

dialysis was run at each concentration. Following equilibration, aliquots of the buffer and plasma compartments were sampled and analyzed by liquid scintillation spectrometry. Sample efficiency was estimated by external standardization.

RESULTS AND DISCUSSION

Data presented in Table I indicate agreement of the binding values in fresh serum and heparinized plasma with those reported earlier² (3, 7). However, the binding of all three drugs significantly decreased in the fresh blood bank plasma, thereby indicating drug displacement from binding sites by the citrate phosphate dextrose preservative. The decreased binding was not due to a change in pH since the pH was maintained at 7.4 after addition of the preservative. The binding of phenytoin and meperidine was also lowered in lyophilized serum while that of bretylium tosylate was not affected. The manufacturer reports that salicylates are added to lyophilized serum. Therefore, a possible explanation may be a change in protein conformation due to freezing and reconstitution, which would affect the more heavily bound drugs (phenytoin and meperidine) or competitive interaction with the salicylates. Bretylium tosylate would be least affected since it is only 10% bound.

Based on these results, studies done with commercial lyophilized serum

should be verified by binding studies in fresh plasma/serum. Furthermore, when blood bank plasma is used for binding studies, the type of preservative used should be stated to allow rigorous evaluation of the pharmacokinetic parameters related to the free fraction and to facilitate duplication of protein binding studies.

REFERENCES

- (1) J. J. Vallner, *J. Pharm. Sci.*, **66**, 447 (1977).
- (2) C. Davidson, in "Fundamentals of Drug Metabolism and Drug Disposition," B. LaDu, H. Mandel, and E. Way, Eds., Williams & Wilkins, Baltimore, Md., 1971, pp. 63-74.
- (3) U. Klotz, T. S. McHorse, G. R. Wilkinson, and S. Schenker, *Clin. Pharmacol. Ther.*, **16**, 667 (1974).
- (4) L. E. Mather, G. T. Tucker, A. E. Pflug, M. J. Lindop, and C. Wilkerson, *ibid.*, **17**, 21 (1975).
- (5) E. Woo and D. J. Greenblatt, *J. Pharm. Sci.*, **68**, 466 (1979).
- (6) P. H. Hinderling, J. Bres, and E. R. Garrett, *ibid.*, **63**, 1684 (1974).
- (7) V. W. Weigan, K. L. Hintze, J. T. Slattery, and G. Levy, *Clin. Pharmacol. Ther.*, **27**, 297 (1980).

Acyclic Puromycin Analogs: 9-[(2-Phenylalanylamidoethoxy)methyl]adenine and 9-(3-Phenylalanylamidopropyl)adenine

JAMES L. KELLEY^{*}, CARL A. MILLER, and HOWARD J. SCHAEFFER

Received January 29, 1981 from Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Accepted for publication April 1, 1981.

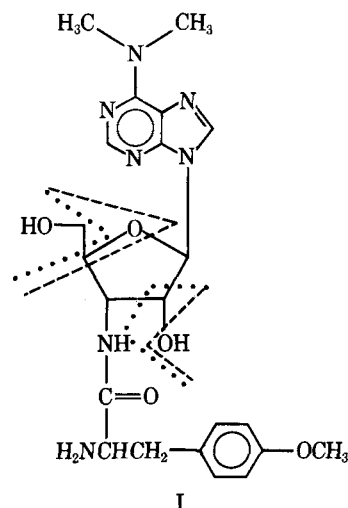
Abstract □ Two acyclic puromycin analogs, in which the 2'- and 5'-hydroxymethyl groups or the 2'-hydroxyl and 5'-hydroxyethoxy portions of the cyclic carbohydrate ring were excised, were synthesized and evaluated for inhibition of an *in vitro* protein-synthesizing system and for antiviral and antibacterial activity. No puromycin-like activity was seen with these conformationally free compounds.

