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A SIMPLE, EFFICIENT SYNTHESIS OF 3'-D_3-MEVALONIC ACID

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SUMMARY

3'-D_3-Mevalonic acid was required to study triterpenoid biosynthesis. It was synthesized by a simple route in a 24% yield from D_3-acetic acid-D. The product was characterized as the crystalline salt: N,N'-dibenzylethylenediammonium bis-(3,5-dihydroxy-3-(D_3-methyl)-pentanoate) and was shown by mass spectroscopy and ^{1}H-NMR to be essentially 100 atom% deuterium labelled.

Key words: Mevalonic acid, 3'-D_3-Mevalonic acid

INTRODUCTION

As a part of our study of the biosynthesis of triterpenoids in *Euphorbia lathyris* latex we wished to incubate a methyl-labelled mevalonic acid. Our goal was to observe the loss of the hydrogen label in the cycloartenol and lanosterol produced from this labelled compound. For this purpose it was necessary that we have 100 atom% label to avoid problems with the kinetic isotope effect.

Due to a great deal of biosynthetic interest there have been many syntheses of carbon- and hydrogen-labelled mevalonic acid (1-4). Very few of these were aimed at labelling the 3' methyl group. There are two reports of the synthesis of 3'-D- or 3'-T-mevalonic acid (2), a synthesis of mevalonic acid with a chiral methyl group (3) and a communication reporting a very good synthesis of 3'-^{13}C-mevalonic acid (4). The previous syntheses of the hydrogen-labelled compounds are not regiospecific, do not produce 100 atom %, or are very inefficient. We wish to report herein the efficient synthesis of the title compound with 100 atom % deuterium involving no chromatography (fig 1).

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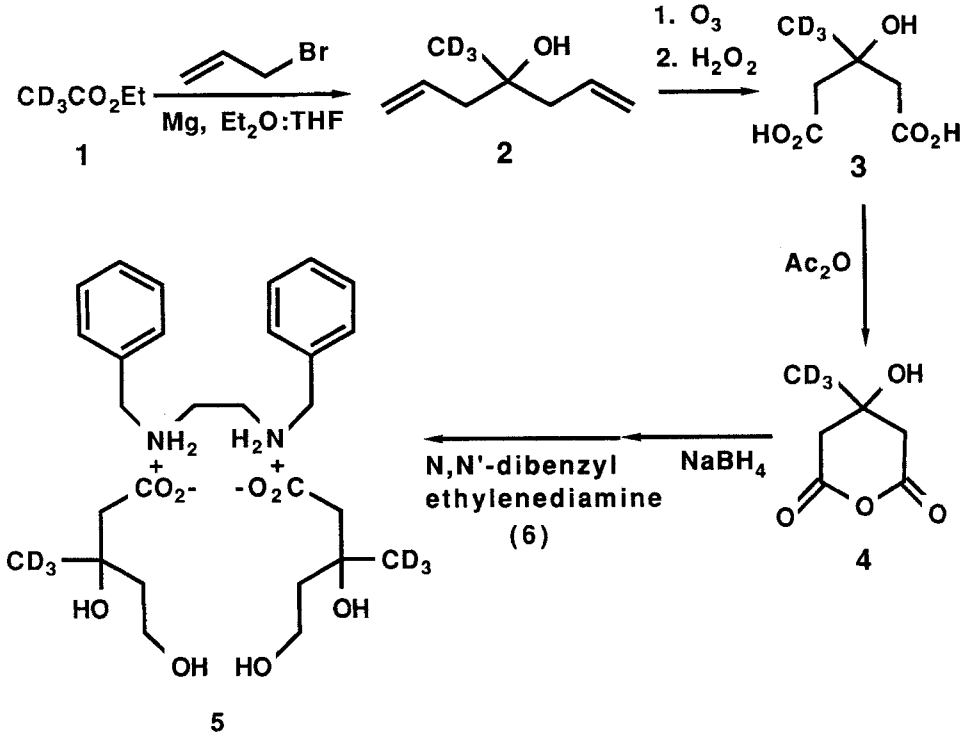

fig. 1

EXPERIMENTAL

D_3-acetic acid-D (99.96 atom%) was obtained from Aldrich. ^1H- and ^{13}C-NMR spectra were determined at 250 and 50.4 MHz, respectively. The purity of most compounds was determined by TLC, HPLC and elemental analysis. Each reaction was also performed using unlabelled compounds.

