against Walker 256 and, unfortunately, more toxic than L-III. Interestingly, both D-III and L-III appear to be more active against Walker 256 and more toxic than III prepared from racemic propylenimine.[4]

Experimental Section[5]

2,5-Bis(D-2-methyl-1-aziridinyl)-*p*-benzoquinone (D-I).—To a solution of 1,4-benzoquinone (10.8 g, 0.10 mole) in 200 ml of EtOH was added 3.86 g (0.066 mole) of D-propylenimine.[2] The solution was cooled to 0° and, after 10 min, the product was filtered off. Recrystallization (MeOH) gave 3.1 g (43%) of orange platelets, mp 168–170°, $[\alpha]^{25}_{5780}$ −178° (*c* 0.5, pyridine). *Anal.* ($C_{12}H_{14}N_2O_2$) C, H, N. (*Cf.* ref 3a.)

L I was prepared in a similar manner using L-propylenimine;[2] mp 168–170°, $[\alpha]^{25}_{5780}$ 180° (*c* 0.5, pyridine). *Anal.* ($C_{12}H_{14}N_2O_2$) C, H, N.

Tris(D-2-methyl-1-aziridinyl)phosphine Sulfide (D-II).—A solution of 8.43 g (0.05 mole) of $PSCl_3$,[6] bp 122–124°, in 50 ml of PhH was added dropwise to a cold (5°), stirred solution of 8.6 g (0.15 mole) of D-propylenimine and 15.2 g (0.15 mole) of Et_3N in 200 ml of PhH. When the addition was complete, the reaction mixture was allowed to warm to room temperature and was stirred for an additional 1 hr. $Et_3N \cdot HCl$ that had precipitated was filtered off and washed with 50 ml of PhH. The filtrate and washings were combined, concentrated to 50 ml, and filtered again. The filtrate was distilled to give 8.0 g (69%) of D-II, bp 120–122° (2 mm), mp 41–43°, $[\alpha]^{25}_{5780}$ −99° (*c* 1.5, C_6H_6). *Anal.* ($C_9H_{18}N_3PS$) C, H, N, P, S. (*Cf.* ref 3b.)

L-II had bp 114–115° (1.6 mm), mp 41–43°, $[\alpha]^{25}_{5780}$ 97° (*c* 1.7, C_6H_6). *Anal.* ($C_9H_{18}N_3PS$) C, H, N.

2,4,6-Tris(D-2-methyl-1-aziridinyl)-*s*-triazine (D-III).—Using the method described by Schaefer,[3c] 3.1 g (0.055 mole) of D-propylenimine was converted to 1.6 g (36%) of D-II, mp 137–138° dec, $[\alpha]^{25}_{D}$ −147° (*c* 1.4, H_2O). *Anal.* ($C_{12}H_{18}N_6$) C, H, N.

L-III had mp 136–138° dec, $[\alpha]^{25}_{D}$ 152° (*c* 1.7, H_2O). *Anal.* ($C_{12}H_{18}N_6$) C, H, N.

(5) Temperatures are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkeley.

(6) Prepared by the method of J. Faschalek, (East) German Patent 10,041 (July 11, 1955); *Chem. Abstr.*, **52**, 20949 (1958).

Syntheses of 8-Bromo-5′-adenylate-Containing Nucleotides[1]

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The synthesis of 8-bromo-5′-adenylic acid (8-bromo-5′-AMP) has been noted recently,[2] but the high apparent yield, slightly low values given for maximum wavelength, and lack of certain assignment of the position substituted warranted further investigation of the direct bromination of 5′-AMP. We now have modified the earlier procedure to obtain in pure form the principal product from alkaline bromination of 5′-AMP, established the structure as the 8-bromo derivative, presented the correct absorption maxima, and shown the compound to be inactive with adenylate deaminase and adenylate kinase. In addition, 8-bromo-5′-AMP has now been used to synthesize the 5′-di- and -triphosphates, which are also inactive with adenylate kinase,

