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The selective introduction of organometallic markers into estrogens. A-ring propargylation of β-estradiol

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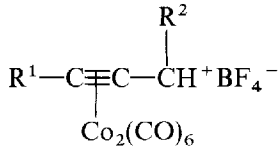
Introduction of the propargyl dicobalt hexacarbonyl moiety onto the estradiol A ring as a potential probe in receptor studies requires protection of the ring phenolic group, and the regioselectivity of the attack (2- versus 4-position) depends on the bulkiness of the organometallic carbenium ion.

Keywords: Estradiol receptor assays, metal carbonyl units, cold bioprobes, selectivity, propargylation, cobalt carbonyl

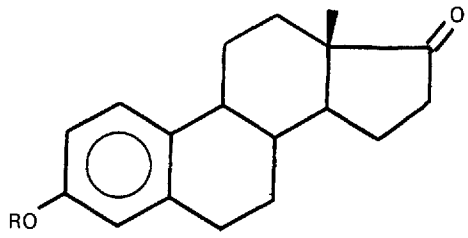
INTRODUCTION

Current biochemical steroid hormone receptor assays, particularly assays for estradiol, are important in therapy but remain expensive, are difficult to perform and require radiolabeled ligands.

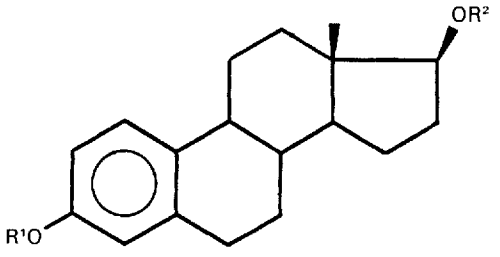
It has recently been demonstrated that metal carbonyl labeled estrogens are of potential use in steroid hormone receptor assay, a new area of application for transition metal carbonyl chemistry.[1] This approach depends on the ready detectability of the M—CO(νCO) stretching frequencies, at very low concentrations and in the presence of virtually any protein, by FT–IR (Fourier transform–infrared) spectroscopy. Further development in this direction depends on the availability of suitable organometallic substrates and hence we have been interested in developing methods for the selective introduction of stable metal carbonyl probes into biologically active molecules. From this point of view a reagent of considerable promise is the (propargyl) dicobalt hexacarbonyl cation **1**, a relatively stable[2] α-carbenium ion, the synthetic potential of which has been demonstrated (see for example Refs 3–5). The reactivity of **1** towards arene rings[6] and enol derivatives[7,8] offers the possibility of direct introduction of the (propargyl) dicobalt hexacarbonyl moiety into certain estrogens; here we describe our results on the synthesis of (propargyl) dicobalt hexacarbonyl derivatives of estradiol.



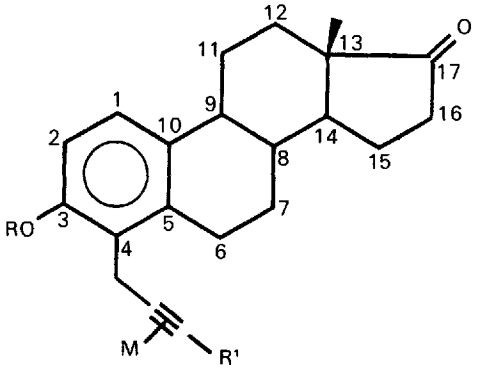
1a $R^1=R^2=H$
b $R^1=CH_3$; $R^2=H$
c $R^1=H$; $R^2=CH_3$



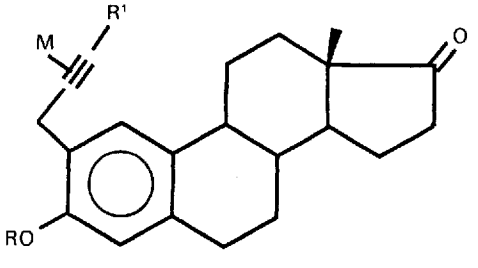
2a $R=CH_3$
b R=TBDMS (*t*-butyldimethylsilyl)



