in the number of reactive sites or the dissociation of a urease–inhibitor complex with the inhibitor assumed to be of natural origin. With the recognition that potassium ion can function as an inhibitor and phosphate ion as an activator of urease it appears that neither of the above explanations are correct and that the dilution effect is simply the consequence of a change in the relative concentrations of enzyme, activator and inhibitor.[18,19]

Experimental

Reagents.—The stock 0.1, 0.2, 0.3, 0.4 and 0.5 *M* buffer solutions were prepared from reagent grade dipotassium hydrogen phosphate and potassium dihydrogen phosphate and from the corresponding sodium salts. In every case irrespective of the concentration of the buffer, the *p*H of the solution after final dilution was 7.0 ± 0.02 at 25°. A 1.0 *M* stock solution was prepared daily from urea which had been recrystallized from ethanol. The crystalline urease was prepared from Arlington jackbean meal by the method of Dounce,[20] all operations subsequent to the initial extraction being conducted at 5°. The thrice recrystallized urease obtained from 400 g. of meal was dissolved in 5 ml. of water 1% saturated with hydrogen sulfide at 0° and this stock solution stored at 5°. The water used for the dilution of the enzyme stock solution was also 1% saturated with hydrogen sulfide at 0°. The water used for all solutions was redistilled from an all-glass apparatus.

Procedure.—In general the procedure used was a modification of that described by Van Slyke and Cullen[9] in which the aeration step was eliminated and the ammonia determined by the method of Conway.[21] In practice 2.0-ml. aliquots of one of the above buffer solutions were placed in eight 5.0-ml. volumetric flasks, 1.0 ml. of 0.016, 0.020, 0.028, 0.032, 0.040, 0.060, 0.10 and 0.20 *M* urea solution added to successive flasks and the latter placed in a bath at 25 ± 0.02°. After thermal equilibrium was obtained 0.78 ml. of a diluted enzyme solution was added to each of the above solutions and the mixtures vigorously stirred with a rod kept in each flask. After 3 minutes 0.5 ml. of 2.0 *N* sulfuric acid was added to each flask, the solution again stirred, the flasks withdrawn from the bath, the stirring rods washed and the volume of solution in each flask made up to 5.0 ml. The diluted enzyme solutions were prepared so as to contain approximately 1 microgram of protein nitrogen per ml. of solution. These solutions which were 0.01 *M* in the appropriate buffer were allowed to stand for 5 hours at 25° prior to use. For the determination of liberated ammonia 1.0-ml. aliquots of approximately 0.005 *N* hydrochloric acid containing Tashiro indicator[21] was placed in the central chamber of a Conway dish, a 1.0-ml. aliquot of one of the above 5.0 ml. solutions placed in the outer chamber, the lid, lubricated with glycerol containing sodium hydroxide, placed in position so as to permit the rapid introduction of 1.0 ml. of saturated potassium carbonate into the outer compartment, the dish sealed, the contents in the outer compartment mixed, and the dish allowed to stand overnight at room temperature. The excess acid remaining in the central compartment was then titrated with approximately 0.005 *N* aqueous barium hydroxide. Suitable blanks were provided for each experiment and it was estimated that for a given set of experiments wherein the same enzyme solution was used a precision of ±1.5% was obtained. A least squares treatment was used for the $1/v$ *versus* $1/[S]_0$ plots and in every case $[S]_0$ was taken as the mean substrate concentration prevailing over the 3 minute reaction time. It should be noted that the slopes of the plots given in Figs. 1, 2, 3, 4 and 6 are dependent upon the concentration of active enzyme and because of the irreversible inactivation of urease with time comparisons of the slopes in the above-mentioned figures should be limited to those experiments which were performed simultaneously, *i.e.*, those given in any separate plot. The curve given in Fig. 5, which is based upon data obtained in separate experiments, was constructed by arbitrarily selecting the curve, given in Fig. 4 (upper plot), which has a slope of 4.7 for a phosphate concentration of 0.159 *M* as a standard and adjusting the coördinates of the other plots so that the slope of the curve representing 0.159 *M* phosphate in each of these plots was equal to 4.7.

(18) O. H. Straus and A. Goldstein, *J. Biol. Chem.*, **26**, 559 (1943).
(19) A. Goldstein, *ibid.*, **27**, 529 (1944).
(20) A. L. Dounce, *J. Biol. Chem.*, **140**, 307 (1941).
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PASADENA, CALIF. RECEIVED AUGUST 16, 1950

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

A New Synthesis of Purine Nucleosides. The Synthesis of Adenosine, Guanosine and 2,6-Diamino-9-β-D-ribofuranosylpurine[1]

BY JOHN DAVOLL[2] AND BERTRAM A. LOWY

A new synthesis of adenosine, starting from adenine, is presented. Syntheses of 2,6-diamino-9-β-D-ribofuranosylpurine and guanosine from 2,6-diaminopurine are described. The method developed involves the condensation of the chloromercuri derivatives of acylaminopurines with acetylglycosyl halides to give fully acylated aminoglycosylpurines which are converted to the desired nucleosides by deacylation and, in the case of guanosine, deamination. The yields obtainable make this synthetic method suitable for the preparation of isotopically labeled nucleosides.

Previously published work from this Laboratory[3] has dealt with the synthesis of isotopically labeled adenine, hypoxanthine, guanine, isoguanine, xanthine, 2,6-diaminopurine and uric acid for studies of the biosynthesis of nucleic acids. An extension of these metabolism studies to the ribofuranosyl derivatives of these purines would be of obvious interest, and the present communication describes syntheses of adenosine, guanosine and 2,6-diamino-9-β-D-ribofuranosylpurine suitable for the preparation of these compounds isotopically labeled in the purine ring.

Three methods have been reported previously for the synthesis of purine nucleosides.

