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121. Yoshihisa Mizuno, Morio Ikehara, Fumiyoshi Ishikawa, and Hiroshi Ikehara : Potential Antimetabolites. V.*1 The Syntheses of 4-, 5-, and 6-Nitrobenzimidazole Ribofuranosides.

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Since Scott, *et al.*[1] found that benzimidazoles substituted by mercapto or nitro group in their benzene ring exerted specific inhibition toward vitamin B_{12} and thymine in some bacterial mutants, the syntheses of ribosides of nitrobenzimidazole were attempted in order to test their anti-cancer activity. From the point of synthetic problem, it seemed of interest to investigate the orientation of N-substitution, especially in the reaction between chloromercury salt of nitrobenzimidazole and 1-chloro-sugar.

The chloromercury salt (II) was obtained in a quantitative yield by stirring equimolar quantities of 5(6)-nitrobenzimidazole[2] (I), sodium hydroxide, and mercuric chloride in hot 10% ethanol. The salt (II) was found to consist of 1:1:1 ratio of the base, mercury, and chlorine as calculated from analytical data. The product (II) was then condensed with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride[3] (III) by the method of Davoll and Lowy,[4] which afforded an amorphous mixture of 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-5- and -6-nitrobenzimidazoles.

The mixture was purified by alumina column chromatography and recrystallized from ether-hexane to leaflets (IV), m.p. 135~138°, in 15.6% yield. Recrystallization from methanol-chloroform raised the melting point to 139~140°, $[\alpha]_D^{15}$ −97.2°. The product (IV) was debenzoylated with methanol-ammonia to a crystalline substance (VI) of m.p. 170°, $[\alpha]_D^{14}$ +13.9°, in 90% yield. The mother liquor of (IV) was evaporated and the residue mostly consisting of (V) was obtained in 24.2% yield. The analytical sample was purified by three recrystallizations from ether-hexane to a substance of m.p. 137~138°, $[\alpha]_D^{15}$ −56.0°. Debenzoylation of (V) gave a crystalline substance (VII), m.p. 200~201°, $[\alpha]_D^{14}$ +27.7°, in 60~70% yield. The ratio of the yields of (VI) and (VII) was ca. 2:3 and these compounds were proved to be β-nucleosides deduced from the optical rotation values and the 1,2-*trans* rule.[5]

According to the report of Fillips,[6] the isomer-ratio in the N-alkylation of 5(6)-nitrobenzimidazole was fairly large. Recently, however, Smith, *et al.*[7] reinvestigated older experiments and found the participation of isomers (VIII) and (IX) almost equal.

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*1 Part IV : This Bulletin, **10**, 665 (1962).

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1) D. B. McNair Scott, M. L. Rogers, C. Rose : J. Am. Chem. Soc., **80**, 2165 (1958).
2) E. Bamberger, B. Berle : Ann., **273**, 340 (1893); O. Fisher, W. Hess : Ber., **36**, 3769 (1903).
3) F. Ishikawa, A. Nomura, T. Ueda, M. Ikehara, Y. Mizuno : This Bulletin, 8, 380 (1960).
4) J. Davoll, B. A. Lowy : J. Am. Chem. Soc., **73**, 1650 (1951).
5) B. R. Baker : J. Org. Chem., **19**, 1786 (1954); Ciba Synposium, **1957**, 120.
6) M. A. Fillips : J. Chem. Soc., **1931**, 1143.
7) J. H. Smith, B. V. Ridd : *Ibid.*, **1960**, 1363.

