[CONTRIBUTION FROM THE SQUIBB INSTITUTE OF MEDICAL RESEARCH]

Streptomycin. XIII. New Derivatives of Streptamine; the Oxidative Degradation of O-Tetramethylstreptamine

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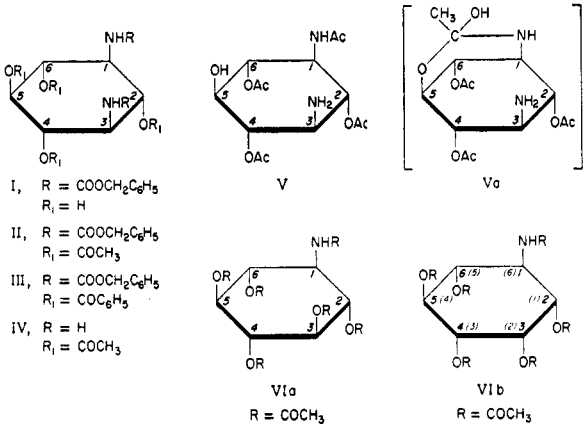
In a search for derivatives of streptamine permitting selective oxidative attack of the molecule at the amino groups O-tetraacetylstreptamine (IV) dihydrochloride has been prepared by catalytic reduction of O-tetraacetyl-N,N′-dicarbobenzoxystreptamine. With larger amounts this reaction leads preponderantly to an O-triacetyl-N-acetylstreptamine which arises from IV by O → N migration of one of the acetyl groups. Stereochemical considerations suggest that the free hydroxyl group in this compound is in position 5 (Formula V). By treatment with nitrous acid followed by acetylation V was converted to an aminocyclohexitol (inosamine) hexacetate VIa or VIb. O-Tetramethylstreptamine (VIII), prepared *via* the N,N′-diacetate VII, on oxidation with aqueous permanganate afforded D,L-dimethoxysuccinic acid, identified by conversion to the diamide and di-N-methylamide. This result shows that the 5-hydroxyl group in streptamine is situated *trans* with respect to those at C_4 and C_6. The 4-carbon acid arises by further oxidation of a 2(3?),4,5-trimethoxy3(2?)-aminoadipic acid (XI or X) which has been isolated in form of its 6 → 3(2?)-lactam-1-methyl ester (XIIIa or XIIa) and can be secured from the latter by alkaline hydrolysis. Of the two alternative formulations the β-amino acid structure XI is favored by the available evidence.

In the course of an investigation undertaken in 1946 to gain insight into the stereochemistry of streptamine we prepared O-tetramethylstreptamine and degraded it to D,L-dimethoxysuccinic acid, thus showing that the hydroxyl group at C_5 is situated *trans* with respect to those at C_4 and C_6. This result was communicated in a preliminary note.[1] The present paper gives a detailed account of this work, and of concomitant experiments which lead to several hitherto undescribed derivatives and transformation products of streptamine.

The general plan of the investigation envisaged oxidative attack at the two carbon atoms carrying the amino groups in suitable O-acylated or O-alkylated derivatives. The preparation of O-acyl derivatives was studied first, and was achieved by intermediate protection of the amino groups by carbobenzoxylation. N,N′-Dicarbobenzoxystreptamine (I),[2] m.p. 249–250°, was obtained by the usual procedure in 80% yield, and afforded on acylation in pyridine without difficulty the corresponding O-tetraacetyl and O-tetrabenzoyl derivatives II and III, melting at 227–228° and 241–242°, respectively. The removal of the carbobenzoxy groups in II by hydrogenation with palladium as the catalyst and dioxane as the solvent proceeded fairly smoothly in three small-scale experiments, yielding on treatment of the crude basic product with the calculated amount of hydrochloric acid the expected O-tetraacetylstreptamine (IV) in form of a crystalline dihydrochloride (m.p. >300°). Anderson and Lardy[3] have recently prepared this compound in amorphous form by hydrolysis of O-tetraacetyl-N,N′-dibenzylidenestreptamine with hydrochloric acid. However, when larger amounts of II were subjected to the catalytic reduction, the dihydrochloride was obtained in small yield only, or not at all. The main product in these runs was an amorphous product[4] which in contradistinction to the dihydrochloride of IV was soluble in ethanol. It showed the analytical composition of a tetraacetylstreptamine monohydrochloride and contained only half of its nitrogen as amino nitrogen, indicating that in its formation acetyl migration from a hydroxyl to an amino group had occurred.



Of the four (racemic) O-triacetyl-N-monoacetylstreptamines which could arise in this manner—depending on which of the 4 O-acetyl groups is transferred to nitrogen—the one represented by formula V deserves preference on steric grounds. In the formation of this isomer the acetyl-donor would be the 5-acetoxy group, and the acceptor could be either of the two amino groups, since they are equidistant from the former. The *cis* relationship of these three groups follows from the work of Wolfrom and his collaborators,[5] whose synthesis of streptamine from D-glucosamine proves unequivocally that the configurations of carbon atoms 1, 3 and 5 are the same and opposite those of carbon atoms 4 and 6, and furthermore renders it highly probable that carbon atom 2 likewise partakes in this "all *trans*" relationship. Consequently, of the four acetoxy groups in IV, that at C_5 is the only one situated on the same side of the cyclohexane ring as the two amino groups and thus in a favorable steric position to form with one of the latter a cyclic orthoester amide (Va), the

(1) O. Wintersteiner and A. Klingsberg, THIS JOURNAL, **70**, 885 (1948).

(2) The spatial formulas employed here take into account the stereochemical characterization of the remainder of the molecule which Wolfrom, Olin and Polglase (ref. 5) have meanwhile achieved by their remarkable synthesis of streptamine from D-glucosamine. It should be understood that those compounds which as depicted here lack a plane of symmetry (V, VIb, IX, X (XI), XII (XIII)) are actually racemates. To save space only one enantiomorph has been written.

(3) L. Anderson and M. A. Lardy, THIS JOURNAL, **72**, 3146 (1950).

(4) In the last experiment of this kind a part of this material was obtained in crystalline form (*cf.* Experimental).

(5) M. L. Wolfrom and S. M. Polglase, Abstracts of Papers, 113th Meeting, Am. Chem. Soc., Chicago, April, 1948, p. 5Q; M. L. Wolfrom, S. M. Olin and W. J. Polglase, THIS JOURNAL, **72**, 1724 (1950).

intermediate form[6] probably involved in the acetyl shift under discussion.

It is true that this argument has lost some of its force by the recent demonstration of Fodor and Kiss[6a] that the O-benzoyl group in *trans*-2-aminocyclohexyl benzoate was capable of migrating, under the influence of alkali, to the adjacent amino group in spite of the latter's *trans* position. Nevertheless, since it stands to reason that in our case migration from the *cis*-oriented 5-acetoxy group would in all likelihood take precedence over the alternative *trans* shifts (actually only 2→1 and 4→3 need to be seriously considered), V remains in our opinion still the most satisfactory expression for the rearranged product. Furthermore two of the three alternative structures (2-OH,1(3)NH_2, and 4(6)-OH,3(1)-NH_2) can be excluded on the ground that the monohydrochloride proved to be inert to periodic acid. The possibility that the *trans*-relationship of the vicinal free amino and hydroxyl groups in these isomers may afford protection against attack can be safely disregarded in view of the fact that vicinal *trans*-glycolic groupings in streptamine derivatives (streptidine, N,N′-diacylstreptamines, N,N′-dibenzoxyl-4-desoxystreptamine[7]) exhibit normal reactivity. An explanation is available[7] for the resistance of the 5,6-*trans*-glycolic grouping in the streptidine portion of streptomycin.[8]

