PREPARATION, SPECTRAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF 5-BROMO-6-METHYL-2′-DEOXYURIDINE AND 5-IODO-6-METHYL-2′-DEOXYURIDINE

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Reaction of 3′,5′-di-O-benzoyl-6-methyl-2′-deoxyuridine (*IIa*) with elementary bromine or iodine afforded 5-halogeno derivatives *IIc* and *IId* which on methanolysis gave 5-bromo-6-methyl-2′-deoxyuridine (*Ic*) and 5-iodo-6-methyl-2′-deoxyuridine (*Id*), respectively. The CD spectra of *Ic*, *Id* and 6-methyl-2′-deoxyuridine (*Ia*) are compared and discussed with regard to determination of the nucleoside conformation. Unlike 5-bromo- and 5-iodo-2′-deoxyuridine, the 6-methyl derivatives *Ic* and *Id* exhibit neither antibacterial nor antiviral activity. Nor do they exert any antimetabolic effect on the *de novo* DNA synthesis in primary rabbit kidney cells.

5-Halogeno derivatives of 2′-deoxyuridine are important antiviral nucleoside analogues. 5-Iodo-2′-deoxyuridine and 5-bromo-2′-deoxyuridine inhibit multiplication of numerous DNA viruses (for literature survey see ref.[1,2]); moreover, the latter compound exhibits a broad-spectrum biological activity and is a potent mutagen[3]. Also, both the remaining 5-halogeno-2′-deoxyuridine derivatives, 5-chloro and 5-fluoro derivative, show antiviral and antibacterial effects, and the last-mentioned compound is endowed with cancerostatic properties[4]. The mechanism of action of these compounds is based on their incorporation into DNA and their interference with the expression of genetic information. Once converted to its 5′-phosphate, the 5-fluoro derivative inhibits the DNA synthesis *de novo* by the block of thymidylate synthetase; the compound can also be cleaved to 5-fluorouracil which will be incorporated into nucleic acids of various kinds and functions[5].

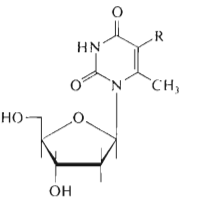
Recently, we described an advantageous synthesis of 6-methyl-2′-deoxyuridine (*Ia*) and its analogues with modified sugar moiety[6–8]. These nucleoside analogues are of interest since the energetic demands for complex formation with enzymes are affected by the presence of a bulky and hydrophobic substituent in immediate vicinity of the nucleoside linkage which might also influence the nucleoside conformation in aqueous solutions[9,10]. The CD-spectrum of 6-methyl-2′-deoxyuridine (*Ia*) differs markedly from that of 6-methyluridine[10], indicating that the compound *Ia* either does not exist predominantly in the *syn*-conformation or, alternatively, that

CD-spectral data cannot be used for assignment of the conformational parameters to the nucleoside.

We decided, therefore, to prepare 5-halogeno substituted 6-methyl-2′-deoxyuridines *Ib – Id* in order to study whether the incorporation of halogen atom (and the resulting bathochromic shifts of the electron transitions) affects the CD spectra of this group of compounds. In addition, we wanted to verify whether the introduction of a methyl group into the position 6 of 5-halogeno-2′-deoxyuridines affects their biological, in particular antiviral activity.

In a previous paper we described the synthesis of 5-fluoro-6-methyl-2′-deoxyuridine (*Ib*) by reaction of protected 6-methyl-2′-deoxyuridine (*IIa*) with elementary fluorine, followed by methanolysis[11]. In addition to the expected product *Ib*, this reaction gave also 5-fluoro-6-fluoromethyl-2′-deoxyuridine, formed by simultaneous fluorination of the 6-methyl group. In this communication we report the preparation of the corresponding 5-bromo and 5-iodo derivatives by halogenation with elementary bromine and iodine, respectively.

We chose 3′,5′-di-O-benzoyl-6-methyl-2′-deoxyuridine (*IIa*) as starting compound for these syntheses because this compound is easily accessible by reductive dehalogenation of the corresponding 2′-chloro-2′-deoxy derivative[6,7]. Reaction of *IIa* with elementary bromine in dioxane or with N-bromosuccinimide in dichloroethane afforded quantitatively the corresponding blocked 5-bromo derivative *IIc* which was characterized by its ^{1}H-NMR spectrum and analysis. Analogously, the 5-iodo derivative *IId* was prepared from the compound *IIa* by reaction with elementary iodine in an acidic medium. No products of halogenation of the 6-methyl group or their hydrolysis products (5-halogeno-6-hydroxymethyl-2′-deoxyuridines) were observed. The substitution on the 6-methyl group is thus characteristic only for the fluorination reaction.



