ISOTOPIC HYDROGEN LABELLING OF STEROIDS BY HOMOGENEOUS PLATINUM CATALYSED EXCHANGE *

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SUMMARY

A number of new homogeneous metal catalysts have been studied for the deuteration and/or tritiation of a range of steroids. Sodium tetrachloroplatinate (II) was the most satisfactory of the catalysts examined. This compound catalysed selective exchange of protons in ring A of aromatic type steroids such as 3-desoxyestrone, oestrone and oestradiol. Conversion of the free steroids to their acetate or benzoate esters gave better labelling since the OH group in the free steroid reacts with the catalyst leading to precipitation of platinum from the homogeneous solution and a reduction in exchange rate. Steroids with hindered double bonds such as cholesterol, testosterone, pregnenolone, desoxycorticosterone acetate and progesterone do not exchange rapidly nor do the cardiac glycosides, digitoxin and digitoxigenin. Exchange with homogeneous platinum is more selective in isotope incorporation than with the corresponding heterogeneous platinum. A mechanism for the homogeneous exchange is proposed in terms of π-bonded intermediates. The present data are a guide for predicting the isotopic hydrogen labelling behaviour of steroids with the recently discovered homogeneous platinum catalyst.

INTRODUCTION

In the preceding paper [1], a representative group of steroids has been labelled using heterogeneous platinum and palladium

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as catalysts for the reaction. In the present work, a similar series of steroids has been labelled with the recently discovered[2] homogeneous sodium tetrachloroplatinate (II) catalyst. Attempts have also been made to use homogeneous palladium, gold, nickel and cobalt catalysts for this labelling work. Previous exchange studies with the homogeneous platinum catalyst have been confined to simple aromatic and aliphatic compounds such as substituted benzenes, alkanes and cyclohexanes.[3-6] This is the first detailed investigation of the use of this homogeneous platinum catalyst with steroids. The results have been interpreted in terms of current catalytic theories. At the conclusion of the paper, a critical evaluation is made of the use of both homogeneous and heterogeneous catalytic methods for labelling steroids and molecules of similar structure of use in the chemical and biological fields.

A preliminary communication of this work has been published.[7]

EXPERIMENTAL

All organic chemicals and heavy water used for the homogeneous exchanges were obtained from the same sources as mentioned in the preceding paper.[1] Sodium chloroplatinite and sodium chloropalladite were supplied by Johnson, Matthey and Co. Limited.

For the actual exchange runs, the quantities of particular reagents used are shown in the relevant Tables I-IV. The general exchange method was a modification of that previously reported for benzene and simpler compounds[8]. The reaction medium was monodeuteroacetic acid (CH_3COOD) which was prepared by mixing the stoichiometric quantities of acetic anhydride with deuterium oxide and carefully refluxing under nitrogen. The catalyst was added to the deuteroacetic acid immediately prior to each series of runs. The organic compound together with any other additives, e.g., HCl, were included in the preconstricted reaction vessel which was then outgassed twice, sealed at 10^{-2} torr and heated for the required time in a

water bath or oven. Fresh preparation of catalyst solution was needed prior to each series of runs, since Pt^{II} disproportionates to Pt^{O} and Pt^{IV} on prolonged standing. The precipitated platinum is capable of catalysing competing homogeneous exchange although previous work with benzene indicates that such heterogeneous deuteration is very slow even at 150°C. The catalyst used in all platinum runs was sodium tetrachloroplatinate (II). The potassium salt of this ligand is more easily purified, however it possesses only limited solubility in the reaction medium and for this reaon was not used in the present project.

The recovery of compounds from this homogeneous medium can be difficult. Those insoluble at room temperature were filtered off on a sintered glass filter, leached through the filter, usually with methanol, and evaporated with further methanol on a water bath under nitrogen to remove labile isotope, especially -OD. Soluble compounds were solvent extracted and the solvent evaporated under nitrogen. The recovered material was dried over silica gel under vacuum at room temperature, then purified by chromatography, recrystallisation and, where applicable, by vacuum sublimation.

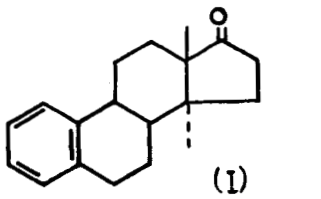
The chemical purity of the products was then checked by physical methods such as infrared spectroscopy, polarimetry, m.p., N.M.R. and mass spectrometry. With all compounds, background runs with non-deuterated medium were performed to check that no catalytic rearrangement, degradation or inversion has occurred during labelling.

