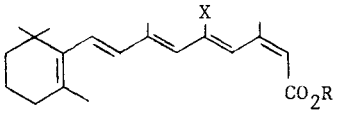
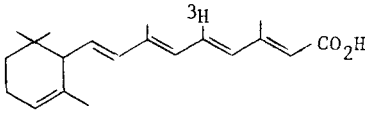
SYNTHESIS OF 13-cis-(11-^{3}H)-RETINOIC ACID

The biological activity of 13-cis-retinoic acid 1a in prevention of epithelial cancer in animals has recently been demonstrated [1]. In connection with a metabolic studies program, we have prepared 13-cis-(11-^{3}H)-retinoic acid 1b from β-ionylideneacetaldehyde via a reaction sequence similar to the one reported by Liebman and co-workers [2] for the synthesis of trans-(11-^{3}H)-α-retinoic acid 2 (see Scheme 1).



1a. R = X = H
b. R = H, X = ^{3}H
c. R = Et, X = ^{3}H



2

The tritiated β-ionylideneacetaldehyde 3 was prepared from the unlabeled aldehyde by reduction with sodium borotritide followed by reoxidation with manganese dioxide, with a net retention of over 75% of the tritium. The phosphonium ylide 4 used in the Wittig reaction was generated in situ from a mixture of cis- and trans-phosphonium chloride 8 by sodium ethoxide in ethanol [3]. Under these reaction conditions, the Wittig condensation of the tritiated aldehyde 3 with the ylide 4 proceeded normally to give a mixture of four isomeric retinoic esters. The major isomer obtained in this reaction was shown to be the desired 13-cis-retinoate 1c. Interestingly, this isomer was formed in even greater amount in the 'hot' experiment than in the 'cold' runs carried out under the same reaction conditions. A small portion of pure 13-cis-retinoate

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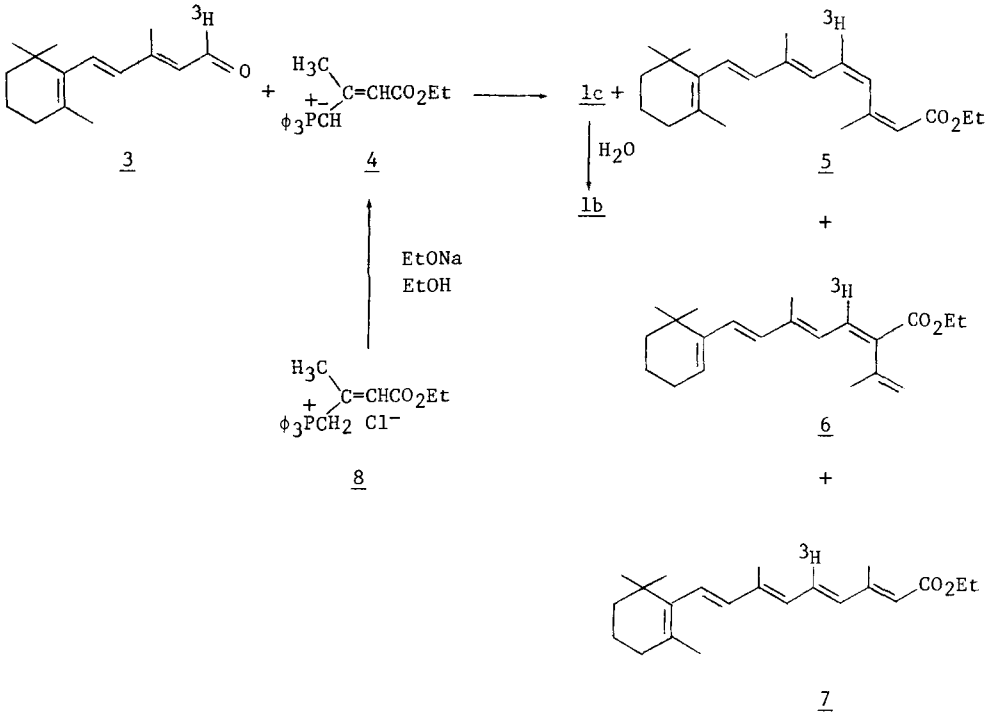
Scheme 1



