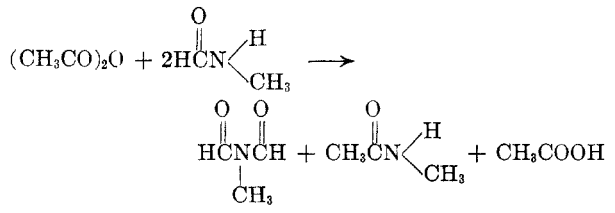


EXPERIMENTAL

A solution of 18 ml. of N-methylformamide (0.31 mole) (b.p. 197°) and 25 ml. of acetic anhydride (0.26 mole)was placed in the pot of a 60 plate fractionating column. Acetic acid was distilled off over a period of 15 minutes without prior reflux. The remaining material was separated into two main fractions (b.p. 183 and b.p. 205° respectively).

N-Methylacetamide was identified by its m.p. 28° and its proton nuclear magnetic resonance spectrum which was identical with that of material prepared by the method of Galat and Elion.[1]

The fraction boiling at 183° with m.p. 13.5 to 14.5° was found to be *diformylmethylamine*. Yield 50%.

*Anal.* Calc'd for $C_3H_5NO_2$: M.W., 87.14; N, 16.09; Moles formate, 2. Found: M.W. (from vapor density at 240°), 86.8; N, 15.85; Moles formate, 1.98.

The diformylmethylamine was further characterized by its proton nuclear magnetic resonance spectrum which consisted of two sharp lines of area ratio 3:2 which is perfectly compatible with the assigned structure.

Use of a higher ratio of acetic anhydride to N-methylformamide resulted in formation of diacetylmethylamine as by-product. Of the several ratios of reactants tried, the one specified above proved to be the optimum. No ratio of reactants employed resulted in formation of any fraction which could be characterized as formylacetylmethylamine.

Diformylmethylamine was also prepared by fractionation of stoichiometric quantities of N-methylformamide and diacetylmethylamine to produce as a by-product, N-methylacetamide. This exchange reaction proved to be very slow.

It is to be noted that the b.p. of N-methylformamide reported by Gautier[2] (180–185°) is not in agreement with that reported in this paper. Gautier's method of preparation of N-methylformamide involved acetic anhydride as a by-product. Thus his products were exactly the starting materials used in the present study. Apparently Gautier's b.p. for N-methylformamide was actually determined on a somewhat impure sample of diformylmethylamine.

*Acknowledgment.* The authors gratefully acknowledge the financial support of this study by the Research Corporation.

DEPARTMENT OF CHEMISTRY
STANFORD UNIVERSITY
STANFORD UNIVERSITY, CALIFORNIA

(1) A. Galat and G. Elion, *J. Am. Chem. Soc.*, **65**, 1566 (1943).

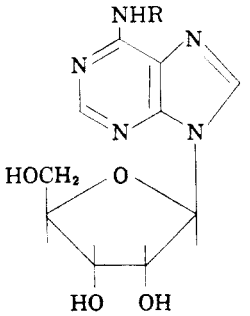
(2) A. Gautier, *Ann.*, **151**, 242 (1869).

## Kinetin Riboside and Related Nucleosides

HENRY M. KISSMAN AND MARTIN J. WEISS

*Received June 6, 1956*

Since Miller and co-workers reported the isolation of kinetin,[1] a cell division factor, from autoclaved DNA samples and its identification as 6-furfurylaminopurine,[2,3] several groups have published on the synthesis of similarly substituted adenine derivatives.[4,5,6] It has been shown[4] that some of these derivatives have kinetin-like activity on plant growth. Because kinetin itself has been isolated from DNA it appeared conceivable that it might actually occur as a riboside or 2-deoxyriboside in nature. In view of our interest in the synthesis of nucleosides and related compounds[7] it seemed pertinent to prepare and test the 9-β-D-ribofuranosyl derivatives (I) of 6-furfurylaminopurine and other substituted adenines.[8] The synthesis of these derivatives, which are listed in Table I, is the subject of this note.



