Synthesis and Structure of Polyarabinouridylic Acid*

Roberto G. Provenzale and Joseph Nagyvary

ABSTRACT: The inversion of the 2′-hydroxyl group in poly-1-β-D-ribofuranosyluracil (poly rU) was effected by the thermal rearrangement of the labile polymeric (2′:3′)→5′-cyclic triester intermediate into poly O^2:2′-cyclouridylate, followed by alkaline hydrolysis. While the degree of ribo–arabino conversion depends on the amount of activating agent used for the generation of the cyclic triesters and a quantitative conversion is possible, an excessive breakdown of the polymer limits the desirable arabino content to around 80%. The heteropolymers of arabinouridylate and ribouridylate, (aU_n,rU_m) and ($aU)_n$-rU, were isolated as the main products in addition to some short homopolymeric arabinouridylates by gel filtration and ion-exchange chromatography. The structure of these compounds were determined by nuclear magnetic resonance, optical rotatory dispersion spectroscopy, and by enzymatic degradations. The outstanding chemical property of polyarabinouridylates is their stability to both acidic and alkaline hydrolysis. According to physical–chemical evidence, none of these polymers exhibits any appreciable degree of secondary structure in the temperature range of 5–60°, and they are also unable to form a complex with poly rA. These results are in accord with the observations of Adler *et al.* (1968) (*Biochemistry 7*, 3836) and Maurizot *et al.* (1968) (*Nature 219*, 377) which were made on dinucleoside monophosphates containing aracytidine. The spatial inhibition of stacking and rotation of the bases by the 2′-hydroxyl groups could be the decisive factor determining the chemistry and structure of polyarabinonucleotides.

Polyarabinonucleotides exemplify the decisive role of the carbohydrate in determining the overall properties of nucleic acids (Adler *et al.*, 1968). A comparative study of the physical–chemical properties of polynucleotides should clarify the intriguing question why the thermodynamically more stable arabinonucleotides and polyarabinonucleotides did not become a part of the evolution of macromolecules. Also, the well-demonstrated therapeutic usefulness of some monomeric aranucleosides (Papac, 1968) suggests that their polymers may possess similar, or even novel effects. The synthesis of this class of compounds requires the development of a new chemical methodology because no enzymatic means are available (Michelson *et al.*, 1962).

The classical approach of linking nucleosides with 3′→5′-phosphodiester linkages in the arabino series involves the problem of suitably protecting the starting materials. An unorthodox method of polymerization was reported by Schramm and Ulmer-Schurnbrand (1967), the mechanism of which is not yet clearly understood. Our concept, first announced in 1967 by Nagyvary eliminates the need for a monomeric starting material altogether. It involves the conversion of the pyrimidine polynucleotides, poly U and poly C, through the labile (2′:3′)→5′-cyclic triester intermediates, into poly O^2:2′-cyclonucleotides (Scheme I, example of poly aU), which are mildly hydrolyzed to poly aU[1] and poly aC. The triesterified intermediate I can be also made *in situ* according to Michelson (1959a,b). The principle of this reaction is very simple but several factors have to be considered to get optimum results for the ribo–arabino conversion and chain length. Our studies on the model compound U-U have already appeared (Nagyvary and Provenzale, 1969); this paper deals with the synthesis and structure of poly aU.

Experimental Section

Materials. Crystalline uridine 2′;3′-cyclic phosphate, pyridinium salt, was prepared by a modification of the original procedure of Michelson (1959a). Bis(tri-*n*-butylammonium)-uridine 2′(3′)-phosphate (Waldhof) was dissolved in pyridine and reacted with 1.1 equiv of ethyl chloroformate for 10 min. Upon concentration the title compound crystallized, the crystals were washed with pyridine–ether mixtures and dried over P_2O_5 (yield 55%). This material remained chromatographically homogeneous for 6 months at −20°.

The "poly U" of Michelson, *i.e.*, a polyuridylate with mixed 2′→5′ and 3′→5′ linkages and with a terminal 2′:3′-cyclic phosphate-[rU2′(3′)p]$_n$U>p- was prepared according to the original procedures (Michelson, 1959a,b). The short oligomers, including trimers, were largely eliminated by dialysis against distilled water, or repeated precipitation from dimethylformamide with ether. The polymer was utilized in the tri-*n*-butylammonium form.

Preparation and purification of tetraphenyl pyrosphosphate, diphenyl phosphorochloridate, *N*,*N*-dimethylforma-

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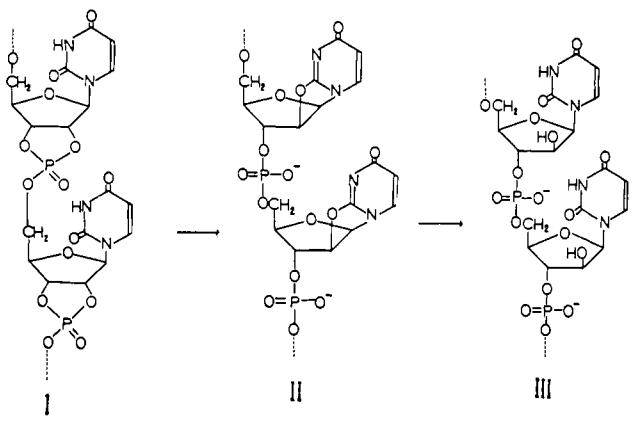
[1] The following abbreviations were employed in accordance with the Extended Rules of the IUPAC–IUB Commission on Biochemical Nomenclature: rU, 1-β-D-ribofuranosyluracil, ribouridine; aU, 1-β-D-arabinofuranosyluracil, arabinouridine; cU, O^2:2′-cyclouridine. The heteropolymer (aU_n,rU_m) contains mixed 2′(3′)→5′ linkages adjacent to the rU sites. Other abbreviations used are: triethylammonium bicarbonate, TEAB; tri-*n*-butylamine, Bu_3N. One optical density unit is the amount of a nucleotidic material which in a 1-cm cell and 1-ml volume exhibits an optical density of 1.00 at a specified wavelength.

SCHEME I



mide, pyridine, dioxane, and tri-*n*-butylamine have been previously described (Nagyvary and Provenzale, 1969). Hexamethylphosphotriamide (Eastman) and analytical grade toluene were filtered through Molecular Sieve (Linde, 4A).

