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Article in *Transition Metal Chemistry* · April 1999

DOI: 10.1023/A:1006906208569

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Iron(II) and nickel(II) mixed-ligand complexes containing 1,10-phenanthroline and 4,7-diphenyl-1,10-phenanthroline

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Summary

Two types of mixed-ligand complexes, *i.e.* $[M(\text{phen})_2(\text{dip})]^{2+}$ and $[M(\text{phen})(\text{dip})_2]^{2+}$ ($M = \text{iron(II)}$ and nickel(II) ; $\text{phen} = 1,10\text{-phenanthroline}$ and $\text{dip} = 4,7\text{-diphenyl-1,10-phenanthroline}$) have been prepared from their related tris-complexes, $[M(\text{phen})_3]^{2+}$ by ligand substitution, and isolated by semi-preparative HPLC. Elemental and chromatographic analyses confirm the purity of the isolated complexes while u.v./vis and i.r. spectra were used to identify and characterize them. ^1H -n.m.r. and room temperature Mössbauer spectra of the iron(III) complexes were also measured and the results are discussed. In addition, our preliminary results on hypochromicity in the MLCT band and circular dichroism (CD) emerging in the u.v./vis region upon addition of CT(calf thymus)-DNA to the racemic complexes indicated that the iron(II) mixed-ligand complexes interact with CT-DNA.

Introduction

Metal complexes of 1,10-phenanthroline (phen) and its derivatives are of increasing interest because of their versatile roles in many fields such as analytical chemistry⁽¹⁾, catalysis⁽²⁾, electrochemical polymerization⁽³⁾ and biochemistry⁽⁴⁾. In recent years, in parallel with the development of metal-DNA chemistry, mixed-ligand complexes containing phen-families have also been widely exploited by workers interested in DNA recognition due to their ability to interact efficiently with helical DNA^(4a,5). However, until recently, mixed-ligand complexes containing phen and its derivatives, such as 4,7-diphenyl-1,10-phenanthroline (dip)⁽⁶⁾, 9,10-phenanthrenequinonediimine (phi)^(6,7) or dipyrido[3,2-a;2',3'-c]phenazine (dppz)⁽⁸⁾ employed for studying their DNA interaction, have been limited to ruthenium complexes. There are several reasons for selecting ruthenium as a metal ion⁽⁹⁾, notably the stability of ruthenium mixed-ligand complexes towards racemization, their intense metal-to-ligand charge transfer (MLCT) band in the visible spectrum and the relatively simple and well-established procedures for their preparation.

Although the methods for the preparation of ruthenium mixed-ligand complexes are well-established^(6a,10), procedures are in general time-consuming because they involve more than two steps and require rather stringent experimental conditions. For example, the procedure developed by Dwyer^(10a) and its modification, by Krause^(10c), for the preparation of $[\text{Ru}(\text{phen})_2(\text{bpy})]^{2+}$ ($\text{bpy} = 2,2'\text{-bipyridine}$) consists of at least three steps; *i.e.* preparing the starting material, $[\text{Ru}(\text{bpy})\text{Cl}_4]$ which

requires 7 days reaction time to achieve a 70% yield, converting it into the intermediate $[\text{Ru}(\text{bpy})(\text{py})_4]^{2+}$ ($\text{py} = \text{pyridine}$, 24 h reflux) and replacing four pyridines by two phenanthrolines (2 days reflux).

Unlike ruthenium complexes, there are few reports on the synthesis of iron(II) and nickel(II) mixed-ligand complexes containing phen and DIP. However, tris-(1,10-phenanthroline)iron(II), as well as tris-(2,2'-bipyridine)iron(II), has been reported to interact with DNA in the same manner as does the tris-ruthenium complex⁽¹¹⁾. Unfortunately, the interaction of both iron(II) and ruthenium(II) tris-complexes with DNA is relatively weak and their binding modes are still controversial⁽¹²⁾, while the interaction of ruthenium mixed-ligand complexes with DNA is, in general, stronger and some of their binding modes are well-understood. Hence, it is anticipated that iron(II) mixed-ligand complexes containing phen and dip will also interact with DNA in the same way as the ruthenium mixed-ligand complexes. Furthermore, the claim that iron(II) complexes of bpy, phen and its derivatives are not suitable for studying their DNA interaction, due to their labile inversion, is no longer a serious problem because a shift in the inversion equilibrium of the enantiomers occurs upon addition of DNA to the racemate of the complexes^(11a-c). This great advantage of the so-called "Pfeiffer effect" would be the fact that stereospecificity of the iron(II) complexes upon binding to DNA can be monitored directly in solution through the CD spectra of the mixture.

On the basis of these criteria, it is likely that the lack of reports on the synthesis of iron(II) and nickel(II) mixed-ligand complexes containing phen and dip arises from the instability of these metal complexes towards racemization. Consequently, their preparation, according to procedures analogous to those of ruthenium(II) mixed-ligand complexes, is probably impossible. For the purpose of examining noncovalent interaction of transition-metal complexes with DNA, we here developed a rapid and simple procedure for the simultaneous preparation of two types of mixed ligand complexes containing phen and dip, *i.e.* $[M(\text{phen})_2(\text{dip})]^{2+}$ and $[M(\text{phen})(\text{dip})_2]^{2+}$ ($M = \text{Fe}^{\text{II}}$ and Ni^{II}). The general structure of the mixed-ligand complexes prepared is shown in Figure 1. The selection of iron(II) and nickel(II) as metal ions is based on the fact that they are more common in the living body than is ruthenium, giving the possibility for *in vivo* study. The mixed-ligand complexes were prepared from the tris-complexes, $[M(\text{phen})_3]^{2+}$ by ligand substitution with dip. The crude products were purified by preparative HPLC to give pure mixed-ligand complexes of the types $[M(\text{phen})_2(\text{dip})]^{2+}$ and $[M(\text{phen})(\text{dip})_2]^{2+}$ and were then characterized by elemental analysis, reversed-phase HPLC, u.v./vis and FT-IR spectroscopy. In addition, ^1H -n.m.r. and Mössbauer spectroscopic analysis were also performed for iron(II) mixed-ligand complexes and the

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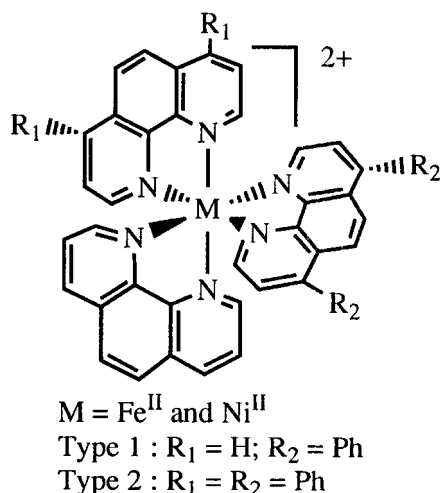


Figure 1. Structure of synthesized mixed-ligand complexes. The complexes have both Λ -(left) and Δ -(right) enantiomers; only Λ -enantiomer is viewed.

results were presented together with the preliminary results of their interaction with DNA.

