1193-82-4; ethyl phenyl sulfoxide, 4170-80-3; methyl benzyl sulfoxide, 824-86-2; phenyl vinyl sulfoxide, 20451-53-0; isoamyl methyl sulfide, 13286-90-3; hexyl methyl sulfide, 20291-60-5; diethyl sulfide, 352-93-2; trimethylene sulfide, 287-27-4; pentamethylene sulfide, 1613-51-0; 1,4-oxathiane, 15980-15-1; 1-thiacyclohexan-4-one, 1072-72-6; 1,4-dithiane, 505-29-3; methyl phenyl sulfide, 100-68-5; isobutyl methyl sulfide, 5008-69-5; thiomorpholine, 123-90-0; 3-hydroxypropionitrile, 109-78-4; butyrolactone, 96-48-0; ϵ-caprolactone, 502-44-3; isovaleryl chloride, 108-12-3; *tert*-butylacetyl chloride, 7065-46-5.

Notes

Synthesis of Thiazole-4-carboxamide Adenine Dinucleotide. A Powerful Inhibitor of IMP Dehydrogenase

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Received October 18, 1982

The chemical synthesis of thiazole-4-carboxamide adenine dinucleotide (TAD), previously identified as the active anabolite of the oncolytic 2-β-D-ribofuranosylthiazole-4-carboxamide (TR), has been achieved by three different approaches: (1) incubation of adenosine 5′-monophosphate (AMP) and 2-β-D-ribofuranosylthiazole-4-carboxamide 5′-monophosphate (TRMP) with excess DCC in aqueous pyridine, (2) reaction of adenosine 5′-phosphoromorpholidate with TRMP in pyridine, and (3) reaction of adenosine-5′-phosphoric di-*n*-butylphosphinothioic anhydride with TRMP in the presence of $AgNO_3$. While the first approach produced only traces of TAD, the last two afforded 31 and 16% yields, respectively, of isolated TAD. The synthetic material was indistinguishable from biosynthesized TAD as judged by its HPLC behavior, NMR, UV and mass spectra, enzymatic resistance to alkaline phosphatase and susceptibility to venom phosphodiesterase, IMP dehydrogenase inhibitory activity, and cytotoxicity. TAD and TR were equally effective against murine P388 leukemia when employed at equimolar doses.

2-β-D-Ribofuranosylthiazole-4-carboxamide (TR, **1a**, Scheme I) shows remarkable activity against several murine tumors, including the Lewis lung carcinoma.[1] In a previous communication from this laboratory, it was reported that this novel C-nucleoside was anabolized to an analogue of NAD that was responsible for its potent inhibition of IMP dehydrogenase (IMPD) and consequent depression of guanine nucleotides.[2] The presence of a phosphodiester linkage in the structure of this anabolite was first surmised from its enzymatic resistance to alkaline phosphatase and susceptibility to venom phosphodiesterase. Subsequently, the structure was completely elucidated by ^1H NMR and mass spectral studies.[2] These results were consistent with an analogue of NAD (**3a**) in which the nicotinamide portion had been replaced by thiazole-4-carboxamide. This anabolite, appropriately abbreviated TAD, became an interesting target for chemical synthesis. Our initially reported biochemical synthesis from ATP and the 5′-monophosphate of TR (**1b**, TRMP) in the presence of NAD pyrophosphorylase proved unwieldy for scale up.[2]

The present work discusses three different approaches to the chemical synthesis of TAD. Initially, TRMP (**1b**), synthesized by the modified procedure of Yoshikawa,[3,4] and AMP (**2a**) were reacted in the presence of excess dicyclohexylcarbodiimide (DCC) in aqueous pyridine. This procedure was identical with the one originally used for the synthesis of NAD;[5] however, when it was used only very small yields of TAD were achieved. An improvement over the first method was achieved, then, by selectively activating one nucleotide before the coupling reaction. To this end, AMP (**2a**) was converted to its phosphoromorpholidate derivative **2b**[6,7] and reacted with TRMP (as the tri-*n*-octylamine salt) in pyridine for 2 h at 60 °C. This procedure afforded pure TAD (**3a**) in 9% yield. Lowering the temperature of the reaction to room temperature and stirring the mixture for 6 days increased the yield of TAD to just 12%. Finally, an intermediate temperature of 45 °C produced the highest yields of TAD (31%) after 5 h.

