Phosphorylated Sugars. Part XIX.[1] Synthesis of 3-Deoxy-D-*glycero*-pent-2-ulosonic Acid 5-(Dihydrogen Phosphate)

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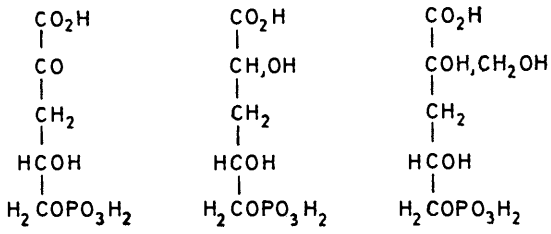
The title compound was obtained both by oxidation of ' xylometasaccharinic acid ' 5-phosphate with sodium chlorate–vanadium(V) oxide and by treatment of ' isosaccharinic acid ' 6-phosphate with periodate. Its stability towards acid and base is similar to that of higher homologues.

3-DEOXY-D- AND L-*glycero*-PENT-2-ULOSONIC ACIDS are known to be intermediates in D- and L-arabinose metabolism in Pseudomonads,[2] the end products being either α-oxoglutaric acid, formed from the ulosonic acid *via* 2,4-dioxopentanoic acid (' α-ketoglutarate semialdehyde ')[3] or pyruvate plus glycolaldehyde[4] or glycolic acid,[1] formed through the action of an aldolase.[4] The synthesis of 3-deoxy-D-*glycero*-pent-2-ulosonic acid 5-phosphate (I) (' 2-keto-3-deoxy-D-arabonic acid 5-phosphate '),

[1] Part XVIII, A. Chiron and P. Szabo, preceding paper.
[2] N. J. Palleroni and H. Doudoroff, *J. Biol. Chem.*, 1956, **223**, 499.
[3] A. C. Stoolmiller and R. H. Abeles, *J. Biol. Chem.*, 1966, **241**, 5764.
[4] A. S. Dahms and R. L. Anderson, *Biochem. Biophys. Res. Comm.*, 1969, **36**, 809.

higher homologues of which have been prepared earlier,[5] is described here.

The phosphorylated 3-deoxypentulosonic acid was prepared by two independent syntheses. In the first, xylometasaccharinic acid ' 5-phosphate [6] (a mixture of 3-deoxy-D-*erythro*- and -D-*threo*-pentonic acid 5-phosphates) (II), obtained by alkaline treatment of D-xylose 3,5-phosphate,[7] was oxidised with chlorate and vanadium(v) oxide [8] and the α-oxo-acid (I) formed was isolated as the lithium salt after ion-exchange chromatography.[5a] In the second, ' isosaccharinic acid ' 6-phosphate [6] [a mixture of 2-*C*-(hydroxymethyl)-D-*erythro*- and -D-*threo*-pentonic acid 6-phosphates] (III), obtained by



alkaline degradation of D-glucose 4,6-phosphate,[6] was treated with 1 mol. equiv. of periodate and the 3-deoxypentulosonic acid phosphate (I) formed was isolated as above. In the semicarbazide test [9] both products gave a molar absorption coefficient value of 10,000 and in the thiobarbiturate reaction,[10] carried out after treatment with an acid phosphatase, a value of 90,000 was obtained, as expected; 1 mol. equiv. of formaldehyde was formed (Figure 1) during the periodate–thiobarbiturate reaction.

When treated with 0·1N-sodium hydroxide at 50°, compound (I) undergoes cleavage of the C(3)–C(4) bond, pyruvic acid and (presumably) glycolaldehyde phosphate

