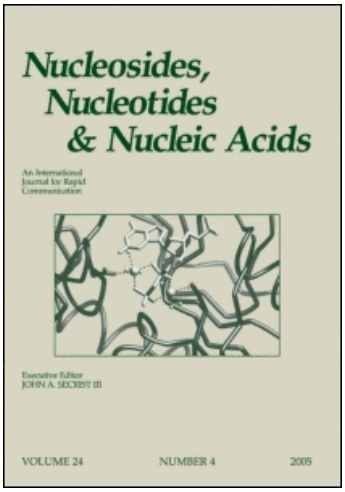

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Kazimierczuk, Zygmunt and Shugar, David(1989) 'Preparation and Properties of the 5,6- and 4,6(5,7)-Dinitro Derivatives of Benzimidazole and their 1-β-D-Ribofuranosides', Nucleosides, Nucleotides and Nucleic Acids, 8: 8, 1379 — 1385
To link to this Article: DOI: 10.1080/07328318908048847
URL: http://dx.doi.org/10.1080/07328318908048847

NUCLEOSIDES & NUCLEOTIDES, 8(8), 1379-1385 (1989)

PREPARATION AND PROPERTIES OF THE 5,6- AND 4,6(5,7)-DINITRO DERIVATIVES OF BENZIMIDAZOLE AND THEIR 1-β-D-RIBOFURANOSIDES

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ABSTRACT. Nitration of benzimidazole leads to the formation of the two isomeric 5,6- and 4,6(5,7)-dinitrobenzimidazoles, which may be isolated by fractional crystallization. The chloromercury salts of these were employed to synthesize the corresponding 1-β-D-ribofuranosides, unequivocally characterized by ^{1}H NMR spectroscopy. Reference is made to the biological significance of these results.

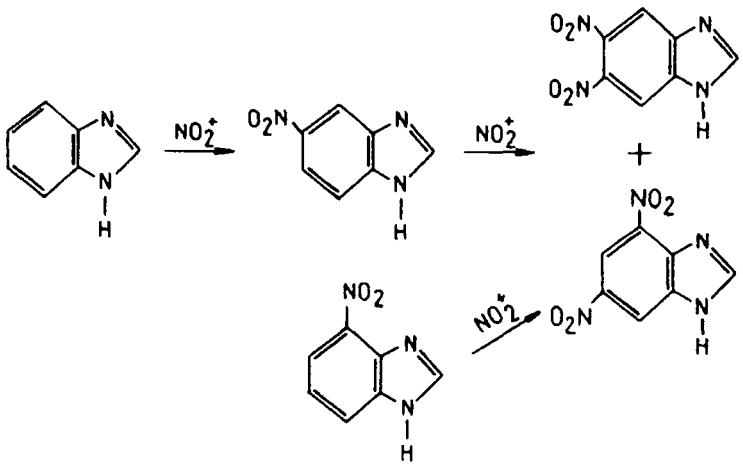
During the course of an investigation on the mechanism of the acid-catalyzed hydrolysis of benzimidazole nucleoside analogues,[1] it appeared desirable to examine the behaviour of the 5,6-dinitro derivative of 1-β-D-ribofuranosylbenzimidazole. The synthesis of 5,6-dinitrobenzimidazole was reported in 1952 by Efros[2] by direct nitration of benzimidazole. Subsequently this synthesis was repeated by Pawelkiewicz & Zodrow,[3] who further demonstrated that the product is incorporated into the vitamin B_{12} of Corynebacterium diphtheriae.

Howeyer, in our hands nitration of benzimidazole by the procedure of Efros[2] led to a product which, when chromatographed on silica gel plates, led to the appearance of two spots with comparable intensities. Recourse was then had to repeated fractional crystallization of the product of nitration with varying proportions of water and

isopropanol. This resulted in isolation of two crystalline products with melting points of 238-240°C and 247-248°C, as compared to the broad melting point of ~186°C reported by both Efros[2] and Pawelkiewicz & Zodrow.[3] They differed in R_f values on silica gel plates, as well as in their UV spectra at different pH values. From the reported[4] marked differences between the coupling constants $J_{4,5}$ and $J_{5,6}$ (7-9 Hz) on the one hand, and $J_{5,7}$ (0.8-3 Hz) on the other, for benzimidazole analogues, it proved possible by means of ^{1}H NMR spectroscopy to identify the two products as the 5,6- and 4,6(5,7)-dinitrobenzimidazoles. An equimolar mixture of the two isolated products melted in the range 192-196°C.

