Labelling of synthetic oestrogens of the trans-stilbene and symmetrical diphenylethane series by catalytic exchange on heterogenous platinum*

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SUMMARY

Synthetic oestrogens of the trans-stilbene and sym. diphenylethane series have been labelled by catalytic exchange with isotopic water in the presence of heterogeneous platinum. Experiment showed that platinum oxide pre-reduced with sodium borohydride produced a more suitable catalyst for the exchange than the alternative of self-activation. Self-activated catalysts accentuated degradation of the substrate. The labelling method was suitable for the tritiation of all ten substances studied and also for their deuteration with the exception of 4,4'-dimethoxy-α-chloro-α-methyl stilbene. Methoxy groups on the aromatic rings sterically hinder exchange in adjacent positions. Steric hindrance to labelling of hydrogens on the bridge carbon atoms was also noted. The results are shown to be consistent with a π-complex adsorption mechanism.

INTRODUCTION

Synthetic (non-steroid) oestrogens of the trans-stilbene and sym. diphenylethane series are a biologically important class of compounds [1]. The present work describes methods for labelling these compounds with deuterium and/or tritium by heterogeneous metal-catalysed exchange with isotopic water. Tritium is commonly used in biological work as a tracer for hydrogen and with

the availability of more sensitive detection methods (particularly mass spectrometry) deuterium also is increasingly being employed for this purpose.

Heterogeneous metal catalysed exchange [2] was chosen as labelling procedure since the method is applicable to deuterium and/or tritium and is therefore more versatile than the corresponding radiation-induced techniques such as the Wilzbach method [3] which, in addition, suffers from problems associated with radiochemical purification of the parent compound [4]. An additional advantage of the catalytic procedure is that deuterium can be used as a tracer for tritium (if due cognisance is taken of possible radiation damage) with the consequent facilitation of experimental work. Deuterium also enables the application of physical methods (N. M. R. and mass spectrometry) for orientation and distribution studies, thus obviating the time-consuming chemical degradations. Of the Group VIII transition metals available as catalysts, platinum is the most useful for general exchange [5]. The most successful types of catalyst activation were utilized in the present experiments (i) sodium borohydride reduction of the inorganic oxide [6] and (ii) self-activation [7] or in situ reduction of the platinum oxide by the organic substrate. Each of the two types of procedures possesses certain advantages during exchange with simpler molecules and it was intended to ascertain whether similar advantages occurred during the labelling of larger molecules

The present labelling results are also of interest to fundamental theory. Recently π-complex mechanisms have been developed for explaining the exchange of aromatic and alkyl hydrogens in simple molecules such as the alkylbenzenes [5, 8–11]. Preliminary experiments have also been reported for the isomerisation of cis- and trans-stilbenes [5]. It is thus important to determine whether steric effects observed in π-bonding with simpler molecules during adsorption are accentuated in the present series containing larger molecules. The present paper concludes with a feasibility discussion of the labelling procedures used for general exchange in the synthetic oestrogens.

EXPERIMENTAL

The purity of all compounds used was checked by m.p., infrared, nuclear magnetic resonance spectroscopy and mass spectrometry. The isotope source was deuterium oxide 99.75 % isotopic purity and the catalyst was platinum oxide (Adam's Catalyst, PtO_2 hydrated). All reagents (including solvents) used were of AR quality.

For the pre-reduced catalyst the platinum oxide was suspended in water in the reaction tube and sodium borohydride (40 mg to each 10 mg of catalyst) added slowly. After effervescence had ceased, the hydrolysis of the borohydride was completed at 70 ºC and the reduced catalyst then washed three times with water, the last washing being checked to ensure neutrality. After reduction of the catalyst the required amount of deuterium oxide and compound were quickly weighed into the tube, the contents frozen and sealed under

vacuum. The reaction was carried out over the temperature range 100-150 °C in a calibrated oven using a specially constructed rotating device. For the self-activation runs the catalyst oxide was weighed into the tube, the deuterium oxide and substrate added and the vessel sealed as before. In this procedure reduction of the catalyst occurs by hydrogen abstraction from the substrate.

