PREPARATION OF 5'-O-CARBOXYMETHYLURIDINE AND ITS 5-HALO DERIVATIVES*

A. HOLÝ[a] and H. PISCHEL[b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*
[b] *Sektion Biowissenschaften-Pharmazie, Karl-Marx-Universität, Leipzig, German Democratic Republic*

Received November 26th, 1976

An improved preparation of 5'-O-carboxymethyluridine (*I*) by reaction of sodium chloroacetate with 2',3'-O-isopropylideneuridine in the presence of sodium hydride and the subsequent acidic hydrolysis is presented along with preparations of 5'-O-carboxymethyl-5-fluorouridine (*II*) by reaction of *I* with elemental fluorine in acetic acid, of 5'-O-carboxymethyl-5-bromouridine (*III*) by bromination of *I* in acetic acid, and of 5'-O-carboxymethyl-5-iodouridine (*IV*) by reaction of *I* with iodine in acidic medium.

In connection with investigation on immunosuppressant conjugates with proteins, a report has been some time ago presented on the preparation of 1-carboxymethyluracil and its 5-halo derivatives and on the preparation and properties of the corresponding compounds of the 2'-deoxyuridine series, namely, of 5'-O-carboxymethyl--2'-deoxyuridine and its 5-halo derivatives[1]. Since some 5-halo derivatives of uridine also exhibit the immunosuppressant activity, attention has been paid to the preparation of these compounds that could be attached to bovine gammaglobulin and human serumalbumin by the method of mixed anhydrides[2]. In the present paper, we wish to report the preparation of the 5'-O-carboxymethyl derivatives of 5-halouridines.

The 5'-O-carboxymethyl derivatives of nucleosides can be most readily prepared by reaction of sodium chloroacetate with the sodium salt of the 5'-hydroxylic function of an appropriately protected nucleoside. However, the reaction conditions obviously do not permit to perform the reaction in the series of 5-halouracil nucleosides in view of potential changes on the heterocyclic base. Consequently, compounds *II* to *IV* were prepared by halogenation of 5'-O-carboxymethyluridine (*I*), *i.e.*, by a method analogous to that used in the 2'-deoxyuridine series[1]. The preparation of the starting compound *I* was effected by modification of a reported procedure[3]. As inferred from the literature[3] and our own experiments in the 2'-deoxyuridine series[1], the use of a monosodium salt of uracil nucleosides protected on all hydroxylic functions

* Part XVI in the series Immunosuppressant Antigene Conjugates; Part XV: J. Org. Chem. *33*, 3593 (1968).

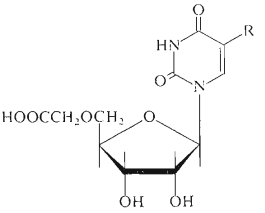
except for that at position 5′ in the reaction with sodium chloroacetate in dimethylformamide or dimethyl sulfoxide results in a predominant substitution of the heterocycle at position N^3 while the required product of the reaction on the alcoholic group at position 5′ can be obtained with the use of the disodium salt of the starting nucleoside. Even under this condition, the required product is accompanied by the $N^3,O^{5'}$-disubstituted by-product, the amount of which can be suppressed by a suitable solvent and molar ratio of the nucleoside to sodium chloroacetate. In the preparation of compound *I*, it was most advantageous to use the disodium salt of 2′,3′-O-isopropylideneuridine (obtained *in situ* by reaction with two equivalents of sodium hydride in dimethyl sulfoxide) and an equimolar amount of sodium chloroacetate. After removal of the protecting group by acidic hydrolysis, the reaction mixture contained 5′-O-carboxymethyluridine (*I*) and a small amount of uridine; no $O^{5'},N^3$-bis(carboxymethyl)uridine was formed under these reaction conditions.