In the first of these Fischer and Helferich[4] condensed silver 2,8-dichloroadenine with tetraacetylglucosyl bromide and deacetylated the product to give 2,8-dichloro-9-β-D-glucopyranosyladenine. Total reductive dehalogenation of this compound gave 9-β-D-glucopyranosyladenine, while partial reduction gave 2-chloro-9-β-D-glucopyranosyladenine (I, R = β-D-glucopyranosyl). Treat-

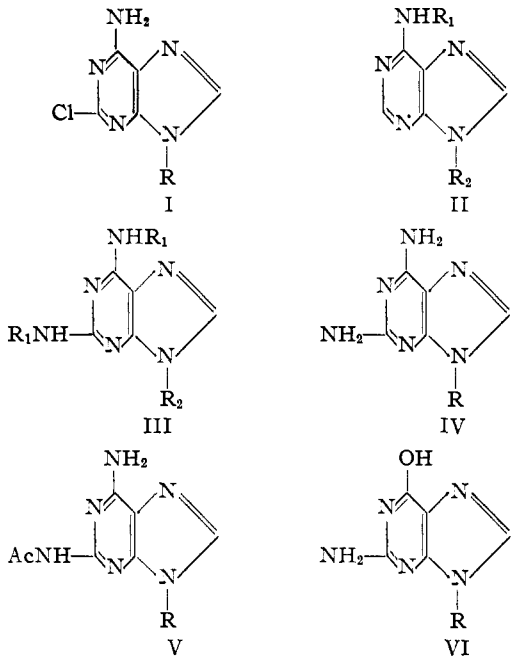
(1) The support of the National Cancer Institute of the United States Public Health Service, The Nutrition Foundation, Inc., and the joint support of the Office of Naval Research and the Atomic Energy Commission, contract N6-ori-99, T.O.-1 is gratefully acknowledged.

(2) Public Health Service Postdoctorate Research Fellow of the National Cancer Institute, United States Public Health Service.

(3) For reviews, see G. B. Brown, *Cold Spring Harbor Symposia on Quantitative Biology*, **13**, 43 (1948), and *Federation Proc.*, **9**, 517 (1950).

(4) E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914).

ment of the latter compound with nitrous acid and then with aqueous ethanolic ammonia at 150° gave 9-β-D-glucopyranosylguanine. When



triacetyl-D-ribofuranosyl chloride became available this synthesis was extended to the preparation of adenosine[5] and guanosine.[6] This synthetic method is unsuitable for the preparation of isotopically labeled nucleosides because of the difficulty of obtaining labeled 2,8-dichloroadenine. The only known synthesis of this compound utilizes uric acid as starting material,[7] and the yield is low. Moreover, the yield of guanosine obtained by this route is only about 3%, based on the 2,8-dichloroadenine used.

Secondly, xanthosine has been prepared from 1-β-D-ribofuranosylglyoxaline-4,5-dicarboxamide by treatment with alkaline hypobromite.[8]

The third synthetic method is exemplified by the preparation of adenosine from 6-amino-4-(5'-benzyl-D-ribofuranosylamino)-5-thioformamido-2-methylthiopyrimidine by cyclization, followed by treatment with Raney nickel.[9]

In the latter two methods the yields are low and, moreover, these procedures do not appear to be readily applicable to the synthesis of nucleosides of guanine or 2,6-diaminopurine.

Isotopic carbon or nitrogen may be incorporated in high yield into adenine[10] or 2,6-diaminopurine[11] and in the present work attention was devoted to the preparation of the required nucleosides from these compounds or from their acyl derivatives.

(5) J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 967 (1948).
(6) J. Davoll, B. Lythgoe and A. R. Todd, *ibid.*, 1685 (1948).
(7) E. Fischer and L. Ach, *Ber.*, **30**, 2208 (1897); E. Fischer, *ibid.*, **30**, 2220, 2226 (1897).
(8) G. A. Howard, A. C. McLean, G. T. Newbold, F. S. Spring and A. R. Todd, *J. Chem. Soc.*, 232 (1949).
(9) G. W. Kenner, C. W. Taylor and A. R. Todd, *ibid.*, 1620 (1949).
(10) G. B. Brown, P. M. Roll, A. A. Plentl and L. F. Cavalieri, *J. Biol. Chem.*, **172**, 469 (1948); V. M. Clark and H. M. Kalckar, *J. Chem. Soc.*, 1029 (1950).
(11) A. Bendich, S. S. Furst and G. B. Brown, *J. Biol. Chem.*, **185**, 423 (1950).

6-Acetamidopurine and 6-benzamidopurine were prepared from adenine substantially as described by Kossel[12]; formation of the diacetyladenine described by Birkofer[13] was not observed. Acylation of 2,6-diaminopurine in the same way with acetic anhydride or benzoic anhydride gave, respectively, 2,6-diacetamidopurine and 2,6-dibenzamidopurine in good yield.

The reactions of various metal derivatives of these purines with acetylglycosyl halides in boiling xylene were studied, and it was found that the chloromercuri derivatives, which have not hitherto been used for this purpose, were the most satisfactory for glycosylpurine synthesis.

The chloromercuri derivative of adenine, $C_5H_4N_5HgCl$, has been previously described[14,15] and analogous derivatives of 2,6-diaminopurine, 6-acetamidopurine, 6-benzamidopurine, 2,6-diacetamidopurine, 2,6-dibenzamidopurine and 2,8-dichloroadenine were prepared by treatment of a solution of the appropriate purine in one equivalent of aqueous or aqueous ethanolic sodium hydroxide with one molecular proportion of mercuric chloride.

In a model experiment chloromercuri-2,8-dichloroadenine was heated in xylene with tetraacetylglucosyl bromide to give 2,8-dichloro-9-tetraacetyl-β-D-glucopyranosyladenine, identical with an authentic specimen,[4] in 47% yield

$$R\text{—}HgCl + BrC_{14}H_{19}O_9 \longrightarrow R\text{—}C_{14}H_{19}O_9 + HgClBr$$

Under similar conditions chloromercuriadenine and chloromercuri-2,6-diaminopurine gave no identifiable products. This may be due to a tendency of the more basic aminopurines to remove the elements of hydrogen halide from the acetylglycosyl halide used, and suggested that the chloromercuri derivatives of the acylaminopurines might give better results than the corresponding aminopurine derivatives. This proved to be the case.