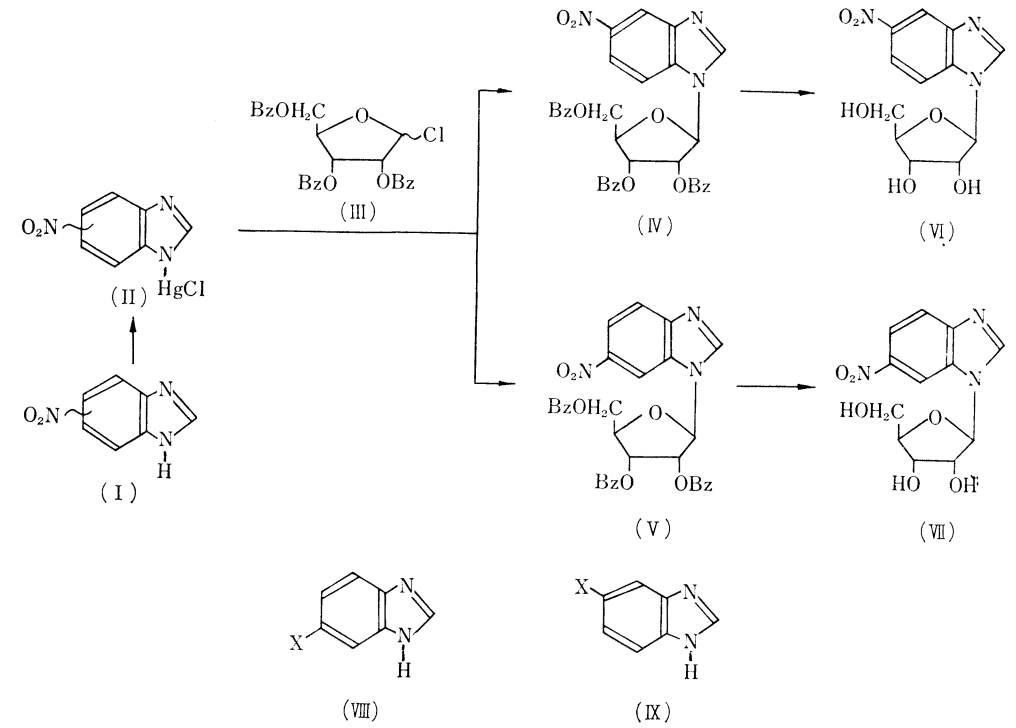


Chart 1.

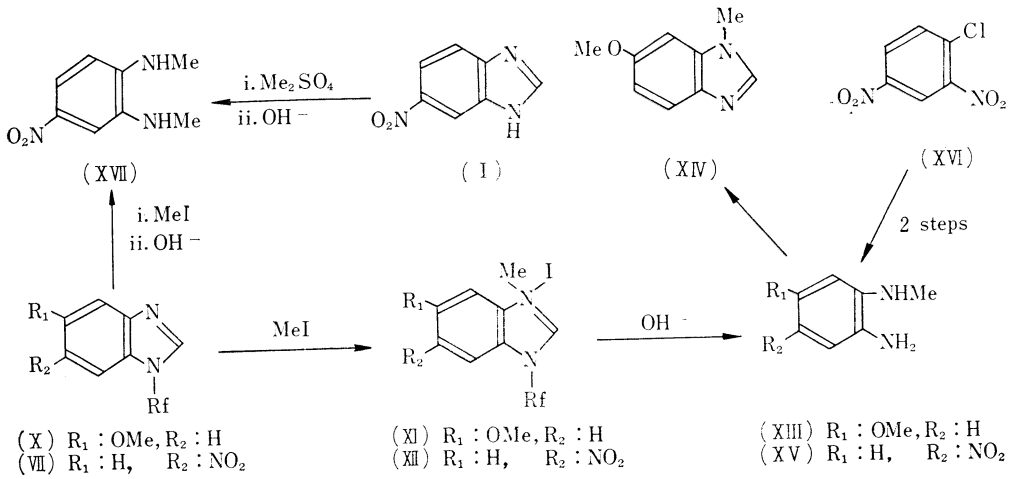


Chart 2.

This conclusion led to the difficulty of determining the structure of isomers from the quantity of their yields.

Folkers, *et al.*[8] elucidated the direction of ribosidation of 5-methoxybenzimidazole

8) C. H. Shunk, F. M. Robinson, J. F. McPherson, M. M. Gasser, K. Folkers: J. Am. Chem. Soc., **78**, 3228 (1956).

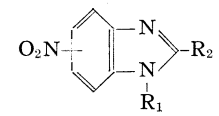
from a series of reactions shown in Chart 2 (X～XIV). In the present series of experiments, attempt was made to establish the structure of (VII) as 1,6-isomer, deduced from its yield and following the reaction scheme of Folkers.

