Tetrahedron. Vol 33, pp. 2551 to 2564 Pergamon Press 1977 Printed in Great Britain

A SIMPLE STEREOCHEMICAL MODEL OF PYRIDOXAL-DEPENDENT ALDOLASE

ASYMMETRIC CONVERSIONS OF THE AMINO ACID FRAGMENT IN CHIRAL COMPLEXES OF POTASSIUM Λ AND Δ BIS-/N-SALICYLIDENEAMINOACIDATO/COBALTATE(III)

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(*Received in UK 7 December 1976; Accepted for publication 22 March 1977*)

Abstract—Several chiral Co^{3+} complexes with Schiff bases of amino acids glycine (**1**), valine (**3**), threonine (**6**), and salicylaldehyde or 3-methylsalicylaldehyde of the same amino acids (**2**, **4** and **5**) have been synthesized.

Diastereomers (**a** and **b**) of compounds **3–6** were separated on Al_2O_3 and enantiomers (**a** and **b**) of compounds **1** and **2** were resolved with brucine and strychnine. The structures of the obtained compounds were determined by elemental analysis, UV, PMR and ORD spectra.

The Λ-absolute configuration of **5a** was established by X-ray structural analysis. On this basis the Λ-absolute configuration was assigned to all **a** isomers and the Δ-absolute configuration to all **b** isomers.

Kinetics and stereochemistry of α-proton exchange of the amino acid fragment in **1–4** was studied. Exchange of both protons of the glycine fragment in **1** proceeds with approximately the same rate, while the exchange of the α-protons in **2** proceed with different rates. The k^R_{ex}/k^S_{ex} ratio was experimentally established to be approximately 10.

Chiral recognition of prochiral groups or faces in molecules is one of the most important biochemical phenomena.[1] A typical example of such recognition is shown by pyridoxalphosphate-dependent enzymes,[2] e.g., serine transhydroxymethylase from mammalian liver. This enzyme selectively exchanges only pro-S-hydrogen of glycine and catalyzes the glycine conversion into S-serine and S-threonine.[3] The mechanism of the action of pyridoxalphosphate-dependent enzymes has been determined in general features on the basis of the investigation of model systems, in particular metal complexes of Schiff bases of amino acids with pirydoxal or salicylaldehyde (Scheme 1).[4]

Until recently only metals that produce stereochemically labile complexes were used in these model systems.[4] We chose to use stereochemically inert octahedral Co^{3+} complexes. These complexes retain their configuration during chemical conversions of the ligand,[5] thus allowing us to introduce another dimension into the study of model reactions.

In the present work we report on the synthesis, resolution, and absolute configuration determination of enantiomeric and diastereomeric chiral Co^{3+} complexes formed by Schiff bases of amino acids and salicylaldehyde.

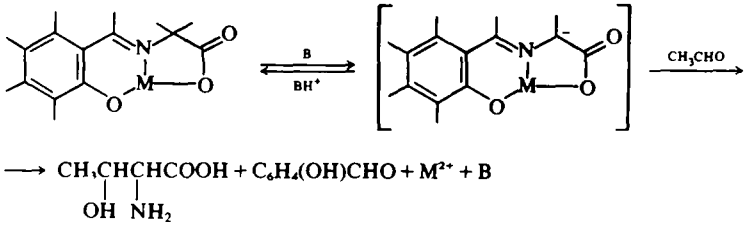
The study of the kinetics and stereochemistry of α-proton exchange in the amino acid fragment of **1–4** and of the stereochemistry of C–C bond formation of the glycine fragment of **1** and **2** with acetaldehyde showed that these complexes represent a simple chemical system which imitates the stereoselectivity of transhydroxymethylase reactions.

EXPERIMENTAL

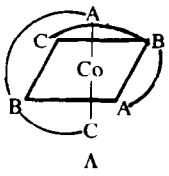
All amino acids were purchased either from "Reachim" or from "Reanal Budapest" and were used without further purification. The enantiomer purity was determined by GLC: S-valine 99.5%, S-threonine 98%. Analytical grade salicylaldehyde was distilled under argon. Pure-grade $CoCl_2 \cdot 6H_2O$ was not additionally purified. The 3-methylsalicylaldehyde was prepared according to the method of Tieman[6] and TU-426-70-grade anhydrous Al_2O_3 was used.

Solns of NaOD in D_2O were prepared by addition of metallic Na under argon to D_2O. The pD values of buffer solutions were determined with a glass electrode on "Radiometer SBR2/SBU1/TTT1" from pD = pH + 0.4, where pH is the observed pH of solution.[7] Concentration of OD ions was calculated from $pK_{D_2O} = pD + pOD$, where $pK_{D_2O} = 14.71$.[7]

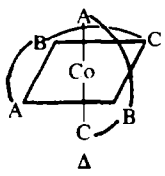
Synthesis of racemic complexes of potassium bis-[N-salicylideneaminoacidato] cobaltate(III) was carried out according to a modification of the technique given in Ref. 8.



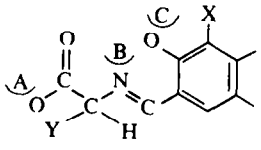
Scheme 1.



Λ



Δ



Compound	Y	X	Abs. configuration
1a	H	H	Λ (OO)
1b	H	H	Δ (OO)
2a	H	CH_3	Λ (OO)
2b	H	CH_3	Δ (OO)
3a	$(CH_3)_2CH$	H	Λ (SS)
3b	$(CH_3)_2CH$	H	Δ (SS)
4a	$(CH_3)_2CH$	CH_3	Λ (SS)
4b	$(CH_3)_2CH$	CH_3	Δ (SS)
5a	$HO(CH_3)_2C$	CH_3	Λ (SS)
5b	$HO(CH_3)_2C$	CH_3	Δ (SS)
6a	$HO(CH_3)_2C$	H	Λ (SS)
6b	$HO(CH_3)_2C$	H	Δ (SS)

A typical synthesis was performed as follows. While stirring, a stream of CO_2-free air was passed for 3 hr through a soln of 0.016 mole of $CoCl_2 \cdot 6H_2O$ and 0.056 mole KOH in 150 ml water containing 0.01 g charcoal. The suspension of cobalt(III) hydroxide was washed with CO_2-free water until neutral. Then a mixture of 0.03 mole of S-valine (containing 99.5% of the pure S form, GLC data) in a small quantity of water, 0.03 mole of salicylaldehyde in a small quantity of alcohol, and a soln of 0.016 mole of KOH in 25 ml water were added. The mixture was stirred at 60° for 30 min, cooled, neutralized with IRC-50 resin (H^+ form) mixed for 15 min, filtered, washed with either and the aqueous layer containing the complexes was evaporated to dryness.

Separation of diastereomers of potassium bis-[N-*salicylidene*-S-*amino-acidato*] *cobaltate*(III) *exemplified by compound* **5**. A mixture of diastereomers obtained in the reaction (starting with 0.022 mole of $CoCl_2 \cdot 6H_2O$) was separated on a column filled with Al_2O_3 (d = 3.7 cm, l = 22 cm, eluent—96% EtOH, flow rate—25 ml/hr). The effectiveness of separation was estimated by TLC. The diastereomers were then additionally purified on a LH-20 Sephadex column eluted with 3:1 EtOH/benzene. The data of the elemental analysis of the compounds are presented in Table 1 (samples were dried *in vacuo* over P_2O_5 before analysis). Compound **1** was obtained and analyzed in Ref. 8.

Resolution of enantiomers of potassium bis-[N-*salicylideneglycinato*] *cobaltate*(III) *and potassium bis*[N-3-*methylsalicylideneglycinato*] *cobaltate*(III)

Compound **1** *was resolved with brucine* according to a modification of the technique given in Ref. 9. Brucine was removed from the active fraction on a column filled with Dowex 50 (Na^+ form). Into the column an alcohol soln of **1** was introduced and the Na^+ salt of the complex was washed out with aqueous alcohol until the eluate became colourless. The eluate was evaporated and the solids were not additionally purified.

Resolution of **1** *with strychnine* was accomplished by the addition of 200 ml of abs alcohol to the dry strychnine salt of **1** (4.78 g) which had been obtained according to Ref. 9. The mixture was boiled and yielded undissolved crystals and soln. The undissolved crystals (2.18 g $[M]_{530} = -13{,}000$) worked up in the following manner. They were converted to the Na form as described for the brucine salt of compound **1**. The Na salt was dissolved in alcohol:benzene (3:1) and left over night. The crystals of the racemic salt were then filtered. The enriched filtrate was purified on LH-20. The Na^+ salt with $[M]_{530} = -17.800$ corresponding to 89% optical purity was obtained.

From the filtrate of the strychnine salt the other isomer with $[M] = +14.000$ was obtained.

Resolution of **2** *with strychnine* was accomplished by the addition of 250 ml of abs alcohol to 3.38 g of the dry strychnine salt. The soln was heated to boiling and left to crystallize at room temp. The resulting crystals contained the enantiomer with a negative Cotton effect associated with the 570 nm band and the filtrate yielded the other enantiomer. These crystals with negative Cotton effect were repeatedly recrystallized from a mixture of abs alcohol and benzene up to a constant specific rotation. The end product had a value of 246. Removal of strychnine and purification of the isomer are described above. The strychnine salt yielded a Na salt with a negative Cotton effect (λ = 570 nm, a = 215, $[M]_{650} = -11.500$ and $[M]_{540} = +10.000$).

UV spectra and ORD curves of **1–6** (Figs. 1 and 3) were recorded respectively on a Hitachi EPS spectrophotometer and a Jasco ORD/UV-5 spectropolarimeter.

Electrochemical reduction Co(III)–Co(II). The electrochemical experiment was carried out in a 0.2 molar soln of KH_2PO_4 at a mercury cathode. The potential was maintained with a P-5827 potentiostat. An AgCl electrode served as a reference electrode. The anode was a Pt spiral wire immersed in a 0.2 M soln of K_2HPO_4. Cathode and anode compartments were separated by a cation-exchange membrane. A typical electrochemical experiment is described below. A complex (0.1 mole) was dissolved in 100 ml of 0.2 M KH_2PO_4 supporting electrolyte and placed into the cathode compartment. The reduction was carried out for 4.5–9 hr at a potentional of −0.6 to −0.86 while the current density varied from 0.4 to 0.04 mA/cm² (pH range of 4.5–6.5). The degree of reduction was estimated from the polarogram of the end product recorded with a LP-7 instrument. After reduction the solution was repeatedly extracted with ether to remove salicylaldehyde. The soln was then passed through a column filled with IRC-50 (Na^+ form) to remove Co(III). The resulting aqueous soln was put onto a column with Dowex 50 (H^+ form). The column was washed with water and the amino acids were then eluated with a 5% ammonia solution. The eluate was evaporated and the dry residue was analyzed by GLC.