The compound was recovered by filtration or solvent extraction depending on its solubility, and evaporated with methanol to remove labile deuterium. The material was then dried under vacuum and the physical properties determined.

For infrared, the KCl disc technique [12] was used throughout on a Perkin-Elmer 221 instrument with potassium chloride purified by the methods of Hales and Kynaston [13]. A Varian A60 instrument was used without temperature control for N. M. R. determinations. Melting points were carried out on a Reichert Kofler block type apparatus and are uncorrected.

DEUTERIUM ANALYSIS.

Extent of incorporation was determined by mass spectrometry using Tamiya's modification [14] of the Graff and Rittenberg [15] method. In this modification the sample (about 3 mg) contained in a sealed reaction vessel fitted with a tail-piece was combusted to water at 600 °C in an atmosphere of oxygen. The water, collected in the tail-piece, was transferred to a second vessel of Corning 1720 glass (impermeable to hydrogen) fitted with a break-seal and containing zinc powder in which the water was reduced by heating to 600 °C (60 min). The gases (H_2, HD, D_2) were analysed on a Metropolitan Vickers MS2G instrument which had previously been calibrated using H_2O-D_2O mixtures of known composition. The method was found to be satisfactory, using the single inlet technique over the range 1-60 atoms % D after which isotope-effects became pronounced [16].

An MS9 (Metropolitan Vickers) mass spectrometer was used with a low voltage technique to determine isotope distribution and to check the incorporation.

RESULTS AND DISCUSSION

The exchange conditions and deuterium incorporations for the compounds are listed in Table I (*trans*-stilbene, stilboestrol derivatives), Table II (*trans*-stilbene derivatives only) and Table III (sym. diphenylethane and derivatives). Isotope orientation in a representative number of compounds is summarised in Table IV whilst in Tables V and VI low voltage mass spectra distributions are shown. Results in Tables V and VI are particularly useful since they support n.m.r orientation results and also check deuterium incorporation data. It will be observed that heterogeneous platinum is a satisfactory catalyst

TABLE I. Heterogeneous exchange of trans-stilbene and stilboestrol derivatives

Compound	Run	Catalyst Activation	Catalyst (g)	D_2O (g)	Compound (g)	Reaction Time (hr)	Temp. (°C)	Recovery (%)	D[c] (%)
trans stilbene	1	S. A.[a]	0.010	2.03	0.050	47	110	80	1.2
	2	S. A.	0.020	2.06	0.050	47	110	66	2.8
	3	$NaBH_4$[b]	0.206	32.9	1.01	48	150	91	44.2
Diethyl	4	S. A.	0.010	2.07	0.050	48	110	88	10.8
stilboestrol	5	S. A.	0,010	2.03	0.050	94	130	58	21.8
	6	S. A.	0.010	2.02	0.050	96.5	152	74	33.5
	7	$NaBH_4$	0.010	2.08	0.050	47	150	66	30.9
	8	$NaBH_4$	0.247	37.0	0.660	48	140	90	43.3
Dimethyl	9	$NaBH_4$	0.044	5.64	0.113	48	140	91	14.7
stilboestrol	10	$NaBH_4$	0.024	5.56	0.049	48	160	72	44.4
	11	$NaBH_4$	0.108	28.1	0.255	48	150	93	35.3

[a] S. A. = self-activation
[b] $NaBH_4$ = Sodium borohydride reduction
[c] By mass spectrometry (Tamiya method).

for the labelling of all compounds in Tables (I), (II) and (III), with deuterium and/or tritium, with the exception of 4,4'-dimethoxy-α-chloro-trans-stilbene (Table II) where tritium incorporation only is feasible.

PURITY OF LABELLED PRODUCTS.

