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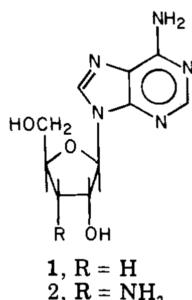
# Nucleosides. 1. 9-(3′-Alkyl-3′-deoxy-β-D-ribofuranosyl)adenines as Lipophilic Analogues of Cordycepin. Synthesis and Preliminary Biological Studies

Andre Rosowsky,* Herbert Lazarus, and Ayako Yamashita

*The Sidney Farber Cancer Institute and Departments of Biological Chemistry and Pathology, Harvard Medical School, Boston, Massachusetts 02115.* *Received March 3, 1976*

A series of lipophilic 9-(3′-alkyl-3′-deoxy-β-D-ribofuranosyl)adenines of increasing chain length was synthesized from the corresponding branched sugars via titanium chloride catalyzed ribosylation of chloromercuri-6-benzamidopurine. Enhanced growth inhibitory activity was observed against CCRF-CEM human lymphoblastic leukemia cells in culture as the length of the alkyl side chain in the sugar and the resultant lipophilic character of the nucleoside were increased. Experiments involving incorporation of radiolabeled uridine, thymidine, and leucine revealed that in contrast to cordycepin (1) the 3′-*n*-butyl and 3′-*n*-hexyl analogues **5** and **6** markedly inhibit not only RNA synthesis but DNA and protein synthesis as well.

Cordycepin (3′-deoxyadenosine, 1) is a nucleoside antibiotic which is produced together with the companion substance 3′-amino-3′-deoxyadenosine (2) by the fungal microorganisms *Cordyceps militaris* and *Aspergillus nidulans*. The isolation, structure elucidation, chemical synthesis, and varied modes of action of cordycepin were reviewed in 1970 by Suhadolnik[1] and more recently by Frederiksen and Klenow.[2] It is generally accepted that the primary biochemical locus of action of this agent following intracellular phosphorylation is at the level of RNA biosynthesis and, probably to a lesser extent, de novo purine biosynthesis.[1,2]




**1**, R = H
**2**, R = $NH_2$

In the last several years an interesting new synthesis of cordycepin has been developed,[3] and many important papers have appeared which deal with the inhibitory effect of this compound on RNA biosynthesis.[4] Other investigations have focused on the ability of cordycepin to interfere with the cell cycle,[5] affect viral replication and virus induced transformation,[6] and produce changes in chromosomal and nucleolar morphology.[7] It has also been reported that cordycepin can impede adenosine transport across the cell membrane,[8] disrupt adrenocorticosteroid hormone mediated phenomena,[9] and perhaps affect feedback regulatory mechanisms involving cAMP.[10]

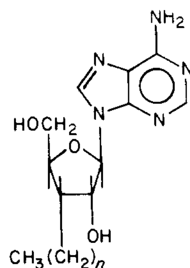
Therapeutic trials with cordycepin have not been extensive, perhaps in part because of the limited amount of material available for such studies. Reports concerning the antitrypanosomal activity of cordycepin have appeared,[11] and one paper has been published in which a low level of antimalarial activity against *Plasmodium berghei* in the mouse is reported.[12]

The antineoplastic activity of cordycepin has also been evaluated experimentally against Ehrlich ascites carcinoma in the mouse.[13] In this tumor system cordycepin shows moderate activity (40% ILS at 188 mg/kg given ip qd 3–6 post implant), but its usefulness is hampered by extensive enzymatic degradation to 3′-deoxyinosine which is inactive. Activity can be enhanced somewhat by giving cordycepin as the $N^1$-oxide derivative.[13] Since the latter is more slowly deaminated than cordycepin and is gradually converted into cordycepin in vivo, it can be regarded as a prodrug.

The appearance in all these preclinical studies of host toxicity at doses very close to the optimum therapeutic level has thus far discouraged further development of cordycepin as a clinical agent. On the other hand, like puromycin which is also considered too toxic for therapeutic use, cordycepin continues to play a significant role in the unraveling of the complicated series of events associated with RNA and protein biosynthesis.[1,2]