Enantiomer and quantitative analysis of amino acids. The amino acid was esterified in a 5% HCl soln of isopropanol (25 ml of soln per mmole of amino acid) for 5 hr at 100°. After evaporation to dryness, the residue was treated for 4 hr at room temp. with a 10% soln of $(CF_3CO)_2O$ in CH_2Cl_2 (25 ml per mmole of amino acid). This mixture was evaporated to 1/10 of the original volume and analyzed by GLC using a (0.5 mm × 90 m) steel capillary column which was filled with the cyclohexyl ester

Table 1. Elemental analysis of compounds **2–6**

Complex	C (%) Found	C (%) Calc.	H (%) Found	H (%) Calc.	N (%) Found	N (%) Calc.
2a $Na[Co(N\text{-}3\text{-}Met\text{-}Sal\text{-}Gly)_2]\cdot 3H_2O$	46.33	46.33	4.60	4.63	5.54	5.41
2b $Na[Co(N\text{-}3\text{-}Met\text{-}Sal\text{-}Gly)_2]\cdot 1.5H_2O$	48.9	48.99	4.48	4.43	5.7	5.46
3a $K[Co(Sal\text{-}S\text{-}Val)_2]\cdot 2H_2O$	50.29	50.35	5.39	5.25	5.08	4.9
3b $K[Co(Sal\text{-}S\text{-}Val)_2]\cdot 2H_2O$	50.38	50.35	4.93	5.25	5.19	4.9
4a $K[Co(N\text{-}3\text{-}Met\text{-}Sal\text{-}S\text{-}Val)_2]\cdot 2H_2O$	51.96	52.0	5.93	5.68	4.75	4.67
4b $K[Co(N\text{-}3\text{-}Met\text{-}Sal\text{-}S\text{-}Val)_2]\cdot 2H_2O$	52.46	52.0	5.65	5.68	5.38	4.67
5a $K[Co(N\text{-}3\text{-}Met\text{-}Sal\text{-}S\text{-}Thr)_2]\cdot 1.5H_2O$	49.39	49.74	4.77	5.01	4.70	4.84
5b $K[Co(N\text{-}3\text{-}Met\text{-}Sal\text{-}S\text{-}Thr)_2]\cdot 1.5H_2O$	49.74	49.74	4.98	5.01	4.75	4.84
6a $K[Co(Sal\text{-}S\text{-}Thr)_2]$	48.83	48.81	4.65	4.07	5.19	5.21
6b $K[Co(Sal\text{-}S\text{-}Thr)_2]\cdot 1.5H_2O$	46.6	46.56	4.43	4.41	4.69	4.94

of N-trifluoroacetyl-S-valyl-S-valine as the stationary phase.[10] The peak areas were determined with a Varian-480 integrator. The accuracy of determination of the amino acid enantiomers ratio was 0.02%.

Quantitative GLC analysis of amino acids was carried out according to the technique described in Ref. 11, using S-alanine as an internal standard.

Determination of thermodynamic equilibrium between diastereomers of **3–6**. Diastereomers of **3–6** were brought to equilibrium in the following manner. To a pair of solns each of which contained one of the diastereomers (0.1 g in 20 ml of alcohol), 0.05 g of charcoal was added. This mixture was boiled until the ratio of the diastereomers in both solns became equal. The solns were cooled, filtered and the filtrate was evaporated. Diastereomer mixtures were separated by preparative TLC, washed with water and the diastereomer ratio at equilibrium was determined spectrophotometrically at 380 nm.

Deuterium exchange of the α-proton of an amino acid moiety in **1–4** *and inversion of the asymmetric carbon in* **3–4**. Proton exchange in **1** and **2** was carried out at pD = 10.59 in a carbonate buffer soln (0.1 mole/l of $NaHCO_3$ and 0.15 mole/l of Na_2CO_3) under Ar. Proton exchange and inversion of the asymmetric carbon in **3** and **4** occur under the action of 0.04 N NaOD in D_2O. The experimental procedure for deuterium exchange was the same for all compounds. A typical experiment of proton exchange is exemplified by **3a**. The compound **3a** (0.3 g) was dissolved in 60 ml of 0.043 N NaOD in D_2O and allowed to stand at 25 ± 0.1°. At given time intervals 10-ml samples were taken, neutralized with 0.43 ml of 1 M H_3PO_4, and evaporated to dryness. The complex was then extracted with a mixture of benzene and alcohol (5:1); the organic phase was evaporated and dried *in vacuo* over P_2O_5. The degree of deuterium exchange was determined by the integration of the resonance PMR signals of the α-proton of the amino acid at 34° in CD_3OD. To eliminate superimposition of the α-proton and OH signals in the case of **2**, PMR spectra were recorded at +45°.

The same samples from the PMR experiments were used for the determination of the degree (%) inversion in the amino acid moiety in **3** and **4**. The samples were decomposed electrochemically, the free amino acid isolated and the relative amount of the two enantiomers was determined.

Calculation of the rate constants of deuterium exchange of **1**, **3** *and* **4** *and carbon inversion in* **3** *and* **4**. The observed psuedo first order rate constant (K_{obs}) for deuterium exchange was determined by a least squares fit of the logarithm of the aldimine/α-proton ratio vs time. The second order rate constant ($K_{ex}^{OD^-}$) is equal to $K_{obs}/[OD^-]$. To determine the rate constant of the α-carbon inversion, a graph of $\ln 100/\beta$ vs time coordinates was plotted, where β is the relative content (in %) of S-valine in the mixture of S and R-forms.

Calculation of rate constants of deuterium exchange in **2**. Resolution of exponents was performed on a "Promin-2" computer as described in Ref. 12.

Asymmetric synthesis of threonine. The procedure is the same both for **1** and **2**. All operations were carried out under Ar. The following is a description of a typical experiment. Compound **1a** (0.3 g, 6.4 mmole) was dissolved in 120 ml of aqueous buffer soln ($NaHCO_3$ = 0.025 mole/l, NaOH = 0.005 mole/l) with pH = 8.5 at 25°. Freshly distilled acetaldehyde (3.6 ml, 0.064 mole) was then added under strong stirring. Samples were taken at certain intervals and neutralized with 1 M H_3PO_4. An internal amino acid standard was added to every sample prior to electrochemical decomposition. Electrochemical decomposition, quantitative analyses and enantiomer analyses of amino acids were described above.

The total quantity of amino acids present as threonine and glycine at the end of these experiments was always equal to the quantity of glycine in the initial complex.

Determination of the rate constants of base-catalyzed acetaldehyde addition to **1** *by PMR*. The compound **1** (0.08 g, 0.174 mmole) was dissolved under Ar in 1 ml of soda buffer in D_2O (0.1 M $NaHCO_3$/0.15 M Na_2CO_3, pD = 10.35) with 0.0154 g (4.1×10^{-4} mole) of CH_3CHO. The mixture was placed in an ampoule and the disappearance of the PMR signals of the acetaldehyde Me group at 2.15 ppm and of the glycine α-proton at 5.0 ppm were recorded simultaneously. The rate constants were calculated assuming pseudo first order kinetics.

Kinetics of threonine formation in H_2O. The rate of condensation of the glycine moiety with acetaldehyde in **1** and **2** was estimated from the amount of Thr formed during the reaction. The concentration of acetaldehyde and of the complex, the temp. and experimental procedure were the same as during the determination of the asymmetric yield of threonine (see above). Aqueous buffer solns (pH = 8.5 and μ = 0.05) were used for **1** and (pH = 9.82 and μ = 0.05) for **2**. Measurements were made on the first 10% of the reaction. The pseudo first order rate constants were calculated according to $\ln [n_\infty/(n_\infty - n_t)] = K_{obs}t$ where n_t and n_∞ are the molar fractions of threonine in the mixture of threonine and glycine formed at time t and after 8 half-lives of the reaction respectively and where t is the time (in sec) at which the sample was taken.

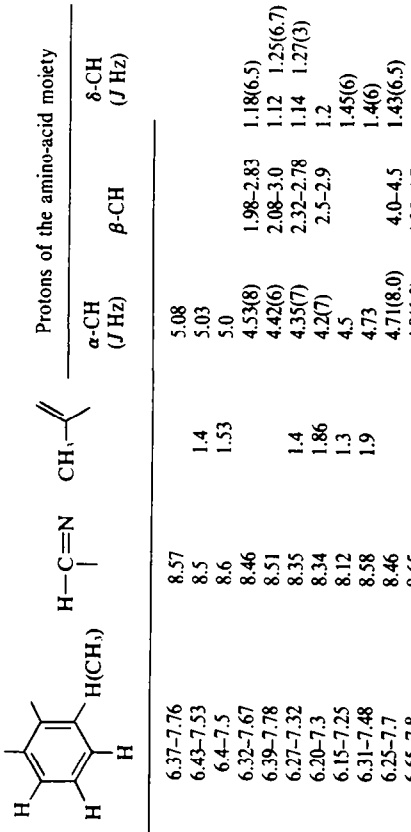
X-ray data for **5a** *crystals*. The crystals which were obtained from abs EtOH were measured on an automatic 4-cycle Hilger–Watts difractometer (Mo–K_α radiation, graphite monochromator) according to the technique in Ref. 13. Crystals of **5a** are monoclinic: a = 8.518(1), b = 23.017(2), c = 7.708(1) Å, β = 104.87(1) Å, d_{ex} = 1.41, d_{calc} = 1.43 g/cm³ for $Z = 2(C_{24}H_{26}N_2O_8Co\cdot C_2H_5OH\ 1.25H_2O)$, space group $P2_1$. The total number of reflections was 2400 ($\vartheta \leq 20°$, $\vartheta/2\vartheta$-scan by the method of ordinate analysis[14]); for structure soln using computer program[15] 2000 reflections with $F^2 \geq 3\sigma$ were used, 1400 of them being independent.

Coordinates of the Co atom were determined from a 3-dimensional Patterson map. All light non-H atoms including those of solvating molecules of water and EtOH were located by several subsequent Fourier syntheses.

The structure was refined by the anisotropic block-diagonal least-square method using published computer programs.[16] Hydrogen atoms were located from a difference synthesis. Besides peaks interpreted as H atoms in this map, a peak was found which exceeded H atom peaks by 2–3 times. From interatomic distances this peak was concluded to correspond to the O atom of the water molecule (W_2) with a population of 0.25 (the H atoms of the W_2 molecule were impossible to locate).

