SYNTHESIS OF A NEW POTENTIAL ANTIVIRAL AGENT — 9-ALLYLOXYMETHYLGUANINE

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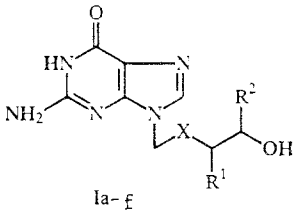
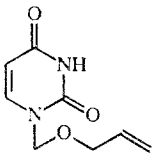
A convenient method has been developed for the synthesis of 9-allyloxymethylguanine. The direct alkylation of the trimethylsilyl derivative of guanine allyloxymethyl chloride gives a 64% yield of 9- and 7-allyloxymethylguanine (3:1). A mixture of 9- and 7-allyloxymethyl-N-acetylguanine (7:4) can be obtained in 56% yield by the condensation of diacetylguanine with allyloxymethyl acetate in dimethyl sulfoxide in the presences of p-toluenesulfonic acid.

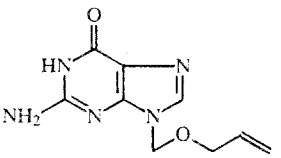
The discovery of the unique antiviral properties of acyclovir [1] has stimulated considerable interest in the synthesis of purine and pyrimidine acyclonucleosides; however, the guaninenucleosides (Ia-f), which are related to acyclovir, are the most effective inhibitors of replication of a variety of herpes viruses.



Ia-f



II



III

Ia X = O, $R^1 = R^2 = H$; b X = CH_2, $R^1 = R^2 = H$; c X = CH_2, R^1 = OH, $R^2 = H$; d X = O, $R^1 = CH_2OH$, $R^2 = H$; e X = CH_2, $R^1 = CH_2OH$, $R^2 = H$; f X = O, $R^1 = H$, $R^2 = CH_2OH$

Despite some differences in metabolism, the mechanism of antiviral action of 9-(2-hydroxyethoxymethyl)- Ia, 9-(4-hydroxybutyl)- (Ib), 9-(3,4-dihydroxybutyl)- (Ic), 9-(1,3-dihydroxypropoxymethyl)- (Id), 9-[1,3-dihydroxy-2(hydroxymethyl)-butyl]- (Ie), 9-(2,3-dihydroxy-1-propoxymethyl)guanine (If), and other compounds which imitate 2′-deoxyguanosine are the same: in cells infected by the herpes viruses, compounds Ia-f are active due to the phosphorylation of viral thymidine kinase, and further, the level of triphosphates inhibits the viral DNA-polymerase [2-5]. The 1-allyloxyalkylpyrimidines that we synthesized earlier [6] do not have hydroxyl groups in the side chain, and consequently cannot be substrates for viral or cellular thymidine kinase; however they show pronounced antiviral activity. Thus, 1-allyloxymethyluracil (II) is active against the simple type I herpes virus, both in vitro and in vivo, and also inhibits the replication of herpes viruses which are stable to the action of acyclovir. In the present work the synthesis of a new potential antiviral agent — 9-allyloxymethylguanine (III) — the purine analog of II is described.

9-Allyloxymethylguanine was obtained by the following route (see top of following page).

The direct alkylation of the trimethylsilyl derivative of guanine with a one-and-one-half excess of allyloxymethyl chloride (IV) in refluxing toluene for 16 h gave a mixture of the 9- and 7-isomers of allyloxymethylguanine (III) in the ratio 5:2 with a total yield of 37.5%. Product yield and regioselectivity of alkylation increased considerably if the toluene solution of the

