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Note

Thin-layer chromatography of purine bases and deoxyribonucleoside analogues. IV

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In a study of the specificity of *Lactobacillus helveticus* nucleoside deoxyribosyltransferase[1], it was observed that 5-aminoimidazole carboxamide was a substrate[2], showing that apart from the imidazole ring a complete fused six-membered ring was not necessary. On the other hand, imidazole did not react, suggesting a role for some substituents at the 4- and/or 5-position in imidazole. In further studies on the specificity of nucleoside deoxyribosyltransferase I and II, we measured the R_F values of a new series of purine analogues and imidazole derivatives in the six developing solvents already used in our previous studies[3]. The R_F values of nine newly synthesized deoxyribonucleosides were also measured.

EXPERIMENTAL

Materials

Most of the analogues were commercial compounds purchased from Aldrich (Milwaukee, Wisc., U.S.A.), Sigma (St. Louis, Mo., U.S.A.) or ICN (Cleveland, Ohio, U.S.A.). When necessary, they were purified by ascending chromatography on Whatman No. 3MM paper using one or several of the developing solvents described here. 4-Hydroxy-1,2,5-thiadiazolo-[3,4-*c*]-pyrimidine, 7-hydroxy-1,2,5-thiadiazolo-[3,4-*b*]-pyrimidine and 7-hydroxy-1,2,5-thiadiazolo-[3,4-*d*]-pyrimidine were prepared in the Department of Natural Products of the Organic Chemical Laboratory, State University, Utrecht, The Netherlands.

Radioactive donors

Deoxyribosyl-[^{14}C]thymidine was prepared as described previously[3] using the transfer reaction:

$$\text{Thy} + [\text{U-}^{14}\text{C}]\text{dIno} \rightarrow \text{deoxyribosyl-}[^{14}\text{C}]\text{Thd} + [\text{U-}^{14}\text{C}]\text{Hyp}$$

catalysed by nucleoside deoxyribosyltransferase II. [U-^{14}C]dIno was obtained by enzymatic deamination of [U-^{14}C]dAdo with adenosine aminohydrolase from intestine mucosa (E.C. 3.5.4.4a; Sigma, 200 U/mg).

Deoxyribonucleoside analogues

The deoxyribonucleoside analogues were prepared from the corresponding

TABLE I

R_F VALUES FOR PURINE BASE ANALOGUES AND DEOXYRIBONUCLEOSIDES

Solvents: I = ethyl acetate–water–formic acid (60:35:5); II = *tert.*-butanol–methyl ethyl ketone–water–12 *N* ammonia (40:30:20:10); III = *n*-butanol–water–12 *N* ammonia (86:10:5); IV = isopropanol–water–12 *N* ammonia (70:20:10); V = 5% Na_2HPO_4 solution (pH 9.3) saturated with isoamyl alcohol; VI = distilled water (pH 6–7). Fluorescence: B = blue, Y = yellow, G = green, O = orange, P = purple, D = dark, p = pale, s = streaking, ss = important streaking, sss = very important streaking.

