Synthesis of Nucleosidic Peptides Related to Amicetin[1]

C. L. STEVENS, T. S. SULKOWSKI, AND M. E. MUNK

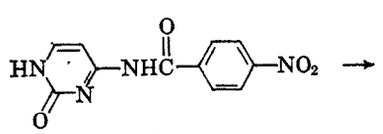
Department of Chemistry, Wayne State University, Detroit, Michigan 48202

Received May 5, 1966

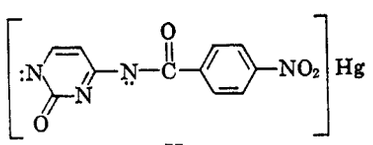
The 1-halo derivatives IIIa, b, and c of D-glucose and D-glucosamine were converted to their corresponding 4-(*p*-nitrobenzoyl)cytosine nucleoside derivatives IVa, b, and c and then reduced to the 4-(*p*-aminobenzoyl)-cytosine nucleosides Va, b, and c. The reactions of Va, b, and c with several types of peptide reagents were studied, and the carbobenzoxyglycyl nucleoside peptides VIb and c were synthesized. The nucleoside linkages were shown to be of β configuration by degradation to known compounds.

The antibiotics amicetin and bamicetin[2] have been shown to have a structure incorporating a nucleosidic peptide moiety. Specifically, in both antibiotics 2,3,6-trideoxy-β-D-*erythro*-hexopyranose[3] is linked *via* a β-glycosylamine bond to the 1 position of 4-(*p*-aminobenzoyl)cytosine. The aminobenzoyl group, in turn, is linked *via* a peptide bond to α-methylserine. The occurrence of the unique 4-(*p*-aminobenzoyl)cytosine peptide system in amicetin led to the exploration of general methods of synthesis of nucleoside peptides. This paper reports the first synthesis of nucleoside peptides of the general type VI, wherein a cytosine nucleoside is linked to a *p*-aminobenzoyl group which is in turn linked *via* a peptide bond to an α-amino acid.

Of the several procedures available for the preparation of pyrimidine nucleosides,[4-6] the mercuric salt procedure of Fox and co-workers[5] appeared to be best suited for the present work. In view of the peptide reactions projected for later in the synthesis, it was felt that the use of a mercury salt of 4-(*p*-nitrobenzoyl)cytosine (II) for the preparation of a nucleoside would allow the facile introduction of a *p*-aminobenzoyl group into the cytosine nucleoside. Thus, the 4-(*p*-nitrobenzoyl)cytosine (I) was prepared in 90% yield from equimolar amounts of cytosine and *p*-nitrobenzoyl chloride. The mercury derivative (II) was prepared by a procedure patterned after the preparation of N_4-acetylcytosinemercury,[5] and was isolated as a hydrophillic colloid which was shown by X-ray fluorescence analysis to contain a 1:1 ratio of mercury and cytosine.



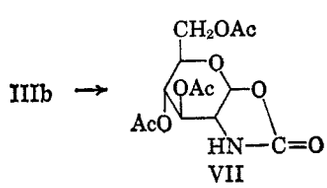
I



II

In the first attempt at nucleoside formation, 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl chloride (α-chloroacetoglucose) was refluxed with II in toluene for 18 hr. Quantitative recovery of II in addition to recovery of 40% of α-chloroacetoglucose indicated that the chloro sugar, while being stable at the reaction temperature, was evidently too unreactive to condense with the mercury salt. Condensation of the more reactive α-bromoacetoglucose (IIIa) with II afforded the desired nucleoside IVa in 58% yield. As observed by Fox[5] in the coupling of 4-acetylcytosinemercury with α-acetobromoglucose, satisfactory yields were obtained only if 2 equiv of halo sugar were used. This is probably due to the instability of the bromo sugar at high temperature. Qualitative comparison of the relative reactivities of N_4-acetylcytosinemercury and II indicate that II is less reactive. Fox reports reaction of the N-acetyl derivative with 1 equiv of IIIa requires only a few minutes in refluxing toluene as evidenced by development of a clear solution. In the case of the N-(*p*-nitrobenzoyl) derivative (II) under identical reaction conditions, development of a clear solution required 5 hr. (See Scheme I.)

In extending the mercury salt procedure to glucosamine derivatives, 1-chloro-3,4,6-tri-O-acetyl-2-deoxy-2-carbobenzoxyamino-α-D-glucopyranose (IIIb) and 1-chloro-3,4,6-tri-O-acetyl-2-deoxy-2-carbomethoxy-α-D-glucopyranose (IIIc) were condensed with II to give their respective nucleosides IVb (11% yield) and IVc (60% yield). In the preparation of nucleoside IVb, N_4-(*p*-nitrobenzoyl)cytosine (I) was recovered in an amount corresponding to 65% of the mercury derivative II. The low yield of IVb could partially be accounted for by the isolation of a side product whose physical constants permitted the assignment of the structure 4,5-(3,4,6-tri-O-acetyl-D-glucopyrano)-2-oxazolone(VII) to the compound. The formation of VII is reasonable when one considers that Konstas[7] and co-workers



VII

prepared oxazolone VII by a reaction sequence which presumably involved a chloro sugar as an intermediate.

In order to provide the amino function for peptide formation, it was necessary to selectively reduce the nitro group of nucleosides IVa, b, and c. Catalytic reduction of IVa with platinum oxide in benzene and with 10% palladium on charcoal in ethanol gave anomalous results. However, catalytic reduction of IVa and c in the presence of 10% palladium on charcoal in

(1) This paper is from the Ph.D. dissertation of T. S. Sulkowski, Wayne State University, 1961.

