LABELLING OF STEROIDS BY EXCHANGE WITH ISOTOPIC WATER ON HETEROGENEOUS PLATINUM AND PALLADIUM: METHODS FOR OVERCOMING COMPETING OXIDATION *

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SUMMARY

A representative group of steroids have been labelled by exchange with isotopic water in the presence of heterogeneous Group VIII transition metals. Platinum and palladium are the best of the metals studied. Steroids examined included oestrogens, keto and hydroxy derivatives, hydrocarbons and two cardiac glycosides. The presence of an OH group in the steroid leads to oxidation simultaneously with isotope incorporation. Esterification of the OH group prior to exchange eliminates oxidation and yields labelled compounds of high chemical purity. The variables affecting optimum exchange have been determined and include solvent, temperature of reaction and method of catalyst activation. Isotope orientation in the labelled steroids supports a mechanism involving the role of π- and σ-bonded intermediates in the exchange reaction.

INTRODUCTION

One-step methods for the labelling of steroids with deuterium and/or tritium are useful, particularly for chemical and biological studies. For tritium labelling which is generally of more value than deuteration, radiation-induced techniques such as the Wilzbach method[1] are of limited applicability because of concurrent radiation degradation.

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Heterogeneous exchange involving isotopic water with Group VIII transition metals has been used extensively to label simple molecules such as substituted aromatics, olefins and saturated hydrocarbons.[2] Preliminary studies with this procedure have previously been performed only on isolated steroids and with platinum as catalyst.[3-6] Data from this earlier work, particularly with cholesterol, indicate that extensive degradation of the molecule will accompany isotope incorporation.[3, 4] In some of these previous studies [6] tritium only was used. No simultaneous deuterium labelling to check degradation or orientation of isotope was performed. It is the purpose of this present investigation to perform a systematic feasibility study of the use of heterogeneous catalysis with isotopic water as a means for labelling steroids. A representative number of saturated and unsaturated steroids containing typical functional groups have been chosen for experiment. A specific attempt will be made to achieve labelling in steroids, such as cholesterol, without concurrent degradation. Finally the results will be rationalised in terms of current theories for heterogeneous catalysis. Deuterium will be used as a tracer for tritium in this work, since N.M.R. and mass spectrometry as well as other conventional instrumental techniques can then be utilized to examine chemical purity and isotope orientation in the tagged compounds.

EXPERIMENTAL

The steroids used were the purest commercially available. Their purity was checked by m.p., infrared, N.M.R., mass spectrometry and where applicable, optical activity. The source of isotope was deuterium oxide (99.7% isotopic abundance supplied by the Australian Institute of Nuclear Science and Engineering). Catalysts employed were platinum oxide (Johnson, Matthey) and palladium oxide (Koch-Light). All reagents, except hydrogen gas, were of AR quality.

For the exchange reactions, two methods of catalyst activation were utilized, namely hydrogen and sodium borohydride.[7] For hydrogen

reduction the weighed catalyst was suspended in half the desired quantity of deuterium oxide in a preweighed, preconstricted reaction tube, then hydrogen bubbled in through a capillary leak (50 ml min^{-1}) for ten minutes. After reduction, excess hydrogen was displaced by oxygen-free nitrogen, the required additional deuterium oxide added, followed by the steroid then the ampoule was sealed under vacuum at $< 5 \times 10^{-2}$ torr after two freeze-thaw cycles. The ampoule was heated in an oven fitted with a rotating device to give uniform agitation. After exchange, the catalyst was centrifuged off and the compound recovered by filtration or solvent extraction depending on its solubility. Labile deuterium was removed by evaporation with methanol. For reduction with the sodium borohydride technique, the procedure previously described was used.[7]

For infrared, the KCI disc method was carried out on a Perkin Elmer 521 spectrometer. N.M.R. spectra were obtained on a Varian A60 instrument. Melting points were determined on a Reichert Kofler block apparatus and are uncorrected. Optical activity measurements were carried out on a Perkin Elmer model 141 photo-electric instrument. For mass spectrometry, the low voltage technique [7] for deuterium analysis was used on an Hitachi-Perkin Elmer RMS-4 instrument. The measured mass spectrum peaks were processed on a Control Data 3200 computer by a Fortran's program which corrected the measured peaks for carbon-13 and oxygen-18, presenting the results as a percentage distribution of species D_o - D_x. From this corrected distribution, the program calculated the average deuterium per molecule as atom % D.

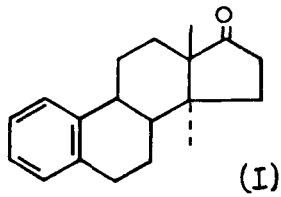
RESULTS AND DISCUSSION

The experimental conditions used and the resulting deuterium incorporated for each compound in the steroid oestrogens, the keto steroids, the hydroxy steroids and their esters, the steroid hydrocarbons and the more complex derivatives, digitoxin and digitoxigenin, are reported in Tables I-VI. The mass spectrometric deuterium distributions for the corresponding labelled steroids are listed in Tables V and VI with

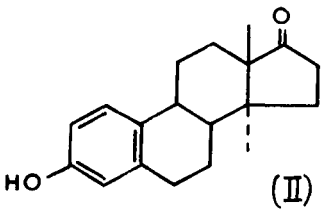
isotope orientation studies from N.M.R. and infrared spectroscopies on a representative number of these steroids summarised in Table VII. With certain compounds, degradation accompanied exchange. Details of these degradation results are shown in Tables VIII and IX.