Experiments designed to replace the amino groups in O-tetraacetylstreptamine (IV) with hydroxyl groups by treating the dihydrochloride with aqueous silver nitrite gave an amorphous product which on acetylation with acetic anhydride in pyridine afforded in moderate yield crystalline material, m.p. 217–221°, having the approximate analytical composition of a pentaacetoxyacetamidocyclohexane, or, to use the term proposed by Carter, *et al.*,[9] for the monoamines derived from cyclohexitols, an inosamine hexaacetate. A similar, but evidently much purer product (m.p. 237–239°) was obtained when this procedure was applied to the monohydrochloride of the O-triacetyl-N-monoacetylstreptamine V described above. Barring the possibility of ring contraction, this compound has to be formulated either as VIa, or, in case that a Walden inversion has occurred during the replacement reaction, as its 3-epimer VIb.[10]

(6) (a) G. Fodor and J. Kiss, THIS JOURNAL, **72**, 3495 (1950); (b) L. H. Welsh, *ibid.*, **71**, 3500 (1940); (c) A. P. Phillips and R. Baltzly, *ibid.*, **69**, 200 (1947).

(7) F. A. Kuehl, R. L. Peck, C. E. Hoffhine and K. Folkers, *ibid.*, **70**, 2325 (1948).

(8) H. E. Carter, Y. H. Loo and P. S. Skell, *J. Biol. Chem.*, **168**, 401 (1947).

(9) H. E. Carter, R. K. Clark, B. Lyttle and G. E. McCasland, *ibid.*, **175**, 683 (1948)

(10) Anderson and Lardy[3] have recently assigned the configuration represented by the parent compound of VIa (1-amino-1-desoxyscyllitol, *scyllo*-inosamine-1) to "inosamine SB," one of the 1-epimeric inosamines which Carter, Clark, Lyttle and McCasland[9] obtained by catalytic reduction of *scyllo-meso*inosose phenylhydrazone. The hexaacetate of this inosamine apparently occurs in two polymorphous modifications melting at 284° and 301°, respectively (Kofler block).[9] The much lower melting point of our hexaacetate (237–239°) would seem to preclude identity with that stereoisomer and thus to favor the alternative formulation VIb, representing the hexaacetate of a *d,l*-6-amino-6-desoxy*meso*inositol or *d,l-meso*inosamine-6 (nomenclature and numbering of carbon atoms according to Anderson and Lardy[3]; *cf.* also B. Magasanik and E. Chargaff, *J. Biol. Chem.*, **174**, 173 (1948). However, we do not wish to commit ourselves to VIb on the basis of such tenuous evidence as the melting point of a single derivative.

Attempts to prepare O-tetrabenzoylstreptamine from the N,N′-dicarbobenzoxy derivative III by the catalytic method were hampered by poor reproducibility, evidently on account of the uncontrollable occurrence of O → N benzoyl migrations. Though some of the crystalline products obtained showed the approximate analytical composition $C_{34}H_{30}O_8N_2$ of a tetrabenzoylstreptamine, they were deficient in, or almost completely devoid of, amino nitrogen. In this case the catalytic reduction had to be carried out in acetic acid instead of in dioxane, because the reaction failed to proceed in the latte solvent, and it is possible that the prolonged contact with the acidic solvent was partly responsible for the greater ease with which acyl migration occurred in this case.

In view of the difficulties encountered in preparing larger amounts of O-tetraacetylstreptamine, and also because partial hydrolysis or acetyl migration could be anticipated to occur in the oxidative reactions to be applied, we resorted to O-methylation as a means of protecting the hydroxyl groups. For the preparation of the first intermediate required, N,N′-diacetylstreptamine, it was found convenient to employ direct N-acetylation with acetic anhydride in methanol[11] rather than the two-step procedure *via* the hexaacetate originally described by Peck, *et al.*[12] Treatment with dimethyl sulfate and under conditions similar to those employed by West and Holden[13] and White[14] for the methylation of D-glucose and N-acetyl-D-glucosamine, respectively, afforded in yields varying from 40 to 66% O-tetramethyl-N,N′-diacetylstreptamine (VII) (m.p. >300°), from which O-tetramethylstreptamine dihydrochloride (m.p. >300°) was secured by hydrolysis with hot *N* hydrochloric acid. This salt could be quantitatively converted by means of silver oxide into the free base (VIII), which in the anhydrous state melted at 83–84° and formed a dipicrate, m.p. 238–239°. It is worth mention that while the dihydrochloride was not attacked appreciably by periodate under conditions specified in a previous paper,[11] the free base consumed up to 4 atoms of oxygen within 72 hours with the liberation of ammonia. The N,N′-diacetyl derivative VII, as expected, showed no uptake.

In the degradation studies the free diamine VIII was oxidized with 4 to 6 molar equivalents of aqueous permanganate at room temperature in a carbon dioxide atmosphere (to buffer the fixed alkali and ammonia liberated). The bulk of the oxidized material consisted of acids which were converted into their methyl esters by treatment with methanolic hydrogen chloride. The esters were then subjected to fractional distillation at 0.1–0.2 mm. between 80 and 160°. Depending on the amount of starting material used in the run, from 3 to 6 cuts were taken, and each ester fraction was treated separately with anhydrous ammonia or methylamine in absolute methanol.

(11) A. E. O. Menzel, M. Moore and O. Wintersteiner, THIS JOURNAL, **71**, 1268 (1949).

(12) R. L. Peck, C. E. Hoffhine, E. W. Peel, R. P. Graber, F. W. Holly, R. Mozingo and K. Folkers, *ibid.*, **68**, 776 (1946).

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

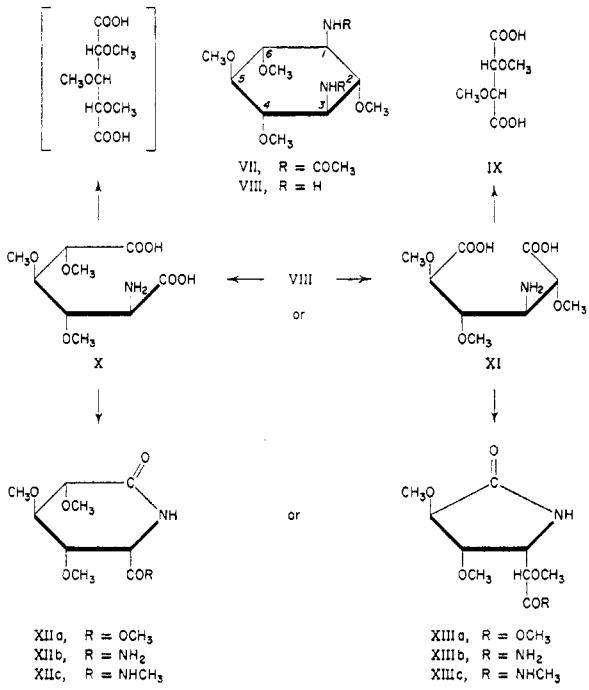
(14) T. White, *J. Chem. Soc.*, 428 (1940).

The lower-boiling fractions with ammonia invariably yielded a crystalline amide which after purification melted at 266–268° (dec.). The analysis of this product and of the methylamide, m.p. 188–189°, obtained from similar fractions showed that these compounds were diamides derived from a dimethoxysuccinic acid. In view of the *meso*-character of streptamine (for which ample evidence existed already at that time) it was clear that the parent acid was either *meso*- or D,L-dimethoxysuccinic acid.

