SYNTHESIS OF 9-*CIS*-RETINOIC ACID AND C-20-[^3H_3C]-9-*CIS*-RETINOIC ACID WITH HIGH SPECIFIC ACTIVITY

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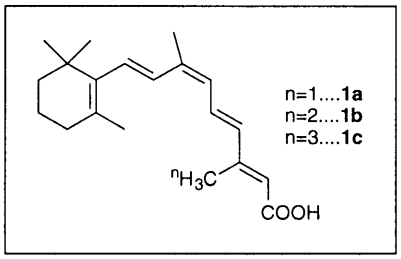
SUMMARY

The synthesis of 9-*cis*-retinoic acid starting from 2,2,6-trimethylcyclohexanone is described. The same methodology was extended for the synthesis of deuterium and tritium labeled 9-*cis*-retinoic acid with high specific activity (73 Ci/mmol). In this methodology, a Grignard reaction was utilized for introducing three tritium atoms simultaneously in the final synthetic steps.

Key words: 9-*cis*-retinoic acid, deuterium, tritium, high specific activity

INTRODUCTION

Following the discovery that 9-*cis*-retinoic acid (9-*cis*-RA) is a ligand for RXR intracellular receptors, there has been a need for high specific activity radiolabeled 9-*cis*-RA. This compound is required to study receptor binding properties, receptor interactions and transcriptional activity. Dawson *et al.*[1] prepared radiolabeled 9-*cis*-RA using photochemical isomerization of commercially available [11,12-$^3H_2$]-all-*trans*-RA. Recently, Boehm *et al.*[2] synthesized [$^3H_2$]-9-*cis*-RA of 29 Ci/mmol specific activity starting from ionylidene acetaldehyde. While our work was in progress, Bennani and Boehm[3] reported an elegant synthesis of tritiated 9-*cis*-RA. In this method, tritium was incorporated at



9-*cis*-RA 1

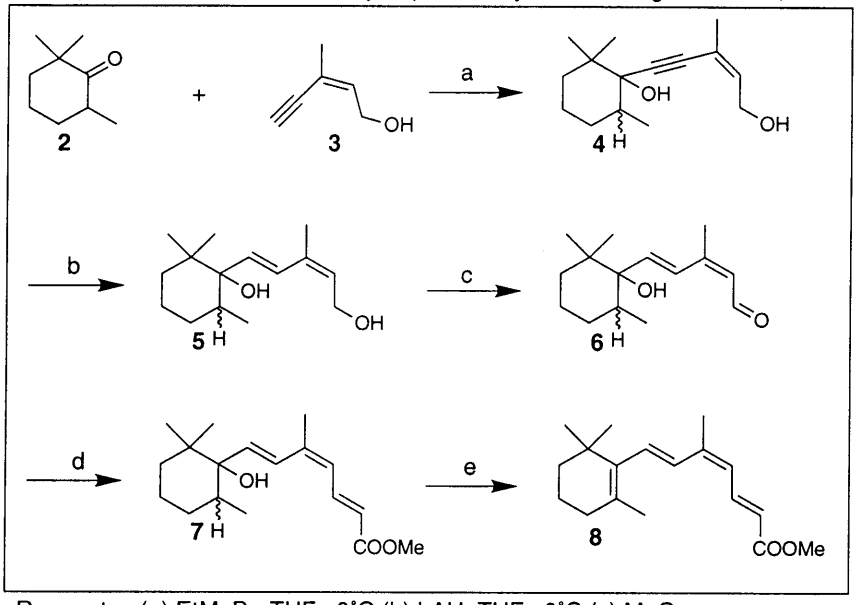
CCC 0362–4803/97/010001–10

Received 2 January 1996
Revised 18 July 1996

either the 2,3- or the 3,4-positions of the ring by taking advantage of regioselective hydrogenation of the endocyclic double bond. For our biological studies we required high specific activity 9-*cis*-RA which prompted us to develop a methodology for the synthesis of 9-*cis*-RA that can be used to prepare the radiolabeled 9-*cis*-RA of high specific activity. Herein we report a methodology for the synthesis of 9-*cis*-RA and demonstrate its use in the preparation of C-20-deuterium or tritium-labeled 9-*cis*-RA with a specific activity of 73 Ci/mmol.