Analytical. Electrophoresis was carried out in a Savant flat-plate apparatus in 0.1 M borate buffer (pH 7). Paper chromatographic separations were performed in 2-propanol–concentrated ammonia–water (7:1:2, A) and in Khorana's solvent system G (Ralph and Khorana, 1961): 1-propanol–concentrated ammonia–water (55:10:35). Eastman Chromagram sheets with fluorescent indicator were used for thin-layer chromatography. For ultraviolet, nuclear magnetic resonance, and optical rotatory dispersion spectral analysis we used Beckman DU, Varian HA 100, and Cary 60 spectrometers. Enzymatic hydrolyses with purified venom phosphodiesterase, and bacterial alkaline phosphatase (Worthington Biochemical Co.) were done according to published procedures (Khorana and Vizsolyi, 1961). Phosphorus was determined according to Ames and Dubin (1960).

For the determination of the chain length, 1 μmole of the oligonucleotide was first dephosphorylated with alkaline phosphatase; after removal of this enzyme by paper chromatography, the oligonucleotides were digested to completion by snake venom phosphodiesterase; the ratio of nucleotide to nucleoside was determined by eluting the corresponding spots from the paper chromatogram and measuring the ultraviolet absorption against appropriate blanks.

Separation Procedures

Separation and chain-length determination of oligomers were performed on Sephadex G-25 and G-50 according to Narang *et al.* (1969) and on G-75 according to Hohn and Schaller (1967). Ion-exchange chromatography on DEAE-cellulose (HCO_3^- form) was done by the standard technic (Staehelin, 1963).

Synthetic Procedures

Method A. Uridine 2′:3′-cyclic phosphate (1 mmole), Bu_3NH^+ salt, was dissolved in a mixture of 10 ml of dioxane and 2 ml of Bu_3N, and the solution was concentrated to a thick syrup; three polymerizations were performed at this scale with 1.2 and 1.4 equiv of tetraphenyl pyrophosphate

TABLE I: Analysis of Sephadex G-25 Chromatography (Figure 4).

Fractions	R_F[a]	aU:rU	Composition (%)
67–77[b]		4.9	$(aU)_5$ (13) and $(aU)_4$-rUp (87)
77	0.37	5.1	$(aU)_4$ (33) and (aU_3)-rUp (67)
82–84	0.51	4.1	$(aU)_3$ (41) and $(aU)_2$-rUp (59)[c]
88–90	0.645	3.6	$(aU)_2$ (56) and aU-rUp (44)[c]
97–101	0.78	3.5	aUp (78) and rUp (22)
113–116	0.985		Not identified
120–122	1.05		Not identified. The compound is not affected by phosphatase.
132–134	1.21		

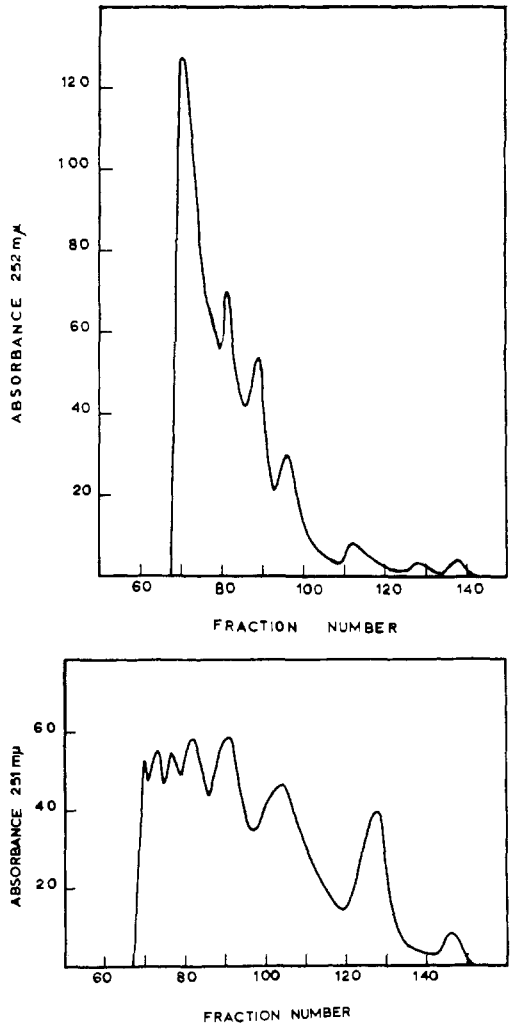
[a] R_F with respect to rUp in solvent G. [b] Further chromatographed on Sephadex G-75. [c] The mixtures were resolved by electrophoresis in borate buffer (2500 V, 2 hr) after 3′-phosphate removal, and the products subsequently identified. Electrophoretical mobilities: aU-rU, 33.0 cm; aU-aU, 23.7; aU-aU-rU, 33.9; aU-aU-aU, 29.6.

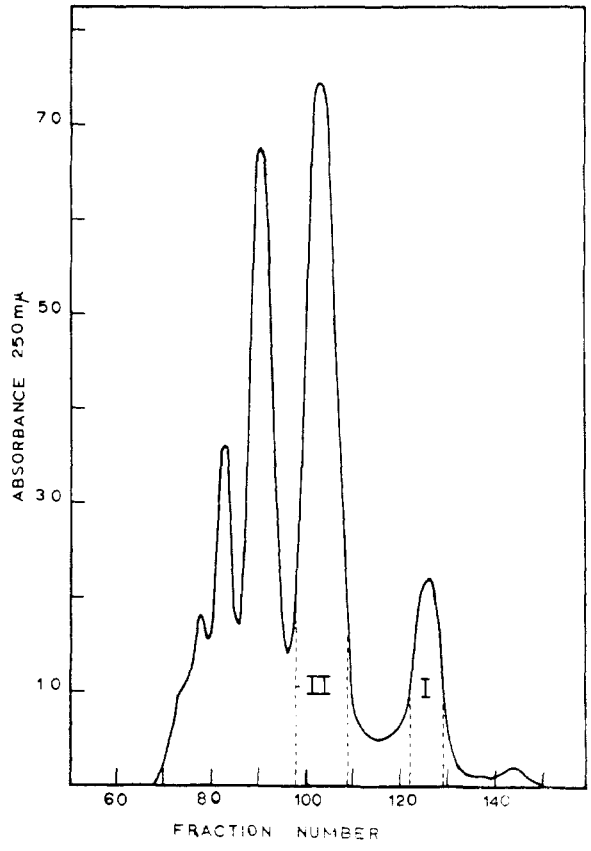
(expt A-1 and A-2) and 1.6 equiv of diphenyl phosphorochloridate (expt A-3), which were added under cooling. The two-phase mixtures were vigorously shaken for several hours and left at room temperature overnight in well-sealed flasks; dry dimethylformamide was then added until clear solutions were obtained, which were kept for 10 hr at 90° or 24 hr at 65°. The hypsochromic shift of the ultraviolet maximum was followed on small aliquots until constancy was reached. After the addition of a small amount of ice, the solutions were concentrated and precipitated with ether. The following ultraviolet maxima were found: A-1, 252 nm (6180 optical density units); A-2, 251 nm (7080 optical density units); A-3, 250 nm (5220 optical density units). All three preparations were passed through columns of Sephadex G-25, superfine (100 × 2.5 cm) in 0.2 M triethylammonium bicarbonate (pH 8) at a rate of 18 ml/hr, and the ultraviolet profile was measured manually (see Figures 1–3).

