CARBOXYMETHYLATED URACIL, 2'-DEOXYURIDINE AND THEIR 5-FLUORO, 5-BROMO, AND 5-IODO DERIVATIVES*

H.PISCHEL[a], A.HOLÝ[b]**, G.WAGNER[a] and D.CECH[c]

[a] *Sektion Biowissenschaften-Pharmazeutische Chemie, Karl-Marx-Universität, Leipzig, D.D.R.*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague and*
[c] *Sektion Chemie der Humboldt-Universität, Berlin, D.D.R.*

Received January 30th, 1975

Halogenation of 1-carboxymethyluracil (*I*) afforded 1-carboxymethyl-5-fluorouracil (*II*) and 1-carboxymethyl-5-iodouracil (*IV*). 2'-Deoxyuridine (*V*) was converted into 5'-O-pivaloyl-2'-deoxyuridine (*VI*) which was treated with dihydropyran and then methanolysed to afford 3'-O-tetrahydropyranyl-2'-deoxyuridine (*VII*). By reaction of compound *VII* with sodium chloroacetate in the presence of sodium hydride and the subsequent acidic hydrolysis there was prepared 5'-O-carboxymethyl-2'-deoxyuridine (*VIII*). Fluorination of compound *VIII* with elemental fluorine in acetic acid furnished 5'-O-carboxymethyl-5-fluoro-2'-deoxyuridine (*X*) while the corresponding 5-bromo derivative *XI* was obtained by bromination and the appropriate 5-iodo derivative *XII* by iodination in acidic medium.

In connection with investigations on conjugates containing various immunosuppressants covalently bound to protein antigenes, some conjugates of the 6-mercaptopurine[1,2], 6-azauridine[3], and 6-azauracil[4] derivatives with bovine γ-globulin and human serumalbumin have been recently prepared. The condensations were performed by the isocyanate method[1,2,4] or the "mixed anhydride" process[3,5]. The latter route proved as particularly suitable for the use in the field of nucleoside antimetabolites[3]; for these purposes, a method for the preparation of the 6-azauridine and 1-(β-D-arabinofuranosyl)cytosine derivatives[3,6] and their bonding to the above mentioned proteins has been developed. The cytostatic, cancerostatic, and virostatic activity has been reported with some members of the series of 5-halo substituted pyrimidine bases such as 5-iodouracil, and the corresponding 2'-deoxyribonucleosides. The immunosuppressant activity has been examined in the case of 5-fluorouracil[7-12], 5-fluoro-2'-deoxyuridine[7,13], 5-bromo-2'-deoxyuridine[7,13,14], and 5-iodo-2'-deoxyuridine[7,13]. It was therefore of interest to prepare the carboxyl derivatives of the

* Part VI in the series Immunosuppressant Antigene Conjugates; Part V: see ref.[4].
** To whom the correspondence should be directed.

above mentioned uracil and 2′-deoxyuridine derivatives and attempt their use in the covalent bonding to proteins by the method of mixed anhydrides.

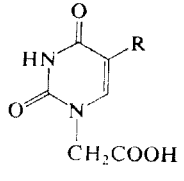
In the uracil series, all the required compounds were prepared from 1-carboxymethyluracil (*I*) as the starting material. Compound *I* is accessible by reaction of uracil with sodium chloroacetate in alkaline medium[15,16]. The reported bromination[17] readily affords 5-bromo-1-carboxymethyluracil (*III*). The fluorination of compound *I* was performed by reaction with elemental fluorine in acetic acid as solvent[18] to afford without any appreciable side reactions the required 1-carboxymethyl-5-fluorouracil (*II*) which was isolated by chromatography on silica gel. The 5-iodo derivative *IV* was prepared by reaction of compound *I* with elemental iodine in aqueous dioxane and in the presence of nitric acid. The structure of the newly prepared compounds was established by elemental analysis as well as by paper chromatography and electrophoresis (the results were in accordance with the expected substituent effects) and also by UV spectra. When compared with compound *I* (λ_{max} 268 nm at pH 7), the absorption maxima of the 5-bromo derivative *III* (285 nm), the 5-fluoro derivative *II* (276 nm), and the 5-iodo derivative *IV* (293 nm) exhibit a bathochromic shift and the spectra display differences in acidic or neutral media and the alkaline medium as typical with 1-substituted derivatives of uracil.

