Phosphorylated Sugars. Part XV.[1] Syntheses of 3-Deoxy-D-*erythro*- and 3-Deoxy-D-*threo*-hexulosonic Acid 6-(Dihydrogen Phosphates)

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The title compounds were obtained by oxidation of glucometasaccharinic acid and 3-deoxy-D-*xylo*-hexonic acid 6-phosphates with vanadium(V) oxide–potassium chlorate and isolated by ion-exchange chromatography Although fairly stable in N-hydrochloric acid at 50°, 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate is rapidly destroyed by 0·1N-hydrochloric acid at 95°. When treated with bases of various strengths the same compound undergoes aldol cleavage between C-3 and -4 and gives pyruvic acid and D-glyceraldehyde phosphate: the latter is immediately transformed into DL-lactic acid and phosphate ion. Concomitant with this reaction, alkali-stable phosphate esters of unknown structure are also formed.

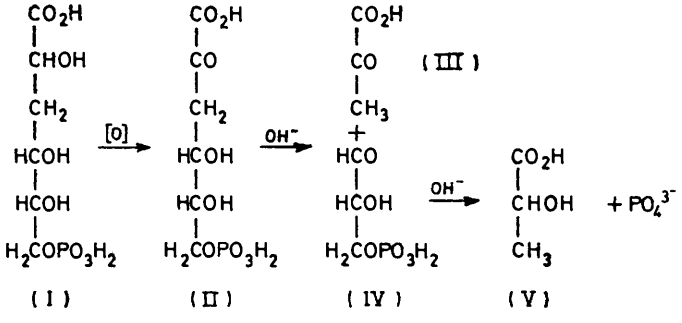
3-DEOXY-D-*erythro*-HEXULOSONIC ACID 6-PHOSPHATE (II) and the D-*threo*-isomer (XVIII) have been identified by Doudoroff and his colleagues as intermediates of D-glucose [2] and D-galactose [3] metabolism in Pseudomonads. Both are cleaved by distinct and specific aldolases [4] between C-3 and -4 to yield equimolar amounts of pyruvate and D-glyceraldehyde phosphate. The 6-phosphate of 3-deoxy-D-*erythro*-hexulosonic acid also appears during the metabolism of D-glucuronic and D-galacturonic acids [5a-e] in bacteria; it is the key intermediate for D-glucose metabolism in those organisms in which the lack of certain enzymes precludes the use of both the pentose phosphate cycle and the glycolytic pathway. Although of importance for biochemical investigations, both phosphorylated acids have, hitherto, only been prepared by enzyme-catalysed reactions [6,7] and are thus not easily accessible; nor have their chemical reactions been studied. The chemical syntheses and some reactions of 3-deoxy-D-*erythro*- and -D-*threo*-hexulosonic acid 6-phosphates are now described.

Of the many synthetic methods elaborated for obtaining aldulosonic acids two have acquired practical interest: in the first, aldonic acids [2,8-13] are treated with chlorate in the presence of a vanadium oxide catalyst; [14a,b] in the second aldehydes are condensed with oxalacetic

[1] Part XIV, P. Szabó, *J.C.S. Perkin I*, 1974, 920.
[2] J. MacGee and M. Doudoroff, *J. Biol. Chem.*, 1954, **210**, 617.
[3] J. DeLey and M. Doudoroff, *J. Biol. Chem.*, 1957, **227**, 745.
[4] C. W. Schuster and M. Doudoroff, *Arch. Mikrobiol.*, 1967, **59**, 279.
[5] (*a*) W. W. Kilgore and M. P. Starr, *J. Biol. Chem.*, 1959, **234**, 2227; (*b*) G. Ashwell, A. J. Wahba, and J. Hickman, *ibid.*, 1960, **235**, 1559; (*c*) J. Hickman and G. Ashwell, *ibid.*, p. 1566; (*d*) J. D. Smiley and G. Ashwell, *ibid.*, p. 1571.
[6] H. P. Meloche and W. A. Wood, *Methods Enzymol.*, 1966, **9**, 51.
[7] J. F. Wilkinson and M. Doudoroff, *Science*, 1964, **144**, 569.
[8] A. Weissbach and J. Hurwitz, *J. Biol. Chem.*, 1959, **234**, 705.
[9] D. B. Sprinson, J. Rothschild, and M. Sprecher, *J. Biol. Chem.*, 1963, **238**, 3170.
[10] D. T. Williams and M. B. Perry, *Canad. J. Biochem.*, 1969, **47**, 491.
[11] D. T. Williams and M. B. Perry, *Canad. J. Biochem.*, 1969, **47**, 983.
[12] M. B. Perry and A. C. Webb, *Canad. J. Chem.*, 1969, **47**, 2893.
[13] (*a*) D. Charon, R. S. Sarfati, D. R. Strobach, and L. Szabó, *European J. Biochem.*, 1969, **11**, 364; (*b*) D. Charon and L. Szabó, *J.C.S. Perkin I*, 1973, 1175.
[14] (*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.

