Urinary excretion of 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman is a major route of elimination of γ-tocopherol in humans

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Abstract **Little is known of the post-absorptive, metabolic fate of γ-tocopherol, the major form of vitamin E in North American diets. The objective of this study was to determine the extent of urinary excretion of 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC), a recently identified metabolite of γ-tocopherol. A method for measurement of urinary γ-CEHC was developed, using gas chromatography–mass spectrometry (GC–MS) with a deuterated internal standard, 2,7,8-trimethyl-2-(β-carboxyethyl)-(3,4-$^2H_2$)-6-hydroxychroman (d_2-γ-CEHC). This standard was synthesized by dehydrogenation of 6-acetyl-γ-CEHC followed by deuteration of the resulting 3,4-double bond. The use of d_2-γ-CEHC resulted in accurate determinations of the concentration of d_0-γ-CEHC in human urine. Urine samples containing added d_2-γ-CEHC were treated with β-glucuronidase, extracted with an organic solvent, and analyzed by GC–MS. Analysis of 24-h urine pools from healthy subjects revealed γ-CEHC concentrations, normalized against creatinine, ranging from 2.5 to 31.5 μmol/g creatinine, or a total of 4.6 to 29.8 μmol per day. These results correspond to 2–12 mg γ-tocopherol excreted daily as γ-CEHC in the urine. Given an estimated mean intake of γ-tocopherol of 20 mg/day, catabolism of γ-tocopherol to γ-CEHC, followed by glucuronide conjugation and urinary excretion, is a major pathway for elimination of γ-tocopherol in humans.—**Swanson, J. E., R. N. Ben, G. W. Burton, and R. S. Parker. **Urinary excretion of 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman is a major route of elimination of γ-tocopherol in humans.** ***J. Lipid Res.*** **1999.** 40: **665–671.**

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The processes of absorption and plasma transport of α- and γ-tocopherols (**1** and **2**, **Fig. 1**), the two members of the vitamin E family of most importance to human nutrition, are known in at least broad outline (1–4). However, knowledge of biotransformation of specific tocopherol vitamers, especially their pathways of catabolism and means of elimination, is rudimentary at best. This is particularly true for γ-tocopherol, as radioactive or stable isotope-labeled forms of this vitamer have not been as readily available for use in metabolic studies. γ-Tocopherol possesses only about one-tenth the vitamin E biopotency of α-tocopherol, as determined by animal bioassays (5, 6). This difference in bioactivities of the vitamers cannot be accounted for by differences in their intrinsic radical trapping efficiencies, because α-tocopherol is only twice as active (7). Neither can the difference in biopotency be attributed to differences in absorption efficiencies, as the two vitamers are taken up equally well from the small intestine (8). However, despite the similarity in absorption efficiencies and higher levels of γ-tocopherol in the North American diet, fasting human plasma concentrations of γ-tocopherol are usually at least 5-fold less than those of α-tocopherol (9, 10). This difference in bioavailability of tocopherol vitamers in humans, as reflected by plasma concentrations, may therefore involve mechanisms of catabolism that result in differential rates of elimination of the tocopherols.

Recently, Wechter et al. (11) reported the presence of a γ-tocopherol metabolite, 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC, **5**, Fig. 1; designated LLU-α by the authors), in human urine, and proposed a physiological role for this substance in the urinary excretion of sodium (natriuresis). The structure of γ-CEHC suggests that it is metabolized via phytyl chain oxidation of γ-tocopherol, without oxidative modification of the chroman

Abbreviations: α-CEHC, 2,5,7,8-tetramethyl-2-(β-carboxyethyl)-6-hydroxychroman; γ-CEHC or d_0-γ-CEHC, 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman; d_2-γ-CEHC, 2,7,8-trimethyl-2-(β-carboxyethyl)-3,4-$^2H_2$)-6-hydroxychroman; δ-CEHC, 2,8-dimethyl-2-(β-carboxyethyl)-6-hydroxychroman; GC–MS, gas chromatography–mass spectrometry; SIM, single ion monitoring; ^{1}H-NMR, proton nuclear magnetic resonance spectrometry; VLDL, very low density lipoprotein; TMS, trimethylsilane; NO, nitric oxide; NO_x, nitrogen oxides; EI, electron impact; SD, standard deviation.

