SYNTHESIS OF ^{13}C-LABELLED (ALL-E,3R,3'R)-β,β-CAROTENE-3,3'-DIOL (ZEAXANTHIN) AT C(12), C(13), C(12'), AND C(13') VIA ALL-E-2,7-DIMETHYLOCTA-2,4,6-TRIENE-1,8-DIAL-$^{13}C_4$

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SUMMARY

The title compound (**10**) has been synthesized from all-E-2,7-dimethylocta-2,4,6-triene-1,8-dial (C_{10}-dialdehyde, **8**) labelled with four ^{13}C from commercially available and relatively inexpensive starting materials. The key starting material in this synthesis, $(EtO)_2P(O)^{13}CHMe^{13}CO_2Et$ (**2**), has been prepared from triethyl phosphonoacetate-$^{13}C_2$. The sodium salt of **2** reacted with fumarylaldehyde dimethylacetal to give ethyl 6,6-dimethoxy-2-methyl-E,E-2,4-hexadienoate (**4**) which was converted to **8** in four steps [(1) acid hydrolysis, (2) **2**/NaH, (3) $LiAlH_4$ reduction, (4) MnO_2 oxidation]. The overall yield of **8** based on phosphonate **1** is 43-46%. The double Wittig reaction of **8** with [(3R-3-hydroxy-β-ionylidene)ethyl]triphenylphosphonium chloride afforded **10**, also known as (all-E,3R,3'R)-zeaxanthin-$^{13}C_4$ (39% based on phosphonate **1**) in high purity. This synthetic method may be extended to prepare other ^{13}C-labelled carotenoids.

Key words: Synthesis of ^{13}C-labelled carotenoids, synthesis of stable isotope of carotenoids, human macular pigment

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INTRODUCTION

Carotenoids are one of the major class of compounds abundant in fruits and vegetables which have been studied for their inhibitory effect against several types of human cancers.[1] In addition to seven previously known dietary carotenoids, we have isolated and characterized fourteen new carotenoids in the extracts from human plasma.[2-4] Among these were low levels of several oxidation products of (3R,3'R)-β,β-carotene-3,3'-diol (zeaxanthin) and (3R,3'R,6'R)-β,ε-carotene-3,3'-diol (lutein) which are two of the most prominent dihydroxycarotenoids found in fruits, vegetables, human plasma, and dissected human retina. In several preliminary human feeding studies with naturally occurring lutein and zeaxanthin, we have established *in vivo* oxidation of these dietary carotenoids to their oxidative metabolites.[5] However, in order to further investigate the absorption, bioavailability, and metabolism of carotenoids in nutritional prevention of cancer, human feeding studies with synthetic carotenoids labelled with a stable isotope (i.e. ^{13}C) are needed. We present a method for the synthesis of ^{13}C-labelled carotenoids, e.g. zeaxanthin, in high purity from commercially available and relatively inexpensive starting materials.

RESULTS AND DISCUSSION

The synthesis of ^{13}C-labelled zeaxanthin was undertaken to study the oxidation-reduction reactions of this compound in humans. The metabolism and the antioxidant function of zeaxanthin is of particular interest, since this compound and its structurally related regio-isomer, lutein, are the only two major macular carotenoids detected in humans. The high intake of fruits and vegetables specifically rich in zeaxanthin and lutein has been associated with a lower risk for advanced age-related macular degeneration.[6] In human metabolic studies with carotenoids, where certain chemical transformations such as oxidation-reduction reactions are of particular interest, the location of the label is not

crucial since the general skeleton of the carotenoid molecule remains unchanged throughout these metabolic reactions. Vitamin A active carotenoids such as α- and β-carotene are exceptions to this rule, since these compounds in part undergo random cleavage across the polyene chain to form vitamin A as well as a number of apocarotenols. However, in metabolic studies with carotenoids, it is imperative that at least four carbons in the carotenoid molecule are labelled. This is because the oxidative metabolites of non-vitamin A active carotenoids are only two mass units lower than their parent compounds. Therefore by increasing the molecular weight in the labelled compound by four mass units, one can readily distinguish carotenoid metabolites from their unlabelled parent compounds. This is normally accomplished by High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) analysis of the serum extracts from human subjects. In the synthesis presented here, the introduction of the label into the carotenoid molecule in general is based on the ease and cost effectiveness of the synthetic pathways. The unlabelled all-E-2,7-dimethylocta-2,4,6-triene-1,8-dial (C_{10}-dialdehyde **8**), a key intermediate in the synthesis of carotenoids[7], has been previously synthesized by many different pathways.[8-16] However these pathways employ starting materials which cannot be readily labelled with ^{13}C atoms and as a result their custom syntheses are quite costly. We have labelled the C_{10}-dialdehyde (**8**) with four ^{13}C atoms employing commercially available triethyl phosphonoacetate-$^{13}C_2$ (**1**) and fumarylaldehyde dimethylacetal in a six step synthesis in an overall yield of 43-46% as shown in Figure 1. Compound **8** has been employed to synthesize (all-E,3R,3'R)-β,β-carotene-3,3'-diol-$^{13}C_4$ (zeaxanthin-$^{13}C_4$, **10**) labelled at C(12), C(13), C(12'), and C(13') in high purity. The synthesis shown in Figure 1 has been recently patented in the US.[17] The first step of this synthesis involves alkylation of **1** (99 atom %^{13}C) with