Experimental

Materials

4,7-Diphenyl-1,10-phenanthroline and 1,10-phenanthroline were obtained from Kanto Chemical Co. Inc. (Tokyo, Japan) and Dojindo Laboratories (Kumamoto, Japan), respectively and were used without further purification. NaClO₄ and KPF₆ for counter ions were obtained from Kanto Chemical Co. Inc. The salts Fe(NH₄)₂(SO₄)₂·6H₂O, NiSO₄·6H₂O and NaCl were purchased from Wako Pure Chemical Industries (Tokyo, Japan). MeCN, Me₂CO, CHCl₃ (all special grade) and distilled H₂O (HPLC grade) were obtained from Katayama Chemical Industries and were used as supplied. Tris(1,10-phenanthroline)iron(II) perchlorate, [Fe(phen)₃](ClO₄)₂ and tris(1,10-phenanthroline)nickel(II) perchlorate, [Ni(phen)₃](ClO₄)₂ were prepared according to the directions of Schilt and Taylor⁽¹³⁾ and the structures of the complexes obtained were confirmed by elemental analysis and u.v./vis spectroscopy before use as starting materials. Calf thymus (CT)-DNA and 2-amino-2-hydroxymethyl-1,3-propanediol (Tris) for DNA buffer were supplied by Sigma Chemical Company (USA) and Junsei Chemical Co. Ltd. (Tokyo, Japan) respectively.

General procedure for preparing [M(phen)₂(dip)]X₂ and [M(phen)(dip)₂]X₂ (M = Fe^{II} and Ni^{II}, X = ClO₄⁻ or PF₆⁻)

Two types of mixed-ligand complexes cited above were synthesized simultaneously by ligand substitution using the following procedure. The complex [Fe(phen)₃](ClO₄)₂ (1.0 g, 1.26 mmol) or [Ni(phen)₃](ClO₄)₂ (1.0 g, 1.25 mmol) was dissolved in Me₂CO (50 cm³) and the mixture was heated to near boiling (ca. 50 °C). A slight molar excess of 4,7-diphenyl-1,10-phenanthroline (0.5 g, 1.50 mmol) in MeOH (10 cm³) was then added dropwise to the hot Me₂CO solution and the reaction mixture was

stirred magnetically at 50 °C, for 3 h and 6 h for the iron(II) complexes and nickel(II) complexes, respectively. The reaction mixture was allowed to cool to room temperature, Me₂CO was removed using a rotary evaporator, to leave crude [M(phen)₂(dip)]X₂ and [M(phen)(dip)₂]X₂ products containing impurities of eliminated phen, unreacted starting material [M(phen)₃]²⁺ and by-product [M(dip)₃]²⁺. The crude products were then fractionated by preparative HPLC on a Develosil ODS column utilizing MeCN-H₂O (80:20, v/v) containing 0.06 M NaClO₄ as a mobile phase at a flow rate of 7.0 cm³/min, followed by immediate evaporation of MeCN and subsequent extraction of the mixed-ligand complexes from the aqueous solution into CHCl₃. Removal of the solvent using a rotary evaporator yielded pure crystalline complexes of bis(1,10-phenanthroline)4,7-diphenyl-1,10-phenanthrolineiron(II) perchlorate [Fe(phen)₂(dip)](ClO₄)₂ and bis(4,7-diphenyl-1,10-phenanthroline)1,10-phenanthrolineiron(II) perchlorate [Fe(phen)(dip)₂](ClO₄)₂ or bis(1,10-phenanthroline)4,7-diphenyl-1,10-phenanthrolineiron(II) perchlorate [Ni(phen)₂(dip)](ClO₄)₂ and bis(4,7-diphenyl-1,10-phenanthroline)1,10-phenanthrolineiron(II) perchlorate [Ni(phen)(dip)₂](ClO₄)₂. The corresponding bis(hexafluorophosphate) complexes were obtained when, KPF₆ instead of NaClO₄ was used as a counter ion reagent during the fractionation of the crude products. Yields were ca. 25% and 35% for [M(phen)₂(dip)]X₂ and [M(phen)(dip)₂]X₂ complexes respectively, giving a net yield of 60% from the starting material [M(phen)₃](ClO₄)₂.

Measurements

Elemental analyses were performed by the Central Laboratory, Faculty of Science and Technology, Keio University (Yokohama, Japan) and the results are displayed in Table 1. U.v./vis and CD spectra (for complex-DNA interaction study) were recorded using a Jasco V-550 UV/VIS spectrophotometer and a Jasco J-720 WI spectropolarimeter, respectively. Molar absorptivities (ϵ) were obtained by constructing calibration graphs of the corresponding compounds utilizing at least 5 individual concentrations. ¹H-n.m.r. spectra were recorded on a 270-MHz Jeol NMR spectrometer using Me₄Si as an internal standard. I.r. spectra (KBr discs) were recorded on a Jasco FT/IR-350 FT-IR spectrometer. The Mössbauer spectra were measured against a ⁵⁷Co source (370 MBq) in a rhodium matrix with a Wissel constant-acceleration transducer and the spectra obtained were fitted to Lorentzian curves using a least-squares fitting program. The isomer shift was referred to the centroid of the sextet of natural iron foil at room temperature.