Deuterium analysis on the products was determined by N.M.R. and mass spectrometry as previously described.[1]

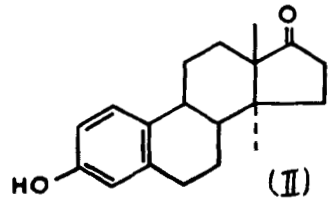
RESULTS AND DISCUSSION

The exchange conditions and isotopic incorporations for 3-desoxyestrone (I), oestrone (II), oestradiol (III), oestradiol monobenzoate (IV) and diacetate (V), testosterone (VI), testosterone acetate (VII) and propionate (VIII), and progesterone (IX) are shown in Tables I and II. The results for desoxycorticosterone

acetate (X), 5-α-dihydrotestosterone (XI), pregnenolone acetate (XII), pregnenolone (XIII), cholesterol (XIV), cholesteryl acetate (XV), and propionate (XVI), digitoxin (XVII) and digitoxigenin (XVIII) are summarised in Table III. In Table IV data for simpler compounds such as benzene (XIX), diphenyl (XX), phenyl cyclohexane (XXI) and hexamethylbenzene (XXII) are shown for mechanistic comparison purposes.



(I)



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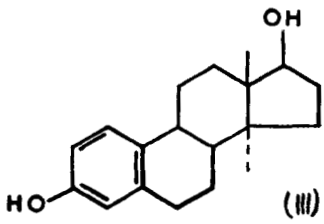

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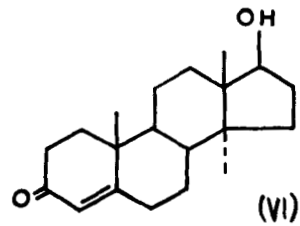

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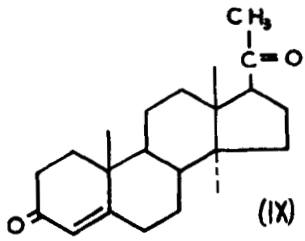
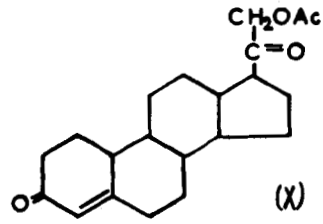
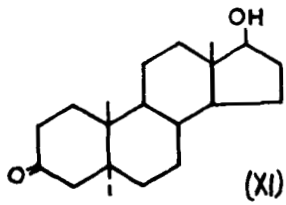


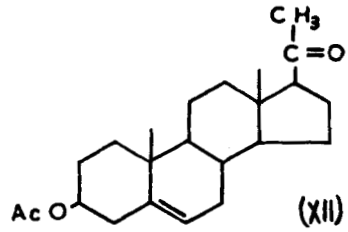
(IX)



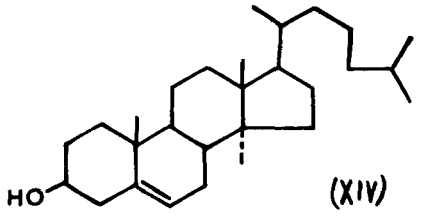
(X)



(XI)



(XII)



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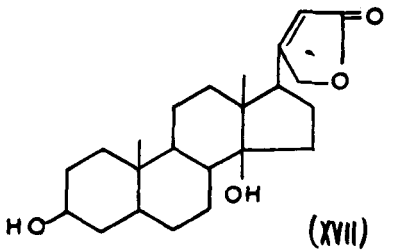
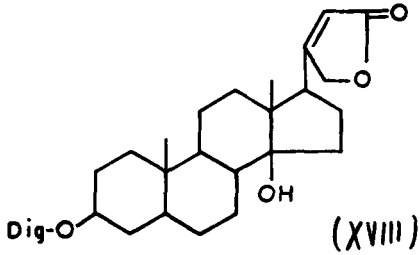


(XVII)



(XVIII)

In Tables V-VII are listed the low voltage mass spectral deuterium distributions for a selected series of runs in **Tables** I-IV. Isotope orientation by N.M.R. on a representative number of the steroids in Tables I-III are summarised in Table VIII. It will be observed in this work that the stability of the homogeneous catalyst is important. Thus there is an upper temperature limit for the actual exchange runs and this leads to significant selectivity in isotope incorporation in those steroids where deuteration was achieved.

