Synthesis and Acidic Degradation of 3-Deoxy-ketoaldonic Acids

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The syntheses of 3-deoxy-D-*threo*-2-hexulosonic acid and 3-deoxy-D-*arabino*-2-heptulosonic acids are described. Upon treatment with acid, 6-, 7-, and 8-carbon 3-deoxy-ketoaldonic acids first yield enolic 1,4-lactones, which are further transformed into 5-(hydroxyalkyl)-2-furoic acids.

It is believed that 3-deoxy-D-*manno*-2-octulosonic acid (I) is an important component of the lipopolysaccharides (endotoxins) elaborated by Gram negative bacteria,[1] and it has been shown that in *Salmonellae* [2] and in *E. coli* [3] this ketoaldonic acid is part of the polysaccharide chain.

Analytical work on such material invariably entails treatment with acid; we have therefore studied the behaviour of 3-deoxy-D-*manno*-2-octulosonic acid and of homologous 3-deoxy-ketoaldonic acids under conditions generally used for the hydrolysis of polysaccharides. The behaviour of a mixture of 3-deoxy-D-*manno*- and D-*gluco*-2-octulosonic acids in acidic medium has also been investigated recently by Dmitriev *et al.*[4]

Three different reaction sequences have been used to obtain 3-deoxy-ketoaldonic acids. In the first, the C-2 hydroxy-group of a 3-deoxyaldonic acid is selectively oxidised to a carbonyl group,[5a-g] with chlorate in the presence of vanadium oxide catalyst.[6a,b] In the second, aldoses are converted into imidolactones of enamines, which are then sequentially hydrolysed to 3-deoxy-ketoaldonic acids,[7a-g] and in the third, aldoses are condensed with oxalacetic acid to yield 3-deoxy-ketoaldonic acids directly.[8a-d] In the present work

[1] O. Lüderitz, O. Westphal, A. M. Staub, and H. Nikaido, in 'Microbial Toxins,' vol. IV, ed. G. Weinbaum, S. Kadis, and S. J. Ajl, Academic Press, New York and London, 1971, p. 145.

[2] M. J. Osborn, *Proc. Nat. Acad. Sci. U.S.A.*, 1963, **50**, 499.

[3] N. A. Fuller and E. C. Heath, *Fed. Proc.*, 1970, **29**, 337 Abs.

[4] B. A. Dmitriev, L. V. Bakinovskii, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1970, **193**, 1304 (*Chem. Abs.*, 1971, **74**, 23,084x)

[5] (*a*) J. MacGee and M. Doudoroff, *J. Biol. Chem.*, 1954, **210**, 617; (*b*) A. Weissbach and J. Hurwitz, *ibid.*, 1959, **234**, 705; (*c*) D. B. Sprinson, J. Rothschild, and M. Sprecher, *ibid.*, 1963, **238**, 3170; (*d*) D. T. Williams and M. B. Perry, *Canad. J. Biochem.*, 1969, **47**, 491; (*e*) M. B. Perry and A. C. Webb, *Canad. J. Chem.*, 1969, **47**, 2893; (*f*) D. T. Williams and M. B Perry, *Canad. J. Biochem.*, 1969, **47**, 983; (*g*) D. Charon, R. S. Sarfati, D. R. Strobach, and L. Szabó, *Eur. J. Biochem.*, 1969, **11**, 364.

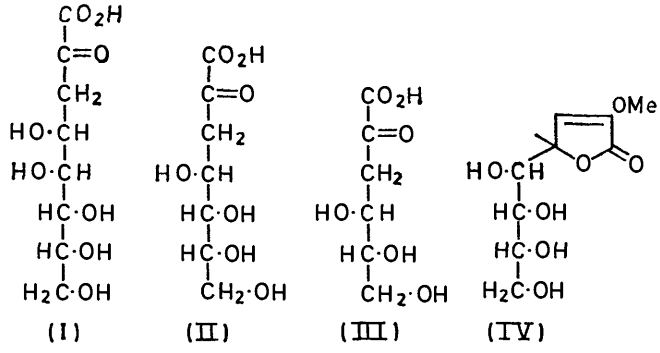
[6] (*a*) P. P. Regna and B. P. Caldwell, *J. Amer. Chem. Soc.*, 1944, **66**, 243; (*b*) H. S. Isbell, *J. Res. Nat. Bur. Stand.*, 1944, **33**, 45.

