SOME 1-β-D-RIBOFURANOSYL-5-PHENYLCYTOSINES AND -5-(2-CHLOROPHENYL)-2-THIOCYTOSINE

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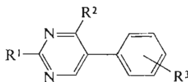
5-Phenylcytidine (*XI*), 5-(4-nitrophenyl)cytidine (*XII*) and 5-(2-chlorophenyl)-2-thiocytidine (*XIII*) were prepared from corresponding trimethylsilyl derivatives *V*—*VII*. Nitro derivative *XII* was converted to amino derivative *XIV* by catalytic reduction. Cytidines *XI*—*XIV* and the starting cytosines *I*—*IV* do not display any *in vitro* inhibitory effect against the influenza virus AWS, virus NDV, vaccinia, herpes simplex and WEE, or *in vivo* effect on mice infected with the herpes simplex virus type 2 (HSV-2) either.

In connection with the investigation of substances with an antiviral effect we prepared a series of 5-phenylcytosines and 5-phenyl-2-thiocytosines, variously substituted on the benzene nucleus, as potential antimetabolites of pyrimidine bases of nucleic acids[1-3]. The substances prepared were submitted to a screening for their antiviral activity, both by the *in vitro* plaque method against the vaccinia virus, the virus of the Newcastle disease (NDV) and the Western equine encephalitis (WEE) and by the *in vivo* method on mice infected with experimental influenza pneumonia[1]. At that time 2-carboxymethylthio-4-amino-5-(4-chlorophenyl)pyrimidine, 5-(4-aminophenyl)cytosine (*III*) and 5-(2-chlorophenyl)-2-thiocytosine (*IV*) seemed promising. However, in consequence of further biological evaluation the effect of the last two

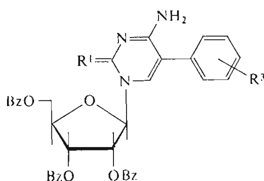


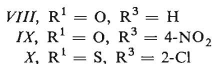
I, R^1 = OH, R^2 = NH_2, R^3 = H
II, R^1 = OH, R^2 = NH_2, R^3 = 4-NO_2
III, R^1 = OH, R^2 = NH_2, R^3 = 4-NH_2
IV, R^1 = SH, R^2 = NH_2, R^3 = 2-Cl
V, R^1 = $OSi(CH_3)_3$, R^2 = $NHSi(CH_3)_3$, R^3 = H
VI, R^1 = $OSi(CH_3)_3$, R^2 = $NHSi(CH_3)_3$, R^3 = 4-NO_2
VII, R^1 = $OSi(CH_3)_3$, R^2 = $NHSi(CH_3)_3$, R^3 = 2-Cl

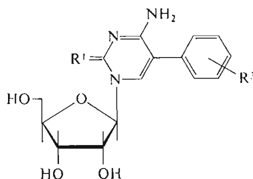
mentioned substances seemed doubtful. Since the assumption that the reason for this could be in the poor solubility of these substances in water seemed probable, we decided to convert them to corresponding ribosides by glycosylation.

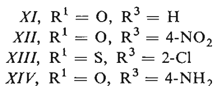
For ribosidation we converted 5-phenylcytosine (*I*), 5-(4-nitrophenyl)-cytosine (*II*) and 5-(2-chlorphenyl)-2-thiocytosine (*IV*) to persilylated derivatives *V*–*VII* using hexamethyldisilazane as reagent. The first two compounds were then glycosylated with 2,3,5-O-tribenzoyl-D-ribofuranosyl chloride in acetonitrile, while in the case of compound *VII* we had to use 2,3,4-O-tribenzoyl-D-ribofuranosyl acetate and stannic chloride. The tribenzoyl-D-ribofuranosyl derivatives *VIII*–*X* formed were isolated by column chromatography on silica gel and then debenzoylated with methanolic ammonia to 5-phenylcytidine (*XI*), 5-(4-nitrophenyl)cytidine (*XII*) and 5-(2-chlorophenyl)-2-thiocytidine (*XIII*). Nitro derivative *XII* was reduced catalytically to 5-(4-aminophenyl)cytidine (*XIV*).

