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The Rhodium Complex of a Tris(bipyridine) Ligand – Its Electrochemical Behaviour and Function as Mediator for the Regeneration of NADH from NAD^+

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Abstract. The tris-bipyridine ligand **3a** and its stoichiometric Rh^{3+} complex have been prepared. Cyclovoltammograms of the complex at pH 7.4 using a glassy carbon disk electrode reveal a strong reduction peak at –620 mV and two weak reduction peaks at more negative voltage. The reduction potential of the new complex is shifted by 300 mV to more positive values as compared to $[Rh(bipy)_3]^{3+}$. There is no reversible reoxidation peak of the Rh(I) complex formed due to the decomplexation of one of the three bipyridine units in the course of the transition Rh(III) → Rh(I). The Rh(III) complex of **3a** was also studied with respect to its function as a possible redox mediator for the electrochemical regeneration of NADH from NAD^+. The preparative electrolysis of the Rh^{3+} complex of **3a** in the presence of NAD^+ yields a selective formation of NADH, whereas NAD dimers were not detected. On the other hand, a significant acceleration of this reaction compared to $[Rh(bipy)_3]^{3+}$ was not observed.

Key words: Bipyridine, bipyridine ligand, complexation, complex chemistry, complex formation, cyclovoltammetry, electrochemical mediator, electrochemical reduction, electrochemistry, NAD^+, NADH, Rh^{3+}, Rh^+, Rh complex.

1. Introduction

Recently we reported some new siderophore type macrocyclic ligands and their use as ferric ion selective complexing agents as well as the properties and stabilities of their metal ion complexes [1]. Whereas in the ligand **1** the complexation of cations is due to the pyrocatechol oxygen donor atoms, in **2** and **3** the metal ions are complexed by the nitrogen atoms of the bipyridine units. It seemed attractive to study the complexation of transition metal ions like Rh and to investigate the possible application of the complexes as mediators for the indirect electrochemical reduction of NAD^+ [2]. While in the macrobicyclic ligands **1** and **2** the central metal ion is strongly complexed and shielded sterically, the open chained ligand **3a** with three bipyridine units held together by a 1,3,5-substituted benzene spacer, seemed to be better suited for this purpose because here a change of the ligand sphere (resp. coordination number) could possibly be achieved by a change of the oxidation number of the central atom. The transition of Rh(III) to Rh(I) by electrochemical reduction should then effect the decomplexation of one of the three bipyridine arms of **3a**. The aim was to show if these assumptions could be experimentally proved.

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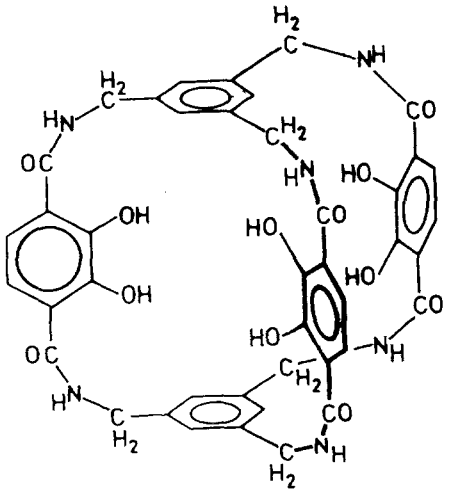

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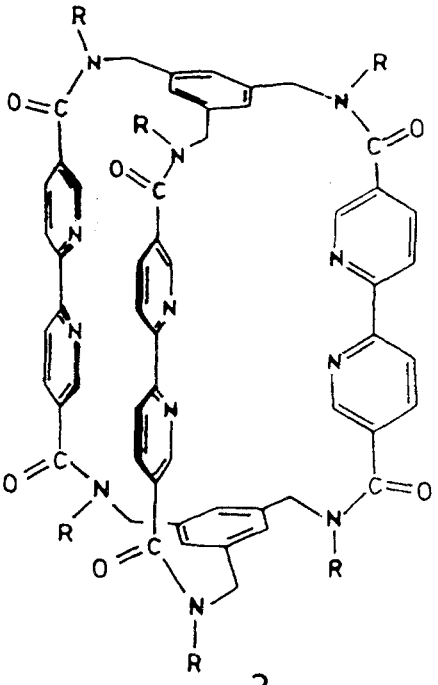

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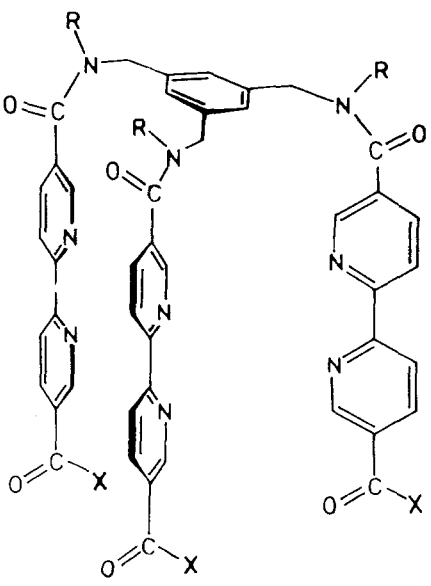
R = -CH$_2$-C$_6$H$_5$