Nature of Catalyst Solution

Because the sodium chloroplatinite catalyst was an untried system for steroid exchanges, extensive preliminary investigations were necessary to determine the effective catalyst conditions. In this respect, a wide range of solvents for the exchange medium were examined to increase both solubility of the compounds and the deuterium content in the reaction solution. Only CH_3COOD gave a satisfactory catalytic solution. Even runs in a two phase system with D_2O were unsatisfactory (Table I, runs 6,7). Complete

reduction of Pt^{II} to Pt^{o} occurred with all other solvents investigated, particularly the alcohols. The presence of hydroxyl and keto groups in the non-aromatic steroids (Tables II, III) also accentuated precipitation of the metal catalyst and is probably responsible for the poor labelling efficiencies of a large number of these compounds since sufficient concentration of catalyst could not be maintained in solution for long periods of time. Previous work[8] with simpler compounds has shown that this precipitated platinum is virtually inactive for heterogeneous aromatic exchange at 100°C.

In general, catalyst stability was found to be a function of time, temperature and nature of the substrate. Since Pt^{II} was known to disproportionate to Pt^{o} and Pt^{IV} after prolonged standing at room temperature[8], a satisfactory catalyst should show some degree of thermal stability. In CH_3COOD, with all compounds investigated, the catalyst was stable for 24 hours at 100°C, although with certain steroids, particularly those in Tables II and III some Pt^{o} was present after this time. At 120°C, complete reduction to Pt^{o} occurred in less than 24 hours with all compounds studied. As a compromise, most runs were performed for 3-4 hours at about 95°C. Similar difficulties with catalyst solution were encountered when sodium chloropalladite was used as catalyst instead of sodium chloroplatinite. Additional homogeneous catalysts examined, but unsuccessfully, in the present work were sodium chloroaurate (square planar), sodium chloropalladate and sodium chloroplatinate (both octahedral), platinum dichloride, nickel acetate and cobalt acetate.

Relationship between Steroid Structure and Labelling Efficiency

All compounds in Table I labelled readily in the aromatic ring using homogeneous platinum. Studies of the orientation of isotope in (I), (II) and (III) showed that both aromatic ring and C-16 hydrogens had exchanged (Table VIII) however back dedeuteration runs with CH_3COOH showed that exchange at C-16 hydrogens was labile. The N.M.R. orientation

effects were confirmed by the m.s. cut-offs in Table V. With oestradiol (III), deuteration of the esters (IV, V) was more facile than the parent compound (Table I, runs 18-21), the diacetate (V) being more efficient than the monobenzoate (IV). The presence of free aliphatic hydroxyl groups tends to precipitate the catalyst through reduction to Pt^{o} and thus the homogeneous exchange rate is decreased. During the deuteration of oestradiol, simultaneous esterification of the compounds was observed, thus it is preferable to exchange the ester rather than the parent compound.

Data in Table I show that it is not possible to obtain a fully deuterated steroid by the homogeneous technique. It is therefore only possible to obtain selectively deuterated (I)-(III) by this procedure. For tritiation work however, despite the orientation effect, the present technique would be satisfactory as a general labelling tool especially for high specific activities, if highly active acetic acid were used.

For the deuteration and/or tritiation of the remaining steroids studied here, the present homogeneous platinum catalysed conditions are unsatisfactory (Tables II, III, V, VI) since back exchange with CH_3COOH and 1MHCl removed all incorporated isotope. These steroids (VI to XVIII) are essentially saturated hydrocarbons which are known to exchange much more slowly than aromatic systems.[3-6] Even those steroids, such as cholesterol (XIV), which possess isolated double bonds did not label satisfactorily with either homogeneous platinum or palladium. Reasons for this lack of exchange will be discussed in the Section associated with the mechanism of the reaction.

It is interesting to note that testosterone (VI) did not exchange, even by keto-enol tautomerism, when reacted with pure CH_3COOD (no catalyst) for 3 hours at 92^{o} (Table II, run 22), consistent with previous steroid keto-enol tautomerism studies in acetic acid.[9] However, if either catalyst was added, or the solution was made 1M in DCl, exchange of "labile" hydrogens occurred. This exchange would be stable for certain tracer experiments, if the pH of the solution were not high, however for most biological work this type of labelling is unsatisfactory.

