Structure–Activity Relations in the Vitamin E Series.[1] II. Derivatives of α-Tocopherol Substituted at the 5-Methyl Group

W. A. SKINNER, R. M. PARKHURST, J. SCHOLLER,[2]

Life Sciences Research, Stanford Research Institute, Menlo Park, California 94025

AND K. SCHWARZ[3]

Laboratory of Experimental Metabolic Diseases, Veteran's Administration Hospital, Long Beach, California

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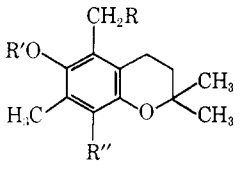
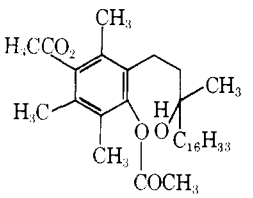
Several derivatives of α-tocopherol substituted at the 5-methyl group were synthesized from the common intermediate, 5-chloromethyl-7,8-dimethyltocol acetate. These derivatives were evaluated for their activities in protecting rabbits against vitamin E deficiency induced muscular dystrophy and rats against dietary necrotic liver degeneration. It was found that the phytyl side chain of *dl*-α-tocopherol contributes to its biological activity in these animal systems, and that the presence of an intact 5-methyl group, although not essential for activity, greatly enhances it. Several of these derivatives were active in both test systems, while others were inactive. Some crossover of activities was seen in muscular dystrophy in the rabbit and dietary liver necrosis in the rat.

In a previous publication,[1] we reported the synthesis of 5-methyl-substituted derivatives of 6-hydroxy-2,2,5,7,8-pentamethylchroman. The biological activity of these compounds was studied in vitamin E deficiency induced liver necrosis in rats, muscular dystrophy in rabbits, chick encephalomalacia, and resorption of embryo in rats as well as *in vitro* respiratory decline of liver slices from vitamin E deficient animals. These vitamin E model compounds were of interest because of their decreased lipid solubility as compared to vitamin E. None of these derivatives showed activity in vitamin E deficiency induced muscular dystrophy in rabbits, chick encephalomalacia, or resorption of embryo in rats. However, significant activity was found to be present in several of these model derivatives with regard to preventing the *in vitro* respiratory decline and liver necrosis associated with a vitamin E deficient diet in rats. Compounds **1**–**4** had some activity in protecting rats against liver necrosis, while **1** and **5** were quite active *in vitro* in preventing respiratory decline. The latter experiments indicated that the phytyl side chain of α-tocopherol was not essential for activity *in vitro*. However, *in vitro* none of the model compounds was as active as was α-tocopherol. These results stimulated us to synthesize the analogous 5-methyl-substituted α-tocopherol derivatives for biological evaluation *in vivo*. This paper reports these synthetic studies and the results of biological evaluation of the derivatives.



1, R = H; R′ = H; R″ = CH_3
2, R = Cl; R′ = $COCH_3$; R″ = CH_3
3, R = OC_2H_5; R′ = H; R″ = CH_3
4, R = $OCH_2C_6H_5$; R′ = H; R″ = CH_3
5, R = H; R′ = H; R″ = H



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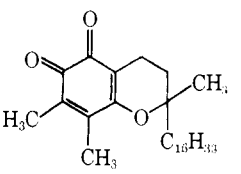

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All of the derivatives of α-tocopherol substituted at the 5-methyl group were prepared from the common intermediate 5-chloromethyl-7,8-dimethyltocol acetate (**8**) which was prepared by a modification of the method previously reported[4] (Table I).

Biological Activities. Vitamin E Deficiency Induced Muscular Dystrophy in Rabbits.—Nutritional muscular dystrophy was produced in New Zealand white rabbits as previously described.[5,6] The compounds were administered to the animals *via* the intravenous route after they were suspended in water with Tween 80 (2 drops/10 ml) *via* sonification. The biological results were evaluated as previously reported.[1] Table II summarizes these results.

Protection of Rats against Dietary Necrotic Liver Degeneration.—These studies were conducted as described in the preceding paper.[1] The tocopherol derivatives were fed in the diet. Male Sprague–Dawley rats were used which had been maintained on a liver necrosis inducing diet for a predepletion period of 14 days after weaning. Table III summarizes these results.

