“Vitamin CE,” a Novel Prodrug Form of Vitamin E

Thomas ROSENAU and Wolf Dieter HABICHER*

Dresden University of Technology, Institute of Organic Chemistry, Mommsenstr. 13, D-01062 Dresden, Germany.
Received November 11, 1996; accepted February 7, 1997

Reaction of 5a-bromo-α-tocopherol with ascorbic acid produces 5a-tocopheryl ascorbate which is designated “vitamin CE.” This novel tocopherol derivative represents an interesting prodrug form of α-tocopherol (vitamin E) that is stable under acidic conditions, but regenerates finely dispersed vitamin E in basic media. The reaction mechanism of the base-induced decomposition of vitamin CE involves elimination of ascorbate and production of an ortho-quinone methide intermediate that oxidizes ascorbate, and is reduced to vitamin E. Kinetic experiments showed the reaction to proceed in the pH range of 8 to 11 under physiological conditions. Tissue culture measurements demonstrated that vitamin E generated from the novel derivative is absorbed at much higher rates than conventional preparations and can even be absorbed under simulated conditions of malabsorption where there is no uptake of conventional vitamin E medications.

Key words 5a-tocopheryl ascorbate; vitamin E; α-tocopherol; ascorbic acid; prodrug; 5a-substituted tocopherol

5a-Bromo-α-tocopherol (1) is obtained in quantitative yield by bromination of α-tocopherol with elemental bromine. The compound is a versatile starting material for the preparation of 5a-substituted tocopherols and can be used as an auxiliary in synthesis, a starting material for new tocopherol compounds, and as a lipophilic drug carrier. In the latter case, it renders the attached drug more lipophilic and, thus, more easily transportable into lipidic tissues, such as membranes.

The reaction of 1 with ascorbic acid (vitamin C, 2) produces 5a-tocopheryl ascorbate (3) which is conveniently named “vitamin CE” (Fig. 1). The preparation of 3 must be carried out under non-basic conditions. Basic media lead to elimination of HBr from the starting material, producing the intermediate ortho-quinone methide (4) that undergoes subsequent reactions, either a hetero-Diels–Alder reaction to form the spiro-dimer of α-tocopherol (5) or a rearrangement to para-tocopheryl quinone (6). Consequently, basic auxiliaries as well as the application of pure sodium ascorbate as the co-reactant have to be avoided.

In the optimized experimental procedure, a mixture of sodium ascorbate with a tenfold excess of ascorbic acid was used to ensure the absence of basic conditions while maintaining a high reactivity. The reaction proceeds with moderate yields in any polar aprotic organic solvent. However, only dimethyl sulfoxide (DMSO) allows a virtually quantitative conversion of the reactants to the product. This behavior can be attributed to the fact that all starting compounds are soluble in DMSO causing a ready Williamson-type reaction, whereas in the case of other polar aprotic solvents at least the ascorbate remains insoluble causing a much slower two-phase reaction with a higher rate of the formation of the above-mentioned byproducts. The product 3, a yellowish wax like α-tocopherol itself, is stable under acidic conditions and remains unaffected even by extended refluxing with 1 N mineral acids. In contrast, basic treatment of 3 leads to immediate deprotonation at the phenolic hydroxyl group followed by elimination of the ascorbate anion and formation of the ortho-quinone methide of α-tocopherol (4). This reaction path is typical of 5a-substituted tocopherols. The intermediacy of 4 has been demonstrated by trapping reactions analogous to those previously reported. In the case of 5a-tocopheryl ascorbate (3), however, the separated moieties of ascorbate and orthoquinone methide join in a redox process in which ascorbate is oxidized to dehydroascorbate while the orthoquinone methide (4) is reduced to α-tocopherol (see Fig. 2), or to the α-tocopherolate anion at a higher pH value. The yield of this reaction, usually above 85%, can be increased by additional ascorbic acid as would be expected from the reaction mechanism. One additional equivalent of ascorbic acid, for instance, allows a 96% reconversion of α-tocopherol.