Analogously to the corresponding 2′-deoxy derivative[1], 5′-O-carboxymethyl--5-fluorouridine (*II*) was prepared by reaction of the lithium salt of compound *I* with an equimolar amount of fluorine in acetic acid. Owing to the presence of the 5-fluorouracil ring, the acidity of the reaction product is higher than that of the starting material. Compound *II* was therefore separated from a small amount of the unreacted starting compound *I* and fluoride ions by chromatography on a column of DEAE-cellulose and obtained as a chromatographically and electrophoretically homogeneous triethylammonium salt exhibiting UV spectrum characteristic of 5-fluorouracil derivatives substituted at position N^1.

Whereas the bromination of 5′-O-carboxymethyl-2′-deoxyuridine with bromine in dimethylformamide readily affords the corresponding 5-bromo derivative[1], complications were surprisingly encountered in the *ribo* series. Thus, the reaction of the lithium salt of compound *I* with elemental bromine in dimethylformamide unexpectedly results in destruction of the molecule and disappearence of the UV absorption indicating a change in the chromophoric system of the heterocycle. The formation of a 4,5-dihydrouridine derivative by addition of bromine to the double bond can be excluded since the UV absorption cannot be restored by refluxing the evaporated reaction mixture in neutral, acidic or alkaline medium or by the action of a tertiary base in an anhydrous aprotic solvent; more likely, an irreversible opening of the heterocycle occurred. Similar cases have been reported[4] in the literature to proceed under more vigorous conditions. The course of the bromination does not change when the free acid *I* is used as the starting compound. The reason of this difference between the ribo and 2-deoxyribo derivatives is not known. 5′-O-Carboxymethyl-5-bromouridine (*III*) was finally prepared by bromination of compound *I* with elemental bromine in 80% aqueous acetic acid. The product was isolated in the form of an analytically pure lithium salt, homogeneous on chromatography and

electrophoresis. The UV spectrum corresponded to those of 5-bromouridine derivatives.

5′-O-Carboxymethyl-5-iodouridine (*IV*) was prepared similarly to the 2′-deoxy analogue by reaction of the acid *I* with elemental iodine in acidic aqueous dioxane solution. The reaction product was isolated in the form of the lithium salt and exhibited the expected UV spectrum corresponding to that of 5-iodouridine. The lithium salt of compound *IV* was analytically pure and homogeneous on chromatography and electrophoresis.



I, R = H
II, R = F
III, R = Br
IV, R = I

The above reported 5′-O-carboxymethyluridine derivatives *II*–*IV* are suitable substrates for the bonding to proteins by the method of activated esters or mixed anhydrides.

EXPERIMENTAL

Solutions were taken down on a rotatory evaporator at 40°C/15 Torr unless stated otherwise. Substances were dried at 0·1 Torr over phosphorus pentoxide. Paper chromatography was performed by the descending technique on paper Whatman No 1 in the solvent system S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). Paper electrophoresis was carried out by the reported technique[5] on paper Whatman No 3 MM (20 V/cm, 1 h) in 0·1M triethylammonium hydrogen carbonate (pH 7·5); the electrophoretical mobility values E_{Up} refer to uridine 3′-phosphate. The chromatography on DEAE-cellulose was performed on a 80 × 4 cm column of the high capacity Cellex D (Calbiochem, Los Angeles, USA) with the use of a linear gradient of triethylammonium hydrogen carbonate (2 l of water in the mixing chamber, 2 l of the buffer solution of the final concentration in the reservoir) at the rate of 3 ml per min, the 30 ml fractions being taken in 10 min intervals, and the course of the elution being checked by the Uvicord apparatus (LKB, Uppsala, Sweden). In the chromatography on a column of Dowex 50 × 8 ion exchange resin (100–200 mesh), the resin was prewashed with 2M-HCl and water to the loss of conductivity and UV absorption. The UV spectra were taken in aqueous solutions on a Specord apparatus (Carl Zeiss, Jena, German Democratic Republic).