Discussion

In the case of biological activity of *dl*-α-tocopherol derivatives in protecting rabbits against vitamin E deficiency muscular dystrophy, we previously had found that model compounds having a methyl group in place of the phytyl side chain were not active.[1] However, significant activities were found with some of these model compounds in protecting rats against

(1) Paper X. For the preceeding papers see W. A. Skinner, R. M. Parkhurst, J. Scholler, P. Alaupovic, Q. E. Crider, and K. Schwarz, *J. Med. Chem.*, **10**, 657 (1967).
(2) Vitamin E deficiency induced muscular dystrophy studies.
(3) Liver necrosis studies.
(4) A. F. Wagner, A. Losi, C. H. Shunk, B. O. Linn, D. E. Wolf, C. H. Hoffman, R. E. Erickson, B. Orison, and K. Folkers, *J. Am. Chem. Soc.*, **85**, 1534 (1963).
(5) J. L. Smith, H. W. Moore, and K. Folkers, *Proc. Soc. Exptl. Biol. Med.*, **118**, 782 (1965).
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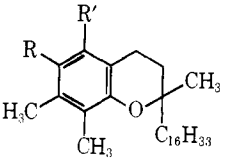
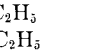
Table I
Derivatives of α-Tocopherol Substituted at the 5-Methyl Group



Compd	R	R′	Mp, °C	R_f[a]	Yield, %[b]	Formula	Analyses
8	CH_3CO_2	CH_2Cl		0.90	40	$C_{31}H_{51}ClO_3$	C, H, Cl
9	CH_3CO_2	$CH_2SCNHNH_2$	HCl, 130–135		19	$C_{32}H_{55}ClN_2O_3S$	C, H, Cl, N, S
10	OH			0.15	25	$C_{34}H_{59}NO_2$	C, H, N
11	OH	CH_2OCH_3		0.65	37	$C_{30}H_{52}O_3$	C, H
12	CH_3CO_2	$CH_2SC_2H_5$		0.75	37	$C_{33}H_{56}O_3S$	C, H
13	OH	$CH_2OC_2H_5$		0.70		$C_{31}H_{54}O_3$	C, H
14	OH	$CH_2OCH_2C_6H_5$		0.80		$C_{36}H_{56}O_3$	C, H
15	OH			0.30		$C_{33}H_{57}NO_3$	C, H
16	OH	$CH_2N(CH_3)_2$	45–46	0.10	32	$C_{31}H_{55}NO_2$	C, H

[a] R_f values on silica gel GF–$CHCl_3$. [b] Yields are for analytical samples isolated from thick silica gel plates.

Table II
Vitamin E Deficiency Induced Muscular Dystrophy in the Rabbit

Compd	Dose, mg/kg iv	No. of rabbits	Av day of death[a]
Negative control		6	7 (4–10)
dl-α-Tocopherol acetate	25	6	26 (20–33)
11	50	4	24 (19–28)
8	50	4	18 (13–21)
16	50	4	16 (12–19)
14	50	2	15 (13, 17)
12	50	4	14 (8–20)
13	50	2	12 (9, 14)
6	50	2	12 (11, 13)
15	50	2	10 (10, 11)
10	50	2	9 (6, 12)

[a] Measured from day of initiation of treatment, when all animals were rated 1+ (controls received saline).

Table III
Preventive Effects of Tocopherol Analogs on Dietary Necrotic Liver Degeneration

Compd	Dose, mg/100 g of diet	No. of animals	No. of survivors	% protection[a]	ED_{50},[b] mg/100 g of diet
dl-α-Tocopherol acetate[c]	0.375	45	8	36	0.53[d]
	0.75	20	19	94	
11	5	20	3	35	7.0
	10	15	9	72	
12	10	10	3	52	9.6
8	10	14	8	69	12.3
	15	10	9	93	
13	10	20	1	15	12.8
	15	10	7	83	
15	10	10	2	21	24.0
9	10	5	0	0	
10	10	10	0	0	
Tocopheryl-quinone	10	10	0	0	
14	10	10	0	0	
16	10	10	0	0	
7	10	10	0	0	

[a] Per cent protection = $100 - 100V_{st}(\text{expt})/V_{st}(\text{control})$, where V_{st} is the reciprocal of the survival time. [b] Dose in mg of compound/100 g of diet to give a 50% protective effect, calculated as previously described: K. Schwarz and C. M. Foltz, *J. Biol. Chem.*, **233**, 245 (1958). [c] Control tests with *dl*-γ-tocopherol acetate, carried out concurrently with assays of the other compounds. [d] This figure is slightly less than previously reported (0.7) because of higher sensitivity of the animals used in the above assays.

dietary liver necrosis. None of the model compounds approached *dl*-α-tocopherol in potency. *In vitro* respiratory decline of liver slices from vitamin E deficient animals was also prevented with some of these derivatives.

