0.2 M HCO_2H. Lyophilize the nucleotide fraction and recrystallize from hot water to give 886 mg of colorless crystals: cellulose TLC (BuOH, AcOH, H_2O 5:2:3) R_f 0.23; UV in 0.05 M phosphate buffer, pH 6.8, 257.5 (10100), 281.2 nm (9930); in 0.01 M HCl, max 253.8 (11300), 292.5 nm (9200).

Registry No. I, 36799-17-4; II, 80326-48-3; III, 80326-49-4; IV, 80326-50-7; $V{\cdot}2H_2O$, 80326-51-8.

Supplementary Material Available: A table of observed and calculated structure factors and full refinement parameters for the heavier atoms (13 pages). Ordering information is given on any current masthead page.

Kinetics of Model Threonine Aldolase Reactions‡

Joseph A. Marcello† and Arthur E. Martell*

Contribution from the Department of Chemistry, Texas A&M University, College Station, Texas 77843. *Received February 27, 1981*

Abstract: Kinetics of dealdolation of Schiff bases of pyridoxal and β-hydroxy amino acids and of their Zn(II), Al(III), and Ga(III) chelates are reported. Disappearance of reactants and appearance of products of the metal-free model systems, and their complexes consisting of 1:1:1 and 2:2:1 molar ratios of pyridoxal:amino acid:metal, were followed by proton NMR. The rates of dealdolation were determined for threonine, β-hydroxyvaline, and β-hydroxyleucine and the corresponding first-order rate constants are reported. Under comparable conditions the rates of Al(III) and Ga(III) chelate catalysis were found to be 2–6 times higher than those of the metal-free systems and of the corresponding Zn(II) Schiff base chelates. The rates of dealdolation of the Zn(II) chelates were approximately equivalent to the rates observed for the proton-catalyzed (metal free) Schiff bases. Catalysis in the 1:1 chelates was found to be stronger than the catalysis observed in the Schiff base chelates having a 2:1 ligand–metal ratio. The electron-donating effect of the methyl groups of β-hydroxyvaline is evident in that dealdolation is observed for the metal-free systems containing β-hydroxyvaline, but not for the analogous L-threonine systems. A reaction mechanism for the dealdolation reaction in these model systems is proposed. The rate constants obtained for the metal-free β-hydroxyvaline–pyridoxal Schiff base were resolved into the specific rate constants for the individual solution components, and the variations obtained for the specific rate constants are interpreted in terms of the proposed reaction mechanism.

Threonine is converted to glycine and acetaldehyde, and serine is converted to glycine and formaldehyde, respectively, in pyridoxal-activated enzyme systems.[1-3] The reaction has been shown to take place with pyridoxal and metal ions albeit at slower rates.[4-8] This reaction also occurs with β-hydroxy amino acids that do not have an α-hydrogen atom.[9]

Two mechanisms have been proposed[9,10] for these model system reactions. The Snell mechanism[9] suggests that the reaction proceeds through direct α–β carbon–carbon cleavage of the reactive Schiff base chelate. Although it was shown that α-methylserine reacts to give α-alanine and formaldehyde under conditions similar to those under which serine and threonine react to give formaldehyde and acetaldehyde, respectively, it has not been demonstrated that the reaction does not proceed at least in part through an α-deprotonated intermediate (in the absence of an α substituent), as has been suggested by Braunstein.[10] In the absence of quantitative kinetic studies, it has not been possible to propose with confidence a reaction mechanism for these model reactions.

This paper reports the first detailed kinetic measurements of the pyridoxal and pyridoxal–metal-ion-catalyzed dealdolation of threonine, β-hydroxyleucine, and β-hydroxyvaline. The rate constants of these model system reactions have now been determined and a probable reaction mechanism is proposed.

Experimental Section

Materials. Pyridoxal hydrochloride was obtained from Mann Laboratories as Mann Analyzed grade and was used without further purification. The following amino acids were obtained from the sources indicated: DL-threonine and DL-serine from Sigma Chemical Co., α-methylserine from Calibochem; L-threonine from Mann Laboratories; β-hydroxyleucine from United States Biochemical; and β-hydroxyvaline from Calbiochem and Dr. T. Wilkinson, Department of Chemistry, Texas A&M University. All of these amino acids were of sufficient quality to use without further purification.

The deuterium oxide used as a solvent for this study was obtained from Aldrich Chemical Co. and was specified as 99.8% deuterium. The aluminum(III) solutions were prepared by dissolving hydrated $Al_2(SO_4)_3$ in D_2O and evaporating to dryness. This procedure was repeated several times to remove residual H_2O. The standard Al(III) solutions were prepared from the deuterated material by dilution to the appropriate concentration with D_2O. The standard Zn(II) solutions were prepared from hydrated $Zn(NO_3)_2$ by a procedure similar to that employed for the Al(III) solutions. The Al(III) and Zn(II) solutions were standardized by conventional chelatometric titrations.[11] The standard Ga(III) solution was prepared by dissolving gallium metal in DCl (20%) and diluting to the appropriate volume. The ionic strengths of the samples used in the measurements were maintained at unity with reagent grade KCl. All of the solutions were initially 0.1000 M in both pyridoxal hydrochloride and amino acid. In the 1:1:1 systems (pyridoxal–amino acid–metal ion) the concentration of the metal ion was 0.1000 M and in the 2:2:1 systems it was 0.0500 M.

Procedures. The NMR spectra were obtained with a Varian HA-100 nuclear magnetic resonance spectrophotometer and were measured at the ambient probe temperature (30 ± 2 °C). The chemical shifts are given

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in Hz relative to an internal capillary of hexamethyldisiloxane (HMDS), the proton resonance of which was used as an internal locking signal. In this paper, pD is the negative logarithm of the deuterium ion concentration. The pD values were obtained by adding 0.40 to the apparent pH,[12] which was measured with a Corning Model 101 digital pH meter equipped with a Beckman miniature combination electrode. The pD was adjusted with NaOD solution in D_2O. The potentiometric apparatus was calibrated with standard buffer solutions through the use of appropriate activity coefficients, and by direct titration of standard HCl solution with standard NaOH solution, so that the pH meter recorded hydrogen ion concentrations directly. The concentrations of individual species in solution were obtained by integration of the resonances pertaining to those species.

Results and Discussion

NMR Spectra of the Schiff Bases. Serine Schiff Base. The NMR spectra of pyridoxal–DL-serine and of pyridoxal–DL-serine–Al(III) Schiff base complexes were determined, and the observed resonances were assigned. The metal-free pyridoxal–DL-serine Schiff base species were studied through the pD range of 4–11. Although a Schiff base concentration of over 70% was attained at pD's above 9.0, no detectable formation of the dealdolation product, formaldehyde, was found under the reaction conditions employed, even though these spectra were followed for several days.

