TABLE I

Time, min.	$NaIO_4$ consumed, moles/mole of substance	Formaldehyde, moles/mole of substance
5	1.22	0.99
15	1.75	1.01
30	1.91	1.01
60	2.29	1.00
120	2.37	1.01
300	2.94	1.01
1440	2.97	1.02

$[\alpha]^{27}D$ +42.9° (*c* 0.55, chloroform). The analytical sample was dried at 30° and 2 mm.

Anal. Calcd. for $C_{29}H_{42}N_2O_{18}$: C, 49.26; H, 5.98; N, 3.96. Found: C, 49.32; H, 6.20; N, 4.30.

N-Acetylhexa-O-acetyl-3-O-(β-D-galactopyranosyl)-D-arabinofuranosylamine (IV).—II (100 mg.) was dissolved in 3.6 ml. of a 1:1 mixture of pyridine–acetic anhydride by heating 5 min. in a boiling-water bath. The mixture was left 24 hr. at room temperature and then evaporated to dryness in a vacum desiccator. The syrup was dissolved in 1.5 ml. of methanol, giving IV as prisms of m.p. 54–56°, $[\alpha]^{20}D$ −11.6° (*c* 0.6, chloroform). The analytical sample was dried at 80° and 2 mm.

Anal. Calcd. for $C_{25}H_{39}NO_{16}$: C, 49.56; H, 5.83; N, 2.31. Found: C, 49.77; H, 5.81; N, 2.63.

Oxidation of 1,1-Diacetamido-1-deoxy-3-O-(β-D-galactopyranosyl)-D-arabinitol.—This substance (3.68 mg.) was dissolved in 3.42 ml. of a 0.015 *M* solution of sodium metaperiodate. The solution was held at 30°. Samples of 0.1 ml. were taken at intervals and diluted with water to 25 ml.; the periodate consumed and the formaldehyde produced were determined according to spectrophotometric methods.[9] Results are given in the Table I.

Methylation of N-Acetyl-3-O-(β-D-galactopyranosyl)-D-arabinofuranosylamine.—Methyl iodide (2.28 g., 1.6×10^{-2} mole) was added to a solution of 80 mg. of II (2.24×10^{-4} mole) in 3 ml. of dimethylformamide which contained 500 mg. of barium oxide (3.25×10^{-3} mole) in suspension. The suspension was shaken for 10 hr. at room temperature and then poured into 100 ml. of chloroform and filtered. The chloroform solution was washed with cold 1 *N* sulfuric acid until no more barium sulfate appeared in the interphase; it was then washed with water, a saturated solution of sodium hydrogen carbonate, and water, dried with anhydrous sodium sulfate, and finally evaporated to dryness. The residual syrup weighed 90 mg. and did not show any spot by development of paper chrmatograms with reagent B.

Hydrolysis of the Methylated N-Acetyl-3-O-(β-D-galactopyranosyl)-D-arabinofuranosylamine.—The methyl derivative of II (90 mg.) obtained as above was dissolved in 5 ml. of 1 *N* sulfuric acid and heated in a boiling-water bath during 6 hr. The solution was neutralized with barium carbonate, filtered, and evaporated to dryness. The residue was taken up with ethyl ether, the solution was evaporated, and the residue was dried exhaustively, yield 78.4 mg. Paper chromatography[15] gave two spots of 2,5-di-*O*-methyl-D-arabinose (R_g 0.80) and 2,3,4,6-tetra-*O*-methyl-D-galactose (R_g 0.86).

The mixture was fractionated on Whatman 3 MM paper and pure 2,5-di-*O*-methyl-D-arabinose of $[\alpha]^{35}D$ +21.3° (*c* 0.32, water) was obtained [lit.[11] $[\alpha]^{20}D$ +20.0° (water)]. 2,3,4,6-Tetra-*O*-methyl-D-galactose was also obtained, $[\alpha]^{32}D$ +115° (final value) (*c* 0.29, water) [lit.[12] $[\alpha]^{20}D$ +150° → +114° (water)].

(15) 2,3,4,6-Tetra-*O*-methyl-D-glucose was employed as standard.

Studies on Condensed Aromatic Nitrogenous Compounds. XXV. Product Distribution in Ribosylation of Purines and Deazapurines by the Mercuri Method[1,2]

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Product distribution in ribosylation of several heterobicycles, including purines, by the mercuri method was closely examined by use of alumina, gas, and paper chromatography. Ribosylation of purine (IV) and 3*H*-imidazo[4,5-*b*]pyridine (V) afforded, in addition to already reported major products, a minor amount of isomeric products, XIX and XVII, respectively. The ribosylation of 6-bromo-3*H*-imidazo[4,5-*b*]pyridine (VI) also gave a minor amount of 7*H* isomer as well as a predominant amount of 9*H* isomer. On the other hand, the 3-deaza analogs of the purines examined (compounds VII, VIII, and IX) gave on ribosylation each of two possible isomeric products in almost equal amounts. A common structural feature of bases whose ribosylation gave one of the two possible isomers in predominant amounts is that they have a nitrogen atom adjacent to the imidazole ring. Bases which have no nitrogen adjacent to the imidazole ring gave rise to each of two possible isomers in about equal amounts. In connection with the present investigation, several new ribonucleosides were prepared, such as XIV, XV, XVII, XIX, XXII, and XXIII.

The synthesis of purine nucleosides by the condensation of poly-*O*-acylglycosyl halides with the heavy metal salts of purines has wide application.[4] One of the main features of this reaction is that position 9 of purines undergoes substitution.[4b] Several exceptions have been known: some of them are theophylline (I),[5] 3-benzylhypoxanthine (IIa),[6a] 6-benzoylamido-3-benzyl- (IIb),[6b] and 6-dimethylamino-2,8-dimethylthiopurine (III).[7] It has been reported that ribosylation of I, IIa, and IIb showed a high preference for respective 7*H* isomers,[5,6] whereas ribosylation of III afforded twice as much 9 isomer as 3 isomer.[7] On the other hand, Mizuno and co-workers[8] have shown that ribosylation of 5-nitrobenzimidazole (VIII) gave

(1) Part XXIV: Y. Mizuno, T. Itoh, and K. Saito, *Chem. Pharm. Bull.* (Tokyo), **12**, 866 (1964).

