melting point about 160–163°, which liberated iodine from an aqueous iodide solution acidified with acetic acid. An additional recrystallization dropped the melting point to 146–*ca.* 159°.

Anal. Calcd. for $C_{15}H_9Br_2NOS$ (mol. wt., 411.06): C, 43.61; H, 2.21. Found: C, 43.37; H, 2.72.

2-Bromo-3-keto-1*H*-pyrido[3,2,1-*kl*]phenothiazine (VII).—When an attempt was made to recrystallize the preceding 2,3-dibromo-2,3-dihydro-3-keto-1*H*-pyrido[3,2,1-*kl*]phenothiazine (VI) from nitromethane on the steam bath, the initially orange-colored solution lightened almost immediately to yellow, and became strongly acidic. After about 15 min. at 80–100°, the solution was cooled and scratched, giving yellow platelets of m.p. 144–146°, raised to 145–147.3° on recrystallization.

Anal. Calcd. for $C_{15}H_8BrNOS$ (mol. wt., 330.21): C, 54.61; H, 2.44. Found: C, 54.93, 55.00; H, 2.53; 2.48.

Acknowledgment.—The author wishes to thank Professor N. Cromwell for a stimulating discussion. Analyses were done by Dr. S. Blackman and his staff.

Studies on Condensed Systems of Aromatic Nitrogenous Series. XXII. Structural Studies of β-D-Ribofuranosylimidazopyridines[1]

YOSHIHISA MIZUNO, MORIO IKEHARA, TOKUO ITOH, AND KAZUKO SAITO

Faculty of Pharmaceutical Sciences, Hokkaido University School of Medicine, Sapporo, Hokkaido, Japan

Received January 28, 1963

3-Methyl-3*H*-imidazo[4,5-*b*]pyridine (I), 1-methyl-1*H*-imidazo[4,5-*b*]pyridine (IV), 1-methyl-1*H*-imidazo[4,5-*c*]pyridine (II), and 3-methyl-3*H*-imidazo[4,5-*c*]pyridine (III) have been prepared. Spectroscopic comparison of a pair of isomers of methyl imidazo[4,5-*b*]pyridines (I and IV) with a nucleoside resulting from condensation of mercuric chloride complex of imidazo[4,5-*b*]pyridine with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (followed by debenzoylation) suggests that the supposed 1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*b*]pyridine is 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine. A nucleoside obtained from 1*H*-imidazo[4,5-*c*]pyridine by an analogous reaction was assigned the 3-β-D-ribofuranosyl-3*H*-imidazo[3,4-*c*]pyridine structure on the basis of spectral comparison with a pair of methylimidazo[4,5-*c*]pyridines.

The synthesis of nucleosides of purines or related heterocyclic bases by condensation reactions is often beset with more than one possibility for the position of attachment of the sugar moiety to the aglycon. In such cases, proof is required for this positional assignment. One technique commonly employed for such proof is to compare the ultraviolet absorption at properly selected pH values of the nucleoside product with appropriate alkyl derivatives of the aglycon.[2,3]

This technique of structural elucidation often makes use of the generally accepted empirical rule: first, in purine ring systems, 7-alkyl substituted purines have the absorption maximum at a longer wave length than corresponding 9-alkyl substituted purines.[4] Secondly, replacement of the alkyl group by a glycosyl moiety would be expected to produce little or no change in the ultraviolet absorption spectrum. Thus, adenosine (9-β-D-ribofuranosyladenine) has an ultraviolet absorption spectrum very similar to that of 9-methyladenine, but not to that of 7-methyladenine,[5] while 7-α-D-ribofuranosyladenine has almost the same absorption maximum as 7-methyladenine.[6] Both of these 7-substituted adenines possess maxima at longer wave lengths than the 9-substituted isomers.[6]

A survey of the literature, however, revealed that the first part of the empirical rule does not always hold, at least in the case of purines possessing no substituents in the pyrimidine moiety. For example, the ultraviolet absorption maximum of 7-methylpurine in cationic form appears at a shorter wave length than that of the cationic form of 9-methylpurine. Yet both have almost identical absorption maxima in their neutral form.[7]

The main purpose of the present investigation is to examine the ultraviolet absorption properties of N-substituted imidazopyridines, with emphasis being laid upon the critical examination of the utility of the aforementioned empirical rule[4] for structural elucidation in the imidazopyridine ring system.

For this purpose, 3-methyl-3*H*-imidazo[4,5-*b*]pyridine (I), 1-methyl-1*H*-imidazo[4,5-*c*]pyridine (II), 3-methyl-3*H*-imidazo[4,5-*c*]pyridine (III), and 1-methyl-1*H*-imidazo[4,5-*b*]pyridine (IV) were required among which I, II, and III have not been described in the literature. Therefore, methods of unambiguous syntheses of these compounds have been devised.

