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

Hans H. Kaegi, James E. Bupp, and Joseph I. DeGraw
Bio-Organic Chemistry Laboratory
SRI International
Menlo Park, California 94025, USA

SUMMARY

trans-Ionylideneacetaldehyde-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 diethyl 3-cyano-2-methylprop-2-enylphosphonate (7) afforded retinonitrile-11-^{3}H (8) which was smoothly reduced to trans-retinal-11-^{3}H (9) with diisobutylaluminum hydride. Reduction of retinal (9) with lithium borohyride followed by acetylation gave retinyl-11-^{3}H-acetate in excellent yield.

Key Words: Emmons-Horner reaction, phase transfer, retino-11-^{3}H-nitrile, retinal-11-^{3}H, diisobutylaluminum hydride, retinyl-11-^{3}H acetate

INTRODUCTION AND DISCUSSION

Metabolic and pharmacologic studies of retinoids related to the prevention of lung cancer and other epithelial cancers required tritiated all-trans-retinal at high specific activity. A synthesis of all-trans-retinal labeled with a tritium on carbon 11 of the side chain was therefore developed.

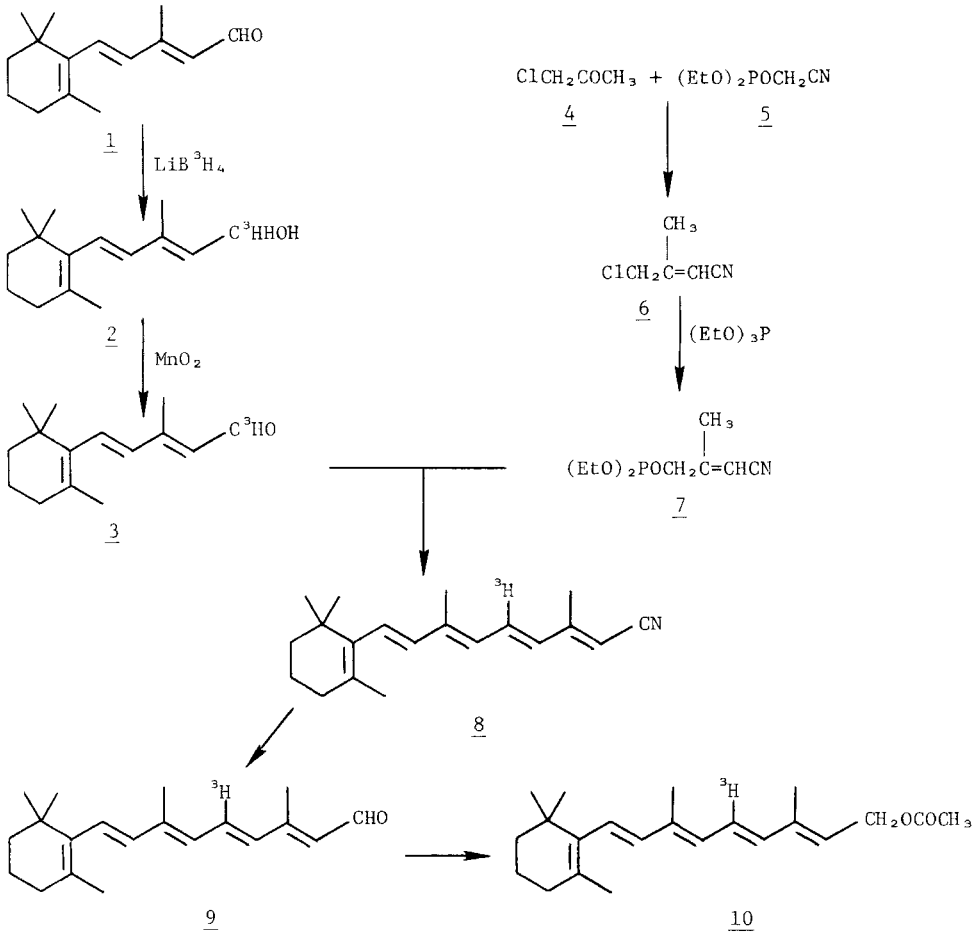
The method, as shown in the scheme below makes use of the Emmons-Horner reaction, a reaction widely used in the synthesis of Vitamin A compounds.[1] The synthesis starts with the β-C_{15}-aldehyde 1, which can be easily labeled with tritium, and forms the retinoid skeleton after a condensation with the C_5-phosphonate 7.