(2) Presented in part at the IUPAC Symposium on the Chemistry of Natural Products, Kyoto, Japan, April 1964; Abstracts of Papers, D 7-3.

(3) (a) To whom inquiries concerning this article should be directed. (b) The Institute of Physical and Chemical Research.

(4) (a) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press Inc., New York, N. Y., 1963, p. 52; (b) J. A. Montgomery and H. J. Thomas, *Advan. Carbohydrate Chem.*, **17**, 301 (1962).

(5) (a) E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914); (b) J. M. Gulland, R. E. Holliday, and T. E. Macrae, *J. Chem. Soc.*, 1639 (1934).

(6) (a) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **28**, 2304 (1963); (b) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.*, **85**, 2673 (1963).

(7) (a) L. B. Townsend, R. K. Robins, R. N. Loepky, and N. J. Leonard, *ibid.*, **86**, 5320 (1964); (b) H. M. Kissman, C. Pidack, and B. R. Baker, *ibid.*, **77**, 18 (1955).

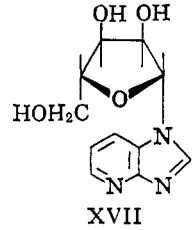
(8) Y. Mizuno, M. Ikehara, F. Ishikawa, and H. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **10**, 761 (1962).

rise to each of two possible isomers.[9] We have also found that, whereas the ribosylation of 3*H*-imidazo-[4,5-*b*]pyridine (V) leads to a predominant formation of one of two possible isomers,[10] that of 1*H*-imidazo-[4,5-*c*]pyridine (VII) gives rise to each possible isomer in comparable amounts.[11]

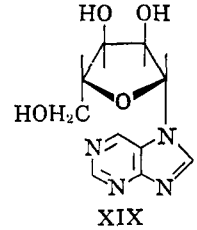
We have been interested in the structural features of these heterobicycles which control the position of entry of the sugar. In order to obtain some information on the mechanism of this reaction, we have studied the ribosylation of several "deaza analogs" of purines and have also re-examined the ribosylation of purine (IV)[12] and 6-chloropurine (X).[12b,13] In this regard emphasis has been laid upon the critical examination of the presence or absence of 7*H* isomer in the products. The deaza analogs examined are shown in Table I. Compounds IV–IX were chosen, since they should have no steric preference for either nitrogen atom in the imidazole ring. Thus, it should be possible to observe only the effect of electron density upon the product distribution from the ribosylation reaction.

Chloromercuri salts of these heterobicycles (bases IV–XI in Table I) were allowed to react with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (XIII) by the standard procedure.[14] The isolation and estimation of the respective products were carried out under comparable conditions, using at least two of three independent techniques; vapor phase chromatography (after prior deblocking and subsequent trimethylsilylation),[15] alumina column chromatography,[13,16] and a combination of alumina column chromatography and subsequent paper chromatography (after prior deblocking). Each isomeric ratio listed in Table I is an average of at least two determinations.

It is worthy of note that the combination of alumina column chromatography and paper chromatography (with BuOH–AcOH–water system[17]) was especially effective for separation of two isomeric nucleosides of bases IV, V, and VI (bases in class A, Table I) where one of a pair of isomers showed a high preponderance over the other. Thus, by this improved technique, the detection of minor products [*viz.*, 1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*b*]pyridine (XVII) and 7-β-D-ribofuranosyl-7*H*-purine (XIX)] which had escaped detection by the former technique[10,12] could now be achieved. On the other hand, gas chromatography



XVII



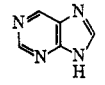
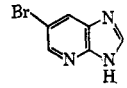
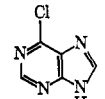
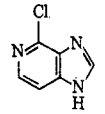
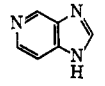
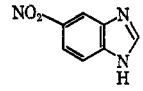
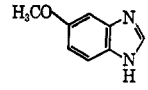
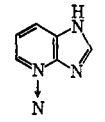
XIX

was the more effective for determining the product ratio in ribosylation of VII, VIII, and IX (bases in class B, Table I).

Identification of the respective products was carried out either by comparison of their spectral properties (in cases of alumina column chromatography) or by

TABLE I

CLASSIFICATION OF BASES EXAMINED ACCORDING TO BEHAVIOR CONCERNING PRODUCT DISTRIBUTION IN THE RIBOSYLATION BY THE MERCURI METHOD

Bases in class A		Yields of isomers, %: 9*H*[a]	7*H*[a]	Isomeric ratios	Method of separation and estimation[b]
	(IV)[c]	34	6	25	A[c]
		47.4	1.9	20	B
	(V)[d]	68	...		A
		68	3.3	20	B
	(VI)	42	13	3.2	C
	(X)[e]	65	...		A
	(XII)	(84)[f]	(16)	5.3	D
Bases in class B					
	(VII)[g]	41	36	1.1	A
		(45)	(55)	0.82	D
	(VIII)[h]	(44)	(56)	0.81	A
	(IX)[i]	(45)	(55)	0.82	D
	(XI)	23.7	20.2	1.17	A

[a] Ref. 18. [b] A, alumina column chromatography; B, a combination of alumina column chromatography and paper chromatography; C, a combination of fractinal crystallization and subsequent paper chromatography; and D, gas chromatography. [c] Ref. 12b. [d] Ref. 10. [e] Ref. 11. [f] Figures in parentheses show relative yield. [g] Ref. 11. [h] Ref. 8. [i] Ref. 9.

