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Bioorganometallic Chemistry – the early years.

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Abstract: Bioorganometallic chemistry is now a maturing field attracting researchers around the world, and also across the Periodic Table. It has found applications in a broad range of areas such as drug discovery, biocatalysis and imaging. We review here the first experiments carried out in the early 1980's at the Ecole Nationale Supérieure de Chimie de Paris under the leadership of Gérard Jaouen, a key figure and visionary in the development of this theme. However, they also required important contributions from Canadian collaborators with expertise in NMR and IR spectroscopy, together with the availability of more powerful and versatile instrumentation. This collaboration found its fulfillment in the demonstration of the use of metal carbonyl complexes (exemplified by an estradiol derivative labeled by a Cr(CO)₃ unit) as non-isotopic tracers allowing the detection of estradiol receptors, an essential marker for setting up a targeted therapy for breast cancer. This approach was based on the premise that the intense metal carbonyl stretching vibrations are found in a window (2100-1850 cm⁻¹) where the protein itself does not absorb. The field was extended to the arena of non-isotopic immunoassays, including the simultaneous detection of multiple antiepileptic drugs bearing metal carbonyl labels. More recently, taking advantage of the new technique of atomic force microscopy-infrared spectroscopy (AFM-IR) it has been possible to accomplish direct tracing of these complexes in cells. The contributions of the original group of workers in the field are highlighted, and placed in the perspective of today's subsequent emphasis on the management of previously untreatable cancers.

Keywords: Metal carbonyls, organometallic tracers, FT-IR spectroscopy, 2D NMR, estrogen receptor, immunoassay,

Dedicated to Gérard Jaouen in recognition of his many seminal contributions to the field.

1. Introduction

Bioorganometallic chemistry is now a well-established field with a large world-wide assemblage of research groups, a continuous flow of publications in major journals and an increasing number of textbooks [1–6] and reviews [7–13]. This was not the case almost four decades ago when the neologism *bioorganometallic chemistry* first appeared in the literature [14]. In a conscious decision to make a break from the already heavily studied medicinal properties of transition metal coordination complexes, as typified by Rosenberg's seminal discovery of the anticancer activity of cisplatin [15], a program was initiated to probe the behaviour of bioactive systems containing direct sigma- or pi-bonded metal-carbon linkages, i.e. organometallic compounds.

It was immediately recognized that a number of potential problems would have to be overcome: for example, would the molecules be unstable, insoluble in water, or too large to pass through cell membranes? Eventually however, despite the lack of funding initially, these difficulties were successfully surmounted and, as the participants in the latest ISBOMC that was held recently in Paris were able to demonstrate, our increasingly sophisticated understanding of the subtleties of the field has led to major advances towards their further applications in medicine. We note, for example, that the use of ferroquine as an antimalarial drug is currently in late-stage clinical trials [16].

A comprehensive review of the founding principles, and subsequent development of bioorganometallic chemistry, which also included the previous studies of Ehrlich et al. on organoarsenicals, and the Cytochrome P450 metabolism studies of organotin compounds by Fish et al.[17,18], appeared in 2005 [19]. However, in this review, we have focused on the first few years of the Ecole Nationale Supérieure de Chimie bioorganometallic chemistry project, when its evident success was less predictable. The initial experiments were apparently somewhat more prosaic, but exemplified the approach that led ultimately to the subsequent breakthroughs.

The genesis of this theme arose in 1983 from the creation at the Ecole Nationale Supérieure de Chimie in Paris of a team of three researchers with complementary skills (Gérard Jaouen, Siden Top and Anne Vessières) who had chosen to work on a multidisciplinary subject linking organometallic chemistry to biology. Gérard Jaouen was a well-recognized researcher in organometallic chemistry for having shown how the temporary complexation of an aromatic ring by a $\text{Cr}(\text{CO})_3$ fragment could be used for innovative regio-

and stereo-specific functionalizations [20,21]. Siden Top was a young post-doctoral researcher whose exceptional talents as a synthetic chemist would soon prove essential to the development of the project. Anne Vessières had just completed a biology thesis in the field of estrogen receptors after having defended a thesis in organometallic chemistry a few years earlier; this enabled her to make the bridge between the two disciplines. Two Canadian researchers quickly proved essential to the successful development of this new theme: Michael McGlinchey, then at McMaster University with a background in organic and organometallic synthesis and NMR, and Ian Butler from McGill University, a specialist in infrared and Raman spectroscopy who encouraged the Paris group to take advantage of the newly developed technique of Fourier transform infrared (FT-IR) spectroscopy available in his laboratory, but not in France at that time.

The first research topic addressed was a feasibility study of the use of a chromium carbonyl complex derived from estradiol as a non-isotopic tracer for the detection, by FT-IR spectroscopy, of estradiol receptors in target tissues (uterus). Gratifyingly, four publications relating to this work established the feasibility of this approach [14,22–24]. But why did we choose such a subject? Knowing that 1 in 8 women in the western world (or developed countries) are likely to develop breast cancer, a new method of assaying the presence of estrogen receptors in breast tumors prior to the use of tamoxifen for the treatment of hormone-dependent cancers was sought.