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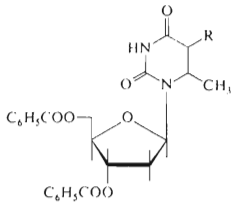

II

In formulae *I*, *II*: *a* R = H, *b* R = F, *c* R = Br, *d* R = I

Methanolysis of the above-described dibenzoates *IIc* and *IId* gave the free nucleosides *Ic* and *Id*. As expected, their UV absorption maxima exhibit bathochromic shifts relative to both 6-methyl-2′-deoxyuridine (*Ia*) and 5-bromo- or 5-iodo--2′-deoxyuridine (Table I). The weakly acidic character of the 5-halogeno-6-methyluracil in the compounds *Ia* and *Id* is manifested by an increased acidity of the nucleoside molecule: The apparent dissociation constants, determined spectrophotometrically, are $pK'_a = 8{\cdot}0$ for *Ic* and $pK'_a = 8{\cdot}5$ for *Id*.

The CD spectra of *Ic*, *Id* and 6-methyl-2′-deoxyuridine (*Ia*) are given in Fig. 1 and Table I. The mentioned bathochromic shift of the long wavelength maximum of *Ic* and *Id* enables to separate the partially overlapping bands which form an envelope in the spectrum of *Ia*. In principle, we can characterize a negative short wavelength band in the region 210–220 nm with a bathochromic shift in the order H → Br → I, and a long wavelength positive band with the same trend of bathochromic shift (and the same sign of molar ellipticity as found for the derivatives containing no 6-methyl group). In the CD spectrum of derivatives *Ia* and *Ic* the third, medium, band at 246–255 nm is obscured and exists only as a positive shoulder; however, in the spectrum of the 5-iodo derivative *Id* this band is completely separated.

It is unlikely that introduction of a halogen atom into position 5 would significantly affect steric situation in the vicinity of the nucleoside bond (*i.e.* the conformation, enforced by the 6-methyl group). Thus, the CD spectra (sign of the bands) of 6-methyluridine and 6-methyl-2′-deoxyuridine (*Ia*) do not offer a true picture of the conformation of the compound[7] as they result from an overlap of two long wavelength bands. In a recent publication, dealing with preparation of a series of 6-substituted uridine derivatives, Japanese authors[12] correlated CD data with con-

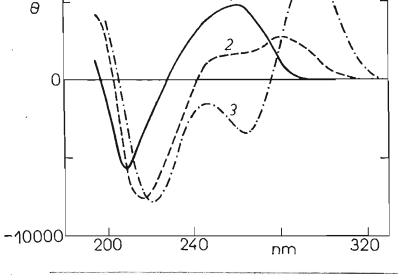

FIG. 1

Circular Dichroism Spectra in Water

1 Compound *Ia*, *2* compound *Ic*, *3* compound *Id*.

formations deduced from ^{1}H-NMR spectra and also stressed that conformational assignments to uracil nucleosides based on their CD spectra can be misleading.

Neither nucleoside *Ic* nor *Id* show any antibacterial activity towards *Escherichia coli* B (synthetic medium with glucose) at concentrations up to 1 mg per 1 ml of medium[13]. Their antiviral activity was studied under standard assay consitions[14] on primary rabbit kidney cells with a set of DNA and RNA viruses, using 5-bromo-2′-deoxyuridine and 5-iodo-2′-deoxyuridine as reference materials. Whereas both the last mentioned compounds exhibited a significant antiviral activity the 6-methyl derivatives *Ic* and *Id* are completely inactive under the conditions of the assay (Table II).

The antimetabolic potentials of the compounds *Ic* and *Id* were assessed in an assay system which consisted of an estimation of DNA synthesis in primary rabbit kidney

TABLE I

Ultraviolet and Circular Dichroism Spectra in Water (wavelength in nm; molar ellipticities in parentheses)

Compound	UV Spectra		CD Spectra				
pH	λ_{max}, nm (ε_{max})	λ_{min}, nm	λ, nm ([Θ], degree cm^2 dmol^{-1})				
Ia	262		261·5	—	246 sh[a]	209	200[b]
7·0	(12 000)	235	(+4 750)	—	(+4 500)	(−5 600)	(−1 600)
Ic	280		281	255 sh[c]	—	217	200[b]
2·0	(11 000)	244	(+2 180)	(+930)	—	(−5 600)	(+1 600)
Ic	280		281·5	255 sh[c]	—	216	200[b]
7·0	(11 000)	244	(+2 800)	(+1 500)	—	(−7 320)	(+4 500)
Ic	280	254	[d]	[d]	[d]	[d]	[d]
12·0	(7 300)						
Id	284	246	297	263	245·5[e]	223	200[b]
2·0	(9 000)		(+4 990)	(−2 430)	(−1 280)	(−4 860)	(+2 100)
Id	284	246	296	205	247·5[e]	221·5	200[b]
7·0	(8 700)		(+6 650)	(−3 320)	(−1 530)	(−7 550)	(+4 000)
Id	280	250	[d]	[d]	[d]	[d]	[d]
12·0	(6 900)						

