

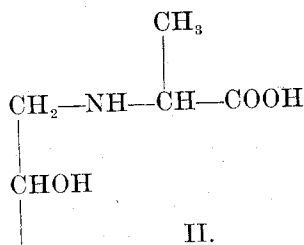
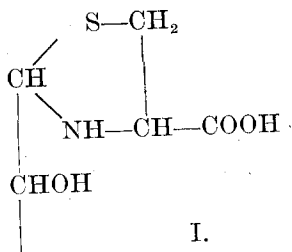
## REACTION PRODUCTS OF ALDOSES WITH CYSTEINE

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The manner in which carbohydrates are attached to proteins in substances such as the glycoproteins is relatively unknown. In order to shed some light on the subject, various workers have studied the reaction of amino acids with carbohydrates. It was soon found that the most stable amino acid-carbohydrate complexes were formed from cysteine and aldoses. Schubert<sup>1</sup> prepared derivatives of reducing sugars with cysteine and assigned a thiazolidine structure (I) to these substances on the basis of a negative sulphydryl test with sodium nitroprusside reagent. At the same time, Agren,<sup>2</sup> working in Copenhagen, prepared derivatives of glucose with various amino acids and showed that only the glucose-cysteine reaction went to completion, i.e. the product gave a negative nitroprusside test and in addition gave a negative test for van Slyke (primary amino) nitrogen.

Since the above results indicated the thiazolidine structure (I) for aldose-cysteine derivatives, we prepared these derivatives from D-mannose and D-galactose in order to subject them to reductive desulfurization with Raney nickel. If the substances possess a thiazolidine structure and are stable, then Raney nickel in aqueous alcohol should desulfurize them to the corresponding N-substituted-1-desoxy-1-glykityl derivatives of alanine (II).

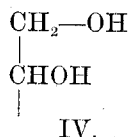
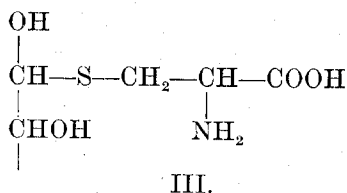
However, reductive desulfurization of the mannose-cysteine derivative actually gave D-mannitol in good yield, and the corresponding galactose derivative gave dulcitol (D-galactitol), i.e. the alcohols corresponding to the original sugars. Assuming the original thiazolidine structure to be correct, these data indicate that under the conditions of reductive desulfurization the aldose-cysteine compounds may be hydrolyzed to their components possibly



via a monothiohemiacetal of formula (III) which would theoretically yield the alcohols (IV) we obtained from the aldose-cysteine derivatives.

<sup>1</sup>M. P. Schubert, J. Biol. Chem. 130, p. 601, 1939.

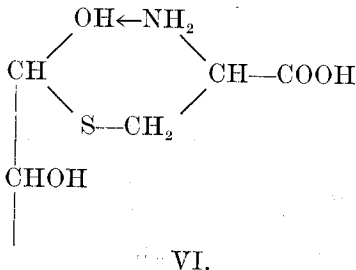
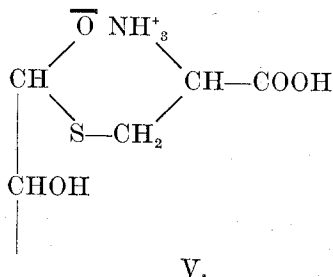
<sup>2</sup>G. Agren, Enzymologia, 9, p. 321, 1941.



Monothiohemiacetals have been described previously. Wolfrom, Weisblat and Hanze<sup>3</sup> succeeded in isolating crystalline *aldehydo-D*-galactose ethyl monothiohemiacetal pentaacetate by treatment of the aldehydo sugar with ethyl mercaptan followed by acetylation. It is entirely possible that aldose-cysteine monothiohemiacetal derivatives would owe their stability to the formation of a zwitterion (V) or a chelated structure (VI) which would likewise be desulfurized to the alcohols we obtained.

V or VI would give a positive amino nitrogen test immediately.