A-1. Fractions 67–95 (4400 optical density units) were freeze dried and hydrolyzed in 1 N KOH for 24 hr at 20°; then the solution was neutralized with perchloric acid and the precipitate was centrifuged. The oligoarabinonucleotides present in the supernatant were separated on the same Sephadex G-25 column. (See Figure 4 and Table I for distribution and composition of the peaks.) The oligonucleotide fractions of pentanucleotide and higher, which comprised the fractions 67–77, were distributed again on a Sephadex G-75 column; conditions are given in Figure 5.

A-2. Fractions 67–85 from a Sephadex G-25 fractionation (Figure 2) containing 2025 optical density units of (cU_n,rU_m) were repeatedly freeze dried from D_2O for nuclear magnetic resonance determination (Figure 6). Then the same sample was hydrolyzed with 0.2 N KOH until the ultraviolet maximum shifted to 262 nm (about 1 hr) and neutralized with hydrochloric acid. The product thus obtained, (aU_n,rU_m), was used for nuclear magnetic resonance (Figure 7).

A-3. The identification of the short oligomers obtained by gel filtration (Figure 3) is given in Results.





FIGURES 1, 2, AND 3: Distribution curves of the primary products (cU_n,rU_m) from expt A-1, A-2, and A-3 on Sephadex G-25 *superfine columns* (100 × 2.5 cm) in 0.2 M TEAB. Fractions of 3 ml were collected every 10 min.

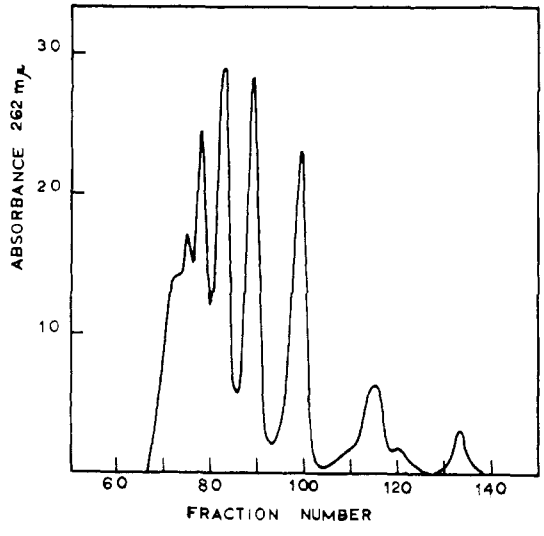


FIGURE 4: Distribution of oligonucleotides type $(aU)_n$-rUp obtained from A-1, fractions 67–95 of Figure 1. The same column and conditions were used.

Method B. The 2′(3′)→5′-poly U (Michelson, 1959a,b) (1 mmole) was dissolved in 5 ml of hexamethylphosphotriamide and dried by repeated addition and evaporation of toluene. Finally, 1 ml of Bu_3N and 1.6 equiv of diphenyl phosphochloridate, or tetraphenyl pyrophosphate, were added, and the sealed reaction mixture was kept at 65° for 24 hr. The polymeric material was precipitated and washed with ether and the residue (0.6–0.7 mmole) was hydrolyzed as in method A and separated on G-25 and G-50 columns (Figures 8 and 9). In another reaction which was carried out under identical conditions and gave about 50% conversion the chemical hydrolysis was followed with phosphatase treatment. The dephosphorylated oligomers were separated on a DEAE-cellulose (HCO_3^-) column (Figure 10a,b) and their properties are given in Tables II and III.

Results

The oligo- and polynucleotides which we obtained as the primary product were heteropolymers of O^2:2′-cyclouridine and ribouridine (cU_n, rU_m), and of arauridine and ribouridine

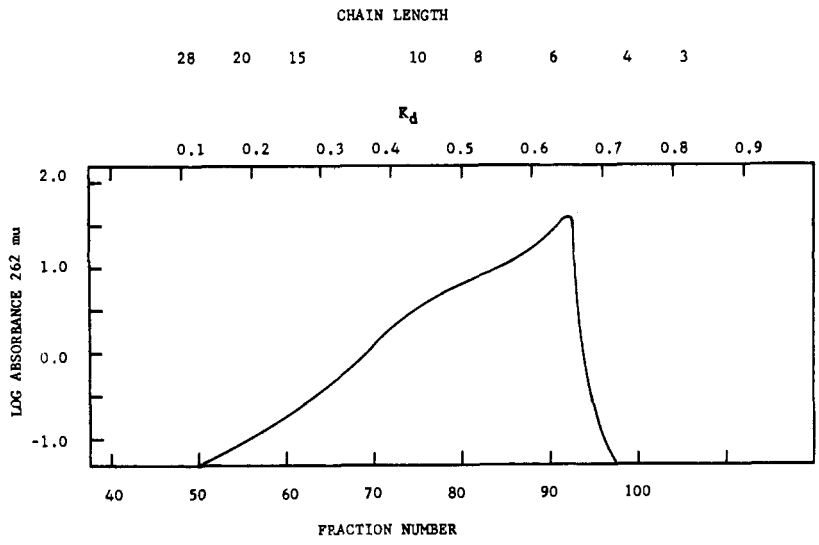


FIGURE 5: Redistribution of the longest oligonucleotides of A-1 from fractions 67–77 (Figure 4) on a Sephadex G-75 column (115 × 2.1 cm) in 0.01 M TEAB. Fractions of 4 ml were collected every 20 min. The void volume (160 ml) and the inner volume (380 ml) were measured with Blue Dextran (Pharmacia) and tritiated water, respectively. Chain lengths were calculated according to Hohn and Schaller (1967).

