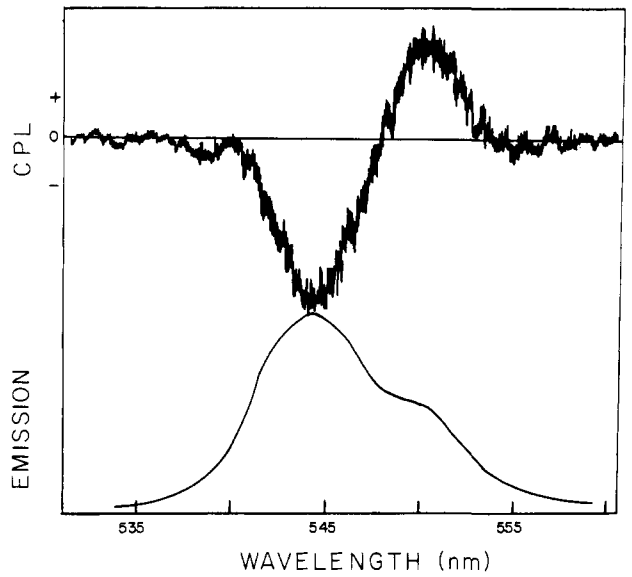

Figure 1. Circularly polarized luminescence (upper) and total luminescence (lower) spectra of $Tb(dpm)_3$ dissolved in neat (*R*)-α-phenethylamine. Both intensity scales are completely arbitrary.

evaluation of the sign of induced optical activity with substrate absolute configuration.

No CPL was observed when resolved 2-butanol, 2-octanol, or diethyl tartrate were used as the chiral solvent, and no Tb(III) emission was observed when using α-(1-naphthyl)ethylamine as the solvent (here the solvent totally absorbed the exciting light at 365 nm and thus made it impossible to excite the Tb(III) chelate).

It was found that the magnitude of total luminescence (TL) and CPL depended strongly on the nature of the solvent used, and in general the Tb(III) emission was at least an order of magnitude more intense in the amine solvents than in the alcohol solvents. Previous work involving emission titrations of Eu(III) chelates has established that the luminescence intensity of the lanthanide ion in a tris(β-diketone) chelate will increase upon adduct formation with a substrate, and that the increase in luminescent intensity can be related to the formation constant of the chelate: substrate adduct.[7] In the cases where induced CPL was not found in the luminescence of a $Tb(dpm)_3$ adduct, it was found that the total emission was simply too weak to be observed.

It is possible to place the CPL results on a quantitative basis by calculating the luminescence dyssymmetry factor, g_{lum} as defined by Richardson and Riehl:[3]

$$g_{lum} = \frac{2(\Delta I)}{I} = \frac{2(I_L - I_R)}{(I_L + I_R)} \quad (1)$$

I_L and I_R refer, respectively, to the intensities of left and right circularly polarized emission, ΔI is the differential emission of left and right circularly polarized light, and I is the mean light intensity. This was done at the two extrema found in the CPL spectra, and the g_{lum} values are found in Table I. It may be noted that the induced CPL is greater in the amine solvents than in the alcohol solvents, and this observation is in accord with stronger amine adducts being formed.

Table I. Comparison of the Luminescence Dissymmetry Factors Observed in the *R* Enantiomers of Various Chiral Solvents

solvent	g_{lum} (544 nm)	g_{lum} (549 nm)
α-phenethylamine	−0.0283	+0.0325
2-aminobutane	−0.0134	+0.0143
2-aminoheptane	−0.0111	+0.0120
α-phenethyl alcohol	−0.0105	+0.00719
propylene glycol	−0.00252	+0.00112

A clear and detailed understanding of the CPL results is not possible at the present time, but the studies presently underway in this laboratory should provide additional insight into the nature of this problem. The present method appears to offer a simple and fairly convenient method to predict the absolute configuration of a substrate capable of forming an adduct with $Tb(dpm)_3$, and is particularly suited to the study of amine solvents.