[a] sh denotes shoulder; [b] ellipticity of the shortest measured wavelength; [c] broad shoulder; [d] not measured; [e] negative minimum belonging to a positive band.

cells, based upon incorporation of labelled thymidine or 2′-deoxyuridine[15]. The reference samples of 5-bromo- and 5-iodo-2′-deoxyuridine again strongly inhibited the incorporation of both metabolites, whereas the 6-methyl derivatives *Ic* and *Id* were completely ineffective (Table III). We can thus conclude that introduction of a methyl group into the position 6 in 5-bromo- or 5-iodo-2′-deoxyuridine results in a complete loss of antiviral and antimetabolic activities of the parent compounds.

TABLE II

Antiviral Activity of 2′-Deoxyuridine Derivatives in Primary Rabbit Kidney Cells

Challenge virus	Minimum inhibitory concentration μg/ml			
	Ic	*Id*	BDU[a]	IDU[b]
Vesicular stomatitis	>400	>400	>400	>400
Vaccinia	>400	400	0·1	0·1
Herpes simples, type 1 (KOS)	>100	>100	0·1	0·1
Herpes simplex, type 1 (McIntyre)	70	>100	0·2	0·2
Herpes simplex, type 1 (F)	>100	>100	0·1	0·1
Herpes simplex, type 1 (Lyons)	>100	>100	0·2	0·4
Herpes simplex, type 2 (G)	>100	>100	0·1	0·2
Herpes simplex, type 2 (196)	>100	>100	0·1	0·1

[a] BDU, 5-bromo-2′-deoxyuridine; [b] IDU, 5-iodo-2′-deoxyuridine.

TABLE III

Antimetabolic Activity of 2′-Deoxyuridine Derivatives in Primary Rabbit Kidney Cells

Incorporation	ID_{50}, μg/ml[a]			
	Ia	*Id*	BDU[b]	IDU[c]
(^{3}H-methyl)dThd	>400	350	0·4	1·1
(^{14}C-2)dUrd	≫400	≫400	0·2	0·6

[a] Dose required to inhibit the incorporation of (^{3}H-methyl)dThd or (^{14}C-2)dUrd into DNA by 50%; [b] BDU, 5-bromo-2′-deoxyuridine; [c] IDU, 5-iodo-2′-deoxyuridine.

EXPERIMENTAL

Unless stated otherwise, the solutions were evaporated at 40°C/2 kPa and the compounds dried over phosphorus pentoxide at 13 Pa. Paper chromatography was performed on a paper Whatman No 1 in system S1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), thin-layer chromatography on plates coated with silica gel (Silufol UV_{235}, Kavalier, Czechoslovakia) in system S2, benzene–ethyl acetate (7 : 3). Preparative chromatography was carried out on loose layers of silica gel (40 × 16 × 0·3 cm), containing a fluorescent indicator. The UV absorption spectra were taken in aqueous solutions on a Specord UV-VIS spectrometer (Carl Zeiss, Jena, G.D.R.), ^{1}H-NMR spectra on a Varian 100 instrument in deuteriochloroform (internal standard hexamethyldisiloxane, chemical shifts given in ppm, coupling constants in Hz). The CD spectra were measured on a Roussel–Jouan Dichrograph, Model II, in aqueous solutions.

3′,5′-Di-O-benzoyl-5-bromo-6-methyl-2′-deoxyuridine (*IIc*)

