50 [H form] resin column.[24] The column was washed with 1:1 methanol–water mixture until the washes showed minimal absorption in the 274 mμ region. The column was then eluted with a 2 *N* ammonium hydroxide in 1:1 methanol–water solution, and the product was obtained by evaporation of the ammoniacal eluates under reduced pressure to yield 360 mg. (41%) of a white glass. Trituration of this glass with 10 cc. of ethyl acetate produced a white, amorphous solid weighing 320 mg., $[\alpha]^{24}D$ −5° (0.58% in MeOH).

(24) B. R. Baker, R. E. Schaub and H. M. Kissman, THIS JOURNAL, **77**, 5911 (1955).

Anal. Calcd. for $C_{13}H_{20}N_6O_4 \cdot {}^1/_2H_2O$: C, 46.83; H, 6.35; N, 25.21; H_2O, 2.70. Found: C, 46.90; H, 6.64; N, 25.15; H_2O, 2.86.

Crystallization and recrystallization from methanol (activated charcoal) gave material melting at 260–261°, $[\alpha]^{25}D$ −16.8° (1.01% in H_2O).

Anal. Calcd. for $C_{13}H_{20}N_6O_4$: C, 48.14; H, 6.22; N, 25.91. Found: C, 48.19; H, 6.40; N, 25.78.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.]

Studies on the Synthesis of 9-(2-Amino-2-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine, an Analog of the Aminonucleoside Derived from Puromycin

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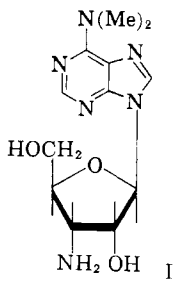
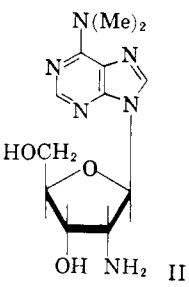
The synthesis of the subject nucleoside (II) was undertaken by a 14-step synthesis from methyl 3-amino-3-deoxy-4,6-*O*-benzylidene-α-D-altropyranoside (III) which is available in quantity from methyl α-D-glucopyranoside. Despite intensive efforts, II could not be obtained in crystalline form. However, the immediate precursor of II, compound XVIII, was crystalline and of unequivocal structure.

Analogs of the aminonucleoside I derived from the antibiotic puromycin[1] are of interest because of the antitumor[2] and trypanocidal[3] activities of I. Among the analogs of I, wherein the aminosugar moiety has been varied, and which have been prepared in our laboratory, are the 9-β,3-aminoarabinofuranosyl,[4] 3-aminoxylofuranosyl,[5] 5-aminoribofuranosyl,[6] 2-aminoallopyranosyl[7] and 3-aminoallopyranosyl[8] derivatives of 6-dimethylaminopurine. This paper describes our efforts to prepare 9-(2-amino-2-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine (II). Although these efforts were not attended with complete success, since the final product could only be obtained as a non-crystalline substance of somewhat doubtful purity, it seems worthwhile to record our results.



I



II

(1) For the chemistry of puromycin see B. R. Baker and co-workers, THIS JOURNAL, **77**, 12 (1955), and preceding papers.

(2) P. L. Bennett, S. L. Halliday, J. J. Oleson and J. H. Williams, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, N. Y., 1954, pp. 766–769.

(3) R. I. Hewitt, A. R. Gumble, W. S. Wallace and J. H. Williams, *Antibiotics and Chemotherapy*, **4**, 1222 (1954).

(4) B. R. Baker and R. E. Schaub, THIS JOURNAL, **77**, 5900 (1955).

(5) R. E. Schaub, M. J. Weiss and B. R. Baker, *ibid.*, **80**, 4692 (1958).

(6) H. M. Kissman and B. R. Baker, Abstracts of Papers 130th Meeting of the A.C.S., Atlantic City, N. J., September, 1956, p. 19D; paper in preparation.

(7) F. J. McEvoy, M. J. Weiss and B. R. Baker, THIS JOURNAL, **82**, 205 (1960).

(8) R. E. Schaub and M. J. Weiss, to be reported.

In principle, the synthesis of II requires the preparation of a properly blocked 2-aminoribose derivative[9] in the furanoid configuration, its conversion to a 1-halo sugar and condensation of this halogenose with a suitable chloromercuripurine derivative. The previously reported[10] methyl 3-amino-3-deoxy-4,6-*O*-benzylidene-α-D-altropyranoside (III) was an attractive starting material since cleavage between C-1 and C-2 would directly afford the required 2-aminoribose structure. In addition, relatively substantial quantities of III can be prepared in four steps from the cheap and abundant methyl α-D-glucopyranoside, 2 kg. of which furnished 800 g. of III.

A convenient procedure for the degradation of a hexose to a pentose is the method of MacDonald and Fischer which proceeds *via* the hexose disulfone.[11] Our initial approach to this problem was an attempt to utilize this procedure. When the 3-aminoaltroside III was shaken with ethyl mercaptan and concentrated hydrochloric acid, there was obtained the diethyl mercaptal (IV) of 3-aminoaltrose · HCl. The crude product was acetylated with acetic anhydride in pyridine and the resultant pentacetate (V) was de-*O*-acetylated with methanolic sodium methoxide to give the crystalline diethyl mercaptal (VI) of 3-acetamidoaltrose in 35% over-all yield from III. Selective N-acylation of IV to give VI was not a satisfactory procedure.

