PREPARATION OF HIGH SPECIFIC ACTIVITY ALL TRANS-α-RETINYL-11-^{3}H ACETATE

Ronnie L. Hale, Walter Burger, Clark W. Perry
and Arnold A. Liebman*
Chemical Research Department, Hoffmann-La Roche, Inc.
Nutley, New Jersey 07110, U. S. A.

Received March 25, 1976
Revised June 28, 1976

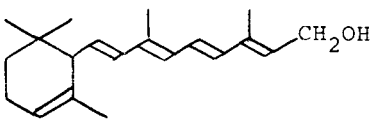
SUMMARY

Lithium borotritide reduction of α-ionylideneacetaldehyde (5) followed by manganese dioxide oxidation provided the tritiated aldehyde (9) which retained over 95% of the label. On treatment with the ylide derived from ethyl 4-chloro-3-methylcrotonate, ethyl α-retinoate-11-^{3}H (14) was obtained which, after purification, was hydrolyzed to α-retinoic-11-^{3}H acid (15). Conversion to the methyl ester (16) followed by lithium aluminum hydride reduction yielded all trans-α-retinol (17) which was isolated as the acetate derivative (18).

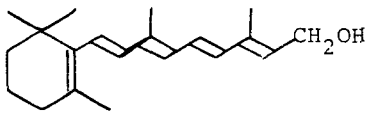
Key Words: Lithium borotritide, Manganese dioxide oxidation, Wittig reaction, α-retinyl-11-^{3}H acetate.

INTRODUCTION AND DISCUSSION

A number of syntheses have been reported [1-3] of α-vitamin A acid and α-vitamin A (1), an isomer of naturally occurring vitamin A (2). Since the natural vitamin (β-retinol) is of the all trans configuration, for biological comparison [4], α-retinol should be similarly of the all trans configuration.



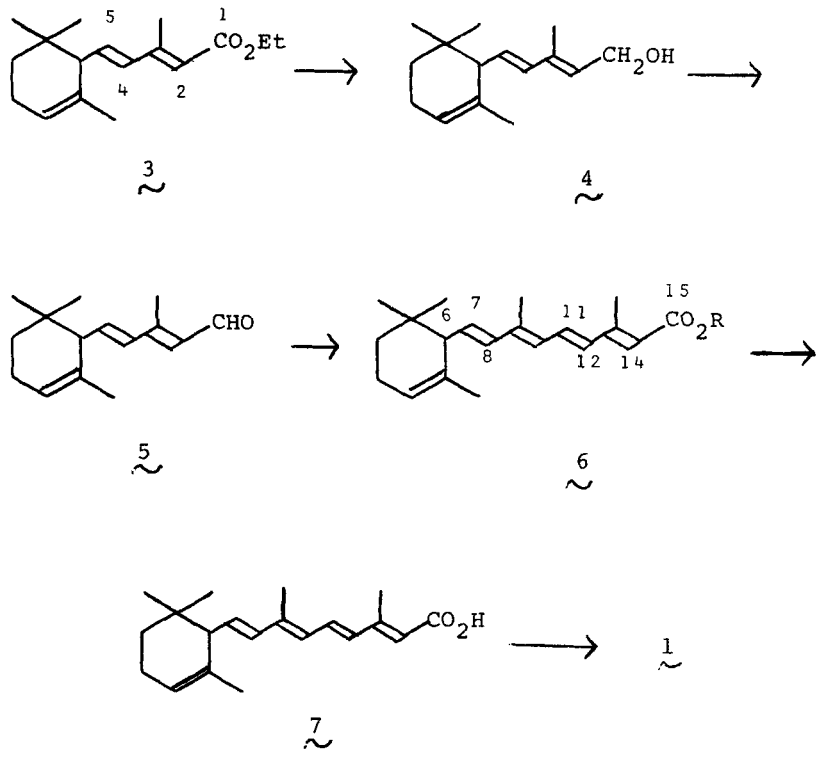
1



2

The reported syntheses offer limited spectral or other analytical data concerning the configuration of the products obtained which most probably consist of mixtures of geometric isomers. We were interested in preparing high specific activity tritium labelled α-retinol of the all trans configuration. The existing procedures were examined in considerable detail and improved in several instances. This we now wish to report along with the methods developed for the isolation and characterization of the desired compounds.