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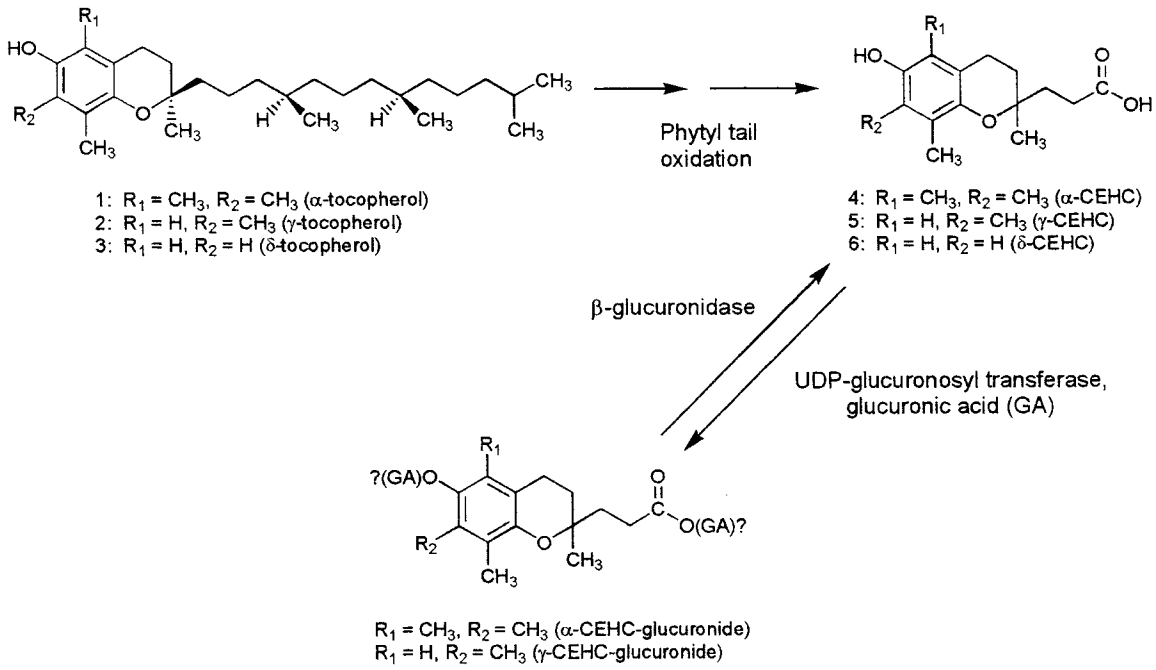

Fig. 1. Proposed scheme for the metabolism of tocopherols to their respective CEHC forms, as found in urine.

ring (Fig. 1). Previously, a phytyl chain shortened form of δ-tocopherol, δ-CEHC (**6**, Fig. 1), was reported in rat urine (12). More recently, the analogous metabolite of α-tocopherol, α-CEHC (**4**, Fig. 1), was identified in human urine by Schonfeld et al. (13) and more extensively studied by Schultz et al. (14).

Interest in the potential biological role of γ-tocopherol has been stimulated recently not only by the identification of γ-CEHC in urine by Wechter et al. (11), but also by reports of the ability of γ-tocopherol, unlike α-tocopherol, to trap the NO radical or secondary reactive intermediates derived from NO_x (15, 16).

We have investigated the quantitative significance of urinary γ-CEHC in the elimination of γ-tocopherol in humans. An important aspect of this work was the synthesis of a suitable deuterium-labeled internal standard, which we report here, along with the use of this standard in obtaining the first quantitative measurements of human urinary γ-CEHC excretion rates.

MATERIALS AND METHODS

General remarks

Confirmatory mass spectra (electron impact, EI) of all synthesized compounds were determined using a Hewlett-Packard 5995 gas chromatograph and mass selective detector equipped with a Hewlett-Packard Ultra 1 capillary column (12 m × 0.25 mm). Mass spectral peak intensities are given in parentheses as a percentage of the base peak value. Proton NMR spectra were recorded using a Bruker 200 MHz instrument using $CDCl_3$ as solvent. Chemical shifts are reported in ppm downfield from TMS as an internal standard. Multiplicities are reported as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

2,3-Dimethyl-1,4-hydroquinone, ethyl levulinate, vinyl magnesium chloride, boron trifluoride diethyl etherate (required for the synthesis of γ-CEHC), and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and deuterium gas (99.8%) were purchased from Aldrich Chemical Co., Milwaukee, WI. All reactions were carried out under a nitrogen atmosphere using oven-dried glassware and anhydrous solvents. Products were recovered using normal extraction procedures; typically, water was added to the reaction mixture, the aqueous phase was extracted at least three times with an appropriate organic solvent (e.g., diethyl ether), the organic phases were combined, washed with saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and the solvent was removed by rotary evaporation. Chromatographic separations were performed using Merck Grade 60 silica gel (230–400 mesh, 600 nm). All yields reported are based on the amount of isolated product obtained after purification.