A survey of the literature revealed that while the diamide[15] and dimethylamide[16] of the *meso*-form were known, there was no record of the preparation of the corresponding D,L-amides. The melting points reported for the *meso*-diamide (245–246°) and the *meso*-di-methylamide (210°) seemed to preclude identity with the compounds derived from streptamine. Nevertheless it was thought advisable to synthesize for comparison not only the hitherto undescribed D,L-derivatives, but also the known *meso*-amides. As anticipated, the synthetic specimens obtained from D,L-tartaric proved to be identical with the amides from streptamine, while the synthetic *meso*-diamide, which in our hands melted at 255–256°, strongly depressed the melting point (266–268°) of the dimethoxysuccinic-diamide from O-tetramethylstreptamine. The synthetic *meso*-di-methylamide melted at 210° as reported by Haworth and Hirst, which *eo ipso* distinguished it from the racemic compound, m.p. 189°. The identity of the oxidation product with D,L-dimethoxysuccinic acid (IX) was thus established beyond doubt.

The higher boiling ester fractions on treatment with methanolic methylamine yielded a compound m.p. 179–180°, which analyzed for $C_{10}H_{18}O_5N_2$ and contained 3 methoxyl groups. Though the analytical data were also compatible with the formula $C_{10}H_{20}O_5N_2$, *i.e.*, that of a trimethoxyglutaric di-methylamide, the melting point precluded identity with the known di-methylamide of *i-xylo*trimethoxyglutaric acid (m.p. 168°),[16] the only isomer which needed to be considered after the identification of the 4-carbon fragment as D,L-dimethoxysuccinic acid. Moreover, the simple diamide (m.p. 242–243°) obtained from a similar ester fraction with ammonia showed the composition $C_9H_{16}O_5N_2$, with three of the carbon atoms again being accounted for by O-methyl groups. It was thus clear that the parent acid contained 6 and not 5 carbon atoms, and, since the methylamide differed from the simple amide by one carbon only, that these compounds were mono- and not di-amides. Consequently the second nitrogen atom was derived from one of the amino groups of streptamine, and in consideration of the empirical formulas and the absence of basic properties, had to be part of a lactam ring.

This conclusion received further support by the subsequent isolation from high-boiling ester fractions, prior to amidation, of a crystalline compound (m.p. 109–110°), whose composition ($C_{10}H_{17}O_6N$), methoxyl content (4 groups) and convertibility by treatment with methylamine to the methylamide, m.p. 180°, left no doubt that it was the monomethyl ester which had given rise to the two amides. Its precursor was obviously a trimethoxyaminoadipic acid which had been formed by scission of the cyclohexane ring proximal to one of the carbon atoms carrying an amino group. Depending on whether this attack took place between positions 1 and 2 or 3 and 4 of the diamine, a 2-amino-3,4,5-trimethoxyadipic acid (X) or a 3-amino-2,4,5-trimethoxyadipic acid (XI) would result. Lactam formation from X followed by esterification would lead to the piperidone derivative XIIa and hence to the amide XIIb and the methylamide XIIc, whereas XI would give rise to the corresponding pyrrolidone structures XIIIa, XIIIb and XIIIc.



In order to secure the parent amino acid (X or XI) the lactam ester was hydrolyzed with hot saturated barium hydroxide solution. Considerable amounts of a crystalline barium salt consisting of large rods invariably deposited as the reaction proceeded. The nature of this product could not be elucidated beyond the fact that it was neither a barium salt of the expected amino acid nor the octahydrate of the reagent. The crystalline amino acid isolated from the filtrate showed variable melting points, probably due to existence of two polymorphous modifications (*cf.* Experimental), but gave analyses conforming with the expected composition. The ninhydrin reaction was negative. The fact that under the conditions used β-alanine likewise failed to react with ninhydrin, whereas O-methyl-D-(−)-threonine,[17] gave the typical color, favors the β-amino acid structure XI. Since the over-all yield from O-tetramethylstreptamine was too small to render feasible structure proof by degradation, resort was taken to a spec-

(15) W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1865 (1926).
(16) W. N. Haworth and D. I. Jones, *ibid.*, 2369 (1927).
(17) We wish to express to Dr. H. E. Carter of The University of Illinois our thanks for the gift of this compound.

trophotometric procedure which seemed to provide a means of differentiating α- and β-amino acids[18] and is based on the following considerations: α-Amino acids are readily convertible with ammonium thiocyanate in boiling 9:1 acetic anhydride–acetic acid into 1-acetylthiohydantoins[19] which can be recognized by their ultraviolet characteristics (maxima at 234 and 279 mμ).[20a] On the other hand, there is no evidence that β-amino acids form under these conditions the analogous 6-membered cyclic thioureas, the 1-acetyl-5,6-dihydrothiouracils. Only certain N-benzyl- or N-cyclohexyl-β-alanine derivatives have been converted in this manner to the corresponding N-substituted dihydrothiouracils.[20b]

Of the three compounds tested spectrophotometrically after treatment with the above reagents (the amino adipic acid from streptamine, β-alanine and O-methyl-D-(−)-threonine) only the last-named give rise to absorption characteristics indicative of the formation of a cyclic thiourea derivative. A preparative experiment carried out on this compound yielded the expected product, 1 - acetyl - 5 - (1 - methoxyethyl) - 2 - thiohydantoin, which showed in its spectrum the characteristic absorption peaks at 234 and 280 mμ. The corresponding experiment with β-alanine did not afford a derivative of the amino acid, but instead a yellow compound (m.p. 212–216°) which was identified by analysis as acetylisoperthiocyanic acid $CH_3CO{\cdot}C_2HNS_3$.[21] These results, taken at their face value, give additional support to the β-amino acid structure XI for the trimethoxyaminoadipic acid, and on this basis the lactam derivatives would have to be formulated as pyrrolidones of the type XIII. However, it should be pointed out that the negative spectrophotometric evidence is not quite conclusive in the present case insofar as under the conditions of the Johnson reaction recyclization to the lactam may have taken precedence over the azlactonization which apparently must precede the reaction with thiocyanic acid leading to the thiohydantoin derivative. Nevertheless we feel that the available evidence justifies assigning provisionally formula XI to the amino acid and formula XIII to the lactam derivatives.

The import of these findings may now be briefly considered. First, it is self-evident from a consideration of the structure of streptamine and of its *meso*-character that the configuration of carbon atom 5 must be opposite to that of carbon atoms 4 and 6 in order to permit the formation of a D,L-succinic acid derivative on degradation. This conclusion is also inherent in the more comprehensive configurational proof of Wolfrom, *et al.*,[5] by synthesis. There it derives from the *trans*-relationship of the 3- and 4-hydroxyl groups in D-glucosamine, which in the course of the synthetic transformation become those at carbon atoms 4 (6) and 5 of streptamine.

Since in O-tetramethylstreptamine the portion comprising C_4, C_5 and C_6 appears least vulnerable to oxidative scission, one would off-hand expect some *i-xylo*trimethoxyglutaric acid to occur among the oxidation products. Had this entity been present in sizeable quantities, it should have been encountered in form of the readily crystallizable amide (m.p. 195°) and methylamide (m.p. 168°) in the high-boiling ester fractions treated with the basic agents. Actually all the fractions so examined yielded the lactam amides; any substantial contamination of the latter with the trimethoxyglutaramides is ruled out by the melting point properties of the crude products. Moreover, the samples treated with ammonia failed to develop the characteristic deep blue color which this reagent produces in methanolic solutions of *xylo*trimethoxyglutaric methyl ester prior to the deposition of the amide crystals.[22] We consider this good evidence for the absence of this acid in the oxidized mixture.

