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The Structure of the Oxazolidine-2-thione Derivatives Synthesized from Some Reducing Sugars

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The structure of the previously described cyclic thione-carbamates of some reducing sugars has been examined by means of their oxidation by periodate and their absorption spectra in infrared and ultraviolet. Results were obtained which contribute to the determination of the structure of the compounds prepared from D-xylose, L-arabinose, and D-fructose.

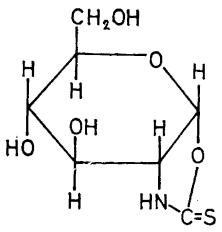
It appeared that they all contain an oxazolidine-2-thione ring fused with the pentoses (through C_1 and C_2) in their furanose form or with D-fructose (through C_2 and C_3) in its pyranose form.

These oxazolidine-2-thione derivatives crystallize in the thione form. In aqueous solution they exhibit uniform acid strength.

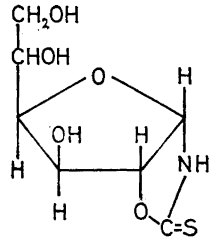
Some reducing sugars, D-glucose [1], D-fructose [2], D-galactose, D-xylose, and L-arabinose [3], have been reported to give with thiocyanic acid crystalline condensation products which presumably all contain an oxazolidine-2-thione ring. Since this cyclic thione-carbamate group in simpler compounds is known to exhibit antithyroid activity [4] and since the structure of these sugar derivatives still presents some unresolved problems, it seemed of interest to examine their structure as far as new methods of approach would allow.

In the present work infrared and ultraviolet absorption spectra as well as a periodate oxidation technique eliminating the disturbing effect of the thione group, have been used to elucidate the structure of the cyclic thione-carbamates of the following sugars: D-xylose, L-arabinose, and D-fructose. Oxazolidine-2-thione, tetrahydrogen-1,3-oxazine-2-thione, and D-gluco-oxazolidine-2-thione have served as reference compounds. All the above-mentioned substances were synthesized according to previously described methods. The D-fructose derivative, not obtainable as a completely homogeneous product by recrystallizations [2], required a chromatographic purification.

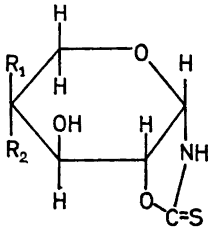
The condensation product between thiocyanic acid and D-glucose, D-gluco-oxazolidine-2-thione, was used as a reference substance because its structure is that most completely examined in this series of sugar derivatives. The formula (II) now accepted differs from the originally suggested structure [1]



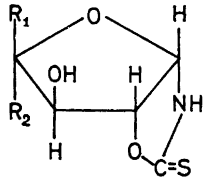
I



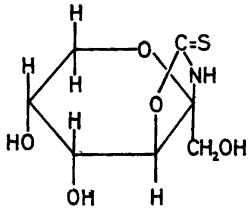
II



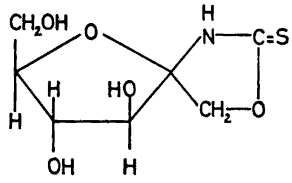
III: $R_1 = H$, $R_2 = OH$
IV: $R_1 = OH$, $R_2 = H$



V : $R_1 = CH_2OH$, $R_2 = H$
VI: $R_1 = H$, $R_2 = CH_2OH$



VII



VIII

(I) by having a furanose ring instead of a pyranose ring and by the mode of attachment of the thione-carbamate group to the sugar chain.

Schwarz [5], studying the oxidation of the D-glucose and the D-galactose derivative by periodate, found that they both yielded one molecule of formaldehyde, a result which is consistent with the furanose form only. Further, the D-glucose compound readily formed an *ortho*formate [5], proving that the thione-carbamate group is incorporated in a five-membered and not in a six-membered ring. Only the hydroxyl group on C_3 and not that on C_2 of the sugar ring is so located as to render the formation of an *ortho*formate sterically possible.

After hydrolytic cleavage of the oxazolidine ring of the D-glucose compound the presence of 2-amino-2-deoxy-D-glucose could not be demonstrated [3]. Edward and Martlew [6] found by paper chromatography of the acid hydrolysate no spots of the very acid-resistant 2-amino-2-deoxy-D-glucose, but 1-aminoglucose and diglucosylamine could be detected. They concluded that the nitrogen of the thione-carbamate group is attached to C_1 as in formula (II) and not to C_2 as previously [1] suggested (I).