A mixture of cis and trans-ionylideneacetaldehyde was separated by preparative HPLC. The trans-isomer 1 was then reduced with lithium borotritide in tetrahydrofuran at ambient temperature to afford the tritiated alcohol 2. Subsequent oxidation of 2 with manganese dioxide afforded the 1-tritio aldehyde 3. The phophononitrile 7[2] was synthesized by Emmons-Horner

0362-4803/82/060745-08$01.00

Received October 6, 1981
Revised January 28, 1982

condensation of chloroacetone (4) with diethyl cyanomethylphosphonate (5) followed by heating the resulting 4-chloro-3-methylcrotonitrile (6) with



triethyl phosphite at 150°C. The phosphonate 7 was obtained as a 50:50 cis-trans mixture. Emmons-Horner condensation of phosphonate 7 with trans-ionylideneacetaldehyde (3), as catalyzed by a phase-transfer[3] reagent gave retinonitrile (8) in greater than 60% yield as a mixture of isomers. Analysis by gas chromatography-mass spectrometry (GC-MS) indicated this material to be 79% all-trans-, 17% 13-cis- and 4% 9-cis-retinonitrile, thus it contained a very favorable percentage of the desired trans-isomer. Reduction of the nitrile 8 at 0-5°C with diisobutylaluminum hydride[4,5] smoothly afforded

retinal. The product was purified by chromatography and then crystallized from hexane at low temperature to yield retinal-11-^{3}H. The crystalline aldehyde was obtained in 35% overall yield, and its melting point and ultraviolet spectrum were in agreement with literature values. The specific activity was 1.97 Ci/mmole, and reverse-phase (Spherisorb ODS) HPLC indicated a purity above 98%. However, TLC indicated the presence of about 8% apparent 13-cis-retinal-11-^{3}H.

After storage for 2 months in toluene at -50°C (DBHQ, 2,5-di-tert-butylhydroquinone, present), the overall retinal content was unchanged, but the isomer ratio had altered to 84% trans:15% 13-cis. The material was purified with heavy losses by minipreparative HPLC (μ Porasil, hexane/diethyl ether 88/12) to afford trans-retinal-11-^{3}H of 98.6% purity. This labeled compound must be used immediately after purification, because its 13-cis-isomer content begins to increase after a few days of storage at low temperature.

Some 13-cis-retinal-11-^{3}H was also isolated from the above HPLC purification. However, this retinoid isomerizes to the all-trans aldehyde even more rapidly. For example, it was found to have altered to 75% cis:25% trans after only 4 days of storage at -15°C.

Reduction of retinal-11-^{3}H with lithium borohydride at ambient temperature, followed by acetylation of the obtained retinol-11-^{3}H with pyridine and acetyl chloride, gave all-trans retinyl-11-^{3}H acetate in good yield. This reduction can be easily executed and proceeds smoothly, avoiding all the complications of the more usual reduction of the methyl ester of retinoic acid with lithium aluminum hydride[6] at low temperature. Reductions of retinal on a 0.1 mmole scale always furnished retinyl-11-^{3}H acetate in yields better than 60% after recrystallization from methanol at low temperature.

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 GF 254. Analyses by HPLC were obtained from a Waters 6000A pump, U6K injector, Model 450 variable wavelength detector and a Spherisorb ODS (4.57 mm x 25 cm) column. 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.

3-(Chloromethyl)-3-methylacrylonitrile (6)

