Studies on Condensed Systems of Aromatic Nitrogenous Series. XXIII. Synthesis of 1-(β-D-Ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridines[1]

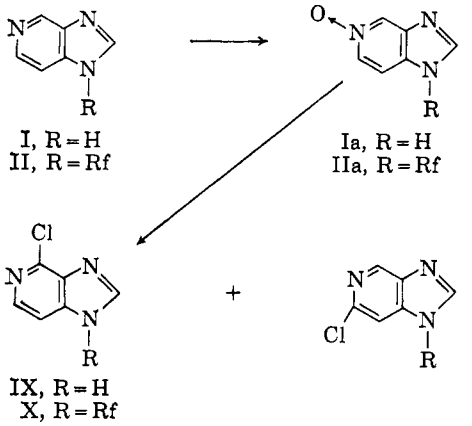
YOSHIHISA MIZUNO, TOKUO ITOH, AND KAZUKO SAITO

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

Received March 25 1964

Condensation of the chloromercuri salt of 1*H*-imidazo[4,5-*c*]pyridine with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride XIII yielded two benzoylated nucleosides which afforded, after alumina column chromatographic separation, two isomeric tri-*O*-benzoylated nucleosides II and III in yields of 26.7 and 22.2%, respectively. Debenzoylation of II afforded a nucleoside IV, m.p. 198–199°, which was assigned the 1 (β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine structure on the basis of spectral properties. Ribosidation of 4-chloro-1*H*-imidazo-[4,5-*c*]pyridine (IX) by the same method also gave a mixture of two isomeric tri-*O*-benzoylated nucleosides X and XI. After separation by alumina column chromatography X and XI yielded 4-chloro-1-(β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine VIII and the corresponding 3*H*-isomer XII, respectively. XII was also prepared from IIIa by the *N*-oxide–phosphoryl chloride reaction which was in turn obtained from III by the usual method.

It has been amply demonstrated[2] that 6-chloro derivatives of glycosyl purines are versatile intermediates for the preparation of other 6-substituted purine nucleosides. By analogy, 4-chloro-1-(β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (VIII, Chart I) may also be useful as a key intermediate for the synthesis of 6-substituted ribonucleosides possessing the 1*H*-imidazo[4,5-*c*]pyridine ring system. Two approaches to VIII appeared promising. In view of the successful synthesis[3] of 4-chloro-3*H*-imidazo[4,5-*c*]-pyridine from I *via* Ia, a similar approach to VIII might be achieved *via* II → IIa → X (see the following diagram).



An alternate approach to VIII would be to employ the Fischer–Helferich method as modified by Davoll and Lowy,[4] starting from the readily available 4-chloro-1*H*-imidazo[4,5-*c*]pyridine.[3,5] The present paper deals with the synthesis of compound VIII by the latter method, as well as with attempts to synthesize VIII by the *N*-oxide–phosphoryl chloride method.[3,6]

1*H*-Imidazo[4,5-*c*]pyridine (I)[7] was converted to its chloromercuri derivative and was condensed with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (XIII)[8] in refluxing xylene. A sirup was obtained which was fractionated by alumina column chromatography and debenzoylated with sodium methoxide. Two isomeric nucleosides were isolated, one of which (22% yield) melted at 200–202°. This nucleoside was identical with 3-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-*c*]pyridine.[1] The second nucleoside obtained (IV) differed from V in melting point (198–199°), and a mixture melting point with V showed a marked depression (179–197°). Nucleoside IV was tentatively assigned the 1*H*-isomer structure. An unambiguous structural elucidation of IV was accomplished by ultraviolet

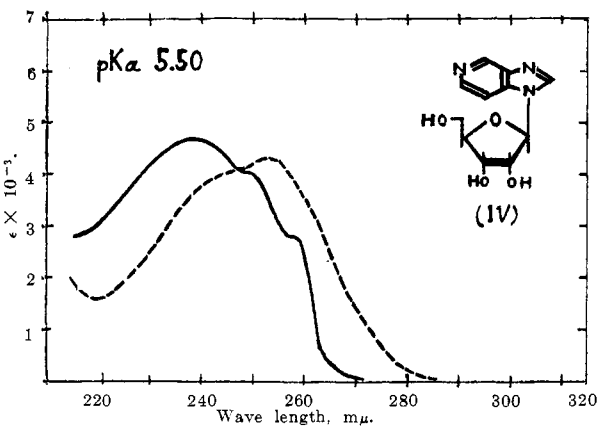


Fig. 1.—Ultraviolet absorption spectra of 1-(β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (IV): ———, pH 12.35; – – – –, pH 1.72.

spectral examination (Fig. 1). The spectra of the neutral (pH 8.50) and cationic (pH 1.72) species were similar to those of 1-methyl-1*H*-imidazo[4,5-*c*]pyridine (VI),[1] but differed markedly from those of the 3*H*-isomer VII.[1] Therefore, the imidazo[4,5-*c*]pyridine nucleoside IV bears the ribofuranosyl moiety on posi-

(1) Part XXII of this series: Y. Mizuno, M. Ikehara, T. Itoh, and K. Saito, *J. Org. Chem.*, **28**, 1837 (1963).