The standard approach involved the use of tritiated estradiol and the usual caveats associated with radiation, (storage, disposal, etc.), and the new idea was to use the natural hormone 17β -estradiol (E_2), **1**, bearing an organometallic fragment detectable by infrared spectroscopy. In the spectra of metal carbonyl complexes, the $\nu(\text{CO})$ stretching vibrations are by far the most intense peaks and, moreover, they lie in the region $2100\text{--}1850\text{ cm}^{-1}$ where proteins have a clear window. Such an approach only became viable with the commercial availability of Fourier-transform (FTIR) spectrometers with their manyfold enhancement in sensitivity and enormously lowered detection limits

The idea of using metal carbonyls for the development of non-isotopic tracers had previously been put forward in 1978 by Michael Cais from the Technion in Israel for carrying out immunoassays [25], but there were no convenient methods allowing one to detect these tracers at the low (nanomolar) concentrations required in these analyses. We considered that it was possible to assemble a group with all the necessary skills to bring a new approach to

this field of non-isotopic tracers applied to an important societal problem, the determination of the hormone-dependent or hormone-independent nature of breast tumors.

2. Synthetic and NMR spectroscopic aspects

The first approach involved the π -complexation of the aromatic A ring in 17β -estradiol by a tricarbonylchromium fragment in the hope that these complexes would still be recognized by the estradiol receptors, and could be used as substitutes for radioactive markers in the estradiol receptor assay. This involved overcoming many potential obstacles. Not only are the half-sandwich chromium complexes known to be photosensitive, but also these lipophilic species are not soluble in biological media consisting of aqueous solutions. It therefore required a good dose of optimism or unconsciousness to embark on such an adventure.

Steroid hormones, and estradiol in particular, are small organic molecules whose mechanism of action is linked to their interaction with a specific receptor present in the nuclei of target cells. Many studies, notably those by researchers from the pharmaceutical company Roussel-Uclaf, had been published on estradiol derivatives with the aim of obtaining molecules that best mimic the behaviour of the natural hormone [26,27]. The estradiol molecule comprises the four-ring backbone (A-D) common to all steroids, and is characterized by a phenolic A ring and an OH substituent at the 17β position (Fig. 1). The presence of the saturated 5- and 6-membered rings causes the three-dimensional structure of estradiol to deviate from planarity, whose stereochemistry is defined in terms of alpha and beta faces, whereby the 17-hydroxy and 13-methyl substituents are neighbours on the β face. The chiral nature of the molecule opens up the possibility of complexation by a $\text{Cr}(\text{CO})_3$ tripod on either face thereby giving rise to a pair of diastereomers. Most of the chemical modifications of estradiol result in a rapid loss of recognition of the molecule by its receptor, especially when one of the OH functions is modified; evidently, they are an essential component of the interactions that allow the correct positioning of the hormone within its receptor site with subsequent triggering of the hormonal effect [28].

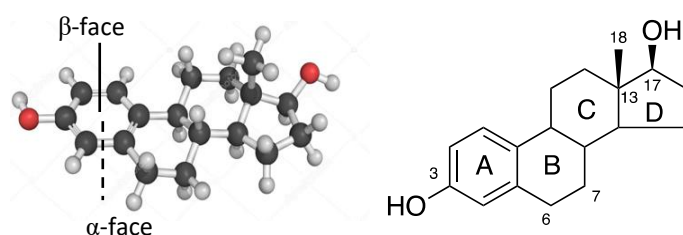
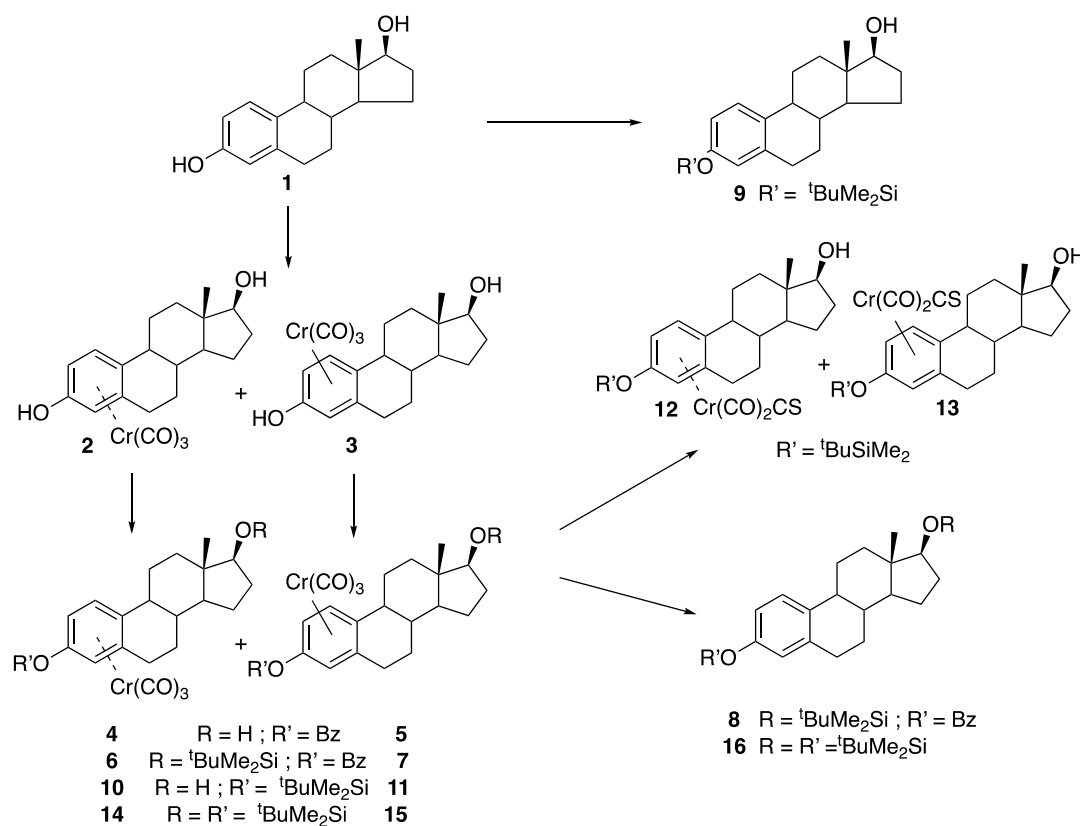


Fig. 1. Three-dimensional structure and atom numbering in 17 β -estradiol (E_2).