Acknowledgments. This work was supported by a Cottrell grant from the Research Corporation (No. 8926) and by the National Science Foundation (Grant CHE 78-03402).

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(2) N. H. Anderson, B. J. Bottino, A. Moore, and J. R. Shaw, *J. Am. Chem. Soc.*, **96**, 604 (1974).
(3) F. S. Richardson and J. P. Riehl, *Chem. Rev.*, **77**, 773 (1977).
(4) H. G. Brittain and R. S. Richardson, *J. Am. Chem. Soc.*, **99**, 65 (1977).
(5) Tb(dpm) was prepared in the manner of K. J. Eisentraut and R. E. Sievers, *Inorg. Synth.*, 11, 94 (1968). Chiral solvents were all obtained from Norse Laboratories.
(6) The CPL spectrometer was constructed in this laboratory and is similar to the one previously described by C. K. Luk and F. S. Richardson, *J. Am. Chem. Soc.*, **97**, 6666 (1975). The luminescent spectra were recorded by exciting the solutions with the 365-nm output of a 200-W Hg-Xe arc lamp and recording the emission at 180° to the exciting beam. The emission was analyzed by a Spex 1870 ½ meter grating monochromator (15-Å band pass) and detected by an EMI 9798B photomultiplier tube (S-20 response). Our optical modulator is a Model PEMFS-3 (Morvue Electronics).
(7) (a) H. G. Brittain and F. S. Richardson, *J. Chem. Soc., Daltons Trans.*, 2253 (1976); (b) H. G. Brittain, *J. Am. Chem. Soc.*, **101**, 1733 (1979); (c) H. G. Brittain, *J. Chem. Soc., Dalton Trans.*, 1187 (1979).

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Orotidine 5′-Monophosphate Decarboxylase Inhibitors Formed by Spontaneous Reaction of Barbituric Acid and Ribose 5-Phosphate, a Surprising Reaction

Sir:

Many drugs used in the treatment of cancer and other proliferative disorders cause an inhibition of RNA and (or) DNA synthesis.[1] We report here determinations of the structures of a series of compounds which result from unusual spontaneous reactions between barbituric acid and D-ribose 5-phosphate in water. All of the products of this reaction thus far tested have been found to behave as potent competitive inhibitors of purified yeast orotidine 5′-monophosphate decarboxylase.[2-4] The decarboxylase catalyzes the final step in the enzymatic sequence for the de novo synthesis of uridine 5′-monophosphate which is eventually incorporated into nucleic acids via its triphosphate.

Barbituric acid (54.1 mg, 0.42 mmol) was incubated with 33.3 mg (0.11 mmol) of D-ribose 5-phosphate disodium salt in 3 mL of deionized water for 2 h at 37 °C, pH 5.5.[2,5] The reaction mixture was then chromatographed on a Sephadex G-10 column (3 × 18 cm) with water, the chromatograph being carried out in a 2 °C cold room to minimize decomposition of the labile product. Monitoring with UV at 260 nm and CD at 290 nm gave the base-line separated peak of product **1** and partly overlapping peaks of barbituric acid and ribose phosphate. The product fraction was lyophilized to afford 39 mg (87%) of practically pure adduct **1**; this was used as such for structural and inhibition studies since it was found that further handling only led to gradual conversion into other products (see below). Adduct **1** strongly and competitively