In the 2′-deoxyuridine series, there are several approaches to the preparation of carboxyl derivatives suitable for the covalent bonding to proteins. The 5′-O-carboxymethyl derivatives, the preparation of which is reported in the present paper, appear especially promising from this standpoint since their character is similar to that of the starting physiologically active nucleosides (certainly more similar than in the case of the corresponding 5′-uronic acids[3]) and since the etherified $C_{(5')}$ position might result in stabilisation of the otherwise rather labile nucleoside bond of the 2′-deoxyuridine series. Despite the relative accessibility of 5-halo-2′-deoxyuridine derivatives, some other substances had to be used as the starting material since the $C_{(5)}$-Hal bond would not probably resist the etherification conditions (*cf.*[3,6]) in the presence of a strong base. Consequently, the present syntheses were performed via halogenation of 5′-O-carboxymethyl-2′-deoxyuridine (*VIII*) with the formation of the corresponding 5-halo derivatives.

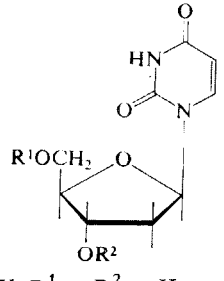
The unambiguous preparation of 5′-O-carboxymethyl-2′-deoxyuridine (*VIII*) required a previous synthesis of such a suitable starting 2′-deoxyuridine derivative which would be substituted at position $C_{(3')}$ by an alkali-stable protecting group. The conventional alkali-labile protecting groups do not survive the reaction conditions[6] and a mixture of products is obtained. In the present case, the 3′-protected derivative was prepared by a two-step procedure. In the first step, 2′-deoxyuridine (*V*) was treated with pivaloyl chloride in pyridine to afford 5′-O-pivaloyl-2′-deoxyuridine (*VI*). The structure of compound *VI* and absence of the isomers was confirmed by analysis and NMR spectra (presence of the free 3′-hydroxylic function). The acid-catalysed reaction of compound *VI* with dihydropyran afforded the fully

protected derivative which was not isolated but directly methanolysed with the formation of 3'-O-tetrahydropyranyl-2'-deoxyuridine (*VII*). In addition to elemental analysis, the structure of compound *VII* was also confirmed by NMR spectra which indicate existence of diastereoisomers on the $C_{(1'')}$ carbon atom of the tetrahydropyran ring.

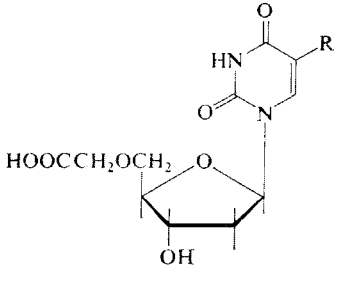
As the most advantageous approach to the preparation of 5'-O-carboxymethyl derivatives (see ref.[3] and quotations given therein), the reaction of compound *VII* with sodium chloroacetate was examined under various conditions. The variant using the sodium alkoxide of compound *VII* prepared *in situ* by reaction with sodium hydride in dimethyl sulfoxide proved as the route of choice. After the reaction with an equimolar amount of dry sodium chloroacetate at room temperature, the protecting tetrahydropyranyl group was removed by the action of 50% aqueous acetic acid without affecting the nucleoside bond under the release of uracil. Composition of the reaction mixture was checked by paper chromatography and electrophoresis. When equimolar amounts of the two reactants (compound *VII* as the di-



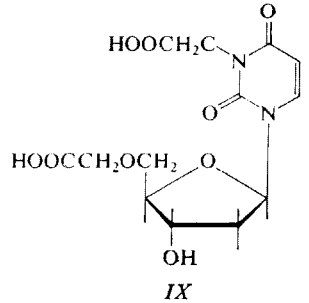
I; R = H
II; R = F
III; R = Br
IV; R = I