Furthermore, nitration under the same conditions of the known 4-nitrobenzimidazole led to a single product, identified as 4,6(5,7)-dinitrobenzimidazole.

Electrophilic substitution on benzimidazole usually proceeds at 5(6) and subsequently at 6(5), e.g. bromination of 1-β-D-ribofuranosylbenzimidazole leads to the 5,6-dibromo analogue via the intermediate monobromo derivative.[5] Nitration of benzimidazole under relatively mild conditions has long been known to lead to the 5(6)-mononitro derivative;[6] but, under the more extreme conditions employed here, the reaction apparently follows a dual pathway, as follows,



with formation of the 5,6-dinitro and 4,6(5,7)-dinitro. It is of interest, in this connection, that some 2-substituted nitrobenzimidazoles are significant radiosensitizing agents.

The foregoing points to the need for a reexamination of the results of Buchel[7] on the synthesis of a variety of analogues of 2-trifluoromethylbenzimidazole, e.g. the 4-chloro-5,6-dinitro derivative exhibited very broad melting in the range 170-190°C, and identification was based solely on results of elementary analysis. However, the broad melting range suggests the possible formation of a mixture of the foregoing with the 4-chloro-5,7-dinitro congener.

Attempts to prepare the ribonucleosides of the two dinitrobenzimidazoles by fusion with 1,2,3,5-tetraacetyl-β-D-ribofuranose were unsuccesful because of the high melting points of the former. Nor did either of the dinitrobenzimidazoles undergo silylation with use of standard conditions i.e. HMDS or HMDS-silyl chloride. However, condensation of the chloromercuri salts with 1-chloro-2,3,5-benzoylribofuranose gave the two ribofuranosides in reasonable yields.

The 5,6-dinitrobenzimidazole, by symmetry, gave a single product. With the 4,6-dinitro analogue, steric hindrance by the 4-nitro accounts for formation of only the 1-β-D-ribofuranoside, as earlier observed for ribosylation of 4-nitrobenzimidazole.[8] The mercuri salt procedure is known to lead to formation of only β-ribosides,[9] further confirmed by conversion of the two nucleosides to their 2',3'-O-isopropylidene derivatives. Their ^{1}H NMR spectra showed a difference in chemical shifts for the isopropylidene methyl groups of 0.23 ppm and 0.24 ppm for the 5,6-dinitro- and 4,6-dinitrobenzimidazole ribofuranosides, consistent only with a β-configuration.[10]

Bearing in mind that the nitration procedure of Efros[2] leads to the two isomeric dinitro benzimidazoles, it becomes clearly desirable to establish whether, in the experiments of Pawelkiewicz and Zodrow,[3] only one or both of these undergo incorporation into vitamin B_{12}. Finally, it should be noted that access to the two dinitro benzimidazole nucleosides may be profited from for the preparation of a variety of nucleosides with lin heterocyclic bases,[11,12] one of which has been identified as a minor component of a vitamin B_{12} analogue.[13]

EXPERIMENTAL

Melting points (uncorr.) were measured with the aid of a Boetius microscope hot stage. Elementary analyses were performed by the Institute of Organic Chemistry, PAN. Thin-layer chromatography was with Merck (Darmstadt, GFR) silica gel 60 F_{254} plates, with chloroform--methanol (85:15, v/v) as solvent. UV spectra were run on a Zeiss (Jena, GDR) VS-2 instrument. ^{1}H NMR spectra were recorded on a Bruker 360 spectrometer on solutions in DMSO-d_6 with TMS as internal standard

