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Streptomyces Antibiotics. XVII. Heptabenzoylstreptidine from Streptomycin

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Streptomycin has been degraded to heptabenzoylstreptidine which served as a key product for the determination of the position of the linkage of streptobiosamine to streptidine.[1] The details of the formation and characterization of heptabenzoylstreptidine and certain of its reactions are described herein.

A suspension of crystalline streptomycin trihydrochloride-calcium chloride double salt[2] in benzoyl chloride and pyridine was heated for about twenty minutes. The crude reaction product was purified by chromatography on charcoal to give a low yield of benzoylated streptomycin as an amorphous white powder. The analytical data were in agreement with the formula for undecabenzoylstreptomycin.

The presence of some dodecabenzoylstreptomycin in the benzoylated streptomycin was possible, since the streptose moiety of streptomycin contains a tertiary hydroxyl group. Although this hydroxyl group is difficult to acylate,[3,4] a bis-*p*-nitrobenzoate of bis-desoxystreptose[3] was obtained. The presence of some of the dodecabenzoyl derivative in the undecabenzoylstreptomycin would be of no consequence in the present study; however, it is essential that the streptidine moiety in the product be completely (hepta) benzoylated.

The molecular weights of undeca- and dodecabenzoylstreptomycin are 1727 and 1831, respectively. Ebullioscopic molecular weight determinations of benzoylated streptomycin in benzene gave values of 1625–1650 ± 165 for two different preparations.

Alkaline hydrolysis of benzoylated streptomycin gave maltol by isolation, and substantiated the presence of the intact streptomycin carbon skeleton[4] in the benzoylated product. This hydrolysis also gave benzoic acid in a yield of 10.9 moles of benzoic acid per mole of undecabenzoylstreptomycin.

The treatment of benzoylated streptomycin with one to three equivalents of hydrogen bromide in chloroform–glacial acetic acid solution at room temperature cleaved the glycosidic linkage between the streptidine and streptobiosamine moieties. Crystalline heptabenzoylstreptidine was obtained from this reaction in 72% yield.

Heptabenzoylstreptidine melted at 256–258° when crystallized from benzene–methanol. When crystallized from benzene a molecule of solvent of crystallization was present which could be removed by drying at 110° *in vacuo*. Analyses and molecular weight measurements were in agreement with the formula, $C_8H_{11}N_6O_4(C_6H_5CO)_7$. Heptabenzoylstreptidine was found to be optically active, $[\alpha]^{25}D$ + 58° (in chloroform), $[\alpha]^{25}D$ −3° (in glacial acetic acid). Further characterization was provided by conversion of heptabenzoylstreptidine to acetylheptabenzoylstreptidine, m. p. 156–160°, $[\alpha]^{25}D$ + 3° (in chloroform), to anisoylheptabenzoylstreptidine, m. p. 213–216°, $[\alpha]^{25}D$ −12° (in chloroform), and to octabenzoylstreptidine, m. p. 263–264°, $[\alpha]D$ 0.

The significance of the optical activity of heptabenzoylstreptidine is detailed in conjunction with the results of the degradation of this compound to α,γ-dibenzamido-β-hydroxyadipaldehyde, which are described in an accompanying paper.[5]

Both heptabenzoylstreptidine and octabenzoylstreptidine yielded a crystalline monobenzoylstreptidine upon treatment with sodium methoxide in pyridine–methanol solution. The isolation of the monobenzoyl derivative may have been due to its insolubility in the reaction medium. Further treatment of this monobenzoyl derivative with acid readily yielded streptidine which was optically inactive, as were streptidine preparations produced by hydrolysis of streptomycin directly.[6,7] It has been observed that octaacetylstreptidine,[6,8] is deacetylated to streptidine at room temperature in methanol containing ammonia.

It was found that streptidine could be benzoylated, under conditions similar to those applied to streptomycin, to give optically inactive octabenzoylstreptidine. A hexabenzoylstreptidine has also been obtained. Octabenzoylstreptidine was distinguished from heptabenzoylstreptidine by melting point and by the fact that only the latter compound (obtained by cleavage of benzoylated streptomycin) is optically active.

Octabenzoylstreptidine was remarkably stable to hydrogen bromide, since it was recovered in nearly quantitative yield after a solution of the substance in glacial acetic acid containing excess hydrogen bromide was refluxed for one hour. Octaacetylstreptidine[6,8] formed a crystalline dihydrobromide when treated with hydrogen bromide in glacial acetic acid at room temperature. This salt hydrolyzed in aqueous solution, and the substance was partially deacetylated.

