Phosphorylated Sugars. Part 22.[1] Synthesis of 3-Deoxy-D-*erythro*- and 3-Deoxy-D-*threo*-hex-2-ulosonic Acid 5-(Dihydrogen Phosphates)

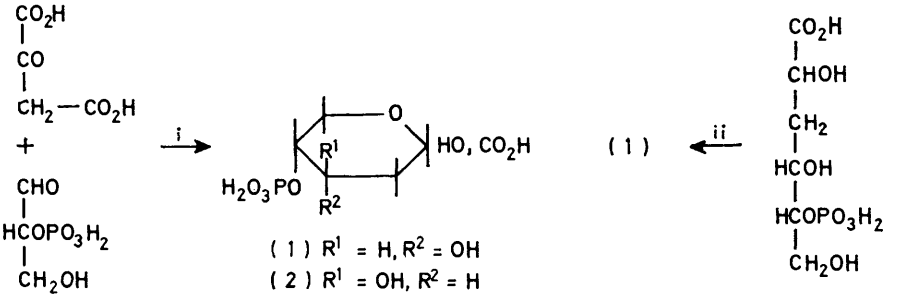
By **François Trigalo,* Robert S. Sarfati, and Ladislas Szabó,*** Equipe de Recherche No. 55 du Centre National de la Recherche Scientifique, Département de Pharmacie Chimique, Centre d'Etudes pharmaceutiques, Université de Paris-Sud, 92290 Châtenay-Malabry, France

D-Glyceraldehyde-2-phosphate (prepared by periodate treatment of D-arabinitol-2-phosphate) when condensed with oxalacetic acid at pH 7 in the presence of Ni^{2+} gave a mixture of the title compounds in high yield. The pure compounds were obtained by ion exchange chromatography.

THE formation of 3-deoxy-D-*erythro*-hex-2-ulosonic acid 5-phosphate during the treatment of glucometasaccharinic acid 6-phosphate with $NaClO_3$–V_2O_5 has been described previously.[2] The 5-phosphate which was a contaminant of preparations of 3-deoxyhexulosonic acid 6-phosphate was not isolated. Its presence was established only by identification of products formed upon its degradation with periodate and borohydride. Syntheses of 3-deoxy-D-*erythro*- and -D-*threo*-hex-2-ulosonic acid 5-phosphates by condensation of D-glyceraldehyde 2-phosphate with oxalacetic acid are now described.

D-Glyceraldehyde 2-phosphate was prepared from D-arabinose 2-phosphate which was first reduced with NaB^3H_4.

Both isomers reacted rapidly in the semicarbazide test[6] and had molar absorbancies of 10.000 cm^2; as expected, neither isomer responded in the thiobarbiturate reaction.[7] Both reduced periodate extremely slowly: 0.25 molar equivalents were reduced within 72 h. As a corollary, when the radioactive acids were treated with 10 equivalents of periodate for 72 h and the material was analysed by paper electrophoresis, only two radioactive compounds were detected. The faster moving (75%, $R_{\text{inorganic phosphate}}$: 1.07 and 1.10 at pH 3.5 and 5, respectively) was unchanged starting material; the slower moving (25%, $R_{\text{inorganic phosphate}}$: 0.80 and 1.0 at



SCHEME Reagents: i, pH 7, Ni^{2+}; ii, pH 4.6, $KClO_3$–V_2O_5

borohydride; the resulting phosphorylated polyol (which need not be isolated) gave, upon treatment with periodate, D-glyceraldehyde 2-phosphate in almost quantitative yield. Attempted isolation of this apparently unstable compound failed, but condensation *in situ* with oxalacetic acid at pH 6—7 (ref. 3) in the presence of Ni^{2+} (ref. 4) led to a mixture of D-*erythro*- and D-*threo*-hex-2-ulosonic acid 5-phosphates in high yield. The mixed lithium salts were isolated by ion exchange chromatography. A sample of the mixed isomers was dephosphorylated enzymatically and then transformed into a mixture of 3-deoxyhexitol acetates by the procedure used previously.[5] Analysis of these by g.l.c. indicated that the ratio of the isomers was 65% D-*erythro* and 35% D-*threo*. Separation of the phosphorylated, isomeric hexulosonic acids was accomplished by a second ion-exchange procedure and the pure isomers were isolated as the magnesium salts in 37 and 23% overall yields, respectively.

Radioactive 3-deoxy-D-*erythro*- and -D-*threo*-hexulosonic acid 5-phosphates labelled with ^3H on C-6 were

pH 3.5 and 5) had the same mobility as 2-deoxy-D-*erythro*-pentonic acid 5-phosphate.[8] While in the conditions used α-hydroxy-acids are almost stable to periodate, α-keto-acids are cleaved fairly rapidly:[9] the half-life of 3-deoxy-D-*glycero*-pentulosonic acid 5-phosphate,[10] a compound necessarily in the straight-chain keto-form, was found to be 2 h. Hence the foregoing observations suggest that in aqueous solution these 3-deoxyhex-2-ulosonic acid 5-phosphates are essentially in the pyranose form which is transformed only very slowly into the straight-chain form.

