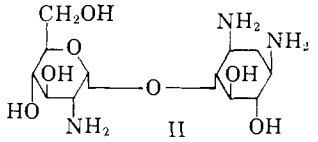
be identical with D-glucosamine hydrochloride by infrared spectrum, X-ray diffraction, optical rotation and comparison of the salicylidene derivatives.

Since the crystalline N,N′,N″-triacetylparomamine [*Anal.* Calcd. for $C_{18}H_{31}N_3O_{10}$ (449.5): C, 48.10; H, 6.95; N, 9.35. Found: C, 48.04; H, 7.03; N, 9.37; $[\alpha]^{25}D$ +108° (*c* 1.0, H_2O); m.p. 300–306° dec.] consumes two moles of periodate[5] with the absence of formaldehyde formation, and paromamine gave negative reduction tests, the structural formula of the latter is represented as II.



II

Since the infrared spectra (KBr) of crystalline paromamine trihydrochloride and paromamine free base show absorption bands at 11.2, 11.91 μ and 11.06, 11.75 μ, respectively, no assignment of anomeric configuration is possible by this method.[6] However, since glucosamine is in the D-series and paromamine trihydrochloride has a high positive molecular rotation (+36,100), an α-D-glycosidic linkage is inferred. Data from methylation experiments described in a subsequent paper have more rigorously established this conclusion.

(5) R. W. Jeanloz and E. Forchielli, *J. Biol. Chem.*, **188**, 361 (1951).
(6) S. A. Barker, E. J. Bourne, M. Stacey and D. H. Whiffen, *J. Chem. Soc.*, 171 (1954); *cf.* "Methods of Biochemical Analysis," Vol. III, Interscience Publishers, Inc., New York, N. Y., 1956, p. 213.

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PAROMOMYCIN. II. PAROMOBIOSAMINE, A DIAMINOHEXOSYL-D-RIBOSE

Sir:

In the previous communication[1] methanolysis of the antibiotic paromomycin was reported to yield paromamine and the α- and β-anomers of methyl paromobiosaminide. This communication deals with preliminary structural studies on the disaccharide moiety, paromobiosamine.

The unresolved anomeric mixture of methyl paromobiosaminides was converted to the amorphous N,N′-dibenzoyl derivative [*Anal.* Calcd. for $C_{11}H_{19}N_2O_7(OCH_3)(COC_6H_5)_2$: C, 58.64; H, 6.06; N, 5.26. Found: C, 58.37; H, 6.25; N, 5.58] melting over a wide range (120–145°). Dilute acid hydrolysis followed by extraction, ion exchange treatment and carbon chromatography afforded a neutral colorless gum which corresponded to ribose in several paper chromatographic systems. Its infrared absorption spectrum in KBr[2] was identical to that of D-ribose. Since it exhibited a negative rotation $[\alpha]^{27}D$ −14° → −18° (18 hr.) the sugar is assigned to the D-series. Reduction with sodium borohydride afforded crystalline ribitol, m.p. 102–103°.

Vigorous acid hydrolysis of methyl paromobiosaminide (6 *N* hydrochloric acid) resulted in complete destruction of the ribose moiety and, following carbon chromatography, afforded an amorphous hygroscopic diaminohexose (paromose) $[\alpha]^{26}D$ +19° (*c* 1.0, H_2O; no mutarotation). Paromose is characterized as its crystalline dipicrate [*Anal.* Calcd. for $C_6H_{14}N_2O_4$ $(C_6H_3N_3O_7)_2$: C, 33.97; H, 3.17; N, 17.61; picric acid, 72. Found: C, 34.15; H, 3.40; N, 17.44; picric acid (via ultraviolet analysis), 70; $[\alpha]^{28}D$ +22° (*c* 0.5, H_2O)] which melted at 126–128° with decomposition. N,N′-Diacetylparomose formed a crystalline *p*-nitrophenylhydrazone (yellow needles) [*Anal.* Calcd. for $C_{16}H_{23}N_5O_7$: C, 48.36; H, 5.83; N, 17.63. Found: C, 48.42; H, 5.82; N, 17.53; $[\alpha]^{28}D$ + 5.9° (*c* 0.4, moist MeOH), m.p. 229–231° dec.]. N-Acetylation of paromose by the method of Roseman and Ludowieg[3] followed by sodium borohydride reduction yielded the crystalline dihydro N,N′-diacetyl derivative [*Anal.* Calcd. for $C_{10}H_{20}N_2O_6$: C, 45.45; H, 7.63; N, 10.60. Found: C, 45.29; H, 7.61; N, 10.50; $[\alpha]^{28}D$ −17.8° (*c* 4.0, 0.2 *M* *p*H 4.5 acetate buffer); m.p. 150.5–151.5°].

