Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 8

CARBON-14 METHYLATION OF THE 2-METHYLBUTYRYL SIDE CHAIN OF MEVINOLIN* AND ITS ANALOGS

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SUMMARY

A one step procedure for the preparation of labeled mevinolin analogs 4, 10 and 15 possessing the 2,2-dimethylbutyryloxy side chain is described. The lactones 1, 7 and 13 were converted into potassium salts of their corresponding di or trihydroxy carboxylic acids from which anionic ester enolates were generated and alkylated with [^{14}C]methyl iodide. Workup and purification by reverse phase HPLC provided radiochemically pure 4, 10 and 15. The labeled lactones were converted into ammonium salts of their corresponding di or trihydroxy acids.

Key Words: HMG-CoA reductase inhibitors, [^{14}C]Methylation of anionic ester enolates, Mevinolin analogs.

INTRODUCTION

Mevinolin is a well known potent HMG-CoA reductase inhibitor. Related compounds possessing the 2,2-dimethylbutyryloxy side chain (4, 10, 15) are highly active inhibitors of HMG-CoA reductase and thus may have potential utility in the treatment of atherosclerosis, hyperlipemia, familial hypercholesterolemia and like disorders.[1] For studies concerning metabolism and tissue distribution of these compounds in experimental animals, carbon-14 labeled tracers were required. We report herein the preparation of three such tracers.

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* The currently accepted generic name for "mevinolin" is "lovastatin".

