JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Organic Synthesis of New Putative Lycopene Metabolites and Preliminary Investigation of Their Cell-Signaling Effects

Eric Reynaud,[†,‡] Gamze Aydemir,[§] Ralph Rühl,[§,∥] Olivier Dangles,[†,‡] and Catherine Caris-Veyrat*[,†,‡]

[†]Safety and Quality of Plant Products, INRA, UMR 408, F-84000 Avignon, France

[‡]University of Avignon, UMR 408, F-84000 Avignon, France

[§]Laboratory of Nutritional Bioactivation and Bioanalysis, Department of Biochemistry, University of Debrecen, Hungary

[∥]Apoptosis and Genomics Research Group of the Hungarian Academy of Science and Molecular Biology, University of Debrecen, Hungary

ABSTRACT: Tomato is the main dietary source of lycopene, a carotenoid that is known to have protective effects on health and whose metabolites could also be involved in bioactivity. Herein we present the first organic synthesis of two potentially bioactive lycopene metabolites, namely, 10′-apolycopen-10′-oic acid (**6**) and 14′-apolycopen-14′-oic acid (**13**), which were obtained in their (all-*E*) stereoisomeric forms using Wittig and Horner−Wadsworth−Emmons type coupling reactions. Both molecules are shown to up-regulate the carotenoid asymmetric cleavage enzyme BCO2 while having no effect on BCO1 expression.

KEYWORDS: Apo-lycopenoic acids, organic synthesis, lycopene, metabolite, β,β-carotene oxygenase, gene expression

INTRODUCTION

Numerous epidemiological studies[1] have found a link between the consumption of tomatoes or tomato-derived products and a lower occurrence of degenerative diseases, particularly prostate cancer[2] and cardiovascular diseases.[3] Lycopene, the red carotenoid pigment found in tomato (Figure 1), is a prime candidate to explain these effects.[4] Furthermore, it is increasingly likely that lycopene metabolites also participate in the biological effects ascribed to lycopene.[5,6]

The first lycopene metabolite identified in humans, namely, epimeric 2,6-cyclolycopene-1,5-diol, was discovered by Khachik et al.[7] Moreover, lycopene derivatives of the apo-lycopenoid type (arising from concomitant oxygenation and cleavage of the hydrocarbon chain) were identified in a limited number of plants: apo-6′- and apo-8′-lycopenals in tomato[8] and 10′-apolycopen-10′-ol and 10′-apolycopen-10′-oic acid in yellow petals of the Maréchal Niel rose hybrid.[9] Apo-lycopenoids were also found in animals fed lycopene-enriched diets, namely, apo-12′- and apo-8′-lycopenals in rat liver[10] and 10′-apolycopen-10′-ol in ferret lung.[11] Recently, Kopec et al.[12] detected various apo-lycopenals in the plasma of humans who had consumed tomato juice. However, since apo-lycopenals were also found in the tomato juice itself, it was unclear whether part of the circulating apo-lycopenoids originated from human metabolism of lycopene.

Apo-carotenoids are known to display important biological properties. Indeed, in vitro studies have shown that asymmetric cleavage products of β-carotene inhibit estrogen receptor-dependent breast tumor cell growth and activator protein-1-mediated transcriptional activation.[13] Moreover, β-carotene and 14′-apo-β-caroten-14′-oic acid prevent the down-regulation of retinoic acid receptor β in benzo[*a*]pyrene-treated normal human bronchial epithelial cells.[14] Hence, we suggest that apo-lycopenoid acids too might bind and activate various nuclear hormone receptors. More generally, the biological activity of lycopene and its metabolites is linked to their antioxidant properties and/or their effect on cell-signaling mechanisms.[15] For instance, the central cleavage product of lycopene, namely, apo-15-lycopenal, has been shown to increase gap junction communication, although only at high concentrations.[16] 10′-Apolycopen-10′-oic acid acid has been shown to inhibit lung carcinogenesis both in vivo and in vitro[17] via the activation of Nrf2 and the induction of phase II detoxifying/antioxidant enzymes.[18] Likewise, Linnewiel et al.[19] have recently shown that diapocarotene dials are more potent than lycopene at activating the antioxidant response element (ARE) transcription system. Thus, lycopene metabolites might induce phase II enzymes by the same mechanism as the one demonstrated for carotenoids[20] and even more efficiently.

Literature data suggest that lycopene is a poor substrate of β,β-carotene 15,15′-monooxygenase (formerly termed β,β-carotene 15,15′-dioxygenase, EC 1.13.11.21) (BCO1).[15] However, β,β-carotene-9′,10′-oxygenase (BCO2), which was cloned and characterized for the first time in mouse[21] and more recently characterized in ferret,[11] has been shown to catalyze the asymmetric cleavage of the (5-*Z*) and (13-*Z*) isomers of lycopene, but not of (all-*E*)-lycopene, to form apo-10′-lycopenal,[11] which may be further reduced into 10′-apolycopen-10′-ol or oxidized into 10′-apolycopen-10′-oic acid by dehydrogenases using NADH or NAD^+ cofactors.

In this context, oxygenated cleavage products of lycopene are of interest either as standards for identification and quantification in plant, food, and biological fluids and tissues or as bioactive compounds for investigations in chemical, cellular, and animal

Received: October 19, 2010
Accepted: December 15, 2010
Revised: December 14, 2010
Published: January 19, 2011

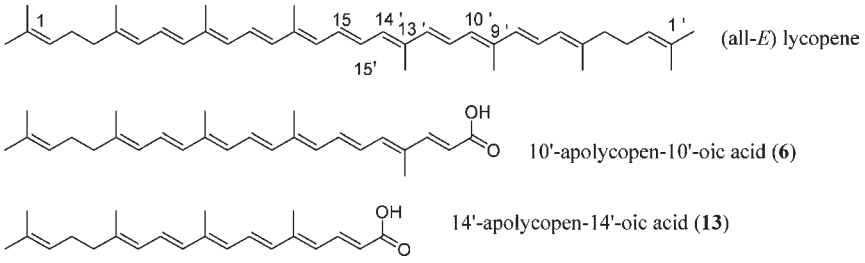

Figure 1. Chemical structures of (all-*E*) lycopene, 10′-apolycopen-10′-oic acid (**6**) and 14′-apolycopen-14′-oic acid (**13**).

models. Herein we describe the first organic synthesis of two apolycopenoic acids that are putative lycopene metabolites, namely, 10′-apolycopen-10′-oic and 14′-apolycopen-14′-oic acids (Figure 1). We also report preliminary bioactivity data in animal models. In particular, using QRT-PCR techniques, we show how expression of the carotenoid-metabolizing enzymes BCO1 and BCO2 is altered in mouse testis in response to treatment with 10′-apolycopen-10′-oic and 14′-apolycopen-14′-oic acids in comparison with lycopene.

MATERIAL AND METHODS

Chemicals. Vinylpseudoionol was provided by BASF (Ludwigshafen, Germany), and (2*E*,4*E*,6*E*)-2,7-dimethylocta-2,4,6-triendial was a gift from DSM Nutritional Products (Kaiseraugst, Switzerland). Manganese dioxide, potassium *tert*-butoxide, sodium methoxide, ammonium formate, triphenylphosphine, sodium, 3 Å molecular sieves, and ethyl 3-methyl-4-oxocrotonate were purchased from Sigma-Aldrich (Saint Quentin-Fallavier, France). Methanesulfonic acid, triethyl phosphonoacetate, benzophenone, and 2,2-dimethylpropan-1,3-diol were purchased from Alfa Aesar. DIBAH was purchased from Acros or Sigma-Aldrich. All reagents were of the highest purity available (95−99%) and were used without further purification. Silica gel (Si 60, 40−63 μm) was obtained from Merck (Limonest, France). All solvents used were of analytical grade. The THF used for chemical reactions was freshly distilled over Na and benzophenone. Ethyl acetate, acetic acid, dichloromethane, and toluene were dried over 3 Å molecular sieves (previously dried at 150 °C for 4 h). All reactions were carried out under dim light and argon atmosphere.

