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A NEW SYNTHESIS OF DOUBLE LABELLED [4,6-$^{13}C_2$] MEVALONOLACTONE.

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SUMMARY

The chemical synthesis of double labelled mevalonolactone with carbon ^{13}C at positions C-4 and C-6 is reported.

Key words: Mevalonolactone, carbon ^{13}C labelling.

INTRODUCTION

The mechanism of biosynthesis of trichothecenes has been postulated to include two methyl shifts and a 1,4-hydride displacement (1). The only proof known derives from the incorporation of [1,2-$^{13}C_2$] acetate into trichothecin (2). In order to demonstrate unambiguously that two methyl shifts are involved in the biosynthesis of 3-acetyldeoxynivalenol an efficient synthesis of double labelled [4,6-$^{13}C_2$] mevalonolactone was needed. The incorporation of this enriched precursor with two simultaneous ^{13}C labels on alternate carbons will yield 3-acetyldeoxynivalenol with vicinal ^{13}C labels on the carbons C(6) and C(15) (3).

[4,6-$^{13}C_2$] Mevalonolactone has been previously synthesized (4) from sodium [2-^{13}C] acetate with a 5.4% overall yield. We developped a new strategy starting from commercially available [1,3-$^{13}C_2$] acetone, which gave the title compound in a 42% overall yield using a facile procedure. The different steps involved in the synthesis are illustrated in scheme 1:

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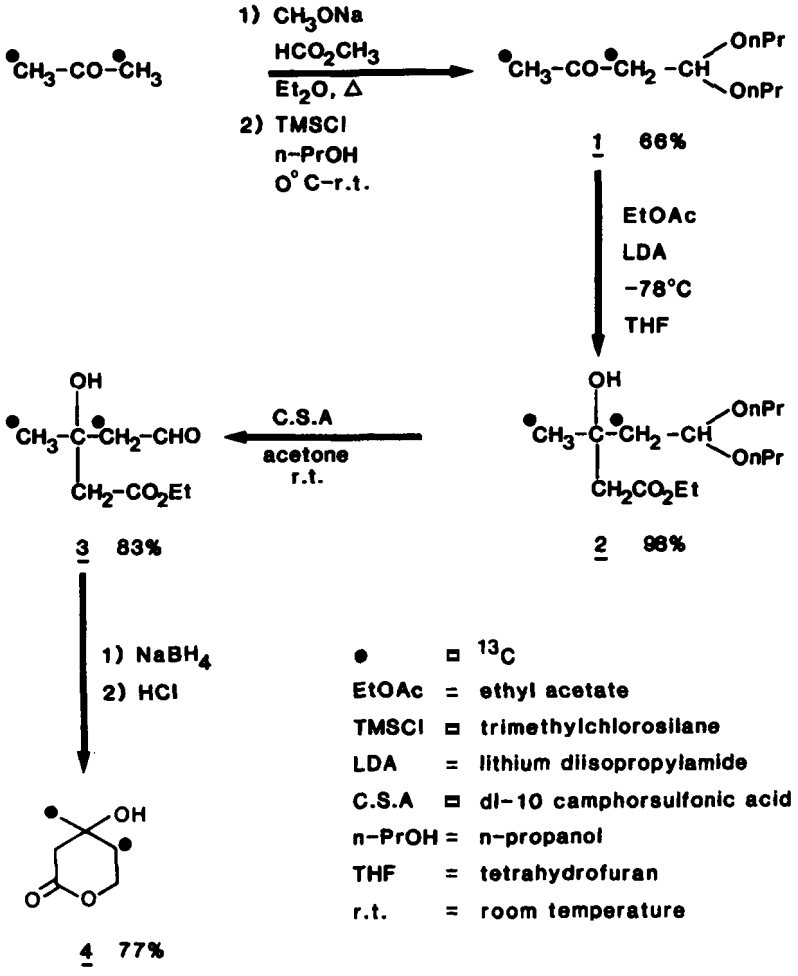

SCHEME 1

The key intermediate in this synthesis was the acetal of [1,3-$^{13}C_2$] acetoacetaldehyde. The dimethyl acetal of acetoacetaldehyde has previously been described (5), but the procedure is not suitable for the small scale needed for labelling since the yield is not high (6). Moreover, the dimethyl acetal of acetoacetaldehyde is volatile. This problem was eliminated by using the di-n-propyl acetal of [1,3-$^{13}C_2$] acetoacetaldehyde (1). (Scheme 1). Thus, by modifying the Brannock procedure (5) by replacing acetone and methanolic hydrogen chloride respectively by [1,3-$^{13}C_2$] acetone and n-propanol/trimethylchlorosilane, we prepared acetal 1

