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VITAMIN E ACTIVITY OF 1-THIO-α-TOCOPHEROL AS MEASURED BY THE RAT CURATIVE MYOPATHY BIOASSAY

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The bioactivity of the acetate of the all-racemic, 1-thio analog of α-tocopherol (*all-rac*-1-thio-α-tocopheryl acetate) has been determined by measuring its ability to decrease plasma levels of pyruvate kinase in vitamin E deficient rats using the curative myopathy bioassay. The thio analog is only 0.22 times as active as *RRR*-α-tocopheryl acetate and is therefore approximately 0.33 times as active as *all-rac*-α-tocopheryl acetate, since the latter has been shown to be 1.47 times less active than *RRR*-α-tocopheryl acetate in the same bioassay (H. Weiser, M. Vecchi and M. Schlachter, *Internat. J. Vit. Nutr. Res.*, **55**, 149–158 (1985)). The 0.33:1.0 ratio is similar to the ratio of 0.41:1.0 measured for the *in vitro* antioxidant activities of the corresponding free phenols. This finding lends further support to our view that the vitamin E activity in the curative myopathy bioassay of close structural analogs of α-tocopherol is determined primarily by the *in vitro* antioxidant activity of the analog relative to α-tocopherol, consistent with the belief that vitamin E functions primarily as a general purpose, lipid-soluble antioxidant in mammals.

KEY WORDS: Vitamin E, antioxidant, 1-thio-α-tocopherol, pyruvate kinase, curative myopathy assay.

INTRODUCTION

In a number of publications we have made use of the rat curative myopathy bioassay to determine the vitamin E activity of some analogs of α-tocopherol, α-**T**.[1–3] We discovered that a dihydrobenzofuran analog (with one less CH_2 group in the heterocyclic ring) is more active than α-**T**[1,2] and that analogs having a non-branched, saturated hydrocarbon "tail" with 11 or 13 carbon atoms are about as active as α-**T**.[3] We have taken these results as providing strong supporting evidence for the prevailing view[3,4] that α-**T** and related phenols (ArOH) owe their biological activity simply and entirely to their ability to inhibit lipid peroxidation via reaction 1. This conclusion would be

$$ROO\cdot + ArOH \xrightarrow{k_1} ROOH + ArO\cdot \qquad (1)$$

further strengthened if it could be demonstrated that vitamin E activity was retained in α-**T** analogs in which the oxygen at the 1-position had been replaced by a different heteroatom. The need to maintain a reasonably good antioxidant activity in such an α-**T** analog (i.e. a k_1 value not too much smaller than that found for α-**T**)[5–8] requires that the new heteroatom have, like oxygen, a *p*-type lone pair of electrons. This will

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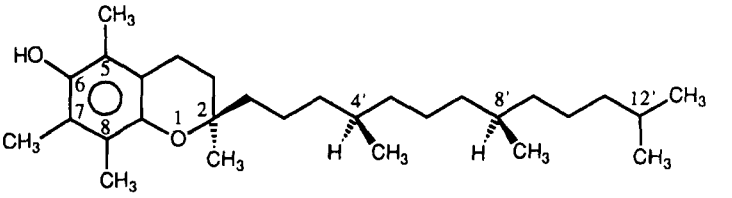
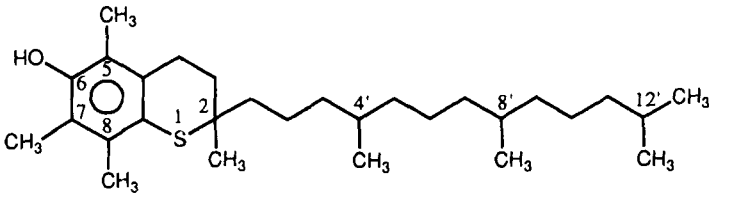
allow the unpaired electron in the ArO· radical to be delocalized onto the heteroatom which provides thermodynamic stabilization to the radical and hence an acceptably high k_1 value.[5-8] The logical replacement heteroatom is sulfur, particularly since sulfur is generally considered to be more effective than oxygen at stabilizing a neighbouring radical center.[9-13] With this in mind we synthesized 2*RS*, 4′*RS*, 8′*RS*-1-thio-α-tocopherol, *all-rac*-**1**.[14,15]



2*R*,4′*R*,8′*R*-α-tocopherol (natural vitamin E), *RRR*-α-**T**



2*RS*,4′*RS*,8′*RS*-1-thio-α-tocopherol, *all-rac*-**1**

An *in vitro* study of the antioxidant activity of *all-rac*-**1** was disappointing in that this compound was found to be a less effective antioxidant than α-**T**.[15] The stoichiometric factor, n (i.e., the number of peroxyl radicals trapped per molecule of ArOH), was between 1.0 and 1.8 for **1** whereas it is 2.0 for α-**T**.[15] Furthermore, the rate constant, k_1, for trapping of the initial peroxyl radical was smaller for **1** than for α-**T**. The effective antioxidant activity of an ArOH is determined by the product of these two quantities, i.e., by nk_1. For **1**, $nk_1 = 2.6 \times 10^6 M^{-1}s^{-1}$ and for α-**T**, $nk_1 = 6.4 \times 10^6 M^{-1}s^{-1}$.[15] Thus, **1** is only ca. 40% as active an antioxidant as α-**T**. Despite this disappointing result we decided to check the vitamin E activity of *all-rac*-**1** using the rat curative myopathy bioassay.[1-3,16]

