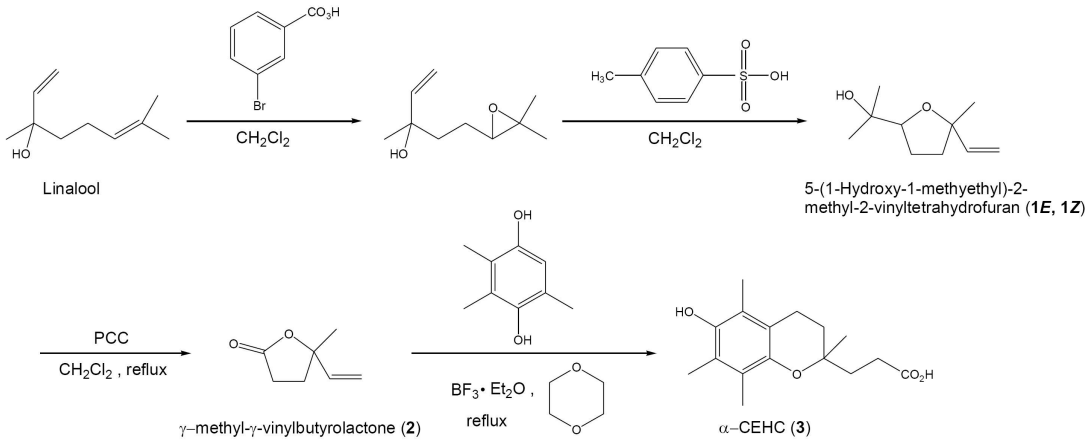
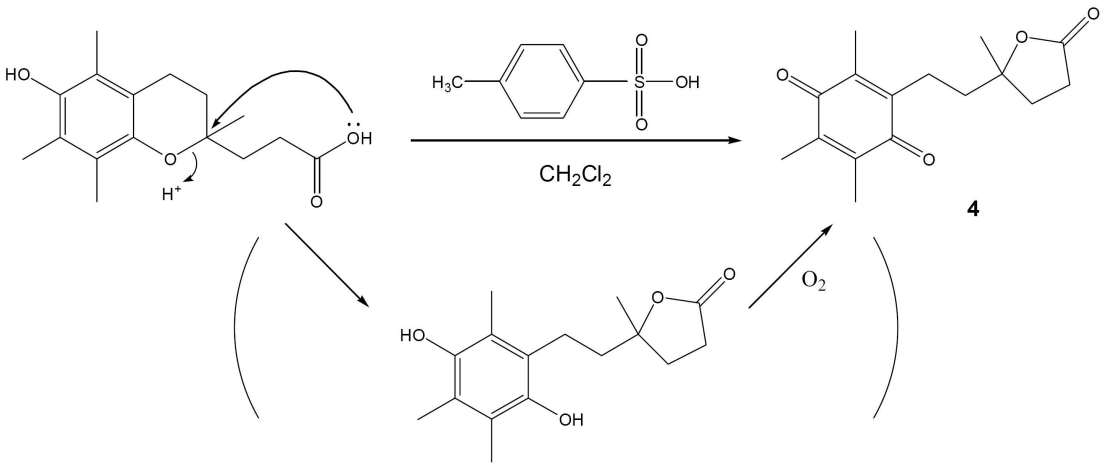
Supporting Information

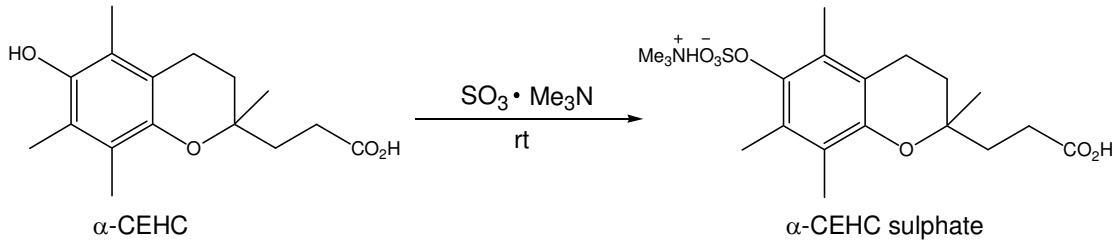
Chemical Syntheses



Scheme S1. Synthesis of α-CEHC.