(2) (a) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953); H. M. Kissman and M. J. Weiss, *J. Org. Chem.*, **21**, 1053 (1956); (c) G. B. Brown and V. S. Weliky, *ibid.*, **23**, 125 (1958); (d) H. J. Schaeffer and H. J. Thomas, *J. Am. Chem. Soc.*, **80**, 4896 (1958).

(3) Y. Mizuno, T. Itoh, and K. Saito, *Chem. Pharm. Bull.* (Tokyo), in press.

(4) (a) E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914); (b) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).

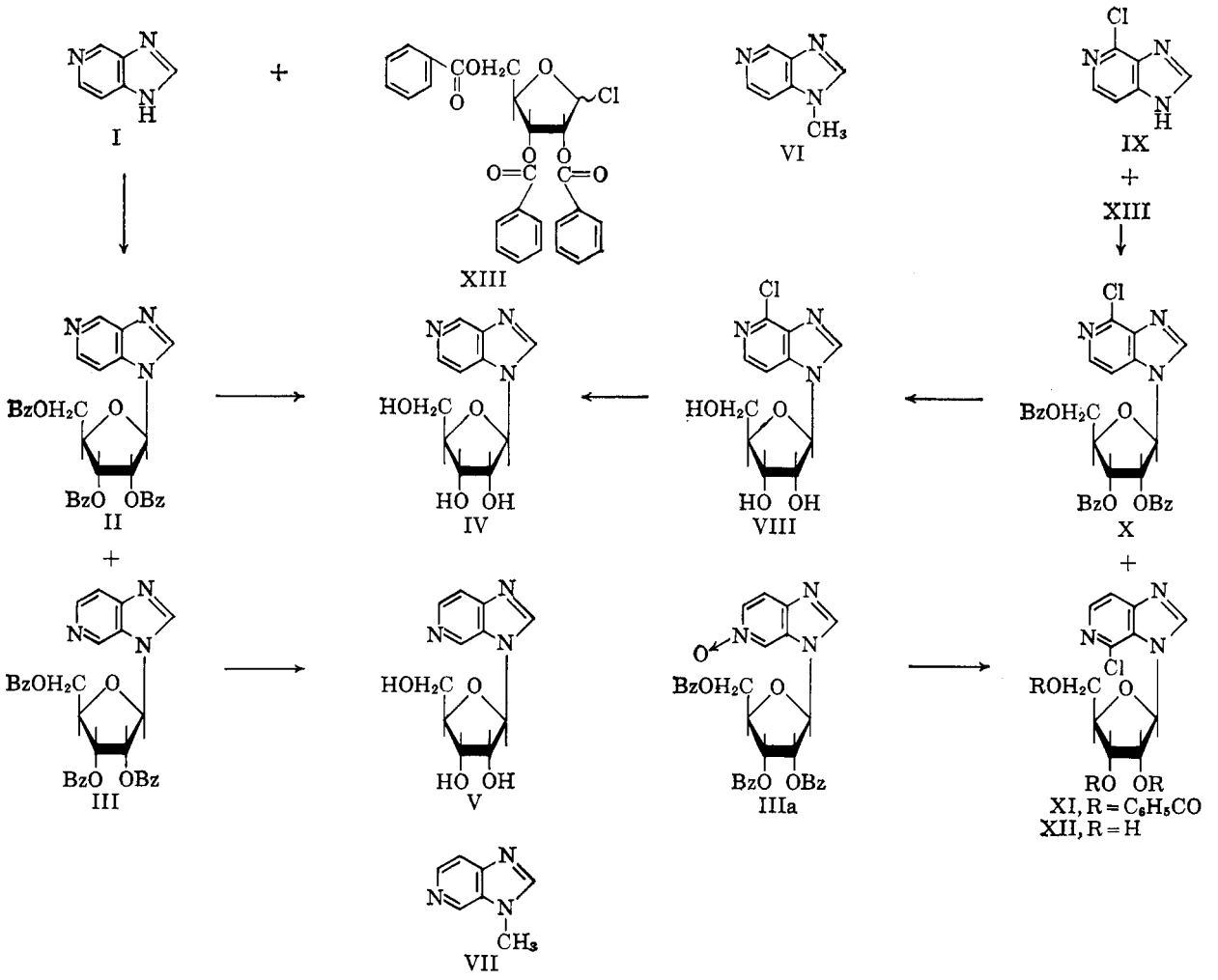
(5) F. Koegle, G. M. van der Want, and C. A. Salemink, *Rec. trav. chim.*, **67**, 29 (1948).

(6) (a) T. Kato, *J. Pharm. Soc. Japan*, **75**, 1236 (1955); (b) Y. Suzuki, *Pharm. Bull.* (Tokyo), **5**, 78 (1957); (c) G. Buchi, R. E. Mannig, and F. A. Hochstein, *J. Am. Chem. Soc.*, **84**, 3393 (1962).

(7) A. Albert and C. Pedersen, *J. Chem. Soc.*, 4683 (1956).

(8) H. M. Kissman, C. P. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955).