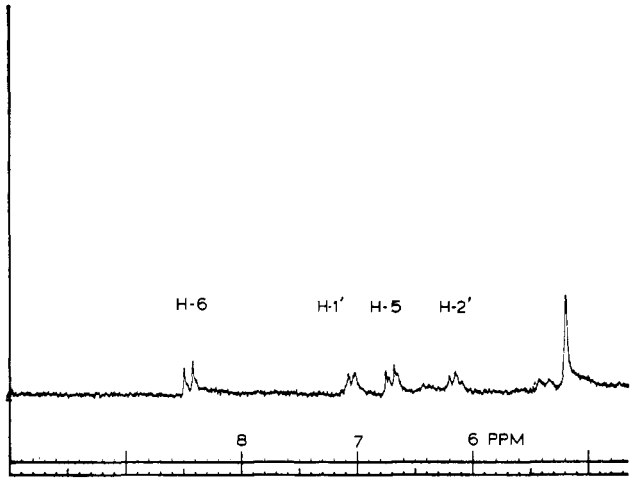


FIGURE 6: The 100-MHz nuclear magnetic resonance spectrum of (cU_n, rU_m), n/m = 9, which was obtained from expt A-2, fractions 67–85 (Figure 2). The sample, 2000 optical density units in 0.3 ml of D_2O, contained a Me_4Si capillary and was kept at 28°.

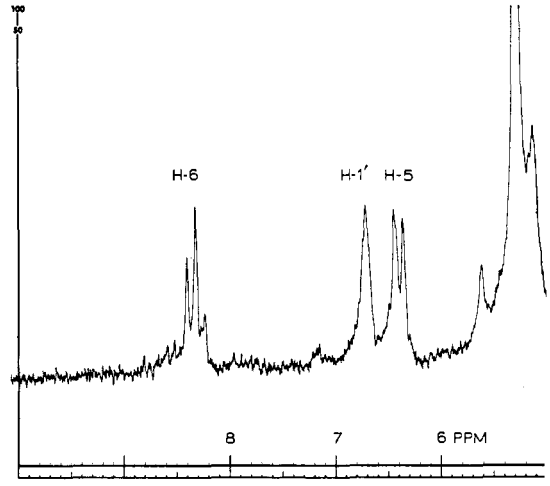
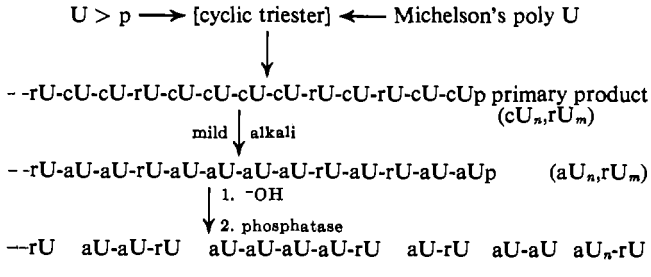


FIGURE 7: The 100-MHz nuclear magnetic resonance spectrum of (aU_n, rU_m) obtained by hydrolysis from the sample described in the legend to Figure 6. The D_2O solution was 0.1 M in KCl.

(aU_n, rU_m), respectively. Alkaline hydrolysis of these polymers gave a mixture of oligonucleotides consisting of homopolymeric stretches of aU with an rUp at the 3′ end. In some cases short homopolymers of cU and aU could also be obtained. A schematic illustration of this process is given in Scheme II.

SCHEME II



The underlying principle of the preparation of these novel polynucleotides is the same in both methods A and B, namely, the formation of polymeric (2′:3′)⟶5′-cyclic triesters and a thermal rearrangement thereof (Scheme I). However, the *de novo* approach is favored over the conversion of preformed poly U for several practical reasons, which will be discussed further below.

We have selected three typical experiments carried out *via* method A which show the influence of the activating agent. In our recent study on rU-rU as a model (Nagyvary and Provenzale, 1969), we found that the optimum yield of cU-rU is obtained when a very exactly equivalent amount of activating agent is employed. The expt A-1, A-2, and A-3 were carried out with increasing amounts of activating agent, from 1.2 to 1.6 equiv. The ultraviolet maxima of the reaction products (cU_n,rU_m) were 252, 251, and 250 nm, indicating an increasing cU content. Figures 1, 2, and 3 reveal, however, that improvement of conversion can only be obtained at the expense of chain length. The reaction mixture A-3 which

TABLE II: Analysis of DEAE-cellulose Chromatography (Figure 10a,b).

Peak	Fractions	R_F[a]	aU:rU	$[R]_D^{20}$[b] Found[d]	$[R]_D^{20}$[b] Theory[e]	Composition
I	8–14[c]					rU
II	46–54[c]	1.16	1.05	165	166.5	aU-rU
III	84–98[c]	0.895	2.1	221	214	$(aU)_2$-rU
IV	128–142[c]	0.644	2.98	238	238.2	$(aU)_3$-rU
V	170–190[c]	0.46	3.95	254	252.6	$(aU)_4$-rU
VI	96–110[f]	0.355				$(aU)_5$-rU
VII	128–140[f]	0.25				$(aU)_6$-rU
VIII	154–170[f]	0.158				$(aU)_7$-rU

[a] Relative to rUp in solvent G. [b] Mean residue rotation in deg cm²/dmole. [c] From Figure 10a. [d] Concentrations of the aqueous solutions were in the range of 30–90 optical density units/ml. [e] Calculated assuming $[M]_D^{20}$ 310° for aU, and 23° for rU. [f] From Figure 10b.

exhibits a pure cU spectrum has only a chain-length range of 1–8, while another experiment with a 50% cU content (not described here in details) afforded polymers containing up to 40–50 nucleotides. A compromise must be accepted between high conversion and high molecular weight, as it is exemplified in expt A-1.

