Polynucleotide Analogues. Copolymers of Vinyl Bases with Acrylic Acid, Methylacrylic Acid, Acrylamide, and 1-Vinylpyrrolidone

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Preliminary studies are reported on the synthesis and testing of substituted vinyl polymers that are designed to have sequence specific affinity for polyribonucleic acids. Copolymers of 1-vinyluracil with acrylic acid, 2-methylacrylic acid, or 1-vinyl-2-pyrrolidone were prepared by γ-irradiation to give the respective polymers 1, **3**, and **4**. Similarly, 9-vinyladenine yielded copolymeric products **5** and **6** with acrylic acid or 2-methylacrylic acid. Radical initiated polymerization of 9-vinyladenine with acrylamide yielded copolymer **7**. The products were characterized by elemental analysis and ultraviolet, infrared, and nuclear magnetic resonance spectroscopy. No hypochromicity could be detected on mixing polymers 1–4 with poly(adenylic acid). The acrylic acid copolymer **2** containing a high ratio of vinyluracil was a potent inhibitor of poly(adenylic acid) coded polylysine synthesis in an in vitro system. Polymers **6** and **7**, containing a high proportion of vinyladenine, inhibited poly(uridylic acid) coded poly(phenylalanine) synthesis.

Many clinically effective drugs exert their action on a disease lesion by either inhibiting a target protein receptor when the excess product of that receptor is toxic or, alternatively, by replacing the deficient product of that receptor. While this approach has been successful in the treatment of many diseases, it does not address the cause of the lesion—that there is an excess or deficiency of a particular receptor protein. If agents can be found that selectively control protein synthesis, they should be invaluable in many disease conditions. Such selectivity for controlling the synthesis of a particular protein can only be achieved if the drug acts at the level of transcription or translation to either inhibit or enhance the expression of the gene containing the message for the target protein.

Advances made in recent years by molecular biologists have shown that many unique mechanisms exist at the level of transcription or translation for the control of gene expression. The basis for such selectivity is the uniqueness of the message in the double-stranded DNA or the mRNA. Thus, it is imperative that any drug designed to inhibit gene expression must have extremely high affinity for the particular sequence of bases in the control region of the target gene or the message. Alternatively, for derepression of a nucleic acid message, a drug must mimic the message to such a degree that it successfully binds to the repressor protein.

Our preliminary studies for the design of agents that have nucleic acid sequence specificity have been directed to a search for an analogue of the ribose phosphate polymeric unit in nucleic acids that will allow specific "reader" molecules attached to this polymer to interact with nucleic acid bases. In these initial studies various copolymers containing the natural readers adenine and uracil have been employed to examine optimal positioning of the readers for complementation to the respective nucleic acids poly(U) or poly(A).

Pitha and co-workers[1] found that radical initiated polymerization of 1-vinyl-4-ethoxypyrimidin-2-one using *N,N'*-azobis(isobutyronitrile) or hydrogen peroxide gave poly(vinyluracil). Kaye[2] examined the free-radical polymerization of 1-vinyluracil, but he detected a substantial amount of dihydrouracil residues in the resulting polymers, caused by a cyclopolymerization reaction. Further studies[3] showed that polymerization of 1-vinyluracil using γ-ray initiation gave little or no evidence of dihydrouracil residues in the polymer product. Our polymerization method employed ^{60}Co γ-ray polymerization of 1-vinyluracil in the presence of co-monomers and was not appreciably different than that reported by Hoffman and co-workers.[4] Copolymerization of an alkaline solution of 1-vinyluracil and acrylic acid by exposure to ^{60}Co radiation gave polymers that were insoluble in methanol, water, and acid media. Reprecipitation from base solution and washing with acid, methanol, and ether removed monomer residues from the product. This material was purified by elution from a G-75 Sephadex column, and the lead fractions containing high-molecular-weight material were collected. After concentration and acidification, the precipitated polymer was dried in vacuo at 60 °C until C, H, and N analysis showed that the water content was stable. Although these are random copolymers, the ratio of monomer units contained in the polymers was determined by C, H, and N analysis. The lack of dihydrouracil residues was confirmed by IR data.