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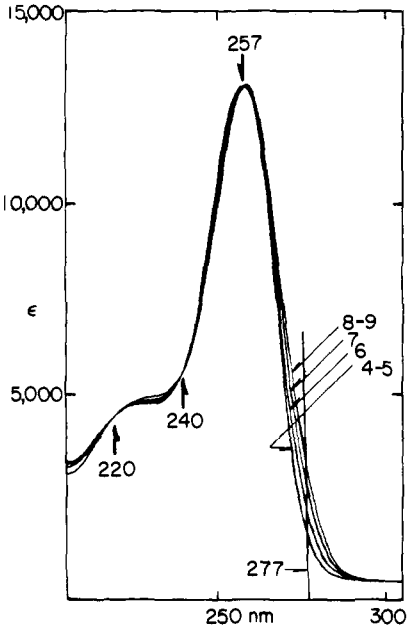
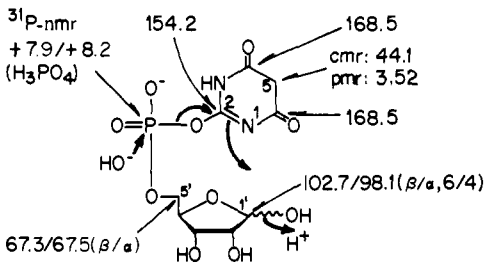


Figure 1. Change in UV of **1** with pH, 50 mM phosphate buffer.

inhibited yeast orotidine 5′-monophosphate decarboxylase with a K_i of 10^{-7} M.[2,6]

The structure of this labile adduct was deduced to be **1** as follows. *Although the UV maximum was at 260 nm* (ε 13 000, in pH 7.0 50 mM phosphate buffer), *there was only a single CD extremum at 290 nm* ($\Delta\epsilon$ −0.20, 50 mM phosphate buffer).[7] Lack of a 260-nm Cotton effect indicates that the chromophore derived from barbituric acid is remote from the ribose chiral centers at C-1′, -2′, and -3′; otherwise product **1** would have shown a CD extremum at ~260 nm.

The barbituric acid moiety hence is linked to the phosphate group. This was borne out by a positive Tollens reaction and the presence of two ^{13}C NMR peaks at 102.7-98.1 ppm[8] (in D_2O, ratio was ~3:2 for β and α anomeric carbons,[9] respectively) (see **1**). Since the solution of **1** in water became discol-



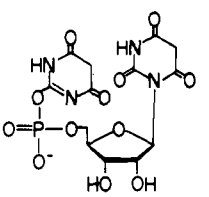
1 uv(H_2O, pH 7): 260 (ε 13,000)
228 (sh, ε 4,800)
cd(H_2O, pH 7): $\Delta\epsilon_{290}$ −0.20
nmr in D_2O, pD 3.5

ored when left at room temperature for 4 h owing to partial decomposition, the ^{13}C NMR was measured at 6 °C; moreover, the ^{13}C NMR was measured in D_2O[10] without buffering (pD ~3.5) since **1** could not be dissolved in pD 7 buffer in a concentration sufficiently high for ^{13}C NMR measurements. In agreement with the presence of an anomeric mixture, two C-5′ ^{13}C NMR signals and ^{31}P NMR signals appeared at 67.3-67.5[7] and 7.9-8.2 ppm,[11] respectively.

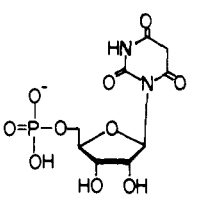
The pK_a' of **1** estimated from the small changes seen in UV absorptions at 277 nm (Figure 1, isosbestic points at 220, 240, and ~257 nm) gives a value of ~6.5. The pK_a of N^1-methylbarbituric acid is 4.2 (pK_2 = 12.8).[12] It is thus unlikely that **1** is substituted at N^1. This is substantiated from the following data. Only three ^{13}C NMR signals for the barbituric acid moiety containing four carbon atoms were present at 44.1, 154.2, and 168.5 ppm (see **1**), the ratio of the latter two sp^2 carbon peaks being ~1:2. This indicates that this moiety is symmetrically substituted, i.e., either at O^2 or at C-5. However, C-5 is unsubstituted since the 44.1 ^{13}C NMR peak (proton noise decoupled) was a singlet (hence no C-P bond); the 3.52 ^1H NMR peak[13] (in D_2O, pD ~3.5[14]) was also a broad singlet indicating the absence of a CH-O-P linkage. The pyrimidine base is therefore linked to the 5′-phosphate through O^2; i.e., the structure of the adduct is as depicted in structure **1**.[15]

The C-5 and 5-H NMR data shown in **1** indicate that, unlike the N^1-substituted barbituric acid ribotide **3** in which the 5-H ^1H NMR peak is absent and the 80-ppm C-5 NMR signal is weak and broad (NMR in D_2O), it exists in the diketo form **1**.[16] The UV absorption is thus due to the conjugated system shown in the structure.

