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The Basic Degradation of D-*ribo*-Hexos-3-ulose and Isolation of 3-Deoxy-D-*glycero*-pentosulose

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From the early stage of the reaction of D-*ribo*-hexos-3-ulose (*1*) with aqueous sodium hydroxide solution, 3-deoxy-D-*glycero*-pentosulose (*2*) was isolated in high yield. In the parallel reaction with calcium hydroxide, the concentration of *2* was much less. After a longer reaction time, *1* (or *2*) was degraded to acids, principally 3-deoxy-D-*threo*-pentonic acid (*10*), but also 3-deoxy-D-*erythro*-pentonic (*9*) and 2-deoxy-D-*glycero*-tetronic acids (*11*).

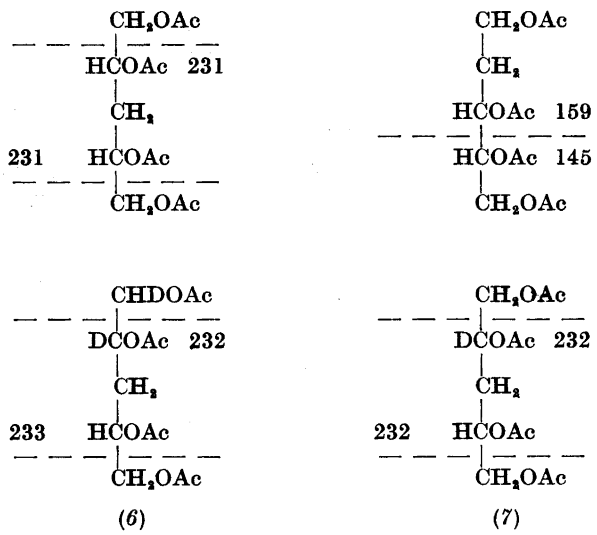
Previous publications [1–5] from this Laboratory have reported the alkaline degradation of dicarbonyl carbohydrates and their derivatives. The alkaline degradation was studied [1–2] of the oxidised glycosides methyl α- and β-D-*ribo*-hexopyranosid-3-ulose (and the 4-*O*-methyl derivative of the latter [3]) and methyl β-D-*arabino*-hexopyranosidulose, which were used as model compounds for intermediates in the oxidative degradation of cellulose and starch. Their extremely rapid breakdown, both is basic solution at room temperature, and at higher temperatures also under neutral and slightly acidic conditions, was shown to proceed *via* dicarbonyl intermediates. 1-Deoxy-D-*erythro*-2,3-hexodiulose, postulated to be an intermediate in glucosaccharinic acid formation, yielded on alkaline treatment [4] predominantly 2-C-methyl-D-ribonic acid ("α"-glucosaccharinic acid), and only traces of the "β"-isomer and the fragmentation product D-erythronic acid. Recently, the alkaline treatment of D-*arabino*-hexosulose (D-glucosone) was studied.[5] The main non-volatile acidic products were the rearrangement acids, mannonic and gluconic acids, with a strong preponderance of the former, and also the fragmentation acids, arabinonic, ribonic, and erythronic acids. Strong support was thus provided that osone units are intermediates in the transformation under oxidative alkaline conditions of reducing sugar end units in polysaccharides into aldonic acid end units.

The present investigation concerns the alkaline treatment of the related hexosulose, D-*ribo*-hexos-3-ulose (*1*), a dicarbonyl carbohydrate, containing non-contiguous carbonyl groups, and the parent sugar to some of the glycosides previously studied.[1,2].

When *1* was treated with 0.04 M aqueous sodium hydroxide solution at room temperature, the product in the early reaction stages contained a large amount of a neutral component (*2*) showing as an elongated spot on paper chromatography. The concentration of this product was at maximum after 10 min, and it persisted in minor concentration up to about 2 h. In a typical preparative experiment, the base treatment was interrupted after 10 min, and after removal of acids the products were fractionated on a cellulose column. A pure fraction was obtained which on concentration yielded a yellowish glass, shown below to be 3-deoxy-D-*glycero*-pentosulose (*2*), in 22 % yield. Also impure fractions containing *2*, and unreacted *1* were obtained.