CHART I



tion 1.[9] The pK_a of IV, determined spectrophotometrically,[11] was 5.50.

4-Chloro-1*H*-imidazo[4,5-*c*]pyridine (IX)[5] in the same condensation reaction, followed by alumina column chromatographic separation, gave two isomeric benzoylated nucleosides X and XI (see Chart I), one of which, X, was obtained in crystalline form, m.p. 100–102°, and other, XI, as a glass. Debenzoylation of XI with methanolic ammonia gave a nucleoside as a solid (m.p. 179–180°). The chromatographic behavior and ultraviolet spectral properties of this solid were found to be identical with those of an authentic sample of 4-chloro-3-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-*c*]pyridine, which was prepared by the scheme outlined in Chart II. Compound III was converted into the corresponding 5-*N*-oxide IIIa with monoperphthalic acid (43% yield), which on treatment with phosphoryl chloride afforded 3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4-chloro-3*H*-imidazo[4,5-*c*]pyridine (XI, 59% yield). Structural assignments for IIIa and XI are based on infrared and ultraviolet spectral data as well as on their hydrolysis experiments: on hydrolysis with perchloric acid, IIIa and XI gave rise to 1*H*-imidazo[4,5-*c*]pyridine 5-oxide (Ia) and 4-chloro-1*H*-imidazo[5-*c*]pyridine (IX), respectively. Debenzoylation of XI afforded (after purification by paper chromatography) pure XII (18% yield).

It is very likely that the crystalline benzoylated nucleoside (m.p. 100–102°) was 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4-chloro-1*H*-imidazo[4,5-*c*]pyridine (X). The unequivocal structural elucidation was based on the fact that deblocking of X to VIII, followed by catalytic dechlorination, afforded 1-(β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (IV).

II was successfully converted into the corresponding N^5-oxide (IIa, 47.7% yield). However, some attempts to convert the *N*-oxide into chloro derivative(s) by the *N*-oxide–phosphoryl chloride reaction[3,6] failed (see Experimental).

In the Fischer–Helferich–Davoll–Lowy method,[4] with few exceptions,[4a,12–14] it is the 9-position of the purine[2,4b] which undergoes ribosidation. In view of the

(9) Jain, Chatterjee, and Anand[10] have carried out a similar ribosidation of I and have isolated only a single nucleosidic product whose properties were almost identical with those of our sample of V, except the values of specific rotation and melting point: reported value, $[\alpha]^{21}D$ −50 (*c* 1, MeOH); our sample, $[\alpha]^{21}D$ −35.8 (*c* 1.45, H_2O); reported melting point, 195°; our sample melted at 200–202° (uncor.). However, they did not refer to the other isomeric nucleoside.

(10) P. C. Jain, S. K. Chatterjee, and N. Anand, *J. Indian Chem.*, **1**, 30 (1963).

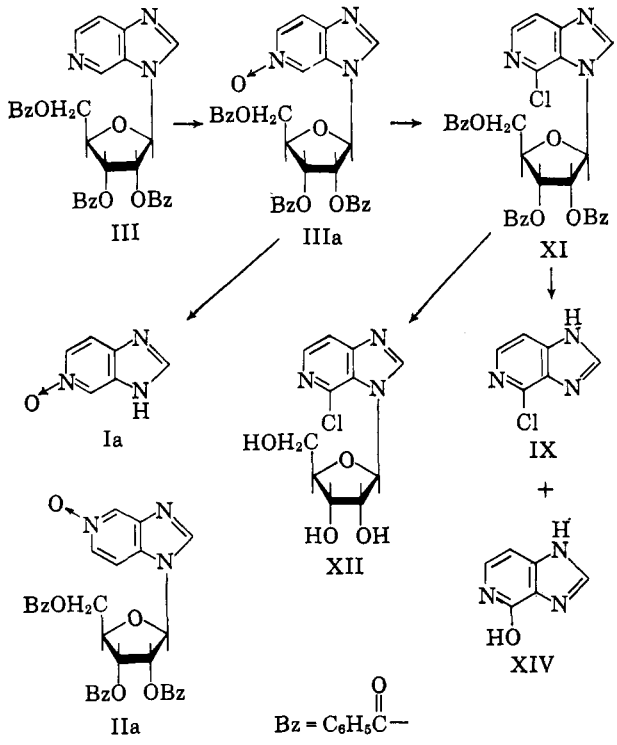
(11) p*K* value was determined essentially according to D. Shugar and J. J. Fox [*Biochim. Biophys. Acta*, **9**, 199 (1952)].

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

(13) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1780 (1954).

(14) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, **85**, 2673 (1963).

CHART II



fact that the ribosidation of 1*H*-imidazo[4,5-*b*]pyridine[1] afforded a high yield (68%) of a single nucleoside among two possible isomeric nucleosides,[15] it is worthy of note that the ribosidation of I and IX by the mercuric method[4b] gave rise to both of two isomeric nucleosides in almost equal amounts.