0362–4803/88/080815–11$05.50

Received November 5, 1987
Revised December 2, 1987

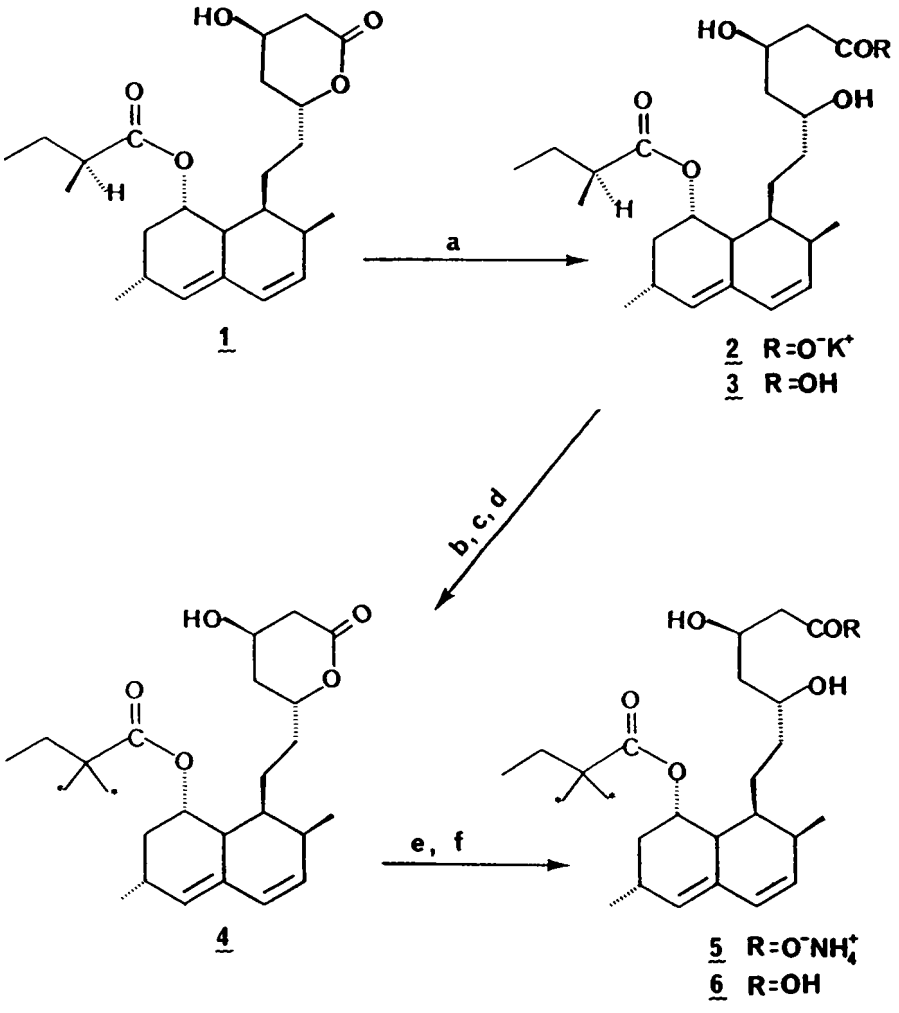
RESULTS AND DISCUSSION

Incorporation of carbon-14 label into the structure of mevinolin was first reported by Willard and Smith.[2] Their procedure is comprised of five steps: (a) de-esterification of the 2(S)-methylbutyrate; (b) protection of the 4-hydroxy group of the tetrahydropyranone ring; (c) re-esterification with (R,S)-2-methyl[1-^{14}C]butyryl chloride; (d) deprotection of the 4-hydroxy group; (e) HPLC separation of diastereomers to yield the desired isomer. The same procedure could also be used to prepare labeled C-methylated mevinolin analogs with appropriate acid chlorides as labeled precursors.

Sletzinger, et al. recently reported a novel one step process for C-methylation of the 2(S)-methylbutyrate moiety of mevinolin and related compounds.[1] This process consists of (a) conversion of the lactone compound into the potassium salt of the corresponding dihydroxy carboxylic acid and (b) generation of the tetra-anionic ester enolate using lithium pyrrolidide followed by alkylation with methyl halide. This route appeared more attractive for the purposes of radiolabeling as it would involve a one step alkylation reaction with easily available [^{14}C]methyl iodide as label carrier. Compounds 4 and 10 were prepared utilizing this procedure (Schemes I & II), and with appropriate modifications furnished the desired tracers in 23 and 35% radiochemical yields, respectively, from labeled methyl iodide.

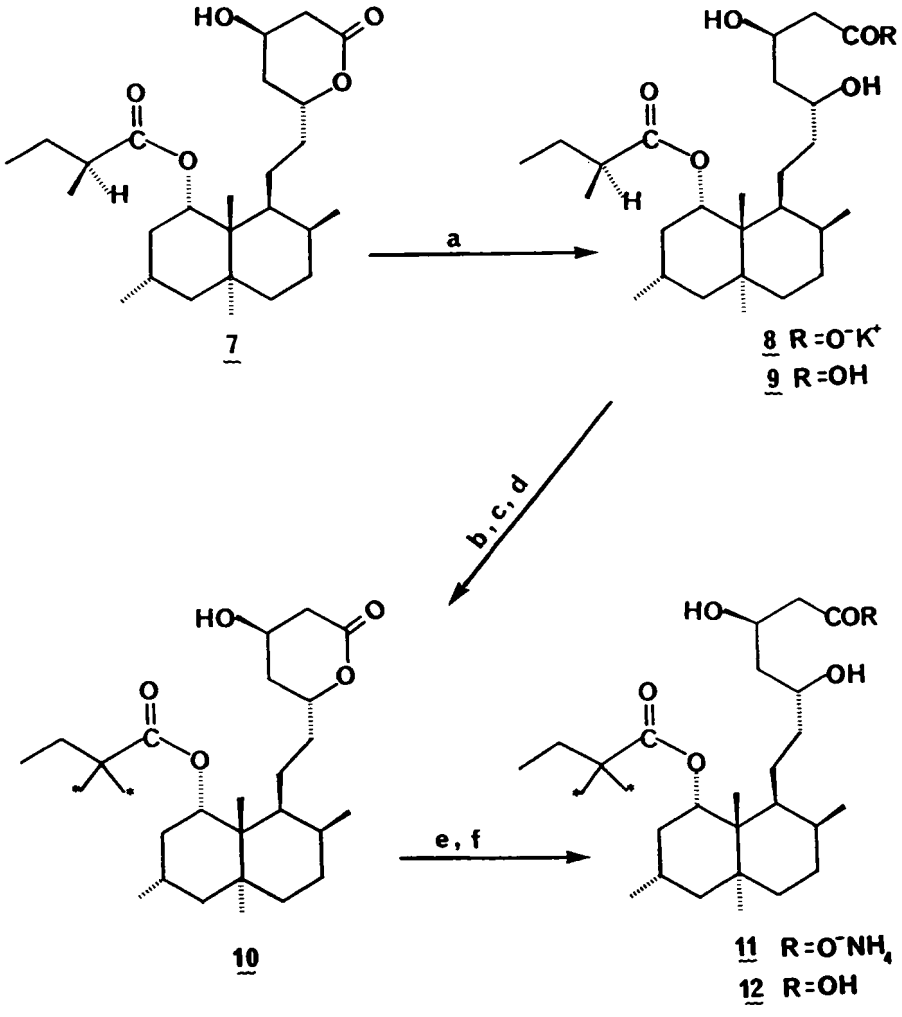
Compound 15 was prepared by Hoffman, et al. using essentially Willard's procedure[2] with 4-acetyloxy-2,2-dimethylbutyryl chloride as esterifying agent[3]. For the preparation of labeled 15 this route would require an early incorporation of the label in a multi-step synthetic sequence. It was therefore thought worthwhile to attempt a penta-anionic ester enolate alkylation of 14[4] following the same procedure utilized for the preparation of 4 and 10. It was also anticipated that during the reaction the side chain could possibly lactonize internally and eliminate, thereby, reducing the yield of the desired alkylated product. When the alkylation reaction of 14 (Scheme III) was carried out using [^{14}C]methyl iodide 15 was obtained in 5% radiochemical yield. The major isolable products were unreacted 13, 15 and 17. The structure of the product 15 was confirmed by ^{1}H NMR and mass spectral analysis and HPLC co-elution with authentic reference material.