Synthetic analogues of cordycepin have been rather few in number, and their preparation has been beset with difficulties. Walton and co-workers prepared 3′-deoxyribonucleosides containing purines other than adenine[14] and also some pyrimidine derivatives.[15] Shigeura and co-workers[16] carried out important structure–activity studies with a number of these compounds in respect to their ease of 5′-O-phosphorylation, as well as their ability to block the de novo purine pathway and inhibit nucleic acid biosynthesis. In 1969 Rosenthal and Sprinzl[17] described the first synthesis of a branched sugar analogue of cordycepin in the form of the compound 9-(3′-deoxy-3′-methyl-β-D-ribofuranosyl)adenine (**3**). More recently Jenkins and Walton[18] prepared 9-(3′-deoxy-3′-methyl-β-D-xylofuranosyl)adenine, the 3′-epimer of

compound **3**, and found it not to be a substrate for adenosine deaminase. These reports stimulated our own interest in the potentialities of other 3′-alkyl-3′-deoxypentofuranosyl nucleosides as medicinal agents. In the present paper we wish to report the preparation of the 9-(3′-alkyl-3′-deoxy-β-D-ribofuranosyl)adenines **3–6** and to present some of our bioassay results for these compounds. Insofar as we are aware, the 3′-ethyl, 3′-*n*-butyl, and 3′-*n*-hexyl analogues **4–6** are the first fraudulent branched sugar nucleosides with a hydrocarbon side chain other than methyl to appear in the chemical literature.

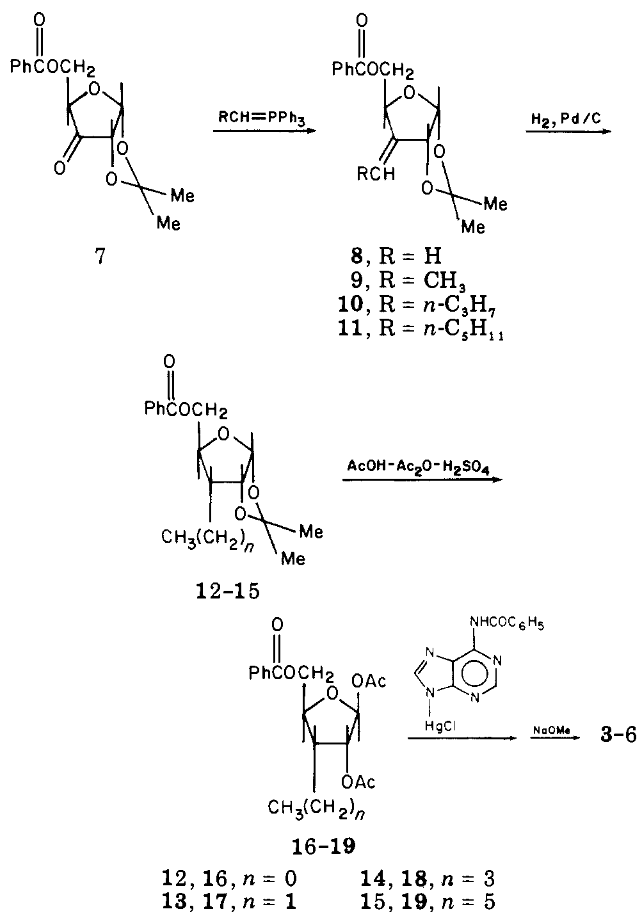



**3**, *n* = 0
**4**, *n* = 1
**5**, *n* = 3
**6**, *n* = 5

**Chemistry.** 1,2-*O*-Isopropylidene-D-xylofuranose was 5-O-benzoylated in pyridine at room temperature[19] and the product oxidized with $Me_2SO$ in acetic anhydride[20] to give the protected ketone **7**, which served as the bulk starting material for all the following steps summarized in Scheme I.

Condensation of **7** with the Wittig reagents derived in situ from methyl-, ethyl-, *n*-butyl-, and *n*-hexyltriphenylphosphonium bromide and *n*-butyllithium in a mixture of ether and hexane afforded the olefins **8–11**, respectively. These compounds were oils at room temperature but could be purified satisfactorily for elemental analysis by column chromatography on silica gel and short-path vacuum distillation. Yields were in the 50–65% range after chromatography. The homogeneity of the products was monitored by thin-layer chromatography and analysis of their NMR spectra. For example, the NMR spectrum of compound **9** in $CDCl_3$ solution showed the anomeric $C_1$ proton as a doublet at τ 4.05 ($J$ = 4.0 Hz), the vinyl proton on the side chain as a multiplet centered at τ 4.13 (clearly discernible under the anomeric proton doublet), the $CH_3C$= protons on the side chain as a doublet at τ 8.24 ($J$ = 7.0 Hz), and the *gem*-dimethyl protons of the 1,2-*O*-isopropylidene group as a pair of singlets at τ 8.53 and 8.60. Although Rosenthal and co-workers[21] have noted the formation of cis and trans olefin mixtures in other Wittig reactions of sugar ketones, NMR spectra of the olefins **8–11** did not reveal more than one double bond isomer and did not allow a conclusive assignment of cis or trans stereochemistry.

