Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No 11, pp. 1357-1360, 1994

0960-894X/94 $7.00+0 00

0960-894X(94)00159-6

THE STRUCTURE OF METHIMAZOLE AND ITS CONSEQUENCES FOR CURRENT THERAPEUTIC MODELS OF GRAVES' DISEASE.

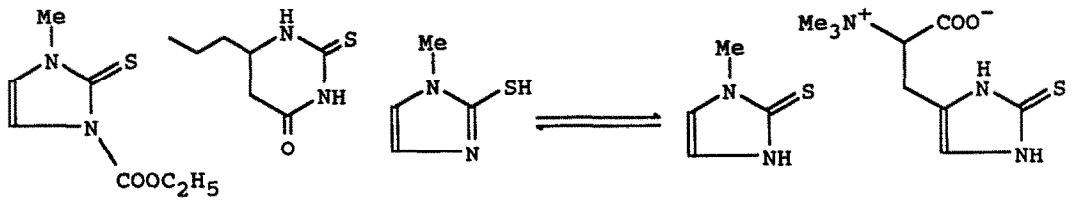
Mark Garner[#], David R. Armstrong[#], John Reglinski[#*], W. Ewen. Smith[#],
Rhoda Wilson[¶] and James H. McKillop[¶]

[#]*Department of Pure & Applied Chemistry*
Strathclyde University, Glasgow G1 1XL, U.K.
[¶]*Department of Medicine*
Glasgow Royal Infirmary, Glasgow G4 0SF

Abstract
NMR spectroscopy indicates that the structure of methimazole is best represented as a thione rather than as a thiol. This structure is predicted by molecular orbital calculations to be the more stable form both in aqueous solution and in lipid. As a consequence, the use of Carbimazole and Propylthiouracil in the threatment of Graves' disease may rely on the hitherto unconsidered chemistry of thiones rather than thiols.

Introduction

Carbimazole (I) and propylthiouracil (II) are thiones widley used in therapy for Graves' disease. In-vitro, many studies make use of the generic compound 2-mercaptomethylimidazole (methimazole, IIIa) as a model for the action of these two species (I, II) [1-3]. With little evidence however, methimazoles' action is believed to centre on the thiol form (IIIa) of the compound rather than the thione tautomer (IIIb) [1-3]. During recent clinical studies using NMR spectroscopy on the effect of carbimazole on the erythrocyte [4], we observed a major change in oxidation-reduction balance of cellular glutathione. However, we also observed a change in ergothioneine (IV). This is also a thione, but one which is present naturally in the cytosol of the erythrocyte. An analogous clinical study on penicillamine in rheumatoid arthritis revealed a similar change in glutathione but no effect on erythrocyte ergothioneine [5]. Although, there are major differences between these two disease states, if both therapies centre on thiolate chemistry, they might be expected to operate through similar mechanisms.



(I) Carbimazole (II) Propylthiouracil (IIIa) Methimazole (IIIb) (IV) Ergothioneine

In the study of Graves' patients [4] treated with Carbimazole, we did not find any behaviour indicative of an active thiolate when erythrocytes were challenged with methimazole. Closer inspection of the current accepted philosophy indicated that there is little chemical evidence to support the hypothesis that methimazole adopts the thiol structure (IIIa) in preference to the thione form (IIIb) under physiological conditions. This dilemma is compounded by the report that in aprotic solvents such as chloroform, methimazole is found to adopt the thione form (IIIb) [7]. This has led us to look more closely at the solution phase structures of methimazole at physiological pH using high field NMR spectroscopy coupled with molecular orbital calculations.

NMR Spectroscopy:

The solution structure of methimazole was identified using a combination of ^{1}H, ^{13}C and ^{15}N NMR spectroscopy [8]. Both the proton spectrum (δ3.63ppm: singlet 3H; Me-, δ7.04ppm: multiplet 2H; -CH) and the carbon spectrum (δ35.7ppm Me-, δ116.8ppm CH, δ122.3ppm CH, 158.1ppm C=S) can be assigned in an ambiguous manner. Of major significance is the resonance found at 158.1ppm which could be interpreted as either the thione (>C=S) or thiol. By comparison with mercaptobenzoic acid (C-S^{-}, 140ppm) and thiourea (>C=S, 185ppm) it could be suggested that the thione tautomer is more likely. Of major importance are the ^{15}N spectra. In DMSO we observe two resonances (NH, δ163.6ppm; J=97.7Hz: Me-N, δ158.6ppm; J=2.0Hz) which are in a region of the spectrum characteristic with tertiary and quaternary aliphatic amines. The strong coupling observed (98Hz) for the N-H resonance in DMSO is indicative of a one bond N-H coupling, i.e. the thione tautomer. Thus, consistent with the reported spectrum of Balestrero et al. [7] methimazole adopts the thione structure. In a physiological buffer, although the larger high frequency coupling constant is removed due to exchange or deprotonation to the anionic form, the resonances shift only slightly to δ165.2ppm (NH) and δ162.3ppm (Me-N) indicating that there is no solvent effect which tautomerises the structure.