with 66% yield after facile work up and purification by flash chromatography. By ^{1}H-NMR, compound 1 showed a doublet of doublet (J_{CH}=128 Hz, J_{CCCH}=2Hz) at 2.13 ppm, assigned to $^{13}CH_3$, and another doublet of doublet (J_{CH}=128 Hz, J_{HH}=6Hz) at 2.73 ppm, characteristic of $^{13}CH_2$. Condensation of 1 with ETOAC-/LDA in THF at -78°C gave ethyl [4,4'-$^{13}C_2$] -3-hydroxy-3-methyl-5,5-di-n-propyloxypentanoate (2) with 98% yield after flash chromatography. The ^{1}H.NMR spectrum of 2 displayed a doublet of doublet (J_{CH}=128 Hz, J_{CCCH}=4Hz) at 1.33 ppm for $^{13}CH_3$, and a doublet of multiplet (J_{CH}=128 Hz, J_{CCCH}=2Hz J_{HH}=6Hz) at 1.96 ppm for $^{13}CH_2$. Deacetalization of compound 2 by dl-10-camphorsulfonic acid in acetone gave ethyl [4,4'-$^{13}C_2$]-3- hydroxy-3-methyl-5-oxo-pentanoate (3) with 83% yield after purification by flash chromatography. In good agreement with its structure, ^{1}H-NMR of 3 showed a doublet of doublet at 1.36 ppm (J_{CH}=128 Hz, J_{CCCH}=4Hz) for $^{13}CH_3$, and a doublet of multiplet (J_{CH}=128Hz) at 2,66 ppm assigned to $^{13}CH_2$. In addition, aldehydic proton at 9,70 ppm was a doublet of triplet (J_{CCH}=22Hz, J_{HH}=2Hz). Finally, compound 3 was reduced with $NaBH_4$ in methanol to the corresponding alcohol which underwent cyclization in acidic methanol to give [4,6-$^{13}C_2$] mevalonolactone (4) with 77% overall yield. The structure of mevalonolactone 4 has been proven by its ^{1}H-NMR, infra red and mass spectra (see experimental section).

EXPERIMENTAL

Materials

[1,3-$^{13}C_2$] Acetone was purchased from M.S.D. Isotopes. Tetrahydrofuran and diisopropylamine were freshly distilled respectively over lithium aluminum hydride and calcium hydride. Flash chromatography was carried out using silica gel (230-400 mesh) purchased from B.D.H. chemicals.

Instrumentation

Infrared (IR) spectra were recorded on a Perkin Elmer 683 spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Varian T-60 spectrometer using internal tetramethylsilane. Mass spectra (M.S.) were taken on a HP5980A mass spectrometer at 70 e.v. under ammonia chemical ionization conditions.

[1,3-$^{13}C_2$] Acetoacetaldehyde-di-n-propylacetal (1)

A mixture of [1,3-$^{13}C_2$] acetone (1 g), methyl formate (10 mL) and dry ether (10 mL) was added slowly at 0°C into a round bottom flask fitted with a condenser, containing 16,66 mmoles of sodium methoxide in suspension in 20 mL of ether. This reaction mixture was then refluxed during 4 hours and carefully evaporated to dryness. n-Propanol (20 mL) was introduced, then cooled at 0°C, followed by addition of trimethylchlorosilane (4.22 mL). The magnetic stirring was continued for 2 hours at room temperature, then the reaction was quenched with 2 mL of concentrated ammonium hydroxide at 0°C. The product was extracted with ether, washed with brine, and dried over magnesium sulfate. After filtration, solvent was evaporated at reduced pressure below 40°C. The crude product was purified by flash chromatography with ethyl acetate-hexane (1:4). Yield: 66%.

^1H NMR δ = 0.90(t), J_{HH} = 7Hz, 6H, $(CH_2-CH_2-\underline{CH_3})_2$; 1.53 (m), 4H, $(CH_2-\underline{CH_2}-CH_3)_2$; 2.13 (dd), J_{CH} = 128 Hz, J_{CCCH} = 2Hz, 3H, ($^{13}CH_3$); 2.73 (dd), J_{CH} = 128 Hz, J_{HH} = 6Hz, 2H, ($^{13}\underline{CH_2}$-CH); 3.46 (m), 4H, $(\underline{CH_2}-CH_2-CH_3)_2$; 4.80 (dt), J_{HH} = 6Hz, J_{CCH} = 1.5 Hz, 1H, ($^{13}CH_2-\underline{CH}$), IR($CHCl_3$) ν = 1705, 2960 cm^{-1}. MS m/e = 208 [M +NH_4]$^+$, 191 [M+1]$^+$, 131 [M-$OCH_2CH_2CH_3$]$^+$, 88 [M-$OCH_2CH_2CH_3$-$^{13}CH_3CO$]$^+$

Ethyl [4,4'-$^{13}C_2$]-3-hydroxy-3-methyl-5,5-di-n-propyloxypentanoate (2)

Under argon atmosphere at -78°C, 33.28 mmoles of n-butyllithium were added dropwise into a round bottom flask containing 33.28 mmoles of diisopropylamine in 35 mL of dry THF. After stirring the resulting mixture for 10 minutes at -78°C, 33.28 mmoles of ethyl acetate in 3 mL of THF were added slowly dropwise, and the stirring was continued for 30 minutes at the same temperature. Next compound 1 (11.09 mmoles) in 5 mL of THF was added slowly. After stirring for 30 minutes more, with the temperature being unchanged, the reaction was quenched with 50 mL of 1N HCl (until neutral pH) and warmed up to room temperature. THF was evaporated under reduced pressure, the product was extracted with ether, washed with brine and dried over magnesium sulfate. Flash chromatography with ethyl acetate-hexane (1:4) gave 3.03 g of compound 2. Yield: 98%.