Analyses. *HPLC−MS.* HPLC−MS analyses were performed using a Hewlett-Packard 1100 apparatus coupled to a UV/vis diode array detector and a Micromass platform LCZ 4000 mass spectrometer. Mass analyses were performed in the positive electrospray ionization mode with a capillary voltage of 15 V and a desolvation temperature of 250 °C. For conditions 1, the column used was a 150 mm × 2.1 mm i.d., 3 μm YMC C30 column with a 10 mm × 2 mm i.d. guard column of the same material (Interchim, Montluçon, France). The temperature of the column was set at 40 °C. The solvent system was a gradient of A (25 mM ammonium formate + 2.6 mM formic acid in water), B (25 mM ammonium formate in methanol), and C (methyl *tert*-butyl ether), with 60% B, 40% A at t = 0; 40% A, 60% B at t = 1.25 min; 30% A, 70% B at t = 2.50 min; 25% A, 70% B, 5% C at t = 3.75 min; 20% A, 70% B, 10% C at t = 5 min; 5% A, 10% B, 85% C at t = 15 min; and 5% A, 10% B, 85% C at t = 25 min (flow rate = 0.5 mL/min). For the analysis of compound **2**, conditions 2 were used:[22] the column used was a 250 mm × 4.6 mm i.d., 5 μm C18 column with a 10 mm × 4.6 mm i.d. guard column of the same material (Macherey-Nagel, Düren, Germany). The temperature of the column was set at 50 °C. The solvent system was an isocratic flow (1 mL/min) of CH_3CN/H_2O (13/7, v/v) containing $(n\text{-Bu})_4NHSO_4$ (0.4%) and sodium dodecyl sulfate (0.2%).

TLC. Analytical thin layer chromatography (TLC) was performed on silica gel plates (Merck Kieselgel 60 F_{254}) with UV detection at 254 or 365 nm.

GC−MS. Volatile products were monitored by GC−MS (CP2010, Shimadzu, Kyoto). Samples (1 μL) were injected onto a UBWAX 52 CB capillary column (30 m, 0.25 mm i.d., 0.5 μm thickness). The injection port was operated in split mode ($R = {}^1/_{10}$). The carrier gas (He) velocity was constant (60 cm/s). The initial oven temperature of 60 °C was increased at a rate of 5 °C per min to 230 °C. This final temperature was maintained for 15 min. The mass spectrometer was operated in the electron impact mode at 70 eV with continuous scans (every 0.5 s) in the range m/z 29−350. Data were analyzed using the GC−MS solution software.

NMR. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance DRX 500 MHz spectrometer. Chemical shifts are given in ppm relative to peak solvent ($CDCl_3$).[23] J values are given in Hz. Signal assignments were deduced from a combination of heteronuclear single quantum coherence (HSQC), distortionless enhancement by polarization transfer quantum (DEPTQ 135), nuclear overhauser effect spectroscopy (NOESY), correlation spectroscopy (COSY), and heteronuclear multiple bond coherence (HMBC) spectra.

HRMS. High-resolution mass analysis was carried out using a QStar Elite (Applied Biosystems SCIEX) with positive electrospray-TOF ionization.

Organic Synthesis. *Triphenyl-(2E,4E,6E,10E)-3,7,11-trimethyldodeca-2,4,6,10-tetraenylphosphonium (**2**).* A mixture of triphenylphosphine (2.13 g, 8.1 mmol), acetic acid (10 mL), and methanesulfonic acid (0.4 mL, 6.3 mmol) was vigorously stirred for 1 h at 10 °C. Vinylpseudoionol (**1**; 1 mL, 6.3 mmol) was then added dropwise over 1 h and stirred for an hour more at 10 °C. The resulting mixture was heated to 45 °C for 20 h. The acetic acid was then distilled off under reduced pressure and the residue taken up in toluene to remove the last traces of acetic acid by azeotropic distillation. The residue was dissolved in CH_2Cl_2 and washed with distilled water (3 × 10 mL), then dried over Na_2SO_4 and concentrated. Purification by silica gel chromatography (eluent CH_2Cl_2/MeOH (9/1, v/v)) gave a mixture of isomers as a light-yellow viscous oil (2.50 g, 71%, (E/Z) = 4). HPLC−UV−MS: t_R (retention time) 21.8 min; λ_{max}/nm 294; m/z 466 ([M− $MeSO^-{}_3$]$^+$).

*Ethyl (2E,4E,6E,8E)-9-Formyl-4-methyldeca-2,4,6,8-tetraenoate (**4**).* (2*E*,4*E*,6*E*)-2,7-Dimethylocta-2,4,6-triendial (**3**) (400 mg, 2.4 mmol) and triethyl phosphonoacetate (0.5 mL, 2.4 mmol) were dissolved in 40 mL of dry THF. A solution of *t*-BuOK (2.4 mmol) in THF (1.4 mL) was then added over 1 h and the mixture stirred for 20 h at room temperature. The reaction was then quenched by addition of 10 mL of a saturated NH_4Cl solution and the product extracted with diethyl ether (3 × 10 mL). The combined organic layers were washed with brine (3 × 10 mL), then dried over Na_2SO_4 and concentrated. The crude product was purified on silica gel (eluent EtOAc/hexane (2/8, v/v)) and crystallized from EtOAc/hexane. Compound **4** was obtained as a yellow powder (215 mg, 38%). δ_H (500 MHz, $CDCl_3$) 1.31 (t, 3, J=7.1 Hz, H_3C−C(ethyl)); 1.90 (s, 3, H_3C−C(9)); 1.99 (s, 3, H_3C−C(4)); 4.23 (q, 2, J=7.1 Hz, H_2C−C(ethyl)); 6.01 (d, 1, J = 15.6 Hz, H−C(2)); 6.54 (d, H, J = 11.7 Hz, H−C(5)); 6.85 (dd, 1, J = 11.7 and 14.3 Hz, H−C(7)); 6.96 (d, 1, J = 11.7 Hz, H−C(8)); 6.99 (dd, 1, J = 11.7 and 14.3 Hz, H−C(6)); 7.37 (d, 1, J = 15.6 Hz, H−C(2)); 9.50 (s, 1, H−C(10)). δ_C (125 MHz, $CDCl_3$) 9.9 (H_3C−C(9)); 13.0 (H_3C−C(4)); 14.5 (H_3C(ethyl)); 60.7 (H_2C(ethyl)); 119.4 (C(2)); 131.0 (C(7)); 136.2 (C(6)); 137.2 (C(5)); 138.4 (C(4)); 139.1 (C(9)); 147.7 (C(8)); 147.9 (C(3)); 167.2 (C(1)); 194.7 (C(10)). HPLC−UV−MS: t_R 5.76 min; λ_{max}/nm 357; m/z 235.1 ([M + H]$^+$).

GC−MS: t_R 37.04 min; m/z 234 ($M^{\bullet+}$). HRMS (electrospray, positive mode): m/z 235.1328 ($[M + H]^+$) (235.1328 calculated for $C_{14}H_{19}O_3$).