DISCUSSION

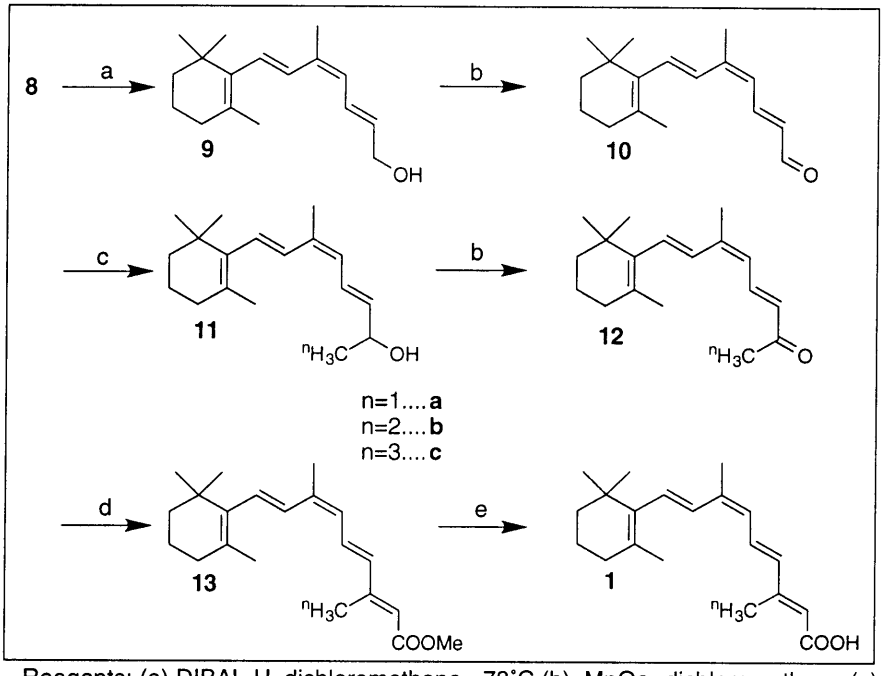
Our synthetic strategy is based on the simultaneous incorporation of three tritium atoms through a methyl Grignard reaction with an appropriate aldehyde to obtain high specific activity. The *cis*-configuration at carbon-9 in the parent RA was fixed using commercially available 95% *cis*-3-methyl-2-penten-4-yn-1-ol **3** reagent. Thus, the bromo-



Reagents: (a) EtMgBr, THF, 0°C (b) LAH, THF, 0°C (c) MnO_2, dichloromethane (d) Ph_3P=CHCOOMe, benzene (e) HCOOH, hexane.

magnesium acetylide of *cis*-3-methyl-2-penten-4-yn-1-ol **3** was added to 2,2,6-trimethylcyclohexanone **2** to obtain diastereomeric diols **4** in 80% yield.[4] The diols **4** were immediately reduced with LAH in THF to yield dienols **5** (68%). The primary hydroxyl group in **5** was oxidized to an aldehyde **6** using MnO_2 in dry dichloromethane and the side chain was extended to an ester **7** by Wittig olefination keeping the tertiary hydroxyl group intact. Subsequently dehydration of the tertiary hydroxyl group was effected smoothly by the treatment with 80% formic acid in hexane to obtain an ester **8** in 69% yield.

The ester **8** was reduced to its alcohol **9** by using two equivalents of DIBAL-H in dry dichloromethane at -78°C, followed by oxidation to an aldehyde **10** with MnO_2. The Grignard reagent of methyl bromide was added to the aldehyde **10** to afford a secondary alcohol **11a** in 55% yield, which was further oxidized to the corresponding ketone **12a.** The ketone **12a** was condensed with methyl diethylphosphonoacetate (NaH, THF) to obtain methyl 9-*cis*-retinoate in 52% yield.(5) Finally, the ester hydrolysis with aq. KOH in methanol furnished a mixture of RA isomers in 80% yield. Reverse phase HPLC analysis



Reagents: (a) DIBAL-H, dichloromethane, -78°C (b) MnO_2, dichloromethane (c) $^nH_3CMgX$,THF, 0°C (d) $(EtO)_2P(O)CH_2COOMe$, NaH, THF (e) aq. KOH, methanol, 50°C.

of the mixture showed three peaks in a 5:11:1 ratio that were identified as 13-*cis*-, 9-*cis*- and all-*trans*-RAs by coeluting them with the authentic samples. The major 9-*cis*-isomer **1a** was selectively crystallized from methanol and its melting point correlated with previously reported data.(6)

