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The Metabolism of Arylthioureas— I. The Metabolism of 1,3-Diphenyl-2-Thiourea (Thiocarbanilide) and its Derivatives

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Aryl derivatives of thiourea are compounds of considerable biological interest. The monoaryl derivatives, $ArNH.CS.NH_2$, are relatively toxic[1] and one of them, α-naphthylthiourea, has been used as a rodenticide,[2] for it appeared to be more toxic to rats than other species. The symmetrical diarylthioureas, ArNH.CS.NHAr, however, are of low toxicity and a number of them have been used as therapeutic agents in tuberculosis and, particularly, in leprosy. Thus, 1-(*p*-butoxyphenyl)-3-(*p*-dimethylaminophenyl)-2-thiourea (SU 1906) has recently received clinical trial in leprosy.[3] It appears that there are considerable differences in metabolism between the toxic monoarylthioureas and the relatively non-toxic diarylthioureas and this has already been reported briefly.[4] This paper is concerned with the metabolism of diarylthioureas; monoarylthioureas are discussed in the succeeding papers.[5–7]

Experimental

Materials. Diphenylthiourea, m.p. 253–254°, was purchased and purified; 1-(*p*-hydroxyphenyl)-3-phenyl-2-thiourea, m.p. 161–162°, was prepared from phenylisothiocyanate and *p*-aminophenol;[8] 1,3-di(*p*-hydroxyphenyl)-2-thiourea, m.p. 235–236°, and 1,3-di(*p*-chlorophenyl)-2-thiourea, m.p. 176°, were prepared from sulphur, carbon disulphide, and *p*-aminophenol or *p*-chloroaniline.[8,9]

Animals. The experimental animals were chinchilla rabbits kept on a constant diet of 100 g of rat cubes (diet No. 41, Associated Flour Millers) and 200 ml of water per day. Compounds

were administered orally suspended in water, and urine was collected daily for analysis.

Analytical Methods. Glucuronic acid and ethereal sulphate in urine were determined as previously described.[10]

Thione (C=S) compounds were determined with Grote's reagent using a modification described by Chesley.[11] The modification was necessary to give consistent results with different arylthioureas.

Buffered Grote's Reagent. To obtain consistent results, Grote's reagent had to be made up with considerable care, and buffered. Sodium nitroferricyanide (1 g) was dissolved in water (30 ml) and hydroxylamine hydrochloride (1 g) was added with swirling. After exactly 2 min, 10 per cent w/v aqueous sodium bicarbonate (20 ml) was added, followed exactly 10 min later by bromine (0·22 ml). After a further 10 min, a 2 per cent (w/v) aqueous solution of phenol (10 ml) was added to the solution and after standing for 10 min, the solution was ready for use. For the estimation of arylthioureas, one volume of Grote's reagent was diluted with 9 vols of 0·05M phosphate buffer of pH 6. Fresh reagent was made up daily. It has been recorded in the literature that Grote's reagent can be kept for several weeks at 0°, but this was contrary to our experience. In order to obtain consistent quantitative results with the reagent, we found that it had to be used within 2 h of being made up. For the estimation of thiourea unbuffered Grote's reagent can be used, but with the arylthioureas the blue colour faded rapidly unless the reagent was buffered. Unbuffered Grote's reagent is apparently sufficiently alkaline to destroy either the coloured complex or the arylthiourea.

Standard curves in water and diluted urine were prepared for each arylthiourea. An aqueous or a 50 per cent (v/v) aqueous ethanolic solution, according to the solubility of the compound (0·5 mmole/100 ml) was prepared and diluted with water or 50 per cent aqueous ethanol so as to obtain a series of solutions containing from $0 \cdot 25 \times 10^{-6}$ to $2 \cdot 0 \times 10^{-6}$ moles of thiourea or the arylthiourea in 3 ml of solution. To each of these, 2 ml of buffered Grote's reagent were added and the solutions mixed. The blue colour was read at the appropriate wavelength (see below) in a Unicam spectrophotometer (S.P. 600) when the maximum colour had developed. The time for maximum colour development varied with the thiourea.