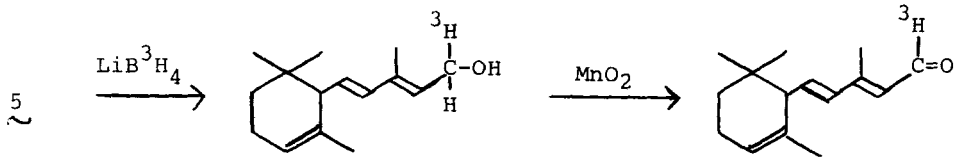
From ethyl α-ionylideneacetate (3)[5] the synthesis of α-vitamin A has been accomplished via a two step conversion to α-ionylideneacetaldehyde (5) which has then been condensed with methyl-β-methylglutaconate [1] or ethyl senecioate[2] or ethyl 4-chloro-3-methylcrotonate[3] to supply the remaining five carbon atoms of the vitamin A skeleton. An ester (6) of α-retinoic acid (7) was obtained which was partially purified by hydrolysis to 7 and the acidic fraction was re-esterified and reduced to α-retinol (1). This sequence is amenable to the preparation of isotopically labelled analogs since the label can be inserted specifically and at a relatively late stage of the synthesis. Condendation of β-ionylideneacetaldehyde with carbon-14 labelled ethyl senecioate has ultimately led to formation of ^{14}C labelled vitamin A and derivatives[6]. Also in the β-series, reduction of the C-15 aldehyde with a deuterium or tritium containing reagent yields the correspondingly labelled β-ionylidene ethanol; reoxidation with manganese dioxide was observed[6] to proceed with a significant isotope effect such that retention of the label occurs in



3 4 5 6 7 1

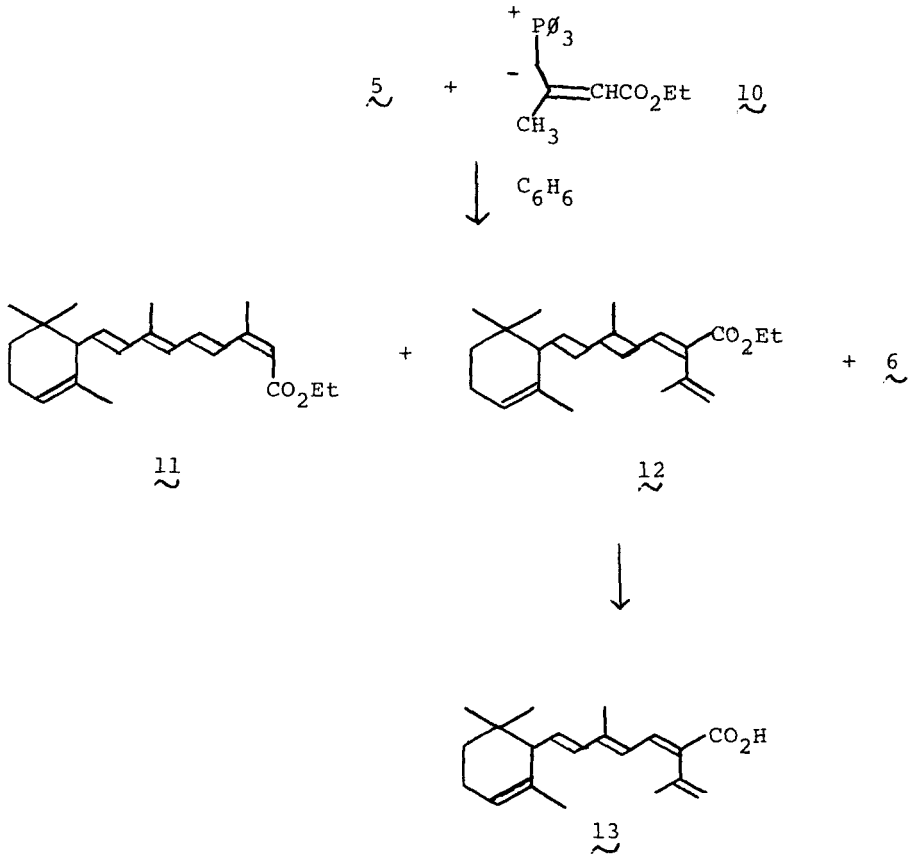
the resulting aldehyde. The above synthesis has been used to prepare vitamin A-11-^{3}H and derivatives (>1 Ci/mmole) after completion of the synthesis *via* the ethyl senecioate condensation.

