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LABELING OF STEROIDS BY ACTIVATED TRITIUM

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SUMMARY

Steroid hormones and derivatives are labeled to high specific activities with activated tritium generated by microwave discharge of tritium gas. The steroid nucleus is not degraded by reactions with tritium; the hydroxyl and oxo groups at 3, 11, 17, and 20 positions of the steroid nucleus are not displaced by tritium but are oxidized or reduced to form the labeled by-products. Saturation of A ring occurs readily, but tritium addition to the isolated C=C double bond occurs in the order of $\Delta^1 > \Delta^4 > \Delta^5$. Steroids with the 21-hydroxyl group are not tritiated. Despite the addition reaction, the final products from the unsaturated steroids may still contain significant amounts of the labeled parents. Specific activities of the labeled by-products steroids can approach 29 Ci/mM.

Key Words: tritium labeling, activated tritium, microwave discharge, steroids, catalyst support, gas chromatography

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INTRODUCTION

Steroids are cell regulators and play an essential role in life processes. Tritium labeled steroids may be prepared by catalytic hydrogenation of dehydro precursors, by catalytic dehalogenation of brominated steroids with tritium gas or by reduction of an oxo steroid with a reducing tritide [1]. Synthesis of these precursors requires time and effort, and many of them are difficult to obtain. Steroids may also be labeled by the gas exposure method [1] or exchange with tritiated water in the presence of catalyst [2,3] but long contact with high levels of tritium increases degradation and lowers the yield. Besides, specific activities of the labeled products are low.

Microwave discharge activation (MDA) of tritium gas creates a low pressure plasma in which electrons, ions, atoms and excited species are present [4]. This method has been used to label a variety of compounds [5,6], including diazo and azido steroids [7]. Depending on the chemical nature of the substrate, the support for dispersion, tritium pressure and microswave power input, labeled products formed from the MDA method by isotopic exchange can attain specific activities in the range of curies per millimole. Other activated tritium species can react with the substrate and lead to the formation of essentially carrier-free by-products or unusual derivatives of the substrate that are difficult to synthesize. Since steroids have rigid molecular structures that can minimize degradation, it is of interest to determine if these steroid molecules can be labeled by energetic tritiums without fragmentation. For this purpose, a number of steroid hormones and their derivatives were labeled by activated tritium, and the labeled products were purified and analyzed by gas-liquid radiochromatography. The results of the study are reported here.

RESULTS AND DISCUSSION

Table 1 lists a number of tritiated steroids, their radioactive yields, percentages of the labeled parents by GLRC and their specific activities. The steroids are derivatives of 5α-androstane, 5β-pregnane, 5α-cholestane,

and estrane. The steroid when labeled while dispersed on Rh catalyst gives a higher crude radioactive yield than on the plain silica-alumina catalyst support. The high crude yield on supported metal catalyst is attributed to chemisorbed oxygen and hydroxy groups on the surface of the catalyst that combine with tritium to form tritiated water. Other supported metal catalysts, such as Pt, Pd, Ru, have also been used but are found to be less effective. The Ni catalyst is more efficient but exhibits a greater tendency to form by-products. Relative yields of the by-products from different catalysts differ slightly. The radiochemical yields of the parents labeled on silica-alumina 980-25 and Rh catalyst are in general in the vicinity of 70 to 100 per cent of the purified, non-labile activity; these labeled parents can have specific activities from several hundred millicuries to curies per millimole. Steroids containing reactive double bonds and hydroxy groups generally give lower radiochemical yields of the labeled parents.

5α-Androstane, 5β-pregnane and 5α-cholestane react with activated tritium to give almost pure labeled parents with specific activities in the range of curies per millimole. The absence of ring scission and ring enlargement in these reactions shows that the steroid nucleus or gonane is stable towards activated tritium. Steroids containing hydroxyl and oxo groups in the 3, 17, and 20 positions and unsaturation in the A and B rings were also studied; the results are given in Tables 2 and 4.