3-Methyl-3*H*-imidazo[4,5-*b*]pyridine (I) was prepared according to two different routes (see Flow Sheet 1). 3-Amino-2-methylaminopyridine (X) was prepared essentially according Schickh, Binz, and Schulz.[8] X was subjected to ring closure with formamidine acetate[9] to I. An improved synthesis of I was obtained when formic acid was employed as condensing agent. For purification, I was converted to its picrate, m.p. 203–203.5°, which, after recrystallization, was converted to I, m.p. 76–78°. I was also prepared by treatment of 1*H*-imidazo[4,5-*b*]pyridine (XIII)[10] with dimethyl sulfate in nitromethane in the presence of acetic acid. After removal of the solvent, conversion of the product to its picrates, followed by fractional recrystallization from aqueous ethanol gave two different picrates, m.p. 203–203.5° and 189–191°. One of them, m.p. 203–203.5°, was found to be identical with that of 3-methyl-3*H*-imidazo[4,5-*b*]pyridine (mixture melting point and infrared absorption spectral comparison). The other picrate was 1-methyl-1*H*-imidazo[4,5-*b*]pyridine (IV). IV was also prepared essentially according to Chatterji,

(1) Part XXI of this series, K. Adachi, *Chem. Pharm. Bull.*, **7**, 479 (1959).

(2) J. Baddiley, "Nucleic Acids," Vol. 1, E. Chargaff and J. Davidson, Ed., Academic Press Inc., New York, N. Y., 1955, pp. 143, 152.

(3) J. M. Gulland, R. E. Holiday, and T. F. Macrae, *J. Chem. Soc.*, 1639 (1934).

(4) J. M. Gulland and L. F. Story, *ibid.*, 692 (1938).

(5) J. M. Gulland and E. R. Holiday, *ibid.*, 765 (1936).

(6) W. Friedrich and K. Bernhauer, *Chem. Ber.*, **89**, 2507 (1956).

(7) A. Bendich, P. J. Russell, and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

(8) O. v. Schickh, A. Binz, and A. Schulz, *Ber.*, **69**, 2593 (1936).

(9) E. C. Taylor and W. A. Ehrhart, *J. Am. Chem. Soc.*, **82**, 3138 (1961).

(10) V. Petrov and J. Saper, *J. Chem. Soc.*, 1389 (1948).

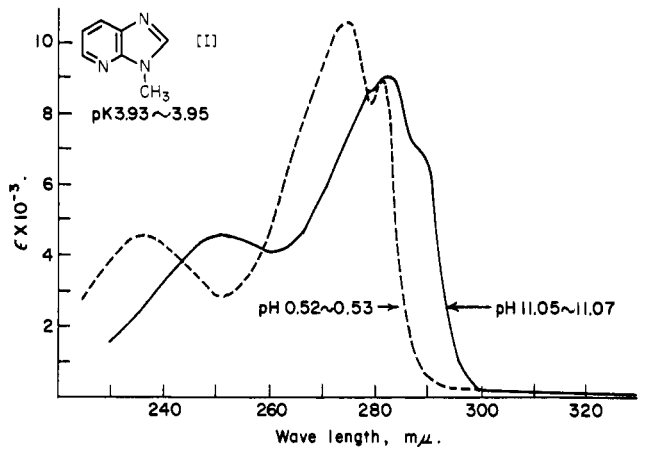


Fig. 1.—3-Methyl-3*H*-imidazo[4,5-*b*]pyridine (I).

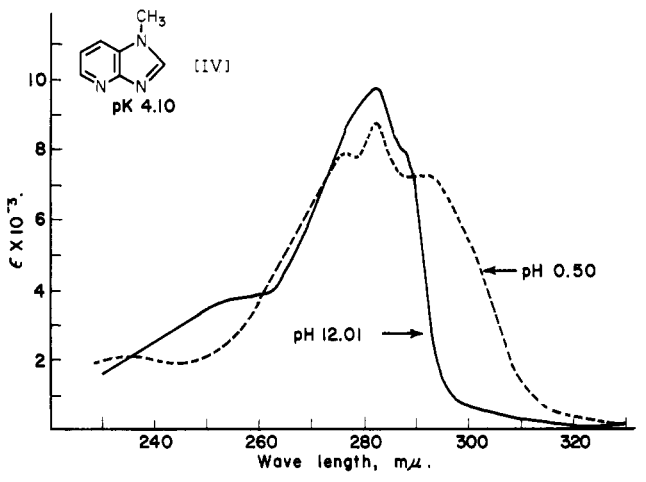


Fig. 2.—1-Methyl-1*H*-imidazo[4,5-*b*]pyridine (IV).

