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Multinuclear NMR (^{1}H, ^{13}C and ^{19}F) spectroscopic re-examination of the solvolytic behaviour of flurazepam dihydrochloride

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Abstract: The dissolution of reference and archival samples of flurazepam dihydrochloride (**2**) was studied in DMSO-d_6 and in D_2O by ^{1}H-, ^{13}C- and ^{19}F-NMR spectroscopy to identify and distinguish solvated species of the parent drug (**2**), the "benzophenone" (**4**) and glycine (**5**) hydrochloride degradation products. In DMSO-d_6, for most samples, only the ring intact form (**2**) could be detected by ^{13}C-NMR whereas the inherently greater sensitivity of ^{19}F-NMR allowed detection of initial trace amounts (<1%) of the open-ring form (**3**). ^{19}F-NMR spectroscopy also afforded the best means of quantifying the various entities in solution, including the increase towards equilibrium levels of the open-ring entity and detection/quantitation of a new equilibrium species, possibly the *cis/trans* rotamer of the open-chain entity (**3**). Various chemical shifts for flurazepam dihydrochloride and USP flurazepam related reference standards C and F are reported for DMSO-d_6 solutions. The bases for ^{1}H- and ^{19}F-NMR assay of DMSO-d_6 solutions of (**2**) for (**4**) are discussed with comparative data. The solvation characteristics of (**2**) in D_2O at 0 and 27°C were found to be too complex to follow by ^{13}C-NMR; however, ^{19}F-NMR studies at these temperatures permitted one to clearly discern that no additional formation of entity (**4**) occurred beyond whatever initial levels were present in degraded samples while the open-ring entity (**3**) was observed to increase to an equilibrium level of 56% over 24 h at 27°C. Dissolution in D_2O at either 0 or 27°C does not contribute to solvolytic degradation of (**2**) to (**4**) over 24 h.

Keywords: *Flurazepam hydrochloride (dihydrochloride); ^{1}H, ^{13}C, and ^{19}F-NMR spectra; Degradation products of flurazepam hydrochloride; ^{1}H- and ^{19}F-NMR assay of flurazepam·2HCl degradation; ^{1}H-NMR chemical shifts (DMSO-d_6) for flurazepam·2HCl; USP flurazepam related reference standards C and F.*

Introduction

Flurazepam dihydrochloride (**2**) (confusingly referred to in the pharmaceutical literature as flurazepam hydrochloride and for which a US Pharmacopeial compendial monograph [1] exists) has already received some study by nuclear magnetic resonance (NMR) spectroscopy employing proton (^{1}H-), carbon-13 (^{13}C-) and fluorine (^{19}F-) nuclei. ^{1}H-NMR spectra (60 MHz) of the "hydrochloride" (in CD_3OD) and its base (in $CDCl_3$), as well as the ^{19}F-NMR spectrum of the hydrochloride (in CH_3OH) were published by Rudy and Senkowski [2]. Kuwayama *et al.* [3] examined (**1**) in 0.5 N DCl by ^{1}H-NMR (100 MHz) but found the spectrum so complex as to preclude assignment; they chose instead to study the system by ^{13}C-NMR (25 MHz) spectroscopy. In acidic aqueous solution (0.5 N DCl), they showed that flurazepam monohydrochloride (**1**) was present in the form of a protonated iminium structure (**2**) immediately after preparation of the solution, and that it subsequently underwent hydrolysis to give a ring-opened benzophenone structure (**3**) (major portion), which was in equilibrium with the ring-closed iminium structure (minor portion). Earlier, Kuwayama and Yashiro [4] had studied the ^{13}C-NMR spectra of diazepam and fludiazepam, also dissolved in 0.5 N DCl, and found each substance to be in the form of a protonated iminium structure immediately after preparation of the solution and subsequently underwent slow hydrolysis of the iminium moiety to give a ring-opened benzophenone structure in equilibrium with the corresponding ring-closed iminium structure. The use of NMR for the detection and quantitation of various benzodiazepines was

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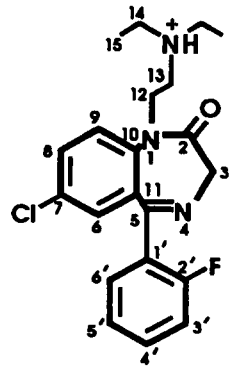
summarized recently by Dawson [5] as one of several categories of abused drugs amenable to this technique.

This study arose as the result of the need for an independent assessment of the state of degradation of some aged (archival) samples of flurazepam hydrochloride [6] which, itself, was an extension of a previous FT-Raman and infrared (IR) study [7] of delorazepam, fludiazepam, tetrazepam, and flurazepam, the fourth part of systematic studies [8–10] of the vibrational spectra, IR and Raman, of a series of benzodiazepines. From the vibrational studies, evidence was produced for the degradation (through natural aging) of flurazepam hydrochloride to the benzophenone (**4**) and glycine hydrochlorides with no apparent residue of the aminoacetylbenzophenone (**3**) precursor that had been identified by Kuwayama *et al.* [3] in 0.5 N DCl solution. The purpose of this paper is to study the solvolytic behaviour of flurazepam hydrochloride more fully by ^{1}H-, ^{13}C and ^{19}F-NMR in different solvents.

Experimental

All NMR spectra were acquired on a Bruker AM400 spectrometer (^{1}H = 400.13 MHz, ^{13}C = 100.6 MHz, ^{19}F = 376.5 MHz) operating at 300 K. Proton and carbon spectra were acquired using a dual ^{13}C/^{1}H 5 mm probe. Fluorine spectra were acquired with a dedicated 5 mm ^{19}F/^{1}H probe and ^{19}F-specific preamplifier, using standard Bruker band-stop and band-pass filters. Proton decoupling for carbon and fluorine spectra was achieved by the use of low power (composite phase) decoupling. The samples were run in DMSO-d_6 or D_2O using standard programs from the Bruker software library (version DISRVK01). ^{19}F chemical shifts were referenced to external $CFCl_3$ at 0.00 ppm. ^{1}H and ^{13}C chemical shifts in DMSO-d_6 were referenced to the solvent peaks (2.49 ppm for the DMSO-d_5 for proton and 39.5 ppm for the centre peak of the DMSO-d_6 for carbon). Chemical shifts for D_2O were referenced to external DSS at 0.00 ppm for both proton and carbon spectra.



