BASE COMPOSITION OF TMV-RNA's

	Substituted	Normal[8]
Adenine	1.23	1.18
Guanine	1.00	1.00
Cytosine	0.71	0.73
Uracil	.66	1.03
5-Fluorouracil	.31	

to uridylic acid were eluted and subjected to electrophoresis at *p*H 9.2 in borate buffer. The material from the diesterase digests separated into the 5′ monophosphates of uridine and 5-fluorouridine which were found in the same proportion as the corresponding 2′ and 3′ nucleotides in the alkaline hydrolysate. The 5′ monophosphates had mobilities slightly greater than those of their counterparts in the alkaline hydrolysate. In neither of these hydrolysates was there any evidence for diphosphates corresponding to end groups such as have been found in the case of 2-thiouracil[6] incorporation into TMV-RNA.

From the above data it is concluded that in TMV grown in the presence of 5-fluorouracil about one-third of the uracil in the virus is replaced by 5-fluorouracil and the total amount of TMV produced is reduced by about 50%. When applied to a local lesion host, however, the same number of lesions were produced by the substituted virus as by a normal virus. Isolated nucleic acid prepared by the detergent treatment[7] also proved to be infective.

Further work on the biological significance of these findings is in progress. The authors are indebted to the Hoffmann–LaRoche Co. of Nutley, New Jersey, for a generous gift of 5-fluorouracil.

(6) H. G. Mandel, R. Markham and R. E. F. Matthews, *Biochim. et Biophys. Acta*, **24**, 205 (1957).

(7) H. Frankel-Conrat, B. Singer and R. C. Williams, *Biochem. et Biophys. Acta*, **25**, 87 (1957).

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THE STRUCTURE OF CRYSTALLINE POLY-(METHYL METHACRYLATE)

Sir:

A recent communication[1] described the preparation and physical properties of methyl methacrylate polymers crystallizable in three different crystal structures. Isotactic, syndiotactic and "block copolymer" chains were tentatively associated with the Type I, Type II and Type III crystal structures respectively. Type II X-ray fiber patterns show some fifty independent reflections. They have been analyzed and the major features of the crystal structure are reported here.

Eight zero layer reflections index reasonably well in the trigonal system using hexagonal axes with a = 12.17 Å. The translation identity distance corresponds to c = 10.55 Å. The calculated density is 1.23 g./ml.[3] on the basis of ten monomer units per cell. This result compares satisfactorily with the observed value of 1.22 g./ml.[3].

(1) T. G. Fox, B. S. Garrett, W. E. Goode, S. Gratch, J. F. Kincaid, A. Spell and J. D. Stroupe, THIS JOURNAL, in press.

Reflections on the four higher layer lines destroy the trigonal symmetry. All data can be approximately indexed on a body-centered orthorhombic lattice with a = 21.08 Å., b = 12.17 Å. and c = 10.55 Å. Fine splitting of many reflections is observed, however, and the true unit cell is triclinic, pseudo-orthorhombic.

The fiber period and the density require that the polymer chains be coiled in a five-fold helix. The 5_2 helix, shown in projection in Fig. 1, accurately fits all the necessary parameters. Each circle on the circumference of the projection of the helix represents a pair of superposed backbone carbon atoms. Both the syndiotactic and the isotactic configurations fit this helix.

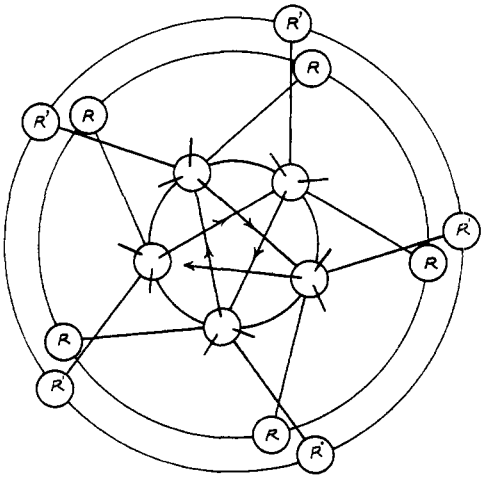

Fig. 1.—Projection of the 5_2 helix of isotactic poly(methyl methacrylate): T = 10.55 Å.; r_h = 0.75 Å.; R = CH_3; R′ = $COOCH_3$.

