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TETRA-N-BUTYLAMMONIUM FLUORIDE AS A NOVEL REAGENT FOR THE PREPARATION OF ACYCLO-NUCLEOSIDES. THE SYNTHESIS OF 9-[(2-HYDROXYETHOXY) METHYL] ADENINE, BITAMYCIN.[1]

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ABSTRACT

The synthesis of the title compound, bitamycin (1), by means of Bu_4NF is described. This new antiviral drug was found to be one of the most powerful and least toxic substances for antiviral therapy in man. The physical and chemical behavior as well as the antiviral activities and clinical properties of this compound were found to be significantly different from that reported by Schaeffer, et al.[1] Although the latter was claimed to have structure 1, we found it to possess structure 2. Bu_4NF was proved to be an effective reagent for the exclusive preparation of N(9)-alkylated products of purines. Condensation reactions and deprotection of the acyl-protecting groups to yield acyclo - nucleoside analogues were performed in a one step reaction.

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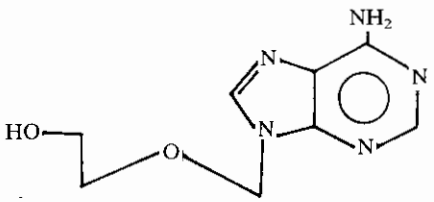
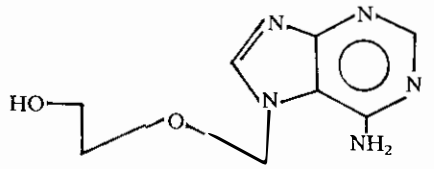
INTRODUCTION

The synthesis of 9-[(2-hydroxyethoxy) methyl] adenine was already claimed.[1] This compound was assumed to possess a conformation which is superimposable with the conformation of adenosine with respect to the adenine group, C(1'), ether oxygen, C(4'), C(5'), and the 5'-OH. In that report it was suggested that through this conformation the aforementioned compound complexes to the enzyme to act as a substrate of adenosine deaminase. This was based on the observation of the special role played by the 5'-OH for the substrate activity in certain adenosine analogues.[2] In fact, not only the 5'-OH but a properly positioned OH-group in other positions can assume the function of the 5'-OH of adenosine.[2-4]

Furthermore, the N(9) and N(7)-alkylated isomers of acycloadenosine analogues, such as compounds 1 and 2, undergo deamination with calf intestinal mucosal adenosine deaminase (see Table III). Therefore, it occurred to us that the reported compound can either possess the structure 1 or 2.



Structure 1



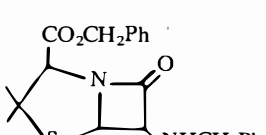
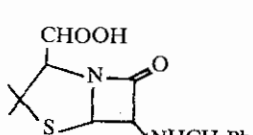
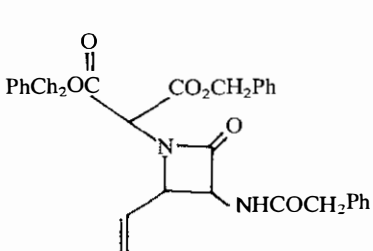
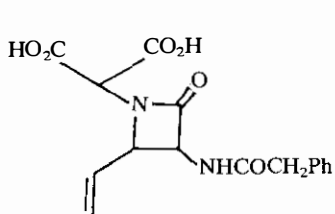
Structure 2

DISCUSSION

In principle, coupling condensations of the chloromethyl ethers with adenine or 6-chloropurine give the

1. Iranian patent, 23291, Sep.6, 1986.

Table I. Removal of Benzyl-Groups with HCl/Silica gel[a]

Starting Material	product[b]	Yield[c][%]
Dibenzyl malonate	malonic acid	95
Dibenzyl maleate	Maleic acid	60
2-Benzyloxyethanol	Glycol	455
1,3-Dibenzyloxy-2-propanol	Glycerol	37
Benzyloxybenzene	Phenol	25
		97
		68

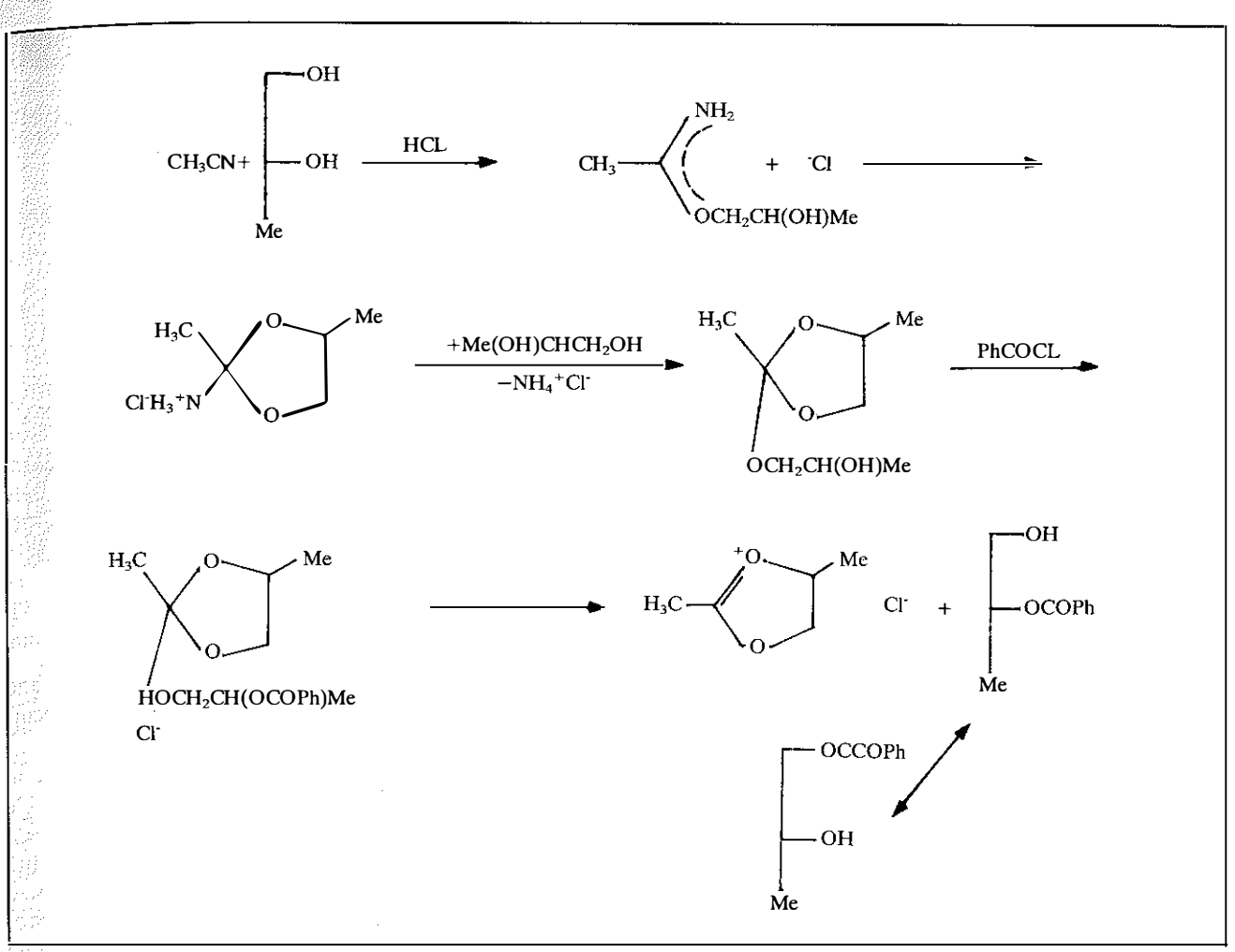
a) The solvent was dry $CHCl_3$ in all cases.
b) The products were characterized by comparison with authentic samples.
C) Yields are based on materials isolated from column chromatography or TLC.