Mechanism of Labelling

From preceding work[8] and exchange of simpler compounds (Tables IV, VII), it is obvious that molecules possessing aromaticity, complex relatively strongly with the homogeneous platinum catalyst. Thus in phenylcyclohexane (Table VII, run 52) under the mild temperature conditions of the present deuteration, exchange is confined predominantly to the aromatic ring. Prolonged exchange of phenylcyclohexane at 120° does give some isotope incorporation into the saturated ring also, however, the aromatic ring still exchanges much faster indicating that in molecules of mixed aromatic-aliphatic character, complexing is preferentially with the aromatic part. The principles of exchange developed with these simpler molecules can now be applied to explain the behaviour of the more complicated compounds such as the steroids studied in the present work.

If benzene is used as a representative aromatic system, from earlier kinetic investigations, exchange can be considered to occur by a homogeneous π-associative process (Figure I).[3,8]

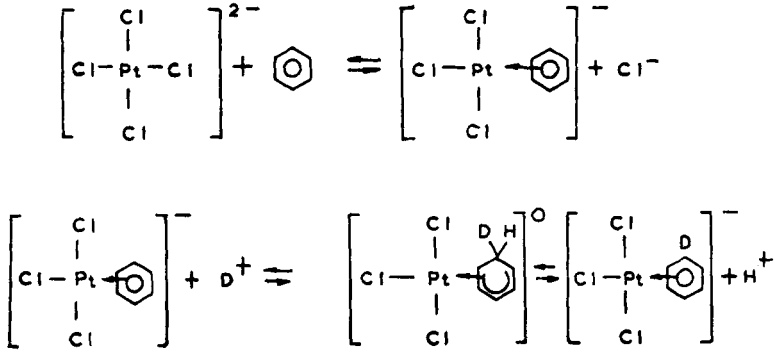


Fig. I. Homogeneous π-associative mechanism for exchange in benzene.

Hence initial formation of a π-complex takes place followed by electrophilic attack of a solvated deuteron (D^+) on the π-complex under the acidic conditions of the exchange to give the associative intermediate shown. Loss of H^+ from the intermediate, followed by rupture of the π-complex, gives monodeuterated benzene. If a number of rapid exchange

cycles of the π-complexed benzene occur before the π-complex breaks, multiple deuteration is observed and explains the pattern of many of the mass spectrometric deuterium distributions found in the present work in Tables V-VII.

However, evidence[8,10] is available from steric considerations to show that the exchange occurs predominantly by a homogeneous π-dissociative process. In this mechanism (Figure 2), exchange is thought to proceed by a dissociative process involving a reversible π-σ

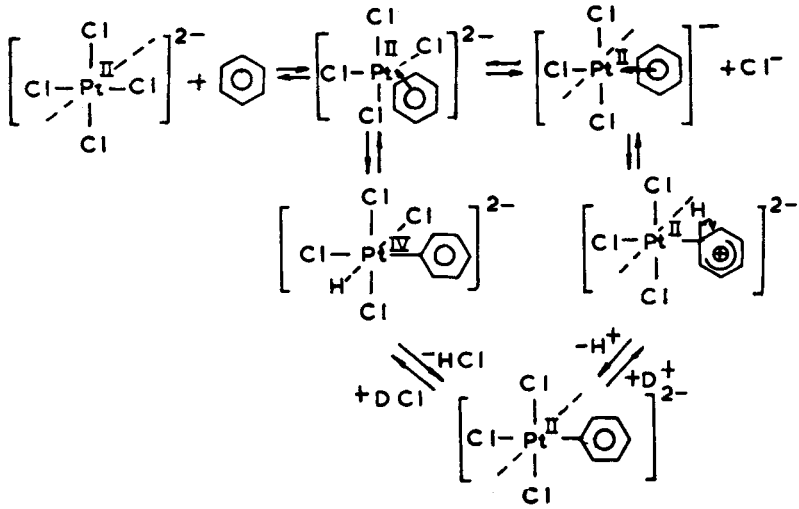


Fig. 2. Homogeneous π-dissociative mechanism.

