[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND THE INSTITUTE FOR ENZYME RESEARCH, UNIVERSITY OF WISCONSIN]

Stereochemical Studies in the Aminodesoxyinositol Series. II. D,L-*myo*-Inosamine-4, D,L-*epi*-Inosamine-2 and Streptamine[1,2]

BY HELGA STRAUBE-RIEKE, HENRY A. LARDY AND LAURENS ANDERSON

RECEIVED JULY 3, 1952

The reduction of D,L-*epi*-inosose-2 oxime with sodium amalgam followed by acetylation of the product yields the hexaacetate of an inosamine which differs from the inosamine-EA of Carter, *et al.* This derivative was found by mixed melting point and X-ray powder pattern determinations to be identical with a hexaacetylinosamine obtained from streptamine by Wintersteiner and Klingsberg. The new inosamine is thereby shown to be D,L-*myo*-inosamine-4, and the *scyllo* (all-*trans*) configuration for streptamine is at the same time finally established. Inosamine-EA is D,L-*epi*-inosamine-2. The kinetic methods previously used to determine configuration in this series were validated by testing them on the new inosamine and its epimer.

The recently proposed[3] *system for the nomenclature and numbering of cyclitols and their derivatives is used throughout this paper.*

In the previous communication,[1] evidence was adduced to show that inosamine-SA and inosamine-SB, the epimeric aminodesoxyinositols obtained from *myo*-inosose-2 by Carter, *et al.*,[4] are *myo*-inosamine-2 and *scyllo*-inosamine, respectively.[5] The *myo* configuration was assigned to inosamine-SA on two grounds: (a) It is the predominant isomer formed when imine derivatives of *myo*-inosose-2 are hydrogenated in glacial acetic acid over platinum catalyst. (b) The alkali-catalyzed O → N acyl migration proceeds more rapidly in the penta-O-acetate of inosamine-SA than in that of inosamine-SB. This configurational assignment subsequently gained further support from an N → O acyl migration study (McCasland[6]) and from a comparison of the rates of periodate and lead tetraacetate oxidation of the two amines (Posternak[7]).

After the study of the amines from *myo*-inosose-2 had been completed, a need arose in our laboratory for a quantity of D,L-*myo*-inosamine-4 (IV), which is one of the two amines theoretically derivable from D,L-*epi*-inosose-2 (I). An inosamine (inosamine-EA) had been prepared by Carter, *et al.*,[4] from the phenylhydrazone and by May and Mosettig[8] from the oxime of I. Since the epimer was not known, it was not possible to study the configuration of this amine by any of the kinetic methods mentioned above. However, the fact that it had in both cases been obtained by catalytic hydrogenation led us to expect it to be D,L-*epi*-inosamine-2 (III). We therefore turned to the sodium amalgam reduction of D,L-*epi*-inosose-2 oxime (II).

The crude amine obtained by this means was converted to the hexaacetyl derivative with a view to purifying it through the N-acetate as described by Carter, *et al.*,[4] for *scyllo*-inosamine. This hexaacetate differed from that of inosamine-EA, and seemed to be a pure compound. It was noticed that its melting point, 236–239°, coincided with that reported by Wintersteiner and Klingsberg[9] for a hexaacetylinosamine which they had obtained by acetylation of the deamination product of N,O,-O′,O″-tetraacetylstreptamine hydrochloride (VIII). This observation presented an unusual opportunity. If it could be shown that the two samples of hexaacetylinosamine were identical, then uncertainties regarding the configurations of both streptamine[10] and the sodium amalgam reduction product would be removed.

