154. The Reaction Mechanism of Chromanol-Ring Formation Catalyzed by Tocopherol Cyclase from *Anabaena variabilis* KÜTZING (*Cyanobacteria*)

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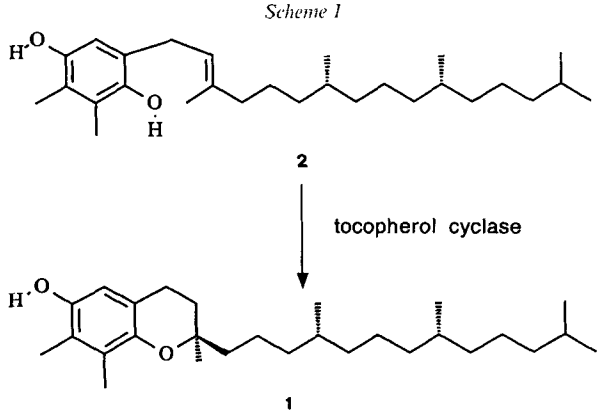
Incubation of the synthetic ^{18}O-labelled phytyl-hydroquinone (O^4-^{18}O)-**2** with the tocopherol cyclase from *Anabaena variabilis* KÜTZING (*Cyanobacteria*) in D_2O furnished the doubly labelled γ-tocopherol, (2*R*,3*S*,4′*R*,8′*R*)-(1-^{18}O,3-^{2}H)-**1**. The chirality at C(3) was determined by two independent routes providing interlocking evidence that the enzyme-catalyzed ring closure proceeds by *si*-protonation of the double bond of **2** and concomitant *re*-attack of the phenolic O-atom.

Introduction. – Recently, we identified a new enzyme in the blue-green algae *Anabaena variabilis* KÜTZING (*Cyanobacteria*) which catalyzes the formation of γ-tocopherol (**1**) from the phytyl-hydroquinone **2** (*Scheme 1*). It was shown that the cyclization was stereospecific and could be driven to nearly quantitative substrate turnover under strictly defined conditions such as incubating the 2,6-di-*O*-methyl-β-cyclodextrin complex of **2** in the presence of ascorbic acid with spheroplasts prepared by lysozyme treatment of intact *Anabaena* cells [1].

Scheme 1

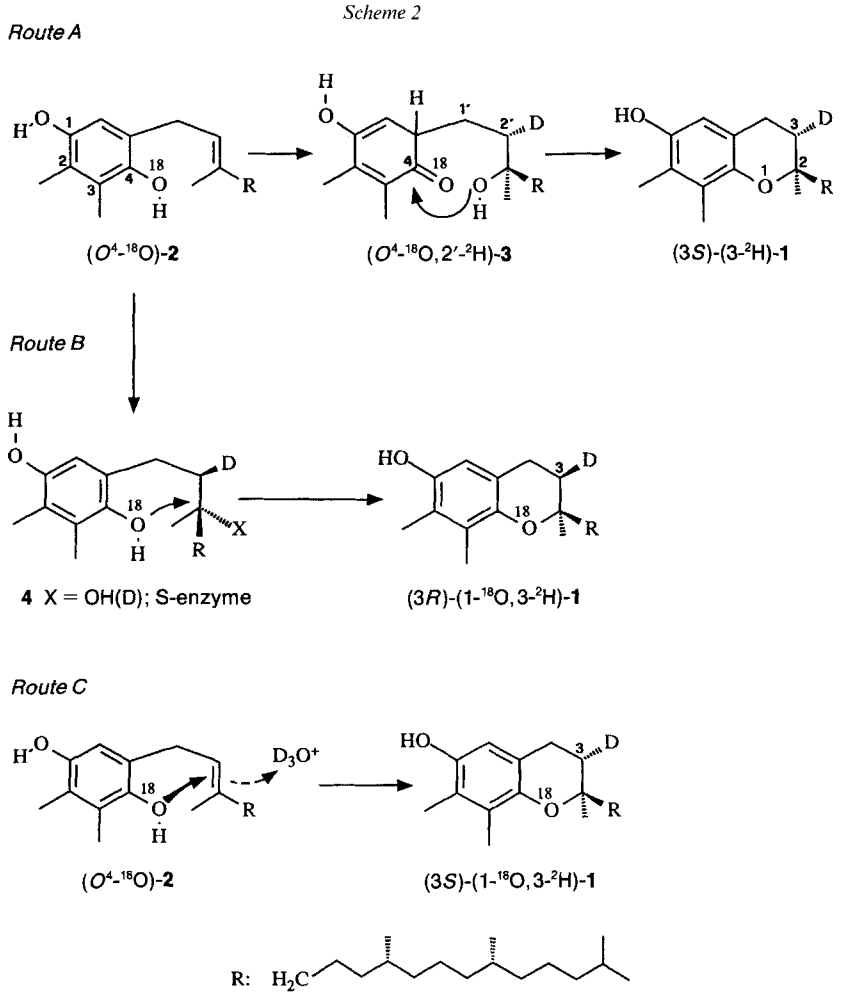


The mechanism of this cyclization, in principle, could proceed through different intermediates, as outlined in *Scheme 2*. *Route A* involves the stereospecific hydration of the (*E*)-double bond of **2** followed by cyclization of **3** (→ **1**) under retention of configuration at the tertiary alcohol. The latter reaction resembles the well established acid-cata-

lyzed ring-closure of **3** in organic solvents, *vide infra* [2]. On the other hand, the reverse addition of H_2O (or any other species like HS-enzyme) to the double bond of **2** would yield an alcohol **4** (or thio adduct) which could cyclize (→ **1**) under inversion of configuration by attack of the phenolic O-atom (*Route B*). The third possibility, *Route C*, simply involves the stereospecific addition of the phenol to the protonated double bond of **2**. *Route A* is distinguished from *Routes B* and *C* by the elimination of the phenolic O-atom and *Routes B* and *C* are distinct with respect to the configuration at C(3) of **1**, if the incubation of **2** is done in deuterated buffer, as shown for convenience in *Scheme 2*.

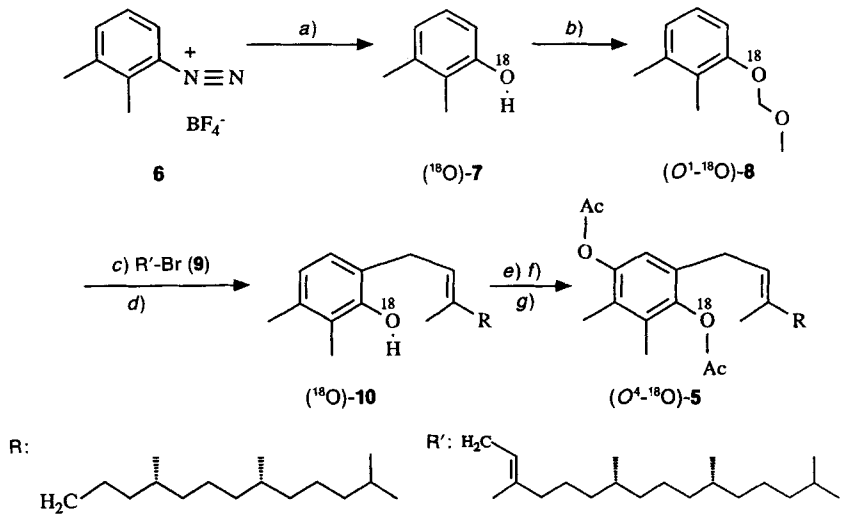
It is obvious that a decisive experiment can be accomplished if *1*) an enzyme fraction can be prepared devoid of H_2O, *2*) a high deuterium incorporation at C(3) is obtained from deuterated buffer, and methods can be developed to determine the absolute configuration at C(3) of **1**, and *3*) a precursor specifically ^{18}O-labelled at C(4) of **2** can be used without exchange of ^{18}O under conditions of incubation.

Scheme 2



Results and Discussion. – To investigate the incorporation of the phenolic O-atom at C(4) of **2**, the benzohydroquinone diacetate (O^4-^{18}O)-**5** was prepared according to *Scheme 3*. The H_2O-free, crystalline diazonium tetrafluoroborate **6** of 2,3-dimethylaniline [3] was treated with $H_2{}^{18}O$ in THF to yield the isotopically substituted phenol (^{18}O)-**7**. The

Scheme 3

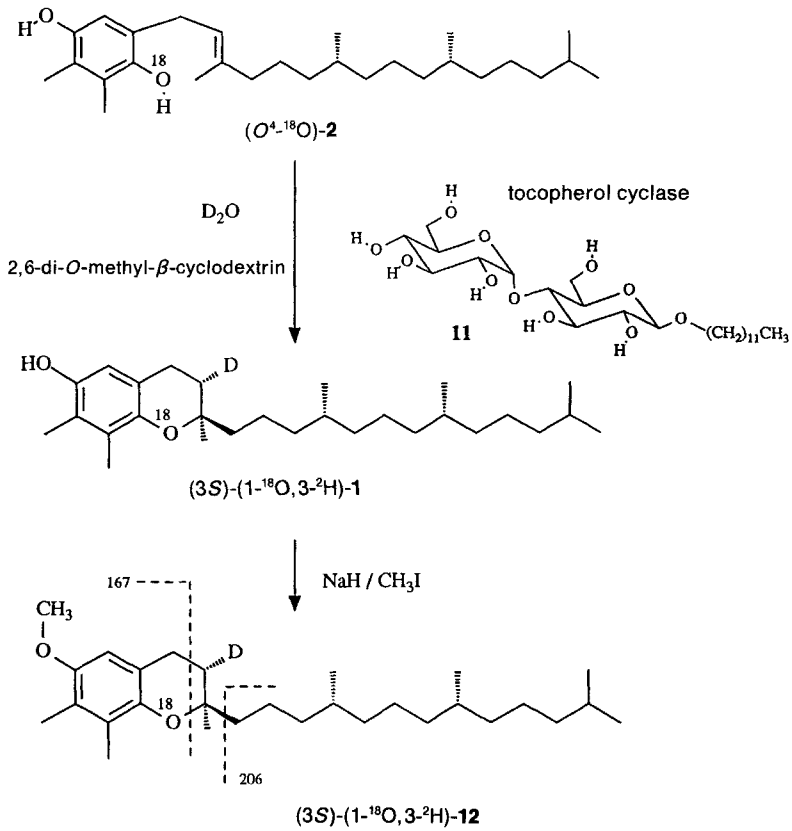


a) $H_2{}^{18}O$/THF, r.t.→65°; 16%. *b*) $ClCH_2OMe$, $(i\text{-}Pr)_2EtN$, 40°; 38%. *c*) 1. BuLi, $Me_2N(CH_2)_2NMe_2$, Et_2O, 2 h; 2. CuBr, **9**, Et_2O, −20°, 4 h. *d*) THF/AcOH/i-PrOH/37% HCl soln., r.t., 4 h; 33% from **8**. *e*) O_2/salcomine, EtOH, r.t. *f*) $Na_2S_2O_4$, H_2O/Et_2O, r.t., 4 h. *g*) Ac_2O/pyridine, $Na_2S_2O_4$; 61% from **10**.

corresponding methoxymethyl ether [4] (O^1-^{18}O)-**8** serves as a guide for regiospecific *ortho*-lithiation and subsequent alkylation with phytyl bromide **9** [5] to give (^{18}O)-**10** after acid-catalyzed deprotection. Salcomine (= bis(salicylidene)ethylenediimino-cobalt(II)) oxidation [6] [1] and reductive acetylation furnished the desired diacetate (O^4-^{18}O)-**5** (95% ^{18}O) in 9% overall yield from **7**. Enzymatic reactions were done with the corresponding hydroquinone (O^4-^{18}O)-**2**, prepared by $LiAlH_4$ reduction of (O^4-^{18}O)-**5**, and subsequent formation of its 2,6-di-*O*-methyl-*β*-cyclodextrin complex [1] in the presence of ascorbic acid.

