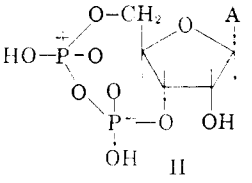
agent)[6]. This suggestion in a slightly modified form also could account for the observed formation of both A-2′-P and A-3′-P.

A-3′-P is formed by the action of barium hydroxide on A-3′:5′-P.[8] It was thought that perhaps A-2′-P and A-3′-P are formed by phosphorylation of adenosine by means of ATP, with the subsequent breakdown of the resultant dinucleoside phosphate to adenosine and a mixture of A-2′-P and A-3′-P. Some evidence against this mechanism for the formation of these two nucleotides was obtained by showing that inosine-2′(3′)-phosphoric acid is not formed when inosine, ATP and aqueous barium hydroxide are heated together. The partial hydrolysis of Ia and Ib by heating with aqueous barium hydroxide also does not serve as an explanation for the origin of A-2′-P and A-3′-P. The diphosphoryl compounds are stable toward hydrolysis under these conditions.

Although the two isomeric diphosphoryladenosines may be formed by the mechanism previously suggested,[6] a more likely pathway for the formation of these two isomers is reaction N-I-βd. Reaction N-I-βe leads to the formation of Ib, but not the 2′,5′-isomer. A study of a Stuart–Briegleb model of P¹,P²-adenosine-3′:5′-pyrophosphoric acid (II) shows that the oxygen atom of the 2′-hydroxyl group cannot come within bonding distance of the phosphorus atoms. The cyclic pyrophosphate cannot be converted by the action of alkali,



II

therefore, into 5′-*O*-phosphoryladenosine-2′:3′-phosphoric acid, which on further hydrolysis would give a mixture of Ia and Ib.

Doubly charged cations are known to form rather stable complexes with ATP.[33] It is assumed that the catalytic effect of barium ion on the rate of hydrolysis of ATP in alkaline solution, like the catalytic effect of other multiply-charged cations,[34] may be explained by the existence of complexes in solution. One function of the barium ion in the complex is to neutralize the negative charges on the oxygen atoms of the triphosphate chain.[35] This increases the positive electrical potential on the phosphorus atoms,[36] which should lead to a more rapid reaction of water molecules, hydroxide ions or sugar hydroxyls with the ATP–barium ion complex. This effect of barium ion complexing on the rate of hydrolysis of ATP is much the same in character as the effect of esterification or the addition of hydrogen ions on the rate of hydrolysis of polyphosphates. The rate of hydrolysis of neutral species such as $R_4P_2O_7$ or $R_5P_3O_{10}$, where R is an alkyl group or hydrogen, is far greater than the rate of hydrolysis of the corresponding negatively charged species (*i.e.*, $H_2P_2O_7^{-2}$, $P_3O_{10}^{-5}$).[37]

Barium ion functions not only as a catalyst for the hydrolysis of ATP, but it also may help determine the character of the hydrolysis products.[36] The product that is obtained by decomposition of a given ATP molecule should depend on the position of the barium ion on the triphosphate chain[34] at the moment that a nucleophilic species (*i.e.*, water molecule, hydroxide ion or alcoholic hydroxyl) combines with the ATP–barium ion complex to give the activated complex of the transition state.

No evidence is available at present to indicate whether or not the complexing of barium ion with the 2′- and 3′-hydroxyl groups of the ribose[38] is of importance in determining the rate and products of the alkaline degradation of ATP.

**Acknowledgment.**—This investigation was supported in part by research grant C-3870 from the National Cancer Institute, Public Health Service. Partial support also was provided by the United States Atomic Energy Commission.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

# 9-β-Lactosyladenine and 2,6-Diamino-9-β-lactosylpurine[1]

BY M. L. WOLFROM, P. MCWAIN, F. SHAFIZADEH AND A. THOMPSON

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Nucleosides containing a disaccharide, 9-β-lactosyladenine and 2,6-diamino-9-β-lactosylpurine, have been prepared and characterized.

