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Metal Complexes of Radiosensitization Drugs: the Characterization of the Metronidazole Adducts of Dirhodium(II)tetracarboxylate Compounds

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Abstract

The compounds $Rh_2(O_2CR)_4 \cdot 2$metronidazole (R = CH_3 (1); $CH_2CH_2CH_2Ph$ (2)) have been prepared by the reaction of $Rh_2(O_2CR)_4$ with an excess of metronidazole. The compounds have been characterized by analytical, spectroscopic, electrochemical and crystallographic measurements. Preliminary biological studies, on 1, have shown the compound to have selective toxic effects on DNA and intact cells under both anoxic and hypoxic conditions.

Introduction

In recent years a number of tetra(μ-carboxylato)-dirhodium(II) compounds have been observed to exhibit a range of antitumour activity [1]. The compound $Rh_2(O_2CCH_3)_4$ has been used in combination with agents such as Arabinosylcytosine to give synergistic inhibition of mouse tumours [2], with no apparent delayed toxicity. Although the precise mechanism of action of these compounds is unknown, it has however been demonstrated that they bind to DNA and act via inhibition of DNA and protein synthesis [1].

Nitroimidazole compounds, especially the 5- and 2-nitroimidazoles, were the first hypoxic cell radiosensitizers to show clinical promise [3–5], because they were of high electron affinity and exhibited relatively low toxicity to non-hypoxic cells. Metronidazole (a 5-nitroimidazole) is widely used in the treatment of anaerobic infections and has been shown to act as a hypoxic cell sensitizer *in vitro* [6, 7] and gives significant sensitization of tumour response in several model murine tumour systems [8].

In this paper we report the synthesis and physicochemical characterization of compounds containing both the tetra(μ-carboxylato)dirhodium(II) moiety and the drug metronidazole, and briefly discuss the results of preliminary biological studies which will be reported in full elsewhere.

Results and Discussion

The compounds $Rh_2(O_2CCH_3)_4 \cdot 2$metronidazole (1) and $Rh_2(O_2CCH_2CH_2CH_2Ph)_4 \cdot 2$metronidazole (2) were prepared in high yield by treating the appropriate tetra(μ-carboxylato)dirhodium(II) compound with an excess of the ligand in methanol. The red compounds are soluble in a wide range of non-polar organic solvents, and are readily recrystallized from chloroform/diethylether mixtures. Integration of the ^1H NMR spectrum of each compound indicates that there are two molecules of metronidazole per tetra(μ-carboxylato)dirhodium(II) unit. Comparison of these NMR spectra with that of uncomplexed metronidazole indicates that the most probable point of coordination of the ligand to the metal is via the unsaturated imidazole nitrogen atom. The infrared spectra contain bands, in the region 1540–1590 cm^{-1}, due to $\nu(NO_2)$. These spectroscopic data, together with the results of microanalysis (C, H, N), are consistent with a molecular formula of $Rh_2(O_2CR)_4 \cdot 2$metro for the products. Prior to undertaking biological testing of these compounds it was important to establish unequivocally their identity, so both have been subjected to a detailed X-ray structural analysis. Details of the solution of the structures are given in 'Experimental'. The important crystallographic parameters are summarized in Table 1 and selected bond lengths and interbond angles for both compounds are presented in Table 2. The structures of 1 and 2 are shown in Figs. 1 and 2 respectively. Each molecule is a dimer lying on a crystallographic inversion centre with each rhodium atom exhibiting distorted octahedral coordination, with angles in the range 87–96°. Each rhodium-to-rhodium bond is bridged by four car-

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TABLE 1. Crystallographic data for two $Rh_2(O_2CR)_4 \cdot 2$metro complexes (R = CH_3 or $CH_2CH_2CH_2Ph$)