Scheme I



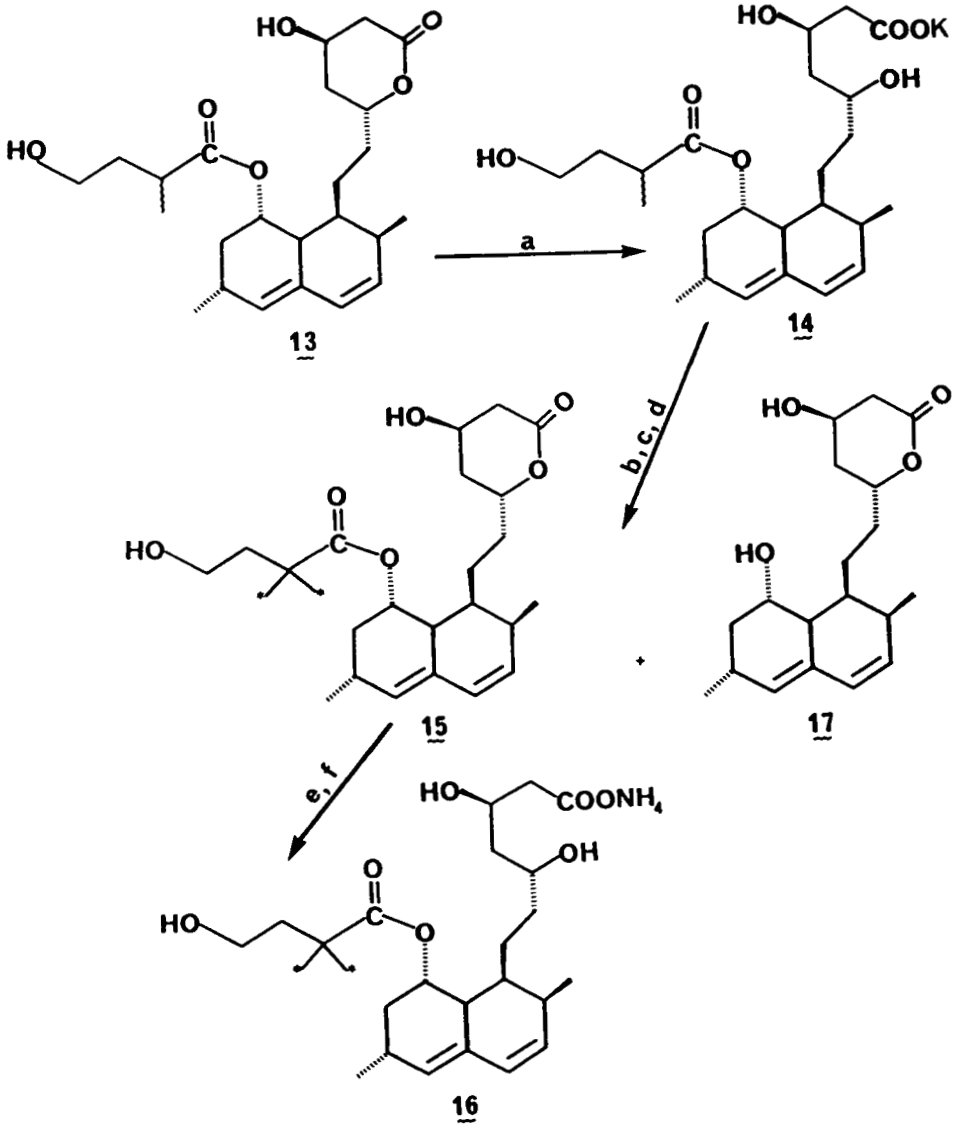
[a] *Cyclohexane, Isopropanol, KOH;* [b] *Pyrrolidine, n-BuLi, THF, [^{14}C]methyl iodide;* [c] *60°C, Toluene;* [d] *Reverse phase HPLC separation;* [e] *(i) NaOH, (ii) HCl, (iii) EtOAc, NH_3;* [f] *CH_3CN-H_2O recrystallization.*

