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Hydrogen-Deuterium Exchange During the Reductive Deuteration of α- and γ-Tocopherol Chromenes

(Short Title: Deuteration of Tocopherol Chromenes)

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Summary:

Reduction of tocopherol chromenes with heterogeneous catalysts and deuterium gas resulted in various degrees of deuterium incorporation despite the use of high purity deuterium gas. Exchange of hydrogens on C-7 of γ-tocopherol was evident by ^{2}H-NMR and could be controlled by consideration of the substrate (chromene) to catalyst ratio, concentration and temperature. Tocopherols deuterated at C3 and C4 were prepared with 94% d_2 incorporation using 10% Pd/C at 0°C in ethyl acetate.

Keywords: α-tocopherol, γ-tocopherol, deuterogenation, hydrogen-deuterium exchange, heterogeneous catalysis

Introduction

Specifically deuterated α-tocopherols have been extremely useful in the investigation of tocopherol biokinetics,(1-5) allowing assessment of the turnover of different pools of plasma and tissue tocopherols,(6) as well as the stereoselective

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nature of the distribution process that occurs after absorption.(6-9) Most of the deuterated tocopherols incorporate deuteromethyl groups on the aryl ring,(10, 11) giving d_3-, d_6-, and d_9-α-tocopherol as possible isotopomers. This is convenient both from the point of view of synthesis as well as for analytical purposes. The increase in molecular mass due to three deuterium atoms makes it possible to detect and distinguish between endogenous, naturally occurring tocopherol and a deuterated dose of the same vitamin.

Virtually all of the work mentioned above was done with α-tocopherol since it is the most biologically active form of vitamin E,(1) despite the fact that γ-tocopherol is more abundant in vegetable oil sources (4) and thus in our diets. Recently, however, the less biologically active γ-tocopherol has attracted greater interest after it was discovered that a product of its metabolism, 2,7,8-trimethyl-(*S*)-2-(β-carboxyethyl)-6-hydroxy chroman (γ-CEHC), may act as a natriuretic hormone. (12, 13) In order to assist biokinetic and metabolic studies of these sort, a specifically deuterated γ-tocopherol is required. Most of the deuterated tocopherols prepared to date have been deuteromethylated α-tocopherols synthesized by the $SnCl_2$ promoted alkylation of tocol starting materials.(10, 11) This is problematic for γ-tocopherol because of the difficulty of achieving the particular dimethyl regiochemistry. For example, methylation of 1,4-hydroquinone produces chiefly the 2,5- and 2,6-dimethyl isomers, and very little of the 2,3-isomer required for construction of the γ-tocopherol ring system. Methylation of tocol (a tocopherol with no aryl methyls) is similarly inefficient. We are currently preparing d_3- and d_6-γ-tocopherol by an indirect route, but in the meantime a more easily prepared material seemed to be available from the reduction of the γ-tocopherol chromene. The same deuteration has been performed with γ-tocopherol under Bouveault-Blanc conditions (Na, $(CH_3)_2CHOD$, 68% yield),(11) and with α-tocopherol (Na, EtOD, 70% yield)(10) and both were stated to have 100% d_2 incorporation. In our hands, deuteration of α-tocopherol chromene under similar conditions resulted in a somewhat lesser yield of 95% d_2-α-tocopherol. Reductive deuteration with D_2 and heterogeneous metal catalysts has been performed(14) but due to lack of stereoselectivity was not pursued and deuterium incorporation was not reported in detail. We report herein our investigation into the reductive deuteration of α- and γ-tocopherol chromenes using such catalysts.

Results and Discussion

The starting material for this work, **1** (Scheme 1), was readily prepared from natural γ-tocopherol via acetylation and oxidation.(11) Reduction of 0.5 g of this material using a balloon of D_2 gas (~24 psi or 165 kPa) and 10% Pd/C provided a quantitative yield of γ-tocopherol acetate, **2**. Mass spectral analysis indicated that only 87.3% of the product contained two deuterium atoms, and some 12.7% contained only one deuterium. (Table 1) This was somewhat irregular since the deuterium gas was of >99 percent purity, but in fact was tolerable for use as a biological tracer. The deuteromethyl-containing α-tocopherols mentioned above were prepared with deuterium incorporations ranging from 75% to 90%.(15) Since we needed over thirty grams of d_2-γ-tocopherol acetate for biological studies we next attempted to reduce 5.0 g of the γ-tocopherol chromene under "similar" conditions. (See Table 1.) While the chemical yields were still excellent, after mass spectral analysis we were disappointed to discover that the isotopic purity was 64.7% d_2-γ-tocopherol acetate and 35.3% d_1. It was clear that some exchange process was diluting the incorporated deuterium.

