Solution Conformation of Benzimidazole Nucleosides with the Aid of Model Analogues

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Z. Naturforsch. **36 c**, 126–134 (1981); received August 19, 1980

Benzimidazole Nucleosides, Synthesis of Halogeno Analogues, Model *syn* and *anti* Conformations, Conformational Analysis

The 4,6-dibromo- and 5,7-dibromo-derivatives of 1-β-D-ribofuranosylbenzimidazole, and the 4,6-dibromo-derivative of 1-α-D-arabinofuranosylbenzimidazole, have been synthesized by condensation of the trimethylsilylated derivative of 4,6-dibromobenzimidazole with the appropriate protected sugar in the presence of $SnCl_4$. Application of the fusion method led to the β-ribofuranoside of 2-methyl-5,6-dichlorobenzimidazole. Two additional analogues of the biologically active ribofuranoside of 5,6-dichlorobenzimidazole, viz. the 2′,3′-O-isopropylidene and the 5′-chloro-5′-deoxy derivatives have been synthesized.

Analyses of the ^{1}H NMR spectra of the foregoing, and of previously synthesized 5(6)-monohalogeno and 5,6-dihalogeno derivatives, were employed to determine solution conformations of these nucleosides. Conformations of the sugar rings and exocyclic groups were evaluated from vicinal proton-proton coupling constants, as for purine nucleosides. The conformation of the benzimidazole ring about the glycosidic bond was determined from the chemical shifts of the sugar protons, principally H(2′), with the aid of model analogues in fixed *syn* and *anti* conformations; and, independently, from the chemical shifts of the benzimidazole ring protons, principally H(4) and H(7). The halogenated benzimidazole ribofuranosides exhibit conformations of the sugar ring similar to those for purine β-nucleosides, but differ from the latter in their preference for the conformation *syn* about the glycosidic bond.

Introduction

Interest in halogenated benzimidazole nucleosides stems in large part from the fact that 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) is a speific and reversible inhibitor of nuclear RNA synthesis [1–3] and a superinducer of interferon production in human fibroblasts [4]. Some bromo derivatives of benzimidazole nucleosides also exhibit enhanced biological activity relative to the corresponding chloro derivatives [4]. It consequently appeared of interest to synthesize, and examine the physicochemical and biological properties of, additional analogues of DRB, including brominated derivatives.

One pertinent aspect of the foregoing is the relationship of the biological activities of benzimidazole nucleosides to their conformations, the latter of which may be determined with the aid of NMR spectroscopy (for review, see 5). In the present study we have applied for this purpose a procedure previously described [6, 7], based on a comparison of the chemical shifts of the sugar protons in nucleosides and nucleotides relative to the chemical shifts of the corresponding protons in models fixed in the *syn* and *anti* conformations, to determine the conformation about the glycosidic bond. This required the synthesis of model benzimidazole nucleosides with a bulky substituent at C(2), which would be expected to sterically constrain the aglycone to the conformation *syn;* and a nucleoside with a bulky substituent at C(7), to constrain the aglycone to the conformation *anti.*

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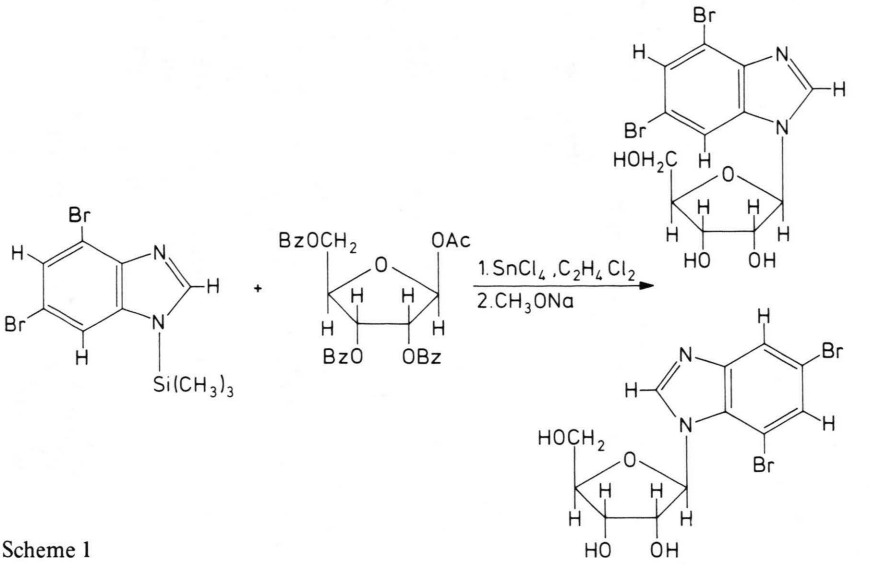
0341-0382/81/0100-0126 $ 01.00/0

