar coupling correlations for the unknown structure. The GMQCOSY pulse sequence encodes proton−proton multiple quantum

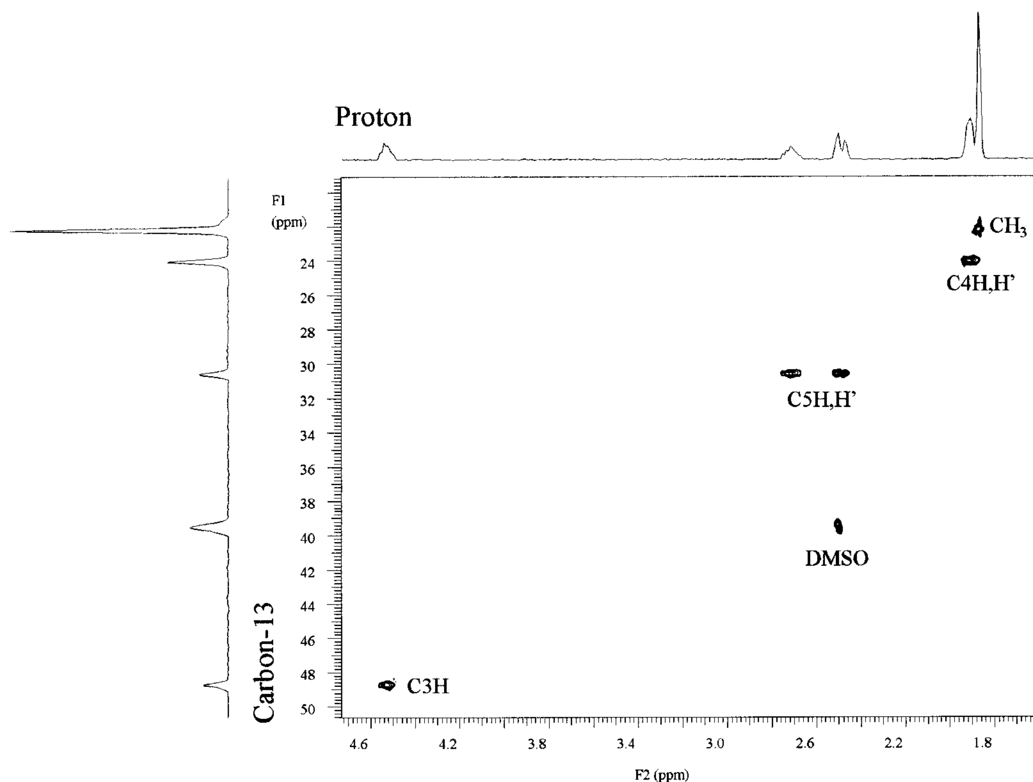

Figure 5. GHSQC spectrum of *N*-(2,6-dioxo-3-piperidinyl) acetamide in DMSO-d_6. Projections have been included for both dimensions. Resonances have been labeled according to the numbering scheme shown in Figure 2.

coherences only during the read pulse. Therefore, both the DMSO-d_6 multiplet and the large singlet (at 2.0 ppm) belonging to the unknown compound (both having non-degenerate or nearly non-degenerate frequencies with unknown peaks) were sufficiently removed from the data set to observe smaller (due to the multiplicity) resonances of the unknown compound. The absence of the 10.7 ppm resonance, N1H, and any of its corresponding off-diagonal peaks indicates that it is also filtered out in the GMQCOSY pulse sequence and is consistent with the lack of coupling observed for this resonance in the one-dimensional proton data set. From these data, the remaining proton assignments were made starting from the second nitrogen proton, N8H, at ~8.2 ppm (see Table 1). These data show near non-degeneracy for the C4 methylene proton peaks; however the C5 methylene proton peaks are clearly resolved in this data set.

A TOCSY trace of the methine proton at the 4.5 ppm resonance (H3) shows scalar coupling relay to all of the protons in the molecule, with the exception of the methyl and N1H resonances (see Figure 4). The TOCSY data indicate that the 8.2 ppm nitrogen proton and the protons of the methine and the two methylenes are of one spin system and support the retention of the NH−CH−CH_2−CH_2 fragment from the parent compound, *N*-acetyl-L-glutamine. No TOCSY correlations were observed for the N1H proton and the methyl resonances.

In Figure 5, a proton−carbon-13 GHSQC data set of the unknown sample is shown in the protonated carbon-13 region, which was determined by DEPT. These data show one-bond scalar coupling correlations between the proton and carbon-13 resonances, thereby aiding in the assignment of the methylene carbons C4 and C5. Utilizing the assignments of the C4 and C5 protons that were obtained from the GMQCOSY and TOCSY (data listed in Table 1), the C4 and C5 carbons are assigned to the carbon-13 resonances at 24.36 and 30.89 ppm, respectively. Improved resolution between the C4 protons from the methyl resonance is achieved in the two-dimensional GHSQC data. Consistent with other NMR data sets (in DMSO-d_6), the C4 resonances were observed to be nearly non-degenerate in the GHSQC data, suggesting a fairly planar structure at this portion of the molecule.

