Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

OXIDATIVE, ALKALINE DEGRADATION OF 3-DEOXYALDOSULOSES*

R. M. ROWELL AND J. GREEN

*Division of Wood Chemistry Research, Forest Products Laboratory**, Forest Service, U. S. Department of Agriculture, Madison, Wisconsin 53705 (U. S. A.)*

(Received February 26th, 1970; in revised form, April 12th, 1970)

ABSTRACT

In alkaline solutions, the 3-deoxyaldosuloses are degraded by two distinct reaction pathways: (1), rearrangements to isomeric saccharinic acids, and (2) to aldonic acids by an oxidative carbon–carbon bond cleavage. Thus, 3-deoxy-D-*erythro*-hexosulose gave 3-deoxy-D-*ribo* and D-*arabino*-hexonic ("α"- and "β"-D-gluco-metasaccharinic) acids in a ratio of approximately 1 to 4 by a benzyl–benzilic acid type of rearrangement and was oxidized to 2-deoxy-D-*erythro*-pentonic acid. Similarly, 3-deoxy-D-*threo*-hexosulose was rearranged to 3-deoxy-D-*xylo* and D-*lyxo*-hexonic ("α"- and "β"-D-galactometasaccharinic) acids in the same ratio and was oxidized to 2-deoxy-D-*threo*-pentonic acid. The preponderant pathway, even under oxidative conditions, was found to be the rearrangement reaction. The effects of cation, alkali concentration, and temperature were also determined.

INTRODUCTION

It has been well established that 3-deoxy-D-*erythro*-hexosulose is an intermediate in the formation of 3-deoxy-D-*ribo* and D-*arabino*-hexonic ("α"-and "β"- D-gluco-metasaccharinic) acids under mild alkaline conditions in an inert atmosphere[1–3]. However, the effect of oxygen on 3-deoxy-D-*erythro*-hexosulose in alkaline solutions has not been investigated. The reactions giving rise to D-glucometasaccharinic acids are important in the field of pulping chemistry, since the formation of D-glucometasaccharinic acid end-units in a cellulose molecule render the polysaccharide more stable to alkali. As a result, losses of cellulosic material during alkaline pulping are decreased. An earlier paper in this series[4] dealt with the alkaline degradation of cellobiose under a variety of conditions. There it was found that D-glucometasaccharinic acids were not formed. Instead, oxidation reactions predominated, giving rise to aldonic acids. It was considered important to degrade 3-deoxy-D-*erythro*-hexosulose under the same non-oxidizing and oxidizing conditions as were used for cellobiose, and determine the influence of oxygen upon the intermediate giving rise to D-gluco-metasaccharinic acids.

*Presented, in part, at the Third Canadian Wood Chemistry Symposium, Vancouver, B.C., June 1970.
**In cooperation with the University of Wisconsin, Madison, Wisconsin.

Vicinal dicarbonyl compounds capable of undergoing the benzyl–benzilic acid type rearrangement[5] to α-hydroxyalkanoic acids can also undergo rupture between the carbonyl groups by an oxidative mechanism and give rise to two shorter-chain carboxylic acids. For example, 4-deoxy-D-*glycero*-2,3-hexo-diulose is rearranged in lime water to"D-glucoisosaccharinic" acid under oxygen-free conditions, and is oxidized by 30% hydrogen peroxide to a mixture of glycolic and 3,4-dihydroxybutyric acids[6,7] Likewise, 3-deoxy-D-*erythro*-hexosulose is rearranged by oxygen-free lime water to D-glucometasaccharinic acids, and is fragmented to formic and 2-deoxy-D-*erythro*-pentonic acids by 30% hydrogen peroxide[1].

