Estrogen derivatives of transition metal carbonyl clusters for analytical detection enhancement

Domenico Osella*, Olimpia Gambino, Roberto Gobetto, Carlo Nervi, Mauro Ravera
Dipartimento di Chimica Inorganica, Chimica Fisica, e Chimica dei Materiali, Università di Torino, via P. Giuria 7, 10125 Turin (Italy)

and Gerard Jaouen
Ecole Nationale Superieure de Chimie de Paris, URA CNRS 403, 75231 Paris (France)

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Abstract

We herein describe the labelling of ethynylestradiol with metal carbonyl fragments, namely $-Co_2(CO)_6$, $-M_3(\mu\text{-}CO)(CO)_9$ (M = Ru, Os) and $-Ru_3(\mu\text{-}H)(CO)_9$. The solution structure of $Ru_3(\mu\text{-}CO)(CO)_9$ (ethynylestradiol) cluster has been studied by means of variable-temperature (VT) ^{13}C NMR spectroscopy. This derivatization can be useful for detection enhancement in high-performance liquid chromatography (HPLC) separation, since the metal carbonyl moieties act as efficient markers for the UV detection mode at the usually employed 254 nm wavelength.

Introduction

Provided an acceptable degree of molecular recognition is retained between the synthesized bio-organometallic compound and the binding proteins, the labelling of a biologically active substance by an organometallic graft may offer analytical alternatives to radioisotopic assay [1]. The organometallic-steroidal series offers an example of this approach [2]. It has been previously reported [3] that the coordination of bi- and trimetallic moieties at the acetylene chain in the 17-α position of ethynylestradiol does not dramatically hinder the recognition properties of the resulting bio-organometallic complexes for the estradiol receptors [3]. The apparent receptor binding activity (*RBA*) values range between 33 and 2.5%, estradiol being taken as 100%. The *RBA* values roughly depend on the steric bulk of the coordinated metal fragments [3]. The determination of hormonal steroids labelled with such organometallic markers has been extensively performed by means of FT-IR spectroscopy, after incubation with cytosol [4]. Recently, we proposed an alternative detection mode in solution of the $Co_2(CO)_6$- and $Mo_2Cp_2(CO)_4$-labelled ethynylestradiol by means of electrochemical techniques [5]. Free estrogens exhibit only ill-defined multielectron oxidation processes; the coordination of such bimetallic moieties, having an accessible LUMO, adds an independent, well-behaved reduction process. The peak current (i_p) of the reduction of such complexes, recorded in linear sweep voltammetry (LSV) mode, is proportional to their concentration in solution [5].

Since only the electrochemical detection (ED) coupled with the HPLC separation indicates sufficient sensitivity for the hormone assay [6], as the first stage of this approach, we have optimized the reversed-phase HPLC separation of free ethynylestradiol from bi- and trimetallic compounds **1**–**4** [7]. In our hands, we have found that the metal carbonyl moieties can also act as markers for the usually employed UV detection ($\lambda = 254$ nm) by virtue of their intense metal to ligand charge transfer (MLCT) transitions.

Experimental

(17α-Ethynyl-1,3,5-estratriene-3,17β-diol)dicobalthexacarbonyl (**1**), (17α-ethynyl-1,3,5-estratriene-3,17β-diol)triruthenium decacarbonyl (**2**), (17α-ethynyl-1,3,5-estratriene-3,17β-diol)triosmium decacarbonyl (**3**) and (17α-ethynyl-1,3,5-estratriene-3,17β-diol)triruthenium-nonacarbonyl hydrido (**4**), hereafter $Co_2(CO)_6(HC_2E)$ (**1**), $M_3(\mu\text{-}CO)(CO)_9(HC_2E)$ (M = Ru (**2**), Os (**3**)) and $Ru_3(\mu\text{-}H)(CO)_9(HC_2E)$ (**4**), respectively (Fig. 1), have been synthesized according to the procedures [2, 3] summarized in the following equations:

*Author to whom correspondence should be addressed.