Formula	$C_{20}H_{30}N_6O_{14}Rh_2$ (**1**)	$C_{52}H_{62}N_6O_{14}Rh_2$ (**2**)
Formula weight	784.38	1198.02
Space group	*Pbca*	$P2_1/c$
a (Å)	8.828(2)	11.814(2)
b (Å)	17.038(5)	14.336(2)
c (Å)	19.311(5)	16.392(4)
α (°)	90.0	90.0
β (°)	90.0	99.79(2)
γ (°)	90.0	90.0
V (Å^3)	2904(1)	2735(1)
Z	4	2
$F(000)$	1576	1236
D_{calc} (g/cm^3)	1.79	1.46
Crystal size (mm)	0.1 × 0.3 × 0.4	0.25 × 0.6 × 0.2
μ (Mo Kα), (cm^{-1})	11.6	6.3
Data collection instrument	Nicolet R3m/V	
Radiation	Mo (λ = 0.71073 Å)	
Orientation reflections: no.; range (2θ)	43, $15 < 2\theta < 30$	30, $11 < 2\theta < 28$
Temperature (°C)	19	
2θ range measured	5–50°	
No. unique data;	3796	6319
Total with $F_o^2 > 3\sigma(F_o^2)$	2326	5567
No. parameters	200	334
R [a]	0.055	0.050
R' [b]	0.052	0.054
Weighting scheme	$w^{-1} = \sigma^2 F + 0.000875F^2$	$w^{-1} = \sigma^2 F + 0.000722F^2$
Largest shift/e.s.d., final cycle	0.006	0.004
Largest peak (e/Å)	0.76	1.03

[a] $R = \Sigma[|F_o| - |F_c|]/\Sigma|F_o|$. [b] $R' = \Sigma[|F_o| - |F_c|w^{1/2}]/\Sigma[F_o w^{1/2}]$.

boxylate ligands to give the familiar 'lantern' structure. The two rhodium–rhodium bond distances, 2.388(1) and 2.394(1) Å for **1** and **2** respectively, are almost indistinguishable. It may however be worth noting in passing that the longer bond length is associated with the compound having the more electron-withdrawing substituent on the carboxylate bridging ligands. The influence of the electronic effects of substituents of bridging ligands on metal–metal bond distances is well understood, and has been discussed in detail previously [9, 10]. The rhodium–rhodium bond length in **1** might also usefully be compared with that observed in $Rh_2(O_2CCH_3)_2 \cdot 2C_5H_5N$, 2.3963(2) Å [11]. Once again the difference is small, and reflects the relative donor power of the pyridine and metronidazole axial ligands. The rhodium–nitrogen bond lengths in the compounds described here do not differ from each other in any statistically significant way. The rhodium–oxygen bond lengths fall within a very narrow range, 2.023 to 2.040 Å, and there is no significant twisting about the rhodium–rhodium bond, the ligating atoms on the metals being virtually eclipsed. The rhodium–rhodium and rhodium–nitrogen bonds are almost colinear, consistent with little steric interaction between bridging and axial ligands. The crystallographic studies confirm that in both compounds the metronidazole ligands are coordinated to the rhodium(II) ions via the imidazole nitrogen atoms. A preference for coordination via the heterocyclic nitrogen atom, rather than an amide nitrogen, is observed in the compounds $Rh_2(O_2CCH_3)_4(admp)_2$ [12].

Both complexes have been investigated by cyclic voltammetry at 25 °C at a platinum electrode in dichloromethane using 0.2 M $[Bu_4N][BF_4]$ as supporting electrolyte. The potential range investigated was +1.8 to −1.6 V. Both compounds exhibit a one-electron reversible oxidation at approximately +1.1 V. The occurrence of a metal-based redox process in the potential range +0.9 to +1.5 V is unsurprising as almost all adducts of tetra(μ-carboxylato)dirhodium(II) compounds have been observed to undergo similar electron-transfer behaviour [13–15].

Metronidazole has been extensively studied by electrochemical means [16, 17]. In aqueous solution it undergoes an irreversible four-electron reduction to yield the hydroxylamine. However, in non-aqueous solvents a reversible one-electron transfer is followed by an irreversible three-electron process. Although the initial reduction is electrochemically reversible the radical anion formed in that process is known to undergo a second order disproportionation reaction,