Thus, when chloromercuri-6-acetamidopurine was heated with tetraacetylglucosyl bromide in xylene, 6-acetamido-9-tetraacetyl-β-D-glucopyranosylpurine (II, R_1 = Ac; R_2 = tetraacetyl-β-D-glucopyranosyl) was obtained in 36% yield. Deacetylation of this with methanolic ammonia gave 9-β-D-glucopyranosyladenine. The structure of this compound was established by comparison with a glucosyladenine of known structure[4,16] prepared from 2,8-dichloro-9-β-D-glucopyranosyladenine by reductive dehalogenation. Similarly, chloromercuri-6-benzamidopurine reacted readily with tetraacetylglucosyl bromide, giving, after deacylation of the crude product (II, R_1 = Bz; R_2 = tetraacetyl-β-D-glucopyranosyl) with methanolic sodium methoxide and addition of picric acid, 9-β-D-glucopyranosyladenine picrate in 44% yield, based on the chloromercuri compound used.

When triacetyl-D-ribofuranosyl chloride was used instead of tetraacetylglucosyl bromide in these reactions, adenosine (9-β-D-ribofuranosyladenine[5,9,17]) was obtained in yields of 27 and 25%,

(12) A. Kossel, *Z. physiol. Chem.*, **12**, 241 (1888).
(13) L. Birkofer, *Ber.*, **76**, 769 (1943).
(14) G. Bruhns, *Z. physiol. Chem.*, **14**, 533 (1890).
(15) M. Krüger, *ibid.*, **18**, 423 (1894).
(16) J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 833 (1946); A. Holland, B. Lythgoe and A. R. Todd, *ibid.*, 965 (1948).
(17) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **94**, 809 (1932).

respectively. Based on the adenine used, the over-all yields were 21 and 14%.

Chloromercuri-2,6-diacetamidopurine and tetraacetylglucosyl bromide reacted together in boiling xylene to give 2,6-diacetamido-9-tetraacetyl-β-D-glucopyranosylpurine (III, R_1 = Ac; R_2 = tetraacetyl-β-D-glucopyranosyl) in 60% yield; deacetylation of this compound with methanolic sodium methoxide gave 2,6-diamino-9-β-D-glucopyranosylpurine (IV, R = β-D-glucopyranosyl), which was also prepared in a similar way from chloromercuri-2,6-dibenzamidopurine.

The structure of this compound was proved by its alternative synthesis from 2-chloro-9-β-D-glucopyranosyladenine (I, R = β-D-glucopyranosyl) by amination with aqueous ethanolic ammonia at 150°.

Sirupy 2,6-diacetamido-9-triacetyl-β-D-ribofuranosylpurine (III, R_1 = Ac; R_2 = triacetyl-β-D-ribofuranosyl) was prepared in 57% yield from chloromercuri-2,6-diacetamidopurine and triacetyl-D-ribofuranosyl chloride, and on deacetylation with methanolic sodium methoxide gave 2,6-diamino-9-β-D-ribofuranosylpurine (IV, R = β-D-ribofuranosyl). The over-all yield of this compound from 2,6-diaminopurine was 20%. When chloromercuri-2,6-dibenzamidopurine was used, the over-all yield was considerably lower.

An attempt was made to prepare the same compound by amination of 2-chloro-9-β-D-ribofuranosyladenine (I, R = β-D-ribofuranosyl). Although the product was not obtained in a pure condition, its ultraviolet absorption spectrum and R_f value on paper chromatograms were similar to those of the material described above.

The ribosylpurine prepared from 2,6-diaminopurine would be expected to be 2,6-diamino-9-β-D-ribofuranosylpurine by analogy with the formation of the corresponding glucose derivative, and this was confirmed by comparison of the ultraviolet absorption spectra of the two compounds, and by conversion of the intermediate 2,6-diacetamido-9-triacetyl-β-D-ribofuranosylpurine into guanosine (9-β-D-ribofuranosylguanine[6]) as described below.

9-β-D-Glucopyranosylguanine and 9-β-D-ribofuranosylguanine (guanosine) were prepared as follows: When 2,6-diacetamido-9-tetraacetyl-β-D-glucopyranosylpurine (III, R_1 = Ac; R_2 = tetraacetyl-β-D-glucopyranosyl) was treated with methanolic ammonia at 0°, a monoacetyl compound was obtained in good yield. Since 6-acetamido-9-tetraacetyl-β-D-glucopyranosylpurine is completely deacetylated under these conditions, the above monoacetyl compound would be expected to have the structure V (R = β-D-glucopyranosyl) and this proved to be the case. The compound was readily deaminated by nitrous acid at room temperature, and deacetylation of the product gave 9-β-D-glucopyranosylguanine (VI, R = β-D-glucopyranosyl) in good over-all yield. The properties of the compound were in agreement with those previously reported.[4,6]

Application of an exactly similar series of reactions to 2,6-diacetamido-9-triacetyl-β-D-ribofuranosylpurine furnished guanosine (VI, R = β-D-ribofuranosyl), identical with the natural nucleoside. The over-all yield from 2,6-diaminopurine was 21%. The anhydrous synthetic nucleoside showed $[\alpha]^{26}D$ −72° in 0.1 *N* sodium hydroxide, in good agreement with $[\alpha]^{23}D$ −70° found for anhydrous natural guanosine in the same medium. These values are considerably higher than those previously reported for guanosine, and it seems possible in view of the extremely hygroscopic nature of the anhydrous nucleoside that earlier determinations may have been made on partially hydrated material.

The condensation of silver purines with acetylglycosyl halides in boiling xylene was also investigated. No glycosylpurine derivatives could be isolated when the silver salts of adenine, 2,6-diaminopurine, 2,6-diacetamidopurine and 2,6-dibenzamidopurine were used, although silver 6-acetamidopurine and silver 6-benzamidopurine did give small quantities of the required 9-glycosylpurines. 9-β-D-Glucopyranosyladenine and adenosine were prepared in this way, although the yields were low (4–9%) and purification of the products was difficult.

