United States Patent [19]

Hoffman et al.

[11] **4,450,171**

[45] * **May 22, 1984**

[54] **ANTIHYPERCHOLESTEROLEMIC COMPOUNDS**

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[*] Notice: The portion of the term of this patent subsequent to Apr. 24, 2001 has been disclaimed.

[21] Appl. No.: **388,372**

[22] Filed: **Jun. 14, 1982**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 217,640, Dec. 18, 1980, which is a continuation-in-part of Ser. No. 175,460, Aug. 5, 1980, abandoned, which is a continuation-in-part of Ser. No. 118,051, Feb. 4, 1980, abandoned.

[51] **Int. Cl.**[3] **C07C 69/74**; A61K 31/335; C07D 309/30

[52] **U.S. Cl.** **424/279**; 549/292; 560/119; 560/256; 424/305; 424/311

[58] **Field of Search** 424/279, 305, 317; 260/343.5; 560/107, 185, 256, 119; 562/501; 549/292

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,983,140	10/1977	Endo et al. .	
4,049,495	6/1978	Endo et al. .	
4,137,322	1/1979	Endo et al. .	
4,231,938	11/1980	Monaghan et al.	549/292
4,293,496	10/1981	Willard et al.	562/501
4,294,846	10/1981	Albers-Schonberg et al.	549/292
4,342,767	8/1982	Albers-Schonberg	549/292
4,351,844	9/1982	Patchett et al.	424/279
4,361,515	11/1982	Terahara et al.	562/501
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OTHER PUBLICATIONS

F. M. Singer et al., Proc. Soc. Exper. Biol. Med., 102, 370, (1959).

Hulcher, Arch. Biochem. Biophys. 146, 422, (1971).

Brown et al., J. Chem. Soc., Perkin I, 1165, (1976).

Endo et al., J. Antibiotics, XXXII, 852, (1979).

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[57] **ABSTRACT**

6(R)-[2-(8'-acyloxy-2'-methyl-6'-methyl (or hydrogen)-polyhydronaphthyl-1')-ethyl]-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-ones are prepared by acylation of the corresponding 8'-hydroxy compounds. The products are strong inhibitors of the biosynthesis of cholesterol.

9 Claims, No Drawings

ANTIHYPERCHOLESTEROLEMIC COMPOUNDS

SUMMARY OF THE INVENTION

This is a continuation-in-part of copending application Ser. No. 217,640, filed Dec. 18, 1980, which is a continuation-in-part of copending application Ser. No. 175,460, filed Aug. 5, 1980, now abandoned, which in turn is a continuation-in-part of copending application Ser. No. 118,051, filed Feb. 4, 1980, (now abandoned).

This invention relates to a group of 6(R)-[2-(8'-acyloxy-2'-methyl-6'-methyl(or hydrogen)-polyhydronaphthyl-1')-ethyl]-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-ones and to the hydroxy acid form of said pyranones, the pharmaceutically acceptable salts of said hydroxy acids and to the lower alkyl and phenyl, dimethylamino or acetylamino substituted lower alkyl esters of said hydroxy acid.

More specifically, this invention relates to a compound of the structure I in Table I, in which the dotted lines X, Y and Z represent possible double bonds, said double bonds being, when any are present, either X and Z together in combination or X, Y or Z alone; R represents 1-ethyl-1-methylpropyl, 1,1-diethylpropyl 1,1-dimethylethyl, 1,1-diethylbutyl, 1,1-dimethylpropyl, C_{3-10} cycloalkyl, C_{2-10} alkenyl, C_{1-10} CF_3-, substituted alkyl, phenyl, halophenyl, phenyl-C_{1-3} alkyl or substituted phenyl-C_{1-3} alkyl, in which the substituent is halo, C_{1-3} alkyl or C_{1-3} alkoxy; and the free hydroxy acids of formula II formed by opening the lactone ring of formula I in Table I.