A sample of our hexaacetate was accordingly forwarded to Dr. Wintersteiner, who kindly performed a mixed melting point determination with his derivative. The melting point was not depressed. Further comparison of the two samples was made through the X-ray powder diagrams. An examination of the patterns (Fig. 2) confirmed the identity of the hexaacetylinosamines from the two sources.[11]

The degradation experiments of Wintersteiner and Klingsberg,[12] followed by the synthesis of streptamine from natural chitosamine[13] (VI) by Wolfrom and co-workers[14] eliminated from consideration all structures for this substance save VII and IX.[15] Careful consideration of formula IX shows that replacement of either one of the amino groups by hydroxyl, *either with or without inversion*, could not possibly give rise to III or IV. Streptamine is therefore VII. The replacement of either one of the two stereochemically equivalent amino groups in VII by hydroxyl should next be considered. If this be done *without inversion* the product is *scyllo*-inosamine (X) which could not

(1) Paper I of this series: L. Anderson and H. A. Lardy, THIS JOURNAL, **72**, 3141 (1950).

(2) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported by grants from the U. S. Public Health Service and the University Research Committee. The authors are grateful to the Corn Products Refining Co. for a generous gift of *myo*-inositol.

(3) H. G. Fletcher, Jr., L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

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

(5) *myo*-Inosamine-2 was called *meso*-inosamine-2 in paper I.

(6) G. E. McCasland, THIS JOURNAL, **73**, 2295 (1951).

(7) T. Posternak, *Helv. Chim. Acta*, **33**, 1597 (1950).

(8) E. L. May and E. Mosettig, *J. Org. Chem.*, **14**, 1137 (1949).

(9) O. Wintersteiner and A. Klingsberg, THIS JOURNAL, **73**, 2917 (1951).

(10) Streptamine is hydrolyzed streptidine, which in turn is one of the three moieties of streptomycin.

(11) The authors are most grateful to Dr. Stanley F. Kern, Lilly Research Laboratories, Indianapolis, who determined the X-ray patterns and furnished the photographs of Fig. 2.

(12) First announced in THIS JOURNAL, **70**, 885 (1948), and described *in extenso* in ref. 9.

(13) Chitosamine has been satisfactorily established as D-glucosamine. For discussion, see A. Neuberger, *Adv. Protein Chem.*, **4**, 315 (1948).

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

(15) N. G. Brink and K. Folkers, *Adv. Enzymology*, **10**, 159 (1950). *Cf.* also Wintersteiner and Klingsberg, ref. 9. It may be noted that Wolfrom, Olin and Polglase (ref. 14) considered VII the more likely of the two structures.

