The high-field (δ 6.74) and low-field (δ 7.20) doublets of the A_2B_2 system in the presence of 20% pyridine-d_5 (in acetone-d_6) were shifted downfield by 3-Hz and 1-Hz, respectively. Under the same conditions, 4-Hz and 3-Hz respective downfield shifts of the aromatic signals at δ 6.35 and 6.44 were observed with no measurable effect on the other protons. These data suggested the free hydroxyl group had two ortho protons and led to the structural assignment seen in Figure 1. Mass spectral confirmation of that assignment was obtained by CAD analysis of the m/z 137 fragment ion (Figure 3, top). The major fragmentation is the loss of formaldehyde ($M^+ - 30$) from the methyl ether. Since this fragmentation must be dependent on regiochemistry, both possible methyl ether isomers were independently synthesized as the benzyl alcohols. Chemical ionization of these benzyl alcohols gives a major $M + 1 - H_2O$ fragment to generate the isomeric benzyl cations which upon CAD give the fragments shown in Figure 3. These data establish the *o*-methoxybenzyl cation as the structure of the m/z 137 fragment of the isolated material.

The new structure **1**, which has oxygen functionality on both rings of the 1,3-diphenylpropene system, can be biogenetically derived from flavanoids. The significance of a flavanoid serving as a host recognition substance for the hemiparasite *Agalinis purpurea* is not entirely clear, but it stands as the first natural host recognition substance for parasitic angiosperms. For that reason we have named it xenognosin or host recognition substance.[13] Studies are under way to determine if this and related substances will function in host recognition for *Striga asiatica* and other root parasites that are of agronomic importance.

Acknowledgment. We thank Professor D. F. Hunt and Mr. W. C. Hutton for helpful discussions and Dr. Mike Thompson, Ms. Barbara Joebstl, and Dr. Marc Cohn for technical assistance. We gratefully acknowledge support from NSF (PCM 78-22889) and USDA (15901-0410-9-0257-0).

(12) Hunt, D. F.; Shabanowitz, J.; Giordani, A. B. *Anal. Chem.* **1980**, *52*, 386–390.

(13) We thank Dr. David Kovacs, Classics Department, University of Virginia for supporting the term xenognostic from the Greek *xenos*, meaning host, and *gignoskein*, meaning to recognize.

Oxygen Chiral Phosphodiesters. 3. Use of ^{17}O NMR Spectroscopy To Demonstrate Configurational Differences in the Diastereomers of Cyclic 2'-Deoxyadenosine 3',5'-[^{17}O,^{18}O]Monophosphate

Jeffrey A. Coderre, Shujaath Mehdi, Peter C. Demou, Richard Weber, Daniel D. Traficante,* and John A. Gerlt*†

Department of Chemistry, Yale University New Haven, Connecticut 06511

Received December 1, 1980

This laboratory recently reported the syntheses of both diastereomers of cyclic 2'-deoxyadenosine 3',5'-[^{16}O,^{18}O]monophosphate (cyclic [^{16}O,^{18}O]dAMP) and assigned their absolute configurations at phosphorus by measuring the ^{18}O perturbations on the phosphorus chemical shifts[1] of the ethyl esters formed by reaction of the chiral diesters with diazoethane.[2] The oxygen chiral phosphodiesters were subsequently reacted with pyrophosphate and glycerol in the presence of a bacterial adenylate cyclase and glycerol kinase to yield oxygen chiral samples of 2'-deoxyadenosine 5'-[α-^{16}O,^{18}O]diphosphate ([α-^{16}O,^{18}O]dADP) and glycerol phosphate; the absolute configurations at the α-phosphorus atoms were assigned by measuring the ^{18}O perturbations on the phosphorus chemical shifts of the $Co_3(NH_3)_4$dADP complexes formed from the chiral samples of [α-^{16}O,^{18}O]dADP.[3]

