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SYNTHESIS OF 2′(3′)-O-AMINOACYL DERIVATIVES OF RIBONUCLEOSIDES AND RIBONUCLEOSIDE-5′-PHOSPHATES VIA ORTHOESTER INTERMEDIATES. THE PREPARATION OF 2′(3′)-O-GLYCYL DERIVATIVES OF URIDINE, ADENOSINE AND ADENOSINE-5′-PHOSPHATE

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The esterification of the hydroxylic function in positions 2′ or 3′ of the terminal adenosine unit of the soluble (transfer) ribonucleic acid by an amino acid represents an important step in the biosynthesis of proteins (1). Although no non-enzymatic methods for the attachment of an aminoacyl residue to the cis-diol grouping of soluble ribonucleic acids or smaller ribo-oligonucleotidic fragments has been hitherto described, considerable effort has been devoted to the preparation of the simplest models, i. e. 2′(3′)-O-aminoacyl-ribonucleosides and ribonucleoside-5′-phosphates. The common drawback of these procedures - which consist in the acylation of ribonucleosides or ribonucleoside-5′-phosphates by amino acid phenylthioester hydrochlorides (2-4), N-benzyloxycarbonyl-amino acid anhydrides (5,6) or N-benzyloxycarbonylamino acids and dicyclohexylcarbodiimide (7,8) in the presence of pyridine - is the lack of selectivity (low yields (3,4), necessity of

protecting the 5´hydroxyl group in ribonucleosides against aminoacylation (5-8) and a simultaneous formation of 2´,3´-di--O-aminoacyl derivatives (6)).

In this paper we report a new method for the preparation of the 2´(3´)-O-aminoacylribonucleosides and ribonucleoside--5´-phosphates, based on the acid catalyzed reaction of carboxylic acid orthoesters (II) with ribonucleosides (I) (9-12) and acid hydrolysis (11) of the ribonucleoside-2´,3´-cyclic orthoesters (III) to the 2´(3´)-O-acylribonucleosides (IV). Treatment of uridine (Ia) with two equivalents of ethyl N-benzyloxycarbonylglycine orthoester[x] (IIa) in the presence of a catalytic amount of methanesulphonic acid in dimethylformamide for 20 hours at room temperature gave rise to uridine-2´,3´-cyclic orthoester* (IIIa) (yield 60 %, m. p. 78°C /diffuse/, ultraviolet and infrared spectra were in accordance with the structure IIIa), which is stable in alkali. Acid hydrolysis of the compound IIIa (25 % acetic acid in 1:4 dioxane - water mixture, 6 hours at room temperature) afforded 2´(3´)-O-(N-benzyloxycarbonyl)glycyluridine* (IVa) (yield 75 %, based on uridine, m. p. 90-100°C /diffuse/, infrared and ultraviolet spectra were in agreement with the structure IVa), which is easily hydrolyzed with alkali (e. g. during paper chromatography in the solvent system 2-propanol - ammonia -

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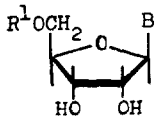
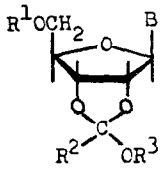
x) Attempted preparations of amino acid orthoesters have failed so far (13). We have now obtained the compound IIa by ethanolysis (14) of ethyl N-benzyloxycarbonylglycine iminoether hydrochloride (15) in ethereal solution (10 hours, 45°C) in 67 % yield (m. p. 38-40°C /light petroleum/, infrared spectrum in agreement with the structure IIa). Satisfactory analytical data were obtained for this compound (and for all marked with*).



I

a: R^1 = H, B = uracil residue
b: R^1 = H, B = adenine residue
c: R^1 = PO_3H_2, B = adenine residue

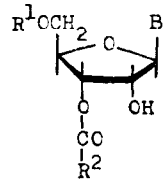
H^+ | $R^2C(OR^3)_3$ II a: R^2 = $CbzNHCH_2$, R^3 = C_2H_5



III

a: R^1 = H, R^2 = $CbzNHCH_2$, R^3 = C_2H_5, B = uracil residue
b: R^1 = H, R^2 = $CbzNHCH_2$, R^3 = C_2H_5, B = adenine residue
c: R^1 = PO_3H_2, R^2 = $CbzNHCH_2$, R^3 = C_2H_5, B = adenine residue
d: R^1 = H, R^2 = NH_2CH_2, R^3 = C_2H_5, B = adenine residue

H^+ | H_2O



IV

a: R^1 = H, R^2 = $CbzNHCH_2$, B = uracil residue
b: R^1 = H, R^2 = $CbzNHCH_2$, B = adenine residue
c: R^1 = PO_3H_2, R^2 = $CbzNHCH_2$, B = adenine residue
d: R^1 = H, R^2 = NH_2CH_2, B = uracil residue
e: R^1 = H, R^2 = NH_2CH_2, B = adenine residue
f: R^1 = PO_3H_2, R^2 = NH_2CH_2, B = adenine residue

+ 2′-isomer

Cbz = $C_6H_5CH_2OCO$

- water 7:1:2) to give uridine (Ia).

Similarly, adenosine (Ib) reacts with the orthoester IIa (4 equivalents) in the presence of more than one equivalent of methanesulphonic acid with the formation of adenosine-2′, 3′-cyclic orthoester* (IIIb) (yield 82 %, amorphous solid, ultraviolet and infrared absorption spectra in accordance with the structure IIIb), which is stable in alkali. The compound IIIb was hydrolyzed by 80 % acetic acid (20 hours at room temperature) to 2′(3′)-O-(N-benzyloxycarbonyl)glycyl-adenosine* (IVb) (yield 75 %, m. p. 151-153°C, ultraviolet and infrared spectra in agreement with the structure proposed), which on treatment with alkali yielded adenosine (Ib).