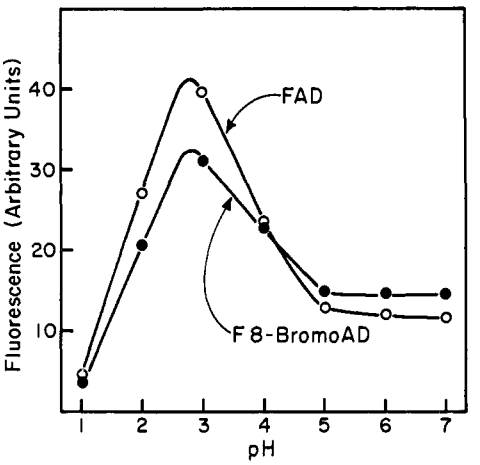

Figure 1.—Effect of pH change on the fluorescence intensities of F8-bromoAD and FAD. Flavins were approximately 10^{-5} *M* in 0.1 *M* buffers of KCl-HCl, 1; glycine-HCl, 2–3; sodium acetate, 4–5; sodium phosphate, 6–7.

and the analog of flavin–adenine dinucleotide (FAD), F8-bromoAD, which like FAD forms an intramolecular complex in solution.

Experimental Section[3]

8-Bromo-5′-adenylic Acid.—Direct bromination of 5′-AMP was accomplished by a modification of the procedure of Ikehara, *et al.*[2] For this, 7.3 g of 5′-AMP·H_2O (20 mmoles) was dissolved in 25 ml of H_2O containing 1.6 g of NaOH (40 mmoles) to make the disodium salt which was further diluted with 225 ml of 0.1 *N* NaOH. To this alkaline solution, 1.0 ml of Br_2 (20 mmoles) in 100 ml of H_2O was dripped in over a 2-hr period and the reaction mixture was stirred at 25° for an additional 5 hr. The solution was poured over a 2.5 × 40 cm column of Dowex 1X2 (formate) and the elution of compounds with a linear gradient from 2 l. of H_2O to 2 l. of 0.5 *N* HCO_2H was followed by absorbancy measurements at 260 mμ. The 8-bromo-5′-AMP was eluted after unreacted 5′-AMP, evaporated to dryness below 50°, dissolved in a small volume of EtOH, and precipitated with 4 vol of Et_2O. The compound was rinsed with Et_2O and dried *in vacuo* to obtain 1.9 g of acid monohydrate; 21% yield. *Anal.* Calcd for ($C_{10}H_{13}BrN_5O_7 \cdot H_2O$): C, 27.4; H, 3.4; Br, 18.0; N, 15.8; P, 7.0. Found: C, 27.8; H, 3.7; Br, 17.4; N, 15.3; P, 6.7. Comparison of nmr spectra of the bromo derivative and authentic AMP, each in DMSO-d_6, revealed an absence of the H-8 at τ 1.60 for the 8-bromo-5′-AMP and the presence of H-2 at τ 1.77.

The value of $\lambda^{pH\ 7}_{max}$ for 8-bromo-5′-AMP is 265 and for 5′-AMP is 259 mμ; $\lambda^{pH\ 2}_{max}$ for 8-bromo-5′-AMP is 262 and for 5′-AMP is 257 mμ.

The 8-bromo-5′-AMP was found to be inactive, both as substrate and as competitive inhibitor, when tested in the usual manner[4] with adenylate deaminase and adenylate kinase.

8-Bromo 5′-Di- and -Triphosphates.—The 5′-phosphoromorpholidate of 8-bromo-5′-AMP was prepared by the general method for nucleoside 5′-phosphoromorpholidates described by Moffatt and Khorana;[5] 93% yield as tetrahydrate. After drying at 100° *in vacuo* a bromine analysis was performed. *Anal.* Calcd for $C_{31}H_{51}BrN_9O_8P$: Br, 10.1. Found: Br, 9.6.

Reaction of 0.39 g of 8-bromoadenosine 5′-phosphoromorpholidate (0.5 mmole) for 5 hr at room temperature with 0.85 g of bis(tri-*n*-butylammonium) pyrophosphate (2.5 mmoles) in 20 ml of anhydrous pyridine was also according to the procedure of

(1) Supported in part by National Institute of Health Grant AM-04585 and by funds from the State University of New York.

(2) M. Ikehara, S. Ueiichi, and M. Kaneko, *Chem. Commun.*, 17 (1967).

(3) Absorption spectra were determined with a Cary Model 14 recording spectrophotometer; nmr spectra with a Varian 60 Mc nmr spectrometer; fluorescence readings with an Aminco-Bowman spectrophotofluorometer set at 450 mμ for activating wavelength and 520 mμ for emission. Elemental analyses were by Schwarzkopf Microanalytical Laboratory, Inc., Woodside, N. Y.