(2) (a) T. H. Haskell, A. Ryder, R. P. Frohardt, S. A. Fusari, S. L. Jakubowski, and Q. R. Bartz, *J. Am. Chem. Soc.*, **80**, 743 (1958); (b) C. L. Stevens, K. Nagarajan, and T. H. Haskell, *J. Org. Chem.*, **27**, 2991 (1962); (c) S. Hanessian and T. H. Haskell, *Tetrahedron Letters*, 2451 (1964), and references cited therein.

(3) C. L. Stevens, P. Blumbergs, and D. L. Wood, *J. Am. Chem. Soc.*, **86**, 3592 (1964).

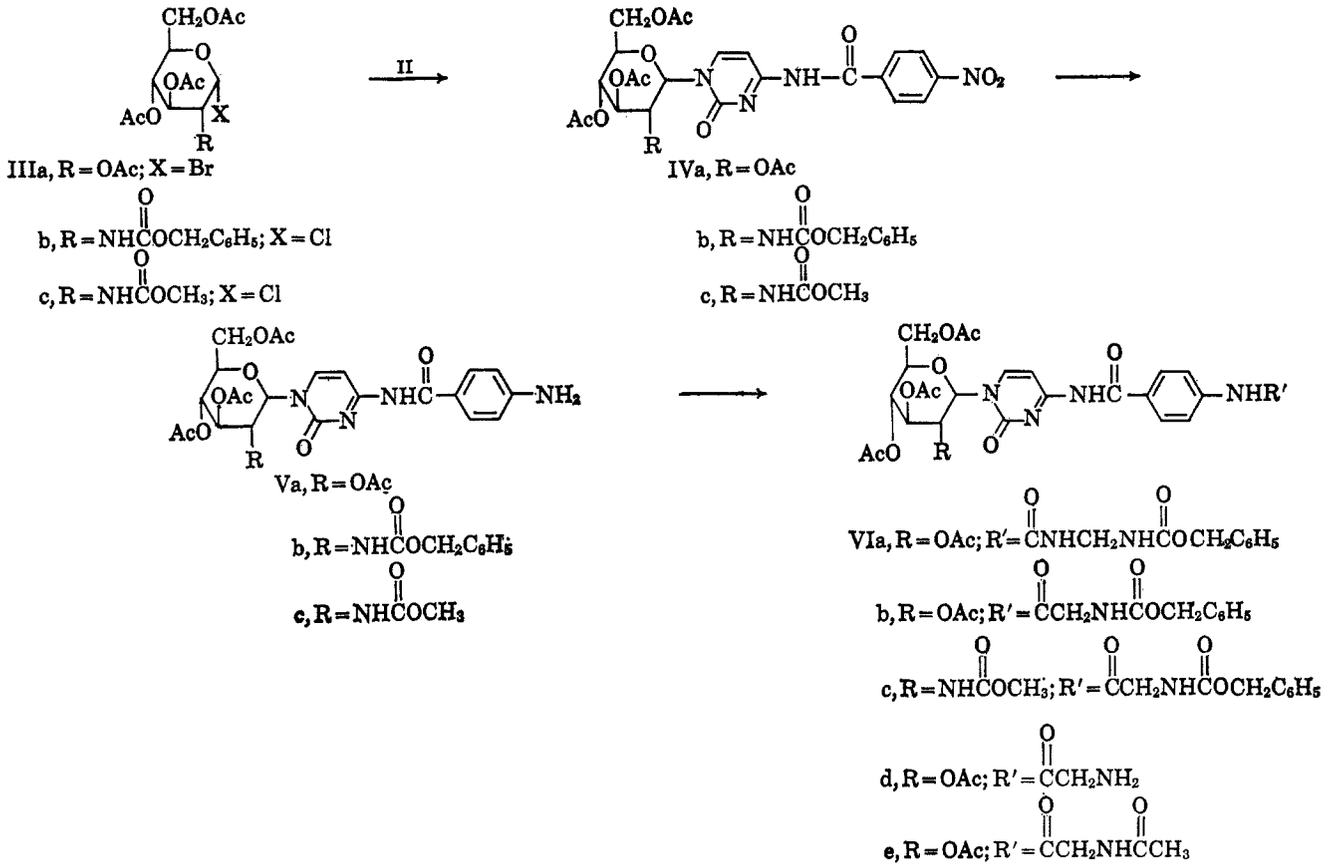
(4) G. E. Hilbert and E. F. Jansen, *ibid.*, **58**, 60 (1936).

(5) J. J. Fox, N. Jung, J. Davall, and G. B. Brown, *ibid.*, **78**, 2117 (1956).

(6) R. K. Ralph and G. Shaw, *J. Chem. Soc.*, 1877 (1956).

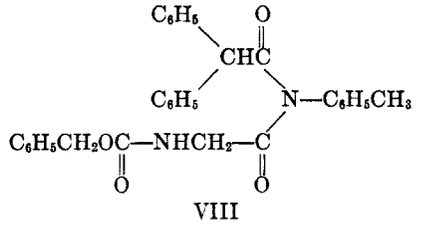
(7) S. Konstas, I. Photaki, and L. Zervas, *Ber.*, **92**, 1288 (1959).

SCHEME I



purified dioxane provided the desired nucleosides Va and c in yields of 80 and 77%, respectively. Extension of this procedure to IVb led to the removal of the carbobenzoxy group and no isolable product was obtained. Reduction with Raney nickel as catalyst in dioxane provided the desired nucleoside Vb.

