SYNTHESIS OF THE CONTRACEPTIVE PROGESTIN LYNESTRENOL-^{3}H FROM TRITIATED ESTR-4-EN-17-ONE.

J.M.G. van Kordelaar*, J.S. Favier** and J.P. Kitcher***

*Department of Pharmacology, University of Nijmegen, Nijmegen, The Netherlands.

**Drug Metabolism R&D Laboratories, Organon Scientific Development Group, Oss, The Netherlands.

***Organic Department, The Radiochemical Centre, Amersham, England.

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SUMMARY

Estrenone-16-^{3}H with a specific activity of 12 Ci/mmol was prepared from estrenone by an exchange procedure with tritiated water in dimethylformamide. Lynestrenol -^{3}H(specific activity 800 mCi/mmol) was obtained from estrenone-^{3}H by reaction with acetylene and potassium t-butoxide in tetrahydrofuran.

INTRODUCTION

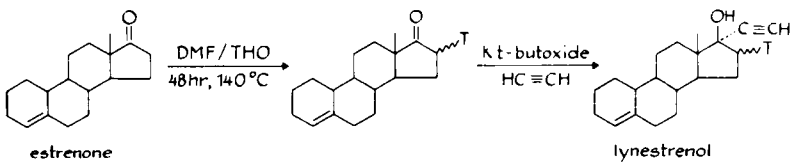
Lynestrenol (17α-ethynyl-estr-4-en-17β-ol) (1) is one of the series of 17α-ethynyl-19-nortestosterone derivatives currently in use as an active principle of many contraceptive preparations.

In the course of studies of its pharmacokinetics and mechanism of action the availability of the tritium labelled compound with a high specific activity was considered to be an indispensable tool.

Although lynestrenol is chemically closely related to norethindrone, norethynodrel and ethynodiol, tritium labelling as described for these compounds by Kepler and Taylor (2) or Chaudhuri and Gut (3), is not possible because of the lack of the

oxygen atom at C_3. This also holds for tritium labelling of norethindrone as described by Rao (4). For the same reason various other methods, based on partial reduction of unsaturated precursors are not feasible. The only other way to obtain a high specific labelled product, apart from a multistep synthesis, would be a halogen-tritium replacement (3, 5), but this again would result in complete saturation of the delta-4 double bond.

The pharmacological studies envisaged required a compound with high specific activity, even at the expense of non-specificity of labelling. We considered that this may be best achieved by tritium labelling of estrenone (estr-4-en-17-one) via exchange with tritiated water (THO) in dimethylformamide followed by an ethynylation reaction at C_{17} to yield lynestrenol. The C_{16} hydrogen atoms of estrenone are able to exchange for tritium under conditions causing enolization of the 17-keto group. After subsequent incorporation of the 17α-ethynyl group these labile tritium atoms will be stabilised, provided they are not lost in the procedure. The introduction of the ethynyl group can be achieved in essentially the same way as described by Djerassi (6), but with some adaptations for radio-isotope work on a micro scale. The sequence of the reactions that was followed is depicted below.



estrenone lynestrenol

The specific activity attained depends very much on the temperature and duration of the exchange reaction (5). For estrenone this appeared to be no problem: heating the non-labelled compound for a period of 16 hours at 160°C in an evacuated ampoule with dimethylformamide and water did not result in the formation of other products.

The contamination of estrenone-^{3}H with estranone-^{3}H after the exchange reaction, although not very probable, required special attention. Even small amounts of estranone-^{3}H, present in estrenone-^{3}H would, if not removed, cause a

serious radiochemical contamination of lynestrenol-^{3}H. The two 17-keto compounds can be separated by TLC on silica gel silver nitrate plates (7), but this method can not be used after the introduction of the 17α-ethynyl group.

MATERIALS

All reagents were of Analar grade.

Estrenone was obtained from Steraloids Ltd., Croydon, U.K.

Lynestrenol, used as a carrier during the identification of lynestrenol-^{3}H was obtained from Organon, Oss, The Netherlands.

Identity and purity of these compounds was authenticated by IR spectroscopy, melting point, mass-spectrometry, and gas-liquid chromatography.

The following solvents were specially purified. Dimethylformamide (DMF) was stored over molecular sieve 4A (BDH Ltd., Poole, Dorset, U.K.) for 2 days, decanted and then distilled. Tetrahydrofuran (THF) was distilled under nitrogen, from lithium aluminium hydride after standing over this compound for 24 hours. The distillate was stored under nitrogen, in the dark, in a phosphorous pentoxide desiccator and used within five days. Potassium t-butoxide was prepared by reacting cleaned potassium (8) with t-butanol under nitrogen. The solution was pumped to dryness on a manifold and the white residue purified by sublimation under reduced pressure. Benzene and ethanol used for storage of tritiated materials in solution were redistilled prior to use.

