This value of k^H/k^D is much larger than is expected for the secondary deuterium IE of the four exchangeable Ls. If the transition state (TS) has the positive charge equally divided between the two oxygens, $L_2O^{+1/2}\cdots CH_3\cdots OL_2^{+1/2}$, then that secondary IE is given by eq 7, where the ϕs are deuterium fractionation factors[10] relative to L_2O for $CH_3OL_2^+$ and the TS. For

$$(k^H/k^D)_{sec} = (\phi_{MeOL_2^+})^2/(\phi_{TS})^4 \quad (7)$$

this symmetric TS, $(k^H/k^D)_{sec}$ thus should be near 1.00.[11] If some positive charge resided on CH_3, then the lower charge on oxygen would increase ϕ_{TS}, causing $(k^H/k^D)_{sec} < 1.00$. Assuming the TS to be unsymmetric does not change these conclusions.[12]

Therefore a factor near 1.57 must be contributed to k^H/k^D by sources other than the substitution of D for H in the internal structures of reactants and TS. Possible other sources are solvent effects, either static or dynamic,[15] and acid/base catalysis.[16] If the standard partial molal free energies for transferring reactants and TS from H_2O into D_2O (without exchange) are not equal, then a *static* solvent effect, $e^{\Delta\Delta\bar{G}^\circ_{Tr}/RT}$, will be present.[15] If charge transfer is strongly coupled to changes in solvent polarization, then a *dynamic* solvent effect will be present.[17-20] If the mechanism involves acid/base catalysis, then coupling of hydron motion into the reaction coordinate and/or strong hydrogen bonding in the TS can increase k^H/k^D.

It is very unlikely that the static solvent effect could be as large as 1.57 at 140 °C. If this value resulted from a static effect with a purely exponential temperature dependence, $k^H/k^D = (1)e^{\Delta\Delta\bar{H}^\circ_{Tr}/RT}$, then $\Delta\Delta\bar{H}^\circ_{Tr} = 0.37$ kcal mol^{-1} and $k^H/k^D = 1.87$ at 25 °C. However, it almost always is true that $|\Delta\bar{H}^\circ_{Tr}| > |\Delta\bar{G}^\circ_{Tr}|$,[21] suggesting that the preexponential factor in k^H/k^D is < 1, so that at 25 °C, a static solvent effect would lead to $k^H/k^D > 1.87$ and $\Delta\Delta\bar{G}^\circ_{Tr} > 0.37$ kcal mol^{-1}. Since $|\Delta\bar{G}^\circ_{Tr}| < \Delta\bar{H}^\circ_{Tr} < 0.03$ kcal mol^{-1} for transfer of H_2O without exchange,[21] the difference between the $\Delta\bar{G}^\circ_{Tr}$ values for TS and $CH_3OH_2^+$ would need to be >0.34 kcal mol^{-1} in order for the static solvent effect to be this large; tabulated values[21] of $\Delta\bar{G}^\circ_{Tr}$ for univalent cations suggest that this difference should not be nearly so large. Thus it is likely that k^H/k^D contains a significant contribution from a dynamic solvent effect or from acid/base catalysis.

Both a large dynamic solvent effect and acid/base catalysis could result from the mechanism predicted for this methyl transfer to L_2O from L_2O. We have proposed[15,22] that methyl transfers to L_2O from other leaving groups (e.g., halide$^-$, RSO_3^-, thiophene) occur via a "partly coupled" mechanism in which the rate-determining step is a solvation change. Our analysis of why those transfers follow that unusual mechanism requires that the symmetric transfer to L_2O from L_2O must follow a different mechanism with a symmetric TS that is tightly coupled to its solvation. In such a "coupled"[19] or "polarization caging"[17] mechanism, the transferring methyl is carried across from leaving group to nucleophile in a potential well created by the solvent polarization, and a dynamic solvent effect results from the polarization change being slower in D_2O than in H_2O. The strong hydrogen bonding expected to be part of that tight coupling could set the stage for coupling hydron motion into the reaction coordinate and shifting the mechanism to simultaneous acid and base catalysis by L_3O^+ and L_2O.