5′-O-Carboxymethyluridine (*I*)

To a stirred solution of 2′,3′-O-isopropylideneuridine[6] (10·0 g; 35 mmol) in dimethyl sulfoxide (180 ml), sodium hydride (1·68 g; 70 mmol) was added, the stirring continued at room temperature under exclusion of atmospheric moisture (calcium chloride tube) and the mixture treated with dry sodium chloroacetate[1] (4·1 g; 35 mmol). The stirring was continued for 30 h at room temperature under calcium chloride tube and ethanol (10 ml) was added. The mixture was neutralised with acetic acid and evaporated at 90°C/0·1 Torr. The residue was refluxed in 80% aqueous acetic acid (100 ml) for 2 h, the mixture evaporated under diminished pressure, and the residue coevaporated with two 50 ml portions of water. The final residue was dissolved in water (50 ml), the solution adjusted to pH 6 by means of triethylamine and applied to a column (200 ml) of Dowex 1 × 2 ion exchange resin in the acetate cycle and prewashed with 1% aqueous acetic acid to the disappearance of the UV absorption of the eluate. The column was eluted with 0·5M acetic acid until the UV absorption of the eluate disappeared. The eluate was evaporated under diminished pressure and the acetic acid removed by coevaporation of the residue with two 50 ml portions of water. The residue was dissolved in water (50 ml) and the solution applied to a column (200 ml) of Dowex 50 × 8 (H^+) ion exchange resin (*vide supra*). The column was eluted with water until the UV absorption of the eluate disappeared. The eluate was evaporated under diminished pressure, the residue coevaporated with three 20 ml portions of water, then dissolved in water (50 ml), the solution adjusted to pH 8 by the addition of 10% aqueous lithium hydroxide, and evaporated under diminished pressure. The residue was coevaporated with three 50 ml portions of ethanol, then dissolved in hot methanol (100 ml), and the solution precipitated with acetone (700 ml). The precipitate was collected with suction, washed with 200 ml portions of acetone and ether, and dried under diminished pressure. Yield, 9·4 g (30·5 mmol; 87%) of the lithium salt of compound *I*; R_F value 0·31 (Urd, R_F 0·44); E_{Up} 0·50. For $C_{11}H_{13}LiN_2O_8$ (308·2) calculated: 42·86% C, 4·25% H, 9·09% N; found: 43·15% C, 4·38% H, 9·24% N. UV spectrum (pH 2, pH 7): λ_{max} 261 nm (ε_{max} 10000). Content (by spectrophotometry): >95%.

5′-O-Carboxymethyl-5-fluorouridine (*II*)

A solution of fluorine (80 mg; 2·1 mmol) in acetic acid was added to a solution of the lithium salt of compound *I* (2 mmol) in acetic acid (50 ml), the mixture kept at room temperature for 1 h, and evaporated under diminished pressure. The residue was coevaporated with acetic acid and ethanol (three 20 ml portions each) and then dissolved in ethanol (20 ml). Triethylamine (0·5 ml) was added, the mixture kept at room temperature for 30 min, and evaporated under diminished pressure. The residue was coevaporated with three 20 ml portions of ethanol and then dissolved in water (50 ml). The aqueous solution was applied to a column of DEAE-cellulose and the elution performed under the above stated conditions (the linear gradient up to 0·2M triethylammonium hydrogen carbonate). The product-containing fraction (0·12M—0·16M) was evaporated under diminished pressure, the residue coevaporated with four 20 ml portions of ethanol, and finally precipitated from ethanol (2 ml) with ether (100 ml). The precipitate was collected with suction, washed with ether, and dried under diminished pressure. Yield, 160 mg (20%) of the triethylammonium salt of compound *II*, homogeneous on chromatography (R_F 0·24) and electrophoresis (E_{Up} 0·76). Content (by spectrophotometry): 83%. UV spectrum (pH 2, pH 7): λ_{max} 270 nm (ε_{max} 8900).