Scheme S2. Synthesis of α-tocopheronolactone



Scheme S3. Synthesis of α-CEHC sulphate

Synthesis of α-CEHC (Scheme S1). α-CEHC was synthesized by the procedure modified from the report of Wechter *et al*. (*1*). γ-Methyl-γ-vinylbutyrolactone, a key intermediate, was first prepared by slowly adding 3-cholorperoxybenzoic acid (*m*-CPBA, 40g) at 0°C to a solution of linalool (20g) in dichloromethane (CH_2Cl_2) for 2 hrs under stirring. *p*-Toluenesulfonic acid monohydrate was then added to the reaction mixture to undergo the cyclize reaction. After 1h the substrate linalool disappeared by the monitor of TLC, the mixture was poured into an excess of water (400 mL) and then extracted with ethyl acetate (400 mL). The organic acid layer was washed with saturated aqueous $NaHCO_3$ to remove *m*-CPBA and *p*-toluenesulfonic acid and then concentrated under vacuum to obtain the crude products (**1*E*** and **1*Z***, 11 g). Pyridinium chlorochromate (PCC, 30g) was added to a solution of **1*E*** and **1*Z*** (11 g) in CH_2Cl_2 (500 mL) and refluxed at 60-70 °C with stirring under N_2 overnight. After cooling, the mixture was filtered by silica gel, the filtrate was concentrated under vacuum to obtain crude product (**2**, 10g). The mixture was purified on a flash silica-gel column eluted with solvent system of ethyl acetate (EA)/*n*-hexane (10:90) to give γ-methyl-γ-vinylbutyrolactone (**2,** 3.18g) as a colorless oil. Product **2** (3.18 g) dissolved in dry dioxane (12 mL) was added, via syringe over 60 min, to a solution of trimethylhydroquinone (TMHQ, 2.56 g) in BF_3-etherate (4 mL) in dioxane (15 mL) which was heated in oil bath (100°C) under N_2. After 3 hrs, the reaction mixture was cooled to room temperature and the dioxane was removed under vacuum. The brown oily residue was poured into water and 3N HCl was added to adjust to pH 2 and then extracted with ethyl acetate (EA), and the organic layer as removed under vacuum. The crude product (3g) was purified on a flash silica-gel column eluted with EA/n-hexane (30:70) to give α-CEHC (**3**) (0.8 g) as a white powder. ^1H and ^{13}C NMR spectra were obtained from a Bruker AC-200, 300 or 400 spectrometer. MS were obtained on a Finnigan TSQ-46C mass spectrometer. IR spectra were recorded on a Bio-Rad FTS-40 spectrophotometer. IR(KBr) 3422, (-OH) 3300–2500 and 1704 (-COOH) cm^{-1}; ^1H NMR (CD_3OD) δ 1.21 (s, 3H, H-2a), 1.75-1.82 (m, 2H, H-3),

1.82-1.88 (m, 1H, H-3'), 1.92-2.01 (m, 1H, H-3'), 2.05 (s, 3H, H-8a), 2.09 (s, 3H, H-5a), 2.13 (s, 3H, H-7a), 2.34-2.52 (m, 2H, H-2'), 2.62 (t, *J*= 6.9 Hz, 2H, H-4); ^{13}C NMR (CD_3OD) δ 11.8 (C-5a), 12.0 (C-8a), 12.8 (C-7a), 21.6 (C-4), 23.6 (C-2a), 29.6 (C-2'), 32.9 (C-3), 35.5 (C-3'), 74.6 (C-2), 118.1 (C-10), 122.1 (C-5), 123.1 (C-8), 124.5 (C-7), 146.2 (C-6), 146.4 (C-9), 177.8 (C-1'); MS (EI) *m/z* 278 (56%, M^+), 165 (100%).

Synthesis of α-tocopheronolactone (4) (Scheme S2). *p*-Toluenesulfonic acid (50 mg) was added to a solution of α-CEHC (50 mg) in CH_2Cl_2 (5 mL) under stirring at N_2 atmosphere. The reaction mixture was stirred overnight and the CH_2Cl_2 was removed under vacuum. The residue was purified on a flash silica gel column eluted with EA / hexane (20:80) to give α-tocopheronolactone (40 mg) as a yellow oil. IR (KBr): 1774, 1642, 1379, 1293, 1259, 1152, 1091, 938 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 3H, H-2a), 1.67-1.73 (m, 2H, H-3), 1.98, 1.99, 2.01 (s, 3H each, H-5a, H-7a, H-8a), 2.00-2.20 (m, 1H, H_a-3'), 2.21-2.28 (m, 1H, H_b-3'), 2.51-2.56 (m, 2H, H-4), 2.58-2.65 (m, 2H, H-2'); ^{13}C NMR ($CDCl_3$) δ: 11.9, 12.2, 12.3, 21.3, 25.4, 28.9, 29.1, 32.5, 39.2, 86.1, 140.3, 140.7 142.8, 176.4, 186.9, 187.4; MS (EI) *m/z* 276 [M^+] (65), 203 (100), 175 (29); UV (MeOH) nm (log ε): 268 (4.32).