Ethyl-D_3-acetate (1). A solution of absolute ethanol (5 mL) and conc. sulfuric acid (5 mL) was heated to near reflux under nitrogen. A solution of ethanol (20 mL) and D_3-acetic acid-D (19g) was added dropwise. The reaction was refluxed for one hour then cooled to room temperature. The product was distilled along with some of the ethanol. The distillate was then washed successively with water, satd. bicarbonate and 40% calcium chloride solution. The product was then dried over $MgSO_4$; yeilding 25.1g (93%) of 1.

3-Hydroxy-3-(D_3-methyl)-glutaric acid (**3**). Using the method described by Tschesche (5), **3** was synthesized from **1** in a 65% yield. Magnesium turnings (18.0g) in a 3-necked round-bottom flask were dried in an oven then cooled to room temperature under nitrogen. To the turnings was added a solution of dry ether:THF 1:1 (60mL). In an addition funnel were placed allyl bromide (52mL), **1** (17.6g), and ether:THF 1:2 (165mL). This solution was added dropwise to the turnings at a rate which kept the vigorous reaction at a mild reflux. After the addition was completed the funnel was rinsed with ether:THF 1:2 (60mL) and this rinse was added to the reaction which was stirred for an additional hour, during which time the reaction mixture solidified. The reaction was cooled to 0°, quenched with cold water and acidified with cold 6 M H_2SO_4. After extraction, the ether layer was dried and the solvent removed *in vacuo* . The product was distilled (bp 72-75, 21mm) yielding 21.4g (85%) of **2**. ^{1}H-NMR ($CDCl_3$) 2.21 (4H, d, J=7.5), 5.1 (4H, m), 5.9 (2H, m). ^{13}C-NMR ($CDCl_3$) 25.49 (septet, J_{C-D}=19.4 Hz), 45.90, 71.39, 118.24, 133.78.

A solution of **2** (5.26g) in CH_2Cl_2:acetic acid 10:1 (200mL) was cooled to -78°. Ozone was bubbled through the solution until a deep blue color persisted. A stream of oxygen was bubbled into the solution to remove the excess ozone and the CH_2Cl_2 was removed at room temperature *in vacuo*. 30% hydrogen peroxide (40mL) and acetic acid (15mL) were added to the remaining solution and it was then refluxed overnight. The solvents were removed *in vacuo* and the crude **3** crystallized. This material was recrystallized from acetone:benzene yielding 5.14g (76%) of **3**. The product was characterized by ^{1}H-NMR, ^{13}C-NMR, elemental analysis and mass spectroscopy.

3-Hydroxy-3-(D_3-methyl)-glutaric anhydride (**4**). **3** was reacted with an excess of acetic anhydride under nitrogen at room temperature overnight. Removal of the solvent at room temperature *in vacuo* gave a quantitative yield of **4**. This material could either be used as is or could be recrystallized from $CHCl_3$. ^{1}H-NMR(d_6-acetone): 2.85 (2H, d, J=16Hz) and 3.02 (2H, d, J=16Hz). ^{13}C-NMR(CD_3COCD_3): 44.13, 67.58, 167.3 (the methyl carbon was obscured due to C-D splitting; it appeared at 27.7 in the unlabelled compound). Analysis: Calc'd for $C_6H_5D_3O_4$: C,43.63; H,6.10; found: C,43.81; H,6.16. MS 148(M^++1), 1.1%; 103, 2.9%; 85, 2.4%; 75, 21.4%; 61, 27.3% b.p.

