63. *Phospholipids. Part IV.** myo*Inositol 2-Phosphate.*

By D. M. Brown and G. E. Hall.

The *myo*inositol monophosphates obtained from phytic acid by chemical hydrolysis and by use of wheat-bran enzyme cannot be distinguished from one another and are considered to be identical.

For comparative structural studies, as many as possible of the *myo*inositol phosphates are necessary. Iselin [1] synthesised a monophosphate, considered to be the 2-isomer, by phosphorylation of 1 : 3 : 4 : 5 : 6-penta-*O*-acetyl*myo*inositol. Monophosphates have also been obtained by chemical [2] and enzymic [3] hydrolysis of phytic acid (*myo*inositol hexaphosphate). On the basis of infrared spectra and *X*-ray powder diagrams Fleury, Desjobert, and Lecocq [4] concluded that the synthetically and enzymically prepared crystalline free acids were identical but different from that obtained by chemical hydrolysis of phytate. The three materials could not be differentiated in other respects.

We have prepared several samples of inositol monophosphate from phytic acid by the chemical and enzymic methods. Their infrared spectra and *X*-ray powder diagrams were identical, as were those of the derived bis*cyclo*hexylammonium salts. Specimens of barium inositol phosphate prepared chemically and enzymically were provided by Dr. Desjobert and converted into the crystalline free acids. Their spectra, and those of a sample of the synthetic inositol phosphate [1] prepared by Dr. Lecocq, were identical with those shown by our own products. We therefore confirm the identity of the synthetic and the enzymically prepared inositol phosphate and can find no evidence which differentiates these from that produced by chemical hydrolysis. Indeed we believe that the evidence allows the conclusion that all samples are identical.†

Experiments based on a personal communication from Dr. Lecocq were carried out, and these indicated that the abnormal spectra recorded [4] for the chemical-hydrolysis product are probably to be ascribed to a difference in crystalline form rather than to the existence of another isomer. In brief, crystallisation from water by addition of five volumes of alcohol gives a product with a different infrared spectrum and *X*-ray powder diagram. This " abnormal " product (which we were unable to obtain from the enzymically prepared acid) gives the usual *cyclo*hexylamine salt and it can also be converted into the normal acid by recrystallisation using ten volumes of ethanol. We avoid, in our own procedure, evaporation to dryness of aqueous solutions of the inositol phosphate to minimise the possibility of acid-catalysed phosphate migration. The isomer isolated is the predominant one present in the barium salts but, particularly in the salt from the enzymic hydrolysis, small amounts of other isomers are probably present.

Finally, there remains some doubt about the actual structure of the inositol phosphate under discussion. The synthetic route adopted by Iselin could only constitute proof of structure if acetyl migration in the intermediate penta-acetylinositol during phosphorylation were excluded. More recently, it has been shown [5] that Purdie methylation of the penta-acetate does involve acetyl migration, by the isolation of the (±)-1-methyl ether, DL-bornesitol, and that, where both the 1- and the 2-hydroxyl group are free, toluene-*p*-sulphonylation occurs on the 1-position.[6]

However, in the hydrolysis of phytic acid at pH values where phosphate migration

* Part III, *J.*, 1958, 1360.

† Dr. C. E. Ballou and Mrs. Frances Pizer (University of California, Berkeley) have informed us that they have reached the same conclusion.

[1] Iselin, *J. Amer. Chem. Soc.*, 1949, **71**, 3822.
[2] Desjobert, *Bull. Soc. Chim. biol.*, 1954, **36**, 1293.
[3] McCormick and Carter, *Biochem. Prep.*, 1952, **2**, 65.
[4] Fleury, Desjobert, and Lecocq, *Bull. Soc. Chim. biol.*, 1954, **36**, 1301.
[5] Anderson and Landel, *J. Amer. Chem. Soc.*, 1954, **76**, 6130.
[6] Angyal, *Quart. Rev.*, 1957, **11**, 212.

cannot be important, five phosphate residues are removed more rapidly than the sixth, because the crude monophosphate is essentially one isomer. In *myo*inositol there is only one axial hydroxyl group so that, by analogy with carboxylic esters, the five equatorial phosphate groups should be hydrolysed most rapidly. The predominant monophosphate should then be the axial or 2-isomer. A similar view has been taken of the enzymic hydrolysis.[7] For this reason we intend to assume the correctness of Iselin's structural assignment until evidence to the contrary is forthcoming.