A mixture of 3′,5′-di-O-benzoyl-6-methyl-2′-deoxyuridine (*IIa*) (2·0 g; 4·45 mmol; see ref.[7]). N-bromosuccinimide (3·5 g; 19·7 mmol) and 1,2-dichloroethane (40 ml) was refluxed (calcium chloride tube) until the reaction was complete, according to chromatography in S2 (3 h). The mixture was diluted with chloroform (200 ml) and washed with 50 ml portions of 10% sodium thiosulfate solution (twice), saturated sodium hydrogen carbonate solution (twice) and with water. The solution was dried over magnesium sulfate, taken down *in vacuo* and the residue chromatographed on two plates of silica gel (*vide supra*) in the system S2. The product bands were eluted with methanol (300 ml), the solvent evaporated and the residue crystallized from ethyl acetate (light petroleum added until the solution became turbid), affording 1·70 g (72·4%) of the product *IIc*, m.p. 151–160°C. For $C_{24}H_{21}BrN_2O_6$ (513·4) calculated: 56·14% C, 4·12% H, 15·57% Br, 5·46% N; found: 56·44% C, 4·09% H, 15·30% Br, 5·70% N. R_F = 0·38 in S2; compound *IIa*, R_F = 0·10 in S2. ^{1}H-NMR spectrum: 2·46 (m, 1 H, $J_{2''1'}$ = 8·5) $H_{2''}$; 2·58 (s, 3 H) 6-CH_3; 3·25 (m, 1 H, $J_{2'1'}$ = 4·5) $H_{2'}$; 4·20–4·85 (m, 3 H) $H_{4'}$ + 2 $H_{5'}$; 5·82 (m, 1 H) $H_{3'}$; 6·23 (dd, 1 H) $H_{1'}$; 7·20–8·20 (m, 10 H) aromatic protons; 9·85 (bs, 1 H) NH.

3′,5′-Di-O-benzoyl-5-iodo-6-methyl-2′-deoxyuridine (*IId*)

A mixture of compound *IIa* (see ref.[7]) (2·25 g; 5 mmol), dioxane (50 ml), chloroform (20 ml), 0·5M nitric acid (10 ml) and iodine (2·6 g; 10 mmol) was refluxed for 4 h, taken down *in vacuo* and codistilled with dioxane (3 . 20 ml). The residue was dissolved in chloroform (200 ml) and worked up as described for the compound *IIc*. The product was purified by chromatography on two layers of silica gel (*vide supra*) in system S2, eluted with methanol (300 ml) and crystallized from ethyl acetate–light petroleum; yield 1·65 g (57·2%) of *IId*, m.p. 169–170°C. R_F = 0·48 in S2. For $C_{24}H_{21}IN_2O_6$ (560·3) calculated: 51·44% C, 3·78% H, 22·65% I, 5·00% N; found: 49·86% C, 3·55% H, 23·09% I, 4·83% N. ^{1}H-NMR spectrum: 2·52 (m, 1 H, $J_{2''1'}$ = 8·5) $H_{2''}$; 2·73 (s, 3 H) 6-CH_3; 3·15 (m, 1 H, $J_{2'1'}$ = 4·5) $H_{2'}$; 4·40–4·82 (m, 3 H) $H_{4'}$ + 2 $H_{5'}$; 5·85 (m, 1 H) $H_{3'}$; 6·28 (dd, 1 H) $H_{1'}$; 7·20–8·20 (m, 10 H) aromatic protons.

5-Bromo-6-methyl-2′-deoxyuridine (*Ic*)

A solution of the dibenzoate *IIc* (1·05 g; 2 mmol) in 0·1M methanolic sodium methoxide (20 ml) was set aside overnight, neutralized with dry Dowex 50 × 8 (H^+-form), filtered, washed with methanol and the filtrate taken down *in vacuo*. The residue was dissolved in water (50 ml), the solution extracted with ether (3 . 25 ml), the aqueous layer taken down *in vacuo*, the residue codistilled three times with ethanol (20 ml portions) and crystallized from ethanol (with addi-

tion of ether). Yield of *Ic* 545 mg (86%), decomposition above 260°C. R_F 0·66 in S1. For $C_{10}H_{13}BrN_2O_5$ (321·2) calculated: 37·39% C, 4·08% H, 24·90% Br, 8·72% N; found: 37·35% C, 3·94% H, 25·18% Br, 8·52% N.

5-Iodo-6-methyl-2′-deoxyuridine (*Id*)

A solution of the dibenzoate *IId* (1·35 g; 2·34 mmol) in 0·1M methanolic sodium methoxide (30 ml) was set aside overnight and worked up as described for *Ic*. Yield 650 mg (75·5%) of the compound *Id*, m.p. 139—141°C. R_F 0·66 in S1. For $C_{10}H_{13}IN_2O_5$ (368·2) calculated: 32·62% C, 3·56% H, 34·48% I, 7·61% N; found: 32·79% C, 3·51% H, 34·95% I, 7·78% N.

Determination of Biological Activity

a) Antiviral activity was determined by the previously described technique[14]. The results are given in Table II, together with the data for 5-bromo- and 5-iodo-2′-deoxyuridine (Fluka), which served as standards.

b) The effect of compounds *Ic* and *Id* on the incorporation of thymidine and 2′-deoxyuridine into DNA in primary rabbit kidney cells was determined by the technique described in ref.[15]. The results, together with the data for 5-bromo- and 5-iodo-2′-deoxyuridine are summarized in Table III.

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