The Use of Tricarbonyl Chromium Hexestrol Derivatives in the Detection of Oestradiol Receptor Sites

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Hexestrol tricarbonyl chromium complexes may be used for oestradiol receptor assay by taking advantage of the high sensitivity of Fourier transform i.r. spectroscopy.

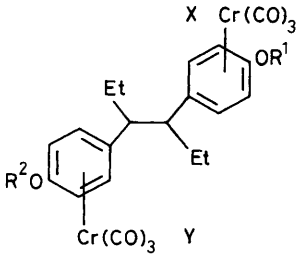
It has recently been suggested that transition metal carbonyl complexes may be used as a new type of marker and therefore obviate the recognized drawbacks of radioelemental analysis in molecular biology.[1] This organometallic labelling technique takes advantage of strong i.r. signals (ν_{CO} stretching frequencies) at about 2100—1850 cm^{-1}, a region where absorption due to proteins is minimal, and benefits from the high sensitivity of Fourier transform i.r. spectrometry.

We now report the synthesis of hexestrol–$Cr(CO)_3$ derivatives together with the examination of their potential use for a metal carbonyl receptor assay. The problem of oestradiol receptor assay is crucial in the hormone-dependent cancer

Table 1. Characteristic data of the hexestrol derivatives.[a]

Compd.	R^1	R^2	$Cr(CO)_3$ units	M.p., *t*/°C	% Yield	R.B.A.[b]
(1)	H	H	None	—	—[c]	140
(2)[d]	$HO[CH_2]_3$	$HO[CH_2]_3$	None	142	58	0.33
(3)[e]	$HO[CH_2]_3$	$HO[CH_2]_3$	X and Y	170	56	10^{-4}
(4)[d]	H	$HO[CH_2]_3$	None	148	36.5	15
(5)[f]	H	$HO[CH_2]_3$	Y	162	14	2
(6)[g]	$SiMe_2Bu^t$	$SiMe_2Bu^t$	None	123.5	71	0.17
(7)[e]	$SiMe_2Bu^t$	$SiMe_2Bu^t$	X	195	31	10^{-3}
(8)[h]	$PhCH_2$	$PhCH_2$	None	218	—	0.12
(9)[f,i]	$PhCH_2$	$PhCH_2$	X	153	39	0

[a] All the new compounds reported here gave satisfactory elemental analyses and spectroscopic data. [b] Relative binding affinities were determined as follows: Lamb uterine cytosol (0.2 ml fractions containing 8 mg protein/ml) was incubated at 0 °C for 3 h with 2 nM [^{3}H]-17β-oestradiol and an increasing amount of competing steroids (10 to 1000 fold excess; 9 concentrations in duplicate). Bound fractions were measured by protamine sulphate precipitation.[7] The R.B.A. of the competitor is taken as the ratio of the concentrations of unlabelled oestradiol/competitor required to inhibit half of the specific [^{3}H]-17β-oestradiol binding with the affinity of oestradiol set at 100%. The higher the value of the R.B.A., the better is the affinity for the oestradiol of the receptor. [c] Commercially available (Sigma). [d] The reaction with 3-bromopropan-1-ol was based on the work of C. M. Brewster and I. J. Putman, Jr., *J. Am. Chem. Soc.*, 1939, **61**, 3083. [e] The complexation was carried out by heating the hexestrol derivative with $Cr(CO)_6$ for 5 h in dibutyl ether under argon. [f] The complexation was carried out first and the modification of the phenolic function second. ^{1}H N.m.r. data for (**5**): Varian XL 100 MHz spectrometer; $(CD_3)_2CO$ solution, relative to $SiMe_4$; δ 3.68 (2H, t, $HOCH_2CH_2CH_2O$–), 4.03 (2H, t, $HOCH_2CH_2CH_2O$–), 5.47 (4H, m, complexed Ar), and 6.83 (4H, m, non-complexed Ar). [g] The protecting group was added according to the general procedure developed by E. J. Corey and A. Venkateswarl, *J. Am. Chem. Soc.*, 1972, **94**, 6190; H. B. Arzeno, D. H. R. Barton, S. G. Davies, X. Lusiach, B. Meunier, and C. Pascard, *Nouv. J. Chim.*, 1980, **4**, 369. [h] Compound (**8**) was obtained by decomposition by sunlight of compound (**9**) in ether solution. [i] The aryl benzyl ether was prepared by treating an alkaline solution of hexestrol with benzyl chloride: T. W. Green, 'Protective Groups in Organic Synthesis,' Wiley–Interscience, New York, 1980, p. 97, and references therein.