It should be borne in mind that the complexation of estrone by $\text{Cr}(\text{CO})_3$ and acetylergosterol by $\text{Fe}(\text{CO})_3$ was published as early as 1963 [29,30] with, on the part of the authors, a stated intention, but no actual use of these complexes for problems of biological interest. Subsequently, in 1980 the complexation of estradiol by $\text{Cr}(\text{CO})_3$ was reported by Pouskouleli and Butler [31]. They described a complex that was not very light- or thermally-stable at room temperature, and therefore not suitable for use in a biological medium; moreover, there was no mention of obtaining diastereomers arising from attachment of the $\text{Cr}(\text{CO})_3$ unit on either the α or β face of estradiol. Initially, the estradiol complexes **2** and **3** bearing a tricarbonylchromium tripod on the alpha or beta side of the molecule were prepared and characterized by NMR spectroscopy, but their stability was insufficient to allow their use in biological media. Subsequently, a series of derivatives of estradiol was prepared, each bearing a $\text{Cr}(\text{CO})_3$ moiety (or a $\text{Cr}(\text{CO})_2\text{CS}$ unit) π -bonded to the aromatic A ring, which also possessed a protected phenol function (Scheme 1). Gratifyingly, the resulting diastereomers, whereby the organometallic unit was positioned on either the α or β face of the steroid, were separable chromatographically [14].



Scheme 1. Structures of an initial series of estradiol derivatives, including molecules complexed by a $\text{Cr}(\text{CO})_3$ or $\text{Cr}(\text{CO})_2\text{CS}$ unit.

However, the question arose to their stereochemical identity as the possibility of carrying out an X-ray crystallographic study in every case would not have been practicable, even though the structure was eventually determined for **12** [24]. (We note that with today's more powerful rotating anode X-ray sources, more sensitive charge-coupled or area detectors, exponentially better computational facilities, and modern direct methods programs, one can solve several structures in a single day, but such was not the case in the early 1980s.) Consequently, during an informal discussion in Paris (Fig. 2), the possibility of using NMR spectroscopy to distinguish the α or β isomers was therefore considered.



Fig. 2. Informal discussions in Paris: from left to right, Michael McGlinchey, Siden Top, Angela Marinetti, Gérard Jaouen.

At that time, although a number of articles concerning the ^{13}C NMR spectra of steroids had been published [32], there were very few for the ^1H regime [33,34] because of severe peak overlap. However, the University of Paris had just acquired a 500 MHz spectrometer (at that time an enormously exciting prospect) and together with the newly-developed two-dimensional NMR techniques, such an approach was thought to be viable. Knowing that protons above an arene ring, as in [10]paracyclophane, are markedly shielded [35], whereas those positioned to the side of the triple bond in cyclooctyne are deshielded [36], one could envisage that the diamagnetic anisotropy of a linear $\text{C}\equiv\text{O}$ fragment would position the 6α and 6β protons of the steroid in very different environments (Fig. 3). To be specific, in the α -isomer, the 6α proton should be strongly deshielded relative to its 6β counterpart, but inversely so in the β -isomer, as indicated in Fig. 4.

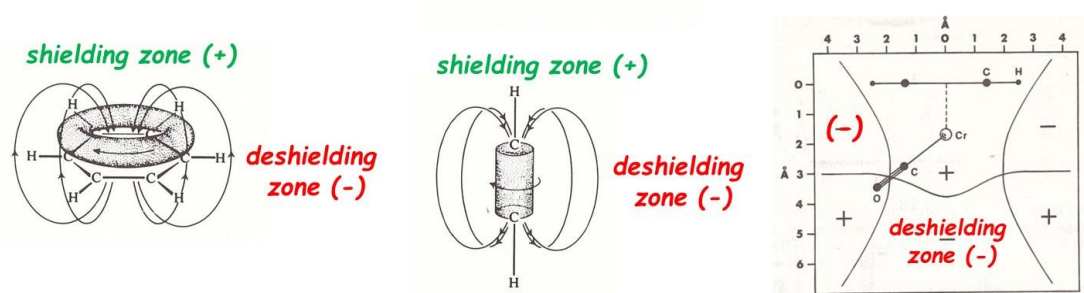


Fig. 3. NMR shielding and deshielding zones in aromatic and linear molecular systems.

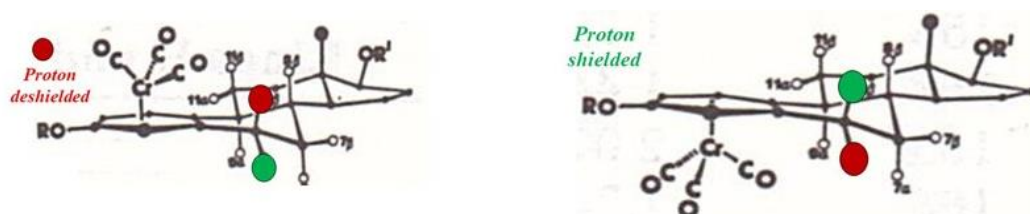


Fig. 4. Predicted shielding and deshielding of the 6α and 6β protons in the β (left) and α (right) estradiol-chromium tricarbonyl isomers.

