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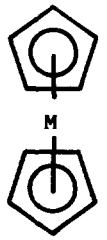
Review

METALLOCENES IN BIOCHEMISTRY, MICROBIOLOGY & MEDICINE

Kenneth E. Dombrowski,* Wendy Baldwin & John E. Sheats
Department of Chemistry, Rider College, Lawrenceville, New Jersey 08648 and *Department of Biochemistry, UMDNJ-Rutgers Medical School, Piscataway, New Jersey 08854

INTRODUCTION

Metallocenes, 1, contain a transition metal ion in the +2 valence state sandwiched between two cyclopentadienyl rings. They are electrically neutral compounds soluble in organic solvents and insoluble in water. Metallocene dihalides, 2, contain a transition metal ion in the +4 valence state with two cyclopentadienyl ligands and two halide or pseudohalide ligands. The metallocene dihalides are soluble in organic solvents without dissociating but in aqueous media undergo slow hydrolysis with release of one or both halide ions.



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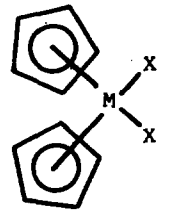

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There are three characteristics of metallocenes and metallocene dihalides that make them novel tools for the study and treatment of metabolic processes both in vivo and in vitro. First, metallocenes are small, rigid, hydrophobic molecules which can easily cross cellular membranes. Even when derivatized with polar substituents, the metallocene can still cross the membrane rapidly, where it can then perform some type of therapeutic or investigational purpose. The second characteristic of metallocenes is that the top surface closely resembles a simple aromatic ring, but the metallocenes are substantially thicker, being

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approximately as thick as they are wide. Thus, they may mimic the binding of simple aromatic compounds but block enzyme active sites because of their extra thickness. The third characteristic is the metal atom, which is bound so tightly it cannot be dislodged without completely destroying the structure and which can serve as a useful tracer, particularly when it is a metal foreign to living systems, such as Ti or Ru, or when it is radioactive.

This review summarizes current metallocene research encompassing three major themes. First, metallocene dihalides as anti-tumor agents where their size and ability to bind to DNA determines their effectiveness, and second, metallocene derivatives as radiopharmaceutical agents where their stability and derivatization are the key to their usefulness. The third theme deals with the general usage of metallocenes and their derivatives in biochemistry, microbiology and medicine [1-2].

METALLOCENE DIHALIDES AS ANTI-TUMOR AGENTS

Metallocene dihalides possess anti-tumor properties resulting principally from their action on the metabolism of DNA, and subsequently, RNA and proteins [3]. The effectiveness of metallocene dihalides as anti-tumor agents varies with the position of the metal atom in the Periodic Table (Table 1). For the d^0 atoms, Ti, Zr and Hf, there is decreasing anti-tumor activity with increasing atomic weight. Similarly, the d^1 atoms and d^2 atoms also show decreasing anti-tumor activity with increasing atomic weight [4]. Optimal activity against tumors *in vivo* leading to 100% cure rates is obtained when the central metal is Ti, V, Nb or Mo [12], indicating a diagonal relationship within the Periodic Table [4].

Titanocene dichloride (TiDC) is the most effective metallocene as an anti-tumor agent with a cure rate in the therapeutic range of over 80% [6]. A single intraperitoneal injection of TiDC at an optimum concentration of 30-60 mg/kg body weight, 24 hours post tumor transplantation (p.t.t.) into mice, achieves survival of 80-100% of the animals until day 180 p.t.t. without tumor manifestation. This is an increase in the mean survival time of 900% in reference to the untreated animal [7-9]. Other compounds of the type $(C_5H_5)_2TiX_2$ where X=F, Br, I, NCS and N_3 show similar tumor inhibiting properties [8]. This is not

surprising since all the counter-ions above can dissociate rapidly in an aqueous medium.

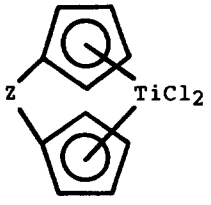
TABLE 1. RELATIVE EFFECTIVENESS OF TRANSITION METAL METALLOCENE DIHALIDES AS ANTI-TUMOR AGENTS

	d^0	d^1	d^2
GROUP	IVb	Vb	VIb
	Ti +++	V +++	--
	Zr 0	Nb +++	Mo +++
	Hf 0	Ta ++	W +

+++ cure rates in excess of 80% in vivo
++ cure rates of 25 to 80% in vivo
+ cure rates less than 25% in vivo
0 no effectiveness in vivo

Modification of the cyclopentadienyl rings of TiDC diminishes the metallocene's cancerostatic effectiveness directly in proportion to the degree of modification [8,10]. Tests in vivo show that the monosubstituted TiDC compounds of the type $(RC_5H_4)(C_5H_5)TiCl_2$, 3, where R is of ethyl or trimethylsilyl have optimal cure rates of 80% and 60%, respectively, which is slightly less than the unsubstituted TiDC. Also, tumor inhibiting activity of the 1,1'-disubstituted, $(RC_5H_4)_2TiCl_2$, 4, and 1,1'- bridged titanocene dichlorides, 5a-g, is much less than TiDC [10]. In addition to its anti-neoplastic activity, TiDC is toxic to mice when administered in doses greater than 80 mg/kg body weight [8].