Scheme I. Synthesis of 9-(3′-Alkyl-3′-deoxy-β-D-ribofuranosyl)adenines




Catalytic hydrogenation of olefins **8–11** in the presence of 10% Pd/C proceeded in 80–100% yield, giving the saturated branched chain sugars **12–15**. These were likewise oils at room temperature but were sufficiently stable for small-scale evaporative distillation [150–165° (0.075–0.25 mmHg)]. The absence of a double bond could be ascertained readily from NMR spectra. Thus, the spectrum of compound **13** in $CDCl_3$ solution showed the $CH_3CH_2$ protons as a multiplet at τ 8.00–9.15 and the anomeric $C_1$ proton as a clean doublet at τ 4.16 ($J$ = 3.8 Hz), with no other absorption in the τ 4.0–4.2 region corresponding to the vinyl proton of **9**. The presence of only one anomeric doublet was in accord with the previous finding that catalytic reduction in this type of compound proceeds with complete stereospecificity; i.e., hydrogen attack occurs from the β side which is not hindered by the 1,2-*O*-isopropylidene group.[17]

Removal of the 1,2-*O*-isopropylidene blocking group in compounds **12–15** was achieved in 70–100% yield at room temperature with acetic acid containing acetic anhydride and sulfuric acid. That acetolysis was complete could be ascertained readily from NMR spectra. For example, compound **16** gave rise to a singlet at τ 3.88 (anomeric $C_1$ proton), a doublet at τ 4.70 ($J$ = 4.5 Hz, $C_2$ proton), and a pair of singlets at τ 7.88 and 8.05 (acetyl groups); the characteristic *gem*-dimethyl singlets in the τ 8.5–8.7 region were absent. Prominent acetate ester absorption was evident in the infrared spectrum at 1760 $cm^{-1}$. Two of the acetolysis products, compounds **16** and **19**, solidified on standing and could be recrystallized from petroleum ether; the others remained syrupy despite all attempts to induce crystallization. However, TLC analysis showed all four acetolysis products to be sufficiently pure for the next step regardless of whether they were crystalline or not. Furthermore, all four compounds appeared to be β-anomers since their NMR spectra showed only a single unsplit anomeric proton signal at τ 3.88 indicative of trans stereochemistry for the $C_1$ and $C_2$ protons. The physical constants and principal NMR spectral features of sugars **8–19** are summarized in Tables I and II.

Chloromercuri-6-benzamidopurine[22,23] was allowed to react with sugars **16–19** in the presence of titanium tetrachloride and Celite and the protecting groups were removed as described previously.[24] Yields of nucleosides **3–6** were 45–65% for the combined coupling and deprotection steps and did not seem to depend on the

Table I. Physical Constants of Sugar Intermediates in the Synthesis of 9-(3'-Alkyl-3'-deoxy-β-D-ribofuranosyl)adenines

| Compd | Method | % yield[a] | Mp or bp (mmHg), °C | Formula[b] |
|---|---|---|---|---|
| 8 | A | 52.5 | 160–185 (0.05)[c] | $C_{16}H_{18}O_5$ |
| 9 | A | 63.9 | 158–160 (0.1) | $C_{17}H_{20}O_5$ |
| 10 | A | 49.7 | 168–170 (0.1) | $C_{19}H_{24}O_5$ |
| 11 | A | 57.1 | 180–220 (0.1)[c] | $C_{21}H_{28}O_5$ |
| 12 | B | 96.0 | 158–160 (0.25) | $C_{16}H_{20}O_5$ |
| 13 | B | 96.1 | 156–159 (0.075) | $C_{17}H_{22}O_5$ |
| 14 | B | 80.5 | 160–164 (0.1) | $C_{19}H_{26}O_5$ |
| 15 | B | 91.5 | *d* | $C_{21}H_{30}O_5$ |
| 16 | C | 88.7 | 87–88 (needles)[e] | $C_{17}H_{20}O_7$ |
| 17 | C | 97.5 | *d* | $C_{18}H_{22}O_7$ |
| 18 | C | 94.8 | *d* | $C_{20}H_{26}O_7$ |
| 19 | C | 70.3 | 69–70 (needles)[e] | $C_{22}H_{30}O_7$ |