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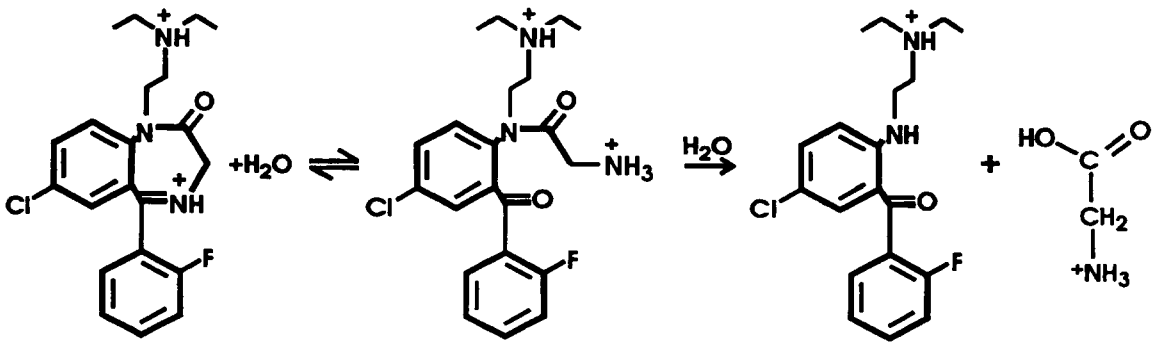


2 **3** **4** **5**

Proton spectra were acquired using 32 K data points over 5618 Hz (Hz/point = 0.343), with a flip angle of about 30°, an acquisition time of 2.916 s and a relaxation delay of 3 s. Carbon spectra had 64 K data points, sweep width of 22727 Hz (Hz/point = 0.694), a flip angle of about 15°, acquisition time of 1.442 s and a relaxation delay of 5 s. Fluorine spectra were acquired with 32 K data points, a sweep width of 38462 Hz (Hz/point = 2.348), a flip angle of 90°, an acquisition time of 0.426 s and a relaxation delay of 3 s.

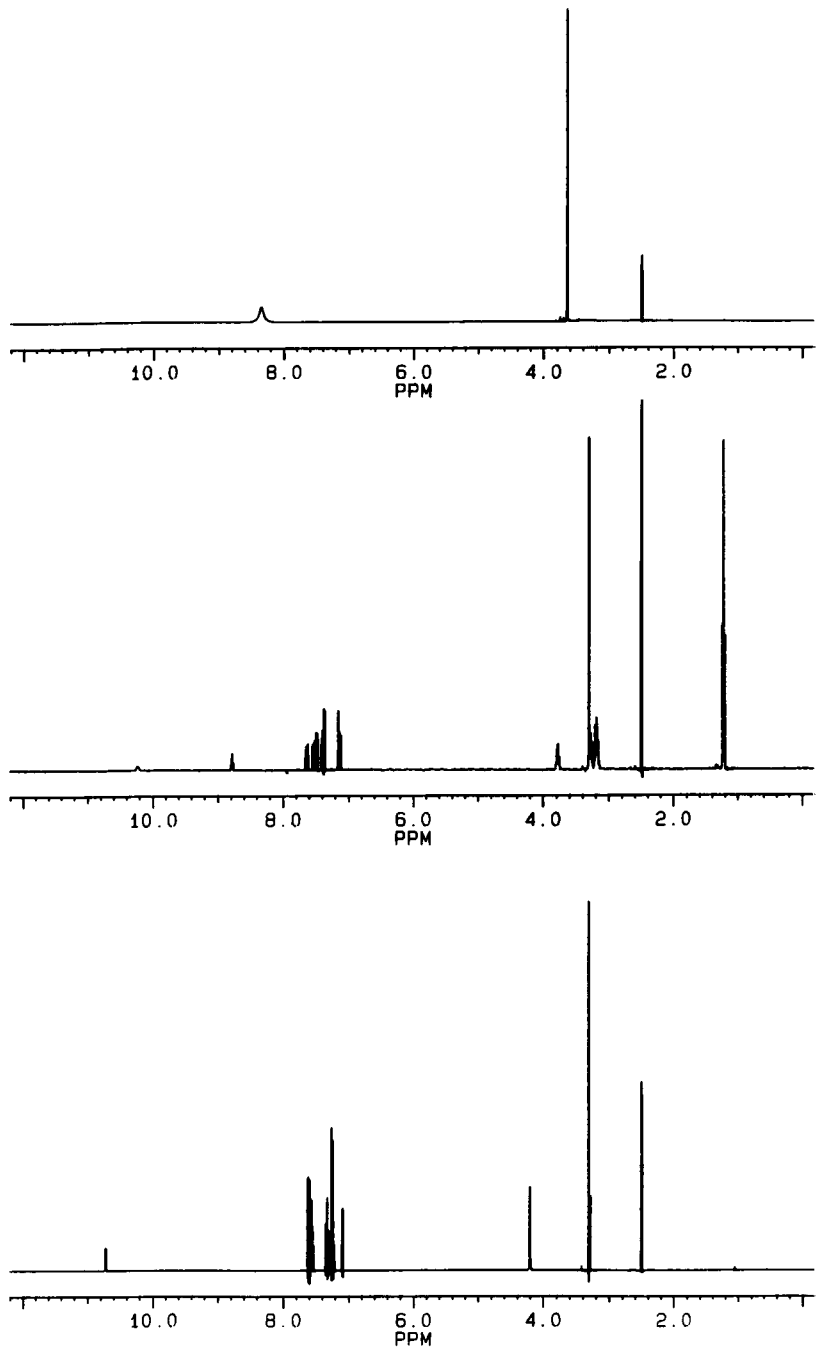


Figure 1
^{1}H-NMR reference spectra obtained from DMSO-d_6 solutions of (top) glycine hydrochloride (Aldrich, Lot 10008JY), (middle) 5-chloro-2-(2-diethylaminoethylamino)-2′-fluorobenzophenone hydrochloride (National Formularly Reference Standard no. 578), and (bottom) 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-[2H]-1,4-benzodiazepine-2-one (USP Flurazepam Related Compound F, Lot G).

Results and Discussion

^{1}H-NMR studies in DMSO-d_6 solution

Before the complex ^{1}H-NMR spectra of degraded (aged) samples of flurazepam hydrochloride, which were determined in deuterodimethyl sulfoxide (DMOS-d_6) solution, could be assessed and their component parts identified, it was necessary to obtain reference spectra of flurazepam hydrochloride (USP Reference Standard, Lot H-2) and of its two hydrolytic end degradation products, glycine hydrochloride (Aldrich, Lot 10008JY) and 5-chloro-2-(2-diethylaminoethylamino)-2′-fluorobenzophenone hydrochloride, a National Formulary (NF) Reference Standard. The NF sample, obtained 15 years earlier, was found to be essentially pure and superior in quality to the current USP Flurazepam Related Compound C (Lot H). In addition to the glycine and "benzophenone" hydrochloride spectra (Fig. 1), a reference spectrum of the desalkyl flurazepam, 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-[2H]-1,4-benzodiazepine-2-one (USP Flurazepam Related Compound F, Lot G) was obtained in the event that some loss of the (diethylamino)ethyl group at position 1 of the flurazepam ring might have occurred during aging of the archival samples.

