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THE REACTIONS OF ISOTHIOCYANATES WITH 2-AMINO SUGARS: ELECTROPHORESIS IN BORATE AND TUNGSTATE BUFFERS, AND SPECTROSCOPY OF THE PRODUCTS

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ABSTRACT

The reaction of 2-amino-2-deoxyaldoses with isothiocyanates goes through three stages, producing a thiourea (**1**), which cyclises to a 5-hydroxyimidazolidine-2-thione (**4**) with concomitant opening of the sugar ring. A molecule of water can then be lost, with the formation of a furano ring, the imidazolidine-2-thione remaining intact in a fused, bicyclic structure (**3**). Products (**3**) derived from 1-amino-1-deoxy-2-ketoses are spiro compounds. Compound **4** is stable in neutral or alkaline solution, but converts quickly into **3** in acid. The conversion into **4** is extremely rapid, and its existence is inferential.

Conversion of **4**, with loss of 1 mole of water per mole, into either an imidazole-2-thione (**6**) or a C-5 substituted imidazolidine-2-thione (**3**) is discussed, utilising the pseudo-basic properties of **4**.

Mixtures of products **4** and **3** are separable by electrophoresis in tungstate and borate buffers. Electrophoretic mobilities of a number of amino-sugar derivatives are given.

INTRODUCTION

In preliminary reports[1,2], it was shown that thioureido compounds initially formed in the reaction of 2-amino-2-deoxy-hexoses and -pentoses with organic isothiocyanates were converted quickly and quantitatively into hydroxyimidazolidine-2-thiones, in which the sugar rings had opened. From more vigorous conditions, crystalline products were isolated[3] which were thought to contain fused imidazolidine and pyranose rings, but which were later shown to possess furanoid structures[4]. The relationships between these compounds are considered in this investigation.

MATERIALS AND METHODS

Materials. — 2-Acetamido-2-deoxy-D-allose, 2-acetamido-2-deoxy-D-ribose, 2-acetamido-2-deoxy-D-arabinose, 2-acetamido-2-deoxy-D-altrose (Professor R. Kuhn *via* Dr. D. A. L. Davies), 2-amino-2-deoxy-D-fucose (Dr. D. A. L. Davies), 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose (Dr. R. W. Jeanloz), 3-amino-3,6-dideoxy-L-talose

(Dr. A. C. Richardson), 1-amino-1-deoxy-D-fructose (Dr. G. Huber), 2-amino-2-deoxy-D-glucitol (Dr. P. W. Kent), phenyl 2-amino-2-deoxy-β-D-glucopyranoside (Dr. D. M. Leaback), and methyl 2-amino-2-deoxy-α-D-glucopyranoside (Dr. R. D. Marshall) were gifts, and 2-deoxy-2-methylamino-L-glucose was prepared by hydrolysing streptomycin.

Compounds originally formulated[3] as "2-deoxy-2-(*p*-tolylcarbamido)-β-D-glucose", "2-deoxy-2-(*p*-tolylthiocarbamido)-β-D-glucose", "2-oxo-3-(*p*-tolyl)-4,5-D-glucopyranotetrahydroimidazole", "2-thio-3(*p*-tolyl)-4,5-D-glucopyranotetrahydroimidazole" were gifts from Dr. C. Morell. 2-Mercapto-4-(D-*arabino*-tetrahydroxybutyl)-1-tolylimidazole was a gift from Dr. G. Huber.

N,N-Dimethyldodecylamine, distilled grade (DM12D) was a gift from Armour Hess Ltd.

Methods. — Ascending chromatography was carried out by using 20:3 isopropyl alcohol–water and Whatman No. 1 paper, with or without impregnation with 50mM sodium molybdate–H_2SO_4 (pH 5.0) and drying.

Electrophoresis was carried out on a horizontal strip of Whatman No. 1 paper, in a Kohn apparatus (Shandon Scientific Co. Ltd.) at 10 volts/cm, with a maximum current of 0.7 mamp/cm width. The paper was soaked in buffer and blotted dry, and aliquots were applied with the paper in position. After 1–2 h, the paper was removed and rapidly dried at 110°.

Borate, molybdate, and tungstate were 50mM as sodium salts, adjusted to the appropriate pH with hydrochloric, sulphuric, or acetic acid. McIlwaine's phosphate–citrate buffers were 50mM.

Thiourea, used as the origin marker, was easily detected by its u.v. absorbance, or with silver nitrate[5], or dichloroquinone–chloroimide[6]. Glycerol was also used as a non-migratory marker during electrophoresis in sodium tungstate.

D-Mannitol or D-glucose were reference compounds in tungstate or borate, respectively.

Detection. — Products of the reaction of isothiocyanates with amines appear as dark spots on a light-blue ground in light of 2537Å (Chromatolite, Shandon). Tungstate and molybdate absorb u.v. light, thus reducing the sensitivity. Alkaline silver nitrate[5] is particularly sensitive for compounds containing a divalent sulphur atom. Molybdate, tungstate, and borate do not interfere. Sulphur-containing products can be detected by passing the dried paper through a 0.1% solution of iodine in chloroform, and air-drying. The dark-brown spots on a pale ground fade in a matter of hours, but can be re-produced on subsequent dipping. Multiple dipping increases the sensitivity, at the expense of increase in the background. Dichloroquinone–chloroimide[6] reacts well with the amino sugar–isothiocyanate derivatives, giving red or orange spots which develop in an hour or two. Spots and background turn grey in a few days. Naphthyl derivatives fluoresce intensely yellow on the *dried* paper under a Chromatolite lamp. The strip is examined immediately after removing from the drying oven. The fluorescence intensity declines in minutes, but can be restored by reheating to 105° for a short time.

U.v. spectra were determined for aqueous solutions by using a Unicam SP500 spectrophotometer fitted with a synthetic quartz prism. Slightly soluble materials were dissolved to 1% in ethanol, and 0.1-ml aliquots were diluted rapidly to 100 ml with distilled water. Appropriate controls containing 0.1% of ethanol were used in these cases.

I.r. spectra were determined for KBr discs on a Perkin–Elmer 137 spectrometer, by Mrs. J. Chittenden and Mr. L. C. Thomas of the Chemical Defence Establishment, Porton.

