SYNTHESIS OF ALL-TRANS-BETA-CAROTENE RETINOIDS AND DERIVATIVES LABELLED WITH ^{14}C

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Summary

In this paper we report the synthesis of all-trans Retinoic acid, Retinal, Retinol specifically labelled with ^{14}C at position 7 and all-trans Beta-carotene labelled at positions 7 and 7', with more than 98 % radiochemical purity. All products were obtained with about 15 % overall yield from ^{14}C sodium cyanide.

Introduction

All-trans-Retinoic acid is a metabolite of vitamin A (retinol) able to support the functions of vitamin A in the maintenance of normal growth and epithelial cell differentiation[1]. Retinoic acid and some of its analogues (retinoids) have recently generated much interest as agents useful for the treatment of skin disorders[2] and as potential cancer chemopreventive or chemotherapeutic compounds[3,4].

To undertake in vivo metabolism of Beta-carotene, we are engaged with other groups in the labelling of carotenoids, retinoids and derivatives by ^{14}C.

Pommer and Kuhn[5] have described a procedure for preparing Beta-carotene from the Beta-ionone-derived triphenyl phosphonium salt. The disadvantages of these procedures include the fact that the triphenylphosphine reactant required for the synthesis is relatively expensive and that the by-products of the reactions, triphenylphosphine, are not water soluble, thus making it difficult to isolate. We use the phosphonate as intermediates which can be synthesised by the reaction of cyclohexyl group - containing ^{14}C through 16C aldehyde, such as 2-methyl-4-(2,6,6 trimethyl-1 cyclohexen-1-yl)-3 butenal (**10**) with a phosphonic acid ester, such as methylen bis phosphonic acid, tetraethyl ester[6].

Synthesis

The preparation of Beta-carotene, and Retinoid derivatives labelled with ^{14}C, was performed according to the reaction sequence depicted in scheme 1 and 2. We show how ^{14}C sodium cyanide can be converted, in a six step sequence, into the labelled Beta-ionone (**8**). Acetonitrile-1-^{14}C was