*Ethyl (2E,4E,6E,8E,10E,12E,14E,16E,20E)-4,9,13,17,21-Pentamethyldocosa-2,4,6,8,10,12,14,16,20-nonaenoate (**5**).* Compound **4** (240 mg, 1 mmol) and phosphonium salt **2** (diastereoisomeric mixture, 710 mg, 1.3 mmol) were dissolved in 5 mL of CH_2Cl_2. A solution of NaOMe (1.3 mmol) in MeOH (2.6 mL) was then slowly added over 30 min at 0 °C and the mixture stirred for 20 h at room temperature. The reaction was stopped by addition of a saturated NH_4Cl solution (1 mL). The organic layer was washed with distilled water (3 × 5 mL), then dried over Na_2SO_4 and concentrated. The crude product was filtered on a silica gel column (eluent EtOAc/hexane (2/8, v/v)), then purified by crystallization from hexane. Compound **5** was obtained as an orange-red powder (74.5 mg, 18%). δ_H (500 MHz, $CDCl_3$) 1.31 (t, 3, J = 7.1 Hz, H_3C−C(ethyl)); 1.61 (s, 3, H_3C−C(21)); 1.69 (s, 3, H_3C(22)); 1.82 (s, 3, H_3C−C(17)); 1.92 (s, 3, H_3C−C(4)); 1.98 (s, 3, H_3C−C(13)); 1.99 (s, 3, H_3C−C(9)); 2.12 (m, 4, H_2−C(18, 19)); 4.22 (q, 2, J = 7.1 Hz, H_2C−C(ethyl)); 5.11 (m, 1, H−C(20)); 5.87 (d, 1, J = 15.5 Hz, H−C(2)); 5.95 (d, 1, J = 11.1 Hz, H−C(16)); 6.18 (d, 1, J = 11.5 Hz, H−C(12)); 6.25 (d, 1, J = 15.2 Hz, H−C(14)); 6.26 (d, 1, J = 11.5 Hz, H−C(8)); 6.35 (d, 1, J = 14.8 Hz, H−C(10)); 6.52 (m, 2, H−C(5, 15)); 6.59 (m, 1, H−C(6)); 6.69 (dd, 1, J = 11.5 and 14.8 Hz, H−C(11)); 6.78 (m, 1, H−C(7)); 7.37 (d, 1, J = 15.5 Hz, H−C(3)). δ_C (125 MHz, $CDCl_3$) 12.7 (H_3C−C(4)); 13.1 (H_3C−C(9)); 13.1 (H_3C−C(13)); 14.5 (H_3C(ethyl)); 17.1 (H_3C−C(17)); 17.9 (H_3C−C(21)); 25.8 (C(22)); 26.8 (C(19)); 40.4 (C(18)); 60.3 (H_2C(ethyl)); 116.5 (C(2)); 124.1 (C(20)); 125.5 (C(15)); 125.8 (C(16)); 126.6 (C(11)); 128.9 (C(6)); 131.4 (C(12)); 131.9 (C(21)); 131.9 (C(8)); 133.6 (C(4)); 133.9 (C(7)); 135.4 (C(14)); 137.1 (C(10)); 137.3 (C(13)); 139.1 (C(9)); 139.4 (C(5)); 140.1 (C(17)); 148.9 (C(3)); 167.7 (C(1)). HPLC−UV−MS: t_R 14.15 min; λ_{max}/nm 442; m/z 421.6 ($[M + H]^+$). HRMS (electrospray, positive mode): m/z 421.3087 ($[M + H]^+$) (421.3101 calculated for $C_{29}H_{41}O_2$).

*(2E,4E,6E,8E,10E,12E,14E,16E,20E)-4,9,13,17,21-Pentamethyldocosa-2,4,6,8,10,12,14,16,20-nonaenoic Acid (**6**).* Compound **5** (300 mg, 0.71 mmol) was dissolved in a solution of NaOH (630 mg, 16 mmol) in EtOH (20 mL). The mixture was stirred for 24 h at 35 °C, then neutralized with HCl (10%, v/v). The product was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic phases were dried over Na_2SO_4 and concentrated. The crude product was purified by crystallization from CH_2Cl_2/hexane. Compound **6** was obtained as a red powder (154 mg, 55%). δ_H (500 MHz, $CDCl_3$) 1.62 (s, 3, H_3C−C(21)); 1.69 (s, 3, H_3C(22)); 1.82 (s, 3, H_3C−C(17)); 1.94 (s, 3, H_3C−C(4)); 1.98 (s, 3, H_3C−C(13)); 2.01 (s, 3, H_3C−C(9)); 2.12 (m, 3, H_2C(18, 19)); 5.11 (m, 1, H−C(20)); 5.87 (d, 1, J = 15.5 Hz, H−C(2)); 5.95 (d, 1, J = 11.0 Hz, H−C(16)); 6.19 (d, 1, J = 11.5 Hz, H−C(12)); 6.25 (d, 1, J = 14.9 Hz, H−C(14)); 6.27 (d, 1, J = 11.5 Hz, H−C(8)); 6.35 (d, 1, J = 14.8 Hz, H−C(10)); 6.52 (m, 2, H−C(5, 15)); 6.59 (m, 1, H−C(6)); 6.72 (dd, 1, J = 11.5 and 14.8 Hz, H−C(11)); 6.80 (m, 1, H−C(7)); 7.46 (d, 1, J = 15.5 Hz, H−C(3)). δ_C (125 MHz, $CDCl_3$) 12.7 (H_3C−C(4)); 13.1 (H_3C−C(9)); 13.1 (H_3C−C(13)); 17.1 (H_3C−C(17)); 17.8 (H_3C−C(21)); 25.8 (C(22)); 26.8 (C(19)); 40.4 (C(18)); 115.3 (C(2)); 124.1 (C(20)); 125.5 (C(15)); 125.8 (C(16)); 126.8 (C(11)); 128.7 (C(6)); 131.4 (C(12)); 131.9 (C(8)); 131.9 (C(21)); 133.4 (C(4)); 134.7 (C(7)); 135.4 (C(14)); 137 (C(10)); 137.4 (C(13)); 139.6 (C(9)); 140.2 (C(17)); 140.6 (C(5)); 151.1 (C(3)); 172.6 (C(1)). HPLC−UV−MS: t_R 12.46 min; λ_{max}/nm <u>430</u> (main absorption band is underlined), 453; m/z 393.4 ($[M + H]^+$). HRMS: m/z 393.2787 ($[M + H]^+$) (393.2788 calculated for $C_{27}H_{37}O_2$).

*Ethyl (E)-3-(5,5-Dimethyl-1,3-dioxan-2-yl)but-2-enoate (**8**).* A solution of ethyl 3-methyl-4-oxocrotonate (**7**) (1.4 mL, 10.3 mmol), 2,2-dimethylpropandiol (5.3 g, 51 mmol) and p-TSA (210 mg, 1.1 mmol) in toluene (25 mL) was heated to reflux in a Dean−Stark apparatus for 4 h. After the mixture was cooled, brine (20 mL) was added. The layers were separated, and the aqueous layer was extracted with diethyl ether (2×20 mL). The combined organic layers were washed with brine (3 × 20 mL), dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel (eluent CH_2Cl_2/MeOH (98/2, v/v)) to afford **8** as a colorless oil (1.90 g, 81%). δ_H (500 MHz, $CDCl_3$) 0.74 (s, 3, $H_{3a}C$−C((5) dioxane)); 1.20 (s, 3, $H_{3e}C$−C((5) dioxane)); 1.26 (t, 3, J = 7.1, CH_3(ethyl)); 2.18 (d, 3, J = 1.7 Hz, H_3C−C(3)); 3.50 (d, 2, J = 10.7 Hz, $H_{2e}C$ (dioxane)); 3.67 (d, 2, J = 10.7, $H_{2a}C$ (dioxane)); 4.16 (q, 2, J = 7.1 Hz, H_2C(ethyl)); 4.73 (s, 1, H−C (4)); 6.04 (s, 1, H−C(2)). δ_C (125 MHz, $CDCl_3$) 14.0 (CH_3−C(3)); 14.2 (H_3C-(ethyl)); 21.8 (CH_{3a}−C((5) dioxane)); 22.9 (CH_{3e}−C((5) dioxane)); 30.2 (C((5)dioxane)); 59.8 (H_2C(ethyl)); 77.3 (2 H_2C(dioxane)); 102.7 (C(4)); 118.1 (C(2)); 152.3 (C(3)); 166.5 (C(1)). GC−MS: t_R 24.34 min; m/z 227 ($[M - H]^{\bullet+}$). HRMS: m/z 229.1433 ($[M + H]^+$) (229.1434 calculated for $C_{12}H_{21}O_4$).