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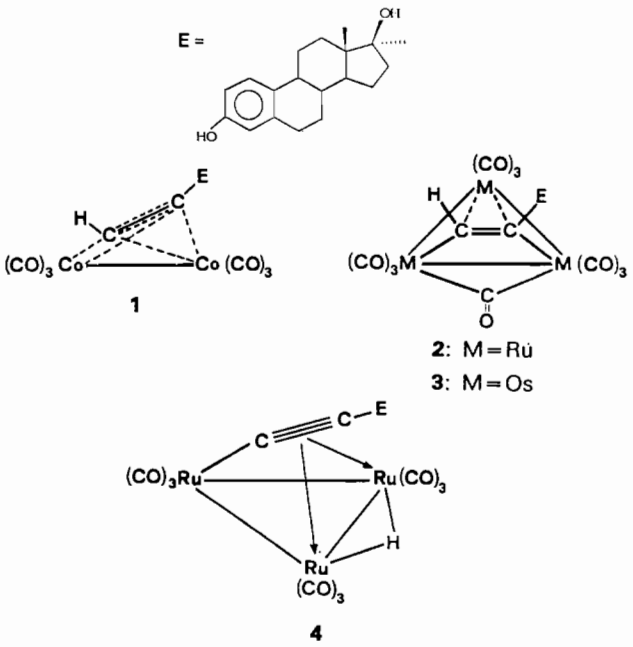

Fig. 1. Sketch of the structures of compounds **1–4**.

$$Co_2(CO)_8 \xrightarrow[(i)]{+HC_2E} Co_2(CO)_6(HC_2E) + 2CO$$

$$M_3(CO)_{12} \xrightarrow[+2MeCN,(ii)]{+2Me_3NO} M_3(CO)_{10}(NCMe)_2 + 2CO_2 + 2Me_3N$$

$$M_3(CO)_{10}(NCMe)_2 \xrightarrow[(ii)]{+HC_2E} M_3(CO)_{10}(HC_2E) + 2MeCN$$

$$Ru_3(CO)_{10}(HC_2E) \xrightarrow[(iii)]{\Delta} Ru_3(H)(CO)_9(C_2E) + CO$$

(i) acetone, ambient temperature, (ii) CH_2Cl_2, ambient temperature, M = Ru, Os; (iii) benzene at the reflux.

Their purity was checked by IR and ^1H NMR spectroscopy [2, 3].

^{13}C NMR spectra were recorded on a Jeol GX270/89 spectrometer at 67.9 MHz; chemical shifts are reported in ppm downfield positive from $SiMe_4$. The separation of the complexes **1–5** was obtained on a Kontron HPLC system (pump model 420, UV detector model 742) interfaced to an IBM microcomputer and a Kontron PC Integration Pack. A LiChrospher 100-RP18 (Merck), 5 μm, 250×4 mm diameter column was employed. All mobile phases employed were of HPLC grade (Merck); 20 μl volumes of acetonitrile solution of bio-organometallic compounds (1×10^{-4} M) were used for each analysis.

The electronic spectra were recorded on a Hitachi 150-20 spectrometer.

Results and discussion

Since the ^{13}C NMR data of such compounds have never been quoted in full [2, 3], we report in Tables 1 and 2, and Fig. 2 selected ^{13}C NMR data of the compounds under study together with that of free ethynylestradiol (HC_2E) and those of the isoelectronic and isostructural alkyne derivatives [8–11].

