Incorporation of 2(S)-Methylbutanoic Acid-1-^{14}C into the Structure of Mevinolin

Alvin K. Willard*[1] and Robert L. Smith

Merck Sharp and Dohme Research Laboratories
West Point, PA 19486 (USA)

SUMMARY

A route to mevinolin (1) bearing (S)-2-methylbutanoic acid -1-^{14}C as the ester side chain has been developed. The structure of mevinolin (1) was degraded and selectively protected to provide the alcohol 3 in three steps. Incorporation of ^{14}C was accomplished by acylation of alcohol 3 with (R,S)-2-methylbutyryl chloride-1-^{14}C. Cleavage of the silyl ether protecting group in the resulting mixture of esters 4 provided the two diastereoisomers 5 and 6 which were separated by reverse-phase liquid chromatography. Mevinolin - ^{14}C (5) was also converted to the ammonium salt 7 of the corresponding dihydroxy acid.

Key Words: Mevinolin-^{14}C, HMG-CoA reductase inhibitor, 2-methylbutyryl chloride-1-^{14}C, preparative reverse-phase HPLC.

Mevinolin (1)[2] is a potent inhibitor of cholesterol biosynthesis at the level of the major rate-limiting enzyme HMG-CoA reductase.[3] The structural elucidation of this natural product, as well as some of the details of its biological activity, was published recently.[2] Metabolism and tissue distribution studies for mevinolin required incorporation of ^{3}H or ^{14}C into the structure.

We chose to pursue a route which would incorporate ^{14}C into the 2-methylbutyric ester group of mevinolin. This required removal of the 2-methylbutyryl group, protection of the lactone hydroxyl and, finally, reacylation with labeled 2-methylbutyric acid. Since acylation with racemic 2-methylbutyric acid or a derivative would result in production of two diastereoisomers, resolution of the labeled 2-methylbutyric acid or separation of the resulting diastereoisomers was anticipated.

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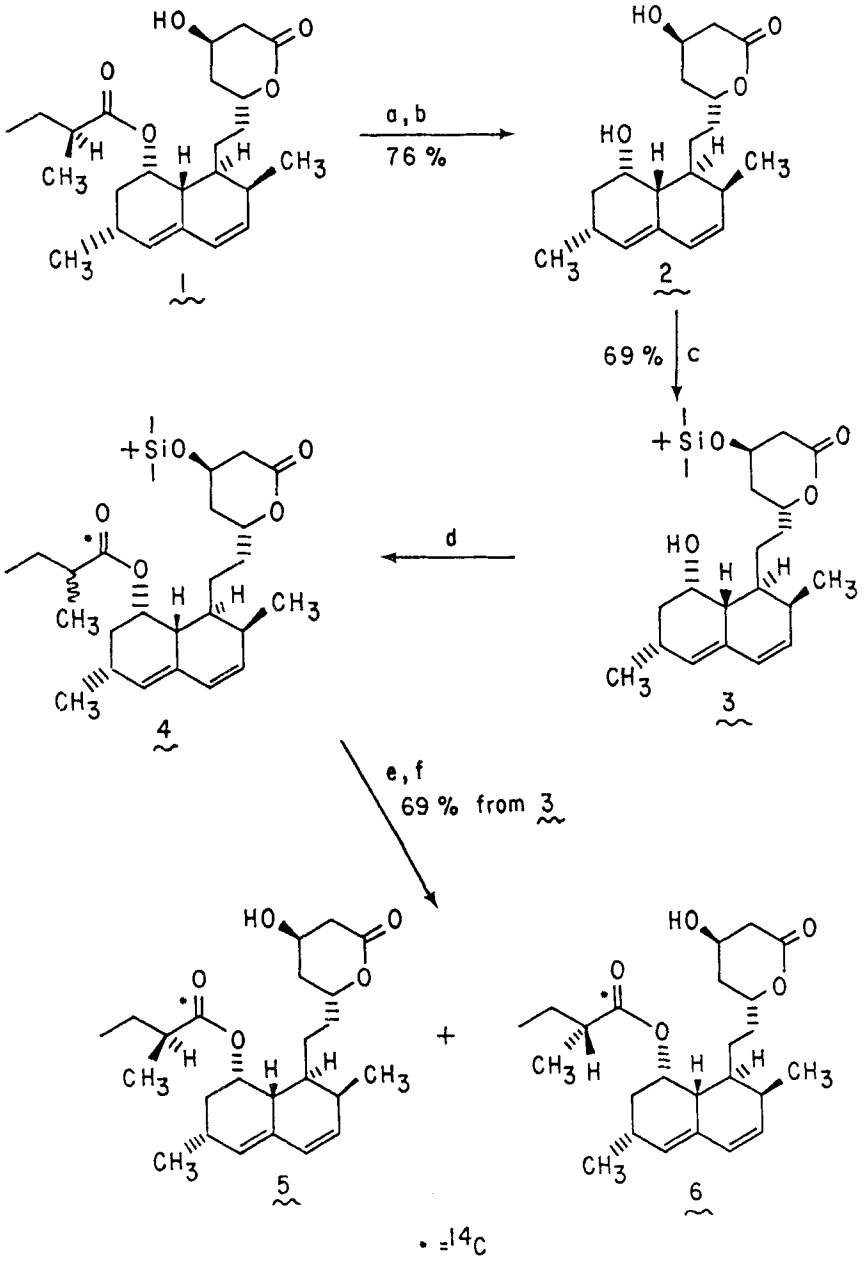
Scheme 1