Cytidines *XI*–*XIV* and the corresponding starting cytosines *I*–*IV* were tested for their antiviral activity. The *in vitro* testing was carried out on tissue cultures of chicken fibroblasts using the method of the inhibition of plaque formation[4–7] against the virus of the influenza type AWS and further against the viruses NDV, vaccinia, herpes simplex type 2 (Janda) and WEE. The criterion of the effect consisted in the prevention of plaque formation or the reduction of their size. As a second method the test tube method was used in which the effect against the virus of herpes 1 (HSV-1) Kupka was observed on tissue cultures of human embryonal









lungs. The effect against the herpes HS-70 (HSV-1) Benda virus was tested on tissue cultures of rabbit fibroblasts. The effect against the virus of influenza A_2 *Victoria* was tested on tissue cultures of monkey kidneys. The evaluation by *in vivo* tests was carried out on mice SPF, weighing 15 g, infected by the tail-method according

to Yoshimura[8] with the virus of herpes simplex type 2 (HSV-2), which served as a model of the virus causing herpes dermatitis and encephalitis. In all the four pairs of the substances tested antiviral activity could not be proved either by *in vitro* or *in vivo* tests.

EXPERIMENTAL

The melting points were determined on a Mettler FP 2 apparatus. The ^{1}H NMR spectra were measured on a Varian XL-200 (200 MHz) spectrometer in hexadeuteriodimethyl sulfoxide, using tetramethylsilane as internal reference. The exchangeable hydroxyl and amino group protons were assigned by measuring the spectra after addition of tetradeuterioacetic acid. Chemical shifts (in ppm) and coupling constants (in Hz) were obtained by first order analysis.

5-Phenylcytidine (*XI*)

A mixture of 5-phenylcytosine (1·12 g, 6 mmol), ammonium sulfate (0·02 g) and hexamethyldisilazane (25 ml) was refluxed until dissolved (10 h). The pure solution was evaporated and this was repeated with two additional 25 ml portions of toluene, under reduced pressure. The residue was dissolved in acetonitrile (10 ml) and then mixed with a solution of 2,3,5-O-tribenzoyl-D-ribofuranosyl chloride in acetonitrile (20 ml). 2,3,5-O-Tribenzoyl-D-ribofuranosyl chloride was prepared by saturation of 1-O-acetyl-2,3,4,5-O-tribenzoyl-D-ribofuranose (3·02 g, 6 mmol) in toluene (60 ml) with dry hydrogen chloride at 0–5°C. The next day the mixture was again saturated with hydrogen chloride and then evaporated under reduced pressure. Toluene (20 ml) was added and the evaporation repeated. The mixture containing 2,3,5-O-tribenzoylribofuranosyl chloride and silylated 5-phenylcytosine was allowed to stand for 2 days, then evaporated under reduced pressure and the residue decomposed with a mixture of methanol (15 ml), water (4 ml) and $NaHCO_3$ (0·6 g). Evaporation under reduced pressure and repeated evaporation with ethanol (3 × 20 ml) gave a semisolid residue which was stirred with dichloroethane (25 ml) and filtered off under suction; the residue on the filter was washed with dichloroethane (5 ml). The combined filtrates were evaporated under reduced pressure and the residue (4·08 g) was dissolved in benzene and chromatographed on a silica gel column (120 g, Lachema L 100/160). The column was washed gradually with benzene, a mixture of benzene with increasing content of chloroform, then with chloroform and finally with a mixture of chloroform and methanol (10 : 1). The course of the chromatography was checked by thin-layer chromatography. The eluates containing the benzoylated nucleoside *VIII* were evaporated under reduced pressure and the residue (3·155 g) was dissolved in methanol and saturated with ammonia at 0–25°C. The debenzoylation course was checked by thin-layer chromatography (DC-Fertigplatte, Merck, benzene–methanol 10 : 1). After 2 days the mixture was evaporated in a vacuum and the residue extracted with tetrachloromethane (10 + 5 ml) in order to eliminate benzamide and methyl benzoate. The residue (1·92 g) was crystallized from ethanol and decolorized with Norite. Yield, 1·14 g (59·5%). An analytical sample had m.p. 141–41·8° (ethanol). For $C_{15}H_{17}N_3O_5$ (319·3) calculated: 56·42% C, 5·37% H, 13·16% N; found: 55·84% C, 5·26% H, 13·09% N. ^{1}H NMR spectrum: 3·55 m, 1 H and 3·69 m, 1 H ($C_{(5')}$—H_2), $J_{5',5'} = 12·2$, $J_{5',OH} = 4·8$, $J_{5',4'} = 2·8$; 3·84 m, 1 H ($C_{(4')}$—H); 4·00 m, 2 H ($C_{(2')}$—H + $C_{(3')}$—H); 4·98 d, 1 H (OH), $J = 5·2$; 5·07 t, 1 H ($C_{(5')}$—OH), $J_{OH,5'} = 4·8$; 5·36 d, 1 H (OH), $J = 5·0$; 5·83 d, 1 H ($C_{(1')}$—H), $J_{1',2'} = 3·4$; 6·33 bs, 1 H (NH); 7·31–7·48 m, 6 H (C_6H_5 + NH); 8·00 s, 1 H ($C_{(6)}$—H).

