(*vide infra*); $[\alpha]^{26}D$ −182.7° (*c* 1.42, chf.). Its infrared spectrum is indistinguishable from that of the sample from 1-bromocodeine.

(b) **From 1-Bromocodeine.**—The oxidation procedure of Homeyer and DeLaMater,[35] when applied to 1-bromocodeine[34] (1.54 g., m.p. 158–161°) yielded 1.00 g. (68.5%) of pure **1-bromocodeinone**, m.p. 202.5–203.5°, $[\alpha]^{25}D$ −180.6° (*c* 1.30, chf.), λ_{max}^{alc} 288 mμ, log ε 3.33, infrared λ_{max} 5.97 μ.

Anal. Calcd. for $C_{18}H_{18}O_3NBr$: C, 57.46; H, 4.82. Found: C, 57.55; H, 5.22.

Its 2,4-dinitrophenylhydrazone, crystallized from ethyl acetate, melted at 224–224.5° and did not depress the m.p. of the sample prepared from 1-bromodihydrothebainone.

Catalytic hydrogenation of 1-bromocodeinone (XXXII) (86.4 mg.) in 5 cc. of alcohol over 15 mg. of Adams catalyst, yielded crude **1-bromodihydrocodeinone** (19 mg., m.p. 174–191°) which after three crystallizations from ethyl acetate had m.p. 207.5–208.5°, undepressed by admixture of authentic 1-bromodihydrocodeinone of m.p. 206.5–208.5°, prepared by bromination of dihydrocodeinone.[29] Its melting point was depressed on admixture with the starting 1-bromocodeinone. This hydrogenation was interrupted after 1 mole of hydrogen had been absorbed, although the uptake showed no sign of slackening at this point. The dihydro base was isolated by chromatography on alumina (3.0 g.) in alcohol-free chloroform and appeared as the most weakly adsorbed fraction.

Codeine (XXXIII).—A mixture of 200 mg. of 1-bromocodeinone, m.p. 202.5–203.5°, 0.5 g. of lithium aluminum hydride and 30 cc. of carefully purified tetrahydrofuran was refluxed for 46 hours. After destruction of the excess reagent with ethyl acetate, the mixture was acidified with 2 *N* hydrochloric acid and extracted with ether. The acid layer was added slowly to a strong potassium hydroxide solution containing Rochelle salt. The resulting alkaline suspension was extracted three times with chloroform, and the chloroform layers were washed, dried, filtered, and concentrated to give 146 mg. (159 mg. theory) of colorless glass which was converted to its **hydrobromide,**[36] 131 mg., m.p. 148–150°, resolidifying, remelting 273–278°. Recrystallization of this hydrobromide gave 110 mg., properties essentially unchanged.

This hydrobromide, dissolved in warm water, on treatment with ammonia gave an oil which rapidly crystallized, 70 mg., m.p. 153–156°. Recrystallization from dilute methanol gave 59 mg., m.p. 156.5–158°, large prisms, hydrated, $[\alpha]^{27}D$ −137° (*c* 1.15, alc.) (reported[48] −135.9°). Its m.p. was undepressed on admixture with authentic codeine of m.p. 157–158.4°,[49] crystallized as above, but was strongly depressed by admixture of 1-bromocodeine of m.p. 161–163°. Its infrared spectrum was indistinguishable from that of an authentic sample.

Morphine (I).—Codeine was demethylated essentially as described by Rapoport and his co-workers,[37] but the processing was rendered simpler (no chromatography or sublimination was necessary) and the yield improved by using hydrosulfite in all alkaline solutions. Morphine was obtained in 34% yield as colorless needles, m.p. 254–256.4°, $[\alpha]^{27}D$ −126° (*c* 2.32, methanol) (reported[49] m.p. 253–254°).

(48) O. Hesse, *Ann.*, **176**, 189 (1875).

(49) R. Kempf, *J. prakt. Chem.*, [2], **78**, 201 (1908), has reported the melting point of anhydrous codeine to be 157°. Various other authors report values in the neighborhood of 155° for either hydrated or anhydrous codeine.

