Kinetics and Stereochemistry of β-Elimination of (*S*)-*O*-Acylthreonine in Λ- and Δ-Bis-[*N*-salicylidene-(*S*)-*O*-acylthreoninato]cobaltate(III) Ions. Implications for Vitamin B_6-catalysed Reactions

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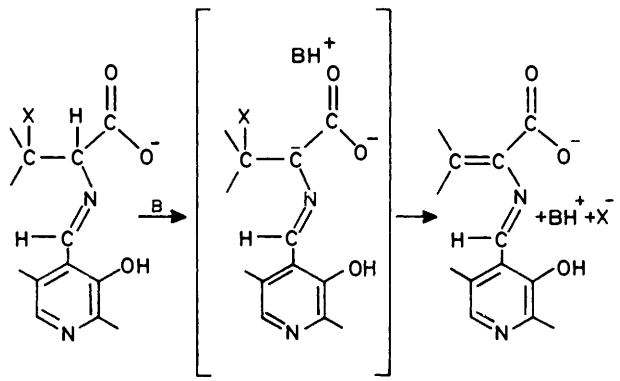
Diastereoisomeric complexes of Λ- and Δ-bis-[*N*-3-R'-salicylidene-(*S*)-*O*-acylthreoninato]cobaltate(III) have been synthesized. Deacylation of the complexes at 25 °C and pH 9—11.0 yields a mixture of isomeric Λ- and Δ-bis-(*N*-3-R'-salicylidene-α-aminodehydrobutyrato)cobaltate(III) ions. Deacylation is subject to general base catalysis. A comparison of the rate constants for epimerization of Λ- and Δ-bis-[*N*-3-R'-salicylidene-(*S*)-threoninato]cobaltate(III) ions with those for deacylation of their acyl derivatives shows that deacylation follows the $(E1cB)_I$ mechanism. The ratio of the products formed *via* the *syn*- or *anti*-elimination mechanism depends on the nature of the base which catalyses the process. The neutral base DABCO catalyses *syn*-elimination predominantly whereas the negatively charged carbonate ion brings about *anti*-elimination. The stereochemistry of pyridoxal-dependent reactions of β-elimination has been discussed along the lines of the data obtained.

β-Elimination of electronegative substituents in amino acids represents a reaction which in nature is catalysed by pyridoxal enzymes. The Schiff's bases of pyridoxal and dehydroamino acids always participate in such transformations as intermediates.[1] Model studies indicate that the formation of such species is described by Scheme 1 according to which an intermediate Schiff's base carbanion of an amino acid (A^-) formed under the action of the base decomposes with the liberation of a negatively charged species X^- and formation of the Schiff's base of a dehydroamino acid.[2] Thus, serine and threonine are dehydrated; tryptophan, tyrosine, and other compounds are decomposed[1] *via* a mechanism of the *E*1cB type.[3] A large primary isotope effect in the series of enzymic β-elimination reactions[4] indicates that at least some of them are described by the $(E1cB)_I$ mechanism in which the abstraction of the amino acid α-hydrogen is the limiting step. The stereochemistry of the reactions proceeding by this mechanism is poorly studied.[3] It is known that in strongly polar solution or in the presence of crown ethers, when ion association can be neglected, *anti*-elimination predominates.[3b] The data available at present for one of the pyridoxal β-eliminating enzymes (tryptophanase), however, can be interpreted in favour of *syn*-elimination of the α-hydrogen of tryptophan and its indole fragment.[5] In other words, this process, like all other reactions of pyridoxal catalysis, takes place on one side of the Schiff's base, whereas its other side is sterically shielded.[5,6]

It is not known in which way the active site of the pyridoxal enzyme makes *syn*-elimination favourable. One may assume, however, that the structure of the main functional group which abstracts an α-proton from an amino acid fragment and/or hydrophobic shielding of one side of the intermediate carbanion are conducive for *syn*-elimination.

In this paper we have tried to determine the stereochemistry of the formation of α-aminodehydrobutyric acid from threonine which represents a model β-elimination reaction typical of pyridoxal catalysis. We also attempted to elucidate the effect of the nature of the base, which catalyses the abstraction of the α-proton from an amino acid fragment and hydrophobic shielding of one side of the intermediate carbanion, on the stereochemistry of elimination. For the purpose, we have synthesized diastereoisomeric ions of Λ- and Δ-bis-[*N*-salicylidene-(*S*)-*O*-acetylthreoninato]cobaltate(III) (SATC) and Λ- and Δ-bis-[*N*-3-methylsalicylidene-(*S*)-*O*-acetylthreoninato]-cobaltate(III) (MSATC) and studied the kinetics and stereochemistry of deacylation of the threonine fragment. Co^{III} complexes were chosen since they are stereochemically inert and water soluble. Chemical transformations of ligands do not change an absolute configuration of Co^{III} complexes[7,8] and their inertness prevents the system of dehydroamino acids in the complexes from decomposition in water to ammonia and keto-acids. In addition, Co^{III} complexes are diamagnetic which makes it possible to use the ^1H n.m.r. method for studying the stereochemistry of the products. Finally, salicylaldehyde in metal complexes of Schiff's bases with amino acids acidifies the α-hydrogen of the amino acid fragment as effectively as pyridoxal deprotonated at the heterocyclic nitrogen does.[9]



Scheme 1.

