SINGLE-STRANDED OLIGOMERS AND POLYMERS OF CYTIDYLIC AND 2'-DEOXYCYTIDYLIC ACIDS: COMPARATIVE OPTICAL ROTATORY STUDIES*

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The use of synthetic polynucleotides as models for RNA and DNA has recently received much attention. Evidence, much of it based upon optical rotatory dispersion (ORD)[1] for detection of asymmetric secondary structures, has accumulated for the importance of base-stacking interactions, as well as for hydrogen bonding, in stabilizing RNA in aqueous solution. One of the remaining problems is to examine the effect of the carbohydrate residue upon macromolecular properties, that is, to compare the structure of ribonucleotides to those of the analogous deoxyribonucleotides.

At neutral pH, polycytidylic acid (poly-rC) exists as a nonprotonated,[2] single-stranded helical structure.[3, 4] This conformation is very different from the half-protonated, double-stranded[5] helix held together by cytosine-cytosine hydrogen bonds in acid solution. The properties of single-stranded poly-rC can be calculated fairly well from consideration of nearest-neighbor base-stacking interactions.[6] Similarly, polydeoxycytidylic acid (poly-dC) undergoes a structural transition during titration, although this polymer differs markedly from poly-rC in its titration curve and hypochromicity,[7] and in the stability of its complexes with poly-rI[8] and with poly-rG.[9]

Several studies (utilizing ORD,[10-12] circular dichroism,[13, 14] and hypochromism[15, 16]) have investigated the forces involved in oligoriboadenylic acids, and have traced the building up of helical structures characteristic of poly A. The present report is concerned with a comparison of the structure and stability of oligocytidylic acids, as well as high-molecular-weight polymers, of both the ribocytidylic and deoxyribocytidylic series. Of these, only the dinucleoside monophosphate cytidyl (3' → 5') cytidine (r-CpC), at room temperature, has been previously studied.[6, 17]

Materials.—CMP-5' was purchased from Schwarz BioResearch, dCMP-5' from Pabst, and r-CpC from Calbiochem. Poly-rC, the K^+ salt from Miles Chemical Co., had $s = 7.18$. Preparation and characterization of rC oligomers (chain length 3 to 14) will be reported.[18] We are grateful to Dr. M. J. Chamberlin for a sample of poly-dC.[8] The dC oligomers, as NH_4^+ salts of chain length 2–10, were a gift from Dr. H. G. Khorana; they were synthesized chemically.[19] Water was redistilled from glass; ethylene glycol was Fisher reagent grade.

Solutions and Concentrations.—The solvent for temperature-dependence studies was 0.08 *M* NaCl, 0.02 *M* tris-tris hydrochloride, pH 8.5. Far-ultraviolet (<225 mμ) spectra and ORD were run in 0.1 *M* NaF, adjusted to a suitable pH with HCl or NaOH. Polymer solutions were heated briefly and slow-cooled to ensure formation of the equilibrium conformation.[3] All solutions were run through Millipore filters. Concentrations were usually in the range of 0.7 to 1.1 $\times 10^{-4}$ *M* cytosine residues, corresponding to a maximum absorbance of 0.4–0.8 for a 1-cm path length. Exceptions to this range occurred during concentration-dependence experiments, and in the ORD scan of poly-dC (Fig. 1, concentration 3.1×10^{-4} *M*, 2-mm path).