MATERIALS AND METHODS

All-rac-**1** was synthesized as described previously,[14,15] and was converted to the acetate for bioassaying. The rat curative myopathy bioassay, which is based on the decrease of the highly elevated pyruvate kinase (PK) activities of vitamin E-deficient rats, was conducted essentially as described previously.[1-3,16] In brief, male weanling (21–22 days old) Sprague Dawley rats were provided with tap water and a vitamin E-free diet *ad libitum*. The diet was based on the AIN 76 formulation[17] but contained 10% tocopherol-stripped corn oil (with 0.02% BHT), a menadione concentration of 500 μg/kg diet, sucrose decreased to 20%, the starch increased to 30% and 10% dyetrose (a

selectively depolymerized corn starch, which permitted the diet to be pelleted). This diet, which was obtained from Dyets Inc., Bethlehem, PA, was fed for 16 weeks prior to use of the animals in the vitamin E bioassay.

Two bioassays were performed. Each bioassay normally employed 36 rats, each receiving one of three doses of either *all-rac*-**1** or *RRR*-α-**T** as their acetates (i.e., 18 rats per compound and 6 per dose level) daily for 4 days. On day 1, before dosing, and on day 5 (23–24 h after the last dose) blood (0.5–1.0 mL) was obtained by cardiac puncture under brief halothane anesthesia (5% in O_2, 1–2 min), mixed with Na_2EDTA (1 mg/mL), chilled on ice and centrifuged at 8,000 × *g* for 1 min to sediment cells. The plasma was retained and stored on ice until assayed (within 1 to 1.5 h) for PK activity, essentially as described by Gutman and Bernt.[18] The rats were ranked in order of initial (day 1) plasma PK and divided sequentially into 6 groups. The 6 rats in each group were than randomly assigned to receive one of the three doses of either test compound. The test compounds were dissolved in tocopherol-stripped corn oil and administered *per os* (250 μL/kg body weight) with a positive displacement pipette. After blood sampling on day 5 the animals were killed by exposure to gaseous CO_2.

For each bioassay the linear regression of plasma PK activity (units/mL) vs ln (dose) of test compound was computed by the method of least squares and, provided that parallelism of the dose response lines was not statistically rejected by analysis of variance, a common slope was calculated and applied to the regressions. The ratio of potencies of *all-rac*-**1** acetate and *RRR*-α-**T** acetate was computed from the horizontal displacement of the two lines.

RESULTS AND DISCUSSION

Two separate bioassay experiments were conducted. They yielded potency ratios for *all-rac*-**1** acetate to *RRR*-α-**T** acetate equal to 0.23:1.0 and 0.22:1.0, respectively (both

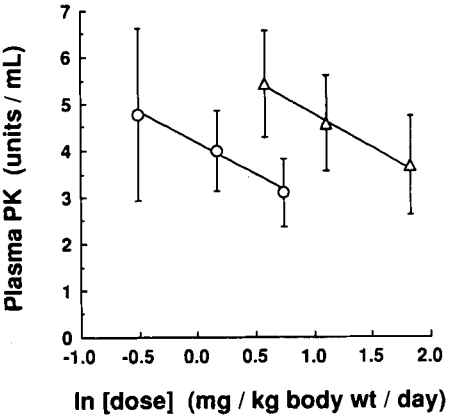


FIGURE 1 Dose-dependence of the decrease in plasma pyruvate kinase (PK) levels measured on day 5 in vitamin E deficient rats after administration of either *RRR*-α-**T**-Ac (○) or *all-rac*-**1**-Ac (△) given once per day for four consecutive days. Each data point is the mean of data from six animals. The potency ratio for *all-rac*-**1** acetate to *RRR*-α-**T** acetate in this experiment was 0.23:1.0 ($p < 0.01$). A duplicate experiment gave a ratio of 0.22:1.0 ($p < 0.01$). The baseline PK level in vitamin E-sufficient rats is about 0.2 units mL^{-1}.[1]

with $p < 0.01$). The results of one of the bioassays are shown in Figure 1. Because of the greater bioactivity of the 2*R* stereoisomer relative to the 2*S* stereoisomer of α-**T**[16,19–25] and the dihydrobenzofuran analog[2] it is probably more appropriate to compare the activity of *all-rac*-**1**-acetate with that of *all-rac*-α-**T** acetate. This is easily done since Weiser *et al.*[26] have employed the rat curative myopathy bioassay to measure the potency ratio for *RRR*-α-**T** acetate vs. *all-rac*-α-**T** acetate for which they obtained a potency ratio of 1.47:1.0. Multiplying the mean *all-rac*-**1** acetate: *RRR*-α-**T** acetate potency ratio of 0.22:1.0 by 1.47 yields the potency ratio for *all-rac*-**1**-acetate:all-rac-α-**T** acetate, viz. 0.33:1.0. This potency ratio is in good agreement with the ratio of the *in vitro* antioxidant activities of **1**:α-**T**, *viz.*,[15] 0.40:1.0. This result lends further support to our view,[1–3] that the vitamin E activity (as measured by the rat curative myopathy bioassay) of close structural analogs of α-**T** is determined primarily by the antioxidant activity of the analog relative to α-**T**.

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