Synthesis and Anti-Deoxyribonucleic Acid Virus Activity of Certain 9-β-D-Arabinofuranosyl-2-substituted Adenine Derivatives

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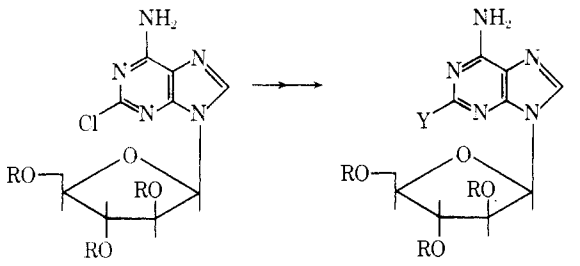
Several 2-substituted *ara*-A derivatives (2-methoxy, 2-methylthio, 2-benzyloxy) were prepared by nucleophilic displacement of chloride from 9-β-D-arabinofuranosyl-2-chloroadenine or 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-chloroadenine and debenzylation when appropriate. *In vitro* anti-DNA virus evaluation revealed 2-chloro-*ara*-A to have activity comparable to *ara*-A. In side-by-side examinations, *ara*-A proved to be more effective than 2-chloro-*ara*-A in the treatment of herpes or vaccinia virus-induced encephalitis in mice. Topical treatment of type 1 herpes simplex virus infections in hamsters with *ara*-A and 2-chloro-*ara*-A also indicated *ara*-A to be more efficacious in increasing the mean survival time.

The antiviral activity of 9-β-D-arabinofuranosyladenine (*ara*-A) against DNA viruses *in vitro* and *in vivo* is well documented.[1-8] It was the purpose of this investigation to determine the effect of 2-substitution of purine in *ara*-A upon the antiviral activity. During the course of our investigation, the synthesis of two 2-substituted *ara*-A derivatives (2-hydroxy and 2-amino) was reported[9] and the compounds were stated to possess anti-DNA virus properties.

Treatment of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-chloroadenine (1)[10] with NaOMe–MeOH gave the benzylated 2-methoxy-*ara*-A derivative **3** which was debenzylated in good yield by hydrogenation over a palladium catalyst to furnish 9-β-D-arabinofuranosyl-2-methoxyadenine (4). A similar displacement of chloride from 1 with sodium benzyl oxide in benzyl alcohol gave the benzylated 2-benzyloxy-*ara*-A intermediate. The carbohydrate benzyl ethers were found to be selectively removed by hydrogenation with 10% palladium on charcoal in a hydrogen atmosphere leaving the benzyl ether of the 2-hydroxyadenine derivative 6 unaffected. Acidic hydrolysis of 6 and chromatographic analysis of the mixture confirmed the presence of D-arabinose and the absence of a hydrolysis product with the properties of an arabinose monobenzyl ether, thereby substantiating the structure of 6 as 9-β-D-arabinofuranosyl-2-benzyloxyadenine.

Displacement of chloride from 9-β-D-arabinofuranosyl-2-chloroadenine[11] (2, 2-chloro-*ara*-A) with NaSMe in MeOH proceeded in excellent yield to furnish 2-methylthio-*ara*-A (5). Preparation of 5 from the benzylated intermediate 1 was avoided due to possible difficulties in effecting debenzylation after introduction of the 2-methylthio group (Scheme I).

