Combination of Vitamin E with a Carotenoid: α-Tocopherol and Trolox Linked to β-Apo-8′-carotenoic Acid

Eva Larsen, Jan Abendroth, Vassilia Partali, Bernhard Schulz, Hans-Richard Sliwka,* and Edward G. K. Quartey

Abstract: Vitamin E and carotenoids show many similar and complementary properties and protect living tissues against a variety of pathological processes. A mixture of both compounds often exhibits a significantly greater effect than the sum of the individual activities. The synthetic linkage of carotenoids with vitamin E might thus increase the synergistic effects. We therefore esterified β-apo-8′-carotenoic acid with all-*rac*-α-tocopherol to give α-tocopheryl-β-apo-8′-carotenoate. The carotenoic acid was also connected to α-tocopherol in a glyceride ether, 1-*O*-(α-tocopheryl)-3-(β-apo-8′-carotenoyl)-glycerol, whereas the water-soluble vitamin E analogue, trolox, was combined with β-apo-8′-carotenoic acid in a diglyceride: 1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-acyl)-3-(β-apo-8′-carotenoyl)-glycerol.

Keywords: carotenoids · condensation reaction · lipids · vitamins · synergism

Introduction

Carotenoids are antioxidants[1] and protect living tissue directly or as immunopotentiators against a variety of diseases.[2–6] They are used as food colorings, internal or external sunscreens, food stabilizers, cosmetics, and as nutritional supplements for animals and humans.[7–15]

β-Apo-8′-carotenoic acid (**1**) is a naturally occurring carotenoid,[16] which is also commercially available as the ethyl ester (C_{30} ester) **2**.[17] Ester **2** is mainly utilized as a food coloring (E160f) and for the pigmentation of poultry and eggs.[16,18,19] In a similar manner to other carotenoids, ester **2** is used in dermatological applications.[20] Acid **1** and ester **2**, despite known vitamin A activity,[16] are not detectably metabolized to retinol in humans.[21,22]

The vitamin E isomers, particularly α-tocopherol, are antioxidants and, like carotenoids, protect against many diseases.[23,24] α-Tocopherol (**3**) is used as a food stabilizer (E307) and as an agent in sunscreens.[25,26] Toxic effects of vitamin E are not known.[27,28] Vitamin E, like C_{30} ester **2**, is added to poultry feed.[29]

[*] H.-R. Sliwka, V. Partali, E. Larsen, J. Abendroth, E. G. K. Quartey
Norwegian University of Science and Technology
Department of Chemistry
Rosenborg, N-7034 Trondheim (Norway)
Fax: Int. code + 47 7359-6255
email: vassilia.partali@chembio.ntnu.no
B. Schulz
Production Fine Chemicals, BASF AG
D-67056 Ludwigshafen (Germany)

The physiological action of vitamin E is generally associated with the free OH group. Protic solvents decrease the antioxidant potential of **3** through formation of solvent hydrogen bonds.[30] Esterification or etherification are assumed to cause complete biological inactivity.[31] Oral applications of tocopherol esters are potent, because they are hydrolyzed in the gut to free tocopherol.[32] Since tocopherol esters are not metabolized to tocopherol on the human skin,[33] the many different topically employed tocopherol esters[34] should therefore be intrinsically inactive. This, however, is in conflict with documented therapeutic effects,[34–36] is contradictory to the widespread utilization of such derivatives, and would reduce vitamin E esters to simple filling ingredients in dermatological and cosmetic formulations. The mechanisms of the topical activity of vitamin E esters still do not seem to be fully understood, and can probably not be ascribed solely to the free phenol group.[31,37]

Ether derivatives of α-tocopherol (**3**) are not expected to be cleaved under physiological conditions and, therefore, should not show activity. It is the etherification of the phenol group which is considered to give tocopherol radical-quenching ability.[38,39] However, recent results demonstrate that α-tocopherol ethers are also effective radical scavengers acting through a charge-transfer mechanism.[40] Consequently, tocopherol ethers provide useful antioxidative protection in cosmetic compositions.[41]

Trolox (**6**), a water-soluble analogue of the lipophilic tocopherols, functions as antioxidant both in aqueous and non-aqueous solutions.[42,43] Trolox is utilized in the treatment of skin, eye, and heart disorders.[44–46]

  0947-6539/98/0401-0113 $ 17.50+.25/0

The similar properties of tocopherol and carotenoids exhibit additive effects when used together.[47] More often, a synergistic effect, due to mutual protection, is observed,[48,49] for example in antioxidants, anticarcinogens, and radioprotective agents.[50–52] Carotenoids are employed together with tocopherol as food protectors, food colorings, preventive medication, sun-tanning formulations, and nutritional supplements in food and feed.[15,18,53–57]

The linkage of vitamin E to carotenoids would provide unique compounds that would eliminate the need for physical mixtures. Such compounds could serve as a single source of both vitamin E and carotenoids. In addition, the close proximity of tocopherol to carotenoids may positively affect the cooperative activity.[58]

We report here on the synthetic combination of α-tocopherol (**3**) and trolox (**6**) with carotenoid **1** by means of direct esterification and linkage to a natural carrier molecule.