(1) Kuehl, Peck, Hoffhine, Peel and Folkers, THIS JOURNAL, **69**, 1234 (1947).

(2) Peck, Brink, Kuehl, Flynn, Walti and Folkers, *ibid.*, **67**, 1866 (1945).

(3) Brink, Kuehl, Flynn and Folkers, *ibid.*, **68**, 2405 (1946).

(4) Kuehl, Flynn, Brink and Folkers, *ibid.*, **68**, 2679 (1946).

(5) Kuehl, Peck, Hoffhine and Folkers, *ibid.*, **70**, 2325 (1948).

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

(7) Carter, Clark, Dickman, Loo, Skell and Strong, *Science*, **103**, 540 (1946).

(8) Fried and Stavely, THIS JOURNAL, **69**, 1549 (1947).

Thus, it is clear that heptabenzoylstreptidine could not have been derived from the presence and hydrolysis of octabenzoylstreptidine as a contaminant in benzoylated streptomycin. The cleavage reaction took place at room temperature, and the yield of heptabenzoylstreptidine was high. It is also clear from the data given above that the atom in the unbenzoylated functional group of heptabenzoylstreptidine is attached to the carbon atom of streptidine which is linked glycosidically to streptobiosamine.

Evidence for the presence of a free hydroxyl group in heptabenzoylstreptidine was provided by the action of chromic acid in 90% acetic acid on octa-, hepta- and hexabenzoylstreptidine at 45°. Only the heptabenzoylstreptidine was affected by this oxidizing reagent and gave crystalline dibenzoylguanidine, m. p. 165–166°.[9] The yield of dibenzoylguanidine was greater than one mole per mole of heptabenzoylstreptidine which showed that each guanido group in the streptidine moiety of benzoylated streptomycin possessed two benzoyl groups. This result provided evidence that streptobiosamine was linked to streptidine through an oxygen atom rather than through a nitrogen atom. Since hexabenzoylstreptidine was unaffected by chromic acid, there is presumably no free hydroxyl group present.

Previous indication that the linkage of streptobiosamine and streptidine was through an oxygen rather than a nitrogen atom was furnished by the ultraviolet absorption spectra of heptabenzoylstreptidine and octabenzoylstreptidine; their spectra were practically identical. Hexabenzoylstreptidine showed distinctly different absorption maxima. These spectra substantiate the interpretation that both guanido groups of heptabenzoylstreptidine contain two benzoyl groups each, as otherwise a change in position of the absorption maxima would be expected.

Dihydrostreptomycin has also been benzoylated under the conditions used for streptomycin. Analyses on benzoylated dihydrostreptomycin were in agreement with the formula for dodecabenzoyldihydrostreptomycin. Cleavage of dodecabenzoyldihydrostreptomycin also yielded heptabenzoylstreptidine.

Experimental

Benzoylation of Streptomycin.—Twenty-five grams of crystalline streptomycin trihydrochloride–calcium chloride double salt was suspended in a mixture of 150 cc. of pyridine and 55 cc. of benzoyl chloride, and the suspension was heated on a steam-bath for ten minutes and then heated at reflux temperature for ten minutes. At the end of the heating period, all of the suspended solid had dissolved. The solution was partially cooled and diluted with 300 cc. of chloroform. The chloroform solution was washed successively with water, dilute hydrochloric acid, sodium bicarbonate solution, and water. The chloroform solution was dried over magnesium sulfate, filtered, concentrated *in vacuo* to a volume of about 200 cc., and then poured with stirring into 2 l. of petroleum ether. The precipitated crude undecabenzoylstreptomycin was collected on a filter and dried; yield, 49 g. For purification, a solution of this material in about 150 cc. of benzene was allowed to flow into a dry chromatographic column containing a mixture of 500 g. of Darco G-60 and 150 g. of filter paper pulp. Fresh benzene was added to the column, and five eluates were collected. A 1:1 acetone–benzene mixture was used next and three more eluates were collected. Chloroform was used to produce the ninth eluate. The eluates were concentrated to *ca.* 25-cc. volume *in vacuo* and the concentrates were poured into about ten volumes of petroleum ether, which caused the precipitation of the benzoylated streptomycin. The details of this chromatographic experiment are summarized in Table I.

