Improving the Yield of α-Tocopherol from Natural Sources—Chemistry versus Molecular Biology

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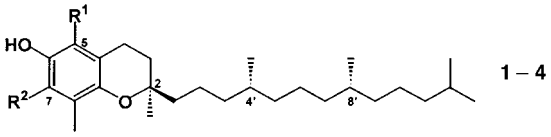
The tocopherols **1**–**4** and the corresponding tocotrienols which can be isolated from photosynthetic organisms are structurally related derivatives of chroman-6-ol displaying vitamin E activity (Table 1).[1] Although the significance of



1 – **4**

Table 1. Comparison of the relative biological activities A_{rel} of tocopherols **1**–**4**.

Compound	R^1	R^2	A_{rel} [%]
α-tocopherol (**1**)	CH_3	CH_3	100
β-tocopherol (**2**)	CH_3	H	50
γ-tocopherol (**3**)	H	CH_3	10
δ-tocopherol (**4**)	H	H	3

these compounds for mammalian nutrition has been known for almost 80 years, our understanding of the basic principle of action of these compounds has only recently evolved. According to Burton and Ingold[2] α-tocopherol (**1**), the biologically most active member of the vitamin E group, is an important lipophilic radical chain breaking antioxidant in living tissues. Substructures such as the lipophilic side chain at C2 allow **1** to be easily incorporated into bilayer membranes and, hence, protect cell walls against oxidative damage.

Since the chirality and the three ring methyl groups of **1** are essential to maximal biological activity,[3] **1** became a target of many synthetic approaches; the *R* configuration at C2 was the most difficult to establish. Though some of these procedures[4] are quite elegant and even efficient, they never became economically competitive with the synthesis of racemic α-tocopherol,[5] which covers about 90 % of the worldwide need of over 22500 t per year,[6] or with isolation of the chiral tocopherols from natural sources such as oil extracts from sun flowers, soya, palm, and wheat. From these plants, however, the tocopherols **1**–**4** are obtained in mixtures of different compositions,[1] posing the principal problem of converting these mixtures in favor of the desired **1**. Two interesting solutions to this problem have been published recently.

The "metabolic engineering" approach of DellaPenna et al.[7] takes the following points into consideration:

a) The methylation of γ-tocopherol (**3**) is the last step in the biosynthesis of α-tocopherol (**1**).[8]
b) The γ-tocopherol methyltransferase (γ-TMT) has been already purified from *Capsicum annuum*, and its primary structure elucidated by Camara et al.[9]
c) Organisms in which γ-TMT could be overexpressed would convert the internal pool of **3**—often the most abundant vitamin E component—into the desired **1**.

Since related biosynthetic genes in bacteria are often organized into operons, DellaPenna et al. speculated that the γ-TMT gene might be located on the operon containing the gene that encodes for *p*-hydroxy-phenylpyruvate dioxygenase (HPPDase).[10] This enzyme catalyzes an important step in the catabolism of aromatic amino acids, that is, the formation of homogentisinic acid, inter alia the precursor of the monomethylated aromatic ring of the vitamin E family.

Two complementary organisms were chosen for the experiments: the cyanobacterium *Synechocystis* PCC6803 and the plant *Arabidopsis thaliana* because the tocopherol pool of the photosynthetic tissues of both species contain greater than 95 % of **1**; however, only about 1 % of **1** is found in the seeds of *Arabidopsis thaliana*. This suggests that γ-TMT activity is not limited in the former parts but is virtually absent in the seeds, which are the major vitamin E source for human nutrition.

Based on sequence data from Camara et al.[9] and the known gene encoding the *Arabidopsis* HPPDase,[10] DellaPenna et al. characterized the γ-TMT genes both in *Synechocystis* PCC6803 and *Arabidopsis thaliana* by construction of a "null mutant" in *Synechocystis* and functional expression of both genes in *E. coli*. The recombinant wild-type γ-TMT proteins catalyzed the transformation of **3** to **1**, whereas the null mutant was devoid of methyltransferase activity. When *Arabidopsis* was transformed with a vector containing its own γ-TMT gene under the control of a seed-specific promotor from carrot, its seeds produced 95 % of α-tocopherol (**1**), 1 % of β-tocopherol (**2**), and 4 % of γ-tocopherol (**3**). This indicated that most of the internal γ- and δ-tocopherols had been methylated; that is, the enzyme γ-TMT catalyzes regiospecifically the methylation at C5 of the chromanol

