UNNATURAL NUCLEOSIDES AND NUCLEOTIDES. III. PREPARATION OF 2-^{14}C AND 4-^{14}C LABELLED 5-ALKYLURACILS AND 5-ALKYL-2'-DEOXYURIDINES*

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SUMMARY

2-^{14}C Labelled 5-alkyluracils were prepared by condensation of the diethylacetals of α-formyl-carbonic acid esters with ^{14}C-thiourea. Compounds labelled at 4-C were synthesized by condensation of the labelled carboxylic acid derivatives with thiourea.

β-Anomers of 5-alkyl-2'-deoxyuridines were obtained in a fairly good radiochemical yield. Alkyl substituents ranged from methyl to tetradecyl, isopropyl and tert-butyl.

Key words: 5-alkyluracils, 5-alkyl-2'-deoxyuridines, CO_2-exchange 2-^{14}C, 4-^{14}C-labelled.

* For Parts I and II see Ref. 3 and 4, respectively.

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INTRODUCTION

The β-isomers of 5-alkyl-2'-deoxyuridines are compounds of different therapeutical interest. For example many of them have antiviral effect against Vaccinia and Herpes simplex and they are not toxic (1,2). The synthesis of the compounds in larger amounts has been elaborated (3,4). Study of the in vitro or in vivo mechanism of activity of these compounds is much more effective in their labelled form. During the metabolism, conversion of the molecules may take place through splitting, therefore better results could be attained by labelling at different positions. With this in view the synthesis was carried out as follows.

The synthesis of 2-^{14}C labelled uracils was based upon the condensation of ^{14}C-thiourea with the diethylacetals of α-formyl-carbonic acid esters. For 4-^{14}C labelling we used the method of Szabolcs, Szammer and Noszkó (5) developed for the exchange of COOH group for ^{14}COOH group in aliphatic carboxylic acids. These acids were used for preparing the corresponding diethylacetals and the bases as described above. We prepared the deoxy-nucleosides by condensation of the protected α,β-halogenated deoxyribose with the silylated base in the presence of $HgBr_2$ and molecular sieves as described earlier by Szabolcs, Sági and Ötvös (3). In this way we could separate the β-anomers of 5-alkyl-2'-deoxy-uridines in good yields.

RESULTS AND DISCUSSION

The generally applied procedure for the preparation of 2-^{14}C-uracils is the condensation of 2-^{14}C-thiourea with the sodium-salt of α-formyl-carboxylic acid esters as described first by Burckhalter and Scarborough (6) and Johnson and Hemingway (7). By this method the yields were rather low (10-20 %) especially in the case of uracils containing longer alkyl chains(6),

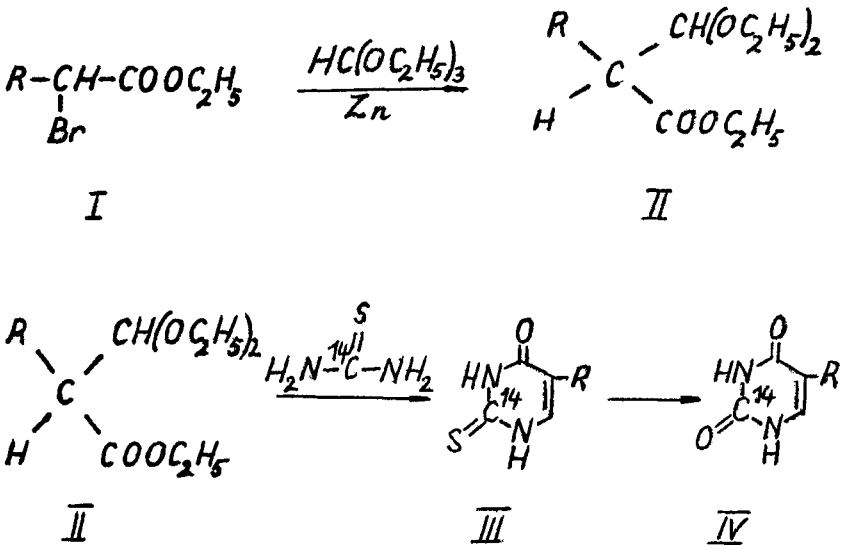
isopropyl or tert-butyl groups (8) in position 5. The method of Deno (9) and Benett (10) applied for the synthesis of 2-thiothymine by condensing thiourea with ethyl 3,3-diethoxy-2-methylpropionate in the presence of sodium ethoxide gave, however, satisfactory results (with 43-55 % yield). This compound was desulphurized by aqueous monochloroacetic acid according to Wheeler and Liddle (11) which produced 2-^{14}C-thymine in 40-50 % yield.