*(E)-3-(5,5-Dimethyl-1,3-dioxan-2-yl)but-2-enal (**9**).* For the reduction step, compound **8** (1.82 g, 8 mmol) was dissolved in dry THF (7 mL), and a solution of DIBAH (21 mmol) in toluene (21 mL) carefully added over 30 min at 0 °C. The mixture was stirred for 1 h. Then a 1/1 MeOH/water mixture (10 mL) was slowly added at 0 °C. The gel formed was broken up by addition of aqueous 2 M potassium sodium tartrate (60 mL) and vigorous stirring for 10 h. The product was then extracted with diethyl ether (3 × 10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to give the crude alcohol as a light-yellow oil (1.1 g, 74%). GC−MS: t_R 18.13 min; m/z 185 ($[M - H]^{\bullet+}$).

For the oxidation step, the crude allyl alcohol (1.1 g, 5.9 mmol) was dissolved in AcOEt (25 mL), and after addition of MnO_2 (12.8 g, 125 mmol), the heterogeneous mixture was vigorously stirred for 45 min. The mixture was then filtered through Celite, dried over Na_2SO_4, and concentrated. The residue was purified by filtration through a silica gel column (eluent hexane/AcOEt (3/7, v/v)) to give compound **9** as a colorless oil (0.83 mg, 76%). δ_H (500 MHz, $CDCl_3$) 0.76 (s, 3, $H_{3a}C$−C((5) dioxane)); 1.21 (s, 3, $H_{3e}C$−C((5) dioxane)); 2.20 (d, 3, J = 1.7 Hz, H_3C−C(3)); 3.52 (d, 2, J = 10.4 Hz, $H_{2a}C$(dioxane)); 3.69 (d, 2, J = 10.4 Hz, $H_{2e}C$(dioxane)); 4.82 (s, 1, H−C (4)); 6.17 (d, 1, J = 7.9 Hz, H−C(2)); 10.09 (d, 1, J = 7.9 Hz, H−C(1)). δ_C (125 MHz, $CDCl_3$) 12.6 (CH_3−C(3)); 21.8 (CH_{3a}-C((5)dioxane)); 22.9 (CH_{3e}−C((5)dioxane)); 30.3 (C((5)dioxane)); 77.3 (2 H_2C(dioxane)); 102.3 (C(4)); 127.6 (C(2)); 155.6 (C(3)); 191.6 (C(1)). GC−MS: t_R 19.98 min; m/z 184 ($M^{\bullet+}$). HRMS: m/z 207.0988 ($[M + Na]^+$) (207.0991 calculated for $C_{10}H_{16}O_3Na$).

*Ethyl (2E,4E)-5-(5,5-Dimethyl-1,3-dioxan-2-yl)hex-2,4-dienoate (**10**).* Compound **9** (1.46 g, 7.9 mmol) and triethyl phosphonoacetate (1.7 mL, 8.6 mmol) were dissolved in 100 mL of dry THF. A solution of *t*-BuOK (8.6 mmol) in THF (8.6 mL) was added over 50 min and the mixture stirred for 24 h at room temperature. The reaction was then quenched with 20 mL of saturated aqueous NH_4Cl solution and the product extracted with diethyl ether (3 × 20 mL). The combined organic layers were washed with brine (3 × 20 mL), dried over Na_2SO_4, and concentrated. The residue was purified on silica gel (eluent EtOAc/hexane (1/9, v/v)) to afford compound **10** as a light-yellow oil (1.1 g, 55%, (*E*/*Z*) = 33). δ_H (500 MHz, $CDCl_3$) 0.75 (s, 3, $H_{3a}C$−C((5)dioxane)); 1.22 (s, 3, H_{3e} C−C((5)dioxane)); 1.30 (t, 3, J = 7.1 Hz, CH_3(ethyl)); 1.95 (d, 3, J = 1.4 Hz, H_3C−C(5)); 3.50 (d, 2, J = 10.5 Hz, $H_{2e}C$(dioxane)); 3.67 (d, 2, J = 10.5 Hz, $H_{2a}C$(dioxane)); 4.21 (q, 2, J = 7.1 Hz, H_2C(ethyl)); 4.78 (s, 1, H−C(6)); 5.92 (d, 1, J = 15.3 Hz, H−C(2)); 6.24 (d, 1, J = 11.4 Hz, H−C(4)); 7.57 (dd, 1, J = 11.4 and 15.3 Hz, H−C(3)). δ_C (125 MHz, $CDCl_3$) 13.0 (CH_3−C(5)); 14.5 (H_3C(ethyl)); 22.1 (CH_{3a}−C((5)dioxane)); 23.2 (CH_{3e}−C((5)dioxane)); 30.5 (C(5)dioxane); 60.6 (H_2C(ethyl)); 77.6 (2 H_2C(dioxane)); 103.7 (C(6)); 123.0 (C(2)); 125.4 (C(4)); 139.7 (C(3)); 143.5 (C(5)); 167.3 (C(1)). HPLC−UV−MS: t_R 4.63 min; λ_{max}/nm 268; m/z 255.1 ($[M + H]^+$). GC−MS: t_R 30.76 min, m/z 254 ($M^{\bullet+}$). HRMS: m/z 255.1587 $[M + H]^+$ (255.1590 calculated for $C_{14}H_{23}O_4$).

*Ethyl (2E,4E)-5-Formylhex-2,4-dienoate (**11**).* A catalytic amount of 12 M HCl (3 drops) was added to a solution of compound **10** (1 g, 3.9 mmol) in 100 mL of H_2O/MeOH (1/1). The resulting solution was stirred for 8 h at room temperature, then neutralized with aqueous $NaHCO_3$ (10%, w/v) and the product extracted with diethyl ether (3 × 30 mL). The combined organic layers were washed with brine (3 × 30 mL), dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel (eluent EtOAc/hexane (2/8, v/v)) to afford compound **11** as a yellow oil (300 mg, 46%). δ_H (500 MHz, $CDCl_3$) 1.33 (t, 3, J = 7.1 Hz, CH_3(ethyl)); 1.96 (d, 3, J = 1.2 Hz, $H_3C-C(5)$); 4.26 (q, 2, J = 7.1 Hz, H_2C(ethyl)); 6.27 (d, 1, J = 15.2 Hz, H−C(2)); 6.92 (d, 1, J = 11.8 Hz, H−C(4)); 7.69 (dd, 1, J = 11.8 and 15.2 Hz, H−C(3)); 9.55 (s, 1, H−C (6)). δ_C (125 MHz, $CDCl_3$) 10.0 ($CH_3-C(5)$); 14.2 (H_3C(ethyl)); 61.0 (H_2C(ethyl)); 128.6 (C(2)); 137.5 (C(4)); 143.8 (C(3)); 143.9 (C(5)); 165.8 (C(1)); 194.4 (C(6)). HPLC−UV−MS: t_R 1.85 min; λ_{max}/nm 283; m/z 169.0 ($[M + H]^+$). GC−MS: t_R 19.35 min; m/z 168 (M^+). HRMS: m/z 169.0857 ($[M + H]^+$) (169.0859 calculated for $C_9H_{13}O_3$).

