the values of 24-h cumulative blood insulin levels were increased by the administration of the drug (Δ, 30-93 μU/mL), resulting in the stimulation of insulin release from pancreatic B cells in all cases. The difference between single and successive administration on cumulative blood glucose levels and cumulative blood insulin levels was not significant in patient C (Table III, footnote *b*).

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Secondary Antithyroid Action of Drugs in Relation to Structure

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Received July 13, 1982, from the *Department of Chimica Therapeutica, Faculty of Medicine and Pharmacy, 87032 Limoges Cedex, France.* Accepted for publication November 29, 1983.

Abstract □ Molecular interactions between iodine and disulfiram, clomethiazole, and tolnaftate were investigated by electron spectroscopy. Iodine forms charge transfer complexes with these molecules, with 1:1 stoichiometry and of the n-σ type. The formation constants were compared with those obtained with antithyroid molecules. Only disulfiram appears to have any effect on the intrathyroid cycle of iodine.

Keyphrases □ Charge transfer complexes—iodine and disulfiram, clomethiazole, tolnaftate □ Disulfiram—charge transfer complexes with iodine □ Clomethiazole—charge transfer complexes, iodine □ Tolnaftate—charge transfer complexes, iodine

It has been shown that molecules possessing an NCS moiety can form charge transfer complexes with iodine (1, 2). Both qualitative and quantitative studies have shown that certain antithyroid drugs (those possessing the NCS function) form charge transfer complexes involving the transfer of charge from the pair of free electrons on the nitrogen and/or the sulfur atoms to the antibonding orbital of the iodine (3, 4). The intensity of this action can be determined from the complex formation constant and the thermodynamic parameters. A correlation has been demonstrated between the constant (K_c) and antithyroid activity (5). A structure-activity relationship has been developed to classify all known antithyroid molecules (6). Iodine fixation by complex formation is one action mechanism of synthetic antithyroid agents.

Synthetic antithyroid drugs can also inhibit peroxidase (7). This enzyme is necessary for the oxidation of circulating iodine, for its integration into thyroglobulin, and for coupling monoiodotyrosines and diiodotyrosines to form triiodotyronines (T_3) and tetraiodothyronines (T_4). While antithyroid agents display variable activity towards peroxidase, they can all complex iodine, so that the latter is unavailable for thyroid hormone synthesis.

This NCS function is found in many drugs belonging to other therapeutic classes. Hence, if we hope to understand biological activity it is important to investigate the possible formation of complexes between these molecules (tolnaftate, disulfiram, and clomethiazole) and iodine. This can help determine whether these molecules possess secondary antithyroid activity.

EXPERIMENTAL SECTION

Materials—An ultrapure iodine was prepared by sublimation and stored in a desiccator containing P_2O_5. Disulfiram[1] [tetraethylthioperoxydicarbonic diamide (I)], tolnaftate[2] [*O*-2-napthyl-*m*,*N*-dimethylthiocarbanilate (II)], and clomethiazole[3] [5-(2-chloroethyl)-4-methylthiazole (III)] were pharmaceutical grade; purity was determined by HPLC[4]. UV-grade carbon tetrachloride[5] was used.

UV and visible spectra were recorded on a double-beam spectrophotometer[6] equipped with a Peltier effect thermoelectric cell holder. Hellma quartz cells with a path length of 1 cm were employed.

Methods—The glassware was thoroughly dried with dry nitrogen to eliminate any effects due to hydration of the complex solutions. Volumetric solutions were prepared from initial solutions obtained by weighing. The spectra were recorded immediately after solution preparation.

[1] Millot; Solac Laboratories, Paris, France.
[2] Unicet; Cetrane Laboratories, Levallois-Perret, France.
[3] Debat Laboratories, Paris, France.
[4] Model 244 U/45; Waters, S.A. Paris, France.
[5] Merck uvasol Art. 2209; E. Merck, Darmstadt.
[6] Model 554 UV-Vis; Perkin-Elmer.

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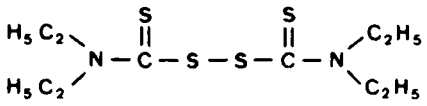

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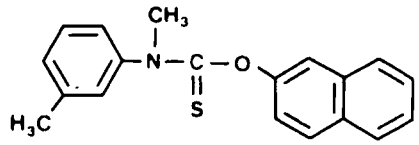
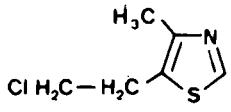


II



III

For all equilibrium constant calculations, a series of complex solutions were used with a constant iodine concentration and a variable donor concentration. The solutions were dilute ($\sim 10^{-4}$ M for iodine, $< 5 \times 10^{-2}$ M for tolnaftate and clomethiazole, and $< 4 \times 10^{-3}$ M for disulfiram). Optical densities were determined at three temperatures, 10°C, 20°C, and 30°C (± 0.1°C).