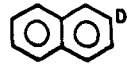
conversion. The exchange step may be a reversible electrophilic displacement of a proton from the π-bonded aromatic or a reversible rearrangement of the π-bonded complex to a six coordinated hydridocomplex with the subsequent exchange involving the hydrido group.

Application of Π-Complex Mechanisms to Steroid Exchange

In considering the possible application of the above homogeneous π-complex mechanisms to the present steroid data, it is significant that, of the steroids studied, only those possessing an aromatic ring have been satisfactorily labelled. Thus in 3-desoxyesterone (I), oestrone (II) and oestradiol (III), the N.M.R. and mass spectra show that deuteration has

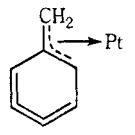
occurred in the C1 to C4 hydrogens and also at C16. In the latter case (C16), exchange was shown to be by keto-enol tautomerism, so that true catalytic deuteration only occurred in the aromatic ring. Exchange at C16 was more difficult than in simple ketones such as acetone, suggesting steric hindrance, however if the solution was 1 M in acid the C16 hydrogens would exchange.

Using 3-desoxyestrone (I) as example, the orientation in ring A indicates that the π-complex occurs preferentially with this part of the molecule. From the mass spectral distributions in Table V, the D_2 followed by D_4 cut-offs suggest that only two positions in the aromatic ring are active since two positions at C16 deuterate leaving a further two from ring A. This result is consistent with exchange in molecules such as naphthalene (XXIII) where previous studies[3,10] have shown that only the



(XXIII)

β positions exchange with the present homogeneous catalyst and the α positions are sterically hindered. From this reasoning, in (I) the hydrogens on C2, C3 would be expected to exchange faster consistent with mass spectral and N.M.R. data. Inclusion of an OH group in the aromatic ring activates the ortho positions to homogeneous catalytic exchange,[10] so that in (II) and (III) position 4 becomes more active to exchange than in (I). In certain of the mass spectral distributions of (II), peaks out to D_6 have been observed in the low voltage mass spectrum. Allowing for two hydrogens at C16 and only three hydrogens left in ring A because of the OH, this suggests that on prolonged exchange, deuteration of the saturated ring adjacent to ring A occurs, presumably at position 6. This would be consistent with the observed deuteration of the methyl group of toluene[10] which involves π-allylic species (XXIV). Because of steric



(XXIV)

hinderance at the C6 position in molecules such as (II), the magnitude of π-allylic exchange in these compounds is not as extensive as in toluene.

Using this π-complex theory, there are a number of reasons why the remaining members (VI) to (XVIII) of the present steroid series do not satisfactorily label with the homogeneous platinum catalyst. Most of VI to XVIII are essentially saturated hydrocarbons, which, even with simple molecules such as methane and cyclohexane, deuterate very much more slowly than aromatic systems.[10] Inclusion of tertiary carbon atoms and condensed ring structures such as in the present steroids reduces the exchange rate even further. The presence of a double bond, e.g. cholesterol (XIV) normally enhances complexing and rate of exchange. If complexing is too strong via such a π-olefin intermediate, then exchange may be irreversibly poisoned. With cholesterol and analogous steroids, however, the double bond is strongly sterically hindered and probably makes no contribution to the complexing and therefore the exchange. The other factor which reduces the exchange rates in these other steroids is the presence of non-hindered, aliphatic hydroxyl and keto groups. These readily reduce the Pt^{II} to Pt^{O}, thus catalyst is lost from solution and the homogeneous exchange terminated.

Comparison of Homogeneous and Heterogeneous Systems

In the preceding paper,[1] heterogeneous associative and dissociative π-complex mechanisms were shown to be valid for the interpretation of the deuteration data for the steroids, the π-dissociative process predominating. In like manner, for the homogeneous exchange of the steroids, homogeneous π-associative and π-dissociative processes appear to be satisfactory, the π-dissociative processes predominating. For the steroids where comparison can be made between homogeneous and heterogeneous systems, i.e. where secondary reactions do not interfere, similar patterns of deuteration exist for both catalytic procedures. This result is consistent with π-complex theory. Thus in 3-desoxyestrone (I), oestrone (II) and oestradiol (III) homogeneous exchange is predominantly and almost