Results

α-Tocopheryl retinoate has been synthesized by condensation with trifluoracetic acid[59] or by reacting α-tocopherol (**3**) with retinoic acid chloride.[5] However, α-tocopheryl-apo-8′-carotenoate (**4**) could not be synthesized with trifluoracetic acid nor with β-apo-8′-carotenoic acid chloride.[60] The reaction of acid **1** with phenol **3** in the presence of triphenylphosphine and CCl_4, a specific phenol ester reagent, produced the ester **4** in insufficient yields.[61] If a polyphosphate ester, another reagent for phenol ester synthesis[34,62] was used, then **1** and **3** reacted to give the carotenoid–tocopherol compound **4** in 64% yield (Scheme 1).

β-Apo-8′-carotenoic acid (**1**) could not be obtained from ester **2** by application of the generally recommended conditions for the hydrolysis of carotenoid esters,[63,64] under which the C_{30} ester proved to be virtually inert. Lipase from *Candida Antarctica* B (CAB) slowly hydrolyzed ester **2** to acid **1** at 40 °C, whereas KOH in *iso*-butanol at 85 °C readily reacted with **2** to provide **1** in high yields.

Trolox–phospholipid derivatives have already been obtained by linking the chroman ring to the phosphate moiety.[65] We tried to react the carboxylic acid group of trolox (**6**) selectively with the hydroxyl group at C3 of the monoglycerol **5**, with dicyclohexylcarbodiimide and dimethylaminopyridine as condensing agents[66] and obtained the 1,3-diglyceride **7** (Scheme 2, cf. ref. [67]).

Diglycerides form stable emulsions with water.[68] Trolox–carotene glyceride **7** may therefore be suitable for the preparation of carotenoid–trolox dispersions with a new functionality, since the activity of vitamin E and carotenoids depends on the partial diffusion of the compounds into the lipid and water phase of the emulsified particles.[43,69,70] The carotene–trolox compound **7** will probably be hydrolyzed in the gut in a similar manner to other diglycerides and, consequently, the constituent compounds of **7** would be released simultaneously at sites which might not be reached by the individual chemicals.

α-Tocopheryl glycidyl ether **9** was formed in a two-phase system from tocopherol **3** and epichlorohydrin (**8**) with NaOH.[71] No reaction was observed with amines as a base.[72] With preformed Na-tocopherolate, only minor amounts of the glycidyl ether **9** were formed.[73] Ring opening to the glycerol ether **10** was achieved with dilute sulfuric acid, while basic conditions failed.[74] Attempts to prepare **10** by hydroxylation of tocopheryl allyl ether were not pursued due to the low yield in the reaction of tocopherol **3** with allyl bromide or iodide. The glycerol ether **10** was esterified with C_{30}-ester **2** by CAB lipase in decaline at 30 °C under reduced pressure and gave the tocopherol–carotene compound **11** in 53% yield[75] (Scheme 3).

The ether bond in **11** can be expected to be inert towards lipase and may only be cleaved by special etherases.[76] It is known that alkyl acylglycerols are metabolically concentrated in tumor cells.[76] Glyceryl ethers have previously been synthesized with cyclic alcohols (sterols) and are used in dermatological prescriptions.[77–79] Alkylglycerols and their ester derivatives are nontoxic, even when ingested in large amounts,[80] and they exhibit antitumor and radioprotective effects.[81] These properties are also shown by carotenoids[2,82,83] and vitamin E.[24,84] Etherlipid **10** may therefore represent a compound in which these qualities are combined.

Experimental Section

General: After reaction, the products were absorbed on silica gel, dried in vacuo, and separated by flash chromatography (silica gel 60, Merck) followed, if necessary, by further purification on preparative or analytical

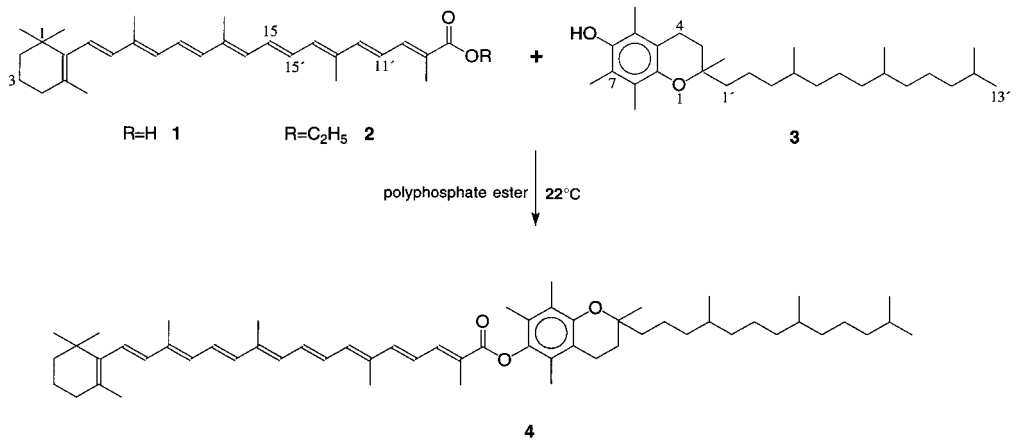
Abstract in German: *Vitamin E und Karotenoide zeigen vielfältige, sich ergänzende Funktionen und schützen Zellen vor einer Vielzahl pathologischer Prozesse. Eine Mischung beider Substanzen erhöht oft die Wirkungsweise weit über eine Addition der Einzelaktivitäten. Die synthetische Verknüpfung von Karotenoiden mit Vitamin E stellt einen Weg dar, den Synergieeffekt möglicherweise zu vergrössern. β-Apo-8′-Karotensäure liess sich direkt mit Tocopherol verestern. Beide Verbindungen konnten auch über ein Äther-Ester-Glycerid miteinander verbunden werden. Das wasserlösliche Vitamin-E-Derivat Trolox wurde mit der Karotensäure in einem Diglycerid vereinigt.*