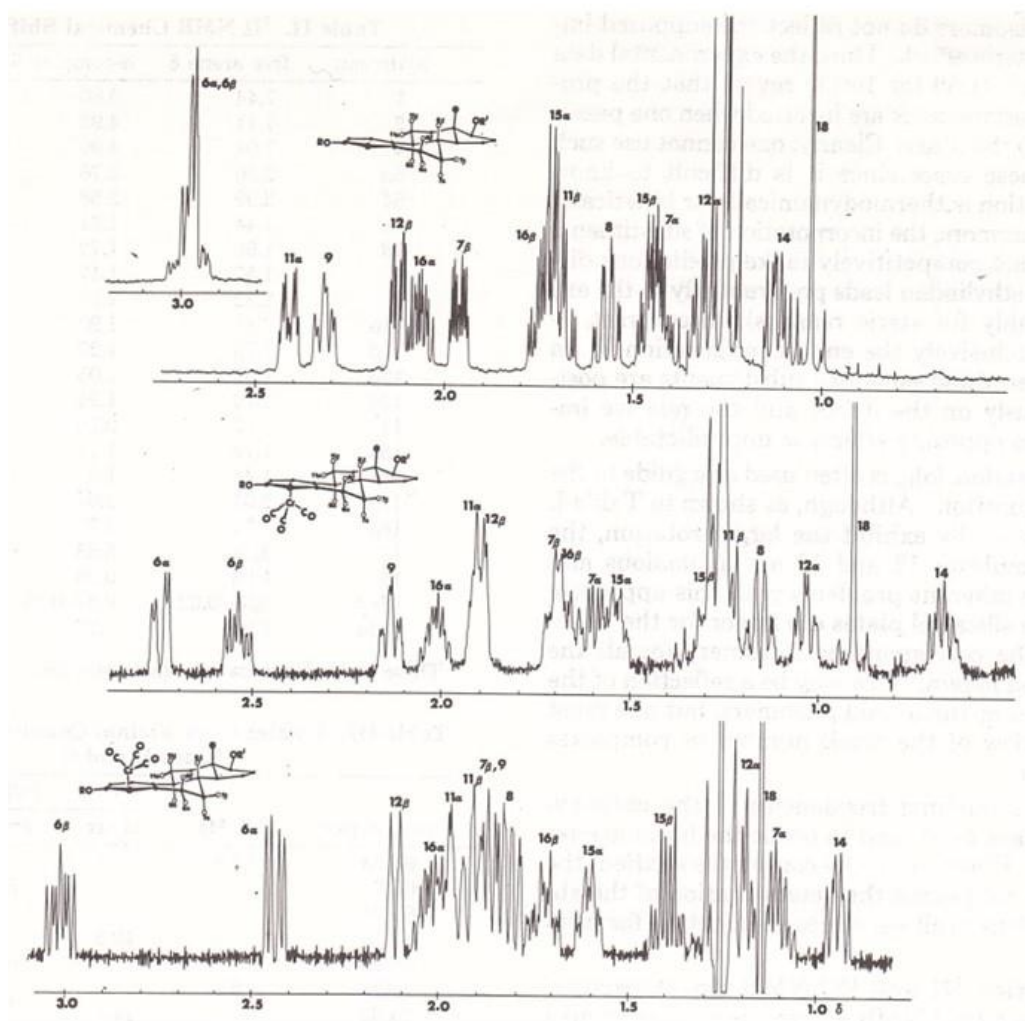
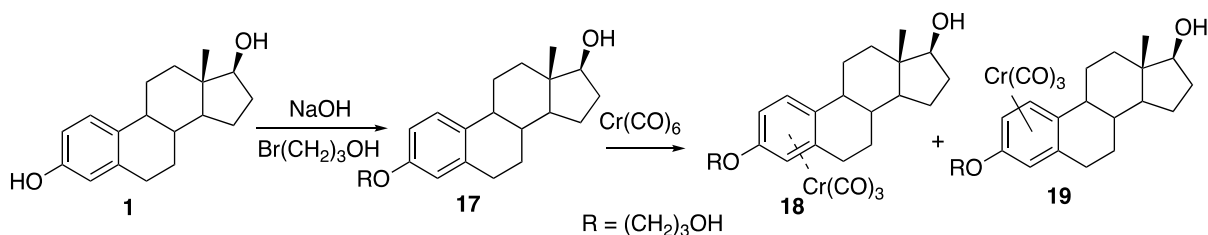


Fig. 5. 500 MHz ^1H NMR spectra of an estradiol derivative and its α and β $\text{Cr}(\text{CO})_3$ complexes ($\text{R} = (\text{CH}_2)_3\text{OH}$, $\text{R}' = \text{SiMe}_2^t\text{Bu}$).

Indeed, the ^1H - ^1H COSY and SECSY data, together with the ^1H - ^{13}C shift-correlated spectra, acquired on the new 500 MHz spectrometer allowed the complete assignment of all hydrogens and carbons in these steroidal-organometallic molecules. As shown in Fig. 5, the strikingly large separations of the 6α and 6β protons provided an unequivocal method of readily distinguishing these pairs of diastereomers. The resulting manuscript [14] was written during a visit by Gérard Jaouen and Anne Vessières to the home of MJM in Canada, during which time the term *bioorganometallic chemistry* was coined and first used in a journal submission.

This approach made it possible to differentiate readily between alpha and beta complexes. However, these complexes lack an OH group at C3, which is known to be essential for the recognition of estradiol molecules by the receptor site. Since complexes **2** and **3** were not stable, the complexes **18** and **19** were prepared in which the OH function is no longer phenolic, but rather is separated from the aromatic ring by a propyl chain (Scheme 2), thereby yielding systems in which 1 mM solutions are stable when kept at 20°C and protected from light.



