Synthesis and Antiperoxidase Activity of Propylthiouracil Derivatives and Metabolites

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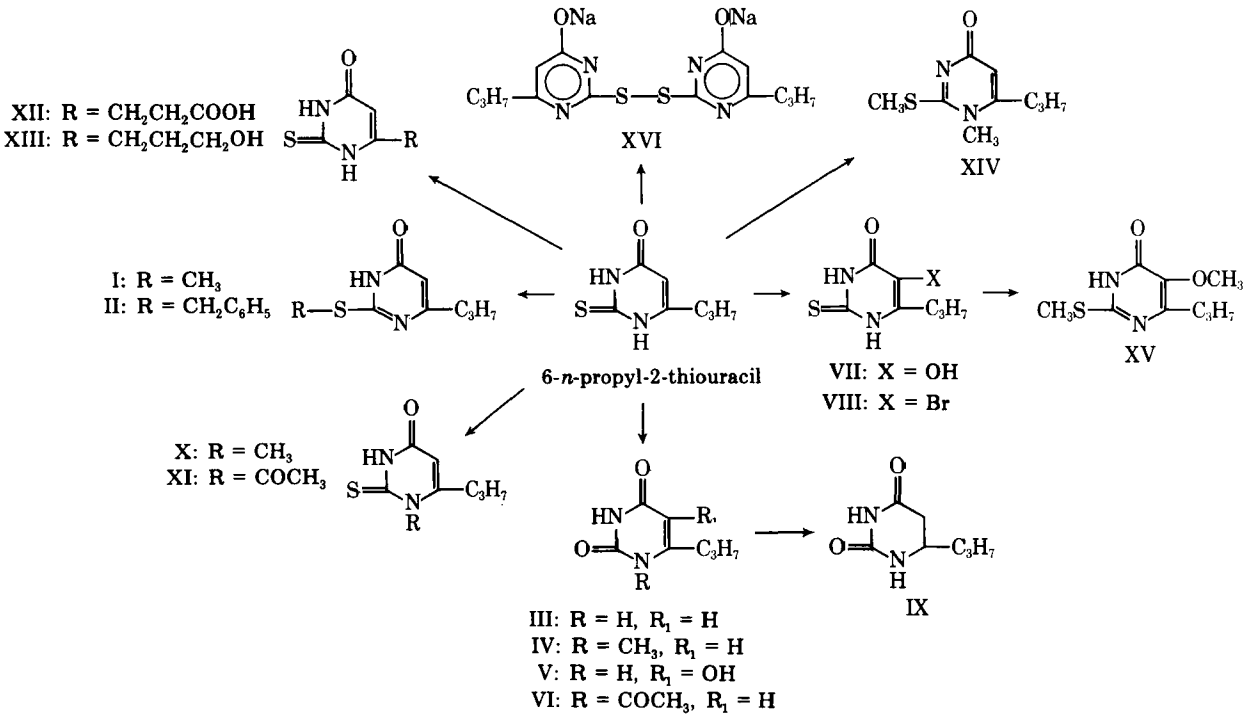
Abstract □ Some metabolites and potential metabolites of the antithyroid drug propylthiouracil (6-*n*-propyl-2-thiouracil) were synthesized and examined for antithyroid peroxidase activity. The compounds positively identified as metabolites of propylthiouracil or implicated in its metabolism were, at the concentrations examined, either completely inactive as thyroid peroxidase inhibitors or only weakly active. Propylthiouracil glucuronide was the most active metabolite, with a potency slightly more than 10% of that of the parent compound. All derivatives in which the sulfur was replaced by oxygen were completely inactive. Assays of propylthiouracil derivatives not implicated in metabolism demonstrated that 1-acetyl-6-*n*-propyl-2-thiouracil and 5-hydroxy-6-*n*-propyl-2-thiouracil were equally as potent as propylthiouracil and that 2-thiouracil-6-propanol and 2-thiouracil-6-propionic acid were weakly active. The data presented demonstrate that biotransformation of propylthiouracil, which occurs primarily at the S group, results in a substantial loss in antiperoxidase activity.