For introduction of the peptide bond several methods of peptide formation were studied. It should be noted that the *p*-aminobenzoyl group is considerably less basic than the amino groups of compounds usually involved in peptide synthesis. Thus five methods of peptide synthesis were explored. Attempts to acylate nucleoside Va with imide VIII[8] in refluxing 80% benzene–20% dimethylformamide gave little or no reaction as indicated by the recovery of 80% of the starting materials. An attempt to acylate nucleoside Va with carbobenzoxyglycine azide[9] also failed. Instead the urea derivative VIa was isolated, presumably being formed by the Curtius rearrangement of the acyl azide to an isocyanate which reacted with the phenyl amino group of the nucleoside. This type of side reaction has previously been reported[10,11] to occur during use of the acyl azide method of peptide synthesis. Acylation of nucleoside Va using the activated cyanomethyl and *p*-nitrophenyl esters[12] of carbobenzyoxy glycine was also unsuccessful. Acylation of Va was accomplished, however, *via* the dicyclohexylcarbodiimide method of Sheehan[13] giving VIb in 18% yield. The mixed anhydride method[14–16] of peptide synthesis proved to be the method of choice for this condensation. Reaction of Va and Vc with the mixed anhydride of carbobenzoxyglycine and ethyl chlorocarbonate afforded the nucleoside peptides VIb and VIc in yields of 60 and 42%, respectively. The difficulty of peptide formation *via* the activated ester and carbodiimide methods was somewhat surprising as Shabarova[17] reported the coupling of amino acids with cytidine and 1-glucosyl cytosine in good yield using these methods.



VIII

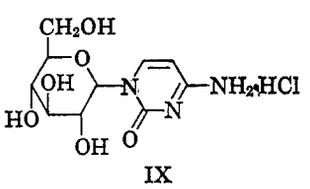
Attempted removal of the N-carbobenzoxy group from nucleoside VIa by reduction gave anomalous results. Hydrogenolysis of VIb in dioxane at atmospheric pressure using 10% palladium-on-charcoal catalyst yielded a crystalline material, mp 195–199° after

(8) C. L. Stevens, and M. E. Munk, *J. Am. Chem. Soc.*, **80**, 4069 (1958).
(9) S. Simmonds, J. I. Harris, and J. S. Fruton, *J. Biol. Chem.*, **188**, 153 (1951).
(10) J. W. Hinman, E. L. Caron, and H. Christiansen, *J. Am. Chem. Soc.*, **72**, 1620 (1950).
(11) C. K. Hooper, H. N. Rydon, J. A. Schofield, and G. S. Heaton, *J. Chem. Soc.*, 3148 (1956).
(12) R. Schwyzer, M. Feurer, and B. Iselin, *Helv. Chim. Acta*, **38**, 69, 83 (1955).
(13) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
(14) R. A. Boissonas, *Helv. Chim. Acta*, **34**, 874 (1951).
(15) J. R. Vaughn, Jr., and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
(16) T. Wieland and H. Bunhard, *Ann.*, **572**, 190 (1951).
(17) (a) Z. A. Shabarova, N. I. Sokolova, L. A. Boikova, and M. A. Prokof'ev, *Zh. Obshch. Khim.*, **29**, 2917 (1959); (b) Z. A. Shabarova, N. I. Sokolova, and M. A. Prokof'ev, *ibid.*, **27**, 2891 (1957); (c) *ibid.*, **29**, 539 (1959).

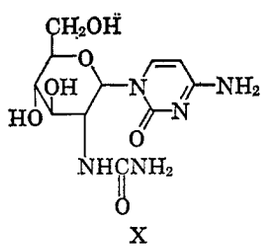
several recrystallizations. Elemental analysis of this did not fit any logical empirical formula which indicated that it was a mixture of compounds. Several further reduction attempts (including the procedure used successfully by Shabarova[17c]) failed to give pure product.

Reduction of VIa in the presence of acetic anhydride produced the N-acetylated derivative VIe in 50% yield. Nucleoside VIe was synthesized alternately by the condensation of N-acetyl glycine with nucleoside Va employing, again, the mixed anhydride method. The isolation of VIe definitely indicates that the unblocked VId was formed in the previous reductions. The inability to isolate VId in a pure form goes, as yet, unexplained.

The anomeric configuration of nucleosides IVa, b, and c was established as β by conversion to known nucleosides. Thus, ammonolysis of IVa afforded cytosine nucleoside IX[18] in 83% yield. In addition, acetylation of IX afforded the known[19] pentaacetyl derivative. Finally, ammonolysis of both nucleosides IVb and c afforded the known nucleoside X[20] in good yield.



IX



X

Experimental Section

N_4-(*p*-Nitrobenzoyl)cytosine (I).—To a stirred, refluxing suspension of 11.1 g (0.10 mole) of finely pulverized cytosine in 800 ml of dry pyridine was added 20.4 g (0.11 mole) of *p*-nitrobenzoyl chloride. A clear solution formed after refluxing for 20 min and almost immediately thereafter a white precipitate began to form. After refluxing for 2 hr the reaction mixture was cooled to room temperature and filtered. The resulting solid was slurried with cold 20% hydrochloric acid, filtered, and washed thoroughly with water, methanol, and acetone. After drying, 23.5 g (90.3%) of off-white solid was obtained: mp 323–325° dec; λ_{max}^{EtOH} 265 mμ (ϵ 18,600), 305 mμ (ϵ 8060).

Anal. Calcd for $C_{11}H_8N_4O_4$: C, 50.77; H, 3.01; N, 21.53. Found: C, 50.99; H, 3.17; N, 21.85.

