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SYNTHESIS OF TRI-, TETRA-, AND PENTA-DEUTERATED FORMS OF VITAMIN A

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SUMMARY

The synthesis of 20,20,20-trideutero, 14,20,20,20-tetradeutero, 12,14,20,20,20-pentadeutero, and 10,19,19,19-tetradeutero analogs of retinoic acid ethyl ester and retinyl acetate, by a modified Wittig-Horner synthesis, is described. Deuterium was introduced by base-catalyzed exchange into appropriate intermediates. The 10,19,19,19-$^{2}H_{4}$-vitamin A, because of its high isotopic integrity (>98% $^{2}H_{4}$), is the preferred analog for biological studies of vitamin A metabolism.

Keywords: Deuterated vitamin A analogues, deuterated retinyl acetate, 20,20,20-$^{2}H_{3}$-retinyl acetate, 14,20,20,20-$^{2}H_{4}$-retinyl acetate, 12,14,20,20,20-$^{2}H_{5}$-retinyl acetate, 10,19,19,19-$^{2}H_{4}$-retinyl acetate, retinoid synthesis.

INTRODUCTION

For studying the metabolism of vitamin A in humans, nonradioactive deuterated or ^{13}C labeled compounds of high purity are desired (1). Because the natural abundance of ^{13}C in retinol, a C-20 compound, is 22% at M+1 and 4.8% at M+2, deuterated compounds should contain three or more deuteriums in metabolically and chemically stable positions to provide adequate analytical sensitivity.

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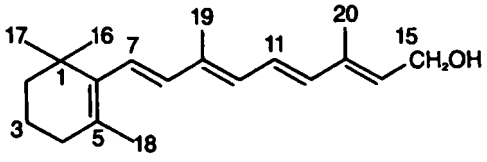


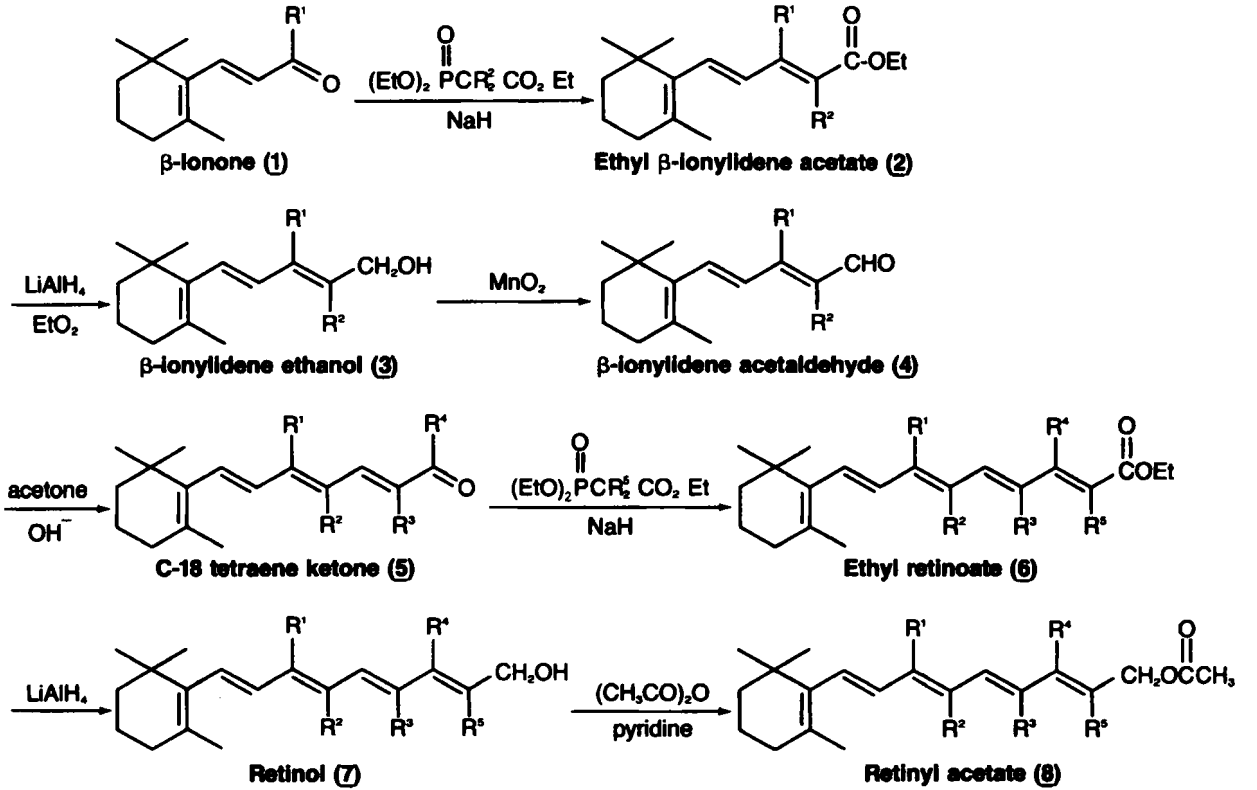
Scheme 1. Numbering system for retinol (vitamin A alcohol).

