1-Methyl-3,5-di-(*p*-methylbenzyl)-4-pyridone from 3,5-Di-(*p*-methylbenzyl)-4H-pyran-4-one.[10-12]—A mixture of 0.5 g. of 3,5-di-(*p*-methylbenzyl)-4H-pyran-4-one and 0.5 g. of methylamine in 6 ml. of absolute methanol was heated in a sealed tube at 160° for 14 hours. The reaction mixture was filtered, yielding 0.5 g. (97%) of colorless needles, m.p. 229.5–233°, identical with 1-methyl-3,5-di-(*p*-methylbenzyl)-4-pyridone, m.p. 231–234°.[1] The melting point was improved by recrystallizing each sample from ethanol as colorless needles, m.p. 236–237°.

Anal. Calcd. for $C_{29}H_{23}NO$: C, 83.23; H, 7.30; N, 4.41. Found: C, 83.22; H, 7.38; N, 4.21.

Substituted 3,5-Dibenzylidenetetrahydro-4H-1-thiapyran-4-ones (III).—Condensation of various aldehydes with tetrahydro-4H-1-thiapyran-4-one[23-25] was carried out more favorably with piperidine acetate in ethanol, as in the general method for the oxygen analogs, than with aqueous ethanolic alkali, which had been used previously.[14] The results are given in Table III.

Substituted 3,5-Dibenzylidenetetrahydro-4H-1-thiapyran-4-one 1,1-Dioxides (IV).—The sulfones IV were made from the corresponding sulfides III in the usual manner. A mixture of 2 g. of the substituted 4H-1-thiapyran-4-one (III), 20 ml. of glacial acetic acid and 3 ml. of 30% hydrogen peroxide was heated under reflux for 10 minutes. The product separated on cooling. The sulfone derivatives are listed in Table IV.

Reaction of 3,5-Dibenzylidenetetrahydro-4H-1-thiapyran-4-one 1,1-Dioxide with Hydrogen Bromide in Acetic Acid.—To a solution of 20 ml. of glacial acetic acid saturated with hydrogen bromide at 25° and containing 0.1 g. of benzoyl peroxide was added 0.5 g. of 3,5-dibenzylidenetetrahydro-4H-1-thiapyran-4-one 1,1-dioxide. The orange-colored solution was maintained at 60° for 22 hours, after which time it had become dark red. Dilution to 100 ml. with water and refrigeration caused the separation of a colorless solid, m.p. 219–220°. Recrystallization from acetic acid gave colorless needles, m.p. 220–220.5°, which contained bromine.

Anal. Calcd. for $C_{15}H_{18}Br_2O_3S$: C, 46.03; H, 3.72. Found: C, 47.11; H, 4.11.

The infrared spectrum (mull) indicated the presence of unconjugated carbonyl (1725 cm.$^{-1}$) along with maxima typical of phenyl and sulfone groupings. The product could not be dehydrobrominated readily. Neither possible structure, 3,5-di-(α-bromobenzyl)-tetrahydro-4H-1-thiapyran-4-one 1,1-dioxide or 3,5-dibenzyl-3,5-dibromotetrahydro-4H-1-thiapyran-4-one 1,1-dioxide, has been rigorously excluded for this product.

URBANA, ILLINOIS

(23) E. A. Fehnel and M. Carmack, THIS JOURNAL, **70**, 1813 (1948).

(24) L. L. Gershbein and C. D. Hurd, *ibid.*, **69**, 241 (1947).

(25) H. M. E. Cardwell, *J. Chem. Soc.*, 715 (1949).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Synthesis of Inosamine and Inosadiamine Derivatives from Inositol Bromohydrins[1]

BY M. L. WOLFROM, JACK RADELL,[2] R. M. HUSBAND[2] AND G. E. MCCASLAND

RECEIVED JULY 13, 1956

The m.p. 240° diastereoisomer of 6-bromoquercitol pentaacetate reacted with ammonia in hot dioxane to give small yields of two diastereoisomeric inosamines, isolated as the hexaacetyl derivatives, m.p. 157° and 280°. The former diastereoisomer appears to be new, and the latter was found to be identical with the *meso*-(1,3,5) ("SB" or "III") inosamine derivative whose preparations from *meso*-(1,3,5)- or "*scyllo*"-inosose, and from 6-deoxy-6-nitro-D-glucose, had previously been reported. Two isomers (m.p. 225° and 130°) of dibromocyclohexanetetrol tetraacetate on similar amination gave two hexaacetylstreptamine diastereoisomers of m.p. 303° and 173°, possibly but not certainly identical with similar isomers previously prepared in other laboratories by different procedures and isolation techniques.