Scheme I

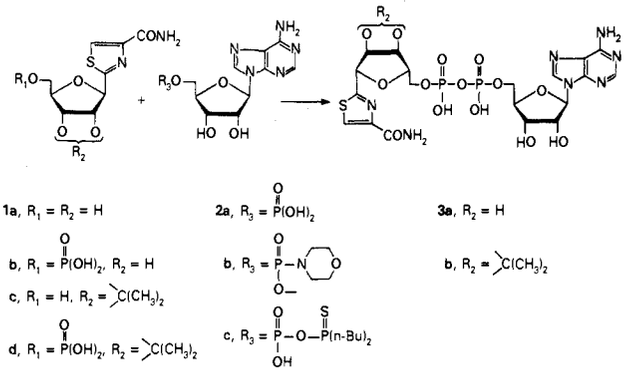

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Table I. Spectral Characteristics of TAD and ITAD

	^{1}H NMR[a]									
	sugar		base: adenosine			protective group: 2',3'-isopropylidene				
compd	adenosine: H1'	TR: H1'	H2	H8	TR: H5	CH_3	CH_3	^{31}P NMR[b]	MS, M	UV, λ_{max}
3a (TAD)	6.06 (d, J = 6)	5.04 (d, J = 4)	7.96 (s)	8.17 (s)	8.40 (s)			−11.17	670[c] 668[d]	252
3b (ITAD)	5.95 (d, J = 5.6)	5.07 (d, J = 2.6)	7.75 (s)	7.95 (s)	8.18 (s)	1.76 (s)	1.56 (s)		710[c]	

[a] Chemical shifts are given in parts per million (δ) relative to TSP. [b] Chemical shifts are given in parts per million (δ) relative to 80% of H_3PO_4. The sample contained ethylenediaminetetraacetic acid (EDTA). [c] M + H, positive-ion FAB mass spectrum. [d] M − H, negative-ion FAB mass spectrum.

Another activated form of AMP, adenosine-5′-phosphoric di-*n*-butylphosphinothioic anhydride (**2c**),[8] afforded 16% of TAD when reacted with TRMP in the presence of $AgNO_3$.

Despite the prior activation of AMP, the last two methods lacked complete selectivity, and yields of TAD, although vastly improved, were still in the low range. In all cases, another pyrophosphate, identified as P^1,P^2-di(adenosine-5′)pyrophosphate, formed in amounts almost equal to TAD. This compound was characterized by its ^{1}H NMR and by HPLC comparison with an authentic sample. It is probable that trace amounts of water hydrolyzed the "activated" AMP to AMP, which in turn competed with TRMP for the remaining activated AMP. Furthermore, a significant amount of TRMP was always recovered in these reactions after workup. This material could arise from unreacted TRMP or from the decomposition of TAD.

TAD was purified by ion-exchange chromatography to afford the final product as the monoammonium salt (see Experimental Section). The only other contaminant present was ammonium formate, which came from the eluent employed in the chromatography. Complete elimination of this salt was achieved by repeated lyophilization. The salient features of the spectral data for TAD are listed in Table I. As expected, chemically synthesized TAD was spectrally equivalent to biochemically synthesized TAD.[2]

The negative-ion fast atom bombardment (FAB) mass spectrum of underivatized TAD is the most structurally indicative. The intense M − H peak at m/z 668 indicates a molecular weight of 669; this is corroborated by a much weaker peak due to the glycerol adduct at m/z 760. FAB mass spectra of nucleotides are characterized by cleavage of the phosphate ester linkages and, in the case of dinucleotides, reveal considerable structural information. For TAD, these base-containing pairs are easily identifiable, since they are separated by 7 mass units. Fragments at m/z 346 and 426 suggest both a phosphodiester linkage and a nucleotide base of mass 134 or adenine. Likewise, fragments at m/z 339 and 419 imply a nucleotide base of mass 127; this corresponds to thiazole-4-carboxamide.

