Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIII, No. 5

EXCHANGE LABELLING STRATEGIES FOR TRITIATED STEROIDS

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SUMMARY

A number of commonly used tritium exchange procedures for steroids are described. Reference is also made to other available methods.

Key words: Tritium, exchange, steroids

INTRODUCTION

There is significant interest in the use of tritium labelled steroids. Steroids are widely used as anti-inflammatory drugs, contraceptives and in receptor studies. Tritium is most frequently chosen as the radiotracer because of its ease of introduction, specific activity and physical characteristics[1].

There are many reductive and halogen displacement methods to introduce tritium into a steroid molecule. However, these can frequently have unattractive problems. For example, the preparation of tritiated 4-ene-3-one steroids from 1,4,6-triene-3-ones. These can result in variable specific activity for preparations of the same compound and between compounds of similar structure. The separation of the desired product from intermediates and by products can also be troublesome. Reintroduction of a 1,2-double bond can also be problematic, establishing the appropriate oxidising agent[2] and resolving decomposition products of the dehydrogenation reaction being time consuming.

0362–4803/93/050403–06$08.00

The traditional method of introducing tritium in the 7 position[3] also proves to be troublesome. The bromination being oxidative and non-specific resulting in significant quantities of by product and the tritiodehalogenation readily resulting in over-reduction and loss of the 5,6-double bond.

Therefore, use of mild one step tritium exchange procedures, producing material at moderate to high specific activity in good yield and purity is very attractive. Particularly in cases of 18-hydroxy steroids and 19-normethyl steroids where double bond introduction is not straight forward due to the chemical instability of these compounds.

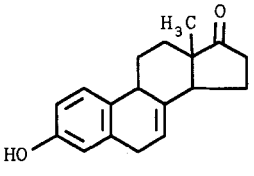
DISCUSSION

Four methods of exchange labelling are generally used. The choice of method depends upon the structure particularly if an aromatic A ring is present.

AROMATIC STEROIDS

Steroids containing an aromatic A ring are readily labelled in the benzylic 6 and 9 positions under mild conditions[4,5]. This is achieved by the technique of exposing the substrate in glacial acetic acid to tritium gas with a palladium catalyst present. The products are of moderate to high specific activities and undergo very little decomposition. This technique is particularly useful for steroids susceptible to chemical decomposition.

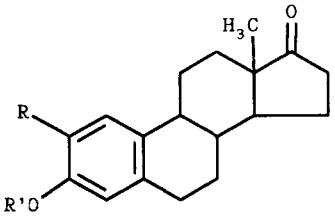
For aromatic steroids containing a reducible functionality the above technique is not appropriate. An exchange procedure using tritiated water, a basic catalyst and a carrier solvent is employed. For example, [^{3}H]equilin in which the 7,8-double bond is susceptible to reduction, exchange in tritiated water and dimethylformamide using sodium hydroxide as a catalyst, yielded a product of 14Ci/mmol.



Equilin

TABLE 1

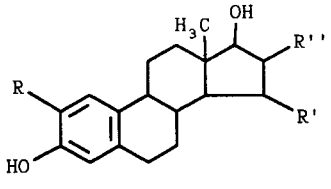
TRITIUM LABELLED OESTRONES BY TRITIUM GAS EXCHANGE



COMPOUND	R	R'	S.A. RANGE Ci/mmol	DISTRIBUTION 6-^{3}H 9-^{3}H
2-Hydroxyoestrone	OH	H	40-60	53 41
Oestrone sulphate	H	SO_3H	30-60	50 50
Oestrone-3(β-D-glucuronide)	H	-GLU	5-15	59 41

TABLE 2

TRITIUM LABELLED OESTROLS BY TRITIUM GAS EXCHANGE



COMPOUND	R	R'	R''	S.A. RANGE	DISTRIBUTION
Oestriol	H	H	OH	40-60	62 38
2-Hydroxyoestradiol	OH	H	H	40-60	58 42
Oestriol-16α-(β-D-glucuronide)	H	H	O-GLU	20-40	56 44
Oestetrol	H	OH	OH	30-50	not measured

NON-AROMATIC STEROIDS

An exchange process involving heating the steroid substrate in dimethylformamide and tritiated water at high temperatures and long exposure times has been reported. The procedure has been successfully employed for steroids with a 17-carbonyl function, for example, estrenone[6,7] (for the synthesis of lyrestrenol) and cardiac glycosides[8].

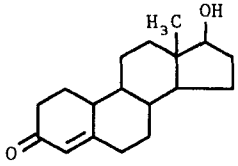
This procedure has been modified in the Author's laboratory for a wider range of steroids. By using a transition metal catalyst (platinum or rhodium) lower temperatures and shorter reaction times are possible. This has allowed labelling of the previously difficult 18-hydroxy steroids and 19-normethyl steroids at moderate specific activities with little decomposition.

TABLE 3

EXAMPLES OF STEROIDS LABELLED USING DMF/TRITIATED WATER

18-Hydroxyprogesterone	6α-Methylprednisolone
18-Hydroxycorticosterone	Dexamethasone-21-phosphate
18-Hydroxy-11-deoxycorticosterone	17α-Hydroxypregnenolone
19-Nortestosterone	Taurocholic acid

The products are labelled in the positions α to carbonyl and conjugated positions for example, 19-[^{3}H]nortestosterone is labelled in the 2,4 and 6 positions.



19-Nortestosterone

The 17 position and side chain 21 position are also labelled in steroids containing 20-ketone.

Labelling can also be achieved α to a carbonyl at high specific activity using base catalysis. Sodium hydroxide or sodium methoxide in dimethylformamide and tritiated water is used. In many instances label in the 16 position only is required and therefore any other carbonyl function must be protected. Specific activities as high as 45Ci/mmol have been achieved.

OTHER METHODS

A number of other methods for exchange labelling steroids have appeared in the literature, a selection is listed in Table 4.

TABLE 4

OTHER HYDROGEN ISOTOPE EXCHANGE PROCEDURES AVAILABLE FOR STEROIDS

STEROID TYPE	CATALYST	SOLVENT	ISOTOPE SOURCE	REF
Various	Pt, Pd	H_2O	CH_3CO_2D, D_2O	9
Various	Na_2PtCl_4 Na_2PdCl_4		CH_3CO_2D	10
Various	BBr_3 $EtAlCl_2$	$CHCl_3, C_6H_{12}$	T_2O	11
Various	Microwave Discharge		T_2	12
Contraceptives	NaOH LiOH	Toluene	T_2O	13

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