The Synthesis of 4-Amino-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (3-Deazaadenosine) and Related Nucleotides

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ABSTRACT: Fusion of 4-chloroimidazo[4,5-*c*]pyridine with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose yielded 4-chloro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (II) as the major product. Compound II was deacetylated to 4-chloro-1-(β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (III), which was treated with hydrazine to give the intermediate 4-hydrazino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (IV). Compound IV was not characterized but was reduced directly with Raney nickel to yield 4-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (V, 3-deazaadenosine). The site of attachment of the ribosyl moiety and the anomeric configuration of these nucleosides were determined by chemical means. Incorporation of 3-deazaadenosine into the terminal position of transfer ribonucleic acid (t-RNA) should be of assistance in elucidating the acceptor function of soluble ribonucleic acid (s-RNA) in protein biosynthesis, since the presence of N^3 in adenosine has been postulated as essential to this process.

The antibiotic tubercidin has been[1,2] shown to be 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine (7-deazaadenosine, Suzuki and Marumo, 1961; Mizuno *et al.* 1963a). Tubercidin substitutes for adenosine in a wide variety of cellular and enzymatic reactions (Acs *et al.*, 1964). Tubercidin (7-deazaadenosine) has been found to be incorporated in both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in a number of biological systems (Acs *et al.*, 1964) and to be highly active toward inhibition of purine biosynthesis *via* a feedback mechanism (Bennett and Smithers, 1964). Tubercidin has shown a striking ability to inhibit the incorporation of nucleosides in a variety of human tumors (Wolberg, 1965). Acs, *et al.* (1964) have shown that tubercidin inhibits the growth of several types of virus. The structure of tubercidin suggests the synthesis of other deaza derivatives of adenosine.

Hecht *et al.* (1959) have shown that the esterification of an amino acid to the 2′- or 3′-hydroxyl of an s-RNA chain does not occur unless the terminal 5′ mononucleotide is an adenylyl residue. This suggests that the adenine moiety plays a significant role in this esterification process. Zamecnik (1962) has proposed that in the terminal adenylyl residue of s-RNA the 2′-hydroxyl proton hydrogen bonds to the N^3 of adenine to give a more nucleophilic oxygen at the 2′ position which can then undergo esterification by an activated amino acid residue. The migration of an acyl group from the 2′ position to the 3′ position is well documented (Kupchan *et al.*, 1962; Reese and Trentham, 1965). Thus, it is quite possible that the presence of N^3 of adenylic acid in s-RNA is vital in the process of protein biosynthesis. In this regard it seemed of interest to prepare 3-deazaadenosine (V). Incorporation of V into the terminal position of s-RNA should give a polymer incapable of accepting an amino acid residue. Thus 3-deazaadenosine (V) could well shed considerable light on the mechanism of protein biosynthesis.

The Synthesis of 4-Amino-1-β-D-ribofuranosylimidazo-[4,5-c]pyridine (V, 3-Deazaadenosine). In planning the chemical synthesis of V, 4-chloroimidazo[4,5-*c*]pyridine (Rousseau and Robins, 1965) was selected as the starting material. Due to the possible contamination of the desired nucleoside with mercuric ions (Bartosek and Šorm, 1962), the new acid-catalyzed fusion synthesis was chosen for attachment of D-ribose (see Robins *et al.*, 1964). 4-Chloroimidazo[4,5-*c*]pyridine (I) (Scheme I) was heated with an excess of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose at 160° *in vacuo* in the presence of chloroacetic acid as a catalyst (Robins and Robins, 1965). 4-Chloro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (II) was isolated from the reaction mixture as a crystalline solid in 72% yield. Deacetylation with alcoholic ammonia gave 4-chloro-1-(β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (III). Another description of the preparation of III in lesser yield *via* the mercury salt procedure has recently appeared (Mizuno *et al.*, 1964). Early attempts to convert 4-chloro-(1-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (III) to 3-deazaadenosine (V) directly by amination with alcoholic ammonia, liquid ammonia, and aqueous ammonia at elevated temperature were unsuccessful. It is noteworthy that the presence of only one nitrogen