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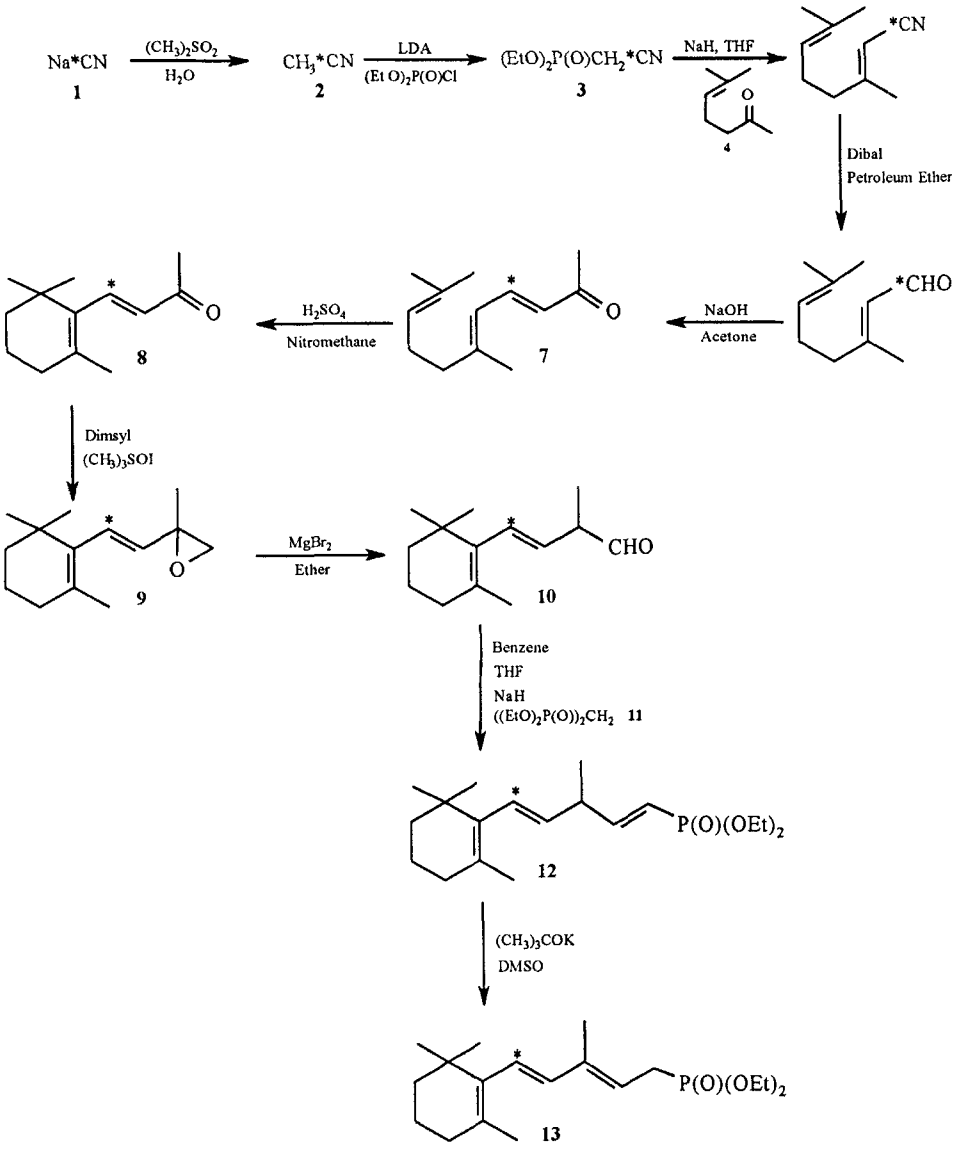
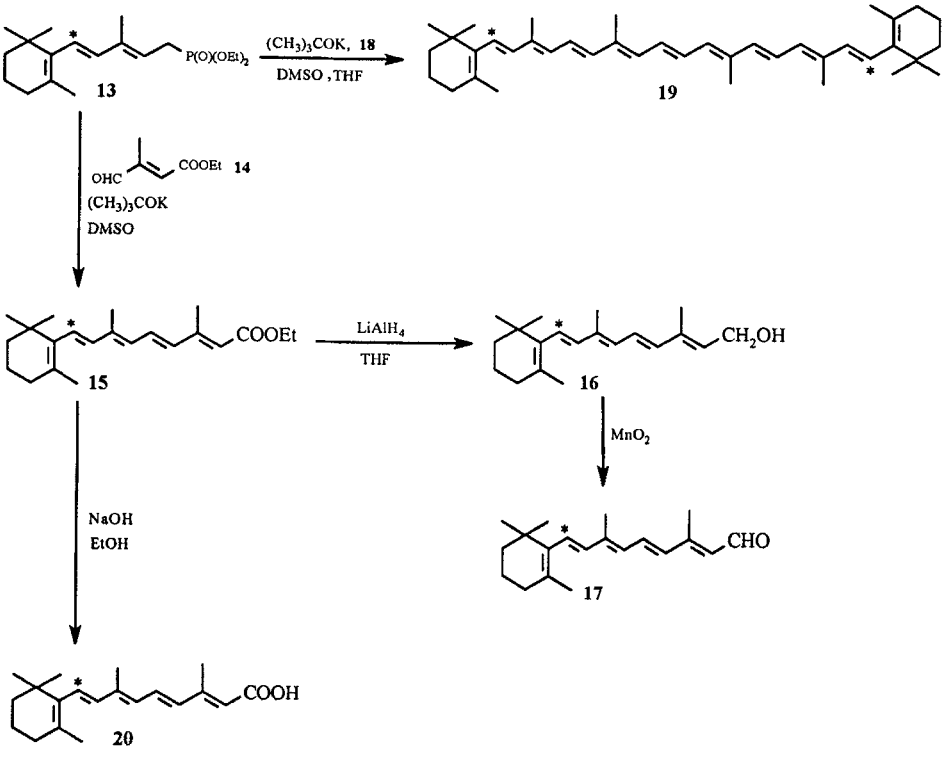
SCHEME 1



prepared from Sodium cyanide ^{14}C by the action of dimethylsulfate; yield 90 %. Diethylchlorophosphate was reacted with acetonitrile anion at -60°C to give required phosphonate (3) in 90 % yield.

Horner-Emmons reaction of (4) with diethylcyanomethylphosphonate and sodium hydride as base, followed by reduction of the nitrile function with diisobutylaluminium hydride, led to the ^{14}C labelled citral (6) as an isomeric mixture in 85 % yield from cyanomethylphosphonate (3). Aldol condensation of citral (6) with acetone and sodium hydroxide as base[7,8] followed by cyclisation in nitromethane with concentrated sulphuric acid at 0°C give ^{14}C labelled Beta-ionone (8) in 67.5 % yield. Epoxide (9) was prepared in quantitative yield by treatment[6] of Beta-ionone (8) with the

SCHEME 2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19
OHC
COOEt 14
(CH3)3COK
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16
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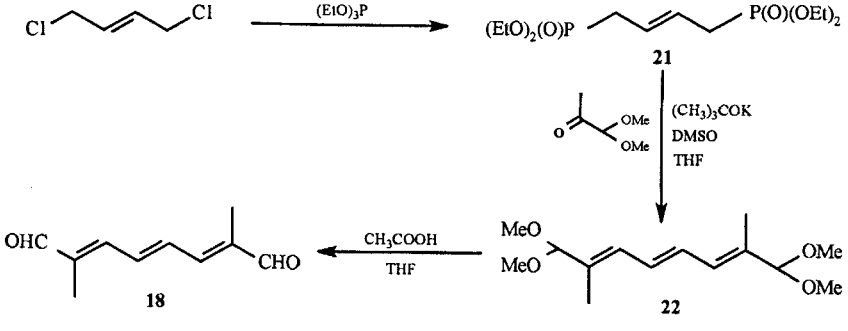
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Cl
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(EtO)2(O)P
P(O)(OEt)2
21
OMe
OMe
(CH3)3COK
DMSO
THF
MeO
MeO
OMe
OMe
22
CH3COOH
THF
OHC
CHO
18