In Fig. 2, ^{1}H-NMR spectra are composed of (A) the flurazepam hydrochloride (~6 mg) reference substance alone, (B) following addition of glycine hydrochloride (~1 mg), (C) following initial spiking of the mixture B with the "benzophenone" (~1 mg), and (D) after addition of another charge (~1.7 mg) of the "benzophenone". By examining the glycine HCl and "benzophenone" HCl spectra separately and later, as successively spiked additions of each of these substances to the DMSO-d_6 solution of the new USP flurazepam HCl reference standard, the complexity of the overall features, particularly the lower field resonance clusters seen at δ 10.8–11.5 and δ

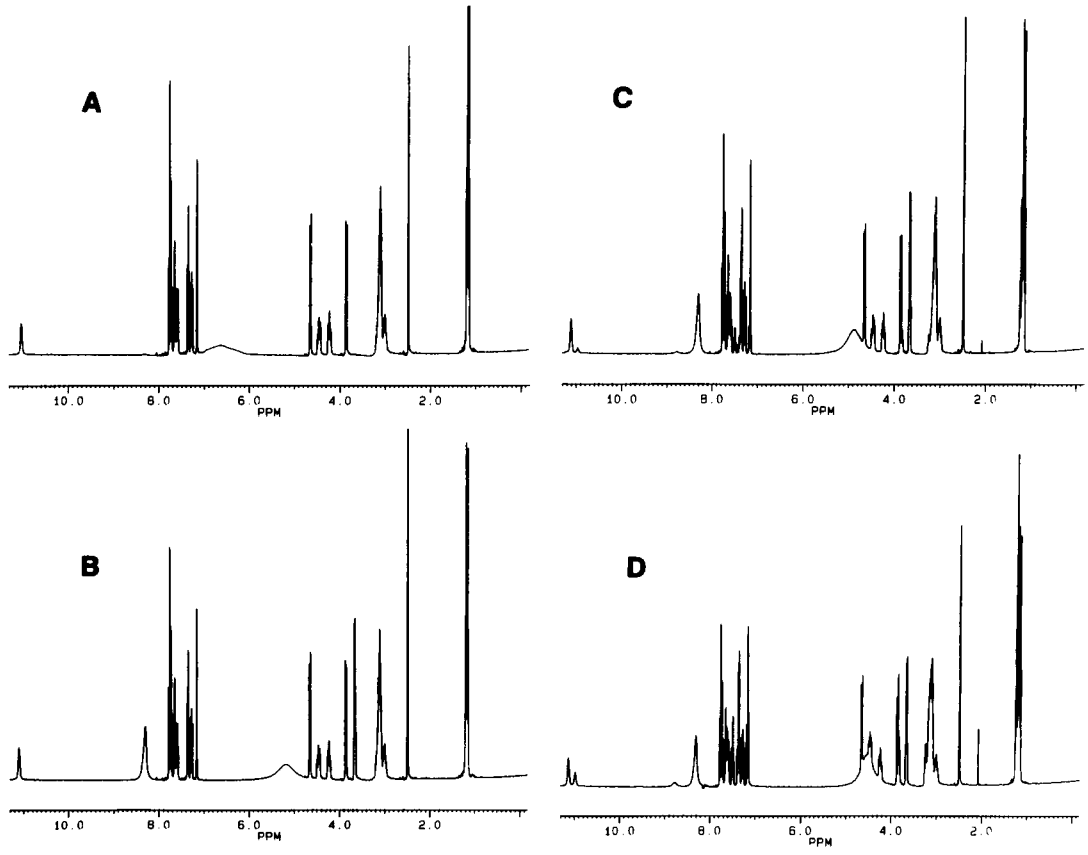


Figure 2
^{1}H-NMR spectra obtained from DMSO-d_6 solutions of (A) flurazepam hydrochloride (~6 mg) (USP, Lot H-2), (B) A following the addition of glycine hydrochloride (~1 mg) (Aldrich, Lot 10008JY), (C) following initial spiking of the mixture B with the "benzophenone" (~1 mg) (USP Flurazepam Related Reference Compound C, Lot H), and (D) C after the addition of another charge (~1.7 mg) of the "benzophenone".

8.2–8.9 could be resolved. The ^{1}H-NMR spectrum of the fresh flurazepam HCl reference material *appeared* on preliminary examination to exist entirely in the open chain form (**3**) with the ethylamino proton resonating near δ 11.1 and the protonated amino group of (**3**) resonating as a broad band centred near δ 6.7 and integrating in a 1:3 ratio, respectively. Re-examination of the sample by ^{13}C-NMR spectroscopy (100 MHz), however, clearly showed from the absence of a carbonyl carbon signal at 196.4 ppm (Fig. 3) that the drug was at least predominantly in the ring closed form (**2**) (i.e. not as an equilibrium of forms (**2**) and (**3**) as reported by Kuwayama *et al.* [3] in aqueous solution). The broad proton resonance at δ 6.7