Our reaction scheme is presented in Figure 1. We used longer refluxing periods both in condensation and desulphurization than the above authors and obtained the compounds IV a-l in better radiochemical yield with an average of 55 %, also in the case of long alkyl and branched chains in position 5 of the uracil ring. The feasibility and good results of these reactions suggest the preference of this method to that of Burckhalter and Scarborough (6).

The exchange of CO_2 for $^{14}CO_2$ in the salts of carboxylic acids is based on the reaction presented in Figure 2 (on top of Fig.). Such reactions were investigated by Szabolcs, Szammer and Noszkó (5). The exchange reaction proceeds as follows: $^{14}CO_2$ is distilled in vacuum on salt. This mixture is then heated at temperatures where the decomposition of the corresponding salt was not more than 1-2 %. The ^{14}C labelled carboxylic acid was liberated by HCl gas. These acids were used then for the synthesis of 4-^{14}C labelled 5-alkyl-uracils according to the reaction scheme in Fig.2 to produce the substituted bases V.a,b,h,l in a yield of 25-35 %.

For the preparation of 5-alkyl-2'-deoxyuridines we used our previous method (3) which is a modification of the "silyl Hilbert-Johnson" reaction. Our method is based on the condensation of 30 % excess of silylated base with 3',5'-di-O-p-chlorobenzoyl-α,β-D-ribofuranosyl chloride in acetonitrile at room temperature for 14 hours by joint application of $HgBr_2$ and molecular sieves (Fig.3). In this way most of the protected β-anomers precipitated

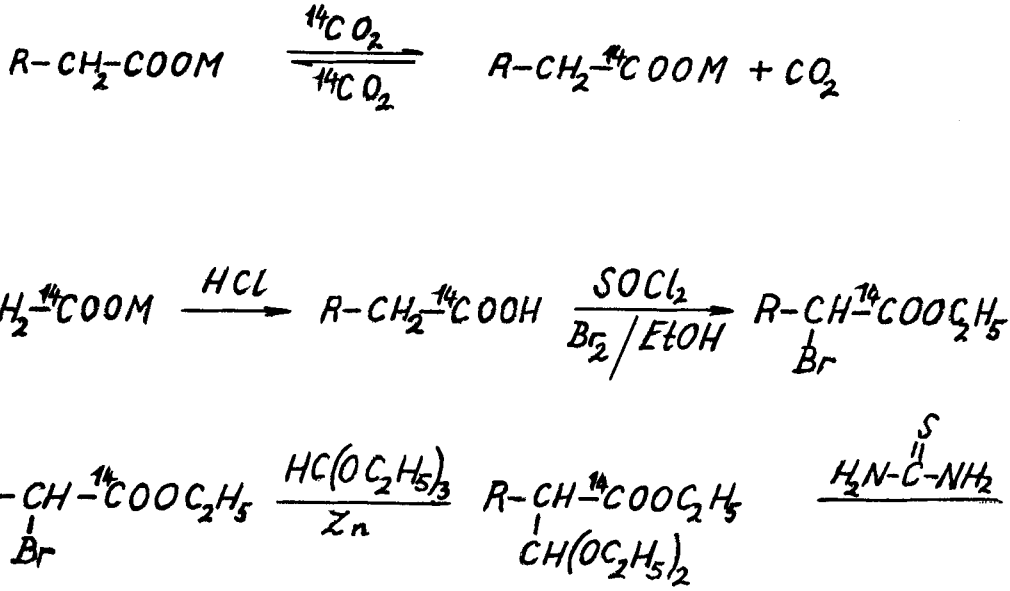
Figure 1.



