[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Stereochemical Studies in the Aminodesoxyinositol Series. *meso*-Inosamine-2 and *scyllo*-Inosamine[1]

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The first representative of a class of compounds which we may call the *aminodesoxyinositols* was encountered three years ago in streptamine,[2] the product of complete hydrolysis of the streptidine moiety of streptomycin. The interest aroused by this discovery is largely responsible for the several studies which have since been carried out in this series. The simplest members of the class have but one amino group, and it seemed likely that chemical study of the monoamines might provide methods by which stereochemical facts could be deduced regarding the more complex diamines such as streptamine.

The present communication is a report of work done on the epimeric monoamines derived from *scyllo-meso*-inosose. This work includes the synthesis of the two amines by sterically selective methods, the preparation of useful new derivatives of these amines and of streptamine, and a study of the acyl migration behavior of the three O-acetyl derivatives. The evidence obtained by synthesis permitted a decision as to the respective configurations of the amino groups in the two "inosamines," making them the first of the six known[3,4] monoaminodesoxyinositols to have complete configurational assignments. The formula (I) shown for streptamine is that proposed by Wolfrom and Olin.[5] Although this investigation was begun independently, it was greatly facilitated by the availability of information regarding the properties of the two epimeric monoamines, which were characterized by Carter and co-workers[3] in 1948.

Nomenclature.—Several systems for the numbering and nomenclature of the inositols and their derivatives are in use.[6] The term *inosose* has been widely accepted as a generic name for the keto compounds, and the authors have adopted in the present paper the recent suggestion[6h] that the aminoinositols be called *inosamines*. A systematic nomenclature for the individual inosamines should be developed along with our knowledge of the stereochemistry of these substances. Since they are amino analogs of the insositols, it seems simplest to designate them. in accordance with recognized practice in the sugar series, by the prefixes used to identify the parent inositols. In six of the eight basic inositol configurations, the different ring positions are not all sterically equivalent, and a numbering system must therefore be employed for distinguishing between isomeric inosamines which share the same configuration but have their amino groups in different positions.

The parent inositols have a monocyclic structure in which all the functions are of equal rank, hence no particular numbering is imposed by the structure itself. Any method of numbering the compounds of this series is accordingly somewhat arbitrary, and it would be highly desirable for some official body to publish a numbering scheme for each of the isomers. In the opinion of the authors, the most workable proposals which have been made to date are those of Magasanik and Chargaff.[6j] Although these authors attribute prior use of the system to Ebel,[6b] they should be credited with formalizing what was no more than implied in Ebel's discussion. In their system, the numbers are assigned so that as many substituents as possible are *cis* to that on carbon 1, with carbons 1 and 6 having their substituents in the *trans* relationship.[7] The numbering is clockwise, and the substituents *cis* to the one on carbon 1 must have the lowest possible numbering.

The inosamines which are the subject of this article have the formulas II and III. Compound II has the *meso*-inositol configuration and its amino group is in position 2 when the ring is numbered according to Magasanik and Chargaff's system. The name *meso*-inosamine-2 is therefore proposed for this substance. Compound III has the scyllitol (all-*trans*) configuration. All of the ring positions are configurationally identical, permitting the assignment of the

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by grants from the Schenley Research Foundation and from the University Research Committee. The results were presented to the American Chemical Society at its 116th National Meeting at Atlantic City, September 18–23, 1949. A more complete account of the experimental work may be found in the Ph.D. thesis of Laurens Anderson, University of Wisconsin, 1950.

(2) (a) Carter, *et al.*, *Science*, **103**, 53 (1946); Fried, Boyack and Wintersteiner, *J. Biol. Chem.*, **162**, 391 (1946); (b) Peck, *et al.*, THIS JOURNAL, **68**, 776 (1946).

(3) Carter, Clark, Lytle and McCasland, *J. Biol. Chem.*, **175**, 683 (1948).

(4) Grosheintz and Fischer, THIS JOURNAL, **70**, 1479 (1948); Iselin and Fischer, *ibid.*, **70**, 3946 (1948).

(5) Wolfrom and Olin, Abstracts, 113th National Meeting, Am. Chem. Soc., Chicago, 1948, p. 5Q; Wolfrom, Olin and Polglase, THIS JOURNAL, **72**, 1724 (1950).

(6) (a) Maquenne, "Les Sucres et Leurs Principaux Dérivés," Gauthier-Villars, Paris, 1900, pp. 14, 190; (b) Ebel, in Freudenberg, "Stereochemie," Franz Deuticke, Leipzig, 1933, p. 619; (c) Posternak, *Helv. Chim. Acta*, **19**, 1333 (1936); (d) Posternak, *ibid.*, **25**, 746 (1942); (e) Fischer, "Harvey Lectures," **40**, 156 (1944–1945); (f) Fleury and Balatre, "Les Inositols," Masson et Cie., Paris, 1947, tableau I; (g) Pigman and Goepp, "Chemistry of the Carbohydrates," Academic Press, 1948, p. 264; (h) Carter, *et al.*, ref. 3, note 3; (i) Carter, *et al.*, *J. Biol. Chem.*, **174**, 415 (1948); (j) Magasanik and Chargaff, *ibid.*, **174**, 173 (1948).

(7) *All-cis* inositol (not yet characterized) presents no problem since all of the positions are configurationally identical. In the amino analog, the carbon carrying the amino group would logically be numbered 1.

number 1 to the carbon carrying the amino group. For it, the name *scyllo*-inosamine-1 or simply *scyllo*-inosamine is proposed. 2-Amino-2-desoxy-*meso*-inositol and 1-amino-1-desoxyscyllitol are likewise acceptable names for these respective structures.