The fact that both *i-xylo*trimethoxyglutaric acid and D-dimethoxysuccinic acid have been obtained at numerous occasions by nitric acid oxidation of methylated glucopyranose derivatives has of course little bearing on the present case, except insofar as it shows that the 4 carbon fragment, since it was optically active, did not arise by further oxidation of the intramolecularly compensated trimethoxyglutaric acid. It is also interesting that in one of these instances, namely, in the degradation studies of Hirst[23] on 2,3,4,6-tetramethyl-D-glucose, a parallel oxidation experiment with permanganate yielded D-dimethoxysuccinic acid and oxalic acid, but no trimethoxyglutaric acid, showing that this oxidant, in contradistinction to nitric acid, had cleaved the carbon chain at only one side of the vulnerable position 5. In our case, however, no oxamide was ever obtained from the low-boiling ammonia-treated ester fractions, although small amounts of oxalic acid could be demonstrated analytically in the oxidized mixture prior to esterification.

On the basis of these facts and considerations we picture the oxidation of O-tetramethylstreptamine to proceed as follows: The primary attack takes place at only one of the amino groups at the time (as would be expected on kinetic grounds); and in such a manner that after the elimination of the nitrogen atom only *one* of the adjacent C–C linkages is cleaved. Presuming that XI is the correct structure of the resulting trimethoxyaminoadipic acid, the linkage severed must be C_3–C_4. Further attack by the oxidant of a part of XI at the β-amino group leads through imino or oximino intermediates to the corresponding β-keto acid, which then undergoes the usual hydrolytic cleavage with the formation of D,L-dimethoxysuccinic acid and O-methylglycolic acid. In regard to the latter acid,

(18) The difference in the rate of liberation of nitrogen in the Van Slyke amino nitrogen determination is far too small to be relied upon for this purpose; *cf.* the comparative measurements on α- and β-alanine by M. S. Dunn and C. L. A. Schmidt, *J. Biol. Chem.*, **53**, 401 (1922).

(19) T. B. Johnson, *ibid.*, **11**, 97 (1912).

(20) (a) Squibb Report **S34**, in Chapter X, V. du Vigneaud and D. B. Melville, "The Chemistry of Penicillin," Princeton University Press, 1949, p. 287; (b) Squibb Report **S45**, *ibid.*, p. 294; Merck Report ***M63***, *ibid.*, p. 307.

(21) M. Nencki and W. Leppert, *Ber.*, **6**, 902 (1873); A. Hantzsch and M. Wolvekamp, *Ann.*, **331**, 263 (1904); for nomenclature *cf.* N. V. Sidgwick "The Organic Chemistry of Nitrogen," Oxford, 1937, p. 355.

(22) E. L. Hirst and C. B. Purves, *J. Chem. Soc.*, **123**, 1352 (1923).

(23) E. L. Hirst, *J. Chem. Soc.*, 350 (1926).

it must be assumed that a small fraction was oxidized to oxalic acid, while the remainder escaped detection in the procedure used (volatility of ester, or unfavorable crystallizing properties of amides). The surviving portion of XI is converted in the isolation procedure to the lactam ester XIIIa.

The less probable structure X would analogously arise by ring cleavage between C_2 and C_3. One would anticipate such a compound, as an α-amino acid, to yield at least some *xylo*trimethoxyglutaric acid on further oxidation. Since according to all indications this degradation product was absent, XI conforms better than X to the observed facts in this respect also.

Experimental

All melting points reported were taken in the capillary and are corrected for stem exposure.

N,N'-Dicarbobenzoxystreptamine (I).—To an ice-cold, mechanically stirred solution of streptamine dihydrochloride (5.01 g., 0.02 mole) in *N* sodium hydroxide (40 cc.) a solution of carbobenzoxy chloride (15 cc., 0.08 mole) in toluene (50 cc.) was added dropwise. The reaction was kept alkaline by the simultaneous dropwise addition at the appropriate rate of *N* sodium hydroxide (80 cc.). During this period and the following 2 hours of continued stirring the temperature was maintained around 5°. The mixture was then shaken at room temperature for 20 hours, whereupon it was placed into the ice-box. The crystalline product was filtered off by suction, washed with water, dried (7.65 g.) and recrystallized from 500 cc. of hot dioxane (5.2 g., 80% of theory, m.p. 249–250°). The derivative is insoluble at room temperature in the usual organic solvents except pyridine. It is sparingly soluble in hot water, but dissolves to the extent of about 5% in boiling dioxane.

Anal. Calcd. for $C_{22}H_{26}O_8N_2$ (466,5): C, 59.18; H, 5.87; N, 6.27. Found: C, 59.28; H, 5.90; N, 5.92, 6.17.

O-Tetraacetyl-N,N'-dicarbobenzoxystreptamine (II).—A solution of N,N'-dicarbobenzoxystreptamine (5 g.) in pyridine (50 cc.) and acetic anhydride (50 cc.) was allowed to stand at room temperature overnight, after which time it had deposited a copious precipitate. After pouring the mixture onto ice, the crystalline product was filtered, washed with water, dried and recrystallized from 500 cc. of 95% ethanol (5.18 g., m.p. 227–228°).

Anal. Calcd. for $C_{30}H_{34}O_{12}N_2$ (614.6): C, 58.61; H, 5.57; N, 4.56. Found: C, 58.44; H, 5.54; N, 4.44.

O-Tetrabenzoyl-N,N'-dicarbobenzoxystreptamine (III).—To an ice-cold solution of N,N'-dicarbobenzoxystreptamine (2.23 g.) in pyridine (20 cc.) benzoyl chloride (4 cc.) was added in several portions. After standing overnight at room temperature the mixture was poured onto ice. The oily product soon became crystalline, and was filtered and washed with water. After drying it was recrystallized twice from warm acetic acid (150 cc.)–water (50 cc.). The pure product (2.56 g.) melted at 241–242° after sintering at 238°. It was soluble in cold chloroform and benzene, and sparingly soluble in hot ethanol.

Anal. Calcd. for $C_{50}H_{42}O_{12}N_2$ (862.8): C, 69.60; H, 4.90; N, 3.25. Found: C, 69.44; H, 4.79; N, 3.35.

O-Tetraacetylstreptamine Dihydrochloride (IV·2HCl).—A suspension of palladium black (100 mg.) in pure dioxane (20 cc.) was saturated with hydrogen at atmospheric pressure, whereupon tetraacetyl-N,N'-dicarbobenzoxystreptamine (614 mg., 1 millimole) in the same solvent (10 cc.) was added. A total of 48.5 cc. of hydrogen (2.15 millimole) was taken up by the substance in 24 hours. The filtered solution was brought to dryness, and the residue was triturated with 0.1 *N* hydrochloric acid (20 cc.). All but a trace went into solution. The acidic solution was evaporated *in vacuo*. The residue (445 mg.) was crystallized by dissolving it in a small amount of water and adding ethanol (5 cc.) and then anhydrous ether to slight turbidity. The crystalline product which deposited on standing at 5° (283 mg.) was recrystallized twice in the same manner. On heating in the capillary the compound turned dark at 250°, but did not melt up to 300°.

Anal. Calcd. for $C_{14}H_{22}O_8N_2 \cdot 2HCl$ (419.3): C, 40.10; H, 5.77; N, 6.68; Cl, 16.91. Found: C, 39.97; H, 5.83; N, 6.67; Cl, 16.63.

In two subsequent experiments starting with 2 and 3 millimoles, respectively, about 25% of the crude basic product remained undissolved on extraction with hydrochloric acid, but the soluble part yielded the crystalline dichloride without difficulty. With larger batches of starting material (5 to 8 millimoles) complications were encountered. In most cases a substantial portion of the reduction product remained insoluble when the calculated amount of hydrochloric acid was added. Usually, but not always, a part of the acid-soluble fraction could be obtained in form of the crystalline, ethanol-insoluble dihydrochloride described above. The remainder consisted essentially of the amorphous, ethanol soluble monohydrochloride of the O-triacetyl-N-acetylstreptamine assigned structure V.