During the last decade much interest has been shown in the diaminocyclohexanetetrols or inosadiamines,[3] and their simpler monoamino analogs, the inosamines. The original stimulus to this interest was the establishment of the diastereoisomer XVI as a component of the antibiotic streptomycin.[4-7] The natural substance XVI is designated streptamine.[5,6] In this communication the words *o*-inosadiamine, *m*-inosadiamine and *p*-inosadiamine will be employed as generic names for the various diastereoisomers of 5,6-, 4,6- and 3,6-diaminocyclohexanetetrol, respectively. The inosadiamine and inosamine ring numbering here used (Figs. 1 and 2) is a departure from the previous usage found in most articles on streptomycin derivatives, but is in conformity with *Chemical Abstracts* practice, which regards the hydroxyl group as the principal function in any amino alcohol. The term inosamine, proposed by Carter and co-workers,[8] has been widely adopted as a generic name for the 20 predictable diastereoisomers of 6-amino-1,2,3,4,5-cyclohexanepentol. Six of these diastereoisomers have been reported previously,[8-13b] and one additional is now recorded herein (VII, Table I). The inosamines are stable, colorless, crystalline solids with poor melting points, and are best characterized as the hexaacetyl derivatives. Even these are best further delineated by their X-ray powder diffraction lines and such data are recorded herein for the substances with which this Laboratory was concerned.

(1) One of a series of articles on streptomycin chemistry by M. L. Wolfrom and co-workers; paper IX on Cyclitols by G. E. McCasland and co-workers, previous communication G. E. McCasland and J. M. Reeves, THIS JOURNAL, **77**, 1812 (1955).

(2) Bristol Laboratories Research Fellow (J. R.) and Research Associate of The Ohio State University Research Foundation (Project 224).

(3) A term suggested by a referee of this communication.

(4) R. U. Lemieux and M. L. Wolfrom, *Advances in Carbohydrate Chem.*, **3**, 337 (1948).

(5) H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, J. S. Meek, P. S. Skell, W. A. Strong, J. T. Albieri, Q. R. Bartz, S. B. Binkley, H. M. Crooks, Jr., I. R. Hooper and Mildred C. Rebstock, *Science*, **103**, 53 (1946).

(6) J. Fried, G. A. Boyack and O. Wintersteiner, *J. Biol. Chem.*, **162**, 391 (1946).

(7) (a) R. L. Peck, C. E. Hoffhine, Jr., Elizabeth W. Peel, R. P. Graber, F. W. Holly, R. Mozingo and K. Folkers, THIS JOURNAL, **68**, 776 (1946); (b) M. L. Wolfrom, S. M. Olin and W. J. Polglase, *ibid.*, **72**, 1724 (1950).

(8) H. E. Carter, R. K. Clark, Betty Lytle and G. E. McCasland, *J. Biol. Chem.*, **175**, 683 (1948).

(9) J. M. Grosheintz and H. O. L. Fischer, THIS JOURNAL, **70**, 1476 (1948).

(10) B. Iselin and H. O. L. Fischer, *ibid.*, **70**, 3946 (1948).

(11) T. Posternak, *Helv. Chim. Acta*, **38**, 1597 (1950).

(12) L. Anderson and H. A. Lardy, THIS JOURNAL, **72**, 3141 (1950).

(13) (a) O. Wintersteiner and Anna Klingsberg, *ibid.*, **73**, 2917 (1951); (b) J. B. Patrick, R. P. Williams, C. W. Waller and B. L. Hutchings, *ibid.*, **78**, 2652 (1956).