3	R	X
a	-CH$_2$-C$_6$H$_5$	OC$_2$H$_5$
b		OH
c		O$^{\ominus}$Na$^{\oplus}$

2. Experimental

2,2'-Bipyridyl-5,5'-bis(ethylcarboxylate) (**5**) [3]: 125 g of Ni/Al alloy were added in portions over 1 h to a solution of 160 g (4.00 mol) sodium hydroxide in 600 ml of distilled water in an open beaker under cooling with ice and vigorous stirring. The temperature of the solution should be kept between 40–80 °C. After the addition the temperature was slowly decreased (strong foaming) under continuous stirring and the suspension was then warmed in a boiling water bath for 30 min. After addition of 600 ml of distilled water and stirring for 10 min the water was decanted from the residue and the modified nickel washed until it was neutral (ten 500 ml portions of distilled water). The wet catalyst was dried first at 10 Torr, then for 3 h at 80 °C/ 0.05 Torr. To the cooled activated nickel catalyst a solution of 53.0 g (351 mmol) of ethyl nicotinate in 220 ml of xylene (dried over $LiAlH_4$) was then added in the absence of air (**warning:** the catalyst (and $LiAlH_4$) may burn on contact with air). After 30 h of reflux the hot reaction mixture was filtered and the residue washed with hot ethanol. The filtrate was evaporated to 200 ml. The product crystallized overnight in colorless needles which were recrystallized from ethanol. For data see Table I.

2,2'-Bipyridyl-5'-ethylcarboxylate-5-carboxylic acid (**6**): To a refluxing solution of 11.52 g (38.4 mmol) of **5** in 500 ml of ethanol 2.15 g (38.4 mmol) potassium hydroxide in 200 ml of ethanol were slowly added dropwise, whereby a colorless precipitate was formed. The mixture was heated under reflux for 6 additional hours. After cooling it was evaporated to dryness, the residue dissolved in water and acidified with dilute hydrochloric acid to a pH of 6. The precipitated flocculent product was filtered by suction and dried in the desiccator. The resulting colorless solid was powdered and once more dried at 95 °C/0.05 Torr. A sample for analytical purposes was obtained by a further purification through extraction with ethyl acetate. For data see Table I.

2,2'-Bipyridyl-5-carboxylic acid chloride-5-ethyl carboxylate (**7**) [4]: 1.36 g (5.00 mmol) of **6** were suspended at room temperature in 500 ml of dichloromethane (dried on P_4O_{10}) and 1.04 g (5.00 mmol) of PCl_5 was added. After 3 h reflux a clear solution was formed which was evaporated *in vacuo*. The residue was dissolved in 50 ml of dry benzene, boiled with 5 g of active carbon and filtered hot. The product which formed colorless platelets, was filtered and dried. For data see Table I.

*1,3,5-Benzene-tri-*N-*benzyl carboxamide* (**10**) [5]: In a 2 l three-necked flask fitted with a gas outlet, a dropping funnel and a mechanical stirrer, 105.9 g (0.99 mol) of distilled benzylamine in 500 ml of toluene were dissolved and 39.8 g (0.15 mol) of trimesylchloride (**9**) in 250 ml of toluene were added dropwise during 2 h under cooling with ice. After completed addition the crystalline mass was stirred for additional 2 h at room temperature, then filtered off. The colorless residue was washed free from chloride with water and dried *in vacuo* over P_4O_{10}. For data see Table I.

1,3,5-Tris[N-*benzyl*(*aminomethyl*)]*benzene* (**11**): 9.54 g (20.0 mmol) trimesinic acid tris(benzylamide) (**10**) were placed in a 500 ml three-necked flask equipped with a serum cap, a gas outlet and a gas inlet tube. After flushing the apparatus with argon, 150 ml of borane etherate (BH_3/THF) were added using a syringe. After heating

