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Chromatographic behaviour of bis(2,2′-bipyridine)ruthenium(II) complexes containing alaninato, phenylalaninato and tyrosinato ligands

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ABSTRACT

Diastereoisomeric resolutions of aminoacidatobis(2,2′-bipyridine)ruthenium(II) complexes, $[Ru(S\text{-am})(bpy)_2]^+$ [am = alaninate (Ala), phenylalaninate (Phe) and tyrosinate anion (Tyr); bpy = 2,2′-bipyridine], were carried out by high-performance liquid chromatography (HPLC) using columns of C_8-bonded silica gel, tartaric acid-immobilizing silica gel and silica gel and by liquid column chromatography using ion exchangers (SP- and CM-Sephadex and SE-Toyopearl). The *Δ-S* isomer was eluted faster than the *Λ-S* isomer on the ion exchangers, whereas the reverse order was found on stationary phases based on silica gel. In the latter instance, adsorption was the major mode of separation. ^1H NMR spectroscopy showed that the aromatic side-chain of the aminoacidato ligand oriented toward the intramolecular amino group bound to ruthenium both in water and in methanol. The results demonstrate that the isomers are distinguished primarily through asymmetric hydrogen bonding between silanolate and the amine proton, which is sterically hindered by the aromatic ring. Sephadex ion exchanger showed enantioselectivity in the elution of $[Ru(RS\text{-Phe})(bpy)_2]Br$.

INTRODUCTION

The photochemical and thermochemical properties of $[Ru(S\text{-am})(bpy)_2]X_n$ (am = aminoacidate anion) have been studied in the last few years [1–6]. Complexes of this kind possess two diastereoisomers, *Δ*-$[Ru(S\text{-am})(bpy)_2]^{n+}$ (*Δ-S*) and *Λ*-$[Ru(S\text{-am})(bpy)_2]^{n+}$ (*Λ-S*), which were partially resolved by liquid chromatography (LC) with SP-Sephadex cation exchanger by Vagg and Williams [1]. In addition, we completely resolved the complex coordinating *S*-Phe or *S*-Tyr anion by LC using SE-Toyopearl cation exchanger [6]. However, there are no reports of the use of high-performance liquid chromatography (HPLC) in the separation of these complexes.

HPLC with a cation-exchange column has been reported to be effective in separating complexes of the type $[RuXY(bpy)_2]^{n+}$ having different XY ligands but the same charge [7,8]. The separation of *cis*- and *trans*-isomers of this type of complex by reversed-phase HPLC has also been reported [9]. Further, optical resolution on a DNA–hydroxyapatite column has been examined for racemic solutions of this kind of complex [10]. These studies have provided useful information, but mechanisms for distinguishing the isomers have not been explained in detail.

We report here a diastereoisomeric separation of bis(2,2′-bipyridine)ruthenium(II) complexes coordinating (*S*)-Ala, (*S*)-Phe and (*S*)-Tyr anions (abbreviated to *S*-Ala, *S*-Phe and *S*-Tyr complexes, respectively) by the use of various stationary phases, and discuss the mechanisms of the separation.

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EXPERIMENTAL

Chemicals and reagents

All materials were of analytical reagent or HPLC grade and used as received. *Λ*- and *Λ*-[Ru(*S*-am)$(bpy)_2]ClO_4$ (am = Ala, Phe and Tyr) and [Ru(*RS*-Phe)$(bpy)_2$]Br were prepared by methods reported previously [6]. The (*S*)-aminoacidato complexes resolved were used as references to identify the chromatographic peaks in HPLC.

^1H NMR measurement

The ^1H NMR spectra of the complexes in ^2H_2O and $C^2H_3O^2H$ were obtained using a JEOL GX270 Fourier transform NMR spectrometer. Chemical shifts (δ) are reported in parts per million relative to sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) (0.015 ppm) as an internal standard. Amine proton signals were assigned in accordance with Vagg and Williams's report [2].

Tartaric acid-immobilizing silica gel (TA-sil)

Four grams of silica gel (Unisil Q30-5) were dried at 110°C for 6 h and suspended in 40 ml of an aqueous solution in which 25 g of (*R,R*)- or (*S,S*)-tartaric acid were dissolved. The solution was stirred moderately for 4 days, then diluted with the same amount of water. The gel in the solution was filtered off with glass-fibre filter (0.5-μm pore size), dried by heating and dehydrated at 180°C for 3 h in a stream of nitrogen. The yellowish powder was washed with a large amount of water by stirring and decantation several times until it became colourless, then filtered off. All the procedures were repeated twice. The almost white gel obtained was washed with methanol and dried at 80°C.