Isothiocyanate derivatives of amino sugars. — Two volumes of a pyridine solution of the isothiocyanate (≮20% excess), also containing 5–10% of collidine, triethylamine, or *N*,*N*-dimethyldodecylamine, were added to one volume of the aqueous sugar solution. After incubation, usually at 55°, for 20–60 min, four volumes of benzene were added with thorough mixing followed by centrifugation. The upper, benzenoid layer was removed, and the extraction was repeated twice to remove pyridine, tertiary amine, and by-products. The following compounds were prepared thus.

(*a*) 5-Hydroxy-1-phenyl-4-(D-*arabino*-tetrahydroxybutyl)imidazolidine-2-thione (**4**, R = phenyl) was obtained by employing solutions of 2-amino-2-deoxy-D-glucose hydrochloride (10%), phenyl isothiocyanate, and triethylamine at 37°. After storage for 16 h *in vacuo* over P_2O_5, the crystals in the syrup were washed rapidly with cold water and dried over P_2O_5 to give material (6%) which "bubbled" at 90–100°, resolidified at 116–118°, and melted at 152–159°. It had λ_{max} (water) 238 nm (ε 20,000) (Found: C, 49.7; H, 5.9; N, 8.9; S, 10.4. $C_{13}H_{18}N_2O_5S$ calc.: C, 49.7; H, 5.7; N, 8.9; S, 10.2%).

(*b*) 5-Hydroxy-1-naphthyl-4-(D-*arabino*-tetrahydroxybutyl)imidazolidine-2-thione (**4**, R = 1-naphthyl) was obtained with solutions of 1-naphthyl isothiocyanate, collidine, and 2-amino-2-deoxy-D-glucose hydrochloride (10%). The aqueous layer set to a mass of fine crystals after a few min at room temperature. The crystals were shaken with water, filtered, and washed with water to give material (77%), m.p. 154–155° (dec.) (from water) (Found: C, 54.2; H, 5.6; N, 7.2; S, 8.3. $C_{17}H_{20}N_2O_5S{\cdot}H_2O$ calc.: C, 53.3; H, 5.8; N, 7.3; S, 8.4%.)

(*c*) 5-Hydroxy-1-naphthyl-4-(D-*lyxo*-tetrahydroxybutyl)imidazolidine-2-thione (**5**, R = 1-naphthyl) (60%) was prepared as in (*b*), using 2-amino-2-deoxy-D-galactose hydrochloride as a 1% solution (Found: C, 55.6; H, 5.5; N, 7.8. $C_{17}H_{20}N_2O_5S$ calc.: C, 56.0; H, 5.5; N, 7.7%.)

(*d*) 4,5-(2-Deoxy-D-glucofurano)-1-phenylimidazolidine-2-thione (**3**, R = phenyl) (43%) was prepared by adjusting an aliquot of an aqueous solution containing **4** (R = phenyl) (see above) to pH 3–4 with hydrochloric acid. After several hours at room temperature, the product separated and had m.p. 191°, λ_{max} (water) 238 nm (ε 19,000) (Found: C, 52.5; H, 5.5; N, 9.6; S, 10.9. $C_{13}H_{16}N_2O_4S$ calc.: C, 52.7; H, 5.4; N, 9.5; S, 10.8%).

Alternatively, an aliquot of the aqueous solution containing **4** (R = phenyl) was freeze-dried. The residue was crystallised from propan-2-ol to give material

TABLE I

SUBSTITUTED THIOUREAS

Amine	*Isothiocyanate*	*Melting point of the thiourea (degrees)*	*Analyses*	ε_{max}	λ_{max} (*nm*)
2-Aminoethanol (1)	phenyl	138[a] lit.[21] 138		14,000	243
2-Aminopropan-1-ol (2)	phenyl	137.5–139[a]	$C_{10}H_{14}N_2OS$ Calc.: C, 57.1; H, 6.8; N, 13.4; S, 15.3. Found: C, 57.4; H, 6.6; N, 13.3; S, 15.2.	13,920	243
3-Aminopropan-1-ol (3)	phenyl	85[a]	$C_{10}H_{14}N_2OS$ Calc.: C, 57.1; H, 6.8; N, 13.4; S, 15.3. Found: C, 56.9; H, 6.5; N, 13.6; S, 15.2.	15,450	242–243
2,2-Diethoxyethylamine (4)	phenyl	95[b] lit.[20] 96		13,325	243–244
2-Amino-2-methyl- (5) propan-1-ol	phenyl	122[a] lit.[7] 127-128		17,050	243–244
2-Amino-2-methyl-propane-1,3-diol (6)	phenyl	127[c]	$C_{11}H_{16}N_2O_2S$ Calc.: C, 55.0; H, 6.7; N, 11,7; S, 13.3. Found: C, 55.1; H, 6.9; N, 11.3; S, 13.0.	17,800	243–244
N-Methylaniline (7)	methyl	114[a]	$C_9H_{12}N_2S$ Calc.: C, 59.9; H, 6.7; N, 15.6; S, 17.8. Found: C, 60.0; H, 6.9; N, 15.6; S, 17.9.	19,250	235

[a]From 50% EtOH–H_2O. [b]From 60%EtOH–H_2O. [c]From 40% EtOH–H_2O.

(40%), m.p. 176–178°, λ_{max} 239 nm (ε 18,750) (Found: C, 53.0; H, 5.6; N, 9.5; S, 11.7%).

*"Anhydro-PTC" (acid-treated "PTC") derivatives**. — These compounds were prepared by acidifying to pH 3–4 aliquots of the aqueous layer containing the "*PTC*" derivative of the amino sugar from the general procedure. In most cases, concentrations were too low to encourage crystal formation, and the solution was used directly for electrophoresis after neutralisation with dilute, aqueous sodium hydroxide.

Substituted thioureas (Table I). — Isothiocyanates were treated in slight excess with amines, in ether where possible, from which the product usually separated quickly at room temperature. Acetone or aqueous acetone were used for amines which were not soluble in ether.

Anilinothiazolines. — 2-Anilinothiazoline (m.p. 162°, lit. m.p. 158–160°) and 2-anilino-4,4-dimethylthiazoline (m.p. 156–157°) were prepared from *N*-(2-hydroxyethyl)-*N'*-phenylthiourea and *N*-(1,1-dimethyl-2-hydroxyethyl)-*N'*-phenylthiourea (see Table I).