Vanadocene dichloride (VDC) has anti-neoplastic properties similar to those of titanocene dichloride. At optimum doses of



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a Z = $(CH_2)_3$
b Z = CH_2
c Z = $CH(CH_3)$
d Z = $SiHCH_3$
e Z = $Si(CH_3)_2$
f Z = $Si(C_2H_5)_2$
g Z = $Ge(CH_3)_2$

80-90 mg/ml body weight VDC causes significant increase, exceeding 125%, in the life span of animals bearing lymphoid leukemias L1210 and P388 [8]. VDC supresses cellular growth of Ehrlich ascites tumors in vitro in 100-fold lower concentrations (5 μM) than does TiDC (500 μM) [11-12].

When the lissamine green dye exclusion test was used during in vitro studies to differentiate living cells from dead cells, it was found that 25% of the cells were irreversibly damaged after administration of 50 μM TiDC and 15% after administration of 1 μM VDC. These concentrations are below those where growth is inhibited significantly. The proportions of dead cells rises when the highest concentration of metallocene dihalide is applied and when the interval after removing the cytotoxic agent is increased. For example, 80% of the cells were dead three days after treatment with a 100-fold excess (5 mM) of TiDC, as were 95% of those treated with 100 μM VDC [12].

Administration of niobocene (NbDC) to mice bearing Ehrlich ascites tumors achieves 100% cure rates with doses of 20-25 mg/kg body weight [4,5] but major side effects are observed. Air oxidation of NbDC changes the niobium from the +4 valence state (paramagnetic) to the +5 (diamagnetic) state to yield the complex $[(C_5H_5)_2NbCl_2]_2O$. This complex has reduced toxicity, particularly in causing hemorrhages, as well as reduced tumor inhibiting properties. Higher doses of 35-40 mg/kg body weight in this case are necessary to achieve the maximum cure rate of 67% [4].

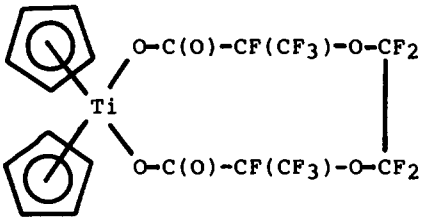
Molybdenocene dichloride (MoDC) is also an effective antitumor agent. Administration of MoDC to mice bearing Ehrlich ascites tumors at a dosage of 75-100 mg/kg body weight 24 hours after transplantation of the tumor achieved 100% tumor inhibition until day 30 p.t.t. [13] and 100% cure rates [5,12]. A concentration of 1 mM slows cell growth, and a concentration of 5 mM stops cell growth in vitro [12].

Tungstocene dichloride (WDC) and tantalocene dichloride (TaDC) exhibit sporadic tumor inhibition both in vivo [4] and in vitro [12]. In the therapeutic dose range of 100-125 mg/kg body weight, less than 10% of the animals were cured after treatment with WDC. Hafnocene dichloride (HfDC) and zirconocene dichloride (ZrDC) have no antineoplastic properties in vivo [7].

One non-halide metallocene derivative, 6, however, has been

synthesized which has exhibited anti-tumor activity using standard oncostatic tests [14].

6



The cytostatic effect of the metallocene dihalides may be due to their ability to interact with the DNA of the tumor cell [3], probably similar to the intrastrand and interstrand cross-linking abilities postulated for cis-dichlorodiammineplatinum (II) (cisplatin), 7. The chloride ion concentration in the blood

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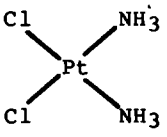


is 103 mM, but inside the cell the concentration of the chloride ion is only 4 mM. Because of this concentration difference, cisplatin dissociates more readily in the cell than in the blood [15]. Also, cisplatin has the advantage that while in the blood it remains a neutral molecule and therefore can cross the cell membranes via a passive diffusion mechanism [16,17]; charged molecules, on the other hand, require a carrier-mediated transport [18].