The 2:2:1 (pyridoxal–serine–Al(III)) Schiff base chelate systems were studied in the pD range 9.0–11.0. However, as in the case of the metal-free system, measurable amounts of formaldehyde were not detected by NMR. Even after several days, resonances corresponding to formaldehyde were not observed. However, when the NMR sample tubes were uncapped, an odor of formaldehyde was detected, indicating that a small amount of the product had formed.

α-Methylserine Schiff Base. The NMR spectra of pyridoxal–DL-α-methylserine and of pyridoxal–DL-α-methylserine–Al(III) Schiff base complexes were determined and the resonances assigned. The NMR spectra taken over a pD range of 3.5–10.0 in the case of the metal-free system and between pD 9.0 and 11.0 in the case of the Al(III) 2:2:1 system did not show resonances corresponding to the dealdolation product, formaldehyde.

Threonine Schiff Base. The NMR spectra of the pyridoxal–L-threonine and the pyridocal–L-threonine–metal ion Schiff base complexes were determined, and the observed resonances were assigned. L-Threonine was used in order to simplify the spectra of the bis complex of the Schiff base, since it was shown[13,14] that the 2-methylpyridine protons give rise to four resonances when the bis complex of the Schiff base of a racemic mixture is used, whereas the pure L or D isomer of threonine gives rise to only two resonances. Since the resonance of the methyl group of acetaldehyde, which is one of the reaction products, is adjacent to those pyridoxal resonances, the use of the racemic mixture of threonine would unnecessarily complicate the spectra and make if difficult to make quantitative measurements of the acetaldehyde formed in the reaction mixture.

The metal-free pyridoxal–L-threonine Schiff base species were studied through the pD range 3–11. Although a Schiff base concentration of over 75% was attained at pD's above 9.0, no detectable formation of the dealdolation product, acetaldehyde, was found under the reaction conditions employed.

The NMR spectra of both the 1:1:1 (pyridoxal–threonine–Zn(II)) and 2:2:1 Schiff base chelate systems were studied. Although substantial amounts of Schiff base were detected at pD 6.00 and above for both the 1:1:1 and 2:2:1 systems, measureable amounts of acetaldehyde were not detected by NMR. Even after several days, resonances corresponding to acetaldehyde were not observed. However, when the NMR sample tubes were uncapped, a slight odor of acetaldehyde was detected, indicating that a small amount of the product had formed.

In the 1:1:1 system (pyridoxal–threonine–Al(III)), Schiff base formation was seen at pD values as low as 3.20. Although these samples were made up with an initial concentration of 0.10 M for all substituents the presence of resonances corresponding to the 2:2:1 complexes was detected at pD values of 4.50 and higher. Above pD 5.50, there were no longer any resonances corresponding to the 1:1:1 complex. Although the spectra in the region between pD 3.20 and 5.50 were observed for several days, no detectable amount of acetaldehyde was observed. Since it was not possible to obtain 1:1:1 complexes above pD 5.50 under the reaction conditions employed, the 2:2:1 complex was used for further measurements.

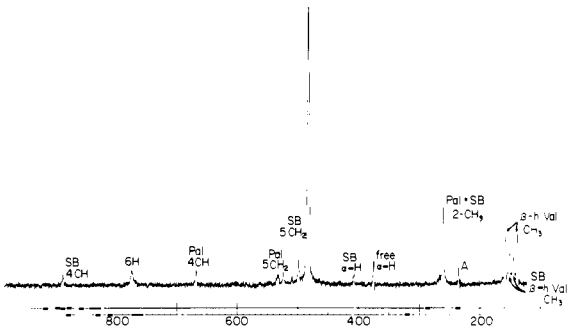

Figure 1. The 100-MHz proton NMR spectrum of pyridoxal–β-hydroxyvaline taken 15 min after mixing at pD 7.76; frequencies are in Hz relative to HMDS, A = acetone, β-Val = β-hydroxyvaline.

The bis Al(III) complex of the threonine Schiff base was formed at pD values as low as 4.00 and complete formation of the Schiff base was detected at pD 8.58. However, in the range of pD 6.90–8.40 the turbidity of the solutions caused considerable broadening of the NMR resonances. Above this range of pD the solutions were again clear and suitable spectra were obtained. As the alkalinity of the solution was increased, the formation of acetaldehyde was detected by NMR at pD 9.12. The rate of the dealdolation reaction was determined over only a narrow pD range because of the tendency of the acetaldehyde to undergo aldol condensation under the reaction conditions employed.

β-Hydroxyvaline Schiff Base. β-Hydroxyvaline was selected as a convenient substitute for threonine because its methyl groups provide an effective NMR probe for the detection of chemical reactions of the amino acid, and because the dealdolation product, acetone, is relatively unreactive in the reaction mixture and thus quantitative measurements may be obtained.

Below pD 6.00 the NMR spectra for the 1:1 (pyridoxal–β-hydroxyvaline) system exhibited resonances entirely attributable to free pyridoxal and β-hydroxyvaline. As the pD was increased, resonances resulting from the formation of the Schiff base were observed. Figure 1 taken 15 min after mixing at pD 7.76 shows the resonances of both the Schiff base and free forms of pyridoxal and β-hydroxyvaline as well as a single sharp resonance due to the formation of acetone. The acetone resonance at 235 Hz with respect to HMDS remained sharp throughout the reaction and the kinetics of dealdolation of β-hydroxyvaline were followed to completion.

In this metal-free system the maximum Schiff base concentration occurs at about pD 9.25. Although the Schiff base concentration never exceeds 66% of the total pyridoxal–amino acid concentration, rapid formation of acetone is observed. Figure 2 shows the percent of Schiff base in solution as a function of pD.

