latter in 200 ml of dry THF was added dropwise during 1.5 hr to a stirred suspension (under N_2) of 9.12 g (0.240 mol) of $LiAlH_4$ in a mixture of 250 ml of anhydrous Et_2O and 250 ml of anhydrous THF. After refluxing for 4 hr, the mixture was cooled in an ice bath and excess $LiAlH_4$ was decomposed under N_2 by the successive dropwise addition of 10 ml of H_2O, 10 ml of 15% NaOH, and 30 ml of H_2O. The insoluble oxides were filtered and washed with THF (500 ml) and PhH (500 ml), and the combined filtrates were evaporated *in vacuo*. The residual orange oil was taken up in 100 ml of 10% HCl and the solution was filtered from insolubles. After washing with PhH, the aqueous layer was cooled and made strongly basic with 10 *N* NaOH. The product was extracted with PhH, and the combined extracts were washed with H_2O and dried (Na_2SO_4). Evaporation *in vacuo* left the free base of **56** as an oil which was converted to its dihydrochloride salt in the usual manner.

Compound **57** was prepared from **41** in an analogous manner. The isomeric bis(urethanes) (crude yield, 69%) were only moderately soluble in CH_2Cl_2 and one of the isomers partly precipitated during the course of the reaction. The reduction was carried out by adding a slurry of the isomeric mixture in THF to the $LiAlH_4$. On recrystallization from 95% EtOH, crude **57** yielded (first crop) a comparatively minor amount of crystalline solid, mp *ca.* 290°. This material was assumed to be isomeric (similar ir) with the major component (mp 274–276°) which was obtained by treatment of the EtOH filtrate with anhydrous Et_2O.

Mono- and Bis(dithianes) (Table IV). Method A. Compounds **58** and **60** were prepared according to the method reported for the corresponding 2-phenyl analog.[10]

Method B. A solution of 1.0 mol of aldehyde, 1.0 mol of 1,3-propanedithiol, and 0.01 mol of *p*-TsOH·H_2O in 600 ml of PhH was refluxed (Dean-Stark trap) for 1 hr. After cooling and diluting with an equal volume of PhH, the solution was washed with 10% NaOH (4 × 400 ml) and H_2O (3 × 400 ml) and dried (Na_2SO_4). The crude product (oil or solid) remaining after evaporation *in vacuo* was triturated under hexane, filtered, dried, and recrystallized.

The aromatic aldehydes required in methods A and B either were commercially available or were prepared by known standard literature methods.

Method C.[4] Under N_2, a stirred solution (or suspension) of 0.5 mol of the dithiane (**58–61**) in 1200 ml of anhydrous THF was kept at −35 ± 5° while adding dropwise during 1.5 hr 345 ml (0.55 mol) of a commercial solution (1.6 *M*) of *n*-BuLi in hexane. After stirring for an additional 1.5 hr at −35° the solution was warmed to −15°. A solution of 0.25 mol of 1,4-dibromobutane (or 1,3-dibromopropane) in 60 ml of anhydrous THF was then added dropwise during 2 hr at −10 ± 5°. After standing overnight in the refrigerator (*ca.* 2–3°) the solution was cooled at −35° and 90 ml of H_2O was added dropwise under N_2 to decompose any excess *n*-BuLi. The residue remaining after evaporation of the solvent *in vacuo* was suspended in 1000 ml of H_2O and the mixture acidified (pH 2–3) by addition of concentrated HCl. The product was extracted with $CHCl_3$ (3 × 500 ml), and the combined extracts were washed with 5% $NaHSO_3$ (3 × 350 ml), 5% KOH (4 × 350 ml), and H_2O (4 × 350 ml) and dried (Na_2SO_4). The $CHCl_3$ was evaporated *in vacuo* and the residue (oil or solid) was triturated under Et_2O or hexane, filtered, dried, and recrystallized.