Steroid Oestrogens

The compounds in this series, namely 3-desoxyestrone (I), oestrone (II) and oestradiol (III) can all be labelled by the present method (Tables I, V, VII). With oestradiol, the parent compound is oxidised to ketonic products during exchange (Tables I and VIII); however, the diacetate



(I)



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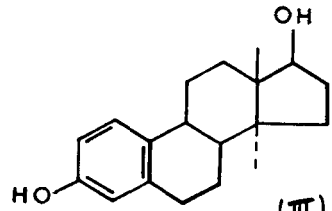


(III)

derivative is stable and deuterates easily. Both PtO_2 and PdO are active catalysts, although both give different isotope orientations during initial rates of exchange (Table VII, runs 5 and 6). Solvent is important in the reaction since in Table 1, D_2O alone with platinum (run 2) gives low incorporation in desoxyestrone which is improved when deuterated acetic acid only is used (run 4) and particularly mixed deuteroacetic acid/heavy water (run 5). Analogous results were obtained with palladium (runs 3 and 6). For either platinum or palladium, the best method of catalyst activation was reduction with hydrogen or sodium borohydride in preference to self-activation [9] which is in situ reduction of the oxide by the organic compound.

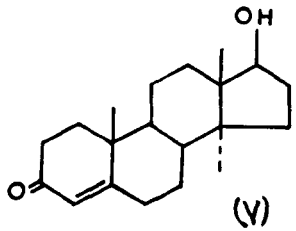
Only platinum and palladium metals were used as catalysts in these runs because of earlier work [7] with simpler compounds which showed that the other Group VIII transition metals catalysed much slower exchange and therefore higher deuteration temperatures would be needed to give significant isotope incorporation in a reasonable period of time. Previous studies also demonstrated that catalysts prepared by activation of the oxides were better than the corresponding material obtained from the chlorides.[10] Preliminary experiments with benzene and cyclohexane confirmed these earlier findings and so only the oxides were used for the present steroid studies.

With platinum and palladium, isotope incorporation occurred in both aromatic and aliphatic positions in all molecules (Table VII). From the mass spectral distributions (Table V), measurable peaks to D_{15} with incipient peaks to D_{18} were observed for a number of runs, thus suggesting that all protons in the molecules were capable of exchange. From the N.M.R. (Table VII), the presence of an OH group in the aromatic ring (run 5,desoxyestrone vs. run 11, oestrone) accentuates aromatic exchange consistent with orientation effects previously observed [7] in simpler molecules (phenol vs. benzene). Conversion to the acetate derivative of these hydroxy compounds such as in oestradiol diacetate (run 16) reduces the relative emphasis of ring deuteration, presumably due to surface steric considerations from the bulky acetate group.[11] The effect of aromatic deuteration is shown by the D_3 cut-off in the mass spectral distribution of desoxyestrone (Table V, run 5) and the D_4 cut-off with oestrone (Table V, run 9).

Keto-and Hydroxy Steroids

Because of the presence of aliphatic ketonic groups in the keto compounds, exchange by keto-enol tautomerism in the deuteroacetic acid catalyst medium occurs. Careful purification of the labelled keto steroids is thus necessary to remove labile isotope and show unequivocally that all incorporated isotope remaining in the molecule,

after back-exchange treatments, was stably bound. Some of the hydroxy steroids in this group were also studied as their esters. The important steroids in this group are testosterone (V) and its esters, acetate (VI) and propionate (VII), progesterone (VIII) and desoxycorticosterone acetate (IX) for which all the exchange data are shown in Tables II, V and VI.



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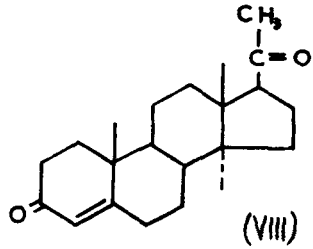

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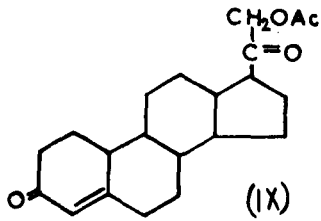
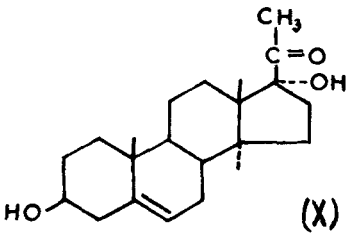
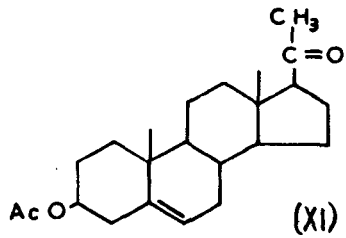


(IX)

In addition, data for 17-hydroxypregnenolone (X), pregnenolone acetate (XI), cholesterol (XII) and its esters (acetate (XIII) and propionate (XIV)), etiocholanolone (XV), androsterone (XVI) and stigmasterol acetate (XVII) are listed in Tables III, IV and VI.