Most of the ^{13}C NMR resonances of the bio-organometallic derivatives are substantially unchanged with respect to those of free HC_2E (Table 2), except that assigned to the carbon atom C(17), bearing the acetylene chain, and especially those assigned to the acetylenic carbon atoms themselves, namely C_α(*) and C_β(△) (Table 1). The downfield shift of the acetylenic resonances and the decrease of the ^1J(CH) values upon coordination to the transition metal tags (coupling which obviously vanishes in the case of the hydrido compound **4**) are in tune with the NMR behaviour previously found for the isostructural alkyne derivatives [8–11] (Table 1). Furthermore, in order to elucidate the solution structure and the dynamic behaviour of the $Ru_3(\mu\text{-}CO)(CO)_9(HC_2E)$ cluster (**2**), we recorded the VT ^{13}C NMR spectra of a ^{13}CO enriched (*c.* 20%) sample of **2** in CD_2Cl_2. The low temperature limiting spectrum at −75 °C exhibits nine resonances at 225.2, 201.4, 198.5, 196.7, 195.8, 195.0, 192.3, 190.8 and 189.6 ppm of integrated intensity 1:1:1:1:1:2:1:1:1, respectively (Fig. 3). By comparing this result with other low temperature limiting spectra of $M_3(\mu\text{-}CO)(CO)_9$(alkyne) (M = Ru, Os) derivatives [11], we propose a ground state solution structure as depicted in Fig. 4, where the presence of a semibridging CO ligand (suggested by the chemical shift value of the downfield resonance at 225.2 ppm and by the IR stretching frequency at 1884 cm^{-1}) is likely conterbalanced by an asymmetric bonding of the alkyne HC_2E. Several structural and spectroscopic investigations [11] on $Os_3(CO)_9L$(alkyne) derivatives (L = CO, PR_3) have indicated that variation in the electronic and steric properties of the alkyne substituents or even substitution of one CO by phosphine ligand can modify the symmetrical structure having the alkyne parallel to one edge of the triangle and a bridging CO to an asymmetric one, having the alkyne twisted away from the parallel orientation and a semibridging CO [11]. In this context, the $Ru_3(\mu\text{-}CO)(CO)_9(HC_2E)$ compound possesses the more dissymmetric alkyne chain and therefore forces the ground state structure to the asymmetric form.

As the temperature is raised (Fig. 3), the resonances at 198.5, 196.7 and one of those at 195.0 ppm broaden and collapse in the base line. This low energy exchange process is interpreted as an axial–equatorial CO scrambling localized at one Ru centre (probably that π-bonded to the alkyne chain). As the temperature is

TABLE 1. Selected ^{13}C NMR data for free ethynylestradiol (HC_2E) and the metal-labelled derivatives **1–4** and the data of isostructural hydrocarbon derivatives recorded in $CDCl_3$ at 67.9 MHz and at 25 °C ($^1J(CH)$ (Hz) in parentheses)

Compound	C(17)	C(α)	C(β)	CO	Reference
HC_2E	87.6	80.0	74.9 (250)		this work[a]
$Co_2(CO)_6(HC_2E)$ (**1**)	85.3	103.7	73.5 (210)	199.5[b]	this work
$Co_2(CO)_6(HC_2H)$		70.8 (223)		199.5[b]	8
$Ru_3(CO)_{10}(HC_2E)$ (**2**)	90.7	192.8	136.7 (150)	198.4[b]	this work
$Ru_3(CO)_{10}(HC_2H)$		147.1 (162)		197.9[b]	9b
$Os_3(CO)_{10}(HC_2E)$ (**3**)	92.5	169.3	115.4 (162)	174.5	this work
$Os_3(CO)_{10}(EtC_2Et)$		141.9		178.4[b]	this work
$HRu_3(CO)_9(C_2E)$ (**4**)	82.5	103.8	162.9	197.1–188.5[c]	this work
$HRu_3(CO)_9(C_2{}^tBu)$		110.6	164.2	196.5–190.1[c]	10

[a]Although known in the literature [2c], the ^{13}C NMR spectrum of HC_2E has been recorded in identical experimental conditions for a better comparison. [b]Averaged resonance of carbonyls in rapid exchange. [c]These resonances, in integrated intensities of 1:2 from low to high field, are assigned to one axial and two equatorial carbonyls of the $Ru(CO)_3$ unit π-bonded to the alkyne; the remaining carbonyls are involved in a moderately fast exchange process at room temperature so that their resonances are too broad to be observed. They eventually merge in a single peak at higher temperature [10].