In fact, although the conversion in A-1 takes place only to an extent of 70–80%, Sephadex G-25 fractionation after alkaline hydrolysis shows the presence of 23% of polymers longer than pentanucleotides (Figure 4, fractions 67–77), and the Sephadex G-75 chromatography indicates that polymers 15–20 nucleotides long are still present (Figure 5).

The remaining fractions of the Sephadex G-25 chromatography were pooled as indicated in Table I and the nature of the various peaks was investigated. The single components were directly examined in solvent G, and all of them gave homogeneous spots. Aliquots were also digested with the combined action of venom phosphodiesterase and alkaline phosphatase, and the ratio aU:rU was determined. These data are given in Table I, from which the percentage of 3′-terminal aU has been calculated. These results clearly indicate the presence of homopolymers $(aU)_n$, from which the shortest ones $(aU)_2$ and $(aU)_3$, could be resolved by electrophoresis in borate buffer and subsequently identified.

The Sephadex G-75 distribution of the polymers $n > 5$ is depicted in Figure 5 with the assignment of chain length according to Hohn and Schaller (1967). The chain length of fractions 76, 84, and 90 was also determined *via* enzymatic degradation and the nucleotide to nucleoside ratios were 8.6, 7.1, and 5.1, respectively, in good agreement with the theoretical values calculated from the distribution curve.

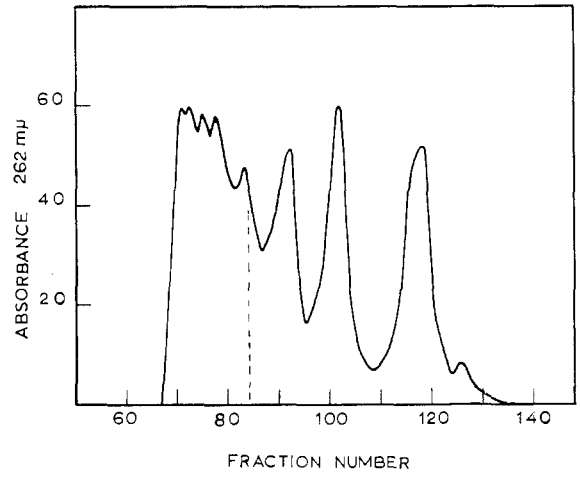


FIGURE 8: Preliminary fractionation of oligomers obtained by method B after alkaline hydrolysis. About 5000 optical density units was applied to a Sephadex G-25 column (100 × 2.5 cm) in 0.2 M TEAB and 2.8-ml fractions were collected every 10 min.

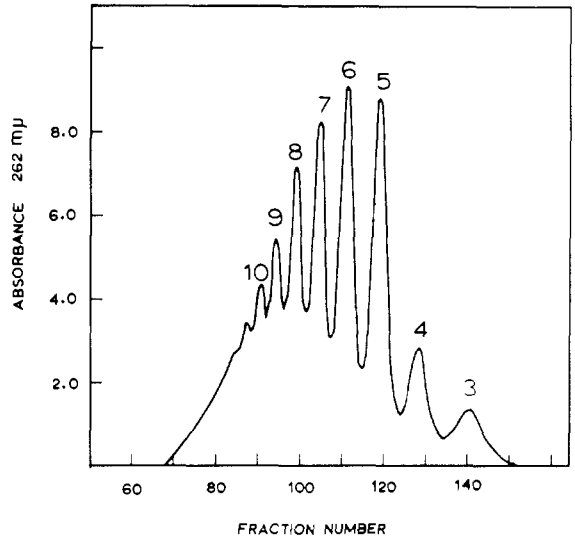


FIGURE 9: Distribution of oligoarauridylates type aU_n-rU on Sephadex G-50 superfine (100 × 2.5 cm) in 0.1 M TEAB; fractions of 2.8 ml were collected every 10 min. The oligonucleotide mixture, about 700 optical density units, was obtained from fractions 66–63 of a preliminary fractionation (Figure 8) and was treated with phosphatase prior to separation. The number of nucleoside units per molecule is indicated over each peak.

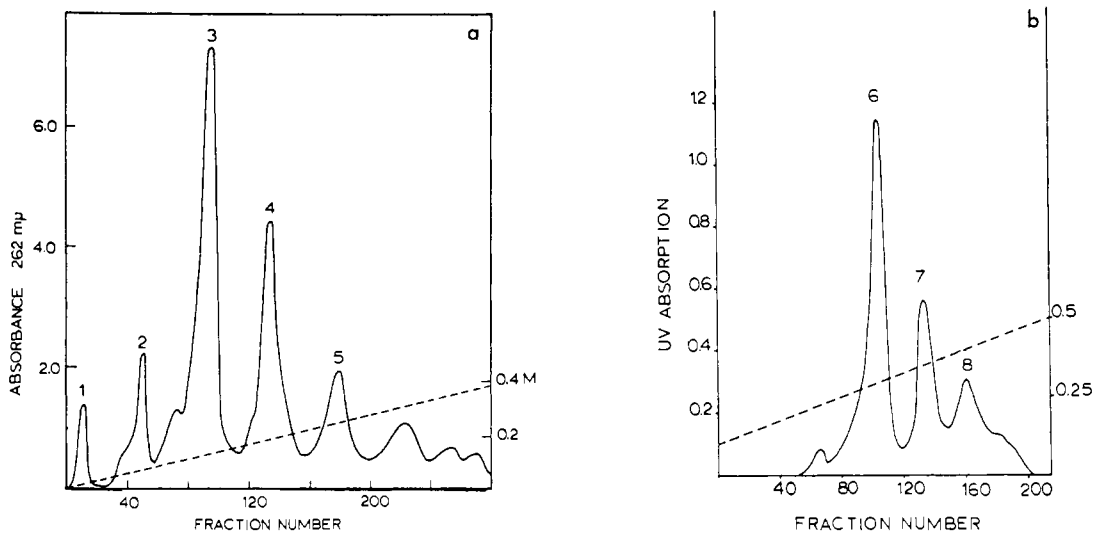


FIGURE 10: Ion-exchange chromatography of oligonucleotides type aU_n-rU. The primary product obtained by method B was repeatedly precipitated from dimethylformamide with ether in order to extract the short oligomers. The residue was hydrolyzed with alkali and with phosphatase, and it was applied to a 50 × 2.2 cm DEAE-cellulose (HCO^-_3) column. The elution was carried out with a linear gradient (2 l. of H_2O–2 l. of 0.4 M) of TEAB, and 14-ml fractions were collected every 10 min (a). The higher oligonucleotides above fraction 200 were pooled and separated again on the same column (2 l. of 0.1 M to 2 l. of 0.5 M TEAB). The numbers above the various peaks in parts a and b correspond to the number of nucleotide units per molecule.