Scheme I



1, R = CH_2Ph
2, R = H

3, Y = OCH_3; R = CH_2Ph
4, Y = OCH_3; R = H
5, Y = SCH_3; R = H
6, Y = OCH_2Ph; R = H

Antiviral Evaluation. *In vitro* antiviral studies were run in disposable plastic microplates.[12] In this system, an 18–24-hr monolayer of human carcinoma of the nasopharynx (KB) or continuous passaged rabbit kidney (RK-13) cells was exposed to 320 $CCID_{50}$ per ml of virus and concentrations of each compound ranging from 1000 to 0.01 μg/ml were added within 15 min after the virus. Antiviral activity was determined by observing inhibition of the viral cytopathic effect (CPE) after a 72-hr incubation at 37°. The degree of CPE inhibition and cytotoxicity exhibited by each compound was given a numerical virus rating (VR) as described previously.[12] A VR of 0.5 or greater was indicative of significant antiviral activity. The viruses used in the study included types 1 and 2 herpes simplex virus, pseudorabies virus, vaccinia virus, myxoma virus, type 3 adenovirus (DNA viruses), type 3 parainfluenza virus, and type 13 rhinovirus (RNA viruses). For comparative purposes, *ara*-A was included in each experiment as a known active control. 2-Chloro-*ara*-A was significantly effective against herpes simplex and vaccinia viruses, with the degree of activity approximately equal to that seen using *ara*-A (Table I). The other compounds tested were at best only weakly to moderately active against DNA viruses in these experiments. Each of the compounds had approximately the same degree of toxicity for KB and

Table I. Comparative *in vitro* Antiviral Activity[a] of 2-Chloro-*ara*-A, 2-Methylthio-*ara*-A, 2-Methoxy-*ara*-A, 2-Benzyloxy-*ara*-A, and *ara*-A

Virus	2-Chloro-*ara*-A	2-Methylthio-*ara*-A	2-Methoxy-*ara*-A	2-Benzyloxy-*ara*-A	*ara*-A
Type 1 herpes simplex	0.9	0.1	0.4	0.1	0.9
Type 2 herpes simplex	0.8	0.4	0.2	0.4	0.7
Pseudorabies	0.1	0.1	0.1	0.5	0.6
Vaccinia	0.9	0.3	0.4	0.0	0.8
Myxoma	0.1	0.4	0.2	0.1	0.8
Type 3 adeno	0.0	0.0	0.0	0.0	0.0
Type 3 parainfluenza	0.2	0.0	0.0	0.0	0.2
Type 13 rhino	0.0	0.0	0.0	0.0	0.1

[a] Antiviral activity was evaluated by the virus rating (VR) method[12] in which CPE development is compared in drug-treated cells (T) and virus control cells (C). In this method, the CPE value (0–4) assigned to T for each drug level was subtracted from that of C, and the differences (C − T) were totaled. If partial toxicity was evident at any drug level, the C − T of that level was divided by 2. The sum of all C − T values was then divided by ten times the number of test cups used per drug level. [b] Average virus rating (VR) of two or more experiments.

Table II. Effect of a Single Intracerebral Injection of 2-Chloro-*ara*-A or *ara*-A on Virus-Induced Encephalitis in Mice

Compound	Dosage,[a] mg/kg	Survivors/total	Survivor increase, P^b	Mean survival time,[c] days	Mean survival increase, P^d
		Herpes Simplex Virus			
2-Chloro-*ara*-A	1.6	4/10	<0.3	13.1	>0.10
ara-A	2.4	8/10	<0.001	18.3	<0.001
Placebo		4/20		10.0	
		Vaccinia Virus			
2-Chloro-*ara*-A	1.6	0/10		7.6	<0.05
ara-A	2.4	5/10	<0.001	15.2	<0.001
Placebo		0/20		6.5	

[a]Maximum obtainable dose as determined by preliminary solubility determinations. [b]Probability (χ^2 analysis). [c]Deaths were recorded for 21 days and survivors considered to have died on day 21. [d]Probability (Student's t test).

Table III. Effect of Topical Treatment of 2-Chloro-*ara*-A or *ara*-A on Type 1 Herpes Simplex Virus-Induced Infections in Hamsters

Compound	Dosage, %	Survivors/total	Survivor increase, P^a	Mean survival time,[b] days	Mean survival increase, P^c
2-Chloro-*ara*-A	20	1/8	>0.3	9.4	>0.05
ara-A	20	4/6	<0.001	12	<0.005
Placebo		0/10		7.3	

[a]Probability (χ^2 analysis). [b]Deaths were recorded for 21 days and survivors considered to have died on day 21. [c]Probability (Student's t test).