ylide derived from trimethylsulfonium methylsulfate in DMSO. Subsequent isomerisation of (9) using a catalytic amount of magnesium bromide according to a known procedure[6] afforded ^{14}C aldehyde (10) in 90 % overall yield from Beta-ionone (8). A modified Horner-Hemmons olefination[9] of the latter compound (10) using tetraethylmethyl-diphosphonate[10] and sodium hydride as base afforded vinylphosphonate (12) in 93 % yield. Isomerisation of vinylphosphonate (12) in basic media afforded the derived allylic phosphonate (13) in 76 % yield. The synthesis of ethyl retinoate (15) proceeded, as expected, via Horner-Emmans olefination of ethyl-trans-3-methyl-4 oxocrotonate with allylic phosphonate (13) and potassium terbutylate in DMSO. The product was isolated in 61% yield. The base-catalysed hydrolyse of ester led to retinoic acid (20) in 90% yield. Reduction of ethyl retinoate (15) led to retinol (16) in 95% yield. Subsequent oxidation of Retinol with manganese dioxide afforded the Retinal in 95%. Beta-carotene was prepared in 61 % yield by treatment of 2,6-dimethyl-2,4,6-octatriendial (18) with vinyl phosphonate (13) in the presence of potassium terbutylate as base.

Experimental

General comments. In the procedures described below, all the reactions were carried out under Ar atmosphere; purified retinals were handled under dim red light. The following solvents were distilled prior to use: THF, from Na, Benzophenone, Petroleum ether (bp 40-60°C) from P_2O_5. All other organic solvents were appropriately purified and/or dried prior to use. All reactions were carried out in flame-dried glassware. The evaporations were performed in vacuum using a rotary evaporator. All starting organic chemicals were obtained from Aldrich chemical co, Kieselgel 60 (230-40 mesh) obtained from E.Merck was used from chromatography. ^1H NMR spectra were recorded on a Bruker W B 400 and 200 AM spectrometer. All ^1H chemical shifts are reported in parts-per-million downfield relative to tetramethylsilane (TMS) as internal standard. NMR signals were assigned by comparison with those of the corresponding unlabelled compounds.

Acetonitrile-1-^{14}C (2)

To a solution of 1.96 g (40 mmoles) (34 mCi) of ^{14}C-sodium cyanide in 4 ml of water were added 3.8 ml of dimethylsulfate in small portions with shaking and cooled in an ice-bath as necessary. The solution is distilled slowly with a micro-distillation apparatus, and the fraction boiling at 76-100°C (3 ml) is collected. The crude product is extracted into ether and dried over magnesium sulphate, yield 90 %.

S A = 31.28 Mbq/mmole, 0.85 mCi/mmole

^1H NMR (200 MHz, $CDCl_3$) : δ 2.00 ppm (s, 3H).

Diethyl (cyanomethyl) phosphonate-2-^{14}C (3)

To a solution of 48.7 mmoles of LDA (1.5 M) in cyclohexane at -60°, 36 mmoles of acetonitrile in ether were added dropwise. After stirring for 30 min, 6.38 g (37 mmoles) of diethyl chlorophosphate were added at -40°C. The mixture was stirred for 2 h at 0°C. Then a saturated NH_4Cl solution was added and the layers were separated. The water layer was extracted three times with ether and the combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered and the solvents were evaporated, yield 90 %.

S A = 31.28 Mbq/mmole, 0.85 mCi/mmole

^{1}H NMR (200 MHz, $CDCl_3$) : δ 4.32 ppm (dq, 4H, ($CH_3C\underline{H}_2$), $J_{H.P}$ 7Hz, $J_{H.H}$ 1.5Hz); 2.85 ppm (dd, 2H, ($C\underline{H}_2$-P), $J_{H.P}$ 19Hz, $J_{H.H}$ 1.9Hz); 1.37 ppm (dt, 6H, ($C\underline{H}_3$-CH_2O), J 7Hz).

3,7-Dimethyl-2,6-octadienal (citral) (6)