2,7,8-Trimethyl-2-(β-carboxyethyl)-6-acetyl-hydroxychroman (7b, Fig. 2)

The scheme for the synthesis of deuterium-labeled γ-CEHC (internal standard) is shown in **Fig. 2**. Racemic γ-CEHC (**7a**) was synthesized as previously described by Gloor et al. (17) and Wechter et al. (11). Acetic anhydride (1 ml, 10.6 mmol) was added dropwise to a stirred solution of γ-CEHC (100 mg; 0.38 mmol) in pyridine (1 ml) at room temperature. The reaction was allowed to proceed until thin-layer chromatography (TLC) indicated that the starting material had been consumed. The pyridine then was removed by rotary evaporation and the resultant residue was extracted with diethyl ether in the manner already described. Purification of the residue by column chromatography (hexanes–ethyl acetate 6:4, v/v) resulted in 90 mg (90% yield) of **7b**. ^{1}H-NMR d: 1.2 (s, 3H), 1.7 (t, 2H), 1.9–2.0 (m, 2H), 2.1 (s, 3H), 2.2 (s, 3H), 2.4 (s, 3H), 2.5 (m, 2H), 2.7 (m, 2H), 6.4 (s, 1H). GC–MS (EI) *m/z* (relative intensity) 307 (M + 1, 4), 306 (20), 264 (56), 151 (100).

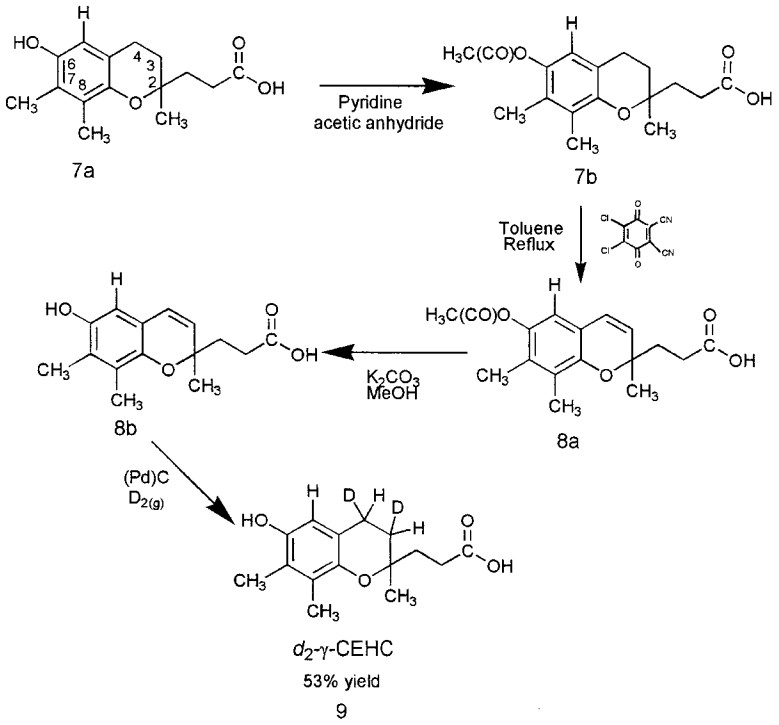

Fig. 2. Scheme of synthesis of 2,7,8-trimethyl-2-(β-carboxyethyl)-(3,4-$^2H_2$)-6-hydroxychroman (d_2-γ-CEHC). (Pd)C, palladium on carbon; D_2(g), deuterium gas.

2,7,8-Trimethyl-3,4-dehydro-2-(β-carboxyethyl)-6-acetylchroman (8a, Fig. 2)

Conversion of **7b** into the title compound was accomplished using a modification of a procedure previously reported (18, 19). A solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.6 mmol in 12 mL toluene) was added by syringe pump over a 2-h period to a solution of **7b** (360 mg; 1.2 mmol) dissolved in toluene (12 mL) and heated to 90°C. The resulting dark, red-brown mixture was refluxed for 18 h. The reaction mixture then was cooled in an ice bath for 15 min, filtered, and the solvent was evaporated to dryness. The resulting yellow oil was purified by flash chromatography using hexanes–ethyl acetate 6:4 (v/v) as an eluant. Racemic **8a** (190 mg; 53% yield) was obtained as a yellow oil. ^{1}H-NMR d: 1.3 (s, 3H), 2.0 (m, 2H), 2.1 (s, 3H), 2.2 (s, 3H), 2.3 (s, 3H), 2.5–2.6 (m, 2H), 5.5 (d, 1H, J = 10 Hz), 6.3 (d, 1H, J = 10 Hz), 6.4 (s, 1H). GC–MS (EI) m/z (relative intensity) 305 (M + 1, 1), 304 (5), 231 (4), 189 (100).

