cis-PLATINUM-*o*-CATECHOLATO CONJUGATE LABELLED WITH A ^{103}[Ru]-RUTHENOCENE RESIDUE

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SUMMARY

The cytostatic platinum complex [4-(2-aminoethyl)-1,2-benzenediolato(2-)-*O,O'*-bis(triphenylphosphine)platinum(II) (**4**) was labelled with ^{103}Ru by attachment of radioactive ruthenocene carboxylic acid (**1e**) via a peptide linkage. The synthesis of [4-[2-[*N*-[^{103}Ru]-ruthenocenecarboxamido(ethyl)]-1,2-benzenediolato(2-)-*O,O'*]-bis(triphenylphosphine)-platinum(II) (**5e**) included the reaction of **1e** with N-hydroxysuccinimide (**2**) followed by treatment with **4**. Organ distribution experiments in mice revealed that the radioactive bimetallic complex **5e** accumulates mainly in the liver and in the spleen.

Key Words: Anti-tumor catecholamine-Pt drug, ^{103}Ru-Labelling, organ distribution measurements.

INTRODUCTION

In search for new efficient anti-tumor drugs with target specificity, we have recently furnished several analogs of *cis*-dichlorodiaminoplatinum(II) with bidentate ligands that can link between the metal atom and biological carriers (1). One of the "chemical handles" that proved both to complex with the platinum to give efficient cytostatic agents, and to bind steroidal hormones as well as other molecule navigators (2) - is 4-(2-aminoethyl)-*o*-catechol (3).

In order to study the organ-distribution of catecholamine-platinum conjugates, we have now investigated the attachment of a radioactive marker to these complexes. Since some ferrocene derivatives could easily be labelled by metal exchange reaction with $^{103}RuCl_3$ (4), we examined this labelling method for introduction of a γ-emitter into the catecholaminoplatinum drugs.

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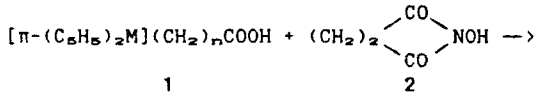
RESULTS AND DISCUSSION

[4-[2-[*N*-Ferrocenecarboxamido(ethyl)]- and [4-[2-[*N*-ferrocenacetamido-(ethyl)]-1,2-benzenediolato(2-)-*O*,*O*']bis(triphenylphosphine)platinum(II) (**5a** and **5b**, respectively) were synthesized as outlined in eqns 1 and 2.



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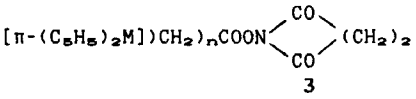

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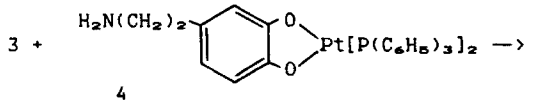

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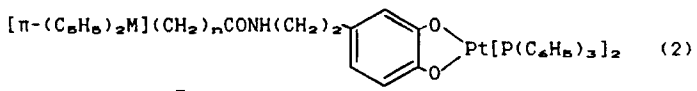

5 (2)

a, M=Fe; n=0 **d**, M=Ru; n=1

b, M=Fe; n=1 **e**, M=^{103}Ru; n=0

c, M=Ru; n=0 **f**, M=^{103}Ru; n=1

The carboxylic acids **1a** and **1b** were converted with the aid of **2** into the corresponding activated esters **3a** and **3b** which, in turn, were reacted with [4-(2-aminoethyl)-1,2-benzenediolato(2-)-*O*,*O*']-bis(triphenylphosphine)-platinum(II) (**4**) (2).

Several attempts to transfer the iron complex **5a** as well as its precursor **3a** directly into the corresponding radioactive compounds **5e** and **3e** led to the destruction of the complexes. Therefore, the radiolabelling was carried out already at the stage of the carboxylic acid **1a**. Treatment of ^{103}Ru-ruthenocene carboxylic acid (**1e**) with *N*-hydroxysuccinic acid followed by coupling with **4** and TLC separation from minor radioactive by-products afforded the radiolabelled platinum conjugate **5e**.

