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The Chemistry of the Tetrose Sugars. II. The Degradation of *d*-Xylose by the Method of Wohl. The Rotation of *d*-Threose[1]

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The first communication of this series[2] described the preparation of a crystalline threose triacetate by the Ruff degradation and recorded for *d*-threose prepared from the purified acetate, an equilibrium levorotation in water of $-12.3°$.[3] This rotation is of a sign opposite to that determined by W. Freudenberg[4] and by Deulofeu.[5] No source of error was discovered in the experiments leading to our measurement, and it was promised that further evidence would be sought in the preparation of *d*-threose by a totally different method of degradation, as one step in the complete resurvey of degradation methods which we announced in the first paper.[2] The method devised by Wohl[6] was selected as the second one to be studied, particularly because Deulofeu used this method in attempting to determine the specific rotation of *l*-threose which he reported as *to the left.*

d-Xylose, when treated with hydroxylamine, yielded a sirupy mixture of oximes of which no constituent has ever been obtained crystalline. Acetylation of this sirup gave a 48% yield of crystalline tetraacetyl-xylonic nitrile. This reaction is very exothermic so that there is great danger of the mixture becoming charred unless an inert diluent such as dioxane is used, and the acetic anhydride is added in small successive quantities.

By warming the tetraacetyl-*d*-xylonic nitrile on a steam-bath with strong aqua ammonia (28%), it is simultaneously degraded and deacetylated. The threose is obtained as a crystalline combination with two molecules of acetamide in a yield of about 78%, which is much greater than the yield obtained by using ammoniacal silver nitrate (17%).[5] Deulofeu reports the isolation of about half a gram of *l*-threose diacetamide to which he attributes a specific rotation in dilute sulfuric acid of $-7.68°$. The *d*-isomer, however, prepared in 50-g. batches, many times recrystallized as colorless, well-built prisms, and analyzed, has a specific rotation in water of $-10.9°$. Obviously the *l*-isomer should have a dextrorotation of equal numerical value and we are forced to the conclusion that Deulofeu did not have pure *l*-threose diacetamide. Moreover, acetylation of the diacetamide compound yields a crystalline triacetate, well-built prisms, m. p. 179-180°, rotation in chloroform, $+74.2°$, which after thorough purification and deacetylation regenerates beautifully crystalline *d*-threose diacetamide rotating $-10.8°$. We consider this good evidence of the purity of the latter.

To determine the equilibrium rotation of the sugar itself, Deulofeu hydrolyzed his acetamide compound to constant rotation with $N/3$ sulfuric acid. He reports a change from specific rotation $-7.68°$ for the diacetamide compound to $-24.6°$, calculated for the sugar, the direct readings being unreported. In a preliminary experiment using about the same quantities, we detected a downward drift barely greater than the experimental error. We were doubtful whether an hydrolysis had occurred until tests showed that the acid-treated solution had a powerful reducing action whereas threose diacetamide fails to reduce Fehling's solution even on boiling. The necessary conclusion that the change of rotation during hydrolysis is very minute was wholly in accord with our previously determined rotations for the diacetamide compound and the sugar, respectively (-10.9 and $-12.3°$).

By refinements of technique, it was possible to follow the course of hydrolysis quantitatively. Control tests indicated that threose may be titrated with consistent results by Cajori's iodine oxidation method,[7] that the diacetamide compound is unaffected by the reagents employed, and that the presence of free acetamide does not interfere with the titration. It was possible, therefore, to follow the course of the hydrolysis by removing samples periodically, dropping them

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(2) Hockett, THIS JOURNAL, **57**, 2260 (1935).

(3) In this paper rotations refer to specific rotations of sodium light at 20° unless otherwise specified.

(4) W. Freudenberg, *Ber.*, **65**, 168 (1932).

(5) Deulofeu, *J. Chem. Soc.*, 2458 (1929).

(6) Wohl, *Ber.*, **32**, 3666 (1899); Maquenne, *Compt. rend.*, **130**, 1402 (1900).

