5'-Halogeno-2',3'-cyclic sulphite isomers in the preparation of 5'-halogeno nucleosides. Synthesis of 5'-deoxyuridine and 5'-deoxy-5-fluorouridine

Hubert Hřebabček and Jiří Beránek

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia

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ABSTRACT

When uridine (Ia) is reacted with thionyl chloride in hexamethylphosphoric triamide a mixture of isomeric 5-chloro-2',3'-sulphites is formed, which can be separated to individual epimers IIa and IIIa, in 45% and 15% yields, respectively. Analogously, crystalline epimers IIb (37%) and IIIb (17%) can be obtained from 5-fluorouridine (Ib). Both isomers IIa, IIIa (or IIb, IIIb) afford a single 5-chloro derivative IVa (or IVb, respectively) if treated with 0.1N sodium methoxide. From the mixture of sulphites IIa and IIIa (or IIb and IIIb) crystalline 5-chlorouridine IVa is formed in 84.5% yield, calculated per starting uridine Ia (or crystalline 5-chloro-5-fluorouridine IVb, 85.5% per starting 5-fluorouridine Ib, respectively). On reduction of 5-chlorouridine IVa with tributyltin hydride 5-deoxyuridine (Va) is formed in 79% yield. During the reduction of 5-chloro-5-fluoro derivative IVb to 5-deoxy-5-fluorouridine (Vb, 57%) a partial reductive elimination of 5-fluorine takes place under formation of 5-deoxyuridine (Va, 9%).

INTRODUCTION

A simple and efficient method has been developed by Kikugawa and Ichino for the preparation of 5-halogeno nucleosides from unsubstituted nucleosides. The procedure is suitable especially for the preparation of cytosine and adenosine analogues (and other nucleosides as well). In contrast to this, direct halogenation according to Dods and Roth with the Vilsmeier-Haack's reagent gives cyclocytidine in the case of cytidine. Direct halogenation of the primary hydroxyl group with the active phosphonium complex, formed from thionyl chloride and hexamethylphosphoric triamide, probably takes place by an ionic mechanism under formation of an alkoxide phosphonium ion, which affords the required alkyl halide on substitution with chloride ion. Hogenkamp assumed.
that a phosphorus-containing cyclic intermediate is formed with the cis diol of the nucleoside, that protects the secondary hydroxyls against halogenation. This assumption was supported by the formation of \(3',5'\)-dihalogeno derivatives from deoxyribonucleosides that cannot afford such a cyclic intermediate.

RESULTS AND DISCUSSION

During the study of the course of the reaction of the thionyl chloride-hexamethylphosphoric triamide reagent with unsubstituted nucleosides it was found that several compounds were formed in the reaction mixture. In addition to \(5'\)-halogeno derivatives, and in contrast to earlier observations, two additional compounds were formed with higher \(R_F\) values in TLC. These substances were sufficiently stable to be isolated, and even though they had very similar \(R_F\) values, they could be separated by chromatography on silica gel. The substances had identical UV spectra and elemental analyses, corresponding to those of \(2',3'\)-sulphites of \(5'\)-halogeno nucleosides. Both substances had closely similar IR spectra, but their NMR spectra and optical rotations were different. On methanolysis with 0.1N sodium methoxide at room temperature a single \(5'\)-halogeno derivative was formed from both isomeric sulphites. The halogeno derivative retained a free cis diol grouping in the molecule and it gave \(5'\)-deoxyribonucleoside on reduction (identical with an authentic sample). Both isomeric sulphites can be further converted to a single \(2,2'\)-anhydro derivative. From what has been said above it follows that both isomeric \(2',3'\)-cyclic sulphites belong to a single configuration of the sugar moiety of the nucleoside, and that the isomerization must involve the atom of sulphur. The formation of two isomeric sulphites was observed for the first time in the reaction with 6-azauridine.

The formation of cyclic sulphinyl derivatives has already been described in organic chemistry, as well as the possibility of the formation and the isolation of individual isomers (cf. and the references therein). Recently cyclic sulphites were also made use of as a protecting group...
for cis diol grouping in the chemistry of ribonucleosides and nucleotides. Nevertheless, in these groups of derivatives individual sulphite isomers were not described. To our knowledge the present paper is the first instance when the isomers of 2',3'-0-sulphinyl derivatives of nucleosides have been isolated and the first case of the preparation of 5'-halogeno-2',3'-cyclic sulphite derivatives of nucleosides. A detailed physico-chemical study of stereochemistry of individual isomers will be the subject of a separate communication. Recently the preparation of 5'-chloro-2',3'-sulphate on reaction of sulphuryl chloride with adenosine has been
described by Kikugawa¹⁵.