Table I. Yields and analytical data of the new compounds

No.	Yield [%]	M.p. [°C] (Solvent)	IR [KBr, cm^{-1}]	^{1}H-NMR (90 MHz, δ-values, ppm)[a]	^{13}C-NMR (90 MHz, δ-values, ppm)[a]	molecular formula/ elemental analysis/MS	C	H	N
5	23	148–149 (ethanol; needles)	3050-2970 (w), 1735 (s) 1600 (s), 1460 (m), 1400 (w), 1380 (m), 1370 (m), 1290 (s), 1270 (s), 1245 (m), 1180 (m), 1120 (s), 1030 (s), 860 (m), 770 (s), 670 (w)	1.44 (t, 6H, CH_3), 4.48 (q, 4H, CH_2), 8.42 (dd, 2H, bipyridyl-H, $^3J = 8.2$ Hz, $^4J = 2$ Hz), 8.62 (dd, 2H, bipyridyl-H, $^3J = 8.4$ Hz, $^5J = 1$ Hz), 9.31 (dd, 2H, —N=C—H, $^4J = 2$ Hz, $^5J =$ 1 Hz)	14.17 (CH_3), 61.40 (CH_2), 121.12, 137.91, 150.47, 158.11 (bipyridyl-C, 165.04 (carbonyl-C)	$C_{16}H_{16}N_2O_4$ (300.32)			
						Calc.	64.00	5.33	9.33
						Found	63.96	5.24	9.22
						MS: Calc. 300.31181 Found 300.1112			
6	79	268–270 (ethyl acetate)	3020-2970 (w), 1720 (s) 1690 (s), 1600 (s), 1480 (w), 1435 (m), 1370 (w), 1315 (m), 1275 (s), 1190 (w), 1140 (w), 1120 (s), 1035 (s), 960 (w), 870 (w), 780 (s)	1.37 (t, 3H, CH_3), 4.44 (q, 2H, CH_2), 8.51 (2 dd, 2H, bipyridyl-H, $^3J = 8.2$ Hz, $^4J = 2$ Hz, $^5J = 1$ Hz), 8.66 (2 dt, 2H, bipyridyl-H, $^3J =$ 8.2 Hz), 9.26 (2 dd, 2H, bipyridyl-H, $^4J = 2$ Hz, $^5J =$ 1 Hz)	14.06 (CH_3), 61.28 (CH_2), 121.10, 126.21, 127.15, 138.21, 138.38, 150.29 (bipyridyl-CH), 157.12, 157.54 (bipyridyl-C_q), 164.44, 165.96 (carbonyl C_q)	$C_{14}H_{12}N_2O_4$ (272.26)			
						Calc.	61.76	4.44	10.29
						Found	61.89	4.30	10.25
						MS: Calc. 272.2639 Found 272.0802			
7	85	166–167 (colorless flakes)	3040-2940 (w), 1785 (s) 1720 (s), 1600 (s), 1560 (w), 1470 (w), 1370 (s), 1280 (s), 1250 (m), 1220 (s), 1150 (s), 1125 (s), 1070 (w), 1035 (s), 880 (s), 860 (s), 805 (w), 775 (m), 760 (s), 705 (s), 670 (s)	1.46 (t, 3H, CH_3), 4.5 (q, 2H, CH_2), 8.52 (dd, 1H, bipyridyl-H, $^3J = 8.5$ Hz, $^4J = 2$ Hz), 8.55 (dd, 1H, bipyridyl-H, $^3J = 8.5$ Hz, $^4J = 2.3$ Hz), 8.68 (dd, 1H, bipyridyl-H, $^3J = 5.8$ Hz, $^5J = 1$ Hz), 8.77 (dd, 1H, bipyridyl-H, $^3J = 6.1$ Hz, $^5J = 1$ Hz), 9.37 (dd, 1H, bipyridyl-H, $^4J = 2$ Hz, $^5J = 0.9$ Hz), 9.42 (dd, 1H, bipyridyl-H, $^4J = 2$ Hz, $^5J = 0.9$ Hz)	14.33 (CH_3), 61.83 (CH_2), 121.97, 122.14, 138.79, 139.82, 150.31, 151.63 (bipyridyl-CH), 127.41, 129.50, 156.89, 159.54, 164.74, 166.81 (bipyridyl-C_q)	$C_{14}H_{11}ClN_2O_3$ (290.71)			
						Calc.	57.84	3.81	9.64
						Found	57.80	3.97	9.39
						MS: Calc. 290.7092 Found 290.0463			
10	94	239–240	3280 (s), 3100 (s), 3070 (m), 2960 (w),	4.51 (d, 6H, CH_2), 7.32 (m, 15H, aromat. H), 8.53 (s,	42.931 (CH_2), 126.92, 127.47, 128.38, 128.90	$C_{30}H_{27}N_3O_3$ (477.57)			

			1655-1645 (s), 1530 (s), 1500 (w), 1480 (s), 1290 (s), 1240 (w), 1090 (m), 1080 (w), 1040 (m), 915 (m), 760 (s), 705 (s)	3H, aromat. H), 9.28 (t, 3H, NH)	(aromat. CH), 135.02, 139.43 (aromat. C_q), 165.573 (carbonyl-C)	Calc. Found MS: Calc. Found	75.47 75.43 477.5688 477.2055	5.66 5.71	8.81 9.04
11	61	oil (pale-yellow)	–	1.58 (s, 3H, NH), 3.76 (s, 12H, CH_2), 7.3 (m, 18H, aromat. H)	–	$C_{30}H_{33}N_3$ (435.61) MS: Calc. Found	435.6281[b] 435.2668		
3a	81	110	3020-2970 (w), 1735 (s), 1650 (s), 1600 (s), 1460 (m), 1425 (m), 1370 (m), 1290 (s), 1130 (s), 1040 (m), 1015 (w), 865 (m), 775 (s), 710 (m)	1.44 (t, 9H, CH_3), 4.37 (q, 6H, CH_2), 4.57 (s, 12H, CH_2), 7.03 (s, 3H, aromat. H), 7.27 (m, 15H, aromat. H), 7.96 (dd, 3H, bipyridyl-H, $^3J = 8.4$ Hz, $^4J = 2.25$ Hz), 8.31 (dd, 3H, bipyridyl-H, $^3J = 8.1$ Hz, $^4J = 2.1$ Hz), 8.42 (dd, 3H, bipyridyl-H, $^3J = 8.4$ Hz, $^5J = 1.05$ Hz), 8.47 (dd, 3H, bipyridyl-H, $^3J = 8.4$ Hz, $^5J = 1.05$ Hz), 8.77 (dd, 3H, bipyridyl-H), 9.1 (dd, 3H, bipyridyl-H)	–	$C_{72}H_{63}N_9O_9$ (1198.36)[c] Calc. Found MS: $MH^{\oplus} = 1198$	72.17 71.91	5.30 5.40	10.52 10.53
3b	99.5	180 (dec.) (colorless powder)	–	4.64 (s, 12H, CH_2), 7.37 (s, 18H, aromat. H), 8.24 (m, 12H, bipyridyl-H), 8.84 (s, 3H, bipyridyl-H), 9.17 (s, 3H, bipyridyl-H)	–	$C_{66}H_{51}N_9O_9$ (1114.20) Calc. Found MS: $[M + H]^+ = 1114$	71.15 –	4.61 –	11.31 10.92
12a	1.5	>300 °C	–	–	–	$C_{66}H_{50}Cl_3N_9Na_3O_{10}Rh$ (1407.40) Calc. Found	56.32 56.11	3.58 4.05	8.96 8.97