[a] Tabulated values are for material that has not been distilled or recrystallized but is homogeneous by TLC on silica gel; in the case of compounds **8–11** the yields represent material that has been purified by silica gel column chromatography (see Experimental Section). [b] Analyses for C and H were within ±0.4% of the theoretical value. [c] Boiling point values for **8** and **11** are bath temperatures at which evaporative distillation was performed. [d] Compounds **15**, **17**, and **18** were syrups that could not be distilled without extensive decomposition but were analytically pure and TLC homogeneous. [e] Crystallized from petroleum ether (bp 40–50°).

anomeric purity of the starting sugars **16–19**. The nucleosides could be purified readily by recrystallization from aqueous methanol, and all of them absorbed in the uv at the expected wavelength of 260 nm in EtOH solution. The CD spectra of the nucleosides exhibited negative Cotton effects consistent with the β configuration predicted by the trans rule.[25] NMR spectra in $Me_2SO$-$d_6$ solution showed the $C_8$ and $C_2$ purine ring protons in the τ 1.5–1.6 and 1.8–1.9 regions, respectively, and the anomeric proton in the τ 4.0–4.1 region. The uv and NMR spectral data for compound **3** were in close accord with the values reported by Rosenthal and Sprinzl,[17] who used a somewhat different synthetic route to prepare this compound. Interestingly, whereas the $C_{1'}$ proton in **3** gave rise to a doublet (τ 4.08, $J$ = 1.5 Hz), as in cordycepin,[26] the corresponding protons in the longer alkyl chain analogues **4–6** had such small coupling constants to the $C_{2'}$ proton as to appear as singlets. The coupling constant $J_{1',2'}$ for the anomeric proton in cordycepin is 2.2 Hz, whereas that for adenosine and 2'-deoxyadenosine is 6.1 and 6.5 Hz, respectively.[26] It has been suggested[26] that the smaller coupling constant in cordycepin compared to adenosine and 2'-deoxyadenosine indicates a greater preference for the $C_{3'}$ endo conformation in solution. The markedly reduced $J_{1',2'}$ values observed in this study for compounds **3–6** may signify that 3'-alkyl substitution enhances this preference still further.

In one instance, involving the coupling reaction of sugar **17**, we chanced to isolate a trace of a slightly more polar nucleoside by-product which melted about 10° below compound **4** and gave a correct microanalysis for the empirical formula $C_{12}H_{17}N_5O_3$. The by-product was assigned the structure 9-(3'-ethyl-3'-deoxy-α-D-ribofuranosyl)adenine (**20**) on the basis of its NMR spectrum in $Me_2SO$-$d_6$, which showed the $C_2$ and $C_8$ purine ring protons as a *single peak* at τ 1.88 (in contrast to the two signals for compound **4**) and the anomeric proton as a doublet centered at τ 3.86 ($J$ = 3 Hz).

The downfield chemical shift for the $C_{1'}$ proton in **20** relative to **4** is consistent with cis stereochemistry,[27] which places the $C_{1'}$ proton relatively far from the region of positive shielding of the 2'-OH group. The upfield chemical shift for the $C_8$ purine proton (fortuitously causing overlap of the $C_2$ and $C_8$ resonances) is likewise consistent with the α-anomeric structure, where the purine

Table II. NMR Data for Sugar Intermediates in the Synthesis of 9-(3'-Alkyl-3'-deoxy-β-D-ribofuranosyl)adenines