acid in a base-catalysed reaction.[15a-j] As it is well known that phosphate ester systems situated β to a carbonyl group, rapidly undergo elimination in alkaline medium,[16a-c] the condensation of D-glyceraldehyde phosphate with oxalacetic acid to give the required phosphorylated 3-deoxyaldulosonic acids was not considered feasible. Therefore the oxidation route, which has already been successfully employed for the synthesis of phosphorylated aldulosonic acids [17] and 3-deoxyaldulosonic acids,[9] was used, the starting materials being the 6-phosphates of glucometasaccharinic acid [16b] (I) and of 3-deoxy-D-*xylo*-hexose [18] (XVI).

In the original method of Regna and Caldwell [14a] the oxidation by chlorate was carried out in the presence of phosphoric acid and catalysed by vanadium pentoxide; the reaction was allowed to proceed for 4 days. Sprinson and his colleagues,[9] when applying this procedure to the oxidation of 3-deoxy-D-*gluco*-heptonate 7-phosphate, obtained erratic results and therefore elaborated a new method for the preparation of the catalyst: they dissolved the vanadium oxide in concentrated hydrochloric acid, added pyridine to the solution, and used the suspension thus obtained after adjustment of its pH to 3·2. The phosphorylated aldulosonic acid was formed in yields of 40—60% after a reaction time of only 16·5 h. The same catalyst has been used at a slightly higher pH value (4·6—4·8) for the oxidation of D-gluconic acid 6-phosphate to D-*arabino*-hexulosonic acid 6-phosphate.[17] When glucometasaccharinic acid 6-phosphate (I) was oxidised with this catalyst, an



α-keto acid was readily formed (judged by the semicarbazide test carried out as described previously [17]). As with gluconic acid 6-phosphate,[17] the formation of inorganic phosphate (Figure 1) and of u.v.-absorbing material was observed when the oxidation was carried out for prolonged periods; the highest yields of α-keto-acid were obtained when the oxidation was allowed to proceed for 20—25 h (Figure 1).

Attempts to isolate the phosphorylated keto-acid after separation by column chromatography on Dowex 2 × 10 ion-exchange resin with a formic acid–ammonium formate system failed, the keto-acid being completely destroyed during the removal of ammonium formate. Clean separation and excellent recovery were, however, obtained when a 0·05M-chloroacetic acid–0·125M-sodium chloroacetate buffer of pH 3·8 was used, the chloroacetic acid being removed batchwise, by ether extraction, after addition of small amounts of an acid ion-exchange

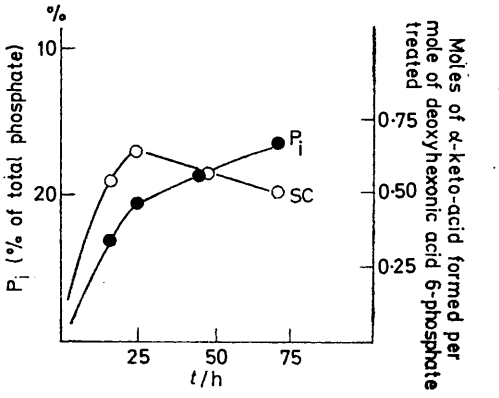


FIGURE 1 Formation of inorganic phosphate (P_i) and of α-keto-acid (SC) during the treatment of glucometasaccharinic acid 6-phosphate wirh $KClO_3$–V_2O_5 (Sprinson type catalyst)

resin to the pooled fractions containing the phosphorylated keto-acid, which was then isolated as the alcohol-insoluble barium salt. It gave satisfactory analytical figures, and, in the semicarbazide test a molar absorbancy of 10,200 (pyruvic and α-ketoglutaric acids: 10,200). However, when submitted to enzymic analysis only 50—60% of the calculated amounts of triose phosphate (IV) and pyruvate (III) were formed. In view of the analytical data it was clear that the preparation consisted, in fact, of a mixture of positional isomers presumably resulting from phosphate migration, and it was possible to demonstrate this by ion-exchange chromatography; [19] however no information regarding the number of isomers present and their structures could be obtained by that method.