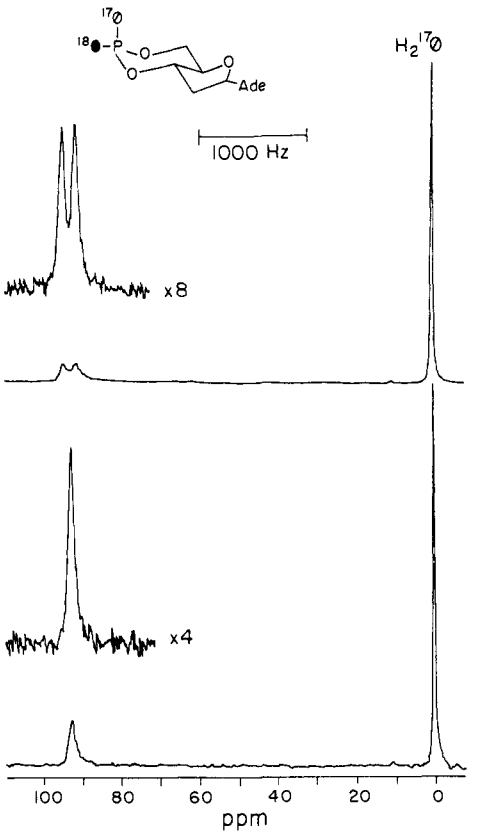

Figure 1. ^{17}O NMR spectra at 36.6 MHz of the R_P diastereomer (axial ^{17}O) of cyclic [^{17}O,^{18}O]dAMP: top, ^{31}P coupled; bottom, ^{31}P decoupled. Sample preparation and spectral acquistion parameters are described in ref 12; a 2-Hz line broadening factor was applied to each free induction decay prior to Fourier transformation.

We are interested in determining the stereochemical course of both enzymatic and nonenzymatic hydrolyses of phosphodiesters; for this reason, we are synthesizing several cyclic and acyclic chiral [^{17}O,^{18}O]phosphodiesters[4] so that the hydrolysis reactions can be most economically and conveniently performed in $H_2{}^{16}O$. In the course of characterizing the diastereomers of cyclic [^{17}O,^{18}O]-dAMP, we discovered that these oxygen chiral phosphodiesters can be distinguished on the basis of their ^{17}O NMR spectra. In particular, the chemical shifts of the diastereomers are sufficiently different that their resonances can be resolved in a ^{31}P decoupled spectrum of a racemic sample. In addition, both the ^{31}P–^{17}O coupling constants and the line widths of the separated diastereomers are significantly different. Thus, ^{17}O NMR spectroscopy is the first spectroscopic technique that has been found to directly detect configurational differences in oxygen chiral phosphate esters.

The diastereomeric ^{17}O-enriched *P*-anilidates of cyclic dAMP were synthesized according to procedures developed in this laboratory for the preparation of unlabeled materials,[5] except that [^{17}O]$POCl_3$[6] was used to prepare the required *o*-(chlorophenyl)-*N*-phenyl[^{17}O]phosphoramidic chloride. The [^{17}O]-*P*-anilidates were reacted separately with a 10-fold excess of 99% enriched $C^{18}O_2$, and the products were purified by chromatography on DEAE-Sephadex A-25.[2] The chiral [^{17}O,^{18}O]phosphodiesters which were obtained[7] were identical with authentic cyclic dAMP

†NIH Research Career Development Awardee (CA-00499), 1978–1983.

(1) Cohn, M.; Hu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978** *75*, 200. Lutz, O.; Nolle, A.; Staschewski, D. *Z. Naturforsch., A* **1978**, *33A*, 380.

(2) Gerlt, J. A.; Coderre, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 4531.

(3) Coderre, J. A.; Gerlt, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 6594.

(4) Gerlt, J. A.; Mehdi, S.; Coderre, J. A.; Rogers, W. O. *Tetrahedron Lett.* **1980**, 2385.

(5) Coderre, J. A.; Gerlt, J. A., unpublished procedures. Our methodology is analogous to that which has been published by W. J. Stec and his collaborators: Lesnikowski, Z. J.; Stec, W. J.; Zielinski, W. J. *Nucleic Acids Res., Spec. Publ. 1978, No. 4*, S49.

(6) Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Bockhoff, F. M.; McLafferty, F. W.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 4323; the isotopic composition was determined by reaction with excess methanol followed by gas chromatography/mass sepctral analysis of the resulting trimethyl phosphate: ^{16}O, 17.1%; ^{17}O, 51.1%; ^{18}O, 31.8%.