Once inside the cell, the chloride ligands dissociate from the complex and the Pt atom then can bind to any electron-rich species present. Particularly important consequences results when the Pt-containing species attacks DNA. Although some attack on the phosphate groups occurs [19], U.V.-visible spectroscopy [20,21], nuclear magnetic resonance [22,23] and X-ray photoelectron spectroscopy [24] have shown that the major sites of cisplatin interactions are interstrand and intrastrand chelation between adenine, guanine and cytosine bases of DNA [25-30]. When intrastrand cross-links form, the length of the DNA strand is decreased [27,31-33]. The normal distance between adjacent guanosines in DNA is 3.4 A; bound to cisplatin this distance becomes 2.9 A [34].

The interstrand and intrastrand cross-linking of DNA results in template inactivation of DNA and accumulation of potentially lethal damage [26]. The preferential toxicity of cisplatin in tumor cells is usually attributed to the fact that tumor cells reproduce more rapidly, and subsequently, replicate DNA more rapidly [35]. However, tumor cells are deficient in DNA repair mechanisms and, hence, are more sensititve to mutations than are normal cells [36,37].

Metallocene dihalides may produce their anti-tumor effects by a mechanism similar to that of cisplatin [5]. They possess a soft, acidic cation with reactivity similar to Pt(II), two strongly electron donating carrier ligands cis to one another and a pair of labile ligands, preferably chlorides. The ability to form chelates with DNA depends on the non-bonding Cl-Cl distance or "bite" [12] which is related to metal-chlorine bond lengths and bond angles. Table 2 gives the atomic radii of the central metal ions and their "bite". The effective anti-tumor agents, cisplatin, TDC, VDC, MoDC and NbDC have "bites" between 3.24 and 3.47 A, close to the 3.4 A distance between adjacent guanosines in DNA; ZrDC and HfDC are poor anti-tumor agents since their "bites" are too large to allow cross-linking.

TABLE 2. RELATIONSHIP BETWEEN THE ATOMIC RADIUS OF THE CENTRAL METAL ATOM AND THE "BITE" OF THE METALLOCENE DICHLORIDE.

Element	Atomic Radius (A)	Metallocene	"Bite" (A)	References
Pt	1.30	cisplatin	3.35	4, 12
Ti	1.32	TiDC	3.47	4, 12
V	1.22	VDC	3.30	12
Mo	1.30	MoDC	3.24	4, 12
Nb	1.34	NbDC	3.36	4
Zr	1.45	ZrDC	3.66	12
Hf	1.44	HfDC	3.60	12

METALLOCENES AS RADIOPHARMACEUTICAL AGENTS

Radioactive metallocenes may have diagnostic applications in nuclear medicine. Some important variables of radiopharmaceutical agents are the physical characteristics of the radionuclide, such as its energy, half-life and types of particles emmited, and the chemical nature of the compound. Ruthenocene derivatives containing [^{97}Ru] or [^{103}Ru] offer the desirable stable combination of a γ-emitter tightly bound to an aromatic organic moiety capable of considerable variation, even to the extent of imparting organ specificity to the compound (Table 3) [38].

A simple one-step process is used to synthesize radioactive substituted metallocenes (Scheme 1). Substituted ferrocenes are heated with radioactive Ru and Os halides to give the corresponding radioactive metallocenes [39-41] via (π-ligand)-(π-ligand) exchange [42]. The reaction is achieved by heating an intimate mixture of a ferrocene derivative and crystalline [^{103}Ru]-$RuCl_3$ above the melting point of the ferrocene derivative. The monosubstituted ruthenocene, 9a-f, is the principal reaction product and the 1,1'-disubstituted ruthenocenes, 10a-f, are obtained as by-products [42a]. Several other labelled metallocene complexes sythesized by this manner were studied extensively including [^{97}Ru]-acetylruthenocene [39],

SCHEME 1. REACTION SCHEME FOR THE SYNTHESIS OF MONOSUBSTITUTED RUTHENOCENES.

$$3\ (C_5H_5)Fe(C_5H_5R) + 3\ [Ru^*]Cl_3 \longrightarrow$$

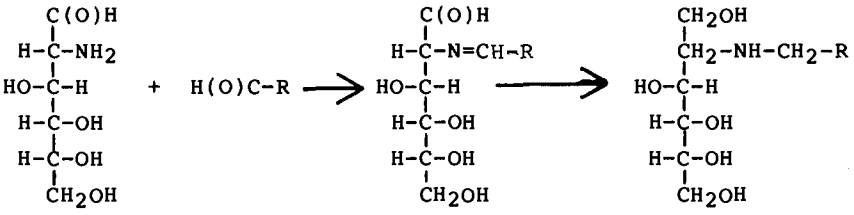
$$[Ru^*](C_5H_5)_2 + (C_5H_5)[Ru^*](C_5H_5R) + (C_5H_5R)_2[Ru^*] + 3\ FeCl_3$$

8 9 10

a R = $-C(O)C_6H_5$ [Ru*] = [^{97}Ru] or [^{103}Ru]
b R = $-C(O)CH_3$
c R = $-C(O)OCH_3$
d R = $-C(O)NH_2$
e R = $-SO_2NH_2$
f R = $-CH{=}CHC(O)CH_3$

[^{103}Ru]-ruthenocene esters of estradiol 17-β and estrone [43,44], [^{103}Ru]-2-chlorobenzoylruthenocene [44] and [^{191}Os]-acetyl-osmocene [45].