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(11) The common assumption[10] is that ϕ_{TS} is the bond order weighted geometric mean of the ϕs for reactant and product. Thus for this TS, $\phi_{TS}^4 = [(\phi_{L_2O})^{1/2}(\phi_{MeOL_2^+})^{1/2}]^4 = (\phi_{MeOL_2^+})^2$.
(12) The Principle of Detailed Balance[13] allows an unsymmetric TS, $L_2O^{\delta+}\cdots CH_3\cdots{}^{18}OL_2^{(1-\delta)+}$, for this symmetric reaction if half of the TSs have this structure and half have its complement, $L_2O^{(1-\delta)+}\cdots CH_3\cdots{}^{18}OL_2^{\delta+}$,[14] so that $\phi_{TS}^4 = [(\phi_{L_2O})^{\delta}(\phi_{MeOL_2^+})^{1-\delta}]^2[(\phi_{L_2O})^{1-\delta}(\phi_{MeOL_2^+})^{\delta}]^2 = (\phi_{MeOL_2^+})^2$.
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(16) For this exchange reaction, detailed balance[12-14] allows only certain kinds of catalysis. Simultaneous acid and base catalysis by L_3O^+ and L_2O ($L_2O + L_2O + CH_3OL + LOL_2^+ \rightarrow L_2OL^+ + LOCH_3 + OL_2 + OL_2$) is allowed, as is half of the reaction proceeding via base catalysis by L_2O ($L_2O + L_2O + CH_3OL_2^+ \rightarrow L_2OL^+ + LOCH_3 + OL_2$) plus half via its reverse, acid catalysis by L_3O^+. It is not allowed for all of the reaction to proceed via either acid or base catalysis alone.
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Stereochemistry of the Visual Cycle

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The stereochemical changes which accompany the processing of vitamin A in the eye as part of the visual cycle are unusual and interesting. The enzymatic isomerization of free *all-trans*-retinol (*1*) (vitamin A), probably via an activated intermediate, to 11-*cis*-retinol (*2*) occurs with inversion of stereochemistry at C-15 (Scheme I)[1] and C–O bond cleavage.[2] The retinol dehydrogenases that oxidize *all-trans*-retinol and 11-*cis*-retinol do so with opposite stereochemistries with respect to the methylene hydroxyl group (Scheme I).[1] In both the bovine (pigment epithelium derived) and amphibian visual systems, the *all-trans*-retinol dehydrogenases are *pro*-R specific, and the 11-*cis*-retinol dehydrogenases are *pro*-S specific.[1] This is also true for the cone only visual system of the lizard Anoleis carolensis.[3] This consistently opposite stereochemistry of the dehydrogenases is intriguing and suggests that the isomerase and dehydrogenases operate from the same face of the vitamin A molecule. Note that although a formal inversion of stereochemistry occurs during the isomerization reaction, this result can easily be accounted for by a mechanism in which C–O bond cleavage and reformation occurs from the same face of the enzyme with retention (Scheme II). In this communication the stereospecificities of the dehydrogenases were examined with respect to the nicotinamide cofactors in order to further explore the stereochemistry of these enzymes.

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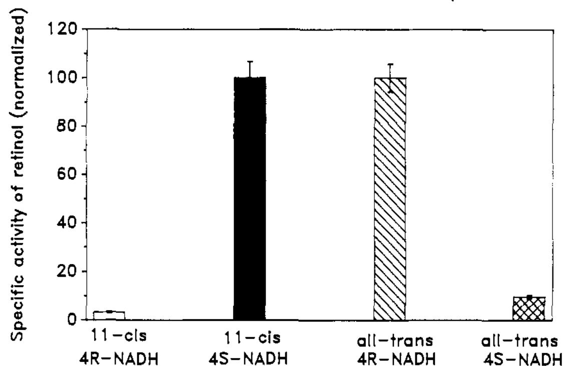


