SYNTHESIS OF 3,4-$^{13}C_2$ STEROIDS*

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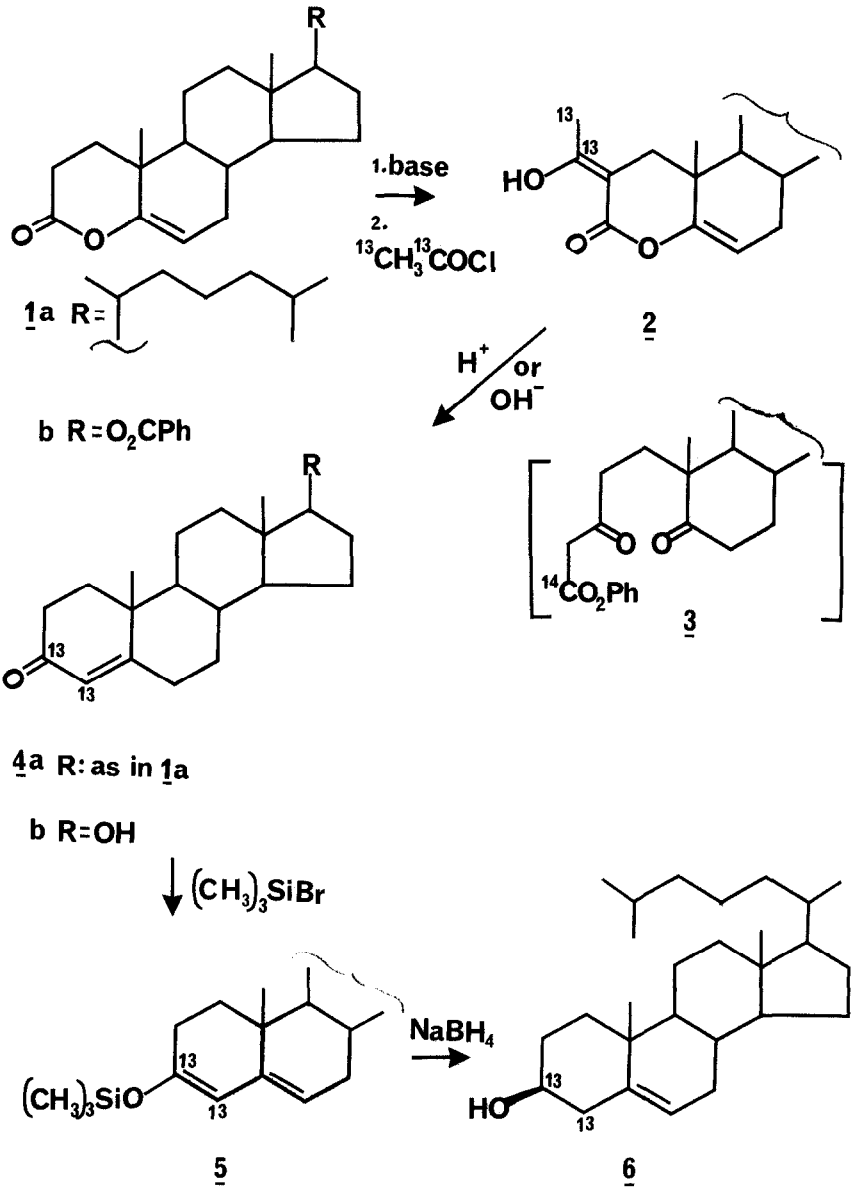
ABSTRACT

A-ring enollactones 1a, 1b or 9 derived from 4-cholesten-3-one, testosterone benzoate or 3-oxo-4-estren-17β-yl benzoate were condensed with [1,2-$^{13}C_2$]acetyl chloride to give intermediates 2a, 2b or 10. 2a and 2b were cyclized by acid or base to give 3,4-$^{13}C_2$-labeled 4-cholesten-3-one and testosterone, respectively. [3,4-$^{13}C_2$]4-Cholesten-3-one was converted via reduction of its trimethylsilyl enol ether to [3,4-$^{13}C_2$]cholesterol. Acetyl enollactone 10 was cyclized in acetic acid to [3,4-$^{13}C_2$]3-oxo-4-estren-17β-yl benzoate followed by aromatization and hydrolysis to produce [3,4-$^{13}C_2$]estradiol-17β. Alternatively, cyclization of 10 with base afforded [3,4-$^{13}C_2$]3-oxo-4-estren-17β-ol directly, which was then oxidized and aromatized to yield [3,4-$^{13}C_2$]estrone. Ozonolysis of progesterone, conversion to the diketal ester 16 and acylation followed by acid hydrolysis furnished [3,4-$^{13}C_2$]progesterone.