The final refinement, anisotropic for nonhydrogen and iso-

Table 2. Chemical shifts of **1–6** (ppm)



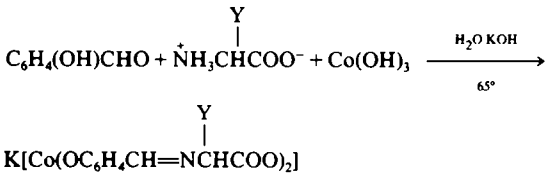
Design	Complex	Internal reference	Solvent	H	H(CH₃)	H—C=N	CH₃	Protons of the amino-acid moiety: α-CH (J Hz)	β-CH	δ-CH (J Hz)
1 rac.	[Co(Sal-Gly)₂]K	DSS	D_2O	6.37–7.76		8.57		5.08		
2a rac.	[Co(N-3-Met-Sal-Gly)₂]Na	DSS	D_2O	6.43–7.53		8.5	1.4	5.03		
2b	Δ[Co(N-3-Met-Sal-Gly)₂]Na	HMDS	CD_3OD	6.4–7.5		8.6	1.53	5.0		
3a	Λ[Co(Sal-S-Val)₂]K	DSS	D_2O	6.32–7.67		8.46		4.53(8)	1.98–2.83	1.18(6.5)
3b	Δ[Co(Sal-S-Val)₂]K	DSS	D_2O	6.39–7.78		8.51		4.42(6)	2.08–3.0	1.12 1.25(6.7)
4a	Λ[Co(N-3-Met-Sal-S-Val)₂]K	HMDS	CD_3OD	6.27–7.32		8.35	1.4	4.35(7)	2.32–2.78	1.14 1.27(3)
4b	Δ[Co(N-3-Met-Sal-S-Val)₂]K	HMDS	CD_3OD	6.20–7.3		8.34	1.86	4.2(7)	2.5–2.9	1.2
5a	Λ[Co(N-3-Met-Sal-S-Thr)₂]K	HMDS	CD_3OD	6.15–7.25		8.12	1.3	4.5		1.45(6)
5b	Δ[Co(N-3-Met-Sal-S-Thr)₂]K	HMDS	CD_3OD	6.31–7.48		8.58	1.9	4.73		1.4(6)
6a	Λ[Co(Sal-S-Thr)₂]K	HMDS	$D_2O + D_2SO_4$	6.25–7.7		8.46		4.71(8.0)	4.0–4.5	1.43(6.5)
6b	Δ[Co(Sal-S-Thr)₂]K	DSS	$D_2O + D_2SO_4$	6.65–7.8		8.65		4.8(6.0)	4.25–4.71	1.55(6.5)

PMR spectra of **1–6** were recorded on Hitachi-Perkin-Elmer R-20 or Perkin-Elmer R-12 instruments.

tropic for hydrogen and O(W_2) atoms gave R = 0.047 (R_w = 0.046). The absolute configuration of the complex was determined by the Hamilton test[17] (for the inverted structure R = 0.057 and R_W = 0.053) and by the Bijvoet method[18] with 10 Friedel pairs. The final coordinates and temperature factors are given in Tables 3 and 4.

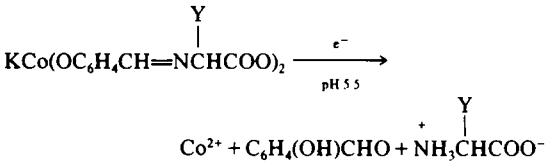
RESULTS

(a) *Synthesis and separation of isomers of* **1–6(a** *and* **b)**. Compounds **1–6** were synthesized by reaction of salicylic or 3-methylsalicylic aldehydes, amino acids, and $Co(OH)_3$ in water according to Scheme 2.



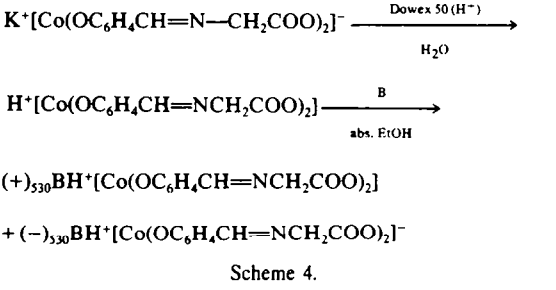
Scheme 2.

All synthesized complexes carry a negative charge according to electrophoresis data. Typical elemental analysis corresponds to potassium bis-[N-salicylideneaminoacidato] cobaltate(III). Electrochemical reduction of Co(III) in these complexes according to Scheme 3 yields salicylaldehyde and amino acid in theoretically expected ratios and the S-amino acids retain their initial optical purity.



Scheme 3.

TLC on Al_2O_3 shows that **1** and **2** are individual substances giving one spot. However, each of the compounds **3–6** were preparatively separated on Al_2O_3 into two individual substances, **a** and **b**, in the order of emergence from the column, that have identical elemental analyses (Table 1), similar UV spectra (see, for instance, Fig. 1), and identical sets of PMR signals differing only by chemical shifts (Table 2). The PMR spectra of **4a** and **4b** are given in Fig. 2. In Fig. 1(a) and (b) the ORD curves of these **3–6** isomers are shown. The brucine and strychnine salts of **1** and **2** obtained according to Scheme 4 were separated into two types—readily and poorly soluble salts.



Scheme 4.

where B is strychnine or brucine.

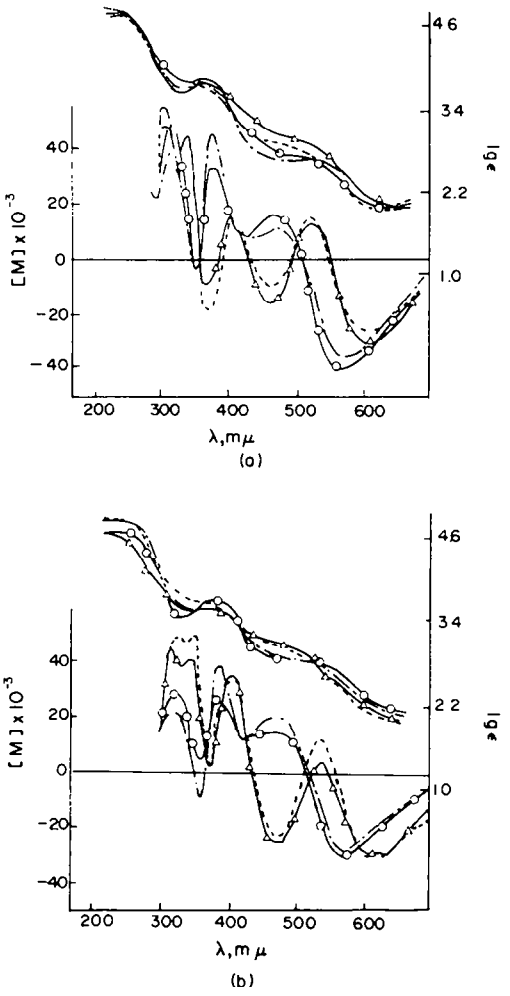


Fig. 1. UV spectra and ORD curves in water at 25°, pH = 6.5. (a) ———, **3a**; —·—, **3b**; —Δ—, **6a**; —○—, **6b**; (b) ———, **4a**; —·—, **4b**; —Δ—, **5a**; —○—, **5b**.

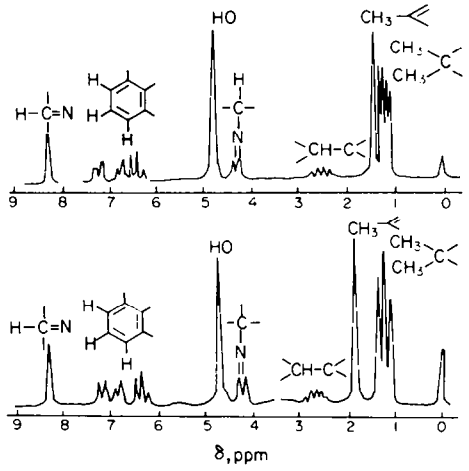


Fig. 2. PMR spectra of **4a** and **4b** recorded in CD_3OD, internal reference HMDS.

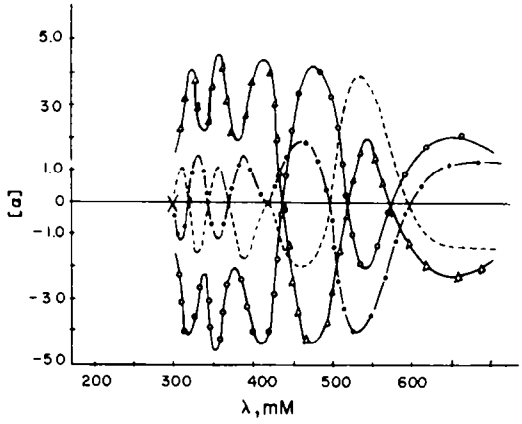


Fig. 3. ORD curves of $(+)_{530}$ and $(-)_{530}$ isomers of I and II recorded in H_2O at 25°. ———, $(+)_{530}$ **1**; —·—, $(-)_{530}$ **1**; —Δ—Δ, $(+)_{530}$ **2**; —Δ—Δ, $(-)_{530}$ **2**.

Brucine or strychnine is readily replaced by other ions according to Scheme 5.

ligands. The distortion of the coordination polyhedron is not very pronounced (Table 7). The mean value of the NCoO(2) angle in the 5-member metalocycle is 83.1(1)°

$$(+)_{530}BH^{+}[Co(OC_6H_4CH{=}NCH_2COO)_2]^{-} + \xrightarrow[H_2O,\ EtOH]{\text{Dowex 50 } (K^+)} (+)_{530}K^{+}[Co(OC_6H_4CH{=}NCH_2COO)_2]$$

Scheme 5.

The ORD curves of $(+)_{530}$ and $(-)_{530}$ isomers of **1** and **2** are presented in Fig. 3. The $(+)_{530}$ isomers of **1** and **2** are designated as (a) and $(-)_{530}$ as (b).

(b) *Crystal structure of* **5a**. The X-ray determined structure is composed of Λ-bis-[N-salicylidene-S-threoninato] cobaltate(III)† anions, Na^+ cations and the solvating molecules of ethanol and water. Bond lengths and angles in the two salicylidenethreoninate ligands are given in Tables 5 and 6. The anion has an approximate non-crystallographic C_2 symmetry. The bond lengths and angles averaged for this symmetry are given in Fig. 4.