Experimental[16]

Chloromercuri Salt of 3*H*-Imidazo[4,5-*c*]pyridine.—To a well-stirred solution of I[7] (7.0 g., 50.5 mmoles) in 28 ml. of 1.5 *N* sodium hydroxide solution was added a solution of mercuric chloride (13.7 g., 1 equiv.) in ethanol (40 ml.) to afford a white precipitate which was collected by centrifugation, washed successively three times with water, four times with ethanol, and finally with dry ether, and dried in 2 mm. at 100°; yield 17.9 g. (96% yield).

Anal. Calcd. for $C_6H_4ClHgN_3$: N, 11.84. Found: N, 11.92.

Ribosidation of the Chloromercuri Salt of I with XIII.—The procedure used in condensation was essentially that reported by Kissman and Weiss.[2b] To an azeotropically dried suspension of the chloromercuri salt of I (14.2 g., 0.04 mole) in dry xylene (500 ml.) was added with stirring 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride,[8] prepared from 20 g. of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose. The mixture was refluxed for 3 hr. and filtered hot. The filtrate was concentrated *in vacuo* to dryness. The residue was taken in chloroform (50 ml.) and washed with 30% potassium iodide solution, then with water and dried. The solvent was removed to leave a residue (22.2 g.) which was dissolved in chloroform (30 ml.), and the solution was applied to acid-washed alumina column (53 × 3 cm.). The first fraction eluted by benzene (2.3 l.) and the subsequent three fractions obtained by washing the column with mixtures of ethyl acetate and benzene (letting the concentration of ethyl acetate gradually increase from 5 to 25%; total volume, 3.5 l.) gave, after evaporation of the solvent, nitrogen-free residues, 0.32 g., 0.20 g., 0.01 g., and 0.08 g., respectively. These fractions were discarded. The subsequent fraction, eluted by 4.0 l. of benzene–ethyl acetate (1:2.3 v./v.), gave, after removal of the solvent, benzoylated nucleoside III, 5.01 g. (22.2 % on the basis of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose); ultraviolet absorption in ethanol: λ_{max} 275 mμ. The subsequent fraction eluted with 2 l. of a mixture of benzene and ethyl acetate (1:1 v./v.) afforded another benzoylated nucleoside II, 6.01 g. (26.9%); ultraviolet absorption in ethanol: λ_{max} 259 mμ.

1-(β-D-Ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (IV).—To a solution of II (0.445 g.) in absolute methanol (30 ml.) was added 1 *N* methanolic solution of sodium methoxide (1 ml.). The solution was refluxed for 1 hr. After cooling, the solution was concentrated to dryness and the residue dissolved in water (20 ml.). The aqueous solution was treated with two 10-ml. portions of chloroform. The aqueous layer was separated and neutralized with Amberlite IRC 50 (H^+ form). After filtration, the filtrate was concentrated to dryness (327 mg.) and crystallized from ethanol containing several drops of water to afford a pure nucleoside, 35 mg., m.p. 198–199° dec; ultraviolet absorption at pH 1.72: λ_{max} 263–264 mμ (ε 4700), λ_{min} 267–268 mμ (sh, ε 4100); pK_a 5.50[11]; $[\alpha]^{21}D$ −36.0 (*c* 1.0, H_2O); R_f 0.34 (*n*-BuOH–H_2O, 84:16 v./v.), 0.67 (H_2O adjusted to pH 10).

Anal. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.58; H, 5.22; N, 16.73. Found: C, 52.64; H, 5.06; N, 16.54.

3-(β-D-Ribofuranosyl)-3*H*-imidazo[4,5-*c*]pyridine (V).—To a solution of III (2.1 g.) in 150 ml. of absolute methanol was added 1.0 *N* methanolic sodium methoxide (2 ml.). The solution was refluxed for 1 hr. and then cooled. The solvent was removed at room temperature to furnish a crude product which was dissolved in water (100 ml.). The solution was treated with two 30-ml. portions of chloroform. The aqueous layer was separated and neutralized with Amberlite IRC 50 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated to dryness. The residue weighed 0.589 g. (62.9%). This was recrystallized from a mixture of methanol and ether, m.p. 200–202°, lit.[10] m.p. 195°; R_f 0.40 (*n*-BuOH–H_2O, 84:16 v./v.),[17] 0.70 (H_2O, adjusted to pH 10 with ammonium hydroxide); $[\alpha]^{21}D$ −35.8 (*c* 1.45, H_2O).

Anal. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.58; H, 5.22; N, 16.73. Found: C, 52.65; H, 5.39; N, 16.29.