From the above mentioned observations the conclusion must be made that the formation of 5'-halogeno derivatives of nucleosides by the method of Kikugawa and Ichino¹ takes place via the primary formation of 2',3'-cyclic sulphite and not via the cyclic phosphorus intermediate, as proposed by Hogenkamp⁵. The instability of isomeric 2',3'-cyclic sulphites during the preparation of 5'-halogeno derivatives was probably the reason why the cyclic sulphites were not observed and isolated in the case of cytidine and adenosine¹.

Thus the reaction of uridine (Ia) with thionyl chloride and hexamethylphosphoramic triamide gave a mixture of isomeric 2',3'-sulphites of 5'-chloronucleoside IIa and IIIa in high yield. In the course of the reaction a primary formation of 2',3'-cyclic sulphite of uridine takes place, followed by a substitution reaction into position 5'. Using chromatography on silica gel both pure epimers IIa and IIIa could be isolated from the mixture of isomeric sulphites, one in the form of a solid foam (IIa, 45%) and the other as an amorphous powder (IIb, 15%).

In the case of 5-fluorouridine (Ib) a mixture of iso-
meric 5'-chloro-2',3'-sulphites IIb and IIIb was obtained in an analogous manner. On chromatography on silica gel both epimers were obtained in crystalline form (IIb, 37%; IIIb, 17%). The epimers also differ in their melting point; one of them (IIb) crystallizes with half a molecule of benzene.

Since chromatographic separation of isomeric sulphites was connected with a loss in yield, and because both isomers IIa and IIIa afford a single 5'-chloro derivative IVa, the mixture of isomeric sulphites IIa and IIIa was used in the preparation of 5'-halogeno derivative IVa. Alkaline methano-
lysis of the mixture IIa, IIIa gave a high yield of crystal-
line 5'-chlororibosyl derivative IVa (84.5%, referred to uridine Ia). Subsequent reduction of 5'-chloro derivative IVa with tributyltin hydride¹⁶ under initiation with 2,2'-azo-
bis(2-methylpropionitrile) (cf.¹⁷ and the references there-
in) gave 5'-deoxyuridine (Va) in 79% yield.
Similarly, methanolysis of both epimers of 5'-chloro-5'-deoxy-2',3'-O-sulphinyl-5-fluorouridine (IIb, IIIb) afforded a single 5'-chloro derivative IVb, in 85.6% yield, calculated per the starting 5'-fluorouridine Ib. On subsequent reduction of fluoro derivative IVb with tributyltin hydride, carried out analogously as in the case of the uridine derivative IVa, 5'-deoxy-5'-fluorouridine (Vb) was prepared in 57% yield. However, during the reaction an unexpected partial reductive cleavage of the fluorine atom in the position 5 of the uracil ring also took place under formation of a small amount of 5'-deoxyuridine (Va; 9%).

In the study by Kuivila, concerning the reductive dehalogenation with alkyltin hydrides, a decreasing reaction rate is mentioned in the series of halogenides: iodine, bromine, chlorine, and tertiary, secondary and primary halogen derivatives. Simultaneously it is mentioned that under these conditions fluorine is stable. A reductive dehalogenation of fluorine was not observed in the reduction of 2'-chloro-2'-deoxy-5'-fluorouridine either. Hence under more drastic conditions necessary for the reduction of the primary 5'-chloro atom partial dehalogenation of the fluorine atom in the position 5 of the nucleobase takes place. It may be assumed that the bond of the fluorine atom on the pyrimidine ring is less firm than the bond in aliphatic fluoro derivatives, and it resembles rather the bond of fluorine to a vinylic group.