Keyphrases □ Puromycin—acyclic analogs, synthesis and analysis, evaluation for inhibition of protein synthesis and antiviral and antibacterial activity □ Analogs—of puromycin, acyclic, synthesis and analysis, evaluation for inhibition of protein synthesis and antiviral and antibacterial activity □ Antineoplastics—puromycin, synthesis and analysis of acyclic analogs, evaluation for inhibition of protein synthesis and antiviral and antibacterial activity

A program initiated to synthesize nucleoside analogs, in which the cyclic carbohydrate moiety is replaced by an acyclic side chain, led to the discovery of the potent anti-herpetic drug acyclovir, 9-[(2-hydroxyethoxy)methyl]guanine¹ (1). This approach to nucleoside modification has been extended to the synthesis of two acyclic analogs of the broad spectrum antibiotic puromycin (I). This nucleoside antibiotic, which is an inhibitor of protein biosynthesis, has not been useful in the treatment of human disease because of its nephrotoxicity (2). It was shown (3-5), however, that toxicity could be alleviated by removal of the 5'-hydroxy group.

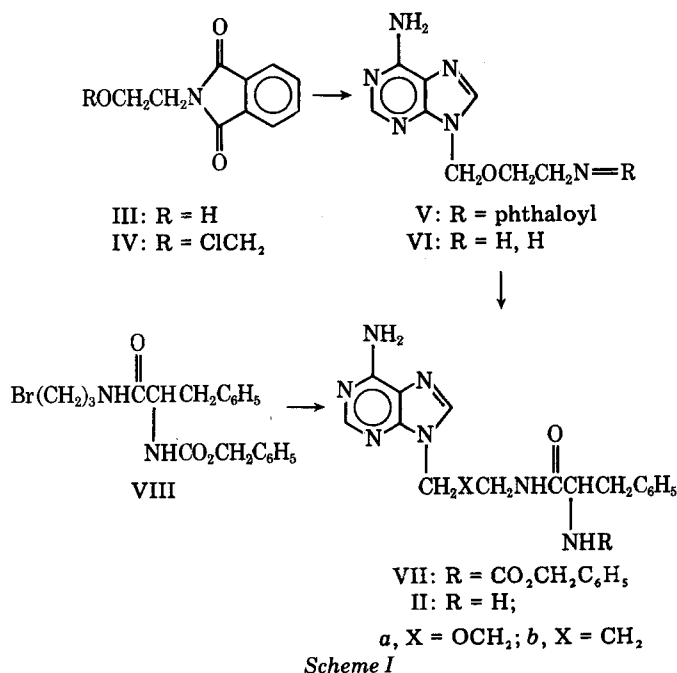
This paper reports an approach to separation of toxicity from activity involving the synthesis of distinct fragments of puromycin in which parts of the cyclic carbohydrate

moiety are excised. Elimination of the 2'- and 5'-hydroxymethyl groups (dotted lines in I) or the 2'-hydroxyl and 5'-hydroxyethoxy portions (broken lines) would leave side chains as in IIa or IIb, respectively². Both IIa and IIb can assume a conformation that is superimposable on puromycin, but they lack the 5'-hydroxyl group implicated in puromycin toxicity (3, 6). Open chain analogs similar to



² In this work, the readily available DL-phenylalanine was used since the phenylalanyl analog of puromycin is nearly as active as puromycin (7, 8). In addition, the N 6-methyl groups of puromycin were shown to be unnecessary for puromycin-like activity (9).

¹ Zovirax.



IIb but containing a 2'-hydroxyl group were reported to have powerful (10) or moderate (11, 12) puromycin-like activity. Evidence on the importance of a 2'-hydroxyl group for puromycin-like activity is conflicting (13-16).

The present paper reports the synthesis of two acyclic analogs of puromycin that lack the 5'- and 2'-hydroxyl groups. These compounds were tested for inhibition of an *in vitro* protein-synthesizing system and for antiviral and antibacterial activity.

RESULTS AND DISCUSSION

The acyclic puromycin analogs IIa and IIb were synthesized as outlined in Scheme I. Chloromethylation (17) of III gave IV, which was reacted with adenine to give V. Identity of the product as a 9-substituted adenine was indicated from the UV spectrum (18). Cleavage of the phthaloyl group gave VI, which was coupled with DL-N-carbobenzoxypheylalanine using the mixed anhydride method (19) to give VIIa. Hydrogenolysis of VIIa gave the acyclic puromycin analog IIa. For preparation of IIb, adenine was alkylated with VIII, followed by cleavage of the carbobenzoxy moiety with hydrogen bromide in acetic acid.