The potential carcinolytic activity of the nucleosides has created an intense interest in the methods of preparation and study of these substances. Since Fischer and Helferich[2] first synthesized a nucleoside by condensing a silver salt of the base with a poly-*O*-acetylglycosyl halide, perhaps the

(1) Supported by Grant No. CY-3232 from the Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Bethesda 14, Md.

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

most significant advance has been the substitution of the chloromercuri[3] for the silver salts of the purine base. A further improvement came with the substitution of the more hydrolytically resistant benzoyl group[4] for acetyl in the glycosyl halide. The importance of using pure chloromercuripurines for the condensation has been noted[5] and methods for preparing them described,[6] and attributed to J. J. Fox.

We wish to report herein the preparation of 9-β-lactosyladenine (VI) and 2,6-diamino-9-β-lactosylpurine (VIII) which we believe are the first nucleosides containing a disaccharide which have been synthesized. We have used essentially the method of Davoll and Lowy,[3,6] condensing hepta-*O*-acetyl-α-lactosyl bromide (I) with the chloromercuri salts of 6-acetamido- (II) and 2,6-diacetamido-purine (III). We also used a small amount of cadmium carbonate in the reaction mixture to protect the product from the hydrolyzing effect of any free acid which may have been formed. The resulting nucleoside acetates (IV and V) were deacetylated with methanolic ammonia or methanolic sodium methoxide. 2,6-Diacetamido-9-β-lactosylpurine was deacetylated stepwise leaving the presumably 2-acetamido group intact (VII). Davoll and Lowy[3] have shown the analogous monoacetamido-β-D-ribofuranosyl derivative to be 2-acetamido-9-β-D-ribofuranosyladenine. The deacetylated products were purified through their crystalline picrate salts and were then crystallized from water. A temperature of 80° aided the crystallization of 9-β-lactosyladenine. The products were characterized by melting points, optical rotations, elementary analysis, X-ray powder diffraction patterns, and infrared and ultraviolet absorption spectra.

## Experimental

**9-β-Lactosyladenine (VI).**—A mixture of 6 g. of chloromercuri-6-acetamidopurine (II),[3,6] 1 g. of Celite[7] and 6 g. of cadmium carbonate were suspended in 400 ml. of xylene and azeotropically dried by distilling a portion (130 ml.) of the solvent. The solution was cooled to 90° and 13 g. of hepta-*O*-acetyl-α-lactosyl bromide (I)[8,9] was added with stirring. The mixture was refluxed with continued stirring for 5.5 hr. A yellow solid collected on the walls of the vessel and was filtered. The filtrate was concentrated under reduced pressure to dryness. The filter cake and the residue were extracted with hot chloroform. The chloroform extracts were combined and washed with potassium iodide solution, water, dried with sodium sulfate and concentrated under reduced pressure to a light yellow sirup; yield 11.5 g. This crude 6-acetamido-9-(hepta-*O*-acetyl-β-lactosyl)-purine (IV, 8 g.) was dissolved in 40 ml. of dry methanol. Anhydrous ammonia was passed into the solution for 1 hr. at 0°. The cold solution was allowed to stand in the refrigerator for 18 hr. and was then evaporated under a stream of air and the sirupy residue dried under reduced pressure. The residue was dissolved in 100 ml. of water, washed with chloroform and evaporated to dryness under reduced pressure. The solid material was triturated twice with hot methanol and once with ethanol. The residue was dissolved in 20 ml. of boiling water, filtered with decolorizing carbon

