PREPARATION OF ALL TRANS-RETINOIC-11-^{3}H ACID AND ALL TRANS-RETINYL-11-^{3}H ACETATE

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SUMMARY

Trans-ionylidineacetaldehyde-1-^{3}H (3) was obtained by reduction of unlabeled aldehyde (1) with lithium borotritide, followed by reoxidation of the tritio alcohol (2). Condensation of aldehyde (3) with triethyl 3-methyl-4-phosphonocrotonate (7), followed by saponification of the retinoic ethyl ester (8), afforded trans-retinoic-11-^{3}H acid (9). Esterification of the crystalline acid with diazomethane, and subsequent reduction of the methyl trans-retinoate-11-^{3}H with lithium aluminum hydride, yielded trans-retinol-11-^{3}H, which was acetylated in situ to give trans-retinyl-11-^{3}H acetate (10).

Key Words: Lithium borotritide reduction, Manganese dioxide, Emmons-Horner reaction, Phase transfer, Retinoic-11-^{3}H acid, Retinyl-11-^{3}H acetate

INTRODUCTION AND DISCUSSION

Within the framework of ongoing metabolic and pharmacologic studies related to the prevention of lung cancer and other epithelial cancers tritiated all-trans-retinyl acetate of high specific activity was required. A synthesis of all-trans-retinoic acid and all-trans-retinyl acetate containing a tritium on carbon 11 of the side chain was therefore developed.

The Wittig and Emmons-Horner reactions have been widely used for the synthesis of Vitamin A compounds.[1] Thus, retinoic acid esters were obtained from the β-C_{15}-aldehyde (1) and the phosphonate[1] (7) or the corresponding phosphonium salt.[2] This sequence is adaptable to the preparation of isotopically labeled analogs and has indeed been used to prepare all-trans-α-retinyl-11-^{3}H acetate[3] from the corresponding α-C_{15}-aldehyde and phosphonium salt.

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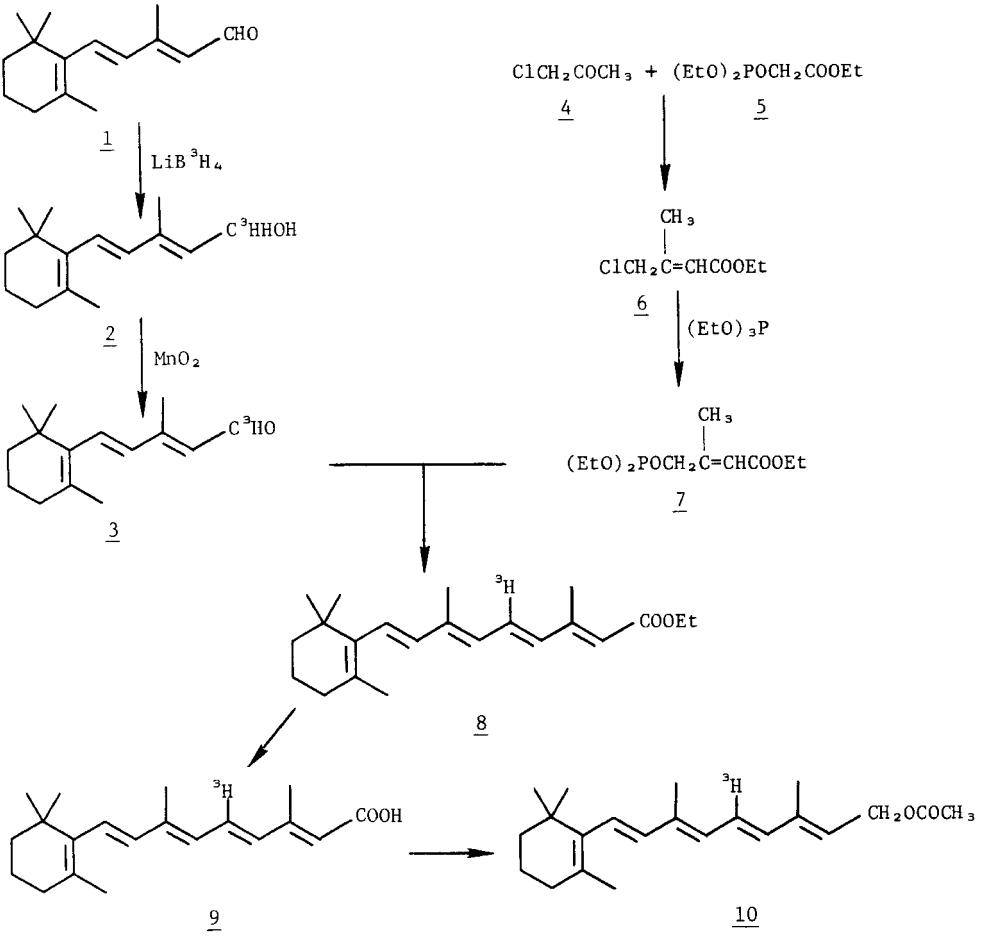
A mixture of cis and trans-ionylidineacetaldehyde was separated by preparative HPLC. The trans-isomer (1) was then reduced with lithium borotritide in tetrahydrofuran at 25° to afford the tritio alcohol (2). Subsequent oxidation of 2 with manganese dioxide afforded the 1-tritio aldehyde (3).