(X)



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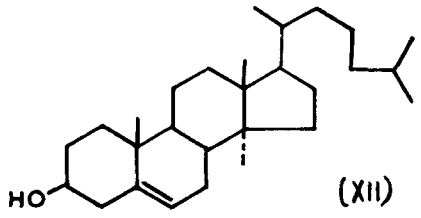

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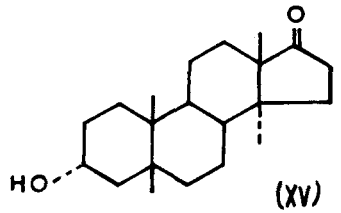

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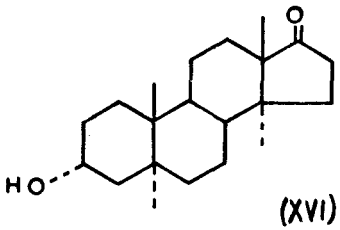

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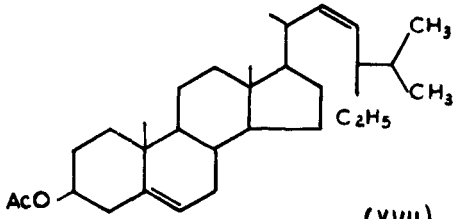
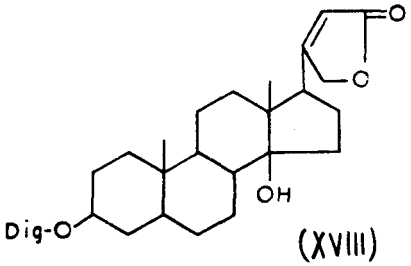


(XVII)

In this series, there are two important features of the results, namely (i) those compounds containing free aliphatic OH groups are degraded during exchange - this aspect will be discussed later in this paper and (ii) the remaining compounds of the series and also the ester derivatives of those materials in (i) are easily labelled by the present technique using either platinum or palladium as catalysts. No hydrolysis of the esters is observed during exchange. Again hydrogen and borohydride reduction, in preference to self-activation, of the inorganic oxides gives a more efficient catalyst. Temperatures of at least 130°C are necessary to achieve significant isotope incorporation in a reasonable time (2 days). In NMR studies of isotope orientation in the labelled compounds, considerable exchange had occurred preferentially at the double bond hydrogens in all steroids. These hydrogens are more active than those attached to saturated carbon atoms. Again, the low voltage mass spectra of these deuterated derivatives (Tables V,VI) indicate that all hydrogens in the acetate are capable of exchanging. The cut-offs in the deuterium distribution patterns of the relevant steroids also confirm the NMR orientations.

Steroid Hydrocarbons and More Complex Steroids

The cardiac glycoside, digitoxin (XVIII) and the aglycone, digitoxigenin (XIX) were investigated because of the general difficulty already experienced in labelling these steroids which possess more complex structures. The last two compounds examined were 5α androstane (XX) and 5βandrostane (XXI). These are hydrocarbons, thus the effect of functional group on the exchange could be estimated.



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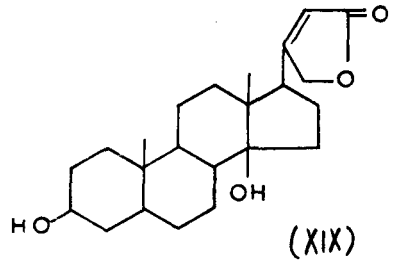

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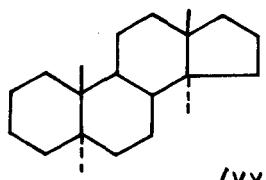
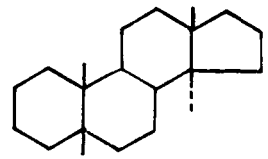


(XX)



(XXI)

Labelling of digitoxin and digitoxigenin was not achieved due to oxidation of the compounds. With digitoxin there was also strong evidence that the C-3 digitose was being hydrolysed. Both saturated hydrocarbons, 5α-and 5β-androstane, exchanged only slowly even at 120°C. This result is consistent with data from simpler molecules such as cyclohexane and benzene. [11] The aromatic compound exchanges much faster than the saturated aliphatic.

Degradation During Exchange

The problem in labelling steroids which contain essentially an aliphatic hydroxyl group is that oxidation and related reactions readily occur with active Group VIII transition metals, particularly

platinum and palladium under the present exchange conditions.[3] Even with the simple alcohols, it has been found that reaction on heterogeneous nickel of some primary alcohols in the liquid phase at temperatures from 140-275°C yields aldehydes, unsaturated hydrocarbons and saturated hydrocarbons. Bond [12] has indicated that the predominant process occurring when alcohols decompose on metal surfaces is dehydrogenation. Thus primary alcohols yield aldehydes and secondary alcohols, ketones. In the only other previous study of this type reported with steroids, Anker and Bloch [3] found that cholesterol in acetic acid was dehydrogenated by heterogeneous platinum.

