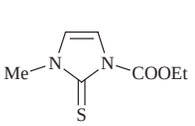
Structure and molecular interactions of anti-thyroid drugs. Part 2. Electron donor properties of carbimazole[1]

Christian Laurence,*[a] Mohamed J. El Ghomari[b] and Michel Berthelot[a]

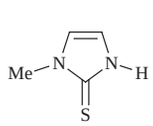
[a] *Laboratoire de Spectrochimie, Faculté des Sciences et des Techniques, Université de Nantes, 2, rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France*
[b] *Laboratoire de Spectroscopie, Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech, Morocco*

The electron donor properties of the anti-thyroid drug carbimazole towards the soft Lewis acid I_2 and the hard hydrogen-bond donor 4-fluorophenol are studied. Diiodine forms only a sulfur coordinated charge-transfer complex with carbimazole (formation constant *ca.* 1400 dm^3 mol^{-1} in CCl_4), while 4-fluorophenol forms both sulfur and carbonyl hydrogen-bonded complexes (formation constants respectively *ca.* 21 and 5 dm^3 mol^{-1} in CCl_4). The lesser hard basicity of carbimazole, and probably also propylthiouracil, compared to methimazole might validate the inactivation of thyroid peroxidase by means of a competitive coordination of these drugs to the ferric site of the enzyme.

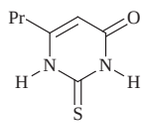
Currently the most commonly employed drugs in the treatment of hyperthyroidism are carbimazole **1** (CBZ), methimazole **2** (MMI) and propylthiouracil **3** (PTU), which all contain the



1



2



3

thiourea pharmacophore.[2,3] Because of a push-pull mechanism in which the nitrogen lone pairs donate electrons to the thiocarbonyl group, this pharmacophore must possess significant electron donor properties at the sulfur atom.

It has been proposed that the donor properties, *i.e.* Lewis basicity, of synthetic anti-thyroid agents (SATs) bearing the thiourea group is at the origin of their anti-thyroid action. In an initial proposed mechanism,[4,5] SATs could form stable electron donor–acceptor complexes with diiodine produced by oxidation of iodides and divert oxidized iodides away from thyroglobulin, which effectively causes thyroid hormones (3,5,3′-triiodothyronine and 3,5,3′,5′-tetraiodothyronine) biosynthesis to cease. In a second mechanism,[6] SATs could interfere with thyroid peroxidase (TPO), a heme enzyme which catalyzes not only the oxidation of iodides, but also the coupling of monoiodotyrosine and diiodotyrosine residues of thyroglobulin. Since the active site of TPO contains the cation Fe^{3+}, the inactivation of TPO could be the result of a coordination of the SAT sulfur to Fe^{3+}, either by increasing the Fe^{3+} coordination number or by ligand displacement.

The measurement of the electron donor properties of SATs seems therefore of great interest, in order to validate the molecular mechanisms of their biological activity. Propylthiouracil **3** is difficult to study because of insolubility in organic solvents. The electron donor properties of methimazole **2** towards the Lewis acid I_2 and the hydrogen-bond donor 4-fluorophenol have already received attention.[7,8] In this paper we report the results of a UV and IR investigation of the sites of complexation of carbimazole **1** and their affinity towards diiodine. However, in the hard and soft acid and base (HSAB) theory,[9] diiodine, with a hardness parameter[10] η of 3.4 eV, is among the softest acid known, and the diiodine basicity of carbimazole will not describe totally its coordinating properties, particularly towards TPO which contains the rather hard acid Fe^{3+} (η = 13.1 eV).[10] We have consequently also measured the hard basicity of carbimazole, by means of stability of its complex with 4-fluorophenol, since hydrogen-bond donors are hard acids in the HSAB classification.[9]

Experimental

Carbimazole (purity 99.4%) was generously given by the laboratoire Roche Nicholas (France). Diiodine was sublimed twice prior to use. 4-Fluorophenol was purified by sublimation. Methanol, CCl_4, CH_2Cl_2 and $CHCl_3$ were Aldrich solvents of spectroscopic grade.