TABLE 2. Selected bond lengths (Å) and angles (°) for **1** and **2**

	1	**2**
Bond lengths		
Rh(1)–Rh(1*)	2.388(1)	2.394(1)
Rh(1)–O(1)	2.037(4)	2.040(3)
Rh(1)–O(2)	2.036(4)	2.036(3)
Rh(1)–O(3)	2.023(5)	2.033(3)
Rh(1)–O(4)	2.023(5)	2.035(3)
Rh(1)–N(1)	2.240(5)	2.232(3)
Bond angles		
Rh(1*)–Rh(1)–O(1)	88.4(1)	87.8(1)
Rh(1*)–Rh(1)–O(2)	87.3(1)	88.0(1)
Rh(1*)–Rh(1)–O(3)	87.4(1)	88.0(1)
Rh(1*)–Rh(1)–O(4)	88.6(1)	87.9(1)
Rh(1*)–Rh(1)–N(1)	177.1(1)	175.9(1)
N(1)–Rh(1)–O(1)	90.7(2)	92.5(1)
N(1)–Rh(1)–O(2)	93.5(2)	96.1(1)
N(1)–Rh(1)–O(3)	95.4(2)	88.0(1)
N(1)–Rh(1)–O(4)	88.6(2)	91.7(1)
O(1)–Rh(1)–O(2)	175.7(2)	90.2(1)
O(1)–Rh(1)–O(3)	90.7(2)	89.0(1)
O(1)–Rh(1)–O(4)	87.8(2)	175.6(1)
O(2)–Rh(1)–O(3)	88.7(2)	175.9(1)
O(2)–Rh(1)–O(4)	92.5(2)	90.7(1)
O(3)–Rh(1)–O(4)	175.7(2)	89.8(1)

Asterisk denotes an atom generated by symmetry.

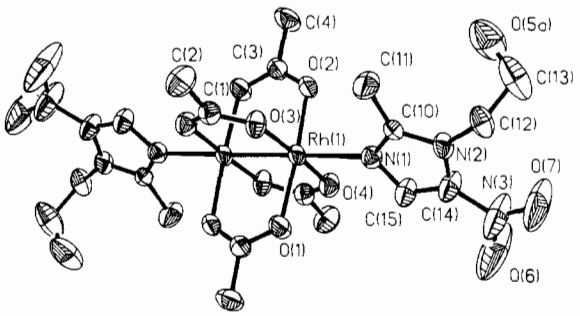

Fig. 1. The molecular structure of **1** showing the atom labelling scheme. Atoms are represented by thermal ellipsoids at the 50% level.

on the basis of electrochemical and pulse radiolysis studies [17, 18].

The electroreduction of both compounds **1** and **2** produced a single reduction peak, at a potential of *c.* −1.0 V, on the forward scan and a coupled oxidation peak on the backward sweep. The substantial peak-to-peak separations and the observation that these separations increase with increasing scan rate was diagnostic of the quasireversible character of the electron transfer reactions. Plots of $i_p/\nu^{1/2}$ were approximately constant over sweep rates of 50–400 mV s^{-1}, indicating diffusion control. Comparison of

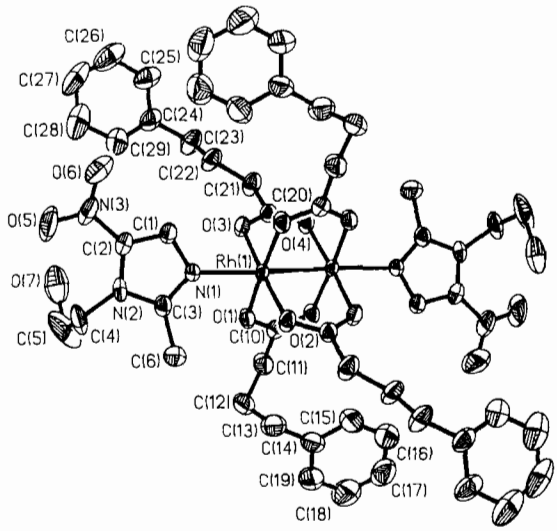

Fig. 2. The molecular structure of **2** showing the atom labelling scheme. Atoms are represented by thermal ellipsoids at the 50% level.

the currents which flow on oxidation and reduction indicate that both coordinated metronidazole ligands undergo simultaneous one-electron reduction reactions. If the voltammograms of the two compounds are compared it is apparent that the potentials for both oxidation and reduction undergo small anodic shifts on increasing the electron-withdrawing ability of the substituent on the bridging carboxylate ligands. Detailed studies into this phenomenon have appeared previously [13].

Biological studies on **1** show that it has selective toxic effects on DNA and intact cells under both anoxic and hypoxic conditions. The compound is 6.5 times more toxic to DNA under anoxic conditions than it is under oxic ones [19]. The ability of **1** to induce DNA SOS repair [20, 21] and the potential mutagenicity of the compound have also been investigated. As there have been no reports of adverse mutagenic or carcinogenic side-effects from the worldwide use of metronidazole in the treatment of protazoal and bacteriological infections it is hoped that these compounds will produce minimal adverse effects.