The results of the present relevant studies reported in Tables VIII and IX are consistent with the above observations. Thus oestradiol, testosterone, 17-hydroxy-pregnenolene, cholesterol, etiocholanolone, androsterone, digitoxin and digitoxigenin were readily oxidised to ketonic and related derivatives under the present catalytic exchange conditions. Oxidation and exchange mechanisms appear to be related since at temperatures of 90° C there was generally no exchange and no oxidation, but at 130°C where exchange is normally fast, oxidation, is, at least, 50% complete with many compounds after 48 hours. In this respect, testosterone is particularly susceptible to ketone formation, since the oxidation is 90% complete after 48 hours at only 110°C (Table VIII, run 23). The important feature of the present work in terms of the above difficulty, is that if all of the above steroids are esterified, the oxidation reaction is eliminated and exchange occurs easily. At the temperature of exchange, even up to 150°C, the esters do not hydrolyse in the solvent used. The present system is thus suitable for labelling all of the above steroids if they are converted to esters prior to exchange. In this respect the importance of doing some reactions in H_2O instead of D_2O in the Tables is apparent, since infrared spectroscopy can be used to monitor the oxidation quantitatively without interference from C-O bands.

Mechanism of Exchange

The mechanism of heterogeneous exchange of organic compounds on metals, such as platinum and palladium is now generally considered to occur in the following manner.[13-16] Aromatic hydrogen atoms deuterate predominantly by a π-dissociative process (Fig.1) with a possible contribution from a π- associative mechanism. Exchange of hydrogens directly attached to olefinic carbon atoms appears to occur by analogous

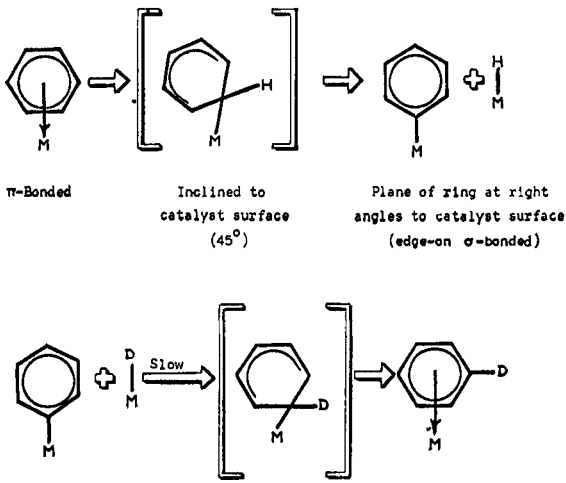
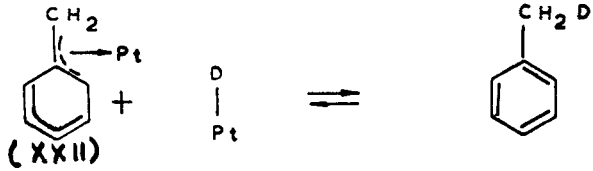


Fig. 1. Dissociative π-complex substitution mechanism

mechanisms involving associative or dissociative intermediates. Deuteration of alkyl groups adjacent to aromatic or olefin bonds is due predominantly to π-olefinic type mechanisms involving π-allylic species (XXII).

Exchange at saturated carbon atoms is consistent with the application of a dissociative type species (XXIII) with also the possibility of a contribution from π-allylic species and intermediates involving delocalised σ-orbitals.[17, 18] For the π-allylic process in saturated hydrocarbons, a chain of three or more carbon atoms, none of which is a quaternary or bridge-head carbon, is required. The mechanism involving delocalised σ-orbitals has recently been proposed



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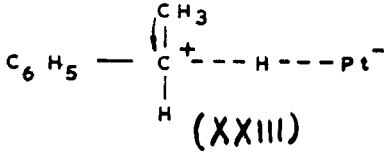

(XXIII)

for heterogeneous exchange at aliphatic saturated carbon atoms because analogous deuteration involving homogeneous platinum has been discovered and delocalised σ-orbitals are suggested as contributing to the bonding between inorganic catalyst and saturated hydrocarbon.[13]

Application of Mechanisms to Steroid Labelling.

