NOTE

Preparation of 9-*cis*-Retinoic Acid [11,12-^{3}H(N)] by Photochemical Isomerization

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SUMMARY

All-*trans*-Retinoic acid [11,12-^{3}H(N)] was photochemically isomerized using a fluorescent source to afford a mixture of isomeric retinoic acids, from which 9-*cis*-retinoic acid[11,12-^{3}H(N)] was isolated by reversed-phase HPLC. The identity of 9-*cis*-retinoic acid [11,12-$^3H_2$] was confirmed by coinjection with the unlabeled acid.

KEY WORDS: 9-*cis*-retinoic acid [11,12-^{3}H(N)], photolysis, all-*trans*-retinoic acid[11,12-^{3}H(N)]

INTRODUCTION

The discoveries that the retinoid X receptor (RXR) influences signalling by the retinoic acid, thyroid hormone, and Vitamin D_3 receptors by forming heterodimers (1-3) and that 9-*cis*-retinoic acid is the natural ligand for RXR (4-5) generated a need for the tritiated isomer of 9-*cis*-retinoic acid that could be readily prepared on a small-scale from commercially available material and then used in biological experiments. We report here a simple method to produce 9-*cis*-retinoic acid [11,12-$^3H_2$(N)] by photoisomerization of all-*trans*-retinoic acid [11,12-$^3H_2$(N)], followed by purification using high performance liquid chromatography (HPLC).

9-*cis*-Retinoic acid can be obtained by multistep synthesis, as a by-product from Vitamin-A preparation, or on a small-scale by photoisomerization of the all-*trans*-isomer. We utilized the latter method for the rapid preparation of small quantities of tritium-labeled 9-*cis*-retinoic acid from all-*trans*-retinoic acid [11,12-^{3}H(N)], which is commercially available with high specific activity.

RESULTS AND DISCUSSION

Retinoids, with their five conjugated double bonds, are easily isomerized by chemical methods, heat, and most importantly light.[6,7] Recently, Motto *et al.*[8] analyzed the photoisomerization of all-*trans*-retinoic acid with time and developed a practical photochemical method, using an improved reversed-phase chromatographic separation of retinoic acid isomers. These isomers were isolated and characterized spectroscopically. We adopted and modified this procedure for small-scale photoisomerization (Figure 1).

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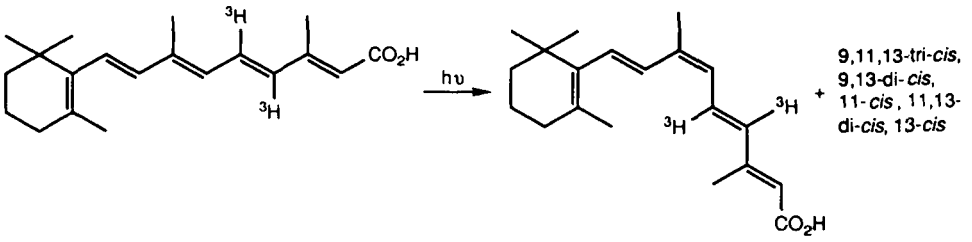

Figure 1. Photoisomerization of all-*trans*-retinoic acid.

All-*trans*-retinoic acid was irradiated in a quartz tube at room temperature and under an argon atmosphere with fluorescent light. Degassed absolute ethanol was used as the solvent. The reaction was followed by HPLC. In Figure 2 is shown the isomer mixture after 1 hour of irradiation. Irradiation for long periods did not produce more 9-*cis*-retinoic acid (8.9%, based on the observed peak area at 345 nm). The peak-height ratios indicated that there was no increase in any of the isomers, but a decrease of all-*trans*-retinoic acid. The reaction mixture was lyophillized and 9-*cis*-retinoic acid was purified by HPLC. Purified 9-*cis*-retinoic acid [11,12-^{3}H(N)] had a total activity of 30 μCi (specific activity of 48.7 Ci/mmole and a radiochemical purity above 96.1%). 11-*cis* Isomer (3.9%) was observed as a left-hand shoulder on the peak of the 9-*cis* acid.