The question also arose as to whether the aldoses themselves might undergo Raney nickel reduction to the corresponding alcohols. Mannitol was obtained by refluxing mannose with Raney nickel in aqueous alcohol. This reaction is being investigated further as a preparative method for sugar alcohols. In the case of Raney nickel desulfurization of the aldose-cysteine derivatives, the question as to whether the polyhydric



Agren actually showed that the crude product of aldoses and cysteine did give positive van-Slyke nitrogen tests but that on purification this amino nitrogen disappeared. We tested our aldose-cysteine products with ninhydrin reagent and obtained a negative test for the amino group but the test became positive after a time indicating slow hydrolysis of the thiazolidine structure. It is questionable whether a structure such as

alcohols arise from an intermediate monothiohemiacetal derivative or from the aldoses themselves is left unanswered. However, it would be of interest to apply the Raney nickel desulfurization reaction to glycoproteins and attempt to isolate the carbohydrate moiety. Identification of polyhydric alcohol in this experiment would seemingly indicate that the carbohydrate is attached to the glycoprotein through a cysteine linkage.

<sup>3</sup> M. L. Wolfrom, D. I. Weisblat and A. R. Hanze, *J. Am. Chem. Soc.* 62, p. 3246, 1940.

## EXPERIMENTAL

*Preparation of Mannose and Galactose Derivatives of Cysteine.*—The thiazolidine derivatives of D-mannose and D-galactose were prepared essentially according to the directions of Schubert<sup>1</sup>. The sugar (3.6 g.) and L-cysteine (3.14 g.) were dissolved in 7 ml. of water with shaking. After standing for forty-eight hours, 1.54 ml. of pyridine was added and the mixture was allowed to stand an additional sixty hours at which time 200 ml. of absolute ethanol was added. The crystals which separated on further standing were dissolved in 35 ml. of warm water, filtered, and 150 ml. of absolute ethanol was added to the filtrate. The crystals were collected by filtration and dried. The mannose-cysteine derivative gave a melting point of 170° and the galactose derivative melted at 134°. Schubert<sup>1</sup> reported melting points of 171° and 138° respectively for these substances.

*Reductive Desulfurization of Mannose-Cysteine Complex.*—One gram of mannose-cysteine compound prepared above was dissolved in 50 ml. of 70 percent aqueous ethanol (7 volumes of ethanol—3 volumes of distilled water) and 10 g. of Raney

nickel catalyst<sup>4</sup> was added. The mixture was refluxed for five hours and filtered hot. The filtrate was concentrated to dryness in a stream of air and the resultant sirup was taken up in 15 ml. of absolute ethanol. The crystals of D-mannitol which separated weighed 0.45 g. (70 percent), gave a melting point of 163-166° and showed a specific rotation of  $-0.6^\circ$  (*C* 1 in water, *D* line). The recorded physical constants of D-mannitol<sup>5</sup> are m.p. 166° and a specific rotation of  $-0.21^\circ$ .

*Anal.* Calcd. for  $C_6H_{14}O_6$  : C, 39.56; H, 7.75. Found: C, 39.60; H, 8.00.

*Reductive Desulfurization of Galactose—Cysteine Complex.*—One gram of the galactose-cysteine complex was desulfurized with Raney nickel in the same manner as the mannose derivative. D-Galactitol (dulcitol) (0.4 g., 62 percent) was obtained with a melting point of 185° and the product showed no optical activity. Authentic D-galactitol has a melting point of 188.5° and is optically inactive.<sup>6</sup>

<sup>4</sup> L. Covert and H. Adkins, *J. Am. Chem. Soc.*, 54, p. 4116, 1932.

<sup>5</sup> W. Pigman and R. Goepf, Jr., *Chemistry of the carbohydrates*; New York, N. Y., Academic Press, p. 238, 1948.

<sup>6</sup> R. Lohmar and R. M. Goepf, Jr., *Advances in Carbohydrate Chem.* 4, p. 219, 1949.