Synthesis of α-CEHC sulphate (Scheme S3). The trimethylamine salt of α-CEHC sulphate was prepared using trimethylamine-SO_3 complex in pyridine according to the method of Pope *et al*. (*2*). α-CEHC (318 mg) in dry pyridine (2 mL) was added, while stirring to a solution of trimethylamine-SO_3 (205 mg) complex in dry pyridine (2 mL) under nitrogen. The reaction was stirred at room temperature for 6-8 hrs and allowed to proceed until TLC indicated that the starting material had been consumed. The salts were precipitated by the addition of excess diethyl ether, solubilized with CH_2Cl_2 with a few drops of water and purified by flash chromatography eluted with methanol/chloroform (30:70) to give α-CEHC sulphate as a beige powder (240 mg).

IR 3300–2500 and 1700 (-COOH), 1232 and 1001 (sulphate) cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.25 (s, 3H, H-2a), 1.76-1.83 (m, 2H, H-3), 1.83-1.89 (m, 1H, H-3'), 1.94-2.03 (m, 1H, H-3'), 2.06 (s, 3H, H-8a), 2.22 (s, 3H, H-5a), 2.25 (s, 3H, H-7a), 2.31-2.46 (m, 2H, H-2'), 2.63 (t, *J*= 6.9 Hz, 2H, H-4), 2.85 (s, 9H, 3× NCH_3); ^{13}C NMR (CD_3OD) δ 12.0, 13.6, 14.6 (C-8a, C-5a, C-7a), 21.6 (C-4), 24.1 (C-2a), 31.3 (C-2'), 32.7 (C-3), 35.7 (C-3'), 75.3 (C-2), 118.5, 123.5, 128.9, 130.6 (C-10, C-8, C-5, C-7), 143.9 (C-9), 149.6 (C-6), 178.0 (C-1'); MS (ESI) *m/z* 357 $[M-H]^-$.

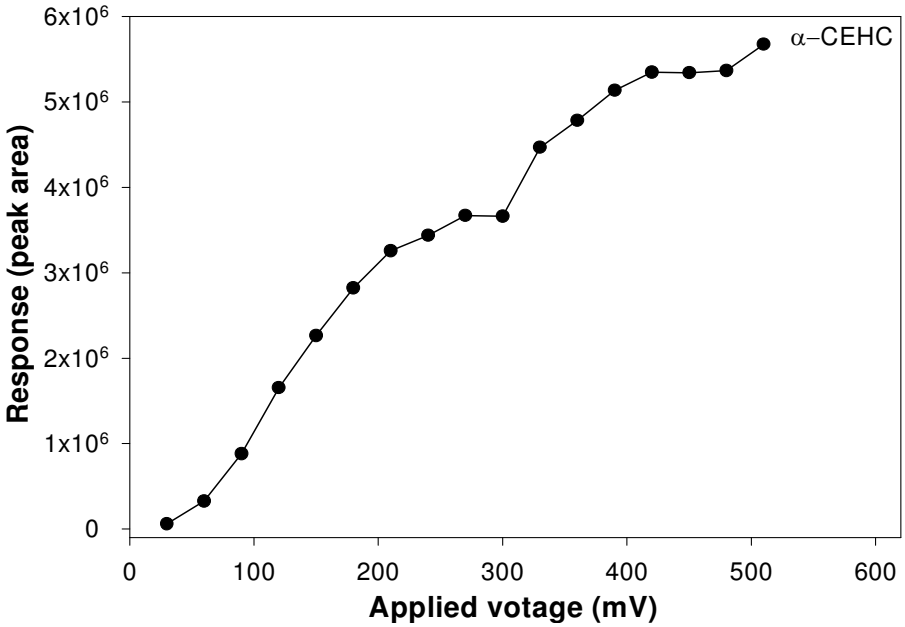


Figure S1. Hydrodynamic voltammograms for α-CEHC standard (2 μg injected).The response (function of peak area) for each peak is shown for each applied potential tested. A potential of 150 mV was found to be optimal because this voltage can oxidize α-CEHC adequately and does not cause fluctuation of baseline and interfering peaks.