Imidazolidine-2-thiones. — 1-(1-Naphthyl)imidazolidine-2-thione and 1-phenylimidazolidine-2-thione (m.p. 164°, lit. m.p. 155°) were prepared from the appropriate 2-(arylamino)ethylamines, *via* the dithiocarbamic acid, by the general method given in Beilstein (System No. 3557).

2-Anilinoethylamine was prepared[8] from aniline and ethylene imine.

N-(1-Naphthyl)imidazolidine-2-thione had m.p. 208° (from acetone) (Found: C, 68.3; H, 5.1; N, 12.5; S, 14.3. $C_{13}H_{12}N_2S$ calc.: C, 68.4; H, 5.3; N, 12.3; S, 14.1%.)

Preparation of a substituted phenyl-2-thiohydantion from 2-amino-2-deoxy-D-gluconic acid, and its reduction with sodium borohydride. — The phenylthiocarbamoyl (PTC) derivative was made from 2-amino-2-deoxy-D-gluconic acid and phenyl isothiocyanate by the general procedure, using triethylamine as the buffer, for 3 h at 37°.

The spectrum of the aqueous layer showed a characteristic phenylthioureide absorption λ_{max} 244 nm (ε 14,220, on the assumption that reaction was quantitative and no other absorbing species was present). Electrophoresis in sodium tungstate at pH 7.0 showed a single u.v.-absorbing component that travelled slightly *faster* than D-mannitol and reacted very sensitively to the silver nitrate reagent.

The solution was freeze-dried and the product (245 mg) was dissolved in 10 ml of M trichloroacetic acid and left for 16 h at room temperature. A few crystals deposited. The supernatant was thrice extracted with ether to remove trichloroacetic acid. The solution showed a characteristic phenylthiohydantoin absorption (λ_{max} 265 nm with a minimum at 245 nm). Electrophoresis in tungstate buffer at pH 7 showed two components, the minor one being immobile, the other moving just *behind* D-mannitol (Table II).

To 3 ml of the solution was added 50 mg of sodium borohydride. After 1 h, the

*PTC = Phenylthiocarbamoyl, used when the structure is not in doubt. "PTC" describes derivatives of amines with isothiocyanates in which the PTC formulation is in doubt.

TABLE II

ELECTROPHORETIC MOBILITIES OF AMINO SUGARS AND THEIR DERIVATIVES IN 50mM TUNGSTATE BUFFER

	$R_{Mannitol}$ (pH 7.0)		$R_{Glucitol}$ (pH 5.0)
	N-*Acetyl*	"*PTC*"	*2-Deoxyhexitols*[10]
2-Amino-2-deoxy-D-glucose	0	0.8	
2-Deoxy-2-methylamino-L-glucose		0.8	
2-Amino-2-deoxy-D-glucitol		0.8	1.0
2-Amino-2-deoxy-D-mannose	0	0.7	
1-Amino-1-deoxy-D-fructose		0.8	
2-Amino-2-deoxy-D-galactose		0.4	0.6
2-Amino-2-deoxy-D-altrose	0	0.7[a]	0.57[b]
2-Amino-2-deoxy-D-allose	0	0.7[b]	
2-Amino-2-deoxy-D-fucose		0.0	
2-Amino-2-deoxy-D-ribose		0.0	
2-Amino-2-deoxy-D-arabinose		0.0	0.0[c]

Other derivatives	
3-Phenyl-5-(D-*arabino*-tetrahydroxybutyl)-2-thiohydantoin (Phenyl-2-thiohydantoin from 2-amino-2-deoxy-D-gluconic acid)	0.8
1-*p*-Tolyl-4-(D-*arabino*-tetrahydroxybutyl)-imidazole-2-thione	
Compound 6 (R = *p*-tolyl)	
"Anhydro-PTC"-2-amino-2-deoxy-D-glucose (3, R = phenyl)	0.0
"Anhydro-PTC"-1-amino-1-deoxy-D-fructose	0.0
"2-deoxy-2-(*p*-tolylthiocarbamido)-D-glucose"	0.8
"2-deoxy-2-(methylthiocarbamido)-D-glucose"	0.8
"2-deoxy-2-(1-naphthylthiocarbamido)-D-glucose"	0.8
4,5-(2-Deoxy-D-glucofurano)-1-*p*-tolylimidazolidine-2-thione[4]	0.0
PTC-3-amino-3,6 dideoxy-L-talose	0.0
PTC-1-amino-1-deoxy-D-lyxose	0.0

[a]Streak. [b]Elongated spot. [c]2-Deoxy-D-*erythro*-pentitol.

thiohydantoin-absorption at 265 nm had disappeared, to be replaced by a somewhat higher peak at 238 nm (see Fig. 1). On electrophoresis in tungstate buffer at pH 7, two u.v.- absorbing components were observed, in the same positions and with the same relative intensities as those present in the solution prior to reduction. The faster spot migrated at the same rate as the reaction product from 2-amino-2-deoxy-D-glucose and phenyl isothiocyanate, prepared by the general procedure.

RESULTS

U.v. spectra (in water). — Spectra of *N'*-substituted *N*-phenylthioureas show maxima at 242–244 nm (Table I), and minima around 225 nm. The long-wave edge of this absorption band declines relatively slowly (Fig. 2). The absorption intensity is more sensitive than the frequency to changes in the nature of the non-phenyl substituent. A secondary or tertiary carbon substituent at the non-phenyl nitrogen e.g., *1–4*, Table I) is associated with an ε_{max} of $13–15 \times 10^3$; ε_{max} for a quaternary carbon substituent (*5* and *6*) increases to $17–18 \times 10^3$.

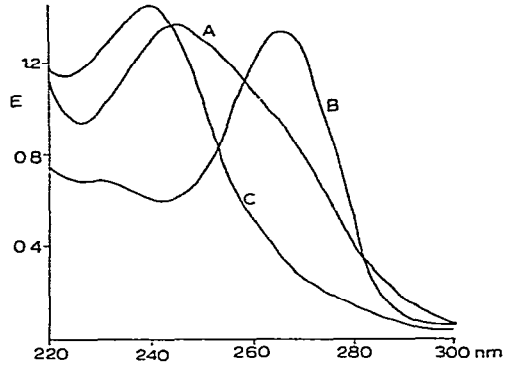


Fig. 1. Conversion of 2-deoxy-2-(3-phenylthioureido)-D-gluconic acid (Curve *A*) into the phenylthiohydantoin derivative (Curve *B*), and reduction with aqueous sodium borohydride to the 5-hydroxyimidazolidine-2-thione (Curve *C*).