Results and Discussion

Syntheses

Treatment of 5,6-dichloro-1-(β-D-ribofuranosyl)-benzimidazole with acetone, in the presence of an acid catalyst according to standard procedures, yielded the 2′,3′-O-isopropylidene derivative in high



Scheme 1

yield. Reaction of the unblocked nucleoside with thionyl chloride in hexamethylphosphoric triamide led to the isolation in almost 70% yield of the 5′-chloro-5′-deoxy derivative of 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole The reaction in this case proceeded more smoothly and rapidly than in the case of purine nucleosides [8] and was complete in 30 min at room temperature.

Fusion of 2-methyl-5,6-dichlorobenzimidazole with 1-O-acetyl-2,3,5-tri-O-benzol-β-D-ribofuranose gave the desired 2-methyl-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole. In this instance the relatively low melting temperatures of benzimidazole derivatives led to a very homogeneous melt with the protected sugar derivatives, so that the reaction proceeded very readily [10, 11].

The synthesis of the 4,6-dibromo and 5,7-dibromo analogues of 1-β-D-ribofuranosylbenzimidazole was based on the procedure of Niedballa & Vorbruggen [12]. The reaction of 4,6-dibromobenzimidazole with the blocked ribose, because of the non-symmetrical structure of 4,6-dibromobenzimidazole, led to a mixture of the isomeric 4,6- and 5,7-dibromo nucleosides (Scheme 1), as shown by NMR spectroscopy. It proved possible to fractionate these by chromatography on Amberlite XAD-4 [13], which has hitherto been little profited from, but which we have found extremely useful for chromatography of both nucleosides and nucleotides [14].

In surprising contrast to the foregoing, the reaction with 1-O-methyl-2,3,5-tri-O-benzoyl-D-arabinofuranose led to formation of a single product, identified unequivocally as the 4,6-dibromo derivative by NMR spectroscopy.

Nucleoside conformations

Analysis with the aid of ^{1}H NMR spectroscopy was employed to determine the conformations of the various nucleoside analogues.

Conformation of sugar rings. The values of the chemical shifts of the sugar protons, and the vicinal proton-proton coupling constants, are listed in Table 1.

Analysis of the conformation of the sugar rings was based on the two-state model [15], with a dynamic equilibrium between the two conformers N, C(3′)*endo* and S, C(2′)*endo*. The results point to a predominance of the form S, from 50% to 75% (Table I). Marked changes in this range result from:

(a) Introduction of a substituent at C(2) of the benzimidazole ring, leading to an increase in population of the S conformer of up to 12%.

(b) Introduction of a bromine substituent at C(7) of the benzimidazole ring, leading to a decrease in population of the S form of 13%. At the same time the benzimidazole ring is sterically constrained to the form *anti* (see below).

Table I. Values for the chemical shifts (in ppm *vs* internal Me_4Si) and vicinal coupling constants (in Hz) for various benzimidazole-β-D-riboside analogues in $(C^2H_3)_2SO$ and, in some instances, also in $C^2H_3O^2H$; and conformational parameters for the sugar rings and exocyclic groups derived from these data.