| | Chemical shifts[a] | | | | | |
|---|---|---|---|---|---|---|
| Compd | $C_1$ proton | $C_2$ proton | -CH=C- | $CH_3CO$- | -C$(CH_3)_2$ | Other protons |
| 8 | 4.07 (d, $J$ = 4.0 Hz) | | 4.60 (d, $J$ = 11 Hz) | | 8.48 (s), 8.60 (s) | |
| 9 | 4.05 (d, $J$ = 4.0 Hz) | | 4.00–4.27 (m) | | 8.53 (s), 8.60 (s) | |
| 10 | 4.03 (d, $J$ = 4.5 Hz) | | 4.05–4.30 (m) | | 8.52 (s), 8.60 (s) | |
| 11 | 4.05 (d, $J$ = 4.5 Hz) | | 4.05–4.30 (m) | | 8.53 (s), 8.60 (s) | |
| 12 | 4.13 (d, $J$ = 4.0 Hz) | | | | 8.48 (s), 8.65 (s) | 8.88 (d, $J$ = 7 Hz, $CH_3CH$-) |
| 13 | 4.16 (d, $J$ = 3.8 Hz) | | | | 8.48 (s), 8.66 (s) | |
| 14 | 4.18 (d, $J$ = 4.0 Hz) | | | | 8.50 (s), 8.67 (s) | |
| 15 | 4.17 (d, $J$ = 4.0 Hz) | | | | 8.50 (s), 8.65 (s) | |
| 16 | 3.88 (s) | 4.78 (d, $J$ = 4.5 Hz) | | 7.88 (s), 8.05 (s) | | 8.90 (d, $J$ = 7 Hz, $CH_3CH$-) |
| 17 | 3.88 (s) | 4.70 (d, $J$ = 4.5 Hz) | | 7.88 (s), 8.05 (s) | | |
| 18 | 3.88 (s) | 4.72 (d, $J$ = 4.5 Hz) | | 7.88 (s), 8.05 (s) | | |
| 19 | 3.88 (s) | 4.75 (d, $J$ = 4.2 Hz) | | 7.88 (s), 8.04 (s) | | |

[a] Chemical shifts are expressed in τ units relative to $Me_4Si$ and were determined in $CDCl_3$ solution.

Table III. Physical Constants of 9-(3′-Alkyl-3′-deoxy-β-D-ribofuranosyl)adenines (and of an α-Anomer)

| Compd | % yield[a] | Mp, °C | Crystn solvent[b] | TLC data[c] | $\lambda_{max}^{MeOH}$, nm ($\epsilon$) | Formula[d] |
|---|---|---|---|---|---|---|
| 3 | 63 | 241-245[e] | A | 0.44 | 260 (15 250) | $C_{11}H_{15}N_5O_3$ |
| 4 | 46 | 228.5-231 | A | 0.48 (0.57)[f] | 260 (15 070) | $C_{12}H_{17}N_5O_3$ |
| 5 | 58 | 209-210.5 | A | 0.55 | 260 (15 160) | $C_{14}H_{21}N_5O_3$ |
| 6 | 52 | 214-216 | B | 0.60 | 260 (16 560) | $C_{16}H_{25}N_5O_3$ |
| 20 | Trace | 228-231 | C | (0.44)[f] | | $C_{12}H_{17}N_5O_3$ |

[a] Tabulated values represent overall yields for the coupling and deblocking steps prior to recrystallization. [b] A, 80% MeOH-$H_2O$; B, 70% MeOH-$H_2O$; C, 50% MeOH-$H_2O$. [c] Unless otherwise specified the tabulated $R_f$ values were determined on fluorescent silica gel sheets (Eastman 6060) using 5:9:2 $C_6H_6$-EtOAc-MeOH as the developing solvent; spots were visualized under uv light. [d] Analyses for C, H, and N were within ±0.4% of the theoretical value. [e] Lit.[17] mp 227-228°. [f] $R_f$ values given in parentheses were obtained with 4:9:2 $C_6H_6$-EtOAc-MeOH as the developing solvent.

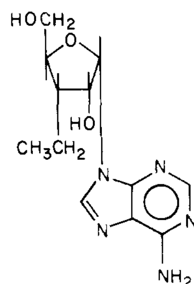
Table IV. NMR Data of 9-(3′-Alkyl-3′-deoxy-β-D-ribofuranosyl)adenines (and of an α-Anomer)

| Compd | Chemical shifts[a] | | | | | |
|---|---|---|---|---|---|---|
| | $C_{1'}$ proton | $C_2$ proton | $C_8$ proton | 6-$NH_2$[b] | 2′-OH[b] | 5′-OH[b] |
| 3 | 4.08 (d, $J$ = 1.5 Hz) | 1.84 (s) | 1.62 (s) | 2.80 (s) | 4.36 (d, $J$ = 4 Hz) | 4.93 (t, $J$ = 5 Hz) |
| 4 | 4.02 (s) | 1.88 (s) | 1.60 (s) | 2.72 (s) | 4.22 (d, $J$ = 4 Hz) | 4.88 (t, $J$ = 5 Hz) |
| 5 | 4.12 (s) | 1.80 (s) | 1.50 (s) | 2.76 (s) | 4.38 (d, $J$ = 4 Hz) | 4.98 (t, $J$ = 5 Hz) |
| 6 | 4.06 (s) | 1.86 (s) | 1.60 (s) | 2.80 (s) | 4.32 (d, $J$ = 4 Hz) | 4.92 (t, $J$ = 5 Hz) |
| 20 | 3.86 (d, $J$ = 3 Hz) | 1.88 (s) | 1.88 (s) | 2.82 (s) | ~4.6 (broad m) | ~5.2 (broad m) |