The compound (VII) was heated with 1.2 molar equivalents of methyl iodide in methanol and treated with metanolic sodium hydroxide to afford red prisms (A) of m.p. 172～173°, in 25% yield, accompanied with 5(6)-nitrobenzimidazole (I). N^1-Methyl-4-nitro-*o*-phenylenediamine (XV), m.p. 176～177°, was synthesized by a standard procedure and compared with (A) by mixed melting point, which showed a large depression. By estimation from elementary analysis data and melting point, this (A) was then compared with N^1,N^2-dimethyl-4-nitro-*o*-phenylenediamine (XVII), synthesized from 5(6)-nitrobenzimidazole (I), and mixed melting point test showed that (A) and (XVII) were identical. This means that previously situated N-alkyl or N-sugar group may have been replaced by the attack of methyl iodide in the quaternarization reaction. From these results, degradation procedure was proved to be unsatisfactory for the present purpose.

For the determination of orientation of nucleoside linkage, it became necessary to examine the physical properties. As shown in Table I, detailed examination of ultraviolet absorption of known 5- and 6-substituted benzimidazole showed that the absorption maxima of 1,5-isomer around 300 and 240 mμ were closer to each other than those of 1,6-isomer, and the ratio of $\varepsilon_{240}/\varepsilon_{300}$ is larger in the former. Therefore, (VI) must be assumed as 1,5-isomer and (VII), as 1,6-isomer.

On the infrared absorption spectra of these isomer, Smith, *et al.*[7] stated that 1,5-isomer has a specific absorption at 11.1 μ, which does not appear in the case of

Table I.



R_1	R_2	Ultraviolet absorption λ_{max} (mμ) ε ($\varepsilon_{240}/\varepsilon_{310}$) 1,5-isomer			1,6-isomer			Ref.
Diethylaminoethyl	Benzyl	310 242	10200 27900	(2.73)	310 240	12000 17700	(1.47)	11
Et	Me	307 241	9500 25000	(2.64)	310 240	10100 19000	(1.88)	9
Me	Me	320 240	9230 16200	(1.78)	321 239	10600 16400	(1.55)	8
Ribofuranosyl	H	302 236	9200 21600	(2.36)	303 235	9900 18600	(1.88)	

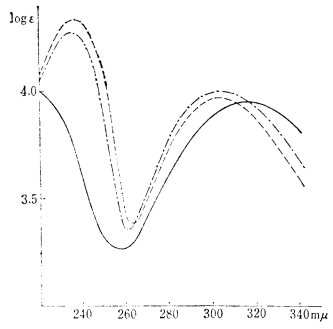


Fig. 1. Ultraviolet Absorption Spectra of Nitro-Substituted Benzimidazole Ribofuranosides

– – – – (VI)
—•—•— (VII)
———— (XXI)

9) R. Foster : J. Chem. Soc., **1957**, 4687.

1,6-isomer. While the infrared absorption spectrum of (IV) has such absorption at 11.1 μ, (V) does not show absorption in this region. From these evidences (VI) was characterized as 1-β-D-ribofuranosyl-5-nitrobenzimidazole and (VII) as 1-β-D-ribofuranosyl-6-nitrobenzimidazole.

The ribosidation of 4(7)-nitrobenzimidazole[10] (XVIII) was achieved in essentially the same manner as stated above. Chloromercury salt (XIX) was condensed with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride (III), purified by alumina column chromatography, and recrystallized from ether-hexane. Yellow crystals (XX), m.p. 124~125°, $[\alpha]_D^{15}$ −94.3°, were obtained as the sole product in 22% yield. Debenzoylation of (XX) gave a crystalline substance (XXI) of m.p. 192~194°, $[\alpha]_D^{14}$ −2.8°, in 84% yield, which has the β-nucleoside linkage deduced from optical rotation value and 1,2-*trans* rule.[5]

