Table I. Selected Bond Lengths and Angles in π-$C_5H_5Zr(hfac)_3$

Bond	Length, Å	Angle	Deg
$Zr-C_1$	2.51	O_1-Zr-O_2	73.5
$Zr-C_2$	2.55	O_1-Zr-O_3	68.2
$Zr-C_3$	2.53	O_3-Zr-O_5	68.1
$Zr-C_4$	2.51	O_5-Zr-O_6	74.5
$Zr-C_5$	2.54	O_6-Zr-O_2	69.7
$Zr-O_1$	2.22		
$Zr-O_2$	2.23	O_4-Zr-O_1	80.9
$Zr-O_3$	2.28	O_4-Zr-O_2	80.2
$Zr-O_4$	2.16	O_4-Zr-O_3	79.6
$Zr-O_5$	2.20	O_4-Zr-O_5	80.6
$Zr-O_6$	2.24	O_4-Zr-O_6	79.6
	Mean Values (Å)		
O-C	1.27	$C-CF_3$	1.49
C-C	1.38	C-F	1.32

clarity. Pertinent bond lengths and angles are listed in Table I; the accuracy of these figures may be judged from the estimated standard deviations in atomic positions obtained from the inverse matrix: Zr = 0.002, F = 0.015, C = 0.021, O = 0.011 Å. The structure is most conveniently described as a pentagonal bipyramid.[4] Two of the bidentate ligands (designated 1, 2 and 5, 6 with reference to the oxygen atoms as labeled in Figure 1) lie in the equatorial plane, while the third ligand (3, 4) occupies one of the axial positions and the remaining equatorial one. The other axial position is occupied by the cyclopentadienyl group,[4] which is symmetrically placed, within error, on the axis of the bipyramid. Presumably as a consequence of the greater steric effect of the cyclopentadienyl group, the five equatorial Zr–O bonds are tilted away by a surprisingly regular 10°.

The temperature variation of the ^{19}F nmr spectrum of π-$C_5H_5Zr(hfac)_3$ is shown in Figure 2. The pentagonal-bipyramidal model predicts four bands of intensity ratio 2:2:1:1, and this is observed at −30°. As the temperature is raised, the more intense pair (due to the equatorial ligands) coalesces, becoming fully averaged near 50°. The less intense pair (due to the 3, 4 ligand) becomes involved in a general exchange process only at higher temperatures. The barrier to exchange of CF_3 groups on the more rigid 3, 4 ligand may be related to the $Zr-O_4$ bond length, which is 0.07 Å shorter than the average of the other five Zr–O bonds.[5]

Both proton and ^{19}F spectra of the unsymmetrical chelate π-$C_5H_5Zr(tfac)_3$ can be similarly interpreted. The room-temperature spectra show that two isomers are present in a molar ratio of *ca.* 3:2; the isomers differ in the orientation of the 3, 4 ligand (*i.e.*, CF_3 equatorial or CF_3 axial). Exchange of CH_3 and CF_3 on the equatorial ligands is rapid at room temperature, so that each isomer is characterized in the CH_3 or CF_3 region by two peaks in 1:2 intensity ratio.

Finally, we have observed in the ^{19}F spectrum of π-$C_5H_5Ti(hfac)_2Cl$ two sets of four equal-intensity bands, with one set relatively weak and due to an isomer. The existence of more than one isomer having all CF_3 groups nonequivalent rules out an octahedral coordination for this molecule in solution. We can confirm, however, the observation[3] that only one isomer of the related molecule π-$C_5H_5Zr(acac)_2Cl$ is observable in its proton nmr spectra.

Acknowledgment. We thank the National Research Council of Canada for financial support.

(5) The statistical significance of the differing Zr–O distances will be finally assessed when the structure is fully refined. It is also worth noting that the two bonds to the unique (3, 4) ligand are 0.06 Å longer and 0.06 Å shorter than the mean of the four Zr–O bonds in the two similar ligands (2.22 Å).