Abstract in Norwegian: *Vitamin E og karotenoider har både like og komplementære egenskaper. En blanding av begge stoffene har ofte større aktivitet enn summen av de enkelte aktivitetene. Den syntetiske kombinasjon av karotenoider og vitamin E kan øke den synergistiske effekt. β-Apo-8′-karotensyre ble direkte forestret med tokoferol. Begge stoffene ble også knyttet sammen i et eter-ester glyserid. Det vannløslige vitamin E derivatet trolox ble kombinert med karotensyren i et diglyserid.*

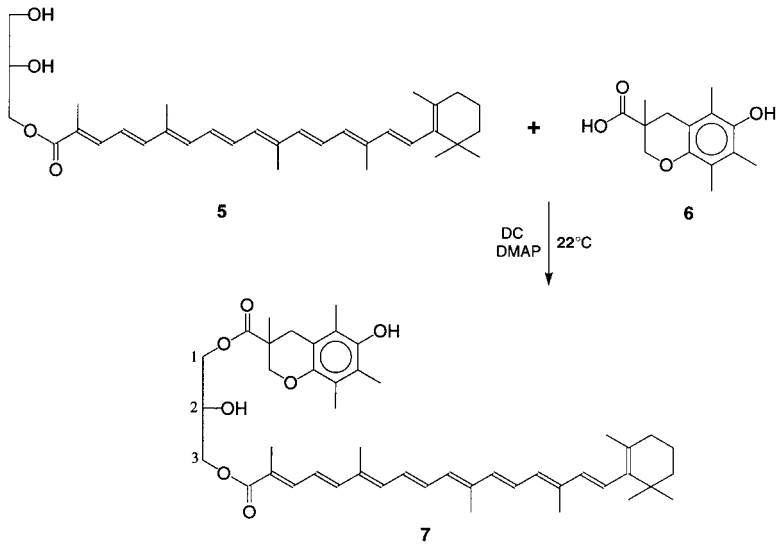
Abstract in Greek:

Η βιταμινη Ε και οι καροτινες εχουν παρομοιες και συμπληρωματικες ιδιοτητες και προστατευουν απο διαφορες παθησεις. Το μειγμα των δυο αυτων χημικων ενωσεων παρουσιαζει συχνα μεγαλυτερη αποτελεσματικοτητα απ′ οτι το αθροισμα των μεμονωμενων αποτελεσματικοτητων. Το συνθετικο ζευγαρωμα της καροτινης με την βιταμινη Ε θα μπορουσε να αυξηση το συνεργειακο φαινομενο των δυο αυτων ουσιων. Το β–Απο–καροτινικο οξυ εστεροποιηθηκε απ′ ευθειας με την βιταμινη Ε. Οι δυο αυτες χημικες ενωσεις συνδυαστηκαν επισης σ′ ενα αιθερα–εστερα–γλυκεριδιο. Το τρολοξ, που ειναι ενα υδατοδιαλυτο αναλογο της βιταμινης Ε, ενωθηκε με το καροτινικο οξυ σ′ ενα διγλυκεριδιο.

0947-6539/98/0401-0114 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 1



Scheme 1. Synthesis of α-tocopheryl-apo-8′-carotenoate (**4**).



Scheme 2. Synthesis of 1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-acyl)-3-(β-apo-8′-carotenoyl)-glycerol **7**.

TLC plates (silica gel 60, Merck) with hexane/heptane – acetone mixtures. Mass spectra (EI, IP 70 eV, 210 °C): only prominent or diagnostically useful peaks are reported. Small yields of carotenoid compounds were determined from the Vis spectra[85] in CH_2Cl_2, if not otherwise stated.

β-Apo-8′-carotenoic acid (**1**):

Method A: C_{30} ester **2** (BASF) (350 mg, 0.76 mmol), lipase CAB (2 g), and water (10 mL) were stirred in decahydronaphthaline (50 mL) for 15 days. Chromatographic work-up afforded **1** (135 mg, 41 %).