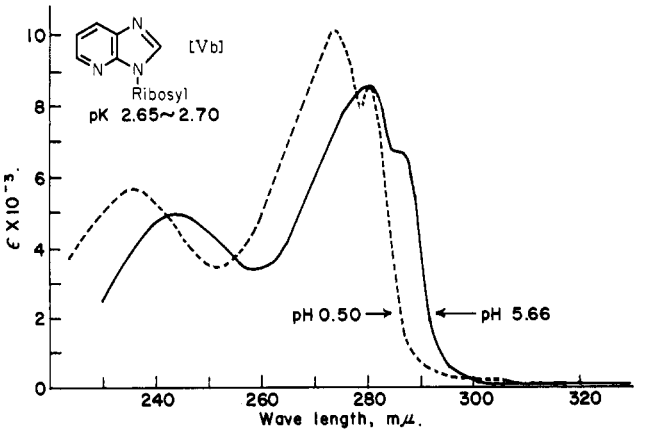


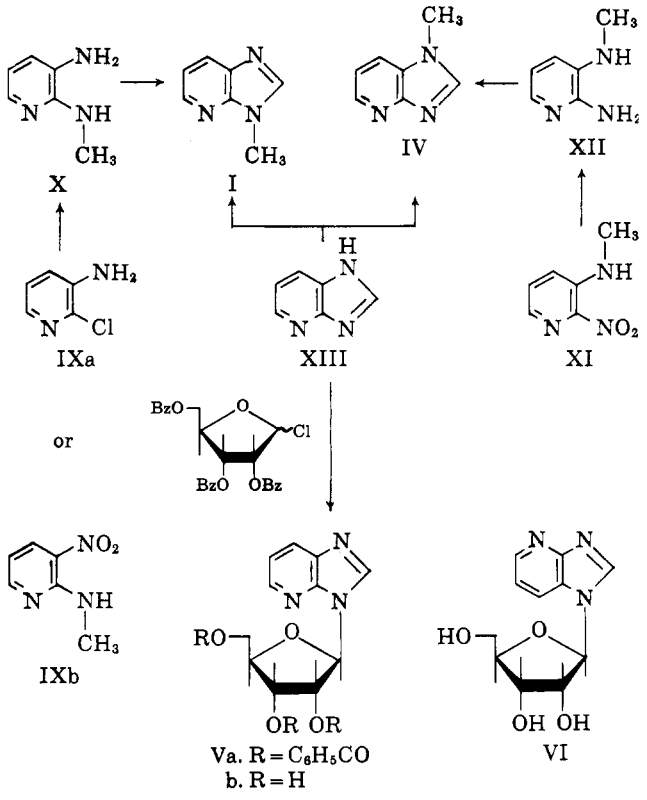
Fig. 3.—3-β-D-Ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (Vb).

Dhar, Anand, and Dhar,[11] from 3-ethoxycarbonyl-*N*-methylamino-2-nitropyridine by way of 2-amino-3-methylaminopyridine.[12] Its picrate had m.p. 189–191° which was found to be identical with that of one of two isomers formed from XIII and dimethyl sulfate.

1-Methyl-1*H*-imidazo[4,5-*c*]pyridine (II) was prepared from 4-chloro-3-nitropyridine through 4-methylamino-3-nitropyridine and 3-amino-4-methylaminopyridine (XIV)[13] (see Flow Sheet 2). The yield of II from XIV was 45% using formic acid as the condensing agent. The base was purified by sublimation *in vacuo*. 3-Methyl-3*H*-imidazo[4,5-*c*]pyridine (III) was prepared from 3-chloro-4-nitropyridine 1-oxide (XV) through 4-amino-3-methylaminopyridine (XVII),[14] by ring closure with formic acid.

The ribosylation of 1*H*-imidazo[4,5-*b*]pyridine (XIII) (see Flow Sheet 1) was carried out as follows: the condensation of the mercuric chloride salt with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[15] in boiling xylene gave rise to a crude benzoyl-blocked nucleoside which afforded (after column chromatographic separation) only a single nucleoside (Va) in good yield, which melted at 154–155°. Debenzoylation of Va gave rise to the free nucleoside (Vb) which melted at 220–222°.

1*H*-Imidazo[4,5-*c*]pyridine in the same condensation (see Flow Sheet 2), followed by separation and subsequent debenzoylation gave two different nucleosides, one of which melted at 200–202°.[16]



Flow Sheet 1

The ultraviolet absorption spectra of these compounds are shown in Fig. 1 through 6 along with their apparent p*K* values, determined spectrophotometrically according to Shugar and Fox.[17] As shown in Fig. 1 and 2, the spectra of a pair of isomers (I and IV) of imidazo[4,5-*b*]pyridine series are pH-dependent and have a broad similarity in their neutral form, although

(11) S. K. Chatterji, M. M. Dhar, N. Anand, and M. L. Dhar, *J. Sci. Ind. Res.* (India), **19c**, 35 (1960).

(12) J. W. Clark-Lewis and M. L. Thompson, *J. Chem. Soc.*, 442 (1957).

(13) O. Bremer, *Ann.*, **518**, 274 (1935).

(14) J. W. Clark-Lewis and R. P. Singh, *J. Chem. Soc.*, 2379 (1962).

(15) J. Davoll, B. Lythgoe, and A. Todd, *ibid.*, 967 (1948); H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955).

(16) Details of this preparation and a structural study of the other nucleoside will appear in a separate communication.[18]

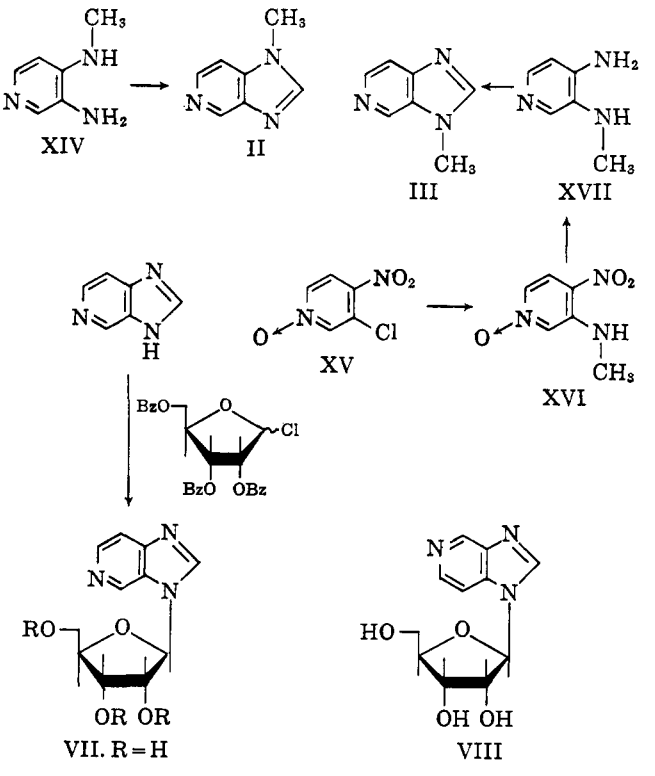
(17) (a) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952); (b) all the p*K* values in this paper were determined by the same method.