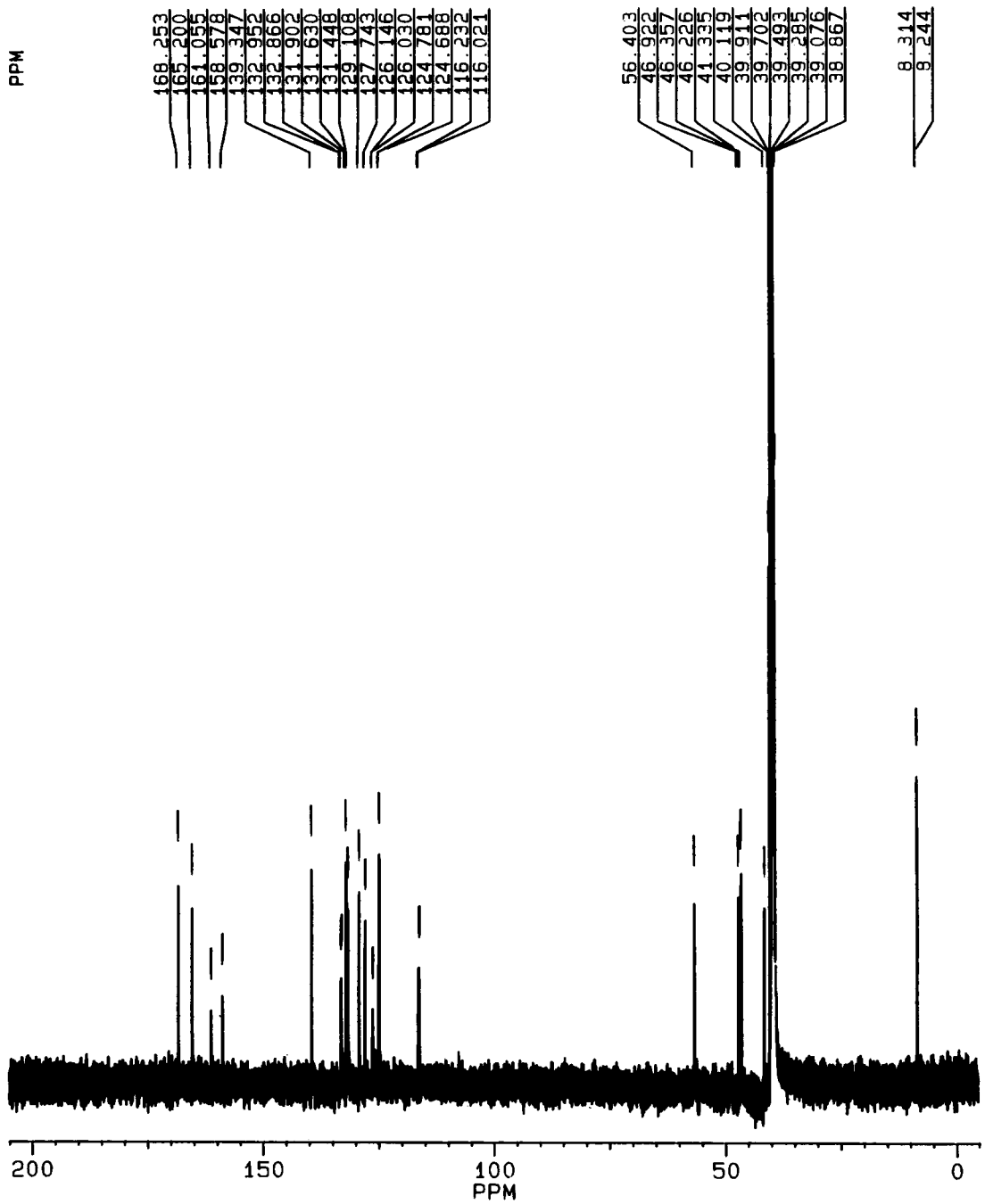


Figure 3
^{13}C-NMR spectrum of flurazepam hydrochloride (USP, Lot H-2) in DMSO-d_6 solution.

was found to be variable in chemical shift and peak area (Fig. 2), and further studies also indicated that its appearance was dependent on the water content of the solvent. When some glycine HCl was added to the fresh DMSO-d_6 solution of flurazepam HCl (Fig. 2B), the protonated amino resonance of (**5**) appeared in the mixed spectrum at δ 8.3 (as it does for glycine HCl alone in Fig. 1A), whereas the protonated amino resonance of (**3**) shifts upfield to ~δ 5.2 as a somewhat less broad band. The methylene proton resonance of (**5**), observed as a sharp singlet from (**5**) alone in DMSO-d_6 at δ 3.6, appears centered at the same chemical shift in the mixed solute as a multiplet (Fig. 2B and C). Addition of some "benzophenone" HCl (**4**) to the mixed solute causes resonances to appear near δ 10.95 and 8.75 for the quaternary and secondary amino protons of (**4**), respectively, (Fig. 2C and D) but not in an exact 1:1 ratio of integrated peaks possibly due to different proton exchange behaviour. Further addition of the benzophenone HCl to the mixed solute does not alter the chemical shifts of the resonances for the amino protons of (**4**), but it does result in further upfield shifting to ~δ 4.5 (with concomitant narrowing) of the resonance for the protonated amino group of (**3**).

Stability of flurazepam HCl in DMSO-d_6

The ^{1}H-NMR spectrum of a degraded specimen of flurazepam HCl (Horner 1979) in DMSO-d_6 solution was recorded initially and again after the solution was allowed to stand at room temperature for 24 h. No spectral change was observed from the original spectrum (Fig. 4A). As a further test of the stability of flurazepam HCl in DMSO solution, the ^{1}H-NMR spectrum of an aged sample of very slightly degraded flurazepam HCl (Roche R-9086) was recorded initially (Fig. 4B), then again after the addition of 10 μl of water (Fig. 4C). Since, after one hour of standing, the treated solution showed no change in its ^{1}H-NMR spectrum, it was heated for 90 h at 50°C.

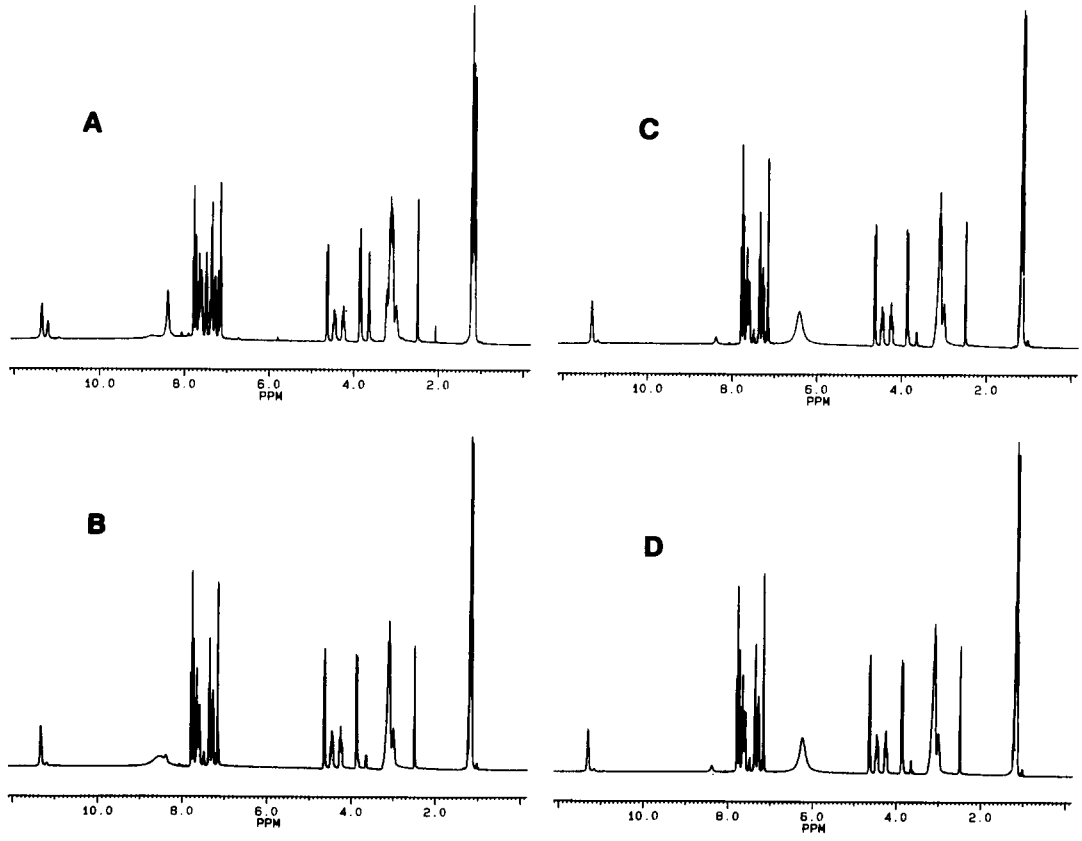


Figure 4
^{1}H-NMR spectra from DMSO-d_6 solutions of (A) degraded flurazepam hydrochloride (Horner, 1979), (B) very slightl degraded flurazepam hydrochloride (Roche R-9086), (C) B 1 h after addition of 10 μl of water, and (D) C after bein heated 90 h at 50°C.