(7) Cajori, *J. Biol. Chem.*, **54**, 622 (1922).

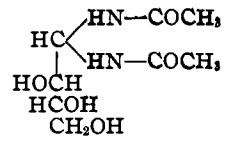
at once into dilute sodium carbonate solutions to stop action and completing the titrations within a reasonable period of time. Figure 1 shows the curve obtained. After removing the last sample for analysis, the remaining solution was immediately cooled and its rotation read. Knowing the concentration of free sugar, the concentration of diacetamide compound (by difference), the rotation of the mixture and the specific rotation of the starting substance, we calculated the equilibrium specific rotation of the sugar in dilute acid as $-12.5°$, a value in excellent agreement with that previously determined for *d*-threose from the acetate. (By accident, the agreement is actually closer than the experimental errors permit us to expect.)

The change in rotation during hydrolysis, though small, is measurable when a 2% soln. is read in a 4-dm. tube. Figure 2 shows the curve obtained in such an experiment. This, in contrast with Deulofeu's report, shows a downward trend, for although the specific rotation of the starting substance is less levorotatory than that of the sugar released, the molecular rotation of the former is much greater. At the end, the solution was titrated and another calculation of the specific rotation of *d*-threose was made, which gave $-10.8°$. This we consider to be in satisfactory agreement. The rates of reaction as determined from the two methods also agree satisfactorily.

In order to prove rigidly that the reducing substances obtained from threose acetate and from threose diacetamide are actually identical, the sugar from hydrolysis of a sample of the latter was reduced to *d*-threitol with sodium amalgam and the latter converted into dibenzylidene-*d*-threitol.[2] By rigid purification of this derivative, it was shown by rotation, melting point and mixed melting point observations to be identical with dibenzylidene-*d*-threitol from threose acetate.

Thus the equilibrium specific rotation of *d*-threose, both in water and in dilute sulfuric acid, appears well established as approximately 12° *to the left*, and sources of error are to be sought in the work of those who have found other values.

The formation by *d*-threose diacetamide of a triacetate indicates the absence of a ring in the former and establishes it as a derivative of aldehydo-*d*-threose:



The subject of ring structure among tetrose derivatives will be elucidated further in another communication.

In the search for cheaper and more efficient methods of making the tetroses, the Wohl method suffers from the disadvantage that every aldose forms several oximes so that it does not appear easy to increase the yield of the isomer necessary for this degradation.[8]

Experimental

Tetraacetyl-*d*-xylonic Nitrile.—Hydroxylamine hydrochloride (25g.) is dissolved in 15 cc. of water and a solution of sodium methylate (8.2 g. sodium in 200 cc. of dry methanol) added to the point of neutrality (phenolphthalein). After chilling the solution and filtering the precipitated sodium chloride, 45 g. of powdered *d*-xylose is added and the mixture allowed to stand overnight, then warmed on the steam-bath as long as is necessary to dissolve all the sugar. After filtering, the solution is concentrated *in vacuo* to a thick sirup, the outer bath not being allowed to rise above 75°. The sirup is dehydrated twice by adding a little dioxane and reconcentrating. Then 12 g. of fused sodium acetate and 25 cc. of dioxane are added, the mixture is warmed to 40° and 10 cc. of acetic anhydride added. The mixture is shaken vigorously until the spontaneous heating has ceased and the flask has begun to cool, then 10 cc. more of acetic anhydride is added, etc., until 120 cc. has reacted. The solution becomes homogeneous when about 20 cc. of acetic anhydride has reacted. At the end, the colorless mixture is warmed gently for an hour and poured over cracked ice. The solution is partially neutralized by stirring in solid sodium bicarbonate and the precipitated sirup soon becomes crystalline; yield, 45 g. or 48% of the theoretical. Tetraacetyl-xylonic nitrile is recrystallized from absolute alcohol as triangular plates of m. p. 81–82° (corr.) and rotation $+50.3°$ (0.7615 g. in 25 cc. of chloroform soln., 3.06° to the right, 2-dm. tube).[9]