In Part XII [20] a method is described by which isomeric phosphate esters of polyhydroxy-compounds can be identified. As applied to sugar phosphates, the method calls for reduction of the carbonyl function (to avoid interference of esters produced from cyclic forms), periodate oxidation of the resulting phosphorylated polyol, borohydride reduction of the aldehyde groups thus formed and, finally, separation of the phosphorylated fragments by paper electrophoresis. The mixture of the isomeric phosphate esters of 3-deoxyhexulosonic acids was analysed by this method.

3-Deoxyhexulosonic acids can form three isomeric

[15] (*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.*, 1968, **241**, 3207; (*c*) C. Hershberger, M. Davis, and S. B. Binkley, *ibid.*, 1968, **243**, 1585; (*d*) D. Charon and L. Szabó, *European J. Biochem.*, 1972, **29**, 184.

[16] (*a*) D. M. Brown, F. Hayes, and A. R. Todd, *Chem. Ber.*, 1957, **90**, 936; (*b*) St. Lewak and L. Szabó, *J. Chem. Soc.*, 1963, 3975; (*c*) W. Jachymczyk, L. Ménager, and L. Szabó, *Tetrahedron*, 1965, **21**, 2049.

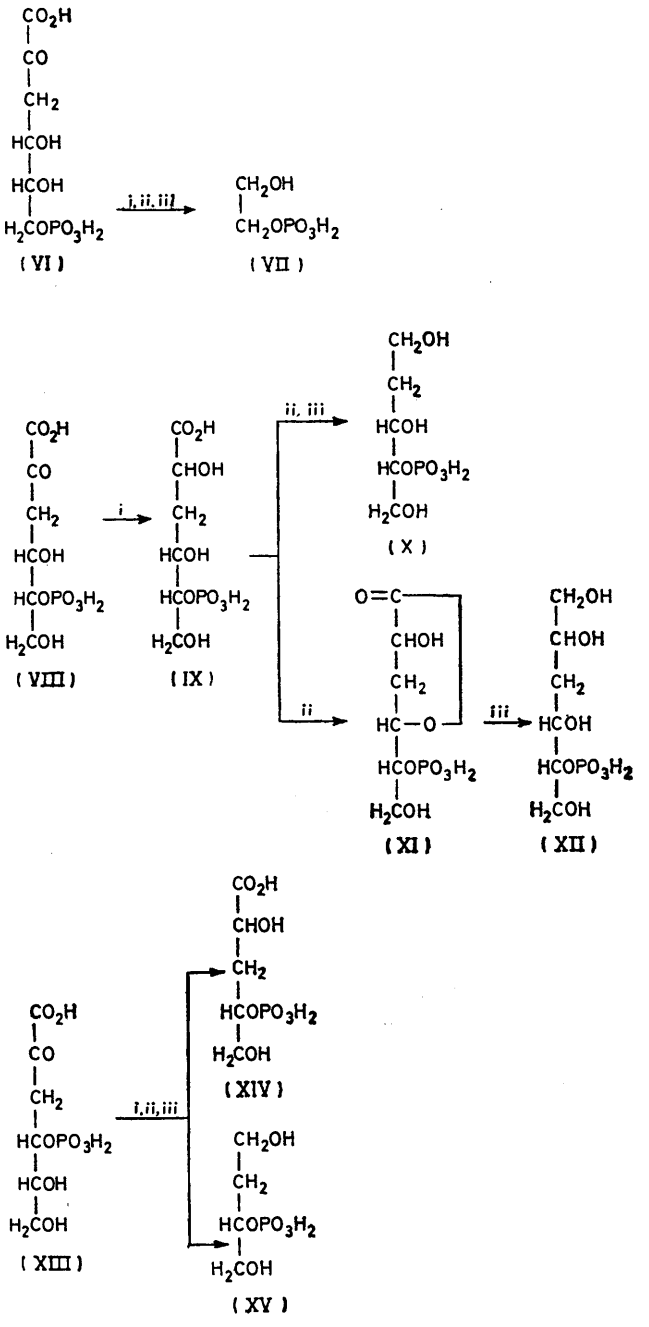
[17] F. Trigalo and L. Szabó, *European J. Biochem.*, 1972, **25**, 336.