Measurement of adsorption capacity of silica gel

A 200-mg amount of the gel stored in a silica gel desiccator was packed in a stainless-steel column (5 cm × 4 mm I.D.) by the slurry method [11] using methanol–water–dichloromethane (6:2:2, v/v/v) as solvent. While the solvent flowed at a rate of 1 ml min^{-1}, an excess of the complex solution containing [Ru(*S*-Phe)$(bpy)_2]ClO_4$ or [Ru(*S*-Ala)$(bpy)_2]ClO_4$ was loaded on the column, which was then washed until no absorption due to the complex was detected in the eluate. The solvent was changed to a solution containing 0.005 mol dm^{-3} lithium chloride so that the complex adsorbed came out of the column. All the eluate containing the complex was collected and evaporated to dryness at 45°C by blowing nitrogen gas. The residue on evaporation was dissolved in 50 ml of water and the absorption at 492 nm was measured (ε = 9040 and 9250 for *S*-Ala and *S*-Phe complexes, respectively). The amounts of the complexes determined are shown in Table I as the adsorption capacity.

TABLE I

ADSORPTION CAPACITIES

Complex	Adsorption capacity (μmol g^{-1} gel)	
	TA-sil	Silica gel
[Ru(*S*-Phe)$(bpy)_2]ClO_4$	6 ± 2	2 ± 0
[Ru(*S*-Ala)$(bpy)_2]ClO_4$	8 ± 2	2 ± 0

HPLC

The HPLC system consisted of a Hitachi Model 638-30 system and a Hitachi L-4200 variable-wavelength spectrophotometric detector with a 17.7-μl flow cell. The packings used were silica gel (Unisil Q30-5, GL Science), tartaric acid-immobilizing silica gel and two kinds of C_8-bonded silica gel (Nucleosil $10C_8$, Macherey–Nagel, and Inertsil C_8-packed column, 25 cm × 4.6 mm I.D., GL Science). In a typical experiment, the gel was packed in a stainless-steel column (25 cm × 4 mm I.D.) by the viscosity method [12] and chromatography was carried out using methanol–water–dichloromethane containing lithium chloride as the mobile phase at a flow-rate of 1 ml min^{-1}. A volume of aqueous solution of the complex (10^{-4}–10^{-5} mol dm^{-3}) less than 20 μl was taken and injected on to the column. The retention time increased with increasing proportion of water in the mobile phase above about 30%. The complexes were irreversibly adsorbed by the gel when a salt such as lithium chloride was not present in the mobile phase.

Liquid chromatography using ion exchangers

The ion exchangers employed were SP- and CM-Sephadex C-25 (Pharmacia) and SE-Toyopearl, which was prepared according to ref. 13. All of the elutions took place in the dark to prevent inversion between the *Δ*- and *Λ*-forms by exposure to light. In

a typical experiment, the exchanger was packed in a glass column (50 cm × 1.0 cm I.D.) equipped with thermostated jacket to keep the temperature at 30.0 ± 0.1°C. The elution curve was recorded at 492 nm, where the absorption maximum of the complexes was observed, and the eluate was collected fractionally if necessary. The closed peaks on the chromatogram were analysed by means of the least-squares method.

RESULTS AND DISCUSSION

Diastereoisomeric resolution of [Ru(S-am)-(bpy)$_2$]ClO$_4$

Typical chromatograms are shown in Fig. 1[a] and the chromatographic data are listed in Table II. In all of the elutions, the *Λ-S* form was eluted faster than the *Δ-S* form.

The Inertsil C_8 column, in which residual silanols were appreciably reduced on the surface of the gel, was also used under the same conditions as with the Nucleosil C_8 column. In this instance, the narrow peak of the complex overlapped with the solvent peak, indicating little interaction between the stationary phase and the complex. Further, when lithium chloride was not added to the mobile phase, Nucleosil C_8 showed irreversible adsorption of the complex, but only a weak interaction was observed on Inertsil C_8, although tailing occurred after the narrow peak. These phenomena seem to be explainable in terms of the amounts of residual silanols on the surface of the gel.

On elution using silica gel (Unisil), irreversible adsorption also occurred using methanol, ethanol, acetonitrile, acetone, chloroform and toluene that did not contain any salt such as lithium chloride[b]. For methanolic solvents, the retention time of each isomer decreased with increasing concentration of LiCl. Including the elution order of the isomers, these trends were similar to those for elution on Nucleosil C_8, in spite of the fact that the surfaces of the two gels possess opposite polarities.

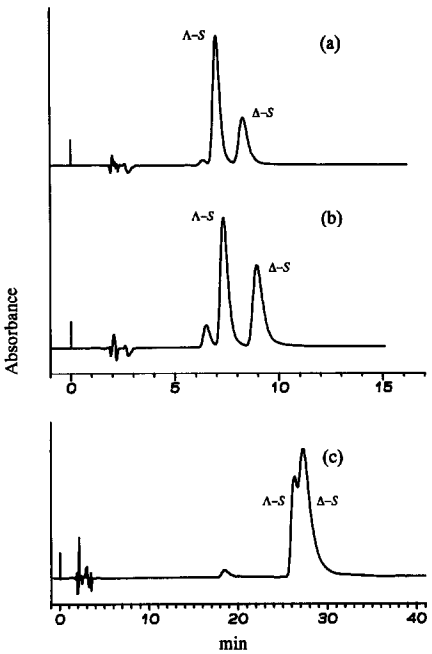

Fig. 1. Chromatograms of (a) [Ru(*S*-Tyr)(bpy)$_2$]ClO$_4$ and (b) [Ru(*S*-Phe)(bpy)$_2$]ClO$_4$ obtained by HPLC on silica gel using as eluent methanol–water–dichloromethane (6:2:2) containing 0.005 mol dm^{-3} of LiCl and (c) [Ru(*S*-Ala)(bpy)$_2$]ClO$_4$ on (*R*)-TA-sil using a 6:1:3 eluent containing 0.01 mol dm^{-3} of LiCl. Flow-rate, 1 ml min^{-1}.