The reaction sequence used for the synthesis of these 3-deoxyhex-2-ulosonic acids did not exclude the possibility that racemisation of the starting D-glyceraldehyde 2-phosphate had occurred before or during the condensation step, nor could the procedure used to identify the *erythro*- and *threo*-isomers reveal the presence of enantiomers in the preparation. In order to eliminate this ambiguity an authentic sample of 3-deoxy-D-*erythro*-hex-2-ulosonic acid 5-phosphate was prepared by treatment of glucometasaccharinic acid 5-phosphate[11] (3)

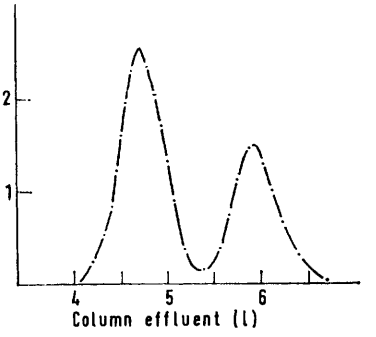
with $KClO_3$ and V_2O_5 according to Regna and Caldwell;[12] a *ca.* 50% yield of 3-deoxy-D-*erythro*-hex-2-ulosonic acid 5-phosphate was obtained. The optical rotation of this compound (−30°) was, within experimental error, identical to that of the D-*erythro*-isomer (−32°) obtained by the condensation reaction. We therefore concluded that no racemisation had taken place and that the compounds obtained by the condensation method represented optically pure isomers.

EXPERIMENTAL

All evaporations were carried out *in vacuo* below 40 °C. pH Values were measured exactly.

3-*Deoxyhex*-2-*ulosonic Acid* 5-(*Dihydrogen Phosphate*) [*Mixed* D-threo *and* D-erythro *Isomers*].—An aqueous solution (4 ml) of sodium borohydride (60 mg) was added to a stirred suspension of D-arabinose 2-(calcium phosphate)[1] (375 mg) in water (4 ml) at room temperature; 20 min later Amberlite IR-120 (H^+) resin (4—5 ml) was added slowly and the mixture poured onto a column (50 ml) of the same resin which was then washed with water until neutrality of the effluent. The effluent was concentrated and methanol (50 ml) was evaporated several times from the residue which was finally dissolved in water (6 ml). Finely ground sodium metaperiodate (800 mg) was added to the stirred solution (pH 1.5) followed 30 min later by 5 drops of glycerol. The pH of the mixture was then brought to exactly 7 (10M-NaOH). Without delay a finely ground mixture of oxalacetic acid (1 g) and $NiCl_2$ (100 mg) (ref. 4) was added portionwise to the stirred solution and the pH maintained between 6 and 7 by addition of 10M-NaOH as required. After all solids had been added, the pH was brought to 7, stirring was maintained for 1 h, and the mixture was stored at room temperature overnight. Solids were removed by centrifugation and the supernatant liquid was combined with the washings (water, 1 × 5 ml); the volume was adjusted to 200 ml with water and the pH brought to 8 with M-NH_4OH. This solution was passed through a column (25 × 1.4 cm) of Dowex 1 × 8 (Cl^-, 100—200 mesh) ion-exchange resin. The column was washed with water (200 ml) and IO_3^- (detection: KI + H^+) and oxalacetic acid (detection: phenol–sulphuric acid[13]) were eluted with 0.06M-LiCl. The phosphorylated hexulosonic acids were then eluted with 0.1M-LiCl and monitored by testing fractions (10 ml) for α-keto-acids,[6] sugars,[13] and phosphate.[14] Appropriate fractions were combined, the pH was brought to 6.9 (M-LiOH), and the volume was reduced (*ca.* 3 ml). The pH was brought to 7.6, ethanol (50 ml) was added, and *ca.* 1 h later the precipitate was collected by centrifugation, washed free of chloride ions with EtOH (3—4 × 50 ml), and finally dried with acetone. The mixed Li-salts (350 mg, 70%) were dehydrated over phosphoric oxide *in vacuo* and then equilibrated with ambient humidity; $[\alpha]_D^{20}$ +10° (*c* 1, water) (Found: C, 21.7; H, 3.9; P, 9.1. $C_6H_8Li_3O_9P{\cdot}3H_2O$ requires C, 21.8; H, 4.2; P, 9.4%).