(18) Presented at the 15th Hokkaido local meeting of the Japanese Pharmaceutical Society, February, 1963, Sapporo, Hokkaido.

differing considerably in the cationic form where the spectrum of IV shows maxima at 289 mμ (ε 7260), 282 mμ (ε 8700), 277 mμ (ε 7870), and 235 mμ (ε 1960), while that of I shows maxima at 281 mμ (ε 8930), 275 mμ (ε 10,640), and 236 mμ (ε 4470).

In connection with the present investigation, the work of the Indian chemists[11] who also investigated the ribosylation of 1*H*-imidazo[4,5-*b*]pyridine (XIII) is pertinent. They found that only a single nucleoside was formed among two possible isomers V and VI and assigned the 1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*b*]-pyridine structure to their product from a comparison only with 1-methyl-1*H*-imidazo[4,5-*b*]pyridine (IV), neglecting a comparison with 3-methyl-3*H*-imidazo-[4,5-*b*]pyridine (I).

However, our spectral data obtained with the pair of isomers (I and IV) shows that spectral comparison with *both* isomers is essential. Moreover, for the comparison to be valid and useful for unambiguous assignment of structure, curves should be selected at pH values sufficiently removed from the p*K*'s to assure pure species in solution.[19] These requirements for



Flow Sheet 2

comparison are met in all figures (1 through 6). It can be seen that the spectrum of the nucleoside Vb (p*K* of 2.65) for the neutral and cationic species is almost identical with that of I and differs appreciably from that of IV indicating that then ucleoside Vb is 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine.[20]

(19) For an extensive discussion on this point, see ref. 17a. We are indebted to J. Fox for his participation in the discussion on this point.

(20) The structure was unequivocally established by us[21] on the basis of a series of reactions: the nucleoside (Vb) was converted to 2',3'-isopropylidene-5'-tosylate. Treatment of the tosylate with boiling acetone gave rise to a water-soluble and intramolecularly quaternized nucleoside. This type of intramolecular quaternization is feasible only with 5'-tosylate of 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine, excluding absolutely the possibility that the nucleoside might be the 1-derivative.

(21) Y. Mizuno, M. Ikehara, T. Itoh, and K. Saito, *Chem. Pharm.*, **11**, 265 (1963).

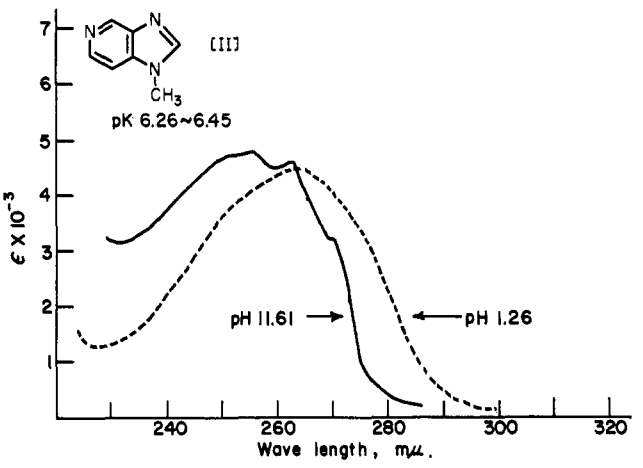


Fig. 4.—1-Methyl-1*H*-imidazo[4,5-*c*]pyridine (II).

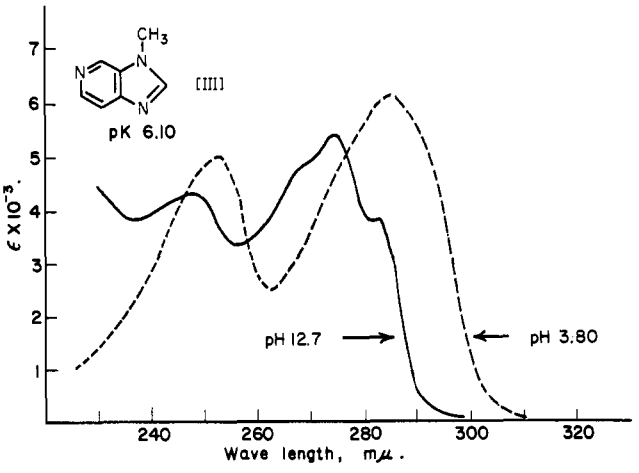


Fig. 5.—3-Methyl-3*H*-imidazo[4,5-*c*]pyridine (III).