INTRODUCTION

Compounds multiply labeled with stable isotopes are needed in the development of definitive GC-MS quantitation methods for drugs and biochemicals(1-3). They also may be used as tracers for in vivo pharmacokinetic and metabolic studies(4,5). Thus, as part of a continuing program to explore these areas, we have synthesized several 3,4-$^{13}C_2$ labeled steroids, e.g. cholesterol, testosterone, estradiol-17β, estrone and progesterone.

Our synthetic strategy is similar to the scheme developed by Turner for the synthesis of 3- or 4-^{14}C-labeled 4-cholesten-3-one and testosterone(6) (Scheme I), wherein enollactone 1 was condensed with phenyl [1-^{14}C]acetate in the presence of sodium hydride. Since the enolate could be generated from either the enollactone 1 or phenyl acetate, two intermediates, 2 and 3, were produced and only intermediate 2 would lead to 3-^{14}C-labeled 4-cholesten-3-one or testosterone. In order to circumvent this loss of label from the acyl group, we chose to preform the enolate of the enol lactone 1 and then to condense it with [1,2-$^{13}C_2$]acetyl chloride to form intermediate 2 exclusively, thus preserving each of the labeled sites.


R
1.base
2.
$^{13}CH_3{}^{13}COCl$
HO
13
13
O
O
1a R=
2
H^+ or OH^-
b R=O_2CPh
R
13
13
O
O
$^{14}CO_2Ph$
3
4a R: as in 1a
b R=OH
$(CH_3)_3SiBr$
$(CH_3)_3SiO$
13
13
$NaBH_4$
HO
13
13
5
6

Scheme I

RESULTS AND DISCUSSION

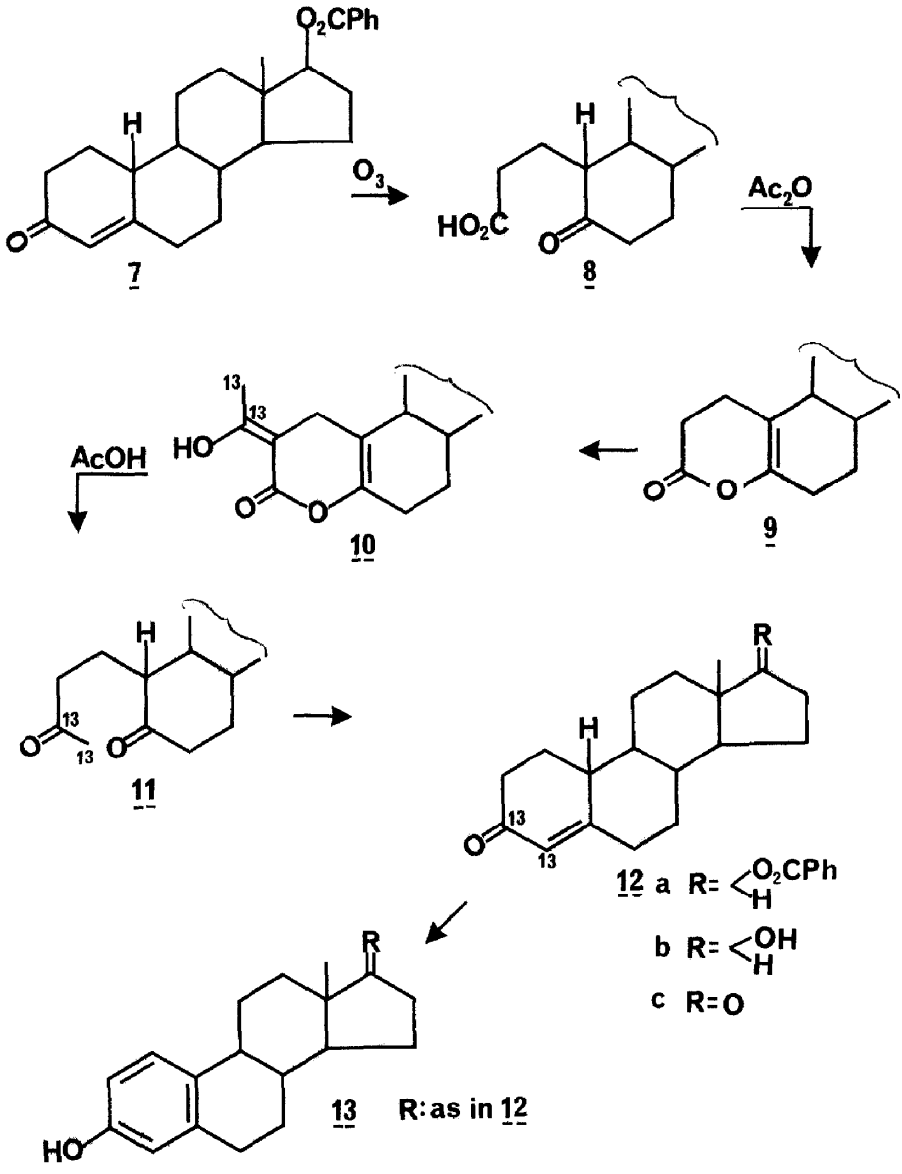
For generating the enolate of the enollactone 1, we found that either lithium bis(trimethylsilyl)amide or N-isopropylcyclohexylamide could be used(7) so that the condensation with [1,2-$^{13}C_2$]acetyl chloride (90% ^{13}C) would proceed with greater than 70% yield to give the product 2. By contrast, reaction with lithium diisopropylamide afforded compound 2 in only 40% yield. The enol configuration of the acetyl derivative 2 was established by its IR spectrum.