The regenerated α-tocopherol is produced in a finely

---

Fig. 1. Preparation of 5a-Tocopheryl Ascorbate (3)

* To whom correspondence should be addressed.

© 1997 Pharmaceutical Society of Japan
dispersed form, which can be observed either phenomenologically or by absorption experiments as described below. The byproducts of the regeneration of vitamin E, mainly para-tocopherol quinone (6) and trace amounts of the spiro-dimer of α-tocopherol (5), are both natural metabolites of vitamin E and fully biologically compatible. Before testing the compound in cell culture experiments, the kinetics of the base-induced decomposition of vitamin CE to α-tocopherol, i.e., the drug release from the prodrug form, was investigated. The dependence of the turnover on the pH value at different reaction times is illustrated in Fig. 3. The pH value required for the reaction to proceed corresponds with the basicity in the intestine, a prerequisite for the use of vitamin CE in cell culture or in in vivo experiments. Vitamin CE (3) becomes an illustrative example of the concept of pH-dependent drug release.

Many of the factors and processes necessary for the absorption of dietary lipids are also required for the absorption of tocopherol. Tocopherol must be emulsified and solubilized before its absorption across the brush-border membrane of the intestinal cells. Emulsification begins in the stomach by mechanical forces that break up large particles into smaller ones. Within the small intestine, chyme is mixed with pancreatic and biliary secretions, which are necessary for the efficient absorption of vitamin E. Pancreatic lipase is required for the hydrolysis of triglycerides to monoglycerides and fatty acids. These lipolytic products—together with bile salts that are produced by the liver and secreted into the small intestine by the gallbladder—form molecular aggregates, so-called mixed micelles. These micelles, with a radius of 15–50 Å, are able to turn in solubilize hydrophobic molecules, such as tocopherol, and are absorbed through the cell membranes by passive diffusion. Consequently, tocopherol can only be absorbed if both the biliary and the pancreatic factors are present in sufficient amounts.  

α-Tocopherol derived from vitamin CE is produced exclusively extracellularly. Once the vitamin E has been generated, it is absorbed in the above-described manner exactly as genuine α-tocopherol. The extracellular decomposition of 3 to vitamin E and ascorbate is proved by the fact that the plasma tocopherol content increases through absorption, whereas the plasma ascorbate/dehy-
The tocopherol level remains unchanged. In contrast to natural \( \alpha \)-tocopherol which must be thoroughly emulsified before forming mixed micelles and being absorbed, vitamin E generated from the new produg form 3 is produced by a chemical reaction as a fine dispersion. Thus, formation of micelles with subsequent absorption is greatly facilitated. It is even conceivable that this thoroughly dispersed form of \( \alpha \)-tocopherol can be absorbed directly without prior formation of mixed micelles, as suggested by the results shown in Fig. 5 (see below). This, however, requires conformation. The facile absorption of \( \alpha \)-tocopherol regeneratated from 5\( \alpha \)-tocopherol ascorbate (3) could be demonstrated in uptake experiments with a small intestinal cell line: the new produg form of vitamin E was compared with traditional vitamin E preparations (Fig. 4).

With the average tocopherol concentration of healthy, normolipidemic intestinal cells set at 100%, uptake of \( \alpha \)-tocopherol or \( \alpha \)-tocopherol acetate under standard conditions for absorption experiments\(^9\) caused an increase of about 10%, in agreement with results previously reported.\(^9,10\) The effect in the case of a blank run—with \( \alpha \)-tocopherol and ascorbic acid added as two separate compounds in an 1:1 ratio—is of similar magnitude. With vitamin CE (3) as the novel produg form of vitamin E, there is a 70% increase in the tocopherol content of the cell, or even more in case of one equivalent of ascorbate added. The latter effect is due to a better reversion of the intermediate ortho-quinone methide (4) to \( \alpha \)-tocopherol by the equivalent of ascorbate as an additional reductant, and results in higher tocopherol concentrations. \( \alpha \)-Tocopherol ascorbate (3) even outperforms tocopherol polyethylene glycol−1000 succinate (TPGS), the only oral preparation of vitamin E known so far that appears to be absorbed by patients in early stages of certain diseases.\(^1\)

