Monatshefte für Chemie 127, 1073–1080 (1996)

Monatshefte für Chemie
Chemical Monthly

1,3-Diphenylpropane-1,3-diamines, X [1]: Synthesis and Biochemical Evaluation of Highly Substituted 1,3-Diphenylpropane-1,3-diamines and Preparation of their Pt(II) Complexes#

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Summary. The ligands of the title complexes **1** and **2** were prepared from the pertinent chalcone **5** and hydrazine hydrate, followed by N-N cleavage. The estrogenic activity of the diamines **11** and **12** was determined by measuring the RBA values (calf uterine cytosol) and by a luciferase test in MCF 7-2a cells. The compounds are by far less active than *Schönenberger*'s most active compound ([*meso*-1,2-*bis*(2,6-dichloro-4-hydroxyphenyl)ethane-1,2-diamine]dichloro-platinum(II), **3**).

Keywords. 1,3-Diphenylpropane-1,3-diamines; Receptor binding affinity; Estrogenic activity.

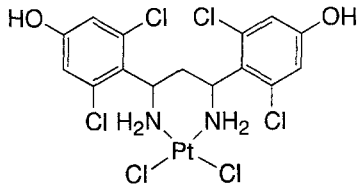
1,3-Diphenylpropan-1,3-diamine, 10 Mitt. [1]: Synthese und biochemische Bewertung hochsubstituierter 1,3-Diphenylpropan-1,3-diamine und Darstellung ihrer Pt(II)-Komplexe

Zusammenfassung. Die Liganden der Titelverbindungen **1** und **2** wurden aus dem entsprechenden Chalcon **5** mit Hydrazinhydrat und anschließende N-N-Spaltung hergestellt. Die oestrogene Wirkung der Diamine **11** und **12** wurde im Rezeptorbindungstest an Kalbsuterus-Zytosol und durch einen Luziferase-Test an MCF 7-2a-Zellen bestimmt. Die Verbindungen wirken viel schwächer als *Schönenbergers* aktivste Substanz ([*meso*-1,2-*Bis*(2,6-dichlor-4-hydroxyphenyl)ethan-1,2-diamin]dichloro-Platin(II), **3**).

Introduction

The cytostatic properties of compounds of the (1,2-diphenyl*ethane*-1,2-diamine)dichloro-platinum(II) type have been thoroughly investigated by *Schönenberger* and his group [2, 3]. In a previous paper [4], we have discussed the conformational flexibility of Pt-complexes of the 1,2-diphenyl*ethane*-1,2-diamine type (five-membered ring) in comparison with that of the homologous (1,3-diphenyl-*propane*-1,3-diamine)-Pt(II) complexes, possessing a six-membered ring. We were especially interested in the biochemical properties of [*meso*-1,3-*bis*(2,6-dichloro-4-

Dedicated with kind regards to Prof. Dr. *G. Seitz*, Marburg/Germany, on the occasion of his 60th birthday



1: meso
2: racemate

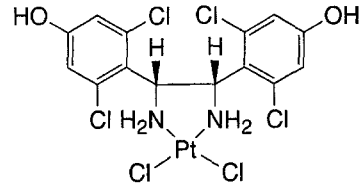


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Fig. 1

hydroxyphenyl)-propane-1,3-diamine]dichloro-platinum(II) (**1**) and the pertinent racemate **2**, because *Schönenberger*'s compound [*meso*-1,2-*bis*(2,6-dichloro-4-hydroxyphenyl)ethane-1,2-diamine]dichloro-platinum(II) (**3**) [2, 3] exhibits low affinity to the estrogen receptor (ER) in comparison with the free ligand. Nevertheless, this Pt complex shows increased endocrinological activity.

Results and Discussion

We have prepared 1,3-diphenylpropane-1,3-diamines by a slightly modified procedure elaborated by *von Auwers* and *Müller* [5]. When we tried to extend this method to the synthesis of 1,3-diphenylpropane-1,3-diamines with highly substituted phenyl groups, 1*H*-aziridines of type **4** arose from the chalcone precursors of type **5** and hydroxylamine [6] (Fig. 2) instead of hydroxyamino-oximes or bisoximes [5].