methyl iodide resulting in a 71% yield of triethyl 2-phosphonopropionate-$^{13}C_2$ (**2**) together with 18% of a side product identified by Gas Chromatography-Mass Spectrometry (GC-MS) as triethyl 2-methyl-2-phosphonopropionate-$^{13}C_2$ (**3**) and 11% of unreacted **1** as shown in Figure 2. The side product **3** does not interfere with the subsequent step of the synthesis. However **1** was separated and recovered almost quantitatively from the mixture by flash column chromatography.

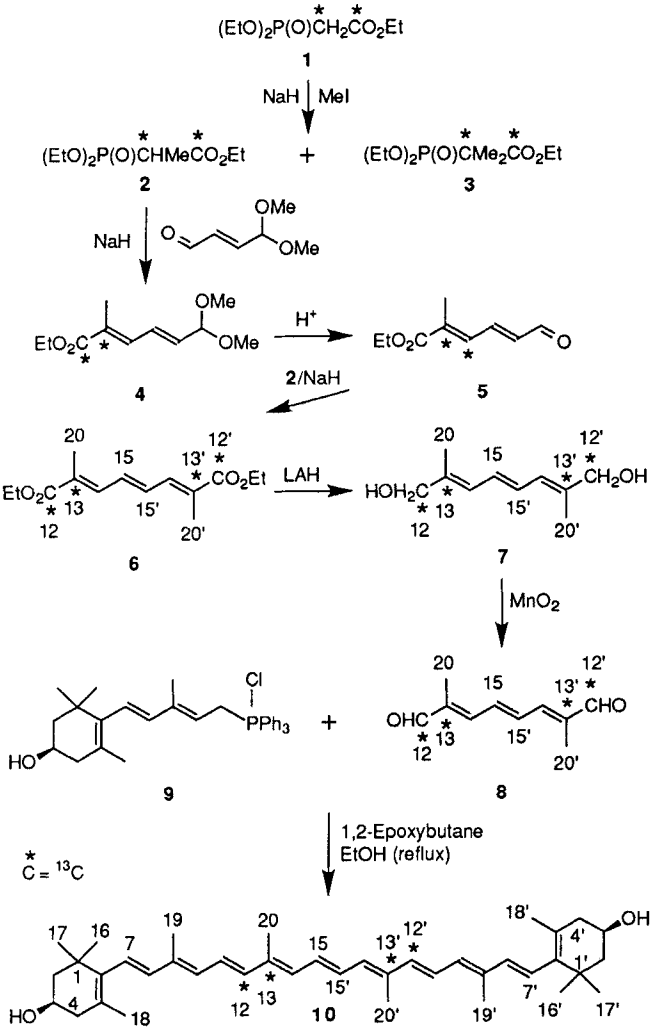

Figure 1. Synthesis of (all-E,3R,3'R)-zeaxanthin-$^{13}C_4$ (**10**). Carotenoid numbering system has been adopted for trienes **6**, **7**, and **8** to relate their structural assignments to that of central polyene chain in **10**.

