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Note

Thin-layer chromatography of purine and pyrimidine bases and deoxyribonucleoside analogues. II.

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Thin-layer chromatography, in parallel with other techniques, was used in our study of the transfer reaction of the deoxyribosyl moiety catalyzed by the enzyme trans-N-deoxyribosylase (E.C. 2.4.2.6). This technique, allowing the separation of reaction products, was particularly useful in defining the specificity of the enzyme[1]. The R_F values of a first series of purine and pyrimidine analogues, as well as a number of deoxyribonucleosides, have been described previously[2]. Some new results have since been obtained under the conditions previously described. The same six developing solvents have been used in the present study, and the R_F values of 23 purine analogues and 27 pyrimidine analogues, as well as 11 deoxyribonucleosides prepared with the transfer enzyme, are reported here.

EXPERIMENTAL

Materials

Analogues. The analogues were purchased from Sigma (St. Louis, Mo., U.S.A.), Fluka (Milan, Italy), Aldrich (Milwaukee, Wisc., U.S.A.), Cyclo Chem. (Los Angeles, Calif., U.S.A.) or Mann Labs. (New York, N.Y., U.S.A.). Some were found too impure to be used as received, and were purified by ascending chromatography on Whatman III paper using one or several of the developing solvents described here. Another series (Table I), synthesized in Dr. Salemink's Laboratory, was used for specificity[1]. Hydroxypyrrolopyrimidine was a gift from Dr. Baltzly (Burroughs Wellcome, Tuckahoe, N.Y., U.S.A.).

Deoxyribonucleosides. These compounds were prepared from the corresponding base and thymidine labelled on the sugar moiety only, according to:

$$B_A + TdR^* \xrightarrow{TNDR} B_AdR^* + T$$

B_A = a base analogue; TdR* = thymidine-^{14}C-deoxyribosyl; TNDR = trans-N-deoxyribosylase; T = thymine.

The enzymic synthesis of thymidine-^{14}C-deoxyribosyl and the precautions used to check the formation of the deoxyribonucleoside analogues (B_AdR^*) have also been described previously. All doubtful results were discarded.

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 saturated with isoamyl alcohol; VI, distilled water (pH ≈ 6–7). Fluorescence: B = blue; G = green; Y = yellow; P = purple. s = Streaking.

Compound	*Solvents*					
	I	*II*	*III*	*IV*	*V*	*VI*
2-amino-4-pteridinol	0.04	0.31	0.04	0.34	0.47 B, s	0.53 B, s
4-aminopyrazolo[3,4-*d*]pyrimidine	0.11 B	0.78 s	0.45 s	0.70	0.30 B	0.29
4-aminopyrazolo[3,4-*d*]pyrimidine deoxyriboside	0.10	0.87	—	0.79	0.55	0.60
benzimidazole	0.24 B, s	0.93 B	0.88 B	0.94 B	—	0.37 B, s
benzimidazole deoxyriboside	0.17	0.92	0.79	0.88	0.57	0.61
5,6-dimethylbenzimidazole	0.34 B, s	—	0.89 B	0.96 B	—	0.12 B
5,6-dimethylbenzimidazole deoxyriboside	0.29	0.93	0.83	0.90	0.34	0.36
4-hydroxypyrazolo[3,4-*d*]pyrimidine	0.23	0.52	0.17	0.58	0.53	0.56
hydroxypyrrolopyrimidine**	0.46	0.75	0.45	0.70	0.46	0.50
1-methylxanthine	0.35	0.58	0.03	0.50	0.51	0.56
3-methylxanthine	0.28	0.60	0.05	0.52	0.48	0.57
7-methylxanthine	0.22	0.50	0.04	0.40	0.58	0.58
9-methylxanthine	0.07	0.54	0.03	0.45	0.58	—
xanthine-3-(β,D-deoxyriboside)	0.14	0.72	0.05	0.58	0.62	0.66
1-deazaadenine, 2 HCl*	0.05 B	0.80 B	0.54 B	—	0.30 B	— s
3-deaza-2-aminopurine*	0.03 B	0.67 B	0.39 B	0.68 B	0.38 B	0.14 B, s
1-deaza-8-azaadenine*	0.10 B	0.40 B	0.12 B	0.50 B	0.32	0.36 B
3-deaza-8-azaadenine*	0.08 B, s	0.49 B	0.15 B	0.55 B	0.44 B	0.41 B
3-deaza-8-azaadenine deoxyriboside	0.03 B	0.87 B	0.49 B	0.77 B	0.55 B	— s
3-deaza-8-aza-2-aminopurine*	0.20 B	—	0.10 B, s	0.47 B, s	0.30 B	0.41 B
3-deaza-8-aza-2-aminopurine deoxyriboside	0.13 B	0.83 B	0.44 B	0.78 B	0.59 B	0.61
3-deaza-6-mercaptopurine*	0.22 Y	0.73 Y	0.34 Y	0.60	0.46 Y	0.43 Y
1,2,5-thiadiazolo[3,4-*b*]pyridine*	0.00	0.00	0.85 B	—	—	0.74
7-chloro-1,2,5-thiadiazolo[3,4-*b*]pyridine*	0.05 B	—	—	—	0.55 Y	0.62 B
4-hydroxy-1,2,5-thiadiazolo[3,4-*c*]pyridine*	0.80 B	0.90 B	0.67 B	0.82 B	0.62 B	0.65 B
1,2,5-selenadiazolo[3,4-*b*]pyridine*	0.84 P	0.86 P	0.70 P	0.78 B	0.60	0.64 P
4-chloro-1,2,5-selenadiazolo[3,4-*c*]pyridine*	0.50 Y	0.80 Y	0.50 Y	0.67 Y	0.52 Y	0.55 Y
7-chloro-1,2,5-selenadiazolo[3,4-*b*]pyridine*	0.94 s	0.85 s	0.79 s	0.81 s	0.45 P	0.49
7-hydroxy-1,2,5-selenadiazolo[3,4-*b*]pyridine*	0.25	0.45 Y	0.24 Y	0.50 Y	0.52 Y	0.55 Y