Table 1. Specific Activities and Radioactive Yields of Tritiated Steroids Labeled on Catalytic Support

Code No.	Steroid	Catalytic Support[a]	Total Radioactivity, mCi Crude	Purified[b]	Radiochemical Yield, %[c]	Specific Activity Ci/mM[d]
5067-6a	5α-Androstane	980-25	24.80	12.72	~ 100	2.32
7087-5	5α-Androstane-3β-ol	980-25	7.20	3.90	93.2	1.01
5197-4	"	0.5% Rh(E)	21.90	3.50	72.9	0.97
7087-4	5α-Androstane-3β,17β-diol	980-25	6.2	3.8	86.9	0.97
5197-8	"	0.5% Rh(E)	24.5	3.2	86.9	0.81
7087-7	5α-Androstane-17β-ol	980-25	9.1	5.1	~ 100	1.41
5197-7	"	0.5% Rh(E)	19.8	3.8	~ 100	1.05
7087-6	5α-Androstane-17β-ol-3-one	980-25	7.5	4.3	66.9	0.84
5197-5	"	0.5% Rh(E)	20.5	1.9	74.5	0.41
6027-3	5α-Androstane-3β-ol-17-one	980-25	3.6	2.6	83.3	0.63
7087-3	5α-Androstane-3,17-dione	980-25	4.6	2.8	86.0	0.70
5197-10	"	0.5% Rh(E)	21.8	1.8	~ 100	0.52
7087-2	5β-Androstane-3,17-dione	980-25	4.5	2.4	71.3	0.50
5197-9	"	0.5% Rh(E)	22.5	1.7	~ 100	0.49
6027-4	Androst-4-en-3,17-dione	980-25	5.8	5.3	31.3	0.48
7017-6	Androst-1,4-diene-17β-ol-3-one	980-25	10.2	6.7	13.2	> 0.25
3187-2b	Androst-4-en-17β-ol-3-one	0.5% Rh(E)	31.7	3.3	57.9	1.06
7087-8	5α-Cholestane	980-25	6.1	4.5	72.5	1.06

6247-5	5α-Cholestane-3β-ol	980-25	14.2	10.2	71.4	2.83
6027-1	Cholest-5-ene-3β-ol	980-25	8.6	4.4	~ 100	1.71
6167-3	Dexamethasone	980-25	7.3	4.9	-	-
6027-5	Estr-1,3,5(10)-triene-3-ol-17-one	980-25	5.3	4.4	39.0	0.76
5277-1b	Estr-1,3,5(10)-triene-3,17-diol	980-25	1.9	1.5	11.1	-
5277-5b	Estr-1,3,4(10)-triene-3,17β-diacetate	980-25	2.0	1.0	33.7	-
6167-2	5β-Pregnane	980-25	7.8	4.7	~ 100	1.36
6167-5	5α-Pregnane-3β,20β-diol	980-25	8.9	7.7	84.3	2.08
6167-7	5α-Pregnane-3β-ol-20-one	980-25	9.1	4.4	69.9	0.98
6247-2	5α-Pregnane-17α-ol-3,20-dione	980-25	6.1	3.4	93.7	1.06
6167-6	5β-Pregnane-21-ol-3,20-dione	980-25	7.0	4.1	-	-
6167-4	5α-Pregnan-3,20-dione	980-25	7.3	6.0	68.4	1.30
5277-1a	Pregn-4-ene-3,20-dione	980-25	1.3	1.1	87.0	1.20
6247-1	5α-Pregnan-3,6,20-trione	980-25	9.5	5.5	86.6	1.58
6247-3	5α-Pregnan-3,11,20-trione	980-25	6.7	3.5	55.3	0.64
4217-5	Pregn-4-en-11β,17α,21-triol-3,20-dione	0.5% Rh(E)	32.3	2.8	18.0	-
6247-4	5β-Pregnan-11β,17α,21-triol-3,20-dione	980-25	13.7	6.2	-	-

[a] 980-25 = silica-alumina #980-25 from Davison Chemical Co. (Baltimore, MD); 0.5% Rh(E) = Rhodium on alumina from Engelhard Industries Division (Newark, NJ).