It is realized that the rules of Magasanik and Chargaff do not form a system which is unequivocally applicable to all of the inositol derivatives and analogs. In particular, the optically active compounds will require further stipulations. In view of the complexity of the subject, however, it would seem that further elaboration would be out of place in this paper. For this reason, no attempt is made to discuss the systematic nomenclature of the diaminodidesoxyinositols, of which streptamine (I) is a representative.

Synthesis of the Amines.—It is well known that the steric direction of a hydroxyl or amino group formed by the reduction of a substituted cycloaliphatic ketone or its oxime is specifically influenced by the reducing agent used and the conditions under which it is applied. The experiments of Skita showed[8] that the strongest influences are exerted by hydrogen over a platinum catalyst in an acid medium, which directs the new group into the *cis* position with respect to its neighboring substituents; and by sodium in alcohol, which favors the *trans* configuration. The rule can be used for predicting the structure of highly substituted compounds only when independent evidence shows that it holds for the type of molecule involved. Fortunately, such evidence is available in the inositol series.

Posternak showed that both *epi-meso*-inosose[6c] and *scyllo-meso*-inosose[9] yield the *cis* alcohols (*epi*-inositol and *meso*-inositol) to the extent of

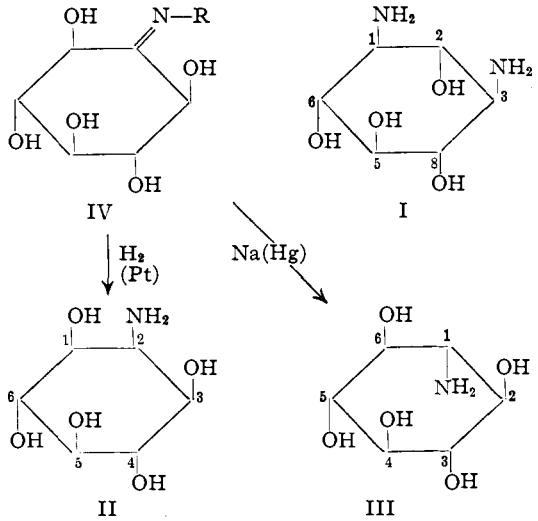

Fig. 1.

90% or better with hydrogen over platinum, even when the reduction is carried out in neutral solution.[10] Sodium amalgam in slightly acid solution gives approximately equal quantities of *cis* alcohol and *trans* alcohol.

Carter, *et al.*,[3] hydrogenated the phenylhydrazone (IV, R = $-NHC_6H_5$) or oxime (IV, R = –OH) of *scyllo-meso*-inosose in aqueous ammonia solution over Raney nickel and obtained a mixture of two primary amines. We found that when glacial acetic acid was used as the solvent and Adams platinum oxide as the catalyst, the primary amine formed from either of these derivatives was almost exclusively a single isomer. The compound is identical with Carter's "inosamine SA." Since the starting material was a keto-inositol of known configuration,[6d] the only point requiring clarification is the configuration of the amino group. The steric selectivity of the conditions employed by us for the synthesis of "inosamine-SA" seems well enough established to permit us to identify it as *meso*-inosamine-2 (II). If this conclusion is correct, "inosamine SB" should be *scyllo*-inosamine (III).

Since inosose derivatives are very alkali-labile, it was not possible to carry out a reduction under the best "*trans*-directing" conditions. However, with sodium amalgam at *p*H 5.5–6.5 the "inosamine-SB"[11] of Carter, *et al.*, accounted for an unexpectedly large proportion (73%) of the primary amine formed. The fact that this isomer, which occurs to a limited extent if at all under "*cis*-directing" conditions, appears as the major product of reduction by a dissolving metal strengthens our conclusions as to the respective configurations of the inosamines from *scyllo-meso*-inosose. The preparation of both epimers under non-enolizing conditions confirms the assumption of the Illinois workers that the hydroxyl groups in both compounds retain the configuration of the parent inosose. The yields obtainable by the procedures described herein are considerably improved over those from the Raney nickel-ammonia reduction. A further advantage of the sodium amalgam method for preparing *scyllo*-inosamine is that a solution of *scyllo-meso*-inosose to which hydroxylamine has been added may be reduced directly after it has stood a short time. This makes unnecessary the two-step isolation of the oxime which may be difficultly obtainable[12] from other inososes.

Derivatives.—The principal aim in preparing the series of derivatives shown in Fig. 2 from each of the three amines, streptamine, *meso*-inosamine-2 and *scyllo*-inosamine, was to obtain the O-acetate hydrochlorides (XI, XII and XIII)

(8) For discussion and references, *cf.* Hückel, *Ann.*, **533**, 1 (1937), and Weidlich, *Die Chemie*, **58**, 30 (1945).

(9) Posternak, *Helv. Chim. Acta*, **24**, 1045 (1941).

(10) *scyllo-meso*-Inosose is hydrogenated to 2-desoxyinositol in aqueous mineral acid (Posternak, ref. 9). Although the compound is nearly insoluble in acetic acid, reduction of a small sample in this solvent (80%) yielded a product which was largely *meso*-inositol.

(11) This amine was known to Carter, *et al.*, only in the form of derivatives. Its isolation in the free state is reported here for the first time.