corresponding acyclopurine nucleosides as a mixture of N(7) and N(9)- alkylated products. Much to our surprise, in the above report, the isolation of one compound was described only. In order to establish the proper structure of the described compound, it was decided to repeat the literature procedure to acquire both isomers for comparison. We experienced considerable difficulty in repeating the procedure reported for the chloromethylation reaction.[1] Therefore, 2-benzyloxyethanol was reacted with 1,3,5-trioxane by means of HCl/silica gel in $CHCl_3$.[5]

Although it was possible to isolate 1-benzyloxy-2-chloromethoxyethane by column chromatography, the yield was very low and it seemed that the benzyl group was easily removed under the reaction conditions. Thus, it occurred to us that the HCl/silica gel may be a useful reagent for mild and effective removal of the benzyl ethers and esters. Indeed, when a series of compounds possessing benzyl ester groups was treated with HCl /silica gel in $CHCl_3$ complete deprotection occurred at 25° after 24h. The results are summerized in Table 1 and show that the benzyl esters cleavage are appreciably faster than those of the benzyl ethers.

Because of the difficulties in preparing 1-benzyloxy-2-chloromethoxyethane, it was decided to prepare 1-benzoyloxy-2-chloromethoxyethane (4). The first step in the synthesis would have been the selective acylation of ethylene glycol. We have found that the benzoylation of ethylene glycol with benzoylchloride in CH_3CN leads to the formation of 2-benzoyloxyethanol in high yield. In order to prove the generality and mildness of this method, a series of polyhydric alcohols was treated with benzoylchloride in CH_3CN at 25°. The results are depicted in Table II and show a remarkable degree of selectivity for a single OH-function. Since the similar reactions in other solvents did not afford the desired selectivity, the remarkable results in CH_3CN exhibited it as a unique solvent and catalyst for the attainment of the desired selectivity on the substrates. The role of CH_3CN was established by the gradual formation of NH_4Cl during the course of the reaction. It should be noted that the best results were obtained when 2 eq. of alcohols were allowed to react with 1 eq. of benzyl chloride. In the case of the substrates such as 1,2-propanediol the reaction leads to the selective protection of the secondary OH-function. However, by refluxing or increasing the reaction time acyl-migration occurred in the selective acylation of the primary OH-group in good yield. With regard to the presence of catalytic amount of HCl in benzoyl chloride the following events are suggested.

It should be noted that in addition to selective acylation, the above method leads to a general procedure for selective alkylation, carbonation, silylation,



sulfonylation and phosphorylation of a single OH-group in polyhydroxyl compounds.[6]

Having established a new method for the high yield preparation of 2-benzoyloxyethanol, we next prepared 1-benzoyloxy-2-chloromethoxyethane (4) by the standard literature procedure.[1] Condensation with 6-chloropurine in DMF containing NEt_3 followed by treatment with $MeOH/NH_3$ gave the corresponding adenine derivative as a single isomer which was found to be identical with the authentic sample.[1] Since by the above procedure we were not able to get both N(7) and N(9)-substituted isomers, it was decided to try other available procedures[5,7,8] involving different catalysts (Bu_4NI, $Hg(CN)_2$, and I_2) and different solvents (benzene, toluene, CH_3CN, and THF) using adenine, N-benzoyladenine, or 6-chloropurine at room or refluxed temperature. Surprisingly, in each case only one single isomer which was identical to the reported compound[1] was isolated. Finally, when adenine was silylated with hexamethyldisilazane (HMDS) and coupled with chloromethyl ether 4 in refluxing benzene, isomeric mixtures N(7) and N(9)alkylated products were formed in 5% yield only. Treatment with $MeOH/NH_3$

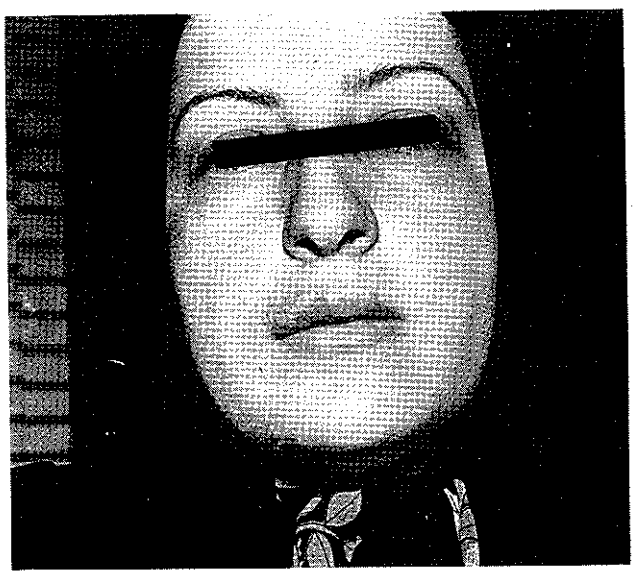


Fig.1. A 33-year-old female with 12 hours pain and vesicular eruptions (24 hours after dental manipulation by a dentist): before treatment.

Fig.2. One day after treatment with Bitamycin, topical: large, yellow vesicles are no more seen and other vesicles are smaller and darker.