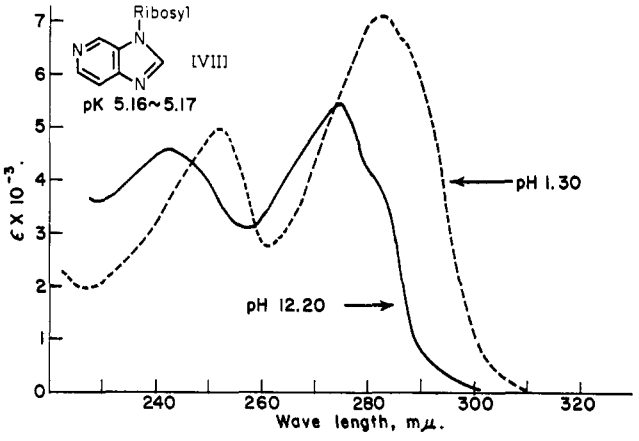


Fig. 6—3-β-D-Ribofuranosyl-3*H*-imidazo[4,5-*c*]pyridine (VII).

As shown in Fig. 4 and 5, 1-methyl-1*H*-imidazo-[4,5-*c*]pyridine (II) and 3-methyl-3*H*-imidazo[4,5-*c*]-pyridine (III), whose p*K*'s are 6.36 and 6.15,[17] respectively, have quite different absorption spectra both in cationic and neutral form. Especially large differences are observed in their absorption maxima in cationic form, III having its maximum at 285 mμ and II at 263 mμ. 3-Methyl-3*H*-imidazo[4,5-*c*]pyridine (III) (7 methyl-3-deazapurine) gives a band at longer wave length than 1-methyl-1*H*-imidazo[4,5-*c*]pyridine (II) (9-methyl-3-deazapurine) showing that the aforementioned empirical rule[4] holds.

The spectral characteristics of the nucleoside (VII) (pK 5.16[17]) derived from 1H-imidazo[4,5-c]pyridine were found to be very similar to that of III in the pH region ranging from 1 to 12, suggesting those the nucleoside (VII) is 3-β-D-ribofuranosyl-3H-imidazo[4,5-c]pyridine.

Experimental[22]

3-Methyl-3H-imidazo[4,5-b]pyridine (I) and Its Picrate. Method A (with Formamidine Acetate).—3-Amino-2-methylaminopyridine[8] (1.10 g.) was treated with formamidine acetate[9] (0.9 g.) in refluxing methoxyethanol (20 ml.) for 1 hr., cooled, and concentrated to dryness. Formamidine acetate was partially removed by sublimation *in vacuo* (90°, 5 mm.) to afford a crude product which was converted to the picrate (500 mg.), needles after two recrystallizations from ethanol, m.p. 203–203.5°.

Anal. Calcd. for $C_{13}H_{10}N_6O_7$: C, 43.10; H, 2.78; N, 23.20. Found: C, 42.97; H, 2.54; N, 22.89.

Method B (with Formic Acid).—2-Methylamino-3-nitropyridine (1Xb, 0.9 g.)[23] in ethanol (10 ml.) was reduced over palladium–carbon (5%, 0.1 g.) to afford 0.59 g. of 3-amino-2-methylaminopyridine (81% yield) which was treated with boiling formic acid for 2 hr. Removal of the solvent gave crude product I. On sublimation *in vacuo* (100–120°, 2 mm.) yield was 0.32 g. (41%), m.p. 203–203.5°; R_f, 0.71.[24]

Anal. Calcd. for $C_7H_7N_3$: C, 63.14; H, 5.30; N, 31.56. Found: C, 62.85; H, 5.30; N, 31.26.

Conversion of the Picrate to Free Base. 3-Methyl-3H-imidazo-[4,5-b]-pyridine (I).—The picrate of 3-methyl-3H-imidazo-[4,5-b]pyridine (450 mg.) in methoxyethanol (10 ml.) was treated with Amberlite IRA 400 (OH^- form, 15 ml.) until the yellow color disappeared. The resin was filtered off and washed twice with water (20 ml.). Concentration of the combined filtrate and washings gave the free base (30 mg.). For further purification the base was sublimed *in vacuo* to give pure product (20 mg.), prisms, m.p. 76–78°. Ultraviolet absorption spectra at pH 0.52–0.53, λ_{max} 236 mμ (ϵ 4470), 275 mμ (ϵ 10,640), 281 mμ (ϵ 8930); λ_{min} 251 mμ (ϵ 2810), 278 mμ (ϵ 8280); at pH 11.05–11.07, λ_{max} 252 mμ (ϵ 4510), 282 mμ (ϵ 9010), 288 mμ (ϵ 6940) (sh); λ_{min} 241 mμ (ϵ 4080).

1-Methyl-1H-imidazo[4,5-b]pyridine (IV).—3-Methylamino-2-nitropyridine (0.89 g.) in methoxyethanol (20 ml.) was hydrogenated over Raney nickel[25] (wet weight, 5 g.) in a hydrogen atomosphere at room temperature. Three hundred and fifty milliliters of hydrogen had been adsorbed at the end of 5 hr. and no further reduction took place after an additional 1 hr. The nickel was filtered, washed twice with methoxyethanol (100 ml.), and filtered. To the combined filtrate was added formamidine acetate (1.9 g.) and the mixture was refluxed for 1.5 hr. The solution was concentrated under reduced pressure to dryness. The solids were purified by distillation *in vacuo* to give the product, m.p. 95–97°, from benzene (lit.[11] m.p. 95–97°), R_f 0.50.[24] Ultraviolet absorption spectra at pH 0.50, λ_{max} 235 mμ (ϵ 1960), 277 mμ (ϵ 7870), 282 mμ (ϵ 8700), 289 mμ (ϵ 7260) (sh); λ_{min} 243 mμ (ϵ 1960), 279 mμ (ϵ 7780); at pH 12.01, λ_{max} 282 mμ (ϵ 9660), 288 mμ (ϵ 7920) (sh). Melting point of the picrate, was 189–191°.