Dilute acid hydrolysis (0.5 *N* hydrochloric acid for 5 hours at 92°) of methyl paromobiosaminide produced, in addition to small amounts of starting material and paromose, the reducing disaccharide paromobiosamine which was isolated as the crystalline dihydrochloride [*Anal.* Calcd. for $C_{11}H_{22}N_2O_8 \cdot 2HCl \cdot CH_3OH$ (415.3): C, 34.71; H, 6.80; N, 6.75; Cl, 17.08. Found: C, 34.53; H, 7.13; N, 6.82; Cl, 16.84; neutral equivalent, 204; $[\alpha]^{27}D$ +25.5° (*c* 1.0, H_2O; no mutarotation)] and as the free base having a mutarotation value of +32° (*c* 1.0, H_2O). The ease of methanolysis (0.32 *N* HCl) of paromomycin to methyl paromobiosaminide which in turn can be hydrolysed with 0.5 *N* HCl to the free disaccharide, paromobiosamine, argues strongly in favor of a diaminohexosyl pentose rather than a pentosyl diaminohexose structure. Rinehart and Woo[4] arrive at the same conclusion with the neobiosamines but base their evidence on the detection of ribitol from hydrolysis of N,N′-dibenzoyldihydroneobiosamine C.

(1) T. H. Haskell, J. C. French and Q. R. Bartz, THIS JOURNAL, **81**, 3480 (1959).
(2) F. E. Resnik, L. S. Harrow, J. C. Holmes, M. E. Bill and F. L. Green, *Anal. Chem.*, **29**, 1874 (1957).
(3) S. Roseman and J. Ludowieg, THIS JOURNAL, **76**, 301 (1954).
(4) K. L. Rinehart, Jr., and P. W. K. Woo, *ibid.*, **80**, 6463 (1958).

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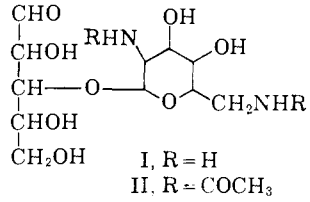
PAROMOMYCIN. III. THE STRUCTURE OF PAROMOBIOSAMINE

Sir:

The characterization of paromobiosamine as an *O*-(diaminohexosyl)-D-ribose was described in the previous communication.[1] This report concerns

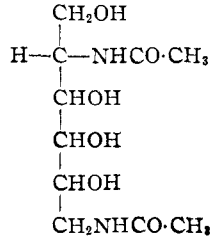
(1) T. H. Haskell, J. C. French and Q. R. Bartz, THIS JOURNAL, **81**, 3481 (1959).

periodate oxidation studies which permit the assignment of structure I to paromobiosamine.



I, R = H
II, R = $COCH_3$

Periodate oxidation[2] of the diaminohexose (paromose) resulted in the rapid consumption of four moles of oxidant with the formation of 0.8 mole of formaldehyde. N,N′-Diacetylparomose also consumed four moles[2] with no formaldehyde production indicating a straight chain hexose with the absence of a hydroxyl function in the 6 position. Crystalline N,N′-diacetyldihydroparomose consumed exactly two moles of oxidant with formation of 0.8 mole of formic acid and no formaldehyde indicating the presence of three contiguous hydroxyl groups in the six carbon chain and an amino group in the 2 position. Bromine oxidation and hydrolysis of the periodate mixture afforded L-serine[3] and glycine identified by paper chromatography and infrared analysis. The isolation of these two amino acids unambiguously allows the assignment of structure III to N,N′-diacetyldihydroparomose.