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Nucleic Acids. 14. Synthesis and Antiviral Activity of Some 5′-Esters of 9-β-D-Arabinofuranosyladenine (Ara-A)[1]

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The synthesis of the 5′-benzoyl and 5′-palmitoyl esters of ara-A is described. Qualitatively, the *in vitro* antiviral activity of 5′-benzoyl-ara-A and ara-A was similar; *i.e.*, both compounds were effective against herpesvirus (a DNA virus) but inactive against several RNA-containing viruses. Ara-A 5′-palmitate was inactive in the plaque inhibition test. In *in vitro* studies comparing the dose of drug with herpesvirus yields, ara-A and the 5′-benzoate had nearly the same activity and were more active than the 5′-palmitate. In *in vivo* studies in mice infected with herpesvirus, ara-A was effective if given intraperitoneally at 125 or 250 mg/kg total daily dose, for 5 days beginning the day after virus inoculation. Both esters of ara-A were devoid of antiviral activity in mice using these conditions of treatment.

The antiviral activity of ara-A was first described by De Rudder and Privat de Garilhe.[2] These authors showed that, *in vitro*, ara-A was inhibitory for the DNA viruses studied (herpes and vaccinia) but was noninhibitory for the RNA viruses (poliomyelitis and measles). Subsequent collaborative studies carried out by the Parke Davis Co. and Southern Research Institute groups expanded and confirmed the *in vitro* studies.[3] These same groups studied the action of ara-A in a variety of model infections in animals and showed that ara-A was active in hamsters and mice infected with herpesvirus[4,5] and in mice infected with vaccinia virus.[6,7]

Recent studies[8,9] comparing the biological activity of ara-C (1-β-D-arabinofuranosylcytosine, cytarabine, Cytosar) with some 5′-esters have provided evidence that the activity of the 5′-esters may be somewhat greater than is ara-C itself. The factors influencing the enhanced biological activity of these derivatives include resistance to pyrimidine nucleoside deaminase, lipophilicity and rate of localization in lipid depots, susceptibility of the ester bond to enzymatic hydrolysis, and dissolution and absorption rates.[9]

We report herein the summary of our findings on the synthesis of two ara-A esters, the 5′-palmitate and the 5′-benzoate, and compare the *in vitro* and *in vivo* antiviral properties of these compounds to that of ara-A.

Methods

Synthesis of 5′-Esters of Ara-adenosine and 5′-Benzoyl-9-β-D-arabinofuranosyladenine. 9-β-D-Arabinofuranosyladenine hydrochloride (5.3 g, 0.017 mol) was dissolved in 50 ml of dimethylacetamide and 4.8 g (0.034 mol) of benzoyl chloride was added. The mixture was allowed to stand at room temperature for 72 hr. The solvent was removed *in*

vacuo and the resultant oil was triturated with ethyl acetate-ether (1:1) and then with 1 *N* $NaHCO_3$. The resultant solid was collected, washed with H_2O, and dried, wt 4.67 g.

The crude product was purified by chromatography over silica gel (Merck-Darmstadt, 0.05-112 mm). The column dimensions were 4.6 × 100 cm and the solvent used was MEK-acetone-H_2O, 72:20:8. After 750 ml of effluent, uv-absorbing material began to emerge, at which point 20-ml fractions were collected. The separation was followed by tlc (silica gel, MEK-acetone-H_2O, 65:20:15). The product appeared in fractions 6-40. This material was recovered and recrystallized from ethanol to obtain 1.1 g (17% yield) of pure material, mp 220-222°. For analysis, a sample was recrystallized from ethanol-H_2O, mp 222-223°. The material was dried at 80° for analysis. The nmr, ir, and uv spectra agreed with the proposed structure. *Anal.* ($C_{17}H_{17}N_5O_5$) C, H, N.

5'-Palmitoyl-9-β-D-arabinofuranosyladenine. Ara-adenosine hydrochloride (0.48 g, 1.6 mmol) was dissolved in 10 ml of DMA and 0.52 g (20% excess) of palmitoyl chloride was added. After about 72 hr reaction at room temperature, the solvent was evaporated *in vacuo* and the resultant oil was triturated with ethyl acetate-ether. The solid was triturated with 1 *N* $NaHCO_3$, collected, washed with water, and dried, yield 0.55 g (6%). The product was recrystallized from ethyl acetate to give 0.4 g, mp 66-68°. The nmr spectrum was completely consistent with structure, including the presence of 0.5 mol of ethyl acetate. *Anal.* ($C_{26}H_{43}N_5O_5 \cdot 0.5EtOAc$) C, H, N.