For the steroid oestrogens such as desoxyestrone (I), oestrone (II) and oestradiol (III), it is proposed that initial adsorption occurs as a π-complex via the aromatic ring. The deuterium distribution and N.M.R. data (Tables V and VII) indicate that during the initial stages of the exchange, the aromatic protons deuterate much faster than the remaining hydrogens. The results are thus consistent with initial strong adsorption though the aromatic ring and the application of the π-dissociative process (Fig.1)

For the steroids containing olefinic bonds, i.e., testosterone (V), progesterone (VIII), desoxycorticosterone acetate (IX), 17-hydroxy-pregnenolone (X), pregnenolone acetate (XI), cholesterol (XII) and stigmasterol acetate (XVII), there was no evidence of double bond migration and initial deuteration was confined predominantly to hydrogens on the double bond carbon atoms. Exchange in these positions can be attributed also to a π-dissociative mechanism. With the olefinic steroids, extensive deuteration was also observed in the allylic positions to the double bond except where precluded

by steric hindrance, thus species such as (XXII) are involved in a π-allylic mechanism in this instance.

The above conclusions are supported by the preliminary early work of Fukushima and Gallagher [4] who exchanged cholesterol with tritium oxide-acetic acid in the presence of hydrogen pre-reduced platinum. These authors [4] ultimately obtained the labelled cholesterol free from the accompanying oxidation products. They then chemically degraded the purified tritiated cholesterol and showed that 40% of the tritium was on C-6 with 52% at C-24, 25, 26 and 27.

With steroids containing the saturated ring structure, i.e. 5α androstane (XX), 5β androstane (XXI), etiocholanolone (XV) and androsterone (XVI), rates of exchange were particularly slow, consistent with observations on the deuteration of simpler molecules such as cyclohexane. For the π-allylic process, a chain of three or more carbon atoms, none of which is quaternary or bridge-head carbon, is required. The polymethylcyclopentanes and 1,1,3,3-tetramethylcyclohexane have structural features in common with the steroids and have been cited as exchanging by a π-allylic mechanism.[15] In the direct α,β dissociative (abstraction) process[11], exchange occurs only on the side of the ring bonded to the catalyst, whereas the π-allylic mechanism allows replacement from either side of the ring with the consequence that inversion is possible. Some change in optical activity did occur with many of the steroids investigated, but all could be ascribed either to decomposition or to isomerisation, e.g., in one run, 17-β-progesterone isomerised to a small degree to 17-α-progesterone during keto-enol tautomerism. All steroids that exchanged were recovered pure by adsorption chromatography which would not separate optical isomers. However optical purity (rotation) where relevant was checked and found to be unchanged. Thus inversion did not occur and a dissociative rather than a π-allylic mechanism is favoured under these conditions. This result is confirmed by the D_2 cut-offs in the mass spectra of (XX) and (XXI). If

inversion did occur with the present series of steroids in heterogeneous exchange, this catalytic method would be completely unsuitable for labelling because of the difficulty of separating optical isomers. In chemical reactions, the less sterically hindered "α face" of the steroids is usually the most reactive. This situation also appears to be valid for heterogeneous catalysis since in no steroid investigated was there exchange in either the C-18 or C-19 angular methyl groups, both of which are β oriented.

The final mechanism which should not be overlooked for the saturated steroids is one analogous to that proposed for the homogeneous metal catalysed exchange of saturated hydrocarbons. Here it is suggested that a preliminary complex between the organic and catalyst is formed involving delocalised σ-orbitals[13,17,18]. This is followed by C-H bond dissociation. No further evidence is available at present to support its relevance in the present heterogeneous work.

Mechanism of oxidation reaction

The intermediates in both oxidation and exchange reactions are probably similar and involve charge-transfer complex formation utilizing the -OH group. The hydroxyl group in an aromatic ring is known to accelerate exchange particularly in the adjacent ortho positions[7], presumably due to increased charge-transfer adsorption involving the oxygen atom. Hydrogen abstraction from the -OH is thus facilitated, particularly in the steroids. Work by Anker and Bloch[3] showed that cholesterol, when reacted with aqueous acetic acid in the presence of active platinum, gave a variety of degradation products, predominantly the ketone and also some hydrogenated derivatives, thus giving a hydrogen balance in the system. In the present studies, a detailed examination of the degradation products was not performed, the major product (ketone) only being identified to confirm the oxidation reaction.

General conclusions

There are a number of significant features of the present results in terms of using a heterogeneous Group VIII transition metal and aqueous acetic

acid as a labelling medium for tagging steroids with isotopic hydrogen. Because of oxidation, the sterols are not labelled satisfactorily by this technique, however if converted to the corresponding esters, efficient exchange occurs without hydrolysis or oxidation even after several days at temperatures of 150°C. Platinum and palladium are the best of the Group VIII transition metals. Sodium borohydride or hydrogen reduction of the metal oxide gives the most active catalyst. Unsaturated steroids, particularly if containing an aromatic ring, exchange much faster than other members of the series. All steroids which did not degrade, were capable of being tritiated to high specific activity by the present technique, however only the most reactive compounds were capable of deuteration in a reasonable period of time, at temperatures of 120-150°C. Thus for complete deuteration a number of equilibrations would be needed under these conditions. However, for most biological work, the demand for tritiated steroids is much higher than for the deuterated derivatives although simultaneous deuteration is valuable in that physical methods such as NMR and mass spectrometry can be readily used to check isotope orientation and chemical purity without recourse to tedious chemical degradation procedures necessitated for tritium labelled compounds. For the present work deuterium is thus an excellent tracer for tritium. Although oxidation is a severe limiting factor in the labelling of the sterols, for tritiation work using high specific activity T_2O, short conversion times may only be necessary and under these conditions it may be possible to separate the tritiated parent from the other degradation products. Radiochemical purification of products under these conditions still remains a problem. It would thus still be preferable to exchange the ester and hydrolyse even if one desired the free sterol tritiated.