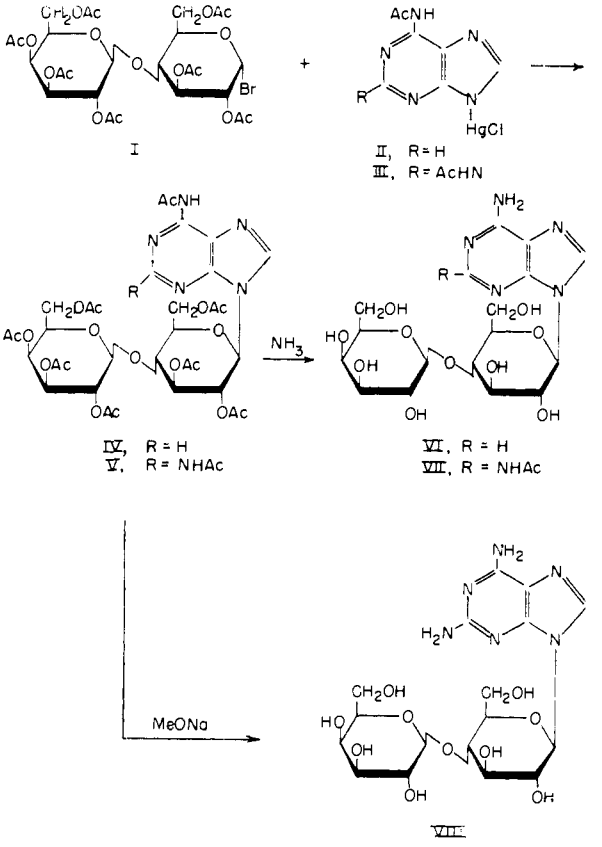


Fig. 1.

and concentrated to dryness to produce crude 9-β-lactosyladenine (VI); yield 1.84 g. (30% based on starting 6-acetamidopurine). An alternative procedure consisted of purification through the picric acid salt as described below for 2,6-diamino-9-β-lactosylpurine. Crystallization was effected by slow evaporation of a saturated aqueous solution in an oven at 85–90°; yield 1.19 g., m.p. 297–301°. Pure material was obtained by recrystallization from water in the same manner, m.p. 309–311°, $[\alpha]^{22}D$ +0.7° (*c* 1, water); absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 260 mμ; $\nu_{max}^{KBr}$ 3500, 3360 cm.$^{-1}$ (OH, NH), 1640 cm.$^{-1}$ ($H_2N$—C=N), 1610, 1570, 1470 cm.$^{-1}$ (NH and purine ring), 1070, 1035, 1010 cm.$^{-1}$ (C-O-C and C-OH); X-ray powder diffraction data[11]: 6.11w, 5.87s(3), 5.68m, 4.96w, 4.72vs(1), 4.42w, 4.02m, 3.74s(2), 3.44vw, 3.32vw, 3.23vw, 3.12w, 3.05vw.

*Anal.* Calcd. for $C_{17}H_{25}N_5O_{10}$: C, 44.44; H, 5.48; N, 15.25. Found: C, 44.72; H, 5.30; N, 15.37.

**2-Acetamido-9-β-lactosyladenine (VII).**—An amount of 6.0 g. of chloromercuri-2,6-diacetamidopurine (III) was treated with hepta-*O*-acetyl-α-lactosyl bromide (I) as described above for the synthesis of VI and the reaction product was isolated in the same manner; yield 19 g. of crude 2,6-diacetamido-9-(hepta-*O*-acetyl-lactosyl)-purine (V) which could be further purified from hot 95% ethanol; m.p. 214–216°; absorption spectra data[10]: $\lambda_{max}^{EtOH}$ 236, 264, 288 mμ; $\nu_{max}^{KBr}$ 3400, 3110 cm.$^{-1}$ (NH), 1740 cm.$^{-1}$ (ester C=O), 1630, 1600 cm.$^{-1}$ (purine ring), 1360 cm.$^{-1}$ (methyl hydrogen), 1220 cm.$^{-1}$ (C-O-C of acetate), 1040, 950, 910 cm.$^{-1}$ (C-O-C of sugar).

(3) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 1650 (1951).

(4) H. M. Kissman, C. Pidacks and B. R. Baker, *ibid.*, **77**, 18 (1955).

(5) J. J. Fox, N. Yung, Iris Wempen and Iris L. Doerr, *ibid.*, **79**, 5060 (1957).