Retinoids and carotenoids are primarily synthesized by the Wittig reaction or some modification of it (2-10). We have used a modified Wittig-Horner procedure in association with the base-catalyzed incorporation of deuterium from $^{2}H_{2}O$ or perdeuteroacetone to synthesize four deuterated retinols with 3 to 5 deuterium atoms. The most useful for biological studies is the 10,19,19,19-$^{2}H_{4}$ labeled compound.

DISCUSSION

The numbering system for retinol is given in Scheme 1 and an outline of the reaction sequence is given in Scheme 2. The advantages of this route for synthesis of 20,20,20-trideuteroretinol (Scheme 2, R^{4}=$C^{2}H_{3}$) are the preferential formation of the all-trans isomer in the Wittig-Horner reactions (essentially 100% in the first reaction), the high yields (>60%) at each step, and the very low quantities of reaction side products. Thus the crude product from one reaction can be used in succeeding reactions without further purification. The overall yield from β-ionone for the sum of cis and trans isomers of 20,20,20-$^{2}H_{3}$-retinyl acetate is 15%. For the trans isomer alone, the yield is approximately 5%.

During the deuterium exchange reaction with the C-18 tetraene ketone, a significant amount (40-60%) of the ketone was destroyed. Replacing dioxane with pyridine as the exchange solvent increased the yield from 40% to 62%, but varying other reaction conditions was not helpful. Other losses occurred



Scheme 2. Synthesis of deuterated forms of vitamin A.
20,20,20-$^2H_3$-Retinoic acid ethyl ester (R^1 = CH_3; R^4 = C^2H_3);
14,20,20,20-$^2H_4$-Retinoic acid ethyl ester (R^1 = CH_3; R^4 = C^2H_3; R^5 = ^2H);
12,14,20,20,20-$^2H_5$-Retinyl acetate (R^1 = CH_3; R^3 = ^2H; R^4 = C^2H_3; R^5 = ^2H);
10,19,19,19-$^2H_4$-Retinyl acetate (R^1 = C^2H_3; R^2 = ^2H; R^4 = CH_3).

during extraction and washing. Whereas a high degree of deuterium incorporation into the trideutrated C-18 tetraene ketone was achieved (>95% $^{2}H_3$), 40% of the deuterium was lost during its subsequent condensation with the triethylphosphonoacetate carbanion. Evidently, the phosphonate ion is a sufficiently strong base to effect enolization of the ketone before condensation occurs (11). Thus, the final product is a mixture of mono-, di-, and tri-deuterated forms.

In the synthesis of 14,20,20,20-$^{2}H_4$-retinyl acetate, exchangeable protons on the phosphonate were completely replaced with deuterium. This procedure not only eliminates deuterium loss from the C-18 ketone but also yields a tetradeuterated retinoid.

The alpha proton of the phosphonate can be exchanged in $^{2}H_2O$ alone without addition of an organic solvent, inasmuch as the phosphonate is water soluble. Exchange occurs easily and yields a product with a high deuterium incorporation. By using deuterated triethyl phosphonoacetate, incorporation into the fully deuterated retinoid increased from 56% in the 20,20,20-$^{2}H_3$-retinyl acetate to 76% in the tetradeuterated retinyl ester. Because the precursor materials are greater than 95% deuterated, the reduced incorporation of deuterium into retinyl acetate probably results from exchange under the basic reaction conditions with protons of side products formed during the reaction.

NMR and mass spectral analysis of the deuterated triethyl phosphonoacetate gave considerably different deuterium incorporation values (>99% by NMR vs. 65% by MS). Because the alpha hydrogens are situated between a phosphono group and a carbonyl group, these two protons are extremely susceptible to exchange. Inasmuch as deuterium incorporation necessarily was measured by analysis of a molecular fragment in the mass spectrum, exchange within the ion source of the deuterated phosphonate or of a fragmentation product could account for the discrepancy between NMR and MS analyses.