5,6-Dinitro- and 4,6(5,7)-dinitro- benzimidazoles. To 40 g (0.34 mole) benzimidazole, cooled on an ice bath, was added 210 ml HNO_3 (d = 1.52), followed by 50 ml H_2SO_4 and 70 ml of 25% oleum. The mixture was heated for 2 hr at 80-90^{o}C, cooled to room temperature, poured onto 1 kg ice pellets and carefully brought to pH ~8 by addition of NH_4OH. The resultant yellow precipitate was collected by filtration, washed with water, and subjected to crystallization initially from 200 ml water + 300 ml isopropanol, then from 50 ml water + 250 ml isopropanol and 50 ml water + 200 ml isopropanol, and finally from 50 ml water + 70 ml dioxane, to yield 8.3 g (12%) of yellow needles of 5,6-dinitrobenzimidazole, m.p. 238-240^{o}C, as compared to a reported 186^{o}C.[2] TLC showed a single spot with R_f = 0.48. Elem. anal.: Calculated for $C_7H_4N_4O_4$: C, 40.40%; H, 1.94%; N, 26.93%. Found: C, 40.25% H, 1.85%; N, 27.05%. UV: $\lambda_{max}^{pH\ 7}$ 237 nm (ε = 14 x 10^3), 285 nm (ε = 5 x 10^3); $\lambda_{max}^{pH\ 12}$ 257 nm (ε = 11.6 x 10^3), 370 nm (ε = 3.3 x 10^3). ^{1}H NMR (DMSO-d_6): 8.49 ppm (2H, s, 4-H and 7-H), 8.77 ppm (1H, s, 2-H), 13.6 ppm (1H, bs, N-H).

The pooled mother liquors from the crystallization steps were brought to dryness, and the residue crystallized initially from 400 ml water + 200 ml isopropanol, and then from 40 ml water + 80 ml isopropanol, to yield 13.6 g (19%) of 4,6(5,7)-dinitrobenzimidazole in the form of yellow needles, m.p. 247-248^{o}C. Chromatographed as above, it exhibited a single spot, R_f = 0.68. Elem. anal.: Calculated for $C_7H_4N_4O_4$: C, 40.40%; H, 1.94%; N, 26.92%. Found: C, 40.21%; H, 1.86%; N, 27.04%. UV: $\lambda_{max}^{pH\ 7}$ 279 nm (ε = 14.9 x10^3), 300 nm (ε = 9.0 x 10^3); $\lambda_{max}^{pH\ 12}$ 312 nm (ε = 12.6 x 10^3), 355 nm (ε = 9.9 x 10^3). ^{1}H NMR (DMSO-d_6): δ 8.76 ppm (1H, s, 2-H); 8.84 ppm (1H, d, 5-H

or 7-H, J = 2 Hz); 8.97 ppm (1H, d, 5-H or 7-H, J = 1.6 Hz), 13.9 ppm (1H, bs, N-H).

4,6(5,7)-Dinitrobenzimidazole: A suspension of 1.3 g (8 mmol) of 4(6)-nitrobenzimidazole[14] in 15 ml fuming HNO_3 and 7 ml conc. H_2SO_4 was heated for 2 hr at 70-80°C, then brought to room temperature and poured onto 60 g ice. The resulting solution was brought to pH ~6 with NH_4OH, leading to a yellow precipitate, which was collected by filtration and washed with water. The residue was crystallized from aqueous ethanol to yield 1.55 g (93%) of the dinitro derivative in the form of needles, m.p. 248-250°C, with spectroscopic and chromatographic properties identical with one of the two products of nitration of benzimidazole, identified above as 4,6(5,7)-dinitrobenzimidazole.