I

A convenient starting material for the preparation of these substituted adenosine derivatives would be a properly blocked 6-chloro-9-β-D-ribofuranosylpurine. The ready replaceability of the 6-chlorine atom in such a nucleoside by ammonia has been demonstrated by Brown and Weliky[9] in their synthesis of adenosine from 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine.[10] However, for our purposes, we have used the corresponding benzoyl blocked 6-chloronucleoside, *i.e.* 6-chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purine (II), because it had been shown previously[11] that the pre-

(1) Miller, Skoog, von Saltza, and Strong, *J. Am. Chem. Soc.*, **77**, 1392 (1955).

(2) Miller, Skoog, Okumura, von Saltza, and Strong, *J. Am. Chem. Soc.*, **77**, 2662 (1955).

(3) Miller, Skoog, Okumura, von Saltza, and Strong, *J. Am. Chem. Soc.*, **78**, 1375 (1956).

(4) Skinner and Shive, *J. Am. Chem. Soc.*, **77**, 6692 (1955).

(5) Daly and Christensen, *J. Org. Chem.*, **21**, 177 (1956).

(6) Bullock, Hand, and Stokstad, *J. Am. Chem. Soc.*, **78**, 3693 (1956).

(7) See for example paper XI of the Puromycin series.[11]

(8) While this work was in progress, R. H. Hall and R. S. de Ropp of these laboratories reported convincing evidence to the effect that kinetin is actually an artifact formed from adenine and 2-deoxy-D-ribose during autoclaving.[15]

(9) Brown and Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(10) L. Goldman, J. Marsico, and R. Angier of these laboratories have successfully effected the reaction of 6-chloro-9-(2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)purine with aliphatic amines (to be published).

(11) Kissman, Pidacks, and Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955).

TABLE I

6-(SUBSTITUTED-AMINO)-9-β-D-RIBOFURANOSYLPURINES (I)

| R[e] | Starting Amine | Formula | Yield, % | M.p., °C. | Optical Rotation $[\alpha]_D^{25-26}$ | $c^d$ | Analyses Calc'd C | H | N | Found C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Furfuryl | Furfurylamine | $C_{15}H_{17}N_5O_5$ | 67[a] | 148–150 | −63.5° | 1.13 | 51.87 | 4.93 | 20.17 | 51.84 | 5.00 | 20.28 |
| Benzyl | Benzylamine | $C_{17}H_{19}N_5O_4$ | 54[b] | 177–179 | −68.6° | 0.55 | 57.13 | 5.36 | 19.60 | 57.04 | 5.66 | 19.70 |
| Thenyl | Thenylamine | $C_{15}H_{17}N_5O_4S$ | 44[a] | 149–150 | −60.7° | 1.05 | 49.58 | 4.72 | 19.23 | 49.78 | 4.91 | 19.26 |
| 3-Pyridylmethyl | 3-Pyridylmethylamine[c] | $C_{16}H_{18}N_6O_4$ | 53[b] | 183–184 | −66.6° | 1.02 | 53.62 | 5.06 | 23.45 | 53.36 | 5.31 | 23.16 |

[a] Recrystallized from methanol. [b] Recrystallized from ethanol. [c] We would like to thank Dr. M. W. Bullock[6] for a sample of this amine. [d] In ethanol. [e] See formula I.

TABLE II

ULTRAVIOLET SPECTRA[a]

| R[b] | Acid $\lambda_{max}$ | $\epsilon$ | $\lambda_{min}$ | $\epsilon$ | Neutral $\lambda_{max}$ | $\epsilon$ | $\lambda_{min}$ | $\epsilon$ | Base $\lambda_{max}$ | $\epsilon$ | $\lambda_{min}$ | $\epsilon$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Furfuryl | 267 | 18460 | 236 | 4580 | 268 | 19000 | 234 | 3195 | 269 | 19140 | 237 | 4030 |
| Benzyl | 266 | 20600 | 235 | 4140 | 268 | 20850 | 233 | 2570 | 269 | 21300 | 236 | 3570 |
| Thenyl | 266 | 18820 | 240 | 10310 | 243 | 9860 | 230 | 9150 | 270 | 20880 | 247 | 9446 |
| | | | | | 270 | 20000 | 247 | 9440 | | | | |
| 3-Pyridylmethyl | 266 | 20520 | 233 | 4300 | 268 | 21650 | 231 | 3440 | 268 | 22230 | 236 | 4440 |

[a] The compounds were dissolved in ethanol. Aliquots were diluted 1:10 with 0.1 *N* hydrochloric acid for the acid spectra and 1:10 with 0.1 *N* sodium hydroxide for the base spectra. [b] See formula I.

cursor sugar, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (III)[11,12] was more readily available[13] and in two instances[11] gave better yields of ribonucleosides than could be obtained with the corresponding tetra-*O*-acetyl-D-ribofuranose.