The rhodium(II)tetracarboxylates $Rh_2(O_2CR)_4$ suggest that additional mixed functionality compounds of this type are worthy of further study, particularly since they often display significant radiosensitizing ability in addition to their anoxic cytotoxicity.

Experimental

Materials

The rhodium(II)tetracarboxylates $Rh_2(O_2CR)_4$ (R = CH_3, $CH_2CH_2CH_2Ph$) were prepared by litera-

ture methods [22]. Metronidazole was supplied by Rhône-Poulenc and used without further purification.

Preparation of $Rh_2(O_2CCH_3)_4$·2metro (1)

$Rh_2(O_2CCH_3)_4$ (0.10 g, 0.23 mmol) and metronidazole (0.40 g, 2.3 mmol) were stirred together at room temperature in CH_3OH (40 cm^3) for 3 h. The initial green solution rapidly became red in colour and a red–brown solid was precipitated. The solid was filtered off, washed with $(C_2H_5)_2O$ and CH_3OH and dried *in vacuo* for 6 h. Yield 0.12 g, 68%. *Anal.*: Found C, 30.3; H, 3.8; N, 10.2. Calc. for $C_{20}H_{30}O_{14}N_6Rh_2$: C, 30.6; H, 3.8; N, 10.7%. ^1H NMR spectrum (CD_2Cl_2, $(CH_3)_4Si$): δ 1.55 (s, 12H), 8.43 (s, 2H), 4.71 (t, $^3J_{H-H}$ = 7.4 Hz, 4H), 4.14 (t, 4H), 2.91 (s, 2H), 1.29 (s, 6H). Infrared spectrum: $\nu(NO_2)$ 1542, 1588 cm^{-1}. Electrochemistry (298 K, CH_2Cl_2/0.2 M $[(n\text{-}C_4H_9)_4N][BF_4]$ electrolyte, Ag/AgCl reference electrode) + 1.08 V (metal-based reversible oxidation) −1.06 V (metronidazole-based quasi-reversible reduction).

Preparation of $Rh_2(O_2CCH_2CH_2CH_2Ph)_4$·2metro (2)

The compound was prepared in a manner closely analogous to that described above. Yield 75%. *Anal.*: Found C, 51.8; H, 5.3; N, 6.8. Calc. for $C_{52}H_{62}O_{14}N_6Rh_2$: C, 52.1; H, 5.2; N, 7.0%. ^1H NMR spectrum (CD_2Cl_2, $(CH_3)_4Si$): δ 7.1–7.9 (m, 20H), 1.9–3.1 (m, 24H), 8.39 (s, 2H), 4.73 (t, $^3J_{H-H}$ = 7.2 Hz, 4H), 4.16 (t, 4H), 2.72 (s, 2H), 1.28 (s, 6H). Infrared spectrum: $\nu(NO_2)$ 1540, 1590 cm^{-1}. Electrochemistry (293 K, CH_2Cl_2/0.2 M $[(n\text{-}C_4H_9)_4N][BF_4]$ electrolyte, Ag/AgCl reference electrode) + 1.10 V (metal-based reversible oxidation) −0.94 (metronidazole-based quasi-reversible reduction).

Measurements

The elemental analyses were by University College London Department of Chemistry. The infrared spectra were recorded as KBr discs on a Perkin-Elmer 983 spectrophotometer. ^1H NMR measurements were obtained on a Varian XL200 spectrometer. Chemical shifts are quoted to high field of tetramethylsilane. Cyclic voltammetric studies employed a Metrohm E506 potentiostat and Metrohm E612 VA Scanner and utilized a 3-electrode cell geometry, as described in detail previously [22].

X-ray Crystallographic Procedures

The molecular structures of **1** and **2** were obtained by general procedures described before [23, 24]. The crystal parameters and basic information pertaining to data collection and structure refinement are summarized in Table 1. Selected bond length and inter-bond angles are presented in Table 2. The fractional atomic coordinates and equivalent isotropic thermal vibration parameters for **1** and **2** are presented in Tables 3 and 4 respectively. The molecular structures