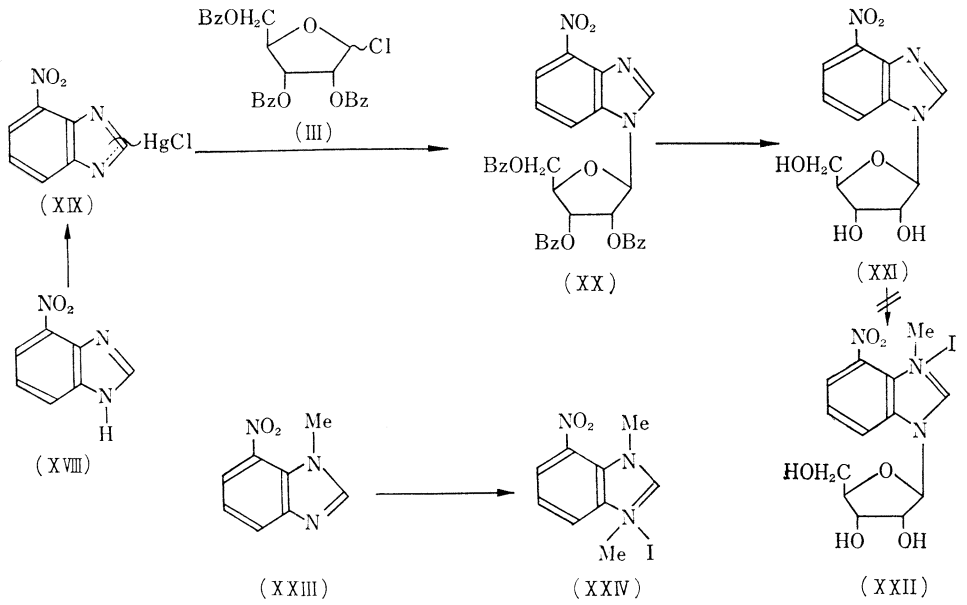


Chart 3.

Hunger, *et al.*[11] also obtained only one 4-nitro isomer in the condensation of sodium salt of 2-*p*-chlorobenzyl-4(7)-nitrobenzimidazole with diethylaminoethyl chloride. If it is assumed that the large nitro group sterically hindered the nitrogen atom in imidazole, the present product may also be 1,4-isomer. This was confirmed by the reaction of (XXI) with methyl iodide at 150°. Despite a long reaction at this temperature, quaternary salt was not obtained. On the other hand, authentic 1-methyl-7-nitrobenzimidazole (XXIII) reacted with methyl iodide smoothly to afford the methiodide (XXIV). Thus, steric effect of the nitro group was proved to be great and the structure of (XXI) was elucidated as 1-β-D-ribofuranosyl-4-nitrobenzimidazole.

None of these ribosides of nitrobenzimidazole revealed any marked activity against tumor cells.

10) G.M. van ver Want : Rec. trav. chim., **67**, 45 (1948); J.L. Rabinowitz, E.C. Wagner : J. Am. Chem. Soc., **73**, 3030 (1951).
11) A. Hunger, J. Kebrle, A. Rossi, K. Hoffmann : Helv. Chim. Acta, **43**, 1032 (1960).

Experimental

Chloromercury Salt of 5(6)-Nitrobenzimidazole (II)——To a solution of 10 g. of 5(6)-nitrobenzimidazole (I) in 1 L. of EtOH-H_2O (1:9) containing 2.5 g. of NaOH, a solution of 16.9 g. of $HgCl_2$ dissolved in 70 cc. of hot EtOH was added with vigorous stirring while hot. Pale yellow precipitation thereby appeared was collected by filtration, washed with H_2O until Cl^- was no longer detected, dehydrated with EtOH and Et_2O, and dried at 105° *in vacuo*. Yield, 24.0 g. (97%). *Anal.* Calcd. for $C_7H_4O_2N_3 \cdot HgCl$: N, 10.55. Found : N, 10.57.