Experimental

Sephadex LH-20 was supplied by Pharmacia Fine Chemicals. All amino acids were supplied by Reanal, Budapest, and used without further purification. DABCO was supplied by Merck, salicylaldehyde (Sal), D_2O, Na_2CO_3, and $NaHCO_3$ by Reakhim, U.S.S.R. 3-Methylsalicylaldehyde was prepared by the procedure described in ref. 10. Al_2O_3 was treated as described in ref. 11.

Diastereoisomers of Sodium Λ- *and* Δ-*Bis*-[N-*salicylidene*-(S)-*threoninato*]*cobaltate*(III) (*BSTC*) *and Sodium* Λ- *and* Δ-*Bis*-[N-3-*methylsalicylidene*-(S)-*threoninato*]*cobaltate*(III) (*MSTC*).—These were prepared by following the procedure

Table 1. Effect of bases on the ratio of diastereoisomers of *E* and *Z* configuration upon deacylation of {Co[3-R'-sal-(*S*)-AcThr]$_2$}Na complexes in water at 25 °C

Run	Substrate	Catalyst	*EE*	*EZ*	*ZZ*	*syn*[b] (%)	*anti*[b] (%)
1[a]	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na	OH^-	1	3.2 ± 0.1	2.2 ± 0.1	40.6	59.3
2[c]	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na	DABCO	2.5	2.8	1	65	38
3[d]	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na	CO_3^{2-}	1	7.1 ± 0.6	10.1 ± 0.1	25	75
4[e]	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na		1	4.2 ± 0.1	6.8 ± 0.1	26	74
5[a]	Δ-{Co[3-MeSal-(*S*)-AcThr]$_2$}Na	OH^-	1	8.8	13.2	23.5	76.5

[a] With the pH stat conditions. [b] Calculated from the ratio of total amounts of *Z*- and *E*-isomers. [c] Under the action of buffer DABCO–DABCO·HCl (0.5:0.2). [d] Under the action of buffer $NaHCO_3$–Na_2CO_3 (0.167:0.83) (pH 9.15). [e] Equilibrium ratio of diastereoisomers obtained by epimerization of Δ-(*ZZ*)-SDBC or Δ-(*EE*)-SDBC.

described in ref. 8. The diastereoisomers were separated by column chromatography on LH-20 with C_6H_6–C_2H_5OH (3:1). Isomer Λ (*SS*) was eluted first; it was followed by Δ (*SS*). The diastereoisomers obtained had the parameters of ^1H n.m.r. and u.v.-visible spectra as well as o.r.d. curves similar to those described earlier.[8]

Sodium Λ- *and* Δ-*Bis*-[N-*salicylidene*-(S)-O-*acetylthreoninato*]*cobaltate*(III) (Λ- *and* Δ-*SATC*) *and Sodium* Λ-*Bis*-[N-3-*methylsalicylidene*-(S)-O-*acetylthreoninato*]*cobaltate*(III) (Λ-*MSATC*).—These were prepared by following the standard procedure described earlier.[12]

Diastereoisomers of Sodium Δ-*Bis*-[N-3-R'-*salicylidene*-α-*aminodehydrobutyrate*]*cobaltate*(III).—These were synthesized using a pH-stat as described earlier.[12]

Kinetics of Deacylation of Δ-*SATC and the Ratio of the Diastereoisomers in Carbonate Buffer Solution and in DABCO–Aqueous Solution at 25* °C.—Δ-SATC·H_2O (6.7 mg, 1.07 × 10^{-5} mol) was dissolved in C_2H_5OH (2 ml) and the solution (0.1 ml) was added to a buffer solution (1.4 ml). The solution was transferred into a spectrophotometer (or polarimeter) cell thermostatted at 25 °C and a change in absorption (or in optical rotation) was recorded at 25 000 cm^{-1} (or 578 nm). The following buffer solutions were used: pH 10 (Na_2CO_3–$NaHCO_3$ 1:1 with μ 3 and Na_2CO_3 concentrations 0.5, 0.16, and 0.05M); pH 9.15 (0.83M-$NaHCO_3$ and 0.17M-Na_2CO_3): pH 10.3 (μ 0.12; 0.01M-$NaHCO_3$ and 0.15M-Na_2CO_3): pH 10.6 (μ 0.12; 0.019M-$NaHCO_3$ and 0.006M-Na_2CO_3): pH 9.4 (μ 0.3, DABCO–DABCO·HCl 0.51:0.24; 0.15:0.12; 0.17:0.08).