Neither acetyl derivative 2a nor 2b needed purification and could be converted directly by hydrolysis-decarboxylation-cyclization(6) in conc HCl-acetic acid or methanolic KOH to give [3,4-$^{13}C_2$]4-cholesten-3-one (4a) or [3,4-$^{13}C_2$]testosterone (4b) in about 40% overall yield after chromatographic isolation. The conversion of 4-cholesten-3-one to cholesterol was accomplished by the following sequence(8): enone 4a was silylated with trimethylsilyl bromide in pyridine to yield trimethylsilyl enol ether 5 which was then reduced with sodium borohydride to give predominantly [3,4-$^{13}C_2$]cholesterol (50% overall yield), along with small amounts of 3α-hydroxy and Δ^4-isomers. Purification of the [3,4-$^{13}C_2$]cholesterol was achieved by chromatography and crystallization.

A similar scheme was employed for the synthesis of a common intermediate 10 which could be converted to either estrone or estradiol-17β (Scheme II). Ozonolysis of benzoate (7) gave the keto acid 8, which in turn was cyclized(9) to yield the enollactone 9 with the double bond located at the 5-10 ring junction.

Condensation of the enolate of 9 with [2,3-$^{13}C_2$]acetyl chloride gave benzoate 10 in only 30% isolated yield. By contrast to the analogous preparation of acetylenollactone 2, apparently the absence of the 19-angular methyl group facilitates self-condensation of the lactone 9 as evinced by the presence of excess high field proton signals in the NMR of the crude product, thus lowering the yield at this step.

In order to convert benzoate 10 to estradiol-17β, the protected 17-hydroxyl needed to be preserved. This was accomplished by heating the benzoate 10 in glacial acetic acid to effect


O2CPh
H
O
7
O3
HO2C
8
Ac2O
9
AcOH
HO
10
11
R
12 a R= O2CPh
b R= OH
c R=O
13 R: as in 12
HO

Scheme II

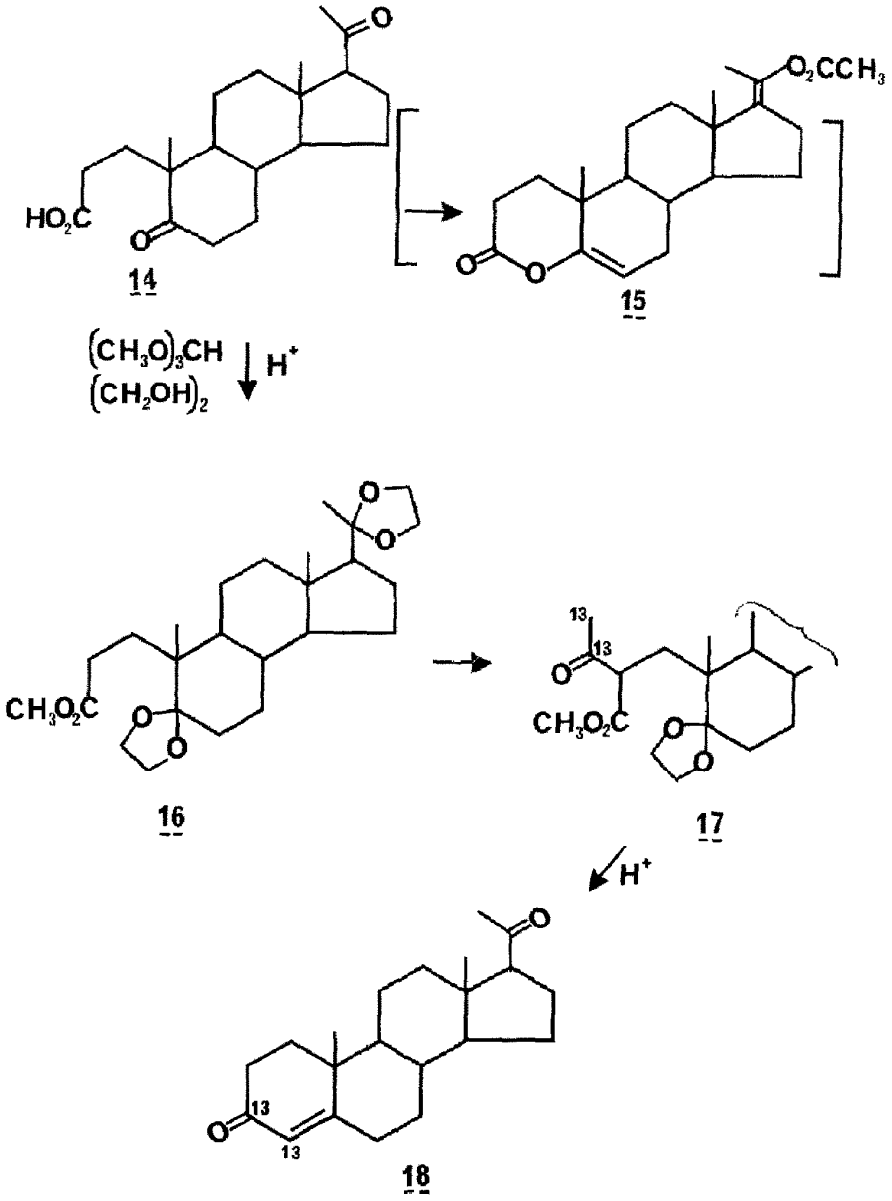
hydrolysis of ring A and decarboxylation to the dione 11 while leaving the benzoate group intact. Further heating cyclized the dione 11 to [3,4-$^{13}C_2$]3-oxo-4-estren-17β-yl benzoate (12a) in 70% yield. Aromatization of the benzoate 12a ($CuBr_2$, LiBr, CH_3CN)(10) and hydrolysis gave [3,4-$^{13}C_2$]estradiol-17 (13b) in 29% overall yield.