Method B: C_{30} ester **2** (40 g, 0.087 mol) was dissolved in 2-methyl-1-propanol (*iso*-butanol, 300 mL). Aqueous KOH (25 %, 100 mL) was added, and the solution stirred at 85 °C. After 2 h the ester **2** was completely hydrolyzed (HPLC evidence). After addition of H_2SO_4 (20 %, 300 mL), the mixture was stirred at 70 °C. The organic layer was separated and washed with H_2O (3×500 mL) at 70 °C. Water was removed by azeotropic distillation with toluene (70 °C, 100 mbar). The residue was stirred in dry *iso*-butanol (200 mL) at 20 °C for 24 h, filtered, and washed with methanol (3×200 mL) to give **1** (33.9 g, 90 %). Recrystallization from 3-pentanone gave pure **1**. MS (ESI): $m/z = 863$ [$2M^+ - H$], 431 [$M^+ - H$], 339 [$M^+ - H - 92$]; Vis (acetone): $\lambda_{max} = 440$ nm; ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 1.03$ (s, 12H, $2 \times CH_3$, C1), 1.46 (m, 2H, C2), 1.59 (m, 2H, C3), 1.69 (s, 3H, CH_3-C5), 1.91 (s, 3H, CH_3, C9′), 1.95 (s, 3H, CH_3, C13′), 1.96 (s, 6H, $2 \times CH_3$, C9, C13), 2.01 (m, 2H, C4), 6.1 – 6.8 (m, 11H, olefinic protons), 7.20 (d, 1H, C10′); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 12.42$ (CH_3, C9′), 12.46 (CH_3, C13), 12.57 (CH_3, C13′), 12.65 (CH_3, C9), 18.74 (C3), 21.40 (CH_3, C5), 28.78 ($2 \times CH_3$, C1), 32.59 (C4), 33.84 (C1), 39.30 (C2), 123.41 (C10′), 125.65 (C11), 126.29 (C7), 126.57 (C9′), 128.94 (C5), 129. 81 (C10), 130.92 (C15′), 131.96 (C15), 132.19 (C14), 135.39 (C14′), 135.48 (C13, C13′), 137.07, 137.20 (C8, C9, C12), 137.41 (C6), 138.0 (C11′), 143.32 (C12′), 169.02 (C8′).

α-Tocopheryl β-apo-8′-carotenoate (**4**):

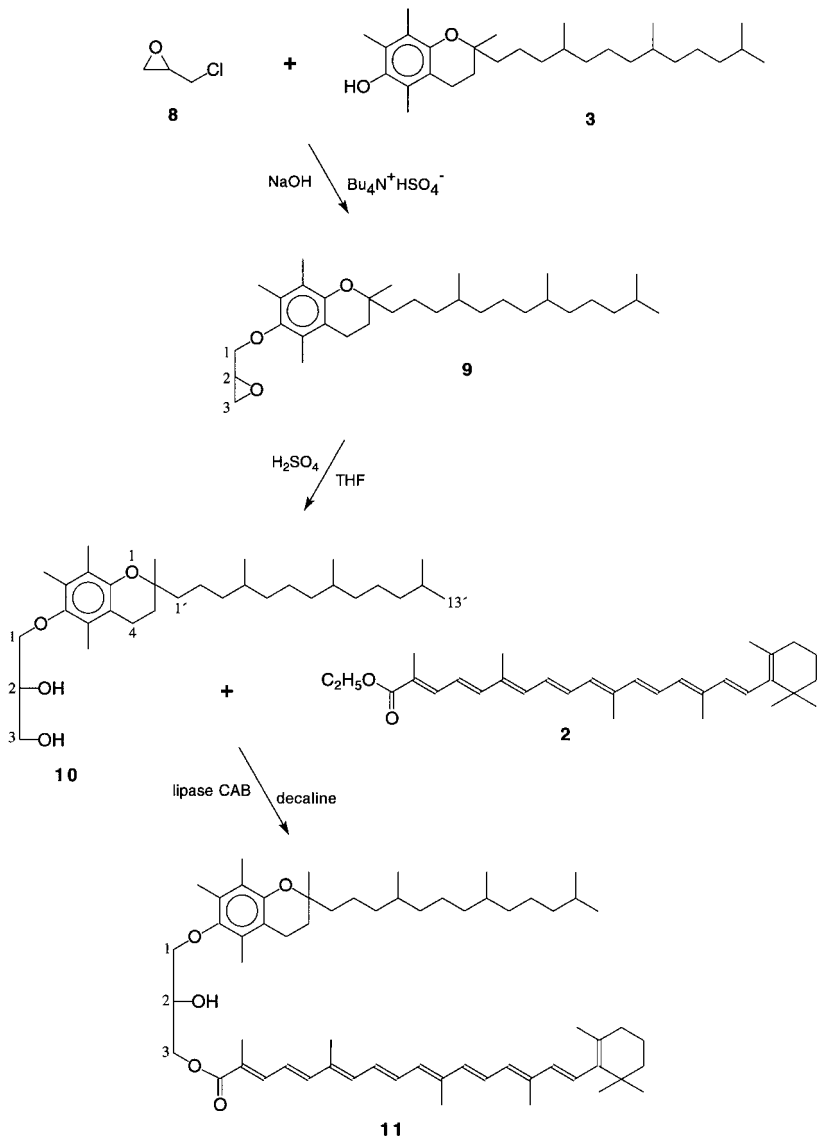
Method A: A solution of β-apo-8′-carotenoic acid (**1**, 140 mg, 0.32 mmol), all-*rac*-α-tocopherol (**3**, 140 mg, 0.32 mmol), and a polyphosphate ester[62] (300 mg) in CH_2Cl_2 (25 mL) were stirred for 2 days at 22 °C. Chromatographic work-up afforded **4** (175 mg, 64 %).