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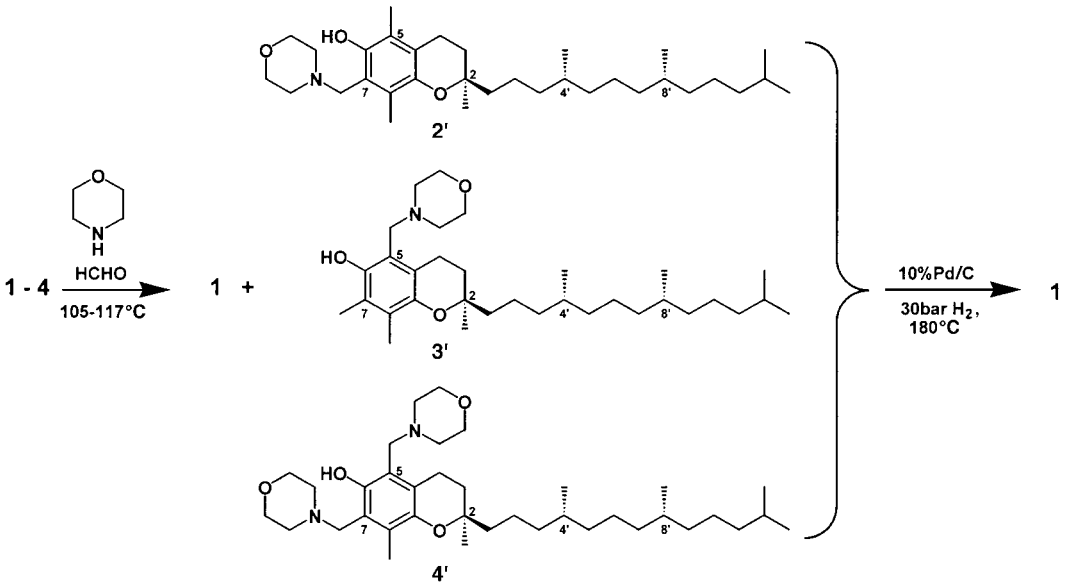
substructure. Given the relative biological activities of the different tocopherols (Table 1) the seeds from *Arabidopsis* lines overexpressing γ-TMT are ninefold more potent than the wild type; the overall vitamin E content, however, remained unchanged.

The aim of the "chemical approach" was to convert any naturally occurring mixture of **1**–**4** into the biologically most significant α-tocopherol (**1**). To accomplish this target Müller and Schneider[11] systematically investigated the Mannich reaction and subsequent catalytic reduction of the intermediate benzylamines. Optimal results were obtained when a mixture of the tocopherols **1**–**4** was heated neat with the Mannich reagent, prepared from morpholin and paraformaldehyde (1:1), and the resulting aminomethylated tocopherols **2′**, **3′**, and **4′** were directly reduced on 10% Pd/C with H_2. The tocopherol yield was nearly quantitative, and the GC analysis revealed the following composition: 98% of **1**, 0.8% of **2**, and 1.2% of **3**. Because any given mixture of natural occurring tocopherols can be converted into **1** in high yield and on a kilogram scale, at present the chemical approach exceeds the method of DellaPenna et al. Both concepts, however, are handicapped with regard to the low level of vitamin E in photosynthetic organisms.



The approach of DellaPenna et al. seemingly opens the door to transgenic plants overproducing **1**. It may be naive, however, to believe this can be accomplished soon since the deregulation of quite a number of enzymatic steps would be required. After all DellaPenna et al. have only modified the last, most simple step of the tocopherol biosynthesis, which is unrelated to the regulation of preceeding enzymatic reactions. Moreover it is quite obscure why his approach was succesful because it seems quite unlikely that functionally and structurally unrelated enzymes such as HPPDase and γTMT should be located on the same operon: His interesting result is a surprise!

To elevate the content of tocopherols in any organism clearly the focus must be shifted to enzymes that occur earlier in the pathway of the tocopherol biosynthesis. Generating a plant that produces a scarce lipophilic metabolite in economically useful concentrations probably needs a joint, long-term effort of plant biologists, chemists, and molecular biologists to investigate every step carefully and define the consequences of overexpression of the individual genes on the plant's life cycle. After all a combination of chemistry and molecular biology approaches might be successful.

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