PREPARATION OF ^{3}H- AND ^{14}C-LABELLED CONTRACEPTIVE STEROIDS

Kenyu Shibata*, Yoshio Osawa and Avery A. Sandberg ▽**
Medical Foundation of Buffalo, Buffalo, New York, 14203, U.S.A.
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SUMMARY

^{3}H- and ^{14}C-Labelled ethynodiol diacetate, chlormadinone acetate, and medroxyprogesterone acetate with one label at the steroid nucleus and the other at the acetoxy group were prepared for use in studies of regio- and stereospecificity of metabolic hydrolysis of the steroid contraceptives. Steroidal secondary, tertiary, equatorial, and axial acetates were labelled under selective conditions using acetic anhydride.

Oral contraceptive steroids such as ethynodiol diacetate[1], chlormadinone acetate, and medroxyprogesterone acetate have been used clinically.[2-7] These steroids contain an acetoxy group in different molecular environments. Ethynodiol diacetate has a secondary quasi-equatorial allylic acetate at 3β and a tertiary equatorial acetate at 17β adjacent to an ethynyl function. Chlormadinone acetate has a tertiary axial acetate at 17α adjacent to a carbonyl function and a remote 6-Cℓ-$\Delta^{4,6}$-3-one system. Medroxyprogesterone acetate has a similar tertiary axial acetate at 17α adjacent to a carbonyl with a remote 6α-methyl-Δ^{4}-3-one system. To assess the rates of metabolic

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*Postdoctoral fellow, 1972-74, on leave from Research Laboratory, Teikoku Hormone Mfg. Co. Ltd., Kawasaki, Japan.

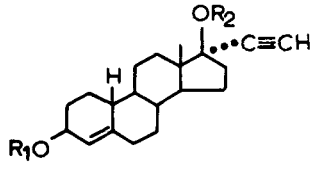
**American Cancer Society Faculty Research Awardee PRA-72; to whom correspondence is to be addressed.

▽Roswell Park Memorial Institute, Buffalo, New York, 14203, U.S.A.

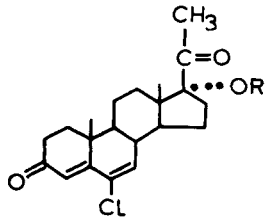
hydrolysis of such contraceptives and aid in the detection and separation of specific acetate carrying metabolites from hydrolyzed ones, the synthesis of steroid acetates with one label at a specific acetate and the other in the steroid nucleus was desired.

17α-Ethynyl-4-estrene-3β,17β-diol (I) was acetylated with tritiated acetic anhydride in pyridine at room temperature to give the 3-monoacetate (II). The labelled 3-monoacetate (II) was further acetylated with unlabelled acetic anhydride under reflux temperature to give the 3-acetoxy labelled diacetate (III) without changing the specific activity of ^{3}H of the 3-acetoxy group. Even though it is well known that acid catalyzed acetylation of tertiary alcohols using p-TsOH [8] or $HClO_4$ [9] gives satisfactory results in preparative synthesis, such conditions were found inappropriate for the micro-scale synthesis of a labelled compound due to undesirable contamination of labelled by-products caused by rearrangement reactions. The use of a reported mild method using p-TsOH-Ac_2O-AcOH [10] was avoided, because of possible unfavorable isotope dilution. Direct heating under reflux of the monoacetate with acetic anhydride gave the most satisfactory product in the labelled synthesis. In reverse order of the labelling, 17α-ethynyl-4-estrene-3β,17β-diol 3-acetate (IV) was acetylated with tritiated acetic anhydride under reflux temperature to yield the 17-acetoxy labelled diacetate (V). Both diacetates (IV and V) were purified in a crystalline form and characterized as identical with an authentic sample [11] by NMR and IR spectroscopy.

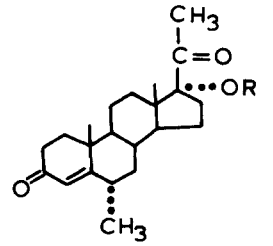
6-Chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione (VI) was heated with [1-^{14}C]acetic anhydride under reflux temperature for 4 hr to give 17-acetoxy labelled chlormadinone acetate (VII). The IR spectrum was identical with that of an authentic sample. [12] 17α-Hydroxy-6α-methyl-4-pregnene-3,20-dione (VIII) was acetylated with [1-^{14}C]acetic anhydride in the same way to give 17-acetoxy labelled medroxyprogesterone acetate (IX). [13]



I. $R_1 = R_2 = H$
II. $R_1 = {}^{3}H\text{-}Ac$, $R_2 = H$
III. $R_1 = {}^{3}H\text{-}Ac$, $R_2 = Ac$
IV. $R_1 = Ac$, $R_2 = H$
V. $R_1 = Ac$, $R_2 = {}^{3}H\text{-}Ac$



VI. R = H
VII. $R = {}^{14}C\text{-}Ac$



VIII. R = H
IX. $R = {}^{14}C\text{-}Ac$

Table 1. Recrystallization after addition of carrier steroid to an aliquot of the labelled acetate.