Method B: A solution of triphenylphosphine (13 mg, 0.05 mmol) and CCl_4 (5 μL, 0.05 mmol) in CH_2Cl_2 was added to a solution of **1** (20 mg, 0.05 mmol), **3** (21 mg, 0.05 mmol), triethylamine (0.1 mL, 0.72 mmol) in CH_2Cl_2.[61] After stirring at 22 °C for 5 days, insufficient formation of **4** was detected by TLC.

Ester 4: MS: $m/z = 844$ [M^+], 752 [$M^+ - 92$], 430 [$M^+ - 414$ (**1** $- H_2O$)], 415 [**3** – 15], 323 [415 – 92]; IR (film on KBr): $\tilde{\nu} = 1700$ cm^{-1} (conj. C=O); Vis: $\lambda_{max} = 455$ nm (in accordance with **2**); ^{1}H NMR (400 MHz, $CDCl_3$): tocopheryl moiety: $\delta = 0.75 - 0.81$ (12H, $4 \times CH_3$,4a′,8a′,12a′,13′), 1.0 – 1.6 (m, 21H, $3 \times$ CH and $9 \times CH_2$, phytyl chain), 1.18 (s, 3H, 2a), 1.75 (m, 2H, C3), 2.02 – 2.07 (9H, $3 \times CH_3$, 5a, 7a, 8b), 2.52 (t, 2H, C4); carotenoyl moiety: $\delta = 0.96$ (s, 6H, $2 \times CH_3$, C1), 1,40 (m, 2H, C2), 1.64 (m, 2H, C3), 1.65 (s, 3H, CH_3, C5), 1.91 – 1.95 (12H, $4 \times CH_3$, olefin chain), 1.97 (m, 2H, C4), 6.1 – 6.8 (m, 12H, olefinic protons); ^{13}C NMR (100 MHz, $CDCl_3$): in accordance with **2** and **3**.

1-(6-hydroxy-2,5,7,8-tetramethylchroman-2-acyl)-3-(β-apo-8′-carotenoyl)-glycerol (**7**): β-Apo-8′-carotenoylglycerol (**5**)[75] (7 mg, 0.014 mmol), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, **6**, 9 mg, 0.036 mmol) were dissolved in a mixture of CH_2Cl_2 (2 mL) and acetone (5 mL). 4-Dimethylaminopyridine (DMAP; 7 mg, 0.06 mmol) and dicyclohexylcarbodiimide (Dcc; 11 mg, 0.104 mmol) dissolved in CH_2Cl_2 (3 mL) were added and the solution stirred for 48 h at 22 °C.[66] Chromatographic work-up gave unreacted **5** (2 mg) and product **7** (1 mg, 14 %). MS: $m/z = 738$ [M^+], 505 [$M^+ - 233$ (troloxacyl)], 430 [$505 - 75$ ($C_3H_7O_2$)]; Vis: $\lambda_{max} =$ 456 nm; ^{1}H NMR (500 MHz, $CDCl_3$): glycerol moiety: $\delta = 4.25$ (m, 1H, C2), 4.16, 3.99, 3.92, 3.84 (m, 4H, C2, C3), 3,66 (m, 2H, C1), 3.77 – 4.0 (m, 2H, C2, C3), 4.14 – 4.27 (m, 1H, C3); trolox moiety: $\delta = 1.22$ (s, CH_3, 2a), 2.09, 2.12 (9H, $3 \times CH_3$, 5a, 7a, 8b), 1.85 (m, 1H, C3), 2.45 (m, 1H, C3), 2.56 (m, 2H, C4); carotenoyl moiety: in accordance with spectra of **2**.

Tocopheryl glycidyl ether (**9**): To a mixture of epichlorohydrin (**8**, 0.63 mL, 8.0 mmol), all-*rac*-α-tocopherol (**3**, 3.1 g, 7.10 mmol) and tetrabutylammonium hydrogensulfate (0.13 g) was added dropwise at 0 °C to an aqueous solution of KOH (50 %, 3.7 mL).[71,78] After stirring for 30 min at 0 °C and 4 h at RT, extractive work-up gave ether **9** (3.1 g, 87 %). ^{1}H NMR ($CDCl_3$, 300 MHz), glycidyl moiety (ABMXY-system): $\delta = 2.71$, 2.87 (dd, 2H, C1, $^2J = 4.9$ Hz, $^3J = 4.7$, 2.7 Hz), 3.35 (m, 2H, C2), 3.65, 3.90 (dd, 2H, C1, $^2J =$



Scheme 3. Synthesis of the tocopherol–carotene compound **11**.

11 Hz, $^3J = 5.9, 3.3$ Hz); tocopheryl moiety: in accordance with **3**; ^{13}C NMR (75 MHz, $CDCl_3$), glycidyl moiety: $\delta = 44.6$ (C1), 50.6 (C2), 73.8 (C3); tocopheryl moiety: in accordance with **3**.

