Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 6

PREPARATION OF DEUTERIUM AND TRITIUM LABELLED NORETHYNODREL, NORETHINDRONE, AND 6-METHYLENEANDROSTENEDIONE

Herbert L. Holland,[1]* Frances M. Brown*, and Liat Tan⊖
Department of Chemistry, Brock University, St. Catharines, Ontario, L2S 3A1, Canada*; Biochemistry Department, University of Sherbrooke Medical Faculty, Fleurimont, Quebec, J1H 5N4, Canada⊖

SUMMARY

A convenient, one step method for the preparation of deuterium or tritium labelled norethynodrel, norethindrone, and 6-methyleneandrostenedione is reported which utilizes water as the source of label. The choice of exchange reagent (lithium or sodium hydroxide) is crucial in controlling rearrangement of the chemically labile β,γ unsaturated carbonyl function of norethynodrel.

Key Words: Norethynodrel, norethindrone, norethisterone

INTRODUCTION

The progestin hormones norethynodrel (1) and norethindrone (norethisterone) (2) are widely used as components of birth control pills: these compounds or their derivatives are in regular use by over 60 million women worldwide (1).

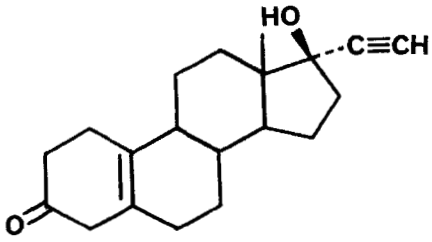
The use of tritium labelling has been pivotal in any study of the metabolic fate of these hormones (2), but in spite of this, neither 1 nor 2 is routinely commercially available in a labelled form, and existing methods for the preparation of labelled samples of these hormones are generally chemically complex.

Although labelled 1 has been prepared by exposure of unlabelled material to tritium gas (2), all other reported methods for the synthesis of labelled 1 or 2 are multi-step procedures starting from labelled estrane derivatives, and proceed in low overall yield (3-6).

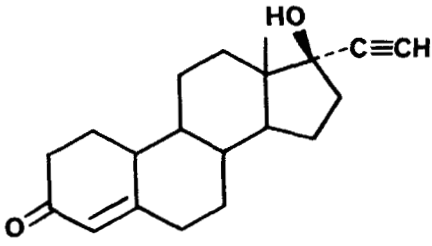
[1]Author to whom enquiries should be directed

0362–4803/89/060629–05$05.00

Received July 15, 1988

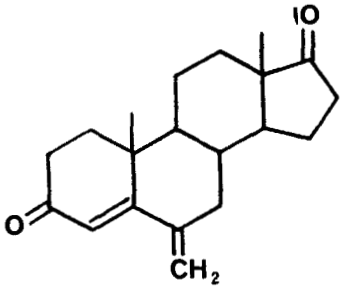


1

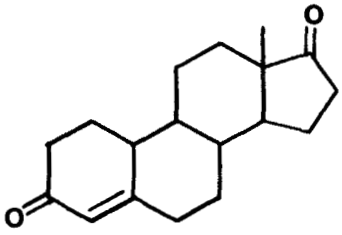


2

We have previously used a phase transfer catalysed exchange procedure for deuterium labelling of steroidal ketones (7), and now report that a modification of this procedure can be successfully used for the preparation of tritium labelled norethindrone (2), and more remarkably, the chemically labile steroids norethynodrel (1) and 6-methyleneandrost-4-ene-3,17-dione (3)



3



4

RESULTS AND DISCUSSION

Although acid or base catalysed enolic exchange is a routine method for deuterium or tritium labelling of carbonyl containing compounds, the method is not generally applicable in cases where the substrate is chemically reactive under the exchange conditions. Such restrictions apply to βγ-unsaturated ketones such as 1 (known to be readily rearranged to 2 under strongly acidic or basic conditions (eg 8,9)), or exo-methylene compounds such as 3, which are susceptible to acid catalysed rearrangements.

The results obtained using the phase transfer catalysed method of exchange of 1-3 with deuterium oxide and lithium or sodium hydroxide are listed in Table 1. Exchange of 3 was carried out at reflux temperature, but 1 and 2 were exchanged at room temperature

as the use of higher temperatures led to the formation of 19-norandrost-4-ene-3,17-dione(4) as a by-product (ca. 40%) in both cases, presumably following base initiated elimination of acetylide from C-17.