To prepare a H_2O-free enzyme fraction, the spheroplasts were cracked by osmolysis at 4° and pH 7.0 and the H_2O-soluble proteins removed by centrifugation, and the resulting pellet was resuspended in buffer. The addition of cold acetone furnished a precipitate which could be washed with cold Et_2O to remove the endogenous tocopherols, ground in a mortar, and dried under high vacuum to give a grey-blue 'acetone powder'. This material was suspended in deuterated buffer in the presence of the non-ionic detergent dodecyl D-maltoside (**11**) and displayed enzymatic activity (90% conversion **2** → **1**) comparable to intact spheroplasts [1]. From an upscaled incubation of **2**, mg quantities of the deuterated tocopherol were isolated, and its corresponding methyl ether (3-^{2}H)-**12** was analyzed by mass and ^{1}H-NMR spectroscopy. Both methods demonstrated a nearly quantitative deuterium incorporation. Due to specific fragmentation in the MS,

Scheme 4



it was evident that only C(3) was monodeuterated, and the ^{1}H-NMR revealed that just one of the diastereotopic positions at C(3) was deuterated. From incubation of (O^4-^{18}O)-**2** under the same conditions in D_2O (see *Scheme 4*), a doubly labelled tocopherol was isolated and its methyl ether (1-^{18}O,3-^{2}H)-**12** prepared for spectroscopical analyses. The ^{1}H-NMR fully confirmed the former experiment; moreover the $M^{+\cdot}$ showed *ca.* 83% enrichment of ^{18}O and ^{2}H (*Fig. 1*). In contrast, no ^{18}O incorporation into **1** and **12** was observed on incubation of the cyclodextrin complex of unlabelled **2** with the 'acetone powder' solubilized with **11** in a buffer containing $H_2{}^{18}O/H_2O$ 1:1, indicating that no exchange of ^{18}O with the medium had occurred. These results exclude *Route A* (*Scheme 2*) as a mechanism of the enzymatic reaction but leave the choice open between *Routes B* and *C*.

In view of the fact that on irradiation of the benzylic protons of **12** in a ^{1}H-NMR experiment the complicated *m* for the protons at C(3) collaps to a well-defined *AB* system, which in the sample of enzymatic origin is even more simplified (→*s* at 1.77 ppm), it was decided to prepare deuterated reference probes of known absolute configuration. Two of the many possible strategies to achieve this goal are reported below.

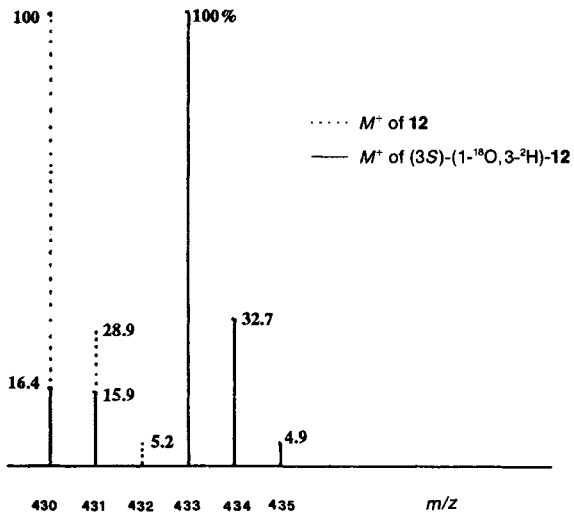


Fig. 1. *Mass spectra of (1-^{18}O,3-^{2}H)-***12** *obtained from tocopherol cyclase treatment of (O^{4}-^{18}O)-***2** *in D_2O and of the corresponding unlabelled* **12**

The concept of the 'Basel route' was to make use of γ-tocopheryl acetate (**13**) as a starting material taking advantage of its easy DDQ (= 2,3-dichloro-5,6-dicyano-1,4-benzoquinone = 4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile) oxidation [7] to the didehydro derivative **14** (67% overall yield) from which, on treatment with *N*-bromoacetamide [8], regioselectively a pair of *trans*-bromohydrins **15** and **16** was prepared and separated by chromatography. Reduction of **15** with $LiEt_3BH$ gave, *via* the intermediate epoxide **17** (*in situ*), alcohol **18**. When the corresponding triflate **19** (Tf = CF_3SO_2) was treated with $LiEt_3BD$, the deuterated γ-tocopheryl methyl ether (3-^{2}H)-**12** (8% yield by GLC) was isolated by HPLC, besides **14** (65%), **18** (19%), and *ca.* 2% of benzofurans produced by rearrangement. The same products, but in a different ratio, were obtained from tosylate **20** in a much slower reaction under forced conditions. Keystones of this sequence are the X-ray analyses of bromohydrin **16** and of tosylate **20** derived from alcohol **18**. It is interesting to note that the cell unit of **16** contains two individual molecules not related by crystallographic symmetry (*Fig. 2*). In the crystal of **20**, most of the alkyl chain is disordered; perspective drawings of the rotamers *A* and *B* obtained from the analysis at 115 K are shown in *Fig. 3*. These results prove the absolute configuration of all chiral centers in question, in particular the (*R*)-configuration at C(3) in both cases.

Since the nucleophilic displacement of the homobenzylic triflate group is of significance to the chirality at C(3) of the final product (3-^{2}H)-**12**, the dideuterated tocopherol derivative (3*R*,4*S*)-(3,4-$^{2}H_2$)-**12** was prepared in three steps from **16** (*Scheme 5*). This compound was also obtained in a 3:5 mixture with (3*S*,4*R*)-(3,4-$^{2}H_2$)-**12** (not shown) from **14** by *cis*-addition of D_2 in the presence of *Wilkinson* catalyst [9]. It was obvious from the ^{1}H-NMR spectra that the protons at C(3) and C(4) display a *cis*-coupling constant of 6.2 Hz in both cases. Almost the same vicinal-coupling constant (6.15 Hz) is observed between H_β at C(3) and H_β at C(4) in the ^{1}H-NMR of (4*R*)-(4-^{2}H)-**12**, prepared in 3 steps

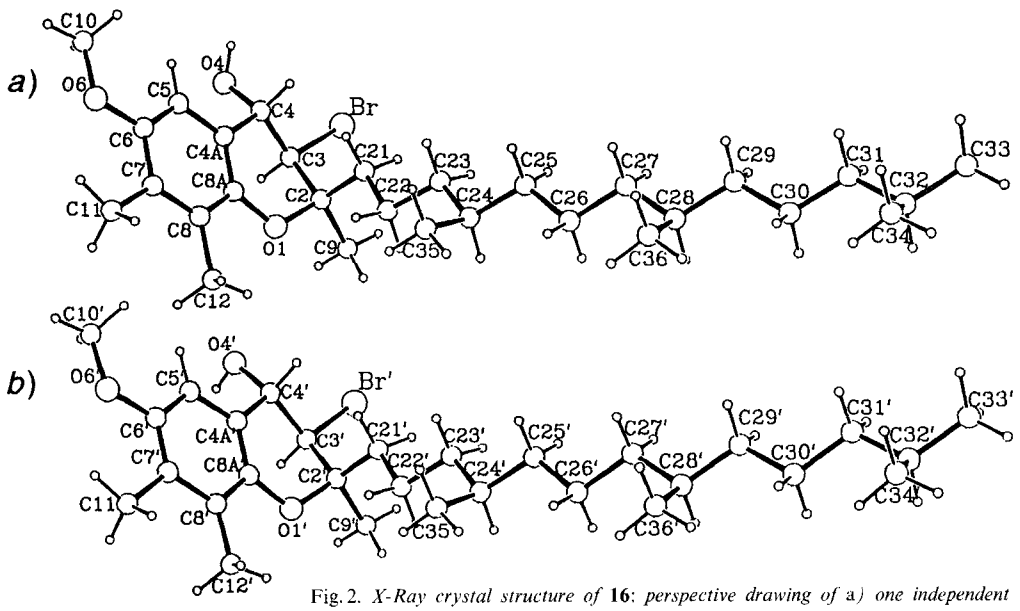


Fig. 2. *X-Ray crystal structure of* **16**: *perspective drawing of* a) *one independent molecule and of* b) *the other independent molecule*. Arbitrary numbering.