Here we describe the synthesis of the Pt(II) complexes **1** and **2** by a different, generally useful approach to 1,3-diphenylpropane-1,3-diamines, characterized by mild reductive N–N cleavage of properly substituted 3,5-diphenyl-4,5-dihydropyrazoles **6**, prepared from the chalcone **5** by reaction with hydrazine hydrate. *Denmark* and *Kim* [7] have published a reductive N–N cleavage leading to 1,3-diphenylpropane-1,3-diamines. The drastic conditions applied by these authors, however, partly dehalogenated the phenyl groups of our compounds. On the other hand, these halogen substituents are important for the high lipophilicity necessary for biological efficacy [8]. Contrary to *Denmark*'s method [7], low diastereoselectivity is an advantage of our protocol because we need the diastereomers **1** and **2** and/or their ligands **11** and **12** for the biological tests.

^{1}H NMR spectroscopy reveals that the ratio of **7** (*meso* form) and **8** (racemate) is 1:4. The diastereomers were identified by their ^{1}H NMR spectra: *Hesse et al.* [9] quote C_2 symmetry for the CH_2 protons of (+/−)-2,4-diaminoglutaric acid, affording homotopic CH_2 protons. *Meso*-2,4-diaminoglutaric acid does not possess this element of symmetry. As a consequence, the CH_2 protons resonate as the AB part of an ABC_2 system. For 1,3-diphenylpropane-1,3-bisacetamides, we have found analogous ^{1}H NMR results [10] which were ensured unequivocally by resolution experiments and chemical transformations [11].

In agreement with these assignments, *rac*-**8** shows a triplet at $\delta = 2.40$ ppm for the CH–CH_2–CH substructure ($^3J = 8.1$ Hz), and the methine protons resonate at 6.03 ppm as a dt ($^3J = 8.1$ Hz; $^{3'}J = 4.6$ Hz). The *meso*-diastereomer **7** leads to two