It is important to note that a large number of preliminary experiments [16] were necessary to find an approach to optimum conditions for deuteration (i.e. minimum chemical decomposition, maximum isotope incorporation). All compounds in both the trans-stilbene and sym. diphenylethane series were purified before isotope analysis. Because physiological actitivity is very sensitive to structure, considerable care was taken to ensure that the recovered compound was identical, after purification, to the original, except for the isotopic substitution. The method adopted was to exchange the compounds

TABLE II. Heterogeneous exchange of dimethoxy *trans*-stilbene derivatives

Compound	Run	Catalyst Activation	Catalyst (g)	D_2O (g)	Compound (g)	Reaction Time (hr)	Temp. (°C)	Recovery (%)	D[a] (%)
4,4'-dimethoxy-α-chloro-*trans*-stilbene	1	NaBH$_4$	0.010	2.16	0.047	48	90	85	0.3
	2	S. A.	0.010	2.07	0.050	47	110	78	3.2
	3	NaBH$_4$	0.031	28.1	0.322	48	120	63	1.6
4,4'-dimethoxy-α-methyl-*trans*-stilbene	4	NaBH$_4$	0.010	2.13	0.053	48	90	87	0.3
	5	S. A.	0.010	2.06	0.050	47	110	78	4.6
	6	NaBH$_4$	0.032	32.1	0.267	48	120	47	5.9
4,4'-dimethoxy-α-ethyl-*trans*-stilbene	7	NaBH$_4$	0.010	2.16	0.050	48	90	86	1.2
	8	S. A.	0.010	2.09	0.050	47	110	84	4.7
	9	NaBH$_4$	0.209	29.9	0.352	24	120	85	5.9

[a] By mass spectrometry (Tamiya method).

using H_2O instead of D_2O. The products from the controls were then processed in a similar manner to the deuterated compounds and their physical properties compared with the original materials. Thus mass and infrared spectra (and to a lesser extent, n.m.r) could be used to compare the structure of the final product from the catalytic exchange with the original compound, without deuterium perturbations.

An additional complication in the recovery of deuterated products from the exchange of the stilbenes was the possibility of thermal isomerisation especially since labelling by heterogeneous catalysis requires elevated temperatures, usually above 100 °C. *Trans*-stilbene is thermodynamically more stable than the *cis*-isomer. Taylor and Murray [17] report that the equilibrium mixture at 200 °C contains 96 % *trans* whilst at 320-340 °C, 92-94 % is present [18]. However with α,α'-dimethyl stilbene, the equilibrium mixture contains 60 % *trans* isomer [19] at 210 °C whereas with α,α'-dichlorostilbene this drops to 25 % *trans* [17]. In the present work, all *trans*-stilbene derivatives were readily recovered pure in high yields after exchange even though some *cis*-isomer may have been present in the crude reaction product.

MECHANISM OF EXCHANGE.

Both groups of compounds studied in the present series contain alkyl and aromatic hydrogens similar to those found in the alkylbenzenes. Exchange

TABLE III. Heterogeneous exchange of Sym. diphenylethane and derivatives

Compound	Run	Catalyst Activation	Catalyst (g)	D_2O (g)	Compound (g)	Reaction Time (hr)	Temp. (°C)	Recovery (%)	D[a] (%)
Sym. diphenylethane	1	S. A.	0.010	2.08	0.050	47	110	90	50
Sym. diphenylethane	2	NaBH₄	0.010	2.06	0.050	47	150	40	90
Sym. diphenylethane	3	NaBH₄	0.209	28.8	1.53	24	120	93	69.1
Hexestrol	4	NaBH₄	0.051	4.61	0.118	48	130	97	27.6
Hexestrol	5	NaBH₄	0.205	43.7	0.696	48	130	96	41.8
Erythro-4,4'-dihydroxy-α-ethyl-α'-methyl sym. diphenyl-ethane	6	S. A.	0.010	2.05	0.050	47	110	92	4.4
	7	NaBH₄	0.010	2.09	0.050	47	150	98	39.8
	8	NaBH₄	0.136	23.6	0.303	48	130	95	35.7
Threo-4,4'-dihydroxy-α-ethyl-α'-methyl-sym. diphenylethane	9	S. A.	0.010	2.05	0.050	47	110	98	45.0
	10	NaBH₄	0.010	2.11	0.050	47	150	70	38.8
	11	NaBH₄	0.138	27.1	0.497	48	130	98	53.1

