solve, was 1.3 g. of a thin brown oil which had an ultraviolet absorption maximum at 320 mμ. This was not further investigated. The next fraction, eluted from the column with benzene, contained the desired product; 3.15 g. of a pale orange oil was obtained upon removal of the solvent. Over-all yield from compound I was 13%. The material remaining adsorbed on the column was removed by washing with ether containing 10% methanol. This gave 4.1 g. of a deep yellow oil which showed general absorption in the 260 to 300 mμ region of the ultraviolet.

Fraction 2 had an ultraviolet absorption curve with maxima at 323, *342*, and 361 mμ (Curve 1, Fig. 1); $E(1\%,1\text{ cm.})$ at 342 mμ = 1650. *Anal.* Calcd. for $C_{18}H_{26}$: C, 89.23; H, 10.77. Found: C, 89.30; H, 10.69.

Preparation of Compounds VIII and XII.—These two compounds were prepared in a manner exactly analogous to that used for compound VII above with the substitution of methallyl bromide and benzyl chloride, respectively, in the initial Grignard condensation with compound I.

Compound VIII.—The over-all yield of chromatographically purified final product from 90 g. of compound I was 4.78 g. (5.1% yield) of a pale orange oil. The ultraviolet absorption curve (Curve 2, Fig. 1) showed three maxima at 332, *348* and 367 mμ with $E(1\%,1\text{ cm.})$ (348 mμ) = 1800. *Anal.* Calcd. for $C_{19}H_{28}$: C, 89.0; H, 11.0. Found: C, 88.67; H, 10.75.

Compound XII.—From 50 g. of compound I, the purified final product was 5.0 g. (8.3% over-all yield) of a light yellow oil. Its ultraviolet absorption spectrum showed a single maximum at 338 mμ (Curve 6, Fig. 2) with $E(1\%,1\text{ cm.})$ = 1875. *Anal.* Calcd. for $C_{22}H_{28}$: C, 90.43; H, 9.57. Found: C, 90.24; H, 9.44.

Preparation of 1-Bromo-4-methoxy-2-butene.—Butadiene (Matheson Co., E. Rutherford, New Jersey) was passed from a cylinder into 1 liter of chloroform at −30° until 162 g. (3.0 moles) had been dissolved. Bromine (480 g., 3.0 moles) was added dropwise with stirring over a period of three hours, maintaining the temperature at −30° with a Dry Ice–acetone-bath. When the bromine addition was complete, the chloroform was removed by distillation, the last traces under diminished pressure. The pressure was lowered to 12 mm. and the bulk of the reaction product distilled over at 80 to 110°. A small amount of the tetrabromide remained in the undistilled residue. The distillate was dissolved in 750 ml. of methanol and chilled slowly with frequent shaking to −50°. The white crystals of *trans*-1,4-dibromo-2-butene were filtered off and recrystallized twice more. This material was dried for sixteen hours in a vacuum desiccator at room temperature, giving 256 g. (40% yield) of crystalline dibromide which melted sharply at 52.5°. *Anal.* Calcd. for $C_4H_6Br_2$: C, 22.43; H, 2.80; Br, 74.77. Found: C, 22.48; H, 2.88; Br, 74.65.

Two hundred and thirty-four grams (1 mole) of the dibromide was dissolved in 200 ml. of methanol. Twenty-three grams (1 mole) of metallic sodium was added to another 100-ml. portion of methanol. The resulting solution of sodium methoxide was added slowly to the dibromide solution and the mixture was refluxed gently for four hours. Five hundred ml. of ether was added to help precipitate the sodium bromide, and the salt was removed by filtration.

The 132 g. of yellow liquid obtained upon removal of the solvent was distilled at 36 mm. pressure in a modified Claisen flask with a 16″ Vigreux sidearm. Eight fractions were collected over a temperature range of 33 to 73°. Analysis showed the lower boiling fractions to contain the dimethyl ether, while the high boiling fractions contained some of the unchanged dibromide. The two main fractions (55–60°, and 60–66°) were combined and refractionated. The main portion (36 g.) distilled at 58 to 65° at 36 mm. This was refractionated at the same pressure once more. The main fraction (27 g.) distilled at 60–63°, representing an over-all yield of purified material from butadiene equal to 5.4%. The product was a clear, water-white, extremely lachrymatory liquid. *Anal.* Calcd. for C_5H_9OBr: C, 36.36; H, 5.45; Br, 48.48. Found: C, 37.12; H, 5.71; Br, 48.90.