In order to ensure the formation of the desired

(9) At the time of this investigation the only known 2-amino-2-deoxypentose was the D-xylose derivative synthesized by Wolfrom and Anno [THIS JOURNAL, **75**, 1038 (1953)] from glucosamine by cleavage of C-6 and reduction of the resulting 5-aldehyde. Since then Wolfrom, Shafizadeh and Armstrong [*ibid.*, **80**, 4885 (1958)] have reported the synthesis of a 2-amino-2-deoxypentose which is probably the L-ribose derivative in view of the work of Lemieux and Chu [*ibid.*, **80**, 4745 (1958)].

(10) (a) W. H. Myers and G. J. Robertson, *ibid.*, **65**, 8 (1943); (b) B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **19**, 646 (1954).

(11) D. L. MacDonald and H. O. L. Fischer, THIS JOURNAL, **74**, 2087 (1952); *Biochim. et Biophys. Acta*, **12**, 203 (1953).

furanoid structure, it was necessary to block the primary hydroxyl group of the altrose to prevent pyranose formation on production of the aldehyde by degradation of the disulfone. Thus, the primary hydroxyl group of VI was tritylated in pyridine with triphenylchloromethane to give a 35% yield of crystalline VII. The trityl group was selected since it was thought that it would later be possible to remove it preferentially in the presence of acetyl-blocking groups, such as had been done in the conversion of 5-trityl-D-ribofuranose triacetate to D-ribofuranose tetraacetate with acetyl bromide.[12] Unfortunately, the MacDonald–Fischer disulfone procedure could not be made to work in our case since all efforts to effect the oxidation of the tritylated diethyl mercaptal VII to the corresponding sulfone were unsuccessful. From the outset, the unexpected extreme acid sensitivity of the trityl group of VII thwarted attempts at oxidation with hydrogen peroxide in acetic acid. In several experiments with this reagent, the only isolatable products were triphenylmethyl compounds containing no nitrogen. To overcome this acid instability of VII, the oxidations with hydrogen peroxide were attempted in alcohol–water mixtures in the presence of barium carbonate. That no oxidation took place under these conditions was shown by no decrease in hydrogen peroxide titer. A hydrogen peroxide oxidation of VI at 52° in water was also unsuccessful. When the oxidation of VII with perbenzoic acid was attempted, the oxidizing agent was consumed, but attempts to carry the reaction mixture through the disulfone cleavage procedure to X gave products which still contained considerable amounts of sulfur or gave no Benedict test for a reducing sugar. When VII was fully acetylated, an attempt at oxidation with neutral permanganate was likewise unsuccessful.

Fortunately, it was possible to develop an alternative synthesis of X. Treatment of the diethyl mercaptal VII with mercuric chloride in the presence of mercuric oxide according to the method of Chang and Lythgoe[13] caused desulfurization to take place and the altropyranose VIII[14] was obtained. The desired one carbon degradation of the altrose to the ribose was accomplished by an oxidative cleavage of the 1,2-glycol with lead tetraacetate, and afforded the crystalline 4-*O*-formyl derivative IX in 32% yield for the two steps. An attempt to effect the cleavage reaction with periodate was unsuccessful. Removal of the formyl group with sodium hydroxide permitted ring closure to the desired furanose X. This product was obtained in 89% yield as a crude gum containing 103% reducing sugar. The remaining free hydroxyl groups of X were then acetylated to give non-crystalline XI.

Detritylation of XI was attempted, as originally planned, by the method of Brederick and Hoepfner, whereby treatment with acetyl bromide results in the replacement of a trityl group with an acetyl group.[12] This procedure afforded a 30% yield of a gummy mixture of 2-acetamido-tri-*O*-acetates from which a crystalline product (A) could be isolated in 12% yield (from X). Attempts to effect detritylation by hydrogenolysis were also unsatisfactory.[15] However, treatment of XI with 80% acetic acid on the steam-bath for 20 minutes followed by acetic anhydride in pyridine afforded an 82% yield of gummy tri-*O*-acetate from which again crystalline A could be isolated (14% from X). Tri-*O*-acetate A was later indicated to have a pyranoside configuration (XV) and the synthesis of the desired nucleoside was carried out with the residual material (B) remaining after the isolation of A. Presumably this material represented a mixture of furanoid and pyranoid ring forms (XIV + XV).

The preparation of the required 1-chloro sugar was attempted by treatment of tri-*O*-acetate mixture B with ethereal hydrogen chloride at −5°.[16] After the usual three- to four-day reaction period there was obtained an *ethylene dichloride-insoluble*, chlorine-containing (73% of theory) gum, which on condensation with chloromercuri-6-chloropurine[17] afforded in 39% yield an amorphous material which by ultraviolet estimation[18] contained approximately 58% 9-substituted purine. When the hydrogen chloride treatment was extended to twelve days, a chlorine-containing (77% of theory) gum was again obtained, but this time the gum was *ethylene dichloride-soluble* and condensation with chloromercuri-6-chloropurine was much more satisfactory affording a 65% yield of a product which according to ultraviolet estimation contained approximately 71% 9-substituted purine.

It is possible to explain these results by assuming the immediate formation of the ethereal hydrogen chloride-soluble hydrochloride salt of the 2-acetamido-tri-*O*-acetate XIV[19] followed by a slow replacement of the 1-acetoxy group by chloride to give XVI. It would be anticipated that replacement of the acetoxy group would be a slow step, since this is an acid-catalyzed reaction requiring protonation of this group (or of the ring oxygen). The ability to achieve such a protonation would be hindered by the presence of the already positively charged acetamido grouping.[21] On the basis of

(12) H. Bredereck and E. Hoepfner, *Ber.*, **81**, 51 (1948).

(13) P. Chang and B. Lythgoe, *J. Chem. Soc.*, 1992 (1950).