Proton−carbon-13 GHMBC data sets yield two-dimensional long-range (≥2 bonds, typically 2−3) scalar coupling, $^{2,3}J$, between protons and carbon-13 nuclei. This is a very important data set for structure identification because it establishes connections across nonprotonated heteroatoms. The GHMBC correlations observed for the unknown are shown in Table 3. All of the long-range scalar coupling correlations were found to be consistent with the structural fragments supported by the data presented thus far. Additionally, GHMBC correlations observed between the carbonyl C9 nucleus and both the adjacent amide N8H and adjacent methyl H12 proton nuclei support the retention of the NAc group. GHMBC correlations observed between the carbonyl C2 and the H3 (α) nuclei support the retention of the carbonyl adjacent to the α carbon and their local nuclei arrangement. Finally, correlations that were observed between carbonyl C6 and its adjacent methylene H5 and N1H protons are consistent with the retention of the carbonyl next to the C5 protons and the corresponding amide group.

Through the application of two-dimensional NMR data, the retention of the original *N*-acetyl-L-glutamine structure, except for H_2O (one N1 proton and the hydroxyl group) in the unknown structure, has been supported. These data are consistent with the proton D_2O data, by which *N*-acetyl-L-glutamine was observed to regenerate in water from a purified sample of the unknown. Final ring formation, via the loss of H_2O, is

supported by the proton and carbon-13 correlation representing a 3-bond scalar coupling, $^3J_{CH}$, between the N1 proton (10.8 ppm) and the C3 carbon (49.0 ppm). This peak would not be expected in the corresponding data for the linear parent *N*-acetyl-L-glutamine structure due to the six bonds between these two nuclei.

The structure produced from the acquisition and interpretation of the collective body of spectral data for the unknown fraction is *N*-(2,6-dioxo-3-piperidinyl) acetamide. This structure could result from a reaction or a series of reactions for which the net result is a nucleophilic attack of the amide nitrogen on the carboxyl group. Amino acids with the amino group on the C4 and C5 carbons are known to form internal amides or lactams when heated (Cram and Cram, 1978). Such a nucleophilic attack would easily form an unrestrained six-membered ring; however, further work will be needed to define the mechanism. The proposed *N*-(2,6-dioxo-3-piperidinyl) acetamide is further substantiated by comparable hydrolysis reported for 3-phenylacetylamino-2,6-piperidinedione, a naturally occurring peptide derivative (Tang, 1996).

The decomposition products of *N*-acetyl-L-glutamine have been identified by NMR and HPLC-MS as *N*-acetyl-L-glutamic acid, *N*-(2,6-dioxo-3-piperidinyl) acetamide, pyroglutamic acid, glutamic acid, and glutamine (listed by decreasing order of percent HPLC total peak area). Their identification allows one to show structurally that the increased stability of *N*-acetyl-L-glutamine is directly related to increased stability of the α-amine due to acetylation. Pyroglutamic acid, a minor decomposition product of *N*-acetyl-L-glutamine at pH 3, is the major decomposition product of glutamine under similar conditions (Airaudo et al., 1987). These data suggest that acetylation (or modification) of the primary α-amine to an amide dramatically slows degradation to pyroglutamic acid. In this study, pyroglutamic acid is a very minor degradation product for *N*-acetyl-L-glutamine and is likely produced only after the relatively slower process of *N*-acetyl-L-glutamine or *N*-acetyl-L-glutamic acid deacetylation. Because the intramolecular cyclization observed with glutamine (forming pyroglutamic acid) is effectively eliminated by acetylation, more minor degradation routes can be observed. This could explain higher levels of *N*-acetyl-L-glutamic acid found in the current work when compared to the corresponding glutamic acid levels observed (0.00 at pH 3 and boiling for 60 min) and reported for glutamine degradation (Airaudo et al., 1987). The absence of the unlikely potential degradation product *N*-acetylpyroglutamic acid in the boiled *N*-acetyl-L-glutamine solution is also consistent with this model. A proposed decomposition scheme for *N*-acetyl-L-glutamine resulting in the five identified decomposition products is shown in Figure 2.

A novel compound, *N*-(2,6-dioxo-3-piperidinyl) acetamide, has been identified as a decomposition product of *N*-acetyl-L-glutamine. The piperidinyl acetamide has been demonstrated to rehydrate to regenerate *N*-acetyl-L-glutamine in aqueous conditions, implicating a dehydration/rehydration equilibrium in its production from *N*-acetyl-L-glutamine. Therefore, the concentration of the piperidinyl acetamide would rapidly reach steady state and then begin to decline as the *N*-acetyl-L-glutamine concentration decreases, causing a shift toward rehydration of the piperidinyl acetamide to form *N*-acetyl-L-glutamine by simple mass action. In this model, the piperidinyl acetamide would eventually disappear in favor of the other degradation products, and finally only pyroglutamic acid would remain. This would, however, be a very slow process, certainly relative to the degradation kinetics of free glutamine.

It is important, however, to remember that the degradation reactions discussed occur at a significant rate only at pH ≤3. Indeed, at neutral pH, even under conditions of heat treatment that would never be encountered in a retort-sterilized liquid food material, we observed no significant degradation of *N*-acetyl-L-glutamine (M. Snowden et al., unpublished results, 2000). This contrasts sharply with glutamine, for which retort sterilization results in a significant loss to pyroglutamic acid and the rest of glutamine lost within a few months of storage. Because retort-sterilized liquid nutritional products typically yield shelf lives of 9−18 months, it is clear that although glutamine is not suitable, *N*-acetyl-L-glutamine is stable enough to be used. It still needs to be determined if *N*-acetyl-L-glutamine is adequately bioavailable, as glutamine, in humans when orally consumed. Further work should address this issue.

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