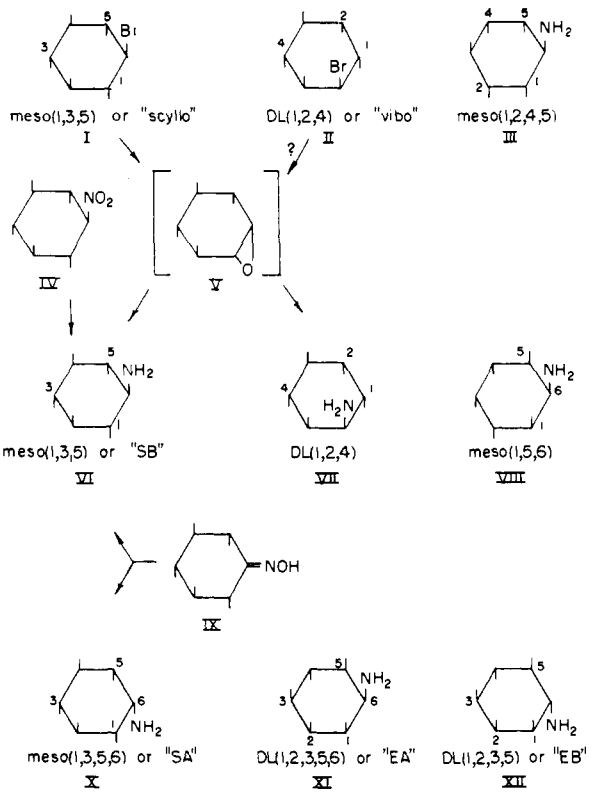

Fig. 1.—Known inosamines, probable configurations and synthesis.

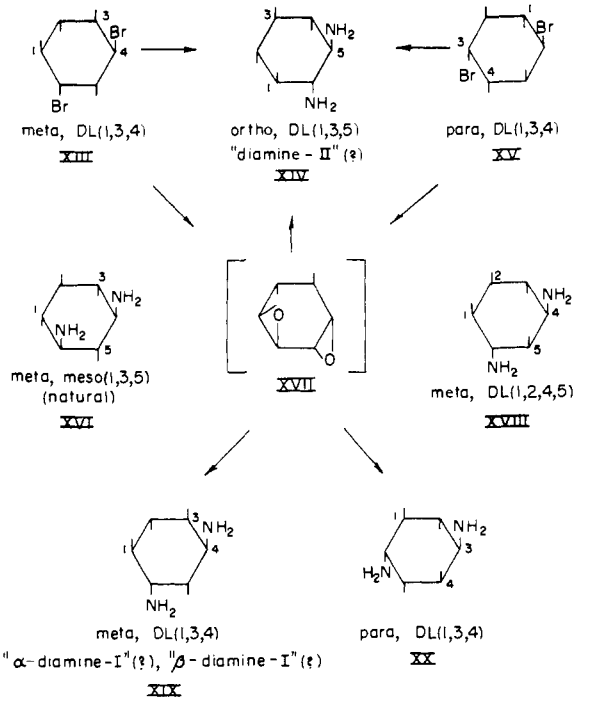

Fig. 2.—Known inosadiamines, probable configurations and synthesis.

Carter and co-workers[8] designated their various inosamine stereoisomers by trivial suffixes, such as "SA," "SB" and "EA" ("S" for *scyllo*, "E" for *epi*). Grosheintz and Fischer[9] used suffixes such as "I," "II" and "III" (not to be confused with the formula numbers in our present article). It was later shown that[11] diastereoisomer "III" is the same as "SB," and that "I" is a mixture consisting partly of "SB." Since most of the configurations are now known, we consider it more informative and convenient to designate each stereoisomer by a systematic numerical prefix,[14] such as *meso*-(1,3,5) for isomer VI (Fig. 1). Here the parenthesized numerals specify the groups which are *cis* to the lowest-numbered group (position 1); the remaining groups will be *trans*. Alternative systems have been proposed.[15]

Methods previously used for inosamine preparation include the partial deamination of natural streptamine,[13b] the reduction of inosose oximes or phenylhydrazones,[8,11,12] and the cyclization and reduction of 6-deoxy-6-nitroaldohexoses.[9] In 1907, Müller[16] found that 6-bromoquercitol pentaacetate (m.p. 240° diastereoisomer) was converted by hot alcoholic ammonia to water-soluble products, presumably inosamines, but none of the products was actually isolated. So far as we are aware, the first successful conversion of bromoquercitols[1,16–22] to inosamines is that now reported.

TABLE I

KNOWN INOSAMINES

Probable formula (Fig. 1)	Hexaacetyl derivatives, m.p., °C.	Source	Reference
VII (?)	157	DL-(1,2,4)-Bromoquercitol pentaacetate	This work
XI	162, 186, 194[a]	*epi*-Inosose, streptamine	8, 13a
III[b]	215, 234[a]	6-Deoxy-6-nitro-D-glucose	9
XII	239	*epi*-Inosose	8
X	247, 261[a]	*scyllo*-Inosose	8
VIII	277.5–278.5	"Antibiotic 1703-18B"	13b
VI[c]	284, 301[a]	*scyllo*-Inosose, 6-deoxy-6-nitro-D-glucose, *meso*-(1,3,5)-bromoquercitol pentaacetate	8, 9, this work

[a] Polymorphs. [b] Designated "II" by Grosheintz and Fischer, ref. 9. [c] Designated "III" by Grosheintz and Fischer, ref. 9.