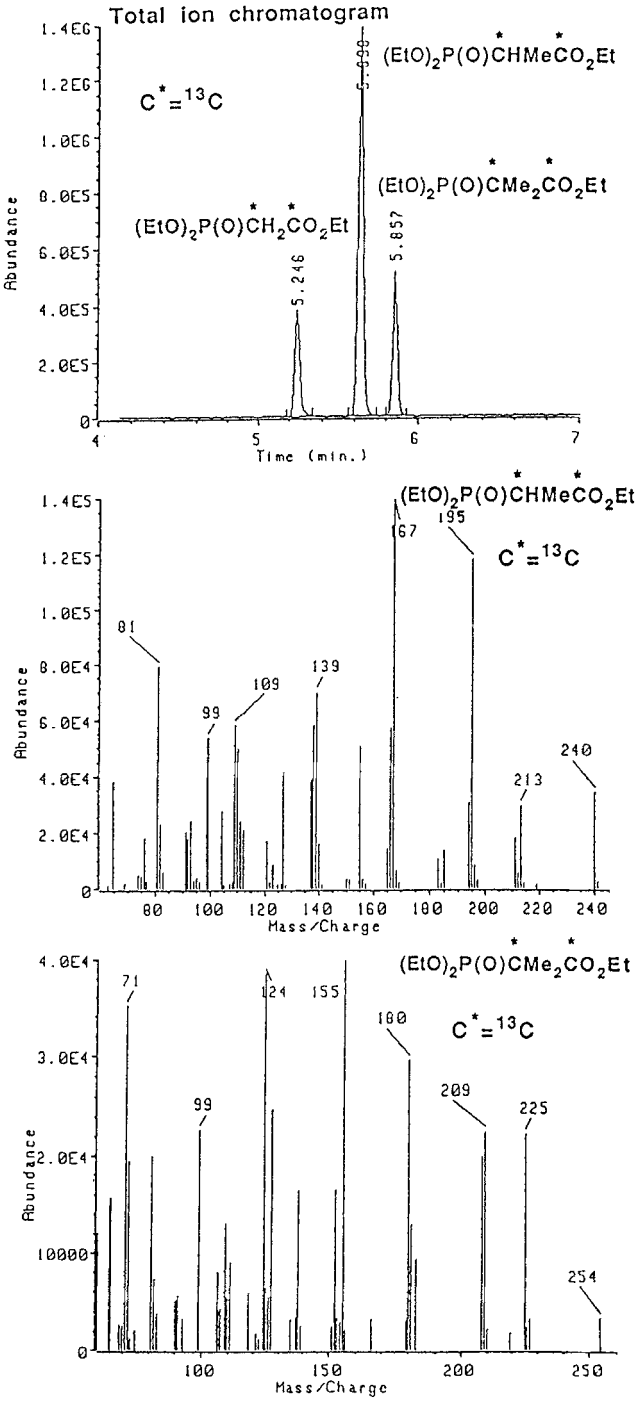

Figure 2. Separation and mass spectral data of ^{13}C-labelled phosphonates **1**, **2**, and **3** by GC-MS. Conditions described in text.

Although only unlabelled MeI was employed at this alkylation step, the use of inexpensive ^{13}C-labelled methyl iodide can allow the introduction of additional label into the molecule. The "Wittig-Horner" reaction between **2** and a mixture of fumarylaldehyde dimethylacetal (95%) and fumarylaldehyde (3%) [obtained from catalytic hydrolysis of fumaryialdehyde bis(dimethylacetal)][18] at -50°C to -60°C resulted in the formation of ethyl 6,6-dimethoxy-2-methyl-E,E-2,4-hexadienoate-$^{13}C_2$ (**4**, 90%) and (all-E)-2,7-dimethylocta-2,4,6-triene-1,8-diacid ethyl ester-$^{13}C_4$ (**6**, 3%). Compound **4** in the crude mixture was hydrolyzed at room temperature to give a 95% yield of ethyl 2-methyl-6-oxo-E,E-2,4-hexadienoate-$^{13}C_2$ (**5**), while **6** remains unchanged. The crude mixture containing **5** (ca. 95%) and **6** (ca. 3%) was then treated with the sodium salt of **2** at -50°C to -60°C to give a 90% yield of **6**, which was purified by crystallization. Reduction of **6** with $LiAlH_4$ similar to published procedures for the unlabeled compound[11] afforded an 82% yield of all-E-2,7-dimethylocta-2,4,6-triene-1,8-diol-$^{13}C_4$ (**7**). Oxidation of **7** with activated manganese dioxide at room temperature similar to a published method for the unlabeled compound[11] resulted in a 95% yield of the desired C_{10}-dialdehyde (**8**). The overall yield of **8** based on **1** was 43-46%. Alternatively, the direct synthesis of **6** from a double Wittig-Horner condensation of **2** with fumarylaldehyde at -50°C to -60°C resulted in poor yields (<30%). This is mainly due to the difficulties associated with preparation and purification of fumarylaldehyde.[19] The C_{10}-dialdehyde (**8**) was employed in a double Wittig reaction with {[(2E,4E)-5[(R)-4-hydroxy-2,6,6-trimethylcyclohex-1-enyl]-3-methylpenta-2,4-dienyl]}triphenyl-phosphonium chloride (Wittig salt **9**)[20-21] to synthesize (all-E,3R,3'R)-zeaxanthin-$^{13}C_4$ (**10**) in 90% yield similar to a published procedure for the