RK-13 cells as *ara*-A (maximum dose causing no visable cytotoxicity, 10 μg/ml).

Since 2-chloro-*ara*-A appeared active *in vitro*, it was then subjected to a series of *in vivo* experiments to further determine its activity in comparison with *ara*-A.

In initial experiments, encephalitis was induced in 18–20-g Swiss mice by intracerebral inoculation of an 80% infectious dose of type 1 herpes simplex virus (strain 123) or 10 LD_{50} of vaccinia virus (Western Reserve strain). A single intracerebral treatment with a compound was administered 6 hr after virus inoculation.[13] The doses used were obtained by injecting 0.03 ml of a saturated solution of the compounds, with actual concentration determined by ultraviolet spectroscopy. *Ara*-A (2.4 mg/kg) significantly increased survivor numbers and survival times in the herpes and vaccinia virus experiments. 2-Chloro-*ara*-A (1.6 mg/kg) increased survivor numbers in the herpes experiment but not in the vaccinia experiment, although it did delay the mean time of death in both herpes and vaccinia virus-infected mice. In this target-organ encephalitis system, antiviral activity depends, in part, on compound solubility; thus the less soluble compound, 2-chloro-*ara*-A, was also the less active (Table II).

2-Chloro-*ara*-A was tested in parallel with *ara*-A against type 1 herpes virus-induced keratitis-encephalitis in hamsters. In this experiment, 40–50-g female Syrian golden hamsters were infected intraocularly[14] with 3.2 LD_{50} of virus and then treated topically twice daily for 15 days beginning 4 hr later. Each compound, in a concentration of 20%, was suspended in Jellene-base ophthalmic ointment containing 1% chloramphenicol (Parke Davis and Co.). The antibiotic, which has no known antiviral properties, was utilized to reduce bacterial infection in the eye. Virus control hamsters were similarly treated with the antibiotic ointment devoid of either nucleoside. The animals were observed daily for signs of keratitis and deaths. 2-Chloro-*ara*-A was weakly effective in this experiment (Table III), with an approximate 2-day delay in signs of keratitis and in mean survival time. One treated animal survived the infection, and it had definite loss of vision in one eye on day 21. *Ara*-A was markedly effective, with two-thirds of the hamsters surviving the infection and showing no signs of keratitis or other eye infection on day 21. Neither drug appeared to be toxic in this experiment. In this virus system, the virus progresses from the cornea to the brain *via* the trigeminal nerve, where it causes a lethal encephalitis.[4,5,14] Topical treatment with *ara*-A apparently stops the infection before it can reach the brain, as reported previously.[4,5] 2-Chloro-*ara*-A slightly prolonged the survival time of the hamsters but was not able to increase the survivor numbers.

Experimental Section

9-(2,3,5-Tri-*O*-benzyl-β-D-arabinofuranosyl)-2-methoxyadenine (3). A solution of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-chloroadenine[10] (1, 2.40 g, 4.2 mmol) and NaOMe (0.6 g) in MeOH (85 ml) was heated for 3 days at reflux. The reaction mixture was cooled and neutralized with glacial acetic acid. The solvent was evaporated *in vacuo* and the residue was dissolved in chloroform (250 ml) and washed with water (3 × 250 ml). The chloroform was evaporated *in vacuo* and the residue was dried by evaporation of successive portions of absolute ethanol. The syrupy residue was dissolved in a minimum amount of chloroform, and the chloroform solution was column chromatographed on silica gel. The product was eluted with ethyl acetate–chloroform (1:9) and crystallized from MeOH to give 2.05 g (86%) of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-methoxyadenine as short needles: mp 103–105°; $[\alpha]^{25}D$ +39.6° (c 1.0, chloroform). *Anal.* ($C_{32}H_{33}N_5O_5$) C, H, N.