[a] By mass spectrometry (Tamiya method).

in the alkylbenzenes has been interpreted predominantly in terms of π-complex adsorption [5, 8, 9, 11]. In aromatic hydrogen exchange, two types of mechanisms have been proposed, viz. the associated and dissociative π-complex substitution mechanisms (fig. 1-3). In the associative mechanism (fig. 1 and 2), the π-bonded aromatic molecule is attacked by a hydrogen atom formed from the dissociative adsorption of water or hydrogen gas. The activation energy in this process is initially due to the partial localisation of one π-electron in the transition state which differs from that of conventional substitution reactions by being π-bonded to the catalyst surface. In the dissociative mechanism (fig. 3), the π-bonded aromatic reacts with a metal radical (active site) by a substitution process which involves a conventional π-σ conversion

TABLE IV. Isotope orientation in labelled trans-stilbene and sym. diphenylethane derivatives [a].

Compound	D (%)	Aromatic Hydrogens (%)	Aliphatic Hydrogens (%)
trans-stilbene	44.2	54.2	α,α' hydrogens not deuterated
Diethyl stilboestrol	43.3	63.1	C_2H_5 — 27.5; CH_3 — 44.9; CH_2 — 1.5
Dimethyl stilboestrol	35.3	67.0	α,α' methyl groups not deuterated
4,4'-dimethoxy-α-ethyl-trans-stilbene	5.9	90 % of isotope incorporation	Insufficient incorporation
4,4'-dimethoxy-α-methyl-trans-stilbene	5.9	90 % of isotope incorporation	Insufficient incorporation
4,4'-dimethoxy-α-chloro-trans-stilbene	1.6	—	—
Sym. diphenylethane	69.1	60.6	α,α' hydrogens 90.8 % deuterated
Threo-4,4'-dihydroxy-α-ethyl-α'-methyl-sym. diphenylethane	53.1	88.5	Total — 25.2; α,α' — nil; α-CH_3 — 13; α'-C_2H_5 (i) CH_2 — 23 (ii) CH_3 — 51.7
Hexestrol	41.8	32.9	Total — 48; α,α' — 55; C_2H_5 — (i) CH_2 — 27 (ii) CH_3 — 59.4
Erythro-4,4'-dihydroxy-α-ethyl-α'-methyl-sym. diphenylethane	35.7	74	Total — 5; 90 % of isotope in CH_3 of C_2H_5

[a] By n.m.r.

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TABLE V. Trans-stilbene series. Intermolecular-distribution of deuterium [a].

Compound	d0	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	d13	d14	d15	d16	d17	d18	d19	d20
trans-stilbene	—	—	—	—	1.2	7.3	26.3	26.3	19.7	10.6	5.4	2.1	1.2	—	—	—					
Dimethyl stilboestrol	—	0.6	0.9	1.9	8.3	17.4	21.7	19.8	12.3	6.6	3.2	2.0	1.4	1.0	0.9	0.8	0.5	0.5	0.4	0.3	
Diethyl stilboestrol						0.7	7.5	14.7	20.4	21.5	17.1	11.1	5.8	0.9	0.3						
4,4'-dimethoxy-α-methyl-*trans*-stilbene	57.9	15.3	21.3	4.7	0.7	—	—	—													
4,4'-dimethoxy-α-methyl-*trans* stilbene	54.5	26.4	11.5	4.6	2.0	0.8															
4,4'-dimethoxy-α-ethyl-*trans*-stilbene	55.2	29.5	11.2	4.0																	

[a] By mass spectrometry.