The coordination polyhedron of the cobalt is a distorted octahedron consisting of four O atoms and two N atoms of the two terdentate salicylidenethreoninate and of the NCoO(1) angle is the 6-member cycle is 92.5(4)°. The angle O(1)CoO(2) is 175.5(4)° which should be compared with the ideal angle of 180°. The corresponding angles in sodium Λ, Δ-bis-[N-salicylideneglycinato] cobaltate(III) trihydrate (**1**) have similar values:[20] 85.8, 96.1 and 176.7°. The mean values of the bond lengths (Co–O(1) = 1.880(8), Co–O(2) = 1.929(4) and Co–N = 1.886(4) Å) are also similar to those found in **1** (1.87, 1.91 and 1.89 Å respectively) and are typical of octahedral amino acid complexes of Co(III). For instance, the lengths of Co–O and Co–N bonds in calcium [bis-L-aspartato] cobaltate decahydrate[21] are in the 1.879–1.931 Å range.

The geometry of the anion in **5a** is characterized by considerable non-planarity of the salicylidenethreoninate ligands, while in **1**, the salicylideneglycinate ligands are practically planar.

†Λ and Δ correspond respectively to left and right spiral arrangement of ligands in relation to C_2 axis.[19]

Table 3. Atomic coordinated ($\times 10^4$) and parameters of temperature factor $T = \exp[-10^{-4}(b_{11}h^2 + b_{22}k^2 + b_{33}l^2 + b_{12}hk + b_{13}hl + b_{23}kl)]$

Atom	X	Y	Z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Co	6962(1)	3/4	6092(1)	75(1)	10(0)	89(2)	−4(1)	20(2)	5(1)
Na	8357(4)	6793(2)	1080(4)	137(6)	23(1)	161(7)	0(4)	51(10)	13(4)
O(1)	7014(6)	8159(2)	4646(6)	120(9)	18(1)	95(10)	0(5)	17(15)	14(6)
O(2)	6722(6)	6822(2)	7465(6)	89(8)	13(1)	116(10)	20(5)	32(14)	3(5)
O(3)	5324(7)	5995(2)	7239(7)	135(10)	12(1)	236(13)	−23(6)	16(19)	32(7)
O(4)	3816(6)	6699(3)	1727(7)	160(10)	24(1)	104(10)	−15(6)	−49(35)	−41(12)
O(1′)	6238(6)	7956(2)	7743(6)	110(9)	12(1)	133(11)	−4(5)	73(15)	0(6)
O(2′)	7867(6)	7035(2)	4496(6)	112(8)	19(1)	98(10)	0(5)	53(14)	−13(5)
O(3′)	10228(6)	6808(3)	3955(7)	133(10)	38(2)	164(12)	23(7)	112(18)	−48(8)
O(4′)	10649(7)	6814(3)	9945(6)	178(10)	28(2)	78(10)	44(7)	77(17)	10(7)
N	4803(7)	7330(3)	4861(8)	87(10)	16(2)	134(12)	−2(6)	72(17)	3(7)
N′	9172(7)	7606(3)	7306(7)	108(10)	11(2)	104(11)	6(6)	−20(17)	−7(7)
C(1)	5532(9)	6479(3)	6663(10)	110(14)	12(2)	102(16)	8(8)	3(22)	−12(8)
C(2)	4387(8)	6713(3)	4991(9)	68(11)	11(2)	110(14)	−6(7)	36(20)	9(8)
C(3)	4497(10)	6366(3)	3317(10)	152(16)	14(2)	126(17)	−19(8)	114(26)	12(9)
C(4)	3579(12)	5824(4)	3136(14)	210(19)	12(2)	283(24)	−15(10)	49(16)	0(6)
C(5)	3715(10)	7707(3)	4054(10)	111(13)	16(2)	104(15)	0(7)	27(23)	22(8)
C(6)	4156(9)	8309(3)	3895(9)	133(14)	12(2)	90(16)	34(8)	−44(23)	14(9)
C(7)	2809(11)	8707(4)	3338(11)	167(18)	27(2)	93(16)	22(10)	23(27)	12(10)
C(8)	3106(12)	9287(4)	3159(13)	247(22)	15(2)	217(21)	46(11)	55(33)	12(11)
C(9)	4774(13)	9474(4)	3504(12)	261(24)	10(2)	150(19)	−25(10)	−28(32)	−18(9)
C(10)	6028(11)	9103(4)	4024(11)	197(17)	15(2)	129(17)	23(9)	20(28)	25(9)
C(11)	5736(10)	8504(4)	4196(10)	178(15)	17(2)	88(16)	−3(9)	14(25)	14(9)
C(12)	7708(12)	9332(3)	4383(13)	247(19)	8(2)	229(17)	−32(9)	−17(28)	−23(9)
C(1′)	9432(9)	6990(4)	4944(10)	112(13)	15(2)	149(17)	−6(8)	−26(24)	2(10)
C(2′)	10253(9)	7155(4)	6863(10)	104(14)	19(2)	111(16)	26(8)	0(23)	−6(9)
C(3′)	10345(10)	6634(3)	8135(10)	157(15)	14(2)	111(15)	11(8)	0(24)	12(9)
C(4′)	11653(12)	6194(5)	7857(11)	224(21)	30(3)	131(18)	103(12)	109(31)	−6(11)
C(5′)	9688(9)	8053(3)	8233(10)	121(14)	14(2)	120(16)	1(8)	21(23)	7(9)
C(6′)	8684(10)	8501(3)	8669(9)	174(16)	10(2)	71(14)	−2(8)	0(22)	−30(8)
C(7′)	9445(10)	9028(4)	9474(10)	176(18)	26(2)	114(17)	−44(10)	51(25)	−16(10)
C(8′)	8536(13)	9462(4)	9933(12)	228(21)	25(2)	143(19)	−37(11)	44(31)	−47(11)
C(9′)	6939(13)	9391(4)	9643(12)	259(23)	18(2)	160(19)	−11(11)	77(32)	−11(10)
C(10′)	6121(10)	8897(3)	8871(10)	187(16)	16(2)	121(16)	−2(9)	98(26)	−24(10)
C(11′)	6998(9)	8442(3)	8407(9)	99(14)	15(2)	71(14)	−7(8)	−20(22)	−14(8)
C(12′)	4307(11)	8807(4)	8719(13)	177(18)	25(3)	259(25)	0(11)	74(34)	−51(13)
O(5)†	7342(10)	5887(3)	637(10)	400(20)	28(2)	261(18)	−25(10)	−173(30)	39(10)
C(13)†	8496(15)	5304(5)	3165(16)	314(29)	21(3)	369(31)	42(14)	−89(49)	61(16)
C(14)†	7600(18)	5324(6)	1229(16)	40(35)	35(4)	289(31)	60(19)	−222(54)	47(19)
$O(W_1)$	6160(7)	7422(3)	938(7)	208(10)	23(2)	213(12)	−35(8)	177(18)	21(9)
$O(W_2)$‡	9110(40)	7960(14)	2460(40)	—	—	—	—	—	—

†Solvating EtOH.
‡This atom was refined isotropically with position multiplicity 0.25 ($B_{iso} = 5.1(7)$ Å^2).

Table 4. Hydrogen atoms coordinates ($\times 10^3$) (temperature factors $B_{iso} = 5.0$ Å^2)

Atom	X	Y	Z	Atom	X	Y	Z
H(2)	314(8)	667(3)	514(9)	H(4′.3)	140(8)	598(3)	650(9)
H(3)	566(8)	629(3)	348(8)	H(5′)	1076(8)	820(3)	874(9)
H(4.1)	238(9)	595(3)	293(9)	H(7′)	1027(9)	908(3)	875(9)
H(4.2)	390(8)	552(3)	355(9)	H(8′)	911(9)	982(3)	1041(9)
H(4.3)	351(8)	563(3)	207(9)	H(9′)	650(8)	971(3)	995(9)
H(5)	269(9)	751(3)	350(9)	H(12′.1)	366(8)	913(3)	807(9)
H(7)	159(9)	856(3)	318(9)	H(12′.2)	431(9)	881(3)	994(9)
H(8)	202(8)	960(3)	312(9)	H(12.′3)	364(8)	858(3)	766(9)
H(9)	484(8)	988(3)	341(9)	H(04′)	1208(9)	727(3)	958(9)
H(12.1)	817(9)	920(3)	370(9)	H(13.1)	786(8)	529(3)	212(9)
H(12.2)	879(8)	935(3)	528(9)	H(13.2)	941(8)	523(3)	272(8)
H(12.3)	802(9)	984(3)	397(9)	H(13.3)	820(9)	549(3)	335(9)
H(04)	411(9)	689(3)	90(9)	H(14.1)	797(8)	557(3)	35(9)
H(2′)	1146(18)	731(3)	680(9)	H(14.2)	690(9)	490(3)	60(9)
H(3′)	926(8)	633(3)	789(3)	H(05)	648(9)	590(3)	7(9)
H(4′.1)	238(8)	618(3)	666(9)	$H(W_1.1)$	596(12)	766(4)	1001(12)
H(4′.2)	221(8)	581(3)	867(9)	$H(W_1.2)$	670(12)	731(4)	682(12)

Table 5. Bond lengths d(Å)

Bond	In L†	In L'†	Average	Bond	In L	In L'	Average
Co–O(1)	1.889(5)	1.872(5)	1.880	C(2)–C(3)	1.54(1)	1.54(1)	1.54
Co–O(2)	1.925(5)	1.933(5)	1.929	C(3)–C(4)	1.46(1)	1.56(1)	1.51
Co–N	1.882(5)	1.890(5)	1.886	C(5)–C(6)	1.45(1)	1.44(1)	1.45
C(1)–O(2)	1.31(1)	1.29(1)	1.30	C(6)–C(7)	1.44(1)	1.43(1)	1.44
C(1)–O(3)	1.23(1)	1.22(1)	1.23	C(7)–C(8)	1.37(1)	1.37(1)	1.37
C(3)–O(4)	1.44(1)	1.42(1)	1.43	C(8)–C(9)	1.44(1)	1.33(1)	1.39
C(11)–O(1)	1.32(1)	1.33(1)	1.33	C(9)–C(10)	1.35(1)	1.39(1)	1.37
C(2)–N	1.47(1)	1.48(1)	1.48	C(10)–C(11)	1.41(1)	1.39(1)	1.40
C(5)–N	1.31(1)	1.27(1)	1.29	C(6)–C(11)	1.38(1)	1.41(1)	1.40
C(1)–C(2)	1.50(1)	1.51(1)	1.51	C(10)–C(12)	1.48(1)	1.53(1)	1.51
				O(5)–C(13)	1.37(1)	—	—
				C(13)–C(14)	1.49(1)	—	—

†L and L'—two ligands of the anion related by the pseudo-C_2 axis.

