Supporting Information

Total Synthesis of (*3R,3'R,6'R*)-Lutein and its Stereoisomers

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General methods.

^{1}H NMR spectra were recorded on a 400 MHz spectrometer with $CDCl_3$ (7.27 ppm) as internal standard. ^{1}H noise-decoupled ^{13}C spectra were recorded on a 400 MHz at 100 MHz with chloroform (77.0 ppm) as an internal standard. High resolution (HRMS) mass spectra were obtained on a AccuTOF CS mass spectrometer (ion source: ESI; needle voltage: 2300 v; flow rate: 100 μl/min; desolvation chamber temperature = 250°C; data acquisition time: 2 min). Circular dichroism (CD) spectra were obtained employing a mixture of hexane, ether, and methanol (10:3:1) as the background solvent.

Reactions were monitored by HPLC employing normal phase and chiral columns under various conditions as described below. The analyses were performed on an HPLC system

equipped with a quaternary solvent delivery system, an autosampler, a thermostat-controlled column compartment, and a photodiode array detector.

Normal phase analytical HPLC separations were carried on a silica-based nitrile bonded column (25 cm length x 4.6 mm i.d.; 5-μm spherical particle). The column was protected with a nitrile bonded guard cartridge (3-cm length x 4.6 mm i.d.; 5-μm particle size). The column flow rate was 0.7 mL/min.

All semipreparative normal phase HPLC separations were carried out on a silica-based nitrile bonded column (25 cm length x 10 mm i.d.; 10-μm spherical particle). The column was protected with a nitrile bonded guard cartridge (3-cm length x 4.6 mm i.d.; 5-μm particle size). Unless otherwise stated, the column flow rate was 3.0 mL/min.

All chiral HPLC separations were carried out on an amylose tris-(3,5-dimethylphenyl-carbamate) analytical column (25 cm length x 4.6 mm i.d., 5-μm), protected with a silica gel guard cartridge (3 cm length x 4.6 mm i.d.; 5-μm particle); flow rate = 0.7 mL/min.

Various mobile phases (eluents) were used with normal phase and chiral HPLC columns. The HPLC eluents for various separations are provided in the Figure Legend of each chromatogram.

All operations and HPLC analyses were conducted under yellow laboratory light to prevent photo-isomerization and degradation of carotenoids and their precursors.

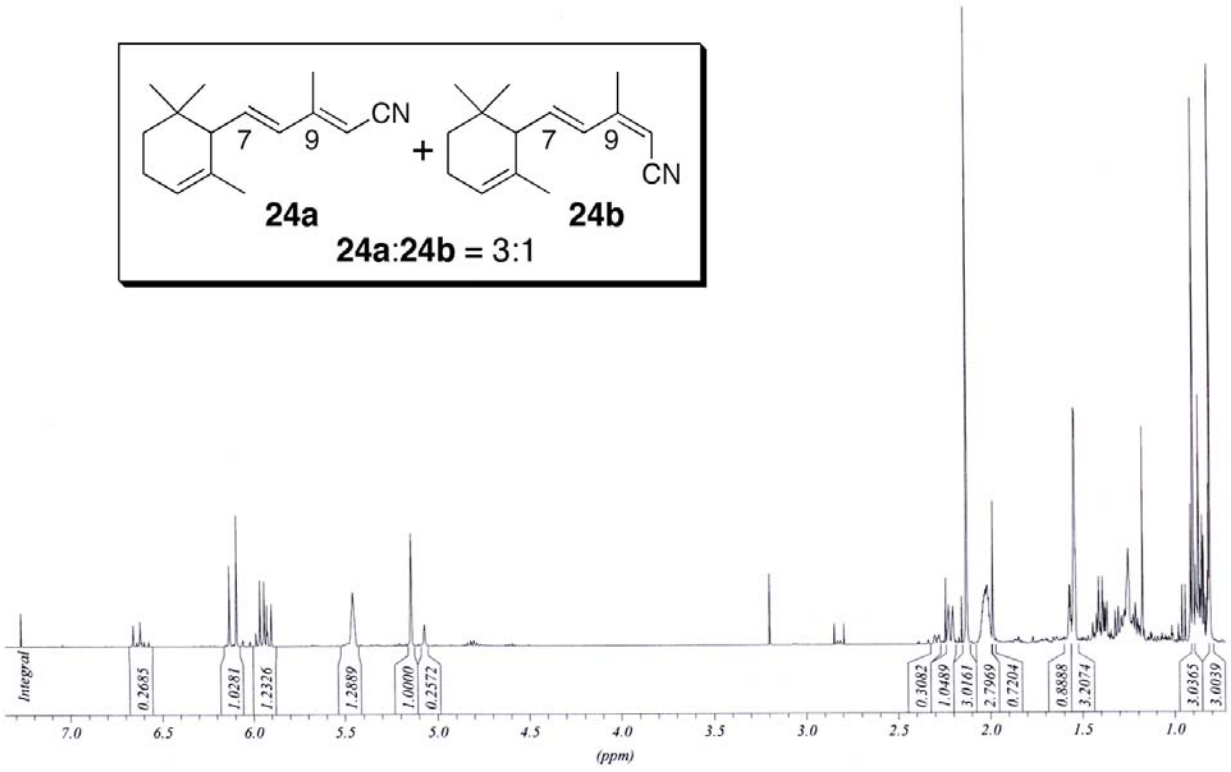
One-Pot Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (23a) to Hydroxyaldehydes 15 – 18. A solution of **23a** (2.40 g, 10.47 mmol) in TBME (20 mL) at -30 °C under N_2 was treated dropwise with a 1M solution of K-Selectride™ in THF (15.2 mL, 15.2 mmol) in TBME (10 mL) in 30 min. After 2h, **23a** was shown by HPLC to have converted to a mixture of (3,6-*trans*)-hydroxynitriles **19**+**20** (86%) and (3,6-*cis*)-hydroxynitriles **21**+**22** (14%). A 1M solution of DIBAL-H in CH_2Cl_2 (26 mL, 26 mmol) was added in 30 min and the reaction

mixture was allowed to stir at -20 °C for 3h. The product was treated with a very slow addition of a homogeneous mixture of 40 g of water absorbed on *n*-silica (1.0 g of water/g of silica) at a rate that the temperature remained below -10 °C; caution: the addition of silica/water results in rapid elevation of the temperature. After stirring at 0 °C for 2h. Na_2SO_4 (6 g) was added and the solids were filtered off and washed with CH_2Cl_2. The organic solution was washed with water, dried, and concentrated to give a pale yellow oil (3.0 g). Column chromatography (hexane:ethyl acetate, 95:5 to 80:20) of the product gave two fractions that were shown by ^{1}H NMR to be identical with previously characterized samples of (3,6-*trans*)-hydroxyaldehydes **15**+**16** (1.75 g, 7.47 mmol, 71%) and (3,6-*cis*)-hydroxyaldehydes **17**+**18** (0.29 g, 1.24 mmol, 12%).

Reduction of (*7E,9E*)-3-Keto-α-Ionylideneacetonitrile (23a) to Hydroxynitriles 19 – 22 with (*R*)-2-Methyl-CBS-Oxazaborolidine. To a solution of (*R*)-2-methyl-CBS-oxazaborolidine (6 mL 1M in toluene, 6 mmol) in TBME (10 mL) was added BH_3.THF (6 mL 1M in THF, 6 mmol) at rt under N_2. The mixture was stirred for 20 min, cooled down to 0 °C, and was treated with a solution of **23a** (1.38 g, 6.02 mmol) in TBME (10 mL). After 1.5 h at 0 °C, the reaction was quenched by slow addition of methanol (10 mL) and the product was sequentially washed with a saturated solution of NH_4Cl, 5% $NaHCO_3$, brine, and water. The organic solution was dried and concentrated to give a colorless oil that was passed through a short silica gel column (hexane:acetone = 97:3) and identified by ^{1}H NMR as 3-hydroxy-α-ionylideneacetonitriles **19** – **22** (1.35 g, 5.84 mmol, 97%). The isomeric ratio of hydroxynitriles (**19**+**20**):(**21**+**22**) = 1:6 was established by normal phase HPLC. Hydroxynitriles **19**+**20** and **21**+**22** were each shown by chiral HPLC to consist of an approximately 1:1 mixture of enantiomers.

Hydroxynitriles **19** – **22** (1.35 g) were subsequently reduced with DIBAL-H similar to the procedure described previously to yield hydroxyaldehydes **15** – **18** (1.09 g, 4.65 mmol, 80%).

^{1}H NMR of α-ionylideneacetonitrile [24a (*7E,9E*):24b (*7E,9Z*) = 3:1]



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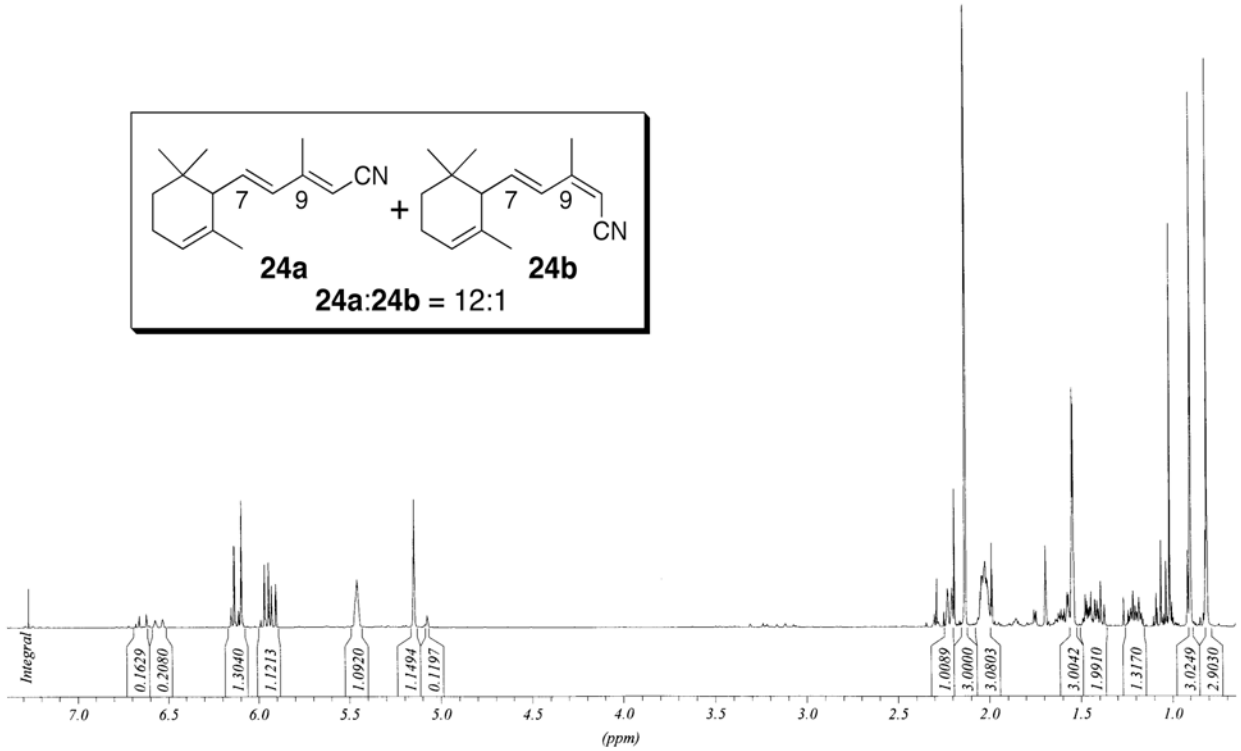

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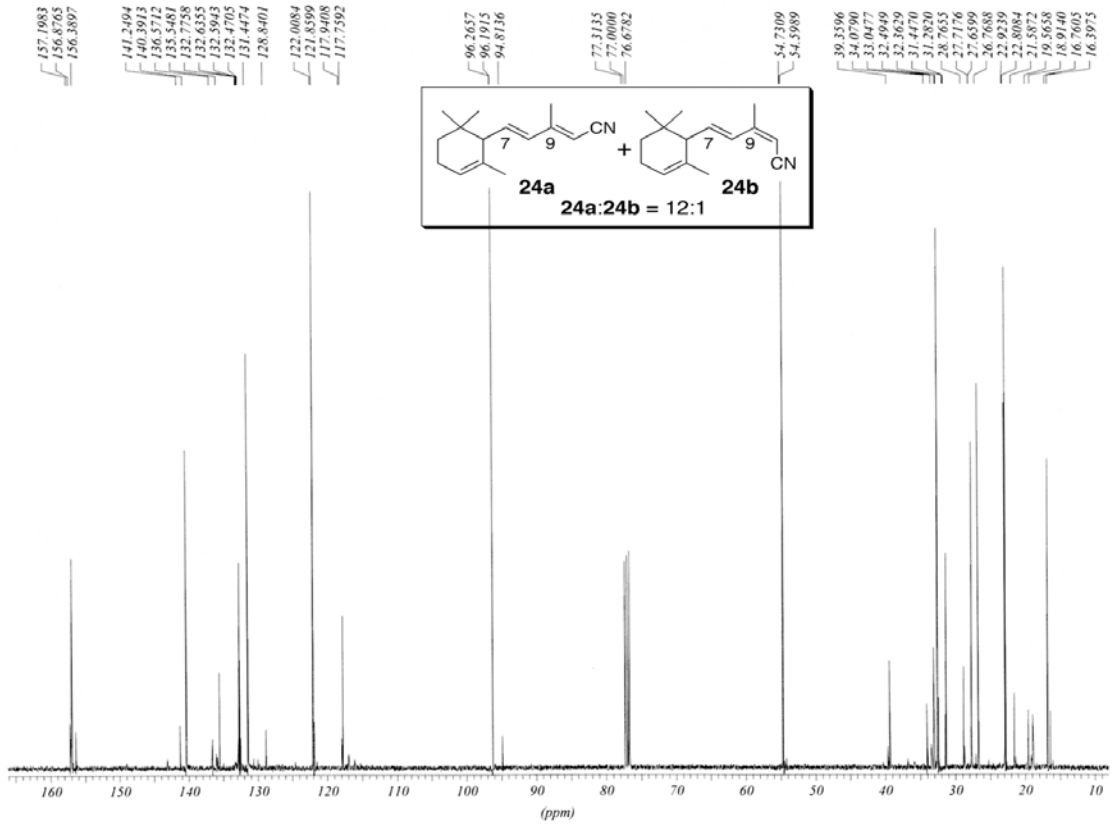
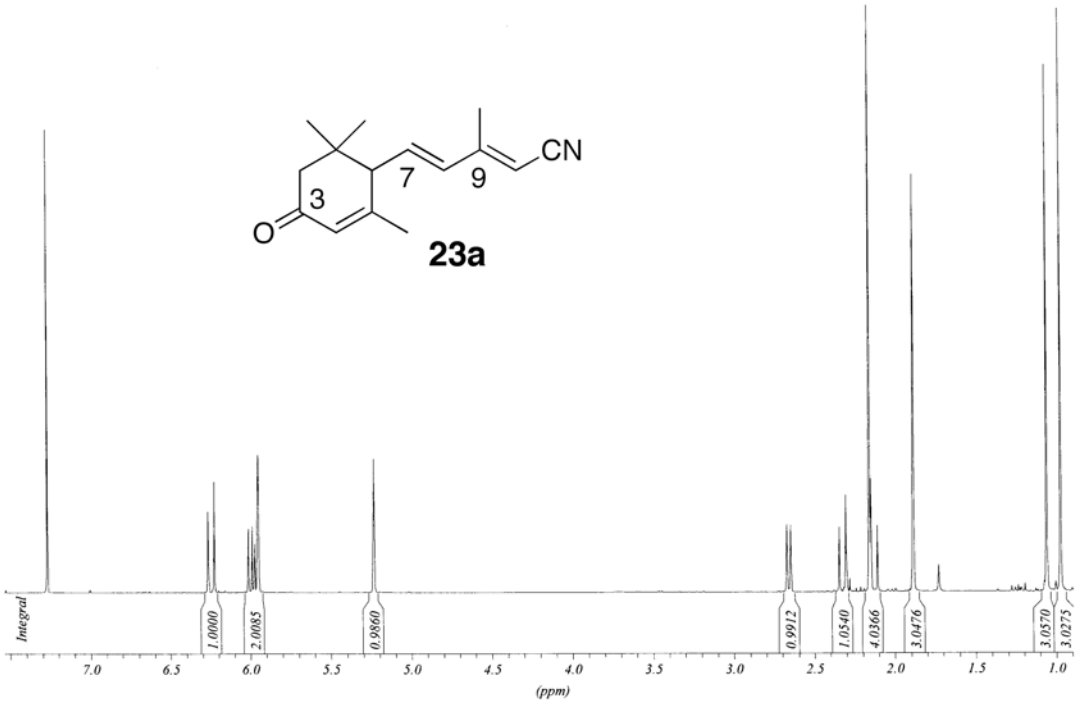


^{1}H NMR of (*7E,9E*)-3-keto-α-ionylideneacetonitrile (23a)



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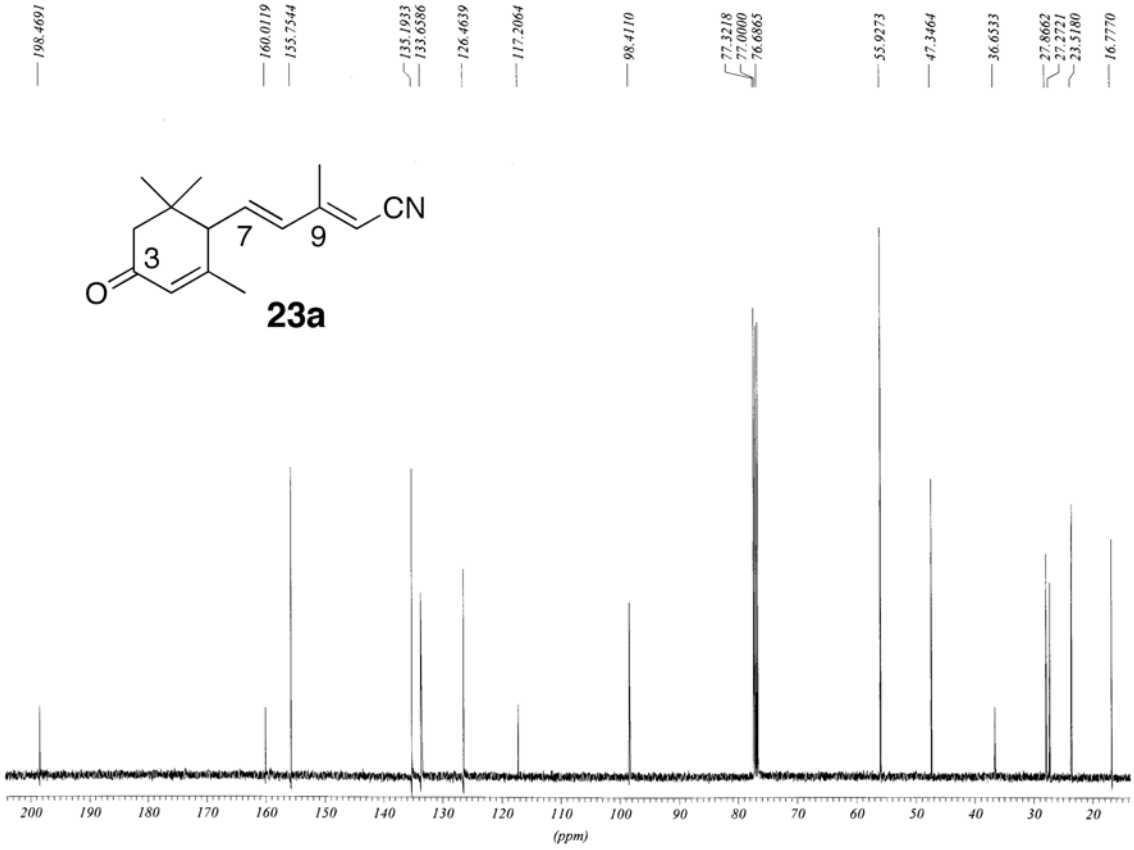

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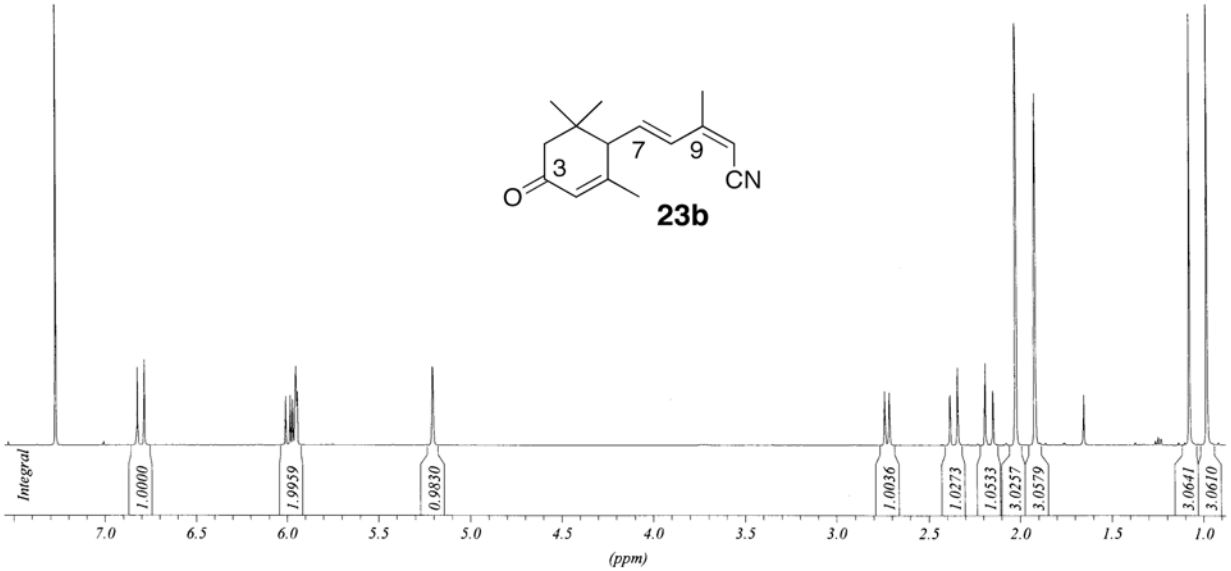