Keyphrases □ Propylthiouracil derivatives and metabolites—synthesis and antiperoxidase activity □ Antithyroid activity—synthesis and evaluation of propylthiouracil derivatives and metabolites □ Antiperoxidase activity—synthesis and evaluation of propylthiouracil derivatives and metabolites □ Biotransformation—propylthiouracil, activity of metabolites

Information relating to the pharmacology of antithyroid drugs was obtained primarily in the 1940's and 1950's with the compounds of interest at that time, thiourea and thiouracil. Because of toxicity, these drugs have been superseded by propylthiouracil (6-*n*-propyl-2-thiouracil) and methimazole, which are currently the drugs of choice in the treatment of hyperthyroidism. Little information is available concerning the pharmacology of these newer compounds and their metabolism is just now receiving attention (1–4).

The primary action of propylthiouracil is on the thyroid gland. It inhibits thyroid peroxidase, resulting in a blockade of iodide utilization for thyroid hormone synthesis (5). Propylthiouracil also inhibits the peripheral action of thyroxine (6) and apparently crosses the placenta, producing adverse effects in the developing fetus (7).

In this study, a number of metabolites and potential metabolites of propylthiouracil were synthesized (Scheme I) and investigated to determine if they inhibit thyroid peroxidase activity. The compounds found to be active in this initial screening test will be assayed *in vivo* for antithyroid activity and for activity as an inhibitor of peripheral thyroxine effects and to determine if the compounds cross the placenta. The ultimate goals are: *(a)* to identify the metabolites of propylthiouracil and to determine if they are biologically active, *(b)* to develop a propylthiouracil analog with potent antithyroid effects but with no inhibitory action on peripheral thyroxine effects, and *(c)* to develop a propylthiouracil analog that retains



Scheme I—6-n-Propyl-2-thiouracil Derivatives and Metabolites

Table I—Data for 6-*n*-Propyl-2-thiouracil and 6-*n*-Propyluracil Derivatives

Compound	Melting Point	Yield, %	UV Absorption Peaks, nm: 0.1 *N* HCl	pH 7.4[a]	0.1 *N* NaOH	Empirical Formula	Analysis, %:	Calc.	Found
I	155–157°	65	223[b] 250 270 (s)	228 248 (s)[c] 274 (s)	247 276	$C_8H_{12}N_2OS$	C H N	52.14 6.58 15.20	52.09 6.66 15.22
II	132–133°	60	253 270 (s)	253 270 (s)	248 277	$C_{14}H_{16}N_2OS$	C H N	64.58 6.20 10.76	64.63 6.22 10.81
III									
Method A	219–221°[d]	93	262	262	272	—		—	—
Method B	219–221°	56	262	262	272	—		—	—
IV	192–193°	70	262	262	282	$C_8H_{12}N_2O_2$	C H N	57.12 7.19 16.66	57.07 7.32 16.54
V	232–234°	86	278	278	288	$C_7H_{10}N_2O_3$	C H N	49.40 5.92 16.47	49.62 5.68 16.59
VI	230–232°	89	262	262	272 281 (s)	$C_9H_{12}N_2O_3$	C H N	55.10 6.17 14.28	54.94 6.27 14.37
VII	249–252°	58	277	269 315 (s)	265 295 (s)	$C_7H_{10}N_2O_2S$	C H N	45.14 5.41 15.06	44.93 5.34 15.11
VIII	226–227°	91	278	270 310 (s)	266 295 (s)	$C_7H_9BrN_2OS$	C H N S	33.75 9.07 11.25 12.87	33.64 3.57 11.31 12.49
IX	215–217°	88	No absorption						
X	170–172°	72	277	277	310 260	$C_8H_{12}N_2OS$	C H N	52.14 6.58 15.20	52.13 6.45 14.97
XI	225–227°	52	274	273	227 260 310 (s)	$C_9H_{12}N_2O_2S$	C H N	50.93 5.70 13.20	49.61 6.06 13.28
XII	282–284°	29	274	274	260 300 (s)	$C_7H_8N_2O_3S$	C H N	41.99 4.03 13.99	41.58 4.26 13.82
XIII	247–249°	36	274	274	260 300 (s)	$C_7H_{10}N_2O_2S$	C H N	45.14 5.41 15.04	45.44 5.22 14.98
XIV	40–42°	57	283 240	288	288 240	$C_9H_{14}N_2OS$	C H N	54.51 7.12 14.13	54.26 7.24 14.06
XV	90–92°	70	235 250 (s) 290	251 290	251 290	$C_9H_{14}N_2O_2S$	C H N	50.44 6.58 13.08	50.52 6.25 13.21
XVI	—	43	Decomposition to propylthiouracil		270 307 (s)	$C_{14}H_{16}N_4Na_2O_2S_2$	C H N	43.97 4.21 14.65	43.39 3.99 14.28
XVII	—	35	Decomposition to thiouracil		275			—	—