Table 6. Bond angles (ω^0)

Angle	In L	In L'	Average	Angle	In L	In L'	Average
O(1)CoO(2)	175.3(3)	175.9(3)	175.6	C(10)C(11)O(1)	117.3(8)	119.7(8)	118.5
O(2)CoN	83.1(3)	83.0(3)	83.1	C(5)C(6)C(7)	115.4	118.6(8)	117.0
O(1)CoN	92.1(3)	92.9(3)	92.5	C(2)NC(5)	121.3(8)	123.5(8)	122.4
CoNC(5)	125.7(4)	122.8(4)	124.3	CoO(2)C(1)	114.1(3)	115.3(3)	114.7
NC(5)C(6)	120.6(7)	125.1(7)	122.8	O(2)C(1)C(2)	116.3(7)	115.3(7)	115.8
C(5)C(6)C(11)	124.0(8)	123.0(8)	123.5	C(1)C(2)N	106.7(7)	104.0(8)	105.4
C(6)C(11)O(1)	123.4(8)	121.1(8)	122.3	C(2)NCo	112.8(4)	113.2(4)	113.0
C(11)O(1)Co	119.8(8)	121.4(8)	120.6	O(2)C(1)O(3)	122.9(7)	124.2(7)	123.6
C(6)C(7)C(8)	119.6(9)	120.4(9)	120.0	C(2)C(1)O(3)	120.8(7)	120.5(7)	120.7
C(7)C(8)C(9)	118.0(9)	119.4(8)	118.7	C(1)C(2)C(3)	111.7(8)	111.2(9)	111.5
C(8)C(9)C(10)	122.4(9)	123.5(9)	123.0	NC(2)C(3)	112.3(5)	109.4(9)	110.7
C(9)C(10)C(11)	120.0(9)	119.1(9)	119.6	C(2)C(3)C(4)	112.3(5)	109.9(9)	111.1
C(10)C(11)C(6)	119.3(9)	119.2(9)	119.3	C(2)C(3)O(4)	110.0(5)	111.4(8)	110.7
C(11)C(6)C(7)	120.6(9)	118.4(9)	119.5	C(4)C(3)O(4)	106.6(7)	111.7(7)	109.2
C(9)C(10)C(12)	119.0(8)	122.0(8)	121.5				
C(11)C(10)C(12)	120.8(8)	118.5(8)	119.7	C(13)C(14)O(5)	111.0(8)	—	—

Table 7. Angles in the coordination polyhedron of Co atom

Angle	ω^0	Angle	ω^0	Angle	ω^0
O(1)CoO(1')	90.7(3)	O(1')CoO(2)	88.9(3)	O(2)CoN	83.1(3)
O(1)CoO(2)	175.3(3)	O(1')CoO(2')	175.9(3)	O(2)CoN'	93.7(3)
O(1)CoO(2')	89.7(3)	O(1')CoN	90.6(3)	O(2')CoN	93.5(4)
O(1)CoN	92.1(3)	O(1')CoN'	92.9(3)	O(2')CoN'	83.0(3)
O(1)CoN'	91.0(3)	O(2)Co(2')	91.0(3)	NCoN'	175.3(3)

It is convenient to describe the ligand conformations in **5a** with respect to the dihedral angles between the planes CoO(1)O(2)N(**1**)†, CoO(1')O(2')N'(**1'**)†, O(1)C(5)–C(11) (**2**), O(1')C(5')–C(11') (**2'**), C(1)C(2)O(2)O(3) (**3**) and C(1')C(2')O(2')O(3') (**3'**) (Table 8, **1/3** 18°, **1'/3'** 16°, **1/2** 33° and **1'/2'** 31°).

An analogous conformation with planar fragments tilted away in one direction from the C(2)NC(5)C(6) plane is observed in terdentate ligands of bis-(pyridoxalidene-DL-valinato)nickel.[22]

The 5-member metalocycles in **5a** differ in their distortion from planarity: the C(1) and C(2) atoms are tilted away from the CoNO(2) plane in the same direction by 0.38 and 0.59 Å respectively, but the C(1') and C(2') atoms are distorted from CoN'O(2') plane by 0.04 and −0.20 Å respectively. Possibly this difference of the

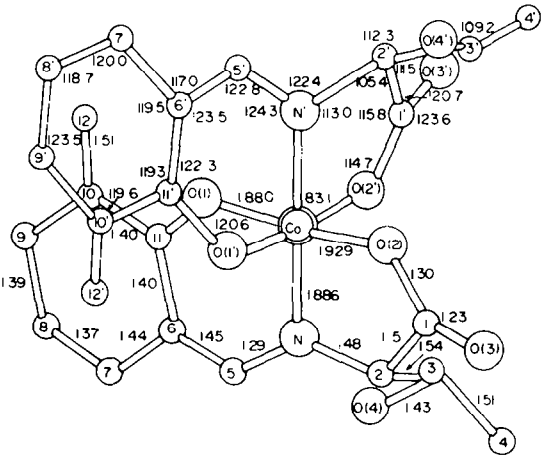


Fig. 4. Structure of complex anion (**5a**) of C_2 symmetry with averaged bond lengths and angles.

†The coordination environment of Co is ideally planar and the ligand projections on these planes are shown in Fig. 5.

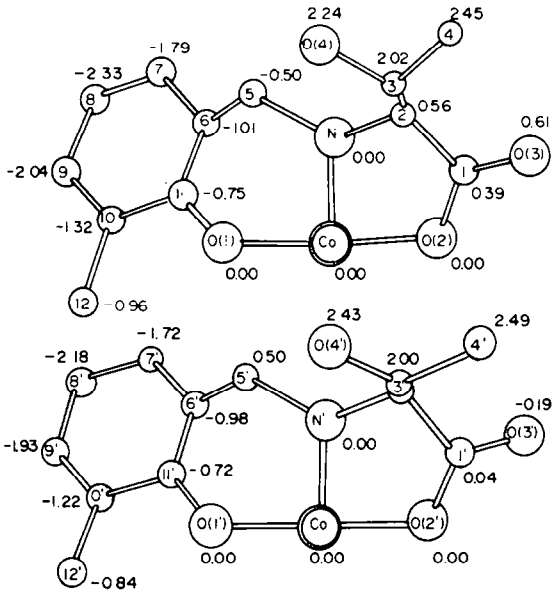


Fig. 5. Projections of **5a** ligands on CoO(1)O(2)N(1) and CoO(1')O(2')N(1') planes.

distortion in the two 5-membered rings is due to a different environment in the crystal: the O(4) atom forms two H-bonds O(4)...O(W_1) (2.78(1) Å) and O(4')...O(4) (2.71(1) Å) while the O(4') atom is additionally coordinated with the cation, Na...O(4') (2.335(8) Å). In both ligands the orientation of hydroxyethyl groups is pseudo-axial (the displacements of C(3) and C(3') atoms from CoNO(2) and CoNO(2') planes are 2.03 and 1.98 Å respectively); H(2) and H(2') atoms are pseudo-equatorial (the corresponding displacements are −0.01 and −0.05 Å).

In **5a** there are 7 independent H atoms capable of forming H-bonds (two from OH groups of the anion, one from the ethanol molecule and four from the two water molecules). According to the O...O distances (Table 9) there are 5 H-bonds. This conclusion is also confirmed by the arrangement of the H atoms. It is difficult to make conclusions about the function of the hydrogens of the water molecule W_2 which has a position population of 0.25. However, this molecule seems to participate in yet another H-bond O(W_2)–H...O(W_1) 2.77 Å. The coordination of the Na^+ is seven fold with four short (2.250–2.375 Å) and three long (2.778–2.900 Å) Na...O distances (Table 9).

Table 9. Hydrogen bonds and Na^+—cation coordination†

Bond	d(Å)	Bond	d (Å)
O(4)–H...O(4) **2**	2.71(1)	Na...O(5) **1**	2.250(8)
O(5)–H...O(3) **4**	2.75(1)	Na...O(4') **4**	2.335(8)
O(W_1)–H...O(1') **4**	2.77(1)	Na...O(W_1) **1**	2.346(8)
O(W_2)–H...O(W_1)	2.77(3)	Na...O(3') **1**	2.375(8)
O(4)–H...O(W_1)	2.78(1)	Na...O(2) **4**	2.778(8)
O(W_1)–H...O(2') **1**	2.89(1)	Na...O(2') **1**	2.826(8)
		Na...O(W_2) **1**	2.900(20)

†Atoms are numbered according to Table 3. The molecules numbering is: **1**: x,y,z; **2**: 1+x,z,1+z; **3**: x,y,1+z; **4**: x,y,z−1.

(c) *Epimerization of* **3–6** *isomers in the presence of charcoal.* Each of the **a** and **b** isomers can be converted into an equilibrium mixture of isomers by boiling in ethanol in the presence of charcoal. The equilibrium ratios of isomers do not depend on chirality of the initial isomers. The respective ratios of isomers are given in Table 10.

(d) *α-Proton exchange and epimerization of the amino acid moiety in* **1–4**. Compounds **1–4** exchange on α-

Table 8. Coefficients in equations Ax + By + Dz = D of some planar anion fragments and displacements of atoms from these planes (in Å)

Plane		1	1'	2	2'	3	3'	4	4'	5	5'
	A	−4.17	1.55	−1.92	8.42	−5.90	1.47	−4.15	1.58	−2.01	5.42
	B	12.58	18.50	3.43	9.09	8.56	2.14	12.66	18.46	4.15	9.56
	C	5.54	−4.47	7.54	−7.08	5.19	−2.57	5.53	−4.48	7.58	−6.89
	D	9.91	12.22	4.98	2.30	5.74	15.09	9.98	12.21	5.60	2.67
Atoms											
Co	Co	0.00	0.00					0.00	0.00		
O(1)	O(1')	0.00	0.00	0.02	0.03					0.10	0.06
O(2)	O(2')	0.00	0.00			0.00	0.00	0.00	0.00		
O(3)	O(3')					0.00	0.00	0.59†	−0.20†		
O(4)	O(4')					1.36†	1.47†	2.12†	2.41†		
N	N'	0.00	0.00					0.00	0.00	−0.16	−0.07
C(1)	C(1')					0.00	−0.01				
C(2)	C(2')					0.00	0.00	0.54†	0.46†		
C(3)	C(3')					1.22†	1.44†	2.03†	1.98†		
C(4)	C(4')					1.25†	2.12†	2.35†	2.46†		
C(5)	C(5')			−0.01	−0.01	−0.76†	−1.49†			0.08	0.11
C(6)	C(6')			−0.01	−0.02	−0.94†	−2.19†			0.03	0.04
C(7)	C(7')			0.01	0.01					0.02	0.05
C(8)	C(8')			0.01	0.01					−0.02	0.00
C(9)	C(9')			0.00	0.01					−0.03	−0.05
C(10)	C(10')			−0.02	−0.02					−0.02	−0.06
C(11)	C(11')			0.00	−0.01	−0.33†	−1.88†			0.04	0.01
C(12)	C(12')									−0.05	0.02
H(2)	H(2')							−0.01†	−0.05†		

Dihedral angles between planes are: **1/1'** = 88°; **1/3** = 18°; **1/3'** = 16°; **1/5** = 33°; **1/5'** = 31°.
†Atoms not included in the calculation at a respective plane equation.

proton of the amino acid moiety in D_2O under the action of OD^-. The rate of the process is determined from the decay of the PMR signal of the α-proton (Fig. 6). Both protons of the glycine fragment in **1** are exchanged with approximately the same rate, while in **2**, the exchange rates differ (Fig. 7). Both rate constants are given in Table 10.