Analogue of 1-β-D-ribofuranosyl-benzimidazole	Solvent	Chemical shifts						Coupling constants						Conformation	
		H(1′)	H(2′)	H(3′)	H(4′)	H(5′)	H(5″)	*J*(1,2′)	*J*(2,3′)	*J*(3,4′)	*J*(4,5′)	*J*(4,5″)	*J*(5,5″)	C(2′)*endo* [%]	*gauche-gauche* [%]
Parent nucleoside	$(C^2H_3)_2SO$	5.86	4.36	4.11	3.97	3.69	3.63	6.2	5.0	3.2	3.4	3.8	−12.0	65	62
	$C^2H_3O^2H$	5.97	4.46	4.28	4.13	3.87	3.79	5.4	5.1	4.2	2.8	3.3	−12.2	55	73
2-α-hydroxybenzyl-[a]	$(C^2H_3)_2SO$	6.09	4.37	4.11	3.87	3.68 [b]		6.9	6.1	3.2	7.5 [b]		[b]	68	57
	$C^2H_3O^2H$	6.10	4.47	4.22	3.90 [b]	3.85 [c]		6.8	6.1	3.2	[c]		[c]	67	[c]
5-Bromo-(6-bromo-)	$(C^2H_3)_2SO$	5.87	4.35	4.12	3.99	3.65 [b]		6.0	4.8	3.5	6.5 [b]		[b]	63	70
5,6-Dibromo-	$(C^2H_3)_2SO$	5.89	4.33	4.15	4.02	3.65 [b]		6.0	4.9	3.5	5.5 [b]		[b]	63	80
	$C^2H_3O^2H$	5.90	4.42	4.27	4.14	3.86	3.80	5.5	5.1	3.8	2.5	2.9	−12.2	60	82
4,6-Dibromo-	$(C^2H_3)_2SO$	5.89	4.32	4.12	4.01	3.66 [b]		5.9	5.0	3.4	5.5 [b]		[b]	63	80
5,7-Dibromo-	$(C^2H_3)_2SO$	6.61	4.45	4.16	3.98	3.71	3.60	4.6	4.7	4.7	3.1	3.1	−12.0	50	72
5,6-Dichloro-	$(C^2H_3)_2SO$	5.90	4.32	4.14	4.02	3.70 [b]		6.1	4.9	3.2	5.5 [b]		[b]	65	80
2-Methyl-5,6-dichloro-	$(C^2H_3)_2SO$	5.77	4.30	4.15	4.02	3.73 [b]		7.4	5.8	2.3	5.5 [b]		[b]	76	82
2′,3′-O-isopropylidene-5,6-dichloro-	$(C^2H_3)_2SO$	6.20	5.19	4.97	4.23	3.53 [b]		3.3	6.1	2.5	8.0		[b]	50% *C(3′)exo*[d]	53
5′-Chloro-5′-deoxy-5,6-dichloro-	$(C^2H_3)_2SO$	5.97	4.47	4.23	4.18	3.95 [b]		6.1	4.9	3.2	8.0 [b]		[b]	63	53

[a] Spectrum supplied by Dr. W. Streicher of Sandoz Forschungsinstitut, Vienna;
[b] The deceptively simple H(4′), H(5′), H(5″) system did not permit of accurate determination of all the parameters; we present here the location of the H(5′), H(5″) signals and the sum $J(4', 5') + J(4', 5'')$.
[c] These parameters could not be individually determinated because of the closely similar values of H(4′) and the system H(5′), H(5″)
[d] The sugar conformation of this compound has been previously discussed (see ref. 6, and references cited therein).

(c) A change in solvent medium. Transfer of the nucleoside from $(C^2H_3)_2SO$ to $C^2H_3O^2H$ results in a decrease in the population S of up to 10%.

Introduction of a halogeno substituent at C(4), C(5) and C(6) of the benzimidazole ring, or replacement of the exocyclic 5′-OH by halogen, is virtually without effect on the sugar ring conformation.

In general the N and S conformer populations of the benzimidazole-β-riboside analogues are similar to those for the corresponding purine β-nucleosides, adenosine and guanosine. As in the case of the 8-substituted purine nucleosides, a 2-substituent leads to an increase in population of the S conformer. The similar values for the *cis*oidal coupling constants, and for the sums $J(1',2') + J(3',4')$, in both classes of compounds unsubstituted at the position C(2) or C(8), points to similar puckering of the sugar rings, $\tau_m = 37°$, and similar values of the pseudorotational angles, *viz.* ${}^{N}P = 20°$ and ${}^{S}P = 165°$.

Introduction of substituents at C(2) in the benzimidazole nucleosides, or the corresponding C(8) in purine nucleosides, results in a characteristic increase in the value of $J(2',3')$ by 0.5–1.0 Hz. The sum $J(1',2') + J(3',4')$ for the benzimidazole ribosides increases by about 0.5 Hz, whereas it is unchanged for the purine nucleosides or is slightly diminished relative to the unsubstituted parent nucleosides. These effects, although small, are at the moment difficult to interpret, since they may be due to some deformation of the sugar ring (*e.g.* flattening) and/or changes in the phase angles of pseudorotation ${}^{N}P$ and ${}^{S}P$.

Conformation of the exocyclic groups. These conformations were determined in the same way as for the corresponding nucleosides [16, 17], assuming a dynamic equilibrium between three classical conformers, *gauche-gauche*, *gauche-trans* and *trans-gauche*. The values for the *gauche-gauche* conformers, the populations of which are predominant, are exhibited in Table I. For most of the nucleosides, the closely similar values for the chemical shifts of H(5′) and H(5″) rendered difficult estimates of the populations of the other two conformers. The predominance of the *gauche-gauche* populations, from 60% to 80% (Table I), is similar to that for the corresponding purine β-nucleosides.