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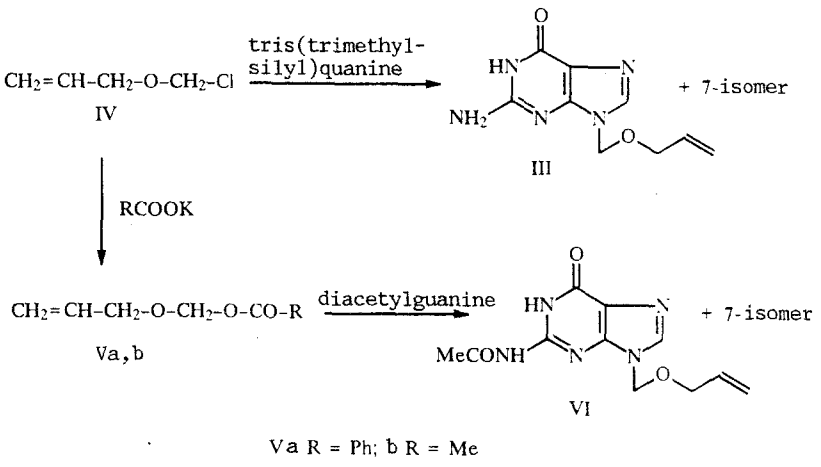
TABLE 1. Total Yield and Ratio of Isomers of Alkylation Products

Substrate*	Alkylating agent	experimental conditions	Total yield, %	Ratio of 9- and 7-isomers
$(Me_3Si)_3G$	$AllOCH_2Cl$	110°C, 16 h	37,5	5 : 2
$(Me_3Si)_3G$	$AllOCH_2Cl$	20°C, 24 h 110°C, 8 h	64,0	3 : 1
Ac_2G	$AllOCH_2OAc$	95...100°C, 16 h	19,6	5 : 2
Ac_2G	$AllOCH_2OAc$	95...100°C, 40 h	55,8	7 : 4
Ac_2G	$AllOCH_2OBz$	95...100°C, 16 h	27,2	5 : 2
Ac_2G	$AllOCH_2OBz$	95...100°C, 40 h	11,6	2 : 1

*$(Me_3Si)_3G$ — tris(trimethylsilyl)guanine, Ac_2G — diacetylguanine.
**$AllOCH_2Cl$ — allyloxymethyl chloride, $AllOCH_2OAc$ — allyloxymethyl benzoate.



Va R = Ph; b R = Me

starting reagents was first mixed for 24 h at room temperature and then refluxed for 8 h. The total yield of the 9- and 7-allyloxymethylguanines in this case was 64% at a molar ratio of the isomers of 3:1 (see Table 1).

The extreme sensitivity of the trimethylsilyl derivative of guanine to traces of moisture and also the instability of allyloxymethyl chloride during storage forced us to use another synthesis, based on the "fusion" method, widely used for the preparation of guanine nucleosides and their acyclic analogs [7-10]. By this method 2-haloalkyl ether is reacted with sodium or potassium acetate in anhydrous dimethylformamide [10], acetone [7], acetic acid [9], or other inert solvent to give the corresponding acetate, which is then condensed with diacetylguanine in a polar solvent or without a solvent in the presence of an acid-type catalyst.

Allyloxymethyl chloride (IV) was obtained in 55% yield by the Henry reaction [11]. Its subsequent reaction with potassium benzoate in dimethylformamide at room temperature gave the allyloxymethyl benzoate (Va) in 66% yield after double distillation in vacuum. The reaction of allyloxymethyl chloride with sodium acetate in anhydrous acetic acid or dimethylformamide also gave the desired allyloxymethyl acetate (Vb), however, because of the closeness of the boiling point of this product and the solvents used we could not obtain an acceptable yield of Vb of sufficient purity. Therefore acylation of allyloxymethyl chloride with sodium acetate was carried out in anhydrous chloroform in the presence of the interphase transfer catalyst — 18-crown-6. The acetate Vb was obtained in 76% yield after double distillation in vacuum.

It is known that the total yield and the ratio of 9- and 7-isomers in the condensation of diacetylguanine with alkoxymethyl acetates is determined to a large extent by the nature of the catalyst and solvent used and also by the temperature of the reaction.

The use of reaction conditions optimum for obtaining acyclovir (stirring a mixture of diacetylguanine, alkoxymethyl acetate, and p-toluenesulfonic acid in the molar ratio 1:2:0.025 in anhydrous dimethyl sulfoxide at 100°C for 16 h [9]), also gave a mixture of 9- and 7-isomers of allyloxymethyl-N-acetylguanine (5:2), however the total yield was 19.6% for allyloxymethyl acetate and 27.2% for allyloxymethyl benzoate. Increasing the reaction time to 40 h increased the total yield of

allyloxymethyl-N-acetylguanines to 55.8% only in the case of allyloxymethyl acetate, with some relative increase in the content of the 7-isomer in the mixture, while for the allyloxymethyl benzoate the yield decreased to less than 10-15%.