TABLE I

CHROMATOGRAPHY OF BENZOYLATED STREPTOMYCIN

Eluate no.	Volume of eluate, cc.	Weight of product, g.	$[\alpha]^{25}D$ (chloroform)
1	300	0.4	+60°
2	500	2.30	+34°
3	1000	2.50	+27.6°
4	1000	1.10	+19.7°
5	1800	0.98	+12.7°
6	1400	0.81	−18.6°
7	700	6.90	−43°
8	800	2.50	−64.5°
9	2100	1.23	−38°

The product from eluate 1 was reprecipitated by dissolving it in 3 cc. of chloroform and pouring the solution dropwise into 80 cc. of boiling petroleum ether. The precipitate was a white amorphous powder; 0.37 g., $[\alpha]^{25}D$ +59° (*c*, 1.0 in chloroform).

Anal. Calcd. for $C_{21}H_{27}N_7O_{12}(C_6H_5CO)_{12}$: C, 68.88; H, 4.79; N, 5.36; mol. wt., 1831: Calcd. for $C_{21}H_{28}N_7O_{12}(C_6H_5CO)_{11}$: C, 68.16; H, 4.85; N, 5.68; mol. wt., 1727. Found: C, 68.53; H, 4.84; N, 5.38; mol. wt. (ebullioscopic in benzene), 1625 ± 163.

A methanol solution of this product showed ultraviolet absorption maxima at 2325 Å. (*E*% 680) and at 2750 Å. (*E*% 285), and a shoulder at 2550 Å. (*E*% 350). A tetrachloroethane solution of this product showed infrared absorption bands at 5.77, 6.25, 6.37, 7.88, 8.50, 9.04, 9.14 and 9.36 μ.

The product from eluate 2 was analyzed without further reprecipitation.

Anal. Found: C, 68.72; H, 5.12; N, 5.38; mol. wt., 1651 ± 165.

The analytical data on the products from eluates 1 and 2 correspond closely to the composition of undecabenzoylstreptomycin and dodecabenzoylstreptomycin. The product obtained from eluate 1 in an analogous experiment had the composition: C, 69.05; H, 4.86; N, 5.46; $[\alpha]^{25}D$ +42° (*c*, 1.0 in chloroform).

The product from eluate 8 was reprecipitated by using chloroform and petroleum ether; 2.1 g.; $[\alpha]^{25}D$ −41° (*c*, 1.0 in chloroform).

Anal. Found: C, 63.17; H, 5.08; N, 6.20; mol. wt., 1100.

The product in eluate 8 was clearly less completely benzoylated.

Cleavage of Undecabenzoylstreptomycin to Maltol.—A sample of undecabenzoylstreptomycin (from eluate 2) weighing 431 mg. was suspended in 5 cc. of methanol and the solution was heated to the reflux temperature. Three cubic centimeters of aqueous 2.5 *N* sodium hydroxide was added to the methanol solution, and the mixture was heated at the reflux temperature for fifteen minutes. The yellow-brown solution was then concen-

(9) Korndörfer (*Arch. Pharm.*, **241**, 449 [1903]) reported dibenzoylguanidine, m. p. 215°, as the product of benzoylation of guanidine in aqueous solution. Walther and Wlodkowski (*J. prakt. Chem.*, **59**, 266 [1899]) and we have found that this reaction gives dibenzoylurea, m. p. 215°.

trated under reduced pressure until the methanol was removed. The residual solution was diluted with 10 cc. of water and extracted three times with chloroform. The chloroform extracts were discarded. The alkaline aqueous solution was then saturated with carbon dioxide, and extracted four times with chloroform. The chloroform extracts were dried and evaporated to dryness to give 28.5 mg. of a crystalline residue. The crystals gave a reddish-purple color with ferric chloride solution which was identical with that given by maltol. The residue was recrystallized from chloroform–ether to give crystals which melted at 159–160°. When this product was mixed with an authentic sample of maltol (m. p. 159–160°), the melting point of the mixture was 159–160°.

The chloroform-extracted aqueous solution was acidified with sulfuric acid to *p*H 2, and extracted three times with chloroform. The extract was dried and evaporated to dryness to yield 332 mg. of benzoic acid (neutral equivalent: calcd., 122; found, 125). This represents a yield of 10.9 moles of benzoic acid per mole of undecabenzoylstreptomycin.

