RAPID TRITIUM LABELLING OF STEROIDS USING ETHYLALUMINIUM CHLORIDE AND BORON TRIBROMIDE CATALYSTS

Ruth A. Brooks, Mervyn A. Long and John L. Garnett.
School of Chemistry, The University of New South Wales.
Kensington, N.S.W. 2033, Australia.

SUMMARY

A representative group of steroids have been labelled by exchange using ethylaluminium chloride and boron tribromide as catalyst and a trace of high specific activity tritiated water as isotope source. Careful choice of experimental conditions and a solvent inert to this exchange system ensures the production of high specific activity steroids with high radiochemical purity.

Key words: Tritium, Exchange, Steroids, Lewis Acid, Catalysis, Labelling.

INTRODUCTION

Single step methods for the labelling of steroids with tritium are useful for producing material for chemical and biological studies. A number of isotope exchange procedures involving radiation and metal catalysis for the tritiation of steroids have been described (1,2) as alternatives to the more common dehalogenation and hydrogenation methods(3).

More recently a simple and rapid tritiation technique involving the use of catalysts such as ethylaluminium chloride and other Lewis acid halides of the type used to promote Friedel Crafts reactions was developed for a variety of simple organic molecules including alkanes containing tertiary carbon atoms (4,5). The successful labelling of compounds such as ethylcyclohexane, decalin and phenol with $EtAlCl_2$ as catalyst and a trace of high specific activity tritiated water as isotope source, suggests that this simple one step procedure may be applicable to steroid molecules since these compounds contain structural features similar to that of the steroid ring systems. No detailed study of the application of these halide catalysts to the tritiation of steroids has previously been reported.

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Earlier exchange methods based on catalysis by platinum and palladium (1) are successful for labelling a variety of steroids including some keto derivatives, hydrocarbons and cardiac gylcosides. However the presence of an hydroxy group in the steroid leads to oxidation simultaneously with isotope incorporation and in such circumstances esterification prior to exchange is necessary (1). Furthermore in those labelling procedures the isotope source is the aqueous solvent itself and hence the specific activity attainable in the steroid is limited to that of the solvent even if exchange equilibrium is reached. Where the mole ratio of substrate to solvent is very low due to solubility limitations, the total activity in the exchange system must be very much higher than that incorporated into the substrate and thus facilities for handling large amounts of tritium are necessary even if only small amounts of high specific activity steroids are to be produced.

In contrast the Lewis acid type catalytic exchange technique (4) involves the use of only a trace of high specific activity water as isotope source and a solvent inert to activation by this type of catalyst. Therefore the technique in principle permits the incorporation of the bulk of the available tritium into the substrate and hence the production of radiochemicals of high specific activity without the use of large total quantities of tritium.

Thus we now report an assessment of this latter catalytic procedure to the tritiation of a representative selection of steroids.

EXPERIMENTAL

The steroid to be labelled (50 mg) was dissolved in an appropriate solvent (0.3 ml) in a dry N_2 atmosphere and approximately 40 mg of catalyst (BBr_3 or $EtAlCl_2$) was added followed by tritiated water (5 µl, 5 Ci g^{-1}). Since a vigorous reaction accompanies the addition of the water to the catalyst solution, the reaction tube was usually frozen in liquid nitrogen prior to the water addition. The sample tube was sealed, and the reaction allowed to proceed for the desired time at the appropriate temperature. Reaction products were

analysed on a radio gas-liquid chromatograph in which a combustion tube and proportional counter were coupled to a post column splitter on the chromatograph (6). An accurate measure of the specific activity of the pure labelled compound was thus obtained. Prior to analysis the steroids were washed repeatedly in IM NaOH to remove labile tritium.

RESULTS AND DISCUSSION

Typical results of the tritiations of representative steroids (Figure 1), chosen in such a way as to include various functional groupings, with $EtAlCl_2$ and BBr_3 as catalysts are contained in the Table. These two catalysts were selected on the basis of the results of previous studies with a range of Lewis acid type halides as being two compounds readily available and most likely to achieve labelling (4). In previous work $EtAlCl_2$ itself proved to be the most effective catalyst in hydrocarbon labelling while BBr_3, although still active, was less vigorous in its reactivity.