N,N'-dibenzylethylenediammonium bis-(3,5-dihydroxy-3-(D_3-methyl)-pentanoate) (5). 4 (0.89g) was dissolved in 30 mL of isopropanol. $NaBH_4$ (0.33g) was added and the reaction was stirred at room temperature for 2 days. Water (10 mL) was added and the solution acidified to pH 1. After stirring at room temperature for 30 min. the solution was freeze dried. The residue was washed with hot $CHCl_3$ which was then filtered to remove the insolubles and dried over $MgSO_4$. Removal of the $CHCl_3$ *in vacuo* gave a viscous oil. This crude lactone (4) was then treated with N,N'-dibenzylethylenediamine (6) as described by Hoffman (6), by dissolving 4 in water (2.5 mL) and adding 6 (0.5g) in methanol (2.5 mL) and allowing the solution to stand overnight. The methanol was removed *in vacuo* and the remaining solution was extracted with $CHCl_3$. The aqueous layer was freeze dried and the residue dissolved in hot methanol (2 mL). Ether (20 mL) was added and the product was allowed to crystallize. The mother liquor and the $CHCl_3$ layer were combined and again treated with 6 and worked up in the same manner. The combined, crystallized yield was 0.66g (40%). ^{1}H-NMR(CD_3OD): 1.82 (4H, t, J=7.4Hz), 2.36 (4H, s), 3.40 (4H, s), 3.73 (4H, t, 7.4Hz), 4.25 (4H, s), 7.5 (10H, m). ^{13}C-NMR(D_2O): 43.1, 43.5, 48.3, 52.1, 58.5, 71.3, 129.9, 130.0, 130.3, 131.3, 180.7 (the methyl group was obscured due to C-D splitting; it appeared at 29.9 in the unlabelled sample). Analysis: Calc'd for $C_{28}H_{38}D_6N_2O_8$: C,61.97; H,8.17; N,5.16. Found: C,61.80; H,8.12; N,5.15. FAB mass spectrum showed a negative ion of mass 150 (labelled mevalonate) and the positive ion of mass 241 ($C_{16}H_{21}N_2^+$).

DISCUSSION

Deuterated acetic acid is inexpensive and is available with 99.96 atom% deuterium, making it the ideal starting material for the synthesis. Labelled ethyl acetate (1) was prepared just prior to running the Grignard reaction and was kept at 0° until used. The Grignard reaction with allyl bromide works quite well, forming allyl magnesium bromide *in situ*. As long as the work-up is kept cool one does not observe any appreciable elimination. The diallyl methyl carbinol (2) thus formed undergoes oxidative ozonolysis to give good yields of methyl-labelled 3-hydroxy-3-methyl glutaric acid (3) and it is fairly simple to prepare 5-10 grams of labelled 3. The major obstacle in the synthesis of

mevalonate from the diacid is the reduction of only one of the two carboxyl groups. This was achieved via the cyclic anhydride.

The formation of the cyclic anhydride (4) is quantitative and the crude product is suitable for the reduction with sodium borohydride. The reduction of 4 with sodium borohydride produces a crude lactone which could be purified as the lactone; however, we found reduced yields upon purification on silica gel which we attributted to irreversible adsorption. Also, in our enzymatic system we have found much better incorporation of the salt of mevalonic acid than with the lactone. The final product can be easily purified by recrystallization and is then very easily characterized. Examination of the final product by ^{1}H-NMR spectroscopy and by mass spectroscopy showed the methyl group to be greater than 99 atom% labelled.

The synthesis of 100 atom% 3'-D_3-mevalonic acid can thus be achieved in 24% overall yield from deuterated acetic acid. The final product is easily purified by crystallization, stable and suitable for use in biological systems. We have reported a very simple, efficient synthesis of the title compound in pure form without chromatography.

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REFERENCES

1. **a.** Cornforth, J.W.; Cornforth, R.H.; Donninger, C. and Popjak, G.--Proc. Roy. Soc. B163: 492 (1966). **b.** Pichat L.; Blagoev, B. and Hardouin, J.C.--Bull. Soc. Chim. Fr. 1968:4489. **c.** Cornforth, R.H. and Popjak, G.--Methods in Enzymol. 15: 359 (1969).
2. **a.** Matsuo, M. and Kasida, Y.--J. Label. Comp. IV: 134 (1967). **b.** Floss, H.G.; Tcheng-Lin, M.; Chang, C.; Naidoo, B.; Blair, G.E.; Abou-Chaar, C.I. and Cassady, J.M.--J. Am. Chem. Soc. 96:1898 (1974).
3. Phillips, G.T.; Clifford, K.H.--Eur. J. Biochem. 61: 271(1976).
4. Scott, A.I. and Shishido, K.--J. C. S. Chem. Comm. 1980: 401.
5. Tschesche, v.R. and Machleidt, H.--Justus Liebig's Ann. Chem. 631:61 (1960).
6. Hoffman, C.H.; Wagner, A.F.; Wilson, A.N.; Walton, E.; Shunk, C.H.; Wolf, D.E.; Holly, F.W. and Folkers, K.--J. Am. Chem. Soc. 79:2316 (1957).