Deacetylation was carried out by the ammonolysis of a mixture of isomers of allyloxymethyl-N-acetylguanine (VI) using a saturated methanolic solution of ammonia at 0°C. Pure 9-allyloxymethylguanine was obtained (in 45.2% yield) by fractional crystallization of mixtures of the isomers from methanol.

Thus, the direct alkylation of trimethylsilyl derivatives of guanine by allyloxymethyl chloride appears the most convenient method of synthesizing of 9-allyloxymethylguanine, since it gives the highest yield, better regioselectivity, and does not require the additional stage of deacetylation.

The antiviral properties of these compounds are presently being studied.

EXPERIMENTAL

PMR spectra were recorded on a Tesla BS-567A (100 MHz) in solutions of acetone-D_6 (compound Va and b) and dimethyl sulfoxide-D_6 (compound III and VI); internal and external standard HMDS. Thin layer chromatography on compound VI was run on silufol UV-254 plates in chloroform and ethanol (10:1), and developed in iodine vapor. Silica gel L40/100 (Czechoslovak Socialist Federated Republic) was used for preparative chromatography. The ratio of the isomeric products of alkylation was determined by PMR spectroscopy and microcolumn liquid chromatography on a Milichrome-2 chromatograph, KAX-3.64.3 column, eluent ethyl acetate–methanol–ethylene glycol (85:5:10) [12].

Diacetylguanine was obtained in 78.7% yield by the acetylation of guanine with acetic anhydride in acetic acid [9].

Allyloxymethyl Chloride (IV, C_4H_7ClO). Dry hydrogen chloride was passed at 0-3°C through a stirred mixture of allyl alcohol (50 ml, 0.73 mmole), paraform (27.5 g, 0.92 mmole), and dichloromethane (100 ml) for a period of 4 h until saturated. The reaction mixture was filtered, the organic layer separated, dried with magnesium sulfate, and distilled; the fraction with bp 60-65°C (100 mm Hg) was collected: n_D^{20} 1.4300; d_4^{20} 1.003. Yield 42.8 g (54.8%).

Allyloxymethyl Benzoate (Va, $C_{11}H_{12}O_3$). Allyloxymethyl chloride (20 g, 0.19 mole) was added over a period of 15 min to a stirred suspension of anhydrous potassium benzoate (64 g, 0.4 mole) in anhydrous dimethylformamide (150 ml) at 20-25°C. The mixture was stirred for 8 h at room temperature, filtered and fractionally distilled in vacuum. bp 105-108°C (3 mm Hg); n_D^{20} 1.5102; d_4^{20} 1.089. Yield 23.7 g (65.6%). PMR Spectrum: 4.12 (2H, dt, J = 5 Hz, J = 1 Hz, O–CH_2–C=C), 4.93-5.35 (2H, m, =CH_2), 5.41 (2H, s, O–CH_2–O), 5.53-6.07 (1H, m, –CH=), 7.12-7.56 (3H, m, phenyl), 7.76-8.12 ppm (2H, m, phenyl).

Allyloxymethyl Acetate (Vb, $C_6H_{10}O_3$). Allyloxymethyl chloride (20.0 g, 0.19 mole) was added over a period of 15 min to a stirred mixture of sodium acetate (40.0 g, 0.49 mole), 18-crown-6 (1.0 g, 4 mmole) and anhydrous chloroform (100 ml) at 20-25°C. The mixture was stirred for 12 h at room temperature, filtered, evaporated, and double-distilled under vacuum. The fraction with bp 65-70°C (50 mm Hg); n_D^{20} 1.4186; d_4^{20} 0.963 was collected. Yield 18.6 g (76.1%). PMR Spectrum: 1.96 (3H, s, CH_3); 4.02 (2H, dt, J = 5 Hz, J = 1 Hz, O–CH_2–C=C), 4.89-5.30 (2H, m, =CH_2), 5.12 (2H, s, O–CH_2–O), 5.52-5.96 ppm (1H, m, –CH=).

