Novel urinary metabolite of d-δ-tocopherol in rats

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Abstract A novel metabolite of d-δ-tocopherol was isolated from the urine of rats given d-3,4-[$^3H_2$]-δ-tocopherol intravenously. The metabolite was collected from the urine of rats given d-δ-tocopherol in the same manner as that of the labeled compound. It was found that the metabolites consisted of sulfate conjugates. The portion of the major metabolite released with sulfatase was determined to be 2,8-dimethyl-2-(2′-carboxyethyl)-6-chromanol by infrared spectra, nuclear magnetic resonance spectra, and mass spectra. The proposed structure was confirmed by comparing the analytical results with those of a synthetically derived compound. As a result of the structural elucidation of this novel metabolite, a pathway for the biological transformation of δ-tocopherol is proposed which is different from that of α-tocopherol. A characteristic feature of the pathway is the absence of any opening of the chroman ring throughout the sequence.—**Chiku, S., K. Hamamura, and T. Nakamura.** Novel urinary metabolite of d-δ-tocopherol in rats. *J. Lipid Res.* 1984. **25:** 40–48.

Supplementary key words d-α-tocopherol • d-3,4-[$^3H_2$]-δ-tocopherol • d-5-methyl-[^{14}C]-α-tocopherol • urinary metabolites • conjugates of sulfuric acid

Tocopherol homologues are distributed widely in nature and occur mainly in a variety of plants. δ-Tocopherol is the most abundant of these homologues occurring naturally. The antioxidative activity of δ-tocopherol is greater than that of α-tocopherol in vitro (1–4), but has less biological activity in vivo (5–9). δ-Tocopherol differs from α-tocopherol in that the former lacks two methyl groups in the aromatic ring of the chroman nucleus. Although studies on the biological transformation of α-tocopherol have been conducted (10–17), investigations into the transformation of δ-tocopherol have not been reported. Simon et al. (18, 19) characterized two metabolites from the urine of rabbits and humans given large doses of α-tocopherol. After hydrolysis these metabolites were identified as a hydroxy acid and its lactone. The isolation of these metabolites provided a basis by which a possible metabolic pathway for tocopherol homologues could be constructed (**Fig. 1**).

Watanabe et al. (20) later characterized minor urinary metabolites in the transformation of α-tocopherol and proposed a modification to the metabolic pathway described by Simon et al. (18, 19). The modified sequence proceeds as follows. α-Tocopheronic acid, formed by the ω-oxidation of α-tocopherol, is in part converted to α-tocopheronolactone and in part dehydrated so that an unsaturated, coenzyme A derivative (E acid-I-CoA) is formed. By β-oxidation and simultaneous reduction of E acid-I, two carbon units are removed to form E acid-II-CoA. Thus, these metabolites are converted to their corresponding hydroquinones and excreted as conjugates. Urinary metabolites are excreted either as mono- or di-conjugates of glucuronic acid or sulfuric acid, or both.

In the present study we describe the separation of a major metabolite of δ-tocopherol from the urine of rats given δ-tocopherol intravenously and the unambiguous determination of the structure of this metabolite. It is suggested that the metabolic pathway of δ-tocopherol is different from that of α-tocopherol.

MATERIALS AND METHODS

Compounds

d-3,4-[$^3H_2$]-δ-Tocopherol (6.7 mCi/mg) was obtained by saturation of d-3,4-dehydrated-δ-tocopherol with tritium gas. The specific radioactivity was adjusted to 10 μCi/mg by addition of unlabeled d-δ-tocopherol. d-5-Methyl-[^{14}C]-α-tocopherol was obtained by methylation of d-γ-tocopherol with [^{14}C]-paraformaldehyde and subsequent reduction (21). The specific radioactivity of labeled d-α-tocopherol was adjusted to 12.4 μCi/mg with unlabeled d-α-tocopherol. Unlabeled d-δ-tocopherol and d-α-tocopherol were obtained from Tama Biochemical Co. (Japan). Their chemical and radiochemical purity

Abbreviations: TLC, thin-layer chromatography; IR, infrared; NMR, nuclear magnetic resonance; MS, mass spectrometry; GLC–MS, gas–liquid chromatography–mass spectrometry; UV, ultraviolet; E acid-I, 2,3,5-trimethyl-6-(5′-carboxy-3′-methyl-2-pentenyl)-1,4-benzoquinone; E acid-II, 2,3,5-trimethyl-6-(3′-carboxy-butyl)-1,4-benzoquinone; δ-tocopheronolactone, 2-methyl-6-(5′-carboxy-3′-hydroxy-3′-methylpentyl)-1,4-benzoquinone lactone; δ acid-I-Me, 2-methyl-6-(5′-methoxycarbonyl-3′-methyl-2′-pentenyl)-1,4-benzoquinone; A-free, 2,8-dimethyl-2-(2′-carboxyethyl)-6-chromanol; A-Me, 2,8-dimethyl-2-(2′-methoxycarbonylethyl)-6-chromanol.