No change was seen in the final ^{1}H-NMR spectrum (Fig. 4D) except for a small upfield shift of the water resonance from ~δ 6.4 to ~δ6.1. These experiments demonstrate that DMSO, because of its high polarity and affinity for water, is able to solvate flurazepam HCl in its intrinsic state and preclude any detectable further hydrolytic degradation of the system.

Assignment of ^{1}H-NMR chemical shifts

Expanded ^{1}H-NMR spectra for the δ 4.8–1.0 and δ 7.9–7.1 regions of appreciably degraded (36%) and slightly degraded (8%) flurazepam HCl, are shown in Fig. 5(A) and (B), respectively. These expansions permit one to more readily identify the contributing resonances of the "benzophenone" degradation product (**4**), i.e. multiplets centred near δ 3.85 and 3.62, and a new methyl triplet centered near δ 1.2. In the aromatic frequency range, multiplets, due to the "benzophenone" can be seen centred near δ 7.48 (2 protons), 7.38, 7.18 and 7.12. Indeed, the window located between δ 7.40 and 7.56 affords a convenient opportunity for assaying (integrating) two aromatic protons of the benzophenone (**4**). The chemical shifts (in DMSO-d_6) for flurazepam hydrochloride and reference standards C and F, are summarized in Table 1; partial ^{1}H-NMR assignments for flurazepam·2HCl (in methanol-d_4 at 60 MHz) as well as full ^{1}H-NMR assignments for flurazepam base (in $CDCl_3$ at 200 MHz) have been reported [11]. The shifts for the salt in DMSO-d_6 (Table 2) agree well with those for the base in $CDCl_3$ [11]. Although close examination of the ^{1}H-NMR spectra allowed for determination of values of the proton chemical shifts, assignments of a number of aromatic resonances were not possible. More sophisticated experiments would be required in order to sort out these assignments.

^{19}F-NMR studies in DMSO-d_6 solution

Whereas ^{1}H-NMR spectra of flurazepam hydrochloride (either in D_2O or DMSO-d_6 solution) are so complex as to have deterred earlier analysis [3], proton decoupled ^{19}F-NMR spectra of this substance should consist

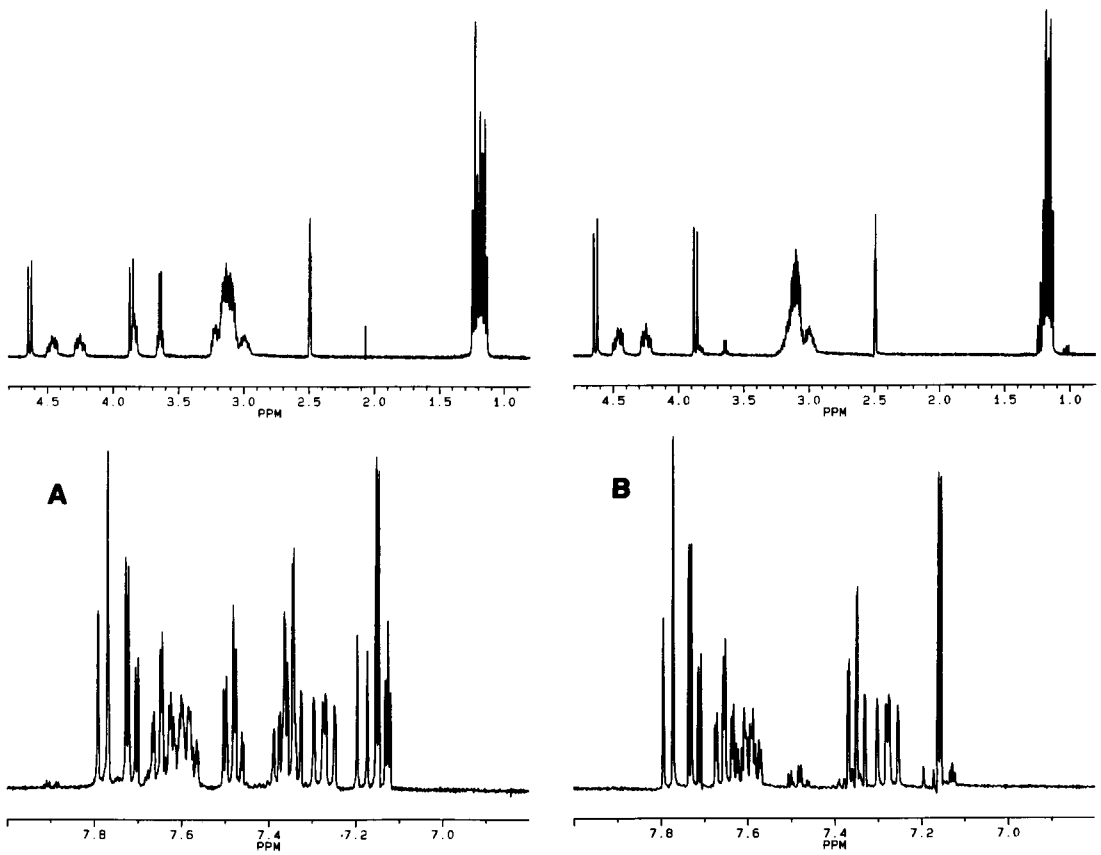
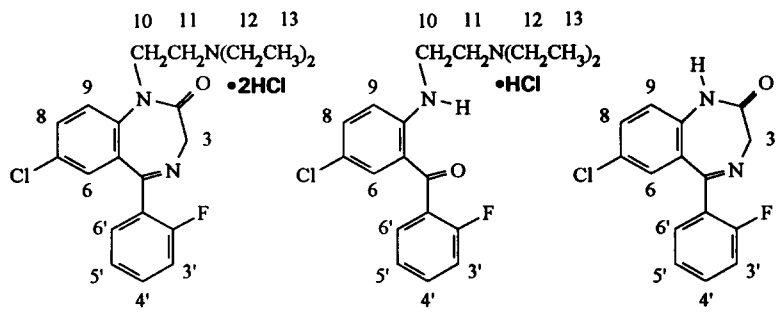


Figure 5
Expanded ^{1}H-NMR spectra for the δ 4.8–1.0 and δ 7.9–7.1 regions of (A) appreciably degraded (Horner, 1979) and (B) very slightly degraded (Roche R-9086) flurazepam hydrochloride in DMSO-d_6 solution.