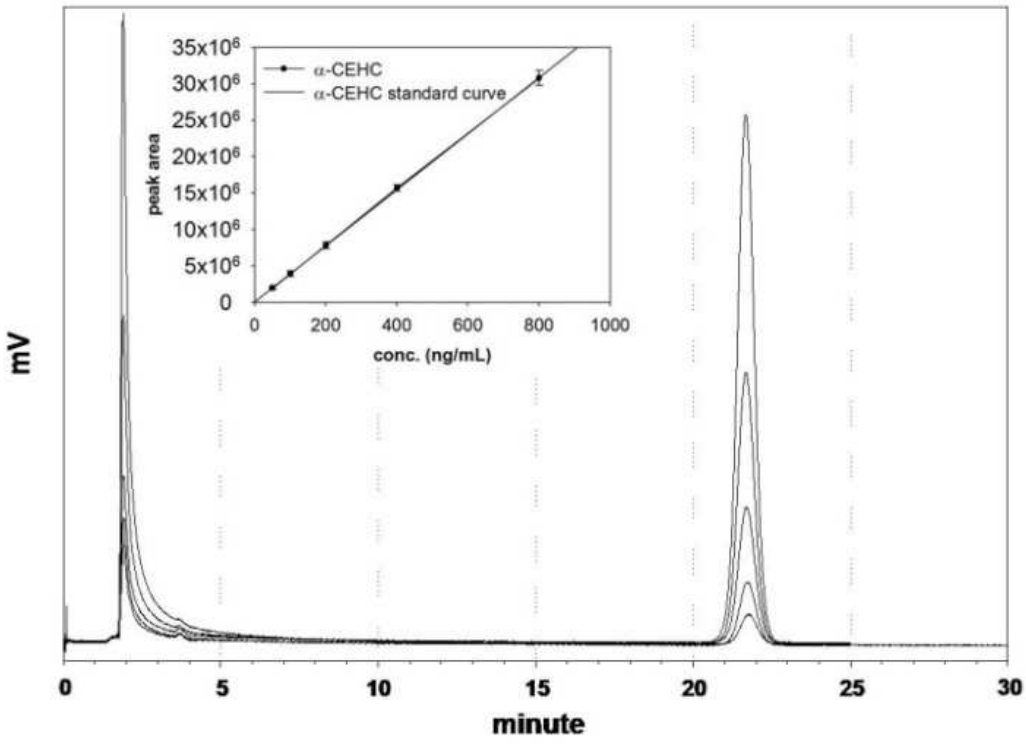


Figure S2. Typical chromatogram and standard calibration curve of α-CEHC. The retention time is 22-23 min for α-CEHC and 800 ng/mL to 50 ng/mL of α-CEHC were injected (volume is 20 μL).

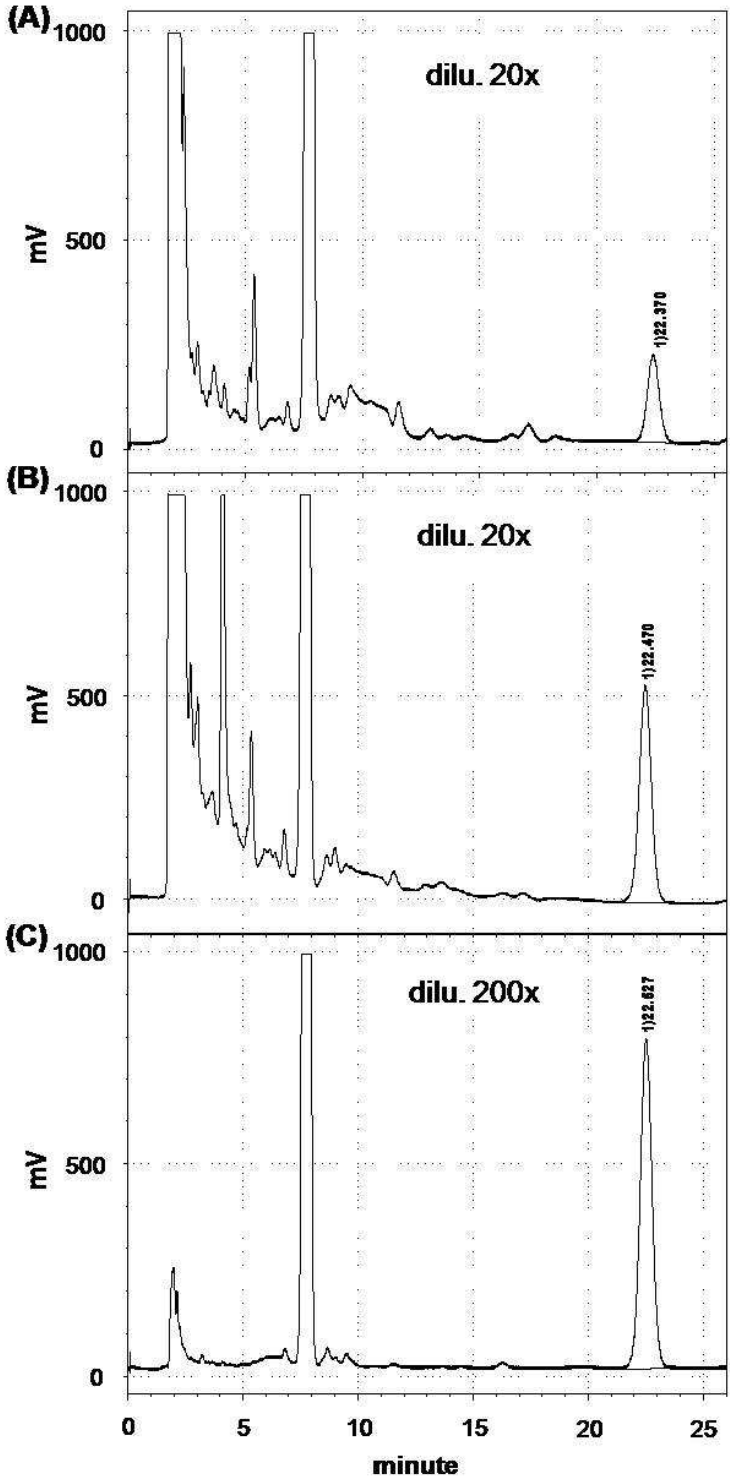
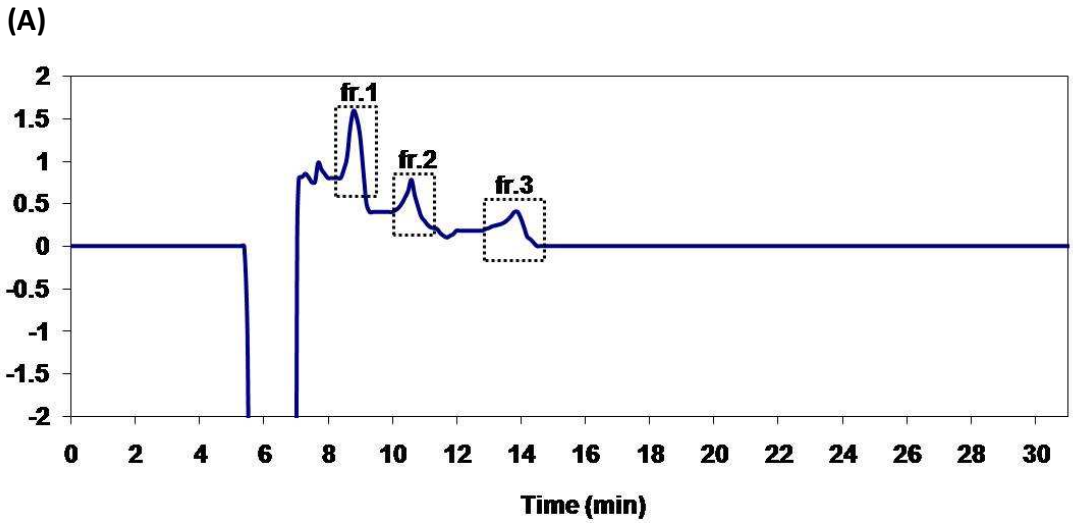