The phosphonate ester[4] (7) was synthesized by Emmons-Horner condensation of chloroacetone with triethylphosphonoacetate followed by heating the resulting 3-methyl-4-chlorocrotonate (6) with triethyl phosphite at 150°. The phosphonocrotonate (7) was obtained as a 50:50 cis-trans mixture, but after Emmons condensation with the tritio aldehyde (3) in a phase transfer medium,[5] the ethyl retinoate (8) so obtained was shown to be 85% trans by HPLC. Hydrolysis of the ester in alcoholic potassium hydroxide afforded the retinoic-11-^{3}H acid (9), which was crystallized from ethanol. The HPLC and UV spectrum were in agreement with authentic material.

It is interesting that the condensation of the approximately 50% trans-phosphonocrotonate (7) with the ionylidineacetaldehyde (4) yielded a product with a very high percentage of trans-isomer. The phase transfer medium was apparently responsible for this result.

The crystalline retinoic-11-^{3}H acid (9) was esterified on an approximately 25μmolar scale by treatment with diazomethane in ether. The methyl ester was then reduced with lithium aluminum hydride in ether at -55°. The reaction mixture was decomposed with ethyl acetate and saturated aqueous ammonium chloride and the retinol-11-^{3}H acetylated with the aid of acetyl chloride and pyridine in ether. The reduction, whose progress was being analyzed by HPLC, proved to be straightforward only when run with inactive material. Attempts to reduce the tritiated methyl retinoate following the published procedures[3,6] gave rise to the formation of an unknown by-product with an R_f very close to retinyl acetate in amounts of up to ∿40% of the product mixture. The formation of this by-product could finally be held to approximately 4% by reversing the usual addition sequence of reducing agent and ester. Chromatography of the crude product on alumina furnished a product of only ∿88% purity. Pure (<97% by HPLC) retinyl acetate could only be obtained by purification on reverse phase HPLC in small



increments. Use of a normal-phase (Waters, μ-Poracil) HPLC column gave rise to formation of probably the same decomposition product with slightly slower elution as already observed earlier during the formation of the retinol.

EXPERIMENTAL

Radioassays were carried out in 10 ml of Scintisol cocktail (Isolab Inc.) with internal standards and counted with a Searle Mark 3 liquid scintillation spectrometer. TLC analyses were conducted on 20-cm TLC plates of Merck Silica Gel 6F 254. Analyses by HPLC were obtained from a Waters 6000A pump, U6K injector, Sperisorb ODS (4.57 mm × 25/cm) column, and Model 450 variable

wavelength detector. Unless otherwise noted, analyses were done with 80:20 acetonitrile-water at 2 ml/min with detection at 280 and 325 nm. GC analyses were obtained from a Hewlett Packard 5710A gas chromatograph on a 10% DC200 Carbowax column.