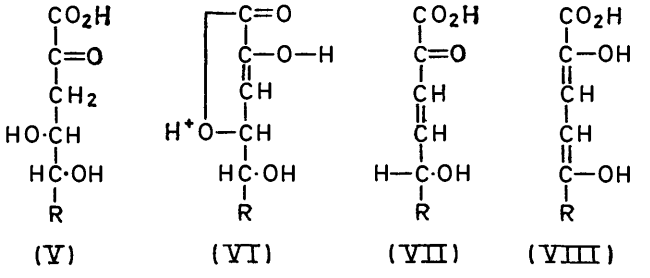
[7] (*a*) R. Kuhn, D. Weiser, and H. Fischer, *Annalen*, 1959, **628**, 207; (*b*) M. Adelsberg and D. B. Sprinson, *Biochemistry*, 1964, **3**, 1855; (*c*) G. B. Paerels, *Rec. Trav. chim.*, 1961, **80**, 985; (*d*) G. B. Paerels and H. W. Geluk, *ibid.*, 1970, **89**, 813; (*e*) N. K. Kochetkov, B. A. Dmitriev, and L. V. Bakinovskii, *Carbohydrate Res.*, 1969, **11**, 193; (*f*) M. N. Mirzanova, L P. Davydova, and G. I. Samokhvalov, *Doklady Akad. Nauk S.S.S.R.*, 1967, **173**, 367; (*g*) G. Baschang and H. Fritz, *Helv. Chim. Acta*, 1969, **52**, 300.

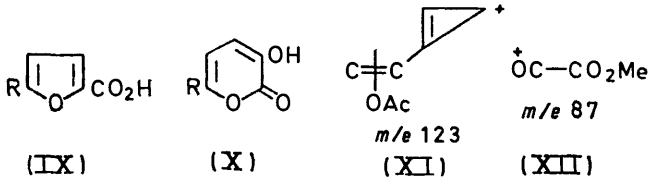
[8] (*a*) J. W. Cornforth, M. E Firth, and A. Gottschalk, *Biochem. J.*, 1958, **88**, 57; (*b*) M. A. Ghalambor, E. M. Levine, and E. C. Heath, *J. Biol. Chem.*, 1966, **241**, 3207; (*c*) C. Hershberger, M. Davis, and S. B. Binkley, *ibid.*, 1968, **243**, 1585; (*d*) D. Charon and L. Szabó, *Eur. J. Biochem.*, 1972, **29**, 184.

3-deoxy-D-*threo*-2-hexulosonic acid (III) and 3-deoxy-D-*arabino*-2-heptulosonic acid (II) were prepared by the oxidation method, and 3-deoxy-D-*manno*-2-octulosonic acid was obtained by condensation of D-arabinose with oxalacetic acid.[8c]

The oxidation method has the advantages that it yields sterically defined products and that the starting material is often readily accessible; its drawbacks







are that the reaction may be slow and that the yields are mediocre. Moreover, the reproducible preparation of the catalyst appears to be difficult: whereas phosphorylated ketoaldonic acids[5c,9] could be obtained in good yields by use of the catalyst prepared according to Sprinson *et al.*,[5c] which has also been used successfully for the oxidation of non-phosphorylated 3-deoxy-aldonic acids,[5d-g] we obtained very low yields of α-keto-acids both from galactometasaccharinic acid and from 3-deoxy-D-*gluco*-heptonic acid; the use of vanadium oxide in the presence of phosphoric acid[6a] gave much better results.