To provide isomerically pure aldehyde (5) in the α-series, the methyl ester of crystalline 2-*trans*, 4-*trans*-α-ionylidene acetic acid was reduced with "Red-Al" to the isomerically pure alcohol (4) which was oxidized with manganese dioxide to afford the aldehyde. Subsequent reduction with sodium borodeuteride and reoxidation with manganese dioxide showed that greater than 95% of the deuterium at C-1 was retained during the oxidation. Similarly, reduction of (5) with lithium borotritide[7] (15 Ci/mmole) and oxidation of the resulting alcohol (8) with manganese dioxide gave the desired C-1 tritiated aldehyde (9) with specific activity of 2-3 Ci/mmole.



8 9

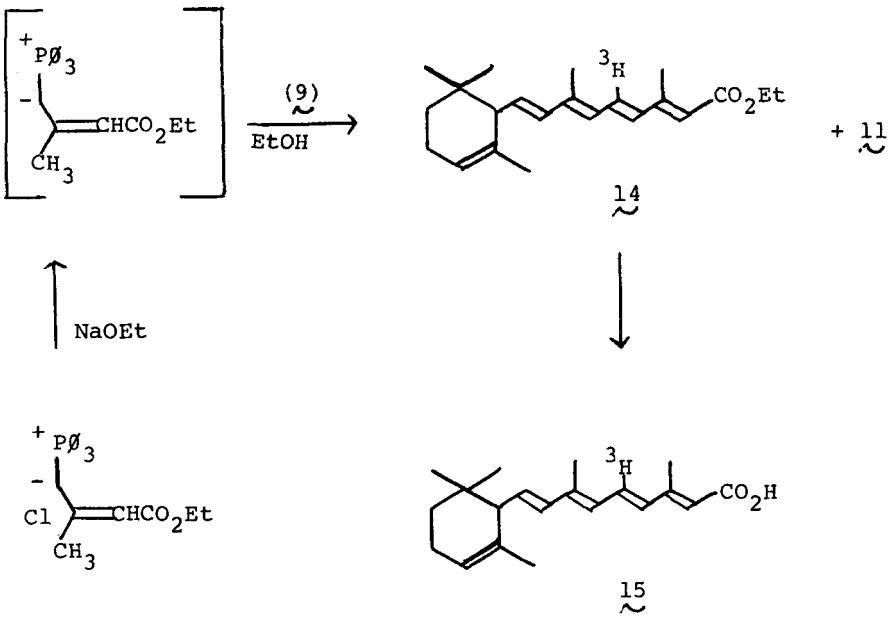
The Wittig condensation of (5) and (10) was found to be highly dependent on solvent and base. With preformed ylide (10) and benzene as solvent, a mixture of isomeric α-retinoic acid esters was formed from which the known 13-cis isomer (11), the desired all trans ester (6) and the anomolous addition product (12) were obtained. The latter was identified as the corresponding acid (13) by hydrolysis of the ester mixture, crystallization and comparison with a sample of (13) synthesized by an alternate route [8]



11 12 13

The formation of some 12 is not unexpected considering the known reactivity of allylic ylides[9].