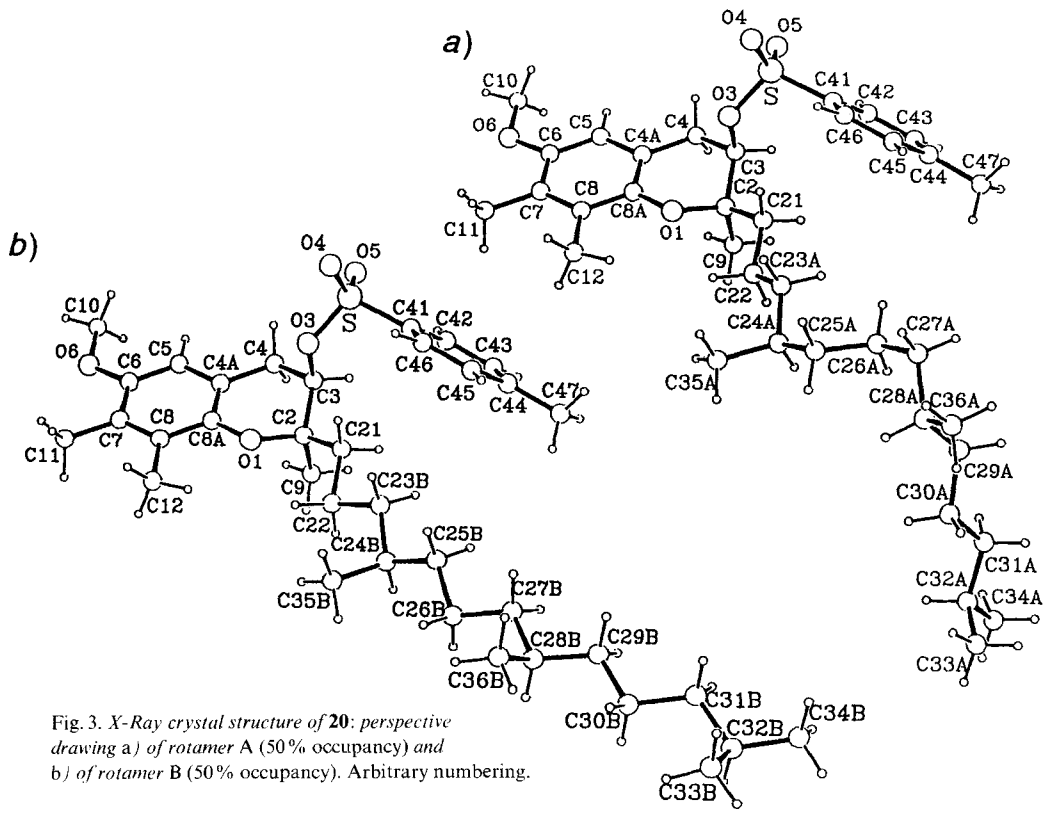
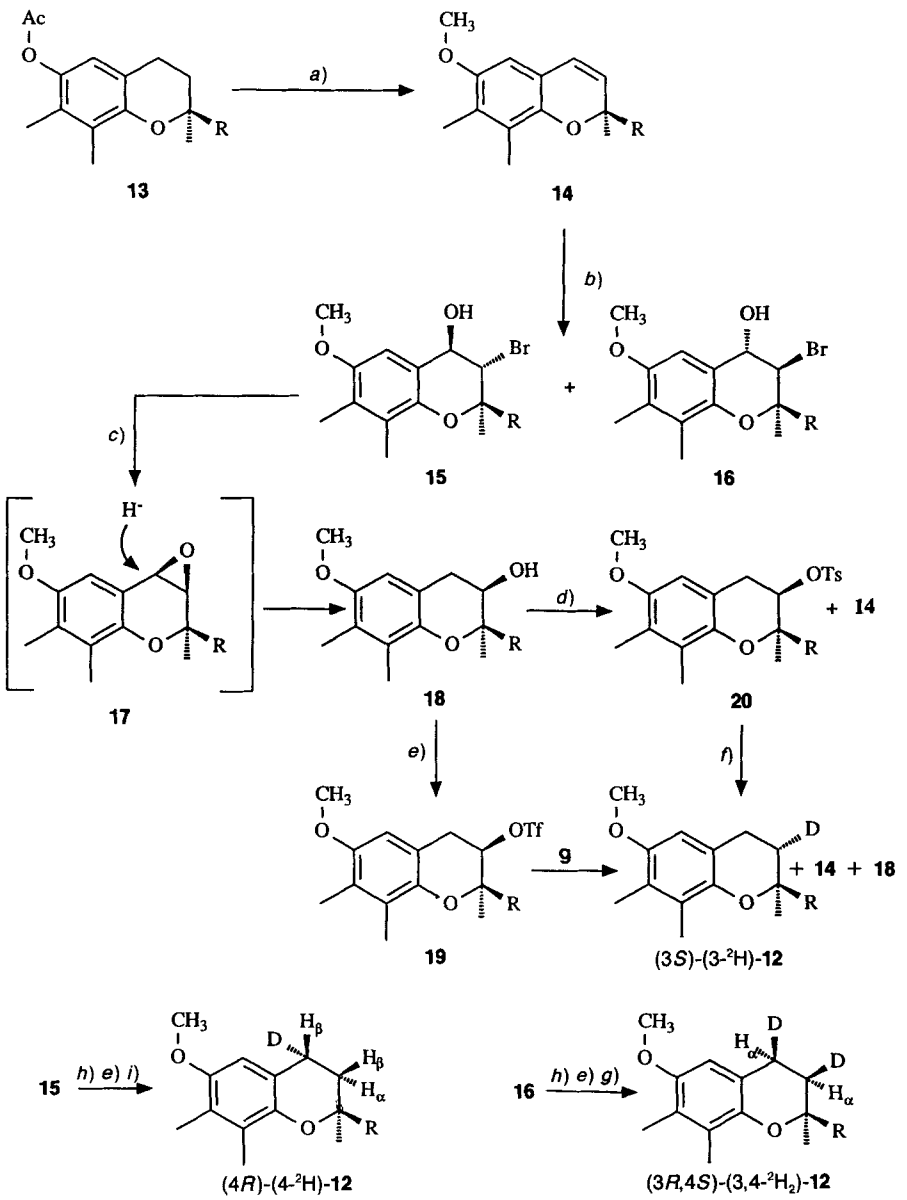


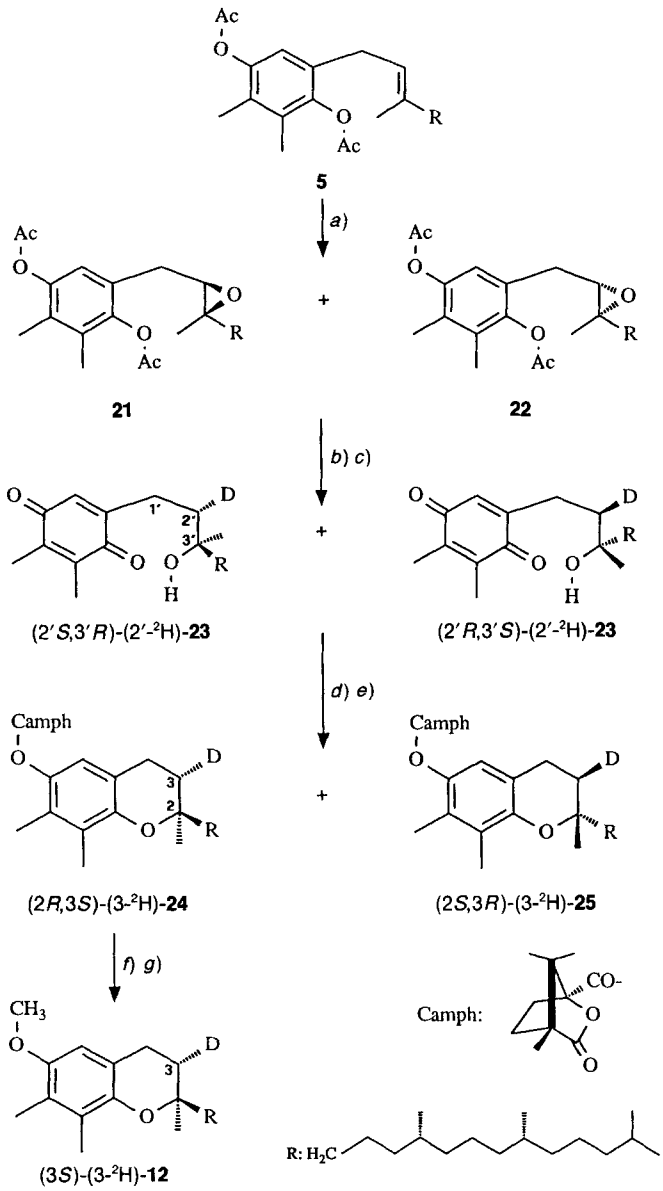
Fig. 3. *X-Ray crystal structure of* **20**: *perspective drawing* a) *of rotamer* A (50% occupancy) *and* b) *of rotamer* B (50% occupancy). Arbitrary numbering.

Scheme 5



a) 1. DDQ, dioxane, reflux; 82%; 2. K_2CO_3, MeOH, r.t.; 96%; 3. NaH, $(MeO)_2SO_2$, THF, r.t.; 85%. *b*) 1. *N*-Bromoacetamide, THF/H_2O, r.t.; 81%; 2. chromatographic separation, SiO_2. *c*) 2.5 Equiv. $LiEt_3BH$, THF, −78°→r.t.; 95–100%. *d*) TsCl, Et_3N, pyridine, reflux; 65%. *e*) Tf_2O, 2,6-dimethylpyridine, Et_2O, −78°→r.t.; *ca.* 100%. *f*) 20 Equiv. $LiEt_3BD$, THF, reflux, 5 d. *g*) 10 Equiv. $LiEt_3BD$, THF, −78°→r.t. *h*) 2.5 Equiv. $LiEt_3BD$, THF, −78°→r.t. *i*) 10 Equiv. $LiEt_3BH$, THF, −78°→r.t.

Scheme 6



a) 3-Chloroperbenzoic acid, CH_2Cl_2/sat. Na_2CO_3 soln., 0°→r.t.; 82%. *b*) $LiAlD_4$, Et_2O, 0°, 2 h. *c*) Air; 83%. *d*) $Na_2S_2O_4$, $CHCl_3$/EtOH/H_2O; TsOH (cat.)/benzene, 80°, 2 h; 87%. *e*) (−)-Camphanoyl chloride, pyridine, r.t., 16 h; 96%; separation on HPLC, see *Fig. 4*. *f*) $LiAlH_4$, Et_2O, r.t., 2 h; 94%. *g*) NaH, MeI, THF, r.t., 10 min; 96%.

from **15**; however, for this monodeuterated compound, the *trans*-coupling constant of 6.85 is also detected. This clearly indicates that removal of the triflate group at C(3) proceeded with inversion of configuration. Together with the X-ray structures of **16** and **20** taken as structural references for the sequence, the (*S*)-configuration at C(3) of (3-^{2}H)-**12** is established, and the compound is suitable for comparison with the sample produced by the enzyme 'tocopherol cyclase'.

The 'Zürich route' takes into account common knowledge [2] concerning the stereospecific ring closure of the tertiary alcohol **3** yielding **1** under retention of configuration (see above, *Scheme 2, Route A*), and the fact that (*R,R,R*)- and (*S,R,R*)-tocopheryl camphanates can be separated by chromatography. Thus, the procedure is very simple (*Scheme 6*).

Epoxidation of the diacetate **5** gave a mixture of diastereoisomeric epoxides **21** and **22** that was immediately treated with $LiAlD_4$ and oxidized to yield the two tertiary alcohols (2′*S*,3′*R*)-(2′-^{2}H)- and (2′*R*,3′*S*)-(2′-^{2}H)-**23**. Without separation, the latter were reduced

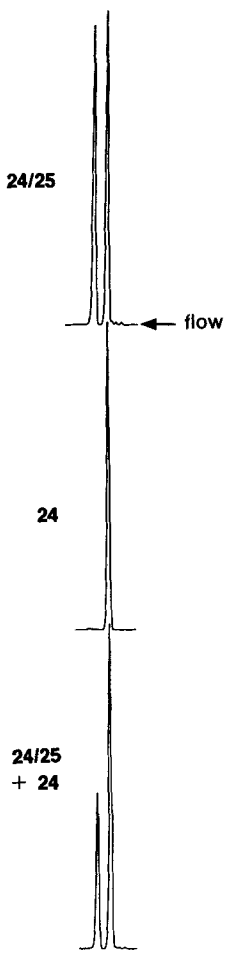


Fig. 4. *HPLC analysis of the diastereoisomeric (−)-camphanates* **24/25** (obtained from (2*RS*,4′*R*,8′*R*)-γ-tocopherol), *of* **24** (obtained from (2*R*,4′*R*,8′*R*)-γ-tocopherol), *and of a mixture of both.* Conditions: *Spherisorb-3S-CN* column, hexane/dioxane 96:4, 250 × 4.0 mm, 62 bar, r.t., flow 1.0 ml/min, 220 nm.

and cyclized [2] to furnish the two γ-tocopherols with opposite configurations at C(2) and C(3). The corresponding camphanates (2*R*,3*S*)-(3-^{2}H)-**24** and (2*S*,3*R*)-(3-^{2}H)-**25** were baseline-separated on HPLC and identified by HPLC comparison with an unlabelled mixture **24**/**25**, prepared from synthetic 2-*ambo*-γ-tocopherol ((2*RS*)-**1**), and coinjection of **24**/**25** with unlabelled **24**, prepared from natural (2*R*)-**1** (*Fig. 4*). Accordingly, the faster-eluting compound proved to be the (2*R*)-configurated isomer **24**. Thus, the corresponding (2*R*,3*S*)-(3-^{2}H)-**24** was hydrolyzed and methylated to finally give the desired reference sample (3*S*)-(3-^{2}H)-**12** (*Scheme 6*).