Antiviral Activity *in Vitro*. a. *In Vitro* Antiviral Activity by Plaque Inhibition. Washed contiguous monolayers of susceptible cells in 60-mm plastic petri dishes were infected with 50-200 plaque forming units of virus as previously described.[10] The host cells employed in this study included embryonic chick kidney monolayers for influenza A and Newcastle disease virus, ML monolayers for Coxsackie A-21, Hep-2 for parainfluenza-3 virus, and primary rabbit kidney monolayers for herpesvirus (MRS). The herpesvirus (MRS) which was used throughout this study was originally isolated from an oral herpetic lesion.[11]

b. *In Vitro* Herpesvirus Multiplication Studies. Washed monolayers of primary rabbit cells in 60-mm plastic petri dishes were infected with herpesvirus (MRS) at a multiplicity of infection of about 2. After 1 hr at 37°, the excess virus was removed and the monolayers were washed twice with 2 ml of Hanks solution. Each infected culture then received 4.5 ml of Eagles medium containing 3% fetal bovine serum and 0.5 ml of Hanks solution containing the drugs at the indicated final concentrations. The cultures were frozen and thawed twice when 50-75% of the cells in the non-treated infected cultures showed viral destruction. The virus titers were determined by the plaque method as previously described.[10]

For the *in vitro* studies, the compounds were prepared in DMSO (10 mg/ml) and further diluted in Hanks solution. The ara-A 5'-palmitate precipitated on dilution in Hanks, whereas the other compounds remained in solution.

Antiviral Activity *in Vivo*. In the studies reported here, ICR mice (Upjohn stock colony) weighed approximately 15 g. Inoculation consisted of giving 0.1 ml of the herpesvirus (MRS) (approximately 10^3 plaque forming units in Hanks solution) into the tail vein at approximately 3 cm from the base of the tail. The animals were observed twice daily for illness and death.

In the initial studies, ara-A was prepared as a suspension in an aqueous vehicle containing per milliliter: carboxymethylcellulose, 10 mg; polysorbate-80, 4 mg; and propylparaben, 0.42 mg. In those studies comparing the activity of ara-A with the 5'-esters, the drugs were prepared as a suspension in 0.9% NaCl. The drugs were given by the intraperitoneal route twice daily at 8:00 a.m. and 4:00 p.m.

Results

In Vitro **Antiviral Studies.** The results of testing ara-A and its 5'-esters *in vitro* against a DNA virus (herpes) and 4 RNA viruses [Coxsackie A-21, parainfluenza-3, influenza A (PR8), and Newcastle disease virus] using the plaque inhibition assay are shown in Table I. In this test, ara-A 5'-palmitate was inactive, whereas ara-A and the ara-A 5'-benzoate were active against herpesvirus and showed no activity against any of the RNA viruses. None of the compounds were cytotoxic to the host cells in this test. These data confirm the previously reported studies[2,3] regarding the selective antiviral activity of ara-A for DNA-containing viruses. This test depends upon the diffusion of the molecule through an agar medium. Hence, any compound which has poor diffusibility would appear to be inactive. Ara-A 5'-palmitate, because of its poor solubility, may be inactive by its poor diffusion.

The effect on final virus yields of different concentrations of each compound in the medium during the replication of herpesvirus is shown in Figure 1. It may be seen that nearly the same virus yields were obtained from those cultures treated with the same concentrations of either ara-A or ara-A 5'-benzoate. These data show that ara-A 5'-palmitate was the least active of the compounds; however, it should be noted that under the conditions of this test, the 5'-palmitate was quite insoluble. Similar observations in identical test systems have been made in our laboratory when ara-C and the ara-C 5'-palmitate were compared, the 5'-palmitate of ara-C being essentially inactive and insoluble.