The described method of preparation of 5'-halogeno-5'-deoxy-2',3'-cyclic sulphites of ribonucleosides represents a simple method of preparation of 5'-halogeno derivatives of ribonucleosides bearing an alkali-labile protecting group on 2',3'-cis diol grouping. These derivatives can be utilized for the preparation of a number of analogues in the field of nucleoside and nucleotide chemistry, in analogy with the papers on 2',3'-cyclic carbonates of ribonucleosides carrying another alkali-labile protecting group on the cis diol grouping. In comparison with the earlier described method of preparation of 5'-halogeno-2',3'-carbonates, the present method of preparation of 5'-halogeno-2',3'-sulphites
is experimentally simpler; it also gives higher yields of the required substance in one reaction step, directly from the unsubstituted nucleoside. In analogy with the earlier described series of papers on 2',3'-cyclic carbonates 17, 19-21 further analogues of nucleosides were prepared 7, 14 using the 5'-halo-

EXPERIMENTAL

The melting points were measured on a heated microscope stage (Kofler block). The ultraviolet spectra were recorded on a CF-4 apparatus (Optica, Milano). The infrared spectra on a UR-20 apparatus (Carl Zeiss, Jena). The 1H-NMR spectra were measured on a Varian HA-100 100 MHz instrument, using hexa-
methyldisiloxane as internal standard; chemical shifts in δ-scale (ppm units) and the coupling constants in Hz. Optical rotations were measured on an automatic Perkin-Elmer 141 MC polarimeter. The mass spectrum was taken on an A.E.I. type MS 902 spectrometer. Analytical samples were dried at 0.5 Torr. Column chromatography was carried out on silica gel according to Pitra, particle size 30-60 μm, produced by Service Laboratories of this Institute. Thin-layer chromato-
graphy was carried out on ready-for-use fluorescent silica gel Silufol plates UV 254 (Kavalier, Czechoslovakia) in the following systems: S1 - benzene-ethyl acetate (1:1) and S2 - ethyl acetate-acetone-ethanol-water (20:2:2:1). Rf values in S1: IIa 0.32, IIb 0.64, IIIa 0.30, IIIb 0.58; in S2: IVa 0.61, IVb 0.76, Vb 0.48, Vb 0.69.

(+)- and (-)-5-Chloro-5-deoxy-2',3'-O-sulphynyluridine (IIa and IIIa)

Uridine (488 mg; 2 mmol) was added to a stirred mixture of hexamethylphosphoronic triamide (4 ml) and thionyl chloride (0.5 ml). After 3 h standing at room temperature the mixture was poured into a saturated sodium hydrogen carbonate solution cooled with ice (100 ml). The mixture was extracted with ethyl acetate (2 x 20 ml) and the extract washed with water (2 x 15 ml) and a saturated aqueous sodium chloride solution (10 ml), dried over magnesium sulphate and evapo-

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of isomers IIa and IIIa.

On chromatography of 0.5 g of the residue on a silica gel column (150 g) in benzene-ethyl acetate (1:1) 205 mg (45%) of the more mobile fraction IIa were obtained in the form of a solid foam. \( \chi_{D}^{25} +25.4^0 \) (c 0.481; ethyl acetate); UV spectrum (methanol): \( \lambda_{\text{max}} 256 \text{ nm} \) (log \( \varepsilon \) 4.03), \( \lambda_{\text{min}} 228 \text{ nm} \) (log \( \varepsilon \) 3.50); IR spectrum (chloroform): c 3.10\(^{-3}\)M: 3388 cm\(^{-1}\) (NH); c 2\%: sh 1720, 1703 cm\(^{-1}\) (C=O), 1638 cm\(^{-1}\) (C=C); KBr: 1219 cm\(^{-1}\) (S=O). For C\(_9\)H\(_{18}\)N\(_2\)O\(_6\)ClS (308.7) calculated: 35.01% C, 2.94% H, 9.08% N, 11.49% Cl, 10.39% S; found: 35.22% C, 3.01% H, 8.97% N, 11.27% Cl, 10.18% S.

Evaporation of the slower moving fraction gave 70 mg (16%) of compound IIIa in the form of an amorphous powder. \( \chi_{D}^{25} -29.7^0 \) (c 0.518; ethyl acetate); UV spectrum (methanol): \( \lambda_{\text{max}} 256 \text{ nm} \) (log \( \varepsilon \) 4.03), \( \lambda_{\text{min}} 228 \text{ nm} \) (log \( \varepsilon \) 3.52); IR spectrum (chloroform): c 3.10\(^{-3}\)M: 3389 cm\(^{-1}\) (NH); c 2\%: sh 1718, 1702 cm\(^{-1}\) (C=O), 1638 cm\(^{-1}\) (C=C); KBr: 1210 cm\(^{-1}\) (S=O). For C\(_9\)H\(_{18}\)N\(_2\)O\(_6\)ClS (308.7) calculated: 35.01% C, 2.94% H, 9.08% N, 11.49% Cl, 10.39% S; found: 35.12% C, 3.16% H, 9.36% N, 11.38% Cl, 10.35% S. Further, 89 mg of a mixture of both substances were obtained.