Below pD 4.0 the NMR spectra of 1:1:1 (pyridoxal–β-hydroxyvaline–Zn(II)) mixtures showed no detectable formation of the Schiff base chelate and thus as expected no dealdolation reaction was observed, even after a 1-week period. At pD values between 4.0 and 6.8, formation of acetone was detected. In this range the spectra of the metal chelate system gave clear evidence for direct α–β carbon–carbon cleavage, since the intensity of the resonance associated with the α proton does not decrease as the formation of acetone occurs. Had the reaction taken place through an α-deprotonated intermediate[10] or had there been an appreciable amount of α-proton exchange, some D_2–glycine would have formed simultaneously with the production of acetone during the reaction. This would have resulted in the loss of intensity of the resonance

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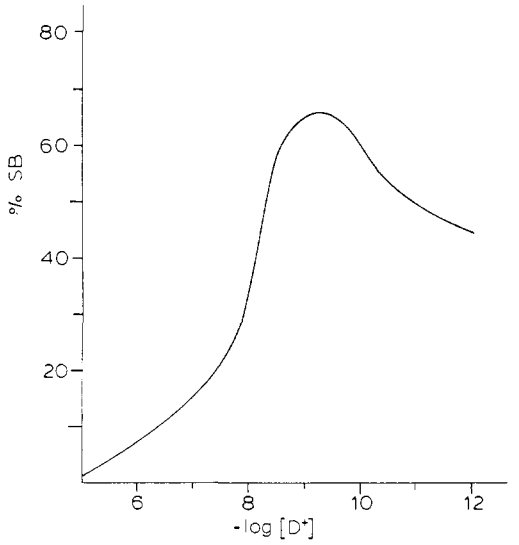

Figure 2. A plot of the percent Schiff base vs. pD for an equimolar solution of pyridoxal and β-hydroxyvaline.

of the α-proton of the Schiff base. Since this did not occur it can be assumed that in this pD range, the reaction involves the formation of the Schiff base followed by the cleavage of the α,β carbon–carbon bond leading to the formation of acetone. There is broadening of the α-proton resonance of the Schiff base during the reaction. When glycine, pyridoxal, and Zn(II) were added to a solution of the β-hydroxyvaline Schiff base, there was no significant chemical shift of the α-proton resonance. Thus one would not expect to be able to monitor the appearance of the HD–glycine Schiff base as a reaction intermediate under the conditions employed.

At pD 6.8 and above for the 1:1:1 Zn(II) Schiff base chelate system, the spectra exhibited resonances associated with a 2:1 Schiff base–metal complex system. At pD 7.3 and above there were no resonances corresponding to the 6-H, 4-CH, 5-CH_2, and 2-CH_3 protons of free pyridoxal, indicating complete Schiff base formation. The only α-proton resonance detectable was that of the Schiff base α proton. This absence of free pyridoxal and amino acid resonances indicates a rapid preequilibrium in the presence of Zn(II), so that above pD 7.3 there is complete formation of Schiff base within a few minutes of mixing. As anticipated, the 2:2:1 system exhibits several resonances assignable to the 2-CH_3 of the Schiff base because of the presence of isomeric forms of the Schiff base chelates.

When the metal ion involved was Al(III), the resonances associated with the 2:2:1 complex were detectable at pD values as low as 4.0, even when the reactants were mixed in 1:1 molar ratios. Thus since it was not possible to form the 1:1:1 Schiff base metal chelate in the absence of the bis Schiff base chelate, only the 2:2:1 system was studied with the Al(III) ion. Between pD values of 6.5 and 8.5 the various samples that were prepared were turbid and it was not possible to obtain quantitative data in this range because of the broadening of all the NMR resonances. However, an overall increase in the Schiff base concentration with time and with increasing pD was observed. In general there also appeared to be an increase in the rate of acetone formation as the pD was increased.

At pD 8.69 and above the various NMR sample solutions were clear. Under these conditions there was a rapid preequilibrium in which the Schiff base intermediate is completely formed within a few minutes of mixing. This was confirmed by the fact that none of the NMR spectra for the 2:2:1 systems at high pD have resonances corresponding to the free pyridoxal or β-hydroxyvaline. The formation of acetone is rapid at these high pD values. Figure 3 shows the variation of the acetone resonance as a function of time.

In the spectra obtained above pD 8.6 the α-proton resonance decreased with time, but the rate of decrease of this resonance was slower than the observed rate of increase in intensity of the

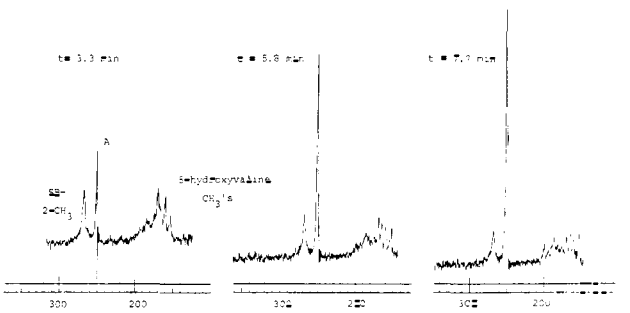

Figure 3. The 100-MHz NMR spectra of the methyl region of a solution of the 2:2:1 (pyridoxal–β-hydroxyvaline–Al(III)) Schiff base chelate system at pD 9.45, showing the growth of the acetone resonance, A, as a function of time. Frequencies are in Hz relative to HMDS.

methyl resonance of acetone. The decrease in the intensity of the α-proton resonance was proportional to the loss of intensity of the Schiff base 4-CH proton, which indicates that the loss of the α-proton resonance is due to proton exchange which may or may not be accompanied by a net transamination reaction. This type of reaction is known to take place at high pD.[15] Since the exchange at the α-proton is slower than the dealdolation reaction, it cannot be a part of the main reaction pathway.

The kinetics of the 1:1:1 (pyridoxal–β-hydroxyvaline–Ga(III)) system was studied by NMR in the range of pD 1.90–2.80. Although the concentration of Schiff base did not exceed 35% under these conditions, the formation of acetone was rapid. At a pD of 3.00 and above, the solutions of the 1:1:1 system became turbid and it was not possible to obtain quantitative data.

The 2:2:1 system with Ga(III) was studied in the pD range 5.80–8.59. In this range there were no resonances corresponding to free pyridoxal or to the free amino acid, thus indicating rapid preequilibrium in the presence of Ga(III) ion and complete Schiff base formation before the first spectrum was taken. The initial spectrum at pD 8.79, which was measured less than 4 min after mixing, indicated that the concentration of acetone was greater than 50%. It was not possible to obtain quantitative kinetic data above pD 8.79 in the presence of Ga(III) ion because in the time required to mix the sample and to obtain the initial spectrum the reaction had proceeded to well over 50% completion.

