SYNTHESIS OF A CONFORMATIONALLY RESTRAINED 4-ARYL-THIOPHENE-3-HEPTENOIC ACID AND ITS EVALUATION AS AN HMG COENZYME A REDUCTASE INHIBITOR

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Abstract: A 4-phenylthiophene tethered to the 5-position of the heterocycle was synthesized from 1-benzosuberone. The key steps in the synthesis were the tandem thiolacetate hydrolysis – Michael addition – intramolecular Wittig reaction (5 → 7) and the chelation-controlled reduction of the hydroxy β-ketoester (12 → 13). The product 14 exhibited nearly identical *in vivo* potency to the non-tethered analogs.

Introduction

Coronary heart disease remains one of the leading causes of death in western civilization. Elevated levels of serum cholesterol, and in particular, low density lipoprotein (LDL) cholesterol are strongly correlated with risk of morbidity and mortality due to this disease (1-3). The primary defense against elevated LDL cholesterol levels is dietary intervention. However, for patients nonresponsive to this intervention pharmacological treatment is indicated.

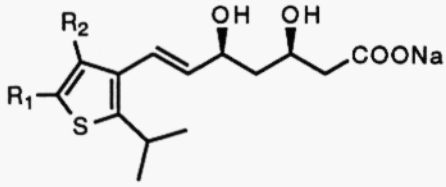
The goal of developing safe and effective therapeutic agents to lower LDL-cholesterol has focused primarily on inhibitors of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase, the key enzyme which catalyzes the conversion of HMG-CoA to mevalonic acid in the cholesterol biosynthetic pathway.

A variety of HMG-CoA reductase inhibitors with a common *erythro*-β,δ-dihydroxyheptenoic acid motif have been reported (4). We recently described the design and biological evaluation of a series of highly functionalized thiophene-based 3,5-dihydroxyheptenoic acid derivatives (1) as inhibitors of HMG-CoA reductase (5). Within this series of compounds we found that the *in vitro* activity was generally higher when the substituent R_2 at C-4 of the thiophene ring was a phenyl group (either substituted or unsubstituted). The biological activity was less sensitive to the nature of the substituent R_1 at C-5. Groups such as phenyl, alkenyl, or small alkyl moieties like methyl or isopropyl were tolerated.

With this data in hand we wished to investigate the effect of tethering the phenyl group at C-4 to the C-5 position of the thiophene ring which would effectively rigidify the conformation of the phenyl with respect to the plane of the thiophene heterocycle (2). We initially considered a one, two, or three-carbon chain as likely candidates, however, we ruled out the one-carbon (CH_2) analog which would possess a highly oxidizable methylene unit between two aromatic groups. The next higher homolog, the two-carbon (CH_2CH_2) analog was also ruled out for synthetic reasons. The conditions used for the aromatization of the thiophene ring (Scheme 1,

step d) would also aromatize the ring containing the methylene carbons. This left the three-carbon tricycle as the most promising analog from a synthetic perspective.

Molecular modeling studies (6) indicate that the angle between the planes of the phenyl ring and the thiophene ring (defined by atoms a-b-c-d in structure **2**) is approximately 51° in this system. Removal of the three-carbon tether followed by energy minimization leads to only a slight change to an angle of 54°, indicating that the orientation of the phenyl ring relative to the thiophene ring in the fused system is similar to that in a reasonable local energy minima in **1** (R_2 = Ph). For two-carbon and one-carbon tethers, leading to six- and five-membered rings, respectively, molecular mechanics calculations suggest tortion angles of approximately 25° and 0°.



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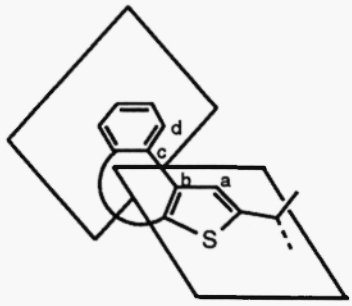

