Preparation of [4-^{3}H]-3-Dehydroretinol by Sodium Borotritide Reduction

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Summary

[4-^{3}H]-Dehydroretinol (5) was prepared from a vitamin A derivative. The procedure involves conversion of methyl retinoate (1) to methyl 4-oxoretinoate (2) by manganese dioxide oxidation. The ^{3}H-label was introduced by sodium borotritide reduction of methyl 4-oxoretinoate (2) followed by acid catalyzed dehydration to methyl [4-^{3}H]-3-dehydroretinoate (4). Reduction of the tritiated methyl dehydroretinoate with lithium aluminium hydride yielded [4-^{3}H]-3-dehydroretinol (5). Final purification was achieved by preparative thin layer chromatography.

Key words: [4-^{3}H]-3-Dehydroretinol, methyl [4-^{3}H]-3-dehydroretinoate
methyl 4-oxoretinoate, sodium borotritide

Introduction and Discussion

3-Dehydroretinol (vitamin A_2) is a naturally occurring analogue of retinol (vitamin A_1). It is the preponderant retinoid in fresh-water vertebrates[1] and has nearly half the activity of retinol in rat growth[2,3]. Although conversion of 3-dehydroretinol to retinol in mammals has been reported in several studies[4-7], the interpretation is not unequivocal[8]. It is, therefore, important to

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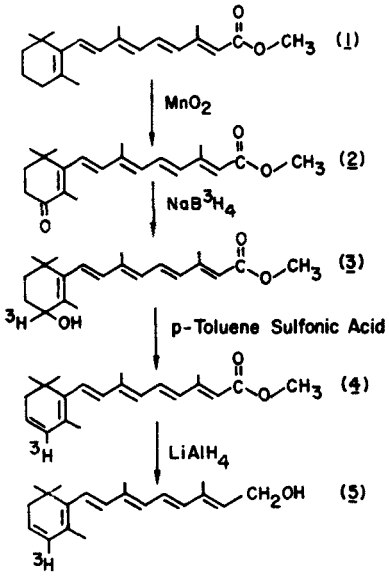
have radioactively labelled 3-dehydroretinol for in vivo studies of vitamin A_2 in mammals. Labelling of the cyclohexyl ring would be desirable so that the label would survive any extensive degradation of the polyene side-chain. We wish to report a method to introduce a ^3H-label into the cyclohexyl ring by sodium borotritide reduction of methyl 4-oxoretinoate (2).



Scheme 1 Preparation of [4-^3H]-3-dehydroretinol from methyl retinoate

The procedure as shown in scheme 1 starts from methyl retinoate (1) which was oxidised by manganese dioxide according to the method of Roa et al[9] providing methyl 4-oxoretinoate (2). Freshly prepared manganese dioxide according to the method of Attenburrow et al[10] is essential in order to obtain a satisfactory yield.

The ^3H-label was introduced by sodium borotritide reduction of methyl 4-oxoretinoate (2) to afford the tritiated methyl 4-hydroxy-retinoate (3) which was converted directly without purification to

methyl [4-^{3}H]-3-dehydroretinoate (4) by dehydration with p-toluene sulphonic acid. After purification by water-deactivated alumina column chromatography methyl [4-^{3}H]-3-dehydroretinoate (4) was reduced with lithium aluminium hydride providing [4-^{3}H]-3-dehydroretinol (5) which was purified by preparative thin layer chromatography.

Experimental

Methyl 4-oxoretinate (2) - Methyl retinoate (1) was prepared from all trans retinoic acid by the procedure of Staab and Mannschreck[11]. A solution of methyl retinoate (500 mg, 1.59 mmol) in light petroleum, b.p. 40°-60° C (100 ml) was allowed to react with freshly prepared active manganese dioxide (6.0 g) for 1 hr, with occasional stirrings. The reaction was carried out at room temperature in the dark. At the end of reaction period the manganese dioxide was filtered off and repeatedly washed with peroxide free diethyl ether. The combined washings were dried over anhydrous $MgSO_4$ and reduced to a small volume under reduced pressure. The concentrate was chromatographed on a column (3 x 30 cm) of 120 g of 10 % (v/w) water-deactivated alumina (E. Merck, No. 1077). The first light yellow band of unreacted methyl retinoate (1) was eluted out with light petroleum, b.p. 40°-60° C. The major deep yellow band of methyl 4-oxoretinoate (2) showing the characteristic UV maxima at 350 nm with a subsidiary band at 280 nm was eluted with 10% (v/v) diethyl ether in light petroleum, b.p. 40°-60° C. Fractions containing (2) were pooled and concentrated under reduced pressure. The residue was re-chromatographed on a column (2 x 30 cm) containing 60 g of 7% water-deactivated alumina (E. Merck No. 1077). The column was eluted stepwise with two column volumes of 5, 10, 15 and 20% (v/v) diethyl ether in light petroleum, b.p. 40°-60° C. Fractions containing (2) which was eluted with 15%