After trying without success many of the usual amination reagents and procedures, we finally obtained useful results by heating acetylated bromohydrins with a dioxane solution of ammonia in sealed tubes. By such treatment of crude 6-bromoquercitol pentaacetate (m.p. 240° diastereoisomer) we were able (after reacetylation) to isolate two diastereoisomers of hexaacetylinosamine. Since this work was done (1950–1952), McCasland and

(14) G. E. McCasland, "Proposed Rules for Naming Stereoisomers," Report of the Advisory Committee on Configurational Nomenclature (July 14, 1953) of the Nomenclature Committee, Division of Organic Chemistry, American Chemical Society; available from Chemical Abstracts Service, The Ohio State University, Columbus 10, Ohio.

(15) H. G. Fletcher, Jr., L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951); S. J. Angyal and C. G. Macdonald, *J. Chem. Soc.*, 686 (1952).

(16) H. Müller, *ibid.*, **91**, 1780 (1907).

(17) K. Kubler, *Arch. Pharm.*, **246**, 620 (1908).

(18) E. G. Griffin and J. M. Nelson, THIS JOURNAL, **37**, 1552 (1915).

(19) A. E. O. Menzel, M. Moore and O. Wintersteiner, *ibid.*, **71**, 1268 (1949).

(20) E. H. Flynn, Dissertation (with H. E. Carter), University of Illinois, 1949.

(21) G. E. McCasland and E. C. Horswill, THIS JOURNAL, **75**, 4020 (1953).

(22) G. E. McCasland and E. C. Horswill, *ibid.*, **76**, 2373 (1954).

Horswill[21] have studied further this preparation of 6-bromoquercitol pentaacetate. It is now evident that our bromoquercitol (I) contained extensive dibromo contaminant, which in all probability was not the precursor of the products isolated; it may also have been contaminated with its diastereoisomer II, which, however, would presumably form the same products, VI and VII (Fig. 1).

The first hexaacetylinosamine derivative, m.p. 157°, clearly does not correspond to any previously known diastereoisomer (Table I). The second was similar in properties to the previously reported *meso*-(1,3,5) diastereoisomer, and a comparison of the X-ray powder diffraction patterns proved that the two compounds were in fact identical. This diastereoisomer had previously been prepared from the inosose oxime IX by Carter, Clark, Lytle and McCasland,[8] who designated it "inosamine-SB," and from 6-deoxy-6-nitro-D-glucose (or -idose) by way of the nitroquercitol IV by Grosheintz and Fischer,[9] who termed it "aminodesoxyinositol-III."

It is probable that the first step in the amination reaction is a rapid deacetylation of the pentaacetate initial material, to give the 6-bromoquercitol, which has previously been shown[21] to have the *meso*-(1,3,5) configuration I. Since the bromo group here has only *trans* neighboring groups, the normal reaction course would lead to the epoxide V, which on subsequent nucleophilic attack by ammonia would produce either diastereoisomer VI or VII of inosamine. Our experimental findings reveal that the *meso*-(1,3,5) product VI actually is formed, although the isolated yield was quite small. There is a good probability that the remaining product has the predicted DL-(1,2,4) configuration VII, but no definite evidence for this has yet been obtained.[22a]

Various isomers (Table II) of *o*-, *m*- and *p*-inosadiamine have been reported. The preparative methods previously employed had included the degradation of streptomycin,[5] the cyclization and reduction of 6-deoxy-6-nitro-D-glucosamine,[7b] the amination of certain dibromohydrins[1,16-22] derived from *myo*-inositol,[19-20] and the reduction of an inososamine[23a] oxime derived from *myo*-inositol.[23b] The inosadiamines resemble inosamines in their properties and are best characterized by their hexaacetyl derivatives.

Müller,[16] in 1907, found that *m*-dibromocyclohexanetetrol[24] tetraacetate (m.p. 130° diastereoisomer) reacts with alcoholic ammonia, liberating ionic bromide, but he did not isolate any product. Griffin and Nelson,[18] in 1915, treated this same isomer with anhydrous liquid ammonia. In their opinion, deacetylation but no amination took place under these conditions. Apparently neither investigator attempted to aminate the *para* isomer.

In 1949, both Carter[20] and Wintersteiner[19] and their respective co-workers treated the unsubstituted *m*-dibromotetrol (m.p. 216°) with hot aqueous ammonia. Carter and Flynn actually isolated only one product, a hexaacetylstreptamine isomer of m.p. 293° (Table II). Wintersteiner and co-workers isolated two products which they designated "β-diamine-I" and "diamine-II"—apparently neither was identical with Carter's product.