A concentrated buffer solution of DABCO in D_2O was prepared by evaporating an aqueous buffer solution (μ 0.3; DABCO–DABCO·HCl 0.51:0.24) to dryness after which small portions of D_2O were repeatedly added and evaporated. Finally, D_2O was added till the volume of the solution was equal to the initial one. Buffer solutions of lower concentrations were prepared by diluting the initial one with 0.3M-KCl solution in D_2O.

Preparative Experiments on determining the Ratio of Diastereoisomers formed in the Deacylation Reaction.—These were carried out in a carbonate buffer with pH 9.15 and in DABCO buffer with pH 9.4. For the purpose, a solution of Δ-SATC (0.15 g, 2.4 × 10^{-4} mol) in buffer (20 ml) was placed into a vessel thermostatted at 25 °C under Ar. After the completion of the experiment controlled by t.l.c., the mixture was neutralized, evaporated, and the ratio of the deacylated fractions thus formed was evaluated as described below.

Electrochemical reduction of complexes and isolation of amino acids were performed as described in ref. 8.

Isomerization of Δ-*Bis*-[N-*salicylidene*-(Z,E)-*dehydroaminobutyrato*]*cobaltate*(III) *Ions* [Δ-(ZZ) *and* Δ-(EE)-*SDBC*].—Δ-(*EE*)-SDBC·$2H_2O$ or Δ-(*ZZ*)-SDBC·$1.5H_2O$ (0.02%) was dissolved in DABCO buffer solution (2 ml) at 25 °C (0.5:0.25 DABCO–DABCO·HCl). After equilibrium had been established (u.v., t.l.c.) (15 h), the ratio of diastereoisomers was determined by t.l.c.: the fractions containing isomers were eluted and the ratio was found spectrophotometrically. The results are given in Table 1.

Isomerization kinetics was studied by following the change in absorption at 430 nm of Δ-(*EE*)-SDBC solution (25 °C) in carbonate and DABCO–DABCO·HCl buffer solutions (0.7:0.25; 0.5:0.25; 0.25:0.25; 0.25:0.12; 0.12:0.12; 0.12:0.05).

^1H *N.m.r. Experiment on Deacylation of* Δ-*SATC.*—A solution of Δ-SATC·H_2O (0.06 g, 9.6 × 10^{-5} mol) in DABCO solution (0.5 mol) (0.5:0.2 DABCO–DABCO·HCl) in D_2O was placed into the ampoule of a ^1H n.m.r. Bruker-200 spectrometer. A change in relative intensities and in character of the acyl group signals (δ 2.03) was recorded for the CH_3 groups of the threonine fragment (δ 1.470) and for the fragment of dehydroaminobutyric acid (δ 2.19 and 2.26 for *E*- and *Z*-isomers, respectively).

^1H N.m.r. spectra were recorded on Tesla-467A and Bruker-200 instruments. Electron spectra and deacylation kinetics were recorded on a Specord UV-Vis instrument. The o.r.d. curves were recorded on a Jasco-ORD/VV-5 instrument. Polarimetric measurements were performed on a Perkin-Elmer-241 polarimeter. pH-Stat experiments were performed on a Radiometer SBR-2/SBU-1/TT-1 apparatus.