The pentadeuterated analogue, 12,14,20,20,20-$^{2}H_5$-retinyl acetate, was prepared by condensing β-ionylidene acetaldehyde with perdeuteroacetone in the presence of base. Although the resulting C-18 ketone is highly labeled,

condensation with dideutero triethyl phosphonate ultimately gave retinyl acetate with only 40% of the desired $^2H_5$ form, together with significant amounts of less deuterated forms. Possible causes for this low yield may be contamination with traces of water or with side products with exchangeable protons.

In the synthesis of 10,19,19,19-$^2H_4$-retinyl acetate, three deuterium atoms were efficiently incorporated into β-ionone from deuterium oxide in the presence of base. Subsequent condensation with dideutero triethyl phosphonoacetate gave ethyl β-ionylidene acetate with >98% $^2H_4$. Subsequent synthetic steps did not result in deuterium loss, as evidenced by both MS and NMR analysis. This highly successful route of synthesis can be attributed to the greater stability of β-ionone relative to the C-18 ketone and to the essential absence of nondeuterated side products in this first reaction step.

EXPERIMENTAL

All synthetic and chromatographic procedures were carried out under yellow fluorescent lights (F40 Gold). Preparative-scale purifications of products were performed by high-pressure liquid chromatography (HPLC) on a Whatman M-20 silica column (22 mm ID x 50 cm L) with hexane:ethyl acetate eluents. Thin-layer chromatography (TLC) was performed on Machery-Nagel Polygram-Sil G/UV_{254} TLC plates using ethyl acetate:hexane (20:80) as the developing solvent. Plates were visualized by using ultraviolet illumination (254 nm and 366 nm). Mass spectroscopy was performed on either a Finnegan Model 4000 or an Extranuclear SpectrEL quadrupole mass spectrometer. Trimethylsilyl derivatives of deuterated retinols for GC-MS analyses were prepared by reaction with trimethylsilyldiethylamine (Aldrich).

Synthesis of 20,20,20-trideuteroretinyl acetate

Freshly distilled ethylbromoacetate (300 g; Aldrich) was added dropwise

to previously distilled triethylphosphite (300 g; Aldrich). After addition of 75 g ethylbromoacetate, an exothermic reaction began and bromoethane began to distill off; the remaining ethylbromoacetate was added slowly to maintain the reaction temperature. The reaction was then heated gently to distill all the bromoethane and further heated at 170° for 5 hr. Vacuum distillation of the product yielded triethylphosphonoacetate in 80-90% yield.

Sodium hydride (37.4 g, 1.56 mol as dry powder) was weighed under an inert atmosphere (nitrogen or argon) and then covered with 300 ml dry diethyl ether. To this stirred suspension was added 350 g (1.56 mol) triethylphosphonoacetate in 100 ml diethyl ether. The solution turned to a clear amber color within approximately 2 hr. To this solution was added dropwise 100 g β-ionone 1 (0.52 mol; Aldrich) in 200 ml diethyl ether, and the reaction mixture was stirred overnight. When all the β-ionone had reacted (as shown by thin-layer chromatography), 250 ml water was added at a rate just slow enough to prevent boiling. The reaction mixture was then poured into 800 ml hexane and washed with several volumes of water. The organic layer was dried ($MgSO_4$), filtered, and concentrated with a rotary evaporator. The yield of all-trans ethyl-beta-ionylideneacetate 2 was 92%.

Lithium aluminum hydride (12.8 g, 0.34 mol) was stirred with 0.5 liter anhydrous diethyl ether on a dry ice-acetone bath at -70°. To this solution was added dropwise 80 g (0.31 mol) ethyl β-ionylideneacetate 2 in 300 ml diethyl ether; this solution was stirred 1 hr and then allowed to warm to room temperature. The reduction of the acid ester to the alcohol was monitored by TLC; if some of the acid ester remained, the solution was again cooled, and additional $LiAlH_4$ was added. When the ester was completely reduced, the mixture was again cooled, and 1 N H_2SO_4 was added dropwise to deactivate the remaining $LiAlH_4$ and to dissolve the voluminous aluminum hydroxide precipitate. The organic layer was then poured into 500 ml diethyl ether and washed several times with 1 N H_2SO_4 and water. The organic layer was dried ($MgSO_4$), filtered,

and evaporated to yield 60 g beta-ionylidene ethanol 3 (90%). Only minor impurities were detected by TLC.