The extinction coefficients which were used for determination of oligomer concentrations are listed in Table 1. They were obtained by measurement of the loss of hypochromicity upon transfer of the nucleotides from pH 8.5 aqueous buffer into 90% ethylene alcohol (other buffer com-

TABLE 1

DERIVED MAXIMUM EXTINCTION COEFFICIENTS OF OLIGOMERS[*]

	$\epsilon_{max} \times 10^{-3}$	
Chain length	Ribo series	Deoxyribo series
1	9.15	9.15
2	8.42[†]	8.62
3	8.14	8.49
4	7.96	8.37
5	7.80	8.28
6	7.68	8.22
7	7.58	8.16
8	7.50	8.12
9	7.43	8.08
10	7.37	8.04
11	7.32	...
12	7.27	...
13	7.24	...
14	7.20	...
Polymer	6.32[‡]	7.62[§]

[*] Per mole of cytosine residues at 22°. The values refer to a solvent of 0.08 *M* NaCl plus 0.02 *M* tris, pH 8.5. The monomer values were averaged from published data, which ranged from 9.0 to 9.3. The others were obtained by the ethylene glycol method outlined in the text, were plotted and obtained from the fitted curve, and are probably reliable to ±4%.

[†] Cf. value of 8.3 for r-CpC found by Warshaw and Tinoco.[17]

[‡] Cf. value of 6.3 for poly-rC (Warner, R. C., *J. Biol. Chem.*, **229**, 711 (1957)).

[§] Cf. value of 7.55 for poly-dC found by Inman.[7]

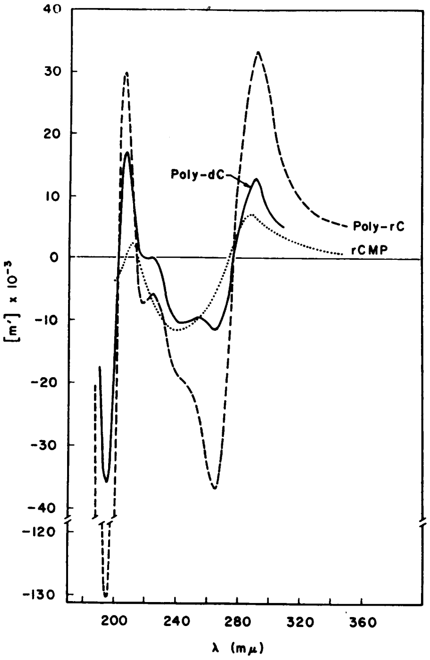
ponents remaining constant). In this solvent mixture, ORD measurements indicated complete loss of secondary structure; therefore, it is assumed that in 90% glycol all oligomers and polymers would have the mononucleotide value of ϵ_{max}. In practice all values were corrected for an apparent 4% residual hypochromicity in glycol, required to have the observed ϵ_{max} for poly-rC coincide with published data determined by more absolute methods.[3]

Methods.—All optical measurements were performed in jacketed, fused quartz cells (Optical Cell Co., Brentwood, Md.). Aliquots of the same solution in the same cell were used for corresponding ORD and absorbance runs. Values of $[m']$ and ϵ were corrected for volume expansion upon heating. Cell temperature was monitored by means of a bimetallic probe (Yellow Springs Instruments) placed in the heating jacket. A Cary model 14 recording spectrophotometer was used for absorbance measurements.

ORD data were obtained with a Cary model 60 recording spectropolarimeter, set for a half-band width of 15 Å. Data are reported in terms of $[m']$, the reduced rotation per mole of cyosine residue, which incorporates the refractive index correction, $3/(n^2 + 2)$.[20] The dispersion of n for ethylene glycol was measured with a Bausch and Lomb refractometer ($n_{589} = 1.4311$, $n_{546} = 1.4330$, $n_{436} = 1.4400$); ultraviolet values were obtained by extrapolation. Values for n for glycol-water mixtures were found to be proportional to glycol concentration. The ORD curves could be read to ±0.0003 degrees in the wavelength range 230–310 mμ. This corresponds to an experimental error of ±500 in $[m']$.