At room temperature, the 3-deoxy-ketoaldonic acids appear to be stable in N-acid for as long as 100 h (as estimated with periodate–thiobarbiturate).[10] Their stability decreases rapidly, however, with increasing temperature: in 0·1N-HCl at 95° almost 50% of the thiobarbiturate-positive material is lost in about 45 min (Figure 1). This apparent destruction is not very sensitive to pH: similar behaviour is observed for 0·01N-acetic acid (pH *ca.* 3·4).

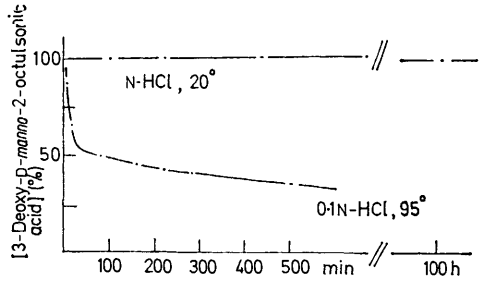


FIGURE 1 Destruction of 3-deoxy-D-*manno*-2-octulosonic acid in acidic medium

In the course of hot acidic treatment, the u.v. spectrum of 3-deoxy-D-*manno*-2-octulosonic acid which, in aqueous acid at room temperature, possesses no absorption band between 220 and 360 nm, undergoes considerable change. Two absorption bands appear sequentially; the first, centred at 230 nm is followed by a much stronger band at 258 nm (Figure 2); their evolution as a function of time is given in Figure 3, the curves being corrected for the mutual contributions of the bands. The rate of appearance is, for both chromophores, of apparent first-order kinetics (Figure 4) if the period before appearance of the 258 nm band is disregarded. These results suggest that the compound absorbing at 230 nm is a precursor of that absorbing

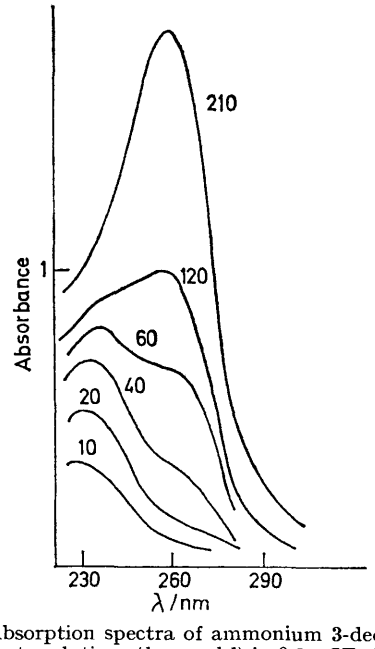


FIGURE 2 Absorption spectra of ammonium 3-deoxy-D-*manno*-2-octulosonate solutions (1 mg ml^{-1}) in 0·1N-CF_3·CO_2H at 100°, taken at the times indicated (min)

at 258 nm. Identical observations were made for the two other 3-deoxy-ketoaldonic acids (II) and (III).

The substance absorbing at 230 nm, derived from

F. Trigalo and L. Szabó, *Eur. J. Biochem.*, 1972, **25**, 336.

[10] A. Weissbach and J. Hurwitz, *J. Biol. Chem.*, 1959, **234**, 705.

3-deoxy-D-*manno*-2-octulosonic acid by treating it with 0·1N-trifluoroacetic acid at 100° for 35 min, was isolated by t.l.c.: upon re-chromatography in the same solvent system it partially re-formed the starting ketoaldonic acid. This is in keeping with the observation [7d] that 3-deoxy-2-hydroxy-D-*arabino*-hept-2-enono-1,4-lactone and similar lactones are unstable. When the acidic solution was immediately treated with diazomethane, a stable, crystalline compound could be isolated. This had a single u.v. absorption band centred at 230 nm, and an i.r. C=O band at 1767 and C=C band at 1656 cm^{-1}. Upon treatment with periodate, 3 mol. equiv. of the oxidant were rapidly