(12) McCasland, private communication.

for the acyl migration studies. The N-arylidene (V, VI and VII) and acetylated N-arylidene (VIII, IX and X) compounds, however, have excellent properties, and derivatives of this type should be useful in separating and identifying mixtures of aminodesoxyinositols. The use of an

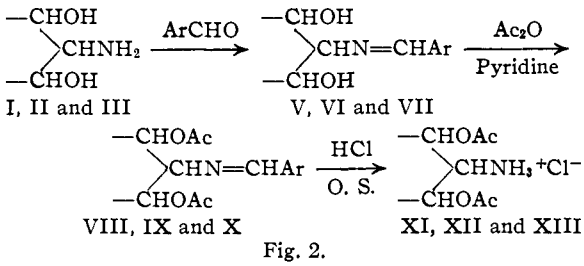

Fig. 2.

arylidene group to block the nitrogen of the amines during acetylation was suggested by the work of Bergmann and Zervas[13] with glucosamine. Di-N-benzylidenestreptamine, originally described by Peck, *et al.*,[2b] proved to be a satisfactory intermediate. Although we eventually settled on salicylaldehyde for preparing the Schiff bases of the inosamines, there is no reason to believe that benzaldehyde would not be equally satisfactory for this purpose.

The hydrochloric acid cleavage of the arylidene groups from the nitrogen atoms of acetylated Schiff bases of glucosamine has been accomplished[13,14] in boiling acetone solution. This method, however, was not entirely satisfactory for the acetylated N-arylidene compounds of the aminoinositol series. The fact that each of the O-acetate hydrochlorides was prepared in a different way is the result of a continuing effort to improve the procedure. The advantages and disadvantages of each variation are indicated in the experimental part. The crude hydrochlorides were used directly for acyl migration experiments. They can be recrystallized but due to their acidic nature tend to lose acetyl groups during this process.

Acyl Migration Behavior.—Recent studies in several different laboratories[15] have led to the conclusion that the migration of acyl groups between neighboring oxygen and nitrogen atoms involves a cyclic intermediate of the type shown in Fig. 3. It occurred us that if this be the case, migration should proceed more rapidly between hydroxyl and amino groups which are *cis* to each other than between those which are *trans*, due to the probable closer approach of the two groups in space. The results of experiments designed to test this hypothesis are shown in Fig. 4.

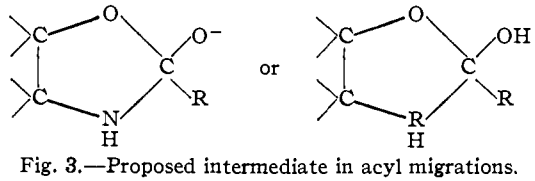

Fig. 3.—Proposed intermediate in acyl migrations.

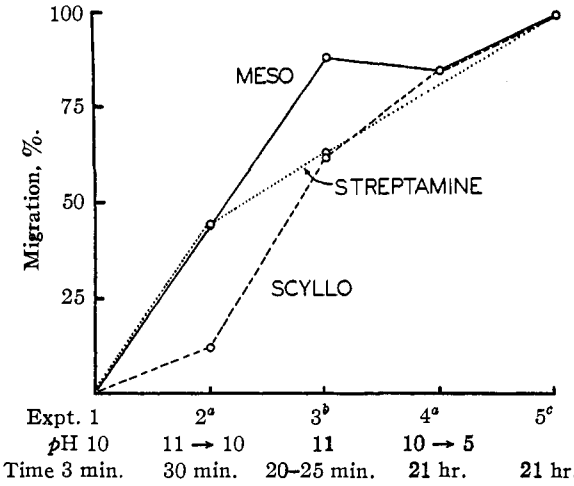

Fig. 4.—Acyl migration in O-acetyl derivatives of aminodesoxyinositols under increasingly severe conditions.[d] [a] The *p*H changed as indicated during the reaction period. [b] The *p*H was held at 11 by the continuous addition of base until no more was required. The consumption ranged from 4.7 to 6.0 molar equivalents. [c] Migration in methanolic ammonia. [d] The values for extent of migration in experiments 1 to 4 were computed by subtracting the number of milliequivalents of acid required for the back-titration from the number of milliequivalents of base used, and dividing this difference by the number of milliequivalents of amino compound present in the solution, The quotient was multiplied by 100 to convert to per cent.

The values from experiments 2 and 3 verify the prediction that a higher rate of migration would be found in the *cis* compound and show that the rate of migration increases with increasing *p*H. Experiment 4 shows that the difference is quantitative only, and that, with sufficient time at a suitable *p*H essentially complete migration takes place in both inosamine derivatives. The hope that the differences would be great enough to permit the estimation of the number of *cis* aminoalcohol groupings in an aminodesoxyinositol of unknown configuration was not realized. The behavior of tetra-O-acetylstreptamine dihydrochloride suggests that the picture is complicated by the presence of two amino groups.

Experimental[16]

Streptamine Hemihydrate (I).—Streptidine sulfate was hydrolyzed to streptamine with barium hydroxide as described by Peck, *et al.*[2b]

(13) Bergmann and Zervas, *Ber.*, **64**, 975 (1931).

(14) White, *J. Chem. Soc.*, 1498 (1938).

(15) (a) Phillips and Baltzly, THIS JOURNAL, **69**, 200 (1947); (b) Isbell and Frush, *ibid.*, **71**, 1579 (1949); (c) LeRosen and Smith, *ibid.*, **71**, 2815 (1949).

