Phosphorylated Sugars. Part XVI.[1] Synthesis of 3-Deoxy-D-*glycero*-hex-3-en-2-ulofuranos(2,5)onic Acid 6-(Dihydrogen Phosphate)

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Treatment of glucometasaccharinic acid 6-phosphate with a Sprinson-type vanadium oxide catalyst gives, besides 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate, 3-deoxy-D-*glycero*-hex-3-en-2-ulofuranos-(2,5)onic acid 6-(dihydrogen phosphate). This, upon hydrogenation over palladium–carbon, yields, apparently stereospecifically, 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate. If [^{3}H]hydrogen is used, 3-deoxy-D-*erythro*-[3,4-$^3H_2$]hexulosonic acid 6-phosphate is obtained.

In the preceding paper[1] it was shown that oxidation of glucometasaccharinic acid 6-phosphate with chlorate in the presence of a Sprinson-type vanadium oxide catalyst[2] led to samples of 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate, which, although chemically almost indistinguishable from authentic material, were recognised (by treatment with the specific aldolase) as being contaminated with at least one other component. It was also noted that the total amount of phosphate esters present in the oxidation mixture submitted to the chromatographic separation was not recovered after the phosphorylated α-keto-acid had been removed with 0·02N-hydrochloric acid. Indeed, if elution was continued with 0·03N-acid, two more phosphate esters could be recovered and isolated (Figure 1, peaks 4 and 5). The amount of this material increased with increasing oxidation time at the expense of the 3-deoxyhexulosonic acid phosphate. Elemental analysis of the lithium salt corresponding to the major peak (peak 5) indicated that its composition was similar to that of a 3-deoxy-2-keto-hexonic acid 6-phosphate. Upon paper electrophoresis (pH 5; 0·2M-pyridinium acetate) it moved only slightly ahead of the latter, but gave a deep

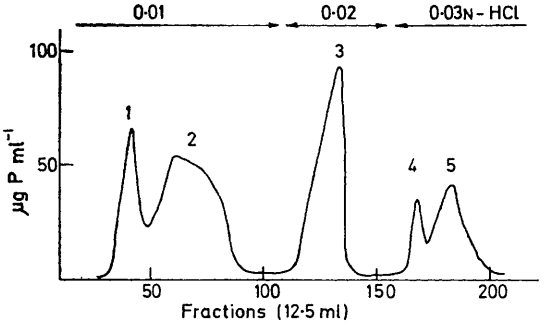


FIGURE 1 Peaks: 1, inorganic phosphate; 2, glucometasaccharinic acid phosphate, 3, 3-deoxy-D-*erythro*-hex-2-ulosonic acid 6-phosphate (with the contaminant); 4, unknown; 5, 3-deoxy-D-*glycero*-hex-3-en-2-ulofuranos(2,5)onic acid 6-phosphate

[1] Part XV, F. Trigalo, W. Jachymczyk, J. C. Young, and L. Szabó, preceding paper.

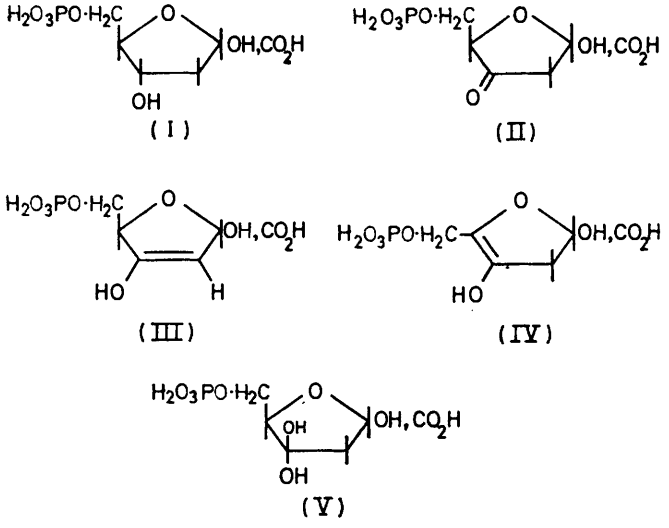
[2] D. B. Sprinson, J. Rothschild, and M. Sprecher, *J. Biol. Chem.*, 1963, **238**, 3170.

violet colour with *o*-phenylenediamine,[3] instead of the fluorescent, yellow quinoxaline derivative characteristic of the α-keto-acid. Although it gave a positive semicarbazide reaction,[4] this was both incomplete (about 75% of the intensity expected for an α-keto-acid) and extremely slow (Figure 2), requiring about 18 h to reach its maximum instead of the usual 15 min. It did not react in the thiobarbiturate reaction[5] either before or after dephosphorylation with acid phosphatase.