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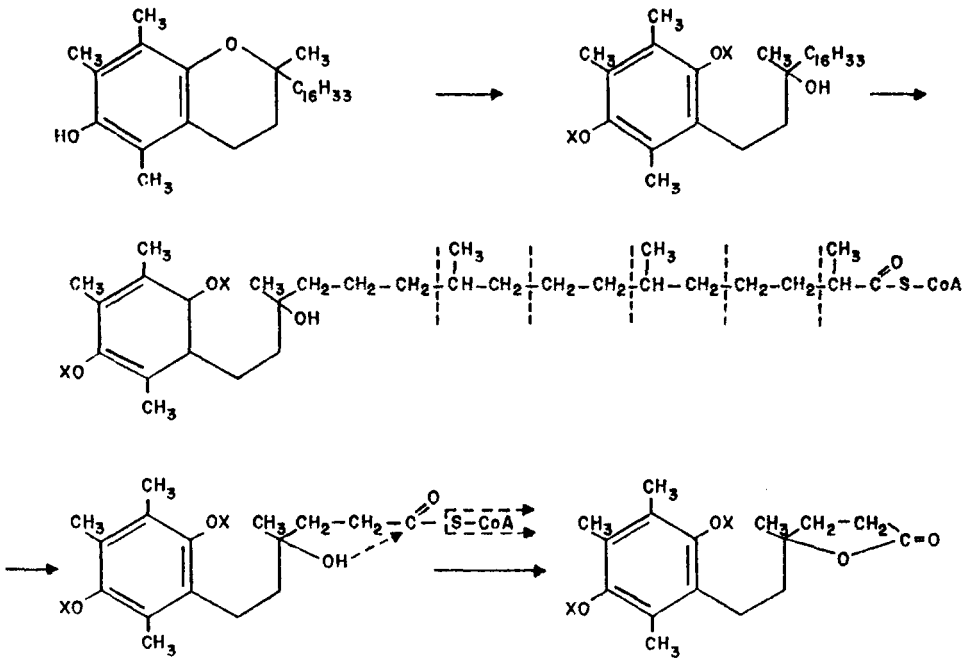

Fig. 1. Hypothetical scheme for the biological formation of the metabolites of α-tocopherol. X may be a hydrogen atom or a glucuronic acid residue (19). Reproduced by permission of the Journal of Biological Chemistry. *J. Biol. Chem.* 1956. **221:** 807–817.

(more than 95%) were examined on TLC in three solvent systems as follows. d-δ-Tocopherol: benzene–ethyl acetate 9:1 (v/v), R_f = 0.45; benzene–chloroform 1:1 (v/v), R_f = 0.14; and benzene–isopropyl ether 1:9 (v/v), R_f = 0.26. d-α-Tocopherol: chloroform only, R_f = 0.55; benzene–ethyl acetate 9:1 (v/v), R_f = 0.64; benzene–ethyl acetate 4:1 (v/v), R_f = 0.59.

Preparation of standard samples

2,8-Dimethyl-2-(2'-carboxyethyl)-6-chromanol (A-free). To a well-stirred mixture of 2-methyl-1,4-benzohydroquinone-4-benzoate (4.6 g), dioxane (50 ml), and BF_3-ether (2.8 g), a solution of γ-vinyl-γ-valerolactone (3.2 g) in dioxane (10 ml) was added dropwise over a period of 2 hr in a stream of N_2 at 70–80°C. The reaction mixture was stirred for another 4 hr at 80°C, diluted with water, and extracted with ether. The extract was hydrolyzed with 2 N NaOH (50 ml) in ethanol (50 ml) at 30°C for 2 hr, diluted with water, and extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4, and the solvent was evaporated in vacuo (in the usual manner hereafter), leaving a yellow oil. The oil was chromatographed on silica gel (100 g) with n-hexane–ethyl acetate as the eluent. The product was recrystallized from ethanol, resulting in material in the form of white needles with a melting point of 52–53°C. The yield was 3.1 g (62%). $IR\upsilon_{max}^{KBr}$ cm^{-1}: 3,400 (OH), 3,000–2,500, 1,720 (COOH). NMR ($CDCl_3$): 1.3 (3H, s), 1.6–1.95 (4H, t), 2.05 (3H, s), 2.4–2.8 (4H, t), 6.4 (2H, d-d), 8.3–8.6 (2H, b). MS: M^+ = 250. Anal. calcd. for $C_{14}H_{18}O_4$: C, 67.18, H, 7.25; found: C, 67,41, H, 7.22.

2,8-Dimethyl-2-(2'-methoxycarbonylethyl)-6-chromanol (A-Me). A-free (500 mg) was treated with an ethereal solution of CH_2N_2 and the solvent was removed in vacuo, yielding a colorless oil (510 mg). $IR\upsilon_{max}^{film}$ cm^{-1}: 3,400 (OH), 1,735 ($COOCH_3$). NMR ($CDCl_3$): 1.32 (3H, s), 1.7–2.0 (4H, t), 2.15 (3H, s), 2.4–2.85 (4H, t), 3.75 (3H, s) 4.95 (1H, b), 6.45 (2H, d-d). MS: M^+ = 264. Anal. calcd. for $C_{15}H_{20}O_4$: C, 68.16, H, 7.63; found: C, 68.44, H, 7.57.