Several attempts to condense this material with compound I employing magnesium and lithium failed completely.

Summary

β-Ionone has been condensed with ethyl formate to give hydroxymethylene β-ionone. This compound, its sodium salt, and its diethyl acetal have been involved in a series of reactions with various metallo-organic compounds. Attempts to form β-ionylideneacetaldehyde by normal addition to the carbonyl group were unsuccessful. By 1,4-addition of various unsaturated Grignard complexes, several compounds related to vitamin A have been synthesized. None of these compounds is biologically active. The relationship between ultraviolet absorption spectrum and structure has been emphasized.

ROCHESTER, NEW YORK RECEIVED AUGUST 29, 1946

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Streptomyces Antibiotics. XII. The Degradation of Streptomycin and Dihydrostreptomycin with Methanol

BY NORMAN G. BRINK, FREDERICK A. KUEHL, JR., EDWIN H. FLYNN AND KARL FOLKERS

Streptomycin hydrochloride was cleaved by the action of anhydrous methanol containing hydrogen chloride into streptidine and methyl streptobiosaminide dimethyl acetal hydrochloride, which upon acetylation gave crystalline methyl tetraacetylstreptobiosaminide dimethyl acetal. It was further demonstrated that the streptobiosamine moiety of streptomycin possessed a methylamino group and also a free or potential carbonyl group, as was shown by the preparation of the oxime and semicarbazone of streptomycin hydrochloride.[1] Details of these investigations and new data on the degradation products and the analogous degradation of dihydrostreptomycin[2] are described in the present publication. Other investigators[3] have studied the reaction of streptomycin and hydrogen chloride in methanol solution and obtained an amorphous, optically active hydrochloride of a base with properties which agreed with

(1) Brink, Kuehl and Folkers, *Science*, **102**, 506 (1945).

(2) Peck, Hoffhine and Folkers, THIS JOURNAL, **68**, 1390 (1946).

(3) Carter, Clark, Dickman, Loo, Meek, Shell, Strong, Alberi, Bartz, Binkley, Crooks, Hooper and Rebstock, *Science*, **103**, 53 (1946).

those described for methyl streptobiosaminide dimethyl acetal hydrochloride.[1]

When a solution of streptomycin hydrochloride in methanol containing about 1% of hydrogen chloride was allowed to stand at room temperature, the specific rotation of the solution changed from a value of $[\alpha]^{25}D$ − 60° soon after mixing to a final, constant value of $[\alpha]^{25}D$ −80° after about twenty hours. The products of the reaction were separated by a chromatographic procedure, using acid-washed alumina. In methanol–ether solution streptidine hydrochloride was adsorbed by the alumina, while the streptobiosamine derivative passed freely through the column. The streptidine hydrochloride was then obtained by elution with methanol, and was characterized as the crystalline picrate. On the basis of the formula $C_{21}H_{39}N_7O_{12}$ for streptomycin,[4] the cleavage of the antibiotic in methanol solution is represented by the equation

$$C_{21}H_{39}N_7O_{12}\cdot 3HCl + 3CH_3OH \longrightarrow C_8H_{18}N_6O_4\cdot 2HCl + C_{13}H_{22}NO_7(OCH_3)_3\cdot HCl + H_2O$$

Methyl streptobiosaminide dimethyl acetal hydrochloride is an amorphous, levorotatory, light tan solid, soluble in water, pyridine, and methanol, but insoluble in most other common organic solvents. No carbonyl absorption could be detected when the infrared spectrum of the material was studied. It seems likely that in this streptobiosamine derivative the reactive carbonyl group has been converted to a dimethyl acetal. The third methoxy group is assumed to be that of a methyl glycoside.

In a Kuhn–Roth determination of methyl groups attached to carbon, 0.83 molar equivalent of acetic acid was obtained, indicating that the compound contained at least one C-methyl group.