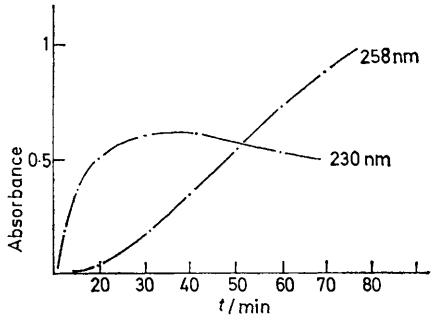


FIGURE 3 Evolution of the absorption bands at 230 and 258 nm of ammonium 3-deoxy-D-*manno*-2-octulosonate (1 mg ml^{-1}) treated with 0·1N-CF_3·CO_2H at 100°

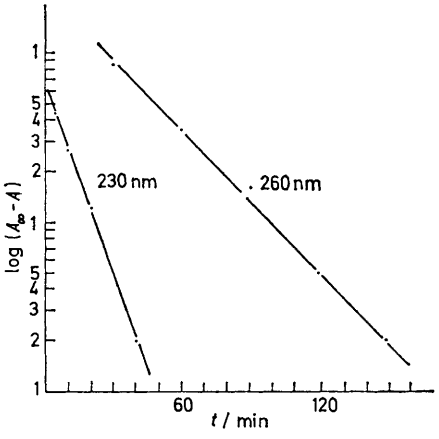


FIGURE 4 Kinetics of the evolution of the absorption bands of acid-treated ammonium 3-deoxy-D-*manno*-2-octulosonate; conditions as for Figures 2 and 3

reduced with the formation of 1 mol. equiv. of formaldehyde, showing the presence of four contiguous hydroxy-groups, one of which was primary. As a corollary, acetylation gave a tetra-acetate whose n.m.r. spectrum showed only one vinylic proton. These data, together with the elemental analysis, clearly defined the substance as the methyl ether of the enolic 3-deoxy-D-*manno*-2-octulosono-1,4-lactone of type (IV). Since under similar conditions all the 3-deoxy-ketoaldonic acids examined showed the same type of spectral and chromatographic behaviour, it was concluded that the formation of enolic lactones is a general reaction for these acids. The reaction is analogous to the formation of ascorbic acids from ketoaldonic acids,[11] but whereas in the latter case the lactones are very stable, the enolic lactones of 3-deoxy-ketoaldonic acids are in reversible equilibrium with the free, non-enolised acids as well as with the enolates observed by Paerels and Geluk [7d] for 3-deoxy-D-*arabino*-2-heptulosono-1,4-lactone and analogous lactones. The formation of an equilibrium mixture between the free acid and its enolic lactone is most likely to be responsible for the kinetics observed (Figure 1) when 3-deoxy-ketoaldonic acids are exposed to mild acidic conditions: the rapidly formed enolic lactone, when treated with periodate, does not yield β-formylpyruvate, which is required for the formation of the red dye in the thiobarbituric acid test. This interpretation is further corroborated by the fact that the rapid phase of disappearance of thiobarbiturate-positive material comes to an end (Figure 1) at about the same time (40 min) as the maximal concentration of the enolic lactone (VI) is attained.

For the isolation of the compound absorbing at 258 nm, 3-deoxy-D-*arabino*-2-heptulosonic acid was treated with N-trifluoroacetic acid at 125° for 1 h; the product was isolated by gel filtration. Trifluoroacetic acid was used because of the ease with which this acid can be removed by evaporation *in vacuo* at low temperature. From its behaviour upon paper electrophoresis, it was evident that the compound had an ionisable proton; its positive reaction with periodate–benzidine [12] indicated that it had a vicinal diol system. Although it was isolated in the solid state, for further manipulations it was found preferable to treat it with diazomethane and then to acetylate the hydroxy-groups. In the n.m.r. spectrum of the crystalline substance thus obtained two ethylenic protons were visible [δ 6·55 and 7·15 p.p.m. (each 1H, d)] together with 3 methyl groups, two of which were due to acetyl substituents. As it is known [13] that in acidic medium aldoses yield 3,4-dideoxy-ald-3-enosuloses, it was expected that in the case of 3-deoxy-ketoaldonic acids [V; in the present case R = D-*glycero*-CH(OH)·CH_2·OH], 3,4-dideoxy-ketoaldonic acid 3-enes (VII; R as before), and the 2,4-dienes (VIII; R as before) (the latter formed by extended enolisation of the former) would appear as intermediates. The diene (VIII) could then either be transformed into the furoic acid derivative (IX; R as before) or else give the lactone (X; R as before). The decision in favour of the furanoid structure (IX) was made on the basis of the compound's mass spectrum, which showed peaks of high intensity [*m/e* 123 and 87, fragments (XI) and (XII)] derived by a well known fragmentation pattern from the furanoid ring.[14] These conclusions were confirmed by chemical means: the