All formulas discussed in the present paper are written in agreement with this conclusion, the nitrogen being attached to the anomeric carbon. Primarily, it is difficult to conceive of a reaction between a reducing sugar and thiocyanic acid resulting in the replacement of a hydroxyl group by nitrogen on any carbon other than the anomeric, whereas a similar replacement on the anomeric carbon might result from a hypothetic sequence of possible reactions already mentioned by Bromund and Herbst [3]: intermediate formation of glycosyl-*iso*thiocyanate undergoing intramolecular urethane formation to a cyclic thione-carbamate with one of the alcohol groups of the sugar moiety, *e. g.* with the adjacent hydroxyl group to an oxazolidine-2-thione ring. Further, the sugar derivatives are found to be of uniform acid strength ($pK_A^{20°} = 9.33$—9.48) and considerably stronger acids than the unsubstituted ring system, oxazolidine-2-thione ($pK_A^{20°} = 11.05$), which according to the present investigation is incorporated in all the sugar derivatives studied. This shows that the interaction of the sugar moiety on the presumably acid NH-group is of similar magnitude throughout the series, indicating that the nitrogen is probably located in an analogous position in all the derivatives, *e. g.* on the anomeric carbon.

According to Schwarz [5] the reaction of D-gluco-oxazolidine-2-thione and of D-galacto-oxazolidine-2-thione with unbuffered sodium periodate is complica-

Table 1. Oxidations by means of periodate of the condensation products between thiocyanic acid and reducing sugars.

Parent sugar	Reaction time at 20° (h)	0.5	1	2	6	22	44
D-Glucose	I [a]	5.02	5.01			5.18	
	II [b]	2.02	2.03			2.05	
D-Fructose	I [a]		3.90	4.32	4.65	5.00	5.03
	II [b]		0.38	0.45	0.83	1.50	1.50
D-Xylose	I [a]	4.05	4.05	4.17	4.32	4.32	
	II [b]	0.19	0.19	0.13	0.18	0.19	
L-Arabinose	I [a]	4.04	4.07	4.15	4.10	4.30	
	II [b]	0.18	0.27	0.27	0.26	0.25	

[a] molecules of periodate reduced.

[b] molecules of potassium cyanide reacting with the oxidation product. The figures are mean values of at least two determinations and are referred to one molecule of the sugar derivative.

ted by the oxidation of the thione group. Iodine was liberated, indicating that periodate was reduced beyond the iodate step, and in most cases the reaction mixture also became cloudy (possibly sulphur), indicating that the sulphur of the thione group was not completely oxidized to sulphate. The authors have overcome this difficulty by carrying out the oxidation with a large excess of periodate in a solution acidified by acetic acid. The organic sulphur is then completely oxidized to sulphate by periodate and no reduction of the resulting iodate occurs. This modification affords the possibility of studying the periodate oxidation of these sulphur compounds by classical analytical methods such as the arsenite titration of excess periodate followed by the cyanide titration of carbonyl groups in the oxidation product (Table 1).

Thus, D-gluco-oxazolidine-2-thione (II) consumes five molecules of periodic acid — four molecules to oxidize the sulphur to sulphate (Found: 0.97 mole) and one molecule to transform a glycol group to two aldehyde groups, one of which is formaldehyde [5].

The two pentose compounds reduce four molecules of periodate, corresponding to the oxidation of sulphur to sulphate (Found: 0.94 mole). The insignificant consumption of potassium cyanide, being apparently independent of the reaction time and the reduction of the oxidant, does not indicate a glycol-splitting reaction.

The fructose compound reduces the four molecules of periodate required to oxidize the thione group to sulphate (Found: 0.99 mole) and one additional molecule of periodate which is consumed in the cleavage of a glycol group liberating two (Found: 1.5) titratable aldehyde functions. Only two possible bicyclic formulas, (VII) and (VIII), are consistent with this result, and formula (VII) has been chosen because it agrees with other experimental evidence.

The cyclic thione-carbamates of D-xylose and L-arabinose were believed to possess a pyranose structure [3] (formulas (III) and (IV), respectively) in complete analogy with the previously suggested structure of D-gluco-oxazolidine-2-thione (formula (I)). The two pentose compounds, however, have no glycol group able to reduce periodate. Formula (IV) of the L-arabinose compound includes a *cis* glycol group, and the actual resistance of the ring system towards periodate, therefore, contradicts this structure. The two vicinal hydroxyl groups in formula (III) of the D-xylose compound are held in a *trans* position which presumably renders this glycol group relatively more resistant to glycol-splitting reagents. Complete resistance of *trans* glycol groups towards periodate has so far been observed only in extremely rigid systems, such as 1-6-anhydro-D-glucofuranose [7]. In case, however, the argument against formula (III) based upon the stability of the sugar ring when exposed to periodate might be considered less decisive, it should be mentioned that a supporting argument favouring the modified formula (V) emerged from the infrared spectrum of the D-xylose compound.