(14) The pyranose configuration (VIII) is assumed without evidence. It is possible that this product was, or contained quantities of, the corresponding altrofuranose, lead tetraacetate treatment of which would have given the 3-*O*-formyl derivative of X rather than IX. However, de-*O*-formylation of either formyl derivative would have given the same product, namely, X.

(15) P. E. Verkade, W. D. Cohen and A. K. Vroege, *Rec. trav. chim.*, **59**, 1123 (1940).

(16) J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 967 (1948).

(17) (a) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953); (b) L. Goldman, J. W. Marsico and R. B. Angier, THIS JOURNAL, **78**, 4173 (1956).

(18) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1780 (1954).

(19) The formation of the ether-insoluble hydrochloride salt of 3-acetamido-3-deoxy-1-*O*-acetyl-2,5-di-*O*-benzoyl-D-ribofuranose has been reported.[20]

(20) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 12 (1955).

(21) The deactivating effect of a protonated amino or acetamido group on acid-catalyzed reactions at C-1 also has been observed for the hydrolysis of the methyl glucosaminides[22-24] and the methyl 3-aminoxylopyranosides,[25] and for the acetolysis of methyl 3-acetamido-3-deoxy-2,4,6-tri-*O*-acetyl-α-D-allopyranoside[8] and methyl 3-acetamido-3-deoxy-2,4-di-*O*-acetyl-β-L-xylopyranoside.[10b]

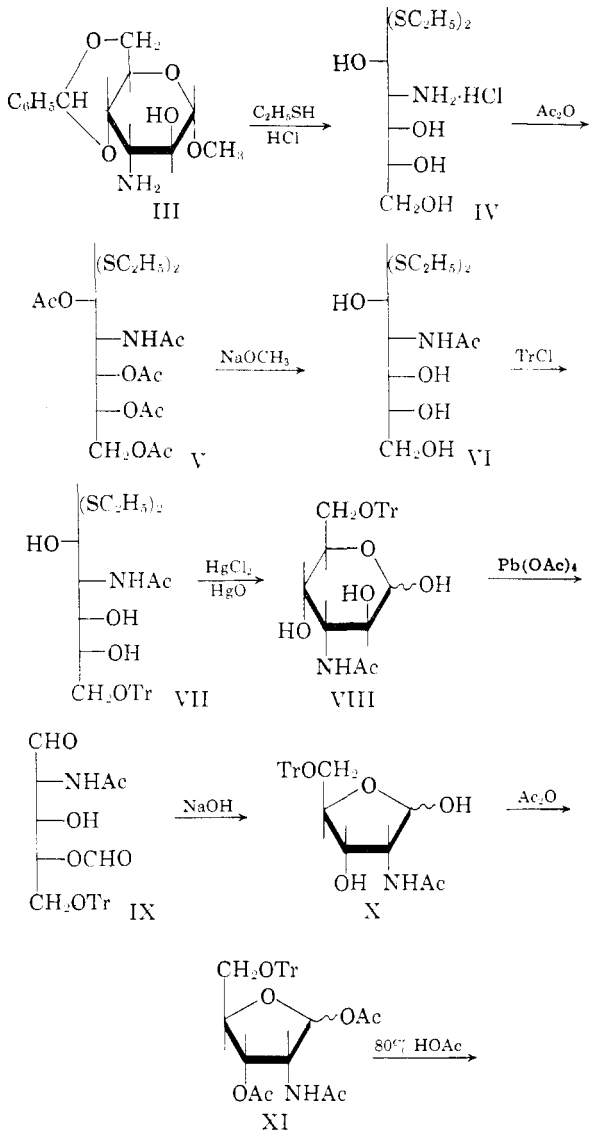
(22) R. C. G. Moggridge and A. Neuberger, *J. Chem. Soc.*, 745 (1938).

(23) A. Neuberger and R. Pitt-Rivers, *ibid.*, 122 (1939).

the chlorine analysis and the ethylene dichloride solubility, it is assumed that the resulting 1-chloro sugar XVI does not form a hydrochloride salt. The apparent inability of the 1-chloro sugar to form a salt is conceivably a result of the greater deactivating influence of chlorine, as compared to acetoxy, on the basicity of the acetamido nitrogen.

The product obtained after condensation of chloromercuri-6-chloropurine with the twelve-day halogenose preparation was presumed to be the blocked 6-chloronucleoside XVII in crude form. Treatment of XVII with dimethylamine resulted in the introduction of the 6-dimethylamino group and the concomitant removal of the *O*-acetates to give the N-acetylnucleoside XVIII,[17] which could be obtained in crystalline form only after partition chromatography. The over-all yield of XVIII from tri-*O*-acetate mixture B was 10% and for the seven steps from IX, the last crystalline intermediate, it was 5%. N-Acetylnucleoside XVIII did not consume any periodate when treated with this oxidant indicating a furanosyl structure for the sugar moiety. Its high negative optical rotation ($[\alpha]^{25}D$ −88°) pointed strongly to a β-configuration. This was further substantiated by the isolation from the partition chromatographic effluent of a second nucleoside fraction which apparently was largely the α-anomer. The amorphous material isolated from this latter fraction contained according to ultraviolet estimation, 93% 9-substituted purine, it did not consume periodate and had $[\alpha]^{25}D$ +22.6°. When this material was again subjected to partition chromatography four fractions were obtained with $[\alpha]^{25}D$ ranging from +9.9 to +26.1°.[26]

Aqueous barium hydroxide hydrolysis of the crystalline N-acetylnucleoside XVIII gave in 76% yield an amorphous product, which after separation from any non-hydrolyzed material *via* absorption on an Amberlite IRC-50 ion exchange column,[27] was subjected to two partition chromatographic purifications. However, the resulting product could not be crystallized and a completely satisfactory analysis was not obtained. Nevertheless, by ultraviolet estimation it was about 96% 9-substituted purine, and it is considered to be essentially the desired 2-aminonucleoside II. Periodate treatment showed overoxidation typical of 3-aminoribofuranosides and presumably to be expected for 2-aminoribofuranosides.[28] The relatively high negative optical rotation value ($[\alpha]^{25}D$ −62°) for the 2-aminonucleoside is in agreement with the presumed β-configuration.



