times were adjusted so that at least four data points were obtained above and below the apparent $t_{50\%}$ value.

Samples of dissolution media, 5 ml, were withdrawn (and replaced with an equal volume of drug-free fluid) in a plastic disposable syringe[3] through an attached 3.81-cm, 22-gauge needle, the top of which was 5 cm beneath the surface of the fluid. These samples were immediately filtered through a 0.45-$\mu$m membrane[4] filter. The samples were then assayed spectrophotometrically[5] at 343 nm. It was determined that there was no interference in the readings due to tablet excipients. The observed concentrations were converted to amounts dissolved and, in turn, to percent dissolved by using the data from the content uniformity determinations reported by Strum *et al.* (1).

## RESULTS AND DISCUSSION

The resulting percent dissolved *versus* time data for each tablet were then plotted on log probability graph paper according to the method of Wagner (2). A line of best fit was obtained by applying least-squares regression analysis to the transformed values of percent dissolved [transformed to $z$ values as suggested by Lippmann (3)] *versus* logarithms of time. Linear plots were obtained in all cases with correlation coefficients ranging from 0.947 to 0.992. Values for $t_{50\%}$ were then calculated from the slopes and intercepts of the lines of best fit.

Means and standard deviations were calculated for each of the four products and are listed in Table I along with previously reported (1) mean disintegration times, $t_{max}$ values, and $k_a$ values. As can be seen from these results, rank-order correlations occur when both dissolution half-time and disintegration time are compared with the two *in vivo* parameters. Specifically, as the dissolution rate decreases, the absorption rate also decreases, as evidenced by decreasing $k_a$ values and increasing $t_{max}$ values. Similarly, increasing disintegration times (slower disintegration) also coincide with decreasing absorption rates. Because of the limited range of values and the lack of statistically significant differences between certain treatment pairs in the *in vivo* data, it was felt that it would be inappropriate to express these findings as linear relationships.

Based on the $k_a$ values, it appears that dissolution $t_{50\%}$ values greater than 27 min and disintegration times greater than 7 min result in substantially decreased absorption rates. Thus, the absorption rates of these four brands of quinidine sulfate tablets apparently are controlled by both disintegration and dissolution.

[3] Becton-Dickinson and Co., Rutherford, N.J.
[4] Millipore Corp., Bedford, Mass.
[5] Model DB-G spectrophotometer, Beckman Instruments, Fullerton, Calif.

**Table I—Means and Standard Deviations of Dissolution Half-Time, Disintegration Time, Absorption Rate Constant, and Time of Peak Serum Concentration for Each of Four Commercially Available Brands of Quinidine Sulfate Tablets**

| Treatment | $t_{50\%}$, min | Disintegration Time, min[a] | $k_a$, hr$^{-1}$[a] | $t_{max}$, hr[a] |
|---|---|---|---|---|
| C | 7.08 ± 0.76 | 1.00 ± 0.0 | 2.91 ± 2.17 | 1.63 ± 0.59 |
| A | 14.49 ± 1.20 | 4.08 ± 0.20 | 2.88 ± 2.47 | 1.84 ± 0.74 |
| B | 27.41 ± 1.63 | 7.16 ± 1.94 | 2.08 ± 2.21 | 2.27 ± 0.71 |
| D | 34.94 ± 3.98 | 17.83 ± 2.22 | 1.12 ± 0.46 | 2.54 ± 0.33 |

[a] Data from Ref. 1.

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# Synthesis and Biological Activity of $N^6$-($n$-Alkylureido)purine Ribonucleosides and Their 5′-Phosphates

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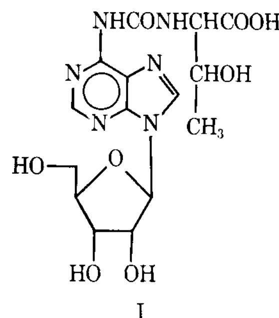
**Abstract** □ Syntheses and biological activities of 12 $N^6$-($n$-alkylureido)purine ribonucleosides (alkyl chain length of 1–10, 16, and 18 carbons) and three $N^6$-($n$-alkylureido)purine ribonucleoside 5′-phosphates (chain length of 4, 9, and 10 carbons) are described. The $N^6$-($n$-alkylureido)purine ribonucleosides were prepared by a reaction of (2′,3′,5′-tri-$O$-acetyl-$\beta$-D-ribofuranosyl)-9$H$-purine-6-carbamate and $n$-alkylamine in refluxing pyridine. The 5′-nucleotides were prepared by direct phosphorylation of the corresponding ribonucleoside with phosphorus oxychloride and triethyl phosphate. Some $N^6$-($n$-alkylureido)purine ribonucleosides ($n$-octyl, $n$-nonyl, and $n$-decyl) and their nucleotides showed a marked antiproliferative activity against L-1210 cells in culture.

