then diluted with 10 mL of H_2O and cooled. The resultant solid was collected, washed with water, and dried: yield 1.83 g (73%); mp 232–238 °C. Recrystallization from THF gave the analytical sample with mp 239–241 °C; TLC (SG) sol 3 and 4; NMR (Me_2SO-d_6) δ 11.72 (br s, 1 H, NH), 8.15 (s, 1 H, C-6), 7.86 (s, 4 H, ArH), 5.08 (s, 2 H, NCH_2O), 3.79 (s, 4 H, CH_2CH_2). Anal. ($C_{15}H_{12}BrN_3O_5$) C, H, N.

1-[(2-Aminoethoxy)methyl]uracil Hydrochloride (5). A mixture of 4.0 mL (124 mmol) of 96% hydrazine, 2.00 g (6.34 mmol) of **2**, and 200 mL of EtOH was refluxed with stirring for 1.25 h. The mixture was cooled and spin evaporated in vacuo at <45 ° C and then four times dispersed in EtOH and evaporated to dryness to remove residual hydrazine. The resultant solid was dispersed in 200 mL of 0.1 N HCl and stirred for 1 h at ambient temperature. The white solid was removed by filtration and washed with water. The combined filtrate and wash were spin evaporated in vacuo to give a light yellow powder, which was recrystallized from aqueous EtOH–C_6H_6. Recrystallization as above gave the analytical sample: yield 0.650 g (46%); mp 226–228 °C; TLC (C) sol 5; UV (0.1 N HCl) λ_{max} 258 nm (ϵ 9900); UV (0.1 N NaOH) λ_{max} 259 nm (ϵ 7000); NMR (Me_2SO-d_6) δ 11.25 (br s, 1 H, NH), 8.31 (br s, 3 H, NH_3^+), 7.81 (d, 1 H, J = 8.0 Hz, C-6), 5.63 (d, 1 H, J = 8.0 Hz, C-5), 5.16 (s, 2 H, NCH_2O), 3.75 (t, 2 H, J = 5.0 Hz, OCH_2C), 2.97 (t, 2 H, J = 5.0 Hz, CCH_2N). Anal. ($C_7H_{11}N_3O_3$·HCl) C, H, N.

1-[(2-Aminoethoxy)methyl]-5-methyluracil Hydrochloride (6). A solution of 3.05 g (9.26 mmol) of **3** and 100 mL of 40% aqueous $MeNH_2$ was stirred at ambient temperature for 48 h and then spin evaporated in vacuo. The residual syrup was thrice dissolved in EtOH and reevaporated. The syrup was dissolved in a minimum of EtOH, and Et_2O was added to incipient tubidity. Ten milliliters of HCl-saturated MeOH was added in 1-mL portions to give solids, which were then diluted with Et_2O to a 240-mL volume. The solvent was decanted from the solids, and the Et_2O wash was thrice repeated. The solids were collected and dried: yield 1.99 g (91%); mp 220–225 °C. Since this material contained some phthalimide impurity, it was dissolved in 50 mL of H_2O and washed with Et_2O. The aqueous solution was spin evaporated in vacuo at <40 °C, EtOH was added to the residue, and it was reevaporated to give a white solid that was recrystallized from EtOH: yield 0.566 g (26%); mp 223–225 °C. A second crop yielded 0.365 g (42% total), mp 222–224 °C. An additional recrystallization gave the analytical sample: mp 226–227 °C; TLC (C) sol 6; UV (0.1 N HCl) λ_{max} 265 nm (ϵ 8600), UV (0.1 N NaOH) λ_{max} 265 nm (ϵ 6400); NMR (Me_2SO-d_6) δ 9.00 (br s, 3 H, NH_3^+), 7.67 (s, 1 H, C-6), 5.13 (s, 2 H, NCH_2O), 3.75 (t, 2 H, J = 5.0 Hz, OCH_2C), 2.97 (t, 2 H, J = 5.0 Hz, CCH_2N), 1.79 (s, 3 H, CH_3). Anal. ($C_8H_{13}N_3O_3$·HCl) C, H, N.