9-β-D-Arabinofuranosyl-2-methoxyadenine (4). A suspension of palladium chloride (1.75 g) in 2-methoxyethanol (25 ml) in a hydrogen atmosphere and 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-methoxyadenine (3, 1.14 g, 2 mmol) in 2-methoxyethanol (30 ml) was added. The mixture was hydrogenated under 3 atm of pressure for 2 hr at room temperature. The palladium catalyst was removed by filtration and washed with a small amount of 2-methoxyethanol. The filtrate was neutralized with 3 *N* NaOMe in MeOH and evaporated to dryness *in vacuo*. The residue was triturated with ice–water and the white solid was collected by filtration, dried, and recrystallized from water. This gave 0.5 g (79%) of 9-β-D-arabinofuranosyl-2-methoxyadenine (4) as needles: mp 239–242°; $[\alpha]^{25}D$ +2.0° (c 1.0, DMSO); uv λ_{max} (pH 1) 274 nm (ε 12,400); λ_{max} (pH 7, 11) 266 nm (ε 12,600). *Anal.* ($C_{11}H_{15}N_5O_5 \cdot H_2O$) C, H, N.

9-β-D-Arabinofuranosyl-2-methylthioadenine (5). To a solution of 9-β-D-arabinofuranosyl-2-chloroadenine[10] (2, 0.50 g, 1.65 mmol) in 2-methoxyethanol (35 ml) was added methanolic 2 *N* sodium methyl mercaptide (15 ml). The reaction mixture was heated at 100° for 3 hr. The solution was then cooled and neutral-

ized with 2 N HCl. The mixture was evaporated *in vacuo* to a small volume (~5 ml). The product was precipitated by addition of water and was collected by filtration. The crude product was recrystallized twice from water to give 0.43 g (83%) of 9-β-D-arabinofuranosyl-2-methylthioadenine (**5**) as needles: mp 245–247°; $[\alpha]^{25}D$ +48.6° (c 1.0, DMF); uv λ_{max} (pH 1) 208 and 268 nm (ε 22,100 and 17,500); λ_{max} (pH 7) 233 and 273 nm (ε 21,600 and 15,600); λ_{max} (pH 11) 234 and 274 nm (ε 21,400 and 15,900). *Anal.* ($C_{11}H_{15}N_5O_4S$) C, H, N.

9-β-D-Arabinofuranoxyl-2-benzyloxyadenine (6). A solution of 9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-2-chloroadenine (1, 1.72 g, 3 mmol) in benzyl alcohol (30 ml) containing sodium (0.21 g) was heated for 19 hr at 160–165°. The reaction mixture was cooled and diluted with chloroform (250 ml). The chloroform solution was washed with water (2 × 250 ml), then with 2 N HCl (2 × 250 ml), and finally with water (3 × 250 ml) before drying over Na_2SO_4. Silica gel (30 g) was added to the chloroform solution, and the mixture was evaporated *in vacuo* to dryness. The crude reaction product absorbed on silica gel was applied to the top of a silica gel column; elution with petroleum ether removed benzyl alcohol and the benzylated product was eluted with ethyl acetate–dichloromethane (3:7). The syrupy benzylated product was dissolved in 2-methoxyethanol (35 ml), and 10% palladium on charcoal (0.3 g) was added to the solution. The mixture was hydrogenated under 3 atm of pressure for 24 hr at room temperature before the palladium catalyst was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in a mixture of 15 ml of concentrated NH_4OH and 300 ml of water. Volume reduction *in vacuo* to 100 ml caused crystallization of the product. Recrystallization in the same manner gave 0.38 g (34%) of 9-β-D-arabinofuranosyl-2-benzyloxyadenine as needles: mp 275–280° dec; $[\alpha]^{25}D$ +6.0° (c 1, DMSO); uv λ_{max} (pH 1) 287 nm (ε 18,800); λ_{max} (pH 7) 248 and 295 nm (ε 9700 and 13,500); λ_{max} (pH 11) 226 and 286 nm (ε 16,100 and 15,300) *Anal.* ($C_{17}H_{19}N_5O_5$) C, H, N.