Scheme II



a-d Same as in Scheme I; [e] *(i) NaOH. (ii) HCl. (iii)* CH_2Cl_2, NH_3; [f] *RP- HPLC.*

Scheme III



[a-e] *Same as in Scheme I;* [f] *RP-HPLC*

The lactones 4, 10 and 15 were converted into their corresponding ammonium salts of the di and trihydroxy carboxylic acids 5, 11 and 16 using standard procedures. These salts were also needed for metabolism and tissue distribution studies. The lactone 4 was hydrolysed using dilute alkali, acidified, and the resulting dihydroxy acid was extracted into ethyl acetate. Gaseous ammonia was bubbled through the dried ethyl acetate extract to precipitate the ammonium salt. The other ammonium salts were made in a similar fashion. These salts were purified either by recrystallization or by preparative reverse phase HPLC to provide >99% radiochemically pure compounds. The buffered eluants containing radiochemically pure ammonium salts 11 and 16 were used as such in biological experiments.

Radiopurity determinations of the labeled compounds were made mainly by HPLC. TLC analyses of these compounds consistently showed lower values indicating decomposition on the plates.[2]

The radiochemical degradation of these compounds posed problems for storage. On one occasion nearly 7% decomposition over a period of six months was noted when 4 at a specific activity of 25 μCi/mg was stored as a solid under nitrogen at -20 °C. On another occasion when 4 was freshly prepared and stored as a solution in toluene under nitrogen at 0 to -5 °C at the same specific activity (25 μCi/mg) nearly 25% decomposition over 3 weeks was observed. The instability of 15 was such that its radiochemical purity had decreased to 80% in three weeks and it had to be repurified just prior to use.

In summary, we have described a one-step carbon-14 methylation of the 2-methylbutyryl side chain of mevinolin (1), trans tetrahydromevinolin (7) and 4-hydroxymevinolin (13), the latter utilizing a penta-anionic ester enolate alkylation.

EXPERIMENTAL

Radioactivity measurements were carried out using Packard Tricarb PLD and 3255 liquid scintillation spectrometers and Instagel (United Technologies). TLC analyses were carried out using Merck silica gel 60 F-254 plates with radiochromatogram scans performed on a Berthold LB 2832 automatic TLC linear analyzer. HPLC analyses were

performed using LDC UV detector at 240nm or 238nm, Hewlett Packard 3388A integrator and Berthold radioactivity monitor LB 503. Preparative HPLC was carried out using Altex pumps and a Beckman UV detector set at 254 nm. The ^{1}H NMR spectra were recorded on varian SC-300 spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Mass spectra were taken on a LKB 9000 spectrometer. [^{14}C]Methyl iodide was obtained from the Amersham corporation. Identities of the labeled compounds were established by co-chromatography with pure unlabeled compounds.[1,3]

6(R)-[2-[8(S)-(2,2-di[^{14}C]methylbutyryloxy)-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 (4)