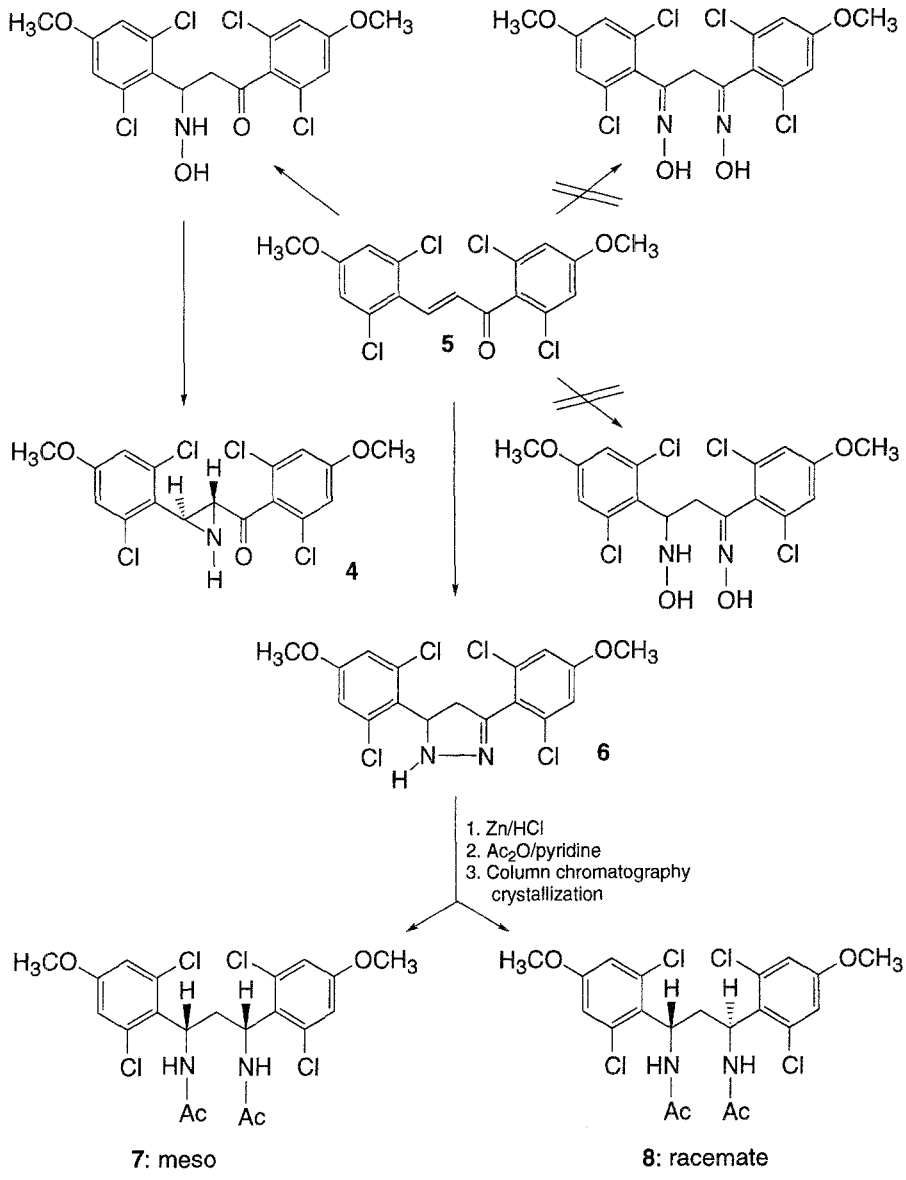

Fig. 2.

separated symmetric multiplets at 2.37 and 2.62 ppm for the CH_2 protons, whereas the CH protons afford a dt at 5.65 ppm ($^3J = 8$ Hz, $^{3'}J = 4.2$ H) similar to the situation of the racemate **8** (Experimental).

The bisacetamides were hydrolyzed by 2 *N* HCl in dioxane [4], yielding the diamines **9** and **10**. Ether cleavage by BBr_3 led to the nicely crystallizing hydrobromides of the diphenols **11** and **12**. Because transformation of these hydrobromides into the corresponding Pt(II) complexes is difficult, the bases **11** and **12** were liberated by column chromatography, dissolved in dil. HCl, and the *pH* of the solution was adjusted to 6.5. Because the ligands are too lipophilic, *tert*-butanol is added (cf. Ref. [2]). From these solutions (Experimental), the Pt(II) complexes **1** and **2** were

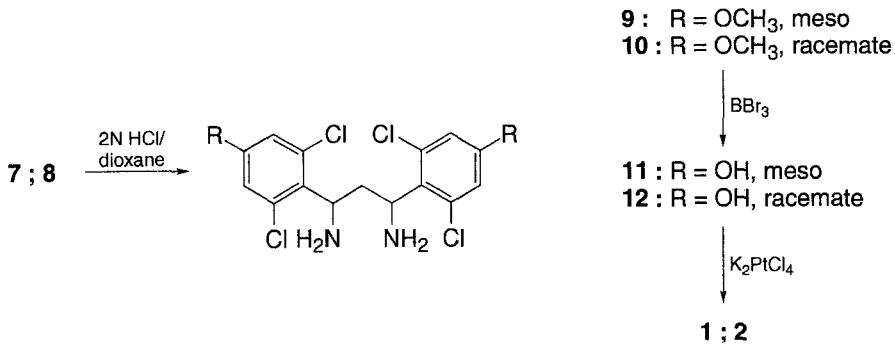

Fig. 3

prepared by addition of potassium tetrachloroplatinate. Complexes **1** and **2** can be purified by dissolving in as little as possible a volume of *DMF* and precipitation by 5% aqueous NaCl solution. The yields rank from 50 to 70%.