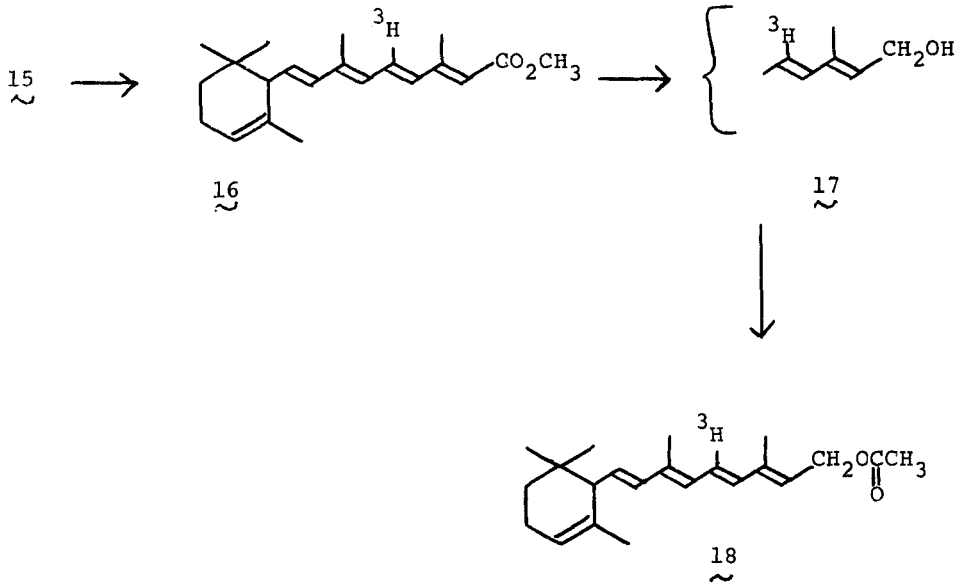
When the ylide (10) was generated with sodium ethoxide in ethanol solution and then added to an ethanol solution of (5), the reaction product consisted of the 13-cis ester (11) and the desired all trans ester (6) in a ratio of about 3:1. To our advantage, this ratio was reversed when high specific activity tritium labelled (5) [aldehyde (9)] was used, thereby providing a reasonable yield of the desired ester (14) labelled at C-11. The ester mixture was chromatographed over silica gel and the eluate highly enriched with 14 was hydrolyzed. The resulting mixture was treated with iodine[10] and the all trans α-retinoic-11-^{3}H



acid (15) was then isolated by crystallization. Interestingly, the relatively high endogenous radiation, which presumably effected a favorable isomer ratio in the Wittig reaction, now influenced iso-

merization of the all trans acid, in solution, to the 13-cis isomer as the mother liquors from which 15 crystallized became increasingly enriched in the 13-cis acid.

The pure all trans acid (15) was converted to the ester (16) with diazomethane and reduced with lithium aluminum hydride at -50° to α-vitamin A-11-^{3}H (17). Attempts to reduce the partially purified ester (14) resulted in a complex mixture of isomeric products. On the purified acid, however, reduction after esterification was straightforward at the low temperature indicated. The acid (15) was found to be moderately stable but the alcohol (17) was quite unstable. The acetate derivative (18) was considerably more stable and was therefore quickly prepared using acetyl chloride and pyridine. The product was purified by chromatography over alumina furnishing pure α-retinyl-11-^{3}H acetate with specific activity of 2.65 Ci/mmole. The α-retinyl acetate prepared in this manner was shown by NMR[4] to be free of β-retinyl acetate.



Experimental

Melting and boiling points are uncorrected. All solvents were distilled prior to use. Radiochemical purity was determined on thin layer chromatograms with a Packard Model 7201 Radiochromatogram Scanner System.