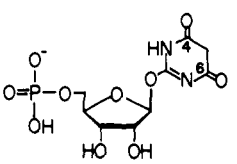
Further incubation of adduct **1** at 37 °C for 1 week at pH 5.5 led to its total disappearance and concomitant formation of a complex mixture. Purification of the mixture by two passages through Sephadex G-10 column (eluted with water), followed by treatments with DEAE-cellulose (exponential gradient elution with aqueous ammonium bicarbonate, 0-300 mM) and Sephadex G-25 (eluted with water), afforded 20 μg of another labile adduct, **2** with $K_i = 10^{-8}$ M[2,6] and an intense



2 uv: 261nm(ε 35,000)
cd: $\Delta\epsilon_{258}$ -17
$\Delta\epsilon_{274}$ +34



3 uv: 262 nm(ε 22,000)
cd: $\Delta\epsilon_{262}$ +0.62



4 uv: 261 nm
cd: 255nm, negative

UV band (H_2O) at 261 nm (ε 35 000). The fact that **2** exhibited a typical split CD of the coupled oscillator type[17] at ~260 nm, i.e., $\Delta\epsilon_{274}$ = +34 and $\Delta\epsilon_{258}$ = −17 (in H_2O), indicates that two barbituric acid moieties are present. The same bis adduct **2** was also formed in 80% yield upon incubation of authentic barbituric acid 5′-ribotide **3** in water for 12 h at 37 °C with a large excess of barbituric acid.[18] Presumably **2** is derived from intramolecular migration of the barbituric acid moiety from the phosphate group to C-1′ (see arrows in structure **1**)[19,20] to give ribotide **3**, which further reacts with a free barbituric acid molecule liberated by decomposition of adduct **1**. Although the complex mixture (see above) appeared to contain a fraction corresponding to ribotide **3**, the amount was insufficient for proper characterization.

A third adduct **4**, UV (H_2O) 261 nm, was isolated in trace quantities (~20 μg) during attempts to further purify the partly decomposed adduct **1** by reverse-phase TLC.[21] Substitution on O^4 (or O^6) to give an enolic system can be discounted because of the lack of an olefinic ^1H NMR signal.[22] In contrast to ribotide **3**, this adduct has a unique *negative* CD extremum; however, it is inconceivable that the adduct is the α anomer of **3** because of unfavorable steric factors. The O^2-yl structure **4** is therefore tentatively assigned to this adduct. Although **4** strongly inhibited the yeast enzyme at a concentration of 10^{-5} M, the quantity was too minute for a quantitative estimation of K_i.

Since the various products appear to be in dynamic equilibria with each other and with the reactants,[2,6] we regard the

measured K_i values given above merely as approximations. It is noteworthy that at least one of the substances under study, **3**, inhibits mammalian as well as yeast decarboxylase.[2,3]