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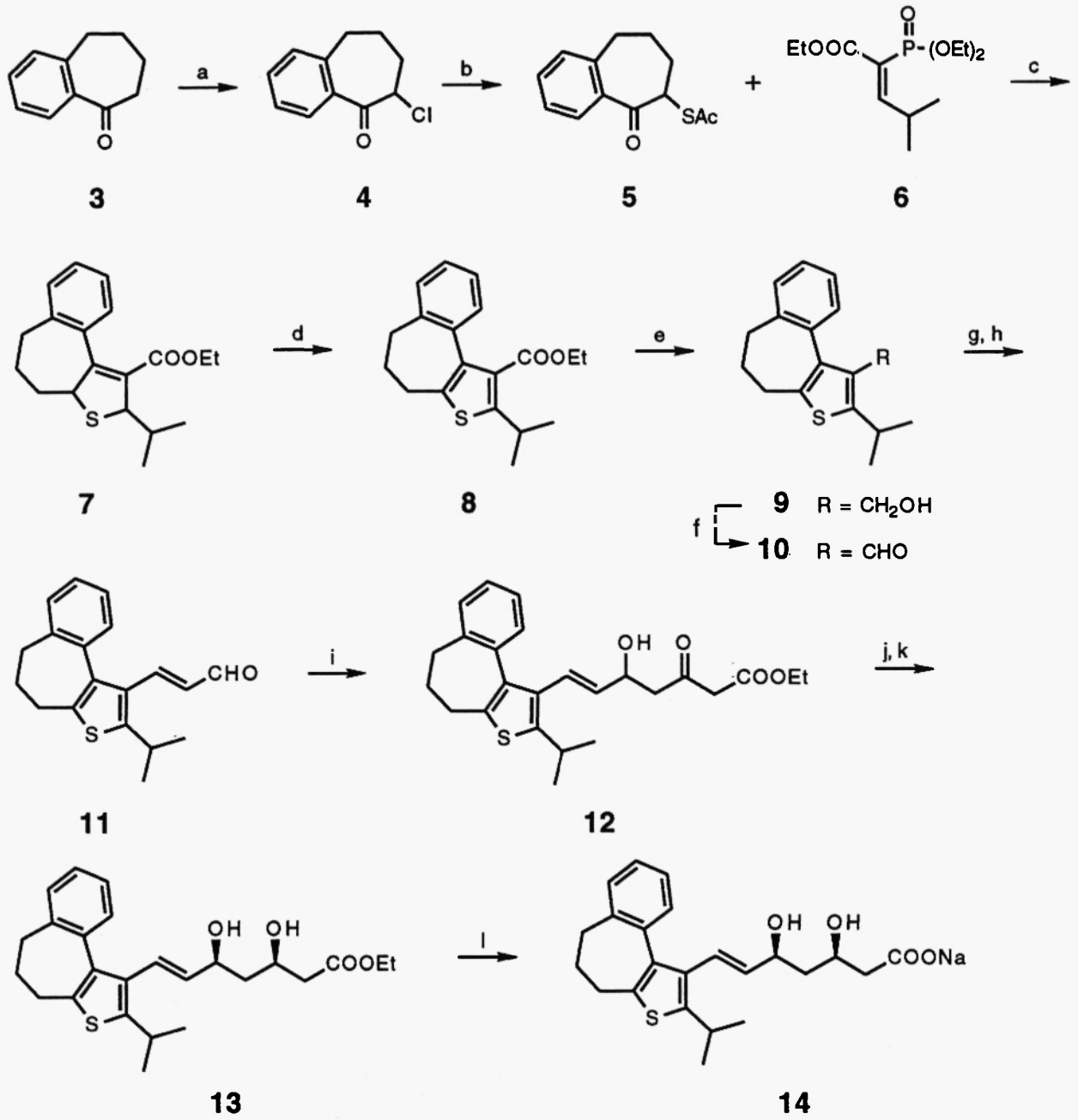
Chemistry

Commercially available 1-benzosuberone (**3**) was chlorinated with sulfuryl chloride after which the chloride was displaced with potassium thiolacetate to give **5** in high yield. In one pot, the acetate was cleaved with lithium ethoxide and the resulting liberated thiolate adds in a Michael fashion to **6** which subsequently undergoes an intramolecular Horner-Emmons olefination to afford the dihydrothiophene tricycle **7**. Aromatization of the thiophene ring was efficiently accomplished with DDQ. Ester **8** was converted to aldehyde **10** *via* reduction to alcohol **9** followed by oxidation using N-methylmorpholine-N-oxide catalyzed by $RuCl_2(PPh_3)_3$ (7).