1-*O*-(α-Tocopherylglycerol (**10**): Ether **9** (2.1 g, 4.30 mmol) was refluxed for 24 h with 3N H_2SO_4 (205 mL, 90% THF, H_2O).[74] Extraction with CH_2Cl_2 and chromatographic work-up with ethyl acetate/hexane 3:7 afforded **10** (1.93 g, 89%). MS: $m/z = 504$ [M^+], 430 [$M^+ - 74$ ($C_3H_6O_2$)], 239 [$M^+ - 265$ [$C_{19}H_{37}$ (phytyl chain) and C2, C3 and C2a], 165 [239 – 74 ($C_3H_6O_2$)]; ^{1}H NMR (400 MHz, $CDCl_3$), glycerol moiety (ABMXY system): $\delta = 3.77$ (m, 2H, C3), 3.83 (m, 2H, C1), 4.08 (m, 1H, C2), 2.45, 3.00 (brs, 2 × OH); tocopheryl moiety: in accordance with **3**; ^{13}C NMR (75 MHz, $CDCl_3$), glycerol moiety: $\delta = 73.9$ (C3), 71.3 (C2), 64.1 (C1); tocopheryl moiety: in accordance with **3**. The position of the glycerol protons was verified by ^{1}H–^{1}H and ^{1}H–^{13}C COSY NMR spectra.

1-*O*-(α-tocopheryl)-3-(β-apo-8′-carotenoyl)glycerol (**11**): Ether **10** (403 mg, 0.799 mmol), **2** (365 mg, 0.798 mmol), and lipase CAB (977 mg) were stirred in decahydronaphthaline (15 mL) at 30 °C at reduced pressure (10 Torr) for 5 days.[75] Chromatographic work-up gave unreacted **2** (247 mg) and product **11** (120 mg, 53%). MS: $m/z = 918$ [M^+], 826 [$M^+ - 92$], 504 [$M^+ - 414$ (**1** – H_2O)], 430 [504 – 74 ($C_3H_6O_2$)], 239 [504 – 265 {$C_{19}H_{37}$ (phytyl chain) and C2, C3 and C2a}], 165 [239 – 74 ($C_3H_6O_2$)]; Vis: $\lambda_{max} = 456$ nm; ^{1}H NMR (500 MHz, $CDCl_3$), glycerol moiety (ABMXY system): $\delta = 3.77$ (m, 2H, C1), 4.29 (m, 1H, C2) 4.42 (m, 2H, C3); tocopheryl moiety: in accordance with **3**; carotenoate moiety: in accordance with **2**; ^{13}C NMR (125 MHz, $CDCl_3$): glyceryl moiety: $\delta = 72.93$ (C1), 69.53 (C2), 65.55 (C3); tocopheryl moiety: in accordance with **3**; carotenoate moiety: in accordance with **2**.

We thank B. Olsrød for the mass spectra and Novo-Nordisk A.S., Copenhagen, for a gift of lipase CAB.

Received: July 7, 1997 [F755]

[1] G. J. Handelmann, in *Handbook of Antioxidants* (Eds.: E. Cadenas, L. Packer), Dekker, New York, **1996**, p. 259.
[2] *Carotenoids in Human Health* (Eds.: L. M. Canfield, N. I. Krinsky, J. A. Olson), *Ann. New York Acad. Sci.*, **1993**, Vol. 691.
[3] *Potential Effects of Reducing Carotenoid Levels on Human Health* (Ed.: D. Anderson), Harvard School of Public Health, **1996.**
[4] S. T. Mayne, *FASEB J.* **1996**, *10*, 690.
[5] C. Eugster, C. H. Eugster, W. Haldemann, G. Rivera (Marigen S.A.), USP 5536504, **1997**.
[6] S. Basanowitz, USP 5455280, **1995**; *Chem. Abstr. 123*, P329983x.
[7] J. C. Bauernfeind, G. B. Grubacher, H. M. Kläui, U. L. Marusich, in *Carotenoids* (Ed.: O. Isler), Birkhäuser, Basel, **1971**, p. 743.
[8] H. P. Gollnick, W. Hopfenmüller, S. Hemmes, S. C. Chun, S. Schmid, K. Sundermeier, H. K. Biesalski, *Eur. J. Dermatol.* **1996**, *6*, 200.
[9] K. Wolff, USP 4642318, **1987**.
[10] J. Olivier-Terras (Cosmetics Development Co.), USP 5554374, **1996**.
[11] Suntan preparation *Carroten*, G. Sarantis S.A., Maroussia (Greece).
[12] W. T. Yang, D. B. Min, *ACS Symp. Ser.* **1994**, *15*.
[13] T. W. Antoshkiw, M. A. Cannalonga, A. Koff (Hoffmann-La Roche A.G.), DEP 2534091 **1976**; *Chem. Abstr. 84*, P163172f.
[14] T. Latscha, *Carotenoids in Animal Nutrition*, Hoffmann-La Roche, Basel (Switzerland), **1990**.
[15] S. Sultenfuss, USP 5514382, **1996**; *Chem. Abstr. 125*, P19052q.
[16] J. C. Bauernfeind, *J. Agr. Food Chem.* **1972**, *20*, 456.
[17] BASF AG, Ludwigshafen (Germany).
[18] K. M. Haila, S. M. Lievona, M. I. Heinonen, *J. Agr. Food. Chem.* **1996**, *44*, 2096.
[19] I. Wildfeuer, L. Acker, A. Mehner, W. Rauch, *Z. Lebensm. Unters. Forsch.* **1968**, *136*, 203.
[20] D. Saint-Leger, A. Bague (L′Oréal S.A.), WOP 85/03225, **1985**; *Chem. Abstr. 103*, P220825h.
[21] J. A. Olson, in *Lipid-Soluble Antioxidants—Biochemistry and Clinical Application* (Eds.: A. S. H. Ong, L. Packer), Birkhäuser, Basel, **1992**, pp. 178, 181.
[22] S. Zeng, H. C. Furr, J. A. Olson, *Meth. Enzymol.* **1993**, *214*, 137.
[23] A. Kamal-Eldin, L.-Å. Appelqvist, *Lipids* **1996**, *31*, 671.
[24] L. Packer, in ref. [21], p. 1.
[25] W. F. Voyt, USP 4144325, **1979**; *Chem. Abstr. 91*, 128902s.
[26] R. A. Kulkarni, G. E. Deckner (Richardson–Vicks), USP 5573754, **1996**.
[27] H. S. Garewal, A. T. Diplock, *Drug Safety* **1995**, *13*, 8.
[28] A. Bendich, L. J. Machlin in *Vitamin E in Health and Disease* (Eds.: L. Packer, J. Fuchs), Dekker, New York, **1993**, p. 411.
[29] A. DeWinne, P. Dirinck, *J. Agr. Food Chem.* **1996**, *44*, 1691.
[30] M. Iwatsuki, J. Tsuchiya, E. Komuro, Y. Yamamoto, E. Niki, *Biochem. Biophys. Acta Gen. Subj.* **1994**, *1200*, 19.