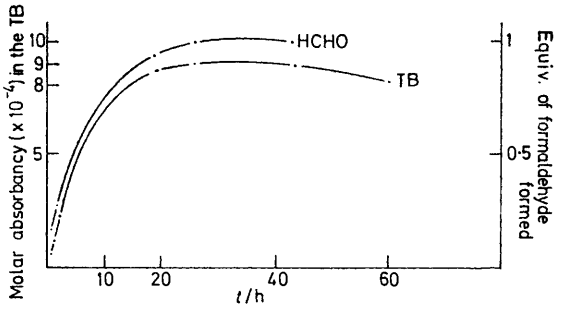


FIGURE 1 Formaldehyde formation and reaction kinetics of 3-deoxy-D-*glycero*-pentulosonic acid 5-phosphate in the periodate–thiobarbiturate test after enzymic dephosphorylation: pentulosonate 6×10^{-4}M, $NaIO_4$ 36×10^{-4}M in 0·1N-H_2SO_4 at 0°

being formed. Although the latter eventually yields inorganic phosphate, pyruvate and mineral phosphate are not produced in parallel (Figure 2), as is the case when 3-deoxy-D-*erythro*-hex-2-ulosonic acid is treated similarly.

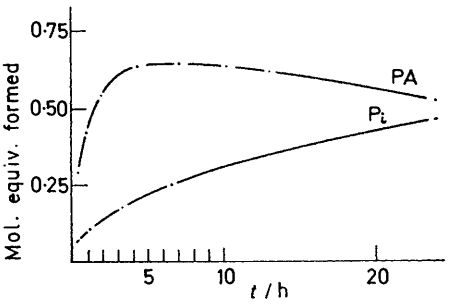


FIGURE 2 Formation of pyruvic acid (PA) and inorganic phosphate (P_i) during the treatment of 3-deoxy-D-*glycero*-pentulosonic acid 5-phosphate with 01N-NaOH at 50°

The discrepancy is explained by the rapid formation of inorganic phosphate from triose phosphate by β-elimination in the latter case, a mechanism is not operative with glycolaldehyde phosphate.

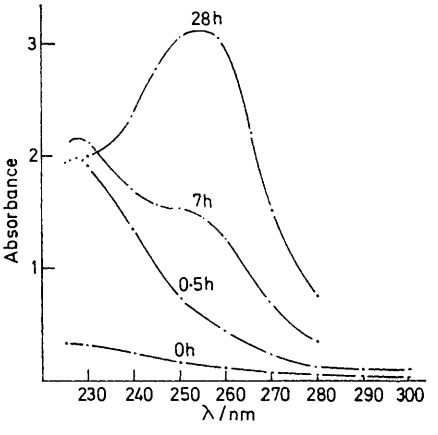


FIGURE 3 Evolution of the absorption spectrum of 3-deoxy-D-*glycero*-pentulosonic acid 5-phosphate (4·15 mg) treated with 0·1N-HCl (25 ml) at 95°; spectra were taken at the times indicated

When exposed to 0·1N-acid at 95°, compounds absorbing at 230 and 255 nm (Figure 3) and inorganic phosphate (Table) are formed, as is the case with 3-deoxy-D-*erythro*-hex-2-ulosonic acid 6-phosphate;[5a] however both reactions proceed much more slowly with the pentulosonate than with the hexulosonate: for instance, half-lives for ester-bound phosphate are about 4 and 9 h for the phosphorylated 3-deoxy-hexulosonic and -pentulosonic acids,

Formation of inorganic phosphate (P_i) from 3-deoxy-D-*glycero*-pent-2-ulosonic acid 5-phosphate (4·15 mg) treated with 0·1N-HCl (25 ml) at 95°

Time (*t*/h)	1	2·5	5·5	7	28
% P_i	7	16·6	38	46	90

[5] (*a*) F. Trigalo, W. Jachymczyk, J. C. Young, and L. Szabó, *J.C.S. Perkin I*, 1975, 593; (*b*) D. B. Sprinson, J. Rothschild, and J. M. Sprecher, *J. Biol. Chem.*, 1963, **238**, 3170; F. Trigalo, M. Level, and L. Szabó, *J.C.S. Perkin I*, 1975, 600.

[6] W. Jachymczyk, L. Ménager, and L. Szabó, *Tetrahedron*, 1965, **21**, 2049.

[7] J. G. Moffat and H. G. Khorana, *J. Amer. Chem. Soc.*, 1957, **79**, 1194.

[8] P. P. Regna and B. P. Caldwell, *J. Amer. Chem. Soc.*, 1944, **66**, 243.

[9] J. McGee and M. Doudoroff, *J. Biol. Chem.*, 1954, **210**, 617.