[b] Labile tritium has been removed from the purified samples by equilibration with CH_3OH, followed by evaporation *in vacuo*.

[c] Per cent radiochemical yield refers to the radioactive peak area of the labeled parent relative to the total radioactive peak areas of labeled parent and by-products.

[d] Calculated from the mass peak and the radioactivity peak.

Table 2 lists the labeled products and by-products of a number of androstane steroids. Among them, 5α-androstane-17β-ol yields almost 100% of the labeled parent, i.e., [^{3}H]5α-androstane-17β-ol, showing that the 17β-hydroxy is stable towards reaction with activated tritium. When 5α-androstane-3β,17β-diol is similarly labeled, [^{3}H]5α-androstane-17β-ol-3-one is formed as the only by-product by oxidation of the 3-hydroxy to the 3-oxo group. Labeling of 5α-androstane-3β-ol-17-one yields 83.3% of the labeled parent and three labeled by-products ranging from 3.7% to 6.7%. These by-products are [^{3}H]5α-androstane-3β,17β-diol, [^{3}H]5β-androstane-3α,17β-diol, and [^{3}H]5β-androstane-3,17-dione, formed, respectively, from the parent steroid by reduction of the 17-oxo group, concerted inversion of the 5H and the 3-hydroxy group, and oxidation of the 3β-hydroxy group. In labeling 5α-pregnane-3β-ol-20-one, the 20-oxo group is reduced to yield the only by-product [^{3}H]5α-pregnane-3β,20β-diol. No labeled des and nor steroids, formed by bond scission of the hydroxy, oxygen, and methyl groups from the steroid nucleus, are observed.

The severest shortcoming of tritium labeling by the MDA of tritium gas is the saturation of the double bonds by activated tritium. Table 3 lists the labeled products and by-products from steroids containing the C=C double bonds. Dehydrotestosterone, i.e., androst-1,4-diene-17β-ol-3-one (I), yields five labeled by-products: testosterone (II), the 5α and 5β forms of the 4,5-dihydro derivative of the parent (III,IV), and the α and β forms of the 3-hydroxy group of the same derivative (V,VI), as shown in Scheme 1. The latter are thus formed by tritium addition to the Δ^4-bond with concomittant reduction of the 3-oxo group in the parent (cf. Table 3). The [^{3}H]testosterone formed has a specific activity approaching 58 Ci/mM, provided that two tritium atoms are added to the Δ^4 double bond.

Table 2. Formation of Labeled Products from Steroids Containing Hydroxy and Keto Groups

Code No.	Steroid		Labeled Parent and By-products[a]	Retention Index[b]	Yield, %[c]
7087-4	5α-Androstane-3β, 17β-diol		[^{3}H]5α-Androstane-3β,17β-diol	2,738	86.9
		a.	[^{3}H]5α-Androstane-17β-ol-3-one	2,912	13.1
6027-3	5α-Androstane-3β-ol-17-one		[^{3}H]5α-Androstane-3β-ol-17-one	2,907	83.3
		a.	[^{3}H]5α-Androstane-3β,17β-diol	2,732	6.7
		b.	[^{3}H]5β-Androstane-3α,17β-diol	2,675	3.7
		c.	[^{3}H]5β-Androstane-3,17-dione	3,036	6.4
5197-4	5α-Androstane-3β-ol		[^{3}H]5α-Androstane-3β-ol	2,499	72.9
		a.	[^{3}H]5α-Androstane-3-one	2,702	27.1
5197-5	5α-Androstane-17β-ol-3-one		[^{3}H]5α-Androstane-17β-ol-3-one	2,935	74.5
		a.	[^{3}H]5β-Androstane-3α,17β-diol	2,628	16.4
		b.	[^{3}H]5α-Androstane-3β,17β-diol	2,727	9.2
5197-7	5α-Androstane 17β-ol		[^{3}H]5α-Androstane-17β-ol	2,617	~ 100
7087-3	5α-Androstane-3,17-dione		[^{3}H]5α-Androstaine-3,17-dione	3,117	86.2
		a.	[^{3}H]5α-Androstane-3β-ol-17-one	2,903	9.6
		b.	[^{3}H]5β-Androstane-3β-ol-17-one	2,782	4.1
7087-2	5β-Androstane 3,17-dione		[^{3}H]5β-Androstane-3,17-dione	3,065	71.3
		a.	[^{3}H]5α-Androstane-3α-ol-17-one	2,823	13.5
		b.	[^{3}H]5β-Androstane-3β-ol-17-one	2,767	6.6
		c.	[^{3}H]5β-Androstane-3β,17β-diol	2,600	8.7
6167-7	5α-Pregnane-3β-ol-20-one		[^{3}H]5α-Pregnane-3β-ol-20-one	3,200	69.9
		a.	[^{3}H]5α-Pregnane-3β,20β-diol	2,947	30.1