V; $R^1 = R^2 = H$
VI; $R^1 = (CH_3)_3CCO$, $R^2 = H$
VII; $R^1 = H$, R^2 = tetrahydropyran-1-yl



VIII; R = H
X; R = F
XI; R = Br
XII; R = I



IX

sodium salt) are used, there is obtained a 62% yield of the product *VIII* along with a 15% yield of compound *IX* as the by-product of the additional substitution at position N^3. With dimethylformamide as solvent (under otherwise analogous conditions), compound *VIII* is obtained in 30% yield only the remainder being the starting material *VII*. Similar differences between reactions in dimethylformamide and dimethyl sulfoxide have been reported by Halford and Jones[19] for the reaction of sodium chloroacetate with 2',3'-O-isopropylideneuridine disodium salt. The N^3-monosubstituted product resulted in that case only when a monosodium salt of the nucleoside derivative was used as the starting material.[19] As shown by electrophoresis under conditions that would favour separation of the two N^1- and N^3-isomers (*i.e.*, at pH higher than the p*K* value of the uracil nucleus), no N^3-monosubstituted isomer of compound *VIII* is formed in the present case. Finally, the $O^{5'}$,N^3-disubstituted derivative *IX* was obtained as the main reaction product when excess sodium chloroacetate was used with respect to the disodium salt of compound *VII*. This situation differs from reactions of 2',3'-O-isopropylidene-6-azauridine disodium salt which afford exclusively the $O^{5'}$-monosubstituted derivative[3] even when excess sodium chloroacetate is used.

On the preparative scale, the products *VIII* and *IX* were separated by chromatography on a column of DEAE-cellulose and isolated in the form of lithium salts.

TABLE I
Chromatography and Electrophoresis

Compound	S_1	S_2	S_3	E_1[a]	E_2[a]	E_3[a]
Uracil	0·62	—	—	0	—	—
Uridine	0·50	0·25	—	0	0·32	0
I	0·42	0·39	0·15	0·74	—	—
II	0·33	0·48		1·05	—	—
III	0·39	0·51	0·23	0·89	—	—
IV	0·42	0·54	0·42	0·74	—	—
V	0·62	0·61	0·11	0	0·34	0
VI	—	0·87	0·67[b]	—	—	—
VII	0·83	—	0·65[c]	—	—	0
VIII	0·44	0·64	—	0·57	0·73	0·50
IX	0·40	0·67	—	0·96	—	0·90
X	0·40	0·69	—	0·89	1·07	—
XI	0·43	0·69	—	0·73	—	—
XII	0·42	0·68	—	0·63	—	—
XIII	0·68	0·74	—	—	—	—

[a] Electrophoretical mobility as referred to uridylic acid; [b] 0·14 in S_4; [c] 0·15 in S_4.

The structure of the two products is in accordance with results of elemental analysis, paper chromatography and electrophoresis indicating with compound *IX* an increased acidity owing to the presence of two carboxylic functions dissociable in weakly alkaline media. The UV absorption spectrum of compound *IX* is characteristic of a N^1,N^3-disubstituted uracil derivative (without hyperchromicity in alkaline media) whereas the hyperchromic effect of the main product *VIII* in alkaline media is in accordance with the structure of N^1-substituted uracil derivatives with a free N^3—H function on the heterocyclic base.

Compound *VIII* was fluorinated analogously to compound *I*, *i.e.*, on treatment with a solution of elemental fluorine in acetic acid[18], to afford the fluoro derivative *X* contaminated with a small amount of compound *VIII*. The pure *X* was isolated as above by preparative chromatography on a column of DEAE-cellulose on the basis of a different acidity of compounds *VIII* and *X* (the increased acidity of compound *X* is due to substitution of the uracil nucleus at position 5 by the electronegative fluoro atom. Compound *X* was isolated as the chromatographically and electrophoretically homogeneous lithium salt and freeze-dried. The ratio of fluorine to nitrogen corresponds to the structure of compound *X* and to the purity higher than 95%.