Conformation of the heterocyclic base

We have previously shown that the populations of the *syn*/*anti* conformers in purine nucleosides may be evaluated by measurements of the chemical shifts of the H(2′) proton relative to those for model nucleosides fixed in the *syn* and *anti* conformations [6, 7].

As in the case of purine nucleosides, the 2-methyl and the 2-α-hydroxybenzyl nucleosides may, to a first approximation, serve as models in the *syn* conformation. An examination, with the aid of CPK models, showed that, as in the case of 8-methyladenosine [6], these substituents are not bulky enough to fully exclude the presence of a small proportion of the form *anti* (but see below). By contrast, CPK models demonstrated, in accordance with expectations that, for the 5,7-dibromo substituted nucleoside, the steric hindrance of the 7-bromo with the pentose ring in the conformation *syn* is sufficiently severe as to exclude this form, so that this nucleoside is a good model for the form *anti*.

The conformations of the various nucleosides were then evaluated by analyses of the chemical shifts of H(1′) and H(2′) and, independently, by examinations of the chemical shifts of the benzimidazole H(4), H(5), H(6) and H(7) protons.

Chemical shifts of sugar protons. For the 2-methyl and 2-α-hydroxybenzyl-nucleosides, the chemical shifts of H(1′) differ by 0.1–0.2 ppm from those for the other nucleosides, with the discrepancies being in the range 0.03 ppm (Table I). This is obviously due to the anisotropic and electrostatic effects of the C(2) substituents which, in the conformation *syn*, are in the vicinity of H(1′).

A characteristic feature is the pronounced deviation of the chemical shift of H(4′) in the 2-α-hydroxybenzyl nucleoside from that for the other nucleosides, as high as 0.2 ppm in methanolic solution (whereas the chemical shift of H(5′) is unaltered). This effect is due to the hydroxybenzyl substituent, which may rotate freely about the single bond to C(2), permitting of its close approach to H(4′), but only when this nucleoside is in the form *syn*. The foregoing two derivatives may consequently serve as models in the *syn* conformation.

The strong deshielding, by 0.8 ppm, of H(1′) in the 5,7-dibromo nucleoside is readily interpretable in terms of the anisotropic affect of the C(7) bromine substituent on H(1′) in the conformation *anti*, pointing to the validity of the use of this nucleoside as a model in the form *anti*.

The values of the chemical shifts for H(2′) in ribofuranosylbenzimidazole and its 5,6-dichloro

analogue are identical with those of the corresponding 2-α-hydroxybenzyl and 2-methyl nucleosides in the model form *syn*, Introduction of bromine substituents at C(4), C(5), C(6), or chlorine substituents at C(5), C(6), *decreases* the value of the chemical shift of H(2′), at most by 0.04 ppm, to a value equal to δH(2′) in the 5,6-dichloro-2-methyl nucleoside. By contrast, introduction of bromine substituents at C(5), C(7), forcing the nucleoside into the anti conformation, *increases* the value of the chemical shift of H(2′) by 0.13 ppm relative to that for the 5,6-dichloro-2-methyl analogue. It may therefore be concluded that benzimidazole riboside, and its 5-bromo-, 6-bromo-, 5,6-dibromo-, 5,6-dichloro- and 4,6-dibromo- derivatives all exhibit a preference for the conformation *syn* in DMSO and methanol. However, a change in conformation from *syn* to *anti* with the benzimidazole nucleosides leads to only small changes in chemical shifts of H(2′), as contrasted to the corresponding marked changes with purine nucleosides (0.5–0.6 ppm, see ref. [6]). As a result of this, it is difficult to quantitatively evaluate the proportions of the *syn/anti* conformers for the benzimidazole nucleosides. It is feasible, at best, to indicate only whether there is a preference for the form *syn*, but not to fully exclude some involvement of the form *anti*.

The differences in the values of δH(2′) of the various analogues are most likely due to the differences in the values of the glycosidic torsion angle assumed by each of them in the conformation *syn*. The differences in δH(3′), up to 0.05 ppm, do not exhibit the same regularity as δH(2′), *i. e.* they are of the same order of magnitude in going from the form *syn* to *anti* as for those analogues in the form *syn*. Most likely these also derive from differences in the glycosidic torsion angles.

