Nmr Spectral Characteristics of N-H Protons in Purine Derivatives

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Received January 15, 1973

Proton chemical shifts are examined in a series of twelve purine and three 5-azapurine derivatives. The assignment of N-H signals in the xanthine and thioxanthine series, as well as the effects of moisture, concentration and temperature on the N-H and C-H nmr spectral characteristics are discussed.

An earlier investigation related to the synthesis and properties of 5-azapurines (1), revealed a problem in locating one N-H nmr absorption signal in certain purine derivatives. Since no systematic survey of N-H absorptions in purines was available, a study was undertaken to catalog these nmr signals in xanthine derivatives while establishing the cause of the missing N-H absorptions.

EXPERIMENTAL

Nmr spectra were measured with a Varian HA-100D spectrometer equipped with a V6040 variable temperature controller. Tetramethylsilane was used as the internal reference. Chemical shifts of C-H are accurate to ±0.01 ppm. The estimated centers of N-H absorptions are less precise because of the broad nature of the peaks.

Dimethyl sulphoxide-d_6 (DMSO-d_6) was used as solvent and was dried by passage through a column of activated molecular sieves (type 4A, 4-8 mesh) under a nitrogen atmosphere. By this method the water content was reduced from *ca.* 0.3% to 0.04%. All samples were prepared under nitrogen in a glove bag.

The compounds were normally studied as 1% solutions. When compound solubility did not permit this, saturated solutions were employed.

The preparation of the azapurines (XIII-XV) has been described previously (1). Compounds IX and X were prepared by Dr. R. K. Robins (2). The other purine derivatives were commercially available compounds which were examined by tlc and found homogeneous enough to be used without further purification.

The moisture content of sample solutions was determined by adding a known quantity of water (1 μl/0.8 ml. DMSO-d_6) to the sample and calculating the concentration from the integral.

Results and Discussion

Spectral Assignments.

The spectra of the purine derivatives studied here (Figure 1) consisted of one or two sharp absorption signals for the aromatic protons between δ 7.5 and 9.2 (Table I),

TABLE I

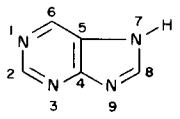
Proton Chemical Shifts of Purine Derivatives

	Chemical Shift (δ)					
Compound	C_2H	C_6H	C_8H	N_1H	N_3H	N_7H
I	8.90	9.12	8.59			13.4
II	8.07		7.94	12.2		13.3
III	8.35		8.15	13.5		13.5
IV		8.48	8.37		13.3	13.3
V			7.89	10.8	11.5	13.2
VI			8.10	13.3	13.3	13.3
VII			8.05	12.2	13.3	13.3
VIII			8.00	12.0	12.0	13.3

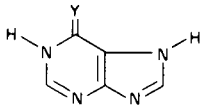
consistant with literature data (3-5,9). The methyl signals, whenever present, (Table II) appeared between δ 3.30-3.82, also consistant with literature values (9). The N-H absorptions (Tables I and II) appeared as one to three broadened signals at low field, usually between δ 10.7-14.2. The data on the few compounds previously studied (3,8) fall into this range. The signals from some of the derivatives containing two N-H protons appeared as a singlet. These protons were characterized by the proper integral intensities and by deuterium oxide exchange experiments.

Due to the ambiguity in assigning the N-H signals in hypoxanthine (II) and other compounds where more than one deuterium oxide exchangeable proton was present, some *N*-methylxanthine derivatives were examined. In 3,7-dimethylxanthine (XI), the N_1H appeared at δ 11.05 as a slightly broadened peak with a half-height width ($\Delta\nu$) of 8 Hz. This signal was independent of the moisture content of the solvent. In 1,3-dimethylxanthine (XII),

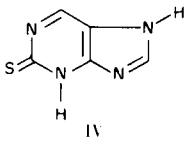
Figure 1



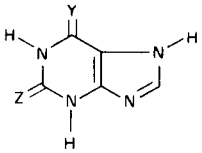
I



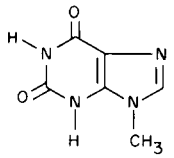
II, Y = O
III, Y = S



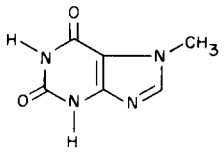
IV



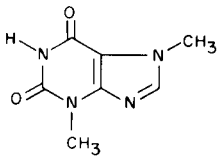
V, Y = O, Z = O
VI, Y = S, Z = S
VII, Y = O, Z = S
VIII, Y = S, Z = O