The five-unit two-turn helix requires the isotactic chain configuration because of the odd number of monomer units per repeat distance. A corresponding 10_4 helix would accommodate syndiotactic chains but would have twice the repeat distance (21.10 Å.). The X-ray data show no evidence for doubling the repeat period. It is, however, possible to develop chain packing arrays which would extinguish all *hkl* reflections for *l* odd. These arrays can be based upon superlattices involving right-handed and left-handed helices or on random distribution of specific dislocations.

Molecular models and calculations show that the syndiotactic 10_4 helix is stiff. This is in accord with the properties of polymers crystallizing in the Type I structure.[1] The isotactic 5_2 helix seems to be somewhat less stiff. Polymers crystallizing in the Type II structure have low glass temperatures and a relatively low melting point.[1] They are readily crystallizable and highly crystalline (*ca.* 90%). They show an extremely small specific volume change on melting.

The above considerations suggest that the Type II crystal structure is based on isotactic 5_2 helices, although interpretation of physical and thermodynamic properties in terms of chain stiffness is not yet complete. Structure refinements are in prog-

ress. The results will be published in a subsequent paper.

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KANAMYCIN. II. THE HEXOSAMINE UNITS

Sir:

The remaining building units of kanamycin[1,2] have now been isolated and characterized as two hexosamines one of which is 6-deoxy-6-amino-D-glucose (I).

Kanamycin was hydrolyzed (4 *N* HCl, 15 min. boiling) to three major ninhydrin-positive substances which could be separated on Whatman 52 paper using *n*-butanol–acetic acid–water 4:1:5. Concentration of the hydrolyzate and addition of ethanol yielded impure 2-deoxystreptamine dihydrochloride,[1] R_f 0.02. Concentration of the mother liquor *in vacuo* yielded crude I hydrochloride, R_f 0.06 (see below). The mother liquor was concentrated and the amorphous ether–ethanol precipitate acetylated (acetic anhydride–pyridine) yielding[3] the pentaacetate (II) of a second hexosamine, which we propose to term kanosamine, m.p. 206–207°, $[\alpha]^{25}D$ +8.1° (*c*, 0.8 in chloroform). *Anal.* Calcd. for $C_{16}H_{23}NO_{10}$: C, 49.4; H, 5.95; N, 3.60; O-acetyl, 44.2; mol. wt., 389. Found: C, 49.4; H, 5.93; N, 3.56; O-acetyl, 43.2; mol. wt. 393.

Pure I hydrochloride, obtained by chromatography on Dowex-50[4] with 0.7 *N* hydrochloric acid decomposed at 161–162°, $[\alpha]^{25}D$ + 23.0° → + 50.1° after 21 hours (*c*, 1.0 in water). *Anal.* Calcd. for $C_6H_{13}NO_5 \cdot HCl$: C, 33.4; H, 6.55; N, 6.50; Cl, 16.4; neut. equiv., 215.6. Found: C, 33.2; H, 6.02; N, 6.63; Cl, 16.4; neut. equiv., 216. Acetylation yielded 6-deoxy-6-amino-β-D-glucopyranose pentaacetate (III), m.p. 114–120°, $[\alpha]^{25}D$ + 9.9° (*c*, 0.8 in chloroform). *Anal.* Calcd. for $C_{16}H_{23}NO_{10}$: C, 49.4; H, 5.95; N, 3.60; O-acetyl, 44.2. Found: C, 49.1; H, 5.94; N, 3.62; O-acetyl, 44.5.