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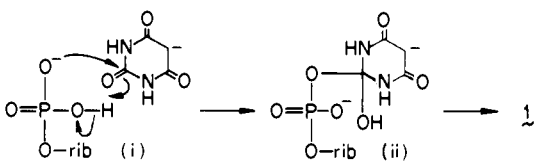
(2) A more detailed discussion of the biochemical properties, possible significance of these results, and implications for the prebiotic synthesis of pyrimidine ribotides will appear elsewhere: Potvin, B. W.; Stern, H. J.; Cooke, J. E.; Krooth, R. S.; Komura, H.; Nakanishi, K., unpublished work.
(3) Several other previously described compounds whose metabolites inhibit the decarboxylase have been shown to possess antineoplastic activity, e.g., Cadman, E. C.; Dix, D. E.; Hill, S. C.; Handschumacher, R. E. *Proc. Am. Assoc. Cancer Res.* **1976**, *17*, 208.
(4) The yeast orotidine 5′-monophosphate was purchased from Sigma Chemical Co., St. Louis, Mo.
(5) Formation of the product was first checked by incubating [2-^{14}C]barbituric acid with cold D-ribose 5-phosphate and monitoring the radioactivity which migrated with an R_f of 0.7 on a descending mode paper chromatograph (in contrast to the R_f of 0.39 for barbituric acid) (see ref 2). When yeast orotidine 5′-monophosphate decarboxylase and its substrate were present in an assay mixture containing barbituric acid, it was found that addition of a large excess of ribose phosphate resulted in maximal inhibition of enzyme activity by the earliest time point (5 min) which could be feasibly measured by the assay. The reaction conditions, however, were modified to those described, when it was found that excessive ribose phosphate led to unnecessary complications in the isolation procedure for product **1**.
(6) The K_m of the yeast orotidine 5′-monophosphate decarboxylase was 5.5×10^{-7} M. All K_i values were computed from double reciprocal plots using the method of least-squares regression analysis. While we can not rigorously prove that the observed inhibitions do not result from a breakdown of **1** (or **2**) into the known potent inhibitor **3** during the incubation period, we believe that this is unlikely for the following reasons. (1) The incubation was carried out at 37 °C for *30 min* at a *pH of 7.4*. It is doubtful that, under these conditions, adduct **3** could accumulate in sufficient quantity to account for the observed inhibition of enzyme activity (note that only a 0.1% yield of **2** and a trace of **3** were produced after incubating **1** for *1 week* at 37 °C at pH 5.5). (2) Whether the incubation was carried out at 4 or at 37 °C, the inhibition of enzyme activity did *not* increase with the time of incubation and was maximal at the earliest time point measured.
(7) D-Ribose and D-ribose 5-phosphate exhibit the following Cotton effects (in water), respectively: $\Delta\epsilon_{282} = -0.0006$ and $\Delta\epsilon_{280} = -0.019$. Note the very small $\Delta\epsilon$ values compared with the value of −0.20 for **1**; this shows that a larger proportion of **1** exists in the aldehydic form; the changes in $\Delta\epsilon$ values accompanying changes in concentration, pH, and buffer ionic strengths of solutions were minimal.
(8) The ^{13}C and ^{1}H chemical shifts were measured from external TSP.
(9) All NMR spectra were measured in D_2O, pD ~3.5, with JEOL FX-100 and Bruker WP-80 instruments. The β and α-anomeric carbons of ribose 5-phosphate appear at 102.0 and 97.2 ppm, respectively (3:1 ratio); the C-5 peaks also appear separately at 64.6 (β)/65.4 (α) ppm: Miwa, M.; Saito, H.; Sakura, H.; Saikawa, N.; Watanabe, F.; Matsushima, T.; Sugimura, T. *Nucleic Acid Res.* **1977**, *4*, 3997.
(10) The solubility of **1** in Me_2SO, DMF, THF, and MeCN was too low for NMR measurements.
(11) D-Ribose 5-phosphate has two ^{31}P NMR signals at 11.4–11.6 ppm, in D_2O. Chemical shifts are from H_3PO_4, the plus signs denoting downfield shifts from the reference.
(12) Fox, J. J.; Shugar, D. *Bull Soc. Chim. Belg.* **1952**, *61*, 44.
(13) The strong HDO solvent peak was removed by the partially relaxed Fourier transform technique.
(14) Since the pK_2' of the chromophore in **1** is ~6.5; it is in the neutral form.
(15) A possible mechanism for the genesis of adduct **1** is shown; since the optimum pH of the reaction was 5.5,[2] whereas the pK_a values of phosphate are ~1.5 and 6, and those of barbituric acid are 3.9 and 12.5, the reaction is depicted as proceeding through two monoanions. We thank Professor F. Ramirez for discussions. Other primary phosphate derivatives of sugars