The deuterium- and tritium-labeled-RAs were synthesized in an analogous manner using either D_3CMgI or $^3H_3CMgI$ in the Grignard reaction to obtain either deuterated **11b** or tritiated secondary alcohol **11c**. The alcohols were then transformed into deuterium- or tritium-labeled RAs by the same sequence of reactions used for the synthesis of 9-*cis*-RA,

incorporating three deuterium or tritium atoms/mole. Radiolabeled 9-*cis*-RA **1c** was directly purified on ODS HPLC using a solvent system of methanol/isopropanol/water/acetonitrile/acetic acid (25/15/30/30/1.2).

In conclusion, a method for the synthesis of 9-*cis*-RA was described. The method was further extended for the preparation of C-20-deuterium or tritium-labeled 9-*cis*-RA by selecting an appropriate Grignard reagent. In the case of C-20-labeled 9-*cis*-RA, a specific activity of 73 Ci/mmol was achieved.

EXPERIMENTAL

General: All solvents and reagents were used as supplied by the manufacturer. Dry solvents were purchased from the Aldrich. ^{1}H NMR spectra were recorded on $CDCl_3$ or CD_3OD solutions using 400 or 500 MHz Bruker DMX spectrometer. Electron impact mass spectra were obtained with a Kratos MS-50 TC instrument equipped with Kratos DS-55 data system. UV spectra were recorded with Perkin-Elmer lambda 3B UV/Vis spectrophotometer.

2(Z)-3-Methyl-5-(1-hydroxy-2,2,6-trimethylcyclohexyl)-pent-2-en-4-yn-1-ol (4): To a stirred solution of 1.12 mL (1.5 equiv) of 95% *cis*-3-methyl-2-penten-4-yn-1-ol **3** reagent in 10 mL THF at 0°C, 7 mL of 3M solution of ethylmagnesiumbromide in diethylether (3 equiv) was added. The solution was stirred for 30 min at room temperature and then a solution of 1 g (1 equiv) of 2,2,6-trimethylcyclohexanone **2** in 5 mL THF was added dropwise. The reaction mixture was stirred overnight and then quenched with saturated solution of NH_4Cl. Crude product was obtained after the usual workup, and impurities from the crude product were removed by passing over silica gel column using hexane and ethyl acetate solvents to yield 1.35 g (80%) of a mixture of diastereomeric diols. ^{1}H NMR ($CDCl_3$) δ: 0.95 (3H, s, CH_3), 0.98 and 1.02 (3H, d, CH_3) (two doublets are for C-6 methyl group of different diastereomeric alcohols), 1.05 (3H, s, CH_3), 1.2-1.65 (6H, m, CH_2), 1.85 (3H, s, CH_3), 4.23 (2H, d, CH_2OH), 5.8 (1H, t, CH). Mass (m/z): 236 (8) (M^+), 218 (28), 203 (36), 147 (58), and 106 (100). UV (ethanol) λ_{max}: 228 nm.

(2Z,4E)-3-Methyl-5-(1-hydroxy-2,2,6-trimethylcyclohexyl)-pent-2,4-dien-1-ol (5): To a stirred mixture of LAH (0.218 g) in THF (10 mL) a solution of ynol **4** (1.35 g) in THF (5 mL) was added dropwise through a syringe. The reaction mixture was stirred overnight at room temperature. The flask was cooled to 0°C, and the excess LAH was quenched with a

saturated solution of Na_2SO_4 and filtered through a celite pad. The residue was washed with dichloromethane several times and solvent was concentrated under reduced pressure. The mixture of dienols **5** was purified from other impurities over a silica gel column using hexane and ethyl acetate solvents and 0.935 g (68%) of product was obtained. ^1H NMR ($CDCl_3$) δ: 0.72 (3H, d, CH_3), 0.81 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.15-1.60 (6H, m, CH_2), 1.85 (3H, s, CH_3), 4.28 (2H, d, CH_2OH), 5.5 (1H, t, CH), 5.69 and 6.0 (1H, d, CH) (olefinic protons for two diastereomeric alcohols), 6.55 and 6.62 (1H, d, CH) (olefinic protons for two diastereomeric alcohols). Mass (m/z) : 238 (8) (M^+), 220 (63), 205 (25), 149 (95) and 69 (100). UV (ethanol) λ_{max}: 240 nm.