Experimental

myo*Inositol 2-Phosphate by Chemical Hydrolysis.*—Sodium phytate (200 g.) (or a solution prepared from the commercial calcium salt) was hydrolysed according to Desjobert's directions [2] and the product was isolated as the barium salt (17·6 g.) (Found, in material dried for 7 hr. at 60° *in vacuo*: C, 15·5; H, 3·7. Calc. for $C_6H_{11}O_9PBa,4H_2O$: C, 15·9 H, 4·1%). The barium salt (3·0 g.) was dissolved in water (50 c.c.) containing a few drops of formic acid, and the clear solution percolated through a column (13 × 3·3 cm.) of Dowex-50 (H^+-form) resin. The solution and washings were evaporated *in vacuo* to *ca.* 5 c.c., and ethanol (50 c.c.) was added; crystallisation began immediately. After 24 hr. at 0° the product was collected, washed with ethanol, then ether, and dried at room temperature [yield 1·42 g., 86%; m. p. 196—198° (decomp.)] (Found: C, 27·5; H, 5·25; P, 11·8. Calc. for $C_6H_{13}O_9P$: C, 27·7; H, 5·0; P, 11·9%). On recrystallisation from aqueous ethanol (1 : 10) the material had an unaltered infrared spectrum and *X*-ray powder diagram.

Hydrolysis with 3N-hydrochloric acid [1] gave *myo*inositol (75%), m. p. and mixed m. p. 226—230° (hexa-acetate, m. p. 222—223°).

*Bis*cyclo*hexylammonium* myo*Inositol 2-Phosphate.*—The above inositol phosphate (100 mg.) in water (5 c.c.) was added to *cyclo*hexylamine (0·2 c.c.), and the mixture extracted with ether (4 × 10 c.c.). The aqueous solution was evaporated *in vacuo* with exclusion of carbon dioxide. Addition of ethanol caused crystallisation, and the *salt* was recrystallised from methanol (10 c.c.) containing a few drops of water and ether (30 c.c.), giving rosettes of needles (125 mg., 62%), m. p. 211—213° (Found, in material dried *in vacuo* at 60° for 10 hr.: C, 40·4; H, 9·0; N, 5·4; P, 6·15. $C_{18}H_{39}O_9N_2P,4H_2O$ requires C, 40·7; H, 8·9; N, 5·3; P, 5·85%). No loss of *cyclo*hexylamine occurred at 100° in 9 hr. *in vacuo* (Found: N, 5·25%). The infrared spectrum showed bands at 3350, 2930, 2860, 2750, 2640, 2610, 2560, 2210, 2020, 1642, 1623, 1555, 1453, 1431, 1389, 1369, 1353, 1318, 1300, 1291, 1264, 1251, 1236 (shoulder), 1202, 1186, 1137, 1115, 1100 (shoulder), 1085 (shoulder), 1053, 1031, 1012, 968, 948, 924, 886, 822, and 728 $cm.^{-1}$. The *X*-ray powder photograph showed lines with $2l$ = 3·63, 4·33, 4·65, 5·12, 5·38, 5·73, 5·99, 6·25, 6·84, 7·16, 7·42, 8·30, 8·71, 9·00, 9·29, 9·56, 9·84, 10·27, and 10·88 cm.

cyclo*Hexylammonium* myo*Inositol Hydrogen Phosphate.*—The above di*cyclo*hexylammonium salt (247 mg.) and inositol phosphate (121 mg.) were dissolved in water, and the solution was evaporated. The residue was recrystallised from hot aqueous ethanol by the addition of ether to turbidity. The *product* formed needles (300 mg.), m. p. 203—205° (Found, in material dried for 6 hr. at 60°/1 mm.: C, 37·95; H, 7·7; N, 3·6. $C_{12}H_{26}O_9NP,H_2O$ requires C, 38·2; H, 7·5; N, 3·7%). Potentiometric titration with alkali gave an equivalent weight 377·5 (theor., 377).

Brucine myo*Inositol Hydrogen Phosphate.*—Brucine (1·0 g.) was warmed with a solution of inositol phosphate (260 mg.) in water (20 c.c.), and undissolved brucine removed. The solution was extracted 3 times with chloroform, and the aqueous layer evaporated under reduced pressure, ethanol being added towards the end to effect crystallisation. The product recrystallised from methanol (25 c.c.) containing a little water. The *salt* (536 mg.) had m. p. 244—247° (decomp.) (Found, in material dried for 4 hr. at 80°/1 mm. over P_2O_5: C, 51·05; H, 6·3; N, 4·1; P, 4·6. $C_{29}H_{39}O_{13}N_2P,1{\cdot}5H_2O$ requires C, 51·05; H, 6·2; N, 4·1; P, 4·55%).

myo*Inositol 2-Phosphate by Enzymic Hydrolysis.*—Sodium phytate (100 g.) was hydrolysed by the wheat-bran enzyme,[3] and the product (4·7 g.) was isolated, as above, as the barium rather than the lead salt. This barium salt (2·65 g.) was converted into the free acid as described for the chemical-hydrolysis product. Crystallisation was much slower and the

[7] Hawthorne, *Biochim. Biophys. Acta*, 1955, **18**, 389.

total yield less [1·0 g., 68%; m. p. 195—197° (decomp.)] (Found, in material dried at room temperature *in vacuo*: C, 27·8; H, 5·4%).