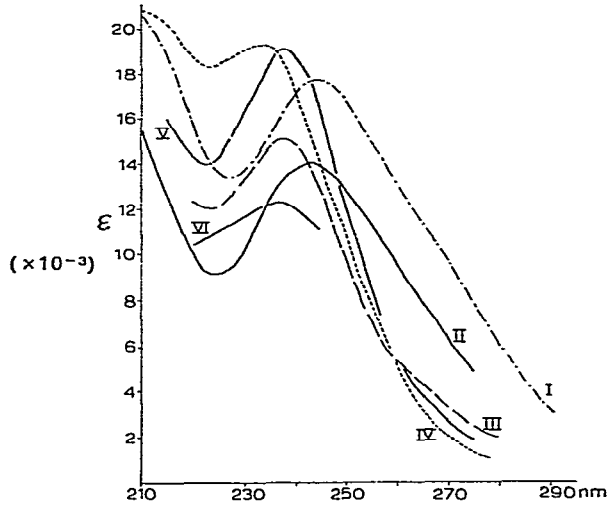


Fig. 2. U.v. spectra (in water) of: I (—·—) *N*-(1,3-dihydroxy-2-methyl)-*N'*-phenylthiourea, II (———) *N'*-(2-hydroxyethyl)-*N'*-phenylthiourea, III (– – –) 5-hydroxy-4,4-dimethyl-1-phenylimidazolidine-2-thione, IV (------) *N*-methyl-*N*-phenyl-*N'*-methylthiourea, V (———) "PTC"-2-amino-2-deoxy-D-glucose, and VI (———) 2-anilino-4,4-dimethylthiazoline.

The spectra of phenylthioureas in which the "anilino" nitrogen atom carries a second substituent show maxima at 238 nm or less (*7*, Table I). In addition to compound *7*, there are[9] 1-phenylimidazolidine-2-thione (ε_{max} 14,400) and 5-hydroxy-4-methyl-1-phenylimidazolidine-2-thione (λ_{max} 237, ε_{max} 17,500). The long-wave edge falls much more sharply than in the symmetrically *NN'*-disubstituted compounds.

2-Anilinothiazoline (ε_{max} 10,350) and 2-anilino-4,4-dimethylthiazoline (ε_{max} 12,250) both absorb maximally at about 238 nm with rather broad peaks.

The spectra of 5-hydroxy-1-phenyl-4-(D-*arabino*-tetrahydroxybutyl)imidazolidine-2-thione (**4**, R = phenyl), its 2-amino-2-deoxy-D-galactose analogue (**5**, R =

phenyl), and 4,5-(2-deoxy-D-glucofurano)-1-phenylimidazolidine-2-thione (3, R = phenyl) were identical, within experimental error, in water, 10mM borate buffer pH 8.95, and 10mM hydrogen carbonate buffer pH 8.95 (λ_{max} 238 nm, ε_{max} 19,000 ±500). The spectrum of "*p*-tolylthiocarbamoyl"-2-amino-2-deoxy-D-glucose showed λ_{max} 238 nm, ε_{max} 18,250.

Electrophoresis. — (*a*) *Citrate–phosphate buffers.* "PTC"-2-amino-2-deoxy-D-glucose and its "anhydro" derivative did not migrate on electrophoresis in McIlwaine citrate–phosphate buffers at pH 7, 5, and 3.2. The substituted phenylthioureas in Table I were also immobile, whereas 2-anilinothiazoline and 2-anilino-4,4-dimethylthiazoline moved rapidly and compactly as cations, at all three pH values.

(*b*) *Borate buffers.* Table III shows mobilities of a series of "PTC" amino sugars, compared with those of the analogous *N*-acetyl derivatives. Mobilities (R_G) are expressed relative to that of D-glucose. All of the "PTC" derivatives move much more rapidly than the *N*-acetyl compounds. Also shown are R_G values of the acid-treated "anhydro-PTC" derivatives of a number of amino sugars, most of which (except PTC-2-amino-2-deoxy-D-glucitol) are drastically reduced following the acid treatment. The R_G of acid-treated "PTC"-2-amino-2-deoxy-D-glucose is the same as that of the product of dissolution of "PTC"-2-amino-2-deoxy-D-glucose in ethanol (see preparative section). The D-glucofurano-imidazolidine derivatives of Morell migrated identically to the "anhydro PTC" derivative of 2-amino-2-deoxy-D-glucose (Table III).

TABLE III

ELECTROPHORETIC MOBILITIES OF AMINO SUGAR DERIVATIVES IN 50mM BORATE BUFFER

	R_G *in borate buffer (pH 9.2)*		
	N-*Acetyl*	*"PTC"*	*Acid-treated "PTC"*
2-Amino-2-deoxy-D-glucose	0.20	0.73	0.65
2-Amino-2-deoxy-D-galactose	0.35	0.71	0.21
2-Amino-2-deoxy-D-mannose	0.6	0.65	0.17
2-Amino-2-deoxy-D-allose	0.37	0.71	
2-Amino-2-deoxy-D-altrose	0.48	0.69	
2-Amino-2-deoxy-D-ribose	0.21	0.49	
2-Amino-2-deoxy-D-arabinose	0.21	0.47	
2-Amino-2-deoxy-3-*O*-methyl-D-glucose		0.4	0.27
1-Amino-1-deoxy-D-fructose		0.73	0.0
2-Amino-2-deoxy-D-glucitol		0.75	0.75
2-Amino-2-deoxy-D-fucose		0.60	
3-Amino-3,6-dideoxy-L-talose		0.80	
4,5-(2-Deoxy-D-glucofurano)-1-*p*-tolylimidazolidine-2-thione[4]			0.63
4,5-(2-Deoxy-D-glucofurano)-1-*p*-tolylimidazolidine-2-one[4]			0.63

"PTC" derivatives of 2-amino-2-deoxy-D-glucose, -D-mannose and -D-galactose migrate at very similar rates, so that mixtures of them could not be resolved by electrophoresis in borate buffer. There is, however, no difficulty in separating acid-

treated "PTC"-2-amino-2-deoxy-D-glucose from analogous derivatives of D-galactose and D-mannose.