ROCHESTER, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND THE INSTITUTE FOR ENZYME RESEARCH, UNIVERSITY OF WISCONSIN]

Phosphoric Esters of Biological Importance. VI. The Synthesis of D-Glucosamine 6-Phosphate and N-Acetyl-D-glucosamine 6-Phosphate[1]

BY FRANK MALEY[2] AND HENRY A. LARDY

RECEIVED OCTOBER 24, 1955

Procedures for the synthesis of glucosamine 6-phosphate and N-acetylglucosamine 6-phosphate have been developed. N-Anisylidene glucosamine in pyridine is treated with diphenyl phosphorochloridate and the product is acetylated before isolation. The anisylidene group is removed by hydrolysis and the 1,3,4-tri-*O*-acetyl-6-diphenylphosphoroglucosamine hydrochloride is converted to glucosamine 6-phosphate by reductive cleavage of the phenyl groups followed by hydrolysis of the acetyl groups. Barium hydrogen glucosamine 6-phosphate is isolated at *p*H 4.0. The barium salt may be separated at *p*H 8 but this treatment results in the conversion of part of the glucosamine 6-phosphate to a more acid-labile compound. Another procedure is described for the conversion of the tri-*O*-acetyl intermediate to N-acetylglucosamine 6-phosphate which is isolated as the crystalline monoammonium salt.

The possible involvement of the phosphoric esters of glucosamine and N-acetylglucosamine in the biosynthesis of the mucopolysaccharides encouraged us to seek a definitive chemical synthesis for these compounds. During the course of this work, Glaser and Brown[3] obtained very good experimental evidence for such involvement and extension of this work should be facilitated by the availability of these synthetic phosphoric esters. Glucosamine 6-phosphate has previously been prepared[4] by the enzymatic phosphorylation of glucosamine using ATP. A chemical synthesis has also been reported[5] but the product was of unknown purity. N-Acetylglucosamine 6-phosphate has been synthesized by the chemical[6] and enzymatic[7] acetylation of glucosamine 6-phosphate.

The synthesis of glucosamine 6-phosphate described in this paper is accomplished by the sequence of reactions shown in Fig. 1. In early experiments, the product was isolated, as the barium salt, at *p*H 8.0 to 8.5. Although the elementary analysis, optical rotation and periodate oxidation, indicated that the compound was pure, the acid-lability of part of the phosphorus indicated the presence of a contaminant. Even after repeated precipitations of the compound, the phosphorus liberated by hydrolysis in 1 *N* HCl at 100° for 30 min-

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(2) Public Health Service Research Fellow of the National Cancer Institute.

(3) L. Glaser and D. H. Brown, *Proc. Nat. Acad. Sci.*, **41**, 253 (1955).

(4) D. H. Brown, *Biochim. Biophys. Acta*, **7**, 487 (1951).

(5) J. M. Anderson and E. Percival, *Chem. and Ind.*, **33**, 1018 (1954).

(6) S. Roseman, *Federation Proc.*, **13**, 283 (1954).

(7) D. H. Brown, *Biochim. Biophys. Acta*, **16**, 429 (1955).

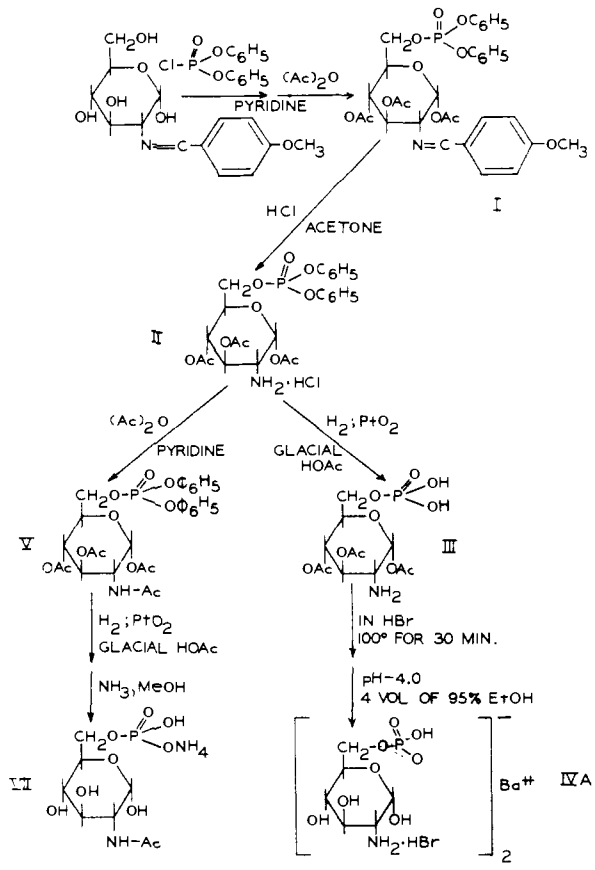

Fig. 1.—Sequence of reactions involved in the synthesis of glucosamine 6-phosphate and N-acetylglucosamine 6-phosphate.