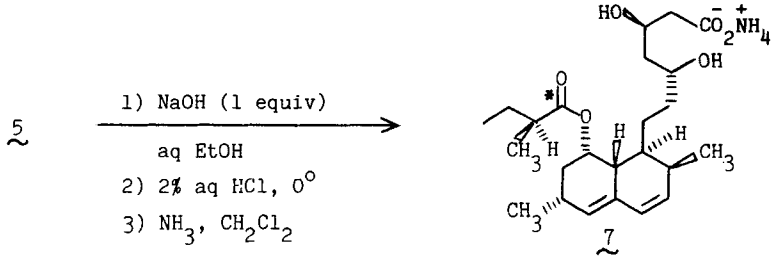


[a]LiOH, H_2O, reflux, 72 h. [b]Toluene, reflux, 2 h. [c]$(CH_3)_3C$ $Si(CH_3)_2Cl$, imidazole, DMF. [d]$CH_3CH_2CH(CH_3)$ $^{14}COCl$, 4-dimethylaminopyridine, pyridine, 20°, 24 h. [e]3 equiv $Bu_4N^+F^-\cdot 3H_2O$, 4 equiv HOAc, THF, 20°, 18 h. [f]HPLC separation of diastereoisomers.

The route which provided mevinolin -^{14}C is shown in Scheme I. Modification of the mevinolin structure to provide alcohol 3, which is properly set-up for the acylation reaction, was accomplished in three steps. Treatment of mevinolin (1) with ten equivalents of lithium hydroxide at reflux in water resulted in rapid opening of the lactone ring followed by slow saponification of the 2-methylbutyric ester over 72 hours. Neutralization provided a dihydroxy acid which, when heated at reflux in toluene for two hours, relactonized to yield lactone 2. Careful analysis of the NMR spectrum of lactone 2 verified that the stereochemical integrity of the lactone hydroxyl had not been compromised. Selective protection of the lactone hydroxyl was accomplished by treatment with tert-butyldimethylchlorosilane[4] and imidazole in N,N-dimethylformamide. The crystalline silyl ether 3 was obtained in 52% yield from mevinolin 1.

Acylation of alcohol 3 was carried out with (R,S)-2-methylbutyryl chloride-1-^{14}C in pyridine in the presence of 4-dimethylaminopyridine[5] as catalyst. The resulting crude mixture of esters 4 was not purified. Treatment of these esters 4 with three equivalents of tetrabutylammonium fluoride trihydrate and four equivalents of acetic acid in tetrahydrofuran caused cleavage of the silyl ether protecting group to give a mixture of lactones 5 and 6. Attempted cleavage of the silyl ethers under the same conditions but omitting acetic acid led to rapid destruction of the lactone ring. The diastereomeric esters 5 and 6 were separated by preparative reverse-phase liquid chromatography.

Treatment of lactone 5 with one equivalent of sodium hydroxide in aqueous ethanol rapidly opened the lactone ring. Careful acidification gave the corresponding dihydroxy acid which, when treated with excess ammonia in dichloromethane, was converted to the stable, highly crystalline ammonium salt 7.