When the crystalline 2-acetamido-tri-*O*-acetate A was treated with ethereal hydrogen chloride at −5° for ten days, there was obtained a crude chloro sugar which on condensation with chloromercuri-6-chloropurine[17] gave a crude nucleoside in 67% yield. This product on reaction with dimethylamine afforded an N-acetylnucleoside in 82% yield (78% 9-substituted purine by ultraviolet estimation). Crystallization of this material was not possible even after purification by partition chromatography. The resulting glass (after chromatography) consumed 0.7 molar equivalent of periodate and had $[\alpha]^{25}D$ +53° indicating it to be largely an α-nucleoside with the sugar in the pyranoid form (XIX). The formation of a pyranosyl nucleoside strongly implied a pyranosyl configuration (XV) for the crystalline tri-*O*-acetate A.

On testing against a transplanted mammary adenocarcinoma of the C_3H mouse, the 2-aminonucleoside II was inactive at a dose of 1.5 mg. per mouse.[29]

(24) A. B. Foster, D. Horton and M. Stacey, *J. Chem. Soc.*, 81 (1957).

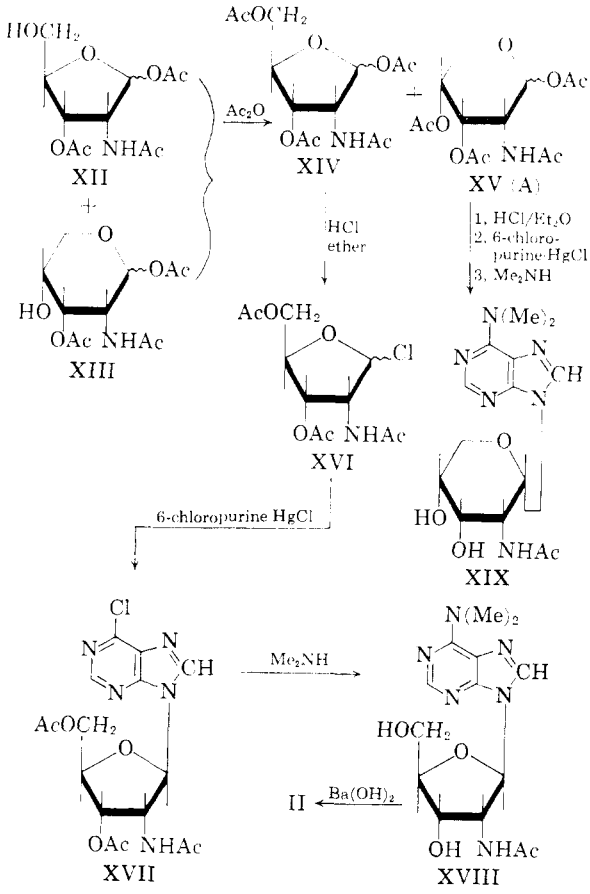
(25) R. E. Schaub and M. J. Weiss, THIS JOURNAL, **80**, 4683 (1958).

(26) The formation of C_1–C_2 *trans* products (in this instance the β-anomer) from the condensation of 2-acetamido-1-chloro sugars with purine mercury salts has been considered to result from an α-chloro sugar by direct displacement or from a β-chloro sugar by a double Walden inversion *via* an intermediate oxazoline [B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954)]. The apparent formation of α-anomer conceivably may be represented as resulting from a direct displacement by purine of a β-chlorine or from an SN1 carbonium ion type mechanism, not intermediated by an oxazoline, which would allow the formation of either α- or β-anomers.

(27) B. R. Baker, R. E. Schaub and H. M. Kissman, THIS JOURNAL, **77**, 5911 (1955).

(28) M. J. Weiss, J. P. Joseph, H. M. Kissman, A. M. Small, R. E. Schaub and F. J. McEvoy, *ibid.*, **81**, 4050 (1959).

(29) Private communication from Miss S. L. Halliday and Dr. J. J. Oleson of these Laboratories.



Acknowledgment.—We are indebted to Mr. Charles Pidacks and staff for the partition chromatography work reported in this paper, to Mr. W. Fulmor and staff for the rotations and spectra, and to Mr. L. Brancone and staff for the microanalyses.

Experimental[30]

3-Amino-3-deoxy-D-altrose Diethylmercaptal·HCl (IV).—To a chilled solution of 1.0 g. of methyl 3-amino-3-deoxy-4,6-*O*-benzylidene-α-D-altropyranoside (III)[10] in 2 cc. of concentrated hydrochloric acid was added 2 cc. of ethyl mercaptan. The mixture was stoppered and shaken at room temperature for 22 hours. The lower acid layer was separated and washed twice with 2-cc. portions of benzene, then evaporated to dryness under reduced pressure at a bath temperature not exceeding 40°. The residue was twice evaporated with 10-cc. portions of ethanol leaving 1.12 g. (98%) of a gum which could not be crystallized.

Anal. Calcd. for $C_{10}H_{23}NO_4S_2 \cdot HCl$: C, 37.3; H, 7.47; N, 4.35. Found: C, 37.5; H, 7.51; N, 4.79.

