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Vitamin E Activity of α-Tocopherol Side Chain Analogs in Selenium-Deficient Chicks[1] (41014)

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Abstract. Three side chain analogs of all-*rac*-α-tocopherol (all-*rac*-α-T) were evaluated for vitamin E activity on the basis of their efficacies in preventing exudative diathesis (ED) and in supporting growth and survival of selenium-deficient chicks. Results showed that 2*RS*,4′*RS*-α-T-C_{11} (lacking one isopentyl unit) and 2*RS*-α-T-nC_{13} (with an unbranched 13-carbon side chain) had the following activities relative to those of all-*rac*-α-T: 69 and 50%, respectively, for supporting growth; 37 and 46%, respectively, for preventing exudative diathesis; 13 and 44%, respectively, for sustaining chick survival. 2*RS*-α-T-C_6 (lacking two isopentyl units) demonstrated no biological activity at the levels fed.

Several different yet related metabolic functions of α-tocopherol (α-T) have been proposed on the basis of the variety of deficiency signs and dietary interactions exhibited in animals (1). In addition to the control of lipid peroxidation, these include roles in respiration and in the metabolism of nucleic acids, ascorbic acid, ubiquinones, and heme. Although the biochemical bases for such functions are not completely understood, each may relate to the activity of α-T as a biological antioxidant.

The vitamin E activities of several analogs of α-T with modifications in and around the chroman ring have been reviewed (2–5). Results of these studies indicate that vitamin E activity depends upon the substituents on the aromatic portion of the chroman ring. Less attention has been given to the role of the hydrocarbon side chain in vitamin E functions. That the saturated isoprenoid side chain of α-T does more than simply "anchor" the molecule in biological membranes is indicated by the relatively low biological activity of 2-*epi*-α-tocopherol (2-*epi*-α-T) (6–11) and the tocotrienols (12, 13). Nevertheless, the exact role of the side chain in conferring biological activity remains unknown.

Early studies (with rats) of modified side chain analogs of all-*rac*-α-T in which the hydrocarbon side chain lacked one (14) or two (15) isopentyl units, had one additional isopentyl unit (16), or was unbranched and contained only 12 carbon atoms (17), showed that changes in the length and branching of the side chain of all-*rac*-α-T markedly affect its biological activity. Although some investigators have reported no significant differences between the biological activities of 2-*ambo*-α-T and all-*rac*-α-T (7, 8, 18, 19), suggesting that the configurations of the 4′ and 8′ carbon atoms are not important for vitamin activity, this conclusion has been questioned (20) and a recent study found a small but significant difference between these two forms (21).

The present study was conducted to evaluate the biological activity of three side chain analogs of all-*rac*-α-T using a very sensitive biological assay—prevention of exudative diathesis (ED), the clinical sign of combined vitamin E and selenium deficiency in the chick.

Materials and Methods. all-*rac*-α-Tocopherol (all-*rac*-α-T) and three side-chain analogs were used in this study (Table I). all-*rac*-α-T and 2*RS*-(4-methylpentyl)-2,5,7,8-tetramethyl-6-hydroxychroman (2*RS*-α-T-C_6) were supplied by Dr. K. Maddy, BASF Wyandotte Corporation, Parsippany, New Jersey. The latter compound was recrystallized from petroleum ether (b.p. 40–60°C) before use. 2*RS*-

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TABLE I. SIDE CHAIN STRUCTURES OF ALL-*rac*-α-TOCOPHEROL AND THREE ANALOGS STUDIED

Notation	Structure[a]
all-*rac*-α-T	R – $(CH_2CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H)_3CH_3$
2*RS*-α-T-C_6	R – $CH_2CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H\ CH_3$
2*RS*,4′*RS*-α-T-C_{11}	R – $(CH_2CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H)_2CH_3$
2*RS*-α-T-nC_{13}	R – $(CH_2)_{12}CH_3$

[a] *R* = 2,5,7,8-tetramethyl-6-hydroxychroman.

(4*RS*,8-dimethylnonyl)-2,5,7,8-tetramethyl-6-hydroxychroman (2*RS*,4′*RS*-α-T-C_{11}) was supplied by Drs. L. Machlin and W. Scott, Hoffmann–LaRoche, Inc., Nutley, New Jersey. 2*RS*-tridecyl-2,5,7,8-tetramethyl-6-hydroxychroman (2*RS*-α-T-nC_{13}) was synthesized[3] and purified by several recrystallizations from petroleum ether. The synthesis involved conversion of myristic acid to 2-pentadecanone using methyllithium. 2-Pentadecanone was reacted with vinylmagnesium bromide to produce 3-methyl-1-hexadecen-3-ol, which was then reacted with trimethylhydroquinone to give 2*RS*-α-T-nC_{13}. Purity was checked by melting point, proton magnetic resonance, and high-pressure liquid chromatography (using a reverse-phase C_{18} column and 2% water in methanol as the mobile phase).