BACKGROUND OF THE INVENTION

It is known that certain mevalonate derivatives inhibit the biosynthesis of cholesterol, c.f. F. M. Singer et al, *Proc. Soc. Exper. Biol. Med.*, 102 370 (1959) and F. H. Hulcher, *Arch. Biochem. Biophys.*, 146, 422 (1971). Nevertheless, the activity of these known compounds has not always been found to be satisfactory, i.e. to have practical application.

Recently, Endo et al, reported (U.S. Pat. Nos. 4,049,495, 4,137,322 and 3,983,140) the production of fermentation products which were quite active in the inhibition of cholesterol biosynthesis. The most active member of this group of natural products, now called compactin, IIIa(R'=H) was reported by Brown et al. [*J. Chem. Soc. Perkin I* 1165 (1976)] to have a complex mevalonolactone structure.

More recently, Monaghan et al in U.S. Pat. No. 4,231,938, which is incorporated herein by reference, reported an inhibitor, designated MK-803 and having the structure III_a (R'=CH_3) in Table I, which was isolated from an entirely different fermentation. Albers-Schonberg et al (U.S. Pat. No. 4,294,846) described a dihydro MK-803, designated III_d (R'=CH_3) in Table I, of about equal potency to MK-803 isolated from the same fermentation as was MK-803. Patchett et al (U.S. Ser. No. 210,826, filed Dec. 1, 1980) describe dihydro and tetrahydro derivatives of MK-803 of different structures ($III_{b,c\ and\ e}$ (R'=CH_3) in Table I), prepared by the catalytic hydrogenation of MK-803. Willard (U.S. Pat. No. 4,293,496), describes the preparation of the 8-hydroxy derivatives (IV_{a-e} (R'=CH_3) in Table I) which are the starting materials for the preparation of some of the novel compounds of this invention.

A tetrahydro analog III_e (R'=H), of compactin was reported in published Japanese Application (Kokai) 55009-024.

Very recently a dihydro-analog of compactin of structure III_d (R=H) was isolated from compactin fermentation broths as reported by Gullo et al, (U.S. application Ser. No. 207,508, filed Nov. 17, 1980).

The preparation of the starting material, III_d, R^1=CH_3) as mentioned previously, is described by Albers-Schonberg et al in U.S. Pat. No. 4,294,846, and is the product of the following fermentation with a strain of *Aspergillus terreus*, ATCC No. 20542, designated MF-4845 in the culture collection of MERCK & CO., Inc., Rahway, N.J.

PREPARATION OF COMPOUND III_d (R'=CH_3)

A. Fermentation

A tube of lyophilized culture MF-4845 was opened aseptically and the contents suspended in an unbaffled 250 ml Erlenmeyer flask (seed flask) containing approximately 10 ml of the Medium which has the following composition:

Medium	
Corn steep liquor	5 g
Tomato paste	40 g
Oatmeal	10 g
Glucose	10 g
Trace Element Solution	10 g
Distilled water	1000 ml
pH 6.8 with NaOH	
Trace Element Solution	
$FeSO_4.7H_2O$	1000 mg
$MnSO_4.4H_2O$	1000 mg
$CuCl_2.2H_2O$	25 mg
$CaCl_2.2H_2O$	100 mg
H_3BO_3	56 mg
$(NH_4)_6Mo_7O_{24}.4H_2O$	19 mg
$ZnSO_4.7H_2O$	200 mg
Distilled Deionized Water	1000 ml

The inoculated flask was incubated for 24 hours at 28° C. on a 220 rpm shaker (2 inch throw). An unbaffled 2 liter Erlenmeyer flask containing 500 ml of the medium was then inoculated with 10 ml of the first stage fermentation growth from the seed mixture. This too was shaken 24 hours at 28° C.

A 200 gallon stainless steel fermentation vat was then charged with 485 liters of a medium comprising:

Cerelose	4.5% wt/vol
Peptonized Milk	2.5% wt/vol
Autolyzed yeast	0.25% wt/vol
Polyglycol P2000	0.25% vol/vol

whose pH was adjusted to 7.0. This was sterilized 15 minutes at 121° C. One liter of the second stage above was then charged and the mixture was incubated at 85 rpm for 12 hours then at 130 rpm for 84 hours at 28° C. with an air flow of 5 cfm for 12 hours then 10 cfm for 84 hours.