Hydrogenation of the compounds IVa and IVb (in 80 % acetic acid over palladium on barium sulphate as catalyst (6,7)) gave the 2′(3′)-O-glycyl derivatives of uridine (IVd) and adenosine (IVe). A portion of the reaction mixture after hydrogenation was subjected to paper electrophoresis at pH 3.4 and paper chromatography in the solvent system 1-butanol - acetic acid - water 5:2:3. The 2′(3′)-O-glycyl-uridine (IVd) (or 2′(3′)-O-glycyladenosine (IVe)) was the sole product present. The product obtained by freeze-drying the reaction mixture contained the compounds IVd and IVe contamined with 10 % of uridine (Ia) or adenosine (Ib), respectively (as determined spectrophotometrically after paper electrophoresis at pH 3.4). The compounds IVd and IVe on paper chromatography in the solvent system 2-propanol - - ammonia - water 7:1:2 underwent alkaline hydrolysis and uridine (Ia) (or adenosine (Ib)), glycine and glycinamide were identified as the only products.

Similar reaction can also be effected with ribonucleoside-5′-phosphates as shown in the case of adenosine-5′-phosphate (Ic), which on treatment with 6.5 equivalent of the ortho-ester IIa and more than one equivalent of methanesulphonic acid (in dimethylformamide in the course of 3 days at room temperature) smoothly afforded the corresponding cyclic ortho-ester derivative* IIIc (yield 60 %, as the bis-triethylammonium salt after chromatography on DEAE-cellulose column; ultra-violet spectrum closely resembles that of adenosine-5′-phosphate (Ic)). The correctness of the structure assigned has been further confirmed by the stability of the compound IIIc in alkali and its behaviour on paper chromatography and paper electrophoresis. Hydrolysis of the substance IIIc with 80 % acetic acid for 20 hours at room temperature afforded 2′(3′)-O-(N-benzyloxycarbonyl)glycyladenosine-5′-phosphate (IVc) chromatographically and electrophoretically pure; this compound is easily hydrolyzed in alkali to adenosine-5′-phosphate (Ic). The N-benzyloxycarbonyl group of the compound IVc was removed by hydrogenation under the conditions mentioned above with the formation of 2′(3′)-O-glycyladenosine-5′-phosphate (IVf), which according to the spectrophotometrical estimation after paper electrophoresis at pH 3.4 contained only C.2 % of adenosine-5′-phosphate (Ic). The structure of the compound IVf has been further confirmed by paper chromatography and alkaline hydrolysis to adenosine-5′-phosphate (Ic) and glycine.

The benzyloxycarbonyl group of the cyclic orthoester IIIb can be readily removed hydrogenolytically in methanol

over palladium-on-charcoal. The resulting orthoester derivative IIId was obtained as an amorphous, chromatografically and electrophoretically pure product giving a positive reaction with ninhydrine. This compound is remarkably stable in alkali as well as in acid (e. g. even in 80 % acetic acid for 7 hours at room temperature). This considerable stability of the cyclic orthoester IIId can be explained by protonation of the free amino group of the glycine moiety. It is reasonable to expect the protonated form to be less reactive in acid hydrolysis. The compound IIId seems to be interesting mainly for the possibility of peptide bond formation by attaching the aminoacyl residues to free amino group of the glycine moiety.

It is hoped that the extension of the present work to other amino acids (preparation of their orthoesters) along with attempts to synthesize some ribo-oligonucleotides carrying 2′(3′)-O-aminoacyl residues will be reported later.

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REFERENCES

1. M. B. Hoagland in E. Chargaff and J. B. Davidson, The Nucleic Acids, Vol. III, p. 349. Academic Press, New York (1960).
2. T. Wieland, F. Jaenicke, H. Merz and M. Ossorio, Ann. 613, 95 (1958).
3. T. Wieland, H. Merz and G. Pfleiderer, Chem. Ber. 93, 1816 (1960).
4. H. G. Zachau, Chem. Ber. 93, 1822 (1960).
5. D. H. Rammler and H. G. Khorana, Federation Proc. 19, 349 (1960).
6. D. H. Rammler and H. G. Khorana, J. Am. Chem. Soc. 85, 1997 (1963).
7. E. Ya. Dreiman, V. A. Dmitrieva, S. G. Kamzolova, Z. A. Shabarova and M. A. Prokof'ev, Zh. Obshch. Khim. 31, 3899 (1961).
8. Z. A. Shabarova, V. D. Smirnov and M. A. Prokof'ev, Biokhimiya 29, 502 (1964).
9. J. Žemlička, Chem. & Ind. 1964, 581.
10. C. B. Reese and M. Jarman, Chem. & Ind. 1964, 1493.
11. C. B. Reese and J. E. Sulston, Proc. Chem. Soc. 1964, 216.
12. F. Eckstein and F. Cramer, Chem. Ber. 98, 995 (1965).
13. H. Vargha, A. Balog and I. Balász, Studia Univ. Babes-Bolyai, Ser. 1, No 2, 153 (1959); C. A. 55, 7306d (1961).
14. S. M. McElvain and J. W. Nelson, J. Am. Chem. Soc. 64, 1825 (1942).
15. M. Mengelberg, Chem. Ber. 89, 1185 (1956).