β-Hydroxyleucine Schiff Base. The NMR spectra of solutions containing pyridoxal and β-hydroxyleucine and of solutions containing pyridoxal, β-hydroxyleucine, and Zn(II) ion were measured and the resonances were assigned. The metal-free system was studied in the range of pD 4.5–10.5. Below a pD of about 6.0 there were no resonances assignable to the Schiff base species. At pD 6.29 and above resonances assignable to the various Schiff base protons were detected. The maximum Schiff base concentration occurs at a pD of about 9.25 at which point the Schiff base is about 68% of the total original pyridoxal–β-hydroxyleucine concentration. At pD 9.48 and above there is a loss in intensity of both the α-proton resonance and of the 4-CH resonance of the Schiff base, indicating that racemization and perhaps transamination have occurred. After several hours a new resonance was observed at 906 Hz, which was assigned to the dealdolation product, isobutyraldehyde. The NMR taken 12 h after mixing at pD 10.02 indicated that isobutyraldehyde had formed to the extent of about 10% of the concentration of the amino acid employed. A spectrum of the same sample taken 4 days later indicated that the intensity of the aldehyde peak had actually decreased in intensity. This is probably due to aldol condensation of the product at this high pD value.

Because of the apparent tendency of isobutyraldehyde to undergo aldol condensation at high pD values, the reaction was studied in the presence of Zn(II) ion at an intermediate pD range. The 100-MHz spectra shown in Figure 4 indicates the formation of isobutyraldehyde and HD–glycine in the presence of Zn(II) ion. In this system both a decrease of intensity of the α-proton

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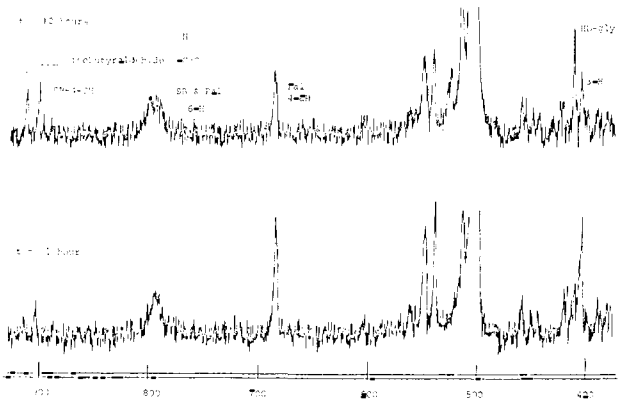


Figure 4. The 100-MHz NMR spectra of a solution of equimolar pyridoxal and β-hydroxyleucine at pD 10.02. HD-gly = HD–glycine Schiff base. Resonances are in Hz relative to HMDS.

resonance of β-hydroxyleucine Schiff base and a concomitant increase in the intensity of the α-proton resonance of the HD–glycine Schiff base were observed. This observation is in accord with the mechanism shown in the reaction scheme.

Treatment of Kinetic Data. The determination of reaction rates was based on measurements of integrated NMR resonances reflecting concentrations of the reaction products, acetaldehyde, acetone, or isobutyraldehyde, as well as the disappearance of the amino acids, threonine, β-hydroxyvaline, or β-hydroxyleucine, respectively.

For the metal-free systems, the rate equations for dealdolation are based upon the following scheme:

$$\text{Pal} + \text{HOAA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{SB} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{Ald} + \text{gly–SB} \qquad (1)$$

for the 1:1 and 1:2 metal ion–Schiff base chelate systems the following reaction sequence was employed.

$$\text{Pal} + \text{HOAA} + \text{M}^{n+1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{M–SB} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{Ald} + \text{M–gly–SB} \quad (2)$$

where Pal = pyridoxal, HOAA = β-hydroxy amino acid, SB = reactive Schiff base intermediate, M–SB = reactive Schiff base metal chelate, Ald = the aldehyde (acetaldehyde or isobutyraldehyde) or ketone (acetone) formed in the reaction, gly–SB = glycine Schiff base, and M–gly–SB = glycine Schiff base metal chelate. In the absence of an appreciable amount of Schiff base the following rate equation involving the steady-state assumption for the concentration of the metal Schiff base chelate intermediate was employed.

$$\frac{-\text{d[HOAA]}}{\text{d}t} = [\text{Pal}][\text{HOAA}][\text{M}^{n+}]\left(\frac{k_1k_2}{k_{-1}+k_{-2}}\right) = k_{\text{obsd}}{}^{\text{M}}[\text{Pal}][\text{HOAA}][\text{M}^{n+}] \quad (3)$$

where $k_{\text{obsd}}{}^{\text{M}}$ = observed rate constant of the metal ion containing system. When a measurable amount of the Schiff base or the Schiff base metal chelate was formed in the reaction, the concentration changes of the Schiff base were followed by NMR. It was then possible to employ the following equation for the metal-free system:

$$\frac{-\text{d[SB]}}{\text{d}t} = k_{\text{obsd}}[\text{SB}] \qquad (4)$$

and in the metal ion containing system:

$$\frac{-\text{d[M–SB]}}{\text{d}t} = k_{\text{obsd}}[\text{M–SB}] \qquad (5)$$

Since byproducts were not formed during the kinetic runs, the reaction stoichiometry over all pD values of the metal-free systems is expressed by:

$$[\text{HOAA}]_0 = [\text{HOAA}] + [\text{Ald}] + [\text{SB}] \qquad (6)$$

Table I. Observed Rate Constants for the Dealdolation of β-Hydroxyvaline–Pyridoxal (Metal-Free System)

pD	k_{obsd}, s^{-1}	pD	k_{obsd}, s^{-1}
6.90	$3.40 \pm 0.05 \times 10^{-4}$	10.20	$7.42 \pm 0.05 \times 10^{-4}$
7.76	$2.93 \pm 0.05 \times 10^{-4}$	10.31	$7.42 \pm 0.05 \times 10^{-4}$
7.97	$2.80 \pm 0.05 \times 10^{-4}$	10.62	$1.00 \pm 0.05 \times 10^{-3}$
8.44	$3.08 \pm 0.05 \times 10^{-4}$	10.76	$1.12 \pm 0.05 \times 10^{-3}$
8.99	$3.45 \pm 0.05 \times 10^{-4}$	10.95	$1.17 \pm 0.05 \times 10^{-3}$
9.65	$5.23 \pm 0.05 \times 10^{-4}$	11.27	$1.20 \pm 0.05 \times 10^{-3}$
9.84	$6.23 \pm 0.05 \times 10^{-4}$	11.63	$1.25 \pm 0.05 \times 10^{-3}$

where $[\text{HOAA}]_0$ is the initial concentration of amino acid. Similarly in metal-ion-catalyzed systems the reaction stoichiometry is expressed by:

$$[\text{HOAA}]_0 = [\text{HOAA}] + [\text{Ald}] + [\text{M–SB}] \qquad (7)$$

where $[\text{HOAA}]_0$ is the initial molar concentration of the amino acid in solution. The values of the first-order rate constants k_{obsd} were found from a plot of d[Ald]/dt vs. [SB] and the $k_{\text{obsd}}{}^{\text{M}}$ were found from a plot of d[Ald]/dt vs. [M–SB] in the metal-ion-containing system. Since the concentration of the Schiff base and of the amino acid may be found by direct integration of the corresponding resonances, the slope of [Ald] vs. time and the corresponding observed rate constants were readily obtained.