[a] Labeled steroids were silylated with Tri-Sil/BSA, Formula "P" (Pierce Chemical Co.) prior to GLRC.

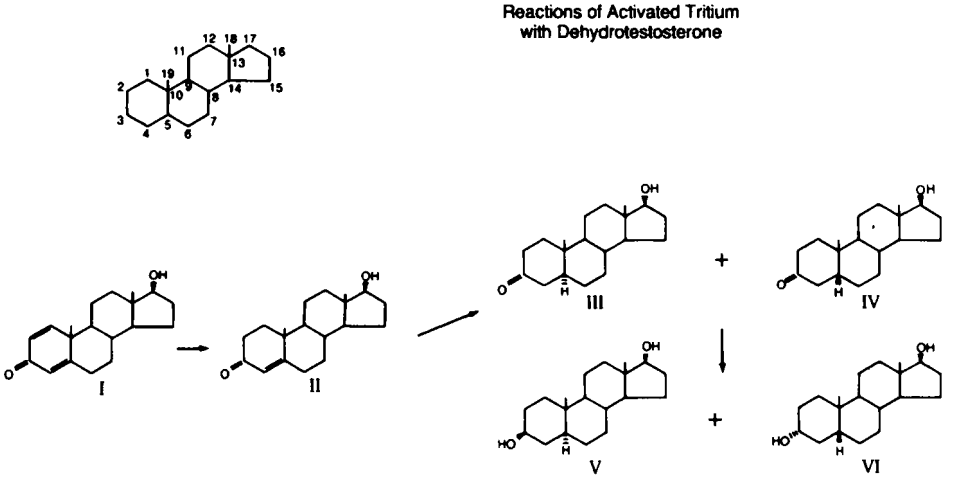
[b] The retention indexes of the hydroxy steroids given are those of their silylated derivatives.

[c] Based on integrated areas of radioactive peaks.

The sequence of reactions of activated tritium with steroids can be illustrated with dehydrotestosterone in Scheme 1; it can account for the formation of all the observed labeled by-products by tritium addition and reduction reactions. From the relative radiochemical yields of the by-products given in Table 3, one may conclude that tritium addition to the C=C double bonds occurs rapidly but is ordered. The Δ^1 double bond has the highest reactivity for tritium addition followed by Δ^4 and then by Δ^5 bond, since the radiochemical yield of the labeled parent increases from 57.9% for testosterone, to 87.0% for progesterone and to approximately 100% for cholesterol. It is also likely that the inductive effect of the alkyl chain at the 17 position of the steroid nucleus may extend its influence on the reactivity of the C=C double bonds towards tritium addition.

Estra-1,3,5(10)-triene-3-ol-17-one and estra-1,3,5(10)-triene-3,17β-diol can be tritiated by the MDA method to give 39.0% and 11.1% of the labeled parents, respectively, as shown in Table 4. The presence of 17-oxo group in the steroid nucleus may slow the rate of saturation in the A ring. Saturation of A ring and oxidation of the 17-hydroxy to 17-oxo group are reactions leading to the formation of labeled by-products in the estrane steroids.