Chemical shifts of benzimidazole protons. These further support the assignment of the preference for the conformation *syn* for all the foregoing nucleosides, with the exception of the 5,7-dibromo analogue. Table II lists the changes in chemical shifts of H(4), H(5), H(6), H(7) and H(2) resulting from:

(a) Introduction on the N(1) position of the benzimidazole ring of β-ribose or α-arabinose, and the resulting difference in chemical shifts between a given halogenobenzimidazole nucleoside and the corresponding free aglycone; and

(b) Introduction of halogeno substituents in the benzene moiety of the benzimidazole ring, and the resulting differences in chemical shifts between the halogeno-substituted bases and the parent bases on the one hand, and between the halogeno-substituted nucleosides and their parent nucleosides on the other.

Introduction of a sugar moiety at N(1) leads to closely similar changes in chemical shifts (~0.1 ppm) of H(4), H(5), H(6) in all the compounds examined. The chemical shifts of H(7) do not exhibit this regularity in the β-ribosides, the deviation from the expected value of 0.1 ppm (for the other protons) being as high as 0.5 ppm. In the α-arabinosides, the changes in δH(7) are close to 0.1 ppm. It follows that the H(7) proton in the β-ribosides is located in a different chemical environment than the remaining protons and also different from that in the aglycone itself, *i. e.* it must be located in the direct vicinity of the sugar ring in the conformation *syn*.

Furthermore, the changes in the chemical shifts in the various analogues testify to differences in the glycosidic torsion angles, so that H(7) is located in different analogues at differing distances from the sugar ring oxygen.

The changes in δH(2) are similar in different analogues, viz. deshielding by about 0.25 ppm on introduction of the ribose ring at N(1), except for the 5,7-dibromo derivative, where it is 0.49 ppm. Since the latter has the conformation *anti*, the remainder must be preferentially *syn*.

Analogous conclusions may be drawn from an analysis of the effect of replacement by a halogen of the other protons in the benzimidazole ring. In the parent benzimidazoles, which are symmetrical systems, the changes in chemical shifts of H(4) and H(7), on introduction of substituents at C(5) and C(6), are identical. In the β-ribosides, on the other hand, while the effect on H(4) is similar to that for the parent aglycone, the changes in δH(7) deviate from these values (by as much as 0.5 ppm). Similarly in the 4,6-dibromo analogue, the change in δH(7) in the aglycone differs from that for the β-riboside. These differences are not due to a disturbance of the symmetry of the system by the sugar ring, since the effects of halogen substituents on H(4) and H(7) in the β-arabinosides are closely similar (in the range 0.05 ppm). The results therefore indicate that, in the β-ribosides, the changes in chemical shifts of H(7) are the additive changes

Table II. Changes in chemical shifts ($\Delta\delta$H, in ppm) of the benzimidazole protons as a result of the substitution: (a) of a sugar on the position N(1); (b) chlorine or bromine at C(5) and C(6); (c) bromine at C(4) and C(6); (d) bromine at C(5) and C(7). The values given represent the differences between the chemical shifts for the given substituted analogue and the unsubstituted parent compound.