A Yanako L-4000 W pump, a Develosil ODS column (10 μ m spherical octadecyl silica, 25 cm $\times$ 3 cm I.D., Nomura Chemical Co., Aichi, Japan) equipped with a Kusano-Kagaku KV-3W loop injector (0.59 cm³), and a Jasco Uvidec 100-III UV detector together with a Dip 3066 pen Recorder from Yokogawa Electric Work Ltd. (Tokyo, Japan) were used for semi-preparative HPLC. The Jasco PU-980 liquid chromatograph, equipped with a Reodyne 7125 (100 μ l loop) injector, an Inertsil ODS column (5 μ m spherical ODS, 250 mm $\times$ 4.6 mm I.D., GL Science Co., Tokyo), and a Jasco MULTI-340 multichannel (diode array) spectrophotometer detector,

Table 1. Elemental analysis, chromatographic and u.v./vis data for tris- and mixed-ligand complexes

Complex	Found (Calcd.) %			HPLC ^a	Purity (%) ^b	u.v./vis ^c $\lambda_{\max}/\text{nm}$ ($\epsilon \times 10^{-4}/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$)
	C	H	N	R _t (min)		
[Fe(phen) ₃](ClO ₄) ₂	54.1(54.4)	2.9(3.0)	10.7(10.6)	3.18	–	268, 510(1.10)
[Fe(phen) ₂ (dip)](ClO ₄) ₂	59.2(59.6)	3.5(3.5)	8.4(8.7)	3.94	96.5	269(13.0), 284(sh) (10.2), 519(1.65)
[Fe(phen)(dip) ₂](ClO ₄) ₂	64.9(64.9)	3.7(3.7)	8.0(7.8)	5.89	98.4	271(sh) (10.1), 285 (11.0), 527(1.91)
[Ni(phen) ₂ (dip)](ClO ₄) ₂ · 5H ₂ O	55.1(55.4)	3.5(4.0)	7.9(8.1)	4.64	97.6	271(11.1), 284(sh) (9.62)
[Ni(phen)(dip) ₂](ClO ₄) ₂ · 6H ₂ O	59.4(59.5)	3.9(4.2)	6.8(6.9)	6.93	98.7	273(sh) (13.6), 284(14.6)
[Ni(phen) ₃](ClO ₄) ₂ · H ₂ O	53.4(53.0)	3.2(3.2)	10.0(10.3)	3.47	–	269

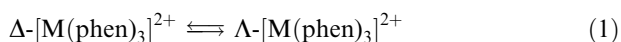
^a Eluent: MeCN-H₂O (80/20, v/v) 0.06 M NaClO₄, column: Inertsil ODS, flow rate: 1 ml/min; ^b determined by injecting each product into HPLC under the conditions mentioned and the purity was justified from the percentage of peak area of each product obtained by integrator; ^c dissolved in MeCN; Abbreviation: phen = 1,10-phenanthroline, dip = 4,7-diphenyl-1,10-phenanthroline and R_t = retention time.

was used for analytical HPLC. The detector was connected to a personal computer (NEC PC-980 VM) for the purpose of peak analysis. The mobile phase for both preparative and analytical HPLC was prepared by volume and was ultrasonically mixed for several minutes before use. All measurements were performed at room temperature (*ca.* 25 °C).

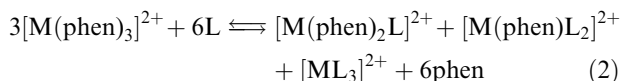
Results and discussion

Synthesis of $[M(\text{phen})_2(\text{dip})]X_2$ and $[M(\text{phen})(\text{dip})_2]X_2$ complexes

In contrast to ruthenium mixed-ligand complexes, usually prepared by reaction of free ligands with a synthetic intermediate, *e.g.* monoaqua or monoacetone complexes^(10d), mono- and di-substituted complexes of iron(II) and nickel(II), $[M(\text{phen})_2(\text{dip})]^{2+}$ and $[M(\text{phen})(\text{dip})_2]^{2+}$, can be prepared simultaneously from their corresponding tris-complexes, $[M(\text{phen})_3]^{2+}$ by the ligand-substitution method. This procedure is based upon the instability of the iron(II) and nickel(II) tris-complexes towards their racemization. Although no optical activity was observed for the racemic solution,



Equation 1 indicates that there are dynamic changes from Δ - to Λ -enantiomer and *vice versa*. This condition gives rise to substitution of one, two or even all of phen ligands for a new ligand L such as dip to yield mono-, di- and tri-substituted complexes most likely by a dissociative mechanism (Equation 2).



On the other hand, such a phenomenon was not observed for ruthenium complexes since, once formed, they were relatively inert towards racemization.

Our procedure is obviously advantageous because we can synthesize both mono- and di-substituted complexes using only one procedure. A product equilibrium was apparently attained after a certain period. This is strongly supported by the fact that there are no remarkable changes in the percentages of the products upon stirring the reaction mixture for longer than 3 h for the preparation of iron(II), or longer than 6 h for the nickel(II) complexes. The differences in reaction time for the synthesis of iron(II) and nickel(II) mixed-ligand complexes arose as a consequence of the differences in the racemization rate between the two complexes. It has

been reported that $[Ni(\text{phen})_3]^{2+}$ racemized slower than $[Fe(\text{phen})_3]^{2+}$ ^(14,15), a result in agreement with the fact that the longer reaction time was needed for the synthesis of nickel(II) complexes, in order to obtain the same results as with the iron(II) complexes.

The effect of various solvents such as acetonitrile, methanol and acetone on ligand substitution revealed that all three solvents may be used for the synthesis. However our results show that acetone was the most suitable solvent. On the basis of this, we propose that the substitution of $[M(\text{phen})_3]^{2+}$ with dip takes place by a dissociative mechanism in which an intermediate with a reduced coordination number, *i.e.* $[M(\text{phen})_2]^{2+}$ is rapidly captured by acetone (solvent molecule) to form other intermediate mixed-ligand complexes containing two molecules of Me₂CO, $[M(\text{phen})_2(\text{Me}_2\text{CO})_2]^{2+}$. This intermediate, in turn, is converted into a product, presumably by a second dissociative process. This explanation is consistent with the conclusions reached by other workers^(10d,16) regarding the role of acetone solvent in the synthesis of mixed-ligand complexes.