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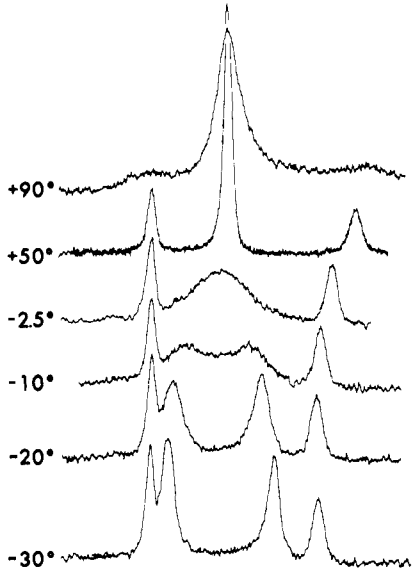

Figure 2. Temperature variation of the ^{19}F nmr spectrum of π-$C_5H_5Zr(hfac)_3$ in methylcyclohexane at 50 and 90° and acetone at lower temperatures. Chemical shifts differ appreciably in the two solvents and the spectra shown have been aligned vertically for illustrative purposes. Spectra were recorded at 56.4 MHz, and a 44-cps segment is shown.

The Structure of Poly-2′-O-methyladenylic Acid at Acidic and Neutral pH

Sir:

The presence of minor components is a highly characteristic feature of the primary structure of RNA, in particular of transfer RNA. Many subtle variations in the three-dimensional structure of RNA may become possible by changing number, distribution, and kind of the minor components in a given sequence of major nucleotides. Such a "fine structure" may be needed for the full biological activity of RNA. As part of our efforts to understand the effects of specific minor nucleotides on RNA conformation[1,2] we have studied the structure of poly-2′-O-methyladenylic acid[3,4] in weakly acidic and neutral solutions. The presence of 2′-O-methylribonucleotides has been detected in transfer,[5] ribosomal,[6] and

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(3) F. Rottman and K. Heinlein, *Biochemistry*, **7**, 2634 (1968).
(4) Abbreviations: poly-2′-O-methyladenylic acid, poly 2′-O-mA; polyadenylic acid, poly A.
(5) R. H. Hall, *Biochemistry*, **3**, 876, (1964).
(6) E. K. Wagner, S. Penman, and V. M. Ingram, *J. Mol. Biol.*, **29**, 371 (1967).