An NMR spectrum of polymer **2** confirmed the absence of dihydrouracil residues, as well as the ratio of uracil to acrylic acid units as determined by the C, H, and N analysis. The spectrum was taken at 143 °C in Me_2SO-d_6 solvent. The 5- and 6-uracil protons appeared at δ 5.5 and 7.24, respectively, and the methine proton nearest the uracil ring appeared at δ 4.22. These three peaks integrated for one proton each. The remaining polymer backbone protons appeared under a broad peak centered at δ 1.98. This peak integrated for 3.2 protons—two methylene protons contributed by the uracil unit and three times 0.4 protons contributed by the acrylic acid unit. Hoffman and co-workers[4] published the NMR spectra of a similar polymer and the assignments agree.

The ratio of the monomer units in the polymer can be controlled to a degree by varying the ratios of the monomers in the reaction mixture. In this way, for example, the high reactivity ratio of acrylic acid can be balanced by using a larger proportion of 1-vinyluracil which apparently has a lower reactivity ratio.[5]

Table I shows the composition of the starting mixtures of the various polymerization reactions. Examination of the extinction coefficients presented in Table I for the uracil copolymers reveals that there is a significant hypochromism present relative to 1-ethyluracil (ϵ 10 300 at 267 nm). Poly(U) solutions at room temperature show only a few percent hypochromism. Pitha and co-workers[1] report an average extinction coefficient value of 6000 at pH 7 for poly(vinyluracil) prepared by a free-radical-induced polymerization reaction. Kaye and Chang[6] report extinction coefficients of 4500 and 6100 at pH 8.5 for two poly(vinyluracil) polymers prepared by x-ray-induced reactions. It is reasonable to assume that the hypochromicity is the result of uracil–uracil base-stacking interactions. Polymer **1** has somewhat less hypochromicity than polymers **2**–**4**. This is not unexpected since polymer **1** contains a great deal more co-monomer units per uracil unit and, therefore, must have greater distances between

Table I. Composition of Copolymers 1-7

Compd	Reactants (mole ratio)		Product ratio of vinyl base-Co-monomer-H_2O	C		H		N		Mol wt of monomer unit[a]	Extinction coeff,[a] nm[b]
	Vinyl base	Co-monomer		Calcd	Found	Calcd	Found	Calcd	Found		
1	1-Vinyluracil (1.0)	Acrylic acid (0.5)	$(C_6H_6N_2O_2)(C_3H_4O_2)_{1.7}(H_2O)_{0.5}$	49.44	49.23	5.16	5.18	10.39	10.43	270	6 600 (260)
2	1-Vinyluracil (1.0)	Acrylic acid (0.33)	$(C_6H_6N_2O_2)(C_3H_4O_2)_{0.4}(H_2O)_{0.9}$	47.21	47.23	5.17	5.28	15.29	15.22	183	5 700 (260)
3	1-Vinyluracil (1.0)	2-Methylacrylic acid (0.37)	$(C_6H_6N_2O_2)(C_4H_6O_2)_{0.7}(H_2O)_{0.9}$	49.31	48.99	5.64	5.25	13.05	13.00	215	5 800 (260)
4	1-Vinyluracil (1.0)	1-Vinyl-2-pyrrolidone (1.25)	$(C_6H_6N_2O_2)(C_6H_9NO)_{1.1}(H_2O)_{0.9}$	54.71	54.64	6.45	6.19	15.70	15.38	277	5 900 (260)
5	9-Vinyladenine (1.0)	Acrylic acid (0.29)	$(C_7H_7N_5)(C_3H_4O_2)(H_2O)_{0.9}$	48.15	48.13	5.17	5.13	28.08	27.98	249	8 700 (257)
6	9-Vinyladenine (1.0)	2-Methylacrylic acid (0.23)	$(C_7H_7N_5)(C_4H_6O_2)_{1.7}(H_2O)_{1.4}$	49.81	49.96	6.06	5.98	21.05	21.00	333	10 100 (257)
7	9-Vinyladenine (1.0)	Acrylamide (0.44)	$(C_7H_7N_5)(C_3H_5NO)_{0.6}(H_2O)_{0.8}$	48.43	48.23	5.36	4.96	35.95	36.01	218	9 000 (257)

