In order to determine the importance of metabolism with respect to $\omega - 1$ oxidation we are now studying longer chain derivatives and are synthesizing other compounds with the $\omega - 1$ carbon atom blocked by various substituents. Studies on the direct measurements of metabolsim by hepatic microsomes have also been initiated.

Experimental Section

5-Ethyl-5-(3,3-dimethylbutyl)barbituric acid (VII) was synthesized as shown in the literature[9] except that 3,3-dimethylbutyl chloride was used instead of the bromide. The melting point was 191–193°. The X-ray structure of our synthetic compound has been recently accomplished.[10]

(9) F. C. Whitmore and M. A. Thorpe, U. S. Patent 2,161,212 (1939).
(10) B. Craven, Department of Crystallography, University of Pittsburgh, personal communication.

Structure–Activity Relationships of Ethylenimines. VIII. Optically Active Methyl-Substituted Ethylenimines[1]

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The reaction of butadiene monoxide and propylenimine gives a 70:30 mixture of 1-(2-methyl-1-aziridinyl)-3-buten-2-ol and 2-(2-methyl-1-aziridinyl)-3-buten-1-ol.[2] Interestingly, the product mixtures obtained from D-propylenimine and L-propylenimine showed different activities against Adenocarcinoma 755, the L mixture being the more active. Although this difference in activity was suggestive of a relationship between biological activity and the absolute configuration of a substituted carbon on the aziridine ring, such an interpretation was considered tenuous because the products were not pure enantiomers.

In order to make possible more suitable examination of the relationship between biological activity and the absolute configuration of a substituted carbon on the aziridine ring, we prepared 2,5-bis(D-2-methyl-aziridinyl)-*p*-benzoquinone (D-I), tris(D-2-methyl-1-aziridinyl)phosphine sulfide (D-II), 2,4,6-tris(D-2-methyl-1-aziridinyl)-*s*-triazine (D-III), and their L enantiomers (L-I–III). Syntheses were accomplished using the appropriate D- or L-propylenimine[2] and methods similar to these described for preparation of the racemic analogs.[3,4] Preliminary screening data, obtained by the Cancer Chemotherapy National Service Center, are summarized in Table I.

Although the paucity of the data does not allow any conclusion regarding the relationship of activity and the absolute configuration of the substituted carbon on the aziridine ring, D-III appears to be more active

TABLE I

ANTITUMOR ACTIVITY[a]

Compd	Test system	Control no.	Dose,[b] mg/kg	Survivors	Wt dif, g. T − C	T/C[c]
D-I[d,e]	WA	329	3.50	6/6[f]	−31	0.2/7.0
	WA	329	1.70	5/6	4	4.9/7.0
	WA	329	0.80	6/6	−7	5.7/7.0
L-I[d,g]	WA	331	1.25	6/6	−8	3.7/8.0
	WA	331	0.62	6/6	−5	8.6/8.0
	WA	331	0.31	6/6	−4	6.2/8.0
L-II[h]	DL	256	12.0	4/6	−19	12.5/13.5
	DL	256	6.00	6/6[i]	−6	30.0/13.5
	DL	256	3.00	6/6	−5	20.0/13.5
	DL	256	1.50	6/6	−3	16.0/13.5
D-III[j–l]	WA	68	1.20	6/6	−17	0.0/10.0
	WA	68	0.60	6/6	−7	0.0/10.0
	WA	68	0.30	6/6	−19	5.4/10.0
	WA	68	0.15	6/6	7	9.0/10.0
	WA	74	0.300	6/6	0	3.3/7.9
	WA	74	0.150[m]	6/6	2	5.3/7.9
	WA	87	1.20[n]	6/6	−16	0.0/8.8
	WA	87	0.60[n]	6/6	−5	0.0/8.8
	WA	87	0.30[n]	6/6	−9	0.7/8.8
	WA	87	0.15[n]	6/6	4	3.7/8.8
	WA	87	0.08[n]	6/6	3	4.8/8.8
L-III[j,l]	WA	68	3.75	5/6	−34	0.0/10.0
	WA	68	1.87	6/6	−25	0.0/10.0
	WA	68	0.94	6/6	−22	9.1/10.0
	WA	68	0.47	6/6	2	3.9/10.0
	WA	74	0.94	6/6	−6	0.0/7.9
	WA	74	0.47	6/6	−26	1.3/7.9
	WA	74	0.24	6/6	−6	5.6/7.9
	WA	74	0.12	6/6	9	5.8/7.9
	WA	87	3.75[n]	4/6	−44	0.0/8.8
	WA	87	1.87[n]	6/6	−27	0.0/8.8
	WA	87	0.94[n]	6/6	−16	0.0/8.8
	WA	87	0.47[n]	6/6	−16	0.0/8.8
	WA	87	0.24[n]	6/6	−4	2.8/8.8
	WA	94	0.47[n]	7/7	−11	2.7/8.8
	WA	94	0.24[m,n]	7/7	−1	4.8/8.8