	Sugar substituent on N(1)					Halogen substituents at C(4) and C(6)			Halogen substituents at C(5) and C(6)			Halogen substituents at C(5) and C(7)		
	$\Delta\delta$H(2)	$\Delta\delta$H(4)	$\Delta\delta$H(5)	$\Delta\delta$H(6)	$\Delta\delta$H(7)	$\Delta\delta$H(2)	$\Delta\delta$H(5)	$\Delta\delta$H(7)	$\Delta\delta$H(2)	$\Delta\delta$H(4)	$\Delta\delta$H(7)	$\Delta\delta$H(2)	$\Delta\delta$H(4)	$\Delta\delta$H(6)
5,6-Dichlorobenzimidazole	–	–	–	–	–	–	–	–	0.14	0.27	0.27	–	–	–
5,6-Dibromobenzimidazole	–	–	–	–	–	–	–	–	0.10	0.40	0.40	–	–	–
2-Methyl-5,6-dichloro-benzimidazole	–	–	–	–	–	–	–	–	–	0.11 [a]	0.11 [a]	–	–	–
4,6(5,7-)-Dibromo-benzimidazole	–	–	–	–	–	0.14	0.39	0.18	–	–	–	0.14	0.18	0.39
1-β-D-ribofuranosyl-benzimidazole	0.27	0.06	0.03	0.06	0.13	–	–	–	–	–	–	–	–	–
1-α-D-arabinofuranosyl-benzimidazole	0.20	0.10	0.10	0.10	0.10	–	–	–	–	–	–	–	–	–
1-β-D-ribofuranosyl-2-hydroxybenzylbenzimidazole	–	0.08	0.07 [b]	0.07 [b]	0.32	–	–	–	–	–	–	–	–	–
1-β-D-ribofuranosyl-5,6-dichlorobenzimidazole	0.24	0.10	–	–	0.36	–	–	–	0.11	0.31	0.50	–	–	–
1-α-D-arabinofuranosyl-5,6-dichlorobenzimidazole	0.16	0.10	–	–	0.14	–	–	–	0.10	0.27	0.31	–	–	–
1-β-D-ribofuranosyl-2-methyl-5,6-dichlorobenzimidazole	–	0.08	–	–	0.68	–	–	–	–	0.13 [a]	0.66 [a]	–	–	–
1-β-D-ribofuranosyl-5,6-dibromobenzimidazole	0.25	0.09	–	–	0.35	–	–	–	0.08	0.43	0.62	–	–	–
1-α-D-arabinofuranosyl-5,6-dibromobenzimidazole	0.19	0.10	–	–	0.14	–	–	–	0.09	0.40	0.44	–	–	–
1-β-D-ribofuranosyl-4,6-dibromobenzimidazole	0.26	–	0.08	–	0.39	0.13	0.44	0.44	–	–	–	–	–	–
1-α-D-arabinofuranosyl-4,6-dibromobenzimidazole	0.16	–	0.08	–	0.17	0.08	0.37	0.25	–	–	–	–	–	–
1-β-D-ribofuranosyl-5,7-dibromobenzimidazole	0.49	0.15	–	0.09	–	–	–	–	–	–	–	0.14	0.27	0.42

[a] The effect of chlorine substitution in this case was measured relative to benzimidazole and benzimidazole-riboside, and not with respect to the 2-methyl derivatives.
[b] Approximate value only because of overlapping of H(5) and H(6) by the benzene ring signals.

resulting from halogen substitution and changes in the glycosidic torsion angles in the *syn* conformation.

The conformation of 1-(β-D-ribofuranosyl)-benzimidazole in liquid N^2H_3 and ^2H_2O has been previously investigated, applying an analysis of coupling constants to determine the conformations of the sugar ring and the exocyclic carbinol group, and measurements of relaxation times (T_1) of protons, together with the Overhauser effect, to determine the conformation about the glycosidic bond [18]. From these, and our results in DMSO, it appears that the sugar conformation is similar in different solvents, with a population of 55–65% of the form S at room temperature. A somewhat larger variation is observed for the *gauche-gauche* population of the exocyclic group, from 55% to 75%. Both the relaxation methods [18], and the chemical shift procedure employed in this study, indicate a marked preference for the *syn* conformation about the glycosidic bond. The 70% population value for the form *syn* calculated from the relaxation method is probably subject to considerable error, as discussed in detail elsewhere [7]. The chemical shift method in this instance does not provide accurate results, and predicts a *syn* population close to 100%; but it is not capable of excluding the presence of a proportion in the form *anti* of as much as 20%.

The benzimidazole β-riboside analogues are conformationally similar to the corresponding purine β-ribosides. The one clear difference relates to the conformation about the glycosidic bond. Purine nucleosides exhibit a marked preference for the conformation *anti* [6, 7], but not to the exclusion of the form *syn* which, in adenosine, may attain a value of 35%.

Like the β-furanosides, the β-arabinofuranosides of benzimidazoles are conformationally "flexible", the conformer populations of the sugar ring and exocyclic group being analogous to those of α-arabinosides of purines (I. Ekiel, in preparation). The results obtained in this study are, however, not adequate to establish unequivocally the conformation about the glycosidic bond, and this problem is being further pursued. X-ray diffraction data testify to the absence of a single rigid conformation, since in the solid state 5,6-dimethylbenzimidazole-α-D-ribofuranoside is in the *syn* conformation [19], whereas the same nucleoside, as a component of vitamin B_{12}, is in the conformation *anti* [20], while in both instances the sugar conformation is C(3′)*endo*.

Experimental

1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose was a product of Pharma Waldhof (Düsseldorf, GFR), and 1-O-methyl-2,3,5-tri-O-benzoylarabinofuranose was prepared according to the procedure of Fletcher [21].

Melting points (uncorr.) were measured on a Boetius microscope hot stage. Elementary analyses were performed on a Perkin-Elmer instrument Model 240 at the Institute of Organic Chemistry. Ultraviolet absorption spectra were run on a Zeiss (Jena, GDR) VSU-2 spectrophotometer.