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*c*]-pyridine 5-Oxide (IIIa).—To a solution of 3-(2,3,5-tri-*O*-benzoyl-β-D-ribosyl)-3*H*-imidazo[4,5-*c*]pyridine (III) (563 mg., 1 mmole) in ether (20 ml.) was added in portions monoperphthalic acid (0.3 *N* solution, 20 ml., 3 equiv.) at 15° and the mixture was kept overnight at the same temperature. During this period, a tar separated and the supernatant liquor was discarded; the tarry substance was washed with two 10-ml. portions of ether and dissolved in chloroform (20 ml.); and the solution was washed with four 100-ml. portions of sodium hydrogen carbonate solution (7%) and with three 30-ml. portions of water, and dried over sodium sulfate. Removal of the solvent *in vacuo* gave a residue, 251 mg. (43.3% yield); ultraviolet absorption in ethanol: λ_{max} 305 and 281 mμ, λ_{min} 299 and 255 mμ[18]; infrared spectrum had a band at 1200 cm.$^{-1}$, characteristic of aromatic *N*-oxide.[19]

Acid Hydrolysis of IIIa. Confirmation of the Structure of IIIa.—A solution of IIIa (11 mg.) in ethanol (0.5 ml.) was treated with hydrochloric acid (1 *N*, 0.1 ml.) at 100°; the process was followed by a paper chromatography (the solvent system: H_2O adjusted to pH 10 with ammonium hydroxide and 1-butanol–pyridine–water, 10:3:3 v./v.). After the solution had been heated for 1 hr., the spot corresponding to the starting material completely disappeared and concomitantly a new, single spot appeared: R_f 0.75 (H_2O, pH 10), 0.29 (*n*-BuOH–pyridine–H_2O) (in the latter solvent system, R_f value of 5-oxide was 0.29; that of I was 0.70[3]). The spot (R_f 0.75) was cut out and extracted with hot ethanol, the extract was cooled to 20°, and the absorption spectrum was determined using an ethanol extract of a blank paper as the control. The spectrum was very similar to that of an authen-

(15) This result does not always indicate that the other isomeric nucleoside was not formed. However, when the recovered sugar is taken into consideration (see Experimental in ref. 1), the yield of the isolated nucleoside is *ca.* 85%. Even a critical examination of the reaction mixture of the ribosidation (by gas chromatographic technique) failed to detect the other isomeric product which must be present in small amounts, if at all.

(16) All melting points are corrected. Ultraviolet absorption spectra were run with a Beckman Model DK-2 recording spectrophotometer. Infrared spectra were determined on a Koken Model DS-301 infrared recording spectrophotometer. Except where noted, the solvent was always removed *in vacuo* (16–19 mm.) by water aspirator. Paper chromatography was performed by use of the ascending technique.

(17) Reported R_f value was 0.45 (*n*-butyl alcohol saturated with H_2O).[10] The spectral properties and p*K* value have been reported by us[1] and Jain, *et al.*,[10] and the reported values are similar to each other.

(18) Ultraviolet absorption maximum in ethanol of III was 273 mμ.

(19) H. Shindo, *Pharm. Bull.* (Tokyo), **7**, 407, 791 (1959).

tic sample of 1*H*-imidazo[4,5-*c*]pyridine 5-oxide (Ia)[3] and differed from that of I.[3]

Chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-3*H*-imidazo-[4,5-*c*]pyridine (XI).—A solution of IIIa (818 mg., 1.41 mmoles) in chloroform (50 ml.) was treated with phosphoryl chloride (1.5 ml.) at reflux temperature for 2.5 hr. and cooled. The solution was poured onto ice–water and the mixture was treated with sodium hydrogen carbonate solution; the aqueous layer was separated and discarded, and the chloroform layer was washed with three 40-ml. portions of 5% sodium hydrogen carbonate solution and with three 20-ml. portions of water and dried over magnesium sulfate; the solution was filtered and the filtrate was concentrated *in vacuo* to afford a glass, 495 mg. (59% yield); ultraviolet absorption in ethanol: λ_{max} 273 and 280 mμ (sh); infrared spectrum showed the absence of the aromatic *N*-oxide group (1200 cm.$^{-1}$)[19] and the presence of carbonyl of benzoate (1730 cm.$^{-1}$).

Confirmation of the Structure of XI.—A solution of XI (10 mg.) in ethanol and benzene (5:1, 0.6 ml.) was treated with hydrochloric acid (1.2 *N*, 0.1 ml.) at 100°. After the mixture had been heated for 20 min., paper chromatography of the mixture gave a single spot (R_f 0.51)[20]; the spot was cut out, extracted with hot water for 3 min., and cooled, and the absorption spectra were determined using an aqueous extract of a blank paper as the control: $\lambda_{max}^{1\ N\ HCl}$ 270 mμ, $\lambda_{max}^{1\ N\ NaOH}$ 280 mμ, $\lambda_{max}^{H_2O}$ 266.5 mμ. An authentic sample of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine[5] had the same spectral properties in three different media.