Ethyl 4-Chloro-3-methylcrotonate (6)

To a mechanically stirred suspension of 12 g (0.25 mol) of 50% sodium hydride oil dispersion in 200 ml of anhydrous ether was added, slowly and under occasional cooling to keep the temperature below 20°C, a solution of 56.05 g (0.25 mmol) of triethyl phosphonoacetate in 100 ml of dry ether. The resulting mixture was stirred at room temperature over night. Then a solution of 27.8 g (0.3 mol) of chloro-2-propanone in 100 ml of ether was added at a rate to keep the temperature below 25°C. The mixture was allowed to stand at room temperature for 2 h and then diluted with 300 ml of water. The phases were separated, and the aqueous layer was extracted two more times with ether. The organic extracts were combined, dried over anhydrous magnesium sulfate and evaporated to dryness. The residue yielded 18.1 g of product, bp 87-89°C/15 mm Hg; GC showed a purity of 88.5% with a 42:58 cis-trans ratio.

Triethyl-3-Methyl-4-phosphonocrotonate (7)

A mixture of 13.29 g (80 mmol) of freshly distilled triethylphosphite and 13 g (80 mmol) of ethyl 4-chloro-3-methylcrotonate was stirred under argon at 150 to 160°C for 3 h. Distillation yielded 15.35 g (72.6%) product, bp 117-119°C/0.55 mm Hg; GC showed a purity of 92.2% with a 46:54 cis-trans ratio.

3-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1-^{3}H-2-trans-4-trans-pentadien-1-ol (2)

A solution of 5.3 mg (0.24 mmol, 3 Ci) of lithium borotritride[7] and 240 mg (1.1 mmol) of 3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-trans-4-trans-pentadienal (1) in 6 ml of anhydrous tetrahydrofuran was stirred at room temperature (under argon) for 21 h. The mixture then was diluted with 20 ml of distilled water and extracted repeatedly with ether. After drying and evaporation of the solvent, 221 mg of the labelled alcohol (2) was obtained.

3-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-trans-4-trans-pentadienal-1-^3H (3)

The alcohol (2) obtained above was dissolved in 25 ml of dichloromethane and stirred under argon together with 2 g of manganese dioxide for 18 h. The mixture was filtered, the solids were thoroughly washed with dichloromethane, and the filtrate was concentrated *in vacuo* to a residue of 215 mg. The product was shown to have the same R_f on TLC (SiO_2, hexane-ethylacetate 95:5) as the unlabeled trans-ionylideneacetaldehyde (1) and to be of ∿90% radiochemical purity. It was used in the next step without further purification.

Ethyl Trans-retinoate-11-^3H (8)

To the tritiated aldehyde (3) dissolved in 12 ml of dichloromethane were added 1.3 g (5 mmol) of diethyl 3-ethoxycarbonyl-2-methylprop-2-enyl phosphonate, 300 mg of trimethyl dodecylammonium bromide and 5 ml of 40% aqueous sodium hydroxide. The mixture was stirred at room temperature under argon for 6 h. The phases then were separated, and the dichloromethane was washed successively with water, 3 N hydrochloric acid, and dilute aqueous sodium hydroxide. All aqueous washes were back-extracted once with dichloromethane. The combined organic extracts were dried over anhydrous magnesium sulfate and evaporated *in vacuo*. The residue (1390 mg) was taken up on 5 ml of hexane/ethyl acetate (95:5) and applied to a short column of 10 g of silica gel. Elution with a total of 115 ml of hexane/ethyl acetate (95:5) yielded 284 mg 8 (78% overall yield). The isomer ratio was found to be about 85:15 ethyl trans-retinoate to ethyl 13-cis-retinoate by HPLC.