[a] Lowest calculated molecular weight of the monomer unit of the polymer containing 1 equiv of uracil or adenine. [b] Solutions contained 0.01 M NaCl and 0.01 M Tris HCl, pH 7.4. Extinction coefficients are calculated from the molarity of the base residues.

the uracil groups resulting in fewer stacking interactions. Hoffman and co-workers[4] reported the synthesis of poly(vinyluracil, acrylic acid) with a 1:1 ratio but did not report the molar extinction coefficient. Compound **4** was also reported by Kondo and co-workers[7] as a 3:2 copolymer (1-vinyluracil, 1-vinyl-2-pyrrolidone) obtained by radical-initiated polymerization. The ultraviolet data for their polymer were not reported.

Copolymers **5** and **6** were prepared by γ-irradiation of 9-vinyladenine with acrylic acid or 2-methylacrylic acid. Compound **5** has been previously prepared by both radical and γ-ray polymerization.[8] Copolymer **7** was prepared by radical-initiated polymerization of a mixture of 9-vinyladenine (1.0) and acrylamide (0.44). Copolymers **5**–**7** were insoluble in mixtures of water and methanol. Monomer residues were removed from the polymers by extensive washing with methanol. Low-molecular-weight fractions were removed from the polymers by sizing on a Sephadex G-75 column. Kondo and co-workers[7] reported the synthesis of **7** by the same procedure to give a copolymer containing a similar ratio (1:0.5) of 9-vinyladenine and acrylamide.

Extinction coefficients for the adenine polymers (**5**–**7**) were in the range 8700–10 000. The extinction coefficient for poly(vinyladenine)[9,10] at maximum ultraviolet absorbance (254 nm) is 9000 which compares to that of poly(adenylic acid) (ϵ 9300). This represents a 37% hypochromic effect due to base-stacking interactions based on the molar extinction coefficient of the monomer, 9-ethyladenine [λ_{max} (pH 7) 260 (ϵ 15 400)]. As in the uracil polymers, our adenine polymers **5** and **7** showed considerable hypochromicity. Polymer **6**, containing the lowest proportion of adenine residues, would have fewer stacking interactions and, therefore, the least hypochromicity.

The uracil copolymers **1**–**4** were examined for the formation of hypochromic complexes with poly(adenylic acid). Job's plots of the ultraviolet absorbance at 260 nm vs. the mole fraction of the polymer under examination and poly(adenylic acid) should show hypochromic deviation from linearity due to ordering (stacking) of the above residues if complex formation takes place. Such continuous variation mixing curves of poly(vinyluracil), prepared by radical-initiated polymerization, and of poly(adenylic acid) showed maximum hypochromic effects at a concentration ratio of 3:1 for the uracil–adenine residues.[6] In contrast, Kaye and Chang[6] noted a hyperchromic effect using the γ-ray polymerized poly(vinyluracil) and poly(adenylic acid) which they attribute to disordering the highly stacked poly(vinyluracil) in the complex formed with the complementary polynucleotide. We failed to detect any deviation from the linear Beer's law in continuous variation mixing curves of polymers **1**, **3**, or **4** with poly(adenylic acid). In contrast, Hoffmann and Witkowski[8] noted a hypochromic effect indicating a 2:1 uracil–adenine complex with their poly(vinyluracil, acrylic acid) and poly(adenylic acid).