(16) Melting points were taken in capillary tubes with NBS calibrated Anschütz thermometers. When the notation "30 sec. dec." is used, it signifies that the compound melted with decomposition 30 ± 5 sec. after the tube was inserted into an oil-bath held constant at the temperature recorded. Nitrogen analyses were performed by the semi-micro Kjeldahl method; other analyses were by the Clark Microanalytical Laboratory, Urbana, Illinois. Exceptions to these statements are specifically noted.

scyllo-meso-Inosose phenylhydrazone was prepared according to Posternak.[9] The compound was recrystallized from 22 volumes of 90% ethanol, dried and finely ground prior to reduction.

scyllo-meso-Inosose oxime was prepared by the method of Carter, *et al.*[6i] We found that recrystallization of the crude sodium inosose oximate from boiling water resulted in considerable decomposition and preferred to convert the salt directly to the free oxime. The product so obtained was 92% pure according to its nitrogen analysis [Found (Friedrich–Kjeldahl), 6.69%; theo., 7.25%] and kept fairly well in the cold room for several weeks. It must be reasonably fresh to be hydrogenated successfully.

Solutions of the oxime suitable for reduction with sodium amalgam were prepared by dissolving 5 g. of sodium acetate and 4 g. of hydroxylamine hydrochloride in 100 ml. of water and adding 10 g. of *scyllo-meso*-inosose. The *p*H was brought to 4.8 with a few drops of concentrated sodium hydroxide and the whole was stirred until the inosose went into solution (two hours). After two more hours, a sample was checked in the Beckman spectrophotometer. It gave a typical oxime curve shown in Fig. 5.

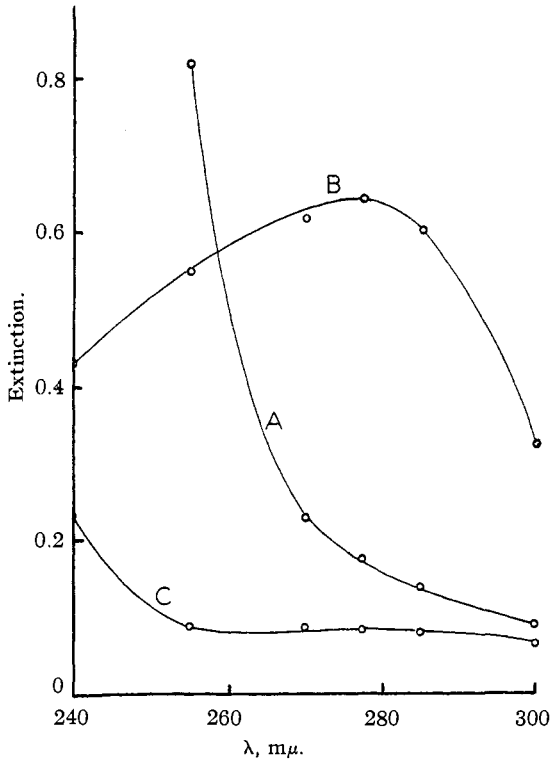

Fig. 5.—Formation and reduction of *scyllo-meso*-inosose oxime in solution: curve A, oxime, 0.2 ml. of solution diluted to 10 ml.; curve B, 0.5 ml. of solution + 0.5 ml. of 6 *N* sulfuric acid + 9.0 ml. of water, before reduction; curve C, same, after reduction.

Catalytic Hydrogenations.—Batches of from 2 to 8 g. of the phenylhydrazone or oxime were suspended in 20 to 25 volumes of glacial acetic acid and shaken with hydrogen at slightly over 1 atmosphere pressure with one-fourth their weight of Adams platinum catalyst. The time of reduction varied from one and one-half to seven hours. The catalyst was removed by filtration and excess acetic acid was evaporated from the filtrates *in vacuo*, leaving a crude sirup of amine acetates. This was taken up in slightly over 1 equivalent of 1.0 *N* sulfuric acid and worked up as described below.

Isolation of the Amines from Acid Solution.[17]—The remaining acetic acid was expelled from the acid solutions by steam distillation. The liquid in the still pot was then made basic with saturated barium hydroxide added at the boiling point, and after standing overnight[18] was filtered from the barium sulfate. If cyclohexylamine (from the phenylhydrazone) was present, this was removed by an additional steam distillation. Finally, excess barium was thrown down by carbonation, the inosamine solution was concentrated at the boiling point and the product was recovered by crystallization or freeze drying.

meso-Inosamine-2 (II). **A. From the Oxime.**—The crude product from the hydrogenation of 10.5 g. of oxime was recrystallized twice to give 5.13 g. of material which corresponded in analysis and solubility with the "inosamine-SA" of Carter, *et al.*[3] For further identification, the hexaacetyl derivative, m. p. 255–256°, and the N-acetyl derivative, m. p.[19] 246–248°, were made. The recorded melting points are 259–260° and 242–248°, respectively, determined with a micro block. For the former, we have observed no values higher than 255–256° using a capillary, even with a very pure sample prepared from the penta-O-acetate hydrochloride. With solubility corrections, the yield quoted accounts for 78% of the nitrogen recovered from the hydrogenation.

B. From the Phenylhydrazone.—Twenty grams of the phenylhydrazone was hydrogenated and worked up. The first crop of crystals, which weighed 7.07 g. and had 7.11% N, was salicylidenated without further purification to give the expected yield of a single derivative, identical with the salicylidene compound from the pure *meso*-inosamine-2, described above. With a solubility correction for losses during crystallization, the first crop accounted for 79% of the nitrogen of the crude product. The mother liquors were worked up for their primary amine content, but yielded only a small amount (0.73 g.) of what was probably the (impure) penta-O-acetate hydrochloride of the *scyllo-epimer*. From this it could be calculated that over 85% of the primary amine formed was *meso*-inosamine-2.

As was observed by Carter, *meso*-inosamine-2 has no definite melting point when heated in the conventional manner. We wish to record a decomposition point of 295° obtained by the thirty-second technique.