[a] Buffer was 0.05 *M* potassium phosphate, pH 7.4. [b] The first peak listed is the λ_{max}. [c] The (s) indicates a shoulder. [d] Lit. (21) mp 221–223°.

its potent antithyroid effects but does not cross the placenta.

EXPERIMENTAL[1]

***S*-Methyl-6-*n*-propyl-2-thiouracil (I)**—Compound I was prepared by the method described by Barrett *et al.* (8) for the synthesis of *S*-methyl-2-thiouracil. A mixture of 8.5 g (0.05 mole) of 6-*n*-propyl-2-thiouracil and 2.2 g of sodium hydroxide was dissolved in a minimum amount of water (about 15 ml) at 70°. The solution was cooled to about 30°, and 30 ml of ethanol and 3.7 ml of methyl iodide were added. The mixture was heated at 70° for 20 min and then cooled. The precipitate that formed was filtered and recrystallized from ethanol.

***S*-Benzyl-6-*n*-propyl-2-thiouracil (II)**—A modification of the procedure of Wheeler and Liddle (9) was adopted. Propylthiouracil (8.5 g, 0.05 mole) and 2.2 g of sodium hydroxide were dissolved in a minimum amount of water, and 5.0 g of benzyl chloride was added. Ethanol was then added until the oil went into solution. The reaction took place immediately and the colorless prisms that formed were filtered and recrystallized from ethanol.

6-*n*-Propyluracil (III)—*Method A*—An adaptation of the Wheeler and Liddle method (9) for the preparation of uracil from 2-thiouracil was used. An aqueous solution of 1.7 g of chloroacetic acid was mixed with 1.5 g (8.8 mmoles) of propylthiouracil. The solution was refluxed for 2 hr and then allowed to cool. The crystalline product was filtered and recrystallized from ethanol. 1-Methyl-6-*n*-propyluracil (IV), 5-hydroxy-6-*n*-propyluracil (V), and 1-acetyl-6-*n*-propyluracil (VI) were also prepared by this procedure using the appropriate propylthiouracil derivative. Table I lists their physical constants.

Method B—A mixture of 2.0 g (7.7 mmoles) of II in 25 ml of 1 *N* HCl was refluxed for 1 hr. The volatile benzyl mercaptan was removed by lyophilization under vacuum. The solid remaining was recrystallized from ethanol to yield III. When I was similarly treat-

[1] Melting points were determined on a Thomas-Hoover Unimelt capillary apparatus and are uncorrected. IR spectra were determined with a Beckman IR-20A spectrophotometer in a KBr phase. UV absorption spectra were measured with a Perkin-Elmer 124 double-beam spectrophotometer in 0.1 *N* NaOH, 0.1 *N* HCl, and 0.05 *M* potassium phosphate buffer, pH 7.4. NMR spectra were obtained with a Varian HA-60 IL using tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standards. All IR and NMR spectra were in agreement with the assigned structure. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by PCR, Inc., Gainesville, Fla.

