Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 237–239

TETRAHEDRON LETTERS

Efficient two-step synthesis of methylphytylbenzoquinones: precursor intermediates in the biosynthesis of vitamin E

Satyamaheshwar Peddibhotla,[a] Zigang Cheng,[b] Dean DellaPenna[b] and Jetze J. Tepe[a,*]

[a]*Department of Chemistry, Michigan State University, East Lansing, MI 48824, USA*
[b]*Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA*

Received 16 October 2002; revised 9 November 2002; accepted 13 November 2002

Abstract—Methylphytylbenzoquinone was synthesized from δ-tocopherol by a simple two-step sequence. Oxidative cleavage of the benzopyran ring with cerium sulfate followed by dehydration using Burgess reagent afforded the methylphytylbenzoquinone as a mixture of positional and geometric isomers which were separated by HPLC. The biological activity of the product corresponds to the natural biosynthetic precursor of vitamin E. The above method is a general procedure applicable to the preparation of any of the tocopherol derivatives. © 2002 Elsevier Science Ltd. All rights reserved.

Tocopherols are a class of lipid-soluble antioxidants synthesized in higher plants and other oxygenic photosynthetic organisms such as cyanobacteria.[1] The four tocopherols synthesized (α, β, δ, and γ-tocopherol) differ only in the number and position of methyl groups on the chroman head group. Tocopherols are important in limiting lipid peroxidation in a variety of organisms and are an essential component of mammalian diets as vitamin E (2*R*,4′*R*,8′*R*-[α]-tocopherol).[2,3] The production of tocopherols for animal nutrition and various industrial purposes has sparked renewed interest in the synthesis and enzymology of the tocopherol biosynthetic enzymes.[2,4–6] Although the tocopherol pathway has been studied for many years,[7] it is only recently that pathway enzymes have been cloned, providing new opportunities to study the substrate specificity and kinetics of the reactions.[5,6,8,9]

The pathway leading to the synthesis of α-tocopherol is shown in Fig. 1. The committed intermediate in the tocopherol biosynthetic pathway is 2-methyl-6-phytyl-1,4-benzoquinone (MPBQ), the product of the condensation of the aromatic compound homogentisate (HGA) and the 20-carbon isoprenoid derived compound, phytyl pyrophosphate.[9,10] MPBQ methyltransferase catalyzes the first methylation reaction in tocopherol synthesis and the methylation of the same ring position of a related compound in plastoquinone synthesis.[11,12]

Access to a variety of methylated phytylbenzoquinone precursors and intermediates is required in order to

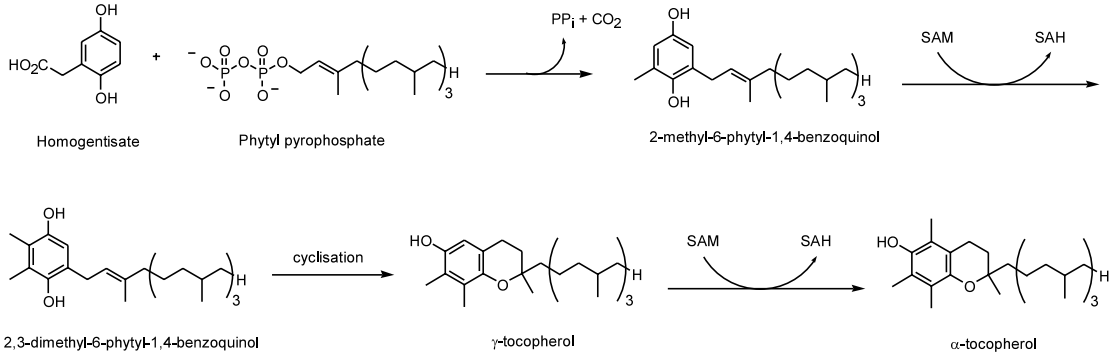


Figure 1. Biosynthesis of α-tocopherol.

* Corresponding author. Tel.: (517) 355-9715; fax: (517) 353-1793; e-mail: tepe@cem.msu.edu