In addition, Wintersteiner and associates aminated the unsubstituted *p*-dibromotetrol (m.p. 190°), obtaining the *same* "diamine-II," and also a product called "α-diamine-I." From the descriptions published, it appears to us that "α-diamine-I" and "β-diamine-I" are identical, except for degree of purity; however, Wintersteiner and co-workers suggest that these isomers are probably, although not certainly, different.

In common with previous authors, we have found that many of the usual amination procedures are unsuitable for preparing inosadiamines. However, by treating the *tetraacetates* of the above dibromotetrols with dry ammonia in hot *dioxane*, we have successfully obtained hexaacetylinosadiamine products of m.p. 173° (from *meta*) and 303° (from *para*). These two isomers are clearly not identical with "natural" hexaacetylstreptamine (XVI), nor with the *meta* isomer, XVIII, of Wolfrom, Olin and Polglase[7b] (Table II).

Our 303° product is quite possibly identical with Carter and Flynn's isomer of similar (293°) melting point. Our 173° product could possibly represent the crystalline form of Wintersteiner's amorphous hexaacetyl derivative of "α-diamine-I" or "β-diamine-I," or it could conceivably be the unknown hexaacetyl derivative of Wintersteiner's "diamine-II."

Unfortunately, despite full coöperation of the previous authors, it has not been possible to correlate our products with theirs, since only extremely minute samples, or none at all, are now available; and those samples which were examined were amorphous (as previously reported), and thus not readily identified by the usual X-ray or melting point techniques.

It is not easy to predict the structures resulting from these dibromohydrin aminations, since the inosadiamine product does not necessarily retain the *ortho*, *meta* or *para* structure of its dibromo precursor. However, it should be noted that one of the isomers in Table II may be assigned the ortho structure on the basis of a normal three-mole periodate uptake for its *N*,*N*'-diacetyl derivative. Three other isomers may be assigned the *meta* structure because of a normal two-mole periodate uptake. The *meta* structure of the 355° isomer is proved by its method of preparation.

Although one might expect that *p*-*N*,*N*'-diacetylinosadiamines also would show a normal two-mole periodate uptake, it is known that inositols with two *para* protective groups tend to show anomalous high uptake ("overoxidation").[22,25] Such anoma-

(22a) ADDED IN PROOF.—Four new diastereoisomers of inosamine have been reported by L. Anderson, *Abstracts Papers Am. Chem. Soc.*, **130**, 27D (1956, Sept.). One of these, which he designates "L-inosamine-2," is perhaps an active form of our new racemic product (hexaacetyl m.p. 157°), but no definite correlation has yet been established.

(23) (a) A term proposed for an amino-deoxy-inosose; compare glucose and glucosamine; (b) K. Heyns and H. Paulsen, *Chem. Ber.*, **89**, 1152 (1956).

(24) In the literature up to 1953 the *para*-dibromo isomer mentioned here was designated "α," and the *m*-dibromo isomer mentioned here, "β."

(25) C. F. Huebner, S. Ames and E. Bubl, THIS JOURNAL, **68**, 1621 (1946).

TABLE II

KNOWN INOSADIAMINES

Probable formula (Fig. 2)	Hexa-acetyl derivative, m.p., °C.	Hexa-benzoyl derivative, m.p., °C.	*N,N'*-Diacetyl derivative M.p., °C.	*N,N'*-Diacetyl derivative IO_4^- uptake, moles/mole	Source	Reference
XIX (?)	146	255	213–215	2	*p*-Dibromocyclohexanetetrol	19
XIX (?)	149	263	208–245 d.	2	*m*-Dibromocyclohexanetetrol	19
(?)	173				*m*-Dibromocyclohexanetetrol tetraacetate	This work
XX (?)	293		305–307 d.	Anomalous	*m*-Dibromocyclohexanetetrol	20
(?)	303				*p*-Dibromocyclohexanetetrol tetraacetate	This work
XVI	345	351	283	2	Streptomycin, 6-deoxy-6-nitro-D-glucosamine, *myo*-inositol	5, 7b, 23b
XVIII	355				6-Deoxy-6-nitro-D-glucoasmine	7b
XIV (?)			269	3	*m*- and *p*-Dibromocyclohexanetetrol	19

lous uptake was actually observed by Carter and Flynn[20] for the *N,N'*-diacetyl derivative of their isomer (hexa-acetyl derivative, m.p. 293°), which thus would appear to have the *para* structure.