[18] K. Antonakis, A. Dowgiallo, and L. Szabó, *Bull. Soc. chim. France*, 1962, 1355.

[19] L. Szabo, Amer. Chem. Soc. Advances in Chemistry Series, 1968, no. 74, p. 85.

[20] F. Trigalo, P. Szabó, and L. Szabó, *J. Chem. Soc.* (*C*), 1968, 901.

phosphate esters (4-, 5-, and 6-phosphates), and each of these can be expected to yield specific phosphate esters in the above reaction sequence. Indeed (see Scheme) the 6-phosphate (VI) should yield, as the sole phosphate ester, ethylene glycol phosphate (VII), and no other



SCHEME Reagents: i, $NaBH_4$; ii, $NaIO_4$; iii $NaBH_4$

isomer can yield this fragment. The 5-phosphate (VIII), bearing no vicinal diol group, will appear largely as glucometasaccharinic acid 5-phosphate (IX); however, as α-hydroxy-acids are cleaved, albeit very slowly, by periodate,[21] 2-deoxyribitol 4-phosphate (X) should also be detectable, as should be a 3-deoxyhexitol phosphate (XII), the latter arising from reduction of the lactone (XI) formed under the acidic conditions of the periodate cleavage during the relatively long (48 h) exposure to this reagent. Neither of these compounds can be derived from the other two isomers. Finally, the 4-phosphate (XIII) should yield a phosphorylated 3-deoxypentonic acid (XIV) together with a small amount of 2-deoxytetritol phosphate (XV).

Paper electrophoresis (pH 5; pyridinium acetate 0·2M) of the products formed from the isomeric mixture of phosphorylated 3-deoxyhexulosonic acids and revelation of phosphate esters gave the following results: besides the major spot of glycol phosphate (M_{P_i} 0·86), which proved the presence of 3-deoxyhexulosonic acid 6-phosphate in the isomeric mixture, phosphate esters having the same mobilities (M) as 2-deoxyribitol phosphate (M_{P_i} 0·64) and glucometasaccharinic acid 5-phosphate (M_{P_i} 1·03) were present, as well as another phosphate ester (detectable with the periodate–benzidine spray [22]) which had a mobility lower than that of the periodate–benzidine-negative 2-deoxypentitol phosphate and identical with that of a 3-deoxyhexitol phosphate. The presence of these esters clearly indicated that 3-deoxy-D-*erythro*-hexulosonic acid 5-phosphate (VIII) was contaminating the 6-phosphate.

The absence of the 4-phosphate (XIII) in the original mixture was ascertained in a separate experiment in which the postulated breakdown products, namely 3-deoxypentonic acid phosphate [16c] and 2-deoxytetritol phosphate, were used as markers: the mobilities of these two compounds (M_{P_i} 1·12 and 0·72 for 3-deoxy-D-*erythro*-pentonic acid phosphate and 2-deoxy-D-*glycero*-tetritol 4-phosphate, respectively) are quite different from those of the phosphate esters present in the mixture.

As it was suspected that phosphate migration occurred during the ion-exchange procedure, isolation of the keto-acid was next carried out on a Dowex 1 × 8 resin (Cl^- form) and the phosphate was isolated as the lithium salt, but again material of only 70—80% purity, as assayed by enzymic analysis, was obtained. No phosphate migration was detected in these preparations but in the thiobarbiturate reaction the enzymically dephosphorylated compounds' molar absorption coefficient was only 80,000 instead of the theoretical [13a] 95,000.