1-[(2-Aminoethoxy)methyl]-5-chlorouracil Hydrochloride Hydrate (7). A magnetically stirred mixture of 0.500 g (2.26 mmol) of **5**, 0.339 g (2.49 mmol) of *N*-chlorosuccinimide, and 3.5 mL of AcOH was heated at ~70 °C for 1.5 h. The mixture was filtered hot and let stand at ambient temperature overnight. The resultant crystals were collected, washed with a minimum of EtOH, and dried to give 0.450 g (73%) of a crystalline solid which was one spot on TLC. The solid was dissolved in aqueous EtOH, decolorized (Norit), and concentrated by boiling with continued addition of C_6H_6. Two recrystallizations in this manner gave analytically pure material: yield 0.249 g (40%); mp 224–226 °C; TLC (C) sol 7; UV (0.1 N HCl) λ_{max} 273 nm (ϵ 8500), UV (0.1 N NaOH) λ_{max} 273 nm (ϵ 5800); NMR (Me_2SO-d_6) δ 9.04 (br s, 3 H, NH_3^+), 8.26 (s, 1 H, C-6), 5.13 (s, 2 H, NCH_2O), 3.73 (t, 2 H, J = 5.0 Hz, OCH_2C), 2.96 (t, 2 H, J = 5.0 Hz, CCH_2N). Anal. ($C_7H_{10}ClN_3O_3$·HCl·H_2O) C, H, N, Cl.

1-[(2-Aminoethoxy)methyl]-5-bromouracil Hydrobromide Hydrate (8). To a magnetically stirred dispersion of 4.00 g (18.0 mmol) of **5** in 35 mL of AcOH was added 6.8 g (21 mmol) of pyridinium hydrobromide perbromide and 10 mL of AcOH. This mixture was heated at ~60 °C for 1 h and cooled on ice. The solids were collected and washed with acetone. Dilution of the filtrate with acetone gave a second crop. The combined crops were recrystallized from aqueous EtOH: yield 4.94 g (75%). Four recrystallizations from aqueous EtOH–C_6H_6 gave the analytically pure material: yield 2.62 g (40%); mp 184–186 °C dec; TLC (C) sol 7; UV (0.1 N HCl) λ_{max} 276 nm (ϵ 8800), UV (0.1 N NaOH) λ_{max} 275 nm (ϵ 5900); NMR (Me_2SO-d_6) δ 8.90 (br s, 3 H, NH_3^+), 8.34 (s, 1 H, C-6), 5.18 (s, 2 H, NCH_2O), 3.75 (t, 2 H, J = 5.0 Hz, OCH_2C), 3.02 (t, 2 H, J = 5.0 Hz, CCH_2N). Anal. ($C_7H_{10}BrN_3O_3$·HBr·H_2O) C, H, N, Br.

1-[(2-Aminoethoxy)methyl]-5-iodouracil Hydrochloride Hydrate (9). A stirred mixture of 1.00 g (4.51 mmol) of **5**, 5.0 mL of AcOH, 7.3 g (45 mmol) of iodine monochloride, and 2 mL of 0.1 N HCl was heated at ~80 °C for 1 h. The mixture was cooled, diluted with 100 mL of H_2O, and extracted with $CHCl_3$ until the halogen color was no longer intense. The remaining halogen was removed by stirring the aqueous solution with cyclohexene. The layers were separated, and the aqueous layer was spin evaporated in vacuo at <40 °C to 20 mL. One-hundred milliliters of EtOH was added, and the solution was concentrated by boiling with the continued addition of C_6H_6. Concentration to 75 mL followed by cooling gave white crystals, which were collected and dried: yield 10.70 g (42%). A second recrystallization gave analytically pure material: yield 0.56 g (34%); mp 236–239 °C; TLC (C) sol 6; UV λ_{max} (0.1 N HCl) 283 nm (ϵ 7200), UV (0.1 N NaOH) λ_{max} 277 nm (ϵ 5300); NMR (Me_2SO-d_6) δ 9.11 (br s, 4 H, NH_3^+, NH), 8.32 (s, 1 H, C-6), 5.15 (s, 2 H, NCH_2O), 3.75 (t, 2 H, J = 5.0 Hz, OCH_2C), 2.97 (t, 2 H, J = 5.0 Hz, CCH_2N). Anal. ($C_7H_{10}IN_3O_3$·HCl·H_2O) C, H, N, Cl, I.

Acknowledgment. The authors are indebted to Dr. D. J. Bauer and his group for the antiviral testing, to Ms. N. K. Cohn for the Detroit 98 and mouse L cells cytotoxicity tests, and to Dr. J. A. Fyfe and Mr. P. M. Keller for the HSV-1 thymidine kinase results. We thank Dr. B. S. Hurlbert and his staff for the elemental analyses and NMR spectra, and we acknowledge the excellent technical assistance of Mrs. A. Melton.