2,7,8-Trimethyl-2-(β-carboxyethyl)-(3,4-d_2)-6-hydroxychroman (d_2-γ-CEHC, 9, Fig. 2)

Anhydrous potassium carbonate (11 mg; 0.6 mmol) was added to **8a** (90 mg; 0.30 mmol) dissolved in methanol (3 mL). The mixture was stirred at room temperature for 18 h. The residual yellow-orange oil obtained by extraction of the reaction mixture with diethyl ether was purified by flash chromatography, using ethyl acetate–hexane–acetic acid 500:300:1 (v/v/v) to yield racemic 2,7,8-trimethyl-3,4-dehydro-2-(β-carboxyethyl)-6-hydroxychroman (**8b**, Fig. 2; 80 mg; 90% yield) as an oil. The purity of the compound was confirmed by GC–MS and NMR. ^{1}H-NMR δ: 1.2 (s, 3H), 2.2–2.0 (m, 8H), 2.7–2.5 (m, 2H), 5.5 (d, 1H, J = 10 Hz), 6.3 (d, 1H, J = 10 Hz), 6.4 (s, 1H).

Activated palladium on carbon (10%; 1–2 mg) was added to **8b** (30 mg; 0.11 mmol) dissolved in the minimal amount of absolute ethanol. The mixture then was placed under one atmosphere of deuterium gas (99.8%) for 1.5 h, after which time TLC and GC–MS analysis showed that the starting material had been consumed. The reaction mixture was transferred to a screw-cap test tube and centrifuged at 500 *g* for 10 min to pellet the palladium catalyst. The clear supernatant was filtered and evaporated to dryness to give 28 mg (92% yield) of **9** (Fig. 2). Analysis by TLC and GC–MS revealed that the crude product was obtained with 95% isotopic purity (95% d_2-, 3.5% d_1- and 1.5% d_0-γ-CEHC). ^{1}H-NMR d: 1.3 (s, 3H), 1.7–1.8 (m, 2H), 2.1 (m, 1H), 2.2 (s, 3H), 2.3 (s, 3H), 2.5 (m, 2H), 2.7 (m, 1H), 6.4 (s, 1H). GC–MS (EI) m/z (relative intensity) 267 (M + 1, 8), 266 (32), 193 (6), 152 (100), **Fig. 3A**. This synthesis was also successfully scaled up with a similar yield using 500 mg of **8b**.

Urine collection

All procedures involving human subjects were approved by the Cornell University Committee on Human Subjects. Seven healthy adults, three females and four males, were recruited from the Cornell community. Plasma lipid, liver function, and kidney function parameters were assessed by standard clinical procedures. Urine samples from each subject were collected into brown plastic containers over three consecutive 24-h periods and were kept refrigerated until delivered to the laboratory. Volumes of individual samples were recorded, and pools of each 24-h period were constructed. Pooled samples were kept frozen at −20°C until analyzed.

Analysis of urinary γ-CEHC

The d_2-γ-CEHC internal standard (12.9 nmol in 25 μL ethanol) was added to 7 ml urine in a screw-cap test tube. *E. coli* β-glucuronidase (Type IX-A, Sigma Chemical Co., St. Louis, MO), 650 units in 0.2 mL of 0.25 m sodium acetate buffer, pH 6.2, was added, the tube was flushed with argon, and the mixture was incubated overnight at 30°C in the dark. The contents of the tube were acidified to pH 1 with 6 N HCl and extracted with 8 ml hexane/*t*-butyl methyl ether (99:1). The solvent was removed from the organic extract by evaporation under a nitrogen stream, and the residue was immediately silylated by dissolving in 100 μl pyridine and 50 μl BSTFA (*N,O*-bis[trimethylsilyl] trifluoroacetamide, Pierce Chemical Co., Rockford, IL) and heating at 50°C for 45 min. The silylation reagents were removed under a nitro-