In a typical experiment 4.91 g. (8 millimoles) of O-tetraacetyl-N,N'-dicarbobenzoxystreptamine was reduced with 500 mg. of palladium black as described above. The residue of the filtered solution was triturated with 0.260 *N* hydrochloric acid added in portions till the reaction remained acidic (22 cc.). After removal of the insoluble part (*ca.* 2 g., after recrystallization m.p. 226–228° apparently starting material) by filtration the acid extract was lyophilized, and the residue crystallized in the usual way. The crystalline product (804 mg.) consisted essentially of the dihydrochloride of IV as shown by analysis (Calcd.: Cl, 16.9; N, 6.67. Found: Cl, 14.7; N, 6.98; amino-N, 6.64). The mother liquor was brought to dryness. The residue (900 mg.), which in contradistinction to the crystalline fraction was soluble in absolute ethanol, was obtained by precipitation from this solvent with anhydrous ether as a gum which solidified on drying in the desiccator.

Anal. Calcd. for $C_{14}H_{22}O_8N_2 \cdot HCl$ (382.8): Cl, 9.26; N, 7.32; amino-N, 3.66. Found: Cl, 9.66; N, 7.07; amino-N, 4.08.

Two other preparations of this description gave, respectively, Cl, 9.42, 9.40; amino-N, 3.68, 3.47. In a later run with 7.3 millimoles and 1 g. of palladium black the monohydrochloride was obtained in crystalline form. In this case almost all of the crude reduction product was soluble in the calculated amount of hydrochloric acid. The freeze-dried residue from the acid extract (2.79 g., calcd. for monohydrochloride, 2.80 g.) was crystallized in the usual manner from water–ethanol–ether, but the crystalline fraction (624 mg.) was deficient in Cl^- (Found: Cl^-, 10.47). On recrystallization from the same solvents the product formed minute blocks (m.p. >300°, gradual darkening about 250°).

Anal. Calcd. for $C_{14}H_{22}O_8N_2 \cdot HCl$: C, 43.92; H, 6.05; Cl, 9.26. Found: C, 43.81; H, 6.45; Cl, 9.42.

The compound did not consume any periodic acid within 48 hours. The amorphous material precipitated from the original mother liquor with excess ether (1.18 g.) contained 10.0% of chlorine. Evidently in this experiment most of the primary reduction product had undergone the acetyl shift.

Inosamine Hexaacetate (VIa or VIb).—To an ice-cold solution of the amorphous O-triacetyl-N-monoacetylstreptamine hydrochloride described above (Cl = 9.66, 645 mg.) in 0.12 *N* hydrochloric acid (4.2 cc.) finely powdered silver nitrite (335 mg., 1.3 molar equivalents) was added in portions with stirring. The mixture was allowed to stand at 5° for six hours and then at room temperature overnight. The amorphous material obtained by lyophilizing the filtered solution (590 mg.) contained no amino-nitrogen. Since it could not be induced to crystallize, a portion (200 mg.) was acetylated with acetic anhydride (2 cc.) and pyridine (2 cc.). The resulting product (169 mg., m.p. 228–230°) was recrystallized three times from absolute ethanol and then melted at 237–239°.

Anal. Calcd. for $C_{18}H_{25}O_{11}N$ (431.4): C, 49.74; H, 5.80; N, 3.22; O-acetyl, 49.9. Found: C, 49.91; H, 5.84; N, 3.30; O-acetyl, 50.3.

A similar experiment had been previously carried out with O-tetraacetylstreptamine dihydrochloride (629 mg., 0.015 mole) as the starting material. The procedure was identical with the above, except that 2.6 molar equivalents of silver nitrite were used, and that after filtering from the silver chloride the excess hydrochloric acid was removed with silver carbonate. The recovered material (248 mg. which in this case was partially crystalline, contained no

amino-nitrogen. On acetylation it yielded a crystalline substance which after three recrystallizations from ethanol melted with decomposition at 217–221°.

Anal. Found: C, 50.13; H, 6.40; N, 2.75.

N-Acetylation of Streptamine.—A mixture of streptamine dihydrochloride (5 g.) and silver acetate (6.68 g.), in dry methanol (50 cc.) and acetic anhydride (6 cc.) was shaken mechanically in the dark for 4 hours and then heated to boiling for 5 minutes. The silver chloride was filtered off and washed with three 100-cc. portions of hot water. The combined filtrates were acidified with 1 *N* hydrochloric acid (1.5 cc.), and after some standing filtered again through a bed of Supercel. The filtrate was concentrated to a small volume and after the addition of methanol (50 cc.) allowed to stand at 5° overnight. The resulting crystalline precipitate (3.70 g., m.p. 280–283°) and an additional amount (1.26 g., m.p. 282–283°) obtained by evaporating the mother liquor to dryness, taking up the residue in warm methanol (30 cc.) and adding excess ether, were dissolved together in water and freed from traces of silver salts still present with hydrogen sulfide. After removal of the silver sulfide the solution was brought to dryness and the residue recrystallized from methanol–ethanol (4.57 g., m.p. 282–283°). The melting point of a mixture with an authentic sample was not depressed.

O-Tetramethyl-N,N′-diacetylstreptamine (VII).—N,N′-Diacetylstreptamine (20 g.) was placed together with 50 cc. of water into a 2-l. 3-neck round bottom flask immersed in an oil-bath and equipped with a stirrer and two dropping funnels. On warming the suspension to 55° the substance went into solution. This temperature was maintained during the subsequent operations, consisting of the addition, in one portion, of dimethyl sulfate (95 cc.) in carbon tetrachloride (100 cc.), and then of 60% sodium hydroxide solution (368 cc.) at the following rates: 1 drop/2 sec. for 5 minutes; 1 drop/sec. during the following 5 minutes; 3 drops/sec. during the addition of the remainder. The temperature was now raised to 75°, and dimethyl sulfate (128 cc.) was admittted at the rate of 3 drops/sec. Finally the mixture was heated as rapidly as possible (within about 5 minutes) to 100°, kept at this temperature for 3 minutes and then quickly cooled. Vigorous stirring was maintained during all these operations. Since preliminary experiments had shown that by far the greater part of the desired product was co-precipitated with the solid sodium sulfate separating out during the reaction, the entire contents were transferred to an expendable round-bottom flask and brought to dryness *in vacuo* on a steam-bath. Several hours of heating under these conditions generally sufficed to render the residual salt cake sufficiently free of water to be broken up and powdered in a mortar. The dry powder was extracted in a soxhlet with chloroform (800 cc.) for 6 hours. This first, strongly pigmented extract was discarded, as previous experience had shown it to contain mostly non-crystallizable oils. Extraction was continued with a new 1-l. portion of chloroform which remained colorless and after about 10 hours began to deposit a copious crystalline precipitate. After 72 hours (which period generally sufficed for complete extraction) the extract was removed and filtered after chilling; the precipitate was washed with 20 cc. of ice-cold chloroform and after drying weighed 15.9 g.

The crystalline product obtained in this manner is sufficiently pure to be used for the subsequent hydrolysis step. It could be recrystallized from absolute ethanol or methanol–ether, from which it formed long fine needles (m.p. > 300°, sublimation at 100°/1 mm.). It is readily soluble in water and methanol, somewhat less in chloroform and benzene. For analysis the crude product was sublimed twice *in vacuo* and then recrystallized from methanol–ether.