TABLE III: Cotton Effect of Oligo- and Polyarauridylates.

	Peak		Crossover, λ (mμ)	Trough		Amplitude,
	λ (mμ)	$[m] \times 10^{-3}$		λ (mμ)	$[m] \times 10^{-3}$	$[m] \times 10^{-3}$
aU-rU[a]	282	9.45	268	252	−13.9	23.35
$(aU)_2$-rU[a]	282	13.45	268	252	−17.0	30.45
$(aU)_3$-rU[a]	282	13.6	268	252	−17.9	31.5
$(aU)_4$-rU[a]	282	14.1	268	252	−19.0	33.1
$(aU)_5$-rU[a]	282	14.7	268	252	−19.3	34.0
$(aU)_n$-rUp[b]	282	9.5[c]	268	252	−15.5[c]	25.0
(n = 15–20)	282	9.5[d]	268	252	−15.0[d]	24.5

[a] In water. [b] In 0.08 M NaCl with 0.01 M phosphate buffer (pH 7.2). [c] At 5°. [d] At 60°.

The $\epsilon(P)$ of the fraction corresponding to $(aU)_{9-11}$-rUp was found to be 9200, which compares favorably with the 8% hyperchromicity experienced following the digestion of the same fraction with snake venom phosphodiesterase.

The reaction mixture A-2 is characterized by a higher, about 90%, conversion as witnessed by the ultraviolet (λ_{max} 251 nm) and nuclear magnetic resonance spectra (Figures 6 and 7). Although the primary product is only about 90% homogeneous, it was possible to assign some of the signals on the basis of our previously taken spectra of the corresponding dinucleoside monophosphates (Nagyvary and Provenzale, 1969). The protons of the cyclouridine moiety appear as follows: H-6, doublet at 8.46 ppm (J = 7.5 cps); H-5, doublet at 6.72 (J = 7.5 cps); H-1′, doublet, at 7.02 (J = 3.6 cps). The corresponding (aU_n, rU_m) which was obtained from the first sample by mild alkaline hydrolysis gave the same signals shifted upfield: H-6, at 8.36 (J = 7.5 cps); H-5, at 6.41 (J = 7.5 cps) and H-1′, at 6.72 (J = 3.6 cps). The major difference between the two spectra is the clear appearance of the H-2′ of the cyclouridine as a triplet at 6.15 (J = 5.2 cps).

Experiment A-3 is interesting because it sheds light to the processes that might cause degradation of the polynucleotide chain. Peaks I and II in Figure 3 were analyzed on paper and thin-layer chromatography prior to and after hydrolysis. The compound in peak I is the monoester of a nucleoside (λ_{max} 250 nm) that after hydrolysis migrates significantly faster on chromatography (benzene–EtOH, 3:1) than aU, while on paper in solvent A their mobilities are about the same. Peak II contains a linear dinucleotide (λ_{max} 250 nm) from which three nucleosides could be isolated after hydrolysis. The major component, about the half, is aU; one of the minor components is the nucleoside from the peak I, and the third nucleoside is the fastest moving on chromatography. This last product could be tentatively identified as 5′-Cl-aU by

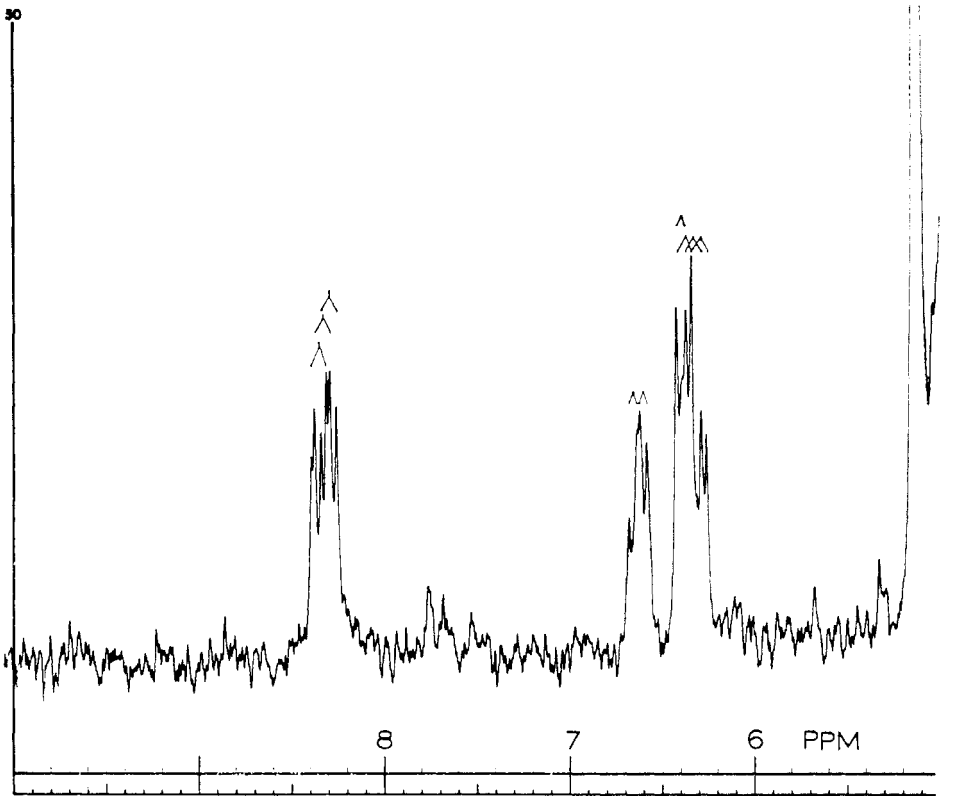


FIGURE 11: The 100-MHz nuclear magnetic resonance spectrum of aU-aU-rU in D_2O relative to Me_4Si as external, standard at 26°.

direct comparison with an authentic sample (R. G. Provenzale, 1969, unpublished results). We assume that the unknown nucleoside is an elimination product bearing a 5′ double bond, but further work will have to prove this point.

