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**Note**

# Thin-layer chromatography of pyrimidine bases and deoxyribonucleoside analogues. III

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An earlier study[1] of the specificity of nucleoside deoxyribosyltransferase (EC 2.4.2.6) in catalyzing the transfer of deoxyribosyl between purine or pyrimidine bases was carried out as described previously[2], using thin-layer chromatography (TLC), to separate the reaction products for identification. Many pyrimidine bases were found to be competent acceptors, and practical rules could be deduced to enable one to predict whether a given base is a suitable substrate. However, some uncertainty remained with regard to the role of the N-3 site, and further studies were necessary to learn more about the positioning of the base on the enzyme at the second stage of this ping-pong bi-bi type reaction[3]. The present paper reports the $R_F$ values of a new series of pyrimidine analogues and the corresponding deoxyribonucleosides in the six developing solvents used in the previous studies[4].

## EXPERIMENTAL

### *Materials*

*Analogues.* The analogues were purchased from Cyclo Chem. (Los Angeles, Calif., U.S.A.), Aldrich (Milwaukee, Wisc., U.S.A.), Fluka (Milan, Italy) or Schwarz (Orangeburg, N.Y., U.S.A.) When necessary, they were purified by ascending chromatography on Whatman III paper using one or several of the developing solvents described here.

*Radioactive donors.* Deoxyribosyl-[$^{14}$C]thymidine was prepared from [U-$^{14}$C]-deoxyinosine obtained by deamination of [U-$^{14}$C]deoxyadenosine (Radioelements, CEA-Saclay, France). The latter reaction was catalyzed by adenosine aminohydrolase from intestine mucosa (EC 3.5.4.4.a; Sigma, St. Louis, Mo., U.S.A.; 200 units/mg) in a 0.05 *M* phosphate buffer (pH 7.5)[5]. The transfer reaction:

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

was run in a 0.01 *M* phosphate buffer (pH 6.0) in the presence of nucleoside deoxyribosyltransferase purified as previously[3]. Xanthine oxidase (EC 1.2.3.2; Boehringer, Mannheim, G.F.R.) was added to oxidize hypoxanthine and displaces the reaction

toward the formation of deoxyribosyl-[$^{14}$C]thymidine. The overall yield was often better than 75%.

*Deoxyribonucleoside analogues.* The deoxyribonucleoside analogues were prepared from the corresponding bases using thymidine labelled in the sugar moiety only as described in the preceding paper of this series[4]. The reaction was catalyzed by nucleoside-deoxyribosyltransferase. However, some deoxyribonucleosides were expected to migrate in the region of thymidine itself, in particular with solvents V and VI, making the interpretation of the results doubtful in a few instances. Hence, the deoxyribonucleosides were also prepared using uniformly labelled deoxyadenosine according to:

$$\text{Pyr} + [U^{14}C]\text{dAdo} \rightarrow \text{deoxyribosyl-}[^{14}C]\text{Pyd} + [U^{14}C]\text{Ade}$$

The radioactive adenine formed and the residual deoxyadenosine have $R_F$ values significantly different from those of most of the expected products. The precautions described in the preceding paper[4] were again used to check the formation of the deoxyribonucleoside analogues.

*Chromatography*

The substances were chromatographed on standard thin-layer plates covered with cellulose MN 300 (thickness 250 $\mu$m) (Macherey, Nagel & Co., Düren, G.F.R.). No activating treatment was performed. The development was carried out at a constant temperature of 21–22° in air-tight jars. All of the 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 (bases) were visualized under UV light (254 nm) either by extinction of the cellulose fluorescence or by their own characteristic fluorescence.

## RESULTS

The $R_F$ values are given in Table I. Each value is the average from 5–7 independent migrations. The characteristic fluorescence is also indicated. Since changes sometimes occurred upon aging, fluorescence was observed at a time when the plate was completely dry, but not later than 24 h after chromatography. Streaking, as previously mentioned, is also a characteristic of substances and solvents and this is again noted to help the selection of a suitable solvent.

