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Note

Separation of the α and β anomers of 5-alkyl-2′-deoxyuridines by high-performance liquid chromatography

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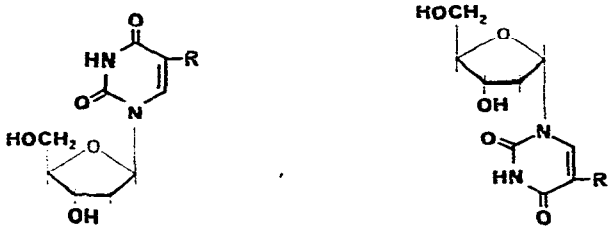
5-Substituted pyrimidine derivatives, *e.g.*, 5-hydroxymethyluracil[1,2], 5-(4,5-dihydroxypentyl)uracil[3] and 5-(4-aminobutylaminomethyl)uracil[4], play an important role among nucleo-base analogues occurring in natural DNA. The synthetic products 5-ethyl-2′-deoxyuridine and 5-iodo-2′-deoxyuridine exert an antiviral effect[5–8], while 5-hexyl-2′-deoxyuridine shows cytostatic activity[9]. In the above biologically active compounds, the configuration of carbon-1 in the sugar moiety is β. However, most synthetic methods yield mixtures of α and β anomers. The ratio of anomers is dependent on the reaction conditions applied. A method for the determination of α and β anomers is therefore necessary.

Nucleoside anomers cannot be resolved by means of paper chromatography[10]. The separation of the α and β anomers was first carried out with protected compounds by thin-layer chromatographic (TLC) techniques[1,11–14]. Recently, Kulikowski and Shugar[15] published a TLC method for the separation of unprotected anomeric nucleosides. We have developed a high-performance liquid chromatographic (HPLC) method for the determination of the α and β anomers of eight pairs of alkyldeoxyuridines.

EXPERIMENTAL

Chromatographic separations were carried out with a Hewlett-Packard 1010B liquid chromatograph equipped with a variable-wavelength UV detector operating at 265 nm. The peak areas were integrated electronically using an Autolab System IV computing integrator. The stainless-steel column (25 cm × 4 mm I.D.) was packed with Merckosorb Si60 (5 μm) (Merck, Darmstadt, G.F.R.). The mobile phase was ethyl acetate at a flow-rate of 1.4 ml/min. Separations were performed at ambient temperatures, the compounds being dissolved in methanol.

Of the eight pairs of compounds (Fig. 1), five were used for a more detailed study of the method (I, IIb–f). Accurate amounts of pure α and β anomers were mixed and five mixtures were prepared from each pair of compounds. A minimum of three independent determinations were made for each mixture. All of the anomeric nucleosides tested were prepared and identified in our laboratory.



I II

Fig. 1. Formulae of 5-alkyl-2′-deoxyuridines investigated: Ia–h = β anomers; IIa–h = α anomers. R = $-CH_3$ (a), $-CH_2-CH_3$ (b), $-(CH_2)_2-CH_3$ (c), $-(CH_2)_3-CH_3$ (d), $-(CH_2)_4-CH_3$ (e), $-(CH_2)_5-CH_3$ (f), $-(CH_2)_6-CH_3$ (g) and $-(CH_2)_9-CH_3$ (h).

TABLE I

RETENTION DATA FOR α AND β ANOMERS OF 5-ALKYL-2′-DEOXYURIDINES

R	*Retention time (sec)*	
	α anomer	*β anomer*
$-CH_3$	886	1156
$-CH_2-CH_3$	555	850
$-(CH_2)_2-CH_3$	437	684
$-(CH_2)_3-CH_3$	380	600
$-(CH_2)_4-CH_3$	351	556
$-(CH_2)_5-CH_3$	309	484
$-(CH_2)_6-CH_3$	298	461
$-(CH_2)_9-CH_3$	278	411

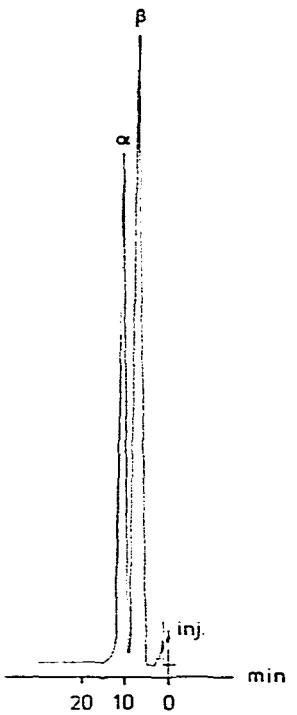


Fig. 2. Chromatogram of the α and β anomers of 5-butyl-2′-deoxyuridine. For conditions see Experimental.

RESULTS AND DISCUSSION

Calibration curves for all five compounds (I, IIb–f) were linear over the range 0.1–10 μg (S.D. varying from $\pm$ 0.29 to $\pm$ 0.62; $r \approx 0.999$). Table I gives chromatographic data for α and β anomers of eight pairs of alkyldeoxyuridines.

Each pair of anomers can be clearly separated by HPLC. As expected, on the polar adsorbent, the length of the alkyl group determines the retention of the compounds. Anomers with longer alkyl groups give shorter retention times. The β anomers always have shorter retention times than the corresponding α anomers. A chromatogram of the α and β anomers of 5-butyl-2'-deoxyuridine is presented in Fig. 2.

Further investigations using other adsorbents and solvent systems are in progress.

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