unlabeled compound.[20] The purity of the intermediates and the final products were determined by GC-MS, HPLC-MS, Thin Layer Chromatography (TLC), and UV-visible spectrophotometry. In the case of compounds **6**, **7**, **8**, and **10**, the structures were further confirmed by ^{1}H- and ^{13}C-NMR spectroscopy. In interpretation of the NMR data for compounds **6**, **7**, and **8**, the numbering system for carotenoids as shown in Figure 1 has been employed for these compounds as opposed to their systematic IUPAC nomenclature. This numbering system is expected to assist the readers to relate the structural assignments for these trienes to that of the central polyene chain in (all-E,3R,3'R)-β,β-carotene-3,3'-diol-$^{13}C_4$ (**10**). The purity of 99 atom %^{13}C label in **10** was elucidated by electron capture negative ionization mass spectrometry, in which the relative abundance of the anion peak at m/z = 573 ($^{13}C_4C_{35}{}^{13}CH_{56}O_2$) was 40% of the relative abundance of the molecular anion peak at m/z = 572 ($^{13}C_4C_{36}H_{56}O_2$). In the synthetic pathways described here, most of the intermediates and products are stable compounds therefore the reactions can be easily handled in large scale operations.

In summary, the introduction of the ^{13}C label into the molecular structure of zeaxanthin (**10**) has been accomplished with the introduction of the label into the C_{10}-dialdehyde (**8**). This is owing to the elaborate synthesis of the Wittig salt end-group (**9**) required for the synthesis of this compound.[20-21] Furthermore, employing the appropriate carotenoid end-group in double Wittig reactions with **8**, other ^{13}C-labelled carotenoids such as lutein, lycopene, α-cryptoxanthin, β-cryptoxanthin, α-carotene, and β-carotene can be readily prepared.

EXPERIMENTAL

Triethyl phosphonoacetate-$^{13}C_2$ and fumarylaldehyde bis(dimethylacetal) were obtained from Aldrich Chemical Co (Milwaukee, WI).

GC-MS system consisted of a Hewlett-Packard (HP) 5890 gas chromatograph which was interfaced with HP 5970B selective electron impact (EI) detector via a capillary direct inlet. A fused silica cross-linked methyl silicone capillary column (25-m length, 0.20-mm i.d., and 0.33-μm film thickness) was used for the analyses. Gas chromatographic conditions were as follows: initial temperature = 140°C increasing at a rate = 5°/min; final temperature = 175°C; total chromatographic run time = 7 min; injection port temperature = 250°C; transfer line temperature = 280°C; helium flow rate = 0.5 mL/min. Mass detector conditions were: ion source temperature = 220°C, ionization energy = 70 eV. Data were acquired with HP 5970 MS Chemstation.

The HPLC-MS system consisted of a Hewlett-Packard 1090 photodiode array detector and a Hewlett Packard Model 5989A mass spectrometer interconnected by a particle beam interface. A silica-based nitrile bonded column (25-cm-length X 4.6-mm i.d.; 5-μm spherical particles) which was protected with .a Brownlee nitrile bonded guard cartridge (3-cm-length X 4.6 mm i.d.) at a flow rate of 0.7 mL/min was used to separate the analytes with this HPLC-MS system. Separations were achieved with an isocratic mixture of hexane (74.65%), dichloromethane (25.0%), methanol (0.25%), and N,N-diisopropylethylamine (0.10%). Electron capture negative ionization (ECNI) and electron capture positive ionization (ECPI) were achieved using methane at a source pressure of 1.2 Torr. Absorption spectra were recorded on a Beckman DU-7 UV/visible spectrophotometer.

The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100.6 MHz) spectra were recorded on a Bruker ARX-400 spectrometer with ASPECT station 1. A pulsed magnetic field z-gradient accessory kit with 10 Amps power amplifier (50 gauss/cm) was available for pulsed magnetic field gradient (PFG) experiments. All spectra were measured in $CDCl_3$ (100% D quality) at ca. 25°C with a 5 mm reverse probe head with optimum sensitivity for protons and reduced sensitivity for carbons.

All the reactions in this report were carried out in a three-necked flask equipped with a magnetic stirrer, condenser, thermometer, addition funnel, and a nitrogen inlet. The flask at the beginning of each experiment was thoroughly flushed with nitrogen to exclude air and was kept under nitrogen atmosphere throughout the course of each experiment. An acetone/dry ice bath, maintained at the range of -50°C to -60°C, was used in low temperature reactions since an exact reaction temperature at this range was not critical. The major safety hazards associated with this synthesis are due to the sensitivity of some of the reagents such as NaH and LAH to air and moisture. Therefore, all reactions with this synthesis involving these reagents have to be carried out under an inert atmosphere such as nitrogen or argon.