When methyl streptobiosaminide dimethyl acetal hydrochloride was heated with aqueous alkali, the formation of maltol in 17% yield was indicated by the ultraviolet absorption of the acidified solution (λ max. = 2750 Å.) and colorimetric determination using ferric ion.[5] The formation of maltol by similar treatment of streptomycin has been described by Schenck and Spielman.[6]

Treatment of methyl streptobiosaminide dimethyl acetal hydrochloride with concentrated aqueous alkali at the reflux temperature of the solution gave methylamine, which was isolated as the hydrochloride and identified by conversion to 2,4-dinitromethylaniline. Since the migration of methyl groups from oxygen to nitrogen under the influence of alkali has been observed,[7] the streptobiosamine derivative was allowed to stand with dilute hydrochloric acid until the methoxy groups had been removed, after which the residue was treated with alkali as before. Methylamine was again isolated and characterized.

Acetylation of methyl streptobiosaminide dimethyl acetal hydrochloride gave crystalline methyl tetraacetylstreptobiosaminide dimethyl acetal, $C_{13}H_{18}NO_7(CH_3CO)_4(OCH_3)_3$. A differential acetyl determination[8] showed that three of the acetyl groups were attached to oxygen atoms, and the fourth to the nitrogen atom. In methanol solution, the ultraviolet absorption spectrum of this compound showed only a low end absorption, with no maximum.

An infrared spectrum of methyl tetraacetylstreptobiosaminide dimethyl acetal in low concentration (*ca.* 5–10%) in tetrachloroethane solution showed strong, symmetrical bands at 5.75 and 6.15 μ. These were attributed to the presence of ester and disubstituted amide groups. A very concentrated solution of a carefully dried sample of the compound in the same solvent showed absorption at 2.75 μ (—OH, > NH region). In a Zerewitinoff active hydrogen determination on this compound, one mole of methane was liberated. There can be no > NH group in this compound, since the nitrogen atom is known to be present as —N(CH$_3$)(CH$_3$CO), and hence these results indicate that methyl tetraacetylstreptobiosaminide dimethyl acetal contains a free hydroxyl group which is resistant to acetylation.

Dihydrostreptomycin hydrochloride[2] was reacted with methanol containing hydrogen chloride and the streptidine hydrochloride was removed chromatographically. Acetylation of the high-rotating amorphous product led to the isolation of two crystalline acetyl derivatives, m. p. 198–198.5° and m. p. 155.5–157°. As expected, both compounds contained one methoxy group and five acetyl groups, four of which were attached to oxygen atoms and one to the nitrogen atom, and appeared on the basis of the analytical data to have the formula $C_{24}H_{37}NO_{14}$.[9] This finding substantiates the interpretation of the nature of the methoxy groups in methyl streptobiosaminide dimethyl acetal hydrochloride; and shows that, as has been suggested,[2] the hydrogenation of streptomycin to dihydrostreptomycin involves the reduction of a carbonyl group to a hydroxyl group.

Both acetyl derivatives gave approximately one mole of methane in the Zerewitinoff active hydrogen determination, and both showed an infrared absorption in the 3 μ region when studied in satu-

(4) Kuehl, Flynn, Brink and Folkers, THIS JOURNAL, **68**, 2096 (1946).

(5) Boxer and Jelinek, Abstracts of Papers, Div. of Biological Chemistry, A. C. S. Meeting, Chicago, Ill., September, 1946, p. 13B. We are indebted to Dr. Boxer for carrying out this determination.

(6) Schenck and Spielman, THIS JOURNAL, **67**, 2276 (1945).

(7) *Cf* Irvine and Hynd, *J. Chem. Soc.*, **101**, 1128 (1912).

(8) Wolfrom, Konigsberg and Soltzberg, THIS JOURNAL, **58**, 490 (1936); Kunz and Hudson, *ibid.*, **48**, 1982 (1926).