(2Z,4E)-3-Methyl-5-(1-hydroxy-2,2,6-trimethylcyclohexyl)-pent-2,4-dien-1-al (6): To the mixture of dienol **5** (0.935 g) in 5 mL of dichloromethane 0.575 g of MnO_2 was added. The reaction mixture was vigorously stirred at room temperature until the reaction was complete as determined by TLC. After completion of the reaction, the product was filtered over a celite pad and the pad was washed with dichloromethane. The solvent was removed under the vacuum and the product was quickly purified over silica gel to obtain 0.740 g (79%) of product and used immediately for the next reaction.

Methyl-(2E,4Z,6E)-5-methyl-7-(1-hydroxy-2,2,6-trimethylcyclohexyl)-hepta-2,4,6-trienoate (7): 2.4 g of methyl (triphenylphosphoranylidene)acetate was added to the solution of 0.70 g of aldehyde **6** in 10mL of benzene. Stirring was continued until the reaction was completed. The solvent was removed under vacuum and the crude product was chromatographed to yield 0.65 g of hydroxy ester (75%). ^1H NMR ($CDCl_3$) δ :0.75 (3H, d, CH_3), 0.82 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.21-1.64 (6H, m, CH_2), 1.92 (1H, m, tert.CH), 2.00 (3H, s, CH_3), 3.75 (3H, s, COOMe), 5.82 (1H, d, CH), 6.07 (1H, d, CH), 6.21 (1H, d, CH), 6.91 (1H, d, CH), 7.89 (1H, dd, CH). Mass (m/z): 292 (43) (M^+), 277 (8), 260 (28), 245 (8), 175 (35), 149 (57), and 69 (100). UV (ethanol) λ_{max}: 306 nm.

Methyl-(2E,4Z,6E)-5-methyl-7-(2,2,6-trimethylcyclohex-1-enyl)-hepta-2,4,6-trienoate (8): To a solution of 0.650 g of hydroxy ester in hexane 0.4 mL of 80% formic acid was added. The mixture was vigorously stirred at room temperature over night. Isolation of the product in the usual manner gave a dehydration product (0.39 g 69% yield). ^1H NMR ($CDCl_3$) δ: 1.02 (6H, s, $2CH_3$), 1.48 (2H, m, CH_2), 1.63 (2H, m, CH_2), 1.73 (3H, s, CH_3), 2.03 (3H, s, CH_3), 2.05 (2H, m, CH_2), 3.75 (3H, s, COOMe), 5.85 (1H, d, CH), 6.06 (1H, d, CH), 6.36 (1H, d, CH), 6.70 (1H, d, CH), 7.79 (1H, dd, CH). Mass (m/z): 274 (57) (M^+), 259 (22), 227 (15), 159 (60), and 123 (100). UV (ethanol) λ_{max}: 320 nm

(2E,4Z,6E)-5-Methyl-7-(2,2,6-trimethylcyclohex-1-enyl)-hepta-2,4,6-triene-1-ol (9): To 0.350 g of ester **8** in 3 mL of dichloromethane at -78°C, 1.88 mL (1.5 molar solution) of DIBAL-H was added. The reaction mixture was stirred at -78°C till the completion of the reaction (monitored by TLC). After completion of the reaction, the contents were poured into a pre-cooled solution of potassium sodium tartrate and extracted repeatedly with dichloromethane. The dichloromethane extract was washed with brine solution and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and 0.200 g (63%) of pure compound was obtained after chromatographic purification. ^1H NMR ($CDCl_3$) δ: 1.00 (3H, s, CH_3), 1.13 (3H, s, CH_3), 1.50 (2H, m, CH_2), 1.58 (3H, s, CH_3), 1.65 (2H, m, CH_2), 1.72 (3H, s, CH_3), 2.05 (2H, m, CH_2), 4.24 (2H, bs, CH_2OH), 5.82 (1H, m, CH), 5.95 (1H, d, CH), 6.17 (1H, d, CH), 6.58 (1H, d, CH), 6.81 (1H, dd, CH). Mass (m/z) : 246 (100) (M^+), 228 (38), 213 (28), 159 (85), and 119 (80). UV (ethanol) λ_{max}: 287 nm.