Cyclohexane was chosen as a solvent because of its inertness towards exchange in the labelling system and hence the absence of loss of tritium from the isotope pool to the solvent. While many steroids would be regarded as insoluble in hydrocarbon solvents even a very slight solubility may be sufficient to lead to labelled compound. However, the more traditional steroid solvent, chloroform, which was free of alcohol stabilizers, was tested as an alternative to cyclohexane.

Both 5α-cholestane and 5α-androstane were labelled in cyclohexane at 60° with $EtAlCl_2$ and BBr_3 as catalysts. No labelled byproducts were observed. This result is in agreement with that of simple branched chain alkanes where general labelling in a molecule such as methylcyclohexane was observed (7). In contrast, the use of chloroform with $EtAlCl_2$ even at ambient temperature for short reaction times led to the production of various labelled byproducts and no recovery of 5α-cholestane. While positive identification of the byproducts was not sought, gas chromatography evidence supported the formation of a number of chlorinated derivatives of cholestane. In general the use of BBr_3 as

Table Specific Activity of Steroids Tritiated by Catalytic Exchange with HTO

Compound		Catalyst	Solvent	Temp. °C	Time h	Compound Recovery %	Specific Activity[a] Ci.mole^{-1}
5α-cholestane	I	$EtAlCl_2$	C_6H_{12}	20	160	82	<0.1
		$EtAlCl_2$	C_6H_{12}	60	3	82	23
		$EtAlCl_2$	$CHCl_3$	20	0.1	0	-[b]
		BBr_3	C_6H_{12}	20	160	100	0.5
		BBr_3	C_6H_{12}	60	3	90	3.7
5α-androstane	II	$EtAlCl_2$	C_6H_{12}	60	3	70	21
		BBr_3	C_6H_{12}	60	3	90	6.1
β-estradiol	III	$EtAlCl_2$	C_6H_{12}	20	2	100	0.3[b]
		$EtAlCl_2$	$CHCl_3$	20	1.5	100	12.1
		BBr_3	C_6H_{12}	20	2	96	34
		BBr_3	$CHCl_3$	20	2	97	7.4[b]
estrone	IV	$EtAlCl_2$	$CHCl_3$	20	2	100	5.6[b]
		BBr_3	$CHCl_3$	20	2	80	9.1[b]
		BBr_3	C_6H_{12}	20	2	95	12
dehydro testosterone	V	$EtAlCl_2$	C_6H_{12}	20	3	93	0.8
		$EtAlCl_2$	$CHCl_3$	20	2	40	3.8
		BBr_3	$CHCl_3$	20	2	56	8.4
Δ^4-androsten-3,17-dione	VI	$EtAlCl_2$	$CHCl_3$	20	0.5	81	2.8[b]
		BBr_3	$CHCl_3$	20	2	60	1.7[b]
$\Delta^{1,4}$-androstendien-3,17-dione	VII	$EtAlCl_2$	$CHCl_3$	20	0.5	94	1.5[b]
		BBr_3	$CHCl_3$	20	2	86	14
5α-pregnan-3β,20β-diol	VIII	$EtAlCl_2$	C_6H_{12}	20	1.5	92	9.6[b]
		$EtAlCl_2$	$CHCl_3$	20	0.2	16	0.8[b]
		BBr_3	C_6H_{12}	20	1.5	78	3.7[b]
		BBr_3	$CHCl_3$	20	0.2	42	0.5[b]
5α-cholestan-3β-ol	IX	$EtAlCl_2$	C_6H_{12}	20	2	75	9.3[b]
		$EtAlCl_2$	$CHCl_3$	20	2	2	1.2[b]
		BBr_3	C_6H_{12}	20	2	90	1.8

a Specific activity of pure steroid.

b In addition to the steroid some active byproducts were detected.