For all systems studied, attempts were made to induce the reverse reaction corresponding to k_{-2} in eq 1 and 2. In these experiments either a metal-free or metal-ion-containing glycine Schiff base was formed and then an excess of the appropriate aldehyde (isobutyraldehyde or acetaldehyde) or acetone was added to the solution. NMR spectra of these solutions were then taken over a period of time. Resonances were not observed for the corresponding β-hydroxy amino acids, and k_{-2} in eq 1 and 2 may be neglected for kinetic analysis of all of these systems.

In the metal-ion-catalyzed reactions, both the rate of and extent of Schiff base formation were greatly increased by the formation of the metal chelate. At high pD in the 2:2:1 metal systems, resonances corresponding to the free forms of pyridoxal and amino acid were not detected and therefore the equilibrium lies far to the right, so that $k_1 \gg k_{-1}$. In addition, under conditions of high pD the rapid preequilibria indicate that the initial Schiff base concentrations are simply equivalent to the initial analytical molar concentrations of the amino acids in the experimental solutions. Under these conditions the value of $k_{\text{obsd}}{}^{\text{M}}$ measured are simply k_2. The values of the first-order rate constants $k_{\text{obsd}}{}^{\text{M}}$ may be found from the slope of ln [M–SB] vs. time. Because it was often difficult to distinguish the α-proton resonances and the 2-CH_3 resonances of the reactive Schiff base (M–SB) from the gly–SB resonances, the values of $k_{\text{obsd}}{}^{\text{M}}$ were determined from the rate of the appearance of product by plotting ln $[\text{Ald}_\infty - \text{Ald}_t]$ vs. time.

As mentioned above, the pyridoxal–threonine systems studied did not show NMR evidence of dealdolation in either the metal-free systems or when Zn(II) ion was employed. Therefore the only rate constants that were determined were those of the 2:2:1 (pyridoxal–L-threonine–Al(III)) Schiff base chelates. Because of the tendency of the acetaldehyde to undergo aldol condensation at high pD, the only rate constants that could be determined with sufficient accuracy were obtained over a narrow pD range. At pD 9.05 $k_{\text{obsd}}{}^{\text{M}}$ is 4.7×10^{-4} s^{-1} and at pD 9.45 $k_{\text{obsd}}{}^{\text{M}}$ is 4.5×10^{-4} s^{-1}.

Since the reaction product, acetone, formed by dealdolation of the pyridoxal–β-hydroxyvaline Schiff base is stable, it was possible to determine dealdolation rate constants over a wide range of pD values. The calculated rate constants for the metal-free and metal-catalyzed reactions are given in Tables I–IV.

The mechanism of the dealdolation reaction illustrated in Scheme I is consistent with the rate behavior found in this investigation. As was mentioned above, the formation of an HD–glycine Schiff base resonance during the reaction indicates that the reaction proceeds via direct carbon–carbon cleavage of the α–β carbon atoms. If appreciable α-proton labilization had occurred as a competing reaction, the reaction products formed during the initial stages of the reaction would have included a

Table II. The Observed Rate Constants for the Zn(II)-Pyridoxal-Catalyzed Dealdolation of β-Hydroxyvaline

pD	k_{obsd}^{M}, s^{-1}	pD	k_{obsd}^{M}, s^{-1}
	1:1:1 system		
6.94	$2.7 \pm 0.01 \times 10^{-4}$	7.98	$3.1 \pm 0.01 \times 10^{-4}$
7.17	$2.8 \pm 0.01 \times 10^{-4}$	8.52	$3.2 \pm 0.01 \times 10^{-4}$
7.56	$3.0 \pm 0.01 \times 10^{-4}$	8.62	$3.3 \pm 0.01 \times 10^{-4}$
	2:2:1 system		
7.35	$0.9 \pm 0.01 \times 10^{-4}$	9.82	$4.5 \pm 0.01 \times 10^{-4}$
8.28	$1.1 \pm 0.01 \times 10^{-4}$	10.22	$6.4 \pm 0.01 \times 10^{-4}$
9.12	$1.9 \pm 0.01 \times 10^{-4}$	10.67	$8.8 \pm 0.01 \times 10^{-4}$
9.44	$2.9 \pm 0.01 \times 10^{-4}$	10.94	$9.3 \pm 0.01 \times 10^{-4}$

Table III. Observed Rate Constants for the Dealdolation of the 2:2:1 Pyridoxal-β-Hydroxyvaline-Al(III)

pD	k_{obsd}^{M}, s^{-1}	pD	k_{obsd}^{M}, s^{-1}
8.69	$8.2 \pm 0.1 \times 10^{-4}$	10.06	$2.9 \pm 0.1 \times 10^{-3}$
9.14	$1.6 \pm 0.1 \times 10^{-3}$	10.30	$3.1 \pm 0.1 \times 10^{-3}$
9.45	$2.1 \pm 0.1 \times 10^{-3}$	10.62	$3.5 \pm 0.1 \times 10^{-3}$
9.78	$2.5 \pm 0.1 \times 10^{-3}$	10.96	$4.0 \pm 0.2 \times 10^{-3}$

Table IV. The Observed Rate Constants for Ga(III) Ion-Pyridoxal-Catalyzed Dealdolation of β-Hydroxyvaline

pD	k_{obsd}^{M}, M^{-2} s^{-1}	k_{obsd}^{M}, s^{-1}
	1:1:1 system	
1.90	$3.45 \pm 0.05 \times 10^{-4}$	
2.23	$4.09 \pm 0.05 \times 10^{-4}$	
2.36		$1.1 \pm 0.1 \times 10^{-4}$
2.66		$1.4 \pm 0.1 \times 10^{-4}$
2.80		$1.7 \pm 0.1 \times 10^{-4}$
	2:2:1 system	
5.80		$4.3 \pm 0.1 \times 10^{-4}$
6.22		$5.6 \pm 0.1 \times 10^{-4}$
7.43		$1.6 \pm 0.1 \times 10^{-3}$
8.59		$3.2 \pm 0.2 \times 10^{-3}$

Table V. The Observed Rate Constants for the Zn(II) Ion-Pyridoxal-Catalyzed Dealdolation of β-Hydroxyleucine (1:1:1 System)