[a] See *Cancer Chemotherapy Rept.*, **25**, 1, 10 (1962). [b] Unless otherwise noted, the vehicle was carboxymethylcellulose, and the route was intraperitoneal. [c] For Walker 256 (WA) test system: tumor weight in grams; for Dunning leukemia (solid) (DL) test system: survival time in days. [d] In tests using KB cells with control numbers 343, 349, and 164 (two different screeners), D-I had ED_{50}'s of <1.0, 0.40 (slope = −0.46), and <0.25 μg/ml, respectively, and L-I had ED_{50}'s of <1.0, 0.36 (slope = −0.45), and 0.23 (slope = −0.78) μg/ml, respectively. [e] Toxic at a dose level of 7.10 mg/kg. [f] Four cures. [g] Toxic at a dose level of 2.50 mg/kg. [h] D-II and L-II, at dose levels of 6.00–48.0 and 3.75–300 mg/kg, respectively, did not produce prolonged survival times when tested against lymphoid leukemia L1210 test system. [i] Six cures. [j] From tumor inhibition *vs.* dosage plots of these data, T/C 0.10 were estimated for D- and L-III as *ca.* 0.5 and 1.4 mg/kg/day, respectively; the approximate 95% confidence limits were 0.2–1.2 and 0.4–4.8 mg/kg/day, respectively. [k] From a tumor inhibition *vs.* dosage plot of similar data (control numbers 36, 87, 94, 103, and 110) for 2,4,6-tris(2-methyl-1-aziridinyl)-*s*-triazine prepared with racemic propylenimine, T/C 0.10 was estimated as *ca.* 12.5 mg/kg/day, with approximate 95% confidence limits of 1.2–12 mg/kg/day. [l] Preliminary LD_{50}'s for D-III, L-III, and III prepared from racemic propylenimine are 1.2, 3.8, and 12.5 mg/kg/day. [m] Inactive at lower dose levels in this series. [n] The vehicle was saline.

(1) (a) Part VII: A. T. Bottini, B. F. Dowden, and R. L. VanEtten, *J. Am. Chem. Soc.*, **87**, 3250 (1965). (b) Supported in part by Grant No. CA-05528 from the National Cancer Institute of the Public Health Service.
(2) A. T. Bottini and V. Dev, *J. Med. Pharm. Chem.*, **5**, 925 (1962).
(3) (a) W. Gauss, S. Petersen, G. Domagk, and C. Hackmann, German Patent 967,344 (Nov 7, 1957); *Chem. Abstr.*, **53**, 13173 (1959); (b) E. Kuh and F. R. Seeger, U. S. Patent 2,670,347 (Feb 23, 1954); *Chem. Abstr.*, **49**, 2181 (1955); (c) F. C. Schaefer, *J. Am. Chem. Soc.*, **77**, 5028 (1955).
(4) Note that use of racemic propylenimine instead of optically pure propylenimine in any of these syntheses gives a mixture of diastereomers. For example, in the absence of asymmetric induction, the reaction of thiophosphoryl chloride with racemic propylenimine will give a 3:1 mixture of the DD,LL and DD,LL diastereomers.

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,

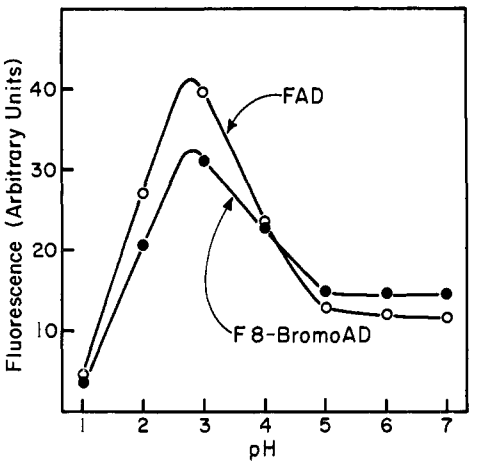

Figure 1.—Effect of pH change on the fluorescence intensities of F8-bromoAD and FAD. Flavins were approximately 10^{-5} *M* in 0.1 *M* buffers of KCl-HCl, 1; glycine-HCl, 2–3; sodium acetate, 4–5; sodium phosphate, 6–7.

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).