TABLE 2. Selected ^{13}C NMR data for free ethynylestradiol (HC_2E) and the metal-labelled derivatives **1–4** recorded in $CDCl_3$ at 67.9 MHz and at 25 °C. The assignments have been confirmed on the bases of the multiplicity of each signal in the ^1H coupled spectrum

Compound	C(3)	C(5)	C(10)	C(1)	C(4)	C(2)	C(17)	C(13)	C(18)
HC_2E	153.4	138.2	132.7	126.5	115.3	112.8	87.6	47.1	12.7
1	153.4	138.2	132.4	124.4	115.3	112.7	85.3	48.8	15.6
2	153.4	138.1	132.2	126.5	115.2	112.7	85.8	48.6	15.1
3	153.5	138.1	132.4	126.5	115.3	112.8	92.5	47.8	15.1
4	153.9	138.1	132.4	126.5	115.2	112.7	90.7	48.6	15.0

further increased, the peaks at 195.8, 190.8, 189.6 ppm, the semibridging carbonyl resonance and the remaining signal at 195.0 begin to broaden. Interestingly, this fluxional process does not involve the resonance at 192.3 until −15 °C. On the basis of these data, we suggest for this intermediate energy exchange process, a pivotal motion of the alkyne coupled with restricted edge to edge migration of the semibridging carbonyl. This motion leaves unchanged only the resonance at 192.3 ppm assigned to the CO group *trans* to the pivotal Ru–C σ-bond (labelled in Fig. 4 by an asterisk). Due to the large difference in steric bulk between the two alkyne substituents (i.e. H and the estradiol E moiety), the pivotal bond should correspond to the Ru–C(*H*) bond (the attribution in Fig. 4 could be however reversed). Finally, at room temperature, the unrestricted rotation of the alkyne over the Ru_3-triangle causes the coalescence of all the signals into an averaged resonance centred at 198.4 ppm. The ligand dynamics in **2** partially differs from those reported for $Os_3(CO)_9(\mu$-$CO)(EtC_2Et)$ [11b] and for $Ru_3(CO)_9(\mu$-$CO)(HC_2H)$ [11b] compounds. The asymmetry induced by the ethynylestradiol chain plays a role in this.

HPLC separation and determination of complexes 1–4

Separation of free ethynylestradiol (HC_2E) from each of the labelled compounds **1–4** was obtained by using reversed-phased C-18 column and an acetonitrile mobile phase at a flow rate of 1 ml/min. In Table 3 the retention volumes and the integrated area ratio are reported along with the molar absorption coefficients at 254 nm for the compounds under investigation. The presence of the bulk organometallic tag increases the retention time of each compound with respect to free HC_2E. As an example the HPLC chromatogram of a mixture of HC_2E, **1** and **2** is depicted in Fig. 5. Clusters **2** and **4** appear to be inseparable by HPLC unless employing a flow rate as low as 0.3 ml/min and acetone/methanol 8:2 as mobile phase. However, in these experimental conditions some fragmentation of the two clusters occurs, testified by a slight decrease in the integrated areas and by the presence of small extra peaks.

The interesting feature of this preliminary HPLC investigation employing a conventional fixed-wavelength (254 nm) UV-spectrophotometric detector is that the metal carbonyl tags increase the detection sensibility of the ethynylestradiol by about 30 times (Table 3). This is due to the high-absorptivity electronic transitions of the metal carbonyl fragments in the UV region ($\leqslant$300 nm). In Fig. 6, the UV spectrum of an equimolar (0.1 mM) solution of HC_2E and cluster **2** in absolute ethanol (where the resolution is better than in acetonitrile) is reported. It is immediately apparent that