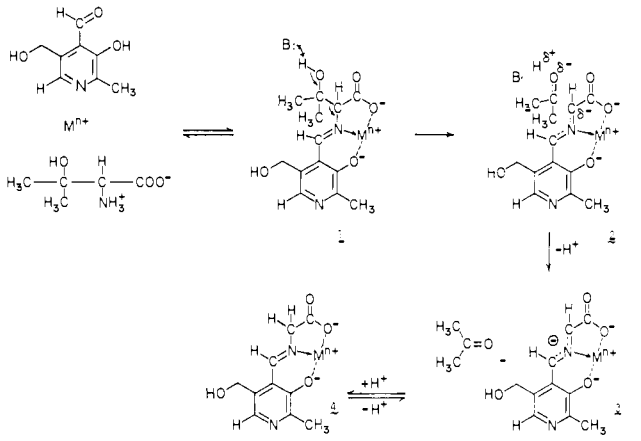
pD	k_{obsd}^{M}, s^{-1}
5.67	$1.2 \pm 0.1 \times 10^{-4}$
5.80	$1.4 \pm 0.1 \times 10^{-4}$
5.90	$1.5 \pm 0.1 \times 10^{-4}$

certain proportion of the D_2-glycine Schiff base.

Evidence for the effect of electron-releasing substituents at the β carbon atom on the rate of dealdolation can be seen from the results obtained with various aliphatic β-hydroxy amino acids. For those Schiff bases without an electron-donating group at the β-carbon atom of the amino acid moiety (serine and α-methylserine) dealdolation was not observed under the reaction conditions employed. Although dealdolation was not observed for the metal-free and Zn(II)-ion-catalyzed pyridoxal–threonine systems, dealdolation was observed in the presence of Al(III) ion, indicating that the methyl group at the β carbon in threonine favors dealdolation to some extent. Dealdolation was observed for the pyridoxal–β-hydroxyleucine Schiff base and for the β-hydroxyleucine–Zn(II) Schiff base chelate, and the observed rate constants are given in Table V. The tendency of β-hydroxyleucine to undergo dealdolation more readily than threonine is probably related to the fact that the isopropyl group at the β-carbon atom is more electron donating than the methyl group.

When the amino acid substrate is β-hydroxyvaline, dealdolation was readily observed for both the metal-free and metal-ion-containing Schiff bases. Here the added electron-donating effect of a second methyl group greatly favors dealdolation. The observed rate constant of dealdolation for the threonine 2:2:1 Schiff base Al(III) chelate at pD 9.45 is 4.5×10^{-4} s^{-1} while the observed

Scheme I. Proposed Mechanism for the Metal-Ion- and Pyridoxal-Catalyzed Dealdolation of β-Hydroxyvaline



rate constant in the β-hydroxyvaline 2:2:1 Al(III) system at pD 9.45 is 21.2×10^{-4} s^{-1}, an almost fivefold increase in the rate constant. The effect of the two methyl groups at the β-carbon is seen in that the rate constant for dealdolation of the β-hydroxyleucine 1:1:1 Schiff base Zn(II) chelate at pD 5.90 is 1.5×10^{-4} s^{-1} while that of the corresponding β-hydroxyvaline 1:1:1 Zn(II) chelate is 2.7×10^{-4} s^{-1} at pD 6.90. (It should be noted that at these pH values there is little pH effect on metal–pyridoxal–Schiff base catalysis so that the comparison is valid.) Another way of rationalizing this effect, which basically is probably equivalent to the above explanation, involves the invoking of the fact that ketones are better leaving groups than aldehydes. This would be the inverse of the greater tendency of aldehydes to undergo addition, relative to ketones.

The increase in the rate of dealdolation resulting from the inductive electron release effects at the β-carbon atom may be considered to be due to an increase in the electron density of the β-carbon atom (formula 2, Scheme I), thus assisting the shift of the electrons in the α–β carbon–carbon bond toward azomethine nitrogen. Electron-releasing groups at the β position may also increase slightly the electron density at the α-carbon atom and slightly decrease the acidity of the α proton. This effect would tend to retard competing reactions which require α-proton dissociation as a first step, such as racemization, α-proton exchange, α,β elimination, and transamination.

From the kinetic data obtained, it is evident that metal ions strongly promote dealdolation of β-hydroxy amino acids. The pyridoxal–threonine system does not undergo dealdolation in the absence of a metal ion under the reaction conditions employed in this investigation. However, the reaction takes place rapidly in the pyridoxal–threonine–Al(III) Schiff base chelate system at pD 9.3 at 30 °C.[8] The effect of metal ions on the dealdolation of β-hydroxyvaline can be seen by comparing the rate constants in Tables I–IV. The values of k_{obsd}^{M} found for the Al(III) and Ga(III) system show a dramatic increase over those of the metal-free systems. The k_{obsd} in the metal-free system at pD 10.31 is 8.8×10^{-4} s^{-1} while for the Al(III) system k_{obsd}^{M} is 3.1×10^{-3} s^{-1} at pD 10.30. When Ga(III) ion is used, the increase is even greater: k_{obsd} at pD 8.99 is 3.5×10^{-4} s^{-1} in the metal-free system while in the Ga(III) system k_{obsd}^{M} is 3.2×10^{-3} s^{-1} at pD 8.50. This rate enhancement is due to the fact that the trivalent Al(III) and Ga(III) ions are more electropositive than the hydrogen ions coordinated to the Schiff base in the metal-free system, and thus are more effective in electron withdrawal from the α–β carbon–carbon bond toward the azomethine nitrogen (formulas 1 and 2, Scheme I).

In general Ga(III) ion forms more stable complexes than Al(III) ion with amino acid ligands.[16] This greater stability would facilitate the movement of electrons toward the Ga(III) ion more

(16) Martell, A. E.; Smith, R. M., Eds. "Critical Stability Constants"; Plenum: New York, 1974; Vol. 1.

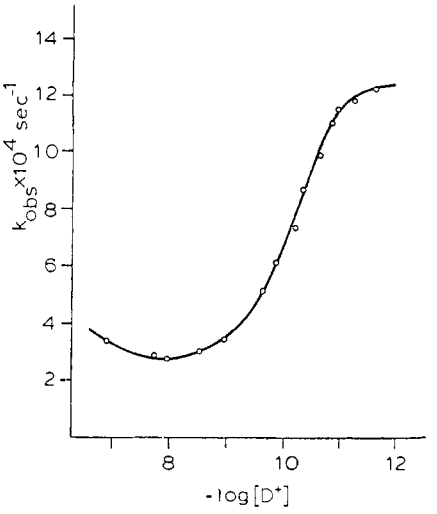


Figure 5. A plot of the observed rate constants, k_{obsd}, for the dealdolation of the pyridoxal–β-hydroxyvaline Schiff base system vs. pD.