B. Isolation

1. Extraction

Two batches of one hundred gallons of whole broth were combined, acidified with stirring to pH 4.1 by careful addition of 800 ml of concentrated hydrochloric acid, and extracted by addition of 75 gal of ethyl acetate and further stirring for two hours.

About 25 lbs of a silicaceous filter aid was then added and the total slurry was pumped through a 24-inch filter press. An additional 75 gal of ethyl acetate was used to wash the press cake and continue the extraction, by reversing the direction of pumping through the press four times. Then all of the wash solvent was discharged from the press and combined with the first filtrate. The two-phase filtrate was allowed to settle, and the water layer removed. The ethyl acetate layer was washed with 10 gal of deionized water, the phases were allowed to separate and the ethyl acetate extracts were concentrated under vacuum to a residue of about 10 gal.

2. Lactonization

Ethyl acetate extracts from an additional three hundred gal of broth were added to the above extract and the volume was reduced to about thirty gal by vacuum distillation. About fifty gal of toluene was added, and the batch was concentrated under vacuum to 32 gal; this step was repeated; then sufficient new toluene was added to bring the volume to 75 gal. Without vacuum, the batch was brought to reflux and maintained there for two hours, with a temperature over 106° C.

This solution was then concentrated under vacuum to a small volume, which was further concentrated to an oily residue in a large rotary evaporator under vacuum.

3. Chromatography on Silica Gel

The extract obtained above was flushed free of other solvents by addition of 2 gal of methylene chloride and reconcentration to an oil.

The oily residue was dissolved in about 5 gal of ethyl acetate-methylene chloride (30/70; v/v) mixture, and a slurry was made by addition of 2.8 kg of silica gel.

The slurry was loaded as a level layer on the top of a 12 in.×50 in. silica gel column packed in the same solvent mixture.

Elution was with ethyl acetate-methylene chloride (40/60; v/v) at 800 ml/min. A forerun of 10 gal, then further fractions of 4 gal each were collected.

Fractions 6–10 inclusive were concentrated under vacuum to an oily residue which was dissolved in hot ethyl acetate, treated with decolorizing carbon, filtered hot, and cooled. Crystals of Compound III_a (R'=CH_3) were filtered off and the mother liquors were concentrated to an oil for further chromatography.

4. Rechromatography on Silica Gel

Mother liquor residues from similar broth extract work-ups equivalent to an additional 600 gal of fermentation production were combined with the above in methylene chloride solution. One-half of this solution was taken for further silica gel chromatography. A small aliquot showed a total solids content of 325 g. The solution was treated with 40 g of decolorizing carbon, filtered, and the cake rinsed with methylene chloride. The combined filtrate and washings were concentrated under vacuum to an oily residue. This was redissolved in 800 ml of ethyl acetate/methylene chloride (30/70; v/v) and slurried with 225 g of silica gel. The slurry was loaded on top of a 14×36 cm column bed of silica gel packed in the same solvent mixture. Development was with ethyl acetate/methylene chloride (40/60; v/v). A forecut of three liters was set aside; then fractions of 800 ml each were collected.

5. Chromatography on Reverse-phase Packing

Forty ml from fraction 12 of the above chromatography were concentrated to an oil weighing 500 mg and the oil redissolved in 5 ml acetonitrile. This acetonitrile solution was charged to a 5/8" OD by 6 ft long stainless steel chromatography column packed with preparative reverse-phase liquid chromatography column packing material "Bondapak C18/PorasilB" (Waters Associates, Inc., Milford, Mass. 01757). The column was eluted with a mixture consisting of (v/v) 55% acetonitrile and 45% 0.05 M ammonium phosphate pH3. The elution volume between 1360 ml and 1700 ml was combined on the basis of refractive index detection. The organic solvent was removed in vacuo and the residual aqueous solution extracted with ethyl acetate. In vacuo removal of the ethyl acetate left 120 mg of compound which crystallized from a concentrated acetonitrile solution yielding crystals of Compound III_d (R'=CH_3), m.p. 129°–131° C.