2,4-Dimethoxypyrimidine, 4-ethoxy-2-hydroxypyrimidine, dihydrothymine and dihydrouracil were not detectable at the concentration used and none formed the corresponding deoxyribonucleosides. $R_F$ values for 2-hydroxypyrimidine, 2-hydroxy-4-methylpyrimidine and 4,6-dihydroxypyrimidine were given previously[4] and are repeated here to accompany their deoxyribosides. $R_F$ values in various solvents not previously available are also shown for 5,6-dimethyluracil.

As illustrated before[4], $R_x$ values[6] may be more reliable when large $R_F$ fluctuations are recorded from one experiment to another. Thymidine was therefore employed as a reference on each plate and used to compute a $R_{Td}$ value in solvents

TABLE I

$R_F$ 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 (4:3:2:1); III = 1-butanol–water–12 *N* ammonia (86:10:5); IV = 2-propanol–water–12 *N* ammonia (70:20:10); V = 5% $Na_2HPO_4$ solution (pH 9.0) saturated with isoamyl alcohol; VI = distilled water (pH 6–7). Fluorescence: B = blue, Y = yellow, G = green, P = purple, D = dark. s = Streaking.

| *Compound* | *Solvent* | | | | | |
|---|---|---|---|---|---|---|
| | *I* | *II* | *III* | *IV* | *V* | *VI* |
| 4-Amino-6-hydroxypyrimidine | 0.24 D | 0.48 D | 0.21 D | 0.47 D | 0.62 D | 0.65 D |
| 4-Amino-6-hydroxypyrimidine deoxyriboside | — | — | 0.28 | 0.60 | 0.71 | 0.75 |
| 4-Aminopyrimidine | 0.05 D | 0.85 D | 0 65 D | 0 80 D | 0 62 D | 0 35 D |
| 2,4-Dihydroxy-3-methylpyrimidine | 0 75 D | 0 82 D | 0 60 D | 0 78 D, s | 0 81 D | 0 83 |
| 2,4-Dihydroxy-3-methylpyrimidine deoxyriboside | 0 66 ss | 0 85 | 0.66 s | 0.89 | 0.84 | 0.85 |
| 4,6-Dihydroxy-2-methylpyrimidine | 0.14 D, s | 0.47 D | 0.08 D, s | 0.51 D | 0.79 D | 0.86 |
| 3,6-Dihydroxypyridazine | 0.52 B | 0.47 B | 0.08 B | 0.49 B | 0.80 B | 0.84 B |
| 3,6-Dihydroxypyridazine deoxyriboside | 0.53 ss | 0.28 | 0.61 | 0.68 | 0.88 | 0.83 |
| 4,6-Dihydroxypyrimidine | 0.16 D | — | 0.05 D | 0.48 D | 0.75 D | 0.87 D |
| 4,6-Dihydroxypyrimidine deoxyriboside | — | 0.60 | 0.15 | 0.68 | 0.90 | 0.92 |
| 1,5-Dimethyluracil | 0.78 D, ss | 0.79 D | 0.61 D | 0.78 D | 0.76 D | 0.80 D |
| 5,6-Dimethyluracil | 0.63 D, s | 0.79 D | 0.58 D | 0.74 D | 0.68 D | 0.74 D |
| 4-Ethoxy-2-hydroxypyrimidine | 0.82 | 0.83 | 0.76 | 0.87 D | 0.80 | 0.86 D |
| 2-Hydroxy-4-methylpyrimidine hydrochloride | 0.12 B | 0.62 B | 0.36 B | 0.65 B | 0.84 B | 0.88 B |
| 2-Hydroxy-4-methylpyrimidine deoxyriboside | — | 0.95 | 0.55 | 0.84 | 0.85 | 0.86 |
| 2-Hydroxypyridine | 0.09 | 0.82 B | 0.71 B | 0.81 B | 0.80 B | 0.83 B |
| 4-Hydroxypyridine | 0.18 D | 0.69 D | 0 51 D | 0.73 D | 0.83 D | 0.82 D |
| 2-Hydroxypyrimidine hydrochloride | 0.14 B | 0.52 B | 0.20 B | 0.55 B | 0.84 B | 0.88 B |
| 2-Hydroxypyrimidine deoxyriboside | 0.48 | 0.82 | 0.38 | 0.84 | 0.86 | 0.74 |
| 4-Hydroxypyrimidine | 0 33 D | 0 58 D | 0.22 D | 0.63 D | 0.78 D | 0.83 D |
| 4-Hydroxypyrimidine deoxyriboside | — | 0.89 | 0.57 | 0.83 | 0.85 | 0.90 |
| 4-Hydroxyquinazoline | 0.80 B s | 0.84 B | 0.68 B | 0.78 B | 0.45 B | 0.54 B |
| 4-Hydroxyquinazoline deoxyriboside | 0.86 ss | 0.97 | 0.84 s | 0.91 | 0.61 | 0.65 |
| 5-Mercaptouracil | 0.08 D | 0.40 D | 0.02 D | 0.39 D | 0.46 D | 0.60 D |
| 5-Mercaptouracil deoxyriboside | — | 0.20 | 0.00 | 0.30 | 0.64 | 0.70 |
| 1-Methylcytosine | 0.07 D | 0.62 D | 0.37 D | 0.65 D | 0.73 D | 0.68 D |
| 3-Methylcytosine | 0.05 D | 0.66 D | 0.44 D | 0.70 D | 0.76 D | 0.17 D |

