Chem. Pharm. Bull. 12 (5) 615 ~ 619

UDC 547.457 : 547.435

88. Chiyoko Satoh (née Takahashi) and Akio Kiyomoto : Studies on Nitrogen-containing Sugars. I. Synthesis of N-Acetyl-D-mannosamine from 1-Nitro-1-deoxy-D-mannitol Pentaacetate.*1

(*Tokyo Research Laboratory, Tanabe Seiyaku Co., Ltd.**2)

Investigation of biochemical functions and metabolism of N-acetylneuraminic acid is one of current problems[1~5] in the field of biochemistry. Since this compound was found to be a condensation product of N-acetyl-D-mannosamine (2-acetamido-2-deoxy-D-mannose) and pyruvic acid,[5~7] biochemical interest in this N-acetylated amino sugar

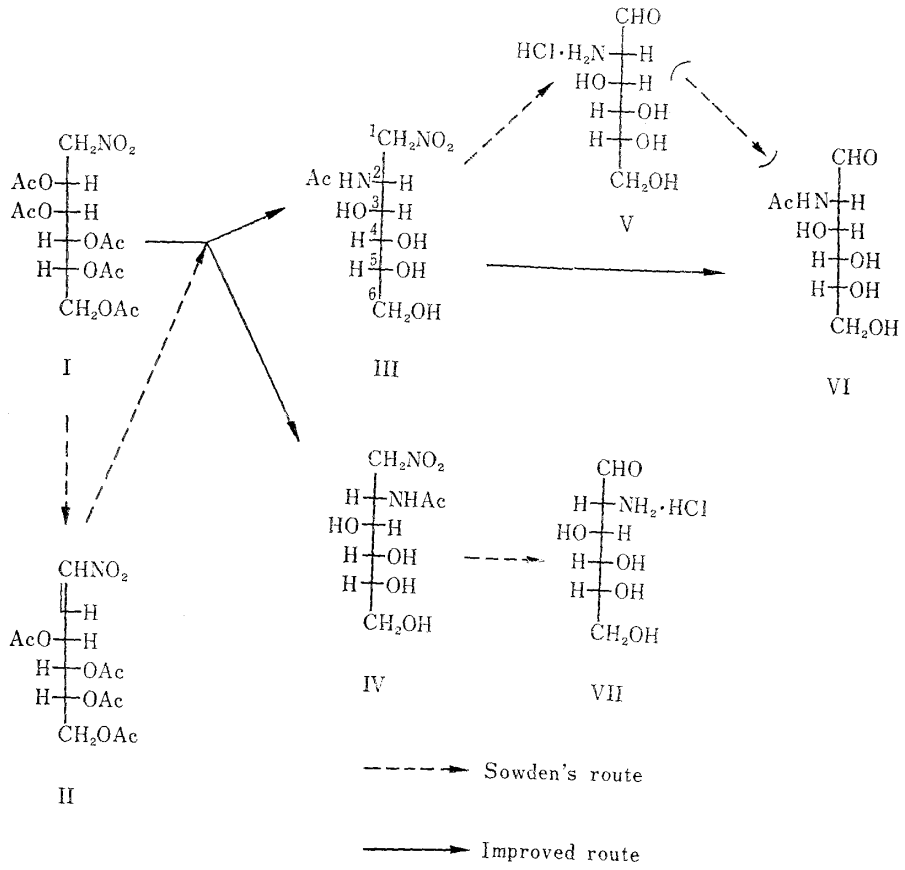

Chart 1.

*1 This work was presented at the 83rd Annual Meeting of Pharmaceutical Society of Japan in Tokyo on November 1, 1963.

*2 Toda-machi, Kita-adachi-gun, Saitama-ken (佐藤(旧姓高橋)千代子, 清本昭夫).