IR measurements were carried out with a Fourier transform spectrometer Bruker IFS 48 using 1 cm^{-1} resolution. Overlapping bands were mathematically deconvoluted into their gausso-lorentzian components by the Curve Fit™ Bruker programme. The diiodine and hydrogen-bonding complexes were studied as CCl_4 solutions in 1 mm KBr and 1 cm infrasil thermoregulated cells, respectively.

UV measurements were carried out with a Cary 219 spectrometer in a 1 cm thermoregulated suprasil cell as CCl_4, CH_2Cl_2 and $CHCl_3$ solutions.

Results and discussion

Diiodine–carbimazole complex

Site of diiodine fixation. There are five possible locations that might be considered as electron donor sites for carbimazole, namely: the nitrogens, the sulfur, the carbonyl oxygen and the ether oxygen lone pairs. However, it is well-established that diiodine coordinates at sulfur in thioureas[11,12] and at the carbonyl oxygen in esters[13] and amides.[14] Moreover, since the formation constants of the complexes of diiodine with carbonyl bases (1–15 dm^3 mol^{-1})[15] are very much lower than with thioureas (13 000 dm^3 mol^{-1}),[12] one might expect carbimazole to be nearly a pure sulfur base towards diiodine.

Conclusive evidence can be obtained from the IR spectra of carbimazole and its complex, by studying the carbonyl bands† between 1700 and 1800 cm^{-1}. We have already shown[1] that carbimazole exists as 90% conformer *E* and 10% conformer *Z* in CCl_4, which explains why its spectrum shows two carbonyl bands at 1747 (*E*) and 1786 (*Z*) cm^{-1}. The progressive addition

† Because of extensive vibrational coupling, the thiocarbonyl stretching absorption is difficult to assign.

Table 1 Carbimazole–diiodine complexes at 298 K in various solvents: CT band, formation constants K_f, $E:Z$ ratio, relative permittivity D and Reichardt parameter E_T

Solvent	λ_{CT}/nm	ε_{CT}/dm^3 mol^{-1} cm^{-1}	K_f[a]/dm^3 mol^{-1}	$Z:E$[b]	D	E_T
CH_2Cl_2	360	34 000	2686	39:61	9.02	40.7
CCl_4	366	30 500	1411	9:91	2.30	32.4
$CHCl_3$	362	30 500	784	36:64	4.89	39.1

[a] Sum of the formation constants of Z and E 1:1 complexes. [b] Ref. 1.

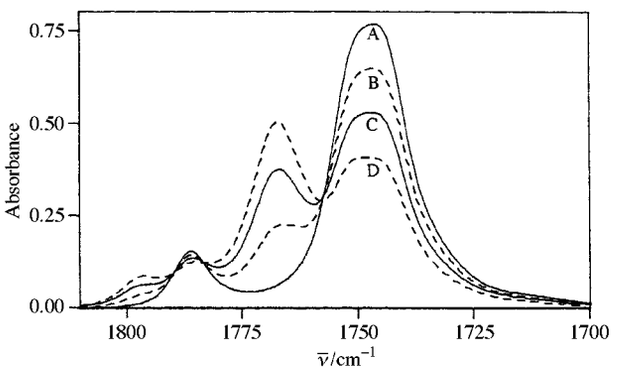


Fig. 1 Influence of diiodine addition to a 0.01 M solution of carbimazole in CCl_4 on the carbonyl bands. I_2 concentrations are: (A) 0, (B) 0.002, (C) 0.004 and (D) 0.006 mol dm^{-3}. Note the existence of isobestic points showing the absence of complexes of stoichiometry higher than 1:1.