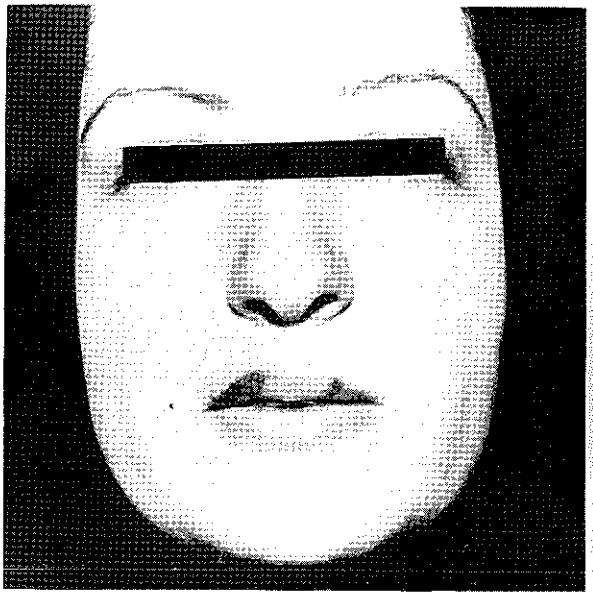


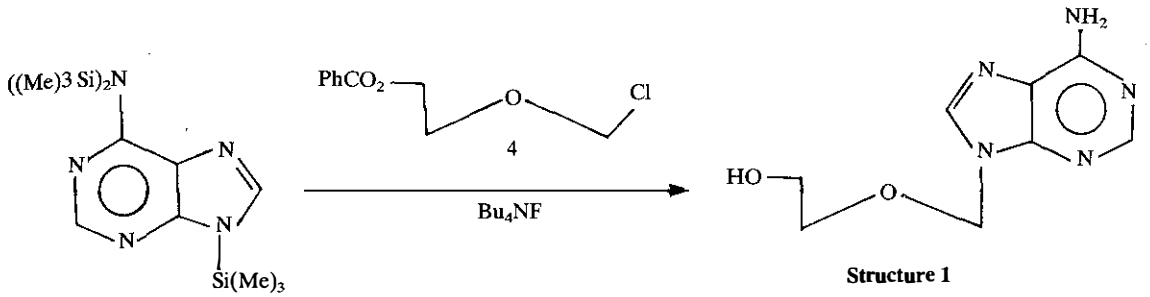
Fig.3. 4th day of therapy: lesions lost their crusts.

indicated that the more polar major product was identical to the reported compound.[1] Because of extremely poor yield observed in the above reaction, we turned our attention to the invention of a new procedure for the preparation of acyclo-nucleoside analogues. We have found a procedure based on the selective removal of a silyl protecting group from the N(9)-position of tris (trimethylsilyl) adenine (3) by means of the fluoridation. To date no procedure has been reported for the selective removal of a silyl group in persilylated compounds of this type. Furthermore, we have noticed that the condensation reaction and deprotection of the acylprotecting group have occurred in a one step reaction.

Alkylation of 3 with 4 in the presence of Bu_4NF in THF at refluxed temperature, after 30 h, gave a single isomer of adenine alkylated product (80%), identical to the less polar minor aforementioned isomer, but different from the one reported.[1] It should be noted that the rate of the ester removal is slower than that of the condensation reaction. Infact, alkylation reaction goes to completion after 3h, but the complete removal

Table II. Selective Benzoylation of Alcohols[a]

Alcohol [mmol]	BZ-Cl [mmo]	Solvent	Product[%]
Glycol (2)	1	CH_3CN	$BzO(CH_2)_2OH$ (95)
Glycol (1)	1	CH_3CN	$BzO(CH_2)_2OH$ (50)
Glycol (2)	1	THF	$BzO(CH_2)_2OH$ (30)
			$BzO(CH_2)_2OBz$ (30)
Glycol (2)	1	C_6H_6	$BzO(CH_2)_2OH$ (20)
			$BzO(CH_2)_2OBz$ (20)
Glycol (2)	1	CH_2Cl_2	$BzO(CH_2)_2OH$ (25)
			$BzO(CH_2)_2OBz$ (20)
Glycol (2)	1	$CH_3CN/AgNO_3$(1)	$BzO(CH_2)_2OBz$ (40)
Glycol (2)	$(Bz)_2O$(1)	CH_3CN	
1,2-Propanediol (2)	1	CH_3CN	$BzOCH_2CH(OH)Me$ (97)
1,2-Butanediol (2)	1	CH_3CN	$BzOCH_2CH(OH)Et$ (97)
1,3-Butanediol (2)	1	CH_3CN	$BzO(CH_2)_2CH(OH)Me$ (90)
1,4-Butanediol (2)	1	CH_3CN	$BzO(CH_2)_4OH$ (100)
1,3-propanediol (2)	1	CH_3CN	$BzO(CH_2)_3OH$ (95)
Glycerol (2)	1	CH_3CN	$BzOCH_2CH(OH)CH_2OH$ (86)
Glycerol[b] (1)	2	CH_3CN	$BzOCH_2CH(OH)CH_2OBz$ (90)
2,3-Butanediol (2)	1	CH_3CN	$CH_3CN(OBz)CH(OH)CH_3$ (97)



Structure 3 **Structure 1**

of the acyl group was achieved by prolonging the reaction time to 30 h. Repeating the same reaction at 25° gave a 20% yield of the isomeric mixtures in 8:2 ratio. The more polar minor product was identical to the authentic sample.[1] However, the reaction in the absence of the fluoridation at refluxed temperature failed and only starting materials were recovered. Thus, Bu_4NF has to be considered as a novel reagent for the preparation of acyclo-nucleoside analogues.

Since the prepared new compound exhibited excellent antiviral activities both *in vitro* and *in vivo* and successfully passed clinical trials, we named it bitamycin. *Bita* is a Persian word which means unique.

At this point, we turned our attention to the structural determination of the prepared isomeric compounds. The spectroscopic data (^{1}H-NMR, UV, IR) of compounds 1 and 2 were too similar to permit differentiation (see experimental part). The only considerable difference was found in the IR of the NH_2 scissoring vibration of the more polar isomer which appears at 1695/cm (cf. data of less polar isomer: 1670/cm, identical to that of the adenosine).

However, the less polar isomer was assigned the stereochemistry of adenosine (9-alkylated product, 1) based on the fact that the NH_2-function of the adenine moiety can release its electrons into the purine ring while the ether oxygen group possesses an opposite direction in the dipole moment of its best conformer. In the case of the mono polar N(7)isomer 2, the direction of the dipole moment of the purine ring and the ether oxygen function were found to be aligned in the same direction. These results were consistent with CPK atomic model studies. When the OH-groups in 1 and 2 were blocked by silylation with (t-Bu) Me_2SiCl, the trend of the polarity of the corresponding silylated products was unchanged. This indicates that the probable hydrogen bonding of the OH-function can not be a reason for the observed difference in polarity of the isomeric compounds 1 and 2.

Next, we tried to find an alternative procedure for the structural determination of bitamycin. It was recently reported that heating the isomeric mixture of 7-{[2-benzyloxy-1-(benzyloxymethy) ethoxy] methyl}-6-chloropurine, (5), and 9-{ [2-benzyloxy-1-(benzyloxymethyl) ethoxy] methyl}-6-chloropurine, (6), results in the complete conversion of 5 to 6. It occurred to us that if Schaeffer's reported compound[1] was actually N(7)-alkylated isomer 2, the corresponding alkylated-6-chloropurine precursor could similarly be isomerized to N(9)-isomer which may as well be transformed to the adenine derivative 1 upon treatment with $MeOH/NH_3$. However, this attempt failed and only starting material was recovered. At this point, an ambiguity occurred concerning the correct assignment of structure 1 to bitamycin. Therefore, it was decided to repeat the literature procedure[9] to prepare compounds 5 and 6 and study their isomerization at elevated temperature. It is interesting to note that the reaction conditions for the preparation of the isomeric mixture 5 and 6 are identical to those reported by Schaeffer, et al[1], in which case a single adenine-alkylated product was isolated.