TABLE VI. Sym. diphenylethane series. Intermolecular distribution [a].

Compound	d0	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	d13	d14	d15	d16	d17	d18	d19	d20
Sym. diphenylethane	—	—	—	—	—	—	0.7	3.4	10.7	22.5	26.3	19.5	11.1	4.3	1.3						
Erythro-4,4'-dihydroxy-α-ethyl-α'-methyl-sym. diphenylethane						1.6	2.0	2.5	3.9	5.9	10.7	16.7	14.7	13.4	12.1	8.7	5.5	2.1			
Threo-4,4'-dihydroxy-α-ethyl-α'-methyl-sym. diphenylethane									2.6	7.7	17.3	23.4	17.9	14.0	10.0	4.6	2.5				
Hexestrol	5.8	6.6	5.6	8.5	10.9	8.2	6.4	4.9	4.0	4.0	4.0	4.6	3.7	4.9	4.2	4.0	3.2	2.5	1.7	1.5	0.8

[a] By mass spectrometry.
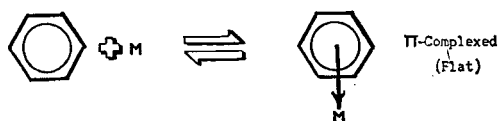
Fig. 1. General π-complex adsorption
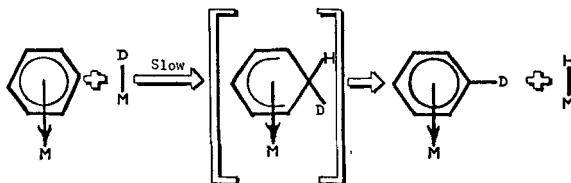
Fig. 2. Associative π-complex substitution mechanism
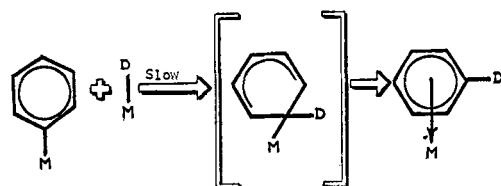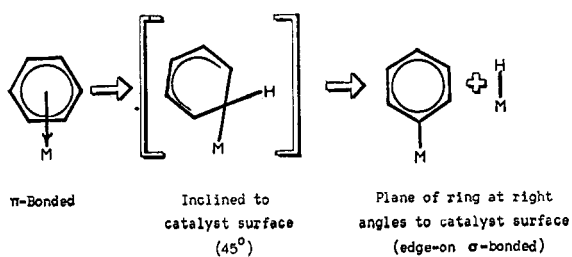
Fig. 3. Dissociative π-complex substitution mechanism

on the catalyst surface. While π-bonded, the aromatic undergoes a second, slower substitution reaction with a chemisorbed deuterium atom at the carbon-metal bond, then returns to the π-bonded state. Multiple exchange will occur if the process is repeated several times before desorption. Present evidence indicates that the dissociative process predominates although this conclusion remains the subject of controversy [5, 11].

EXCHANGE OF AROMATIC HYDROGENS.

The important experimental evidence used to differentiate between the above two mechanisms for aromatic hydrogen exchange involved ortho deactivation effects with the monosubstituted benzenes where the substituent was catalytically inert, e.g. the alkylbenzenes. In this series, methyl and ethyl groups exert *severe* ortho deactivation whereas bulky groups such as *t*-butyl exhibit *complete* ortho deactivation.

Generally the orientation of aromatic deuterium in the present sym. diphenylethane and stilbene series is consistent with the alkylbenzene interpretation. In the *trans*-stilbene series, the two benzene rings and the double bond in the parent compound are co-planar, thus delocalisation occurs throughout the whole molecule. Inclusion of α-alkyl substituents leads to a degree of non-planarity in the molecule with subsequent deactivation of the ring positions ortho to the allylic group. With the *dialkyl stilboestrols* (Table I) a compensation effect occurs in that the positions adjacent to the -OH group are activated as in phenol. Thus large differences are not observed for the total deuterium incorporated in the aromatic positions in trans-stilbene, diethyl stilboestrol and dimethyl stilboestrol (Table I).