1H-Imidazo[4,5-b]pyridine (XIII).—The procedure used was essentially that reported by Petrow and Saper.[13] Purified base was obtained in 54% yield, m.p. 146–147° (lit.[13] m.p. 153–154°). Sublimation *in vacuo*, followed by recrystallization from acetone, gave product unchanged in melting point, R_f 0.63[24]; melting point of picrate was 188–189°.

Methylation of 1H-imidazo[4,5-b]pyridine. Preparation of 1-Methyl-1H-Imidazo[4,5-b]pyridine (IV) and 3-Methyl-3H-imidazo[4,5-b]pyridine (I).—To a mechanically stirred solution of 1H-Imidazo[4,5-b]pyridine (2.0 g.) in nitromethane (20 ml.) and acetic acid (5 ml.) was added dropwise at 63–65° dimethy sulfate (2.5 g.). The internal temperature rose spontaneously to 75° and then went down to 65° which required 15 min. After the addition was complete, the temperature was raised to 90–93° and the solution was maintained at the same temperature for 30 min. and cooled. The solution was concentrated to dryness. To the residue was added 15 ml. of ethanol which was distilled *in vacuo*. This process was repeated until all trace of acetic acid was removed. The residue was dissolved in water (20 ml.), made alkaline with 4 ml. of 10 N sodium hydroxide pH 11 and then rapidly and repeatedly extracted with ethyl acetate (600 ml.); a yellow fluorescent organic layer was separated, washed with water, dried, and filtered. The filtrate was concentrated to oily solids.[26] For separation and purification, the residue (0.44 g.) was converted to picrates in the following way. One-third (0.252 g., 1.1 mmoles) of the picric acid (0.765 g., 3.3 mmoles), required to convert the entire methyl[4,5 b]pyridine (0.44 g., 3.3 mmoles) to picrates, was dissolved in ethanol (2 ml.) and added to the base (0.44 g.) in ethanol (2 ml.). The reaction mixture was kept standing at room temperature overnight. The precipitate was filtered, and recrystallized from ethanol to give a picrate having m.p. of 203–203.5° which was found to be the picrate of I by comparison with an authentic sample, described earlier. To the filtrate another one-third of the picric acid (0.252 g.) was added to precipitate the second crop which melted after two recrystallizations from ethanol at 203–203.5° and did not depress the melting point of the picrate of I. The combined picrates (m.p. 203–203.5°) weighed 245 mg. By using the second filtrate, the same process was repeated to give a third crop (15 mg.) which melted at 189–191° after two recrystallizations from aqueous methanol and did not depress melting point of picrate of IV.

3-Methyl-3H-imidazo[4,5-b]pyridine Hydrochloride (I·2HCl).—Dry hydrogen chloride gas was passed through a solution of the picrate of I (200 mg.) in absolute ethanol (14 ml.). Ethanol was removed *in vacuo* and the picric acid liberated was extracted repeatedly with ether to give the hydrochloride of I which was dissolved in a minimal amount of absolute methanol and to the solution was added a mixture of dry ether and dioxane to deposit pure hydrochloride.

Anal. Calcd. for $C_7H_7N_3$·2HCl: C, 40.81; H, 4.41; N, 20.38. Found: C, 41.00; H, 4.28; N, 20.25.

3-Amino-4-methylaminopyridine (XIV).—4-Methylamino-3-nitropyridine was prepared according to Bremer[13] as golden yellow needles, m.p. 157–158° (lit.[13,14] m.p. 162–163°). 4-Methylamino-3-nitropyridine (1.65 g.) was hydrogenated over palladium-carbon prepared by reduction of a mixture of palladium chloride (20 ml. of 1% solution) to give 1.1 g. (83.3%) of product, m.p. 169° (lit.[27] m.p. 169°); the picrate melted at 184° (lit.[24] m.p. 185°).

1-Methyl-1H-imidazo[4,5-c]pyridine (II).—3-Amino-4-methylaminopyridine (0.7 g.) was treated with refluxing formic acid (1 ml.) for 1 hr. and cooled. Excess formic acid was removed under reduced pressure. The residue was dissolved in ethanol and the solution was treated with calcium carbonate (500 mg.), filtered, and concentrated to dryness under reduced pressure. The residue was subjected to sublimation *in vacuo* to give 40% of product, prisms, m.p. 111.5–112.5°. Ultraviolet absorption spectra at pH 1.26, λ_{max} 263 mμ (ϵ 4400); at pH 11.6, λ_{max} 255 mμ (ϵ 4800), 263[16] mμ (ϵ 4600); λ_{min} 261 mμ (ϵ 4500). pK_a of II was 6.26–6.46.[17b]

Anal. Calcd. for $C_7H_7N_3$: C, 63.14; H, 5.30; N, 31.56. Found: C, 63.26; H, 5.43; N, 31.23.