To a mechanically stirred suspension of 50% NaH-oil dispersion (14.5 g, 0.3 mol) in anhydrous ether (300 ml) in a 500-ml 3-neck flask fitted with a thermometer, argon inlet-outlet tubes, and an addition funnel, was added dropwise, a solution of diethyl cyanomethylphosphonate (53.1 g,0.3 mol) in anhydrous ether (100 ml) over 30 min at 13-15°C with ice-bath cooling. The ice bath was removed 15 min after the phosphonate was added, and the resulting thick white paste was stirred 20 hr at room temperature to give the sodium salt. Then a solution of freshly distilled (bp 119-120°C) chloroacetone (29 ml, 0.36 mol) in dry ether (100 ml) was added dropwise to the above mixture over 1 hr at 10-12°C with ice-bath cooling and rapid stirring. The reaction mixture darkened and thinned during the addition period. The ice bath was removed, and stirring was continued for 2 hr at room temperature; however, stirring stopped some time in this period when the heavy product separated and solidified. Workup was accomplished by adding water (300 ml) to dissolve the solid, and the product was extracted into ether. The ether-extract was washed first with water and then with brine, dried over Na_2SO_4, and evaporated at 20 mm Hg, leaving crude product. Fractionation through a 3 in Vigreaux column gave 25 g (72%) of a colorless liquid, bp 96-100°C/31 mm Hg, as a 95% pure (GC) mixture of isomers. Both GC and NMR showed an isomer ratio of 66:34. IR and NMR spectra were consistent with the desired product.[2]

Diethyl 3-cyano-2-methylprop-2-enylphosphonate (7)

A magnetically stirred solution of triethyl phosphite (11.6 g, 0.1 mol) and the 34:66 isomeric mixture of cis,trans-3-(chloromethyl)-3-

methylacrylonitrile (6), (16.6 g, 0.1 mol) was heated to 150°C over 30 min, with evolution of ethyl chloride beginning about 125°C. Following the course of the reaction by GC showed 95% completion and 91% product after 2-1/2 hr at 150°C. The reaction solution darkened only slightly. The product was fractionated directly from the reaction vessel to give 16.2 g of colorless liquid, bp 99-100°C/0.08 mm Hg, which GC analysis showed to be 99% pure, with a 50:50 isomer 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 6.7 mg (0.31 mmole; 3 Ci) lithium borotritide[7] and 240 mg (1.1 mmole) 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 an atmosphere of argon for 24 hr. The mixture was then diluted with 10 ml of distillled water and the solvents were removed by vacuum transfer. To the residue was added 5 ml of ethanol, which was also removed by vacuum transfer. After repeating this procedure once more the residue was distributed between 5 ml of water and 10 ml of ether and the aqueous phase was repeatedly extracted with ether. The organic extracts were combined and, after drying over anhydrous magnesium sulfate, evaporated to dryness. The 248 mg of alcohol 2 so obtained contained approximately 2.2 Ci of tritium.

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

A solution of the alcohol 2 in 25 ml dichloromethane was stirred, under argon, with 2 g of manganese dioxide for 18 hr. The mixture was filtered, the solids were thoroughly washed with dichloromethane and the filtrate concentrated *in vacuo* to a residue of 234 mg. The product was shown to have the same R_f on TLC (SiO_2, hexane-ethyl acetate 95:5) as the unlabeled trans-ionylidene acetaldehyde (1) and to be of ~90% radiochemical purity. It was used in the next step without further purification.

trans-Retino-11-^{3}H nitrile (8)

To the tritiated trans-ionylidineacetaldehyde (3), 1 g (4.6 mmol) of diethyl cis- and trans-3-cyano-2-methylprop-2-enylphosphonate (7) and 200 mg (0.87 mmol) of benzyltriethylammonium chloride in 10 ml of dichloromethane was added 5 ml of 50% aqueous sodium hydroxide. The mixture was allowed to stir at room temperature under an argon atmosphere for 2 hr. The phases were separated and the dichloromethane washed successively with water, 1 N hydrochloric acid, and water. 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 (1.05 g) was applied to a short column of 10 g of silica gel. Elution with a total of 75 ml of hexane/ethyl acetate, 95:5, yielded 234 mg (78%) of retinonitrile. Analysis of the retinonitrile by GC-MS, obtained the same way with inactive material, showed it to be 79% all-trans-, 17% 13-cis-, and 4% 9-cis-retinoitrile. The mass spectra for all three compounds gave the same molecular ion, m/e 281.

all-trans-Retinal-11-^{3}H (9)