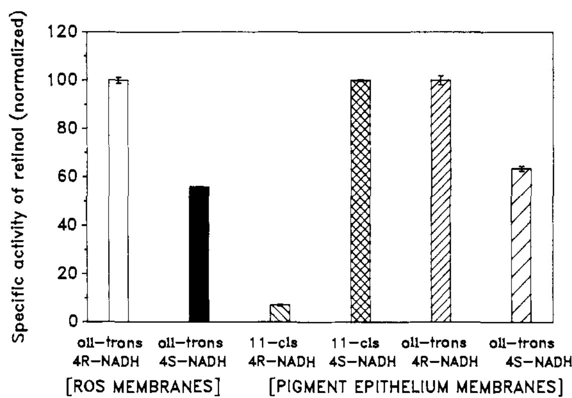


Figure 1. A and B: Reduction of the retinals by the ocular retinol dehydrogenases. The experiments were conducted as described in the text. Since the 4(*S*)- and 4(*R*)-[4-^{3}H]-NADHs had different specific activities, the specific activities of the [^{3}H]-retinols formed were normalized so that the results for each pair of experiments were comparable. ROS refers to rod outer segments and PE to pigment epithelium.

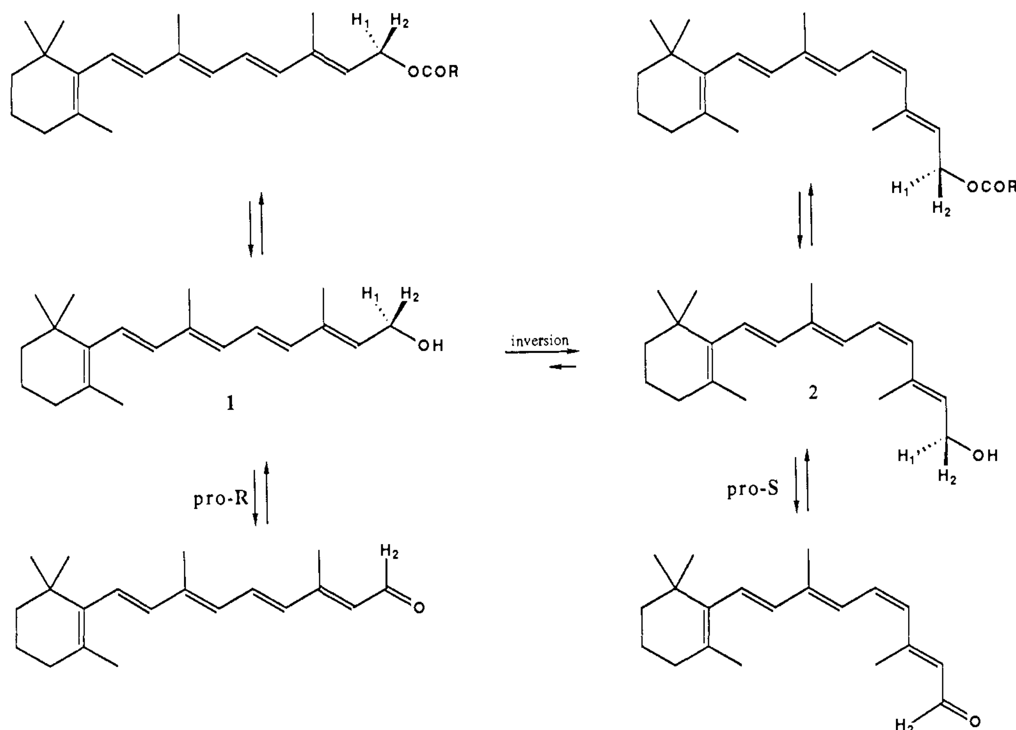
Table I. Aldehyde-Alcohol Equilibrium Constants for 11-*cis*-Retinoids and *all-trans*-Retinoids[b]

retinol/retinal	K_{eq}[a]	$-\log E_{eq}$
11-*cis*-retinoid (n = 4)	$(2.7 \pm 0.1) \times 10^{-9}$	8.6
all-trans-retinoid (n = 4)	$(5.2 \pm 1.0) \times 10^{-9}$	8.3