was separated from the mixture by careful column chromatography over silica gel. Saponification of the ester with alcoholic potassium hydroxide readily afforded 13-cis-(11-^{3}H)-retinoic acid 1b. After dilution with unlabeled 13-cis-retinoic acid and recrystallization, the final product had a specific activity of 1.75 Ci/mmole. Assay by reverse phase HPLC [4] showed that both the chemical and radiochemical purity was greater than 98%.

The $^{3}H\{^{1}H\}$ NMR spectrum of 13-cis-(11-^{3}H)-retinoic acid (Figure 1) shows one major resonance at 6.88 ppm, which may be assigned to tritium at the 11 position [5]. The assignment was confirmed by obtaining the proton coupled spectrum (not shown) which consists of a doublet of doublets with coupling

constants of approximately 12 and 15 Hz. These data are also consistent with those recorded in previous studies. The minor resonances are due to decomposition impurities. It should be noted that the minor resonances were not present in the $^{3}H\{^{1}H\}$ NMR of this same sample taken 3 months earlier.

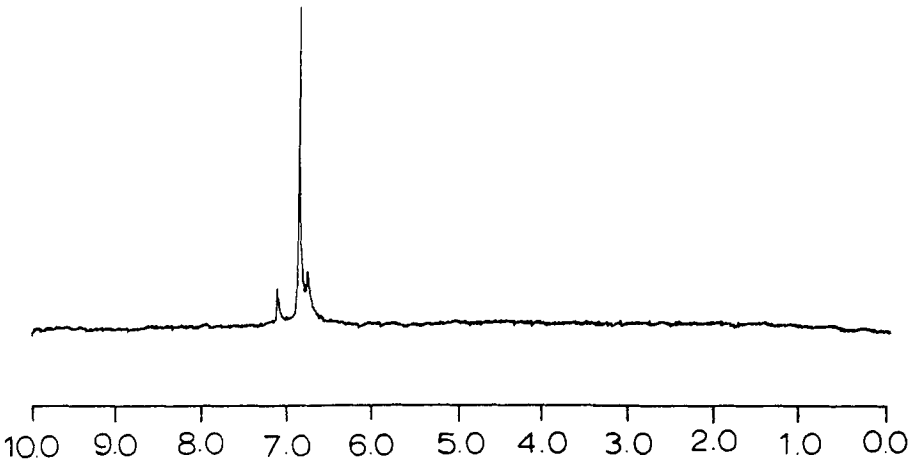


Figure 1 - The $^{3}H\{^{1}H\}$ NMR spectrum of 13-cis-(11-^{3}H)-retinoic acid. The conditions used to obtain this spectrum were: spectral window: 1,500 Hz; data acquisition time: 8.0 sec; data points accumulated: 24,000; pulse width: 72° (100 μ sec); broadband proton noise decoupling was employed and 10,000 transients were accumulated. The spectrum is plotted on a p.p.m. scale with TMS[1-^{3}H] at 0.0 p.p.m.

In addition to the 13-cis-retinoate 1c, three other isomeric products were also formed in the Wittig reaction, but in much smaller amounts. Two of these were identified as the 11,13-di-cis isomer 5 and the trans-retinoate 7 by HPLC analysis and comparison with authentic unlabeled standards. The other product is probably the isomer 6 by analogy with the result of Liebman and co-workers [2].