It is also possible to change the substituent on the cyclopentadienyl ring once it has been bound to the radioactive metal. Glucosamine, 11, reacts with ferrocene carboxaldehyde or ruthenocene carboxaldehyde to yield the corresponding Schiff's base, 12. This is reduced at the C=N bond and also at the aldehyde functionality of the sugar moiety, 13 [46]. Similar derivatives have been prepared with galactosamine, 14, and mannosamine, 15.



11 12 13

R = Fc or Rc

Fc = $(C_5H_5)_2Fe$

Rc = $(C_5H_5)_2Ru$

14 15

Many of the metallocene complexes demonstrate organ specificity which is principally determined by the substituents on the cyclopentadienyl moiety (Table 3). Also, organ specificity is partly determined by the method of administration and the drug treatment history of the individual. For example, the accumulation of [^{103}Ru]-cinnamoyl ruthenocene following an intravenous injection is highest in the liver, lungs and spleen. Intraperitoneal injection of this complex, however, leads to its accu-

mulation in the thymus gland [39,47]. The affinity of this complex for the thymus can be diminished by corticoid pretreatment and increased by inhibition of phagocytosis [48]. This compound, however, was also found to accumulate to a significant extent in both the liver and lungs, but this probably only represents the metabolism of the compound and not a specific target organ [47]. Ferrocene vapor containing [^{59}Fe], administered by inhalation, has an affinity for the upper respiratory tract and the lungs and is tenaciously retained in the nasal and pulmonary areas [49].

METABOLISM OF METALLOCENES

Metallocene complexes are metabolized and excreted from the bodies of mice and rats in a manner similar to that of various aromatic compounds by hydroxylation of the aromatic moieties followed by glucuronide or sulfate conjugation and its subsequent excretion in the bile and urine. The detoxification reaction primarily occurs inside the liver microsomes via the cytochrome P-450 system. This process involves the hydroxylation of the aromatic moieties using molecular oxygen and reducing equivalents in the form of NADPH. The hydroxylated product is usually conjugated with either a molecule of glucuronic acid from UDP-glucuronate (UDP = uridine diphosphate) or sulfatolyzed by transfer of an active sulfate from 3'-phosphoadenosine-5'-phosphosulfate, resulting in a highly polar water soluble compound, excretable in the bile and urine. This process was demonstrated to be P-450 linked for metallocenes by studying the metabolism of [^{59}Fe]-ferrocene [62]. By pretreating laboratory animals with phenobarbital, a drug known to induce the P-450 enzymes, a sevenfold stimulation of the metabolism of ferrocene was obtained. Ferrocene metabolism is inhibited by CO which competes with O_2 for binding to the heme center of the cytochrome P-450 system [62].

The hydroxylation of the metallocenes accounts for the instability of the normally stable metallocene (i.e. ferrocene) in vivo, since hydroxymetallocenes are unstable in air. Hydroxylation of the metallocene is followed by partial degradation, liberating Fe^{+2}, and by partial conjugation to the stable O-glucuronide [38,63-66]. The ferrous ion is recovered and stored in the ferric form as complexes with ferritin and hemosiderin.

TABLE 3. CHARACTERISTIC ORGAN SPECIFICITIES OF METALLOCENE DERIVATIVES AS RADIOPHARMACEUTICAL AGENTS

Metallocene Derivative	Administration Route *	Primary Target Organ	Excretion Conjugate	References
Acetyl	PO, IV	Adrenals Kidneys	Glucuronide	45, 50
Carboxylate	IP	Kidneys	NR **	53-57
Carboxaldehyde	IP	Kidneys	NR	
Cinnamoyl-Rc ***	IV	Liver, Lungs Spleen	NR	39-47
Cinnamoyl-Rc	IP	Thymus	NR	39, 47, 48
Estrogen Esters of Carboxylates	IP	Adrenals	NR	43, 44, 59
Ferrocene	IH	Lungs	Glucuronide	49
Hexosamine	SQ	Kidneys Liver	Hexosamine	60

Hydroxyacetyl	IV	Adrenals Kidneys	NR	51
Hydrazone	IV	Lungs	NR	61
Hydroxyacetyl Glucuronide	IV	Liver Kidneys	NR	51 , 52