In contrast to the smooth transformation of **1a** to **1e**, the reaction of ferrocenacetic acid **1b** with $^{103}RuCl_3$ resulted in the formation of **1f** in a poor yield which was too low for the synthesis of **5f**.

The purity of the labelled compounds was determined by comparing their physical properties with those of the unlabelled ruthenium complexes

synthesized from cold ruthenocenyl derivatives by the route given in eqns. 1 and 2.

The radioactive [4-[2-[*N*-[^{103}Ru]-ruthenocenecarboxamido(ethyl)]-1,2-benzenediolato(2-)-*O,O'*]bis(triphenylphosphine)platinum(II) (**5e**) was subjected to biological tests in mice. The measured organ-distribution summarized in Table 1 indicates that the ruthenocene moiety serves as a selective biological carrier and directs most of the platinum drug to the liver and the spleen.

EXPERIMENTAL

$^{103}RuCl_3$ (ca. 500 mCi/mol) was purchased from Buchler-Amersham, Braunschweig.

[4-(2-Aminoethyl)-1,2-benzenediolato(2-)-*O,O'*]bis(triphenylphosphine)-platinum(II) (**4**) (2), ferrocenecarboxylic acid (**1a**) (5), ferrocenacetic acid (**1b**) (6), and ruthenocencarboxylic acid (**1c**) (5) were obtained as described previously.

Ruthenocenacetic acid (1d) was prepared analogously to **1b** by treatment of the methiodide of [(*N,N*-dimethylamino)methyl]ruthenocene (7)(8) with KCN followed by hydrolysis (6). Yield 71%; cream colored crystals; mp 141 °C; IR (KBr) 1703 cm^{-1} (C=O), 200 MHz ^1H NMR ($CDCl_3$) δ 3.300 (s, 2H, CH_2), 4.530 (t, 2H, J=1.6 Hz, cpH3, cpH4), 4.542 (s, 5H, C_5H_5), 4.651 (t, 2H, J=1.6 Hz, cpH2, cpH5). *Anal.* Calcd for $C_{12}H_{12}O_2Ru$: C, 49.82; H, 4.18. Found: C, 49.72; H, 4.33.

[4-[2-[*N*-Ferrocenecarboxamido(ethyl)]-1,2-benzenediolato(2-)-*O,O'*]-bis(triphenylphosphine)platinum(II) (5a).

A. Under argon atmosphere 200 mg (0.87 mmol) of **1a** was converted into the active ester **3a** by treatment with 100 mg (0.87 mmol) of 2 and 179 mg (0.87 mmol) of *N,N*-dicyclohexylcarbodiimide (DCC) in 3.5 ml of freshly dried THF (9); A mixture of 30 mg (0.092 mmol) of **3a** (freshly chromatographed on alumina of activity I, which had been deactivated with 16% of H_2O, using CH_2Cl_2 as eluent), 76 µl (0.55 mmol) of triethylamine, 80 mg of **4** and 1.6 ml of dry THF was stirred under exclusion of air and light for 40 h. The mixture was filtered, the filtrate evaporated under reduced pressure to dryness and the residue chromatoraphed on a silica gel PLC plate using initially a MeOH-CH_2Cl_2

TABLE 1. Organ-Distribution of the ^{103}Ru-Labelled Platinum-Catecholate Conjugate 5e in Mice ($x \pm \sigma$)*

Time after injection (min.) [n]	^{103}Ru-Concentration (% inj.dose/% body weight)							
	Muscle	Blood	Lung	Liver	Kidney	Adrenal	Heart	Spleen
15 [4]	0.096	1.410	2.740	10.40	0.965	0.258	0.443	3.790
	±0.024	±0.046	±0.504	±1.04	±0.095	±0.006	±0.026	±0.454
60 [3]	0.040	0.369	1.310	11.300	0.317	0.036	0.163	4.62
	±0.005	±0.053	±0.189	±0.414	±0.014	±0.002	±0.003	±0.348