In patients with liver diseases or biliary obstruction, luminal bile salt concentrations are less than the critical micellar concentration. Therefore, tocopherol cannot be solubilized in the small intestine.\(^2\) Also, patients with pancreatic insufficiency, i.e., patients secreting a reduced amount of pancreatic enzymes, show drastically impaired absorption of vitamin E, presumably caused by a failure to hydrolyze triglycerides and thereby to form mixed micelles.\(^3\) Both groups of patients exhibit very low serum concentrations of \( \alpha \)-tocopherol, but are unfortunately unable to absorb conventional oral preparations of vitamin E.\(^4\) In these cases, the new produg form, vitamin CE (3), promises a solution: as already indicated, the compound is able to regenerate \( \alpha \)-tocopherol in a fast chemical reaction as a finely dispersed form, almost on a molecular level. The resulting facilitated absorption in small intestinal cells has been demonstrated in another experiment, as illustrated in Fig. 5.

Here, conditions of vitamin E malabsorption were simulated by depleting the intestinal volume of factors necessary for the formation of mixed tocopherol micelles. These substances are mainly bile salts, besides fatty acids and monoglycerides that are formed by the action of lipolytic pancreatic enzymes. Surprisingly, in the absence of biliary or pancreatic factors, when normal vitamin E preparations and even TPGS cannot be absorbed, there is still uptake of \( \alpha \)-tocopherol generated from vitamin CE (see Fig. 5). This opens the way for new, convenient vitamin E medications that can replace conventional treatments, such as extremely large oral vitamin doses, replacement therapies, or non-oral administration.

In summary, we have described the synthesis of a novel produg form of vitamin E, 5\( \alpha \)-tocopherol ascorbate (3). The compound does not contain \( \alpha \)-tocopherol in the traditional form of an ester or ether, but as a 5\( \alpha \)-substituted derivative that generates finely dispersed \( \alpha \)-tocopherol under basic conditions. The kinetics of this drug release has been investigated. Absorption experiments demonstrated that the new vitamin E preparation was superior to conventional vitamin E preparations.

**Experimental**

H-NMR spectra were recorded at 300 MHz and \(^{13}\)C-NMR spectra at 75 MHz on a Bruker AC-300P. Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) experiments were carried out on a Shimadzu time-of-flight instrument with linear geometry (pulsed \( \mathrm{N}_2 \) laser, 337 nm, pulse duration 3 ns, acceleration voltage 20 kV) with gentisic acid as the matrix. GC-MS was performed on a Hewlett Packard (5890 Series II, E1, 70 eV). Nomenclature and numbering of the C atoms...
in tocopherol derivatives follows IUPAC recommendations.141 Chemicals as well as ingredients for culture medium, stock solutions and tocopherol preparations were obtained from Sigma–Aldrich Chemie GmbH, unless otherwise indicated.

