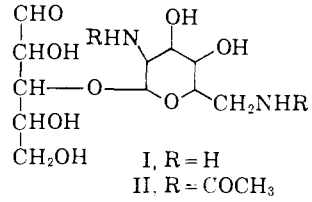
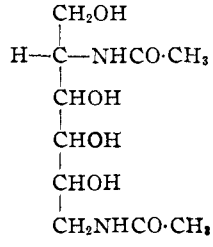
periodate oxidation studies which permit the assignment of structure I to paromobiosamine.



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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RECEIVED MAY 5, 1959

PAROMOMYCIN. IV. STRUCTURAL STUDIES

Sir:

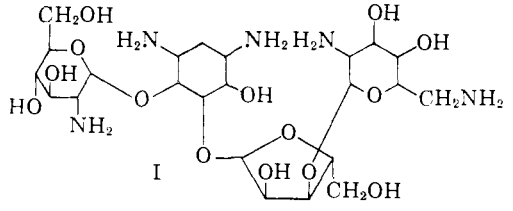
Previous communications[1] have described the structural elucidation of the two fragments, paromamine and paromobiosamine, obtained from the degradation of paromomycin. We now wish to report the position of attachment of paromobiosamine to paromamine thus completing the gross structure for paromomycin.

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).

to adjacent hydroxyls in deoxystreptamine as shown in I.



I

Additional support for a 5,6(4,5) linkage also can be rationalized on the basis of the extreme ease of methanolysis of paromomycin (0.32 *N* HCl) in contrast to kanamycin which is unaffected under these conditions and has been shown to be a 4,6-disubstituted deoxystreptamine.[2] Final proof for structure I was obtained from methylation and subsequent hydrolysis experiments.

When N-pentacetylparomomycin was methylated by a modified procedure of West and Holden,[3] a product containing 22.2% methoxyl (26.4% for full methylation) was obtained. Acid hydrolysis followed by ion exchange and cellulose chromatography afforded an optically active mono-*O*-methyldeoxystreptamine isolated as the crystalline N,N'-diacetyl derivative. *Anal.* Found: C, 50.38; H, 7.76; N, 10.72; $[\alpha]^{27}D$ +15° (*c* 1.0, H_2O); m.p. 280–282° dec. The product consumed 1.0 mole of periodate in 24 hours at 5°. The isolation of an optically active *O*-monosubstituted deoxystreptamine conclusively places the methoxyl in either the 4 or 6 position since 5 substitution produces a *meso* form.[4]

From the neutral fraction of the above hydrolyzate there was isolated a sugar which corresponded to 2,5-di-*O*-methyl-D-ribose by paper chromatography and electrophoresis.[5] Final characterization was accomplished by conversion to the crystalline *p*-bromophenylosazone which melted at 183–184° and showed no depression on mixing with an authentic sample.[6] *Anal.* Found: C, 42.89; H, 4.14; N, 11.09. The isolation of this disubstituted ribose proves the presence of a furanose ring structure in the intact antibiotic and confirms the glycosidic attachment of paromose to the third carbon of D-ribose.

Since the molecular rotation of an unsymmetrically monosubstituted deoxystreptamine is now known (+3900) it is possible to calculate by Hudson's rules the anomeric contribution (A_G) of the glycosidic linkage in paromamine. Since 4 and 6 monosubstituted deoxystreptamines are enantiomorphs, the contribution of the deoxystreptamine moiety ($[M]_D$) in paromamine must be of opposite sign to the methoxy derivative. By appropriate substitution into the equation $[M]_p = [M]_D + A_G + B_G$ and solving for A_G, a value of +27,070 is obtained. This is in agreement with the calculated values (± 25,000 ± 5,000)[7,8] for the contribution of anomeric centers in alkyl glycosides. Since the sugar is in the D-series, the positive value establishes an α-D-linkage in paromamine.

(2) M. J. Cron, O. B. Fardig, D. L. Johnson, D. F. Whitehead, I. R. Hooper and R. U. Lemieux, THIS JOURNAL, **80**, 4115 (1958).

(3) E. S. West and R. F. Holden, *ibid.*, **56**, 930 (1934).

(4) S. Umezawa, Y. Ito and S. Fukatsu, *J. Antibiotics (Japan)*, **A11**, 162 (1958).

(5) D. M. Brown, D. I. Magrath and A. R. Todd, *J. Chem. Soc.*, 1442 (1954).

(6) The authors are indebted to Professor A. R. Todd and coworkers for this service.

(7) C. S. Hudson, THIS JOURNAL, **31**, 66 (1909).

(8) R. U. Lemieux, C. W. DeWalt and M. L. Wolfrom, *ibid.*, **69**, 1838 (1947).

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RECEIVED MAY 5, 1959

ENZYMATIC FORMATION OF L-GLUTAMIC ACID AND ACETIC ACID FROM KYNURENIC ACID[1]

Sir:

Kynurenic acid (KA) has been shown to be degraded by resting cell suspensions and crude extracts of *Pseudomonas*,[2,3] but precise metabolic pathways have not yet been elucidated. We now are reporting the identification of L-glutamate and acetate as products of the degradation of KA by a partially purified enzyme preparation from a tryptophan adapted *Pseudomonas* sp. (ATCC 11299B).

Cells were grown and extracts were made as previously described.[4] Crude extracts then were centrifuged at 100,000 X *g* for one hour. The incubation mixture contained, in a final volume of 55.5 ml., 52 ml. of the supernatant fraction (468 mg. protein), 0.40 μM. of KA-3-C^{14} [5] (758,000 c.p.m.) and 304.0 μM. of unlabeled KA. The reaction mixture was incubated at 36° for 2.5 hours with continuous reciprocal shaking, deproteinized with cold 3 % H_2SO_4 and was extracted with 3 vol. of ether. The ether soluble fraction, which contained approximately 40% of the original counts, was subjected to partition chromatography on a Celite column.[6] A radioactive compound was identified tentatively as acetic acid by its titration curve, an enzymatic assay using acetokinase of *E. coli*[7] and by the melting point of *p*-bromophenacyl ester (85°).[8]

The water layer was neutralized with $Ba(OH)_2$ and centrifuged. The supernatant was subjected to ion exchange chromatography on Dowex-1 and Dowex-50 columns and L-glutamic acid hydro-

(1) This investigation was supported in part by research grants from the National Institutes of Health (C-4222), the Rockefeller Foundation and the Jane Coffin Childs Memorial Fund for Medical Research.

(2) R. Y. Stanier and O. Hayaishi, *Science*, **114**, 326 (1951).

(3) E. J. Behrman and T. Tanaka, *Fed. Proc.*, **18**, 189 (1959).

(4) O. Hayaishi, *Biochem. Preparations*, **3**, 108 (1953).

(5) DL-Tryptophan-3-C^{14} was converted enzymatically to kynurenine-3-C^{14},[4] which was further converted to KA-3-C^{14} by Pseudomonas transaminase in the presence of α-ketoglutarate (I. L. Miller, M. Tsuchida and E. A. Adelberg, *J. Biol. Chem.*, **203**, 205 (1953). KA was then purified by ion exchange chromatography and was shown to be chromatographically pure on paper using three different solvent systems. Details of this procedure will be published elsewhere.

(6) M. H. Peterson and M. J. Johnson, *ibid.*, **174**, 775 (1948).

(7) I. A. Rose, M. Grunberg-Manago, S. P. Korey and S. Ochoa, *ibid.*, **211**, 737 (1954).

(8) W. L. Judefind and E. E. Reid, THIS JOURNAL, **42**, 1043 (1920).