The base was converted to hydrochloride by passing dry hydrogen chloride gas into an ethanol solution of the base.

Anal. Calcd. for $C_7H_7N_3$·2HCl: C, 40.79; H, 4.41; N, 20.38. Found: C, 39.92; H, 4.98; N, 19.01. Picrate of II, m.p. 217–218°.

Anal. Calcd. for $C_{13}H_{10}N_6O_7$: C, 43.10; H, 2.78; N, 23.20. Found: C, 39.82; H, 3.22; N, 23.41.

3-Chlorpyridine 1-oxide.—3-Chloropyridine 1-oxide was prepared by a standard procedure.[28] The oxide (11.0 g.) was ob-

(22) All melting points are uncorrected. Ultraviolet absorption spectra were run with the Beckmann Model DK 11 recording spectrophotometer. Molecular extinction coefficients were determined with a Shimadzu manual spectrophotometer. Except where noted sublimation *in vacuo* was done with a "Sublimatometer," devised by E. Shibata (for leading reference, E. Shibata and S. Saito, *Nippon Kagaku Zasshi*, **80**, 604 (1959).

(23) A. E. Chichibabin and A. W. Kirssanow, *Ber.*, **61B**, 1223 (1928).

(24) Paper chromatography was performed using ascending technique; solvent system employed, n-butyl alcohol saturated with water.

(25) D. J. Brown, *J. Soc. Chem. Ind.*, **69**, 353 (1950).

(26) Paper chromatography revealed[24] that the ethyl acetate layer contained two components (R_f 0.73 and 0.53) in addition to starting material (R_f 0.63).

(27) R. Weidenhagen, G. Train, H. Wegner, and L. Nordstrom, *Ber.*, **75**, 1936 (1942).

(28) E. Ochiai, *J. Org. Chem.*, **18**, 535 (1953).

tained from 3-chloropyridine (12.35 g.) in a yield of 78%, m.p. 59–60°, from ether (lit.[29] m.p. 59–60°).

3-Chloro-4-nitropyridine 1-Oxide (XV).—To a solution of 3-chloropyridine 1-oxide (7.2 g.) in concentrated sulfuric acid (sp. gr. 1.80, 16 ml.) was added dropwise with stirring a mixture of fuming nitric acid (sp. gr. 1.54, 24 ml.) and concentrated sulfuric acid (sp. gr. 1.80, 20 ml.). After the addition was complete, the internal temperature was raised to 90° and the mixture was kept at the same temperature for 1.5 hr. after which it was cooled and poured into ice-water, and neutralized with sodium carbonate to deposit a small amount of sodium sulfate which was filtered off. The filtrate was extracted with chloroform. The chloroform layer was separated, washed with water, dried over sodium sulfate, and filtered. The filtrate was concentrated to dryness and recrystallized from acetone to give yellow crystals, m.p. 103–110°; yield was 6.1 g. (64%).

Anal. Calcd. for $C_5H_3N_2O_3Cl$: C, 34.41; H, 1.73; N, 15.96. Found: C, 34.32; H, 1.80; N, 16.10.

3-Methylamino-4-nitropyridine 1-Oxide (XVI).—3-Chloro-4-nitropyridine 1-oxide (8.3 g.) was treated for 20 min. with a refluxing methanol solution of methylamine (5%, 50 ml.) on the steam bath. In a few minutes solids separated. Heating was continued for another 20 min. and the solution cooled. After cooling, a precipitate was collected by filtration. Recrystallization from ethanol gave pure product (3.65 g., 45.4%), m.p. 227°, from ethanol (lit.[14] m.p. 227°).

4-Amino-3-methylaminopyridine (XVII).—Methylamino-4-nitropyridine 1-oxide was reduced to 4-amino-3-methylaminopyridine over Raney nickel according to the procedure of Clark-Lewis and Sigh.[14] Reduction of 3-methylamino-4-nitropyridine (3.5 g.) gave 2.4 g. (quantitative yield) of product, needles, m.p. 112° (lit.[14] m.p. 114°). The picrate melted at 225–228°.

3-Methyl-3*H*-imidazo[4,5-*c*]pyridine (III).—4-Amino-3-methylaminopyridine (130 mg.) was refluxed with 10 ml. of freshly distilled formic acid for 4 hr. and cooled. After cooling formic acid was removed *in vacuo* to dryness and to the residue was added to ethanol which was distilled *in vacuo*. The process was repeated until all trace of formic acid was removed, to give 4-formylamino-3-methylaminopyridine. Picrate melted at 177° (from ethanol).

Anal. Calcd. for $C_{13}H_{12}N_6O_8$: C, 41.05; H, 3.15; N, 22.10. Found: C, 40.92; H, 3.34; N, 22.00.

For ring closure the solid residue was sublimed *in vacuo* to give the desired product (65 mg., 46% on the basis of XVII); needles from ethanol, m.p. 101–101.5°. Ultraviolet absorption spectra at pH 3.80, λ_{max} 253 mμ (ε 5000), 285 mμ (ε 6100); λ_{min} 263 mμ (ε 2500); at pH 12.7, λ_{max} 249 mμ (ε 4300), 275 mμ (ε 5400); λ_{min} 257 mμ (ε 3300). pK_a of III was 6.10.[17b]

Anal. Calcd. for $C_7H_7N_3$: C, 63.14; H, 5.30; N, 31.56. Found: C, 63.26; H, 5.43; N, 31.50.