(9) H. Shunk, F. M. Robinson, J. F. McPherson, M. M. Gasser, and K. Folkers [*J. Am. Chem. Soc.*, **78**, 3228 (1956)] also reported in preliminary form the formation of each of two possible isomers on ribosylation of 5-methoxybenzimidazole. Details of their experiments, especially the product ratio, were not given.

(10) Y. Mizuno, M. Ikehara, T. Itoh, and K. Saito, *J. Org. Chem.*, **28**, 1837 (1963).

(11) Y. Mizuno, T. Itoh, and K. Saito, *ibid.*, **29**, 2611 (1964).

(12) (a) Ribosylation of purine (IV) has been done by Brown and Weliky[12b] who have isolated only the 9*H* isomer in 34% yield. (b) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

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

(14) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).

(15) H. T. Miles and H. M. Fales [*Anal. Chem.*, **34**, 960 (1962)] have reported in preliminary form the application of gas chromatography to the separation of nucleoside acetates. More successful separation was obtained for the trimethylsilyl derivatives by R. L. Hancok and D. L. Coleman [*Anal. Biochem.*, **10**, 365 (1965)].

(16) The procedure was essentially similar to that of Kissman and Weiss.[13] The estimation was done by weight. The probable error was *ca.* 5%.

(17) Of the several solvent systems examined, the BuOH–AcOH–water system was found to be the most effective for the isolation of isomeric mixture of nucleosides of bases IV, V, and VI by paper chromatography. The R_f value of 7*H* isomer[18] was without exception smaller than that of 9*H* isomer[18] by *ca.* 0.1 (see Experimental Section).

(18) For the sake of simplicity, 3-*N*-glycosides of 3*H*-imidazo[4,5-*b*]-pyridines, 1-*N*-glycosides of 1*H*-imidazo[4,5-*c*]pyridines, and 1-*N*-glycosides of 5-substituted benzimidazoles were referred to as "9-*H*-glycosides," by analogy with 9 isomers of purines. Accordingly, the respective isomeric *N*-glycosides are referred to as "7*H* isomers."

TABLE II

R_f VALUES AND RELATIVE RETENTION TIMES OF NUCLEOSIDES[a]

Riboside	R_f[b]	Riboside	R_f[b]
XVI	0.61	XIV	0.77
XVII	0.44	XV	0.69
XVIII[c]	0.50	XXII[d]	0.40
XIX	0.40	XXIII[d]	0.21
Riboside	Retention time[e]	Riboside	Retention time[e]
XXIV[f]	0.54	XXVIII[g]	0.73
XXV[h]	0.43	XXIX[i]	0.65
XXVI[j]	1.45	XXX[k]	0.99
XXVII[l]	0.95	XXXI[m]	0.53

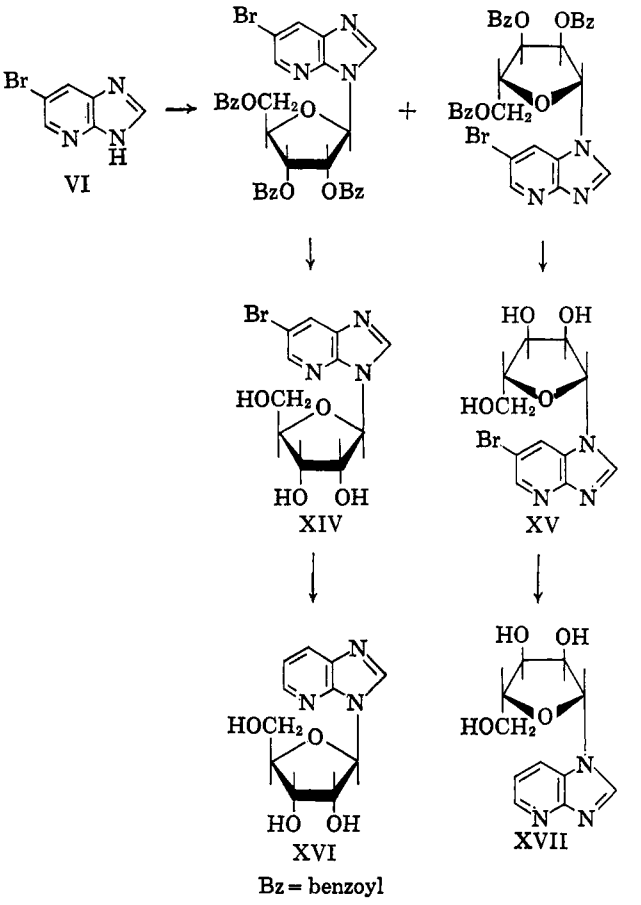
[a] Retention times were determined for the trimethylsilyated nucleosides. [b] R_f values in solvent A, Toyo filter paper 51A, and see also footnotes 17 and 20. [c] 9-β-D-Ribofuranosyl-9*H*-purine. [d] XII is the 3-ribofuranoside of XI; XXIII is the corresponding 1-riboside. [e] Retention times relative to cholestane (15.5 min.) being 1.00. Column: 1.5% SE-30 on Chromosorb W (80–100 mesh), 150 cm. × 4 mm. i.d.; temperature, 212°; carrier gas, N_2 (75 cc./min.). [f] 1-Riboside of VII. [g] 1-Riboside of IX. By analogy to the fact that "9*H* isomer" (XXIV, XXX, and XXVI) has longer retention time than the corresponding "7*H* isomer" (XXV, XXXI, and XXVII), an isomer having longer retention time was tentatively assigned the "9*H* isomer" structure, *viz.*, the structure XXVIII; the corresponding isomer was assigned the structure XXIX. [h] 3-Riboside of VII. [i] 3-Riboside of IX. [j] 1-Riboside of VIII. [k] 1-Riboside of XII. [l] 3-Riboside of VIII. [m] 3-Riboside of XII.