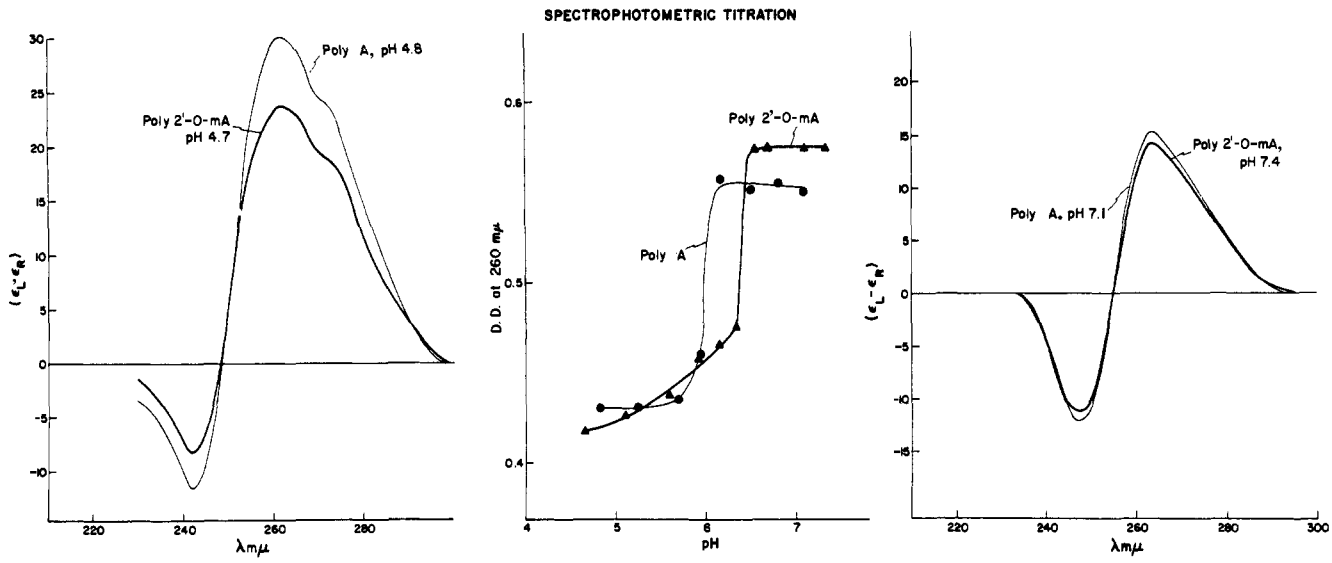

Figure 1. Spectrophotometric titration of poly A and poly 2′-O-mA. A sample (3 ml) of a slightly alkaline solution of each polymer in 0.15 *M* KCl containing 0.16–0.20 μmol of nucleotide was titrated with appropriate amounts of 0.01–0.05 *N* HCl. The absorption spectra were measured after equilibrating the solutions. The maxima and minima in the absorption spectra at a pH above and below the sharp transition pH were the same for both polymers. CD spectra were taken before and after the titrations with a Cary 60 recording spectropolarimeter, equipped with a 6001 circular dichroism accessory. CD results are reported in terms of the differential dichroism absorption ($\varepsilon_L - \varepsilon_R$) and are given on a "per nucleotide residue" basis. The polymer preparations were high molecular weight fractions of low dispersity obtained by chromatography on Sephadex or by sedimentation in a sucrose gradient.

rhapidosomal[7] RNA.

As has been well documented for poly A[8] and will be shown for poly 2′-O-mA in this paper these polymers are able to form double-stranded, helical structures at acidic pH but assume single-stranded structures at neutrality at room temperature. Figure 1 shows the results of a spectrophotometric titration of poly 2′-O-mA and poly A at 25°. A sharp drop in the absorbance at 260 mμ occurring within less than 0.25 pH unit is observed for both polymers in going from neutrality to pH 4.5. This reflects the transition of the single-stranded to the double-stranded structure of poly A[8] and in analogy of poly 2′-O-mA, a conclusion supported by the circular dichroism (CD) spectra of the two forms of both polymers (see below). While the over-all shape of the titration curves is similar, the following significant differences are observed: (1) the midpoint of the transition of poly 2′-O-mA is shifted to higher pH by approximately 0.4 pH unit; (2) the total change in absorbance at 260 mμ in going from pH 7 to 4.5 is larger for poly 2′-O-mA than for poly A.

The CD spectra of poly 2′-O-mA and poly A at 28° are shown in the right and left side of Figure 1. The positions of the maxima, minima, shoulders, and crossing points are nearly identical for both polymers at a pH close to 7 on one hand and a pH below 5 on the other (0.15 *M* KCl). The conservative and nonconservative spectra are representative for the single- and double-stranded structures of poly A[9] and, by analogy, of poly 2′-O-mA. A slightly lower rotational strength is found for the positive and negative bands in the spectrum of poly 2′-O-mA at acidic pH as compared to poly A.

Table I. T_m[a] of the Cooperative Part of the Denaturation of Poly 2′-O-mA and Poly A as a Function of pH in 0.15 *M* KCl[b]

pH	Poly 2′-O-mA	Poly A
4.6	86.0	79.5
5.4	59.5	48.5
6.3	32.0	9.5
7.0	13.5	

[a] In degrees. [b] A 0.01 *M* cacodylate buffer was used for the melting curves at pH 6.3 and 7.0 and a 0.05 *M* acetate buffer at pH 4.6 and 5.4. The solutions were 0.15 *M* in KCl and the concentration of the polymers was 40–60 mμmol of nucleotide/ml. The change in the absorbance at 260 mμ with temperature was measured using a thermostated cell holder. The temperature was determined with a calibrated thermometer at the eflux of the heating block in the cell compartment. The shifts in the absorption maxima and minima as a function of temperature were the same for both polymers.