After successive treatments with periodate and with borohydride, paper electrophoresis showed the presence of glycol phosphate only: this clearly indicated that the phosphate was attached to the primary alcohol function on C-6 and that there was a secondary alcohol group on C-5.

Reduction over palladium–carbon led to a 3-deoxy-2-keto-hexonic acid 6-phosphate, qualitatively and quantitatively identical with 3-deoxy-D-*erythro*-hex-2-ulosonic acid 6-phosphate in its reactions with semicarbazide, *o*-phenylenediamine, and thiobarbiturate, both before and after treatment with acid phosphatase.

These observations strongly suggested that the new compound was formed from 3-deoxy-D-*erythro*-hex-2-ulosonic acid 6-phosphate by oxidation of the secondary OH group on C-4. However, the keto-structure (II) was considered to be unlikely (i) because the compound reacts extremely slowly with semicarbazide, while the diulosonic acid (II) could be expected to give a rapid and intense reaction in that test, and (ii) because it would not account for the compound's increased acidity as compared with the aldulosonic acid (I). On the other hand, the enolic forms, (III) and (IV), being more acidic, appeared to be in agreement with the compound's observed properties. As in the reaction sequence leading to the establishment of the phosphate group's position the exclusive formation of glycol phosphate was observed, it was concluded that the enolisation involved C-3 and -4 rather than C-4 and -5, as in the latter case this reaction sequence would have led to glycolic acid phosphate.[6] This conclusion was confirmed when the compound was successively reduced with ^3H and borohydride, then oxidised with periodate and again reduced with borohydride:[7] the glycol phosphate obtained was devoid of radioactivity, but the 2,4-dihydroxybutyric acid simultaneously formed was strongly labelled.



The formation of the 2,4-diketo-acid (II) during the oxidation of glucometasaccharinic acid 6-phosphate with Sprinson's catalyst[2] is most likely to be the explanation of the presence of an undetermined contaminant in the samples of 3-deoxy-D-*erythro*-hex-2-ulosonic acid 6-phosphate mentioned in the previous paper. Indeed, as aldosulose derivatives are known to form stable hydrates,[8] it would be expected that the 2,4-diulose derivative (II) forms, concomitantly with the enol (III), a stable hydrate (V) which, having the same acidity as the 3-deoxyaldulosonic acid phosphate (I), would be eluted together with the latter during ion-exchange chromatography. As the aldulosonic acid (I) is isolated as a hydrated salt, the two types of water of hydration would not be distinguished. This interpretation is confirmed by the fact that the sample recognised as impure by the aldolase consistently had a thiobarbiturate value lower than required by theory, whereas the figures in the semicarbazide test were those expected.

The catalytic reduction of the enol could lead either to 3-deoxy-D-*erythro*- (I) or 3-deoxy-D-*threo*-hex-2-ulosonic acid 6-phosphate (VI). Examination of models led us to the conclusion that the formation of the D-*erythro*-isomer was strongly favoured. This conclusion was borne out by experiment: the acid obtained by catalytic reduction of the enol (III) was dephosphorylated with acid phosphatase and the carbonyl function reduced with borohydride; after esterification the carboxy-group was reduced in turn and the peracetylated mixture analysed by g.l.c. under conditions in which epimeric alditol acetates derived from glucometasaccharinic acid emerged as a peak well separated from the epimeric pair of alditol acetates derived from galactometasaccharinic acid (5% XE 60 on Varaport 30, 100—120 mesh, 1/8 in × 10 ft, 210°, isothermal). The appearance of a single peak having a retention time identical with that of the alditol acetates derived from glucometasaccharinic acid proved that the product obtained by reduction of the enol (II) had the D-*erythro*-configuration. This conclusion was further confirmed by enzymic analysis, which showed that over 98% of the compound was of the D-*erythro*-configuration.

[3] M. C. Lanning and S. S. Cohen, *J. Biol. Chem.*, 1951, **189**, 109.
[4] J. MacGee and M. Doudoroff, *J. Biol. Chem.*, 1954, **210**, 617.
[5] A. Weissbach and J. Hurwitz, *J. Biol. Chem.*, 1959, **234**, 716; D. Charon, R. S. Sarfati, D. R. Strobach, and L. Szabó, *European J. Biochem.*, 1969, **11**, 364.
[6] P. Szabó and L. Szabó, *Carbohydrate Res.*, 1967, **4**, 206.
[7] F. Trigalo, P. Szabó, and L. Szabó, *J. Chem. Soc.* (*C*), 1968, 901.
[8] O. Theander, *Acta Chem. Scand.*, 1964, **18**, 2209; P. J. Beynon, P. M. Collins, P. T. Doganges, and W. G. Overend, *J. Chem. Soc.* (*C*), 1966, 1131.