5-(4-Nitrophenyl)cytidine (*XII*)

5-(4-Nitrophenyl)cytosine (1·96 g, 8 mmol) was silylated by refluxing with hexamethyldisilazane (60 ml) and ammonium sulfate (30 mg) for 9 h. Further procedure was the same as in the preceding case. 2,3,5-O-Tribenzoyl-D-ribofuranosyl chloride was prepared from 2,3,5-O-tribenzoyl--D-ribofuranosyl acetate (4·05 g, 8 mmol). Ribosidation of the silylated derivative *II* was carried out in acetonitrile (40 ml) and its course was followed by thin layer chromatography (DC Fertigplatte, Merck, benzene–methanol 10 : 1). After 48 h standing at room temperature the mixture was worked up and the residue (6·19 g) chromatographed on a silica gel column (165 g, Lachema L 100/160). Elution was carried out gradually with benzene, then benzene with increasing amount of chloroform and finally with chloroform with 2% of ethanol. Yield, 4·91 g of crude *IX* which was debenzoylated in methanol (150 ml) saturated with ammonia, by standing overnight, Crystalline *XII* was filtered off under suction and washed with methanol. Yield: 1·14 g (39·1%). An analytical sample had m.p. 230·8–233·1°C (methanol). For $C_{15}H_{16}N_4O_7$ (364·3) calculated: 49·45% C, 4·43% H, 15·37% N; found: 49·16%C, 4·50% H, 15·36% N. ^{1}H NMR spectrum: 3·46 ddd, 1 H; 3·71 ddd, 1 H ($C_{(5')}$—H_2), $J_{5',5'} = 12·2$, $J_{5',OH} = 5·0$, $J_{5',4'} = 2·8$; 3·86 m, 1 H ($C_{(4')}$—H); 4·02 m, 2 H ($C_{(2')}$—H + $C_{(3')}$—H); 4·96 d, 1 H (OH), $J = 5·6$; 5·11 t, 1 H ($C_{5'}$—OH), $J_{5',OH} = 5·0$; 5·39 d, 1 H (OH), $J = 5·0$; 5·81 d, ($C_{(1')}$—H), $J_{1',2'} = 2·6$; 6·79 bs, 1 H (NH); 7·45 bs, 1 H (NH); 7·62 m, 2 H ($C_{(2'')}$—H + $C_{(6'')}$—H); 8·24 s, 1 H ($C_{(6)}$—H); 8·25 m, 2 H ($C_{(3'')}$—H + $C_{5'')}$—H).

5-(4-Aminophenyl)cytidine (*XIV*)