Preparation of 3-Hydroxy-5-(1,2-dihydroxystyryl)-4-[6-hydroxy-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-chroman-5-ylmethoxy]-furane 2(5-f)-one (5a-Tocopherol Acetate, 3) In a 250 ml flask equipped with an efficient magnetic stirrer, a mixture of dry and finely powdered sodium acetate (10 mmol, 1.981 g), powdered ascorbic acid (50 mmol, 8.806 g), and DMSO (50 ml) was stirred at 60 °C for 2 h and then cooled to room temperature. Under stirring, a solution of 5a-bromo-a-tocopherol (1 10 mmol, 0.996 g) in tetrahydrofuran (THF) (10 ml) and DMSO (10 ml) was added within 2 h in an inert atmosphere. The mixture was stirred at 50 °C for 3 h and cooled to room temperature. Then 100 ml of n-hexane and 50 ml of water were added, and the phases were separated. The aqueous layer containing the major portion of the DMSO was extracted twice with n-hexane, and the combined organic phases were washed once with 20 ml of 1 N HCl, and then with water until they were free of DMSO, normally 8 to 10 times. The obtained solution was carefully dried over sodium sulfate and absorbed on acidic aluminum oxide (60 g). The yellowish aluminum oxide was washed with diethyl ether until the filtrate was colorless. Finally, the filtrate was dried and 10 ml of solvents was removed by methanol. The obtained solution contained pure vitamin CE. Removal of the solvent under reduced pressure at room temperature produced pure 3 as a yellow wax in 63% yield. 11-NMR (DMSO-d$_6$) (δ): 2.11 (s, 3H, CH$_2$Ar), 2.13 (s, 3H, CH$_3$Ar), 2.63 (t, 2H, Ar-CH$_2$-CH$_3$), 3.6 (d, 2H, CH(OH)-CH$_2$(OH)), 3.7 (m, 1H, CH-CH$_2$(OH)-CH$_2$(OH)), 4.63 (d, 2H, Ar-CH$_2$-O), 4.77 (d, 1H, CH$_2$-CH(OH)-CH$_2$(OH)). The strong resonances of the isopropyl side chain below the ppm range are not listed.

Kinetic Measurements of the Base-Induced Decomposition of 3 A 100 mg sample of 3 in 5 ml of aqueous methanol (v/v=1:1) was added to 10 ml of buffer solution and 2 g of coarse-grained SiO$_2$. The pH was set to integral values between 6 and 14 with citrate/dichromephosphate buffer according to McIlvaine (pH 6–7, glycine/borate buffer according to Sörensen for pH 8–12, and NaOH in brine for pH 13–14). The mixture was vigorously shaken over a specific period of time. Afterwards, the reaction was stopped by addition of 5 ml of 1 N HCl. The solids were filtered off and washed with acetone (2 cm$^3$), and the washing was added to the filtrate. The resulting aqueous mixture was extracted twice with 5 ml of n-hexane and the phases were carefully separated. The n-hexane phase was washed with 5 ml of water, 20 ml of 10 mmol/L Na$_2$SO$_4$, and analyzed by GC. The ratio of a-tocopherol and para-tocopherol quinone was determined as the average value of two GC runs. Unreacted 3 that was also present in the organic phase cannot be detected by GC, and did not interfere with the measurement. The aqueous phase was divided into two equal volumes to allow verification by a second run. Ascorbic acid and dehydroascorbic acid were quantified according to the standard procedures13 by redox titration with 2,6-dichlorophenolindophenol. The total of ascorbic acid and dehydroascorbic acid is equal to the turnover, i.e., the amount of 3 that reacted. The ratio of a-tocopherol and para-tocopherol quinone obtained from GC experiments, and the ratio of dehydroascorbic acid and ascorbic acid determined by titration must be equal according to the underlying reaction mechanism. In all cases, the two corresponding values obtained differed from each other by less than 3%, thereby proving the accuracy of the procedure applied (cf. Fig. 3).

Absorption Experiments Cell Culture Absorption Media and Tocopherol Preparations A human intestinal cell line (Caco-2 cells) in tissue culture was used in absorption experiments according to the method of Traber et al.10 The cells were cultured in medium consisting of Dulbecco’s minimal essential medium (DMEM) with 10% fetal calf serum (FCS), 20 mm HEPES pH 7.3, 200 mg/ml streptomycin, 20000 U/l penicillin and 1 mm a-tocopherol. Seven days before the absorption experiments the cells were dissociated and replated. For the absorption measurements 2 ml of medium (approximately 2 x 10$^5$ cells) was used on

References and Notes