Labeling of steroids by the excited tritium depends not only on the functional groups in the steroid molecule but also upon the catalytic surfaces and the solvent used for dissolution and dispersion of the substrate. Table 5 shows the influence of the solvents THF, ethanol, and a mixture of ethylamine in ethanol (1:1) as solvents on radiochemical yields, formation of by-products and product specific activity in the case of testosterone. Among these solvents, ethanol gives the highest specific activity and the highest yield of labeled testosterone, while ethylamine in ethanol gives the lowest. The latter also yields the by-product [^{3}H]4-androsten-3,17-dione.



Scheme 1. Reactions of tritium with dehydrotestosterone. I = [^{3}H]dehydrotestosterone = [^{3}H]androstane-1,4-diene-17β-ol-3-one. II = [^{3}H]androst-4-ene-17β-ol-3-one. III = [^{3}H]5α-androstane-17β-ol-3-one. IV = [^{3}H]5β-androstane-17β-ol-3-one. V = [^{3}H]5α-androstane-3β,17β-diol. VI = [^{3}H]5β-androstane-3α,17β-diol.

Table 3. Formation of Labeled Products from Steroids Containing Isolated C=C Double Bond(s)

Code No.	Steroid	Labeled Parent and By-Products[a]	Retention Index[b]	Yield %[c]
3187-2b	Androst-4-en-17β-ol-3-one	[^{3}H]Androst-4-en 17β-ol-3-one	3,029	57.9
		a. [^{3}H]5β-Androstane-3α, 17β-diol	2,623	15.0
		b. [^{3}H]5α-Androstane-3β, 17β-diol	2,730	2.8
		c. [^{3}H]5β-Androstane-17β-ol-3-one	2,874	14.2
		d. [^{3}H]5α-Androstane-17β-ol-3-one	2,924	10.2

7017-6	Androst-1,4-diene 17β-ol-3-one	[^{3}H]Androst-1,4-diene 17β-ol-3-one	3,081	13.2
		a. [^{3}H]Androst-4-ene-17β-ol-3-one	3,040	8.5
		b. [^{3}H]5α-Androstane-17β-ol-3-one	2,970	38.1
		c. [^{3}H]5β-Androstane-17β-ol-3-one	2,891	10.9
		d. [^{3}H]5α-Androstane-3β, 17β-diol	2,770	16.9
		e. [^{3}H]5β-Androstane-3α, 17β-diol	2,700	12.4
6027-4	Androst-4-ene 3,17-dione	[^{3}H]Androst-4-ene-3,17-dione	3,238	31.3
		a. [^{3}H]5α-Androstane-3,17-dione	3,133	47.0
		b. [^{3}H]5β-Androstane-3,17-dione	3,078	21.7
9999-0	Pregn-4-ene-3,20-dione	[^{3}H]Pregn-4-ene-3,20-dione	3,400	87.0
		a. [^{3}H]5α-Pregnane-3,20-dione	3,269	13.0
6027-12	Cholest-5-ene 3β-ol	[^{3}H]Cholest-5-ene-3β-ol	3,266	~ 100

[a,b,c] See Footnotes a, b, and c in Table 2.

Table 4. Formation of Labeled Products from Estrogens

Code No.	Steroid	Labeled Products and By-Products[a]	Retention Index[b]	Yield, %[c]
6027-5	Estra-1,3,5(10)-triene-3-ol-17-one	[^{3}H]Estra-1,3,5(10)-triene-3-ol-17-one	3,064	39.0
		a. [^{3}H]5α-19-nor-androstane-3β-ol-17-one	2,803	46.9
5277-1b	Estra-1,3,5(10)-triene-3,17β-diol	[^{3}H]Estra-1,3,5(10)-triene-3,17β-diol	2,900	11.1
		a. [^{3}H]5α-19-nor-Androstane-3β,17β-diol	2,791	33.1
		b. [^{3}H]5β-19-nor-Androstane-3β,17β-diol	2,632	29.0
		c. [^{3}H]Estra-1,3,5(10)-triene-3-ol-17-one	3,053	26.8

5277-5b	Estra-1,3,5(10)-triene-3,17-di acetate	[3H]Estra-1,3,5(10)-triene-3,17-diacetate	3,400	33.7

a,b,c See Footnotes a, b, and c in Table 2.