Phenylthiourea developed maximum colour in 90 min in water and 50 min in urine (see below); two hours was required by thiourea in water or urine, whilst diphenylthiourea required 6 h in water and 4 h in urine. The accelerating effect of urine is not understood. Blanks consisted of 3 ml of water and 2 ml of buffered Grote's reagent. Standard curves were also constructed with added urine, each 3 ml of test solution containing 0·5 ml of rabbit urine, and in this case the blank consisted of 0·5 ml of rabbit urine, 2·5 ml of water and 2 ml of buffered Grote's reagent. The curves obtained with or without urine were similar except that with urine the curves became non-linear above an optical density of 0·8. The absorption peaks of the colour obtained varied with the thiourea and were as follows; thiourea 580 mμ, phenylthiourea and *p*-hydroxyphenylthiourea 590 mμ, and diphenylthiourea 595 mμ, and for these four compounds 10^{-6} mole in 3 ml of solution produced an optical density of 0·45–0·48. The colour obtained with 1,3-di(*p*-hydroxyphenyl)-2-thiourea was unstable and gradually changed from blue to magenta. Recoveries of phenylthiourea added to rabbit urine were 104 per cent (range 99–108) and of diphenylthiourea added to a mixture of equal volumes of ethanol and rabbit urine were 101 per cent (range 99–105).

Chromatography. R_f values and colour reactions for the compounds and metabolites involved in this work are shown in Table I.

Detection, Isolation and Synthesis of Metabolites

(*a*) *1,3-Diphenyl-2-thiourea.* A total of 2·94 g of diphenylthiourea was fed to 3 rabbits and a 24-h urine specimen (400 ml) collected. The urine was normal in colour and had a pH of about 6, but its volume was greater than normal. Tests with ferric chloride and for *p*-aminophenol were negative, but strong positive reactions were obtained with Grote's reagent and naphthoresorcinol. These latter tests suggested the presence of a glucuronide containing a CS group. Paper chromatography of the urine in solvent A (see Table I) revealed a large spot R_f 0·5 giving positive Grote's and naphthoresorcinol tests. Tests for diphenylthiourea and its free 4-hydroxy- and 4,4′-dihydroxy-derivatives were negative. The urine also gave an unidentified spot of R_f 0·17 reacting weakly with Grote's reagent—this was probably an

Table I. R_f values and colour reactions of diarylthioureas and diarylureas

	R_f in solvent				Colour reactions					
								Brentamine[b]		Ultra-violet light[c]
	A	B	C	D	Grote's	Silver nitrate	Iodine–Na azide	Red GG	Red RC	
Diphenylthiourea	0·93	0·94	--	0·93	blue	brown	white spot	none	none	d.s.
Diphenylurea	0·89	0·75–0·9	—	0·89	none	none	no[a] change	none	none	d.s.
1-(*p*-Hydroxyphenyl)-3-phenylthiourea	0·92	0·36–0·56	0·93	0·27	blue	brown	white spot	pink	pink	d.s.
1-(*p*-Hydroxyphenyl)-3-phenylurea	0·97	0·03–0·24	0·91	0·05–0·16	none	none	no[a] change	brownish-green	yellowish pink	d.s.
1,3-Di(*p*-hydroxyphenyl)-thiourea	0·86	0	0·82	0	blue	brown	white spot	yellow, then brown after Na_2CO_3	grey	d.s.
1,3-Di-(*p*-hydroxyphenyl)-urea	0·87	0	0·6–0·7	0	none	none	no[a] change	pink, then olive green after Na_2CO_3	pink	d.s.
1-(*p*-Glucosiduronophenyl)-3-phenylthiourea	0·56	0	—	0	blue	brown	white spot	none	none	d.s.
1-(*p*-Glucosiduronophenyl)-3-(*p*-hydroxyphenyl)-thiourea	0·38	0	—	0	blue	brown	white spot	orange pink	pink	d.s.