Sodium Amalgam Reductions.—Portions of 100 ml. of 10% solution of *scyllo-meso*-inosose oxime were used. The reaction was carried out with mechanical stirring in a 250-ml. beaker set in a water-bath which was maintained at 25° with running tap water. Three per cent. sodium amalgam was added in 50-g. batches, new additions being made after gas evolution from the previous batch had ceased and the solution had cooled to 25°. The *p*H was read with a glass electrode and was held between 5.5 and 6.5 by additions of glacial acetic acid (*ca.* 25 ml. required).

The progress of the reaction was followed by acidifying samples of the solution to hydrolyze the unreduced oxime and plotting their absorption in the spectrophotometer. The presence of variable amounts of absorbing impurities made it impractical to attempt to calculate the amount of free inosose liberated in the acid solution from the optical density at its wave length of maximum absorption, 282 mμ. Instead, the disappearance of the "hump" from the plot of the extinctions between 240 and 300 mμ was used as a criterion of completeness of reduction. A typical initial and final curve are shown in Fig. 5. The requirement for amalgam was 5 to 6 batches, 250–300 g. in all.

Upon completion of the reduction, the amine solution was filtered from the mercury and acidified with 40 ml. of

(17) Exploratory experiments indicate that the involved procedure given here for ridding the product of all components except the inosamine may be unnecessary for the *meso*-compound, which can be precipitated from either acidic or basic solutions by simple neutralization. The *scyllo*-epimer does not appear to separate satisfactorily from solutions containing salts.

(18) If *meso*-insoamine-2 is being worked up, care must be taken to maintain adequate volume after the solution has been neutralized, since the compound has a low solubility (0.7 g./100 ml.) in the cold.

(19) Uncalibrated, temperature rise 10°/min.

concentrated hydrochloric acid, then evaporated almost to dryness *in vacuo*. The caked solid residue was broken up and the flask was placed in a vacuum desiccator over sulfuric acid for one or two days to complete the drying. Ten grams of anhydrous sodium acetate was added and the whole was refluxed two hours with 200 ml. of acetic anhydride. When the flask was cool, the solid material, consisting of sodium chloride plus a portion of the acetylated product, was filtered off. The residue obtained by evaporating the filtrate to dryness *in vacuo* was triturated with water till granular and added to that obtained by filtration and the whole was washed thoroughly and dried.

scyllo-Inosamine (III).—The crude mixed hexaacetyl derivative obtained from a total of 35 g. of starting material was recrystallized from 100 volumes of ethanol after decolorization with Nuchar GL, yield, 51.4 g. This recrystallization eliminates some dark colored impurities but does not alter the ratio of the epimers. The product was suspended in 5 liters of methanol and saponified according to Carter.[20] There resulted 17.0 g. of pure N-acetyl derivative which melted[19] at 288–290° and did not depress the m. p. of a sample of N-acetyl "inosamine-SB" supplied by Dr. McCasland. The identity of the compound was further confirmed by reacetylating a small sample, which then exactly duplicated the complex melting behavior (micro block) described by Carter for hexaacetyl "inosamine-SB."

In addition to the pure N-acetyl derivative there was isolated from the mother liquors 4.6 g. of a mixture which was 50% *scyllo*-epimer, bringing the amount identified to 19.3 g. or 73% of the theoretical. At least 73% of the primary amine from the sodium amalgam reduction was therefore *scyllo*-inosamine.

The N-acetyl compound (only the pure fraction was used) was refluxed twelve hours with a 4% excess of 1.0 *N* sulfuric acid and the acid solution was worked up as previously described. On recrystallization of the crude product from 100 ml. of boiling water (Nuchar C-190 N), 9.5 g. of crystals (elongated blades) was obtained. This was apparently the hemihydrate (found: N, 7.31, 7.36; theo. for the hemihydrate, 7.44), for although the nitrogen content could not be raised by repeated recrystallization nor by drying at 100° in an Abderhalden drier, it increased on three months standing in an ordinary reagent bottle in the cupboard. An additional 2.3 g. of pure hemihydrate was obtained by working up the mother liquors. Samples of the stored material were dried in high vacuum for analysis. *scyllo*-Inosamine hemihydrate is soluble in 16 parts of cold or 5 parts of boiling water. It darkens but does not melt below 300°.

Anal. Calcd. for $C_6H_{13}O_5N$ (179.17): C, 40.22; H, 7.31; N, 7.82. Found: C, 40.19, 39.92; H, 7.21, 6.96; N, 7.57, 7.77.

Di-N-benzylidenestreptamine (V) was prepared as described by Peck, *et al.*,[2b] by heating streptamine hemihydrate with excess benzaldehyde. The compound was isolated by diluting the crystal brei with Skellysolve A, filtering and washing the crystals repeatedly on the filter with the same solvent. The crude product, which was obtained in 92% yield, melted at 224–225° (uncalibrated) and was used without further purification.

N-Salicylidene-*meso*-inosamine-2 (VI).—*meso*-Inosamine-2 does not react with either benzaldehyde or salicylaldehyde when heated with the pure reagents. Four and one-half g. of the amine (from the phenylhydrazone) was suspended in 900 ml. of absolute ethanol, 22.5 ml. of redistilled salicylaldehyde was added, and the mixture was refluxed twenty-six hours under a calcium chloride tube. The yellow solution which resulted was filtered while hot to remove the undissolved material (0.80 g.), then set aside overnight. The bright yellow crystalline product, which was recovered by filtration and washing with cold absolute ethanol, weighed 4.53 g. and had m. p. 240–243° (dec.). An additional 0.98 g. was recovered from the filtrate by azeotropically replacing the water with benzene and the ethanol with Skellysolve C; total yield 5.51 g. (93% based on the amount of material which went into solution). Two recrystallizations from pyridine (20 volumes) raised the m. p. to a constant value of 243.5–245°. On analysis, however, the recrystallized material was found to be the monopyridinate.