Compound	Recryst.	Specific Activity	
		dpm $^{3}H x 10^{4}$/mg	dpm $^{14}C x 10^{3}$/mg
[17-OAc-^{3}H-4-^{14}C]EDA	1st.	3.71	1.71
	2nd.	3.76	1.74
	3rd.	3.72	1.74
[3-OAc-^{3}H-4-^{14}C]EDA	1st.	1.74	1.58
	2nd.	1.70	1.59
	3rd.	1.71	1.63
[17-OAc-^{14}C-1-^{3}H]CAP	1st.	2.52	1.19
	2nd.	2.34	1.20
	3rd.	2.30	1.17
[17-OAc-^{14}C-1,2-^{3}H]MAP	1st.	1.75	0.829
	2nd.	1.72	0.842
	3rd.	1.72	0.850

The acetoxy labelled compound thus synthesized was mixed with the steroid nucleus labelled counterpart: III and V with [4-^{14}C]ethynodiol diacetate,[14] VII with [1-^{3}H]chlormadinone acetate,[15] and IX with [1,2-^{3}H]medroxyprogesterone acetate.[16] The mixed doubly labelled compound was purified by thin layer chromatography and recrystallization. The purity was further analyzed by recrystallization to constant specific activity, after addition of carrier to an aliquot of the prepared labelled compound, as shown in Table 1.

EXPERIMENTAL SECTION

Melting points were measured on a Fisher-Jones melting point apparatus and are uncorrected. Nuclear magnetic resonance (nmr) spectra were obtained with a Varian EM-360 spectrometer using deuteriochloroform as solvent and the chemical shifts are reported in parts per million (ppm) on the δ scale (tetramethylsilane = 0). Infrared (ir) spectra were recorded with a Perkin-Elmer 267 spectrophotometer on KBr disks. Silica gel GF254 was used for thin layer chormatography (tlc). [^{3}H]Acetic anhydride (100 mCi/mmole) was purchased from the Amersham Searle Corp. (Arlington Heights, Ill.). [^{14}C]Acetic anhydride was purchased from Amersham Searle (19 mCi/mmole) and New England Nuclear Corp. (Boston, Mass.) (5.0 mCi/mmole). [4-^{14}C] Ethynodiol diacetate was given by Dr. Kenneth King of Searle Laboratories (Chicago, Ill.), [1-^{3}H]chlormadinone acetate was a gift from Dr. R. Forchielli of Syntex Research (Palo Alto, Cal.) and [1,2-^{3}H]medroxyprogesterone acetate was given by Dr. C. Wayne Bardin of Milton S. Hershey Medical Center (Hershey, Pa.).

[3-OAc-^{3}H-4-^{14}C]Ethynodiol Diacetate

17α-Ethynyl-4-estrene-3β,17β-diol (I) (30mg) was acetylated with [^{3}H]acetic anhydride (0.06 ml, 20 mCi/mmole) in pyridine (0.24 ml) at room temperature for 20 hr. The product was purified by tlc (benzene-acetone, 19:1) followed by recrystallizations from aqueous methanol to give [3-OAc-^{3}H]17α-ethynyl-4-estrene-3β,17β-diol 3-acetate (II) as

colorless leaflets, mp. 75-80°, (25.63 mg, 7.4 mCi/mmole) ir (ν_{max} cm^{-1}) 3540 (OH), 3300 (-C≡CH), 1720 (-$COCH_3$), 1258 (-O-$COCH_3$); nmr δ 0.88 (18-CH_3, 3H, s.), 2.05 (OAc, 3H, s.), 2.56 (-C≡CH, 1H, s.), 5.22 (3α-H, 1H, m.), 5.33 (4-H, 1H, b.s.). The 3-monoacetate II (15.34 mg) was heated with acetic anhydride (0.15 ml) under reflux for 1 hr. The mixture was poured into ice water and the product was purified by tlc (benzene-acetone, 19:1) followed by recrystallizations from n-hexane yielding [3-OAc-^{3}H]ethynodiol diacetate (III) as colorless prisms, mp 127-129° (14.3 mg, 7.3 mCi/mmole), ir 3300 (-C≡CH), 1745 (-$COCH_3$), 1733 (-$COCH_3$), 1247 (-O-$COCH_3$); nmr δ 0.88 (18-CH_3, 3H, s.), 2.05 (OAc, 6H, s.), 2.56 (-C≡CH, 1H, s.), 5.22 (3α-H, 1H, m.), 5.33 (4-H, 1H, b.s.). The diacetate III was mixed with [4-^{14}C]ethynodiol diacetate (13.9 μCi, 7.54 mCi/mmole) and purified by tlc followed by recrystallizations from n-hexane. The doubly labelled diacetate, mp 127-129°, 11.4 mg, was obtained with a specific activity of 7.0 mCi/mmole for ^{3}H and 318 μCi/mmole for ^{14}C.