The characterization of inosadiamines is further complicated by the numerous diastereoisomers possible for each structure (20 for *ortho*, 20 for *meta*, 14 for *para*). McCasland and Horswill[22] have pointed out that both the *m*- and *p*-dibromotetrols here mentioned would on amination probably form the *same* diepoxide intermediate XVII which would tend to form three principal isomers, XIV, XIX and XX, with the structures and configurations indicated (Fig. 2). While this explanation seems to be in accord with the available experimental facts, most of the configurational assignments (Table II) must still be regarded as tentative.

In the course of our experimental work, two acetylated derivatives containing 8–9% nitrogen were encountered and were not identified. Among possible structures for these substances are: (1) a tetraacetylinosadiamine, (2) an acetylated inosadiamine bisoxazoline, (3) a diaminocyclohexenediol derivative.

In the hope of obtaining a new type of derivative suitable for characterizing inosadiamines, we prepared the *N,N'*-bis-(2,4-dinitrophenyl) derivative of natural streptamine. A bright yellow crystalline product was easily obtained, but was found to have inconvenient solubility and melting point characteristics.

Acknowledgment.—The help derived from the preliminary experiments of Dr. Hubert M. Hill in this Laboratory is gratefully acknowledged. We wish to thank Professor P. M. Harris and Dr. A. Thompson for assistance with X-ray diffraction patterns. Drs. G. Allen, J. Patrick and J. Brockman of the Lederle Laboratories very kindly provided unpublished information regarding a new inosamine isomer isolated by J. Patrick and coworkers.[13b] We are obliged to Dr. H. E. Carter and Dr. O. Wintersteiner, and their respective co-workers, for information and samples.

Experimental

Preparation of Bromoquercitols and Dibromocyclohexanetetrols.—A mixture of 50 g. of dry *myo*-inositol and 100 g. of acetyl bromide was heated in a sealed tube at 130° for 20 hr. The contents of the cooled, opened tube was distilled under reduced pressure and to the residue was added 250 ml. of acetic anhydride and 25 g. of anhydrous sodium acetate. After 1 hr. at reflux temperature, the mixture was cooled and poured onto 250 g. of chopped ice. The precipitate was fractionally crystallized from 95% ethanol (decolorizing carbon).

Fraction A consisted of 15 g. (11%) of the *p*-dibromocyclohexanetetrol tetraacetate, colorless crystals, m.p.[26] 218–220° (reported[22] 225°). This *para* diastereoisomer has previously been assigned[22] the DL-(1,3,4) configuration XV.

Anal. Calcd. for $C_{14}H_{18}Br_2O_8$: C, 35.5; H, 3.83. Found: C, 36.0; H, 3.74.

Fraction B consisted of 5 g. (4%) of the *m*-dibromocyclohexanetetrol tetraacetate, colorless crystals, m.p. 120–121° (reported[22] 130°). This meta diastereoisomer has previously been assigned[22] the DL-(1,3,4) configuration XIII.

Anal. Calcd. for $C_{14}H_{18}Br_2O_8$: C, 35.5; H, 3.83. Found: C, 35.5; H, 3.73.

Fraction C consisted of 4 g. (3%; total yield of fractions A, B, C, 18%) of the bromoquercitol pentaacetate of m.p. 230–233° (reported[19] 240–241°). This diastereoisomer has previously been assigned the "*scyllo*-A" or *meso*-(1,3,5) configuration, I.

An amount of 1.33 g. of the above *p*-dibromo tetraacetate (m.p. 218–220°) was further purified by passing it in 5% benzene solution over a 208 × 45 (diam.) mm. column of Magnesol[27]–Celite[28] (5:1 by wt.), prewet with 40 ml. of benzene. After development with 300 ml. of benzene–ethanol (abs.) (100:1 by vol.) and extrusion, a permanganate streak[29] showed a main zone midway on the column, the contents of which were eluted with acetone; yield 1.00 g. Pure material was obtained on recrystallization from benzene; m.p. 224–225°.

N-Acetylpenta-*O*-acetyl-6-aminocyclohexanepentol (*N*-Acetylpenta-*O*-acetylinosamine), *meso*-(1,3,5) Diastereoisomer (M.p. 280°).—The starting material used was a partially purified mixture[30] containing 60–70% of 6-bromoquercitol pentaacetate (m.p. 240° diastereoisomer) and 30–40% of *p*-dibromocyclohexanetetrol tetraacetate. A 3.8-g. portion of this mixture was dissolved in 50 ml. of dioxane and the solution sealed into a Pyrex tube at −80° with 12 ml. of anhydrous liquid ammonia. After 13 hr. at 75–80°, the contents of the chilled, opened (*caution*) tube were distilled under reduced pressure, and to the residue was added 50 ml. of acetic anhydride and 3 g. of anhydrous sodium acetate. After 25 min. under reflux, the cooled mixture was poured onto 100 g. of chopped ice, and the precipitate (1.2 g., m.p. 219–221°) of unreacted *p*-dibromo impurity was removed by filtration.