ACKNOWLEDGEMENTS

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TABLE I

Exchange of 3-desoxyestrone (I), oestrone (II), oestradiol (III) and oestradiol diacetate (IV) on platinum and palladium.

Compound	Run	Catalyst [a]		D_2O	H_2O	DAc [b]	Compound	Reaction Time	Temp	D [c]
		Act	(g)	(ml)	(ml)	(ml)	(g)	(hr)	(°C)	(%)
(I)	1	H_2	0.036	4.0			0.218	18	101	0
	2	H_2	0.038	4.0			0.114	43	120	0.2
	3 [a]	H_2	0.053			7.0	0.150	43	130	5.8
	4	H_2	0.042			7.0	0.108	43	130	5.3
	5	H_2	0.040	2.0		5.0	0.101	43	120	15.0
	6 [a]	H_2	0.044	2.0		5.0	0.107	43	120	34.1
(II)	7	S.A.	0.010	1.85			0.050	48	110	0.7
	8	$NaBH_4$	0.010	2.0			0.051	48	110	2.5
	9	H_2	0.042	2.0		4.0	0.131	42	120	18.9
	10 [a]	H_2	0.041	4.0			0.111	42	120	0
	11	$NaBH_4$	0.198	27.2			0.501	48	160	39.9
(III)	12	$NaBH_4$	0.010	2.0			0.054	48	110	2 [d]
	13	$NaBH_4$	0.011	1.9			0.056	24	130	13.9 [d]
	14	$NaBH_4$	0.022		1.8		0.110	48	167	- [d]
	15	$NaBH_4$	0.010	1.9			0.046	48	167	51.2 [d]
(IV)	16	H_2	0.038	2.0		5.0	0.127	43	130	14.9
	17	H_2	0.047			7.0	0.114	43	120	0
	18	H_2	0.043	4.0			0.103	42	120	0

[a] Catalyst was PtO_2 except for runs 3, 6, 10 when PdO used; S.A. = self activation.

[b] DAc = CH_3COOD

[c] Determined by low voltage mass spectrometry (Table V) except runs 12-15 where Tamiya method used. (8)

[d] Significant conversion to 17 ketone (Table VIII).

TABLE II

Exchange of testosterone (V), testosterone acetate (VI), testosterone propionate (VII), progesterone (VIII) and desoxycorticosterone acetate (IX) on platinum.

Compound	Run	Catalyst		D_2O	H_2O	DAc[a]	Compound	Reaction Time	Temp	D
		Act.	(g)	(ml)	(ml)	(ml)	(g)	(hr)	(°C)	(%)
(V)	19	$NaBH_4$	0.010	1.9		0.5	0.050	48	90	4.3[b], [c]
	20	$NaBH_4$	0.010	1.9			0.050	48	90	1.2[b], [c]
	21	S.A.	0.020		4.0		0.100	46.5	150	- [c]
	22	S.A.	0.020		4.0		0.100	26	150	- [c]
	23	S.A.	0.020		4.0		0.100	48	110	- [c]
(IV)	24	H_2	0.036		3.6		0.121	42	120	11.9
(VII)	25	$NaBH_4$	0.256		30.4		0.601	48	140	16.2
	26	$NaBH_4$	0.010		1.8		0.050	48	90	6.3
	27	$NaBH_4$	0.010		1.9		0.050	48	152	14.7
(VIII)	28	$NaBH_4$	0.010		1.9		0.051	48	130	13.8
	29	$NaBH_4$	0.010		1.9		0.051	24	130	12.3
	30	H_2	0.037		3.6		1.131	43	120	10.1
	31	H_2	0.045			7.0	0.106	43	120	11.4
	32	$NaBH_4$	0.010		2.0		0.053	48	167	14.5
(IX)	33	H_2	0.054			7.0	0.095	43	120	12.3

a DAc = CH_3OOD

b By low voltage mass spectrometry (Tables V, VI), except runs 1 and 2 where Tamiya method used. (8)

c Significant conversion from -OH to C = O derivative (Table VIII).

TABLE III

Exchange of 17-hydroxypregnenolone (X), pregnenolone acetate (XI), cholesterol (XII), cholesteryl acetate (XIII) and cholesteryl propionate (XIV) on platinum, palladium and nickel.