2-Methyl-6-(5'-methoxycarbonyl-3'-methyl-2'-pentenyl)-1,4-benzoquinone (δ Acid-I-Me). To a well-stirred mixture of 2-methyl-1,4-benzohydroquinone-4-benzoate (2.3 g), dioxane (30 ml), and BF_3-ether (1.5 g), a solution of *trans* methyl-6-hydroxy-4-methyl-4-hexenoate (2 g) in dioxane (10 ml) was added dropwise over a period of 2 hr in a stream of N_2 at room temperature. The reaction mixture was stirred for another 6 hr, diluted with water, and extracted with ether. The extract was hydrolyzed with 2 N NaOH (30 ml) in ethanol (30 ml) at 30–35°C for 2 hr, diluted with water, and extracted with ethyl acetate. The extract was shaken with 10% $FeCl_3$ (50 ml), washed with saturated NaCl solution, and treated with an ethereal solution of CH_2N_2. The solvent was worked up in the usual manner and the residue was chromatographed on silica gel (80 g) with n-hexane–ether as the eluent, yielding an orange oil. The yield was 1.1 g (42%). $IR\upsilon_{max}^{film}$ cm^{-1}: 1,720 ($COOCH_3$), 1,640 (quinone). NMR ($CDCl_3$): 1.7 (3H, s), 1.95 (3H, s) 2.3 (4H, b), 3.1 (2H, d), 3.6 (3H,

s), 4.9 (1H, t), 6.5 (2H, d-d). MS: M^+ = 262. Anal. calcd. for $C_{15}H_{18}O_4$: C, 68.68, H, 6.92; found: C, 68.48, H, 7.10.

2-Methyl-6-(5'-carboxy-3'-hydroxy-3'-methylpentyl)-1,4-benzoquinone lactone (δ-tocopheronolactone). To a solution of A-free (2 g) in methanol (500 ml), a solution of ceric sulfate (2 g) in 5% H_2SO_4 (10 ml) was added dropwise with stirring at 0°C. The reaction mixture was stirred for another 2 hr, diluted with water, and extracted with ether. The extract was worked up in the usual manner and the residue was chromatographed on silica gel (60 g) with n-hexane–ether as the eluent. The product was recrystallized from ethanol, leaving a residue in the form of yellow needles with a melting point of 46–47°C. The yield was 0.8 g (40%). IR υ_{max}^{KBr} cm^{-1}: 1,770 (γ-lactone), 1,640 (quinone). NMR ($CDCl_3$): 1.25 (3H, s), 1.5–1.8 (4H, m), 1.95 (3H, s), 2.2–2.6 (4H, m), 6.5 (2H, d-d). MS: M^+ = 248. Anal. calcd. for $C_{14}H_{16}O_4$: C, 67.73, H, 6.50; found: C, 67.92, H, 6.54.

Apparatus and conditions

IR spectra were obtained with a Hitachi 215 Gating Infrared Spectrophotometer. NMR spectra were obtained on an R-24 Hitachi High Resolution NMR Spectrometer. Mass spectra of metabolites treated with methanolic-HCl were obtained on a JMS-01SG MS with the following operating conditions: ion source temperature, 250°C; ionizing voltage, 75 eV; ionizing current, 300 μA. GLC–MS of methylated and reductive acetylated derivatives of hydrolyzed metabolites were obtained on JMS D-300 MS (JEOL, Japan) with the following operating conditions: ion source temperature, 300°C; ionizing voltage, 30 eV; ionizing current, 300 μA. TLC: the metabolites were separated on silica gel GF_{254} plates (0.25-mm thickness for the analysis and 0.75-mm for the preparation) (E. Merck Co., Germany). The spots or bands on TLC were visualized by UV light (253.6 nm) in the dark or by spraying on an Emmerie-Engel reagent (first with α,α'-dipyridyl in ethanol and then with $FeCl_3$ solution). HPTLC plate RP-8 (E. Merck Co., Germany) was used as a reversed-phase silica gel plate.

Measurement of radioactivity

Radioactivity was measured in a Liquid Scintillation Spectrometer (LSC 653) (Aloka, Japan) with 5 ml of scintillation fluid consisting of 16 g PPO, 0.8 g dimethyl POPOP, 400 g naphthalene, 400 ml ethyl cellosolve, 600 ml toluene, and 3,000 ml dioxane. Radioactivity on TLC plates was detected by Radioscanner and radioautography using Marco Radioautograph film ^3H type (Sakura, Japan). Radioactivity of feces was determined after combustion (Automatic Sample Combustion System ASC-111, Aloka, Japan) using 30 mg of dried feces for one determination.

Isolation of metabolites

For excretion experiments, d-δ-tocopherol, 10 mg/kg body weight, was injected into the femoral vein of male Sprague-Dawley rats (weighing about 300 g). For metabolite isolation studies, 20 mg/kg of d-δ-tocopherol was injected. The micelle solution of tocopherol was prepared by sonicating a mixture of 1 mg of tocopherol, 6 mg of polyoxyethylene [60] hydrogenated castor oil (Nikkol HCO-60) (Nikko Chem. Co., Japan), and 0.1 ml of water. A volume of 1 ml/kg was injected into rats. Urine was collected for 24 hr after injection and filtered in vacuo. The structure of metabolites was determined by three independent procedures (A, B, and C) as follows. (A) The filtrate was lyophilized to a powder. The urine powder was extracted three times with methanol. Pooled extracts (7.80×10^6 dpm) were concentrated and kept overnight at −20°C. Precipitated salts were removed. Conjugates were separated on TLC plates developed in ethyl acetate–methanol 2:1 (v/v), and were hydrolyzed for 2 hr with sulfatase (Type H-1) (Sigma, USA) in 0.1 M acetate buffer, pH 6.2. The hydrolyzed mixture was lyophilized and extracted with methanol. The hydrolysates were purified by TLC in ether–n-hexane–methanol 12:3:1 (v/v) and methylated with diazomethane in ether. Two methylated metabolites, A-Me and B-Me, were separated by TLC in benzene–ethyl acetate 4:1 (v/v).