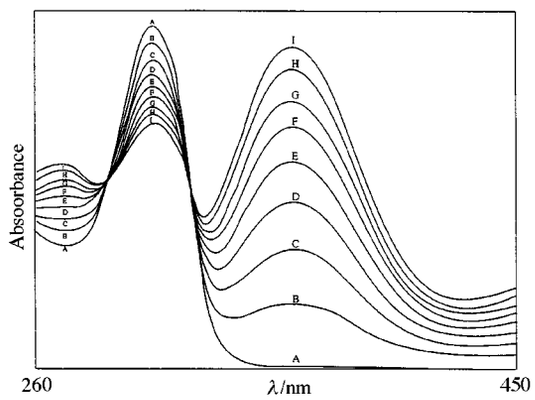


Fig. 2 (A) $\pi\rightarrow\pi^*$ transition of carbimazole in CCl_4 at 309 nm. The addition of increasing quantities of diiodine [spectra (B)–(I)] causes the appearance of two new bands at 270 (blue shifted $\pi\rightarrow\pi^*$ band) and 366 nm (CT band). The existence of isobestic points indicates an 1:1 complex.

of diiodine to a carbimazole solution in CCl_4 causes the disappearance of the 1747 cm^{-1} band and the appearance of a new band at 1770 cm^{-1}, whereas no new band appears below 1747 cm^{-1} (Fig. 1). Since the coordination of diiodine to a carbonyl group lowers the carbonyl frequency,[14] it is evident that diiodine is not bonded to the carbonyl oxygen of *E*-carbimazole. The 23 cm^{-1} increase of the carbonyl band position in the complex can only be interpreted as S-coordination making the bonded thiocarbonyl more electron-withdrawing than free thiocarbonyl. The same phenomenon occurs for the 1786 cm^{-1} carbonyl band of the *Z* conformer, the frequency of which also increases in the complex. The lower basicity of the sulfur in the *Z* conformer (more exposed to the electron-withdrawing field of the carbonyl dipole) probably explains the decreased change in band position for the *Z* (12 cm^{-1}) than for the *E* conformer (23 cm^{-1}).

UV determination of the formation constant. The UV spectrum of carbimazole in CCl_4 shows an intense band at 309 nm (ε = 14 790 dm^3 mol^{-1} cm^{-1}). From previous UV studies[16,17] on Δ^4-imidazoline-2-thiones, we interpret this band as the $\pi\rightarrow\pi^*$ transition of the thiocarbonyl chromophore. This transition decreases the charge of the sulfur atom,[17] and the ground state is more stabilized by hydrogen-bonding donor solvents than the excited state.[16,17] We indeed observe a blue shift of the 309 nm band, which is found at 298 nm in methanol and 291 nm in water.

The progressive addition of diiodine to a CCl_4 solution of carbimazole causes a decrease of the 309 nm band and the appearance of two new bands at 270 and 366 nm (Fig. 2). For the same reason (lower basicity of sulfur in the excited state) as hydrogen bonding, halogen bonding to sulfur must provoke a blue shift of the $\pi\rightarrow\pi^*$ transition, and we attribute the 270 nm band as the blue-shifted $\pi\rightarrow\pi^*$ transition of the bound thiocarbonyl chromophore. So the 366 nm band must be the charge-transfer ‡ (CT) band of the Mulliken theory of charge-transfer complexes,[18] which is generally found in this region for the complexes of diiodine with thioureas.[12]

The graphical method of Rose and Drago[19] allows the simultaneous determination of the formation constant of the complex and of the molar extinction coefficient of its charge-transfer band, the two unknowns of the Rose and Drago equations[19] relating absorbances of the CT band to initial carbimazole and diiodine concentrations. We have measured the equilibrium constant K_f in three solvents, namely CCl_4, CH_2Cl_2 and $CHCl_3$. The results are summarized in Table 1.

Table 1 shows that K_f does not increase regularly with the solvent polarity, measured by the relative permittivity D or the Reichardt parameter E_T.[20] The regular increase of K_f with D or E_T, generally found[12,21,22] for the diiodine complexes of thiocarbonyl bases because of a stronger solvation of the polar complex as compared to the uncomplexed reactants,[21,22] is disturbed by the influence of solvent on the conformational equilibrium $Z \rightleftharpoons E$. In fact the solvent polarity also increases the *Z* conformer population,[1] which with the lower basicity of the conformer *Z* (*vide supra*) should cause a decrease of K_f with solvent polarity. The first solvent effect predominates in going from CCl_4 to CH_2Cl_2 while the second one prevails from CCl_4 to $CHCl_3$.