Again the CH_2 protons of the racemic complex **2** resonate as a triplet at 2.58 ppm ($^3J = 8.6$ Hz), whereas the *meso* complex **1** shows two separated multiplets at 3.40 and 3.60 ppm, respectively, for the CH_2 protons.

Biochemical assays

For evaluation of the estrogenic properties, the 1,3-diaminopropanes **11** and **12** were tested in two *in vitro* assays. The binding to the estrogen receptor (RBA) [12], prerequisite for estrogenic activity, was determined in a competition experiment with estradiol. In contrast to the diasteromeric 1,2-*bis*(2,6-dichloro-4-hydroxyphenyl)-ethane-1,2-diamines (*meso*: RBA = 0.45%; *D*, *L*: 0.1%), the homologous diamines **11** and **12** do not show affinity to the estrogen receptor (data not shown). This may be the consequence of different conformations of the diamines. If so, due to the CH_2 group, diamines **11** and **12** cannot adopt a shape comparable to synthetic estrogens like hexestrol or diethylstilbestrol, which consequently leads to only very low activation in the luciferase assay. This *in vitro* assay was developed by *Hafner et al.* [13] and replaces the uterine weight test: *Hafner* transfected hormone sensitive MCF 7-2a cells with the plasmid EREwtc luc containing the enhancer sequence of the estrogen responsive element. Molecules with affinity to the estrogen receptor and estrogenic properties lead to luciferase expression which correlates with the hormone potency. *Meso*-1,2-*Bis*(2,6-dichloro-4-hydroxyphenyl)ethane with the profile of a true estrogen reached the activity of estradiol (100%) at a concentration of about 10^{-6} mol/l. In a tenfold higher concentration, for amines **11** and **12** an activation of 11.5 and 2.5%, respectively, was found. The corresponding platinum complexes **1** and **2** were excluded from the tests since no estrogenic activity was expected.

Experimental

For general remarks, cf. Ref. [1]; for the sake of clarity, mass spectroscopic data are related to ^{35}Cl and ^{195}Pt only; *MNBA*: *m*-nitro-benzyl alcohol.

3,5-Bis(2,6-dichloro-4-methoxyphenyl)-4,5-dihydropyrazole (**6**)

4 g (10 mmol) *trans*-1,3-*bis*(2,6-dichloro-4-methoxyphenyl)-2-propen-1-one (**5**) [6] and 40 ml hydrazine hydrate in 100 ml of EtOH 96% are heated under reflux for 12 h. After cooling, about 50% of the solvent is removed *in vacuo*; the remaining suspension is poured onto 100 ml of ice water and extracted with AcOEt (3 × 50 ml). The org. phase is washed with satd. NaCl solution (2 × 30 ml), dried over $MgSO_4$, and evaporated, affording a colourless oil which is purified by CC (silica; CH_2Cl_2): colourless foam (2.75 g; 65%) which decomposes upon contact with air (brownish discolouration).

FT-IR (KBr): $\nu = 3278$ (NH), 3087 (C–H), 1603 (C=N), 1555 and 1474 (C=C), 1045 (C–Cl arom) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 3.15$–3.40 (dd; $^3J = 12$ Hz, $^2J_{HCH} = 5$ Hz, 2H, CH_2CHN), 3.83 (s; 6H, OCH_3), 5.72 (t; $^3J = 12$ Hz, 1H, CH_2CHN), 6.87 and 6.83 (2s; 4H arom), 7.80 (s, Br; 1H, NH, exch.) ppm; EI-MS (70 eV): m/z (%) = 418 (50; $M^{+\cdot}$), 383 (18; $M - Cl)^+$).