Another factor which seems to play an important part in the benzil–benzilic acid type of rearrangement is the cation employed in the alkali. Divalent cations such as calcium and barium give the highest yields of the rearrangement products, whereas monovalent cations such as sodium and potassium favor fragmentation of the carbon skeleton. There has been speculation that this is due to an internal complex-formation with divalent cations[8]. Thus, 1-deoxy-D-*erythro*-2,3-hexodiulose in calcium hydroxide gives predominantly the rearrangement product, "α"-D-glucosaccharinic acid, whereas in sodium hydroxide the major products are acetic and D-erythronic acids[9]. Likewise, D-*arabino*-hexosulose in calcium hydroxide forms D-mannonic acid by a stereospecific rearrangment, whereas in sodium hydroxide D-arabinonic acid is the major product[10]. It is clear that two distinct types of reaction occur with the dicarbonyl intermediates in alkali: (*a*) rearrangements to isomeric saccharinic acids, and (*b*) oxidative chain-cleavage to aldonic acids. The extent of these two reactions on 3-deoxyaldosuloses in non-oxidizing and mildly oxidizing· alkaline media are studied herein.

Since the formation of 3-deoxyaldosuloses involves the loss of two asymmetric centers, only two structures are possible for the products in the D-series, the D-*erythro* configuration, in which the hydroxyl groups at C-4 and C-5 are *cis*, and the D-*threo* configuration in which these two hydroxyl groups are *trans*. If C-4, C-5, and C-6 have any influence on the rearrangement reaction, these differences should be observed in the degradation of 3-deoxy-D-*erythro*-hexosulose and its D-*threo* analog.

EXPERIMENTAL

3-Deoxy-D-erythro-*hexosulose*. — The following procedure is a modification of the one originally given by Kato[11]. To 30 g of D-glucose in absolute methanol (20 ml) was added 17 ml of butylamine, and the mixture was stirred for 30 min at 65°. The light yellow solution was cooled quickly to 25°, 10 ml of glacial acetic acid was added, and the mixture was again stirred for 30 min at 65°. The resulting black solution was cooled quickly to 25° and diluted with 300 ml of distilled water. Decolorizing carbon was added, and the mixture was stirred for 1 h. After filtering the mixture through a bed of Celite, the straw-colored filtrate contained three components, as indicated by t.l.c. in 6:3:1 (v/v) ethyl acetate–ethanol–water. The major product, having $R_{Glucose}$ 0.35 was found to be the Amadori product[12], 1-butylamino-1-deoxy-D-fructose[13]. Minor spots having $R_{Glucose}$ 1.0 and 1.3 were found to be D-glucose and

3-deoxy-D-*erythro*-hexosulose, respectively. To remove the nitrogenous material, the aqueous solution was passed through a column of Bio-Rad AG 50w–X8 (H^+) resin (4 × 20 cm bed of resin). The effluent was evaporated under diminished pressure below 40° to a thick, light-yellow syrup weighing 6.2 g. This syrup was dissolved in a minimum amount of ethanol and applied to the top of an Avicel* column (3 × 60 cm) and chromatographed in the 6:3:1 solvent system. The pure fractions containing 3-deoxy-D-*erythro*-hexosulose were combined and evaporated below 40° to a thick syrup weighing 0.87 g.

3-Deoxy-D-threo-hexosulose. — By the above procedure, 30 g of D-galactose gave 0.94 g of chromatographically pure 3-deoxy-D-*threo*-hexosulose, $R_{Galactose}$ 1.8 in the 6:3:1 solvent.

Degradation of 3-deoxyaldosuloses. — *Run A.* Purified nitrogen gas was bubbled through 25 ml of 0.02M $Ba(OH)_2$ (prepared from deoxygenated, distilled water) in a 100-ml bubbling tower equipped with a closed side-arm and two vacuum-sealed stopcocks. Nitrogen was allowed to pass through the solution for 15 min, and was then closed off. A vacuum was employed to evacuate the reaction vessel, which was then filled again with nitrogen. This procedure was repeated four times, and then 0.035 g of 3-deoxy-D-*erythro*-hexosulose was added from the side arm inside the flask. The flask was shaken. Nitrogen gas was bubbled through the solution throughout the degradation. A temperature of 25° was maintained by using a constant-temperature water-bath. Aliquots of 3 ml were removed, under nitrogen, at different time-intervals and the reaction products were determined.

Run B. The procedure was that given for Run *A*, except that no special precautions were used to exclude air from the flask when samples were removed. The flask was opened to the atmosphere for sampling, and aliquots were withdrawn by means of a disposable pipet.