Triethyl 2-Phosphonopropionate-$^{13}C_2$ (2): To a suspension of 95% NaH (0.90 g 95% = 0.86 g, 0.036 mol) in tetrahydrofuran (THF, 20 mL dried over Na) cooled at 10°C, triethyl phosphonoacetate-$^{13}C_2$ (**1**, 8.0 g, 0.035 mol) in THF (20 mL) was added during 15 minutes. The reaction mixture was allowed to warm up to room temperature and stirred at this temperature for 2.5 hr. The mixture was cooled to 0°C and methyl iodide (5.20 g, 0.037 mol) in THF (20 mL) was added at -5°C during 10 minutes. The cold bath was removed and the mixture was allowed to warm-up to room temperature and stirred at this temperature for 1 hr. The mixture was cooled in an ice-bath, water (30 mL) was added, and the product was partitioned between water (100 mL/salt) and dichloromethane (100 mL). The organic layer was removed and the water layer was washed with dichloromethane (2 x 50 mL). The organic layers were combined and dried over Na_2SO_4. After evaporation 8.62 g of a colorless oil was obtained. The crude oil was examined by GC-MS (Fig 2) and was shown to consist of unreacted **1** (11%; GC retention time, t_R=5.246 min; Mass=226), **2** (71%; t_R=5.638 min; Mass=240), and a side product identified as triethyl 2-methyl-2-phosphonopropionate-$^{13}C_2$ (**3**, 18%; t_R=5.857 min; Mass=254).

Purification of triethyl 2-phosphonopropionate-$^{13}C_2$ (2) by flash column chromatography: A flash chromatographic column[22] (40 cm x 4 cm) was packed with 80 g of n-silica gel [60-200 mesh, Baker Analyzed, average particle diameter (APD) ~ 63-200 μm, mean pore diameter ~60 Å] using petroleum ether (PE, b.p. 30-60°C). This corresponded to the height of 27 cm for the packing. The mixture (8.62 g) was loaded onto the column using 5 mL of PE and the column was sequentially eluted with 100 mL of PE and 200 mL of PE (90%)-ether (10%) at the rate of 1 inch/min. No product was shown by GC-MS to have eluted from the column at this time. The column was then eluted with PE-ether:9-1 (fractions 1-12), PE-ether:4-1 (fractions 13-25), PE-ether:2.3-1 (fractions 25-70), and PE-ether:0.43-1 (fractions 71-80), respectively. The volume of each fraction was 75 mL. All of the fractions were monitored by a GC-MS equipped with

an autosampler to determine fractions containing **2**. Fractions 16-70 (7 g) were combined and shown by GC-MS (EI) to consist of a mixture of **2** [m/z=240 for $^{13}C_2C_7H_{19}O_5P$](6 g, 86%) and **3** [m/z=254 for $^{13}C_2C_8H_{21}O_5P$] (1g, 14%). Fractions 10-12 were shown to consist of pure **3** (0.5 g). Combined fractions 71-80 (0.80 g) consisted of a mixture of **1** [m/z=226 for $^{13}C_2C_6H_{17}O_5P$](90%) and **2** (10%).

Ethyl 6,6-dimethoxy-2-methyl-E,E-2,4-hexadienoate-$^{13}C_2$ (4): To a suspension of 95% sodium hydride (0.36 g 95% = 0.34 g, 0.014 mol) in THF (20 mL dried over Na) cooled at 10°C, **2** (3.4 g, 86% pure ~2.92 g, 0.0122 mol) in THF (15 mL) was added during 15 minutes. The reaction mixture was allowed to warm-up to room temperature and stirred at this temperature for 2.5 hr. Fumarylaldehyde dimethylacetal[18] (1.82 g, 0.014 mol) in 30 mL of THF was added in 1.5 hr at -50 to -60°C. After the addition was completed, the mixture was stirred at -50°C to -60°C for an additional hour. The mixture was allowed to warm up to room temperature and was refluxed for 1 hr. The product was cooled in an ice-bath, water (30 mL) was added, and the crude mixture was partitioned between dichloromethane (100 mL) and water (100 mL/salt). The organic layer was removed and the water layer was washed with dichloromethane (2 x 50 mL). The combined dichloromethane layers was dried over sodium sulfate and evaporated to dryness to give a crude oil (6 g), which was shown to consist of mainly **4** (2.37 g, 0.011 mol, 90%): UV-visible, λ_{max} (hexane)=256 nm, $E^{1\%}$=1040; HPLC-MS [ECPI, ion source temperature = 150°C, $(M+H)^+$ at m/z=217 for $^{13}C_2C_9H_{18}O_4$] and low levels of (all-E)-2,7-dimethylocta-2,4,6-triene-1,8-diacid ethyl ester-$^{13}C_2$ (**6**, 0.032 g, 0.00013 mol): UV-visible, λ_{max} (hexane)=314 nm, $E^{1\%}$=2739; HPLC-MS [$(M+H)^+$ at m/z=257 for $^{13}C_4C_{10}H_{20}O_4$].