A very small hypochromic effect (2%) was observed with compound **2** and poly(adenylic acid). However, this polymer, being enriched in vinyluracil, would be expected to contain some longer tracts of poly(vinyluracil) and hence show some hypochromic effects similar to Kaye's[2] or Pitha's[1] poly(vinyluracil).

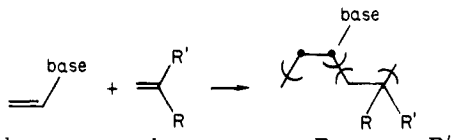
Few comments can be made on the detailed structure of these copolymers because of the random nature of the polymerization reaction. The fact that resonance, polar, and steric effects contribute to the reaction[11] suggests that the copolymers prepared in this study are head to tail polymers in a random sequence of the two monomers as

Table II. Inhibition of in Vitro Polypeptide Synthesis by Copolymers of 1-Vinyluracil or 9-Vinyladenine[a]

Compd	Poly(phenylalanine) synthesis: Ratio of (bases in polymer)/(uracil in message)	Poly(phenylalanine) synthesis: % inhibn	Polylysine synthesis: Ratio of (bases in polymer)/(adenine in message)	Polylysine synthesis: % inhibn
1, poly(vU, AA)	11.1	0	20.6	0
2, poly(vU, AA)	57	26	2.6	10
			6.9	30
			13.6	80
3, poly(vU, MAA)	40	0	37.2	0
4, poly(vU, vP)			13.4	8
			28.9	39
5, poly(vA, AA)	25.7	0		
6, poly(vA, MAA)	3.1	92	9.3	0
	6.0	100		
7, poly(vA, AAmide)	26.0	34		
	52.0	66		

[a] In vitro translation assays coded by poly(U) or poly(A) are described in the Experimental Section. Normal control assays showed 80 pmol of [^{3}H]-Phe and 48 pmol of [^{3}H]-Lys incorporated in acid-insoluble material.

Scheme I



copolymer	base	R	R'
1	1-uracil	COOH	H
2	1-uracil	COOH	H
3	1-uracil	COOH	CH_3
4	1-uracil		H
5	9-adenine	COOH	H
6	9-adenine	COOH	CH_3
7	9-adenine	$CONH_2$	H

shown in the general reaction in Scheme I. Apparently, the randomness of these copolymers, both with regard to the distance between the uracil bases and their atactic orientation, does not allow for enough structural regularity in the polymers to form a complex with poly(adenylic acid).

Some synthetic analogues of nucleic acids have shown interesting biological properties. Poly(vinyladenine) and poly(vinyluracil) inhibited in vitro ribosomal binding and protein synthesis coded by the complementary polynucleotides.[12] Antiviral effects[13] and inhibition of RNA-dependent DNA polymerase[14,15] are also noted for these polymers in addition to interferon priming action.[8]

Preliminary biological studies using polymers of 1-vinyluracil or 9-vinyladenine with maleic acid or acrylic acid revealed that these compounds are potent stimulants of in vitro poly(uridylic acid) coded poly(phenylalanine) synthesis using a cell-free system purified from *Escherichia coli* MRE 600.[16] These unexpected results were studied, and it was determined that the action is not due to preservation of the message via ribonuclease inhibition. Rather, the stimulation afforded by these copolymers is through inhibition of a ribosomal bound protein that appears to inhibit poly(phenylalanine) synthesis.[17] When this inhibitory protein was removed from the ribosomes by washing in high salt buffers the unusual stimulation is not observed.

The copolymers prepared in this study were examined for inhibition of in vitro protein synthesis in a similar system using washed ribosomes. The results (Table II) show that compound **2** is the most effective of the uracil series in inhibiting poly(adenylic acid) coded polylysine synthesis. Poly(1-vinyluracil, 1-vinylpyrrolidone) (**4**) also showed activity, but considerably less than that afforded by **2**.