*Methyl (2E,4E,6E,8E,10E,12E,16E)-5,9,13,17-Tetramethyloctadeca-2,4,6,8,10,12,16-heptaenoate (**12**).* Compound **11** (0.57 g, 3.4 mmol) and phosphonium salt **2** (2.2 g, 4 mmol, diastereoisomeric mixture) were dissolved in 5 mL of CH_2Cl_2. Then a solution of NaOMe (4 mmol) in MeOH (8 mL) was slowly added over 30 min at 0 °C and the mixture stirred for 20 h at room temperature. The reaction was subsequently quenched with saturated aqueous NH_4Cl (5 mL). The organic layer was washed with distilled water (3 × 5 mL), dried over Na_2SO_4, and concentrated. The crude product was filtered on a silica gel column (eluent EtOAc/hexane (2/8, v/v)) and purified by crystallization from hexane to give compound **12** as an orange powder (280 mg, 24%). δ_H (500 MHz, $CDCl_3$) 1.61 (s, 3, $H_3C-C(17)$); 1.69 (s, 3, $H_3-C(18)$); 1.83 (s, 3, $H_3C-C(13)$); 1.99 (s, 3, $H_3C-C(9)$); 2.06 (s, 3, $H_3C-C(5)$); 2.12 (m, 4, $H_2-C(14, 15)$); 3.76 (s, 3, H_3C(methyl)); 5.11 (m, 1, H−C(16)); 5.88 (d, 1, J = 15.0 Hz, H−C(2)); 5.96 (d, 1, J = 11.0 Hz, H−C(12)); 6.18 (d, 1, J = 11.3 Hz, H−C(8)); 6.22 (d, 1, J = 12.1 Hz, H−C(4)); 6.25 (d, 1, J = 14.8 Hz, H−C(10)); 6.35 (d, 1, J = 14.8 Hz, H−C(6)); 6.55 (dd, 1, J = 11.3 and 14.8 Hz, H−C(11)); 6.81 (dd, 1, J = 11.3 and 14.8 Hz, H−C(7)); 7.70 (dd, 1, J = 12.1 and 15 Hz, H−C(3)). δ_C (125 MHz, $CDCl_3$) 13.1 ($H_3C-C(9)$); 13.3 ($H_3C-C(5)$); 17.2 ($H_3C-C(13)$); 17.9 ($H_3C-C(17)$); 25.8 (C(18)); 26.8 (C(15)); 40.4 (C(14)); 51.6 (H_3C(methyl)); 119.9 (C(2)); 124.0 (C(16)); 125.7 (C(12)); 126.2 (C(11)); 128.7 (C(4)); 128.7 (C(7)); 130.9 (C(8)); 132.0 (C(17)); 135.2 (C10)); 136.1 (C(6)); 138.7 (C(9)); 140.7 (C(13)); 140.8 (C(3)); 144.7 (C(5)); 168.0 (C(1)). HPLC−UV−MS: t_R 12.71 min; λ_{max}/nm 406; m/z 341.4 ($[M + H]^+$). HRMS: m/z 341.2465 ($[M + H]^+$) (341.2475 calculated for $C_{23}H_{33}O_2$).

*(2E,4E,6E,8E,10E,12E,16E)-5,9,13,17-Tetramethyloctadeca-2,4,6,8,10,12,16-heptaenoic Acid (**13**).* Compound **12** (250 mg, 0.74 mmol) was dissolved in a solution of NaOH (720 mg, 18 mmol) in EtOH (20 mL). The resulting mixture was stirred for 24 h at 35 °C, then acidified with aqueous HCl (10%, v/v), and the product was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, concentrated and the residue was purified by crystallization from CH_2Cl_2/hexane to give compound **13** as an orange powder (120 mg, 49%). δ_H (500 MHz, $CDCl_3$) 1.62 (s, 3, $H_3C-C(17)$); 1.69 (s, 3, $H_3C(18)$); 1.83 (s, 3, $H_3C-C(13)$); 1.99 (s, 3, $H_3C-C(9)$); 2.08 (s, 3, $H_3C-C(5)$); 2.12 (m, 4, $H_2C(14, 15)$); 5.11 (m, 1, H−C(16)); 5.88 (d, 1, J = 14.9 Hz, H−C(2)); 5.96 (d, 1, J = 11.0 Hz, H−C(12)); 6.19 (d, 1, J = 11.4 Hz, H−C(8)); 6.24 (d, 1, J = 12.1 Hz, H−C(4)); 6.25 (d, 1, J = 15.0 Hz, H−C(10)); 6.36 (d, 1, J = 15.0 Hz, H−C(6)); 6.57 (dd, 1, J = 11.0 and 15.0 Hz, H−C(11)); 6.85 (dd, 1, J = 11.4 and 15.0 Hz, H−C(7)); 7.79 (dd, 1, J = 12.1 and 14.9 Hz, H−C(3)). δ_C (125 MHz, $CDCl_3$) 13.2 ($H_3C-C(9)$); 13.4 ($H_3C-C(5)$); 17.2 ($H_3C-C(13)$); 17.9 ($H_3C-C(17)$); 25.9 (C(18)); 26.8 (C(15)); 40.4 (C(14)); 119.3 (C(2)); 124.0 (C(16)); 125.8 (C(12)); 126.4 (C(11)); 128.5 (C(4)); 129.3 (C(7)); 130.8 (C(8)); 132.0 (C(17)); 135.1 (C10)); 136.0 (C(6)); 139.1 (C(9)); 140.9 (C(13)); 142.8 (C(3)); 145.8 (C(5)); 172.8 (C(1)). HPLC−UV−MS: t_R 10.22 min; λ_{max}/nm 399; m/z 327.3 ($[M + H]^+$). HRMS: m/z 327.2320 ($[M + H]^+$) (327.2319 calculated for $C_{22}H_{31}O_2$).

Animal Supplementation Studies and Subsequent QRT-PCR Analysis. Animal experiments were performed at the Laboratory Animal Core Facility at the University of Debrecen in Hungary in accordance with Hungarian ethical guidelines. Male mice (C57/BL6) aged 10−12 weeks received a standard pelleted laboratory animal diet from Altromin, VRF 1 (Charles River, Budapest, Hungary). Lycopene-containing beadlets (100 mg lycopene/kg bw) and the same amount of control beadlets (beadlets without lycopene) were given to eight animals by oral gavage. Equimolar amounts of 10′-apolycopen-10′-oic acid (4.9 mg/kg bw) or 14′-apolycopen-14′-oic acid (4 mg/kg bw) were incorporated in the diet via a DMSO solution (200 μL). The negative control animal group was treated with the same volume of DMSO. Each of the 10′-apolycopen-10′-oic acid-treated and 14′-apolycopen-14′-oic acid-treated groups included n = 4−5 animals. Mice were killed by cervical dislocation 16 h later and their organs collected for QRT-PCR. All organs were stored at −80 °C until RNA isolation. Total RNA was isolated from mouse tissue using the standard Trizol method according to the manufacturer's instructions (Invitrogen). After a check of the concentration and purity, RNA samples were reverse-transcribed into cDNA according to the supplier's protocol under the following conditions: 10 min at 25 °C, 120 min at 42 °C, 5 min at 72 °C, and 10 min at 4 °C (Applied Biosystems, 2720 thermal cycler). QRT-PCR was performed using an ABI PRISM 7900 sequence detection system (Applied Biosystems) as follows: 1 min at 94 °C, followed by 40 cycles of 12 s at 94 °C and 30 s at 60 °C. mRNA levels were normalized to the level of cyclophilin, which served as an internal control for the amount of RNA used in each reaction. The resulting normalized values were plotted as a bar graph ± the standard error. Student's t test was used for biostatistical analysis.