The infrared spectra of all substances enumerated in Table 2 have been studied by the potassium bromide plate technique. The main purpose of this examination was to obtain information about the size of the N-heterocyclic ring in the various sugar derivatives.

The infrared absorption of different series of oxazolidine-2-thione derivatives has been investigated by Ettlinger [8] (samples in chloroform) and Skulski *et al.*[9] (samples in Nujol).

The strong absorption band of the five-membered cyclic thione-carbamate in oxazolidine-2-thione (1 525 cm^{-1}) and in D-gluco-oxazolidine-2-thione (1 510 cm^{-1}), tentatively attributed to a NH bending vibration [9], is found in the same region of the spectra of all the sugar derivatives studied, whereas this band of the six-membered cyclic thione-carbamate, tetrahydrogen-1,3-oxazine-2-thione, is displaced to a higher frequency (1 575 cm^{-1}). This is a strong indication of the presence of a five-membered ring (oxazolidine-2-thione) not only in the D-glucose compound but in all the sugar derivatives examined.

It also emerged from the infrared spectra of the sugar derivatives that, in analogy with the unsubstituted oxazolidine-2-thione and some of its substitution derivatives [9], they crystallize in the thione form (and not in the tautomeric thiol form). Absorption due to SH stretching or to C=N bonds did not occur (Table 2).

Further, on comparing the infrared spectra of the oxazolidine-2-thiones of the two homomorphous [10] sugars D-glucose and D-xylose, it was noticed that the absorption curves exhibited a striking parallelism between 4 000 cm^{-1} and 900 cm^{-1}. This may be taken as an indication of the presence of the same fused ring system in both sugar derivatives, formula (II) and (V).

The ultraviolet absorption of the unsubstituted N-heterocyclic rings and the sugar derivatives was also recorded. Results tabulated in Table 3 support the above conclusion that the cyclic thione-carbamates of various sugars examined all contain this group in a five-membered ring.

In order to choose between the two D-fructo-oxazolidine-2-thione formulas (VII) and (VIII) the reducing product of the periodate oxidation was examined by methods which have proved to be reliable in the study of oligo-saccharides, *e. g.* by Courtois and Wickstrøm [11]: paper chromatographic analysis of carboxylic acids formed by bromine oxidation of the aldehyde groups and subsequent acid hydrolysis. D-Fructo-oxazolidine-2-thione yielded glycolic acid in about 50 % of the theoretical amount. The glycolic acid is considered to contain C_6 and C_5 of the fructose moiety of formula (VII), whereas a substance of the formula (VIII) by the same sequence of reactions should yield D-glyceric acid (from C_6, C_5, and C_4). Only one acid spot was observed, probably as a result of destruction of the N-heterocyclic moiety during hydrolysis.

All experimental results agree with the formulas (V) and (VI) for the D-xylose and the L-arabinose derivative, respectively, and with formula (VII) for the D-fructose derivative. According to these formulas the two pentose compounds have a structure analogous to that of the D-gluco-oxazolidine-2-thione (II) and not to the erroneous formula of this compound (I). The size of the rings in formula (VII) of the D-fructose compound is in agreement with the structure attributed to this substance by Zemplén *et al.*[2]

Table 2. Infrared absorption from 1 500 to 4 000 cm^{-1}.

Assignment	NH or OH stretching vibration (bonded) cm^{-1}	NH bending [a] cm^{-1}
Substance		
Oxazolidine-2-thione	3 220	1 525
Tetrahydrogen-1,3-oxazine-2-thione	3 210	1 575
D-Gluco-oxazolidine-2-thione	3 410 3 200	1 510
D-Fructo-oxazolidine-2-thione	3 370 3 180	1 505
D-Xylo-oxazolidine-2-thione	3 420 3 170	1 525
L-Arabo-oxazolidine-2-thione	3 380 3 270	1 485

Table 3. Ultraviolet absorption.

Substance	Aqueous solution		Solution in 0.1 N NaOH [b]	
	λ_{max} (mμ)	$\varepsilon \times 10^{-3}$	λ_{max} (mμ)	$\varepsilon \times 10^{-3}$
Oxazolidine-2-thione [a]	239	16.0	228	12.3
Tetrahydrogen-1,3-oxazine-2-thione	244	14.9	242	11.8
D-Gluco-oxazolidine-2-thione	241	19.7	233	14.5
D-Fructo-oxazolidine-2-thione	241	21.5	233	16.8
D-Xylo-oxazolidine-2-thione	241	20.3	233	14.6
L-Arabo-oxazolidine-2-thione	241	19.6	233	14.3

[a] the ultraviolet absorption found is in agreement with previously published curves for this compound [4].