Heating the mixture of 5, R_f (AcOEt/ether 2:3) 0.61, and 6, R_f (AcOET/ether 2:3) 0.71, at 90° for 24 h results in the disappearance of N(7)-substituted isomer 5, but the appearance of three new spots having R_f values of 0.36, 0.09, and 0.01 as evidenced by TLC developement in the above mix-solvents. When the TLC was developed in AcOEt/MeOH 8:3, the lowest spot appeared at 0.30. These suggest that the N(7)-alkylated isomer 5 is not converted to the N(9)- substituted compound 6, but to a series of other compounds. In order to understand this phenomenon better, compounds 5 and 6 were separated by thick-layer chromatography and were applied separately to the above pyrolysis conditions. Compound 6 was partly transformed to debenzylated product 8, R_f 0.36, while 5 was completely converted to 7, R_f 0.09, and 9, R_f 0.01. These results clearly indicate that the rate of the pyrolysis of 5 is faster than that of 6 and this was misleading in the interpretation of the thermal conversion of 5 → 6. It should be noted that the complete conversion of 7 → 9 was achieved after 54 h at 110°. In this regard it is of interest to consider that the appropriate substituents, which make the departure of the leaving group easier, cause large rate increase from a remote position.[10] Ether oxygen in the N(7)-alkylated product 7 acts as an internal nucleophile and displaces

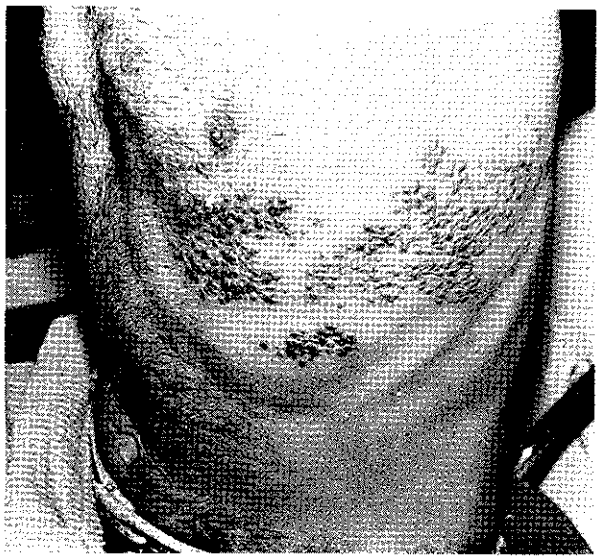


Fig.4. 50-year-old male, known case of polycythemia vera for 5 years, on Busulphan in the last 2 years. He had 5 days zona on T4/5/6 left, anterior and posterior. Bitamycin 30 mg intravenous was given for 6 days: before treatment.

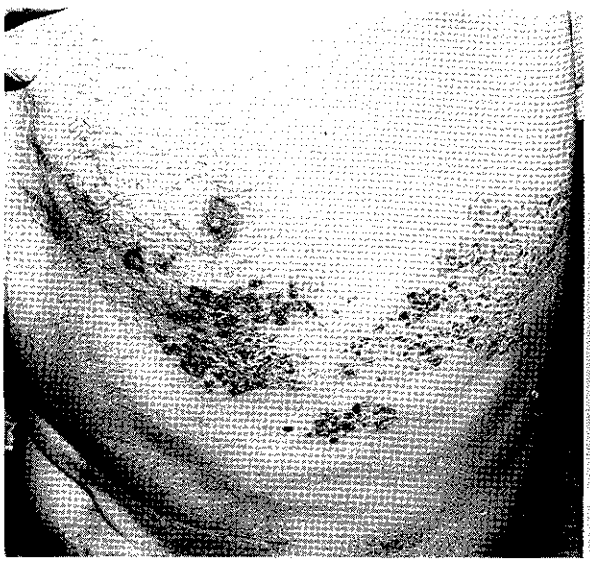


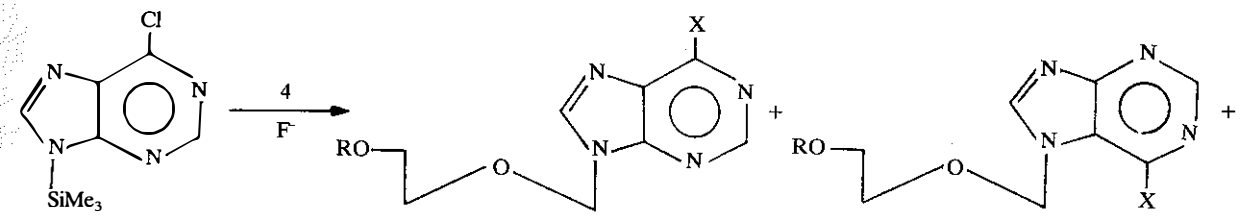
Fig.5. 4th day of therapy: crusted lesions.

the Cl-atom via a 5-membered transition state to form an oxonium ion intermediate 10 which in turn is rapidly attacked by the internal OH-function to give, after further degradation, compound 9.

At this stage in the development of a practical route for the structural elucidation of N(7) and N(9)-substituted isomers of adenine nucleosides it became essential to take advantage of the above observations. Therefore, it was decided to study the relative nucleophilic displacements of the Cl-functions in 5 and 6. A methanolic solution of the isomeric compounds 5 and 6 containing NaN_3 was stirred at 25°. 7-{[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl}-6-methoxypurine, (11), was the exclusive reaction product after 20 h. Compound 6 did not react under the conditions employed. When 5 and 6 were solvolyzed separately, complete transformations of 5 → 11 and 6 → 12 occurred after 20 and 49 h respectively. Similarly the nucleophilic displacements of the Cl-functions in 5 and 6 with NaSH in MeOH were studied and the relative products 13 and 14 were obtained (ca.100%) within 5 minutes. The former results clearly indicate that the ether oxygen group in N(7)-alkylated product 5 can assist nucleophilic replacement of the Cl-function via the favorable 5-membered oxonium ion intermediate to afford 11. In the latter case, the reactions of the Cl-functions in 5 and 6 with NaSH were too fast to be able to detect the effect of the oxonium ion intermediate in the transformation 5 → 13.

We next proceeded to apply this method to the structural confirmation of bitamycin. The route chosen to the adenine derivative 1 involved 6-chloropurine in the condensation step. Using the conditions described for the preparation of bitamycin (for general procedure see experimental part), silylated 6-chloropurine was reacted with 4 by means of Bu_4NF. After 1 h compounds 15-17 were formed which were separated by thick-layer chromatography and were characterized as their respective adenine derivatives 1-2, and 18 ($MeOH/NH_3$ [5], 60%) in 2:7.5:0 ratio respectively. The ^1H-NMR spectrum of 18 [m.p. 130-135, $R_f(CH_2CL_2/MeOH\ 7:3)$ 0.29] showed some significant differences from those of 1 and 2 (see exper. part). The absorption for the exo-cyclic amino group was observed as a broad peak at 8.12, which constitutes a shift down field of 0.84 ppm. The protons on C(2) and C(8) appeared at 8.23 and 8.50pp, (△ 15 Hz). This has indicated that the introduction of a substituent on a ring-nitrogen atom in the pyrimidine portion of purine results in pyrimidine ring that appears to be a π-deficient pyrimidinic ring and an apparently π-excessive imidazole ring. This situation would result in a deshielding effect for the proton at C(2) and would produce a shielding effect for the proton at C(8), as well as a larger π than for compounds 1-2 in which the effect is essentially nonexistent (π for protons at C(2) and C(8) in 1-2, 5-6 Hz). However, the similar solvolysis reactions of 15-16 ($MeOH/NaN_3$) gave 19-20 after 50 and 20h respectively. These clearly indicate that the displacement reaction of N(7)-substituted isomer 16 is appreciably faster than that of the N(9)-alkylated product 15. It should be noted that the treatment of 15-16 with NaSH/MeOH afforded 21-22 respectively in about 8 minutes.