Anal. Calcd. for $C_{14}H_{26}O_6N_2$ (318.4): C, 52.81; H, 8.23; N, 8.81; OCH_3, 39.1. Found: C, 52.99; H, 8.28; N, 8.67; OCH_3, 40.1.

It should be mentioned that the yield in the experiment described above (66%) was considerably above average (*ca.* 45%). Attempts to recover additional amounts by remethylating the oily products present in the first chloroform extract and in the mother liquor from the crude crystalline material were not successful.

O-Tetramethylstreptamine Dihydrochloride (VIII·2 HCl).—A solution of O-tetramethyl-N,N′-diacetylstreptamine (8.15 g.) in a N-hydrochloric acid (750 cc.) was boiled under reflux for 12 hours and then brought to dryness *in vacuo*. The residue was freed from excess acid by dissolving it in water, removing the latter by distilling, and once repeating this procedure. The slightly yellow product was treated in aqueous solution (5 cc.) with charcoal. On addition to the colorless filtrate of ethanol (50 cc.) and ether (80 cc.) and scratching a crystalline precipitate formed, increased on standing at 4° overnight. The product was collected by filtration and washed with ether (4.92 g.). An additional amount (2.68 g.) was obtained by taking up the residue from the evaporated mother liquor in water (2 cc.) and adding ethanol (30 cc.) and ether (70 cc.). For analysis a sample was recrystallized 3 times from the same solvents (m.p. > 300°, partial sublimation).

Anal. Calcd. for $C_{10}H_{22}O_4N_2 \cdot 2HCl$ (307.2): C, 39.09; H, 7.87; N, 9.12; Cl, 23.08; OCH_3, 40.4. Found: C, 38.76; H, 7.71; N, 8.98; Cl, 23.4; OCH_3, 40.4.

A sample treated with excess potassium periodate showed negligible uptake (0.24 molar equivalent in 15 minutes, constant to 48 hours).

O-Tetramethylstreptamine (VIII).—A solution of the dihydrochloride (6.7 g.) in water (50 cc.) was shaken with silver oxide (5.07 g.) for 10 minutes. The precipitate was removed by filtration and thoroughly washed with water. The filtrates and washing were treated with hydrogen sulfide, filtered, aerated and lyophilized. The crystalline material thus obtained (4.9 g., m.p. 52–54°) was shown to consist of a hemihydrate which loses its water of crystallization on drying at 56° (2 mm.) or on standing several days *in vacuo* over calcium chloride. It is readily soluble in water, ethanol, benzene and dioxane. It was purified for analysis by sublimation at 80° (5 mm.), and subsequent recrystallization from warm ligroin (b.p. 90–100°), from which it formed long needles melting at 83–84°.

Anal. Calcd. for $C_{10}H_{22}O_4N_2$ (234.3): C, 51.26; H, 9.46; N, 11.96; OCH_3, 53.0. Found: C, 51.18; H, 9.38; N, 11.71; OCH_3, 52.5.

The free diamine is fairly readily attacked by periodate, as shown by the following figures, which represent atoms of oxygen consumed per mole and the reaction time in hours (in parentheses): Expt. I: 2.40 (21), 3.54 (66); Expt. II: 3.96 (18), 4.52 (42); Expt. III: 1.26 (7), 3.85 (72). Ammonia was liberated already during the initial stage of the oxidation. (Found: 0.57 mole after 55 minutes.)

The dipicrate was prepared by heating the base (50 mg., 0.22 millimole) with a concentrated solution of picric acid (1.15 g., 0.48 millimole, in minimum volume of warm water) on the steam-bath for a few minutes. The solution was evaporated almost to dryness, and the residue was recrystallized from ethanol (206 mg., m.p. 233–237° (dec.)). After 3 more recrystallizations the product melted at 238–239° (dec., darkening at 233°).

Anal. Calcd. for $C_{22}H_{28}O_{18}N_8$ (692.5): C, 38.17; H, 4.07; N, 16.18. Found: C, 38.21; H, 4.20; N, 16.2.

Oxidation of O-Tetramethylstreptamine with Permanganate. Isolation and Fractionation of Esters.—The results reported in the theoretical part represent the composite findings of three small-scale experiments, the first two of which were of more or less orienting character. Only the third experiment (A) is described here in full but with the inclusion of a few analytical and other data from the preceding runs. In later experiments, which were undertaken primarily for the purpose of securing larger amounts of the lactam ester (XIIa or XIIIa), essentially identical results were obtained (*cf.* B below).

A. To an ice-cooled solution of O-tetramethylstreptamine (1.76 g., 7 millimoles) in water (10 cc.) potassium permanganate (6.63 g., 42 millimoles) dissolved in water (250 cc.) was added dropwise with mechanical stirring, while a stream of carbon dioxide was passed through the solution. The mixture was then kept in the refrigerator for 2 days in a closed vessel filled with carbon dioxide. Titration of a 2-cc. aliquot with oxalic acid showed that all but 2% of the oxidant had been consumed; the excess was destroyed by the addition of ethanol (30 cc.). (In all other runs the oxidant was added, and the mixture was allowed to stand, at room temperature. Under these conditions standing overnight sufficed for complete reduction of the permanganate. To judge from the yields of crude and fractionated esters, the low temperature procedure offers no advantage.) The *p*H was adjusted to about 9 with *N* potassium hydroxide (20 cc.), whereupon the solution was freed from the manganese dioxide by centrifugation. The precipitate was washed

several times with warm water, and the combined supernatants were distilled to dryness *in vacuo*. (Titration of the distillates with acid in the first two experiments showed that 0.55 and 0.70 mole, respectively, of ammonia per mole of diamine were present.) The residue was taken up in water and subjected to continuous extraction with chloroform overnight. The extract yielded 126 mg. of amorphous material, which was not further examined.

The aqueous phase was acidified to *p*H 3 with *N* hydrochloric acid (evolution of nitrous gases) and brought to dryness under reduced pressure. The dark brown residue was extracted with 140 cc. of 3% methanolic hydrogen chloride in 7 equal portions, leaving 3.5 g. of insoluble, mostly inorganic material. The combined extracts were boiled under reflux for two hours and evaporated to dryness. The residue was taken up in methanol (50 cc.) and treated with silver carbonate in portions till the reaction of the supernatant was neutral. Removal of the solvent from the Cl^--free filtrate yielded a brown sirup, which was taken up in a few cc. of dry acetone for the removal of some insoluble inorganic material still present. The residue of the filtered acetone extract was dissolved in water, and the solution was extracted with chloroform (3 × 60 cc.). The combined chloroform phases were washed consecutively with 5 cc. each of dilute hydrochloric acid, sodium bicarbonate solution and water. (Neither the residue of the original water phase, a brown gum weighing 555 mg., nor the acid-extractable material (43 mg.) yielded any crystalline products.) The residue obtained by evaporation of the dried chloroform solution, a dark-brown oil weighing 579 mg., was transferred with ether into a small retort-like distillation vessel filled with glass wool and provided with a short, V-shaped side arm and a central tubule accommodating a hair-fine capillary. Its shape permitted immersion into a metal-bath to a point about 1.5 cm. below the junction of neck and side arm, an arrangement necessitated by the high boiling point and viscosity of the lactam ester. After removal of the ether the esters were fractionally distilled in a high vacuum, while the bath temperature was raised at the rate of about 20° per hour. Three cuts were taken: Fraction I, 128 mg. colorless, mobile, 75–100° (0.25 mm.); Fraction II, 123 mg., slightly yellow, mobile, 100–125° (0.25 mm.); Fraction III, 61 mg., yellow, viscous, 168–185° (0.1 mm.) (for further treatment *cf.* following sections).