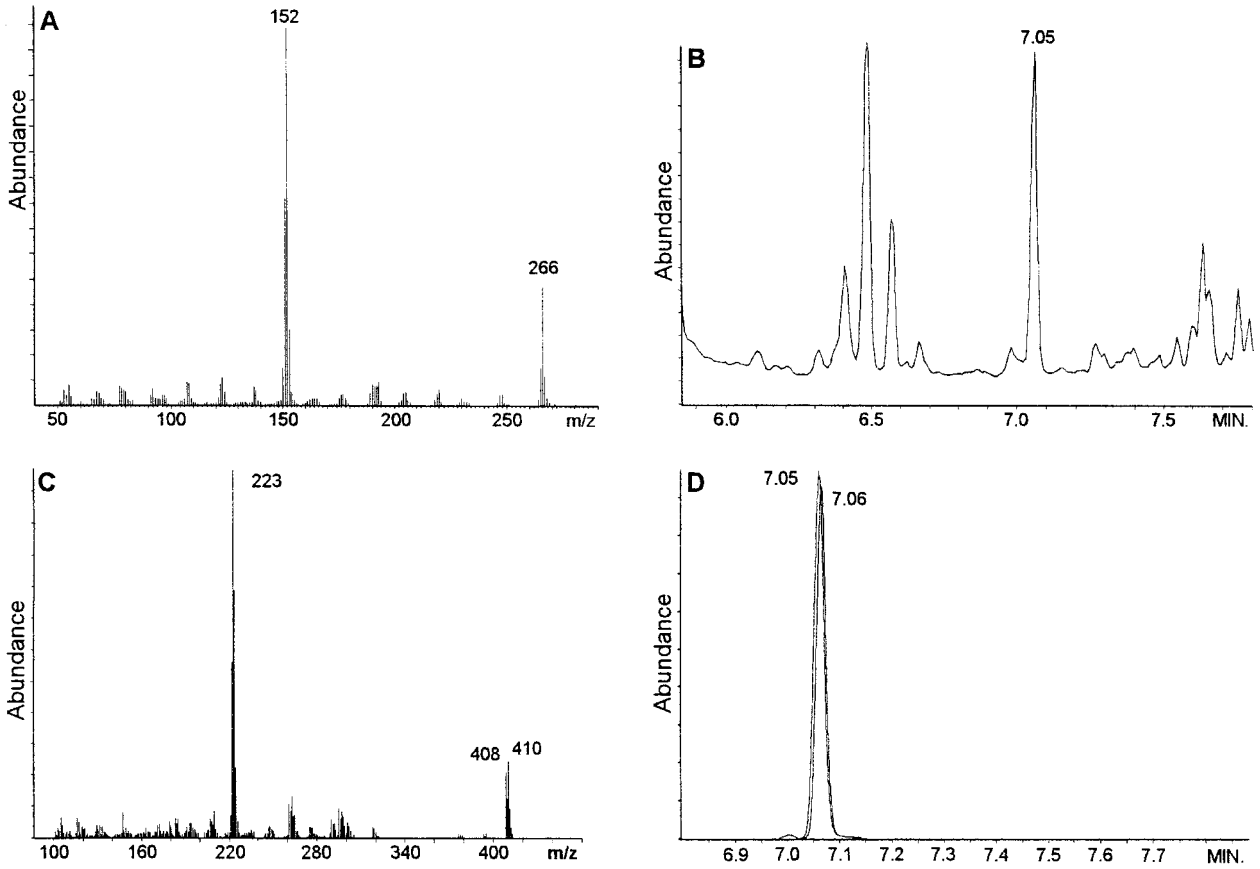

Fig. 3. Panel A: GC–MS (EI) of the synthetic d_2-γ-CEHC internal standard. Conditions were as described in Materials and Methods. Panel B: Total ion chromatogram (scan mode, *m*/*z* 100–550) of silylated extract of β-glucuronidase-treated urine sample containing d_2-γ-CEHC standard. GC–MS conditions were as described in Materials and Methods. Panel C: Mass spectrum (EI) of the 7.05 min peak of chromatogram shown in panel B, corresponding to a mixture of the disilyl forms of d_0-CEHC (molecular ion *m*/*z* 408) and d_2-γ-CEHC (molecular ion *m*/*z* 410). Panel D: GC–MS chromatogram of sample shown in panel B, obtained in selected ion monitoring (SIM) mode, monitoring *m*/*z* 408 and 410. The peak eluting at 7.05 min corresponds to disilyl-d_2-γ-CEHC (*m*/*z* 410), and that at 7.06 to disylyl-d_0-γ-CEHC (*m*/*z* 408).

gen stream and the residue was redissolved in 100 μL heptane for GC–MS analysis. Replicate analyses of silylated samples performed at different times indicated that the samples were stable at −20°C for at least several weeks. Confirmation of β-glucuronidase activity (and the absence of arylsulfatase activity) in urine of the *E. coli* β-glucuronidase preparation was obtained separately using para-nitrophenol glucuronide and para-nitrocatechol sulfate (Aldrich Chemical Co., Milwaukee, WI) as substrates, and assaying for the deconjugated products by GC–MS after carrying out incubation, extraction, and silylation of samples as described above.