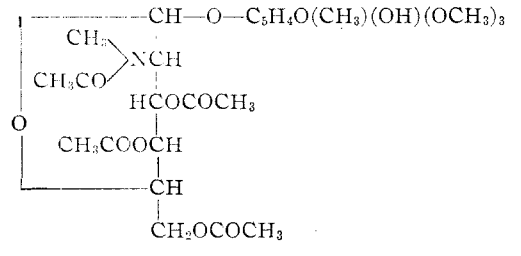
(9) Footnote added in proof: The preparation of the higher melting acetyl derivative of methyl dihydrostreptobiosaminide has been described by two other groups of investigators, Fried and Wintersteiner, Abstracts of Papers, Division of Biological Chemistry, A. C. S. Meeting, Chicago, Ill., September, 1946, p. 15B; and Bartz, Controulis, Crooks and Rebstock, *ibid.*, Div. of Medicinal Chemistry, p. 8K. Fried and Wintersteiner's formulation of the compound was in agreement with that presented here, whereas Bartz, *et al.*, described the compound as a hexaacetyl derivative.

rated solution in tetrachloroethane. It is thus evident that the unacetylatable hydroxyl group observed in methyl tetraacetylstreptobiosaminide dimethyl acetal is also present in the isomeric methyl pentaacetyldihydrostreptobiosaminides.

To investigate the nature of the isomerism involved in these two compounds, they were separately treated with ethyl mercaptan and hydrogen chloride to replace the methoxy groups by ethylmercapto groups, and were then treated, after reacetylation, with Raney nickel in order to substitute hydrogen atoms for the ethylmercapto groups. Both isomeric methyl glycosides gave the same final crystalline product, pentaacetyldihydrodesoxystreptobiosamine. Thus, it appears that the two compounds differ sterically at the carbon atom which bears the glycosidic methoxy group, since destruction of the asymmetry at this carbon atom led to the isolation of a common compound. The compounds have been designated α-methyl pentaacetyldihydrostreptobiosaminide, m. p. 198–198.5°, and β-methyl pentaacetyldihydrostreptobiosaminide, m.p. 155.5–157°.

The hydrolysis of methyl streptobiosaminide dimethyl acetal hydrochloride to yield N-methyl-L-glucosamine hydrochloride has been reported.[10] α-Methyl pentaacetyldihydrostreptobiosaminide was dissolved in hot 10% hydrochloric acid, and the solution was refluxed for three hours. The reaction products were reacetylated and a low yield of pentaacetyl-N-methyl-L-glucosamine[10] was secured by a chromatographic fractionation. This observation substantiates the conclusion[1] that the reactive carbonyl group of streptomycin resides in the streptose (nitrogen-free) portion of the molecule.

It has been shown[1] that the N-methyl-L-glucosamine portion of streptobiosamine is linked to the streptose moiety through carbon atom one (the reducing group) of the methylamino hexose. A partial structural formula for methyl tetraacetylstreptobiosaminide dimethyl acetal based on the accumulated evidence is as follows.



Experimental

Methyl Streptobiosaminide Dimethyl Acetal Hydrochloride.—A solution of 1.97 g. of pure, anhydrous streptomycin hydrochloride in 100 ml. of anhydrous methanol containing 1.1% of hydrogen chloride was allowed to stand for nineteen hours at room temperature, during which time the rotation changed from $[\alpha]^{25}D$ −60° immediately after mixing to a final value of $[\alpha]^{25}D$ −80°. The solvent was removed *in vacuo* and the residue evaporated repeatedly with toluene. The product, a light pink powder, weighed 2.15 g.

The material was dissolved in 154 ml. of methanol, 96 ml. of ether was added, and the solution was put on a column of 44 g. of acid-washed alumina prepared with a 2:1 methanol–ether mixture. The column was then washed with 193 ml. of a 3:2 methanol–ether mixture. Removal of the solvent from the eluate *in vacuo* gave 553 mg. of a light tan powder, $[\alpha]^{25}D$ −143° ± 2° (*c*, 1.02 in methanol). A second elution with 190 ml. of 3:2 methanol–ether afforded 241 mg. of somewhat less pure material, $[\alpha]^{25}D$ −113° ± 2° (*c*, 0.930 in methanol). A sample of the higher-rotating fraction was dried in a weighing pig at room temperature and analyzed.

Anal. Calcd. for $C_{13}H_{22}NO_7(OCH_3)_3 \cdot HCl$: C, 44.29; H, 7.57; N, 3.19; OCH_3, 21.5; C-methyl, 3.5. Found: C, 44.35; H, 7.13; N, 4.00; OCH_3, 19.1; C-methyl, 2.9; amino-nitrogen (Van Slyke), none.