[11] F. Smith, *Adv. Carbohydrate Chem.*, 1946, **2**, 83.
[12] J. A. Cifonelli and F. Smith, *Analyt. Chem.*, 1954, **26**, 1132.
[13] E. F. L. J. Anet, *Adv. Carbohydrate Chem.*, 1964, **19**, 181.
[14] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Interpretation of Mass Spectra of Organic Compounds,' Holden-Day, San Francisco, 1965, p. 226.

acid [IX; R = D-*glycero*-CH(OH)·CH_2·OH] isolated after gel filtration was first treated with periodate and then with borohydride; the material thus obtained had chromatographic properties identical with those of an authentic sample of 5-hydroxymethyl-2-furoic acid (IX; R = CH_2·OH) prepared from D-fructose.[15] The same furoic acid derivative was obtained from 3-deoxy-D-*manno*-2-octulosonic acid (I) when it was degraded by acid and the side chain of the 5-(D-*erythro*-1,2,3-trihydroxypropyl)-2-furoic acid formed was shortened by periodate oxidation and borohydride reduction. 5-Hydroxymethyl-2-furoic acid was obtained directly in 70% yield from 3-deoxy-D-*threo*-2-hexulosonic acid. The formation of the hydroxypropylfuroic acid upon acidic treatment of 3-deoxy-octulosonic acids has been observed by Dmitriev *et al.*[4]

Thus the formation of furoic acid derivatives by acidic treatment of 3-deoxy-ketoaldonic acids is a general reaction. The suggested mechanism is based upon that proposed by Isbell [16] and further elaborated by Anet [13] for the acidic degradation of aldoses. In contrast with the relatively harsh conditions required for the formation of furfural derivatives from unsubstituted aldoses, the transformation of 3-deoxy-ketoaldonic acids into 5-(hydroxyalkyl)-2-furoic acids takes place with great ease and occurs, for instance, under the relatively mild conditions usually employed for the hydrolysis of polysaccharides. The explanation for this is probably connected with the initial, rapid formation of enolic γ-lactones. Indeed, whereas in the Isbell–Anet mechanism the aldoses, after initial enol formation, yield 3-deoxy-ketoaldoses by elimination of an OH group (usually difficult to eliminate), 3-deoxy-ketoaldonic acids first form enolic γ-lactones (VI) and then, in the subsequent step, easily eliminate the carboxy-group.

Dmitriev *et al.*[4] observed, after acidic treatment (pH 3; 100°; 2 h) of the mixed 3-deoxy-D-*gluco*- and D-*manno*-2-octulosonic acids, the formation of a compound $\lambda_{max.}$ 233 and 301 nm which they identified as 3-hydroxy-6-(D-*erythro*-1,2,3-trihydroxypropyl)-2-pyrone [X; R = CH(OH)·CH(OH)·CH_2·OH] on the basis of the n.m.r. spectrum of a derivative obtained after treatment with diazomethane followed by acetylation. Under our conditions of acidic treatment the formation of this compound was not observed; its presence as a major component of the reaction mixture can reasonably be excluded on the basis of the absorption spectrum shown in Figure 2.

