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Synthesis of All the Four Possible Stereoisomers of Acaterin, Naturally Occurring ACAT Inhibitor, and the Determination of Its Absolute Configuration†.

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Key word: Acaterin; ACAT inhibitor; Enantioselective synthesis; Determination of absolute configuration

Abstract: Enantioselective synthesis of all the possible stereoisomers of acaterin **1**, naturally occurring ACAT inhibitor with acetogenin-type skeleton, was accomplished starting from both the enantiomers of ethyl 3-hydroxy butanoate **3**. Stereochemistry of synthetic samples **1** and pseudo-**1** was unambiguously assigned by converting to the authentic compound. The absolute configuration of natural acaterin was determined as (4*R*, 1'*R*) by careful comparison of TLC behavior and spectral and optical data.

INTRODUCTION

Inhibitors of acyl-CoA: cholesterol acyltransferase (ACAT) activity are expected to be effective for treatment of atherosclerosis and hypercholesterolemia[1,2]. Although several synthetic ACAT inhibitors are known[3,4,5], those of natural origin have rarely been reported[6,7]. Among them, acaterin **1** was isolated from a culture broth of *Pseudomonas* sp. A92 by Endo and co-workers[7]. Its structure was proposed as **1** by spectroscopic analysis, but the stereochemistry remained unknown. Acaterin contains butenolide skeleton with alkyl chain at C-2 position, which is related to Annonaceous acetogenins[8], such as uvaricin **2**[9], with remarkable antitumor activity. As a lead compound, ACAT inhibitors from natural origin must be very useful and important and for that work, it is necessary to clarify the stereochemistry of acaterin.

During the course of our continuing studies on the synthesis of enzyme inhibitors, we started the synthesis of all the possible stereoisomers of acaterin to determine the absolute configuration of the natural product. Our synthetic plan was based on the construction of α-alkylthio-γ-lactones **A** from readily available chiral source, ethyl 3-hydroxybutanoate, (*S*)- and (*R*)-**3**. Acylation of **A** should give **B**, from which those stereoisomers **1** and pseudo-**1** would be derived. Described below is our synthesis and characterization of the

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synthetic samples to determine the absolute configuration.

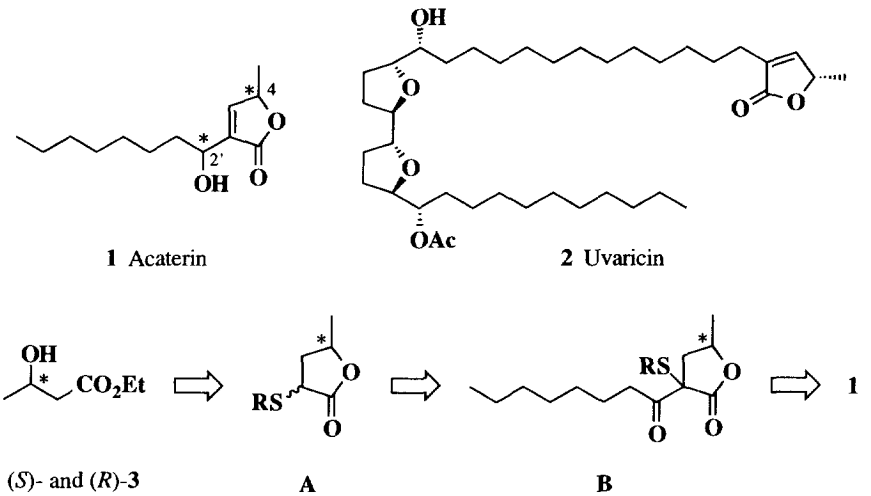


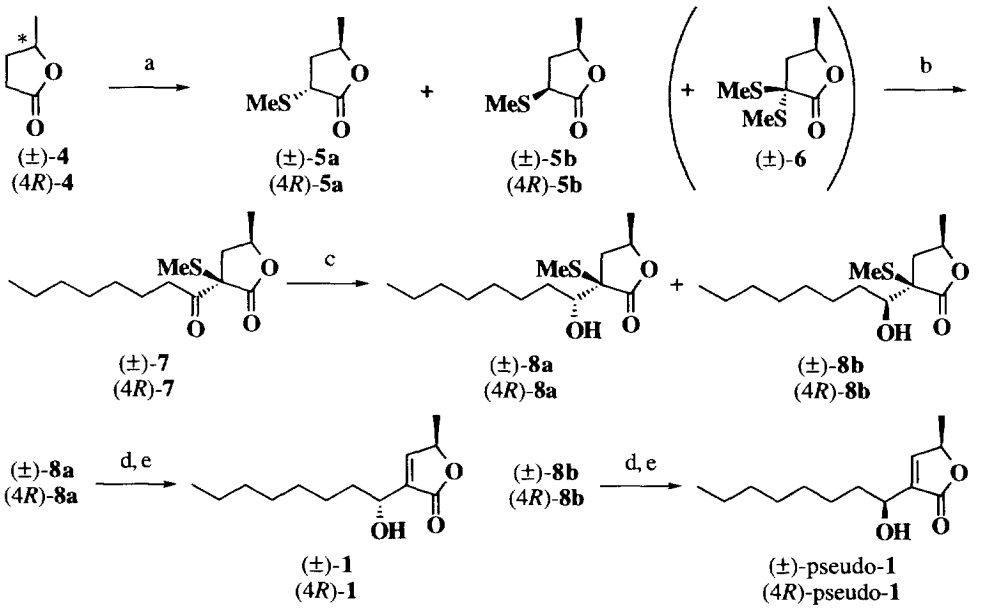
Fig. 1.