Trans-retinoic-11-^3H Acid (9)

The preceding mixture of all-trans and 13-cis ethyl retinoate-11-^3H was hydrolyzed by stirring with 0.5 g of potassium hydroxide in 9 ml of ethanol and 1 ml of water for 1 h at 60°C under an argon 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 sulfuric acid, and extracted repeatedly with methylene chloride. The extracts were dried over anhydrous magnesium

sulfate and evaporated to dryness. The resulting yellow solid was recrystallized from ethanol, yielding a total of 89.3 mg of retinoic-11-^{3}H acid (27% overall yield) as yellow needles with a specific activity of 3.02 Ci/mmol. The chemical and radiochemical purity was determined by reverse-phase HPLC on a Spherisorb ODS column using acetonitrile/0.1% aqueous acetic acid, 4:1, as the eluting solvent. The composition of the 11-^{3}H retinoic acid was found to be 97.1% all-trans- and 2.2% 13-cis-retinoic acid.

After unsuccessful attempts to separate the isomers by chromatography on silica gel with benzene/methanol/ether, 200:1:10, the mother liquor (42.2 mg) was dissolved in 20 ml of ether. To this was added 10 ml of ether containing 1 mg of iodine, and the mixture was allowed to stand at room temperature in the dark for 5 h. HPLC analyses showed an improvement from about 50:50 all-trans/13-cis retinoic acid at the start to about 60:40. The ether solution then was washed with 10 ml of 5% sodium thiosulfate, dried, and concentrated. The residue was crystallized from ethanol, yielding an additional 8.5 mg of retinoic acid, but it contained 10% of the 13-cis isomer.

Trans-Retinyl-11-^{3}H acetate (10)

Retinoic-11-^{3}H acid (6.86 mg, 0.023 mmol, 66 mCi) was dissolved in 2 ml of dry ether and treated with an excess of diazomethane in ether for 30 min at room temperature. After the solution was filtered through 2 g of silica gel, the solvent was removed in vacuo, yielding methyl retinoate-11-^{3}H, which was not purified further. The ester was dissolved in 5 ml of dry ether, and the solution was cooled to -50°C while being kept under a blanket of argon. At this temperature, 1 ml of 1 M lithium aluminum hydride in ether was added, with stirring over 1 min. The reaction mixture was stirred at -55°C for 30 min and then quenched by the addition of 1 ml of ethyl acetate, followed 5 min later by 0.4 ml of saturated aqueous ammonium chloride. The mixture was allowed to warm to room temperature and then filtered through anhydrous magnesium sulfate. After the drying agent was washed with three 5-ml portions of ether, the solvent was removed in vacuo. The residue was taken up in 10 ml of dry ether and cooled

under argon in an ice bath. To this was added, with stirring, 1 ml (12.4 mmol) of pyridine. Then 1 ml (14 mmol) of acetyl chloride was added carefully. The resulting mixture was stirred for 2 h in an ice bath, followed by 1 h at room temperature. After 5 ml of ice water was added, the ether solution was extracted with 1 N hydrochloric acid, then washed repeatedly in cold 1% sodium carbonate. After it was dried over magnesium sulfate, the organic phase was concentrated and the residue purified by column chromatography over activity II alumina (Woelm). Fractions containing retinyl acetate were pooled to provide 4.08 mg or 36 mCi. The radiochemical purity of this product was found to be 87.5% (excluding 4.2% 13-cis-retinyl-11-^{3}H acetate) by reverse-phase HPLC. Half of the above retinyl-11-^{3}H acetate was purified successfully in small increments using a "normal-phase" HPLC column (Waters, μ-Porasil) and hexane/ethyl acetate (97:3) as the solvent. The 8.6 mCi (8.8 mCi/mg, 2.9 Ci/mmol) so obtained were found to be 97% radiochemically pure (excluding 2% 13-cis isomer) by HPLC.

In subsequent preparations of retinyl-11-^{3}H acetate it was found to be more suitable to use a reverse-phase HPLC column and acetonitrile-water 4:1 as the solvent. Using this system all-trans retinyl-11-^{3}H acetate of purities <97% could be obtained without complications.

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