The reaction of lead adenine, $C_5H_3N_5Pb$,[15] with two molecular proportions of tetraacetylglucosyl bromide in xylene, followed by brief acid hydrolysis to remove any 6-glucosylaminopurine derivative, gave a 3% yield of 9-β-D-glucopyranosyladenine, isolated as the pentaacetyl derivative. 2,6-Diaminopurine did not form an insoluble lead salt under the conditions used for the preparation of lead adenine.

In the course of this work a convenient method of regenerating adenosine and other glycosyladenines from their picrates by the use of an anion-exchange resin was discovered; in the case of adenosine the recovery of once-recrystallized material was 82%.

Experimental

Melting points were determined on a heated microscope stage and are uncorrected. All evaporations were carried out at 10–20 mm. pressure. The ultraviolet absorption spectra were determined on a Beckman model DU spectrophotometer.

2,6-Diaminopurine.—The hydrochloride (10.0 g.) was dissolved in 400 ml. of boiling water containing one equivalent of sodium hydroxide. The solution was treated with Norit and allowed to cool, giving 7.8 g. (97%) of 2,6-diaminopurine as brownish, cubic crystals.

6-Acetamidopurine.—A mixture of adenine (1.35 g.) and 8 ml. of acetic anhydride was refluxed for 3 hours, then cooled and the crystalline product collected and washed with ether; yield 1.58 g. (89%), sublimes above 260°. Birkofer[13] gives m.p. 195° (dec.) for diacetyladenine, and above 280° (dec.) for monoacetyladenine.

2,6-Diacetamidopurine.—2,6-Diaminopurine (1.0 g.) and 10 ml. of acetic anhydride were refluxed together for 2.5 hours, then cooled and the crystalline product collected and washed with ether and ethanol; yield 1.3 g. (83%), m.p. 295–300° (dec.). A sample recrystallized from ethanol–acetone–water formed irregular prisms, m.p. 295–306° (dec.).

Anal. Calcd. for $C_9H_{10}O_2N_6$: C, 46.15; H, 4.30; N, 35.90. Found: C, 46.09; H, 4.33; N, 36.22.

2,6-Dibenzamidopurine.—A mixture of 0.80 g. of 2,6-diaminopurine and 5.0 g. of benzoic anhydride was heated over a free flame until a clear melt was obtained, and for a further 15 minutes. The cooled mixture was boiled with 75 ml. of ethanol and allowed to stand, when the compound was obtained as a mass of fine needles; yield 1.51 g. (79%), m.p. 318°. A sample recrystallized from ethanol–acetone had m.p. 320°.

Anal. Calcd. for $C_{19}H_{14}O_2N_6$: C, 63.70; H, 3.94; N, 23.45. Found: C, 64.15; H, 4.07; N, 23.66.

Condensation of Metal Purine Derivatives with Acetylglycosyl Halides.—In all cases the finely powdered purine derivative was suspended in pure xylene and the mixture slowly distilled until the distillate was clear. To the residual suspension tetraacetylglucosyl bromide or a solution of triacetyl-D-ribofuranosyl chloride[5] in xylene was added. The latter compound was obtained from tetraacetyl-D-ribofuranose prepared either according to Bredereck and Höpfner[18] or Zinner.[19]

The resulting mixture was refluxed gently for the specified time, with exclusion of moisture and with care to avoid local overheating of the separated material on the sides of the flask.

Silver Purines.—Adenine and 2,6-diaminopurine were dissolved in boiling water, and their acyl derivatives in boiling 50% ethanol, and to the hot solutions dilute aqueous ammonia was added, followed immediately by an aqueous solution of one equivalent of silver nitrate. After cooling, the precipitates were collected and dried *in vacuo* at room temperature, with exclusion of light.

Silver 6-Acetamidopurine and Tetraacetylglucosyl Bromide.—To a suspension of 1.15 g. of the silver salt in 40 ml. of xylene was added 1.73 g. of tetraacetylglucosyl bromide. The mixture was refluxed for 2.5 hours, then filtered hot and 200 ml. of petroleum ether (b.p. 30–60°) added to the cooled filtrate. The resulting powdery precipitate was washed with petroleum ether, dried, and dissolved in 15 ml. of methanolic ammonia, saturated at 0°. The solution was kept overnight at 0°, then evaporated to dryness and the residue dissolved in 25 ml. of hot water and treated with Norit. To the hot filtrate was added a solution of 0.60 g. of picric acid in 20 ml. of warm ethanol; on cooling 9-β-D-glucopyranosyladenine picrate separated and was recrystallized from water; yield 0.145 g. (7%), m.p. 247° (dec.), undepressed by admixture with an authentic sample.

Silver 6-Benzamidopurine and Tetraacetylglucosyl Bromide.—The silver salt (1.40 g.) and 1.73 g. of tetraacetylglucosyl bromide were condensed together as in the above experiment. The precipitate obtained on adding petroleum ether was dissolved in 30 ml. of methanol and treated with a solution of 0.43 g. of sodium in 20 ml. of methanol. The mixture was kept overnight at room temperature, then refluxed for 15 minutes. A hot solution of 1.31 g. of citric acid in 50 ml. of ethanol was added, sodium citrate was collected and the filtrate was evaporated to dryness. The residue was dissolved in water and treated with ethanolic picric acid, giving, after recrystallization from water, 9-β-D-glucopyranosyladenine picrate; yield 0.20 g. (9%), m.p. 252° (dec.), undepressed by admixture with an authentic sample.