Compound	*Solvent*					
	I	*II*	*III*	*IV*	*V*	*VI*
3-Amino-4-carbethoxypyrazole	0.92 (pB)	0.97 (pB)	0.84 (Bs)	0.93 (B)	0.64 (pB)	0.71 (B)
4-Amino-6-hydroxypyrazolo-[3,4-*d*]-pyrimidine	0.02 (B)	0.28 (Bss)	0.10 (B)	0.29 (B)	0.40 (B)	0.26 (B)
4-Amino-6-hydroxypyrazolo-[3,4-*d*]-pyrimidine deoxyriboside	0.02	0.51	0.09	0.47	0.74	0.68
5-Aminoindazole	0.08 (B)	0.90 (B)	0.77 (Bs)	0.80 (B)	0.38 (B)	0.28 (Bs)
6-Aminoindazole	0.47 (Bss)	0.93 (B)	0.71 (B)	0.80 (B)	0.30 (B)	0.40 (B)
7-Aminoindazole	—	0.96 (B)	0.84 (B)	0.90 (B)	0.35 (B)	0.44 (B)
3-Aminopyrazole	0.25 (ss)	—	0.53 (D)	0.75 (D)	0.76 (pB)	0.75 (D)
3-Aminopyrazole deoxyriboside	0.01	0.44	0.15	0.44	0.82	0.80
1-Hydroxyisoguanine	0.03 (B)	0.13 (B)	0.01 (B)	0.14 (B)	0.45 (B)	0.60 (B)
1-Hydroxyisoguanine deoxyriboside	0.02	0.20	0.02	0.22	0.70	0.65
4-Hydroxymethylimidazole	—	—	—	—	—	—
4-Hydroxymethylimidazole deoxyriboside	0.00	0.80	0.45	0.80	0.86	0.68
2-Hydroxy-6-methylpurine	0.08 (B)	0.48 (B)	0.15 (B)	0.51 (B)	0.62 (B)	0.67 (B)
2-Hydroxy-6-methylpurine deoxyriboside	0.04	0.48	0.15	0.53	0.80	0.82
2-Hydroxypurine	0.06 (B)	0.50 (B)	0.10 (B)	0.44 (B)	0.63 (B)	0.65 (B)
2-Hydroxypurine deoxyriboside	0.02	0.50	0.12	0.48	0.78	0.79
4-Nitroimidazole	—	0.92 (D)	0.51 (D)	0.85 (D)	0.72 (pB)	0.78 (D)
1,2-Naphthotriazole	0.90 (BYsss)	0.92 (sss)	0.97 (Yss)	0.85 (sss)	0.11 (Y)	0.15 (B)
4-Phenylimidazole	0.37 (pB)	0.56 (D)	0.87 (pB)	—	0.32 (pB)	0.32 (pB)
4-Phenylimidazole deoxyriboside	0.26 (ss)	0.95	0.85 (ss)	0.92	0.43	0.48
Pyrimidazole	0.09 (B)	0.97 (B)	0.86 (Bs)	0.91 (B)	0.57 (B)	0.32 (Bs)
4-Hydroxy-1,2,5-thiadiazolo-[3,4-*c*]-pyridine	0.95 (GY)	0.86 (G)	0.75 (GY)	0.84 (G)	0.66 (GY)	0.70 (GY)
7-Hydroxy-1,2,5-thiadiazolo-[3,4-*b*)-pyridine	0.60 (Ys)	0.75 (B)	0.40 (B)	0.73 (pB)	0.55 (Y)	0.73 (pB)
7-Hydroxy-1,2,5-thiadiazolo-[3,4-*b*]-pyridine deoxyriboside	0.41	0.81	0.60	0.73	0.81	0.76
7-Hydroxy-1,2,5-thiadiazolo-[3,4-*d*]-pyrimidine	0.63 (Os)	0.65 (O)	0.25 (O)	0.67 (O)	0.57 (B)	0.74 (Y)
7-Hydroxy-1,2,5-thiadiazolo-[3,4,*d*]-pyrimidine deoxyriboside	—	0.86	0.60	0.80	0.82	0.78

bases using deoxyribosyl-[^{14}C]thymidine as described previously[3]. Some of the reactions were very slow and the small amounts of products formed gave only faint radioactive spots which could be confused with trace impurities occasionally present in the radioactive reference compounds. Moreover, in solvents V and VI some deoxyribonucleosides were expected to migrate in the region of thymidine itself. To overcome these difficulties, the deoxyribonucleosides were also prepared (a) with low specific radioactivity [U-^{14}C]deoxyadenosine using deoxyribosyl donor and acceptor in equal amounts and incubating with nucleoside deoxyribosyltransferase II for 15 min, 2 h and 4 h, and (b) with [U-^{14}C]deoxyadenosine at the specific radioactivity of 300 mCi mmole^{-1} in a concentration approximately one sixtieth that of the acceptor base. In the latter procedure the transfer reaction was very sensitive, but presented the risk of product formation from trace impurities left in the acceptor base even after purification. All doubtful results were discarded.

Chromatography

All conditions given in the preceding paper[3] were used without modification.

RESULTS

The R_F values are given in Table I. Each value is the average of five to seven independent migrations. As in the previous report, fluorescence and occasional streaking are indicated.

4-Hydroxymethylmidazole was not detected at the concentration used in any of the solvents, but it gave a deoxyribonucleoside that could be detected on the autoradiograph. Indazole was undetectable and gave no significant reaction under the conditions used.

TABLE II

R_{dThd} VALUES FOR PURINE BASE ANALOGUES AND DEOXYRIBONUCLEOSIDES

$R_{dThd} = R_F$ value relative to thymidine. Solvents as in Table I.