4,6-Dinitro-1-(β-D-ribofuranosyl)benzimidazole: To a solution of 4.16 g (20 mmol) of 4,6-dinitrobenzimidazole in 100 ml ethanol and 400 ml water was added 20 ml 1 N NaOH and, dropwise with stirring, a solution of 5.48 g (20.2 mmol) $HgCl_2$ in 100 ml ethanol. To the resulting gelatinous mass was added 3 g NH_4Cl and 5 ml conc. NH_4OH, leading to a pale yellow precipitate, which was washed with water, ethanol and ether to yield 8.05 g of the mercurichloride salt. This was ground to a powder, suspended in 300 ml anhydrous xylene, and the volume reduced to 250 ml by distillation. To this suspension under reflux was added 1-chloro-2,3,5-tri-O-benzoylribofuranose prepared from 10.12 g (20 mmol) of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in 100 ml toluene, with simultaneous removal by distillation of 100 ml solvent. The mixture was refluxed for an additional 2.5 hr. Insoluble material was removed by filtration, and the filtrate brought to dryness. The residue was dissolved in 200 ml $CHCl_3$, which was washed successively with 2 x 100 ml 30% KI, 2 x 150 ml 0.5 M $NaHCO_3$ and 3 x 200 ml water. The organic phase was dried over Na_2SO_4, deposited on a 3.5 x 25 cm silica gel column, and elution carried out successively with benzene (0.5 l), benzene-methylene chloride (1:1, 0.5 l) and methylene chloride (1 l). The fractions containing the benzoylated nucleoside were pooled and brought to dryness in the form of a foam (7.2 g, 52%). This was taken up in 100 ml methanol and 10 ml of 1 N sodium

methoxylate and stirred at room temperature for 2 hr. It was brought to neutrality with acetic acid, taken to dryness, and dried again several times from water. Crystallization of the residue from 50 ml water gave 1.62 g (23%) of yellow needles, m.p. 190-192°C, and R_f = 0.33. Elem. anal.: Calculated for $C_{12}H_{12}O_8N_4$: C, 42.36%: H, 3.56%; N, 16.47%. Found: C, 42.18%; H, 3.62%; N, 16.31%. UV (pH 2 - 12): λ_{max} 280 nm (ε_{max} 13.6 x 10^3), 315 nm (ε_{max} 9.0 x 10^3). ^{1}H NMR (DMSO-d_6): δ 3.70 ppm (2H, m, 5'-H and 5''-H), 4.09 ppm (1H, d, 4'-H), 4.17 ppm (1H, d, 3'-H), 4.37 ppm (1H, q, 2'-H), 5.30 ppm (2H, m, 3'-OH and 5'-OH), 5.65 ppm (1H, d, 2'-OH), 6.17 ppm (1H, d, 1'-H, $J_{1',2'}$ = 5.8 Hz), 8.83 and 9.31 ppm (2H, 2 x d, 5-H and 7-H, J~2 Hz), 9.11 ppm (1H, s, 2-H).

5,6-Dinitro-1-(β-D-ribofuranosyl)benzimidazole: This was prepared from 5,6-dinitrobenzimidazole, following the procedure for the 4,6-dinitro nucleoside in the previous section, to yield the product in the form of yellow needles (35%), m.p. 176-178°C, and R_f = 0.27. Elem. anal.: Calculated for $C_{12}H_{12}O_8N_4$: C, 42.36%; H, 3.56%; N, 16.47% Found: C, 42.20%: H, 3.63%; N, 16.35%. UV (pH 2 - 12): λ_{max} 240 nm (ε_{max} 15.3 x 10^3), 305 nm (ε_{max} 5.0 x 10^3). ^{1}H NMR (DMSO-d_6): δ 3.69 ppm (2H, m, 5'-H and 5''-H), 4.06 ppm (1H, d, 4'-H), 4.15 ppm (1H, d, 3'-H), 4.36 ppm (1H, q, 2'-H), 5.31 ppm (2H, m, 3'-OH and 5'-OH), 5.60 ppm (1H, d, 2'-OH), 6.07 ppm (1H, d, 1'-H, $J_{1',2'}$ = 6.2 Hz), 8.61 ppm (1H, s, 2-H), 8.89 and 9.01 ppm (2H, 2 x s, 4-H and 7-H).

ACKNOWLEDGMENTS

This investigation profited from the support of the Polish Cancer Research Program (CPBR-11.5) and the Polish Academy of Sciences (CPBR-3.13).

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Received March 24, 1988.