2.59 g of NaH (64.8 mmoles, 60% dispersion in oil) were washed three times with dry petroleum ether and suspended in 60 ml of THF. The suspension was cooled to 0°C and 5.73 g (32.4 mmoles) of diethylcyanomethylphosphonate ^{14}C (3) in 60 ml of THF were added dropwise. After stirring at room temperature for 1 h, the clear solution was again cooled to 0°C and 4.08 g (32.4 mmoles) of 6-methyl-5-heptene-2-one (4) in 6 ml of THF were added. Stirring at room temperature for 1 h led to complete conversion of the starting ketone. Work up was accomplished by adding water, extraction with petroleum ether three times. The combined organic layers were washed with saturated brine, after which it was dried over anhydrous magnesium sulphate. Removal of the petroleum ether under reduced pressure afforded the unsaturated nitrile (5). This nitrile 4.01 g (27.5 mmoles) was dissolved in 300 ml of dry petroleum ether and the solution was cooled to -60°C. Using a syringe, 70 ml of 1 M Dibal-H solution in hexane were added. The mixture was allowed to warm slowly to 10°C over 1.5 h with stirring. Hydrolysis was realised by adding a suspension of 90 g of silica gel and 14.4 ml of water at -30°C. After stirring for 1 h at 0°C, $MgSO_4$ and K_2CO_3 were added and stirring was continued for another 5 min. The filtrate was evaporated and the crude citral (6) was purified using a column of silica gel, elution with (20 % ether/petroleum ether), yield 4.19 g (27.5 mmoles) as a 2E/Z mixture (this is 85 % based on (3)). The pure all-E isomer was obtained from silicagel column chromatography in 75% yield from (3).

^{1}H NMR (200 MHz, $CDCl_3$) : trans citral, δ 9.95 ppm (d, 1H, (7-CH), J 9Hz); 5.84 ppm (d, 1H, (6-CH), J. 9Hz); 5.04 ppm (t, 1H, (2-CH)); 2.19 ppm (m, 4H, (3-CH_2, 4-CH_2)); 2.14 ppm (s, 3H, (19-CH_3)); 1.65 ppm (s, 3H, (17-CH_3)); 1.56 ppm (s, 3H, (16-CH_3)).

^{1}H NMR (200 MHz, $CDCl_3$) : 5 cis citral, δ 9.86 ppm (d, 1H, (7-CH), J 8Hz); 5.85 ppm (d, 1H, (6-CH), J 8Hz)); 5.07 ppm (dt, 1H, (2-CH)); 2.55 ppm (t, 2H, (4-CH_2)); 2.20 ppm (q, 2H, (3-CH_2)); 1.95 ppm (s, 3H, (19-CH_3)); 1.65 ppm (s, 3H, (17-CH_3)); 1.65 ppm (s, 3H, (17-CH_3)); 1.56 ppm (s, 3H, (16-CH_3)).

4-(2,6,6-Trimethyl-1- cyclohexen-1-yl)-3-buten-2-one (β ionone) (8) starting from citral (6)

To a solution of citral (6) (3.70 g, 24.3 mmoles) in 100 ml of acetone, 25 ml of NaOH (2N) were added. After stirring at room temperature for 2.5 h the reaction media was treated as follow: 110 ml of petroleum ether and 170 ml of saturated NH_4Cl solution were successively added, the organic layer was separated and the water layer extracted 3 times with petroleum ether. The combined organic layers were dried ($MgSO_4$). After evaporation pseudo-ionone (7) was dissolved in 50 ml of nitromethane and 50 ml of concentrated sulphuric acid were added dropwise at such a rate that the temperature of the reaction mixture remain below 10°C. The colour of the reaction turned reddish-brow. Stirring was continued for 15 min at 0°C. After this reaction time, 500 ml of ice-water were added and the mixture was neutralised with a stoeichiometric amount of NaOH. The resulting mixture was extracted 4 times with ether. The combined organic layers were washed with saturated brine, after which it was dried over anhydrous magnesium sulphate and subsequently filtered. Removal of the ether by evaporation under reduced pressure, followed by purification through a column of silicagel (elution with 10 % ether/petroleum ether) yielded to 3.15 g (16.4 mmoles ; 67.5 %) of Beta-ionone (8) as a light-yellow oil.

S A = 31.28 Mbq/mmole, 0.85 mCi/mmole

^{1}H NMR (200 MHz, $CDCl_3$) : δ 7.27 ppm (d, 1H, (7-CH), J 16.4Hz); 6.11 ppm (d, 1H, (8-CH), J 16.4Hz); 6.11 ppm (d, 1H, (8-CH), J 16.4Hz); 2.29 ppm (s, 3H, (19-CH_3)); 2.07 ppm (t, 2H,(4-CH_2), J 6Hz); 1.76 ppm (s, 3H, (18-CH_3)); 1.63 ppm (m, 2H, (3-CH_2)); 1.48 ppm (m, 2H, (2-CH_2)); 1.07 ppm (s, 6H, (16,17-CH_3)).

2-methyl-2-[2-(2,6,6-trimethyl-1 cyclohexen-1-yl) ethenyl] oxirane (9)