RESULTS

Visible Region— Iodine in solutions of carbon tetrachloride displayed an absorption peak at 515 nm. The donors displayed negligible absorption in the 350–700-nm region. All the complex solution spectra showed a disturbance of the visible band of the halogen consisting of a hypsochromic displacement ($\Delta\lambda$) of the $\pi g \rightarrow \tau_0$ transition of iodine (Fig. 1).

The stability of the complexes was examined by making new recordings after 24 h. Each of the three systems exhibited new absorption bands at 290 and 360 nm, characterizing the presence of I_3^- ions. The changes were greater with higher donor concentrations.

The 1:1 stoichiometry of the complexes was confirmed by analysis of the absorption bands, the accuracy of measurements, and the isosbestic points observed. However, this stoichiometry was checked experimentally by Job's method of continuous variations (8). To do this, the optical densities of the series of complex solutions with constant donor and iodine concentrations were recorded. A correction was made by subtracting from the optical densities recorded, the optical densities of donor and iodine solutions of the same concentration as in the complex solution:

$$A_{corr} = A_{obs} - \epsilon_D \cdot [D] - \epsilon_{I_2} \cdot [I_2] \qquad \text{(Eq. 1)}$$

where A_{corr} is the corrected optical density of the complex, A_{obs} is the optical density of the donor–iodine mixture, ϵ_D and ϵ_{I_2} are the molar absorption coefficients of the donor and iodine, and [D] and [I_2] are the donor and acceptor concentrations, respectively.

The curve obtained by plotting these optical densities for each complex solution against the iodine molar fractions displays a maximum for equal donor and iodine concentrations. The position of the maximum and the perfect symmetry of the curve confirms the presence of a complex with 1:1 stoichiometry and excludes the presence of higher-order complexes (Fig. 2).

The formation constants (K_c) and the absorption coefficients (ϵ_c) of the three donor–iodine systems investigated are given in Table I. These values were determined by using:

$$K_c = \frac{[C]}{([A_0] - [C])([D_0] - [C])} \qquad \text{(Eq. 2)}$$

where [C] is the complex concentration, [A_0] the initial iodine concentration, and [D_0] is the initial donor concentration. [C] can be replaced by the term d_c/ϵ_c leading to:

$$\frac{[A_0][D_0]}{d_c} = \left([A_0] + [D_0] - \frac{d_c}{\epsilon_c}\right) \cdot \frac{1}{\epsilon_c} + \frac{1}{K_c \epsilon_c} \qquad \text{(Eq. 3)}$$

where, at the wavelength investigated, d_c is the absorption of the complex only, ϵ_c is the molar absorption coefficient of the complex, and K_c is the complex formation constant. Equation (3) was resolved by computer using the least-squares method. Graphic representation of the term $[A_0][D_0]/d_c$ as a function of $[A_0] + [D_0] - (d_c/\epsilon_c)$ produces a line with a slope of $1/\epsilon_c$ and an intercept of $1/K_c$ (Fig. 3).

Graphic representation of $R \log K_c$ *versus* $1/T$ yields a line whose slope provides the value of $\Delta H°$, and the intercept determines the value of $\Delta S°$ (Fig.