For the synthesis of estrone, the intermediate 10 was treated with methanolic KOH to yield [3,4-$^{13}C_2$]3-oxo-4-estren-17β-ol (12b) (62%). This was oxidized by CrO_3 to give dione 12c (78%)(11), and then dione 12c was aromatized to give [3,4-$^{13}C_2$]estrone (13c) (53% yield).

[3-^{14}C]Progesterone had been prepared by Gut(12) using Turner's approach by condensing the enollactone-20-enol acetate 15 with phenyl [1-^{14}C]acetate. The yield was very low because of label losses in the formation of an intermediate analogous to diketoester 3 and because of consumption of labeled reagent by reaction with the 20-enol acetate. To avoid these complications in our work, the known diketo acid 14 produced from ozonization of progesterone(12) was treated with ethylene glycol-trimethyl orthoformate p-toluenesulfonic acid(13) to generate a diketal ester 16 (55%) (Scheme III). Acylation of ester 16 by acetyl chloride could only proceed with the use of lithium N-isopropylcyclohexylamide and not with bis(trimethylsilyl)amide. The condensation product 17 was obtained in 75% yield and, surprisingly, it existed in the keto form. Treatment of acetyl derivative 17 with conc HCl in acetic acid then afforded [3,4-$^{13}C_2$]progesterone (18) in 70% yield.

EXPERIMENTAL

Materials and Methods: [1,2-$^{13}C_2$]Acetyl chloride, 90% ^{13}C, was synthesized using previously published methods(14). 4-Cholesten-3-one, testosterone benzoate, progesterone and 3-oxo-4-estren-17β-ol were purchased from Searle Chemicals, Inc., Chicago, IL. These were ozonized and then converted to their enollactones according to the previously published methods(6,9,12). ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Varian EM360A and Brucker HFX-100 NMR spectrometer. IR spectra were recorded on a Perkin-Elmer 727IR spectrophotometer. Only those spectral signals affected by ^{13}C were interpreted. Identities of the synthetic steroids were checked against authentic samples by TLC. Melting points were measured on a Fisher-Johns apparatus and were uncorrected.


HO2C
O
O
14
O2CCH3
O
O
15
(CH3O)3CH
(CH2OH)2
H+
CH3O2C
O
O
16
13
13
O
CH3O2C
O
O
17
H+
O
13
O
13
18

Scheme III

Optical rotations were measured in a Rudolph-62 polarimeter (C=2 dioxane). Literature values were from US Pharmacopeia XX, 1980.