Ethyl 2-methyl-6-oxo-E,E-2,4-hexadienoate-$^{13}C_2$ (5): Crude product from the previous reaction (total weight 6 g, containing 2.37 g of

4) was dissolved in 100 mL of THF and 10 mL of dilute H_2SO_4 (from 1.80 mL of conc. H_2SO_4/100 mL of H_2O, saturated with Na_2SO_4). After 1 hr, the product was partitioned between dichloromethane (100 mL) and water (100 mL). The organic layer was removed, dried over sodium sulfate and evaporated to dryness to give 3.95 g of a crude product which was identified as a mixture of **5** (1.78 g, 0.0105 mol, 95%): UV-visible, λ_{max} (CH_2Cl_2)=284 nm, $E^{1\%}$=1962; λ_{max} (hexane)=278 nm, $E^{1\%}$=2069; HPLC-MS (ECNI, ion source temperature = 150°C, molecular parent ion at m/z=170 for $^{13}C_2C_7H_{12}O_3$) and **6** (0.032 g, 0.00013 mole).

(All-E)-2,7-dimethylocta-2,4,6-triene-1,8-diacid ethyl ester-$^{13}C_4$ (6): To a suspension of 95% NaH (0.29 g 95% = 0.28 g, 0.012 mol) in THF (15 mL dried over Na) cooled at 10°C, **2** (3.14 g, 86% pure ~ 2.70 g, 0.0112 mol) in 15 mL of THF was added during 15 minutes. The reaction mixture was allowed to warm-up to room temperature and stirred at this temperature for 2.5 hr. Compound **5** (1.78 g, 0.0105 mol) in THF (20 mL) was added in 1.5 hr at -50° to -60°C. After the addition was completed, the reaction mixture was stirred at the above temperature range for an additional hour, allowed to warm up to room temperature, and refluxed for 1 hr. The product was worked-up as described above for compound **4**. After drying and evaporation a crude oil (4.50 g) was obtained, which was crystallized from EtOH to give **6** (2.42 g, 0.0095 mole, ~90%): UV-visible: λ_{max} (hexane) = 305 (inflection), 314 (main absorption), 329 nm, $E^{1\%}$ at 314 nm = 2078; HPLC-MS (ECPI, ion source temperature = 150°C) molecular positive ion $[M+H]^+$ at m/z = 257 for $^{13}C_4C_{10}H_{20}O_4$; ^{1}H- NMR: 1.323 [6H, t, J_{HH} = 7.2 Hz, CH_3], 4.233 [4H, dd, $^3J_{CH}$ = 3.0 Hz, J_{HH} = 7.2 Hz, CH_2], 2.011 [6H, dd, ≈ 6.5 and 5 Hz, Me(20,20')], ≈ 6.805 [2H, m, H-C(15,15')], and ≈ 7.286 ppm [2H, m, H-C(14,14')]; ^{13}C-NMR: 11.94 [d, J_{CC} = 43.9 Hz, C(20,20')], 13.28 [s, C(methyl)], 59.78 [s, C(methylene)], 129.20

[d, J_{CC} = 71.9 Hz, C(13,13')], 132.56 [≈ 8.1 Hz, C(15,15')], 136.09 [d, J_{CC} = 71.3 Hz, C(14,14')], and 167.00 ppm [d, J_{CC} = 71.9 Hz, C(12,12')].