**Keyphrases** □ Purine ribonucleosides, substituted—synthesized, evaluated for cytotoxic activity *in vitro* □ Cytotoxic activity—various substituted purine ribonucleosides and 5′-phosphates evaluated *in vitro* □ Structure-activity relationships—various substituted purine ribonucleosides and 5′-phosphates evaluated for cytotoxic activity *in vitro*

In view of the growth-inhibitory activities by analogs of the anticodon-adjacent modified ribonucleoside $N$-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside (I) against cells of leukemic origin grown in culture (1, 2), a series of $N^6$-($n$-alkylureido)purine ribonucleosides of varying chain length (1–10, 16, and 18 carbons) were prepared for determination of structure–activity relationships.

Since the 5′-nucleotides had shown greater water solubility (3), as well as the ability to cross cell membranes (4), and acted as sustained-release forms for the nucleosides



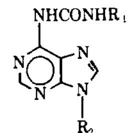



**Table I—$N^6$-(*n*-Alkylureido)purine Ribonucleosides and 5′-Phosphates**

| Compound | $R_1$ | $R_2$ | Method | Yield, % | Melting Point[a] | Formula | Analysis, % | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Calc. | Found |
| II[b] | $CH_3$ | Ribosyl | A | 92 | 199–200° | $C_{12}H_{16}N_6O_5 \cdot 0.5H_2O$ | C 43.24<br>H 5.13<br>N 25.21 | 43.41<br>5.15<br>25.00 |
| III | $CH_2CH_3$ | Ribosyl | A | 97.5 | 198° | $C_{13}H_{18}N_6O_5$ | C 46.14<br>H 5.36<br>N 24.84 | 45.92<br>5.53<br>24.62 |
| IV | $CH_2CH_2CH_3$ | Ribosyl | A | 98 | 184–185° | $C_{14}H_{20}N_6O_5$ | C 47.71<br>H 5.72<br>N 23.85 | 47.74<br>5.92<br>23.63 |
| V | $CH_2CH_2CH_2CH_3$ | Ribosyl | A | 54.5 | 127–129° s<br>174–175° | $C_{15}H_{22}N_6O_5$ | C 49.17<br>H 6.05<br>N 22.94 | 49.22<br>6.28<br>23.05 |
| VI | $CH_2(CH_2)_3CH_3$ | Ribosyl | B | 49 | 113–115° s<br>142–144° | $C_{16}H_{24}N_6O_5 \cdot 0.5H_2O$ | C 49.34<br>H 6.46<br>N 21.58 | 49.38<br>6.61<br>21.64 |
| VII | $CH_2(CH_2)_4CH_3$ | Ribosyl | A | 36 | 115–117° s<br>37–139° | $C_{17}H_{26}N_6O_5 \cdot 0.5H_2O$ | C 50.60<br>H 6.74<br>N 20.83 | 50.38<br>6.74<br>20.78 |
| VIII | $CH_2(CH_2)_5CH_3$ | Ribosyl | B | 70 | 81–83° | $C_{18}H_{28}N_6O_5$ | C 52.93<br>H 6.91<br>N 20.58 | 52.76<br>7.04<br>20.82 |
| IX[c] | $CH_2(CH_2)_6CH_3$ | Ribosyl | B | 84 | 96–98° | $C_{19}H_{30}N_6O_5 \cdot H_2O$ | C 51.80<br>H 7.31<br>N 19.08 | 51.51<br>7.20<br>19.13 |
| X | $CH_2(CH_2)_7CH_3$ | Ribosyl | B | 59.5 | 92–94° s<br>110–112° | $C_{20}H_{32}N_6O_5$ | C 55.03<br>H 7.39<br>N 19.25 | 55.19<br>7.57<br>19.45 |
| XI | $CH_2(CH_2)_8CH_3$ | Ribosyl | B | 46 | 127–128° | $C_{21}H_{34}N_6O_5$ | C 55.98<br>H 7.61<br>N 18.65 | 55.95<br>7.82<br>18.88 |
| XII | $CH_2(CH_2)_{14}CH_3$ | Ribosyl | B | 89 | 105–107° | $C_{27}H_{46}N_6O_5$ | C 60.64<br>H 8.67<br>N 15.71 | 60.58<br>8.71<br>15.88 |
| XIII | $CH_2(CH_2)_{16}CH_3$ | Ribosyl | B | 85 | 115–120° s<br>135–137° | $C_{29}H_{50}N_6O_5$ | C 61.89<br>H 8.95<br>N 14.93 | 61.61<br>9.05<br>14.80 |
| XIV | $CH_2CH_2CH_2CH_3$ | Ribose 5′-phosphate | C | 70 | — | $C_{15}H_{21}BaN_6O_8P$ | C 30.97<br>H 3.64<br>N 14.45<br>P 5.33 | 30.88<br>3.82<br>14.47<br>5.39 |
| XV | $CH_2(CH_2)_7CH_3$ | Ribose 5′-phosphate | C | 69 | — | $C_{20}H_{31}BaN_6O_8P$ | C 36.85<br>H 4.79<br>N 12.89<br>P 4.75 | 38.24<br>5.21<br>13.29<br>5.02 |
| XVI | $CH_2(CH_2)_8CH_3$ | Ribose 5′-phosphate | C | 87.5 | — | $C_{21}H_{33}BaN_6O_8P$ | C 37.88<br>H 5.00<br>N 12.62<br>P 4.65 | 37.77<br>5.19<br>12.78<br>4.74 |

