important monocrotaline system and analogous structures of other ring sizes. Only one example (integerrimine; Narasaka et al. ~30% in the activation-cyclization-isolation sequence)[13] of a 12-membered pyrrolizidine dilactone synthesis is reported to date. We will describe additional studies in these areas shortly.

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Registry No. (±)-**1**, 89772-18-9; (±)-**1** (methoxymethyl ether), 89772-21-4; (±)-**2**, 89772-17-8; **8**, 89710-44-1; (±)-**9**, 89710-45-2; **7**, 89710-46-3; (±)-**11**, 89710-47-4; (±)-**12** (isomer 1), 89710-48-5; (±)-**14**, 89725-98-4; (±)-**15**, 89710-49-6; (±)-**16**, 89772-16-7; (±)-**17**, 89772-19-0; (±)-**18**, 89772-20-3; (±)-**18** (methoxymethyl ether), 89772-22-5; **19**, 41478-07-3; (*E*)-**20**, 19980-31-5; (*Z*)-**20**, 19980-29-1; **21**, 89710-53-2; **22**, 89710-54-3; (±)-**23**, 89710-55-4; $(CH_3)_2C(OH)CN(Br)_2$, 24482-83-5; $(CH_3)_2AlOCH_2CH_2Si(CH_3)_3$, 89710-50-9; fulvinic acid, 89710-51-0; fulvinic anhydride methoxymethyl ether, 89710-52-1; crispatic acid, 41478-08-4; methyl ketene, 6004-44-0; (±)-**12** (isomer 2), 89772-23-6.

Supplementary Material Available: NMR data for synthetic crispatine and fulvine isomers and key precursors (2 pages). Ordering information is given on any current masthead page.

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Stereoselectivity of Enzymatic Transfer of Hydrogen from Nicotinamide Coenzymes: A Stereochemical Imperative?

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Pyridine coenzyme-dependent dehydrogenases can be placed into two discrete groups based on stereoselectivity of reduction, namely those that transfer the *pro-R* hydrogen and those that transfer the *pro-S* hydrogen of the reduced nicotinamide ring. Many attempts have been made to provide a rational explanation for the stereochemical preferences of dehydrogenases.[1] Unfortunately, all the approaches thus far have suffered either from significant exceptions or, in the case of the empirical correlations, from having no demonstrable underlying mechanistic principle to explain the pattern.

Recently Benner and co-workers have proposed a correlation between the stereospecificity of dehydrogenases that reduce unconjugated carbonyls and the value of the equilibrium constant for the catalyzed reactions.[2-4] This proposal has two attractive features. First, they report an apparently strong correlation; that is, reactions with a value of $-\log E_{eq} > 11.2$ are *pro-R* specific and those with a value of $-\log E_{eq} < 11.2$ are *pro-S* specific. Second, a mechanistic rational is provided to explain their correlation. They argue that in order for a dehydrogenase to proceed with optimum efficiency, the redox potentials of the substrate and coenzyme must be matched. This is to be achieved by having the *pro-R*-specific dehydrogenases bind the coenzyme with the dihydronicotinamide ring anti and the *pro-S*-specific enzymes binding the ring syn. The proposed differences in microscopic redox potential engendered by these conformations, especially in regard to puckering of the ring, would then result in the desired matching of potentials.

The criteria for selecting enzymes to consider for their correlation are as follows:[3] "the enzyme must catalyze the interconversion of "simple" unconjugated carbonyls[5] with their corresponding alcohols, its natural substrate must be well-defined, and the equilibrium constant for the overall reaction of that substrate must lie at least 1 log unit away from the position of the "break" between *pro-R*- and *pro-S*-specific enzymes. *Any enzyme conforming to these criteria and not fitting the correlation we consider a violation of the correlation, necessarily prompting the reevaluation of the correlation and the theories supporting it.*" (Italics added.)

This communication reviews the published data for three dehydrogenases that meet the required criteria outlined by Benner and co-workers but do not fit their correlation. A reevaluation of the underlying theories is therefore justified and is also presented.