comparison of R_f values (Table II) or their retention times (Table II) with those of the authentic samples. In cases where new products were isolated (ribosylation of IV–VI and XI), structural elucidation was carried out either by spectral comparison with the corresponding *N*-methyl counterparts[19] or by their conversion to known nucleosides. Thus, the structure of the ribosyl derivatives of VI was determined as shown in Chart I. The benzoylated nucleosides obtained were deblocked and separated by fractional crystallization and subsequent preparative paper chromatography. Reductive debromination of each nucleoside, followed by paper chromatography and spectral examination showed that XIV and XV were 6-bromo-3-β-D-ribofuranosylimidazo[4,5-*b*]pyridine and the corresponding 1*H* isomer, respectively. The condensation of the chloromercuri salt of XI with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (XIII), followed by alumina column chromatography, gave two isomeric benzoylated nucleosides (see Chart II), one of which (XX) was obtained as an analytically pure glass (23% yield), and other (XXI) in crystalline form, m.p. 198.5–200° (20% yield). Reductive deoxygenation of each deblocked nucleoside (XXII, m.p. 258–259°, and XXIII, m.p. 340°), followed by spectral examination, showed that XXII and XXIII were 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine 4-oxide and the corresponding 1*H* isomer, respectively. Even by the use of our improved separation technique, no nucleosidic products other than a reported main product, 9-β-D-ribofuranosyl-6-chloropurine,[13] could be detected in the ribosylation of 6-chloropurine.

The results obtained are summarized in Table I where the bases examined are classified into two groups, class A and class B. Ribosylation of the bases in class A

(19) (a) The structure determination of XVII and XIX rests upon spectral comparison with the corresponding *N*-methyl counterparts: 1-methyl-1*H*-imidazo[4,5-*b*]pyridine[10] and 7-methyl-7H-purine.[19b] (b) A. Bendich, P. J. Russel, and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

CHART I

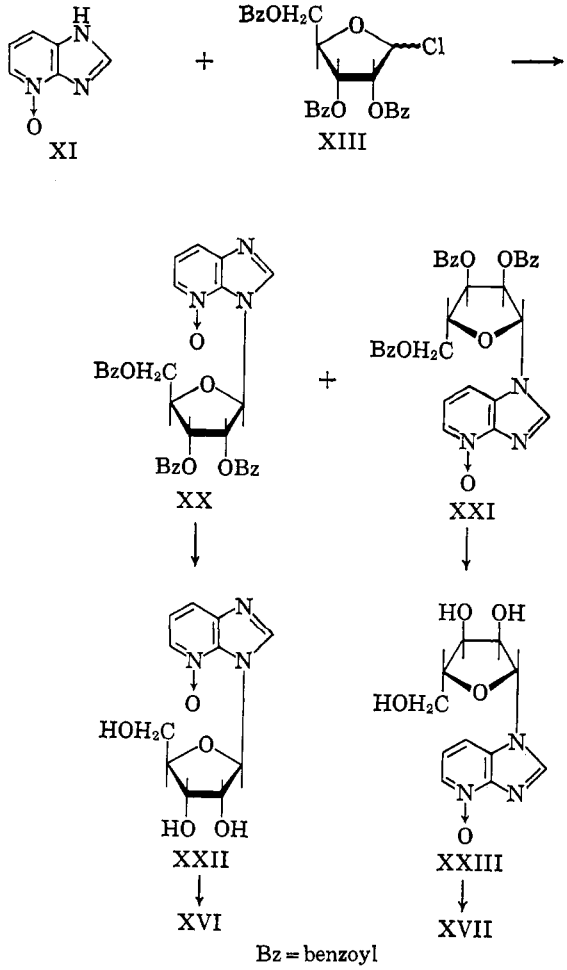


gave rise to one of two possible isomers in predominant amounts or failed to afford a detectable amount of one of two possible isomers. With exception of XII, a common structural feature of these bases in class A is that they have a nitrogen atom adjacent to the imidazole ring. Ribosylation of bases in class B which have no ring nitrogen atom adjacent to the imidazole ring gave rise to each of two possible isomers in about equal amounts, with exception of XI whose nitrogen atom carries a substituent. It is to be noted that, in ribosylation of 5-substituted benzimidazoles VIII and IX and "3-deazapurine" (VII), the two possible products are formed in approximately equal amounts. These results suggest that the influence of the substituent in position 5 (benzimidazoles) or "1"-nitrogen in "3-deazapurine" on the determination of the isomeric ratio is unimportant, even if the substituent were electron attracting (nitro group or doubly bonded ring nitrogen atom) or electron releasing (methoxy group).

On the other hand, with exception of XII, a common structural feature of the bases in class A, as mentioned above, is that they possess a nitrogen atom adjacent to the imidazole ring. It is strongly indicated that the nitrogen atom, presumably through its electron-attracting inductive effect (−I effect), exerts directive influence in the distribution of the products of the ribosylation, insofar as the steric effect on both nitrogen atoms in the imidazole ring were equal.

Our lack of knowledge on the structure of the chloromercuri salts of these bases and the reaction mechanism involved prevents us from clarifying the precise mode of contribution of this −I effect.

CHART II



Experimental Section[20]

1*H*-Imidazo[4,5-*b*]pyridine 4-Oxide (XI).—Compound XI was prepared by a standard procedure.[21] The oxide (1.2 g.) was obtained as monoacetate from 1.1 g. of 1*H*-imidazo[4,5-*b*]-pyridine (V)[22] in 66% yield.

Anal. Calcd. for $C_8H_9N_3O_3$: C, 49.23; H, 4.65; N, 21.53. Found: C, 48.96; H, 4.85; N, 21.67.

The acetate (0.84 g.) was treated with calcium carbonate (500 mg.) in refluxing ethanol for 3 hr. The solution was filtered; the filtrate was concentrated to dryness. The residue was crystallized from ethanol, m.p. 250–253° dec., yield 0.45 g.

Anal. Calcd. for $C_6H_5N_3O$: C, 53.33; H, 3.73; N, 31.10. Found: C, 53.10; H, 4.03; H, 31.11.