Scheme 2. synthesis of (3-O-(3-hydroxypropyl)-17 β -estradiol chromium tricarbonyl (alpha and beta isomers).

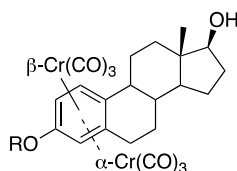
3. Measurement of Relative Binding Affinities

These lipophilic complexes are not soluble in biological media, but the addition, in biological media, of these complexes in alcoholic solution in order to obtain a final concentration of 5% in ethanol allows their solubilization and the study of their biological properties, in particular their affinity for the estradiol receptor. With the need to have biological extracts containing estradiol receptors, and being unable to gain convenient access to rat uteri, we chose to work instead on sheep uteri that we would collect once a year from the slaughterhouse at Mantes la Jolie, not far from Paris. This allowed us to perform experiments with uteri from young animals whose receptors are not already occupied by

circulating estradiol, and also to work with a homogeneous batch of animals allowing good reproducibility of the results. Since estrogen receptors degrade rapidly at room temperature, it was essential to freeze them quickly in liquid nitrogen after sampling, thereby allowing their conservation for a year or more).

The studies began with the determination of the relative binding affinity of complexes **10**, **11**, **17-19** (Table 1) to see whether a molecule is still recognized by the estradiol receptor (Rc-E₂). To obtain this value, in the presence of a fixed quantity of receptor, a fixed concentration of tritiated estradiol ([6,7]³H-E₂; 6 x 10⁻⁹ M) and variable concentrations of the complexes were tested (1 x 10⁻¹⁰ to 1 x 10⁻⁶ M). The quantity of tritiated estradiol bound to Rc-E₂ decreases according to the quantity of the complex to be tested and its affinity for the receptor. For each product, the concentration required to displace 50% tritiated estradiol is determined. This value is compared with that obtained for non-radioactive estradiol. The ratio between these two values constitutes the Relative Binding Affinity (RBA) of the complex for Rc-E₂. The RBA of the natural hormone estradiol (E₂) is, by definition, 100%; the lower this value, the less the complex is recognized by the estradiol receptor.

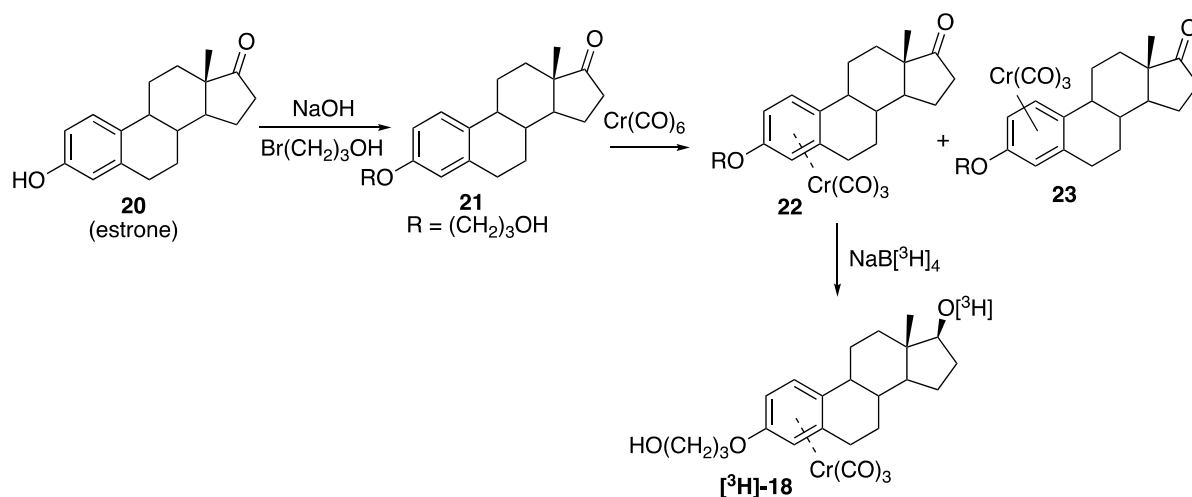
Table 1. Relative Binding Affinity (RBA) values of a selection of complexes [24]



R	H 17β- estradiol	Si(CH ₃) ₂ tBu 9	Si(CH ₃) ₂ tBu α-Cr(CO) ₃ 10	Si(CH ₃) ₂ tBu β-Cr(CO) ₃ 11	HO(CH ₂) ₃ O 17	HO(CH ₂) ₃ O α-Cr(CO) ₃ 18	HO(CH ₂) ₃ O β-Cr(CO) ₃ 19
RBA	100	11	1.1	0.4	37	28	1.8

The data revealed that all the complexes tested retain some affinity for Rc-E₂. Unsurprisingly, the silyl complex **9**, which has a 17β OH, but no phenolic OH at C3, has a rather low affinity of 11%. This decreases significantly upon incorporation of the α-complexed Cr(CO)₃ moiety in **10** (1.1%), and even more so for its β-complexed counterpart **11** (0.4%). In contrast, when the substituent at C3 is the HO(CH₂)₃O unit, as in **17**, the RBA value of 37% indicates that the folding of the propyl chain allows a correct positioning of the OH within Rc-E₂. This value drops only slightly in the corresponding α-Cr(CO)₃ system (RBA = 28% for **18**), whereas it falls dramatically for the β-complexed diastereomer

(RBA = 1.8% for **19**). To carry out the necessary biochemical experiments with complex **18**, and thereby study its interactions with the receptor site, required the preparation of a radioactive derivative. As shown in Scheme 3, the corresponding estrone complex **22** was stereospecifically reduced with sodium borohydride, then available in its tritiated form ($\text{NaB}[^3\text{H}]_4$), to furnish the desired 17β -OH derivative.