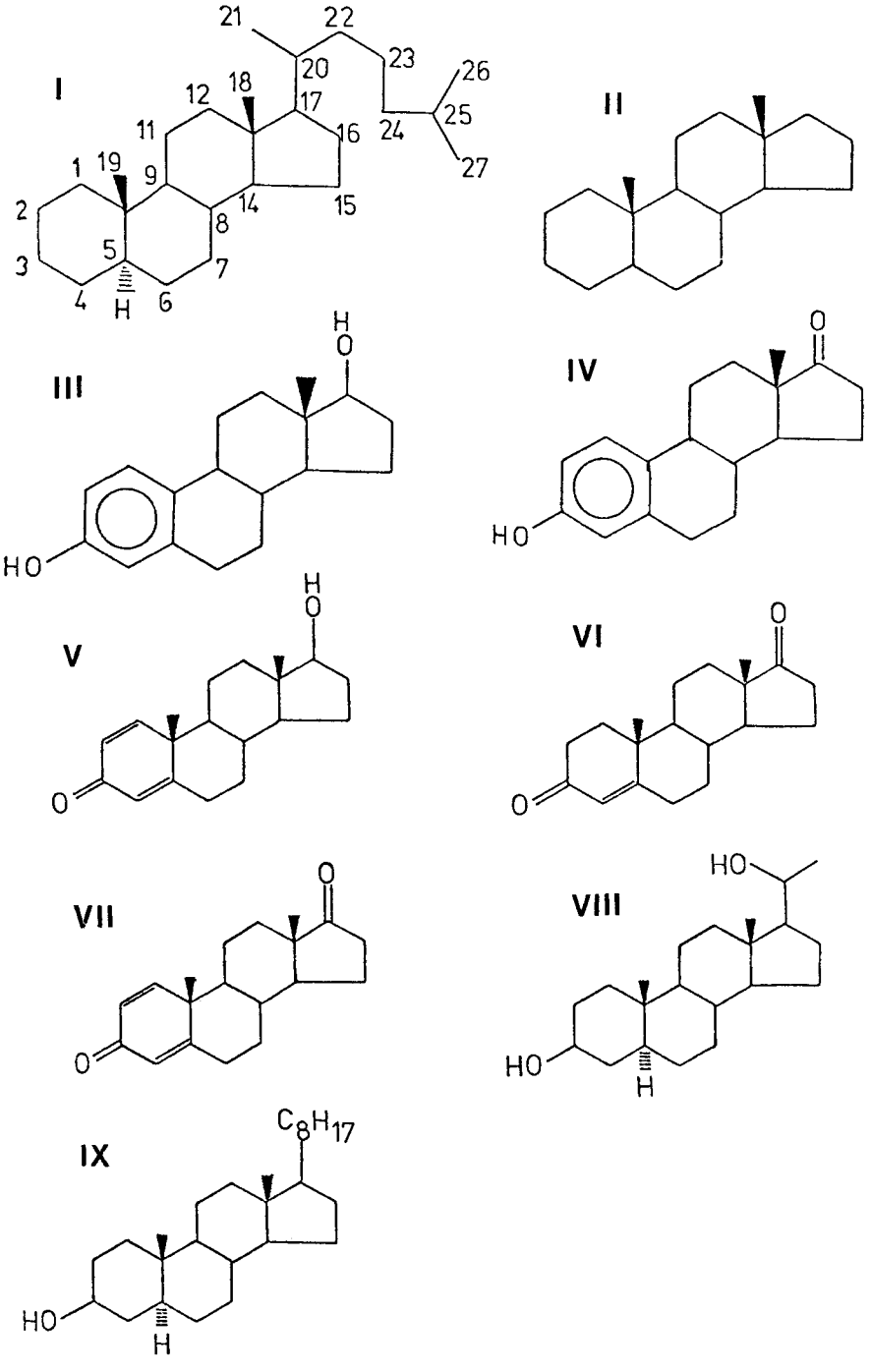

Figure 1. Structure of steroids reported in Table.

catalyst instead of $EtAlCl_2$ led to higher recoveries of the substrate and higher levels of activity.

The aromatic steroids β-estradiol and estrone were substantially labelled in both catalyst systems at ambient temperatures with recovery of the steroid from the reaction mixture being high (Table). BBr_3 again provided the most efficient procedure. Some low activity byproducts were observed in most cases, particularly when chloroform was used as a solvent. In additional experiments at elevated temperatures substantial loss of the substrate was observed. The conclusion that rapid and adequate exchange is achieved at ambient temperatures is in accordance with the ease of labelling of simple aromatic hydrocarbons(8). While some competing rearrangement reactions of some polysubstituted aromatics have previously been observed in the $EtAlCl_2$ system (9) no such side reactions appear to have accompanied the exchange of the estrogens.