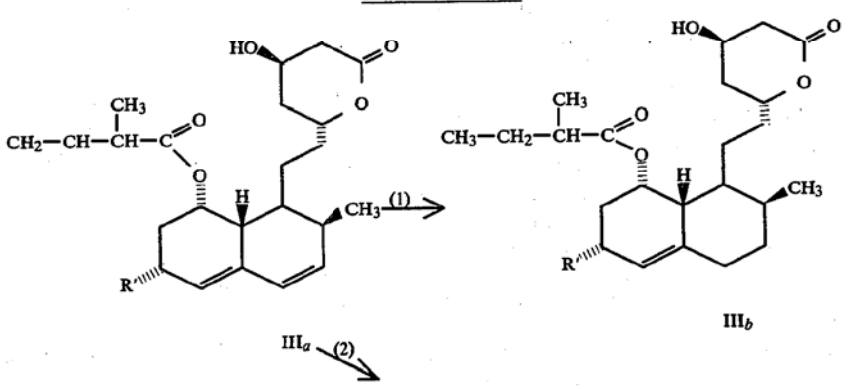
PREPARATION OF COMPOUNDS $III_{b, c, e}$

Starting materials III_b, III_c and III_e (R'=CH_3) as mentioned above are described in U.S. application, Ser. No. 210,826, filed Dec. 1, 1980 by Patchett et al., in accordance with the following Flow Sheet and preparative methods extracted therefrom.

The desmethyl analogs, III_b, III_c and III_e (R'=H) are obtained substantially as described by Patchett et al. but starting with III_a (R'=H) in each case.

For the preparation of III_e it is advantageous to reduce III_d inasmuch as the desired trans fusion of the perhydronaphthalene ring, present in the starting materials, is retained in the final product, and the need to separate isomers is avoided.

FLOW SHEET



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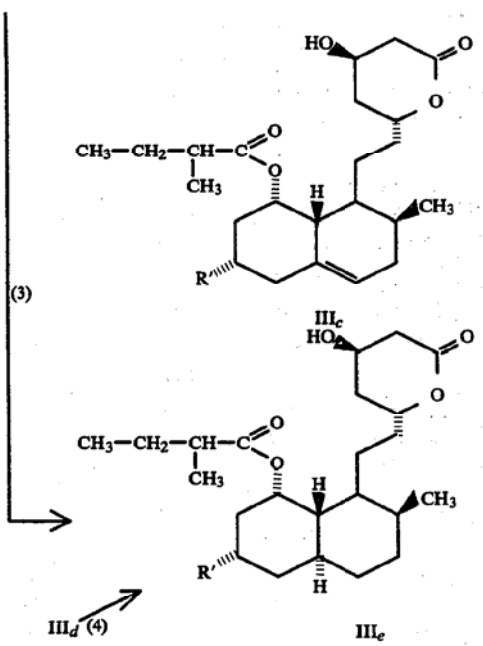
FLOW SHEET



Reactions and Reagents

1. Hydrogenation at about 20°–75° C. and about atmospheric pressure to about 4 atmospheres over tris-(triphenylphosphine)chlororhodium in an aromatic solvent such as benzene, toluene or xylene, preferably toluene. Preferred conditions are about 40° C. and about 2–7 atmospheres in toluene.
2. Hydrogenation at about 20°–25° C. and about atmospheric pressure over 5% palladium on calcium carbonate in a lower alkanol such as a C_{1-3} alkanol, especially ethanol.
3. Hydrogenation at about 20°–25° C. and atmospheric pressure over platinum oxide in ethyl acetate.
4. Hydrogenation at about 20°–25° C. and atmospheric pressure over 10% Palladium on charcoal in ethyl acetate.