Reactivity

NMR spectroscopy clearly demonstrates that the predominant form adopted by methimazole in aqueous solution is the thionate tautomer (IIIb). With the large dynamic range of ^{13}C NMR any contribution from the thiolate form would be detected as an additional set of resonances at a concentration down to 0.1%. Should the thiol (IIIa) be present at concentrations less than those detectible by ^{13}C-NMR spectroscopy it could still determine the reactivity of methimazole due a dynamic equilibrium betweeen IIIa and IIIb. As such, a chemical test of the nature of the thiol function using thiol-disulphide exchange with Ellmans reagent was carried out (equation 1) [9]. This would allow the minor thiol component to dominate the chemistry of methimazole.

$$[Met{=}S]^{-} \rightleftharpoons [Met\text{-}S]^{-} + ESSE \rightleftharpoons Met\text{-}SSE + ES^{-} \qquad -(1)$$

Where Met = methimazole and ESSE = Ellmans reagents

Even when the methimazole was present at a ten fold excess over the Ellmans reagent, little Ellmans anion is generated. This is in contrast to glutathione where almost stoichiometric conversion of Ellmans reagent to Ellmans anion is achieved. Thus even if the thiol form represents less than 0.1% of the methimazole in aqueous solution it has minimal reactivity.

Ab-initio molecular orbital calculations

Ab-initio calculations which include geometry optimistation proceedures [10] were carried out using a 6-31G basis set [11] plus a set of d functions on sulphur for the three forms of methimazole, i.e. the thiol, the thione and the anionic form in the gas phase. In the latter case no preference was introduced into the calculation for the thiolate or thionate form. Consistent with the NMR study of Balestrero et al. [7] the thione (IIIb) tautomer is found to energetically more favourable compared to the thiol (IIIa) by 21.7kcal/mol. Repeating these calculations using factors which mimic solvents of low (e.g. lipid) and high (e.g. water) dielectric constants indicates that the environment of the molecule is unimportant to its preferred structure, the thione form. This form is calculated to be 23.2 kcal/mol and 25.4 kcal/mol more stable in non-polar and polar domains respectively. In a similar manner the introduction of electron correlation effects via the Moller Plesset method [12] at the MP2 level also showed that thione configuration is preferred by 16.8kcal/mol.

By removing the acidic proton and re-optimising the structure, it is possible to gain some impression of any re-organisation to attain the thiolate\thionate form. All the parameters (table 1) indicate that the methimazole framework does not re-organise to form a thiolate, but that the charge remains distributed over the three atoms ($S\text{-}C_1\text{-}N_1$). This interpretation is consistant with the observed unreactivity of methimazole to Ellmans reagent (equation 1).

	Bond lengths			Charges			Bond indices			Predicted
	C_1-S	C_1-N_1	N_1-H_1	S	C_1	N_1	C_1-S	C_1-N_1	N_1-H_1	ν(XH)
Thiol	1.757	1.306	---	+0.02	0.42	-0.56	1.13	1.69	---	2,650 cm^{-1}
Thione	1.687	1.357	0.990	-0.36	0.66	-0.92	1.49	1.27	0.86	3,510 cm^{-1}
Anion	1.742	1.339	---	-0.59	0.50	-0.63	1.28	1.61	---	------

Table 1. The bond lengths, charges on each atom, the bond indices and the predicted stretching frequencies (Raman) for methimazole in the thiol, thione and anionic form, derived from SCF calculations.

To re-confirm the conclusion derived from the above calculations, further calculations were carried out to predict the position of the intense bands in the Raman spectra. Both spectra are predicted to have 17 intense bands. The key feature which confirms the conclusions drawn on the basis of NMR and calculations is the absence of a band between 2,300-2,900 cm^{-1} (-SH) but the prescence of a band (3,550 cm^{-1}) indicative of an NH stretch.