The presence of methoxy groups (dimethoxy stilbenes, Table II) leads to *complete* deactivation of the ortho positions adjacent to the $-OCH_3$ as in anisole[11], thus this group of compounds exchanges relatively slowly on heterogeneous platinum. The presence of a chlorine atom in the α-position accentuates the toxicity of a molecule in exchange, thus 4,4'-dimethoxy-α-chloro-*trans*-stilbene was the least reactive of the stilbene series presumably due to increased chemisorption via the chlorine atom leading to increased reagent displacement effects and a corresponding reduction in exchange rate, as is observed with chlorobenzene.

Deuteration of the aromatic positions in the sym. diphenylethane series (Table III) follows a similar pattern to that observed for the stilbenes. The presence of alkyl groups in the side chain leads to ortho steric effects which are compensated for by enhanced activation due to hydroxyl groups in the 4,4' positions. Thus all compounds in Table III readily exchange, hexestrol with two α-ethyl groups being the least reactive whilst sym. diphenylethane with no alkyl substituents is the most reactive.

EXCHANGE OF ALKYL HYDROGENS.

Catalytic deuteration in the alkyl hydrogens of compounds such as the alkylbenzenes has been interpreted by two type mechanisms, each of which appears to contribute to the overall exchange [10, 21]. Using toluene as representative compound, figure 4 depicts the π-allylic mechanism proposed to explain deuteration in the methyl group. It is suggested that the π-allyl platinum shown in figure 4 is formed via the intermediate π-olefin type complex (species I). The second mechanism [21] proposed to explain echange in the
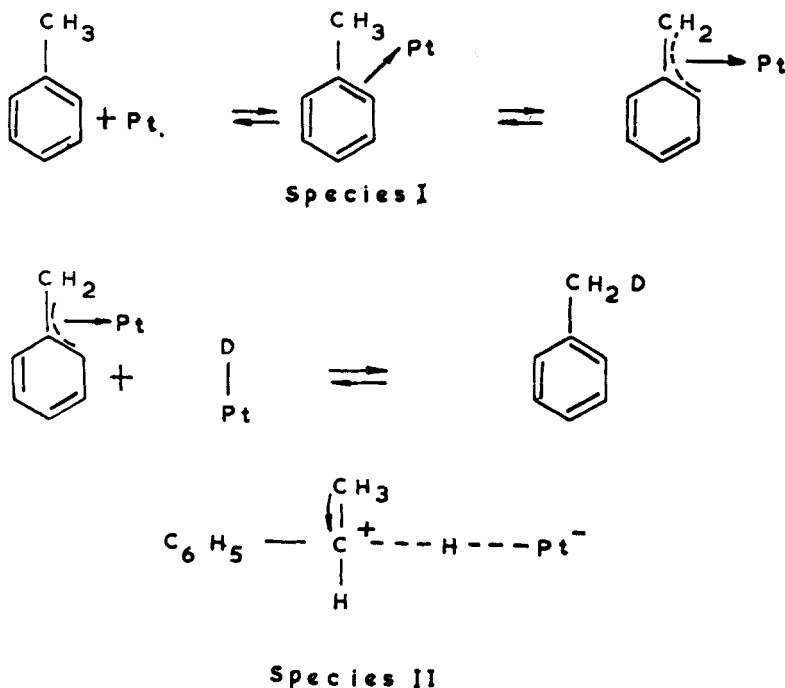
Species I

Species II

Fig. 4

alkyl group of the alkylbenzenes, particularly deuteration of the hydrogens on the α-carbon atom involves an abstraction reaction which proceeds through a transition state similar to the one postulated by Huyser [22] for an electrophilic radical abstraction process (species II).

