amino acids VIII. R_f 0.22, mp 219-222°C, yield 0.40 g. Found, %: C 48.58; H 8.87; N 9.43. $C_6H_{13}NO_3$. Calculated, %: C 48.98; H 8.84; N 9.52. IR spectrum (ν, cm^{-1}): 3380 (OH), 1590 (COO^-), 1520 (NH_3^+).

EXPERIMENTAL BIOLOGY

The toxicity of the compounds was determined on white nonpedigree mice, weight 18-20 g each at a single intraperitoneal administration. The antitumorigenic activity was studied on mice of the C57B1/6 line and nonpedigree mice with sarcoma C-37 and C-180, adenocarcinoma of the mammary gland (AK-755), leucoses L-1210 and La. The compounds were administered in doses tested to the animals daily, five times intraperitoneally, in the form of a suspension prepared in a 3% starch solution. In the experiments 220 mice were used. The experimental results were processed statistically.

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SYNTHESIS AND ANTILEUKEMIC ACTIVITY OF ARA-CYTIDYLYL[5' → 5']-6-METHYLPURINE RIBOSIDE

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The antileukemic activity of cytosine arabinoside (ara-C) is due to its conversion in the cell successively to the 5'-mono-, di-, and triphosphates [1]. This reaction competes with the catabolic deactivation of ara-C by deamination in the presence of cytidine deaminase [1]. Thus, the conversion of ara-C to its 5'-monophosphate (ara-CMP) is of crucial importance for the occurrence of physiological activity. This is the main reason for the interest of investigators in the antileukemic activity of ara-CMP [2, 3] and various derivatives at the phosphate group [4-10]. It is also known that the combination of ara-C with thiopurines [11] and their ribosides [12] results in higher antileukemic activity than either of the compounds possess individually. These considerations stimulated our interest in the synthesis and study of the antileukemic activity of compounds containing ara-C and thiopurine nucleosides linked by a phosphate ester bond.

We here describe the synthesis and antileukemic activity of ara-cytidylyl[5' → 5']-6-methylthiopurine riboside (VIII). The dinucleoside monophosphate (VII) was synthesized by the triester method [13], using as the condensing agent quinolylsulfonyl-3-nitro-1,2,4-triazole [14], which was generated in the reaction flask from a mixture of quinoline-8-sulfonyl chloride [15] and 3-nitro-1,2,4-triazole [16]. One of the starting materials was 2'=O, 3'=O,N(4)-triacetylarabinofuranosylcytosine (V) [10]. For the synthesis of the phosphate compound, 2',3'-di-O-isopropylidene-6-methylthiopurine riboside (II) was chosen, obtained in 77% yield from 6-methylthiopurine riboside (I) [17] and acetone in the presence of perchloric acid as catalyst. Phosphorylation of (II) by reaction with 2-cyanoethanol, and chromatography on silica gel gave 2',3'-di-O-isopropylidene-6-methylmercaptopurine riboside-5'-(2-chlorophenyl or 2-cyanoethyl) phosphate (III) in 84% yield. Treatment of the triester (III) with a mixture of pyridine and triethylamine (1:1) [19] gave 2',3'-di-O-isopropylidine-6-methyl-