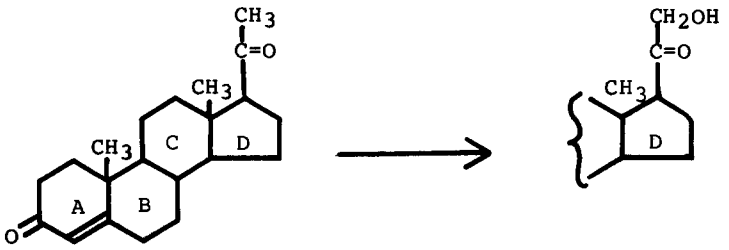
* IV - Intravenous
IP - Intraperitoneal
IH - Inhalation
PO - Orally
SQ - Subcutaneously

** NR - The metabolism and excretion of the metallocene derivative has not been reported in the literature. However, most metallocenes are detoxified by the liver microsomal P-450 system and usually excreted as the glucuronide or sulfate conjugates.

*** Rc - Ruthenocene

The fate of free Ru and Os liberated during metabolism of ruthenocene and osmocene, respectively, is unknown, but most of the [^{103}Ru] is excreted within 24-48 hours [38].

Since ruthenocene is metabolized by the same paths as aromatic compounds such as benzene and phenol, it was expected that acetylruthenocene, like acetophenone, would be detoxified and excreted as a glucuronide or sulfate conjugate after first reduction of the keto group. A second, minor pathway exists in which a hippuric acid conjugate is formed after oxidation and decarboxylation of the methyl group [45,67]. However, the extra bulk of the metallocene "sandwich" prevents reduction of the keto group. Instead, the metabolism of acetylruthenocene proceeds by oxidation of the methyl group to a primary alcohol followed by conjugation with glucuronic acid [51], inactivating any possible toxic side-effects of the metallocene and facilitating its removal from the body. This pathway is similar to the method of degradation of progesterone, 16, which is oxidized, 17, conjugated with glucuronic acid and excreted.



16 17

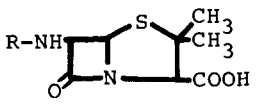
From the above discussion it is not surprising that metallocenes and their derivatives exhibit specific organ affinities. Metallocenes derivatized with polar R groups, irrespective of the method of administration, will accumulate in both the liver and kidneys since both or these organs are involved in the excretion of metabolic by-products either through the bile, such as is bilirubin diglucuronide, or in the urine as water soluble compounds. Similarly, hydrophobic or acetyl R groups will accumulate in the adrenal glands, probably because of this organ's ability to detoxify acetylated steroid hormones.

Derivatization of the cyclopentadienyl ligands of metallocenes, for the most part,explains the various organ specificities

of the metallocene derivatives. However, there are inconsistencies with this hypothesis. In the case where acetylruthenocene will accumulate in the adrenal glands, [^{191}Os]-acetylosmocene has no affinity for the adrenal glands [45]. From this, it would appear that the central metal may be involved in this selection process. The change in binding may result from a change in the size and shape of the molecule as a whole rather than from a specific effect of either metallocene or side chain [1a,1b,3]. Therefore, more study is required before a positive correlation between the role of the central metal atom and organ specficity can be firmly established.

CELLULAR APPLICATIONS OF METALLOCENES

Metallocene derivatives have proven effective as agents to modify antibiotics. Antibiotic-resistant strains of bacteria are becoming increasingly prevalent and pose a serious problem to the treatment of bacterial infections. These bacteria produce β-lactamases which degrade penicillin and cepalosporin antibiotics into inactive compounds. Edwards and co-workers [68-72] have developed a series of ferrocenyl-penicillins, 18c-f, and ferrocenyl-cephalosporins, 19c-f, as β-lactamase inhibitors in which the usual phenyl, 18a, (benzyl penicillin or Penicillin G) or heteroaromatic, 19b, (cephalothin) group has been replaced by the ferrocene moiety. The structural features which make these classes of antimicrobial agents unique are that a metal atom is placed in close proximity to the β-lactam ring and, more impor-



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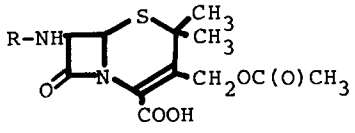

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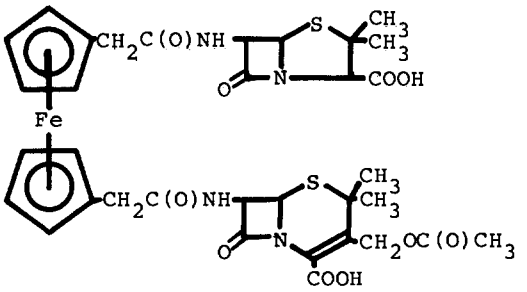
a R = C_6H_5-CH_2-C(O)-
b R = $C_4H_3SCH_2CO$-
c R = Fc-C(O)-
d R = Fc-CH_2-C(O)-
e R = Fc-$CH(CH_3)$C(O)-
f R = Fc-$C(CH_3)_2$C(O)-

tantly, that the second cyclopentadiene ring modifies the compound in the third dimension without disrupting the molecular profile necessary for antimicrobial activity [68-72].