In view of its pyrophosphate linkage, TAD was expected to show the same AB quartet that is characteristic of NAD in its ^{31}P NMR spectrum. However, the two phosphate groups appeared to be magnetically equivalent, and only one signal was observed. Curiously this was also the case with the reduced form of NAD, and it is probable that in this respect TAD more resembles NADH than NAD.[9,10]

Table II. Biological Activity of TAD

source of TAD	IMP dehydrogenase inhib act.:[a] K_i, μM	cytotoxicity against P388 cells in culture:[a] IC_{50}, μM
enzymatic	0.30 ± 0.18	2.1 ± 0.5
chemical	0.12 ± 0.10	3.1 ± 0.4

[a] Mean plus or minus standard deviation.

Table III. In Vivo Antitumor Activity of TR and TAD

treatment	survival, days ± SD	% T/C
saline	9.3 ± 0.7	
TR	12.6 ± 1.6	135
TAD	12.6 ± 0.5	135

Despite the lack of definitive proof that the ^{31}P NMR was supposed to have provided, all of the chemical, biochemical, and other spectral data indicated that the structure of TAD was correct as formulated. However, it was still possible that a 5′→3′(2′) phosphate bearing both TR and adenosine fragments and with two accidentally equivalent phosphate groups could have formed and been mistakenly identified as TAD. These products could be envisioned as resulting from the attack of either the 2′- or 3′-hydroxy groups of TRMP on the activated AMP. In order to rule out such a possibility, the isopropylidene derivative of TRMP (**1d**), obtained after phosphorylation of the known isopropylidene-substituted-TR (**1c**),[4,11] was reacted with **2b** under the same conditions. Following an identical chromatographic procedure, the isopropylidene analogue of TAD (**3b**, ITAD) was isolated and fully characterized (Table I). Moreover, deprotection of ITAD with 10% acetic acid under mild heating afforded TAD identical in all respects with the material previously obtained. This experiment demonstrated, in conjunction with the spectral data, that TAD possessed a 5′→5′ pyrophosphate linkage.

Biological Activity. A rigorously desalted preparation of TAD was examined for its ability to inhibit a partially purified preparation of IMP dehydrogenase from P388 leukemic cells. On kinetic analysis, with NAD as the variable substrate, formally noncompetitive inhibition was observed with a $K_i \simeq 0.12$ μM (Table II). This value was in good agreement with the K_i's of the dinucleotide isolated ex vivo and with that synthesized enzymatically.[2,12] In-

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terestingly, both chemically as well as biologically synthesized TAD proved to be cytotoxic to P388 cells in culture with a median inhibitory concentration of 3.1 and 2.1 μM, respectively (Table II). These values are not significantly different. In vivo studies with the synthetic material carried out in mice bearing the same tumor showed that TAD was as effective as TR at equimolar doses (Table III). It remains to be determined whether the bulky dinucleotide itself enters the cell or whether it must undergo prior hydrolysis, at the plasma membrane for example, followed by reassembly within the cell. Support for the latter of these possibilities is provided by the finding that TAD is, like TR, noncytotoxic at 1 mM to a variant of P388 leukemia rendered resistant to the parent drug.[13] Since this variant lacks the capacity to synthesize TAD, it might not be able to reassemble the molecule from its breakdown products.