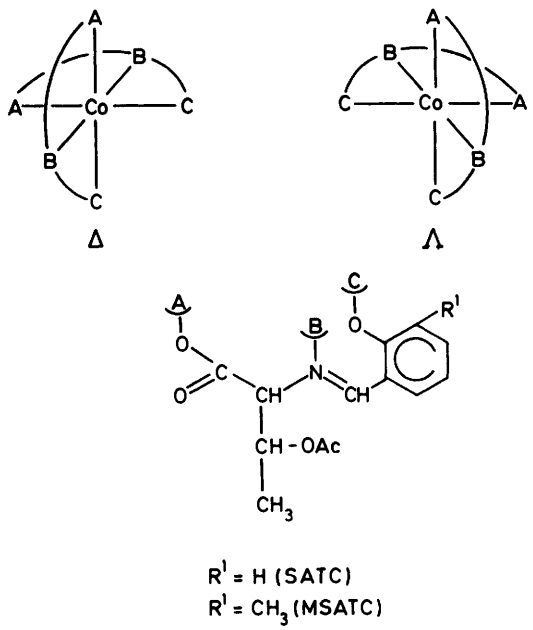
Results

(1) *Synthesis and Separation of* Λ- *and* Δ-*Bis*-[N-3-R'-*salicylidene*-(S)-O-*acylthreoninato*]*cobaltate*(III) *Ions.*—A mixture of the initial diastereoisomeric complexes of sodium Λ- and Δ-bis-[*N*-salicylidene-(*S*)-threoninato]cobaltate(III) (BSTC) or sodium Λ- and Δ-bis-[*N*-3-methylsalicylidene-(*S*)-threoninato]-cobaltate(III) (MSTC) was prepared from $Na_3Co(CO_3)_3·3H_2O$, 3-R'-C_6H_3(OH)CHO, and (*S*)-threonine as described in ref. 11. The mixture was then separated on Sephadex LH-20 or Al_2O_3. Pure diastereoisomers were obtained which differ in their Λ- or Δ-complex configuration * [8] and have the same configuration of the amino acid fragment.

Each diastereoisomer was acylated with acetic anhydride in acetonitrile in the presence of catalytic amounts of pyridine as described earlier.[12]

In the course of the isolation of acylated products partial deacylation takes place which yields complexes containing the dehydroaminobutyric acid fragment. Λ- and Δ-SATC were purified on LH-20. The Δ-MSATC isomer was not separated in

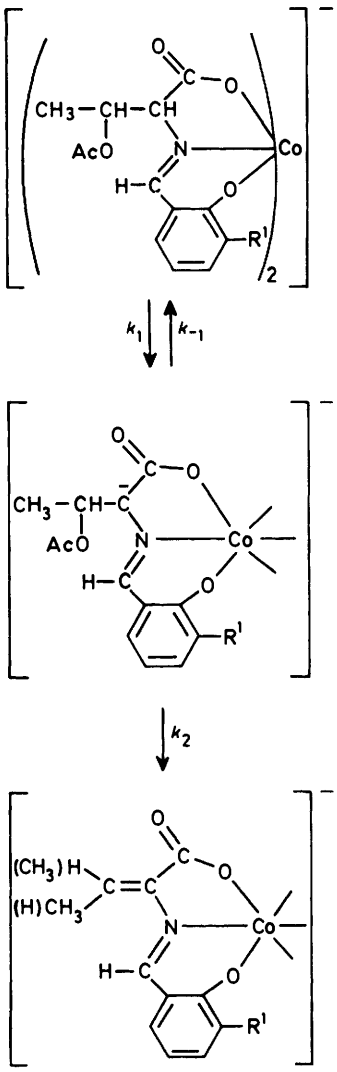
* Λ and Δ correspond to left and right spiral arrangement of ligands relative to the C_2 axis.



a pure form because of the deacylation reaction. It was obtained directly in the ampoule of the ^1H n.m.r. spectrometer and its transformations were studied without isolation with the use of the reaction mixture after acylation. A comparison of the o.r.d. curves of the initial[8] and acylated isomers indicates that during acylation the absolute configuration of the complexes remains unchanged. Threonine obtained from acylated complexes after their electrochemical reduction and hydrolysis of the acyl derivative did not contain allothreonine (according to t.l.c. on cellulose) which indicated that no epimerization of the amino acid fragment took place in the course of acylation.

(2) *Deacylation of* Λ- *and* Δ-*Bis*-[N-3-R′-*salicylidene*-(S)-O-*acylthreoninato*]*cobaltate*(III) *Ions in Water under the Effect of Bases.*—Deacylation of Λ-and Δ-SATC and Λ- and Δ-MSATC under the action of bases in H_2O gives a set of diastereoisomeric Co^{III} complexes containing the Schiff's bases of *Z*- and *E*-dehydroaminobutyric acid according to Scheme 2.

Deacylation proceeds faster than epimerization of the amino acid fragment which is confirmed by the absence of allothreonine in threonine isolated after partial deacylation and by the absence of the exchange of the α-hydrogen in the threonine fragment during deacylation in D_2O. One pure SATC or MSATC diastereoisomer gives all possible isomers of the *ZZ*, *EE*, and *ZE* configuration. Diastereoisomers are readily separated by preparative t.l.c. on Al_2O_3. The isomers differ from the initial complexes of SATC and MSATC in their electronic spectra,[12] ^1H n.m.r. spectra,[12] and the o.r.d. curves.[12] After electrochemical reduction of SDBC and MSDBC no trace of amino acids was detected in the reaction mixture which means that deacylation was complete. No side-products were found during deacylation of Λ- and Δ-SATC and Λ- and Δ-MSATC under the action of OH^-, CO_3^{2-}, and DABCO (^1H n.m.r. experiment). The volume of the titrant consumed during the SATC or MSATC deacylation reaction under pH-stat conditions corresponds to the expected one. The structure and configuration of deacylated compounds was established earlier.[12] The R_F value of deacylated diastereoisomers on Al_2O_3 decreases in the order Δ(*EE*), Δ(*ZE*), and Δ(*ZZ*). The ratio of *EE*, *EZ*, and *ZZ* isomers obtained in deacylation depends on the nature of the base catalysing the process. Table 1