In Vivo **Antiviral Studies.** Several preliminary studies were carried out with ara-A itself in mice inoculated with herpesvirus in an effort to maximize the antiviral activity. The reported activity of ara-A indicates that the compound shows good activity in herpesvirus infected mice at 250 mg/kg total daily dose when the compound is given twice daily.[5] The data in Figure 2 are from a study in which drug treatment was initiated at different times following virus inoculation and treatment in each case was continued for a total of 5 days. It can be seen that when the therapy was started on the day of virus inoculation, none of the treated mice died, whereas 65% of the control mice died. When the drug treatment was initiated on day 1 following virus inoculation, 20 and 65% of the mice died in the drug treated and control groups, respectively. When drug therapy was delayed

Table I. Inhibition of Plaque Formation of Viruses by Ara-A and Its 5'-Esters[a]

Virus	Cell	Ara-A	Ara-A 5'-benzoate	Ara-A 5'-palmitate
Coxsackie A-21	ML	0	0	0
Parainfluenza-3	Hep-2	0	0	0
Influenza A (PR8)	CK	0	0	0
Newcastle disease virus	CK	0	0	0
Herpesvirus (MRS)	RK	3	2	0

[a] Monolayers of susceptible cells were infected with 50-200 plaque forming units of virus and covered with Medium 199 containing 5% fetal calf serum and 1% agar. The drugs (approximately 0.05 ml of 1 mg/ml suspensions prepared as described in text) were placed in wells cut in the agar. After incubation at 37° for 2-4 days to allow for plaque formation, the degree of plaque inhibition was scored from 0 to 4, depending upon the diameter of the zone of plaque inhibition.

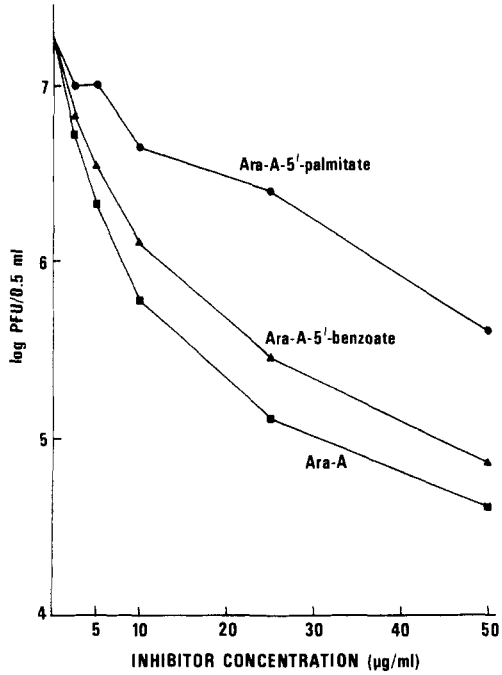

Figure 1. Effect of different concentrations of ara-A and its 5'-esters on herpesvirus yields in primary rabbit kidney monolayers. Details are given in the text.

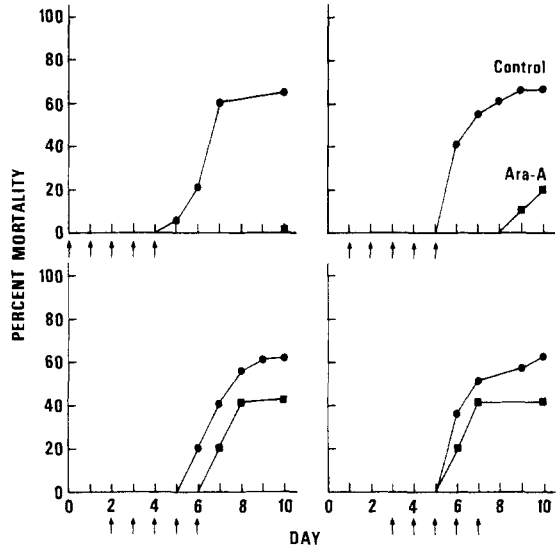

Figure 2. Effect of ara-A treatment, when instituted at different times following intravenous virus inoculation, on mortality of mice infected with herpesvirus. Virus was inoculated intravenously on day 0 followed by ip injection (two times per day) on the days indicated by the arrows. Control groups (20 mice) received vehicle only (•). Ara-A treated groups (ten mice) received ara-A (250 mg/kg total daily dose) in CMC (▪).

until the second or third days following virus inoculation, the mortality rates were not significantly different in the two groups; *i.e.*, 40% of the mice died in the drug treated groups and 60% of the mice died in the control groups. These data show that the antiviral activity of ara-A in herpesvirus infected mice may be augmented by initiating treatment early after inoculation of virus. In the studies which follow, drug treatment was initiated on day 1 following inoculation of virus.