Column chromatography was carried out with Servachrome (Heidelberg, GFR) XAD-4, 100–200 μ, and with Merck (Darmstadt, GFR) Kieselgel 60, 70–230 mesh. Thin-layer chromatography was with Merck silica gel 60 F_{254} and PF_{254} plates.

^1H NMR spectra were recorded on a JEOL JNM 4H 100 and on a Brucker-90 at room temperature, using 0.2 M solutions in $(C^2H_3)_2SO$ or $C^2H_3O^2H$, with $(CH_3)_4Si$ as internal standard. The spectra of the 2-α-hydroxybenzyl derivative of benzimidazole riboside were kindly provided by Dr. W. Streicher of Sandoz Forschungsinstitut, Vienna. Chemical shifts were determined to an accuracy of 0.01 ppm and coupling constants to an accuracy of 0.2 Hz. The accuracy is slightly less in the case of the unresolved mixture of the 5-bromo- and 6-bromo- derivatives of benzimidazole riboside.

*4,6-dibromo- and 5,7-dibromo-1-β-*D-ribofuranosylbenzimidazole. A mixture of 2.70 g (9.4 mmol) of 4,6-dibromobenzimidazole, 8 ml hexamethyldisilazane and a crystal of $(NH_4)_2SO_4$ was heated under reflux for 15 min. The solution was brought carefully to dryness and the residue taken up in 60 ml anhydrous ethylene chloride. To this was added 5.04 g (10 mmol) 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranoside and 1.7 ml anhydrous $SnCl_4$, and the mixture stirred for 24 h at room temperature. Chloroform (150 ml) was added followed by 100 ml of saturated $NaHCO_3$, and the mixture vigorously shaken. The resulting emulsion was filtered through Celite-501. The organic phase was removed, washed twice with 100 ml water, and dried over anhydrous Na_2SO_4. It was then concentrated to small volume and loaded on a 40 × 5 cm column of silica gel. Elution was then performed with benzene, followed by 1 → 5% acetone in benzene. The pooled fractions containing the blocked nucleosides were brought to

dryness (foam), and the residue taken up in 100 ml methanolic 0.1 M $NaOCH_3$, and heated under reflux for 30 min. The mixture was brought to dryness, and the residue taken to dryness several times from water to remove methyl benzoate. The residue was crystallized from aqueous methanol to yield 1.41 g (35%) of a mixture of the 4,6-dibromo- and 5,7-dibromoisomeric nucleosides in the ratio 7:3 (from ^{1}H NMR analysis).

A sample of the mixed isomers (120 mg) in 10 ml methanol was deposited on a 45 × 2 cm column of Servachrom XAD-4, and elution carried out with a linear gradient of water and 70% aqueous isopropanol (2 L). The nucleosides were eluted in an asymmetric peak at about 60% isopropanol. The fractions from this peak were divided into three portions. The first two portions were pooled, brought to dryness, and the residue crystallized from a small volume of aqueous isopropanol to yield 60 mg of the hydrate of the 4,6-dibromo nucleoside in the form of needles, m.p. 183–185 °C. Elem. anal.: Calculated for $C_{12}H_{12}O_4N_2Br_2 \cdot H_2O$: C, 33.96%, H, 3.30%; N, 6.60%; Found: C, 33.91%; H, 3.28%; N, 6.66%.

The third portion of the peak was brought to dryness and also crystallized from aqueous isopropanol to yield 45 mg of the hydrate of the 5,7-dibromo derivative in the form of needles, m.p. 159–162 °C. Elem. anal.: Calculated for $C_{12}H_{12}O_4Br_2 \cdot H_2O$: C, 33.96%; H, 3.30%; Å, 6.60%; Found: C, 34.13%; H, 3.29%; N. 6.53%.