(B) The lyophilized urine powder was hydrolyzed with 3 N methanolic HCl at room temperature for 20 hr under a stream of N_2 and diluted with water and extracted with ethyl acetate (20). The extract was concentrated in vacuo. The crude extract was chromatographed on a column of silica gel with a mixture of n-hexane and ethyl acetate. From a fraction eluted with 20% ethyl acetate in n-hexane, three Emmerie-Engel reactive substances appeared on TLC when developed in the solvent system acetone–benzene 3:7 (v/v). A major Emmerie-Engel reactive substance was isolated and analyzed by IR spectrometry, NMR, and MS.

(C) The filtered urine was applied to a column of octadecylsilyl silica (Sep Pak C_{18}) (Waters Associates, USA) (22) to remove salts. The conjugates were eluted from the column with water. A fraction eluted with water was lyophilized to powder. The powder was extracted two times with methanol. The combined extracts were concentrated in vacuo and diluted with a small quantity of methanol and treated with diazomethane in ether to obtain methylated conjugates. The methylated conjugates were hydrolyzed with sulfatase in 0.1 M acetate buffer (pH 6.2) for 5 hr and lyophilized. The dry hydrolyzed sample was diluted with water and applied to a column of octadecylsilyl silica to remove salts. The methylated metabolites were eluted with methanol and ethanol from the column. The combined eluates with methanol and

ethanol were methylated completely with diazomethane. The methylated metabolites were kept under a reductive acetylation condition with acetic anhydride, sodium acetate, acetic acid, and zinc dust at 90°C for 3 hr (23). The reaction mixture was neutralized with sodium bicarbonate and then sodium hydroxide, and extracted with ethyl acetate. The extract was evaporated to dryness and diluted with methanol and extracted with n-hexane. The n-hexane layer was washed with methanol–water and concentrated. The residue was analyzed by GLC–MS.

RESULTS

The cumulative excretion patterns of radioactivity in urine and feces after intravenous injection of δ-tocopherol or α-tocopherol are shown in **Fig. 2**. The urinary excretion of radioactivity derived from δ-tocopherol is clearly larger than that from α-tocopherol. Mild treatment was used to separate metabolites as conjugates from the urine. The urine recovered from rats injected with labeled δ-tocopherol was lyophilized and extracted with methanol. Ninety-nine percent of the radioactivity in the urine was recovered in the methanol extract. The radioactive substances were analyzed on TLC (**Fig. 3**). The major metabolite scraped off the TLC plate corresponded to 91.9% of the applied radioactivity. Under the solvent system employed, the R_f value of the compound seemed to indicate a more polar substance. **Fig. 4** shows the radioscan patterns of TLC plates after hydrolysis of conjugates with sulfatase or β-glucuronidase in various conditions. By treatment with sulfatase in acetate buffer for 60 min, radioactivity moved far from the origin; but in buffer containing phosphate ion, a sulfatase inhibitor (24), radioactivity remained at the origin. With β-glucoronidase treatment, radioactivity remained at the origin. These results indicated that the urinary metabolites of δ-tocopherol were excreted as conjugates of sulfuric acid. They were treated with diazomethane to examine whether the hydrolysates had carboxylic acid at the terminal end of the side chain of the metabolites. Before methylation the hydrolysates were able to be separated by two-dimensional TLC in two solvent systems (**Fig. 5a**). After methylation two mobile radioactive substances appeared under one-dimensional TLC conditions (Fig. 5b). The radioactivity of the major metabolite (A-Me) was 75% of the total radioactivity developed on the TLC plate and the minor metabolite (B-Me) was 19%.

Table 1 shows the R_f values of metabolite A, metabolite B, methylated metabolite A (A-Me), and presumed standards (δ-tocopheronolactone and δ acid-I-Me) which were analyzed in the various solvent systems. The R_f value of a mixture of metabolites A and B was different from that of δ-tocopheronolactone. A-Me and B-Me were different from δ acid-I-Me and δ-tocopheronolactone. The corresponding metabolites were collected from the urine of rats given unlabeled δ-tocopherol to perform further structural analyses. On separation of unlabeled metabolites, the labeled metabolites described above were used as markers.

For separation of Emmerie-Engel reactive substances, method (B) was used. The conjugates were hydrolyzed and simultaneously converted into the methyl ester with methanolic HCl. After treatment, the ethyl acetate extract was chromatographed on a column of silica gel to separate Emmerie-Engel reactive substances. A major Emmerie-Engel reactive substance was identical to the A-Me that was obtained by method (A) on TLC analyses. The isolated Emmerie-Engel reactive substance was analyzed by IR spectrometry, NMR, and MS. The results are presented in **Fig. 6**. The evidence for the presence of a chromanol ring, three carbons in the side chain and methyl carboxylate at the terminal end of the side chain was shown clearly by IR spectra, NMR, and MS.