Anal. Calcd. for $C_{13}H_{17}O_6N \cdot C_5H_5N$ (362.38): C, 59.66; H, 6.12; N, 7.73. Found: C, 59.28, 59.47; H, 5.94, 6.08; N (Dumas, Clark Lab.), 7.31, 7.25; (Kjeldahl), 6.70, 6.65.

Recrystallization from ethanol regenerated the unsolvated Schiff base.

A sample of the compound was prepared from very pure *meso*-inosamine-2. On recrystallization from 135 volumes of absolute ethanol, blades of pure N-salicylidene-*meso*-inosamine-2 aggregated in lustrous lemon yellow scales. The compound darkens and melts to a brown liquid at 243–244°.

Anal. Calcd. for $C_{13}H_{17}O_6N$ (283.28): C, 55.11; H, 6.05; N, 4.94. Found: C, 54.78, 54.92; H, 6.29, 6.03; N, 4.92, 4.90.

N-Salicylidene-*scyllo*-inosamine (VII).—The salicylidenation was carried out as described for the *meso*-derivative except that more ethanol (350 ml./g. of amine) was required to effect complete solution. A correspondingly greater amount of salicylaldehyde was used. The crude product was very finely divided and difficult to filter, but crystallized nicely in the form of lemon yellow rectangular plates from methanol. According to their nitrogen analysis and loss of weight sustained on drying at 64°, these crystals were the monomethanolate. Somewhat to our surprise, we found that the compound can be recrystallized from boiling water. Droplets of salicylaldehyde appear during this process, but the recovered Schiff base is of good quality. The long rectangular plates which separate are apparently the monohydrate. N-Salicylidene-*scyllo*-inosamine begins to darken at *ca.* 255° and melts at 264.5–265.5°.

Anal. Calcd. for $C_{13}H_{17}O_6N \cdot H_2O$ (301.29): C, 51.82; H, 6.36; N, 4.65. Found: C, 53.41, 53.44; H, 6.82, 6.55; N, 4.75, 4.65.

The Schiff bases V, VI and VII were acetylated by reacting them with acetic anhydride in dry pyridine at room temperature (the solutions were cooled before addition of the reagent) for three days. The products precipitated in solid form when the reaction mixtures were poured into 10 volumes of ice water, and were isolated by filtration.[21]

Di-N-benzylidenetetra-O-acetylstreptamine (VIII).—From V, 0.87 g. pyridine, 5 ml., and acetic anhydride, 2.5 ml.; part of the product crystallized during the reaction; yield, 69%. In the cold, the compound is insoluble in water and Skellysolve B; slightly soluble in n-butanol, isopropyl ether, isopropyl alcohol, ethanol, benzene, methanol, ethyl acetate and ethyl ether; and somewhat more soluble in acetone, dioxane, chloroform, methyl ethyl ketone and pyridine. It is best recrystallized from hot benzene. A sample which had been recrystallized first from pyridine then benzene melted at 247.5–248.5°.

Anal. Calcd. for $C_{28}H_{30}O_8N_2$ (522.54): C, 64.35; H, 5.79; N, 5.36. Found: C, 64.42, 64.15; H, 5.91, 5.69; N (Dumas, Clark Lab.), 5.58, 5.64.

N-Acetylsalicylidenepenta-O-acetyl-*meso*-inosamine-2 (IX).—From VI, 4.53 g., pyridine, 90 ml., and acetic anhydride, 18 ml., three hours shaking was required to dissolve the starting material; yield, 87%. One recrystallization from 6 volumes of boiling dioxane brought the m. p. to a constant value of 243–244°. The pure white substance melts without decomposition. It was found advantageous to work up the dioxane mother liquors to avoid excessive losses. The compound is slightly soluble in Skellysolve B, isopropyl alcohol, *n*-butanol and ethanol in the cold and somewhat more soluble in benzene, ethyl acetate, dioxane and pyridine.

Anal. Calcd. for $C_{25}H_{28}O_{12}N$ (535.49): C, 56.07; H,

(20) *Cf.* "N-Acetylinosamine-SB," p. 687, *loc. cit.*, note 8.

(21) A neutral desiccant should be used when drying these compounds, since they are very acid-labile.

5.46; N, 2.62. Found: C, 55.89, 56.04; H, 5.18, 5.08; N, 2.59, 2.62.

N-Acetylsalicylidenepenta-O-acetyl-*scyllo*-inosamine (X).—From VII, 5 g.; pyridine, 25 ml. and acetic anhydride, 12.5 ml.; yield, 96%; solubility, slight in Skellysolve B, isopropyl alcohol, *n*-butanol, *n*-propanol and isopropyl ether; moderate in ethanol, carbon tetrachloride and methanol; soluble in benzene and extremely soluble in ethyl acetate, dioxane, pyridine and tetrahydrofuran. One recrystallization from 25 volumes of carbon tetrachloride served to raise the melting point from 200–201° to 206–206.5°, which latter value remained constant. The long fine needles tenaciously retained a small amount of carbon tetrachloride, and in order to obtain analytically pure material it was necessary to recrystallize the compound from absolute ethanol (20 volumes). Recrystallization from ethanol without prior recrystallization from carbon tetrachloride causes a slight decomposition of the product.