[a] Chemical shifts are expressed in $\tau$ units relative to $Me_4Si$ and were determined in $Me_2SO$-$d_6$ solution. [b] Addition of $D_2O$ to the $Me_2SO$-$d_6$ solution caused these signals to disappear.

and ribofuranose rings are in the anti conformation and the $C_8$ proton falls within the shielding space of the 2′-OH substituent. It should also be noted that, while the $J_{1',2'}$ value for compound **20** was slightly larger than that for compound **4**, this difference alone cannot be used as the basis of structure assignment in purine nucleosides.

The physical constants and principal NMR spectral features for nucleosides **3–6** and **20** are given in Tables III and IV.




**20**

**Biological Studies.** Cordycepin (**1**) and the 3′-alkyl analogues **3–6** were subjected to preliminary bioassay in vitro using human lymphoblastic leukemia cells (CCRF-CEM) in suspension culture. The previously described assay method[28] was used except that horse serum was added instead of fetal calf serum in order to avoid enzymatic deamination of the nucleosides in the growth medium prior to absorption into the cells.

As indicated in Table V the $ID_{50}$ dose for cordycepin in this system was $2.7 \times 10^{-4}$ M. This value was consistent with results obtained earlier by Rich and co-workers[29] using another line of human tumor cells (H.Ep. 1) in culture. Whereas the 3′-methyl and 3′-ethyl analogues **3** and **4** were relatively inactive in our assay system, the 3′-$n$-butyl and 3′-$n$-hexyl analogues **5** and **6** proved to be four to six times more growth inhibitory on a molar basis than cordycepin. It is of interest to note that the $ID_{50}$ value for cordycepin was not greatly different from those obtained with adenosine and 2′-deoxyadenosine.

Table V. In Vitro Bioassay of 9-(3′-Alkyl-3′-deoxy-β-D-ribofuranosyl)adenines against CCRF-CEM Human Lymphoblastic Leukemia Cells in Culture[a]

| Compd | $ID_{50} \times 10^{-4}$ mol/l. |
|---|---|
| 1 (cordycepin) | 2.7 |
| 3 | 5.0+ |
| 4 | 3.8 |
| 5 | 0.63 |
| 6 | 0.42 |
| Adenosine | 1.4 |
| 2′-Deoxyadenosine | 2.5 |

[a] Actively dividing cells were suspended in Eagle's minimal essential medium supplemented with 10% horse serum and incubated at 37° to give a final population of ca. $5 \times 10^5$ cells per milliliter of medium at the time of addition of the test compound, dissolved in $Me_2SO$. $ID_{50}$ values were determined from plots of mean cell counts after 48 h. Assays were carried out in triplicate, with appropriate controls.

In order to determine how a long-chain 3′-alkyl analogue might differ from cordycepin at the biochemical level, CCRF-CEM cells were incubated with cordycepin or the $n$-hexyl derivative **6** for 2 h and then with [$^3$H]uridine, [$^3$H]thymidine, or [$^3$H]leucine for 1 h. The extent of incorporation of radiolabel into the RNA, DNA, and protein of treated cells was compared with the corresponding values for untreated controls. The results are shown in Table VI. At a concentration of $5 \times 10^{-4}$ M, cordycepin caused 50% inhibition of RNA synthesis in CCRF-CEM cells but no inhibition of DNA or protein synthesis within the time span of the experiment. In sharp contrast, compound **6** at the same concentration not only caused >95% inhibition of RNA synthesis but also led to >95% inhibition of DNA synthesis and >90% inhibition of protein synthesis. In a separate experiment, in which the radioactive precursors were given at the same time as the test drugs, incorporation measurements after just 1 h revealed negligible inhibition of RNA, DNA, or protein