1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-5-nitrobenzimidazole (IV) and 1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-6-nitrobenzimidazole (V)——A solution of 6.2 g. of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose dissolved in dry Et_2O, previously saturated with dry HCl gas at 0°, was kept in a refrigerator with exclusion of moisture for 7-10 days. Et_2O was evaporated at room temperature *in vacuo*, residual syrup was azeotropically freed from HCl by the addition and evaporation of 30 cc. of dry benzene 4 times. The residue was taken up in 30 cc. of dehyd. xylene and added into 5.3 g. of the chloromercury salt (II) of 5(6)-nitrobenzimidazole suspended in 80 cc. xylene. The solution was concentrated from 125 cc. of initial volume by azeotropic drying, with vigorous stirring and refluxing. The reaction mixture gradually became transluscent and at the end of 2.5 hr., a precipitate appeared. Insoluble material was removed while warm, mother liquor was evaporated to dryness at 40 *in vacuo*. The residue was taken up in 75 cc. of $CHCl_3$, which was filtered, washed twice with 30% KI solution (20 cc.) and twice with H_2O, and dried over Na_2SO_4. On evaporation of the solvent, 7.2 g. of crude nucleoside was obtained, which was taken up in 5 cc. of dehyd. benzene and applied to alumina (ca. 100 g.) column chromatography. The column was eluted with AcOEt-benzene and the fractions eluted with 20~50% (v/v) AcOEt were collected.*3 Residual vitreous substance (5.1 g.) was triturated with Et_2O and afforded a solid product 3.07 g. or 39.8% from (III). Recrystallization from a large volume of Et_2O-hexane gave the 5-nitro derivative (IV) of m.p, 135~138°, (1.15 g. or 15.6%), as the first crop, which was further recrystallized from $CHCl_3$-MeOH to give 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-5-nitrobenzimidazole in a pure state, m.p. 139~140°, $[\alpha]_D^{15}$ −83.4° (c=1.22, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{25}O_9N_3$: C, 65.23; H, 4.15; N, 6.92. Found : C, 65.16; H, 3.99; N, 6.79.

Mother liquor of above recrystallization was evaporated *in vacuo* and residual solid consisting almost solely of (V) (1.92 g. or 24.2%), m.p. 109~118°, was recrystallized several times from Et_2O-hexane to 1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-6-nitrobenzimidazole (V) in a pure state, m.p. 137~138°, $[\alpha]_D^{15}$ −56.0° (c=1.03, $CHCl_3$).

1-β-D-Ribofuranosyl-5-nitrobenzimidazole (VI)——A solution of 0.58 g. of the above protected nucleoside (IV) dissolved in 50 cc. of warm dehyd. MeOH was added to 50 cc. of dehyd. MeOH previously saturated with dry NH_3, the mixture was set aside at room temperature overnight, and the solvent was evaporated. Residual white vitreous solid was washed several times with $CHCl_3$ to remove methyl benzoate and benzamide. The crude product (270 mg.) was recrystallized from 5~6 cc. of H_2O to a substance of m.p. 169~170°, $[\alpha]_D^{14}$ +13.9° (c=1.28, *N* HCl). Yield, 240 mg. (90%). *Anal.* Calcd. for $C_{12}H_{13}O_6N_3$: C, 48.81; H, 4.48; N, 14.23. Found : C, 48.70; H, 4.50; N, 14.32.

1-β-D-Ribofuranosyl-6-nitrobenzimidazole (VII)——A crude substance (V) (1.8 g.) was debenzoylated with 150 cc. of dehyd. MeOH saturated with NH_3 at 0°. A crude nucleoside (0.82 g.) was obtained, and recrystallized several times from H_2O to furnish a product of m.p. 200~201°, $[\alpha]_D^{14}$ +27.7° (c=1.63, *N* HCl). Yield, 0.64 g. *Anal.* Calcd. for $C_{12}H_{13}O_6N_3$: C, 48.82; H, 4.48; N, 14.23. Found: C, 49.03; H, 4.36; N, 14.37.

N^1,N^2-Dimethyl-4-nitro-*o*-phenylenediamine (XVII)——1) A mixture of 200 mg. of 1-β-D-ribofuranosyl-6-nitrobenzimidazole (VII), 115 mg. (1.2 mole equiv.) of MeI, and dehyd. MeOH was sealed in a glass tube and heated at 120~130° for 5 hr. After MeOH was evaporated, residual vitreous substance (290 mg.) was washed several times with dehyd. Et_2O. The whole was added with MeOH-NaOH (1 g. NaOH in 12 cc. MeOH), the reaction mixture was refluxed for 30 min., the solvent was evaporated *in vacuo*, and the rad residue was dissolved in 10 cc. of H_2O. Crystalline product that appeared, was collected on a filter and recrystallized from MeOH to a substance of m.p. 172~173°. Yield, 30 mg. (24.2%). *Anal.* Calcd. for $C_8H_{11}O_2N_3$: C, 53.02; H, 6.12; N, 23.28. Found : C, 53.18; H, 6.17; N, 23.33.