utes was 6 to 7% of the total organic phosphorus. This figure was higher than expected since triacetyl glucosamine 6-phosphate is hydrolyzed to the extent of only 1% under these conditions. These anomalous results appear to be related to the final step in the isolation and not to differences between the enzymatic and synthetic compounds, for the enzymatically prepared glucosamine 6-phosphate precipitated at *p*H 8 and dried as the Ba salt also contained the high concentration of acid labile phosphorus. Therefore, other conditions for the precipitation of the compound were sought. The product obtained by precipitation at *p*H 4.0 had an acid labile phosphorus content of 1.5% or less, which is more in accord with expectations. Figure 2 reveals the difference in phosphorus lability between the compounds precipitated at *p*H 8.0 and at *p*H 4.0. The latter appears to be Ba diglucosamine 6-phosphate dihydrobromide.

To obtain additional evidence concerning the acid-labile impurity in the product precipitated at *p*H 8.0, paper chromatography was employed. Utilizing the solvent system of Axelrod and Bandurski,[8] only one phosphorus containing spot was detected, but the acid system of Leloir and Palladini[9] separated the compound into two phosphorus containing components (Fig. 3). Only a single

(8) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(9) A. C. Palladini and L. F. Leloir, *Biochem. J.*, **51**, 426 (1952).

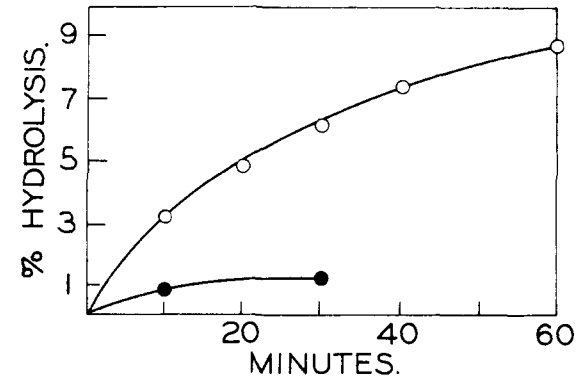

Fig. 2.—Inorganic phosphorus liberated by acid hydrolysis at 100° in 1 *N* HCl of barium glucosamine 6-phosphate precipitated at *p*H 8.0 (○) and *p*H 4.0 (●).

component was found in the preparation precipitated at *p*H 4.0.

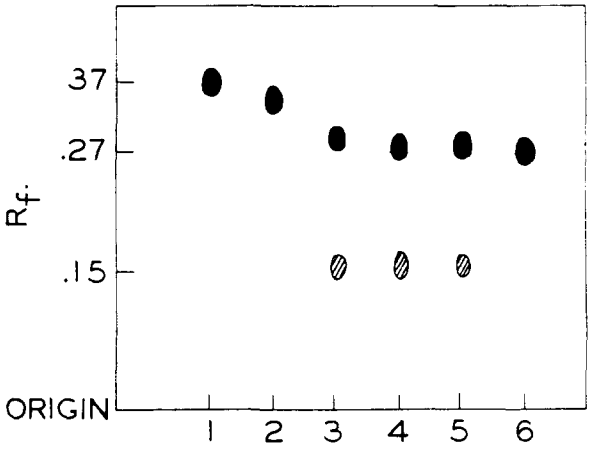

Fig. 3.—Paper chromatogram according to the procedure of Palladini and Leloir; (1) glucose 1-phosphate; (2) glucose 6-phosphate; (3) enzymatically prepared glucosamine 6-phosphate precipitated as the barium salt at *p*H 8.0; (4) chemically prepared glucosamine 6-phosphate precipitated at *p*H 4.0, then reprecipitated at *p*H 8.0; (5) chemically prepared glucosamine 6-phosphate precipitated at *p*H 8.0; (6) chemically prepared glucosamine 6-phosphate precipitated at *p*H 4.0.

Other differences noted between the two compounds were (1) the inorganic phosphorus content of the compound precipitated at *p*H 8.0 was always about 1 to 2% of the total phosphorus, whereas that of the compound precipitated at *p*H 4.0 was always less than 1%; (2) during storage, the *p*H 8.0 compound discolored much more rapidly than the *p*H 4.0 compound; (3) while repeated precipitations at *p*H 8.0 were required to obtain a product of high purity[4,5] a single precipitation at *p*H 4.0 yielded a product containing 92% or more of glucosamine 6-phosphate. Reprecipitation at *p*H 4.0 resulted in a colloidal suspension and greatly reduced the yield. The product, obtained by centrifuging at high speed, contained no detectable bromide ion and appeared to be Ba diglucosamine 6-phosphate.