[b] the absorption in alkaline solution is due to the ionized heterocyclics because of the occurrence of an isosbestic point between the curve in 0.1 N NaOH and curves recorded at several lower pH values.

EXPERIMENTAL

All melting points are micro melting points (Kofler's hot-stage microscope).

1. Preparations

(a) *Cyclic thione-carbamates* were prepared from the various reducing sugars (D-glucose, D-xylose, L-arabinose, D-fructose) according to methods previously described [1-3]. The products obtained from the aldoses were found to be identical with the compounds described in the literature by determination of their melting points, their optical rotation in water, and their reduction of hypoiodite [12] (8 equiv.): D-glucose compound, m. p. 170°, $[\alpha]_D^{20°} = +33.9°$ ($c = 2$), 99.7 %; D-xylose compound, m. p. 131°, $[\alpha]_D^{28°} = +13.2°$ ($c = 2$), 99.5 %; L-arabinose compound, m. p. 136°, $[\alpha]_D^{20°} = +11.8°$ ($c = 2$), 98.7 %.

The heterocyclic product obtained from D-fructose and thiocyanic acid (Zemplén's "Fructose Rhodanverbindung 2") is contaminated with variable amounts of Zemplén's

"Fructose Rhodanverbindung 1", presumably an open chain addition product, m. p. 217°, which is extremely difficult to remove completely by recrystallizations [2]. The two products may be separated on paper chromatograms with the solvent system acetone, *n*-propanol, water (45:45:10 v/v) and the spots ($R_F^{20°}$ = 0.53 (1) and 0.66 (2)) revealed by silver nitrate and ethanolic sodium hydroxide [13]. From columns packed with Whatman standard grade cellulose powder (4.6 × 37 cm) and developed with the above-mentioned solvent system, a poor yield of chromatographically pure "Fructose Rhodanverbindung 2" was obtained, *e. g.* from 0.5 g of the crude mixture (m. p. 172—76°, containing about 10 % of the "Fructose Rhodanverbindung 1") 0.05 g of chromatographically pure "Fructose Rhodanverbindung 2" was obtained. It was further purified by recrystallization from methanol, m. p. 185° (decomp.), $[\alpha]_D^{20} = -50.7°$ (c = 1, water), iodometric titration [2], 97.8 %.

(b) *Oxazolidine-2-thione*, m. p. 97.5°, was prepared from ethanolamine according to Rosen [14], and tetrahydrogen-1,3-oxazine-2-thione was synthesized by a similar procedure starting with 3-amino-propanol; the method described by Rosen [14] for the preparation of 4,4,6-trimethyl-tetrahydrogen-1,3-oxazine-2-thione from 4-amino-4-methyl-pentanol-2 was followed without any essential modification. Repeated crystallization from *n*-butanol gave long, colourless needles (only 3 % yield), m. p. 127°, titration with hypoiodite [12] 99.7 %. (Found: C 41.01; H 5.95; N 11.98; S 28.17. Calc. for C_4H_7ONS: C 41.02; H 6.03; N 11.95; S. 27.36.)

2. Oxidations with periodate

(a) *Titration of periodate uptake and liberated aldehydes.* A 0.05 M periodate reagent (A) of pH about 4 was prepared by dissolving sodium *para*-periodate (Hopkin and Williams Ltd) (7.5 g) in N acetic acid (200 ml) and water (about 200 ml), filtering the solution after some days through sintered glass and adding water to 500 ml. The samples (10 ml of 0.005 M solutions) were oxidized with this reagent (20 ml) at room temperature in the dark. Iodic acid, not iodine or hydroiodic acid, was formed by reduction of the periodic acid. After neutralization with sodium bicarbonate the periodate uptake was determined by the classical arsenite method [15] and titratable carbonyl groups were estimated according to the previously described technique [11]. For oxidation of the fructose compound 0.01 M periodate was used. Results are shown in Table 1.