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6) D. G. Comb, S. Roseman : J. Am. Chem. Soc., **80**, 497 (1958); S. Roseman, D. G. Comb : *Ibid.*, **80**, 3166 (1958).
7) R. Kuhn, R. Brossmer : Liebigs Ann. Chem., **616**, 221 (1958).

has been aroused,[1~3,8] and its chemical synthesis in a high yield by a simple route is now desirable.

The Sowden's synthetic method for amino sugars,[9] which is illustrated in Chart 1, appears to be the best route to date,[10~13] and it consists of (1) preparation of D-*arabino*-1-nitro-3,4,5,6-tetraacetoxy-1-hexene (II) by heating an epimeric mixture of D-*arabino*-1-nitro-1-deoxy-hexitol pentaacetates in benzene solution in the presence of sodium hydrogen carbonate, (2) treatment of II with methanolic ammonia to produce 1-nitro-2-acetamido-1,2-dideoxy-D-hexitols (III and IV), and (3) conversion of the nitro-methyl group of III to an aldehydic group by application of the Nef reaction. At the last step in this method, solution of sodium salt of the *aci*-nitro compound is poured into concentrated hydrochloric acid and the reaction mixture is briefly boiled. Then, after cooling to room temperature, the mixture is saturated with hydrochloric acid in order to precipitate sodium chloride, and the filtrate is concentrated at reduced pressure. During this step the N-acetyl group is hydrolyzed, the final product being D-mannosamine hydrochloride (V). For synthesis of N-acetyl-D-mannosamine (VI) a further step of acetylation is required.

The present paper describes a method of synthesis of VI in two steps in a high yield starting from 1-nitro-1-deoxy-D-mannitol pentaacetate (I) as well as from the acetylated mixture of 1-nitro-1-deoxy-D-mannitol and corresponding D-glucitol. The first step involves treating 1-nitro-1-deoxy-D-mannitol pentaacetate (I) with ammonia in methanol. I is converted directly to 1-nitro-2-acetamido-1,2-dideoxy-D-mannitol (III) and its epimeric D-glucitol derivative (IV) and the two products are separated from each other by fractional crystallization. The yields of III and IV were 70% and 8% respectively. The second step brings about direct formation of VI from III. The reaction sequences are illustrated in Chart 1.

Sowden[9] reported that the acidic hydrolysis of the N-acetyl group was much faster in N-acetyl-D-mannosamine than in N-acetyl-D-glucosamine. This was verified by comparing rate of hydrolysis between the two N-acetyl-D-hexosamines. The material was dissolved in 0.5*N* hydrochloric acid and heated in a boiling water bath for a period of time. N-acetyl-D-hexosamine remaining unhydrolyzed was colorimetrically estimated according to the method of Leloir-Reissig[14] based on the Morgan-Elson reaction. The results are shown in Fig. 1. The hydrolysis of N-acetyl-D-mannosamine is about five times faster than that of N-acetyl-D-glucosamine. The above results being taken into account, conditions of the Nef reaction were sought, in which VI would be directly obtained from III with minimal deacetylation by the acid. The

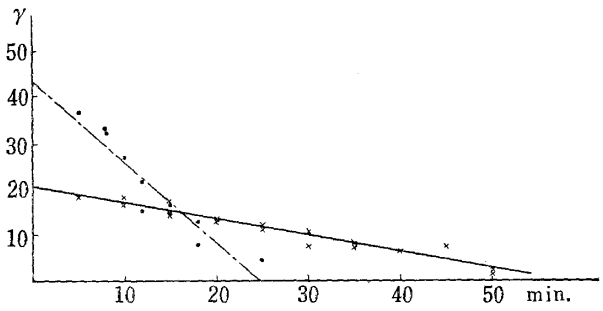

Fig. 1. Removal of N-Acetyl Groups by Acid Hydrolysis

------ N-Acetyl-D-mannosamine
———— N-Acetyl-D-glucosamine

8) S. Roseman, F. Hayes, S. Ghosh : Federation Proc., **19**, 85 (1960).
9) J. C. Sowden, M. L. Oftedahl : J. Am. Chem. Soc., **82**, 2303 (1960); J. C. Sowden, H. O. L. Fischer : *Ibid.*, **69**, 1048 (1947).
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best result was obtained by addition of an aqueous barium hydroxide solution of III to an excessive amount of cold dilute sulfuric acid, and by subsequent neutralization of the mixture with barium hydroxide at a controlled temperature (20°). The yield of N-acetyl-D-mannosamine thus obtained was 66%.