RESULTS AND DISCUSSION

In order to establish the synthetic scheme and relative stereochemistry, both racemates of natural isomer (±)-**1** and its diastereomer (±)-pseudo-**1** were first synthesized. γ-Valerolactone (±)-**4** was treated with LDA and methyl methanethiolsulfonate to give a mixture of *trans*-sulfide (±)-**5a** (45.7%), *cis*-sulfide (±)-**5b** (22.8%) and a by-product, bis-methylthio derivative, (±)-**6** (10.1%). It was a separable mixture and each isomer was fully characterized, but both sulfides, (±)-**5a** and **5b**, were treated with LDA and octanoyl chloride to afford single β-keto lactone (±)-**7** in 72.5% yield. Stereochemical assignment of (±)-**5** and (±)-**7** was definitely executed by the following ^{1}H NMR data: In those whose methylthio group locates *cis*-position to methyl substituent, chemical shifts of methyl protons are 1.46 ppm (**5b** and **7**) and 1.45 ppm (**6**). On the other hand, those signals are at higher field, 1.41 ppm in the case of *trans*-located isomer **5a** and non-substituted lactone **4**.

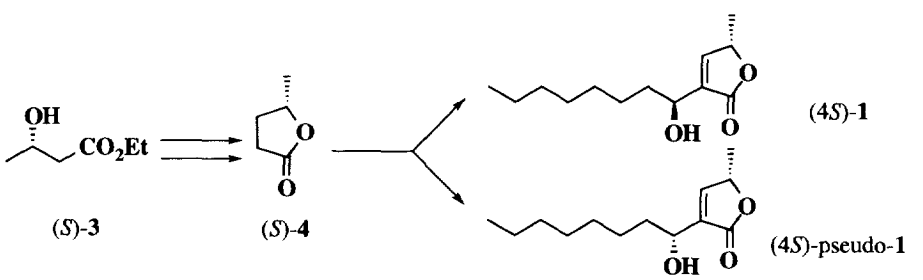
Reduction of (±)-**7** with $NaBH_4$ in aqueous THF afforded approximately 1.2 to 1 mixture of diastereomeric alcohols (±)-**8a** (44.1%) and (±)-**8b** (36.7%). Each diastereomer was separated and pure (±)-**8a** and (±)-**8b** were submitted to MCPBA oxidation followed by pyrolysis in toluene eliminating of methylthio group to give natural (±)-**1** (63.5%, Rf = 0.13, Hexane / EtOAc = 4 / 1) and (±)-pseudo-**1** (62.8%, Rf = 0.20), respectively. Although relative configurations of **8a**, **8b**, (±)-**1** and (±)-pseudo-**1** were still unknown and they were elucidated as shown in the scheme I later on, the polar isomer (±)-**1** was proved to be identical with natural acaterin by TLC behavior and spectral analysis. the isomer (±)-**1** was crystalline compound melting at 38.8 ~ 39.2°C, but unfortunately not suitable for X-ray crystallography. Thus, we turned to synthesize optically active compounds via this route to determine both relative and absolute stereochemistry.

(*R*)-γ-Valerolactone (*R*)-**4**[10] was prepared from ethyl (*R*)-3-hydroxybutanoate (*R*)-**3**[11,12] with ~100% e.e. via one carbon-extension in 28.4% yield through 5 steps[10h]. The lactone was converted to the β-keto lactone (2*R*, 4*R*)-**7** in 52.5% yield for 2 steps, which was reduced as above to give a 1.1 to 1 mixture of diastereomers (2*R*, 4*R*)-**8a** (42.3%) and (2*R*, 4*R*)-**8b** (40.1%). Elimination of methylthio group afforded (4*R*)-**1**, $[\alpha]_D^{20}$ −19.7 ($CHCl_3$), and (4*R*)-pseudo-**1**, $[\alpha]_D^{20}$ −63.7 ($CHCl_3$), respectively.