Compound	Run	Catalyst[a]		D_2O	H_2O	DAc	Compound	Reaction Time	Temp	D[b]
		Act.	(g)	(ml)	(ml)	(ml)	(g)	(hr)	(0^oC)	(%)
(X)	34	$NaBH_4$	0.032	20.0			0.445	48	120	5.5[c]
	35	$NaBH_4$	0.010	1.9			0.050	48	152	16.2[c]
	36	$NaBH_4$	0.021		5.0		0.094	48	120	- [c]
(XI)	37	H_2	0.041			7.0	0.108	43	130	9.2
	38	H_2	0.041	3.6			0.124	43	120	0
(XII)	39	$NaBH_4$	0.010	1.9			0.050	48	90	0[c]
	40	$NaBH_4$	0.010	2.9			0.050	48	90	0[c]
	41	$NaBH_4$	0.010	1.95		0.5	0.050	48	130	0[c]
	42	$NaBH_4$	0.020		4.0		0.100	26	150	0[c]
(XIII)	43	H_2	0.334	1.8			0.178	44	130	0
	44	H_2	0.083	3.6			0.206	44	130	0
	45	H_2	0.041	1.8		4.0	0.100	42	120	3.4
	46	H_2	0.040	1.8		5.0	0.116	43	130	5.3
(XIV)	47	$NaBH_4$	0.020	5.25			0.169	48	130	2.5
	48	H_2	0.034	3.6			0.151	41	120	0

[a] PtO_2 used as catalyst except for runs 43 (nickel powder) and 44 (PdO).

[b] By low voltage mass spectrometry (Table VI) except runs 1-3 where Tamiya[8] method used.

[c] Significant conversion from -OH to C=O derivative (Tables VIII and IX).

TABLE IV

Exchange of etiocholanolone (XV), androsterone (XVI), stigmasterol acetate (XVII), digitoxin (XVIII), digitoxigenin (XIX), 5α androstane (XX) and 5β androstane (XXI) on platinum.

Compound	Run	Catalyst Act	Catalyst (g)	D_2O (ml)	H_2O (ml)	DAc (ml)	Compound (g)	Reaction Time (hr)	Temp (0^oC)	D[a] (%)
(XV)	49	$NaBH_4$	0.010	2.0			0.050	48	90	2.1[b]
	50	$NaBH_4$	0.010	1.9			0.050	46.5	133	5.8[b]
(XVI)	51	$NaBH_4$	0.010	2.0			0.047	48	90	1.5[b]
	52	$NaBH_4$	0.010	2.0			0.053	24	130	12.0[b]
	53	$NaBH_4$	0.010	2.0			0.050	46.5	150	14.2[b]
	54	$NaBH_4$	0.027		5.0		0.050	48	120	-[c]
(XVII)	55	H_2	0.035	2.0		4.0	0.107	43	120	2.2
	56	H_2	0.039			7.0	0.139	43	120	0
	57	H_2	0.042	4.0			0.124	48	120	0
(XVIII)	58	$NaBH_4$	0.010	2.1			0.029	48	90	0.2[b]
	59	$NaBH_4$	0.034	3.9			0.100	48	130	0.9[b]
	60	$NaBH_4$	0.044		5.0		0.104	48	130	-[b]
(XIX)	61	$NaBH_4$	0.016	3.9			0.056	48	130	2.3[b]
(XX)	62	H_2	0.010	2.0			0.106	24	120	0.7
(XXI)	63	H_2	0.011	2.0			0.100	24	120	0.6

a By low voltage mass spectrometry (Table VI) except runs 1-5, 10,11 where Tamiya[8] method used.

b Significant conversion from -OH to C=O derivative (Table IX).

TABLE V

Deuterium distribution[a] in desoxyestrone (I), oestrone (II), oestradiol diacetate (IV), testosterone acetate (VI) and testosterone propionate (VII) from Tables I and II.

Compound	Run	Table	D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9	D_{10}
I	2	I	95.1	4.9									
	3	I	25.3	37.1	25.5	10.3	1.7						
	4	I	23.1	45.5	27.1	4.3							
	5	I	2.0	4.4	22.1	33.6	22.6	8.8	3.5	2.0	1.2		
	6	I	1.0	2.2	2.5	4.7	7.4	7.8	8.8	11.0	14.6	14.7	10.6[b]
	7	I	86.2	12.7	1.1								
II	8	I	68.2	15.0	11.3	4.2	1.2						
	9	I	1.3	3.1	9.9	21.5	24.7	20.5	11.8	4.9	1.8	0.6	
	11	I	0	0	2.1	2.5	3.8	5.0	6.9	9.4	12.2	14.3	15.7[c]
IV	16	I	4.7	10.4	17.3	24.5	19.6	13.8	6.9	2.3	0.6		
VI	24	II	2.3	9.6	16.2	24.9	21.7	14.1	4.2	4.1	1.3	1.4	
	25	II	0.9	0.7	3.5	14.7	21.0	20.5	16.7	8.6	7.6	3.4	1.5[d]
VII	26	II	5.0	29.1	35.9	22.2	7.4	0.5					
	27	II	1.2	4.4	8.8	17.5	18.2	18.4	12.6	7.0	6.1	3.9	1.5[e]

a By low voltage mass spectrometry;

b D_{11}=7.8; D_{12}=3.9; D_{13}=1.5; D_{14}=1.0; D_{15}=0.6

c D_{11}=13.0; D_{12}=8.5; D_{13}=3.9; D_{14}=2.0; D_{15}=0.7

d D_{11}=0.8

e D_{11}=0.5.