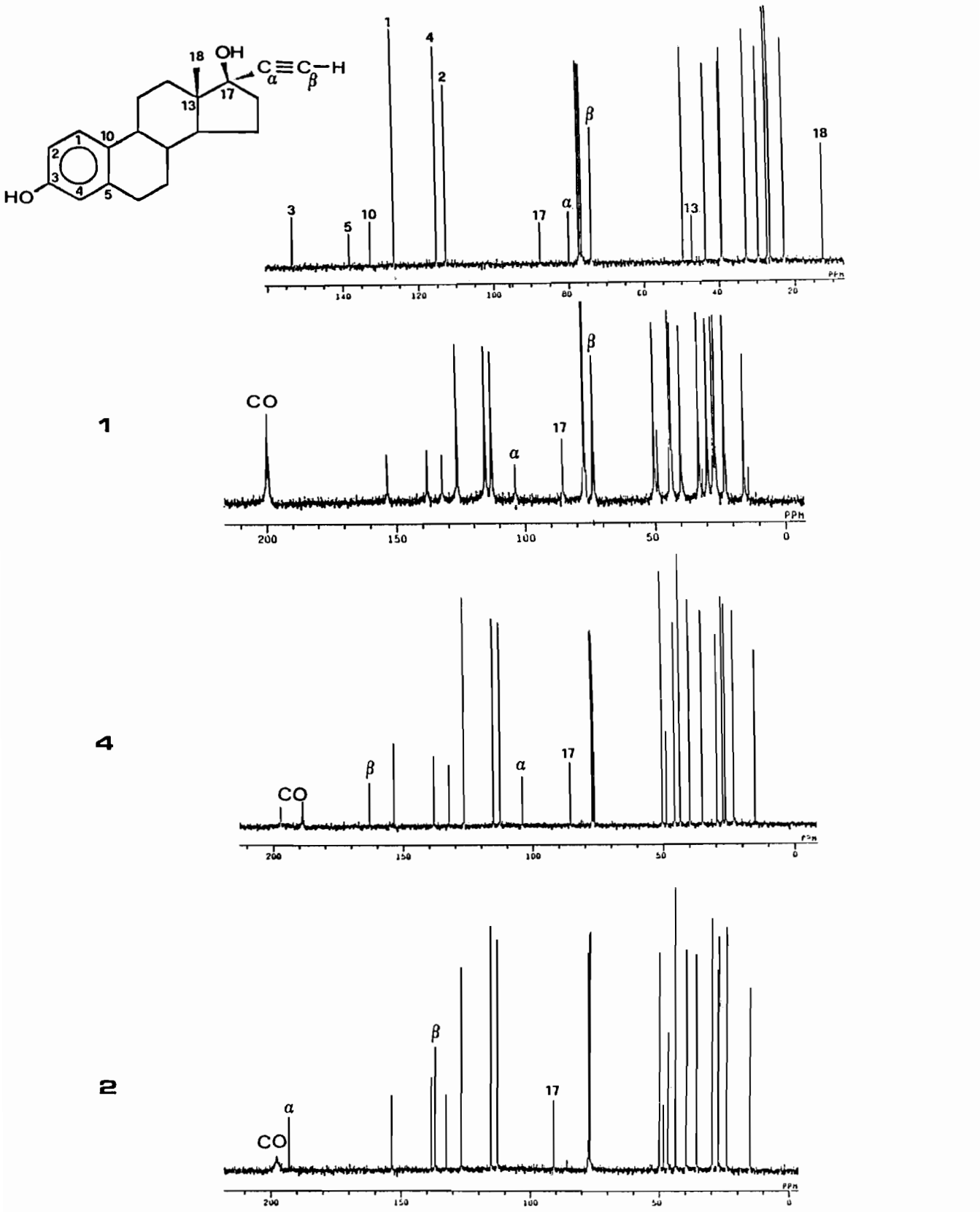

Fig. 2. ^{13}C NMR spectra of HC_2E, and of compounds **1**, **2** and **4** recorded in $CDCl_3$ at 67.9 MHz. Sketch of the structure of HC_2E, together with the steroid numbering scheme.