A mixture of (1.65 g, 41.4 mmoles) sodium hydride (60 % dispersion in mineral oil, which was removed by washing with hexane prior to the addition of DMSO) and 17 ml of anhydrous dimethyl sulfoxide (DMSO) were heated, protected from atmospheric moisture, at a bath temperature of 65°C for approximately 45 min until evolution of hydrogen had ceased. After cooling this mixture to room temperature,the above dimsyl sodium solution was added dropwise over a period of 10 minutes to a stirred slurry of (8.57 g, 42 mmoles) of trimethylsulfonium iodide in 35 ml of 1:1 (v/v) anhydrous DMSO: tetrahydrofuran, protected from atmospheric moisture and kept cold in an ice-brine bath at -5°C. The resulting grey suspension was stirred for an additional 5 min after which a solution of (3.15 g, 16.4 mmoles) beta-ionone (8) in 9 ml of anhydrous THF were added dropwise rapidly. This mixture was subsequently stirred at 0°C for 2 h, after which it was allowed to warm to room temperature. 4 ml of water were added to quench the reaction. The product was isolated by dilution of the mixture with 100 ml of pentane and 130 ml of 10 % aqueous sodium chloride. Separation of the layers was followed by washing the organic layer with 10 % aqueous sodium chloride (2 x 130 ml). The organic extracts were then dried over anhydrous magnesium sulphate and subsequently filtered. Removal of the pentane and tetrahydrofuran by evaporation at reduced pressure afforded 3.38 g (16.4 mmoles) 100 % yield of the desired epoxide (9).

S A = 31.28 Mbq/mmole, 0.85 mCi/mmole

^{1}H NMR (200 MHz, $CDCl_3$) : δ 6.15 ppm (d, 1H, (7-CH)), J 16.2 Hz); 5.20 ppm (d, 1H, (8-CH), J 16.2Hz); 2.76 ppm (dd, 2H, (10-CH), J 5 Hz); 1.94 ppm (m, 2H, (4-CH_2)); 1.62 ppm (s, 3H, (18-CH_3)); 1.55 ppm (m, 2H, (3-CH_2)); 1.47 ppm (s, 3H, (18-CH_3)); 1.42 ppm (m, 2H, (2-CH_2)); 0.95 ppm (s, 6H, (16,17-CH_3)).

2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3 butenal (10)

A solution of 3.38 g, (16.4 mmoles) of the epoxide (9) in 12 ml of anhydrous ether was added dropwise over 5 min to a stirred suspension of magnesium bromide [prepared in situ from (732 mg, 3.87 mmoles) of 1,2-dibromoethane and 99 mg of magnesium turnings] in 6 ml of anhydrous ether, protected from atmospheric moisture, at -10°C. The resulting mixture was stirred at -10°C for an additional 5 min, after which it was diluted with 20 ml of ether. The organic layer was washed in successive order with 22 ml portions of water and saturated brine, after which it was dried over anhydrous magnesium sulphate and subsequently filtered. Removal of the ether by evaporation at reduced pressure afforded 3.04 g (14.8 mmoles, 90 % yield) of the desired aldehyde.

S A = 31.28 Mbq/mmole, 0.85 mCi/mmole

^{1}H NMR (200 MHz, $CDCl_3$) : δ 9.60 ppm (d, 1H, (10-CH), J 1.8Hz); 6.01 ppm (d, 1H, (7-CH), J 16Hz); 5.28 ppm (dd, 1H, (8-CH), J 16Hz); 1.95 ppm (t, 2H, (4-CH_2)); 1.64 ppm (m, 4 H, (9-CH, 5-CH_3)); 1.56 ppm (m, 2H, (3-CH_2)); 1.43 ppm (m, 2H, (2-CH_2)); 1.21 ppm (d, 3H, (18-CH_3); J 7Hz); 0.96 ppm (s, 6H, (16,17-CH_3)).

3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-pentadienyl phosphonic acid, diethyl ester (12)

A solution of 6.53 g (22.64 mmoles) methylen bisphosphonic acid tetraethyl ester (11) in 32 ml of benzene and 19 ml of anhydrous tetrahydrofuran was added dropwise over 5 min to a stirred mixture of 888 mg (21.9 mmoles) of sodium hydride (60 % dispersion in mineral oil, which was removed prior to the addition by washing with hexane) and 13 ml of benzene, protected from atmospheric moisture and maintained at a temperature of 15-20°C by use of an external cold water bath. This mixture was stirred for an additional 15 min, after which a solution 3.04 g (14.8 mmoles) of aldehyde (10) in 32 ml of benzene was added dropwise rapidly. This reaction media was stirred at room temperature for 25 min, and then diluted by 180 ml solution of 1 : 1 (v/v) pentane : ether then the organic media was successively washed by 2 x 360 ml solution of 1M aqueous sodium hydroxide : methyl alcohol 7 : 3 (v/v) and 360 ml of saturated brine. The organic layer was then dried over anhydrous magnesium sulphate and subsequently filtered. Removal of the pentane, ether, and benzene by evaporation at reduced pressure afforded 4.68 g (13.7 mmoles, 93 % yield) of desired vinyl phosphonate (12).