The inhibition afforded by compound **2** is thought to be due to the high ratio of the vinyluracil monomer in the polymer. In this random polymer it is probable that there are reasonably long stretches of poly(vinyluracil), which is known to inhibit polylysine synthesis. This is confirmed by the observation that the corresponding polymer with a high ratio of acrylic acid (polymer **1**) was not active. On the other hand, compound **4**, a polymer with almost a 1:1 ratio of the monomers, does have activity. However, the low order of activity, 40% inhibition at a 30:1 ratio of uracil residues in the polymer to adenine residues in the message, is not encouraging.

In the adenine series weak inhibition was observed for compound **7**. Like **2** this could be due to poly(vinylbase) sequences in the copolymer. Poly(9-vinyladenine, 2-methylacrylic acid) (**6**), however, was a potent inhibitor of poly(uridylic acid) coded poly(phenylalanine) synthesis. The strong inhibition afforded using a relatively low total concentration of adenine residues in copolymer **6** suggests that the inhibition is message specific. This was supported by the observation that **6** had no effect on poly(adenylic acid) coded synthesis. The inhibition, 90% using a 3:1 ratio of copolymer adenines to message uracils, is considered significant. This is particularly evident since the carboxyl analogue **5** was inactive at a much higher concentration. The original intention in preparing the acrylic acid copolymers was to examine the possible ordering effect the carboxylate group would have on the polymer structure. Also, the presence of a negative charge would produce a polymer more analogous to the natural polynucleotides. Evidently, the charge neither orders the chain nor makes **5** a closer analogue of poly(adenylic acid).

On first inspection of the activity of **6** it appears that the addition of the methyl group (compare to **5**) contributed to activity, perhaps by ordering the chain by a steric effect. However, the random nature of the polymer required further analysis of **6**. Fractionation a second time on Sephadex G-75 was done using water and the major peak was cut into three portions, each of which was tested separately. Fraction 1 and 2 were found to be inactive (Table III) while fraction 3 was very potent; 100% inhibition was found using a concentration ratio of 1.7 adenine residues in the inhibitor to 1 uracil residue in the message. Elemental analysis, Table III, showed fraction 3 to be high in vinyladenine. Presumably the high activity of fraction 3 is due to poly(vinyladenine) sequences in the copolymer.

Reynolds and co-workers[14] failed to find message activity in poly(vinyluracil) and poly(vinyladenine), and they attributed this to the close spacing of the bases and lack

Table III. Inhibition of in Vitro Polypeptide Synthesis by G-75 Resolved Polymers 6[a]

Compd	G-75 fraction	Ratio of (adenine polymer)/ (uracil in message)	% inhibn
6, poly(vA, MAA)	1	3.7	0
	2	3.7	0
	3[b]	1.7	100

[a] Methods used in poly(phenylalanine) synthesis are described in the Experimental Section. [b] Elemental analysis of this copolymer was calculated for the following ratio (9-vinyladenine)$_{1.0}$(2-methylacrylic acid)$_{0.4}$(water)$_{1.2}$. Anal. Calcd: C, 47.55; H, 5.48; N, 32.24. Found: C, 47.53; H, 5.02; N, 32.15.

of a negative charge on the backbone. Compounds 1–3, 5, and 6 are polycarboxylate polymers. However, they did not code for poly(phenylalanine) (1–3) or polylysine (5, 6) synthesis.

Experimental Section

Poly(U) was purchased from Boehringer and poly(A) from Miles Laboratories. [^{3}H]- and [^{14}C]phenylalanine and [^{3}H]lysine were products of Schwarz/Mann. *E. coli* B tRNA was purchased from Plenum Scientific; DNase and other common chemicals were purchased from Sigma. *E. coli* MRE 600 cells were furnished by the Enzyme Laboratory, University of Kansas.