β-ionylidene ethanol 3 (55 g, 0.25 mol) in 1.5 l hexane was oxidized to beta-ionylidene acetaldehyde 4 by stirring with 275 g manganese (VI) oxide (Aldrich) overnight. The MnO_2 was removed by vacuum filtration and washed with dichloromethane. The filtrate was rotary-evaporated to yield 51.5 g beta-ionylidene acetaldehyde (94.5% yield).

Beta-ionylidene acetaldehyde 4 (45 g, 0.21 mol) in 1500 ml acetone was treated with 100 ml 1 N NaOH and stirred for 5 hr. The solution was then divided in half, and each half was poured into hexane, washed with water (6 x 1 l), dried ($MgSO_4$), and filtered. The solutions were recombined, and solvent was removed by rotary evaporation to yield 48.9 g C-18 tetraene ketone 5 (20% cis, 80% trans), a 92% yield.

Either all-trans C-18 tetraene ketone (11 g, 0.04 mol) purified by normal-phase HPLC (hexane:ethyl acetate, 98:2) or the crude cis/trans mixture (ca. 80% trans) was dissolved in 60 ml freshly distilled pyridine. Deuterium oxide (99.8%, 28 g; Columbia Organic Chemicals Co., Columbia, SC) and 10 drops 40% NaO^2H (Aldrich) were added, and the solution was stirred for 1 hr. The solution turned a dark red; as indicated by TLC, some of the ketone is degraded during this step. The reaction mixture was poured into 400 ml hexane and repeatedly washed with water (5 x 400 ml) until the pyridine was removed, then dried ($MgSO_4$), and concentrated by rotary evaporation. The reaction was repeated until the deuterium incorporation was >98% (as determined by NMR or MS). Overall yield (after purification) was 6.8 g (62%) trideutero C-18 tetraene ketone.

Sodium hydride (1.38 g, 0.058 mol) was weighed in an inert atmosphere and stirred with 250 ml anhydrous diethyl ether. Triethylphosphonoacetate (12.88 g, 0.057 mol) was added dropwise in 100 ml diethyl ether at a rate allowing for slow evolution of hydrogen gas. The initial cloudy suspension became clear and amber after stirring for 2 hr. 18,18,18-$^2H_3$-Tetraene ketone

5 (5 g, 0.019 mol) was added dropwise in 50 ml anhydrous diethyl ether. The reaction mixture was stirred overnight, and 100 ml water was then added to quench the reaction. The solution was poured into 1 l of hexane and washed with water (5 x 700 ml). The organic layer was dried ($MgSO_4$) and concentrated to yield 20,20,20-$^2H_3$-retinoic acid ethyl ester 6 (4 g, 0.012 moles) in 65% yield (60% trans, 40% cis) as determined by UV absorption (E=45,600 at 350 nm). Deuterium incorporation was judged to be: $^2H_0$, 6.3%; $^2H_1$, 6.8% $^2H_2$, 30.0%; $^2H_3$, 56.9%.

20,20,20-$^2H_3$-Retinol 7 was prepared by adding 20,20,20-$^2H_3$-retinoicacid ethyl ester (5 g, 0.015 mol, in 100 ml diethyl ether) dropwise to 0.57 (0.015 mol) $LiAlH_4$ in 250 ml anhydrous diethyl ether at -70°, with stirring. After being stirred 30 min at -70°, the solution was allowed to warm to room temperature; if reduction was not complete as judged by TLC, the solution was cooled, and more $LiAlH_4$ added. The reaction was quenched by careful addition of 1 N H_2SO_4 to the cooled solution. The mixture was warmed to room temperature, washed with 1 N H_2SO_4 (2 x 200 ml) and then with water (3 x 200 ml), dried ($MgSO_4$), and rotary evaporated to give 3.65 g (85% yield) of crude product.

20,20,20-$^2H_3$-Retinol 7 (1 g, 0.003 mol) was dissolved in 10 ml pyridine and cooled in an ice bath. Acetic anhydride (0.28 ml, 0.003 mol) was added dropwise. After being stirred 15 min, the solution was allowed to warm to room temperature and checked by TLC; if acetylation was not complete, the solution was cooled, and more acetic anhydride was added. Ice was then added to destroy residual acetic anhydride. The solution was poured into 250 ml hexane and washed with water (6 x 250 ml) to remove pyridine. The organic layer was dried ($MgSO_4$) and rotory-evaporated to give an overall crude yield of 60% (40% cis, 60% all-trans) 20,20,20-$^2H_3$-retinyl acetate 8. The isomers were separated by HPLC (hexane:ethyl acetate, 98:2).