Anal. Calcd. for $C_{25}H_{29}O_{12}N$ (535.49): C, 56.07; H, 5.46; N, 2.62. Found: C, 56.11, 55.89; H, 5.76, 5.42; N, 2.60, 2.58.

Tetra-O-acetylstreptamine Dihydrochloride (XI).—To a boiling solution of 4.60 g. of VIII in 55 ml. of acetone was added 2 molar equivalents (2.7 ml.) of 6.56 *N* HCl. The gelatinous precipitate which separated within a few seconds was filtered off, washed twice with acetone and air dried. The product was not completely hydrolyzed, since a considerable amount of benzaldehyde was liberated when it was heated with acid. It was, therefore, redissolved by gentle heating in 12 ml. of water. After the addition of 1.5 ml. of 6 *N* HCl the solution was filtered and the hydrochloride was precipitated in amorphous form from the filtrate by the addition of 10 volumes of acetone. The amount of air dried material so obtained was 2.7 g. With allowance for samples taken out prior to the reprecipitation, this represents a yield of 75% of theoretical, m. p. 260° (30 sec. dec.).

Anal. Calcd. for $C_{14}H_{24}O_8N_2Cl_2 \cdot H_2O$ (437.28): N, 6.41; Cl, 16.22; loss on drying, 4.12. Found: N, 6.85, 6.95; Cl (author), 17.2, 17.1; loss on drying (100°) 4.76.

Penta-O-acetyl-*meso*-inosamine-2 Hydrochloride (XII).—The compound did not precipitate from boiling solutions of IX in acetone or ethanol on the addition of hydrochloric acid. On continued boiling the acetone solution turned a deep red, presumably from condensation with the liberated salicylaldehyde. The use of dioxane as the solvent for IX eliminated these difficulties. A solution of 1 g. of IX in 20 ml. of dioxane was treated for thirty minutes with a rapid stream of dry hydrogen chloride. The hydrochloride, which precipitated gradually during this time, was filtered off and washed on the filter, once with dioxane and twice with ether; yield 0.74 g., 93% of theoretical. A slight molar excess of 6 *N* aqueous hydrochloric acid may be used instead of the dry gas. The product prepared in either way is free of unhydrolyzed material. A sample was recrystallized (irregular plates) from 7 volumes of water for analysis; m. p. 220° (30 sec. dec.).

Anal. Calcd. for $C_{16}H_{24}O_{10}NCl$ (425.82): C, 45.13; H, 5.68; N, 3.29; acetyl, 50.54. Found: C, 46.57, 46.41; H, 5.82, 5.85; N, 3.35, 3.36; acetyl, 49.16.

Penta-O-acetyl-*scyllo*-inosamine Hydrochloride (XIII).—When this compound was prepared in the manner described for the *meso* epimer, it developed considerable yellow color on solution in methanolic ammonia, indicating that unhydrolyzed material was present. A better product, still not completely free of starting material, was obtained by dissolving X in three volumes of dioxane and adding nearly as much 6 *N* hydrochloric acid. After a few minutes wait, additional dioxane was added, and the precipitate was filtered off. Unfortunately, the optimum amount of dioxane for complete precipitation could not be used, because two liquid phases[22] are formed unless very small or very large volumes are taken. The best yield was obtained when the solvent was tetrahydrofuran, which is miscible with 6 *N* hydrochloric acid in all proportions. X (0.625 g.) was dissolved by heating with 3 volumes of tetrahydrofuran. The hydrochloride precipitated immediately on the addition of 1.5 volumes of 6 *N* hydrochloric acid to the cooled solution. After one-half hour, 27 volumes of tetrahydrofuran was added, and after further standing to insure complete precipitation, the solid was filtered off, washed with tetrahydrofuran, and dried in a desiccator. The yield was 0.446 g. (90%); the product was contaminated with unhydrolyzed material. A small amount of the compound dissolved in six volumes of boiling water precipitated on cooling in a very finely divided, apparently amorphous form. It was hygroscopic and was dried at 64° to constant weight for analysis; m. p. 270° (30 sec. dec.).

Anal. Calcd. for $C_{16}H_{24}O_{10}NCl$ (425.82): C, 45.13; H, 5.68; N, 3.29. Found: C, 44.19, 44.08; H, 5.96, 5.85; N, 3.30, 3.23.

Acyl Migration Experiments.—Experiments 1 to 4 were carried out in aqueous solution by the technique devised by Phillips and Baltzly.[15a] Samples of 0.25 or 0.5 milliequivalent of the O-acetate hydrochlorides were used. The *p*H of the ester hydrochloride solutions was 3.1; this value was therefore used as the end-point in the back titrations. In most of these experiments saponification, with the concomitant production of sodium acetate, was a competing reaction. Fortunately, sodium acetate titrates quantitatively as a base at the end-point used and therefore does not interfere with the measurement.

Since both the penta-O-acetylinosamines precipitate when dilute aqueous solutions of their hydrochlorides are made basic, it was necessary to add an organic solvent to the system. Ethanol was first employed, but dioxane was later adopted to rule out the possibility of a transesterification reaction. One experiment was carried out with ethanol, then repeated with dioxane. The results were identical within experimental error. Although larger quantities were used in most of the runs, it was eventually found that an amount of dioxane equivalent to 5% of the final volume was sufficient to hold the bases in solution.