Chloromercuri Salts of 1*H*-Imidazo[4,5-*b*]pyridine 4-Oxide (XI) and 6-Bromo-3*H*-imidazo[4,5-*b*]pyridine (VI).—1*H*-Imidazo[4,5-*b*]pyridine 4-oxide acetate (3.0 g., 15.4 mmoles) was converted by a standard procedure into the chloromercuri salt (5.46 g., 96%).

Anal. Calcd. for $C_6H_4ClHgN_3O$: N, 11.35. Found: N, 11.21.

6-Bromo-3*H*-imidazo[4,5-*b*]pyridine (VI, 728 mg.) was converted into the chloromercuri salt (1.4 g., 92%).

Anal. Calcd. for $C_6H_3BrClHgN_3$: N, 9.90. Found: N, 9.76.

Ribosylation of Chloromercuri Salts under Comparable Conditions.—The chloromercuri salt (1 equiv.) was treated with 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride (XIII), prepared from 1.01 equiv. of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose by a standard procedure.[14] Yields of crude ribosylated products are summarized in Table III.

TABLE III

YIELDS IN RIBOSYLATION BY THE MERCURI METHOD

Bases	Chloromercuri salts, g. (mmoles)	Yields of crude tri-*O*-benzoyl-D-ribofuranosides, g. (%)
IV	3.55 (10)	5.20 (92)
V[a]	13.9 (39)	20.02 (92)
VI	1.47 (3.4)	2.19 (100)
VII[b]	3.16 (8.9)	4.44 (88)
VIII[c]	3.98 (10)	5.65 (93)
IX[d]	3.01 (7.9)	4.43 (95)
X[e]	5.46 (14)[f]	
XI	7.40 (20)	9.90 (86)

[a] Ref. 10. [b] Ref. 11. [c] Ref. 8. [d] Ref. 9. [e] Ref. 13. [f] An excess (1.4 equiv.) of 1-*O*-acetyl-2,3-5-tri-*O*-benzoyl-D-ribose was used.

Separation of Isomeric Benzoylated Nucleosides by Alumina Column Chromatography.—The procedure of separation of the crude ribosylated products (Table III) was similar to that reported before.[10,11] The elution pattern was made by plotting the weight of each eluate (after removal of solvent) against the tube number. Except in cases of 2,3,5-tri-*O*-benzoyl-β-D-ribosyl-5(6)-nitrobenzimidazoles or isomeric benzoylated purine nucleosides, where separation was only partially achieved, the elution pattern consisted of two main peaks, corresponding to each isomer and identification of eluates was done according to the original paper.[11,12b] Results obtained are shown in Table I.

Separation of Minor Nucleosidic Products by a Combination of Alumina Column Chromatographic and Paper Chromatographic Technique.—Fractions obtained by alumina column chromatography were deblocked by usual way[10,11] and the mixture of resulting isomeric nucleosides was subjected to paper chromatography primarily by use of solvent A. Clear-cut separation was achieved in cases of isomeric mixtures of bases in class A.[17] The spots were detected both under the ultraviolet light and the metaperiodate spray reagent. R_f values in solvent A are shown in Table II.

For estimation of each isomeric ratio, the spots in the paper chromatograms were excised and extracted with 4 ml. of water, the optical density of the extract at the respective maximum was determined, and the ratio was calculated. The accuracy of the estimation was checked by use of an artificial mixture of the known isomeric ratio and the error was found to be within 5%.

Estimation of Isomeric Ratios by Gas Chromatography. General Procedure.—Prior to gas chromatography, the nucleoside was trimethylsilated by a modification of Sweeley and coworkers' method.[23] In a micro test tube with a stopper, 2 mg. of the nucleoside was treated with 0.2 ml. of dry pyridine, 0.1 ml. of hexamethyldisilazane, and 0.1 ml. of trimethylchlorosilane. After shaking vigorously for 2 min. and allowing to stand at room temperature for about 5 min., 1–3 μl. of the reaction mixture was injected with a 10-μl. Hamilton syringe directly onto a chromatograph. A Shimadzu gas chromatograph, Model GC-1B, equipped with a hydrogen detector, a dual column, and a differential flame system was employed throughout the experiments. The column employed consisted of a 150 cm. × 4 mm. i.d. U-shaped tube of stainless steel. The supported material consisted of acid-washed and siliconized Chromosorb W (80–100 mesh), prepared by the filtration technique; the stationary phase used was the silicone polymer SE-30. The ratio of SE-30 to the support was 1.5%. After packing, the column was conditioned at 270° for 36 hr. with a slow nitrogen flow. The standard operating conditions were as follows: column temperature, 212°; detector temperature, 240°; temperature of the sample heater, 270°; and carrier gas flow rate, 75 cc./min. of N_2. The retention times of nucleosides relative to cholestane were determined and are listed in Table II.

Analysis of Reaction Products.—To a solution of 200–300 mg. of crude benzoyl-blocked nucleoside (Table III) in 7 ml. of abso-

(20) All melting points are corrected. Ultraviolet spectra were recorded with a Hitachi recording spectrophotometer. Except where noted, removal of solvent was performed by use of the water aspirator (18 mm.). Paper chromatography was performed by use of the ascending technique: solvent A, BuOH–AcOH–water (4:1:2, upper phase); solvent B, BuOH–water (84:16); solvent C, *i*-C_3H_7OH–NH_3–water (7:1:2); solvent D, *n*-C_3H_7OH–NH_3–water (6:3:1); and solvent E, water, adjusted to pH 10 with NH_3.

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lute methanol was added 1 *N* methanolic sodium methoxide (1 ml.); the solution was refluxed for 1 hr., cooled, neutralized with Amberlite IRC 50 (H^+ form), filtered, and concentrated to dryness. The residue was dried to constant weight. Out of the dried nucleoside, a portion (3 mg.) was taken up in dry pyridine, trimethylsilylated, and gas chromatographed. Relative yields of ribosylation products were calculated from the peak areas in the chromatograms, measured with a planimeter. Accuracy of the estimation was checked by use of an artificial mixture of the known ratio and error was found to be within 2%. Results obtained are shown in Table I.