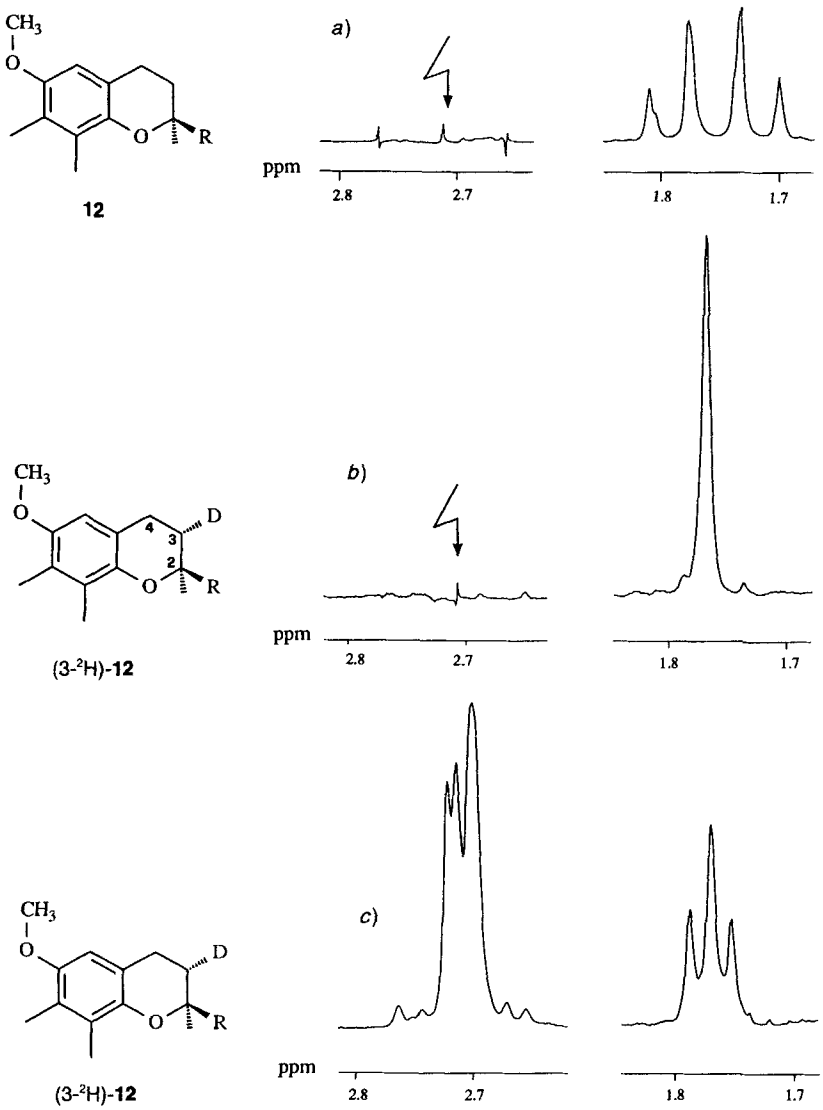


Fig. 5. *Partial 400-MHz ^{1}H-NMR spectra* (region of H–C(3) and H–C(4)) *of* a) *unlabelled* **12**, *selectively decoupled, and of* b) c) *deuterated (3S)-(3-^{2}H)-***12**, *with and without decoupling, respectively*

Spectroscopic comparison of the two synthetic probes of (3-^{2}H)-**12** with the compound of enzymatic origin for a partial ^{1}H-NMR-spectrum (see *Fig. 5*), revealed the identity of the three samples in every respect, and thus firmly establishes the mechanism of the enzymatic reaction catalyzed by 'tocopherol cyclase' to operate by *si*-protonation of the double bond of **2** and concomitant *re*-attack of the phenolic O-atom (*Route C* in *Scheme 2*). Whether the transition state of the cyclization is early or late on the reaction coordinate will be investigated by testing the binding profile of different possible transition-state analogues to the purified enzyme [10].

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Experimental Part

General. See [1]. THF was freshly distilled from Na/benzophenone under Ar. $H_2{}^{18}O$ ($\geq 99.5\%$ ^{18}O) was purchased from *Ventron*, natural *trans*-phytol from *Eisai Co., Ltd.*, Tokyo, Japan; lot No. 590'222, assay: 87.7% by GLC. The latter was further purified by column chromatography on silica gel (1 kg for 25 g of phytol, hexane/Et_2O 1:1). The residue was dissolved in hexane and washed twice with $NaHCO_3$ soln., the hexane soln. evaporated, and the residue distilled (bulb-to-bulb, *ca.* 120°/0.1 mm) to give trans-*phytol* (= *(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-enol*), purity $\geq 98\%$ (GLC). This was subsequently condensed with 2,3-dimethylhydroquinone in HCOOH [11] [12] to yield *2*-ambo-*γ-tocopherol* ((*2RS,4'R,8'R*)-**1**; see optimized procedure below). Natural *γ-tocopherol* was isolated from the commercially available tocopherol concentrate '*d*-mixed tocopherols' (*Bizen Chemical Co., Ltd.*, Kumayama, Akaiwa, Okayama, 709-07, Japan; composition according to GLC: γ-tocopherol (40%), δ-tocopherol (23%), α-tocopherol (8%), β-tocopherol (0.8%)) by column chromatography (3.5 kg *Merck* silica gel *60*, particle size 0.063–0.200 mm for 60 g '*d*-mixed tocopherols', hexane/AcOEt 88:12) to yield *ca.* 97% pure material which was acetylated (Ac_2O/pyridine), chromatographed (SiO_2, hexane/AcOEt 95:5), and distilled (bulb-to-bulb): purity of the acetate **13** *ca.* 98% (GLC). The corresponding methyl ether derivative **12** was shown to be stereoisomerically pure (> 99.5% by HPLC) [13]. In addition: TLC: Detection of substances by UV (254/366 nm) and ammonium molybdate/$Ce(SO_4)_2$ in H_2O/H_2SO_4 with subsequent heating. GLC: *PS-086* capillary column, 14 and 15 m. Supercritical fluid chromatography (SFC): Instrument *Lee Scientific*, model *600* (*Dionex*, Salt Lake City, UT, USA), FID at 380°, fused-silica capillary colum coated with biphenyl-30, length 10 m, internal diameter 50 μm, mobile phase CO_2, 100°, 0.2–0.75 g·ml^{-1}, linear ramp at 0.01–0.005 g·ml^{-1}·min^{-1} [16]. Melting points (uncorrected): *Büchi-510* apparatus. Optical rotations: *Perkin-Elmer-241* polarimeter, *c* in g/100 ml.

'Acetone Powder' Containing Tocopherol Cyclase. Spheroplasts were obtained from intact cells of *Anabaena variabilis* KÜTZING (*Cyanobacteria*) according to [1]. The spheroplasts (52 g) were suspended twice in 300 ml of ice-cold phosphate buffer (10 mM, pH 7.0) and centrifuged at 1500 g/15 min. The resulting pellet was suspended in 40 ml of the same phosphate buffer at 4°, 1 l of acetone (−20°) was added to the vigorously stirred medium, and the insoluble proteins were collected by centrifugation at 1500 g/2 min. The org. phase was removed and the protein pellet extracted for the lipids with 500 ml of ice-cold Et_2O (10 s vigorous shaking by hand). Finally the suspension was filtered through cotton and the residue dried under high vacuum for 3 h. After grinding in a mortar, 2.1 g of a slightly blue 'acetone powder' were obtained, which could be stored at −80° for *ca.* 2 months without loosing more than 20% of the enzymatic activity. According to HPLC analysis, extracts of the 'acetone powder' contained no traces of tocopherols.

Tocopherol-Cyclase Activity of the 'Acetone Powder'. The 'acetone powder' (4.5 g) was suspended at 4° in buffer (pH 7.0, 200 ml) containing dodecyl D-maltoside (**11**; 15 mM), potassium phosphate (100 mM), and DL-dithiothreitol (2 mM). After stirring for 1 h at 4° with a magnetic bar, the suspension was centrifuged at 100.000 *g* for 1 h

and the supernatant (186 ml) used for incubations as follows. Buffer (40 µl) containing the substrate inclusion complex [1] was added to the solubilisate (1 ml) such that the following final concentrations were obtained: 96 µM for **2** (40 µg), 1.8 mM for 2,6-di-*O*-methyl-β-cyclodextrin, and 10 mM for ascorbic acid. Incubation was performed at 35° for 15 h to yield, after usual workup [1] and detection by HPLC (internal standard/fluorescence det.), 36 µg (90%) of **1**.

Incubation of **2** *with Solubilized 'Acetone Powder' in* D_2O. Both substrate buffer and 'acetone powder' (4 g) were prepared and solubilized, respectively, in D_2O ($\geq$ 99.8% D). Incubation of **2** (7.5 mg, 18.0 µmol) furnished (3*S*)-(3-^{2}H)-**1** (6.29 mg, 83.9%) which was immediately methylated by treatment with an excess of NaH/MeI in abs. THF [1]. Workup and purification on TLC (silica gel, hexane/CH_2Cl_2 2:1) gave γ-tocopheryl methyl ether (3*S*)-(3-^{2}H)-**12** (5.6 mg, 71%), pure both by GLC (97.5%) and HPLC (99.4%). ^{1}H-NMR (400 MHz): 6.43 (*s*, H–C(5)); 3.76 (*s*, MeO); 2.72 (br. *d*, *J* = 7.0, 2 H–C(4)); 2.13 (*s*, Me–C(7)); 2.12 (*s*, Me–C(8)); 1.78 (*t*, *J* = 7.0, H–C(3)); 1.61–1.02 (*m*, 21 H); 1.25 (*s*, Me–C(2)); 0.88–0.84 (*m*, 12 H). MS: 433 (6), 432 (36), 431 (100, $M^{+\cdot}$), 206 (12, $[M - C_{16}H_{33}]^+$), 166 (18), 165 (100, $[M - C_{19}H_{37}]^+$), 164 (27).

Incubation of (O^4-^{18}O*)-***2** *with Solubilized 'Actone Powder' in* D_2O. 'Acetone powder' (1.7 g) and substrate buffer were prepared in D_2O ($\geq$ 94% D) and (O^4-^{18}O)-**2** (2.0 mg, 4.8 µmol) incubated at 35° for 15 h. After workup and purification, (3*S*)-(1-^{18}O,3-^{2}H)-**1** (0.4 mg, 20%) was isolated and directly methylated as described [1] to yield (3*S*)-(1-^{18}O,3-^{2}H)-**12**, pure by HPLC (97.7%). MS: 435 (5), 434 (33), 433 (100, $M^{+\cdot}$), 431 (16), 430 (16), 208 (5, $[M - C_{16}H_{33}]^+$), 168 (8), 167 (56, $[M - C_{19}H_{37}]^+$), 166 (14), 165 (14), 164 (3).

Incubation of **2** *with 'Acetone Powder' Solubilized in* $H_2{}^{18}O$. A control incubation of **2** (0.5 mg, 2 µmol) in substrate buffer/solubilized 'acetone powder', both prepared in $H_2{}^{18}O$ (50% ^{18}O), gave γ-tocopherol (**1**) and its corresponding methyl ether **12**, purified by HPLC. The MS of this sample was completely identical both with respect to peak distribution and peak integration with the MS of unlabelled **12**. MS: 432 (5), 431 (29), 430 (100, $M^{+\cdot}$), 205 (9, $[M - C_{16}H_{33}]^+$), 166 (9), 165 (75, $[M - C_{19}H_{37}]^+$), 164 (18).