Condensation of enolates with [1,2-$^{13}C_2$]acetyl chloride: Under N_2, n-butyllithium (2M in n-hexane, 0.2 mole) was added slowly to 0.2 mol of bis(trimethylsilyl)amine or N-isopropylcyclohexylamine in 500mL of dry tetrahydrofuran (THF) chilled with a -20°C ice-salt bath. After stirring for 1 h, the temperature was lowered to -78°C (dry ice-acetone) and 0.1 mole of the enollactones 1a, 1b, 9 or diketal ester 16 in 1L of dry THF was added slowly so as to keep the solution at -70°C. The reaction was stirred for an additional 1 h, and then 0.1 mole of [1,2-^{13}C]acetyl chloride in 20mL of THF was added dropwise to keep temperature below -70°C. One hour later, 0.2 mole of conc HCl was added, followed by 100mL of water. After warming up to room temperature, the organic layer was separated, washed with satd NaCl soln and then dried over Na_2SO_4. Evaporation of the solvent gave crude products 2a, 2b, 10 and 17, respectively.

2-[1',2'-$^{13}C_2$]Acetyl-4-oxa-5-cholesten-3-one (2a): The crude 2a could be purified by column chromatography on silica gel (10cm diameter x 20cm) using $CHCl_3$ as eluent to give 70mmol, (70%) of 2a. ^{1}H-NMR ($CDCl_3$): δ 0.7, 0.8, 0.9 and 1.1 (methyl groups), 2.1 (3H, dd, J_{CH}=130 Hz, J_{CCH}=6 Hz, $^{13}CH_3\,^{13}CO$, only one branch at 3.1 can be discerned), 5.3 (1H, d with fine splitting, 6-CH) and 13ppm (1H, b, enol OH). IR (KBr): 3350 (OH), 1725 (^{13}enollactone conjugated with enol), 1640 (C=C-O) and 1580 cm^{-1} (HO-^{13}C=C).

[3,4-$^{13}C_2$]4-Cholesten-3-one (4a): Crude 2a was heated to reflux with 80mL of conc HCl in 500mL of glacial acetic acid for 16 h. The acid was evaporated and the residue was chromatographed on silica gel using chloroform as eluent. The product fraction was evaporated and recrystallized from acetone to give 19g of 4a (50%). ^{1}H-NMR ($CDCl_3$): δ 0.7, 0.8, 0.9, 1.2 (methyl groups), 1.0-2.0 (m, methylene and methine protons), 2.0-2.6 (4H, m, 2-CH_2 and 6-CH_2) and 5.7ppm (1H, d, J_{CH}=160 Hz with fine splitting, 4-^{13}CH). IR (KBr): 1670 (sh, ^{12}C=O), 1630 (^{13}C=O) and 1585 cm^{-1} (^{13}C=C). mp 78° (lit. 79°).

[3,4-$^{13}C_2$]5-Cholesten-3β-ol (6): Under N_2, 100mL of dry pyridine, 100mL of bis(trimethylsilyl)amine and 10mL (80mmol) of bromotrimethylsilane were premixed in a 1L flask. A solution of 7.72g (20mmol) of 4a in 50mL of pyridine was added and the mixture was stirred at room temperature (rt) for 4 h. Dry ether (500mL) was then added and the supernatant was decanted into a 1L flask. The ether solution was evaporated to dryness to give 9g of 5 (100%). ^{1}H-NMR ($CDCl_3$): δ 0.2 (9H, s, $(CH_3)_3Si$), 0.7, 0.8, 0.9, 0.95 (methyl groups), 3.2 (1H, m, 6-CH) and 5.2ppm (1H, dd, J_{CH}=150 Hz, J_{CCH}=4 Hz, 4-^{13}CH). The silyl ether was again dissolved in 100mL of ether, and then 2.5g of $NaBH_4$ in 100mL of 2-propanol was added. After stirring at rt for 4 h, the excess hydride was destroyed by adding 1N HCl. The product was extracted into pentane-ether and then washed with satd $NaHCO_3$

solution. After drying (Na_2SO_4) and evaporation of the solvent, the crude product was chromatographed on a silica gel dry column using toluene:ethyl acetate=7:1. The cholesterol fraction was collected and then recrystallized from ethanol to give 3.8g of 6 (50%). ^{1}H-NMR ($CDCl_3$): δ0.65, 0.85, 0.9, 1.0 (methyl groups), 1.0-2.2 (m, methylene and methine protons), 2.2 (2H, dm, J_{CH}=140 Hz, only the signal at 3.3 is discernible, 4-$^{13}CH_2$), 3.5 (1H, dm, J_{CH}=140 Hz, 3-^{13}CH) and 5.3ppm (1H, m, 6-CH). ^{13}C-NMR ($CDCl_3$): δ 42 (d, 9% uncoupled, J_{CC}=35 Hz, 4-^{13}C) and 71ppm (d, 9% uncoupled, J_{CC}=35 Hz, 3-^{13}C), indicating the presence of 91% ^{13}C at each position. mp 147° (lit. 147-149°). $[\alpha]_D^{25°}$=-34° (lit. -34°). GC of its trimethylsilyl ether(8) indicated the presence of 1.5% each of α-OH and Δ^4-isomers.