Anal. Calcd. for $C_{14}H_{18}Br_2O_8$: C, 35.5; H, 3.69. Found: C, 35.4; H, 3.69.

The aqueous acetic acid filtrate was adjusted with sodium bicarbonate to *p*H 6, and extracted with four 50-ml. portions of chloroform. The combined extract, after concentration to 35 ml., was dried and passed through a 225 × 45 mm.

(26) All melting points are corrected; Fisher–Johns apparatus.

(27) A synthetic magnesium silicate produced by the Westvaco Chemical Division of the Food Machinery and Chemical Corp., South Charleston, W. Va.

(28) A siliceous filter-aid produced by the Johns-Manville Co., New York, N. Y.

(29) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(30) *Cf.* ref. 21.

(diam.) column of Magnesol–Celite (5:1), prewet with 100 ml. of chloroform. After development with chloroform (850 ml.) and extrusion, four zones were detected by permanganate streaking.

The *N*-acetylpenta-*O*-acetylinosamine eluted by acetone from the top zone (30–49 mm. down) was recrystallized from ethyl acetate; yield 9 mg., m.p. 275–280°.

Anal. Calcd. for $C_{18}H_{25}NO_{11}$: N, 3.25. Found: N, 3.20.

The X-ray powder diffraction lines on the above crystals were obtained and found to be identical with those given by a sample[31] of "hexaacetylinosamine-SB" prepared from inosose oxime. The principal interplanar spacings were as follows: 9.61[32]-1.0,[33] 5.90-0.8, 4.80-0.8, 4.53-0.4, 4.09-0.6, 3.93-0.2, 3.62-0.4, 3.51-0.4, 3.26-0.4, 3.19-0.4, 3.07-0.4, 2.95-0.2, 2.80-0.2.

N-Acetylpenta-O-acetyl-6-aminocyclohexanepentol (N-Acetylpenta-O-acetylinosamine), Diastereoisomer of M.p. 157°.—The above ethyl acetate mother liquor, on concentration under reduced pressure, gave crystals of a second diastereoisomer of *N*-acetylpenta-*O*-acetylinosamine; yield 122 mg., m.p. 155–157° (softening at 145°); X-ray powder diffraction data: 7.71-1.0, 6.37-0.7, 5.65-0.6, 5.08-0.5, 4.66-0.8, 4.08-0.8, 3.89-0.5, 3.66-0.8, 3.40-0.6, 3.26-0.1, 3.09-0.1, 2.84-0.2, 2.71-0.1, 2.45-0.2, 2.35-0.2, 2.24-0.1.

Anal. Calcd. for $C_{18}H_{25}NO_{11}$: C, 50.11; H, 5.84; N, 3.25. Found: C, 50.43; H, 5.79; N, 3.18.

Di-N-acetyltetra-O-acetyldiaminocyclohexanetetrol Isomer of M.p. 303° (Diastereoisomer of *o*-, *m*- or *p*-Inosadiamine).—Five grams of the above *p*-dibromotetrol tetraacetate (m.p. 218–220°) in 40 ml. of dry dioxane was sealed in a Pyrex tube at −80° with 15 ml. of liquid anhydrous ammonia, and the tube was heated for 68 hr. at 50–53°. The contents of the chilled, opened (*caution*) tube, on distillation under reduced pressure, left a sirup to which was added 60 ml. of acetic anhydride and 5 g. of anhydrous sodium acetate. After 1 hr. under reflux the mixture was cooled and poured onto 200 g. of crushed ice. The precipitate of unreacted starting material was removed by filtration; yield 1.6 g. The material was recrystallized from 95% ethanol; m.p. 218–220°.

The aqueous acetic acid filtrate was adjusted to *p*H 6, and extracted with chloroform. The extract, after concentration to 25 ml., was passed through a 261 × 79 mm. (diam.) column of Magnesol–Celite (5:1), prewet with 100 ml. of chloroform. After development with chloroform (4000 ml.), the extruded and permanganate-streaked column showed only one zone, about one-third of the way down. The material eluted from this zone with acetone was recrystallized from 4 ml. of ethyl acetate. Three crops of crystals were collected. The first and second crops were combined, and again recrystallized from ethyl acetate; yield 270 mg., m.p. (constant), 156–158°.