It has been reported that a neutral complex, [Ru(CN)$_2$(bpy)$_2$]°, was developed by methanol alone on a silica gel thin-layer plate, and the appropriate adsorption was explained by the dipole moment of the complex [14]. Hence it is suggested that the irreversible adsorption of the aminoacidato complexes occurs on silica gel in elution with methanol alone because of the aminoacidato ligand and/or the overall charge of the complexes. In preliminary experiments, it was found that an acidic solvent reduced the adsorption ability of the complex toward silica gel without changing α as compared with a neutral solvent containing lithium chloride. The adsorbent used was Unisil Q30. When the *S*-Phe complex was eluted with mobile phase (6:2:2) (Table II) solution containing 0.005 mol dm^{-3} lithium chloride, k'_1 = 2.1, HETP = 360 μm, R_s = 1.1 and α = 1.28; with a solution containing 0.005 mol dm^{-3} hydrochloric acid, k'_1 = 1.4, HETP = 420 μm, R_s

[a] A peak preceding the peak of *Λ*-S seems to be that of a decomposition product, which is not discussed in this paper.

[b] To obtain appropriate elution conditions, LiCl, $LiClO_4$, NaCl, $NaClO_4$, NaI and Na_2(R,R)-tart or acetic acid were used as additives. Of these, Na_2(R,R)-tart showed the largest R_s value but long tailing peaks, whereas LiCl and $LiClO_4$ showed sharper and more clearly separated peaks.

TABLE II

DATA FOR THE CHROMATOGRAPHIC SEPARATION OF THE DIASTEREOISOMERS BY HPLC[a]

Separation factor (α), resolution (R_s) and height equivalent to a theoretical plate (HETP) are obtained by the following equations:
$\alpha = (V_{R2}-V_O)/(V_{R1}-V_O)$
$R_s = 1.18\,(V_{R2}-V_{R1})/(W_1 + W_2)$
$\mathrm{HETP} = L/[5.54(V_{R1}/W_1)^2]$
where V_{R1} and W_1 represent retention volume and peak half-width of an earlier eluted peak, respectively, and V_{R2} and W_2 those of a later eluted peak, L is the height of the column and V_O is the void volume, which was approximated by the retention volume of the solvent peak in HPLC and by the column bed volume in LC on ion exchangers.

Complex[b]	Adsorbent	Mobile phase[c]		k'_1[d]	HETP (μm)	R_s	α[e]
S-Phe	Nucleosil C_8[f]	A (7:3)	0.017 *M* LiCl	1.1	270	0.85[g]	1.18
	Unisil[h]	A (8:2)	0.03 *M* LiCl	4.2	130	2.4	1.31
			0.03 *M* $LiClO_4$	3.4	140	1.9	1.27
		B (6:2:2)	0.005 *M* LiCl	3.6	170	1.9	1.29
		B (6:1:3)	0.003 *M* $LiClO_4$	3.5	170	2.1	1.32
			0.005 *M* LiCl	2.5	280	1.5	1.33
		C (6:2:2)	0.03 *M* LiCl	2.1	170	2.8	1.50
	(*R*)-TA-sil[i]	B (6:2:2)	0.01 *M* LiCl	2.4	86	2.5	1.31
		B (6:1:3)	0.01 *M* LiCl	3.7	81	2.7	1.28
	(*S*)-TA-sil	B (6:1:3)	0.01 *M* LiCl	3.1	93	2.7	1.30
S-Tyr	Nucleosil C_8	A (65:35)	0.024 *M* LiCl	0.67	250	1.1[g]	1.28
	Unisil	A (8:2)	0.03 *M* LiCl	2.8	130	2.1	1.35
		A (6:4)	0.05 *M* LiCl	5.2	100	2.6	1.27
		A (5:5)	0.05 *M* LiCl	5.5	140	2.4	1.32
		B (6:2:2)	0.005 *M* LiCl	2.6	170	1.6	1.27
		B (6:1:3)	0.005 *M* LiCl	2.6	280	1.3	1.28
		C (6:2:2)	0.03 *M* LiCl	2.0	150	2.6	1.45
	(*R*)-TA-sil	B (6:2:2)	0.01 *M* LiCl	2.3	86	2.1	1.25
		B (6:1:3)	0.01 *M* LiCl	2.5	89	2.1	1.22
	(*S*)-TA-sil	B (6:1:3)	0.01 *M* LiCl	3.3	89	2.2	1.24
S-Ala	Unisil	B (6:2:2)	0.01 *M* LiCl	5.7	250	0.4	1.05
		B (6:1:3)	0.004 *M* LiCl	9.2	250	0.5	1.07
	(*R*)-TA-sil	B (6:2:2)	0.01 *M* LiCl	7.3	80	0.4	1.03
		B (6:1:3)	0.01 *M* LiCl	13	80	0.4	1.03
	(*S*)-TA-sil	B (6:1:3)	0.01 *M* LiCl	9.0	100	0.6	1.06