To a slurry of mevinolin 1 (404 mg, 1 mmol) in cyclohexane (7 mL) and isopropyl alcohol (0.4 mL) was added an aqueous solution of potassium hydroxide (0.22 mL, 4.65 M), and the resulting mixture was stirred at room temperature under nitrogen for 30 min. The mixture was concentrated by distillation. Additional cyclohexane was added (5.5 mL) and the mixture was again concentrated to a thick syrup and dried in vacuo overnight to yield the potassium carboxylate 2. HPLC: 70% CH_3CN in water with 0.3% H_3PO_4, 1ml/min, 240nm, ODS-3, RT=5.25 min, UV purity 93%.

An oven dried 50 mL three necked flask fitted with a nitrogen inlet, thermometer and a stopper was evacuated and purged with nitrogen three times. The stopper was then quickly replaced with a rubber septum. The flask was charged with pyrrolidine (96 mg, 1.36 mmol), THF (2 mL) and a solution of the aforedescribed potassium carboxylate in THF (4.5 mL). The solution was cooled to -55 ^{o}C and n-butyllithium in hexane (2.2 mL, 1.52 M, 3.34 mmol) was added over 15 min. The solution was warmed to -30 ^{o}C and aged between -30 to-40 ^{o}C for 75 min, recooled to -55 to -60 ^{o}C and [^{14}C]methyl iodide (45 mCi, 59 mCi/mmol, 0.76 mmol; + carrier 117 mg, 0.82 mmol, total 1.58 mmol, 28.5 mCi/mmol) in THF (4 mL) was added over 10 min. The mixture was aged at -55 ^{o}C for 30 min and at -40 to -50 ^{o}C for 45 min, then quenched with water (8 mL). The temperature of the quenched mixture was raised to 0 to 5 ^{o}C, and the pH of the reaction mixture was adjusted to near 2 with 3 N hydrochloric acid. The mixture was extracted (3x) with ethyl acetate and the combined organic extracts were washed with water and

dried (sodium sulfate and sodium bisulfite), filtered and concentrated. HPLC analysis of the crude isolate (70% CH_3CN in water with 0.3% phosphoric acid, 1 mL/min, Zorbax ODS-3, 240 nm) revealed the presence of four major components: 6, RT 6.40 min, 50%; 3, RT 5.2 min, 17%; 4, RT 10.1 min, 11% and 1, RT 8 min, 2.2%. The crude product (535 mg, 24 mCi) was dissolved in toluene (12 mL) and heated at 60 °C with continuous subsurface bubbling of nitrogen through the solution for 5 h. The toluene was evaporated in vacuo, the crude product was divided into three portions and preparative HPLC was carried out on each of them separately (Magnum 20 Whatman ODS-3, 60% CH_3CN in water, 254 nm). Appropriate fractions containing 4 were pooled and worked up by removing acetonitrile and extracting the product into ethyl acetate. The ethyl acetate extracts were dried ($MgSO_4$) and concentrated to give a combined yield of 10.5 mCi of 4. This compound was diluted with 252 mg (0.6 mmol) of carrier 4 in methanol (5 mL). Water was added to the point of cloudiness (2.5 mL) to initiate crystallization. The mixture was stirred at room temperature for 30 min and at 0 °C for 30 min. The product was collected, washed with 15 mL of cold methanol-water (1:1) and dried in vacuo to provide pure 4 (365 mg, 24.1 μCi/mg, 10.1 mCi/mmol, 8.8 mCi). HPLC: 75% CH_3CN in water, Zorbax ODS-3, RT 8.51 min, radiochemical purity >99%.

6(R)-[2-[8(S)-(2,2-Di[^{14}C]methylbutyryloxy)-2(S),6(S)-dimethyl-1,2,3,4,4a(S),5,6,7,8,8a(S)-decahydronaphthyl-1(S)]ethyl]-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one (10)

Following the procedure described for 4, the title compound was prepared from 7 in 35% radiochemical yield. From 15 mCi of [^{14}C]methyl iodide , 5.3 mCi of pure 10 was obtained. HPLC: 70% CH_3CN in water, Zorbax ODS-3, 220 nm, radiochemical purity >98%.