The proton magnetic resonance spectrum[5] of III indicated a straight-chain aldose with a diaxial arrangement for the 1- and 2-hydrogens. The presence of a single band for the acetyl hydrogens indicated the absence of axial acetyl groups, indicating a *gluco*-configuration. Anomerization in acetic anhydride–acetic acid with perchloric acid catalyst gave the α-anomer (IV), m.p. 141–142°, $[\alpha]^{23}D$ +92.6° (*c*, 0.4 in chloroform).

O-Deacetylation of the hexosamine pentaacetates (III and II) over Amberlite IR 410 (OH^-)[6] yielded N-acetyl I (V) m.p. 196–198° (dec.), $[\alpha]^{25}D$ +44.0° → + 34.9° after 22 hours (*c*, 1.0 in water) and N-acetylkanosamine (VI), m.p. 199–202° (dec.) $[\alpha]^{25}D$ +43° (*c*, 1.0 in water). *Anal.* Calcd. for $C_8H_{15}O_6N$: C, 43.4; H, 6.84; N, 6.34; N-acetyl, 19.4. Found for V: C, 43.4; H, 7.00; N, 6.14. Found for VI: C, 43.3; H, 6.96; N, 6.38; N-acetyl, 19.3.

Both I and V consumed four moles of periodate, producing three moles of formic acid and no formaldehyde. Nitrous acid deamination of tetra-*O*-acetyl I (acetic anhydride–acetic acid–perchloric acid acetylation) and reacetylation gave α-D-glucopyranose pentaacetate. Thus, I must be 6-deoxy-6-amino-D-glucose, a conclusion verified by m.p. and infrared comparison of III and synthetic pentaacetate.[7] Kanamycin thus appears to be composed of deoxystreptamine, 6-deoxy-6-amino-D-glucose, and a hexosamine, $C_6H_{13}NO_5$, termed kanosamine.

(1) M. J. Cron, D. L. Johnson, F. M. Palermiti, Y. Perron, H. D. Taylor, D. F. Whitehead and I. R. Hooper, THIS JOURNAL, **80**, 752 (1958).

(2) T. Takeuchi, T. Hikiji, K. Nitta, S. Yamazuki, S. Abe, H. Takayama and H. Umezawa, *J. Antibiotics*, Ser. A, **10**, 107 (1957).

(3) All crystallizations were from methanol–ethanol.

(4) A product of the Dow Chemical Co.

(5) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, THIS JOURNAL, **79**, 1005 (1957).

(6) A product of Rohm and Haas Company.

(7) H. Ohle and L. v. Vargha, *Ber.*, **63**, 2905 (1930).

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HEAVY-ATOM DYES FOR CRYSTALLOGRAPHIC STUDIES OF PROTEINS. I. A BIS-AZOMETHINE COMPLEX OF URANYL

Sir:

Compounds which contain a heavy element incorporated within the molecule and which bind to proteins are valuable in a number of fields of research, among them protein crystal-structure analysis and electron microscopy. Such compounds we shall refer to as heavy-atom dyes, regardless of whether the heavy element is bound by a simple covalent bond, as in the organic mercurials or iodine compounds, or is chelated. Heavy-atom dyes may be synthesized with wide variations in molecular size and shape, charge distribution and identity of the heavy atom, in order to make possible a search for compounds binding specifically to fixed sites on the surfaces of molecules of a given protein. Such specific binding is required in the isomorphous-substitution method of crystal-structure analysis,[1] which is based on a comparison of the X-ray diffraction intensities obtained from two crystals having structures identical except for the substitution of atoms of different elements at certain specific crystallographic positions.

The series of metal chelates of bis-azomethine prepared from substituted salicylaldehydes and *o*-diamines has the valuable characteristics of ease of preparation, stability and variability through choice of initial components. Of especial interest as heavy-atom dyes are the chelates having such charged groups as $-SO_3^-$; representatives of such compounds are the chelates of bis-(sulfosalicylal) ethylenediamine prepared by Mukherjee and Rây.[2]

(1) C. A. Beevers and H. Lipson, *Proc. Roy. Soc.* (*London*), **A146**, 570 (1934).

(2) A. K. Mukherjee and P. Rây, *J. Indian Chem. Soc.*, **32**, 633 (1955).