Run C. Oxygen gas was bubbled through 25 ml of 0.02M $Ba(OH)_2$ for 0.5 h, and then 0.035 g of 3-deoxy-D-*erythro*-hexosulose was added. A temperature of 25° was maintained and oxygen gas was bubbled through the solution throughout the degradation. Aliquots of 3 ml were removed at various time intervals.

Run D. The procedure was as given for Run C, except that 0.04M NaOH was used.

Run E. The procedure was as given for Run C, except the temperature was maintained at 50°.

Run F. The procedure was as given for Run E, except that 0.2M $Ba(OH)_2$ was used.

Run G. The procedure was as given for Run C, except that the temperature was maintained at 100°.

Run H. The procedure was given for Run C, except that 0.035 g of 3-deoxy-D-*threo*-pentosulose was used.

Automated detection of products. — The aliquots from the various reactions

*Microcrystalline cellulose available from FMC Corporation, American Viscose Division, Newark, Delaware.

were neutralized with Bio-Rad AG 50w–X8 (H^+) resin. The solutions were decanted into small test tubes and frozen until needed. It is important to note in this neutralization procedure that the resin should be added, the mixture shaken, and the pH be determined quickly. Once neutral the solution should be separated immediately, because lactones form rapidly in the presence of the acid resin and the losses of free acids are significant. Corbett and Liddle[14] also found this to be a problem in the determination by the resin methods of total acids produced in the alkaline degradation of D-glucose. In those solutions where lactonization accounted for more than 1% of the total products (as determined by the area under recorded peaks described later), 0.02 ml of 2M KOH was added to ensure complete conversion into the acid salts.

After warming the solution to 25°, a sample (0.6 ml) was pumped (0.6 ml/min) by means of a peristaltic pump onto a column (0.6 × 72 cm) packed with Bio-Rad AG 1-X8 (200–400 mesh) resin in the acetate form. The column was eluted with water (1.8 ml) and the acids eluted with 2M acetic acid. The eluate from the bottom of the column was divided into two fractions. One part went to a fraction collector and the other was analyzed by the automated periodate–pentane-2,4-dione assay[15] for released formaldehyde, employing a Technicon Autoanalyzer. The yellow color developed in the assay, due to the formation of 3,5-diacetyl-1,4-dihydro-2,6-lutidine, was recorded at 420 nm. The amount of each compound was determined by comparison of areas under peaks relative to standard runs. The results are given in Tables I and II.

DISCUSSION

The alkaline degradation of the 3-deoxyaldosuloses provides an opportunity to determine the effects of oxygen on the intermediate that gives rise to 3-deoxyhexonic

TABLE I

RETENTION TIMES OF COMPOUNDS ON BIO-RAD AG 1-X8 (200–400 MESH) RESIN[a]

Compound	*Time to peak center (min)*		
	Standard	*3-deoxy-D-erythro-hexosulose*	*3-deoxy-D-threo-hexosulose*
3-Deoxy-D-*erythro*-hexosulose	42–44	42	
3-Deoxy-D-*threo*-hexosulose	42–44		43
Lactones of acids listed below	50–52	50	50
2-Deoxy-D-*erythro*-pentonic acid	64–66	64	
2-Deoxy-D-*threo*-pentonic acid	64–66		65
3-Deoxy-D-*ribo*-hexonic ("α"-D-glucometasaccharinic) acid	84–86	84	
3-Deoxy-D-*arabino*-hexonic ("β"-D-glucometasaccharinic) acid	100–104	100	
3-Deoxy-D-*xylo*-hexonic ("α"-D-galactometasaccharinic) acid	85–87		86
3-Deoxy-D-*lyxo*-hexonic ("β"-D-galactometasaccharinic) acid	98–102		98

[a]Acetate form, elution with 2M acetic acid.