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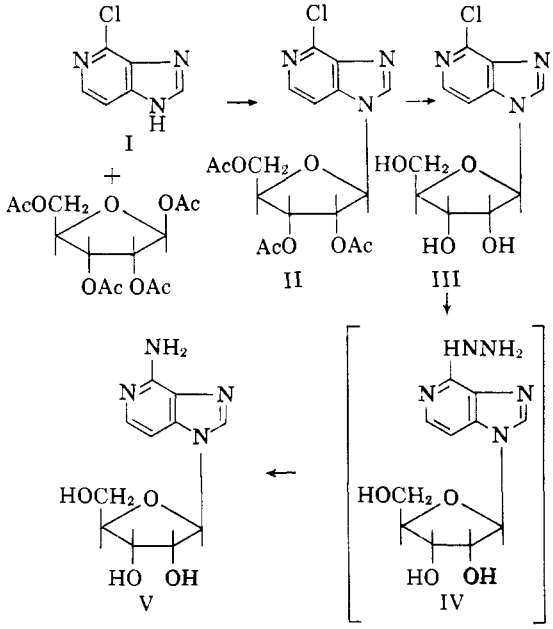


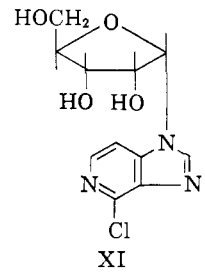
SCHEME I

(N^5) in the six-membered ring greatly reduced the susceptibility of the 4-chloro group to nucleophilic displacement. The corresponding purine derivative by comparison (6-chloro-9-β-D-ribofuranosylpurine, Robins, 1960) is readily converted to adenosine with alcoholic ammonia heated in an open vessel on the steam bath. However, when III was treated with hydrazine, in a nitrogen atmosphere, nucleophilic substitution readily occurred to yield the corresponding 4-hydrazino derivative IV. Attempts to purify IV resulted in considerable colorization and decomposition of the products. Therefore IV was converted directly with Raney nickel to the desired 3-deazaadenosine (V) in approximately 60% yield.

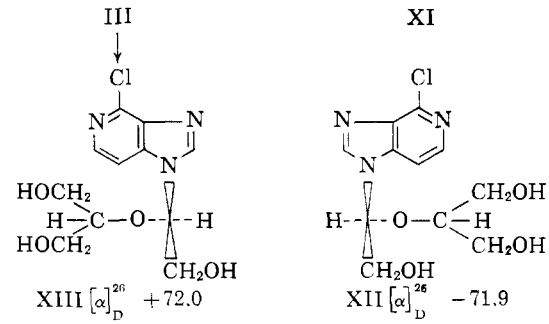
Acid hydrolysis of V gave 4-aminoimidazo[4,5-*c*]pyridine as judged by rigorous comparison with an authentic sample (Salemink and Van der Want, 1949). D-Ribose was also confirmed as being present among the hydrolysis products.

The position of attachment of the D-ribose moiety in 4-chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (III) was rigorously established as position one by catalytic dehalogenation to yield 1-β-D-ribofuranosylimidazo-[4,5-*c*]pyridine. The structure of this latter compound had been previously determined with regard to the site of the sugar (Mizuno *et al.*, 1963b, 1964). It is interesting to note that the mercuric salt coupling reaction of I gave an approximately equal amount of nucleoside product with the D-ribose moiety at position 1 and position 3 (Mizuno *et al.*, 1964). The fusion procedure, on the other hand, gave no evidence of sugar attachment at any position other than position 1.