Compound *2*, ($[\alpha]_D^{22}+7°$) gave an immediate precipitate with phenylhydrazine or 2-4-dinitrophenylhydrazine, suggestive, together with its high electrophoretic mobility in bisulphite buffer,[6] of the presence of a carbonyl group or groups. Reduction of the product with excess of sodium borohydride gave a material, which on paper chromatography clearly was seen to consist of two compounds in approximately equal amounts, having mobilities similar to deoxypentitols. Acetylation of this reduction product mixture, followed by GLC (ECNSS column at 150°) showed the two component acetates to be in equal amounts and to have the same retention times as authentic samples of 3-deoxy-D-*erythro*-pentitol (*3*) and 3-deoxy-D-*threo*-pentitol (*4*). The retention times did not coincide with those of the acetates of erythritol, threitol, 2-deoxy-D-*erythro*-pentitol (*5*), or the hexitols. The identity of the reduction products was confirmed as *3* and *4*, since the mass spectra of their acetates were identical with those of the authentic samples.

The mass spectra are as expected from previous experience,[7] showing C – C cleavage at C1 – C2 (C4 – C5), but not at bonds adjacent to the methyl-



(*6*) (*7*)

Fig. 1. C – C Cleavage in mass spectra (only *erythro*-isomers shown; *m/e* values identical for *threo*-isomers).

ene group (Fig. 1). Other peaks in the spectra can be satisfactorily accounted for by the single or sequential loss of acetic acid, and ketene from the (unobserved) molecular ion or the *m/e* 231 (C1 – C2 cleavage) ion. The mass spectrum of the acetate of *5* was different from those of *3* and *4*, showing significant peaks at *m/e* 159 and 145 for C3 – C4 cleavage, which is not observed for *3* and *4*. The *m/e* 231 ion is not observed in acetylated *5*, but a peak at 129 probably results from the loss of acetic acid and ketene from the 231 ion.

The reduction data show that the product contains a non-terminal carbonyl group. The question of whether it also contained a terminal carbonyl group was resolved by reduction with sodium borodeuteride, followed by acetylation and GLC-mass spectrometry of the product. The possible shifts expected in *m/e* values from the normal reduction product are shown in Fig. 1 (*6* and *7*). The spectra of the isomers of the deuterium-containing acetate showed equally intense peaks at *m/e* 232 and 233 (highest mass peaks), thus showing the incorporation of two atoms of deuterium and hence two carbonyl groups in the original compound. That the compound was the 1,2- rather than the 1,4-dicarbonyl was shown by the isolation of the 2,4-dinitrophenylosazone, which had similar m.p. to that published,[8] and which gave a violet colour with sodium hydroxide solution, showing it to be a bis-2,4-dinitrophenylhydrazone of an α-dicarbonyl compound.[9,10]

The optical rotation of the previously isolated [8] material *2*, a colourless solid, was $[\alpha]_D^{22}$ $-13° \rightarrow -19°$, whereas it was $+7°$ in the present case. It is possible that this discrepancy results from slight impurity in the isolated material. Further purification by chromatography is difficult, due to the tendency

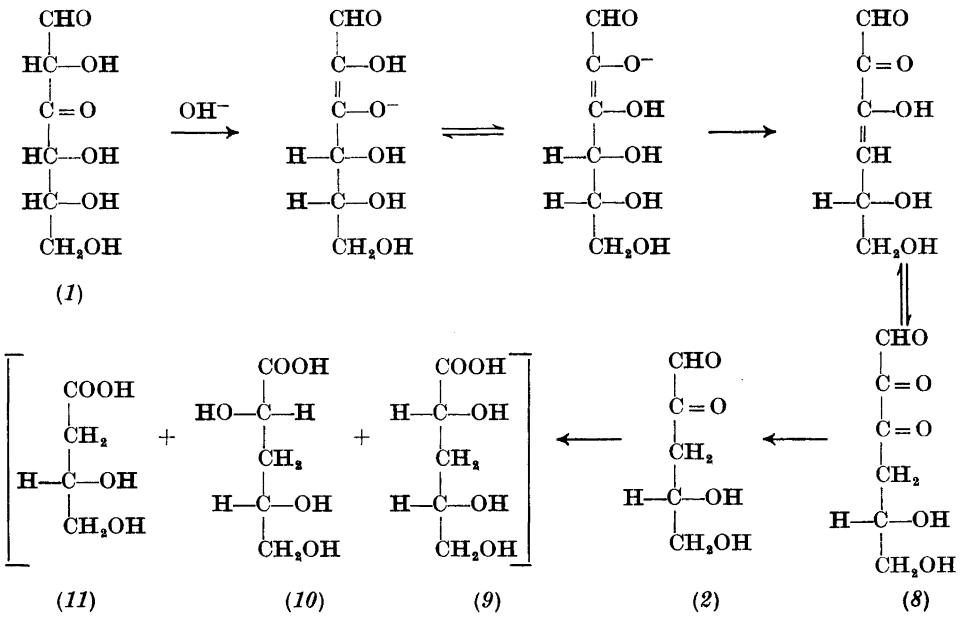


Fig. 2. Alkaline degradation of D-*ribo*-hexos-3-ulose.

of *2* to streak and to its lability. The contaminating material is probably not present in high amount, since the GLC trace, of the product of reduction and acetylation does not show any significant peaks, except those for the *3* and *4* acetates. It has previously been noted [11] that a dicarbonyl carbohydrate may have widely different rotation when isolated from different sources.