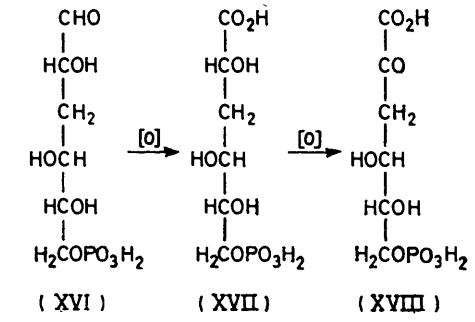
It was then considered that the very active catalyst might have initiated some unidentified reaction (*cf.* following paper). The oxidation was therefore carried out under Regna and Caldwell's conditions [14a] previously used for obtaining 3-deoxy-D-*manno*-octulosonic,[13a] 3-deoxy-D-*arabino*-heptulosonic,[13b] and 3-deoxy-D-*threo*-hexulosonic acids.[13b] Although it was necessary to allow the oxidation to proceed for about 5 days to obtain reasonable yields (30—40%), the α-keto-acid, which was separated from other material by ion-exchange chromatography in the chloride system and isolated as

[21] P. F. Fleury, G. Poirot, and Y. Fiévet, *Compt. rend.*, 1945, **220**, 664; P. F. Fleury, J. E. Courtois, R. Perlès, and L. Le Dizet, *Bull. Soc. chim. France*, 1954, 347.

[22] J. A. Cifonelli and F. Smith, *Analyt. Chem.*, 1954, **26**, 1132.

the lithium salt, gave correct elemental and functional analyses and also assayed for more than 98% of 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate when treated with the specific aldolase.

For obtaining 3-deoxy-D-*threo*-hexulosonic acid 6-phosphate, 3-deoxy-D-*xylo*-hexose 6-phosphate [18] (XVI) was first oxidised by bromine to the corresponding



phosphorylated deoxyaldonic acid (XVII), which was then further oxidised under Regna and Caldwell's conditions to the phosphorylated aldulosonic acid (XVIII); it gave correct analytical figures both as regards composition and functional groups, but the purity of the compound could not be checked by enzymic methods because the pure, specific aldolase was not available.

Both compounds gave only very weak reactions with periodate–thiobarbiturate indicating that no free vicinal 4,5-diol system was present; however, after treatment with acid phosphatase both compounds gave the

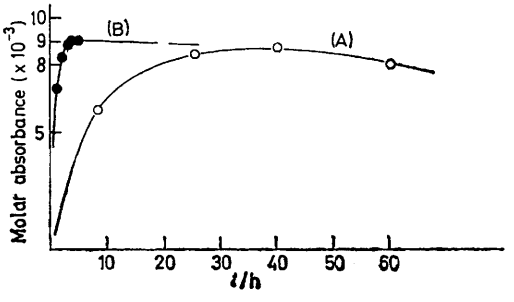


FIGURE 2 Kinetics of (A) 3-deoxy-D-*erythro*- and (B) 3-deoxy-D-*threo*-hexulosonic acid 6-phosphates in the periodate–thiobarbiturate test [13a] after enzymic dephosphorylation

theoretical molar absorption coefficient of 93×10^3 in the thiobarbituric acid test carried out as previously described; [13a] but while the compound with D-*erythro*-configuration reached this value within 5—6 h, the *threo*-analogue required 40 h exposure to periodate (Figure 2); their rates of reaction in this test may thus be used to distinguish between the two isomers.

As measured by the semicarbazide test,[2] 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate is relatively stable in N-hydrochloric acid at 50° (Figure 3); no measurable amount of inorganic phosphate is formed during 7 h. However, at higher temperature (*e.g.* at 95° in 0·1N-HCl), phenomena similar to those seen with non-phosphorylated deoxyaldulosonic acids [13b] (appearance of absorption bands at 230 and 260 nm, accompanied in the present case by formation of inorganic

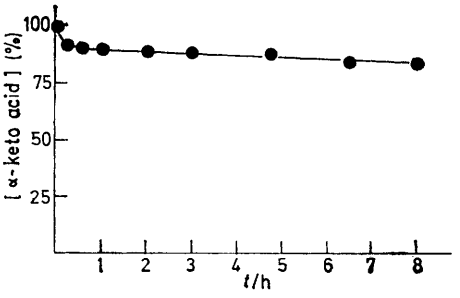


FIGURE 3 Destruction of α-keto-acid during the treatment of 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate with N-HCl at 50°

phosphate) are observed, indicating that similar reactions occur in both cases. Formation of inorganic phosphate parallels that of the substance absorbing at 260 nm (Figure 4).