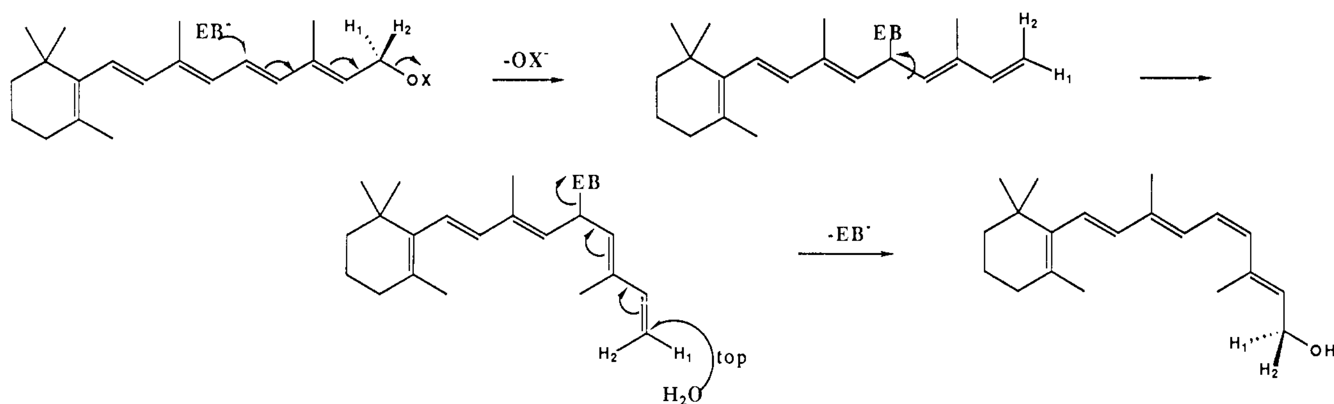
[a] K_{eq} = [aldehyde][NADH][H$^+$]/[alcohol][NAD$^+$]. [b] The incubation mixture contained 5 μM of [^{3}H]-*all-trans*-retinol, 3.3 mM of NAD$^+$, 0.165 mM of NADH, 0.3 mg of horse liver alcohol dehydrogenase, and 0.01% of Tween 80 in 300 μL of 50 mM sodium phosphate buffer (pH 7.2). The incubations were carried out at 37 °C for 30 min. At the end of the incubations, 300 μL of MeOH and 100 μL of 0.1 M NH_2OH in Tris buffer (pH 6.5) were added to convert the retinals to retinyl oximes.[4,5] The radioactive retinols and retinyl oximes were extracted with CH_2Cl_2 and separated by HPLC (7% dioxane in hexane as eluant) and counted on a Berthold LB 506 C online radioactivity counter to obtain the retinal-to-retinol ratios. The K_{eq} was then calculated. A similar protocol was followed for the 11-*cis*-retinoids except that 0.5 mg of alcohol dehydrogenase was used.

The washed and sonicated membrane fractions, consisting of the whole retina/pigment epithelium in the amphibian[4] and these two organs separately in the bovine case,[5] were suspended in 0.5 mL of 0.1 M sodium acetate buffer (pH 5.0). The membrane homogenate was then mixed with 5 μCi of either 4(*S*)- or 4-(*R*)-[^{3}H]-NADH,[6] and the pH of the mixture was adjusted to 5.0 with concentrated acetic acid. To the above mixture was added 5 nCi of [15-^{14}C]-11-*cis*- or *all-trans*-retinal (5 mCi/mmol) and 25 μL of 10% BSA. The incubation was then carried out at 37 °C for 0.5 h. The extraction and analysis of the retinol/retinal isomers were performed as described before.[4,5] A control incubation, which excluded [^{3}H]-NADH but was otherwise identical, was carried out to demonstrate that there was insignificant reduction of [^{14}C]-retinal in the absence of [^{3}H]-NADH. In Figure 1A are shown data for the stereochemistry of the reduction of retinals by membranes from the amphibian retina/pigment epithelium. Within experimental error, the 11-*cis*-retinol dehydrogenase(s) is *pro*-S specific and the *all-trans*-retinol dehydrogenase(s) is *pro*-R specific. That is, they again show opposite stereochemistries. The unavoidable chemical isomerization of the retinoids during the incubation period probably gives rise to the somewhat less than complete stereospecificity observed.