6(R)-[2-[8(S)-(4-Hydroxy-2,2-di[^{14}C]methylbutyryloxy)-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 (15)

An aqueous solution of potassium hydroxide (0.103 mL, 5.06 N, 0.514 mmol) was added to a suspension of 13 (211 mg, 0.5028 mmol) in cyclohexane (3.5 mL) and isopropyl alcohol (0.2 mL). The mixture was stirred at room temperature for 30 min and then concentrated by distillation. Additional cyclohexane (4 mL) was added and the

concentratrion repeated until a thick syrup was obtained which was further dried in vacuo for 48 h to provide 14.

To a solution of 14 (0.5 mmol) in THF (4 mL), pyrrolidine (0.052 mL, 0.623 mmol, 1.25 eq) was added. This solution was cooled to -70 °C and n-butyllithium in hexane (1.4 mL, 1.5 M, 4.2 eq) was added dropwise. THF (4 mL) was added and the mixture was stirred at the same temperature for 1 h. [^{14}C]Methyl iodide (14 mCi, 60 mCi/mmol, 0.23 mmol + .025 mL of carrier methyl iodide, 0.4 mmol, total 0.63 mmol, 22.2 mCi/mmol) in THF (2 mL) was added dropwise over 10 min and then the mixture was aged at -60 °C for 1 h. HPLC analysis (40% acetonitrile in water, 240 nm, C8, 1mL/min) of an aliquot of the reaction mixture (quenched with phosphoric acid) indicated a 5:1 ratio of 13:15. No significant change in this ratio was observed on raising the temperature of the reaction mixture to -40 °C and stirring at that temperature for 15 min. The mixture was cooled to -60 °C and then quenched by the addition of 10 mL of water. The mixture was brought to 0 °C, pH was adjusted to 2.6 by the addition of 3 N HCl, and extracted (5x) with ethyl acetate. The combined ethyl acetate extracts were washed with water, dried(Na_2SO_4), and concentrated in vacuo. HPLC analysis of the residue indicated a uv profile of 17:13:15 in a ratio of 2.5:5:1 and a radioactivity profile in which 33% of the radioactivity was associated with 15 and >56% of the radioactivity with column wash (100% CH_3CN).[5] The crude product mixture was subjected to preparative HPLC (Magnum-9, C8 column, 3 mL/min, 254 nm) and appropriate fractions were combined and worked up to provide 63 mg of 13, 25 mg of 17, and 11 mg of 15 (523 μCi, 20.6 mCi/mmol). The radiochemical purity of 15 as determined by HPLC was greater than 99%. The NMR and mass spectra were obtained on a sample made similarly using unlabeled methyl iodide and 13 and were identical with those of authentic reference material. ^{1}H NMR ($CDCl_3$) ∂0.9 (d, 3 H, J=7 Hz, CH_3), 1.1 (d, 3 H, J=7 Hz, CH_3), 1.21 (s, 3 H, CH_3), 1.24 (s, 3 H, CH_3), 2.7 (m, 2 H, pyran C-3H's), 3.7 (m, 2 H, CH_2OH), 4.39 (m, 1 H, pyran C-4H), 4.68 (m, 1 H, pyran C-6H), 5.39 (m, 1 H, C-8H), 5.56 (m, 1 H, C-5H), 5.82 (dd, 1 H, J=6,10 Hz, C-3H), 6.04 (d,1 H, J=10 Hz, C-4H); MS (FAB); m/z 435 (M+1), 303, 285, 267, 243, 225, 199. The labeled sample was stored in toluene at 5 °C and after 3 weeks the radiochemical purity had decreased to 80%. This sample was repurified by preparative HPLC (as before) and diluted with carrier 15 to afford 30 mg of 15 (257 μCi, 8.6 μCi/mg, 3.7 mCi/mmol, radiochemical purity >99%).