D,L-3-Methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-trans, 4-trans-pentadienoic acid, (2-trans, 4-trans-α-ionylidene acetic acid).- A solution of potassium hydroxide (5g) in the minimum amount of water was flushed with argon and then treated with a solution of 5g (19.1 mmole) of ethyl α-ionylidene acetate (approximately 60% trans-40% cis) in 150 ml of methanol. The resulting mixture was refluxed for one hour, cooled then added to cracked ice. Hydrochloric acid was added to pH3 and the crude product was then extracted with $CHCl_3$. Removing the solvent under reduced pressure left a residual oil which was crystallized from acetonitrile (3 crops) providing 2.79g (58%) of all trans product, m.p. 98-99° (Found: C, 77.1; H, 9.5. $C_{15}H_{22}O_2$ theoretical: C, 76.9; H, 9.5%) λmax (ethanol) 254 nm ε=24,800. The acid was treated with diazomethane to give the methyl ester, which was used without further purification.

D,L-3-Methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-trans, 4- trans-pentadien-1-01 (2-trans, 4-trans-α-ionylidene ethanol (4).- A solution of 2.4g of the methyl ester, prepared as described above, in 12 ml of benzene was cooled to 5°C and with stirring was treated dropwise with 3.5 ml of a 70% benzene solution of sodium dihydrido-bis (2-methoxy ethoxy) aluminate. The resulting mixture was stirred for two hours at room temperature and then treated with 10 ml of water-saturated ether followed by 50 ml of water. The mixture was filtered and the filtrate was extracted with ether which was then concentrated to a residual oil of 2.05g (9.3 mmole). By thin later chromatography (silica gel; benzene-ethyl acetate,

9:1), the product consisted of >99% alcohol (4)[1-3] at R_f0.33 and a trace of the starting ester at R_f0.8.

D,L-3-Methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-trans, 4-trans-α-ionylidene acetaldehyde) (5).- The alcohol (4) obtained above was dissolved in 50 ml of dichloromethane and stirred with 11.5g of manganese dioxide at room temperature for 16 hr. After filtration the product was purified by chromatography over silica gel (E. Merck No. 7734, 100g) eluting with hexane-ethyl acetate, 35:1. Molecular distillation of the product (90-100°C, 0.01 mm) yielded 1.77g of the aldehyde which was 97-98% pure by tlc. Reduction of (5) with sodium borodeuteride (>95% d_4) in tetrahydrofuran yielded the 1-deutero derivative of (4) which was purified by column chromatography (silica gel; hexane-ethyl acetate, 7:1) with d_1 content of 89.8%. Reoxidation with MnO_2 as described[6] yielded the 1-deutereo derivative of (5) with the same d_1 content (90.4%) after purification.

Lithium borotritide.- A heavy-walled pyrex tube (5x20 cm) fitted with a high-vacuum stopcock was charged with 11 mg (0.5 mmole) of lithium borohydride. The tube was flushed with hydrogen, evacuated then heated to 230°C. After cooling, hydrogen was added to a pressure of 45 cm of Hg and the tube was again heated to 230° where it was kept for 1 hour. After cooling, the tube was again evacuated to <1μ and 20 Ci of carrier-free tritium gas was admitted. The tube was closed and heated to 225° where it was maintained for 3 1/2 days when no further exchange occurred. During this period, the exchange was monitored as described[7] using benzaldehyde or other readily reducible carbonyl. Final specific activity was approximately 15 Ci/mmole.

D,L-3-Methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-^{3}H-2-

trans, 4-trans-pentadien-1-ol (8).- The lithium borotritide obtained above was dissolved in 0.5 ml of peroxide free tetrahydrofuran in the tube used for the exchange. The solution was cooled to 5° and with magnetic stirring a solution of the all trans aldehyde (5), 460 mg (2.1 mmole) in 2 ml of THF was added and the resulting mixture was stirred at room temperature for 20 hours when a thin layer chromatographic probe (silica gel, benzene elution) showed the reaction to be complete. The mixture was transferred to a liquid-liquid extractor, treated with water, then continuously extracted with ether which on evaporation, provided the labelled alcohol (8) as a light yellow oil which was not further purified.