***d*-Threose Diacetamide.**—Thirty grams of tetraacetyl-*d*-xylonic nitrile is mixed with 300 cc. of concentrated aqua ammonia (28–29%), warmed on a water-bath until the solid is all dissolved and allowed to stand for three hours. The solution is then concentrated *in vacuo* to a thick sirup, taken up in aqua ammonia and reconcentrated. The final thick sirup is dissolved out of the flask with absolute alcohol, ether is added to turbidity and the mixture is refrigerated for forty-eight hours; yield, 16.5 g. or 78% of the theoretical. The substance is recrystallized by dissolving in two volumes of warm 75% alcohol, filtering through carbon and adding an equal volume of absolute alcohol. It crystallizes slowly, forming clear, sharp needles or prisms in rosets. When purified these show m. p.

(8) Deulofeu, Wolfrom, Cattaneo, Christman and Georges, THIS JOURNAL, **55**, 3488 (1933).

(9) *Cf.* Deulofeu, *Nature*, **131**, 548 (1933).

165–167° (corr.) and rotate −10.86° (0.5979 g. in 25 cc. of aqueous soln.; 0.519° to the left; 2-dm. tube). The substance sometimes requires frequent recrystallization to get rid entirely of a gummy substance which contaminates the crude compound. A sample of the compound made by deacetylating purified triacetyl-*d*-threose diacetamide rotated −10.8° (0.9495 g. in 25 cc. of aqueous soln., 0.817° to the left; 2-dm. tube). The substance is exceedingly soluble in water, nearly insoluble in absolute alcohol, methanol, ether and chloroform.

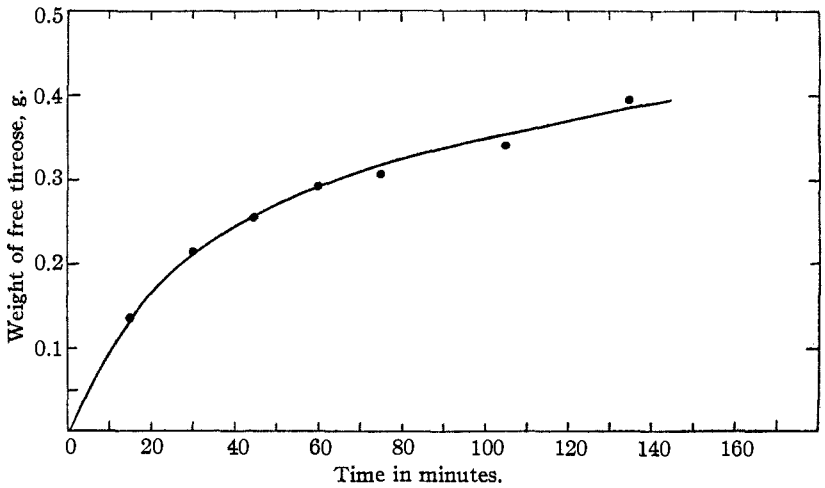


Fig. 1.—Hydrolysis of threose diacetamide by 0.100 *N* sulfuric acid (iodine titration).

Anal. Calcd. for $C_8H_{16}O_5N_2$: N, 12.72. Found: N, 12.65, 12.72.

Triacetyl-*d*-threose Diacetamide.—Two grams of purified threose diacetamide is treated with 10 cc. of acetic anhydride and 10 cc. of pyridine, warmed until the crystals dissolve and let stand overnight. The mixture is filtered through carbon, then concentrated to dryness *in vacuo*. The remaining material which crystallizes solid in the flask is dissolved in absolute alcohol and reconcentrated to dryness to remove additional pyridine; then the crystals are dissolved out of the flask with absolute alcohol, from which clear needles separate on the addition of ether. Recrystallized from the same solvents until free from pyridine, the substance shows m. p. 179–180° (corr.) and rotates +74.2° (0.3434 g. in 25 cc. of chloroform; 2.04° to the right; 2-dm. tube). The crystals are fairly soluble in water and alcohol, soluble in chloroform and practically insoluble in ether.