Table 5. Influence of Solvents on the Yield's of Labeled Products of Testosterone

Code No.		7157-1	7157-2	7157-3
Solvent[a]	(RI)[b]	I	II	III
Yield,[c] Crude, mCi		9.2	11.1	7.9
Purified, mCi		5.8	6.4	3.7
[3H]Testosterone, % relative yield[d]	3,026	13.5	13.7	17.9
Specific activity, mCi/mM		78.6	258.5	136.6
% Relative yields of labeled by-products				
a. [3H]5β-Androstane-3α,17β-diol	2,650	9.3	5.6	7.7
b. [3H]5α-Androstane-3β,17β-diol	2,742	8.0	2.6	4.2
c. [3H]5β-Androstane-17β-ol-3-one	2,868	17.3	25.6	23.1
d. [3H]5α-Androstane-17β-ol-3-one	2,934	37.2	43.4	47.1
e. [3H]5β-Androstane-3,17-dione	3,069	-	1.9	-
f. [3H]5α-Androstane-3,17-dione	3,131	-	7.1	-
g. [3H]Androst-4-ene-3,17-dione	3,273	14.6	- -	
Solvent, % of activity		65.4	18.7	28.0

[a] Solvent used for solubilizing the steroid for dispersion on silica-alumina catalyst support (980-25): I = ethylamine:ethanol (1:1); II = 95% ethanol, and III = tetrahydrofuran.

[b] RI = Retention index.

[c] Purified yield refers to the yield after removal of labile tritium.

[d] The term "% relative yield" refers to the yield of individual component relative to the total yield of labeled testosterone and derivatives.

[e] The term "% of activity" refers to the % of the injected activity.

A number of steroids containing the 21-hydroxyl group have been labeled with activated tritium but no tritiated products could be detected by GLRC.

CONCLUSION

Steroids are derivatives of gonane with methyl groups at 18 and 19, alkyl chain at 17, and functional hydroxyl and oxo groups at 3, 11, 17, 20, and 21 positions. All these substituent groups are rigid with the exception of 21-hydroxy group which can freely rotate. The tight form of steroid can absorb the impact of activated tritium species during reaction and dissipate the excitation energy intramolecularly without resorting to ring opening, ring scission or bond rupture. Hydroxy and oxo groups attached to the steroid nucleus at 3, 11, 17, and 20 positions are oxidized or reduced but are not replaced by tritium. The oxo group appears to be more stable than the hydroxy group. The stability of 5α-androstane, 5α-cholestane, β-estradiol and estrone towards catalytic exchange with HTO has also been noted [3].

Aromatic A ring and isolated C=C double bonds in the steroids are readily saturated by activated tritium. The brief reaction time allows substantial amounts of the unsaturated steroids to survive the addition reaction in the final product. Saturation of A ring occurs rapidly and no partially saturated intermediates have been observed. For C=C double bonds in steroids the order of reactivity towards additon is $\Delta^1 > \Delta^4 > \Delta^5$. The Δ^4 and Δ^5 double bonds are hindered and react slowly in the addition reaction. Saturation of the Δ^4 double bond gives rise to 5α and 5β dihydro derivatives. In testosterone, formation of 5β is favored over that of 5α, and in progesterone, only 5α dihydro derivative is formed. [^{3}H]Estra-1,3,5(10)-triene-3-ol-17-one can be formed directly from labeling of the parent as well as a by-product from labeling of the estra-1,3,5(10)-triene-3,17β-diol. The by-product is formed with a specific activity approaching 29 Ci/mM. Table 6 shows the relative stability of functional groups in steroids in tritium labeling.

The fact that the 21-hydroxy steroids are not labeled by the activated

tritium may be attributed to free rotation of the 21-hydroxy group which enters into isotopic T-for-H exchange reactions with activated tritium much more readily than the protiums on the steroid nucleus. The 21 hydroxy group forms the -OT group with tritium which can back exchange and become lost during purification.