2,3-Dimethylbenzene-1-diazonium Tetrafluoroborate (**6**)[1]). Slowly 2,3-dimethylaniline (12.1 g, 0.1 mol) was added to a mixture of 37% aq. HCl soln. (25 ml, 0.3 mol) and H_2O (50 ml) at 10°. After 10 min, a soln. of $NaNO_2$ (7 g, 0.1 mol) in H_2O (100 ml) was added dropwise to the mechanically stirred precipitate at −5°. After 1 h stirring at 0°, $NaBF_4$ (11.6 g, 0.102 mol) in H_2O (100 ml) was added in 30 min. Then the beige precipitate was filtered, washed with cold (0°) H_2O (2 × 25 ml), cold (−20°) EtOH (4 × 25 ml), and Et_2O (8 × 50 ml), and dried at 0°/1 Torr: **6** (12.0 g, 55%). Beige crystals. M.p. *ca.* 80° (dec.). Anal.: H_2O-content < 0.1%.

2,3-Dimethyl(^{18}O*)phenol* ((^{18}O)-**7**). A suspension of **6** (4.0 g, *ca.* 18 mmol) in THF (15 ml)/$H_2{}^{18}O$ (1 ml; $\geq$ 99.5% ^{18}O) was stirred at r.t. for 1 h, then at 35° for 2 h, and at 65° for 30 min. Then the mixture was poured into hexane/Et_2O 3:1 (200 ml) and extracted with 2M NaOH (4 × 100 ml). Neutralization of the aq. phase with 37% aq. HCl soln., extraction with Et_2O (3 × 100 ml), evaporation, and chromatography (SiO_2 (60 g), hexane/AcOEt 4:1) gave (^{18}O)-**7** (350 mg, 16%). Beige crystals. M.p. 69.5°. GLC Purity 99.6%. ^{1}H-NMR (250 MHz): 2.16, 2.27 (2*s*, Me–C(2), Me–C(3)); 4.59 (*s*, OH); 6.63, 6.76 (2*d*, *J* ≈ 8, H–C(4), H–C(6)); 6.97 (*t*, *J* ≈ 8, H–C(5)). MS: 124 (74, M^+, 96% ^{18}O), 109 (100), 91 (25), 79 (26), 77 (36), 57 (26).

(O^1-^{18}O*)-1-(Methoxymethoxy)-2,3-dimethylbenzene* ((O^1-^{18}O)-**8**). To a cold (0°) soln. of (^{18}O)-**7** (330 mg, 2.6 mmol, 96% ^{18}O) and (i-Pr)$_2$EtN (5 ml, 30 mmol) in CH_2Cl (5 ml) was added 6.1M $ClCH_2OMe$ (4 ml, 24.4 mmol in AcOMe; *in situ* prepared [4]). After 5 h stirring at 40°, 25% aq. NH_3 soln. (5 ml) was added at r.t. Then the mixture was poured into Et_2O (50 ml) and the org. phase washed with 3N HCl, sat. $NaHCO_3$ soln., and brine, dried (Na_2SO_4), and evaporated: 300 mg of yellow oil. Bulb-to-bulb distillation at *ca.* 50°/0.1 mbar afforded pure (O^1-^{18}O)-**8** (170 mg, 38%). Colourless oil. GLC Purity 99.6%. ^{1}H-NMR (250 MHz): 2.18, 2.27 (2*s*, Me–C(2), Me–C(3)); 3.49 (*s*, MeO); 5.18 (*s*, OCH_2O); 6.82, 6.92 (2*d*, *J* ≈ 7, H–C(4), H–C(6)); 7.05 (*t*, *J* ≈ 7, H–C(5)). MS: 168 (15, M^+, *ca.* 100% ^{18}O), 138 (8), 123 (5), 105 (6), 91 (5), 77 (7), 45 (100).

2,3-Dimethyl-6-phytyl(^{18}O*)phenol* ((^{18}O)-**10**). BuLi (1.6M in hexane; 0.8 ml, 1.3 mmol) was added to a cold (0°) soln. of (O^1-^{18}O)-**8** (170 mg, 1 mmol) in *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (0.2 ml, 1.3 mmol) and Et_2O (2 ml). After 2 h, CuBr (15 mg) and a soln. of phytyl bromide (**9**; 0.5 g, 1.4 mmol; prepared from *trans*-phytol according to [5]) in Et_2O (2 ml) was added at −20°. After 4 h at −20°, the mixture was mixed with Et_2O (50 ml), washed with 2N H_2SO_4/MeOH 1:1, H_2O/MeOH 1:1, and brine to give, after evaporation, a yellow oil (450 mg) which was treated at r.t. with freshly prepared THF/AcOH/i-PrOH/37% aq. HCl soln. 10:10:10:1 (5 ml). After 4 h, the mixture was diluted with hexane (50 ml) and washed with 1/2-sat. $NaHCO_3$ soln./MeOH 1:1 (30 ml). Usual evaporation and chromatography (SiO_2, hexane/AcOEt 19:1) yielded (^{18}O)-**10** (160 mg, 33%). Yellow oil. GLC Purity: (*E*) + (*Z*) 83.5%, (*E*)/(*Z*) 98:2. MS: 402 (12, M^+), 177 (37), 149 (13), 137 (100, 96% ^{18}O).

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[1]) Preparation of **6** according to a general procedure described in [3]; it decomposes slowly at r.t. and should be stored at −20°, if necessary.

(O⁴-[18]O)-2,3-Dimethyl-5-phytyl-1,4-hydroquinone Diacetate (= (O⁴-[18]O)-2,3-Dimethyl-5-phytylbenzene-1,4-diyl Diacetate; (*O⁴*-[18]O)-**5**). A soln. of ([18]O)-**10** (150 mg, 0.31 mmol; GC: 83.5%) and salcomine [6] (15 mg, 0.05 mmol) in EtOH (5 ml) was stirred under O_2 (1.2–1.4 atm) for 4 h at r.t. Evaporation and chromatography (SiO_2, toluene) gave a yellow oil ((*O⁴*-[18]O)-2,3-dimethyl-5-phytyl-1,4-benzoquinone, 95 mg; HPLC purity: 88%), which was dissolved in Et_2O (5 ml) and treated with a soln. of $Na_2S_2O_4$ (300 mg, 1.7 mmol) in H_2O (3 ml). After stirring for 4 h at r.t., the mixture was extracted with Et_2O (75 ml) and the extract washed with brine and evaporated. The remaining oil was acetylated with Ac_2O (0.5 ml) and pyridine (4 ml) in the presence of $Na_2S_2O_4$ (200 mg). Usual workup gave a yellow oil (120 mg) which was chromatographed twice on SiO_2 (30 g, toluene; 30 g, hexane/AcOEt 6:1): (*O⁴*-[18]O)-**5** (60 mg, 61%). Slightly yellow oil. GLC Purity: (*E*) + (*Z*) 95.4%, (*E*)/(*Z*) 96:4. ^{1}H-NMR (250 MHz): *ca.* 0.85 (*m*, Me–C(7′), Me–C(11′), Me–C(15′), Me(16′)); 1.0–1.6 (*m*, 19 H); 1.64 (*s*, Me–C(3′)); 2.0 (*t*, $J \approx 7$, CH_2(3′)); 2.05, 2.06 (2*s*, Me–C(2), Me–C(3)); 2.31, 2.32 (2*s*, 2 Ac); 3.16 (*d*, $J \approx 7$, CH_2(1′)); 5.2 (*t*, $J \approx 7$, H–C(2′)); 6.74 (*s*, H–C(5)). MS: 502 (3, M^+), 459 (18), 418 (100, 95% [18]O), 195 (21), 153 (68), 152 (16).

*(2R)-6-Methoxy-2,7,8-trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-2*H*-1-benzopyran (= (2R,4′R,8′R)-3,4-Didehydro-6-*O*-methyl-γ-tocopherol*; **14**). The soln. of (2*R*,4′*R*,8′*R*)-γ-tocopheryl acetate (**13**; obtained from natural sources; 45.90 g), and DDQ (34.05 g) in dioxane (600 ml) was stirred under refluxed for 6 h [7]. After stirring for additional 15 h at r.t., the mixture was filtered and the residue washed with hexane (200 ml). The combined filtrate was diluted with hexane (500 ml) and washed twice with H_2O (1000 ml), the H_2O phase extracted with hexane (500 and 1000 ml), the combined org. phase dried ($MgSO_4$) and evaporated, and the crude black oil (*ca.* 52 g) purified by chromatography (silica gel (2 kg), hexane/Et_2O 9:1; TLC: R_f 0.39 (**13**), 0.33 (didehydro acetate)): 37.65 g (82%) of *(2R,4′R,8′R)-3,4-didehydro-γ-tocopheryl actate* [7] as a pale yellow oil, purity (GLC) 98.7–98.8%[2]).

To a soln. of this material (22.84 g, 50.0 mmol) in MeOH (220 ml), K_2CO_3 (3.4 g, *ca.* 25 mmol) was added at r.t. After *ca.* 1 h of vigorous stirring (TLC control (CH_2Cl_2): R_f 0.65 (didehydro acetate), 0.45 (didehydrophenol)), the brown mixture was evaporated, the residue dissolved in Et_2O (300 ml) and the soln. washed with sat. NaCl soln. and H_2O (100 ml each), dried ($MgSO_4$), and evaporated: 19.90–20.70 g (96–100%) of *(2R,4′R,8′R)-3,4-didehydro-γ-tocopherol* [7] as a brownish oil, purity (GLC) 97.7%[2]), which was used without further purification in the next step.

The soln. of this material (19.98 g, 48.2 mmol) in THF (75 ml) was added dropwise to the stirred suspension of NaH (1.45 g, 60.4 mmol, 1.25 equiv.; prepared from 55 or 80% NaH/mineral oil by washing with hexane several times) in THF (100 ml) at r.t. during 5 min. After completion of H_2 evolution (1.5–2 h), Me_2SO_4 (6.00 ml, 63.0 mmol, 1.3 mol-equiv.) was added dropwise and stirring continued for 2.5 h (TLC control, (hexane/AcOEt 9:1): R_f (**14**) 0.59, R_f (didehydrophenol) 0.29). Then 2N NaOH (50 ml) was added carefully, the mixture stirred for 1 h and then extracted with Et_2O (250, 200, 100 ml), the combined extract washed with H_2O (100 ml), dried ($MgSO_4$), and evaporated and the crude yellowish oil (*ca.* 20 g) chromatographed (silica gel (220 g), hexane/AcOEt 195:5): 17.46–19.15 g (85–93%) of **14** [7]. Pale yellow oil. Purity (GLC) 97.6. $[\alpha]_{589}^{20} = -44.1$ ($c = 1.0$, $CHCl_3$). The anal. data given below resulted from a distilled (250°/1–2 mbar, bulb-to-bulb distillation, 618→521 mg) sample which, however, contained both the (2*R*)- and the (2*S*)-stereoisomer due to thermal epimerization at C(2)[2]). Colourless oil. Purity (GLC) 98.2%. IR (film): 2927*s*, 2867*m*, 1462*s*, 1120*m*, 1100*m*, 845*w*. ^{1}H-NMR (250 MHz, $CDCl_3$): 0.84 (*d*, $J = 7$, 2 *Me*CH); 0.86 (*d*, $J = 7$, 2 *Me*CH); 0.93–1.69 (*m*, 21 aliph. H); 1.34 (*s*, Me–C(2)); 2.12 (*s*, Me–C(7), Me–C(8)); 3.76 (*s*, MeO); 5.58, 6.28 (2*d*, *J*(3,4) = 9.7, H–C(3), H–C(4)); 6.37 (*s*, arom. H). MS: 428 (3.5, M^+), 413 (4, $[M - Me]^+$), 203 (100, $[M - C_{16}H_{33}]^+$), 165 (3, $[C_{10}H_{13}O_2]^+$). Anal. calc. for $C_{29}H_{48}O_2$ (428.70): C 81.25, H 11.29; found: C 81.09, H 11.30.