Table VI. Inhibition of Macromolecular Synthesis in CCRF-CEM Cells by Cordycepin (1) and 9-(3'-Deoxy-3'-*n*-hexyl-β-D-ribofuranosyl)adenine (6)[a]

| Compd | Time, min | % inhibn of [$^3$H] uptake into acid-insoluble fraction | | |
|---|---|---|---|---|
| | | [$^3$H]-Urd | [$^3$H]-TdR | [$^3$H]-Leu |
| 1 | 60 | 26 | 4 | 3 |
| | 120 | 36 | 2 | 2 |
| | 180 | 47 | 2 | 5 |
| 6 | 60 | 98 | 99 | 90 |
| | 120 | 99 | 99 | 95 |
| | 180 | 99 | 100 | 98 |

[a] Time was measured from a point 2 h after exposure of cells to $5 \times 10^{-4}$ M drug. Inhibition data are percentage values relative to control cells receiving no drug. Cells were grown in Eagle's minimal essential medium supplemented with 10% horse serum.

synthesis by cordycepin but very significant inhibition of all three by compound **6**. The effect of compound **5** was similar to that of compound **6**, though somewhat less pronounced, in agreement with its slightly higher $ID_{50}$ value.

The foregoing evidence suggests that long-chain 3'-alkyl analogues such as **5** and **6** may be blocking RNA synthesis so efficiently as to allow protein and even DNA synthesis to be inhibited in a short-term incubation. Several interlocking factors may be involved in this enhanced activity, including, for example, the transport of the nucleosides across the cell membrane, the rate of inactivation by adenosine deaminase, and the ability of kinases to bring about phosphorylation to the nucleotide level. Further studies are planned in order to shed light on these problems.

Compounds **3**–**6** were evaluated for antimalarial activity against *P. berghei* in the mouse as part of the Walter Reed Army Institute program. Tests were carried out according to the published method,[30] which involves ip injection of a single dose of each compound to mice 3 days after infection. No increase in survival was seen at doses of 40, 160, and 640 mg/kg, and there was no evidence of toxicity.

## Experimental Section

Melting points (uncorrected) were determined in Pyrex capillary tubes by means of a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) at a heating rate of approximately 2°/min. Ir spectra were recorded on a Perkin-Elmer Model 137B double-beam spectrophotometer and quantitative uv spectra were determined on Cary Model 11 and Model 15 instruments. NMR spectra were taken by means of Varian A-60 and T-60A spectrometers with tetramethylsilane as the reference. Thin-layer chromatography was carried out on silica gel sheets (Eastman 6060) containing a fluorescent indicator. Unless otherwise specified, solutions were dried over anhydrous $Na_2SO_4$ and dry solvents were stored over Linde 3A or 4A molecular sieves. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

**5-*O*-Benzoyl-3-deoxy-3-ethylidene-1,2-*O*-isopropylidene-α-D-ribofuranose (9). Method A.** Ethyltriphenylphosphonium bromide (Aldrich Chemical Co., Milwaukee, Wis.) (30 g, 0.081 mol) was added in small portions with magnetic stirring to a mixture of dry ether (300 ml) and 2.0 M *n*-BuLi in hexane (45 ml, 0.090 mol) under $N_2$. After 3 h the solution of ylide was added dropwise to a solution of ketone **7** (20 g, 0.069 mol) in $CH_2Cl_2$ (60 ml) and ether (140 ml), and stirring was continued for 3 h at room temperature followed by another 3 h under reflux. After cooling, $H_2O$ (200 ml) was added, the organic layer was separated, the aqueous layer was extracted three times with ether, and the combined organic layers were washed with saturated NaCl solution, dried, and evaporated under reduced pressure. The resultant pale yellow syrup was purified by column chromatography on silica gel (Baker 5-3405, 60–200 mesh), with 23:2 petroleum ether (bp 40–50°)–acetone as the eluent. Material obtained in this fashion was sufficiently pure to be used directly in the next step. A small analytical sample was prepared by evaporative distillation (see Table I).

**5-*O*-Benzoyl-3-*n*-butyl-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (14). Method B.** Compound **10** (7.1 g, 0.021 mol) in EtOH (250 ml) was hydrogenated in the presence of 10% Pd/C (2 g) in a Parr apparatus at room temperature for 24 h. Filtration of the catalyst and solvent evaporation under reduced pressure left a pale yellow syrup of sufficient purity (TLC and NMR) for the next step. A microanalytical sample was prepared by small-scale evaporative distillation (see Table I).