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EXPERIMENTAL

General. All reactions were carried out under a nitrogen atmosphere. "Dry" pyridine and tetrahydrofuran (THF) were dried over 4A molecular sieves. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian SC-300 Spectrometer and infrared (IR) spectra were determined on a Perkin-Elmer 621 Grating Infrared Spectrophotometer. Flash chromatography was performed according to the method of W. C. Still[6] on columns of silica gel (230-400 mesh, E. Merck) of the indicated diameter. Radiochemical purity was determined on thin-layer chromatograms by scraping bands and determining radioactivity by the liquid scintillation technique. Alternatively, radiopurity determinations were done by liquid chromatography. Radioactivity in elution fractions was determined by liquid scintillation.

6(R)-{2-[8(S)-Hydroxy-2(S),6(R)-dimethyl-1,2,6,7,8,8a(R)-hexahydronaphthyl-1(S)]-ethyl}-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyrane-2-one (2). A mixture of 20.0 g (49.4 mmol) of mevinolin (1) and 20.7 g (0.493 mol) of $LiOH \cdot H_2O$ in 1.5 L of water was stirred at reflux for 72 h. The reaction mixture was cooled to 0°C, acidified by addition of 50 mL of conc HCl and then extracted with ether (3X500 mL). The combined extracts were washed with water (3X500 mL) and satd brine (500 mL), dried ($MgSO_4$) and evaporated to give a white solid. This solid was dissolved in 300 mL of toluene and heated at reflux for 2 h in a Dean-Stark apparatus for azeotropic removal of water. After evaporation of the toluene, the residual oily solid was heated at reflux in hexane (150 mL) for 30 min. After cooling to 0°C, the hexane solution was filtered and the collected solid was dried in air. This amounted to 12.05 g (76%) of an off-white powder. An analytical sample was prepared by recrystallization of a portion of this material from 1-chlorobutane to give white clusters: mp 128-131°C (vac); NMR ($CDCl_3$) δ 0.87 (d, 3, J=7Hz, CH_3), 1.16 (d, 3, J=7Hz, CH_3), 2.64 (m, 2, pyran C-3H's), 4.27 (br m, 1, naphthalene C-8H), 4.37 (m, 1, pyran C-4H), 4.71 (m, 1, pyran C-6H), 5.56 (m, 1, napth C-5H, 5.79 (dd, 1, J=6, 10 Hz, napth C-3H), 6.03 (d, 1, J=10Hz, napth C-4H); IR ($CHCl_3$) 3400 (OH), 1725 (C=O), 1240, 1120, 1080 cm^{-1}.

Anal. Calcd for $C_{19}H_{28}O_4 \cdot 0.1 \cdot C_4H_9Cl$: C, 70.67; H, 8.84. Found: C, 70.77; H, 8.75.

6(R)-{2-[8(S)-Hydroxy-2(S),6(R)-dimethyl-1,2,6,7,8,8a(R)-hexahydronaphthyl-1(S)]-ethyl}-4(R)-tert-butyldimethylsilyloxy-3,4,5,6-tetrahydro-2H-pyran-2-one (3). A mixture of 18.3 g (57.1 mmol) of the alcohol 2, 21.5 g (142.8 mmol) of tert-butyldimethylchlorosilane and 19.4 g (285.6 mmol) of imidazole in 200 mL of DMF was stirred at 20°C for 18 h. The reaction mixture was diluted with 1.5 L of ether and washed successively with water, 2% aq HCl, water and satd aq $NaHCO_3$. The ether solution was dried ($MgSO_4$), filtered and reduced to a volume of 1 L. After addition of 600 mL of hexane, the volume was reduced to 600 mL on a steam bath. Crystallization at room temperature provided 13.7 g of crystals. The mother liquors were reduced to 250 mL and a second crop of crystals was isolated after this solution stood at 0°C overnight. The combined yield was 17.13 g (69%) of the silyl ether 3 as a white, cottony solid: mp 142-144°C (vac); NMR ($CDCl_3$) 0.10 (s, 6, $(CH_3)_2Si$), 0.90 (s, 9, $(CH_3)_3CSi$), 1.19 (d, 3, J=7Hz, CH_3), 2.58 (d, 2, J=4Hz, pyran C-3H's), 4.3 (m, 2, pyran C-4H and naphthalene C-8H), 4.70 (m, 1, pyran C-6H), 5.57 (m, 1, napth C-5H), 5.58 (dd, 1, J=6, 10Hz, napth C-3H), 6.03 , 1, J=10Hz, napth C-4H).