Silver 6-Acetamidopurine and Triacetyl-D-ribofuranosyl Chloride.—The reaction was carried out using 1.11 g. of silver salt and the triacetyl-D-ribofuranosyl chloride prepared from 1.60 g. of tetraacetylribose; the method was the same as that used for the corresponding reaction with tetraacetylglucosyl bromide. The picrate obtained was kept overnight at room temperature with 2 ml. of pyridine and 1 ml. of acetic anhydride; ethanol was then added and after 30 minutes the mixture was evaporated to dryness and the residue dissolved in chloroform. This solution was washed with sodium bicarbonate solution until free of yellow color, dried, and evaporated. The residue was kept overnight at 0° with 20 ml. of methanolic ammonia (saturated at 0°), evaporated to dryness and the residue crystallized from 1 ml. of water to give 9-β-D-ribofuranosyladenine; yield 45 mg. (4%), m.p. 236°, undepressed by admixture with natural adenosine.

Silver 6-Benzamidopurine and Triacetyl-D-ribofuranosyl Chloride.—The silver salt (1.0 g.) was condensed with the triacetyl-D-ribofuranosyl chloride prepared from 1.3 g. of the tetraacetate, and the crude reaction product deacylated with sodium methoxide as described for the corresponding glucose derivative. After purification through the picrate 40 mg. (5%) of adenosine, m.p. 234° alone or in admixture with the natural nucleoside, was obtained.

Lead Adenine and Tetraacetylglucosyl Bromide.—Lead adenine (1.0 g.) and 2.5 g. of tetraacetylglucosyl bromide in 40 ml. of xylene were refluxed for 2 hours. The hot suspension was filtered and the cooled filtrate diluted with 200 ml. of petroleum ether to give 2.0 g. of a brown powder. This was heated for 30 minutes on the steam-bath with 15 ml. of 50% acetic acid. The solution was evaporated to dryness and the dark-red residue deacetylated with methanolic ammonia. Addition of ethanolic picric acid to an aqueous solution of the product gave a small amount of a picrate, which was acetylated with acetic anhydride–pyridine. A chloroform solution of the product was washed with sodium bicarbonate solution until free of picric acid, then evaporated to dryness and the residue recrystallized twice from ethanol, giving 42 mg. (3%) of 6-acetamido-9-tetraacetyl-β-D-glucopyranosylpurine, m.p. 226° alone or in admixture with an authentic sample.

Chloromercuri-purines.—2,8-Dichloroadenine, adenine and 2,6-diaminopurine were dissolved in hot water containing one equivalent of sodium hydroxide and treated with one molecular proportion of mercuric chloride in a small volume of hot ethanol. The acyl derivatives were dissolved in boiling 50% ethanol and treated with one equivalent of sodium hydroxide, followed immediately by an ethanolic solution of one molecular proportion of mercuric chloride. The amorphous or microcrystalline precipitates were collected after cooling by filtration or centrifugation, dried *in vacuo* at room temperature, and finely powdered.

The yields of chloromercuri derivative obtained were as follows: 2,8-dichloroadenine, 87%; adenine, 100%; 2,6-diaminopurine, 93%; 6-acetamidopurine, 88%; 6-benzamidopurine, 72%; 2,6-diacetamidopurine, 83%; 2,6-dibenzamidopurine, 90%.

Analysis showed most of the compounds to contain somewhat less chlorine than would be expected from the formula RHgCl; this may be due to slight contamination with mercury purine derivatives of the type R_2Hg.

The derivatives were dried by distillation with xylene before addition of the acetylglycosyl halide, as described for the reactions using silver purines.

Chloromercuri-2,8-dichloroadenine and Tetraacetylglucosyl Bromide.—The chloromercuri derivative (1.87 g.) and 1.90 g. of tetraacetylglucosyl bromide in 40 ml. of xylene were refluxed for 1 hour; the resulting yellow solution was then cooled and treated with 250 ml. of petroleum ether (b.p. 30–60°). Crystallization of the precipitate from 10 ml. of glacial acetic acid gave 1.06 g. (47%) of 2,8-dichloro-9-tetraacetyl-β-D-glucopyranosyladenine. Recrystallization of this from glacial acetic acid gave 0.76 g., m.p. 215°, undepressed by admixture with an authentic sample.[4]

Chloromercuri-6-acetamidopurine and Tetraacetylglucosyl Bromide.—The chloromercuri derivative (1.54 g.) and 2.0 g. of tetraacetylglucosyl bromide in 100 ml. of xylene were refluxed with stirring for 1 hour. Without filtering, the mixture was evaporated to about 30 ml. and treated with 150 ml. of petroleum ether (b.p. 30–60°). The precipitate was collected and dried, then extracted with 60 ml. of warm chloroform in three portions. The extract was washed with 30% aqueous potassium iodide solution and with water, dried over sodium sulfate, and evaporated to dryness. Crystallization of the residue from 50 ml. of ethanol gave 0.68 g. (36%) of 6-acetamido-9-tetraacetyl-β-D-glucopyranosylpurine as fine needles, m.p. 226°. Recrystallization from ethanol raised the melting point to 227°; this was not depressed by admixture with the compound obtained by acetylation of 9-β-D-glucopyranosyladenine.

Anal. Calcd. for $C_{21}H_{25}O_{10}N_5$: C, 49.70; H, 4.96; N, 13.81. Found: C, 49.97; H, 5.00; N, 13.56.

A solution of 0.68 g. of the above pentaacetate in 25 ml. of boiling methanol was cooled rapidly to room temperature, and treated with an equal volume of methanolic ammonia, saturated at 0°. After standing 18 hours at 0° the solution was evaporated to dryness and the residue crystallized from water to give 0.245 g. (61%) of 9-β-D-glucopyranosyladenine, m.p. 239° (rapid heating).

Anal. Calcd. for $C_{11}H_{15}O_5N_5$: N, 23.56. Found: N, 23.42.

9-β-D-Glucopyranosyladenine from 2,8-Dichloro-9-β-D-glucopyranosyladenine.—The dichloroglucosyladenine was subjected to reductive dehalogenation as described for the ribose analog,[5] and the product isolated as the picrate, which formed yellow needles, m.p. 252° (dec.) (rapid heating). Removal of picric acid with an anion-exchange resin, as described later for adenosine picrate, gave 9-β-D-glucopyranosyladenine, m.p. 205–207°, followed by resolidification

(18) H. Bredereck and E. Höpfner, *Ber.*, **81**, 51 (1948).