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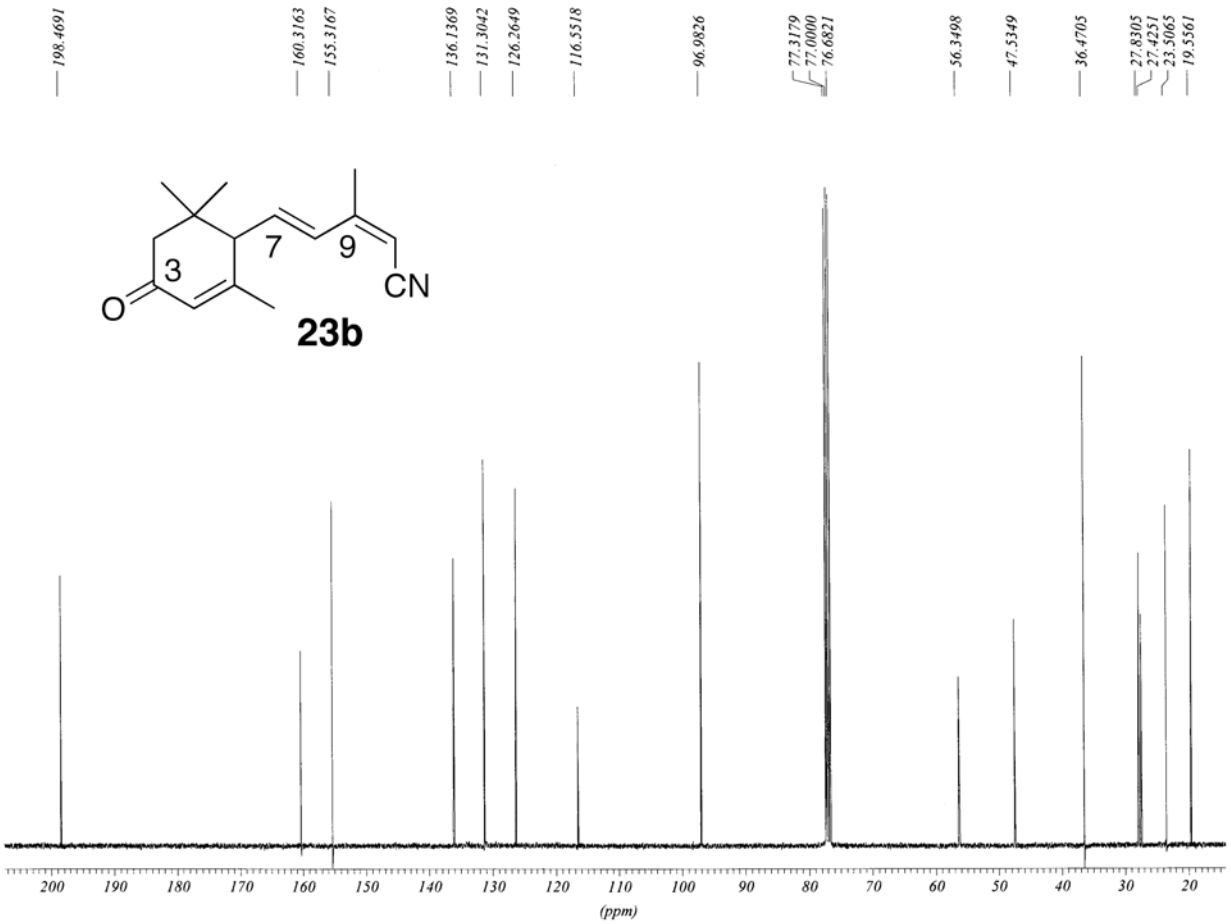
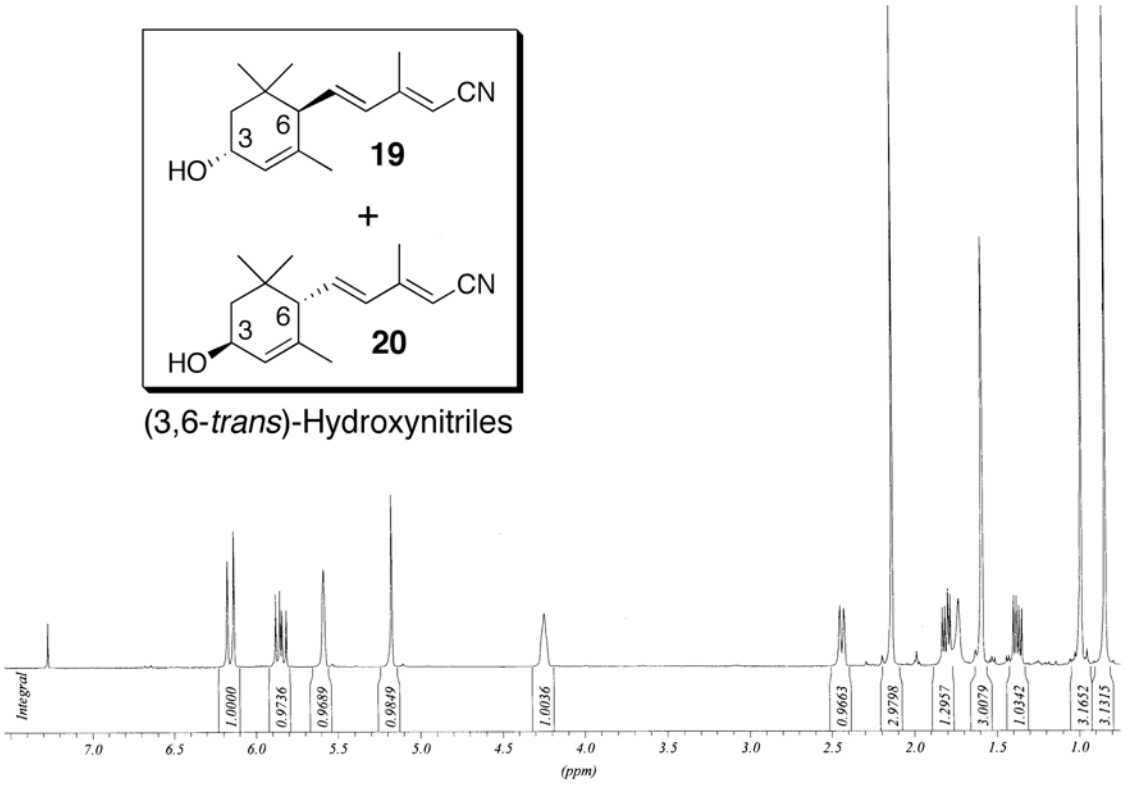


[1]H NMR of (*3,6-trans, 7E,9E*)-3-hydroxy-α-ionylideneacetonitrile (19+20)



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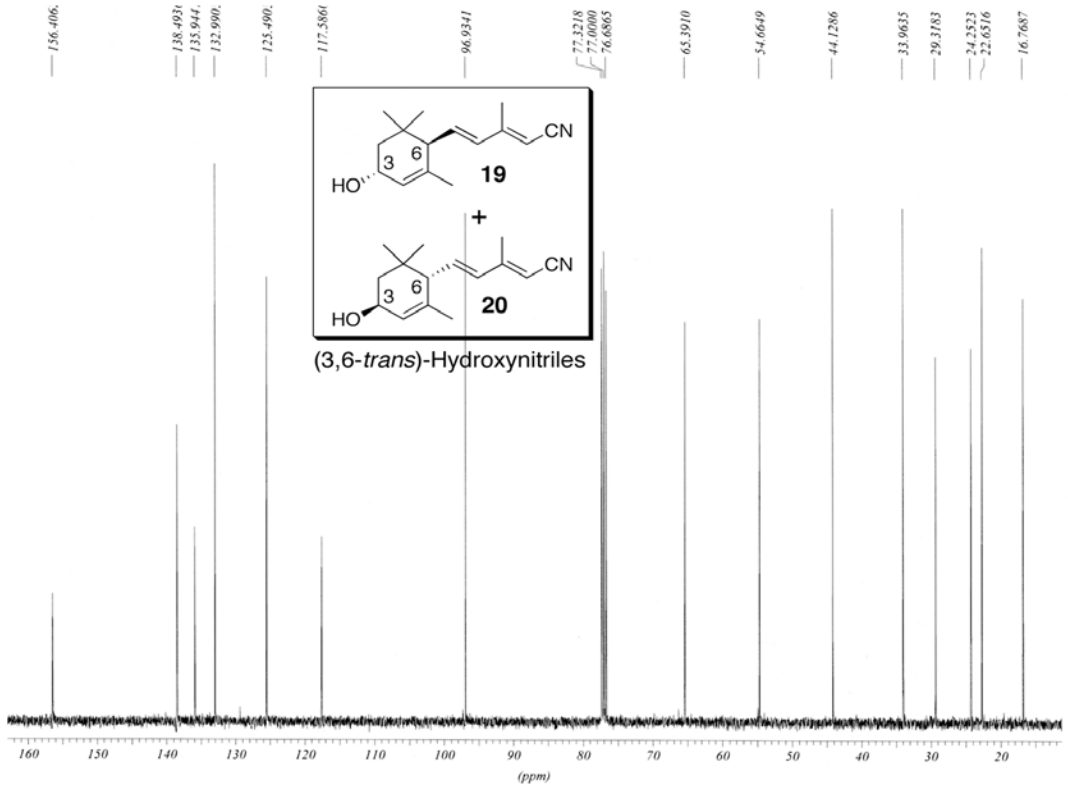

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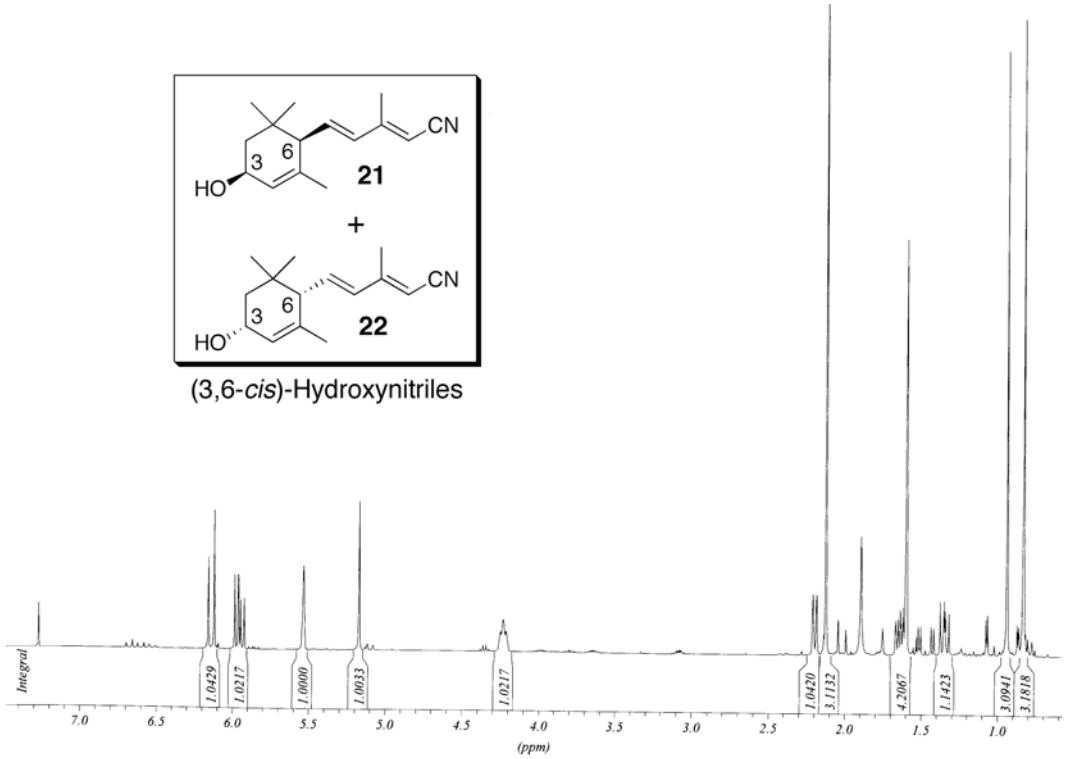

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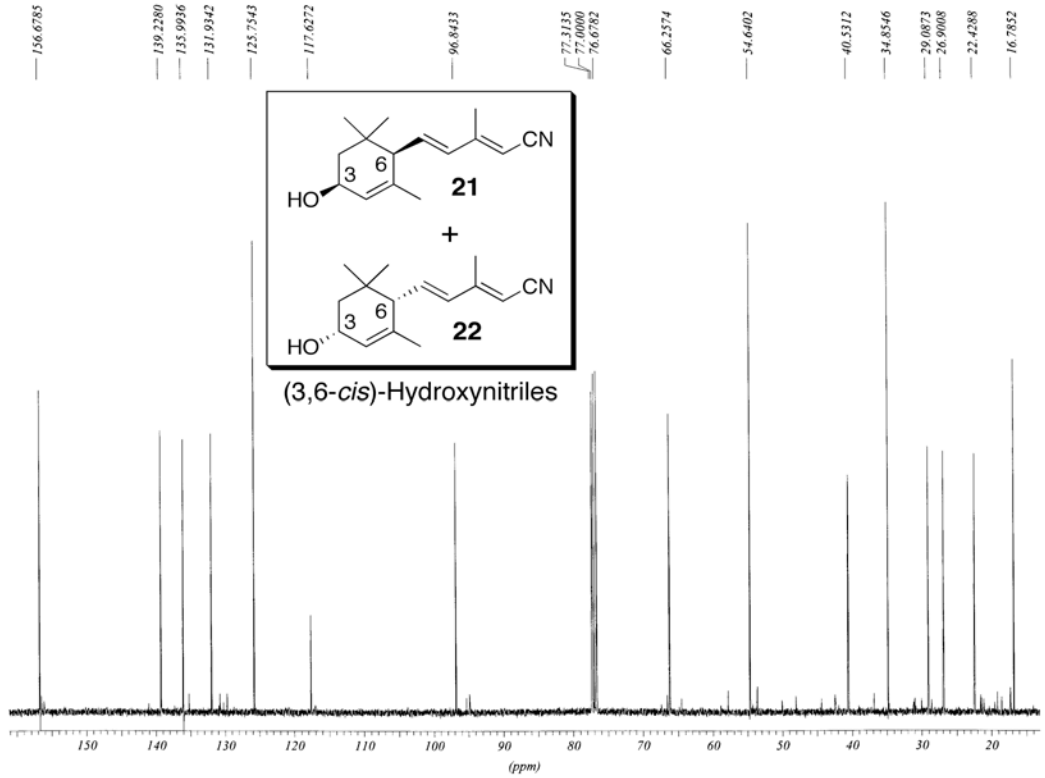

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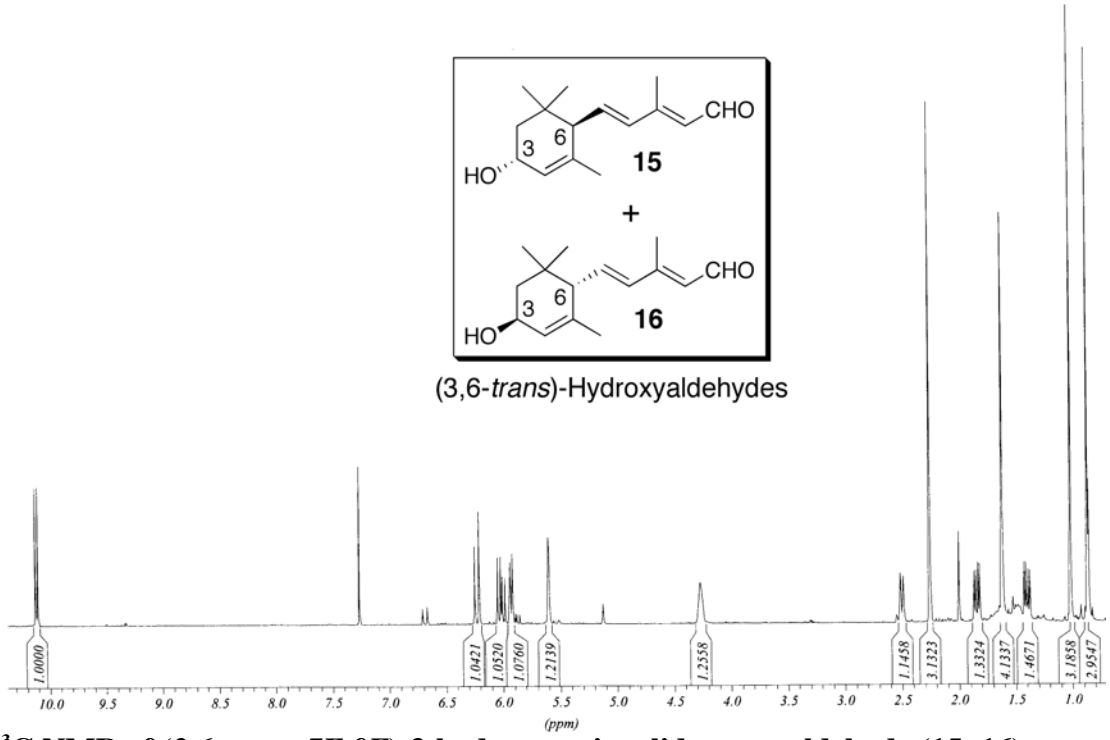

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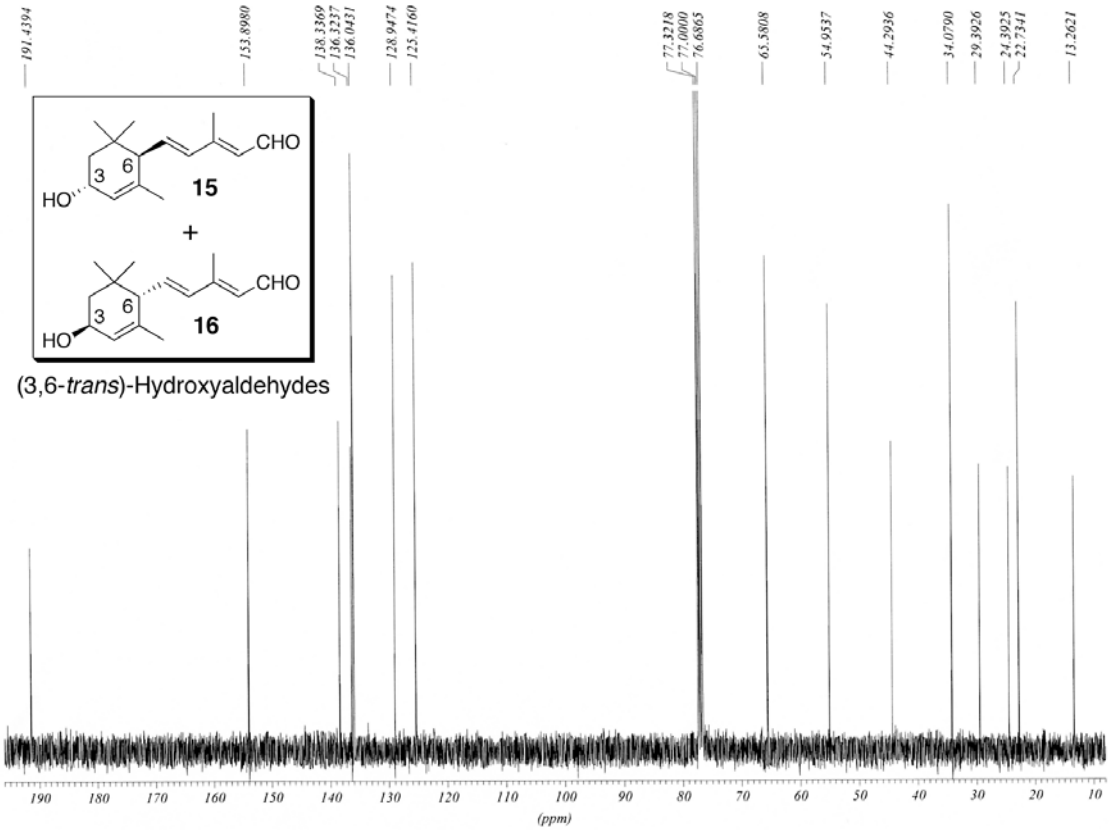