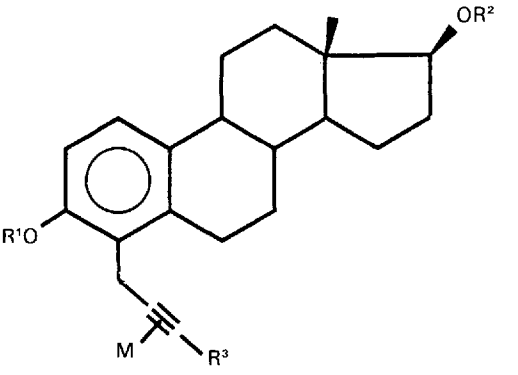
3a R^1=CH_3; R^2=CH_3
b R^1=TBDMS; R^2=TBDMS
c R^1=$CH_2CH_2CH_2OH$; R^2=H
d R^1=R^2=H (estradiol)



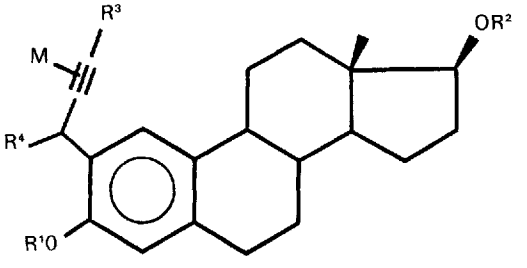
4a R=CH_3; R^1=H
b R=TBMS; R^1=H
M=$Co_2(CO)_6$



5a R=CH_3; R^1=H
b R=TBMS; R^1=H



6a R^1=R^2=CH_3; R^3=H
b R^1=TBDMS; R^2=H; R^3=H
c R^1=$CH_2CH_2CH_2OH$; R^2=H; R^3=CH_3
d R^1=TBDMS; R^2=$CH_2C{\equiv}CH$; R^3=H
M



7a R^1=R^2=CH_3; R^1=H; R^4=H
b R^1=TBDMS; R^2=H; R^3=H=R^4
c R^1=$CH_2CH_2CH_2OH$; R^2=H=R^4; R^3=CH_3
d R^1=TBDMS; R^2=$CH_2C{\equiv}CH$; R^3=H=R^4
M
e R^1=R^2=R^3=H; R^4=CH_3

RESULTS AND DISCUSSIONS

The (propargyl) dicobalt hexacarbonyl cations were either prepared in propionic anhydride according to the method of Nicholas[2] or by room-temperature addition of excess $HBF_4 \cdot OEt_2$ to an ether solution of the corresponding propargylic alcohol complex. The precipitated cation was thoroughly washed with ether and dried *in vacuo* before use.

Addition of estrone methyl ether **2a** or estradiol dimethyl ether **3a** to a suspension of cation **1a** (dichloromethane, −20°C) gave, after aqueous work-up, a mixture of the corresponding *ortho*-substituted derivatives, **4a**/**5a** and **6a**/**7a**. No differentiation between the two possible sites of attack was observed (**4a**:**5a** = **6a**:**7a** = 1:1). However, reaction of estrone silyl ether **2b** with **1a** led to partial desilylation (which may have been promoted by the presence of BF_4^- which has been shown to cleave trimethylsilyl ethers[9]) and considerable decomposition of the metal carbonyl complex. Similarly, cleavage of the silyl protecting group (Bu_4NF in 1:1 THF/water) in **6b** or **7b**, which were obtained from alkylation of **3b**, was accompanied by decomplexation, resulting in a mixture of organic products that could not be recomplexed[10] by $Co_2(CO)_8$. These and other observations (for instant, direct reaction of cation **1a** with estrone itself was observed to produce a deep-red solution, the color of which was immediately discharged on exposure to air) lead us to believe that in the C-2 and C-4 ring A substituted propargyl cobalt compounds the proximity of the propargyl metal carbonyl moiety to a free phenolic group results in attack by the phenol oxygen leading to destruction of the complex and loss of the acetylene function. These complexes are stable while the C-3 hydroxyl function is protected, but are destroyed by deprotection and release of the phenol.