Cleavage of Undecabenzoylstreptomycin to Heptabenzoylstreptidine.—One hundred thirty-five milligrams of undecabenzoylstreptomycin (from eluate 1) was dissolved in 3.0 cc. of chloroform, and to the solution was added 0.11 cc. of a 16% solution of hydrogen bromide in glacial acetic acid. The solution was allowed to stand at room temperature for twenty-four hours, diluted with 5 cc. of chloroform, and the mixture was extracted twice with saturated aqueous sodium bicarbonate solution. The chloroform solution was washed with water, dried and evaporated *in vacuo* to give 124.5 mg. of an amorphous glassy residue. The residue was dissolved in 1 cc. of warm benzene, and diluted with 2 cc. of methanol. A white granular crystalline precipitate quickly separated. The precipitate was collected on a filter and washed with methanol; yield, 55.7 mg. (yield, 72%); m. p. 245–252° (micro-block). Recrystallization of this product from benzene–methanol yielded heptabenzoylstreptidine of melting point 256–258° (micro-block); $[\alpha]^{25}D$ +58° (*c*, 1.24 in chloroform).

Anal. Calcd. for $C_{57}H_{46}N_6O_{11}$: C, 69.15; H, 4.68; N, 8.49; mol. wt., 991. Found: C, 69.10; H, 4.71; N, 8.72; mol. wt., (ebullioscopic in benzene), 1116 ± 112.

In the ultraviolet, an ethanol solution of heptabenzoylstreptidine showed maxima at 2350 Å. (E_M 59,000), 2530 Å. (E_M 53,000) and 2770 Å. (E_M 43,500).

Heptabenzoylstreptidine contained benzene of crystallization when it was recrystallized from benzene–acetone solution upon removal of acetone by evaporation.

Anal. Calcd. for $C_{57}H_{46}N_6O_{11}\cdot C_6H_6$: C, 70.77; H, 4.90; solvent of crystallization, 7.3. Found: C, 70.45; H, 4.89; weight loss on drying two hours at 110° *in vacuo*, 7.3.

Acetylheptabenzoylstreptidine.—A solution of 428 mg. of heptabenzoylstreptidine in 10 cc. of acetic anhydride was refluxed for forty-five minutes. The solution was evaporated *in vacuo* to a thick glassy residue. The residue was dissolved in hot benzene, and the solution was diluted with methanol, whereupon crystals separated quickly; yield, 400 mg.; m. p. 156–160° (micro-block); $[\alpha]^{25}D$ +3° (*c*, 2.98 in chloroform). This product contained benzene of crystallization.

Anal. Calcd. for $C_{59}H_{48}N_6O_{12}\cdot 2C_6H_6$: C, 71.71; H, 5.09. Found: C, 71.48; H, 4.54.

Anisoylheptabenzoylstreptidine.—A mixture of 507 mg. of heptabenzoylstreptidine, 1.0 cc. of anisoyl chloride and 10 cc. of pyridine was heated at the reflux temperature for thirty minutes, cooled, and diluted with chloroform. The chloroform solution was washed and evaporated to a volume of 5 cc., and poured into 50 cc. of petroleum ether. The precipitate of crude anisoylheptabenzoylstreptidine was dissolved in 10 cc. of benzene and the solution was chromatographed on Darco G-60 to yield anisoylheptabenzoylstreptidine in the first eluates. The first eluates on evaporation left a crystalline residue which was recrystallized from ether–methanol; yield, 251 mg.; m. p. 212–216° (micro-block). One further crystallization from acetone–methanol gave material which melted at 213–216° (micro-block), and which showed $[\alpha]^{25}D$ −12° (*c*, 2.29 in chloroform).

Anal. Calcd. for $C_{65}H_{52}N_6O_{13}$: C, 69.38; H, 4.66; N, 7.47; OCH_3, 2.76. Found: C, 69.22; H, 4.40; N, 7.49; OCH_3, 3.44.

A chromatographic fraction which was crystallized from acetone melted at 218–220° (micro-block), and contained acetone of crystallization.

Anal. Calcd. for $C_{74}H_{70}N_6O_{16}$: C, 68.40; H, 5.43; OCH_3, 2.39. Found: C, 68.32; H, 5.16; OCH_3, 2.08.

Octabenzoylstreptidine from Heptabenzoylstreptidine.—One hundred twenty milligrams of heptabenzoylstreptidine was dissolved in a mixture of 2 cc. of pyridine and 0.2 cc. of benzoyl chloride. The solution was refluxed for thirty minutes, diluted with chloroform, washed with dilute acid and aqueous sodium bicarbonate and then water. The chloroform solution was dried and evaporated *in vacuo*. The residue was crystallized from benzene–methanol to give 54 mg. of octabenzoylstreptidine which melted at 261–263° (micro-block). When this product was mixed with heptabenzoylstreptidine, the melting point of the mixture was depressed. When this product was mixed with octabenzoylstreptidine, which was prepared by benzoylation of streptidine, the melting point was unchanged.