I II II III IV

R = a CH_3-
b CH_3-CH_2-
c $CH_3-(CH_2)_2-$
d $(CH_3)_2CH-$
e $CH_3-(CH_2)_3-$
f $(CH_3)_3C-$
g $CH_3-(CH_2)_4-$
h $CH_3-(CH_2)_5-$
i $CH_3-(CH_2)_6-$
j $CH_3-(CH_2)_7-$
k $CH_3-(CH_2)_9-$
l $CH_3-(CH_2)_{13}-$

Figure 2.


R-CH2-COOM
14CO2
14CO2
R-CH2-14COOM + CO2
R-CH2-14COOM
HCl
R-CH2-14COOH
SOCl2
Br2/EtOH
R-CH(Br)-14COOC2H5
R-CH(Br)-14COOC2H5
HC(OC2H5)3
Zn
R-CH(CH(OC2H5)2)-14COOC2H5
H2N-C(=S)-NH2
ClCH2-COOH
H2O
R
HN
N
H
S
O
14C

V

from the solvent in crystalline form in the course of the reaction. Deacylation of the compounds was performed with sodium methoxide. This method was used for condensing both 2-^{14}C and 4-^{14}C labelled base analogues with protected halogenated deoxyribose. For better radiochemical yields two additional steps were added to the procedure. The first was the recovery and re-use of excess unreacted labelled bases, recovered by continuous alcoholic extraction in 25-30 % yield. The second procedure was separation of the unprotected anomeric mixture of 5-alkyl-2'-deoxyuridines by column chromatography. This was the extension of a TLC separation method described previously (3).

EXPERIMENTAL

We used silica gel HF_{254} (Merck, Darmstadt) for thin-layer chromatography and 75-250 mesh silica gel for the column chromatographic separation of anomeric nucleosides. Radioactivity measurements were carried out in a Carbon-Tritium Automatic Gas Analyzer Typ OE-973 (CHINOIN RT, Hungary). All melting points and boiling points are uncorrected.

α-Bromo-carboxylic acid ethyl ester (Ia-i)

To 0.3 mole of carboxylic acid, 38 ml of freshly distilled thionyl chloride was added carefully. The mixture was then warmed up slowly to boiling and was refluxed for 2 hours to remove SO_2 and HCl. After the mixtures cooled to room temperature, 1-2 crystals of I_2 and then 16.1 ml of Br_2 were added dropwise in the course of 3 hours. This was followed by a refluxing period of 10-12 hours in order to remove HBr; 57 ml of EtOH was then added carefully at room temperature and the mixture was refluxed again for 40 minutes, to remove HCl. The cool solution was poured into a separatory funnel and extracted twice with 50 ml of distilled water. After separation the organic phase was distilled under

Figure 3.