0002-7863/81/1503-1870$01.25/0

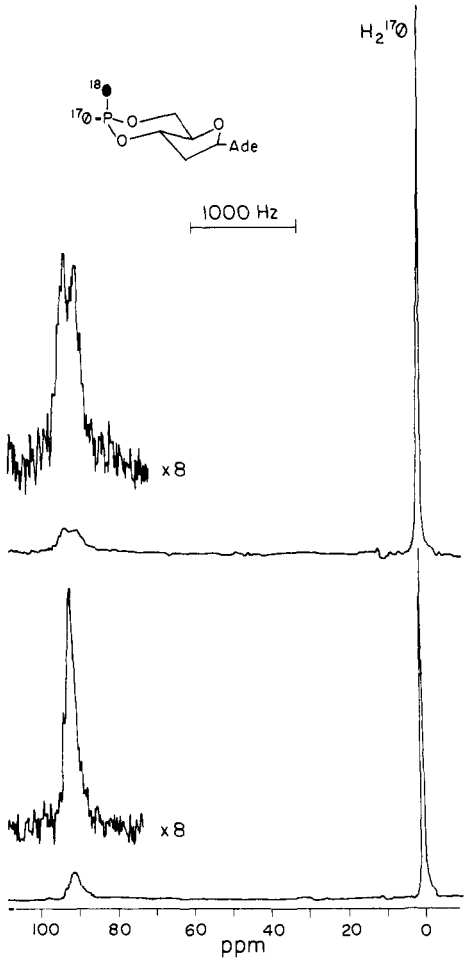

Figure 2. ^{17}O NMR spectra at 36.6 MHz of the S_P diastereomer (equatorial ^{17}O) of cyclic [^{17}O,^{18}O]dAMP: top, ^{31}P coupled; bottom, ^{31}P decoupled. Sample preparation and spectral acquisition parameters are described in ref 12; a 5-Hz line broadening factor was applied to each free induction decay prior to Fourier transformation.

Table I. ^{17}O NMR Spectral Data for the Diastereomers of Cyclic [^{17}O,^{18}O]dAMP

configuration	chemical shift[a]	J_{PO}[b]	line width[b,c]
R_P (axial ^{17}O)	92.8	130	50
S_P (equatorial ^{17}O)	91.2	102	82

[a] In ppm from $H_2{}^{17}O$, with a positive value indicating lower shielding; estimated error, ±0.2 ppm. [b] In Hz; estimated error, ±7 Hz. [c] Corrected for line broadening factors.

by using the criteria of TLC and ^1H NMR spectroscopy at 270 MHz; the ^{31}P spectra at 81.0 MHz revealed the expected ratio of ^{16}O,^{18}O- and ^{18}O,^{18}O-resonances.[8]

Oxygen-17 NMR spectra at 36.6 MHz[9] of aqueous solutions of the tetraethylammonium salts[10] of the diastereomers of cyclic

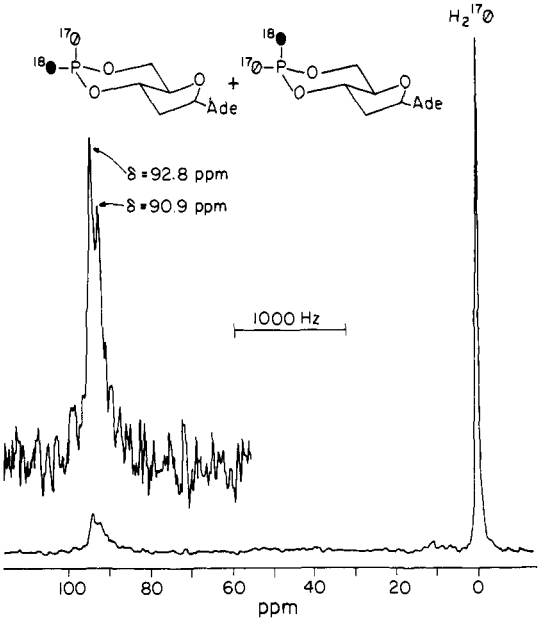

Figure 3. ^{17}O NMR spectra at 36.6 MHz of a racemic sample of cyclic [^{17}O,^{18}O]dAMP with ^{31}P decoupling. Sample preparation and spectral acquisition parameters are described in ref 12; a 10-Hz line broadening factor was applied to the free induction decay prior to Fourier transformation.