TABLE VI

Deuterium distribution[a] in progesterone (VIII), desoxycorticosterone acetate (IX), pregnenolone acetate (XI), cholesteryl acetate (XIII), cholesteryl propionate (XIV), stigmasterol acetate (XVII), 5α-androstane (XX) and 5β-androstane (XXI) from Tables III and IV.

Compound	Run	Table	D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9	D_{10}
VIII	28	II	0	2.9	9.8	21.1	27.8	25.4	10.0	3.0			
	29	II	3.0	5.3	17.6	23.2	24.6	12.3	7.1	2.6	2.3	1.3	0.5
	30	II	1.4	7.1	31.6	33.2	14.1	6.6	2.6	2.4	1.1		
	31	II	1.5	5.0	14.9	29.0	31.9	15.9	1.1	0.6			
	32	II	0	1.9	6.8	17.5	32.9	25.6	8.4	3.2	2.5	1.3	
IX	33	II	0	3.7	20.7	19.5	23.7	16.0	9.5	4.6	2.3		
XI	37	III	13.4	23.7	32.4	19.8	7.8	2.8					
XIII	45	III	27.8	56.3	12.7	3.0							
	46	III	13.4	58.0	13.9	7.5	3.7	2.0	1.5				
XIV	47	III	47.8	41.5	7.5	1.7	1.5						
XVII	55	IV	15.2	61.7	12.9	5.5	1.8						
XX	62	IV	82.3	13.2	4.5								
XXI	63	IV	84.7	11.5	3.8								

a by low voltage mass spectrometry.

TABLE VII

Isotope orientation[a] in labelled 3-desoxyestrone (I), oestrone (II) and oestradiol diacetate (IV).

Compound	Run	Table	Catalyst	D (%)	% of Total D.	
					Aromatic Hydrogens	Aliphatic Hydrogens
I	5	I	$P+O_2$	15.0	88.5	11.5[b]
	6	I	PdO	34.1	53.4	46.6[b]
II	11	I	$P+O_2$	39.9	34.2	65.8[b]
IV	16	I	$P+O_2$	14.9	35.0	65.0

a By N.M.R.

b Angular methyl (C-18) not deuterated.

TABLE VIII

Degradation during the exchange of oestradiol (III), testosterone (V) and 17-hydroxypregnenolone (X) in Tables I - III.

Compound	Run	Table	Reaction		Remarks
			Time (hr)	Temp. (°C)	
	12	I	48	110	17-OH region almost intact (infrared)[a]
III	13	I	24	130	large C=O peak
	14	I	48	167	complete conversion 17-OH to C=O[b]
	15	I	48	167	complete conversion 17-OH to C=O
	19	II	48	90	small conversion -OH to C=O
V	20	II	48	90	small conversion -OH to C=O
	21	II	46.5	150	complete conversion -OH to C=O (infrared)
	22	II	26	150	complete conversion -OH to C=O (infrared)
	23	II	48	110	90% conversion -OH to C=O (infrared)
	34	III	48	120	5% conversion -OH to C=O
X	35	III	48	152	both OH groups completely oxidised to C=O[c]
	36	III	48	120	small conversion -OH to C=O

a α_D +78.0° (MeOH) for parent compound; this sample α_D +79.5°.

b α_D +110°.

c 3β (secondary) hydroxyls oxidised faster than both 17α (tertiary).

TABLE IX

Degradation during the exchange of cholesterol (XII), etiocholanolone (XV), androsterone (XVI), digitoxin (XVIII) and digitoxigenin (XIX).

Compound	Run	Table	Reaction Time (hr)	Reaction Temp (°C)	Remarks
	39	III	48	90	No deuteration, no oxidation
XII	40	III	48	90	No deuteration, no oxidation
	41	III	48	130	20% conversion of 3-OH to C=O
	42	III	26	150	90% conversion of 3-OH to C=O
XV	49	IV	48	90	no oxidation
	50	IV	46.5	133	50% conversion 3αOH to C=O[b]
	51	IV	48	90	no oxidation
XVI	52	IV	24	130	50% conversion of 3αOH to C=O[c]
	53	IV	46.5	150	80% conversion of 3αOH to C=O[c]
	54	IV	48	120	Two sets of angular methyls present (N.M.R.)
	58	IV	48	90	No oxidation
XVIII	59	IV	48	130	50% conversion of -OH to C=O[d]
	60	IV	48	130	50% conversion of -OH to C=O[d]
XIX	61	IV	48	130	50% conversion of -OH to C=O

[a] α_D -39.3° ($CHCl_3$) for parent compound; this sample α_D +49.7°

[b] Estimated from infrared (1720 cm^{-1}); 48 hr at 150°C, conversion 90%.

[c] α_D +93.6° (MeOH) for parent compound; this sample α_D +85.5°

[d] α_D +19.5° (MeOH) for parent compound; this sample α_D +12.5°.

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