2-[1',2'-$^{13}C_2$]Acetyl-3-oxo-4-oxa-5-androsten-17β-yl benzoate (2b): Crude 2b was obtained in 90% yield. ^{1}H-NMR ($CDCl_3$): δ1.0 (3H, s, 18-CH_3), 1.2-2.6 (m, methylene and methine protons), 2.1 (3H, dd, J_{CH}=128 Hz, J_{CCH}=6 Hz, $^{13}CH_3{}^{13}CO$), 4.9 (1H, 17-CH), 5.3 (1H, d, J=4 Hz, with fine splitting, 6-CH), 7.5-8.1 Hz (5H, m, aromatic). IR (KBr): 3350 (enol OH), 1720 (enollactone conj with enol and benzoate), 1640 (C=C-O) and 1590 cm^{-1} (HO-^{13}C=C).

[3,4-$^{13}C_2$]17β-Hydroxy-4-androsten-3-one (4b): Crude 2b was heated to reflux with 11g of KOH in 250mL of water and 1L of methanol for 24 h. Methanol was removed *in vacuo* and the solid crude product was isolated on a filter. Recrystallization from acetone gave 15g (50% yield) of 4b. ^{1}H-NMR ($CDCl_3$): δ0.8 (3H, s, 18-CH_3), 1.2 (3H, s, 19-CH_3), 1.0-2.1 (m, methylene and methine protons), 2.0 (1H, s, OH), 2.1-2.6 (4H, m, 2-CH_2 and 6-CH_2) and 5.75 (1H, d with fine splitting, J_{CH}=158 Hz, 4-^{13}CH). ^{13}C-NMR ($CDCl_3$): δ 124 (d, J_{CC}=51 Hz, 7% uncoupled, 4-^{13}C) and 199ppm (d, J_{CC}=51 Hz, 10% uncoupled, 3-^{13}C) indicating the presence of >90% ^{13}C at each position. IR (KBr): 3500 (OH), 1660 (sh, ^{12}C=O), 1620 (^{13}C=O) and 1590 cm^{-1} (^{13}C=C). mp 150° (lit. 153°). $[\alpha]_D^{25°}$=+97° (lit. 101°).

3-Oxo-4-oxa-5(10)-estren-17β-yl benzoate (9): 3-Oxo-4-estren-17β-ol was esterified with benzoyl chloride in pyridine to give benzoate 7 in 75% yield. This was ozonized to give gummy keto acid 8 (90%) (6). This crude keto acid was lactonized at 0° by acetic anhydride-perchloric acid in ethyl acetate(9), but 1/5 the prescribed amount of ethyl acetate was used so as to minimize the reaction volume. Pyridine was added to neutralize the solution, and the pyridine hydroperchlorate was removed by filteration. Most of the ethyl acetate was removed *in vacuo* and the product 9 crystallized out (33% overall from 7). ^{1}H-NMR ($CDCl_3$): δ0.96 (3H, s, 18-CH_3), 1.2-2.8 (m, methylene and methine protons), 4.9 (1H, t, J=6 Hz, 17-CH), 7.4-8.0ppm (5H, aromatic). IR (KBr): 1765 (enollactone) and 1720 cm^{-1} (benzoate).

2-[1',2'-$^{13}C_2$]Acetyl-3-oxo-4-oxa-5(10)-estren-17β-yl benzoate (10): The crude product from the condensation reaction was recrystallized from ethyl acetate to give 10 to 12g of 10 (25-30% yield).

^{1}H-NMR ($CDCl_3$): δ0.97 (3H, s, 18-CH_3), 1.2-2.6 (m, methylene and methine protons), 2.1 (3H, dd, J_{CH}=128 Hz, J_{CCH}=6 Hz, $^{13}CH_3$), 2.9 (2H, m, 1-CH_2) 4.9 (1H, t, J=6 Hz, 17-CH), 7.5-8.1 (5H, m, aromatic) and 10.4ppm (1H, m, OH). IR (KBr): 3450 (OH), 1705 (enollactone and benzoate), 1640 (C=C-O) and 1580 cm^{-1} (HO-^{13}C=C).