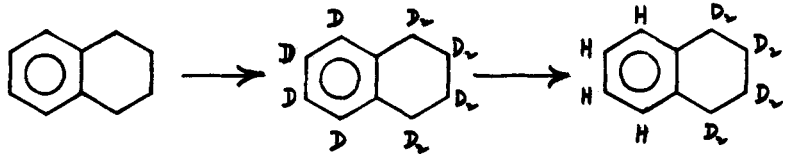
exclusively in ring A. For the heterogeneous process, the ring A also exchanges preferentially, but after prolonged deuteration, the other hydrogens also exchange. This pattern of activity parallels what has been observed for simple molecules. Thus the present observed relationships are further support for the concept that the manner of bond formation involving adsorbed molecules and the chemistry of inorganic co-ordination complexes are intimately related, presumably through π-complex formation.[10] The additional important mechanistic feature of the present work is that homogeneous platinum catalysed exchange in the steroids is generally more selective in a molecule than the corresponding heterogeneous reaction. Under heterogeneous conditions, exchange ultimately occurs over the whole molecule, although initially much faster near the unsaturated positions. If the homogeneous data are subtracted from the corresponding heterogeneous results, a significant fraction of the deuterium remains in the molecule. This fraction must be incorporated by processes that are surface catalysed only. Such mechanisms probably involve hydrogen abstraction processes as previously proposed for simple molecules.[10] In this respect the present biological compounds illustrate an extreme example, since they are relatively large molecules and can spread over a number of active sites on a heterogeneous surface.

Homogeneous and Heterogeneous Catalysis as Complementary Labelling Systems

The fact that the homogeneous method gives selectively deuterated and/or tritiated compounds whereas the heterogeneous technique ultimately exchanges all positions can be utilized to increase the range of selectively labelled compounds capable of being obtained by the present procedures.

Using tetralin to represent rings A and B of steroids (I) - (III), it is possible to homogeneously back exchange fully deuterated compound (XXV) from a heterogeneous reaction to give

selectively deuterated (XXVI) which would normally be difficult to prepare.



(XXV) (XXVI)

In conclusion, it should be possible now to predict the behaviour of a particular steroid when being labelled by either the homogeneous or heterogeneous techniques. The data show the conditions under which the procedures are powerful deuteration and/or titration labelling tools. They obviously possess advantages when compared with the alternate radiation-induced methods,[11] for tritium labelling.

ACKNOWLEDGEMENTS

Acknowledgement is made by one of us (J.L.G.) to the Australian Research Grants Committee and the Australian Institute of Nuclear Science and Engineering for the support of this research. We thank Drs. Collins and Hobbs of the Department of Veterinary Physiology, University of Sydney, for supplying several of the compounds investigated, for tritium labelling.

See the following pages for

Tables I-VIII and References

TABLE I

Exchange of 3-desoxyestrone (I) oestrone (II), oestradiol (III), oestradiol monobenzoate (IV) and oestradiol diacetate (V) with homogeneous platinum and palladium catalysts.

Compound	Run	Wt (g)	Catalyst soln[a] (ml)	DAc[c] (ml)	Additional Reagents (g)	Temp (°C)	Time (hr)	D (%)
I	1	0.097	10			102	3	8.0
	2	0.130	10			102	25	13.6
	3	0.177	5	10		102	18.5	9.8
	4	0.153	20[b]			102	18.5	0
II	5	0.061	3			92	2.5	14.6
	6	0.265			4[d]	120	3	0
	7	0.256			4[d]	120	3	0
	8	0.211	4			92	3	0
	9	0.226		5		92	3	0
	10	0.110		20	0.131[e]	95	4.7	8.4
	11	0.106		20	0.118[e]	95	42.5	13.2
	12	0.111	20			95	4.7	10.8
	13	0.114	20			95	24	11.9
	14	0.097	20		1.0[f]	95	4	7.9
	15	0.107	20			95	4	0
	16	0.115			20[g]	95	4	8.1
	17	0.131	10	10		102	25	15.8
III	18	0.181	3[b]			92	3	0
	19	0.193	3			92	3	1.1[h]
IV	20	0.059	3			92	2.5	3.4
V	21	0.260	3			92	3	6.0

a Na_2PtCl_4 (0.02 M) in CH_3COOD b Na_2PdCl_4 (0.02 M) in CH_3COOD
c DAc = CH_3COOD d D_2O contains 0.02 M Na_2PtCl_4 e Na_2PtCl_4
f 1 M HCl g CH_3COOH, to examine degradation with no isotope present
h oestradiol acetylated during exchange.

