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Synthesis of a triantioxidant compound: combination of β-apo-8′-carotenoic acid, selenacapryloic acid and trolox in a triglyceride

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Abstract

Carotenoids, vitamin-E and selenium show similar or complementary physiological properties and protect against a variety of pathological processes. Mixtures of these antioxidants are found in nutritional supplements and are used to prevent several diseases. The synthetic connection of carotenoids, vitamin-E and selenium may increase the chemopreventive activity of the individual compounds. A carotenoic acid, a selena fatty acid and the vitamin-E derivative trolox were successively esterified with glycerol to 1-(β-apo-8′carotenoyl)-2-(7-selenaoctanoyl)-3-(6-hydroxy-2,5,7,8-tetramethylchroman-2-acyl)-glycerol. This triantioxidant compound revealed, in the DPPH (1,1-diphenyl-2-picrylhydrazyl) test, an additive effect, consisting of the radical quenching activity of the carotenoid and trolox. The DPPH test was not sensitive for the Se moiety in the triantioxidant compound.

Keywords: Antioxidants; Carotenoids; Condensation reactions; Selenium; Structured lipids; Vitamin E

1. Introduction

Several antioxidants together provide increased protection against toxic processes (Chen and Tappel, 1996). Thus, the mutual administration of vitamin-E, selenium and β-carotene offers a more effective prophylaxis than the individual compounds alone (Tominga et al., 1992; Chen and Tappel, 1993; Chen et al., 1993). An epidemiological investigation has verified that the combination, particularly of vitamin-E, β-carotene and selenium (from selenium yeast), reduced the risk of cancer (Blot et al., 1993). The combined application of these three antioxidants is suggested for the treatment of certain diseases (Rombi, 1993; Sokol, 1997). Mixtures of selenium, β-carotene and vitamin-E are available as food supplements (PolyANTOX). The close proximity of different antioxidants can be of importance for their activity (Young and Gregoriadis, 1996). Chalcogen

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modified lipids may be used to prepare self-assembled monolayers on noble metal surfaces (Ulman, 1996; Ion et al., 2001).

We describe here the synthesis of a derivative, which contains three biologically important antioxidants covalently bound to the physiologically active carrier molecule glycerol. Monoantioxidant and diantioxidant glycerides have previously been synthesized (Hoving et al., 1975; Weithmann et al., 1994; Morizaki and Ozaki, 1996; Partali et al., 1996; Larsen et al., 1998).