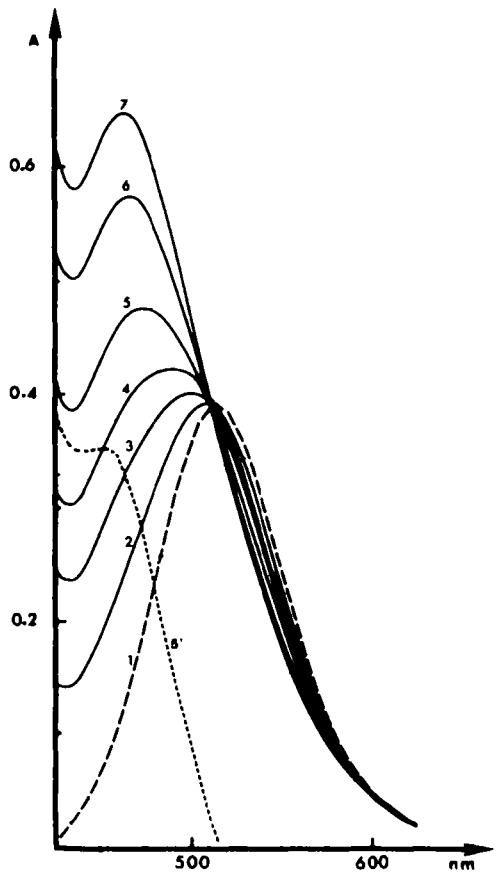


Figure 1—*Visible absorption spectra of disulfiram–iodine complex (solvent, carbon tetrachloride, temperature 20 ± 0.1°C). Key: (1) 4.242 × 10^{-4} M iodine; (2) 4.242 × 10^{-4} M iodine and 0.434 × 10^{-4} M disulfiram; (3) 4.242 × 10^{-4} M iodine and 0.868 × 10^{-3} M disulfiram; (4) 4.242 × 10^{-4} M iodine and 1.216 × 10^{-3} M disulfiram; (5) 4.242 × 10^{-4} M iodine and 1.737 × 10^{-3} M disulfiram; (6) 4.242 × 10^{-4} M iodine and 2.605 × 10^{-3} M disulfiram; (7) 4.242 × 10^{-4} M iodine and 3.473 × 10^{-3} M disulfiram; (5′) absorption curve of complex obtained for solution 5 by placing a 4.242 × 10^{-4} M iodine solution in the reference beam.*

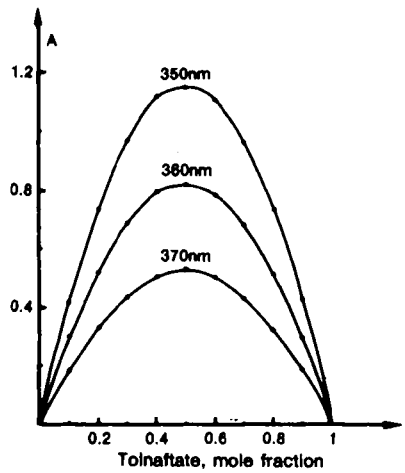


Figure 2—*Determination of stoichiometry of the tolnaftate–iodine complex by the method of continuous variations (solvent, carbon tetrachloride, temperature 20 ± 0.1°C). For each complex solution, the sum of tolnaftate and iodine concentrations is constant and equal to 18.8 × 10^{-4} M.*

Table I—Formation Constants and Molar Extinction Coefficients for Iodine Complexes in Solution in Carbon Tetrachloride[a]

Donor	nm	C, $L \cdot M^{-1} cm^{-1}$ [e]	Complex Formation Constant (K_c), $L \cdot M^{-1}$ [e]	Mean K_c
Tolnaftate[b]	455	57.38	2136	
	460	57.07	2110	
	465	55.78	2040	
	470	57.88	1855	56.65 ± 0.96
	475	55.76	1692	
	480	55.69	1466	
	485	56.56	1209	
Disulfiram[c]	460	300.51	2599	
	465	298.39	2484	
	470	288.81	2340	296.78 ± 4.44
	475	298.36	2068	
	480	298.45	1799	
	485	292.40	1520	
	490	300.51	1195	
Clomethiazole[d]	410	24.96	1231	
	415	24.84	1305	
	420	24.84	1337	
	425	25.32	1343	25.00 ± 0.19
	430	25.17	1321	
	435	24.83	1283	
	440	25.07	1203	

[a] At 20 ± 0.1°C. [b] Six different tolnaftate-I_2 solutions; $[I_2]$ 4.438 × 10^{-4} M; [tolnaftate] varied from 0.229 × 10^{-2} M to 1.836 × 10^{-2} M. [c] Six different disulfiram-I_2 solutions; $[I_2]$ 4.242 × 10^{-4} M; [disulfiram] varied from 0.434 × 10^{-3} M to 3.473 × 10^{-4} M. [d] Five different clomethiazole-I_2 solutions; $[I_2]$ 4.580 × 10^{-4} M; [clomethiazole] varied from 1.093 × 10^{-2} M to 5.467 × 10^{-2} M. [e] Values were calculated from absorption data in the visible region.

4). The thermodynamic parameters related to the formation of the complex are given in Table II.