Table 1
^{1}H-NMR Chemical Shifts (DMSO-d_6) of flurazepam dihydrochloride, and the flurazepam related substance standards C and F



Position	Flurazepam dihydrochloride	Ref. std C	Ref. std F
3	4.63, 3.87	3.80	4.20
6	7.16	7.63*	7.08
8	7.72	7.51*	7.60
9	7.78	7.48*	7.24
10	4.45, 4.25	3.77†	—
11	2.98	3.27†	—
12	3.12	3.20†	—
13	1.18	1.21	—
3′	7.66‡	7.39*	7.56§
4′	7.59‡	7.37*	7.54§
5′	7.35‡	7.15*	7.32§
6′	7.28‡	7.12*	7.24§

*, †, ‡, § — chemical shifts may be interchanged (see text).

Table 2
Comparison of integral count vs peak height in ^{19}F-NMR assay of aged flurazepam hydrochloride samples for the "benzophenone" degradation product (BP)

	Integral Counts					Peak Height			
Flurazepam HCl	Open	Intact	BP	%BP	(*)	Open	Intact	BP	%BP
NF Ref. 0475F	0.23	12.27	1.14	8.4	(9.2)	0.40	15.00	1.82	10.6
Roche R-8434	0.13	11.80	1.06	8.2	(7.2)	2.27	150.0	16.06	9.5
Apotex SX4070	0.73	17.67	3.42	15.7	(16.7)	0.80	15.00	3.96	20.0
Horner 56-2	0.10	10.91	0.75	6.4	(6.6)	1.75	150.0	13.50	8.2
Roche 9086	0.03	11.12	0.91	7.5	(8.4)	0.43	150.0	15.62	9.4
USP H-2	0.06	15.24	0.15	1.0	(3.1)	0.39	150.0	2.07	1.4
Horner 6/79	0.78	16.70	9.17	34.4	(35.5)	0.67	15.0	9.17	36.9
Novo J559	0.05	15.03	0.42	2.7	(4.5)	0.50	150.0	4.45	2.9

* %BP determined by proton integration in the "aromatic window".

of a single singlet for the sole fluorine atom of the molecule. This is indeed the case for the flurazepam related compounds C and F, whose ^{19}F singlet resonances occur at −113 and −112 ppm, respectively. When the well preserved but aged Roche 9086 flurazepam hydrochloride was examined by ^{19}F-NMR in DMSO-d_6 solution, three singlets were observed: the principal one at −111.6 ppm for the unchanged drug (90.3%), a second resonance at −113 ppm for the related "benzophenone" degradation product (**4**) (9.4%), and a trace resonance at −108.5 ppm for the open-ring form (**3**) (0.3%). After refrigeration (4°C) of this tightly capped NMR tube for 10 days, its ^{19}F-NMR spectrum showed increase of the open-ring entity to 16.6% and essentially constant "benzophenone" entity at 9.0% with reduction of the parent drug to 72.6%. In addition, a small quantity (1.7%) of a new species was seen in the form of a singlet at −108.1 ppm, which is most likely due to a minor equilibrium

level of the *cis/trans* rotomer of the open-ring entity (**3**). Similar examination of the aged but more degraded Horner 79 flurazepam hydrochloride material showed initially 2.7% open-ring (**3**), 36.9% "benzophenone" (**4**) and 60.4% ring-closed (intact) drug (**2**). Ten days later, the same solution showed increased level of the open-ring entity (14.1%), no significant change in the "benzophenone" level (36.3%), and corresponding reduction of the parent drug level to 47.9%. A small quantity (1.6%) of the other new entity was also seen here after 10 days standing of the solution under refrigeration. Proof that flurazepam related substance F (the desalkyl compound) could be observed (resolved) from the ^{19}F-NMR resonance of flurazepam hydrochloride itself (−111.6) was obtained by spiking the solution with some "F" material whose ^{19}F-NMR resonance was seen clearly resolved at −111.7 ppm. An example of this monitoring is shown in Fig. 6, as discussed below. These studies indicate that some open-ring entity (**3**) is present in aged samples of flurazepam hydrochloride, and that on standing in DMSO-d_6 solution it slowly rises in concentration until equilibrium is obtained with the ring-intact (parent) substance. Various initial levels of the open-ring entity are discussed below in the context of ^{19}F-NMR assay of the aged flurazepam hydrochloride samples for levels of the "benzophenone" (**4**) entity. It should also be noted from these studies that the concentration of "benzophenone" does not change appreciably as the DMSO-d_6 solutions are allowed to stand for days, i.e. the level detected represents the level of (**4**) in the aged samples at the time of dissolution.

^{13}C- and ^{19}F-NMR studies in D_2O solution

When flurazepam hydrochloride was examined by ^{13}C-NMR spectroscopy at 0°C in D_2O solution (following its dissolution also at 0°C to form a solution at hydrolytic pH), a spectrum showing 21 resonances, of the possible 26–28 resonances (depending on the number of resolvable ^{13}C-^{19}F couplings) for the flurazepam molecule, was obtained. Perhaps due to better resolution than achievable by Kuwayama *et al.* [3] (who, incidentally reported only 19 ^{13}C-NMR resonances), this spectrum showed two closely spaced resonances (δ 14.60 and 14.70) for the ethyl methyl carbon and five mid–high resonances (δ 49.84, 54.37, 54.47, 55.20, and 58.13) in place of the single, and four corresponding resonances reported by Kuwayama *et al.* [3] for flurazepam hydrochloride in 0.5 N DCl at 0°C. At 0°C, the lower field region shows three resonances (δ 165.89, 175.12, and 178.72) free of any of the open-ring "benzophenone" entity which gives a carbonyl resonance at δ 200.70. When, however, the solution is heated *in situ* to 27°C, the low field region consists of only two resonances, δ 175.53 (ring-intact) and 200.70 (ring-open). At the same time, in place of 2 ethyl methyl resonances, there are now five, and in place of the five mid–high resonances of 0°C there are now seven resonances at 27°C. Because of the complexity of the ^{13}C-NMR spectra with changing temperature as well as the inherent low sensitivity of ^{13}C nuclei, studies of the behavior of flurazepam hydrochloride in D_2O were continued instead using ^{19}F-NMR spectroscopy by which a 10^4 gain in sensitivity was realizable.