N_4-(*p*-Nitrobenzoyl)cytosinemercury (II).—To a stirred solution of 750 ml of water and 750 ml of ethanol was added 7.8 g (0.03 mole) of I and 30 ml (0.03 mole) of 1 *N* sodium hydroxide. After stirring until solution was complete, the yellow solution was filtered to remove trace amounts of insoluble material. Addition of an ethanolic solution of 8.15 g (0.03 mole) of mercuric chloride yielded a precipitate. The neutral reaction mixture was warmed to 70° to coagulate the fine precipitate and then cooled to 40° and treated with an additional 30 ml of 1 *N* sodium hydroxide. After reheating to 70° the mixture was cooled to room temperature, filtered, and washed with water until the filtrate was free of chloride ion. Washing with methanol and acetone followed by drying yielded 11.2 g (81%) of a yellow solid which melted above 325°.

Anal. Calcd for $C_{11}H_6HgN_4O_4$: Hg, 43.73. Found: Hg, 45 (by X-ray fluorescence analysis).

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-4-(*p*-nitrobenzamido)-2-(1H)-pyrimidinone (IVa).—A vigorously stirred suspension of 2.76 g (0.006 mole) of II in 150 ml of toluene was dried by azeotropic distillation of 35 ml of solvent. Then a solution of 2.5 g (0.006 mole) of acetobromoglucose (III) was added to the refluxing mixture. After refluxing for 45 min, another 2.5-g portion of acetobromoglucose was added. Almost immediately a clear solution began to form and after 20 min the solution was clear with some gummy material on the sides of the flask. After refluxing for 3 hr the solvent was decanted from the gummy solid and cooled to room temperature. Addition of 600 ml of petroleum ether gave a solid which was filtered, dissolved in chloroform, washed with 30% potassium iodide and water, and dried over sodium sulfate. Removal of the chloroform *in vacuo* left a viscous syrup which crystallized on trituration with warm absolute ethanol. Recrystallization from absolute ethanol gave 2.05 g (57.9%) of long, white needles: mp 238°, pK'_a = 10 (50% methanol), $[\alpha]^{25}D$ −19.6° (*c* 1.0, $CHCl_3$), $\lambda_{max}^{0.1\ N\ HCl}$ 269 mμ (ϵ 24,980), $\lambda_{max}^{pH\ 7}$ 269 mμ (ϵ 24,390), $\lambda_{max}^{0.1\ N\ NaOH}$ 217 mμ (ϵ 17,210), λ_{max}^{EtOH} 268 mμ (ϵ 25,750).

Anal. Calcd for $C_{25}H_{26}N_4O_{13}$: C, 50.83; H, 4.44; N, 9.49. Found: C, 50.79; H, 4.53; N, 9.79.

1-(3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-carbobenzoxyamino-β-D-glucopyranosyl)-4-(*p*-nitrobenzamido)-2(1H)-pyrimidinone (IVb).—A vigorously stirred suspension of 1.84 g (4.0 mmoles) of II in 100 ml of toluene was dried by azeotropic distillation of 25 ml of solvent. A solution of 1.93 g (4.2 mmoles) of chloro sugar IIIb[20] in 8 ml of dry toluene was added to the stirred, refluxing suspension. After 1.5 hr at reflux temperature, another equal portion of chloro sugar was added. The mixture was refluxed for 20 hr and then filtered to remove unreacted solids (680 mg). The filtrate was cooled, diluted with 500 ml of petroleum ether, and filtered. The precipitate was dissolved in chloroform and washed with 30% potassium iodide solution, and water, and dried over sodium sulfate. Removal of chloroform *in vacuo* left a gummy residue. This was dissolved in 25 ml of hot ethanol and allowed to cool to room temperature overnight. The solid was filtered and recrystallized from absolute ethanol. After drying, 360 mg (11%) of small, cream-colored needles, mp 220°, was obtained: $[\alpha]^{25}D$ −37.5° (1%, chloroform); $\lambda_{max}^{0.1\ N\ NaOH}$ 325 mμ (ϵ 15,290), 275 mμ (ϵ 13,060); $\lambda_{max}^{pH\ 7}$ 270 mμ (ϵ 17,440); $\lambda_{max}^{0.1\ N\ HCl}$ 278 mμ (ϵ 19,060).

Anal. Calcd for $C_{31}H_{31}N_5O_{13}$ (mol wt, 681.60): C, 54.62; H, 4.59; N, 10.28. Found: C, 54.65; H, 4.55; N, 10.40.

The insoluble material filtered from the reaction mixture was identified as *p*-nitrobenzoylcytosine (I) by the infrared absorption spectrum. This was equivalent to 65% of the starting amount of *p*-nitrobenzoylcytosine mercury.

Concentration of the original mother liquor yielded 210 mg of crystals, mp 170–173°. After recrystallization from ethanol, 175 mg of long, white needles, mp 174–175°, was obtained, $[\alpha]^{25}D$ +49.8° (2%, chloroform). This compound apparently is identical with oxazolone VII, mp 175°, $[\alpha]^{25}D$ +50.3° (2%, chloroform), recently reported by Konstas, Photaki, and Zervas.[7]

Anal. Calcd for $C_{13}H_{17}NO_2$ (mol wt, 219.28): C, 47.11; H, 5.18; N, 4.23. Found: C, 46.89; H, 5.24; N, 4.24.