Ultraviolet Region—The donors displayed strong absorption in the UV region (Table III), whereas iodine exhibited weak absorption. The high values of the molar extinction coefficients of the complexes in this spectral region permit the use of much lower donor concentrations than those required for the visible spectra.

The absorption bands due to the intermolecular charge transfer around 300 nm are superimposed on iodine and especially for donor absorption (Fig. 5). Analysis of these bands is therefore inaccurate and is limited only to qualitative results. Charge transfer bands, calculated graphically, are located at ~338 nm for the tolnaftate-iodine complex, at 310 nm for the disulfiram-iodine complex, and at 276 nm for the clomethiazole-iodine complex. The absorption intensity of the charge transfer bands increases with donor concentration and declines sharply with increasing temperature (Fig. 6). After 24 h the spectra

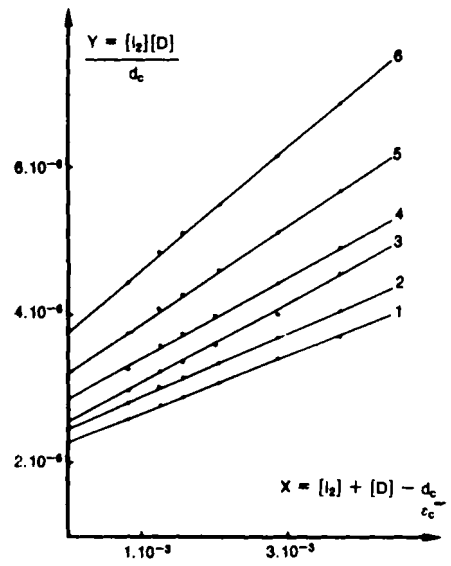


Figure 3—*Graphic representation of Eq. 3 obtained for the disulfiram-iodine complex. Lines 1, 2, 3, 4, 5, and 6 were obtained at 460, 470, 475, 480, 485, and 490 nm, respectively:* $x = [I_2] + [D] - d_c/\epsilon_c$ *and* $y = [I_2][D]/d_c$.

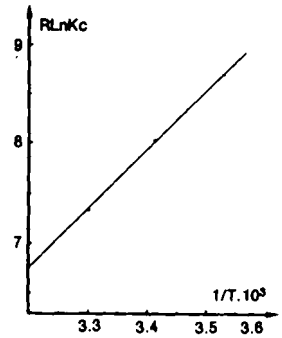


Figure 4—*Determination of the thermodynamic parameters of the tolnaftate-iodine complex. The linear regression line has the equation* y = 5.8 x − 11.77 (r = 0.999).

Table II—Thermodynamic Parameters Obtained for the Iodine Complexes

	$-\Delta H°$, kcal · mol^{-1}	$-\Delta S°$, cal · mol^{-1} K^{-1}	$-\Delta G°_{293}$, kcal · mol^{-1}
Tolnaftate[a]	5.80 ± 0.12	11.79 ± 0.42	2.35 ± 0.01
Disulfiram[b]	6.96 ± 0.30	12.48 ± 1.04	3.32 ± 0.01
Clomethiazole[c]	7.08 ± 0.14	17.76 ± 0.50	1.87 ± 0.005

[a] $[I_2]$ 4.438 × 10^{-4} M; [tolnaftate] varied from 0.229 × 10^{-2} M to 1.836 × 10^{-2} M. [b] $[I_2]$ 4.242 × 10^{-4} M; [disulfiram] varied from 0.434 × 10^{-3} M to 3.473 × 10^{-4} M. [c] $[I_2]$ 4.580 × 10^{-4} M; [clomethiazole] varied from 1.093 × 10^{-2} M to 5.467 × 10^{-2} M.

Table III—Molar Extinction Coefficients of Donors Obtained in the Ultraviolet Region[a]

Donor	λ_{max}, nm	log ϵ_{max}
Tolnaftate	263	4.38
	320.5	2.92
Disulfiram	259	4.53
Clomethiazole	256.4	3.42

[a] Solvent: carbon tetrachloride; temperature: 20 ± 0.1°C.

displayed the appearance of two new bands at 290 and 360 nm, respectively, characteristic of the formation of I_3^- ions in the complex solutions. Graphic extrapolation for the disulfiram-iodine complex (Fig. 5) helped to reveal the