Copolymerization of 1-Vinyluracil with Acrylic Acid (1 and 2). The general procedure described by Hoffmann and co-workers[1] was used in this and other 1-vinyluracil copolymer preparations. 1-Vinyluracil[18] (100 mg, 0.72 mmol) was dissolved in 1.5 mL of deoxygenated 3 M potassium hydroxide in a soft glass ampule. Acrylic acid (26 mg, 0.36 mmol) was added; the ampule was flushed with argon and sealed. This was irradiated for 19 h using a ^{60}Co source rated at 39 000 rads/h. The clear solution was added slowly to 50 mL of methanol and the solid crude polymer was collected. This was dissolved in 2 mL of water and acidified by adding 1 M HCl, and the solid 1 was collected. After washing repeatedly with methanol and ether 30 mg of 1 was dissolved in 2 mL of 1 M Tris HCl buffer, pH 8.4, and sized on a 25 × 0.9 cm column of Sephadex G-75 by elution with water. The lead fractions containing high-molecular-weight material were concentrated and acidified, and the precipitated polymer (1) was collected, washed with methanol and ether, and dried to a constant water content. Elemental analysis (Table I) showed a ratio of 1:1.7:0.5 for vinyluracil–acrylic acid–water which calculates to ~270 mol wt for the smallest monomer unit in the polymer that contains 1 equiv of uracil. The molar extinction coefficient at 260 nm is 6600 for a mol wt of 270.

Polymer 2 was prepared in the same way as described for 1 using a higher ratio of 1-vinyluracil (150 mg, 1.08 mmol) and 26 mg of acrylic acid (0.36 mmol). The product 2 analyzed (Table I) for a ratio of 1:0.4:0.9 for vinyluracil–acrylic acid–water. On this calculated monomer molecular weight (~183) the molar extinction coefficient was 5700 at 260 nm.

This procedure also was used in the synthesis of 3 and 4.

Copolymerization of 9-Vinyladenine with Acrylic Acid (5). 9-Vinyladenine[9] (125 mg, 1 mmol) was dissolved in 3 mL of 1 M HCl in a soft glass ampule and the solution deoxygenated by bubbling argon gas through the solution. Freshly distilled acrylic acid (21 mg, 0.29 mmol) was added, the solution again deoxygenated with argon, and the ampule sealed. Irradiation using a ^{60}Co source at 39 000 rads/h was done for 19 h. Polymeric material was precipitated by the addition of sodium hydroxide (2 M) to the clear solution to a pH of 6. The solid was collected and washed extensively with methanol and ether.

Low-molecular-weight material was removed from the product by gel filtration. A 2-mL solution of 40 mg of polymer in 1 M Tris HCl, pH 8.4, was placed on a 25 × 0.9 cm column of Sephadex G-75.

After elution with 0.5 M triethylammonium bicarbonate (pH 7.5) the lead fractions containing high-molecular-weight material were combined and concentrated in vacuo and hydrochloric acid was added to a pH of 6. The precipitated polymer was collected and washed extensively with methanol and ether. The solid 5 was dried for several days in vacuo until, from elemental analysis, no further water could be removed. According to C, H, and N analysis (Table I) compound 5 contained a 1:1 copolymer ratio and 0.9 equiv of water. On this basis the lowest unit molecular weight containing 1 equiv of adenine in the polymer is 249 and the molar extinction coefficient (at maximum 257 nm) is 8700.

This procedure also was used in the synthesis of 6.

Copolymerization of 9-Vinyladenine with Acrylamide (7). 9-Vinyladenine[9] (100 mg, 0.79 mmol) and 25 mg of recrystallized acrylamide (0.35 mmol) were placed in a pressure bottle and 6 mL of deoxygenated methanol and 8 mg of α, α'-azodiisobutyronitrile was added. After flushing with argon the bottle was sealed and heated to 60 °C for 2 days. The precipitated polymer was washed extensively with methanol and ether followed by sizing on Sephadex G-75 as described in the preparation of 5 using water. The elemental analysis of 7 (Table I) calculates to give a ratio of 1:0.6:0.8 for the base–amide–water. The molar extinction coefficient for the unit monomer containing one adenine (mol wt ~218) is 9000 at 257 nm, the maximum absorbance.