Another advantage of our procedure is that the crude product can be readily purified, by preparative HPLC in one step process, to yield relatively pure mixed-ligand complexes of the $[M(\text{phen})_2(\text{dip})]^{2+}$ and $[M(\text{phen})(\text{dip})_2]^{2+}$ type. The complexes were dark-red [iron(II)] and pale red-rose [nickel(II)]. Hexafluorophosphate salts appeared to be generally lighter than the perchlorate salts. Nickel(II) complexes were found to contain molecules of water while the deviation in some of the observed elemental analyses data (Table 1) from the required values may be attributed to solvent contamination in the crystals; such deviations are quite common in these types of complexes^(3a,17). The complexes are very stable in the solid state and show no changes in their absorption spectra in the dissolved state (Tris-HCl buffer solution) even after three days of dissolution, suggesting that no redistribution of the ligands takes place during the period.

Preparative and analytical chromatography

In order to obtain very pure $[M(\text{phen})_2(\text{dip})]^{2+}$ and $[M(\text{phen})(\text{dip})_2]^{2+}$, it was necessary to employ chromatography. Our results are described here in some detail because they may be of general interest in the context of separations involving other cationic mixed-ligand complexes. Product purification was carried out by semi-preparative HPLC using conditions described in the Experimental section. The selection of MeCN-H₂O (80:20, v/v) containing 0.06 M NaClO₄ as a mobile

phase was based upon our previous experience in separating of iron(II) complexes of 1,10-phenanthroline and its derivatives^(1b,c), except that HClO₄ was replaced by NaClO₄ or KPF₆. By using this mobile phase composition, all components (products and contaminants) were well-separated from each other and eluted after less than 1 h at a flow rate of 7.0 cm³ min⁻¹. First, two free ligands of phen and dip (if any) were eluted consecutively, followed by unreacted [M(phen)₃]²⁺ and two types of mixed-ligand complexes, *i.e.* [M(phen)₂(dip)]²⁺ and [M(phen)(dip)₂]²⁺. The by-product, [M(dip)₃]²⁺, appeared after elution of [M(phen)(dip)₂]²⁺, however three or four runs were repeated in order to obtain an ample amount of product due to limitation of the sample size that can be loaded into the injector (0.59 cm³).

As an additive to produce a counter ion in the mobile phase, HClO₄ generally gave sharper peaks and eluted faster than NaClO₄. This is probably due to the role of the H⁺ ions from HClO₄ in preventing the surface of residual silanol of the column packing materials from adsorbing the products, so that tailing is minimized and the products traverse the column faster. However, the use of HClO₄ as the counter ion is not recommended because the products tend to dissociate in acidic solution (Equation 3)⁽¹⁴⁾ and, moreover, a low pH in the mobile phase solution may also damage the column packing.



Immediate evaporation of acetonitrile from the solution obtained by fractionation is also suggested in order to avoid possible substitution for the solvent molecules or dissociation of the complexes. Otherwise, products of low purity will be obtained (*e.g.* <95 % by HPLC).

Figure 2 gives the chromatograms of each isolated mixed-ligand complex eluted on analytical HPLC under the conditions described in the Experimental section. The retention times (*R_t*/min) of each complex are collected in Table 1. The increase in *R_t* of the complexes with the number of dip ligands suggests that the hydrophobicity of the complexes also increases in the same order. This effect is undoubtedly generated by substitution of the two phenyl groups in the dip ligand. It is clear that the synthesized mixed-ligand complexes are > 95 % pure, as indicated by the detection of only a single peak for each complex and by good elemental analyses.

Absorption spectra

The absorption spectra of [Ni(phen)₂(dip)]²⁺ and [Ni(phen)(dip)₂]²⁺ in acetonitrile are shown in Figure 3. Spectra, relatively similar to those of the nickel(II) complexes, were obtained for the related iron(II) complexes with an additional intense absorption band characteristic of phen-iron(II) complexes in the visible region. The band assignment for the reported complexes was made by analogy with the ruthenium complexes^(10b). Inspection of Figure 3 as well as Table 1 reveals that, while the spectra of the tris-complexes show only one peak in the 250–300 nm range, the spectra of related mixed-ligand complexes exhibit two distinct peaks in this region: one at *ca.* 271 nm and the other at 284 nm. The mono-substituted complexes [M(phen)₂(dip)]²⁺ exhibit an absorption maximum (*λ_{max}*) at the first peak and a shoulder at the second, while the reverse pattern is observed for the di-substituted complexes [M(phen)(dip)₂]²⁺. These two

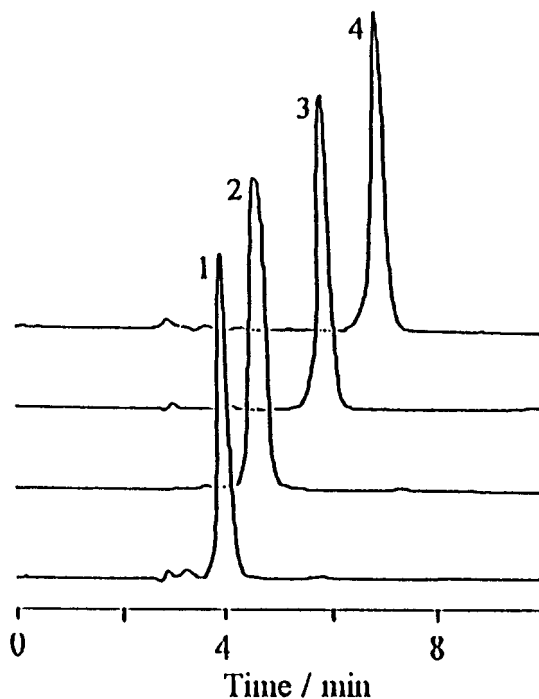


Figure 2. Chromatogram of each isolated mixed-ligand complex eluted on Inertsil ODS column using MeCN-H₂O (80/20, v/v) containing 0.06 M NaClO₄ as a mobile phase and detected at a wavelength of 280 nm: 1, [Fe(phen)₂(dip)]²⁺, 2, [Ni(phen)₂(dip)]²⁺, 3, [Fe(phen)(dip)₂]²⁺ and 4, [Ni(phen)(dip)₂]²⁺. The detection of a single peak confirms the purity of the complex.