Homologation of the aldehyde to the α,β–unsaturated aldehyde **11** proceeded by a two-step sequence which involved addition of *cis*-2-ethoxyvinyllithium (8) to **10** followed by acidic hydrolysis of the resulting intermediate hydroxy enol ether. An aldol condensation of **11** with ethyl acetoacetate dianion produced adduct **12** in quantitative yield. A stereoselective chelation-controlled reduction of the β–ketoester function using the Merck modification (9) of Narasaka's procedure (10) afforded the *erythro* dihydroxy ester **13** in 44% yield with >96% *syn*-selectivity. Hydrolysis of the ester with one equivalent of 1.0N NaOH followed by lyophilization gave the sodium carboxylate **14** in 77% yield.

Scheme 1



Reagents and Conditions: (a) $SOCl_2$, CCl_4; (b) KSAc, EtOH; (c) LiOEt, THF; (d) DDQ, CH_2Cl_2; (e) $LiAlH_4$, THF; (f) $(Ph_3P)_3RuCl_2$, NMO, acetone; (g) *cis*-2-ethoxyvinyllithium, THF; (h) PTSA, THF-H_2O; (i) ethyl acetoacetate, 2 LDA, THF; (j) Et_3B, THF-MeOH; (k) MeOH; (l) NaOH, EtOH

Biology

The compounds were tested in a rat liver microsomal preparation as inhibitors of HMG-CoA reductase as described by Ackerman,*et al.* (11). Data are represented as IC_{50}s, which are the concentrations found to produce a 50% inhibition of the incorporation of labelled HMG-CoA into mevalonate. The *in vivo* studies were performed

using male Sprague-Dawley rats with 6 animals per dose group. The results are expressed as ED_{50}s, which are the doses (in mg/kg) necessary to produce a 50% inhibition of the incorporation of ^{14}C-acetate into cholesterol.

The biological activity of the tethered thiophene **14** was compared to its closest non-tethered (5-methyl-4-phenyl) thiophene analog as well as compactin (12), a fungal metabolite with potent HMG-CoA reductase inhibitory activity (Table 1). Comparison of the inhibitory activity of **14** and **1** showed the two compounds to be approximately equivalent in both *in vitro* and *in vivo* assays. These results suggest that the accessible conformations of **14** may be relatively close to the bioactive conformation of the non-tethered analog **1**, a result consistent with the molecular mechanics calculations.

Comparison of **14** with compactin showed its *in vitro* activity was approximately 7 times as potent as the lactone form but roughly equivalent to the saponified form. The *in vivo* potency of **14** was 22 times that of compactin lactone and 7 times that of the saponified form.

Table 1. *In Vitro* HMG-CoA Reductase Inhibitory and *In Vivo* Cholesterol Synthesis Inhibitory Activities of Tethered and Non-tethered Thiophenes

Compound	*In Vitro* IC_{50} (μM)[a]	*In Vivo* ED_{50} (mg/kg)[c]
1 $R_1 = CH_3$, $R_2 = Ph$	0.094 ± .016 (5)	0.19 ± .06 (9)
14	0.17 ± .003 (4)	0.18 ± .1 (9)
Compactin (lactone)	1.15 ± .03 (232)[b]	4.02 ± 1.13 (29)
Compactin (Na salt)	0.11 ± .026 (12)[b]	1.3 ± .4 (12)

[a] Parameters were calculated using logistic curve fit of dose response data with the indicated number of dose points (in parentheses). The response at each dose point is the mean response of duplicate determinations. [b]Mean and S.E. from the indicated number of separate dose response studies. [c]The response at each dose was the mean response of a group of 6 animals

Experimental

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. The infrared spectra were recorded on an Analect FX-6200 spectrometer. Absorption frequencies are quoted in reciprocal centimeters. Nuclear magnetic resonance spectra were recorded on Jeol FX-90Q and Jeol FX-200 spectrometers using tetramethylsilane as an internal reference. Chemical shifts are quoted in parts per million (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). All carbanion generating reactions were conducted under argon atmosphere using tetrahydrofuran which was freshly distilled over lithium aluminum hydride. No attempt has been made to optimize the yields of the described reactions.

2-Chloro-1-benzosuberone (4)

A mixture of 8.0 g (50 mmole) of 1-benzosuberone and 7.5 g (55 mmole) of sulfuryl chloride in 100 ml of carbon tetrachloride was stirred at 70° C for 18 hr. The solvent was removed under reduced pressure to give 9.5 g (98%) of **4** as an oil. The product was used in the next step without purification; ir (neat) 1696 cm^{-1}; ^1H nmr ($CDCl_3$): δ 7.70-7.06 (m, 4H), 4.82 (dd, 1H), 3.09-2.84 (m, 2H), 2.47-1.78 (m, 4H).