The results shown in Figure 3 are from a study designed to establish the length of time necessary for treating herpesvirus inoculated mice with ara-A. The total daily dose of

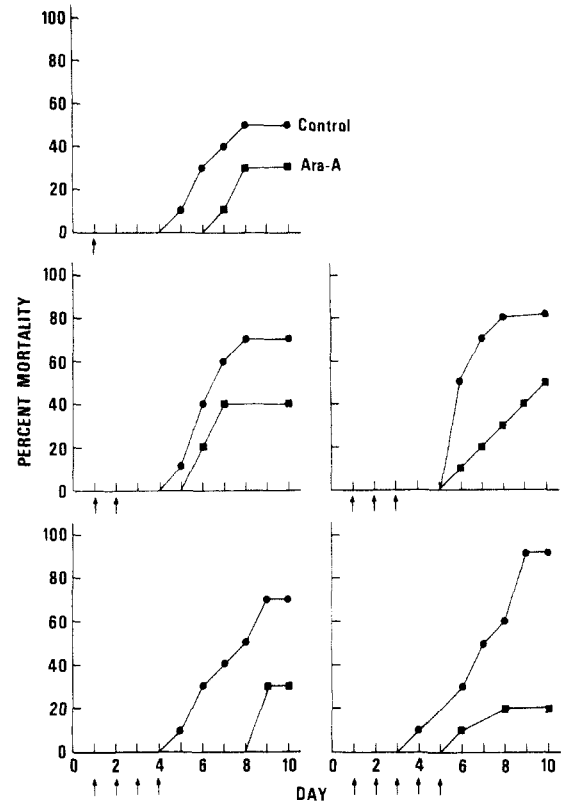

Figure 3. Effect of ara-A treatment, when given for different time intervals, on mortality of mice infected with herpesvirus. Virus inoculation was as described in Figure 2. Ara-A was given by ip injection (two times per day) on the days indicated by the arrows. The total daily dose of ara-A was 250 mg/kg.

ara-A given was 250 mg/kg, and the drug was given twice daily. The results show that the greatest antiviral activity was observed when drug treatment was allowed to continue for 5 days. Paradoxically, the percentage of control animals which died increased with increased handling of the animals. For example, 50% of the animals died in the control group treated for 1 day; however, when the animals were treated for 5 days, 90% of the control animals died. The reason(s) underlying this increased mortality with increased handling is not known. For the studies which follow, ara-A was given twice per day for 5 days beginning on day 1 following virus inoculation.

The activity of ara-A (250 and 125 mg/kg total daily dose) compared with that of ara-A 5'-palmitate (250 mg/kg total daily dose) and ara-A 5'-benzoate (125 mg/kg total daily dose) in mice inoculated with herpesvirus is shown in Figure 4. The data presented in Figure 4 show the cumulative percentages of mice which are healthy, ill, or dead. In this study, all of the animals appeared to be healthy during the first 3 days following virus inoculation; by the fourth day, 50% of the mice in the saline control group were ill. Of the mice treated with ara-A 5'-palmitate, 20% were ill, but all of the mice in the other groups were healthy. On day 6, ill or dead mice were found in all groups of mice. Of the mice 50% died between days 6 and 7 in the saline control group and in the groups treated with ara-A 5'-palmitate as well as the 5'-benzoate, whereas 50% death was not observed until the 9th day in the group treated with ara-A at 125 mg/kg and until the 14th day in the group treated with ara-A at 250 mg/kg. From these data, it is concluded that ara-A is active when given at a total daily dose of 125 and 250 mg/kg but that neither the ara-A 5'-benzoate nor the ara-A 5'-palmitate was active at the doses studied in this system.