Interestingly, reaction of estradiol (**3d**) with the methyl-substituted complex **1c** afforded an unstable substitution product **7e** (32%) whose NMR spectrum clearly indicated selective propargylation of C-2 (two singlets in the aromatic region), a result which was further supported by NMR and MS analysis of the demetalated derivative. A second lesser product (22%) also showed an aromatic NMR resonance pattern characteristic of C-2 attack, possibly the epimer of the major product, but its solution instability prevented a more definite characterization. No evidence of C-4 alkylation was obtained. The differing regiochemical results between the cations **1a** and **1c** are noteworthy and may provide the basis for a general method of regiospecific 'tagging' of complex bioactive molecules. We assume this effect is steric in origin; a similar effect has been seen earlier in the varying *ortho*/*para* product ratios from the alkylation of anisole by a series of the cation **1**.[6]

In order to circumvent this apparent destabilization by the proximate hydroxyl group we have turned our attention to 3-*O*-(3-hydroxypropyl)-estradiol **3c**, in which biological activity is retained[1] but where the free hydroxyl group is further removed from the aromatic ring. Alkylation of the C-2 and C-4 positions to give a mixture of **6c** and **7c** can be obtained by stirring **3c** with one equivalent of the cation **1b** in the presence of excess $HBF_4 \cdot OEt_2$ at a warm temperature for several hours. Initially rapid alkylation by **1b** of the two free hydroxyl groups also occurs, but in a strongly acidic medium this is reversible since protonation of the resulting ether linkage is able to regenerate the stable cation. Similarly *O*-alkylation is observed in the reaction of the cation **1a** with estradiol disilyl ether **3b** to give **6d**/**7d** among the products (*in situ* cleavage of the relatively labile aliphatic silyl ether having occurred). Treatment of **6d**/**7d** with $HBF_4 \cdot OEt_2$ (ether solution, room temperature) results in cleavage of the C-17 ether linkage; in this case the insoluble cation is precipitated from solution. The slower irreversible aromatic alkylation can be followed by TLC until all the cation is consumed (TLC monitoring shows the initial formation of at least three relatively non-polar products; during the course of the reaction these compounds disappear to be replaced by the two more polar products **6c** and **7c** which are eventually isolated). The 2- and 4-(propargyl) dicobalt hexacarbonyl estradiol derivatives **6c** and **7c** are isolated and separated in a combined yield of 50%. The biological activity of **6c** and **7c** towards the estradiol receptor is currently being determined; preliminary results suggest that these organometallic hormones retain sufficient affinity for the receptor for them to be of use in receptor assay.

EXPERIMENTAL

Reaction of protected estrone and estradiol with 1a and 1b

The same procedure was used for **2a**, **2b**, **3a** and **3b**. To a solution containing 0.4 g (1.2 mmol) of complexed propargyl alcohol in 2 cm^3 of dry Et_2O was added 1 cm^3 of $HBF_4 \cdot Et_2O$ in 2 cm^3 of Et_2O at −10°C. The resulting red oily product was washed once with Et_2O and 2 cm^3 CH_2Cl_2 was added. The product became crystalline, and was washed with Et_2O until the washings were colorless. Residual solvent was removed *in vacuo*: 2 cm^3 of dry CH_2Cl_2 was added to the propargylcobalt cation **1a** or **1b**, followed by