In experiment 5, the O-acetate hydrochlorides reacted with absolute methanolic ammonia (40 volumes, saturated at 0°) for twenty-four hours at room temperature. The following products were isolated in nearly quantitative yields, indicating complete or nearly complete migration in all cases: N-acetyl-*meso*-inosamine-2, m. p.[19] alone and mixed with an authentic sample 246–248°. N-Acetyl-*scyllo*-inosamine, m. p.[19] 291–293°, mixed with an authentic sample, 289–291°. Mixed mono- and di-N-acetylstreptamine melting at 280–285° dec. (uncalibrated). Found: acetyl, 22.83. Di-N-acetylstreptamine melts[25] at 283–284° and has 32.83% acetyl. These results parallel those obtained by White[14] with the corresponding derivative of glucosamine.

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Summary

1. The epimeric inosamines related to *scyllo-meso*-inosose have been synthesized by methods which permit tentative decision as to the configuration of the amino groups.

2. The rates of acyl migration (O → N) in the

(22) Dioxane and 6 *N* hydrochloric acid form a two phase system when the ratio of acid to dioxane lies between the approximate limits 1:3 and 1:50 at room temperature.

acetates of the two inosamines have been compared and the results tend to confirm the configurational assignments made. Acyl migration data for tetra-O-acetylstreptamine are also reported.

3. The nomenclature of these compounds is discussed. The use of the names *meso*-inosamine-2 for the epimer having the configuration of *meso*-inositol and *scyllo*-inosamine for the one having the configuration of scyllitol is suggested.

4. *meso*-Inosamine-2 is identical with the "inosamine-SA" and *scyllo*-inosamine with the "inosamine-SB" of Carter, Clark, Lytle and McCasland.

5. A series of new derivatives of these two inosamines and of streptamine has been prepared. These derivatives should be useful for separating and identifying inosamine mixtures.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Preparation and Alkali Sensitivity of Some New Enol Glucosides and Glucosides of β-Hydroxy Carbonyl Compounds[1]

BY CLINTON E. BALLOU AND KARL PAUL LINK

Alkali lability of glycosides has been attributed to a unique activation of the glycosidic linkage by the aglycon.[2,3,4] Electronic interpretation of this phenomenon usually involves the formation of a cationoid center on the aglycon or the anomeric carbon atom of the sugar. The induced shift of the free electron pair on the glycosidic oxygen will govern the approach of the cleaving anion, and this effect is related to certain properties of the aglycon. Hibbert[5] ascribed this property to aglycon acidity. Though aglycon acidity may be useful in correlating relative rates of alkaline cleavage of some phenolic glycosides, all of the types of alkaline cleavage of glycosides cannot thus be rationalized.[3,4]

In previous papers we have reported the alkaline methanolysis of β-D-glycosides of 3-phenyl-4-hydroxycoumarin[3,6] and the glucoside of theobromine.[4] The former undergo methanolysis to the aglycon and a methyl α-D-glycoside, indicating cleavage between the glycosidic oxygen and the sugar residue. However, when theobromine D-glucoside tetraacetate is cleaved in the same manner, glucose and a methoxy theobromine are obtained.

The alkali sensitivity of these glycosides may be associated with the activating conjugated carbonyl structure of the aglycon. The lability of the glucoside of salicylic acid[7] was thus rationalized.[2] It has been suggested[8] that the biosidic structure, Gl—O—C=C—, is sufficient to labilize the glycosidic linkage to alkali. This paper concerns studies on the basic activating system, Gl—O—C=C—C=O.

A series of glucosides containing variations of the minimum activating system as represented above was synthesized. The glucoside of the enolic form of ethyl acetoacetate is representative.[9] The Koenigs–Knorr conditions produced uncrystallizable sirups. However, a modification of the Robertson synthesis[10] introduced in this Laboratory by Dr. C. F. Huebner[6] gave success. It was first used in the synthesis of the acetylated diglucoside of 3,3′-methylenebis-(4-hydroxycoumarin) (Dicumarol^{R}), and consists of treating tetraacetyl-D-glucosyl bromide (I) with the enolic aglucon, in the presence of silver oxide and a *trace* of quinoline. In the synthesis of glucosides of phenol Robertson[10] employed a relatively large amount of quinoline. The success of the syntheses reported below depends largely on the amount of quinoline used, an observation previously made.[6]

Inspection of the structural formula of the glucoside of ethyl acetoacetate reveals that *cis–trans* isomerism is possible, and two forms of the glucoside were isolated. The method of separation involved fractional crystallization. The yields were low, and it was difficult to obtain the fractions in a pure form. Chromatographic separation on Silene EF, following the procedure of Binkley and Wolfrom,[11] was used with success, and offered a more reliable method of separation.

Since it is not known which of the compounds is *cis* and which is *trans*, the higher melting product was arbitrarily designated *trans*(?), and the

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(3) Spero, Ballou and Link, THIS JOURNAL, **71**, 3740 (1949).

(4) Ballou and Link, *ibid.*, **71**, 3743 (1949).

(5) Fisher, Hawkins and Hibbert, *ibid.*, **63**, 3031 (1941).

(6) Huebner, Karjala, Sullivan and Link, *ibid.*, **66**, 906 (1944).

(7) Helferich and Lutzmann, *Ann.*, **537**, 11-21 (1938).

(8) Evans and Benoy, THIS JOURNAL, **52**, 294 (1930); Gehman, Kreider and Evans, *ibid.*, **58**, 2388 (1936).

(9) The preparation of a glucoside of ethyl acetoacetate, m. p. 170–171°, was reported by Gonzales and Aparicio, *Anales fis y quim*, **41**, 846–859 (1945). Neither rotation nor elemental analysis are given in the paper. Thus the structure of their product is questionable.

(10) Robertson and Waters, *J. Chem. Soc.*, 2730 (1930).

(11) Binkley and Wolfrom, Sugar Res. Found., Inc., Scientific Report Series No. 10, August, 1948.