[3,4-$^{13}C_2$]3-Oxo-4-estren-17β-yl benzoate (12a): Intermediate 10 (8g, 19mmol) was heated to reflux in 80mL of glacial acetic acid. Carbon dioxide evolution started after 1 h, and an aliquot of the solution showed the following ^{1}H-NMR ($CDCl_3$) for 11 (not isolated): δ1.0 (3H, s, 18-CH_3), 1.0-2.7 (m, methylene and methine protons), 2.1 (3H, dd, J_{CH}=126 Hz, J_{CCH}=6 Hz, $^{13}CH_3$), 4.8 (1H, t, J=6.5 Hz, 17-CH) and 7.3-8.2ppm (5H, m, aromatic). After 24 h of heating, the acid was removed under reduced pressure, and the residue was recrystallized from ethanol to give 5.1g (70%) of 12a. ^{1}H-NMR ($CDCl_3$): δ1.0 (3H, s, 18-CH_3), 1.1-2.7 (m, methylene and methine protons), 4.8 (1H, t, J=7 Hz, 17-CH), 5.8 (1H, d with fine splitting, J_{CH}=162 Hz, 4-^{13}CH) and 7.3-8.1ppm (5H, m, aromatic). IR (KBr): 1715 (benzoate), 1675 (w, ^{12}C=O), 1635 (^{13}C=O) and 1595 cm^{-1} (^{13}C=C).

[3,4-$^{13}C_2$]3-Hydroxy-1,3,5(10)-estratrien-17β-yl benzoate (13a): Under N_2, a solution of 12a (5.1g, 14mmol) in 20mL of acetonitrile was added to a mixture of cupric bromide (6.3g, 28mmol) and lithium bromide (1.2g, 28mmol) in 50mL of acetonitrile. The mixture was heated to reflux for 1 h. (The literature(10) does not give any experimental details). Then, approximately 50g of ice chips were added and the reaction flask was transferred to an evaporator for removal of the acetonitrile under partial vacuum. Chloroform (100mL) was added to the concentrated reaction mixture, and the insoluble material was removed by filtration. The chloroform layer was dried (Na_2SO_4) and evaporated to give 2.6g of crude 13a (56%). ^{1}H-NMR ($CDCl_3$): δ1.0 (3H, s, 18-CH_3), 1.1-2.5 (m, methylene and methine protons), 2.8 (3H, m, 6-CH_2 and 9-CH), 4.91 (1H, t, J=7 Hz, 17-CH), 6.6 (1H, dm, J_{CH}=153 Hz, 4-CH) and 6.7-8.2ppm (7H, m, aromatic). IR: virtually identical to IR of unlabeled authentic sample.

[3,4-$^{13}C_2$]1,3,5(10)-Estratriene-3,17β-diol (13b): The crude 13a (2.6g, 6.9mmol) was heated to reflux with 6g of potassium hydroxide in 500mL of 50% aqueous ethanol. After 16 h, the solution was evaporated to remove most of the alcohol and then acidified with dil HCl. The organic material was extracted into CH_2Cl_2, and this was in turn washed with 4x100mL of satd $NaHCO_3$ solution. The CH_2Cl_2 solution was dried (Na_2SO_4) and evaporated. The crude product was purified on a dry silica gel column using 10% acetone in chloroform as eluent to give 1.5g (80%) of 13b. ^{1}H-NMR (acetone-d_6): δ0.8 (3H, s, 18-CH_3), 1.1-2.6 (m, methylene and methine protons), 2.75 (3H, 6-CH_2 and 9-CH), 2.9 (2H, s, OH), 3.7 (1H, t, J=8 Hz, 17-CH), 6.5 (1H, dm, J_{CH}=152 Hz, 4-^{13}CH, integration of these ^{13}C splitted doublet vs other aromatic signals showed the presence of 90% ^{13}C), 6.6 (1H, m, 2-CH) and 7.1ppm (1H, m, 1-CH). IR: virtually identical to IR of unlabeled authen-

tic sample. mp 170° (lit. 173°). $[\alpha]_D^{25°}$=+75° (lit. 76°).

[3,4-$^{13}C_2$]17β-Hydroxy-4-estren-3-one (12b): Intermediate 10 (20g, 50mmol) was heated to reflux with 8g of potasium hydroxide in 1L of 75% methanol in water for 16 h. Most of the methanol was removed by evaporation, and the product was extracted into ether. The ether solution was dried (Na_2SO_4) and evaporated. The crude oil was crystallized from acetone-hexane to give 8g (62%) of 12b. ^{1}H-NMR ($CDCl_3$): δ 0.8 (3H, s, 18-CH_3), 0.9-2.6 (m, methylene and methine protons), 3.65 (1H, m, 17-CH) and 5.8ppm (1H, dm, J_{CH}=160 Hz, 4-^{13}CH). IR (KBr): 3420 (OH), 1660 (sh, ^{12}C=O), 1615 (^{13}C=O) and 1595 cm^{-1} (^{13}C=C).