The base was converted to picrate which melted after recrystallization from ethanol at 199.5–200°.

Anal. Calcd. for $C_{13}H_{10}N_6O_7$: C, 43.10; H, 2.78; N, 23.20. Found: C, 43.08; H, 2.93; N, 23.15.

Mercuric Chloride Salt of 3*H*-Imidazo[4,5-*b*]pyridine.—To a well stirred solution of 3*H*-imidazo[4,5-*b*]pyridine (2.66 g.) in 15 ml. of 1.5 *N* sodium hydroxide was added a solution of mercuric chloride (6.1 g.) in ethanol (20 ml.) to give rise to white precipitates which were collected by centrifugation, washed successively three times with water, four times with ethanol, and finally with dry ether, and dried *in vacuo* at 100° (2 mm.). The mercuric chloride obtained was stable to heat, up to 250°; yield was 7.7 g. (97%).

Anal. Calcd. for $C_6H_4N_3HgCl$: N, 11.84. Found: N, 12.01.

Ribosylation of the Mercuric Chloride Salt of 3*H*-Imidazo[4,5-*b*]pyridine with 2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl Chloride.—The procedure used in condensation was essentially that reported by Kissman and Weiss.[30] The mercuric chloride salt (13.9 g.) and 2,3,5-tri-*O*-benzoylribofuranosyl chloride prepared by a standard method[15] from 1-*O*-acetyl-2,3,5-tri-o-benzoyl-β-b-ribofuranose (20 g.) gave a crude benzoyl-blocked nucleoside(s), which was applied to acid-washed alumina (2.5 × 55 cm.). The first seven fractions (200 ml. each) eluted by benzene (1.5 l.) contained only a sugar derivative (total weight, 3.2 g.[31]) and were discarded. Each fraction of the subsequent seven fractions (200 ml. each) eluted by a mixture of ethyl acetate and benzene (1:5 v./v., 1.5 l.) afforded after evaporation of the solvent the same nucleoside,[32] total weight, 8.75 g. The eluting solvent was switched to ethyl acetate (seven 200-ml. portions) which eluted seven fractions, each of which was concentrated to dryness. The residues were found to be identical with each other (total weight, 6.46 g.) and also with preceding nucleoside. On washing the alumina with ethanol (660 ml.) 2.05 g. of 3*H*-imidazo-[4,5-*b*]pyridine was recovered in the form of the free base. The combined benzoyl-blocked nucleoside weighed 15.2 g. (68%), m.p. 154–155°.

Anal. Calcd. for $C_{32}H_{25}N_3O_7$: C, 68.20; H, 4.47; N, 7.46. Found: C, 68.10; H, 4.51; N, 7.42.

3-β-D-Ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (Vb).—To a solution of the benzoyl-blocked nucleoside (Va, 0.7 g.) in absolute methanol (70 ml.) was added a 1 *N* methanol solution of sodium methoxide (1 ml.); the solution was refluxed for 1 hr. and cooled. After cooling the solvent was removed *in vacuo* at room temperature to furnish a product which was dissolved in water (20 ml.). The aqueous layer was separated and concentrated *in vacuo* to dryness. Recrystallization from water gave colorless needles, 0.22 g., 71%, m.p. 220–222° (lit.[11] m.p. 220°). Ultraviolet absorption spectra at pH 0.50, λ_{max} 236 mμ (ε 5600), 275 mμ (ε 10,120), 281 mμ (ε 8600); λ_{min} 251–252 mμ (ε 3440), 279 mμ (ε 7,900); at pH 5.66, λ_{max} 243 mμ (ε 4940), 281 mμ (ε 8540) 287 mμ (ε 6620) (sh); λ_{min} 259 mμ (ε 3350). pK_a[17] was 2.65–2.70.

Absorption spectra of 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*c*]-pyridine (VII) at pH 1.30, λ_{max} 252 mμ (ε 5000), 283 mμ (ε 7200); λ_{min} 261 mμ (ε 2800); at pH 12.20, λ_{max} 242 mμ (ε 5600), 275 mμ (ε 5500); λ_{min} 257 mμ (ε 3100). pK_a[17] of VII was 5.16–5.17.

Acknowledgment.—We wish to thank Dr. Fox of the Sloan-Kettering Institute for reviewing our manuscript and for his constructive suggestions. We also wish to thank Mrs. T. Tohma for the microanalyses. This research was supported in part by grant-in-aid from Ministry of Education, Japan, to which our thanks are due.

(29) R. E. Evans and H. C. Brown, *J. Org. Chem.* **27**, 1329 (1962).

(30) H. G. Kissman and M. J. Weiss, *J. Org. Chem.*, **21**, 1053 (1956).

(31) The substance was nitrogen free and infrared spectra were very similar to that of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose which suggested that conversion of this to the corresponding 1-chloride was incomplete.

(32) Identity was based on criteria of infrared and ultraviolet absorption spectral comparisons and on the fact that after recrystallization from ethanol (recovery of the recrystallization was approximately 70%) the mixture melting point with each other were not depressed.