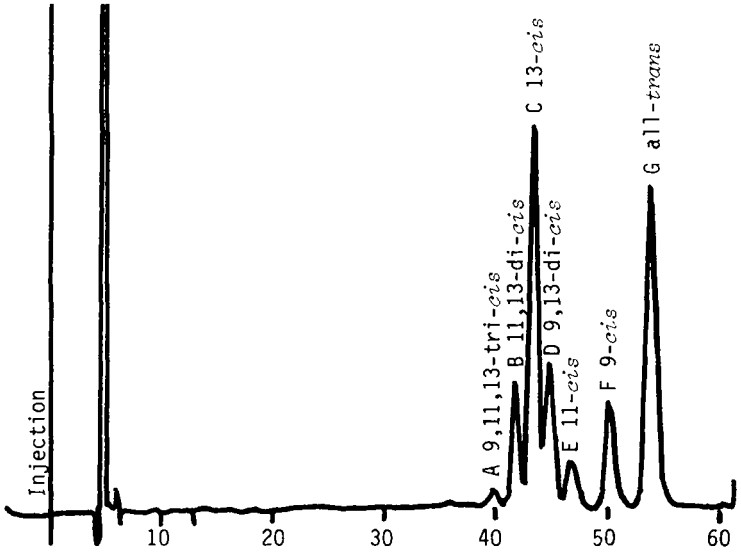

Figure 2. Reversed-phase HPLC chromatogram of ^{3}H-labeled retinoic acid isomer mixture on Nova-Pak C_{18}. The isomer mixture was produced by irradiation of all-*trans*-retinoic acid [11,12-^{3}H(N)] for 60 min in EtOH with fluorescent light. Peak F (9-*cis*-retinoic acid) and G (all-*trans*-retinoic acid) were identified by co-elution with authentic samples. Assignments of other retinoic acid isomers (*i.e.*, peaks A through E) are based on the chromatographic profile described in Reference 10.

EXPERIMENTAL

All-*trans*-retinoic acid [11,12-^{3}H(N)] (500 μCi, 48.7 Ci/mmole) was purchased from NEN Research products (Boston, MA), all-*trans*-retinoic acid from Eastman (Rochester,

N.Y.), and 9-*cis*-retinal from Aldrich (Milwaukee, WI). Radioassays were carried out in 10 mL of Ecolite cocktail (ICN, Irvine, CA) with an internal standard and counted with a Beckman LS-250 liquid scintillation system. HPLC analyses were obtained using a Waters 6000A solvent delivery system, U6K injector, Model 450 variable wavelength detector, and a Berthold HPLC radioactivity monitor LB305 with a flow cell. Analyses were performed on a Nova-Pak C_{18} stainless steel column (60Å, 4 μm, 3.9 × 300 mm), and for convenience preparative separations were carried out on a Radial-Pak cartridge (Waters, C_{18}, 8 × 100 mm), using the same HPLC eluant (CH_3CN/MeOH/$(CH_3)_2CHOH$/H_2O/HOAc 30/25/15/22.5/1). The detection wavelength was 345 nm.

9-*cis*-Retinoic Acid [11,12-^{3}H(N)]. All-*trans*-retinoic acid [11,12-^{3}H(N) (500 μCi in 500 μL of 90% EtOH) was transferred to a 5-mL quartz tube and diluted to 1 mL by addition of degassed EtOH. The solution was stirred at room temperature under a blanket of argon and irradiated with two circular fluorescent lamps (Sylvania FC 12T10-CW-RS, 34 watts). Isomerization was monitored by HPLC. After 60 min of irradiation, the solvent was removed by lyophilization, and the residue was redissolved in 500 μL of HPLC eluant. Ten aliquots (50-μL each) were then sequentially injected onto the column for separation.

The products were eluted with degassed solvent (0.5 mL/min), with UV detection at 345 nm and 0.04 AUFs. 9-*cis*-Retinoic acid fractions (t_R 49 to 51 min) were collected with light excluded. The collected fractions were combined, lyophilized to dryness, taken up in 10 mL of EtOH to give 100 μCi of tritiated 9-*cis*-retinoic acid having a radiochemical purity of 80%. Purification of this material under the same HPLC conditions gave 30 μCi of 9-*cis*-retinoic acid [11,12-^{3}H(N)] (6% overall radiochemical yield) with a radiochemical purity of 96.1% determined by HPLC radioactivity.

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