Table II.[a] Velocities and Isotope Effects for COMT-Catalyzed Methylation of DHA by AdoHcy-CH_3 and AdoHcy-CD_3

Cofactor[b]	10^9V(M min^{-1})[c]	V_H/V_D[d]
AdoHcy-CD_3-I	3045 ± 4	0.823
AdoHcy-CH_3-I	2507 ± 3	0.803
AdoHcy-CD_3-I	3123 ± 4	0.844
AdoHcy-CH_3-I	2636 ± 3	0.861
AdoHcy-CD_3-I	3063 ± 2	0.865
AdoHcy-CH_3-I	2651 ± 4	0.870
AdoHcy-CD_3-I	3047 ± 3	0.789
AdoHcy-CH_3-I	2403 ± 3	0.798
AdoHcy-CD_3-I	3011 ± 3	0.875
AdoHcy-CH_3-I	2634 ± 7	
AdoHcy-CD_3-II	2817 ± 4	0.772
AdoHcy-CH_3-II	2172 ± 5	0.795
AdoHcy-CD_3-I	2732 ± 6	
AdoHcy-CD_3-II	2780 ± 4	0.789
AdoHcy-CH_3-II	2192 ± 5	0.778
AdoHcy-CD_3-I	2819 ± 5	
AdoHcy-CD_3-II	2865 ± 5	0.920
AdoHcy-CH_3-I	2636 ± 5	0.919
AdoHcy-CD_3-II	2869 ± 4	0.831
AdoHcy-CH_3-I	2384 ± 4	0.836
AdoHcy-CD_3-II	2851 ± 5	0.810
AdoHcy-CH_3-I	2309 ± 6	
	Mean 0.832 ± 0.045	

[a] [AdoMet] = 10^{-3} M. Experimental conditions other than [AdoMet] are same as in Table I. [b] The suffixes I and II refer to completely independent biological preparations of the labeled and unlabeled cofactors. [c] Error limits are standard deviations within the single run. The three data sets were obtained on separate days. Differences in rates may reflect slight changes in enzyme activity. [d] Calculated as the ratio of adjacent measurements.

binding isotope effect. A substantial isotope effect is, however, observable for the maximum-velocity term: $V^H_{max}/V^D_{max} = 0.86 \pm 0.04$. For confirmation and further definition of this effect, carefully matched sets of velocities were obtained for CH_3 and CD_3 cofactors at [AdoMet] = 10^{-3} M $\simeq 20K_m$. These are shown in Table II, and yield a mean value $V_H/V_D = 0.832 \pm 0.045$.

For these experiments, AdoHcy-CD_3 was prepared by biological adenosylation of [methyl-$^2H_3$]-L-methionine (made from [methyl-$^2H_3$]methyl iodide and *S*-benzyl-L-homocysteine in sodium–liquid ammonia;[6] extent of deuteration (NMR): 90 ± 5% in cofactor), using a preparation of the yeast *Saccharomyces cerevisiae*.[7] Protiated AdoMet (AdoHcy-CH_3) was prepared in the same way and two completely independent preparations of AdoHcy-CH_3 gave indistinguishable velocities, while two completely independent preparations of AdoHcy-CD_3 gave identical velocities, quite distinct from those for the AdoHcy-CH_3 preparations (Table II).

Although the results strongly imply a trigonal-bipyramidal transition-state structure (as in **3**), they cannot indicate the nature of the methyl donor and acceptor structures X and Y. Our data are consistent with (1) rate-determining methyl transfer directly from AdoMet to DHA, or (2) methyl transfer from AdoMet to enzyme followed by enzyme-to-DHA transfer, with either or both steps determining the rate. Kinetic and inhibition studies are currently in conflict as to the likely involvement of a methylated-enzyme intermediate.[8] If two or more sequential steps or parallel pathways (as in meta and para methylation of DHA) contribute to rate limitation, the observed isotope effect will be a weighted average. The highest free-energy activated complex will be weighted most heavily for sequential processes and the lowest free-energy activated complex will be weighted most heavily for parallel processes. The large magnitude of the isotope effect observed here strongly suggests a "tight" SN2 character[3] for all contributing transition states.