[a] Compounds **5**, **7**, **11**: $CDCl_3/TMS_{int.}$; compounds **6**, **10**, **3a**, **3b**: DMSO-$d_6/TMS_{int.}$.
[b] R_F: 0.24 (TLC alumina foil, Al_2O_3 neutral, CH_2Cl_2/CH_3OH 100 : 1).
[c] R_F: 0.7 (TLC alumina foil, silica gel 60 F_{254}, ethyl acetate).

for 6 h under reflux the suspension was converted into a clear red solution which turned to pale-yellow after 2 h and finally became colorless. Then 100 ml of water were added carefully, the THF was distilled off and the residue was heated under reflux after addition of 50 ml of half-conc. hydrochloric acid for 2 h. After standing over night the precipitated amine hydrochloride was filtered off, washed with water and dissolved in 2N NaOH. After 6 h of stirring the solution was extracted several times with chloroform, dried on Na_2SO_4 and evaporated to dryness. For data see Table I.

1,3,5-Tris[*5-*(*ethoxycarbonyl*)*-2,2'-bipyridyl-5'-yl-carbonyl-*(N-*benzyl*)*aminomethyl*] *benzene* (**3a**): 5.50 ml (39.6 mmol) of dry ethylamine and 3.44 g (7.92 mmol) 1,3,5-tris[*N*-benzyl(aminomethyl)]benzene (**11**) were dissolved in 50 ml of *N, N*-dimethylacetamide and added to a suspension of 6.90 g (23.75 mmol) of 5,5'-bipyridyl-5-carboxylic acid ethylester-5'-carboxylic acid chloride (**7**) in 150 ml of *N, N*-dimethylacetamide. After short reflux a clear solution was formed which was stirred overnight at room temperature. The precipitated ammonium salts were filtered off and the solvent evaporated to dryness. The pale-yellow residue was chromatographed on silica gel using ethylacetate. For data see Table I.

1,3,5-Tris[*5-*(*carboxy*)*-2,2'-bipyridyl-5'-yl-carbonyl-*(N)*-benzyl*) *aminomethyl*]*benzene* (**3b**): 13.3 g (11.1 mmol) of the triester **3a** were heated under reflux in 500 ml of ethanol for ca. 30 min. A solution of 1.57 g (39.3 mmol) NaOH p.a. in 20 ml of water/180 ml of ethanol was added to the clear solution which formed. After the addition the solution was refluxed for additional 24 h. The solvent was distilled off in the rotary evaporator whereby a viscous product was formed. The trisodium salt **3c** was dissolved in water and the free tricarboxylic acid **3b** was precipitated by addition of 2 NHCl. The precipitate was filtered off using a frit, washed well with water and dried on P_4O_{10} *in vacuo*. The powder was dried completely at 70 °C/0.05 Torr. For data see Table I.

Preparation of the **3c** · *Rh complex* · H_2O (**12a**): The preparation followed the procedure described in [6] for the corresponding complex of unsubstituted 2,2'-bipyridine: In a 10 ml threenecked flask, fitted with a reflux condenser and internal thermometer, 65.6 mg (0.25 mmol) $RhCl_3 \cdot 3\ H_2O$, 294 mg (2.50 mmol) of the sodium salt **3c** and 2.2 µl of *N*-methylmorpholine were mixed and 1 ml of ethanol/water (1 : 1) was added. The mixture was stirred at 80 °C for 1 h. After cooling the reaction mixture was filtered on celite and then evaporated to dryness. The resulting yellow-brown precipitate was recrystallized from ethanol/water (3 : 1). As UV spectra still showed the characteristic absorption of the free ligand **3c** at 296 nm, a further purification on a short column (RP 18, Merck) with ethanol/water (9 : 1) as solvent was carried out. After recrystallization from water/ethanol/butanol (2 : 2 : 1) deep yellow crystals were obtained. For data see Table I.

Cyclic voltammetry: The cyclovoltammograms were obtained using an Amel potentiostat model 553 combined with a function generator, model 8200 (Kontron) and with an XY-recorder, model 70145A (Hewlett Packard). The analytical cell (Metrohm EA 875-20) was fitted with a glassy carbon rotating disc working electrode ($\emptyset = 3$ mm), a platinum counter electrode (2.2×0.3 mm) and a Ag/AgCl (0.1M) reference

electrode, for measuring the potential of the working electrode via a Luggin capillary [7].

Preparative electrolysis: A potentiostat, model NTN 700, M 200 (FUG-Rosenheim) served as a current source. The electrolysis was carried out in a cell of type EA-805-20 (Metrohm) fitted with a graphite cathode (6 cm^2 surface) and a glass cylinder as the anode compartment (volume 1 ml) containing a platinum net as the anode. The cathode and anode compartments were separated by a G3 frit. The electrolysis was carried out under Argon atmosphere. Before the electrolysis, catholyte and anolyte were freed from dissolved oxygen by flushing with argon. Potential measurements were carried out using a Ag/AgCl reference electrode. The electrolysis was performed at room temperature under magnetic stirring. The charge consumption was determined with a digital current integrator.