The above procedure for preparation of III can be carried out with the syrupy mixture of the two D-*arabino*-1-nitro-1-deoxy-hexitol pentaacetates (I and its epimer), which are possibly contaminated with acetylated D-arabinose.

An interesting phenomenon was observed on the optical properties of the two 1-nitro-2-acetamido-1,2-dideoxy-D-hexitols, III and IV. Both compounds were levorotatory at the sodium D-line ($[\alpha]_D^{25}$ in water: III −13.0°, IV −8.1°). However, in their optical rotatory dispersion, III exhibited a negative Cotton effect with a trough at 334 mμ, whereas its epimer IV showed a positive Cotton effect with a peak at 346 mμ. The optical rotatory dispersion curves and ultraviolet spectra of III and IV are shown in Figs. 2 and 3 respectively.*3 Evidently the marked difference in the optical rotatory

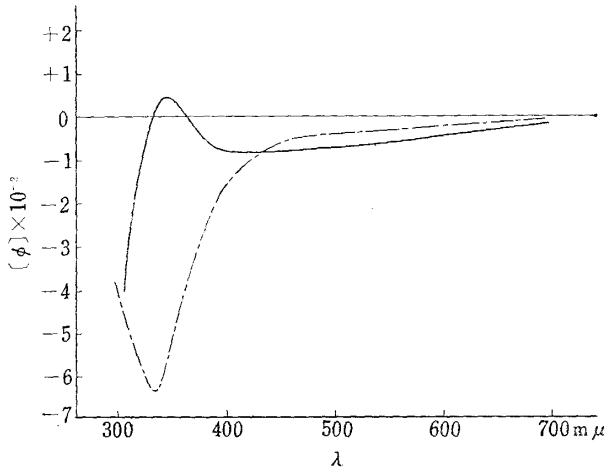

Fig. 2. Optical Rotatory Dispersion Curves (in water) of III and IV

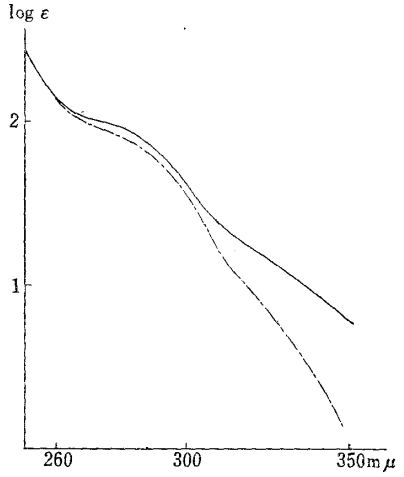

Fig. 3. Ultraviolet Absorption Spectra (in water) of III and IV

----- III ——— IV

dispersion curves of III and IV is due to the difference in the absolute configuration at C-2 adjacent to the nitro-methyl group. Regarding the optical rotatory dispersion of compounds having nitro chromophores, several papers were published on nitrooctane,[15] dinitrocamphane[16] and a series of nitrosteroids.[17] However, there are few reports dealing with optical rotatory dispersion of carbohydrates. The present findings on the two 1-nitro-2-acetamido-1,2-dideoxy-D-hexitols might give a clue to the problem of absolute configuration in aldosugars and corresponding amino sugars. This point will be the subject of a separate paper to be published later.