Both IIa and IIb were tested against an *Escherichia coli*-f₂-RNA protein-synthesizing system and were inactive at 10⁻⁴ M. Under the same conditions, puromycin showed 50% inhibition at 10⁻⁶ M. The lack of puromycin-like activity in IIa or IIb further substantiates the earlier conclusion that a conformationally rigid system, such as that provided by a cyclopentane ring, is necessary for puromycin-like activity (4, 11, 20).

All six of the 9-substituted adenines, V, VI, VIIa, VIIb, IIa, and IIb, were tested by means of plaque inhibition tests against three DNA viruses (vaccinia virus, adenovirus type 5, and herpes simplex virus type 1) and six RNA viruses (influenza NWS, measles, Bunyamwera, Semliki Forest, rhino 1B, and corona) (1, 21, 22). Compounds IIb, VIIa, and VIIb showed weak activity at 50 µg/disk against one DNA virus, herpes simplex type 1, although none were sufficiently active to warrant secondary testing. None of the compounds was active at 50 µg/disk against the RNA viruses.

The six adenines were also tested for *in vitro* antibacterial activity (23) against *Streptococcus pyogenes* (CN10), *Streptococcus faecalis* (CN478), *Staphylococcus aureus* (CN491), *E. coli* (CN314), *Salmonella typhosa* (CN512), *Shigella dysenteriae* (CN1513), *Klebsiella pneumoniae* (CN3632), *Enterobacter aerogenes* (2200/86), *Enterobacter cloacae* (2200/87), *Citrobacter freundii* (2200/77), *Proteus vulgaris* (CN329), *Proteus mirabilis* (S2409), *Pseudomonas aeruginosa* (CN200), and *Candida albicans* (CN1863). None of the compounds was inhibitory at 100 µg/ml.

EXPERIMENTAL

N-[(2-Chloromethoxy)ethyl]phthalimide (IV)—A stirred dispersion of 19.12 g (0.10 mole) of III and 3.00 g (0.10 mole) of paraformaldehyde in 250 ml of ethylene dichloride was saturated with dry hydrogen chloride at -10°. After 4 hr, the solution was dried with calcium chloride, filtered, and spin-evaporated *in vacuo* to afford a white solid (21.94 g, 91%), mp 69-72°. Recrystallization from ether gave the analytical sample, mp 69-70°; NMR (CDCl₃): δ 7.80 (m, 4H, aromatic H), 5.45 (s, 2H, ClCH₂O), and 3.95 (s, 4H, CH₂CH₂N).

Anal.—Calc. for C₁₁H₁₀ClNO₃: C, 55.1; H, 4.21; N, 5.85. Found: C, 55.9; H, 4.34; N, 5.84.

9-[(2-Phthalimidoethoxy)methyl]adenine (V)—This compound was prepared from adenine and IV as described for VIIb on a 100-mmol scale, except that the reaction solution was poured over 2 liters of ice water; the product was collected by filtration to yield 20.0 g (59%), mp 252-256°. A portion was recrystallized twice from 2-methoxyethanol and once from dimethylformamide to give the analytical sample, mp 256-258°; UV: λ_{max}^{0.1N HCl} 258 nm (ε 10,800); UV: λ_{max}^{0.1N NaOH} 259 nm (ε 15,000); NMR (trifluoroacetic acid): δ 9.20 (s, 1H, purine H), 8.70 (s, 1H, purine H), 7.98 (s, 4H, aromatic H), 6.03 (s, 2H, NCH₂O), and 4.17 (s, 4H, CH₂CH₂N).

Anal.—Calc. for C₁₆H₁₄N₆O₃: C, 56.8; H, 4.17; N, 24.8. Found: C, 56.8; H, 4.28; N, 24.7.