*Dose: 3 µmol/kg

mixture (1:24) and then a MeOH-$CHCl_3$ mixture (1:1) as eluent. Traces of dicyclohexylurea were removed from the eluted solid by fractional crystallization from CH_2Cl_2 at 0 °C to give 11 mg (11%) of **5a**; orange crystals; mp 186-188 °C (from ether); IR (KBr) 1650, 1635, 1560 (HNC=O), 1480, 1270 cm^{-1} (C-O); 250 MHz ^1H NMR ($CDCl_3$) δ 2.679 (t, 2H, J=5.9 Hz, $NHCH_2CH_2$), 3.509 (dt, 2H, J_d=5.1 Hz, J_t=5.9 Hz, $NHCH_2CH_2$), 4.105 (s, 5H, C_5H_5), 4.234 (t, 2H, J=1.8 Hz, cpH3, cpH4), 4.539 (t, 2H, J=1.8 Hz, cpH2, cpH5), 5.787 (t, 1H, J=5.1 Hz, NH), 6.263 (dd, 1H, $J_{3,5}$=1.6 Hz, $J_{5,6}$=7.9 Hz, ArH5), 6.354 (d, 1H, $J_{3,5}$=1.6 Hz, ArH3), 6.446 (d, 1H, $J_{5,6}$=7.9 Hz, ArH6), 7.126-7.574 (m, 30H, PC_6H_5); EI MS (80 eV, 40 °C): calcd for $[C_{55}H_{47}{}^{56}FeNO_3P_2{}^{195}Pt]^{+\cdot}$ 1082.2028, recorded by peak match method 1082.1943; *m/z* (relative intensity) (10) 1077-1084 [$M^{+\cdot}$ and $(M-H)^+$, 35], 715-722 ($Pt[P(C_6H_5)_3]_2^{+\cdot}$, 25), 365 ($C_{19}H_{19}{}^{56}FeNO_3^+$, 9), 363 ($[M-{}^{195}Pt[P(C_6H_5)_3]_2)^{+\cdot}$, 6], 262 $[P(C_6H_5)_3^{+\cdot}$, 100], 261 ($C_{18}H_{14}P^+$, 23), 229 ($C_{11}H_{11}{}^{56}FeNO^+$, 12), 213 ($C_{11}H_9{}^{56}FeO^+$, 7), 185 ($C_{12}H_{10}P^+$, $C_{10}H_9{}^{56}Fe^+$, 14), 184 ($C_{12}H_9P^{+\cdot}$, 14), 183 ($C_{12}H_8P^+$, 48), 154 ($C_{12}H_{10}^{+\cdot}$, 9), 153 ($C_{12}H_9^+$, 7), 108 ($C_6H_5P^{+\cdot}$, 43), 107 ($C_6H_4P^+$, 10). *Anal.* Calcd for $C_{55}H_{47}FeNO_3P_2Pt$: C, 61.00; H, 4.37; N, 1.29. Found: C, 60.97; H, 4.25; H, 1.42.

B. Ferrocenecarboxylic acid (**1a**) could also be converted directly into **5a** in 5% yield when a solution of 1.03 mmol of the carboxylic acid, 1.25 mmol of DCC and 0.600 mmol of **4** in 6.5 ml of THF was stirred at room temperature for 72 h under exclusion of air and light, followed by the work-up described above.

[4-[2-[*N*-Ferroceneacetamido(ethyl)]-1,2-benzenediolato(2-)-*O,O'*]-bis(triphenylphosphine)]platinum(II) (5b).

A solution of 169 mg (0.82 mmol) of DCC in 1 ml of dry THF was syringed under argon at 0 °C into a solution of 200 mg (0.82 mmol) of **1b** and 94 mg (0.82 mmol) of **2** in 6 ml of the same solvent. The mixture was stirred at 0 °C, for 2 h and then for 16 h at 25 °C. The colorless precipitate of dicyclohexylurea was filtered off and washed with CH_2Cl_2. The filtrate was evaporated to dryness and the residue treated with cold CH_2Cl_2, filtered and concentrated once again. The resulting tan colored active ester **3b** [IR (KBR) 1805, 1780, 1735 cm^{-1} (C=O)] was dried at 1 mm. A solution of the crude active