(All-E)-2,7-dimethylocta-2,4,6-triene-1,8-diol-$^{13}C_4$ (7): This compound was prepared similar to the published method for the synthesis of the unlabelled **7**.[11] Lithium aluminum hydride (LAH, 4.0 g) was heated at reflux in ether (280 mL) for 15 minutes. The decanted solution (160 mL) was transferred into a three-necked flask and cooled to -15°C. Compound **6** (1.20 g, 0.00468 mol) in ether (100 mL) was added as fast as possible (~5-10 min) so that the temperature did not rise above -10°C. The mixture was stirred for 15 minutes at -15°C and methanol (30 mL) was added slowly at -20°C, until the excess of LAH was destroyed. This was followed by the addition of half-saturated NH_4Cl-H_2O (200 mL). The organic layer was separated and the water layer was washed with ether (3 x 150 mL). The combined ethereal layer was dried and evaporated to dryness to give a white solid identified as **7** (0.66 g, 0.0038 mol, 82%): UV-visible: λ_{max} (ether) = 273 (inflection), 281 (main absorption), 293 nm; λ_{max} (methanol) = 271 (inflection), 280 (main absorption), 292 nm; HPLC-MS (ECNI, ion source temperature = 150°C) molecular anion at m/z = 172 for $^{13}C_4C_6H_{16}O_2$; ^{1}H-NMR: 1.807 [6H, ≈ t, $^2J_{CH} \approx {}^3J_{CH} \approx$ 5.5 Hz, Me(20,20')], 4.090 [4H, dd, $^1J_{CH}$ = 141.6 Hz, $^2J_{CH}$ = 4.5 Hz, CH_2(12,12')], ≈ 6.156 [2H, m, H-C(14,14')], and ≈ 6.450 ppm [2H, m, H-C(15,15')]; ^{13}C-NMR: 14.28 (dd, $^1J_{CC}$ = 42.9 Hz, $^2J_{CC}$ = 4.3 Hz, C-20, C-20'), 68.41 (d, $^1J_{CC}$ = 45.7 Hz, C-12, C-12'), 131.78 (d, $^1J_{CC}$ = 47.2 Hz, C-14, C-14'), and 137.40 ppm (d, $^1J_{CC}$ = 45.7 Hz, C-13, C-13'). NMR data for C-15, C-15' could not be assigned.

(All-E)-2,7-dimethylocta-2,4,6-triene-1,8-dial-$^{13}C_4$ (8): This compound was prepared similar to the published method for the synthesis of the unlabelled **8**.[11] Diol **7** (0.66 g, 0038 mol) in acetone (600 mL) was treated with activated Mn (IV) oxide (17.5 g) and the mixture was stirred

for 3 hr. The solution was filtered through celite and evaporated to dryness to give a yellowish crude product (0.60 g). This was dissolved in ~15 mL of dichloromethane and filtered through a 0.45-μm disposable filter assembly (Baxter, Scientific Products Division, MacGaw Park, IL). The solvent was evaporated and the pale yellow solid was crystallized from dichloromethane-ether to give **8** (0.61 g, 0.0036 mol, 95%): UV-visible: λ_{max} (hexane) = 325 nm; HPLC-MS (ECNI, ion source temperature = 150°C) molecular anion at m/z = 168 for $^{13}C_4C_6H_{12}O_2$; ^{1}H-NMR: 1.952 [6H, d.d, $^2J_{CH}$, $^3J_{CH}$ ≈ 6.5 3.8 Hz, Me(20,20')], ≈ 7.00 [2H, m, H-C(14,14')], ≈ 7.09 [2H, m, H-C(15,15')], and 9.543 ppm [2H, dd, $^1J_{CH}$ = 174 Hz, $^2J_{CH}$ =24.4 Hz, H-C(12,12')]; ^{13}C-NMR: 9.90 (d, $^1J_{CC}$ = 44.5 Hz, C-20, C-20'), 134.43 (≈ t, ≈ 6.8 Hz, C-15, C-15'), 141.05 (d, $^1J_{CC}$ = 51.7 Hz, C-13, C-13'), 146.07 (dd, $^1J_{CC}$ = 69.9 Hz, $^2J_{CC}$ = 7.2 Hz, C-14, C-14'), and 194.44 ppm (d, $^1J_{CC}$ = 51.0 Hz, C-12, C-12').

(All-E, 3R,3'R)-β,β-carotene-3,3'-diol-$^{13}C_4$ (10): This compound was prepared similar to the published method for the synthesis of the unlabelled **10**.[20] A solution of {(2E,4E)-5-[(R)-4-hydroxy-2,6,6-trimethyl-cyclohex-1-enyl]-3-methylpenta-2,4-dienyl}triphenylphosphonium chloride (Wittig salt **9**)[20-21] (1.034 g, 0.002 mol), dial **8** (0.168 g, 0.001 mol), and 1,2-epoxybutane (0.60 g, 0.008 mol) in ethanol (10 mL) was stirred at reflux for 20 hr. The suspension was cooled to -10°C and the product was filtered, washed with ethanol (10 mL, -15°C), and crystallized from dichloromethane (containing 1% of Et_3N)-hexane and dried *in vacuo* at 60°C, 0.1 mm-Hg for 3 days. This was shown by HPLC-MS to consist of more than 99% of pure all-E isomer of **10** (0.52 g, 0.0009 mol, 90%): UV-visible: λ_{max} (CH_2Cl_2) = 461 (main absorption), 488 nm; λ_{max} (hexane) = 451 (main absorption), 479 nm; HPLC-MS (ECNI, ion source