Δ (*ZZ*) ; Δ (*EZ*) ; Δ (*EE*) R¹ = H ; SDBC
Λ (*ZZ*) ; Λ (*EZ*) ; Λ (*EE*) R¹ = CH₃ ; MSDBC

Scheme 2.

summarizes the ratios of diastereoisomers obtained under different experimental conditions.

(3) *Deacylation Kinetics.*—To measure the deacylation rate, use was made of spectrophotometric, polarimetric, and pH-stat procedures. In all cases deacylation followed pseudo-first-order kinetics up to 80% transformation. The results obtained spectrophotometrically agreed with those obtained with the aid of the polarimetric procedure. The deacylation rate increased with an increase in the base concentration of the solution. In 0.01M-HCl no deacylation is observed for 5 days. The relationship between the deacylation rate constant observed (k_{obs}) and the concentration of the buffer components is described by the equation $k_{obs} = K^{OH} \cdot a_{OH} + k^B B$ where *B* is the concentration of free base present in the solution, and a_{OH} is the activity of the hydroxide ion in solution. Extrapolation of the k_{obs}–*B* relationship at different pH yields a value of k^{OH} which agrees with that determined by the pH-stat method. Curves of k_{obs} *versus B* or a_{OH} are straight lines for all buffer solutions in

Table 2. Deacylation rate constants of diastereoisomers of {Co[3-R'-Sal-(*S*)-AcThr]$_2$}Na at 25 °C in water

Run	Substrate	Catalyst	k^b l mol^{-1} s^{-1} [a]
1	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na	H_2O	v. slow
2	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na	OH^b	1.9 ± 0.04
3	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na	CO_3^{2-}	1.22×10^{-4}
4	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na	DABCO[c]	$(1.30 \pm 0.03) \times 10^{-2}$
5	Δ-{Co[Sal-(*S*)-AcThr]$_2$}Na	DABCO[c,d]	$(0.8 \pm 0.03) \times 10^{-2}$
6	Δ-{Co[3-MeSal-(*S*)-AcThr]$_2$}Na	OH^{-e}	1.97 ± 0.04
7	Λ-{Co[3-MeSal-(*S*)-AcThr]$_2$}Na	OH^{-e}	0.26 ± 0.02
8	Λ-{Co[Sal-(*S*)-AcThr]$_2$}Na	OH^{-e}	1.2 ± 0.1

[a] Second-order rate constants. [b] Obtained from the dependence of k_{obs}/s^{-1} on a_{OH} for five points (spectrophotometric determination and pH-stat measurements). [c] Four buffer solutions with different buffer capacity at μ 0.3. [d] In D_2O. [e] Spectrophotometric determination in a carbonate buffer solution at pH 10.8 when $k_{obs} \approx k^{OH} . a_{OH}$.

H_2O or D_2O. Table 2 lists the second-order rate constants for deacylation of complexes under the action of OH^- ion (for Δ-SATC), DABCO (in H_2O and D_2O), and CO_3^{2-}.

Discussion

The absence of any noticeable deacylation of complexes in water at pH < 4.0, the general base catalysis of the deacylation reaction, as well as the absence of α-hydrogen exchange and epimerization of the threonine fragment in the course of the reaction show that deacylation follows an E_2 or $(E1cB)_I$ mechanism.[3] This is also confirmed by the absence of an inverse solvent isotope effect[13] during deacylation in D_2O with the use of DABCO buffer solutions (see Table 2, runs 4 and 5). The $(E1cB)_I$ mechanism with abstraction of the α-proton of the amino acid as the rate-limiting stage is supported by the closeness of the second-order rate constants of Λ-SATC and Λ-MSATC deacylation under the action of OH^- (Table 2) to the rate constants for the exchange of the α-proton of the threonine fragment in Λ-bis-[*N*-salicylidene-(*S*)-threoninato]cobaltate-(III) ion and Λ-bis-[*N*-3-methylsalicylidene-(*S*)-threoninato]-cobaltate(III) ion calculated as double the rate constants for the inversion of the amino acid fragment[14] and equal to 0.5 l mol^{-1} s^{-1} and 0.16 l mol^{-1} s^{-1}, respectively. The fact that the deacylation rate is 1.5—4 times greater may be the result of a higher electron-withdrawing effect of the $OCOCH_3$ group compared with that of OH.[15] A decrease in the rate constant when passing from Λ-SATC to Λ-MSATC is similar to that of the deuterium exchange rate constant of the (*S*)-valine fragment when passing from Λ-bis-[*N*-salicylidene-(*S*)-valinato]-cobaltate(III) ion to Λ-bis-[*N*-3-methylsalicylidene-(*S*)-valinato]-cobaltate(III) ion[8] and may be attributed to steric hindrance of OH^- attack on the α-hydrogen of the amino acid fragment caused by its shielding with the methyl group of the neighbouring ligand in Λ-MSATC (Figure 1). Thus, deacylation of SATC and MSATC is described by the $(E1cB)_I$ mechanism which corresponds to $k_2 \gg k_{-1}$ (Scheme 2).