The degradation product is formed most probably by the route shown in Fig. 2, which is related to the accepted scheme of metasaccharinic acid formation. Compound *2* was also detected when 0.02 M calcium hydroxide was used in place of sodium hydroxide, but its concentration was much lower in the reaction with calcium hydroxide, and its presence was more transient. It was hardly detectable after 15 min at room temperature. It would not be expected that the postulated tricarbonyl intermediate *8* would exist long enough to be detected by chromatography.

The acids formed on base treatment of *1* and *2* were identified by comparison with authentic samples by paper chromatography and conversion to the lactones followed by GLC of their trimethylsilyl derivatives. The *erythro-* and *threo-*isomers of 3-deoxy-pentonic acid (*9* and *10*) and 2-deoxy-D-*glycero*-tetronic acid (*11*) were formed. Some other acids (not yet investigated) were formed, of which one with a retention time between the tetronic acid and the two pentonic acids was present in a significant amount, particularly when formed from *1*, where it amounted to more than *9* did. Compounds *9*, *10*, and *11* are expected products from *2*, having been previously [12] obtained by alkaline treatment of D-xylose, and they are analogous to the acids formed by alkaline treatment of 3-deoxy-D-*erythro*-hexosulose.[13] The relatively high amount of the unidentified acid mentioned above, as well as some minor ones, suggests the existence of other modes of degradation.

Table 1. Relative proportions of acids formed on alkaline treatment of D-*ribo*-hexos-3-ulose (*1*) and 3-deoxy-D-*glycero*-pentosulose (*2*)

Acid	Treatment with NaOH		Treatment with $Ca(OH)_2$	
	1	*2*	*1*	*2*
3-Deoxy-D-*threo*-pentonic acid	81	77	66	69
3-Deoxy-D-*erythro*-pentonic acid	13	15	14	21
2-Deoxy-*glycero*-tetronic acid	6	8	20	10

The relative proportions of the three identified acids, estimated by measuring the GLC peak areas of their corresponding lactone trimethylsilyl ethers are given in Table 1. Whilst the detailed study of the acid products is at present incomplete, it is clear that *1* is rapidly and predominantly degraded to acids by fragmentation to the intermediate *2*, the expected intermediate for formation of C-5 metasaccharinic acids. It is, however, notable that the rearrangement acids *9* and *10* clearly predominated over the fragmentation acid *11* in either sodium or calcium hydroxide treatment of both *1* and *2*, as was

observed [13] in studies of glucometasaccharinic acid formation. It is also evident that the *threo*-isomer *10* strongly predominates over the *erythro*-isomer *9* in all four experiments, as it did also in the alkaline treatment of D-xylose.[12] A comparable preferential formation of β-D-glucometasaccharinic acid over the α-isomer has been reported.[13] Furthermore, the preference of *10* over *9* is greater for sodium hydroxide than for calcium hydroxide, which also parallels previous findings.[13]

The reason for the preference of *10* to *9* is not clear. Asymmetric induction has been evoked in some other cases,[4,5] where there is an asymmetric centre adjacent to one of the carbonyl groups, but that is not the case in *2*. Relative thermodynamic stability of the product acids might play a part, but there was no appreciable epimerisation when 3-deoxy-D-*erythro*-pentonic acid was kept in 0.04 M sodium hydroxide solution at room temperature for 4 h. At higher temperature, however, the *erythro* and *threo* acids may be interconverted [12]

EXPERIMENTAL

General. Concentrations were carried out at reduced pressure below 40°. Paper chromatograms were run on Whatman 1 papers in the following solvent systems: (a) butanol-ethanol-water (4 : 1 : 5); (b) ethyl acetate-acetic acid-water (3 : 1 : 1); (c) water-saturated methyl ethyl ketone. Electrophoresis was done in bisulphite buffer [6] (pH 4.7) at 50°. Conventional spraying reagents were used for reducing sugars, polyols, acids and lactones. The melting points are corrected.

D-*ribo*-Hexos-3-ulose (*1*) was synthesised by the catalytic oxidation of 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose with ruthenium dioxide-potassium periodate,[14] followed by hydrolysis of the purified product with trifluoroacetic acid-water 9 : 1.[15] The product was purified on a cellulose column (solvent a).