Bromination of compound *VIII* with elemental bromine in dimethylformamide afforded 5-bromo-5′-O-carboxymethyl-2′-deoxyuridine (*XI*) in a fair yield. The applicability of various methods for iodination of uracil nucleosides and nucleotides to our purposes has been examined on 2′-deoxyuridine (*V*) as the model substance, with a special respect to the yield and extent of the nucleoside bond cleavage. Thus, a modification of the reported[20] procedure afforded 5-iodo-2′-deoxyuridine (*XIII*) of high purity and in fair yield. Under analogous conditions, *i.e.*, on treatment with iodine in aqueous dioxane in the presence of dilute nitric acid and at an elevated temperature, compound *VIII* was successfully converted into 5′-O-carboxymethyl--5-iodo-2′-deoxyuridine (*XII*) which was isolated by chromatography on silica gel and freeze-dried in the form of the ammonium salt.

The chromatographical and electrophoretical behaviour of the 5-bromo derivative *XI* and the 5-iodo derivative *XII* is in accordance with substitution effects (increased acidity). Analogously to compounds *I*–*IV*, the halogenation at position 5 of 5′-O-carboxymethyluridine derivatives results in a bathochromic shift of the ultraviolet maximum; while the unsubstituted compound *VIII* like all the uracil nucleosides exhibits the maximum at 260 nm in neutral solution, the other maxima are situated at 269 nm (the 5-fluoro derivative X), at 282 nm (the 5-bromo derivative *XI*) and at 288 nm (the 5-iodo derivative *XII*). Compounds *X*–*XII* also display a typical hyperchromic effect in alkaline media corresponding to N^1-substituted uracil derivatives with a free N^3—H group.

All the above mentioned properties confirm the correct structure of 5′-O-carboxymethyl-2′-deoxyuridine (*VIII*) and its 5-halo derivatives *X*–*XII*.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Boetius apparatus) and are uncorrected. Unless stated otherwise, solutions were taken down on a rotatory evaporator at 40°C/15 Torr and substances were dried over phosphorus pentoxide at 0·1 Torr. Anhydrous dimethylformamide, dimethyl sulfoxide, and acetic acid were prepared by distillation over phosphorus pentoxide under diminished pressure.

Paper chromatography was performed by the descending technique on paper Whatman No 1 in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2), and S_2, 1-butanol–acetic acid–water (5 : 2 : 3). Thin layer chromatography was carried out on ready-for-use Silufol UV_{254} (Kavalier Glassworks, Czechoslovakia) silica gel sheets in the solvent systems S_3, chloroform–ethanol (9 : 1), and S_4, chloroform–ethanol (95 : 5). Preparative runs were performed on 40 × 16 × 0·3 cm layers of loose fluorescent silica gel (produced by Service Laboratories, Prague - Suchdol, of this Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague). Paper electrophoresis was performed by the technique of Markham and Smith[21] on paper Whatman No 3 MM at 20 V/cm for 1 h in the buffer solutions E_1, 0·1M triethylammonium hydrogen carbonate (pH 7·5), E_2, 0·05M sodium tetraborate (pH 10·5), and E_3, 1M acetic acid. The R_F values and electrophoretical mobilities are summarised in Table I. The DEAE-cellulose chromatography was performed on a column (80 × 4 cm) of Cellex D (standard capacity, Calbiochem, Los Angeles, USA) (HCO_3^-); elution with a linear gradient of triethylammonium hydrogen carbonate (pH 7·5) (2 l of water in the mixing chamber and 2 l of the 0·2M buffer solution in the reservoir); elution rate, 3 ml per min; the fractions were taken in 10 min intervals. The course of elution was checked by the Uvicord apparatus; the corresponding fractions were pooled, taken down, and the volatile buffer removed by coevaporation of the residue with methanol.