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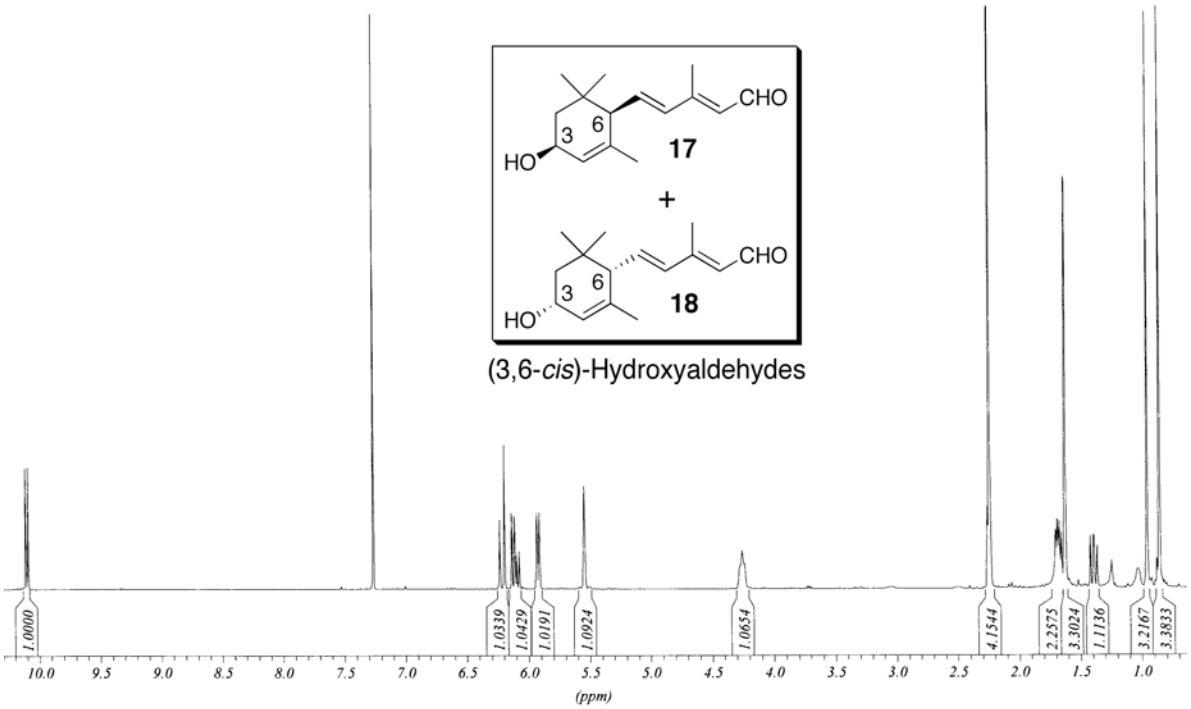

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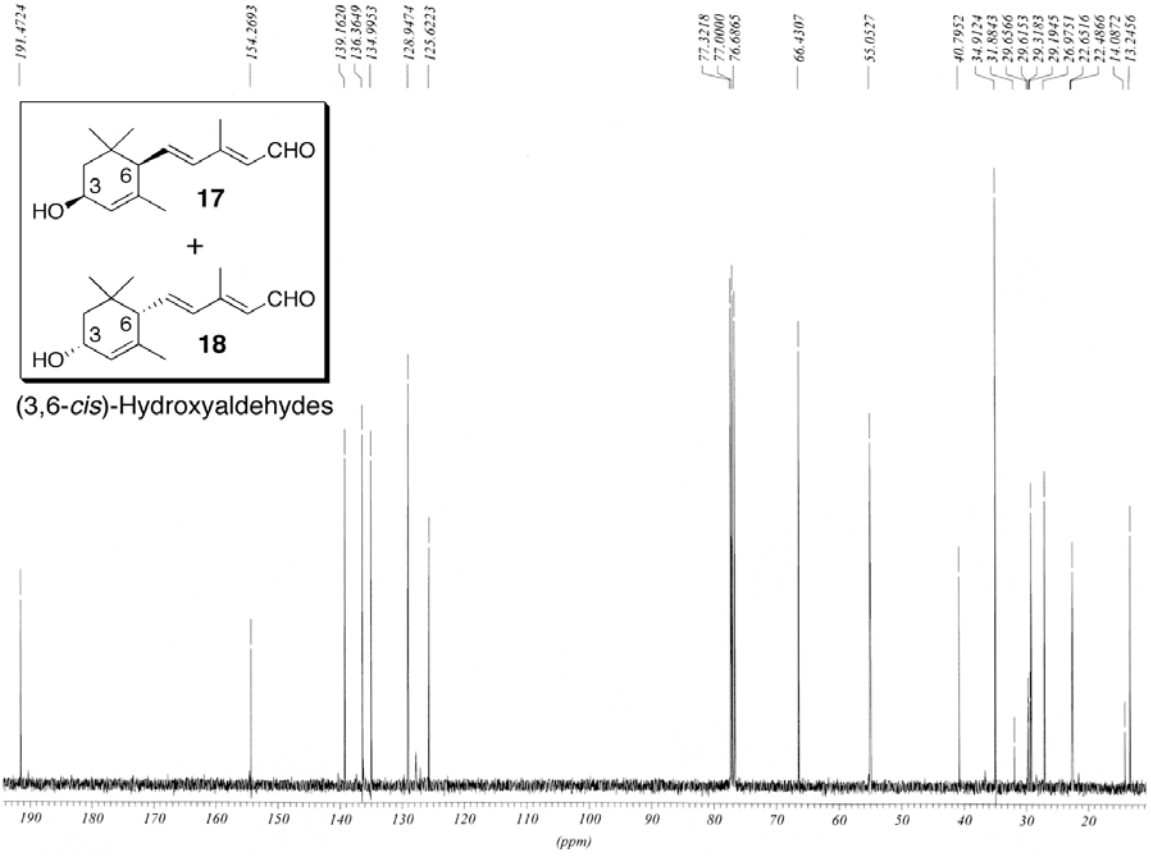

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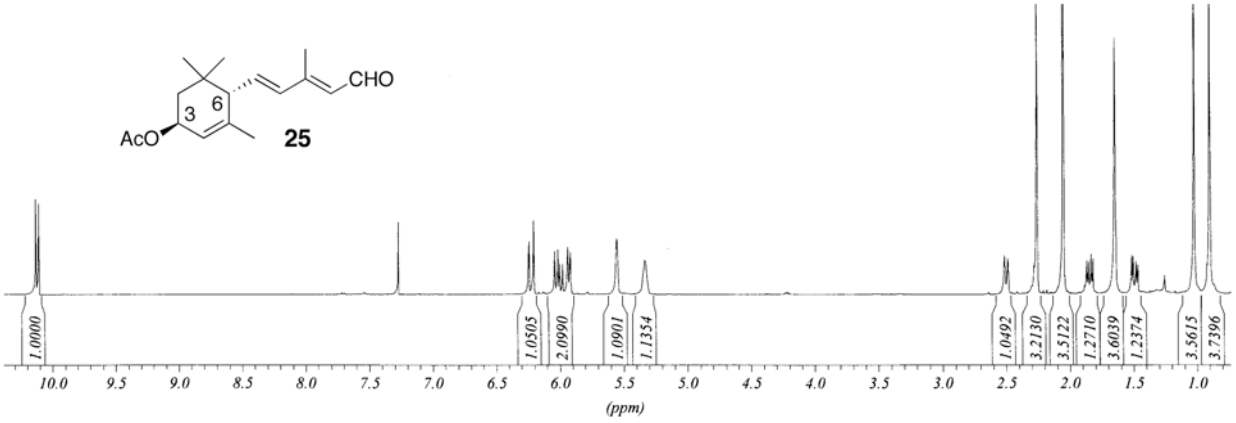

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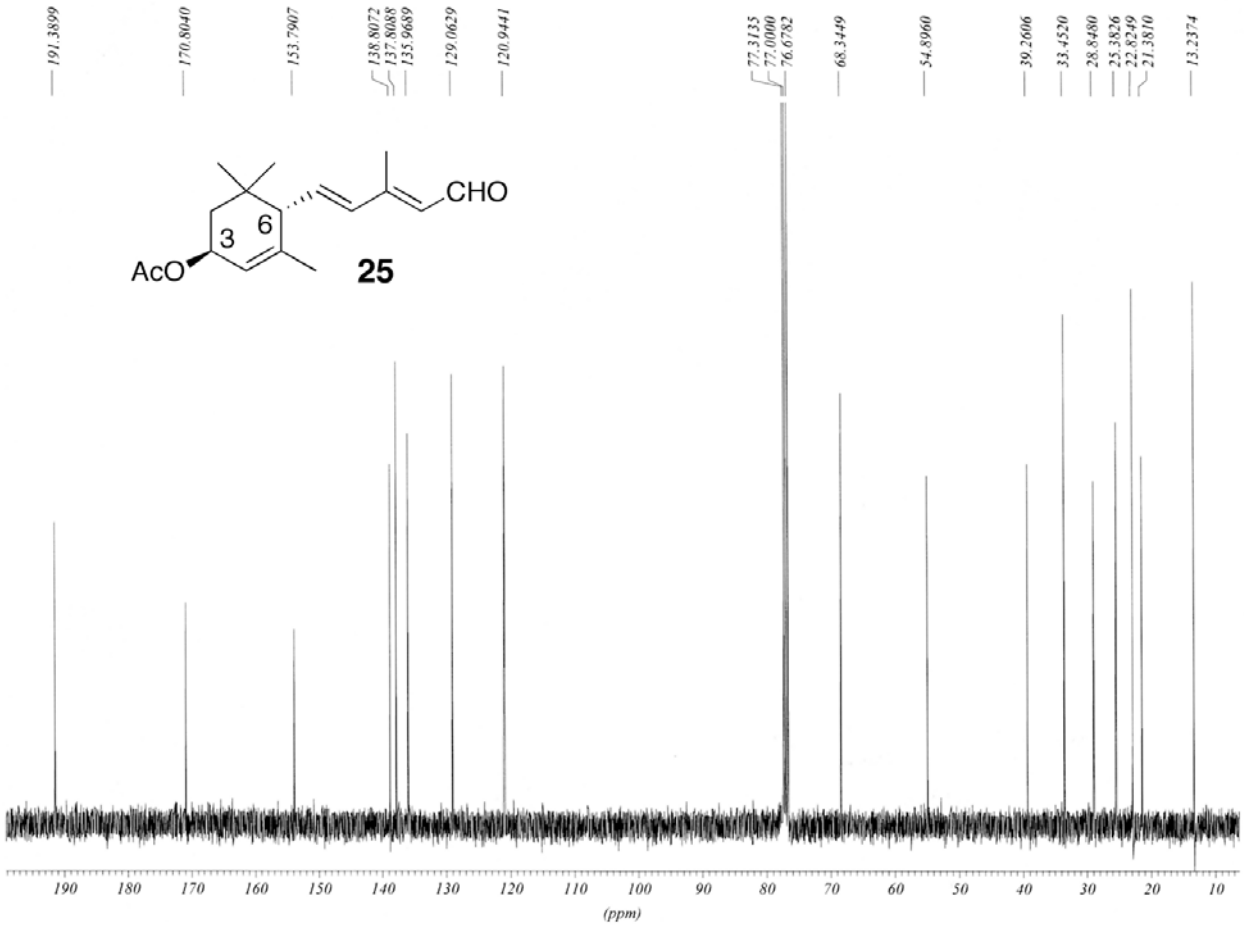

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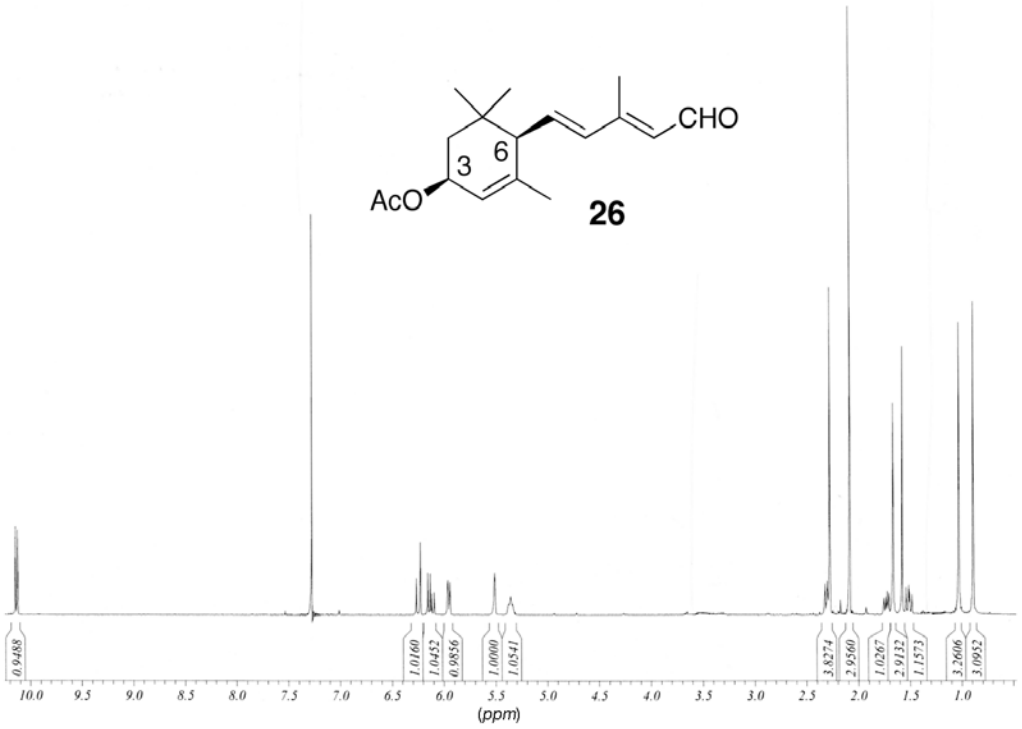

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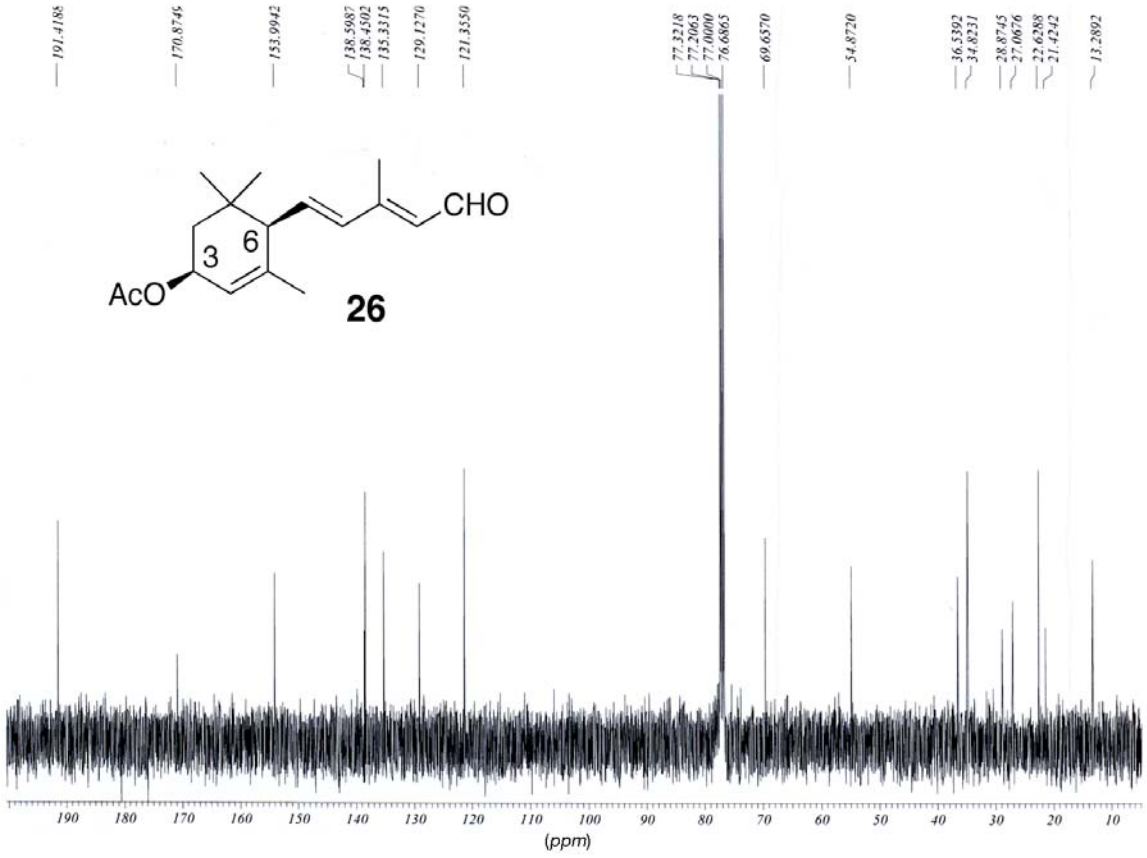
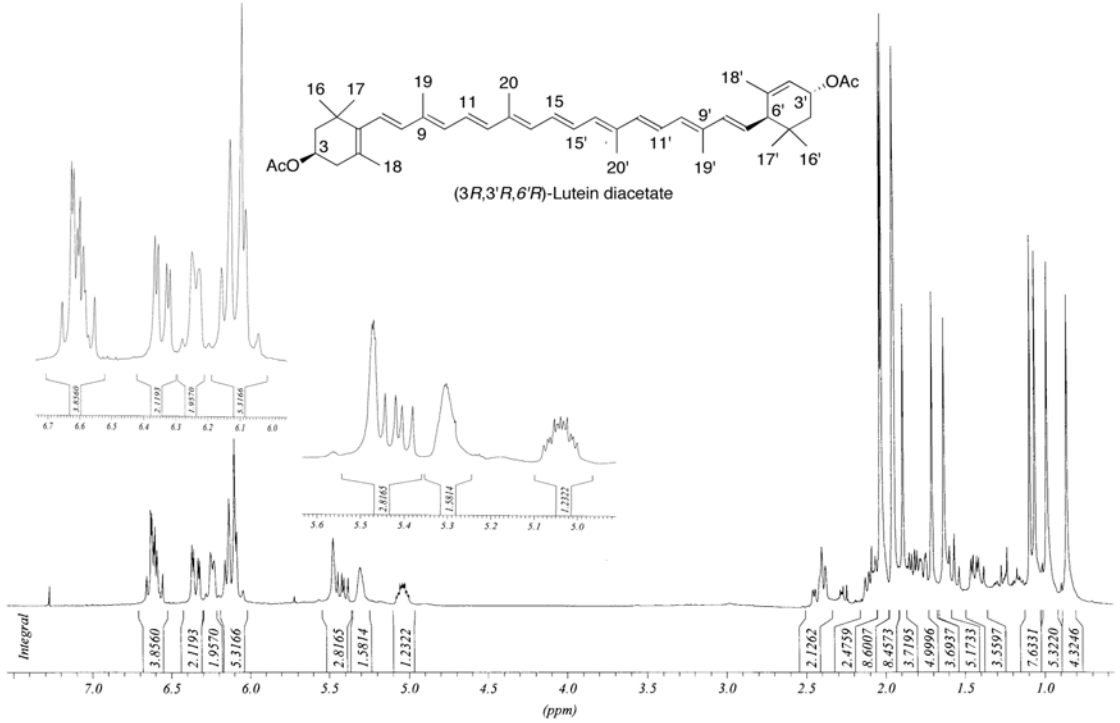


^{1}H NMR of (*3R,3'R,6'R*)-lutein diacetate



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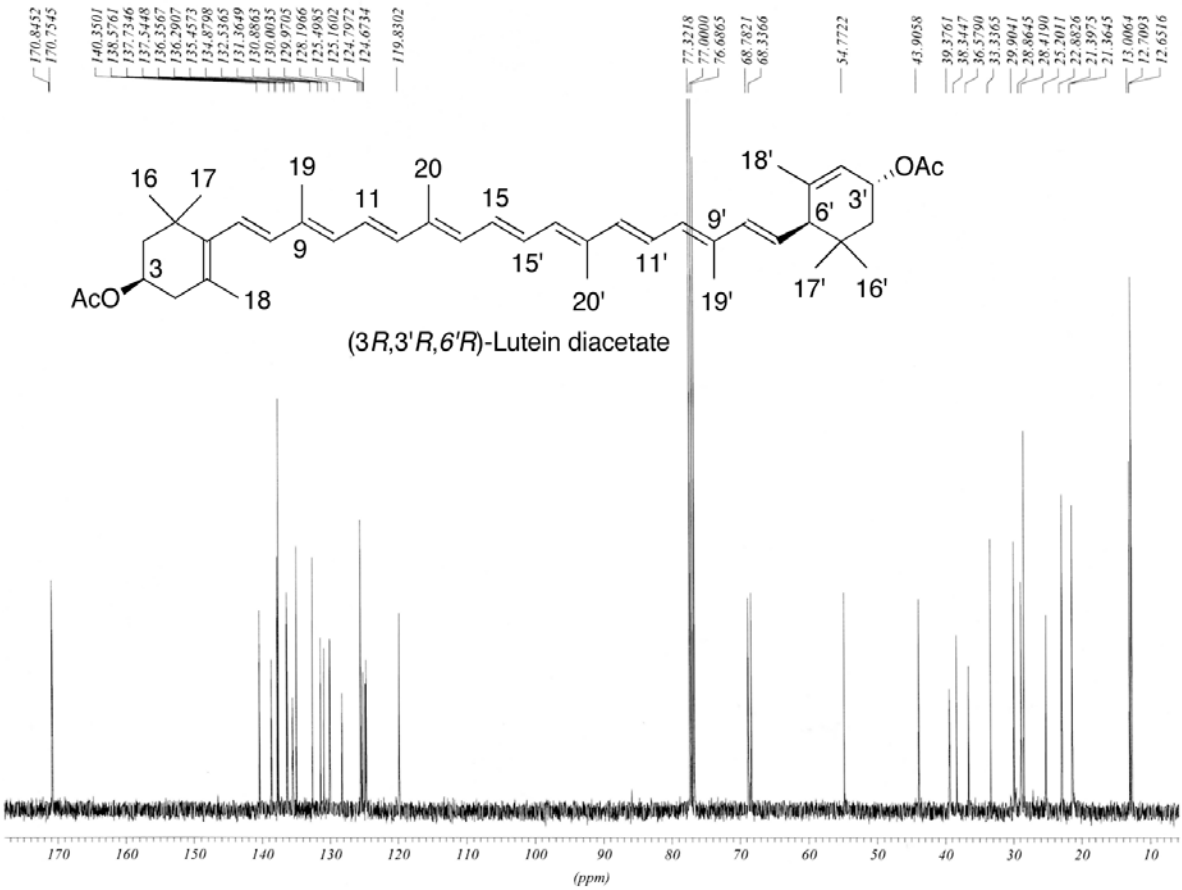