(19) H. Zinner, *ibid.*, **83**, 153 (1950).

and remelting at 236°. The melting point behavior varied considerably with the rate of heating; the preliminary melting at 205–207°, described by Fischer and Helferich[4] for this compound, was not observed in samples prepared from 6-acetamido- and 6-benzamidopurine.

The pentaacetate was more suitable for comparison purposes. The above glucosyladenine (0.2 g.) was heated on the steam-bath for 1 hour with 2 ml. of pyridine and 1 ml. of acetic anhydride, then kept overnight at room temperature. Excess acetic anhydride was then decomposed by addition of ethanol, and the mixture was evaporated to dryness. Crystallization of the residue from ethanol gave 6-acetamido-9-tetraacetyl-β-D-glucopyranosylpurine as a mass of fine needles, m.p. 226–227°.

Anal. Calcd. for $C_{21}H_{25}O_{10}N_5$: N, 13.81. Found: N, 13.85.

Chloromercuri-6-benzamidopurine and Tetraacetylglucosyl Bromide.—The chloromercuri derivative (1.03 g.) and 1.3 g. of tetraacetylglucosyl bromide in 40 ml. of xylene were refluxed for 2 hours, and the product isolated as described for the corresponding condensation of chloromercuri-6-acetamidopurine. It was obtained as a colorless glass, yield 1.0 g. (81%). A solution of this material in 15 ml. of methanol was treated with a solution of 0.215 g. of sodium in 10 ml. of methanol. The mixture was kept overnight at room temperature, then refluxed for 15 minutes. A solution of 0.654 g. of citric acid in 50 ml. of boiling ethanol was added, and after removal of sodium citrate the filtrate was evaporated to dryness, then re-evaporated with water to remove methyl benzoate. Treatment of an aqueous solution of the residue with ethanolic picric acid gave 0.51 g. (44%, from chloromercuri derivative) of 9-β-D-glucopyranosyladenine picrate, m.p. 252° (dec.), undepressed by admixture with an authentic sample.

Treatment of the picrate in hot aqueous solution with Dowex No. 1 anion-exchange resin in the chloride form, followed by neutralization with sodium hydroxide and evaporation to small volume gave 9-β-D-glucopyranosyladenine, m.p. 238–239° (rapid heating).

Anal. Calcd. for $C_{11}H_{15}O_5N_5$: N, 23.56. Found: N, 23.52.

Chloromercuri-6-acetamidopurine and Triacetyl-D-ribofuranosyl Chloride.—The chloromercuri derivative (1.78 g.) and triacetyl-D-ribofuranosyl chloride, prepared from 1.7 g. of the tetraacetate, in 85 ml. of xylene were refluxed for 1 hour and the product isolated exactly as described for the corresponding glucose derivative. Deacetylation of the sirupy tetraacetyl compound with methanolic ammonia and crystallization of the product from water gave, after working up the mother liquors, a total of 0.307 g. (27%) of adenosine. Recrystallization from water gave 0.18 g., m.p. 235–236°, alone or in admixture with natural adenosine; $[\alpha]^{28}D$ −65.5° (*c*, 0.6% in water).

Anal. Calcd. for $C_{10}H_{13}O_4N_5$: N, 26.22. Found: N, 26.34.

Chloromercuri-6-benzamidopurine and Triacetyl-D-ribofuranosyl Chloride.—The chloromercuri derivative (1.0 g.) and triacetyl-D-ribofuranosyl chloride, prepared from 1.0 g. of the tetraacetate, were condensed together and the product isolated, deacylated, and converted to the picrate as described for the corresponding glucose derivative. Treatment of the picrate with an anion-exchange resin as described later gave 0.14 g. (25%, from chloromercuri derivative) of adenosine, m.p. 233–235° alone or in admixture with the natural nucleoside; $[\alpha]^{28}D$ −62.7° (*c*, 0.55% in water).

Anal. Calcd. for $C_{10}H_{13}O_4N_5$: N, 26.22. Found: N, 26.42.

Chloromercuri-2,6-diacetamidopurine and Tetraacetylglucosyl Bromide.—The chloromercuri derivative (1.17 g.) and 1.2 g. of tetraacetylglucosyl bromide in 50 ml. of xylene were refluxed for 1 hour. The product was isolated in the usual way; evaporation of the chloroform solution left a crystalline residue, which on recrystallization from 10 ml. of ethanol gave 0.85 g. (60%) of 2,6-diacetamido-9-tetraacetyl-β-D-glucopyranosylpurine, m.p. 176–178°. Recrystallization from ethanol gave 0.65 g. of colorless blade-shaped crystals, m.p. 179°.

Anal. Calcd. for $C_{23}H_{28}O_{11}N_6$: C, 48.90; H, 5.00; N, 14.90. Found: C, 48.99; H, 5.14; N, 14.80.

2,6-Diamino-9-β-D-glucopyranosylpurine.—A solution of 1.68 g. of the above hexaacetate in 30 ml. of boiling methanol was treated with a hot solution of 0.36 g. of sodium in 30 ml. of methanol. The mixture was kept overnight at room temperature, then refluxed for 30 minutes, neutralized with acetic acid, and evaporated to dryness. Crystallization of the residue from 6 ml. of water yielded 0.86 g. (92%) of product in the form of small prisms, m.p. 305–307° (dec.). Recrystallization from water raised the melting point to 308–309° (dec.); $[\alpha]^{26}D$ −6° (*c*, 0.73% in 0.1 *N* HCl). The ultraviolet absorption spectrum, 25.65 mg. per liter in 0.05 *M* phosphate buffer, *p*H 6.7, showed three maxima at 215 mμ (ϵ_m 25,200), 255 mμ (ϵ_m 9,600), and 280 mμ (ϵ_m 10,030).