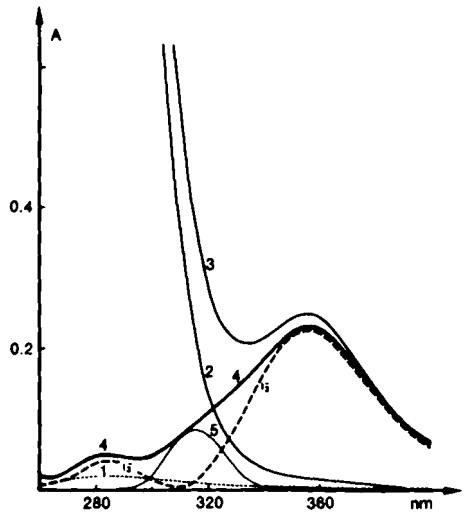


Figure 5—*Ultraviolet spectra of the disulfiram-iodine complex. Key: (1)* 4.242 × 10^{-4} *M iodine; (2)* 4.342 × 10^{-5} *M disulfiram; (3)* 4.242 × 10^{-4} *M iodine and* 4.342 × 10^{-5} *M disulfiram; (4) spectral recording obtained by placing a* 4.242 × 10^{-4} *M iodine solution and a* 4.342 × 10^{-5} *M disulfiram solution in the reference beam; (5) charge transfer band obtained by graphic extrapolation.*

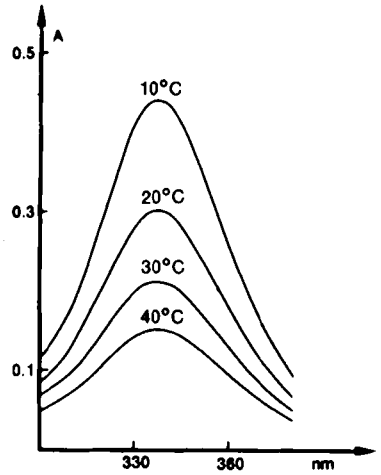


Figure 6—*Variation of the charge transfer band of a tolnaftate–iodine complex solution with temperature (4.438 × 10^{-4} M iodine and 4.682 × 10^{-4} M tolnaftate).*

three absorption bands located at 290 and 360 nm (I_3^- ions) and 310 nm (charge transfer band).

DISCUSSION

Spectroscopic observations in the visible and UV regions confirm the n-σ character of the charge transfer complexes formed by iodine with disulfiram, tolnaftate, and clomethiazole. The 1:1 stoichiometry of these complexes is confirmed by analysis of the absorption bands, measurement accuracy, isosbestic points observed, and Job's method of continuous variations.

In agreement with Mulliken (9) and Popov and Deskin (10), the formation of I_3^- ions is caused by the conversion of an outer complex into an inner complex, releasing I^- ions which act on the free molecular iodine:

1. Disulfiram + I_2 ⇄ disulfiram-I_2 (outer complex)
2. Disulfiram-I_2 ⇄ disulfiram-I^+I^-
3. Disulfiram-I^+I^- ⇄ (disulfiram-I)$^+$ + I^- (inner complex)
4. I^- + I_2 ⇄ I_3^-

Reaction 2 occurs slowly, while the other three reactions display rapid kinetics.

Table IV—Comparison of Formation Constants of Iodine Complexes Obtained with Antithyroid Drugs and with Clomethiazole, Tolnaftate, and Disulfiram

	K_c at 20°C, L · mol^{-1}	Solvent
Potassium thiocyanate	94 (at 22°C)[a]	H_2O
2-Thiazoline-2-thiol	2527 ± 118[a]	CCl_4
Thiourea	8825 ± 505[a]	CH_2Cl_2
Tetramethylthiourea	13215 ± 1150[a]	*n*-Heptane
2-Mercapto-1-methylimidazole	23194 ± 667[a]	CCl_4
Clomethiazole	25.00 ± 0.19	CCl_4
Tolnaftate	56.65 ± 0.96	CCl_4
Disulfiram	296.78 ± 4.44	CCl_4

[a] From Ref. 5.

The formation constants K_c of the iodine complexes of tolnaftate, disulfiram, and clomethiazole were compared with those obtained for antithyroid molecules (Table IV).

Antithyroid compounds are stronger donors for iodine than tolnaftate, disulfiram, and clomethiazole. Only disulfiram displayed a constant higher than that of potassium thiocyanate, whose antithyroid action is well known. Without totally inhibiting the synthesis of thyroid hormones, disulfiram appears to diminish this formation and interferes with it, causing the formation of a goiter. This property has been reported in a clinical study (11).

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