(2E,4Z,6E)-5-Methyl-7-(2,2,6-trimethylcyclohex-1-enyl)-hepta-2,4,6-triene-1-al (10): To 0.150 g of alcohol in 3 mL of dichloromethane, 0.058 g of MnO_2 was added . The reaction mixture was vigorously stirred at room temperature until the reaction was complete as determined by TLC. After completion of the reaction, the product was filtered through a celite pad and the celite pad was washed with dichloromethane. The solvent was removed under reduced pressure and 0.110 g of pure compound was obtained after chromatographic purification, giving a 74% yield. ^1H NMR ($CDCl_3$) δ: 1.00 (6H, s, 2 CH_3), 1.48 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.70 (3H, s, CH_3), 2.03 (2H, m, CH_2), 2.1 (3H, s, CH_3), 6.2 (2H, m, 2 CH), 6.25 (1H, d, CH), 6.5 (1H, d, CH), 7.51 (1H, dd, CH), 9.6 (1H, d, CHO). Mass (m/z): 244 (100) (M^+), 229 (32), 159 (59), 145 (72), and 119 (65). UV (Ethanol) λ_{max}: 336 nm.

(2E,4Z,6E)-1,5-Dimethyl-7-(2,2,6-trimethylcyclohex-1-enyl)-hepta-2,4,6-triene-1-ol (11a): To a stirred solution of 0.100 g aldehyde in 2 mL THF at 0°C, 0.05 mL of MeMgBr was added. The solution was stirred for 30 min at 0°C. The completed reaction was quenched with saturated solution of NH_4Cl and extracted with ether. The ether extract was washed with water, brine solution and dried over anhydrous $NaSO_4$. The solvent was removed under vacuum and after chromatographic purification, the compound was obtained at a 55% yield. ^1H NMR ($CDCl_3$) δ: 1.00 (6H, s, 2CH_3), 1.30 (3H, d, CH_3),1.45 (2H, m, CH_2), 1.56 (2H, m, CH_2), 1.73 (3H, s, CH_3), 1.94 (3H, s, CH_2), 2.01 (2H, m, CH_2),

4.41 (1H, d, CH_2OH), 5.66 (1H, dd, CH), 5.92 (1H, d, CH), 6.14 (1H, d, CH), 6.56 (1H, d, CH), 6.66 (1H, dd, CH). Mass (m/z) : 260 (20) (M^+), 242 (100), 227 (25), 157 (55), and 119 (75). UV (ethanol) λ_{max}: 282 nm.

(2E,4Z,6E)-1,5-Dimethyl-7-(2,2,6-trimethylcyclohex-1-enyl)-hepta-2,4,6-triene-1-one (12a): To 0.050 g of alcohol in 2 mL of dichloromethane, 0.018 g of MnO_2 was added. The reaction mixture was vigorously stirred at room temperature until the reaction was complete as determined by TLC. After completion of the reaction, the product was filtered through a celite pad and the celite pad was washed with dichloromethane. The solvent was removed under vacuum, and 0.039 g (79%) of pure compound was obtained following chromatographic purification. ^1H NMR ($CDCl_3$) δ: 1.05 (6H, s, $2CH_3$), 1.50 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.75 (3H, s, CH_3), 2.00 (3H, s, CH_3), 2.05 (2H, m, CH_2), 2.25 (3H, s, CH_3), 6.05 (2H, two doublets merged, CH), 6.35 (1H, d, CH), 6.70 (1H, d, CH), 7.65 (1H, dd, CH). Mass (m/z): 258 (30) (M^+), 243 (10), 149 (40), 129 (30), and 119 (32). UV (ethanol) λ_{max}: 332 nm.