The disilyl derivative of urinary γ-CEHC was quantified by gas chromatography–mass spectrometry (GC–MS) using a Hewlett-Packard 6990 gas chromatograph coupled to a Hewlett-Packard 5972A mass selective detector. The GC was equipped with an HP-1 methyl siloxane capillary column (30 m × 0.25 mm), operated in split injection mode (10:1) using helium as the carrier gas (1.0 mL/min) and an injection volume of 1 μL. The oven temperature was programmed to ramp from 200°C (2 min hold) to 240°C at 10°C/min, then to 285°C at 25°C/min (final 8 min hold). In selected ion mode (SIM) the following ions were monitored: *m*/*z* 408 (d_0-γ-CEHC) and 410 (d_2-γ-CEHC; reference peak). The retention times of d_0- and d_2-disilyl-γ-CEHC isotopomers were 7.05 and 7.06 min, respectively (Figs. 3B and 3D). Disilyl-d_2-γ-CEHC peak areas were corrected for contributions by heavy isotopes of carbon and silicon present in unlabeled (d_0)-γ-CEHC. The urinary concentration of d_0-γ-CEHC was calculated using the corrected d_0/d_2 peak area ratio and the concentration of d_2-γ-CEHC internal standard initially present in the urine sample. Replicate analyses of urine samples yielded a coefficient of variation of less than 5%.

The concentration of creatinine in each pooled urine sample was determined by spectrophotometry using Sigma Diagnostics Creatinine Kit #555A (Sigma Chemical Co., St. Louis, MO), used according to the manufacturer's instructions as developed by Slot (20) and Heinegard and Tiderstrom (21).

RESULTS

The synthetic procedure yielded a product made up of 95% d_2-γ-CEHC and 5% d_1- and d_0-γ-CEHC, as determined by GC–MS. The mass spectrum of the product was similar to that reported by Wechter et al. (11), allowing for the presence of two deuterium atoms at carbons 3 and 4 of the chroman ring (Fig. 3A). The primary differences between the d_2-γ-CEHC and d_0-γ-CEHC spectra are the addition of 2 mass units to the parent ion (266 vs. 264, respectively) and of one mass unit to the base peak (152 vs. 151, respectively, reflecting fragmentation between carbons 3 and 4). NMR analyses confirmed the identity of the product; ^{1}H-NMR peaks corresponding to the olefinic hydrogens in unsaturated γ-CEHC (8b, Fig. 2) disappear

completely after deuteration with deuterium gas yielding d_2-γ-CEHC (9, Fig. 2).

β-Glucuronidase treatment of 7 mL of human urine yielded amounts of γ-CEHC that were easily quantitated by GC–MS in SIM mode, without the need for HPLC prepurification (Fig. 3D). Inclusion of arylsulfatase (Type VI, from *Aerobacter aerogenes*, Sigma Chemical Co., St. Louis, MO) did not increase the yield of d_0-γ-CEHC, nor did treatment with 2 N HCl overnight at 25°C. The identity of the disilyl-γ-CEHC derivative extracted from human urine was readily confirmed by GC–MS in scan mode (Fig. 3C). Examination of urine samples deliberately subjected to oxidizing conditions by the addition of ferric chloride revealed essentially complete loss of γ-CEHC, with the concomitant appearance, at a retention time of 5.2 min, of a product whose mass spectrum was consistent with a quinone structure. However, there was no evidence for any such γ-CEHC-derived quinone product in any of the urine samples subjected to the described processing conditions, indicating that oxidation of γ-CEHC, both in vivo and artifactually during sample processing and work-up, probably does not occur to any measurable extent.

The concentrations of γ-CEHC in three consecutive 24-h urine pools obtained from seven subjects, with and without normalization against urinary creatinine, are presented in **Table 1**. Between-subject variation in excretion rates was generally larger than within-subject (day-to-day) variation.