Sulfonate Analogues of Adenosine Nucleotides as Inhibitors of Nucleotide-Binding Enzymes

Paul H. C. Mundill, Richard W. Fries, Christoph Woenckhaus, and Bryce V. Plapp*

Department of Biochemistry, The University of Iowa, Iowa City, Iowa 52242. *Received October 15, 1980*

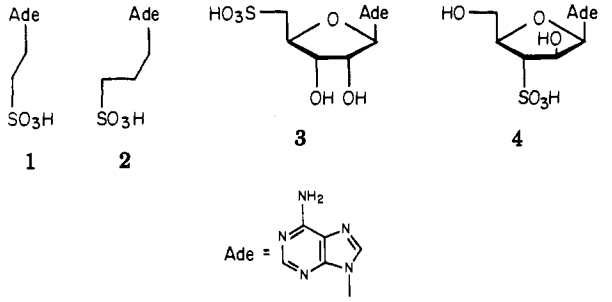
2-(Adenin-9-yl)ethanesulfonic acid (**1**), 3-(adenin-9-yl)propanesulfonic acid (**2**), 9-(5-deoxy-β-D-ribofuranosyl)-adenine-5′-sulfonic acid (**3**), and 9-(3-deoxy-β-D-arabinofuranosyl)adenine-3′-sulfonic acid (**4**) were prepared by reaction of the corresponding chlorides by sodium sulfite (**1**–**3**) or by reaction of an epoxide with sodium hydrogen sulfite (**4**). They inhibited a typical nucleotide-binding enzyme, horse liver alcohol dehydrogenase, with inhibition constants in the range of 0.18–4.9 mM at pH 8, 25 °C.

Adenosine nucleotide analogues in which the monophosphate ester is replaced by a sulfonate group are of interest as inhibitors of nucleotide-binding enzymes. Furthermore, they are precursors of sulfonate esters, which are mild alkylators and could be active-site-directed alkylators of such enzymes.[1,2] Under physiological condi-

0022-2623/81/1824-0474$01.25/0

tions, the sulfonate analogues, or derivatives thereof, may be able to penetrate cell membranes.[3,4] It may be noted also that the sulfonate group cannot be removed by hydrolysis, and therefore the nucleoside sulfonates should be more resistant to metabolism than nucleoside monophosphates. Thus, these sulfonate analogues have potential applications in chemotherapy.[5-7]

The simplest adenosine analogues are the aliphatic derivatives of adenine. Since these analogues can be relatively easily synthesized[8,9] and their monophosphate esters are often inhibitors, competitive against the parent nucleotide,[9,10] they are attractive for initial studies. An examination of space-filling models indicates that attaching a sulfonate group to the 9 position of adenine by either a two-carbon (1) or three-carbon (2) chain would give rise



to analogues that could mimic 2'- and 3'-AMP. On an alkyl chain, the sulfonate group will have considerable freedom of movement (as compared to the phosphate group on a furanose ring of AMP) and should have the flexibility to fit into a nucleotide-binding pocket. We accordingly synthesized and evaluated two analogues of this type. Being encouraged by the biological data, we then synthesized and evaluated 3 and 4, which are isosteric with the nucleoside monophosphates, except for the lack of an atom between the sulfur and the sugar.

Chemistry. Using established procedures,[11] the sodium salt of adenine was allowed to react in situ with either 1-bromo-2-chloroethane or 1-bromo-3-chloropropane to give the corresponding alkyl chlorides. The crude chlorides were then treated with sodium sulfite[12] in refluxing aqueous methanol to give the desired sulfonic acids 1 and 2, respectively. An examination of their UV spectra (λ_{max} = 260 nm) confirmed that only the desired adenin-9-yl isomers had been formed.[13]

Our strategy for the more complex adenosine analogues 3 and 4 required as a key step an efficacious nucleophilic displacement at the 5' and 3' positions, respectively. Unfortunately, one of the major problems in such a transformation at the 5' position in the adenosine series has been the concomitant formation of the $N^3,C^{5'}$-cyclonucleoside.[14] This pathway is favored if, during the transition state, there is a buildup of positive charge at $C_{5'}$.[15] Thus, by using a nonpolar medium and conducting the displacement under S_N2 conditions, this side reaction should be minimized. We decided to use 5'-deoxy-5'-chloroadenosine as the precursor to the sulfonic acid. Although chloride is a poor leaving group, we reasoned that this would help promote an S_N2 pathway and, by using an excess of a good nucleophile, the internal cyclization pathway would be minimized.