[a] s = soften. [b] This compound was prepared by the urethane method (1). [c] This compound was prepared by the isocyanate method (2).

(5), some potent ribosides were converted into their 5′-phosphates.

## RESULTS AND DISCUSSION

The $N^6$-(*n*-alkylureido)purine ribonucleosides (II–V and VII) (Table I) were prepared in good yields by a reaction of 2′,3′,5′-tri-*O*-acetyladenosine and excess *n*-alkyl isocyanates in pyridine at 85–90° overnight, followed by deblocking of the acetyl groups in 4 *N* methanolic ammonia at room temperature (1). When the isocyanates were not available, the ureidopurine ribonucleosides (VI and VIII–XIII) were prepared by displacement of the ethoxy group of the urethane, ethyl 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (6), by 2 equivalents of *n*-alkylamines in pyridine at 120° overnight, followed by removal of the acetyl groups in methanolic ammonia.

$N^6$-(*n*-Alkylureido)purine riboside 5′-phosphates (XIV–XVI) were prepared by direct phosphorylation of the corresponding ribonucleosides with phosphorus oxychloride and triethyl phosphate at 0° in 50–90% yields (3, 7). Nonyl and decyl compounds (XV and XVI) precipitated out when the reaction mixtures were diluted with ice water. Indeed, the nucleotides improved the solubility in water (2 mg/ml, 10 times more soluble) as compared to that of the ribosides. The UV spectra of the alkylureido compounds were similar to those of I (Table II).

The compounds were tested for growth-inhibitory activity toward mammalian cells in axenic culture derived from mouse L-1210 leukemic cells (Table II). To relate the magnitude of the antiproliferative activity, compounds were assigned as >++, ++, +, ±, and NA at $1 \times 10^{-4}$ and $1 \times 10^{-5}$ *M* as indicated in Table II. Compounds with an alkyl chain 2–5 carbons long (III–VI) were marginally active against L-1210, while II (methyl) showed good activity. In VII–XI, the growth-inhibitory activity increased as the alkyl chain increased from 6 to 10 carbons; IX (*n*-octyl) (2), X (*n*-nonyl), and XI (*n*-decyl) were quite potent.