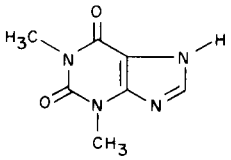
IX



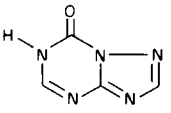
X



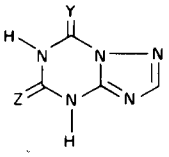
XI



XII



XIII



XIV, Y = O, Z = S
XV, Y = S, Z = O

the N_7H of the 5-membered ring gave a signal at 13.5 with $\Delta\nu$ 16 Hz in very dry solvent only. Two N-H signals at δ 10.8 ($\Delta\nu$ = 4 Hz) and 11.4 ($\Delta\nu$ = 6 Hz) were observed for 7-methylxanthine (X) in molecular sieve dried solvent, while only one signal spread over 100 Hz was observed in undried DMSO-d_6. The N-H signals for the 9-methyl derivative (XI) appeared at δ 10.7 ($\Delta\nu$ = 8 Hz) and δ 11.9, the lower field signal being very broad. The higher field signal was assigned to the N_1H absorption and the lower field signal to the N_3H for the following reasons. The N-H of the pyrimidine ring in xanthine and its derivatives are situated in the positive shielding region of the neighboring carbonyl groups (11). The N_1H is influenced by two carbonyl groups adjacent to it, whereas the N_3H is affected by one. The situation is similar to that observed for the methyl absorption frequencies in methylxanthines (9). The N_3H is one bond closer to the imidazole ring than the N_1H, and is more exposed to the deshielding ring current of the 5-member ring.

TABLE II

Proton Chemical Shifts of Methylxanthine Derivatives

Compound	Chemical Shift (δ)							
	C_8H	N_1H	N_3H	N_7H	Me_1	Me_3	Me_7	Me_9
IX	7.57	10.7	11.9					3.60
X	7.85	10.8	11.4				3.82	
XI	7.94	11.05				3.33	3.84	
XII	7.98			13.5	3.24	3.44		

TABLE III

Proton Chemical Shifts of 5-Azapurine Derivatives

Compound	Chemical Shift (δ) C_2H	C_8H	N_1H	N_3H
XIII	8.36	8.36	13.2	
XIV		8.13	13.0	14.2 (a)
XV		8.10	13.1	13.1

(a) Could not be observed at 100 MHz. Measured at 220 MHz.

When the inductive effects of substituents are taken into consideration, the previous assignments get further support. Since a C=S group is more deshielding than a C=O group (10,12), any N-H adjacent to a C=S rather than a C=O group would be shifted towards lower field. This was observed in the following cases. The N_1H appeared at δ 10.8 and δ 13.3 in xanthine (V) and 2,6-dithioxanthine (VI), respectively. In 2-thioxanthine (VII) and 6-thioxanthine (VIII) where the N_1 is flanked by one C=O and one C=S group, the N_1H signal appeared at an intermediate position of δ 12 in both compounds. The N_3H, observed at δ 11.5 in xanthine, was shifted to δ 12.0 in 6-thioxanthine and to δ 13.3 in 2-thioxanthine and 2,6-dithioxanthine (VI). This approximately 1 ppm downfield shift caused by replacing a C=O with a C=S group was also observed between hypoxanthine (II) and 6-mercaptopurine (III). Therefore, when three distinct N-H signals were observed for xanthine, the highest field signal at δ 10.8 ($\Delta\nu$ = 4 Hz) was assigned to the N_1H, the lowest signal at δ 13.2 ($\Delta\nu$ = 20 Hz) was assigned to the N_7H of the imidazole ring and the intermediate signal at δ 11.5 ($\Delta\nu$ = 8 Hz) to the N_3H.

Effect of Moisture.

The water content of the nmr solvent affects the line width of N-H signals greatly. As reported previously in the 5-azapurine series (1), when samples were prepared in ordinary DMSO-d_6 (not chromatographed, but stored over molecular sieves in a desiccator), one of the N-H signals often was not observed. Only in carefully dried solvent were all of the N-H signals seen, either as separated peaks, or as one peak which integrated for the correct number of protons. In order to confirm the extent that the water content of the solvent did affect the non-observation of the N-H signals, a controlled amount of water was added to a dried solvent sample. As a general rule, when the moisture content of the DMSO-d_6 sample solution reached 0.15%, one of the N-H signals could not be detected. However, xanthine (V) and its methylated derivatives form a special case. The two N-H signals of 7-methylxanthine (X) were not affected by the presence of moisture up to 0.2%. In unsubstituted xanthine, the moisture required to collapse the N_7H signal was about 0.6%. Although the N_3H started to show broadening when the moisture content of the sample reached 0.3%, a broad signal ($\Delta\nu$ = 38 Hz) was still observable at 3.6%. The N_1H signal was unaffected by a water content up to 3.6%. It is, therefore, quite evident that proton exchange between the sample molecule and traces of water in the solvent is a major cause for non-observation of N-H signals in the purines studied. These observations also provide evidence that the N_7H of the imidazole portion of the purine molecule is the proton most susceptible to exchange, and that N_1H is the least labile.