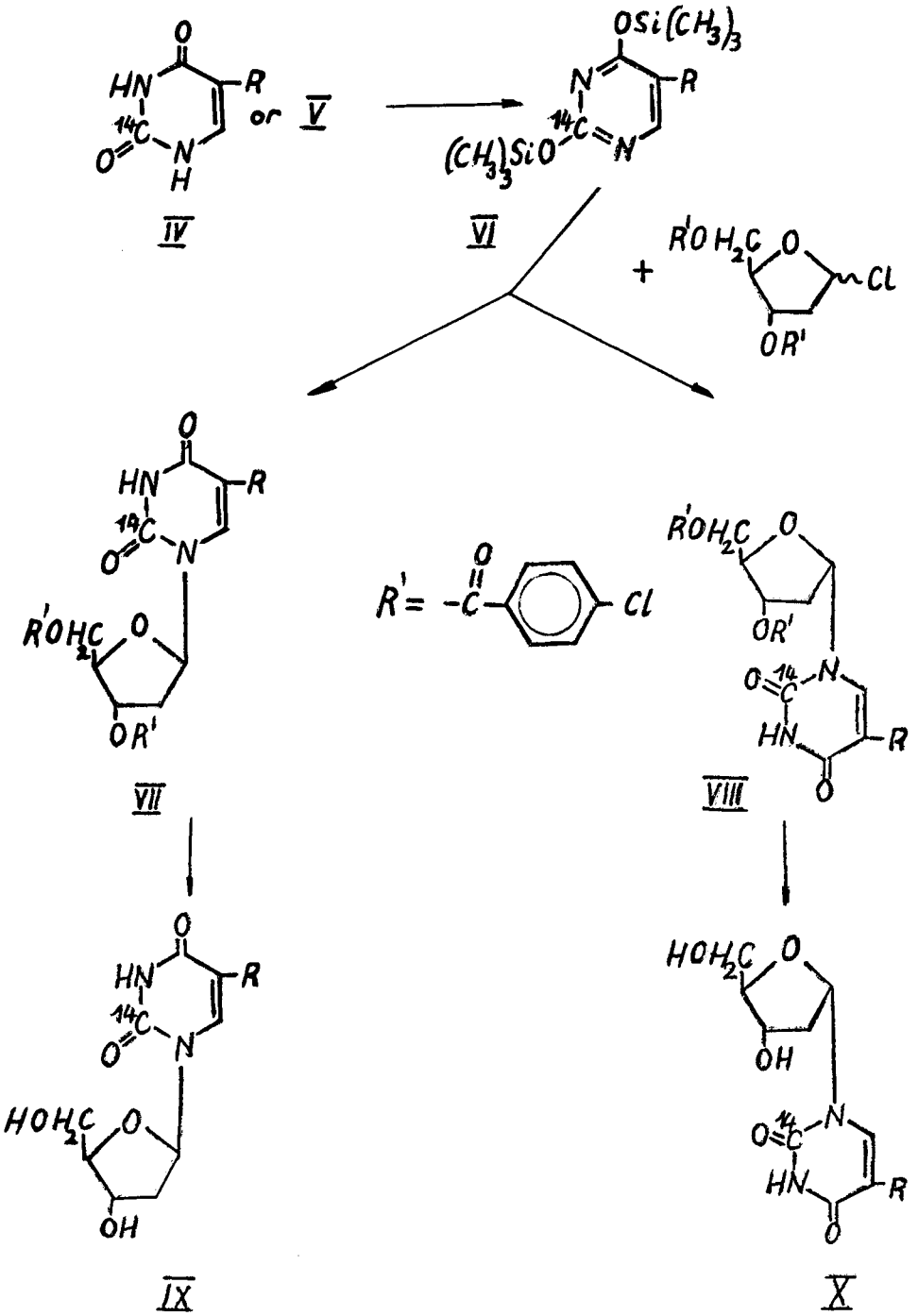


Table 1 Properties and yields of compounds I-IV presented in Fig. 1

Compounds	I		II		IV		
R	B.p. (Hgmm)	Yield[1] (%)	B.p. (Hgmm)	Yield[2] (%)	Radiochemical yield[3] (%)	M.p.(°C)	Rf[4]
a	159/745	89.0	92-6/12	55-60	56.2	315	0.53
b	71/14	90.0	105-9/14	54-58	57.2	300	0.57
c	85/16	87.0	124-8/20	52-55	58.1	292	0.64
d	82/14	90.0	112-5/14	53-57	60.3	310-1	0.60
e	108/22	84.0	125-9/12	58-62	58.7	291	0.71
f	82/12	59.0	115-9/12	50-52	36.3	198-200	0.82
g	125/22	85.0	131-5/10	52-54	66.3	291-2	0.67
h	130/15	81.0	149-53/13	58-60	60.9	273-4	0.72
i	151/20	80.0	118-25/1	58-61	61.1	281-2	0.75
j	148/7	87.0	158-64/7	50-52	58.5	285-7	0.72
k	165/10	75.0	126-34/0.1	54-57	55.5	258-9	-
l	168/3	98.0	146-52/0.1	42-44	52.8	250-2	-

[1] based on starting carboxylic acid

[2] based on I

[3] based on ^{14}C-thiourea (spec. act.: 25 mCi/mmole)

[4] Solvent: water saturated n-butanol:15 N NH_4OH= = 100:1

reduced pressure. Table 1 shows the boiling points and yields of the products.