20α-Hydroxysteroid Dehydrogenase (1.1.1.149). In 1960, Wiest and Wilcox[6] isolated from rat ovaries an NADP-requiring 20α-hydroxysteriod dehydrogenase that transferred the *pro-R* hydrogen.[7] This enzyme conducts the crucial conversion of progesterone to 20α-hydroxy-4-pregnen-3-one based upon the hormone dependence of the enzyme levels.[8] The enzyme has a narrow range of substrate specificity and the value of $-\log E_{eq}$ for this reaction is 6.8.[6] A 20α-hydroxysteroid dehydrogenase has also been isolated form porcine testes[9] and it too conducts transfer of the *pro-R* hydrogen.[10]

3α-Hydroxysteroid Dehydrogenase (1.1.1.50). In 1967, an NADP-requiring 3α-hydroxysteroid dehydrogenase was isolated from rat liver and shown to have *pro-R* specificity.[11-13] The enzyme is specific for reduction of the 3-keto group of steroids. No equilibrium constant was measured for the reaction catalyzed by this particular enzyme; however, the reaction is the same (except for the sterochemistry of the hydrogen transfer) as that of 3α-hydroxysteroid dehydrogenase from *P. testosteroni* (1.1.1.50) where $-\log E_{eq} = 8.0$.[3] In summary, both the 3α- and 20α-hydroxysteroid dehydrogenases, which are *pro-R* specific, have equilibrium constants that are well within the "*pro-S* range" as defined by Benner and co-workers.

21-Hydroxysteroid Dehydrogenase (1.1.1.150). In 1963, Monder and White[14] purified a bovine adrenal 21-hydroxysteroid dehydrogenase that utilizes NAD and conducts a stereospecific transfer of the *pro-S* hydrogen.[15] The restricted specificity suggests that the 21-hydroxysteroid dehydrogenase plays a role in corticosteroid metabolism.[16] The value of $-\log E_{eq}$ for the reaction catalyzed by this *pro-S* enzyme is between 13.5 and 14,[14] placing it solidly within the "*pro-R* range" as defined by Benner and co-workers.

The lack of conformity of these enzymes to the correlation presented by Benner and co-workers calls for an objective look at the enzymes that *do* fit the pattern. First it should be noted

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(4) The initial impression made by Benner and co-workers in ref 3 was one of offering a solution to the puzzle of the stereochemical choices made by dehydrogenases. In reality the correlation is for only one, albeit important, category of dehydrogenases, the alcohol dehydrogenases. The category has been further narrowed by exclusion of polyol dehydrogenases and enzymes that reduce aldehydes or ketones conjugated to olefins.

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that of the 24 enzymes listed in their paper, 12 are within ±1 log of the break point; thus, estimating their specificity should be difficult. It is curious that for the reactions with values of -log E_{eq} near 11.2, where according to their hypothesis the energy difference between binding modes should be minimal, there is still complete conservation of stereochemistry for a given reaction; e.g., all glycerol-3-phosphate dehydrogenases (-log E_{eq} = 11.1) are *pro-S* specific and all glycerol-2-dehydrogenases (-log E_{eq} = 11.3) are *pro-R* specific.

The seven *pro-S*-specific enzymes with the smallest values of -log E_{eq} can be grouped into two broad categories on the basis of their substrate specificity: steroid dehydrogenases and 3-hydroxycarboxylic acid dehydrogenases. Further limitations arise because the prokaryotic steroid dehydrogenases from *P. testosteroni*, β-hydroxysteroid dehydrogenase (1.1.1.51) and 3α-hydroxysteroid dehydrogenase (1.1.1.50),[17] are adaptive enzymes. They are derived from a prokaryotic source that has no intrinsic role for sterols other than in this case the fortuitous battery of degradative enzymes necessary to grow on testosterone as a sole carbon source. The bacterial β-hydroxysteroid dehydrogenase is also quite nonselective, oxidizing 3β-, 17β-, and even 16β-hydroxy sterols. Therefore the "natural substrates" for these two enzymes must be considered as undefined. Exclusion of the two bacterial enzymes and inclusion of the two *pro-R* α-hydroxysteroid dehydrogenases dispels the correlation for reactions with -log E_{eq} < 11.2.

Finally, the range of reactions considered for enzymes with -log E_{eq} >12 (*pro-R*) is limited, with the general category of dehydrogenases utilizing α-hydroxy carboxylic acids predominating. All these observations suggest that within the various groups of enzymes the sterochemical preference may derive from some aspect of their evolutionary origin. Such relationships, if true, would greatly diminish the significance of any apparent mechanism-based correlation.

Without a unique correlation the mechanistic proposals of Benner and co-workers become no longer pertinent. Note that the essential features of their proposal regarding the mechanistic implication of distorting the dihydronicotinamide ring have been discussed previously[18] as have the relationship between stereochemistry of hydrogen transfer and torsional conformation around the nicotinamide–ribosidic bond.[19] Finally, the activated intermediate they propose leads, in the extreme, to a valence tautomer with a planar imminium ion at the anomeric position, a form that can be viewed as deactivating the dihydronicotinamide ring for hydrogen transfer.