**1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*n*-hexyl-β-D-ribofuranose (19). Method C.** Compound **15** (9.7 g, 0.027 mol) was dissolved in glacial AcOH (150 ml) and acetic anhydride (15 ml), and concentrated $H_2SO_4$ (9 ml) was added dropwise while cooling below 5°. After being stored at room temperature for 4 days the mixture was poured with vigorous stirring into ice and water (200 ml), and the product was extracted into $CHCl_3$ (three times). The combined $CHCl_3$ extracts were washed with water and aqueous $NaHCO_3$, rinsed to neutrality with water, dried ($MgSO_4$), and evaporated under reduced pressure to an oil which solidified immediately. Recrystallization from petroleum ether (bp 40–50°) afforded analytically pure colorless needles. For the subsequent fusion reaction it was found that the syrupy product recovered from the mother liquor was just as satisfactory as the crystalline material.

**9-(3'-Deoxy-3'-ethyl-β-D-ribofuranosyl)adenine (4). Method D.** A mixture of compound **17** (3.4 g, 0.096 mol), chloromercuri-6-benzamidopurine (5.6 g, 0.012 mol),[22,23] Celite (6 g), and 1,2-dichloroethane (500 ml) was distilled under anhydrous conditions until 100 ml of distillate had been collected. To the partially cooled mixture was added dropwise 1.3 ml (0.012 mol) of $TiCl_4$, and the mixture was stirred under reflux for 24 h with exclusion of moisture. While the mixture was still warm, saturated aqueous $NaHCO_3$ solution (170 ml) was added with vigorous stirring. After 2 h of stirring, the mixture was filtered through Celite, the filter cake was washed with $CHCl_3$, and the organic layer was separated and concentrated nearly to dryness on the rotary evaporator (40° bath temperature). The volume was adjusted to 200 ml with $CHCl_3$ and the solution was washed with 30% aqueous KI (2 × 100 ml), rinsed with $H_2O$, dried ($MgSO_4$), and evaporated to a yellowish foam under reduced pressure (60° bath temperature). The crude esterified nucleoside (4.5 g, 88% crude yield) was taken up directly in 80 ml of 1 M NaOMe in MeOH (80 ml) and refluxed for 24 h. The reaction mixture was cooled, neutralized with glacial AcOH, left to stand overnight at 0°, and filtered, and the solid was washed with a little cold MeOH and dried in vacuo. The analytical sample was prepared by recrystallizing twice from 4:1 MeOH–$H_2O$.

**9-(3'-Deoxy-3'-ethyl-α-D-ribofuranosyl)adenine (20).** In one instance, during the preparation of nucleoside **4**, the NMR spectrum of the crude deacetylated product showed evidence of contamination by a trace (<5%) of a by-product. Preparative thin-layer chromatography on glass plates coated with silica gel (Analtech, 1000 μ thick), with 5:9:2 $C_6H_6$–EtOAc–MeOH as the developing solvent, allowed this by-product to be isolated in sufficient quantity for chemical characterization. The analytical specimen was prepared by recrystallization from 1:1 MeOH–$H_2O$. It should be noted, however, that **20** was the only α-anomer isolated in this series and that its isolation was apparently a fortuitous event due to some minor variation in experimental procedure which has not been identified.

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# Phenylalanyl Transfer Ribonucleic Acid Synthetase from *Escherichia coli* B. Potent Inhibition by Analogues of *N*-Benzyl-2-phenylethylamine

Robert T. Anderson, Jr., and Daniel V. Santi*

*Department of Pharmaceutical Chemistry and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143.* *Received March 24, 1976*

A potent new class of inhibitors of phenylalanyl-tRNA synthetase from *Escherichia coli* B is described. *N*-Benzyl-2-phenylethylamine is a competitive inhibitor with respect to L-phenylalanine and appears to possess the structural features required for near-optimal binding. Hydrophobic substituents at the ortho position of either ring appear to be well tolerated, but substituents on both rings lead to large losses in binding. Poor noncompetitive inhibitors result from alkylation of the secondary nitrogen, further separation of the *N*-benzyl group from the nitrogen, or alkylation at the $\alpha$ position of the *N*-benzyl moiety. In contrast, placement of a methyl group at the 1 position of the 2-phenylethylamine moiety to give *N*-benzyl-D-amphetamine results in the most potent inhibitor yet described for this enzyme.

Each of the aminoacyl-tRNA (aa-tRNA) synthetases is responsible for the attachment of an amino acid to the tRNA's which recognize the codons for that amino acid. Should an uncorrected mistake occur at this stage, the