RESULTS AND DISCUSSION

Organic Synthesis of 10′-Apolycopen-10′-oic Acid and 14′-Apolycopen-14′-oic Acid. Apo-lycopenals and diapocarotene dials have already been synthesized in our laboratory by oxidation of lycopene with $KMnO_4$.[24] However, this route gave complex mixtures of molecules and was not suitable for preparation of the pure products required for biological testing. Thus, we developed another synthetic strategy based on previous work describing the total synthesis of retinoids and carotenoids,[25] of (all-*E*)-lycopene,[26] and of (*Z*) isomers of lycopene and apo-12′-lycopenal.[22] We used chemical reactions classically utilized in carotenoid synthesis, such as the Horner−Wadsworth−Emmons (HWE) and Wittig coupling reactions, to build the hydrocarbon skeleton of the apo-lycopenoic acids. A critical point in the synthesis of the two apo-lycopenoic acids was the diastereoisomeric control of the carbon−carbon double bonds formed in these steps. Our aim was to obtain the apo-lycopenoids in a pure (*E*) configuration. Therefore, we optimized the experimental conditions in order to minimize the formation of (*Z*) carbon−carbon double bonds.

The triphenylphosphonium methanesulfonate salt **2** is a common building block for the two targeted apo-lycopenoic acids (Figures 2 and 3). Although this phosphonium salt has been prepared previously from vinylpseudoionol (**1**) via a S_N1 reaction with hydrobromic acid and triphenylphosphine in methanol,[27] we obtained only a poor stereoselectivity [(*E*/*Z*) = 2.4] and numerous byproducts. Another method for converting **1** into the corresponding phosphonium methanesulfonate **2** involves the use of methanesulfonic acid in

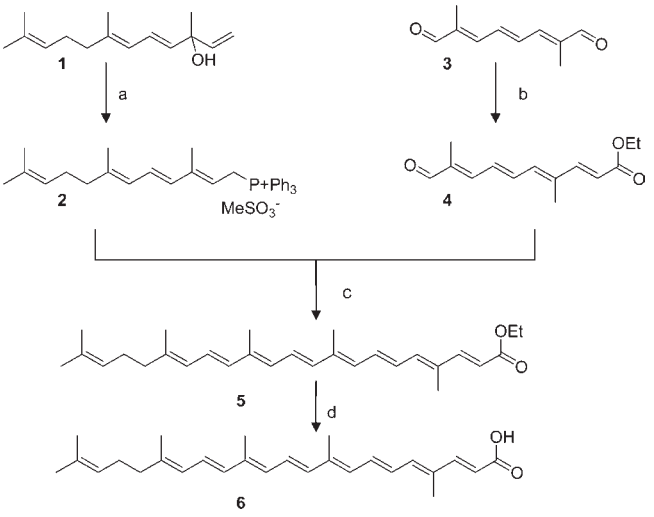

Figure 2. Organic synthesis of 10′-apolycopen-10′-oic acid (**6**). Reagents and conditions are as follows: (a) PPh_3, $MeSO_3H$, AcOH, 10 °C → 45 °C, 71%; (b) $(EtO)_2OPCH_2COOEt$, *t*-BuOK, THF, 38%; (c) NaOMe, CH_2Cl_2, 18%; (d) NaOH, EtOH, 55%.

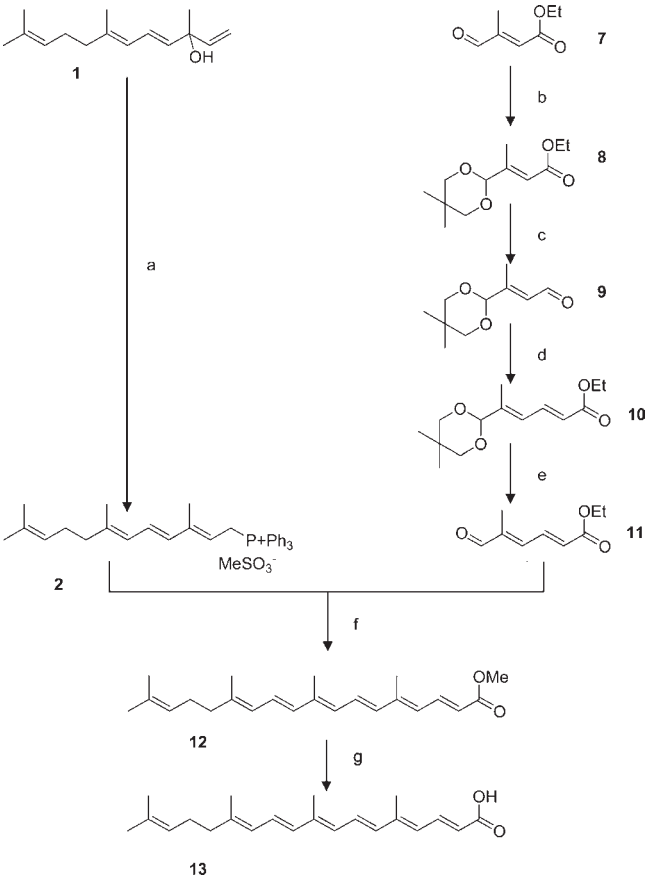

Figure 3. Organic synthesis of 14′-apolycopen-14′-oic acid (**13**). Reagents and conditions are as follows: (a) PPh_3, $MeSO_3H$, AcOH, 10 °C → 45 °C, 71%; (b) 2,2-dimethylpropanediol, p-TSA, toluene, reflux, 81%; (c) (i) DIBAH, THF, (ii) MnO_2, EtOAc 56%; (d) $(EtO)_2OPCH_2COOEt$, *t*-BuOK, THF, 55%; (e) HCl, $MeOH/H_2O$, 46%; (f) NaOMe, CH_2Cl_2, 24%; (g) NaOH, EtOH, 49%.

glacial acetic acid[26,28] (Figures 2 and 3). Under these experimental conditions, we were able to improve the stereoselectivity to (*E*/*Z*) = 4 and obtain compound **2** with a yield of 71% (Figures 2 and 3). According to Wegner et al.,[28] this increased stereoselectivity is due to the initial formation of the tertiary phosphonium cation at 10 °C, followed by its allylic transposition when the temperature is increased to 45 °C.

Compound **4**, the other building block needed to synthesize 10′-apolycopen-10′-oic acid, was prepared by elongation of one side of dialdehyde **3** upon treatment with 1 equiv of triethylphosphonoacetate in a HWE reaction.[29] The mechanism of this reaction involves an oxaphosphetane intermediate and leads to a new carbon−carbon double bond with a high diastereoisomeric (*E*/*Z*) ratio of 25. Compound **4** was separated from the byproduct formed as a result of double coupling by open column chromatography and was subsequently crystallized in pure (*E*) form with a global yield of 38%. Ethyl 10′-apo-10′-lycopenoate (**5**) was obtained by a Wittig coupling[29] between **2** and **4**, both of which were used as geometric mixtures. Thus, the ylide formed upon deprotonation of **2** with sodium methoxide was treated with **4** to form the new carbon−carbon double bond mainly in an (*E*) configuration. Compound **5** was obtained in pure (*E*) form with a yield of 18% upon crystallization. The first target molecule 10′-apolycopen-10′-oic acid (**6**) was subsequently obtained in 55% yield by saponification of **5** with sodium hydroxide in ethanol.