[^{17}O,^{18}O]dAMP and a mixture of diastereomers were obtained at 95 °C;[11] spectra were recorded with and without decoupling of the phosphorus nuclei.[12] The spectra so obtained are presented in Figures 1–3. Chemical shifts, ^{31}P–^{17}O coupling constants, and line widths are summarized in Table I.

One bond ^{31}P–^{17}O coupling is evident in the ^{31}P coupled spectrum of each diastereomer (Figures 1 and 2), with the coupling constant measured to the axially positioned ^{17}O being larger than that measured to the equatorially positioned ^{17}O (Table I). This finding is in contrast to the empirical rule which Stec and his co-workers have formulated for coupling between phosphorus and several $I = {}^1/_2$ nuclei in the 1,3,2-dioxaphosphorinane ring system; for ^1H, ^{13}C, ^{15}N, ^{19}F, and ^{77}Se, the coupling to the axially positioned magnetically active nucleus is smaller than that to the equatorially positioned nucleus.[13] Oxygen-17 is, of course, a quadrupolar nucleus with $I = {}^5/_2$ and need not obey Stec's rule. Gorenstein has postulated that coupling constants in this and related ring systems depend on bond angle and torsional angle considerations;[14] conformational differences may explain the deviation of our observation from those reported previously.

In the presence of ^{31}P decoupling, differences in the line widths of the resonances of the two diastereomers can be detected. In ^{17}O NMR spectra, the line widths are the result of quadrupolar

(7) The configuration of the presumed R_P diastereomer was confirmed by measuring the ^{18}O perturbations on the phosphorus chemical shifts of the ethyl esters formed by reaction of the ^{17}O,^{18}O-labeled material with diazoethane.[2]

(8) Since the carbon dioxide used to prepare the chiral [^{17}O,^{18}O]-phosphodiesters was 99% enriched we only see resonances arising from the ^{16}O and ^{18}O present in the ^{17}O-enriched water.

(9) The spectra were obtained on a Bruker HX-270 spectrometer equipped with a probe modified for time-shared multinuclear capabilities. The details for construction of the probe were presented at the 21st Experimental NMR Conference (ENC) in Tallahassee, FL, March 1980, and will be published elsewhere.

(10) Tetraethylammonium salts were used because these samples had previously been dissolved in methanol and subjected to ^{17}O NMR analysis. In order to conserve material, the counterion was not exchanged prior to obtaining spectra in aqueous solution.

(11) This temperature was used to effect line narrowing. A higher temperature would decrease the rotational correlation time and lead to an increase in the spin–lattice relaxation time.

(12) The ^{17}O-labeled phosphodiesters were dissolved in 20% D_2O and treated with Chelex-100 (tetraethylammonium); the resin was removed by centrifugation and washed with additional solvent. The total volume of each sample was about 2.5 mL and the final concentration of phosphodiester was about 50 mM. All glassware and NMR tubes were soaked in a 1:1 mixture of concentrated nitric and sulfuric acids and rinsed with deionized water to remove metal ions. Since the line widths were observed to decrease with increasing temperature, the spectra were obtained at as high a temperature as the solvent would permit. A spectral width of 14085 Hz and an acquistion time of 0.14 s were used; no delay was used between pulses, so the recycle time was equal to the acquisition time. Ten thousand transients were collected by using 4K total data points. A line broadening factor was applied to each free induction decay prior to Fourier transformation, and the values used are specified in the legends to the figures. Chemical shifts are measured relative to natural abundance $H_2{}^{17}O$, which is the sharp resonance at 0 ppm in the spectra in Figures 1–3.

(13) Stec, W. J.; Okruszek, A.; Uznanski, B.; Michalski, J. *Phosphorus* **1972**, *2*, 97. Stec, W. J.; Kinas, R.; Okruszek, A. *Z. Naturforsch., B* **1976**, *31B*, 393. Stec, W. J., private communication.