Hydrogen-bonding complexes of carbimazole

Sites of hydrogen bonding. Previous hydrogen bonding studies have demonstrated that hydrogen-bonding occurs on the carbonyl oxygen of carbamates[23] and on the sulfur of thioureas.[8] The hydrogen-bonding basicities of carbamates and thioureas being of the same order of magnitude,[8,23] we expect that carbimazole behaves both as a sulfur and a carbonyl hydrogen-bonding base.

If we add carbimazole to a solution of 4-fluorophenol in CCl_4 and study the IR O–H stretching region, there appears, to the detriment of the 3614 cm^{-1} O–H band of free 4-fluorophenol, two new broad bands at 3480 cm^{-1} (shift $\Delta\nu_1$ = 134 cm^{-1}) and 3323 cm^{-1} (shift $\Delta\nu_2$ = 291 cm^{-1}) (Fig. 3). A similar spectrum is obtained with methanol as hydrogen-bond donor: the 3644 cm^{-1} O–H band of free methanol undergoes shifts of 58 and 164 cm^{-1}. From shifts previously observed with thioureas[8] and carbamates,[23] the first broad band (smaller shift) is assigned to the O-complex and the second one (greater shift) to

‡ From the carbimazole HOMO to the diiodine LUMO (σ_u^*).

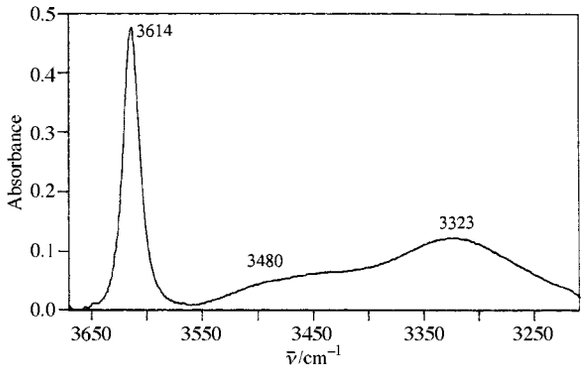


Fig. 3 IR spectrum of a solution of 4-fluorophenol (0.004 mol dm^{-3}) in CCl_4 after addition of carbimazole (0.017 mol dm^{-3}). The 3614 cm^{-1} sharp band is the ν(OH) band of free FP, while the 3480 and 3323 cm^{-1} broad bands are, respectively, the ν(OH···O) and ν(OH···S) bands of hydrogen-bonded FP.

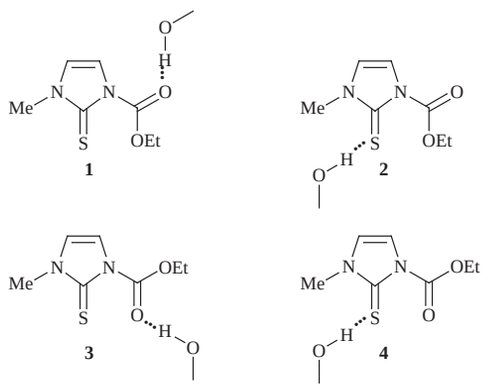


Fig. 4 Schematic structures of the four possible S and O hydrogen-bonded complexes of FP with the *E* and *Z* conformers of carbimazole

the S-complex. The higher intensity of the S-complex band indicates the higher stability of this complex.

These fixation sites are confirmed by the study of the carbonyl stretching region. The addition of 4-fluorophenol to a solution of carbimazole in CCl_4 brings about an absorbance decrease of the 1747 cm^{-1} band of the *E* conformer of free carbimazole, at the benefit of not only a new band at 1720 cm^{-1}, which signifies a carbonyl complex, but also of a new band at 1760 cm^{-1}, attributed to a S-fixation (*vide supra*). The 1786 cm^{-1} band of the *Z* conformer behaves similarly, revealing also O- and S-fixations. Fig. 4 shows schematic structures of the four complexes of carbimazole with O–H hydrogen-bond donors.