The substituted phenylthioureas in Table I were all immobile in borate buffer.

(*c*) *Tungstate and molybdate buffers.* The structural features required of polyhydroxy compounds in order to complex with both molybdate and tungstate are identical[10]. Tungstate has been used most in this investigation, because it is effective at pH 7.0 (compared with pH 5.0 for molybdate), it does not absorb u.v. light as strongly, and electrode decomposition was not so noticeable. Whenever comparisons were made, results in molybdate and tungstate were similar.

In complete contrast to the behaviour of "PTC"-2-amino-2-deoxyhexoses, *N*-acetyl derivatives did not migrate in tungstate buffers (Table II). The mobilities (R_M) of "PTC"-2-amino-2-deoxyhexoses, relative to that of D-mannitol, fall into three very distinct groups, *i.e.*, *ca.* 0.8, 0.4, or 0.0. Migration is generally rapid and compact.

Chromatography with a variety of solvents, using untreated paper or paper previously soaked in borate buffer, did not result in any useful separations of the various "PTC"-hexosamines. However, chromatography on paper previously treated with 1% sodium molybdate solution, at pH 5.0, resulted in very clear resolution of 2-amino-2-deoxy-D-glucose–2-amino-2-deoxy-D-galactose mixtures. The "PTC"-derivatives move more rapidly than the parent amino sugars in all solvent systems.

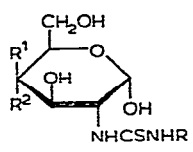
DISCUSSION

The evidence for the structure of "PTC"-2-amino-2-deoxyaldoses is of three kinds: (1) elemental analysis of the isolated product, (2) behaviour on complexing with borate and tungstate, (3) u.v. and i.r. spectroscopy.

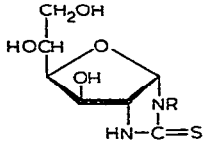
The isolation of "PTC"-2-amino-2-deoxy-D-glucose is not easy. A rapid change takes place on dissolution in many organic solvents, with the appearance of new compounds characterised by completely different electrophoretic mobility, both in tungstate and borate buffers. It is therefore necessary to work in aqueous solution, in which the compound is very soluble. Crystals are obtained from concentrated syrups, but in rather low yield. Their analysis corresponds to 2-deoxy-2-(3-phenylthioureido)-D-glucose (**1**, R = phenyl). The isolation and recrystallisation of 1-naphthyl derivatives in high yield is easy, because of a lower solubility in water. Elemental analysis of the 2-amino-2-deoxy-D-galactose derivative corresponds to a naphthylthioureido compound (**2**, R = naphthyl), whereas the D-glucose analogue is apparently a monohydrate. The melting points of both "crude" and purified products are not very meaningful. Vigorous evolution of a gas occurs at quite low temperatures, and although crystals are reformed, the subsequent melting ranges are not well defined.

Elemental analysis suggests that 2-(3-arylthioureido)-2-deoxyaldoses have been obtained, but this formulation is incompatible with the pattern of complexing in tungstate and borate buffers. The comparison with 2-acetamido-2-deoxyhexoses

is particularly interesting, in that they are totally immobile in tungstate buffer, whereas the "PTC" derivatives migrate almost as rapidly as D-mannitol. It is pertinent that pyranosides of D-glucose, D-galactose, and D-mannose do not complex with tungstate[10].



1 R^1=H, R^2=OH
2 R^1=OH, R^2=H



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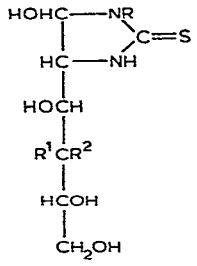
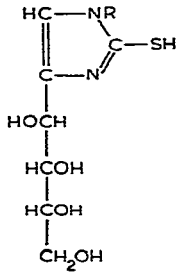
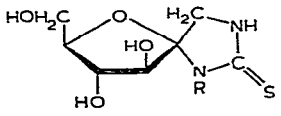


4 R^1=H R^2=OH
5 R^1=OH R^2=H



6 R=*p*-tolyl



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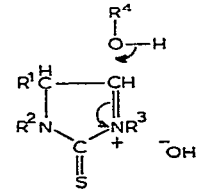

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The arylthioureido moiety is not responsible for complex formation, since none of the model compounds (Table I) showed any mobility in tungstate buffer. Alternatively, the thiocarbamoyl group could take part in a complex involving hydroxyl groups or some other part of the sugar moiety. This seems unlikely, since the PTC derivatives of phenyl 2-amino-2-deoxy-α-D-glucopyranoside and methyl 2-amino-2-deoxy-α-D-glucopyranoside do not complex with tungstate, nor do the "PTC" derivatives of 2-amino-2-deoxypentoses, 3-amino-3,6-dideoxy- and 2-amino-2,6-dideoxyhexoses, or 2-amino-2-deoxy-3-*O*-methyl-D-glucose shown in Tables II and III. If the sugar ring remains intact, then every position in the hexosamine molecule is somehow involved in the tungstate complex, and yet such a complex occurs with seven different reducing amino-sugars listed in Table II. A more reasonable alternative is that the sugar ring has opened, producing a polyhydroxy chain, since at least three suitably placed (usually vicinal) hydroxyl groups are required for formation of a tungstate complex[10]. As a first assumption, since *N*-aryl-*N'*-substituted thioureas do not complex with tungstate, the "PTC" grouping may be replaced by *O*-methyl, or hydrogen, without materially affecting the complexing ability of the rest of the molecule. When this is done, the behaviour of the appropriately 2-substituted hexitols exactly parallels those of the "PTC" derivatives of 2-amino-2-deoxyhexoses (Table II).

Furthermore, the electrophoretic behaviour of acyclic derivatives of 2-amino-2-deoxy-D-glucose ("PTC"-2-amino-2-deoxy-D-glucitol, the phenylthiohydantoin from 2-amino-2-deoxy-D-gluconic acid, and compound **6**, Table II) is identical with that of the "PTC"-2-amino-2-deoxy-D-glucose.