In a number of the compounds studied, the N_3 and N_7 protons appeared together as one broad peak in an averaged position (Tables I and II). In hypoxanthine (II), the separate peaks at δ 12.2 (N_1H) and δ 13.3 (N_7H) were observed on one occasion as one peak centered at δ 12.6.

Concentration Effects.

The concentration dependence of the chemical shifts was studied in two purine compounds and was found to be small. For both purine (I) and 6-mercaptopurine (III), a maximum 10 Hz upfield C-H shift was observed over a 12% to 1% solution concentration range. Practically no shift was detected for the N-H signals. The small shift observed for the C-H absorption is in good agreement with the observations of Montgomery *et al.* (3) for purine. The concentration independence of the N-H absorption, however, is contrary to the sizable shift they observed. No obvious reason for this difference is apparent.

Temperature Effects.

An investigation of the temperature dependence of the line shape provided evidence that intermediate exchange rates (7) have a predominant effect on N-H line broadening. Line widths increased with increasing temperature and the peaks collapsed at 100° in both 6-mercaptopurine (III) and purine (I). This is contrary to the phenomenon Roberts (7) reported for pyrrole in the neat liquid state. The proton tautomerism possible between the N_7 and N_9 positions of the imidazole ring could be responsible for the prominent broadening of the N_7H signal although this tautomerism was not considered important in a study of medium effects on the position of C_8H absorption (5).

While increased temperature caused a line broadening of the N-H signal in 6-mercaptopurine (III), a sharpening of the δ 8.35 aromatic C-H signal was noticed at the same time. This observation provided the information for assigning the two C-H signals. Although it has been established (3) that the shielding order in purine (I) is $H_8 > H_2 > H_6$, there is no reason to assume that this order

must be followed after conversion of the 6-position to a carbonyl or thiocarbonyl group. The two C-H signals of 6-mercaptopurine at concentrations lower than 3% were of the same intensity but showed differences in line width and height. The signal at δ 8.35 was broader than the one at δ 8.15. The broadening of the signal could be a result of coupling with the neighboring *N*-protons. As discussed previously, the N_1H is less labile than the N_7H. Therefore, the coupling between the N_1H and C_2H is more likely to be observed than the N_7H-C_8H coupling which should be averaged out due to the high exchange rates of the N_7H. At elevated temperature, the exchange rates of the N_1H should be increased and cause a decrease in line width of the C_2H signal. This was observed at temperatures above 70°. Accordingly, the δ 8.35 signal was assigned to be the C_2H and the δ 8.15 to the C_8H. The same line broadening due to weak coupling was also observed in hypoxanthine (II).

An examination of the C_8H chemical shifts in the purine and xanthine derivatives shown in Table I reveals the following: The C_8H of purine is the least shielded of all the C_8H studied, regardless of whether strong electron-withdrawing groups (C=O or C=S) were present. In addition, the C_8H in xanthine (V) (two C=O) is more shielded than the corresponding proton in hypoxanthine (II) (one C=O), contrary to what might be expected from inductive effects. However, this observation might be explained when the deshielding contribution of the ring current is taken into consideration. Insertion of a carbonyl (or thiocarbonyl) group into the 2- or 6-position reduces the aromaticity of the pyrimidine ring which causes a decrease in ring current that, in turn, has a shielding effect on the neighboring imidazole protons. Shielding effects of this type have been observed in the pyrimidine series (12). We must conclude that the ring current is of major importance in determining chemical shifts in these heterocyclic systems, and that substituent electronic effects play an important role when ring currents are comparable. This is seen in a comparison of the chemical shifts of the C_8H in xanthine (V) and 2-thioxanthine (VII). Replacing a C=O by a C=S group causes a deshielding, in agreement with the work of Lichtenberg *et al.* (10).

Protons in the 5-azapurine series are deshielded relative to their purine counterparts (Table III). Whether this is due to differences in ring currents, inductive effects or an anisotropic effect of the additional nitrogen atom is presently not certain.

Acknowledgement.

The authors would like to thank Dr. R. K. Robins, ICN Corporation, for making available samples of 7- and 9-methylxanthine, and Dr. Robert B. Bradley, NIAMD, NIH, for determining the 220 MHz spectrum of compound XIV.

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