D,L-3-Methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-^{3}H-2-trans, 4-trans-pentadienal (9).- The alcohol (8) obtained above, dissolved in 15 ml of dichloromethane, was treated with 2.5 g of manganese dioxide and the resulting mixture was stirred at room temperature for 16 hr. The mixture was filtered, and the filtrate was concentrated in vacuo to a residue of 420 mg (1.92 mmole) of (9), 91%, homogenous and >98% radiochemically pure by tlc.

D,L-2-Isopropenyl-5-methyl-7-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-trans, 4-trans, 6-trans-heptatrienoic acid (13).- The ylide (10), 550 mg, 1.4 mmole) was prepared from the corresponding phosphonium salt (680 mg, 1.6 mmole) by exactly neutralizing an aqueous solution of the salt with 0.1N sodium hydroxide at 0° and filtering the bright yellow solid which immediately precipitated. The ylide was dried under a stream of dry nitrogen and then dissolved in 25 ml of benzene. A solution of (5),(308 mg, 14 mmole) in 5 ml of benzene was added and the resulting mixture then heated to 80° where it was held for 3 hr. The mixture was evaporated to about one-half the original volume, cooled, and

treated with 10 ml of cold hexane to precipitate triphenylphosphine oxide (345 mg, 1.2 mmole, obtained in two crops). Evaporation of the filtrate provided a residual oil which on filtration chromatography through 10 g of silica (E. Merck No. 7734) packed in hexane , separated 75 mg of unreacted (5) and 228 mg of an ester mixture. By gas chromatography (all glass 6' column, 3% UCW-98 liquid phase at 178° and He as mobile phase), the mixture contained approximately 10% phosphine, 60% of an unknown material and about 15% each of the 13 cis isomer (11) and the desired all trans isomer (6). Further column chromatography on 11.4 g of silica packed in benzene and elution with benzene effectively separated these 4 compounds. The unknown material was treated with methanolic potassium hydroxide (2g in 3 ml) for 1 hour at 60-70°, cooled, diluted with water and extracted with ether. The aqueous solution was then acidified to pH2 (H_3PO_4) and again extracted with ether. The extract was dried and concentrated in vacuo to a solid residue which was crystallized to provide 119 mg of product, m.p. 160-161° (from hexane-ether) λmax 323 nm (hexane) ε39,500, $\delta(CDCl_3)$ compatible with theory including chemical shifts at 5.24 and 4.89, dd, $>C=C<^{H}_{H}$

D,L-Ethyl α-retinoate-11-^{3}H (14).- The phosphonium salt, (trans-3-ethoxy carbonyl-2-methyl allyl) triphenylphosphonium chloride, 1.27 g, 3 mmole, was dissolved in 5 ml of dry ethanol, cooled to 0° and treated with the dropwise addition of 5 ml of 0.54 N sodium ethoxide solution. The tritiated aldehyde (9), obtained above, in 2 ml of ethanol, was then treated with the ylide solution and the resulting mixture then stirred at room temperature for 16 hr. The mixture was treated with water and then continuously extracted with petroleum ether (b.p. 30-60°) to a constant level of radioactivity. The extract was concentrated in vacuo to a residual oil which was chromatographed on silica gel using (E. Merck No. 7734) 50 times the weight of residue packed and eluted with benzene, which separated the 13-cis ester (11)

from the desired all trans ester (14). The chromatography was carried out in an inert atmosphere to maintain stability and the products were kept in solution and not further purified.

D,L α-retinoic-11-^{3}H acid (15). - The fractions containing trans ester (14) obtained above were combined in an inert atmosphere then concentrated in vacuo. The residue, dissolved in about 1 ml of absolute ethanol, was treated with a solution of 500 mg of potassium hydroxide in 0.5 ml of water and 5 ml of absolute ethanol with stirring at 60° for 1 hr. The mixture was cooled, acidified with phosphoric acid and continuously extracted with ether. After concentrating the extract to a small volume, 1 ml of hexane containing 1 mg of iodine was added and the resulting mixture was maintained at room temperature for 3 hr, cooled to -20° for 16 hr. The resulting crystals were filtered off and washed with cold hexane, dissolved in ether then treated with solid sodium thiosulfate (3-5 mg) for 10 minutes. The mixture was filtered, the filtrate concentrated to a solid which was again crystallized from ether-hexane at -20° to provide 35.7 mg (0.12 mmole) of product (15), homogeneous by tlc (silica gel; benzene-ethyl acetate, 8:2) and having specific activity of 2.65 Ci/mmole.

