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:

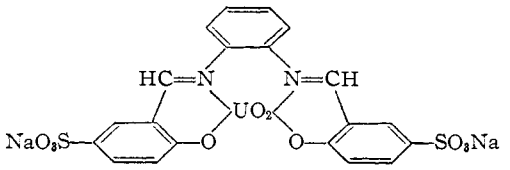
Compounds which contain a heavy element incorporated within the molecule and which bind to proteins are valuable in a number of fields of research, among them protein crystal-structure analysis and electron microscopy. Such compounds we shall refer to as heavy-atom dyes, regardless of whether the heavy 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).

We wish to report here the synthesis of the complex, uranyl bis-(sulfosalicylal)-*o*-phenylenediamine, which was prepared in pure crystalline form as the sodium salt, and is being applied as a heavy-atom dye in the crystal-structure analysis of the protein bovine pancreatic ribonuclease. The complex is taken up readily by crystals of ribonuclease from a 2.5×10^{-4} *M* solution in 75 volume % 2-methyl-2,4-pentanediol. This complex is presumed to have the structure



The method of preparation involves simply the mixing in aqueous solution of sulfosalicylaldehyde (prepared according to Blau[3]), *o*-phenylenediamine, uranyl acetate and sodium hydroxide in the molar ratio 2:1:1:2, and crystallization from aqueous isopropyl alcohol. The analytical results for uranium are in accord with the proposed composition (Calcd. for $C_{20}H_{12}N_2S_2O_{10}Na_2U$: U, 30.3. Found: U, 31.5).[4] In the absence of further analytical data or degradative studies, the proposed structure cannot be proved, but is rendered highly probable by analogy with known complexes and consideration of steric requirements. The assigned octahedral coördination of uranium(VI) is not the most usual for this element, but has been observed previously in crystalline compounds, *e.g.*, $BaUO_4$.[5]

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(3) F. Blau, *Monatsh.*, **18**, 123 (1897).

(4) Virginia Hong, Master's Thesis, Polytechnic Institute of Brooklyn, June, 1957.

(5) S. Samson and L. G. Sillen, *Ark. f. Kem. Min. och Geol.*, **25A**, No. 21, 1 (1947).

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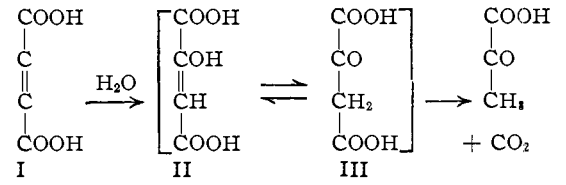
ENZYMATIC UTILIZATION OF AN ACETYLENIC COMPOUND

Sir:

A soluble enzyme system catalyzing the conversion of acetylenedicarboxylic acid (I) to equimolar quantities of pyruvic acid and carbon dioxide has been partially purified from sonic extracts of a species of *Pseudomonas* isolated from soil. The enzyme system has been purified 50-fold by protamine treatment, ammonium sulfate and acetone precipitation and by adsorption and elution from calcium phosphate gel. The most active preparations catalyze the formation of 55 μmoles of pyruvate[1] per minute per mg. protein at 25° and *p*H 7.3.

After incubation of the enzyme with I, the 2,4-dinitrophenylhydrazone of pyruvic acid was isolated (m.p. 215°). The reaction is experimentally irreversible as demonstrated by the lack of incorporation of C^{14} into acid-stable linkages when $C^{14}O_2$ is incubated with the enzyme and either I or pyruvate. In a preliminary report Eimhjellen[2] has noted that oxalacetate (III) is formed by resting cells, and pyruvate by extracts, of an enterobacterium exposed to ADA. This finding would suggest the pathway shown



However, results with the purified enzyme from *Pseudomonas* clearly eliminate *free* oxalacetate as an

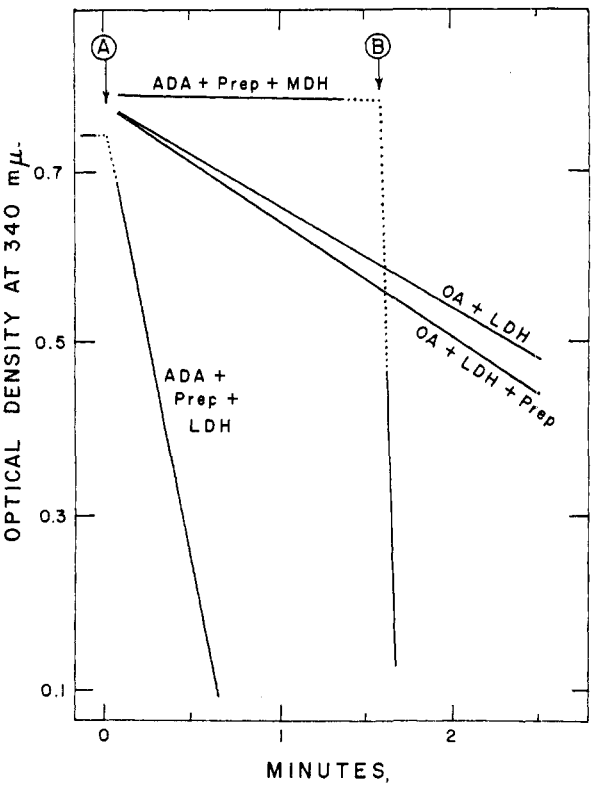


Fig. 1.—Tracing obtained from a Cary recording spectrophotometer of the rate of reduced pyridinenucleotide (DPNH) oxidation. All incubation mixtures contain 50 μmoles of phosphate at pH 7.3 and 0.1 μmoles of DPNH per ml. When applicable the following were added to a total volume of 1.0 ml.: ADA, acetylene dicarboxylate, 10 μmoles, Prep, *Pseudomonas* enzyme, 2 μg.; OA, oxalacetate freshly prepared, 2 μmoles; LDH and MDH, an excess of lactic dehydrogenase and malic dehydrogenase, respectively. Arrows A and B denote the addition of substrate and oxalacetate, respectively.

(1) Assayed by the method of T. E. Friedemann and G. E. Haugen, *J. Biol. Chem.*, **147**, 415 (1943).

(2) K. E. Eimhjellen, *Biochem. J.*, **64**, 4 p. (1956).