[a] *A*-*S* was eluted early.
[b] *S*-am represents $[Ru(S\text{-am})(bpy)_2]ClO_4$.
[c] A = methanol–water; B = methanol–water–dichloromethane; C = ethanol–water–dichloromethane. The volumetric proportion of the components are given in parentheses. 1 *M* = 1 mol dm^{-3}.
[d] Capacity factor of *A*-*S* peak.
[e] Deviation of α was roughly estimated to be ± 0.04.
[f] C_8-bonded silica gel.
[g] Column height was 500 mm.
[h] Silica gel.
[i] (*R*)-TA-sil represents the silica gel immobilizing (*R*,*R*)-tartaric acid.

= 0.9 and α = 1.29. When *S*-Tyr complex was eluted with 0.005 mol dm^{-3} lithium chloride solution, k'_1 = 1.7, HETP = 380 μm, R_s = 0.9 and α = 1.24; with 0.005 mol dm^{-3} hydrochloric acid, k'_1 = 1.1 and the other parameters were not measurable, as the two peaks were very close. This can be attributed to the extent of dissociation of surface silanols. All of the observations mentioned above imply the presence of a direct interaction between the complex and surface silanols, and hence that normal-phase adsorption is the major separation mode in the present chromatography with silica gel. The occurrence of peak tailing also supports this conclusion (Fig. 1).

Table II shows that the separation factor α for each complex is approximately constant and is not much affected by the adsorbents and the composition of the solvents, except ethanolic solvents. It was also independent of the concentration of lithium chloride in the mobile phase, within experimental error. The values for the *S*-Ala complex were smaller than those of the other complexes. The α value can be regarded as the ratio of distribution coefficients for the two isomers. It is therefore suggested that differences in the eluent conditions hardly changed the fundamental distinction mechanism, which determines the value of α. For the *S*-Phe complex, the resolution R_s increased in the order C_8-bonded silica gel < silica gel < TA-sil, whereas the height equivalent to a theoretical plate (HETP) tended to become shorter in this order. Similar trends were also observed for *S*-Tyr and *S*-Ala complexes.

The main reason why high resolutions were found for TA-sil was not stereospecific interaction with tartaric acid on the gel, but the increase in the number of theoretical plates caused by immobilization of tartaric acid, as the effect of TA-sil appeared markedly in HETP but scarcely in α. This is also supported by the data in Table I, where the adsorption capacity increases three to four times for TA-sil in comparison with silica gel.

Chromatographic separations using ion exchangers were examined for the eluents involving $K_2(R,R)$-tart and $K_2[Sb_2(R,R)\text{-tart}_2]$, which are representative anionic selectors [15], as listed in Table III. The elution order of the diastereoisomers was the opposite to that using adsorbents based on silica gel; *Δ-S* moved faster than *Λ-S* on the ion exchangers. For the elution of *S*-Phe complex on a CM-Sephadex column, the R_s values were 0.50, 0.63 and 0.80 for eluents containing KCl, $K_2(R,R)$-tart and $K_2[Sb_2(R,R)\text{-tart}_2]$, respectively. Nevertheless, the corresponding α values were the same, although HETP decreased keeping the same elution order. Moreover, it was shown that R_s or α took the same values when using either (*R,R*)- or (*S,S*)-tartrate salt as an eluting agent. The elution on SP-Sephadex also showed almost the same α values as with CM-Sephadex, giving a higher resolution and shorter HETP. However, in aqueous media, the α value for SE-Toyopearl was different from those for CM- and SP-Sephadex. It seems that the complexes interacted more weakly with such anions than with