9-[(2-Aminoethoxy)methyl]adenine (VI)—A mixture of 3.38 g (10 mmoles) of V, 1.0 ml (20 mmoles) of hydrazine hydrate, 250 ml of ethanol, and 75 ml of 2-methoxyethanol was refluxed with stirring for 4 hr. The cooled reaction mixture was spin-evaporated *in vacuo*. The residual solid was dispersed in 100 ml of 1 N HCl, stirred at ambient temperature for 0.5 hr, and then left overnight at 0°. The mixture was filtered and thoroughly washed with water. The combined filtrates were spin-evaporated *in vacuo* to give a residue, which was dissolved in 100 ml of water and stirred with ion-exchange resin³ until a negative silver nitrate test was obtained. The mixture was filtered and spin-evaporated *in vacuo* to give a white powder (1.25g, 60%), mp 168-170°. Recrystallization from 2-propanol gave analytically pure material (0.80 g, 38%), mp 170-171°; UV: λ_{max}^{0.1N HCl} 256 nm (ε 13,900); UV: λ_{max}^{0.1N NaOH} 259 nm (ε 14,100); NMR (dimethyl sulfoxide-d₆): δ 8.31 (s, 1H, purine H), 8.22 (s, 1H, purine H), 7.34 (broad s, 2H, purine NH₂), 5.60 (s, 2H, NCH₂O), 3.48 (t, 2H, OCH₂CH₂), 2.64 (t, 2H, CH₂CH₂N), and 1.60 (broad s, 2H, aliphatic NH₂).

Anal.—Calc. for C₈H₁₂N₆O: C, 46.1; H, 5.81; N, 40.4. Found: C, 46.3; H, 5.86; N, 40.2.

9-[(2-N-Carbobenzoxypheylalanyl)amidoethoxy)methyl]adenine (VIIa)—To a stirred, ice bath cooled solution of 7.78 g (26.0 mmoles) of DL-N-carbobenzoxypheylalanine and 2.62 g (26.0 mmoles) of triethylamine in 100 ml of tetrahydrofuran was added 2.70 g (25.0 mmoles) of ethyl chloroformate. A precipitate formed within a few minutes; after 1 hr, 2.70 g (13.0 mmoles) of VI was added. After 2 hr at ambient temperature, 20 ml of water was added and the reaction was spin-evaporated *in vacuo*. The residue was dissolved in 250 ml of chloroform and washed with four 50-ml portions of water, four 50-ml portions of 5% aqueous sodium bicarbonate, 50 ml of water, and 50 ml of brine. Then it was dried and spin-evaporated *in vacuo*.

The product was slurried in 2-propanol and collected (5.25 g, 82%), mp 207-208°. The analytical sample was recrystallized from 2-propanol, mp 208-210°; UV: λ_{max}^{0.1N HCl} 257 nm (ε 14,000); UV: λ_{max}^{0.1N NaOH} 259 nm (ε 14,600); NMR (dimethyl sulfoxide-d₆): δ 8.26 (s, 1H, purine H), 8.17 (s, 1H, purine H), 8.10 (t, 1H, CH₂NH), 7.45 (d, 1H, CHNH), 7.28 and 7.23 (s, 12H, aromatic H, NH₂), 5.53 (s, 2H, NCH₂O), 4.93 (s, 2H, OCH₂C₆H₅), 4.19 (m, 1H, CH), 3.47 (m, 2H, OCH₂CH₂), 3.22 (m, 2H, CH₂CH₂NH), and 2.90 and 2.70 (m, 2H, CHCH₂C₆H₅).

Anal.—Calc. for C₂₅H₂₇N₇O₄: C, 61.3; H, 5.56; N, 20.0. Found: C, 61.5; H, 5.62; N, 20.0.

9-[(2-Phenylalanyl)amidoethoxy)methyl]adenine Fumarate (IIa)—A mixture of 0.98 g (2.0 mmoles) of VIIa dissolved in 50 ml of acetic acid and 0.30 g of 10% palladium-on-carbon was shaken in the presence of hydrogen at 2-3 atm for 3 hr. The mixture was filtered and spin-evaporated *in vacuo*. The residual oil was combined with 0.232 g (2.0 mmoles) of fumaric acid in 10 ml of ethanol, warmed to effect solution, and allowed to cool (0.660 g, 93%), mp 188-189° (effervescent). The analytical sample was recrystallized from ethanol, mp 190-191° (effervescent).

³ Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. NMR data were recorded on Varian XL-100-15-FT and T-60 spectrometers with tetramethylsilane as an internal standard. UV spectra were obtained on a Unicam SP 800 spectrophotometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on TLC.

vescent); UV: $\lambda_{\max}^{0.1N\text{ HCl}}$ 257 nm (ϵ 12,000); UV: $\lambda_{\max}^{0.1N\text{ NaOH}}$ 259 nm (ϵ 12,200); NMR (dimethyl sulfoxide- d_6): δ 8.26 (s, 1H, purine H), 8.17 (s, 1H, purine H), 8.11 (t, 1H, CH_2NH), 7.29 (s, 2H, purine NH_2), 7.21 (s, 5H, aromatic H), 6.51 (s, 1H, fumarate CH), 5.52 (s, 2H, NCH_2O), \sim 5.5 (broad s, CHNH_2 , fumarate OH), 3.54 (m, 1H, CH), 3.44 (m, 2H, OCH_2CH_2), 3.19 (m, 2H, CH_2NH), and 2.91 and 2.71 (m, 2H, $\text{CH}_2\text{C}_6\text{H}_5$).

Anal.—Calc. for $\text{C}_{17}\text{H}_{21}\text{N}_7\text{O}_2 \cdot 0.5\text{ C}_4\text{H}_4\text{O}_4$: C, 55.2; H, 5.61; N, 23.7. Found: C, 54.9; H, 5.73; N, 23.5.

3 - N - Carbobenzoxypheylalaninamidopropyl Bromide (VIII)—To a stirred, ice-salt bath cooled solution of 29.93 g (0.10 mol) of DL-N-carbobenzoxypheylalanine and 10.12 g (0.10 mole) of triethylamine in 325 ml of chloroform was added 10.85 g (0.10 mole) of ethyl chloroformate in 20 ml of chloroform. After 0.25 hr, a solution of 21.89 g (0.10 mole) of 3-bromopropylamine hydrobromide in 70 ml of water was added, followed by 25 ml of 4 N NaOH with vigorous stirring. After 96 hr at ambient temperature, the two layers were separated. The chloroform layer was washed with two 50-ml portions of 0.1 N HCl, two 50-ml portions of water, and once with brine. It then was dried and spin-evaporated *in vacuo*. The residual solid was slurried with hexane and collected (22.25 g, 53%), mp 123–125°. A portion was recrystallized from ethyl acetate-hexane to give the analytical sample, mp 125–126° (resolidified and remelted 129–130°).

Anal.—Calc. for $\text{C}_{20}\text{H}_{23}\text{BrN}_2\text{O}_3$: C, 57.3; H, 5.53; N, 6.68. Found: C, 57.3; H, 5.46; N, 6.60.

9-(3-N-Carbobenzoxypheylalaninamidopropyl)adenine Hydrochloride (VIIb)—To a stirred, ice bath cooled dispersion of 1.81 g (45.2 mmoles) of sodium hydride (60.2% dispersion in mineral oil) in 70 ml of dimethylformamide was added 6.08 g (45.0 mmoles) of adenine. After 10 min, the cold bath was removed and the mixture was stirred at ambient temperature for 1 hr, when salt formation was complete. A solution of 19.08 g (45.5 mmoles) of VIII in 100 ml of dimethylformamide was added; after 42 hr at ambient temperature, the reaction was filtered and spin-evaporated *in vacuo*. The residue was partitioned between 600 ml of 5% methanol in chloroform and 50 ml of water, and the layers were separated.