The ferrocenyl-penicillin, 18a-f, and cephalosporin, 19c-f, derivatives [68] were tested in vitro for antibiotic activity against a variety of bacteria using Penicillin G, 18a, or methicillin as controls for the penicillins and cephalothin, 19b, for the cephalosporins. When the penicillin and cephalosporins were derivatized with only the ferrocenoyl moiety, 18c, 19c, no antibiotic activity was observed. This was attributed to the steric hindrance of the bulky ferrocenyl moiety being too close to the β-lactam ring. In contrast, when the ferrocenyl moiety is displaced further away from the β-lactam ring by the addition of a methylene group, 18d, 19d [68], high activities equivalent to control values were obtained. Substitution of the methylene hydrogens with methyl groups decreased activity with increasing substitution. The ferrocenyl-penicillins, and to a lesser extent, the ferrocenyl-cephalosporins proved to be effective as β-lactamase inhibitors. The effectiveness increased with increasing substitution of the α-ferrocenyl carbon atom with the dimethyl compound, 18f, having the greatest inhibitory effect. Thus, in this series, good antibiotics are poor β-lactamase inhibitors. An additional unique compound having antibiotic activity combines both the penicillin and cephalosporin pharmacophores in an α,α'-ferrocenyl linkage, 20, [69].



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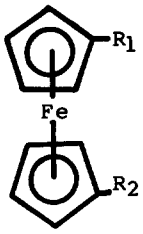
Several other ferrocene derivatives, 21a-e, containing two or three carbon acyl side chains or derivatives thereof, but not penicillin or cephalosporin groups, have been shown to exhibit

antimicrobial activity against a variety of bacteria, yeasts and fungi [73].



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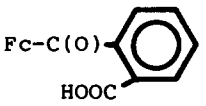
	R_1	R_2
a	-C(O)CH=CHCl	-H
b	-C(O)C≡CH	-H
c	-CCl=CH-CHO	-H
d	$-C(O)CH_2CH_2Cl$	$-C(O)CH_2CH_2Cl$
e	-C(O)CH=CHPh	$-C(O)CH_2Cl$

In addition to possible in vivo treatment of bacterial infections, the ferrocene derivative, o-carboxybenzoyl ferrocene, 22, has proven effective in the treatment of the gum diseases Parodontosis dystropica, Parodontitis superificialis and Parodontitis profunda. This ferrocene derivative appears to stimulate the activity of certain groups of enzymes taking part in tissue metabolism (the enzymes directly effected were not adequately described by the authors of this paper). This compound is a beneficial therapeutic agent since, when it is taken orally, it is easily tolerated by patients suffering from oral cavity, as well as gastro-intestinal, diseases [74].

22



Also, in the realm of metallocenes as therapeutic agents, a series of ferrocenylpolyamines were synthesized for the possible chemoimmunotherapy of cancer. It was discovered by Rosenberg [75] and his co-workers [76] that nucleic acids were present on the surface of tumor cells and not on the surface of normal cells. To exploit this phenomenon, a series of polyamines were synthesized to bind to the nucleic acids, since naturally occuring polyamines are involved in the binding to DNA [77], to elicit an antibody response [78,79]. It was the hope that these compounds would have advantages over conventional antineoplastic agents, including metallocene dihalides, which need to be administered in high, and often toxic, doses to interfere with cellu-

lar processes.

To the polyamine, an insulating group was connected, usually $(CH_2)_n$ where $n \leq 4$, and a hapten. The hapten choosen was ferrocene, due to its ability to elicit a strong antigenic response when bound to polypeptides [80,121]. When these compounds were screened for antitumor activity against lymphocytic leukemia P-388, administered via intraperitoneal injection as the hydrobromide salt, all were inactive and toxic in doses greater than 12.5 mg/kg body weight. The free base of the polyamine $Fc-(CH_2)_4-NH-(CH_2)_4-NH_2$ showed a decrease in toxicity (25 mg/kg) but still no anti-tumor activity. Suprisingly, though, the di-, tri- and tetraamide precursors of the compound exhibited low, yet significant antitumor acitvity [80].