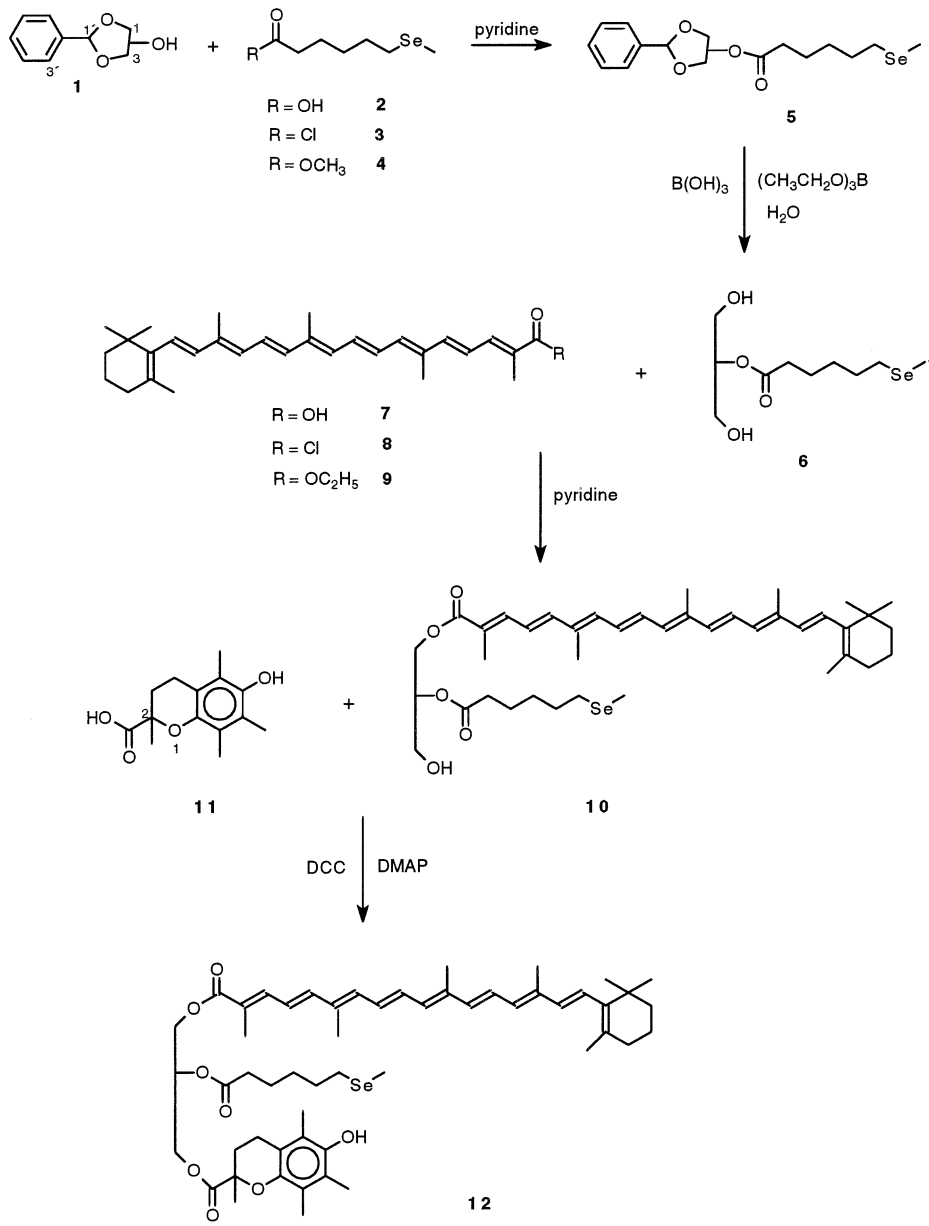
2. Results

The synthesis of regio-isomerically pure unsymmetrical triglycerides implies that the constituents (here vitamin-E, carotenoid, selenium) occur as 'fatty' acids. For the carotenoid we selected the naturally occurring and synthetically available apocarotenoic acid **7** (Bauernfeind, 1972). Trolox (**11**) stood for the vitamin-E part of the molecule (Cynshi et al., 1995). The selenium containing segment came from selenacaprylic acid **2** (Lie, 1993).

7-Selenaoctanoyl chloride (**3**), obtained from 7-selena-caprylic acid (**2**) by reaction with oxalyl chloride, (Wood et al., 1944) reacted with benzylideneglycerol (**1**) (Mattson and Volpenheim, 1962; Serdarevich, 1967) to selenaoctanoyl benzylideneglycerol (**5**) (Scheme 1). [Direct esterification (Neises and Steglich, 1978) of selenaacid **2** with the protected glycerol **1** formed only traces of **5**.] The opening of the acetal **5** with isomerization preventing triethylborate (Martin, 1953; Serdarevich, 1967) gave 2-selenaoctanoyl-glycerol (**6**) in 11% yield. Carotenoyl chloride **8**, prepared from acid **7** with oxalylchloride, reacted with monoglyceride **6** to 1,2-diglyceride **10**, which, with trolox (**11**), DMAP and DCC (Kodali, 1987) formed the triantioxidant triglyceride **12** in 24% yield. 2-Monoglycerides and 1,2-diglycerides such as **6** and **10** easily convert into other positional isomers (Sjursnes and Anthonsen, 1994; Boswinkel et al., 1996). However, the ^{1}H NMR spectra, recorded directly after the work-up procedure, indicated no significant acyl migration for **6** and **10** (Sonnet and Dudley, 1994). In the mass spectra the characteristic molecular ion of **12** was detected, in accordance with the calculated isotope pattern. The UV/VIS spectra of **12** resembled that of carotenoic ester **9**. In the ^{1}H NMR spectra the glycerides **6**, **10** and **12** displayed the distinct patterns of the mono-, di- and triglyceryl protons (Haraldsson et al., 1995) and the carbon atoms of the glyceryl backbone in these compounds presented the characteristic shift values in the ^{13}C NMR spectra (Chapman and Goñi, 1994).