Preparation of 6α-[2-(8′-β-2-(S)-methylbutyryloxy-2′β,6′α-dimethyl-1′,2′,3′,4′,6′,7′,8′,8′a-octahydronaphthyl-1)ethyl]-4β-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one, III_b ($R'=CH_3$)

A mixture of 50 mg (0.1236 mmol) of Compound III_a ($R'=CH_3$) and an equal molar amount (114.35 mg, 0.1236 mmol) of tris(triphenylphosphine)chlororhodium in 10 ml of dry toluene was hydrogenated at room temperature for 6 days, with a total uptake of 14.6 ml of hydrogen. The mixture was evaporated in vacuo to dryness. The red residue was subjected to preparative thin-layer chromatography on silver nitrate impregnated silica plates and was developed twice in the 10% ethyl acetate-ether system. The yield of Compound III_b ($R'=CH_3$) was 22.3 mg.

Mass spectrum (M/e): 406 (m^+), 304 (m-102), 286 (m-102–18)

nmr ($CDCl_3$, 300 MHz): δ 4.37 (m, 1H), 4.60 (m, 1H), 5.34 (d of t, J=2.5 Hz, 1H), 5.41 (m, 1H)

Preparation of 6α-[2-(8′β-2-(S)-methylbutyryloxy-2′β,6′α-dimethyl-1′,2′,3′,5′,6′,7′,8′,8′a-octahydronaphthyl-1)ethyl]-4β-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one, III_c ($R'=CH_3$)

A solution of 80.91 mg (0.2 mmol) of Compound III_a ($R'=CH_3$) in 10 ml of absolute ethanol, in the presence of an equal weight of 5% Pd on $CaCO_3$ was hydrogenated at 1 atmosphere until an uptake of one mole equivalent of hydrogen was observed. The catalyst was then removed by filtration and the filtrate was evaporated to dryness (81 mg). After a purification by preparative thin-layer chromatography to remove a small amount of by-product tetrahydro compound, 72 mg of the 1,4 reduction product III_c ($R'=CH_3$) was isolated.

Mass spectrum (M/e): 406 (m^+), 304 (m-102), 286 (304-H_2O)

nmr ($CDCl_3$, 300 MHz): δ 4.38 (m, 1H), 4.64 (m, 1H), 5.28 (d of t, J=3.5 Hz, 1H), 5.48 (m, 1H)

Preparation of 6α-[2-(8′β-2(S)-methylbutyryloxy-2′α, bαβ-dimethyl-1′,2′,3′,4′,4′aα,5′,6′,7′,8′,8′a-decahydronaphthyl-1)ethyl]-4β-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one, III_e ($R'=CH_3$)

A solution of 80.91 mg (0.2 mmol) of Compound III_a ($R'=CH_3$) in 10 ml of ethyl acetate was hydrogenated in the presence of an equal weight of platinum oxide at one atmosphere. An exact 2 mole equivalent of hydrogen was consumed within 1 hour. The catalyst was removed by filtration and the filtrate was concentrated to dryness to give an oil. The cis and trans isomers were separated by preparative thin-layer chromatography on silica gel plates (10% ethyl acetate-ether system, bands detected by water spray). The trans isomer III_e ($R'=CH_3$) appears as the more polar spot, compared to the cis isomer, and 60 mg was isolated.

Mass spectrum (M/e): 408 (m^+), 323 (m-85), 306 (m-102)

nmr ($CDCl_3$, 300 MHz): δ 4.36 (broad singlet, 1H), 4.59 (m, 1H), 5.19 (d of t, J=2.5 Hz, 1H)

Fermentative Production of Compound III_d (R'=H)

A. Fermentation

A natural isolate of *Penicillium citrinum*, NRRL 8082 was used to prepare a yeast-malt extract (YME) slant which was incubated for 2 weeks at 28° C.

A portion (1/5) of the slant (MF-4870a) was used to inoculate each of 5 unbaffled seed flasks (250 ml) containing 44 ml of KF seed medium with $CaCl_2$. They were incubated for 3 days at 28° C., and 220 rpm. A portion of the seed growth (about 1.5 ml) was used to inoculate each of 100 production medium flasks (250 ml unbaffled) containing 40 ml of LM Production Medium Without Malt Extract. The production flasks were incubated for 4 days at 25° C.

Another group of production medium flasks (140), each containing 40 ml of LM Production Medium Without Modification were inoculated and incubated under the same conditions as previously described. The broths from both fermentations were combined.