The condensation of the 1-chloro sugar derived from III[11] with the chloromercuri derivative of 6-chloropurine[9] was carried out in refluxing xylene and the blocked 6-chloronucleoside (II) was obtained as a fluffed glass after chromatography on acid-washed alumina. The β-configuration of this material was established by its conversion in 61% yield through ammonolysis[9] to a crystalline solid which was shown to be identical with adenosine. It may be noted that the yield of adenosine over-all from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (III) was 35% whereas the reported yield over-all from tetra-*O*-acetyl-D-ribofuranose was 22%.[9]

The transformation of the 6-chloronucleoside (II) to the compounds described in Table I was carried out in two stages in satisfactory over-all yields. Compound II was heated with the required amine in 2-methoxyethanol solution[6,10] and the crude reaction product—presumably the 6-(substituted-amino)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-purine—was de-*O*-benzoylated with catalytic amounts of sodium methoxide in methanol. The ultraviolet absorption maxima and minima of the final products are given in Table II.

Preliminary testing results[14] indicate that the substituted adenosine derivatives listed in Table I had kinetin-like activity.[15] Details of these tests will be published elsewhere.

## EXPERIMENTAL[16]

*Chloromercuri derivatives of 6-chloropurine.* The chloromercuri derivative of 6-chloropurine[17] was prepared as described by Brown and Weliky[9] except that 4.87 g. of Celite was added to the sodium hydroxide solution of the 6-chloropurine before addition of the mercuric chloride solution.[18]

*6-Chloro-9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine* (II). A solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride,[11] obtained from 10.33 g. (0.025 mole) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose,[11,12] in 20 ml. of anhydrous xylene was added to an azeotropically-dried suspension of the 6-chloropurine chloromercuri derivative on Celite [12.46 g. of the mixture containing 7.95 g. (0.024 mole) of the purine salt] in 500 ml. of xylene. The mixture was stirred under reflux for three hours and then was filtered. The filtrate was evaporated under reduced pressure and the

(12) Ness, Diehl, and Fletcher, Jr., *J. Am. Chem. Soc.*, **76**, 763 (1954).

(13) See also Wright and Khorana, *J. Am. Chem. Soc.*, **78**, 811 (1956).

(14) Private communication from Dr. R. S. de Ropp of these laboratories.

(15) Hall and de Ropp, *J. Am. Chem. Soc.*, **77**, 6400 (1955).

(16) Melting points were taken on a Kofler micro hot-stage and are corrected. Ultraviolet absorption spectra were determined on a Cary recording spectrophotometer.

(17) 6-Chloropurine was obtained from The Francis Earle Laboratories Inc.

(18) Baker, Joseph, and Schaub, *J. Org. Chem.*, **19**, 1780 (1954).

residue was dissolved in a mixture of 100 ml. of chloroform and 20 ml. of a 30% aqueous potassium iodide solution. The layers were separated and the organic phase was washed with another 15-ml. portion of the potassium iodide solution and then with 20 ml. of water. The chloroform layer was dried over magnesium sulfate, filtered, and evaporated *in vacuo*. This left 11.3 g. of yellow glassy material, which was dissolved in 12 ml. of benzene and was chromatographed on a column (28 × 3 cm.) of acid-washed alumina.[19] The column was washed with 600 ml. of benzene and these washings were discarded. Elution with 1000 ml. of 20% ethyl acetate in benzene afforded, after evaporation *in vacuo*, a faintly yellow glass which was further purified by solution in ether, filtration through Darco and evaporation *in vacuo*. There was obtained 7.01 g. (57%); $\lambda_{max}^{ethanol}$ 265 mμ (ε 9900).