ester in 6 ml of dry THF was then syringed under argon at 0 °C into a solution of 561 mg (0.645 mmol) of **4** and 1.1 ml (8 mmol) of triethylamine in 9 ml of the same solvent. After 2 h at this temperature the mixture was allowed to warm up to 25 °C and stirring was continued under exclusion of light for 6 h. A small amount of solid particles was filtered off, washed with THF and the filtrate evaporated to dryness in vacuo. Successive washing of the residue with H_2O followed by column chromatography on alumina (activity I), which had been deactivated with 15% of H_2O (CH_2Cl_2 serving a eluent), yielded orange crystals that were washed with a small amount of ether to give 271 mg (38%) of **5b**; mp 150 °C; IR (KBr) 1640, 1555 (HNC=O), 1480, 1265 cm^{-1} (C-O); 200 MHz ^1H NMR ($CDCl_3$) δ 2.511 (t, 2H, J=5.4 Hz, $NHCH_2CH_2$), 3.314 (dt, 2H, J_d=4.4 Hz, J_t=5.4 Hz, $NHCH_2CH_2$), 3.207 (s, 2H, CH_2CO), 4.077 (br s., 9H, C_5H_4, C_5H_5), 5.654 (t, 1H, J=4.4 Hz, NH), 6.043 (dd, 1H, $J_{3,5}$=1.9 Hz, $J_{5,6}$=7.9 Hz, ArH_5), 6.188 (d, 1H, $J_{3,5}$=1.9 Hz, ArH_3), 6.347 (d, 1H, $J_{5,6}$=7.9 Hz, ArH_6), 7.131-7.562 (m, 30H, PC_6H_5); EI MS (80 eV, 40 °C) calcd for $[C_{56}H_{49}{}^{56}FeNO_3P_2{}^{195}Pt]^{+\cdot}$ 1096.2185, recorded by peak match method 1096.2076; *m/z* (relative intensity) 1091-1098 [$M^{+\cdot}$ and $(M-H^+)$, 45], 715-722 ($Pt[P(C_6H_5)_3]^{+\cdot}$; 100), 379 ($C_{20}H_{21}{}^{56}FeNO_3^{+\cdot}$, 17), 377 ($[M-{}^{195}Pt[P(C_6H_5)_3]_2)^{+\cdot}$, 17], 262 $[P(C_6H_5)_3^{+\cdot}$, 100], 185 ($C_{12}H_{10}P^+$, $C_{10}H_9{}^{56}Fe$, 23), 184 ($C_{12}H_9P^{+\cdot}$, 16), 183 ($C_{12}H_8P^+$, 76), 154 ($C_{12}H_{10}^{+\cdot}$, 31), 153 ($C_{12}H_9^+$, 17), 152 ($C_{12}H_8^{+\cdot}$, 9), 108 ($C_6H_5P^{+\cdot}$, 38), 107 ($C_6H_4P^+$, 26). *Anal.* Calcd for $C_{56}H_{49}FeNO_3P_2Pt$: C, 61.32; H, 4.50; N, 1.27. Found: C, 61.35; H, 4.50; N, 1.66.

[4-[2-[*N*-Ruthenocenecarboxamido(ethyl)]-1,2-benzenediolato(2-)-*O,O'*]-bis(triphenylphosphine)platinum(II) (5c).

By applying procedure A described above for the synthesis of **5a**, the ruthenium complex **5c** was obtained in 30% yield. Direct transformation of the carboxylic acid **1c** to **5c** (method B) gave only 12% yield; mp 218 °C; IR (KBr) 1655, 1620, 1565 (HNC=O), 1480, 1270 cm^{-1} (C-O); 300 MHz ^1H NMR ($CDCl_3$) δ 2.611 (t, 2H, J=6.0 Hz, $NHCH_2CH_2$), 3.416 (dt, 2H, J_d=4.6 Hz, J_t=6.0 Hz, $NHCH_2CH_2$), 4.459 (s, 5H, C_5H_5), 4.562 (t, 2H, J=1.7 Hz, cpH3, cpH4), 4.898 (t, 2H, J=1.7 Hz, cpH2, cpH5), 5.706 (t, 1H, J=4.6 Hz, NH), 6.204 (dd, 1H, $J_{3,5}$=2.0 Hz, $J_{5,6}$=7.8 Hz, ArH5), 6.275 (d, 1H, $J_{3,5}$=2.0 Hz, ArH3), 6.426 (d,