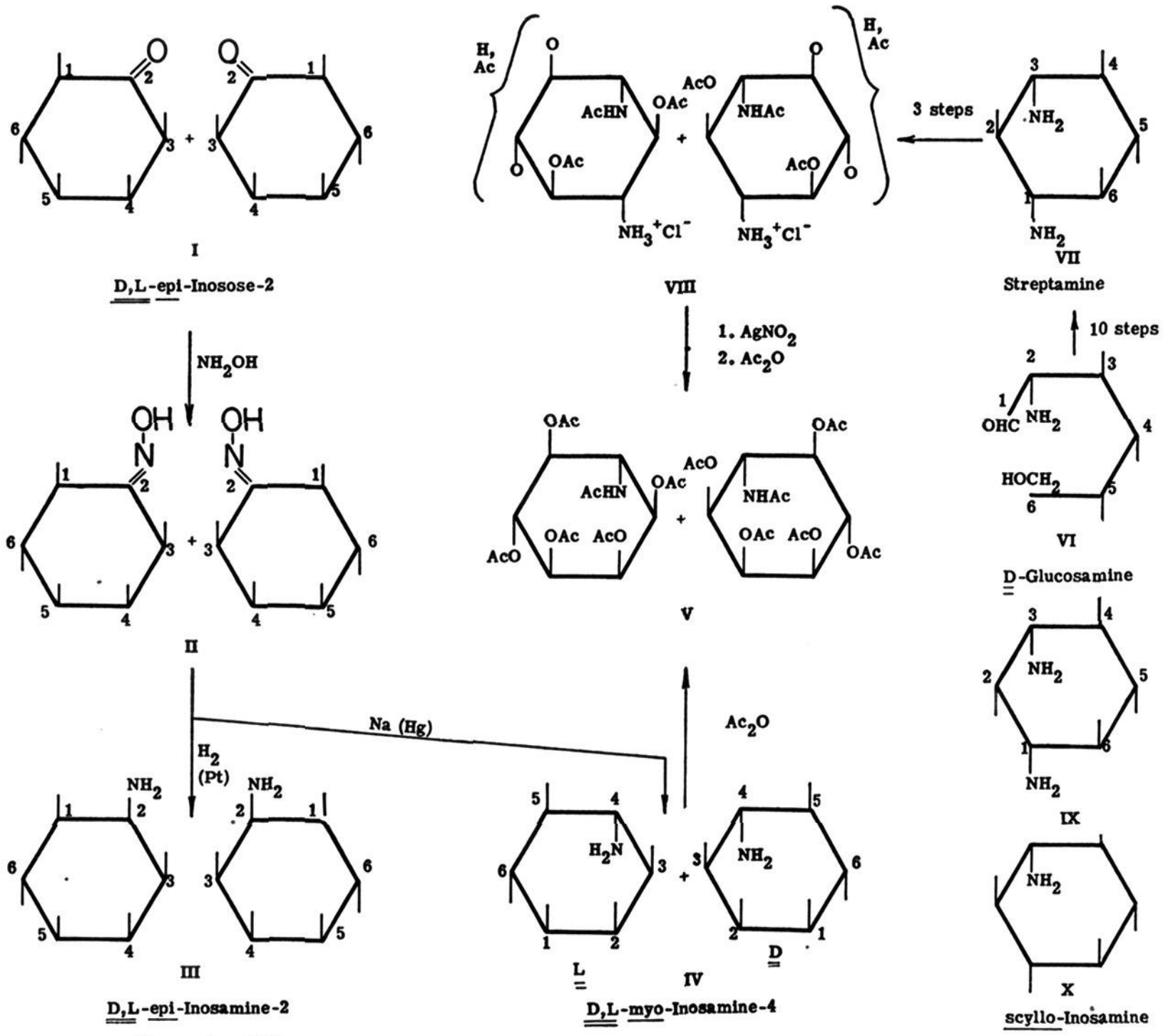


Fig. 1.

arise by the reduction of II. On the other hand replacement of one amino group of VII by hydroxyl *with inversion* gives IV; the deamination of streptamine could not give III. Since a common derivative was obtained from II and from VII, the deamination must have taken place with inversion and the new inosamine must be D,L-*myo*-inosamine-4 (IV).[16] Inosamine-EA is then D,L-*epi*-inosamine-2 (III).

With the epimeric inosamines from *epi*-inosose-2 thus independently identified, it was possible to test the validity of the glycol cleavage and acyl migration methods for determining inosamine configuration. D,L-*epi*-Inosamine-2 was accordingly synthesized, and a portion of it converted to the N-acetate. D,L-*myo*-Inosamine-4 was prepared by the ammonolysis of the hexaacetate to the N-acetate, followed by hydrolysis of this derivative to the free base. Periodate oxidation (free bases) and acetyl migration (N-acetates) studies were then carried out. As was expected, both these processes were more rapid in the *cis* epimer III. The configurational assignments previously made[1] for the *myo*-2–*scyllo* pair are thereby strengthened.

(16) The configuration of I (and therefore of II) was established by T. Posternak, *Helv. Chim. Acta*, **29**, 1991 (1946). In the light of the same author's later work (ref. 7), inversion is to be expected in the deamination of such compounds as VIII.