Several methods are employed for the characterization of the radical quenching activity of individual antioxidant compounds. Nevertheless, a reliable technique which determines concurrently the activity of different kinds of antioxidants has not yet been developed (Pryor et al., 1993). A widely used assay measures the scavenging of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical (Bondet et al., 1997). The results obtained with DPPH are in good agreement with more biologically related assays such as peroxidizing of lipids in viable hepatocytes (Malterud et al., 1993). The DPPH test has already been adopted to determine the antioxidant activity of carotenoids (Nomura et al., 1997) and retinoids (Yamanu and Ito, 1998). However, the apocarotenoate **9** reacted considerably slower with DPPH than trolox (**11**), (Fig. 1). The 50%-inhibitor concentration (IC_{50}) for trolox (**11**) was found to be 30 μM and for triglyceride **12** 23 μM, (Fig. 1). The improved value for **12** resulted mainly from the addition of the inhibitor activity of C_{30}-ester **9** and trolox (**11**). Thus, the antioxidant activity of carotenoid **9** and trolox (**11**) is not influenced by esterification with glycerol. The DPPH test was not sensitive for the selenium moiety in **12**: selenaoctanoate **4** did not react with the DPPH radical. However, in sunflower oil oxidation tests selenadodecylglycerol-1-ether acts synergistically with vitamin E (Yanishlieva et al., 2001a). Also, carotenoic acid **7**, ester **9** and 1-β-apo-8′-carotenoylglycerol prevented synergistically with vitamin E, sunflower oil oxidation (Yanishlieva et al., 2001b). So far, the combination of vitamin E with vitamin A or C has resulted in few compounds with increased effects (Suetsugu et al., 1994; Makishima et al., 1998). Other antioxidant-glyceride derivatives such as tocopheryl-ascorbyl- or BHT (butylated hydroxy-

toluene)-glycerides show, at best, the same activity as one of the single antioxidant component, with DPPH even an antagonistic effect was observed (Weithmann et al., 1994; Morizaki and Ozaki, 1996). 3-Retinoyl ascorbate is only half as active in the DPPH-test as vitamin-A or vitamin-C in its radical inhibitor capacity (Yamanu and Ito, 1998). Triglyceride **12** represents a combined antioxidant-carotenoid derivative, where an additive effect was found by a radical test. Whether the triantioxidant-triglyceride **12** will be active in vivo has still to be demonstrated.



Scheme 1.

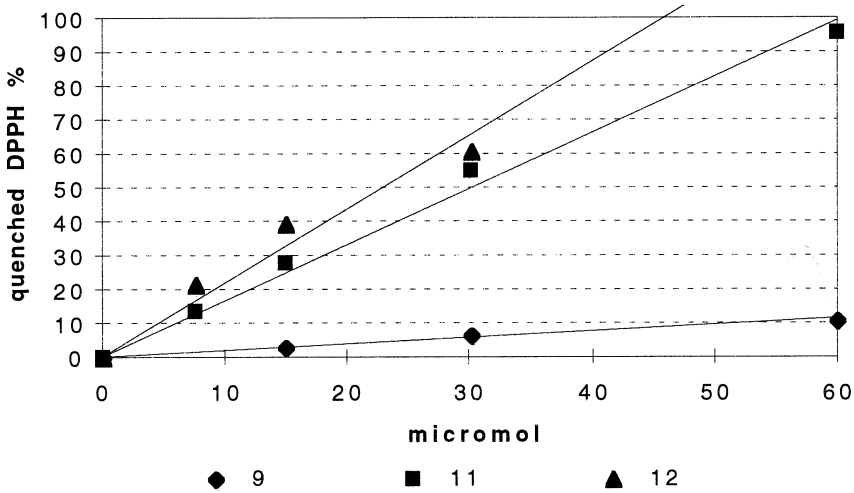


Fig. 1. Decrease of DPPH with ethyl apocarotenoate **9**, trolox (**11**) and triglyceride **12.** 50%-inhibiting concentration: **11** $IC_{50} = 30$ mM, **12** $IC_{50} = 23$ mM.

3. Experimental

3.1. General

After reaction the products were adsorbed on silicagel, dried in vacuo and separated by flash chromatography (silica 60, Merck). If necessary, separation was also carried out on preparative or analytical TLC plates with heptane–acetone mixtures. The peaks of the mass spectra (EI, IP 70 eV, 220 °C) are related to ^{80}Se. Small yields of carotenoid compounds were determined from the VIS spectra in CH_2Cl_2 (Davies, 1976). For the determination of DPPH scavenging (Malterud et al., 1993) the test substances, dissolved in DMSO (50 ml), were added to a methanolic solution of DPPH (2.95 ml) The DPPH concentration was sufficient to give an A_{517} of 1). The mixture was stirred for a few seconds and the decrease in absorbance was measured over a 15 min period. Percentage scavenging was calculated as $100 \times (A_0 - A_t)/A_0$, where A_0 is the initial absorbance and A_t the absorbance at time t. The values are corrected for dilution factors, absorption of apocarotenoid and of the DPPH reaction product diphenylhydrazine.