Confirmation of the identity of the synthetic glucosamine 6-phosphate was sought through enzymatic studies. Direct evidence could not be obtained

for the conversion of synthetic glucosamine 6-phosphate to glucosamine 1-phosphate by partially purified phosphoglucomutase using the assay procedures of Brown[10] or Najjar.[11] It is possible that the enzyme preparation used required glucose 1,6-diphosphate as activator when the synthetic substrate was employed; its activity with enzymatically prepared glucose 1-phosphate may have depended on the presence of some of the diphosphate in the latter.[12] Since glucosamine 6-phosphate hydrobromide was used as substrate after separation of barium ion as the sulfate, bromide ion was tested as a possible inhibitor of phosphoglucomutase. It was found not to inhibit at the concentrations present in the assay but did so beginning at concentrations two to three times higher. Evidence for the combination of glucosamine 6-phosphate with phosphoglucomutase is presented in Table I.

TABLE I

INHIBITION OF GLUCOSE 1-PHOSPHATE CONVERSION TO GLUCOSE 6-PHOSPHATE BY GLUCOSAMINE 6-PHOSPHATE

The incubation mixture contained aside from a suitable amount of phosphoglucomutase; 3.4×10^{-3} *M* glucose 1-phosphate; 1.7×10^{-2} *M* cysteine, *p*H 7.5; 2.0×10^{-3} *M* $MgCl_2$; vol., 0.6 ml.; *t*, 30°; time of incubation, 5 min. Concn. of glucose-1-PO_4 = 3.4×10^{-3} *M*.

Glucosamine-6-PO_4, *M*	μmoles Glucose-6-PO_4 formed
0	1.28
7.9×10^{-4}	1.11
1.6×10^{-3}	0.99
3.2×10^{-3}	0.88
6.4×10^{-3}	0.68

More encouraging results were obtained with the N-acetylase system of yeast,[7] as can be seen from Fig. 4. The conversion of glucosamine 6-phosphate to N-acetylglucosamine 6-phosphate, employing the N-acetylglucosamine assay of Aminoff, *et al.*,[13] appears to be almost complete. In these assays synthetic N-acetylglucosamine 6-phosphate was used as a standard reference substance in the colorimetric assays. Maximum color developed after seven to nine minutes of heating with either the synthetic or enzymatically prepared N-acetylglucosamine 6-phosphate. The molar extinction coefficient was 5.72×10^3. N-Acetylglucosamine was not used as the reference standard because maximum color (molar extinction coefficient = 7.14×10^3) developed after four minutes of heating and decreased with further heating.[13]

N-Acetylglucosamine 6-phosphate was synthesized by the deacetylation of tetraacetylglucosamine 6-phosphate in ammoniacal methanol (Fig. 1). This procedure is somewhat more practical than the combined enzymatic and chemical method of Roseman.[6] Some of the amorphous precipitates contained a higher acid-labile (1 *N* HCl at 100° for 30 minutes) phosphorus content than others. This probably resulted from over-exposure to ammonia;

(10) D. H. Brown, *J. Biol. Chem.*, **204**, 877 (1953).

(11) V. A. Najjar, *ibid.*, **175**, 281 (1948).

(12) We are indebted to Dr. D. H. Brown for assaying, with phosphoglucomutase, a crude sample of glucosamine 6-phosphate precipitated at *p*H 8. He found that this sample was mutated to an extent of 45% of that expected had the reaction gone to completion.[10]

(13) D. Aminoff, W. T. J. Morgan and W. M. Watkins, *Biochem. J.*, **51**, 379 (1952).