Results and Discussion.—Optical properties at room temperature: The comparison of the ORD spectra of nonprotonated poly-dC, poly-rC, and rCMP is seen in Figure 1; dCMP appears in Figure 2. Because of the different pK$_a$'s for the rC and dC polymers, different pH's were chosen to ensure that the neutral cytosine species existed.[2, 7] The double Cotton effect in the wavelength region above 220 mμ, which is characteristic of polynucleotides displaying stacking interactions between bases, is present in both of the polymers, and in all the dC and rC oligonucleotides, but not in the monomers. The peak and trough wavelengths are identical for poly-dC and poly-rC, thus indicating that both polymers have the same type of helical conformation. However, the magnitudes of the Cotton effects are twofold to threefold greater in poly-rC, implying a higher degree of stacking at room temperature. Similar augmented rotation is seen in all rC oligomers when they are compared to dC oligomers of the same chain length (Table 2). Other ORD data on poly-dC,[30] poly-rC,[3, 4, 17] r-CpC,[17] and the two monomers[21] agree well with the presented curves, which have been extended to lower wavelengths. Yang and co-workers[21] report data on d-CpC at pH 7.5, under which conditions the first Cotton effect is red-shifted by 8 mμ from the data for 2′-deoxycytidine dinucleotide [d(pC)$_2$] at pH 8.5 reported here; the d-CpC may be in the half-protonated form, since the pK$_a$ is 7.5 for this transition in poly-dC.[7]

All members of the rC series (except the monomer) exhibit greater absorbance



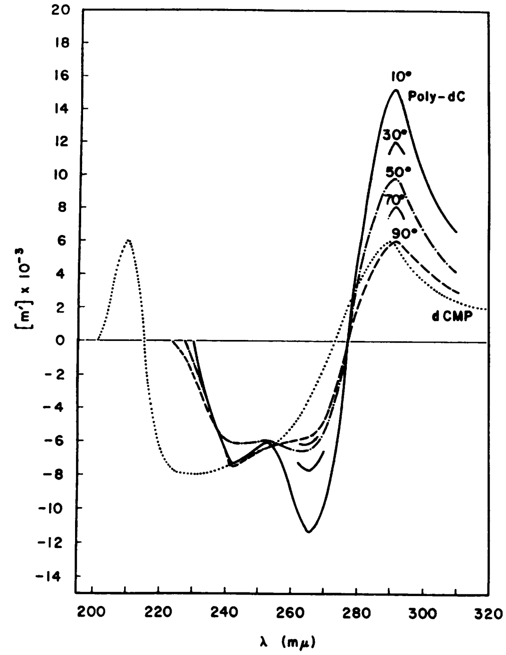


Fig. 1.—Optical rotatory dispersion of poly-dC, poly-rC, and rCMP in their nonprotonated states. Solvent 0.1 *M* NaF, 22°C. Poly-dC at pH 8.8, ———; poly-rC at pH 7.1, – – – –; CMP-5′ at pH 6.9, ... [*m*′] is the reduced residue rotation per cytosine.

Fig. 2.—Optical rotatory dispersion of poly-dC as a function of temperature at pH 8.5 in 0.02 *M* tris buffer with 0.08 *M* NaCl. 10°, ———; 50°, – – – –; 90°, -·-·-. The monomer, dCMP-5′, at 22° in 0.1 *M* NaF, pH 7.0, is shown for comparison, ...

hypochromism than do the corresponding dC nucleotides (see Table 1). The ultraviolet spectra (187–320 mμ) of single-stranded poly-rC and poly-dC are nearly identical to each other, and are very similar to the spectra reported[22] for rCMP and dCMP at neutral pH. The only differences are the extinction coefficients of the polymers at their 269-mμ peak, and a 3-mμ blue shift of each entire polymer spectrum, relative to the monomers. In poly-dC, the 195-mμ peak appears only as a shoulder.

The circular dichroism spectrum of poly-rC was measured in the solvent noted in Figure 1. A Cary attachment for the model 60 polarimeter enabled measurement farther into the ultraviolet than previously reported.[23] Three CD peaks were found, whose positions coincide with the inflection points of the three Cotton effects discernible in the ORD, plus an additional negative peak at 214 mμ. The positions and residue ellipticities, $[\theta] \times 10^{-3}$, of these extrema are: 276 mμ, +70; 234 mμ, −6; 214 mμ, −7; and 200 mμ, +110.