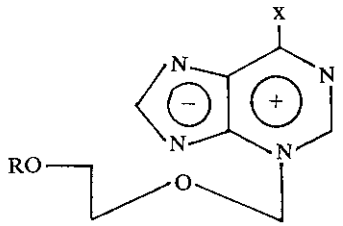
The most available and appropriate method for the confirmation of bitamycin structure is the following: 3,9-(ethanoxymethano) adenin-3-ium chloride (35) has recently been reported[5] to have been converted to



Substance 15 X = Cl , R = Bzl
Substance 19 X = OMe, R = Bzl
Substance 21 X = SH , R = Bzl
Substance 1 X = NH_2, R = H

Substance 16 X = Cl , R = Bzl
Substance 20 X = OMe, R = Bzl
Substance 22 X = SH , R = Bzl
Substance 2 X = NH_2 , R = H

Bzl = PhCO



Substance 17 X = Cl , R = Bzl
Substance 18 X = NH_2, R = H

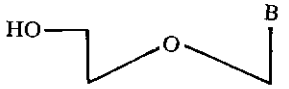
9-[(2-azidoethoxy)methyl] adenine (36).

The mesylation of the hydroxyl functions in compounds 1 and 2, and the subsequent displacements of the relative mesyl derivatives with NaN_3 can clearly indicate which compound, 1 or 2, is converted to N(9)-alkylated product 36. These transformations can demonstrate the actual stereochemistry of each compound.

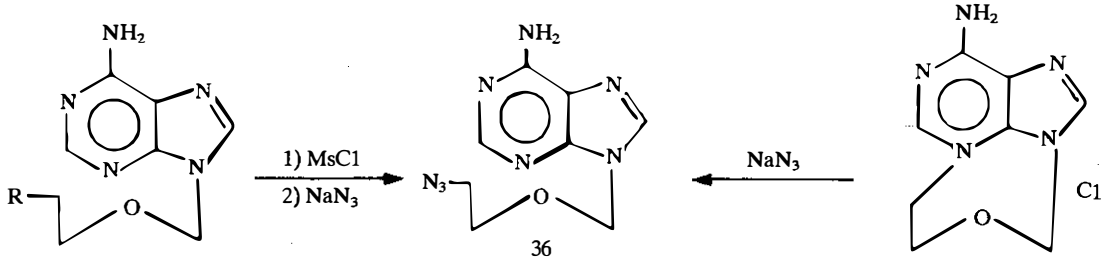
Reaction of bitamycin with MsCl in refluxing CH_3CN/DMF (5:1)[6] gave compound 37 which was treated with NaN_3 in DMF at 90°to afford 36, identical with authentic sample[5]. Therefore, we conclude that 1 has to be the structural formula of bitamycin.

Having established the bitamycin structure 1, it was decided to examine the capability of the fluoride methodology in preparing the N(9)-alkylated products of adenine acyclo-nucleosides. Thus, treatment of 1,3-dibenzyloxy-2-chloromethoxypropane (23), 1,3-di-benzoyloxy-2-chloromethoxypropane (24), 1,3-dichloro-2-chloromethoxy-propane (25), and 2-benzoyloxychloro (carbomethox) methoxyethane (26) with tris (trimethylsilyl) adenine (3) gave the corresponding N(9) alkylated products 27-30 by means of Bu_4NF in THF. Deprotection of 27-28[5] and the nucleophilic displacements of the Cl-functions in 29[11] gave the N(9)-alkylated product 31 which was found to be identical with an authentic sample[9]. It is of great interest to note that in all cases the N(7)-alkylated products of adenine were found to be more polar than the corresponding N(9)-substituted isomers. This is consistant with the aforementioned dipole moment discussions.

We next decided to apply the fluoride method to other nucleobases. Thus, guanine, uracil, and cytosine were silylated with HMDS and coupled with 4 in refluxing THF using Bu_4NF to give the corresponding desired products 32-33 (ca.80%) which were found to be identical with authentic samples.[12-13] In the case of cytosine we were not able to use the above conditions for the successful condensation to obtain 34.



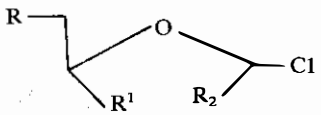
substance 32 B = guanine
substance 33 B = uracil
substance 34 B = cytosine



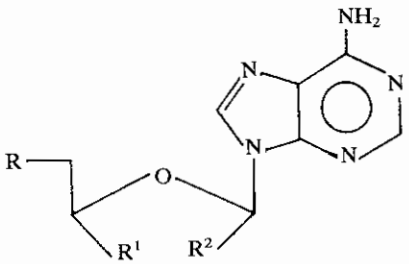
1 R = OH
37 R = OSO_2Me

36

35



Substance 23 R=OBZ1, $R^1=CH_2OBz1$, $R^2=H$
Substance 24 R=OBZ, $R^1=CH_2OBz$, $R^2=H$
Substance 25 R=Cl, $R^1=CH_2Cl$, $R^2=H$
Substance 26 R=OBz, $R^1=H$, $R^2=CO_2Me$



Substance 27 R=OBZ1, $R^1=CH_2OBz1$, $R^2=H$
Substance 28 R=OH, $R^1=CH_2OBz$, $R^2=H$
Substance 29 R=Cl, $R^1=CH_2Cl$, $R^2=H$
Substance 30 R=OBz, $R^1=H$, $R^2=CO_2H$
Substance 31 R=OH, $R^1=CH_2OH$, $R^2=H$

Adenosine-Deaminase Studies on Acyclo-nucleosides. The rate of deamination of 1,2,31, and adenosine in the presence of calf mucosal adenosine deaminase in buffered solutions of varying substrate concentration were determined according to the procedure described.[9] The inhibition studies on the above substrates were also carried out following the procedure. The results are shown in Table III.

The acyclo-nucleosides 1,2, and 31 are weakly bound to the enzyme relative to adenosine. The substrates 1,2, and 31 are also weak competitive inhibitors of adenosine deaminase. Because of the extremely poor inhibitory properties of 2 relative to 1 and 31 one might be able to assume a conformation for 2 (N(7)-alkylated isomer) which is not superimposable with the conformation of adenosine. However, enzymatic evaluation of compounds 1 and 2 revealed that these substrates would be recognized better by adenosine deaminase than 31, whereas, the V_{max} for 1,2, and 31 are respectively 0.019/,1.38%, and 2.55% that of adenosine.