For analysis, this material was dissolved in hot isopropyl alcohol and was collected as an amorphous solid on cooling. The dried substance showed the following characteristics[20]: $\lambda_{max}$ 265 mμ (ε 9850 acid); 265 mμ (ε 9620 neutral); 263 mμ (ε 9350 base); $[\alpha]_D^{25}$ −64.0° (*c*, 0.79 in ethanol).

*Anal.* Calc'd for $C_{31}H_{23}ClN_4O_7$: C, 62.16; H, 3.87; N, 9.35. Found: C, 62.11; H, 4.52; N, 9.03.

*Adenosine.* A mixture of 1.2 g. of the 6-chloronucleoside (II) and 35 ml. of methanolic ammonia (saturated at 0°) was heated in a stainless steel bomb on the steam-bath for five hours. The bomb contents were evaporated to dryness *in vacuo* and the partially crystalline residue was mixed with 25 ml. of water. The mixture was extracted with three 10-ml. portions of ether and the aqueous phase was evaporated *in vacuo* to a small volume. The solid which crystallized on cooling was collected and dried to yield 0.386 g., m.p. 223–226°. It was recrystallized from a small amount of water and dried *in vacuo* at 100° for four hours; 0.325 g. (61%); m.p. 229–230°; $[\alpha]_D^{24}$ −61.2° (*c*, 1.01 in water). The m.p. was not depressed by admixture of an authentic sample[21] of adenosine. Identity was further confirmed by comparison of the ultraviolet and infrared spectra.

*6-(Substituted-amino)-9-β-D-ribofuranosylpurines* (I). The compounds which are summarized in Table I were prepared by reacting 6-chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purine (II) with the appropriate amine according to the procedure described below for the 6-furfurylamino derivative.

For the preparation of *6-thenylamino-9-β-D-ribofuranosylpurine* it was necessary, due to the decreased water solubility of the final product, to change the work up slightly as follows. The reaction mixture, after debenzoylation, was evaporated *in vacuo* and the residue was simply triturated with ether, collected by filtration, and then was washed with more ether and finally with a little water. The dried product was recrystallized from methanol.

*6-Furfurylamino-9-β-D-ribofuranosylpurine.* To a solution of 0.6 g. (0.001 mole) of the 6-chloronucleoside (II) in 15 ml. of 2-methoxyethanol was added 1.5 ml. of freshly distilled furfurylamine and the mixture was heated at the reflux point for one hour. It was then evaporated *in vacuo* and the oily residue was taken up in 20 ml. of chloroform. The solution was washed with saturated aqueous sodium bicarbonate solution and then with water. The organic phase was dried over magnesium sulfate, filtered, and freed from solvents *in vacuo* to leave 0.69 g. of ether-soluble gum. This was dissolved in 20 ml. of anhydrous methanol and after addition of 0.2 ml. of 1 *N* methanolic sodium methoxide solution, the mixture was allowed to reflux for one hour, during which time the solution remained between *p*H 8 and 9. Evaporation *in vacuo* afforded a partially solid mixture to which was added 30 ml. of water. Following extraction with three 10-ml. portions of ether, the aqueous phase was concentrated to a small volume *in vacuo*. The solid, which formed on cooling in the refrigerator overnight, was collected and washed with a small amount of ice-water. The dried substance (0.269 g., m.p. 137–135°) was recrystallized from methanol to afford 0.231 g. (67%) with m.p. 148–150°.

*Acknowledgment.* We would like to thank Dr. M. W. Bullock for helpful discussions. Microanalyses were carried out by Mr. L. Brancone and staff and spectroscopic and polarimetric work by Mr. W. Fulmor and staff.

ORGANIC CHEMICAL RESEARCH SECTION
RESEARCH DIVISION
AMERICAN CYANAMID COMPANY
LEDERLE LABORATORIES
PEARL RIVER, NEW YORK

(19) Reagent Grade alumina (Merck & Co., Inc.) was washed with 1 *N* hydrochloric acid and then with water until neutral. It was dried at 170–180° for 24 hours.

(20) The acid spectrum was determined in methanol:0.1 *N* hydrochloric acid (1:1); the base spectrum was run in methanol:0.1 *N* sodium hydroxide (1:1); the neutral spectrum was run in methanol.

(21) Authentic adenosine, m.p. 228–230°, was obtained from Nutritional Biochemicals Corporation.