Further confirmation of the structure of A-Me as 2,8-dimethyl-2-(2′-methoxycarbonyl ethyl)-6-chromanol and

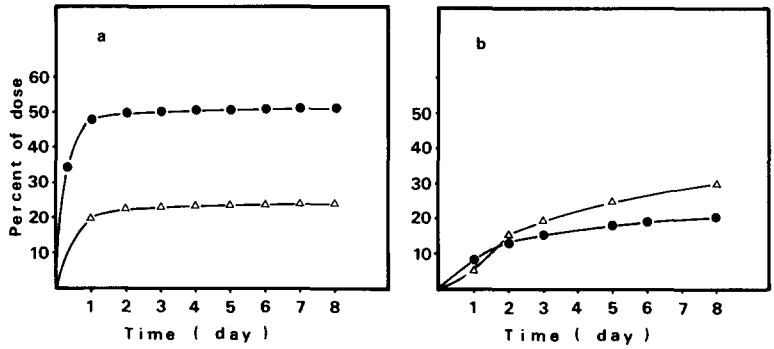

Fig. 2. Cumulative excretion of radioactivity in urine (●) and feces (△) after intravenous injection of 10 mg, 100 μCi/kg, of labeled δ-tocopherol (a) or 10 mg, 124 μCi/kg of labeled α-tocopherol (b). Results are shown as the average of two or three rats.

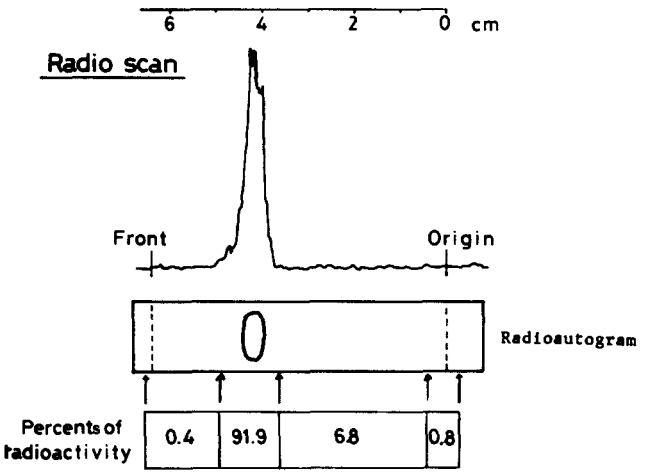

Fig. 3. Analysis of radioactive substances in methanol extract by TLC. The solvent system was ethyl acetate–methanol 2:1 (v/v). Percent of radioactivity was calculated after extraction of radioactivity from each zone.

A-free as 2,8-dimethyl-2-(2′-carboxyethyl)-6-chromanol was carried out by synthesizing the two compounds. No difference could be found on analysis by IR spectrometry, NMR, and MS between the metabolite isolated from urine and the synthesized one.

The third procedure (procedure C) for separation of urinary metabolites, a very mild treatment, was carried out to confirm the presence of a chromanol ring in the structure of the major metabolite of δ-tocopherol. The procedure consisted of methylation of conjugates, hydrolysis of methylated conjugates with sulfatase, and reductive acetylation. A sample prepared by this procedure was analyzed by GLC–MS. GLC–MS spectra are shown in **Fig. 7**. The appearance of the major mass fragments of the derivatives were m/z 306, 264, and 137. These fragments were assigned as shown in the figure. The findings supported the conclusion that the metabolite was 2,8-dimethyl-2-(2′-methoxycarbonylethyl)-6-chromanyl acetate.

DISCUSSION

A major metabolite of δ-tocopherol was isolated from the urine of rats given δ-tocopherol, and its structure was determined. The metabolites excreted in the urine were predominately in conjugated form, and their recovery was over 90% of the total radioactivity. The facile hydrolysis of the conjugate with sulfatase and inhibition by the addition of phosphate ion (a sulfatase inhibitor) in-