Experimental Section

General Methods. HPLC analyses were performed at ambient temperature on a Waters Associates Model 204W chromatograph by using a 4.6 × 250 mm Partisil 10-SAX column eluted with a linear gradient of 0.01 to 0.5 M $(NH_4)H_2PO_4$ at 1.0 mL/min over 30 min. Compounds of interest were monitored by their UV absorbance at 254 nm. ^1H NMR spectra were recorded on a Varian XL-200 spectrometer; 3-(trimethylsilyl)-1-propanesulfonic acid (TSP) in D_2O was used as an internal reference. Positive- and negative-ion mass spectra were obtained on a VG Analytical 7070E mass spectrometer equipped with a VG fast atom bombardment ion source operated at an accelerating voltage of 6 kV. Glycerol was used as a sample support medium, and ionization was effected by a beam of argon atoms derived by charge exchange neutralization of a 1-mA bean of argon ions accelerated through 8 kV. Spectra were acquired at a scan speed of 10 s/decade by using a VG Analytical 2035 data system, and the background due to the glycerol matrix was automatically subtracted. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Biological Methods. IMP dehydrogenase inhibtion by TAD was determined essentially as described previously for the parent thiazole nucleoside (TR).[14] Details of the kinetic studies of IMPD inhibition by TAD are described in ref 12. Cytotoxicity studies were performed as described previously,[15] by using P388 cells in log phase growth. For in vivo studies, groups of BDF_1 mice (five per group for TAD and ten per group for the rest) were injected ip with 10^6 cells/mouse of P388 cells. Intraperitoneal treatment with the drugs was started 24 h later from day 1 to day 5 (D = 1 × 5). Mice were regularly weighed and mortalities were noted. Equimolar doses of 200 and 523 mg/kg were used for TR and TAD, respectively.

Diammonium Salt of 2-β-D-Ribofuranosylthiazole-4-carboxamide 5′-Phosphate (1b). Phosphoryl chloride (0.36 mL, 4 mmol) was added to triethyl phosphate (6 mL) and cooled to 0 °C. Water (0.036 mL) was then added, and the mixture was stirred for 5 min. Immediately after stirring, **1a** (1.04 g, 4 mmol) was added, and stirring was continued for 3 h at 0 °C. An equal amount of water-treated phosphoryl chloride was again added, and the reaction mixture was stored for 2 days at 4 °C. Water (3 mL) was added, and stirring was continued for 2 h. The reaction mixture was then passed through a column of Dowex 50W-X8 (H^+ form, 100–200 mesh, 2.5 × 13 cm) with water as the eluting solvent. The fractions containing TRMP were combined, and the solution was lyophilized. The resulting gum was dissolved in 5 mL of water and passed through a column of Bio-Rad AG1-X2 (CO_3^{2-} form, 100–200 mesh, 2.5 × 1.2 cm) and eluted first with 500 mL of water, followed by 1000 mL of 0.7 M ammonium carbonate. Lyophilization of the first 500 mL of the ammonium carbonate eluent gave 0.97 g (71%) of a white solid, which was homogeneous on HPLC. In addition, the material was identical with an authentic sample of TRMP.[16]

2-(2′,3′-Isopropylidene-β-D-ribofuranosyl)thiazole-4-carboxamide (1c). To a solution of **1a** (1.0 g, 3.8 mmol) and triethyl orthoformate (0.6 g, 4 mmol) in acetone (25 mL) was added 0.5 mL of 1 N HCl in ethyl ether, and the reaction mixture was stirred at room temperature overnight. After neutralization with concentrated ammonium hydroxide, the reaction mixture was concentrated in vacuo and dissolved in 15 mL of water. The aqueous solution was then extracted with ethyl acetate (7 × 50 mL), and the resulting organic solution was dried (Na_2SO_4) and concentrated in vacuo. The recovered oil was purified by preparative HPLC over silica by using ethyl acetate as the eluting solvent (Waters LC-500, Prep Pak-500/silica). The fraction corresponding to the product was concentrated and recrystallized from ethyl acetate to give 0.83 g (73%) of **1c** as white cubes, mp 119–120 °C (lit.[11] mp 119–120 °C).