The UV spectra (Table II) were taken in aqueous solutions on a Zeiss Specord spectrophoto-

TABLE II

Ultraviolet Absorption Spectra (in water)

Compound	pH 7			pH 12		H, %[c]
	λ_{max},nm	λ_{min},nm	ε_{max}	λ_{max},nm	λ_{min},nm	
I	269	234	10 100	267	244	19
II[a]	276	237	9 750	273	251	19
III	285	245	8 600	281	253	18
IV	293	248	9 300	284	254	21
VIII	260	230	10 000[b]	260	252	18
IX	260	234	10 000[b]	260	236	0
X	269	238	8 900[b]	267	236	19
XI	282	244	9 240[b]	276	253	23
XII	288	248	6 600[d]	278	254	22
XIII	288	248	6 700	280	253	26

[a] Prepared as described in [17], m.p. 260°C (decomp.) (water); [b] values used tentatively for quantitative determinations; [c] hypochromicity at λ_{max}, %; [d] at 280 nm.

meter and (quantitative runs) on a Spectromom 204 apparatus. The NMR spectra were recorded in hexadeuteriodimethyl sulfoxide on a Varian 100 apparatus (chemical shifts in p.p.m., the coupling constants in Hz).

1-Carboxymethyl-5-fluorouracil (*II*)

1-Carboxymethyluracil[15,16] (*I*; m.p. 295–296°C; crystallised from water; 0·68 g; 4 mmol) was dissolved by heating in acetic acid (500 ml) and the solution allowed to attain the room temperature. A saturated solution of fluorine (152 mg of F_2; 4·0 mmol) in acetic acid was then added, the mixture kept at room temperature for 1 h, evaporated under diminished pressure, and the residue coevaporated with three 40 ml portions of ethanol. The final residue was applied (in methanol) to seven plates of silica gel F-254-Merck and chromatographed in the solvent system S_1. The UV-absorbing band of R_F 0·40 was eluted with methanol, the eluate evaporated, the residue applied (in a little water) to a column (30 ml) of Dowex 50 X 8 (H^+) ion exchange resin, and the column washed with water. The effluent was evaporated under diminished pressure and the residue crystallised from ethanol–light petroleum to afford 0·52 g (70%) of compound *II*, m.p. 273 to 276°C (sublimation above 220°C). For $C_6H_5FN_2O_4$ (188·1) calculated: 38·30% C, 2·68% H, 10·10% F, 14·89% N; found: 38·46% C, 2·72% H, 9·95% F, 14·91% N. Mass spectrum: mol. peak *m/e* 188·0233.

1-Carboxymethyl-5-iodouracil (*IV*)

To a solution of 1-carboxymethyluracil (*I*; 0·42 g; 2·5 mmol) in 0·5M nitric acid (5 ml) and dioxane (20 ml), there was added iodine (1·30 g; 5 mmol) and the whole refluxed for 1 h. The mixture was then evaporated under diminished pressure and the excess iodine removed by repeated coevaporations of the residue with ethanol under diminished pressure. The final residue was triturated with a little water, the solid collected with suction, and washed with water. Yield, 0·56 g (75%) of compound *IV*, m.p. 248–249°C (water) (sublimation above 220°C). For $C_6H_5IN_2O_4$ (296·0) calculated: 24·34% C, 1·71% H, 9·46% N, 42·88% I; found: 24·67% C, 1·71% H, 9·22% N, 43·20% I.

5'-O-Pivaloyl-2'-deoxyuridine (*VI*)

To a mixture of 2'-deoxyuridine (*V*; 6·0 g; 26·3 mmol) and pyridine (50 ml), there was added dropwise pivaloyl chloride (3·92 g; 4 ml), the mixture stirred at room temperature until the solid dissolved, and the resulting solution kept overnight. Water (5 ml) was then added, the mixture kept at room temperature for 1 h, evaporated under diminished pressure, and the residue coevaporated with four 25 ml portions of toluene. The final residue was dissolved in chloroform (100 ml), the solution wahed with water (three 20 ml portions), and the aqueous phase extracted with chloroform (20 ml). The chloroform solutions were combined, dried over anhydrous magnesium sulfate, evaporated under diminished pressure, and the residue coevaporated with toluene and ethanol. The final residue was dissolved in the minimum amount of hot ethanol and the solution treated with light petroleum until turbid. Crystallisation at 5 °C yielded 6·0 g (73%) of compound *VI*, m.p. 166–167°C. For $C_{14}H_{20}N_2O_6$ (312·3) calculated: 53·83% C, 6·45% H, 8·97% N; found: 53·47% C, 6·66% H, 8·65% N. NMR spectrum: 2·0 (pent, 1 H; $J_{2'',1'} = J_{2'',3'} = 6{\cdot}5$, $J_{2',2''} = 13{\cdot}0$) $H_{2''}$; 2·72 (dq, 1 H; $J_{2',1'} = 6{\cdot}5$, $J_{2',3'} = 4{\cdot}0$) $H_{2'}$; 3·90–4·30 (m, 4 H) $H_{3'} + H_{4'} + 2\,H_{5'}$; 5·55 (brd, 1 H; $J_{H_5,NH} \leq 1$, $J_{5,6} = 8{\cdot}0$) H_5; 6·14 (t, 1 H; $J_{1',2'} = J_{1',2''} = 6{\cdot}5$) $H_{1'}$; 7·46 (d, 1 H) H_6; 4·0 (br. 1 H) OH; 1·17 (s, 9 H) CH_3.