Solubility in Water and Lipophilicity of Adenine Derivatives 1 and2. The N(9)-substituted isomer 1 and the N(7)-alkylated product 2 showed similar solubility in H_2O (Table IV). The lipophilicities were also determined via a pentanol/H_2O distribution according to the methods described.[14] Compound 1 exhibited fair increases of lipophilicity compared with 2 (Table IV).

Biological Activity. The compounds 1 and 18 were tested for activity against *Herpes simplex* type-1 virus (HSV-1) and found to possess ED_{50} value vs.HSV-1 of 0.30 μg/ml, and 5.12μg/ml, respectively. The control experiments showed 2 and 9-(α-D-arabinofuranosyl) adenine (ara-A) to be active against HSV-1 with ED_{50} values of 8.10 μg/ml and 6.38μg/ml, respectively. The increased activity of bitamycin (1) over ara-A and the decreased activity of 2 relative to ara-A reflect the fact that the N(9)-alkylated product 1, having a conformation similar to that of the adenosine, has more pronounced antiviral activity than the corresponding N(7)-alkylated isomer 2.

Bitamycin (1) was also active against herpes-simplex-type-2 virus (HSV-2) and herpes-zoster virus with ED_{50} values of 0.17 μg/ml and 1.2 μg/ml, respectively.

Bitamycin (1) did not show any detectable toxicity toward cells at concentration up to 500 μg/ml. In studies of the acute toxicity of 1, the LD_{50} in mice given the

Table III. Substrate Activities and Inhibitory Properties against Adenosine Deaminase Enzyme

Substrate	K_m (M.10^5)	Rel. V_{max}	K_i
Adenosine	4.57	1	-
31	183	$2.55.10^{-2}$	$2.00.10^{-4}$
2	14.55	$1.38.10^{-2}$	$1.74.10^{-6}$
1	22.41	$1.93.10^{-4}$	$2.30.10^{-4}$

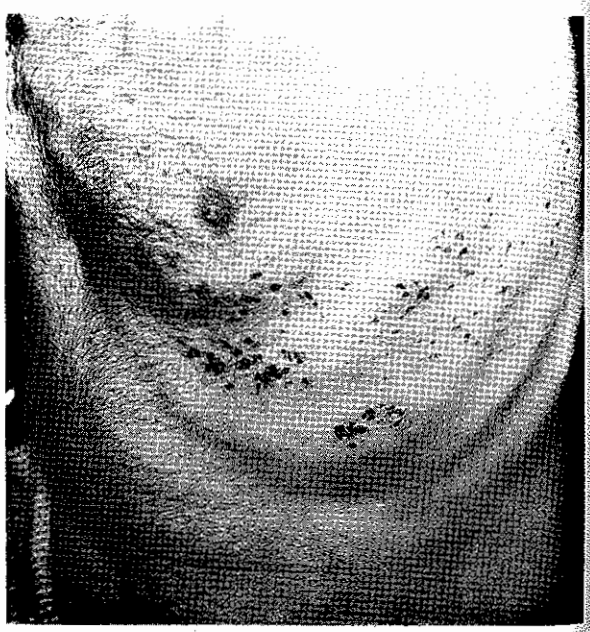


Fig.6. One day after completion of treatment: total scabbing.

Table IV. Solubility in H_2O and Lipophilicity of Nucleoside Analogues

Substrate	Solubility in H_2O [mg/ml]	Log P(1-pentanol/H_2O)[a]
ara-A	0.40	-0.47
1	1.95	2.99
2	2.03	0.98

a) Partition coefficients were calculated as P = [substrate] pentanol/ [substrate]$_{H_2O}$

drug intravenously was greater than 500 mg/kg. It should be noted that, in a 30-day study in mice, daily oral administration of 300 mg/kg of bitamycin produced no toxicity.

Clinical trials of Bitamycin (1). The clinical studies of the new antiviral drug 1 during April 1986-April 1987 revealed that it is curative in low doses for genital, oral, and other cutaneous herpes infections as well as herpes simplex encephalitis and meningoencephalitis. The dosages applied were; 20-40 mg/day intravenously (i.v.) for 4-5 days for zona, 50mg/day i.v. for 10 days for disseminated herpes zoster, and 50 mg/12h i.v. for 10 days for herpes simplex encephalitis and meningoencephalitis. It should be noted that the drug was dissolved in H_2O and used intravenously. In topical form bitamycin was used 2 times/day for 1-6 days in cases of herpes simplex lesions, zona, erythema multiform and epidermolysis. Of 89 patients with herpes labialis, facialis, and eye lids 20, 22, 14, and 13 were respectively treated topically with compounds 1, 2, 18, and acyclovir. The 20 chosen control cases received calamin lotion. In 90% of patients with herpes labialis and facialis, shrinkage of active vesicles were noted in 1-12 h when bitamycin was used. 49% of patients have responded (drying of lesions) to compound 2 and 32% responded to 18 but only 8% responded to acyclovir. It should be noted that 77% of cases on acyclovir have responded to therapy after 30 h and 75% of control group have responded after 37-72 h.

Forty eight cases with severe herpes zoster infections were divided to six groups and treated respectively with bitamycin (1) intravenously (i.v.), bitamycin topically, compounds 2,18, and acyclovir topically; calamin lotion was used as control. 80% of patients had total crusting with bitamycin i.v. With bitamycin topically 99% of patients had total crusting within 1-5 days. Accordingly, 60% of patients responded to compound 2 in 12 days and 83% of patients responded to compound 18 in 5 days while 17% of patients and total crusting with acyclovir within 8-13 days. There was no difference between those who were on acyclovir and the ones on control; both were treated for 10-13 days. It should be noted that 2 days after starting intravenous therapy with bitamycin in a leukemic patient with disseminated zona, all vesicles, spread all over his body, were dried out and flattened and 6 days later they

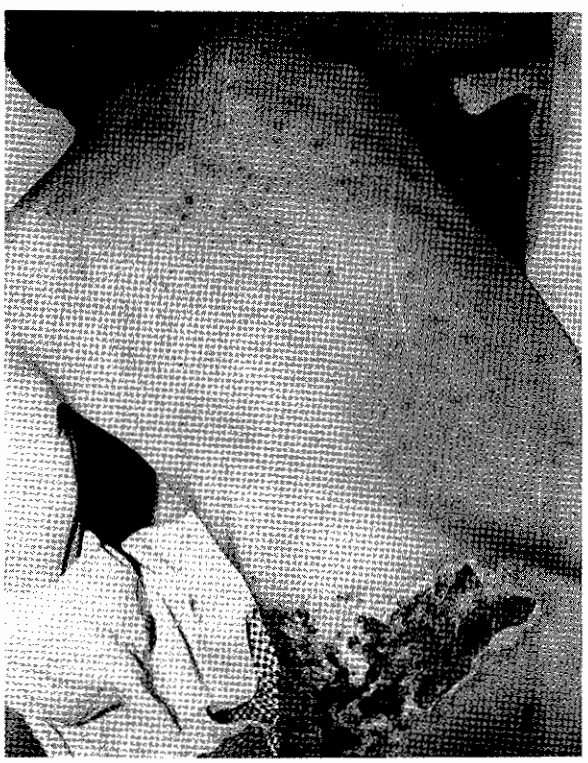


Fig.7. A 57-year-old chronic lymphocytic leukemia male, on Chlorambucil and Prednisolone for 6 months. 13 days zona on TII/12 left, anterior and posterior and 3 days disseminated zoster all over body except palms and soles. Bitamycin 50 mg intravenous every 12 hours was given for six days: before treatment.

healed. He had ulceration at zona sites for up to 2 weeks post therapy.