Diammonium Salt of 2-(2′,3′-Isopropylidene-β-D-ribofuranosyl)thiazole-4-carboxamide 5′-Phosphate (1d). Phosphoryl chloride (0.9 g, 2 mmol) was added to a stirred solution of **1c** (0.6 g, 2 mmol) in triethyl phosphate (3 mL) at 4 °C. The reaction mixture was then stirred for 3 h and stored in the freezer (−20 °C) for 3 days. After the reaction was washed with hexane (3 × 50 mL), 50 mL of water was added, the the pH was adjusted to ca. 9 by using concentrated ammonium hydroxide. The resulting solution was then applied to a column of Bio-Rad AG1-X2 (CO_3^{2-} form, 100–200 mesh, 1.5 × 80 cm) and eluted with 500 mL of water, followed by a 0.5 M ammonium carbonate solution. The first 700 mL of the ammonium carbonate eluent contained 0.32 g of a white solid, which was obtained after lyophilization. This material was found to be a mixture of TRMP (t_R = 9.5 min) and the desired isopropylidene analogue of TRMP (t_R = 11.5 min) by HPLC. The next 1300 mL contained 0.065 g of the desired compound (t_R = 11.5 min), which was obtained after lyophilization. This material gave the correct ^1H NMR spectrum and was used as such for the synthesis of ITAD. When the initial fraction containing both products was heated (70 °C) with 10% acetic acid (50 mL) for 2 h, the only compound detected by HPLC was the deprotected TRMP.

***N,N′*-Dicyclohexyl-4-morpholinecarboxamidinium Salt of Adenosine 5′-Phosphoromorpholidate (2b).**[6,7] This material was prepared in 94% yield as reported previously. HPLC analysis showed a single peak (t_R = 7 min). AMP had a retention time of 10 min.

Adenosine-5′-phosphoric Di-*n*-butylphosphinothioic Anhydride (2c). This material was prepared in 71% yield following the methodology of Furusawa et al.[8]

4-Carboxamido-2-β-D-ribofuranosylthiazolyl(5′→5′)-adenosine Pyrophosphate (3a). Method A. A solution of the diammonium salt of **1b** (0.681 g, 1.81 mmol) in water (100 mL) was treated with 15 g of cation-exchange resin [Bio-Rad AG 50W-X8 (H^+ form)] to obtain TRMP as the free monophosphate. After the resin was removed, the aqueous solution was lyophilized, and the resulting solid residue was dissolved in 50% aqueous pyridine (100 mL). To this solution was added tri-*n*-octylamine (0.713 g, 2 mmol), and the resulting mixture was lyophilized overnight. A separate solution of **2b** (1.426 g, 2 mmol) in pyridine (100 mL) was then added to the lyophilized TRMP tri-*n*-octylammonium salt. The solvent was removed under vacuum at room temperature, and the residue was again redissolved in pyridine (100 mL) and reduced to dryness once more. Finally, 100 mL of anhydrous pyridine was added, the temperature was raised to 45 °C, and the reaction mixture was stirred until HPLC analysis revealed that all of the morpholidate **2b** had been consumed (5 h). The reaction mixture was reduced to dryness and partitioned between $CHCl_3$ (50 mL) and water (50 mL). The aqueous layer was then treated with sodium acetate (0.450 g) and extracted twice with ether (2 × 50 mL). After removing all traces of ether from the aqueous layer, it was loaded onto a Hamilton HA-X4 an-

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(16) A sample of authentic TRMP was kindly supplied by Dr. Ven Narayanan, Drug Synthesis and Chemistry Branch, DTP, DCT, NCI, Silver Spring, MD 20910.

ion-exchange column (HCO_2^- form, 0.8 × 28 cm) maintained at 37 °C and eluted with a linear gradient of water (270 mL) and a mixture of 250 mL of ammonium formate plus formic acid (final molarities 2 and 2.6 M, respectively). Fractions (7.5 mL) were collected at a flow rate of 1.5 mL/min and monitored by UV at 290 nm. Fractions 26–28 were combined and lyophilized several times to eliminate the volatile buffer. This procedure afforded 0.420 g (31%) of pure TAD according to HPLC, NMR, and elemental analysis.