Experimental*4

1) 1-Nitro-2-acetamido-1,2-dideoxy-D-mannitol (III) and 1-Nitro-2-acetamido-1,2-dideoxy-D-glucitol (IV)——a) From 1-deoxy-1-nitro-D-mannitol pentaacetate[9] (I): I (13.1 g.) was dissolved in MeOH (140

*3 Japan Spectroscopic Manufacturing Co. (Nippon Bunko) recording spectropolarimeter (JASCO model ORD/UV-5), and Hitachi Co. recording spectrophotometer (Type EPS-2U) were employed in ORD and UV measurements, respectively.

*4 All melting points were measured in H_2SO_4 bath and not corrected.

15) C. Djerassi: "Optical Rotatory Dispersion; Applications to Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, page 194~195.
16) S. Mitchell, R. R. Gordon: J. Chem. Soc. **1936**, 853.
17) C. Djerassi, H. Wolf, E. Bunnenberg: J. Am. Chem. Soc., **85**, 2835 (1963).

ml.) with slight warming and the resulting solution was cooled to 0° with salted ice H_2O. Anhyd. NH_3 gas was passed into the solution. After 3 to 4 min. some crystals appeared, which redissolved in next few min. The solution turned red and vigorous uptake of NH_3 continued. After approximate saturation with NH_3 (about in 2 hr.), the solution was left to stand overnight at room temperature under protection from moisture with a drying tube, and concentrated *in vacuo* to a semicrystalline mass. Filtration and washing with cold EtOH gave 5.47 g. of III, m.p. 168~172°. The mother liquor and washings were concentrated to a syrup, which was then extracted several times by trituration with $CHCl_3$ in order to remove acetamide. The residue was crystallized from EtOH to yield additional 0.4 g. of III, m.p. 165~171° (total yield, 74.8%), and the mother liquor, after partial concentration, gave 0.78 g. of IV, m.p. 152~153°. Recrystallization of the total product (5.87 g.) of III from EtOH gave 5.53 g. (70.5%) of needles, m.p. 173° and $[\alpha]_D^{25}$ −13.0° (c=2.6, H_2O). *Anal.* Calcd. for $C_8H_{16}O_7N_2$: C, 38.11; H, 6.39; N, 11.10. Found: C, 38.04; H, 6.46; N, 10.99. ORD in H_2O (c=0.745): $[\phi]_{700}$ 0°; $[\phi]_{589}$ −33.9°; $[\phi]_{500}$ −40.7°; $[\phi]_{334}$ −638.0° (trough); $[\phi]_{300}$ −407.3°.

Recrystallization of the crude product of IV (0.78 g.) from EtOH yielded 0.65 g. (8.3%) of needles, m.p. 153~155° and $[\alpha]_D^{25}$ −8.1° (c=2.28, H_2O). *Anal.* Calcd. for $C_8H_{16}O_7N_2$: C, 38.11; H, 6.39; N, 11.10. Found: C, 38.22; H, 6.39; N, 11.16. ORD in H_2O (c=0.50); $[\phi]_{700}$ −29.9°; $[\phi]_{589}$ −59.7°; $[\phi]_{364}$ 0°; $[\phi]_{355}$ +39.8°; $[\phi]_{346}$ +49.8° (peak); $[\phi]_{335}$ 0°; $[\phi]_{308}$ −417.9°.

b) From D-arabinose: According to the procedure reported by Sowden,[9] mixed pentaacetates of 1-nitro-1-deoxy-D-mannitol and its epimer were synthesized from D-arabinose by condensation with CH_3NO_2, deionization with ionexchange resin (Dowex-50, H^+-form), acetylation, and extraction with $CHCl_3$. The concentrate of the dried $CHCl_3$ extract was syrupy in most cases. Ten grams of D-arabinose gave about 22 g. of syrup containing both pentaacetates, possibly contaminated with tetraacetyl-D-arabinose. When treated similarly as described in a), 7.9 g. of the above acetate mixture gave 1.52 g. (26.5% from D-arabinose) of III, m.p. 170~173°, and 0.19 g. (3.3% from D-arabinose) of IV, m.p. 153~155°.