Anal. Calcd. for $C_{11}H_{16}O_5N_6$: C, 42.30; H, 5.16; N, 26.93. Found: C, 42.16; H, 5.15; N, 26.70.

2,6-Diamino-9-β-D-glucopyranosylpurine from 2-Chloro-9-β-D-glucopyranosyladenine.—A mixture of 0.35 g. of the chloroglucosyladenine, 2.5 ml. of concentrated aqueous ammonia and 7.5 ml. of saturated ethanolic ammonia was heated in a sealed tube for 6 hours at 150°. The resulting orange-colored solution was evaporated to dryness and the residue dissolved in 10 ml. of hot water and treated with Norit. Addition of ethanolic picric acid gave a picrate which was collected and treated in hot aqueous solution with Dowex No. 1 anion-exchange resin in the chloride form. The filtrate was made slightly alkaline with sodium hydroxide and evaporated to dryness. Three recrystallizations of the residue from water gave 41 mg. of small prisms, m.p. 301–303° (dec.), and m.p. 303° (dec.) in admixture with the compound previously obtained. The ultraviolet absorption spectrum, 24.64 mg. per liter in 0.05 *M* phosphate buffer, *p*H 6.7, showed three maxima at 215 mμ (ϵ_m 24,600), 255 mμ (ϵ_m 9,400), and 280 mμ (ϵ_m 9,950).

Anal. Calcd. for $C_{11}H_{16}O_5N_6$: N, 26.93. Found: N, 26.70.

2-Acetamido-6-amino-9-β-D-glucopyranosylpurine.—A solution of 1.0 g. of 2,6-diacetamido-9-tetraacetyl-β-D-glucopyranosylpurine in 20 ml. of boiling methanol was cooled rapidly to room temperature and treated with 40 ml. of methanolic ammonia (saturated at 0°). The mixture was kept overnight at 0°, then evaporated to dryness and the crystalline residue recrystallized from 8 ml. of water to give 0.495 g. (79%) of small, fine needles, m.p. 191–193° (to a gum). Recrystallization from water raised the melting point to 192–194° (to a gum).

Anal. Calcd. for $C_{13}H_{18}O_6N_6$: C, 44.07; H, 5.11; N, 23.73. Found: C, 43.98; H, 5.12; N, 23.50.

9-β-D-Glucopyranosylguanine.—A solution of 0.35 g. of the above monoacetyl compound and 0.80 g. of sodium nitrite in 2.5 ml. of hot water was cooled rapidly to room temperature. Glacial acetic acid (0.80 ml.) was added and the mixture shaken until a clear solution was obtained. After 1 hour an equal volume of water was added; the mixture was then kept overnight at room temperature. The solution was then diluted with 20 ml. of water and treated with an excess of lead acetate solution and aqueous ammonia. The precipitate was collected, washed, and dissolved in 30 ml. of 20% acetic acid. Lead was removed by treatment with hydrogen sulfide; evaporation of the filtrate left 0.26 g. (74%) of a white powder. This was refluxed for 1 hour with a solution of 0.10 g. of sodium in 15 ml. of methanol; water was then added until a clear solution was obtained and the mixture was refluxed for 45 minutes. After neutralization with acetic acid the product was isolated through the lead salt in the usual way, to give, after crystallization from 20 ml. of water, 0.17 g. (55% from monoacetyl compound) of 9-β-D-glucopyranosylguanine, m.p. 296–297° (dec.). Recrystallization from water raised the melting point to 296–300° (dec.); $[\alpha]^{28}D$ −41.8° (*c*, 0.62% in *N* NaOH). Reported values are m.p. 298° (dec.); $[\alpha]^{15}D$ −41.6° (*c*, 7.37 in *N* NaOH)[4], and m.p. 295–305° (dec.); $[\alpha]^{16}D$ −44.6° (*c*, 0.68% in *N* NaOH).[6]

The ultraviolet absorption spectrum, 26.19 mg. per liter in 0.05 *M* phosphate buffer, *p*H 6.7, showed a single maximum at 250 mμ (ϵ_m 13,900).

Anal. Calcd. for $C_{11}H_{15}O_6N_5$: C, 42.16; H, 4.82; N, 22.35. Found: C, 41.87; H, 5.04; N, 22.67.

Chloromercuri-2,6-dibenzamidopurine and Tetraacetylglucosyl Bromide.—The chloromercuri derivative (1.60 g.) and 1.50 g. of tetraacetylglucosyl bromide in 50 ml. of xylene were refluxed for 1.5 hours, and the product isolated

in the usual way as a yellow glass; yield 1.55 g. (83%). Deacylation with methanolic sodium methoxide as described for the corresponding acetyl compound gave 0.42 g. (50% from chloromercuri compound) of 2,6-diamino-9-β-D-glucopyranosylpurine, m.p. 307–308° (dec.).

Anal. Calcd. for $C_{11}H_{16}O_5N_6$: N, 26.93. Found: N, 27.30.

Chloromercuri-2,6-diacetamidopurine and Triacetyl-D-ribofuranosyl Chloride.—The chloromercuri derivative (6.0 g.) and triacetyl-D-ribofuranosyl chloride, prepared from 4.0 g. of the tetraacetate, in 400 ml. of xylene were refluxed with stirring for 3 hours. After cooling, the crude product was filtered off and extracted with chloroform. The extract was washed and worked up in the usual way, to yield 3.57 g. (57%) of crude 2,6-diacetamido-9-triacetyl-β-D-ribofuranosylpurine as a yellow glass.

2,6-Diamino-9-β-D-ribofuranosylpurine.—A solution of 2.56 g. of the above pentaacetate in 20 ml. of warm methanol was treated with a solution of 0.40 g. of sodium in 40 ml. of methanol. After 4 hours at room temperature the mixture was refluxed for 30 minutes, neutralized with acetic acid, and evaporated to dryness. Crystallization of the residue from water, using Norit, gave 0.76 g. (52%) of 2,6-diamino-9-β-D-ribofuranosylpurine as flat, rectangular plates, m.p. 245–247°. Recrystallization from water raised the melting point to 248°; $[\alpha]^{28}D$ −40.5° (*c*, 0.7% in 0.1 *N* HCl).