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(7) A product of the Johns-Manville Co., New York, N. Y.

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(9) C. S. Hudson and A. Kunz, THIS JOURNAL, **47**, 2052 (1925).

(10) The ultraviolet absorption analyses were made on a Carey Recording Spectrophotometer, Model 10, Applied Physics Corp., Pasadena, Calif. The infrared spectral data were obtained by an Infrared Recording Spectrophotometer, Model B, Baird Associates, Inc., Cambridge, Mass. Structural assignments were made following W. B. Neely, *Advances in Carbohydrate Chem.*, **12**, 13 (1957) and B. R. Baker (see ref. 6 and other publications of B. R. Baker and co-workers).

(11) Interplanar spacing, Å., CuKα radiation. Relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. Parenthetical numerals indicate order of three most intense lines; 1, most intense.

Anhydrous ammonia was passed for 1 hr. at 0° into 25 ml. of methanol containing 3.38 g. of V. The solution was kept in a refrigerator for 21 hr. and was then evaporated under reduced pressure to a sirup. The sirup was dissolved in 60 ml. of water and washed with chloroform. The water extract was concentrated to dryness. The solid material was twice triturated with methanol. The methanol solution was decolorized and allowed to stand overnight at room temperature. Two crops of crystalline 2-acetamido-9-lactosyladenine (VII) were obtained; combined yield 400 mg., m.p. 263–267°; absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 224, 270 mμ; $\nu_{max}^{KBr}$ 3400, 3320 cm.$^{-1}$ (OH, NH), 1650 cm.$^{-1}$ (amide carbonyl or C=C), 1620 cm.$^{-1}$ ($NH_2$ and $H_2N$—C=N), 1120, 1075, 1030, 990 cm.$^{-1}$ (C-O-C and C-O-H).

*Anal.* Calcd. for $C_{19}H_{28}N_6O_{11}$: C, 44.17; H, 5.48; N, 16.27. Found: C, 44.26; H, 5.50; N, 16.29.

**2,6-Diamino-9-β-lactosylpurine (VIII).**—Complete deacetylation of 2,6-diacetamido-9-(hepta-*O*-acetyl-β-lactosyl)-purine (V) was effected by boiling with an excess of sodium methoxide in methanol.[12] A small pellet of freshly cut sodium was added to a solution of 12 g. of V in 40 ml. of dry methanol containing 60 ml. of 0.1 *N* sodium methoxide. The mixture was refluxed for 2 hr., cooled and neutralized with acetic acid. The solution was evaporated to dryness under reduced pressure, the residue was dissolved in 100 ml. of water and washed with chloroform. After concentration to 20 ml., 60 ml. of a 10% methanolic solution of picric acid was added. The crystalline picrate complex formed immediately; yield 8.0 g., dec. 270–275°.

The yellow crystalline picrate (8.0 g.), was suspended in 200 ml. of warm water and stirred with Dowex-1 (carbonate form) anion exchange resin[13] until the solution became colorless. The solution was filtered and concentrated to dryness to produce crude crystalline 2,6-diamino-9-β-lactosylpurine (VIII); yield 2.09 g. (45%). The pure material was obtained upon recrystallization from water; m.p. 283–285°, $[\alpha]^{22}D$ −5.5° (*c* 0.5, water); absorption spectra data[10]: $\lambda_{max}^{H_2O}$ 256, 280 mμ; $\nu_{max}^{KBr}$ 3440, 3380, 2900 cm.$^{-1}$ (OH, NH), 1640, 1615 cm.$^{-1}$ ($NH_2$ and purine ring), 1088, 1065, 1040, 1005 cm.$^{-1}$ (C-O-C and C-OH); X-ray powder diffraction data[11]: 11.6m, 7.34vw, 6.03m, 5.75vs(1), 5.27m(2), 5.03w, 4.77vw, 4.51w, 4.23vs(1), 3.96vw, 3.78m(3), 3.61vs-(1).