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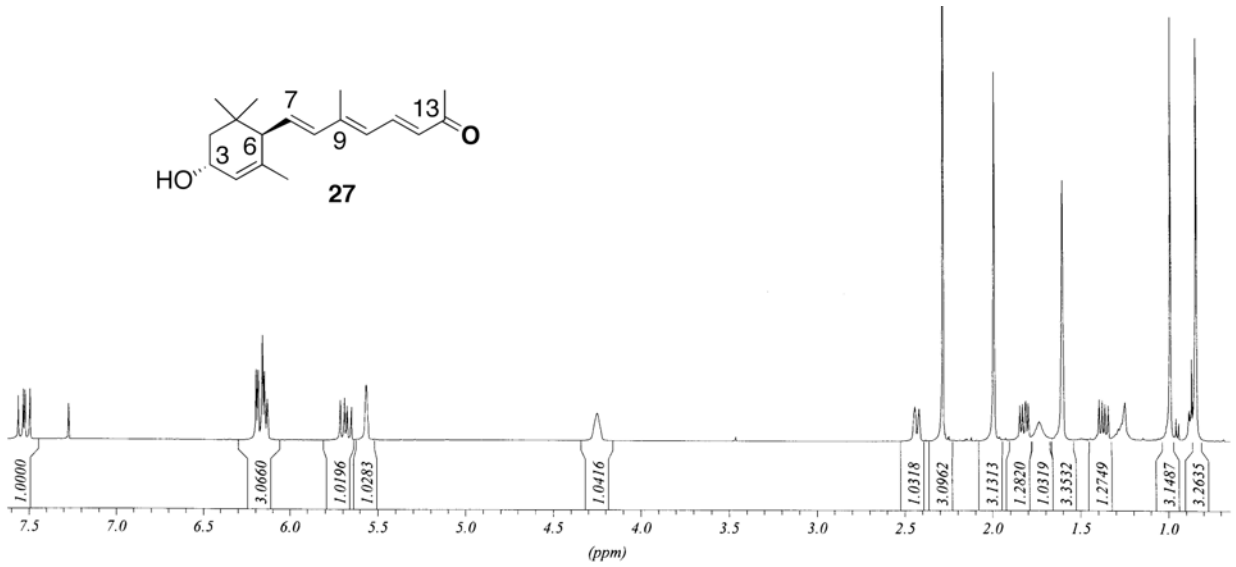

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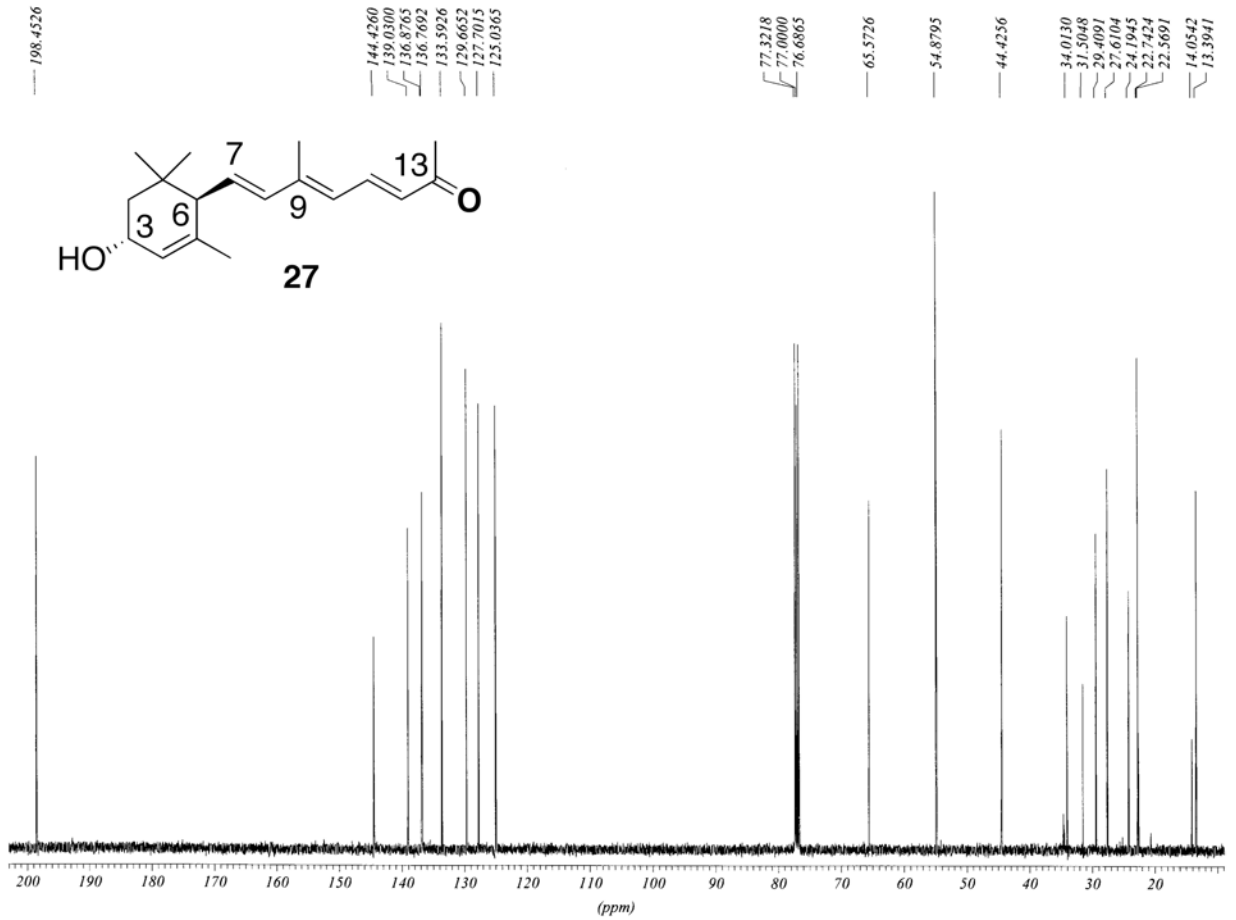

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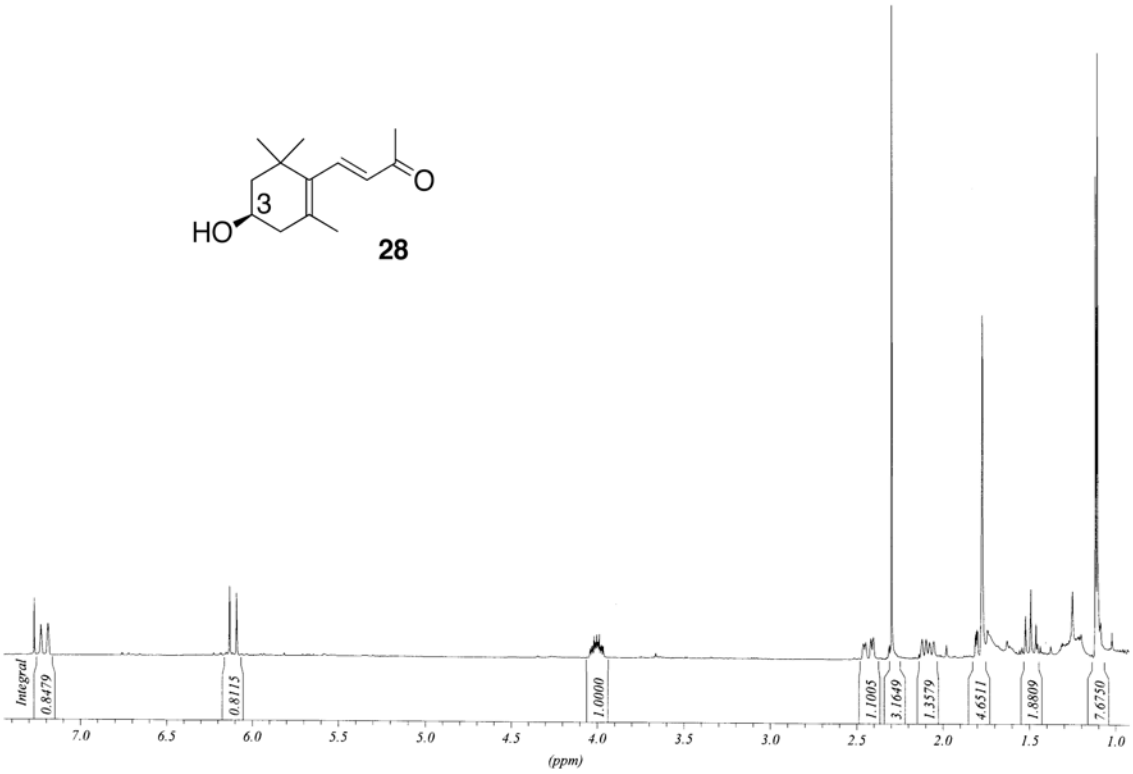

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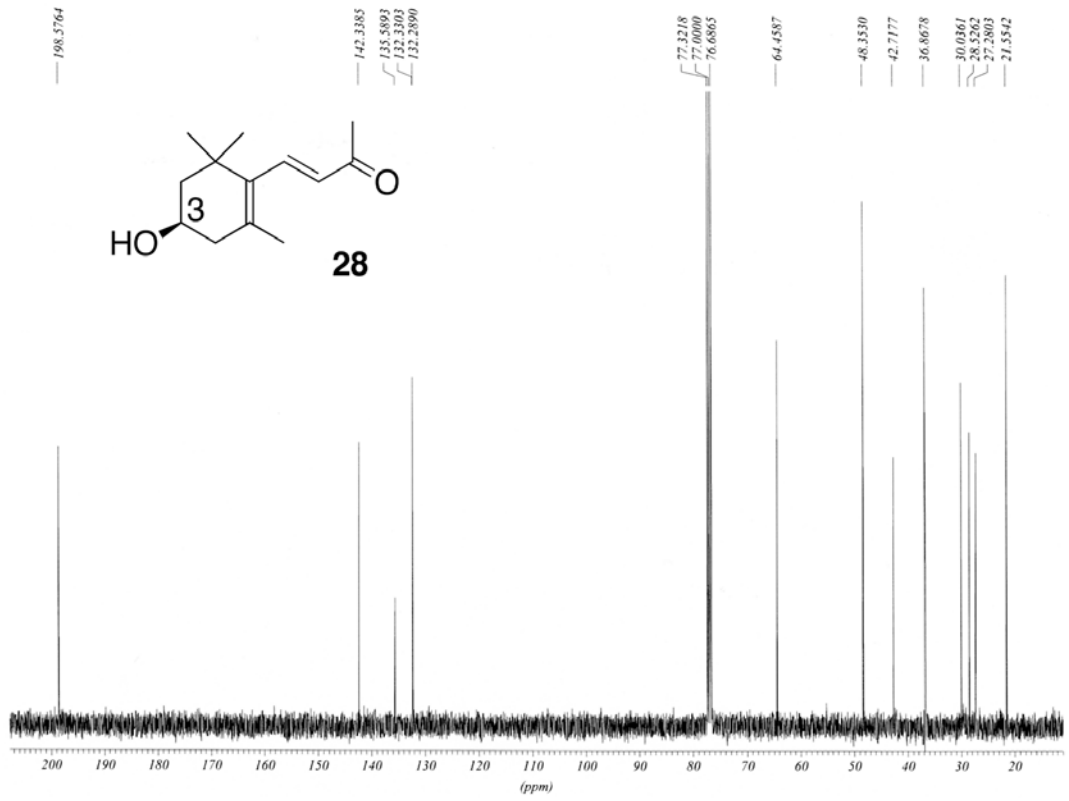

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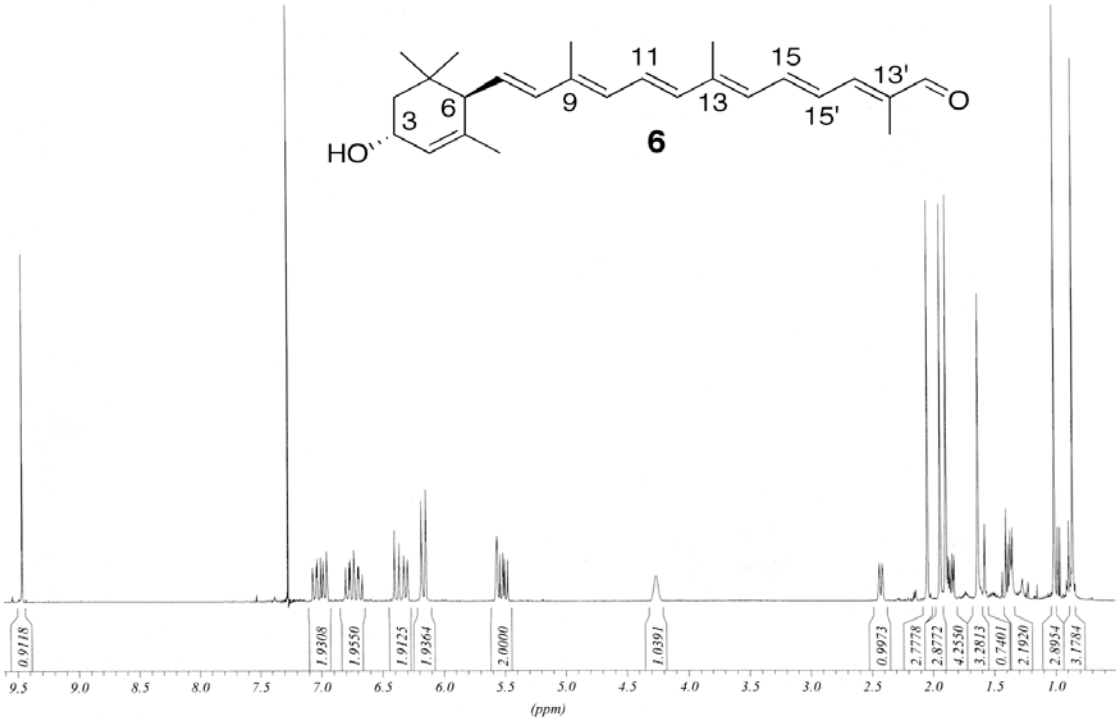

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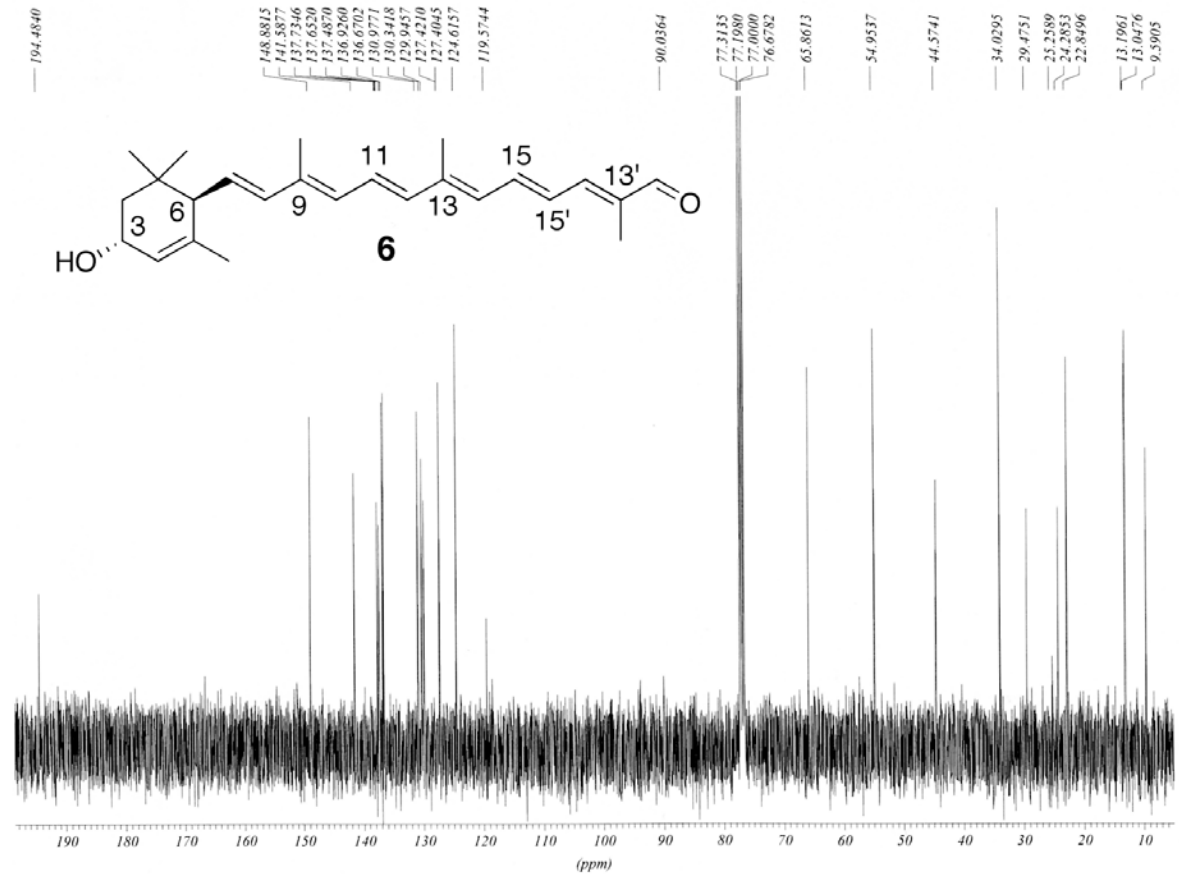

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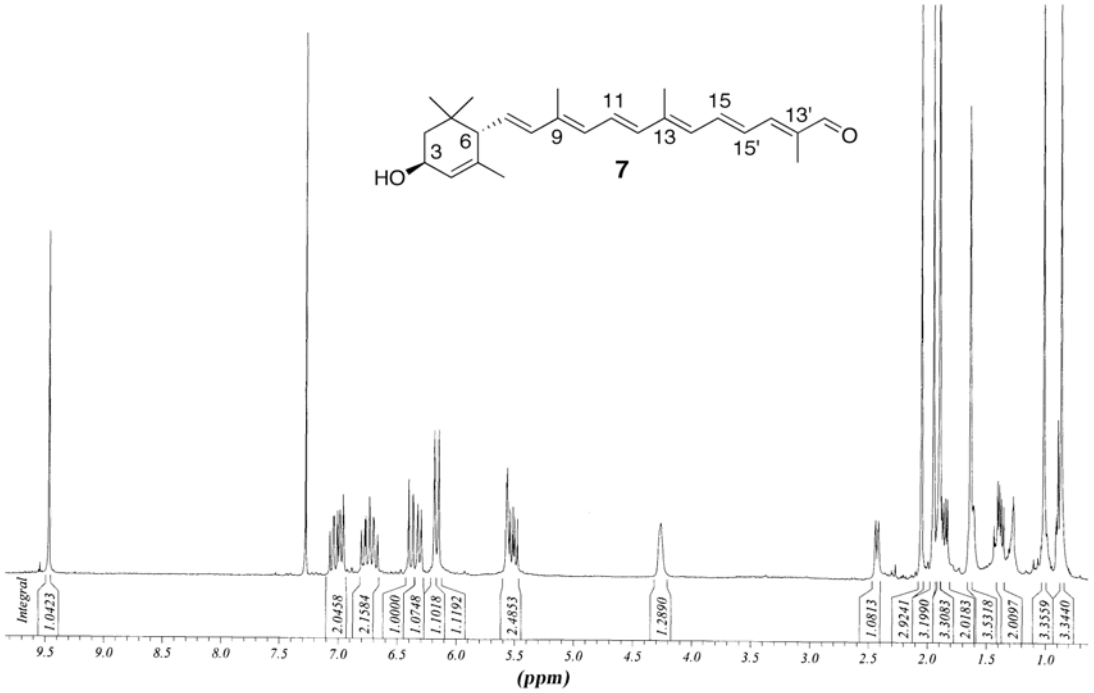

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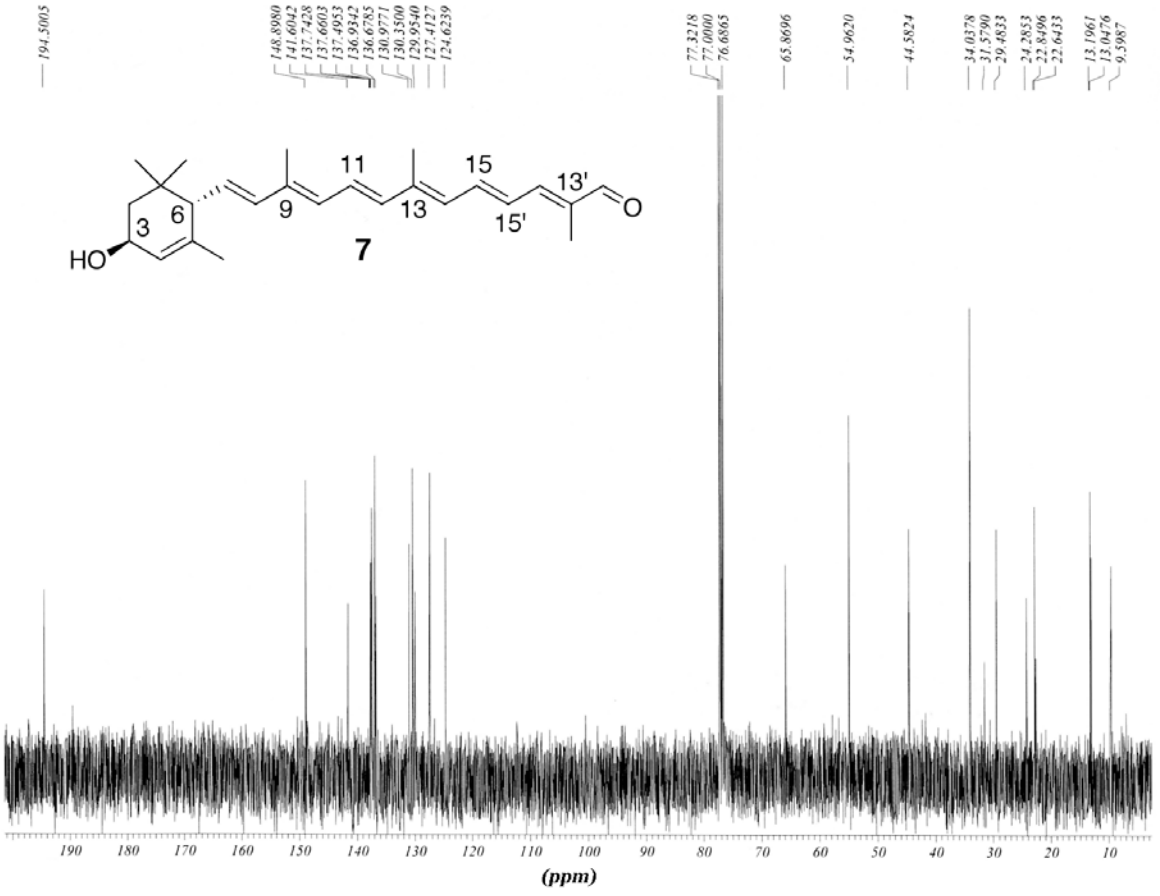