N,N′-Bisacetyl-1,3-bis(2,6-dichloro-4-methoxyphenyl)propane-1,3-diamines (**7** *and* **8**)

In a three-necked flask, a solution of 2 g (5 mmol) **6** in 40 ml of absol. MeOH is added dropwise to a suspension of 2 g (30 mmol) Zn in 75 ml of absol. MeOH with ice cooling. Simultaneously, 9.5 ml of conc. HCl are added so that the mixture is boiling gently. When the addition is complete, the ice bath is removed and the mixture is stirred at first for 1 h at room temp., then for 4–6 h under reflux (tlc control). After cooling to room temp., about 50% of the MeOH is distilled off, the residue is poured onto 100 ml of ice/2 *N* HCl (1:1), and the mixture is extracted with ether (2 × 30 ml). The aqueous phase is made alkaline with conc. ammonia and extracted with ether overnight. The etheral phase is washed with 30 ml of water and 30 ml of satd. NaCl solution, dried over Na_2SO_4, and evaporated *in vacuo*, affording a colourless oil, crystallizing at the oil pump: 1.36 g (68%) of a mixture of diastereomers which is directly acetylated. The mixture is dissolved in 25 ml of absol. CH_2Cl_2; to this solution 0.4 g (5 mmol) pyridine and 0.5 g (5 mmol) Ac_2O are added dropwise with ice cooling. After stirring for 3 h at room temp., the solution is washed twice with 50 ml of 2 *N* HCl and 50 ml of satd. NaCl solution, dried over Na_2SO_4, and evaporated *in vacuo*, leading to 1.2 g (75%) of a mixture of the diastereomers **7** and **8** (1:4 by ^1H NMR) as a colourless foam, obtained after flush chromatography (silica; CH_2Cl_2/acetone 3/1). The diastereomers are separated by column chromatography (CC) (silica; CH_2Cl_2/MeOH/acetone 19/1/3). Racemate **8** (644 mg; 53%) is obtained as colourless crystals from MeOH/$CHCl_3$ (1/1); m.p.: 240–241.5 °C.

IR (KBr): $\nu = 3444$ (NH, br), 3058, 3008 (C–H), 1667 (C=O), 1557, 1497, 1468 (C=C), 1041 (C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 1.97$ (s; 6H, $NHCOCH_3$), 2.40 (t; $^3J = 8.1$ Hz, 2H, $CHCH_2CH$), 3.77 (s; 6H, OCH_3), 6.03 (dt; $^3J = 8.1$ Hz, $^{3'}J = 4.6$ Hz, 2H, CH_2CHNH), 6.37 (d; $^{3'}J = 4.6$ Hz, 2H, $CHNHCOCH_3$), 6.84 (s; 4H, arom) ppm; MS (70 eV): m/z (%) = 471 (40, $M-Cl)^+$, *ortho*-effect); FAB-MS (CH_2Cl_2/*MNBA*): m/z (%) = 507 (60, MH^+), 471 (20, $(M-Cl)^+$); $C_{21}H_{22}Cl_4N_2O_4$ (508.2); calcd.: C 49.62, H 4.36, N 5.51; found: C 49.34, H 4.87, N 5.54.

Meso-N,N′-Bisacetyl-1,3-bis(2,6-dichloro-4-methoxyphenyl)propane-1,3-diamine (**7**)

Diastereomer **7** (67 mg; 5%) is obtained after repeated CC as indicated for diamide **8** and three- to fourfold recrystallization from absol. MeOH; colourless crystals, m.p.: 235–238 °C.

IR (KBr): $\nu = 3444$ (NH, br), 3067 (C–H), 1655 (C=O), 1580, 1562, 1437 (C=C), 1036 (C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 1.96$ (s; 6H, $NHCOCH_3$), 2.37 and 2.62 (2m; 2H, $CHCH_2CH$), 3.76 (s; 6H, OCH_3), 5.65 (dt; $^3J = 8$ Hz, $^{3'}J = 4.2$ Hz, 2H, CH_2CHNH), 6.70 (d; $^{3'}J = 4.2$ Hz, 2H, $CHNHCOCH_3$), 6.83 (s; 4H, arom) ppm; EI-MS (70 eV): m/z (%) = 471 (8; $(M-Cl)^+$, *ortho*-effect); $C_{21}H_{22}Cl_4N_2O_4$ (508.2); calcd.: C 49.62, H 4.36, N 5.51; found: C 49.19, H 4.91, N 5.04.