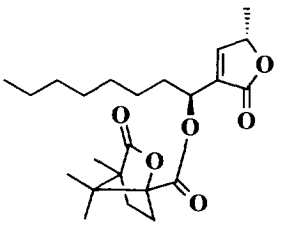
Scheme 1. a) LDA, $MeSSO_2Me$, THF, −78°C → −20°C, 2.5 h, 68.5%. b) LDA, *n*-$C_7H_{15}COCl$, THF, −78°C → −10°C, 2.5 h, 72.5%. c) $NaBH_4$, THF-H_2O (10 : 1), −5°C, 1 h, 44.1% (**8a**), 36.7% (**8b**). d) MCPBA, CH_2Cl_2, −78°C, 15 min. e) $CaCO_3$, toluene, reflux, 3 h, 63.5% in 2 steps.

In the same manner, ethyl (*S*)-3-hydroxybutanoate (*S*)-**3** (98.7% e.e.)[12,13] was transformed to (*S*)-γ-valerolactone (*S*)-**4**, which was submitted to the same procedure to give (4*S*)-**1**, $[\alpha]_D^{19}$ +19.6 ($CHCl_3$), and (4*S*)-pseudo-**1**, $[\alpha]_D^{19}$ +62.6 ($CHCl_3$) in overall 7.2% and 6.4% yield through 10 steps.



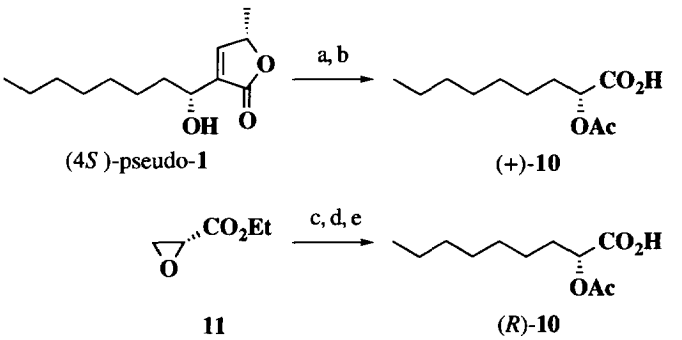
Scheme 2.

As $[\alpha]_D$ value of natural acaterin is –17 ($CHCl_3$) at 19°C[14], it was concluded that (4*R*)-**1** was identical with natural product, but the absolute configuration at C-1' position still remained unknown. Those optically active isomers were oily, so several crystalline derivatives were prepared and (1*R*, 4*S*)-camphanate **9** of (4*S*)-**1** gave nice crystals of long needles (m.p. 82.0 ~ 82.3°C), but it was again not suitable for X-ray analysis. Thus , we attempted to correlate one of the synthetic isomer to the standard sample via degradation.



9

Acetylation of (4*S*)-pseudo-**1** was followed by ozonization and oxidative workup with H_2O_2 to give (+)-2-acetoxynonanoic acid (+)-**10** in 36% yield: $[\alpha]_D^{19}$ +17 ($CHCl_3$). The authentic sample was prepared by the following route. Ethyl (*R*)-(+)-glycidate **11**[15] was treated with hexylmagnesium bromide in the presence of cuprous bromide to give α-hydroxy ester. Alkaline hydrolysis and subsequent acetylation afforded (*R*)-(+)-2-acetoxynonanoic acid (*R*)-(+)-**10**, $[\alpha]_D^{19}$ +22 ($CHCl_3$) in overall 32% yield from **11**. Both the degradation product (+)-**10** from (4*S*)-pseudo-**1** and the authentic sample (*R*)-(+)-**10** were entirely identical including positive sign of $[\alpha]_D$ value. Thus, original (4*S*)-pseudo-acaterin should have (4*S*, 1'*R*)-configuration and consequently, natural acaterin was proved to be (4*R*, 1'*R*)-(–)-2-(1'-hydroxyoctyl)-4-pent-2-enolide (4*R*, 1'*R*)-**1**.

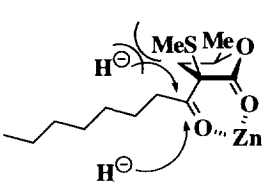


Scheme 3. a) Ac_2O, C_5H_5N, r. t., overnight, 97%. b) O_3, AcOH-EtOAc, –10°C, then H_2O_2, r. t., overnight, 37%. c) *n*-$C_6H_{13}MgBr$, CuBr-Me_2S, Et_2O, –78°C, 2 h, 47%. d) NaOH aq., r. t., 30 min. e) Ac_2O, DMAP, CH_2Cl_2, r. t., 30 min, 68% in 2 steps.