Figure S3. Chromatograms of rat urine samples after treatment with enzyme or acid hydrolysis. (A) Without hydrolysis, (B) Hydrolysis with β-glucuronidase and (C) Hydrolysis with HCl. The rat urine were collected from rats fed an AIN-76 modified diet containing 500 mg/kg *all-rac*-α-tocopheryl acetate.



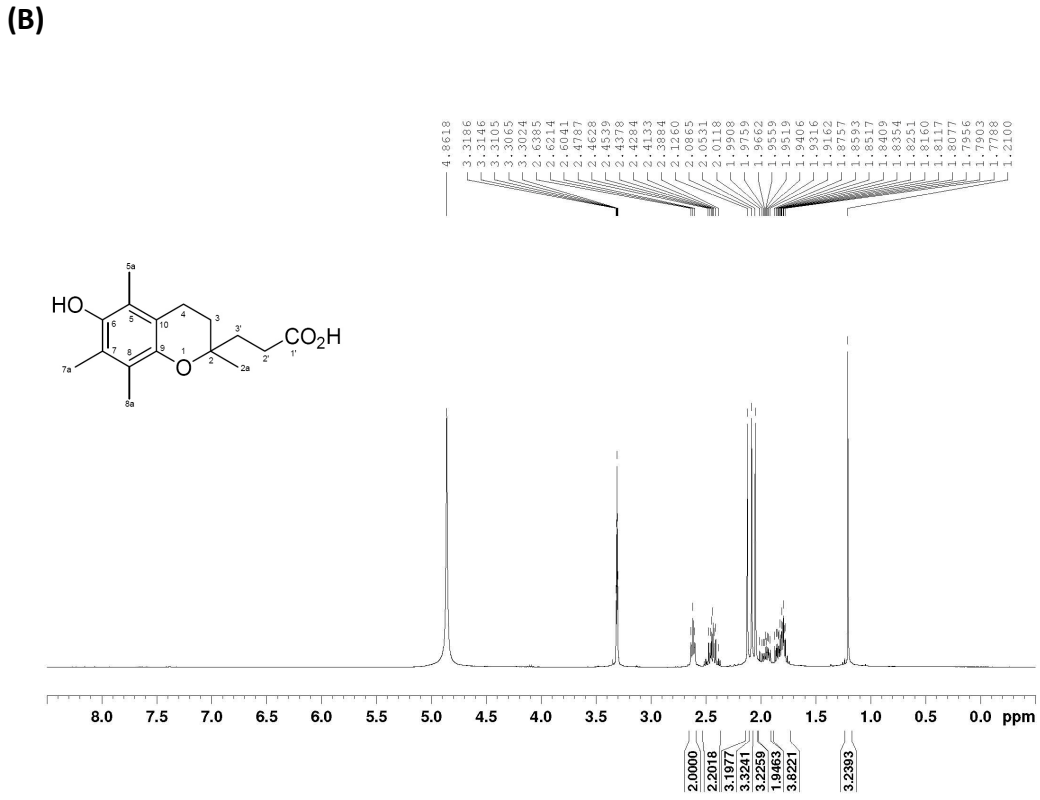


Figure S4. Rat urine was mixed thoroughly with 6 N HCl and immediately extracted twice with diethyl ether. The crude extracts were separated by a preparative HPLC with a RP-18 column and eluted with MeOH/H_2O (70:30, v/v) containing 50 mM sodium acetate (pH 4.5) solvent system at a flow rate of 2 ml/min. A α-CEHC