2,4,5,6-Tetra-*O*-acetyl-3-acetamido-3-deoxy-D-altrose Diethylmercaptal (V).—To a cooled solution of 6.5 g. of 3-amino-3-deoxy-D-altrose diethylmercaptal hydrochloride (IV) in a mixture of 60 cc. of acetic anhydride and 60 cc. of pyridine was added 2.9 g. of sodium acetate. After standing at room temperature for 19 hours, the solution was poured into 300 cc. of ice-water. The aqueous solution was extracted with two 100-cc. portions of chloroform. The combined extracts were dried over magnesium sulfate and evaporated to dryness under reduced pressure. The residue was twice evaporated with 65-cc. portions of toluene leaving an amber gum weighing 7.5 g. (85%) which was not further purified.

3-Acetamido-3-deoxy-D-altrose Diethylmercaptal (VI).—To a solution of 7.5 g. of crude 2,4,5,6-tetra-*O*-acetyl-3-acetamido-3-deoxy-D-altrose diethylmercaptal (V) in 85 cc. of methanol was added 4.0 cc. of 1 *N* sodium methoxide. The solution was refluxed for 30 minutes, then evaporated to dryness under reduced pressure leaving an amber gum weighing 4.9 g. The gum was dissolved in 49 cc. of a hot solution of 10:1.1 ethyl acetate–absolute alcohol and the solution chilled in an ice-bath for 1 hour. The product was collected and recrystallized from 175 cc. of the same solvent mixture to yield 2.1 g. (35% based on III) of white crystals, m.p. 145–146°. A similar preparation [m.p. 146–147°, $[\alpha]^{24}D$ +31.4° (1% in methanol)] was analyzed.

Anal. Calcd. for $C_{12}H_{25}NO_5S_2$: C, 44.0; H, 7.65; N, 4.28; S, 19.6 Found: C, 44.4; H, 7.89; N, 4.91; S, 19.8.

3-Acetamido-3-deoxy-6-*O*-trityl-D-altrose Diethylmercaptal (VII).—To a solution of 2.0 g. of 3-acetamido-3-deoxy-D-altrose diethylmercaptal (VI) in 4.0 cc. of pyridine was added 1.86 g. of triphenylchloromethane. After standing at 37° for 24 hours in a closed flask the solution was poured into 80 cc. of water. The aqueous solution was extracted with two 40-cc. portions of chloroform and the combined extracts, dried with magnesium sulfate, were evaporated to dryness under reduced pressure at a bath temperature of 45–50°. The residue was evaporated to dryness under reduced pressure with 40 cc. of toluene and the residual gum crystallized from methanol–water yielding 1.2 g. (35%) of white crystals, m.p. 91–93°.

A similar preparation, m.p. 91–92°, was analyzed; $[\alpha]^{24}D$ −9.7° (0.5% in chloroform).

Anal. Calcd. for $C_{31}H_{39}NO_5S_2$: C, 65.4; H, 6.85; N, 2.46. Found: C, 65.1; H, 7.25; N, 2.57.

2,4,5-Tri-*O*-acetyl-3-acetamido-3-deoxy-6-*O*-trityl-D-altrose Diethylmercaptal.—A solution of 130 mg. of 3-acetamido-3-deoxy-6-*O*-trityl-D-altrose diethylmercaptal (VII) in 2.6 cc. of 1:1 pyridine–acetic anhydride was heated on the steam-bath for 2 hours and then poured into 10 cc. of ice-water. The aqueous solution was extracted with three 5-cc. portions of chloroform. The combined extracts were dried over magnesium sulfate and evaporated to dryness under reduced pressure. The evaporation was repeated with 5 cc. of toluene leaving an amber glass weighing 150 mg. (94%) which was analyzed; $[\alpha]^{24}D$ +30.9 (0.8% in chloroform).

Anal. Calcd. for $C_{36}H_{45}NO_8S_2$: C, 63.2; H, 6.59; N, 2.05. Found: C, 63.3; H, 6.68; N, 2.28.

3-Acetamido-3-deoxy-6-*O*-trityl-D-altropyranose (VIII).[14]—To a solution of 200 mg. of 3-acetamido-3-deoxy-6-*O*-trityl-D-altrose diethylmercaptal (VII) in 5.0 cc. of 10% aqueous acetone was added 335 mg. of mercuric oxide. To this suspension was added a solution of 380 mg. of mercuric chloride in 1.4 cc. of acetone.[13] The mixture was stirred for 21 hours at room temperature, then filtered through Celite.[31] The filtrate was diluted with 10 cc. of water and extracted with two 10-cc. portions of chloroform. The combined chloroform extracts were washed with 10 cc. of 40% potassium iodide solution, dried over magnesium sulfate, and evaporated to dryness under reduced pressure. The residual white glass gave a positive Benedict test and was analyzed.

Anal. Calcd. for $C_{27}H_{29}NO_6$: C, 70.0; H, 6.26; N, 3.02; S, 0.0. Found: C, 70.7; H, 6.67; N, 2.56; S, trace.

2-Acetamido-2-deoxy-4-*O*-formyl-5-*O*-trityl-D-ribose (IX).[14]—To a stirred solution made from 510 mg. of 3-acetamido-3-deoxy-6-*O*-trityl-D-altropyranose (VIII) in 12 cc. of methylene chloride was added 220 mg. of lead tetraacetate. The solution was stirred for 1 hour and an additional 220 mg. of lead tetraacetate was added. After stirring for 30 minutes, 0.5 cc. of ethylene glycol was added and the solution was then washed with two 10-cc. portions of water. The solution was next washed with 10 cc. of 5% sodium thiosulfate solution, dried over magnesium sulfate, and evaporated to dryness under reduced pressure. The residual gum was crystallized from toluene yielding 135 mg. (32%) of white crystals, m.p. 149–150°. Recrystallization from toluene raised the melting point to 152–153°, $[\alpha]^{24}D$ +23.8° (1.1% in chloroform).