5′-O-Carboxymethyl-5-bromouridine (*III*)

To a solution of the lithium salt of compound *I* (0·62 g; 2 mmol) in 80% aqueous acetic acid (40 ml), bromine (0·3 ml; 0·942 g; 5·9 mmol) was added, the mixture kept at room temperature

for 5 h, and evaporated under diminished pressure. The residue was coevaporated with four 20 ml portions of water and then dissolved in water (5 ml). The solution was adjusted to pH 8·0 by the addition of 5% aqueous lithium hydroxide, evaporated under diminished pressure, and the residue coevaporated with two 20 ml portions of ethanol. The final residue was dissolved in methanol (4 ml), the solution precipitated with acetone (100 ml), the precipitate collected with suction, washed with acetone and ether, and dried under diminished pressure. Yield, 0·64 g (79%) of the monohydrate of the lithium salt of compound *III*, homogeneous on chromatography (R_F 0·33) and electrophoresis (E_{Up} 0·70). For $C_{11}H_{14}BrLiN_2O_9$ (405·1) calculated: 19·74% Br, 6·92% N; found: 19·79% Br, 6·33% N. UV spectrum (pH 2): λ_{max} 278 nm, λ_{min} 242 nm, ε_{max} 9100.

5′-O-Carboxymethyl-5-iodouridine (*IV*)

A solution of the lithium salt of compound *I* (1·54 g; 5 mmol) in water (5 ml) was applied to a column (50 ml) of Dowex 50 X 8 (H^+) ion exchange resin and the column eluted with water until the UV absorption disappeared. The eluate was evaporated under diminished pressure and the residue coevaporated with dioxane (50 ml). A mixture of the residual foam, dioxane (20 ml), water (5 ml), 65% nitric acid (0·2 ml), and iodine (1·3 g; 5·1 milligramatom) was refluxed for 1 h, diluted with water (200 ml), neutralised with triethylamine, and extracted with four 50 ml portions of chloroform. The aqueous solution was evaporated under diminished pressure and the residue (in 10 ml of water) applied to a column (50 ml) of Dowex 50 × 8 (H^+) ion exchange resin. The column was eluted with water until the UV absorption of the eluate disappeared. The eluate was evaporated under diminished pressure, the aqueous solution of the residue neutralised with 10% aqueous lithium hydroxide, evaporated, the residue coevaporated with three 20 ml portions of ethanol, and finally precipitated from methanol (50 ml) with acetone (500 ml). The precipitate was collected with suction, washed with acetone and ether, and dried under diminished pressure. Yield, 2·1 g of the lithium salt of compound *IV*, homogeneous on chromatography (R_F 0·31) and electrophoresis (E_{Up} 0·60). For $C_{11}H_{12}ILiN_2O_8$ (434·1) calculated: 6·45% N, 29·24% I; found: 6·76% N, 29·50% I. UV spectrum (pH 2): λ_{max} 288 nm, λ_{min} 246 nm. Content (by spectrophotometry, ε_{280} 6600): 80%.

The authors wish to thank Professor Dr V. Herout, Director of the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, for making possible to one of them (H. P.) the work in the Institute. Further thanks are due to DrD. Cech, Humboldt-Universität, Berlin, German Democratic Republic, for the fluorination, and Mrs B. Kopecká for the excellent technical assistance.

REFERENCES

1. Pischel H., Holý A., Wagner G., Cech D.: This Journal *40*, 2689 (1975).
2. Pischel H., Holý A., Wagner G.: This Journal *39*, 3773 (1974).
3. Halford M. H., Jones A. S.: J. Chem. Soc. *1968*, 2667.
4. Otter B. A., Falco E. A., Fox J. J.: J. Org. Chem. *33*, 3593 (1968).
5. Markham R., Smith J. G.: J. Biochem. *52*, 552 (1952).
6. Fromageot H. P. M., Griffin B. E., Reese C. B., Sulston J. E.: Tetrahedron *23*, 2315 (1967).
7. Nuhn P., Schilling E., Wagner G., Hemmerling J., Malberg K., Schimke R., Stülpner H., Ambrosius H.: Pharmazie, in press.

Translated by J. Pliml.