5'-Deoxy-5'-chloroadenosine was prepared[16] in one step from adenosine in good yield (90%). Unfortunately, due to solubility problems it was not possible to conduct the displacement reaction in a nonpolar medium. However, treatment of 5'-deoxy-5'-chloroadenosine with an excess of sodium sulfite in refluxing methanol–water (2:3) gave a satisfactory yield (50–60%) of the 5'-sulfonic acid (3). As expected,[17] the ^1H NMR spectra revealed that the signals corresponding to the 5' protons had moved upfield (by 0.3 ppm) with respect to adenosine. A similar effect was observed in the ^{13}C NMR spectra[18] (upfield shift being 6 ppm), thus confirming the introduction of a sulfonate group at the $C_{5'}$ position.

Continuing with the same strategy we attempted to prepare the 3' analogue by direct nucleophilic displacement. Such displacements are reported[19] to be difficult to achieve, often not occurring at all and when they do only in low yield. In order to test the feasibility of this strategy, 2',3',5'-trideoxy-3',5'-dichloroadenosine was prepared[20] in one step from 2'-deoxyadenosine and used as a model compound. The displacement reaction was attempted with a variety of nucleophiles (CH_3COS^-, SCN^-, SO_3^{2-}, and HSO_3^-) in a number of different solvent systems (DMF, CH_3OH/H_2O, CH_3CN/H_2O). In no case was evidence obtained to indicate that displacement had occurred at the 3' position. We therefore examined an alternative approach in which the known epoxide 9-(2,3-anhydro-β-D-lyxofuranosyl)adenine[21] would be a key intermediate. Opening of this epoxide with the appropriate nucleophile would be expected to give predominantly the 3' isomer.[21,22] Although the 2'-hydroxyl group would be in the arabino configuration, it could be inverted,[22] if required, to give the 3'-AMP analogue.

The key epoxide was prepared in moderate yield following literature procedures.[21] The opening of this epoxide proved to be a little more difficult than expected. Treatment with potassium thiocyanate, which has been reported[23] to open similar epoxides and give predominantly

(1) B. V. Plapp, *J. Biol. Chem.*, **248**, 4896 (1973).
(2) Y. Nakagawa and M. L. Bender, *Biochemistry*, **9**, 259 (1970).
(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors", Wiley, New York, 1967, pp 10 and 265.
(4) J. A. Montgomery, G. L. Dixon, E. A. Dulmage, H. J. Thomas, R. W. Brockman and H. E. Skipper, *Nature (London)*, **199**, 769 (1963).
(5) E. De Clercq and A. Holý, *J. Med. Chem.*, **22**, 510 (1979).
(6) P. Langen, "Antimetabolites of Nucleic Acid Metabolism", Gordon and Breach, New York, 1975.
(7) R. N. Prasad, D. S. Bariana, A. Fung, M. Savic, K. Tietje, H. H. Stein, H. Brondyk, and R. S. Egan. *J. Med. Chem.*, **23**, 313 (1980) and references therein.
(8) K. H. Scheit, "Nucleotide Analogs, Synthesis and Biological Function", Wiley, New York, 1980, pp 179–184.
(9) J. D. Bryant, G. E. Keyser, and J. R. Barrio, *J. Org. Chem.*, **44**, 3733 (1979).
(10) V. S. Prasolov, A. M. Kritsyn, S. N. Mikhailov, and V. L. Florent'ev, *Doklad. Biochem.*, **221**, 176 (1975).
(11) N. J. Leonard, T. G. Scott, and P. C. Huang, *J. Am. Chem. Soc.*, **89**, 7137 (1967).
(12) A. Lambert and J. D. Rose, *J. Chem. Soc.*, 46 (1949).
(13) N. J. Leonard, K. L. Carraway, and J. P. Helgeson, *J. Heterocyl. Chem.*, **2**, 291 (1965).
(14) M. MacCoss, E. K. Ryu, R. S. White, and R. L. Last, *J. Org. Chem.*, **45**, 788 (1980) and references therein.
(15) S. D. Dimitrijevich, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, **44**, 400 (1979).
(16) D. E. Gibbs and J. G. Verkade, *Synth. Commun.*, **6**, 563 (1976).
(17) J. M. Purcell and H. Susi, *Appl. Spectrosc.*, **19**, 105 (1965).
(18) The $C_{5'}$ signal was easily assigned from the off resonance and gated-1 spectra as it is the only doublet.
(19) C. A. Dekker and L. Goodman in "The Carbohydrates", Volume IIA, W. Pigman and D. Horton, Ed., Academic Press, New York, 1970, p 1.
(20) H. P. C. Hogenkamp, *Biochemistry*, **13**, 2736 (1974).
(21) M. J. Robins, Y. Fouron, and R. Mengel, *J. Org. Chem.*, **39**, 1564 (1974) and references therein.
(22) R. Mengel and H. Wiedner, *Chem. Ber.*, **109**, 433 (1976).