The mean daily excretion rate of γ-CEHC over the 3-day collection period and the corresponding total mass of γ-tocopherol excreted daily as γ-CEHC are presented in **Table 2**. Day-to-day variations in total excretion of γ-CEHC and γ-tocopherol were greater between subjects than within subjects. Daily total excretion of γ-tocopherol, as the γ-CEHC metabolite, ranged from 1.9 ± 0.7 to 12.7 ± 3.0 mg.

TABLE 2. Mean daily excretion of γ-CEHC and equivalent daily excretion of γ-tocopherol in seven subjects

Subject	γ-CEHC[a]	γ-Tocopherol[b]
	μmol/day	*mg/day*
1	19.9 ± 4.0	8.3 ± 1.7
2	23.5 ± 4.8	9.8 ± 2.0
3	16.2 ± 2.7	6.8 ± 1.2
4	11.6 ± 3.0	4.8 ± 1.2
5	29.8 ± 6.8	12.4 ± 2.8
6	18.3 ± 5.7	7.6 ± 2.4
7	4.6 ± 1.3	1.9 ± 0.5

[a] Values represent the mean ± SD of three consecutive 24-h urine collections, calculated from the 24-h pool concentrations and the total daily urine volumes.

[b] Values represent the mean ± SD of three consecutive 24-h urine collections. Equivalent γ-tocopherol elimination rates were calculated from the mean daily excretion rates of γ-CEHC.

DISCUSSION

In the U.S., intake of γ-tocopherol exceeds that of α-tocopherol, due in part to the use of oils containing predominantly γ-tocopherol, e.g., corn and soybean oils (22). However, plasma levels of γ-tocopherol in fasting subjects are usually only about 20% those of α-tocopherol (9, 10), despite their similar absorption efficiencies (8). Although γ-tocopherol levels, as a proportion of total tocopherol, tend to be higher in some tissues than in plasma (4), it can be reasonably concluded that overall retention of γ-tocopherol is substantially less than that of α-tocopherol. Less efficient hepatic secretion of γ-tocopherol in very low density lipoprotein (VLDL), compared to α-tocopherol, has been reported by Traber et al. (8, 23). This effect has been attributed to a 31 kD hepatic tocopherol binding protein (24, 25), which appears to bind α-tocopherol preferentially to γ-tocopherol and which has been proposed to play a role in transferring tocopherols to hepatic nascent VLDL. A defect in the gene coding for this binding protein has been linked with vitamin E deficiency in humans (26). Increased rates of uptake and turnover of γ-tocopherol relative to α-tocopherol have also been reported in human plasma and cultured endothelial cells (27, 28), and γ-tocopherol disappears more rapidly from plasma than α-tocopherol after infusion of the two vitamers directly into the bloodstream of humans (29). While these findings suggest that γ-tocopherol may be rapidly eliminated via the urine or bile, studies directly addressing this issue have not been reported. Tocopherols are known to be secreted by the liver into the bile (8, 30). However, biliary tocopherol composition closely resembles that of plasma, i.e., favoring α-tocopherol (8), and therefore does not represent a selective means of elimination

TABLE 1. Concentrations of γ-CEHC in 24-h urine pools obtained from seven subjects over three consecutive days

	γ-CEHC			γ-CEHC		
Subject	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
	μmol/L			*μmol/g creatinine*		
1	13.9 ± 0.9	7.0 ± 0.04	14.5 ± 0.6	19.3 ± 1.2	16.2 ± 0.1	17.1 ± 0.8
2	32.0 ± 1.9	26.0 ± 0.9	12.3 ± 0.1	31.5 ± 1.8	22.6 ± 0.7	17.2 ± 0.1
3	13.8 ± 0.1	11.6 ± 0.2	13.1 ± 0.2	11.1 ± 0.1	10.2 ± 0.1	15.1 ± 0.2
4	10.3 ± 0.1	4.7 ± 0.6	8.5 ± 1.8	10.6 ± 0.3	6.2 ± 0.8	8.5 ± 1.8
5	32.6 ± 0.3	25.7 ± 0.3	21.1 ± 0.6	24.8 ± 0.2	28.2 ± 0.4	18.4 ± 0.5
6	5.6 ± 0.5	7.8 ± 0.9	7.5 ± 0.6	16.4 ± 1.4	21.2 ± 2.3	15.3 ± 1.3
7	5.0 ± 0.6	3.8 ± 0.4	3.6 ± 0.2	5.2 ± 0.6	3.0 ± 0.02	2.5 ± 0.1

Values represent the mean ± difference from the mean of two independent urine analyses.

of γ-tocopherol. In addition, biliary tocopherol secretion would be expected to be an inefficient excretory route due to tocopherol reabsorption in the small intestine.