To the rest of the mixture was added 60% perchloric acid (0.1 ml.) and heating was continued for another 4 hr.; paper chromatography of this mixture gave another, single spot (R_f 0.61)[20]; ultraviolet spectra (after a similar treatment as described above): $\lambda_{max}^{pH\ 6.0}$ 256 mμ, $\lambda_{max}^{1\ N\ HCl}$ 263 mμ, $\lambda_{max}^{1\ N\ NaOH}$ 269 mμ. In three different media the patterns of the absorption curves of the hydrolytic product were very similar to those of 4-hydroxy-1*H*-imidazo-[4,5-*c*]pyridine (XIV).[3,5]

4-Chloro-3-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-*c*]pyridine (XII). Method A.—To a solution of XI (273 mg., 0.456 mmole) in absolute methanol (27 ml.) was added a methanolic solution of sodium methoxide (1 *N*, 1 ml.). The solution was refluxed for 1 hr., and the removal of the solvent *in vacuo* gave a residue[21] which was taken in water (20 ml.). The aqueous solution was treated with two 10-ml. portions of ether and washed with ethyl acetate (10 ml.). The aqueous layer was neutralized with IRC 50 (H^+ form) and filtered. Concentration of the filtrate left a residue (304 mg.), which was extracted with absolute ethanol (*ca.* 100 ml.). (The residue was discarded.) The solution was concentrated to 1 ml. After the solution had been kept at room temperature overnight, a solid (sodium benzoate) separated. The solid was filtered off. The filtrate was dissolved in methanol (2 ml.); the solution was submitted to a preparative paper chromatography using 1-butanol–water (84:16 v./v.)(Toyo filter paper 51A, 40 × 40 cm.). The band corresponding to XII (R_f 0.58) was cut out and extracted with methanol (50 ml.). Removal of the solvent left a solid, m.p. 168–170°, 250 mg.; ultraviolet absorption: $\lambda_{max}^{pH\ 1.5}$ 273 mμ (ε 5400), $\lambda_{min}^{pH\ 1.5}$ 254–255 mμ (ε 3800), $\lambda_{max}^{pH\ 5.6}$ 274–275 mμ (ε 5200), $\lambda_{max}^{pH\ 5.6}$ 257–258 mμ (ε 3200); pK_a[11] 2.50; isosbestic points: 250.5, 276, and 293 mμ; R_f in 1-BuOH–H_2O (84:16 v./v.) 0.58; $[\alpha]^{20}D$ +12 (*c* 1.25, MeOH); infrared spectrum was well compatible with the assigned structure (XII).

Preparation of IIa and Confirmation of the Structure.—IIa was prepared essentially as described for the preparation of IIIa. From 2.03 g. (563 mmoles) of II, IIa was obtained in a yield of 47.7% (0.975 g.); ultraviolet absorption in ethanol: λ_{max} 271.5 mμ. No shift in the maximum was observed in both 0.1 *N* HCl and 0.1 *N* NaOH; infrared absorption spectrum had a band at 1200 cm.$^{-1}$, characteristic of an aromatic *N*-oxide.[19,22]

To 0.5 ml. of methanolic solution of IIa (10 mg.) was added 0.2 ml. of concentrated hydrochloric acid and the solution was refluxed. The process of the reaction was followed by examining paper chromatograms prepared from aliquots, removed from the reaction mixture at increasing time intervals. After the solution had been heated for 5 hr., the spot corresponding to the starting material disappeared, and the formation of a new spot corresponding to hydrolytic product (R_f 0.2 or 0.78)[23] was observed. The spot (R_f 0.2 or 0.78) was cut out and extracted with water. The extract had an ultraviolet absorption spectrum (λ_{max} 271.5 mμ) very similar to that of an authentic sample of 1*H*-imidazo-[4,5-*c*]pyridine 5-oxide[3] (Ia) and different from that of I.[1]

Attempted Synthesis of X from IIa.—A chloroform solution (10 ml.) of IIa (100 mg.) was treated with 0.2 ml. of phosphoryl chloride at refluxing temperature. The process of the reaction was followed spectrophotometrically at the increasing time intervals. Even after heating had been continued for 2 days, no remarkable shift in ultraviolet absorption was observed. After the solution had been refluxed for 100 hr., IIa decomposed considerably to an intracterable product(s).

Synthesis of X and XI by Ribosidation of Chloromercuri Salt of IX.—To a solution of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine[3,5] (IX, 3.6 g.) in 48.8 ml. of 0.5 *N* sodium hydroxide solution (1 equiv.) was added with stirring 100 ml. of ethanol solution of mercuric chloride (6.37 g., 1 equiv.) to afford a gel which was subjected to centrifugation. The supernatant liquor was discarded and the precipitate was washed successively with water, ethanol, and finally with ether and dried, 7.3 g. (80%). To an azeotropically dried suspension of 7.3 g. (18.9 mmoles) of the chloromercuri salt of IX in 100 ml. of dry xylene was added with stirring XIII,[8] prepared from 10 g. of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose. The suspension was refluxed for 3 hr., filtered hot, and cooled. The filtrate was concentrated to dryness. The residue was taken in 100 ml. of chloroform. The solution was washed with three 50-ml. portions of 30% potassium iodide solution and with three 50-ml. portions of water and dried. The solvent was removed to leave a residue, 9.54 g. (81%). The residue was dissolved in 50 ml. of chloroform and the solution was applied to acid-washed alumina (177 g., 25 × 3 cm.) and treated in a manner described above. After removal of the solvent fraction 1 (eluted by 2 l. of 5 ~ 10% benzene solution of ethyl acetate), 3.9 g. of a solid was obtained. This benzoylated nucleoside melted, after recrystallization from ethanol, at 100.5–102°, 1.85 g.