such as D-glyceraldehyde, D-ribulose, and four others reacted with barbituric acid under conditions similar to that of the formation of adduct **1**. For details see ref 2.
(16) The 5-methylene group, however, undergoes slow exchange. Thus the intensity of the 3.52-ppm 5-H peak is reduced to one half when the D_2O solution of **1** is left for 85 h at room temperature, pD 3.5, and then intensifies when **1** is further reprotonated for a period of 48 h. These changes however are accompanied by partial decomposition of the adduct.
(17) Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257. Harada, N.; Chen, S.-M.L.; Nakanishi, K. *J. Am. Chem. Soc.* **1975**, *97*, 5345.
(18) The split CD of bis adduct **2** disappears upon leaving its aqueous solution for a few days. The inhibitory activity of the aqueous solution against orotidine 5′-monophosphate decarboxylase however *increases*. Although the minute quantity precluded direct chemical identification of the decomposition product(s), it is nearly certain that **2** had been converted into barbituric acid and its ribotide **3**, which has a higher K_i of $\sim 10^{-9}$ M.
(19) The intramolecular rearrangement is necessary to account for the spontaneous formation of the *N*-riboside linkage without prior activation of the ribose lactol function.
(20) A similar mechanism has been proposed in the biosynthesis of *N*-carboxybiotin: Kluger, R.; Adawadkar, P. D. *J. Am. Chem. Soc.* **1976**, *98*, 3741. We thank Professor D. Arigoni for bringing this to our attention.
(21) Whatman KC_{18} plate, developed with *i*-PrOH–H_2O (75:25 v/v). R_f values of adducts 1 and **4** were 0.66 and 0.80, respectively.
(22) We thank Dr. L. Johnson, Nicolet Instrument Corp., for this measurement at 200 MHz.
(23) Potvin, B. W.; Stern, H. J.; May, S. R.; Lam, G. F.; Krooth, R. S. *Biochem. Pharmacol.* **1978**, *27*, 655.
(24) Deceased Oct. 20, 1979.

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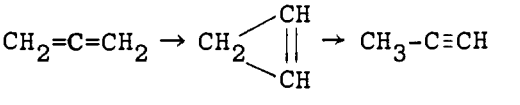
Concerning the Role of Cyclopropene in the Allene to Propyne Isomerization. A Study of the Thermal Rearrangements of C_3H_3D Isomers[1]

Sir:

One of us[2] has recently suggested, on thermochemical kinetic grounds, that cyclopropene may be a possible intermediate in the thermal reversible isomerization of allene to propyne (methylacetylene) as shown in Scheme I. It was previously proposed[3] that this rearrangement occurred via a concerted process involving a direct 1,3-H shift. It is not possible to distinguish between these mechanisms with either unlabeled allene or propyne, since the stationary level of cyclopropene required by Scheme I is below the level of analytical detectability. However, alternative processes can *in principle* be distinguished by means of a study starting with deuteriopropyne. From propyne-*1*-d_1 (**1**), for instance, a concerted process would predict allene-d_1 (**3**) as the sole initial product. The mechanism via cyclopropene (Scheme II) on the other hand is likely to produce propyne-*3*-d_1 (**2**) in addition to allene-d_1. This is because the intermediate cyclopropene-*1*-d_1 (**4**) can revert to propyne-d_1 *in two ways* which are equivalent by symmetry (apart from the deuterium label) to produce **2** as well as **1**. This latter process is likely to be in effective competition with formation of **3** since it is known that cyclopropene isomerization favors the formation of propyne rather than allene.[4]

The necessary test materials were prepared as follows. **1** was made by reaction of propyne with ethylmagnesium bromide in THF solution at −50 °C, followed by hydrolysis with D_2O

Scheme I



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