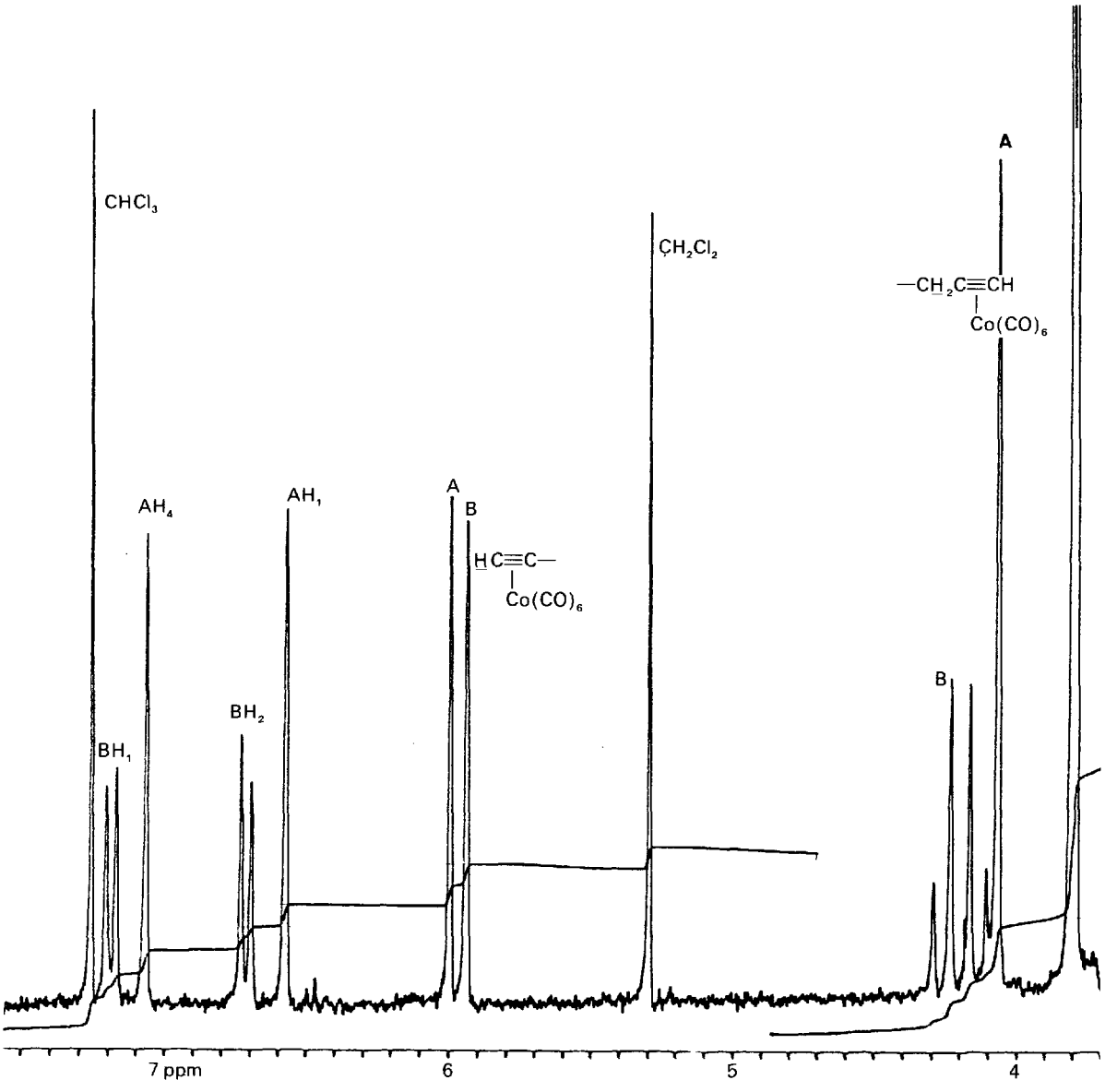