Isomeric 6-Bromo-β-D-ribofuranosylimidazo[4,5-*b*]pyridines (XIV and XV).—To a hot solution of crude benzoylated nucleoside (1.92 g., Table III) in 70 ml. of absolute methanol was added 1 *N* methanolic sodium methoxide (1 ml.). The solution was refluxed for 1 hr., cooled, neutralized with Amberlite IRC 50 (H^+ form) resin, and filtered. The filtrate was concentrated to dryness. The residue was dissolved in 20 ml. of water and filtered, and the solvent was removed *in vacuo*. The residue weighed 541 mg. (54.7%). Recrystallization from water afforded a pure product, m.p. 186–188.5°. The mother liquor was subjected to paper chromatography (see below). After another recrystallization, the melting point did not change; the R_f (in solvent A) was 0.78; ultraviolet spectra $\lambda_{max}^{pH\ 0.46}$ 291.5 mμ (ε 10,800), 240 mμ (ε 3100); $\lambda_{min}^{pH\ 0.46}$ 259 mμ (ε 1450); $\lambda_{max}^{pH\ 4.93}$ 295 (ε 10,300), 250 mμ (ε 3220); $\lambda_{min}^{pH\ 4.93}$ 267 mμ (ε 1970).

Anal. Calcd. for $C_{11}H_{12}BrN_3O_4$: C, 40.01; H, 3.36; N, 12.72. Found: C, 39.75; H, 3.73; N, 12.78.

Paper chromatography (solvent A) of the mother liquor showed the presence of two nucleosidic products (spots could be detected both under the ultraviolet lamp and the periodate spray reagent); R_f values of the two spots (in the same solvent) were 0.78 and 0.69. Each spot was extracted with 4 ml. of water. Ultraviolet spectra of each extract showed that the compound having R_f 0.69 was XV. The ratio of the optical density of 9*H* isomer XIV to 7*H* isomer XV in the mother liquor was 7:5.

Structural Elucidation of Isomeric 6-Bromo-(β-D-ribofuranosyl)-imidazo[4,5-*b*]pyridines.—Crystalline nucleoside XIV (11 mg., m.p. 186–188°) was dissolved in 15 ml. of 50% ethanol. To the solution was added a small amount of 5% palladium on carbon. Catalytic debromination was performed in an atmospheric pressure for 8 hr. at room temperature and the mixture was filtered. The filtrate was concentrated to dryness to afford 6 mg. of nucleoside. Paper chromatography (solvent A) of the nucleoside showed the presence of a new spot (R_f 0.61) in addition to the starting material. The spot (R_f 0.61)[24] was cut out and extracted with 4 ml. of water. The ultraviolet spectrum of the extract was identical with that of XVI.[10]

An aliquot of the above-mentioned mother liquor was similarly hydrogenated for 8 hr. Paper chromatography of the resulting mixture showed the presence of XVII (R_f in solvent A 0.44) in addition to a compound of R_f 0.61. On the basis of the optical density, the mother liquor was a 7:5 mixture of 9*H* isomer XVI and 7*H* isomer XV. Therefore, ribosylation of VI gave rise to a 42% yield of XIV and a 13% yield of XV.

Separation of Ribosylated Products of XI.—Crude benzoyl-blocked nucleosides (9.90 g., Table III) were fractionated by alumina column chromatography (200 g. of alumina, 3.0 × 36 cm. column, 300-ml. fractions) into 32 parts. Fractions 9–18 afforded, after removal of solvent, XX as a glass: 2.46 g. (22.6%); $[\alpha]^{22}D$ +28° (*c* 2, $CHCl_3$); ultraviolet absorption λ_{max}^{MeOH} 275.5, 310 mμ; λ_{min}^{MeOH} 256.5, 294 mμ.

Anal. Calcd. for $C_{32}H_{25}N_3O_8$: C, 66.34; H, 4.35; N, 7.25. Found: C, 66.62; H, 4.54, N, 7.35.

Fractions 19–32 afforded 1-(2,3,5-tri-*O*-benzoyl-β-D-ribosyl)-1*H*-imidazo[4,5-*b*]pyridine 4-oxide (XXI, 2.13 g., 20.2%) as crystals: $[\alpha]^{22}D$ −94° (*c* 2, $CHCl_3$); ultraviolet absorption λ_{max}^{MeOH} 286 (shoulder), 300 mμ. An analytically pure sample of XXI, recrystallized from ethyl acetate, melted at 198.5–200.5°.

Anal. Calcd. for $C_{32}H_{25}N_3O_8$: C, 66.34; H, 4.35; N, 7.25. Found: C, 66.36; H, 4.43; N, 7.29.

Preparation of 3-(β-D-Ribosyl)-3H-imidazo[4,5-*b*]pyridine 4-Oxide (XXII).—To a solution of XX (404 mg., 0.699 mmole) in 50 ml. of absolute methanol was added 1 *N* methanolic sodium methoxide (0.5 ml.). The solution was refluxed for 1 hr. and cooled, neutralized with IRC resin (H^+ form). The resin was filtered off and the filtrate was concentrated to dryness. The residue was purified by preparative paper chromatography (solvent A): m.p. 258–259° dec.; $[\alpha]^{18}D$ +26.7° (*c* 1.3, water); R_f 0.40 (in solvent A), 0.14–0.15 (in solvent B), 0.81–0.82 (in solvent E); $\lambda_{max}^{pH\ 1}$ 276 (shoulder), 295 mμ; $\lambda_{max}^{H_2O}$ 273.5, 304 mμ; $\lambda_{max}^{pH\ 10}$ 273.5, 306 mμ.