2) **N-Acetyl-D-mannosamine (VI)**——A solution containing $Ba(OH)_2 \cdot 8H_2O$ (13.78 g.) in H_2O (220 ml.) was prepared and cooled. III (11.24 g.) was dissolved in the $Ba(OH)_2$ solution and the solution was added dropwise with vigorous stirring to cold dil. H_2SO_4 consisting of 23 g. of conc. H_2SO_4 and 96 ml. of H_2O. After stirring for 4 hr. at 20°, the reaction mixture was left to stand at 20° for 15 hr. The solution was neutralized with saturated $Ba(OH)_2$ solution and centrifuged. The precipitates were washed twice with H_2O in order to minimize the loss of VI. Combined supernatants were concentrated *in vacuo* to a syrup, which was then extracted with 30 ml. of 95% EtOH. This EtOH extract was concentrated under reduced pressure to a syrup. This syrup was extracted with EtOH and concentration was repeated. The resulting hygroscopic syrup was dissolved in 40 ml. of 50% EtOH, and Me_2CO was slowly added until the solution became slightly turbid. When stored in a refrigerator overnight, the crystallization was complete. The resulting crystalline VI-monohydrate was collected and washed with EtOH. The mother liquor and washings were combined and concentrated to a syrup, which was then dissolved in 50% EtOH, and the addition of Me_2CO for crystallization was repeated. Combined preparations of crude VI-monohydrate were recrystallized from 50% EtOH by addition of Me_2CO to yield 7.1 g. (66.3%) of VI-monohydrate as platelets (leaflets), m.p. 108~110° and $[\alpha]_D^{25}$ −6.5° (7 min.) to +7.2° (2 hr.) (c=2.315, H_2O). *Anal.* Calcd. for $C_8H_{15}O_6N \cdot H_2O$: C, 40.16; H, 7.16; N, 5.85. Found: C, 40.71; H, 7.06; N, 5.90.

3) **Hydrolysis of N-Acetyl-D-hexosamines**——Determination of N-acetyl-D-hexosamine was carried out according to the Leloir-Reissig's method[14] with slight modification.

a) Reagents for quantitative determination of N-acetyl-D-hexosamines: Borax solution; 40 ml. of 0.05*M* $Na_2B_4O_7$ was mixed with 1.725 ml. of 2*N* NaOH. (When 1 ml. of this solution was mixed with 0.2 ml. of 0.5*N* HCl, the pH was 8.9).

Reissig's dimethylamino benzaldehyde (DMAB) reagent; 10 g. of *p*-DMAB was dissolved in 100 ml. of analytically pure glacial AcOH contg. 12.5% (v/v) of 10*N* HCl (analytical reagent). Shortly before use the solution was diluted with 9 volumes of glacial AcOH of reagent grade.

b) Hydrolysis and assay procedure: A solution of N-acetyl-D-glucosamine (10.15 mg.) in 0.5*N* HCl (100 ml.) and a solution of N-acetyl-D-mannosamine monohydrate (9.4 mg., corresponding to 8.686 mg. of anhydrous substance) in 0.5*N* HCl (40 ml.) were used for the test. Glass stoppered test tubes, each containing 0.2 ml. of the above test solution, were heated in a boiling water bath. At various intervals the tubes were taken out and cooled in ice H_2O. One milliliter each of the cold borax solution was added. The tubes were kept in ice H_2O till the last tube was treated in the above way. Then, the tubes were heated in a vigorously boiling water bath for exactly 3 min. and cooled with tap H_2O. Three milliliters of DMAB reagent was added, and, immediately after mixing, the tubes were placed in a bath at 37°. After 20 min. the tubes were cooled with tap H_2O and optical density values of solutions were read without delay at 585 mμ with Hitachi (EPU-2) spectrophotometer. The results are presented in Fig. 1 as the amounts of N-acetyl-D-hexosamine remaining in the solutions against time length of acidic hydrolysis. The accuracy of the present colorimetric determination was ascertained beforehand by observing that N-acetyl-D-hexosamines dissolved in 0.5*N* HCl (10 to 50 γ of N-acetyl-D-glucosamine, and 20 to 100 γ of N-acetyl-D-mannosamine) gave optical densities exactly proportional to the amounts present in the solutions tested.