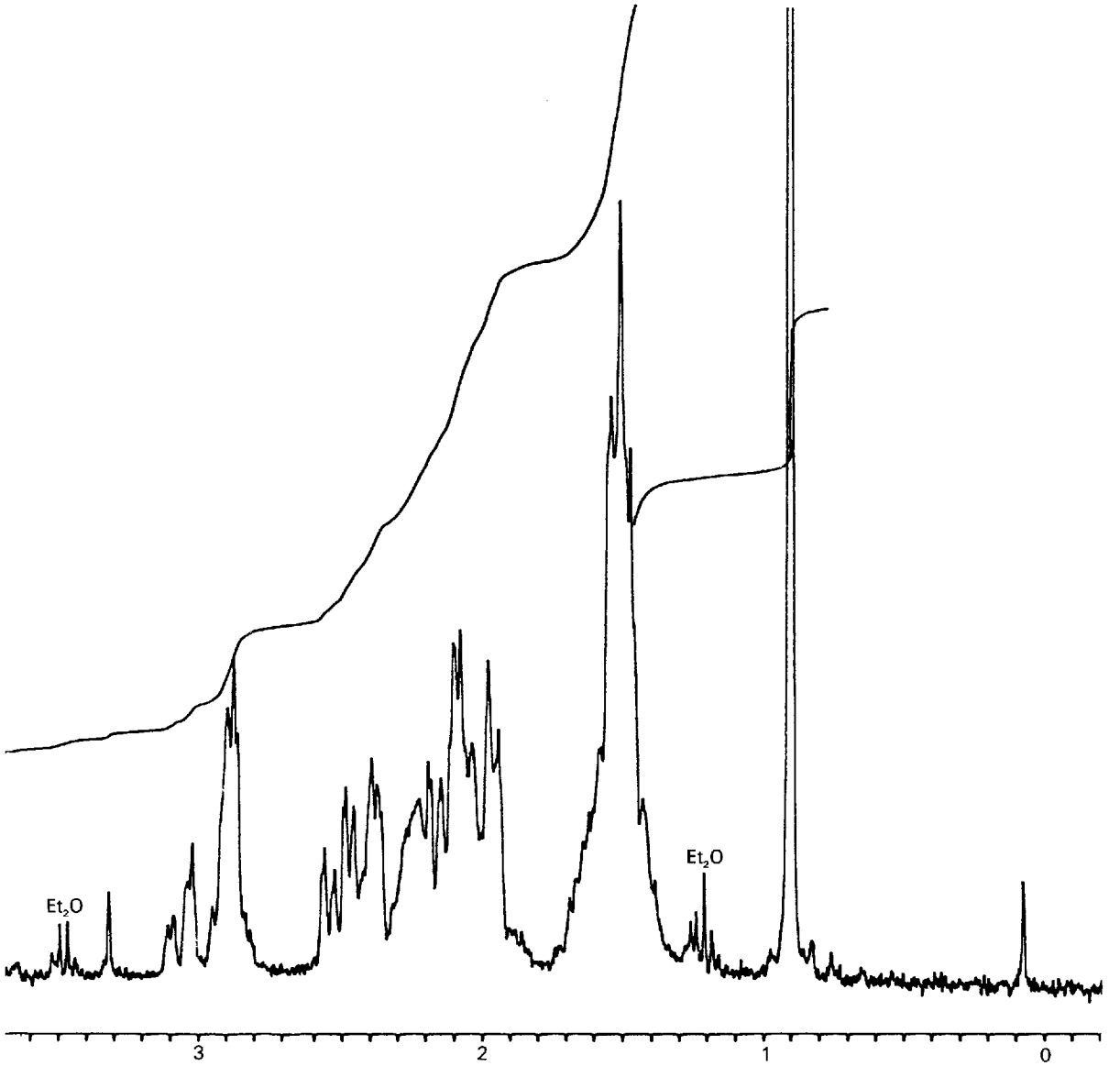