Day-old male vitamin E- and selenium-depleted Single-Comb White Leghorn chicks, produced as described previously (22), were reared to 2 weeks of age in thermostatically controlled battery brooders with raised wire floors. Feed and water were provided *ad libitum*. Chicks were fed a semipurified basal diet based on torula yeast, isolated soya protein, casein, and glucose monohydrate (23). This diet contained less than 25 ppb total selenium by fluorometric analysis (24), and was essentially free of tocopherols, the only source of fat being "stripped" corn oil processed by molecular distillation to remove tocopherols. Chicks were randomly assigned in duplicate lots of 10 chicks each per treatment. Dietary treatments consisted of the basal diet alone or the basal diet supplemented with graded levels of all-*rac*-α-T or of each side-chain analog. Gain in body weight, feed consumption, mortality, and incidence of ED were recorded for individual lots. At 14 days of age blood was obtained from each of four chicks per treatment by anterior cardiac puncture using a heparinized syringe. Plasma was prepared by centrifugation (1000*g* for 10 min); vitamin E activity in plasma was estimated by determination of total fat-soluble reducing substances by a ferric reduction method using bathophenanthroline as the chromagen and all-*rac*-α-T as the standard (25).

All data were evaluated by linear regression analysis using log or log-probit transformations. Treatment means were ranked according to a 5% multiple range test.[4]

Results and Discussion. Chicks reared for 2 weeks using the selenium-deficient diet showed significant ($P < 0.05$) increases in gain, efficiency of feed utilization (feed/gain), prevention of ED, survival, and total fat-soluble reducing substances in plasma in response to dietary supplementation with all-*rac*-α-T (Table II). Of these variables, prevention of ED appeared to be most sensitive to dietary vitamin E concentration ($Y_{ED} = -1.939\,X + 8.937$, $r^2 = -0.866$; $Y_G = 17.844\,X + 9.118$, $r^2 = 0.777$; $Y_D = -1.085\,X + 5.709$, $r^2 = -0.762$; where X = log dietary all-*rac*-α-T, Y_{ED} = probit of incidence of ED, Y_G = gain and Y_D = probit of death rate). Supplementation of the basal diet with 2*RS*-α-T-C_6 did not result in significant ($P > 0.05$) changes in these variables, whereas supplementation with high levels of 2*RS*,4′*RS*-α-T-C_{11} or 2*RS*-α-T-nC_{13} did so (for 2*RS*,4′*RS*-α-T-C_{11}: $Y_{ED} = -0.725\,X + 8.482$, $r^2 = -0.672$; $Y_G = 12.316\,X + 10.238$, $r^2 = 0.733$; $Y_D = -0.140\,X + 5.081$, $r^2 = -0.523$; for 2*RS*-α-T-nC_{13}: $Y_{ED} = -0.884\,X + 8.591$, $r^2 = -0.674$; Y_G =

[3] Details of the synthesis are available upon request.

[4] Statistical Analysis System, SAS Institute, Cary, N.C.

TABLE II. INFLUENCES OF VITAMIN E ANALOGS ON 2-WEEK PERFORMANCE OF SELENIUM-DEFICIENT CHICKS: DIETARY TREATMENT

Source	Level (μmole·kg^{-1})	Gain (g/chick)	Feed/Gain	Exudative diathesis (%)	Mortality (%)	Total reducing substances in plasma (mg·dl^{-1})[a]
None	0	20.8 ± 5.4$^{C\,b,c}$	3.31 ± .60$^{ABC\,b,c}$	100.0 ± 0$^{A\,b,c}$	50.0 ± 10.0$^{AB\,b,c}$	.153 ± .023$^{D\,c,d}$
all-*rac*-α-T[e]	21	22.1 ± 8.4^{C}	3.43 ± 1.02^{AB}	95.0 ± 5.0^{A}	65.0 ± 15.0^{A}	.192 ± .088^{CD}
	63	22.8 ± 4.5^{C}	3.28 ± .36^{ABC}	90.0 ± 0.0^{A}	20.0 ± 10.0^{BC}	.197 ± .042^{D}
	211	53.8 ± 7.1^{B}	2.03 ± .04^{BC}	55.0 ± 15.0^{C}	10.0 ± 10.0^{BC}	.842 ± .033^{B}
	633	73.4 ± 0.5^{A}	1.87 ± .05^{C}	0 ± 0^{D}	0 ± 0^{C}	2.549 ± .140^{A}
2*RS*-α-T-C$_6$[f]	63	21.8 ± 3.7^{C}	2.98 ± .08^{ABC}	100.0 ± 0^{A}	35.0 ± 25.0^{ABC}	.144 ± .015^{D}
	211	21.3 ± 4.7^{C}	3.19 ± .68^{ABC}	100.0 ± 0^{A}	30.0 ± 10.0^{ABC}	.183 ± .016^{D}
	633	31.3 ± 0.1^{C}	2.78 ± .02^{ABC}	95.0 ± 5.0^{A}	40.0 ± 10.0^{ABC}	.163 ± .030^{D}
2*RS*,4′*RS*-α-T-C$_{11}$[g]	63	27.2 ± 1.9^{C}	2.49 ± .04^{BC}	100.0 ± 0^{A}	65.0 ± 5.0^{A}	.188 ± .025^{D}
	211	26.8 ± 6.3^{C}	2.82 ± .41^{ABC}	95.0 ± 5.0^{A}	45.0 ± 5.0^{ABC}	.122 ± .022^{D}
	633	57.2 ± 5.9^{B}	1.93 ± .02^{BC}	70.0 ± 10.0^{B}	30.0 ± 0.0^{ABC}	.189 ± .022^{D}
2*RS*-α-T-nC$_{13}$[h]	63	14.9 ± 0.1^{C}	4.24 ± .24^{A}	100.0 ± 0.0^{A}	65.0 ± 25.0^{A}	.244 ± .034^{D}
	211	27.6 ± 6.0^{C}	2.94 ± .25^{ABC}	95.0 ± 5.0^{A}	15.0 ± 15.0^{BC}	.207 ± .020^{D}
	633	55.0 ± 3.7^{B}	1.97 ± .01^{BC}	45.0 ± 5.0^{C}	15.0 ± 5.0^{BC}	.344 ± .014^{C}