The increasing occurrence of aU at the 3′ end with increasing activation deserves a special emphasis. The elimination of a fully activated pyrophosphate in the middle of a $(cU)_n$-rUp could be one mechanism that would lead to the formation of short homopolymeric $(cU)_n$. On the other hand, the terminal ribouridine 2′:3′-cyclic phosphate can apparently also rearrange during the prolonged activation as the diphenyl phosphoryl anhydride. While the rearrangement of such cyclic intermediates is much more facile in the case of cytidine derivatives (Nagyvary and Tapiero, 1969), nevertheless, we were able to demonstrate it on 5′-*O*-mesyluridine 2′:3′-cyclic phosphate to an extent of about 10% (R. G. Provenzale and J. Nagyvary, unpublished). The direct conversion of the 2′:3′-cyclic phosphate ends is obviously the case in expt A-1 which did not contain excessive activating agent. The occurrence of the 3′-aUp decreases with the chain length as is shown in Table I. This is not unexpected, since the longer homopolymeric stretches are randomly distributed within the chains.

The only by-products in these reactions were 5′-modified oligonucleotides; 3′-end modifications, which are theoretically feasible (for example, symmetrical pyrophosphates), could not be detected.

The shortest oligomers in the primary product contain a relatively higher degree of modifications; therefore, we discarded these fractions prior to hydrolysis.

Method B. THE CONVERSION OF POLY rU INTO POLY aU. The principle of this approach is similar to the previous one; however, the reproducibility of results is erratic. Therefore, only a few experiments were carried out, which gave generally short oligomers as illustrated in Figures 8 and 9. A conversion of 50% is considered a good average in this approach. All the oligomers obtained after hydrolysis contain rU moieties at the 3′ end, and the aU:rU ratios corresponded to the theoretical values. The residue rotation values $[R]_D^{20}$ of the series $(aU)_n$-rU were in agreement with those calculated from the rotation of the monomers (Table II). The amplitudes in optical rotatory dispersion (Table III) follow the same tendency, although they are less sensitive to the change.

The obvious reason for the poor reproducibility of occasional good conversions by method B lies in the uncertainty with regard to the actual degree of activation. As we have shown in our previous paper, an excess of activating agent inhibits the conversion because it causes substitution of N-3 and thus diminishes the nucleophilicity of the 2-carbonyl group. A lower degree of activation means *a priori* a low conversion. While in method A the presence of polymeric cyclic triesters is proportional to the degree of observed polymerization, no such simple assay is available in method B. Since the drying of poly rU cannot be well standardized, the outcome of the experiment depends greatly on chance. Portionwise addition of the activating agent, followed each time by a 24-hr reaction period is one way to improve the conversion, but the original chain length cannot be maintained on prolonged heating because of the lability of the diester linkage.

Structure of Polyarabinouridylic Acid

The stability of the internucleotide linkage between two aranucleosides was first emphasized by Wechter (1967). He found that the mild alkaline treatment (0.1 N KOH, 37°, 16 hr) which degrades RNA left the 3′-arabinonucleotidyl esters completely unaffected. We could confirm the stability of the interarabinonucleotide linkage toward alkali under similar conditions in case of aUaUrU. The same trinucleotide proved to be completely stable also in 0.1 N HCl at 60° for 18 hr, and in water at 90° for the same period of time. This finding is less self-evident than it first appears. Although it is impossible to form 2′:3′-cyclic phosphates of aranucleotides, on the other hand, 2′:5′-cyclic phosphates can be obtained, and they are very stable (Wechter, 1969). The lack of nucleophilic attack by the 2′-hydroxyl on the 5′-ester, which is in the *cis* position, may be due to steric hindrance, which does not exist in monomeric aranucleotides.

The mutual shielding and interaction of the uracil base and the 2′-OH in polyarauridylate is apparently a major factor in determining the overall characteristics of this polynucleotide analog. The structure of dinucleoside monophosphates containing one or two arabinosyl moieties has been studied by physical-chemical methods such as optical rotatory dispersion (Maurizot *et al.*, 1968), ultraviolet and circular dichroism (Adler *et al.*, 1968). Practically no stacking interaction could be demonstrated between the two bases. Our measurements of $[\alpha]_D$ and optical rotatory dispersion of a series of the oligonucleotides $(aU)_n$-rU show that these data can be derived directly from the monomeric rotations (Tables II and III). The optical rotatory dispersion curves of the longest polynucleotide analyzed by us, $(aU)_{15-20}$-rUp, were found identical at 5 and 60° (Table III). The related polynucleotides poly rU and poly dT exhibit a significant change of their optical rotatory dispersion amplitudes in the same temperature range (Ts'o *et al.*, 1966). It seems that the increase in chain length of aranucleotides from $n = 2$ to 15–20 does not confer an increased tendency for stacking. This conclusion was corroborated by the results of ultraviolet measurements on the same sample ($n = 15$–20) that showed only 1% change from 2 to 60°.

The nuclear magnetic resonance spectra of arabinonucleotides are characterized by a general deshielding of the H-6, H-5, and H-1′ protons as compared with the analogous ribonucleotides (Wechter, 1967; Nagyvary and Provenzale, 1969). Unfortunately, major conclusions gained from the nuclear magnetic resonance of oligoribonucleotides do not seem to be valid in the arabino series, and the interpretation of these spectra is quite tentative. For example, no specific deshielding of the H-6 by the 5′-phosphate of aracytidine as compared with 3′-ara-CMP is apparent from the data of Wechter (1967), although this is very typical of the natural nucleotides in the *anti* conformation (Ts'o *et al.*, 1969). Yet it is hardly conceivable that all aranucleotides would be locked in the energetically and sterically unfavorable *syn* conformation.