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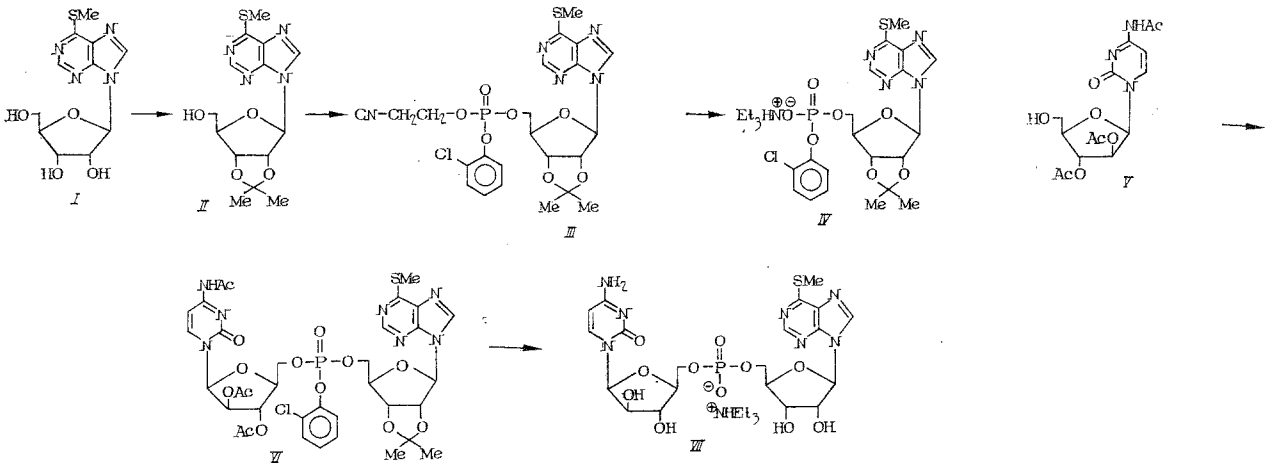
thiopurine riboside-5'-(2-chlorophenyl)phosphate (IV), which was used for the subsequent condensation without further purification. Condensation of the triacetate (V) with the diester (IV) in pyridine in the presence of a mixture of quinoline-8-sulfonyl chloride and 3-nitro-1,2,4-triazole followed by chromatography on silica gel afforded 2'-O,3'-O-N(4)-triacetylarabinofuranosylcytidylyl-5'-[2',3'-di-O-isopropylidine-6-methylthiopurine riboside,5'-(2-chlorphenyl)phosphate (VI) in 90% yield. Successive treatments of the triester (VI) with p-nitrobenzaldoxime [20] to selectively remove the 2-chlorphenyl protecting group from the phosphate, with saturated methanolic ammonia to remove the acetyl protecting groups, and with 80% acetic acid to remove the isopropylidene groups, followed by chromatography on DEAE-Sephadex A-25 in a gradient of 0.001–0.3 M triethylammonium bicarbonate (TEAB) gave the phosphate (VII) as its triethylammonium salt, in 56.7% yield.



The structure of (VII) was confirmed by a combination of spectral methods.

EXPERIMENTAL CHEMISTRY

UV spectra were recorded on a Specord UV-VIS spectrophotometer (East Germany), and the PMR spectrum on a Bruker WM-360 spectrometer (West Germany). TLC was carried out on silica gel F 1500 LS 254 and cellulose F 1440 LS 254 plates (Schleiher and Schüll, West Germany). Column chromatography was carried out on L 40/100 silica gel (Czech SSR).

2',3'-Di-O-isopropylidene-6-methylthiopurine Riboside (II). To a suspension of 2.6 g of 6-methylthiopurine riboside (I) in 100 ml of anhydrous acetone was added with stirring and cooling 1 ml of 60% perchloric acid. Stirring was continued for 2 h at ambient temperature, then the mixture was neutralized with solid $NaHCO_3$ to pH 7.0. The solid was filtered off, and the filtrate evaporated to dryness. The residue after evaporation was chromatographed on silica gel (100 cm^3), and the products eluted with $CHCl_3$ followed by a mixture of $CHCl_3$ and methanol (24:1). The fractions containing the product were combined, evaporated, and crystallized from ether to give 2.27 g (77.3%) of (II), mp 103-104°C (from ether). UV spectrum, λ_{max}, nm (log ε) (CH_3OH): 284 (4.29), 290 (4.28); R_f 0.72 ($CHCl_3$–MeOH, 9:1).

2'-3'-Di-O-isopropylidene-6-methylthiopurine Riboside 5'-(2-Chlorophenyl, 2-cyanoethyl)-phosphate (III). To a solution of 0.61 g of 1,2,4-thiazole (8.88 mmole) in 12 ml of dry pyridine was added 1.01 g (4.14 mole) of o-chlorophenyl phosphodichloridate, the mixture was stirred at ambient temperature for 10 min, cooled to 0°C, and gradually treated over a period of 30 min with a solution of 1 g (2.96 mmole) of (II) in 9 ml of pyridine. Stirring was continued at 0°C for a further 1 h, then 2.10 g (29.6 mmole) of β-cyanoethanol was added, and the solution was stirred at ambient temperature for 16 h. The solution was evaporated to dryness, and co-evaporated with toluene (2 × 5 ml). The residue was dissolved in 150 ml of $CHCl_3$, and extracted with a phosphate buffer of pH 7.0 (2 × 100 ml). The chloroform layer was dried over anhydrous Na_2SO_4, evaporated, and chromatographed on a column of silica gel (60 cm^3), the products being eluted with chloroform. The fractions containing the product were combined and evaporated to give 1.45 g (84.3%) of (III). UV spectrum,