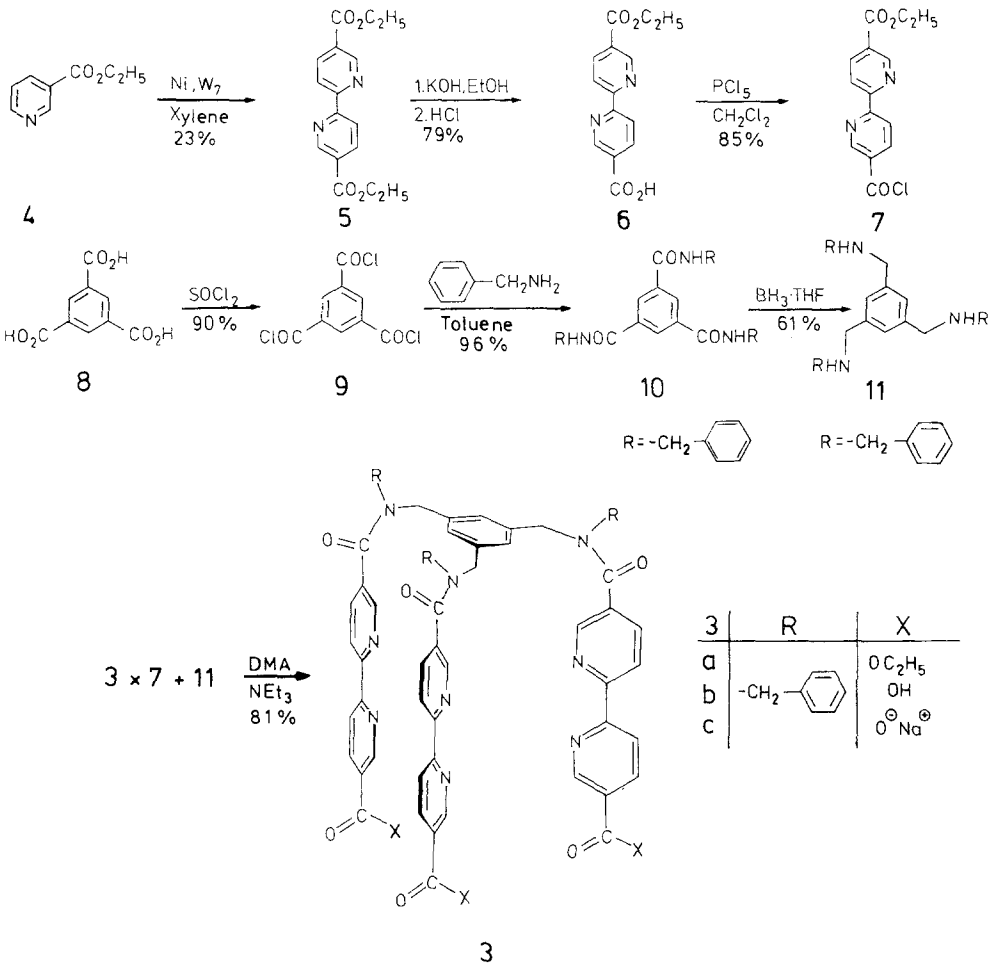
Preparative electrolysis of **12a** *in a discontinuous mode*: The electrolysis was carried out at –620 mV using an Ag/AgCl reference electrode. The catholyte was composed of 20 ml Tris–H_2SO_4 buffer solution of pH 7.4 containing 3.25 mg (0.0023 mmol) **3c** as well as 51 mg (0.76 mmol) NAD^+. The electrolysis was carried out over a period of 88 h, whereby the current at the beginning was near 1.1 mA. After consumption of 303 As the solution became yellow. Thin layer chromatography (silica gel 60 F_{254}, Merck, ammonium acetate/ethanol 9 : 4) showed that NAD^+ and NADH were present. The catholyte was transferred into a 100 ml flask. 50 mg (0.51 mmol) of cyclohexanone and 2.50 mg HLADH added and the mixture stirred under argon atmosphere. After 1 h a 4 ml sample was taken and extracted three times with 1 ml of diethyl ether. The organic phase was investigated for cyclohexanol by gas-liquid chromatography [8].

3. Results and Discussion

3.1. SYNTHESES

Nicotinic acid ethyl ester (**4**) was dimerized to 2,2′-bipyridyl-5,5′-dicarboxylic acid diethylester (**5**) by activated Raney nickel catalysis. The selective hydrolysis of one of the two ester groups yielding **6** is achieved using equimolar amounts of KOH in ethanol. By reaction with PCl_5 one obtained the monoacid chloride **7**. Trimesinic acid (**8**) is converted into its tris-acid chloride **9** by means of thionyl chloride and is converted into the trimesinic acid tribenzyl amide **10**. Reduction with BH_3 in THF yields 1,3,5-tris[*N*-benzyl(aminomethyl)]benzene (**11**). Reaction of three equivalents of **7** with **11** in dimethylacetamide as solvent leads to the tripode ligand **3a** in 81% yield. Hydrolysis leads to the tris-sodium salt **3c** and neutralisation yields the water soluble ligand **3b**.

The preparation of the rhodium complex **12a** follows that of $[Rh(bipy)_3]^{3+}$ [6] by reaction of the ligand **3c** with rhodium chloride under catalysis with *N*-ethylmorpholine in a water/ethanol (1 : 1) mixture. Successive purification of the raw product to recover the unreacted ligand by chromatography on a RP 18 column yields the rhodium complex **12a** with chloride as the counter ion. After recrystallization from water/ethanol/butanol (2 : 2 : 1) yellow crystals are obtained.



Scheme 1.