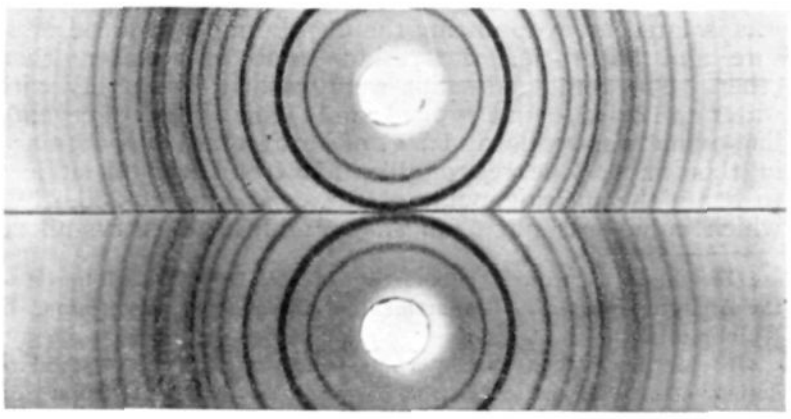


Fig. 2.—X-Ray powder patterns of hexaacetyl-D,L-*myo*-inosamine-4: top, from D,L-*epi*-inosose-2; bottom, from streptamine.

Infrared spectroscopy was considered as a possible additional tool for obtaining information about

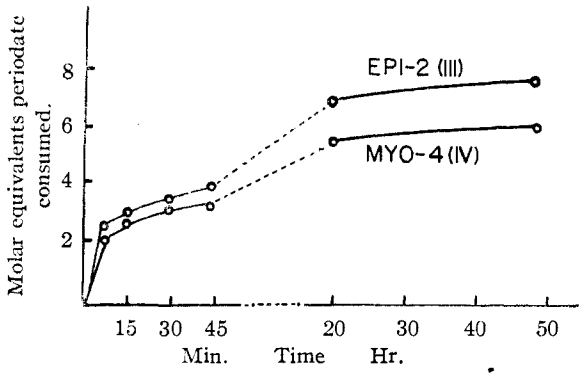


Fig. 3.—Course of the periodate oxidation of the two amines.

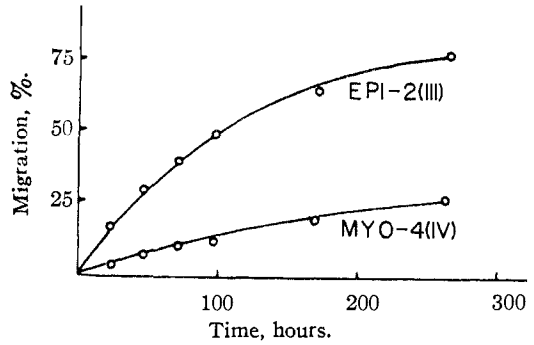


Fig. 4.—Course of the N → O acyl migration.

the configuration of compounds of this type. An examination of the spectra[17] of the two available epimeric pairs of inosamines showed that a strong band at 9.50 μ, tentatively assigned to C–N stretching, is slightly displaced toward the higher wave lengths when the amino group is *cis* to the neighboring hydroxyls. The recorded positions, in μ, of the band were: D,L-*myo*-inosamine-4, 9.50; *scyllo*-inosamine, 9.51; D,L-*epi*-inosamine-2, 9.59 and *myo*-inosamine-2, 9.65. An evaluation of the usefulness of this observation must await the recording of more spectra.

Finally, the N-salicylidene-, N-acetylsalicylidenepenta-O-acetyl- and penta-O-acetate hydrochloride derivatives of *myo*-inosamine-4 were prepared.

Experimental[18]

D,L-*epi*-Inosose-2 (I) was prepared by Posternak's[19,20] procedure. In making the oxime (II), the directions of May and Mosettig[8] were followed carefully. With some batches, the crystallization step was omitted and the oxime solution was treated directly with sodium amalgam.

D,L-*epi*-**Inosamine-2** (III).—Crystalline II was hydrogenated as described by May and Mosettig.[8] The amorphous amine thus obtained was used directly for the periodate oxidation experiment and for conversion to the **hexaacetate.**[8] From the latter the **N-acetate**[8] was obtained for the acyl migration study.