The assignment of anomeric structure to 4-chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (III) has previously been made (Mizuno *et al.*, 1964) without proof. The recent report of the synthesis of α and β anomers of adenosine from the acid-catalyzed fusion reaction of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose and N^6-acetyladenine (Pichat *et al.*, 1964) suggested that a proof of the β configuration would be desirable. The pooled filtrates from the isolation of II gave after careful work-up with alcoholic ammonia a small amount of a compound XI isomeric with III. D-Ribose was assigned



to position 1 by inspection of the ultraviolet absorption spectrum of XI. Since compounds III and XI were suspected to be an anomeric pair, an effort was made to obtain supporting evidence. In the absence of an N^3 nitrogen (in the purine ring) the usual method of establishing β configuration by cyclonucleoside formation was impossible. In an effort to assign configuration on the basis of the coupling constants of the anomeric proton, 4-chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]-pyridine (III) and 4-chloro-1-α-D-ribofuranosylimidazo-[4,5-*c*]pyridine (XI) were converted to their respective 2′,3′,-*O*-isopropylidene derivatives by the method of Hampton (1961). The coupling constant for the anomeric proton of 4-chloro-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine in deuteriochloroform was found to be 2.4 cps as compared to 3.3 cps for the anomeric proton of 4-chloro-1-(2′,3′,-



O-isopropylidene-α-D-ribofuranosyl)imidazo[4,5-*c*]pyridine in the same solvent. The assignment of the lower coupling constant to the β anomer is in accord with previous studies (Lemieux and Lawn, 1963; Leonard and Laursen, 1963). However the comparatively low value for the α nucleoside and the relatively small difference between the coupling constants observed suggested further evidence would be desirable. To establish conclusively that compounds III and XI are indeed an anomeric pair, each derivative was subjected to periodate oxidation followed by reduction with sodium borohydride according to the procedure of Wright *et al.* (1958). By this procedure the dialde-

hydes are reduced to the corresponding alcohols and the anomeric ribonucleosides are converted to a *dl* pair which should possess optical rotations of equal magnitude and opposite sign. Thus 4-chloro-1-α-D-ribofuranosylimidazo[4,5-*c*]pyridine (XI), $[\alpha]_D^{26}$ +2.7°, gave compound XII, $[\alpha]_D^{28}$ −71.9°, and 4-chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (III), $[\alpha]_D^{27}$ −39.1°, gave compound XIII, $[\alpha]_D^{26}$ +72.0. This is to be compared to α- and β-adenosine. α-Adenosine ($[\alpha]_D$ 24°) and β-adenosine ($[\alpha]_D$ −60.4°) gave a *dl* pair with rotation of −66 and +66°, respectively (Wright *et al.*, 1958). This information established III and XI as β and α anomers and lends considerable support to the assignment of III as the β anomer. Since 3-deazaadenosine (V) was prepared from III, it follows that this evidence is equally applicable to the assignment of V as the β anomer.

In view of the recent synthesis and biochemical properties of nicotinamide–1-deazapurine dinucleotide (Woenckhaus and Pfleiderer, 1965) it would be of considerable interest to study 3-deazaadenosine as a component of the various coenzyme systems, since N^1 and/or N^3 have been postulated as binding sites for NAD (Woenckhaus and Pfleiderer, 1965). The incorporation of 3-deazaadenosine into nucleic acid may be extremely helpful in the investigation of the template functions and binding properties of nucleic acids. This could be especially useful in uncovering any special role in which N^3 functions as a binding site or as a nucleophilic center such as that postulated in protein biosynthesis. In addition the absence of N^3 should increase the basicity of the remaining nitrogen N^1 which would then undergo protonation and/or hydrogen bonding more readily. R_F values of these interesting new nucleoside derivatives are given in Table I and the ultraviolet absorption spectral data are recorded in Table II.

TABLE I: R_F Values for Various 4-Substituted Imidazo-[4,5-*c*]pyridine Nucleosides.

Compound	Solvent Systems A	B	C
4-Chloro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[4,5-*c*]pyridine (II)		0.87	0.92
4-Chloro-1-β-D-ribofuranosyl-imidazo[4,5-*c*]pyridine (III)	0.59	0.73	0.31
4-Chloro-1-α-D-ribofuranosyl-imidazo[4,5-*c*]pyridine (XI)	0.60	0.74	0.25
4-Amino-1-β-D-ribofuranosyl-imidazo[4,5-*c*]pyridine (V)	0.44	0.56	0.02
4-Chloro-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-imidazo[4,5-*c*]pyridine	0.65	0.83	0.90
4-Chloro-1-(2′,3′-*O*-isopropylidene-α-D-ribofuranosyl)-imidazo[4,5-*c*]pyridine	0.66	0.86	0.91

TABLE II: Ultraviolet Spectra[a] of 4-Substituted Imidazo-[4,5-*c*]pyridine Nucleosides.