diethyl ether in light petroleum were pooled. The solvent was removed under reduced pressure leaving a residual oil which was crystallized from light petroleum (b.p. 40°-60° C) at -20° C yielding 120 mg (24%) of methyl 4-oxoretinoate (2); UV absorption (in light petroleum) at 350 nm, $E^{1\%}_{1cm}$ = 1,800 and at 280 nm, $E^{1\%}_{1cm}$ = 355 was in agreement with literature[9].

Methyl [4-^{3}H]-3-dehydroretinoate (4) - A methanol (25 ml) solution of methyl 4-oxoretinoate (20 mg, 0.061 mmol) was treated with sodium borotritide (100 mCi, 5 Ci/mmol, New England Nuclear) at room temperature for 30 min. Inactive sodium borohydride (100 mg) was added, and the reaction was allowed to proceed for a further 5 min. Ice-cold water (25 ml) was added and the resulting mixture was extracted with light petroleum, b.p. 40°-60° C (3 x 25 ml). The combined extracts were dried over anhydrous $MgSO_4$ and removal of solvent under reduced pressure left a light yellow residue of methyl [4-^{3}H]-4-hydroxyretinoate (3). Without purification the residue in dry benzene (5 ml) was treated with p-toluene sulphonic acid (3 mg) for 3 min at 50° C. An ice-cold 1% $NaHCO_3$ solution (10 ml) was added and the resulting mixture was extracted with light petroleum, b.p. 40°-60° C (3 x 10 ml). The combined extracts were dried over anhydrous $MgSO_4$ and the solvent was removed uder reduced pressure. The residue obtained was chromatographed on a column (1 x 15 cm) containing 10 g of 5% (v/w) water-deactivated alumina (E. Merck No. 1077). The fast moving yellow band of methyl [4-^{3}H]-3-dehydroretinoate (4) showing UV maxima at 307 nm and 375 nm was eluted with light petroleum, b.p. 40°-60° C. Removal of solvent under reduced pressure left a yellow residue of 15 mg (0.048 mmol) 79% yield of (4) with a specific radioactivity of 0.85 Ci/mmol.

[4-^{3}H]-3-Dehydroretinol (5) - The tritiated methyl retinoate (12 mg, 0.038 mmol) was reduced with lithium aluminium hydride (200 mg) in

dried peroxide free diethyl ether (10 ml) at 0° C. After 30 min excess lithium aluminium hydride was decomposed by careful addition of ethyl acetate. Ice-cold water (10 ml) was added followed by 10 ml of light petroleum, b.p. 40°-60° C. The resulting organic layer was separated, dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by preparative thin layer chromatography (5 plates, 20 x 20 cm, 0.5 cm of silica gel 60 G, E. Merck No. 7731 and 10% acetone in light petroleum, b.p. 40°-60° C for development). Fractions of silica gel with Rf 0.2-0.3 which showed fluorescence under UV light were scraped off and the compound was eluted with ethanol (4 x 7.5 ml). A yield of 0.027 mmol (total activity: 23 mCi, specific radioactivity: 0.85 Ci/mmol) was obtained. Greater than 99% of the radioactivity of (5) co-chromatographed with standard 3-dehydroretinol on thin layer chromatography using the following solvent system for development: diethyl ether: benzene: ethanol: acetic acid (40: 40: 2: 0.2) (Rf = 0.51). The UV absorption in ethanol at 351 nm ($E^{1\%}_{1cm}$ = 1455), 286 nm ($E^{1\%}_{1cm}$ = 715) and 277 nm ($E^{1\%}_{1cm}$ = 555) was in agreement with literature[12].

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