1-(3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-carbomethoxyamino-β-D-glucopyranosyl)-4-(*p*-nitrobenzamido)-2(1H)-pyrimidinone (IVc).—A vigorously stirred suspension of 8.0 g (0.0175 mole) of II in 575 ml of toluene was dried by azeotropic distillation of 140 ml of solvent. A solution of 6.87 g (0.0175 mole) of chloro sugar IIIc[20] in 20 ml of dry toluene was added to the suspension. After 20 min at reflux temperature, an equal portion of chloro sugar was added. The mixture was refluxed for 20 hr and filtered to remove the residue (700 mg). The filtrate was evaporated *in vacuo* to an oil. The oil was dissolved in chloroform and washed with 30% potassium iodide solution, with cold water, and then dried over sodium sulfate. Removal of chloroform *in vacuo* left a tan residue. About 200 ml of absolute ethanol was added and heated to reflux. The suspension was filtered while still warm and washed with alcohol. After drying, 6.0 g (56.6%) of slightly tan solid was obtained, mp 180°; a second crop (0.4 g, 3.8% mp 178–180°) was obtained on cooling the mother liquor. The analytical sample was prepared from absolute ethanol yielding long, silky, pale yellow needles: mp 180°; pK'_a = 9.9 (66% dimethylformamide); $[\alpha]^{25}D$ −15.3° (1%, chloroform); $\lambda_{max}^{0.1\ N\ NaOH}$ 322 mμ (ϵ 12,840), 271 mμ (ϵ 15,320); $\lambda_{max}^{pH\ 7}$ 269 mμ (ϵ 22,525); $\lambda_{max}^{0.1\ N\ HCl}$ 269 mμ (ϵ 22,340).

Anal. Calcd for $C_{25}H_{27}N_5O_{13}$ (mol wt, 605.51): C, 49.58; H, 4.49; N, 11.57. Found: C, 49.79; H, 4.79; N, 11.32.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-4-(*p*-aminobenzamido)-2(1H)-pyrimidinone (Va).—A solution of 2.36 g (4.0 mmoles) of IVa in 18 ml of purified, acid-free dioxane was subjected to hydrogenation at atmospheric pressure, using 1.0 g of 10%

(18) J. Davoll, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 883 (1946).

(19) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *J. Am. Chem. Soc.*, **79**, 5060 (1957).

(20) C. L. Stevens and K. Nagarajan, *J. Med. Pharm. Chem.*, **5**, 1124 (1962).

palladium-on-charcoal catalyst. The theoretical uptake of hydrogen was complete after 35 min. The catalyst was removed by filtration and washed with hot dioxane. The filtrate was evaporated to dryness *in vacuo* leaving a solid residue. About 100 ml of absolute alcohol was added to the residue and heated to reflux. The suspension was filtered while still hot and the solid was washed with hot alcohol. After drying, 1.8 g (80.4%) of cream-colored solid was obtained, mp 260–261°. The analytical sample was recrystallized from alcohol giving fine needles: mp 262–263°; $[\alpha]^{25}D$ −30.3° (1%, dioxane); $\lambda_{max}^{0.1\ N\ HCl}$ 306 mμ (ε 12,270), 260 mμ (ε 20,460); $\lambda_{max}^{pH\ 7}$ 323 mμ (ε 24,210), 250 mμ (ε 14,240); $\lambda_{max}^{0.1\ N\ NaOH}$ 330 mμ (ε 29,710).

Anal. Calcd for $C_{15}H_{23}N_4O_{11}$ (mol wt, 560.5): C, 53.57; H, 5.03; N, 10.00. Found: C, 53.57; H, 5.36; N, 9.99.

1-(3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-carbobenzoxyamino-β-D-glucopyranosyl)-4-(*p*-aminobenzamido)-2(1H)-pyrimidinone (Vb).—A solution of 150 mg (0.22 mmole) of IVc in 8 ml of purified, acid-free dioxane was subjected to hydrogenation at atmospheric pressure, using 300 mg of specially washed Raney nickel catalyst. (Commercial Raney nickel was thoroughly washed with water, with alcohol, and finally with dioxane before use.) Uptake of hydrogen stopped at 90% of theoretical after 75 min. The catalyst was removed by filtration and washed with hot dioxane. The filtrate was concentrated *in vacuo* to a yellow oil. The oil was dissolved in a minimum of hot *n*-butyl alcohol and left at room temperature overnight. The solid was filtered and recrystallized from *n*-butyl alcohol with charcoal treatment. After drying, 69.8 mg (48.2%) of yellow, crystalline solid was obtained: mp 245–247°; $[\alpha]^{25}D$ −45.2° (1%, chloroform); pK'_a = 11.0 (50%, methanol); $\lambda_{max}^{0.1\ N\ HCl}$ 309 mμ (ε 10,900), 260 mμ (ε 16,970); $\lambda_{max}^{pH\ 7}$ 324 mμ (ε 25,230), 252 mμ (ε 19,770); $\lambda_{max}^{0.1\ N\ NaOH}$ 332 mμ (ε 30,800), 223 mμ (ε 11,090).

Anal. Calcd for $C_{31}H_{33}N_5O_{11}$ (mol wt, 651.61): C, 57.14; H, 5.10; N, 10.75. Found: C, 56.87; H, 5.40; N, 10.52.

1-(3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-carbomethoxyamino-β-D-glucopyranosyl)-4-(*p*-aminobenzamido)-2(1H)-pyrimidinone (Vc).—A solution of 2.21 g (3.65 mmoles) of IVc in 15 ml of purified, acid-free dioxane was subjected to hydrogenation at atmospheric pressure, using 1.5 g of 10% palladium-on-charcoal catalyst. The theoretical uptake of hydrogen was complete after 1.5 hr. The catalyst was filtered off and washed with hot dioxane. The filtrate was evaporated *in vacuo* to an oil. The oil was dissolved in a minimum of hot absolute ethanol, treated with charcoal, and filtered, then left at room temperature overnight. On standing, 1.62 g (77%) of light tan needles precipitated: mp 215°, pK'_a = 10.8 (66% dimethylformamide); $[\alpha]^{25}D$ −24.6° (1%, chloroform); $\lambda_{max}^{0.1\ N\ HCl}$ 309 mμ (ε 11,160), 260 mμ (ε 20,370); $\lambda_{max}^{pH\ 7}$ 323 mμ (ε 26,590), 252 mμ (ε 14,560); $\lambda_{max}^{0.1\ N\ NaOH}$ 331 mμ (ε 28,490).