Compound	pH 1 λ_{max}	pH 1 ϵ_{max}	pH 11 λ_{max}	pH 11 ϵ_{max}
4-Chloro-1-(2′,3′,-5′-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazo[4,5-*c*]-pyridine (II)	255 265 272	5,300 6,200 6,300	254 265 272	6,700 6,100 4,200
4-Chloro-1-β-D-ribofuranosyl-imidazo[4,5-*c*]-pyridine (III)	253 266 273	5,600 5,770 4,770	252 265 273	7,140 5,940 4,750
4-Chloro-1-α-D-ribofuranosyl-imidazo[4,5-*c*]-pyridine (XI)	256[c] 270	4,550 6,300	255 265 273	7,400 6,850 5,700
4-Amino-1-β-D-ribofuranosyl-imidazo[4,5-*c*]-pyridine (V)	262 267–280[b]	10,300 9,500	265	10,800
4-Chloro-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-imidazo[4,5-*c*]-pyridine	255 267 272	5,850 6,500 6,500	253 264 272	7,800 6,500 5,200
4-Chloro-1-(2′,3′-*O*-isopropylidene-α-D-ribofuranosyl)-imidazo[4,5-*c*]-pyridine	257[b] 267 273	4,250 6,500 6,500	251 265 272	6,850 5,550 4,550

[a] Determined on a Beckman DK-2 spectrophotometer. [b] Shoulder. [c] Broad peak.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Optical rotations were taken with a Sargent polarimeter with a 2-dm path length. Chromatograms were developed using Whatman No. 1 chromatography paper in the following solvent systems: A, 20% ammonium carbonate, aqueous, descending; B, ethanol–water (7:3, v/v), descending; C, 1-butanol saturated with water, ascending.

4-Chloro-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-imidazo[4,5-c]pyridine (II). 4-Chloroimidazo[4,5-*c*]pyridine (I) (Rousseau and Robins, 1965) (5.0 g) was mixed with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (Zinner, 1953) (20.0 g) in a pear-shaped flask and heated in an oil bath (bath temperature 175°). After melting was observed, chloroacetic acid (25 mg) was added and

heating was continued until a homogeneous melt was obtained (inside temperature 160°). Vacuum (1 mm) was applied and the melt was heated until nearly all acetic acid was removed (0.5 hr). After the melt had cooled it was dissolved in methanol (100 ml) and a small amount of dark material was removed by filtration through celite. The celite pad was washed with methanol and the filtrate was evaporated to dryness *in vacuo*. The syrup was dissolved in methylene chloride (50 ml) and slowly poured into anhydrous ether (1 l.). The light tan flocculent precipitate, which readily darkened when exposed to air, was removed by filtration and discarded. The light yellow filtrate was evaporated to a syrup *in vacuo*, and this syrup was triturated with anhydrous benzene (50 ml), whereupon unreacted 4-chloroimidazo[4,5-*c*]pyridine (2.3 g) crystallized and was collected by filtration. The filtrate was extracted four times with cold saturated aqueous sodium bicarbonate solution (50-ml portions), and once with cold water. A total of 3.0-g of 4-chloroimidazo[4,5-*c*]-pyridine was removed as unreacted starting material. The benzene phase was dried over anhydrous sodium sulfate and applied to a column prepared with 300 g of alumina (Merck Aluminum Oxide acid washed, suitable for chromatographic absorption) and eluted with 5 l. of benzene. This fraction was found to contain unreacted 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose. The column was then eluted with 5 l. of 20% ethyl acetate–80% benzene mixture and finally with 1 l. of ethanol. The combined light yellow eluents were evaporated to a syrup *in vacuo* (the first 500-ml eluent of the ethyl acetate–benzene mixture was found to contain a small amount of unreacted tetra-*O*-acetyl-β-D-ribofuranose). The residual syrup was dissolved in a minimal amount of ethanol and allowed to stand overnight at 10°. A colorless crystalline material was collected by filtration (3.8 g) to give a yield (based on recovered I) of 72%. The solid crystalline material was recrystallized from a mixture of acetone and *n*-heptane to give an analytically pure sample of 4-chloro-1-(2′,3′,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (II), mp 158.5–159°.