B. After it had become clear that crystalline products could be realized only from the ester fraction, a much simpler procedure was substituted for the one initially used. Thus, in an experiment starting with 4.8 g. (20 millimoles) of diamine and 12.64 g. (80 millimoles) of permanganate (r. temp., overnight), the mixture was freed from the manganese dioxide without prior addition of potassium hydroxide. The filtrate was concentrated to 100 cc. and acidified with hydrochloric acid to *p*H 3 before bringing it to dryness. The residue was dried over phosphorus pentoxide and extracted in portions with 1% methanolic hydrogen chloride (160 cc.). The addition of dry ether (150 cc.) to the extract precipitated most of the inorganic material present. The latter was removed by filtration, and ethereal diazomethane (3.7 g.) was added to the filtrate. After 30 minutes the ether was removed on the steam-bath, and the chloroform solution (275 cc.) of the residue was washed with small volumes of dilute sodium bicarbonate solution and water. Fractional distillation of the esters (2.42 g.) yielded in the first 3 fractions distilling at 0.01 mm. between 80 and 120°, 780 mg. of dimethyl dimethoxysuccinate, identified by the refractive index $n^{28}D$ 1.4312 ($n^{27.5}D$ of reference sample 1.4320); and in fractions 4 to 6 (140–165° (0.01 mm.), 1.33 g.) 406 mg. of crude crystalline lactam ester, after purification 202 mg., m.p. 107–109°, in capillary.

D,L-Dimethoxysuccinic Acid Diamide.—A part of ester fraction I from Experiment A (86.5 mg.) was dissolved in 0.9 cc. of absolute methanol which had been saturated with dry ammonia gas at 0°. On allowing the solution to stand for 70 hours at room temperature it deposited a crystalline product (47 mg., m.p. 256–260°) which was recrystallized twice from water (prisms, m.p. 266–268° (dec.)). The melting point was not depressed upon admixture of an authentic sample of D,L-dimethoxysuccinic acid diamide (m.p. 266–268°), whereas a mixture with the *meso* diamide (m.p. 255–257° (dec.)) melted at 227–231°.

Anal. Calcd. for $C_6H_{12}O_4N_2$ (176.2): C, 40.90; H, 6.87; N, 15.90; OCH_3, 35.2. Found: C, 41.14; H, 6.76; N, 16.19; OCH_3, 35.0.

The diamide prepared in the same manner from authentic dimethyl D,L-dimethoxysuccinate was indistinguishable from the amide derived from O-tetramethylstreptamine in melting point, crystal shape and analytical properties (Found: C, 40.70; H, 6.64; N, 16.08; OCH_3, 35.2.) The ester was obtained by Purdie methylation of silver D,L-tartrate,[24] which in turn was derived from calcium D,L-tartrate secured by racemization of L(+)-tartaric acid. The same procedure was followed for the preparation of the known[15] *meso*dimethoxysuccinic acid diamide (m.p. 255–257°, dec.) from calcium *meso*tartrate.[25]

D,L-Dimethoxysuccinic Acid Di-N-methylamide.—The remainder of ester fraction I from Experiment A (40.6 mg.) was treated at room temperature with 0.5 cc. of a methanolic solution of dry methylamine prepared by saturation at 0°. As no crystals had appeared after 70 hours, the solution was taken to dryness. The crystalline residue was recrystallized from hot ethyl acetate (2 cc.), yielded 22 mg. of small prisms melting at 178–181°. Two more recrystallizations raised the melting point to 188–189°, undepressed by admixture of a synthetic specimen.

Anal. (Sample from a previous experiment): Calcd. for $C_8H_{16}O_4N_2$ (204.2): C, 47.04; H, 7.90; N, 13.72; OCH_3, 30.4. Found: C, 47.46; H, 8.05; N, 13.96; OCH_3, 30.5.

The specimen prepared from authentic dimethyl D,L-dimethoxysuccinate melted at 188–189° (Found: C, 47.26; H, 7.94; N, 13.97; OCH_3, 30.1).

2(3?),4,5-Trimethoxy-3(2?)-aminoadipic Lactam Amide (XIIIa or XIIa).—A 66-mg. sample of an ester fraction b.p. 110–120° (0.1 mm.) from a preliminary experiment was treated with methanolic ammonia (0.70 cc.). After standing for 7 days at room temperature the dark yellow solution was taken to dryness, and the residue was dissolved in 2 cc. of warm ethanol. On cooling, rosettes of needles 28 mg. (m.p. 239–242°) separated out, which were recrystallized twice from the same solvent and then melted at 242–243° after sintering at 237°.

Anal. Calcd. for $C_9H_{16}O_5N_2$ (232.2): C, 46.94; H, 6.94; N, 12.07; OCH_3, 40.9. Found: C, 46.80; H, 7.13; N, 11.83; OCH_3, 37.5.

Fraction II from Experiment A (123 mg.) yielded on similar treatment 7.8 mg. of the crude amide, which after recrystallization melted at 242–243° alone and in mixture with the above specimen.

2(3?),4,5-Trimethoxy-3(2?)-aminoadipic Lactam N-methylamide (XIIIb or XIIb).—An 85-mg. sample of the ester fraction b.p. 110–120° (0.1 mm.) which had afforded the above amide was dissolved in 0.85 cc. of methanolic methylamine. After standing for 4 days the clear solution was brought to dryness. The gummy residue could be crystallized from warm ethyl acetate, yielding 43 mg. of a crystalline product m.p. 163–167°. On recrystallization various crops of crystals differing in appearance (needles and prisms) but showing the same melting point separately and in mixture (170–172°) were obtained. The combined fractions were repeatedly recrystallized till the melting point became constant at 179–180°.

Anal. Calcd. for $C_{10}H_{18}O_5N_2$ (246.2): C, 48.77; H, 7.37; N, 11.38; OCH_3, 37.8. Found: C, 49.02; H, 7.19; N, 11.02; OCH_3, 37.3.

2(3?),4,5-Trimethoxy-3(2?)-aminoadipic Lactam Methyl Ester (XIIIc or XIIc).—Ester Fraction III from Experiment A on standing for a week had deposited a crop of crystalline material which could be recrystallized from warm absolute ether (16 mg., m.p. 93.5–94°). On recrystallization the melting point rose to 109–110°. On heating on the microscope stage, the substance partially sublimed above 104°, and then melted at 109–110°.

Anal. Calcd. for $C_{10}H_{17}O_6N$ (247.2): C, 48.57; H, 6.93; N, 5.67; OCH_3, 50.2. Found: C, 48.74; H, 6.87; N, 5.65; OCH_3 49.2.

In subsequent experiments larger amounts of the lactam ester were obtained from appropriate ester fractions by dissolving them in the minimum amount of ether and seeding. The crude products thus obtained usually melted at 102–

(24) As described by T. S. Patterson and D. C. Patterson, *J. Chem. Soc.*, **107**, 154 (1915), for the preparation of the *meso* ester.

(25) We wish to express our sincere appreciation to Dr. R. Pasternack of the Chas. Pfizer Co. for making available to us 158 g. of this salt.

106°. For the conversion to the N-methylamide 15 mg. of the ester was treated with 0.15 cc. of methanolic methylamine for four days. The residue of the evaporated solution was recrystallized twice from ethyl acetate, yielding 9 mg. of needles m.p. 179–180°, undepressed by admixture of the lactam N-methylamide described above.