3′-O-Tetrahydropyranyl-2′-deoxyuridine (*VII*)

To a solution of compound *VI* (2·80 g; 9 mmol) in dioxane (20 ml) there was added dihydropyran (7·5 ml) and then with stirring 0·5 g of *p*-toluenesulfonic acid monohydrate (without cooling). After 15 min, the reaction was quantitative as indicated by thin-layer chromatography in S_3. The mixture was neutralised with 1M methanolic sodium methoxide and evaporated. The residue was triturated with chloroform (100 ml), the extract filtered through Cellite, and the material on the filter washed with chloroform (50 ml). The filtrate and washings were combined, evaporated under diminished pressure, and the residue kept at room temperature in 0·5M methanolic sodium methoxide (30 ml) overnight. The mixture was neutralised by the addition of dry Dowex 50 X 8 (H^+) ion exchange resin, filtered, and the resin washed with methanol (200 ml). The filtrate and washings were combined, treated with triethylamine (0·5 ml), and evaporated under diminished pressure. The residue was mixed with silica gel according to Pitra (particle size, 30–60 micron; 25 g) and chloroform; the resulting suspension was applied to a column of the same silica gel (100 g) packed in chloroform. The column was washed with chloroform and then with a mixture of ethanol and chloroform (5 : 95). The fractions of compounds *VII* were pooled, evaporated under diminished pressure, and the residue coevaporated with ethanol (35 ml). The final residue was dissolved in a minimum amount of hot ethanol, the solution treated with light petroleum until turbid, and allowed to deposit crystals at 5°C. Yield, 2·1 g (76%) of the chromatographically (S_4) homogeneous compound *VII*, m.p. 151–152°C. For $C_{14}H_{15}N_2O_6$ (307·3) calculated: 54·72% C, 4·92% H, 9·15% N; found: 54·65% C, 5·02% H, 9·04% N. NMR spectrum: 5·65 (d, 1 H) H_5; 6·13 (td, 1 H) $H_{1'}$; 7·66 (dd, 1 H) H_6; 9·48 (br d, 1 H) NH.

5′-O-Carboxymethyl-2′-deoxyuridine (*VIII*) and $O^{5'}$,N^3-Bis(carboxymethyl)-2′-deoxyuridine (*IX*)