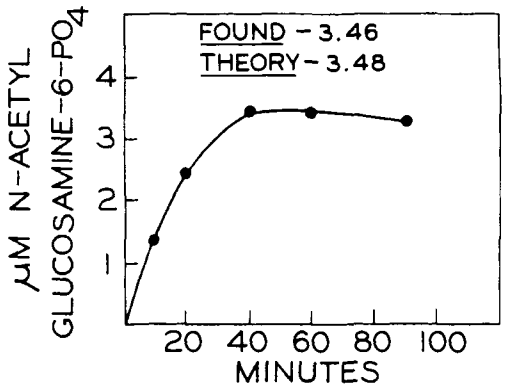

Fig. 4.—Acetylation of glucosamine 6-phosphate by the N-acetylase system of Fleischman's yeast.

most of the contaminant could be removed by treatment with charcoal. A crystalline product was also obtained which contained no impurities detectable by chromatography. With an ascending solvent system composed of 7.5 volumes of 95% ethanol and 3 volumes 1 *M* ammonium acetate brought to *p*H 3.1 with concd. HCl, synthetic N-acetylglucosamine 6-phosphate had an R_f of 0.5 as compared to 0.32 for glucosamine 6-phosphate. If unacetylated glucosamine was present in this preparation, it could not be detected by absorption at 275 mμ, a characteristic property of the decomposition products of glucosamine,[4] or by the Dische[14] hexosamine test.

The specificity of glucose 6-phosphatase toward glucosamine 6-phosphate and a number of other substrates was also determined. The results obtained (Table II) are analogous to those obtained by Crane.[15]

TABLE II

EFFECT OF RAT LIVER MICROSOMAL GLUCOSE 6-PHOSPHATASE ON VARIOUS HEXOSE PHOSPHATES

The numerical values represent the ratio of hexose phosphate hydrolyzed to glucose 6-phosphate hydrolyzed X100. The microsomes were isolated by the method of Schneider.[16] In addition to a suitable aliquot of the microsomal fraction, the incubation mixtures contained from 8.0×10^{-3} *M* to 1.3×10^{-2} *M* of the hexose phosphates and 3.0×10^{-2} *M* to 4.2×10^{-2} *M* sodium citrate, the incubation times varied from 15 to 21 minutes. The reaction was terminated by the addition of 10% trichloroacetic acid.

Substrate	% Hydrolysis relative to glucose-6-PO_4
Glucose-6-PO_4	100
2-Deoxyglucose-6-PO_4	20, 27
Glucose-1-PO_4	3.2
Mannose-6-PO_4	32
Sorbose-1-PO_4	35
Tagatose-6-PO_4	11
Glucasamine-6-PO_4 (Chemical)	8.5, 14
Glucosamine-6-PO_4 (Enzymatic)	7.3
N-Acetylglucosamine-6-PO_4 (Chemical)	2.2

Experimental

I. N-Anisylidene 1,3,4-Tri-*O*-acetyl-6-diphenylphosphoroglucosamine.—To 4.58 g. of anisylidene glucosamine[17]

(14) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **184**, 517 (1950).

(15) R. K. Crane, *Biochim. Biophys. Acta*, **17**, 443 (1955).

(16) W. C. Schneider, *ibid.*, **176**, 259 (1948).

(17) F. Bergmann and L. Zervas, *Ber.*, **64B**, 975 (1931).

dissolved in 170 ml. of dry pyridine[18] and cooled to −20° or lower, 4.0 ml. of diphenyl phosphorochloridate was added and the resultant solution kept at −5° for about 15 hours. After this period, 9 ml. of acetic anhydride was added and the mixture was kept at room temperature for 24 hours. The pyridine solution was then poured into one liter of ice-water with stirring. About 10 hours were required for the precipitate to solidify, at the end of which time the product was collected on a buchner funnel and washed thoroughly with water followed by 25 ml. of ice-cold ethanol. The yield after drying *in vacuo* over P_2O_5 was 6.15 g. (Yields varied from 40 to 65%.)

The compound could be recrystallized from 95% ethanol by adding water slowly while scratching the flask with a glass rod. When the solution became turbid it was placed at 5° until crystallization was complete. The melting point was 134 to 135°; $[\alpha]^{20}D$ +96.8 ± 0.5° (*c* 0.940, chloroform).

Anal. Calcd. for $C_{32}H_{34}O_{12}NP$ (655.6): C, 58.60; H, 5.19; N, 2.14; P, 4.73. Found: C, 58.61; H, 5.38; N, 2.24; P, 4.4.

II. **1,3,4-Tri-*O*-acetyl-6-diphenylphosphoroglucosamine. HCl.**—The crude I (6.15 g.) was dissolved in about 100 ml. of acetone and filtered. The clear solution was then heated to boiling, 3 ml. of 4 *N* HCl was added, and heating was continued for 1 minute more. A precipitate formed as the solution cooled. The solution was placed in the refrigerator at −5° until precipitation was completed. The yield of the dried compound was 4.52 g. It melted with decomposition at 190 to 191° (yields varied from 80 to 85%); $[\alpha]^{20}D$ + 49.6 ± 0.2° (*c* 4.05, methanol or 50% ethanol).