In a similar experiment, 194 mg. of the streptomycin methanolysis product was chomatographed on 4 g. of alumina. After eluting most of the high-rotating material with methanol–ether mixtures, as described above, a 1:1 acetone–methanol mixture and then a portion of pure methanol were passed through the column, giving 22 mg. and 63 mg. of solids respectively. The material of the last fraction had a rotation of $[\alpha]^{25}D$ −20° (*c*, 1.17 in water), and consisted mainly of streptidine hydrochloride, as was shown by its conversion in 72% yield to crystalline streptidine picrate, m. p. 281–282°.[3,11]

Methylamine from Methyl Streptobiosaminide Dimethyl Acetal Hydrochloride.—One-half gram of methyl streptobiosaminide dimethyl acetal hydrochloride was allowed to stand in solution in 15 ml. of 3 *N* hydrochloric acid for six days (room temperature), after which the hydrochloric acid was removed *in vacuo*. The methoxyl-free residue (*Anal.* Found: OCH, none), was refluxed for two hours in 15 ml. of 20% potassium hydroxide solution, during which time a stream of nitrogen was bubbled through the solution. The exit gases after passing through the reflux condenser, were led into 5 ml. of 1 *N* hydrochloric acid. Evaporation of the hydrochloric acid left 14 mg. (18%) of a glassy residue. This was dissolved in 0.8 ml. of alcohol and 1.5 ml. of 0.13 *N* methanolic potassium hydroxide added. The resulting solution was added to 60 mg. of 2,4-dinitrochlorobenzene dissolved in 0.8 ml. of hot alcohol. The solution was concentrated to a volume of 1 ml. After thirty minutes the crystals which had formed were collected, washed, and recrystallized from hot alcohol, giving 10 mg., m. p. 181.5–182°. There was no depression of the melting point when this material was mixed with an authentic sample of 2,4-dinitromethylaniline of m. p. 180.5–182°.[12]

Anal. Calcd. for $C_7H_7N_3O_4$: C, 42.64; H, 4.51; N, 21.31. Found: C, 42.88; H, 3.87; N, 20.89.

In another experiment methyl streptobiosaminide dimethyl acetal hydrochloride (0.46 g.) was treated directly with 20% alkali and the methylamine (7 mg.) isolated as described above. Again, reaction with 2,4-dinitrochlorobenzene gave 2,4-dinitromethylaniline, m. p. and mixed m. p. 180–181°.

Methyl Tetraacetylstreptobiosaminide Dimethyl Acetal.—A solution of 919 mg. of crude, amorphous methyl streptobiosaminide dimethyl acetal hydrochloride in 6 ml. of pyridine was cooled to 0° and 4.7 ml. of acetic anhydride added. The solution was held at 0° for three to four hours and was then allowed to come slowly to room temperature and stand overnight. Water was added to decompose the excess acetic anhydride, the solution was evaporated to dryness at room temperature, and the residue taken up in chloroform and washed with water, 5% sulfuric acid, and again with water. The chloroform solu-

(10) Kuehl, Flynn, Holly, Mozingo and Folkers, THIS JOURNAL, **68**, 536 (1946).

(11) Peck, Graber, Walti, Peel, Hoffhine and Folkers, *ibid.*, **68**, 29 (1946).

(12) Romburgh, *Chem. Ztg.*, **35**, 200 (1911), gives the melting point of 2,4-dinitromethylaniline as 178°.

tion was dried and the solvent removed, giving 1.20 g. of oil. This was crystallized from about ten parts of ether, and yielded 711 mg. of needles, m. p. 122–123°, with previous softening. Recrystallization from ether gave 620 mg. (70%) of crystals, m. p. 123.5–126°. The first time this preparation was done, crystalline material was obtained by chromatographing the acetylation product on alumina.

A sample for analysis was recrystallized from ether and from benzene–petroleum ether mixtures to a constant melting point of 124.5–126° (micro-block). The pure substance had a rotation $[\alpha]^{25}D$ −124 ± 1° (*c*, 1.07 in chloroform).

Anal. Calcd. for $C_{13}H_{18}NO_7(CH_3CO)_4(OCH_3)_3$: C, 50.97; H, 6.95; N, 2.48; CH_3CO, 30.5; OCH_3, 16.5; mol. wt., 565. Found: C, 50.88, 51.20; H, 7.09, 6.95; N, 2.55; CH_3CO, 29.7; OCH_3, 15.4; mol. wt., 530 (ebullioscopic in benzene). A determination of O-acetyl[8] gave a value of 22.4. The calculated value for three CH_3CO is 22.9. A Zerewitinoff determination carried out in anisole solution at room temperature gave 0.95 mole of methane. At 95°, the determination showed 1.2 moles of methane produced.