standard (retention time was 13.5 min) was used as a reference in the refractive index detector (RI) and the fraction eluted at the same retention time was collected, desalted and then identified by its characteristics in NMR spectroscopy. (A) Three peaks, peak 1, peak 2 and peak 3, were collected in the HPLC system and the retention time of the peak 3 is as the same as α-CEHC standard. (B) The ^{1}H NMR spectrum of the peak 3, which was as the same as α-CEHC.

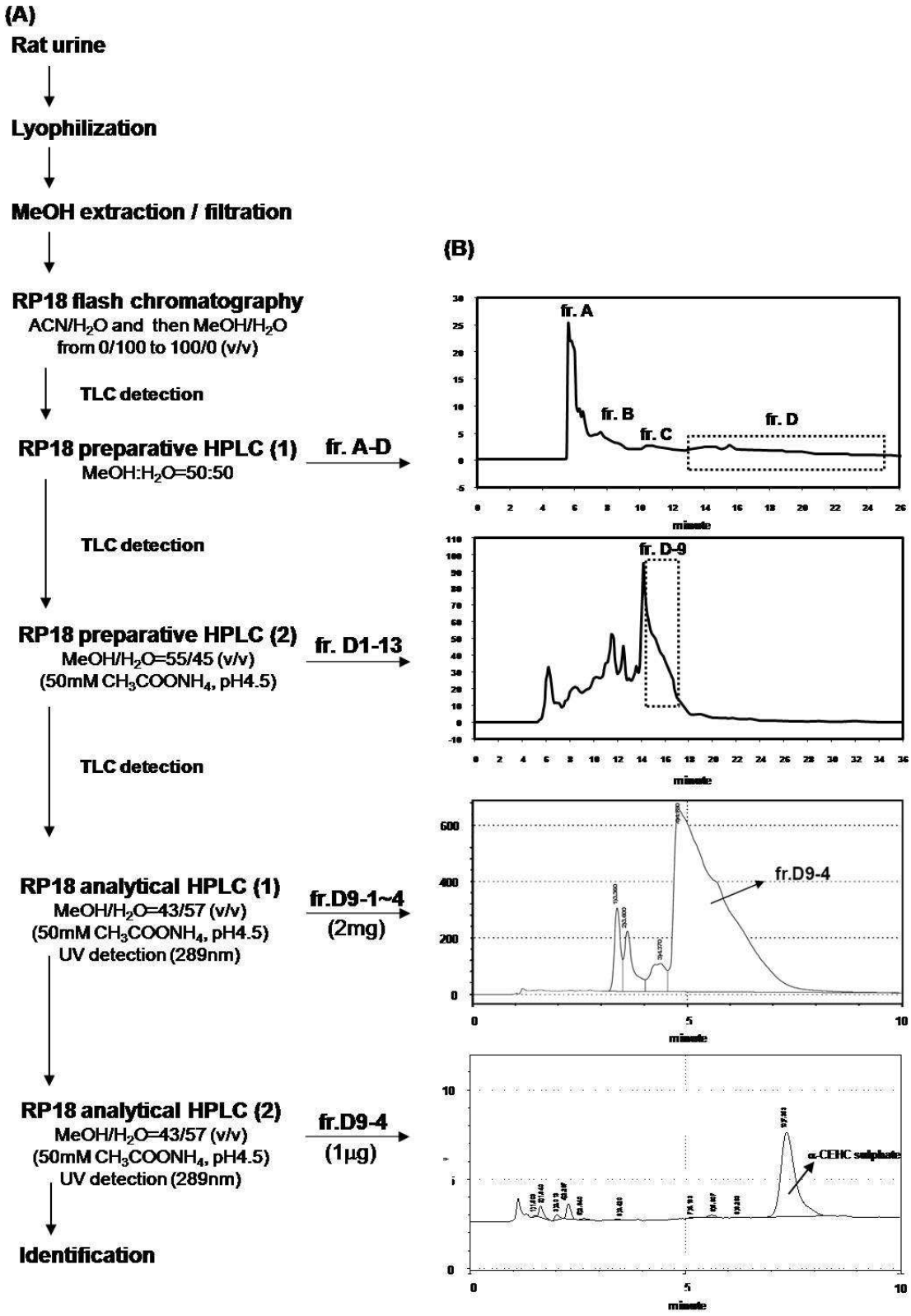
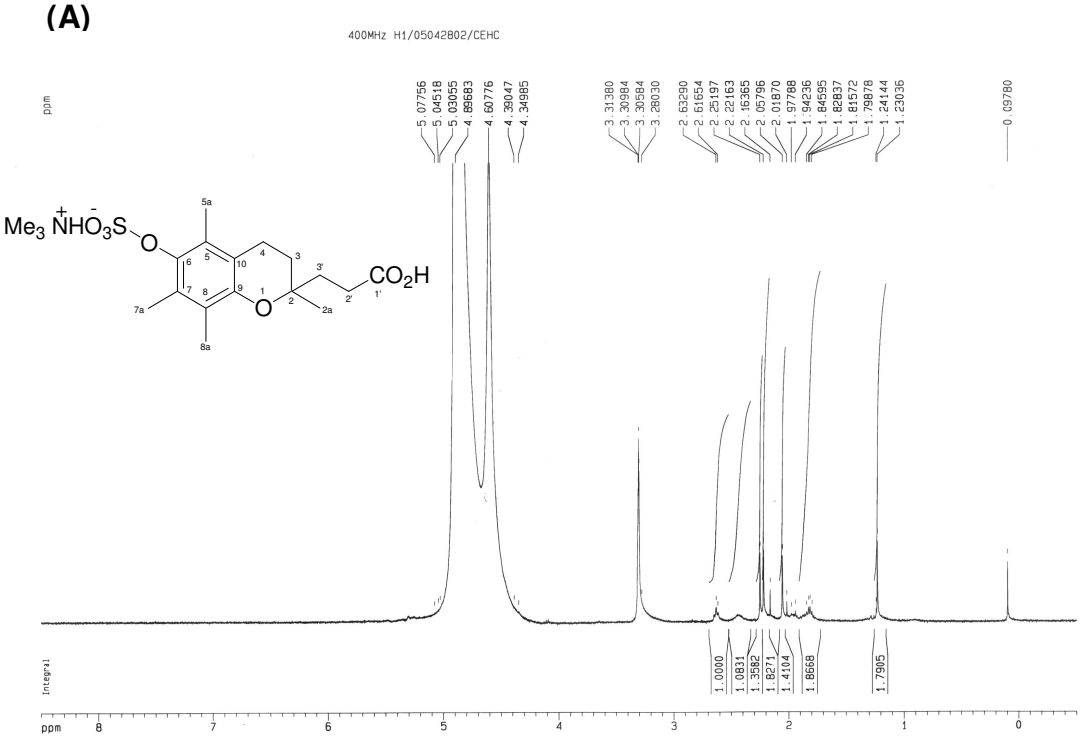