With respect to exchange at the α,α' carbon atoms of the sym. diphenylethane series (Table III) significant deuteration in these positions was observed presumably by analogous mechanisms to those proposed for the alkyl hydrogens in the alkylbenzenes. These results contrast markedly with the deactivation of the vinyl hydrogens in the *trans*-stilbene series (Table IV), little deuteration being observed in α,α' hydrogens of the parent compound, also the methyl hydrogens in dimethyl stilboestrol and the methylene hydrogens of diethyl stilboestrol. This deactivation effect is considered to be steric since scale models of the molecule show that the vinyl hydrogens in *trans*-stilbene are sterically hindered in π-bonded chemisorption. This difficulty can only be overcome if the vinyl group twists out of the conjugational plane. A similar argument can be applied to the α,α' hydrogens in dimethyl stilboestrol, although models show that the effect is not so pronounced as with *trans*-stilbene. However, in diethyl stilboestrol, the α,α' ethyl groups were 27.5 % deuterated. Models show that the terminal methyl groups project beyond the plane of the benzene ring and are thus not sterically hindered. This postulate is confirmed

by the distribution of deuterium in the ethyl groups where the terminal methyl is 44.9 % deuterated whilst the methylene group which is in the same position (bond distance) as the α-methyl of dimethyl stilboestrol is only 1.5 % deuterated.

The extensive exchange of the substituents of the α,α' carbon atoms of the sym. diphenylethane series is attributed to some degree of rotation about the bond joining these atoms. At some point in the rotation steric hindrance from the aromatic rings will be a minimum and the groups can then bond to the catalyst.

Comparison of Deuterium Analytical Procedures.

Because of the intermittent availability of a heated inlet mass spectro-meter during the initial stages of the investigation, the Tamiya method was adopted for routine deuterium analysis on a Metropolitan Vickers MS-2 instrument. Samples were subsequently analysed again by the low voltage technique on a double focussing MS-9 unit. Using standard water samples, the MS-2 instrument was calibrated for the Tamiya method over the range 1-100 % D. Above 60 atom % D, the presence of a progressively increasing isotope effect severely limited the accuracy of the method.

The precision of the method was $\pm$ 5 % over the range 1 to 55 atom % D. However, some significant discrepancies were observed between certain Tamiya results and analyses from the low voltage technique. These differences (all at reasonably high deuterium contents) are attributed to isotope effects in the first conversion procedure to water. The Tamiya method involves combustion at 600 ºC to water followed by reduction of the water by zinc to hydrogen. Using *standard* water as calibrant, it is not possible to correct for the isotope effect in the first combustion step, however the present data indicate that it can be a source of serious error in the overall analytical deu-terium figure, particularly for atom % D in excess of 40 %. The Tamiya method has also been criticised by Eisenberg [23] on the basis of incomplete oxidation of multiply deuterated groups.

Biological Significance of the Exchange Results.

For biological work, the ability to label the present series of compounds is important since the only other plausible alternative is the radiation-induced Wilzbach procedure with its subsequent radiochemical purification difficulties. A further advantage is that the compounds have been labelled in known positions, an important requirement for some biological applications. A knowledge of the exchange mechanism is also of considerable importance since it enables prediction of the labelling behaviour. Choice of catalyst activation procedure requires comment, i.e. reduction with sodium borohy-dride or self-activation of the platinum oxide. The self-activation method

produces a very active catalyst, in some instances being more active than the sodium borohydride product. However, this catalyst generally resulted in greater decomposition of the substrate and often yielded a coloured product which was more difficult to purify. Thus, particularly for tritium labelling, the sodium borohydride method is to be preferred.

The reason for the often greater reactivity of the self-activated catalyst is not known. It may be that this method produces a catalyst with smaller particles possessing a greater surface area and presumably more active sites. Although no *in situ* surface area measurements could be performed, the appearance of the catalysts after reduction gave some support to this hypothesis since the borohydride pre-reduced catalyst appeared more granular than the corresponding self-activated material.

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