D,L-Methyl α-retinoate-11-^{3}H (16).-The filtrate from which pure 15 crystallized was shown by tlc to contain additional all trans-α-retinoic acid and 13-cis-α-retinoic acids. After concentration to a small volume, this mixture was applied to a column of 100 times its weight of silica gel (E. Merck No. 7734) packed in benzene and flushed with dry argon. Elution with benzene, methanol, ether (100:5:15) and collection into amber glass tubes resulted in separating the mixture and obtaining an additional 100 mCi of pure 15. After concentration to dryness in vacuo, the residue was treated with an excess of diazomethane in ether for 10 minutes at room temperature, filtered through a cm^3 of silica gel then concentrated to dryness in vacuo yielding 16 which was not further purified.

D,L α-retinyl-11-^{3}H acetate (18). - The ester (16) obtained above, dissolved in 2 ml of dry ether, was added over a 5 minute period at -80° to 0.5 ml of 0.2 M $LiAlH_4$ in 2 ml of dry ether. The resulting mixture was then warmed to -60° over a 15 minute period and then stirred at -55° for 1 hr. Ice cold saturated NH_4Cl solution was added cautiously followed by 5 ml of petroleum ether. The resulting organic layer was separated and evaporated to a residue (17) which was immediately dissolved in 2 ml of dry ether.

The solution, under argon, was cooled to 5° and with stirring 0.2 ml anhydrous pyridine and 22 mg (0.28 mmole) acetyl chloride was added. The resulting mixture was stirred at 5° for 10 min then at 25° for 30 min then refrigerated overnight. The mixture was successively extracted with 10 ml of ice-water, 10 ml of cold 1% Na_2CO_3 solution, 10 ml of ice-water. The organic phase was then concentrated and the residue purified by column chromatography over activity III alumina (Woelm) (1:500) eluting with benzene. Fractions containing only (18) were pooled to provide 62 mCi (0.023 mmole @ 2.65 Ci/mmole). Radiochemical purity exceeded 99%.

Nonradioactive 18 was obtained in 66.5% yield from pure α-retinoic acid by the above procedures providing a colorless solid of m.p. 43-43.5°.

Acknowledgement

We thank Dr. R. P. W. Scott and his staff in our Physical Chemistry Department, in particular, Dr. W. Benz for mass spectra, Dr. F. Scheidl for microanalysis, Dr. V. Toome for u.v. spectra, and Dr. T. Williams for NMR spectra.

References

1. Robeson C.D., Cawley J.D., Weisler L., Stern M.H., Eddinger C.C. and Chechak A.J. - J.Amer.Chem.Soc. 77:4111 (1955)

2. Manchand P.S., Rüegg R., Schwieter U., Siddons P.T. and Weedon B.C.L. - J.Chem.Soc. 2019 (1965)

3. Rigassi N. - unpublished data

4. Clamon G.H., Sporn M.B., Smith J.M. and Henderson W.R.- J.Nutrition 105: 215 (1975)

5. Young W.G., Andrews L.J. and Cristol S.J. - J.Amer.Chem. Soc. 66: 520 (1944)

6. Würsch J. - unpublished data

7. Smith N.H., Wilzbach K.E. and Braun W.G. - J.Amer.Chem. Soc. 77: 1033 (1955)

8. Pawson B. - unpublished data

9. Howe R.K. - J.Amer.Chem.Soc. 93: 3457 (1971)

10. Garbers C.F., Eugster C.H. and Karrer P. - Helv.Chim. Acta 36: 562 (1953)