Hexaacetyl-D,L-*myo*-inosamine-4 (V).—The sodium amalgam reduction of II was carried out exactly as described[1] for *myo*-inosose-2 oxime, except for variations in batch size. The salt cake obtained by concentrating the solution *in vacuo* after completion of the reduction was dried first over calcium chloride then over phosphorus pentoxide.[21] Fused sodium acetate and acetic anhydride were then added and the whole refluxed for 1.5 hours. After cooling, the solids were removed by filtration and the remainder of the product was isolated by evaporating the filtrate under reduced pressure and triturating the residue first with water, then ether. The combined solids were washed thoroughly with water and dried. In a typical run in which 2.5 g. of crystalline oxime was reduced, 1.7 g. of hexaacetate was obtained in this way. One recrystallization from ethanol sufficed to bring the melting point of the compound to 236–239°, which value was not changed by additional recrystallizations. The recovery in the crystallization step is nearly quantitative if the mother liquors are worked up. The material which was furnished to Dr. Wintersteiner and later submitted for X-ray analysis was a twice recrystallized sample. A mixture of this hexaacetate and that of D,L-*epi*-inosamine-2 melted over a wide range beginning at 150°.

Additional crude material (1.9 g. in the run described) was isolated by chloroform extraction of the aqueous wash liquors, but it is not known whether it can be purified sufficiently for further use.

Anal. Calcd. for $C_{18}H_{25}O_{11}N$ (431.39): C, 50.11; H, 5.84; N, 3.25. Found: C, 49.93; H, 5.88; N, 3.02, 2.94.

N-Acetyl-D,L-*myo*-inosamine-4.—The hexaacetyl compound (1.62 g.) was dissolved in 50 ml. of methanolic ammonia (saturated at 25°). After standing 12 hours at 25°, the solution was kept in the cold room at −3° overnight. The N-acetyl-D,L-*myo*-inosamine-4 which separated was recrystallized by suspending it in *ca.* 20 volumes of boiling 95% ethanol and adding water dropwise until solution took place, then filtering and allowing the solution to stand. The amount of pure product obtained on one recrystallization with working up of the mother liquors was 0.78 g. (94%), m.p. 270–271° (dec.).

Anal. Calcd. for $C_8H_{15}O_6N$ (221.21): C, 43.43; H, 6.84; N, 6.33. Found: C, 43.20; H, 6.95; N, 6.36.

D,L-*myo*-**Inosamine-4** (IV).—The hydrolysis of 1.6 g. of the N-acetyl derivative and the removal of the sulfuric and acetic acids from the solutions were carried out as described[1] for *scyllo*-inosamine. Alternatively, a strong base anion-exchange resin may be used for acid removal. On concentration of the acid-free solution *in vacuo* the amine crystallized out. The product was recrystallized as described above for the N-acetyl derivative. The yield of pure, once recrystallized material was 0.83 g. (64%). D,L-*myo*-Inosamine-4 begins to darken at around 180° and gradually melts with decomposition between 210 and 250°. It is extremely soluble in water.

Anal. Calcd. for $C_6H_{13}O_5N$ (179.17): C, 40.22; H, 7.31. Found: C, 39.97; H, 7.45.

By following the procedures used for the corresponding *myo*-inosamine-2 derivatives, D,L-*myo*-inosamine-4 was successively converted to the salicylaldehyde Schiff base, the acetylsalicylidenepenta-O-acetate and the penta-O-acetate hydrochloride. No effort was made to obtain optimum yields of these derivatives.

N-Salicylidene-D,L-*myo*-inosamine-4.—Yield (crude), 59%. After two recrystallizations from ethanol–water the compound was pure, m.p. 219–221°.

Anal. Calcd. for $C_{13}H_{17}O_6N$ (283.28): C, 55.11; H, 6.05; N, 4.94. Found: C, 54.93; H, 6.18; N, 4.84.

N-Acetylsalicylidenepenta-O-acetyl-D,L-*myo*-inosamine-4.—Yield (crude), 0.45 g. from 0.5 g. of N-salicylidene derivative. The compound is insoluble in ether; slightly soluble in isopropyl alcohol, carbon tetrachloride and Skellysolve B; moderately soluble in ethanol and very soluble in dioxane, benzene and pyridine. A satisfactory analysis was not obtained, due to the decomposition which occurs when the compound is recrystallized from ethanol. One recrystallized sample, when heated in a capillary, underwent a transition at 156–158° and melted at 181–182°.