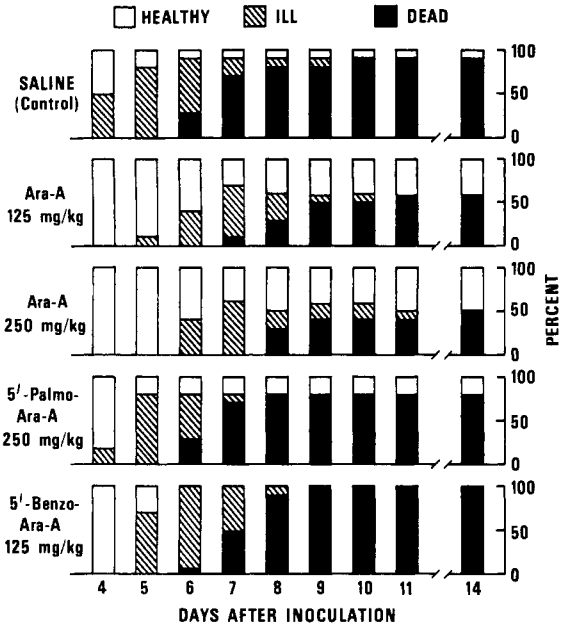

Figure 4. Comparison of ara-A and the 5'-esters in mice inoculated with herpesvirus. Virus inoculation was as described in Figure 2. The drugs were prepared in saline and given twice daily at the indicated total daily dose by ip injection. Drug treatments were initiated on the day after virus inoculation and continued for 5 days; *i.e.*, each animal received a total of ten treatments.

Discussion

The results of the studies reported herein confirm the previous reports regarding the antiviral activity of ara-A. Ara-A has been shown to be active against herpesvirus *in vitro* but not against several RNA viruses. Comparisons of virus yields from herpesvirus infected primary rabbit kidney cultures showed that ara-A did reduce the virus yield when incorporated into the growth medium. Our previous data with ara-C[10] would indicate that ara-A is less active in this *in vitro* test system than is ara-C. The 5'-benzoyl ester of ara-A shared *in vitro* properties with ara-A; *i.e.*, it was nearly as active as ara-A in the *in vitro* tests. However, the 5'-palmitoyl ester of ara-A was considerably less active than ara-A; perhaps this decreased *in vitro* activity is associated with the extreme insolubility of this compound.

Ara-A was shown to be effective in treating mice infected with herpesvirus. It was shown that the activity of ara-A could be maximized by initiating therapy early after virus inoculation and continuing drug treatment twice per day for 5 days with total daily doses of 250 mg/kg. Under these conditions where ara-A was shown to be effective, the 5'-esters of ara-A were devoid of *in vivo* antiviral activity. These observations are in contrast to the early studies with the 5'-esters of ara-C which were shown to have enhanced activity over ara-C itself.[9]

The reasons underlying the loss of *in vivo* antiviral activity of ara-A following esterification at the 5' position of this purine antagonist are unknown. It has been suggested[9] that the enhanced *in vivo* biological activities which have been observed with the 5'-esters of ara-C result from the fact that the ester is slowly hydrolyzed, thereby maintaining low levels of ara-C for long time periods. It is known that the triphosphate of ara-A is an effective inhibitor of DNA polymerase.[12] If indeed the mechanism of antiviral activity of ara-A is dependent upon the conversion (phosphorylation) of the nucleoside to the nucleotide with subsequent inhibition of viral DNA synthesis, then it is tempting to speculate that the esterases of the host are less effective in releasing ara-A from its 5'-acylates than they are in performing the comparable conversion of ara-C esters to the nucleoside.

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Synthesis and Antiinflammatory Evaluation of Certain 5-Alkoxy-2,7-dialkyltryptamines

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A variety of 5-methoxy-2,7-dialkyltryptamines was prepared and evaluated for central nervous system and antiinflammatory activites. Of these, the azabicyclononane derivative IX proved most interesting as a potential antiinflammatory agent. Various congeners of IX were synthesized but none was as active as IX. The Nenitzescu condensation between 2,5-dichlorobenzoquinone and alkyl 3-aminocrotonates to give 4,7-dichloro-5-hydroxy-2-methylindole-3-carboxylates is discussed.

The important place held by the indole nucleus in pharmaceutical chemistry and in animal as well as plant biochemistry is too well recognized to require documentation here (for reviews see *inter alia* ref 1). Suffice to note that a strong rationale is provided for the exploratory efforts of medicinal chemists seeking to develop novel pharmaceutical agents. Thus, when in the course of our synthesis of indoloquinone congeners of the mitomycin antibiotics, entry to the hitherto unrecorded 7-methyl-5-hydroxyindole series became available to us,[2] we undertook the preparation of a number of representative derivatives of this class for biological screening, and the results of this effort constitute the subject of this