In this study we have found that various structural changes introduced at the 5-methyl group of *dl*-α-tocopherol all decrease its biological activity against both of these disorders. Contrary to the results obtained with the model compounds, several of the 5-methyl-substituted derivatives of *dl*-α-tocopherol do exhibit some *in vivo* biological activity in the rabbit. The most active of the series tested (Table II) was the 5-methoxymethyl derivative, followed by the 5-chloromethyl, the dimethylaminomethyl, the benzyloxymethyl, and the ethylthiomethyl derivatives. The latter compound doubled the life expectancy of dystrophic rabbits over the control group when it was given to them intravenously at 50 mg/kg. It is difficult to explain the variation in activity with structure except to point out that even the most active derivative, **11**, has to be given at twice the dose of *dl*-α-tocopherol acetate in order to attain comparable activity. This would seem to point out the importance of having an intact 5-methyl group. The difference between activity of the methoxy and ethoxy derivatives is surprising.

A comparison of the activity of these derivatives in the dystrophic rabbit with their activities in dietary liver necrosis in the rat is interesting. There is some correlation of activities in the two disorders. Compound **11**, for example, is the most active of the derivatives in both test systems, and **8** is also quite active in both tests. Compound **12** is more active in the rat than in the rabbit, whereas **16** is active in the rabbit and not in the rat. A possible explanation for the lack of activity of **16** in the rat is its reduced absorption when given orally. It is a quite polar compound as evidenced by its R_f of 0.10 on silica gel tlc. As in the rabbit test, the 5-methyl group of *dl*-α-tocopherol is quite important

for activity in preventing dietary necrotic liver degeneration in the rat. In dietary liver necrosis even the most active of the 5-methyl-substituted derivatives is far less potent than *dl*-α-tocopherol itself; it has only approximately $^1/_{13}$ of the activity of the latter.

It is noteworthy that in prevention of liver necrosis in the rat the activity of all 5-methyl-substituted derivatives of *dl*-α-tocopherol investigated here is less than that of γ-tocopherol which lacks the methyl group in the 5 position. γ-Tocopherol shows approximately one-fifth of the potency of α-tocopherol in a variety of biological test systems, such as resorption sterility[7] and respiratory decline of liver homogenates of vitamin E deficient animals.[8] In muscular dystrophy in rabbits, natural γ-tocopherol has been reported to be 30% as active as natural α-tocopherol while synthetic γ-tocopherol was much less effective.[9]

It is interesting to compare the activity of the model series with the tocopherol series in dietary liver necrosis in the rat. Compounds **1**–**4** were the most active of the model series. These correspond to *dl*-α-tocopherol, **8**, **13**, and **14** of the tocopherol series. The methoxy derivative of the model series, corresponding to **11**, was not very active, giving only 15% protection at 10 mg % concentration. The model of **12**, the thioethyl derivative, was not evaluated in previous tests so no comparison can be made. In the model series, again the importance of the 5-methyl group becomes apparent.

Experimental Section[10]

5-Chloromethyl-7,8-dimethyltocol Acetate (8).—α-Tocopherol (7.8 g) in 100 ml of 95% EtOH and 10 g of $AgNO_3$ in 100 ml of 95% EtOH were mixed and allowed to stand for 0.5 hr at 50°. During this time the mixture turned yellow and metallic Ag was precipitated. The solution was decanted and the Ag was washed with a small amount of EtOH. H_2O was added to the combined solutions which were then extracted with petroleum ether (bp 30–60°). The solvent was removed *in vacuo* and H_2O and EtOH were removed by azeotropic distillation with PhH. The intermediate α-tocopherylquinone was purified by column chromatography on silica gel and Florisil using Et_2O and petroleum ether mixtures as solvents. The center-cut material was about 75% of the theoretical and constituted pure α-tocopherylquinone. The solvent was evaporated, the material was redissolved in PhH (100 ml), and excess AcCl was added. After standing overnight at room temperature, the mixture was evaporated again *in vacuo* and the crude chloromethyl derivative was purified by silica gel column chromatography. While the crude yields were essentially quantitative and silica gel tlc showed these products to be reasonably pure, rather high losses were tolerated during chromatographic purification in order to obtain analytically pure materials in later steps. The over-all yield for the above procedure was about 40%.