Table II—Inhibition of Thyroid Peroxidase by 6-*n*-Propyl-2-thiouracil and Its Metabolites

Compound	Concentration, $\times 10^{-5}$ *M*	Specific Activity of Peroxidase	Inhibition, %
None	—	1.95 ± 0.11	—
6-*n*-Propyl-2-thiouracil	50	0.22 ± 0.01	88.7
	10	1.17 ± 0.08	40.0
	5	1.52 ± 0.08	22.1
6-*n*-Propyluracil	50	1.96 ± 0.14	0
	10	1.90 ± 0.08	2.5
	5	1.93 ± 0.14	1.0
S-Methylpropyl-thiouracil	100	2.01 ± 0.12	0
	50	1.98 ± 0.11	0
	10	1.99 ± 0.12	0
Propylthiouracil disulfide	50	1.72 ± 0.05	11.8
	10	1.83 ± 0.01	6.2
	5	1.88 ± 0.05	3.6
Propylthiouracil glucuronide	50	1.34 ± 0.08	30.8
	10	2.06 ± 0.08	0
	5	2.04 ± 0.08	0

ed with 1 *N* HCl, it was quantitatively converted to III with the evolution of methyl mercaptan.

5-Hydroxy-6-*n*-propyluracil (V)—Following the procedure described by Wang (10) for the conversion of 5-bromouracil to 5-hydroxyuracil, a mixture of 3.0 g (12.9 mmoles) of 5-bromo-6-*n*-propyluracil, prepared according to the procedure of Baker and Kawazu (11), and 100 ml 10% sodium bicarbonate solution was refluxed for 12 hr under nitrogen. The solution was then acidified to about pH 2 with hydrochloric acid and cooled. The white crystalline solid that formed was filtered and recrystallized from aqueous ethanol.

5-Hydroxy-6-*n*-propyl-2-thiouracil (VII)—The procedure followed was essentially the same as that used to prepare V, using 1.4 g (5.6 mmoles) of 5-bromo-6-*n*-propyl-2-thiouracil (VIII) and 50 ml of 10% sodium bicarbonate solution. The white crystalline solid obtained was recrystallized from aqueous ethanol.

5,6-Dihydro-6-*n*-propyluracil (IX)—A modification of the method of DiCarlo *et al.* (12) for the preparation of 5,6-dihydrouracil was used. A solution of 1.23 g (7.99 mmoles) of III in acetic acid was subjected to catalytic hydrogenation at room temperature for 72 hr under an initial pressure of 50 psi, using platinum oxide as a catalyst. After the reduction was complete (as indicated by the loss of UV absorption at 262 nm), the catalyst was filtered and the solvent was removed under vacuum. The product was recrystallized twice from water. Repeated attempts to synthesize 5,6-dihydro-6-*n*-propyl-2-thiouracil by catalytic reduction of *S*-benzyl- or *S*-methylpropylthiouracil using platinum oxide or 5% rhodium on powdered alumina were unsuccessful.

1-Methyl-6-*n*-propyl-2-thiouracil (X)—A modified procedure of Anderson *et al.* (13) for the preparation of 6-*n*-propyl-2-thiouracil was used. A solution of sodium ethoxide was prepared by dissolving 2.3 g (0.1 g-atom) of sodium metal in 50 ml of absolute ethanol; then 6.3 g (0.07 mole) of *N*-methylthiourea and 7.91 g (0.05 mole) of ethyl butyrylacetate were added simultaneously. The mixture was refluxed for 24 hr and the alcohol was removed by distillation under vacuum. The residue was dissolved in 50 ml of water and acidified to pH 3 with concentrated hydrochloric acid. The white precipitate was filtered, washed, and recrystallized from water.

A similar procedure was used to prepare 1-acetyl-6-*n*-propyl-2-thiouracil (XI), by condensing *N*-acetylthiourea and ethyl butyrylacetate, and 2-thiouracil-6-propionic acid (XII), by condensing thiourea and ethylmethyl-3-ketoadipate prepared by the method of Korman (14).

2-Thiouracil-6-propanol (XIII)—An ethanolic solution of 1.14 g (5 mmoles) of an ethyl ester of XII was reduced with 3.78 g (0.1 mole) of sodium borohydride. The solution was refluxed for 2 hr after the evolution of hydrogen ceased. The solvent was removed under vacuum and the residue was dissolved in 100 ml of water. The pH was adjusted to 3 with hydrochloric acid, and the precipitate obtained was filtered and recrystallized from aqueous ethanol.