*(2R,3S,4R)-3-Bromo-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-2*H*-1-benzopyran-4-ol* (**15**) *and (2R,3R,4S)-3-Bromo-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-2*H*-1-benzopyran-4-ol* (**16**). *N*-Bromoacetamide (4.14 g, 30.0 mmol, 1.2 equiv.) was added to the colourless soln. of **14** (10.72 g, 25.0 mmol) in THF (625 ml) and H_2O (250 ml) [8]. After 1 h stirring at r.t., the soln. turned yellow. TLC Control (CH_2Cl_2): R_f 0.78 (**14**), 0.32 (**15**), 0.23 (**16**). After a total of 6 h stirring at r.t., H_2O (500 ml) was added, the mixture extracted with AcOEt (3 × 200 ml), and the org. phase washed with H_2O (200 ml), dried ($MgSO_4$), and evaporated. Careful chromatography of the dark-brown smelly oil (13.25 g; **15**/**16** 8:6, t_R 32.3 (**15**), 30.6 min (**16**), SFC) on silica gel (1 kg, hexane/AcOEt 98:2→97:3; TLC (hexane/AcOEt 3:1); R_f 0.47 (**15**), 0.43 (**16**)) gave first 5.83 g (44%) of **15** as a pale yellow viscous oil (96.1% chemical and 99.8% diastereoisomeric purity[3]),

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[2]) Caution: heating (vacuum distillation, 250°) of this compound results in epimerization at C(2).

[3]) Chemical purity means content of **15**/**16**, diastereoisomeric purity corresponds to the ratio of the two stereoisomers **15** and **16** in this procedure.

SFC), then 1.17 g (9%) of oily **15**/**16** (*ca.* 1:3), and then 3.69 g (28%) of **16** as yellowish tacky crystals (97.3% chemical and 99.2% diastereoisomeric purity[3]). Total yield 81%. Pure **16** was obtained by recrystallization of crude **16** (9.5 g; from several batches) from pentane (150 ml): 100% chemical and 99.5% diastereoisomeric purity[3]).

Data of **15** (from another experiment which gave material of 95.4 chemical and 100% diastereoisomeric purity[3])): $[\alpha]_{589}^{20} = +14.0$ ($c = 1.0$, CH_2Cl_2). IR (film): 3278*s* (OH), 2926*s*, 1463*s*, 1422*s*, 1379*m*, 1266*m*, 1238*m* (aryl ether), 1127*s*, 1102*s* (OH), 811*w*. ^{1}H-NMR (250 MHz, $CDCl_3$): 0.85 (*d*, $J = 6.5$, *Me*CH); 0.87 (*d*, $J = 6.5$, 2 *Me*CH); 0.88 (*d*, $J = 6.5$, *Me*CH); 0.96–1.62 (*m*, 19 aliph. H); 1.32 (*s*, Me–C(2)); 1.84 (*t*, $J = 8$, CH_2(1′)); 2.09, 2.13 (2*s*, Me–C(7), Me–C(8)); 2.41 (*d*, $J = 5$, OH); 3.79 (*s*, MeO); 4.19 (*d*, $J = 9$, CHBr); 4.94 (*dd*, $J = 9$, 5, C*H*OH); 6.81 (*s*, arom. H). MS: 526, 524 (18, 17, M^+), 444 (10, $[M - HBr]^+$), 4.15 (13, $[M - HBr - CHO]^+$), 283, 281 (3, 3, $[M - C_{16}H_{33} - H_2O]^+$), 219 (11, $[M - C_{16}H_{33} - HBr]^+$), 203 (33, $[M - C_{16}H_{33} - HOBr]^+$), 181 (100), 180 ($C_{10}H_{12}O_3$), 165 (21, $C_{10}H_{13}O_2$), 153 (13). Anal. calc. for $C_{29}H_{49}BrO_3$ (525.61): C 66.27, H 9.40, Br 15.20; found: C 66.14, H 9.55, Br 15.24.

Data of **16**: Pale yellow crystals. M.p. 75.0°. $[\alpha]_{589}^{20} = +6.6$ ($c = 1.0$, CH_2Cl_2). IR (nujol): 3391*s* (OH), 2925*s*, 1466*s*, 1380*m*, 1268*m*, 1234*m*, 1127*s*, 1104*m* (OH); 838*w*. ^{1}H-NMR (250 MHz, $CDCl_3$): 0.80 (*d*, $J = 6.5$, *Me*CH); 0.82 (*d*, $J = 6.5$, *Me*CH); 0.86 (*d*, $J = 6.5$, 2 *Me*CH); 0.90–1.45 (*m*, 19 aliph. H); 1.45–1.65 (*m*, H_B–C(1′)); 1.54 (*s*, Me–C(2)); 1.80 (*ddd*, H_A–C(1′)); 2.11, 2.13 (2*s*, Me–C(7), Me–C(8)); 2.38 (*d*, $J = 5$, OH); 3.79 (*s*, MeO); 4.16 (*d*, $J = 9$, CHBr); 4.94 (*dd*, $J = 9$, 5, C*H*OH); 6.78 (*s*, arom. H). MS: No difference to the MS of **15**. Anal. calc. for $C_{29}H_{49}BrO_3$ (525.61): C 66.27, H 9.40, Br 15.20; found: C 66.33, H 9.49, Br 15.13.

Recrystallization of a sample of **16** from MeOH/H_2O gave colourless long needles for X-ray analysis. M.p. 76.0°.

*(2*R*,3*R*)-3,4-Dihydro-6-methoxy-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-2*H*-1-benzopyran-3-ol* (**18**). 1M $LiEt_3BH$ in THF (36 ml, 3 equiv. of hydride) was added dropwise into the stirred soln. of **15** (6.31 g, 12.0 mmol; *i.e.* 6.61 g of 95.4% material) in THF (100 ml) at −78° during 20 min. The soln. was allowed to warm up to r.t. overnight (16–20 h), and H_2O (5 ml) and AcOH (10 ml) were added subsequently to hydrolyze the intermediates (boric esters/complexes, detected by NMR and MS in isolated products from previous experiments). After stirring for 1 h at r.t., the mixture was diluted with Et_2O (200 ml), the org. phase washed with 2N NaOH (2 × 100 ml) and H_2O (100 ml), the aq. phase extracted with Et_2O (150 and 100 ml), the combined org. extract dried ($MgSO_4$) and evaporated, and the crude oil (*ca.* 6.5 g) chromatographed (silica gel (800 g), hexane/AcOEt 19:1→9:1; TLC (hexane/AcOEt 3:1): R_f 0.47 (**15**), 0.35 (**18**)): 5.11–5.36 g (95–100%; 1-mmol experiments gave 70–81%) of **18** as a colourless oil; chemical purity 99.5–99.8% (GLC), diastereoisomeric purity at C(3) > 99.7% (HPLC, 3.5% AcOEt +0.2% i-PrOH in hexane). $[\alpha]_{589}^{20} = -0.71$ ($c = 0.7$, CH_2Cl_2). IR (film): 3361*s* (OH), 2926*s*, 1464*s*, 1423*m*, 1378*m*, 1237*m* (aryl ether), 1126*m*, 1098*m*, 1056*w*, 829*w*. UV (hexane): 291 (3700). ^{1}H-NMR (250 MHz, $CDCl_3$): 0.84, 0.87 (2*d*, 4 *Me*CH); 0.96–1.62 (*m*, *ca.* 19 aliph. H); 1.19 (*s*, Me–C(2)); 1.69 (*t*′, $J = 7.5$, CH_2(1′)); 1.74 (*s*, OH); 2.12, 2.13 (2*s*, Me–C(7), Me–C(8)); 2.74 (*dd*, H_β–C(4)); 3.05 (*dd*, H_α–C(4)); 3.75 (*s*, MeO); 3.80 (*t*′, C*H*OH); 6.40 (*s*, arom. H); $J(3,4\alpha) = 4.7$, $J(3,4\beta) = 4.2$, $J(4\alpha,4\beta) = 17.0$. MS: 446 (24, M^+), 428 (8, $[M - H_2O]^+$), 413 (2, $[M - H_2O - Me]^+$), 203 (65, $[M - C_{16}H_{33} - H_2O]^+$), 165 (100, $[C_{10}H_{13}O_2]^+$). Anal. calc. for $C_{29}H_{50}O_3$ (446.72): C 77.97, H 11.28; found: C 77.82, H 11.49.

*(2*R*,3*R*)-3,4-Dihydro-6-methoxy-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-2*H*-1-benzopyran-3-yl Trifluoromethanesulfonate* (**19**). Trifluormethanesulfonic anhydride (0.20 ml, 339 mg, 1.2 mmol) was added to the stirred soln. of **18** (447 mg, 1.0 mmol) and 2,6-dimethylpyridine (0.17 ml, 161 mg, 1.5 mmol) at −78° (→white precipitate). The mixture was allowed to warm up to 0° and stirred for 1 h (TLC control (hexane/AcOEt 9:1): R_f 0.49 (**19**), 0.10 (**18**)), diluted with cold Et_2O (100 ml), and washed in turn with 2N H_2SO_4, sat. $NaHCO_3$ soln., and H_2O (50 ml of 0° each). The aq. phases were extracted with Et_2O (50 ml) and the combined org. extracts dried ($MgSO_4$) and evaporated (temp. < 20°). The crude turbid colourless oil (560–600 mg, *ca.* 100%) was characterized and used without further purification in the next step. Attempted column chromatography (silica gel), workup at higher temp., and standing at r.t. led to rapid decomposition. IR (film): 2927*s*, 1465*m*, 1414*s*, 1245*m*, 1211*s* (SO_2), 1146*s*, 914*s*, 609*w*. ^{1}H-NMR (250 MHz, $CDCl_3$): 0.84 (*d*, $J \approx 6.5$, *Me*CH); 0.85 (*d*, $J \approx 6.5$, *Me*CH); 0.87 (2*d*, $J \approx 6.5$, 2 *Me*CH); 0.93–1.80 (*m*, *ca.* 21 aliph. H); 1.35 (*s*, Me–C(2)); 2.12 (*s*, Me–C(7), Me–C(8)); 3.11 (*dd*, H_B–C(4)); 3.27 (*dd*, H_A–C(4)); 3.75 (*s*, MeO); 5.08 (*t*′, CH–O); 6.38 (*s*, arom. H); $J(3,4A) \approx J(3,4B) \approx 6.5$, $J(4A,4B) = 17$. MS: 578 (1.5, M^+), 428 (38, $[M - HOSO_2CF_3]^+$), 413 (5, $[M - Me - HOSO_2CF_3]^+$), 203 (100, $[M - C_{16}H_{33} - HOSO_2CF_3]^+$), 165 (36, $[C_{10}H_{13}O_2]^+$), 69 (40, $[CF_3]^+$).