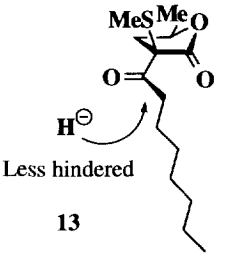
In order to obtain natural enantiomer selectively, various reagents were examined for the reduction of the keto lactone (2*R*, 4*R*)-**7**. Reduction with $Zn(BH_4)_2$ gave pseudo-type isomer predominantly (**8a** / **8b** = 29 / 71) probably via a chelating transition state **12**, in which hydride preferably approaches from less hindered α-face (opposite to angular methylthio group) to give **8b** as a major isomer. On the other hand , using sterically hindered reagent, DIBAL-BHT[16], natural isomer **8a** was predominated (**8a** / **8b** = 63 / 37). The best ratio (**8a** / **8b** = 73 / 27) was obtained by reduction with $LiAl(O^tBu)_3H$ at –65°C. It might be rational that both carbonyl groups are existing at opposite direction like **13** without chelation because of dipole-dipole interaction. Bulky reagents, therefore, should approach preferentially from less hindered front face to give natural isomer **8a**.

Entry	Reagent	Condition	Ratio (**8a** : **8b**)	Yield
1	$NaBH_4$	THF-H_2O, –5°C,1 h	51 : 49	82.4%
2	$Zn(BH_4)_2$	toluene, –78°C, 3 h	29 : 71	83.5%
3	DIBAL-BHT	toluene, –78°C, 2 h	63 : 37	91.9%
4	BH_3-THF	THF, –5°C ~ 5°C, 8 h	65 : 35	57.6%
5	$LiAl(O^tBu)_3H$	THF, –65°C, 1 h	73 : 27	81.4%
6	$LiBH_4$	THF, –78°C, 2 h	25 : 75	83.4%

Table 1. Stereoselective Reduction of (2*R*, 4*R*)-**7**.



12 Chelating transition



13

ACAT inhibition activity of four stereoisomers of acaterin was assayed using microsomes prepared from rat liver. Interestingly, ACAT inhibitions by four stereoisomers were much the same.

Compound	Concentration (μM)	ACAT activity ($pmol \cdot min^{-1} \cdot mg^{-1}$)
None	—	522 (100)*
Acaterin (natural product)	200	121 (23)
Synthetic (4*R*, 1'*R*)	200	132 (25)
(4*S*, 1'*S*)	200	136 (26)
(4*R*, 1'*S*)	200	107 (20)
(4*S*, 1'*R*)	200	121 (23)

Table 2. ACAT Inhibitions by Four Stereoisomers of Acaterin. (* Percent of control value.)

In conclusion, enantioselective synthesis of all the possible four stereoisomers of acaterin, naturally occurring ACAT inhibitor, was accomplished and the absolute configuration of the natural product was unambiguously determined as (4*R*, 1'*R*) via correlation to the authentic sample. Thus stereochemistry of butenolide core in acaterin from microbial origin is opposite to that of Annonaceous acetogenin from plant kingdom. Relationship between stereostructure and inhibiting activity is very interesting and now under investigation. Results will be reported in due course.

EXPERIMENTAL

IR: Jasco A-102 spectrometer. — ^{1}H NMR (in $CDCl_3$): Jeol JNM EX-90 spectrometer (90 MHz) or Bruker AC-300 spectrometer (300 MHz). — Optical rotations: Jasco DIP-371 polarimeter. — Column chromatography: Merck Kieselgel 60 (Art. Nr. 7734). — Boiling points and melting points: uncorrected values.

4-Methyl-2-(methylthio)-γ-butyrolactone (**5**). — (a) *Racemate:* A solution of *n*-butyllithium in hexane (1.71 M, 1.75 ml, 2.99 mmol) was added to a solution of diisopropylamine (0.42 ml, 303 mg, 3.00 mmol) in tetrahydrofuran (3.4 ml) at 0°C under argon. After stirring this solution at 0°C for 1 h, a solution of (±)-**4** (300 mg, 3.00 mmol) in tetrahydrofuran (4.7 ml) was added at –78°C and stirred at –78°C for 30 min. Then, to this solution, a solution of methyl methanethiolsulfonate (378 mg, 3.00 mmol) in tetrahydrofuran (1.5 ml) was added at –78°C and stirred at –78°C for 30 min and at –20 ~ –30°C for 2 h. The reaction mixture was poured into 1 N hydrochloric acid and extracted with ether. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (25 g). Elution with hexane / ethyl acetate (8 : 1) gave (±)-**5** (300 mg, 68.5%, **5a** : **5b** = 2 : 1) and **6** (58 mg, 10.1%). — (±)-**5a**: n_D^{23} = 1.4930. IR (film): ν = 1760, 1195, 1185, 1115, 1090, 1055 cm^{-1}. ^{1}H NMR: δ = 1.41 (3H, d, *J* = 6.0 Hz, 4-Me), 2.20 (2H, dd, *J* = 7.5, 5.3 Hz, 3-H), 2.28 (3H, s, -SMe), 3.46 (1H, t, *J* = 5.3 Hz, 2-H), 4.78 (1H, tq, *J* = 7.5, 6.0 Hz, 4-H). Anal. Calcd. for $C_6H_{10}O_2S$: C, 49.31; H, 6.90. Found: C, 49.66; H, 7.03.