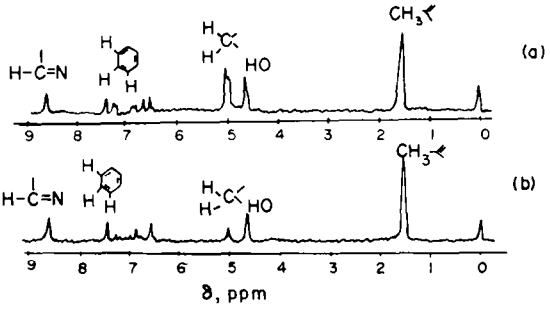


Fig. 6. PMR spectra of **2** (in CD_3OD) after reaction in D_2O (pD = 10.59, 25°) (a) beginning of the experiment, (b) after 70 min.

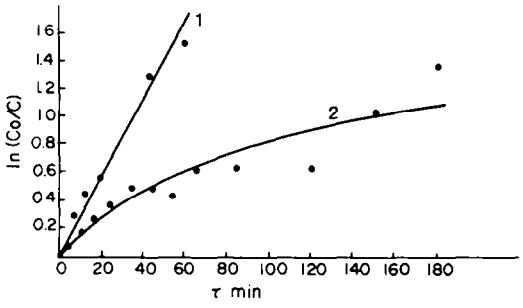


Fig. 7. Kinetic plots of α-proton exchange on the glycine fragment of 1 and 2 in D_2O (pD = 10.59, 25°). Solid line is the theoretically calculated curve.

In the case of **3** and **4** the process is accompanied by eperimization of the S-amino acid moiety. Figure 8 shows the evolution of the ORD curve of **3b** in 0.01 N KOH at 35°. At 535 min a non-zero isorotational point is observed. The racemization degree of the amino acid fragment was determined by GLC after an electrochemical reduction of complexes. No losses of amino acids occured, which was checked by introducing the standard amino acid S-ala into the reaction mixture directly after

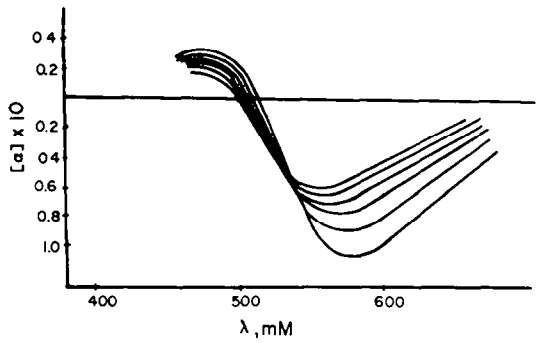


Fig. 8. Variation of ORD curve of **3** in 0.01 N KOH and 25°.

electrochemical reduction. Deuterium exchange rate constants of the α-proton of the amino acid moieties in **1–4** (K_{ex}) are presented in Table 10. The rates of amino acid α-carbon inversion (K_{inv}) determined from the first 10% of conversion are given for **3** and **4** also in Table 10.

(e) *Asymmetric synthesis of S-threonine and allothreonine.* Interaction of **1a**, **1b**, **2a** and **2b** with acetaldehyde at pH 8.5 and 25° proceeds according to Scheme 6.

$$K^+[Co(OC_6H_4CH{=}NCH_2COO)_2]^- + CH_3CHO \xrightarrow[H_2O]{OH^-}$$

$$\left[\begin{array}{c}\text{mixture of diastereomeric}\\ \text{threonine complexes}\end{array}\right] \xrightarrow[pH\,5\,0]{e^-} C_6H_4(OH)CHO$$

$$+ CH_3OH(OH)CH(NH_2)COOH + Co^{2+}$$

Scheme 6.

The reaction at pH values up to pH 11.2 was not accompanied by side processes and the only product is a mixture of diastereomeric threonines. The rate of threonine accumulation was measured for the first 10% conversion with the ratio acetaldehyde/complex equal to 100/1. The rate constants of acetaldehyde addition to **1** and **2** are given in Table 11. When reacting **1** (0.5 M, pD = 10.35 in D_2O) with acetaldehyde at a reagent ration of 1:2, the rate of disappearence of the acetaldehyde Me group and the glycine α-proton are equal.

The change of the asymmetrical yield of threonine and allothreonine, determined by GLC, during the reaction of **1a** and **2a** with acetaldehyde is shown in Fig. 9.

As expected, at the same degree of conversion **1b** and

Table 10. Deuterium exchange rate of the α-proton in the glycine fragment of **1–4**, inversion of amino acid moieties of **3** and **4** in D_2O at 25°, and the equilibrium ratio of **a** and **b** isomers in boiling ethanol

N	Compound	$K_{ex} \cdot 10^2$ ($M^{-1}s^{-1}$)	$K_{inv} \cdot 10^2$ ($M^{-1}s^{-1}$)	$\frac{K_{-S}}{K_{-R}}$	a/b ratio
1	K[Co(Sal-Gly)$_2$]	626 ± 34			1
2	K[Co(N-3-Met-Sal-Gly)$_2$]	K_1 = 547‡			
		K_2 = 48‡			1
3a	K[Co(Sal-S-Val)$_2$]	0.9 ± 0.2	0.48†	1	
3b		1.3 ± 0.2	0.54†	1	0.56
4a	K[Co(N-3-Met-Sal-S-Val)$_2$]	0.2 ± 0.01	0.1 ± 0.02	1	
4b		2.0 ± 0.1	0.17 ± 0.02	10	1.22
5	K[Co(N-3-Met-Sal-S-Thr)$_2$]				2.35
6	K[Co(Sal-S-Thr)$_2$]				0.15

†One-point determination.
‡The kinetic parameters were resolved on a "Promin-2" computer.[12]

Table 11. Rate constants of acetaldehyde addition to 1 and 2 at 25° in water

N	Compound†	pH	K_{obs} (s^{-1})	K_{obs}/OH^- ($M^{-1}s^{-1}$)
1	K[Co(Sal-Gly)$_2$]	8.5	$(0.33 \pm 0.016) \times 10^{-4}$	10.3 ± 0.5
2	K[Co(N-3-Met-Sal-Gly)$_2$]	9.82	$(1.23 \pm 0.05) \times 10^{-4}$	1.86 ± 0.08

†The ratio of acetaldehyde to complex was 100 to 1. The concentration of the metal complex was equal to $4.6–4.9 \times 10^{-3}$ M.

Table 12. Comparison of enantioselectivity of reactions of **1a** and **1b** with acetaldehyde and of **2a** and **2b** with acetaldehyde

% of threonines in amino acid mixtures	°C	Complex	S-Thr (%)	R-Thr (%)	S-allo Thr (%)	R-allo Thr (%)
95	25	**1a**	36.5	63.5	22.85	87.15
95	11.5	**1b**	73	27	77.5	22.5
85	25	**2a**	80	20	56	44
85	25	**2b**	13.5	86.5	40	60

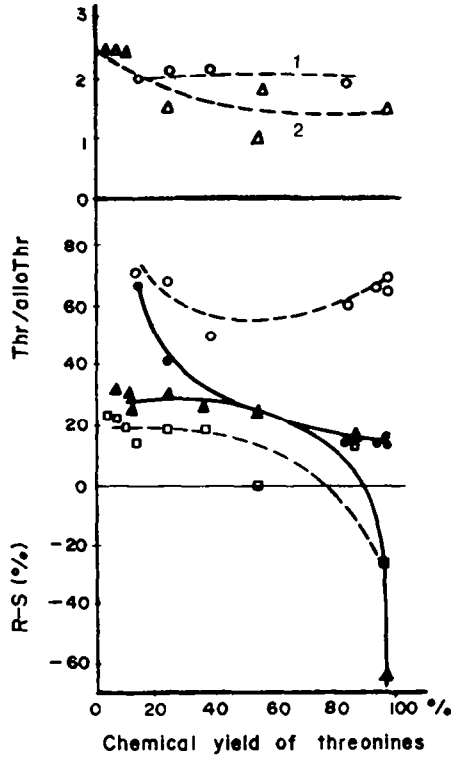


Fig. 9. Variation of the allo-threo ratio and of the asymmetric yield of threonine (– – –) and allothreonine (———) during the reaction of **2b** (upper curves) and **1b** (lower curves) with acetaldehyde.

2b give an excess of an enantiomeric form of threonine differing from that obtained from **1a** and **2a** (see Table 12).

DISCUSSION

Schiff bases of amino acids with aromatic aldehydes form planar terdentate ligands. In an octahedral complex only two such ligands may be arranged perpendicularly to each other. Complexes of Ni^{2+} and Zn^{2+} with N-pyridoxalidene-S-valine,[22] compound **1**,[20] and compound **5a** possess this structure. Any other octahedral arrangement of these ligands requires an energetically unfavourable twisting of the C=N bond. Therefore, undoubtedly compounds **1–6** have the same basic structure. Since the only symmetry element is the C_2 axis, complexes **1–4** are chiral. Depending on the left- or right-hand arrangement of ligands in relation to the C_2 axis the configuration of these complexes is designated Λ or Δ.[19] The compounds **1** and **2** may be separated into enantiomers, while **3–4**, due to the presence of their asymmetric amino acid moieties, may be separated into diastereomers, Λ(SS) and Δ(SS).