Anal. Calcd. for $C_{24}H_{29}O_{11}NPCl$ (573.9): C, 50.21; H, 5.09; N, 2.44; P, 5.40. Found: C, 50.13; H, 5.29; N, 2.43; P, 5.1.

III. **1,3,4-Tri-*O*-acetylglucosamine-6-phosphoric Acid.**—A solution of 1.012 g. of II in about 50 ml. of dry glacial acetic acid was hydrogenated at atmospheric pressure and room temperature with 0.1 g. of PtO_2 as the catalyst. About 2.5 hours were required for uptake of the theoretical amount of hydrogen; the solution was then filtered and the flask and filter paper rinsed with 95% ethanol. The alcohol-acetic acid solution was concentrated to about 5 ml. and 95% ethanol was added slowly with scratching. After about 100 ml. of ethanol was added the turbid solution was placed at −5° until crystallization was complete. The filtered solution was then reconcentrated and the crystallization procedure repeated until no additional product could be obtained.[19] The total yield after drying *in vacuo* over P_2O_5 was 0.58 g. (Yields varied from 70 to 75%.) After two recrystallizations from a minimum of glacial acetic acid, by the slow addition of 95% ethanol, the compound melted with decomposition at 166 to 167°, $[\alpha]^{20}D$ +48.9 ± 0.2° (*c* 2.67 in 1.02 *N* HCl). The analyses revealed that the compound crystallized with 2 molecules of water which could be removed by thorough drying.

Anal. Calcd. for $C_{12}H_{20}O_{11}NP \cdot 2H_2O$ (421.3): C, 34.20; H, 5.75; N, 3.33; P, 7.36. Found: C, 34.29; H, 5.77; N, 3.4; P, 7.2. After drying at 100°: Calcd. for $C_{12}H_{20}O_{11}NP$ (385.3): C, 37.41; H, 5.22; N, 3.63. Found: C, 37.10; H, 5.28; N, 3.44.

IVa. Barium Di-(glucosamine 6-Hydrogenphosphate Hydrobromide).—A solution of 140 mg. of III in 1 *N* HBr was heated at 100° for 30 minutes. The solution was then cooled to room temperature and the *p*H raised to 4.0 to 4.1 by the addition of solid $Ba(OH)_2$ with rapid stirring. Four volumes of 95% ethanol were added and the precipitate, after being allowed to settle for a few hours, was collected by centrifugation, washed once with 100% ethanol and twice with anhydrous ether and immediately placed in a vacuum desiccator over P_2O_5. The dried precipitate weighed about 90 mg. (weighing was difficult due to the hygroscopic nature of the compound). Bromide was detected with $AgNO_3$ and also by permanganate oxidation.

Anal. Calcd. for $C_{12}H_{28}O_{16}N_2P_2Br_2Ba$ (815.5): C, 17.67; H, 3.46; N, 3.43; P, 7.60. Found: C, 17.57; H, 3.73; N, 3.14; P, 7.4.

IVb. Barium Di-(glucosamine 6-Hydrogenphosphate).—Reprecipitation of IVa at *p*H 4.0 yielded a colloidal suspension which could be spun down only at high speeds. The recoveries were poor and bromide ion could not be detected.

Anal. Calcd. for $C_{12}H_{26}O_{16}N_2P_2Ba$ (653.7): C, 22.04; H, 4.01; N, 4.28. Found: C, 21.24; H, 4.13; N, 4.34.

IVc. Barium Glucosamine 6-Phosphate.—After deacetylating III as described above, the solution was brought to about *p*H 7.5 with solid $Ba(OH)_2$ and the barium salt of the phosphoric ester precipitated with four volumes of 95% ethanol. Three reprecipitations were required before satisfactory P and N analyses could be obtained. However, the H analyses were always high (the H analysis reported by Percival[5] is also high); other properties of this compound presented in the discussion, illustrate that though it appears to be pure, closer examination reveals this not to be the case; $[\alpha]^{20}D$ +49.6 ± 1.1° (*c* 4.33 of dipolar ion, H_2O at *p*H 2.52). The reported value[10] for the enzymatically prepared compound is +48.5°.