Table I. Binding of Adenine Derivatives to Liver Alcohol Dehydrogenase

no.	inhibitor	inhibn constant, mM
1	2-(adenin-9-yl)ethanesulfonate	4.3 ± 0.7
2	3-(adenin-9-yl)propanesulfonate	4.9 ± 0.8
3	9-(5-deoxy-β-D-ribofuranosyl)-adenine-5′-sulfonate	0.18 ± 0.03
4	9-(3-deoxy-β-D-arabinofuranosyl)-adenine-3′-sulfonate	1.2 ± 0.3
5	adenosine 5′-monophosphate	0.038 ± 0.002
6	adenosine 5′-monophosphate methyl ester	0.44 ± 0.04
7	adenosine cyclic 3′,5′-monophosphate	0.78 ± 0.13

the 3′-substituted product, gave several products. Since no major product was obtained, we did not pursue this reaction. Instead, we examined the effect of using sodium sulfite[24] and sodium hydrogen sulfite[12] as nucleophiles. When sodium sulfite was used, only a small amount (10%) of the desired sulfonic acid could be detected, the balance of material being the starting epoxide. However, use of sodium hydrogen sulfite gave a much better yield (65%). Although sodium sulfite should be the better nucleophile, sodium hydrogen sulfite may facilitate ring opening by giving increased protonation of the epoxide ring, since the pH of the latter reaction mixture was much more acidic (pH 5) than the former (pH 8.5). Chromatographic (high-pressure LC and TLC) analysis on the crude acid indicated that more than 90% of the product was one major isomer. The ^1H NMR spectrum of the major isomer did not allow assignment of the configuration, since $J_{1',2'}$ was 5 Hz.[25] By comparison with other nucleoside analogues it was possible to assign the 1′, 4′, and 5′ protons on the basis of chemical shift.[21,22] The 2′ proton was assigned to the resonance at 4.93 ppm on the basis of a double irradiation experiment in which this signal collapsed to a doublet from a triplet upon saturation of the 1′ proton. The 3′ proton could then be assigned to the resonance at 3.69 ppm, which due to its position at relatively high field must be the proton attached to the carbon bearing the sulfonate group. Thus, the major isomer is the expected 4.

Biochemical Evaluation. The nucleotide analogues, 1–4, were evaluated as inhibitors of horse liver alcohol dehydrogenase and found to have competitive inhibition constants in the range 0.18–4.9 mM (Table I). Compared with adenosine nucleotide analogues (entries 5–7) they appeared to be good inhibitors, the 5′- and 3′-sulfonate derivatives (3 and 4) being the best. The better inhibition by 3 as compared to 4 can be attributed to the better similarity of 3 to 5′-AMP, where the hydroxyl group can interact with the carboxylate of aspartic acid residue 223 and the amino group of lysine residue 228, while the sulfonate could interact with the guanidino group of arginine residue 47, as is observed for the binding of other adenine nucleotides.[26] It is interesting to note that in the phosphate ester series inhibition is reduced by a factor of 10 on going from a dianionic to a monoanionic species (entries 5 and 6, Table I), which is similar to the reduction of affinity of the monoanionic sulfonates as compared to dianionic 5′-AMP. Furthermore, the 3′-sulfonate analogue (4) and cyclic AMP (7) have similar inhibition constants. Thus, the two sulfonates compare very favorably with their phosphate analogues and probably bind to the enzyme in a similar manner. In contrast, the acyclic analogues, 1 and 2, were weaker inhibitors, which is probably due to the lack of a furanose ring that positions the anionic sulfonate properly for good binding. In conclusion, the sulfonate analogues are effective inhibitors of one nucleotide-binding enzyme and should be effective in other systems.