Anal. Calcd. for $C_{27}H_{27}NO_6$: C, 70.3; H, 5.85; N, 3.04. Found: C, 70.1; H, 6.20; N, 2.94.

In subsequent experiments it was found that reproducible results could best be obtained if the reaction was carried out in methylene chloride containing 5% pyridine.

(30) Melting points are uncorrected.

(31) Celite is the trade-mark for Johns-Manville's diatomaceous silica products.

2-Acetamido-2-deoxy-5-*O*-trityl-D-ribofuranose (X).—A solution of 300 mg. of 2-acetamido-2-deoxy-4-*O*-formyl-5-*O*-trityl-D-ribose (IX) in 6.0 cc. of methanol and 1.05 cc. of 10% sodium hydroxide solution was allowed to stand at room temperature for 30 minutes. After dilution with 25 cc. of water, the solution was extracted with two 25-cc. portions of chloroform. The combined extracts, dried with magnesium sulfate, were evaporated to dryness under reduced pressure leaving a white glass weighing 250 mg. (89%) which could not be crystallized. This gum showed 103% reducing sugar by Hanes modification of the Hagedorn–Jensen titration technique.[32]

Anal. Calcd. for $C_{26}H_{27}NO_5$: C, 72.0; H, 6.23; N, 3.23. Found: C, 72.3; H, 6.52; N, 2.71.

2-Acetamido-2-deoxy-1,3-di-*O*-acetyl-5-*O*-trityl-D-ribofuranose (XI).—A solution of 220 mg. of 2-acetamido-2-deoxy-5-*O*-trityl-D-ribofuranose (X) in 5 cc. of 1:1 pyridine–acetic anhydride mixture was heated on the steam-bath for 2 hours, then poured into 20 cc. of ice-water. The aqueous solution was extracted with two 15-cc. portions of chloroform and the combined extracts dried over magnesium sulfate. The extracts were evaporated to dryness under reduced pressure, the residual oil dissolved in 5 cc. of toluene, and the evaporation repeated leaving an amber gum weighing 240 mg. (92%) which was analyzed.

Anal. Calcd. for $C_{30}H_{31}NO_7$: C, 69.6; H, 6.00; N, 2.71. Found: C, 70.4; H, 6.39; N, 2.61.

2-Acetamido-2-deoxy-1,3,5-tri-*O*-acetyl-D-ribofuranose (XIV) and 2-Acetamido-2-deoxy-1,3,4-tri-*O*-acetyl-D-ribopyranose (A, XV); Mixture B. (A) Acetyl Bromide Detritylation.[12]—To a solution of 2.2 g. of 2-acetamido-2-deoxy-1,3-di-*O*-acetyl-5-*O*-trityl-D-ribofuranose (XI) in 5.0 cc. of acetic anhydride was added 0.63 cc. of acetyl bromide. The solution was allowed to stand at room temperature for 30 minutes then poured into 100 cc. of ice-water. The 1.02 g. (92%) of precipitated tritylcarbinol was discarded. The filtrate was extracted with two 50-cc. portions of chloroform. The combined extracts were washed with excess saturated sodium bicarbonate solution, dried over magnesium sulfate, and evaporated to dryness under reduced pressure leaving a clear gum weighing 410 mg. (30%). Crystallization from ethyl acetate–heptane gave 166 mg. (12%) of white crystals (A), m.p. 156–158°, whose melting point was raised by recrystallization from ethyl acetate to 159–160°, $[\alpha]^{24}D$ −46.7° (2% in chloroform). This product is probably the pyranoside XV.

Anal. Calcd. for $C_{13}H_{19}NO_8$: C, 49.2; H, 5.99; N, 4.42. Found: C, 49.2; H, 6.21; N, 4.30.

The crystallization mother liquor, on evaporation to dryness, left a clear gum weighing 192 mg. (14%), $[\alpha]^{24}D$ + 36.9° (1.6% in chloroform).

Anal. Calcd. for $C_{13}H_{19}NO_8$: C, 49.2; H, 5.99; N, 4.42. Found: C, 49.5; H, 6.43; N, 4.35.

(B) Acetic Acid Detritylation Followed by Acetylation to Give Crystalline A and Mixture B.—A solution of 1.8 g. of 2-acetamido-2-deoxy-1,3-di-*O*-acetyl-5-*O*-trityl-D-ribofuranose (XI) in 18 cc. of 80% acetic acid was heated on a steam-bath for 20 minutes. The solution was cooled and 38 cc. of acetic anhydride was added. After stirring for 5 minutes, 5.5 cc. of pyridine was added and the solution was heated on the steam-bath for 2 hours. The solution was stirred with 190 cc. of ice-water for 15 minutes, then decanted from gummy triphenylmethylcarbinol. The aqueous solution was saturated with sodium chloride and extracted with two 135-cc. portions of chloroform. The combined extracts were washed with excess sodium bicarbonate solution, dried over magnesium sulfate and evaporated to dryness under reduced pressure. The residual oil was dissolved in 20 cc. of toluene and the evaporation repeated leaving an amber gum weighing 900 mg. (82%). Crystallization from ethyl acetate–heptane gave 160 mg. (14%) of white crystals (A, XV), m.p. 156–157°. Evaporation of the filtrate gave 680 mg. (62%) of a gum (B). Gum B was presumed to contain substantial amounts of the desired furanosyl tri-*O*-acetate XIV and was used for the synthesis of nucleoside II (below).

