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Dissociation, solubility and lipophilicity of azathioprine

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Summary

The thermodynamic pK_a values of 7.87 and 7.99 were determined for azathioprine (I) at 25°C by spectrophotometric and solubility methods, respectively. The partition coefficient of undissociated I between 1-octanol and 0.01 N acetic acid (pH = 3.6) at 25°C was 1.25, and the partition ratio of I between 0.04 M phosphate buffer (pH = 7.4) and 1-octanol at 37°C was 1.04.

The solubility of I in water was 0.130 mg/ml and the intrinsic solubility at pH 4.08 in 0.02 N acetate buffer was 0.124 mg/ml at 25°C.

Intramolecular hydrogen bonding between the N-7 hydrogen and C-6 sulfur atoms in 6-mercaptopurine (II) accounts for the approximately 1000 times greater K_a of the tautomeric proton at positions 7 and 9 in I compared to the N-7 proton in II.

Introduction

Azathioprine (I) is a mercaptopurine (II) derivative that suppresses T-lymphocyte-borne delayed immune responses and is used primarily to prevent rejection of kidney transplants (Elion and Hitchings, 1975).

The poor distribution of I to lipid tissues in mice (Elion and Hitchings, 1975) and the clinical use of sterile lyophilized I sodium diluted in intravenous fluids (Johnson and Porter, 1981) prompted the determination of the pK_a, partition coefficient and aqueous solubility of I.

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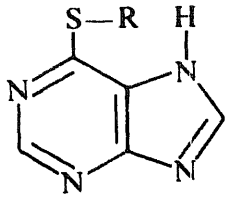
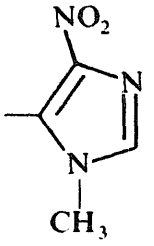


I, R =  II, R = H

Materials and methods

The I specimen was dried at 105°C for 5 h, II monohydrate was used as received, and deionized water was obtained with a resisitivity of $> 10^7$ Ωcm. Other chemicals were analytical reagent grade.

Partion coefficient determination

The partition coefficients of undissociated I and II were determined at 25°C by equilibrating quadruplicate samples of approximately 3.7×10^{-5} M between mutually presaturated 10-ml volumes of 0.01 N acetic acid (pH 3.58) and 1-octanol. After 15 min at 45 rpm in a rotating bottle apparatus, the aqueous phases were allowed to separate and assayed with a Beckman DB-GT spectrophotometer for I or II. The partition ratio of I was similarly determined using 0.04 M phosphate buffer (pH 7.40) at 37°C.

Aqueous solubility of I and II

Excess I and II were equilibrated with water in triplicate samples for 120 h at 25°C. The samples were filtered through 0.45 μm cellulose ester membrane (MF-Millipore) and the solubilities were determined gravimetrically for I and spectrophotometrically for II.

pK_a determination of I

The pK_a of I was determined by solubility and spectrophotometric methods. Triplicate samples of I in masses that were incompletely soluble were equilibrated at 25°C for 48 h with 10 ml of 0.02 N buffer at pH 4.00 and with 10 ml of 0.025 M tromethamine buffers at six pH values from 7.00 to 8.60. The pH values of samples at 25°C were recorded, then the filtrates were diluted and I concentrations were determined from absorbances at 280 nm by comparison to known I solutions at the same pH values.

Seven 2.89×10^{-5} M I solutions in 0.025 M tromethamine buffers were prepared

over the range of pH 7.30–8.40 at 25°C. Absorbance values of samples were determined at 226 nm, the wavelength of maximum separation between spectra of the undissociated and anionic species.

Results

Partitioning studies

The partition coefficients of undissociated I and II at pH 4.08 were 1.25 ± 0.01 and 0.72 ± 0.01, respectively. The partition ratio of I at pH 7.4 was 1.04 ± 0.07, uncorrected for dissociation.

Solubility and pK_a values

The water solubilities of I and II were 0.130 and 0.124 mg/ml, respectively. The intrinsic solubility, S_0, of undissociated (>99.9%) I was 0.124 mg/ml at pH 4.08. The pK'_a of I, 7.94 ± 0.04, is shown as the ordinate intercept in Fig. 1 that was plotted according to Eqn. I after Krebs and Speakman (1945).

$$pH = pK'_a + \log[(S/S_0) - 1] \quad (1)$$

where pK'_a is the value uncorrected for ionic strength, S is the I solubility in the tromethamine buffers, and S_0 is the intrinsic solubility of I. A thermodynamic pK_a value of 7.99 was calculated for zero ionic strength (Albert and Serjeant, 1971). The pK_a value of I by spectrophotometry was 7.87 ± 0.04 calculated according to the method of Albert and Serjeant (1971).