Temperature dependence: The ORD spectrum of poly-dC at several temperatures is shown in Figure 2. As the temperature is increased, the gradual decrease of the first Cotton effect (with peak at 291 mμ), finally attaining the monomer rotation while the trough at 242 mμ remains nearly constant, is characteristic of all the oligomers in this study. Attention will be focused on the amplitude of the highest-wavelength Cotton effect, and upon the extinction coefficient of the corresponding

TABLE 2

Optical Rotation and Absorption Parameters of Cytosine and Deoxycytosine Nucleotides: Temperature Dependence at pH 8.5[a]

	Temperature °(C)												
Compound	10	20	25	30	40	50	55	60	70	80	90	Wavelength (mμ)[c] λ_{peak}	λ_{trough}
	Rotation: Amplitude[b] of Longest Wavelength Cotton Effects $\Delta[m']_{peak\text{-}to\text{-}trough} \times 10^{-3}$												
rCMP-5′	16.4	...	...	...	...	16.4	...	...	...	...	16.0	288	240
r-CpC	38.9	...	31.1	...	26.3	...	22.1	...	19.4	...	16.2	291	266
r-C_3p_2[d]	27.6	...	21.5	...	17.2	...	14.9	...	12.2	...	10.8	291.5	266
r-C_5p_4	38.8	...	32.8	...	27.4	...	22.5	...	18.2	...	14.4	292	266
r-C_8p_7	45.8	...	39.5	...	32.9	...	26.9	...	21.8	...	17.0	292	265.5
r-$C_{14}p_{13}$	45.8	...	40.0	...	33.2	...	27.8	...	22.4	...	16.0	292	265.5
poly-rC[e]	...	71.0	...	...	61.0	...	...	45.9	...	...	29.0	292	265
dCMP-5′	13.0	12.6	...	12.6	12.7	12.9	...	12.6	12.1	12.2	12.4	289	228
d(pC)$_2$	13.9	...	12.6	...	11.9	...	10.9	...	11.0	...	10.8	290	262
d(pC)$_4$	20.4	...	16.9	...	14.6	...	13.5	...	12.2	...	11.2	291	264
d(pC)$_6$	16.7[h]	15.0	...	13.2	12.9	11.5	...	11.1	...	9.8	9.4	291.5	265
d(pC)$_8$	20.5	18.3	...	16.5	14.9	13.9	...	12.8	13.0	11.7	12.3	291.5	265
d-C_8p_7	...	18.3	...	...	...	14.2	...	...	...	...	...	291.5	265
d(pC)$_{10}$	20.1	17.4	...	15.4	14.4	13.1	...	12.8	12.4	11.9	12.1	291.5	265
poly-dC	26.6	22.9	...	19.7	17.9	16.2	...	15.3	14.2	13.2	12.1	291.5	265.5
	Absorption: Maximum Extinction Coefficient, $\epsilon_{max} \times 10^{-3}$											λ_{max}[g] at 10°	90°
rCMP-5′	9.26	...	...	...	...	9.00	...	...	...	...	8.94 (−3)	272	272.5
r-CpC	8.26	...	8.42	...	8.48	...	8.46	...	8.45	...	8.53 (3)	269.5	271.5
r-C_3p_2	8.11	...	8.14	...	8.22	...	8.30	...	8.37	...	8.46 (4)	269	271
r-C_5p_4	7.75	...	7.82	...	7.94	...	8.08	...	8.19	...	8.29 (7)	270	72
r-C_8p_7	7.43	...	7.52	...	7.68	...	7.84	...	7.96	...	8.10 (9)	269	271.5
r-$C_{14}p_3$	7.12	...	7.22	...	7.38	...	7.55	...	7.72	...	7.95[j] (11)	269	271
poly-rC[e]	...	...	...	...	...	...	...	...	...	...	... (13)	...	...
dCMP-5′	9.23	9.17	...	9.08	9.07	9.00	...	8.98	8.98	8.92	9.07 (−3)	271.5	273
d(dC)$_2$	8.66	...	8.62	...	8.59	...	8.53	...	8.50	...	8.38 (−3)	270.5	272
d(pC)$_4$	8.35	...	8.37	...	8.43	...	8.43	...	8.49	...	8.49 (2)	269.5	272
d(pC)$_6$	8.22	8.22	...	8.23	8.25	8.29	...	8.31	8.31	8.35	8.44 (3)	268	271.5
d(pC)$_8$	8.08	8.13	...	8.14	8.13	8.14	...	8.20	8.23	8.18	8.23 (2)	269	271.5
d-C_8p_7	...	8.12	...	...	...	8.13	...	...	...	...	8.26 ...	269	271.5
d(pC)$_{10}$	8.01	8.04	...	8.07	8.11	8.12	...	8.17	8.22	8.20	8.30 (4)	268.5	271
poly-dC	7.47	7.55	...	7.63	7.68	7.76	...	7.83	7.90	7.94	8.03 (7)	268.5	271.5