When an aged, moderately decomposed sample of flurazepam hydrochloride (Roche) was dissolved in D_2O at 0°C and examined 3 min later while maintained at 0°c, the ^{19}F-NMR spectrum showed three resonances: a major peak (91.0%) at δ −112.47 for the intact drug, a minor peak (7.7%) at δ −116.13 for the "benzophenone" degradation product and a much smaller peak (1.6%) at δ −111.97 for the open-ring entity. As the temperature of this solution was maintained at 0°C and its solvolytic behaviour examined over a 5-h period, the "benzophenone" content did not increase from the initial dissolution level, but the level of the open-ring entity increased to 24.0% as it approached equilibrium conditions after 5 h while the intact drug content decreased to 68.9%.

When a fresh solution of the same aged and moderately degraded Roche flurazepam hydrochloride was prepared in D_2O and examined over a 24-h period while the solution was maintained at 27°C, similar but more dramatic observations were made. No increase beyond the initial level (7.3%) of the "benzophenone" degradation product was observed to occur over 24 h indicating that its level had been achieved earlier by years of slow formation *in situ* in the solid state. An initial level of open-ring entity (5.7%) at 5 min was observed to increase to an equilibrium level of 56.2% over 24 h at the expense of the intact drug which declined from an initial 87.0 to 35.8%. After 15 min at 27°C, the solution

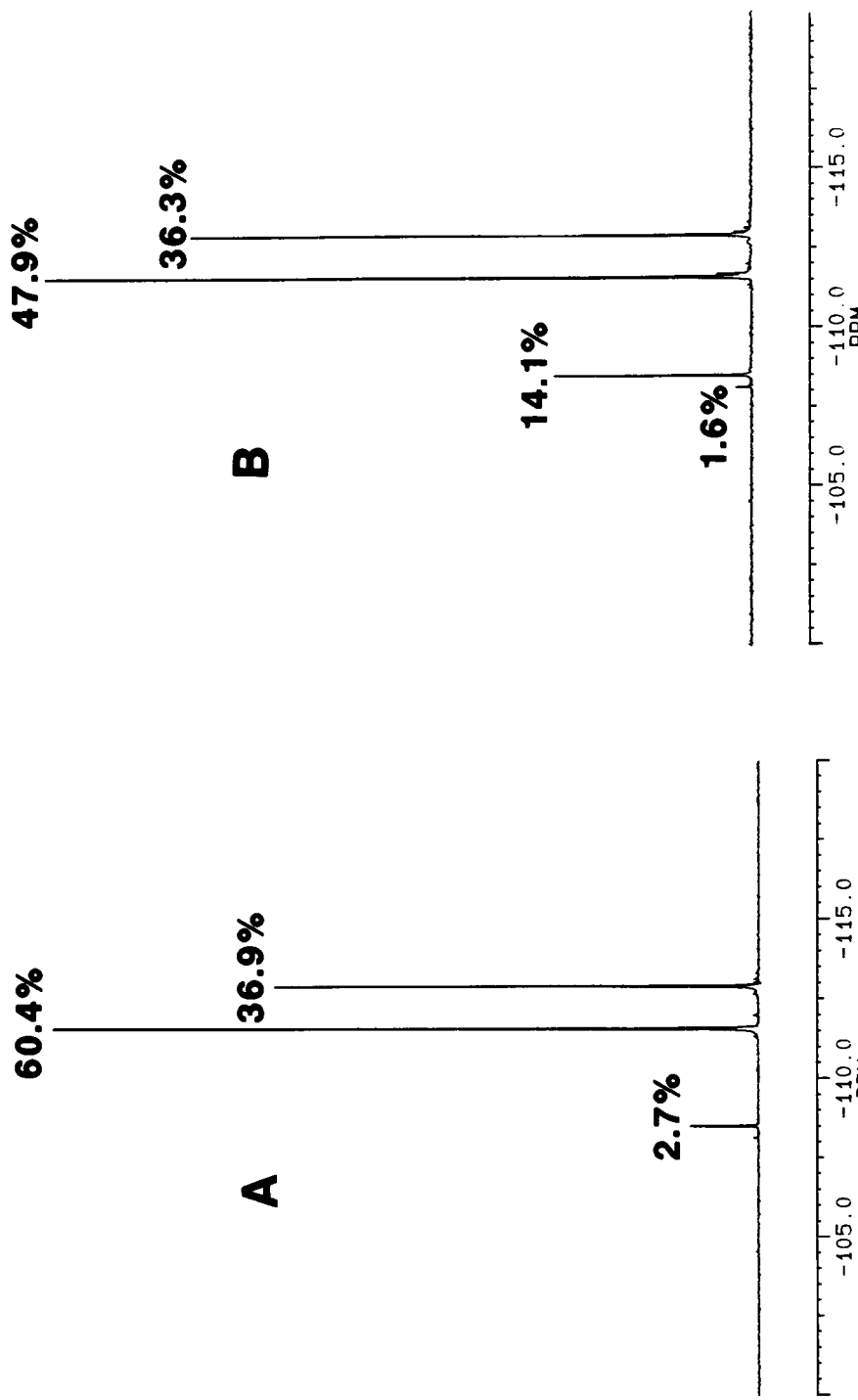


Figure 6
^{19}F-NMR spectra from a DMSO-d_6 solution of aged, degraded (Horner, 1979) flurazepam hydrochloride (A) upon initial dissolution, and (B) after having been stored under refrigeration for 10 days. The indicated percentages of entities were determined by measurement of peak heights of the ^{19}F-NMR resonances.