1,3-Di(*p*-chlorophenyl)-thiourea	—	- -	0·95	0·92	blue	brown	white spot	none	none	d.s.
1,3-Di(*p*-chlorophenyl)urea	—	—	0·95	0·95	none	none	no[a] change	none	none	d.s.

[a] In brown background. [b] I.C.I. Ltd. (stabilized diazo compounds). Brentamine Fast Blue 2B was also used. [c] d.s. = dark spot.

Descending chromatography was used with Whatman No. 4 paper

Solvent systems: A, butanol–acetic acid–water (4 : 1 : 5 by vol.); B, benzene saturated with formic acid (98%); C, benzene–butanol–water (5 : 1 : 1 by vol.); and D, benzene–acetic acid–water (1 : 1 : 2 by vol.).

Spraying reagents: (*a*) Buffered Grote's reagent, prepared as described in the text, was diluted with an equal volume of water. After spraying, the paper was warmed, and C=S compounds showed up as blue spots. (*b*) Five per cent (w/v) aqueous silver nitrate; this gives a brown stain with thioureas, but not with ureas. (*c*) Iodine–sodium azide spray of Feigl.[14] A solution of 3 g of sodium azide in 100 ml of 0·1N iodine. This gives white spots on a brown background with C=S compounds. (*d*) Hydroxyaryl-ureas and -thioureas were detected by spraying with 1% (w/v) of Brentamine Fast Red GG or Brentamine Fast Red RC or Brentamine Fast Blue 2B (stabilized diazo compounds, I.C.I. Ltd.) followed by 5% (w/v) aqueous Na_2CO_3. Glucuronides were detected by spraying with a 1% (w/v) solution of naphthoresorcinol in 50% (v/v) aqueous ethanol diluted with an equal volume of syrupy phosphoric acid just before spraying. The chromatograms were heated in a hot air oven for 5 min, and the glucuronides showed up as blue spots. (*f*) The thioureas and ureas quenched ultra-violet light and appeared as dark spots when the paper was viewed under ultraviolet light. This was the only way of detecting diphenylurea (carbanilide).

ethereal sulphate. On keeping some of the urine for 3 days at 0° and then chromatographing it, traces of free 4-hydroxydiphenylthiourea were detected. The urine thus contained two metabolites, a main one which from the tests appears to be the glucuronide of 4-hydroxydiphenylthiourea, and a minor one, probably the corresponding ethereal sulphate.

It was discovered in preliminary experiments that systematic lead acetate precipitation[12] could not be used for isolating glucuronides of the arylthioureas owing to desulphuration. A portion (120 ml) of the above urine was filtered through glass wool and treated with concentrated HCl (30 ml), and then kept at 0° for 3 h. The pinkish-brown deposit which formed was filtered, washed with water and recrystallized (charcoal) from hot water (yield 0·45 g or 27 per cent of the dose). It was repeatedly recrystallized from hot ethanol (charcoal) to form very small white needles of m.p. 170–171° with $[\alpha]_D^{21} - 61 \cdot 5 \pm 1°$ (*c*, 0·6 in water as the Na salt). The compound gave a positive Grote's and naphthoresorcinol test and was the *monohydrate* of 1-(p-*glucosiduronophenyl*)-3-*phenyl-2-thiourea* identical with a synthetic sample (see below).

Anal. Calcd. for $C_{19}H_{20}N_2O_7S \cdot H_2O$: C, 52·05; H, 5·1; N, 6·4; S, 7·3; H_2O, 4·1; equiv. wt, 438. Found: C, 51·9; H, 5·1; N, 6·8; S, 7·2; H_2O, 4·4; equiv. wt (by titration), 441.