3.2. COMPARISON OF THE Rh^{3+} COMPLEX (**12a**) WITH THE Fe^{2+} COMPLEX (**12b**)

Figure 1 shows the UV spectra of the Rh^{3+} (**12a**) and Fe^{2+} (**12b**) complexes. Whereas the maxima at 260 and 320 nm can be referred to the absorption of the ligand itself, the red Fe^{2+} complex **12b** exhibits a maximum at $\lambda = 546$ nm and the yellow Rh complex **12a** a characteristic shoulder at 330 nm (*cf.* $\lambda_{max} = 318$ nm for $[Rh(bipy)_3]^{3+}$) [8].

3.3. ELECTROANALYTICAL INVESTIGATIONS

The cyclovoltammograms of **12a** were obtained using a 0.1M Tris-H_2SO_4 buffer of pH 7.4 at room temperature. A disk electrode made from glassy carbon ($\emptyset = 3$ mm) served as the working electrode. The potential measurement was done versus a Ag/AgCl reference electrode.

As can be seen from Figure 2, the rhodium complex **12a** shows a strong reduction peak at −620 mV (E_{P_1}) as well as two weak peaks at −715 (E_{P_2}) and −865 mV

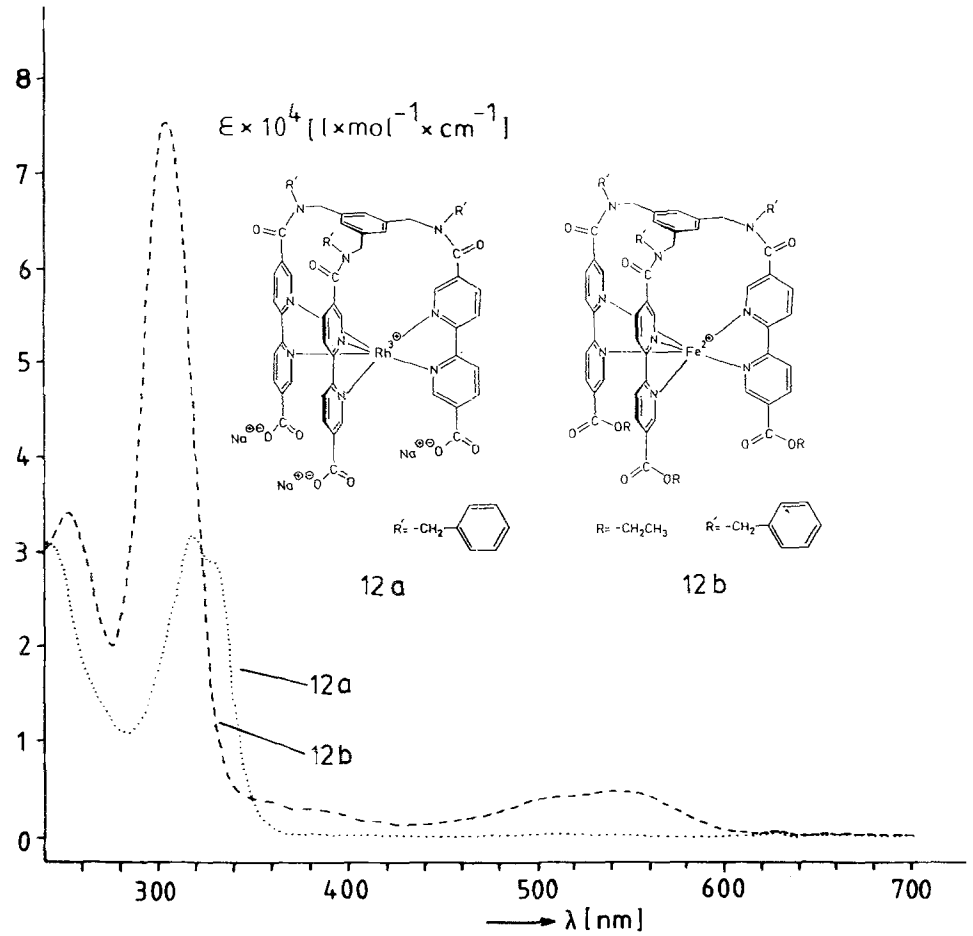


Fig. 1. UV/Vis spectra of the Rh^{3+} (**12a**) and Fe^{2+} (**12b**) complexes.

(E_{P_3}): In contrast to $[Rh(bipy)_3]^{3+}$ [2] a reoxidation peak cannot be observed in the potential range studied.

Due to the tendency of the complex **12a** to deposit on the electrode, at the beginning of each measurement the working electrode has to be cleaned. Compared to $[Rh(bipy)_3]^{3+}$ the reduction potential of the complex **12a** is shifted by 300 mV to more positive values. This can be referred to the electron withdrawing influence of the carboxyl and carboxamide functions at the 2,2'-bipyridine part of the ligand **3**.

In the course of the reduction of Rh(III) to Rh(I) one of the three bipyridine units of **12a** must be set free because it is known that Rh(I) prefers a coordination by only two bipyridine units, in contrast to Rh(III) which is known to be coordinated in an octahedral arrangement by three bipyridine units. It is plausible therefore that in the $[Rh(bipy)_3]^{3+}/[Rh(bipy)_2]^{+}$ redox system no reversible reoxidation peak is observed [the non-reversible oxidation peak at 200 mV (see Figure 2) is most likely due to the oxidation of $[Rh(bipy)_2]^{+}$ or $[RhH(bipy)_2(H_2O)]^{2+}$ to $[Rh(bipy)_2(H_2O)_2]^{3+}$ [6, 8].