TABLE II

ALKALINE DEGRADATION OF 3-DEOXYALDOSULOSES[a]

Compound and run no.	*Time of degradation*			
	15 min	*2 h*	*6 h*	*End*[b]
3-Deoxy-D-*erythro*-hexosulose				
N_2, 25°, 0.02M $Ba(OH)_2$	68.2	28.2		
Trace O_2, 25°, 0.02M $Ba(OH)_2$	66.8	25.6	22.6	7.1
O_2, 25°, 0.02M $Ba(OH)_2$	56.0	23.1	19.4	2.3
O_2, 25°, 0.04M NaOH	63.1	19.9	18.2	4.6
O_2, 50°, 0.02M $Ba(OH)_2$	18.9	9.9	5.4	3.8
O_2, 50°, 0. 2M $Ba(OH)_2$	14.2	7.1	3.C	
O_2, 100°, 0.02M $Ba(OH)_2$	3.1			
2-Deoxy-D-*erythro*-pentonic acid				
N_2, 25°, 0.02M $Ba(OH)_2$	1.2	2.7		
Trace O_2, 25°, 0.02M $Ba(OH)_2$	7.8	8.9	9.5	14.8
O_2, 25°, 0.02M $Ba(OH)_2$	15.6	31.0	35.9	41.5
O_2, 25°, 0.04M NaOH	7.0	14.6	16.8	22.6
O_2, 50°, 0.02M $Ba(OH)_2$	20.0	26.8	23.9	24.1
O_2, 50°, 0. 2M $Ba(OH)_2$	20.3	25.2	21.2	
O_2, 100°, 0.02M $Ba(OH)_2$	16.8			
"α"-D-Glucometasaccharinic acid				
N_2, 25°, 0.02M $Ba(OH)_2$	8.7	17.5		
Trace O_2, 25°, 0.02M $Ba(OH)_2$	7.2	16.4	17.0	19.5
O_2, 25°, 0.02M $Ba(OH)_2$	8.4	8.6	8.5	11.5
O_2, 25°, 0.04M NaOH	6.0	12.7	12.3	13.9
O_2, 50°, 0.02M $Ba(OH)_2$	12.9	15.1	17.5	16.2
O_2, 50°, 0. 2M $Ba(OH)_2$	26.1	27.6	31.3	
O_2, 100°, 0.02M $Ba(OH)_2$	24.4			
"β"-D-Glucometasaccharinic acid				
N_2, 25°, 0.02M $Ba(OH)_2$	21.9	51.6		
Trace O_2, 25°, 0.02M $Ba(OH)_2$	18.2	49.0	51.0	58.6
O_2, 25°, 0.02M $Ba(OH)_2$	20.0	37.3	36.2	44.6
O_2, 25°, 0.04M NaOH	23.8	52.8	52.6	58.8
O_2, 50°, 0.02M $Ba(OH)_2$	48.1	48.2	53.2	55.9
O_2, 50°, 0. 2M $Ba(OH)_2$	39.4	40.2	44.4	
O_2, 100°, 0.02M $Ba(OH)_2$	55.6			
3-Deoxy-D-*threo*-hexosulose				
O_2, 25°, 0.02M $Ba(OH)_2$	35.4	28.9		3.6
2-Deoxy-D-*threo*-pentonic acid				
O_2, 25°, 0.02M $Ba(OH)_2$	22.4	24.4		36.3
"α"-D-Galactometasaccharinic acid				
O_2, 25°, 0.02M $Ba(OH)_2$	10.2	11.1		15.7
"β"-D-Galactometasaccharinic acid				
O_2, 25°, 0.02M $Ba(OH)_2$	32.1	35.5		44.4

[a]Expressed as molar percent yields. [b]Periods of B, 4 days; C, 9 days; D, 11 days; E, 2 days; and H, 9 days.

(metasaccharinic) acids. Since D-glucometassaccharinic acid is not formed to any great extent in the degradation of cellobiose[4] or cellulose[16] in the presence of oxygen, it is not known whether this is due to the lack of formation of the 3-deoxyaldosulose or whether the 3-deoxyaldosulose, once formed, is degraded to other products.

Table II clearly shows that non-oxidative benzil–benzilic type of rearrangement to the metasaccharinic acids is the preponderant reaction, even in the presence of oxygen. Run A demonstrates that the pathway by oxidative carbon–carbon bond cleavage to 2-deoxy-D-*erythro*-pentonic acid can effectively be eliminated by excluding oxygen. Run B shows how sensitive these reactions are to oxygen and that alkaline 30% hydrogen peroxide is not required. The controls in this run closely resemble the conditions most often referred to in the literature as an "oxygen-free" environment; in this case 15% of the total products are lost in oxidation products.