Mutual transformation of the *Z* and *E* isomers under the experimental conditions was not observed. Concentrated DABCO solutions in which slow epimerization does take place under the action of a free base are exceptional. The isomerization of Δ-(*ZZ*)- or Δ-(*EE*)-SDBC is base catalysed with a rate constant $(7.1 \pm 0.8) \times 10^{-5}$ l mol^{-1} s^{-1} at 25 °C in H_2O which is still more than two orders of magnitude smaller than the deacylation rate constant of Δ-SATC under the effect of DABCO (see Table 2, run 4).

Thus, the configuration of the dehydroamino acids obtained is kinetically controlled.

Formally, the *Z*-configuration of α-aminodehydrobutyric acid results from *anti*-elimination of α-hydrogen and acetate ions whereas *E*-α-aminodehydrobutyric acid is the result of *syn*-elimination from *O*-acetylthreonine (Scheme 3).

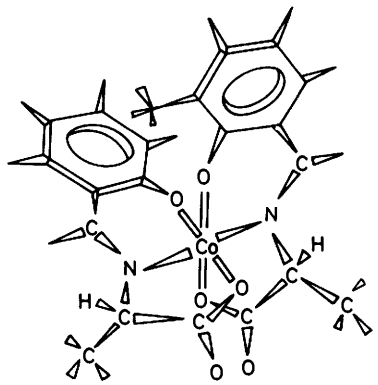
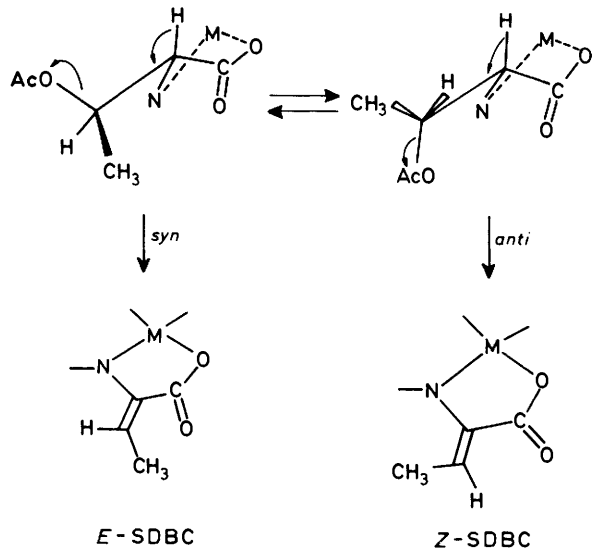
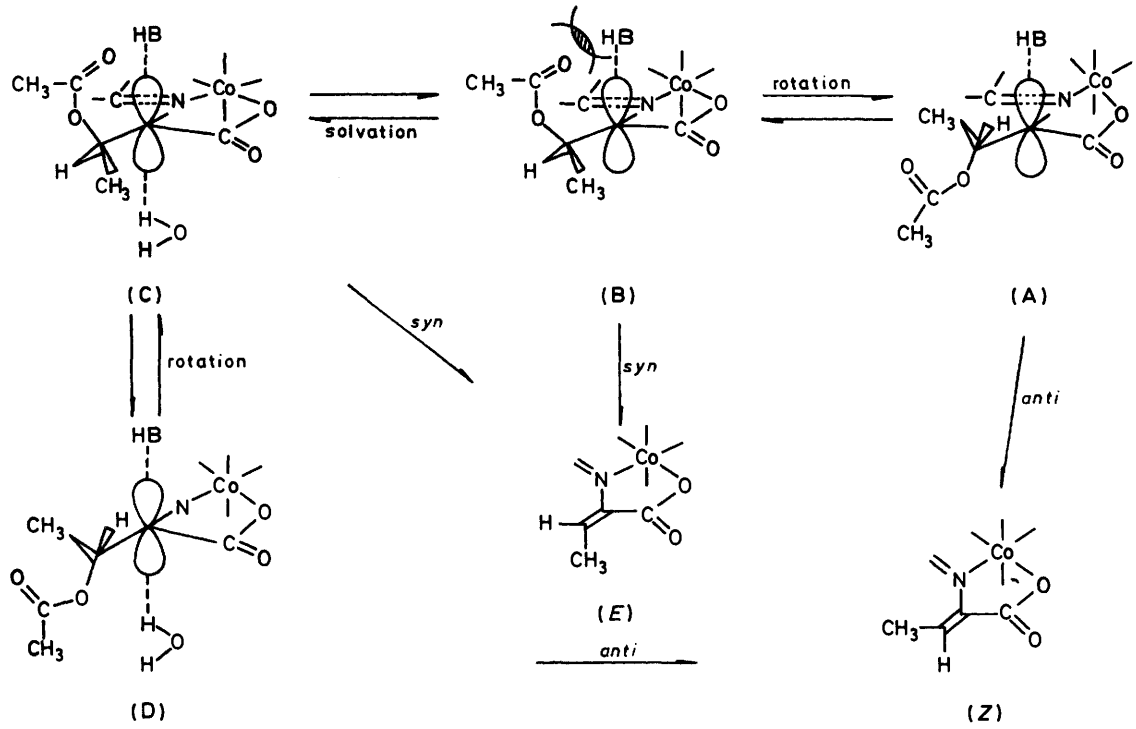