Anal. Calcd for $C_{17}H_{18}ClN_3O_7$: C, 49.7; H, 4.39; N, 10.2. Found: C, 49.7; H, 4.63; N, 10.0.

4-Chloro-1-β-D-ribofuranosylimidazo[4,5-c]pyridine (III). 4-Chloro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (II) (1.0 g) was added to methanolic ammonia saturated at 0° (50 ml) and the solution was stirred for 14 hr at room temperature. The solution was then evaporated to a syrup *in vacuo*, leaving a glass. This product was dissolved in a small amount of absolute ethanol to which a few drops of ethyl acetate was added. After the solution had cooled, crystals formed which were collected and recrystallized from a mixture of ethanol and *n*-heptane to yield 700 mg of colorless needles, mp 192.5–193.5°. Optical rotation observed $[\alpha]_D^{27}$ −39.1° (*c* 1.02, methanol) (Mizuno *et al.*, 1964, record mp 189–190°, optical rotation $[\alpha]_D^{19}$ −41.6° (*c* 1.25, methanol)).

Anal. Calcd for $C_{11}H_{12}ClN_3O_4$: C, 46.3; H, 4.21; N, 14.7. Found: C, 46.4; H, 4.17; N, 14.8.

4-Chloro-1-α-D-ribofuranosylimidazo[4,5-c]pyridine (XI). The collected filtrates from the crystallization of II from four 5-g reactions of 4-chloroimidazo[4,5-*c*]-pyridine with tetra-*O*-acetyl-β-D-ribofuranose was evaporated to a syrup *in vacuo*. The residue was stirred at room temperature for 24 hr in 150 ml of methanolic ammonia (methanol saturated with ammonia at 0°). This solution was evaporated to a syrup *in vacuo*, and the syrup dissolved in ethanol. Ethyl acetate was added to a cloud point and the brown solution was allowed to stand until a tan precipitate was noted. This solid (500 mg) was collected by filtration (mp 215° dec) and recrystallized from water. The colorless needles which resulted were analytically pure, mp 234–235°, $[\alpha]_D^{26}$ −2.7° (*c* 1.0, pH 13.5).

Anal. Calcd for $C_{11}H_{12}ClN_3O_4$: C, 46.3; H, 4.21; N, 14.7. Found: C, 46.5; H, 4.41; N, 14.8.

4-Chloro-1-(2′,3′-O-isopropylidene-α-D-ribofuranosyl)-imidazo[4,5-c]pyridine. *p*-Toluenesulfonic acid monohydrate (6.6 g) was added to a stirred suspension of 4-chloro-1-α-D-ribofuranosylimidazo[4,5-*c*]pyridine (1.0 g) in anhydrous acetone (100 ml) with the exclusion of moisture. After 2 hr the solution was poured slowly with stirring into aqueous 0.5 N sodium bicarbonate. After 0.5 hr the combined mixture was evaporated *in vacuo* and the residual solid azeotropically dried with benzene. The residual solid was boiled in 100 ml of benzene and filtered. The filtrate was concentrated to about 25 ml *in vacuo* and cooled overnight at 10° and a heavy syrup was noted. The syrup was scratched with a spatula and crystals formed (0.8 g, 71%). This solid was recrystallized from benzene (small amount of chloroform added) to yield a colorless crystalline solid which softened at 110° and melted at 118–119°.

Anal. Calcd for $C_{14}H_{16}ClN_3O_4$: C, 51.6; H, 4.92; N, 12.9. Found: C, 51.3; H, 5.07; N, 12.9.

4-Chloro-1-(2′,3′-O-isopropylidene)-β-D-ribofuranosylimidazo[4,5-c]pyridine. 4-Chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (III, 1.0 g) was treated with *p*-toluenesulfonic acid as for the preparation of the α anomer. The product (1.0 g, 88.8%) was recrystallized from water to give heavy colorless crystals, mp 173–174°.