Its absorption spectrum was almost the same as that of diphenylthiourea.[7]

The above glucuronide was synthesized as follows. To a solution of *p*-aminophenyl glucuronide[13] (303 mg) in water (10 ml), sodium bicarbonate (84 mg) was added and the solution warmed. To this, acetone (10 ml) and phenylisothiocyanate (0·135 ml) was added and the mixture kept on a boiling water-bath for 30 min and then at room temperature overnight. The solution was filtered, acidified with 10 per cent (v/v) HCl and the precipitate which formed was collected and dried (yield, 350 mg or 79 per cent). On recrystallization from hot water (charcoal), the glucuronide formed small white needles, m.p. 170–170·5°, not depressing the m.p. of the biosynthetic glucuronide obtained by feeding diphenylthiourea (above) or 4-hydroxydiphenylthiourea (see below). It showed $[\alpha]_D^{22} - 59 \cdot 5 \pm 1°$ (*c*, 1 in water as Na salt) and was identical in all respects with the biosynthetic samples.

The faeces of the three rabbits dosed with diphenylthiourea were collected for 6 days and extracted by boiling with acetone. The extract gave a positive Grote's test, and diphenylthiourea was found to be present by paper chromatography. This suggests that some of the compound escapes absorption.

(*b*) *1-*(p-*Hydroxyphenyl*)*-3-phenyl-2-thiourea.* The hydroxy compound (1·13 g) was administered orally to a rabbit and a 24-h urine specimen (200 ml including cage washings) was collected. The urine was acid (pH 6) and tests for thione compounds and glucuronic acid were positive, but tests for *p*-aminophenol were negative. Paper chromatography in solvent A (see Table I) gave the same spots as in diphenylthiourea urine. Tests for other compounds such as *p*-hydroxyphenylurea were negative. The glucuronide was isolated by acidification as above (yield 1·2 g or 73 per cent of the dose). It had m.p. and mixed m.p. 169·5–170·5° after recrystallization and $[\alpha]_D^{21} - 60 \cdot 7 \pm 1°$ (*c*, 0·6 in water as Na salt) and was identical with 1-(*p*-glucosiduronophenyl)-3-phenyl-2-thiourea.

Anal. Found: S, 7·2 per cent; equiv. wt, by titration, 438·5.

(*c*) *1,3-Di*(p-*hydroxyphenyl*)*-2-thiourea.* A total of 3·2 g of the compound was fed to 3 rabbits and a 24-h urine specimen collected. The urine had pH 5·6 and gave positive Grote's and naphthoresorcinol tests, but no test for *p*-aminophenol. Paper chromatography in solvent A revealed three Grote-positive spots of R_f 0·06 (weak), 0·28–0·46 (large) and 0·85 (strong). The last spot corresponded to the compound fed in all tests tried (see Table I). The large spot gave a positive naphthoresorcinol test and a pink colour with the diazo reagent Brentamine Fast Blue 2B (I.C.I. Ltd.) and aqueous Na_2CO_3. This suggested that it was a glucuronide of a thione compound with a free phenolic group. The weak spot also gave a pink colour with the Brentamine but no naphthoresorcinol test; this may be due to an ethereal sulphate of the dihydroxy compound fed. Chromatography in another solvent (C, see Table I) revealed the presence of 1,3-di(*p*-hydroxyphenyl)-2-thiourea, but not of 1,3-di(*p*-hydroxyphenyl)urea. Positive Grote's tests were obtained on the urines for three days after dosing, possibly indicating slow absorption.

The filtered urine of the first day (400 ml) was acidified (20 ml of conc. HCl) and kept at 0° for two days. Brown crystals (0·2 g)

separated which gave a positive Grote's and naphthoresorcinol test. Recrystallization from hot water (charcoal) yielded small white needles, m.p. 177–178° and $[\alpha]_D^{22} - 56°$ (*c*, 0·5 in water as the Na salt). The compound analysed as a *hydrate* of 1-(p-*glucosiduronophenyl*)-3-(p-*hydroxyphenyl*)-2-*thiourea*.