(b) *Determination of sulphuric acid liberated by periodate oxidation.* The samples (5×10^{-5} molecule in 10 ml of water) were oxidized by the periodate reagent (A) (20 ml). The oxidation was stopped by adding 2 N hydrochloric acid (1.5—2 ml) and then hydrazine hydrate (50 % w/w) dropwise, stirring constantly until the colour of the reaction mixture changed from violet to yellow (0.5—1 ml). The solution was heated on a water bath till the colour (iodine) disappeared completely. Barium sulphate was precipitated from the boiling solution by adding barium chloride in slight excess. The following molecular yields of sulphuric acid per molecule of oxidized sugar derivative were found as mean values of repeated gravimetric determinations (reaction time with periodate in brackets): D-gluco-oxazolidine-2-thione, 0.97 (30 min), D-xylo-oxazolidine-2-thione, 0.94 (60 min), L-arabo-oxazolidine-2-thione, 0.94 (60 min), D-fructo-oxazolidine-2-thione, 0.99 (20 h).

(c) *Examination of the reducing product formed by periodate oxidation of the* D-*fructo-oxazolidine-2-thione.* The sample (25 mg) was oxidized with free periodic acid (500 mg) in aqueous solution at room temperature for 20 h. The solution was neutralized with strontium hydroxide to pH 5—6 and filtered. The filtrate was treated with a weak excess of bromine at room temperature until an aliquot failed to reduce the Nessler reagent (6—8 days). Bromine was then removed by aeration and the solution evaporated to dryness (reduced pressure). The residue was taken up in 2 N sulphuric acid (1.50 ml) and heated in a boiling water bath for one hour. Sulphuric acid was removed by treatment with strontium carbonate and the hydrolysate examined by paper chromatography in the following solvent systems: I, ethyl acetate-formic acid-water (10:2:5 v/v), and II, *n*-butanol-formic acid-water (10:1:5 v/v), which clearly separated authentic samples of glycolic acid ($R_F^{20°}$ = 0.52 (I) and 0.67 (II)) and D-glyceric acid ($R_F^{20°}$ = 0.35 (I) and 0.55

(II)). In repeated experiments only spots corresponding to glycolic acid were observed (bromophenol blue spray). By comparison with a series of standard solutions of glycolic acid (0.1 to 0.5 %) the amount of glycolic acid recovered from the fructose compound was evaluated to about 50 % of the theoretical, indicating its formation by a main reaction. The heterocyclic moiety of the oxidized fructose compound was probably undergoing destruction during hydrolysis (gas evolution).

3. Determination of acid strength

The apparent pK_A values at 20° were calculated from pH measurements on a Metrohm pH-meter E 196 S of 0.01 M aqueous (carbon dioxide free) solutions half neutralized with barium hydroxide. The following mean values were obtained: oxazolidine-2-thione, 11.05 (5,5-dimethyl-oxazolidine-2-thione [4], 11.05); D-glucooxazolidine-2-thione, 9.33; D-xylo-oxazolidine-2-thione, 9.48; L-arabo-oxazolidine-2-thione, 9.48; D-fructo-ozaxolidine-2-thione, 9.40.

4. Absorption spectra

The ultraviolet absorption spectra were recorded on a Beckman DU spectrophotometer using about 2×10^{-5} M solutions of the samples (Table 3). The infrared spectra of the samples (Table 2) in potassium bromide plates were recorded with a Perkin-Elmer Model 21 double beam spectrophotometer.

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REFERENCES

1. Zemplén, G., Gerecs, A. and Rados, M. *Ber.* **69** (1936) 748.
2. Zemplén, G., Gerecs, A. and Illés, E. *Ber.* **71** (1938) 590.
3. Bromund, W. H. and Herbst, R. M. *J. Org. Chem.* **10** (1945) 267.
4. Astwood, E. B., Greer, M. A. and Ettlinger, M. G. *J. Biol. Chem.* **181** (1949) 121.
5. Schwarz, J. P. C. *J. Chem. Soc.* **1954** 2644.
6. Edward, J. T. and Martlew, E. F. *Chem. & Ind. London* **1952** 1034.
7. Dimler, R. J. *Advances in Carbohydrate Chem.* **7** (1952) 37.
8. Ettlinger, M. G. *J. Am. Chem. Soc.* **72** (1950) 4699.
9. Skulski, M., Garmaise, D. L. and McKay, A. F. *Can. J. Chem.* **34** (1956) 815.
10. Pigman, W. *The Carbohydrates*, New York, 1957.
11. Courtois, J. and Wickstrøm, A. *Bull. soc. chim. biol.* **32** (1950) 759.
12. Wojahn, H. *Arch. Pharm.* **284** (1951) 243.
13. Trevelyan, W. E., Procter, D. P. and Harrison, J. S. *Nature* **166** (1950) 444.
14. Rosen, A. A. *J. Am. Chem. Soc.* **74** (1952) 2994.
15. Fleury, P. and Lange, J. *J. pharm. & chim.* **17** (1933) 107.

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