* Synthesized in Dr. Salemink's laboratory.

** A gift from Dr. Baltzly.

TABLE II

R_F VALUES FOR PYRIMIDINE 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 saturated with isoamyl alcohol; VI, distilled water (pH ≈ 6–7). Fluorescence: B = blue; Y = yellow. Streaking: s.

Compounds	*Solvents*					
	I	*II*	*III*	*IV*	*V*	*VI*
2-amino-5-nitropyrimidine	—	0.90 B	0.66	0.78	0.56	0.60
4-aminopyrimidine	0.03	0.81	0.65	0.78	0.63	—
5-aminouracil	0.03 B	0.34 B	0.09 B	0.08 Y	0.73 B	0.70 B
6-azathymine	0.76	0.53	0.13	—	0.75	0.78
6-azathymine deoxyriboside	—	—	0.17	—	—	—
5-carboxycytosine	—	0.41	—	0.51	—	0.80
5-carboxyuracil	0.36	0.11	0.00	0.11 B	0.74 B	0.90
2-chloro-4,5-diaminopyrimidine	0.67 B	0.90 B	0.66 B	0.85 B	0.50 B	0.53 B
2,3-diaminopyridine	0.13	0.85 B	0.62 B	0.79 B	0.62 B	0.15 B, s
3,4-diaminopyridine	0.05 B	0.80 B	0.52 B	0.73 B	0.76 B	0.06 B
5-diazouracil	0.23	0.72 B	0.19	0.62	—	—
1,3-dimethyluracil	0.45 s	0.60	0.09	0.63	0.76 B	0.77
5,6-dimethyluracil	—	0.78	0.57	0.76	0.68	0.73
5-fluorouracil	0.49	0.55	0.13	—	—	0.76
5-hydroxymethyluracil	0.12	—	0.12	0.54	0.80	0.77
5-hydroxymethyluracil deoxyriboside	0.06	0.44	0.17	0.52	—	—
2-hydroxypyrimidine	—	—	—	0.66 B	—	—
4-hydroxypyrimidine	0.33	0.53	0.19	0.66	0.78	0.80
5-methylcytosine	0.05	—	0.29	0.65	0.69	—
6-methyl-2-thiouracil	— s	0.58 s	0.21	0.65 s	0.61 B	0.63 s
6-methyluracil	0.35	0.56	—	0.63	0.73	0.75
5-nitrouracil	0.43	0.71	0.14	—	0.56	0.82
6-phenyl-2-thiouracil	0.90 Y, s	0.82 Y	0.38 Y	0.80 Y	0.25 Y	0.40 Y, s
6-propyl-2-thiouracil	—	0.85	0.57	0.87	0.64	0.67
pyridazine	0.73	0.90	—	0.89	—	0.86
5-sulfoaminouracil	0.00 B	0.32 B	0.00 B	0.37 B	0.80 B	0.91 B
2-thiocytosine	0.08	0.56	0.26	0.52	0.61	0.61
2-thiocytosine deoxyriboside	0.08	0.76	—	—	0.67	0.66
2-thiouracil	0.59	0.57	0.17	0.58	0.65 B	0.63
2-thiouracil deoxyriboside	—	0.54	0.21	0.52	0.74	0.67