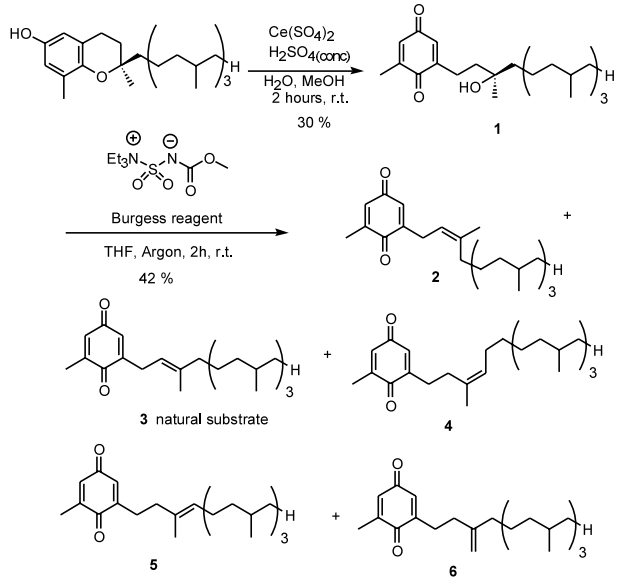
PII: S0040-4039(02)02564-9

elucidate the regulation and the activity of MPBQ/MSBQ methyltransferase.[11] Previously reported methods provide some of these substrates in relatively low yields and as multiple isomers.[12–14] The development of a short and efficient synthetic method applicable to generating a wide range of these substrates has therefore become an integral part of elucidation of the biosynthetic pathway. We report herein an efficient two-step synthesis of methylphytylbenzoquinones (MPBQs) to study the kinetics and substrate specificity of MPBQ methyltransferase.

The earlier reported chemical synthesis of MPBQ involved oxidation of commercially available methylated phenols to methyl quinones followed by coupling of the phytyl side chain in the presence of $BF_3{\cdot}OEt_2$ resulting in a mixture of positional isomers.[10] Oxidation of the resulting quinols with Ag_2O provided a mixture of isomers, which were separated by repeated thin-layer chromatography.

Unfortunately, due to the limited availability of the required isophytol side chain, the generation of wide mixtures of isomers, and corresponding low yielding reactions, this procedure was quite limited as a routine method for the large scale preparation of the many different tocopherol precursors. We report here an alternative preparation of MPBQ derivatives from the naturally available tocopherols to provide more efficient access to these biosynthetic precursors.

Oxidative cleavage of a chroman ring with cerium sulfate affords the quinone alcohol.[15] The well established $FeCl_3$ oxidation as described by Cohen has also successfully been used in the conversion of α-tocopherol to α-tocopheroquinones.[16] Cerium sulfate oxidation of δ-tocopherol resulted in a mixture of products from which the quinone alcohol **1** was readily isolated in 30% yield at multigram scale. The quinone alcohol **1** was subjected to dehydration with Burgess reagent under argon atmosphere to afford a mixture of methylphytylquinones **2**–**6** (Scheme 1).[17,18]



Scheme 1. Synthesis of methylphytylbenzoquinone.

The positional and geometric isomers **2**–**6** were isolated by flash chromatography, followed by two rounds of HPLC purification using a silica column with hexane/isopropyl ether (992:8) as the eluant. Compounds **2**–**6** were isolated in a relative ratio of 1:2:1.1:1.5:2.2. The positional and geometric isomers from the dehydration will be further used as competitive inhibitors for evaluating the reaction mechanism and kinetic parameters of the MPBQ methyltransferase.[11]

Purification of MPBQ and its isomers: The mixture of isomeric methylphytylbenzoquinones was injected on a ReliaSil Silica, 5 μm, 250×4.6 mm normal phase column (Column Engineering, Ontario, CA) with a guard column containing the same matrix. Chromatography was performed at 30°C with a mobile phase of 992:8 (v/v) hexane:isopropyl ether flowing at 1.0 mL/min. For large-scale purification, a preparative column of the same matrix (Column Engineering, Ontario, CA) was used. The collected fractions were dried down under vacuum before a small portion was injected onto the analytical column to monitor the purity. The natural substrate **3** was eluted as the second HPLC peak.[19]

Enzyme Assay: Assays of MPBQ methyltransferase activity were carried out in a 100 μL reaction containing 10 μL of solubilized *E. coli* extract (50 and 75 μg protein) expressing the MPBQ methyltransferase of Synechocystis PCC6803,[11] 50 mM Tris pH 8.0, 10 mM DTT, 80 μM [^{14}C-methyl]*S*-adenosylmethionine (56 mCi/mmol), 40 μM unlabeled *S*-adenosylmethioine, and 100 μM each of the individual MPBQ compounds **2**–**6**. After incubation at 30°C for 2 h, the reactions were stopped by the addition of 200 μL of 0.9% (w:v) NaCl, extracted with 450 μL of 2:1 (v:v) methanol:chloroform, centrifuged, and the organic phase collected and dried under a stream of nitrogen gas. The resulting residue was resuspended in hexane and fractionated on 250 μ 60A KF6 silica gel TLC plates (Whatman, Clifton, NJ, USA) developed with

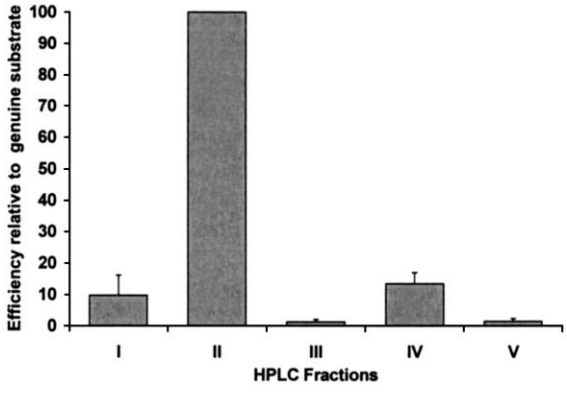


Figure 2. Efficacy of isomers **2**–**6** compared to natural MPBQ as substrates for methyltransferase activity. The percent efficacy of the isomers was based on the average of three independent assays.