Hydrolysis of **6** with 1.75 N hydrochloric acid for 15 min at 65° and paper chromatographic analysis of the hydrolysis mixture [Whatman No. 1, descending, 4:1:5 (upper phase) *l*-butanol–ethanol–water] showed the presence of D-arabinose and absence of any benzylated D-arabinose.

Acknowledgments. The authors wish to thank Dr. Charles G. Tindall, Jr., for valuable suggestions and assistance. Thanks are also expressed to Nancy Campbell, Mary Patterson, Ana M. Shuman, and Nora Tung for their technical assistance.

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Notes

Synthesis and Antifungal Properties of 3-Substituted *as*-Triazino[5,6-*c*]quinolines

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Several 3-substituted *as*-triazino[5,6-*c*]quinolines (**1**, **2**, and **3**) have been found to possess antifungal activity[1] (Table II). The synthesis of the *as*-triazino[5,6-*c*]quinolines, which represent a new heterocyclic ring structure, is also described in this paper.

Chemistry. A synthetic route (Scheme I) to the 3-substituted *as*-triazino[5,6-*c*]quinolines (**1**, **2**, and **3**), similar to that used in the preparation of pyrimido[5,4-*e*]-*as*-triazines,[2] has been developed. The reaction of ethyl carbazate with the known 3-nitro-4-chloroquinoline (**4**) (a modification of the procedure of Bachman, *et al.*[3]) afforded the intermediate ethyl 3-(3-nitro-4-quinolyl)carbazate hydrochloride (**5**). Subsequent reduction of the nitro group of **5** gave ethyl 3-(3-amino-4-quinolyl)carbazate hydrochloride (**6**). Ring closure of **6** to *as*-triazino[5,6-*c*]quinolin-3(4*H*)-one (**9**) was accomplished by two different routes.

Treatment of **6** in EtOH with NaOMe in the presence of air afforded the sodium salt **7**. Subsequent acidification of **7** with AcOH gave **9**.

The second route to **9** was through the ring closure of **6** in hot AcOH to the intermediate 1,2-dihydro-*as*-triazino-[5,6-*c*]quinolin-3(4*H*)-one hydrochloride (**8**). Oxidation of **8** with $Pb(OAc)_4$ in AcOH gave **9**.

The chlorination (with $POCl_3$) of **9** provided the intermediate 3-chloro-*as*-triazine[5,6-*c*]quinoline (**1**), from which the 3-amino-*as*-triazino[5,6-*c*]quinolines (**2**) were prepared by facile chloro displacement with the appropriate amine. The compound 3-methoxy-*as*-triazino[5,6-*c*]quinoline (**3**) was obtained by chloro displacement of **1** with NaOMe in MeOH.

Mycology. The 3-substituted *as*-triazino[5,6-*c*]quinolines (**1**, **2a–c**, and **3**) (Table I), initially screened against *Candida albicans* and *Microsporum canis* by the agar diffusion–cylinder cup method,[4] were found to inhibit the growth of these organisms (Table II).

Compounds **1**, **2a–c**, and **3** were also screened in a minimum inhibitory concentration test against the following species of yeasts: *Torulopsis glabrata, Candida tropicalis, C. krusei, C. guillermondi,* and *C. albicans* (Table II). Against all organisms the 3-chloro (**1**) compound was consistently more active than the other compounds (**2a–c** and **3**), all of which showed a similar degree of activity. Also, compound **1** was as active as the known antifungal agent Nystatin.

In an agar dilution test[5] the 3-chloro (**1**) derivative showed complete inhibition of *Aspergillus niger* at 10