0947-6539/98/0401-0116 $ 17.50+.50/0

[31] J. R. Trevithick, H. Xiong, S. Lee, D. T. Shum, S. E. Sanford, S. J. Karlik, C. Norley, G. R. Dilworth, *Arch. Biochem. Biophys.* **1992**, *296*, 575.
[32] M. G. Traber, W. Cohn, D. P. R. Muller, in ref. [28], p. 35.
[33] D. S. Alberts, R. Goldman, M. J. Xu, R. T. Dorr, J. Quinn, K. Welch, J.Guillen-Rodriguez, M. Aickin, Y. M. Peng, L. Loescher, H. Gensler, *Nutr. Cancer Int. J.* **1996**, *26*, 193.
[34] D. L. Bisset, R. D. Bush (Procter & Gamble Co.), USP 4847072, **1989**; *Chem. Abstr*. *112*, P 104589w.
[35] B. A. Jurkiwicz, D. L. Bisset, G. R. Buettner, *J. Invest. Dermatol*. **1995**, *104*, 484.
[36] B. J. Hall, J. A. Baur, G. E. Deckner (Procter & Gamble Co.), USP 5545407, **1996.**
[37] K. Pehr, R. R. Forsey, *Can. Med. Assoc. J.* **1993**, *149,* 1247.
[38] S. Urano, S. Yamaoi, Y. Hattori, M. Matsuo, *Lipids* **1997**, *12*, 105.
[39] R. Yamauchi, N. Miyake, K. Kato, Y. Ueno, *Lipids* **1993**, *28*, 201.
[40] M. P. S. Ishar, R. Kaur, G. Kaur, R. P. Gandhi, *Ind. J. Chem.* **1996**, *35B*, 641.
[41] Y. D. Kim, C. K. Kim, C. N. Lee, B. J. Ha (Pacific Co.), DEP 4232686, **1992**; *Chem. Abstr*. *120*, 37812p.
[42] O. Cynshi, Y. Takashima, Y. Katoh, K. Tamura, M. Sato, Y. Fujira, *J. Biolum. Chemolum.* **1995**, *10*, 261.
[43] S.-W. Huang, E. N. Frankel, J. B. German, *J. Agr. Food Chem*. **1995**, *43*, 2345.
[44] G. E. Deckner, A. C. Georgalas (Charles of the Ritz Group) EP 238302, **1986**; *Chem. Abstr*. *108*, p 81826x.
[45] N. H. Ansari, A. Bhatnagar, E. Fulep, P. Khanna, S. K. Srivasta, *Res. Commun. Chem. Pathol. Pharm*. **1994**, *84*, 93.
[46] D. A. G. Mickle, T. W. Wu (Eastman Kodak), USP 4877810, **1989;** *Chem. Abstr*. *112*, P 104873j.
[47] L. A. Lambert, W. G. Wamer, R. R. Wei, S. Lavu, S. J. Chirtel, A. Kornhauser, *Nutr. Cancer Int. J.* **1994**, *21*, 1.
[48] F. Böhm, R. Edge, E. J. Land, D. J. McGarvey, T. G. Truscott, *J. Am. Chem. Soc*. **1997**, *119*, 621.
[49] D. Perrin, M. Eloualja, R. Martin, *J. Chim. Phys.* **1996**, *93*, 1462.
[50] Z.-L. Li, L.-M. Wu, L.-P. Ma, Y.-C. Liu, Z.-L. Liu, *J. Phys. Org. Chem*. **1995**, *8*, 774.
[51] G. Shklar, J. Schwartz, *Internat. J. Oncology* **1994**, *4*, 215.
[52] U. Funegard, I. Johansson, B. Malmer, R. Hendrikson, T. Ericson, *Eur. J. Cancer* **1995**, *31A*, 2347.
[53] P. S. Kirk (BASF Co.), USP 5023095, **1991.**
[54] J. M. Teikari, *Investigat. Ophthalmol. Vis. Sci*. **1992,** *33*, 1307.
[55] H. S. Garewal, *J. Cell Biochem*. **1993**, 262**.**
[56] Suntan preparation BellaCarotin, 3M Medica GmbH, Borken (Germany).
[57] S. Sigurgisladottir, C. C. Parish, S. P. Lall, R. G. Ackman, *Food Res. Int.* **1994**, *27*, 23.