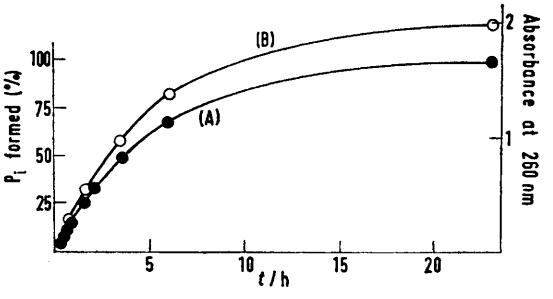


FIGURE 4 Formation of (A) inorganic phosphate and (B) material absorbing at 260 nm during acid treatment (0·1N-HCl; 95°) of 3-deoxy-D-*arabino*-hexulosonic acid 6-phosphate

The same phosphorylated deoxyaldulosonic acid is very unstable towards alkali: the kinetics of inorganic phosphate formation, catalysed by different concentrations of sodium hydroxide at 50° are shown in Figure 5. The appearance of inorganic phosphate in the early stages of the reaction at a rate incompatible with the generally low rate of base-catalysed hydrolysis of phosphomonoesters [23] (the latter is observed during the later stages of the reaction) suggested that an

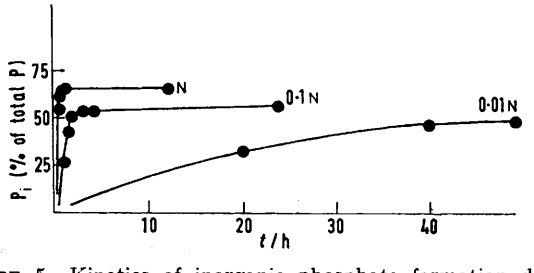


FIGURE 5 Kinetics of inorganic phosphate formation during the treatment of 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate with various concentrations of NaOH at 50°

elimination reaction may be occurring. Such an elimination reaction could occur if, as is the case with

[23] C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vernon, *J. Chem. Soc.*, 1958, 3574.

D-*arabino*-hexulosonic acid 6-phosphate,[17] pyruvate (III) and D-glyceraldehyde 3-phosphate (IV) were formed from the phosphorylated 3-deoxyhexulosonic acid by aldol cleavage, the phosphorylated fragment than undergoing a β-elimination of the phosphate to yield DL-lactic acid (V) and inorganic phosphate. That this is indeed the case can be seen from the Table: formation of inorganic

	Mol of		
	inorganic phosphate	pyruvic acid	lactic acid
formed per mol of 3-deoxy-D-*erythro*-hexalulosonic acid			
t/h	in 0·1N-NaOH at 50°		
1	0·395	0·34	0·328
2	0·557	0·49	0·498
3·75	0·714	0·62	0·60
4·5	0·749	0·66	0·64
5·5	0·776	0·676	0·656
	in 0·1N-NH_4OH at 50°		
18	0·12	0·10	0·03
24	0·14	0·13	0·05
40	0·21	0·175	0·11

phosphate is accompanied by the appearance of equivalent amounts of pyruvic and lactic acids as estimated by the appropriate enzymes (as the enzyme used is specific for L-lactic acid, the amount shown is twice that actually measured).

Towards 0·1N-ammonium hydroxide at 50°, the compound is far more stable (Table): 12% of inorganic phosphate and 10% of pyruvic acid, but only 3% of DL-lactic acid are formed in 18 h. The presence of equimolar amounts of pyruvate and inorganic phosphate indicates that here, too, a retro-aldol reaction is operative. The formation of small amounts of lactic acid could be accounted for on the basis of the observation that although 3-*O*-methyl-D-glucose is degraded to 3-deoxy-D-*erythro*-hexosulose, the ultimate product is an imidazole derivative rather than metasaccharinic acid, the reaction of the intermediary α-dicarbonyl compound with ammonia to yield the heterocycle being faster than the benzylic acid type of rearrangement.[24] Inorganic phosphate is also liberated when the phosphorylated deoxyaldulosonic acid is treated, at 50°, with 0·1N-cyclohexylamine or tetramethyl- or tetra-n-butylammonium hydroxide.