Identification of Oxalic Acid.—In an oxidation experiment starting with 4.8 g. of O-tetramethylstreptamine the oxidized mixture, after removal of the manganese dioxide, was brought to *p*H 6.7 by the addition of hydrochloric acid and lyophilized. A portion of the residue (600 mg. corresponding to 0.24 g. of the starting product) was dissolved in a few cc. of water (*p*H 5.4), and a 20% calcium chloride solution (2 cc.) was added. The resulting precipitate (12 mg.) was purified by reprecipitation from dilute hydrochloric acid with ammonia. The salt reduced permanganate in the presence of dilute sulfuric acid.

Anal. (1) (Sample dried at 100° (2 mm.) for 6 hours): Calcd. for $Ca(COO)_2 \cdot H_2O$: Ca, 25.6. Found: Ca, 25.4; (2) (Sample dried at 150° (2 mm.) to constant weight): Calcd. for $Ca(COO)_2 \cdot {}^1/_2H_2O$: C, 17.52; H, 0.75. Found: C, 17.62; H, 1.20.

2(3?),4,5-Trimethoxy-3(2?)-aminoadipic Acid (XI or X) (a).—A 100-mg. sample of the lactam methyl ester (XIIIc or XIIc) was hydrolyzed with 6 cc. of a saturated (0.289 *N*) aqueous barium hydroxide solution at reflux temperature for 2 hours, while nitrogen gas free of carbon dioxide was passed through the boiling solution. After about 20 minutes a heavy crystalline precipitate began to form, which seemed to increase in amount during the remainder of the hydrolysis period. After 2 hours the contents of the flask were cooled to room temperature, and the precipitate was quickly filtered by suction and washed with 20 cc. of carbon dioxide-free water in 5 portions (39 mg., see below). The combined filtrate and washings were freed from barium with sulfuric acid, and the excess of the latter was carefully removed with barium hydroxide. The barium sulfate was centrifuged off, and the supernatant, now free from inorganic ions, was lyophilized. The residue (73 mg.) was extracted with 10 cc. of warm absolute methanol. The insoluble portion (14.5 mg.) was filtered off, and the filtrate concentrated to 4 cc. On the gradual addition of 8 cc. of dry acetone a gelatinous precipitate formed. Without removing the latter the solution was concentrated in an air current to about 1 cc., whereby the precipitate turned partly into small crystals. More crystalline material deposited on standing at 4°. The product was collected by centrifuging, washed with acetone and dried (32 mg., m.p. 160–161° after sintering at 158°). Recrystallization by dissolving the material in 10 cc. of warm methanol and concentrating to a small volume yielded tiny prisms (23.8 mg.) melting at 155–156° (dec.). When the melting point was redetermined 2 years later, the preparation melted at 167–170°. Polymorphism is obviously involved.

Anal. Calcd. for $C_9H_{17}O_7N$ (251.2): C, 43.02; H, 6.82; OCH_3, 37.1. Found: C, 42.82; H, 6.89; OCH_3, 38.0.

(b) In a previous experiment starting with 60 mg. of the lactam ester, in which no precautions were taken to exclude carbon dioxide, the crystalline precipitate formed during the hydrolysis weighed 70 mg. (see below) and the residue of the barium-free filtrate 40 mg. The methanol extract of the latter was filtered and concentrated to 0.4 cc., and excess ethyl acetate was added dropwise. The resulting flocculent material was dissolved in 0.2 cc. of water. Dropwise addition of dioxane precipitated an oil which solidified to a microcrystalline product on scratching (24 mg., m.p. 165–168°, dec.). Since attempts to recrystallize it in the same manner or from water–ethanol yielded only small amounts of crystalline material, the solvents were removed, and the partially crystalline residue analyzed after drying to constant weight at 100° (2 mm.) (6 hours, 6.2% weight loss).

Anal. Calcd. for $C_9H_{17}O_7N$ (251.2): C, 43.03; H, 6.82; N, 5.58. Found: C, 43.74; H, 6.94; N, 5.59.

The ninhydrin reaction was negative: under the same conditions O-methylthreonine yielded the typical violet-blue color, while β-alanine failed to react.

A 4.25 mg. sample of the specimen m.p. 154–156°, ammonium thiocyanate (1.60 mg., 1.2 molar equivalents) and acetic anhydride–acetic acid (9:1, 0.2 cc.) were heated together at 100° for 20 minutes. O-Methyl-D(−)-threonine (4.84 mg.) and β-alanine (4.15 mg.) were similarly treated with 1.2 molar equivalents of ammonium thiocyanate in 0.2 cc. of the solvent mixture. After dilution of the samples with ether to 5.0 cc. their ultraviolet absorption spectra were determined. The curves showed the following characteristics: (ε based on molecular weight of substance treated): Trimethoxyaminoadipic acid: No discernible maxima or shoulders, but only extensive end-absorption (ε at 240 mμ 10,500, ε at 280 mε 1800). O-Methylthreonine: maxima at 235 mμ (ε 11,200) and 267 mμ (ε 13,700). β-Alanine: maximum at 265 mμ (ε 5800), minimum at 250 mμ (ε 4500).

The barium salt formed during the hydrolysis in Experiment A consisted of fairly large prisms which were slightly soluble in cold water, imparting on it alkaline reaction. The analysis of a sample dried to constant weight at 150°/2 mm. (weight loss 2.13%) indicated the composition $C_2H_2O_6Ba_2$, which is that of a basic barium oxalate $Ba(COO)_2 \cdot Ba(OH)_2$. However, to the best of our knowledge there is no record of such a salt in the literature. When a corresponding amount of oxalic acid was subjected to the conditions used in the hydrolysis experiment, barium oxalate (Ba, calcd., 60.9; found, 61.5) was obtained in form of a fine precipitate, quite dissimilar to the crystalline salt in question.

Anal. Calcd. for $C_2H_2O_6Ba_2$ (396): C, 5.91; H, 0.50; Ba, 69.2. Found: C, 5.82; H, 0.64; Ba, 68.2.

The crystalline precipitate obtained in Experiment B (70 mg.) was triturated with 10% acetic acid (4 cc.) till the evolution of carbon dioxide had ceased. The insoluble residue (18 mg.) was dried for analysis to constant weight at 110° (2 mm.) (weight loss 3.87%). The analysis (C, 8.14; H, 1.51; Ba, 57.3), though unsatisfactory, suggested again that oxalic acid was involved (calcd. for $Ba(COO)_2 \cdot H_2O$: C, 9.87; H, 0.82; Ba, 56.4).

1-Acetyl-5-(1-methoxyethyl)-2-thiohydantoin.—O-Methyl-D-(−)-threonine[17] (70 mg.) and an equal amount of ammonium thiocyanate was dissolved at room temperature (occasional shaking) in 1 cc. of a 9:1 mixture of acetic anhydride and acetic acid. After 45 minutes the solution was placed into the refrigerator overnight, whereupon ice-water (6 cc.) was added gradually in small portions. The crystals formed on further standing at 4° were collected after 24 hours (19 mg.). The melting point (152–163° after sintering at 139°), was not materially improved by recrystallization from aqueous ethanol. (Inhomogeneity was to be expected in this case, since thiohydantoin formation from optically active amino acids is invariably associated with partial or total racemization at the α-carbon atom; when, as in threonine, a second assymetric center is present, a mixture of two optically active thiohydantoins necessarily results.) The recrystallized preparation after drying for 3 hours at 100° (2 mm.) (7.1% weight loss) melted at 152–160° after sintering at 148°. The ultraviolet absorption curve exhibited the typical maxima at 234 mμ (ε 12,300) and 280 mμ (ε 18,200).

Anal. Calcd. for $C_8H_{12}O_3N_2S$: N, 12.96; S, 14.82. Found: N, 12.87; S, 15.22.

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