3:7 (v:v) diethylether:petroleum ether. The TLC plates were exposed on a Molecular Dynamics low energy phosphor screen (Amersham) to determine the incorporation of ^{14}C into the products.

Interestingly, MPBQ methyltransferase was found to utilize all five MPBQ isomers (**2**–**6**), however the activity with the genuine substrate **3** (shown as fraction II in Fig. 2) results in specific activity that is 10–100 times higher than those of its isomers. The method described in this report has also been successfully applied to large-scale preparation of phytylquinone precursors of α-tocopherol (data not shown) and is applicable to a wide range of methylphytylbenzoquinone precursors.

References

1. Kamal-Eldin, A.; Appelqvist, L. A. *Lipids* **1996**, *31*, 671–701.
2. Fryer, M. J. *Plant Cell Environ*. **1992**, *15*, 381–392.
3. Topinka, J.; Binkova, B.; Sram, R. J.; Erin, A. N. *Mutat. Res*. **1989**, *225*, 131–136.
4. Weber, P.; Bendich, A.; Machlin, L. J. *Nutrition* **1997**, *13*, 450–460.
5. Grusak, M. A.; DellaPenna, D. *Annu. Rev. Physiol. Plant Mol. Biol*. **1999**, *50*, 133–161.
6. DellaPenna, D. *Science* **1999**, *285*, 375–379.
7. Trelfall, D. R.; Whistance, G. R. *Aspects of Terpenoid Chemistry and Biochemistry*; Academic Press: London, 1971.
8. Garcia, I.; Rodgers, M.; Lenne, C.; Rolland, A.; Sailland, A.; Matringe, M. *Biochem. J*. **1997**, *325*, 761–769.
9. Shintani, D.; DellaPenna, D. *Science* **1998**, *282*, 2098–2100.
10. Soll, J.; Schultz, G. *Phytochemistry* **1980**, *19*, 215–218.
11. Shintani, D. K.; Cheng, Z.; DellaPenna, D. *FEBS Lett*. **2002**, *511*, 1–5.
12. Chenevert, R.; Courchesne, G. *Tetrahedron Lett*. **2002**, *43*, 7971–7973.
13. Soll, J. *Methods Enzymol*. **1987**, *248*, 383–387.
14. Henry, A.; Powls, R.; Pennock, J. F. *Biochem. J*. **1987**, *242*, 367–373.
15. Cohen, N.; Lopresti, R. J.; Saucy, G. *J. Am. Chem. Soc*. **1979**, *101*, 6710–6716.
16. Cohen, N.; Lopresti, R. J.; Neukom, C. *J. Org. Chem*. **1981**, *46*, 2445–2450.
17. Burgess, E. M.; Penton, H. R. J.; Taylor, E. A. *J. Org. Chem*. **1973**, *38*, 26–31.
18. **Procedure for the dehydration of methylphytyl benzoquinol 1:** The quinone alcohol **1** (0.4 g, 0.9 mmol) was weighed into a flame dried flask under argon atmosphere and dry THF (50 mL) was added. To this solution was added Burgess reagent (0.4 g, 1.8 mmol) and the mixture stirred at room temperature overnight. The reaction mixture was then diluted with ether (200 mL) and extracted twice with water and the organic layer was dried over anhydrous sodium sulfate and the ether layer was evaporated to obtain a yellow residue. The residue was flash chromatographed on silica-gel (5% ether:hexane) to obtain the methylphytylbenzoquinone as a mixture of five isomers (0.16 g, 42%) They were further separated by HPLC.
19. **Spectral data: 3;** ^1H NMR (500 MHz, acetone-d_6) δ 6.6 (1H, td, $J_1=1.5$ Hz, $J_2=4.5$ Hz), 6.46 (1H, td, $J_1=1.5$ Hz, $J_2=3.5$ Hz), 5.22 (1H, qt, $J_1=1.5$ Hz, $J_2=7.5$ Hz), 3.14 (2H, d, $J=7.5$ Hz), 2.06–2.07 (3H, m), 2.02 (2H, d, $J=1.5$ Hz), 1.66 (3H, s), 1.0–1.6 (18H, m), 0.85–0.9 (12H, m); ^{13}C NMR (125 MHz, acetone-d_6) δ 187.61, 148.56, 146.07, 139.55, 133.01, 132.04, 118.87, 39.85, 39.41, 37.43, 37.42, 37.31, 36.56, 32.81, 32.66, 28.82, 28.67, 27.99, 27.48, 25.25, 24.8, 24.43, 22.29, 22.2, 19.4, 15.36, 15.15; IR; IR (neat): 1670 cm^{-1}, 1600 cm^{-1}. MS (EI): calculated for $C_{27}H_{44}O_2$ $[M]^+$ 400.64, found $[M]^+$ 400.33.