A Simple Synthesis of [1,3-$^{13}C_2$]4-(2,6,6-Trimethylcyclohexen-1-yl)buten-2-one (ß-Ionone)

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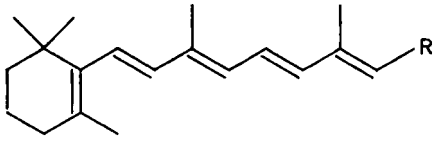
SUMMARY

A convenient, one-step synthesis of doubly ^{13}C-labelled ß-ionone was accomplished by aqueous sodium hydroxide catalyzed aldol condensation of 1,3-$^{13}C_2$-acetone with the recently commercially available ß-cyclocitral. This retinol-like compound obtained in ca. 60% yield, should prove useful in NMR studies of retinoid binding to its transport proteins.

Key Words: Synthesis, ß-ionone, carbon-13, retinoid, NMR

INTRODUCTION

Recently, interest has developed in the vitamin A metabolite retinoic acid (1) and its analogues because of their utility in treating skin diseases and as cancer chemopreventive agents (1). Retinal (2), the aldehyde metabolite of the alcohol vitamin A (retinol; 3), is also essential as the visual pigment chromophore in mammalian vision (2). For a number of years, isotope-labelled retinals have been employed in NMR studies of the association of 2 with opsin and its bacterial counterpart bacterioopsin (3). We have developed ligands for the affinity chromatographic purification of proteins which bind 3 (4) and 1 (5). For further



1 R = COOH

2 R = CHO

3 R = CH_2OH

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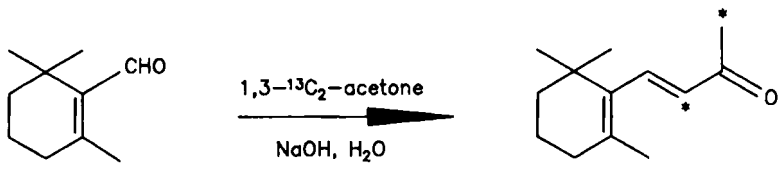

5 4

efforts in these areas, we intend to employ recently introduced isotope-edited NMR techniques (6) which should assist us in gathering more detailed knowledge about the conformation of protein-bound retinoid and the nature of the binding site (7).

Because of its greater stability and ease of manipulation than 1-3, we have chosen to exploit 4-(2,6,6-trimethylcyclohexen-1-yl)buten-2-one (ß-ionone; 4) as a ligand for these studies. This terpene binds to the 21 kDa serum transport protein for 3, the serum retinol-binding protein (4). Thus, this simplified retinoid should serve as a useful tool to probe the conformation of protein-bound retinoids, particularly with respect to the relationship between the polyene side chain and the trimethylcyclohexene ring. Earlier NMR and theoretical studies (8) suggested a non-protein bound conformational preference for 4 and 2 similar to that we have recently found for 1 and its analogues (9).

The ^{13}C-labeling of 4 at the C-1 methyl and C-3 vinyl carbons presented here provides two distinct classes of selectively observable protons (vinylic and aliphatic) which can be used to assess the conformation of protein-bound 4 and perhaps the nature of spatially close amino acid residues in the protein binding site using isotope-edited NOESY techniques (7). The recent commercial availability of ß-cyclocitral (5) has permitted the straightforward preparation of doubly ^{13}C-labelled 4 presented herein.

RESULTS AND DISCUSSION

Methods for the preparation of $^{13}C_2$-4 were initially developed following the procedure of Surmatis and co-workers (10) using unlabelled acetone. Thus, aqueous potassium hydroxide mediated aldol condensation of 5 with a large molar excess of acetone readily provided 4 after column chromatography in a 60% yield. Complete,

unambiguous ^{1}H- and ^{13}C-NMR resonance assignments were made on this synthetic material or commercially available 4 using ^{1}H-^{13}C shift correlation (11) experiments and COLOC (COrrelation by LOng range Coupling) spectra (12) where necessary.

Reaction scale down to eventually employ 1,3-$^{13}C_2$-acetone was performed using unlabelled acetone in a 5 mL conical vial at 65°C. The rate of reaction was considerably slower using limited quantities of acetone, no doubt because the reaction mixture now becomes biphasic with a small water layer evident. Periodic sampling of the reaction mixture by capillary gas chromatography showed consumption of 5 required ca. 9 days. The same reaction time course was observed using 1,3-$^{13}C_2$-acetone. When using labeled acetone, reaction work-up was initiated by short path distillation to recover about one-third of the unconsumed 1,3-$^{13}C_2$-acetone. While most of the remaining acetone could probably have been recovered, we have not routinely done this to avoid potential problems with thermal cyclization of 4 (13). Further work-up provided $^{13}C_2$-4 in about 58% yield after column chromatographic purification. By ^{1}H-NMR analysis, the resulting $^{13}C_2$-4 appears to be approximately 94% trans isomer consistent with the known thermodynamic instability of 7-cis retinoids (13). If necessary, rigorously purified $^{13}C_2$-4 can be obtained by reverse-phase HPLC.