TABLE III

DATA FOR THE CHROMATOGRAPHIC SEPARATION OF THE DIASTEREOISOMERS ON ION EXCHANGERS

Complex[a]	Ion exchanger	Eluent[b]	Retention volume (bed volumes)		HETP (μm)	R_s	α[c]
			Δ-S	*Λ-S*			
S-Phe	CM-Sephadex	0.01 *M* $K_2[Sb_2(R,R)\text{-tart}_2]$	22.3	25.1	670	0.80	1.13
		0.01 *M* $K_2(R,R)$-tart	6.6	7.3	840	0.63	1.12
		0.01 *M* $K_2(S,S)$-tart	6.9	7.6	910	0.61	1.12
		0.02 *M* KCl	10.7	11.8	1400	0.50	1.12
	SP-Sephadex	0.05 *M* $Na_2(R,R)$-tart	4.3	4.9	630	1.5	1.15
	SE-Toyopearl	0.05 *M* $Na_2(R,R)$-tart	13.3	17.4	220	2.6	1.33
		0.1 *M* KCl	11.3	14.7	160	2.6	1.33
		Methanol–0.02 *M* LiCl	11.8	16.0	350	1.8	1.39
	HW-40[d]	0.01 *M* KCl	8.0	9.5	387	1.0	1.22
		Methanol–water (9:1)	65.4	65.4	650	0.0	1.00
S-Ala	CM-Sephadex	0.008 *M* $Na_2(R,R)$-tart	23.4	24.1	200	0.5	1.05
	SP-Sephadex	0.04 *M* $Na_2(R,R)$-tart	4.1	4.2	580	0.62	1.05

[a] *S*-Phe and *S*-Ala represent $[Ru(S\text{-Phe})(bpy)_2]ClO_4$ and $[Ru(S\text{-Ala})(bpy)_2]ClO_4$, respectively.
[b] Aqueous solution. 1 *M* = 1 mol dm^{-3}.
[c] Deviation of α was roughly estimated to be ±0.04.
[d] TSK-GEL Toyopearl HW-40F (Toyo Soda).

the ion-exchange resin. In other words, these observations indicate that the stereospecific character of $[(R,R)\text{-tart}]^{2-}$ and $[Sb_2(R,R)\text{-tart}_2]^{2-}$ anions in the eluents did not exert an intrinsic effect for discrimination of the isomers. A similar situation was also observed when TA-sil was used as an adsorbent.

We define a value A which is the ratio of distribution coefficients of the two isomers, as follows:

$$A = K_\Delta/K_\Lambda$$

where K_Δ is the distribution coefficient of the Δ-S isomer,

$$K_\Delta = [\Delta\text{-}S]_{\text{stationary}}/[\Delta\text{-}S]_{\text{mobile}}$$

and K_Λ similarly that of the Λ-S isomer. A has the same significance as α when Λ-S elutes faster than Δ-S, but for the contrary case $A = 1/\alpha$. The A values obtained for each adsorbent are listed in Table IV. A is transformed as

$$A = Ks_s/Ks_m$$

where $Ks_s = [\Delta\text{-}S]_{\text{stationary}}/[\Lambda\text{-}S]_{\text{stationary}}$ and $Ks_m = [\Delta\text{-}S]_{\text{mobile}}/[\Lambda\text{-}S]_{\text{mobile}}$. For instance, when A is less than unity, Ks_s becomes smaller than Ks_m, which means that the stationary phase prefers Λ-S to Δ-S in comparison with the mobile phase. Table IV therefore shows that a more effective interaction occurred on Λ-S with the ion exchangers and on Δ-S with the adsorbents based on silica gel. This trend was unchanged even if the components of the eluent were modified to some extent.

With elution on silica gel, hydrogen bonding, charge transfer, π–π, dipole–dipole and Van der Waals interactions and steric effects would be expected in the adsorption of the complexes in the present experiments. The elution of the $[RuCl_2(bpy)_2]Cl$ complex on silica gel gave a peak at a capacity factor $k' = 0.6$, whereas peaks were found at 2.05 for Λ-S and 2.68 for Δ-S of the S-Phe complex under the same elution conditions. The dichloro complex dissolved in dichloromethane was injected on to the column of Unisil Q30-5. Methanol–water–dichloromethane (6:1:3) containing 0.005 mol dm^{-3} of lithium chloride was used as the mobile phase. The peak at $k' = 0.6$ showed extensive tailing and a shoulder appeared at $k' = 1.4$ on the tailing peak. The latter peak grew strongly when a methanol solution of the complex was used, and was larger than the peak at $k' = 0.6$ when an aqueous solution was injected. It is known that the complex is easily hydrolysed in water. Hence the peak at $k' = 1.4$ seemed to be one of the hydrolysis products of the complex. The fact that the small k' value was derived from chloro ligands implies that a significant interaction was accomplished by hydrophilic groups of the aminoacidato ligand. The aminoacidato complex would interact with silica gel mainly through three forces: hydrogen bonding, dipole–di-

TABLE IV

VALUES OF A OBTAINED FOR EACH ADSORBENT IN METHANOL–WATER SYSTEM

$A = K_\Delta/K_\Lambda$, where $K_\Delta = [\Delta\text{-}S]_{\text{stationary}}/[\Delta\text{-}S]_{\text{mobile}}$ and K_Λ similarly that of Λ-S.