temperature = 250°C) molecular anion at m/z = 572 for $^{13}C_4C_{36}H_{56}O_2$; ^{1}H-NMR: 1.074 [12H, Me(16,16',17,17'), 1.35 [2H, d, J_{OH} = 4.8 Hz, OH-C(3) and OH-C(3')], 1.48 [2H, dd, J_{gem} = 12 Hz, $J_{2ax,3ax}$ ≈ 11.8 Hz, H_{ax}-C(2) and H_{ax}-C(2')], 1.738 [6H, Me(18,18')], 1.77 [2H, ddd, J_{gem} = 12 Hz, $J_{2eq,3ax}$ ≈ 2.5 Hz, $J_{2eq,4eq}$ ≈ 1.8 Hz, H_{eq}-C(2) and H_{eq}-C(2')], 1.968 [12H, Me(19,19',20,20')], 2.05 [2H, dd, J_{gem} =17 Hz, $J_{4ax,3ax}$ = 9.8 Hz, H_{ax}-C(4) and H_{ax}-C(4')], 2.39 [2H, ddd, J_{gem} =17 Hz, $J_{4eq,3ax}$ = 6 Hz, $J_{4eq,2eq}$ = 1.6 Hz, H_{eq}-C(4) and H_{eq}-C(4')], 4.00 [2H, H_{ax}-C(3) and H_{ax}-C(3')], 6.10 [2H, H-C(7) and H-C(7')], 6.13 [2H, H-C(8) and H-C(8')], 6.16 [2H, d, $J_{10,11}$ = 11 Hz, H-C(10) and H-C(10')], 6.25 [2H, H-C(14) and H-C(14')], ≈ 6.36* [2H, ddd, $^1J^{13}_{C,H}$ ≈ 150 Hz, $^2J^{13}_{C\text{-}13,}{}^{13}_{C\text{-}12}$ ≈ 5-10 Hz, $J_{H\text{-}11,H\text{-}12}$ ≈ 15 Hz, H-C(12) and H-C(12')], 6.63 [2H, m, H-C(15) and H-C(15')], 6.64 [2H, ddd, $J_{H\text{-}11,H\text{-}12}$ = 15 Hz, $J_{10,11}$ = 11.2 Hz, $J_{H\text{-}11,\ ^{13}C\text{-}12}$ = 5.6 Hz, H-C(11) and H-C(11')]. *Signals due to H-C(12) and H-C(12') could not be assigned with certainty due to complications resulting from partial deviation from first-order interpretation and strong overlap of the subspectra belonging to the spin up and down states of carbon. ^{13}C-NMR: 12.77 (s, C-19, C-19'), 12.85 (dd, $^1J_{CC}$ = 40 Hz, $^2J_{CC}$ = 4 Hz, C-20, C-20'), 21.63 (s, C-18, C-18'), 28.74 (s, C_{ax}-16, C_{ax}-16'), 30.27 (s, C_{eq}-17, C_{eq}-17'), 37.14 (s, C-1, C-1'), 42.60 (s, C-4, C-4'), 48.48 (s, C-2, C-2'), 65.11 (s, C-3, C-3'), 124.93 (d, $^1J_{CC}$ = 66 Hz, C-11, C-11'), 125.60 (s, C-7, C-7'), 126.18 (s, C-5, C-5'), 130.10 (t, ≈ 8 Hz, C-15, C-15'), 131.32 (d, $^2J_{CC}$ = 7.3 Hz, C-10, C-10'), 132.60 (dd, $^1J_{CC}$ = 66.7 Hz, $^2J_{CC}$ ≈ 5 Hz, C-14, C-14'), ≈ 135.82 (s , C-9, C-9'), 136.42 (d, $^1J_{CC}$ = 57.4 Hz, C-13, C-13'), 137.65 (d, $^1J_{CC}$ = 57.4 Hz, C-12, C-12'), 137.78 (s, C-6, C-6'), and 138.51 ppm (s, C-8, C-8'). The assignments of chemical shifts, axial/equatorial/geminal protons, and coupling constants were in agreement with the ^{1}H and ^{13}C NMR data for the unlabelled (all-E,3R,3'R)-zeaxanthin reported previously.[23]

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