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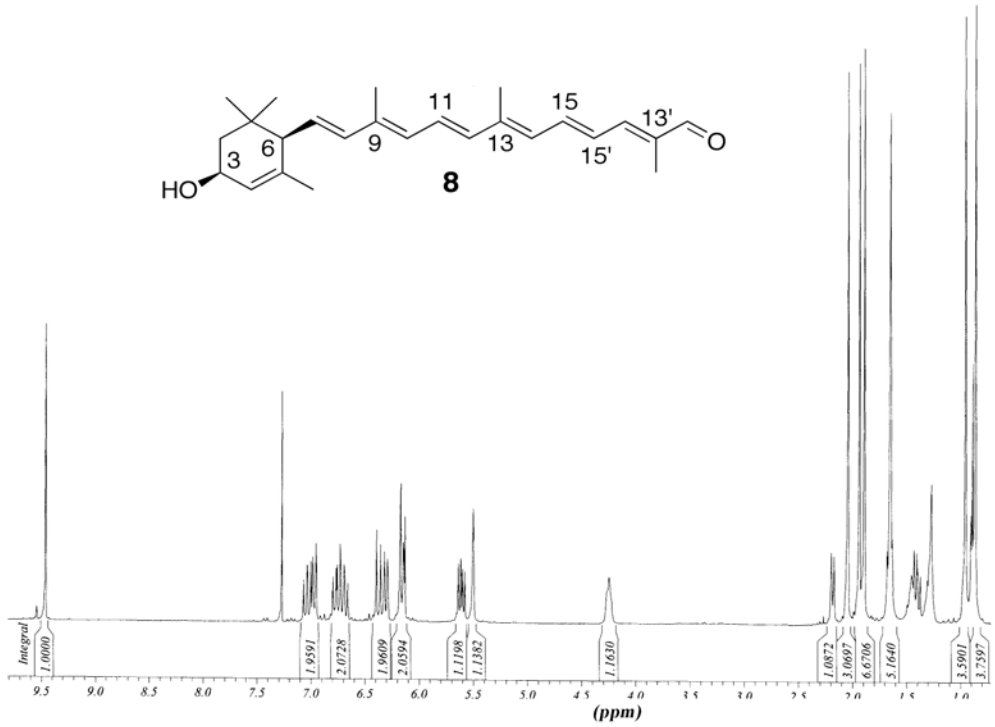

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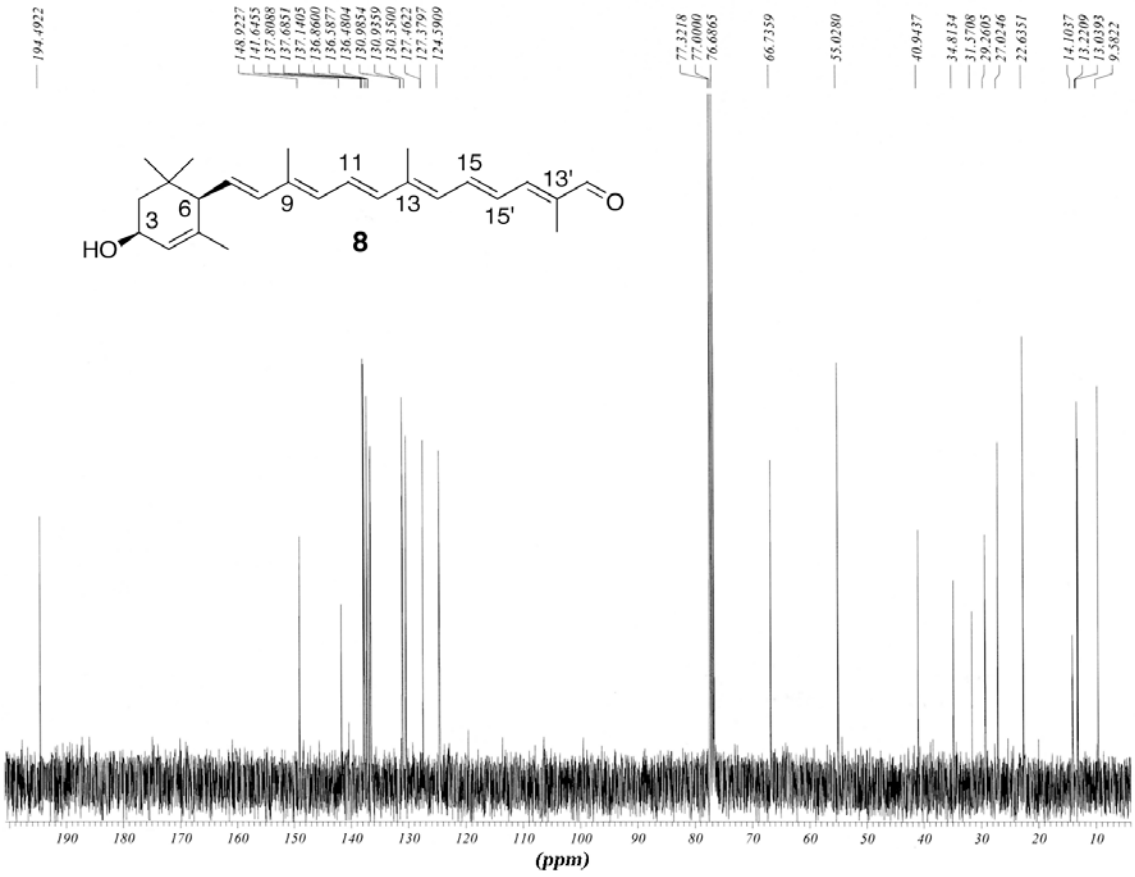

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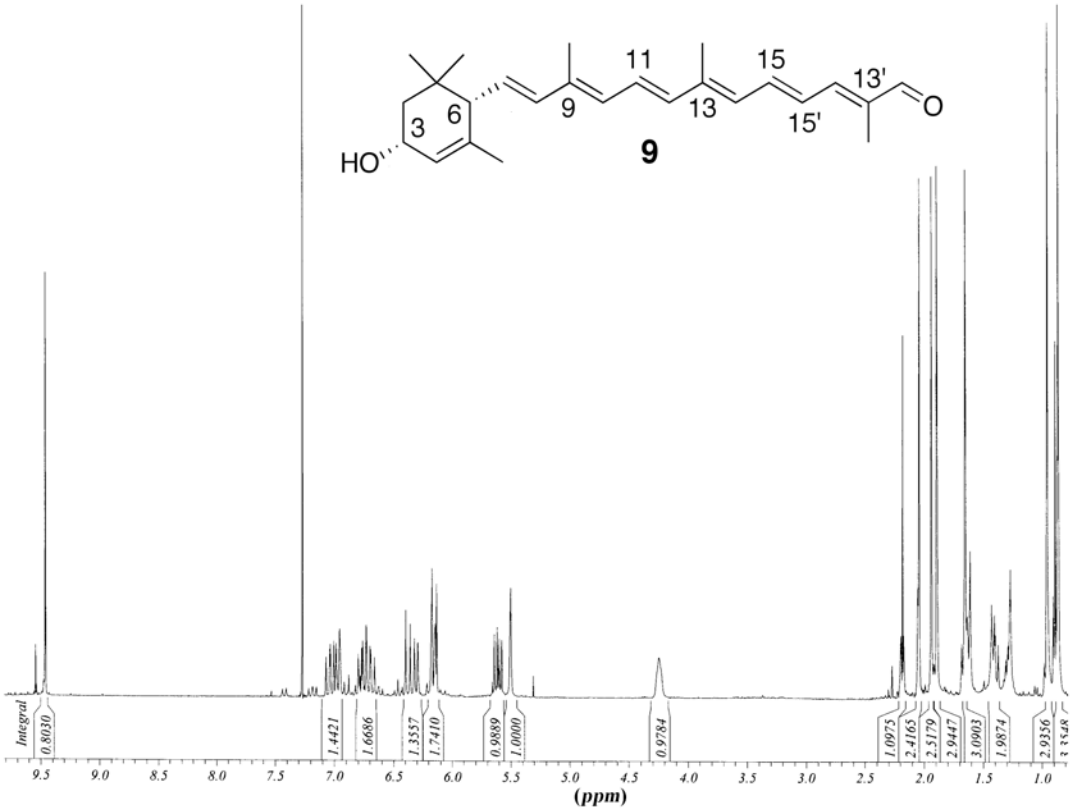

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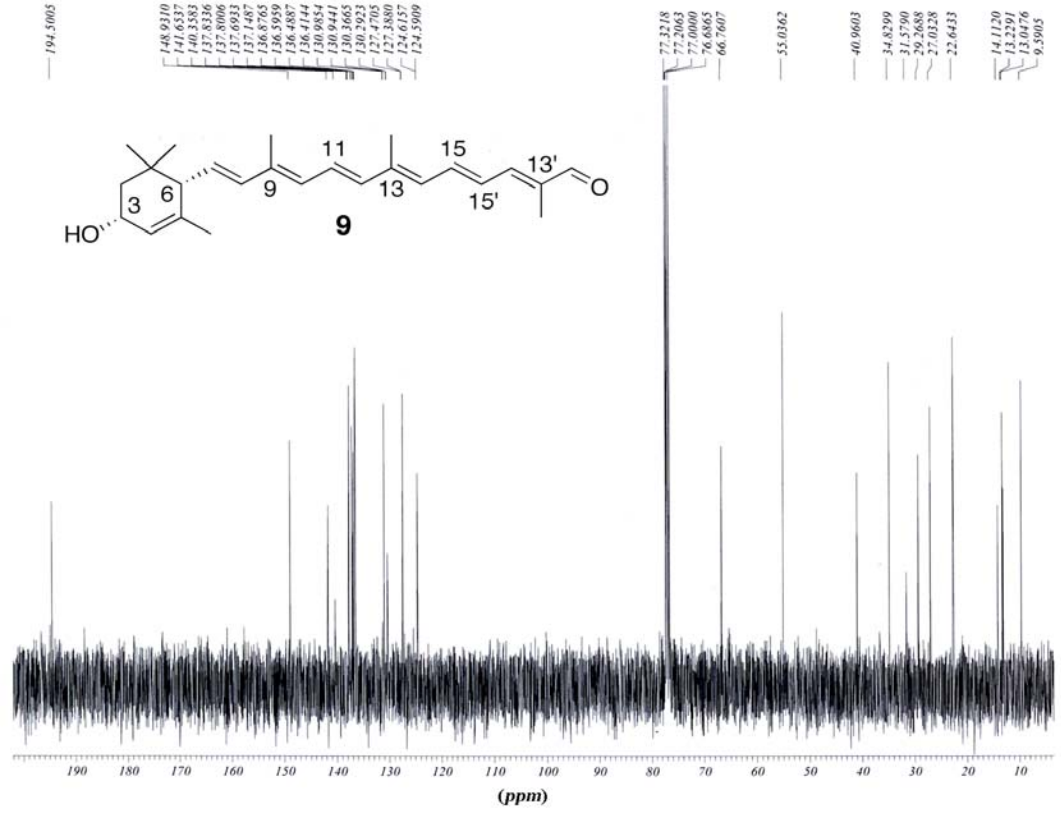

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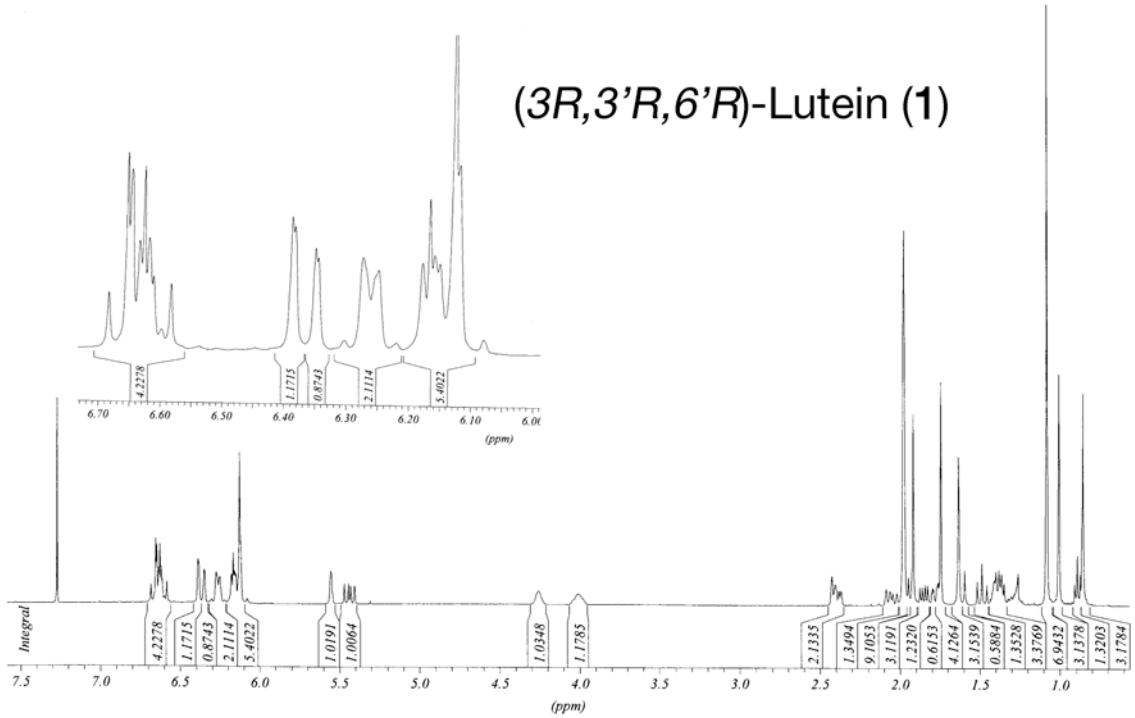

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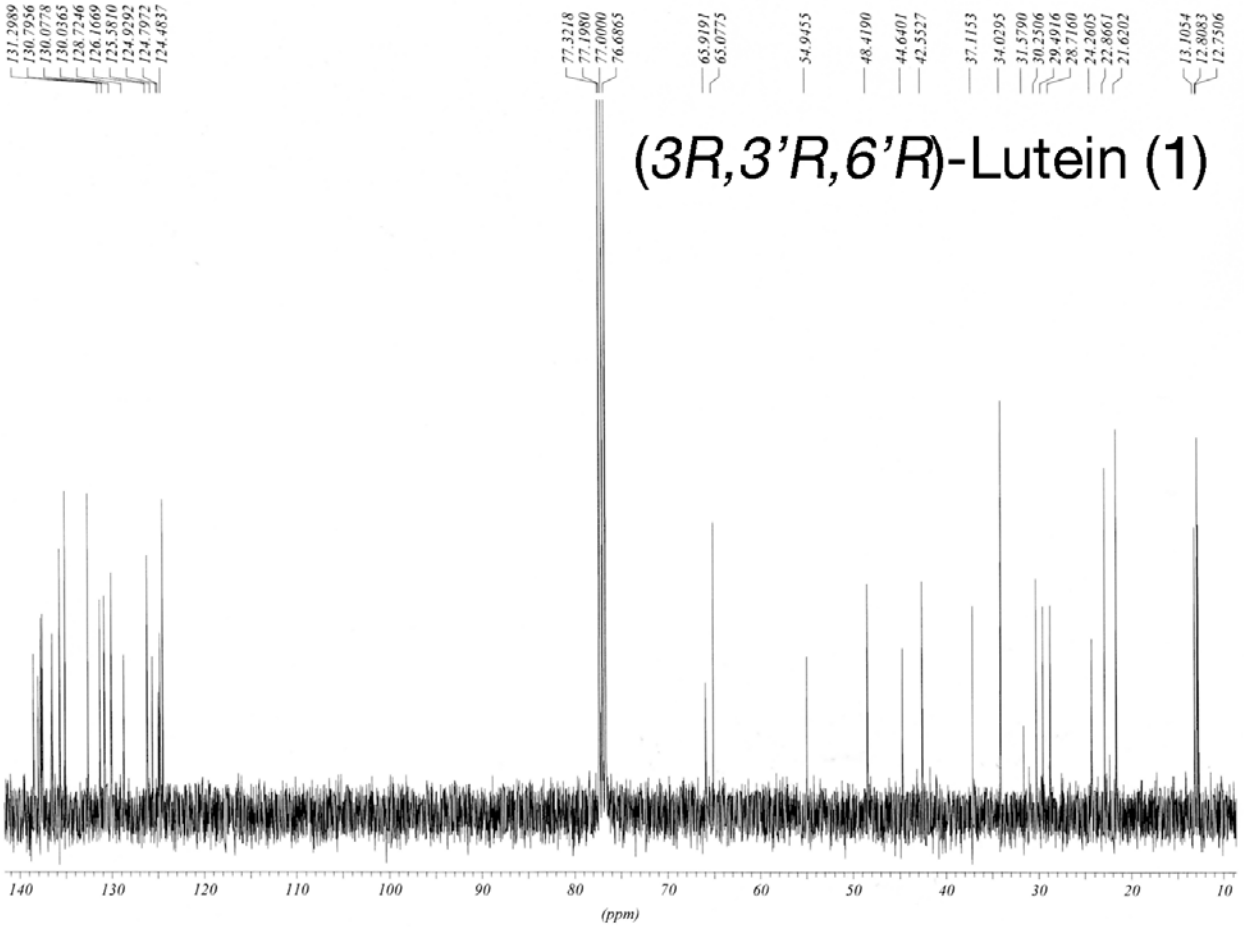

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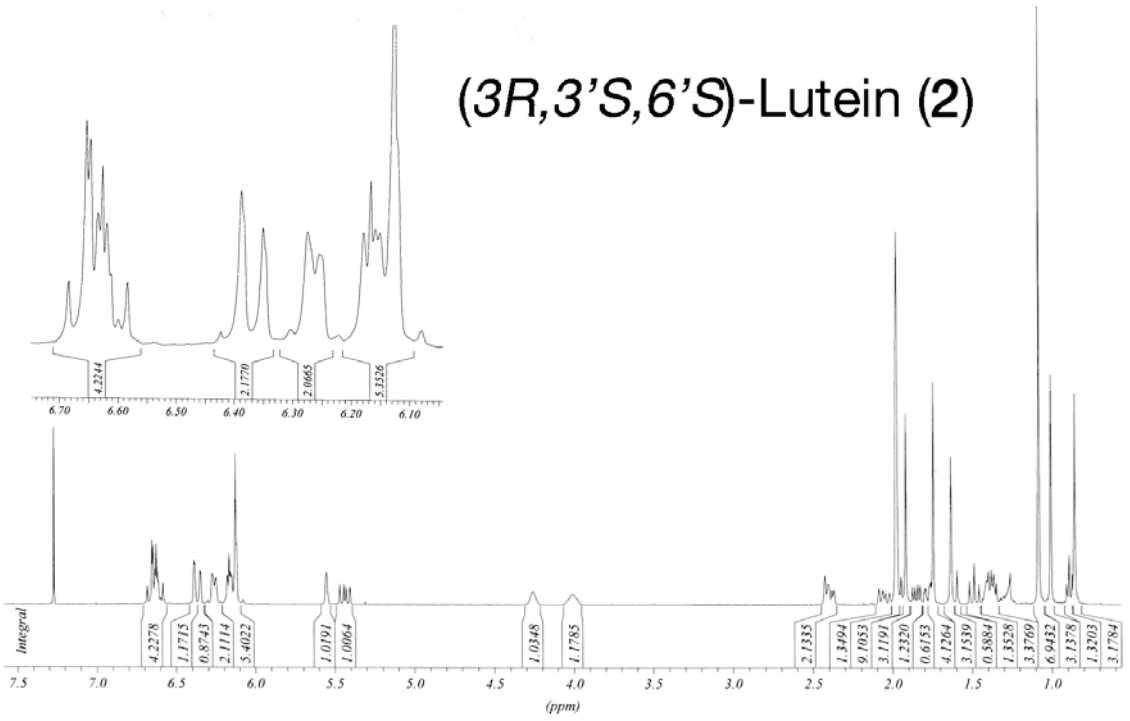