All-trans $^2H_3$-retinyl acetate could be prepared from the cis isomers by dissolving up to 1 g of the cis isomers in 100 ml hexane and adding a small

crystal of iodine. After being stirred 1 hr, the organic layer was washed with 100 ml 5% $NaHSO_3$, then with water (2 x 100 ml), and was then dried ($MgSO_4$). The all-trans ester was purified from the equilibrium mixture (55% all-trans, 35% 13-cis, 10% other cis forms) by HPLC.

Preparation of 14,20,20,20-tetradeutero retinyl acetate

The preparation of this tetradeuterated species is identical to that of 20,20,20-$^2H_3$-retinyl acetate, with the exception that dideuterotriethylphosphonoacetate is used in place of the phosphonate carbanion in the final Wittig-Horner reaction of the synthesis scheme. Triethylphosphonoacetate (175 g, 0.78 mol) was stirred with 310 g deuterium oxide and 5 ml 40% NaO^2H for 2 hr. The reaction mixture was poured into 500 ml diethyl ether, and the aqueous layer was extracted with diethyl ether (5 x 300 ml). The combined ether extracts were dried ($MgSO_4$) twice. The deuterated phosphonate, recovered after rotary evaporation of the solvent, was again dissolved in deuterium oxide (200 g) and stirred with 70 drops NaO^2H for 2 hr. After extraction with diethyl ether by the same procedure, 152 g (87%) dideuterotriethylphosphonoacetate was recovered; NMR showed >99% dideuterated species.

When dideuterated triethylphosphonoacetate was condensed with the trideutero C-18 tetraene ketone, 14,20,20,20-$^2H_4$ retinoic acid ethyl ester 6 was formed with the following deuterium content, as determined by MS: $^2H_4$, 76%; $^2H_3$, 21%; $^2H_2$, 3%.

Preparation of 12,14,20,20,20 pentadeutero retinyl acetate

The procedure outlined for the synthesis of 14,20,20,20-$^2H_4$-retinyl acetate was used with the following modification. β-Ionylidene acetaldehyde 4 (5 g, 0.023 mol) was dissolved in 80 g (1.25 mol) $^2H_6$-acetone (Norrell, Inc., Landisville, NJ) and stirred during the addition of 2 ml 1 N $NaO^2H/^2H_2O$. The solution was stirred for 12 hr and then poured into 300 ml hexane and 20 ml 1 N

H_2SO_4. The hexane layer was dried ($MgSO_4$), filtered, and rotary-evaporated under high vacuum. The product, tetradeuterated C-18 tetraene ketone 5, was reacted with the deuterated triethylphosphonoacetate carbanion to yield pentadeuterated ethyl retinoate. After reduction and acetylation, the resulting 12,14,20,20,20-pentadeutero retinyl acetate 8 showed the following deuterium incorporation by MS: $^2H_0$, 0.1%; $^2H_1$, 3.8%; $^2H_2$, 11.3%; $^2H_3$, 18.3%; $^2H_4$, 27.0%; $^2H_5$, 39.5%.

Preparation of 10,19,19,19-$^2H_4$-retinyl acetate

Trideuterated β-ionone 1 was synthesized according to the procedure of Johansen and Liaaen-Jensen (11). Briefly, 50 g (0.26 mol) β-ionone was stirred with 156 g (7.8 mol) deuterium oxide. The emulsion that formed was dispersed by adding freshly distilled pyridine until the solution cleared. Then, 50 drops of 40% NaO^2H were added, and the solution was stirred for 3 hr. The solution was poured into 500 ml hexane, and the aqueous layer was removed. The organic layer was washed repeatedly (6 x 400 ml water) to remove pyridine, then was dried ($MgSO_4$), filtered, and rotoevaporated. The deuteration reaction was repeated once. The yield was 45 g (90% yield) of 13,13,13 trideutero beta-ionone, with deuterium incorporation of >98% $^2H_3$, as measured by NMR.

The tri-deuterated β-ionone 1 was used in a Wittig-Horner reaction with deuterated phosphonate to give tetradeuterated ethyl β-ionylidene acetate 2. The methods for the synthesis of 20,20,20-$^2H_3$-retinyl acetate were used to complete the synthesis, except that nondeuterated reactants were employed for the remaining addition reactions. Deuterium incorporation, as determined by NMR and GLC-MS, was >98% $^2H_4$ in the final product, 10,19,19,19-$^2H_4$-retinyl acetate 8.

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