Conclusions

The thesis presented here that the active form of methimazole is the thione tautomer has two basic implications. Interpretations of the action of methimazole in relation to thiolate chemistry (i.e. oxidation-reduction chemistry and free radical scavenging) although numerous [1-3] may be incorrect, misleading and may arise from artifactual chemistry. Furthermore, the ab-initio calculations predict that the thione form will predominate irrespective of the biological environment that methimazole adopts. Thus, it is unlikely that the thiolate

form will be produced in any biological environment. The proposal that the activity propylthiouracil and carbimazole (I, II) as pro-drugs which generate a reactive thiol seems to be unlikely as even if a thiolate were produced in small quantities, its reactivity could at best be described as poor (equation 1). Assigning methimazole as a thione creates a more realistic series of compounds, suggesting that chemical transformation of I and II prior to or during therapeutic action is not necessary. However, it re-opens the debate as to whether there is a biochemical function of thiones such as ergothioneine (V) and with what biochemical pathways thiones such as propylthiouracil, carbimazole and methimazole interact.

Acknowledgements:

MG would like to thank the Scottish Home & Health Department for financial support.

References

1. J.J.Taylor, R.L.Wilson and P.Kendall-Taylor FEBS Lett (1984), 176, 337-340
2. B.L.Hallman and P.K.Bondy Am. J.Med (1951), 11, 724-729
3. A.P.Weatman, M.C.Holt, A.K.Campbell, R.Hall and A.M.N.McGregor Brit. Med. J (1984) 288, 518-520
4. J.Reglinski, W.E.Smith, R.Wilson, L.M.Buchanan, J.H.McKillop and J.A.Thomson Clin Endocrinol. (1992), 37, 319-324
5. J.Reglinski, W.E.Smith, M.Brzeski, M.Marabani and R.D.Sturrock J.Med. Chem. (1992) 35, 2134-2137
6. Merck Index M.Windholtz ed Merck Co Ltd p 857 (1983)
7. R.S.Balestrero, D.M.Forkey and J.G.Russell (1986), 24, 651-655
8. All spectra were recorded on a Bruker AMX400 operating at 400.13 MHz for ^{1}H, 100.6 MHz for ^{13}C and 40.55 MHz for ^{15}N. Spectra were recorded in methylsulphoxide ($(CD_3)_2S=O$) and (phosphate balance saline, 0.1M Na_2HPO_4/0.154M NaCl; pH adjusted to 7.4 with NaOH) water ($^{2}H_2O$ at pH 7.4) using the residual solvent (H_2O), added tert-butanol and external ^{15}N labelled glycine as reference materials for the respective nuclei (^{1}H, ^{13}C, ^{15}N).
9. G.Ellman Arch. Biochem. Biophys. (1959), 82, 70-77
10. M.J.Frisch, G.W.Trucks, M.Head-Gordon, P.M.W.Gill, M.W.Wong, J.B.Foresman, B.G.Johnson, H.B.Schlegel, M.A.Robb, E.S.Replogle, R.Gomperts, J.L.Andres, K.Raghavachari, J.S.Binkley, C.Gonzalez, R.L.Martin, D.J.Fox, D.J.Defress, J.Baker, J.J.P.Stewart and J.A.Pople Gaussian Inc. Pittsburg PA (1992)
11. W.J.Hehre, R.Ditchfield and J.A.Pople J. Chem. Phys. (1972), 56, 2257-2261, P.C.Hariharn and J.A.Pople Theor. Chim. Acta. (1973), 28, 213-222 M.S.Gordon, Chem Phys. Lett. (1980), 76, 163-168
12. M.W.Wong, M.J.Frisch and K.B.Wiberg J.Amer. Chem. Soc. (1991), 113, 4776-4782 M.W.Wong, K.B.Wiberg and M.J.Frisch J. Chem. Phys. (1991), 95, 8991-8998, M.W.Wong, K.B.Wiberg and M.J.Frisch J. Amer. Chem. Soc. (1992), 114, 523-529 M.W.Wong, K.B.Wiberg and M.J.Frisch J. Amer. Chem. Soc. (1992), 114, 1645-1652,
13. C.Moller and M.S.Plesset Phys. Rev. (1934), 46, 618-622, M.J.Frisch, M.Head-Gordon and J.A.Pople Chem. Phys. Lett. (1990), 166, 281-288

(*Received in USA* 24 *February* 1994; *accepted* 26 *April* 1994)