These original reactions took several hours to complete and were monitored by TLC. Thinking that perhaps the H/D exchange occurred after prolonged exposure of

exp.	substrate and amount	cat/sub (mg/mg)	[substrate] (mg/mL)	T (°C)	solvent	% d_2
1	0.5 g γ-toc	1:5	50	20	EtOAc	87.3
2	5.0 g γ-toc	1:20	125	20	EtOAc	64.7
3	0.5 g γ-toc	1:5	50	0	EtOAc	94.9
4	0.5 g γ-toc	2:1	50	20	EtOAc	81.3
5	1.5 g γ-toc	1:5	50	0	EtOAc	89.3
6	0.5 g γ-toc	1:5	50	20	Benzene	77.0
7	0.5 g γ-toc	1:5 (Rh)[a]	50	20	EtOAc	–
8	0.5 g γ-toc	1:5 (Pt)	50	20	EtOAc	51.0
9	0.5 g α-toc	1:5	50	0	EtOAc	93.7
10	0.5 g α-toc	1:20	125	20	EtOAc	75.3
11	0.3 g α-toc	1:1	43	0	EtOAc	93.5

Table 1. Experimental trials for the reduction of γ- and α-tocopherol acetates using various catalysts. (a) The homogenous rhodium-containing Wilkinson's catalyst was used at a ratio that maintained the molar amount of metal provided in the heterogeneous examples.

the product to the reducing conditions, we placed the 87.3% purity d_2-γ-tocopherol acetate in the presence of either D_2 or H_2 and fresh palladium catalyst for overnight. No change in the deuterium incorporation was evident by mass spectral analysis (87.2% in both cases). This suggested that the exchange was occurring on the γ-tocopherol chromene itself, either prior to or during the reduction mechanism.

The ^{1}H-NMR spectrum of the product showed a clear loss of the alkene proton resonances at 5.55 and 6.28 ppm. The proton on C-3 gave an unequal pair of doublets at 1.78 and 1.71 ppm (J=6Hz) in a ratio of about 2.5:1. The C-4 proton resonance appears as a doublet at 2.68 ppm (J=6Hz) with a small peak at 2.72 suggesting an overlapped doublet. The reduction product is thus a mixture of stereoisomers resulting from the reduction having occurred from both faces of the alkene. Variable temperature ^{1}H-NMR (20-75°C, Cl_2CCCl_2) showed that these signals did not coalesce nor broaden as the temperature was increased, strong indication that they are stereoisomers and not interconverting conformational isomers. The integration of the resonances corresponding to C-3 and C-4 protons was very close to 1:1. ^{13}C-NMR showed the characteristic triplets for carbons attached to deuterium at 22.2 (C-3) and 30.9 ppm (C-4), but a residual C-H resonance was still evident, although very small.

Since the ratio of hydrogen on C-3 and C-4 was nearly 1:1 then deuterium was either lost equally from both sites after reduction (which would be unlikely), or the double bond was reduced non-regioselectively with H-D produced by an exchange process that occurred prior to the completion of reduction. The most likely place for an exchange would be the aryl methyls at C-7 or C-8. Integration of these signals in the ^{1}H-NMR spectrum is not accurate enough for analysis of these experiments as they overlap with each other to some extent.

The ^{2}H-NMR of the 87.3% d_2-γ-tocopherol acetate (Exp. 1 in Figure 1) showed two broad resonances at 1.74 and 2.73 ppm corresponding to C-3 and C-4 respectively. A shoulder on the C-3 resonance was just discernable. The ^{2}H-NMR of the d_2-γ-tocopherol recovered from Exp. 4 (81.3% d_2), clearly shows a resonance, now enhanced, at the same place as the shoulder in the d_2-γ-tocopherol sample from Exp 1. (87.3 % d_2). The chemical shift (2.07 ppm) identifies it as a deuterium on C-7.