Figure 1 ^{1}H NMR spectrum of **4a** (B peaks) and **5a** (A peaks) at 250 MHz in CD_2Cl_2, where $M = Co_2(CO)_6$. The presence of both 2- and 4-substitution is clearly seen in the aromatic region (two doublets for **4a**, two singlets for **5a**). Note also the non-equivalence of the propargylic protons in **4a** (AB quartet at 4.2 ppm). Resonances centred at 1.2 ppm and 3.5 ppm are due to ether contaminant.


Et2O
Et2O
3
2
1
0

hormonal derivatives **2a** or **2b** and **3a** or **3b** (1 mmol) in 2 cm^3 of CH_2Cl_2. The mixture was allowed to warm to room temperature. The reaction was monitored by TLC on silica gel 60F254, layer thickness 0.2 mm. After about 30 min the solution was washed with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, concentrated, and the crude product was chromatographed on silica (eluted with pentane/Et_2O, 2:1). For **3c**, the cation **1a** or **1b** was used without removing $HBF_4 \cdot Et_2O$. Each product was identified by its ^1H NMR, IR and mass spectra.

4a

^1H NMR, 250 MHz, $CDCl_3$: 7.19 (1H, d, 9 Hz); 6.71 (1H, d, 9 Hz); 5.94 (1H, s); 4.20 (2H, A, B, 15 Hz); 3.79 (3H, s); 3.2 to 1.45 (15H); 0.90 (3H, s). See Fig. 1.

MS (desorption chemical ionization with NH_3 as reactant gas—DCI/NH_3): M + 1, 609; M + 18, 626.

IR (CH_2Cl_2): 2090, 2050, 2020 cm^{-1} νCO(M—CO); 1735 cm^{-1} νCO.

5a

^1H NMR, 250 MHz ($CDCl_3$): 7.07 (1H, s); 6.57 (1H, s); 5.99 (1H, s); 4.07 (2H, s); 3.80 (3H, s); 3.2 to 1.45 (15H); 0.90 (3H, s). See Fig 1.

MS (DCI/NH_3): M + 1, 609; M + 18, 626.

IR (CH_2Cl_2): 2090, 2050, 2020 cm^{-1} νCO(M—CO); 1735 cm^{-1} νCO.

4b

^1H NMR, 250 MHz ($CDCl_3$): 7.07 (1H, d, 9 Hz); 6.57 (1H, s); 6.00 (1H, s); 4.07 (2H, A, B, 16 Hz); 3.2 to 1.4 (15H); 1.03 (9H, s); 0.90 (3H, s); 0.34 (6H, s).

MS (DCI/NH_3): M + 1, 708; M + 18, 726.

IR (CH_2Cl_2): 2095, 2060, 2030 cm^{-1} νCO(M—CO); 1738 cm^{-1} νCO.

5b

^1H NMR, 250 MHz ($CDCl_3$): 7.09 (1H, s); 6.51 1H, s); 6.03 (1H, s); 4.19 (2H, s); 3.2 to 1.4 (15H); 1.03 (9H, s); 0.90 (3H, s); 0.34 (6H, s).

MS (DCI/NH_3): M + 1, 7.08; M + 18, 726.

IR (CH_2Cl_2): 2095, 2060, 2030 cm^{-1} νCO(M—CO); 1738 cm^{-1} νCO.

6a

^1H NMR, 60 MHz ($CDCl_3$): 7.14 (1H, d, 9 Hz); 6.68 (1H, d, 9 Hz); 5.95 (1H, s); 4.2 (2H, s); 3.83 (3H, s); 3.43 (3H, s); 3.35 (1H, m); 3.2 to 1.45 (15H); 0.90 (3H, s).

MS (DCI/NH_3): M + 1, 625; M + 18, 642.

IR (CH_2Cl_2): 2090, 2050, 2020 cm^{-1} νCO(M—CO).

7a

^1H NMR, 60 MHz ($CDCl_3$): 7.00 (1H, s); 6.67 (1H, s); 6.00 (1H, s); 4.00 (2H, s); 3.83 (3H, s); 3.43 (3H, s); 3.35 (1H, m); 3.2 to 1.45 (15H); 0.90 (3H, s).

MS (DCI/NH_3): M + 1, 625; M + 18, 642.

IR (CH_2Cl_2): 2090, 2050, 2020 cm^{-1} νCO(M—CO).