In summary, a simple, one-step procedure for the preparation of doubly ^{13}C-labelled 4 by aldol condensation of 5 and 1,3-$^{13}C_2$-acetone has been described. This retinoid should prove useful for NMR studies of protein-bound retinoid conformation.

EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were recorded on $CDCl_3$ solutions using an IBM AF250 spectrometer operating at 250 MHz for proton and 62.5 MHz for carbon measurements with residual $CHCl_3$ as the internal standard for the proton spectra. UV spectra were recorded with a Beckman DU-40 spectrophotometer. Electron-impact mass spectra were obtained with a Kratos MS-25RFA spectrometer. TLC was performed on 0.2 mm silica gel 60 F_{254} precoated aluminum plates from EM Science. Column chromatography was performed on silica gel (70-230 mesh, EM Science). Capillary gas chromatography was conducted on a Varian 3300 gas chromatograph equipped with a flame ionization detector, helium carrier gas, and 30 m x 0.32 mm SPB-1 column (Supelco, Inc.).

The 1,3-$^{13}C_2$-acetone was obtained from Isotec, Inc., the ß-cyclocitral from Sigma Chemical Co., and the reference ß-ionone from Aldrich Chemical Co. All manipulations of these compounds were performed under gold fluorescent light.

Synthesis of ß-Ionone (4). To a three-necked round bottom flask equipped with stir bar, condenser, drying tube and argon inlet was added 100 mg (0.66 mmol) of ß-cyclocitral (purified by column chromatography), 25 mL (340 mmol) of acetone, and 3 mL of 10% aqueous KOH. The reaction mixture was gently refluxed for 12 hr, the acetone removed under reduced pressure, and the residue partitioned between ether and water. The ether layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated to afford 92 mg (73%) of 4 as a light yellow oil. Column chromatography (25% ethyl acetate/hexane) afforded 76 mg (60%) of purified 4:TLC (25% ethyl acetate/hexane):R_f = 0.5; UV (CH_2Cl_2) λ_{max} 293 nm; ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 6, $C(CH_3)_2$), 1.44-1.48 (m, 2, CCH_2), 1.57-1.63 (m, 2, $CH_2\underline{CH_2}CH_2$), 1.74 (s, 3, $=CCH_3$), 2.05 (m, 2, $=CCH_2$), 2.28 (s, 3, $COCH_3$), 6.09 (d, 1, CHCO, J = 16.4 Hz), 7.25 (d, 1, =CCH=, J = 16.4 Hz); ^{13}C NMR ($CDCl_3$) δ 18.8 ($C_{4'}$), 21.5 ($C_{2'}$-CH_3), 27.0 (C_1), 28.7 ($C_{6'}$-$(CH_3)_2$), 33.4 ($C_{3'}$), 34.0 ($C_{6'}$), 39.7 ($C_{5'}$), 131.5 (C_3), 135.6 ($C_{2'}$), 135.9 ($C_{1'}$), 142.8 (C_4), 196.5 (C_2); MS m/z (%) 192 (M^+, 8.5), 177 (100).

Synthesis of 1,3-$^{13}C_2$-ß-Ionone ($^{13}C_2$-4). In a 5 mL conical reaction vial equipped with a stir bar was mixed 100 mg (0.66 mmol) of ß-cyclocitral, 3 gm (50 mmol) of 1,3-$^{13}C_2$-acetone, and 525 µL of 10% aqueous NaOH. The vial was sealed with a septum cap and immersed in a 63°C oil bath and stirred for 9 days at which time periodic sampling by GC showed 5 had been consumed. The contents were transferred to a short-path still and 1 mL of unreacted $^{13}C_2$-acetone recovered by distillation. The residue was partitioned between ethyl acetate and water and the ethyl acetate layer worked-up as was the ether layer for 4 above to afford 178 mg of a light orange oil. Column chromatography (25% ethyl acetate/hexane) afforded 73 mg (58%) of purified $^{13}C_2$-4 as a light yellow oil with the properties of 4 above, as well as the following: ^{1}H NMR ($CDCl_3$) δ 2.28 (d, 3, $COCH_3$, J_{CH} = 124 Hz), 6.09 (dd, 1, J_{HH} = 16.4 Hz, J_{CH} = 1.66 Hz); ^{13}C NMR ($CDCl_3$) 27.1 (d, CH_3, J_{CC} = 15 Hz), 131.6 (d, =CH, J_{CC} = 15 Hz); MS m/z (%) 194 (M^+, 16.9), 177 (100).

Temperature programmed gas chromatography of $^{13}C_2$-4 (100 to 175°C at 5°C/min) showed t_R = 11.5 min (reference 4 t_R = 11.5 min, reference 5 t_R = 5.4 min).

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