TABLE II

Exchange of testosterone (VI), testosterone acetate (VII), testosterone propionate (VIII) and progesterone (IX) with homogeneous platinum and palladium catalysts.

Compound	Run	Wt (g)	Catalyst soln [a] (ml)	DAc [b] (ml)	Additional Reagents (g)	Temp °C	Time (hr)	D %
VI	22	0.239	3	5		92	3	0
	23	0.206	3			92.5	3	15.3
	24	0.194		4	0.045 [c]	92.5	3	0
	25	0.215		5	0.142 [d]	92	3	17.9
	26	0.070			3 [e]	92	3	0
	27	0.181		3	0.05 [f]	92	3	17.7
	28	0.065			3 [e]	92	3	0
	29	0.252		3	.053 [d]	92.5	3	0
VII	30	0.237	3			92	3	10.4
	31	0.266	4			120	3	7.3
	32	0.134			3 [e]	95	3	0
VIII	33	0.251	3			92	3	7.8
	34	0.195			3 [e]	92	3	0
IX	35	0.195	3			92	3	11.9
	36	0.254	3			120	3	13.4
	37	0.150			3 [e]	92	3	0

[a] Na_2PtCl_4 (0.02 M) in CH_3COOD

[b] DAc = CH_3COOD

[c] Na_2PdCl_4

[d] Na_2PtCl_4

[e] 0.05 ml 1D NHCl added to HAc; these were dedeuteration runs to check for incorporation by keto-enol tautomerism.

[f] 1D NHCl

TABLE III

Exchange of desoxycorticosterone acetate (X), 5-α-dihydrotestosterone (XI), pregnenolone acetate (XII), pregnenolone (XIII), cholesterol (XIV), cholesteryl acetate (XV), cholesteryl propionate (XVI), digitoxin (XVII), and digitoxigenin (XVIII) with homogeneous platinum and palladium catalysts.

Compound	Run	Wt (g)	Catalyst soln[a] (ml)	DAc[b] (ml)	Additional Reagents (g)	Temp °C	Time (hr)	D (%)
X	38	0.292	3			96	1.5	9.0
	39	0.156			3[c]	92	3	0
XI	40	0.201	3			92	3	10.9
	41	0.130			3[c]	92	3	0
XII	42	0.209	3			92	3	12.2
XIII	43	0.126	3			92	3	8.9
XIV	44	0.204		4	0.030[d]	92.5	3	0
	45	0.211		4	0.035[e]	92.5	3	0
XV	46	0.274	4			92.5	3	0
XVI	47	0.257	4			92.5	3	0
XVII	48	0.130	5			95	4	0
XVIII	49	0.132	5			95	4	0

a Na_2PtCl_4 (0.02 M) in CH_3COOD

b DAc = CH_3COOD

c 0.05 ml 10 NHCl added to HAc; these were dedeuteration runs to check for incorporation by keto-enol tautomerism.

d Na_2PdCl_4

e Na_2PtCl_4

TABLE IV

Exchange of benzene (XIX), diphenyl (XX), phenycyclohexane (XXI) and hexamethylbenzene (XXII) with homogeneous platinum and palladium catalysts.

Compound	Run	Wt (g)	Catalyst soln[a] (ml)	DAc[c] (ml)	Additional Reagents (g)	Temp (°C)	Time (hr)	D (%)
XIX	50	1.6		20	0.120[d]	95	20	38.7
XX	51	0.156	20			102	18.5	15.2
XXI	52	0.16	20			95	20	9.0
	53	0.16	20[b]			95	20	0
XXII	54	0.156	20			102	3	0.1
	55	0.163	20			102	25	0.7
	56	0.145			5.0[e]	102	18	0

a Na_2PtCl_4 (0.02 M) in CH_3COOD
b Na_2PdCl_4 (0.02M) in CH_3COOD
c DAc = CH_3COOD
d Na_2PtCl_4
e D_2O+Na_2PtCl_4 (0.130g)

TABLE V

Deuterium distributions for 3-desoxyestrone (I) oestrone (II), oestradiol (III) and its monobenzoate (IV) and diacetate (V) reported in Table 1