9-(2-Acetamido-2-deoxy-3,5-di-*O*-acetyl-β-D-ribofuranosyl)-6-chloropurine (XVII).—To a solution of 400 mg. of the above described gum B in 2.0 cc. of acetyl chloride was added 40 cc. of saturated ethereal hydrogen chloride. The solution was kept at −5° for 12 days, then evaporated to dryness under reduced pressure at a bath temperature not exceeding 40°. The evaporation was repeated several times with 20-cc. portions of benzene leaving an amber gum. The gum was dissolved in 40 cc. of ethylene dichloride and added to an azeotropically dried suspension of 1.08 g. of chloromercuri-6-chloropurine[17] in 40 cc. of ethylene dichloride. The mixture was stirred and refluxed for 18 hours and then filtered while hot. The filtrate was evaporated to dryness under reduced pressure. The 0.9 g. of residual gum was dissolved in a mixture of 35 cc. of chloroform and 25 cc. of 30% potassium iodide solution. The organic layer was dried with magnesium sulfate and evaporated to dryness under reduced pressure leaving a gum weighing 340 mg. (65%) whose ultraviolet absorption spectra indicated 71% of a 9-substituted purine having the molecular weight of XVII.[18] This gum was undoubtedly contaminated with the corresponding α-nucleoside (see Discussion).

9-(2-Acetamido-2-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine (XVIII).—To a solution of 2.6 g. of crude 9-(2-acetamido-2-deoxy-3,5-di-*O*-acetyl-β-D-ribofuranosyl)-6-chloropurine (XVII) in 30 cc. of absolute methanol was added 6.0 cc. of anhydrous dimethylamine.[17] The solution was heated in an autoclave at 100° for 2 hours, then evaporated to dryness under reduced pressure. The residual gum was dissolved in 130 cc. of 50% aqueous methanol and stirred with 20 g. of Amberlite IRA-400[33] ion exchange resin for 30 minutes. The resin was separated by filtration and the solvent removed under reduced pressure. The residue was evaporated to dryness with ethanol several times under reduced pressure and the resultant gum was subjected to partition chromatography on Celite with ethyl acetate saturated with water.[34] The main fraction (hold-back volumes 4–8) on evaporation to dryness *in vacuo* yielded 444 mg. of gum. Trituration with 45 cc. of cold toluene afforded 330 mg. of white crystals, m.p. 173–175°. Recrystallization from ethyl acetate raised the melting point to 178–179°, $[\alpha]^{25}D$ −88.1° (0.7% in methanol), λ_{max}^{MeOH} 274 mμ (ε 18,800). This substance did not consume periodate.

Anal. Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.0; H, 5.95; N, 25.0. Found: C, 50.3; H, 6.18; N, 24.8.

The second major fraction (hold-back volumes 9–16) produced a gum weighing 213 mg., $[\alpha]^{25}D$ +22.6° (1% in ethanol). It did not consume periodate. Similar material having $[\alpha]^{25}D$ +22.6° (1% in ethanol), obtained from other experiments, was rechromatographed and a symmetrical curve was obtained. After discarding a small forerun, the effluent was divided into four fractions, all of which gave amorphous solids on evaporation to dryness under reduced pressure. The following physical constants were recorded. These fractions apparently represent anomeric mixtures.

Fraction	2% in ethanol $[\alpha]^{25}D$	λ_{max}^{MeOH}	ε
I	+ 9.9°	274	15,700
II	+15.2	274	16,150
III	+26.1	274	17,750
IV	+25.5	274	17,470

9-(2-Amino-2-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine (II).—A solution of 800 mg. of 9-(2-acetamido-2-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine (XVIII), having $[\alpha]^{24}D$ −80.2° (1% in ethanol), in 80 cc. of saturated barium hydroxide was heated on the steam-bath for 20 hours. The solution was cooled, saturated with carbon dioxide and filtered. The precipitate was washed several times with ethanol. The filtrate and washings were evaporated to dryness *in vacuo* leaving a gum weighing 539 mg. (76%). The gum was dissolved in 50 cc. of 50% aqueous methanol and placed on a Amberlite IRC-50[33] ion exchange column.[27] The column was washed with 50% aqueous methanol until the eluate was free of ultraviolet absorbing material as measured at 274 mμ. The column was then eluted with 2 *N* ammonium hydroxide in 50% aqueous methanol. Upon evaporation under reduced pressure, the ammonia eluates yielded 484 mg. (69%) of a brown glass. The crude glass was partition chromatographed[34] on Celite with an ethyl

(32) C. S. Hanes, *Biochem. J.*, **23**, 99 (1929).

(33) Amberlite IRA-400 and Amberlite IRC-50 are the trade-marks of the Rohm and Haas Co.

(34) H. M. Kissman, C. Pidacks and B. R. Baker, THIS JOURNAL, **77**, 18 (1955). This paper contains detailed procedures for the partition chromatography separations.

acetate–petroleum ether (90–100°)–methanol–water (4:1:1:2) system, and the main fraction (hold-back volumes 6-7) yielded a glass weighing 320 mg. This material was rechromatographed on Celite with an ethyl acetate–petroleum ether (90–100°)–methanol–water (8:1:2:4) system, and the product collected from hold-back volumes 3–4 by evaporation to dryness under reduced pressure was a glass, $[\alpha]^{25}D$ −62° (0.8% in methanol), λ_{max}^{MeOH} 274 mμ with an ε value of 18,100, indicating[18] *ca.* 96% 9-substituted purine having a molecular weight corresponding to II.

Anal. Calcd. for $C_{12}H_{18}N_6O_3$: C, 49.0; H, 6.12; N, 28.6. Found: C, 50.0; H, 7.16; N, 27.9.