No data are given in this table for ORD at shorter wavelength. A second, negative, Cotton effect, absent in the monomers, appears in all higher nucleotides. Its trough is at 242 mμ for all compounds except d(pC)$_2$ (238 mμ) and d(pC)$_4$ (240 mμ). The [m′] trough is the same (−8,000 to −10,000) for all compounds but poly-rC (−18,000). Neither the position nor the [m′] of this trough is significantly affected by heat or by ethylene glycol.

[a] The solvent used was 0.08 *M* NaCl, 0.02 *M* tris-tris hydrochloride (pH 8.5). [b] This Cotton effect is nearly symmetric; $[m']p \simeq -[m']t$ under all conditions. [c] λp and λt do not vary with temperature for a given compound. [d] For r-C_3p_2 all $\Delta[m']$ data are probably low. However, their dependence upon temperature is meaningful. [e] Additional data on poly- rC are available in Fig. 4 and ref. 3. [f] Loss of hypochromism (%) upon heating from 10 to 90°. [g] The red shift of λ_{max} is gradual upon heating. [h] 19.0 at 3°. [i] At 75°. [j] At 80°.

absorption band. The first of these parameters is the more sensitive indicator of helical structure; the second is included because of its relation to base-stacking interactions, even though $\epsilon_{\max}$ does not approach the monomer value at 90°. (See Table 2 and Figures 3 and 4.)

All heating profiles were reversible upon cooling, thus showing that neither hydrolysis nor helix imperfection[3] was a problem. Both ORD and absorbance were proportional to concentration (10^{-4} to 10^{-3} *M* cytosine residue) for poly-rC, r-C_5p_4, and $d(pC)_8$ throughout the temperature transition. This finding rules out any intermolecular complex formation (such as occurs at acid pH), and confirms the single-stranded, intramolecular nature of the nonprotonated structures, even for small oligomers. The removal of the terminal 5′-phosphate of $d(pC)_8$, by means of *E. coli* alkaline phosphatase, had negligible effect upon the optical properties of this oligomer (see Table 2); thus the additional phosphate group in the dC oligomers is not the source of their decreased stability.