[10] D. Charon, R. S. Sarfati, D. R. Strobach, and L. Szabó, *European J. Biochem.*, 1969, **11**, 364.

respectively. Since, according to the mechanism proposed [11] for the acid-catalysed degradation of 3-deoxyaldulosonic acids, the formation of 2-furoic acid must be preceded by the departure of the phosphate group in the case of 3-deoxypentulosonic acids, the relatively low rate of acid-catalysed hydrolysis of the phosphate group may be the factor responsible for the overall slackening of the degradation.

EXPERIMENTAL

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

3-*Deoxy*-D-glycero-*pent*-2-*ulosonic Acid* 5-(*Dihydrogen Phosphate*) (I).—(*a*) A suspension of ' xylometasaccharinic acid ' 5-phosphate, barium salt [6] (450 mg) in water (10 ml) was treated with Amberlite IR120 (H^+) resin (15 ml); the mixture was stirred (5 min) and the solids were filtered off and washed. N-Lithium hydroxide was added to the filtrate, which was then heated on a water-bath until the pH remained constant at 9. The solvents were then removed and commercial vanadium(v) oxide (3 mg) and water (1 ml), containing phosphoric acid (85%; 0·035 ml; *d* 1·71), were added to the dry residue. The pH of the solution was adjusted to 4·6—4·8 with pyridine or 8·5% phosphoric acid and the mixture was stirred in a closed vessel for 5 days. It was then passed through a column (30 ml) of Amberlite IR120 (H^+) resin and the pH of the acid effluent and washings (50 ml) was adjusted to 7·5 with aqueous N-ammonia. As estimated with semicarbazide,[9] the solution contained 0·35 mmol of α-oxo-acid. It was passed through a column (5 ml) of Dowex 1 × 8 resin (Cl^-; 100—200 mesh); the column was washed with water and eluted with 0·01N-hydrochloric acid (60 ml h^{-1}). Collected fractions (each 12·5 ml) were tested for phosphorus content.[12] When this was nil, the elution was continued with 0·02N-hydrochloric acid and the α-oxo-acid and phosphorus contents of the fractions were estimated. Those containing the title compound were pooled, brought to pH 6·9 with lithium hydroxide, and concentrated to 3 ml. The pH was adjusted to 7·2 with N-lithium hydroxide and the *lithium salt* of the phosphorylated pentulosonic acid was precipitated with ethanol (50 ml). The precipitate (80 mg, 30%) was collected by centrifugation, washed with ethanol until free of chloride (3—4 times) and once with acetone, dried *in vacuo*, and equilibrated in air. It had $[\alpha]_D^{22}$ +1·6° (*c* 0·5 in water) (Found: C, 22·65; H, 2·8; P, 11·5. $C_5H_6Li_3O_8P,H_2O$ requires C, 22·7; H, 3·0; P, 11·7%).

(*b*) To a stirred suspension of ' isosaccharinic acid ' 6-phosphate,[6] barium salt, (481 mg, 1 mmol) in water (10 ml), Amberlite IR120 (H^+) resin (15 ml) was added; 15 min later the resin was filtered off and washed. N-Lithium hydroxide was added to the pooled effluent and washings, which were then heated on a water-bath until the pH remained constant at 9. The solvent was then removed and the residue dissolved in water (5 ml); to the stirred solution an aqueous solution (10 ml) of sodium periodate (214 mg) was added dropwise during 30 min. Stirring was continued for another 60 min, and the solution was passed through a column of Dowex 1 × 8 resin (Cl^-; 100—200 mesh; 10 ml). The column was washed with water (50 ml) and then eluted (60 ml h^{-1}) with 0·01N-hydrochloric acid. After the removal of iodate, 20 more fractions (each 12·5 ml) were collected and discarded; the product (200 mg, 76%) was then eluted with 0·02N-hydrochloric acid and isolated as in (*a*) (Found: C, 22·7; H, 2·8; P, 11·6%).

[4/1295 *Received*, 28*th June*, 1974]

[11] D. Charon and L. Szabó, *J.C.S. Perkin I*, 1973, 1175.
[12] M. Macheboeuf and J. Delsal, *Bull. Soc. chim. biol.*, 1943, **25**, 116.