To reconcile a cyclic sugar derivative with the behaviour of the "PTC" compounds in borate buffer is equally difficult. The increase in electrophoretic mobility of "PTC" derivatives as compared with *N*-acetyl derivatives is unexpected, because of the larger size of the PTC substituent. Somewhat similar arguments to those used in the tungstate series are applicable. The arylthioureas of Table I do not complex with borate, so the great increase in complexing ability, shown throughout the series of aminodeoxy-hexoses and -pentoses, and *O*-methyl and deoxy derivatives can only be explained on the basis of a cyclic sugar by postulating novel and varied structures for the borate complex. With the assumption that the sugar ring has opened, the results are easily explicable. New glycol groups thus produced offer sites for additional types of complex with borate. It is concluded that an acyclic sugar derivative is present during electrophoresis in borate, if not before. In contrast, *N*-acetyl derivatives migrate as expected for 2-substituted pyranoses (with the single exception of 2-amino-2-deoxy-D-mannose, Table III).

A series of phenylthiocarbamoyl compounds containing various structural features of the "PTC"-aminodeoxyaldoses were prepared and examined spectroscopically (Table I). Remarkable constancy of λ_{max} (242–244 nm) and of the spectrum shape is observed, despite great changes in the nature of the non-phenyl substituent. It is therefore pertinent that λ_{max} of "PTC"-2-amino-2-deoxy-D-glucose is 5–6 nm lower, at 238 nm, and that the spectrum is somewhat dissimilar in shape (Fig. 2). Particularly significant is the difference in λ_{max} between "PTC"-2-amino-2-deoxy-D-glucose and PTC-2-amino-2-deoxy-D-glucitol, PTC-2-amino-2-deoxy-D-gluconic acid, and PTC-(methyl 2-amino-2-deoxy-D-glucopyranoside); the last three compounds, for which the PTC formulation seems beyond doubt, have characteristic PTC maxima at 243 nm. Moreover, λ_{max} of "PTC"-2-amino-2-deoxy-D-glucose is *ca.* 30% higher than for all of the relevant PTC model compounds. The u.v. spectrum is therefore inconsistent with a simple PTC group in "PTC"-2-amino-2-deoxy-D-glucose, and, by implication, also with an acyclic PTC aldehyde form.

On the other hand, phenylthioureas having a second substituent on the "anilino" nitrogen atom (including the *N*-phenylimidazolidine-2-thiones) have identical λ_{max}, and the spectral curves are of a very similar shape, to those of the "PTC" compounds.

The reaction of C-1 with the thioureido sulphur atom (*cf.* ref. 11), giving anilinothiazolines with the required λ_{max}, can be excluded, since "PTC"-2-amino-2-deoxy-D-glucose and its analogues are not cationic on electrophoresis in citrate–phosphate buffer at pH 3–7. Anilinothiazolines are basic by virtue of their "isothiouronium" structure.

The interpretation of i.r. spectra of compounds containing the N–C = S system is not completely clear[12]. Nevertheless, in the region 1500–1625 cm^{-1}, there is a strong band which is present in all of the relevant model compounds. The

N-phenyl, symmetrically disubstituted thioureas of Table I, and 18 other symmetrically disubstituted thioureas (not all *N*-phenyl) from the literature (Sadtler spectra) have this band at 1500–1550 cm^{-1}. Since it occurs at 1610 cm^{-1} in the spectrum of "PTC"-2-amino-2-deoxy-D-glucose, the PTC formulation (**1**, R = phenyl) is contra-indicated. 1-Phenylimidazolidine-2-thione, 5-hydroxy-4,4-dimethyl-1-phenylimidazolidine-2-thione and 5-hydroxy-4-methyl-1-phenylimidazolidine-2-thione show the band at 1560–1590 cm^{-1}, and so does 1-naphthylimidazolidine-2-thione. The occurrence of this band at 1560–6125 cm^{-1} in all of the sugar derivatives (naphthyl, phenyl, or *p*-tolyl, both "PTC" and "anhydro-PTC") is compatible with the imidazolidine-2-thione formulation.

U.v. spectra and elemental analyses are incompatible with a phenylimidazole-2-thione (**6**, R = phenyl, Neuberg and Wolf[13]), which lacks a molecule of water, and has a λ_{max} at *ca.* 265 nm (see, *e.g.*, Ref. 9). Since the spectrum is unchanged in borate at pH 8.9, the state of combination of the "PTC" group is unlikely to be affected by borate-complex formation.

In summary, u.v. and i.r. spectral evidence is against a simple PTC formulation, with or without a cyclic sugar. The electrophoretic data are most easily interpreted on the basis of an acyclic sugar. The u.v. spectra suggest that the "anilino" nitrogen atom has acquired a second substituent, *before* contact with the complexing anions. Analyses indicate that no water is lost or gained, compared with the PTC form (**1**). The structure which satisfies these considerations is **4**, R = phenyl.

Structure **4**, a 4(5)-hydroxyimidazolidine-2-thione, is the reduced form of a 2-thiohydantoin. This reduction is easily affected by borohydrides in mild conditions[9] (Scheme 1). The phenylthiohydantoin of 2-amino-2-deoxy-D-gluconic acid (Scheme 1, R′ = D-*arabino*-tetrahydroxybutyl, R^2 = H, R^3 = phenyl), characterised by the method of preparation, ultraviolet spectrum, and mobility in tungstate buffer, was reduced by sodium borohydride in aqueous solution at room temperature to a compound having the spectral and electrophoretic properties of "PTC"-2-amino-2-deoxy-D-glucose. The spectral change on reduction should be compared with the practically identical result of the reduction[9] of 5-methyl-4-phenyl-2-thiohydantoin (Fig. 1). This evidence strongly supports the imidazolidine-2-thione formulation of "PTC"-2-amino-2-deoxy-D-glucose.

It is concluded that **4** (R = phenyl) is the first stable, isolable product of reaction of isothiocyanates with 2-amino-2-deoxyaldoses from aqueous solution. I.r. spectra suggest that **4** is also the structure in the solid state.