Figure S5. Isolation and purification of the HCl-releasable α-CEHC conjugate in rat urine. (A) The flowchart showing the procedure of the isolation and purification. (B) Chromatograms of HPLC separation for the last four steps.

(A)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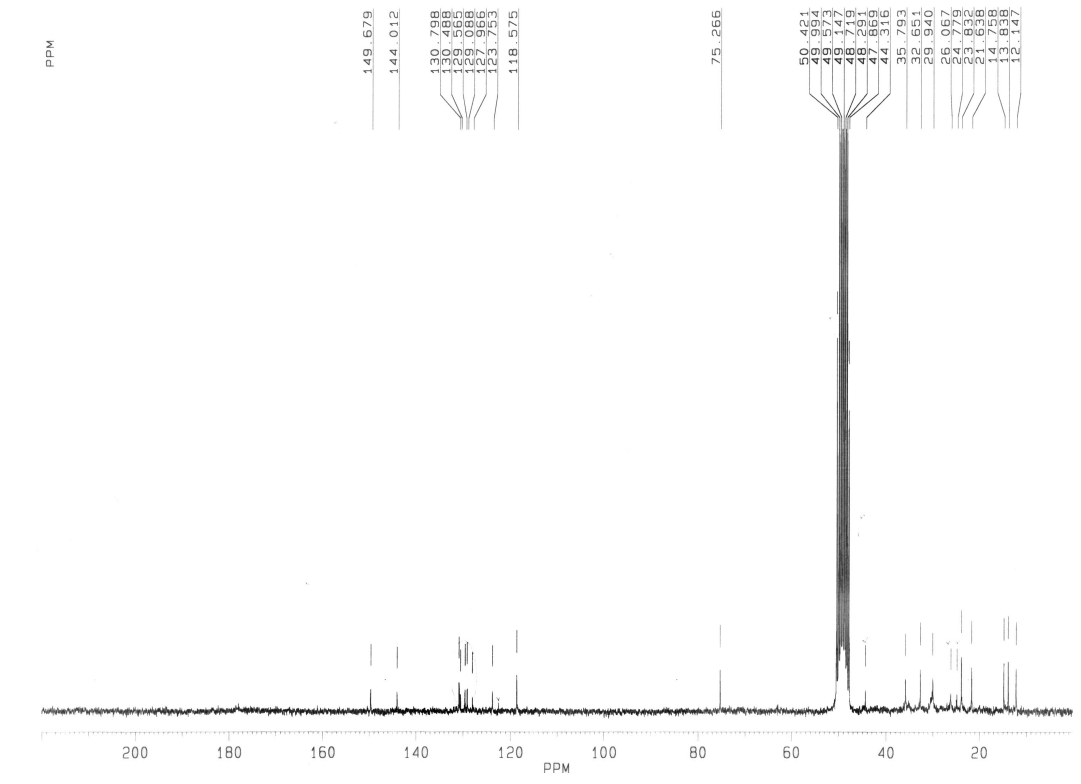
(B)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PPM