*(2*R*,3*R*)-3,4-Dihydro-6-methoxy-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-2*H*-1-benzopyran-3-yl Toluene-4-sulfonate* (**20**). The soln. of **18** (447 mg, 1.0 mmol), Et_3N (0.42 ml, 304 mg, 3.0 mmol), and TsCl (381 mg, 2.0 mmol) in pyridine (20 ml) was refluxed for 3 h (TLC control (hexane/AcOEt 9:1): R_f 0.66 (**14**), 0.23 (**20**), 0.08 (**18**)). Additonal TsCl (191 mg, 1.0 mmol) was added and heating continued for 9 h. The dark-brown mixture

was diluted with CH_2Cl_2 (50 ml), washed successively with 2N H_2SO_4, sat. $NaHCO_3$ soln., and H_2O (100 ml each), the aq. phase extracted with CH_2Cl_2 (50 ml), and the combined org. extract dried ($MgSO_4$) and evaporated. Column chromatography (silica gel (200 g), hexane/AcOEt 9:1) afforded, after a fraction containing **14**, 393 mg (65%) of **20** as a colourless oil which crystallized upon standing: off-white crystals. M.p. 84–85°. Purity 98.6% (SFC). Data given below originate from this material. $[\alpha]_{589}^{20} = -16.2$ ($c = 1.0$, CH_2Cl_2). IR (KBr): 2924*s*, 1596*w*, 1465*m*, 1366*m*, 1175*s* (aryl-SO_2), 1099*m*, 896*s*, 814*m* (*p*-disubst. benzene), 738*m*, 664*m*, 554*m*. ^{1}H-NMR (250 MHz, $CDCl_3$): 0.81 (*d*, *Me*CH); 0.84 (*d*, *Me*CH); 0.87 (2*d*, 2 *Me*CH); 0.92–1.64 (*m*, *ca.* 21 aliph. H); 1.13 (*s*, Me–C(2)); 2.07, 2.09 (2*s*, Me–C(7), Me–C(8)); 2.45 (*s*, *Me*C_6H_4); 2.94 (*dd*, H_B–C(4)); 3.06 (*dd*, H_A–C(4)); 3.72 (*s*, MeO); 4.63 (*dd*, CH–O); 6.28 (*s*, arom. H); 7.33 (*XX'* of *AA'XX'*, 2 H); 7.79 (*AA'* of *AA'XX'*, 2 H); $J(3,4A) = 6$, $J(3,4B) = 7$, $J(4A,4B) = 17$. MS: 600 (14, M^+), 428 (22, $[M - TsOH]^+$), 413 (2, $[M - TsOH - Me]^+$), 203 (100, $[M - C_{16}H_{33} - TsOH]^+$), 165 (32, $[C_{10}H_{13}O_2]^+$), 91 (16, $[C_7H_7]^+$). Anal. calc. for $C_{36}H_{56}O_5S$ (600.90): C 71.96, H 9.39, S 5.34; found: C 71.77, H 9.26, S 5.40.

Recrystallization of a sample of **20** from MeOH gave colourless, thin plates for X-ray analysis. M.p. 85.5–86.0°.

Single-Crystal X-Ray Analyses of **16** *and* **20**[4]). The intensity data for the single-crystal X-ray analyses were measured on an *Enraf-Nonius-CAD-4* diffractometer (graphite-monochromated CuK_α radiation, ω-2θ scans). The data were corrected for absorption. The structures were solved by a multiple-solution procedure [14] and refined by full-matrix least squares. In the final refinement, the non-H-atoms were refined anisotropically. The H-atoms were included in the structure-factor calculations, but their parameters were not refined. The absolute configurations of **16** and **20** were based on the anomalous scattering of the Br- and S-atom, resp., and were established by refining both enantiomers in each case. The final weighted R values were 0.0559 and 0.0708, resp., for the configurations shown in *Figs. 2* and *3*, and 0.0581 and 0.0731 for their corresponding antipodes. Thus, by *Hamilton*'s test [15], the configurations shown in *Figs. 2* and *3* are absolute.

Bromoalcohol **16** crystallized in the monoclinic space group $P2_1$ with $a = 5.529(1)$, $b = 49.866(4)$, $c = 11.039(1)$ Å, $\beta = 103.98(1)°$, $V = 2953.3(6.3)$ Å^3, $Z = 4$, $d_{calc} = 1.182$ g·cm^{-3}, μ (CuK_α) = 20.8 cm^{-1}. The cell unit contained two independent molecules, *i.e.* two molecules not related by crystallographic symmetry. The size of the crystal used for data collection was *ca.* 0.04 × 0.08 × 0.39 mm. Of the 4444 independent reflections for $\theta < 60°$, 3181 were considered observed ($I > 2.0\,\sigma(I)$). Three reflections which were strongly affected by extinction were excluded from the final refinement and difference map. The final discrepancy indices were $R = 0.058$ and $wR = 0.056$ for the 3178 observed reflections. The major peaks (< 0.6 e·Å^{-3}) of the final difference map are near the Br-atoms.

Tosylate **20** crystallized in the monoclinic space group $P2_1$ with $a = 10.005(7)$, $b = 8.667(2)$, $c = 20.004(14)$ Å, $\beta = 93.76(6)°$, $V = 1730.8(6.7)$ Å^3, $Z = 2$, $d_{calc} = 1.153$ g·cm^{-3}, μ (CuK_α) = 10.9 cm^{-1}. In the crystal, most of the alkyl chain was disordered. Perspective drawings of rotamers *A* and *B* are shown in *Fig. 3*. The crystal used for data collection, *ca.* 0.06 × 0.22 × 0.42 mm, was cooled with a N_2 gas stream to 115 K. Of the 3809 independent reflections for $\theta < 75°$, 3078 were considered observed ($I > 3.0\,\sigma(I)$). Twelve reflections which were strongly affected by extinction were excluded from the final refinement and difference map. The final discrepancy indices were $R = 0.060$ and $wR = 0.071$ for the 3066 observed reflections. The major peaks (< 1.1 e·Å^{-3}) of the final difference map were near the S-atom.

*(2*R*,3*S*)-3,4-Dihydro-6-methoxy-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-(3-^{2}H)-2*H*-1-benzopyran* (= *(2*R*,3*S*,4'*R*,8'*R*)-6-O-Methyl-(3-^{2}H)-γ-tocopherol*; (3*S*)-(3-^{2}H)-**12**). Dropwise 1M *Super Deuteride* in THF (5 ml, 5 mmol, 10 equiv. of $LiEt_3BD$) was added to the stirred soln. of crude **19** (278 mg, *ca.* 0.48 mmol) at –78°. The soln. was allowed to warm up to r.t. overnight (21 h). TLC control (hexane/AcOEt 9:1): R_f 0.54 (**14** and **12**; **14** turns red immediately, **12** yellow slowly upon heating the plate treated with cerium(IV) sulfate/ammonium heptamolybdate reagent), 0.44 (**19**), 0.06 (**18**). After careful hydrolysis with 2N H_2SO_4 (5 ml) at 0°, the mixture was diluted with Et_2O (30 ml) and washed subsequently with H_2O and sat. $NaHCO_3$ soln. (30 ml each). The aq. layers were extracted with Et_2O (30 ml) and the combined org. extracts dried ($MgSO_4$) and evaporated. The crude colourless oil (*ca.* 230 mg) was chromatographed (silica gel (50 g), hexane/AcOEt 9:1) to give first 156 mg of colourless oil containing **14** (yield 65% by GLC), (3-^{2}H)-**12** (yield 8% by GLC), and three benzofurans (yield *ca.* 2% by GLC), and then 41 mg (19%) of **18** as a colourless oil. A part of the oil from the first fraction was separated by HPLC (*Hibar* 25 cm × 4 mm column, *Spherisorb Si 60* 5 μm (*E. Merck*), flow 1 ml·min^{-1}, 1% $(i\text{-}Pr)_2O$ in hexane, detection at 284 nm) to afford a pure sample of (3-^{2}H)-**12**.

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[4]) Details of the crystal-structure investigations (table of final atomic parameters, anisotropic thermal parameters, bond distances, and angles) are available on request from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, England.

Treatment of tosylate **20** with 20 equiv. of $LiEt_3BD$ (5 d, reflux) gave, after identical workup, 32% of **14**, 8% of (3-^{2}H)-**12**, 3% of starting material **20**, and 51% of **18**.

*Data of (3-$^2H_1$)-***12** (3*S*): colourless oil. ^{1}H-NMR (400.1 MHz, $CDCl_3$): 0.84 (*d*, *J* = 6.6, *Me*CH); 0.85 (*d*, *J* = 6.4, *Me*CH); 0.86 (*d*, *J* = 6.3, 2 *Me*CH); 0.96–1.62 (*m*, *ca.* 21 aliph. H); 1.24 (*s*, Me–C(2)); 1.77 ('*t*', $J(3,4\alpha) + J(3,4\beta) = 14$, H_β–C(3)); 2.11, 2.12 (2*s*, Me–C(7), Me–C(8)); 2.69 (*AB*, *J* = 6.5, 16, H_β–C(4)); 2.73 (*AB*, *J* = 7.5, 16, H_α–C(4)); 3.75 (*s*, MeO); 6.42 (*s*, arom. H). MS: 431 (48, M^+), 430 (2, M^+ undeuterated), 206 (10, $[M - C_{16}H_{33}]^+$), 165 (100, $[C_{10}H_{13}O_2]^+$), 164 (29, $[C_{10}H_{12}O_2]^+$); D-content > 95%.

*(2*R*,4*R*)-3,4-Dihydro-6-methoxy-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-(4-^{2}H)-2*H*-1-benzopyran* ((4*R*)-(4-^{2}H)-**12**). Obtained from **15** in 12% yield by consecutive treatment with *1*) $LiEt_3BD$, *2*) Tf_2O/2,6-dimethylpyridine, and *3*) $LiEt_3BH$, as described above for (3*S*)-(3-^{2}H)-**12**. Colourless oil. ^{1}H-NMR (400.1 MHz, $CDCl_3$): 0.84 (*d*, *J* = 6.5, *Me*CH); 0.85 (*d*, *J* = 6.3, *Me*CH); 0.87 (*d*, *J* = 6.5, 2 *Me*CH); 0.96–1.63 (*m*, *ca.* 21 aliph. H); 1.24 (*s*, Me–C(2)); 1.71 (*dd*, *AB*, H_α–C(3)); 1.79 (*dd*, *AB*, H_β–C(3)); 2.11, 2.12 (2*s*, Me–C(7), Me–C(8)); 2.68 ('*t*', H–C(4)); 3.75 (*s*, MeO); 6.42 (*s*, arom. H); $J(3\alpha,3\beta) = 13.4$, $J(3\alpha,4) = 6.85$, $J(3\beta,4) = 6.15$. MS: 431 (64, M^+), 430 (2, M^+ undeuterated), 206 (9, $[M - C_{16}H_{33}]^+$), 166 (100, $[C_{10}H_{12}DO_2]^+$), 165 (34, $[C_{10}H_{11}DO_2]^+$); D-content > 96%.