TABLE 3. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for **1**

	x	*y*	*z*	U_{eq}
Rh(1)	225(1)	274(1)	560(1)	30(1)
O(1)	1999(5)	880(3)	148(2)	44(2)
O(2)	−1565(5)	−372(3)	902(2)	40(1)
O(3)	1582(5)	−658(3)	749(2)	40(1)
O(4)	−1118(5)	1188(3)	295(2)	41(1)
C(1)	1682(8)	−1201(4)	304(3)	39(2)
C(2)	2513(10)	−1932(5)	524(4)	58(3)
C(3)	−2260(7)	−809(4)	492(3)	39(2)
C(4)	−3525(10)	−1288(6)	788(4)	62(3)
N(1)	599(6)	847(3)	1590(3)	34(2)
N(2)	1087(6)	1153(3)	2681(3)	38(2)
N(3)	715(13)	2593(5)	2607(5)	98(4)
C(10)	992(7)	576(4)	2212(3)	36(2)
C(11)	1275(13)	−277(4)	2367(4)	73(4)
C(12)	1461(8)	1039(5)	3416(3)	49(2)
C(13)	56(12)	924(9)	3854(5)	88(5)
C(14)	746(9)	1823(4)	2319(4)	50(2)
C(15)	451(9)	1627(4)	1654(4)	46(2)
O(5A)[a]	−769(11)	341(7)	3692(5)	98(5)
O(5B)	−950(30)	1403(17)	3822(14)	78(11)
O(6)	508(17)	3132(5)	2206(5)	171(6)
O(7)	901(14)	2677(5)	3231(4)	135(5)

[a]Disordered hydroxyl oxygen atoms; refinement based on 75% occupancy for O(5A), 25% occupancy for O(5B).

TABLE 4. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for **2**

	x	*y*	*z*	U_{eq}
Rh(1)	944(1)	−90(1)	5394(1)	25(1)
O(1)	1491(2)	887(2)	4644(2)	36(1)
O(2)	550(2)	936(2)	6158(2)	38(1)
O(3)	1229(2)	−1093(2)	4576(2)	37(1)
O(4)	286(2)	−1072(2)	6078(2)	37(1)
O(5)	6012(3)	−1785(3)	7112(3)	88(2)
O(6)	4603(4)	−2696(3)	6704(4)	114(2)
O(7)	6279(6)	−495(7)	5605(5)	97(4)
N(1)	2722(3)	−344(2)	6071(2)	31(1)
N(2)	4578(3)	−235(3)	6590(2)	37(1)
N(3)	5028(3)	−1916(3)	6783(3)	66(2)
C(1)	3152(3)	−1219(3)	6188(3)	37(1)
C(2)	4293(3)	−1152(3)	6507(3)	40(1)
C(3)	3589(3)	238(3)	6314(2)	36(1)
C(4)	5699(4)	209(4)	6837(4)	61(2)
C(5)	6230(6)	436(7)	5914(8)	144(5)
C(6)	3485(4)	1280(3)	6298(4)	60(2)
C(10)	763(3)	1268(3)	4088(2)	34(1)
C(11)	1202(4)	2019(3)	3572(3)	49(1)
C(12)	1914(4)	2764(4)	4121(4)	66(2)
C(13)	1239(5)	3238(4)	4723(4)	66(2)
C(14)	248(5)	3805(3)	4288(3)	58(2)
C(15)	−884(5)	3463(4)	4224(4)	72(2)

(continued)

TABLE 4. *(continued)*

	x	y	z	U_{eq}
C(16)	−1796(6)	3994(6)	3832(5)	96(3)
C(17)	−1595(7)	4863(6)	3519(6)	98(3)
C(18)	−493(8)	5196(5)	3581(6)	102(4)
C(19)	420(6)	4681(4)	3952(5)	76(2)
C(20)	440(3)	−1290(3)	3977(2)	33(1)
C(21)	730(4)	−1978(3)	3351(3)	50(1)
C(22)	1741(4)	−2634(3)	3679(3)	49(1)
C(23)	2207(5)	−3120(4)	2980(3)	59(2)
C(24)	3215(4)	−3741(3)	3319(3)	54(2)
C(25)	3129(6)	−4701(4)	3222(4)	69(2)
C(26)	4064(9)	−5275(5)	3537(5)	94(3)
C(27)	5055(8)	−4871(6)	3952(6)	102(4)
C(28)	5167(6)	−3910(7)	4055(5)	106(4)
C(29)	4225(5)	−3356(5)	3731(5)	81(3)

of **1** and **2** are illustrated in Figs. 1 and 2 respectively. Crystallographic calculations used the SHELXTL PLUS program package [25].

Supplementary Material

Complete tables of bond lengths and angles, together with tables of anisotropic thermal parameters, and observed and calculated structure factors are available from the authors on request.

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