5-Oxo-6,7,8,9-tetrahydro-5H-benzo[a]cyclohepten-6-yl Ethanethioate (5)

A mixture of 9.5 g (48 mmole) of 4 and 6.5 g (57 mmole) of potassium thiolacetate in 100 ml of ethanol was stirred at room temperature for 48 hr. The solvent was removed under reduced pressure and water was added to the residue. The mixture was extracted into methyl *t*-butyl ether (2x) and the combined organic phases were dried over sodium sulfate. The solvent was removed under reduced pressure to give 11.0 g (97%) of 5 as an oil; ir (neat) 1683, 1200, 1121 cm^{-1}; ^1H nmr ($CDCl_3$): δ 7.74-7.09 (m, 4H), 4.80-4.54 (m, 1H), 3.13-2.91 (m, 2H), 2.37 (s, 3H), 2.37-1.74 (m, 4H); Anal. Calcd. for $C_{13}H_{14}O_2S$: C, 66.64; H, 6.02; S, 13.68. Found: C, 66.81; H, 5.89; S, 13.56.

Ethyl 2-Isopropyl-3a,4,5,6-tetrahydro-2H-benzo[3,4]cyclohepta[b]thiophene Carboxylate (7)

To a solution of 1.15 g (25 mmole) of ethanol in 75 ml of tetrahydrofuran at –60° C was added dropwise 1.6 g (25 mmole) of *n*-butyllithium (1.6M in hexane). After stirring the mixture at –60° C for 20 min, a solution of 5.7g (24 mmole) of 5 in 30 ml of tetrahydrofuran was added dropwise. The mixture was stirred at –60° C for 6 hr then a solution of 6.8 g (24 mmole) of 6 in 30 ml of tetrahydrofuran was added dropwise. The mixture was stirred at –60°C for 1 hr then was allowed to warm to room temperature overnight. The reaction was poured into water and was extracted into methyl *t*-butyl ether (1x) and methylene chloride (1x). The organic solutions were combined and dried over sodium sulfate and the solvent was removed under reduced pressure. The residue was chromatographed on a Waters Prep-500 apparatus using 10% ethyl acetate/hexane to elute the product, 1.5 g (20%) of 7 as an oil; ir (neat) 1704 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.18 (m, 4H), 4.78 (dd, 1H), 4.07 (m, 1H), 3.98 (q, 2H), 2.91-1.27 (m, 7H), 1.00 (d, 6H), 0.94 (t, 3H).

Ethyl 2-Isopropyl-5,6-dihydro-4H-benzo[3,4]cyclohepta[b]thiophene-1-carboxylate (8)

To a suspension of 1.2 g (5.3 mmole) of DDQ in 30 ml of methylene chloride was added a solution of 1.5 g (4.8 mmole) of 7 in 20 ml of methylene chloride. After stirring the mixture at room temperature for 24 hr, it was poured into 10% aqueous sodium bicarbonate. The organic phase was separated and the solvent was removed under reduced pressure. The residue was passed through a pad of silica gel using methylene chloride as eluent. Removal of the solvent under reduced pressure gave 1.35 g (90%) of 8 as an oil; ir (chloroform) 1707 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.31-7.07 (m, 4H), 4.17 (q, 2H), 3.76 (m, 1H), 2.75-2.45 (m, 4H), 2.40-2.12 (m, 2H), 1.38 (d, J=7 Hz, 6H), 1.09 (t, 3H); Anal. Calcd. for $C_{19}H_{22}O_2S$: C, 72.57; H, 7.05. Found: C, 73.02; H, 7.25.

(2-Isopropyl-5,6-dihydro-4H-benzo[3,4]cyclohepta[b]thiophene-1-yl)methanol (9)

To a solution of 1.2 g (32 mmole) of lithium aluminum hydride in 200 ml of tetrahydrofuran was added dropwise a solution of 7.9 g (25 mmole) of 8 in 50 ml of tetrahydrofuran. The mixture was stirred at room temperature for 3 hr then saturated sodium sulfate solution was added carefully until a thick precipitate formed. The solid was filtered and was washed well with tetrahydrofuran. The filtrate was evaporated under reduced pressure and the residual oil was chromatographed on a Waters Prep-500 apparatus using methylene chloride as eluent to give 2.2 g (32%) of 9 as a solid. An analytical sample was crystallized from cyclohexane, mp 115-118° C; ir ($CHCl_3$) 3609, 3452 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.57-7.12 (m, 4H), 4.57 (d, J=5.5 Hz, 2H), 3.49 (m, 1H), 2.71-2.40 (m, 4H), 2.40-2.01 (m, 2H), 1.40 (m, 1H), 1.38 (d, J=7 Hz, 6H); Anal. Calcd. for $C_{17}H_{20}OS$: C, 74.95; H, 7.40; S, 11.77. Found: C, 74.79; H, 7.41; S, 11.68.