For the synthesis of 14′-apolycopen-14′-oic acid (**13**), building block **2** was prepared as described previously and the other building block, **11**, was obtained in a four-step pathway from ethyl 3-methyl-4-oxocrotonate (**7**). Thus, the aldehyde group of **7** was first protected as a cyclic acetal under acid catalysis. Then the ethyl ester of acetal **8** was reduced to the corresponding allyl alcohol by treatment with DIBAH. This alcohol was then oxidized with manganese dioxide to form aldehyde **9** without intermediate purification (overall yield = 56%). The carbon chain of **9** was then elongated by two carbon atoms in a HWE coupling to afford **10** in 55% yield and with a high diastereoisomeric (*E*/*Z*) ratio of 33. Finally, the target building block **11** was obtained by deprotection of the aldehyde of **10** by acid-catalyzed hydrolysis. Compound **11** was obtained with a yield of 46% after crystallization. Methyl 14′-apolycopen-14′-oate (**12**) was prepared by a Wittig coupling between **2** and **11** as described previously for compound **5**. The ethyl ester of **12** was converted into a methyl ester during the reaction. The pure diastereoisomer of **12** was obtained in 24% yield upon crystallization, and subsequent saponification gave 14′-apolycopen-14′-oic acid (**13**) with all carbon−carbon double bonds under (*E*) configuration.

Influence of 10′-Apolycopen-10′-oic Acid or 14′-Apolycopen-14′-oic Acid on BCO1 and BCO2 Expression in Mouse Testis in Comparison with Lycopene. The expression of BCO1 and BCO2 in mouse testis was found to be significantly lower after lycopene treatment (Figure 4). However, treatment with an equimolar amount of 10′-apolycopen-10′-oic acid or 14′-apolycopen-14′-oic acid had no effect on BCO1 expression, thereby suggesting that BCO1 is not regulated by these two putative lycopene metabolites or their downstream metabolites. In contrast, both these metabolites were found to up-regulate BCO2 in a statistically significant manner for 14′-apolycopen-14′-oic acid, whereas (all-*E*)-lycopene down-regulates the expression of this enzyme (Figure 4).

It is still unclear which nuclear hormone receptor-mediated pathways are responsible for this effect, although further work on this topic is currently underway in our laboratories. BCO1 is regulated by nuclear hormone receptor response pathways involving the retinoic acid receptor (RAR)[30] and the peroxisome proliferator activated

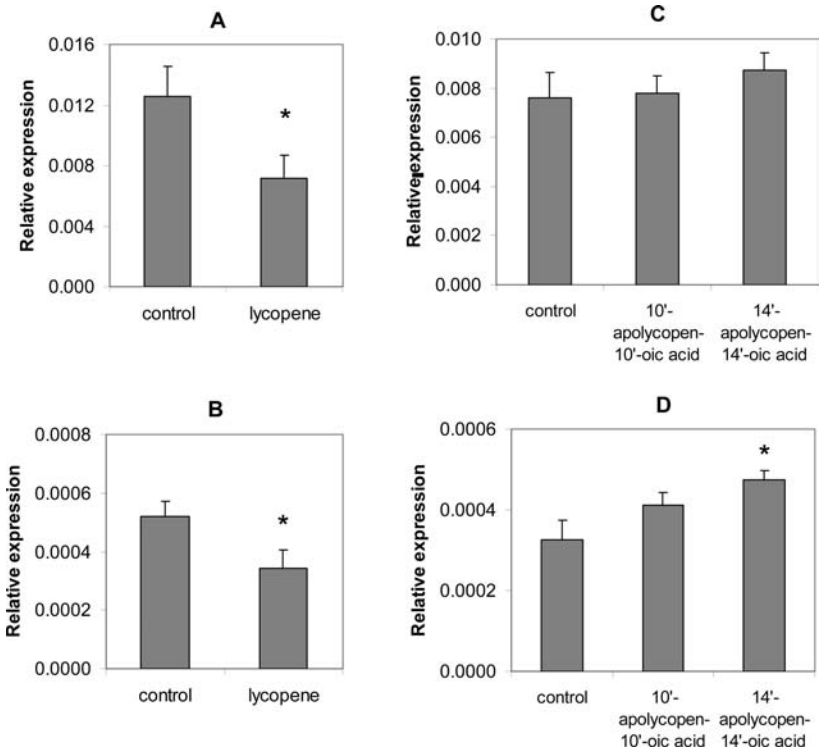

Figure 4. QRT-PCR expression analysis from mouse testis of the two carotenoid-metabolizing enzymes BCO1 (**A** and **C**) and BCO2 (**B** and **D**). Mice treated with (all-*E*)-lycopene (100 mg/kg bw) (**A** and **B** respectively), 14′-apolycopen-14′-oic acid (**13**) (4.0 mg/kg bw), or 10′-apolycopen-10′-oic acid (**6**) (4.9 mg/kg bw) in comparison with controls (**C** and **D**). All animals were treated via oral gavage, $n = 4-5$ animals were used for 14′-apolycopen-14′-oic acid (**13**)-treated and 10′-apolycopen-10′-oic acid (**6**)-treated groups, $n = 8$ were used for (all-*E*)-lycopene and control groups. Gene expression (*y*-axis) is expressed versus cyclophilin: ∗, $p < 0.05$ indicating a significant difference compared to the control.

receptor (PPAR).[31] In particular, β-carotene and several of its metabolites were shown to down-regulate BCO1 expression in rat intestinal cells (with the exception of β-apo-12′-carotenal for which an up-regulation was observed) while being inactive in liver cells.[30] No such data are available with BCO2. We simply suggest that the lycopene-mediated down-regulation observed with BCO2 might involve lycopene metabolites distinct from apo-lycopenoic acids such as apo-lycopenals. Studies about lycopene and apo-lycopenoic acids metabolism in mice and about BCO1/BCO2-mediated pathways of lycopene cleavage to apo-lycopenoids are underway in our laboratories.

In conclusion, we have reported the first organic synthesis of 10′-apolycopen-10′-oic acid and 14′-apolycopen-14′-oic acid, both of which are bioactive lycopene derivatives with a remarkable ability to up-regulate BCO2 expression while (all-*E*)-lycopene induces a down-regulation of the enzyme. We suggest that lycopene metabolism into apo-lycopenoids is an important preliminary step toward the expression of lycopene bioactivity.

AUTHOR INFORMATION

Corresponding Author

*To whom correspondence should be addressed. Phone: +33 4 32 72 24 89. Fax: +33 4 32 72 24 92. E-mail: catherine.caris@avignon.inra.fr.

Funding Sources

This work was supported by grants from the European Community's Sixth Framework Program (Lycocard, Integrated European Project No. 016213). The work is supported by the TÁMOP 4.2.1./B-09/1/KONV-2010-0007 project. The project is implemented through the New Hungary Development Plan, cofinanced by the European Social Fund.

ACKNOWLEDGMENT

We thank Hansgeorg Ernst (BASF Aktiengesellschaft, Fine Chemicals, and Biocatalysis Research, Ludwigshafen, Germany), Bettina Wüstenberg, and Simon Werner (DSM Nutritional Products Ltd., Kaiseraugst, Switzerland) for their generous gifts of chemicals. We also thank Christian Giniès for his help with GC-MS analyses and the Spectropole in Marseille for their assistance in NMR and HRMS analyses.

NOTE ADDED AFTER ASAP PUBLICATION

There was an error in the caption of Figure 4 in the version published ASAP January 19, 2011; the corrected version was published ASAP January 28, 2011.

REFERENCES

(1) Rao, A. V. R.; Agarwal, S. Role of antioxidant lycopene in cancer and heart disease. *J. Am. Coll. Nutr.* **2000**, *19*, 563–569.

(2) Giovannucci, E. Tomato products, lycopene, and prostate cancer: a review of the epidemiological literature. *J. Nutr.* **2005**, *135*, 2030S–2031S.