The reasonably high precision within each set of enzymatic rates obtained here is due in part to the excellent stability of the COMT preparation and in part to the use of an automated spectrophotometric data-acquisition system. During each kinetic run, this system collects 1000 kinetic points (absorbances at 360 nm, determined by direct observation of the thermostated reaction mixture) each at least 15-fold time-averaged and in the current work 900-fold time-averaged, by direct digitization of the photomultiplier signal of the Cary 16 spectrophotometer. The data are stored in a Hewlett-Packard 2100A computer and fit to the appropriate rate law by a general least-squares procedure.

Acknowledgment. The critical contributions to this work of the University of Kansas Enzyme Laboratory (Dr. S. Osaki, Director) are acknowledged with gratitude.

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(3) Most methyl transfer reactions show $k_H/k_D \sim 0.88$–0.97 (e.g., 14 examples such as hydrolyses of methyl derivatives and Menschutkin reactions in benzene, tabulated by S. Seltzer and A. A. Zavitsas, *Can. J. Chem.*, **45**, 2023 (1967), and the aqueous reactions of methyl iodide with acetate, azide, cyanide, and thiosulfate ions studied by A. V. Willi's group [C. M. Won and A. V. Willi, *J. Phys. Chem.*, **76**, 427 (1972)]). A few methyl transfers give normal isotope effects ($k_H/k_D > 1$) but these probably have unusually "loose" transition states (cf. J. Bron, *Can J. Chem.*, **52**, 903 (1974)). The closest available isotope-effect model for the COMT reaction is the reaction of $(CH_3)_3S^+$ and $(CD_3)_3S^+$ with phenoxide ion ($k_H/k_D = 1.21$, 76°), ethoxide ion ($k_H/k_D = 1.07$, 76°), and thiophenoxide ion ($k_H/k_D = 0.91$, 59°) in water (C.-Y Wu and R. E. Robertson, *Chem. Ind. (London)*, 1803 (1964)). The isotope-effect contribution from deuteration of the leaving group is probably small although this is not certain. Presumably the thiophenoxide ion forms the "tightest" transition state, and the magnitude of the enzymic effect also suggests a reasonably "tight" disposition of entering and leaving groups. Although these organic reactions were largely examined at higher temperatures than the enzymic one, the temperature corrections involved are small. For example, the largest expected temperature dependence would convert an effect of 0.85 at 25° to 0.87 at 100°.
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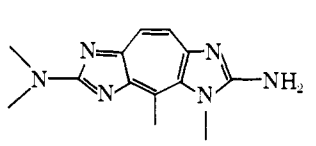
The Synthesis of Zoanthoxanthins

Sir:

Zoanthoxanthins[1-5] are highly fluorescent metabolites of colonial anthozoans, marine animals belonging to the order of Zoanthidae. The pigments thus far identified belong to either the parazoanthoxanthin(1,3,5,7-tetrazacyclopent[*f*]azulene) or the pseudozoanthoxanthin(1,3,7,9-tetrazacyclo-

pent[*e*]azulene) group. Within the two series the metabolites differ only in the number and position of *N*-methyl groups. Zoanthoxanthin[1,2] (**1**) and paragracine[6,7] (**2**) have received most attention, and their structures were established mainly by x-ray crystallography.



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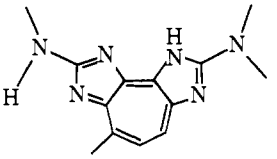
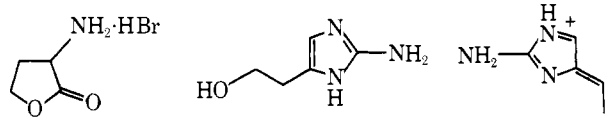


2

Experimental evidence relating to the biosynthesis of the zoanthoxanthins is lacking but the Italian workers, who deserve credit for their pioneering studies on this novel class of natural products, first postulated the intermediacy of two arginine derived C_5N_3 units.[4] We describe a short laboratory synthesis of the two simplest zoanthoxanthins that embodies this principle.