Compounds having analyses less by one molecule of water as compared with **4** were investigated by García González and co-workers[14] who, with Morell[3], concluded that these were fused-ring compounds of imidazolidine-2-thione and a pyranose. Recently, a re-investigation[4] by the n.m.r. method has shown that the sugar ring is furanoid (**3**). The compounds derived from **4**, by treatment with dilute acid or organic solvents, are also formulated as 4,5-furanoimidazolidine-2-thiones (**3**), since (*a*) a molecule of water was lost on formation from **4**; (*b*) the u.v. spectrum is identical in detail with that of **4** (R = phenyl); (*c*) the i.r. spectrum band at 1610 cm^{-1}

supports an imidazolidine-2-thione ring formulation; (*d*) the products are *immobile* in tungstate buffer, indicating a change of structure at C-3, C-4, C-5, or C-6; (*e*) the PTC derivative of 2-amino-2-deoxy-D-glucitol does not convert, suggesting that another part of the molecule must be involved in addition to the tetrahydroxybutyl chain; and (*f*) since "PTC"-2-amino-2-deoxy-3-*O*-methyl-D-glucose converts, HO-3 is not involved in the change.

Points (*a*)–(*e*) establish that a molecule of water was lost, not solely from the tetrahydroxybutyl chain, without affecting the imidazolidine-2-thione ring. There remains ring formation involving C-1 of the sugar chain (*i.e.*, HO-5 of the imidazolidine-2-thione). The electrophoretic properties of the "anhydro-PTC" derivatives of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and 2-amino-2-deoxy-3-*O*-methyl-D-glucose, assuming that a similar reaction has taken place in each case, tend to rule out the pyranoid configuration, since (*a*) the 3-*O*-methyl derivative (R_G 0.27) would not be expected to migrate (*cf.* 2,3-di-*O*-methyl-D-glucopyranose[15], R_G 0.12); (*b*) the mobility of the 2-amino-2-deoxy-D-glucose derivative (R_G 0.65) should be much lower (*cf.* 2-*O*-methyl-D-glucose[15], R_G 0.23; and (*c*) the 2-amino-2-deoxy-D-galactose derivative (R_G 0.21) should have a higher R_G than the 2-amino-2-deoxy-D-glucose derivative (*cf.* 2-acetamido-2-deoxy-D-galactose, R_G 0.35, with 2-acetamido-2-deoxy-D-glucose, R_G 0.20).

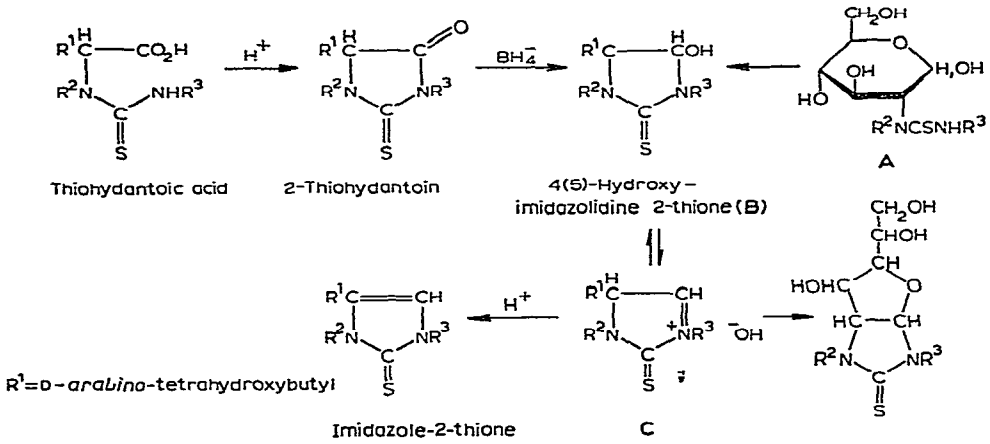
A furanoid structure fits the observed properties. The comparable 1,2-*O*-isopropylidene-α-D-glucofuranose, to which the borate is assumed to be attached at C-3, C-5, and C-6, migrates rapidly[15] (R_G 0.73). Significantly, the "PTC"-3-*O*-methyl derivative, in which this complex could not form, has a low R_G value. Their electrophoretic behaviour in both tungstate and borate buffers is identical (Tables II and III) with thio and oxo analogues (kindly given by Dr. C. Morell) which were shown by n.m.r. to be glucofurano-imidazolidine derivatives (**3**, R = *p*-tolyl). The evidence therefore strongly supports **3** as a typical structure of the acid- or solvent-treated **4**.

The anhydro-compound derived from "PTC"-1-amino-1-deoxy-D-fructose has particular interest as a spiro compound (**7**). It was not isolated. It also is represented as a furano derivative, since, in the pyrano form, there is an available *cis*-glycol group at C-4–C-5 which, since $R_G = 0$ in borate, is unlikely to be present [*cf.* comparable methyl α- or β-D-arabinofuranosides (R_G 0.04), whereas the alternative methyl-D-arabinopyranosides have R_G 0.38 (Ref. 15)].

The anhydro-PTC compounds of this investigation are therefore similar to, if not identical with, those described by Morell. Since C-5 of the hydroxyimidazolidine-2-thione (C-1 of the sugar chain) is asymmetric, there should be two epimers of each compound. It is not possible to state which form has been isolated; in some cases, mixtures of both may have been prepared. This could explain the variability of some melting points.

The reaction of isothiocyanates with 2-amino-2-deoxyaldoses therefore goes through at least 3 stages (Scheme 1). Attempts to demonstrate that PTC-2-amino-2-deoxyaldoses exist in tungstate buffer failed, and the formation of **1** (R = phenyl) is,

therefore, inferential. It is assumed that the conversion **1**→**4** is very rapid. Since neither methyl 2-amino-2-deoxy-α- nor phenyl 2-amino-2-deoxy-β-D-glucopyranosides showed any tendency to change from PTC forms to imidazolidine-2-thiones, it is suggested that the open-chain form is the reaction intermediate.



Scheme 1.