1H, $J_{5,6}$=7.8 Hz, ArH6), 7.122-7.549 (m, 30H, PC_6H_5); DCI MS (isobutane, 50 eV; sample dissolved in DMSO) *m/z* (relative intenity) 1118-1132 (MH^+, 2), 405-413 ($C_{19}H_{19}NO_3Ru^{+\cdot}$ and [M-Pt[$P(C_6H_5)_3$]$_2$, 18), 269-277 ($C_{11}H_{11}NORu^{+\cdot}$; 36); 262 [$P(C_6H_5)_3^{+\cdot}$, 100], 253-261 ($C_{11}H_9ORu^+$, 41), 225-244 ($C_{10}H_9Ru^{+\cdot}$ 24), 185 ($C_{12}H_{10}P^+$, 7), 184 ($C_{12}H_9P^{+\cdot}$, 9), 183 ($C_{12}H_8P^{+\cdot}$, 49), 154 ($C_{12}H_{10}^{+\cdot}$, 73), 153 ($C_{12}H_9^+$, 16), 152 ($C_{12}H_8^{+\cdot}$, 17), 108 ($C_6H_5P^{+\cdot}$, 24), 107 ($C_6H_4P^+$, 11). *Anal.* Calcd for $C_{55}H_{47}NO_3P_2PtRu$: C, 58.56; H, 4.20; N, 1.24. Found: C, 58.28; H, 4.41; N, 1.51.

[4-[2-[*N*-Ruthenoceneacetamido(ethyl)]-1,2-benzenediolato(2-)-*O,O'*]-bis(triphenylphosphine)]platinum(II) (5d).

In a similar manner to the preparation of **3b**, 363 mg (1.25 mmol) of **1d** was converted into the active ester **3d** with the aid of 144 mg (1.25 mmol) of **2** and 258 mg (1.25 mmol) of DCC in 2.3 ml of THF. [IR (KBr) 1810, 1780, 1735, 1725 cm^{-1} (C=O)]. After separation of the dicyclohexylurea, the crude ester was stirred together with 922 mg of **4** and 1.5 ml of triethylamine in 11 ml of THF for 18 h at room temperature. Crude **5d** was chromatographed on alumina (activity I), deactivated with 15% H_2O, using mixtures of CH_2Cl_2 and MeOH, from pure CH_2Cl_2 to MeOH-CH_2Cl_2 (1:25) as eluents, to give after washing with ether 293 mg (24%) of orange crystals; mp 134-138 °C; IR (KBr) 1650, 1555 (HNC=O), 1480, 1265 cm^{-1} (C-O); 300 MHz ^1H NMR ($CDCl_3$) δ 2.576 (t, 2H, J=6.7 Hz, NHC$H_2$$CH_2$), 3.079 (s, 2H, CH_2CO), 3.383 (dt, 2H, J_d=5.7 Hz, J_t=6.7 Hz, NHC$H_2$$CH_2$), 4.455 (t, 2H, J=1.6 Hz, cpH3, cpH4), 4.467 (s, 5H, C_5H_5), 4.512 (t, 2H, J=1.6 Hz, cpH2, cpH5), 6.119 (t, 1H, J=5.7 Hz, N*H*), 6.135 (dd, 1H, $J_{3,5}$=1.8 Hz, $J_{5,6}$=7.9 Hz, ArH5), 6.239 (d, 1H, $J_{3,5}$=1.8 Hz, ArH3), 6.374 (d, 1H, $J_{5,6}$=7.9 Hz, ArH6), 7.126-7.542 (m, 30H, PC_6H_5); EI MS (80 eV, 40 °C); calcd for $[C_{56}H_{49}NO_3P_2{}^{195}Pt^{102}Ru]^{+\cdot}$ 1142.1879, recorded by peak match method 1142.1913; *m/z* (relative intensity) 1135-1146 [$M^{+\cdot}$ and $(M-H)^+$, 20], 715-722 ($Pt[P(C_6H_5)_3]_2^{+\cdot}$, 12), 419-427 ($C_{20}H_{21}NO_3Ru^{+\cdot}$ and [M-Pt[$P(C_6H_5)_3$]$_2^{+\cdot}$, 39), 262 [$P(C_6H_5)_3^{+\cdot}$, 100], 261 ($C_{18}H_{14}P^+$, 16), 185 ($C_{12}H_{10}P^+$, 14), 184 ($C_{12}H_9P^{+\cdot}$, 16), 183 ($C_{12}H_8P^+$, 53), 154 ($C_{12}H_{10}^{+\cdot}$, 20), 153 ($C_{12}H_9^+$, 12), 152 ($C_{12}H_8^{+\cdot}$, 20), 108 ($C_6H_5P^{+\cdot}$. 24), 107 ($C_6H_4P^+$, 7). *Anal.* Calcd for $C_{56}H_{49}NO_3P_2PtRu$: C, 58.89; H, 4.32; N, 1.22. Found: C, 58.84; H, 4.56; N, 1.37.