EXPERIMENTAL

General.—Solutions were concentrated *in vacuo*. N.m.r. spectra were obtained at 60 MHz with tetramethylsilane as internal standard for solutions in [^{2}H]chloroform. Periodate was estimated either volumetrically with thiosulphate [17] or colorimetrically; [18] formaldehyde was determined with chromotropic acid.[19]

Ammonium 3-*Deoxy*-D-arabino-2-*heptulosonate* (II).—Methyl 3-deoxy-D-*gluco*-heptonate [5c] (4·5 g) was dissolved in slightly less than the calculated amount of 2N-sodium hydroxide; when the pH of the solution had dropped to 7 small amounts of the base were added until the pH remained constant at 8. This solution was added to a mixture of sodium chlorate (740 mg), commercial vanadium oxide (120 mg), and 85% phosphoric acid (0·1 ml) and the mixture was shaken for 5 days at room temperature: the colour, originally orange-yellow, turned progressively to blue-green. Sufficient aqueous barium hydroxide was added to bring the pH to 9 and the precipitate was centrifuged off: the supernatant (which gave no precipitate upon addition of a drop of aqueous barium hydroxide) was percolated through a column of Amberlite 120 (H^+) resin (3·5 × 35 cm); the effluent, after neutralisation with ammonium hydroxide solution, was concentrated to a thick, yellow syrup. The syrup, placed on a column (4 × 40 cm) of cellulose powder [Whatman CF11 fine–CF1 coarse (1 : 1)] was eluted with acetone–water (85 : 15 v/v). The fractions (10 ml) containing the deoxy-ketoaldonic acid (thiobarbiturate test [20]) were pooled and concentrated to a syrup, which, when diluted with its own volume of methanol and left at +4° for 7 days (the compound crystallises easily but very slowly) yielded the title *compound* (1 g). A sample crystallised from methanol had m.p. 96°, $[\alpha]_D^{22}$ +42·4° (*c* 2·2 in H_2O) at 4 and 30 min (Found: C, 34·8; H, 7·0; N, 5·7. $C_7H_{15}NO_7,H_2O$ requires C, 34·55; H, 7·0; N, 5·75%). In the semicarbazide test [5a] its molar absorption coefficient was 10,000, attained after 60 min at 40° (pyruvic acid: 10,200 after 15 min at 40°). In the thiobarbituric acid test [5g] the molar absorption coefficient was 95 ± 4 × 10^3. Under the conditions of the cold acid method [5g] 3 mol. equiv. of periodate were reduced and 1 mol. equiv. of formaldehyde was formed within 30 h, a slow overoxidation being observed afterwards.

Ammonium 3-*Deoxy*-D-threo-2-*hexulosonate* (III).—The lactone (2 g) of ' galactometasaccharinic acid ' (a mixture of 3-deoxy-D-*xylo*- and D-*lyxo*-hexonic acids) [21] was treated in the same way as methyl 3-deoxy-D-*gluco*-heptonate to give a 33% yield of crystalline *ammonium salt*, m.p. 145°, $[\alpha]_D^{20}$ +16° (5 min) and +14° (at equilibrium) (*c* 1·8 in H_2O) (Found: C, 37·15; H, 6·9; N, 7·3. $C_6H_{13}NO_6$ requires C, 36·9; H, 6·7; N, 7·2%). In the semicarbazide test [5a] the molar absorption coefficient was 10,000 (15 min heating) and in the thiobarbituric acid test [5g] 95 ± 5 × 10^3. In cold acid [5g] the compound reduced 2 mol. equiv. of periodate within 48 h (slow overoxidation afterwards) and yielded 1 mol. equiv. of formaldehyde.

3-*Deoxy*-2-O-*methyl*-D-manno-*oct*-2-*enono*-1,4-*lactone* (IV).—An aqueous solution of ammonium 3-deoxy-D-*manno*-octulosonic acid (500 mg) was decationised with Amberlite IR 120 (H^+) resin; the solution, made 0·1N with respect to trifluoroacetic acid (total vol. 125 ml) was kept at 100° for 35 min. To the cooled (0°) solution, diluted with cold methanol (200 ml), diazomethane in ether was added until a faint yellow colour persisted. Two hours later the solvents