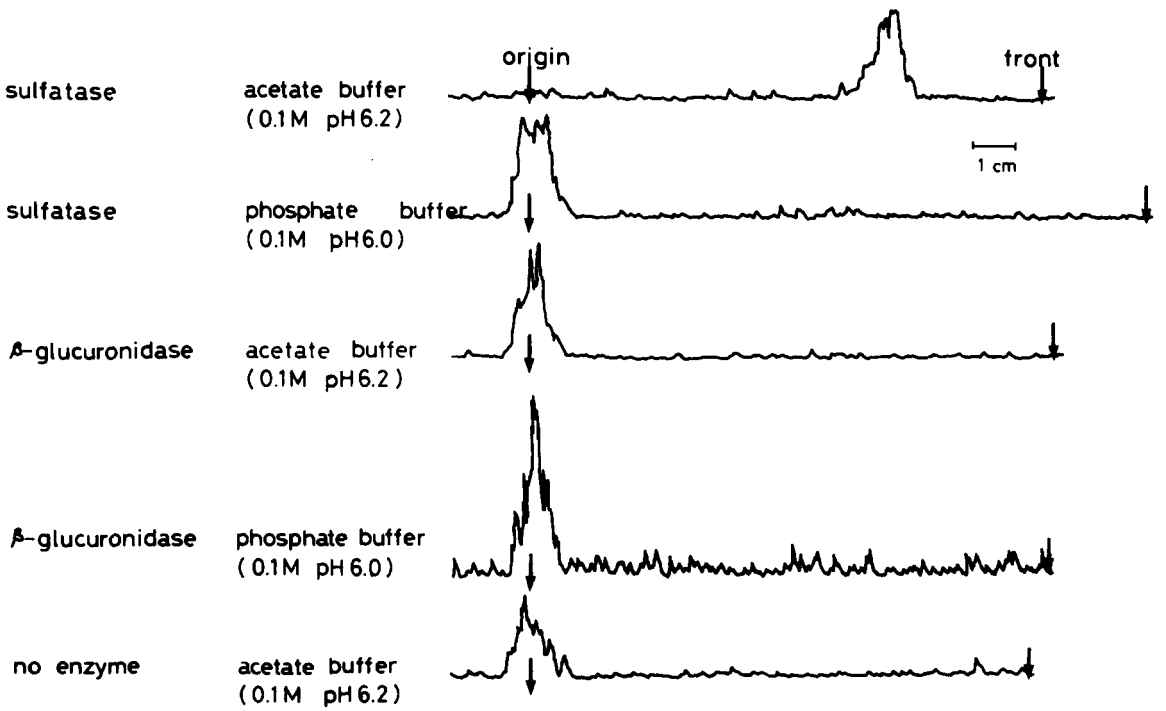

Fig. 4. Detection of radioactive substances by TLC after enzymatic hydrolysis. The sample was obtained by procedure A (before methylation). The solvent system was ether–n-hexane–methanol 12:3:1 (v/v). Incubation media consisted of 0.1 ml of enzyme solution (containing sulfatase, 246 units/ml, or β-glucuronidase, 12,202 units/ml), 3.0 ml of buffer, and 0.2 ml of sample solution (containing conjugated metabolites in water, 5×10^5 dpm). The mixture was preincubated without sample at 37°C for 5 min. The sample was added and incubated for 60 min. The incubated mixture was cooled and lyophilized. The lyophilized powders were extracted with methanol. The extracts were analyzed on TLC.

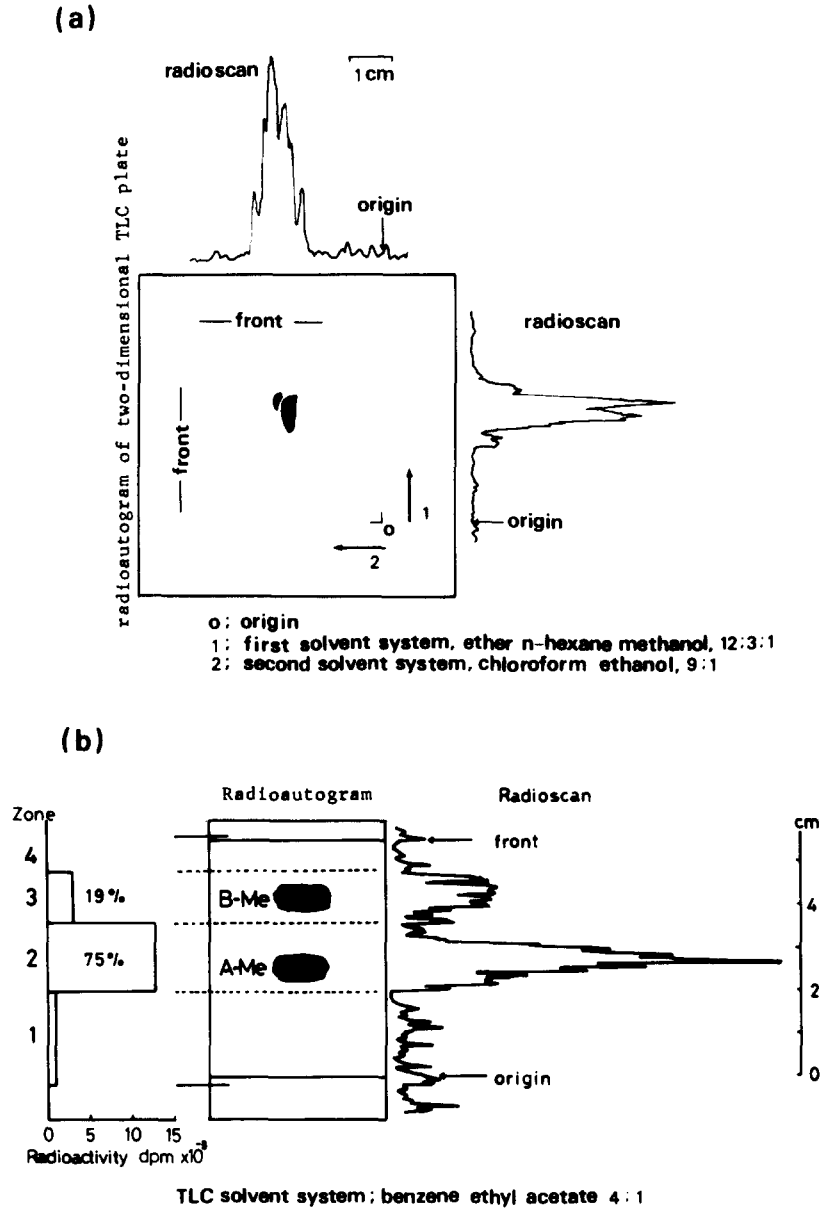

Fig. 5. Separation of radioactive substances by TLC before (a) and after (b) methylation. The sample for analysis was obtained by procedure A. (a) Radioactive substances before methylation were subjected to two-dimensional TLC. The developed plate was examined by radioautography and by radioscanning. (b) Methylated samples were chromatographed on TLC plates. The scraped zones from the plates were determined by the pattern of the radioautogram of the developed plate.

dicated that the metabolites were excreted as a conjugate of sulfuric acid.