Scheme 3. Synthesis of **18** in its tritiated form, via the reduction of **22**, the corresponding estrone, with $\text{NaB}[^3\text{H}]_4$.

The preparation of **18** represented a real challenge that required working on such small quantities (5×10^{-5} M of **22**, or 9.4 mg). However, it was possible to purchase 4×10^{-6} mol of $\text{NaB}[^3\text{H}]_4$ (i.e. 100 μCi of $\text{NaB}[^3\text{H}]_4$ with a specific activity of 25 Ci/mmol). Moreover, thanks to the good yield (94%), it was finally possible to obtain 7 mg of the $[\text{³H}]\text{-18}$ complex as a yellow solid with a specific activity of 4.1 Ci/mmol that was stable when kept in the cold and protected from light. Although markedly lower than that of commercial tritiated estradiol ($[\text{6.7-}^3\text{H}]\text{-E2}$, AS = 58 Ci/mmol), it was sufficient to obtain a saturation curve for the complex with the estradiol receptor, demonstrating that this hormone binds specifically to Rc-E2 with a low level of non-specific binding (Fig. 6).

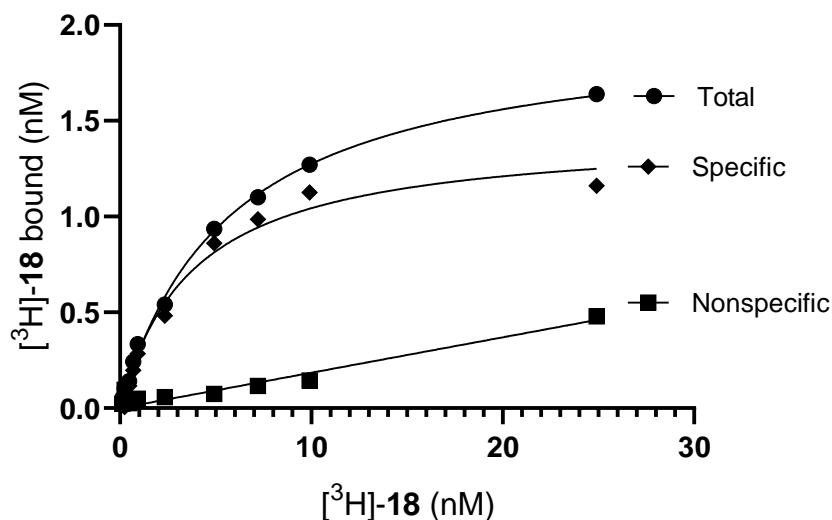


Fig. 6. Interaction of [³H]-**18** with uterine cytosol used as a source of Rc-E₂. Lamb uterine cytosol was incubated with increasing concentrations of [³H]-**18**, alone or together with a 100-fold excess of unlabeled diethylstilbestrol (DES) (to determine the non-specific binding)

The data from this curve made it possible to calculate the affinity constant of this complex for the estradiol receptor. Interestingly, the K_D value obtained for [³H]-**18** is very comparable to that found for [³H]-E₂ (0.85 nM and 0.67 nM respectively) [24]. We note that this type of preparation of a such a tritium-labeled derivative would not currently be possible. Indeed, the rules concerning the use of radioactive compounds have led to the ban on the routine use of tritiated NaBH₄ in synthetic chemistry laboratories. Furthermore, the reduction of a ketone by NaBH₄, as shown in Scheme 3 leads to the formation of potentially hazardous tritiated water. Nevertheless, at that stage, we had an estradiol derivative bearing a π -complexed Cr(CO)₃ moiety having a strong affinity for the estradiol receptor. It was therefore a viable proposition to consider taking advantage of the particular infrared properties of metal carbonyl complexes to proceed to the last step of this project, a quantification of the estradiol receptors present in sheep uterus cytosols.

4. An infrared spectroscopic approach towards bioassays

As indicated above, this work was carried out in the course of a France-Canada collaboration (Fig. 7, photos from 1984) with Prof. Ian Butler and Ashraf Ismail, a doctoral student at McGill University in Montreal, on a Nicolet 6000-FT-IR spectrometer, one of the

first to use the Fourier transform infrared technique, which made it possible to markedly increase the sensitivity of the analyses.



Fig. 7. (Left) Gérard Jaouen and Ian Butler on a bridge near Notre Dame cathedral in Paris; (right) Anne Vessières in her office at ENSCP.

In these analyses, fractions of sheep uterus (6-8 mL) containing estradiol receptors were incubated for 2 hours at 4°C in the presence of 10 nM of complex **18**. At the end of the incubation, addition of a basic solution of protamine sulphate brought about precipitation of the acidic proteins (including Rc-E₂) that were recovered by centrifugation and lyophilized. These solid samples were pressed into 3-mm pellet for IR-analysis. The spectra shown in Fig.8 are the result of the collection of 10,000 scans, in sets of 300 scans at a time, and then co-added using the Nicolet software [24].