III

To prove unequivocally that paromose is an aldose rather than a ketose,[4] it was converted into crystalline derivatives: paromose dibenzyldithioacetal dihydrochloride [*Anal.* Calcd. for $C_{26}H_{28}N_2O_3S_2 \cdot 2HCl \cdot H_2O$ (499.5): C, 48.09; H, 6.46; N, 5.61; S, 12.84; Cl, 14.20. Found: C, 47.91; H, 6.69; N, 5.73; S, 13.01; Cl, 14.51]; N,N′-dibenzoylparomose dibenzyldithioacetal [*Anal.* Found: C, 65.92; H, 6.04; N, 4.42; S, 10.55; m.p. 162–163°]; and N,N′-dibenzoyl-1-deoxyparomose [*Anal.* Found: C, 64.21; H, 6.42]. The last product consumed exactly two moles of periodate which indicated an aldose structure for paromose. To confirm this further, the oxidation products were extracted into ethyl acetate, oxidized with bromine and hydrolyzed with acid to yield alanine and glycine which were identified by paper chromatography.

(2) For overoxidation of N-acetylamino sugars see R. W. Jeanloz and E. Forchielli, *J. Biol. Chem.*, **188**, 361 (1951).

(3) We are indebted to Dr. O. D. Bird and Miss Barbara Hall of these Laboratories for determining this configuration by microbiological assay.

(4) The evidence accumulated up to this point does not rule out the possibility of paromose being $CH_2OH—CH(NH_2)—CHOH—CHOH—C(=O)—CH_2NH_2$.

The observation that methyl paromobiosaminide dihydrochloride consumed 2.8 moles of periodate with formation of 0.7 mole of ammonia and no formaldehyde establishes a pyranose ring structure in the paromose moiety of the disaccharide.

To determine the positional linkage of paromose to D-ribose, N,N′-diacetylparomobiosamine (II) [*Anal.* Found: C, 45.50; H, 6.96; N, 7.01; $[\alpha]^{28}D$ + 42.2° (*c* 0.95, H_2O)] upon oxidation with periodate consumed 1.4 moles of oxidant liberating a trace of formaldehyde.[5] However, upon borohydride reduction of this material, the corresponding dihydro derivative [*Anal.* Calcd. for $C_{15}H_{28}N_2O_{10} \cdot H_2O$ (414.4): C, 43.47; H, 7.30; N, 6.76. Found: C, 43.56; H, 7.21; N, 6.96; $[\alpha]^{27}D$ +57° (*c* 1.0, H_2O)] consumed 4.8 moles of oxidant in 1 hour with the liberation of 2.0 moles of formaldehyde. The glycosidic linkage of paromose is therefore on the third carbon atom of D-ribose. This conclusion was further substantiated by the isolation of 2,5-di-*O*-methyl-D-ribose[6] from the acid hydrolyzate of methylated N-acetylparomomycin.

(5) Methyl N,N′-diacetylparomobiosaminide as well as the corresponding N,N′-dibenzoyl derivative consumed less than one-half mole of oxidant in 3 days under standard conditions. However, by using 0.05 *M* periodate rather than 0.005 *M* these two compounds consumed one mole of oxidant in 22 hours.

(6) T. H. Haskell, J. C. French and Q. R. Bartz, THIS JOURNAL, **81**, 3481 (1959).

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PAROMOMYCIN. IV. STRUCTURAL STUDIES

Sir:

Previous communications[1] have described the structural elucidation of the two fragments, paromamine and paromobiosamine, obtained from the degradation of paromomycin. We now wish to report the position of attachment of paromobiosamine to paromamine thus completing the gross structure for paromomycin.

N-Pentacetylparomomycin [*Anal.* Calcd. for $C_{33}H_{55}N_5O_{19} \cdot H_2O$ (843.8): C, 46.97; H, 6.81; N, 8.30. Found: C, 46.64; H, 7.25; N, 8.17; $[\alpha]^{27}D$ +64° (*c* 1.5, H_2O)] in 0.05 *M* periodate solution consumed 1.94 moles in 22 hours. After periodate removal and strong acid hydrolysis deoxystreptamine was isolated in 83% yield indicating that the ribose moiety is glycosidically linked to one of the two hydroxyls in the cyclohexane rather than the glucosamine portion. No glucosamine could be detected in the hydrolyzate.

Quantitative ammonia liberation studies then were conducted on periodate oxidized samples of paromomycin, paromamine and methyl paromobiosaminide. Under identical conditions ammonia was liberated in molar ratios of 3:2:1, respectively. These facts indicate that the ribose and glucosamine moieties are glycosidically linked

(1) The preceding paper in this series is T. H. Haskell, J. C. French and Q. R. Bartz, THIS JOURNAL, **81**, 3482 (1959).