cis-(±)-**5b**: n_D^{22} = 1.4918. IR (film): ν = 1760, 1190, 1175, 1135, 1120, 1055 cm^{-1}. ^{1}H NMR: δ = 1.46 (3H, d, *J* = 6.6 Hz, 4-Me), 1.78 (1H, ddd, *J* = 8.1, 9.4, 13.1 Hz, 3-H), 2.28 (3H, s, -SMe), 2.74 (1H, ddd, *J* = 6.6, 9.4, 13.1 Hz, 3-H), 3.56 (1H, t, *J* = 9.4 Hz, 2-H), 4.58 (1H, ddq, *J* = 8.1, 6.6, 6.6 Hz, 4-H). Anal. Calcd. for $C_6H_{10}O_2S$: C, 49.31; H, 6.90. Found: C, 49.62; H, 7.02.

(b) *(4R)-Isomer:* In the same manner as described above, (*R*)-**4** (1.30 g, 13.0 mmol, ~100% e.e., prepared from (*R*)-3-hydroxybutanoate[10h,11,12]) gave (4*R*)-**5** (1.37 g, 72.2%, **5a** : **5b** = 2 : 1) . — (4*R*)-**5a**: n_D^{22} = 1.4933, $[\alpha]_D^{22}$ = +62.1 (*c* = 1.16 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**5a**. Anal. Calcd. for $C_6H_{10}O_2S$: C, 49.31; H, 6.90. Found: C, 48.85; H, 6.88.

(4*R*)-**5b**: n_D^{22} = 1.4971, $[\alpha]_D^{19}$ = –20.2 (*c* = 1.16 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**5b**. Anal. Calcd. for $C_6H_{10}O_2S$: C, 49.31; H, 6.90. Found: C, 48.81; H, 7.08.

(c) *(4S)-Isomer:* In the same manner as described above, (*S*)-**4** (2.08 g, 20.8 mmol, 98.7% e.e., prepared from (*S*)-3-hydroxybutanoate[10h,12,13]) gave (4*S*)-**5** (2.07 g, 68.2%, **5a** : **5b** = 3.5 : 1). — (4*S*)-**5a**: n_D^{19} = 1.4958, $[\alpha]_D^{19}$ = –57.0 (*c* = 1.25 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**5a**. Anal. Calcd. for $C_6H_{10}O_2S$: C, 49.31; H, 6.90. Found: C, 49.73; H, 6.93.

(4*S*)-**5b**: n_D^{19} = 1.4947, $[\alpha]_D^{19}$ = +22.4 (*c* = 1.11 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**5b**. Anal. Calcd. for $C_6H_{10}O_2S$: C, 49.31; H, 6.90. Found: C, 49.27; H, 6.95.

4-Methyl-2-(methylthio)-2-octanoyl-γ-butyrolactone (**7**) — (a) *Racemate:* A solution of *n*-butyllithium in hexane (1.70 M, 3.18 ml, 5.40 mmol) was added to a solution of diisopropylamine (0.755 ml, 545 mg, 5.40 mmol) in tetrahydrofuran (6.3 ml) at 0°C under argon. After stirring this solution at 0°C for 1 h, a solution of (±)-**5** (788 mg, 5.40 mmol, mixture of **5a** and **5b**) in tetrahydrofuran (8.3 ml) was added dropwise at –78°C and stirred at –78°C for 30 min. Then, to this solution, a solution of *n*-octanoyl chloride (878 mg, 5.40 mmol) in tetrahydrofuran (3.9 ml) was added at –78°C, stirred at –78°C for 30 min and warmed slowly to –10°C within 2 h. The reaction mixture was poured into 1 N hydrochloric acid and extracted with ether. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (100 g). Elution with hexane / ethyl acetate (100 : 1 ~ 50 : 1) gave (±)-**7** (1.01 g, 68.8%) and recovered (±)-**5** (40 mg, 5.1%); n_D^{18} = 1.4841. IR (film): ν = 1765, 1710, 1190, 1155, 1105 cm^{-1}. ^{1}H NMR (300 MHz): δ = 0.87 (3H, d, *J* = 6.6 Hz, 8'-H), 1.20 ~ 1.37 (10H, m, 3'-, 4'-, 5'-, 6'- and 7'-H), 1.46 (3H, d, *J* = 6.0 Hz, 4-Me), 1.58 (2H, ddt, *J* = 7.2, 7.5, 7.9 Hz, 3'-H), 1.96 (1H, dd, *J* = 9.5, 12.9 Hz, 3-H), 2.03 (3H, s, -SMe), 2.70 (1H, dt, *J* = 17.4, 7.2 Hz, 2'-H), 3.03 (1H, dt, *J* = 17.4, 7.5 Hz, 2'-H), 3.20 (1H, dd, *J* = 5.7, 12.9 Hz, 3-H), 4.43 (1H, ddq, *J* = 5.7, 9.5, 6.0 Hz, 4-H). Anal. Calcd. for $C_{14}H_{24}O_3S$: C, 61.74; H, 8.88. Found: C, 61.25; H, 8.83.