Tocopherols lack sufficient water solubility to be excreted directly in urine. However, there have been reports of urinary excretion of water-soluble conjugates of δ-CEHC by rats (12) and of α-CEHC by humans (13, 14). The occurrence of low concentrations of unconjugated γ-CEHC in human urine was recently reported (11). It was therefore clear that the urine may represent a physiologically significant route of elimination of tocopherols, and that methods to quantitate rates of excretion of tocopherol metabolites were required.

We have begun to do this by developing a method for measuring urinary excretion of a γ-tocopherol metabolite, γ-CEHC. The method that previously had been used to synthesize α-tocopherol with 3, 6, or 9 deuterium atoms per molecule, namely by deuteriomethylation of the aromatic ring of γ-tocopherol, δ-tocopherol or tocol, respectively (18, 19), is not easily adapted to produce analogously labeled γ-CEHC or γ-tocopherol. Instead, we synthesized γ-CEHC selectively labeled with two deuterium atoms at the 3- and 4-positions of the chroman ring by the method described herein, which is an adaptation of our previously used method involving γ-tocopherol (18, 19). The successful formation of the labeled product permits, for the first time, accurate quantitation of urinary γ-CEHC.

Wechter et al. (11), by using non-hydrolyzed urine extracts, estimated the concentration of unconjugated γ-CEHC at about 3 nmol/L. In contrast, total γ-CEHC concentrations measured in our subjects, after β-glucuronidase treatment, ranged from roughly 4,000 to 33,000 nmol/L. Addition of sulfatase did not increase the yield, indicating that γ-CEHC is excreted by humans largely, if not exclusively, as a glucuronide conjugate. The chemical structure of the conjugated form of γ-CEHC remains to be determined.

Accurate quantitative data regarding daily intake of specific tocopherol vitamers is not available. However, in North America, intake of γ-tocopherol has been estimated to exceed α-tocopherol by a factor of two to four (31–35). Recent estimates of total vitamin E intake in the U.S. indicate that adults consume, on average, about 8 mg tocopherol equivalents (TE) per day (36). Assuming a γ-tocopherol:α-tocopherol intake ratio of 3:1, and using vitamer biopotencies of 0.1 and 1.0 mg per TE for γ- and α-tocopherol, respectively, the calculated mean intake of γ-tocopherol would be approximately 18 mg per day. The mean daily urinary excretion of γ-tocopherol, as γ-CEHC, in the seven subjects studied here, was 7.6 mg, or roughly half the estimated mean daily intake of γ-tocopherol. It should be noted, however, that these estimates of γ-tocopherol elimination are based solely on rates of excretion of γ-CEHC. While this 3′-carboxylic acid metabolite likely is the terminal oxidation product of phytyl tail truncation, the existence of γ-CEHC homologs with longer residual tails is possible. Consequently, urinary γ-tocopherol elimination rates reported here may underestimate the actual extent of elimination of this vitamer by this route. Irrespective, these data indicate that phytyl tail oxidation, followed by glucuronidation and urinary excretion, represents a major, if not the primary, route of elimination of γ-tocopherol in humans.

We have also developed a similar method for the quantitation of the corresponding metabolite of α-tocopherol, α-CEHC, which will be reported separately. It is apparent from preliminary studies that the rate of urinary excretion of α-CEHC is very much less than that of γ-CEHC. This finding provides strong support for the idea that in humans there is a mechanism that actively promotes the retention of α-tocopherol. Preferential binding of α-tocopherol by the cytosolic liver tocopherol binding/transfer protein, which may effectively shield α-tocopherol from phytyl tail oxidation, is a likely effector of this selective retention. However, differential tocopherol binding affinities or substrate turnover of the enzyme(s) responsible for the phytyl tail oxidation leading to γ-CEHC synthesis cannot be ruled out as an additional contributing factor.

In summary, we have synthesized a deuterated analog of γ-CEHC, a catabolite of γ-tocopherol, the predominant dietary tocopherol vitamer in North American diets. Using this internal standard we have determined that urinary excretion of a glucuronide conjugate of γ-CEHC represents a major route of elimination of γ-tocopherol in the human. These findings in large part explain the ultimate fate of this, and perhaps other, tocopherols which are efficiently absorbed yet poorly retained in blood and tissues.

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