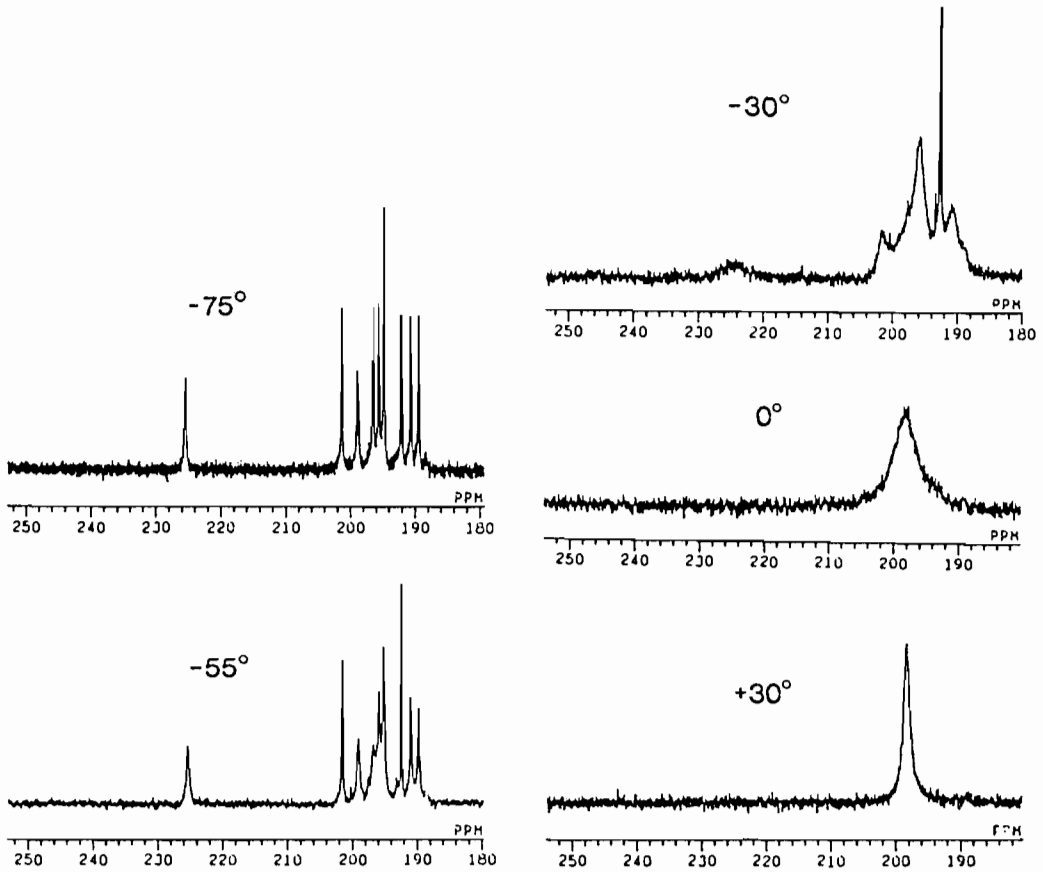

Fig. 3. VT ^{13}C NMR spectra of a ^{13}CO enriched sample of **2** recorded in CD_2Cl_2 at 67.9 MHz.

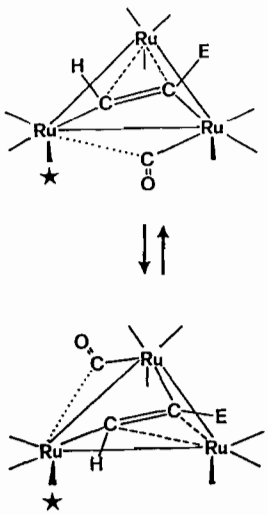

Fig. 4. Sketch of the intermediate energy exchange process in **2**.