Methyl-(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,2,6-trimethylcyclohex-1-enyl)-nona-2,4,6,8-trienoate (13a): To a suspension of 50% oil dispersion of NaH (4 mg) in THF, 0.014 mL of methyl diethylphosphonoacetate was added. The reaction was stirred until cool and clear. Then 0.10 g of ketone in THF was added dropwise and the reaction was stirred at room temperature until completion of the reaction. Excess of NaH was quenched with water and the reaction mixture was extracted with ether. The organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was distilled off under reduced pressure and chromatographic purification yielded methyl retinoate. ^1H NMR ($CDCl_3$) δ: 1.03 (6H, s, $2CH_3$), 1.46 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.73 (3H, s, 18-CH_3), 2.00 (3H, s, 19-CH_3), 2.07 (2H, m, CH_2), 2.34 (3H, s, 20-CH_3), 3.71 (3H, s, $COOCH_3$), 5.77 (1H, s, 14-CH), 6.03 (1h, d, 10 or 12-CH), 6.21 (1H, d, 10 or 12-CH), 6.28 (1H, d, 8-CH), 6.66 (1H, d, 7-CH), 7.09 (1H, dd, 11-CH). Mass (m/z): 314 (100) (M^+), 299 (14), 255 (26), 177 (30), and 133 (32). UV (ethanol) λ_{max}: 348 nm.

(2E,4E,6Z,8E)-3,7-Dimethyl-9-(2,2,6-trimethylcyclohex-1-enyl)-nona-2,4,6,8-tetraenoic acid (9-*cis*-RA) (1a): To the ester **13a** in 5 mL of MeOH, 0.5 mL of 5N KOH was added and the reaction mixture was heated to 60°C for 1 h. After the hydrolysis was complete (by TLC), the solution was cooled to 0°C and acidified using 1N HCl. The mixture was extracted with ether, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The

product was purified by crystallization to give pure 9-*cis*-RA. m.p. 188-190° C, lit[5] 189-190°C. ^{1}H NMR ($CDCl_3$) δ: 1.06 (6H, s, 2CH3), 1.54 (2H, m, CH2), 1.67 (2H, m, CH2), 1.77 (3H, s, 18-CH3), 2.00 (3H, s, 19-CH3), 2.09 (2H, m, CH2), 2.32 (3H, s, 20-CH3), 5.67 (1H, s, 14-CH), 6.12 (1H, d, 10-CH), 6.32 (2H, br.d, 7CH+12CH), 6.71 (1H, d, 8-CH), 7.13 (1H, dd, 11-CH). Mass (m/z): 300 (72) (M^+), 285 (12), 255 (10), 244 (10), 149 (100), and 119 (30). UV (ethanol): λ_{max}: 335 nm.

(20-CD_3)-(2E,4E,6Z,8E)-3,7-dimethyl-9-(2,2,6-trimethylcyclohex-1-enyl)-nona-2,4,6,8-tetraenoic acid ([20-CD_3]-9-*cis*-RA) (1b): (20-CD_3)-9-*cis*-RA was prepared in similar manner as 9-*cis*-RA except that CD_3MgI was used in the Grignard reaction instead of MeMgBr. ^{1}H NMR spectra of **11b,12b,13b** and **1b** are similar to that of **11a,12a,13a** and **1a** except C-20 methyl signal is not present.

Mass m/z: for **11b** 263 (50) (M^+), 245 (35), 215 (15), 187 (45), and 145 (100); **12b** 261 (12) (M^+), 246 (6), 177 (29), and 159 (18); **13b** 317 (100) (M^+), 302 (11), 286 (5), 274 (15), 258 (22), and 119 (26); **1b** 303 (57) (M^+), 288 (11), 258 (10), 123 (61), and 69 (100).

(2E,4Z,6E)-1-^3H_3C,5-Methyl-7-(2,2,6-trimethylcyclohex-1-enyl)-hepta-2,4,6-triene-1-ol (11C): 12.5 mg (0.051 mmol) of (2E,4Z,6E)-5-methyl-7-(2,6,6-trimethylcyclohex-1-enyl)-hepta-2,4,6-triene-1-al was dissolved in 0.8 mL of dry tetrahydrofuran. This mixture was injected by syringe into a freshly prepared solution of [^{3}H] MeMgI (1.0 mmol) in 3.0 mL of diethyl ether and stirred at 0°C. The reaction was warmed to room temperature and stirring was continued for 2 h. The reaction was terminated by the addition of 2.0 mL of 1% aq. ammonium chloride solution followed by 2.0 mL of 2% sodium thiosulfate solution. Volatile solvents and labile tritium products were removed by vacuum transfer. The aqueous phase was extracted with 3 X 5 mL of diethyl ether. The combined ether extracts were dried with anhydrous sodium sulfate and filtered. A near quantitative recovery of product was obtained at 90% radiochemical purity.