The double-stranded acid form of poly-dC (pH 5.2, 0.02 *M* acetate, 0.08 *M*

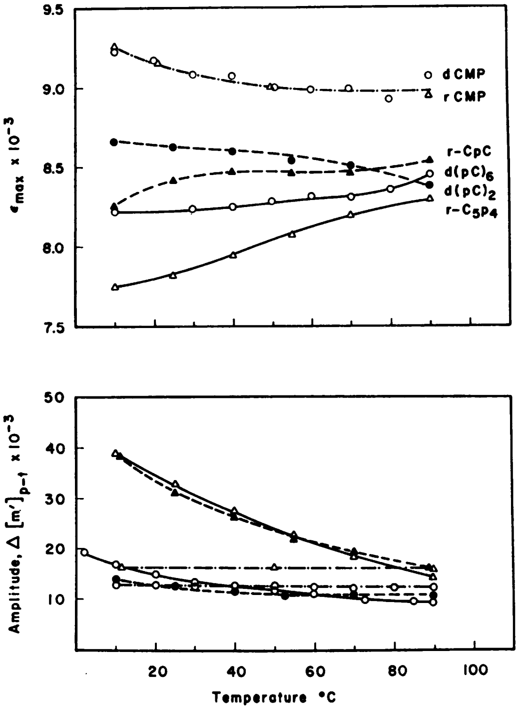


Fig. 3.—Temperature-optical rotatory dispersion and ultraviolet absorption profiles for monomers, dimers, and small oligomers. Upper curves show maximum extinction coefficients, per cytosine, for the absorption band near 270 mμ; $\lambda_{\max}$ increased slightly with temperature. Lower curves show the amplitude (peak-to-trough [*m*′]) of the longest wavelength Cotton effect. The solvent was 0.02 *M* tris buffer, pH 8.5, with 0.08 *M* NaCl. ○—·—○, dCMP-5′; ●--●, $d(pC)_2$; ○—○, $d(pC)_6$; △—·—△, rCMP-5′; ▲--▲, r-CpC; △—△, r-C_5p_4.

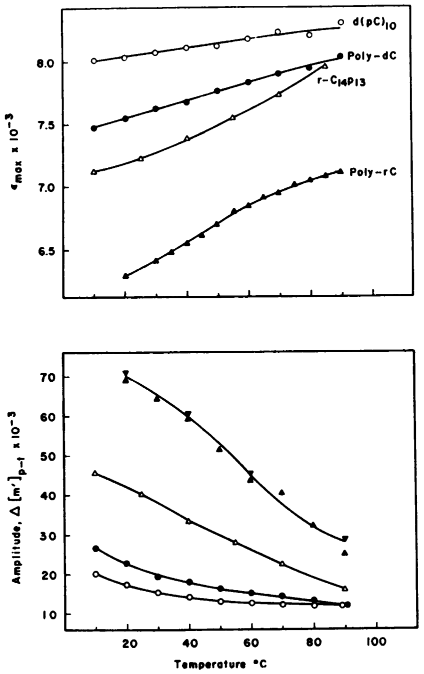


Fig. 4.—Temperature-optical rotatory dispersion and ultraviolet absorption profiles for large oligomers and for polymers. The format is the same as for Fig. 3. ○—○, $d(pC)_{10}$; ●—●, poly-dC; △—△, r-$C_{14}p_{13}$; ▼—▼, poly-rC; all in pH 8.5 tris. ▲—▲, Data for poly-rC taken from ref. 3, in which the solvent was 0.15 *M* NaCl plus 0.015 *M* sodium citrate, pH 7.0.

NaCl) has a sharp melting temperature (*Tm*) of 96° by both ϵ_{max} and ORD, in agreement with Inman's data[7], which is higher than that found for poly-rC at the same degree of protonation (0.56),[2, 3] not in agreement with the data of Ts'o *et al.*[30]