(4) D. B. McCormick, *Biochemistry*, **5**, 746 (1966).

(5) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 649 (1961).

Moffatt and Khorana[5] for the similar preparation of 5′-ADP and 5′-ATP from 5′-AMP. After separation of the nucleotides by chromatography on a 2.5 × 20 cm column of Dowex 1X8 (chloride) with elution by 2 l. of 0.003 *N* HCl to 2 l. of the same plus 0.5 *N* LiCl, extraneous lithium pyrophosphate was removed from the 8-bromo-5′-ATP and especially the 8-bromo-5′-ADP by dissolving the crude materials in 25 ml of cold H_2O, adjusting the pH to 2.5 with 0.1 *N* HCl, and adsorbing the nucleotides on 1 g of HCl-washed Norit. The pure nucleotides were eluted from the charcoal by twice stirring with 25-ml portions of 50% EtOH–H_2O, adjusting the pH to 7.5 with 0.1 *N* LiOH, and filtering off the charcoal. The filtrates were then evaporated to dryness for 65 mg of 8-bromo-5′-ADP and 46 mg of 8-bromo-5′-ATP, both as the tri- and tetralithium polyhydrates, respectively.

The relative positions of elution from Dowex 1X8 (chloride) and, as given in Table I, R_f values upon ascending paper chromatography identify the 8-bromonucleotides.

TABLE I

CHROMATOGRAPHIC CHARACTERIZATIONS (PAPER) AND YIELDS OBTAINED (COLUMN) OF 8-BROMO NUCLEOTIDES

Compound	R_f value (isobutyric acid–NH_4OH–H_2O, 66:1:33)	Yield, %
8-Bromo-5′-AMP	0.51	27
5′-AMP	0.48	
8-Bromo-5′-ADP	0.31	44
5′-ADP	0.29	
8-Bromo-5′-ATP	0.24	28
5′-ATP	0.22	

With adenylate kinase, 8-bromo-5′-ADP was found not to be phosphorylated by ATP, and 8-bromo-5′-ADP could not form the corresponding bromo derivatives of 5′-AMP and 5′-ATP in the reverse reaction.

Flavin-8-Bromoadenine Dinucleotide.—Condensation of 0.59 g of 8-bromoadenosine 5′-phosphoromorpholidate (0.5 mmole) with 0.81 g of tri-*n*-octylammonium FMN (1 mmole) in 100 ml of anhydrous pyridine plus 5 ml of DMF was done by a modification of the method for FAD synthesis by Moffatt and Khorana.[6] After 1 week at room temperature, the pyridine was evaporated off, the residue was dissolved in 25 ml of H_2O, and the solution was extracted twice with 25 ml of Et_2O. The aqueous phase was carefully neutralized with 1 *N* NH_4OH and the solution was poured over a 2.5 × 40 cm column of DEAE-cellulose (Cl^-). The small amount of riboflavin was washed through with 2 l. of H_2O, and the elution of flavin phosphates with a linear gradient from 2 l. of 0.003 *N* HCl to the same plus 0.1 *M* LiCl was followed by absorbancy measurements at 260 and 450 mμ. The F8-bromoAD which exhibits a 260/450 ratio near 3.7, was eluted after 8-bromo-5′-AMP and FMN, the pH was adjusted to 6 with 0.1 *N* LiOH, and the solution was lyophilized. The residue was stirred in 20 ml of MeOH and the Li_2 salt of F8-bromoAD was precipitated with 200 ml of Me_2CO plus 20 ml of Et_2O. The yellow-orange precipitate was collected by centrifugation and washed twice more to remove all LiCl by resuspending in 3 ml of MeOH and reprecipitating with 30 ml of Me_2CO plus 3 ml of Et_2O. This material was dissolved in 10 ml of H_2O, filtered, and lyophilized for 130 mg; 28% yield based on initial phosphoromorpholidate. After drying at 50° *in vacuo*, a bromine analysis was performed. *Anal.* Calcd for $C_{27}H_{32}BrLi_2N_9O_{15}P_2 \cdot 2H_2O$: Br, 8.6. Found: Br, 8.1. Tlc on MN silica gel S-HR with *n*-BuOH–AcOH–H_2O (2:1:1) as ascending solvent gave R_f values of 0.23 and 0.18 for F8-bromoAD and FAD, respectively (fluorescent uv). The $\lambda_{max}^{neutral}$ values for the F8-bromoAD are the same as for FAD at 450 and 375 mμ, but the bromo compound evidences a slight red shift of 267 mμ from the 264-mμ maximum of FAD.