The organic phase was washed with two 100-ml portions of water and once with brine. It then was dried and spin evaporated *in vacuo*. The residue was dissolved in ethanol and diluted with hydrogen chloride-saturated ethanol to give a crystalline product (14.20 g, 62%), mp 195–197°. The analytical sample was obtained from ethanol, mp 196–197°; UV $\lambda_{\max}^{0.1N\text{ HCl}}$ 262 nm (ϵ 12,200); UV: $\lambda_{\max}^{0.1N\text{ NaOH}}$ 262 nm (ϵ 12,200); NMR (dimethylsulfoxide- d_6): δ 8.45 (s, 2H, purine H), 8.20 (t, 1H, CH_2NH), 7.57 (d, 1H, CHNH), 7.26 (s, 10H, aromatic H), 4.95 (s, 2H, $\text{OCH}_2\text{C}_6\text{H}_5$), \sim 4.2 (m, 5H, NH_2 , purine CH_2 , CH), 3.0–2.8 (m, 4H, CH_2NH , $\text{CHCH}_2\text{C}_6\text{H}_5$), and 1.92 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$).

Anal.—Calc. for $\text{C}_{25}\text{H}_{28}\text{ClN}_7\text{O}_3$: C, 58.8; H, 5.53; N, 19.2. Found: C, 59.2; H, 5.61; N, 19.1.

9-(3-Phenylalaninamidopropyl)adenine (IIb)—To a stirred solution of 8.16 g (16.0 mmoles) of VIIb in 100 ml of acetic acid was added 30 ml of 30–32% hydrogen bromide in acetic acid. After 18 hr, the reaction mixture was diluted with 500 ml of ether, the solvent was decanted, and the solids were washed with ether. The crude hydrobromide was dissolved in water and passed through a column containing an excess of ion-exchange resin³. The eluates were spin-evaporated *in vacuo* to give a syrup, which crystallized under ether and was recrystallized from 2-propanol (2.57 g, 47%), mp 143–145°. The analytical sample had a melting point of 145–146°; UV: $\lambda_{\max}^{0.1N\text{ HCl} + 10\%\text{ C}_2\text{H}_5\text{OH}}$ 259 nm (ϵ 13,700); UV: $\lambda_{\max}^{0.1N\text{ NaOH} + 10\%\text{ C}_2\text{H}_5\text{OH}}$ 261 nm (ϵ 13,700); NMR (dimethyl sulfoxide- d_6): δ 8.13 (s, 1H, purine H), 8.10 (s, 1H, purine H), 7.96 (t, 1H, CH_2NH), 7.22 (s, 7H, aromatic H and purine NH_2), 4.04 (t, 2H, purine CH_2), 3.39 (q, 1H, CH), 3.03 (q, 2H, CH_2NH), 2.92 and 2.65 (m, 2H, $\text{CH}_2\text{C}_6\text{H}_5$), 1.88 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), and 1.65 (s, 2H, CHNH_2).

Anal.—Calc. for $\text{C}_{17}\text{H}_{21}\text{N}_7\text{O}$: C, 60.2; H, 6.24; N, 28.9. Found: C, 60.0; H, 6.23; N, 28.8.

Inhibition of Protein Synthesis Assay—For measurement of protein synthesis, extracts were prepared from cells of *E. coli* D10 by the method of Nirenberg (24) as modified by Godson and Sinsheimer (26). RNA was prepared from bacteriophage- f_2 as described previously (25). Reaction mixtures (0.1 ml) were prepared as described (24) with [4,5- ^3H]-L-leucine⁴ as the radioactive amino acid. Compounds to be tested were added as

neutral solutions. The reaction was started by addition of 10 μg of f_2 -RNA. After incubation at 37° for 10 min, the reaction was stopped by the addition of sodium hydroxide to a final concentration of 0.1 M. After a further 15 min at 37°, 10 volumes of 10% trichloroacetic acid were added; then the mixture was heated at 85° for 20 min and cooled. The precipitates were collected on filters⁵ and washed with 5% trichloroacetic acid, and the precipitated radioactivity was counted as described previously (27). In each trial, puromycin⁶ was tested as a standard inhibitor and gave 50% inhibition at $\sim 10^{-6}$ M.