Adsorbent	Component[a]	A	Solvent
SE-Toyopearl	Polyvinyl derivative containing OH groups (sulphoethyl groups)	0.76 (0.72)[b]	Water
CM-, SP-Sephadex	Dextran (carboxymethyl or sulphopropyl groups)	0.87–0.89	
HW-40	Polyvinyl derivative containing OH groups	0.82 (1.0)[b]	
Nucleosil C_8	C_8-bonded silica gel	1.2	Methanol–water–dichloromethane
Unisil	Silica gel	1.2–1.3	
TA-sil	Tartaric acid-immobilizing silica gel	1.2–1.3	

[a] Ion-exchange group is given in parentheses.
[b] Methanol as solvent.

pole and weak ion–ion interactions. Considering an ion-exchange process, hydrogen bonding would reinforce the weak ion–ion interaction. For the aminoacidato complex cation, one of the amine protons would be able to approach a silanolate to form a hydrogen bond, the surface of the gel being electrically neutralized. On the other hand, no available hydrogen is present in the dichloro complex, which therefore cannot form any effective hydrogen bond.

Vagg and Williams [2] reported the ^1H NMR spectra of the *S*-Ala complex in ^2H_2O. Significant low-field shifts were observed on the resonance peaks of amine protons of the *S*-Ala ligand, and were explained in terms of asymmetric solvation of the coordinated NH_2 group through hydrogen bonding (asymmetric hydrogen bonding). Further, deuterium exchange rates of the protons were measured, and it was clarified that H_a (Fig. 2) in the *Δ-S* form was exchanged faster than H_b in the *Λ-S* form between the two less sterically hindered protons, which were accessible to the solvent to interact with it through asymmetric hydrogen bonding in each isomer. In our NMR measurements, similar chemical shifts were observed for the *S*-Phe and *S*-Tyr complexes, as shown in Table V, and it was confirmed that asymmetric hydrogen bonding also occurred on the two complexes in a similar manner.

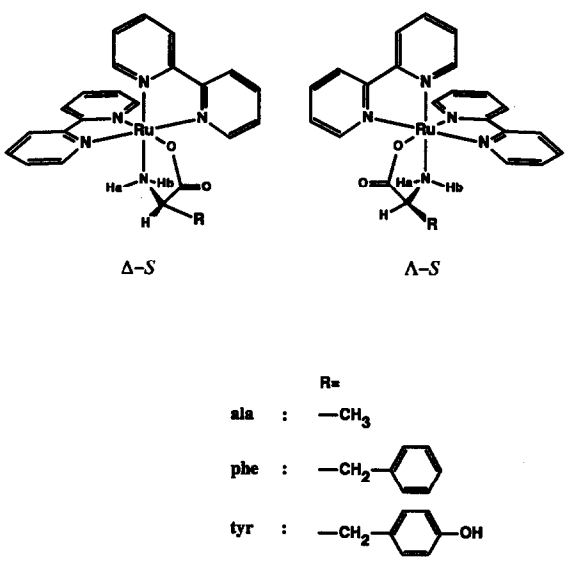

Fig. 2. Structures of *Δ*- and *Λ*-[Ru(*S*-am)(bpy)$_2$]$^+$.

TABLE V

CHEMICAL SHIFTS OF AMINE PROTONS OF AMINOACIDATO LIGAND

^2H_2O was used as a solvent.

Ligand[a]	δ (ppm)			
	Δ-S		*Λ-S*	
	H_a	H_b	H_a	H_b
S-Ala	4.4	4.1	3.6	5.2
S-Phe	4.3	3.5	2.8	5.2
S-Tyr	4.3	3.5	2.7	5.2

[a] *S*-am represents [Ru(*S*-am)(bpy)$_2$]ClO$_4$.

This is not inconsistent with the fact that the *Δ-S* isomer of the *S*-Phe complex was more soluble than the *Λ-S* isomer in water (the solubilities of the *Δ-S*-Phe and *Λ-S*-Phe complexes were 1100 and 830 mg, respectively, in 100 ml of water at 45°C). Considering asymmetric hydrogen bonding with silica gel, the interaction would be stronger for the *Δ-S* than for the *Λ-S* isomer. This trend accounts for the elution order of the isomers in HPLC using silica gel, but cannot solely explain the difference in α between *S*-Ala and the other two complexes.

The NMR spectra in the resonance region of phenol ring protons were obtained for the *S*-Tyr complex as shown in Fig. 3. The resonance peaks of phenol protons in *Δ-S* appeared at almost the same positions as those in free tyrosine, whereas the corresponding peaks of *Λ-S* shifted to higher field. Considering a model in which the phenol ring orients to an intramolecular amino group as shown in Fig. 4, the upfield shift was explainable in terms of the shielding effect induced by the ring current on the pyridine ring that faced on the phenol ring in the *Λ-S* isomer. A similar orientation of the aromatic ring was expected in *Δ-S*, as not much difference was observed between the isomers in the coupling constants of methylene protons bound to the carbon that adjoined the aromatic ring ($^3J_{HH_1}$, $^3J_{HH_2}$ = 2.7, 5.5 Hz for *Λ-S*-Tyr, 2.4, 5.7 Hz for *Δ-S*-Tyr, 4.4, 5.1 Hz for *Λ-S*-Phe and 3.4, 5.6 Hz for *Δ-S*-Phe). The peaks of the phenol protons of *Λ-S* in $C^2H_3O^2H$ showed similar upfield shifts, which reflected that the aromatic group retained the orientation even in the methanolic solvent. The point to