(b) *(R)-Isomer:* In the same manner as described above, (4*R*)-**5** (1.25 g, 8.56 mmol, mixture of **5a** and **5b**) gave (4*R*)-**7** (1.58 g, 67.8%) and recovered (4*R*)-**5** (85 mg, 6.8%); n_D^{21} = 1.4827, $[\alpha]_D^{21}$ = +94.4 (*c* = 1.10 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**7**. Anal. Calcd. for $C_{14}H_{24}O_3S$: C, 61.74; H, 8.88. Found: C, 61.52; H, 8.93.

(c) *(S)-Isomer:* In the same manner as described above, (4*S*)-**5** (1.96 g, 13.4 mmol, mixture of **5a** and **5b**) gave (4*S*)-**7** (2.87 g, 78.6%) and recovered (4*S*)-**5** (300 mg, 15.3%); n_D^{18} = 1.4841, $[\alpha]_D^{18}$ = –96.9 (*c* = 1.04 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**7**. Anal. Calcd. for $C_{14}H_{24}O_3S$: C, 61.74; H, 8.88. Found: C, 61.60; H, 8.97.

4-Methyl-2-(1-hydroxyoctyl)-2-(methylthio)-γ-butyrolactone (**8**). — (a) *Racemate:* Sodium tetrahydroborate (50 mg, 1.32 mmol) was added to a solution of (±)-**7** (500 mg, 1.84 mmol) in tetrahydrofuran (10 ml) and water (1 ml) at -5°C. The mixture was stirred at –5°C for 1 h, then poured into water and extracted with ether. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (37 g). Elution with hexane / ethyl acetate (30 : 1 ~ 15 : 1) gave **8a** (221 mg, 44.1%) and **8b** (186 mg, 36.7%) as colorless oil. — **8a**; n_D^{23} = 1.4858. IR (film): ν = 3460, 1740, 1195, 1115, 1080, 1050 cm^{-1}. ^{1}H NMR (300 MHz): δ = 0.88 (3H, d, *J* = 6.5 Hz, 8'-H), 1.19 ~ 1.42 (11H, m, 2'-, 3'-, 4'-, 5'-, 6'- and 7'-H), 1.51 (3H, d, *J* = 6.3 Hz, 4-Me), 1.58 (1H, dd, *J* = 3.2, 13.9Hz, 3-H), 1.96 (1H, m, 2'-H), 2.15 (1H, d, *J* = 4.9 Hz, OH), 2.18 (3H, s, -SMe), 2.91 (1H, dd, *J* = 9.0, 13.9 Hz, 3'-H), 4.03 (1H, dd, *J* = 4.9, 10.5 Hz, 1'-H), 4.66 (1H, ddq, *J* = 3.2, 9.0, 6.3 Hz, 4-H). Anal. Calcd. for $C_{14}H_{26}O_3S$: C, 61.29; H, 9.55. Found: C, 61.73; H, 9.52.

8b; n_D^{23} = 1.4883,. IR (film): ν = 3500, 1755, 1195, 1125, 1115, 1080, 1055 cm^{-1}. ^{1}H NMR (300 MHz): δ = 0.87 (3H, d, *J* = 6.5 Hz, 8'-H), 1.22 ~ 1.68 (12H, m, 2'-, 3'-, 4'-, 5'-, 6'- and 7'-H), 1.51 (3H, d, *J* = 6.5 Hz, 4-Me), 1.61 (1H, dd, *J* = 4.4, 14.4 Hz, 3-H), 2.26 (3H, s, -SMe), 2.74 (1H, dd, *J* = 8.7, 14.4 Hz, 3'-H), 2.85

(1H, br, OH), 3.83 (1H, brd, J = 9.6 Hz, 1'-H), 4.64 (1H, ddq, J = 4.4, 8.7, 6.5 Hz, 4-H). Anal. Calcd. for $C_{14}H_{26}O_3S$: C, 61.29; H, 9.55. Found: C, 61.68; H, 9.59.