REFERENCES

- (1) H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer, and P. Collins, *Nature*, **272**, 583 (1978).
- (2) D. Nathans, in "Antibiotics," vol. I, D. Gottlieb and P. D. Shaw, Eds., Springer-Verlag, New York, N.Y., 1967, pp. 259–277.
- (3) R. G. Almquist and R. Vince, *J. Med. Chem.*, **16**, 1396 (1973).
- (4) S. Daluge and R. Vince, *ibid.*, **15**, 171 (1972).
- (5) R. Vince, R. G. Almquist, C. L. Ritter, F. N. Shiota, and H. T. Nagasawa, *Life Sci.*, **18**, 345 (1976).
- (6) E. Kmetec and A. Tirpack, *Biochem. Pharmacol.*, **19**, 1493 (1970).
- (7) D. Nathans and A. Neidle, *Nature (London)*, **197**, 1076 (1963).
- (8) B. L. Hutchings in "Chemistry and Biology of Purines," CIBA Foundation Symposium, G. E. W. Wolstenholme and C. M. O'Connor, Eds., Little, Brown, Boston, Mass. 1957, p. 177.
- (9) F. W. Lichtenthaler, E. Cuny, T. Morina, and I. Rychlik, *Chem. Ber.*, **112**, 2588 (1979).
- (10) B. P. Gottikh, A. A. Kraevskii, M. K. Kukhanova, A. A., Yatsyna, A. M. Kritsyn, and V. L. Florentiev, *Mol. Biol. Rep.*, **1**, 173 (1973).
- (11) R. Vince and R. G. Isakson, *J. Med. Chem.*, **16**, 37 (1973).
- (12) A. Holy, J. Cerna, and I. Rychlik, *Nucleic Acid Res.*, **1**, 1221 (1974).
- (13) I. Rychlik, J. Cerna, S. Chladek, J. Zemlicka, and Z. Haladova, *J. Mol. Biol.*, **43**, 13 (1969).
- (14) M. K. Kukhanova, V. A. Pozdnyakov, L. V. Nikolaeva, A. A. Kraevskii, Y. U. Mitin, and B. P. Gottikh, *Mol. Biol. (Moscow)*, **8**, 389 (1974); through *Chem. Abstr.*, **81**, 14700m (1974).
- (15) J. Robins, R. A. Jones, and M. MacCross, *Biochemistry*, **13**, 553 (1974).
- (16) M. Sprinzl and F. Cramer, *Proc. Natl. Acad. Sci. USA*, **72**, 3049 (1975).
- (17) A. J. Hill and D. T. Keach, *J. Am. Chem. Soc.*, **48**, 257 (1926).
- (18) N. J. Leonard and J. A. Deyrup, *ibid.*, **84**, 2148 (1962).
- (19) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *ibid.*, **89**, 5012 (1967).
- (20) R. Vince and S. Daluge, *J. Med. Chem.*, **20**, 930 (1977).
- (21) F. C. Herrmann, *Proc. Soc. Exp. Biol. Med.*, **107**, 142 (1961).
- (22) B. Rada, D. Blašković, F. Sorm, and J. Skoda, *Experientia*, **16**, 487 (1960).
- (23) S. R. M. Bushby and G. H. Hitchings, *Br. J. Pharmacol. Chemother.*, **33**, 72 (1968).
- (24) M. W. Nirenberg, "Methods in Enzymology," vol. VI, Academic, New York, N.Y., 1963, p. 17.
- (25) G. N. Godson and R. L. Sinsheimer, *Biochim. Biophys. Acta*, **149**, 476 (1967).
- (26) R. F. Gesteland and H. Boedtker, *J. Mol. Biol.*, **8**, 496 (1964).
- (27) R. J. Harvey, *J. Bacteriol.*, **101**, 574 (1970).

ACKNOWLEDGMENTS

The authors are indebted to Dr. R. J. Harvey for the inhibition of protein synthesis test results, to Dr. D. J. Bauer and his group for the antiviral testing, to Dr. S. R. M. Bushby for the antibacterial screening. They also thank Dr. B. S. Hurlbert and his staff for the elemental analyses and 60-MHz NMR spectra, Mr. Aris Ragouzeous for assistance with the 100-MHz NMR analysis, and Mrs. A. Melton for excellent technical assistance. J. L. Kelley thanks Dr. B. Roth and Dr. S. Daluge for helpful discussions.

³ Rexyn 201(OH).

⁴ New England Nuclear.

⁵ Millipore.

⁶ Nutritional Biochemical Corp.