Anal. Calcd for $C_{25}H_{29}N_5O_{11}$ (mol wt, 575.52): C, 52.17; H, 5.08; N, 12.17. Found: C, 52.20; H, 5.35; N, 12.08.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-4-[*p*-(carbobenzoxyglycylamino)benzamido]-2(1H)-pyrimidinone (VIb). Method A. Mixed Anhydride.—A solution of 942 mg (4.5 mmoles) of carbobenzoxyglycine, 0.610 ml of triethylamine, and 6 ml of dimethylformamide was cooled to −5°. After addition of 0.468 ml of ethyl chlorocarbonate, the mixture was stirred occasionally and kept between 0 and −5° for 20 min. A cold solution of 1.68 g (3.0 mmoles) of Va in 10 ml of dimethylformamide was added and the mixture was stirred at −5° for 15 min. The reaction flask was stoppered and stored at 0° for 18 hr then left at room temperature for an additional 18 hr. The mixture was evaporated to dryness *in vacuo* and the residue was dissolved in chloroform. The chloroform solution was thoroughly washed with saturated sodium bicarbonate solution, water, cold 20% hydrochloric acid, and again with water. After drying over sodium sulfate, the chloroform was evaporated leaving a solid residue. The solid was dissolved in a minimum amount of hot 95% ethanol and left at room temperature overnight. The resulting solid was filtered and recrystallized from absolute ethanol with charcoal treatment. After drying, 1.495 g (62%) of fine, white solid was obtained: mp 246–248°, $[\alpha]^{25}D$ −24.3° (1%, chloroform), λ_{max}^{EtOH} 304 mμ (ε 34,430).

Anal. Calcd for $C_{35}H_{37}N_5O_{14}$ (mol wt, 751.69): C, 55.92; H, 4.96; N, 9.32. Found: C, 56.01; H, 5.23; N, 9.24.

Method B. Carbodiimide Method.—To a solution of 84 mg (0.15 mmole) of Va and 31.4 mg of carbobenzoxyglycine in 15 ml of dioxane was added 32.5 mg of dicyclohexylcarbodiimide. The solution was left at room temperature for 36 hr, then evaporated without heat to a volume of 7 ml. The precipitated solid was filtered and dried to give 6 mg of white needles, mp 223–224°. The infrared spectrum of this solid was identical with spectrum of an authentic sample of dicyclohexylurea.

The filtrate was evaporated to dryness and dissolved in the minimum of hot ethanol. After standing overnight, the solid was filtered off and recrystallized from absolute alcohol. After drying, 21 mg (18.6%) of fine, white solid was obtained, mp 246°. The mixture melting point with the peptide obtained by method A was not depressed. The infrared spectra of the peptide from methods A and B were identical.

Combining and evaporating the mother liquors from above gave 45 mg (54%) of starting nucleoside Va, mp 261–263°.

Method C. Azide Method.—To a solution of 134 mg of carbobenzoxyglycine hydrazide in 10 ml of 0.1 *N* hydrochloric acid cooled to −2° was added a solution of 41.4 mg of sodium nitrite in 2 ml of water. After 10 min at ice-bath temperature, the mixture was extracted with cold ethyl acetate. The ethyl acetate solution was washed with cold, 5% potassium bicarbonate solution and with cold water, then dried over magnesium sulfate in the cold. The dried solution was added to a solution of 112 mg (0.2 mmole) of Va dissolved in 3 ml of dimethylformamide at 0°. After 34 hr at 0°, the solution was evaporated to an oil. A chloroform solution of the oil was washed with saturated bicarbonate solution, cold 0.1 *N* hydrochloric acid, water, then dried over magnesium sulfate. Evaporation of the solvent left a solid residue. After recrystallization from alcohol, 59 mg of fine, white solid was obtained: mp 194–195°; λ_{max}^{EtOH} 253 mμ (ε 14,200), 309 mμ (ε 37,240).

Microanalytical data indicated that the product was the urea VIa formed by reaction of Va with the isocyanate of the carbobenzoxyglycine azide.

Anal. Calcd for $C_{35}H_{38}N_6O_{14}$: C, 54.83; H, 5.00; N, 10.96. Found: C, 54.41; H, 5.16; N, 10.77.

1-(3′,4′,6′-Tri-O-acetyl-2′-deoxy-2′-carbomethoxyamino-β-D-glucopyranosyl) - 4 - [*p* - (carbobenzoxyglycylamino)benzamido]-2(1H)-pyrimidinone (VIc).—To a solution of 419 mg (2.0 mmoles) of carbobenzoxyglycine and 0.42 ml of triethylamine in 4 ml of dimethylformamide cooled to −5° was added 0.32 ml of ethyl chlorocarbonate. The mixture was stirred occasionally and kept between 0 and −5° for 20 min. A cold solution of 575.5 mg (1.0 mmole) of Vc in 5 ml of dimethylformamide was added and the mixture was kept at −5° for 15 min with occasional stirring. The flask was stoppered and stored at 0° for 18 hr then at room temperature for 18 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in chloroform. The chloroform solution was washed successively with saturated sodium bicarbonate solution, water, cold 20% hydrochloric acid, and water, then dried over sodium sulfate. Solvent was evaporated *in vacuo*. The residue was dissolved in hot 95% ethanol, treated with charcoal, filtered, and left at room temperature overnight. On standing, 323 mg (42%) of white solid precipitated: mp 203–204°; pK'_a = 10.75 (66% dimethylformamide); $[\alpha]^{25}D$ +3.5° (1%, chloroform); $\lambda_{max}^{0.1\ N\ NaOH}$ 324 mμ (ε 19,930), 267 mμ (ε 18,630); $\lambda_{max}^{pH\ 7}$ 303 mμ (ε 28,060); $\lambda_{max}^{0.1\ N\ HCl}$ 309 mμ (ε 26,070).