Figure 1. Structure of Λ-bis-[*N*-3-methylsalicylidene-(*S*)-aminoacidato]-cobaltate(III) ion



Scheme 3.

Table 1 shows that both *syn*- and *anti*-elimination take place independently of the structure of the initial diastereoisomers. If the $(E1cB)_I$ mechanistic hypothesis is correct, results recorded in Table 1 may only be explained taking into account the



Scheme 4.

stereochemical reactivity and structure of the intermediate carbanion of *O*-acylthreonine fragment (see Scheme 4). One may assume that the carbanion of the amino acid fragment in the Schiff's bases is planar which is supported by the calculations[16] and by the identical rates of deuterium exchange and racemization of the amino acid fragment in the structurally related Co^{3+} complexes studied earlier.[8] Solvation of such a carbanion may, however, be asymmetrical with the conjugate acid of the catalyst base (BH) still retaining some association with the carbanion in the transition state of elimination as shown in Scheme 4. The dependence of the ratio of the *Z*- and *E*-isomers formed on the nature of the base used provides support for this hypothesis (see Table 1, runs 1—3).

Scheme 4 represents a set of all possible reactive conformations where the mutual orientation of the unshared electron pair in the carbanion and the C–O–Ac bond being broken (dihedral angles 0 and 180°) optimum for β-elimination is realized. Different types of solvation of this carbanion are also given in Scheme 4.

Evidently, the translocation of BH and the leaving Ac group [as in structure (A) leading to the *Z*-isomer] is sterically most favourable for elimination in the initial structures where only one side of the carbanion is solvated with the BH group formed from the base and the α-proton of the amino acid fragment. In structure (B) (leading to the *E* isomer) there is a sterically unfavourable non-bonding interaction of the leaving acyl group and BH. Thus, formation of the *Z*-isomer (*anti*-elimination) is favoured on the basis of purely steric arguments. However if DABCO is used at pH 9.4 (0.51:0.24 DABCO–DABCO·HCl), when the contribution to catalysis from OH^- ion is negligibly small compared with that from DABCO, the favoured stereochemistry of elimination in the case of Δ-SATC tends to become *syn* and an *EE* : *ZZ* ratio of 2.5:1 is observed (Table 1, run 2). Probably, the use of DABCO enhances *syn*-elimination because the non-bonding interaction between the leaving group and the DABCO conjugate acid (which solvates the carbanion) is partially compensated by their electrostatic attraction [carbanions (B) and (C)]. The electrostatic effects are even more pronounced when we pass from hydroxide ion catalysis to that by CO_3^{2-} ion. In a carbonate buffer solution (pH 9.15) where 56% of deacylation proceeds under the action of OH^- and 44% under the action of CO_3^{2-} (see Table 2), the total ratio between the *syn*- and *anti*-elimination is 1:3. This corresponds to > 95% *anti*-elimination upon deacylation under the effect of CO_3^{2-}. This means that the catalyst yielding a negatively charged conjugate acid tends to locate itself in the *anti*-position with respect to the negatively charged leaving group Ac^- [carbanions (D) and (A)].