Scheme I



Scheme II. Same Face C–O Cleavage and Reformation Causes Apparent Stereochemical Inversion



Is the same situation found in the bovine case? Here the situation is more complex because there are likely to be multiple *all-trans*-retinol dehydrogenases.[7] However, the situation is somewhat simpler for the 11-*cis*-retinol dehydrogenase, where only one enzyme is expected. No significant 11-*cis*-retinol dehydrogenase activity was detected in the bovine rod outer segment membranes. All of the enzymatic activity was found in the pigment epithelium. This dehydrogenase behaved as in the amphibian case, with the enzyme being *pro*-S specific (Figure 1B). The *all-trans*-retinol dehydrogenases, both from the rod outer segments and from the pigment epithelium, were *pro*-R selective, but substantial processing of the *pro*-S H also occurred. This suggests that the different *all-trans*-retinol dehydrogenases show different stereochemistries with respect to the nicotinamide cofactor. Since it is clear that the stereochemistries for the *all-trans*- and 11-*cis*-retinol dehydrogenases can be opposite, it was of interest to determine the equilibrium constants for the two redox reactions. This measurement was made because a controversial but mechanistically appealing proposal has recently been made which argues that in alcohol dehydrogenases whose natural substrates are known—at least those that are simple and unconjugated—a formal relationship exists between the stereochemistry of the reducing hydrogen of NADH and the redox equilibrium constants of the substrates.[8,9] "Thermodynamically unstable carbonyls are reduced with the *pro*-R hydrogen of NADH, while thermodynamically stable carbonyls are reduced with the *pro*-S hydrogen."[8] It was of interest to determine if this relationship is relevant to the retinals. The redox equilibrium constants were measured for *all-trans*- and 11-*cis*-retinol and they proved to be virtually identical and belong to the thermodynamically stable category (Table I). Therefore, at least for the retinols, it is not apparent what catalytic significance the stereochemistry of reduction might have.

Acknowledgment. This work was supported by United States Public Health Service Grant EY 04096.

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Book Reviews*

Fluorescence and Phosphorescence Spectroscopy: Analytical Chemistry by Open Learning. By David Rendell (Wolverhampton Polytechnic). John Wiley & Sons: New York, 1987. xix + 419 pp. $29.95. ISBN 0-471-91381-2 (paperback).

This book constitutes a thorough introductory treatment of the theory, instrumental and chemical methods, and practical considerations involved in luminescence spectroscopy. It is a self-learning guide and so is not a text book in the traditional sense. The book is divided into five chapters including an introduction followed by more specific sections covering differential practical aspects. The introduction is a general overview of photophysics, which by the way is quite good considering the more simplified approach used. The nature of excited states, solvent interactions, and energy-transfer phenomena are all discussed in enough detail to allow the reader to make predictions as to experimental outcomes. The second chapter addresses the instrumentation required to perform fluorescence and phosphorescence spectroscopy. The analysis of basic spectrofluorimeter designs, the types of sample cells required, etc. are covered quite adequately.

The next two chapters cover all aspects of photoluminescence methods of analysis. The first of these deals wtih quantitative fluorimetry. Discussed here are calibration curves, inner filter effects, quenching effects, photodecomposition, and limits of detection as related to blank luminescence and scattering. The second of these two chapters is the chemical approach to solving quantitative analysis problems. Direct, derivative, and quenching methods are each discussed in turn. Taken as a whole, these two chapters apparently sum up the important aspects of quantitative fluorimetry.

The final chapter is really quite a surprise for an introductory type text. The author has included methods that are not "tried and trued". Addressed are pre- and post-column derivative techniques used for chromatography, use of micellar solutions for fluorescence and phosphorescence, and cyclodextrin systems.

This book is intended to teach basic analytical photoluminescence methods without taking a formal class. As such, there are numerous problems throughout the text that readers can use to tests themselves before reading on. I find this book to be very practical for independent study and will (have) recommend it to both graduate and undergraduate students. Although there are many graphs and data sets that the reader must interpret, thereby gaining a working knowledge without actually using an instrument, there are also four experiments in the last chapter. These experiments are particularly well detailed.

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*Unsigned book reviews are by the Book Review Editor.