Anal. Calcd. for $C_{64}H_{50}N_6O_{12}$: C, 70.19; H, 4.60. Found: C, 70.23; H, 4.79.

Octabenzoylstreptidine.—Four grams of streptidine dihydrochloride was mixed with 67 cc. of pyridine and 27 cc. of benzoyl chloride. The mixture was heated at the reflux temperature for twenty minutes, evaporated *in vacuo* to a thick sirup, and then the residue was dissolved in 50 cc. of chloroform. The chloroform solution was washed with dilute hydrochloric acid, saturated aqueous sodium bicarbonate, and water. It was then dried over magnesium sulfate and filtered through a layer of Darco G-60. The filtrate was poured into 900 cc. of petroleum ether to give a granular, tan precipitate. The crude product, which weighed 13.9 g., was dissolved in 30 cc. of benzene and the solution was allowed to flow through a column containing 135 g. of aluminum oxide. The first 200 cc. of eluate yielded 12 g. of solid on evaporation. This product was dissolved in 25 cc. of hot benzene and 60 cc. of methanol was added. On cooling, crystals separated; yield, 7 g. (three crops). This product melted and resolidified in the range of 120–140° and on continued heating melted at about 250–260° (micro-block). Rechromatography of this product and recrystallization of the major chromatographic fraction from benzene–methanol gave 4.65 g. of crystals which showed a transition of 125–140° and then melted at 261–264°. Further recrystallization of this product from benzene–methanol gave octabenzoylstreptidine which melted constantly at 263–264°; $[\alpha]_D$ 0.

Anal. Calcd. for $C_{64}H_{50}N_6O_{12}$: C, 70.19; H, 4.60; N, 7.68; mol. wt., 1095. Found: C, 70.74; H, 4.65; N, 7.69; mol. wt. (ebullioscopic in benzene), 1267 (±10%).

In the ultraviolet, an ethanol solution of octabenzoylstreptidine showed maxima at 2375 Å. (E_M 77,800), 2550 Å. (E_M 63,500) and 2750 Å. (E_M 50,400).

Hexabenzoylstreptidine (1,3-benzoylguanido-2,4,5,6-tetrabenzoyloxycyclohexane).—The chromatograph columns used for the purification of octabenzoylstreptidine were subsequently eluted with chloroform. Evaporation of the chloroform extracts to dryness, and crystallization of the residue from acetone and from acetone–benzene gave a white crystalline product. This material melted partially at 165–175° and then melted fully at 220–225° when heated on the micro-block; $[\alpha]_D$ 0 (*c*, 2.0 in acetone).

Anal. Calcd. for $C_8H_{12}N_6O_4(C_7H_5O)_6$: C, 67.71; H, 4.77; N, 9.47. Found: C, 67.20; H, 4.90; N, 9.88.

This substance showed no evidence of oxidation when treated with chromic acid in 90% acetic acid solution. An ethanol solution showed a single maximum in the ultraviolet at 2625 Å. with E_M 35,000.

This evidence is in agreement with a 1,3-benzoylguanido-2,4,5,6-tetrabenzoyloxycyclohexane for the structure of hexabenzoylstreptidine.

Treatment of Octabenzoylstreptidine with Hydrogen Bromide.—One and one-half grams of octabenzoylstreptidine was dissolved in 15 cc. of chloroform and 15 cc. of 2.8 *N* hydrogen bromide in glacial acetic acid was added. The solution was refluxed (76°) for one hour, cooled, diluted with 25 cc. of chloroform, washed with saturated aqueous sodium bicarbonate and with water. The dried chloroform solution was evaporated *in vacuo* to give 1.5 g. of residue. This residue was crystallized from a mixture of 3 cc. of benzene and 10 cc. of methanol to give 1.35 g. of crystals which showed a transition at 120–160° and melted at 255–263°. One further crystallization gave 1.2 g. of recovered octabenzoylstreptidine which showed the transition at 120–150° and melted at 261–263° (micro-block).

Anal. Calcd. for $C_{84}H_{50}N_6O_{12}$: C, 70.19; H, 4.60. Found: C, 70.20; H, 4.57.