To a solution of compound *VII* (2·0 g; 6·5 mmol in dimethyl sulfoxide (40 ml) there was added with stirring sodium hydride (0·31 g; 13 mmol) and the stirring was continued at room temperature for 15 min under exclusion of atmospheric moisture. Sodium chloroacetate (dried over phosphorus pentoxide at 90°C/0·1 Torr; 0·76 g; 6·5 mmol) was then added, the mixture stirred at room temperature for 16 h under exclusion of atmospheric moisture, and evaporated at 50°C/0·1 Torr to remove dimethyl sulfoxide. The residue was coevaporated under the same conditions with dimethylformamide (50 ml) and two 25 ml portions of toluene. The final residue was heated in 50% aqueous acetic acid (50 ml) for 3 h at 50°C, the mixture evaporated under diminished pressure, and the residue coevaporated with three 50 ml portions of water. The final residue was dissolved in water, the solution adjusted to pH 8·5 with aqueous ammonia, filtered through Cellite, and the filtrate applied to a column of DEAE-cellulose (see above). The column was washed with water to the drop of the UV absorption of the neutral eluate and then eluted with the linear gradient of the buffer solution. Fractions containing compound *VIII* (0·02–0·07M) and compound *IX* (0·08–0·14M) were pooled and worked up as usual. The residue containing compound *VIII* and chloride ions as contaminants was chromatographed on two preparative layers of silica gel in the solvent system S_1. The product was eluted with 1% aqueous ammonia (250 ml), evaporated, the residue dissolved in water (50 ml), the solution filtered to remove silica gel, and the filtrate applied to a column (150 ml) of Dowex 50 X 8 (Li^+ cycle) ion exchange resin. The lithium salt of compound *VIII* was eluted with water using the Uvicord apparatus. The effluent was evaporated under diminished pressure, the residue dissolved in methanol (5 ml), and the solution diluted successively with ethanol (20 ml), acetone (200 ml), and ether (200 ml). The precipitate was collected with suction, washed with ether, and dried under diminished pressure to afford 1·6 g (62%) of compound *VIII* (spectrophotometrical content, 75%). For $C_{11}H_{13}LiN_2O_7.5\ H_2O$ (382·3) calculated: 7·33% N; found: 7·31% N.

Fractions containing compound *IX* were evaporated, the residue 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 of the effluent dropped. The effluent was neutralised with 10% aqueous lithium hydroxide, the mixture evaporated, the residue dissolved in methanol (5 ml), and the solution precipitated successively with ethanol (100 ml) and acetone (100 ml). The precipitate was collected with suction, washed with ether, and dried under diminished pressure to afford 0·54 g (15·4%) of the lithium salt of compound *IX* homogeneous on chromatography (solvent systems S_1 and S_2) and electrophoresis (buffer solutions E_1 and E_2); content, 77% of *IX* (as determined spectrophotometrically). For $C_{13}H_{14}Li_2N_2O_9.6\,H_2O$ (464·2) calculated: 6·03% N; found: 5·85% N.

Effect of rection conditions. To a solution of compound *VI* (0·5 mmol) in dimethylformamide or dimethyl sulfoxide (3 ml each) there was added sodium hydride (25 mg; 1 mmol) and then, after stirring for 15 min under exclusion of atmospheric moisture, sodium chloroacetate (0·15 g; 1·25 mmol). The mixture was stirred at room temperature for 24 h, diluted with ethanol (5 ml), neutralised with acetic acid, and an aliquot analysed by electrophoresis in the buffer solution E_3. With dimethylformamide as solvent, the mixture contained 70% of compound *VII* and 30% of compound *VIII* while in dimethyl sulfoxide the content was 36% of compound *VIII* and 64% of compound *IX* (as determined spectrophotometrically at 260 nm after elution of spots with 0·01M hydrochloric acid).

5-Fluoro-5'-O-carboxymethyl-2'-deoxyuridine (*X*)

A solution of the lithium salt of compound *VIII* (0·49 g; 1 mmol) in acetic acid (250 ml) was treated with a saturated solution of fluorine (46 mg; 1·2 mmol) in acetic acid, the resulting mixture kept at room temperature for 2 h, and evaporated at 35°C/15 Torr. The residue was coevaporated under the same conditions with three 20 ml portions of ethanol and the final residue was dissolved in water (50 ml). The aqueous solution was adjusted with water to pH 8·5 and applied to a column of DEAE-cellulose (see above). The product was isolated from the 0·12—0·15M fraction and converted to the lithium salt analogously to compound *VIII*. Freeze-drying afforded 122 mg (39·5%) of the lithium salt of compound *X*, homogeneous on chromatography (solvent systems S_1 and S_2) and electrophoresis (buffer solution E_1); content, 95% (referred to compound *X*), as determined spectrophotometrically. For $C_{11}H_{12}FLiN_2O_7$ (310·2) calculated: 6·13% F, 9·03% N; found: 5·69% F, 8·99% N.