Table 1. Exchange of 1-3 with D_2O

starting material	base	product (%)	Deuterium content (%) d_0	d_1	d_2	d_3	d_4	d_5	d_6
1	NaOH	1 (60)	-	-	2.5	20.1	23.6	30.8	23.0
1	LiOH	2 (68)	-	-	-	10.3	21.9	38.9	28.9
2	NaOH	2 (80)	27.3	20.7	11.8	15.5	13.6	7.6	3.5
2	LiOH	2 (72)	30.7	10.2	10.3	14.0	19.0	11.6	4.2
3	NaOH	3 (76)	-	-	-	16	84	-	-

Exchange of the exo-methylene compound 3 was efficient and proceeded without rearrangement. Norethindrone (2) was only poorly exchanged under these conditions, but norethynodrel was efficiently exchanged by either sodium or lithium hydroxide; choice of catalyst allowed control of the rearrangement such that the combination norethynodrel/LiOH/D_2O can be used to produce a good yield of norethindrone with an acceptable level of label, whereas labelled norethynodrel can be obtained using sodium hydroxide as catalyst. In both cases, an isomeric mixture of 1 and 2 is formed, (readily separable by chromatography). The proportions of 1 and 2 in this mixture are presumably a reflection of the position of the rearrangement equilibrium, in turn controlled by the site of protonation of an enolate anion, a factor known to be dependent upon the nature of the base counterion involved (10, 11).

In the presence of tritium labelled water, the preparation of tritium labelled 1-3 proceeded smoothly, as summarized in Table 2. In all cases, isolated yields were similar to those listed in Table 1 for the analogous experiments using deuterium oxide.

Table 2. Exchange of 1-3 with tritiated water*

starting material	base	product	S.A., (mCi/mg)
1	NaOH	1	1.2
1	LiOH	2	1.0
3	NaOH	3	1.04

*S.A. 11 mCi/mL

The exchange method described herein thus provides a rapid and simple procedure for the preparation of tritium labelled keto steroids such as 1 whose chemical reactivity precludes the use of normal enolic exchange procedures. In addition, it can be used as a routine method for the labelling of other carbonyl containing compounds.

EXPERIMENTAL

Deuterium content was determined by mass spectrometry (AEI MS30/Kratos DS55, EI mode) following appropriate corrections for natural abundance ^{13}C. Tritium determination was by LSC (Searle Delta 55). Thin layer (0.2 mm) and preparative layer (2.0 mm) chromatography used Merck silica gel 60-F_{254} plates and ether/hexane (7:3) as solvent. Two elutions gave Rf values of 0.61 (1) and 0.42 (2). Labelled steroids were identified by spectral comparison (i.r., n.m.r. ms) with authentic unlabelled samples (Sigma). 6-Methyleneandrost-4-ene-3,17-dione (3) was obtained by Jones' oxidation of 6-methylenetestosterone (12).

Exchange procedure:

Dry toluene (10 mL) was placed in a 25 mL round-bottomed flask. To this was added 1.0 mL of labelled water, followed by either sodium (0.05 g, CARE!!) or lithium (0.05 g). When the reaction with the metal was complete, steroid (0.1 g) and tetra n.butylammonium bromide (0.05 g) were added. The mixture was then stirred magnetically, and (in the case of 3 only), heated to reflux. The exchange was allowed to proceed for 3 days (18 hr at reflux for 3), after which time the mixture was allowed to cool (if appropriate), and stirring was discontinued. The toluene layer was then removed by pipette, filtered through anhydrous sodium sulphate, and evaporated without further treatment to give the product directly. In the case of 1 and 2, final purification was achieved by preparative layer chromatography. Details of yields are given in Table 1 and refer to isolated, purified material.

ACKNOWLEDGEMENTS

Financial support was provided by the Medical Research Council of Canada and the Natural Sciences and Engineering Research Council of Canada. We are grateful to Mr. T. Jones for assistance with spectral analysis.

REFERENCES

1. Stokes B. - Popul. Rep. J. 20: 639 (1978).
2. Arai K., Golab T., Layne D.S., and Pincus G. - Endocrinology 71: 639 (1962).
3. Schulze P.E. - J. Labelled Comp. 5: 312 (1969).

4. Kepler J.A. and Taylor G.F. - J. Labelled Comp. 7: 545 (1971).
5. Rao, P.N. - Steroids 18: 219 (1971).
6. Merrill E.J. and Vernice G.G. - J. Labelled Comp. 9: 43 (1973).
7. Holland H.L. and Taylor G.J. - Can. J. Chem. 59: 2809 (1981).
8. Johns W.F. - J. Amer. Chem. Soc. 80: 6456 (1958).
9. Nelson N.A. and Garland R.B. - J. Amer. Chem. Soc. 79: 6313 (1957).
10. Stork G., Rosen P., Goldman N., Coombs R.V., and Tsuji J. - J. Amer. Chem. Soc. 87: 275 (1965).
11. Schaub R.E. and Weiss M.J. - Chem. Ind. 2003 (1961).
12. Holland H.L., Chenchaiah P.C., Thomas E.M., Mader B., and Dennis M.J. - Can. J. Chem. 62: 2740 (1984).