(C)

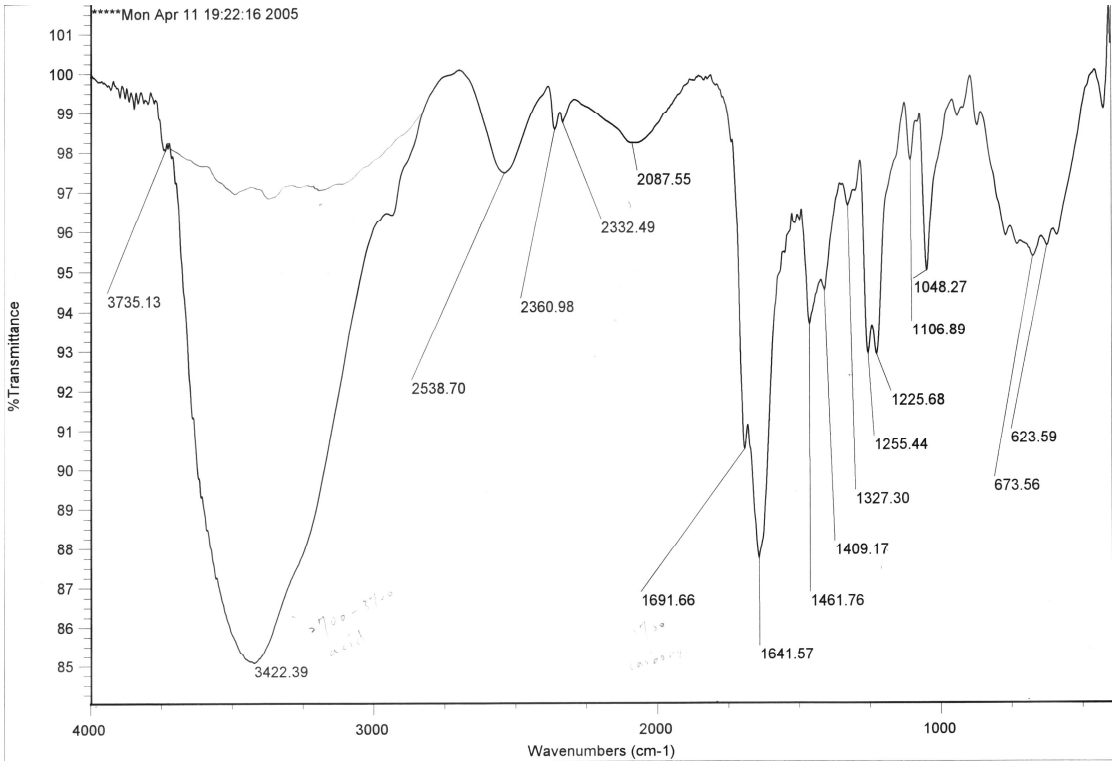

Figure S6. Spectroscopic analysis of conjugated α-CEHC, 6-*O*-sulphated α-CEHC. (A) ^{1}H NMR; (B) ^{13}C NMR; a similarity of the chemical shift to those of various tocopherol derivatives was noted. (C) Infrared spectrum.

1. Wechter, W. J.; Kantoci, D.; Murray, E. D., Jr.; D'Amico, D. C.; Jung, M. E.; Wang, W. H., A new endogenous natriuretic factor: LLU-alpha. *Proc Natl Acad Sci U S A* **1996,** 93, (12), 6002-7.
2. Pope, S. A.; Burtin, G. E.; Clayton, P. T.; Madge, D. J.; Muller, D. P., Synthesis and analysis of conjugates of the major vitamin E metabolite, alpha-CEHC. *Free Radic Biol Med* **2002,** 33, (6), 807-17.