Comparison of the oligomer series: Perhaps the most striking contrast to be noted from Table 2 is that the nonprotonated rC oligonucleotides exhibit much larger rotations and greater loss of hypochromism upon heating than do the corresponding dC compounds. A comparison of the two dimers [r-CpC, $d(pC)_2$] is of interest. Ribo-CpC shares many properties with the rC polymer: a large Cotton effect (greater than twice that of the monomer), loss of hypochromism upon heating, ORD extrema at 292, 265, and 242 mμ, and absorption maximum at 269 mμ. On the other hand, $d(pC)_2$ has an ORD amplitude only slightly greater than that of dCMP (although the dimer does show thermal denaturation behavior), and displays a slight decrease in ϵ upon heating (like the monomer) (see Fig. 3). In the dC series, the wavelengths characteristic of the polymer are not attained until the hexamer. In neither series did any of the oligomers studied approach the rotation or hypochromicity of the polymers. (In single-stranded oligoriboadenylic acids, this limit is reached by chain length 30, but not 12.[14])

The thermal transitions of the two series are distinct. In the rC series the temperature profiles begin to assume a sigmoidal shape at the trimer, using absorbance as the criterion, or at the octamer, using ORD measurements. For higher rC oligomers the inflection point, or mid-point of the melting curve, occurs at about 50°, as viewed by both criteria (see Fig. 4). The limiting values of $\Delta[m']_{p-t}$ (ref. 1) at low temperature are probably near 60,000 for r-$C_{14}p_{13}$ and near 100,000 for poly-rC. The situation is very different for the dC series. The inflection points appear to be much lower than the lowest temperature measured (10 or 2°); the melting curves do not approach sigmoidal shape even for poly-dC. However, a slow increase in melting temperature with increasing chain length is apparent from the shapes of the curves. No estimate can be made of limiting $[m']$ values. It is noted that in the single-stranded rA series even the dimer has a melting temperature of about 25°;[13] adenine is a larger ring system than cytosine, and may have greater stacking interactions. For both rC and dC oligomers the melting temperature increases rather strongly with chain length. Thus, the conclusion that base stacking is noncooperative,[16] or only slightly cooperative,[11, 14, 15] in rA oligomers at neutral pH may not be as valid for cytosine stacking. In any case, the temperature profiles provide additional evidence that the single-stranded helical structure is more stable in rC than in dC, and that the stacked structure builds up more rapidly in the former, as its chain length increases.

Effect of ethylene glycol upon poly-dC: In contrast to the very gradual temperature profiles of poly-dC, the addition of ethylene glycol causes a rather abrupt loss of structure (Fig. 5), with the steepest part of the transition occurring at about 35 per cent glycol. Poly-rC is more stable towards ethylene glycol; its denaturation takes place most markedly at 50–60 per cent glycol.[3] The expected effect of an organic solvent of low dielectric constant, such as glycol,[24] is to weaken base stacking and other hydrophobic interactions, while strengthening solute hydrogen bonds. It is concluded from this experiment that poly-rC derives its additional structural stability, in comparison to poly-dC, not from increased stacking *per se*, but perhaps from a hydrogen bond involving the ribose-2′-hydroxyl group.

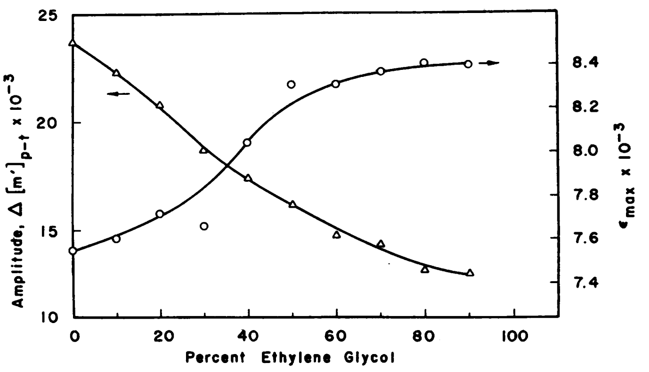


FIG. 5.—Effect of ethylene glycol concentration upon optical rotatory dispersion and absorption of poly-dC at pH 8.5 (0.02 *M* tris buffer with 0.08 *M* NaCl). Glycol concentration is given in volume per cent. Amplitude (Δ—Δ) and ϵ_{max} (O—O) have the same meaning as in Fig. 3. The λ_{max} was 269 mμ in aqueous solution, and shifted gradually to 272 mμ as the glycol concentration was increased. The peak (291.5 mμ) and trough (265 mμ) wavelengths of the first Cotton effect remained constant. Neither the position (242 mμ) nor the [*m'*] (−10,000) of the second trough varied.