(2-Isopropyl-5,6-dihydro-4H-benzo[3,4]cyclohepta[b]thiophene-1-carboxaldehyde (10)

To a mixture of 1.6 g (5.9 mmole) of **9** and 1.6 g (13.6 mmole) of N-methylmorpholine N-oxide in 50 ml of dry acetone was added 0.16 g of tris(triphenylphosphine)-ruthenium(II) dichloride. After stirring the mixture at room temperature for 3 hr, the solvent was removed under reduced pressure.The residue was taken up in methyl *t*-butyl ether and the solution was washed with 1N hydrochloric acid, 10% sodium bicarbonate solution, and saturated sodium chloride. The organic solution was dried over sodium sulfate and the solvent was removed under reduced pressure to give 1.6 g (100%) of **10** as an oil. This was used in the next step without further purification; ir ($CHCl_3$) 1675 cm^{-1}; ^1H nmr ($CDCl_3$) δ 9.93 (s, 1H), 7.77-7.09 (m, 4H), 4.11 (m, 1H), 2.74-2.11 (m, 6H), 1.39 (d, J=7 Hz, 6H).

(E)-3-(2-isopropyl-5,6-dihydro-4H-benzo[3,4]cyclohepta[b]thiophen-1-yl)-2-propenal (11)

To a solution of 1.9 g (12.5 mmole) of *cis*-1-bromo-2-ethoxyethylene in 25 ml of tetrahydrofuran (under argon) at –78° C was added dropwise 1.62 g (25.3 mmole) of *t*-butyllithium (14.8 ml of a 1.7M solution in pentane). After stirring the mixture at –78° C for 2.5 hr, a solution of 1.7 g (6.3 mmole) of **10** in 25 ml of tetrahydrofuran was added dropwise and stirring was continued at –78° C for 1 hr. The mixture was then quenched with saturated ammonium chloride and extracted with methyl *t*-butyl ether. The solvent was removed under reduced pressure and the residual oil was dissolved in 50 ml of tetrahydrofuran/water (9:1). To this was added 0.5 g (2.6 mmole) of *p*-toluenesulfonic acid and the mixture was stirred at room temperature for 2 hr. The mixture was poured into 10% sodium bicarbonate solution and was extracted into methyl *t*-butyl ether. The organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The residual oil was chromatographed on a Waters Prep-500 apparatus using methylene chloride as eluent to give 0.65 g (35%) of **11** as an oil; ir ($CHCl_3$) 1673, 1614 cm^{-1}; ^1H nmr ($CDCl_3$) δ 9.52 (d, J=8 Hz, 1H), 7.45 (d, J=16 Hz, 1H), 7.31-7.04 (m, 4H), 6.25 (dd, 1H), 3.53 (m, 1H), 2.73-2.40 (m, 4H), 2.40-2.05 (m, 2H), 1.40 (d, J=7 Hz, 6H).

Ethyl (E)-5-hydroxy-7-(2-isopropyl-5,6-dihydro-4H-benzo[3,4]cyclohepta[b]thiophen-1-yl)-3-oxa-6-heptanoate (12)

To a solution of 0.65 g (6.5 mmole) of diisopropylamine in 10 ml of tetrahydrofuran (under argon) at 0° C was added 0.42 g (6.56 mmole) of *n*-butyllithium (4.2 ml of a 1.6M solution in hexane). To this solution was added dropwise a solution of 0.42 g (3.2 mmole) of ethyl acetoacetate in 5 ml of tetrahydrofuran. After stirring the mixture at 0° C for 2 hr, the temperature was lowered to –25° C then a solution of 0.64 g (2.2 mmole) of **11** in 5 ml of tetrahydrofuran was added dropwise. After stirring the mixture at –25° C for 2 hr, it was quenched with saturated NH_4Cl solution and was then extracted into methyl *t*-butyl ether (2x). The organic phase was dried over sodium sulfate and the solvent was removed under reduced pressure to give 0.92 g (100%) of **12** as an oil. This was used without further purification in the following step.