METALLOCENES IN HISTOLOGY AND IMMUNOLOGY

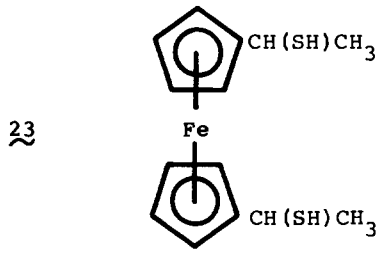
Metallocenes have also been useful tools for histochemical and immunochemical analyses. Commonly employed immunochemical methods used to visualize surface cellular antigenic determinants include treatment of the sample with ferritin (a 440 kilodalton iron-containing protein) labelled antibodies for use in electron microscopy [81] and the peroxidase method [82]. When conjugated with a 40 kilodalton dextran, ferrocene has been helpful in the indirect identification of lectin receptors via electron microscopy. The ferritin and peroxidase methods are at a disadvantage, though, to identify intracellular structure, since they are large molecules and cannot traverse the cell membrane unless the cell is first treated with disrupting agents [84,85] or subjected to enzymatic digestion of membrane proteins [86], creating holes in the membrane, thus allowing the compounds to enter more easily. These methods, however, lead to shape distortion of the cell. In order to visualize cytoplasmic actin, Tiggemann and Govidan [87] were able to label the Fab-fragment of an anti-actin antibody with ferrocene monocarboxylic acid (FMCA). This method of identifying intracellular structures via electron microscopy was superior since, first, the coupling process did not influence the binding capacity of the conjugate, and second, they were able to identify intracellular antigens without background effects using a procedure that did not result in the destruction of the cellular ultrastructure.

Non-isotopic metalloimmunoassays have been developed by Cais

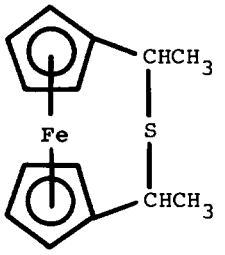
[88] and co-workers [89] that employ metal atoms, in the form of their organometallic or coordination complexes (Fe and ferrocene, respectively, for this example), as a method for the quantitiation of both antigens and antibodies. This method is a modification of the precipitin reaction [90] and is performed by allowing a known amount of FMCA-labelled antigen or antibody to react with its corresponding antibody or antigen, respectively. After precipitation is completed the solid is collected, washed free of inert materials, and the amount of antigen or antibody is quantitated by determining its Fe content by atomic absorption spectroscopy.

IN VIVO APPLICATIONS OF METALLOCENES

Metallocenes, particularly ferrocene, have been investigated in man and several other species [91-94] and display a variety of effects. Due to their lipophilic nature they have been valuable tools in studying the absorption, distribution and utilization of iron. The iron was administered as compound, 23 or 24, through the gastrointestinal tract where there is less mucosal regulation of Fe^{+2} when it is administered as a hydrophobic compound than as a ferrous salt [2,95-96a]. Upon metabolism of the hydrocarbon moieties, the Fe^{+2} is stored primarily in the liver for later use, particularly for hemoglobin and cytochrome synthesis.



23



24

Other interesting observations in the liver were also made using ferrocene and its derivatives. Neopentyl-ferrocene, 25, led to liver enlargement one week after a single massive dosage; however, no changes in liver function were observed [93]. The chronic oral administration of high levels of ferrocene to dogs resulted in cirrhosis of the liver, a reversible decrease in hemoglobin, erythrocyte count, packed cell volume and testicu-

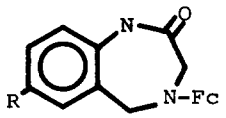
lar hypoplasia [94]. The hepatotoxicity was attributed to the hydrocarbon moiety of the metallocene [94]. There have been two reports in which a metallocene of undisclosed structure was shown to increase liver cell metabolism in rats [97] and where 1,1'-diacetylferrocene, 26, stimulated liver regeneration in partially hepatectomized rats [3]. In contrast to the properties of the latter compound, ferrocene and acetylferrocene were ineffective in promoting liver regeneration [3]. Also, ferrocene has been shown to inhibit the growth of Chinese Hamster Ovary cells and the compound 1-ferrocenyl-1-phenyl-4-methyl-1,4-dihydroxy-pent-2-yne, 27, inhibits the growth of cultured chick embryo cells. The latter effect, though, has been associated with the anticarcinogenic activity of the molecule [98]. Therapeutic use of ferrocene to correct iron deficiency anemias would require long periods of time to replenish iron storage. However, failure of regulation of Fe^{+2} absorption is of toxicological concern since normally little Fe^{+2} is excreted. The testicular hypoplasia mentioned above was apparently a manifestation of prolonged iron overload [100].