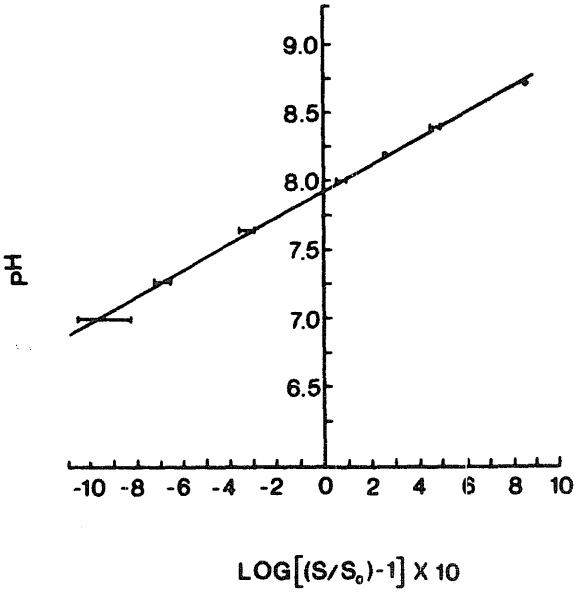


Fig. 1. Plot of pH versus solubility according to Eqn. 1 for triplicate samples of I at 25°C.

Discussion

The substituent constant, π, of the 1-methyl-4-nitroimidazole group on I was calculated as 0.24 from Eqn. 2 (Leo et al., 1971)

$$\pi = \log P_x - \log P_H \quad (2)$$

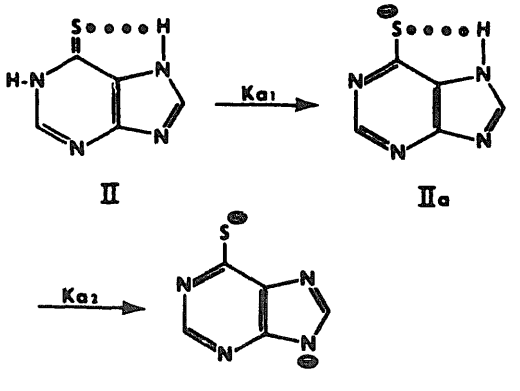
where P_x and P_H are the partition coefficients of I and II, respectively. There is only a 1.7-fold increase in the lipophilicity of I over II. The ratio of II/I water solubilities is 1.56; which is 4.3% less than the ratio of I/II formula weights and corroborates the weakly lipophilic or hydrophobic nature of the 1-methyl-4-nitroimidazole group on I. Based on these data, sorption of I by plastic containers and tubing would not be expected.

There was a 0.12 unit difference in the I pK_a by the solubility and spectrophotometric methods. However, the accuracy of the latter method was limited by only 0.24 absorbance units difference between the anionic and undissociated I species. The pK_a of about 8.0 for I can be used in Eqn. 2 to predict the solubility, S, of I at various pH values, such as in intravenous admixture solutions

$$S = S_0[1 + 10^{(pH - pK_a)}] \quad (3)$$

The sodium salt of I would probably react with the salts or conjugate acids of bases with $pK_a \geq 8$ in aqueous mixtures to cause precipitation of I and/or the free-base, depending on the solution pH and ionic strength.

The pK_{a1} value of II was determined to be 7.77 (Albert and Brown, 1954) and 7.7 (Fox et al., 1958) for dissociation of the 6-SH group, which is slightly more acidic than I. A proton magnetic resonance spectrum of I showed that the acidic proton exists in tautomeric equilibrium between N-7 and N-9 atoms in the purine ring. The pK_{a2} for the N-7 proton in II was reported as 10.84 (Albert and Brown, 1954) and 11.17 (Fox et al., 1958), an average of 965 times less acidic than I. The decreased acidity of the N-7 proton in II is attributed to its stabilization by an intramolecular hydrogen bond with the 6-S^- group illustrated in Scheme 1 where IIa and IIb are



Scheme 1

the mono- and dianions, respectively. Hydrogen bonding between the N-7 hydrogen and 6-thioxo sulfur atoms in crystalline II monohydrate was shown by Sletten et al. (1969).

Acknowledgments

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References

Albert, A. and Brown, D.J., Purine studies. Part 1. Stability to acid and alkali. Solubility. Ionization. Comparison with pteridines. J. Chem. Soc., (1954) 2060–2071.

Albert, A. and Serjeant, E.P., The Determination of Ionization Constants, 2nd edn., Chapman and Hall, London, 1971, pp. 28, 29, 44, 45.

Elion, G.B. and Hitchings, G.H., In Sartorelli, A.C. and Johns, D.G. (Eds.), Handbook of Experimental Pharmacology, Vol. XXXVIII, Part 2, Springer-Verlag, New York, 1975, pp. 404–425.

Fox, J.J., Wempem, I., Hampton, A. and Doerr, I.L., Thiation of nucleosides. I. Synthesis of 2-amino-6-mercapto-9-β-D-ribofuranosylpurine ('Thioguanosine') and related purine nucleosides. J. Am. Chem. Soc., 80 (1958) 1669–1675.

Johnson, C.A. and Porter, W.A., Compatibility of azathioprine sodium with intravenous fluids. Am. J. Hosp. Pharm., 38 (1981) 871–875.

Krebs, H.A. and Speakman, J.C., Dissociation constant, solubility, and the pH value of the solvent. J. Chem. Soc., (1945) 593–595.

Leo, A., Hansch, C. and Elkins, D., Partition coefficients and their uses. Chem. Rev., 71 (1971) 525–616.

Sletten, E., Sletten, J. and Jensen, L., Crystal and molecular structure of 6-mercaptopurine monohydrate. Acta Crystallogr., Sect. B, 25 (1969) 1330–1338.