Carbohydrate involvement: The reported data show that the ribose-2'-hydroxyl confers additional structural stability upon single-stranded oligo- and poly-cytidylate nucleotides, relative to their 2'-deoxy analogues. A likely source of this effect is an intrachain hydrogen bond between the 2'-OH group and a neighboring phosphate or ribose-ether oxygen atom. Furthermore, Brahms and Sadron[25] find that GMP-5' (but neither GMP-2' or dGMP) forms an ordered gel and exhibits strong circular dichroism. The authors postulate a "vertical" 2'-OH—O-phosphate hydrogen bond in GMP-5'. A similar stabilizing effect of ribose is observed in double-stranded polynucleotide complexes at neutral pH.[8, 9] In each of the series C:I, C:G, A:T, and AU copolymers, the r:r complex is more stable than either the r:d or the d:d. Since this effect is so general, a hydrogen bond between the 2'-OH and electron-donating groups on specific bases does not seem likely. In particular, if the 2-C=O of cytosine were involved in such a bond, the competition between lateral and vertical hydrogen bonding might be expected to result in a lowered stability of rC:rG.[9] This effect is not found; the melting temperatures are higher than and analogous to those in the C:I series,[8] which does not utilize the cytosine carbonyl for base pairing.

This sugar interaction might supplement base stacking and pairing in causing the ordered folding of RNA. There is some evidence for the involvement of the 2'-OH group in the structure of natural RNA. Rhapidosome RNA,[26] which contains about 80 per cent 2'-O-methyl ribose, has chemical properties more like DNA than like RNA. Nuclear magnetic resonance[27] of transfer RNA at neutral pH shows that the bases show rotational freedom at a lower temperature than do the ribose units. X-ray studies of natural, double-stranded RNA samples indicate that the 2'-hydroxyl may impose an extra rigidity upon RNA.[28] A recent investigation of reovirus and fragmented yeast RNA[29] indicates that in the solid state (but not necessarily in solution) intermolecular hydrogen bonds may be formed between 2'-hydroxyl groups and phosphate oxygens on neighboring helices.

Summary.—The ultraviolet optical rotatory dispersion and absorbance properties of oligo- and polycytidylic acids, and of their 2'-deoxy analogues, have been studied. The structural stabilities of the oligonucleotides in their single-stranded conformation at neutral pH are compared over a wide temperature range. The ribopolymer and oligomers display greater rotatory power, hypochromicity, and heat stability than do the comparable deoxy compounds, although a similar type of helical base stacking is indicated by the positions of the Cotton effects. Comparison of the two oligomer series shows that asymmetric macromolecular structure is attained more readily, as a function of chain length, in the ribo-oligonucleotides. The possible role of the 2′-hydroxyl group in stabilizing the single-stranded secondary structure of cytidylate nucleotides and of RNA is discussed.

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[1] Abbreviations: ORD, optical rotatory dispersion; rC, cytidylate; dC, 2′-deoxycytidylate; r-CpC, cytidyl ($3' \rightarrow 5'$) cytidine; r-C_3p_2, cytidine trinucleoside diphosphate, with $3' \rightarrow 5'$ linkages (similar nomenclature for longer ribo-oligomers); poly-rC, polycytidylic acid; d$(pC)_2$, 2′-deoxycytidine dinucleotide, etc.; poly-dC, polydeoxycytidylic acid; $\Delta[m']_{p-t}$, amplitude (peak-to-trough) of longest wavelength Cotton effect.

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