Binding Affinity Determinations. Analysis for hypochromic effects was done by determination of the ultraviolet absorbance of solutions containing varying mole fractions of the polymers described in this study with the complementary single-stranded ribonucleic acid. In this way polymers 1–4 were analyzed for binding to poly(adenylic acid).

In a typical experiment a 2.14×10^{-4} M solution of 2 (based on the unit monomer of mol wt 183) and a 2.14×10^{-4} M solution of poly(adenylic acid) were used; the solution also contained 0.01 M sodium chloride and 0.01 M Tris HCl buffer, pH 7.4. Plots of absorbance at 260 nm vs. mole fraction of a mixture of 2 and poly(A) were determined at each 0.1 mol fraction for the range of 0–1.0 mole fraction of 2. The plots obtained for polymers 1–4 did not deviate from linearity and the conclusion is that there is little or no double-stranded complex formation. In contrast, a 28% hypochromic effect was observed in similar experiments using solutions of poly(U) and poly(A).

Preparation of *E. coli* Ribosomes and S-100. All isolations were carried out at 2 °C. Thawed *E. coli* MRE 600 cells (30 g) were disrupted by grinding with 45 g of washed sand and 20 mL of buffer A: 50 mM Tris HCl (pH 7.8) with 10 mM magnesium chloride, 6 mM 2-mercaptoethanol, and 60 mM ammonium chloride. Alternatively, larger quantities were disrupted by sonication using the same buffer. The mixture was centrifuged (10 000 g) for 15 min, DNase (5 μg/mL) and 2-mercaptoethanol (6 μmol/mL) were added to the supernatant, and the solution was stirred for 15 min. The solution was clarified by centrifugation for 20 min at 30 000g. Ribosomes were collected by centrifugation of the S-30 supernatant at 105 000g for 2 h. The S-100 fraction was frozen and stored in small aliquots at −70 °C. Ribosomes were resuspended in 30 mL of buffer containing 1.0 M ammonium chloride, 20 mM Tris HCl (pH 7.8), 10 mM magnesium acetate, and 10 mM 2-mercaptoethanol. The solution was stirred gently overnight. The washed ribosomes were collected by centrifugation as described, resuspended in buffer A, and stored in small aliquots at −70 °C.

Assay of Poly(U)-Dependent Poly(phenylalanine) Synthesis. The reaction mixture contained 50 mM Tris HCl (pH 7.8), 56 mM ammonium chloride, 6 mM 2-mercaptoethanol, 5 mM ATP, 0.5 mM GTP, 15 mM magnesium chloride, 120 μg of *E. coli* B tRNA, 5 μL of S-100, three A_{260} units of washed ribosomes, poly(U) as indicated, 30 μM [^{3}H]phenylalanine (sp act 0.5 mCi/Mmol), and polymer added as indicated in a total volume of 0.1 mL. Polymer was preincubated with message for 8 min at 25 °C. After that S-100 and ribosomes were added and reaction was continued 20 min at 37 °C. The reaction was terminated by adding approximately 2 mL of 10% trichloroacetic acid. The samples were heated to 95 °C for 10 min, cooled, and filtered on glass filter pads, and the pads were washed three times with 5% trichloroacetic acid and finally with ethanol. The dried filter pads were counted in a Beckman scintillation counter.

Assay of Poly(A)-Dependent Polylysine Synthesis. The reaction mixture contained in final volume of 0.1 ml: 50 mM Tris HCl (pH 7.8), 56 mM ammonium chloride, 6 mM 2-mercaptoethanol, 5 mM ATP, 0.5 mM GTP, 15 mM magnesium chloride, 120 μg of *E. coli* tRNA, 5 μL of S-100, three A_{260} units of washed ribosomes, poly(A) as indicated, 15 μM [^{3}H]lysine (sp act 1

mCi/μmol), and polymer. Polymer was preincubated with poly(A) for 10 min at 25 °C. After that S-100 and ribosomes were added and reaction was carried out for 20 min at 37 °C. Polylysine (200 μg) was added and reaction was stopped by adding 0.1 mL of 1 N KOH. The solution was incubated for 20 min at 37 °C after which 25 μL of 100% trichloroacetic acid and 3 mL of 5% trichloroacetic acid–0.25% sodium tungstate, pH 2.0, were added. Samples were filtered and counted as described for poly(U) translation.