Anal. Calcd for $C_{35}H_{38}N_6O_{14}$ (mol wt, 766.70): C, 54.83; H, 4.99; N, 10.96. Found: C, 54.53; H, 5.17; N, 10.66.

Attempted Removal of Carbobenzoxy Protecting Group from VIb. Method A.—A solution of 498 mg (0.662 mmole) of VIb in 6 ml of purified, acid-free dioxane was subjected to hydrogenation at atmospheric pressure, using 300 mg of 10% palladium-on-charcoal catalyst. Theoretical uptake of hydrogen was complete after 2.5 hr. The catalyst was filtered off and washed with dioxane. Evaporation of the solvent at room temperature left a gummy solid. Five recrystallizations from ethyl acetate gave a white solid, mp 195–199°. A pure compound was not obtained on further purification.

Anal. Calcd for $C_{27}H_{31}N_5O_{12}$: C, 52.51; H, 5.06; N, 11.34. Found: C, 52.11; H, 5.32; N, 10.05.

Method B.—Hydrogen gas was bubbled through a suspension of 250 mg of nucleoside VIb and 100 mg of 10% palladium on charcoal in 50 ml of purified, acid-free dioxane at 90°. After 2.5 hr, the effluent gas no longer caused turbidity when passed through barium hydroxide solution. Removal of catalyst and evaporation of solvent afforded an amorphous solid. After several recrystallizations from ethanol, a fine, white solid, mp 180–187°, was obtained. A pure compound was not obtained on further purification.

Anal. Calcd for $C_{27}H_{31}N_5O_{12}$: C, 52.51; H, 5.06; N, 11.34. Found: C, 52.52; H, 6.60; N, 9.33.

Method C. Preparation of VIe.—A solution of 225 mg (0.4 mmole) of VIb and 0.5 ml of acetic anhydride in 5 ml of purified

dioxane was subjected to hydrogenation at atmospheric pressure, using 200 mg of 10% palladium-on-charcoal catalyst. Reduction was allowed to proceed for 4 hr. The catalyst was removed by filtration and removal of solvent *in vacuo* left a solid residue. Two recrystallizations from 95% ethanol afforded 107 mg (53%) of a light yellow, crystalline solid: mp 263–265° dec, $[\alpha]^{25}D$ −6.8° (0.5%, EtOH), λ_{max}^{EtOH} 304 mμ (ε 13,060).

Anal. Calcd for $C_{29}H_{33}N_5O_{13}$: C, 52.80; H, 5.04; N, 10.62. Calcd for $C_{29}H_{33}N_5O_{13}\cdot 0.5H_2O$: C, 51.97; H, 5.11; N, 10.47. Found: C, 52.24; H, 5.36; N, 10.47.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-4-[*p*-(acetylglycylamino)benzamido]-2(1H)-pyrimidinone (VIe).—A solution of 118 mg (1.0 mmole) of acetylglycine, 0.140 ml of triethylamine, and 4 ml of dimethylformamide was cooled to −5°. After addition of 0.095 ml of ethyl chlorocarbonate, the mixture was stirred occasionally and kept between 0 and −5° for 20 min. A cold solution of 280 mg (0.5 mmole) of Va in 4 ml of dimethylformamide was added and the mixture was stirred at −5° for 15 min. The reaction flask was stoppered and stored at 0° for 18 hr, then left at room temperature for an additional 18 hr. The mixture was evaporated to dryness *in vacuo* and the residue was dissolved in chloroform. The chloroform solution was extracted with saturated sodium bicarbonate solution, cold 20% hydrochloric acid, and water. After drying over sodium sulfate, evaporation of chloroform left a solid residue. Recrystallization from 95% ethanol gave 125 mg (38% yield) of slightly yellow, crystalline solid: mp 265° dec, $[\alpha]^{25}D$ −6.2° (0.5%, EtOH), λ_{max}^{EtOH} 304 mμ (ε 12,000).

Anal. Calcd for $C_{29}H_{33}N_5O_{13}$: C, 52.80; H, 5.04; N, 10.62. Calcd for $C_{29}H_{33}N_5O_{13}\cdot 0.5H_2O$: C, 51.97; H, 5.11; N, 10.47. Found: C, 52.24; H, 5.20; N, 10.43; loss on drying, 1.35.