2',3'-Di-O-isopropylidene-6-methylthioopurine riboside-5'-(2-chlorophenyl)phosphate (IV). A solution of 1.37 g of (III) in 30 ml of a mixture of pyridine and triethylamine (1:1) was kept at ambient temperature for 20 h, then evaporated to dryness and co-evaporated with pyridine (10 ml) to give 1.48 g of (IV), which was used for the next step without further purification. UV spectrum, λ_{max}, nm (log ε) (CH_3OH): 284 (4.29), 290 (4.28); R_f 0.22 ($CHCl_3$–MeOH, 9:1).

2'-O-3'-O,N(4)-Triacetylarabinofuranosylcytosine-5'-(2',3'-di-O-isopropylidene-6-methylthiopurine riboside)phosphate (VI). A mixture of 0.43 g (0.68 mmole) of the diester (IV) and 0.21 g (0.56 mmole) of 2'-O-3'-O,N(4)-triacetylarabinofuranosylcytosine (V) was co-evaporated with pyridine (2 × 10 ml), dissolved in 0.56 ml of pyridine, and 0.46 g (4.03 mmole) of 3-nitro-1,2,4-triazole and 0.31 g (1.34 mmole) of quinolinesulfonyl chloride were added. The mixture was stirred at ambient temperature for 20 h, diluted with $CHCl_3$ (100 ml), and extracted with a phosphate buffer of pH 7.0 (2 × 50 ml). The organic layer was dried over anhydrous Na_2SO_4, evaporated, and co-evaporated with toluene (2 × 10 ml), The residue was chromatographed on a column of silica gel (50 cm^3), and the products eluted with chloroform, followed by a gradient of $CHCl_3$ and a mixture of $CHCl_3$–methanol (24:1). The fractions containing the product were combined and evaporated to give 0.44 g (90%) of (VI). UV spectrum, λ_{max}, nm (log ε) (CH_3OH): 251 (4.25), 284 (4.32), 291 (4.33); R_f 0.58 ($CHCl_3$–MeOH, 9:1).

Ara-cytidyl(5' → 5')-6-methylthiopurine Riboside (VII). A mixture of 0.30 g (0.34 mmole) of the triester (VI) and 0.56 g (3.40 mmole) of p-nitrobenzaldoxime was dissolved in 20.4 ml of a mixture of dioxane, triethylamine, and water (1:1:1). The mixture was kept for 20 h at ambient temperature, then evaporated to dryness. The residue was dissolved in 40 ml of a saturated solution of ammonia in methanol, and the mixture was kept for 2 h at ambient temperature, then evaporated to dryness. The residue was treated with 100 ml of a mixture of chloroform and water (1:1), the aqueous layer separated, evaporated to dryness, treated with 20 ml of 80% acetic acid, and heated at 100°C for 1 h. The solution was evaporated, dissolved in 30 ml of an 0.001 M solution of triethylammonium bicarbonate, and chromatographed on DEAE-Sephadex A-25 (40 cm^3) in the HCO_3^- form, the product being eluted with a gradient of 0.001-0.3 M TEAB. The fractions containing the product were combined, evaporated, and co-evaporated with ethanol (3 × 20 ml). The residue was dissolved in 4 ml of a mixture of ethanol and water (1:1), and precipitated in 100 ml of a mixture of ether and ethanol (1:1). The solid was filtered off and dried, to give 0.14 g (56.7%) of (VII), UV spectrum, λ_{max}, nm (log ε) (CH_3OH): 286 (4.32) 293 (shoulder) (4.29); R_f 0.26 (cellulose, n-BuOH–AcOH–H_2O, 5:3:2). PMR spectrum (D_2O + DSS), δ, ppm: 8.61 s and 8.56 s (1H each, H-2,8), 7.46 d (1H, H-6, $J_{6,5}$ = 7.5 Hz), 6.16 d (1H, H-1', $J_{1',2'}$ = 5.0 Hz), 5.94 d (1H, H-1', $J_{1',2'}$ = 5.0 Hz), 5.71 d (1H, H-5, $J_{5,6}$ = 7.5 Hz), 4.91 dd (1H, H-2', $J_{2',3'}$ = 5.0 Hz), 4.80-4.65 (H-3', overlapped by water signal), 4.58 dd (1H, H-3', $J_{3',2'} = J_{3',4'}$ = 5.0 Hz), 4.40 m (1H, H-4'), 4.35 dd (1H, H-2', $J_{2',1'} = J_{2',3'}$ = 5 Hz, 4.19 m (1H, H-4'), 4.10-3.87 m [4H, 2(H-5,5')], 2.65 s (3H, SCH_3).