rac-1,3-Bis(2,6-dichloro-4-methoxyphenyl)propane-1,3-diamine (**10**)

350 mg (0.7 mmol) **8** are refluxed for 12 h in 8 ml of dioxane and 18 ml of 2 *N* HCl. Then, about 75% of the solvents are distilled off *in vacuo*. The residue is alkalized with conc. ammonia and extracted with

25 ml of ether three times. The organic phase is washed with 20 ml of satd. NaCl solution, dried over Na_2SO_4, and evaporated *in vacuo*, yielding a colourless oil, solidifying as a wax at the oil pump. Crystallization from absol. EtOH leads to colourless crystals; 250 mg (84%), m.p.: 142–142.5 °C.

IR (KBr): $\nu = 3388$ (NH_2, br), 3004 (C–H), 1555, 1466, 1424 (C=C), 1047 (C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 1.86$ (s, br; 4H, NH_2, exch.), 2.39 (t; $^3J = 7.5$ Hz, 2H, $CHCH_2CH$), 3.77 (s; 6H, OCH_3), 4.90 (t; $^3J = 7.5$ Hz, 2H, CH_2CHNH_2), 6.83 (s; 4H, arom) ppm; FAB-MS (CH_2Cl_2/*MNBA*): *m/z* (%) = 423 (35, MH^+), 847 (20, M_2H^+); $C_{17}H_{18}Cl_4N_2O_2$ (424.2); calcd.: C 48.14, H 4.28, N 6.61; found: C 48.28, H 4.45, N 6.83.

meso-1,3-Bis(2,6-dichloro-4-methoxyphenyl)propane-1,3-diamine (**9**)

Diastereomer **9** was prepared as described for **10**, starting from 100 mg of diamide **7**; 66 mg (80%), m.p.: 135–137.5 °C (absol. EtOH).

IR (KBr): $\nu = 3388$ (NH_2, br), 2940 (C–H), 1595, 1545, 1460, 1424 (C=C), 1054 (C–Cl) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 1.97$ (s, br; 4H, NH_2, exch.), 2.39–2.49 (m; 1H, $CHCH_2CH$, overlapped with NH_2), 2.62–2.72 (m; 1H, $CHCH_2CH$), 3.76 (s; 6H, OCH_3), 4.62–4.68 (dd; $^3J = 7.2$ Hz, $^{3'}J = 3.2$ Hz, 2H, CH_2CHNH_2), 6.82 (s; 4H, arom) ppm; $C_{17}H_{18}Cl_4N_2O_2$ (424.2); calcd.: C 48.14, H 4.28, N 6.61; found: C 48.08, H 4.50, N 6.66.

rac-1,3-Bis(2,6-dichloro-4-hydroxyphenyl)propane-1,3-diamine dihydrobromide (**12**)

Under N_2, 300 mg (0.7 mmol) of **10** are dissolved in 5 ml of absol. CH_2Cl_2 and cooled to −78 °C. Then, 1.80 g (7 mmol) of BBr_3 in 5 ml of absol. CH_2Cl_2 are added dropwise with vigorous stirring. After slow warming to room temp., stirring for 12 h under reflux, and cooling to room temp., 15 ml of absol. MeOH are added dropwise. After stirring for 20 min, the solvent is removed *in vacuo* below 40 °C. After addition of 3–5 ml of acetone and stirring for 15 min under ice cooling, **12**-hydrobromide is sucked off and washed with a small volume of ice cold acetone; 270 mg (70%), m.p.: 184–185 °C (decomp.).