Experimental Section

Thin-layer chromatography was performed on Eastman cellulose sheets in 70% 2-propanol, 20% concentrated ammonium hydroxide, and 10% water. Electrophoresis was carried out on Eastman cellulose sheets in 0.025 M sodium phosphate buffer, pH 8.0, and 20 V/cm. The rates of migration are relative to AMP. High-pressure liquid chromatography was performed with either an Altex C_{18} or a Varian AX-10 anion-exchange column using water/acetonitrile (0–20%) and potassium phosphate buffer (0.01 M, pH 5.5)/acetonitrile (20%), respectively. Ultraviolet spectra were recorded in aqueous solution of neutral pH with a Cary 118C spectrophotometer. NMR spectra were obtained on a JOEL FX90Q, for proton, and a Bruker 90, for carbon, in D_2O using 3-(trimethylsilyl)-1-propanesulfonic acid as an internal standard. Elemental analyses for C, H, N, and S were carried out by Galbraith Laboratories. Melting points are uncorrected. The adenosine 5′-monophosphate and the adenosine cyclic 3′,5′-monophosphate were purchased from Sigma. The adenosine 5′-monophosphate methyl ester was made by standard procedures.[27]

2-(Adenin-9-yl)ethanesulfonic Acid (1). A suspension of the sodium salt of adenine (1.96 g, 14.5 mmol) in anhydrous DMF and 1-bromo-2-chloroethane (2.62 g, 18.3 mmol) was stirred overnight at ambient temperature with the exclusion of water and filtered; the filtrate was evaporated to dryness, giving a residue that was washed with ether and recrystallized from methanol/water: yield 1.03 g (36%); mp 199–202 °C; UV λ_{max} 260 nm (ε 13 500).

The chloride[11] (2 g, 10 mmol) was suspended in a solution of sodium sulfite (2 g, 16 mmol) in water (100 mL) and heated under reflux. When no starting material was left, as judged by TLC, the solution was concentrated and applied to a column (2 × 30 cm) of Dowex 50 (H^+ form, 200–400 mesh). The product was eluted with water and crystallized upon concentration of the solution. As an alternative to isolation by ion-exchange chromatography, the reaction mixture could be acidified to pH 1 with dilute hydrochloric acid and evaporated to dryness; the residue was dissolved in dilute hydrochloric acid and once more evaporated to dryness. This residue was then suspended in water (50 mL) and brought into solution with dilute sodium hydroxide. After filtration, the solution was adjusted to pH 2 with dilute hydrochloric acid, whereupon the sulfonate crystallized (yield 65%): mp >300 °C; UV λ_{max} 260 nm (ε 15 400). Anal. ($C_7H_9N_5SO_3$) C, H, N, S.

3-(Adenin-9-yl)propanesulfonic Acid (2). Using the above procedure, adenine was converted to the propyl chloride[11] derivative (mp 198 °C) and to the sulfonic acid: mp >300 °C; UV λ_{max} 260 nm (ε 15 400). Anal. ($C_8H_{11}N_5SO_3$) C, H, N, S.

9-(5-Deoxy-β-D-ribofuranosyl)adenine-5′-sulfonic Acid (3). A stirred suspension of 5′-deoxy-5′-chloroadenosine (2.0 g, 7.4 mmol) and sodium sulfite (1.26 g, 10 mmol) in a mixture of methanol (20 mL) and water (80 mL) was heated to reflux for 48 h. The resulting solution was cooled and concentrated to give a cream-colored residue. The crude product was washed with ethanol (to remove unreacted chloroadenosine), taken up in the minimum of water, and applied to a column (2 × 50 cm) of Dowex 1 × 8 (formate, 200–400 mesh) which was developed with a linear

(23) T. Ueda, A. Matsuda, T. Asano, and H. Inoue in "Chemistry and Biology of Nucleosides and Nucleotides", R. E. Harmon, R. K. Robins, and L. B. Townsend, Ed., Academic Press, New York, 1978, p 397.
(24) S. G. Traynor, B. J. Kane, M. F. Betkouski, and L. M. Hirschy, J. Org. Chem., 44, 1557 (1979).
(25) If the $H_{1'}$ and $H_{2'}$ protons are in a trans relationship, J = 5–8 Hz; if in a cis relationship, J = 1–4 Hz. See ref 21 and 22.
(26) C.-I. Brändén, H. Jörnvall, H. Eklund, and B. Furugren, Enzymes, 3rd Ed., 11, 103 (1975).
(27) M. Smith, J. G. Moffatt, and H. G. Khorana, J. Am. Chem. Soc., 80, 6204 (1958).