The differences between Runs C and D contradict previous findings of the effect of monovalent versus divalent cations. It would be expected that the most severe conditions favoring fragmentation would be with sodium hydroxide in an oxygen atmosphere. The greatest proportion of the fragmentation product, 2-deoxy-D-*erythro*-pentonic acid is formed, however, in the presence of oxygen and divalent barium. This is inconsistent with existing theories of the participation of divalent ions in the benzil–benzilic type rearrangement discussed earlier and is under further investigation.

In the rearrangement reaction, "α"-and "β"-D-glucometasaccharinic acids are produced in a ratio of approximately 1 to 4, indicating some stereospecificity in the reaction. This was also found with "D-galactometasaccharinic" acid, indicating C-4, C-5, and C-6 exert no stereochemical influence on the rearrangement. In the alkaline degradation of D-*erythro*-hexosulose, Lindberg and Theander[10] found a very selective, stereospecific rearrangement to D-mannonic acid in preference to D-gluconic acid, probably due to the influence of the asymmetric center at C-3. Since the 3-deoxyaldosuloses do not contain this asymmetric center it is not clear what are the controlling factors to produce this one-sided ratio. Since the same ratio is observed when monovalent sodium is used, it can be assumed that the complexing nature of divalent barium is not the explanation for the stereochemical preference. It is interesting to note that at high alkali concentrations (0.2M, Run F) the ratio of "α" to "β" is reduced to 1 to 1.5.

The effect of increasing the temperature in these reactions is less pronounced than was found for cellobiose[4]. The yield of the oxidation product decreases as the temperature is increased and slightly more "α" D-glucometasaccharinic acid appears at higher temperatures.

Finally, the data prove that in the previous degradation of cellobiose[4], the 3-deoxy-D-glucosyl-D-*erythro*-hexosulose was not formed. If it had been produced as an intermediate from cellobiose, the D-glucosyl-D-glucometasaccharinic acid would have been observed, since the rearrangement is the predominating reaction pathway under all conditions employed.

REFERENCES

1 G. MACHELL AND G. N. RICHARDS, *J. Chem. Soc.*, 1938 (1960).
2 E. F. L. J. ANET, *J. Amer. Chem. Soc.*, 82 (1960) 1502.
3 E. F. L. J. ANET, *Aust. J. Chem.*, 14 (1961) 295.
4 R. M. ROWELL, P. J. SOMERS, S. A. BARKER, AND M. STACEY, *Carbohyd. Res.*, 11 (1969) 17.
5 A. LACHMAN, *J. Amer. Chem. Soc*, 45 (1923) 1509.
6 M. J. BLEARS, G. MACHELL, AND G. N. RICHARDS, *Chem. Ind.* (London), 1150 (1957).
7 G. MACHELL AND G. N. RICHARDS, *J. Chem. Soc.*, 1932 (1960).
8 J. KENNER AND G. N. RICHARDS, *J. Chem. Soc.*, 3019 (1957).
9 A. ISHIZU, B. LINDBERG, AND O. THEANDER, *Carbohyd. Res.*, 5 (1967) 329.
10 B. LINDBERG AND O. THEANDER, *Acta Chem. Scand.*, 22 (1968) 1782.
11 H. KATO, *Agr. Biol. Chem.*, 26 (1962) 187.
12 J. E. HODGE, *Advan. Carbohyd. Chem.*, 10 (1955) 169.
13 F. MICHEEL AND G. HAGEMANN, *Ber.*, 92 (1959) 2836; 93 (1960) 2381.
14 W. M. CORBETT AND A. M. LIDDLE, *J. Chem. Soc.*, 531 (1961).
15 O. SAMUELSON AND H. STRÖMBERG, *Carbohyd. Res.*, 3 (1966) 89.
16 O. SAMUELSON AND L. STOLPE, *Tappi*, 52 (1969) 1709.