Having confirmed the structure of the major metabolite, the following changes might have taken place in the injected δ-tocopherol; shortening of the side chain to three carbons by ω-oxidation and subsequent β-oxidations. During the metabolic processes the chroman ring was maintained. The retention of the chroman ring throughout the metabolic sequence of δ-tocopherol was the main reason for the presence of metabolites different from those of α-tocopherol. This difference in the metabolic pathway might be explained by the number of methyl groups substituted in the chroman ring. It has been speculated that the influence of steric hindrance to the chroman ring is larger in α-tocopherol due to the presence of two more methyl groups in the chroman ring of α-tocopherol (25). Steric hindrance of the chroman ring in α-tocopherol might increase the susceptibility of the ring to open and yield α-tocopheryl quinone or its more completely metabolized compounds. δ-Tocopherol is stable enough to maintain the chroman ring. On chemical oxidation of δ-tocopherol and its model compound, no δ-

TABLE 1. R_f values of metabolites in several solvent systems on TLC

Solvent System	Metabolites		Derivatives		δ-Tocopher-onolactone	δAcid-I-Me
	Conjugates	A + B	A-Me	B-Me		
Ethyl acetate–methanol (2:1) (a)	0.66					
Ether–n-hexane–methanol (12:3:1) (a)		0.55	0.76	0.76	0.51	0.87
Benzene–ethyl acetate (4:1) (a)		0.13 broad	0.44	0.73	0.23	0.56
Chloroform–ethanol (9:1) (a)		0.42			0.54	
Acetone–water (1:1) (b)			0.17		0.32	0.12
Emmerie-Engel reaction	−	+	+	+	−	−

(a), Silica gel plate; (b), reversed-phase silica gel plate.

tocopheryl quinone was formed, but several dimers retaining the chroman ring have been isolated (26–28). It was reported in a previous paper (29) that the formation of tocopheryl quinone is not indispensable for antioxidative activity in vivo.

Three procedures for the elucidation of urinary metabolites of δ-tocopherol were used. These procedures were used to prove that the metabolite was not the result of an artifact due to the isolation procedure employed. A mild procedure which consisted of hydrolysis with sulfatase (procedure A) yielded chromanol derivatives. A second procedure involved treatment with methanolic HCl, which yielded hydrolyzed and methylated metabolites. This procedure made it possible to obtain larger amounts of sample to analyze the structure of the metabolite. The procedure has been employed for the determination of the urinary metabolites of vitamin K or coenzyme Q_7 to prevent the formation of γ-lactone as

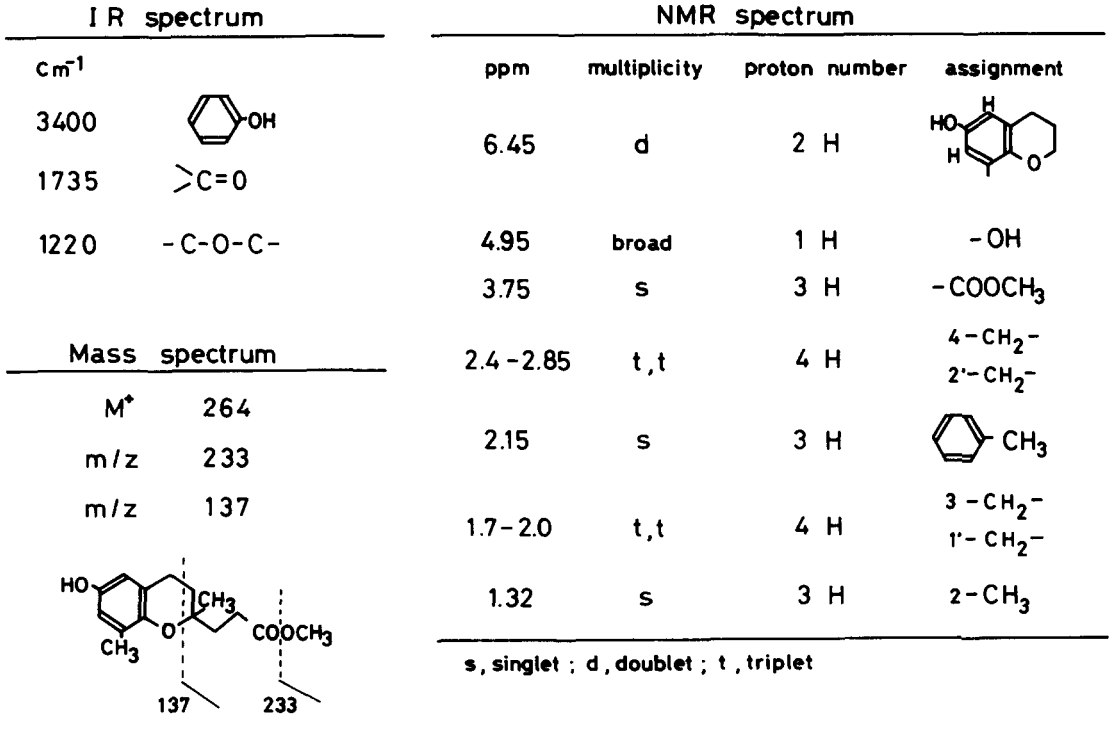

Fig. 6. Analyses of an Emmerie-Engel reactive substance (A-Me).