The ultraviolet absorption spectrum, 27.26 mg. per liter in 0.05 *M* phosphate buffer, *p*H 6.7, showed three maxima at 215 mμ (ϵ_m 25,200), 255 mμ (ϵ_m 9,450), and 280 mμ (ϵ_m 10,000).

Anal. Calcd. for $C_{10}H_{14}O_4N_6$: C, 42.55; H, 5.00; N, 29.78. Found: C, 42.67; H, 4.91; N, 29.95.

Amination of 2-Chloro-9-β-D-ribofuranosyladenine.—A solution of the chloro compound in 15 ml. of water, prepared by partial dehalogenation of 0.80 g. of 2,8-dichloro-9-β-D-ribofuranosyladenine,[6] was saturated with ammonia at room temperature, mixed with an equal volume of saturated ethanolic ammonia, and heated in a sealed tube for 6 hours at 150°. The resulting dark solution was evaporated to dryness and an aqueous solution of the residue treated with ethanolic picric acid. The picrate was collected and treated in hot aqueous solution with Dowex No. 1 anion-exchange resin in the chloride form. The neutralized filtrate was evaporated to dryness and the residue recrystallized repeatedly from water, to give a small quantity of material, m.p. 216–226° (dec.), R_f value 0.26 on a paper chromatogram in *n*-butanol–diethylene glycol. The ultraviolet absorption spectrum in 0.05 *M* phosphate buffer, *p*H 6.7, showed maxima at 215, 255 and 280 mμ. A qualitative test showed the presence of halogen-containing material.

2-Acetamido-6-amino-9-β-D-ribofuranosylpurine.—A solution of 1.96 g. of crude 2,6-diacetamido-9-triacetyl-β-D-ribofuranosylpurine in 20 ml. of hot methanol was cooled to room temperature and treated with 40 ml. of methanolic ammonia (saturated at 0°). The mixture was kept overnight at 0°, then evaporated to dryness and the residue recrystallized from 15 ml. of water, using Norit, to give 1.0 g. (77%) of the monoacetyl compound as fine leaflets, melting indefinitely between 140° and 150°. Recrystallization from water did not sharpen the melting point.

Anal. Calcd. for $C_{12}H_{16}O_5N_6$: C, 44.45; H, 4.97; N, 25.93. Found: C, 44.60; H, 4.85; N, 26.35.

9-β-D-Ribofuranosylguanine (Guanosine).—The above monoacetyl compound (0.454 g.) was deaminated and deacetylated exactly as described for the preparation of 9-β-D-glucopyranosylguanine. Purification of the final product through the lead salt was unnecessary; the neutralized solution was evaporated to dryness and a solution of the residue in 25 ml. of water was seeded with natural guanosine, when crystallization occurred rapidly; yield 0.28 g. (71%). Recrystallization from 7 ml. of water gave 0.223 g., the compound charred without melting at 235° alone or in admixture with natural guanosine; $[\alpha]^{26}D$ −72° (*c*, 1.4% in 0.1 *N* NaOH). A sample of anhydrous natural guanosine had $[\alpha]^{23}D$ −70° (*c*, 1.15% in 0.1 *N* NaOH).

The ultraviolet absorption spectra in water, 0.1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid, respectively, were identical with those of natural guanosine under similar conditions, and were in agreement with previously reported values.[20]

Anal. Calcd. for $C_{10}H_{13}O_5N_5$: C, 42.40; H, 4.63; N, 24.73. Found: C, 42.42; H, 4.55; N, 25.00.

Chloromercuri-2,6-dibenzamidopurine and Triacetyl-D-ribofuranosyl Chloride.—The chloromercuri derivative (9.2 g.) and triacetyl-D-ribofuranosyl chloride, prepared from 5.0 g. of the tetraacetate, in 400 ml. of xylene were refluxed with stirring for 3 hours. The mixture was cooled and the separated solid collected and extracted with chloroform. The extract was washed as usual and evaporated, to give 6.85 g. of sirupy material. This was deacylated as described for the corresponding acetyl compound and the product recrystallized three times from water, to give 0.60 g. (14%) of 2,6-diamino-9-β-D-ribofuranosylpurine, m.p. 243–244°, undepressed by admixture with the compound prepared from 2,6-diacetamidopurine.

Chromatography of Glycosylpurines on Paper.—This was used to confirm the identity of the natural and synthetic nucleosides, and the identity of glycosylpurines obtained by different routes. Using an *n*-butanol–diethylene glycol–water mixture in an ammonia atmosphere,[21] the following R_f values were obtained: adenosine, 0.42; 9-β-D-glucopyranosyladenine, 0.22; 2,6-diamino-9-β-D-ribofuranosylpurine, 0.26; 2,6-diamino-9-β-D-glucopyranosylpurine, 0.11; guanosine, 0.27; 9-β-D-glucopyranosylguanine, 0.22.

Recovery of Adenosine from Its Picrate.—A solution of 0.40 g. of adenosine picrate in 80 ml. of hot water was treated with a small quantity of Dowex No. 1 anion-exchange resin in the chloride form. The mixture was filtered and the filtrate again treated with resin and filtered. One equivalent proportion of sodium hydroxide was added to the colorless filtrate, which was then evaporated to 4 ml., to give 0.176 g. (82%) of adenosine, m.p. 236°.

Acknowledgment.—The authors wish to thank Roscoe C. Funk, Jr., and John Deonarine for the microanalyses, Dr. George H. Hitchings, of the Wellcome Research Laboratories, for a generous gift of 2,6-diaminopurine hydrochloride, and Dr. George Bosworth Brown for his continued interest in this work.

NEW YORK 21, N. Y. RECEIVED OCTOBER 9, 1950

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