[15] W. N. Haworth, E. L. Hirst, and V. S. Nicholson, *J. Chem. Soc.*, 1927, 1525.
[16] H. S. Isbell, *J. Res. Nat. Bur. Stand.*, 1944, **32**, 45.
[17] L. Malaprade, *Bull. Soc. chim. France*, 1928, **34**, 683; *cf. Methods Carbohydrate Chem.*, 1962, **1**, 439.
[18] G. Avigad, *Carbohydrate Res.*, 1969, **11**, 119.
[19] D. A. MacFadyen, *J. Biol. Chem.*, 1945, **158**, 107.
[20] L. Warren, *Nature*, 1960, **186**, 237.
[21] H. Kiliani and H. Sanda, *Ber.*, 1893, **26**, 1469; *cf. Adv. Carbohydrate Chem.*, 1957, **12**, 54.

were evaporated off to yield a yellow, slowly crystallising oil. To this an amount of ethyl acetate–methanol (1 : 1) sufficient to dissolve the oil but not the crystals was added. The supernatant was decanted from the crystals which, after recrystallisation from the minimal amount of hot water, gave the title *compound* (50 mg), m.p. 208°, $[\alpha]_D^{23}$ +36° (*c* 0·5 in H_2O) (Found: C, 45·9; H, 6·0; O, 47·8. Calc. for $C_9H_{14}O_{16}$: C, 46·35; H, 6·0; O, 47·9%), $\nu_{max.}$ (KBr) 1767s (C=O) and 1656s cm^{-1} (C=C), $\lambda_{max.}$ (H_2O) 230 (ε 8300). In unbuffered solution at room temperature the compound reduced 3 mol. equiv. of periodate and set free 1 mol. equiv. of formaldehyde.

Methyl 5-(D-glycero-1,2-*Diacetoxyethyl*)-2-*furoate* [IX; R = CH(OAc)·CH_2·OAc].—An aqueous solution of ammonium 3-deoxy-D-*arabino*-2-heptulosonate (1·2 g) was decationised with Amberlite IR 120 (H^+) resin and the solution was made N with respect to trifluoroacetic acid (total volume 160 ml). The mixture was heated in a sealed tube at 125° for 1 h, cooled, and concentrated to a yellow syrup which was placed on a Sephadex G10 gel column (1 × 90 cm) and eluted with water. The fractions (2 ml) were analysed for u.v. absorption and those absorbing at 260 nm were subjected to t.l.c. on cellulose (Macherey and Nagel 300) with butan-1-ol–acetic acid–water (4 : 1 : 5 v/v, upper phase) as solvent. Homogeneous fractions were pooled and lyophilised: 5-(D-*glycero*-1,2-dihydroxyethyl)-2-furoic acid (304 mg) was obtained as a white solid. This was treated with a slight excess of ethereal diazomethane and, after removal of the solvent, acetylated with acetic anhydride–pyridine (1 : 1; 50 ml) overnight at room temperature. After the usual work-up an almost colourless syrup was obtained. Preparative t.l.c. [silica gel (Merck H); benzene–methanol (97 : 3)] led to a crystalline *product*, m.p. 45° and $[\alpha]_D^{23}$ +2° (*c* 0·5 in $CHCl_3$) (Found: C, 53·4; H, 5·0; O, 41·3. $C_{12}H_{14}O_7$ requires C, 53·3; H, 5·2; O, 41·5%), δ 2·1 (6H 2s, 2 × Ac), 3·9 (3H, s, CO_2Me), 4·5 (2H, m, CH_2·OAc), 6·12 (1H, q, *J* 5 and 6 Hz, C*H*·OAc), 6·55 (1H, d, *J* 3·5 Hz, H-3), and 7·15 (1H, d, *J* 3·5 Hz, H-4).

5-*Hydroxymethyl*-2-*furoic Acid* (IX; R = CH_2·OH).—Ammonium 3-deoxy-D-*threo*-2-hexulosonic acid (372 mg) was decationised and treated with N-trifluoroacetic acid as before. Concentration of the acidic mixture gave a crystalline residue, which, after recrystallisation from ethanol–toluene (1 : 1) yielded the pure title compound (200 mg), m.p. 165° (lit.,[15] 165·5—167°), identical (i.r. and u.v. spectra) with an authentic sample.[15]

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