Octaacetylstreptidine Dihydrobromide.—A solution of 178 mg. of octaacetylstreptidine[6,8] in 1 cc. of glacial acetic acid containing 15% of hydrogen bromide was allowed to stand at room temperature, and white crystals began to separate in about ten minutes. After twenty-four hours, the mixture was diluted with 6 cc. of chloroform, and evaporated *in vacuo*. The residue was recrystallized from methanol-ether solution to give 139 mg. of crystals which melted at 175–185° (micro-block). A second crop of crystals weighing 61 mg. was obtained from the mother liquors. After recrystallization from methanol-ether, the product weighed 100 mg. and melted over the range 183–190°. The substance appeared to be octaacetylstreptidine dihydrobromide.

Anal. Calcd. for $C_8H_{10}N_6O_4(C_2H_3O)_8 \cdot 2HBr$: C, 37.92; H, 4.77; N, 11.05; Br, 21.02; CH_3CO, 45.23. Found: C, 38.91; H, 4.97; N, 10.89; Br, 21.7; CH_3CO, 40.4.

The ultraviolet absorption spectrum of the product in ethanol showed maxima at 2150 Å. (E_M 22,200) and 2550 Å. (E_M 27,500).

A solution of 1 g. of octaacetylstreptidine in 6 cc. of glacial acetic acid containing 15% of hydrogen bromide deposited crystals. The product was collected on a filter, washed with ether, and dried; yield, 702 mg., m. p. 180–195° (micro-block).

Anal. Calcd. for $C_8H_{10}N_6O_4(C_2H_3O)_8 \cdot 2HBr$: C, 37.92; H, 4.77; Br, 21.02. Found: C, 37.34; H, 4.84; Br, 20.85.

A 545-mg. portion of octaacetylstreptidine dihydrobromide was treated with a little water. Some crystalline material remained which was collected on a filter and washed with water. These crystals weighed 4.4 mg., and melted at 250–257° (micro-block) corresponding to octaacetylstreptidine. The aqueous filtrate was neutralized with sodium bicarbonate, and the precipitate was extracted with chloroform. The chloroform extract was evaporated to give 245 mg. of crystalline residue which melted at 170–230°. This material appeared to be partially deacetylated.

Hydrolysis of Octaacetylstreptidine to Streptidine Carbonate.—A solution of 229 mg. of octaacetylstreptidine in 100 cc. of absolute methanol was saturated at 0° with gaseous ammonia. After three hours, the solution was evaporated *in vacuo*, and the residue was extracted with chloroform to remove acetamide. The chloroform-insoluble residue weighed 110 mg. and appeared to be a mixture of streptidine and streptidine carbonate. A portion of this residue was converted to the picrate, which melted at 281–283° (micro-block). The melting point of this product on admixture with authentic streptidine picrate was not depressed. Twenty-eight milligrams of the residue was dissolved in 1.5 cc. of water; the solution was strongly alkaline. The addition of 2 cc. of methanol caused the separation of a crystalline product, which weighed 14 mg. and decomposed gradually above 240° (micro-block).

Anal. Calcd. for $C_8H_{18}N_6O_4 \cdot H_2CO_3 \cdot H_2O$: C, 31.58; H, 6.48; N, 24.55. Found: C, 31.25; H, 6.31; N, 24.54; acetyl, nil.

Stepwise Hydrolysis of Octabenzoylstreptidine to Monobenzoylstreptidine and Streptidine Dihydrochloride.—A solution of 504 mg. of octabenzoylstreptidine in 10 cc. of dry pyridine was mixed with 10 cc. of absolute methanol containing approximately 3 mg. of sodium methoxide. The solution was heated at the reflux temperature for three hours, during which time a crystalline solid separated. The crystals were removed, washed with methanol, and dried; weight 158 mg.; m. p. 232–235° (dec.) (micro-block). An aqueous solution of the product was alkaline and showed an ultraviolet absorption maximum at 2575 Å. (E_M 15,700).

Anal. Calcd. for $C_8H_{17}N_6O_4(C_6H_5CO)$: C, 49.17; H, 6.05; N, 22.94. Found: C, 48.50; H, 6.16; N, 22.09.

Nineteen milligrams of monobenzoylstreptidine was converted to a crystalline sulfate by treatment with a dilute sulfuric acid–acetone solution; yield, 24 mg. The crystals decomposed gradually when heated above 245° (micro-block).