1-β-D-Glucopyranosylcytosine Hydrochloride (IX). From Ammonolysis of IVa.—A suspension of 820 mg (1.39 mmoles) of IVa in 30 ml of absolute ethanol was saturated with ammonia at 0°. The mixture was heated in a glass-lined bomb for 36 hr at 95–100°. The cloudy solution was concentrated to dryness *in vacuo*. The white residue was dissolved in 30 ml of hot 95% ethanol and treated with charcoal. The hot solution was acidified to pH 2 with concentrated hydrochloric acid and cooled. The precipitate was filtered and thoroughly washed with warm absolute ethanol. Recrystallization from 95% ethanol gave 360 mg (83%) of white, crystalline solid: mp 205° dec, $[\alpha]^{25}D$ +23.3° (2%, water), λ_{max}^{EtOH} 281 mμ (ε 10,930). For comparison, an authentic sample was prepared independently following known procedures.[21,22]

Acetylation of IX.—A mixture of 125 mg (0.4 mmole) of IX, 3 ml of dry pyridine, and 3 ml of acetic anhydride was warmed until complete solution occurred. After standing at room temperature for 20 hr, the solution was quenched into 20 ml of ice–water. The solid was collected by filtration and recrystallized from alcohol with charcoal treatment. After drying, 145 mg (72%) of fine needles was obtained, mp 218.5–219.5° (lit.[19] mp 218.5°).

1-(2′-Deoxy-2′-ureido)-β-D-glucopyranosylcytosine (X). A. From Ammonolysis of IVc.—A suspension of 325 mg (0.54 mmole) of IVc in 30 ml of absolute ethanol was saturated with ammonia at 0°. The mixture was heated in a glass-lined bomb for 48 hr at 115°. The mixture was evaporated to dryness *in vacuo*, dissolved in hot 90% ethanol, and treated with charcoal. A white precipitate formed on standing. After drying, 91.8 mg (54%) of white, crystalline solid was obtained: mp 279–280° dec, $[\alpha]^{25}D$ +71.2° (0.9%, water), $\lambda_{max}^{0.1\ N\ HCl}$ 277 mμ (ε 11,670).

B. From Ammonolysis of IVb.—Ammonolysis of 350 mg (0.51 mmole) of IVb was carried out as described for IVc. After recrystallization from 90% ethanol, 94.5 mg (59%) of white, crystalline solid was obtained: mp 279–280° dec, $[\alpha]^{25}D$ +70.9° (0.9%, water). The physical constants of X agreed with those previously reported.[20]

Acknowledgment.—The authors wish to acknowledge the numerous helpful suggestions of Dr. K. Nagarajan and the financial support of the National Institutes of Health (Grant CA 03772).

(21) G. E. Hilbert and E. F. Jansen, *J. Am. Chem. Soc.*, **58**, 60 (1936).
(22) J. J. Fox and I. Goodman, *ibid.*, **73**, 3256 (1951).

Relationships between Some Uronic Acids and Their Decarboxylation Products[1]

M. S. FEATHER AND J. F. HARRIS

Forest Products Laboratory,[2] Forest Service, U. S. Department of Agriculture, Madison, Wisconsin
Received June 6, 1966

2-Furaldehyde, 5-formyl-2-furoic acid, and reductic acid (2,3-dihydroxy-2-cyclopenten-1-one), all products from the decarboxylation of hexuronic acids, were found to be essentially end products in the reaction. Structural relationships between the 2-furaldehyde and reductic acid and the parent uronic acids were obtained by preparation of D-galacturonic-2-C^{14}, -1-C^{14}, and D-glucuronic acid-1-C^{14} from which pertinent C^{14}-labeled decomposition products were isolated. The 2-furaldehyde arising from the 1-C^{14}-labeled uronic acids contained over 99% of the activity in the aldehyde group. D-Galacturonic acid-2-C^{14} was converted to reductic acid-C^{14} which was shown to be a mixture of the reductic acids-1-C^{14} and -2-C^{14} in the approximate ratio 9:1. Conversely, the reductic acid-C^{14} from D-galacturonic acid-1-C^{14} was found to be a mixture of reductic acids-1-C^{14} and -2-C^{14} in the ratios 1:9. Thus, reductic acid is formed from hexuronic acids *via* two different mechanisms. In addition to isotopic-labeling experiments, a series of carbohydrate derivatives was examined for their potential as a source of reductic acid.

The decarboxylation of uronic acids and glycuronans in hot aqueous acid to give near-stoichiometric quantities of carbon dioxide is a well-known reaction of considerable practical importance, forming the basis for various analytical procedures which determine uronic acids in the presence of other carbohydrate materials. Although it has been the subject of extensive studies by various investigators,[3–5] the reaction sequence is still a matter of some controversy, and in recent years several mechanisms have been suggested.[4–7] The basis of these proposals rests largely on kinetic studies which have compared decarboxylation rates of uronic acids with other carbohydrate and organic acids. Recently, however, Anderson and Garbutt[8] have verified that the carbon dioxide evolved in this reaction arises from C-6 of the uronic acid; they used D-glucuronic acid-6-C^{14} as a representative model.

Although the above proposals are consistent with the observed reaction kinetics and products, they remain largely untested and unevaluated. This paper pre-

(1) Presented at the 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 11–16, 1966.
(2) Maintained at Madison, Wis., in cooperation with the University of Wisconsin.
(3) E. W. Taylor, W. F. Fowler, Jr., P. A. McGee, and W. O. Kenyon, *J. Am. Chem. Soc.*, **69**, 342 (1947).
(4) D. M. W. Anderson and S. Garbutt, *J. Chem. Soc.*, 3204 (1963), and earlier papers in that series.
(5) E. Stutz and H. Deuel, *Helv. Chim. Acta*, **41**, 1722 (1958), and earlier papers.
(6) H. S. Isbell, *J. Res. Natl. Bur. Std.*, **33**, 45 (1944).
(7) V. P. Kiseleva, A. A. Konkin, and Z. A. Rogovin, *Zh. Prikl. Khim.*, **27**, 1133 (1954).
(8) D. M. W. Anderson and S. Garbutt, *Talanta*, **8**, 605 (1961).