Apparently, an exchange of D for H on C-7 was accompanied by a loss of deuterium incorporation at C3 and C-4. Hydrogen-deuterium exchange at benzylic

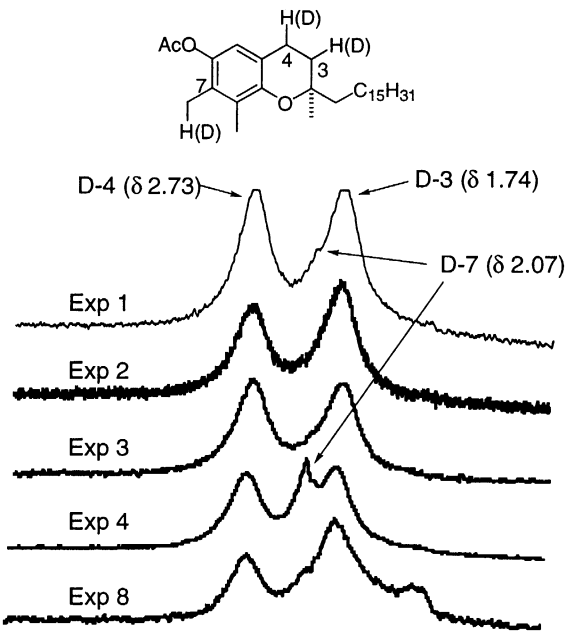
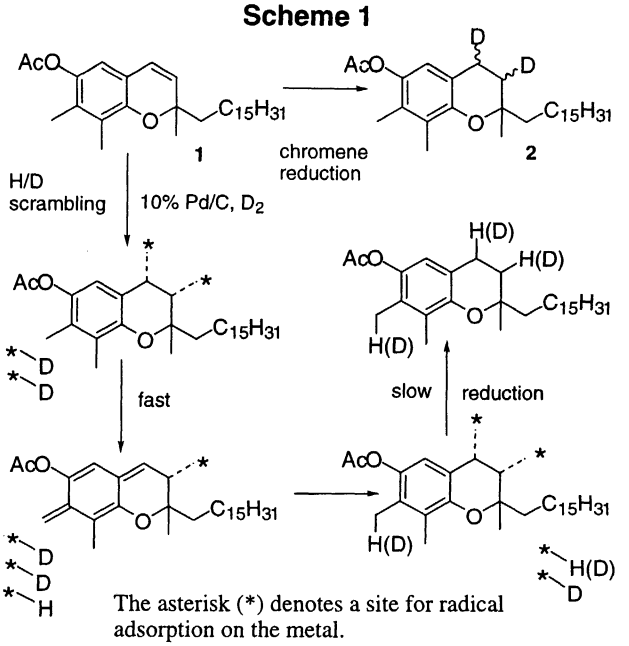


Figure 1. ^{2}H-NMR spectra of deuterated γ-tocopherol acetates obtained from experimental conditions described in Table 1. Spectra were obtained at 45 MHz with $CDCl_3$ as the internal standard.

positions has been observed many times during deuterogenations(16-18) but facile exchange most often occurs at higher temperatures and pressures(19) or in the gas phase.(20) A recent account by Azran *et al* has described the deuterium-hydrogen exchange of bibenzyl in solution at room temperature using palladium on carbon.(21)

A possible explanation for our results is outlined in Scheme 1. Here, the exchange only takes place on the chromene that is bound to the metal surface in a manner following the hydrogenation mechanism for heterogeneous catalysts proposed long ago by Horiuti and Polanyi(22) where * represents an active site for radical adsorption on the heterogeneous catalyst. The methyl group at C-7 is activated for exchange during this process by virtue of a possible resonance structure across the aromatic ring. Exchange of a C-7 hydrogen atom leaves it adsorbed to the catalyst (H–*). Either D–* or H–* can be redelivered to C-7 generating the species that is ultimately reduced. The mix of H/D adsorbed on the catalyst by this exchange can

Scheme 1



The asterisk (*) denotes a site for radical adsorption on the metal.

presumably be delivered to the alkene at C-3/C-4 with no regiochemical bias of addition, keeping the ratio of hydrogen on these carbons near 1:1. The slightly greater amount of deuterium at C-3 after Exp. 2 suggests that when substrate is in large excess there may be a preference for redelivery of H to C-4 at the beginning of the reaction.