Compound XII (*n*-hexadecyl) was as active as IX–XI, and XIII was somewhat less active compared to the activity of IX–XI in the culture medium[1], which remained as a suspension during the assay. The 5′-nucleotides XV (*n*-nonyl) and XVI (*n*-decyl) were more water soluble and showed stronger antiproliferative activity than the corresponding nucleosides. The nucleotide XIV (*n*-butyl), like its nucleoside (V), was inactive. Evaluation of the octyl (IX), nonyl (X), and decyl (XI) compounds in mice bearing L-1210 leukemia is in progress.

[1] Dimethyl sulfoxide (0.5%) in Roswell Park Memorial Institute Medium 1640 plus 10% fetal calf serum.

**Table II—UV Data and Biological Activity**

| Compound | $UV_{max}$, nm ($\epsilon \times 10^{-3}$) | | | Biological Activity (L-1210)[b] | |
|---|---|---|---|---|---|
| | 0.1 *N* HCl[a] | 70% Ethanol | 0.1 *N* NaOH | $1 \times 10^{-4}$ *M* | $1 \times 10^{-5}$ *M* |
| II | 277 (25.2) | 269 (24.0)<br>276 | 278 (18.3)<br>298 (18.5) | ++ | |
| III | 277 (24.2) | 269 (23.5)<br>276 (19.3) | 270 (13.9)<br>278 (14.5)<br>298 (16.1) | + | |
| IV | 277 (24.0) | 269 (22.2)<br>276 (18.3) | 269 (20.9)<br>276 (18.1)<br>298 (15.7) | NA | |
| V | 277 (24.9) | 269 (24.1)<br>276 (20.1) | 269 (22.0)<br>277 (19.4)<br>298 (16.5) | NA | |
| VI | 277 (25.1) | 269 (23.6)<br>276 (19.7) | 270 (21.0)<br>277 (18.6)<br>298 (15.4) | + | |
| VII | 277 (23.5) | 270 (22.3)<br>276 (18.6) | 269 (20.5)<br>277 (17.9)<br>299 (14.1) | ++ | |
| VIII | 277 (23.9) | 269 (22.6)<br>276 (18.9) | 269 (19.7)<br>277 (17.5)<br>298 (14.0) | >++ | |
| IX | 270 (23.5)<br>277 (21.9) | 269 (24.1)<br>277 (20.2) | 271 (16.3)<br>278 (16.4)<br>298 (14.5) | >++ | + |
| X | 270 (22.4)<br>277 (21.1) | 269 (23.9)<br>276 (19.9) | 271 (15.2)<br>278 (15.5) | >++ | + |
| XI | 270 (23.3)<br>277 (21.9) | 270 (24.3)<br>276 (20.3) | 269 (13.1)<br>277 (13.3)<br>298 (11.5) | >++ | ++ |
| XII | 270 (21.9)<br>276 (20.5) | 269 (22.6)<br>275 (19.0) | 261 (15.1)<br>282 sh[c] (7.6) | >++ | |
| XIII | 268 (11.4) | 270 (19.8)<br>276 (16.6) | 261 (15.7)<br>283 sh[c] (7.1) | + | |
| XIV | 270 (20.3)<br>277 (19.4) | 269 (20.8)<br>277 (17.4) | 280 (16.7)<br>278 (16.1)<br>298 (9.9) | NA | |
| XV | 270 (21.6)<br>277 (20.9) | 269 (23.2)<br>277 (19.3) | 270 (17.4)<br>277 (16.5)<br>298 (9.4) | | >++ |
| XVI | 270 (21.9)<br>277 (21.2) | 269 (22.8)<br>277 (19.1) | 270 (17.6)<br>277 (16.5)<br>198 (8.9) | | >++ |

[a] Spectra for II–VIII were determined in aqueous 0.1 *N* HCl; spectra for IX–XVI were determined in 0.1 *N* HCl in 70% ethanol. [b] The notation represents the viable cell number relative to the controls at $1 \times 10^{-4}$ and $1 \times 10^{-5}$ *M* after 72 hr of incubation: >++, 0–30%; ++, 30–60%; +, 60–80%; ±, 80–90%; and NA, 90–110%. [c] sh = shoulder.

## EXPERIMENTAL[2]

***N*[6]-[(Ethylamino)carbonyl]adenosine (III): Method A**—A stirred mixture of 2.76 g (7 mmoles) of 2′,3′,5′-tri-*O*-acetyladenosine and 2 ml of ethyl isocyanate in 50 ml of anhydrous pyridine was heated in a glass bomb at 85–90° overnight. After evaporating to dryness, the residue was azeotroped with toluene (20 ml) and stirred in 200 ml of 4 *N* methanolic ammonia at room temperature for 5 hr. The solvent was then evaporated to dryness, and the residue was dissolved in 100 ml of hot ethanol.