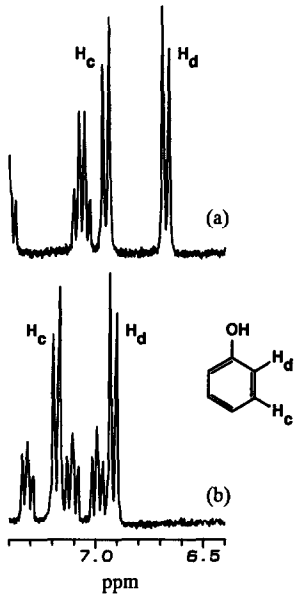

Fig. 3. Proton NMR spectra in the range δ 6.4–7.4 ppm: (a) Λ-[Ru(*S*-Tyr)(bpy)$_2$]ClO$_4$ and (b) Δ-[Ru(*S*-Tyr)(bpy)$_2$]ClO$_4$, dissolved in ^2H_2O.

be emphasized is that the aromatic ring orients toward not the carboxyl group but the amino group in the *S*-Tyr and, probably, *S*-Phe ligands. Because of this orientation, steric interference would be expected to act against the asymmetric hydrogen bonding between amine protons and the surface silanolates on the silica gel. This consideration is supported by the fact that the retention times of both the *S*-Tyr and *S*-Phe complexes were shorter than that of the *S*-Ala complex, being about 6–10 min for the former complexes and about 15–20 min for the latter in typical elutions on Unisil.

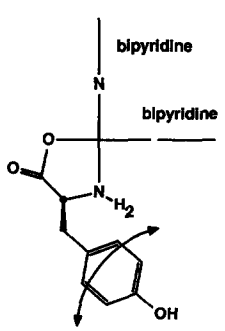

Fig. 4. Schematic representation of the orientation of the aromatic ring of the *S*-Tyr ligand in Λ-[Ru(*S*-Tyr)(bpy)$_2$]$^+$.

Table V shows that, for the *S*-Ala complex, the difference in the chemical shifts between H_a and H_b in Λ-*S* ($\Delta\delta$ = 1.6) is larger than that in Δ-*S* ($\Delta\delta$ = 0.3). Further, for the *S*-Phe and *S*-Tyr complexes, the difference in Λ-*S* ($\Delta\delta$ = 2.5) becomes even larger than that in Δ-*S* ($\Delta\delta$ = 0.8). The effect of the aromatic ring appears more marked in Λ-*S* than in Λ-*S*, suggesting that the aromatic ring is located closer to the amine protons in Λ-*S* than in Δ-*S*. The amine protons in Λ-*S* would, therefore, be less accessible to the surface silanolate. This situation can provide the possibility of distinguishing the isomers, and enhance the discrimination by the asymmetric hydrogen bonding in the diastereoisomeric separation observed for the *S*-Ala complex. Large α values for the *S*-Tyr and *S*-Phe complexes can be explained reasonably also in terms of these effects.

On the other hand, with elution on the ion exchangers, it was found that the complex isomers did not interact distinctively with the anionic selectors. In aqueous media, elution on SE-Toyopearl showed that the *S*-Ala complex was eluted earlier than the *S*-Phe complex. This can be explained by hydrophobic interactions between the complex and the framework of the exchanger.

Noteworthy α values were observed in elution on HW-40 (Table III). HW-40 is the parent gel of SE-Toyopearl, and consists of a polyvinyl derivative containing hydroxyl groups and having no ion-exchange group. HW-40 showed irreversible adsorption of the complex in elution with water alone, but with aqueous potassium chloride solution it gave sufficient α values to resolve the isomers, which were eluted in the same order as with the ion exchangers. It is obvious that matrix effects contributed significantly to elution on the ion exchangers. As with silica gel, it was expected that the complex would interact with surface hydroxyl groups, but the interaction would be weak owing to the low acidity of the C–OH hydroxyl group [16]. Instead of this, hydrophobic interactions would be effective because of the low polarity of the framework of HW-40. Elutions with methanol alone or methanol–water (9:1) showed reversible adsorption of the complex, indicating a decrease in adsorption ability. This could be explained by the idea that the hydrophobic interaction with the stationary phase was diminished owing to low polarity of methanol. Hence the system of HW-40 with a methanolic sol-

vent showed no selectivity for the diastereoisomers.

Sephadex G-10, which is the parent gel of Sephadex ion exchangers, also revealed irreversible adsorption in water containing no salt, but the adsorption ability was substantially decreased when a small amount of potassium chloride was dissolved in the mobile phase. Of the Λ- and Δ-*S* isomers, Λ-*S* would be more hydrophobic than Δ-*S*, as the amine proton in Λ-*S* is less accessible to water as described above. The elution order of the isomers can therefore be explained by hydrophobic interaction with the ion exchangers. The difference in *A* values between Toyopearl and Sephadex may be due to matrix effects, but further discussion is not possible owing to a lack of information on the structure of HW-40.