3.2. 7-Selenaoctanoic acid (2)

2 was prepared according to Lie (1993). Recrystallisation of the crude product from hexane at 20 °C gave **2** at a yield of 70%.

3.3. 2-(7-Selenaoctanoyl)-1,3-benzylidenglycerol (5)

To a solution of 7-selenaoctanoylchloride (**3**) [prepared from acid **2** with oxalylchloride (Wood et al., 1944)] (2.2 g, 9.5 mmol) in CH_2Cl_2 (6 ml) was added, under a N_2-atmosphere, 1,3-benzylideneglycerol (Mattson and Volpenheim, 1962) (**1**) (2.1 g, 11.4 mmol) in dry $CHCl_3$ (4 ml) and pyridin (4 ml). The solution was stirred for 48 h at room temperature (Martin, 1953). Extraction with CH_2Cl_2 and chromatographic work-up with heptane:acetone 7:3 afforded **5** (*cis* and *trans* 1:2) (2.4 g, 68%), whose formation was confirmed by spectral data from MS, ^1H NMR and ^{13}C NMR (Serdarevich, 1967).

3.4. 2-(7-Selenaoctanoyl)-glycerol (6)

To 2-selenaoctanoyl-1,3-benzylidenglycerol **5**

(1.5 g, 4 mmol), dissolved in triethyl-borate (15 ml), finely powdered boric acid (0.78 g, 12.4 mmol) was added. The mixture was stirred for 5 min at 100 °C. The solvent was evaporated (100 °C, 2–5 Torr) and heating under vacuum continued for 10 min. Diethylether (50 ml) was added, the ether solution was washed four times with 20 ml portions of H_2O, dried over Na-sulfate and the ether evaporated under vacuum at 25 °C (Martin, 1953). Chromatographic work-up of the residue with heptane:acetone 6:4 afforded **6** (61 mg, 11% based on reacted **5**). $C_{10}H_{20}O_4Se$, 28.1% Se.

MS: *m/z* 284 [M^+], 210 [M^+—74 (propandiol), Se isotopic pattern], 193 [M^+—91 (glycerol-H), Se isotopic pattern]. ^{1}H NMR (300 MHz, d-acetone, immediately recorded after work-up procedure): glycerol part ($A_2A_2'\times$ system) $\delta = 3.71$ and 3.65 (both dxd, 2H, A C1, C3, 2H, A′ C1, C3, $J_{AA'} = 11.6$, $J_{AX} = 6.4$ Hz), 4.88 (qui, 1H, C2, $J_{ax} = 6.4$ Hz); selenaoctanoyl part $\delta = 2.55$ (*t*, 2H, C6), 2.33 (*t*, 2H, C2), 1.96 (*s*, 3H, Se–CH_3), 1.57–1.75 (m, 4H, C3, C5), 1.37–1.50 (*m*, 2H, C4).

-C^{13}C NMR (100 MHz, d-acetone): glycerol part $\delta = 61.5$ (C1, C3), 76.3 (C2); selenaoctanoyl part $\delta = 173.5$ (C = O), 34.7 (C2), 32.0 (C5), 30.6 (C4), 25.4 (C6), 25.2 (C3), 3.5 (Se–CH_3).

3.5. β-Apo-8′-carotenoic acid chloride (8)

To an ice cold solution of apocarotenoic acid **7** (3.0 g, 6.9 mmol) in CCl_4 (20 ml) was added, under N_2 atmosphere, oxalylchloride (0.7 ml, 8.3 mmol). The mixture was heated at 70 °C for 24 h. The solvent and excess oxalylchloride were evaporated under reduced pressure (Wood et al., 1944). The residual acid chloride **8** was used without further purification.

3.6. 1-(β-Apo-8′-carotenoyl)-2-(7-selenaoctanoyl)-glycerol (10)

To an ice cold solution of apocarotenoyl chloride (**8**) (93 mg, 0.2 mmol) in CH_2Cl_2 was added, under N_2 atmosphere, 2-selenaoctanoyl-glycerol **6** (61 mg, 0.2 mmol) and pyridine (0.2 ml). The mixture was stirred at 40–45 °C for 3 h. Extraction with CH_2Cl_2 and chromatographic work-up with hexane:acetone 6:4 afforded **10** (47 mg, 31%). $C_{40}H_{58}O_5Se$, 11.4% Se.