It could be expected that an advantage of the hexadentate ligand **3c** compared to

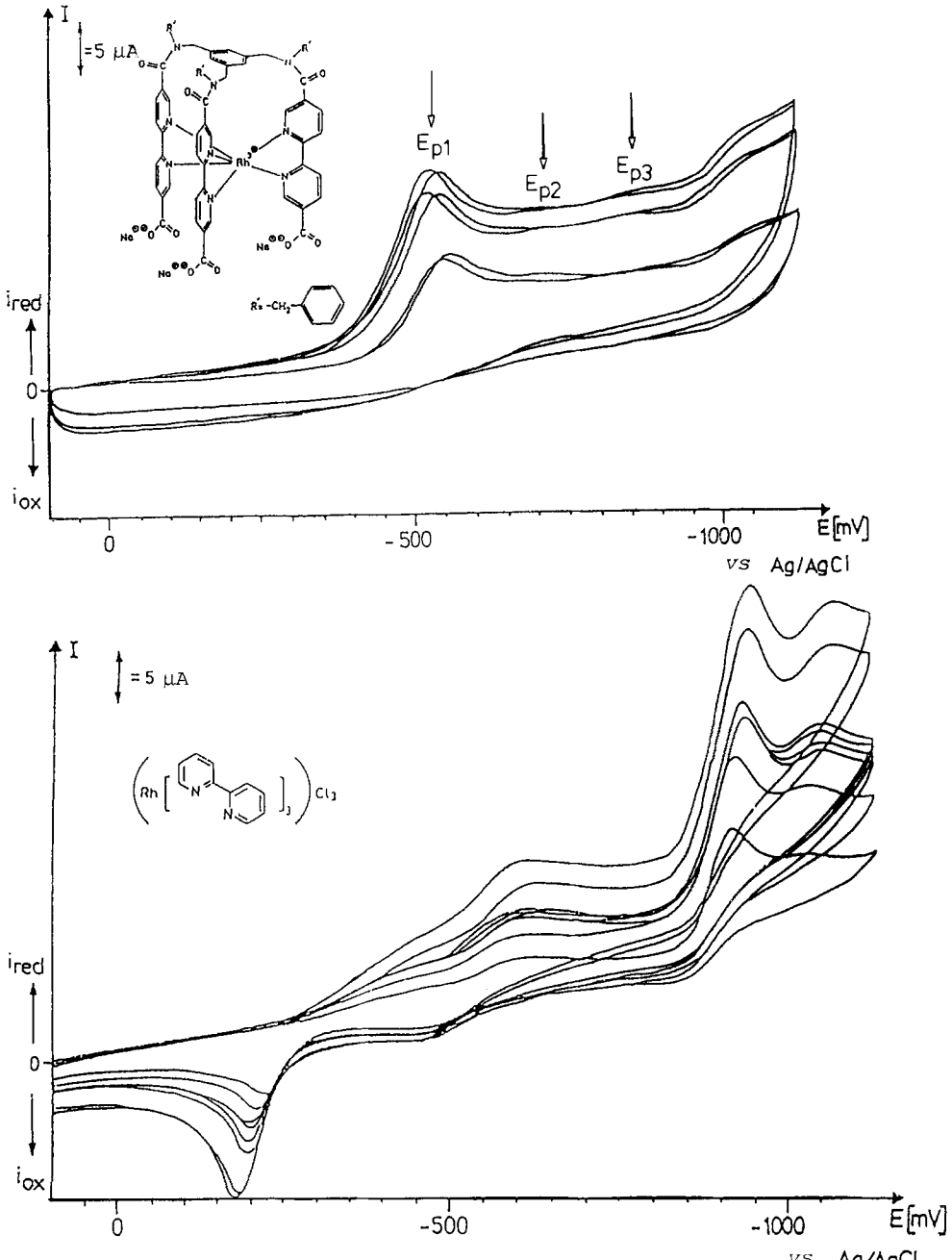
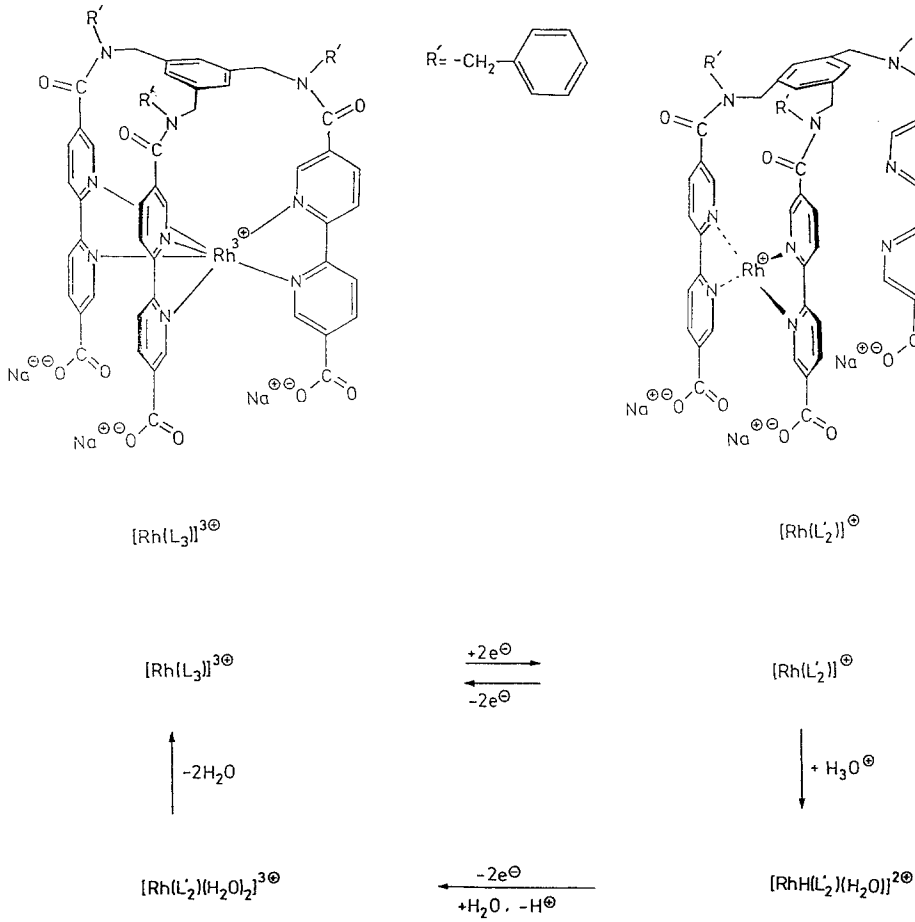