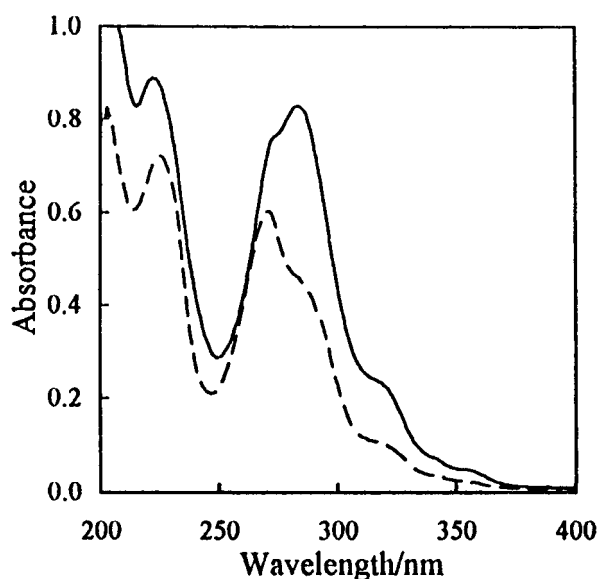


Figure 3. U.v./vis spectra of [Ni(phen)(dip)₂]²⁺ (full lines) and [Ni(phen)₂(dip)]²⁺ (broken lines) in MeCN. Two *λ_{max}* in the u.v. region at 271 nm and 284 nm correspond to *π-π** transitions of phen and dip, respectively.

peaks are obviously ascribed to the localized or intraligand *π-π** transitions (ILT) of phen and dip ligands, respectively^(10b-d,17). The same transition is found at 265 nm for free phen and at 275 nm for free dip, suggesting that upon coordination of the ligands to the metal ions, the *λ_{max}* of the mixed-ligand complexes is shifted to longer wavelength by at least 5 nm.

The intense visible absorption band of the iron(II) complexes in the 375–600 nm region is mainly due to a metal-to-ligand charge transfer (MLCT) transition involving the promotion of electrons from the filled *d* orbital of the iron to the vacant π^* orbital of the ligands ($d\text{-}\pi^*$)^(10b,d). The λ_{max} of the complexes in this region gives rise to a regular red shift (*ca.* 5 nm) as the number of dip ligands in the complexes increase. The intensity of the band, as indicated by the value of its molar absorptivity (ϵ), also increases significantly with the number of dip substituents. For example, the ϵ value of the tris-dip complex is twice as high as that of the tris-phen complex. This result is in agreement with previous reports regarding the effect of phenyl substitution on the intensity of the CT bands⁽¹⁸⁾. These absorption spectral results demonstrate that the synthesized metal-complexes are of the mixed-ligand type containing phen and dip and that the absorption spectra of the complexes are very useful for identification purposes.

Vibrational spectra

As regards their gross features, the i.r. spectra of the iron(II) and nickel(II) mixed-ligand complexes are similar and there are no major differences in their vibrational frequencies. These results are not surprising because both metal ions have the same valence and number of coordinated ligands. However, more detailed examination shows that the spectra of iron(II) and nickel(II) mixed-ligand complexes can be easily distinguished from those of either the free ligands or the tris-complexes, thus providing a satisfactory method for identifying them.

In general, the spectra of tris- and mixed-ligand complexes are simpler than those of the free ligands especially in the 600–900 cm^{-1} region, which is associated with the ring deformation of the ligands^(10c,13,19). Perchlorate bands which appear at *ca.* 1090 cm^{-1} , 930 cm^{-1} and 640 cm^{-1} belong to an ionic species^(19a,20). This means that the perchlorate ion is not involved in the metal-ligand coordination and only acts as a counter ion.

Differentiation of both iron(II) and nickel(II) mixed-ligand complexes from the related tris-complexes requires more careful examination of the spectra in the region specified in order to obtain an unequivocal answer. A detailed comparison of the spectra in the 1400–1650 cm^{-1} and 700–900 cm^{-1} regions appears more suitable for this differentiation⁽¹³⁾. The spectra of the $[\text{M}(\text{phen})_3]^{2+}$ moiety in the 1400–1650 cm^{-1} region, which is concerned with the ring modes (ν_{CC} and ν_{CN}), consist of three intense bands; one close to 1510 cm^{-1} , the second in the neighbourhood of 1600 cm^{-1} and the third at *ca.* 1420 cm^{-1} (Figure not shown). The first and the second bands usually split into doublet and triplet bands respectively. The same spectral pattern is brought about by the $[\text{M}(\text{dip})_3]^{2+}$ moiety, except that an additional intense band most likely due to the phenyl substituent appears at *ca.* 1560 cm^{-1} . All the mixed-ligand complexes also exhibit a relatively intense band at *ca.* 1560 cm^{-1} besides those characteristic of phen, unequivocally suggesting the presence of both phen and dip ligands in the complexes. Moreover, a general shift of the ring vibrations in this region to higher frequencies, compared with those of the free ligands, confirms that both phen and dip ligands are coordinated to the metal ions.

Additional valuable evidence can be obtained by carefully examining the spectra of the tris- and mixed-ligand complexes in the 600–900 cm^{-1} region. The representative spectra of iron(II) complexes in this region are given in Figure 4. The tris-complexes $[\text{M}(\text{phen})_3]^{2+}$ consistently exhibit two intense bands at *ca.* 725 cm^{-1} , assigned to the out-of-plane motion of the hydrogen atoms on the heterocyclic rings and at *ca.* 845 cm^{-1} assigned to the hydrogen in the center ring^(13,19a). These two bands must be expected because there are two distinct groups of hydrogen atoms in the ring system, *i.e.* six hydrogen atoms bound to the heterocyclic rings and, two hydrogen atoms to the center ring. In $[\text{M}(\text{dip})_3]^{2+}$, the former band is shifted to lower frequency (702 cm^{-1}), while the latter becomes extremely weak, but new intense bands observed at 738 cm^{-1} and 767 cm^{-1} , are probably due to the presence of phenyl substituents. Again, the fact that all the mixed-ligand complexes exhibit intense bands at all wavenumbers mentioned above confirms that the complexes should contain both phen and dip ligands and thus obviously mixed-ligand complexes.