In conclusion, our studies have shown that steroid hormones, with the exception of 21-hydroxy steroids, can be readily labeled with activated tritium to high specific activities. The steroid nuclei androstane, cholestane, pregnane, are difficult to label by synthesis or by catalytic exchange [3] can be readily tritiated with activated tritium.

MATERIALS AND METHODS

Steroids were obtained commercially from the Sigma Chemical Company and used as supplied. The silica-alumina catalyst support of known composition [8] was obtained from Davison Chemical Company. The Rh, Ru, Pd, and Pt catalysts were purchased from the Engelhard Industries Division. The Ni catalysts were prepared as previously described [9].

Steroid samples were prepared for tritium labeling by dispersion on solid supports to provide high surface area for gas phase reaction. The following procedure was used. Pellets (1/8" in diameter) of plain silica-alumina catalyst support and supported metal (Rh, Ru, Pt, or Ni) catalysts were impregnated with a solution of steroid in redistilled tetrahydrofuran (THF) or ethanol so that each pellet contained about 0.2 mg of the steroid. Five such pellets were prepared for each steroid sample. The impregnated pellets were dried in vacuo overnight before tritiation.

Tritium gas free from the decay product ^3He was freshly generated by heating uranium tritide to 350°-400°C and was introduced to a highly evacuated tritiation system to a pressure of approximately 2-5 torrs, corresponding to about 0.8 Ci of tritium gas. The steroid sample was cooled with liquid nitrogen (-196°C). About 100 W of microwave power was transmitted to the tritium gas through an Evenson microwave cavity situated 6-8 cm upstream from the sample, and a discharge was initiated with a Tesla

coil. After 5 minutes, the discharge was terminated, and the spent tritium gas pumped to a reservoir for radioactive waste disposal. The apparatus was repeatedly flushed with helium and evacuated to remove completely any residual tritium before opened to atmosphere to retrieve the labeled pellets.

Labeled steroids were eluted off from the irradiated pellets with THF or ethanol. The solution, after dilution, was radioassayed for tritium activity by liquid scintillation counting. Labile, exchangeable tritiums and tritiated water were removed by repeatedly drying the sample in vacuo after equilibration with ethanol. The purified sample was analyzed by gas-liquid radiochromatography (GLRC) on a HP 5890 Gas Chromatograph using a 10 m HP-17 (0.53 mm i.d. x 2 μm 50% phenylmethylpoly-oxisilane) fused silica capillary column. Radioactivity in the column effluent was detected by a heated 70-ml proportional counter operated at 300°C in series with the chromatographic column, using a mixture of n-propane and helium (2:1) as counting gas. The efficiency of the counter was calibrated with standardized tritiated toluene.

The hydroxy derivatives of the steroids were silylated with Tri-Sil/BSA reagent (Formula "P", Pierce Chemical Co.), according to the procedure of Chambaz and Horning [10] prior to analysis by GLRC.

Chromatography of the Steroids

The steroids and their silylated derivatives were chromatographed on HP-17 fused silica capillary column, using a temperature program maintained initially at 200°C for 2 minutes, increasing to 230°C at a rate of 30°C/min for 7 minutes and then to 260°C at 15°C/min for 10 minutes. The entire chromatographic run requires about 19 minutes and will resolve the steroid hormones and their closely related derivatives, as shown in the radiochromatogram in Figure 1a for [^{3}H]dehydrotestosterone and its by-products. Figure 1b shows the radiochromatogram of [^{3}H]progesterone labeled while dispersed on Rh (980-25) catalyst and at an oven temperature programmed from 200°C to 230°C. The steroid sample is co-injected with a sample of n-alkanes (C_{22}, C_{24}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{32}, C_{33}, C_{34}, and

C_{36}) as standard. The retention index of the steroid is calculated from the retention times of the injected steroid sample and two n-alkanes, one eluting before and the other eluting after the steroid [11]. It should be noted that the chromatographic peaks of the steroids are well resolved but closely spaced, and any components of the injectant when present in a slightly larger amount than the optimal, can displace the closely adjacent peaks by crowding to affect the retention index. Steroids formed as by-products are tentatively identified by retention index, when known standards of these products are unavailable for co-chromatography. Specific activities of the labeled parent steroids are calculated from the integrated mass peak and the intensity of the corresponding radioactive peak.