When the catalytic reduction was carried out with [^{3}H]hydrogen, 3-deoxy-D-*erythro*-[3,4-$^3H_2$]hex-2-ulosonic acid 6-phosphate was obtained.

The phosphate ester eluted as the minor peak (peak 4, Figure 1) was also isolated as the trihydrate of its lithium salt. This had $[\alpha]_D^{22}$ −5·9° (*c* 0·5 in water), its analytical figures (Found: C, 21·75; H, 3·2; P, 9·15%) were identical with those of the major compound, but its reaction with semicarbazide was much slower (Figure 2). Like its isomer it did not respond in the

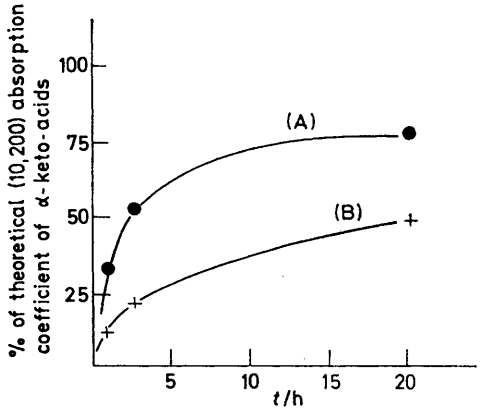


FIGURE 2 Reaction kinetics of the unsaturated acid (' peak 5 ') (A) and of the unknown compound (' peak 4 ') (B) in the semicarbazide test

thiobarbiturate test [5] either before or after enzymic dephosphorylation and it, too, had the phosphate ester attached to C-6. The exact structure of this compound was not established.

Oxidation of 3-deoxy-D-*xylo*-hexonic acid 6-phosphate [1] with Sprinson's catalyst did not lead to detectable amounts of the diulosonate, nor was the hydroxy-group of 1,2:5,6-di-*O*-isopropylidene-D-gluco- or D-allofuranose transformed into a keto-group.

EXPERIMENTAL

All evaporations were carried out under reduced pressure below 40°. 3-*Deoxy*-D-glycero-*hex*-3-*en*-2-*ulofuranos*(2,5)-*onic Acid* 6-*Phosphate* (III).—Glucometasaccharinic acid 6-phosphate lithium salt dihydrate (940 mg) was treated with a Sprinson-type catalyst as described in the preceding paper. After elution of the phosphorylated α-keto-acid with 0·02N-hydrochloric acid, elution was continued with 0·03N-acid and the phosphorus contents of the fractions were estimated. From the pooled fractions of peak 5 (Figure 1) the *lithium salt* of the title compound was isolated by the procedure used [1] for the isolation of the lithium salt of 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate; $[\alpha]_D^{22}$ −11° (*c* 0·5 in H_2O) (Found: C, 21·25; H, 3·2; P, 9·1. $C_6H_5Li_4O_9P,3H_2O$ requires C, 21·6; H, 3·3; P, 9·3%). The *barium salt* had $[\alpha]_D^{22}$ −0·4° (*c* 0·5 in 0·1N-HCl) (Found: C, 11·6; H, 2·4; P, 5·1. $C_6H_5Ba_2O_9P,5H_2O$ requires C, 11·7; H, 2·4; P, 5·0%).

3-*Deoxy*-D-erythro[3,4-$^3H_2$]*hex*-2-*ulosonic Acid* 6-(*Dilithium Phosphate*).—An aqueous solution (1·5 ml) of the above olefinic acid (50 mg) was treated with [^{3}H]hydrogen (65% ^{3}H) in the presence of 5% palladium–carbon (50 mg). When the theoretical amount of hydrogen gas was absorbed (2 h) the catalyst was removed and the solution evaporated to dryness. The residue was dissolved in water and the solvent again removed; this procedure was repeated five times. The dry residue was then taken up in water (50 ml), the solution passed through a column (12 × 55 mm) of Dowex 1 × 8 resin (Cl^-) and the column washed with water (50 ml). It was then eluted first with 0·01N-hydrochloric acid (20 × 10 ml) and then with 0·02N-acid. Fractions containing the title compound (24—35) were pooled, their pH adjusted to 6·95 with N-lithium hydroxide and the volume reduced to *ca.* 1 ml. The precipitate obtained by addition of ethanol (20 ml) was collected by centrifugation, washed [ethanol (2 × 10 ml); acetone (1 × 10 ml)] and then dried *in vacuo* (P_2O_5). After equilibration in air the yield was 30 mg. On the basis of its P content (7·8%. Calc. for $C_6H_8Li_3O_9P,2H_2O$: P, 10·1%) and as estimated with the specific aldolase, it contained 78% (by weight) of the title compound. The ratio of phosphorus to α-keto-acid [4] content was 1 : 1. The compound's specific activity was 52 μCi μmol^{-1}.

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