The change of absorbance at 260 mμ in going from 5 to 90° in the pH range 4.6–7 was compared for poly 2′-O-mA and poly A in 0.15 *M* KCl. In all cases, with the exception of poly A at pH 7, a substantial portion of the total change in absorbance occurred within a narrow temperature range. At pH 5.4, 6.3, and 7 for poly 2′-O-mA and at pH 5.4 and 6.3 for poly A, however, the steep (cooperative) change in absorbance is followed by a gradual (noncooperative) increase which starts to level off near 90°.[10] The midpoints (T_m's) of the cooperative part of the melting curves are listed in Table I. Substantially higher T_m's were found for poly 2′-O-mA than for poly A under the same conditions. A T_m of 13.5° for the cooperative melting of poly 2′-O-mA was determined at

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(8) J. R. Fresco and E. Klemperer, *Ann. N. Y. Acad. Sci.*, **81**, 730 (1959); R. F. Steiner and R. F. Beers, *Biochim. Biophys. Acta*, **32**, 166 (1959); A. Rich, D. R. Davies, F. H. C. Crick, and J. D. Watson, *J. Mol. Biol.*, **3**, 71 (1961); C. Stevens and G. Felsenfeld, *Biopolymers*, **2**, 293 (1964).

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(10) The spectroscopic characteristica (uv, CD) of the cooperatively melting forms of poly A at pH 5.4 and 6.3 and of poly 2′-O-mA at pH 5.4, 6.3, and 7.0 in 0.15 *M* KCl differ from the ones observed at pH 4.6. This may reflect the formation of intermediate structures of poly A and poly 2′-O-mA under these conditions and will be discussed in a forthcoming paper.

pH 7 while poly A melted in a noncooperative manner at this pH.

The following general conclusions can be reached: methylation of the backbone in poly A does not interfere with the formation of a double-stranded structure at weakly acidic pH at intermediate temperatures and a partially ordered single-stranded structure at a pH close to 7. The absorption spectra (not shown) and more significantly the CD spectra of both forms of poly A and poly 2′-O-mA indicate a close similarity of the dissymmetric structures of the single- and double-stranded conformations of both polymers. The most significant difference between the two polymers, which is reflected in the melting and titration curves, is the considerable higher tendency of poly 2′-O-mA to form a double-stranded structure as compared to poly A under the same conditions. A number of reasons may be responsible for this difference. These include a change in solvation entropy, a shift in the position of the equilibrium between the *exo* and *endo* conformation of the ribose residues, and a change in the preferred orientation of the heterocyclic portion relative to the sugar due to the methylation of the backbone.

It has been suggested that the 2′-hydroxyl group may participate in the stabilization of the ordered structures of polyribonucleotides by the formation of hydrogen bonds to the phosphate groups of the backbone[11] or to various sites of the heterocyclic bases.[12] Beyond eliminating the 2′-hydroxyl groups as potential hydrogen bond donors, methylation of the backbone may change the relative importance of other factors contributing to the stabilization of polynucleotide structure in solution. The data so far obtained with poly 2′-O-mA therefore neither support nor clearly eliminate this hypothesis.

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The Synthesis of the Fungal Sex Hormone Antheridiol[1]

Sir:

The substance antheridiol is the first specific functioning steroidal sex hormone to be identified in the plant kingdom. This unique natural product, secreted by the female plant of the aquatic fungus *Achlya bisexualis*, induces the growth of antheridial hyphae in the male plant, thus initiating sexual reproduction in this species.[2]

The structure of antheridiol, depicted by **1**, was deduced by McMorris and collaborators mainly on the basis of spectroscopic evidence.[3] We wish to report the synthesis of two of the four possible 22,23 isomers of **1**. One of our synthetic compounds exhibits physical and biological properties in good agreement with the natural hormone, thereby confirming the structure (**1**) proposed for antheridiol.