Anal. Calcd for $C_{14}H_{16}ClN_3O_4$: C, 51.6; H, 4.92; N, 12.9. Found: C, 51.7; H, 5.19; N, 12.8.

4-Amino-1-β-D-ribofuranosylimidazo[4,5-c]pyridine (V). 4-Chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (1.0 g) in 30 ml of anhydrous hydrazine was heated on a steam bath for 1 hr under a nitrogen atmosphere. The reaction mixture was evaporated to a glass *in vacuo*, and the residue was dissolved in 30 ml of oxygen-free water. Raney nickel catalyst (3.0 g) was added and the mixture was refluxed for 1 hr. The catalyst was removed by filtration and washed well with boiling water, and the filtrate was evaporated to a sticky solid *in vacuo*. The material was dissolved in a minimum amount of aqueous 20% ammonium carbonate solution, applied to a cellulose column (20 × 150 cm, Whatman CF-11), and eluted with 20% ammonium carbonate solution. The initial 300 ml collected contained no ultraviolet absorbing material. The next 40 ml of eluent contained a

small amount of impurity. The following fractions (85 ml) were found to contain pure 4-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (V). These fractions were boiled until the ammonium carbonate had decomposed, then evaporated to dryness *in vacuo*, and recrystallized from a small amount of water to give 490 mg (53%) of colorless crystalline needles, mp 225–226°. The specific rotation was $[\alpha]_D^{26}$ −48.3° (*c* 1.03, H_2O).

Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.7; H, 5.27; N, 21.0. Found: C, 49.8; H, 5.41; N, 20.9.

This compound was converted to 4-aminoimidazo-[4,5-*c*]pyridine and D-ribose by treatment with boiling 1 N HCl for 5 min. Comparison of this hydrolysate with R_F values of authentic samples in solvent systems A, B, and C as well as ultraviolet absorption data afforded satisfactory results.

1-β-D-Ribofuranosylimidazo[4,5-c]pyridine. 4-Chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (500 mg) was hydrogenated in anhydrous methanol (100 ml) and anhydrous sodium acetate (1 g) for 12 hr at 35 psi in a Parr hydrogenation apparatus. Palladium chloride (500 mg) was used as catalyst. The palladium was removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residue was boiled in anhydrous ethyl acetate (50 ml) and the solution was decanted and allowed to cool. Colorless crystals (85 mg) which melted at 199–200° resulted. The initial residue was then extracted with ethyl acetate using a Soxhlet extraction apparatus. An additional yield of 310 mg was obtained. The combined residues of 1-β-D-ribofuranosylimidazo[4,5-*c*]-pyridine were recrystallized from a small amount of water and gave colorless needles which melted 202–203°. The total yield was 70% (Mizuno *et al.*, 1964, report mp 198–199°). The specific rotation observed was $[\alpha]_D^{26}$ −38.0° (water) (Mizuno *et al.*, 1964, report $[\alpha]_D^{27}$ −36° (water)). The ultraviolet absorption spectra were identical with that previously reported (Mizuno *et al.*, 1964).

Anal. Calcd for $C_{11}H_{13}N_3O_4$: C, 52.6; H, 5.18; N, 16.7. Found: C, 52.4; H, 4.97; N, 17.1.

Periodate Oxidation and Sodium Borohydride Reduction of 4-Chloro-1-α- and -β-D-ribofuranosylimidazo[4,5-c]pyridines (XI and III). 4-Chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (III, 60 mg) was treated with 5.0-ml of 0.08 M sodium periodate solution and the mixture was kept at room temperature for 15 min. Sodium borohydride (130 mg) was then added followed after 0.5 hr by the slow addition of 1.7 ml of 10% acetic acid. When evolution of gas had ceased, optical rotation was determined as $[\alpha]_D^{26}$ +72° based on the original weight of III. Similarly 22 mg of XI and 1.5 ml of 0.08 M sodium periodate followed by 40 mg of sodium borohydride resulted in a solution after neutralization which gave $[\alpha]_D^{26}$ −71.9° based on the original weight of XI.

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