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).