\((+)-\) and \((-)-5\text{-Chloro-5'-deoxy-2',3'-O-sulphinyl-5-fluoro-uridine (IIb and IIIb)}\)

Reaction of 5-fluorouridine\(^22\) (524 mg; 2 mmol) with thionyl chloride (0.5 ml) in hexamethylphosphoric triamide (4 ml), carried out in the same manner as in the case of uridine, gave 750 mg of a mixture of sulphites IIb and IIIb (dry residue).

Chromatography of 0.5 g of the residue on a silica gel column (150 g) in benzene-ethyl acetate (5:2) gave 222 mg (45.5%) of a faster and 115 mg (26.4%) of a slower fraction, and 40 mg of a mixture of both fractions. Crystallization of the first fraction from benzene with 1% of methanol afforded 180 mg (37%) of compound IIb, m.p. 115-120°C. \( \chi_{D}^{25} +19.1^0 \) (c 0.513; ethyl acetate); UV spectrum (methanol): \( \lambda_{\text{max}} 262 \text{ nm} \) (log \( \varepsilon \) 4.00), \( \lambda_{\text{min}} 232 \text{ nm} \) (log \( \varepsilon \) 3.53); IR spectrum (chloroform): c 1.10\(^{-3}\)M: 3381 cm\(^{-1}\) (NH), c 2\%: sh 1733, 1721 cm\(^{-1}\).
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(C=O); KBr: 3188 cm⁻¹ (NH), 1710, sh 1695 cm⁻¹ (C=O), 1225 cm⁻¹ (S=O); ¹H-NMR spectrum (hexadeuteriomethyl sulfoxide with deuteriochloroform): 12.03 (bd, 1H, NH, J NH,F = 4), 8.13 (d, 1H, H1, J1,F = 7), 5.80 - 6.00 (m, 2H, H1', H2'), 5.63 (m, 1H, H3'), 4.34 (m, 1H, H4'), 3.85 (m, 2H, 2H4O!)

Mass spectrum: M⁺ 326, m/e: 262 (M - SO₂), 227 (262 - Cl), 213 (262 - CH₂Cl), 197 (M - base), 133 (197 - SO₂). For C₉H₈N₂O₆ClFS·1/2 C₆H₆ (365.75) calculated: 39.40% C, 3.03% H, 7.66% N, 9.69% Cl, 5.19% F, 8.77% S; found: 39.54% C, 3.04% H, 7.40% N, 9.68% Cl, 5.38% F, 9.00% S.

On crystallization of the residue of the slower moving fraction from benzene containing 1% of methanol 75 mg (17%) of compound IIIb were obtained, m.p. 174°C, under decomposition. [α]D²⁵ -45.9° (c 0.38; ethyl acetate); UV spectrum (methanol): λ max 264 nm (log ε 4.01), λ min 234 nm (log ε 3.47); IR spectrum (chloroform): c 3.30 - 3.10: 3382 cm⁻¹ (NH); c 2.9: sh 1748, sh 1732, 1761 cm⁻¹ (C=O); KBr: 3195 cm⁻¹ (NH), 1732, 1712, sh 1695 cm⁻¹ (C=O), 1208 cm⁻¹ (S=O); ¹H-NMR spectrum (hexadeuteriomethyl sulfoxide with deuteriochloroform): 12.06 (bd, 1H, NH, J NH,F = 4), 8.21 (d, 1H, H1, J1,F = 7), 6.17 (d, 1H, H1', J1',2' = 1.5), 5.82 (dd, 1H, H2', J2',1' = 1.5, J2',3' = 7.5), 5.52 (dd, 1H, H3', J3',2' = 7.5, J3',4' = 4), 4.67 (dt, 1H, H4', J4',3' = 4, J4',5' = J4',6' = 6.5), 3.87 (m, 2H, 2H4O!) For C₉H₈N₂O₆ClFS (326.7) calculated: 33.09% C, 2.47% H, 8.58% N, 10.85% Cl, 5.81% F, 9.62% S; found: 33.18% C, 2.67% H, 8.57% N, 11.04% Cl, 5.94% F, 10.07% S.