The various media employed in the foregoing fermentations are:

YME Slant	
Dextrose	4 g./l.
Malt Extract	10 g./l.
Yeast Extract	4 g./l.
Agar	20 g./l/
Dist. Water	to 1 liter
pH	7.0
KF Seed Medium with $CaCl_2$	
$CaCl_2$	10 g
Corn steep liquor	5 g
Tomato paste	40 g
Oatmeal	10 g
Cerelose	10 g
Trace Element Mix	10 ml
Distilled water	1000 ml
pH	6.8
Trace Element Mix	
$FeSO_4.7H_2O$	1 g
$MnSO_4.4H_2O$	1 g
$CuCl_2.2H_2O$	25 mg
$CaCl_2$	100 mg
H_3BO_3	56 mg
$(NH_4)_6Mo_7O_{24}.4H_2O$	19 mg
$ZnSO_4.7H_2O$	200 mg
Distilled Water	1000 ml
LM Production Medium Without Malt Extract	
Dextrose	20 g
Glycerol	20 ml
Ardamine pH	10 g
$CoCl_2.6H_2O$	8 mg
Polyglycol p 2000	0.25%
Distilled Water	1000 ml
pH	7.0
LM Production Medium Without Modification	
Dextrose	20 g
Glycerol	20 ml
Ardamine pH	10 g
Malt Extract	20 g
$CoCl_2.6H_2O$	8 mg
Polyglycol p 2000	0.25%
Distilled Water	1000 ml
pH	7.0

B. Isolation

The combined whole broth (10.3 liters) was filtered and the mycelia cake was washed with 2.5 liters of deionized water. The combined filtrate and wash was adjusted to pH 4.0 with 1 N hydrochloric acid. The aqueous solution was extracted with 7 liters of ethyl acetate and the extract was back-extracted with 3×2 liters of aqueous sodium hydroxide solution. The combined sodium hydroxide extract was adjusted to pH 3.8 with 1 N hydrochloric acid and extracted with 2 liters and 1 liter of ethyl acetate. The combined ethyl acetate solution was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness. The oily residue was dissolved in toluene and refluxed for 1 hour. The toluene solution was concentrated to dryness and the residue was dissolved in 18 ml of a mixture of n-hexane/toluene/methanol (4/1/1 by volume). This solution was loaded onto a 30 mm (ID)×40 cm. Sephadex LH-20 column equilibrated in the same solvent system. After eluting with 300 ml of solvent, a 10 ml fraction was obtained which was concentrated to an oil. High performance liquid chromatography (HPLC) on an ES Industries Chromega ® column (9 mm×50 cm) using a mixture of acetonitrile/water (60/40 by volume) as the eluting solvent yielded 45 mg of dihydrocompactin (Compound III_d, R'=H), m.w. 392.2560 by mass spectrum (calculated for $C_{23}H_{36}O_5$, 392.2558).

In KBr, the major IR peaks obtained from a Fourier Transform-IR (FTIR, Nicolet, Model 7199) are at 1724, 1704, 1258, 1078 and 1070 cm^{-1}. Of significance is a peak at 3005 cm^{-1} and the absence of a peak at 3030 cm^{-1}.

A nuclear magnetic resonance spectrum was obtained in $CDCl_3$, ~1 mg/0.5 ml) on a Varian SC-300 superconducting nmr spectrometer. The following are the peak positions given in ppm (δ) relative to internal tetramethylsilane (TMS).

δ	Assignment
5.62 d,d,d (2.17, 4.5, 10.0)	$H_{3'}$ (d?)
5.43 d (10)	$H_{4'}$ (c?)
5.20 m	$H_{8'}$
4.63 m	H_6
4.39 m	H_4
2.75 d,d (17.5, 5.5)	
2.63 d,d,d (17.5, 4.0, 1.5)	3-CH_2
2.39 m	$CH_3HCC(=O)$
2.29 m	$H_{4a'}$ + $H_{5'}$
1.14 d	$CH_3CHC(=O)$
0.90 t	CH_3CH_2
0.84 d	$CH_3H_{2'}$

d: doublet;
m: multiplet;
t: triplet

The evidence indicates the structure to be:

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