9-(2-Acetamido-2-deoxy-3,5-di-*O*-acetyl-D-ribopyranosyl)-6-chloropurine.—To a suspension of 1.8 g. of crystalline 2-acetamido-tri-*O*-acetate A in 9 cc. of acetyl chloride was added 165 cc. of saturated ethereal hydrogen chloride. The solution was kept at −5° for 10 days, then evaporated to dryness under reduced pressure at a bath temperature not exceeding 40°. The evaporation was repeated twice with 50-cc. portions of benzene. The residue was dissolved in 140 cc. of ethylene dichloride and added to an azeotropically dried suspension of 4.6 g. of chloromercuri-6-chloropurine[17] in 120 cc. of ethylene dichloride. The mixture was stirred and refluxed for 20 hours, then filtered hot and the solids washed with hot chloroform. The filtrate and washings were combined and evaporated to dryness under reduced pressure. The residual gum was dissolved in a mixture of 150 cc. of chloroform and 100 cc. of 30% potassium iodide solution. The organic layer was separated, washed with 50 cc. of 30% potassium iodide solution, and dried over magnesium sulfate. Evaporation of the chloroform solution to dryness under reduced pressure left a brown gum weighing 1.57 g. (67%), ultraviolet absorption spectra of which indicated[18] it to be approximately 80% 9-substituted purine.

9-(2-Acetamido-2-deoxy-D-ribopyranosyl)-6-dimethylaminopurine (XIX).—To a solution of 1.57 g. of crude 9-(2-acetamido-2-deoxy-3,5-di-*O*-acetyl-D-ribopyranosyl)-6-chloropurine in 18 cc. of methanol was added 3.6 cc. of anhydrous dimethylamine in 5 cc. of methanol.[17] The solution was heated in an autoclave at 100° for 2 hours, then evaporated to dryness under reduced pressure. The residue was dissolved in 60 cc. of 50% aqueous methanol and stirred with 12 g. of Amberlite IRA-400 ion exchange resin for 30 minutes. The resin was removed by filtration and the solvent removed under reduced pressure. The residue was evaporated to dryness with ethanol several times under reduced pressure leaving an amber glass weighing 1.05 g. (82%). By ultraviolet absorption spectra, the glass was about 78% 9-substituted purine.[18] The crude material was partition chromatograped on Celite with an ethyl acetate saturated with water system.[34] The main peak at hold-back volumes 8–10, on evaporation to dryness under reduced pressure, yielded 210 mg. of glass. Re-chromatography of this material with the same system or with an ethyl acetate–petroleum ether (90-100°)–methanol–water (8:1:4:2) system afforded no further purification; $[\alpha]^{25}D$ +53.0° (0.85% in ethanol), λ_{max}^{MeOH} 274 mμ (ε 17,700). This product consumed 0.7 molar equivalent of periodate. It is probably mainly the α-pyranoside XIX.

Anal. Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.0; H, 5.95 N, 25.0. Found: C, 48.9; H, 6.55; N, 23.0

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMICAL PATHOLOGY, ST. MARY'S HOSPITAL MEDICAL SCHOOL]

The Synthesis and Properties of γ-Aminoacetoacetic Acid

BY J. B. NEILANDS,[1] A. NEUBERGER AND J. J. SCOTT

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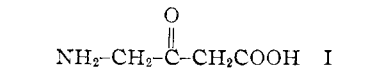
A method is described for the preparation of γ-aminoacetoacetic acid from acetonedicarboxylic acid *via* γ-oximinoacetoacetic acid.

The amino acid, γ-aminoacetoacetic acid (glycylacetic acid; 3-keto-4-aminobutyric acid (I)) is a substance which could arise biologically in a number of ways. Possible biosynthetic pathways leading to the formation of I would include the condensation of the Coenzyme A derivatives of glycine and acetic acid, the condensation of malonyl Coenzyme A and glycine (with concomitant loss of CO_2 from the glycine carboxyl), the reductive amination of oxaloacetic semialdehyde or finally, through a fatty acid-like oxidation of γ-aminobutyric acid.



I

Either I or its trimethylammonium analog might serve as an immediate precursor of *l*-carnitine, a physiologically important substance of unknown biogenesis.[2] Very recently, chromatographic evidence has been obtained that the reduced form of I, γ-amino-β-hydroxybutyric acid, first synthesized in 1923 by Tomita,[3] may occur in mammalian brain tissue where it is believed to act under certain circumstances as a natural regulator of the motor activity.[4,5] Direct decarboxylation of I, as contrasted to the oxidation of threonine,[6] would provide a second mechanism for the formation of aminoacetone. In addition, as the next lower homolog of δ-aminolevulinic acid, the effect of I on porphyrin metabolism would be worthy of investigation.

Since I is a β-keto acid as well as an α-keto amine, it was of interest to compare the stability and reactions of a compound of this type with those of a number of closely related substances previously studied in this Laboratory.[7] Also, by further reduction and N-trimethylation, I could easily be converted successively into optically inactive γ-amino-β-hydroxybutyric acid and carnitine.

Although the preparation of I might be attempted through a variety of alternative routes, the controlled nitrosation of free acetonedicarboxylic acid followed by reduction of the resultant γ-oximinoacetoacetic acid (II) appealed to the authors as a relatively direct and simple procedure.

(1) Fellow of the John Simon Guggenheim Foundation; on sabbatical leave from the University of California, Berkeley, 1958–1959.

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(3) M. Tomita, *Z. physiol. Chem.*, **124**, 253 (1923).

(4) T. Hayashi, *J. Physiol.*, **145**, 570 (1959).

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(6) W. H. Elliott, *Nature*, **183**, 1051 (1959).

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