5-Chloro-5'-deoxyuridine (IVA)

A solution of the mixture of sulphites IIa and IIIa prepared from 244 mg; 1 mmol of uridine in a 0.1M solution of sodium methoxide (10 ml) was allowed to stand at room temperature for 15 min. After neutralization with Dowex 50 (H⁺) prewashed with methanol, the exchanger was filtered off and washed with methanol (3 x 10 ml). The combined filtrates were evaporated in a vacuum. Crystallization of the residue from 2-propanol gave 203 mg (77%) of 5-chlorouridine (IVA), m.p. 176-178°C. Another 20 mg (7.5%) of the same compound
were obtained from the mother liquors. $[\alpha]_{D}^{25} +4.0^\circ$ (c 0.362; water). For C$_{9}$H$_{11}$N$_{2}$O$_{5}$Cl (262.65) calculated: 41.15% C, 4.22% H, 10.67% N, 13.50% Cl; found: 41.03% C, 4.41% H, 10.91% N, 13.73% Cl.

5-Chloro-5-deoxy-5-fluorouridine (IVb)

From the mixture of sulphites IIb and IIIb (prepared from 262 mg; 1 mmol of 5-fluorouridine Ib) 5-chloro-5-deoxy-5-fluorouridine (IVb) was prepared in the same manner as in the case of 5-chloro-5-deoxyuridine (IVA). 240 mg (85.5%) of compound IVb were obtained after crystallization from 2-propanol, m.p. 181-182°C. $[\alpha]_{D}^{25} +8.8^\circ$ (c 0.477; water); UV spectrum (water): $\lambda_{\text{max}}$ 269 nm (log $\varepsilon$ 4.06), $\lambda_{\text{min}}$ 235 nm (log $\varepsilon$ 3.37); IR spectrum (KBr): sh 3380, 3280, sh 3220 cm$^{-1}$ (OH, NH), 1722, 1691, 1666 cm$^{-1}$ (C=O). For C$_{9}$H$_{10}$N$_{2}$O$_{5}$ClF (280.65) calculated: 38.51% C, 3.59% H, 9.98% N, 12.63% Cl, 6.77% F; found: 38.74% C, 3.59% H, 10.02% N, 12.81% Cl, 6.75% F.

5-Deoxyuridine (IVA)

A 1M solution of tri-n-butyltin hydride in benzene (12 ml, in 3 ml portions) and 2,2-azobis(2-methylpropionitrile) (40 mg, in 10 mg doses) were added gradually over 25 h to a refluxing solution of 5-chloro-5-deoxyuridine (263 mg; 1 mmol) in methanol (6 ml). The mixture was evaporated in a vacuum and the residue triturated with light petroleum (10 ml) and extracted with water (25 ml). The aqueous solution was washed with light petroleum (2 x 10 ml) and evaporated under reduced pressure. Crystallization of the residue from 2-propanol gave 181 mg (79%) of 5-deoxyuridine, m.p. 181-183°C, identical with an authentic sample. For C$_{9}$H$_{12}$N$_{2}$O$_{5}$ (228.2) calculated: 47.36% C, 5.30% H, 12.28% N; found: 47.37% C, 5.27% H, 12.30% N.

5-Deoxy-5-fluorouridine (Vb)

A 1M solution of tri-n-butyltin hydride in benzene (12 ml, in 3 ml portions) and 2,2-azobis(2-methylpropionitrile) (40 mg, in 10 mg portions) were added gradually over 25 h to a refluxing solution of 5-chloro-5-deoxy-5-
-fluorouridine (281 μg; 1 mmol) in methanol (6 ml). The mixture was evaporated in a vacuum and the residue was partitioned between water (25 ml) and light petroleum (10 ml). The aqueous layer was washed with light petroleum (2 x 10 ml) and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (75 g) with ethyl acetate–ethanol–acetone–water (20:2:3:1). Crystallization from 2-propanol of the residue of the main UV absorbing fraction gave 140 μg (57%) of 5-deoxy-5-fluorouridine. m.p. 186–188°C. [α]D 25 +18.4° (c 0.419; water); IR spectrum (KBr): sh 3373, 3345, 3175 cm⁻¹ (OH, NH), 1723, 1693 cm⁻¹ (C=O), 1678, sh 1668 cm⁻¹ (C=C), 1378 cm⁻¹ (CH₃).

For C₉H₁₁N₂O₅F (246.2) calculated: 43.90% C, 4.50% H, 11.38% N, 7.72% F; found: 44.18% C, 4.66% H, 11.29% N, 8.00% F. On crystallization of the residue of the slower moving fraction from 2-propanol 20 μg (9%) of 5-deoxyuridine (Va) were obtained.

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