siderable value, and an improved synthesis of the isosteric analogue (I) of glucose 6-phosphate (II) is described in the Appendix (Roach *et al.*, 1981).

We thank the Science Research Council for a research studentship (to D. J. W. R.) and Mr. P. R. Adams for helpful advice.

References

- Adams, P. R., Harrison, R. & Inch, T. D. (1974) *Biochem. J.* **141**, 729–732
- Adams, P. R., Harrison, R., Inch, T. D. & Rich, P. (1976) *Biochem. J.* **155**, 1–4
- Afolayan, A. (1972) *Biochemistry* **11**, 4172–4178
- Ames, B. N. & Dubin, D. T. (1960) *J. Biol. Chem.* **235**, 769–775
- Bliss, C. I. (1967) *Statistics in Biology*, vol. 1, pp. 438–439, McGraw-Hill, New York
- Chen, P. S., Toribara, T. Y. & Warner, H. (1956) *Anal. Chem.* **28**, 1756–1758
- Cornish-Bowden, A. (1976) *Principles of Enzyme Kinetics*, pp. 168–193, Butterworths, London
- Dixon, H. B. F. & Sparkes, M. J. (1974) *Biochem. J.* **141**, 715–719
- Dixon, H. B. F. & Sparkes, M. J. (1976) *Biochem. J.* **155**, 440–441
- Engel, R. (1977) *Chem. Rev.* **77**, 349–367
- Florini, J. R. & Vestling, C. S. (1957) *Biochim. Biophys. Acta* **25**, 575–578
- Inch, T. D. (1978) *Ciba Found. Symp. New Ser.* **57**, 135–154
- Lefevre, M., Gonzalez, N. S. & Pontis, H. G. (1964) *J. Chromatogr.* **15**, 499–500
- Le Marechal, P., Froussios, C., Level, M. & Azerad, R. (1980a) *Biochem. Biophys. Res. Commun.* **92**, 1097–1103
- Le Marechal, P., Froussios, C., Level, M. & Azerad, R. (1980b) *Biochem. Biophys. Res. Commun.* **92**, 1104–1109
- Loesche, W., Butlitz, R., Horn, A., Koehler, W., Peterman, H. & Tul, U. (1974) *J. Chromatogr.* **92**, 166–170
- Lowry, O. H., Passonneau, J. V., Schulz, D. W. & Rock, M. K. (1961) *J. Biol. Chem.* **236**, 2746–2755
- Orr, G. A. & Knowles, J. R. (1974) *Biochem. J.* **141**, 721–723
- Roach, D. J. W. (1980) Ph.D. Thesis, University of Bath
- Roach, D. J. W., Harrison, R., Hall, C. R. & Inch, T. D. (1981) *Biochem. J.* **197**, 735–737
- Stribling, G. (1974) *Biochem. J.* **141**, 725–728
- Unger, F. M., Stix, D., Moderndorfet, E. & Hammer-schmid, F. (1978) *Carbohydr. Res.* **67**, 349–356
- Webster, D., Jondorf, W. R. & Dixon, H. B. F. (1976) *Biochem. J.* **155**, 433–440

APPENDIX

An improved synthesis of 6,7-dideoxy- α -D-glucopyranose 7-phosphonic acid

Daniel J. W. ROACH,* Roger HARRISON,* C. Richard HALL† and Thomas D. INCH†

*Department of Biochemistry, University of Bath, Bath BA2 7AY, U.K., and †Chemical Defence Establishment, Porton, Salisbury, Wilts. SP4 0JQ, U.K.

6,7-Dideoxy- α -D-glucopyranose 7-phosphonic acid (I), the isosteric phosphonate analogue of glucose 6-phosphate (II), was previously synthesized (Adams *et al.*, 1976) by treatment of the crude 6-aldehyde sugar benzyl 2,3,4-tri-O-benzyl- α -D-glucopyranoside (III) with the Wittig reagent diphenyl triphenylphosphoranylidene-methylphosphonate (Jones *et al.*, 1968), followed by transesterification of the reaction product to the fully benzylated compound and catalytic hydrogenolysis to the free phosphonic acid (I). The method suffers from the major disadvantage that diphenyl triphenylphosphoranylidene-methylphosphonate is difficult to prepare, involving reaction of the unreactive triphenylphosphine with diphenyl chloromethylphosphonate, itself not readily available. Moreover, the transesterification step, necessary because of the unpredictable behaviour of phenyl esters in hydrogenolysis reactions (Adams *et al.*,

1974, 1976), requires a column-chromatographic separation of product from benzyl alcohol and proceeds in relatively low yield.

An alternative synthetic route to the phosphonate (I) is now described (Scheme 1). This avoids the diphenyl ester intermediate, proceeding instead via the corresponding diethyl derivative (IV), which is more readily prepared and undergoes ready dealkylation to the phosphonic acid in the presence of bromotrimethylsilane.

Method

Benzyl 2,3,4-tri-O-benzyl-6,7-dideoxy- α -D-glucopyranose 7-phosphonic acid diethyl ester (IV)

n-Butyl-lithium (15 ml) was added dropwise to a solution of tetraethyl methylene-diphosphonate (9 g) [prepared by the procedure of Ford-Moore &

Scheme 1. *Synthesis of 6,7-dideoxy- α -D-gluco-heptose 7-phosphonic acid*
 (1) $(\text{EtO})_2\text{PO}-\text{CH}_2-\text{PO}(\text{OEt})_2$; (2) Me_3SiBr ; (3) catalytic hydrogenation. $\text{R} = \text{CH}_2-\text{C}_6\text{H}_5$.