EXPERIMENTAL PHARMACOLOGY

The antileukemic activity of the dinucleoside monophosphate (VII) was examined in experiments on F_1 hybrid mice (CVA/2 × C_5B1) with transplanted L-1210 leukemia. The drug was administered intraperitoneally in solution, by the following regimes: doses of 5 and 10 mg/kg dialy for six days, and doses of 25 and 50 mg/kg for 3 days. The greatest antileukemic effect was obtained with the dose of 50 mg/kg. The leukocyte count in the peripheral blood of the experimental animals decreased by a factor of 2.1 in comparison with the controls (11.6 ± 0.9 and 24.8 ± 3.2 thousand per 1 mm^3, respectively), together with the numbers of undifferentiated cells (90 ± 60 and 590 ± 120) therein. Leukemic cells characteristic of the L-1210 model leukemia were not found in the treated animals, whereas in the controls there were 490 ± 110 per 1 mm^3 of peripheral blood.

Studies of the bone marrow of the experimental animals likewise showed the absence of leukemic cells, and a reduction in the percentage of undifferentiated hemopoietic elements.

The development of ascitic leukemic tumors in animals treated with the drug was retarded while treatment was continued. The lifespan of the experimental animals was increased at the 25 and 50 mg/kg dose levels, by 39.9 and 54.1%, respectively, in comparison with the controls.

In mouse experiments, (VII) was less active against leukemia than ara-C and ara-CMP [2].

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME 3-FORMYL-8-METHYL-6H-IMIDAZO[1,2-f]XANTHINES

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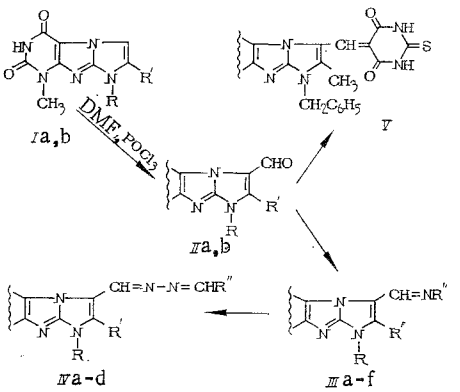
Xanthines annelated at side f by the imidazole ring display various types of biological activity [1-4]. Imidazo[1,2-f]xanthines undergo electrophilic substitution to give a variety of functional derivatives [5]. This communication describes an examination of the formulation of 1,8-dimethyl-2-phenyl- and 1-benzyl-2,8-dimethyl-6H-imidazo[1,2-f]xanthine (Ia, b) and nucleophilic addition reactions of the resulting aldehydes (IIa, b), together with a study of the biological activities of the compounds obtained.

Heating the tricyclic compounds (Ia, b) in a mixture of DMF and phosphoryl chloride (the Vilsmeier reaction) [5] affords the 3-formyl derivatives (IIa, b), the structures of which were confirmed by PMR spectroscopy (disappearance of the C_8 proton). The PMR spectrum of (IIa) (in CF_3COOH) contained signals for the following protons: N_1 and N_8 methyl groups (3.87 and 3.93 ppm, s, 3H), the phenyl substituent (7.7-7.83 ppm, m, 5H), and the formyl group (9.3 ppm, s, 1H). The presence of the formyl group resulted in a low-field shift of the PMR signals in comparison with those of the original compound (Ia) [6].



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