A number of non-aromatic keto- and hydroxy-steroids were successfully labelled with both $EtAlCl_2$ and BBr_3 as catalysts. In general a substantial portion of the substrate was recovered although the use of longer reaction times and/or elevated reaction temperatures in an attempt to increase the labelling level led to high substrate loss. Of particular significance is the observation that steroids with free hydroxyl groups may be labelled by this method since aqueous exchange techniques using platinum and palladium catalysts (1) led to substantial oxidation of the steroid unless esterification was used to protect the molecule.

In general the inert solvent, cyclohexane, was most satisfactory for the production of pure tritiated substrates, but the low activity in some cases probably results from the extreme insolubility of those particular compounds in cyclohexane. Chloroform frequently is an adequate alternative solvent provided reaction times are kept short, as indicated in the Table, to minimise labelled byproduct formation.

The specific activities quoted in the Table indicate the levels of

activity attainable by the use of 5 $Ci.g^{-1}$ tritiated water. In an additional experiment in which tritium gas was converted catalytically to yield 500 $Ci.g^{-1}$ water which in turn was used in the labelling of estradiol with BBr_3 catalyst, a specific activity of approximately 3000 $Ci.mole^{-1}$ was obtained. This result demonstrates that since the activity attainable is limited by the specific activity of the water available as isotope source, steroids of high specific activity can be produced by this method if suitable tritium gas handling facilities are available.

The mechanism by which Lewis acid type catalysts promote exchange labelling in simple aromatic compounds has been discussed elsewhere (4). In Figure 2 the adaptation of this mechanism to the labelling of estrone is shown.

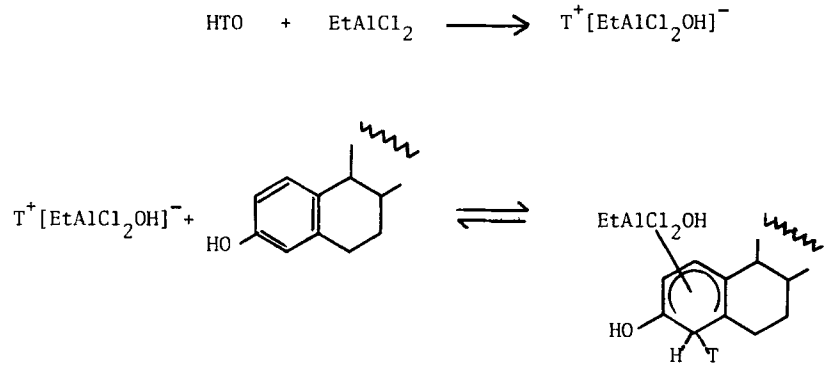

Figure 2. Mechanism for exchange of estrone.

While considerable controversy surrounds the nature of the proton substitution step (4) and the possibility of the involvement of various carbonium ion intermediates (10), it is never-the-less clear that charge transfer complex formation between an aromatic centre and the catalyst occurs in these systems. It is possible that interconversion of π- and σ- bonded aromatic species may lead to isotope exchange as described previously for the ethylaluminium system (4) and for other metal catalysed exchange reactions (11). The consequence of

such a mechanism is that substitution is likely to take place preferentially within the aromatic centre of the molecule.

In non-aromatic substances it is generally accepted that a carbonium ion mechanism involving hydride transfer reactions propagates exchange (10,12). The distribution of isotope incorporated into the molecule might therefore be expected to depend on the relative stability of various possible carbonium ions, and a knowledge of these would be necessary to predict the isotope distribution. However, where it has been possible with simple alkanes to measure the isotope distribution by ^{3}H n.m.r. following Lewis acid catalysis (7) the results suggest a reasonably general labelling pattern. It is likely therefore, that the tritium within steroids other than the estrogens is widely distributed throughout the molecules.

CONCLUSION

The more active Lewis acid type catalysts such as $EtAlCl_2$ and BBr_3 may be used as a convenient and rapid technique for tritiation of a variety of steroids. It probably represents the fastest general labelling procedure. The purity of the product and the levels of activity attainable depend on the choice of solvent, reaction time and temperature. Careful choice of these reaction conditions can yield pure high specific activity compounds. The simplicity of the experimental technique renders it an attractive alternative to the more conventional exchange methods involving the use of heterogeneous or homogeneous platinum catalysts.

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