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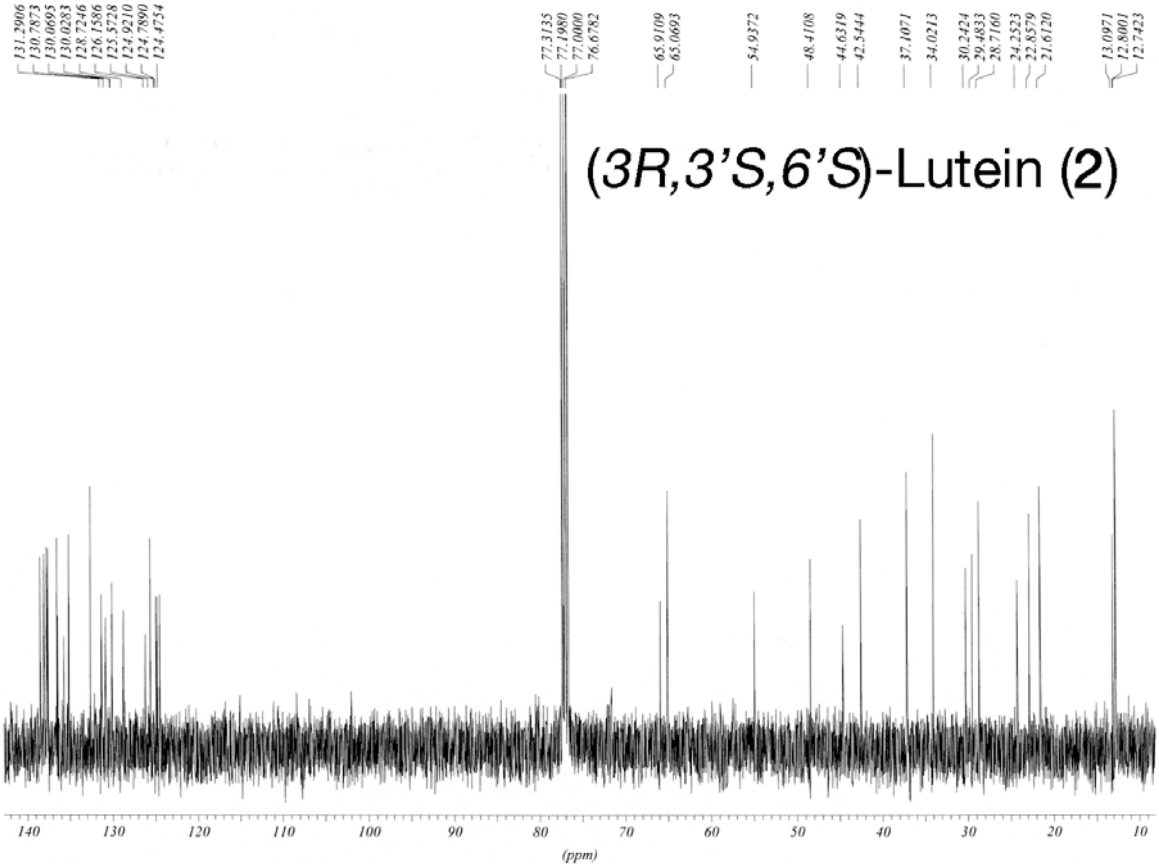

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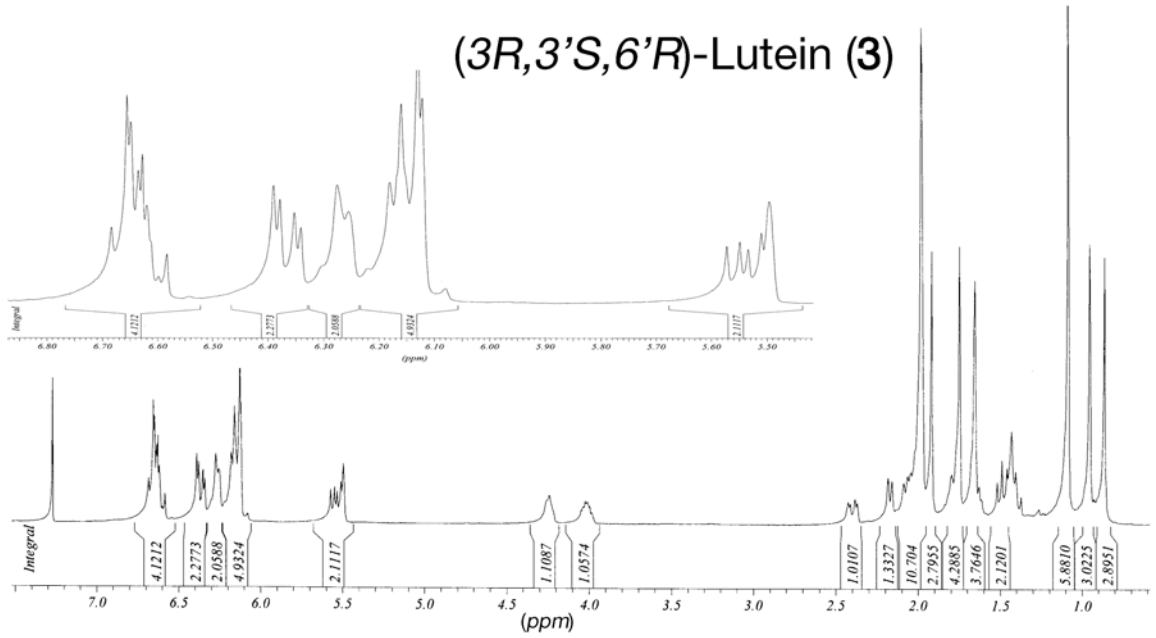

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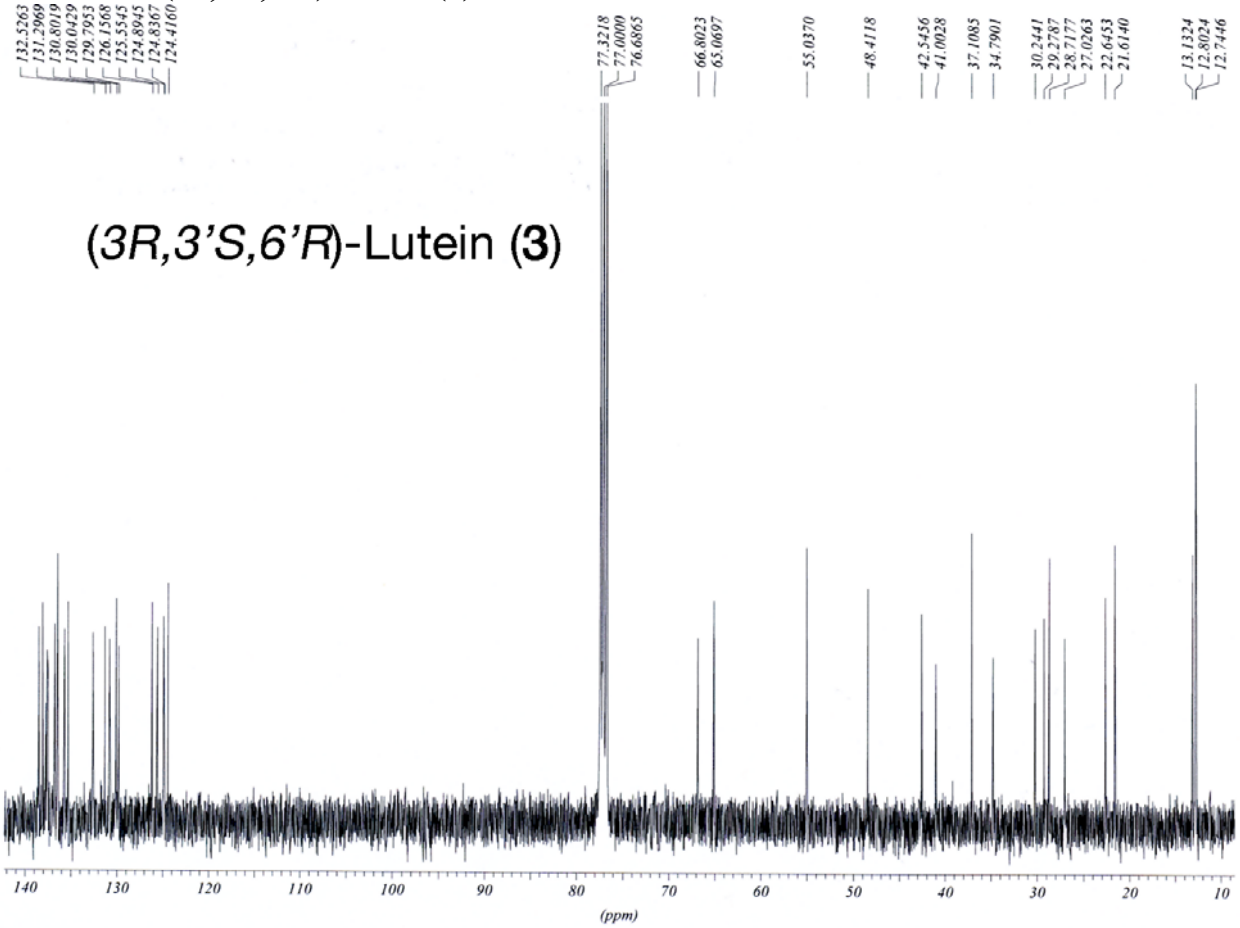
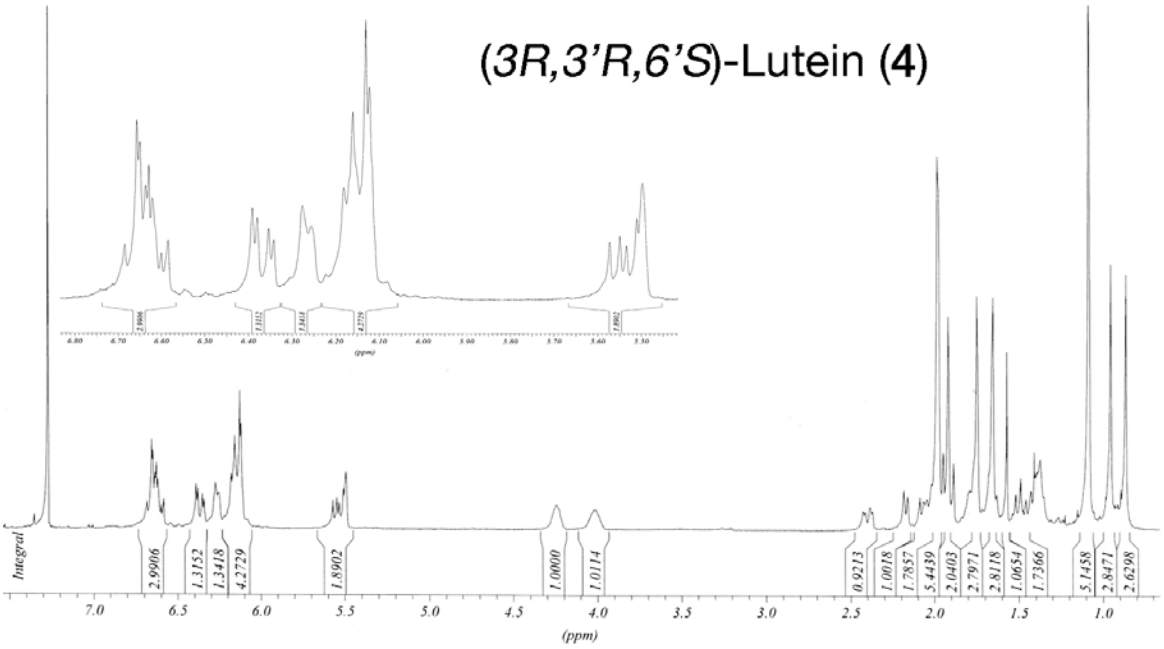
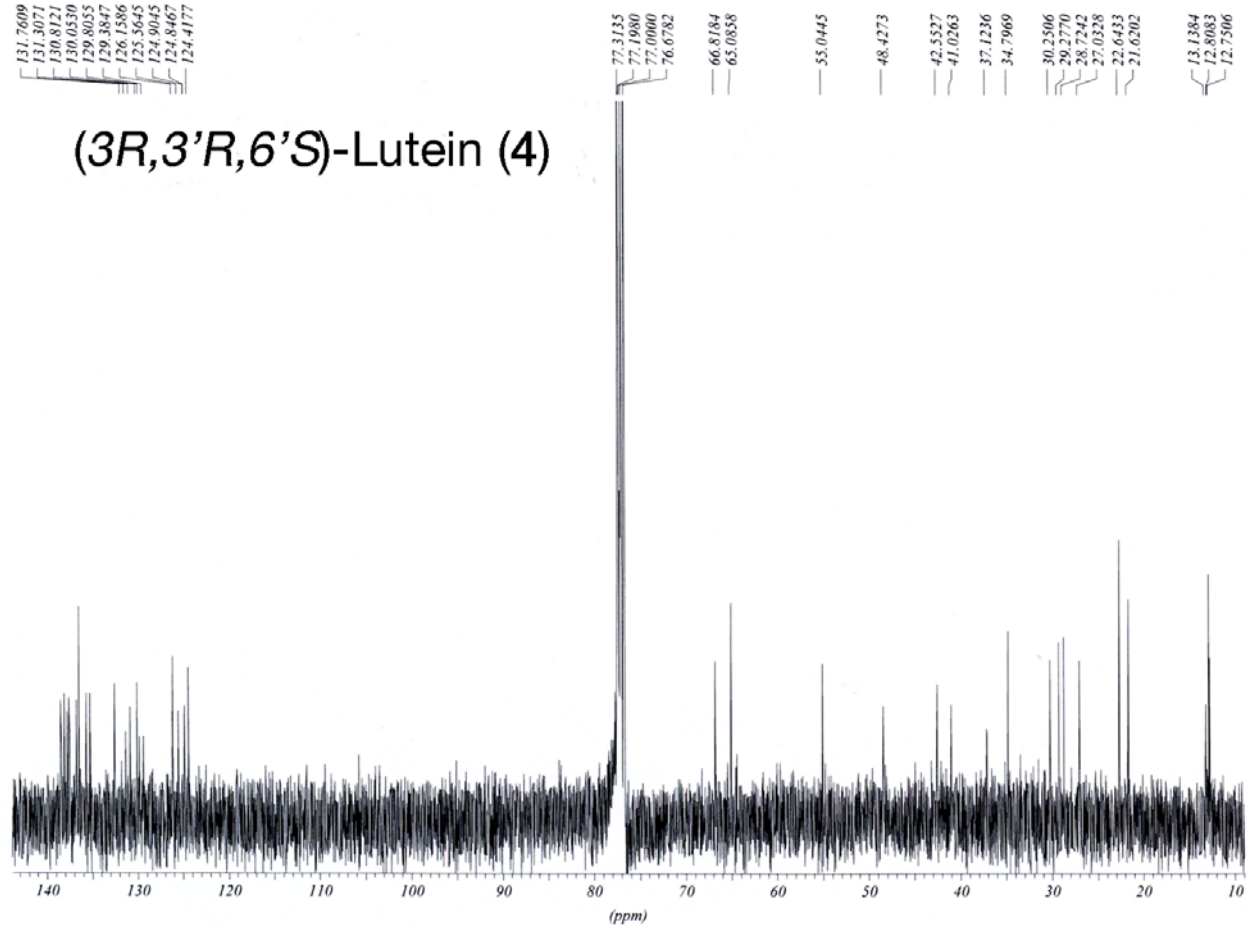


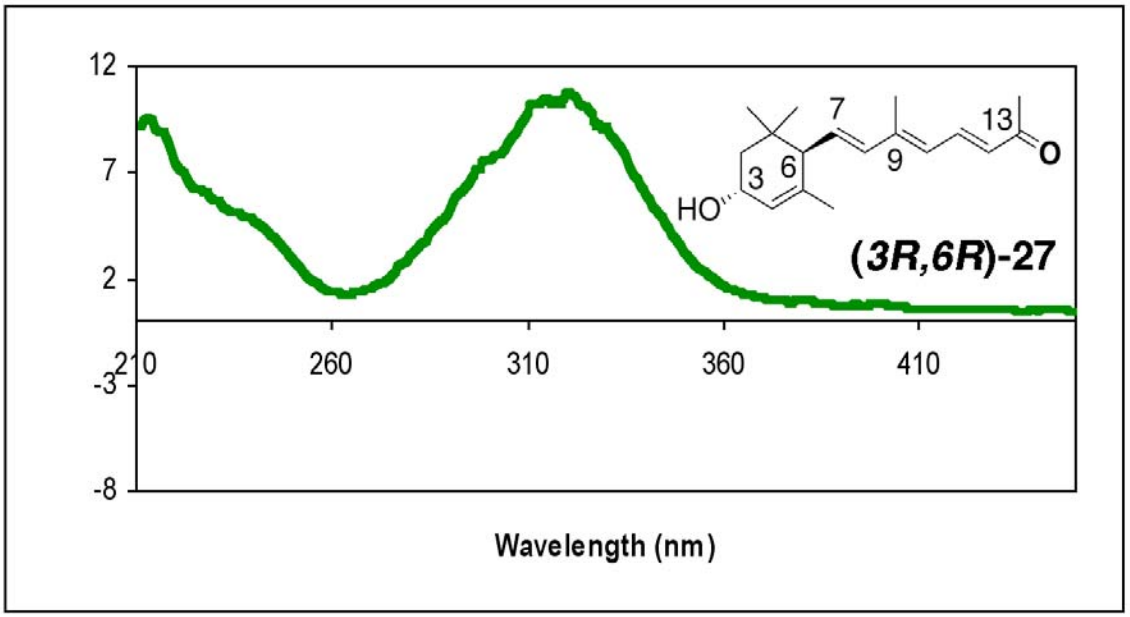
^{1}H NMR of (*3R,3'R,6'S*)-Lutein (4)

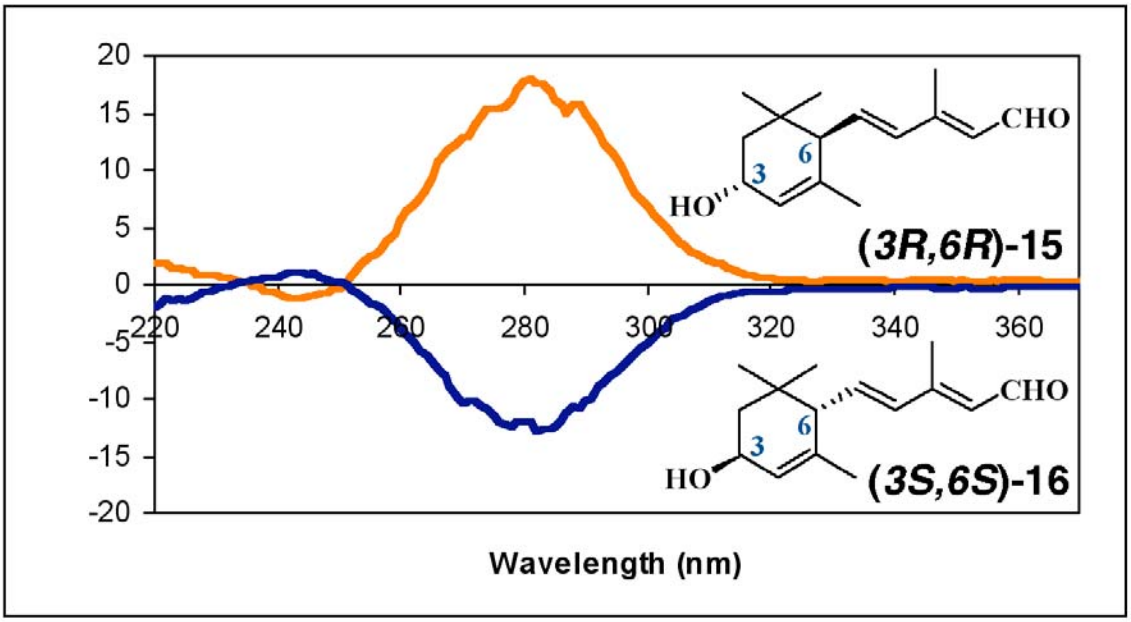


^{13}C NMR of (*3R,3'R,6'S*)-Lutein (4)

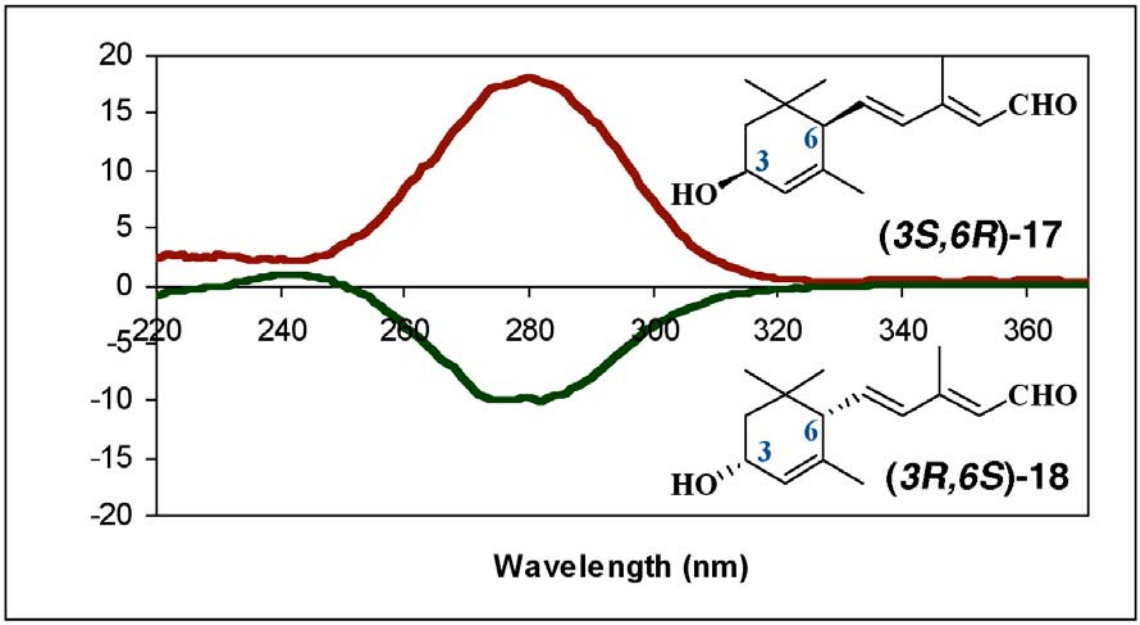


CD spectra of (*3R,6R*)-3-hydroxy-13-apo-ε-caroten-13-one (27), (*3R,6R*)-15, and (*3S,6S*)-16