Acetylene was purified by passing it through a cold (-80°C) trap, a mercury safety valve, an empty bottle, concentrated sulphuric acid, and a soda-lime tower.

RADIOACTIVITY MEASUREMENTS

Radioactivity measurements were made with a Nuclear-Chicago Mark I counter using the toluene: triton X100 scintillator of Patterson & Greene (9).

THIN LAYER CHROMATOGRAPHY

Analytical chromatography was performed on Merck 0.25 mm silica gel pre-

coated plates, or with plates spread with 10% silver nitrate impregnated silica gel (thickness 0.25 mm). Active regions were located by scanning the developed plate with a windowless gas flow proportional counter.

Steroid markers were visualized by spraying with a 2.5% w/v solution of ceric sulphate in 2N-sulphuric acid and heating at 110°C for about 10 minutes. Preparative chromatography was performed on 1 mm silica gel PF_{254} coated plates. Active regions were located on developed plates by autoradiography. Labelled steroids were recovered by scraping off the silica gel and extracting with benzene: ethanol (9:1).

Table I shows the solvent systems which were used together with typical Rf values of steroids likely to occur in the crude reaction product.

TABLE 1
TLC chromatographic data steroids

system	solvents		adsorbent	Rf values steroids*			
				I	II	III	IV
A	benzene/ethanol	99/1	silica gel	0.38	0.23	0.60	--
B	cyclohexane/ ethyl acetate	9/1	silica gel	0.32	0.20	0.54	--
C	cyclohexane/ acetone	95/5	silica gel	0.29	0.23	0.60	--
D	cyclohexane/ acetone	95/5	silica gel/ $AgNO_3$	0.00	0.32	0.66	0.85

*I.lynestrenol, II.estrenol, III.estrenone, IV.estranone

ESTRENONE-16-^{3}H

Estrenone (144 mg) was dissolved in a mixture of purified dimethylformamide (1 ml) and tritiated water (0.3 ml, 200 Ci). This mixture was sealed in an ampoule under vacuum and heated at 140°C for 48 hours. After cooling the solvent was removed under vacuum, followed by removal of labile tritium with benzene:ethanol (4:1). The residue was taken up in chloroform (1 ml) and purified by TLC on two

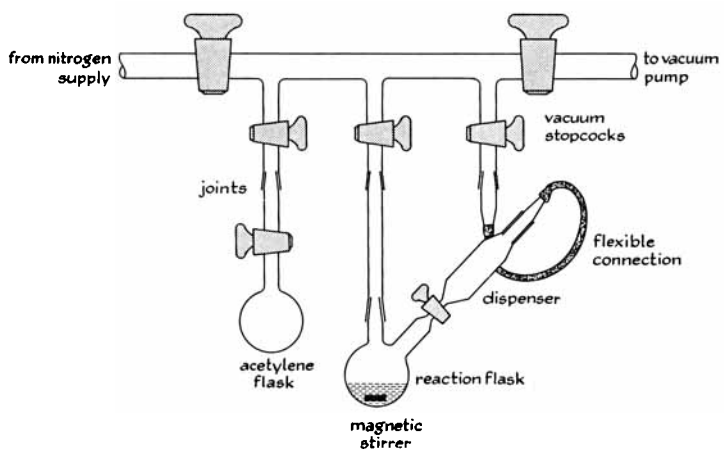
1 mm silica gel plates, developed in system C.

The weight of estrenone-^{3}H in the whole sample was measured after rotary evaporation of the benzene:ethanol (9:1) solution to dryness and storage in a vacuum desiccator until the weight remained constant. The sample was redissolved in benzene:ethanol (9:1), counted and its specific activity calculated. The solution of estrenone-^{3}H was analysed by TLC in systems A, B, C and D.

Yield:	Estrenone-^{3}H	1.8 Ci 40.5 mg
	Specific activity	12 Ci/mmol
	Radiochemical purity	98% in systems A, B, C and D.

LYNESTRENOL-^{3}H

The reagents required for the conversion of estrenone-^{3}H to lynestrenol-^{3}H were manipulated in an enclosure filled with dry nitrogen. A solution of potassium t-butoxide in purified tetrahydrofuran (THF) (20 ml) was prepared. Aliquots (2 ml) of this were removed from the enclosure, diluted with water (1 ml) and then evaporated under a nitrogen stream to remove excess THF. These samples were then titrated against 0.1M hydrochloric acid to determine the base concentration.