4,6-dibromo-1-(α-D-arabinofuranosyl)-benzimidazole. A solution consisting of 3.48 g (10 mmol) of silylated 4,6-dibromobenzimidazole, 4.77 g (10 mmol) of 1-O-methyl-2,3,5-tri-O-benzoylarabinofuranose, and 3 ml (26 mmol) $SnCl_4$ in 50 ml of 1,2-dichloroethane was refluxed for 6 h and then left overnight at room temperature. The reaction mixture was then worked up as for the synthesis of 4,6-dibromo-1-(β-D-ribofuranosyl)-benzimidazole. The benzoylated nucleoside, contaminated with unreacted sugar, was dissolved in benzene and deposited on a column of 500 g of Merck (Darmstadt, GFR) Kieselgel 60, 70–230 mesh. Elution was carried out with benzene solutions of isopropanol with increasing concentrations of the latter: 2% (500 ml), 5% (500 ml), and 10% (1000 ml), and collection of 25-ml fractions. The fractions containing the nucleoside (60–72) were pooled, brought to dryness under reduced pressure and debenzoylated in methanolic sodium methoxylate as above. The resulting solution was brought to neutrality with acetic acid and brought to dryness. The resulting oily residue was extracted several times with chloroform, and crystallized from aqueous ethanol to yield 612 mg (15%) of the free nucleoside in the form of fine needles, m.p. 189–191 °C. Elem. anal.: Calculated for $C_{12}H_{12}N_2O_4Br_2$: C, 35.33%; H, 2.94%; N, 6.86%; Found: C, 35.24%; H, 2.81%; N, 6.71%.

5,6-dichloro-2-methyl-1-β-D-ribofuranosylbenzimidazole. A mixture of 370 mg (1.95 mmol) of 5,6-dichloro-2-methylbenzimidazole [9], 1.05 g (2.1 mmol) 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose, and 10 mg of toluenosulfonic acid hydrate, was ground to a fine powder. The mixture was heated under vacuum (water pump) on an oil bath (160–165 °C) for 40 min. The mixture was cooled, taken up in 10 ml chloroform, and subjected to chromatography on Merck PF_{254} silica gel plates with chloroform containing 1% methanol (R_f of blocked nucleoside 0.60). The band corresponding to the nucleoside was eluted with a 1:1 mixture of chloroform-isopropanol. The eluate was brought to dryness, and the residue taken up in 30 ml methanolic 0.1 M $NaOCH_3$. This was heated under reflux for 15 min and then brought to dryness. The residue was taken up in 20 ml water, the solution brought to pH 5 with acetic acid, and brought to dryness several times from water to remove methyl benzoate. The final residue was dissolved in 20 ml hot water, and crystallized at room temperature to yield 160 mg (25%) of bipyramids, m.p. 193°–196 °C. Recrystallization from water gave an analytical sample, m.p. 194°–195 °C. Elementary analysis: Calculated for $C_{13}H_{14}O_4N_2Cl_2$: C, 47.00%; H, 4.24%; N, 8.43%; Found: C, 46.84%; H, 4.21%; N, 8.36%.

5,6-dichloro-(2,3-O-isopropylidene-1-β-D-ribofuranosyl)benzimidazole. To 1.05 g (3.3 mmol) 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole in 25 ml acetone and 5 ml 2,2-dimethoxypropane was added, portionwise and with constant stirring over the course of 1 h at room temperature, 2.1 g (11 mmol) *p*-toluenosulfonic acid. Stirring was continued for an additional 2 h. The mixture was then poured into 150 ml water, the pH brought to 8 with triethylamine, followed by heating, and addition of 2–3 ml methanol to produce a clear solution. Storage at room temperature led to formation of crystals in the form of needles, (1.05 g, 90% yield), chromatographically homogeneous. An analytical sample was obtained by recrystallization from 33% aqueous meth-

anol, m. p. 168 °C. Elementary analysis: Calculated for $C_{15}H_{15}N_2O_4Cl_2$: C, 50.26%; H, 4.22%; N, 7.82%; Found: C, 49.94%; H, 4.33%; N, 7.76%.

5,6-dichloro-1-(5-chloro-5-deoxy-β-D-ribofuranosyl) benzimidazole. To a solution of 1.32 g (7 mmol) 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole in 13.5 ml of hexamethylphosphoric triamide was added 2.64 ml (36.5 mmol) freshly distilled thionyl chloride. Chromatography showed disappearance of starting substance after 1 h at room temperature. The mixture was poured into 200 ml water and filtered. The filtrate was loaded on a 2 × 20 cm column of Dowex 50 W × 8 (H^+), and the column washed with 500 ml of methanol-water-conc. NH_4OH 2:1:1, v/v). The effluent was brought to dryness and the residue crystallized from water to yield fine needles (0.94 gm, 68%), m. p. 210–211 °C. Elementary analysis: Calculated for $C_{12}H_{11}N_2O_3Cl_2$: C, 42.41%, H, 3.26%; N, 8.25%; Found: C, 42.53%; H, 3.15%; N, 8.14%.

Acknowledgements

This investigation profited from the support of the Polish Academy of Sciences (Project 09.3.1), the Polish National Cancer Research Program (Project PR-6), and the Ministry of Higher Education, Science and Technology (MR-I/5).

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