gradient of hydrochloric acid (0 to 1 M in 2 L). The effluent material with absorbance at 280 nm was collected, concentrated, and precipitated with anhydrous ethanol. The white solid was filtered and dried in vacuo: yield 1.21 g (52%); mp 230–232 °C; R_f (AMP) 0.67; UV λ_{max} 259 nm (ϵ 14970); ^{1}H NMR (D_2O, pD 8) δ 3.43 (m, 2 H, $H_{5'}$), 4.5 (m, 2 H, $H_{2'}$ and $H_{4'}$), 4.7 (s, HOD and $H_{3'}$), 6.03 (d, 1 H, J = 5.1 Hz, $H_{1'}$) 8.15 (s, 1 H, H_8), 8.3 (s, 1 H, H_2); ^{13}C NMR (D_2O, pD 8) 56.76 ($C_{5'}$), 76.64 and 77.03 ($C_{2'}$, $C_{3'}$), 83.1 ($C_{4'}$), 91.26 ($C_{1'}$), 121.31 (C_5), 142.78 (C_8), 151.4 (C_4), 155.26 (C_2), 157.96 (C_6). Anal. ($C_{10}H_{13}N_5O_6S\cdot0.5H_2O$) C, H, N.

9-(3-Deoxy-β-D-arabinofuranosyl)adenine-3′-sulfonic Acid (4). 9-(2,3-Anhydro-β-D-lyxofuranosyl)adenine[21] (0.23 g, 1 mmol) and sodium hydrogen sulfite (0.5 g, 5 mmol) were suspended in a mixture of acetonitrile (20 mL) and water (10 mL). This mixture was heated to 80 °C, to give a homogeneous solution, under an atmosphere of nitrogen for 48 h. The reaction mixture was cooled to ambient temperature and acidified (pH 2–3) with 10% hydrochloric acid (to decompose excess sodium hydrogen sulfite). The solution was then concentrated, under reduced pressure, to about one-third its original volume and applied to a column (1 × 20 cm) of Dowex 50 (H^+ form, 200–400 mesh). Development with water resolved two peaks (as determined by A_{280}). The first one had a λ_{max} of 250 nm and an R_f (AMP) of 0 by electrophoresis and the second had a λ_{max} of 258 nm and an R_f (AMP) of 0.52 by electrophoresis. The fractions corresponding to the second peak were pooled and concentrated, under reduced pressure, to give yellowish white crystals (0.18 g, 65%). A portion was recrystallized from water to give a 50% yield of white crystals, mp >260 °C dec; UV (pH 4.9) λ_{max} 258 nm (ϵ 13780); ^{1}H NMR (D_2O, pD 8) δ 3.69 (dd, 1 H, $H_{3'}$, J = 5 Hz), 4.04 (m, 2 H, $H_{5'}$), 4.47 (m, 1 H, $H_{4'}$), 4.93 (t, 1 H, $H_{2'}$, J = 5 Hz), 6.3 (d, 1 H, $H_{1'}$, J = 5.1 Hz), 8.1 (s, 1 H, H_8) and 8.3 (s, 1 H, H_2); ^{13}C NMR (D_2O, pD 8) 64.71 (t, $C_{5'}$, J = 138.25, 142.66 Hz), 67.64 (d, $C_{3'}$, J = 138.25 Hz), 75.5 (d, $C_{2'}$, J = 150 Hz), 82.06 (dd, $C_{4'}$, J = 148.9 and 5.15 Hz), 87.85 (d, $C_{1'}$, J = 167.66 Hz), 120.53 (d, C_5, J = 10.29 Hz), 143.86 (d, C_8, J = 216.93 Hz), 151.0 (C_4), 155.16 (d, C_2, J = 202.93 Hz), 157.89 (d, C_6, J = 13.97 Hz). Anal. ($C_{10}H_{13}N_5SO_6$) C, H, N, S.