3-Deoxy-D-*erythro*-pentitol and 3-deoxy-D-*threo*-pentitol were made by the reduction (sodium borohydride) of authentic samples of lactones of 3-deoxy-D-*erythro*-pentonic and 3-deoxy-D-*threo*-pentonic acids. 2-Deoxy-D-*erythro*-pentitol was made by reduction of 2-deoxy-D-*erythro*-pentose.

Alkaline treatment of 1. On preliminary experiments, *1* was treated with 0.04 M sodium hydroxide solution or 0.02 M calcium hydroxide solution, and samples withdrawn at intervals, dealkalinified (Dowex 50 (H^+)), and the acids formed by degradation, removed by treatment with Dowex 3 (free base). The resulting neutral solutions were examined by paper chromatopraphy (solvent a) and by electrophoresis. A major component, which streaked on the chromatograms (R_G 1.6 – 4.3) and had $M_{vanillin}$ [6] 2.5 on electrophoretograms, was present in maximum concentration after 10 min, when sodium hydroxide was used. In a typical preparative experiment, *1* (600 mg) was treated with 270 ml of the sodium hydroxide solution for 10 min at room temperature, deionised as above, and concentrated. Chromatography on a cellulose column (solvent a) gave in the early fractions a yellowish glass (100 mg) $[\alpha]_D^{22}$ +7°, which was shown to be 3-deoxy-D-*glycero*-pentosulose (*2*). The compound *2* showed on chromatography as a strong orange spot with, 2,4-dinitrophenylhydrazine reagent, dark red with *p*-anisidine hydrochloride, grey with resorcinol, and dark brown with silver nitrate-sodium hydroxide.

The identity of the product 2 was proved by reduction (excess sodium borohydride) followed by (*i*) paper chromatography (solvent a), and (ii) acetylation (acetic anhydride-pyridine), followed by GLC (ECNSS 3 % column at 150°) or GLC-mass spectrometry. The GLC trace showed two peaks of equal intensity, having retention times of 16.5 and 18.5 min (*cf.* acetates of 3-deoxy-D-*erythro*-pentitol 18.5 min, 3-deoxy-D-*threo*-pentitol 16.5 min, 2-deoxy-D-*erythro*-pentitol 13.4 min). Mass spectral data: acetates of 3-deoxy-*erythro*- and *threo*-pentitols (essentially identical and also identical with the acetates produced above from the degradation product *2*), *m/e* 231 (10 % of base peak), 184 (4), 171 (5), 142 (18), 129 (62), 103 (10), 100 (9), 87 (9), 83 (11), 82 (15), 69 (off scale, arbitrarily 100), 43 (off scale, arbitrarily 100), 28 (90). Degradation product *2* reduced

with sodium borodeuteride and acetylated: 233 (3), 232 (4), 186 (1.5), 173 (2), 172 (2), 145 (3), 144 (7), 143 (3), 131 (15), 130 (15), 71 (30), 70 (30), 43 (100), 28 (76). Acetate of 2-deoxy-*erythro*-pentitol: 159 (6), 145 (6), 142 (3), 129 (4), 117 (5), 103 (7), 99 (6), 43 (50), 36 (10), 32 (21), 28 (100).

Treatment of the degradation product with 2,4-dinitrophenylhydrazine solution [16] gave a 2,4-dinitrophenylosazone, m.p. 252–260° (lit. [7] 257°). Addition to this of a 2 M sodium hydroxide solution [9,10] caused a strong violet colour.

Acid formation. Compound *1* (5 mg) was treated at room temperature with 0.04 M sodium hydroxide solution (1 ml) for 4 h. The solution was dealkalinified by addition of Dowex 50 (H^+) and the product acids extracted on to Dowex 3 (free base), whence they were regenerated free from neutral material by the addition of 50 % ammonium hydroxide. After evaporation, the ammonium salts of the acids were converted to the free acids by a small amount of Dowex 50 (H^+) added to the aqueous solution. Evaporation caused spontaneous lactonisation. Similar procedures were used for *2* with sodium hydroxide, and for *1* and *2* with 0.02 M calcium hydroxide. The lactones were (i) trimethylsilylated and examined by GLC on an OV 17 column at 140° (Table 1), and (ii) examined by paper chromatography (solvents b and c). 3-Deoxy-D-*threo*-pentonic acid was seen as a strong spot with periodate-benzidine reagent, silver nitrate-sodium hydroxide, or lactone spray. 3-Deoxy-D-*erythro*-pentonic and 2-deoxy-D-*glycero*-tetronic acids were detected in smaller amounts.

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