Anal. Calcd. for $C_8H_{17}N_6O_4(C_6H_5CO) \cdot H_2SO_4 \cdot 2H_2O$: C, 36.00; H, 5.64; N, 16.79; S, 6.41. Found: C, 36.03; H, 6.62; N, 16.02; S, 5.63.

Another sample of the monobenzoylstreptidine was heated for two hours at the reflux temperature with 6 *N* hydrochloric acid. The hydrolysis mixture yielded benzoic acid and streptidine dihydrochloride. The latter was identified by conversion, in good yield, to octaacetylstreptidine.

Hydrolysis of Heptabenzoylstreptidine to Monobenzoylstreptidine.—Using the procedure described for the hydrolysis of octabenzoylstreptidine, a sample of heptabenzoylstreptidine weighing 2.004 g. was debenzoylated with methanol and sodium methoxide. The monobenzoyl derivative, which separated, was converted to the crystalline sulfate; yield, 379 mg. The crystalline salt decomposed gradually above 240° (micro-block).

Anal. Calcd. for $C_8H_{17}N_6O_4(C_6H_5CO) \cdot H_2SO_4 \cdot 2H_2O$: C, 36.00; H, 5.64; N, 16.79. Found: C, 36.17; H, 5.13; N, 17.41.

This product showed an ultraviolet absorption maximum which shifted with a change in the *p*H of the solution. In *N* hydrochloric acid, the maximum appeared at 2400 Å. (E_M 15,500). At *p*H 7, the maximum was at 2550 Å. (E_M 12,000). In 0.2 *N* sodium hydroxide, the maximum was at 2600 Å. (E_M 12,500). An aqueous solution of the compound showed a maximum at 2575 Å. (E_M 18,700).

Chromic Acid Oxidation of Heptabenzoylstreptidine; Isolation of Dibenzoylguanidine.—One and two-tenths grams of heptabenzoylstreptidine was dissolved in 20 cc. of acetic acid and the solution was mixed with 90 cc. of a 10% solution of chromic acid in 90% acetic acid. The solution was allowed to stand at 45° for five hours, and at room temperature for sixteen hours, and was then frozen and dried from the frozen state. The residue was dissolved in water, and the solution was neutralized to *p*H 7.0 with sodium bicarbonate and extracted four times with chloroform. The chloroform extract was washed with water, dried over magnesium sulfate, and evaporated *in vacuo* to give a crystalline residue weighing 357 mg. The residue was dissolved in hot benzene, and the solution was filtered through a small pad of Darco G-60. The filtrate deposited white needles which weighed 100 mg., m. p. 163–164° (micro-block).

Anal. Calcd. for $CH_3N_3(C_7H_5O)_2$: C, 67.40; H, 4.90; N, 15.72; mol. wt., 267.3. Found: C, 67.25; H, 4.86; N, 15.44; mol. wt. (ebullioscopic in benzene), 264 ± 3.

Further recrystallizations from benzene and from

benzene–petroleum ether gave material which melted constantly at 165–166° (micro-block).

Anal. Calcd. for $CH_3N_3(C_7H_5O)_2$: C, 67.40; H, 4.90; N, 15.72. Found: C, 67.58; H, 5.15; N, 15.63.

This substance exhibited two maxima in the ultraviolet at 2450 Å. with E_M 32,000 and 2750 Å. with E_M 32,000 in methanol solution.

The data obtained on this oxidation product were in agreement with a dibenzoylquinidine, but the melting point was lower than that reported in the literature (m. p. 215°; Korndörfer, *Arch Pharm.*, 241, 449 [1903]). Accordingly, a 28.7-mg. sample was dissolved in 3.0 cc. of concentrated hydrochloric acid and the solution was refluxed for ninety minutes. The hydrolysis solution was diluted with water and extracted with ether. Evaporation of the ether extracts left a crystalline residue of practically pure benzoic acid; yield, 23.7 mg. The residual acid aqueous solution was evaporated to dryness to give a crystalline residue which was dissolved in 3.0 cc. of water. Addition of 2.0 cc. of saturated aqueous picric acid caused a crystalline precipitate to separate. The picrate was recrystallized once from hot water; yield, 25.9 mg., m. p. 335–336° (micro-block). Admixture with authentic guanidine picrate (m. p. 335–336°) did not alter the melting point of the product.

Anal. Calcd. for $CH_5N_3 \cdot C_6H_3N_3O_7$: C, 29.17; H, 2.80; N, 29.17. Found: C, 29.33; H, 3.10; N, 29.39.