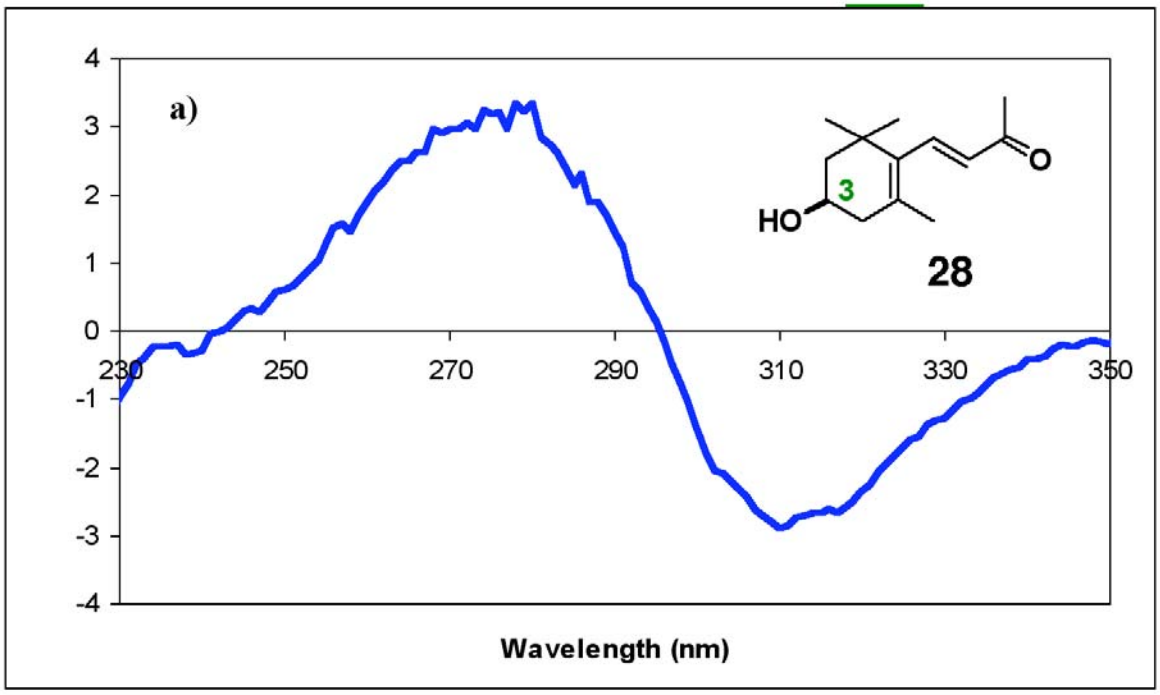




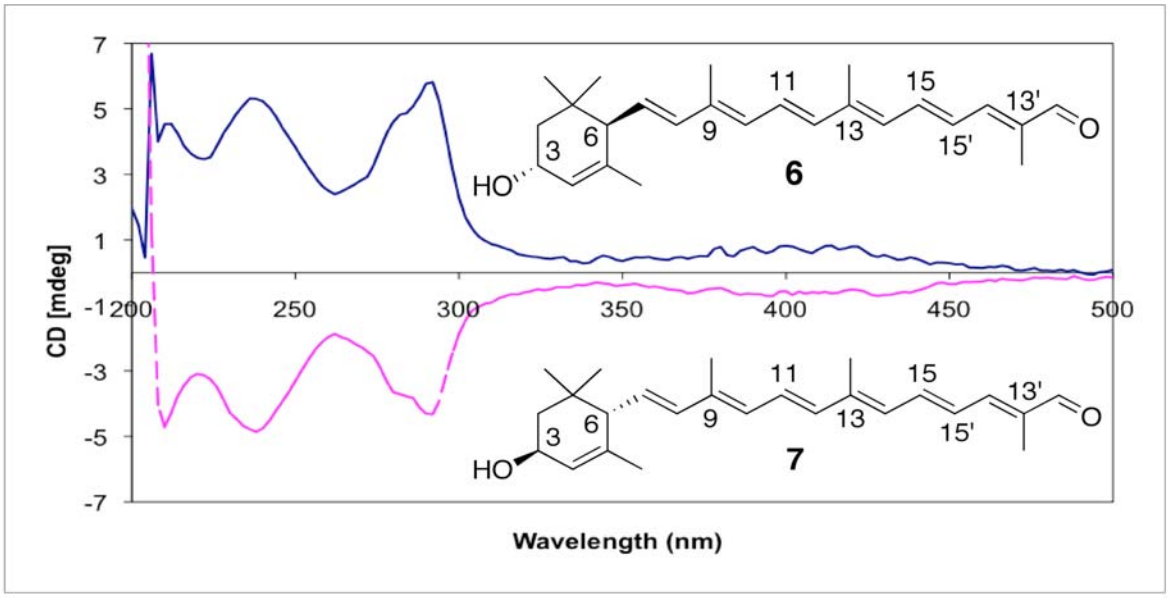
CD spectra of (*3S,6R*)-17, (*3R,6S*)-18, and (*3R*)-3-Hydroxy-β-ionone (28)



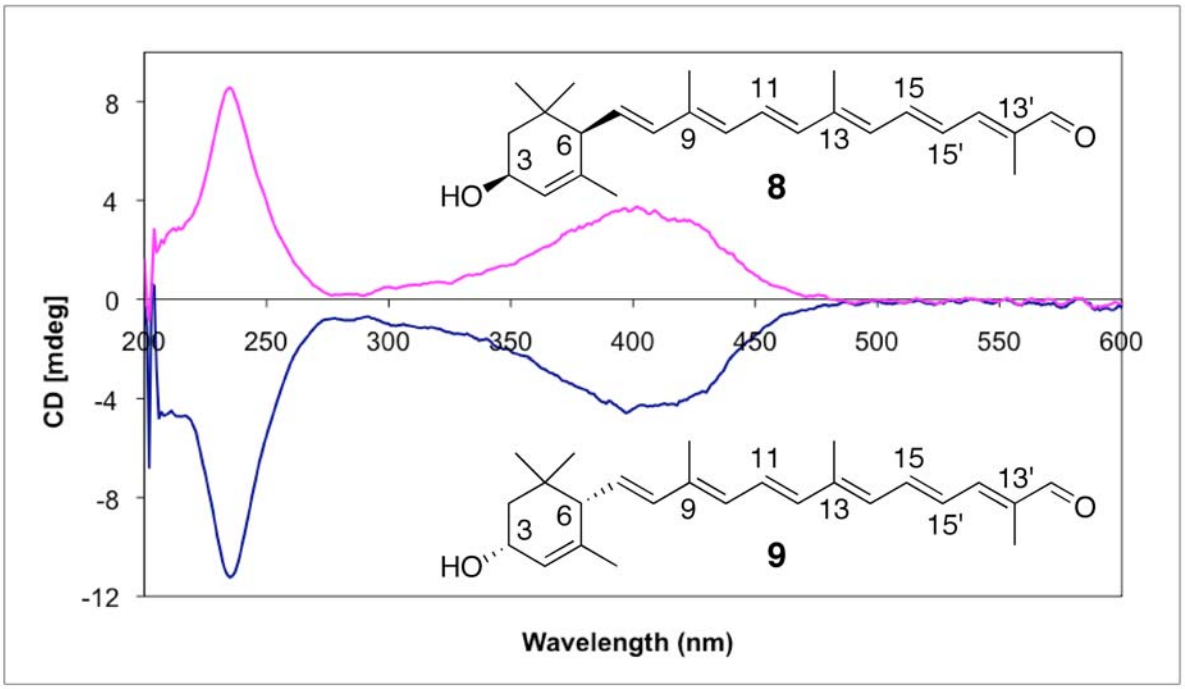
CD spectrum of (*3R*)-3-hydroxy-β-ionone (28)



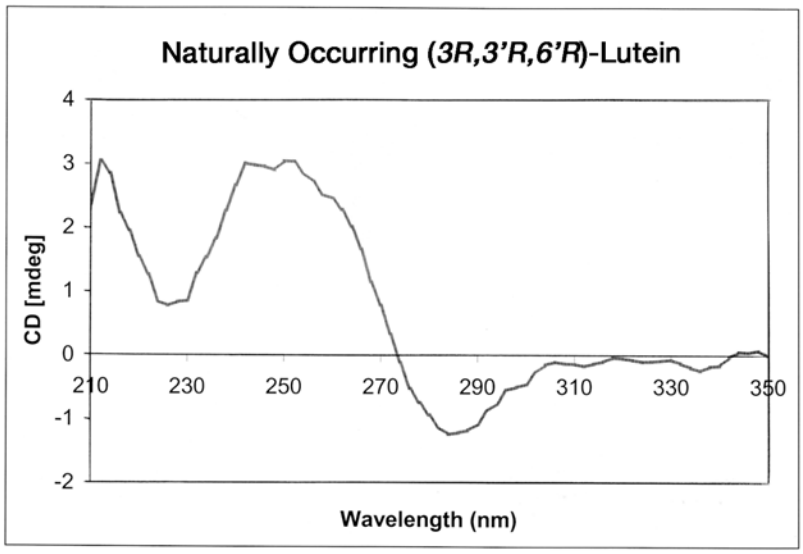
CD spectra of (*3R,6R*)-3-hydroxy-12'-apo-ε-caroten-12'-al (6) and (*3S,6S*)-3-hydroxy-12'-apo-ε-caroten-12'-al (7)



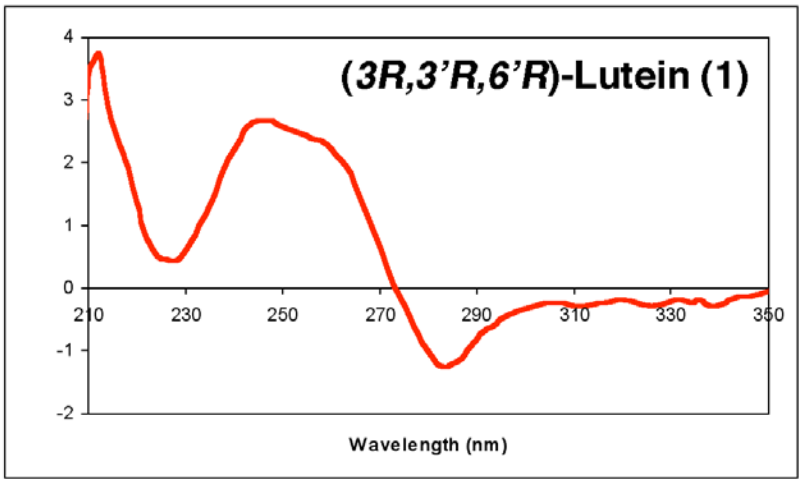
CD spectra of (*3S,6R*)-3-hydroxy-12'-apo-ε-caroten-12'-al (8) and (*3R,6S*)-3-hydroxy-12'-apo-ε-caroten-12'-al (9)

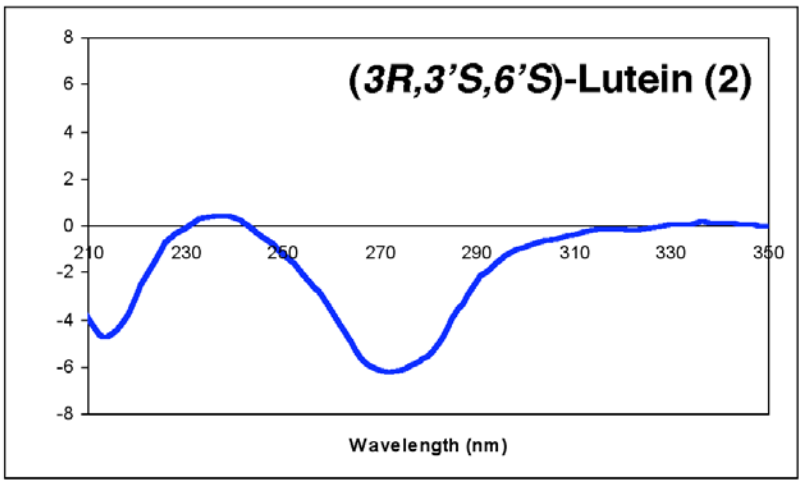


CD spectrum of naturally occurring (*3R,3'R,6'R*)-lutein

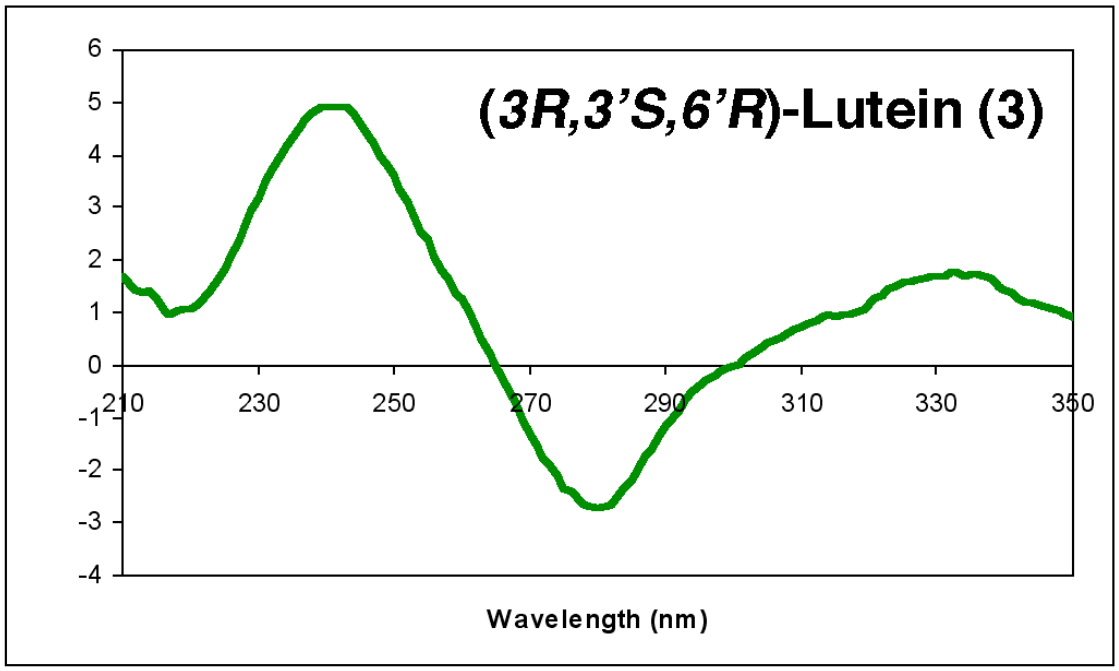


CD spectra of synthetic (*3R,3'R,6'R*)-lutein (1) and (*3R,3'S,6'S*)-lutein (2)





CD spectra of synthetic (*3R,3'S,6'R*)-lutein (3) and (*3R,3'R,6'S*)-lutein (4)



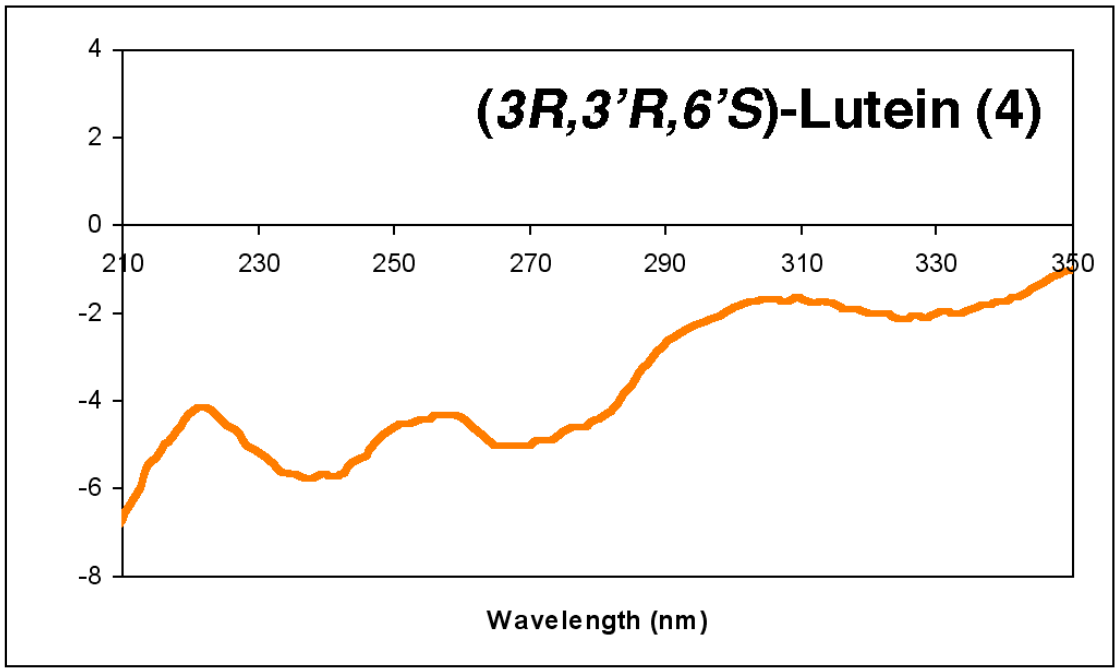
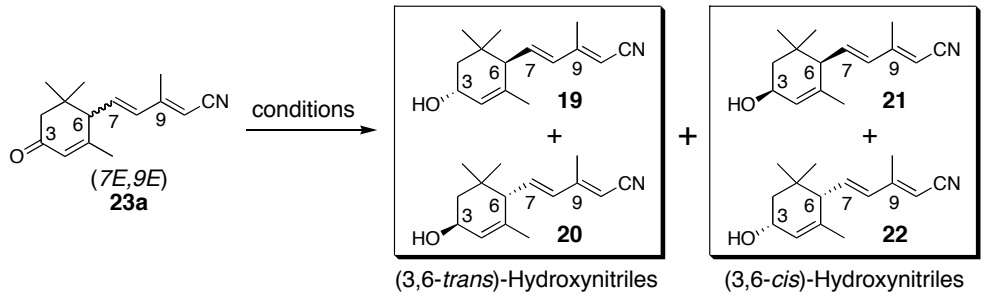


Table 1S. Reduction of ketotonitrile **23a** to hydroxynitriles **19** – **22**



Entry	Conditions	(**19**+**20**):(**21**+**22**) (3,6-*trans*:3,6-*cis*)[a]	Conversion (%)[b]
1	$NaBH_4$, EtOH, H_2O, 0 °C to rt, 24h	1.0 : 1.0	97 [c]
2	Triisobutylaluminum (TIBA), Toluene, -40 °C to rt, 1h	2.0 : 3.0	95
3	$NaBH_4$/*d*-Tartaric acid (3/1), EtOH, -10 to -15 °C, 2h	3.0 : 1.0	94
4	$NaBH_4$/*l*-Tartaric acid (3/1), EtOH, -10 to -15 °C, 2h	3.0 : 1.0	94
5	$NaBH_4$/Dibenzoyl-*d*-tartaric acid (3/1), EtOH, -10 to -15 °C, 2h	3.0 : 1.0	96
6	$NaBH_4$/*dl*-Tartaric acid (3/1), EtOH, -10 to -15 °C, 2h	3.0 : 1.0	94
7	Sodium bis(2-methoxyethoxy)aluminum hydride, $NaAlH_2(OCH_2CH_2OMe)_2$ = (Red-Al™), TBME, -5 to 0 °C, 1h	1.3 : 1.0	95
8	Lithium tri-*sec*-butylborohydride, $LiB[CHMeCH_2CH_3]_3H$ (L-Selectride™), TBME, -30°C, 0.5h	1.2 : 1.0	93
9	Sodium tri-*sec*-butylborohydride, $NaB[CHMeCH_2CH_3]_3H$ (N-Selectride™), TBME, -30 °C, 0.5h	2.5 : 1.0	92
10	Potassium trisiamylborohydride, $KB[CHMeCHMe_2]_3H$ (KS-Selectride™), TBME, -30 to 0 °C, 2h	2.2 : 1.0	91
11	**Potassium tri-*sec*-butylborohydride, $KB[CHMeCH_2CH_3]_3H$ (K-Selectride™), TBME, -30 °C, 0.5h**	**6.0 : 1.0**	96[c]
12	**BH_3/(*R*)-2-methyl-CBS-oxazaborolidine, TBME, 0 °C, 1.5h**	**1.0 : 6.0**	90[c]
13	BH_3/(*S*)-2-methyl-CBS-oxazaborolidine, TBME, 0 °C, 1.5h	1.0 : 3.0	93

[a] Ratios were determined by HPLC on a silica-based nitrile bonded column and a chiral column (Eluent A and Eluent C, Table 2S). [b] Conversion to product was determined by HPLC (Eluent A). [c] Isolated yield after chromatography.

***Table 2S.** HPLC conditions for separation of the synthetic compounds.

HPLC Columns	**HPLC Eluents**		**Compounds separated, monitoring wavelength (nm)**
Silica-based nitrile bonded column (analytical & semipreparative)	A	Hexane 75%, CH_2Cl_2 25%, MeOH 0.5%	**24a** & **24b**, 260 nm; **23a** & **23b**, 260 nm; **19** – **22**, 260 nm; **15** – **18**, 280 nm; **6** – **9**, 412 nm
Silica-based nitrile bonded column (semipreparative)	B	Hexane, Isopropanol 0.1%	**24a** & **24b**, 260 nm
Chiral, amylose tris-(3,5-dimethylphenylcarbamate)	C	Hexane 95%, Isopropanol 5%, CH_3CN 0.75%	**19** – **22**, 260 nm
Chiral, amylose tris-(3,5-dimethylphenylcarbamate)	D	CH_3CN 98%, Isopropanol 2%	**15** – **18**, 280 nm **6** – **9**, 412 nm
Silica-based nitrile bonded column (semipreparative)	E	Hexane 75%, CH_2Cl_2 25%, MeOH 0.35%	**27**, 324 nm & **28**, 286 nm
Chiral, amylose tris-(3,5-dimethylphenylcarbamate)	F	Hexane 87%, *tert*-Amyl alcohol 13%	**1** & **2**, 446 nm
Chiral, amylose tris-(3,5-dimethylphenylcarbamate)	G	Hexane 90%, 2-Pentanol 10%	**3** & **4**, 446 nm

*Details of the HPLC systems, columns, and flow rates are described in the general methods.