Aldol condensation of 3-tetrahydropyran-2′-yloxy-22,23-bisnorchol-5-en-24-al (**2a**)[4,5] [mp 137–139°; $[\alpha]_D$ −39°] at −78° in tetrahydrofuran with the anion of ethyl *trans*-3,4-dimethyl-2-pentenoate (**3**)[7] [bp 46–48° (0.7 mm)] generated by treatment of **3** with lithium triphenylmethide in tetrahydrofuran at −20° followed by warming the reaction mixture to 0° (45–90 min) and work-up by acidification afforded the α,β-unsaturated lactone **4** (23%) [mp 208–212°; $[\alpha]_D$ −62°; ν_{max} 1710, 1630 cm^{-1}; nmr 0.71 (18-H), 1.02 (19-H), 1.12 (d, J = 7 Hz, isopropyl CH_3), 5.80 ppm (24′-H)]. Sequential treatment of lactone **4** with boiling 2% methanolic sodium hydroxide (5 hr) and dilute hydrochloric acid in aqueous methanol (15 min at 20°) provided the key intermediate, 3β-hydroxy-22,23-*trans*-dienoic acid **5a** (86%) [mp 213–215°; $[\alpha]d$ −55°; λ_{max} 262 mμ (log ε 4.28); ν_{max} 1680, 1635, 1595 cm^{-1}; nmr (100 MHz) in DMSO-d_6 0.69 (18-H), 0.94 (19-H), 1.04 (*d*, J = 6.5 Hz, 21-H and isopropyl CH_3), 5.50 (24′-H), 5.98 (q, $J_{20,22}$ = 9 Hz, $J_{22,23}$ = 16 Hz, 22-H), 7.35 ppm (d, $J_{22,23}$ = 16 Hz, 23-H].

Retention of the natural 20*R* stereochemistry was confirmed by ozonolysis of the 5α,6α-epoxydienoic acid **5b**[9] to the amorphous 3β-hydroxy-5α,6α-oxido-22,23-bisnorcholan-24-al (**2b**) [$[\alpha]_D$ −54°; ν_{max} 2675, 1720 cm^{-1}; nmr (100 MHz) 0.66 (18-H), 1.06 (19-H), 1.09 (d, J = 7.0 Hz, 21-H): 2.91 (d, J = 4.0 Hz, 6β-H), 9.54 ppm (d, J = 3.0 Hz, 24-H); *m/e* 346 (M^+)] which was identical in all respects with the aldehyde obtained by ozonolysis of

(1) Publication No. 355 from the Syntex Institute of Organic Chemistry.

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(4) This substance was prepared by reducing methyl 3-tetrahydropyran-2′-yloxy-22,23-bisnorchol-5-en-24-oate with lithium aluminum hydride to the corresponding alcohol followed by oxidation with dimethyl sulfoxide–dicyclohexylcarbodiimide.[6]

(5) Satisfactory elemental analyses were obtained for all fully characterized compounds. Nmr spectra were obtained on Varian A-60 and HA-100 spectrometers in deuteriochloroform solutions (10% w/v) containing tetramethylsilane as internal reference. Chemical shifts are reported as parts per million on the δ scale. We thank Miss J. Tremble for these determinations. In the presentation of data d = doublet, q = quartet, m = multiplet. Specific rotations are measured in chloroform solution, ultraviolet spectra in 95% ethanol, and infrared spectra in KBr disks. The mass spectra were obtained with an Atlas-werke CH-4 spectrometer equipped with a direct inlet system. Spectra were measured at an ionizing potential of 70 eV and an acceleration voltage of 3 kV. We thank Mr. J. Smith and Dr. L. Tökes for assistance with these measurements.

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(7) This ester was prepared by Emmons alkylation of methyl isopropyl ketone with the anion of triethyl phosphonoacetate.[8]

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(9) This epoxide, prepared by the reaction of **5a** with 1 molar equiv of *m*-chloroperbenzoic acid in methylene dichloride, showed mp 196–197°; $[\alpha]_D$ −65°; λ_{max} 265 mμ (log ε 4.27); ν_{max} 1685, 1635, 1595 cm^{-1}.