[17-OAc-^{3}H-4-^{14}C]Ethynodiol Diacetate

17α-Ethynyl-4-estrene-3β,17β-diol 3-acetate (IV) (12.55 mg) was heated with [^{3}H]acetic anhydride (0.125 ml, 8.2 mCi/mmole) under reflux for 1 hr. The diacetate V was obtained after purification by tlc (benzene-acetone, 19:1) followed by recrystallizations from n-hexane as colorless prisms, mp 127-129° (6.31 mg, 8.2 mCi/mmole). The ir and nmr spectra were identical with those of the [3-OAc-^{3}H] compound obtained above.

Compound V, [4-^{14}C]ethynodiol diacetate (13.5 μCi, 7.65 mCi/mmole) and carrier (4.02 mg) were mixed and purified by tlc (benzene-acetone, 19:1) followed by recrystallizations from n-hexane. The doubly labelled diacetate (5.72 mg) was obtained with a specific activity of 2.74 mCi/mmole for ^{3}H and 248 μCi/mmole for ^{14}C.

[1-^{3}H-17-OAc-^{14}C]Chlormadinone Acetate

A solution of 6-chloro-17α-hydroxy-4,6-pregnadiene-3,20-dione

(VI) (10 mg) in [1-^{14}C]acetic anhydride (51 µl, 460 µCi/mmole) was heated under reflux for 4 hr. The mixture was poured into ice water and the product was purified by tlc (benzene-acetone, 19:1) to give 5.52 mg of chlormadinone acetate (VII) (224 µCi/mmole), ir 1738 (OAc), 1713 (-$COCH_3$), 1657 (C=O), 1244 (O-AC). Compound VII and [1-^{3}H]chlormadinone acetate (61.2 µCi, 9 Ci/mmole), were mixed and purified by tlc (benzene-acetate, 19:1) to give crystalline chlormadinone acetate (5.30 mg, 4.76 mCi/mmole for ^{3}H and 225 µCi/mmole for ^{14}C.

[1,2-^{3}H-17-OAc-^{14}C]Medroxyprogesterone Acetate

17α-Hydroxy-6α-methyl-4-pregnene-3,20-dione (VIII) (14.85 mg) was acetylated with [1-^{14}C]acetic anhydride (54 µl, 3.78 mCi/mmole) under reflux for 4 hr. The acetylated compound was isolated and purified by repeated tlc (benzene-acetone, 9:1) to give [17-OAc-^{14}C] medroxyprogesterone acetate (IX) (1.2 mg, 5.85 µCi). It was mixed with [1,2-^{3}H]medroxyprogesterone acetate (118 µCi, 58 Ci/mmole) and further purified by tlc (benzene-acetone, 19:1) to show a constant ratio of ^{3}H/^{14}C = 21.1 (88.5 µCi^{3}H and 4.2 µCi^{14}C; 38.4 mCi^{3}H and 1.82 mCi^{14}C/mmole). Constant specific activities were obtained when these doubly labelled acetates were recrystallized after addition of carrier (see Table 1).

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REFERENCES AND NOTES

1. Abbreviations and trivial names used are ethynodiol diacetate, EDA, 3β,17β-diacetoxy-17α-ethynylestr-4-ene; chlormadinone acetate, CAP, 6-chloro-17α-acetoxy-4,6-pregnadiene-3,20-dione; medroxyprogesterone acetate, MAP, 17α-acetoxy-6α-methyl-4-pregnene-3,20-dione.

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14. [4-^{14}C]Ethynodiol diacetate was given by Dr. Kenneth King of G. D. Searle Co.

15. [1-^{3}H]Chlormadinone acetate was a gift from Dr. R. Forchielli of Syntex Research.

16. [1,2-^{3}H]Medroxyprogesterone acetate was given by Dr. C. Wayne Bardin, Milton S. Hershey Medical Center.