Structural Confirmation of XXII. Preparation of XVI from XXII.—Compound XXII (30 mg.) was dissolved in 30 ml. of 50% ethanol was subjected to catalytic deoxygenation in the presence of a small quantity of Raney nickel in a hydrogen atmosphere for 2 hr. at room temperature. The catalyst was filtered off and the filtrate was concentrated to dryness (9 mg., 32%). Recrystallization from water gave colorless needles, m.p. and m.m.p. (with an authentic sample[10]) 220–222°.

1-β-D-Ribosyl-1H-imidazo[4,5-*b*]pyridine 4-Oxide (XXIII).—Compound XXI (882 mg.), obtained from fraction 19, was deblocked as described above: yield 285 mg. (70.5%); m.p. 340°; $[\alpha]^{21}D$ −13.0 (*c* 1.15, water); R_f 0.21 (in solvent A), 0.05 (in solvent B), 0.73 (in solvent E); $\lambda_{max}^{pH\ 1.0}$ 294 mμ; $\lambda_{min}^{pH\ 1.0}$ 251 mμ; $\lambda_{max}^{H_2O}$ 297 mμ; $\lambda_{min}^{H_2O}$ 249 mμ; $\lambda_{max}^{pH\ 10}$ 298 mμ; $\lambda_{min}^{pH\ 10}$ 248 mμ.

Anal. Calcd. for $C_{11}H_{13}N_3O_5$: C, 49.43; H, 4.86; N, 15.43. Found: C, 49.51; H, 4.72; N, 15.40.

Structural Confirmation of XXIII. Preparation of XVII from XXIII.—Compound XXIII (37 mg.) was catalytically deoxygenated to XVII as described above: yield 30 mg. (96%); R_f 0.54 (in solvent A) (R_f of XXIII 0.23 in solvent A). The ultraviolet absorption spectra were identical in every detail with those of 1-methyl-1*H*-imidazo[4,5-*b*]pyridine[10]: $\lambda_{max}^{pH\ 0.11}$ 278, 283, 289 mμ; $\lambda_{max}^{pH\ 5.60}$ 247, 282, 288 (shoulder) mμ; pK_a = 2.95.

Anal. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.58; H, 5.17; N, 25.50. Found: C, 52.51; H, 5.20; N, 25.38.

Ribosylation of the Chloromercuri Salt of Purine and Separation of the Isomeric Benzoylated Nucleosides.[25]—A crude benzoyl-blocked nucleoside (4.08 g.) was applied to alumina column (100 g. of alumina) collecting 100-ml. fractions. Fractions 4–23, eluted by a mixture of benzene and ethyl acetate, gave on evaporation of the solvent 2.0 g. of tri-*O*-benzoylated 9-β-D-ribosylpurine (see below).

Fractions 29–32, eluted by 400 ml. of a 1:4 (v./v.) mixture of ethanol and ethyl acetate gave 168 mg. of a 1:1 mixture of 7*H* isomer and 9*H* isomer.

9-β-D-Ribofuranosyl-9*H*-purine (XVIII, Nebularine).—To a hot solution of 340 mg. (0.6 mmole) of 9-(tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-purine (combined fractions 9 and 23) in absolute methanol (50 ml.) was added 2 *N* methanolic sodium methoxide (0.1 ml., 0.2 mmole) and the solution was refluxed for 30 min. and cooled. Worked up as usual, crude XVIII (103 mg., 67.7%) was obtained. Crystallization from 10 ml. of ethanol gave 77 mg. of pure product: m.p. 175–177° (lit.[12b] m.p. 181–182°); R_f 0.38 (in solvent B), 0.76 (in solvent C), 0.76 (in solvent D), 0.50 (in solvent A); $\lambda_{max}^{pH\ 4}$ 262.5 mμ; $\lambda_{max}^{pH\ 0}$ 262.5 mμ; pK_a = 2.10.

7-β-D-Ribofuranosyl-7*H*-purine (XIX).—Fractions 29–32 had a slightly different ultraviolet spectra from those of the preceding fractions and weighed 131 mg. (232 mmoles). These fractions were deblocked as described above to afford 59 mg. (99%) of nucleosidic products. Paper chromatography showed the presence of two spots which were detected by both the ultraviolet light and the periodate spray reagent: R_f values in solvent A were 0.49 and 0.39. Each spot was cut out and extracted with water. Ultraviolet spectral examination of the extract showed that the spot of R_f 0.40 corresponded to 7*H*-riboside and that of R_f 0.49 to 9*H*-riboside (nebularine). Based on the total optical density, this sample was a 1:1 mixture of 7*H* isomer XIX and 9*H* isomer XVIII. The 1:1 mixture (37 mg.) was applied to 40-cm. wide Toyo filter paper 51A and developed for 25 hr. with solvent A. A band corresponding to XIX (R_f 0.40) was excised and extracted with 25 ml. of water. Removal of the solvent gave 13 mg. of the residue. Crystallization from aqueous ethanol afforded 9 mg. of XIX: paper chromatography in solvent A showed an R_f value of 0.36 (a single spot); $\lambda_{max}^{pH\ 5}$ 265.0 mμ; $\lambda_{max}^{pH\ 0}$ 258 mμ. The ultraviolet absorption spectra were identical with those of N^7-methylpurine.[19b]

Anal. Calcd. for $C_{10}H_{12}N_4O_4$: C, 47.62; H, 4.76; N, 22.22. Found: C, 47.58; H, 4.59; N, 21.19.

(24) The R_f value in solvent *A* of an authentic sample of 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XVI) was 0.61. That of the 1*H* isomer was 0.44.