Method B. TRMP (**1b**; 0.27 g, 0.72 mmol) was dissolved in formamide (3 mL) and added to a solution of **2c** (0.7 g, 1.43 mmol) in pyridine (50 mL). The resulting solution was concentrated in vacuo and dissolved in 6 mL of dry pyridine. Silver nitrate (0.91 g, 5.36 mmol) was added, and the mixture was stirred for 36 h. After the addition of water (70 mL), H_2S was bubbled into the reaction mixture, and the black precipitate that formed was removed by filtration. The filtrate was lyophilized, and the resulting syrupy liquid was diluted with 5 mL of water and applied to a column of Bio-Rad AG1-X2 (HCO_2^- form, 100–200 mesh, 1.5 × 5 cm) and eluted with water (50 mL), followed by 2 M ammonium formate (50 mL). The ammonium formate fraction was lyophilized several times, redissolved in water, and precipitated with ethanol. The precipitate that formed was saved, and the filtrate was concentrated and treated again with ethanol. The combined precipitates (0.325 g) were chromatographed on a Hamilton HA-X4 column as in method A to afford 0.08 g of TAD, which eluted as a single peak. An extra 5 mg of TAD was obtained from a third precipitation of the mother solution, bringing the total yield to 0.085 g (16%); negative-ion FAB mass spectrum, m/z (relative intensity) 760 (M^+, glycerol − H, 1.8), 668 (M − H, 100), 426 (34), 419 (30), 408 (7.3), 401 (7.1), 346 (13), 339 (11). Anal. Calcd for $C_{19}H_{29}N_8O_{14}P_2S \cdot 2.5H_2O$ (monoammonium salt): C, 31.14; H, 4.51; N, 15.30; P, 8.47; S, 4.37. Found: C, 31.16; H, 4.63; N, 15.67; P, 8.53; S, 4.14. These results were reproducible and are consistent with the monoammonium salt of TAD. The remaining phosphate anion must accordingly form an inner salt with a basic amino group of either TR or adenosine.

4-Carboxamido-2-(2′,3′-isopropylidene-β-D-ribofuranosyl)thiazolyl(5→5′)adenosine Pyrophosphate (3b). Compound **1d** (0.030 g, 75 μmol) was treated with the adenosine phosphoromorpholidate **2b** (0.048 g, 65 μmol) and tri-*n*-octylamine (0.0266 g, 25 μmol) in an analogous manner as in the synthesis of TAD. The reaction mixture was kept for 2 h at 60 °C and overnight at room temperature. It was then diluted with water (20 mL), treated with sodium acetate (0.022 g), and extracted twice with ether (2 × 20 mL). After lyophilization, the aqueous layer afforded a yellowish solid, which was purified by passing it through a Hamilton HA-X4 (HCO_2^- form) column in the same manner as for TAD, to afford ITAD (11.4 mg) as a white solid. Progress of this reaction was followed by HPLC monitoring of the peak with retention time of 16 min. Other peaks with retention times of 14 and 15 min corresponded to TAD and P_1,P_2-di(adenosine-5′)pyrophosphate, respectively. Treatment of ITAD (2 mg) with 10% acetic acid for 25 min at 70 °C afforded 1.7 mg of a white fluffy solid after lyophilization. This material was identical with TAD according to chemical, spectral, and biological criteria.