¹H-n.m.r. and Mössbauer spectra of iron(II) complexes

The ¹H-n.m.r. data of the iron(II) mixed-ligand complexes, $[\text{Fe}(\text{phen})_2(\text{dip})]^{2+}$ and $[\text{Fe}(\text{phen})(\text{dip})_2]^{2+}$, in DMSO are presented in Table 2 together with those of their corresponding tris-complexes and free ligands for comparison. As expected, the spectra of both free ligands (either phen or dip) and tris-complexes exhibit

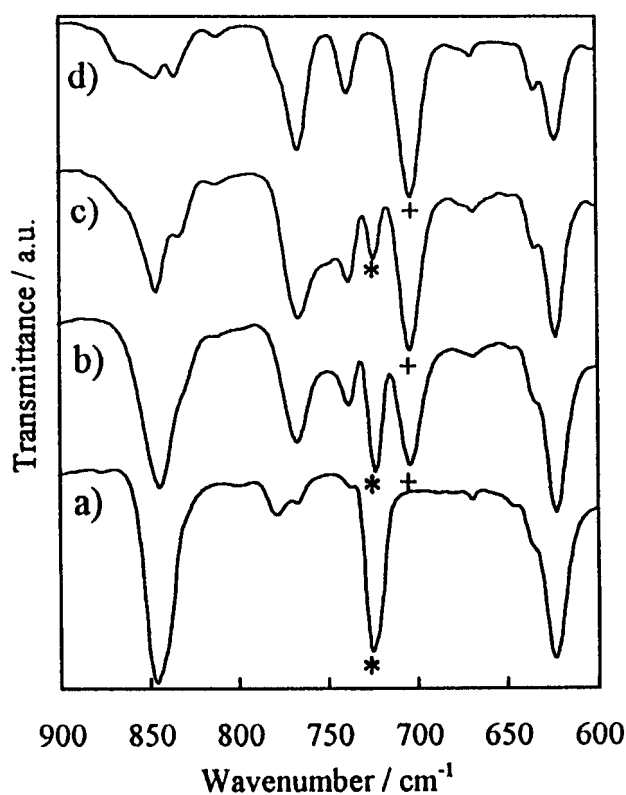


Figure 4. I.r. spectra of (a) $[\text{Fe}(\text{phen})_3]^{2+}$, (b) $[\text{Fe}(\text{phen})_2(\text{dip})]^{2+}$, (c) $[\text{Fe}(\text{phen})(\text{dip})_2]^{2+}$ and (d) $[\text{Fe}(\text{dip})_3]^{2+}$ complexes in the region between 600–900 cm^{-1} . “*” denotes the γ -CH (out-of-plane mode of hydrogen atom in the heterocyclic rings) of phen and “+” corresponds to that of dip. It is clear that the mixed-ligand complexes (b and c) contain both of them.

four distinct resonance patterns owing to the symmetrical structure of the molecules. The assignment of the various peaks to the proton is either straightforward or analogous to those of ruthenium complexes which have been extensively studied^(6,10d,12c,21). The splitting will consist of two doublets, H_{2,9} and H_{4,7}, one singlet, H_{5,6} and one multiplet, H_{3,8} (see Table 2 for carbon numbering).

Although the spectra of the iron(II) mixed-ligand complexes are more complicated, no major differences in the resonance patterns are observed when one or two phen of the tris-complexes are replaced by dip ligands. This is probably due to the slight difference in the structure between phen and dip. Therefore, as shown in Table 2, eight patterns of resonance contributed from phen (4 patterns) and dip (also 4 patterns) are readily distinguishable. Closer examination of the spectra, however, reveals that some asymmetries in resonance peaks are detected because the aromatic rings of each ligand are not purely equivalent in their magnetic environment, due to the presence of phenyl substituents on the dip ligand. For example, the H_{5,6} resonance of either phen or dip in [Fe(phen)₂(dip)]²⁺ and [Fe(phen)(dip)₂]²⁺ is no longer a pure singlet but is split into one major singlet flanked by two small peaks. For the same reason, such splitting patterns have also been observed for ruthenium mixed-ligand complexes containing bipyridine and other ligands^(10d,21a,b).

A general chemical shift (δ) downfield in the protons occurs upon chelation of the ligand to iron(II) ion except for the H_{2,9} protons. This deshielding effect can be attributed to the decrease in electron density at the individual carbon nuclei as a result of the interaction with the positive iron(II) ion^(21b). Unlike the other protons, the H_{2,9} protons show a dramatic upfield shift upon chelation of the ligands to the iron(II) ion. The magnitude of the upfield shifts is between 1.27–1.36 ppm and 1.24–1.30 ppm in phen and dip, respectively. This shielding effect is readily explained, given the structural basis of the complexes^(21a). These protons lie within the shielding cone of the heterocyclic ring of the adjacent

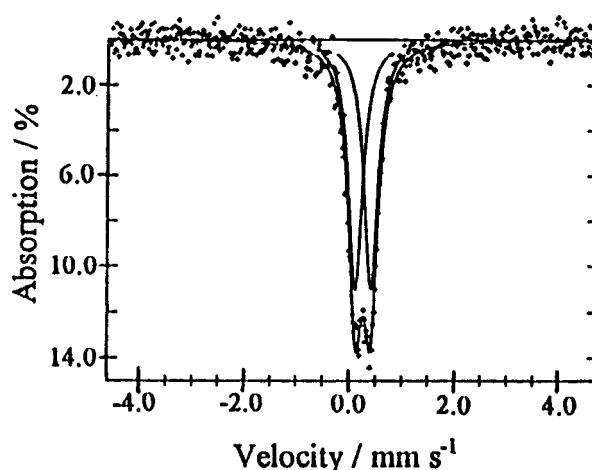


Figure 5. Room temperature Mössbauer spectrum of [Fe(phen)(dip)₂](PF₆)₂ complex exhibiting a doublet with a small quadrupole splitting (ΔE_Q).

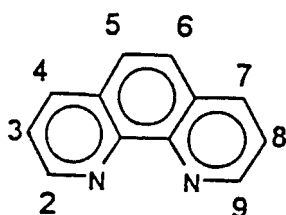
ligand. Castellano *et al.*⁽²²⁾ called this a diamagnetic anisotropic effect of the aromatic ring of the adjacent ligand. Furthermore, upon coordination of the ligands to the iron(II) ion, it is likely that the interaction between these protons and the lone pair of electrons in the nitrogen atom of the ligand is negated^(2a). Therefore, it is not surprising that the magnitude of the upfield shifts is relatively large. The relative ratio of phen protons to dip protons is 16:16 in [Fe(phen)₂(dip)]²⁺ and 8:32 in [Fe(phen)(dip)₂]²⁺, giving unequivocal evidence for the proposed structural assignments.