MS: *m/z* 698 [M^+, Se isotopic pattern], 606 [M^+—92 (toluene), Se isotope pattern], 505 [M^+—193 (Se acylium)], 488 [M^+—210 (Se acid)], 413 [606–193 (Se acylium)], 432 [C_{30} acid], 267 [M^+—431 (C_{30} acid -H, Se isotopic pattern)].

^{1}H NMR (400 MHz, $CDCl_3$): glycerol part (ABMXY system) $\delta = 4.35$ and 4.40 (both d × d, 2H, C1), 3.77–3.78 (*m*, 2H, C3), 5.17 (qui, 1H, C2); selenaoctanoyl part: $\delta = 2.54$ (*t*, 2H, C6), 2.38 (*t*, 2H, C2), 1.99 (*s*, 3H, Se-CH_3), 1.60–1.78 (*m*, 4H, C3, C5), 1.40–1.50 (*m*, 2H, C4); carotenoyl part $\delta = 1.03$ (*s*, 6H, 2 × CH_3, C1), 1.40 (*m*, 2H, C2), 1.59 (*s*, 2H, C3), 1.72 (*s*, 3H, CH_3, C5), 1.98 (*s*, 12H, 4 × CH_3, polyene chain), 6.15–6.73 (*m*, 12H, polyene chain).

^{13}C NMR (100 MHz, $CDCl_3$): glycerol part $\delta = 72.3$ (C2), 62.2 (C1), 61.5 (C3); selenaoctanoyl part $\delta = 173.6$ (C = O, C1), 33.9 (C2), 29.7 (C5), 29.2 (C4), 25.1 (C6), 24.4 (C3), 4.0 (Se–CH_3); carotenoyl part $\delta = 168.4$ (C = O), 122.8–141.3 (16C, polyene chain), 39.6 (C2), 34.3 (C1), 33.1 (C4), 29.0 (2 × CH_3, C1), 20.8 (CH_3, C5), 19.2 (C3), 13.0 (4 × CH_3, polyene chain). A ^{13}C NMR spectra recorded after several weeks indicated substantial acyl migration.

3.7. 1-(β-Apo-8′-carotenoyl)-2-(7-selenaoctanoyl)-3-(6-hydroxy-2,5,7,8-tetramethylchroman-2-acyl)-glycerol (12)

To a solution of 1-apocarotenoyl-2-selenaoctanoyl-glycerol **10** (32 mg, 0.046 mmol) and trolox (**11**) (30 mg, 0.12 mmol) in CH_2Cl_2 (4 ml) was added, under N_2 atmosphere, dicyclohexylcarbodiimide (55 mg, 0.3 mmol) and 4-dimethylaminopyridin (32 mg, 0.3 mmol) (Kodali, 1987). The mixture was stirred for 5 h at room temperature. Chromatographic work-up with heptane:acetone 4:6 afforded **12** (10 mg, 24%). $C_{54}H_{74}O_8Se$, 8.5% Se.

VIS: $\lambda_{max} = 458$ nm.

MS: *m/z* 930 [M^+, Se isotopic pattern], 838 [M^+—92 (toluene), Se isotopic pattern)], 737 [M^+—193 (Se acylium)], 588 [838-250 (trolox), Se isotopic pattern], 515 [M^+—415 (C_{30} acylium), Se isotopic pattern)], 499 [M^+—431 (C_{30} acid -H, Se isotopic pattern)], 432 [C_{30} acid], 250 [trolox].

^{1}H NMR (400 MHz, $CDCl_3$): glycerol part $\delta = 4.21$ and 4.40 (both d × d, 4H, C1, C3),?5.22

(*m*, 1H, C2); trolox part $\delta = 1.25$ (*s*, 3H, CH_3, C2a), 2.17 (*s*, 3H, CH_3, C5a), 2.15 (*s*, 3H, CH_3, C7a), 2.10 (*s*, 3H, CH_3, C8b), 1.86 (*m*, 1H, C3), 2.46 (*m*, 1H, C3), 2.60 (*m*, 2H, C4); selenaoctanoyl and carotenoyl part in accordance with **10**.

^{13}C NMR (100 MHz, $CDCl_3$): glycerol part $\delta = 68.8$ (C2), 62.5 (C3), 62.0 (C1); trolox part $\delta = 173.7$ (C = O), 145.5 (C6), 30.7 (C3), 21.9 (CH_3, C2a), 12.3 (CH_3, C7a), 11.8 (CH_3, C8b), 11.3 (CH_3, C5a); selenaoctanoyl and carotenoyl part in accordance with **10**.

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