Dietnyl-acetals of α-formyl-carboxylic acid etnyl esters (IIa-1)

For this type of reactions activated zinc was used. Activation was performed by shaking 65 g of zinc chip in 100 ml of 10 % HCl. The zinc was washed by decantation 3 times with water, and then with methyl alcohol; 1-2 crystals of I_2 were added and the zinc was dried at 100 °C in vacuum for 10-15 minutes; 12.5 ml of abs. benzene was added to 50 g of activated Zn, with stirring; 0.1 mole of α-bromo-carboxylic acid ethyl ester (Ia-1) and 0.12 mole (20 ml) of orthoformic ester in 35 ml abs. benzene were added dropwise in 40-50 minutes. The mixture should be warmed if necessary. Once the reaction has started, it requires ice-cooling. In a few minutes an elastic material precipitated. Stirring was stopped and an additional 12.5 g of activated zinc was added, followed by an 10-12 hour refluxing period. The cool solution was poured by decantation into a flask containing a 50 ml mixture of diethyl ether and ice. The zinc slurry was washed with 30 ml of ether wich was then combined with the solvent mixture. This was acidified at 0 °C with acetic acid, the organic layer was separated and washed with $KHCO_3$ solution (10 %). After drying over $MgSO_4$, the ether was removed by evaporation and the residue was distilled under reduced pressure. Data on b.p. and yields of products are given in Table 1.

[2-^{14}C]2-Thio-5-alkyluracils (IIIa-1)

To 4.41 mmole of IIa-1, 0.3 g (18 mCi) of ^{14}C-thiourea (^{14}C-thiourea was prepared from $Ba^{14}CO_3$ according to reference (12) and had a specific activity of 25 mCi/mmole) and a solution of sodium ethoxide (0.15 g sodium in 7.7 ml of abs. EtOH) were added and refluxed for 40-48 hours, then cooled. Most of the

ethanol was removed from the mixture by evaporation in vacuum. The semisolid residue, was dissolved in 10-15 ml of water and acidified to pH=1 by conc. HCl. The solution was kept at 0 °C for several hours. The crystalline material was filtered off, then washed with water and petroleum ether; 0.3 g of the corresponding 2-thio-5-alkyluracil was dissolved in the remaining solvent by adjusting the pH value to 8-9 with NaOH then the solution was acidified again to pH=1. In this way an additional 0.5 mCi of the labelled product, altogether 8-10 mCi of [2-^{14}C] 2-thio-5-alkyluracil could be obtained.

[2-^{14}C]5-Alkyluracils (IVa-l)

5.0 mmole of IIIa-l in 10 % monochloroacetic acid (7.5 mmole) was refluxed for 20-24 hours, then kept at 0 °C for a few hours. The crystals were filtered off and washed with cold water. The bases obtained in 80-90 % yield were used for the synthesis of deoxynucleosides without further purification. Physical parameters are presented in column IV of Table 1.

[4-^{14}C] 5-Alkyluracils (Va,b,h,l)

10.0 mmole of carboxylic acid potassium salt was placed in a thick-walled glass tube of 10 ml volume on a vacuum stand. The compound was dried in vacuum at 150-200 °C. The tube was then cooled with liquid N_2, $^{14}CO_2$ obtained from 1 mmole (0.197 g) $Ba^{14}CO_3$ and conc. H_2SO_4, was distilled on the salt, the tube was sealed in vacuum. The sealed tube was placed in a metal bomb under 3-4 atm N_2 pressure to balance the inner pressure of the glass tube (4-5 atm). This metal tube was kept in a metal bath for 1-2 hours at an appropriate temperature. The tube was then cooled in liquid N_2, opened and the excess $^{14}CO_2$ was recovered as $Ba^{14}CO_3$. The labelled carboxylic acid salt was washed with water into a flask and dried by evaporation. An equivalent amount of HCl gas obtained from dry NaCl and H_2SO_4 was distilled on the