Bitamycin was curative in patients with first attack of genital herpes infection including a patient who had been having recurrent attacks for 20 years. In another case resistant to acyclovir it widened the interval of attacks which were initially every few days to every 3 months. The following are representative examples of the therapeutic effect of bitamycin on two patients with genital herpes infection.

An 18 year old insulin dependent female who was on gold and prednisolone therapy for rheumatoid arthritis was treated with bitamycin. In her first attack of labia majora and minora, herpes vesicles got smaller after 10 h of topical bitamycin therapy and healed after 3 days.

A 30 year old male with recurrent attacks of penile herpes lasted for 3 years had taken acyclovir tablets for 18 months, but had developed face edema, urine incontinence and abnormal behavior. Therefore, he was administered topical acylovir. He responded after 3 days and within 8 days the vesicles were healed. However he referred to us when it was 7 months from the time he had shown no response to acyclovir. With bitamycin, he responded after 12 h to topical administration, and all vesicles dried out after 30 h.

Bitamycin also exhibited to possess remarkable

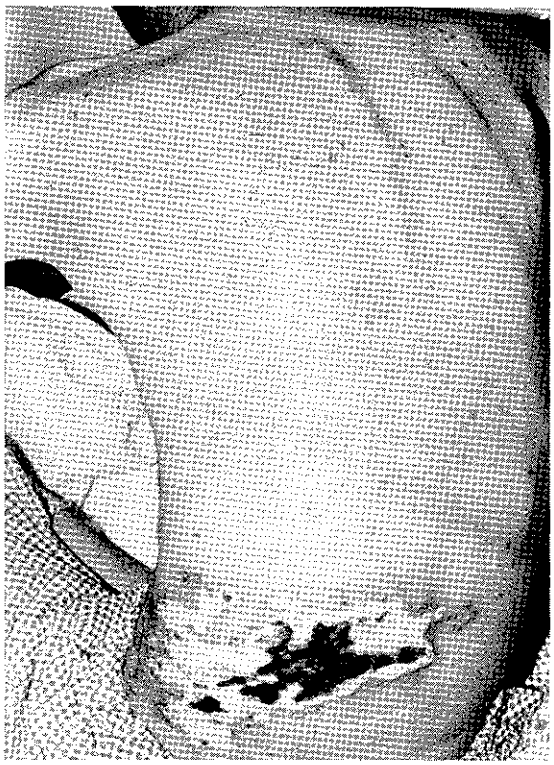


Fig.8. 2nd day of treatment: all vesicles dried out.

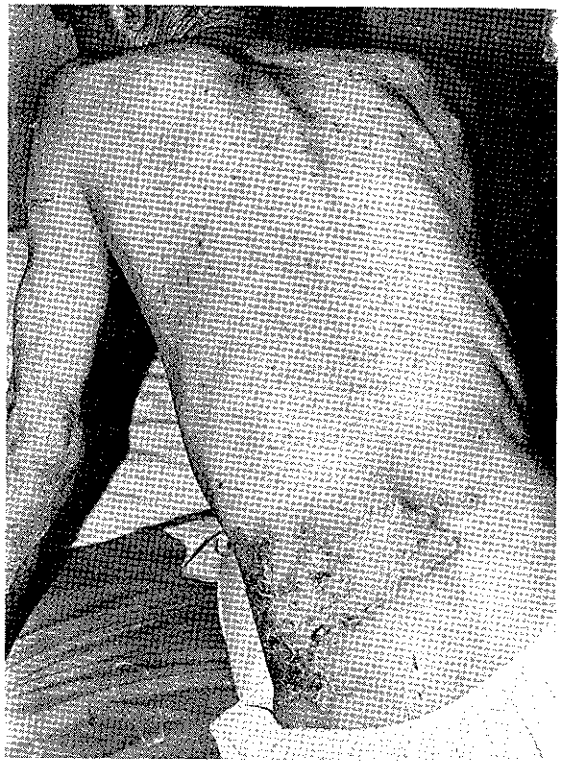


Fig.9. 6th day of therapy: healed vesicles with hyperpigmentation.

curative effect on encephalitis. The following cases are examples of bitamycin effects on herpes simplex encephlitis and meningoencephalitits respectively. The diagnosis was established by focal neurological signs and specific findings of cerebrospinal fluids, electroencephalogram, brain CT scan and brain biopsy. It should be noted that bitamycin can pass easily from the brain barrier as evidenced by the drug detection from spinal fluid less than 1 h of administration intravenously.

A 17 year old male with 15 days fever, headache, flu like symptoms, who had been in stuporous state and deep coma for 7 days while having had decerebrate posture to painful stimuli with focal neurological deficit on left side, was put on bitamycin 40 mg/12 h i.v. for 10 days. He developed blinking, responded to verbal stimuli, and had spontaneous movement of extremities respectively after 4,6, and 7 days from the onset of therapy. He left hospital with completely normal neurological exam.

A 19 year old male with 12 days photophobia, headache, fever, neck rigidity, visual hallucination, tremor of lips and hands, disorientation to person, horizontal nystagmus, abnormal two point discrimination and proprioception and decreased power of lower extremities, was put on bitamycin 60 mg/12 h i.v. for 10 days. On the second day of therapy he had less hallucination, less lips and face movement and more power of lower extremities with normal proprioception and two point discrimination. On the third day of therapy he had decreased nuchal rigidity with no more hallucination and disorientation but had mild tremor of tongue, lips, and extremities. On the fifth day of therapy he had completely normal neurological findings.

Bitamycin had promising effects on patients having erythema multiform, hepatitis, persistent hepatitis, epidermolysis, multiple sclerosis and genital warts. This drug had no effects on hepatitis B virus carriers and patients having rabies, vitiligo, acute myelogenous leukemia, and acute lymphocytic leukemia.

Bitamycin normalized the blood glucose of a patient with maturity onset diabetes mellitus for as long as the patient was on this drug but had no effect on another case with juvenile onset diabetes mellitus.