[58] A. M. Young, G. Gregoriadis, *Photochem. Photobiol.* **1996**, *63*, 344.
[59] H. Fukawa, K. Tanaka (Nisshin Flour Milling Co.), JP 73469, **1971**; *Chem. Abstr*. *78*, P 111126a.
[60] V. Partali, H.-R. Sliwka, unpublished results.
[61] S. Hashimoto, I. Furukawa, *Bull. Chem. Soc. Jpn.* **1981**, *54*, 2227.
[62] Y. Kanaoka, O. Yonemitsu, K. Tanizawa, K. Matsozaki, Y. Ban, *Chem. Ind. (London)* **1964**, 2102.
[63] C. H. Eugster, in *Carotenoids, Vol. 1A* (Ed.: G. Britton, S. Liaaen-Jensen, H. Pfander) Birkhäuser, Basel, **1995**, p. 72.
[64] K. Schiedt, S. Liaaen-Jensen, in ref. [63], p. 95.
[65] T. Koga (Noda Inst. Scientific Res.), USP 5364564, **1994**; *Chem. Abstr*. *122*, P 240347z.
[66] D. R. Kodali, *J. Lipid Res.* **1987**, *28*, 464.
[67] H. J. Nestler (Hoechst AG), DEP 3336199, **1985**; *Chem. Abstr*. *104*, P 19415b.
[68] M. Berger, M. Schneider, *Fett. Wiss. Technol*. **1993**, *95*, 169.
[69] T. Koga, J. Terao, *Biosci. Biotech. Biochem*. **1996**, *60*, 1043.
[70] L. R. C. Barclay, J. D. Artz, J. J. Mowat, *Biochem. Biophys. Acta, Biomembranes* **1995**, *1237*, 77.
[71] S. Takano, Y. Sekiguchi, M. Setho, T. Yoshimitsu, K. Inomata, M. Takahashi, K. Ogasawara, *Heterocycles* **1990**, *31*, 1715.
[72] H. S. Bevinakatti, A. A. Banerji, *J. Org. Chem.* **1991**, *56*, 5372.
[73] E.-L. Wang, H.-Y. Chen, C.-L. Tzeng, *J. Chin. Chem. Soc.* **1993**, *40*, 73.
[74] B. Berti, M. Macchia, F. Macchia, *Tetrahedron Lett.* **1965**, *38*, 3421.
[75] V. Partali, L. Kvittingen, H.-R. Sliwka, T. Anthonsen, *Angew. Chem.* **1996**, *108*, 342; *Angew. Chem. Int. Ed. Engl*. **1996**, *35*, 329.
[76] F. Spener, in *Ether Lipids—Biochemical and Biomedical Aspects* (Eds.: H. K. Mangold, F. Palthauf), Academic Press, New York, **1983**, p. 239.
[77] K. Urata, S. Yano, A. Kawamata, N. T. Takaishi, Y. Inamoto, *J. Am. Oil Chem. Soc.* **1988**, *65*, 1299.
[78] S. Yano, A. Kawamata (Kao Soap Co.), JP 02/104546, **1990**; *Chem. Abstr*. *113*, P 103225h.
[79] K. Urata, N. Takaishi, *J. Am. Oil Chem. Soc.* **1996**, *73*, 819.
[80] H. K. Mangold, in ref. [76], p. 231.
[81] B. Hallgren, in ref. [76], p. 261.
[82] K. Umegaki, S. Ikegami, K. Inoue, T. Ichikawa, S. Kobayashi, N. Soeno, K. Tomabechi, *Am. J. Clin. Nutr.* **1994**, *59*, 409.
[83] A. Ben Amotz, B. Rachmilevich, S. Greenberg, S. Sela, Z. Weshler, *Rad. Environ. Biophysics* **1996**, *35*, 285.
[84] I. Felemovicius, M. E. Bonsack, J. P. Delaney, *Ann. Surgery* **1995**, *222*, 504.
[85] B. H. Davies, in *Chemistry and Biochemistry of Plant Pigments* (Ed.: T. W. Goodwin), Academic Press, London, **1976**, pp. 38, 149.

  0947-6539/98/0401-0117 $ 17.50+.25/0