The oxidation product is thus established to be dibenzoylguanidine. The yield of dibenzoylguanidine from heptabenzoylstreptidine would be 28% if one dibenzoylguanido moiety were present in the latter molecule, or 56% if both guanido groups were di-benzoylated. The yields of dibenzoylguanidine obtained in three oxidation experiments were 48, 30 and 43%, respectively.

Dodecabenzoyldihydrostreptomycin.—Benzoylation of 2.80 g. of dihydrostreptomycin trihydrochloride, using the conditions described above for streptomycin, gave 5.82 g. of buff-colored benzoylated dihydrostreptomycin. Chromatographic purification of 5.5 g. gave a first-eluate fraction in the form of a white amorphous powder weighing 0.5 g., $[\alpha]^{25}D$ +51° (*c*, 1.78 in chloroform).

Anal. Calcd. for $C_{21}H_{29}N_7O_{12}(C_7H_5O)_{12}$: C, 68.81; H, 4.90; N, 5.35. Found: C, 68.40; H, 5.25; N, 5.66.

Heptabenzoylstreptidine from Dodecabenzoyldihydrostreptomycin.—A 347-mg. sample of dodecabenzoyldihydrostreptomycin was dissolved in 6.62 cc. of chloroform and 0.16 cc. of 30% hydrogen bromide in glacial acetic acid was added. The solution was allowed to stand overnight, then diluted with chloroform and extracted with water and aqueous sodium bicarbonate. The chloroform solution was dried, evaporated to dryness, and the residue was dissolved in 0.5 cc. of benzene and 2.5 cc. of methanol. On standing, there was deposited 101 mg. of crystalline precipitate. Recrystallization of the precipitate from benzene–acetone and from benzene–methanol gave 22 mg. of white crystals of heptabenzoylstreptidine which melted at 256–258°, and showed $[\alpha]^{32}D$ +54° (*c*, 0.98 in chloroform). There was no change in the melting point of this material when mixed with heptabenzoylstreptidine (m. p. 256–258°) obtained from undecabenzoylstreptomycin.

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Summary

Streptomycin was benzoylated to give undecabenzoylstreptomycin, which was degraded by alkali to maltol and by hydrogen bromide to heptabenzoylstreptidine.

Dihydrostreptomycin was also benzoylated to give dodecabenzoyldihydrostreptomycin, which was likewise cleaved by hydrogen bromide to give heptabenzoylstreptidine.

Heptabenzoylstreptidine was further characterized by acetyl and anisoyl derivatives and by conversion to octabenzoylstreptidine. A hexabenzoylstreptidine and a monobenzoylstreptidine were also characterized. Hydrolysis of octabenzoyl-, heptabenzoyl- and octaacetylstreptidine yielded streptidine, showing that acylation and hydrolysis reactions involved no change in the structure of streptidine.

Heptabenzoylstreptidine gave more than one equivalent of dibenzoylguanidine upon chromic acid oxidation showing that streptobiosamine is linked to streptidine through an oxygen atom.

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Streptomyces Antibiotics. XVIII. Structure of Streptomycin

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The degradation of streptomycin to N,N′-dibenzoyldesoxystreptamine, and the oxidation of this degradation product to show that streptobiosamine is linked glycosidically at position 4 of streptidine, have been reported.[1]

The unbenzoylated functional group of heptabenzoylstreptidine[2] is attached to the carbon atom of streptidine which is linked glycosidically to streptobiosamine. This unbenzoylated functional group was considered to be a hydroxyl group because of the facile methanolysis[3] of streptomycin, and because of the formation of more than one mole of dibenzoylguanidine[2] per mole of heptabenzoylstreptidine upon chromic acid oxidation. Conclusive evidence excluding a nitrogen-atom linkage was obtained in the present study.

Streptidine and octabenzoylstreptidine[2] are optically inactive, showing that they are *meso* forms and have *cis* guanido groups; however, heptabenzoylstreptidine is optically active.[2] This optical activity proves that the unbenzoylated hy-

(1) Kuehl, Peck, Hoffhine, Peel and Folkers, THIS JOURNAL, **69**, 1234 (1947).

(2) Peck, Kuehl, Hoffhine, Peel and Folkers, *ibid.*, **70**, 2321 (1948).

(3) Brink, Kuehl and Folkers, *Science*, **102**, 2655 (1945); Brink, Kuehl, Flynn and Folkers, THIS JOURNAL, **68**, 2557 (1946).