(**1**)—(**9**) (see Table 1)

area.[2] Hexestrol (**1**) offers distinct advantages over steroidal oestrogen derivatives such as oestradiol: a higher binding affinity with respect to the oestradiol receptor and a simpler chemistry.[3]

The first requirements to be met in the preparation of organometallic hormones are that the compounds should be stable in solution and exhibit good binding affinities for the specific receptor. An obvious site of complexation of hexestrol is the aromatic ring and a suitable organometallic unit for a six-electron ligand is the $Cr(CO)_3$ moiety.[4] However, the resulting phenolic complexes (with one or two complexed rings) rapidly decompose in solution, giving chromium salts and regenerating hexestrol, and a modification of the hydroxy function is necessary to yield sufficiently stable complexes. The characteristic data for the newly synthesized hexestrol derivatives are summarized in Table 1.

Table 1 reveals that, as previously observed,[5] substitution of the phenolic hydrogen invariably decreases the relative binding affinity (R.B.A.). However, good affinity is regained when this hydrogen is replaced by the –OH bearing chain $-[CH_2]_3-OH$ [R.B.A. = 15% for compound (**4**)]. Complexation of the aromatic ring also decreases the receptor affinity. Nevertheless, replacement of one of the two phenolic hydrogens by the $-[CH_2]_3-OH$ chain and complexation of this same aromatic ring led to a chromium carbonyl hexestrol derivative (**5**), whose affinity for the oestradiol receptor reaches 2%. According to Katzenellenbogen[6] compounds for which the affinity lies in the 0.5—5% range might be useful for labelling semipurified preparations of proteins and thus compound (**5**) could be a marker for oestradiol receptor detection.

Compound (**5**) was therefore selected as the most likely compound of those in Table 1 to demonstrate the feasibility of oestradiol receptor detection. The Fourier-transform spectrum of lamb uterine cytosol incubated with oestradiol and precipitated by protamine sulphate[8] reveals a 'window' at *ca.* 2000 cm^{-1} [Figure 1(a)], and the chromium carbonyl hexestrol complex (**5**) exhibits the two characteristic modes of vibration of the $Cr(CO)_3$ moiety in the 1850—2100 cm^{-1} region [Figure 1(b)], *i.e.* in the region free from absorption due to the protein. Figure 1(c) shows the Fourier-transform i.r. spectrum of the precipitated proteins following incubation with compound (**5**). This spectrum results from the computer expansion of the 1850—2100 cm^{-1} region, and the two peaks at 1957 and