After cooling at 0° overnight, the product was collected on a filter and washed with cold ethanol and ether; the yield was 2.3 g (97%). The analytical sample was recrystallized from ethanol, mp 198°; IR: 1680 (ureido C=O) and 1620 and 1550 (C=C, C=N) cm$^{-1}$.

[2] Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. IR spectra were measured using Perkin-Elmer 457 and 137B spectrophotometers. UV spectra were recorded on Cary model 14 and Beckman Acta V spectrophotometers. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, TN 37921, and by Heterocyclic Chemical Corp., Harrisonville, MO 64701.

***N*[6]-[(*n*-Nonylamino)carbonyl]adenosine (X): Method B**—A mixture of 3.26 g (7 mmoles) of ethyl 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (6), 2 g (14 mmoles) of *n*-nonylamine, and 200 ml of anhydrous pyridine was refluxed with stirring for 20 hr. After evaporating to dryness, the residue was azeotroped with toluene (20 ml) and then stirred in 200 ml of 4 *N* methanolic ammonia at room temperature for 5 hr. The solvent was evaporated to dryness, and the residue was dissolved into 100 ml of hot ethanol.

After cooling at room temperature for 1 hr, the white crystals were filtered and washed with cold ethanol and ether; the yield was 861 mg. Additional product was obtained from the filtrate in a yield of 950 mg; the total yield was 1.811 g (59.5%). The analytical sample was recrystallized from ethanol, mp 92–94° soften, 110–112° melted; IR: 3000 (CH), 1730, 1700 (ureido C=O), and 1625 (C=C, C=N) cm$^{-1}$.

***N*[6]-[(*n*-Nonylamino)carbonyl]adenosine 5′-Phosphate (XV): Method C**—To a cooled mixture of 2 ml (~14 mmoles) of phosphorus oxychloride and 80 ml of triethyl phosphate was added 3.05 g (7 mmoles) of X at 0°. The mixture was stirred at 0° for 18 hr and diluted with ice water (350 ml). The white solid, formed within a few minutes, was filtered and washed with cold water. The product was dried at room temperature, and the yield was 2.49 g (69%).

The product was then converted to the ammonium salt by passing the solution of the product in 2-propanol–concentrated ammonia–water (7:1:2) through a cellulose column (2). The analytical sample was obtained as the barium salt after equimolar amounts of the aqueous ammonium salt and barium iodide were mixed, followed by precipitating with acetone; IR: 2950 (CH), 1700 (ureido C=O), 1625, 1560 (C=C, C=N), 1260 (P=O), and 1070 (POC) cm$^{-1}$.

**Growth-Inhibition Assays**—The compounds were dissolved in dimethyl sulfoxide and allowed to remain at 37° overnight; this step achieved sterilization. Sterile growth medium was then added to bring the concentration of dimethyl sulfoxide to 0.5% and that of a compound to $10^{-4}$ and $10^{-5}$ *M*. At this dimethyl sulfoxide concentration, growth of control cultures was unaffected.

The assays were performed in test tubes without agitation. The tubes were inoculated with mouse leukemia cells (L-1210) from stock cultures in logarithmic growth to a starting density previously determined to be near the minimum required for initiation of logarithmic growth of control cultures (between 2 and $5 \times 10^5$ cells/ml). At 24-hr intervals, 0.2 ml was removed and mixed with trypan blue to a final concentration of 0.05%. Then the total and viable cells were counted in a hemocytometer. Cells able to exclude the dye were scored as viable. The compounds were rated as shown in Table II to indicate the following viable cell densities relative to controls after 72 hr of incubation: >++, 0–30%; ++, 30–60%; +, 60–80%; ±, 80–90%; and NA (not active), 90–110%.

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## ACKNOWLEDGMENTS

Supported by U.S. Public Health Service Grant CA 14185.

The authors thank Dr. E. D. Holyoke for his interest and encouragement in this work and Dr. G. L. Tritsch for help in assaying these compounds.