A solution of *IX* (0·728 g, 2 mmol) in ethanol (40 ml) and water (20 ml) was hydrogenated on 5% Pd—C catalyst (0·2 g) at room temperature and 11·7 kPa pressure. The hydrogenation was over after 30 min, the catalyst was filtered off and the filtrate evaporated. The crude product was crystallized from 20% ethanol. Yield: 0·51 g (76·3%) An analytical sample had m.p. 254·6 to 255·2°C (decomp.) from 50% ethanol. For $C_{15}H_{18}N_4O_5$ (334·4) calculated: 53·89% C, 5·42% H, 16·76% N; found: 53·80% C, 5·70% H, 16·89% N. ^{1}H NMR spectrum: 3·54 m, 1 H and 3·72 m, 1 H ($C_{5'}$—H_2), $J_{5',5'} = 12·4$, $J_{5',4'} = 2·2$, $J_{5',OH} = 4·6$; 3·90–4·15 m, 3 H ($C_{(2')}$—H + + $C_{(3')}$—H + $C_{(4')}$—H); 4·98 d, 1 H (OH), $J = 5$; 5·04 t, 1 H ($C_{5'}$—OH), $J_{5',OH} = 4·6$; 5·40 d, 1 H (OH), $J = 4·8$; 6·66 bs, 2 H (NH + $C_{(1')}$—H); 7·33–7·58 m, 4 H ($C_{(3'')}$—H + $C_{(4'')}$—H + + $C_{(5'')}$—H + $C_{(6'')}$—H); 7·87 bs, 1 H (NH); 8·37 s, 1 H ($C_{(6)}$—H).

5-(2-Chlorophenyl)-2-thiocytidine (*XIII*)

Compound *X* (2·38 g, 10 mmol) was refluxed for 30 h in hexamethyldisilazane (100 ml) in the presence of ammonium sulfate (25 mg). The mixture was evaporated in a vacuum, the residue dissolved in dichloroethane (30 ml), freshly distilled $SnCl_4$ (1·5 ml) was added to it and the solution formed was added dropwise at 18–20°C and over 5 min into a solution of 2,3,5-O-tribenzoyl-D-ribofuranosyl acetate (4·6 g) in dichloroethane (100 ml). The reaction course was followed by thin-layer chromatography (Silufol, toluene–acetic acid–water 5 : 5 : 0·5). After 4 h standing the mixture was poured into a saturated solution of $NaHCO_3$ in water (40 ml), shaken with Hyflocel (2·5 g), filtered off under suction and the precipitate on the filter was washed with dichloroethane (4 × 10 ml). The organic layer was separated, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The residue (7·5 g) was freed from traces of humidity by distillation with benzene (50 ml), then dissolved in benzene and put onto a silica gel column (230 g of silica gel Merck). Elution with benzene with a gradient of chloroform and the concentration *in vacuo* of the required fractions gave crude *IX* (2·84 g). This was dissolved in 30 ml

of boiling methanol from which an almost pure product crystallized out after several days standing in an ice-box. Debenzoylation was carried out in a closed pressure vessel with methanolic ammonia. After 20 h standing at room temperature the mixture was evaporated under reduced pressure, the residue was extracted with cold benzene (4 × 10 ml) and then crystallized from 14% ethanol. Yield: 0·906 g (26·9%), m.p. of an analytical sample was 134·6–136·6°C (14% ethanol). For $C_{15}H_{16}ClN_3O_4S$ (369·8) calculated: 48·72% C, 4·36% H, 9·59% Cl, 11·36% H, 8·67% S; found: 48·37% C, 4·45% H, 9·53% Cl, 10·93% N, 8·62% S. ^{1}H NMR spectrum: 3·54 m, 1 H and 3·67 m, 1 H ($C_{(5')}$—H_2), $J_{5',5'} = 12·1$, $J_{5',OH} = 4·8$, $J_{5',4'} = 2·8$; 3·85 m, 1 H ($C_{(4')}$ to H); 4·00 m, 2 H ($C_{(2')}$—H + $C_{(3')}$—H); 5·00 d, 1 H (OH), $J = 5·0$; 5·06 t,1 H ($C_{(5')}$—OH), $J_{OH,5'} = 4·8$; 5·20 bs, 2 H (NH_2); 5·34 d, 1 H (OH), $J = 5·0$; 5·83 d, 1 H ($C_{(1')}$—H), $J_{1',2'} = 3·6$; 6·13 bs, 1 H (NH); 6·62 m, 2 H ($C_{(3'')}$—H + $C_{(5'')}$—H); 6·98 m, 2 H ($C_{(2'')}$—H + $C_{(6'')}$—H); 7·27 bs, 1 H (NH); 7·81 s, 1 H ($C_{(6)}$—H).

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