The authors express their gratitude to Prof. S. Akamatsu, Professor Emeritus of Biochemistry, Chiba University Medical School, for his kind advices, and to Dr. K. Abe, Dr. T. Okuda for their encouragement throughout the studies. They are indebted to the staff of Central Analysis Room of Tokyo Research Laboratory, Tanabe Seiyaku Co., Ltd., for measurements of UV spectra and ORD and elemental analysis.

Summary

The Sowden's synthetic procedure of D-mannosamine hydrochloride was simplified, and modified in order to prepare N-acetyl-D-mannosamine. By the action of ammonia on 1-nitro-1-deoxy-D-mannitol pentaacetate (I), I was directly transformed in a yield of 70.5% to 1-nitro-2-acetamido-1,2-dideoxy-D-mannitol (III) without isolating the intermediate, D-*arabino*-1-nitro-tetraacetoxy-1-hexene (II). 1-Nitro-2-acetamido-1,2-dideoxy--D-glucitol (IV) was produced as a by-product in a yield of 8.3%. The barium salt of *aci*-form of III was decomposed with sulfuric acid in the cold in order to prevent hydrolysis of the N-acetyl group and facilitate separation of the product from the inorganic salt. The yield of N-acetyl-D-mannosamine was 66.3%. An unfractionated mixture of the two epimeric D-*arabino*-1-nitro-1-deoxy-hexitols which had been obtained from D-arabinose was acetylated and transformed to III in a yield of 26.5% from D-arabinose.

III and IV exhibited a negative and a positive Cotton effect respectively in their optical rotatory dispersion curves.

(Received January 14, 1964)

[Chem. Pharm. Bull. 12 (5) 619 ~ 623]

UDC 547.852.7 : 542.943

89. Ikuo Suzuki and Toshiaki Nakashima : Studies on Cinnolines. I. N-Oxidation of 4-Chlorocinnoline and 4-Methoxycinnoline.

(*National Institute of Hygienic Sciences*[*1])

It has been reported[1] that cinnoline and 4-methylcinnoline gave their 1-oxide and 2-oxide on treatment with hydrogen peroxide in acetic acid, and the ratio of 1-oxide to 2-oxide was 1:2 in both cases.

The present paper deals with N-oxidation of 4-chlorocinnoline and 4-methoxycinnoline, and syntheses of 4-hydroxycinnoline N-oxides.

When 4-chlorocinnoline (I) was treated with phthalic monoperacid in ethereal solution at a room temperature for two weeks, two kinds of new N-oxide (III, m.p. 94~94.5°, colorless needles, and IV, m.p. 150~151°, yellow needles) were isolated in 28% and 43% yields, respectively. III and IV gave analytical values corresponding to 4-chlorocinnoline mono-N-oxide. Catalytic reduction of 4-chlorocinnoline gave mainly 4,4'-bicinnoline,[2] but catalytic hydrogenation of III over palladium charcoal in ethanolic solution containing sodium hydroxide gave known cinnoline 1-oxide (V),[1] m.p. 111~112°, in 62% yield,

*1 Tamagawa-yoga, Setagaya, Tokyo (鈴木郁生, 中島利章).

1) M. Ogata, *et al.* : This Bulletin, **11**, 1527 (1963).
2) J. S. Morley : J. Chem. Soc., **1951**, 1971.