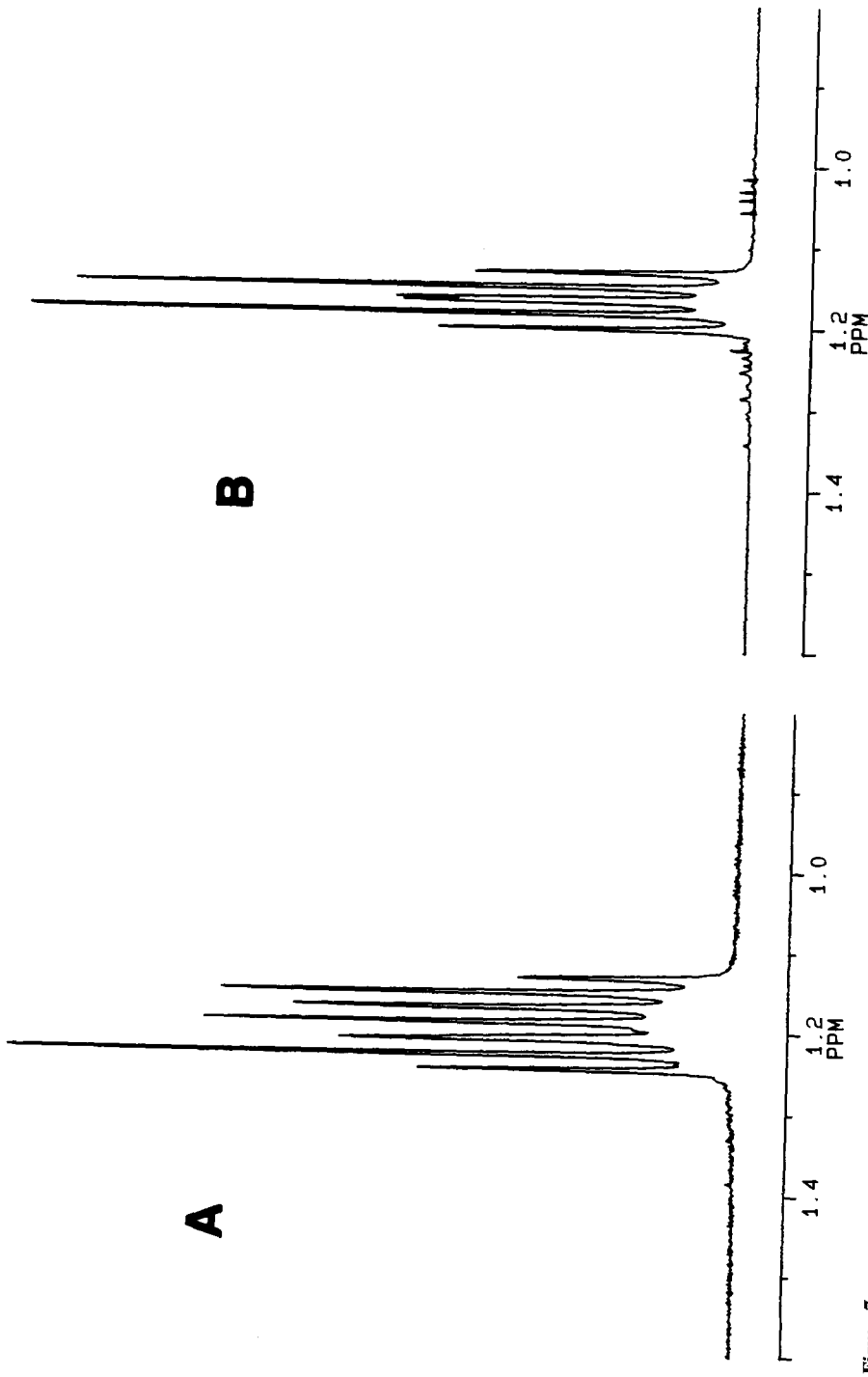


Figure 7
Expanded ^{1}H-NMR spectra of degraded (Horner 1979) (A) and reference (USP, H-2) (B) flurazepam hydrochloride in DMSO-d_6 solution showing the methyl proton resonances of the ethylamino moieties from both the "benzophenone" and parent substance in the degraded sample.

began to reveal a minor open-chain entity (probably the other amide rotamer) (0.5%) whose level at no time exceeded 1.7%. It is clear from these studies that dissolution of flurazepam hydrochloride in D_2O at 0 or 27°C does not contribute to solvolytic degradation of the molecule to the "benzophenone" entity, a conclusion consistent with the observation of Kawayama *et al.* [3] that no end hydrolysates (**4** and **5**) were formed upon dissolving **1** in 0.5 N DCl.

Bases for ^1H- and ^{19}F-NMR assay of the "benzophenone" degradation product in DMSO-d_6 solution

Owing to the complexity of nitrogen proton exchange in the mixed (degraded) system and the possibility of some minor further decomposition products as indicated by the trace resonance at δ 10.9 and the shoulder at the base on the high-field side of the glycine amino resonance near δ 8.3 (Fig. 4A), the aromatic proton multiplet centered near δ 7.48, arising from two aromatic protons of the benzophenone (**4**), and appearing in a window for the aromatic protons of (**2**) can be used as a more reliable basis (than the protonated amino resonances) for estimating the degree of degradation of (**2**) to (**4**). Alternatively, the methyl triplets (Fig. 7) can be used for ratio determination. The expanded spectra for the methyl proton resonances of the ethylamino moieties from both the benzophenone and parent substance (Fig. 7) show two methyl triplets of essentially equal intensity centered at δ 1.70 and 1.85 which can be attributed to the diethylamino group of the intact flurazepam hydrochloride because of the steric hindrance its ethyl groups would experience (and thus be magnetically non-equivalent) possibly from intramolecular hydrogen bonding in (**2**) (and perhaps (**3**)) but not in (**4**). The other component of this methyl proton cluster is seen to consist of virtually a single methyl triplet centred at δ 1.21 and having approximately twice the intensity of either of the other methyl triplets. The triplet centered at δ 1.21, therefore, would be consistent with the diethylamino group of the benzophenone (**4**) whose opened ring structure would allow for less hindered rotation of the N-ethyl substituents (and apparent magnetic equivalence). The incomplete separation of the methyl triplet signals, however, makes accurate quantitation difficult; thus, the aromatic window was used for ^1H-NMR quantitation. On these bases, the benzophenone (**4**) content of the aged samples (Table 2) was found to vary from ~3% to 36%. The data of Table 2 provide the "aromatic window" assay values for comparison with those from the ^{19}F analyses discussed below.

Owing to the relative simplicity of the ^{19}F-NMR spectra compared to ^1H-NMR, and the much greater intrinsic sensitivity of the ^{19}F nucleus compared to ^{13}C-NMR spectroscopy, the state of degradation of the various aged flurazepam hydrochloride samples is more easily and more accurately determined by integration (or measurement of peak heights, Fig. 6) of the ^{19}F-NMR resonances. These data are shown in Table 2 compared with those obtained from the ^1H-NMR integrals. In addition to accurately determining the level of the "benzophenone" degradation product in the aged samples, ^{19}F-NMR spectrosocpy permits the initial level of the open-ring entity (**3**) to be determined. The percentage "benzophenone" degradation, therefore, whether determined from integrals or peak heights, requires that the "benzophenone" (BP) level be divided by the sum of the levels (integral counts or peak heights) of the open-ring, the intact ring (parent compound) and the BP entities by the following formula

$$\% \text{ BP} = \left(\frac{\text{BP}}{\text{open} + \text{intact} + \text{BP}}\right) \times 100.$$

Conclusions

^{19}F-NMR spectroscopy (with ^1H decoupling), for those who have access to it, affords the easiest and best means of assaying flurazepam dihydrochloride samples for the "benzophenone" degradation product (i.e. the USP flurazepam related compound C). Alternatively, ^1H-NMR spectroscopy can be used for such assay with comparable results providing care is taken in analysing the complex resonance patterns.

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