Anal. Calcd for $C_{25}H_{42}O_4Si$: C, 69.08; H, 9.74. Found: C, 69.46; H, 9.83.

6(R)-{2-[8(S)-Hydroxy-2(S),6(R)-dimethyl-1,2,6,7,8,8a(R)-hexahydronaphthyl-1(S)]-ethyl}-4(R)-tert-butyldimethylsilyloxy-3,4,5,6-tetrahydro-2H-pyran-2-one, 8-(R,S)-2-methylbutanoic ester-1-^{14}C (4). A solution of 500 mg (1.15 mmol) of the alcohol 3 and 50 mg of 4-dimethylaminopyridine[5] in 4 mL of dry pyridine was stirred at 0°C. To this stirred solution was added 198 mg (1.64 mmol, 9 mCi) of (R,S)-2-methylbutyryl chloride-1-^{14}C[7] over 1 min. This solution was stirred at 0°C for 15 min and then at 20°C for 8 h. The reaction mixture was treated with 140 µL (138 mg, 1.15 mmol) of unlabeled 2-methylbutyryl chloride and stirred at 20°C for an additional 2.5 h. The reaction mixture was diluted with 100 mL of ether, washed with 2% aq HCl (3 x 25 mL) and satd aq $NaHCO_3$ (2 x 25 mL) and dried ($MgSO_4$). Evaporation of the ether solution left 670 mg of the crude mixture of esters 4. TLC analysis (silica gel, 60% ether/hexane) indicated that there was no starting alcohol 3 in the mixture.

6(R)-[2-[8(S)-Hydroxy-2(S),6(R)-dimethyl-1,2,6,7,8,8a(R)-hexahydronaphthyl-1(S)]-ethyl]-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one,8-[2(S)-methylbutyric ester-1-^{14}C] (5) and 8-[2(R)-methylbutyric ester-1-^{14}C] (6). A solution of 670 mg of the crude mixture of esters 4 in 5 mL of dry THF was treated with 263 μL (276 mg, 4.60 mmol) of acetic acid. To this stirred solution was added 5.13 mL (3.45 mmol) of a solution of tetrabutlyammonium fluoride•trihydrate in THF. The reaction mixture was stirred at 20°C for 11 h. TLC analysis (silica gel, 10% ether/dichloromethane or 20% acetone/-dichloromethane) indicated that the desilylation was complete. The reaction mixture was diluted with 100 mL of ether, washed with 2% aq HCl (1 x 25 mL) and satd aq $NaHCO_3$ (2 x 25 mL) and dried ($MgSO_4$). The solvent was evaporated to give 630 mg of a light orange oil. This oil was purified by flash chromatography[6] on a 30 mm column of silica gel with 15% acetone/dichloromethane. Fractions containing the pure mixture of esters were combined and the solvent was evaporated. The residue was triturated with 2 mL of hexane to give 320 mg (69% from alcohol 3) of the mixture of esters 5 and 6 as a white powder: R_f = 0.27 (silica gel, 15% acetone/dichloromethane).

HPLC Separation of Diastereomeric Esters 5 and 6

The diastereomeric esters 5 and 6 were separated by chromatography on a Beckman Ultrasphere-octyl, 5 μ column (10 mm ID x 25 cm length) using an Altex Model 330 isocratic liquid chromatograph. The mobile phase, acetonitrile-water (45:55), was pumped at a flow rate of 5.1 mL/min; the UV detector monitored 254 nm. These HPLC conditions allowed 5 mg of the mixture to be separated per injection: ester 5, retention time = 43.4 min; ester 6, 45.6 min. Overlapping injections could be made every 12 min.