Table III—Inhibition of Thyroid Peroxidase by Derivatives of 6-*n*-Propyl-2-thiouracil

Compound	Concentration, $\times 10^{-5}$ *M*	Specific Activity of Peroxidase	Inhibition, %
None	—	1.93 ± 0.11	—
6-*n*-Propyl-2-thiouracil	50	0.19 ± 0.02	90.2
	10	1.11 ± 0.07	42.5
	5	1.47 ± 0.07	24.0
X	100	1.62 ± 0.07	16.0
	50	1.79 ± 0.13	7.3
	10	1.92 ± 0.13	0
XI	50	0.14 ± 0.01	92.8
	10	1.00 ± 0.05	48.2
	5	1.31 ± 0.08	21.3
V	50	0.24 ± 0.05	87.5
	10	1.18 ± 0.02	39.0
	5	1.37 ± 0.07	29.0
XV	100	1.97 ± 0.08	0
	50	1.84 ± 0.09	4.7
	10	1.89 ± 0.05	2.1
XII	10	1.82 ± 0.05	5.7
	5	1.90 ± 0.08	1.6
	1	1.93 ± 0.10	0
XIII	10	1.75 ± 0.07	9.3
	5	1.86 ± 0.08	3.4
	1	1.94 ± 0.05	0

1,2-Dimethyl-6-*n*-propyl-2-thiouracil (XIV)—A solution of 0.85 g (4.0 mmoles) of X in 10 ml of 0.5 *N* NaOH was treated with a solution of 0.45 ml of methyl iodide in 20 ml of ethanol. The mixture was stirred for 2 hr at 70°, acidified with hydrochloric acid, and extracted with chloroform. The extract was dried over anhydrous magnesium sulfate, filtered, and condensed under vacuum to yield an oily substance which solidified after several hours.

5-Methoxy-2-methyl-6-*n*-propyl-2-thiouracil (XV)—Compound VII (500 mg, 2.7 mmoles) was dissolved in 1 ml of water containing 0.11 g of sodium hydroxide, 0.4 ml of dimethyl sulfate was added, and the mixture was stirred for 10 min at room temperature. The mixture was acidified with hydrochloric acid and extracted with chloroform. The chloroform extract was dried and filtered, and the solvent was removed under vacuum to yield a white solid.

6-*n*-Propyl-2-thiouracil Disulfide (XVI) and 2-Thiouracil Disulfide (XVII)—These compounds were synthesized by an adaptation of the procedure of Miller *et al.* (15) and were recrystallized from a water–acetone mixture. Several attempts to synthesize 6-*n*-propyl-2-thiouracil glucuronide were unsuccessful. However, this compound was isolated from rat bile after 6-*n*-propyl-2-thiouracil administration and subsequent column chromatography[2] and also preparative chromatography on cellulose TLC plates.

The purity of each compound was verified by TLC on precoated plastic sheets[3] run in 0.05 *M* ammonium carbonate, on cellulose sheets[4] run in 1 *M* ammonium acetate–ethanol (15:75), or on silica gel sheets[5] run in benzene–isopropanol (6:1).

Peroxidase Assay—Porcine thyroid peroxidase was prepared as previously outlined (16) and assayed by a modification of the guaiacol test (17). The assay medium contained 190 μmoles of guaiacol, 108 μmoles of tromethamine hydrochloride (pH 7.4), 1.0 mg of enzyme protein, and various concentrations of propylthiouracil derivative (in 0.3 ml of ethanol or water) in a final volume of 3.0 ml. The reaction was initiated by the addition of 2 μmoles of hydrogen peroxide. The increase in absorbance at 470 nm was measured for 15 sec during the initial linear reaction using a recording spectrophotometer[6]. Protein was determined by the method of Lowry *et al.* (18).