We have taken the nuclear magnetic resonance spectrum of aU-aU-rU (Figure 11) for comparison with the previously described (aU_n,rU_m) spectrum (Figure 7). The good resolution of the corresponding protons in the terminal and central nucleosides is remarkable. Our tentative assignment of the signals in each multiplet is based on the assumption that the twice-esterified central aU should experience the strongest deshielding, while rU is the most shielded nucleoside. Accordingly, the doublets H-6 (at 8.30, 8.34, and 8.36 ppm), H-5 (at 6.30, 6.34, and 6.38 (J = 7.5–8.0 cps)), and H-1′ (at 6.40, 6.61, and 6.66 (J = 3.5–4.0 cps)) originate from the rU, central aU, and terminal aU, respectively. The comparison of Figures 7 and 11 shows no apparent polymer shift.

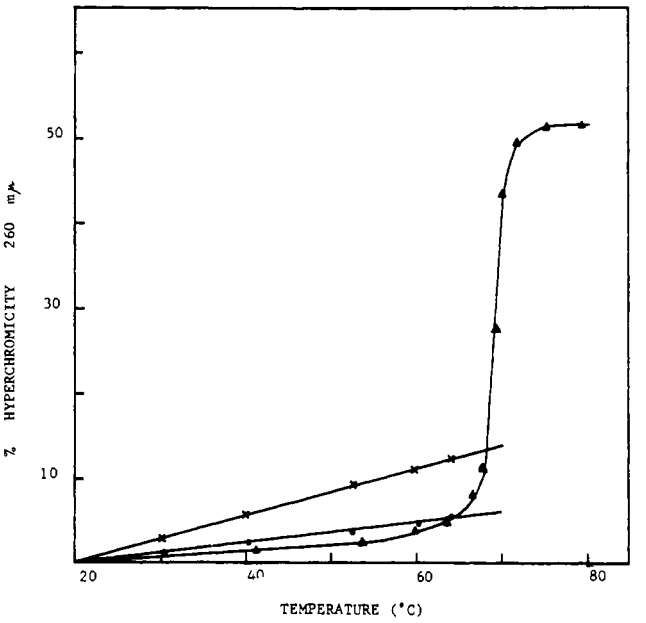


FIGURE 12: Ultraviolet absorbance–temperature profile of poly rA (-×-×-×-), poly rA·poly aU (-●-●-●-), and poly rA·poly rU (-▲-▲-▲-) in 0.05 M cacodylate buffer (pH 7). The solutions were 0.1 M in NaCl and 0.01 M in $MgCl_2$, and the concentration of the polymers was 60 mμmoles of nucleotide/ml. The per cent change of A_{260} was measured in a thermostated cell holder; the temperature was determined with a Gilford thermocouple in one of the cells. No correction was made for thermal dilatation.

The H-6 and H-5 protons of both aU and oligo aU are deshielded relative to rU. It is likely that this shift is due to the interaction of the 2′-OH with the uracil base. The nature of this interaction could be a weak bonding to the $C_{5,6}$ double bond or a general perturbation of the whole electronic structure. A small angle H bonding to the 2-carbonyl is less likely, although other authors (Ts'o *et al.*, 1969; Brahms *et al.*, 1969) have made this assumption based on the findings in the ribo series. Since the pK of the NH group is expected to reflect the changes in hydrogen bonding, we have measured these pK's for aU and $(aU)_{15-20}$-rUp. The spectrophotometric determination was carried out in 0.1 M NaCl–0.05 M sodium cacodylate and gave the values 9.32 for aU and 9.73 for the polymer. This is practically the same as the pK's of ribouridine, 9.2, and poly rU, 9.7, given by Richards *et al.* (1963). If one assumes that the close similarity of the pK values in the ribo and arabino compounds is due to a similar extent of the C=O···HO interactions, one would not expect such a marked chemical shift of the H-6 and H-5 protons in the nuclear magnetic resonance spectra. For this reason, a rigid hydrogen bonding to the carbonyl group is not likely. The supposed perturbation of the ring current might be one factor prohibiting the stacking of bases. The other, and probably the major factor could be the spatial shielding of

the bases by the 2′-OH, as was pointed out by us (Nagyvary *et al.*, 1968) and more explicitly by Ts'o *et al.* (1969) on the basis of model building. More significant than the absence of any ordered structure in poly aU is its inability to be drawn into a helix by poly A (Nagyvary *et al.*, 1968). The mixing of equimolar quantities of poly A and (aU_{15-20}-rUp in 0.1 M sodium cacodylate (pH 7.6), and 0.01 M $MgCl_2$ failed to reveal any hypochromicity. The change of absorbancy in the range of 8–65° was due to the poly A only, as it is shown in Figure 12. The lack of double-strand formation does not immediately follow from the spatial inhibition of stacking by the 2′-hydroxyl groups, since both the 4-keto and 3-NH groups are available for hydrogen bonding with adenine. Thus poly aU may be a good example to show that hydrogen bonding alone is not sufficient to hold a double-stranded complex. One is tempted to think, however, that, in spite of the availability of the usual electron-donor and -acceptor groups, even the intermolecular hydrogen-bonding capability of the system could be affected by the neighboring hydroxyl group.

Another possible factor contributing to the randomness of the structure of poly aU is the hindrance to rotation of the bases around the glycosidic linkage. The bases might be fixed in a manner which would allow only a very limited rotation. At the present time we cannot exclude the presence of a certain number of *syn* rotational isomers in these polymers, although the *anti* isomers should be predominant. In our knowledge, the existence of *syn* aranucleosides has not yet been seriously considered in the literature. However, the aranucleoside moiety in our poly aU was derived from a polymeric precursor, and the exact stereochemistry of its formation is not really clear. Perhaps, it might be advisable to consider poly aU preparations which were obtained *via* conversion and *de novo* under separate label until their identity will be more strictly proven.

In conclusion, poly aU can be formed directly from a poly rU intermediate in addition to *de novo* approaches. Evidence obtained by ultraviolet, optical rotatory dispersion, and nuclear magnetic resonance studies indicate that our poly aU is completely devoid of secondary structure. The stability of this compound to hydrolysis, as compared with poly rU, could be primarily due to the alteration of a kinetic factor, *i.e.*, the lack of neighboring group participation. The thermodynamic consequences of the inversion should also be kept in mind in considering the imposed rotational rigidity of arabinonucleosides and the less rigid organization of ribonucleosides in contrast to the differing capability of their polymers to assume organized structure. Although this phenomenon can be best understood in terms of direct inhibition of stacking interactions, it is also possible that an excessive decrease in the rotational entropy of the mononucleotide precludes the further lowering of total entropy that would follow the process of helical organization.

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