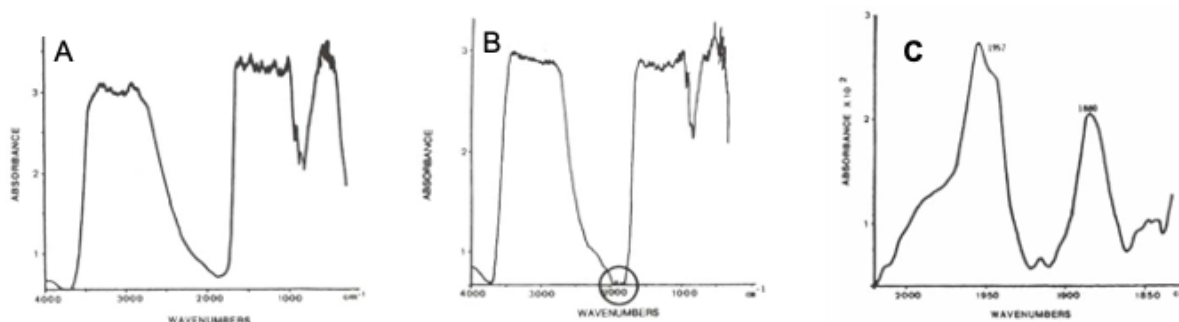


Fig. 8. FT-IR spectra of mini-pellet of lyophilized proteins (from lamb uterine cytosol). **A**, cytosol incubated with E₂, off scale; **B**, cytosol incubated with 10 nM of **18**, off scale; **C**, as in spectrum **B**, but expanded in the metal carbonyl stretching region.

The spectrum in Fig. 8A highlights the existence of a window in the IR spectrum of proteins in the region 2100-1850 cm⁻¹. After incubation in the presence of 10 nM of **18**, the resonances attributable to the metal complex are visible (spectrum 8B), but given the small

amount of Rc-E2 contained in the fractions (in the fmol mg⁻¹ protein range) the peaks are of low intensity. Nevertheless, expansion of the metal carbonyl stretching region (spectrum 8C) confirmed that these resonances indeed correspond to those of complex **18**.

This work demonstrated the feasibility of our approach, a first in the field of the use of metal carbonyl complexes as non-radioactive tracers. However, because of the very small quantities of estrogen receptors to be assayed, it was not possible to develop this technique into a routine assay. Consequently, we then turned to the development of immunological assays with a much higher sensitivity range, and chose to focus on the dosages of three antiepileptic drugs, carbamazepine, phenobarbital and DPH (diphenylhydantoin) whose toxic doses are close to the effective doses. Three organometallic tracers carrying different metal carbonyl fragments [Co₂(CO)₆, Mn(CO)₃, Cr(CO)₃] (**24-26**) were prepared (Fig. 9).

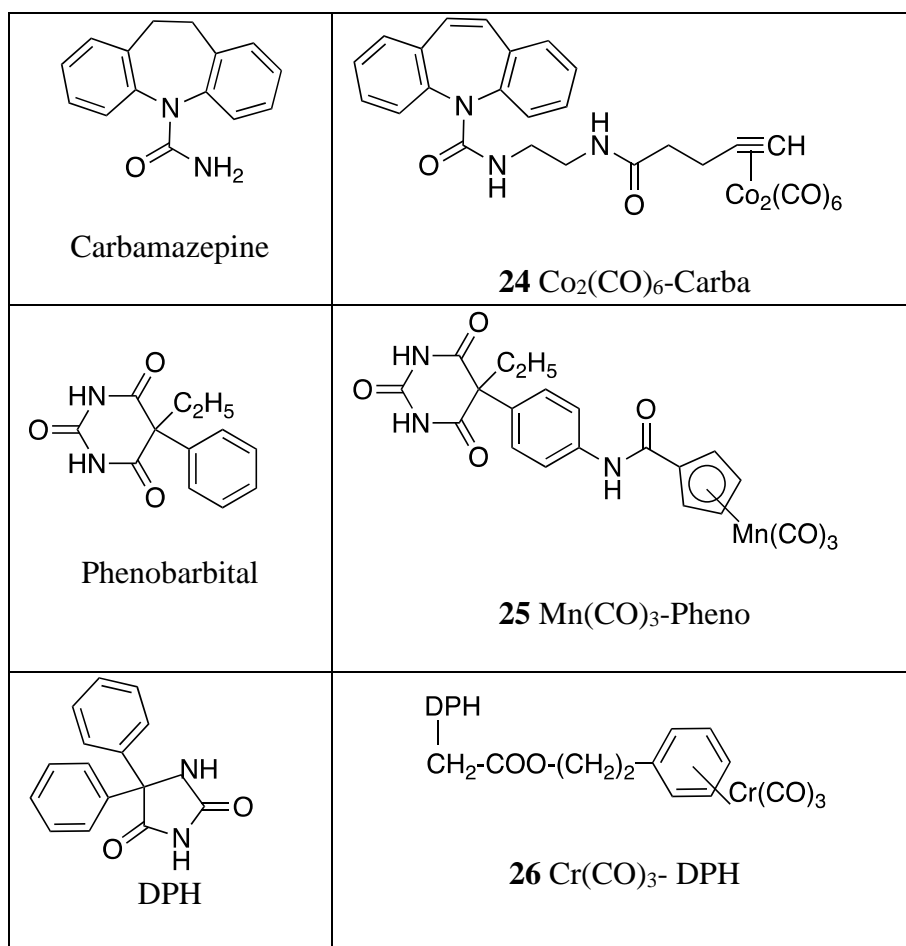


Fig. 9. Antiepileptic drugs and their organometallic tracers.

Each of these tracers has characteristic IR bands, $\nu_{(\text{CO})}$, in the 1850-2100 cm⁻¹ region and, since their positions, intensities and number of characteristic peaks depend on the

identity of the metal and the local symmetry of the $M(CO)_x$ entity, this opened the way to the simultaneous detection of the three tracers (Fig. 10) [37].