Reduction of the commercially available lactone **3** in aqueous ethanol (45:20) with 2.5% sodium amalgam[8] (12 equiv of sodium) at pH 2.5–3.5 (3–7°; 2 h), followed by addition of 4–5 equiv of cyanamide[9] at pH 4.5 (60–70°; 2 h) and exposure of the crude product to 15% aqueous hydrochloric acid (20°; 30 min) gave 2-amino-4(5)-hydroxyethylimidazole (**4**) conveniently purified as the crystalline picrate, mp 177–179° (C_2H_5OH). A standard procedure[10] was used to reconvert the picrate to the pure, yet oily hydrochloride (64% overall yield from **3**): NMR (D_2O; sodium 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate) δ 6.55 (s, 1), 3.80 (t, 2, J = 7 Hz), 2.75 (t, 2, J = 7 Hz); uv(max) (C_2H_5OH) 216 nm (ϵ 8150); *m/e* 127 (M^+ of free base), 109, 97, 96. Conversion of the imidazole **4** to the two zoanthoxanthins **7** and **8** was effected simply by heating a 10% solution of the hydrochloride in concentrated sulfuric acid (90–95°; 17 h). The reaction mixture was diluted with water and basified to pH 12 with barium hydroxide. Filtration and concentration of the solution afforded crude zoanthoxanthins which were purified by thin layer chromatography (silica gel, $CHCl_3$–CH_3OH–concentrated NH_4OH (80:20:3). The more polar isomer (R_f 1.8) (15% yield) (mp >310°; NMR (CF_3COOH, Me_4Si) δ 3.30 (s, 3), 8.94 (s, 2); uv(max) (CH_3OH) 295 nm (ϵ 30 400), 403 (10 600); uv(max) (CH_3OH; HCl) 286 nm (ϵ 33 200), 381 (9450); *m/e* found 214.09556 calcd for $C_{10}H_{10}N_6$ 214.09669[11] was identical with parazoanthoxanthin A[4] (**7**) previously isolated from *Parazoanthus axinellae*. The less polar isomer (R_f 2.4) (8% yield) (mp >310°, NMR (CF_3COOH, Me_4Si) δ 3.13 (s, 3), 8.61 (AB quartet, J = 11 Hz); uv(max) (CH_3OH) 250 nm (ϵ 8600), 297 (24 000), 360 (4000), 400 (6700); uv(max) (CH_3OH; HCl) 237 nm (ϵ 6650), 286 (27 600), ~340 broad, 394 (8500); *m/e* found 214.09631) is a new compound. According to its uv and NMR spectra it belongs to the pseudo series and we propose the name pseudozoanthoxanthin A (**8**).



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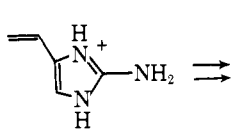

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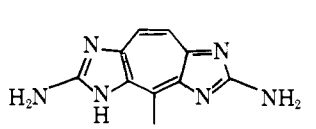

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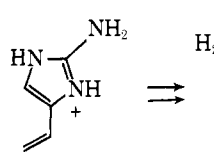

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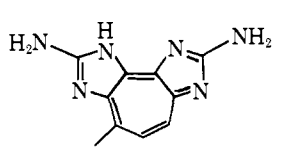

8

Sulfuric acid serves both as an oxidant and an acid catalyst in this oxidative dimerization. It may not be the agent of choice but other acids and oxidizing agents remain to be tested. 2-Amino-4(5)-vinylimidazole (**6**) undoubtedly is involved in the transformation and [6 + 4] cycloadditions[12] of **5** and **6** in the manner indicated account for the eventual formation of isomers **7** and **8**. Methods for alkylation of both ring and side chain nitrogen atoms have been devised[3] and homologous zoanthoxanthins can thus be synthesized from the prototypes **7** and **8**.

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The Structure of the Heteropolytungstate $(NH_4)_{17}Na[NaW_{21}Sb_9O_{86}]\cdot 14H_2O$. An Inorganic Cryptate

Sir:

Although the chemistry and structure of hetero and isopolyanions have been studied for a long time,[1] new properties of known compounds and ions possessing new structural units are still being found. Thus, Jasmin and co-workers have recently shown that silico-12-tungstates have in vitro antiviral properties.[2] This study prompted a systematic screening of similar compounds and a recently prepared antimoniotungstate[3] was found whose in vitro activity was much higher than that of the silico-12-tungstates. This compound is active against a broad spectrum of viral strains and, in vivo, against Friend leukemia virus.[4] Its toxicity is extremely low.

We undertook the x-ray structure analysis of this compound in order to firmly establish its geometry and composition.

Crystals of the title compound are hexagonal with $a = b$ = 17.791 (3) Å and c = 22.709 (5) Å. The observed density is 3.72 g/cm^3; the calculated value is 3.65 g/cm^3 for Z = 2, M = 6396.6. Possible space groups are $P\bar{6}2c$ and $P6_3/mmc$. Data