As can be seen from Figure 5, only 50—60% of the total phosphate is released by this mechanism and it can be shown by paper electrophoresis that, at the time when the rapid phase of phosphate release is terminated, two phosphate esters are present which do not give the characteristic reactions of the starting material and which both have an absorption maximum at 256 nm. Their structure is being investigated.

EXPERIMENTAL

All evaporations were carried out under reduced pressure below 40°.

3-*Deoxy*-D-xylo-*hexonic Acid* 6-*Phosphate* (XVII).—To a stirred, aqueous solution (10 ml) of 3-deoxy-D-*xylo*-hexose 6-(dilithium phosphate) dihydrate (obtained from the calcium salt [18]) (1·25 g) were added barium carbonate (2 g) and then, dropwise, saturated aqueous bromine, until a slight colour persisted. Stirring was continued for 1 h. The mixture was kept overnight at 4°, then centrifuged; the sediment was washed with water (2 × 15 ml) and the combined supernatant liquid was decationised on a column of Amberlite IR120 (H^+) resin (50 ml). The effluent was neutralised with barium hydroxide solution, then heated (60—70°), and the pH of the solution was simultaneously adjusted to 7·5 until no further addition of base was necessary. The solution was concentrated (10 ml) and the product precipitated with ethanol. The solid (1·5 g) collected by centrifugation and washed free of bromide with ethanol had $[\alpha]_D^{22}$ +8·8° (*c* 1 in 0·1N-HCl) (Found: C, 14·1; H, 3·0; P, 6·0. Calc. for $C_6H_{10}Ba_{1\cdot5}O_9,3H_2O$: C, 13·9; H, 3·1; P, 6·0%). The calcium salt, prepared by neutralising the decationised solution with aqueous calcium hydroxide as above, had $[\alpha]_D^{22}$ +11·2° (*c* 1 in 0·1N-HCl) (Found: C, 19·3; H, 4·1; P, 8·35. Calc. for $C_6H_{10}Ca_{1\cdot5}O_9P,3H_2O$: C, 19·4; H, 4·3; P, 8·35%).

3-*Deoxy*-D-erythro-*hexulosonic Acid* 6-*Phosphate* [(II), (VI)].—(a) *Oxidation with vanadium*(V) *oxide*. A mixture of glucometasaccharinic acid 6-phosphate trilithium salt (940 mg, 3 mmol), commercial vanadium(V) oxide (9 mg), and potassium chlorate (129 mg, 0·99 mmol) was treated with water (3 ml) containing phosphoric acid (0·105 ml; 85%; *d* 1·71). The pH of the mixture was adjusted to 4·6—4·8 with either pyridine or 85% phosphoric acid and the reaction was allowed to proceed in a closed tube with constant stirring for 5 days. The mixture was then percolated through a column (2·8 × 15 cm) of Amberlite IR120 (H^+) resin and the pH of the effluent brought to 7·5 by addition of concentrated ammonium hydroxide solution (*ca.* 300 ml). The solution, which by the semicarbazide test [2] contained about 1 mmol of α-keto-acid, was slowly percolated through a column (1·2 × 8 cm) of Dowex 1 × 8 resin (100—200 mesh; Cl^-) and the column was washed with water (*ca.* 100 ml). It was first eluted with 0·01N-hydrochloric acid (80 ml h^{-1}) and the fractions (12·5 ml) were analysed for total phosphorus.[25] When the phosphorus content of the fractions became negligible, elution was continued with 0·02N-hydrochloric acid, the fractions being analysed for both phosphorus and α-keto-acid. The pooled fractions containing the keto-acid were neutralised (pH 6·9) with N-lithium hydroxide and concentrated (to *ca.* 5 ml). The pH of the solution was adjusted to 7·6 and the lithium salt of the title compound precipitated with ethanol (100 ml), collected by centrifugation, washed free of lithium chloride with ethanol, washed with acetone, and dried *in vacuo* at room temperature (P_2O_5). After equilibration in air [16b] the compound (300 mg) had $[\alpha]_D^{22}$ +6° (*c* 1 in H_2O) (Found: C, 23·3; H, 3·7; P, 10·1. Calc. for $C_6H_8Li_3O_9P,2H_2O$: C, 23·1; H, 3·85; P, 9·9%). When treated with 2-keto-3-deoxy-6-phosphogluconic aldolase it yielded 0·98—0·99 mol. equiv. of both pyruvate and D-glyceraldehyde phosphate. In the thiobarbituric acid test it had, after enzymic dephosphorylation, a molar absorption coefficient of 93 × 10^3.