Anal. Calcd. for $C_{32}H_{24}ClN_3O_7$: C, 64.27; H, 4.05; N, 7.03. Found: C, 64.40; H, 4.25; N, 7.36.

The subsequent fraction (fraction 2) eluted by ethyl acetate–benzene of concentrations ranging from 10 to 20% gave, after removal of the solvent, 1.2 g. of a mixture of two isomeric products (X and XI). Fraction (fraction 3) eluted by a mixture of ethyl acetate and benzene (1:3 ~ 1:1 v./v., 2 l.) gave, after removal of the solvent, a residue, 2.51 g. (22.8%). The infrared spectrum of fraction 3 was almost identical with that of a sample prepared from IIIa by *N*-oxide–phosphoryl chloride reaction and quite different from that of fraction 1.

4-Chloro-1-(β-D-ribofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (VIII).—To a solution of X (598 mg.) in absolute methanol (25 ml.) was added 2.97 g. (30 equiv.) of cyclohexylamine. The solution was kept in a desiccator for 2 days at room temperature and finally refluxed for 30 min. and cooled. Removal of the solvent left a residue which was dissolved in methanol, the solvent was removed, and the process was repeated three times to afford a white solid, which was recrystallized from ethanol, 239 mg. (83.6%), m.p. 189–190°, $[\alpha]^{19.5}D$ −41.6 (*c* 1.25, MeOH), R_f (1-BuOH–H_2O, 84:16) 0.38.

Anal. Calcd. for $C_{11}H_{12}ClN_3O_4$: C, 46.22; H, 4.23; N, 14.71. Found: C, 46.32; H, 4.23; N, 15.05.

Hydrogenation of VIII to IV (Confirmation of Structure of VIII).—To a suspension of 400 mg. of 20% palladium on carbon in 20 ml. of methanol was added 20 mg. of VIII and hydrogenated at atmospheric pressure at room temperature for 1 hr. The catalyst was removed and the filtrate was concentrated to dryness (semi-solid); paper chromatography of this sample: R_f (H_2O adjusted to pH 10 with NH_4OH) 0.66, R_f (1-BuOH–H_2O, 84:16 v./v.) 0.30; absorption spectra of the spots: $\lambda_{max}^{pH\ 2.0}$ 263 mμ, $\lambda_{max}^{pH\ 12}$ 249 mμ. The infrared spectrum of this sample (dried at 1 mm. at 100°) was found to be identical with that of a sample of IV, described above.

(20) The solvent system employed was water adjusted to pH 10 with ammonium hydroxide; the compound on the developed chromatograms was detected under an ultraviolet lamp; spots other than R_f 0.51 failed to be detected; in this solvent system, R_f values of IX and XIV were 0.51 and 0.61, respectively.

(21) Paper chromatography in water of the residue showed two spots (whose R_f values were 0.74 and 0.65) which were detected by both the periodate spray reagent and the ultraviolet light. Each spot was cut out and extracted with water, and the absorption spectra were determined. The extract of the spot of R_f 0.74 had the ultraviolet absorption spectrum expected for XII.

(22) R. H. Wilet and S. C. Slagmaker, *J. Am. Chem. Soc.*, **79**, 2233 (1957).

(23) Solvent systems employed were 1-BuOH–H_2O (84:16 v./v.) or H_2O adjusted to pH 10 with NH_4OH.

4-Chloro-3-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-*c*]pyridine (XII). Method B.—The procedure was essentially that used for the synthesis of VIII from X. To a solution of XI (obtained by ribosidation, followed by separation; 2.35 g.) in 80 ml. of absolute methanol was added cyclohexylamine (11.6 g.). The solution was kept at room temperature for 2 days and finally refluxed for 1 hr. (until the paper chromatography in H_2O, adjusted to pH 10 had a single spot: R_f 0.60). The solution was concentrated to *ca.* 5 ml. To the solution 20 ml. of water was added and the mixture was treated with chloroform (30 ml.). The aqueous layer was separated and concentrated to dryness. The residue was taken in methanol (20 ml.), and methanol was removed *in vacuo*. The process was repeated twice to afford crystalline nucleoside, 942 mg. (84%), m.p. 177–178°. After recrystallization from aqueous ethanol, m.p. 179–180°; R_f (BuOH–H_2O, 84:16 v./v.) 0.54; ultraviolet absorption: λ_{max}^{EtOH} 275 mμ (ε 5300).

Anal. Calcd. for $C_{11}H_{12}ClN_3O_4$: C, 46.22; H, 4.23; N, 14.71. Found: C, 46.40; H, 4.06; N, 14.95.

Acknowledgment.—We wish to thank Dr. Jack J. Fox of the Sloan-Kettering Institute for reviewing our manuscript and for his valuable suggestions. We also wish to thank Mrs. Toyoko Tohma for the elementary microanalyses.