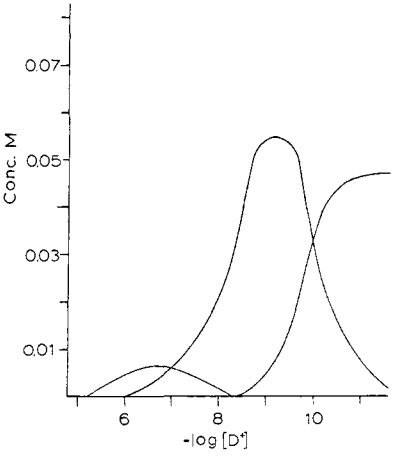


Figure 6. The species distribution of the various forms of the pyridoxal–β-hydroxyvaline (1:1) Schiff base system as a function of the pD. The initial concentrations of pyridoxal and amino acid were 0.10 M.

than would be the case with the Al(III) ion. Thus Ga(III) ion is expected to be more effective than Al(III) ion in catalyzing pyridoxal–metal Schiff base catalyzed dealdolation. A comparison of the values of the observed rate constants for the pyridoxal–metal ion catalyzed dealdolation of β-hydroxyvaline shows this to be the case. The k_{obsd}^{M} at pD 8.59 is 3.2×10^{-3} s^{-1} for the Ga(III) 2:2:1 system while the k_{obsd}^{M} at pD 8.69 is 8.2×10^{-4} s^{-1} in the Al(III) system.

The model systems in which Zn(II) is the central metal ion have rate contants that are lower than those of either Al(III) or Ga(III) systems, in agreement with the lower charge of the Zn(II) ion. In fact Zn(II) exhibits about the same catalytic effect as measured by k_{obsd} as the azomethine proton in the metal-free systems. The strong catalytic effect of the azomethine proton was pointed out by Martell[17] for Schiff base systems undergoing transamination. It was found that in the Zn(II) systems above pD 9.0 appreciable α-proton exchange (racemization) and transamination were also detected. Since transamination and proton exchange proceed through an α-deprotonated Schiff base intermediate they constitute competing reactions that may lessen the catalytic effect of the Zn(II) ion on dealdolation. As indicated above α-deprotonated intermediates are not in the dealdolation reaction pathway. Loss of the α proton would withdraw some of the starting material in the dealdolation reaction if the amount of α-deprotonated intermediate were to accumulate in appreciable concentrations. The possibility that such intermediates may under suitable conditions be present in measurable amounts has been suggested by Abbott and Martell.[18]

The values of the observed rate constants in the metal-free pyridoxal–β-hydroxyvaline system show a general increase in dealdolation rate with an increase in pD, but not in a regular fashion. Figure 5 shows a slight decrease in the value of k_{obsd} from pD 6.90 to pD 7.97, followed by a slow increase to about pD 10, above which a sharp increase in k_{obsd} with pD occurs. Analysis of this behavior requires consideration of several factors that influence the rate of dealdolation. The ability of the Schiff base to delocalize and thus stabilize the negative charge through the extended π-bond system of the pyridine ring (formula 3, Scheme I) is dependent on the degree of protonation of the Schiff base. One would expect a Schiff base protonated at the azomethine nitrogen to have an additional favorable inductive effect toward dealdolation. Thus the monoprotonated Schiff base (protonated at the azomethine nitrogen) and the diprotonated Schiff base (protonated at the azomethine and pyridine nitrogens) should both be reactive intermediates in the dealdolation reaction. While the diprotonated form should exert a higher catalytic effect than the monoprotonated species, the observed rate should also vary with the changing concentrations of these species with increasing pH. In addition, base catalysis by hydroxide ion would assist the removal of a proton from the aliphatic hydroxyl group (formula 2, Scheme I).

The observed rate constant, k_{obsd}, for the metal-free system is the summation of the individual rate constants associated with the three forms of the Schiff base: the diprotonated form (H_2SB), the monoprotonated form (HSB), and the deprotonated form (SB). Thus the overall rate constant k_{obsd} may be expressed as:

$$k_{obsd}[SB_T] = k^{I}[H_2SB] + k^{II}[HSB] + k^{III}[SB] \quad (8)$$

where $[SB_T]$ is the total concentration of Schiff base species. Since the total concentration of Schiff base varies with pH it is more convenient to express eq 8 in the following way:

$$k_{obsd} = k^{I}\frac{[H_2SB]}{[SB_T]} + k^{II}\frac{[HSB]}{[SB_T]} + k^{III}\frac{[SB]}{[SB_T]} \quad (9)$$

The mole fraction of each species can be determined as a function of pH with the aid of the appropriate pK_a values of the Schiff base. At high pH values where the hydroxide ion becomes significant, allowance for the contribution of the hydroxide ion should be incorporated in the rate law. Thus the specific rate constants may be expressed by:

$$k_{obsd} = k^{I}\frac{[H_2SB]}{[SB_T]} + k^{II}\frac{[HSB]}{[SB_T]} + k^{IV}\frac{[HSB][OH^-]}{[SB_T]} \quad (10)$$

It should be noted that the specific rate constant corresponding to $k^{III}[SB]/[SB_T]$ has been omitted since it is related to k^{III}-$[HSB][OH^-]/[SB_T]$ by the constant factor k_{HSB}^{H}/k_w where k_{HSB}^{H} is the dissociation constant of HSB and k_w is the ion product constant of water. Thus the increase in reaction at high pH may be ascribed to either [SB] or $[HSB][OH^-]$, since the corresponding reaction pathways are kinetically indistinguishable. The latter second-order mechanism is selected for the purpose of this discussion, since the "push–pull" dual catalytic effects of H^+ and OH^- seem more reasonable. Another high-pH pathway corresponding to $k^{V}[SB][OH^-]/[SB_T]$ represents a higher order of base catalysis (kinetically equivalent to $K^{VI}[HSB][OH^-]^2$). This mechanistic pathway is not considered significant for the systems under consideration because of the lack of significant increases in the rates of dealdolation at very high pH. At pD 10 and above the increase in k_{obsd} levels off, as might be expected if the major reactive intermediate is the monoprotonated Schiff base. The decrease in its concentration as the pH is increased is cancelled by the increase in $[OH^-]$, resulting in a relatively constant con-

(17) Martell, A. E. In "Chemical and Biological Aspects of Pyridoxal Catalysis", Snell, E. E., Fasella, P. M., Braunsteln, A., Rossi Fanelli, A., Eds.; Pergamon Press: New York, 1963; p 13.
(18) Abbott, E. H.; Martell, A. E. *J. Am. Chem. Soc.* **1973**, *95*, 5014.