IR determination of the formation constant. Four simultaneous equilibria, corresponding to the formation of the four complexes **1**–**4**, occur in dilute CCl_4 solutions of 4-fluorophenol (*ca.* 4×10^{-3} mol dm^{-3}) (FP) on the addition of carbimazole (CBZ) (8 to 30×10^{-3} mol dm^{-3}) [eqns. (1)–(4)].

$$\text{CBZ} + \text{pFP} \rightleftharpoons \mathbf{1} \qquad K_1 = [\mathbf{1}]/[\text{FP}][\text{CBZ}] \qquad (1)$$

$$\text{CBZ} + \text{pFP} \rightleftharpoons \mathbf{2} \qquad K_2 = [\mathbf{2}]/[\text{FP}][\text{CBZ}] \qquad (2)$$

$$\text{CBZ} + \text{pFP} \rightleftharpoons \mathbf{3} \qquad K_3 = [\mathbf{3}]/[\text{FP}][\text{CBZ}] \qquad (3)$$

$$\text{CBZ} + \text{pFP} \rightleftharpoons \mathbf{4} \qquad K_4 = [\mathbf{4}]/[\text{FP}][\text{CBZ}] \qquad (4)$$

The classical method,[24] which follows the IR absorbance decrease of the 3614 cm^{-1} O–H band of FP on the addition of increasing quantities of CBZ, is only able to give the total concentration of hydrogen-bonded complexes [**1**] + [**2**] + [**3**] + [**4**], *i.e.* the global constant K_t as shown by eqn. (5). The mean of three determinations gives a value of 25.9 dm^3 mol^{-1}.

$$K_t = ([\mathbf{1}] + [\mathbf{2}] + [\mathbf{3}] + [\mathbf{4}])/[\text{FP}][\text{CBZ}] = K_1 + K_2 + K_3 + K_4 \qquad (5)$$

Let us write $K_1 + K_3 = K_O$ and $K_2 + K_4 = K_S$. The value of K_O can be estimated from a relationship established[25] between the IR shift of the O–H band of methanol hydrogen-bonded to esters and the logarithm of the formation constant of the complex of FP with esters. From a shift of 58 cm^{-1} (*vide supra*), we calculate $K_O = 5.2$ dm^3 mol^{-1} and we deduce $K_S = K_t - K_O = 20.7$ dm^3 mol^{-1}. This method of determination of the sulfur and carbonyl hydrogen-bond basicities of CBZ is approximate since we suppose that complexes **1** and **3** have the same OH frequencies. However it furnishes a ratio $K_S/K_O = 4$ which compares well to the ratio $A_S/A_O = 3.4$ of the absorbances of the OH bands of S- and O-complexes and to the ratio $B_S/B_O = 5.7$ of the integrated intensities of the same bands (Fig. 3).

Conclusion

The hard basicity of the thiourea pharmacophore of CBZ ($K = 20.7$ dm^3 mol^{-1}; $\Delta G^\circ = -7.5$ kJ mol^{-1}) is significantly lower than that of MMI[8] ($K = 129$ dm^3 mol^{-1}; $\Delta G^\circ = -12.0$ kJ mol^{-1}). This basicity decrease is largely explainable by the electron-withdrawing effect of the carbonyl group on the N^3 ring atom. For solubility reasons, we could not measure the basicity of PTU, but the carbonyl group also on N^3 in the thiouracil ring must produce a similar effect. Then we assume the following hard basicity order: MMI > CBZ ~ PTU. The higher stability constants of ferric complexes of MMI, compared to thiouracil,[26] validates this hypothesis. Interestingly, *in vitro* the inhibitory effects of CBZ, MMI and PTU on TPO catalysed oxidation of guaiacol follow the order[27] MMI > CBZ ~ PTU, namely the hard basicity order. This agrees with a possible mechanism of TPO inactivation by coordination of SATs to the metallic site of TPO. The situation is more complex *in vivo* since CBZ is transformed to MMI,[27] and will be considered in the third part of this series which will focus on the diiodine basicity of MMI and related compounds.

Acknowledgements

We thank the laboratoire Roche Nicholas (France) for providing us with carbimazole, and Dr M. Helbert (Nantes) for recording IR spectra.