TABLE 3. Retention volumes, integrated area ratio and UV spectral data of free HC_2E and compounds **1–4**

Compound	No.	V_R (ml)	A_R[a] (λ=254 μm)	ϵ (mol^{-1} cm^{-1}) (λ=254 μm)
HC_2E		2.55	1	345
$Co_2(CO)_6(HC_2E)$	**1**	3.67	31	11700
$Os_3(CO)_{10}(HC_2E)$	**3**	3.95	27	10200
$Ru_3(CO)_{10}(HC_2E)$	**2**	4.25	27	10400
$HRu_3(CO)_9(C_2E)$	**4**	4.27	29	10700

[a] A_R is the peak area ratio of the complexes under study and that of free ethynylestradiol recorded on equimolar mixture.

the metal carbonyl moiety adds two bands to the spectrum of the free estradiol: one weak at low energy (c. 440 nm) associated with the Ru–Ru bonds in the metallic triangle [12], like in $Ru_3(CO)_{12}$, and a second quite intense and broad band, whose tail starts at wavenumbers $\leqslant$300 nm. Although theoretical treatments and detailed assignments of electronic transitions in metal carbonyls are scarce [12], it is generally accepted that the intense absorptions in the 200–350 nm range could be attributed to $d(M) \rightarrow \pi^*(CO)$, metal-to-ligand charge transfer (MLCT) transitions. This UV detection enhancement is not enough for the estradiol assay at real physiological concentration (namely from nmol/l

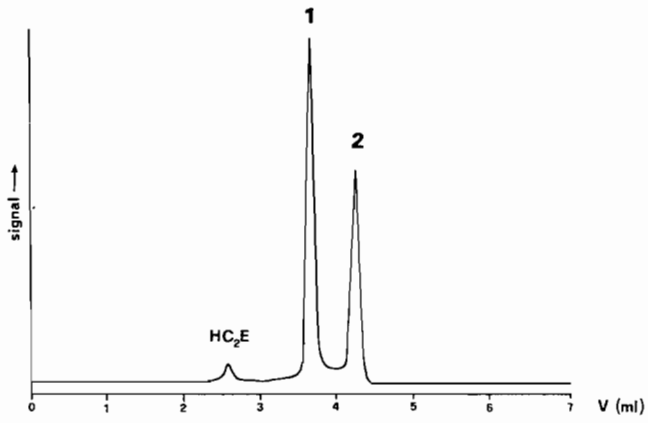

Fig. 5. Reversed-phase HPLC chromatogram of HC_2E, **1** and **2**. Mobile phase: acetonitrile; column: LiChrosorb 100-RP18, 240×4 mm; detector wavelength: 254 nm; temperature: ambient; flow rate: 1 ml/min.

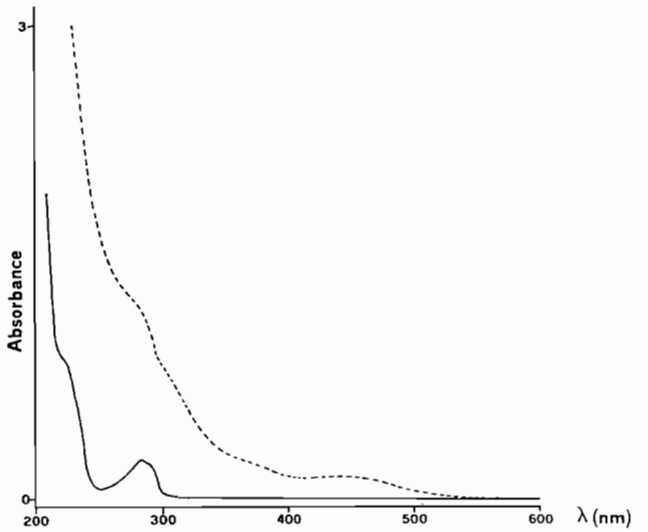

Fig. 6. UV absorption spectra (200–600 nm) of ethanol solutions (1×10^{-4} M) of HC_2E (——) and **2** (– – – –).

to pmol/l [13]), but can be useful for pharmacological analyses.

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