Methanolysis of Dihydrostreptomycin Hydrochloride.—Two grams of dihydrostreptomycin hydrochloide was dissolved in 100 ml. of methanol containing 1% of hydrogen chloride. The rotation of the solution changed from an initial value of $[\alpha]^{25}D$ −60° to a constant value of $[\alpha]^{25}D$ −68° on standing overnight (seventeen hours). The solvent was removed *in vacuo*, giving 2.12 g. of amorphous residue. This was dissolved in 154 ml. of methanol, 93 ml. of ether added, and the solution put on a column of 42.5 g. of acid-washed alumina prepared with a 2:1 methanol–ether mixture. The column was then washed with 187 ml. of a 3:2 methanol–ether mixture. The eluate was evaporated to dryness under reduced pressure, giving 425 mg. of amorphous, tan residue, $[\alpha]^{25}D$ −122° (*c*, 1.49 in methanol). This product consisted of a mixture of α-methyl dihydrostreptobiosaminide hydrochloride and β-methyl dihydrostreptobiosaminide hydrochloride, as shown by its conversion to the crystalline isomeric acetyl derivatives.

α-Methyl Pentaacetyldihydrostreptobiosaminide.—A 1.27-g. portion of the mixture of amorphous methyl dihydrostreptobiosaminide hydrochlorides was acetylated overnight at room temperature with 7 ml. of acetic anhydride and 9 ml. of pyridine. Water was then added and the solution evaporated to dryness *in vacuo*. The product was dissolved in chloroform and the chloroform solution washed with water, dilute sulfuric acid, and with water. The chloroform was distilled, and the white solid residue boiled with 100 ml. of ether for about two minutes. The ethereal solution was decanted from the undissolved material.

The ether-insoluble fraction was crystallized from a chloroform–ether mixture, yielding 1.02 g., m. p. 195–196°. Recrystallization from chloroform–ether followed by two recrystallizations from methanol gave pure α-methyl pentaacetyldihydrostreptobiosaminide, m. p. 198–198.5°, $[\alpha]^{25}D$ −117° (*c*, 0.865 in chloroform).

Anal. Calcd. for $C_{13}H_{19}NO_8(CH_3CO)_5(OCH_3)$: C, 51.15; H, 6.62; N, 2.49; CH_3CO, 38.19; CH_3O, 5.51; mol. wt., 564. Found: C, 51.18, 51.29; H, 6.46, 6.70; N, 2.56; CH_3CO, 38.6; CH_3O, 4.96; mol. wt., 571 (ebullioscopic in benzene). A determination of O-acetyl[8] gave a value of 30.62; calcd. for four CH_3CO, 30.55. A Zerewitinoff determination in anisole solution gave at room temperature 0.8 mole of methane, and at 95°, 1.2 moles.

A saturated solution of this compound in tetrachloroethane showed infrared absorption at 2.90, 5.75 and 6.13 μ.

β-Methyl Pentaacetyldihydrostreptobiosaminide.—Addition of petroleum ether to the ether-soluble fraction of the acetylation product (in ether solution) gave 259 mg. of crystals, m. p. 149–153°. Recrystallization from chloroform–ether and then from methanol gave pure β-methyl pentaacetyldihydrostreptobiosaminide, m. p. 155.5–157°; $[\alpha]^{25}D$ −34° (*c*, 0.935 in chloroform).

Anal. Found: C, 51.25; H, 6.33; N, 2.84; CH_3O, 5.47; active hydrogen, 1.1 moles.

A saturated solution of the material in tetrachloroethane solution showed infrared absorption at 2.85, 5.75 and 6.12 μ.