Anal. Calcd. for $C_6H_{12}O_8BaPN$: C, 18.27; H, 3.07; N, 3.55; P, 7.85. Found: C, 18.21; H, 3.82; N, 3.5; P, 8.0.

V. N-Acetyl-1,3,4-tri-*O*-acetyl-6-diphenylphosphoroglucosamine.—To 1.014 g. of II dissolved in 35 ml. of dry pyridine, 0.6 ml. of acetic anhydride was added. After 24 hours at room temperature, petroleum ether was added slowly until turbidity appeared at which point an additional 150 ml. of petroleum ether was added and the solution placed at −5° until crystallization was complete. The precipitate was washed thoroughly on a sintered glass funnel with water to remove pyridine hydrochloride (until a negative test is obtained with $AgNO_3$). The yield after drying was 0.839 g. (Yields varied from 80 to 90%.) Recrystallization was accomplished by dissolving V in a small volume of chloroform and adding petroleum ether slowly, with scratching. The compound melted at 141 to 142°; $[\alpha]^{20}D$ +20.7 ± 0.2° (*c* 5.07, chloroform).

Anal. Calcd. for $C_{26}H_{30}O_{12}NP$ (579.5): C, 53.90; H, 5.21; N, 2.42; P, 5.35. Found: C, 53.48; H, 5.22; N, 2.28; P, 5.19.

VI. N-Acetyl-1,3,4-tri-*O*-acetylglucosamine 6-phosphoric Acid.—A solution of 1.071 g. of V in 50 ml. of dry glacial acetic acid was hydrogenated over 0.1 g. of PtO_2 at room temperature and atmospheric pressure. One hour was required for the theoretical uptake of hydrogen, after which the solution was filtered and the flask and filter paper rinsed with 95% ethanol. The ethanol-acetic acid solution was concentrated to about 5 ml. and petroleum ether added slowly with scratching. After crystals began to form a total of about 200 ml. of petroleum ether was added and the suspension kept at −5° until crystallization was complete. The yield after drying was 0.716 g. (Yields varied from 85 to 90%.) After two recrystallizations from absolute methanol by the addition of ethyl ether, the compound melted at 157 to 158° with decomposition; $[\alpha]^{20}D$ +24° (*c* 3.16, methanol).

Anal. Calcd. for $C_{14}H_{22}O_{12}NP$ (427.3): C, 39.35; H, 5.19; N, 3.28; P, 7.25. Found: C, 39.32; H, 5.26; N, 2.96; P, 7.1.

VIIa. Mono-ammonium-N-acetylglucosamine 6-Phosphate.—A solution of 310 mg. of VI in 40 ml. of absolute methanol was cooled to 5° and anhydrous NH_3 was bubbled in for about 15 minutes. The ammoniated methanol solution was allowed to stand at room temperature for three to four hours; gel formation occurred after about an hour. Methanol was added to dissolve the gel and the solution was concentrated and dried under vacuum to remove the ammonia. The dry powder was dissolved in methanol, filtered and precipitated with an equal volume of ethyl ether. The product, collected by centrifugation after three precipitations and dried *in vacuo* over P_2O_5, weighed 84 mg. Nitrogen analysis revealed it to be the mono-ammonium salt of N-acetylglucosamine 6-phosphate.

Anal. Calcd. for $C_8H_{19}O_9N_2P$ (318.2): C, 30.20; H, 6.02; N, 8.81; P, 9.74. Found: C, 31.35; H, 5.86; N, 8.60; P, 9.77.

VIIb. Crystalline Ammonium N-Acetylglucosamine 6-Phosphate.—Approximately 60 mg. of VIIa was dissolved in 8 ml. of water and treated with 50 mg. of charcoal (Nu-

(18) Variable results were obtained with different lots of pyridine. A lot of pyridine that was ineffective in the preparation of I was made effective by refluxing with solid KOH for six hours, allowing to cool and distilling. The pyridine was then stored over solid KOH.

(19) Since the free phosphoric acid esters are extremely hygroscopic, filtering may be difficult when the humidity is high. In such cases the compounds can be collected by centrifuging.

char C-190). To the colorless filtrate about 35 ml. of absolute methanol was added followed by 30 ml. of ethyl ether. The turbid solution was placed at 5° for about 24 hours; the crystals were collected by centrifugation and dried *in vacuo* over P_2O_5. The compound melted with decomposition at 146.5 to 147.5°.

Anal. Found: C, 29.44; H, 6.36; N, 7.30.