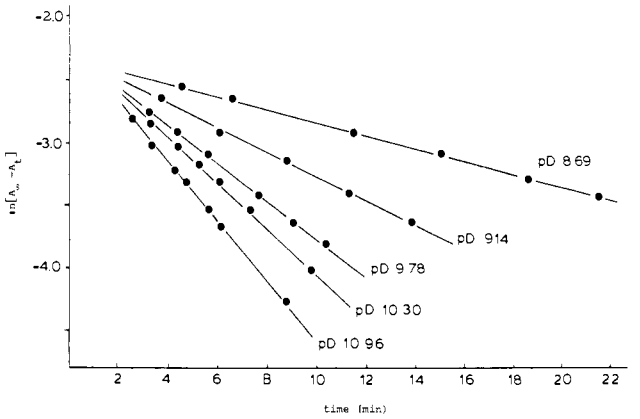


Figure 7. First-order plots (ln aldimine concentrations vs. time) for the pyridoxal and proton-catalyzed dealdolation of β-hydroxyvaline as a function of pD.

tribution from $k^{IV}[HSB][OH^-]$ to the observed rate. The values of k^I, k^{II}, and k^{IV} were obtained by regression analysis[19] of the sum of the squares of the errors, U, by minimizing U, defined as:

$$U = (k_{obsd}[SB_T] - k^I[H_2SB] - k^{II}[HSB] - k^{IV}[HSB][OH^-])^2 \quad (11)$$

The specific rate constants for the pyridoxal–β-hydroxyvaline Schiff base system were found to be: $k^I = 4.25 \times 10^{-4}$ s^{-1}; $k^{II} = 2.62 \times 10^{-4}$ s^{-1}; $k^{III} = 15.6$ M^{-1} s^{-1}. The protonation constants for the Schiff base species were determined spectrophotometrically and found to be $pK_1 = 6.65$; $pK_2 = 9.70$. The species distribution is shown in Figure 6. The specific rate constants were used with the concentrations of the individual species to determine calculated values of k_{obsd} as a function of pD. The small differences between the experimental and observed rate constants, illustrated in Figure 5, indicate that the model chosen to estimate the calculated rates is in accord with the experimental data.

Between pD values of 6 to about 9.5 the hydroxide ion concentration is low and thus the net contribution of k^{IV} on k_{obsd} is negligible. Thus in the pD range the value of k_{obsd} is due primarily to k^I and k^{II}. The diprotonated Schiff base, which is the most reactive species, is present in amounts less than 20% of the total Schiff base in this entire region of pD and its concentration decreases with increasing pD, thus explaining the slight initial drop in k_{obsd}. The major contributor to dealdolation in the pD range 7–10 is the monoprotonated species HSB. Between pD 9.5 and 11.0, the value of k_{obsd} increases rapidly with pD as illustrated in Figure 7, and then levels off, indicating a transfer of the main contribution to the reaction pathway from HSB^- to HSB^- and OH^-, and a corresponding shift of the reaction from mainly first-order to mainly second-order kinetics.

Registry No. Threonine, 72-19-5; β-hydroxyvaline, 2280-28-6; β-hydroxyleucine, 28908-11-4; pyridoxal, 66-72-8; Zn, 7440-66-6; Al, 7429-90-5; Ga, 7440-55-3.

(19) Brookes, C. J.; Bettely, I. G.; Loxston, S. M. "Mathematics and Statistics for Chemists"; Wiley & Sons: New York, 1966; Chapter 15.

Communications to the Editor

Resolution of Chiral Olefinic Hydrocarbons and Sulfoxides by High-Performance Liquid Chromatography via Diastereomeric Platinum Complexes

M. Goldman,† Z. Kustanovich, S. Weinstein, A. Tishbee, and E. Gil-Av*

Department of Organic Chemistry
The Weizmann Institute of Science, Rehovot, Israel

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Asymmetric olefinic hydrocarbons, unless found in nature, are not readily available in well-defined optical purity. Resolution of racemic olefins, e.g., by repeated crystallization of their diastereomeric Pt complexes,[1] does not guarantee that the final product is optically pure. Also, the determination of the optical purity of olefins of unknown specific rotation is not readily accomplished. An obvious approach is functionalization of the double bond and conversion to compounds of known $[\alpha]_D$ value. This method, except for being time-consuming, may involve racemization to an unknown extent, and the relevant information on the products formed may not be available.

It is, therefore, not surprising that the data in the literature on the chirooptical properties and the magnitude of inductive effects in olefin synthesis and conversion reactions may be seriously in error.[2] In the present communication we wish to report on the resolution of this class of chiral compounds[3] by HPLC of

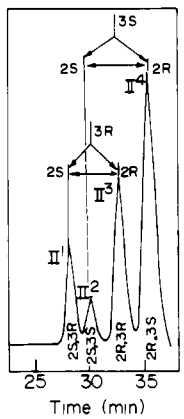

Figure 1. Chromatogram of the platinum complexes of 3-methylpent-1-ene (II); mobile phase: *n*-hexane/CH_2Cl_2/2-butanol, 60/40/1.

diastereomeric Pt complexes as well as on the extension of this approach to sulfoxides.

Diastereomeric *trans*-dichloro((*R*)-α-phenlyethylamine)(olefin)platinum (II) complexes, used for the resolution of olefins by crystallization,[1] could not be separated by HPLC under all con-

†Agricultural Research Organization, Volcani Center, Bet Dagan, Israel.

(1) Cope, A. C.; Ganellin, C. R.; Johnson, H. W., Jr.; van Auken, R. V.; Winkler, H. J. S. *J. Am. Chem. Soc.* **1963**, *85*, 3276.

(2) Schurig, V.; Gil-Av, E. *Isr. J. Chem.* **1976/77**, *15*, 96.

(3) Resolution by GC on dicarbonylrhodium(I) 3-(trifluoroacetyl)-1-(*R*)-camphorate [Schurig, V. *Angew. Chem.* **1977**, *89*, 113, see also ref 2] was found to be limited to 3-methyl- and 3-ethylcyclopentene. Sporadic reports on the separation of enantiomeric olefins by LC include the resolution of *cis*- and *trans*-1,1′,2,2′,3,3′-hexahydro-4,4′-biphenanthrylidene through charge-transfer complexation on a support coated with a chiral acceptor [Ferringa, B.; Wynberg, H. J. Am. Chem. Soc. **1977**, *99*, 602] and resolution of 3-methylcyclohexene, 3- and 5-phenylnorbornene on triacetylcellulose [Hesse, G.; Hagel, R. *Liebigs Ann. Chem.* **1976**, 996].

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