Fig. 2. Cyclovoltammogram of **12a** (and for comparison of $[Rh(bipy)_3]^{3+}$) [9] in Tris-H_2SO_4 buffer (pH 7.4).

the $[Rh(bipy)_3]^{3+}$ system is that in the course of the transition of Rh(III) to Rh(I) the one bipyridine arm which is no longer coordinated, remains in spatial neighbourhood to the central ion, so that in the reoxidation to Rh(III) this bipyridine arm is already preoriented to facilitate the recoordination (see Scheme 2).

The expected higher chemical reversibility in the tripode complex **12a** should

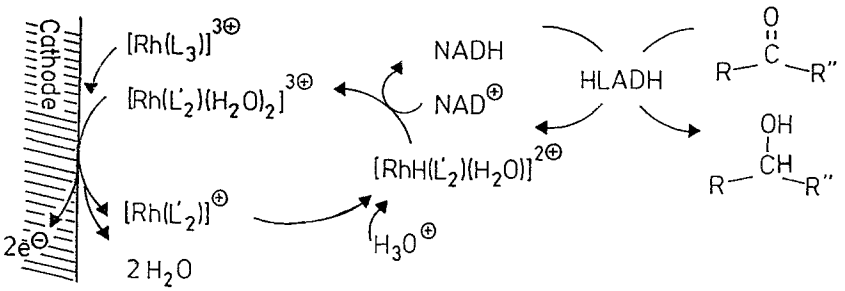


Scheme 2. Possible recoordination in the course of the reoxidation.

result in a shift of the oxidation peak in the cyclovoltammogram to more negative values, compared to the $[Rh(bipy)_3]^{3+}$ complex. This surprisingly is not the case. Therefore it seems unlikely that in the time scale of the CV measurement the one bipyridine arm set free in the course of the reduction is ligated again during the oxidation of the Rh(I) to the Rh(III) complex.

3.4. APPLICATION OF THE Rh COMPLEX **12a** AS A REDOX CATALYST FOR THE ELECTROCHEMICAL REGENERATION OF NADH FROM NAD^+

In previous studies [2, 8] electrochemically generated and regenerated $[Rh(bipy)_2]^+$ has been successfully used for the indirect electrochemical regeneration of NADH from NAD^+. It is likely that thereby the rhodium hydride, e.g. $[RhH(bipy)_2(H_2O)]^{2+}$ which is formed as an intermediate from $[Rh(bipy)_2]^+$ acts as a hydride transfer reagent [6]. For the first time this method opened up an effective pathway for the selective electrochemical regeneration of the coenzyme NADH in the presence of a NADH dependent enzyme (HLADH); see Scheme 3.



Scheme 3. Selective electrochemical regeneration of the enzyme NADH in the presence of a NADH dependent enzyme (HLADH).

However, with the redox system $[Rh(bipy)_3]^{3+}/[Rh(bipy)_2]^{+}$ the following problems arose [2, 8]:

(a) electrode covering by intermediate deposition of $[RhH(bipy)_2(H_2O)]^{2+}$,
(b) the negative potential of the rhodium complex (–923 mV *vs.* SCE) compared to the standard potential of $NAD^+/NADH$ (–560 mV *vs.* SCE) leads to a minor direct cathodic reduction of NAD^+ under formation of the enzymatical inactive dimer NAD_2,
(c) the speed of the overall reaction is too low to be useful for technical applications.

The search for a modified rhodium complex is important to overcome the drawbacks mentioned above. As the rhodium complex **12a** with −620 mV shows a significant, more positive potential than $[Rh(bipy)_3]^{3+}$, it should be suitable at least to solve the second problem (b). Indeed, the preparative electrolysis of **12a** (2.3. μmol) in the presence of NAD^+ (76 μmol) led to the selective formation of NADH (18.9 μmol, determined enzymatically). In the course of this reaction no NAD dimers could be detected. Up to this point the rhodium complex had undergone 8.2 regeneration cycles. On the other hand, a significant acceleration of the reaction in comparison to $[Rh(bipy)_3]^{3+}$ was not observed.

To sum up **3b** offers a favourable "podand" for the complexation of rhodium. This investigation also demonstrates with respect to the indirect electrochemical regeneration of NADH from NAD^+ a further optimization of the redox catalyst on the basis of rhodium complexes will be necessary in future.

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