It is interesting that, among the elutions on the ion exchangers, the largest α value was observed in elution where a methanolic solution of lithium chloride was used on SE-Toyopearl (Table III). In this elution, the mobile phase reduced the adsorption ability of the complex, and the retention time of the *S*-Ala complex became the same as that of the *S*-Phe complex. From the results of elution on HW-40 with methanol–water (9:1), it is likely that the hydrophobic effect on the matrix is almost cancelled by the methanolic solvents. In this instance it is necessary to consider a different mechanism such as ion-pair chromatography, but it could not be elucidated solely from the present data.

In these experiments, primarily hydrogen bonding explained the chromatography on silica gel and hydrophobic interaction that on ion exchangers. These interactions apply in substantially opposite directions but have the same implication, as the complexes would interact with the mobile phase through hydrophobic moieties in HPLC on silica gel and through hydrogen bonding in LC on ion exchangers. It is reasonable to deduce that the opposite relationship causes the reversed order in the elution of the diastereoisomers between the two kinds of chromatography, and this implication is demonstrated by the α values, which are roughly comparable to each other for the corresponding complexes in Tables II and III.

Enantiomeric resolution of [Ru(RS-Phe)(bpy)$_2$]Br

Sephadex ion exchangers showed enantioselectivity when [Ru(*RS*-Phe)(bpy)$_2$]Br was eluted with an aqueous solution. In addition to Δ-*S* and Λ-*S* isomers, Λ-*R* and Δ-*R* isomers exist in this complex. The four isomers were partially resolved by CM-Sephadex column chromatography using $K_2[Sb_2(R,R)\text{-tart}_2]$ as an eluting agent. The elution curve is shown in Fig. 5, together with the curve for $[Ru(S\text{-Phe})(bpy)_2]ClO_4$ eluted under the same conditions. From absorption and circular dichroism measurements on the fraction of the eluate, it was found that the order of elution of the isomers was Δ-*S* > Λ-*R* ≈ Δ-*R* > Λ-*S*. The same elution order and α values were obtained on elution with $K_2(R,R)$-tart instead of $K_2[Sb_2(R,R)\text{-tart}_2]$. It is interesting that Λ-*R* and Δ-*R* isomers containing an *R*-Phe ligand were hardly resolved. This indicates that the ability for diastereoisomeric discrimination was cancelled by that for enantiomeric discrimination and that the two kinds of discrimination mechanisms are energetically comparable.

It has been reported that for both $[Ru(bpy)_3]^{2+}$ and $[Ru(bpy)_2(\text{di-2-pyridylamine})]^{2+}$ complex ions, the Δ form was eluted faster than the Λ form in SP-Sephadex column chromatography with the use of either $Na_2[Sb_2(R,R)\text{-tart}_2]$, $Na_2(R,R)$-tart or NaCl as eluting agent [17]. This order accorded with the results of the elutions in the present experiments.

As regards SE-Toyopearl, elution of [Ru(*RS*-Phe)(bpy)$_2$]Br with $Na_2(R,R)$-tart showed no enantioselectivity, giving two completely resolved peaks, the faster consisting of Δ-*S* and Λ-*R* and the later one Λ-*S* and Δ-*R* [6]. Moreover, two peaks were

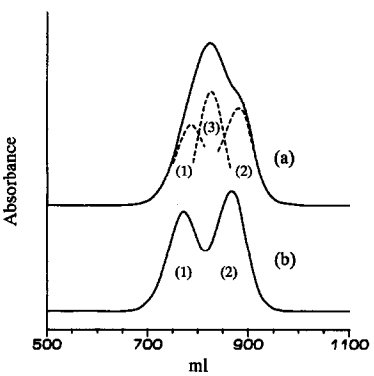

Fig. 5. Chromatograms of (a) [Ru(*RS*-Phe)(bpy)$_2$]Br and (b) [Ru(*S*-Phe)(bpy)$_2$]ClO_4 obtained by LC on CM-Sephadex C-25 (column, 43 cm × 1 cm I.D.) with 0.01 mol dm^{-3} aqueous $K_2[Sb_2(R,R)\text{-tart}_2]$ as eluent. Peak 1 = Δ-*S*-isomer, peak 2 = Λ-*S* isomer and peak 3 = Λ- and Δ-*R* isomers.

also obtained in HPLC on silica gel or TA-sil, but the faster peak consisted of *Λ*-*S* and *Δ*-*R* and the later one *Δ*-*S* and *Λ*-*R*.

The enantioselectivity shown by Sephadex ion exchangers in these experiments can be explained in terms of its characteristic asymmetric framework. Also in the diastereoisomeric resolution, the difference in the frameworks of Sephadex and Toyopearl was reflected in the *A* values (Table IV). It is certain that some interactions occur between the complexes and the framework of the resin. However, as there is not a particular interaction with the aminoacidato complexes, it would be necessary to obtain data for other monovalent cationic bis(2,2′-bipyridine)ruthenium(II) complexes.

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