A solution of 1 mmole of diisobutyl aluminum hydride in 1.5 ml of hexane was added slowly over 10 min under stirring and under a blanket of argon to an ice-cooled solution of 234 mg (0.8 mmole) retinonitrile-11-^{3}H in 10 ml of dry benzene. Stirring was continued for 10 min, while cooling in ice, followed by 40 min at room temperature. The reaction mixture was cooled again in an ice-bath and then decomposed by the slow addition of 5 ml of 15% aqueous acetic acid. After stirring for another 15 min at 4°C, the mixture was allowed to warm to ambient temperature. The phases were separated and the organic layer was washed in succession with water, saturated sodium bicarbonate, and saturated sodium chloride solution. All aqueous phases were back-extracted twice with benzene. The organic phases were combined, dried over anhydrous magnesium sulfate, and evaporated to dryness. The residue, 250 mg of reddish oil was dissolved in a small amount of benzene, and applied to a column of 15 g silica gel packed in benzene and flushed with argon. Elution with benzene (50 ml), followed by benzene/1% ethyl acetate (85 ml) and, finally, benzene/2%

ethyl acetate (75 ml) furnished 165 mg of retinal as an oil (visible on the column as a red band). The oily material was crystallized from 0.6 ml of hexane at -10°C, yielding 112 mg of crystalline retinal-11-^{3}H in two crops; UV λ_{max} (EtOH) 379-380, ε 41600, in agreement with the literature.[8] The radiopurity was found to be 97.6% by HPLC (reverse phase) and the specific activity, 6.95 mCi/mg (1.97 Ci/mmole). TLC indicated the presence of about 8% apparent 13-cis-retinal-11-^{3}H. All-trans-retinal (98.6%) was obtained by chromatography in small increments using an HPLC column (Waters, μ-Porasil) and hexane/diethyl ether, 88/12, as the solvent. Even though stored in toluene (DBHQ present) at -50°C the retinal rearranged slowly to form increasing amounts of 13-cis-isomer, although it was apparently stable under the storage conditions.

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

Retinal-11-^{3}H (21.6 mg; 0.076 mmole) dissolved in 13 ml dry ether was added slowly to a stirred suspension of 5 mg (0.23 mmole) lithium borohydride in 2 ml of dry ether kept at room temperature and under a blanket of argon. Stirring was continued for an additional 20 min and then 10 ml of distilled water was added. After vigorous stirring for 10 min the phases were separated and the aqueous layer extracted twice with a total of 15 ml ether. The ether extracts were combined and dried over anhydrous magnesium sulfate.

The solution, under argon, was cooled in an ice-bath and with stirring, 1 ml pyridine (12.4 mmole) and 1 ml acetyl chloride (14 mmole) was added. The ice-bath was removed after 30 min and stirring continued at ambient temperature for 2 1/2 hr. The mixture was then successively extracted with 10 ml water and twice with 10 ml 5% sodium carbonate solution. All aqueous phases were back-extracted twice with 10 ml of ether. The organic extracts were dried over anhydrous magnesium sulfate, combined, and concentrated. The residue, a light yellow oil, was recrystallized from methanol at -15°C, yielding 20.5 mg crystalline retinol-11-^{3}H acetate. The radiopurity was determined to be 96.7% by HPLC and the specific activity 5.95 mCi/mg (1.95

Ci/mmole). Nonradioactive retinyl acetate obtained by the above procedure from retinal provided light yellow crystals, m.p. 58.9-59°C.

ACKNOWLEDGEMENT

This work was supported by Contract No. NO1-CP-75911-56 from the National Cancer Institute.

REFERENCES

1. Pommer, H. Ang. Chem. 72, 811 (1960)
2. Fujiwara, K., Takahashi, H., and Ohta, M. Bull. Chem. Soc. Japan 35, 1743 (1962)
3. Piechucki, C. Synthesis 1974, 869
4. N. V. Philips' Gloeilampenfabrieken, Brit. Pat. 811,527 (1959); Chem. Abstr. 53, 17177 (1959)
5. Samokhvalov, G. I. Davydova, L. P., Zakharkin, L. I., Khorlina, I. M., Vakulova, L. A., Zhikhareva, L. T., and Preobrazhenskii, N. A. J. Gen. Chem. USSR 30, 1806 (1960)
6. Schwarzkopf, O., Cahnmann, H. J., Lewis, A. D., Swidinsky, J., and Wuest, H. M. Helv. Chim. Acta. 32, 443 (1949)
7. Purchased from Dynapol, Palo Alto, California
8. The Vitamins Vol. 1. Sebrell, W. H., and Harris, R. S., Eds., Academic Press, New York, 2nd Ed. 1967, page 14