Separation of 3-*Deoxy*-D-erythro- (1), *and* 3-*Deoxy*-D-threo- (2) *hex*-2-*ulosonic Acid* 5-(*Dihydrogen Phosphates*).—An aqueous solution (20 ml) of the mixed Li-salts (1 g) was percolated through a column (45 × 2 cm) of Dowex 1 × 2 (monochloracetate, 100—200 mesh) resin which was then eluted with a solution of monochloracetic acid (11.75 g/l) brought to pH 4.7 with 10M-NaOH. Fractions (10 ml) were collected and the phosphate content of aliquots (100 μl) of each fifth fraction was estimated.[14] The elution pattern shown in the Figure was obtained. Appropriate fractions were combined, their pH was adjusted to 7.5 with ammonium hydroxide and the solutions of both isomers were diluted with water to 5 l. These solutions were percolated through columns (*ca.* 40 ml) of Dowex 1 × 8 (Cl, 100—200 mesh) resins; the columns were washed with water (300 ml) and then successively with 0.01M HCl (1 l) and 0.02M-HCl (500 ml). The phosphorylated acids were then eluted with 0.03M-HCl (500 ml). MgO (light) was added to the eluate until the pH turned slightly alkaline, any solid remaining was filtered off, the pH was adjusted to 6.9 with dilute HCl, and the volume was reduced (*ca.* 5 ml). The Mg-salts of the phosphorylated hexulosonic acids were precipitated with EtOH (100 ml), collected by centrifugation, washed free of chloride ions with EtOH, dried *in vacuo* over phosphoric oxide, and then equilibrated with ambient humidity. The D-*erythro*-isomer (480 mg) had $[\alpha]_D^{22}$ −32° (*c* 1, water) (Found: C, 17.6; H, 4.9; P, 8.0. $C_6H_8Mg_{3/2}O_9P{\cdot}6H_2O$ requires C, 18.0; H, 5.0; P, 7.8%). The D-*threo*-isomer (270 mg) had $[\alpha]_D^{22}$ +21° (*c* 1, water) (Found: C, 18.9; H, 4.6; P, 8.0. $C_6H_8Mg_{3/2}O_9P{\cdot}5H_2O$ requires C, 18.9; H, 4.7; P, 8.1%).



3-*Deoxy*-D-erythro-*hex*-2-*ulosonic Acid* 5-(*Dihydrogen Phosphate*) (1) *from Glucometasaccharinic Acid* 5-*Phosphate*.—Amberlite-IR 120 (H^+) resin (10 ml) was added to a stirred, aqueous (10 ml) suspension of αβ-glucometasaccharinic acid 5-(barium phosphate) (500 mg).[11] When all the salt had dissolved the acidic solution was filtered off, the filtrate and washings were combined and M-LiOH was added until the pH of the mixture maintained on a boiling water-bath remained constant at 9. The solvent was removed and water (1 ml), phosphoric acid (85%, 35 μl), V_2O_5 (3 mg), and $KClO_3$ (43 mg) were added to the dry residue. The pH of the mixture was brought to 4.6 with pyridine or dilute (8.5%) phosphoric acid as required; it was stirred at room temperature for 5 days and then percolated through a column (50 ml) of Amberlite IR-120 (H^+) resin; the pH of the effluent was brought to 8 with M-NH_4OH and the solution was passed through a column (8 × 1.2 cm) of Dowex 1 × 8 (Cl^-, 100—200 mesh) resin. The column was washed with water (50 ml) and then eluted with 10mM-HCl (80 ml/h). Fractions (10 ml) containing the phosphorylated α-keto-acid (650—110 ml) were combined, the pH was brought to 6.9 with magnesium oxide (light powder), and the volume reduced (*ca.* 3 ml). The Mg-*salt* of the title compound (200 mg, *ca.* 50%) precipitated with EtOH (50 ml), was collected by centrifugation, washed free of chloride ions with EtOH, and dried

in vacuo over phosphoric oxide. After equilibration with ambient humidity it had $[\alpha]_D^{22}$ −30° (*c* 1, water) (Found: C, 18.9; H, 4.8; P, 8.1. $C_6H_8Mg_{2/3}O_9P{\cdot}5H_2O$ requires C, 18.9; H, 4.7; P, 8.1%).

[8/272 *Received*, 16*th February*, 1978]

REFERENCES

[1] Part 21, R. S. Sarfati, M. Mondange, and L. Szabó, *J.C.S. Perkin I*, 1977, 2074.
[2] F. Trigalo, W. Jachymczyk, J. C. Young, and L. Szabó, *J.C.S. Perkin I*, 1975, 595.
[3] A. C. Stoolmiller and R. H. Abeles, *J. Biol. Chem.*, 1966, **241**, 5764.
[4] K. M. Herrman and M. D. Poling, *J. Biol. Chem.*, 1975, **250** 6817.
[5] D. Charon and L. Szabó, *J.C.S. Perkin I*, 1976, 1628.
[6] J. MacGee and M. Doudoroff, *J. Biol. Chem.*, 1954, **210**, 617.
[7] A. Weissbach and J. Hurwitz, *J. Biol. Chem.*, 1959, **234**, 705.
[8] P. Szabó and L. Szabó, *J. Chem. Soc.*, 1964, 5139.
[9] D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, 1946, **164**, 433.
[10] F. Trigalo and L. Szabó, *J.C.S. Perkin I*, 1975, 604.
[11] S. Lewak and L. Szabó, *J. Chem. Soc.*, 1963, 3975.
[12] P. P. Regna and B. P. Caldwell, *J. Amer. Chem. Soc.*, 1944, **66**, 243; H. S. Isbell, *J. Res. Nat. Bur. Std.*, 1944, **33**, 45.
[13] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Analyt. Chem.*, 1956, **28**, 350.
[14] M. Macheboeuf and J. Delsal, *Bull. Soc. Chim. biol.*, 1943, **25** 116.