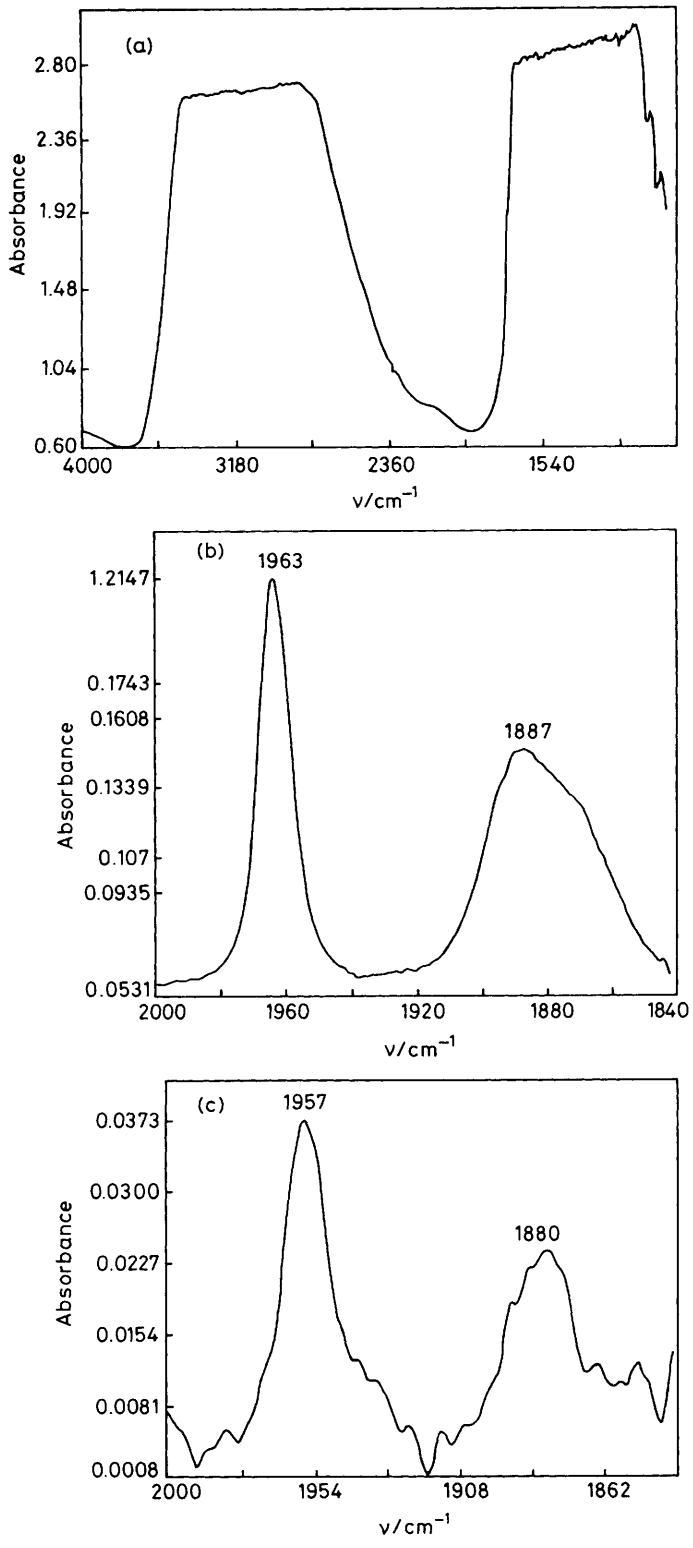


Figure 1. Fourier transform i.r. spectra: †(a) lamb uterine cytosol (micropellet of the lyophilized precipitate); (b) compound (**5**) in $CHCl_3$; expansion of the metal carbonyl region; (c) lamb uterine cytosol incubated with compound (**5**), following base-line correction and subtraction of the spectrum in (a).

† Lamb uterine cytosol (1 ml fractions containing about 300 fmol/ml of oestradiol binding sites, as determined by protamine sulphate precipitation assay) obtained after partial purification by ammonium sulphate precipitation, 35% wt/vol, which removes the bulk of non-specific binding proteins,[7] was incubated at 0 °C for 3 h with either 2 μM of unlabelled oestradiol [Figure 1(a)] or 2 μM of organometallic complex (**5**) [Figure 1(c)]. Following incubation, protamine sulphate precipitation, washing, and lyophilization, the white powder was pressed into 3 mm micropellets. I.r. spectra were obtained using either a Nicolet 6000 or a Bruker 45 Fourier transform i.r. spectrometer equipped with a mercury–cadmium telluride liquid nitrogen-cooled detector. To enhance the signal/noise ratio, >2000 scans were collected at 4 cm^{-1} resolution.

1880 cm^{-1}, corresponding to the two ν_{CO} (a_1 + e) peaks of the organometallic marker, are seen clearly. This spectrum establishes the stability of the compound (**5**) in the biological system and the potential use of hexestrol–$Cr(CO)_3$ derivatives for oestradiol receptor detection and assay.

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