The acid (100 mg.) was converted into its bis*cyclo*hexylammonium salt as described above. This was recrystallised by dissolving in hot aqueous ethanol (1 : 1; 5 c.c.) and adding ether to turbidity and formed needles (150 mg., 74%), m. p. and mixed m. p. 210—212° (Found, in material dried at 60°/1 mm. for 3·5 hr.: C, 40·6; H, 9·1; N, 5·25%). The infrared spectrum and *X*-ray powder diagram were identical with those of the bis*cyclo*hexylamine salt of the inositol phosphate prepared by chemical hydrolysis.

Infrared and X-Ray Comparisons of Chemically and Enzymically Produced Inositol Phosphates.—Infrared spectra were obtained by using a Perkin-Elmer Model 21 with sodium chloride optics and Nujol and hexachlorobutadiene mulls. *X*-Ray powder diagrams were obtained by using a 19·0 cm. diameter camera and Cu-K_α radiation: the figures ($2l$) recorded are the distances between the two lines symmetrically disposed about the *X*-ray beam and corresponding to the same reflection, as measured on the film.

*myo*Inositol phosphate, prepared by either procedure, had infrared bands at 3340, 2910, 2300 (b), 1474, 1448, 1403, 1370, 1350 (shoulder), 1337, 1319, 1286, 1243, 1206, 1190 (shoulder), 1148, 1119, 1106, 1054, 1031, 990, 935, 819, 770 (b), and 712 cm.$^{-1}$, and lines on the *X*-ray powder photograph at $2l$ = 3·50, 5·03, 5·49, 5·80, 6·16, 6·54, 7·01, 7·35, 8·02, 8·33, 9·01, 9·30, 9·84, 10·20, 10·58, 10·88, 11·81, 12·29, 12·89, 13·50, and 13·86 cm. These spacings correspond closely to those reported by Fleury *et al.*[4] for the enzymic and the synthetic product.

These are described as type A spectra.

Samples of barium inositol phosphate kindly supplied by Dr. A. Desjobert and prepared from phytate chemically and enzymically were converted into the free acids as described above. A sample of synthetic *myo*inositol 2-phosphate prepared and kindly supplied by Dr. J. Lecocq was also studied. All gave type A infrared and *X*-ray spectra.

The following experiment based on information provided by Dr. Lecocq was carried out. Barium inositol phosphate (chemical hydrolysate) (0·97 g.) was shaken in water (55 c.c.) for 4 hr. Undissolved material (35 mg.) was filtered off, and barium removed by passage of the solution through a Dowex-50 (H^+-form) column. The solution was divided equally. The first part was evaporated to 5 c.c., and ethanol (25 c.c.) added. The inositol phosphate (184 mg.) (Found: C, 27·55; H, 5·4%) had spectra of type B, *viz.*, infrared bands at 3340, 2910, 2320 (b), 1470 (shoulder), 1448, 1401, 1382, 1371, 1335, 1315, 1284, 1265 (shoulder), 1240, 1203, 1146, 1117, 1105 (shoulder), 1050, 1033 (shoulder), 990, 935, 816, 785 (b), 718 (shoulder), and 710 cm.$^{-1}$.

The spacings on the *X*-ray powder diagram were similar to those given as type A, except that lines with $2l$ = 4·38, 8·64, and 13·01 appeared, whilst those with $2l$ = 6·54, 9·84, 12·29, and 12·39 cm. were absent. There were also intensity changes.

On recrystallisation (31 mg.) from aqueous ethanol (1 : 10) the product (26 mg., 84%) showed type A spectra. Preparation of the bis*cyclo*hexylammonium salt from unrecrystallised acid (type B spectra) (63 mg.) gave recrystallised material (67% yield) which had the same spectra as those recorded above for this salt.

The second part was taken to dryness *in vacuo*. The crystalline residue (240 mg.) had spectra of type A. This (158 mg.) was recrystallised by dissolving it in water (0·4 c.c.) and carefully adding ethanol (2·0 c.c.) to form a layer on top of the aqueous phase. After 60 hr. at room temperature, the large crystals (70 mg.) were filtered off, washed with aqueous ethanol (1 : 5), and dried *in vacuo*; the product gave spectra of type B. When this material (20 mg.) recrystallised from aqueous ethanol (1 : 10) at 0°, the product gave type A spectra.

This work was carried out during the tenure of a D.S.I.R. Award (by G. E. H.). We thank Sir Alexander Todd, F.R.S., for his interest, and Drs. Lecocq and Desjobert for their kindness in providing some inositol phosphate samples and for helpful personal communications. We are grateful to Ciba Laboratories Ltd., Horsham, for a generous gift of sodium phytate.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, August 6th,* 1958.]