Fractions containing the more mobile component from 44 injections were combined (550 mL) and the acetonitrile was evaporated. The aqueous solution was satd with NaCl and extracted with dichloromethane (3 x 50 mL). The combined extracts were washed with satd brine, dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography[6] on a column (10 mm x 15 cm) of silica gel with 15% acetone/dichloromethane. Fractions containing the lactone were combined and evaporated. The residual oil was crystallized from a mixture of 120 μL of acetonitrile and 150 μL of water to give 68 mg of mevinolin-^{14}C (5) as white needles. The retention

time of mevinolin-^{14}C (5) on analytical HPLC[8] was identical to that of mevinolin (1); less than 1% of the epimeric ester 6 was present. The radiopurity of ester 5 was at least[9] 96.8% (TLC, silica gel, toluene-methanol, 4:1). Radiopurity of 99% was determined by HPLC on a Whatman Partisil 10 PAC column with 10% isopropanol/hexane as the eluant. The specific activity of mevinolin-^{14}C was 10.2 μCi/mg.

Similar treatment of the fractions containing the less mobile component gave 52 mg of ester 6 as long, white needles after recrystallization from ether. The sample contained less than 4% of the epimeric ester 5. Radiopurity was determined to be at least[9] 94%. Specific activity was 11.5 μCi/mg.

7-{1(S),2(S),6(R),7,8(S)8a,(R)-Hexahydro-2,6-dimethyl-8-[2(S)-methylbutyrloxy-1-^{14}C]naphthalenyl-1}-3(R),5(R)-dihydroxyheptanoic acid, ammonium salt (7). A suspension of 45.8 mg (0.113 mmol) of mevinolin-^{14}C (5) in 1.3 mL of absolute ethanol was treated with 1.36 mL (0.136 mmol) of 0.1N aq NaOH. This mixture was stirred for 10 min at 20°C. The homogeneous solution was then cooled to 0° and acidified by dropwise addition of 2% aq HCl. The reaction mixture was diluted with 25 mL of ether, washed with water (3 x 10 mL) and saturated brine (1 x 10 mL) and dried ($MgSO_4$). The solvent was removed by rotary evaporation at 20°. The above operations were carried out as quickly as possible to minimize spontaneous lactonization of the dihydroxy acid. The residue was dissolved in 10 mL of dichloromethane which was then satd with anhydrous ammonia. Ether (10 mL) was added and after 10 min the deposited fine white solid was collected on a medium porosity sintered-glass funnel to afford 42 mg of a solid. This material was dissolved in 1.5 mL of boiling isopropanol/conc aq NH_4OH (95:5) and allowed to crystallize overnight to provide 32 mg of the ammonium salt 7 as white needles. The identity of 7 was established by TLC comparison with authentic, unlabeled 7 (silica gel, chloroform-methanol-conc aq NH_4OH (80:30:5) or dichloromethane-acetic acid (85:15). Radiopurity was determined to be 98.5% using the first eluant. The specific activity of ammonium salt 7 was 9.4 μCi/mg.

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References and Notes

1. Current address: Biomedical Research Department, ICI Americas, Wilmington, DE, 19897.

2. Alberts A. W., Chen J., Kuron G., Hunt V., Huff J., Hoffman C., Rothrock J., Lopez M., Joshua H., Harris E., Patchett A., Monaghan R., Currie S., Stapley E., Albers-Schonberg G., Hensens O., Hirshfield J., Hoogsteen K., Liesch J., and Springer J., Proc. Nat. Acad. Sci. U.S.A. 77: 3957-3961 (1980).

3. Rodwell V. W., Nordstrom J. L., and Mitschell J. J., Adv. Lipid Res. 14: 1-74 (1976).

4. Corey E. J., Venkateswarlu A., J. Amer. Chem. Soc. 94: 6190-6192 (1972).

5. Hofle G., Steglich W., and Vorbruggen H., Ang. Chem. Int. Ed. Engl. 17: 569-583 (1978).

6. Still W. C., Kahn M., and Mitra A. J., Org. Chem. 43: 2923-2925 (1978).

7. The 2-methylbutyryl chloride-1-^{14}C was purchased from American Radiochemical Corp., P.O. Box 1938, Sanford, Florida 32771.

8. Analytical HPLC was performed on an Altex-Ultrasphere-octyl column (4.6 mm x 25 cm) with acetonitrile-water (45:55) pumped at 2:0 mL/min with UV detection at 254 nm. Retention times: 5, 26.0 min; 6, 27.2 min.

9. Two-dimensional TLC analysis indicated that some decomposition of 5 was occurring under these analysis conditions.