7-[1,2,6,7,8,8a(R)-Hexahydro-2(S),6(R)-dimethyl-8(S)-(2,2-di[^{14}C]methylbutyryloxy)-naphthalenyl-1(S)]-3(R),5(R)-dihydroxyheptanoic acid, ammonium salt (5)

To a solution of 4 (42.3 mg, 1 mCi, 10 mCi/mmol) in isopropyl alcohol (1.2 mL) was added an aqueous solution of sodium hydroxide (1.2 mL, 0.1 N). The mixture was stirred at room temperature for 15 min, cooled and acidified with 0.4 mL of 0.5 N HCl. The resulting dihydroxy acid was extracted into 15 mL of ethyl acetate, the extract was washed with water, brine, dried ($MgSO_4$) and filtered. Ammonia gas was bubbled through the ethyl acetate solution at ice bath temperature for 10 min. The precipitated ammonium salt was collected, washed with ethyl acetate and dried (30 mg, 68%). This material (24.4 mg) along with carrier 5 (42.9 mg) was dissolved in hot acetonitrile-water (1:1, 60 ^{o}C, 1.4 mL). The solution was allowed to cool, diluted with acetonitrile (4.5 mL), stirred at room temperature for 1 h and at -5 ^{o}C for 30 min, and filtered. The collected product was washed with acetonitrile (2 mL) and dried in vacuo to yield pure 5 (56 mg, 8.85 μCi/mg, 4 mCi/mmol). The radiochemical purity as determined by HPLC (Zorbax ODS-3, 40% CH_3CN in 0.05 M NH_4OAc, pH 7.6, 1 mL/min, 238 nm) was greater than 99 %.

7-[1,2,3,4,4a(S),5,6,7,8,8a(S)-Decahydro-2(S),6(S)-dimethyl-8(S)-(2,2-di[^{14}C]methyl-butyryloxy)-naphthyl-1(S)]-3(R),5(R)-dihydroxyheptanoic acid, ammonium salt (11)

To a solution of 10 (47.1 mg, 1.276 mCi) in 1.4 mL of ethanol was added an aqueous solution of sodium hydroxide (1.25 mL, 0.1 M). The mixture was stirred at room temperature for 15 min, cooled and diluted with 5 mL of water and acidified with 0.07 mL of 2.5 N HCl. The resulting dihydroxy acid 12 was extracted into methylene chloride (25 mL). The methylene chloride extract was dried over $MgSO_4$, filtered and the filtrate saturated with gaseous ammonia. Evaporation of methylene chloride provided the crude ammonium salt which solidified upon standing. This salt was purified by preparative HPLC in three portions (Whatman M9, ODS-3, 40% CH_3CN in 0.01 M NH_4OAc, 2.5 mL/min, 205 nm). Pure 11 was recovered as a solution in column eluant (850 μCi, 27 μ Ci/mg, radiochemical purity >98.5 %).

7-[1,2,6,7,8,8a(R)-Hexahydro-2(S),6(R)-dimethyl-8(S)-(4-hydroxy-2,2-di[^{14}C]methyl-butyryloxy)-naphthalenyl-1(S)]-3(R),5(R)-dihydroxyheptanoic acid, ammonium salt (16)

To a solution of 15 (9 mg, 68 μCi, radiochemical purity, 84%) in 0.25 mL of 2-propanol was added 0.25 mL of 0.083 M sodium hydroxide solution. The mixture was stirred at room temperature for 15 min, cooled, acidified with 0.3 mL of 0.1 N HCl and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried ($MgSO_4$) and filtered. Ammonia gas was bubbled through ethyl acetate solution at 0 °C. Evaporation of ethyl acetate provided the crude ammonium salt which was subjected to preparative reverse phase HPLC (Magnum 9, ODS-3, 22.5% CH_3CN in 0.05M NH_4OAc, 3 mL/min, 254 nm). Pure 16 was recovered as a solution in column eluant (37 μCi, 8.6 μCi/mg, 4 mCi/mmol, radiochemical purity 99%).

ACKNOWLEDGEMENT

We are grateful to Dr. T. R. Verhoeven and Mr. W. F. Hoffman for their help during the course of this work. Thanks are due to Dr. B. H. Arison for the NMR spectra and to Mr. J. L. Smith for the mass spectra.

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3. Hoffman, W. F., Smith, R. L., Scolnick, E. M.-EP 0211416 (1987); US Patent 4,668,699 (1987).
4. We thank Mr. W. F. Hoffman for providing a sample of 13 (precursor of 14) which was prepared from dihydro-3- methyl-2(3H)-furanone.
5. The products eluting in the column wash were not identified.