[3,4-$^{13}C_2$]3-Hydroxy-1,3,5(10)-estratrien-17-one (13c): Compound 12b was oxidized(11) to give [3,4-$^{13}C_2$]4-estren-3,17-dione (12c) in 78% yield. ^{1}H-NMR ($CDCl_3$): δ 0.9 (3H, s, 18-CH_3), 1.1-2.7 (m, methylene and methine protons), 5.8ppm (1H, dm, J_{CH}=160 Hz, 4-^{13}CH). IR (KBr): 1640 (17-C=O), 1660 (w, 3-^{12}C=O), 1630 (^{13}C=O) and 1595 cm^{-1} (^{13}C=C). Aromatization followed by chromatography, as reported for 13a, gave 13c in only 53% yield (lit.(10) claimed 85%). ^{1}H-NMR (acetone-d_6): δ 0.9 (3H, s, 18-CH_3), 1.2-2.6 (m, methylene and methine protons), 2.7 (1H, s, OH), 2.8 (3H, m, 6-CH_2 and 9-CH), 6.5 (1H, dm, J_{CH}=156 Hz, 4-^{13}CH, integration showed the presence of 90% ^{13}C), 6.6 (1H, m, 2-CH) and 7.1 (1H, m, 1-CH). IR: virtually identical to IR of unlabeled authentic sample. mp 250° (lit. 255°). $[\alpha]_D^{25°}$=+154° (lit. 158°).

Methyl 5,20-bis(1',2'-ethylendioxy)-4,5-seco-4-norpregnan-3-oate (16): Diketo acid 14 (49g) was stirred at rt with 300mL of trimethyl orthoformate, 300mL of ethylene glycol and 1g of p-toluenesulfonic acid for 16 h. Enough satd sodium carbonate solution was added to neutralize the acid, and this solution was diluted further with 300mL of water. The insoluble phase was extracted into CH_2Cl_2, and the organic solution was dried over Na_2SO_4. Evaporation of the solvent gave a crude solid which was recrystallized from methanol to give 34g of 16 (56%). ^{1}H-NMR ($CDCl_3$): δ 0.8 (3H, s, 18-CH_3), 1.0 (3H, s, 19-CH_3) 1.3 (3H, s, 21-CH_3), 1.0-2.1 (m, methylene and methine protons), 2.4 (2H, m, 2-CH_2), 3.65 (3H, s, OCH_3) and 3.9ppm (8H, s, OCH_2CH_2O). IR (KBr): 1730 (O-C=O), 1050-1250 cm^{-1} (C-O-C).

[3,4$^{13}C_2$]Methyl 5,20-bis(1',2'-ethylenedioxy)-3-oxo-4,5-secopregnane-2-carboxylate (17): The diketal ester 16 (44g) was condensed with acetyl chloride-1,2-$^{13}C_2$ to give 49g of oily 17. This was purified by chromatography on silica gel using ether-hexane (1:2) to give crystalline 17 in 70% yield. ^{1}H-NMR ($CDCl_3$): δ 0.8 (3H, s, 18-CH_3), 1.0 (3H, s, 19-CH_3), 1.3 (3H, s, 21-CH_3), 1.0-2.5 (m, methylene and methine protons), 2.2 (3H, dd, J_{CH}=130 Hz, J_{CCH}=6 Hz, $^{13}CH_3$) 3.2 (1H, d, J_{CCH}=6 Hz, 2-CH), 3.7 (3H, s, OCH_3) and 3.9ppm (8H, m, ketal). IR (KBr): 1740 (O-C=O) and 1680 cm^{-1} (^{13}C=O).

[3,4-$^{13}C_2$]4-Pregnene-3,20-dione (18): Crude 17 was heated to reflux with 100mL of conc HCl in 500mL of acetic acid for 16 h.

Acetic acid was removed in vacuo and the residue was taken up in 500mL of ether. The ether solution was washed 3x with 200mL of water, to extract the glycol diacetate, then dried and evaporated. The crude product was chromatographed on a silica gel dry column using ether-hexane (1:2), and the isolated fraction was recrystallized from ethanol to give 15g (49%) of 18. ^{1}H-NMR ($CDCl_3$): δ 0.7 (3H, s, 18-CH_3), 1.2 (3H, s, 19-CH_3), 0.9-2.7 (m, methylene and methine protons), 2.1 (3H, s, 21-CH_3) and 5.7ppm (1H, d with fine splitting, J_{CH}=160 Hz, 4-^{13}CH). ^{13}C-NMR ($CDCl_3$): δ124 (d, 9% uncoupled, $^{13}J_{CC}$=52 Hz, 4-^{13}C) and 199ppm (d, 9% uncoupled, J_{CC}=53 Hz, 3-^{13}C) indicating 91% ^{13}C at each position. IR (KBr): 1700 (C=O), 1660 (sh, 3-^{12}C=O), 1625 (^{13}C=O) and 1895 cm^{-1} (^{13}C=C). mp 125° (lit. 125°). $[\alpha]_D^{25}$=+174° (lit. 175°).

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