6b

^1H NMR, 60 MHz ($CDCl_3$): 7.02 (1H, d, 8 Hz); 6.61 (1H, d, 8 Hz); 5.99 (1H, s); 4.06 (2H, s); 3.66 (1H, m); 3.2 to 1.4 (broad envelope); 0.96 (9H, s); 0.76 (3H, s); 0.21 (6H, s).

7b

^1H NMR, 60 MHz ($CDCl_3$): 7.06 (1H, s); 6.54 (1H, s); 6.03 (1H, s); 4.16 (2H, s); 3.66 (1H, m); 3.2 to 1.4 (broad envelope); 0.96 (9H, s); 0.76 (3H, s); 0.21 (6H, s).

6c

^1H NMR, 60 MHz ($CDCl_3$): 7.18 (1H, d, 9 Hz); 6.72 (1H, d, 9 Hz); 4.20 (2H, s); 4.11 (2H, t, 6 Hz); 2.93 (2H, m); 2.55 (3H, s); 0.77 (3H, s).

IR (CH_2Cl_2): 2085, 2045, 2017 cm^{-1} νCO(M—CO).

7c

^1H NMR, 60 MHz ($CDCl_3$): 7.10 (1H, s); 6.60 (1H, s); 4.15 (2H, t, 6 Hz); 4.10 (2H, s); 2.83 (2H, m); 2.60 (3H, s); 0.77 (3H, s).

IR (CH_2Cl_2): 2086, 2045, 2017 cm^{-1} ν(CO(M—CO).

6d

^1H NMR, 60 MHz ($CDCl_3$): 7.02 (1H, d, 8 Hz); 6.61 (1H, d, 8 Hz); 6.00 (2H, s); 4.64 (2H, s); 4.06 (2H, s); 3.66 (1H, m); 3.2 to 1.4 (broad envelope); 0.96 (9H, s); 0.84 (3H, s); 0.21 (6H, s).

7d

^1H NMR, 60 MHz ($CDCl_3$): 7.06 (1H, s); 6.54 (1H, s); 6.00 (2H, s); 4.16 (2H, s); 3.66 (1H, m); 3.2 to 1.4 (broad envelope); 0.96 (9H, s); 0.85 (3H, s); 0.21 (6H, s).

Reaction of estradiol (3d) with 1c

To a solution containing 1.1 g (2.6 mmol) of salt **1c** in 25 cm^3 of dry CH_2Cl_2 was added 0.60 g (2.2 mmol) of estradiol (**3d**). The resulting mixture was stirred at 0°C under N_2 for 12 h. The mixture was quenched with 50 cm^3 of saturated aqueous $NaHCO_3$, extracted thrice with CH_2Cl_2 (50 cm^3), the organic extracts dried over $MgSO_4$, concentrated, and the residue chromatographed over silica. Elution with ethyl ether/petroleum ether (60:40) afforded two closely spaced dark red fractions. The less polar, minor fraction (22%) exhibited aromatic ^1H NMR absorptions at 7.1 (s) and 6.4 (s) ppm, a complexed acetylenic resonance at 6.0 (s) ppm, plus a broad high-field envelope, and decomposed noticeably within minutes in solution. The more polar fraction (33%) had aromatic resonances at 7.1 (s) and 6.4 (s) ppm and a complexed acetylenic resonance at 6.0 (s) ppm. Demetalation of the latter complex was carried out by treatment with excess $Fe(NO_3)_3 9H_2O$ in 95% ethanol (0°C, 3 h) and standard aqueous extractive work-up. 2-(3-Butynyl)-estradiol was thus obtained as a colorless solid IR(KBr): 3500–3600, 2100 cm^{-1}. MS (70 eV): 324(M^+). ^1H NMR (acetone-d_6): 8.3 (1H, s); 7.4 (1H, s); 6.5 (1H, s); 2.4 (1H, s); 1.4 (3H, d, J = 7 Hz), 0.9–4.0 (broad envelope).

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