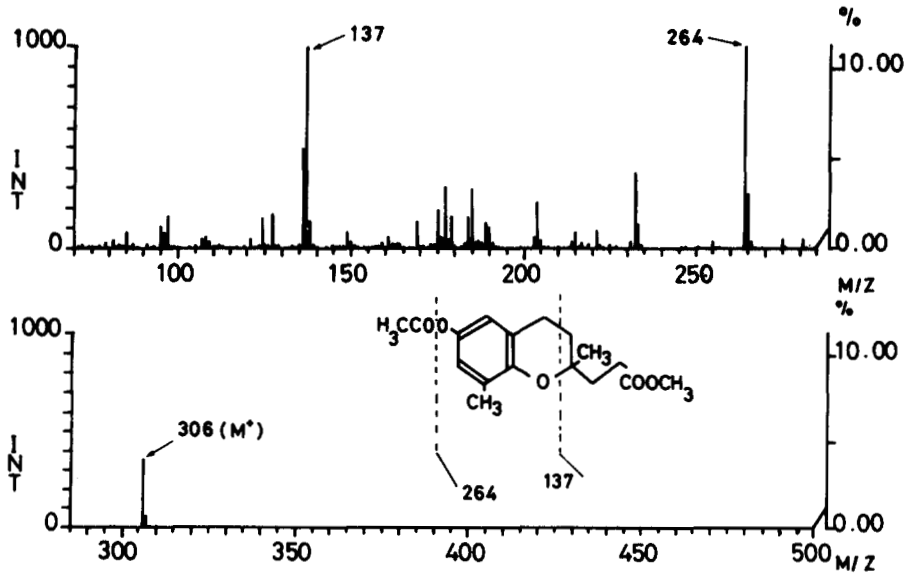

Fig. 7. Mass spectrum of methylated and reductive acetylated derivative of the hydrolyzed metabolite of δ-tocopherol in urine and assignment of the fragment ions.

an artifact (20). However, the procedure does not indicate any information on the conjugation of the metabolites.

Although a metabolite of lesser significance (metabolite B) was not analyzed further, it was found that metabolite B and its methylated ester (B-Me) did not correspond to so-called δ-tocopheronolactone or δ acid I-Me which were synthetically prepared as the same type of metabolite of α-tocopherol.

The proposed metabolic pathway of δ-tocopherol and chemical treatments used for the separation of the metabolite are presented in **Fig. 8**. The proposed metabolic pathway for δ-tocopherol might be explained also by the

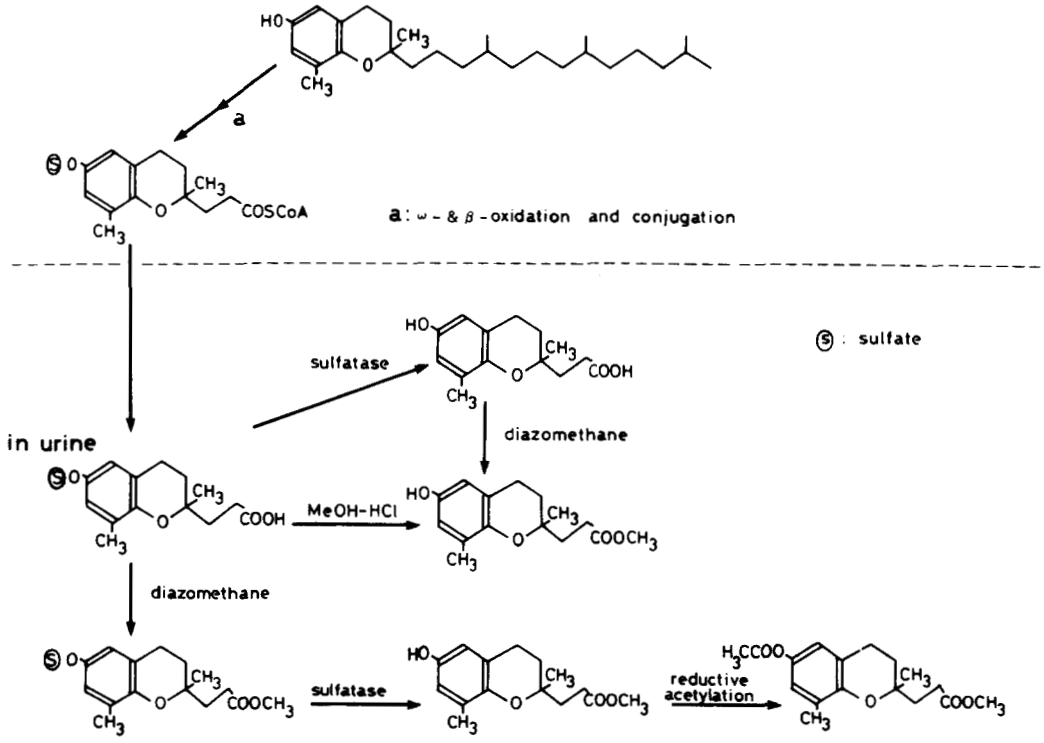

Fig. 8. Schematic representation of the metabolic pathway of δ-tocopherol and chemical treatments.

difference in the excretion percentage of radioactivity between δ-tocopherol and α-tocopherol (Fig. 2). The lesser affinity of δ-tocopherol when compared with α-tocopherol in biomembranes may be the reason for the excretion of large amounts of δ-metabolites in urine and feces. It has been shown that δ-tocopherol has less physiological activity due to less retention in tissues than α-tocopherol (8, 30). Urinary metabolites of α-tocopherol are excreted either as mono- or di-conjugates of glucuronic acid or sulfuric acid, or both, after opening of the chroman ring (20). On the other hand, the major metabolite of δ-tocopherol was a mono-conjugate of sulfuric acid retaining the chroman ring.

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