5-Bromo-5'-O-carboxymethyl-2'-deoxyuridine (*XI*)

To a solution of the lithium salt of compound *VIII* (0·39 g; 1 mmol) in dimethylformamide (2 ml) there was added with stirring and ice-cooling bromine (0·32 g; 2 mmol), the mixture stirred at 0°C for 3 h, and treated with 10 ml of 0·4M triethylammonium hydrogen carbonate (pH 7·5). The whole mixture was then kept at room temperature for 1 h, evaporated under diminished pressure, and the residue chromatographed on a layer of silica gel (see above) in the solvent system S_1. The UV-absorbing band of compound *XI* (R_F 0·40) was eluted with 1% aqueous ammonia (200 ml), the eluate evaporated, and the residue dissolved in methanol (50 ml) to deposit silica gel which was filtered off. The filtrate was concentrated under diminished pressure to the volume of about 3 ml. The concentrate was made alkaline with methanolic ammonia and diluted with ether (100 ml) to deposit the ammonium salt of compound *XI*. The salt was kept in a refrigerator overnight, collected with suction, washed with ether, and dried under diminished pressure. Yield, 0·25 g (55%) of the ammonium salt of compound *XI*; content, 82% (spectrophotometrically, referred to *XI*). For $C_{11}H_{16}BrN_3O_7.5\,H_2O$ (472·3) calculated: 16·96% Br, 8·90% N, N/Br 3·00; found: 16·79% Br, 9·20% N, N/Br 3·12.

5-Iodo-5′-O-carboxymethyl-2′-deoxyuridine (*XII*)

A solution of the lithium salt of compound *VIII* (0·39 g; 1 mmol) in water (5 ml) was applied to a column (50 ml) of Dowex 50 X 8 (H^+) ion exchange resin and the column was eluted with water to the drop of the UV absorption. The eluate containing the free acid *VIII* was evaporated under diminished pressure and the residue coevaporated with dioxane (20 ml). A mixture of the final residue, 0·5M nitric acid (1 ml), dioxane (4 ml), and iodine (0·26 g; 1 mmol) was then refluxed for 1 h, cooled down, adjusted to pH 8 by the addition of triethylamine, and evaporated under diminished pressure. The residue was dissolved in water (40 ml), the aqueous solution extracted with chloroform (20 ml portions) to remove iodine, and then evaporated under diminished pressure. The residue was chromatographed on a layer of silica gel in the solvent system S_1. Band of the product (R_F 0·40) was eluted with 1% aqueous ammonia (200 ml), the eluate evaporated, and the residue dissolved in methanol (50 ml) to deposit silica gel which was filtered off. The filtrate was concentrated under diminished pressure to the volume of about 3 ml and the concentrate made alkaline by the addition of methanolic ammonia to deposit the product in the form of a gel which was dissolved with hot methanol. Precipitation with ether (100 ml) afforded the ammonium salt which was collected with suction, washed with ether, and dried under diminished pressure. Yield, 0·38 g (73%) of the ammonium salt of compound *XII* homogeneous on chromatography (solvent system S_1 and S_2) and electrophoresis (buffer solution E_1); content, 83% (on spectrophotometry, referred to *XII*). For $C_{11}H_{16}IN_3O_7.5\,H_2O$ (519·3) calculated: 8·09% N, 24·46% I, N/I 3·00; found: 8·25% N, 24·38% I, N/I 3·10.

5-Iodo-2′-deoxyuridine (*XIII*)

A solution of 2′-deoxyuridine (*V*; 0·11 g; 0·5 mmol) in 0·5M nitric acid (1 ml) and dioxane (4 ml) was treated with iodine (0·26 g; 1 mmol), the whole refluxed for 1 h, evaporated, and the residue coevaporated with ethanol. The final residue was recrystallised from water to afford 0·12 g (68%) of compound *XIII*, m.p. 185°C (decomp.); reported[22], m.p. 160°C. For $C_9H_{11}IN_2O_5$ (354·1) calculated: 30·52% C, 3·13% H, 35·84% I, 7·91% N; found: 30·92% C, 3·34% H, 36·01% I, 8·17% N.

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 scientific visit of H. P. in the Institute. The excellent technical assistance of Mrs B. Kopecká and Mrs T. Morgner is gratefully acknowledged.

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Translated by J. Pliml.