In conclusion, discerning ordered patterns from chaos is a noble goal for scientists. The dehydrogenases, however, remain as intractable as ever and have yet to yield the secrets of their underlying principles.

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Registry No. NADH, 58-68-4; NADPH, 53-57-6; EC 1.1.1.149, 9040-08-8; EC 1.1.1.50, 9028-56-2; EC 1.1.1.150, 37250-75-2; dehydrogenase, 9035-82-9.

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(18) The potential influence of puckering on the stereospecificity of dehydrogenases was first proposed by Levy and Vennesland (Levy, H. R.; Vennesland, B. *J. Biol. Chem.* **1957**, *228*, 85–96) and more recently the results of NMR experiments have been discussed in terms of the specific activation of the axial N4 proton in a puckered conformation (Oppenheimer, N. J.; Arnold, L. J., Jr.; Kaplan, N. O. *Biochemistry* **1978**, *17*, 2613–2619).

(19) Rossman and co-workers were the first to discuss seriously the effects of syn vs. anti binding on the stereospecificity of dehydrogenases (Rossman, M. G.; Liljas, A.; Branden, C. I.; Banaszak, L. J. *in* "The Enzymes", 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1975: Vol. XI, pp 61–102. See also ref 1e). Note, however, implicit in the argument that syn yields *pro-S* and anti yields *pro-R* is the assumption that the coenzyme binds first, with the substrate on top. If, instead, the coenzyme were to bind on top of the substrate, then the opposite correlation would hold. Glutathione reductase represents such an example (Pai, E. F.; Schulz, G. E., *J. Biol. Chem.* **1983**, *258*, 1752–1757). It is a *pro-S*-specific enzyme that binds the dihydronicotinamide ring anti, on top of the flavin ring to be reduced.

Mechanism of Methylthiolation of the Fungicide Pentachloronitrobenzene by *Tetrahymena thermophila*

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Pentachloronitrobenzene (**1**) is a fungicide in widespread use, and residues of **1** and its transformation products have been found in a variety of foodstuffs.[1] Major metabolites of **1** are pentachloro(methylthio)benzene (**2**) and pentachloroaniline.[2] We have been investigating the biotransformation of **1** to **2** in the free living protozoan *Tetrahymena thermophila*[2] and have suggested that the pathway for replacement of a nitro group by a SCH_3 group (i.e., methylthiolation) is as follows, via the thiol pentachlorobenzenethiol (**3**):

1 → *S*-(pentachlorophenyl)glutathione →→ *S*-(pentachlorophenyl)cysteine → **3** → **2**

Methylthiolation has recently been described in several biological systems with a variety of drugs and foreign compounds,[3] and it has been proposed that the SCH_3 group can be derived from methionine or methionine derivatives (R_2–SCH_3) by a pathway involving sulfonium intermediates (path a) or direct transfer of a SCH_3 group (path b),[4] where R_1–X is a foreign compound with an electrophilic center (Scheme I). Pathway c is the suggested route for conversion of **1** to **2** in *T. thermophila*[2] and onion[5] and for certain drugs in rat liver,[6] where an intermediate thiol (R_1–SH) is methylated by *S*-adenosylmethionine (AdoMet).

To distinguish unequivocally between these pathways, we have used dual isotopic labeling with [*methyl*-^{3}H]methionine and [^{35}S]methionine to label the methionine and AdoMet pools of *T. thermophila*. In addition, since this organism converts methionine to cysteine via transsulfuration,[7] cells exposed to [^{35}S]methionine contain [^{35}S]cysteine and [^{35}S]glutathione (GSH); the later thiol is responsible for initiating metabolism of **1** in *T. thermophila*.[2] If cells, radiolabeled to constant specific activity, are exposed to **1** and then **2** is isolated, the ^{3}H/^{35}S ratio of **2** should equal that of the methionine pool if pathway a or b is operative. If pathway c is used exclusively, the ^{3}H/^{35}S ratio of **2** should be equal to the ratio of cellular [^{3}H]AdoMet/cellular [^{35}S]GSH, assuming single pools of these compounds.[8]

T. thermophila was grown in defined medium[9] in the presence of L-[*methyl*-^{3}H]methionine (10.9 mCi/mmol) and L-[^{35}S]-methionine (3.5 mCi/mmol)[10] and transferred 3 times to assure uniform labeling of cellular pools. Labeled cells were incubated with **1**, and the resulting **2** and cellular methionine were isolated by a procedure developed to give pure **2** and methionine, each

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(10) Radiolabeled methionine was obtained from Amersham, Arlington Heights, IL. Cells were transferred 3 times in medium containing [^{3}H, ^{35}S]-methionine to assure uniform labeling of cellular pools.

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