We obtained in pure form the compounds **1–6** which was confirmed by elemental analyses UV (Fig. 1) and PMR (Table 2) spectra, and ORD curves (Figs. 1 and 3). For isomers of **1** and **2** the ORD curves are mirror images (Fig. 3) indicating enantiomeric chirality. In **3–6**, in addition to the configurational component, the asymmetric C atoms of amino acids also contribute to the observed rotation (vicinal contribution). Consequently, the ORD curves of the **a** and **b** isomers are not mirror images (Fig. 1).

A "configurational" ORD curve of **3a** calculated assuming the additivity of the configurational and vicinal contributions[23] is presented in Fig. 10. Comparison of this curve with the ORD curve of **1a** shows that both compounds have the same absolute configuration. Analagously, it can be shown that **2a–6a** possess the same absolute configuration; **2b–6b** form the series with the opposite absolute configuration. Equilibrium of **a** and **b** induced by charcoal, a known catalyst of complex epimerization at Co^{3+},[24] confirm that the isomers are diastereomers differing only in the absolute configuration of the complex and having the same S-configuration of the amino acid (Scheme 7).

$$\Lambda(SS) \underset{C_2H_5OH,\ 78°}{\overset{\text{charcoal}}{\rightleftharpoons}} \Delta(SS)$$

Scheme 7.

Thus it is sufficient to determine the absolute configuration of only one compound in either the **a** or **b** series to assign absolute configuration to all the isomers.

According to X-ray structural data, the compound **5a** has a Λ-configuration (Fig. 4). Therefore, all **a** isomers

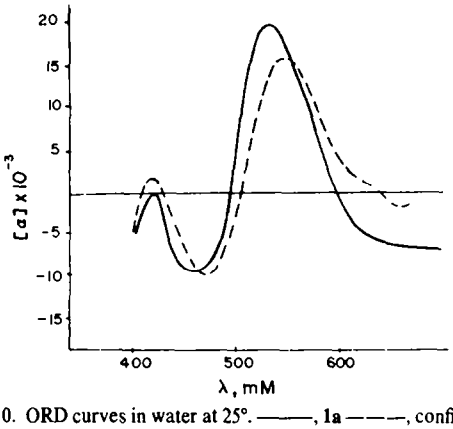


Fig. 10. ORD curves in water at 25°. ———, **1a** – – –, configurational curve of **3a**.

also have the Λ-absolute configuration and all the **b** isomers the Δ-configuration.

The strong distortion of the chelate rings of **5a** leading to the pseudo-axial arrangement of the threonine hydroxyethyl group is not caused by the intramolecular H-bonds (Table 9). Possibly these distortions are a result of non-binding intramolecular interactions involving the hydroxyethyl group of the threonine moiety and the aldimine proton of the same ligand. Minimization of these Van der Waals interactions with small distortions of bond angles with no variance of the bond lengths might lead to rotation in bonds between the phenoxy and carboxy groups around the Co^{3+} which results in the drawing together of the phenyl rings of the neighboring ligands (Fig. 11). The same pattern of distortions should be observed in the complexes with Δ-absolute configuration. This conclusion is confirmed by the structure of the complex ion of Δ-bis-[N-pyridoxalidene-S-valinate] nickel(II).[22] However, in the complexes with Δ-absolute configuration, distortion of the chelate rings leads to an increase in the distance between the phenyl rings of the neighboring ligands.

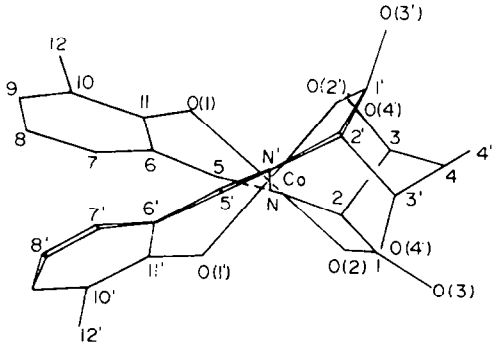


Fig. 11. Distortion of chelate rings in Va viewed from the N–Co–N' axis.

The fact that these distortions are preserved in the solution is verified by the position of the 3-Me substituents of the phenyl rings signals in the PMR spectra of **2**, **4** and **5** (**a** and **b**). In the absence of any distortions, the chemical shift of this group should have been the same for all these compounds.

However, if twisting around the Co–O bonds is retained in solution, the 3-Me group in the Λ(SS) isomers (**4a** and **5a**) is shielded by the magnetic anisotropy of the phenyl ring of the neighboring ligand and its signal should be shifted up field. For the Δ(SS) isomers (**4b** and **5b**) the Me group is out of the area shielded by the aldimine double bond of the adjacent ligand and its signal should be shifted down field.[25] This behaviour was indeed observed. In the case of **4b** and **5b**, the shifts observed were 0.33 and 0.37 ppm down field, while for **4a** and **5a**, the shifts were measured to be 0.13 and 0.23 ppm up field with respect to the 3-Me signal in **2**. These results confirm the retention of conformation of the chelate rings in the crystals and in solution.

The system under consideration exhibits pronounced stereoselectivity (Table 10). In a mixture equilibrated with charcoal the Δ(SS) isomer predominates over the Λ(SS) in the case of **3** and **6**. The energy differences between the isomers are −0.45 kcal/mole for **3** and −1.32 kcal/mole for **6** (at 78°).

These effects may be caused by the energetically unfavorable interaction of the phenyl rings in the isomers of Λ(SS) configuration.

Introduction of a Me group into salicylaldehyde at position 3 reverses stereoselectivity. The Λ(SS) isomer now becomes energetically favorable. The energy difference is +0.145 kcal/mole for **4** and +0.595 kcal/mole for **5**. This reversal is explained by the non-binding interaction of the alkyl substituent of the amino acid fragment and the 3-Me group in the salicylaldehyde ring in the complexes of Δ(SS) configuration (Fig. 12). This interaction is not present in the Λ(SS) complexes. The relative energy difference between the Λ(SS) and the Δ(SS) isomers caused by the introduction of a Me group is 0.6 kcal/mole for the S-valine complexes and 1.9 kcal/mole in the case of the S-threonine complexes.

The base catalyzed replacement of the α-proton of amino acid moiety by deuterium in **1**–**6** takes place under mild conditions and is not accompanied by transamination between the salicylaldehyde and the amino acid. The absence of transamination is indicated by the fact that the relative area of the aldimine proton signal remains constant during the process (Fig. 6). Also, the absolute configurations of all the complexes studied does not change. This is confirmed by the absence of any appreciable changes in the ORD curves of **1** and **2** during the exchange and by the presence of nonzero isorotational points in ORD curves of **3** and **4** (see Fig. 8).

The epimerization rate of the S-valine fragment in **3** (**a** and **b**) practically does not differ from the exchange rate of the α-proton in these compounds and the rates of exchange of both protons in **1** are very similar. A simple scheme of exchange with the intermediate formation of a planar sp^2 or rapidly inverting sp^3 carbanion completely explains these facts.

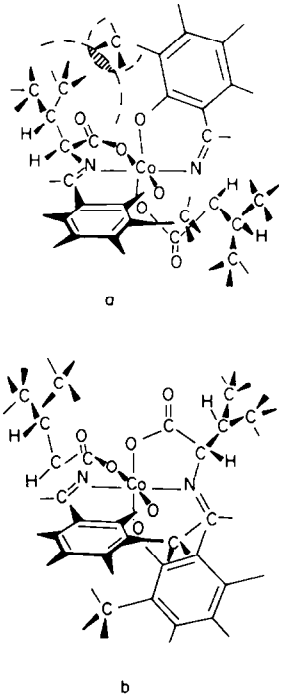
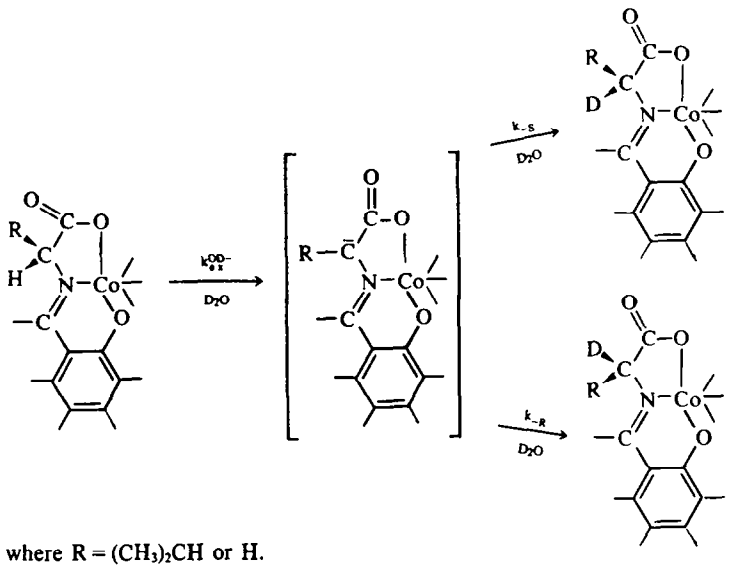


Fig. 12. (a) Schematic representation of intramolecular interaction between the isopropyl group of the valine fragment and the 3-Me group in salicylaldehyde ring of the neighboring **4b** ligand. (b) Schematic representation with absence of such interaction in **4a**.



where R = $(CH_3)_2CH$ or H.

Scheme 8.

The rate constant of α-carbon inversion is given by

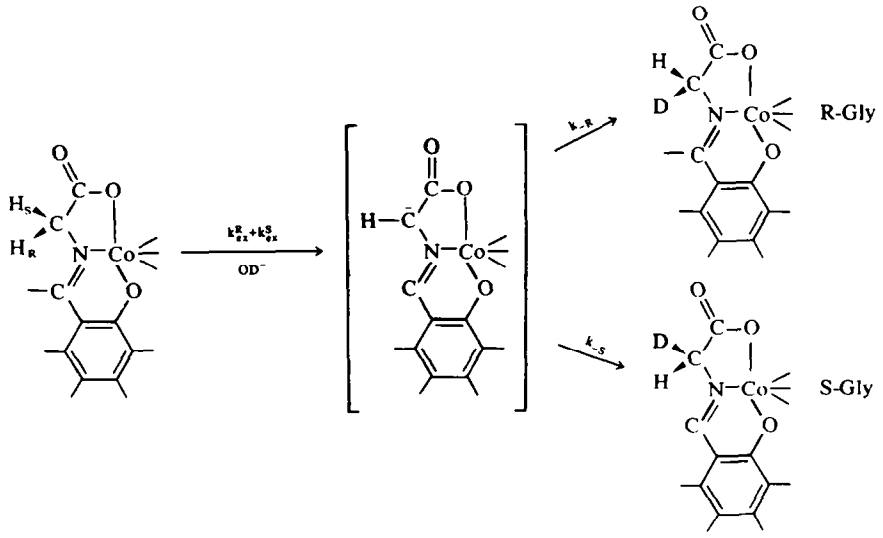
$$K_{inv} = \frac{K_{ex}^{OD^-}}{1 + \frac{K_{-S}}{K_{-R}}}$$

where $K_{ex}^{OD^-}$ is the rate constant of amino acid carbanion formation which is equal to the rate constant of deuterium exchange and where K_{-S} and K_{-R} are the deuterization rate constants of the intermediate carbanion which result in retention or inversion respectively of the absolute configuration of the amino acid α-carbon.