[a] Total fat-soluble reducing substances expressed as *all-rac*-α-tocopherol equivalents.
[b] Mean ± SE for duplicate lots of 10 chicks each.
[c] Means with similar superscripts are not significantly different ($P > 0.05$).
[d] Mean ± SE for four chicks per treatment.
[e] all-*rac*-α-tocopherol; dietary levels used are equivalent to 10, 30, 100, and 300 IU·kg^{-1}.
[f] 2*RS*-(4-methylpentyl)-2,5,7,8-tetramethyl-6-hydroxychroman.
[g] 2*RS*-(4*RS*,8-dimethylnonyl)-2,5,7,8-tetramethyl-6-hydroxychroman.
[h] 2*RS*-tridecyl-2,5,7,8-tetramethyl-6-hydroxychroman.

8.943 X + 14.093, r^2 = 0.593; Y_D = −0.476 X + 5.249, r^2 = −0.435). Comparison of the activities of 2*RS*,4′*RS*-α-T-C_{11} and 2*RS*-α-T-nC_{13} (by the ratios of slopes of the linear regressions) for supporting growth indicated the following activities relative to those of all-*rac*-α-T: 69% for 2*RS*,4′*RS*-α-T-C_{11} and 50% for 2*RS*-α-T-nC_{13}. Such comparisons of the efficacies of those analogs showed that they had about 37 and 46%, respectively, of the activity of all-*rac*-α-T for preventing ED, and about 13 and 44%, respectively, relative activities in sustaining chick survival. Because no significant responses were observed for 2*RS*-α-T-C_6, it is concluded that this analog is not biologically active, or that its activity is lower than the limits of detection when fed at levels up to 633 μmole·kg^{-1}. The dietary concentration of all-*rac*-α-T that produced 50% ED was 107 μmole·kg^{-1}. This parameter was clearly much greater for each analog; however, those calculations have questionable validity as they are extrapolations from the present data. In spite of the positive responses to 2*RS*,4′*RS*-α-T-C_{11} and 2*RS*-α-T-nC_{13} in the chick, only the highest level (633 μmole·kg^{-1}) of the latter analog produced a significant ($P < 0.05$) increase in total fat-soluble reducing substances in chick plasma.

It has been suggested that the long, branched hydrocarbon side chain of α-T is necessary for its vitamin E activity (26, 27). If this is true, then α-T analogs with shorter or unbranched side chains would be expected to have less vitamin E activity than all-*rac*-α-T. The data presented here are consistent with that hypothesis. Removal of one isopentyl unit (2*RS*,4′*RS*-α-T-C_{11}) reduced vitamin E activity by approximately 60% (based on the average of the three responses measured); removal of two isopentyl units (2*RS*-α-T-C_6) reduced vitamin E activity to an undetectably low level. An analog with an unbranched side chain (2*RS*-α-T-nC_{13}) was about 47% as active as all-*rac*-α-T. These results are consistent with previous reports of diminished activities of 2*RS*-α-T-C_6, 2*RS*,4′*RS*-α-T-C_{11} and 2 *RS*-α-T-nC_{12} in rat fetal resorption tests (14, 15, 17), and of 2*RS*-α-T-C_6 and 2*RS*,4′*RS*-α-T-C_{11} in erythrocyte hemolysis tests (28). It is therefore clear that both the length and the branching of the side chain of α-T are important for vitamin E activity.

Reduced vitamin E activities of these side-chain analogs may be due to their impaired absorption and transport, rather than to their impaired metabolic function, as each has the same chroman structure to participate directly in chemical antioxidant activities. The reduced concentrations of total fat-soluble reducing substances in plasma, as determined using a method which measures chemical antioxidant activity without discriminating between tocopherols, support this hypothesis. That 2*RS*,4′*RS*-α-T-C_{11} and low levels of 2*RS*-α-T-nC_{13} were biologically effective without producing increased total fat-soluble reducing substance in plasma indicates that the metabolic functions of these analogs are important in tissues other than plasma.

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