Williams (1947) as modified by Kosolapoff (1953)] in tetrahydrofuran (150 ml) at -10°C . After 20 min the crude 6-aldehyde derivative, benzyl 2,3,4-tri-*O*-benzyl- α -D-glucopyranoside 6-aldehyde (III) (16 g) (Adams *et al.*, 1976), was added to the reaction mixture, which was gently shaken and stored overnight at 22°C under an atmosphere of N_2 . Production of the diethyl phosphonate was monitored by t.l.c. $\{R_F 0.3$ [light petroleum (b.p. $60-80^\circ\text{C}$)/ether, 1:3, v/v], and portions of *n*-butyllithium were added until the reaction appeared to be complete. The reaction mixture was poured into water and extracted with ether. The ether extracts were combined, dried (over MgSO_4) and concentrated to give the crude diethyl ester, which was applied to a column (40 cm $\times$ 3 cm) of silica gel and eluted with light petroleum (b.p. $60-80^\circ\text{C}$)/ether (1:3, v/v). Fractions containing the product (monitored by t.l.c.) were collected and concentrated to give benzyl 2,3,4-tri-*O*-benzyl-6,7-dideoxy- α -D-glucopyranoside 7-phosphonic acid diethyl ester (IV) (7.0 g, yield 96%). N.m.r. data (C^2HCl_3): δ 7.30 p.p.m. (phenyl); 6–7 p.p.m. ($\text{CH}=\text{CH}$); 4.1, 1.3 p.p.m. (OEt). I.r. data: $8.0\mu\text{m}$ ($\text{P}=\text{O}$); 9.7, $10.3\mu\text{m}$ ($\text{P}-\text{OEt}$).

Benzyl 2,3,4-tri-O-benzyl-6,7-dideoxy- α -D-glucopyranoside 7-phosphonic acid (V)

The partially purified diethyl ester (IV) (7.0 g) was mixed with bromotrimethylsilane (2.3 g, 1.5 mol. equiv.) (Gilliam *et al.*, 1946) and stored at 22°C for 16 h under an atmosphere of N_2 . Completion of the reaction, as judged by disappearance of starting material from t.l.c. chromatograms, was assured by

portion-wise further addition of bromotrimethylsilane. The completed reaction mixture was poured into a large excess of water (300 ml), and acetone was added to obtain a homogeneous solution, which was extracted with chloroform. The combined chloroform extracts were dried (over MgSO_4) and concentrated to give a brown residue, which was crystallized from benzene/light petroleum (b.p. $60-80^\circ\text{C}$) to give benzyl 2,3,4-tri-*O*-benzyl-6,7-dideoxy- α -D-glucopyranoside 7-phosphonic acid (V) (3.55 g, yield 59%), m.p. $165-168^\circ\text{C}$, $[\alpha]_D^{20} +83.5 \pm 1.0^\circ$ (c 2.0 in chloroform). N.m.r. data (C^2HCl_3): δ 7.2 p.p.m. (phenyl); 6–7 p.p.m. ($\text{CH}=\text{CH}$). I.r. data: 3–5 μm ($\text{P}-\text{OH}$).

6,7-Dideoxy- α -D-gluco-heptose 7-phosphonic acid (I)

A solution of the benzyl 2,3,4-tri-*O*-benzyl-6,7-dideoxy- α -D-glucopyranoside 7-phosphonic acid (3.4 g) in ethanol (150 ml) containing a few drops of trifluoroacetic acid was hydrogenolysed over 10% palladium on charcoal at room temperature and atmospheric pressure until H_2 uptake ceased (uptake 700 ml, theoretical value 653 ml). The solution was filtered through Celite and concentrated to give 6,7-dideoxy- α -D-gluco-heptose 7-phosphonic acid (I) (1.3 g, yield 90%) as a hygroscopic white solid. Both n.m.r. and i.r. spectra showed a complete absence of benzyl groups and were indistinguishable from those of the compound previously prepared by Adams *et al.* (1976). Moreover, the product (I) behaved as a substrate for yeast glucose 6-phosphate dehydrogenase, showing kinetic parameters identical with those obtained for

the compound synthesized by the previously described method.

Discussion

The improved synthesis avoids a number of problems associated with the preparation and deblocking of the unsaturated diphenyl phosphonate (Adams *et al.*, 1976) by using the stable diethyl ester. Dialkyl esters of this type are traditionally difficult to convert into the free acid, but bromotrimethylsilane, used by McKenna *et al.* (1977) to de-esterify a range of simple dialkyl phosphonates, was found also in the present case to yield smoothly the phosphonic acid, which was readily hydrolysed to the required analogue of glucose 6-phosphate. The overall synthesis from D-glucose proceeded in 23% yield [cf. 14% (Adams *et al.*,

1976)] and proved to be both faster and more reliable than the previously described method.

References

- Adams, P. R., Harrison, R. & Inch, T. D. (1974) *Biochem. J.* **141**, 729–732
Adams, P. R., Harrison, R., Inch, T. D. & Rich, P. (1976) *Biochem. J.* **155**, 1–4
Ford-Moore, A. H. & Williams, J. H. (1947) *J. Chem. Soc.* 1465–1467
Gilliam, W. F., Meals, R. N. & Sauer, R. O. (1946) *J. Am. Chem. Soc.* **68**, 1161–1163
Jones, G. H., Hamamura, E. K. & Moffat, J. G. (1968) *Tetrahedron Lett.* **55**, 5731–5734
Kosolapoff, G. M. (1953) *J. Am. Chem. Soc.* **75**, 1500–1501
McKenna, C. E., Higa, M. T., Cheung, N. H. & McKenna, M. C. (1977) *Tetrahedron Lett.* **2**, 155–158