IR (KBr): $\nu = 3600$–3100 (H_2O, OH, NH_3^+), 3001 (C–H), 1574, 1476 (C=C), 1057 (C–Cl) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 3.31$ (t; $^3J = 8.8$ Hz, 2H, $CHCH_2CH$, after exch. of water of crystallization), 4.51 (t; $^3J = 8.8$ Hz, 2H, $CH_2CHNH_3^+$), 6.81 (d; $^4J = 2.4$ Hz, 2H, arom), 6.96 (d; $^4J = 2.4$ Hz, 2H, arom), 8.63 (s; 4H, NH_2, exch.), 10.75 (s; 2H, OH, exch.) ppm; FAB-MS (CH_2Cl_2/*MNBA*): *m/z* (%) = 395 (78, MH^+), 548 (10, $(MH + MNBA)^+$); $C_{15}H_{14}Cl_4N_2O_2 \times 2HBr \times 2H_2O$ (593.9); calcd.: C 30.33, H 3.39, N 4.72; found: C 30.26, H 3.62, N 4.48.

meso-1,3-Bis(2,6-dichloro-4-hydroxyphenyl)propane-1,3-diamine dihydrobromide (**11**)

Diamine **11** was prepared from 140 mg (0.33 mmol) of **9** as described for aminophenol **12**, but additional recrystallization from acetone is necessary; colourless crystals, 115 mg (60%), m.p.: 171–173.5 °C (decomp.).

IR (KBr): $\nu = 3450$–3100 (H_2O, OH, NH_3^+), 3002 (C–H), 1602 (NH_3^+), 1574, 1474 (C=C), 1060 (C–Cl) cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 2.32$–2.43 (m; 1H, $CHCH_2CH$), 2.57–2.68 (m; 1H, $CHCH_2CH$), 4.63 (t; $^3J = 9$ Hz, 2H, $CH_2CHNH_3^+$), 6.85 (s; 4H, arom), 5.10–8.50 (br; OH and NH_3^+, exch.) ppm; $C_{15}H_{14}Cl_4N_2O_2 \times 2HBr \times H_2O$ (576.0); calcd.: C 31.28, H 3.15, N 4.86; found: C 31.55, H 3.38, N 4.89.

[rac-1,3-Bis(2,6-dichloro-4-hydroxyphenyl)propane-1,3-diamine]dichloro-platinum(II) (**2**)
(cf. Ref. [15])

From 83 mg (0.15 mmol) of the dihydrobromide **12**, the corresponding diamine is liberated by CC (silica; MeOH/conc. NH_3 100/1.5 (v/v)). The solvent is removed *in vacuo* below 40 °C (bath temp.), and the residue is dried for 3 h at the oil pump. The base is suspended in 10 ml of water and dissolved by addition of 1 ml of 2 *N* HCl. If the solution gets turbid again, it is filtered. After warming to 40 °C the *pH*

value is adjusted to 6.5 (*pH*-meter). Turbidity is removed by addition of 10 ml of *tert*-butanol. Now 208 mg (0.5 mmol) of K_2PtCl_4 in 5 ml of water are added dropwise during 10 min with stirring. Stirring is continued in the dark at 40 °C, and the *pH* is adjusted to 6.5 every 45 min by addition of 0.1 *N* NaOH. When the *pH* value is constant (approximately after 15 h), the precipitated complex is filtered off, thoroughly washed with water, and dried overnight in the desiccator. For purification, the complex is dissolved in the minimum quantity of *DMF*, reprecipitated with 5% NaCl solution, and dried at 70 °C for 5 d at the oil pump over P_2O_5; ochreous powder, 63 mg (62%), m.p.: 217–219 °C (decomp.).

IR (KBr): $\nu = 3500–3200$ (OH, br), 3267 (NH_2, partly covered by OH), 2925 (C–H), 1601 (NH_2), 1565, 1508, 1460 (C=C), 1068 (C–Cl) cm^{-1}; ^1H NMR (*DMF*-d_7): $\delta = 2.58$ (t; $^3J = 8.6$ Hz, 2H, $CHCH_2CH$, overlapped with *DMF*), 5.10–5.20 (m; 2H, $CHNH_2$), 5.48–5.56 (m; 2H, $CHNH_2$), 5.89–6.02 (m; 2H, CH_2CHNH_2), 6.92 (s; 4H, arom), 10.77 (s; 2H, OH, exch.) ppm; NI-FAB-MS (*DMSO*/glycerol): *m/z* 659 (M^-), 623 $(M–HCl)^-$; $C_{15}H_{14}Cl_6N_2O_2Pt \times 5\ H_2O$ (752.3); calcd.: C 23.95, H 3.21, N 3.72; found: C 24.12, H 2.83, N 3.39.