Compound (Table I)	Run (Table I)	D (%)	Distribution (%)*						
			D_0	D_1	D_2	D_3	D_4	D_5	D_6
I	1	8.0	5.0	38.8	41.3	10.9	4.0		
	2	13.6	6.1	25.2	37.1	28.4	2.7	0.4	
	3	9.8	4.3	21.8	41.1	22.4	9.0	1.4	
II	5	14.6	0	3.3	15.7	39.4	40.5	1.0	0.2
	10	8.4	2.6	14.4	60.6	16.8	0.4		
	11	13.2	0	5.1	23.2	35.9	23.4	5.5	1.9
	12	10.8	1.7	8.8	42.9	43.8	2.7	0.1	
	13	11.9	1.8	6.2	29.8	52.6	8.9	0.6	
	14	7.9	3.9	18.2	76.3				
	16	8.1	4.2	23.6	65.3	5.9	0.9		
	17	15.8	0	2.4	10.6	35.0	46.1	4.5	1.4
III	19	1.1	84.3	8.8	4.7	2.1			
IV	20	3.4	58.2	16.1	14.8	4.8	4.7	1.2	
V	21	6.0	18.1	43.5	26.7	11.7			

* By low voltage mass spectrometry.

TABLE VI

Deuterium distributions for testosterone (VI), its acetate (VII) and propionate (VIII), progesterone (IX), desoxycorticosterone acetate (X), dihydrotestosterone (XI), pregnenolone acetate (XII) and pregnenolone (XIII) reported in Tables II and III.

Compound (Tables II & III)	Run (Tables II & III)	D (%)	Distribution (%) [a]							Remarks
			D_0	D_1	D_2	D_3	D_4	D_5	D_6	D_7
	23	15.3	1.4	5.5	18.9	33.4	27.6	10.1	2.0	0.7
VI	25	17.9	0	0	5.8	27.4	55.5	7.2	4.0	
	27	17.7	0	1.3	7.1	25.6	54.3	7.2	4.5	
VII	30	10.4	0.8	5.1	21.2	37.4	28.5	6.5	0.5	
	31	7.3	7.3	23.0	33.6	24.0	9.4	2.7		b
VIII	33	7.8	2.8	16.6	32.9	29.9	15.0	2.2	0.6	
IX	35	11.9	1.8	5.6	12.6	25.4	29.7	18.8	6.0	
	36	13.4	1.8	4.7	12.9	18.0	25.8	22.5	10.6	3.7 b
X	38	9.0	6.7	12.7	23.5	24.5	20.1	8.7	3.9	
XI	40	10.9	0.6	2.7	12.7	38.3	44.6	1.2		
XII	42	12.2	4.0	11.4	29.4	37.0	16.8	1.5		
XIII	43	8.9	7.4	25.4	40.2	25.4	1.4			

a By low voltage mass spectrometry

b Catalyst unstable

TABLE VII

Deuterium distributions and isotope orientation for benzene (XIX), diphenyl (XX), phenylcyclohexane (XXI) and hexamethylbenzene (XXII), reported in Table IV

Compound (Table IV)	Run (Table IV)	D (%)	Distribution (%) [a]							Isotope Orientation [b]
			D_o	D_1	D_2	D_3	D_4	D_5	D_6	
XIX	50	38.7	35.5	6.1	12.3	13.3	12.1	10.1	10.4	
XX	51	15.2	37.9	21.4	15.8	12.1	6.1	4.0	2.7	3,4,5,3',4',5'
XXI	52	9.0	25.8	27.9	26.5	17.8	2.0			Aromatic
XXII	54	0.1	97.8	2.2						
	55	0.7	89.0	11.0						

a By low voltage mass spectrometry

b By N.M.R.

TABLE VIII

Orientation of deuterium in 3-desoxyestrone (I), oestrone (II) and oestradiol (III) and its benzoate (IV) and diacetate (III)[a]

Compound	Run	D (%)	C-1 to C-4 Hydrogens (%)	C-16 Aliphatic Hydrogens (b)
I	1	8.0	5.0	80.0
	2	13.6	30.0	95.0
II	17	15.8	51.3	97.0
III	19	1.1	All D incorporated	
IV	20	3.4	Predominantly	
V	21	6.0	All D incorporated	

a By N.M.R.

b This deuterium was labile since back exchange occurred using reaction solution containing protium and no catalyst.

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