(b) *(4R)-Isomer:* In the same manner as described above, (4*R*)-**7** (1.15 g, 4.23 mmol) gave (4*R*)-**8a** (490 mg, 42.3%) and (4*R*)-**8b** (465 mg, 40.1%). — (4*R*)-**8a**; n_D^{22} = 1.4864, $[\alpha]_D^{22}$ = +43.0 (c = 0.93 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**8a**. Anal. Calcd. for $C_{14}H_{26}O_3S$: C, 61.29; H, 9.55. Found: C, 61.87; H, 9.57.

(4*R*)-**8b**; n_D^{20} = 1.4895, $[\alpha]_D^{21}$ = –23.0 (c = 0.79 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**8b**. Anal. Calcd. for $C_{14}H_{26}O_3S$: C, 61.29; H, 9.55. Found: C, 61.10; H, 9.60.

(c) *(4S)-Isomer:* In the same manner as described above, (4*S*)-**7** (1.96 g, 7.21 mmol) gave (4*S*)-**8a** (950 mg, 48.1%) and (4*S*)-**8b** (826 mg, 41.8%). — (4*S*)-**8a**; n_D^{18} = 1.4855, $[\alpha]_D^{18}$ = –42.2 (c = 1.06 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**8a**. Anal. Calcd. for $C_{14}H_{26}O_3S$: C, 61.29; H, 9.55. Found: C, 61.06 H, 9.64.

(4*R*)-**8b**; n_D^{17} = 1.4903, $[\alpha]_D^{17}$ = +22.4 (c = 1.06 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**8b**. Anal. Calcd. for $C_{14}H_{26}O_3S$: C, 61.29; H, 9.55. Found: C, 61.12; H, 9.62.

Acaterin (**1**). — (a) *Racemate:* A solution of *m*-chloroperoxybenzoic acid (55%, 240 mg, 0.77 mmol) in dichloromethane (4 ml) was added dropwise to a solution of (±)-**8a** (210 mg, 0.77 mmol) in dichloromethane (16 ml) at –78°C under argon. Stirred for 15 min, the reaction mixture was poured into 10% aqueous sodium thiosulfate solution and extracted with dichloromethane. The extraction was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was dissolved in toluene (6 ml) and refluxed in the presence of calcium carbonate (77 mg, 0.77 mmol) for 3 h. After a filtration and a concentration of the reaction mixture, the residue was chromatographed over silica gel (4 g). Elution with hexane / ethyl acetate (8 : 1) yielded crude (±)-**1**, which was distilled to give (±)-**1** (110 mg, 63.5%) as colorless crystal; b.p. 123 ~ 125 °C / 4 Torr, m.p. 38.8-39.2°C. IR (film): ν = 3450, 1745, 1200, 1120, 1080, 1020 cm^{-1}. ^{1}H NMR (300 MHz in pyridine-d_5): δ = 0.82 (3H, d, J = 6.6 Hz, 8'-H), 1.13 ~ 1.41 (8H, m, 4'-, 5'-, 6'- and 7'-H), 1.29 (3H, d, J = 6.8 Hz, 4-Me), 1.51 - 1.78 (2H, m, 3'-H), 1.91 (1H, dddd, J = 5.0, 8.0, 10.1, 13.2 Hz, 2'-H), 2.09 (1H, dddd, J = 4.0, 6.2, 10.1, 13.7 Hz, 2'-H), 4.88 (1H, dddd, J = 1.6, 2.0, 4.0, 8.0 Hz, 1'-H), 5.04 (1H, ddq, J = 1.6, 2.0, 6.8 Hz, 4-H), 7.55 (1H, t, J = 1.6 Hz, 3-H).

^{13}C NMR (75 MHz in pyridine-d_5): δ = 14.22 (C-8'), 19.20 (4-Me), 22.87 (C-7'), 25.81 (C-3'), 29.53 (C-5'), 29.82 (C-4'), 32.03 (C-6'), 36.50 (C-2'), 66.48 (C-1'), 77.88 (C-4), 138.66 (C-2), 150.49 (C-3), 172.66 (C-1). Anal. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80. Found: C, 68.79; H, 9.78.

(b) *(4R)-Isomer:* In the same manner as described above, (4*R*)-**8a** (470 mg, 1.72 mmol) gave (4*R*)-**1** (300 mg, 77.4%) as colorless oil; b.p. 133.0 ~ 135.0°C / 5 Torr, n_D^{20} = 1.4720, $[\alpha]_D^{20}$ = –19.7 (c = 0.61 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**1**. Anal. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80. Found: C, 68.78; H, 9.85.

(c) *(4S)-Isomer:* In the same manner as described above, (4*S*)-**8a** (890 mg, 3.25 mmol) gave (4*S*)-**1** (445 mg, 60.6%) as colorless oil; b.p. 123.0 ~ 125.0°C / 4 Torr, n_D^{17} = 1.4732, $[a]_D^{19}$ = +19.6 (c = 1.04 in chloroform). Its IR and ^{1}H NMR spectra were identical with those of (±)-**1**. Anal. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80. Found: C, 68.70; H, 9.87.

