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SYNTHESIS OF DEUTERATED β-CAROTENE

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SUMMARY

The synthesis of 10,10',19,19,19,19',19',19'-$^{2}H_{8}$-β-carotene is described. The double condensation (C_{15}+C_{10}+C_{15}=C_{40}) of the Wittig salt of 9,9,9,10-$^{2}H_{4}$-β-ionylidene ethanol with the symmetrical C_{10} dial 2,7-dimethyl-2,4,6-octatrienedial led directly to $^{2}H_{8}$-β-carotene. The symmetrical labelling pattern of this deuterated β-carotene will function as a tracer for the study of β-carotene metabolism in humans.

Keywords: Deuterated β-carotene; 10,10',19,19,19,19',19',19'-$^{2}H_{8}$-β-carotene; 9,9,9,10-$^{2}H_{4}$-β-ionylidene ethanol; 9,9,9,10-$^{2}H_{4}$-β-ionylideneethyl triphenyl phosphonium bromide; carotenoid synthesis.

INTRODUCTION

Among the many carotenoids present in nature all-trans-β-carotene exhibits the greatest provitamin A activity (1). Dietary β-carotene is thus a nutritionally important source of vitamin A. The central cleavage of β-carotene in the gut to yield vitamin A has been previously characterized (2,3). However, the extent of any asymmetric cleavage has yet to be determined. Recent epidemiological studies indicate that diets high in carotenoids are correlated with a lower cancer risk (4). The mechanism by which this protective effect is exerted is just beginning to be elucidated (5). To define

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more clearly several aspects of β-carotene metabolism in humans, we have synthesized 10,10',19,19,19,19',19',19'-$^2H_8$-β-carotene.

DISCUSSION

We have previously reported the synthesis of deuterated analogues of vitamin A (6). One of the key intermediates in the synthesis of 10,19,19,19-$^2H_4$-retinol is the tetra deuterated C-15 component 10,15,15,15-$^2H_4$-β-ionylidene ethanol 1 (figure 1). Conversion of this deuterated C-15

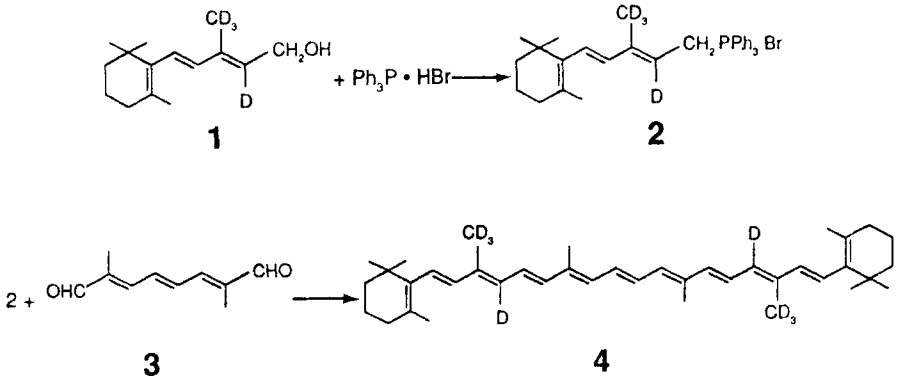

Figure 1. Synthesis scheme for $^2H_8$-β-carotene

alcohol to the Wittig salt 2 and the double condensation ($C_{15} + C_{10} + C_{15} = C_{40}$) with the C-10 dial 2,7-dimethyl-2,4,6-octatriene-dial 3 led directly to 10,10',19,19,19,19', 19',19'-$^2H_8$-β-carotene 4. Yields for the synthesis of the deuterated β-ionylidene ethanol from β-ionone were approximately 75%. Conversion of the C-15 alcohol 1 to the Wittig salt was accomplished by the equimolar addition of triphenyl phosphonium hydrobromide to a stirred solution of the $^2H_4$-C-15 alcohol in dry methanol. A crude product (97%) was obtained that crystallized from THF. The condensation of the C-15 Wittig salt with the C-10 dial 3 in a 2:1 molar

ratio led directly to $^{2}H_{8}$-β-carotene in a 25% yield. Alternatively, adding an excess amount of the C-10 dial to the $^{2}H_{4}$-C-15 ylide gave 10,19,19,19-$^{2}H_{4}$-12'-apocarotenal (data not shown).