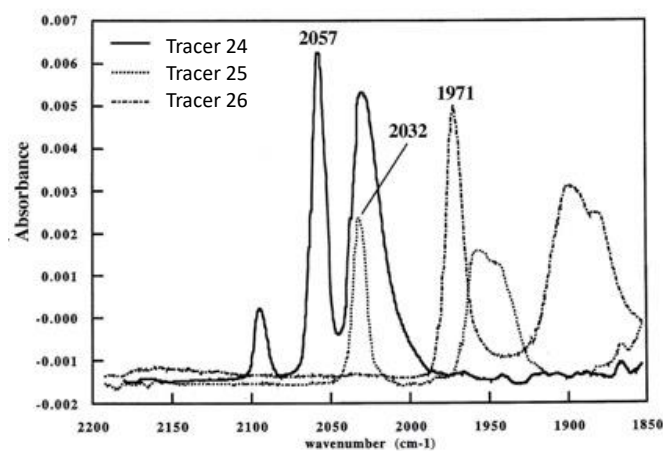


Fig. 10. Superimposition of the IR spectra of the three metal carbonyl tracers.

The development of benchtop infrared spectrometers equipped with a liquid nitrogen cooled, InSb (indium antimony) detector and the design of an ultra-low volume gold light-pipe with a long optical pathway (2 cm) and a low fill volume (23 μ L) [38] allowed us to perform quantitative assays of these tracers with detection limits as low as 300 femtomoles for the cobalt carbonyl complex, **24** [37]. With the collaboration of Michèle Salmain, at that time a thesis student in the laboratory, and Prof. Pierre Brossier in Dijon who provided us with specific antibodies, we were able to demonstrate the feasibility of this approach christened Carbonyl Metallo-Immuno Assay (CMIA) [39] for these three epileptic drugs carbamazepine [40], phenobarbital [39] and DPH [41]. It was also found possible to carry out simultaneous multidosages of two [41] or three molecules [42]; we note that analytical techniques allowing this kind of approach are rare [43].

More recently, in an extension of the original project, the complex **27** (Fig. 11), in which the ferrocenyl substituent in a ferrocifen-type system was replaced by the (cyclopentadienyl)tricarbonylrhenium moiety, has been localized and located inside a breast cancer cell, thanks to the use of AFM-IR (atomic force microscopy-infrared spectroscopy) a cutting-edge technique developed by Prof. Alexandre Dazzi at Université Paris-Sud, [44,45]. This technique is based on the coupling of an atomic force microscope (AFM) with a tunable pulsed infrared laser emitting in the mid-infrared region (1200-3200 cm^{-1}), and makes it possible to obtain images with a resolution between 20 and 50 nm, whereas that of a

conventional optical infrared microscope is $2.5 \mu\text{M}$ at 2000 cm^{-1} . It is therefore possible to carry out mapping of the complex in a cell whose size is of the order of $20 \mu\text{M}$.

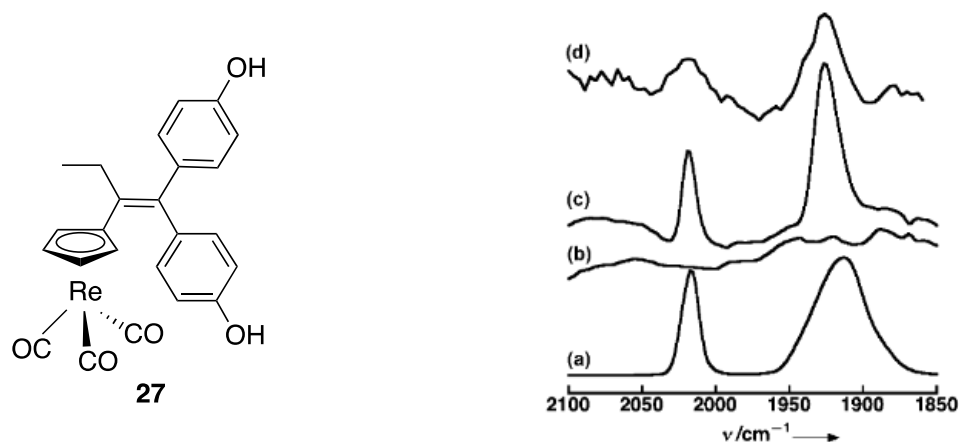


Fig.11. Complex **27** was localized in the nucleus of single cell (MDA-MB231 cancer cells). a) FT-IR spectrum of pure **1**; b) FT-IR spectrum of control cells; c) FT-IR spectrum of a collection of cells incubated with **27** ($50 \mu\text{M}$, 1 h); d) AFM-IR spectrum at the nucleus of a single cell treated with of **27** ($10 \mu\text{M}$, 1 h).

This work was done in collaboration with Prof. Clotilde Policar at the Ecole Normale Supérieure de Paris whereby incubation of MDA-MB-231 breast cancer cells in the presence of complex **27** ($10 \mu\text{M}$, 1 h) revealed that the complex entered the cells well, and that it was located essentially in the nucleus (Fig. 11) [46].

5. Concluding remarks

In summary, this review takes stock of the development of metal carbonyl complexes as tracers in biology, based on their particular infrared spectroscopic properties. This project made it possible to validate this approach to Bioorganometallic Chemistry, initially in the field of hormone receptors. It was then extended by the development of the non-isotopic immunoassay CMIA before more recently finding a new application for the tracing of these complexes in cells.

The subsequent emphasis on the syntheses and bioactivity of ferrocifens, in which a phenyl group in Tamoxifen, the current first line treatment for hormone-dependent breast cancer, has been replaced by a ferrocenyl sandwich moiety has been comprehensively reviewed elsewhere [11]. All these results demonstrate that multidisciplinary research led by a visionary leader keen on innovation, involving teams at the forefront of innovation in their respective fields, can lead to major scientific advances.

Acknowledgements

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