5-Isothiocarbamidomethyl-6-acetoxy-7,8-dimethyltocol Hydrochloride (9).—The 5-chloromethyl compound **8** (2.27 g) and 350 mg of thiourea were added to 25 ml of EtOH and the mixture refluxed under N_2 for several hours. The solvent was removed *in vacuo* and the product was chromatographed on a silica gel column using a series of solvents starting with petroleum ether and finally removing the product with EtOH. Rechromatography of this center cut of EtOH eluent on thick-layer silica gel GF plates with EtOH gave a pure product showing only one spot on tlc (silica gel GF, EtOH; R_f 0.60–0.67). The EtOH eluent from the thick layer was evaporated and Et_2O was added to the oily product which precipitated on standing. The yield was 500 mg of white amorphous powder (19%): mp 130–135° (poorly defined); λ_{max}^{Nujol} (μ) 3.04, 3.14, 3.27 (NH), 5.69 (CO, acetate), 6.05, 6.21 (aryl), 9.28 (COC, chroman).

5-Piperidinomethyl-7,8-dimethyltocol (10).—The 5-chloromethyl compound (4 g) was warmed (70°) with excess piperidine in dioxane overnight under N_2. A strong KOH solution was added and stirring was continued for several more hours. The mixture was poured into cold H_2O and extracted (Et_2O), and the Et_2O was washed, dried (Na_2SO_4), and evaporated *in vacuo*. The brown oil residue was chromatographed on a silica gel column and the center cut was again chromatographed twice on silica gel thick-layer plates. About 1 g (25%) of an analytical sample was recovered from the plates, which showed one spot on tlc.

5-Methoxymethyl-7,8-dimethyltocol (11).—Na (0.5 g) was dissolved in 25 ml of MeOH and 5 g of **8** was added. The mixture was refluxed for several hours under N_2 and then poured into 100 ml of cold H_2O. It was then extracted (Et_2O), washed, dried, and evaporated *in vacuo*. The brown oily product was chromatographed on a silica gel column with ether–petroleum ether mixtures. The center cut was rechromatographed on Florisil. The center cut from the Florisil column was then chromatographed on thick-layer silica gel GF plates with $CHCl_3$. The analytical sample, 1.5 g (37%), was obtained as a straw-colored viscous oil, which gave only one spot on tlc.

5-Ethylthiomethyl-7,8-dimethyltocol Acetate (12).—Na (0.5 g) was placed in 100 ml of dry PhH containing excess EtSH. After the Na dissolved and only a cloudy precipitate remained, 5 g of **8** was added and the mixture refluxed for 7 hr under N_2. It was then poured into ice–water, the product was extracted (PhH), washed, and dried, and the solvent was removed *in vacuo*. The crude product was chromatographed on silica gel GF unibars using $CHCl_3$ as a solvent. The product was eluted from the silica gel and chromatographed again on silica gel GF thick-layer plates using petroleum ether–$CHCl_3$ mixtures. The analytical sample obtained was a pale yellow, almost colorless viscous oil, which showed only one spot on tlc silica gel GF–$CHCl_3$; yield 2 g (37%).

5-Ethoxymethyl-7,8-dimethyltocol (13) was prepared by the same procedure as that used for **11**. The product showed one spot on silica gel GF–$CHCl_3$.

5-Benzyloxymethyl-7,8-dimethyltocol (14) was prepared by the same procedure as that used for **11** except that some residual benzyl alcohol remained in the crude product. This was easily removed during the chromatographic procedure, however, since it is much more strongly adsorbed. The final product showed only one spot on thin-layer silica gel GF–$CHCl_3$.

5-Morpholinomethyl-7,8-dimethyltocol (15) was prepared as was the 5-piperidinomethyl compound (**10**). The product, a straw-colored viscous oil, gave only one spot on thin-layer silica gel GF–$CHCl_3$.

5-Dimethylaminomethyl-7,8-dimethyltocol (16).—The 5-chloromethyl compound (5 g) was dissolved in 100 ml of PhH and stirred while Me_2NH was bubbled through the cold solution overnight. The temperature was then slowly raised to reflux without interrupting the flow of amine. After a few hours at reflux, the solvent was removed *in vacuo* and the sticky-brown, oily product was chromatographed on a silica GF unibar using $CHCl_3$ as a solvent. Rechromatography on silica gel GF thick-layer plates using petroleum ether–$CHCl_3$ mixtures gave a thick oil which crystallized on standing to a waxy solid, mp 45–46°, yield 1.5 g (32%).

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(8) K. Schwarz, *Vitamins Hormones*, **20**, 463 (1962).
(9) E. L. Hove and P. L. Harris, *J. Nutr.*, **33**, 95 (1947).
(10) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.