[2] Bio-Gel P-2.
[3] CEL 300 DEAE, Brinkmann Instruments, Inc.
[4] Eastman Kodak 6064.
[5] Eastman Kodak 13179.
[6] Perkin-Elmer 124.

RESULTS AND DISCUSSION

Some derivatives of propylthiouracil were synthesized and pertinent physical constants are shown in Table I. These compounds were used as authentic standards in studies of propylthiouracil metabolism. Several radioactive metabolites of propylthiouracil in bile and urine were identified by comparing the chromatographic properties of the compounds in Table I with those of radioactive metabolites isolated and purified by chromatographic methods (19).

In recent studies (1, 2) two metabolites of propylthiouracil were identified. Propylthiouracil glucuronide was reported in human urine and plasma and in rat bile and plasma. Sulfate was also found in human urine and thyroid and in rat bile, thyroid, and plasma. Subsequently, propylthiouracil glucuronide was reported (4) to be the major metabolite of propylthiouracil in both rat urine and bile. Results obtained in this laboratory (19) confirmed the presence of propylthiouracil glucuronide in rat bile and urine and identified three other urinary metabolites as sulfate, *S*-methylpropylthiouracil, and 6-*n*-propyluracil. In addition to these metabolites which have been positively identified, Desbarats-Schönbaum *et al.* (3) reported that highly alkalinized guinea pig urine contained a metabolite with the same R_f value as propylthiouracil disulfide in a TLC system. Several metabolites in bile and urine have chromatographic properties different from any compound shown in Table I and remain unidentified.

The metabolites of propylthiouracil were examined to determine their effects on thyroid peroxidase, the primary site of the antithyroidal action of propylthiouracil in the thyroid gland (Table II). The nature of the metabolites makes it immediately apparent that a major site of metabolism in the propylthiouracil molecule is the S group. In addition to the compounds obviously formed by alteration of the S, preliminary data (20) indicate that propylthiouracil glucuronide is a S conjugate. None of the metabolites was as active as the parent compound. Metabolic replacement of the sulfur with oxygen (6-*n*-propyluracil) and conjugation of the sulfur with a methyl group abolished the inhibitory effect on peroxidase. The disulfide was slightly active but was less than 10% as potent as propylthiouracil. Although the material used was chromatographically pure, this compound is very unstable, readily forming propylthiouracil. Part of the activity of the disulfide may be due to formation of the parent compound. The glucuronide of propylthiouracil was the most active metabolite and appears to be slightly more than 10% as active as propylthiouracil.

Although all of the metabolites in rat bile and urine have not been identified, the S group of propylthiouracil appears to be the major site of alteration; biotransformation at this site results in a total or major loss of antiperoxidase activity. All compounds shown in Table I in which the sulfur of propylthiouracil was replaced by oxygen to form the 6-*n*-propyluracil derivative were completely inactive as inhibitors of thyroid peroxidase. This demonstrates that sulfur is essential for the antiperoxidase activity to be expressed. However, the oxygen analogs are useful for identification of the sulfur analogs, which can be readily desulfurated.

The results of assays of the antiperoxidase activity of propylthiouracil derivatives which have not been implicated in metabolism are presented in Table III. Methylation of the 1-*N* group (Compound X) reduced antiperoxidase activity to less than 5% of that of propylthiouracil. However, substitution at the same position with an acetyl group (Compound XI) produced no alteration in antiperoxidase activity. 5-Hydroxy-6-*n*-propylthiouracil is also equally as active as the parent compound. The 6-*n*-propyl side chain was converted to a 6-propionic acid group (Compound XII) or a 6-*n*-propanol side chain (Compound XIII) with a marked reduction in antiperoxidase activity. Higher concentrations of these two substances could not be assayed due to their limited solubilities in aqueous solutions.

The results presented demonstrate that the antiperoxidase activities of all known metabolites of propylthiouracil are substantially lower than that of the parent compound, demonstrating that this biological effect is reduced or eliminated upon biotransformation. None of the 6-*n*-propyluracil derivatives was an inhibitor of peroxidase and only two propylthiouracil derivatives, XI and VII, were as potent as the parent compound.

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