Fig 1S. The HPLC profile of a mixture of ketonitriles **23a** and **23b** on a silica-based nitrile bonded column employing eluent A (Table 2S), flow rate = 0.7 mL/min.

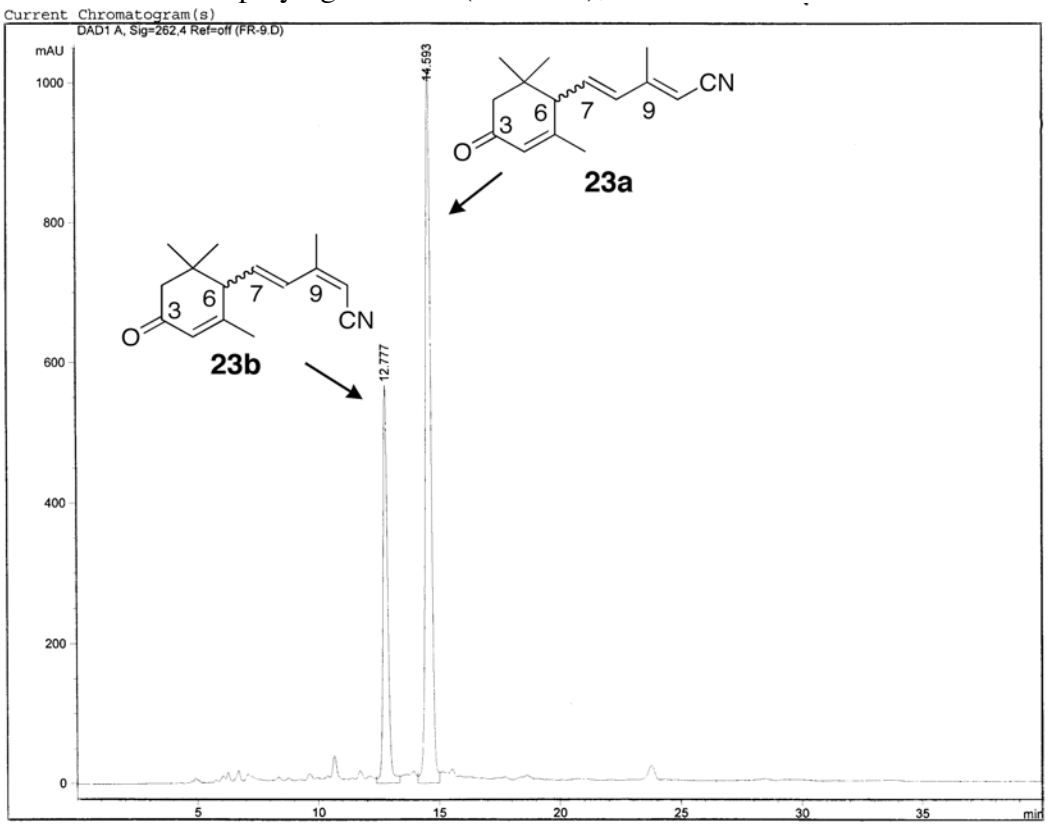


Fig 2S. The HPLC profile of a racemic mixture of hydroxynitriles **19** – **22** on a silica-based nitrile bonded column employing eluent A (Table 2S), flow rate = 0.7 mL/min.

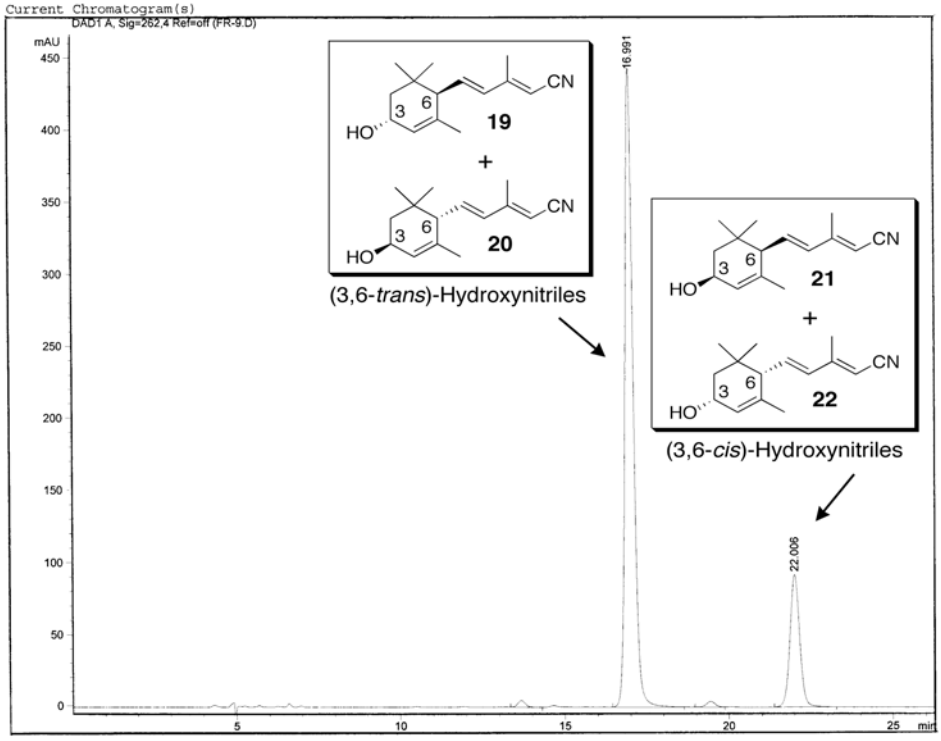


Fig 3S. The HPLC profile of a racemic mixture of hydroxyaldehydes **15** – **18** on a silica-based nitrile bonded column employing eluent A (Table 2S), flow rate = 0.7 mL/min.

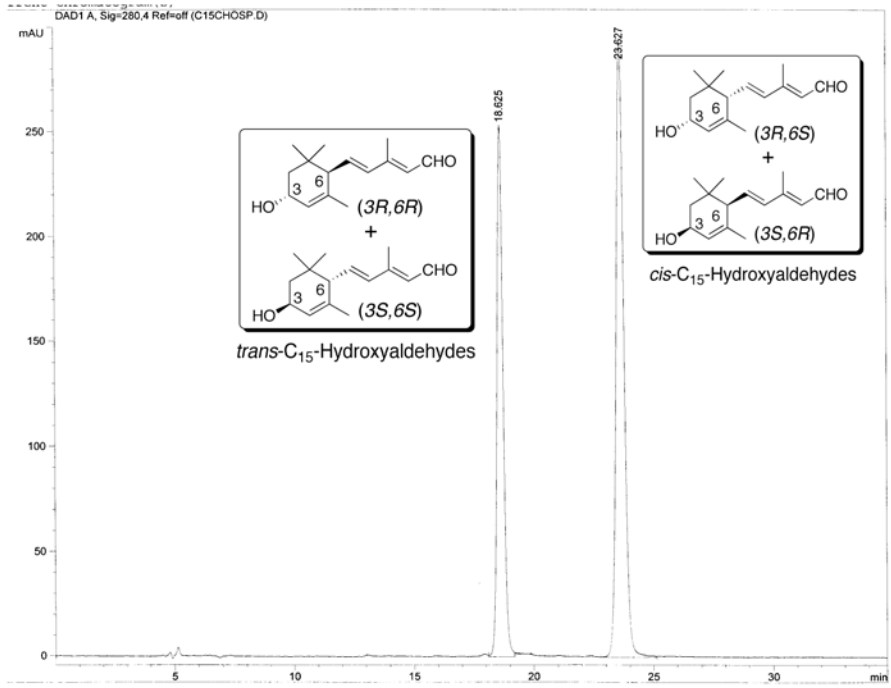


Fig 4S. The HPLC profile of a racemic mixture of hydroxyaldehydes **15** – **18** on an amylose tris-(3,5-dimethylphenylcarbamate) chiral column employing eluent D (Table 2S), flow rate = 0.7 mL/min. Enantiomeric aldehydes were well separated while simultaneous separation of all four aldehydes resulted in partial separation between diastereomeric aldehydes.

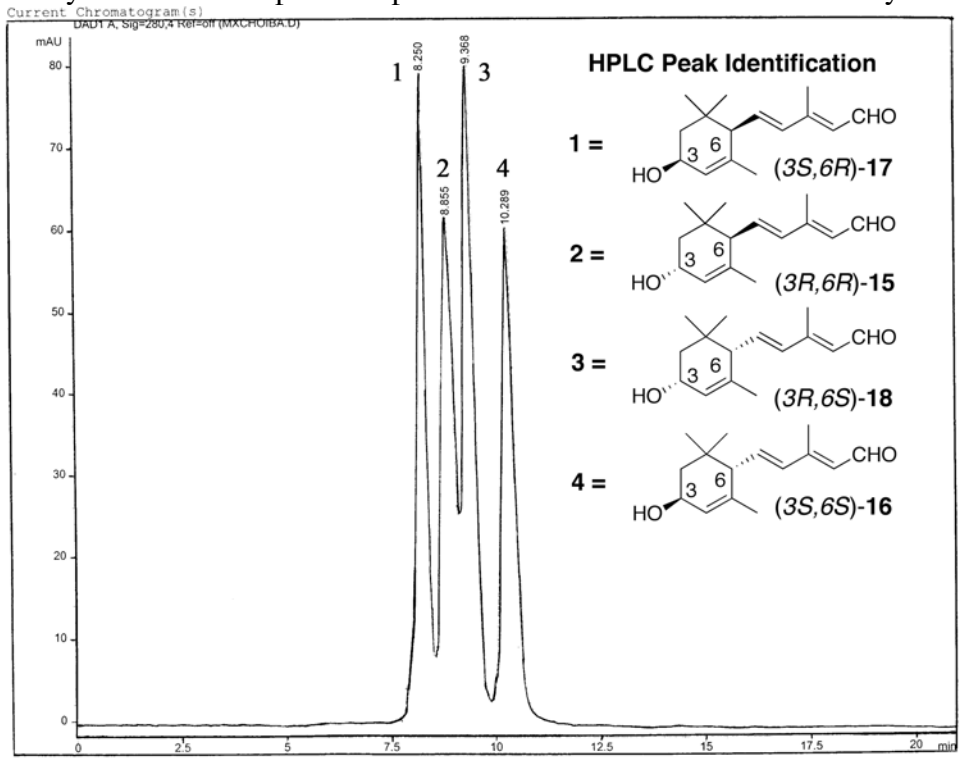


Fig 5S. The HPLC profile of a racemic mixture of C_{25}-hydroxyaldehydes **6** – **9** on an amylose tris-(3,5-dimethylphenylcarbamate) chiral column employing eluent D (Table 2S), flow rate = 0.7 mL/min. Enantiomeric C_{25}-hydroxyaldehydes were well separated while simultaneous separation of all four aldehydes resulted in partial separation between diastereomeric aldehydes.

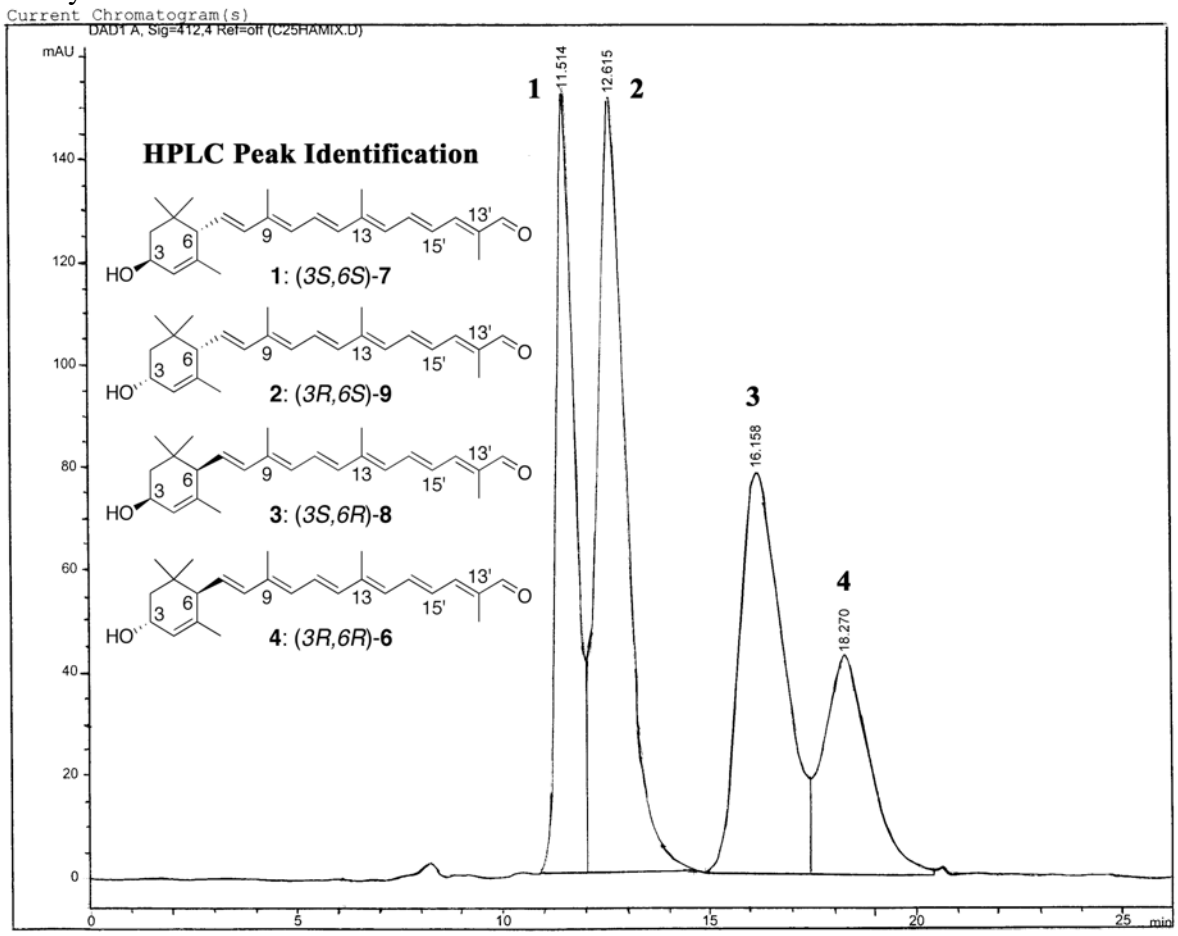


Fig 6S. The HPLC profile of synthetic (*3R,3'R,6'R*)-lutein (**1**) on an amylose tris-(3,5-dimethylphenylcarbamate) chiral column employing eluent F (Table 2S), flow rate = 0.7 mL/min.

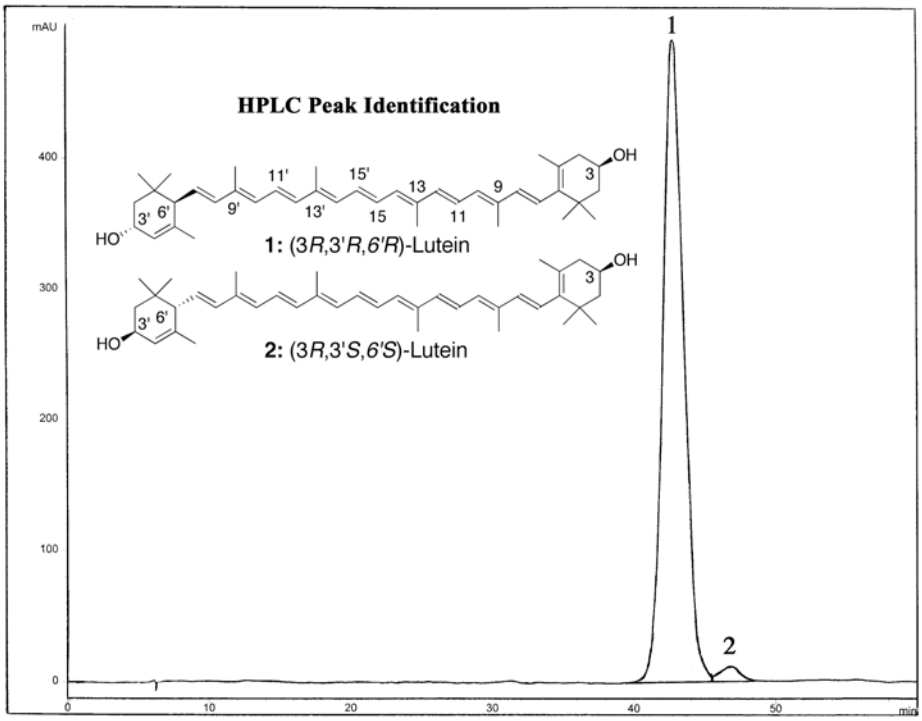


Fig 7S. The HPLC profile of synthetic (*3R,3'S,6'S*)-lutein (**2**) on an amylose tris-(3,5-dimethylphenylcarbamate) chiral column employing eluent F (Table 2S), flow rate = 0.7 mL/min.

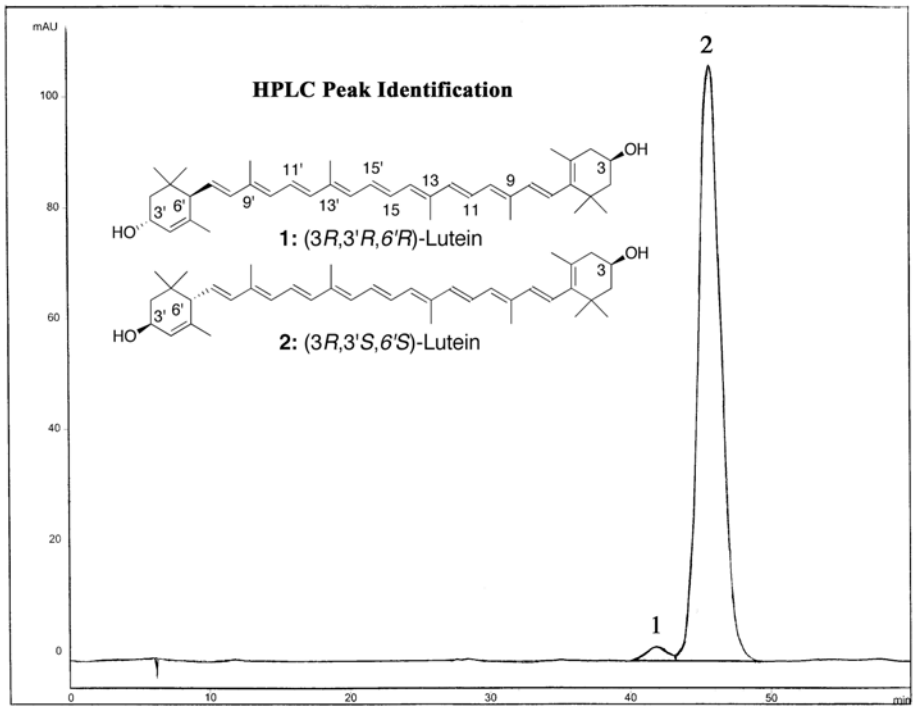


Fig 8S. The HPLC profile of synthetic (*3R,3'S,6'R*)-lutein (**3**) on an amylose tris-(3,5-dimethylphenylcarbamate) chiral column employing eluent G (Table 2S), flow rate = 0.7 mL/min.

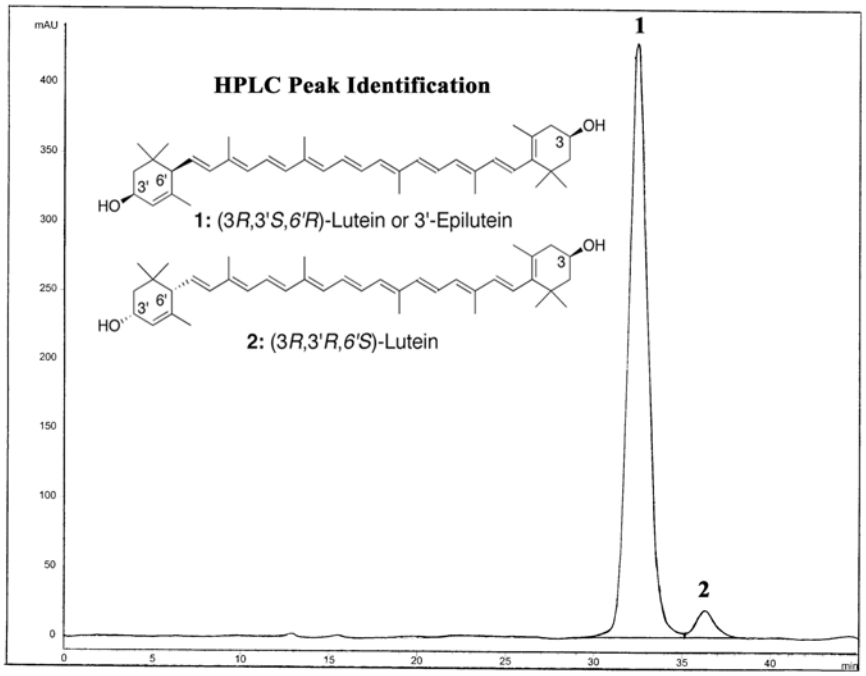


Fig 9S. The HPLC profile of synthetic (*3R,3'R,6'S*)-lutein (**4**) on an amylose tris-(3,5-dimethylphenylcarbamate) chiral column employing eluent G (Table 2S), flow rate = 0.7 mL/min.