Penta-O-acetyl-D,L-*myo*-inosamine-4 Hydrochloride.—Yield 0.092 g. from 0.363 g. of the acetylated Schiff base. The ether washed product was dried and analyzed directly,

(17) Obtained through the courtesy of Mr. Donald R. Johnson of the Analytical Division, Department of Chemistry. Determined in Nujol mulls.

(18) Melting points were determined in a copper block of standard design, using capillary tubes and a stock thermometer (76 mm. immersion). Elemental analyses by Micro-Tech Laboratories, Skokie, Illinois. All new compounds were recrystallized to constant melting point.

(19) "Biochemical Preparations," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1952, p. 57.

(20) T. Posternak, *Helv. Chim. Acta*, **19**, 1333 (1936).

(21) The product darkened excessively when sulfuric acid was used as the desiccant.

m.p. 223–225°. The compound is very soluble in water and moderately soluble in neutral dioxane.

Anal. Calcd. for $C_{16}H_{24}O_{10}NCl$ (425.82): C, 45.13; H, 5.68; N, 3.29. Found: C, 45.18; H, 5.77; N, 3.26.

Periodate Oxidation.—Measured quantities (0.005 mmole) of the two free inosamines were treated with 7.65 molar equivalents of sodium metaperiodate heavily buffered with sodium bicarbonate. The rates of periodate consumption are plotted in Fig. 3.

Acyl Migration.—Two-millimole samples of the N-acetyl derivatives were dissolved in 25 ml. of *N* hydrochloric acid. The solutions were maintained at 30 ± 0.5° in glass stoppered flasks and analyzed periodically for free amino groups by the Van Slyke method. The results are plotted in Fig. 4. Control samples of the free amines gave the theoretical quantities of nitrogen in the Van Slyke apparatus.

MADISON 6, WISCONSIN

[CONTRIBUTION FROM THE AVERY LABORATORY OF THE UNIVERSITY OF NEBRASKA]

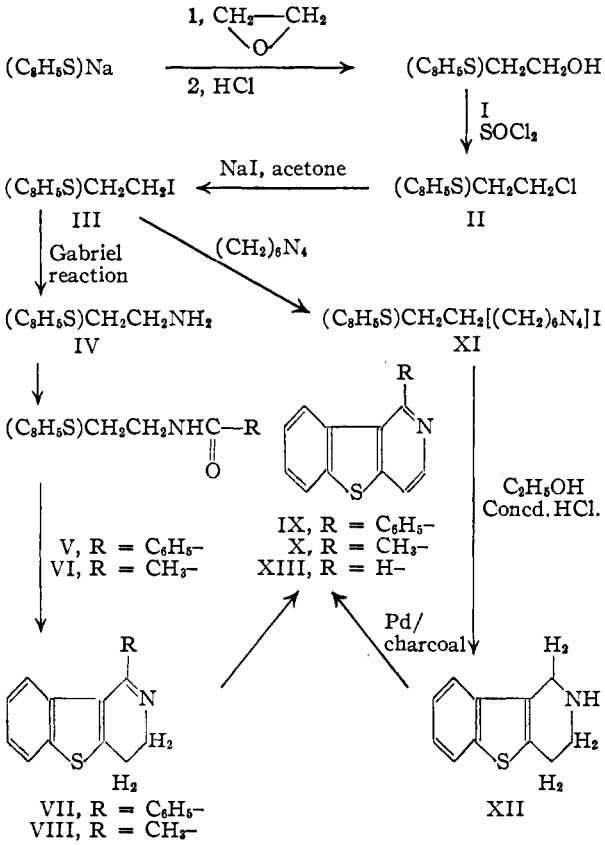
Thianaphtheno[3,2-c]pyridine and Certain Derivatives