In the case of OH^- catalysis the contribution of electrostatic effects is negligible because the conjugate acid of the base is a neutral H_2O molecule. In this case for the asymmetrically solvated initially formed carbanion a large contribution from *anti*-elimination would result in a large excess of *Z*- over *E*-isomer. But, as observed in the case of Δ-SATC, deacylation ratio of *Z*- and *E*-isomer of Δ-SDBC (Table 1, run 1) is even smaller than in their thermodynamically equilibrated mixture (Table 1, run 4). Probably the initially formed carbanions (A) and (B) survive long enough to become symmetrically solvated [carbanions (C) and (D)] before the final expulsion of the Ac^- group occurs. The difference in steric energy between carbanions (C) and (D) is evidently not very significant which results in a relatively low predominance of one over the other at equilibrium and as a consequence a low *anti*:*syn* elimination ratio. However, steric shielding of an intermediate carbanion from the side opposite to the abstracted α-proton ought to increase the degree of *anti*-elimination since the approach of the second solvating water molecule with the formation of a symmetrically solvated carbanion would either be slowed or be sterically unfavourable. This situation is realized in deacylation of Δ-MSATC where the methyl substituent in position 3 of the neighbouring ligand effectively shields the *si*-side of an intermediate carbanion (see Figure 2). As expected the extent of

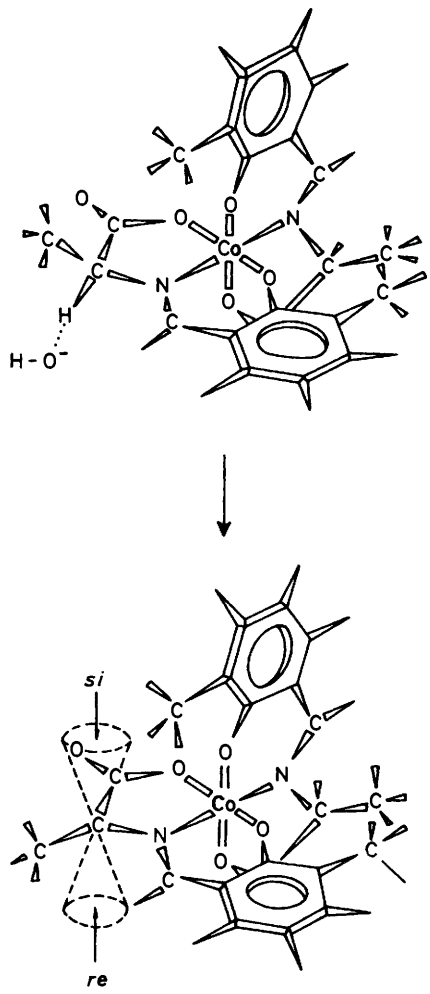


Figure 2. Schematic presentation of sterically hindered approach of the reagent from the *si*-side of the carbanion formed from sodium Δ-bis-[*N*-3-methylsalicylidene-(*S*)-aminoacidato]cobaltate(III)

anti-elimination in the case of Δ-MSATC is greater than that for Δ-SATC (see Table 1, runs 1 and 5). The ratio of the *EE*:*ZZ* isomers changes from 1:2.2 in OH^--catalysed deacylation of Δ-SATC to 1:13 in the case of Δ-MSATC.

We believe that the data presented in this paper have some relevance for an understanding of the mechanism of the pyridoxal-dependent enzymatic reactions.

According to the Dunathan hypothesis, all phenomena related to proton transfer in a reactive Schiff's base formed from pyridoxal and amino acid take place on one side of the cofactor–substrate imine.[6] Intramolecular transfer of the deuterium label from the α-position of tryptophan onto the leaving indole fragment in the course of β-elimination catalysed by tryptophanase (a pyridoxal-dependent enzyme) shows that at least in this case elimination is of the *syn*-type and involves a single basic group of the enzyme active site. It is known that the active sites of many pyridoxal enzymes contain the ε-amino group of lysine or the imidazole fragment of histidine which function as a catalyst of α-proton abstraction from amino acids.[17] Our data indicate that β-elimination of amino acids catalysed by pyridoxal enzymes can be of a *syn*-type due to electrostatic stabilization of the leaving group by a positively charged conjugate acid of the basic group of the active site. A decrease of dielectric constant in the active site of the enzymes (a well known phenomenon)[18] must contribute to the predominance of the *syn*-process.

Dehydration of (*S*)-threonine under the action of threonine dehydrase gives an intermediate α-aminodehydrobutyric acid with a configuration different from that of the same acid produced as an intermediate in preparation of (*S*)-threonine from homoserine under the action of threonine synthetase enzyme.[6a] Evidently, in one of these enzymes the addition (or abstraction) of H_2O follows the *syn*-mechanism and in the other the *anti*-mechanism.

Our results demonstrate that hydrophobic shielding of one site of the carbanion and the location in the enzyme of a negative group responsible for the basic catalysis can change completely the stereochemistry of β-elimination (or addition) in pyridoxal-dependent reactions.

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