Ethyl (3R*,5S*,6E)-3,5-dihydroxy-7-(2-isopropyl-5,6-dihydro-4H-benzo[3,4]cyclohepta[b]thiophen-1-yl)-6-heptenoate (13)

To a solution of 0.92 g (2.1 mmole) of **12** in 25 ml of tetrahydrofuran/methanol (4:1) (under argon) was added dropwise 2.3 ml of triethylborane (as a 1.0 M solution in tetrahydrofuran). The solution was stirred at room temperature for 2 hr then was cooled to –78° C. To this was added 0.1 g (2.6 mmole) of pulverized sodium

borohydride then the mixture was stirred at –78° C for 4 hr. Saturated NH_4Cl solution was carefully added then the mixture was extracted into methyl *t*-butyl ether (2x). The solvent was removed under reduced pressure and the residue was methanolized by adding 100 ml of methanol and removing it under reduced pressure (3x). The residual oil was flash chromatographed using 2% methanol/methylene chloride as eluent to give 0.4 g (44%) of **13** as an oil which slowly crystallizes upon standing. A sample was triturated with pentane, mp 111-113° C; ir ($CHCl_3$) 3495, 1723 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.31-7.11 (m, 4H), 6.50 (d, J=17 Hz, 1H), 5.58 (dd, 1H), 4.45 (m, 1H), 4.25 (m, 1H), 4.19 (q, 2H), 3.71 (s, broad, 1H), 3.45 (m, 1H), 3.04 (s, broad, 1H), 2.65-2.45 (m, 6H), 2.29-2.13 (m, 2H), 1.81-1.55 (m, 2H), 1.33 (d, J=7Hz, 6H), 1.29 (t, 3H); Anal. Calcd. for $C_{25}H_{32}O_4S$: C, 70.06; H, 7.53; S, 7.48. Found: C, 70.24; H, 7.50; S, 7.51.

(3R*,5S*,6E)-3,5-Dihydroxy-7-(2-isopropyl-5,6-dihydro-4H-benzo[3,4]cyclohepta[b]thiophen-1-yl)-6-heptenoic Acid Sodium Salt (14)

To a solution of 0.3 g (0.7 mmole) of **13** in 10 ml of ethanol at 60° C was added 0.7 ml of 1.0N aqueous sodium hydroxide. After stirring the mixture at 60° C for 3 hr, the solvent was removed under reduced pressure. The residue was dissolved in water and the solution was washed with methyl *t*-butyl ether. The aqueous phase was lyophilized to give 0.233 g (77%) of **14** as an amorphous solid, mp 225-228° C (wets at 200°); ^1H nmr (DMSO-d_6) δ 7.35-7.10 (m, 4H), 6.33 (d, J=17 Hz, 1H), 5.56 (dd, 1H), 4.18 (m, 1H), 3.73 (m, 1H), 3.45 (m, 1H), 2.56-2.34 (m, 6H), 2.22-1.79 (m, 4H), 1.63-1.20 (m, 2H), 1.28 (d, J=7Hz, 6H).

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References

(1) R.I. Levy, Circulation 72, 686 (1985)
(2) J. Stamler, Acta Med. Scand. 207, 433 (1980)
(3) Lipid Research Clinics Program, J. Am. Med. Assoc. 251, 351 and 365 (1984)
(4) F.G. Kathawala, Med. Res. Rev. 11, 121 (1991)
(5) G.M. Coppola, R.E. Damon, H. Yu, R.G. Engstrom, T.J. Scallen, Biomed. Chem Lett. 7, 549 (1997).
(6) Modeling studies were performed using Sybyl software from Tripos Associates, St. Louis, MO. All energy minimizations were done using the Tripos force field.
(7) R. Baker, M.A. Brimble, Tetrahedron Lett 27, 3311 (1986)
(8) R.H. Wollenberg, K.F. Albizati, R. Peries, J. Am. Chem. Soc. 99, 7365 (1977)
(9) M. Sletzinger, T.R. Verhoeven, R.P. Volante, J.M. McNamara, E.G. Corley, T.M.H. Liu, Tetrahedron Lett. 26, 2951 (1985)
(10) K. Narasaka, F. Pai, Tetrahedron 40, 2233 (1984)
(11) M.E. Ackerman, W.L. Redd, C.D. Tormanen, J.E. Hargrave, T.J. Scallen, J. Lipid Res. 18, 408 (1977)
(12) A. Endo, M. Kuroda, Y. Tsujita, J. Antibiot. 29, 1346 (1976)

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