Radioactive synthesis

[^{103}Ru]-Ruthenocenecarboxylic acid (**1e**) was prepared either in 20-50% yield of the administrated radioactivity from methyl ferrocenecarboxylate and $^{103}RuCl_3$ followed by base mediated hydrolysis as reported previously (4a), or in a somewhat lower yield directly from **1a** as described below.

In a typical run, 5 mg of **1a** and 20 µCi of hydrated $^{103}RuCl_3$ in 0.15 ml of dioxane-hydrochloric acid mixture (97:3) was heated at 130 °C in a sealed pressure tube for 60 min. The cooled reaction mixture was adsorbed on a 0.2 mm thick silica gel PLC plate, and the components separated with a mixture of CH_2Cl_2-MeOH (9:1). The band of R_f=0.45 was extracted with a mixture of $CHCl_3$-MeOH (1:1) and the resulting solution of ferrocene- and ruthenocene-carboxylic acid (radioactivity 3.3 µCi) was evaporated with a stream of N_2 to dryness. The mixture containing **1e** was then treated for 20 h with 15 mg of **2** and 30 mg of DCC in 0.3 ml of THF to give **3e** (R_f=0.55 on silica gel, using 2% MeOH in CH_2Cl_2 as eluent). The dicyclohexylurea was removed by centrifugation, and 70 µl of the resulting solution (radioactivity: 0.7 µCi) was stirred for 40 h at room temperature with 15 mg of **4** and 10 µl of Et_3N. Separation of **5e** from the mixture was accomplished by PLC on silica gel, using 4% MeOH in CH_2Cl_2 as eluent. The band at R_f=0.38 was shown to be **5e** with radioactivity of 0.5 µCi. The specific radioactivity of the sample was calculated (on the basis of the yields obtained for **5c**) to be 2.2 µCi/µmol metallocene derivative. The ferrocene derivative **5a** could not be separated from the ^{103}Ru-labelled compound **5e**.

The ^{103}Ru-radioactivity was measured directly on the TLC and PLC plates by a Berthold-Scanner LB 2273 (Labor Berthold, Wildbad) with an efficiency of 45% for the β and Auger electrons emitted (11).

Organ-distribution measurements

Typically, the radioactive compounds were dissolved in 1,2-propylene glycole and 50 µl of the solution was injected i.v. A dose of 3 µmol/kg was administrated. Immediately after the injection the total body radioactivity was measured by placing the mice in a plastic container which was then inserted into the well of a NaI scintillation counter. Extraction percentages

were calculated from the change in the radioactivity measured for each animal. The mice were killed with $CHCl_3$ and muscle samples were taken from the hind leg. Excised organs were bottled free of blood, weighed and assayed in an automatic γ-counter (Labor Berthold, Wildbad) with an efficiency of 27% for ^{103}Ru (12).

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