Attack of a D_2O molecule on the S-valine carbanion is practically not hindered both from the si and re sides, therefore $K_{-S} \approx K_{-R}$ (see Table 10). However, in **2** and **4** the 3-Me substituent of salicylaldehyde should sterically hinder the attack of any electrophile on one side of the intermediate carbanion. In complexes with the Λ-absolute configuration this shielding must take place from the re side and in the Δ-complexes from the si side (Fig. 13). The attack of a base on the pro-R proton in **2a** and the pro-S proton in **2b** should also be selectively hindered. Depending on the structure of the transition state two limiting cases may exist.

(a) In the first case, the transition state of the process is similar in structure to the intermediate carbanion. In this case the process is the "steric approach control" type (SAC)[26] and is mainly determined by steric factors in the transition state and not by the final state of the reaction. Consequently, the exchange of the α-proton on **4b** should proceed with retention of the amino acid configuration. In other words, a "counter-thermodynamic" formation of the C–H bond should be observed (Figs. 12 and 13).

(b) In the second case, the transition state is similar structurally to the end product. The stereochemistry of the reaction is determined by the energy of the final state and the process is related to the "product development" type.[26] It can be expected that the stereochemistry of initial and final stages of the reaction will be determined by thermodynamic properties of the final state. The



Scheme 9.

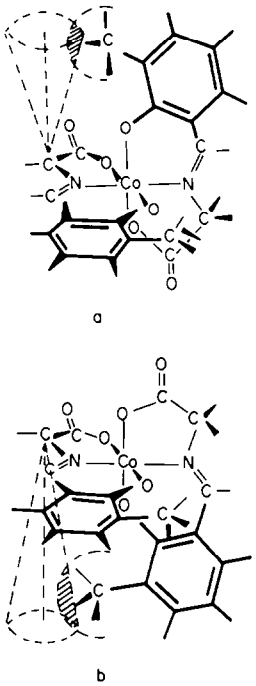


Fig. 13. Schematic representation of steric screening by the Me group of the neighboring ligand of the electrophilic attack on the amino acid carbanion in complexes: (a) Δ-absolute configuration from the si side, (b) Λ-absolute configuration from the re side.

requires an energetically unfavorable eclipsed arrangement of the alkyl group of the amino acid and of the aldimine proton of the same ligand (Fig. 14). This situation is very similar to the 1,3 interaction of axial substituents of cyclohexane.[28] Since the present and the cyclohexane systems may be analogous, the expected energy difference between an isopropyl–proton interaction and a proton–proton interaction is on the order of 2.2 kcal/mole.[28] Further, since a C=N bond is shorter than a C–C bond, the difference in energy of the transition states of the C–H bond ionization of structurally related glycine and valine complexes (**1** and **3**, **2** and **4**) should be slightly larger than 2.2 kcal/mole. The observed $K_{ex}^{OD^-}$ difference of the glycine and valine complexes is between 2.5 and 3.0 kcal/mole.

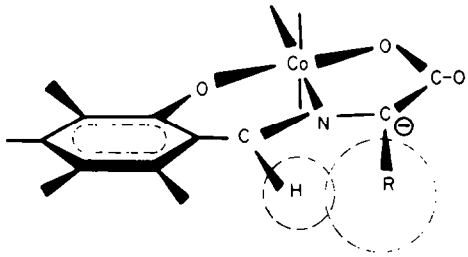


Fig. 14. Schematic representation of intramolecular steric interaction between the isopropyl group of the valine fragment and aldimine protons in transition state of exchange of α-protons in **3** and **4**.

exchange of the α-proton in **4b** should proceed with inversion of configuration and the exchange of both glycine α-protons in **2** should proceed with the same rate.

However, exchange of the α-protons in **2** occurs with different rates (Fig. 7 and Table 10). The reaction sequence may be described by Scheme 9.

Applying the method of steady state kinetics[27] to this system, we found that the rate ratio of the disappearance of the H_R and H_S glycine protons is virtually proportional to the K_{ex}^R/K_{ex}^S ratio. According to experimental data, this ratio is approximately equal to 10 (Table 10). Thus, proton exchange in **2** belongs to the group of SAC reactions (version **a**).

In the absence of ligand distortion in **4** the ration of K_{-S} and K_{-R} (Scheme 8) of both isomers should be the same as for **2**. However, the distortion of the chelate rings (see X-ray section) leads to different shielding of the neighboring amino acid moiety by the Me group in **4a** and **4b**. Accordingly, **4b** exchanges its proton with retention of configuration ($K_{-S}/K_{-R} = 10$) while the exchange of the proton in **4a** occurs with complete racemization ($K_{-S}/K_{-R} = 1$).

Hence, the stereochemistry of proton exchange in **4** also belongs to the SAC group.

Additional confirmation of a planar carbanion transition state comes from a comparison of the rate constants of proton elimination from **4a** and **4b**. The steric strain in the initial state of **4b** due to interaction of 3-Me group of one ligand and the isopropyl group of the valine fragment of the neighboring ligand is absent in **4a** (Fig. 12). The disappearance of this interaction in the transition state of the **4b** ionization explains the 10-fold increase of K_{ex}^{OD} in **4b** as compared with $K_{ex}^{OD^-}$ in **4a**.

Formation of an intermediate planar carbanion

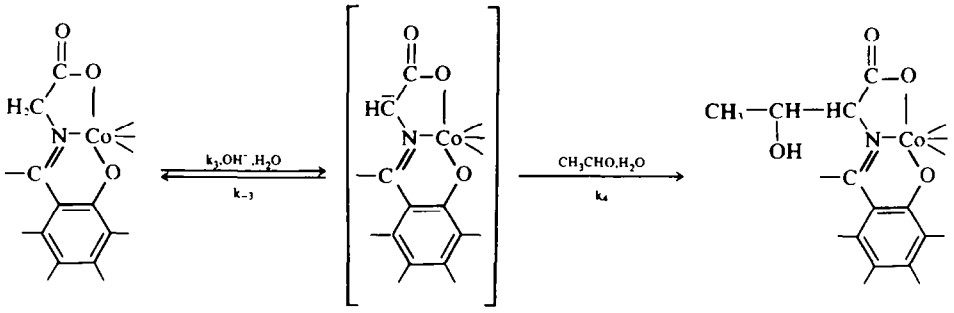
In conclusion of this part of the investigation, it is to be noted that the simple system studied makes it possible to carry out stereospecific exchange of the α-protons of glycine and other amino acids in the same manner and according to the same mechanism which is specific for serine transhydroxymethylase.

Another important reaction of this enzyme is stereospecific hydroxyalkylation. We selected a typical natural reaction of threonine formation from glycine and acetaldehyde[3] for investigating the regularities of C–C bond formation.

The general route of threonine synthesis by the reaction of **1** and **2** with acetaldehyde is shown in Scheme 6. At high concentrations of aldehyde and **1** (0.5 M) or at high ratios of aldehyde to complex (100/1, complex concentration = 5×10^{-3} M), the rate limiting step is elimination of the amino acid α-proton (Scheme 10). This is indicated by essentially equal rate constants of α-proton exchange in the glycine fragment of **1** in D_2O (Table 10) and of the threonine formation from the glycine fragment of **1** and acetaldehyde in H_2O (Table 11). The equality of the rates of the PMR signal decay of the glycine α-proton and of the acetaldehyde Me group by the reaction of **1** with acetaldehyde in D_2O at pD = 10.35 is a direct proof of this mechanism.

The reaction mechanism is shown in Scheme 10. To be consistent with the mechanism, $K_4[CH_3CHO]$ must be much greater than K_{-3}. Allowing for concentrations of acetaldehyde and water in the solution, the affinity of the intermediate carbanion of **1** for the carbon of the CO group is higher by at least two orders of magnitude than for the proton of water.

Introduction of a Me group into the 3-position of salicylaldehyde (conversion from **1** to **2**) presumably leads to a change of the rate limiting step of the process. The rate constant of deuterium exchange of the first



Scheme 10.

proton in **2** is approximately three times higher than the rate constant of C–C bond formation (Tables 10 and 11). Thus, $K_4[CH_3CHO] < K_{-3}$ and the interaction of the carbanion with acetaldehyde becomes the rate limiting step. However, we can not satisfactorily explain the difference behavior of **1** and **2** in this reaction.

The stereochemistry of C–C bond formation obeys the same rules as C–H bond formation. Shielding of the intermediate carbanion of the amino acid by substituents of the neighboring ligand (hydrogen in **1** and Me group in **2**) determines the stereochemistry of C–C bond formation at the initial stages of the reaction (Fig. 13). The set of diastereomers formed is not the most favorable thermodynamically. The subsequent rupture of the initially formed bonds and formation of new C–C and C–H bonds leads to the prevalence in the final mixture of the thermodynamically favorable diastereomeric complexes, Λ(RR) or Δ(SS) (see **6a**/**6b** ratio in Table 10). This mechanism explains why an excess of the S-form of threonine is formed at the initial stages of the reaction of **1a** with acetaldehyde (~20% A.Y.) (R-form for **1b**). As the reaction proceeds the sign of asymmetric yield reverses and an excess of the R-form (40–60% A.Y.) for **1a** and of the S-form for **1b** is formed in the mixture (Fig. 7).

The difference of kinetic and thermodynamic stereoselectivity is not retained in **2** (Fig. 9). With the introduction of a Me group into the 3-position of salicylaldehyde, no reversal in the sign of the asymmetric yield is observed. The 3-Me substituent drastically raises the energy of the Δ(SS) isomer as compared with that of the Λ(SS) isomer.

The threo/allo ratio also changes in going from **1** to **2**. However, the changes are too small to justify any conclusions about the structure of the corresponding transition states.

Results presented in this work are the first example of applying concepts of pyridoxal catalysis to the asymmetric synthesis of amino acids.

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