Anal. Calcd. for $C_{14}H_{22}O_8N_2$: N, 8.10; acetyl, 37.29. Found: N, 8.17, 8.17; acetyl, 37.18, 37.14.

Hydrolysis of *d*-Threose Diacetamide.—After showing by control experiments that threose may be titrated quantitatively with hypoiodite, and that threose diacetamide and free acetamide are unaffected by the reagents used, the following experiment was performed. A sample of 0.9437 g. of pure threose diacetamide was made up to 50 cc. with 0.100 *N* sulfuric acid. This solution was poured by funnel into a 200-cc. flask submerged to the middle of the neck in a bath of boiling water and the time noted. At intervals 5-cc. samples were removed by pipet and run into Erlenmeyer flasks containing 5 cc. of water and 2 cc. of 15% sodium carbonate each, the time being noted and a glass stopper inserted as promptly as possible. As soon as convenient, the free sugar in these solutions was determined by adding 25 cc. of iodine–potassium iodide solution, letting stand twenty minutes in the dark, acidifying with 3 cc. of 10% sulfuric acid and titrating back with standard sodium thiosulfate using starch paste indicator. The results are shown in the curve of Fig. 1. The curve corresponds approximately to the unimolecular law; on the assumption that the reaction is unimolecular, k (minutes and decimal logarithms) = 0.0063 ± 0.0013.

The Equilibrium Rotation of *d*-Threose.—At withdrawal of the last sample, the whole solution was cooled and its rotation at 20° was found by two independent observers to be −0.29°. Titration showed 0.0395 g. of tetrose in 5 cc., equivalent to 0.395 g. of tetrose in a volume of 50 cc., after one hundred and thirty-five minutes of hydrolysis at 100°. At complete hydrolysis 0.515 g. of tetrose would be present. At one hundred and thirty-five minutes, therefore, 76.7% of the threose diacetamide was

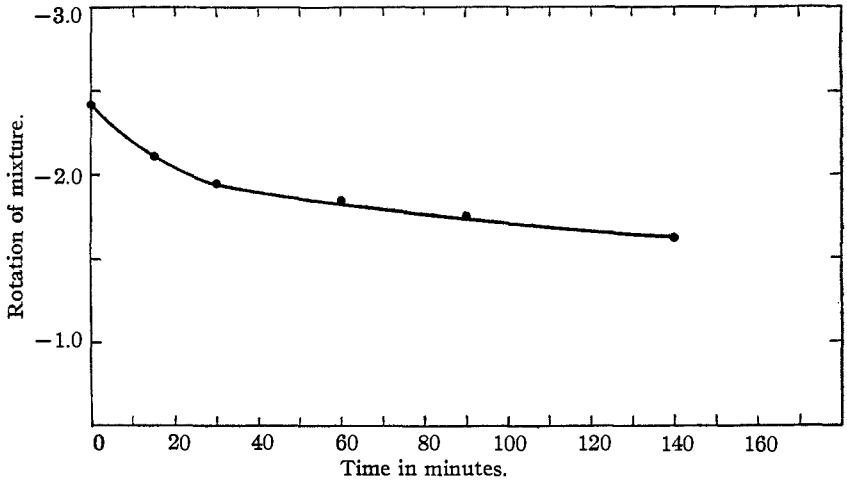


Fig. 2.—Hydrolysis of threose diacetamide by 0.100 *N* sulfuric acid (change of rotation).

hydrolyzed and 23.3% or 0.220 g. unchanged. The specific rotation of threose diacetamide is −10.9°. Therefore

$$-10.9° = X \times 50/2 \times 0.220$$

and $X = -0.096°$, the rotation due to unchanged threose diacetamide. Subtracting this from −0.29°, we find −0.198° contributed by the 0.395 g. of tetrose present in 50 cc.

$$[\alpha]^{20}D = -0.198° \times 50/2 \times 0.395 = -12.5°$$

This value for the specific rotation of *d*-threose agrees well with the value −12.3° announced in the previous paper. No difference between the rotations in pure water and in dilute acid was observed.