9- and 7-Allyloxymethyl-N-acetylguanine (VI, $C_{11}H_{13}N_5O_3$). A mixture of diacetylguanine (1.2 g, 5.1 mmole), allyloxymethyl acetate (1.35 ml, 10.3 mmole), p-toluenesulfonic acid monohydrate (0.05 g, 0.25 mmole), and anhydrous dimethyl sulfoxide (10 ml) was stirred in a nitrogen atmosphere at 95-100°C for 40 h, filtered, and the filtrate evaporated under vacuum. The residue was chromatographed on a silica-gel column (20 × 1.5 cm) and eluted with chloroform–ethanol (10:1). The eluate was concentrated to give a bright yellow oil which was dissolved in warm 96% ethanol (5 ml), and then cooled for 24 h at –10°C; the material which precipitated was filtered off and repeatedly recrystallized from ethanol (5 ml) to give 0.75 g (55.8%) of a mixture of 9- and 7-allyloxymethyl-N-acetylguanine (7:4), mp 168-170°C. PMR Spectrum: 2.37 (3H, s, CH_3); 4.22 (2H, dt, J = 5 Hz, J = 1 Hz, O–CH_2–C=C), 5.19-6.14 (3H, m, –CH=CH_2), 5.69 (1.25H, s, N–CH_2–O, 9-isomer), 5.89 (0.75H, s, N–CH_2–O, 7-isomer), 8.33 (0.65H, s, H-8, 9-isomer), 8.57 (0.35H, s, H-8, 7-isomer), 11.84 (0.35H, br.s, NH, 7-isomer), 12.01 (0.65H, br.s, NH, 9-isomer), 12.31 ppm (1H, br.s, NH–Ac).

Pure 9-allyloxy-N-acetylguanine was obtained from a mixture of the isomers by fractional crystallization from ethanol, mp 151-153°C.

9-Allyloxymethylguanine (III, $C_9H_{11}N_5O_2$). A mixture of isomers of allyloxymethyl-N-acetylguanine (0.5 g, 3.8 mmole) in methanol (15 ml) was saturated at 0°C with ammonia, and then kept at room temperature for 24 h. The solution was evaporated to dryness, the residue washed with diethyl ether (3 × 5 ml), recrystallized first from a mixture of ethanol and acetic

acid (8:1) and then from methanol to give 0.19 g (45.2%) of 9-allyloxymethylguanine, containing, according to PMR spectroscopy, not more than 5% of the 7-isomer, mp 244-246°C. PMR Spectrum: 4.21 (2H, dt, J = 5 Hz, J = 1 Hz, $O-CH_2-C=C$), 5.21-5.70 (2H, m, $=CH_2$), 5.55 (2H, s, $N-CH_2-O$), 5.74-6.12 (1H, m, $-CH=$), 6.78 (2H, br.s, NH_2), 8.03 (1H, s, H-8), 10.96 ppm (1H, br.s, NH).

9- and 7-Allyloxymethylguanine (III, $C_9H_{11}N_5O_2$). Allyloxymethyl chloride (0.9 g, 8.5 mmole) was added to a stirred solution of the trimethylsilyl derivative of guanine (2.0 g, 5.4 mmole) in absolute toluene (10 ml) in an atmosphere protected from atmospheric moisture. After stirring for 24 h at room temperature, the reaction mixture was refluxed for 8 h, and the solution concentrated under vacuum. The residue was refluxed for 1 h with 96% ethanol (10 ml) and dimethyl sulfoxide (5 ml), the hot solution filtered, and the filtrate evaporated to dryness. Diethyl ether (25 ml) was added to the residue and the mixture kept at −10°C for 24 h. The solid material was filtered off, washed with cold ethanol (5 ml), and dried in air to give 0.77 g (64%) of light-yellow crystals which were shown by PMR spectroscopy to be a mixture of the 9- and 7-isomers of allyloxymethylguanine (3:1), mp 216-220°C.

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