(14) Gorenstein, D. G. *J. Am. Chem. Soc.* **1977**, *99*, 2254.

broadening, and it is apparent that the magnitude of this broadening also depends on the orientation of the ^{17}O nucleus relative to the cyclic phosphate ring system (Table I). We do not believe that the magnitude of the quadrupolar broadening depends only on the π-bond character of the P-^{17}O bond, since the line widths for the diastereomeric [^{17}O]-*P*-anilidates in methanolic solution depend significantly on the orientation of the ^{17}O nucleus relative to the cyclic phosphate ring system.[15]

A comparison of the ^{31}P decoupled spectra of the separated diastereomers reveals a difference of 2 ppm in their chemical shifts, with the resonance for the equatorially positioned ^{17}O nucleus being less shielded (Figures 1 and 2); this chemical shift difference is also observed in the ^{31}P decoupled spectrum of a mixture of the two diastereomers, since two resonances can be resolved (Figure 3).

The ^{17}O NMR spectral observations which we report in this communication constitute the first *direct* physical evidence for configurational differences in oxygen chiral phosphate ester anions. Previous demonstrations of oxygen chirality by NMR methodology have been reported by our laboratory[2,3] and the laboratories of Trentham,[16] Tsai,[17] Gorenstein,[18] and Knowles;[19] these required chemical modification of the chiral molecule so that the ^{18}O perturbations on the phosphorus chemical shift could be used to indirectly demonstrate chirality. Knowles and his collaborators have described a general method for the configurational analysis of oxygen chiral phosphate monoesters by using metastable ion mass spectroscopic techniques, but this approach also requires chemical modification reactions.[6] Although Cullis and Lowe have reported that (S_P)-methyl [^{16}O,^{17}O,^{18}O]phosphate has a circular dichroism maximum at 208 nm,[20] the validity of this observation must be viewed with caution unitl spectral properties are reported for the R_P enantiomer.

The differences in spectral properties that we have observed for the diastereomers of cyclic [^{17}O,^{18}O]dAMP should be present in the diastereomers of other ^{17}O-labeled cyclic nucleotides and, presumably, six-membered-ring cyclic phosphates, in general. Thus, ^{17}O NMR spectroscopy will be useful in making configurational assignments and complements the approach based on ^{18}O perturbations of phosphorus chemical shifts that we and others have used previously.

Our results also demonstrate that high-field NMR spectrometers with the capability to decouple phosphorus nuclei permit direct observation of ^{17}O NMR resonances with sufficient resolution to provide useful chemical and biochemical information. This instrumentation will be particularly useful if compounds that are stereospecifically labeled with a single ^{17}O atom are available for study; a number of such phosphate esters can be obtained via the chemical and enzymatic procedures that we[2,3] and Stec and his co-workers[21] have developed. Thus, the methodology described in this paper complements that recently reported by Tsai et al.[22] in which the line broadening effects of ^{17}O on ^{31}P resonances were used to indirectly monitor changes in the ^{17}O line widths.

Acknowledgment. We thank Dr. Wojciech J. Stec for useful discussions. The research was supported by a grant (GM-22350) from the National Institutes of Health. The high-field NMR spectrometer used in this research (Bruker HX-270) is supported by a grant from the National Science Foundation (CHE-7916120).

(15) In methanolic solution at 50 °C, the line width of the axially positioned ^{17}O resonance was about 170 Hz and that of the equatorially positioned ^{17}O resonance was about 270 Hz; similar line widths were observed for the cyclic [^{17}O,^{18}O]dAMP diastereomers.

(16) Webb, M. R.; Trentham, D. R. *J. Biol. Chem.* **1980**, *255*, 1775.

(17) Tsai, M. D.; Chang, T. T. *J. Am. Chem. Soc.* **1980**, *102*, 5418.

(18) Gorenstein, D. G.; Rowell, R. *J. Am. Chem. Soc.* **1980**, *102*, 6166.

(19) Buchwald, S. L.; Knowles, J. R. *J. Am. Chem. Soc.* **1980**, *102*, 6601.

(20) Cullis, P. M.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1978**, 512. In this communication and two others which have appeared subsequently (Jarvest, R. L.; Lowe, G.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1980**, 1142. Jarvest, R. L.; Lowe, G. *Ibid.* **1980**, 1145.), Lowe and his collaborators have described the syntheses of and stereochemical studies of displacement reactions at chiral [$^{16}O$$^{17}O$,$^{18}O$]-phosphate monoesters. Recent work in our laboratory (Coderre, J. A.; Mehdi, S.; Gerlt, J. A. *J. Am. Chem. Soc.* following paper in this issue) and in Lowe's laboratory (G. Lowe, personal communication) has shown that the published configurational assignments of all of the oxygen chiral phosphate monoesters which have been prepared in Lowe's laboratory are in error, i.e., the correct absolute configurations are S_P rather than R_P.