3-methyl-5-(2,6,6, trimethyl-1-cyclohexen-1-yl-2,4-pentadienylphosphonic Acid, Diethyl Ester (13)

4.68g (13.7 mmoles) of Vinyl phosphonate (12) and 447 mg (3.96 mmoles) of potassium tert-butoxide in 54 ml of anhydrous dimethyl sulfoxide (DMSO) were stirred, protected from atmospheric moisture, at 20°C for 80 min. The product was isolated by dilution of the reaction mixture with 320 ml of ether and subsequent washing with 380 ml of 10 % aqueous sodium chloride (4 x 380 ml). The organic layer was then dried over anhydrous magnesium sulphate and filtered. Removal of the ether by evaporation at reduced pressure afforded 3.54 g (10.4 mmoles, 76 % yield) of the desired allylic phosphonate (13), whose structural integrity was confirmed by NMR analysis [δ 2.75 ppm (dd, 2H, (11-CH_2), J_{H-H} 8Hz, J_{P-H} 22.9Hz)]

All trans Beta-carotene (19)

To a solution of 1.0 g (2.94 mmoles) of 3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadienylphosphonic acid, diethyl ester (13) and 213 mg (1.3 mmoles) of 2,7-dimethyl-2,4,6-octatrienedial (18) in 13 ml of 8 : 1 (v/v) anhydrous tetrahydrofuran : dimethyl sulfoxide (protected from atmospheric moisture and maintained at 5°) was added 307 mg (2.7 mmoles) of potassium tert-butoxide. This mixture was subsequently stirred in the cold for 15 min and then at room temperature for 3.5 h. The product was isolated by dilution of the mixture with 130 ml of 4 : 1 (v/v) ether : dichloromethane and subsequent washing of the organic layer with 130 ml portions of 10 % aqueous sodium chloride (3 x130 ml). The organic layer was then dried over anhydrous magnesium sulphate and filtered. Removal of the volatile organic solvents by evaporation at reduced pressure, followed by filtration through a small column of silica gel, (elution with 3 : 1 (v/v) hexane : benzene to remove unreacted starting materials) afforded 426 mg (1.72 mmoles, 61 % yield) of deep-purple crystals (19).

mp 183-185°C

S A = 62.56 Mbq/mmole 1.7 mCi/mmole

^{1}H NMR (400 MHz, $CDCl_3$) : δ 6.64 ppm (dd, 2H,(11-CH, 11'-CH), J 14.9 Hz, J 11Hz); 6.62 ppm (dd, 2H, (15-CH, 15'-CH), J 11.7Hz, J 14.2Hz); 6.34 ppm (d, 2H, (12-CH, 12'-CH), J 14.9Hz); 6.24 ppm (d, 2H, (14-CH, 14'-CH), J 11.7Hz); 6.17 ppm (d, 2H, (7-CH, 7'-CH), J 15.9Hz); 6.14 ppm (d, 2H, (10-CH, 10'-CH), J 11Hz); 6.12 ppm (d, 2H, (8-CH, 8'-CH), J 15.9Hz); 2.02 ppm (m, 4 H, (4-CH_2, 4'-CH_2)); 1.97 ppm (s, 12 H, (9-CH_3, 9'-CH_3, 13-CH_3, 13'-CH_3)); 1.71 ppm (s, 6H, (5-CH_3, 5'-CH_3)); 1.62 ppm (m, 4H, (3-CH_2, 3'-CH_2)); 1.46 ppm (m, 4 H, (2-CH_2, 2'-CH_2)); 1.02 ppm (s,12 H, (1-CH_3, 1'-CH_3)).

All-trans Retinoic Acid, Ethyl Ester (15)

To a solution 431 mg (3.03 mmoles) of ethyl 3-methyl-4 oxobutenoate (**14**) and 966 mg (2.84 mmoles) of 3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadienylphosphonic acid diethylester (**13**) in 26 ml of 6 : 1 (v/v) anhydrous tetrahydrofuran : dimethylsulfoxide, (protected from atmospheric moisture and maintained at 5°C), were added 346 mg (2.87 mmoles) of potassium ter-butoxide. This mixture was successivly stirred in the cold for 10 min and then at room temperature for 6 h. The product was isolated by dilution of the mixture with 220 ml of 1 : 1 (v/v) pentane : ether and subsequent washing the organic layer with 3x200 ml portions of 10 % aqueous sodium chloride The organic layer was then dried over anhydrous magnesium sulphate and filtered. Removal of the volatile organic solvents by evaporation at reduced pressure, followed by filtration through a small column of silicagel,(elution with 96 : 4 (v/v) hexane, ether, to remove any unreacted starting materials) afforded 569 mg (1.73mmoles, 61 % yield) of ethyl retinoate (**15**).

S A = 31.28 Mbq/mmole 0.85 mCi/mmole

^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.03 ppm (dd, 1H, (11-CH), J 15Hz, J 10Hz); 6.32 ppm (d, 1H, (12-CH), J 15.2Hz); 6.30 ppm (d, 1H, (7-CH), J 16Hz); 6.16 ppm (d, 1H, (10-CH), J 11.6Hz); 6.15 ppm (d, 1H, (8-CH), J 16Hz); 5.80 ppm (s, 1H, (14-CH)); 4.20 ppm (q, 2H, (CH_2-O)); 2.37 ppm (s, 3H, (13-CH_3)); 2.04 ppm (s, 2H, (4-CH_2)); 2.02 ppm (s, 3H, (9-CH_3)); 1.72 ppm (s, 3H, (5-CH_3)); 1.61 ppm (m, 2H, (3-CH_2)); 1.48 ppm (m, 2H, (2-CH_2)); 1.28 ppm (t, 3H, (CH_3-CH_2-O)); 1.03 ppm (s, 6H, (1-CH_3)).