Pentaacetyldihydrodesoxystreptobiosamine.—A solution of 453 mg. of α-methyl pentaacetyldihydrostreptobiosaminide in 25 ml. of ethyl mercaptan was saturated with hydrogen chloride and allowed to stand overnight at room temperature. After removal of the excess ethyl mercaptan, the residue was dissolved in 20 ml. of acetic anhydride and heated on the steam-bath with 2 g. of anhydrous sodium acetate for one hour. The excess acetic anhydride was removed *in vacuo* and the residue was dissolved in water. Chloroform extraction of the aqueous solution gave 463 mg. of the crude mercaptal acetate. This product was refluxed in 20 ml. of 70% ethanol containing 5 ml. of freshly prepared Raney nickel for two hours. The catalyst was removed by centrifugation and washed twice with hot alcohol. The supernatant and combined washings were concentrated to a residue *in vacuo* and dissolved in water. Extraction of this aqueous solution with chloroform gave the crude reduction product as an oil, 219 mg. The material was dissolved in 5 ml. of 1:4 chloroform–ether and chromatographed on 5 g. of alumina. The crystalline fractions, 90 mg., were combined and recrystallized three times from ether. The product melted at 136–136.5°, $[\alpha]^{25}D$ −81° (*c*, 0.40 in chloroform).

Anal. Calcd. for $C_{27}H_{35}O_{13}N$: C, 51.83; H, 6.62; N, 2.63. Found: C, 51.84; H, 6.85; N, 2.67.

Pentaacetyldesoxydihydrostreptobiosamine from β-Methyl Pentaacetyldihydrostreptobiosaminide.—A solution of 294 mg. of the β-isomer in 10 ml. of ethyl mercaptan saturated with hydrogen chloride was allowed to stand overnight at room temperature. After removal of the excess ethyl mercaptan the product was acetylated with acetic anhydride–sodium acetate. The crude acetylation product, 268 mg., was refluxed for two hours in 10 ml. of 70% ethanol containing 3 ml. of freshly prepared Raney nickel. The reduction product was worked up in the manner described above to give in all, 42 mg. of crystalline product, m. p. 128–130°. Upon recrystallization the compound melted at 133–134°, $[\alpha]^{25}D$ −84° (*c*, 1.40 in chloroform) and did not depress the melting point of pentaacetyldesoxydihydrostreptobiosamine of m. p. 136–136.5°.

Acid Hydrolysis of α-Methyl Pentaacetyldihydrostreptobiosaminide.—A solution of 130 mg. of α-methyl pentaacetyldihydrostreptobiosaminide in 10 ml. of 10% hydrochloric acid was refluxed for three hours. After cooling, the light brown solution was decolorized with charcoal and evaporated to dryness *in vacuo*. The residue was acetylated with acetic anhydride and pyridine at room temperature, giving a pale yellow product, 105 mg. This was chromatographed on 2 g. of acid-washed alumina. The column was prepared with petroleum ether and the acetylation product was adsorbed from solution in a 7:3 benzene–petroleum ether mixture. A 3:2 benzene–chloroform mixture eluted material which crystallized when moistened with ether; yield 15 mg. Recrystallization from chloroform–ether gave needles, m. p. and mixed m. p. with the pentaacetyl derivative of N-methyl-L-glucosamine,[10] 161–162°.

Streptomycin Oxime Hydrochloride.—A solution of 300 mg. of streptomycin hydrochloride, 28.8 mg. of hydroxylamine hydrochloride, and 45 mg. of pyridine in 9 ml. of water was allowed to stand overnight at room temperature. After removal of the solvent, the residual colorless glass was dissolved in 20 ml. of methanol and 220 ml. of acetone was added. The curdy white precipitate was centrifuged, washed with acetone, and dried. The product, a white powder, weighed 270 mg., and had a rotation of $[\alpha]^{25}D$ −82° (*c*, 0.985 in water). A sample for analysis was reprecipitated from methanol containing a drop of concentrated hydrochloric acid by the addition of acetone, washed with acetone, dried *in vacuo*, and dried in a weighing pig at 56°.

Anal. Calcd. for $C_{21}H_{40}N_8O_{12}\cdot 3HCl$: C, 35.72; H, 6.14; N, 15.87. Found: C, 35.67; H, 6.09; N, 15.45.

Streptomycin Semicarbazone Hydrochloride.—A mixture of 401 mg. of streptomycin hydrochloride, 60.1 mg. of semicarbazide hydrochloride, and 55 mg. of pyridine dissolved in 10 ml. of water was allowed to stand overnight. The amorphous semicarbazone was isolated as described above for the oxime. The product had a rotation of $[\alpha]^{25}D$ −70° (*c*, 1.08 in water).