Table 2 Properties of compounds presented in Fig. 3

	V	IX				
Compounds R	Radiochemical yield[1] (%)	Radiochemical yield[2] (%) β(IX)	Total (IX+X)	Specific activity (mCi/mmole)	M.p.(°C)	Rf[3]
a	30-32	40.1	54.5	4.1	188	0.21
b	32-35	42.1	56.2	3.2	152-3	0.28
c		43.4	59.2	2.5	167.5	0.31
d		45.6	60.8	2.5	183	0.28
e		45.2	62.1	2.6	118-20	0.38
f		-	-	-	163	0.36
g		42.5	60.2	3.0	109-10	0.41
h	28-30	40.3	55.16	2.9	101	0.45
i		35.5	50.1	3.4	133	0.48
j		36.8	54.6	2.9	130	0.50
k		-	-	-	137	0.54
l	24-25	-	-	-	137.5	0.55

[1] based on starting labelled carboxylic acid

[2] based on IV and V

[3] Solvent: Ethyl acetate: methyl alcohol = 95:5

salt and the liberated labelled acid was lyophilised in vacuum in 80-90 %. These labelled acids were used as starting materials for the preparation of [4-^{14}C]5-alkyluracils. Reaction steps were as described above: acid, α-bromocarboxylic acid ester, diethylacetals, 2-thio-5-alkyluracils, 5-alkyluracils. Radiochemical yields of the products are shown in Table 2.

[2-^{14}C] and [4-^{14}C]2,4-bis-O-(Trimethylsilyl)-5-alkyluracils (VIa-l)

These derivatives were prepared according to Nishimura et al. (13) in 85-90 % yield. The compounds were used for further synthesis without distillation.

[2-^{14}C] and [4-^{14}C] 3',5'-Di-O-p-chlorobenzoyl-5-alkyl-2'-deoxy-uridines (VIIa-l and VIIIa-l)

The coupling reactions were carried out as described earlier by Szabolcs et al. (3) with the only modification that the cloroform-insoluble materials of the precipitated protected β-anomer and that of the protected α,β-anomeric mixture were combined and extracted with EtOH in a Soxhlet extractor for 6-8 hours. The alcoholic solvent was evaporated and the labelled 5-alkyluracils were recovered in 20-25 % yields.

[2-^{14}C] and [4-^{14}C]5-Alkyl-2'-deoxyuridines, β-isomers (IXa-l)

Deacylation of the compounds VIIa-l and VIIIa-l were carried out according to Prystas and Sorm (14). Compounds IXa-f were recrystallized from ethanol-petroleum ether, and IXi-k from ethyl acetate. Yields were between 35-45 %, specific activities between 2.5-4.1 mCi/mmole. The physical constants and radiochemical yields of the products are shown in Table 2 (the starting thiourea had a specific activity of 25 mCi/mmole).

Separation of the α/β -anomeric labelled nucleosides (IXa-l and Xa-l) by column chromatography

The column (2.5 cm x 50 cm) was packed with 200-210 ml of 75-250 mesh silica gel and washed with 2-3 volumes of ethyl acetate; 0.5 g of the deacylated mixture of α,β-nucleosides was dissolved in 10-15 ml of EtOH; 10-15 g of silica gel was added to this solution and the EtOH evaporated in vacuum. Ethyl acetate evaporation was repeated 2-3 times. This silica gel containing the mixture of nucleosides was packed on the column which was washed with 2 volumes of EtOAc. The compounds were separated by elution with EtOAc containing 5 % EtOH; 10-15 ml fractions were collected. Separation was controlled in each fraction on TLC with EtOAc:EtOH= =95:5 solvent mixture. The fractions were then evaporated to small volumes, petroleum ether was added and the precipitate was filtered off. In this way 0.2-0.25 g of α- and 0.1-0.15 g of β-nucleosides could be obtained depending on the ratio of anomeric nucleosides in the starting mixture.

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