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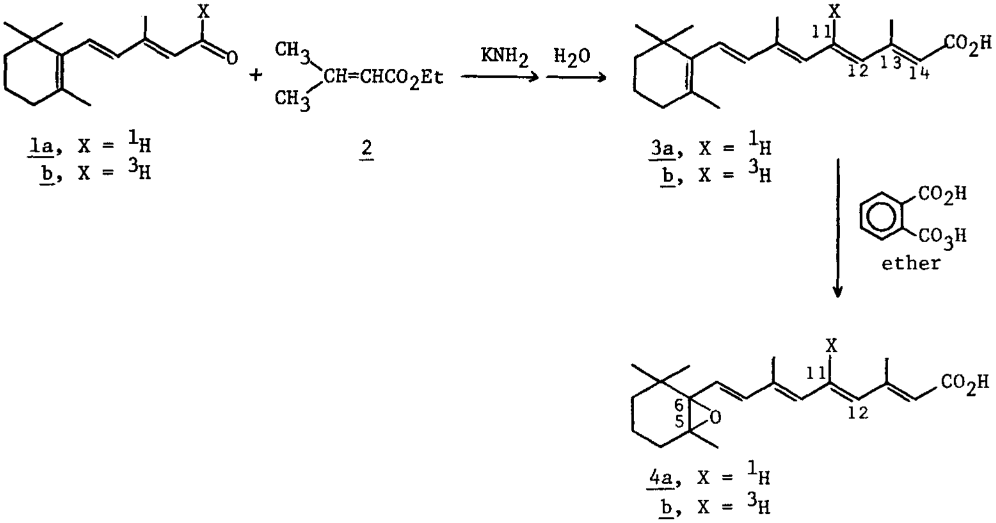
SYNTHESIS OF *trans*-[11-^{3}H]-RETINOIC ACID AND ITS 5,6-EPOXIDE

Recently, *trans*-[13,14-$^{14}C_2$]-retinoic acid was synthesized in our laboratory *via* condensation of β-ionylideneacetaldehyde (1a) with methyl senecioate-$^{14}C_2$ [1] using the reaction conditions reported by Matsui and co-workers [2]. Herein, we report the use of this versatile reaction for the synthesis of *trans*-[11-^{3}H]-retinoic acid (3b). The tritiated intermediate, β-ionylideneacetaldehyde (1b), required for this work was prepared from the unlabeled aldehyde by the method reported earlier [3]. Upon treatment of this aldehyde with ethyl 3-methyl-2-butenoate (2) in the presence of potassium amide in ether, a mixture of retinoic acid esters was obtained, which upon hydrolysis gave a 79% yield of crude retinoic acids consisting of approximately 80% of the desired *trans*-retinoic acid, 15% of the 13-*cis* isomer, and 5% of two unidentified compounds as indicated by reverse phase HPLC analysis [4]. One rapid recrystallization of the crude product from methanol then furnished ≥ 98% pure *trans*-[11-^{3}H]-retinoic acid (3b) with a specific activity of 1.14 Ci/mmol.



1a, X = ^{1}H
b, X = ^{3}H

2

3a, X = ^{1}H
b, X = ^{3}H

4a, X = ^{1}H
b, X = ^{3}H

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The tritium-labeled 5,6-epoxy-trans-retinoic acid (4b) was readily obtained, in good yield, by epoxidation of trans-[11-^{3}H]-retinoic acid with monoperphthalic acid [5,6,7] under reaction conditions which had been carefully worked out with unlabeled retinoic acid 3a prior to the experiment using labeled material. This compound was also characterized by reverse phase HPLC analysis and its radiochemical purity was found to be greater than 97%. The specific activity was 1.14 Ci/mmol.

EXPERIMENTAL

trans-[11-^{3}H]-Retinoic Acid (3b)

To a stirred suspension of potassium amide, prepared just prior to use from 0.51 g of potassium and 25 ml of anhydrous ammonia [1], in 10 ml of ether was added, under argon at -65°, a mixture of freshly prepared tritiated β-ionylideneactealdehyde (1b) (545 mg, 2.5 mmol, 3 Ci) [3] and 486 mg (3.8 mmol) of ethyl 3-methyl-2-butenoate (2) in 15 ml of ether. The mixture was stirred at -65° for 1 hr and then at room temperature for 14 hr. It was then treated with 4 ml of methanol followed by 20 ml of water. After stirring for 1 hr the aqueous layer was removed and the ether layer was extracted [8] with 5 ml of 3 N sodium hydroxide. The combined aqueous solution was acidified with 3 N phosphoric acid to pH 3 and extracted with ether (100 ml). Surprisingly, on evaporation of the solvent, there were only traces of residue remaining [8]. To effect saponification of the ester, the ether solution, which contained neutral substances, was treated with 5 ml of 3 N sodium hydroxide solution and 30 ml of methanol, and the resulting mixture was warmed at 60° for 3 hr while ether was allowed to evaporate. Methanol was removed by evaporation and ether (50 ml) was added.

The aqueous layer was separated and the ether solution was extracted with 5 ml of 3 N sodium hydroxide. The combined aqueous solution was acidified with 3 N phosphoric acid to pH 3 and the product extracted with ether (3 x 30 ml). The combined ether extract was washed with water (3X), dried ($MgSO_4$) and evaporated to give 0.59 g of residue (79% yeild), which, on analysis by reverse phase HPLC on a Whatman Partisil PXS 10/25 ODS-2 column, was shown to contain about 80% of trans-[11-^{3}H]-retinoic acid, 15% of the 13-cis isomer and 5% of two other compounds. The crude product was recrystallized once from methanol to give 155 mg (589 mCi) of $\geq$ 98% pure trans-[11-^{3}H]-retinoic acid (specific activity 1.14 Ci/mmol). The overall radiochemical yield was 20%. No attempt was made to recover more product from the mother liquor.

5,6-Epoxy-trans-[11-^{3}H]-retinoic Acid (4b)

A mixture of 100 mg (0.33 mmol) of trans-[11-^{3}H]-retinoic acid and 2.6 ml of 1.44 M monoperphthalic acid in ether was stirred in the dark, under argon, for 30 min. Upon dilution with ether (30 ml), the mixture was washed successively with 2% sodium bicarbonate (3 x 15 ml) and water (2 x 15 ml). The ether solution was dried ($MgSO_4$) and evaporated. The residue was recrystallized from cyclohexane to give 65 mg (64%) of the desired 5,6-epoxy-trans-[11-^{3}H]-retinoic acid as pale yellow needles. It was characterized by HPLC analysis using a fully identified (elemental analysis, NMR, MS) unlabeled epoxy-retinoic acid 4a as standard. The radiochemical purity of this product was determined by HPLC to be greater than 97%. The specific activity was 1.14 Ci/mmol.

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8. Usually, retinoic acids are formed directly in this reaction. However, in this particular experiment the product was found to be retinoic esters which therefore remained in the ether solution.