Table 1 details the other conditions and methods that were tried to raise the deuterium incorporation. What is most apparent is that the extent of deuterium incorporation is dependent on the catalyst to alkene ratio, and on the concentration of the substrate. A similar observation was made by Azran *et al*(21) although in their case H/D exchange was the only possible result with bibenzyl. Dilution of deuterium incorporation due to H/D exchange has also been observed for Pd-catalyzed reduction of cyclopentene.(23) Using palladium-on-carbon at a ratio to substrate of 1:5, with substrate concentration at 50 mg/mL (~109 and 106 mM for γ and α-tocopherol respectively) the yield of d_2-γ-tocopherol acetate was 87.3% (Exp.1). Deuterium incorporation could be increased to ~94% for both d_2-γ and d_2-α-tocopherol acetates by performing the reduction at 0° C (Exp. 3). Decreasing the catalyst to substrate ratio to 1:20 and increasing the concentration of substrate to 125 mg/mL (~270 mM) lowered the deuterium incorporation to 64.7% for d_2-γ-tocopherol acetate (Exp. 2) and

75.3% for d_2-α-tocopherol acetate (Exp.10). The solubility of deuterium in organic solvents such as benzene is about 2.3 mM at 1 atm and 298 K(24) and this, coupled with the amount of deuterium that could be adsorbed on the metal catalyst, is negligible compared to the amount of substrate that is present at the beginning of the reaction. During the large scale attempt to reduce **2** (Exp. 2) the delivery of D_2 to the solution and the catalyst must be rate determining so that C-7 H/D exchange occurs faster than the complete reduction.

Palladium was the best catalyst for this reductive deuteration. Platinum (as 10% Pt/C) had fine chemical yields, but rapidly catalyzed the exchange process and yielded only 51% of d_2-γ-tocopherol acetate. ^{2}H-NMR showed that platinum also catalyzed the exchange of deuterium with sites on the phytyl sidechain, a somwhat surprising result at such low temperatures.(17) This is not acceptable for the *in vivo* use of these products since it is metabolism of the sidechain to produce γ-CEHC that is of interest in proposed work and C–D bonds in the sidechain could affect the rates of oxidative metabolism. The homogeneous Wilkinson's catalyst reduced at a much lower rate such that after five days the reaction was still not complete, having only reduced 38% of the chromene as judged by ^{1}H-NMR. No attempt was made to analyze the percent deuterium incorporation since the remaining starting material interferes with the d_1 peak intensities.

Experimental

General procedure for reduction of d_2-γ-tocopherol chromene acetate: To 0.50 g (1.1 mmol) of **1** in 10.0 mL of EtOAc at 0°C was added 100 mg of 10% Pd/C. The solution was degassed at ~20 mm Hg for several minutes then the flask was connected to a D_2 gas-filled balloon. When the reaction was complete (on TLC the spot for the chromene chars brown with 1% H_2SO_4 in MeOH and heating, while the starting material turns yellow) the catalyst was filtered and solvent evaporated to give 0.510 g (100%) of d_2-γ-tocopherol acetate. TLC R_f = 0.41 (hexane:ethyl acetate = 10:1); ^{1}H NMR (300 MHz) 6.68 (s, 1H), 2.68 (overlapped doublets, 1H, J=6 Hz), 2.31 (s, 3H), 2.16 (s, 3H), 2.07 (s, 3H), 1.78 and 1.71 (both doublets, J=6 Hz, ratio = 2.5:1), 1.65-1.05 (br m, 25 H), 0.88 (m, 12H). ^{13}C NMR 170.53, 150.01, 141.95, 127.40, 126.21,

119.25, 118.75, 76.35, 40.64, 39.78, 37.83, 37.69, 33.20, 33.09, 30.91 (t, J_{D-C} =19 Hz), 28.39, 25.21, 24.85, 24.64, 23.13, 23.04, 22.21 (t, J_{D-C} = 19 Hz), 21.40, 21.25, 20.15, 20.06, 13.09, 12.37. EI-MS m/z 460 (M^+, 2D, 22.5), 459 (1D, 1.1), 418 (100), 194 (14), 152(42).

Non-deuterated γ-tocopherol acetate does not have an M-1 peak on the EI mass spectrum. Deuterium incorporations of d_2-products were calculated as 1-[M-1/ (M-1)+M^+] and were not corrected for isotopic abundance contributions from M-1 on M^+.

Conclusions

We have prepared d_2-α-and d_2-γ-tocopherol acetates with high deuterium incorporation suitable for use as *in vivo* labels. Because of a hydrogen-deuterium exchange phenomenon, the syntheses were best accomplished by restricting the concentration of substrate and performing the reductions at 0° C in ethyl acetate with Pd/C.

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