Trans [7-^{14}C]-retinol (16)

222 mg (0.676 mmole) of Methyl trans-(7-^{14}C)-retinoate (**15**) were dissolved in 8 ml of diethyl ether in a 40 ml flask containing a magnetic stirrer bead and set up for work under Ar atmosphere. The flask was shielded from light in tin foil and cooled in a dry ice-acetone bath at -60°C. The solution of LAH in ether (0.676 ml, 0.676 mmoles) was added with stirring, keeping the temperature below - 30°C. After 20 min, the solution was cooled again to -60°C and 4ml of Analar methanol were added dropwise. The resulting solution was partitioned between 40-60°C petroleum ether (66 ml) and 1N H_2SO_4 (16 ml), precooled to 0°C. The organic phase was speedily washed with ice water (2 x 16 ml), dried ($MgSO_4$) and evaporated to yield 193 mg (0.67 mmoles, 95 %) of trans-[7-^{14}C]-retinol (**16**).

S A = 31.28 Mbq/mmole 0,85 mCi/mmole

^{1}H NMR (200 MHz, $CDCl_3$) : δ 6.59ppm (dd, 1H, (11-CH)); 6.32ppm (d, 1H, (12-CH)); 6.30ppm (d, 1H, (7-CH)); 6.16ppm (d, 1H, (10-CH)); 6.14ppm (d, 1H, (8-CH)); 5.70ppm (t, 1H, (14-CH)); 4.28ppm (d, 2H, (15-CH_2), J 7Hz); 2.37ppm (s, 3H, (13-CH_3)); 2.04ppm (s, 2H, (4-CH_2)); 2.02ppm (s, 3H, (9-CH_3)); 1.72ppm (s, 3H, (5-CH_3)); 1.61ppm (m, 2H, (3-CH_2)); 1.48ppm (m, 2H, (2-CH_2)); 1.03ppm (s, 6H, (1-CH_3)).

Trans [7-^{14}C]-retinal (17)

420 mg of manganese dioxide were added in small portions to 7 ml of Analar methanol cooled to 0°C. A solution of Trans [7-^{14}C]-retinol (16) 92mg (0.32mmole) in 1 ml of methanol was added dropwise to the vigorously-stirred reaction left in the dark for 4 h. The reaction mixture was filtered with gentle suction through a N°3 Sinter, and the MnO_2 residue washed with methanol until the washings were clear to UV. The filtrate and washings were evaporated to low bulk, centrifuged, and the supernatant evaporated to dryness on a rotary evaporator. This gave 88 mg (0.31 mmole, 97 %) of retinal (17).

S A = 31.28 Mbq/mmole 0.85 mCi/mmole

^{1}H NMR (400 MHz, $CDCl_3$) : δ 10.10 ppm (d, 1 H, (15-CH), J 8.1Hz); 7.14 ppm (dd, 1H, (11-CH), J 15.2Hz, 11.5Hz); 6.37 ppm (d, 1H, (12-CH), J 15.2Hz); 6.35 ppm (d, 1H, (7-CH), J 16Hz); 6.19 ppm (d, 1H, (CO-CH), J 11.5Hz); 6.17 ppm (d, 1H, (8-CH) , J 16Hz); 5.97 ppm (d, 1H, (14-CH), J 8.1Hz); 2.33 ppm (m, 3H, (13-CH_3)); 1.62 ppm (m, 2H, (3-CH_2)); 1.47 ppm (m, 2H, (2-CH_2)); 1.04 ppm (s. 6H. (1-CH_3)).

Trans [7-^{14}C]-retinoic Acid (20)

The all-Trans ethyl retinoate-7-^{14}C (15) 121 mg (0.37 mmole) was hydrolysed by stirring with 41 mg (0.74 mmole) of potassium hydroxide in 6 ml of ethanol and 1 ml of water for 3 h at 60°C under an Ar atmosphere. The mixture was allowed to cool, diluted with water, and extracted three times with ether. After back extracting the organic extracts with 1 N sodium hydroxide, all aqueous layers were combined, acidified with concentrated sulphuric acid, and extracted repeatedly with methylene chloride, The extracts were dried over anhydrous magnesium sulphate and evaporated to dryness. The resulting yellow solid was recrystallised from ethanol, yielding a total of 100 mg (0.33 mmole) of retinoic-7-^{14}C-acid (20) (90 % overall yield) as yellow needles.

mp 180-181°

S A = 31.28 Mbq/mmole 0.85 mCi/mmole

^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.05 ppm (dd, 1H, (11-CH), J 10.4Hz, J 15Hz); 6.32 ppm (d, 1H, (12-CH), J 15.2Hz); 6.30 ppm (d, 1H, (7-CH), J 16Hz); 6.16 ppm (d, 1H, (10-CH), J 11.6Hz); 6.15 ppm (d, 1H, (8-CH), J 16Hz); 5.81 ppm (s, 1H, (14-CH)); 2.37 ppm (s, 3H, (13-CH_3)); 2.04 ppm (s, 2H, (4-CH_2)); 2.02 ppm (s, 3H, (9-CH_3)); 1.72 ppm (s, 3H, (5-CH_3)); 1.61 ppm (m, 2H, (3-CH_2)); 1.48 ppm (m, 2H, (2-CH_2); 1.03 ppm (s, 6H, (1-CH_3)).