It was pointed out[9] that 4(5)-hydroxyimidazolidine-2-thiones are pseudo-bases, in which three tautomeric forms (an amino-aldehyde, a carbinolamine, and a mesomeric quaternary ammonium cation (Scheme 1) are in equilibrium. Ether-formation, and ether-exchange reactions, in which alkoxy groups are interchanged freely with alcohols, are characteristic of pseudo-bases (see, *e.g.*, ref. 16). The formation of the furano-ring (an internal ether) from **4** in mild conditions is exemplary. It is suggested that ether formation from **4** is a reaction of the quaternary ammonium cation (Scheme 1) derived from the pseudo-bases, proceeding by the mechanism shown (**8**). This mechanism accounts for the pH dependence of the formation of furanose rings, which are formed in weak acid. At low concentrations of OH^-, the equilibrium would be displaced (Scheme 1) from forms **A** and **B** into the mesomeric ammonium cation **C**, and thence into the ether. Alkaline conditions would suppress ionisation of the mesomeric ammonium hydroxide, thus stabilising 4(5)-hydroxyimidazolidine-2-thiones, as found in this investigation.

A second reaction of the ammonium cation is its ready conversion into imidazole-2-thiones by the loss[9] of a proton (which is finally derived from C-4). The ratio of imidazole-2-thione to 4(5)-alkoxyimidazolidine-2-thione obtained must then be decided by the relative rates of proton loss and alkoxy gain. Presumably because of steric factors, HO-2 of the tetrahydroxybutyl chain reacts at the greater rate, so that imidazole-2-thione is formed in small proportions, if at all. This is compared with the reaction of 1-arylamino-1-deoxyfructoses with ammonium thiocyanate to form imidazole-2-thiones[17]. Presumably, this reaction went *via* a thiourea, and an intermediate 4-hydroxyimidazolidine-2-thione[9]. In contrast to the present investigation, the aryl substituent was not at the mesomeric ammonium nitrogen, which instead

carried a proton (Scheme 1; **C**, $R^3 = H$) likely to dissociate at a much greater rate than H-4. Thus, when the pseudo-basic hydroxyl group and the *N* substituent (Scheme 1; $R' =$ tetrahydroxybutyl, $R^3 = H$) are vicinal, cyclic ethers should be formed faster than the deprotonation from C-4 to an imidazole-2-thione, but when the pseudo-basic hydroxyl group is vicinal to an unsubstituted $\succ NH$ ($R^3 = H$), the faster deprotonation from $\gtrless N^+H$ should produce an imidazole-2-thione. In agreement with this hypothesis, the "PTC"-1-amino-1-deoxy-D-fructose derivative (*N*-substituent vicinal to the pseudobasic hydroxyl group), on treatment with acid, produced an anhydro derivative having the electrophoretic behaviour of a cyclic (furanose) compound (**7**, R = phenyl), quite different from the imidazole-2-thione prepared from the corresponding non-vicinal derivative (**6**, R = phenyl), kindly supplied by Dr. Huber (see Tables II and III). It appears likely, therefore, that Neuberg and Wolf[2] prepared the "anhydro-PTC" [*i.e.*, glucofuranoimidazolidine-2-thione (**3**)] derivative, since they started from hexosamine and isothiocyanate. The analyses would be identical with those of the hoped-for imidazole-2-thione. Their melting point (208°) differs considerably from that (179–181°) of the authentic phenylimidazole-2-thione[17] (**6**), but is identical with that of the compound formulated as an imidazolidine-2-thione having λ_{max} 238 nm. Thus, the reaction analogy by which Neuberg and Wolff assigned an acyclic sugar ring and an imidazole-2-thione structure to the 2-amino-2-deoxy-D-glucose–isothiocyanate product was unsound.

The conversion in alcohols of "PTC"-2-amino-2-deoxy-D-glucose into a cyclic ether is interesting, since Morrell's "p-tolyl thiocarbamoyl" compounds survived prolonged treatment in methanolic ammonia without splitting off water. Two explanations are possible. (*1*) In the completely non-aqueous synthesis of Morell's compounds, the aldehydo sugar (which is suggested to be necessary for the conversion of **1** into **4**) was not present. If so, Morell's compounds would be "thiocarbamoyl" derivatives similar to **1**. The i.r. evidence is against this; Morell's "2-deoxy-2-*p*-tolylthiocarbamido-D-glucose" absorbs at the imidazolidine-2-thione frequency of 1560 cm^{-1}. (*2*) It is more likely that the alkalinity of methanolic ammonia suppressed the ionisation of the mesomeric ammonium hydroxide, so that no reactive ammonium cation was formed (by analogy with the situation in water).

It can, nevertheless, be predicted that if true PTC-2-amino-2-deoxyaldoses could be made, they would be converted into hydroxyimidazolidine-2-thiones on contact with any solvent in which mutarotation (and hence the aldehydo form) occurred.

Although this paper has dealt exclusively with thio compounds, the conclusions probably also hold for oxo compounds (*e.g.*, naphthyl isocyanate reacts with 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose to give compounds migrating in molybdate buffer[18]).

Resolution of mixtures of amino sugars. — 2-Amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose are the two naturally occurring amino sugars which it is most commonly necessary to separate and measure. Fortunately, the difference in configuration of the respective tetrahydroxybutyl chains is such that the complex

of the former with tungstate has only half the mobility of the latter. Equally clear separations would be expected of the derivatives of any *threo–erythro* pair of hexosamines, *e.g.*, 2-amino-2-deoxy-D-talose and 2-amino-2-deoxy-D-mannose. On the other hand, electrophoretic separation of PTC-aminodeoxyhexoses differing only at C-2 would not be expected. The resolving power is similar to that of the ninhydrin degradation-to-pentose procedure[19] in which C-1 is removed. However, on further treatment of the "PTC" derivative with dilute acid, the anhydro "PTC" derivative is formed, and pairs previously inseparable in tungstate or borate (*e.g.*, 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-mannose) become easily separable in borate buffer.

Clear distinctions are possible between the "PTC"-2-amino-2-deoxypentoses (which do not migrate) and the 2-amino-2-deoxyhexoses, and, in principle, between 2-amino-2-deoxyglycosides (which would not be expected to migrate) and the parent PTC-2-deoxy-2-aminoglycose. The presence at C-3, C-4, C-5, or C-6 of a substituent at a hydroxyl group would be expected to greatly decrease or eliminate the electrophoretic mobility in tungstate (*cf.* ref. 10).

The combination of the isothiocyanate reaction with electrophoresis of the product is very advantageous in the assay and resolution of mixtures of naturally occurring amino sugars, in speed, convenience, and sensitivity. This aspect will be dealt with in a later paper.

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