Chromatography

The substances were chromatographed on standard thin-layer plates covered with cellulose MN 300 (thickness: 250 μm). The plates were carefully cleaned before use but otherwise no activating treatment was performed. The development was carried out at a constant temperature between 22 and 24° in air-tight glass jars. All solvents were freshly prepared and an equilibration period of 4–6 h was allowed. The radioactive substances were located by autoradiography (Kodirex film). The non-radioactive substances were visualized under UV light (254 nm) either by extinction of the cellulose fluorescence or by their own characteristic fluorescence.

RESULTS

R_F values for the different compounds are given in Tables I and II. Each value is the average of 6–10 independent migrations. In the case of solvents II and IV, some extreme values were discarded for reasons briefly discussed below. In those cases where most of the R_F values were scattered, or the location of the substance could not be ascertained, no indications are given.

Two extra items of information, fluorescence and streaking, are also reported. Fluorescence may be a useful characteristic when a given compound must be identified in a mixture. Streaking is also a characteristic of some of the substances studied here. This latter phenomenon is most frequently encountered in solvent I, and whenever possible it is advisable to avoid using this solvent.

In solvents II and III, the R_F values were sometimes very broadly distributed as can be seen from results given in Tables III and IV (1st and 2nd columns), in which two extreme values for two independent migrations are recorded. (These values were not normally taken into consideration in the computation of the corresponding average R_F values given in Tables I and II). Such a scattering of the R_F values may be due to a special sensitivity to temperature and solvent composition, and possibly some influence of the migration time. To overcome this drawback we suggest adding

TABLE III

COMPARISON OF VALUES OF R_F AND R_{Td} IN SOLVENT II

Compound	R_F		*Scattering**	R_{Td}		*Scattering**
	min.	*max.*		*min.*	*max.*	
2-amino-4-pteridinol	0.29	0.32	0.098	0.38	0.40	0.050
4-aminopyrazolo[3,4-*d*]pyrimidine	0.76	0.84	0.100	1.01	1.04	0.029
6-azathymine	0.51	0.63	0.210	0.76	0.78	0.026
2,3-diaminopyridine	0.83	0.91	0.092	1.26	1.27	0.008
3,4-diaminopyridine	0.75	0.85	0.125	1.15	1.17	0.017
4-hydroxypyrazolo[3,4-*d*]pyrimidine	0.50	0.58	0.148	0.68	0.70	0.029
2-hydroxypyrimidine	0.52	0.66	0.237	0.67	0.76	0.126
5-nitrouracil	0.64	0.71	0.104	0.98	0.99	0.010
2-thiocytosine	0.51	0.71	0.328	0.78	0.81	0.038
2-thiouracil	0.57	0.71	0.218	0.78	0.81	0.038

* Scattering expressed as $\Delta R_F/R_F$ or $\Delta R_{Td}/R_{Td}$.

TABLE IV
COMPARISON OF VALUES OF R_F AND R_{Td} IN SOLVENT IV

Compound	R_F		*Scattering**	R_{Td}		*Scattering**
	min.	*max.*		*min.*	*max.*	
2-amino-4-pteridinol	0.33	0.37	0.114	0.48	0.50	0.041
4-aminopyrazolo[3,4-*d*]pyrimidine	0.68	0.79	0.150	0.99	0.99	0.000
6-azathymine	0.55	0.69	0.226	0.77	0.80	0.038
2,3-diaminopyridine	0.77	0.88	0.133	1.02	1.08	0.057
3,4-diaminopyridine	0.72	0.83	0.142	0.97	1.01	0.040
4-hydroxypyrazolo[3,4-*d*]pyrimidine	0.56	0.65	0.149	0.81	0.81	0.000
2-hydroxypyrimidine	0.63	0.70	0.105	0.84	0.84	0.000
5-nitrouracil	0.55	0.68	0.211	0.81	0.81	0.000
2-thiocytosine	0.51	0.64	0.226	0.69	0.77	0.110

* Scattering expressed as $\Delta R_F/R_F$ or $\Delta R_{Td}/R_{Td}$.

thymidine as a reference substance and expressing the mobility of each substance as the R_{Td} value[3]. These values are given in Tables III and IV. The much better reproducibility is seen from a comparison of the scatter shown in the third and sixth column of Tables III and IV. With this technique, solvents II and IV are also very valuable systems for identifying the purine and pyrimidine bases and deoxyribonucleosides.

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