EXPERIMENTAL

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

In a 100-ml two-neck flask fitted with a stopcock and a rubber septum was placed ∿ 10 Ci of sodium borotritide (purchased from Amersham Corporation with a specific activity of 15 Ci/mmole). After the flask was evacuated, 5 ml of THF was added via a syringe, immediately followed by a solution of 2.1 mmoles of 3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)-2-trans-4-trans-pentadienal [6] in 10 ml of the same solvent. The mixture was stirred at room temperature for 20 hr. Water (5 ml) was then added and all of the volatile substances removed in vacuo. The residue was treated with 5 ml of methanol and evaporated to dryness again in vacuo. This procedure was repeated again and the residue thus obtained was extracted with a total of 200 ml of ether. TLC examination of the extract indicated total absence of the starting aldehyde. The ether extract was dried ($MgSO_4$) and evaporated under reduced pressure. The crude reduction product was dissolved in 100 ml of hexane and stirred overnight, under nitrogen, at room temperature with 2.6 g of manganese dioxide. The oxidizing agent was filtered off and thoroughly washed with hexane. The combined hexane solution was evaporated to give a nearly quantitative yield of the tritiated aldehyde with a radiochemical purity estimated at 90-95% by TLC/radiochromatogram scanning. This aldehyde appeared to deteriorate rapidly on standing in the absence of solvent.

13-cis-(11-^{3}H)-Retinoic Acid (1b)

A solution of 1.89 g (4.5 mmoles) of (3-ethoxycarbonyl-2-methylallyl)-triphenylphosphonium chloride (obtained from a mixture of cis- and trans-3-ethoxycarbonyl-2-methylallyl chloride) in 5 ml of ethanol was treated under

nitrogen at ice bath temperature with a sodium ethoxide solution freshly prepared from 92 mg (4 mg-atoms) of sodium and 5 ml of ethanol. After 5 min stirring, a solution of the tritiated aldehyde (3) in 20 ml of ethanol was added together with 1 mmole of unlabeled aldehyde. The reaction mixture was stirred overnight at room temperature, diluted with water (40 ml), and continuously extracted with hexane for 2 hr. The hexane extract was removed and the extraction was continued with fresh hexane for four more hours. The combined hexane extract was evaporated under reduced pressure to give a residue containing triphenylphosphine oxide and a mixture of isomeric retinoic esters. The esters, after trituration with a little hexane, were separated from triphenylphosphine oxide, which was washed several times with small amounts of hexane. The combined hexane solution was then chromatographed over 110 g of silica gel. The column was eluted first with a mixture of 4:1 hexane:chloroform and then with 3:1 mixture of the same solvent pair according to a pattern developed in runs using unlabeled materials. The elute was monitored by TLC and it was found that the first 30 retinoate fractions (total volume $\sim$ 60 ml) were homogeneous and contained the desired 13-cis-retinoate. These fractions were combined and evaporated to dryness under reduced pressure. The tritiated ethyl 13-cis-retinoate thus obtained was immediately hydrolyzed by boiling with 0.64 g of potassium hydroxide in 30 ml of ethanol and 2 ml of water for 2 hr under a nitrogen blanket. After the reaction mixture was diluted with water (20 ml), the ethanol was evaporated under reduced pressure. The aqueous residue was extracted with ether (50 ml) and the ether extract was washed once with dilute potassium hydroxide. The combined aqueous solution was chilled and acidified with 3N phosphoric acid to pH 3 and then extracted with ether (4 x 50 ml). The combined ether extract was washed with water (3 x 20 ml), dried ($MgSO_4$) and evaporated

to give 60 mg of slightly contaminated 1b. The product was mixed with 10 mg of unlabeled 13-cis-retinoic acid and recrystallized once from methanol to give 45 mg of 13-cis-(11-^{3}H)-retinoic acid as orange yellow needles with a specific activity of 1.75 Ci/mmole. The chemical purity of this product was shown to be greater than 98% by reverse phase HPLC [4] on a Whatman Partisil PXS 10/25 ODS-2 column. The radiochemical purity was determined by a similar HPLC procedure in which 200 fractions of ∿ 0.2 ml each were collected from the column and counted individually. Over 98% of the total activity was found in a sharp single peak corresponding to that of 13-cis-(11-^{3}H)-retinoic acid. The $^{3}H\{^{1}H\}$ NMR spectrum, obtained at 106.7 MHz on a Varian XL-100-12 FT spectrometer especially equipped to handle radioactive samples, showed one resonance at 6.88 ppm.

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