Registry No. **1a**, 60084-10-8; **1b**·$2NH_3$, 85221-10-9; **1c**, 60084-11-9; **1d**·$2NH_3$, 85221-11-0; **2b** *N,N′*-dicyclohexyl-4-morpholinecarboxamidine salt, 24558-92-7; **2c**, 57816-25-8; **3a**, 83285-83-0; **3a**·NH_3, 85221-12-1; **3b**, 85221-13-2; IMP dehydrogenase, 9028-93-7.

Book Reviews

Chemical Approaches to Understanding Enzyme Catalysis: Biomimetic Chemistry and Transition-State Analogs. Edited by B. S. Green, Y. Ashani, and D. Chipman. Elsevier Scientific Publishing Co., Amsterdam. 1982. xv + 355 pp. 17 × 24.5 cm. ISBN 0-444-42063-0. $100.00.

This volume contains the Proceedings of the 26th OHOLO Conference held in Zichron Yaacov, Israel, on March 22–25, 1981. The primary objective of this conference was to bring together those researchers who are trying to understand how enzymes function, those who are trying to imitate enzyme-like catalysis using simple organic or inorganic models, and those interested in the design and synthesis of transition-state analogues. The book contains 25 papers by some of the leading scientists in the areas of enzyme catalysis, biomimetic systems, and transition-state analogues. Some of the topics of potential interest to medicinal chemists include concertedness and enzyme catalysis (Jencks), asymmetric reaction with chiral bridged 1,4-dihydropyridines (Kellogg), stereochemistry and thermodynamics in alcohol dehydrogenase (Benner and Stackhouse), stereochemical principles in the design and function of synthetic molecule receptors (Laidler et al.), biomimetic reactions inside the channels of the choleic acids (Leiserowitz et al.), models of the receptor sites of enzymes (Wulff and Sarhan), space and directionality in bioorganic chemistry (Menger), models for reactive intermediates or transition states of biological interest (Martin and Ross), organophosphorous inhibitors of acetylcholinesterase as transition-state analogues (Ashani and Green), mechanistic studies and transition-state analogue inhibitors of alkyltransferases (Coward), stereochemical aspects of micelle-catalyzed esterolysis (Moss and Lee), binding forces in lysozyme catalysis (Chipman and Schindler), correlation of X-ray structures of serine proteases with their catalytic activity (Warshel et al.), functionalized cyclodextrins as artificial enzymes (Tabushi), distortion and purification with transition-state analogues (Wolfenden and Andersson), structure–reactivity relationship in the cyclodextrin-catalyzed hydrolysis of oxazolones (Daffe and Fastrez), hydroxo complexes of Hg^{2+} chelates that mimic the active site of carbonic anhydrase (Werber), and enzyme-like activity of macrotricyclic ammonium salts (Schmidtchen).

An extremely useful feature of this book was the inclusion by the editors of the discussion that followed each presentation at the conference. These discussions were very informative and often thought provoking. This volume would be a useful addition to any academic or industrial library.

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Molecular Enzymology. By Christopher W. Wharton and Robert Eisenthal. Halsted Press (a Division of Wiley), New York. 1981. ix + 326 pp. 15 × 21 cm. ISBN 0470-27152-3. $46.95.

The text is directed at biochemistry or biology students who have had basic biochemistry and chemistry and who wish to deepen their understanding of enzyme kinetics and the chemical basis for enzymatic reactions. The sections on kinetics appear to be adequate; the discussion of chymotrypsin seems thorough and up-to-date. However, the chemical discussions tend toward the superficial, even for a nonchemical audience, and there are shortcomings that cast doubt on the usefulness of the book. The transition-state free energy is derived from the rate constant at a given temperature via the Eyring equation; it is the enthalpy of activation that is derived from the temperature dependence of the rate constant (p 2). The free energy vs. reaction coordinate diagrams are drawn with straight lines and sharp breaks, depriving the student of the intuition about energy surfaces. The derivation of the Eyring equation (pp 4–5) is obscure, since the factor kT/h