[meso-1,3-Bis(2,6-dichloro-4-hydroxyphenyl)propane-1,3-diamine]dichloro-platinum(II) (**1**)

Prepared from 83 mg (0.15 mmol) of diamine **11** as described for complex **2**; ochreous powder, m.p.: 214–217 °C, yield 46 mg (59%).

IR (KBr): $\nu = 3450–3250\ cm^{-1}$ (OH, br), 3255 (NH_2, overlapped with OH), 3075 (C–H), 1601 (NH_2), 1545, 1508, 1468 (C=C) 1055 (C–Cl) cm^{-1}; ^1H NMR (*DMF*-d_7): $\delta = 3.40$ and 3.60 (2m; 2H, $CHCH_2CH$, overlapped with *DMF*), 5.04–5.24 (m; 4H, $CHNH_2$), 6.06–6.19 (m; 2H, CH_2CHNH_2), 6.93 (s; 4H, arom), 10.83 (s; 2H, OH, exch.) ppm; NI-FAB-MS (*DMSO*/glycerol): $m/z = 659$ (M^-), 623 $(M–HCl)^-$ $C_{15}H_{14}Cl_6N_2O_2Pt \times 2\ H_2O$ (698.3); calcd:. C 25.80, H 2.60, N 4.01; found: C 26.25, H 2.46, N 3.89.

Estrogen receptor binding assay of diamines **11** *and* **12**

The relative binding affinity (RBA) was determined according to *Hartmann et al.* [12] by studying the replacement of 17β-[^{3}H]estradiol (*β-Es*). In brief: at 4 °C 6–8 appropriate concentrations of the test compound in EtOH are shaken with calf uterine cytosol (100 µl) and *β-Es* (0.5 pmol; specific activity 90–115 Ci/nmol) for 16 h. The incubation is stopped by dextran-coated charcoal, and after centrifugation, the radioactivity of a 100 µl supernatant aliquot (total volume: 500 µl) is counted. The binding of *β-Es* is estimated in the same way. To estimate the non-specifically bound quantity of *β-Es*, an excess of non-radioactive 17β-estradiol (2 nmol) is added in order to displace the specifically bound amount of (radioactive) *β-Es*. The percentage of bound *β-Es vs.* the concentration of the competing test compound is plotted half-logarithmically. At least six concentrations of the test compound are chosen to get a linear graph. From this plot molar concentrations of unlabeled 17β-estradiol and of the test compound are determined which reduce the binding of the radioligand by 50%.

Estrogenic activity of diamines **11** *and* **12**

The pertinent *in vitro* assay was described by *Hafner* [13]. In brief: MCF 7-2a cells are seeded (100 µl/well) in six well flat bottomed plates (Flacon Plastics 3075). After 24 h, the test compound is added as freshly 1000-fold concentrated solution in EtOH, and the cells are incubated for 48–50 h. The culture medium is removed by suction, and the cells in each well are washed with 2 ml of phosphate buffered saline (PBS). 200 µl of cell lysis buffer is added, respectively. After 15 min, centrifugation leads to a pellet of cell fragments. 30 µl of the supernatant are mixed with 100 µl of substrate (luciferine) of the luciferase assay (Fa. Promega), and the development of light (RLU: relative light units) is measured in a luminometer for 10 s. In order to standardize the results, the quantity of proteins of the cell extracts [14] was correlated with the mass of luciferase [13]: log [mass of luciferase (pg)] = (log

[RLU] – 5.1607)/0.866325. The estrogenic activity is expressed as % activation of a 10^{-8} molar estradiol control.

Acknowledgements

We thank Prof. Dr. *von Angerer* for helpful discussions and Mrs. *Liebl* for skilful technical assistance with the biochemical tests.

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Received April 3, 1996. Accepted April 15, 1996