The double condensation of the deuterium-labelled C-15 Wittig salt with the C-10 dial is an extension of the approach first used by G. Wittig (7) for the synthesis of β-carotene and, later, in the commercial synthesis of β-carotene (8).

Deuterium incorporation was 8.5% $^{2}H_{6}$, 29.4% $^{2}H_{7}$, 58.0% $^{2}H_{8}$, for a total of 96% as $^{2}H_{6}$, $^{2}H_{7}$, and $^{2}H_{8}$. Less than 0.06% of the product had a m/e of 536 (i. e., that of naturally occurring β-carotene). The central cleavage of this symmetrically labeled $^{2}H_{8}$-β-carotene in vivo should yield two molecules of vitamin A with identical labeling.

EXPERIMENTAL

All reactions were performed under F-40 Gold fluorescent lamps. Mass spectral analysis was performed on a Finnegan 4000 quadrupole mass spectrometer. NMR data was collected on a Nicolet 300-MHz instrument. Tetrahydrofuran (THF) was used after distillation from lithium aluminum hydride (LAH) under an inert atmosphere. All reactions were performed under argon whenever possible.

Preparation of 9,9,9,10-$^{2}H_{4}$-β-ionylideneethyl triphenyl phosphonium bromide

9,9,9,10-$^{2}H_{4}$-β-Ionylidene ethanol was prepared as previously described (6,9). To 0.045 moles (9.86 g) of $^{2}H_{4}$-β-ionylidene ethanol in 100 ml freshly distilled methanol was added 0.045 moles (15.3 g) triphenyl phosphonium hydrobromide (Alfa Products, Danvers, MA). The solution was

stirred for 1 h at room temperature. TLC analysis (Machery-Nagel Polygram-Sil G/UV_{254}, 5% ethylacetate/hexane) showed complete conversion of the alcohol to the salt. The solution was rotary evaporated at <30°C, and the residual oil washed with 2 X 100 ml hexane. Any remaining solvent was removed under vacuum (0.5 mm Hg), after which 23.9 g (0.044 moles, 97%) of a crude crystalline solid was recovered. This crude salt crystallized from a minimum volume of THF to give white crystals of 9,9,9,10-$^2H_4$-β-ionylideneethyl triphenyl phosphonium bromide, mp 133-136°C (c.w. lit. 135-137°C) (10).

Preparation of $^2H_8$-β-carotene

A stirred solution of recrystallized 9,9,9,10-$^2H_4$-β-ionylideneethyl triphenylphosphonium bromide (20.6 g, 37.8 mmoles) in 1 liter of THF was cooled to -70°C in a dry ice/acetone bath. Butyllithium (37.4 mmoles, Aldrich, Milwaukee, WI) in hexane was added dropwise. The solution was stirred for 2 h, at which time 2.95 g (18.0 mmoles) of 2,7-dimethyl-2,4,6-octatrienedial 3 (recrystallized from ethyl acetate) in 25 ml THF was added dropwise. The reaction mixture was stirred overnight and allowed to warm to room temperature. TLC analysis showed β-carotene as a major product with the mono-addition compound (12'-apo-carotenal) as a minor product. The reaction mixture was poured into hexane and washed successively with water (2 X 500 ml), methanol/H_2O (95/5, 2 X 1000 ml) and brine. Drying ($MgSO_4$) and recrystallization of the residue from hexane/methanol (1/2) yielded 2.5 g of crystalline 10,10',19,19,19,19',19',19'-$^2H_8$-β-carotene (25%).

Comparison of the 300-MHz ^1H NMR of $^2H_8$-β-carotene with an authentic sample of β-carotene confirmed the structure of

the deuterated compound. The only differences were a reduction of intensity at 1.97 ppm (9,9'-C^2H_3), the absence of a signal at 6.14 ppm (d, J=11Hz, 10,10'-C^2H) and the collapse of a multiplet at 6.65 ppm (11,11'-CH) due to replacement of the protons at 10,10'-CH. The UV spectrum was identical to β-carotene, λ_{max} 448,476,425 nm. HPLC analysis (Waters Resolve, 5um: 5% CH_2CL_2 in MeOH) revealed only trace amounts of cis isomers. Mass spectral analysis showed m/e 536 (D_o), 0.05%; 537 (D_1), 0.13%; 538 (D_2), 0.27%; 539 (D_3), 0.55%; 540 (D_4), 0.78%; 541 (D_5), 2.2%; 542 (D_6), 8.5%; 543 (D_7), 29.4%; 544 (D_8), 58.0%.

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