*Anal.* Calcd. for $C_{17}H_{26}N_6O_{10}$: C, 43.03; H, 5.53; N, 17.72. Found: C, 42.91; H, 5.48; N, 17.73.

(12) B. R. Baker and Kathleen Hewson, *J. Org. Chem.*, **22**, 959 (1957).

(13) A product of the Dow Chemical Co., Midland, Mich.

COLUMBUS 10, OHIO

# COMMUNICATIONS TO THE EDITOR

## THE CONFIGURATION OF B-NORSTEROID DERIVATIVES

*Sir:*

In 1956,[1] this laboratory reported results in the B-norsteroid series which when compared to those in normal steroids indicated that the stable configuration of the A/B ring juncture in the former series was *trans*. Subsequently, on the basis of rotatory dispersion measurements, Djerassi, Marshall and Nakano[2] suggested a *cis* arrangement and recently on the basis of chemical transformations Goto and Fieser[3] arrived at a similar conclusion. We wish to report the results of our continuing study which are in agreement with the latter conclusion and which define certain other stereochemical aspects of this system.

Hydrogenation of B-norcholesteryl acetate (I) in acetic acid over platinum and then saponification yielded 70% of 3β-hydroxy-B-norcoprostane (II, m.p. 77°, $[\alpha]D$ +16°, formerly called B-norcholestanol) and 15% of 3β-hydroxy-B-norcholestane (III, m.p. 132°, $[\alpha]D$ +8°). Oxidation of II and III yielded the corresponding 3-keto derivatives (m.p. 76°, $[\alpha]D$ +16°; m.p. 96°, $[\alpha]D$ +35°), the rotatory dispersion curves of which were practically identical with coprostanone and cholestanone, respectively.[4] Reduction of B-norcoprostane-3-one with LAH gave rise to 25% of II and 75% of the 3α epimer IV (m.p. 96°, $[\alpha]D$ +16°); previously we had reported a predominance of II. Thus, on the basis of the dispersion curves and the stereochemistry of the hydride reduction, II is A/B *cis* and III is A/B *trans*. This hydrogenation result differs greatly from that of cholesteryl acetate which yields mostly the A/B *trans* material.

The direct chemical method employed by Goto and Fieser to establish a *cis* configuration in B-norcoprostane-3,6-dione (V) was the conversion with $NaBH_4$ of the dione V to a 3,6-diol (VI) assumed to be 3β,6α, which, in turn, was transformed into a 3α,6α-oxide (VII). The diketone V has only been related to the B-nor series obtained by hydrogenation of I on the basis of Wolff-Kishner reduction to the same hydrocarbon (VIII) obtained from B-norcoprostanone. Since it is known that such a reduction of a carbonyl group can affect adjacent asymmetric centers,[5] a stereospecific conversion method must be used before the oxide data can be employed for the configurational determination of II. LAH reduction of 3,6-dione V yielded four diols, epimeric only at the hydroxyl functions, two of these diols (VI, m.p. 141° and IX, m.p. 171°) having been reported by Goto and Fieser. The diol IX was converted to a ditosylate (m.p. 121°) which upon reduction with LAH yielded B-norcoprostane VIII, thus specifically relating II and V.

Finally, Goto and Fieser postulated conformation XI as the preferred conformation of compounds in this series on the basis of our earlier LAH reduction results. In addition, they also preferred XI on energetic grounds and postulated that oxide VII was derived directly from conformation XI, ring A remaining as a chair. We prefer conformation X, the usual steroidal conformation,

(1) W. G. Dauben and G. J. Fonken, THIS JOURNAL, **78**, 4386 (1956).

(2) C. Djerassi, D. Marshall and T. Nakano, *ibid.*, **80**, 4856 (1958).

(3) T. Goto and L. F. Fieser, *ibid.*, **81**, 2276 (1959).

(4) Kindly performed by Professor C. Djerassi.

(5) C. Djerassi, R. Riniker and B. Riniker, THIS JOURNAL, **78**, 6362 (1956).