2,4-Dinitrobenzenesulfenyl as a Blocking Group for Hydroxyl Functions in Nucleosides[1]

R. L. LETSINGER, J. FONTAINE,[2] V. MAHADEVAN, D. A. SCHEXNAYDER, AND R. E. LEONE[3]

Department of Chemistry, Northwestern University, Evanston, Illinois

Received March 12, 1964

The dinitrobenzenesulfenic esters of butanol and cyclohexanol were found to be relatively stable in acetic acid and in pyridine solutions containing acetic anhydride, *p*-toluenesulfonyl chloride, and dicyclohexylcarbodiimide; however, they reacted readily at room temperature with solutions containing polarizable nucleophiles such as thiosulfate, cyanide, or thiophenol. As a consequence of the stability of the esters in pyridine and the lability in solutions containing active nucleophiles, the 2,4-dinitrobenzenesulfenyl group offers promise as a blocking agent for hydroxyl functions, supplementing acyl groups, which are removed by alkaline treatment, and trityl or tetrahydropyranyl groups, which are removed by acid treatment. The feasibility of using the 2,4-dinitrobenzenesulfenyl group in nucleoside work was explored by preparing derivatives of thymidine and 5′-*O*-tritylthymidine, removing the trityl and dinitrobenzenesulfenyl groups in stepwise fashion, and acetylating one of the dinitrobenzenesulfenyl derivatives.

The groups most frequently used to protect hydroxyl functions in nucleosides and nucleotides are acetyl,[4] benzyl,[5] triarylmethyl,[6–8] benzoyl,[7,8] tetrahydropyranyl,[7] and isopropylidene.[8] Hydroxyl groups protected by esterification are unblocked by alkaline hydrolysis; those protected by ether formation are unblocked by acid hydrolysis. For use in the synthesis of oligonucleotides on polymer supports[9] it was desirable to have available a blocking group which would withstand the conditions employed in forming internucleotide bonds, yet which could be selectively removed under mild conditions in a neutral medium or in a pyridine solution. Ability to remove the blocking group in an aprotic solvent was especially desirable, since in that case both the formation of internucleotide bonds and unblocking of hydroxyl functions could be carried out without changing the nature of the solvent.

With these considerations in mind we selected the 2,4-dinitrobenzenesulfenyl group for study as a potential blocking agent in nucleotide syntheses. Three lines of evidence supported this choice. (a) Kharasch, McQuarrie, and Buess showed that relatively stable esters could be prepared in high yield from the reaction of 2,4-dinitrobenzenesulfenyl chloride with simple aliphatic alcohols.[10] (b) Edwards and Pearson[11] pointed out that nucleophilicity toward divalent sulfur is largely governed by the polarizability of the attacking nucleophile; accordingly, it appeared likely that facile cleavage of the sulfenic esters might be realized with weakly basic but highly polarizable nucleophiles. (c) Foss[12] described an analytical method for determination of closely related compounds, the 2,4-dinitrobenzenesulfenamides, which involved the quantitative conversion of sulfenamides to amines by reaction with a polarizable ion, thiosulfate, in dilute acid at room temperature.

Preliminary experiments with 2,4-dinitrobenzenesulfenic esters of butanol and cyclohexanol revealed that the dinitrobenzenesulfenic esters were sufficiently stable to serve as blocking groups in synthetic work. Thus, the esters were recovered unchanged after standing 48 hr. in pyridine solutions containing either acetic anhydride or *p*-toluenesulfonyl chloride. Furthermore, chromatographic evidence indicated that cyclohexyl 2,4-dinitrobenzenesulfenate was not affected in a solution in which pyridinium thymidine 5′-phosphate was converted to a mixture of oligonucleotides by dicyclohexylcarbodiimide[13]; that is, the sulfenic ester survived in the presence of the active phosphate comprising the phosphorylating agent.

(1) This work was supported by the Division of General Medical Sciences, National Institutes of Health, Grant GM 10265-01. It also benefitted from a Public Health Service training grant, 5TI-626, from the National Institute of General Medical Science, Public Health Service.

(2) Abbott Fellow of Northwestern University, 1962.

(3) National Science Foundation Summer Research Participant, 1963.

(4) P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6212 (1958).

(5) B. E. Griffin and A. R. Todd, *J. Chem. Soc.*, 1389 (1958).

(6) G. Weimann and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 4329 (1962).

(7) M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *ibid.*, **84**, 430 (1962).

(8) P. Levene and R. Tipson, *J. Biol. Chem.*, **121**, 131 (1937).

(9) R. L. Letsinger and M. J. Kornet, *J. Am. Chem. Soc.*, **85**, 3045 (1963).

(10) N. Kharasch, D. P. McQuarrie, and C. M. Buess, *ibid.*, **75**, 2658 (1953).

(11) J. O. Edwards and R. G. Pearson, *ibid.*, **84**, 16 (1962). The conclusions were based on data on disulfide reactions assembled by A. J. Parker and N. Kharasch, *Chem. Rev.*, **59**, 583 (1959).

(12) O. Foss, *Acta Chem. Scand.*, **1**, 307 (1947).

(13) Preparation of thymidine oligonucleotides by the action of dicyclohexylcarbodiimide in pyridine on pyridinium thymidine 5-phosphate was described by G. M. Tener, H. G. Khorana, R. Markham, and E. H. Pol, *J. Am. Chem. Soc.*, **80**, 6223 (1958).