Anal. Calcd. for $C_{19}H_{20}N_2O_8S \cdot 1{\cdot}5H_2O$: C, 49·3; H, 5·0; N, 6·05; H_2O, 5·8; equiv. wt 463. Found: C, 49·7; H, 5·3; N, 6·45; H_2O, 6·1; equiv. wt, by titration, 459.

Direct separation of the compound from the urine by acidification was only occasionally successful. However, it readily separated on acidification if the urine were first reduced to less than one-tenth its volume by evaporation under reduced pressure. In this way yields of nearly 33 per cent based on the weight of the compound fed could be obtained.

The faeces of the above rabbits were collected for 7 days after dosing. They were extracted in a Soxhlet apparatus with acetone for 8 h. The acetone extract on paper chromatography was shown to contain much of the original compound fed. The acetone extract (450 ml) was reduced to 50 ml by distillation under reduced pressure and treated with light petroleum (b.p. 40–60°). An aqueous layer separated and this was concentrated to 10 ml. On cooling, crystals separated (0·3 g, or 9·4 per cent of the dose) which were identified as 1,3-di(*p*-hydroxyphenyl)-2-thiourea by chromatography in three solvents and by m.p. and mixed m.p. 235–236°, after recrystallization from 50 per cent aqueous ethanol.

(*d*) *1,3-Di*(p-*chlorophenyl*)-*2-thiourea*. A total of 3·7 g of the compound was fed to three rabbits. The 24-h urine specimen collected gave a negative Grote's test and the naphthoresorcinol reaction was normal. Traces of the compound were found in the urine by continuously extracting the urine with ether and chromatographing the concentrated extract. The faeces of the above animals were collected for 3 days and triturated with acetone. The acetone extract was reduced *in vacuo* to a small volume and light petroleum (b.p. 40–60°) added to throw out water and inorganic salts. The light petroleum fraction was evaporated and the residue recrystallized (charcoal) repeatedly from aqueous ethanol. 4,4′-Dichlorodiphenylthiourea (100 mg after purification, i.e. 2·7 per cent of the dose), m.p. and mixed m.p. 176°, was

recovered from the faeces. 1,3-Di-(*p*-chlorophenyl)urea was not detected in the urine or faeces. It appears that 1,3-di(*p*-chlorophenyl)-2-thiourea is only slightly absorbed and no evidence was obtained to suggest that it was metabolized.

Results and Discussion

The quantitative aspects of the metabolism of 1,3-diphenyl-2-thiourea and its derivatives are shown in Table II. About

Table II. The metabolism of diphenylthiourea and its derivatives in rabbits

Compound	Dose, mg/kg	% of dose excreted in urine in 2 days as[a]			Faecal excretion
		Thione compounds	Glucuronide	Ethereal sulphate	
1,3-Diphenyl-2-thiourea	60	65 (61–68)	—	—	
	100	66 (62–69)	—	—	
	400	73 (72–74)	62 (58–69)	5 (3–7)	detected in faeces (by colour tests and chromatography
1-(*p*-Hydroxyphenyl)-3-phenylthiourea	400	82 (81–83)	67 (63–72)	2 (0·5–2·5)	—
1,3-Di(*p*-hydroxyphenyl)-thiourea	400	> 50[b]	60 (56–62)	2·5 (1·5–3)	isolated from faeces (9·5% of dose isolated)
1,3-Di(*p*-chlorophenyl)-thiourea	400	0 (0–0)	8 (7–11)	6 (4–8)	isolated from faeces (3% isolated in pure state)

[a] The values given are the average for 3 animals, the ranges being given in parentheses.
[b] The colour obtained with the dihydroxy compound and Grote's reagent is unstable.