In all cases physical exams were done daily during treatment period, then monthly for three months and every three months there after. Liver and kidney function tests, blood glucose, blood urea nitrogen, serum calcium and phosphate, urinalysis and complete blood count were done on all patients. In a few patients tissue biopsy including brain biopsy were also done. The only side effect which was noted on two patients was transient increase in transaminases which was returned to normal a few days after cessation of therapy. To determine the possible biotransformation

of bitamycin in man, the analysis of the urine of a few patients was performed and unmetabolized material was recovered. Presumably, there was no cleavage of compound 1 in man. Fig. 1-9 shows the effect of bitamycin on some herpes virus infections in man.

The complete biological studies of bitamycin in vitro and in vivo and details of clinical trials against the various manifestations of herpes virus infections in man as well as other viral infections will be described elsewhere.

Experimental part

General Remarks. See [5].

General Procedure for the Removal of Benzyl Groups. All compounds (Table 1) possessing benzyl groups were debenzylated in the same manner. Their structures were proven by comparison with authentic samples. The following is a representative procedure: To a stirred solution of 1 mmol of substrate in 15 ml dry $CHCl_3$ was added 3 g of HCl/silica gel [5]. After 24 h at 25°, the solid material was filtered and washed with $CHCl_3$ (3× 10 ml). The combined filtrate and washings were evaporated and the product was purified by chromatography on silica gel.

General Procedure for Selective Benzoylation of Alcohols. All compounds (Table 2) having OH-groups were benzoylated to the correspondings esters in the same manner. Their structures were proven by IR, and ^{1}H-NMR or by comparison with an authentic sample. The following is a representative procedure: To a solution of 2 mmol substrate in 7 ml of dry CH_3CN at 25° was added 1 mmol of benzoyl chloride with stirring. After 24 h, the precipitate ($NH^+_4Cl^-$) was filtered off and Et_2O was added to the filtrate. The ethereal solution was washed with H_2O, dried, and evaporated. The product was purified by chromatography on silica gel.

9-[(2-Hydroxyethoxy)methyl] adenine (bitamycin,1) and 9[(2-benzoyloxyethoxy) carboxy-methyl)] adenine (30). Representative procedure: Adenine (1.35 g, 0.01 mol) and $(NH_4)_2SO_4$ (50 mg) were weighed into a 500 ml round-bottom flask. Hexamethyldisilazane (100 ml) was added and the mixture was heated under reflux until a clear solution was obtained (12 h). The solvent was removed at reduced pressure and the residue (compound 3) was dissolved in 300 ml of freshly distilled dry THF. Dried Bu_4NF (2.62 g, 0.01 mol) in benzene (25 ml) was added dropwise during 2 h at reflux temperature. Then, chloromethyl ether 4 (3.22 g, 0.015 mol) was added at the same temperature. After 30 h, the soln. was diluted with AcOEt (250 ml) and H_2O (200 ml). The organic layer was separated and washed with H_2O (2×100 ml), dried (Na_2SO_4), filtered, and evaporated. The residue was chromatographed on silica gel and the impurities were eluted with CH_2Cl_2. Elution with acetone afforded 1 (80%) as a white precipitate, m.p. 150°. R_f CH_2Cl_2/MeOH 7:3, 0.80. UV (EtOH): 259. IR (nujol): 3300 br., 3110 br., 1670 s, 1579 m, 1600 s, 1105 s. ^{1}H-NMR (D_6) DMSO): 3.30-3.71 (m, OCH_2CH_2O); 5.51 (s, OCH_2N); 7.28 (br.s, NH_2 exchanged with D_2O); 8.20, 8.29 (2s, H-C (2) and H-C(8)). Anal. calc. for $C_8H_{11}N_5O_2$ (209.20): C 45.93, H 5.30, N 33.48; found C 45.85, H 5.28, N 33.38.

This compound was claimed [1] to have m.p. 198-199°. No spectroscopic data is given. However we repeated the literature procedure to obtain 2, m.p. 198.3°, R_f CH_2Cl_2/MeOH 7:3, 0.38. UV (EtOH): 259. IR (nujol): 3000-3335 br., 1695 s, 1580 m, 1615 s, 1115 s. ^{1}H-NMR ((D_6)DMSO): 3.50 (br.s, OCH_2CH_2O); other signals were identical to that of 1.

Compound 30 was prepared like 1 except that compound 26 was used in place of 4. M.P. 210°. R_f AcOEt/MeOH 4:1, 0.31. UV (EtOH): 262. IR (nujol): 3340 br., 3130 br., 1710 s, 1660 s, 1580 s, 1120 s. ^{1}H-NMR ((D_6) DMSO): 4.59 (br.s, OCH_2CH_2O); 7.00-8.10 (m, CHCO, NH_2 exchanged with D_2O, and Ph); 8.20, 8.30 (2s, 2H, H-C (2) and H-C (8)).

Pyrolysis of 7-{[2-benzyloxy-1-(benzyloxymethyl) ethoxyl] methyl} -6-chloropurine (5) and 9-{[2-benzyloxy-1-(benzyloxymethyl) ethoxy] methyl} -6-chloropurine (6). Compounds 5 (1 mmol) and 6 (1 mmol) were separately heated in an oil bath at 90° for 24 h. Purification of the residue of each reaction by thick layer chromatography revealed that the compound 6 is converted to debenzylated product 8 (25%), m.p. 127°, which was found to be identical with an authentic sample [9]. Compound 5 was pyrolyzed to 7 (70%), m.p. 158°, and 9 (15%). Treatment of 5 with the 1N BCl_3 in CH_2Cl_2 [9] also gave 7 (60%) which was identical to that obtained from pyrolysis of 5. Pyrolysis of 5, 6, or 7 at 110° for 54 h resulted in the transformation of 6 → 8 (70%) and 5 or 7 → 9 (60%), m.p. 280° (dec.). R_f AcOEt/MeOH 8:3, 0.30. UV (EtOH): 257. IR (nujol): 3200-3680 br., 1615 s, 1575 s, 1200 m, 1100 s. 1H-NMR ((D_6)DMSO): 3.40-4.11 (br.m, $CHCH_2OH$ exchanged OH with D_2O); 4.51 (br.d. OCH_2); 5.81 (br., OH exchangeable with D_2O); 7.41, 7.58 (2br.s, H-C (2) and H-C (8)); 8.54-9.41 (br., NH, exchangeable with D_2O). MS (20 ev, 97°) (%): 210 (15, M^+).

Enzyme Assays. The procedures used for adenosine deaminase have been described previously [9]. The results are collected in Table 3.

Determination of Solubiltiy. An excess of each compound (see Table 4, 10 mg) was agitated for 20 h in a 25-ml volumetric flask with 5 ml of 0.1 M phosphate buffer. The soln. was filtered from undissolved solids through a 4-5.5 mesh (ASTM) sintered glass funnel and the concentration of the solute determined by UV absorbance (Table 4).

Determination of Partition Coefficients (Lipophili-

cities) A soln. of each compound (see Table 4, 10 ml) in 0.1 M phosphate buffer possessing an absorbance of 2.2-2.9 at 260 nm was shaken with pentanol (10 ml) in a 50 ml separatory funnel for 1 h.

The layers were separated and their concentrations determined by UV. The partition coefficient was calculated as $P=[S]$ pentanol/$[S]_{H2O}$ (Table 4).

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