Change in Rotation during Hydrolysis of *d*-Threose Diacetamide.—A sample of 0.9843 g. of threose diacetamide was made up to 50 cc. in a calibrated flask with 0.100 *N* sulfuric acid and poured at a noted time into a flask submerged in boiling water. At noted intervals, the whole solution was cooled and its rotation read in a 4-dm. tube. The curve of Fig. 2 shows the readings obtained. At the time of the last reading, a titration was made showing the presence of 0.4056 g. of free tetrose in 50 cc. (75.5% hydrolysis) and 0.2411 g. of diacetamide remaining. The rotation due to the latter was −0.21° and that due to the sugar −0.35°, which corresponds to a specific rotation of −10.8°. The velocity constant could not be determined with great accuracy because the range of the change is so small but the value of *k* (minutes and decimal logs) calculates to 0.0093 ± 0.0023, which is of the same order of magnitude as the constant more accurately determined by the other method.

Dibenzylidene-*d*-threitol.—The solutions remaining from the hydrolysis experiments were combined, freed from sulfuric acid with barium hydroxide and repeatedly extracted with ether to remove acetamide. Then the solution was shaken with 2% sodium amalgam under mildly acid conditions until 100 g. of amalgam had been used and all reducing action had disappeared. The dibenzylidenethreitol was prepared just as described in the previous paper.[2] After several slow recrystallizations from warm chloroform by the addition of benzene, the new sample melted from 220–222° (corr.). The old sample from threose acetate, after several recrystallizations, melted from 218–222° (corr.) and an intimate mixture of the two gave m. p. 220–223° (corr.). The new sample, after the first recrystallization, rotated −77.9° (0.1167 g. in 25 cc. of chloroform soln.; 0.727° to the left; 2-dm. tube). The value previously found was −78.2°.

***d*-Erythrosazone.**—A small volume of hydrolysate, when treated with phenylhydrazine in the usual way,[2] yielded *d*-erythrosazone of m. p. 164° (corr.).

We wish to express to Dr. C. S. Hudson, Chief of the Division of Chemistry, deep gratitude for the sympathetic and patient interest which permitted these investigations to be carried out. Our thanks are due also to Mr. C. G. Remsburg of this Laboratory for nitrogen analyses.

Summary

1. Wohl's method of degrading sugars has been applied to *d*-xylose, and *d*-threose diacetamide has been prepared in relatively large quantity.

2. This *d*-threose derivative is found to have a specific levorotation in water of −11°, which is opposite in sign to that indicated by Deulofeu, who found −7.68° for *l*-threose diacetamide.

3. Quantitative hydrolysis of *d*-threose diacetamide with 0.100 *N* sulfuric acid at 100° shows a rate corresponding to *k* (minutes and decimal logs) = 0.0063 ±0.0013 and permits determination of the equilibrium specific rotation of *d*-threose in dilute acid as −12.5°, which is in agreement with our previously determined value and contrary to the findings of W. Freudenberg and Deulofeu.

4. During hydrolysis of *d*-threose diacetamide, the rotation is found to drift toward a smaller levorotation, in opposition to the reported observation of Deulofeu, and in agreement with the rotations determined by us for *d*-threose diacetamide and for *d*-threose.

5. The formation of a *tri*acetate by threose diacetamide indicates an aldehydo structure for this compound.

6. The preparation of *d*-erythrosazone and dibenzylidene-*d*-threitol from threose diacetamide hydrolysates, shows the identity of the sugar formed with that obtained by deacetylation of *d*-threose triacetate.

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