65–75 per cent of diphenylthiourea is excreted as thione compounds in the urine, and this appears to be made up almost entirely of conjugates of 1-(*p*-hydroxyphenyl)-3-phenyl-2-thiourea, mainly the glucuronide. The fact that some of the unchanged compound can be detected in the faeces suggests that it may not be completely absorbed. No evidence was obtained of the formation of 1,3-diphenylurea derivatives and it can be concluded that diphenylthiourea does not lose its sulphur *in vivo*. Its main metabolic

reaction is *para*-hydroxylation and subsequent conjugation with glucuronic acid.

$$C_6H_5\cdot NH\cdot CS\cdot NH\cdot C_6H_5 \longrightarrow C_6H_5\cdot NH\cdot CS\cdot NH\cdot C_6H_4OH \longrightarrow C_6H_5NH\cdot CS\cdot NH\cdot C_6H_4OC_6H_9O_6$$

The fate of 1-(*p*-hydroxyphenyl)-3-phenylthiourea is relatively simple. Over 80 per cent of it is excreted with the thione group intact and no evidence of desulphuration to urea derivatives was found. Its main metabolite is the corresponding glucuronide which accounts for nearly 70 per cent of the dose; small amounts of the ethereal sulphate and the unchanged compound are also excreted in the urine. Its glucuronide is identical with that obtained from the metabolism of diphenylthiourea.

1,3-Di-(*p*-hydroxyphenyl)-2-thiourea again is not desulphurized. It is not entirely absorbed since significant amounts (nearly 10 per cent by isolation) of the unchanged compound can be isolated from the faeces. Its main urinary metabolite, which accounts for 60 per cent of the dose, is the corresponding monoglucuronide. Small amounts of the ethereal sulphate and the unchanged compound are also found in the urine.

1,3-Di(*p*-chlorophenyl)-2-thiourea may not be readily absorbed for it can be detected in the faeces in appreciable amounts judging by the yield of crude material, although only 3 per cent was isolated in the pure state from the faeces. Only traces of the unchanged compound are found in the urine and there is no evidence to show that it is desulphurized. Table II shows small rises in glucuronide and ethereal sulphate output after feeding the compound, but it is doubtful whether or not these values have any significance.

None of the diarylthioureas used here had any toxic effects on rabbits in doses of 400 mg/kg and there was no evidence that they were desulphurized *in vivo*. This is in contrast with the monoarylthioureas which are discussed in the succeeding papers.[5–7]

Summary. The fate of 1,3-diphenyl-2-thiourea and its *p*-hydroxy-, *p,p'*-dihydroxy- and *p,p'*-dichloro-derivatives in rabbits has been studied. Diphenylthiourea (dose 0·4 g/kg orally) is mainly hydroxylated in the body to 1-(*p*-hydroxyphenyl)-3-phenyl-2-thiourea which is excreted mainly as the corresponding *O*-glucuronide which was isolated and characterized. This glucuronide was identical with an authentic sample synthesized from *p*-

aminophenyl glucuronide and phenylisothiocyanate. 1-(*p*-Hydroxyphenyl)-3-phenyl-2-thiourea (0·4 g/kg orally) is metabolized mainly by conjugation with glucuronic acid to give the same glucuronide as obtained by feeding diphenylthiourea.

1,3-Di(*p*-hydroxyphenyl)thiourea (0·4 g/kg orally) is excreted mainly as a monoglucuronide, which was isolated and identified as 1-(*p*-glucosiduronophenyl)-3-(*p*-hydroxyphenyl)-2-thiourea. Some of the unchanged compound was found in the faeces. The above three compounds also form small amounts of ethereal sulphates (2–5 per cent of the dose), but they were not desulphurized *in vivo* because the corresponding 1,3-diphenylureas could not be detected in the urine.

1,3-Di(*p*-chlorophenyl)-2-thiourea (0·4 g/kg orally) is probably excreted mainly unchanged in the faeces and does not appear to be metabolized.

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