ACHROMYCIN.[1] THE STRUCTURE OF THE ANTIBIOTIC PUROMYCIN.[2] I.

Sir:

The new antibiotic, Puromycin, isolated from the mold *Streptomyces alboniger*, has been found to be active against certain bacteria and Trypanosomes.[3]

Puromycin, I, m.p. 175.5–177°, $[\alpha]^{25}D$ − 11° (ethanol), *Anal.* Calcd. for $C_{22}H_{29}N_7O_5$: C, 56.04; H, 6.20; N, 20.79. Found: C, 56.12; H, 6.48; N, 21.12, is a diacidic base and readily forms a dihydrochloride or a monosulfate. Titration and molecular weight data are in agreement with the above empirical formula. Group analyses show the presence of one amino group (Van Slyke), one methoxyl group, two N-methyl groups and five active hydrogens. A carbonyl group is indicated by a band at 6.05 μ in the infrared spectrum. This band may be assigned to a carboxamide grouping. The compound absorbs ultraviolet light with maxima in 0.1 *N* sodium hydroxide at 275 mμ (*E* 20,300) and in 0.1 *N* hydrochloric acid at 267.5 mμ (*E* 19,500). On acid hydrolysis the ultraviolet absorption maxima are shifted 5 to 10 millimicrons to the longer wave length. Concomitantly, the biological activity of the compound is destroyed.

On alcoholysis with ethanolic hydrogen chloride I is cleaved into three fragments. One of these, II, is an amphoteric compound that precipitates as a dihydrochloride, m.p. 225–227° (dec.), from the cleavage mixture. The free base melts at 257–258°, *Anal.* Calcd. for $C_7H_9N_5$: C, 51.52; H, 5.56; N, 42.92; N-methyl, 9.21; mol. wt., 163.2. Found: C, 51.56; H, 5.76; N, 43.05; N-methyl, 14.65; mol. wt. (Rast), 169. The analytical data, the ultraviolet and infrared spectra and the amphoteric nature of II suggest a dimethylamino purine. Compound II was identified as 6-dimethylaminopurine by comparison with an authentic sample.[4]

The second fragment, compound III, was identified as O-methyl-L-tyrosine by analysis and by comparison of its melting point, rotation and spectra with those of an authentic sample. The compound was further characterized by representative derivatives.[5]

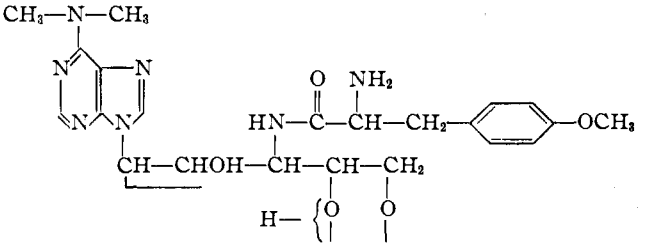
Compound IV, the third fragment, when isolated as its hydrochloride melts at 158–158.5° (dec.), $[\alpha]^{25}D$ −24.6° (water), *Anal.* Calcd. for $C_5H_{11}NO_4 \cdot HCl$: C, 32.35; H, 6.52; N, 7.55; Cl, 19.10; mol. wt., 185.6. Found: C, 32.57; H, 6.72; N, 7.62; amino nitrogen (Van Slyke), 7.70; Cl, 19.45; neut. equiv., 190.7. The characterizing reactions of IV show a positive Fehling test, a positive Brady's reaction, a negative ninhydrin test[6] and the formation of furfural on deamination and subsequent treatment with phosphoric acid. While IV consumes 3.8 moles of periodic acid in three hours, its N-acetyl derivative reacts with 2.0 moles in the same period. The absence of a carbonyl band in the infrared absorption spectrum of IV (in nujol) and the above chemical and analytical data permit the postulation of IV as a hemiacetal form of a 3- or 4-aminopentose.

The formation of a triacetate of I and its subsequent partial deacetylation with alcoholic ammonia to N-acetylpuromycin permits the postulation of two free alcoholic groups in I. The free amino group in I is placed in the O-methyltyrosine moiety by the failure of I to consume periodic acid. This failure to consume periodic acid by I also eliminates a 4-aminopentose structure for compound IV.

The 3-aminopentose was identified as D-3-aminoribose by comparison of its infrared spectrum, melting point and rotation with a synthetic sample.[7]

A negative Brady's test until after hydrolysis indicates a glycosidic linkage in Puromycin. A comparison of the ultraviolet absorption spectra of I with those of 7- and 9-ethyl-6-dimethylaminopurines[8] establishes this linkage to be at the 9 position of the purine.

Partial structure V is proposed for Puromycin, I.



Puromycin V

Structural features of I to be considered in a future communication are: (1) the α- or β-linkage of the glycoside and (2) the furanosidic or pyranosidic nature of the sugar portion.

COY W. WALLER
LEDERLE LABORATORIES DIVISION PETER W. FRYTH
AMERICAN CYANAMID COMPANY BRIAN L. HUTCHINGS
PEARL RIVER, NEW YORK JAMES H. WILLIAMS

RECEIVED MARCH 26, 1953

(1) American Cyanamid Company Trademark for Puromycin.

(2) Puromycin is the generic name for Achromycin.

(3) J. N. Porter, R. I. Hewitt, C. W. Hesseltine, G. Krupka, J. A. Lowery, W. S. Wallace, N. Bohonos and J. H. Williams, *Antibiotics and Chemotherapy*, **2**, 409 (1952).

(4) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, to be published.

(5) L. Behr and H. T. Clark, THIS JOURNAL, **54**, 1630 (1932).

(6) 2-Amino sugars give positive ninhydrin test.

(7) B. R. Baker, *et al.*, to be published.

(8) The ultraviolet absorption spectra data for these purines are: 6-dimethylamino-9-ethylpurine $\gamma^{0.1N\ NaOH}_{max}$ 277.5 (*E* 18,300); $\gamma^{01.N\ HCl}_{max}$ 270 (*E* 17,500); and 6-dimethylamino-7-ethylpurine $\gamma^{0.1N\ NaCl}_{max}$ 295 (*E* 19,400), $\gamma^{0.1N HCl}_{max}$ 290 (*E* 23,300), see reference 4.