The ⁵⁷Fe Mössbauer spectra of the iron(II) mixed-ligand complexes and related tris-complexes were studied using powder samples at room temperature (298 K). Figure 5 gives the Mössbauer spectrum of one of the complexes studied, *i.e.* [Fe(phen)(DIP)₂](PF₆)₂ as an example. Both tris- and mixed-ligand complexes show a quadrupole doublet with small splitting energy. Calculation from the spectra gives an isomer shift, δ , of 0.29 ± 0.02 mm s⁻¹ and quadrupole splitting, ΔE_Q ,

Table 2. ¹H-n.m.r. and Mössbauer spectroscopic data for the ligands and iron(II) complexes

Compounds ^a	¹ H-n.m.r. Chemical shift (δ_H) (270 MHz, Me ₂ SO, SiMe ₄)				Mössbauer ^b δ /mm s ⁻¹	ΔE_Q /mm s ⁻¹
	H _{2,9}	H _{3,8}	H _{4,7} /Ph _{4,7}	H _{5,6}		
phen	9.13(2H, dd)	7.80(2H, m)	8.52(2H, dd)	8.02(2H, s)	—	—
dip	9.14(2H, d)	7.69(2H, d)	7.56(10H, m)	7.88(2H, s)	—	—
[Fe(phen) ₃] ²⁺	7.77(6H, dd)	7.72(6H, m)	8.92(6H, dd)	8.41(6H, s)	0.32	0.28
[Fe(phen) ₂ (dip)] ²⁺	7.83(4H, dd)	7.75(4H, m)	8.84(4H, dd)	8.44(4H, s)	0.28	0.27
	7.88(2H, d)	8.02(2H, d)	7.67(10H, m)	8.27(2H, s)		
[Fe(phen)(dip) ₂] ²⁺	7.86(2H, dd)	7.81(2H, m)	8.88(2H, dd)	8.46(2H, s)	0.27	0.29
	7.90(4H, d)	8.02(4H, d)	7.68(20H, m)	8.29(4H, s)		
[Fe(dip) ₃] ²⁺	7.84(6H, d)	8.02(6H, d)	7.68(30H, m)	8.35(6H, s)	0.29	0.26

^a As perchlorates; ^b δ = isomer shift and ΔE_Q = quadrupole splitting at room temp (298 K). Protons are assigned as follows:



varying between 0.26 and 0.29 mm s⁻¹; the detailed results of each complex are collected in Table 2. These results differ significantly from those measured on Fe(diimine)₂X₂ compounds in the ⁵T₂ (O_h) ground state (S=2), *i.e.* the high spin state of iron(II), where δ commonly lies between 0.96 and 1.09 mm s⁻¹ and ΔE_Q between 2.40 and 3.40 mm s⁻¹(23). The Mössbauer spectral parameters obtained are similar to those of Fe(diimine)₂X₂ compounds in the ¹A₁ (O_h) ground state (S=0); *e.g.*, for [Fe(phen)₂(CN)₂]·H₂O δ = 0.20 and ΔE_Q = 0.59 mm s⁻¹, and for [Fe(phen)₂(NO₂)₂] δ = 0.28 and ΔE_Q = 0.38 mm s⁻¹(23,a,b). Furthermore, the isomer shift and quadrupole splitting of the complexes studied are also comparable with δ = 0.33 ± 0.04 mm s⁻¹, and ΔE_Q between 0.18 and 0.31 mm s⁻¹ for [Fe(diimine)₂X₂] complexes, where diimine is phen, bpy or 4,7-dimethyl-phen and X₂ stands for bidentate oxalato, malonato or two fluoro ligands(24). This evidence undoubtedly suggests that all of the complexes studied, either mixed-ligand or tris-complexes, are in the ¹A₁ (O_h) ground state (S=0), *i.e.* the

low spin state of iron(II) complexes. The low ΔE_Q values for the complexes imply a relatively small electric field gradient (efg) and thus an almost spherical d-electron distribution(24). In addition, the almost constant ΔE_Q values indicate that the substitution of phen in [Fe(phen)₃]²⁺ by dip does not cause any distortion in the spherical symmetry of the complexes and thus no significant geometrical distortion is expected(25). This conclusion agrees well with the n.m.r. results discussed above.

Interaction of iron(II) complexes with CT-DNA

The interaction of [Fe(phen)₂(dip)]²⁺ and [Fe(phen)(dip)₂]²⁺ with CT-DNA was studied by using u.v./vis and circular dichroism (CD) spectroscopy and the results were compared with those of the parent complex, [Fe(phen)₃]²⁺. The visible absorption and CD spectra of the complexes were measured in the absence and presence of CT-DNA and are compared in Figure 6 and 7. Our preliminary results, *i.e.* the values of their binding