The analyst reported that the compound was hygroscopic and each portion taken for analysis was specially dried at 100°. The low nitrogen can perhaps be accounted for by this drying procedure, since the nitrogen analysis of the sample prior to shipment to the analyst was 9.00%.

ADDED IN PROOF.—Since this paper was submitted, J. L. Reissig, J. L. Strominger and L. F. Leloir, *J. Biol. Chem.*, **217**, 959 (1955), have published a modification of the Morgan method which yields identical extinction coefficients for N-acetylglucosamine and N-acetylglucosamine 6-phosphate. However, we have found it necessary to heat N-acetylglucosamine 6-phosphate in the borate solution for 8 minutes, instead of 3, to obtain maximal color development. With this method, the ratio of phosphate to acetylglucosamine in the synthetic N-acetylglucosamine 6-phosphate was found to be 1.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE CHEMISTRY DIVISION, CANADA DEPARTMENT OF AGRICULTURE]

The Differential Thermal Properties of Bacterial Dextrans[1]

BY HIROKAZU MORITA

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Differential thermal analyses of some bacterial dextrans were undertaken in order to elucidate the relation between their thermographic features and molecular constitution. Specific types of dextrans exhibit characteristic thermal behavior, which also reflects changes in physical properties resulting from the method of preparation. As a result of this study it is concluded that thermal analysis, when supplemented by other data, affords valuable information relating the constitution of the dextrans with their chemical and physical properties.

This paper is the third in a series of studies pertaining to the use of differential thermal analysis and the elucidation of the factors which determine the differential thermal properties exhibited by organic substances. Previous endeavors[2,3] showed that, aside from the obvious influence of elemental composition, differential thermal reactions of certain polymers were dependent upon molecular configuration, as for example, the type of polymer linkages. The behavior of cellulose in contrast to starch, and that of amylose and amylopectin were specific instances.

In order to ascertain further the relation between molecular constitution and differential thermal property, analyses were undertaken on some bacterial dextrans representative of distinct structural types. The results constitute the main theme of this communication.

The samples described below were chosen for two reasons. First, since data relating to their chemical and physical properties were available, it was hoped some correlation might be established between these and those provided by thermal analysis. Second, there is, at present, considerable interest in the fundamental properties of the dextrans among polymer and biological chemists.

Experimental

Details of the analytical technique have been described earlier.[2] In this investigation, 100-mg. samples were used to prepare the "sandwich" packing in calcined alumina. The dextrans were provided by the Northern Utilization Research Branch and are designated by the number assigned in the NRRL Culture Collection to the bacterial strain by which the dextran was produced. Each sample was vacuum dried at 100° and 0.01 mm. pressure to constant weight. Details on the physical and chemical properties of most of the dextran samples used have been reported by Jeanes and co-workers.[4]

Results and Discussion

It has been observed[3] that the array of endotherms in the 130 to 310° region is the most prominent thermographic feature shown by glucopyranose polymers having α-1,4-linkages. Analogous patterns (Fig. 1, curves A and B) are shown by typical preparations of the water soluble, native dextran from *Leuconostoc mesenteroides* NRRL B-512. These dextrans, which were produced by the whole-culture process, contain 95% α-1,6-linked units. The salient thermographic features are the sharp endotherms at 200 and 295° followed by a diminished one at 310°.

Modifications of the B-512 thermograms, shown in Fig. 1, curves C and D, were observed for a B-1308 and an enzymatically synthesized B-512 dextran.[5] The former gave endotherms at 220, 235 and 285°, while in the latter they appeared at 215 and 280°. The only other significant way these preparations are known to differ from those of curves A and B are in intrinsic viscosity, the values corresponding to curves A, B, C and D being 1.23, 1.02, 0.463 and 1.37, respectively.[4]

Further variations of the B-512 thermograms are portrayed in Fig. 2. While the dextrans, shown by curves A and B in Fig. 2, have analogous anhydroglucose linkages, they differ markedly from those in Fig. 1 in their intrinsic viscosities, the values corresponding to curves A and B being 0.75 and 0.811. The dextrans represented by curves C and D, on the other hand, are distinct from those in Fig. 1 by virtue of their linkages, periodate analysis indicating these to be 7% 1,4-like, 3% 1,3-like and 90% 1,6-anhydroglucose units.

In view of the thermographic changes caused by pretreatment of other polysaccharides,[3] a more detailed study was made of the relation between purity and differential thermal property. In most instances, purification involving treatments to reduce ash content had negligible effects. An exception is

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