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Synthesis and Antibacterial Activity of Some Derivatives of Tolypomycinone. Relationship between Structure and Activity in Ansamycins

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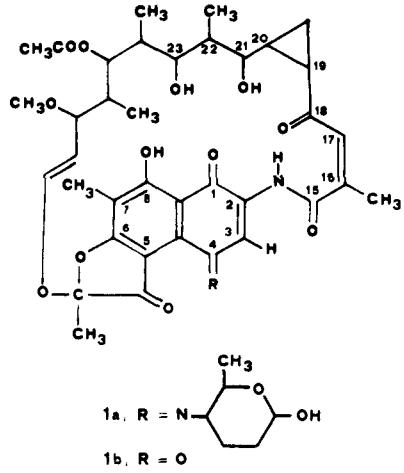
3-Aminotolypomycinones and 3,16-diamino-16,17-dihydrotolypomycinones are formed by the addition of primary and secondary amines to tolypomycinone, obtained by mild hydrolysis of the antibiotic tolypomycin Y. 3-Amino-16,17-dihydrotolypomycinones are formed by the addition of primary and secondary amines to 16,17-dihydrotolypomycinone. In vitro microbiological tests showed high antibacterial activity in compounds obtained by the addition of primary amines, which must be unbranched in the α position to the nitrogen atom, to position 3 of the naphthoquinone ring. The relationship between structure and activity is described, and evidence is presented that hydrogen bonding between the amino NH bonded to C_3 and the amide CO of tolypomycinone is very important for biological activity.

Tolypomycin Y (**1a**) is an antibiotic isolated from fermentation broths of *Streptomyces tolypophorus*.[1-3] The antibiotic has high antimicrobial activity against gram-positive bacteria and is active to some extent also against gram-negative bacteria.[4] Its ansa structure[5-7] is similar to that previously determined for the rifamycins[8,9] and streptovaricins.[10,11] It has also been demonstrated that activity of tolypomycin is due to inhibition of bacterial RNA polymerase as for the other naphthalenic ansamycins.[12,13]



Tolypomycin Y is unstable both to acids and alkalies;[4] mild acid hydrolysis of tolypomycin Y leads to tolypomycinone (**1b**) that is structurally related to rifamycin S but differs from it in having a considerably lower antibacterial activity. We therefore undertook the task of preparing derivatives more stable than tolypomycin Y and with a higher antibacterial activity than tolypomycinone.

From structure–activity relationship (SAR) studies carried out on rifamycin derivatives,[14,15] the hypothesis was advanced that the presence of two free hydroxy groups on C_{21} and C_{23} and of aromatic nucleus with oxygen atoms on C_1 and C_8 is required for antibacterial activity. Also it was hypothesized that only if the hydroxyls on C_{21} and C_{23} have the proper orientation will the derivative be able to form, with bacterial RNA polymerase, the complex responsible for antibiotic activity.[15]

From crystallographic studies of rifamycin SV *p*-iodoanilide and rifampicin,[16] both very active rifamycin derivatives, it has been evidenced that the hydroxy groups on C_{21} and C_{23} are almost parallel to the plane of the naphthohydroquinone nucleus and oriented to the same direction as the phenolic hydroxyls on C_1 and C_8.

The 8,21,23-tri-*m*-bromobenzoate of tolypomycinone, the structure of which has also been determined by x rays,[7] is completely inactive because the hydroxy groups on C_8, C_{21}, and C_{23} are esterified, but the conformation of the ansa corresponding to the C_{21} and C_{23} atoms is very close to that