*(2*R*,3*R*,4*S*)-3,4-Dihydro-6-methoxy-2,7,8-trimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-(3,4-$^2H_2$)-2*H*-1-benzopyran* ((3*R*,4*S*)-(3,4-$^2H_2$)-**12**). Obtained from **16** in 15% yield by consecutive treatment with *1*) $LiEt_3BD$, *2*) Tf_2O/2,6-dimethylpyridine and *3*) $LiEt_3BD$, as described above for (3*S*)-(3-^{2}H)-**12**. Colourless oil. ^{1}H-NMR (400.1 MHz, $CDCl_3$): 0.84 (*d*, *J* = 6.5, *Me*CH); 0.85 (*d*, *J* = 6.3, *Me*CH); 0.87 (*d*, *J* = 6.4, 2 *Me*CH); 0.95–1.62 (*m*, *ca.* 21 aliph. H); 1.24 (*s*, Me–C(2)); 1.70 (*d*, $J(3\alpha,4\alpha) = 6.2$, H_α–C(3)); 2.11, 2.12 (2*s*, Me–C(7), Me–C(8)); 2.70 (*d*, H_α–C(4)); 3.75 (*s*, MeO); 6.42 (*s*, arom. H). MS: 432 (67, M^+), 431 (5, M^+ monodeuterated), 207 (10, $[M - C_{16}H_{33}]^+$), 166 (100, $[C_{10}H_{12}DO_2]^+$), 165 (32, $[C_{10}H_{11}DO_2]^+$); D_2-content 93%, D_1-content 7%.

*(2*RS*,4′*R*,8′*R*)-2-*ambo*-γ-Tocopherol* ((2*RS*,4′*R*,8′*R*)-**1**). To a stirred soln. of 2,3-dimethylhydroquinone (13.8 g, 0.1 mol) in HCOOH (70 ml) was added over 2 h at 50° natural *trans*-phytol (29.65 g, 0.1 mol). The mixture was refluxed for 2.5 h, cooled to r.t., transferred to a round-bottom flask using toluene (100 ml), and concentrated at 45° under reduced pressure. The concentrate was diluted with MeOH (130 ml) and 5N H_2SO_4 (27 ml), and the mixture refluxed for 2 h and cooled to r.t. After the addition of hexane (200 ml), the mixture was extracted 3 times with *ca.* 20 ml each of 80% aq. MeOH. The MeOH phases were discarded and the upper layers combined, dried ($MgSO_4$), and evaporated. The dark brown oil (41.2 g) was chromatographed (silica gel (1 kg), hexane/Et_2O 9:1): 31.7 g (*ca.* 75%) of (2*RS*,4′*R*,8′*R*)-**1**. Red-brown oil. Purity 98.8% (GLC). This material was used directly for the next step (preparation of the camphanates **24**/**25**).

*5-[(7′*R*,11′*R*)-2′,3′-Epoxyphytyl]-2,3-dimethyl-1,4-hydroquinone Diacetate* (= *5-[(7′*R*,11′*R*)-2′,3′-Epoxyphytyl]-2,3-dimethylbenzene-1,4-diyl Diacetate*; **21**/**22**). To a 2-phase system consisting of CH_2Cl_2 and sat. Na_2CO_3 soln. (1 ml each) were added at 0° 80% 3-chloroperbenzoic acid (27 mg, 0.13 mmol) and **5** (45 mg, 0.09 mmol; obtained by acetylation of **2** [1]). The cooling bath was removed and the mixture stirred at r.t. After 20 min, the mixture was extracted with CH_2Cl_2 (3 × 10 ml), the combined org. phase dried ($MgSO_4$) and evaporated, and the residue purified on 2 anal. TLC plates (*Merck*, F_{254}, 20 by 20 cm, hexane/AcOEt 8:2): **21**/**22** (38 mg, 82%). Colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 6.78 (*s*, H–C(6)); 2.83 (*t*, $^3J = 5.88$, H–C(2′)); 2.69 (*dd*, $^2J(1a',1b') = 15.10$, $^3J(1a',2') = 5.92$, H_a–C(1′)); 2.59 (*dd*, $^2J(1a',1b') = 15.10$, $^3J(1b',2') = 5.59$, H_b–C(1′)); 2.29, 2.24 (2*s*, 2 Ac); 2.00 (*s*, Me–C(3), Me–C(4)); 1.44–0.98 (*m*, 25 H); 0.80–0.75 (*m*, Me–C(7′), Me–C(11′), Me–C(15′), Me(16′)). CI-MS: 536 (7), 535 (37), 534 (100, $[M + NH_4]^+$), 409 (6), 408 (23), 330 (7), 308 (7), 296 (8), 262 (16), 69 (9).

*5-[(3′*RS*,7′*R*,11′*R*)-3′-Hydroxyphytyl]-2,3-dimethyl-1,4-benzoquinone* ((3′*RS*)-**23**). To a soln. of **21**/**22** (5 mg, 9.7 μmol) in dry Et_2O (1 ml) was added $LiAlH_4$ (2 mg, 53 μmol) under Ar and stirred at 0° for 2 h. The reaction was then quenched with ice (1 g) and sat. *Seignette*-salt soln. (1 ml) and stirring continued for a further 10 min. Extraction of the mixture with Et_2O (2 × 10 ml), drying of the combined org. layers, evaporation, purification of the residue on 1 anal. TLC plate (*Merck*, F_{254}, 20 by 20 cm, hexane/AcOEt 8:2), and oxidation by air furnished 3.5 mg (83%) of (3′*RS*)-**23**. Yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 6.46 (*s*, H–C(6)); 2.42 (*m*, 2 H–C(1′)); 1.96, 1.94 (2*s*, Me–C(3), Me–C(4)); 1.57–0.97 (*m*, 26 H); 0.81–0.76 (*m*, Me–C(7′), Me–C(11′), Me–C(15′), Me(16′)). EI-MS 432 (0.8, $M^{+\cdot}$), 415 (1.3, $[M - OH]^+$), 207 (17), 164 (66), 43 (100).

The labelled compounds (2′*S*,3′*R*)-(2′-^{2}H)-**23**/(2′*R*,3′*S*)-(2′-^{2}H)-**23** were prepared by using $LiAlD_4$ and transformed further by *1*) $Na_2S_2O_4$ treatment (stirring in $CHCl_3$/EtOH/H_2O), *2*) cyclization with a cat. amount of TsOH [2], *3*) separation *via* the camphanates (*vide infra*), *4*) $LiAlH_4$ treatment in Et_2O, and *5*) methylation (MeI, NaH, THF) to finally yield (3*S*)-(3-^{2}H)-**12** (3.0 mg, 65% based on **23**).

Camphanate **24** *of γ-Tocopherol*. A soln. of natural γ-tocopherol (**1**; 58 mg, 0.14 mmol) and (−)-camphanoyl chloride (45 mg, 0.21 mmol, 1.5 equiv.) in dry pyridine (2 ml) was stirred at r.t. overnight. The mixture was then

diluted with Et_2O (50 ml) and washed with 2M HCl (3 × 50 ml). The org. layer was dried ($MgSO_4$), evaporated, and purified by FC (SiO_2, hexane/AcOEt 8:2): **24** (88 mg, 0.14 mmol, 100%). Colourless solid. Pure by TLC (hexane/Et_2O 4:1, R_f 0.14; CH_2Cl_2/hexane 9:1 (2 times), R_f 0.30) and HPLC (*Fig. 4*). M.p. 52–55°.

Camphanates **24**/**25** were prepared by the same procedure from 2-*ambo*-γ-tocopherol (2-*ambo*-**1**). TLC (hexane/Et_2O 4:1): 1 spot. TLC (CH_2Cl_2/hexane 9:1 (2 times)): R_f 0.30 (**24**), 0.27 (**25**); most conveniently, **24**/**25** was separated by HPLC (*Fig. 4*).

Data of **24**: ^{1}H-NMR (300 MHz, $CDCl_3$): 6.51 (*s*, H–C(5)); 2.64 (*t*, ^3J = 6.71, 2 H–C(4)); 2.54–2.45 (*m*, camph.); 2.18–2.09 (*m*, camph.); 2.04, 1.97 (2*s*, Me–C(7), Me–C(8)); 1.95–1.87 (*m*, camph.); 1.78–1.60 (*m*, camph., 2 H–C(3)); 1.46–0.97 (*m*, 20 H); 0.81–0.76 (*m*, Me–C(4′), Me–C(8′), Me–C(12′), Me(13′)). CI-MS: 615 (33), 614 (100, $[M + NH_4]^+$), 597 (5, $[M + H]^+$), 596 (8), 434 (9), 417 (8).

REFERENCES

[1] A. Stocker, A. Rüttimann, W.-D. Woggon, *Helv. Chim. Acta* **1993,** *76,* 1729.

[2] P. Schudel, H. Mayer, J. Metzger, R. Rüegg, O. Isler, *Helv. Chim. Acta* **1963,** *46,* 333; N. Cohen, R.J. Lopresti, Ch. Neukom, *J. Org. Chem.* **1981,** *46,* 2445; J.-M. Santer, R.K. Müller, unpublished results.

[3] B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell, Eds., 'Vogel's Textbook of Practical Organic Chemistry', 5th edn., Longman Scientific & Technical, Harlow, Essex, 1989, p. 939.

[4] J.S. Amato, S. Karady, M. Sletzinger, L.M. Weinstock, *Synthesis* **1979,** 970.

[5] O. Isler, R. Rüegg, L. Chopard-dit-Jean, H. Wagner, K. Bernhard, *Helv. Chim. Acta* **1956,** *39,* 897.

[6] H. Diehl, C.C. Hach, *Inorg. Synth.* **1950,** *3,* 196.

[7] H. Mayer, O. Isler, *Methods Enzymol.* **1971,** *18,* 241; L. Hughes, M. Slaby, G.W. Burton, K.U. Ingold, *J. Labelled Compd. Radiopharm.* **1990,** *28,* 1049.

[8] Sh.K. Agarwal, D.R. Boyd, R. Dunlop, W.B. Jennings, *J. Chem. Soc., Perkin Trans. 1* **1988,** 3013; R.E. Lehr, P.L. Kole, M. Singh, K.D. Tschappat, *J. Org. Chem.* **1989,** *54,* 850.

[9] A.J. Birch, K.A.M. Walker, *Tetrahedron Lett.* **1966,** 4939; A.J. Birch, K.A.M. Walker, *J. Chem. Soc. (C)* **1966,** 1894; J.-F. Biellmann, H. Liesenfelt, *Bull. Soc. Chim. Fr.* **1966,** 4029; J.R. Morandi, H.B. Jensen, *J. Org. Chem.* **1969,** *34,* 1889; J.G. Atkinson, M.O. Luke, *Can. J. Chem.* **1970,** *48,* 3580.

[10] G. Derungs, W.-D. Woggon, in preparation.

[11] P. Karrer, H. Fritzsche, *Helv. Chim. Acta* **1938,** *21,* 1234.

[12] P. Schudel, G. Ryser, M. Imfeld (*F. Hoffmann-La Roche AG*, Basel), unpublished results.

[13] Th. Netscher, P. Bohrer, R.A. Outten, H. Schneider, R.K. Müller, E. Glinz, W. Walther, U.B. Ranalder, Ischia Advanced School of Organic Chemistry VI, Napoli, Italy, Sept. 25–30, 1994.

[14] P. Main, S. Fiske, S. Hull, L. Lessinger, G. Germain, J.P. Declercq, M. Woolfson, 'MULTAN 11/82', University of York, England, and University of Louvain, Belgium.

[15] W.C. Hamilton, *Acta Crystallogr.* **1965,** *18,* 502.

[16] W. Walther, Th. Netscher, *J. Chromatogr. Sci.*, in press.