2-Butenyl-1,4-bis phosphonic Acid, Tetraethyl Ester (21)

A solution of 2 ml (18.9 mmoles) of trans 1,4-dichloro-2-butene in 3 ml (17.5 mmoles) of triethyl phosphite was added dropwise over 25 min to a flask containing 5 ml (29.2 mmoles) of triethyl phosphite,maintained at a temperature of approximately 140°C. This mixture was heated at 140°C for 12 h, during this time ethyl chloride was continuously distilled out of the reaction flask.after that, the external oil bath temperature was allowed to raise 180°C to distil most of the remaining triethyl phosphite. The desired product was then obtained by fractional distillation under reduced pressure, affording 5.4 g (87.5 % yield) of bis-phosphonate (21) : bp 161-184°C, 0.25 mm.

^{1}H NMR (200 MHz, $CDCl_3$) : δ 5.61 ppm (t, 2H, (2-CH, 2'-CH)); 4.10 ppm (q, 8H, ($C\underline{H_2}$-O), J 7Hz); 2.60 ppm (dd, 4H, (1-CH_2, 1'-CH_2), J_{P-H} 16Hz, J_{H-H} 4Hz); 1.32 ppm (t, 12 H, ($C\underline{H_3}$-CH_2), J 7Hz).

1,1,8,8-Tetramethoxy-2,7-dimethyl-2,4,6-octatriene (22)

To a solution of 312 mg (0.95 mmoles) of 2-butenyl-1,4-bis phosphonic acid tetraethyl ester (**21**), and 0.25 ml (2.07 mmoles) of pyruvic aldehyde dimethylacetal in 3.25 ml of 12 : 1 (v/v) anhydrous tetrahydrofuran : Dimethylsulfoxide (protected from atmospheric moisture and maintained at a temperature of 5°C) was added 211 mg (1.88 mmoles) of potassium tert-butoxide. This mixture was successivly stirred in the cold for 15 min and then at room temperature for 7 h. The product was isolated by dilution of the mixture with 30 ml of 1 : 1 (v/v) ether : pentane and washing the organic layer with 10% aqueous sodium chloride (3 x 30 ml). The organic layer was then dried over anhydrous magnesium sulphate and filtered. Removal of the volatile organic solvents by evaporation at reduced pressure afforded 151 mg (0.59 mmole, 62 % yield) of bisacetal (**22**).

^{1}H NMR (200 MHz, $CDCl_3$) : δ 6.55 ppm (dd, 2H, (4-CH, 4'-CH), J 7.6Hz, J 3.2Hz); 6.30 ppm (d, 2H, (3-CH, 3'-CH), J 6.4Hz); 4.60 ppm (s, 2H, (1-CH, 1'-CH)); 3.33 ppm (s, 12H, ($C\underline{H_3}$O)); 1.78 ppm (s, 6H, (2-CH_3, 2'-CH_3)).

2,7-Dimethyl-2,4,6-octatrienedial (18)

A solution of 150 mg (0.585 mmole) of 1,1,8,8-tetramethoxy-2,7 dimethyl-2,4,6-octatriene (**22**), in 35 ml of 4 : 2 : 1 (v/v/v) (glacial acetic acid : THF : water) was heated at 45°C for 3 hours. The product was isolated by dilution of the mixture with (2 x 25 ml) of 4 : 1 (v/v) (ether : dichloromethane) and the organic layer was washed in successive order with (2 x 25 ml) of 1:1 (v/v) (saturated brine : 1 M aqueous sodium hydroxide) and 25ml of saturated brine. The organic layer was then dried over anhydrous magnesium sulphate and filtered. Removal of the volatile organic solvents by evaporation at reduced pressure afforded 86 mg (0.52 mmole, 90 % yield) of the desired bis dialdehyde (**18**).

^{1}H NMR (200 MHz, $CDCl_3$) : δ 9.48 ppm (s, 2H, (1CHO, 1'CHO)); 6.97 ppm (m, 4H, (3-CH, 3'-CH, 4-CH, 4'-CH)); 1.68 ppm (s, 6H, (2-CH_3, 2'-CH_3)).

CONCLUSION

This synthetic scheme which led to synthesis of Retinoic acid in 14%, Retinol, Retinal and beta-carotene in 16% yield calculated from sodium cyanide, can also be used for the labelling of the cycle in position **6**.

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