Compound	*Solvent*				
	II	*III*	*IV*	*V*	*VI*
3-Amino-4-carbethoxypyrazole	1.08	1.58	1.19	0.77	0.80
4-Amino-6-hydroxypyrazolo-[3,4-*d*]-pyrimidine	0.41	0.19	0.41	0.46	0.29
5-Aminoindazole	—	1.32	1.06	0.45	0.24
6-Aminoindazole	1.20	1.39	1.05	0.34	0.44
7-Aminoindazole	1.23	1.59	1.11	0.39	0.49
3-Aminopyrazole	—	1.00	0.96	0.90	0.83
1-Hydroxyisoguanine	0.17	0.00	0.17	0.57	0.66
2-Hydroxy-6-methylpurine	0.62	0.26	0.67	0.74	0.74
2-Hydroxypurine	0.60	0.19	0.59	0.79	0.72
7-Hydroxy-1,2,5-thiadiazole-[3,4-*b*]-pyridine	0.96	0.81	0.96	0.65	0.82
7-Hydroxy-1,2,5-thiadiazolo-[3,4-*d*]-pyrimidine	0.84	0.56	0.91	0.69	0.83
4-Nitroimidazole	1.06	1.08	1.06	0.82	0.87
1,2-Naphthotriazole	—	—	—	0.12	0.15
4-Phenylimidazole	0.64	1.81	—	0.36	0.36
Pyrimidazole	1.21	1.59	1.20	0.67	0.36

TABLE III

R_{Ade} VALUES FOR PURINE BASE ANALOGUES AND DEOXYRIBONUCLEOSIDES

$R_{Ade} = R_F$ value relative to adenine. Solvents as in Table I.

Compound	*Solvent*				
	II	*III*	*IV*	*V*	*VI*
3-Amino-4-carbethoxypyrazole	—	3.50	1.73	1.78	2.24
4-Amino-6-hydroxypyrazolo-[3,4-*d*]-pyrimidine	0.51	0.27	0.48	1.17	0.89
4-Amino-6-hydroxypyrazolo-[3,4-*d*]-pyrimidine deoxyriboside	1.00	0.32	0.81	2.06	1.91
5-Aminoindazole	1.60	2.20	1.60	0.87	0.97
6-Aminoindazole	1.80	2.29	1.50	1.00	1.09
7-Aminoindazole	1.90	2.84	1.73	1.18	1.20
3-Aminopyrazole	—	—	—	2.11	—
3-Aminopyrazole deoxyriboside	0.86	0.63	0.85	2.25	2.66
1-Hydroxyisoguanine	0.25	0.13	0.25	1.50	—
1-Hydroxyisoguanine deoxyriboside	0.42	0.16	0.40	1.97	1.82
2-Hydroxy-6-methylpurine	0.85	0.48	0.96	1.64	1.88
2-Hydroxy-6-methylpurine deoxyriboside	1.00	0.52	1.00	2.08	2.29
2-Hydroxypurine	0.81	0.39	0.83	1.59	1.88
2-Hydroxypurine deoxyriboside	0.94	0.39	0.94	1.97	2.26
4-Hydroxy-1,2,5-thiadiazolo-[3,4-*c*]-pyridine	1.69	2.50	1.45	1.83	2.00
7-Hydroxy-1,2,5-thiadiazolo-[3,4-*b*]-pyridine	1.33	1.33	1.26	1.69	2.03
7-Hydroxy-1,2,5-thiadiazolo-[3,4-*b*]-pyridine deoxyriboside	1.59	2.00	1.26	2.25	2.17
7-Hydroxy-1,2,5-thiadiazolo-[3,4-*d*]-pyrimidine	1.30	0.90	1.21	1.71	2.03
7-Hydroxy-1,2,5-thiadiazolo-[3,4-*d*]-pyrimidine deoxyriboside	—	1.93	—	2.41	2.14
4-Nitroimidazole	1.60	1.55	1.45	2.03	2.03
4-Phenylimidazole	—	—	1.65	0.85	0.76
Pyrimidazole	1.88	3.75	1.73	1.86	—

Thymidine, adenosine and deoxyadenosine were systematically employed as references on each plate and were used to calculate the corresponding R_X values[4] given in Tables II and III.

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