(2E,4Z,6E)-1-^3H_3C,5-Methyl-7-(2,2,6-trimethylcyclohex-1-enyl)-hepta-2,4,6-triene-1-one (12C): The secondary alcohol obtained in the previous step was immediately reconstituted in 2.0 mL of dry dichloromethane in a 10 mL reaction flask fitted with a drying tube and a stir bar. 30 mg of activated manganese dioxide was added to this solution while stirring. The reaction was stirred at room temperature for 1 h and then filtered through a small bed of celite and rinsed with dichloromethane. The solvent was removed by rotary evaporation. The crude product was purified by silica gel column

chromatography (1.0 cm. X 15.0 cm.) in hexane/ethyl acetate (9/1). Pure fractions were combined to give 4.5 mg (0.017 mmol) of product. The specific activity of this intermediate was found to be 85.1 Ci/mmol as determined by mass spectral analysis.

Methyl-(2E,4E,6Z,8E)-3-$^{3}H_3C$,7-methyl-9-(2,2,6-trimethylcyclohex-1-enyl)-nona-2,4,6,8-tetraenoate (13C): 6.7 mg (0.174 mmol) of sodium hydride (60% oil dispersion), 200 μL of dry tetrahydrofuran, and 32 μL (0.174 mmol) of methyl diethylphosphonoacetate were combined in a 5 mL septum seal flask fitted with a drying tube and a stir bar. This mixture was stirred at room temperature for 15 min. The 4.5 mg (0.017 mmol) of **12C** dissolved in 100 μL of dry tetrahydrofuran was added by syringe to the stirring mixture. The reaction was stirred in darkness at room temperature for 18 h. The reaction was terminated by the addition of 2.0 mL of diethyl ether and 1mL of water. The aqueous phase was extracted with 3X3.0 mL of diethyl ether. Ether extracts were combined, dried over sodium sulfate and filtered to give 4.0 mg (0.013 mmol) of crude labeled ester (76% radiochemical yield).

(2E,4E,6Z,8E)-3-$^{3}H_3C$,7-Methyl-9-(2,2,6-trimethylcyclohex-1-enyl)-nona-2,4,6,8-tetraenoic acid (1C): The crude labeled retinoic ester was reconstituted in 3.0 mL of methanol in a 10 mL reaction flask. 0.3 mL of 7.0 N aq. potassium hydroxide was added and the mixture was heated at reflux for 2 h. After cooling, the reaction was neutralized by the addition of 2.0 N aq. HCl solution. The mixture was extracted with 3 X 5 mL of diethyl ether. Ether extracts were combined dried over sodium sulfate and filtered. The crude product was purified by HPLC using Zorbax ODS column (25 cm X 9.4 mm) in a system of methanol/ isopropanol/water/acetonitrile/acetic acid, (25/15/30/30/1.2). Fractions of pure [20 methyl-^{3}H] 9-*cis*-RA were combined to give 1.5 mg of pure product (38% radiochemical yield). The specific activity of (C-20 methyl ^{3}H) 9-*cis*-RA was found to be 73 Ci/mmol as determined by mass spectral analysis.

ACKNOWLEDGMENTS

This work was supported a fund from the National Foundation for Cancer Research and a fund from the Wisconsin Alumni Research Foundation. We acknowledge the National Magnetic Resonance facility at Madison for their help in obtaining the spectral data.

REFERENCES

1. Dawson, M.I., Hobbs, P.D., Cameron, J.F. and Rhee, S.W. - *J. Lab. Comp. Radiopharma.* 33: 245 (1993).

2. Boehm, M.F., McClurg, M.R., Pathirana, C., Mangelsdorf, D., White, S.K., Hebert, J., Winn, D., Goldmann, M.E. and Heyman, R.A. - *J. Med. Chem.* 37: 408 (1994).

3. Bennani, Y.L. and Boehm, M.F. - *J. Org. Chem.*. 60: 1195 (1995).

4. Olson, G.L., Cheung, H.C., Morgan, K.D., Borer, R. and Saucy, G. - *Helv. Chim. Acta* 59: 567 (1976).

5. Bu Lock, J.D., Quarrie, S.A., and Taylor, D. A. - *J. Labeled Compounds* 9: 311 (1973).

6. Robeson, C.D., Cawley, J. D., Weisler, L., Stern, M.H., Eddenger, C.C. and Chechak, A.J - *J. Am. Chem. Soc.* 77: 4111 (1955).