From the mother liquor of this compound, (XVII) and 5-nitrobenzimidazole (I) were extracted with Et_2O. Though it was quite difficult to isolate the two compounds in a pure state, the latter was confirmed by UV spectrum.

2) According to the method of Smith, *et al.*,[5] 5-nitrobenzimidazole was heated with slight excess

*3 The other fractions were a trace of xylene, moiety of unreacted ribose, and oily substances. These substances were not the desired products.

of Me_2SO_4 and decomposed by treatment with NaOH to give a product of m.p. 171～172°, (70%). Mixed melting point with that obtained in (1) showed no depression.

Chloromercury Salt of 4(7)-Nitrobenzimidazole (XIX)——Obtained from 10 g. of 4(7)-nitrobenzimidazole (XVIII) by an analogous treatment as that for 5(6)-nitrobenzimidazole (I). Yield, 23.0 g. (95%). *Anal.* Calcd. for $C_7H_4O_2N_3 \cdot HgCl$: N, 10.55. Found : N, 10.95.

1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-4-nitrobenzimidazole (XX)——Condensation of 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride (III) (from 10 g. of 1-O-acetyl derivative) with chloromercury salt of 4(7)-nitrobenzimidazole (XIX) was achieved by essentially analogous manner as stated above. The crude product (11.0 g.) was purified by alumina column chromatography. Fractions eluted by 20～50% (v/v) of AcOEt-benzene were collected and the solvents were evaporated. Glassy substance (3.9 g.) thus obtained was triturated with Et_2O to give a crystalline solid. Yield, 2.6 g. (21.7%). A sample for analysis was recrystallized from Et_2O to m.p. 124～125°, $[\alpha]_D^{15}$ −94.3° (c=1.21, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{25}O_9N_3$: C, 65.23; H, 4.15; N, 6.92. Found : C, 65.22; H, 4.06; N, 6.85.

Further efforts to obtain 7-isomer were unsuccessful.

1-β-D-Ribofuranosyl-4-nitrobenzimidazole (XXI)——To 1.0 g. of the above product (XX) dissolved in 120 cc. of warm dehyd. MeOH, 120 cc. of dehyd. MeOH saturated with NH_3 was added, and the mixture was set aside at room temperature overnight. The solvent was removed *in vacuo*. The residue was washed with $CHCl_3$, and recrystallized from H_2O to yellow needles, m.p. 192～194° (420 mg., 84%); $[\alpha]_D^{14}$ −2.8° (c=1.44, *N* HCl). *Anal.* Calcd. for $C_{12}H_{13}O_6N_3$: C, 48.81; H, 4.48; N, 14.23. Found : C, 48.25; H, 4.57; N, 14.46.

Attempt to obtain Methiodide of 1-β-D-ribofuranosyl-4-nitrobenzimidazole (XXII)——To 100 mg. of 1-β-D-ribofuranosyl-4-nitrobenzimidazole (XXI), 55 mg. of MeI (1.1 mole equiv.) dissolved in 2 cc. of dehyd. MeOH was added and heated at 140～150° for 7 hr. in a sealed tube. Residual crystalline substance (ca. 100 mg.) was directly compared with the starting material and confirmed to be identical.

1-Methyl-7-nitrobenzimidazole 3-Methiodide (XXIV)——To 89 mg. of 1-methyl-7-nitrobenzimidazole (XXIII), 78 mg. (1.1 mole equiv.) of MeI dissolved in 3 cc. of MeOH was added and the reaction mixture was heated at 120～130° for 4 hr. The residue was recrystallized from MeOH to small orange plates, m.p. 210° (decomp.), yield, 50%. *Anal.* Calcd. for $C_9H_{10}O_2N_3I$: C, 33.87; H, 3.16; N, 13.17. Found : C, 33.79; H, 2.79; N, 12.94.

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Summary

5- and 6-Nitrobenzimidazole ribofuranosides were synthesized from chloromercury salt of 5(6)-nitrobenzimidazole and 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride in 15.6% and 24.2% yield respectively. The structure of both compounds was elucidated physicochemically. 4-Nitrobenzimidazole ribofuranoside was also synthesized by an analogous reaction.

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