^1H NMR($CDCl_3$) δ = 0.93(t), J_{HH} = 7Hz, 6H, $(CH_2-CH_2-\underline{CH_3})_2$; 1.26(t), J_{HH} = 7Hz, 3H, $(CH_2-\underline{CH_3})$; 1.33(dd), J_{CH} = 128Hz, J_{CCCH} = 4Hz, 3H,

($^{13}CH_3$); 1.53(m), 4H, (CH_2-$C\underline{H}_2$-CH_3)$_2$; 1.96(dm), J_{CH} = 128Hz, J_{CCCH} = 2Hz, JHH=6Hz, 2H, ($^{13}CH_2$); 2.53(t), J_{CCCH} = J_{CCCH} = 4Hz, 2H, ($C\underline{H}_2$-CO_2Et); 3.46(m), 4H, ($C\underline{H}_2$-CH_2-CH_3)$_2$; 4.03(s), 1H, (OH); 4.16(q), J_{HH} = 7Hz, 2H, ($C\underline{H}_2$-CH_3); 4.76(t), J_{HH} = 6Hz, 1H, ($^{13}CH_2$-$C\underline{H}$).

IR($CHCl_3$) υ = 1720, 2950, 3470 cm^{-1}.

MS m/e = 219 $[M-OCH_2CH_2CH_3]^+$, 201 $[M-H_2O-OCH_2CH_2CH_2]^+$

Ethyl [4,4'-$^{13}C_2$]-3-hydroxy-3--methyl-5-oxopentanoate (3)

At room temperature, 1.21 g of dl-10-camphorsulfonic acid was added in one portion to a flask containing 3.039 g (10.93 mmoles) of compound 2 in 120 mL of acetone. The mixture obtained was vigorously stirred for 10 minutes, then quickly neutralized by the addition of anhydrous $NaHCO_3$ (12.14 g). Filtration on celite was followed by evaporation under reduced pressure to dryness. Flash chromatography with ethyl acetate-hexane (1:2) gave pure aldehyde 3 (1.607 g, 83% yield).

^1H NMR δ = 1.26(t), J_{HH} = 7Hz, 3H, (CH_2-$C\underline{H}_3$); 1.36(dd), J_{CH} = 128Hz, J_{CCCH} = 4Hz, 3H, ($^{13}CH_3$); 2.56(t), J_{CCCH} = J_{CCCH} = 2Hz, 2H, ($C\underline{H}_2$-CO_2Et); 2.66(dm), J_{CH} = 128Hz, 2H, ($^{13}C\underline{H}_2$-CHO); 4.13(q), J_{HH} = 7Hz, 2H, ($C\underline{H}_2$-CH_3); 9.70(dt), J_{CCH} = 22Hz, JHH = 2Hz, 1H, ($C\underline{H}$O).

IR ($CHCl_3$) υ = 1715, 3500 cm^{-1}.

MS m/e = 194 $[M + NH_4]^+$, 177 [M+1], 176 $[M]^+$, 159 $[M-H_2O+1]^+$

[4,6-$^{13}C_2$] Mevalonolactone (4)

1.555 g (8.83 mmoles) of aldehyde 3 were dissolved in 21 mL of methanol. This solution was cooled to 0°C in an ice bath, $NaBH_4$ (231 mg) in 16 mL of methanol was added dropwise and the mixture was magnetically stirred for 20 minutes at 0°C, then at room temperature for an additional hour. Neutralization with 1N HCl was followed by evaporation to dryness under vacuum. The crude product was dissolved in 7 mL of methanol, then acidified to pH 2 with 1N HCl. After stirring at room temperature for 2 hours, triethylamine was added to the mixture to give a pH of 7. After evaporation to dryness, the resulting product was dissolved in ethyl acetate and filtered through a celite column. Flash chromatography with ethyl acetate-hexane (2:1) gave pure mevalonolactone 4. Yield: 77%.

^1H NMR δ = 1.36(dd), J_{CH} = 128Hz, J_{CCCH} = 4Hz, 3H, ($^{13}CH_3$); 1.93(dm), J_{CH} = 128Hz, 2H, ($^{13}CH_2$); 2.56(m), 2H, (CH_2-CO); 2.63(s), 1H, (OH); 4.46(m), 2H, (CH_2-O).

IR($CHCl_3$) ν = 1720, 3420 cm^{-1}.

MS m/e = 150 $[M+NH_4]^+$, 133 $[M+1]^+$

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