(21) Baraniak, J.; Lesiak, K.; Sochacki, M.; Stec, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 4533.

(22) Tsai, M. D.; Huang, S. L.; Kozlowski, J. F.; Chang, C. C. *Biochemistry* **1980**, *19*, 3531.

Oxygen Chiral Phosphodiesters. 4. Stereochemical Course of the Hydrolysis of 2′-Deoxyadenosine 3′,5′-[^{17}O,^{18}O]Monophosphate in $H_2{}^{16}O$ Catalyzed by Bovine Heart Cyclic Nucleotide Phosphodiesterase

Jeffrey A. Coderre, Shujaath Mehdi, and John A. Gerlt*†

Department of Chemistry, Yale University
New Haven, Connecticut 06511

Received January 12, 1981

The classical approach for determining the stereochemical course of nucleophilic displacement reactions at phosphorus in enzymology is to employ phosphorothioate analogues of the natural substrates,[1,2] since the syntheses and configurational analyses of phosphorothioate mono- and diesters usually can be accomplished more easily than can the syntheses and configurational analyses of oxygen chiral phosphate mono- and diesters. It can be argued, however, that the results of stereochemical experiments utilizing phosphorothioate analogues are subject to mechanistic ambiguity, since these substrates are usually processed by enzymes at rates less than those observed for the natural materials. However, the results of recent research reported by Knowles' laboratory[3,4] and this laboratory[5,6] suggest that these mechanistic concerns may be ill-founded; the reactions catalyzed by the glycerol kinase obtained from yeast[3,4] and the adenylate cyclase isolated from *Brevibacterium liquefaciens*[5,6] proceed with the identical stereochemical course whether phosphorothioates or oxygen chiral substrates are used.

We therefore viewed with considerable caution the recent report from Lowe's laboratory that the stereochemical course of the bovine heart cyclic nucleotide phosphodiesterase reaction differs with oxygen chiral and phosphorothioate substrates.[7,8] Lowe and his collaborators synthesized chiral 5′-[^{16}O,^{17}O,^{18}O]AMP according to the procedure published by Cullis and Lowe for the preparation of the R_P diastereomer of methyl [^{16}O,^{17}O,^{18}O]-phosphate,[9] except that 2′,3′-diacetyladenosine was substituted for methanol. The labeled 5′-AMP was reacted with diphenyl phosphorochloridate, and the resulting diphenyl ester of labeled ADP was cyclized in the presence of potassium *tert*-butoxide to yield an equimolar mixture of the three types of oxygen chiral cyclic AMP, cyclic [^{16}O,^{17}O]AMP, cyclic [^{17}O, ^{18}O]AMP, and cyclic [^{16}O, ^{18}O]AMP. By assuming that the starting [^{16}O,^{17}O,^{18}O]AMP had the R_P configuration and after determining the configuration of the cyclic [^{16}O, ^{18}O]AMP present in the mixture of cyclic AMP molecules (by measurement of the [^{18}O] perturbations on the phosphorus chemical shifts of the equatorial and axial methyl esters[10,11]), Lowe et al. concluded that the cy-

† NIH Career Development Awardee (CA-00499), 1978-1983.

(1) Eckstein, F. *Acc. Chem. Res.* **1979**, *12*, 204.

(2) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877.

(3) Blättler, W. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1980**, *102*, 510.

(4) Pliura, D. H.; Schomburg, D.; Richard, J. P.; Frey, P. A.; Knowles, J. R. *Biochemistry* **1980**, *19*, 325.

(5) Gerlt, J. A.; Coderre, J. A.; Wolin, M. S. *J. Biol. Chem.* **1980**, *255*, 331.

(6) Coderre, J. A.; Gerlt, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 6594.

(7) Jarvest, R. L.; Lowe, G.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1980**, 1142.

(8) Jarvest, R. L.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1980**, 1145.

(9) Cullis, P. M.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1978**, 512.

(10) Gerlt, J. A.; Coderre, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 4531.

0002-7863/81/1503-1872$01.25/0