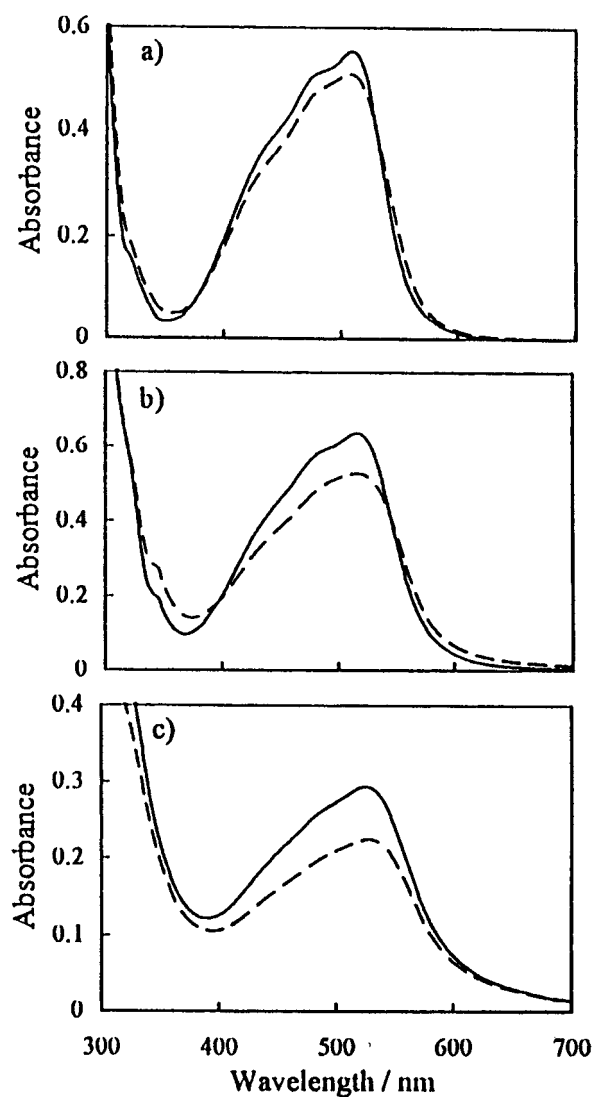


Figure 6. Absorption spectra of (a) [Fe(phen)₃]²⁺ (50.3 μM), (b) [Fe(phen)₂(dip)]²⁺ (39.2 μM) and (c) [Fe(phen)(dip)₂]²⁺ (16.3 μM) in the metal-to-ligand charge transfer (MLCT) region in the absence (full lines) and in the presence (broken lines) of calf-thymus (CT) DNA at [complex]/[CT-DNA] = 0.1. Hypochromicities in these bands increase with the content of dip ligand.

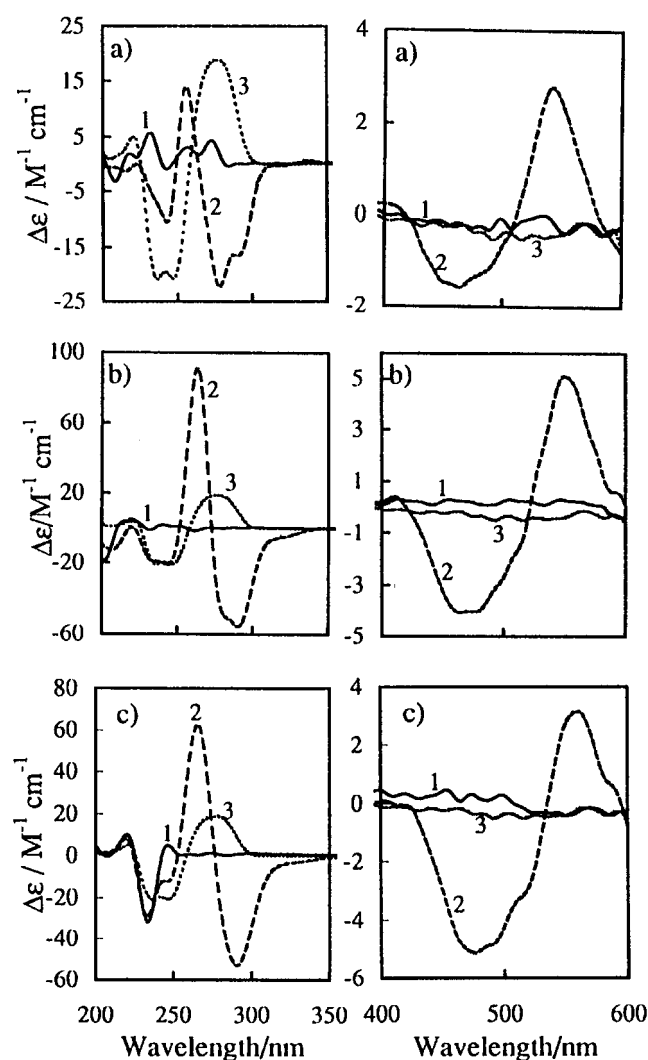


Figure 7. Circular dichroism (CD) spectra of racemic (a) [Fe(phen)₃]²⁺ (50.3 μM), (b) [Fe(phen)₂(dip)]²⁺ (24.5 μM) and (c) [Fe(phen)(dip)₂]²⁺ (20.1 μM) complexes dissolved in Tris buffer pH = 7.2 (μ = 0.05 M NaCl, 25 °C) in the u.v./vis region: 1, in the absence of CT-DNA, 2, in the presence of CT-DNA (at [complex]/[CT-DNA] = 0.15) and 3, free CT-DNA. A strong CD spectrum develops upon addition of CT-DNA to the racemic complex.

constants (data were not included), hypochromic shift in the MLCT bands and the developed CD spectra of the complexes upon binding to DNA show that both iron(II) mixed-ligand complexes interact with CT-DNA more strongly than does the parent complex. The increasing order of the binding constants and hypochromic shift in the MLCT bands, i.e.: $[\text{Fe}(\text{phen})_3]^{2+} < [\text{Fe}(\text{phen})_2(\text{dip})]^{2+} < [\text{Fe}(\text{phen})(\text{dip})_2]^{2+}$, is consistent with the hydrophobic properties of the complexes. In addition, CD spectroscopic studies reveal that an intense spectrum develops in the u.v./vis region upon the addition of CT-DNA to racemic $[\text{Fe}(\text{phen})_2(\text{dip})]^{2+}$ or to $[\text{Fe}(\text{phen})(\text{dip})_2]^{2+}$. In fact, no CD spectrum in the u.v./vis region is detected for the racemate of these complexes in the absence of CT-DNA. These results provide evidence for the existence of the interaction of the complexes with CT-DNA. Moreover, a striking resemblance in the profile of the CD spectra (after equilibration) to that of Δ -enantiomer⁽²⁶⁾ indicates that the spectra correspond to the diastereomers of $\{\text{CT-DNA}; \Delta\text{-}[\text{Fe}(\text{L})_3]^{2+}\}$ complexes, which may be enriched by the chiral equilibrium shift⁽²⁷⁾. This directly suggests that Δ -enantiomers of iron(II) complexes are preferentially bound to CT-DNA. Such phenomena have been observed previously by some workers^(11a,b,c) for $[\text{Fe}(\text{bpy})_3]^{2+}$ and $[\text{Fe}(\text{phen})_3]^{2+}$. On the basis of u.v./vis and CD spectroscopic results, it is clear that the iron(II) mixed-ligand complexes reported here interact with CT-DNA quite strongly. Detailed studies on the interaction of these complexes with CT-DNA as well as with other polynucleotides are ongoing subjects and the results will be reported elsewhere.

Acknowledgements

The first author wishes to thank The Hitachi Scholarship Foundation, Tokyo, Japan for financial support. Authors also thank the editor for intensively editing the manuscript.

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(Received 15 June 1998;

Accepted 20 August 1998)

TMC 4281