As shown in Figure 1, F8-bromoAD like FAD has maximal fluorescence between pH 2.5 and 3.0 and decreases toward more nearly neutral pH due to intramolecular complexing with quenching of the isoalloxazine fluorescence by the purine moiety.[7]

The coenzymatic reactivity of F8-bromoAD is being investigated with D-amino acid apooxidase, and an attempt will be made to form crystals of this analog suitable for X-ray studies of the three-dimensional structure.

(6) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 3756 (1958).

(7) D. B. McCormick in "Molecular Associations in Biology," B. Pullman, Ed., Academic Press, New York, N. Y., 1968, p 377.

Synthesis of Potential Antineoplastic Agents. Substituted Phenylcyclohexenes[1]

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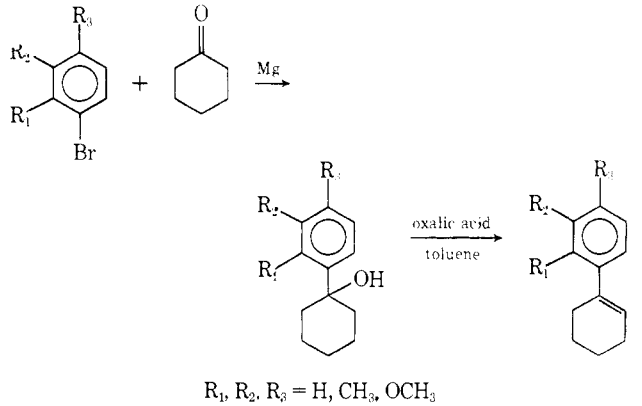
The finding of limited antitumor activity of related intermediates, 1-(2,3-dimethoxyphenyl)cyclohexene and 1-(2-methoxyphenyl)cyclohexene, prompted the attempt to synthesize similar compounds with more potent antitumor activity and relate this activity to chemical activity.

Compounds I–VII were synthesized according to Scheme I. The formation of all Grignard reagents was

SCHEME I

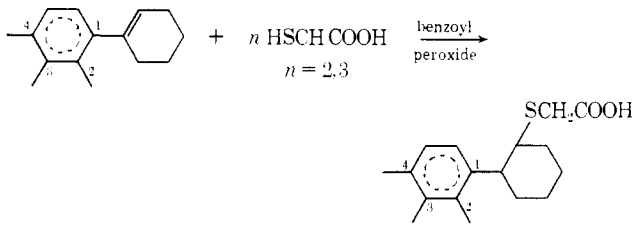


R_1, R_2, R_3 = H, CH_3, OCH_3

spontaneous and vigorous, except that of 1-bromo-2,3,4-trimethoxybenzene which required heating. Condensation of the Grignard reagents with cyclohexanone gave

TABLE I

RELATIVE REACTIVITIES OF SUBSTITUTED PHENYLCYCLOHEXENES TOWARD MERCAPTOACETIC ACID AT 30°



Compd	Ring substituent	Rel reactivity, n = 2	No. of runs	Rel reactivity, n = 3	No. of runs
I	4-OCH_3	1.33 ± 0.06	2	1.32 ± 0.02	2
III	2-OCH_3	1.26 ± 0.06	2	1.33 ± 0.06	2
II	3-OCH_3	1.00 ± 0.01	2	1.07 ± 0.01	2
IV	4-CH_3	1.12 ± 0.02	2	1.14 ± 0.02	2
V	3-CH_3	1.09 ± 0.05	2	1.03 ± 0.02	2
VIII	None	1.00	2	1.00	
VII	2,3,4-$(OCH_3)_3$	0.95 ± 0.02	2	...	
IX	2,3-$(OCH_3)_2$	0.71 ± 0.00	2	...	
VI	2-CH_3	0.52 ± 0.04	2	0.58 ± 0.06	2

(1) Supported in part by an Institutional General Research Cancer Grant (IN-76D), National Institutes of Health Training Grant 5 T1 GM1171-04, and National Institutes of Health Research Grant 1 R01 CA10270-01.

(2) A portion of this work was abstracted from the Ph.D. dissertation of S. Core.