Table 6. Relative Stability of Functional Groups in Tritium Labeling of Steroids[a]

Steroid Nucleus	Unstable Group and its Conversion	Stable Groups
Androstane	1,4-diene ⟶ 4-ene	3,17-dione
	4-ene ⟶ 5α(H),5β(H)	17β-ol
	17-one ⟶ 17β-ol	
	3-ol ⟶ 3-one	
	3-one ⟶ 3α-ol,3β-ol	
Pregnane	20-one ⟶ 20β-ol	3β-ol
		3,20-dione
		6-one
		11-one
		4-ene
Estrane	1,3,5(10) triene ⟶ complete saturated A ring	3β-ol
	3-ol ⟶ 3β-ol	17-one
	17β-ol ⟶ 17-one	
5α-Cholestane		5-ene
		3β-ol

[a] Based on the steroid hormones studied.

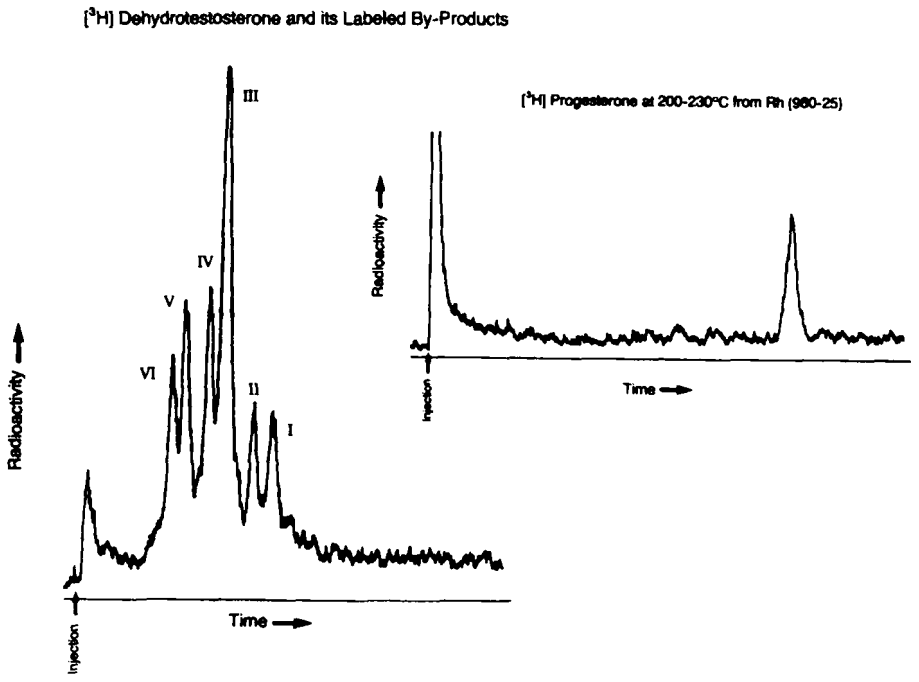


Figure 1. a. Radiochromatogram of [^{3}H]dehydrotestosterone and its by-products at 200°-260°C on a 10-m HP-17 fused silica column. I = [^{3}H]dehydrotestosterone; II = [^{3}H]testosterone; III = [^{3}H]5α-androstane-17β-ol-3-one; IV = [^{3}H]5β-androstane-17β-ol-3-one; V = [^{3}H]5α-androstane-3β,17β-diol and VI = [^{3}H]5β-androstane-3α,17β-diol.

b. Radiochromatogram of [^{3}H]progesterone at 200-230°C from Rh (980-25) on the same column as above.

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