Estrenone-^{3}H solution was evaporated to dryness and 0.12 mmol (31.5 mg) of this residue was dissolved in purified THF (3 ml). This together with a rinsing of THF (2 ml) was transferred to a small separating funnel. The acetylene flask was evacuated and filled with acetylene (volume about 20 ml = 1 mmol). Potassium t-butoxide (0.2 mmol of base) in THF (5 ml) was placed in the reaction flask (see figure), which was temporarily frozen with liquid nitrogen. The space above the solution in the separating funnel was partially evacuated and the main system was evacuated to a final pressure between 0.1 and 0.05 torr. The acetylene was condensed into the reaction flask and the stopcocks closed. The system was warmed to 0°C and with vigorous stirring the estrenone-^{3}H solution was added from the separating funnel. This mixture was stirred for 3 hours. Nitrogen was admitted to the system and THF:water, 20:1 (0.1 ml) was added to the reaction flask, cooled to 0°C. About 1 minute later Amberlite AG50 H^{+} resin (0.5 g) was added and the mixture was stirred for a further 20 minutes, whereupon it was filtered and the filter washed with THF and benzene. The combined filtrate was rotary evaporated under reduced pressure to dryness. The residue was taken up in chloroform, a sample was taken for analysis, and the bulk of this solution applied to two 1 mm silica gel plates. These and also the analytical plate were developed with system B.

Lynestrenol-^{3}H and estrenone-^{3}H were removed separately from the plate and eluted from the silica. The resulting lynestrenol-^{3}H solution was rotary evaporated to small bulk and transferred to a tared flask. The solvent was removed in vacuo at room temperature and pumped to constant weight. The crystalline residue was dissolved in benzene, assayed and analysed in systems A, B and C.

Yield:	Lynestrenol-^{3}H	29 mCi	10.4 mg
	Specific activity	800 mCi/mmol	
	Recovered estrenone-^{3}H	52 mCi	

Radiochemical Purity

After determination of the specific activity the lynestrenol-^{3}H, cochromatographed with carrier, was 98% pure in systems A and B, 97% pure in system C. The impurities were minor decomposition products which had arisen during the

manipulation of the sample.

The recovered estrenone-^{3}H was 95% pure in system C. The principle impurity was lynestrenol-^{3}H.

DISCUSSION

The dimethylformamide/tritiated water exchange reaction was carried out three times in all, the quality (age and previous use) of the solvent mixture being varied. The best result (Exp. 3) was obtained with a freshly prepared solution. These results together with the yields in the subsequent ethynylation reactions are shown in table 2. The conditions of tritiation in all cases resulted in loss of the starting material estrenone, but this was acceptable for the resultant decomposition products exhibited low Rf values in system C and were easily separated from estrenone-^{3}H.

TABLE 2

Yields of Estrenone-H3 and Lynestrenol-H3

Experiment	Quantity Estrenone	Yield of Estrenone-H3			Yield of Lynestrenol-H3			Recovered Estrenone-H3	
		by weight	activity	specific activity	by weight	activity	specific activity	activity	specific activity
	mg	%	mCi	mCi/mmol	%	mCi	mCi/mmol	mCi	mCi/mmol
1	220	61	440	880	23	23.5	230	34	240
2	230	60	1020	2000	60	77	270	28	--
3	144	30	1700	12000	32	28	800	52	--

In all cases the ethynylation reaction was accompanied by considerable loss of tritium from the 16 position. This is demonstrated by the activity yields of both lynestrenol-^{3}H and estrenone-^{3}H shown in table 2. In the case where the specific activity of the recovered estrenone-^{3}H was measured it was found to be similar to the product, lynestrenol-^{3}H. This result indicates that the activity found in the

products after the ethynylation reaction could be in positions other than C-16. This non-specific introduction of tritium is perhaps to be expected, considering the vigorous conditions (140°C, 2 days) of the initial tritiation.

In addition to the DMF catalysed exchange reactions, an attempt was made to tritiate estrenone by heating it at 135°C for 16 hours in the presence of acetic acid (carboxyl-^{3}H) and platinium black. The product from this reaction contained much activity and superficially behaved like estrenone. On careful analysis in solvent systems A, B and C the activity separated from the estrenone marker. No further work was conducted with this product.

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