Enzymology. The horse liver alcohol dehydrogenase enzyme (EE isoenzyme) was purified and assayed as previously described.[28] The inhibition constants of the nucleotide analogues were determined by competition against varied concentrations (4–20 μM) of purified NAD^+ at a constant concentration of ethanol (5 mM) at 25 °C, in 33 mM sodium phosphate buffer, pH 8. A Hitachi MPF2A fluorimeter was used with excitation at 340 nm and emission at 420 nm. The data were fitted to the equation for competitive inhibition.[29]

Acknowledgment. This work was supported by Research Grant AA00279 and Research Scientist Development Award AA00010 from the National Institute on Alcohol Abuse and Alcoholism, United States Public Health Service, to B.V.P. and by a special fellowship from the Deutsches Forschungsgemeinschaft to C. W. We thank David P. Bohlken and Dr. Yvonne Oey Tjung for assistance with some of these experiments.

(28) R. T. Dworschack and B. V. Plapp, *Biochemistry*, **16**, 111 (1977).
(29) W. W. Cleland, *Adv. Enzymol.*, **29**, 1 (1967).

Book Reviews

Anthracyclines: Current Status and New Developments. Edited by Stanley T. Crooke and Steven D. Reich. Academic Press, New York. 1980. xiii + 444 pp. 16 × 23.5 cm. $27.50.

The anthracyclines, typified by adriamycin and daunomycin, are widely employed agents in the treatment of human cancer. According to the editors, the purpose of this book is to provide an overview of recent progress in the study of anthracyclines and to provide a framework for developing new directions for future research. In 28 chapters the book is partially, but not completely, successful at meeting these objectives. Because the prototype agents have proven so useful in cancer chemotherapy, there has been a high level of activity in the development of anthracycline analogues. This book makes accessible in one place a definitive amount of facts on the chemistry, pharmacology, and antitumor properties of the most important of the newer drugs. Also, a great deal of attention is paid to the toxicities of this class of agents, especially to the most serious problem of cardiac toxicity. There are several chapters dealing with model systems for evaluating cardiac damage, giving a good overview of both the problems in this area and the promising reduction in cardiotoxicity of several of the newer agents.

The weakness of the volume is that none of the authors takes a broad view of the whole anthracycline field. There is no attempt to review and tie together all the various lines of research into these drugs or to speculate on unifying principles. Also, the absence of an index makes it difficult for the reader to do his or her own cross-referencing. Some topics are not covered, for example, drug transport, drug resistance, or cell cycle effects. Moreover, there is a heavy thread throughout of DNA as the preeminent anthracycline receptor, with relatively little mention of the evidence that other targets, such as cell membranes, may also be important in the biochemical mechanism. These limitations aside, the book is useful because most of the chapters are clearly written and much important and current information is presented. Anyone in the immediate field, or in the more general area of cancer chemotherapy, will find useful material here.

Department of Pharmacology **Thomas R. Tritton**
Yale Medical School
New Haven, Connecticut 06510

Advances in Neurology. Volume 27. Antiepileptic Drugs. Mechanisms of Action. Edited by G. H. Glaser, J. K. Penry, and Dixon M. Woodbury. Raven Press, New York. 1980. iii + 728 pp. 18 × 26 cm. $69.00.

This massive volume is the end product of a sequential process initiated by the Epilepsy Advisory Committee of the National Institute of Neurological and Communicative Disorders and Stroke. The progression from subcommittee (1975) via organizing committee and closed workshop (1976) to an open symposium (1977) yielded (1980) this 400 000 word publication.

Many chapters were originally written in 1977. In some cases, minor revisions and additions were made in 1979; thus, certain recent developments are not adequately covered.

The nine introductory chapters on basic neurobiology (185 pages) are stimulating and will serve to update the background knowledge of researchers in this area. They are followed by three chapters on structure and activity relations in convulsant and anticonvulsant drugs, and a chapter on the mechanism of action of convulsant drugs. The latter enjoys the advantage of presenting definitive data and, thus, shines in comparison with the subsequent chapters.

The 26 chapters on anticonvulsant drugs are divided into 12 on the phenytoins, 8 on barbiturates, and 1 each on carbamazepine, oxazolidinediones, succinimides, benzodiazepines, carbonic anhydrase inhibitors, ketogenic diet, and valproate. This lack of balance may be a reflection of the total research effort in the last 40 years. It would be unfortunate if it, through the influence of this volume, determined the pattern of future research.