Anal. Found: N, 8.08.

The third crop was again recrystallized from ethyl acetate; yield 30 mg., m.p. 298–303° with a transition at 240–255° from large crystals to fine needles; X-ray powder diffraction data: 9.09-0.9, 7.47-1.0, 6.66-0.3, 5.95-0.4, 5.22-0.4, 4.72-0.8, 4.23-0.9, 3.82-0.8, 3.56-0.8, 3.31-0.7, 2.98-0.8, 2.81-0.4, 2.57-0.4, 2.49-0.4, 2.33-0.5, 2.20-0.8, 2.13-0.3, 2.06-0.5, 1.98-0.3, 1.89-0.4, 1.83-0.1, 1.78-0.1, 1.72-0.1, 1.68-0.1. This product was a diastereoisomer of di-*N*-acetyltetra-*O*-acetylinosadiamine.

Anal. Calcd. for $C_{18}H_{26}N_2O_{10}$: C, 50.24; H, 6.09; N, 6.51. Found: C, 49.95; H, 5.90; N, 6.48.

Di-N-acetyltetra-O-acetyldiaminocyclohexanetetrol Isomer of M.p. 173° (Diastereoisomer of *o*-, *m*- or *p*-Inosadiamine).—A 5-g. portion of the above *m*-dibromo tetraacetate was aminated as above, except that heating was maintained for 60 hr. at 75°. Only a trace of starting material was precipitated after acetylation and dilution. The chloroform extract was concentrated to 100 ml. and chromatographed as above, employing 350 ml. of chloroform as the developer. The chromatogram showed four zones.

The eluate from the main zone (second from the column top) was recrystallized from ethyl acetate; yield 134 mg., m.p. 170–173° with softening at 167°; X-ray powder diffraction data: 8.80-0.2, 7.18-0.7, 6.28-1.0, 4.95-0.4, 4.27-1.0, 3.87-0.9, 3.51-0.4, 3.24-0.1, 3.00-0.3, 2.80-0.1, 2.61-0.2, 2.37-0.2, 2.24-0.2, 2.19-0.9, 2.14-0.1, 2.03-0.1. The substance was a diastereoisomer of di-*N*-acetyltetra-*O*-acetylinosadiamine.

Anal. Calcd. for $C_{18}H_{26}N_2O_{10}$: C, 50.24; H, 6.09; N, 6.51. Found: C, 50.49; H, 6.13; N, 6.37.

The eluates from the third and fourth zones from the column top in the above chromatogram gave positive halogen and negative nitrogen tests and were not further investigated.

The eluate from the top zone gave a sirup that was crystallized from butanone; yield 60 mg., m.p. 168–170° with sintering at 150°.

Anal. Found: N, 8.71.

***N,N'*-Bis-(2,4-dinitrophenyl)-streptamine.**—A mixture of 1.38 g. of finely powdered streptamine sulfate[5] with 1.0 g. of sodium bicarbonate and 25 ml. of water was heated to boiling (effervescence), and the clear solution cooled to 25° (*p*H 9–10). A second portion (1.52 g.) of sodium bicarbonate was added. A 2.79-g. amount of 2,4-dinitrofluorobenzene (m.p. 25–27°) was dissolved in 50 ml. of absolute ethanol at 30–40° (fume hood). The clear yellow solution was added to the above streptamine solution and the mixture was stirred overnight. The yellow crystals which had separated were collected and washed successively with absolute alcohol, water and again with absolute alcohol (2 × 10 ml. of each); yield 2.50 g. (98%), m.p. 300–310° dec. (Köfler stage).

The bis-(dinitrophenyl) derivative was insoluble in *N* hydrochloric acid and in nearly all organic solvents. It was recrystallized from 40 parts of nitrobenzene by solution at 190°. The bright yellow crystals which separated on cooling were washed with nitrobenzene (5 ml.) and then with four 5-ml. portions of absolute ethanol; yield 0.45 g., m.p. 315–317° dec.

Anal. Calcd. for $C_{18}H_{18}N_6O_{12}$: C, 42.36; H, 3.55. Found: C, 42.68; H, 3.79.

COLUMBUS 10, OHIO

(31) Prepared in 1948 by Prof. H. E. Carter and co-workers of the University of Illinois, Urbana, Ill.

(32) CuK_α radiation, Å.

(33) Relative intensity, estimated visually; 1.0 the strongest.