25 $Fc-CH_2-C(CH_3)_2-CH_3$

26 $[CH_3C(O)C_5H_4]_2Fe$

27 $Fc-C(C_6H_5)(OH)-C\equiv C-C(OH)(CH_3)-CH_3$

Ferrocene complexes of benzodiazopine have been tested for activity as muscle relaxants and anticonvulsants in mice. The 2-hydro derivative of 1,3-dihydro-5-ferrocenyl-1-methyl-1,4-benzodiazepine-2-one, 28a, was, ineffective as a tranquilizer. Similarly, the 2-iodo derivative, 28b, which is reported to enhance the activity of benzodiazapines, was also ineffective [100].



28 a R = H
b R = I

Metallocenes and metallocene dichlorides have also been shown to effect the metabolism of certain drugs. Paralysis induced in rats by the drug zaxozalamine (2-amino-5-chlorobenzoxazole) was prolonged by injection of NiDC, TDC, VDC, HfDC, ZrDC, 1-dimethylaminomethyl ferrocene, 29, 1-butyroyl ferrocene, 30, and 1-salicoyl ferrocene, 31. This is probably a result of competition for the detoxification system of the P-450 system between the drug and the metallocene as discussed previously [101].

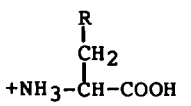
29 $Fc-CH_2-N(CH_3)_2$

30 $Fc-C(O)CH_2CH_2CH_3$

31 

METALLOCENES AS ENZYME INHIBITORS

Since metallocene derivatives were shown to inhibit certain metabolizing enzymes, they offer a unique method of enzyme active site studies and receptor site studies in three-dimensions rather than in two-dimensions as is usually done by adding substituents to a soluble substrate. For example, β-ferrocenylalanine, 32a, due to its structural similarity to phenylalanine, 32b, was employed to study the aromatic hydroxylation reaction of phenylalanine hydroxylase and phenylalanine decarboxylase and also its ability to support growth of phenylalanine requiring bacteria in the absence of phenylalanine. Although this compound does not support growth of the organism in the absence of phenylalanine, it is a non-competitive inhibitor with respect to L-phenylalanine and a mixed inhibitor with respect to $DMPH_4$ in the phenylalanine hydroxylase system. β-Ferrocenylalanine is a competitive inhibitor of aromatic L-amino acid decarboxylases with respect to phenylalanine [64,102]. Similarly, ferrocenylcholine very effectively inhibits the in vitro hydrolysis of butyrylcholine by Horse Serum butyrylcholinesterase with a $K_i = 9.63 \times 10^{-8}$ M [103].



32 a R = Fc-

b R = C_6H_5-

In addition to their effectiveness as enzyme inhibitors metallocenes and metallocene-like compounds offer an additional

approach in studying the incorporation of extrinsic metals into proteins [106-113]. It has been estimated that one-fourth to one-third of all proteins known either contain metals or require metals for their biological activity [114]. The metallocenes offer a variety of reactivities and spectral characteristics, principally due to the different ring systems, ligands and metals available, for the study of metallo-proteins and other biological macromolecules.

Ferrocene may be oxidized to the ferricinium cation, $(C_5H_5)_2Fe^+$, by horseradish peroxidase in the presence of hydrogen peroxide [114a]. The uncatalyzed reaction of ferrocene with hydrogen peroxide proceeds extremely slowly. The hydrogen peroxide may be replaced by the combination of glucose oxidase and glucose, which readily generate hydrogen peroxide. It is also possible that other oxidase enzymes may attack ferrocene derivatives but no other examples have been reported.

METALLOCENES AS CATALYSTS

A new and exciting use of metallocenes is in their use as catalysts in synthetic enzymes [115,116]. Basically, the synthetic enzyme consists of a synthetic molecule, termed a cavitand [117], with a cavity containing the catalyst capable of binding substrate. Breslow and his colleagues [118] have observed the greatest rate increase thus far. They achieved catalytic activity in the hydrolysis of m-tert-butylphenyl acetate by the attachment of the catalytic p-nitrophenyl ester of ferrocene acrylic acid to β-cyclodextrin, a doughnut shaped molecule consisting of seven glucose molecules. The catalyst is sterically constrained in that the acrylic side chain is incorporated into a fused ring system and by attaching the ester to the ring through a double bond. This modification holds the ester more rigidly near the catalytic hydroxyl of the cavitand with the reaction proceeding 5.9×10^6 faster than the uncatalyzed reaction.

CONCLUDING REMARKS

Although there may be other uses of metallocenes in the biosciences which have not been mentioned, the purpose of this review is to gather together the preliminary results available in several major areas in the hope of persuading organometallic chemists to think in biochemical terms and biochemists to realize

that organo-transition metal compounds may have important applications in their field.

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