References

1 Part 1: C. Laurence, M. J. El Ghomari and M. Luçon, *J. Chem. Soc., Perkin Trans. 2*, 1998, 1159.
2 J. Buxeraud, *Les médicaments des dysfonctionnements thyroïdiens*, in *Traité de chimie thérapeutique*, Tec et Doc Lavoisier, Paris, 1995, vol. 4.
3 J. Leclere, J. Orgiazzi, B. Rousser, J. L. Schlienger and J. L. Wemeau, *La thyroïde*, Expansion scientifique française, Paris, 1992.
4 J. Buxeraud, A. C. Absil, J. Claude, C. Raby, G. Catanzano and C. Beck, *Eur. J. Med. Chem.*, 1985, **20**, 43.
5 C. Raby, J. F. Lagorce, A. C. Jambut-Absil, J. Buxeraud and G. Catanzano, *Endocrinology*, 1990, **126**, 1683.
6 R. Basosi, N. Niccolai and C. Rossi, *Biophys. Chem.*, 1978, **8**, 61.
7 C. Raby, J. Claude, C. Moesch and J. Buxeraud, *C. R. Hebd. Seances Acad. Sci.*, 1979, **288**, 319.
8 C. Laurence, M. Berthelot, J. Y. Le Questel and M. J. El Ghomari, *J. Chem. Soc., Perkin Trans. 2*, 1995, 2075.
9 R. G. Pearson, *J. Am. Chem. Soc.*, 1963, **85**, 3533.
10 R. G. Parr and R. G. Pearson, *J. Am. Chem. Soc.*, 1983, **105**, 7512.
11 R. P. Lang, *J. Am. Chem. Soc.*, 1962, **84**, 1185.
12 R. P. Lang, *J. Phys. Chem.*, 1968, **72**, 2129.
13 D. G. Brown, R. S. Drago and T. F. Bolles, *J. Am. Chem. Soc.*, 1968, **90**, 5706.

14 C. D. Schmulbach and R. S. Drago, *J. Am. Chem. Soc.*, 1960, **82**, 4484.
15 C. Laurence, G. Guihéneuf and B. Wojtkowiak, *J. Am. Chem. Soc.*, 1979, **101**, 4793.
16 G. Assef, D. Bouin-Roubaud, J. Kister and J. Metzger, *C. R. Hebd. Seances Acad. Sci.*, 1976, **283**, 143.
17 D. Bouin-Roubaud, J. Kister, M. Rajzmann and L. Bouscasse, *Can. J. Chem.*, 1981, **59**, 2884.
18 R. S. Mulliken and W. B. Person, *Molecular complexes*, Wiley, New York, 1969.
19 N. J. Rose and R. S. Drago, *J. Am. Chem. Soc.*, 1959, **81**, 6138.
20 C. Reichardt, *Solvents and solvent effects in organic chemistry*, VCH, Weinheim, 1988.
21 G. Guihéneuf, J. L. M. Abboud and W. Bouab, *Can. J. Chem.*, 1987, **65**, 2106.
22 H. Mollendal, J. Grundnes and E. Augdahl, *Acta Chem. Scand.*, 1969, **23**, 3525.
23 J. Y. Le Questel, C. Laurence, A. Lachkar, M. Helbert and M. Berthelot, *J. Chem. Soc.*, *Perkin Trans. 2*, 1992, 2091.
24 E. M. Arnett, L. Joris, E. Mitchell, T. S. S. R. Murty, T. M. Gorrie and P. v. R. Schleyer, *J. Am. Chem. Soc.*, 1970, **92**, 2365.
25 F. Besseau, C. Laurence and M. Berthelot, *J. Chem. Soc.*, *Perkin Trans. 2*, 1994, 485.
26 W. O. Foye and J. R. Lo, *J. Pharm. Sci.*, 1972, **61**, 1209.
27 T. Nakashima and A. Taurog, *Clin. Endocrinol.*, 1979, **10**, 637.

Paper 8/00902C
Received 2*nd February* 1998
Accepted 26*th February* 1998