(25) Ribosylation of the chloromercuri salt of purine with 2,3,5-tri-*O*-acetyl-D-ribosyl chloride, but not with XIII, has been reported by Brown and Weliky.[12b]

1-β-D-Ribofuranosyl-1*H*-imidazo[4,5-*b*]pyridine (XVII).—The mother liquor resulting from crystallization of XVI[10] gave, on evaporation of the solvent, 930 mg. of residue. The crude products were deblocked as described above. The deblocked products (350 mg.) contained nucleosidic compounds whose R_f values in solvent A were 0.60 and 0.43. The extract of the spot of R_f 0.60 had ultraviolet spectra identical with those of an authentic sample of 3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (XVI).[10] The ratio of the total optical density of the two spots (XVII to XVI) was 4:1, which means ribosylation of V gave rise to 68% yield of 9*H* isomer XVI and 3.3% of 7*H* isomer XVII. The mixture (345 mg.) was dissolved in 10 ml. of water and the solution was treated with activated carbon and filtered. The filtrate was concentrated to dryness (197 mg.) and suspended in 10 ml. of ethanol. To the suspension was added water until the residue almost dissolved. On standing for 39 days at room temperature, crystals deposited: m.p. 226–227.5; $\lambda_{max}^{pH\ 0.11}$ (cationic form) 278 mμ (ε 8890) (shoulder), 283.5 (10,000), 289 (ε 9700) (shoulder); $\lambda_{min}^{pH\ 5.6}$ (neutral form) 259 mμ (ε 3640); $pK_a = 2.95 \pm 0.02$; isosbestic points at 259.5, 271–272, and 281 mμ. Ultraviolet spectra of this nucleoside (XVII) for the neutral and cationic species were identical with those of 1-methyl-1*H*-imidazo[4,5-*b*]pyridine and differed appreciably from those of the 3-methyl-3*H* isomer.[10]

Anal. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.58; H, 5.22; N, 16.73. Found: C, 52.61; H, 5.16; N, 16.49.

Examination of Products Other Than the 9*H* Isomer in Ribosylation Products of Chloromercuri Salt of 6-Chloropurine (X).—Chloromercuri-6-chloropurine[13] (5.45 g., 14 mmoles) was treated with XIII, prepared from 10 g. (19.8 mmoles) of 1-*O*-acetyl-2,3-5-tri-*O*-benzoyl-D-ribose by the standard procedure.[14] The residue (10.46 g.) obtained was fractionated into 39 parts on an alumina column. Each fraction was put into a bomb of stainless steel and treated with 5 ml. of methanolic ammonia (saturated at 0°) at 100–110° (bath temperature) for 4 hr. The solution was evaporated to dryness. To the residue was added 5 ml. of methanol and the solvent was removed *in vacuo*. The process was repeated four times to remove methyl benzoate and benzamide, and the resulting residue was subjected to paper chromatographic examination (solvent A). Each fraction contained, if any, only a single nucleosidic product, adenosine.

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Ferrocene-Containing Polymers. X. Isomeric Bis(ferrocenylmethyl)ferrocenes

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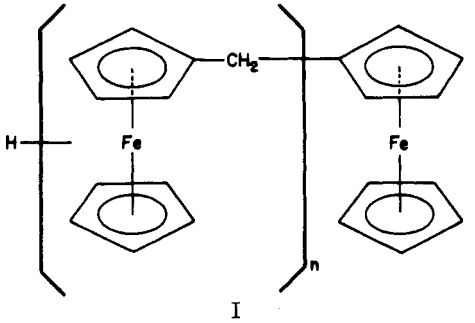
From a trinuclear fraction of the methylene-bridged ferrocene polymer I, obtained in earlier investigations, the isomeric 1,2-, 1,3-, and 1,1′-bis(ferrocenylmethyl)ferrocenes IIa–c are separated by fractional crystallization in the approximate ratio, 1:5:4. The three isomers are identified by elemental analyses and spectroscopic techniques. Their structural assignments are confirmed by independent syntheses *via* Clemmensen reduction of the corresponding monoketones IIIa–c, obtained from ferrocenoyl chloride and diferrocenylmethane under Friedel–Crafts conditions. The established ratio of isomers II, while at best a very crude approximation, corroborates earlier qualitative predictions with regard to the sequence distribution of 1,2-, 1,3-, and 1,1′-substituted recurring units along the backbone of polymer I.

It was shown recently[2a] that the ferrocenyl Mannich base, N,N-dimethylaminomethylferrocene, can be cocondensed with ferrocene in the presence of a zinc chloride–hydrochloric acid catalyst system to give polymeric products possessing the idealized structure I.[2b] In this reaction (and, similarly so, in the self-



I

condensation of the Mannich base[3]), slightly prevailing homoannular substitution along the polymer chain of I was predicted on the basis of earlier publications demonstrating enhanced (inductive and hyperconjugative) activation of the substituted, compared with the unsubstituted, π-cyclopentadienyl rings of alkyl ferrocenes in acylation reactions[4] and was evidenced spectroscopically by a quantitative evaluation of the 9-μ absorption[5,6] of a series of polyhomologous fractions.

To obtain a more precise measure of this prevailing homoannularity in I, in this earlier study[2a] a monodisperse fraction composed of the three trinuclear (I, $n = 2$) isomers II was separated chromatographically. For this fraction, on which the distribution of the three substitution orientations could be studied without interference by branching (such interference has to be anticipated in homologs with $n \geq 3$), a 59.6% content of homoannular isomers was determined by the same quantitative spectroscopic technique. While the preponderance of homoannular bonding in the trinuclear isomer mixture (and, by inference, also in the chain of polymeric I) was thus clearly established, no efforts were made to separate this isomer mixture further into the individual components IIa–c.

In the present investigation, the earlier work was resumed, and this isolation of isomers IIa–c[7] was

(1) (a) Douglas Aircraft Co., Inc.; (b) California Research Corp.

(2) (a) E. W. Neuse and K. Koda, paper submitted for publication. (b) As in the earlier work (ref. 2a and 3), the centered substituent link on the left of this simplified scheme denotes a random sequence distribution along the polymer backbone of 1,2-, 1,3-, and 1,1′-substituted units.

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