Anal. Calcd. for $C_{22}H_{42}N_{10}O_{12}\cdot 3HCl$: C, 35.32; H, 6.06; N, 18.73. Found: C, 35.66; H, 6.14; N, 18.16.

Acknowledgment.—The authors wish to thank Dr. N. R. Trenner and Mrs. R. C. Anderson for the infrared and ultraviolet absorption measurements, Dr. J. B. Conn for the molecular weight determinations and Mr. Richard N. Boos and his associates for microanalyses.

Summary

Streptomycin hydrochloride has been degraded by methanol containing hydrogen chloride to streptidine and methyl streptobiosaminide dimethyl acetal hydrochloride, a derivative of the disaccharide-like molecule streptobiosamine, $C_{13}H_{23}NO_9$. The disaccharide derivative was further characterized by conversion to crystalline methyl tetraacetylstreptobiosaminide dimethyl acetal. In like manner, degradation of dihydrostreptomycin gave two isomeric methyl glycosides, α- and β-methyl dihydrostreptobiosaminide, which were separated and characterized as the crystalline pentaacetyl derivatives. The preparation of the oxime and semicarbazone of streptomycin hydrochloride has been described.

It has been shown that the streptobiosamine portion of streptomycin contains a reactive carbonyl group, a C-methyl group, a methylamino group, three acetylatable hydroxyl groups, and one hydroxyl group which is resistant to acetylation.

RAHWAY, NEW JERSEY RECEIVED AUGUST 10, 1946

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF COLORADO]

A Microbiological Synthesis of 2-Thiophenecarbinol

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Erlenmeyer[2] first pointed out the isosteric relationship between the vinylene group and a divalent organic sulfur atom. Many biologically important compounds have since been prepared wherein the vinylene group and the sulfide group have been interchanged. One group of the isosters resulting from such an exchange produces inhibition of the normal biological processes[3,4,5]; whereas the other group retains some of the natural biological activity.[6,7,8,9] It therefore seemed desirable to investigate whether the substitution of a thiophene ring for a benzene ring would alter the synthetic abilities of a fermenting yeast system. Neuberg and co-workers[10,11] demonstrated that yeast could synthesize benzyl alcohol and acetylphenylcarbinol from benzaldehyde; Lintner and Liebig[12] showed that 2-furfuryl alcohol was obtained when yeast acted on 2-furaldehyde. In this report are presented the results of studies of the effect of fermenting yeast on 2-thiophenealdehyde.

For the microbiological synthesis herein reported, a suspension of fermenting yeast was prepared in a manner similar to that employed by Neuberg, *et al.*[10,11] With the Budweiser strain of yeast and under the conditions used in this investigation a large part of the 2-thiophenealdehyde was converted to thiophenecarbinol. In this respect 2-thiophenaldehyde is attacked by the yeast system in a manner analogous to the reaction with benzaldehyde and furaldehyde. The thiophenecarbinol was isolated by precipitation of the 5-chloromercuri-2-thiophenecarb nol derivative. The mercury could be removed with hydrogen sulfide, liberating the thiophenecarbinol.

To establish the identity of the carbinol produced microbiologically, thiophenecarbinol was synthesized from 2-thiophenealdehyde by the crossed Cannizzaro reaction with formaldehyde This procedure, developed by Davidson and Bogert[13] as a general one for aromatic alcohols, was found to apply equally as well to the thiophene compound. The carbinols prepared synthetically and microbiologically were compared by mixed melting points of the phenylurethan and α-naphthylurethan derivatives and by elementary analysis. The thiophenecarbinol produced by the fermenting yeast was found to be identical in every respect with the thiophenecarbinol prepared by chemical synthesis.

Experimental[14]

5-Chloromercuri-2-thiophenecarbinol.—The microbiological synthesis was carried out with a mixture of 50 g. of Budweiser baker's yeast, 50 g. of sucrose, 1250 cc. of water and 5 g. of thiophenealdehyde. At the end of four days the yeast was removed by filtration, and 500 cc. of 5% mercuric chloride was added to the aqueous solution. At the end of several days, when precipitation was complete, the supernatant liquid was decanted and the pre-

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