II–VI (Table II). In solvent I, thymidine exhibits considerable streaking and was unsuitable as a reference.

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TABLE II

$R_{Td}$ VALUES FOR PYRIMIDINE BASE ANALOUGES AND DEOXYRIBONUCLEOSIDES

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

| *Compound* | *Solvent* | | | | |
|---|---|---|---|---|---|
| | *II* | *III* | *IV* | *V* | *VI* |
| 4-Amino-6-hydroxypyrimidine | 0.74 | 0.42 | 0.65 | 0.76 | 0.79 |
| 4-Amino-6-hydroxypyrimidine deoxyriboside | — | 0.49 | 0.71 | 0.84 | 0.96 |
| 2-Amino-5-nitropyrimidine | — | 1.14 | 0.87 | 0.66 | 0.77 |
| 4-Aminopyrimidine | 1.18 | 1.28 | 1.10 | 0.75 | — |
| 2,4-Dihydroxy-3-methylpyrimidine | 0.99 | 1.24 | 1.01 | 1.00 | 1.07 |
| 2,4-Dihydroxy-3-methylpyrimidine deoxyriboside | 1.05 | 1.32 | 1.17 | 0.99 | 1.12 |
| 4,6-Dihydroxy-2-methylpyrimidine | 0.60 | 0.18 | 0.75 | 0.96 | 1.12 |
| 4,6-Dihydroxypyrimidine | 0.56 | 0.12 | 0.64 | 0.94 | 0.95 |
| 4,6-Dihydroxypyrimidine deoxyriboside | 0.88 | 0.30 | 0.97 | 1.08 | 1.07 |
| 1,5-Dimethyluracil | 1.08 | 1.37 | 1.04 | 0.96 | 1.02 |
| 5,6-Dimethyluracil | 1.05 | 1.16 | 1.03 | 0.86 | 0.93 |
| 4-Ethoxy-2-hydroxypyrimidine | — | — | — | 1.08 | 1.18 |
| 2-Hydroxy-4-methylpyrimidine hydrochloride | 0.86 | 0.85 | — | 1.13 | 1.19 |
| 2-Hydroxy-4-methylpyrimidine deoxyriboside | 0.99 | 1.34 | 1.12 | 1.18 | 1.15 |
| 4-Hydroxypyrimidine | 0.73 | 0.48 | 0.86 | 0.99 | 1.14 |
| 4-Hydroxypyrimidine deoxyriboside | 1.22 | 1.36 | 1.12 | 1.12 | 1.18 |
| 5-Mercaptouracil | 0.54 | 0.04 | 0.53 | 0.60 | 0.79 |
| 5-Mercaptouracil deoxyriboside | 0.27 | 0.00 | 0.41 | 0.76 | 0.86 |
| 1-Methylcytosine | 0.85 | 0.95 | 0.88 | 0.92 | 0.78 |
| 3-Methylcytosine | 0.92 | 0.90 | 0.96 | 0.90 | — |
| 2-Thiouracil | 0.75 | 0.42 | 0.76 | 0.81 | 0.75 |

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