(b) *Oxidation with a Sprinson-type catalyst.*[9] A suspension of commercial vanadium(V) oxide (150 mg) in concentrated hydrochloric acid (*d* 1·19; 9 ml) was stirred for 1 h. To the red solution pyridine (9 ml) was added slowly, whereupon the colour changed through green to

[24] M. R. Grimmett, R. Hodges, and E. L. Richards, *Austral. J. Chem.*, 1968, **21**, 505.

[25] M. Macheboeuf and J. Delsal, *Bull. Soc. Chim. biol.*, 1943, **25**, 116.

blue and a slight precipitate appeared. The catalyst was used immediately. A solution of glucometasaccharinic acid 6-phosphate lithium salt dihydrate (940 mg, 3 mmol) was decationised with Amberlite IR120 (H^+) resin, the pH of the solution was brought to 9 with cyclohexylamine, and the mixture was heated on a water-bath, the pH being re-adjusted to 9 until it remained constant. The solution was then concentrated (15 ml) (5 ml per mmol of metasaccharinic acid phosphate), sodium chlorate (150 mg, 0·47 mmol per mmol of substrate) and catalyst (4·5 ml) were added and, if necessary, the pH of the mixture was adjusted to 4·6—4·8 with pyridine or concentrated hydrochloric acid as required. The deep green mixture was stirred in a closed vessel at room temperature for 24 h. The pale green solution was passed through a column (3 × 30 cm) of Amberlite IR120 (H^+) resin and the percolate and washings were pooled, brought to pH 6·9 with 2N-lithium hydroxide and concentrated (10 ml). After adjustment of the pH to 7, ethanol (150 ml) was added and the precipitate was collected by centrifugation, washed free of lithium chloride with ethanol and dried. The mixed lithium salts (*ca.* 850 mg), which contained 400—450 mg of α-keto-acid (semicarbazide test) were dissolved in water (250 ml) and passed through a column (1·2 × 8 cm) of Dowex 1 × 8 resin (100—200 mesh; Cl^-). The resin was washed with water (100 ml) and eluted (80 ml h^{-1}) with 0·01N- and 0·02N-hydrochloric acid and the *phosphorylated α-keto-acid* (300 mg) was isolated as above; $[\alpha]_D^{22}$ +6·5° (*c* 1 in H_2O) (Found: C, 23·0; H, 4·0; P, 10·1. $C_6H_8Li_3O_9P,2H_2O$ requires C, 23·1; H, 3·85; P, 9·9%). When treated with the specific aldolase it yielded 0·6—0·8 mol. equiv. of pyruvate, and in the thiobarbituric acid test it had, after dephosphorylation, a molar absorbance of 75—85 × 10^3.

3-*Deoxy*-D-threo-*hexulosonic Acid* 6-*Phosphate* (XVIII).—The lithium salt of the title compound was obtained from 3-deoxy-D-*xylo*-hexonic acid 6-phosphate (lithium salt; 630 mg, *ca.* 2 mmol) by the procedure (a) described above. It has $[\alpha]_D^{22}$ −8·2° (*c* 0·5 in H_2O) (Found: C, 23·0; H, 3·6; P, 9·8. Calc. for $C_6H_8Li_3O_9P,2H_2O$: C, 23·0; H, 3·9; P, 9·9%). The barium salt, prepared by passing a solution of the lithium salt through a column of Amberlite IR120 (H^+) resin, raising the pH of the acid effluent to 7 with barium hydroxide solution, and precipitating the salt with ethanol, had $[\alpha]_D^{22}$ +3·4° (*c* 0·5 in 0·1N-HCl) (Found: C, 14·5; H, 2·4; P, 6·0. Calc. for $C_6H_8Ba_{1.5}O_9P,2H_2O$: C, 14·5; H, 2·4; P, 6·2%).

We thank Drs. M. Doudoroff and H. P. Meloche for the enzymic analyses and the French Government for fellowships (to W. J. and J. C. Y.).

[4/1026 *Received*, 29*th May*, 1974]