BY DAVID B. CAPPS[1] AND CLIFF S. HAMILTON

RECEIVED SEPTEMBER 10, 1952

2-Thianaphthenyl sodium was treated with ethylene oxide, giving 2-thianaphthene-ethanol. The alcohol was converted to its chloro analog and the latter to the iodo compound, which gave 2-thianaphthene-ethylamine by Gabriel's phthalimide reaction. 2-Thianaphthene-ethylamine was benzoylated and acetylated yielding amides which were converted to the corresponding dihydroisoquinoline-like compounds by the Bischler–Napieralski reaction. These amines were then aromatized giving 1-phenyl- and 1-methylthianaphtheno[3,2-c]pyridine. 2-(2-Iodoethyl)-thianaphthene formed an addition compound with hexamethylenetetramine which gave 1,2,3,4-tetrahydrothianaphtheno[3,2-c]pyridine upon alcoholysis in acid, instead of the expected primary amine. Dehydrogenation of this base provided thianaphtheno[3,2-c]pyridine.

Several simple 2-position derivatives of thianaphthene have not been reported. Therefore, it was of interest to synthesize 2-thianaphthene-ethanol and from it, derivatives leading to thianaphtheno[3,2-c]pyridines. The accompanying diagram illustrates the method of synthesis.



2-Thianaphthenyl sodium[2] gave 2-thianaphthene-ethanol (I) on treatment with ethylene oxide. The alcohol was converted to 2-(2-chloroethyl)-thianaphthene (II), and the latter to 2-(2-iodoethyl)-thianaphthene[3] (III). The iodide proved to be the better halide for use in Gabriel's phthalimide reaction.[4] The intermediate substituted phthalimide was hydrolyzed by a modification[5] of Ing and Manske's[6] hydrazine hydrolysis. Benzoylation and acetylation of 2-thianaphthene-ethylamine gave amides (V and VI) which underwent the Bischler–Napieralski reaction[7a] giving 1-phenyl- and 1-methyl-3,4-dihydrothianaphtheno[3,2-c]pyridine (VII and VIII). The ring closures were effected both by using phosphorus pentoxide at elevated temperatures and phosphorus pentachloride at room temperature.[8] Attempts to cyclize the benzamide (V) using phosphorus pentoxide suspended in hot xylene failed until glass balls were introduced into the stirred reaction mixture to prevent the deposit of the phosphate salt of the basic product, insoluble in xylene, on the suspended particles of phosphorus pentoxide.

When phosphorus oxychloride in boiling xylene was used to effect ring closure of the benzamide derivative, the fully aromatized product (IX) was obtained, not the dihydro derivative (VII) which ample precedent[7a] would lead one to expect.

1-Phenyl-3,4-dihydrothianaphtheno[3,2-c]pyridine (VII) was aromatized by heating its xylene solution under reflux in the presence of activated carbon (Nuchar). The methyl analog was dehydrogenated in the presence of palladized charcoal.

Attempts were made to prepare 2-thianaphthene-ethylamine by the Delépine reaction.[9] Treatment of the addition compound (XI) of hexamethylenetetramine and 2-(2-iodoethyl)-thianaphthene

(1) Parke, Davis and Company Fellow.

(2) A. Schönberg, E. Petersen and H. Kaltschmitt, *Ber.*, **66**, 233 (1933).

(3) Procedure patterned after that of F. F. Blicke and J. H. Burckhalter, THIS JOURNAL, **64**, 480 (1942), for 3-(α-thienyl)-propyl iodide.

(4) S. Gabriel, *Ber.*, **20**, 2224 (1887).

(5) H. J. Barber and W. R. Wragg, *J. Chem. Soc.*, 1331 (1947).

(6) H. R. Ing and R. H. F. Manske, *ibid.*, 2348 (1926).

(7) (a) R. Adams, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 74; (b) p. 151.

(8) J. M. Gulland and R. D. Haworth, *J. Chem. Soc.*, 581 (1928).

(9) Delépine, *Bull. soc. chim. France*, **13**, 358 (1895).