(3) Sesso, H. D.; Liu, S. M.; Gaziano, J. M.; Buring, J. E. Dietary lycopene, tomato-based food products and cardiovascular disease in women. *J. Nutr.* **2003**, *133*, 2336–2341.

(4) Sesso, H. D.; Buring, J. E.; Norkus, E. P.; Gaziano, J. M. Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in women. *Am. J. Clin. Nutr.* **2004**, *79*, 47–53.

(5) Wang, X. D. Carotenoid Oxidative/Degradative Products and Their Biological Activities. In *Carotenoids in Health and Disease*; Krinsky, N. I., Mayne, S. T., Sies, H., Eds.; Marcel Dekker: New York, 2004; pp 313−335.

(6) Lindshield, B. L.; Canene-Adams, K.; Erdman, J. W. Lycopenoids: Are lycopene metabolites bioactive?. *Arch. Biochem. Biophys.* **2007**, *458*, 136–140.

(7) Khachik, F.; Spangler, C. J.; Smith, J. C.; Canfield, L. M.; Steck, A.; Pfander, H. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Anal. Chem.* **1997**, *69*, 1873–1881.

(8) Ben-Aziz, A.; Britton, G.; Goodwin, T. W. Carotene epoxides of *Lycopersicon esculentum*. *Phytochemistry* **1973**, *12*, 2759–2764.

(9) Märki-Fisher, E.; Eugster, C. H. Dyes from roses. 5. Isolation of 10′-apo-beta-carotene-10′-ol and (3*R*)-10′-apo-beta-carotene-3, 10′-diol (galloxanthin) from rose flowers. *Helv. Chim. Acta* **1987**, *70*, 1994–2002.

(10) Gajic, M.; Zaripheh, S.; Sun, F. R.; Erdman, J. W. Apo-8 ′-lycopenal and apo-12′-lycopenal are metabolic products of lycopene in rat liver. *J. Nutr.* **2006**, *136*, 1552–1557.

(11) Hu, K. Q.; Liu, C.; Ernst, H.; Krinsky, N. I.; Russell, R. M.; Wang, X. D. The biochemical characterization of ferret carotene-9′,10′-monooxygenase catalyzing cleavage of carotenoids in vitro and in vivo. *J. Biol. Chem.* **2006**, *281*, 19327–19338.

(12) Kopec, R. E.; Riedl, K. M.; Harrison, E. H.; Curley, R. W.; Hruszkewycz, D. P.; Clinton, S. K.; Schwartz, S. J. Identification and quantification of apo-lycopenals in fruits, vegetables, and human plasma. *J. Agric. Food Chem.* **2010**, *58*, 3290–3296.

(13) Tibaduiza, E. C.; Fleet, J. C.; Russell, R. M.; Krinsky, N. I. Excentric cleavage products of beta-carotene inhibit estrogen receptor positive and negative breast tumor cell growth in vitro and inhibit activator protein-1-mediated transcriptional activation. *J. Nutr.* **2002**, *132*, 1368–1375.

(14) Prakash, P.; Liu, C.; Hu, K. Q.; Krinsky, N. I.; Russell, R. M.; Wang, X. D. Beta-carotene and beta-apo-14′-carotenoic acid prevent the reduction of retinoic acid receptor beta in benzo a pyrene-treated normal human bronchial epithelial cells. *J. Nutr.* **2004**, *134*, 667–673.

(15) Mein, R.; Wang, X.-D. Oxidative Metabolites of Lycopene and Their Biological Functions. In *Carotenoids. Physical, Chemical, and Biological Functions and Properties*; Landrum, J. T., Ed.; CRC Press: Boca Raton, FL, 2010; pp 417−435.

(16) Stahl, W.; von Laar, J.; Martin, H.-D.; Emmerich, T.; Sies, H. Stimulation of gap junctional communication: comparison of acycloretinoic acid and lycopene. *Arch. Biochem. Biophys.* **2000**, *373*, 271–274.

(17) Lian, F. Z.; Smith, D. E.; Ernst, H.; Russell, R. M.; Wang, X. D. Apo-10′-lycopenoic acid inhihits lung cancer cell growth in vitro, and suppresses lung tumorigenesis in the A/J mouse model in vivo. *Carcinogenesis* **2007**, *28*, 1567–1574.

(18) Lian, F. Z.; Wang, X. D. Enzymatic metabolites of lycopene induce Nrf2-mediated expression of phase II detoxifying/antioxidant enzymes in human bronchial epithelial cells. *Int. J. Cancer* **2008**, *123*, 1262–1268.

(19) Linnewiel, K.; Ernst, H.; Caris-Veyrat, C.; Ben-Dor, A.; Kampf, A.; Salman, H.; Danilenko, M.; Levy, J.; Sharoni, Y. Structure activity relationship of carotenoid derivatives in activation of the electrophile/antioxidant response element transcription system. *Free Radical Biol. Med.* **2009**, *47*, 659–667.

(20) Ben-Dor, A.; Steiner, M.; Gheber, L.; Danilenko, M.; Dubi, N.; Linnewiel, K.; Zick, A.; Sharoni, Y.; Levy, J. Carotenoids activate the antioxidant response element transcription system. *Mol. Cancer Ther.* **2005**, *4*, 177–186.

(21) Kiefer, C.; Hessel, S.; Lampert, J. M.; Vogt, K.; Lederer, M. O.; Breithaupt, D. E.; von Lintig, J. Identification and characterization of a mammalian enzyme catalyzing the asymmetric oxidative cleavage of provitamin A. *J. Biol. Chem.* **2001**, *276*, 14110–14116.

(22) Hengartner, U.; Bernhard, K.; Meyer, K.; Englert, G.; Glinz, E. Synthesis, isolation, and NMR-spectroscopic characterization of 14 (*Z*)-isomers of lycopene and of some acetylenic didehydrolycopenes and tetradehydrolycopenes. *Helv. Chim. Acta* **1992**, *75*, 1848–1865.

(23) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR chemical shifts of common laboratory solvents as trace impurities. *J. Org. Chem.* **1997**, *62*, 7512–7515.

(24) Caris-Veyrat, C.; Schmid, A.; Carail, M.; Bohm, V. Cleavage products of lycopene produced by in vitro oxidations: characterization and mechanisms of formation. *J. Agric. Food Chem.* **2003**, *51*, 7318–7325.

(25) Paust, J. Recent progress in commercial retinoids and carotenoids. *Pure Appl. Chem.* **1991**, *63*, 45–48.

(26) Ernst, H. Recent advances in industrial carotenoid synthesis. *Pure Appl. Chem.* **2002**, *74*, 2213–2226.

(27) Muller, R. K.; Bernhard, K.; Giger, A.; Moine, G.; Hengartner, U. (*E*/*Z*)-Isomeric carotenes. *Pure Appl. Chem.* **1997**, *69*, 2039–2046.

(28) Wegner, C.; John, M. U.S. Patent 6,423,873, 2002.

(29) Takeda, T. *Modern Carbonyl Olefination*; Wiley-VCH: Weinheim, Germany, 2004; p 349.

(30) Bachmann, H.; Desbarats, A.; Pattison, P.; Sedgewick, M.; Riss, G.; Wyss, A.; Cardinault, N.; Duszka, C.; Goralczyk, R.; Grolier, P. Feedback regulation of beta,beta-carotene 15,15′-monooxygenase by retinoic acid in rats and chickens. *J. Nutr.* **2002**, *132*, 3616–3622.

(31) Boulanger, A.; McLemore, P.; Copeland, N. G.; Gilbert, D. J.; Jenkins, N. A.; Yu, S. S.; Gentleman, S.; Redmond, T. M. Identification of beta-carotene 15,15′-monooxygenase as a peroxisome proliferator-activated receptor target gene. *FASEB J.* **2003**, *17*, 1304–1306.