Pseudo-acaterin (pseudo-**1**). — (a) *Racemate:* In the same manner as described above, (±)-**8b** (170 mg, 0.62 mmol) gave (±)-pseudo-**1** (88 mg, 62.8%) as colorless oil; b.p. 115 ~ 117 °C / 4 Torr, n_D^{22} = 1.4717. IR (film): ν = 3450, 1745, 1200, 1120, 1085, 1025 cm^{-1}. ^1H NMR (300 MHz in pyridine-d_5): δ = 0.81 (3H, d, *J* = 6.7 Hz, 8'-H), 1.13 ~ 1.41(8H, m, 4'-, 5'-, 6'- and 7'-H), 1.23 (3H, d, *J* = 6.8 Hz, 4-Me), 1.51 - 1.79 (2H, m, 3'-H), 1.88 (1H, dddd, *J* = 5.0, 8.2, 10.1, 13.3 Hz, 2'-H), 2.08 (1H, dddd, *J* = 4.0, 6.0, 10.1, 13.7 Hz, 2'-H), 4.89 (1H, dddd, *J* = 1.2, 1.4, 4.0, 8.2 Hz, 1'-H), 5.08 (1H, ddq, *J* = 1.2, 1.4, 6.8 Hz, 4-H), 7.55 (1H, t, *J* = 1.4 Hz, 3-H).

^{13}C NMR (75 MHz in pyridine-d_5): δ = 14.27 (C-8'), 19.12 (4-Me), 22.92 (C-7'), 25.95 (C-3'), 29.59 (C-5'), 29.86 (C-4'), 32.09 (C-6'), 36.51 (C-2'), 66.58 (C-1'), 77.97 (C-4), 138.68 (C-2), 150.60 (C-3), 172.80 (C-1). Anal. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80. Found: C, 68.70; H, 9.91.

(b) *(4R)-Isomer:* In the same manner as described above, (4*R*)-**8b** (440 mg, 1.61 mmol) gave (4*R*)-pseudo-**1** (203 mg, 55.9%) as colorless oil; b.p. 117 ~ 119°C / 3 Torr, n_D^{20} = 1.4729, $[\alpha]_D^{20}$ = –63.7 (*c* = 0.53 in chloroform). Its IR and ^1H NMR spectra were identical with those of (±)-pseudo-**1**. Anal. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80. Found: C, 68.84 H, 9.84.

(c) *(4S)-Isomer:* In the same manner as described above, (4*S*)-**8b** (786 mg, 2.87 mmol) gave (4*S*)-pseudo-**1** (420 mg, 64.8 %) as colorless oil; b.p. 133.0 ~ 135.0°C / 5 Torr, n_D^{21} = 1.4737, $[\alpha]_D^{21}$ = +62.6 (*c* = 1.06 in chloroform). Its IR and ^1H NMR spectra were identical with those of (±)-pseudo-**1**. Anal. Calcd. for $C_{13}H_{22}O_3$: C, 68.99; H, 9.80. Found: C, 68.99; H, 9.87.

Degradation of (4S)-pseudo-acaterin. — (i) *Acetylation of (4S)-pseudo-1*; Acetic anhydride (170 µl, 184 mg, 1.8 mmol) was added to a ice-cooled solution of (4*S*)-pseudo-**1** (200 mg, 0.885 mmol) in pyridine (350 µl) and the mixture was stirred at room temp. overnight. After addition of ethanol (0.5 ml) and further stirring for 1 h, the reaction mixture was poured into 1 N hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (8 g). Elution with hexane / ethyl acetate (6 : 1) gave acetyl (4*S*)-pseudo-acaterin (230 mg, 97%) as colorless oil; n_D^{20} = 1.4610, $[\alpha]_D^{21}$ = +68 (*c* = 0.61 in chloroform). IR (film): ν = 1755, 1235, 1210, 1120, 1085, 1030 cm^{-1}. ^1H NMR: δ = 0.87 (3H, t, *J* = 6 Hz, 8'-H), 1.05 ~ 2.30 (12H, m, 2'-, 3'-, 4'-, 5'-, 6'- and 7'-H), 1..42 (3H, d, *J* = 7 Hz, 4-Me), 2.10 (3H, s, Ac), 5.05 (1H, brq, *J* = 7 Hz, 4-H), 5.59 (1H, brt, *J* = 7 Hz, 1'-H), 7.17 (1H, br, 3-H). Anal. Calcd. for $C_{15}H_{24}O_4$: C, 67.13; H, 9.02. Found: C, 67.06; H, 9.16.

(ii) *Ozonolysis of acetyl (4S)-pseudo-acaterin;* Ozone, from ON-3-2 ozonator (Nippon Ozone Co., Ltd.), was passed into a solution of acetyl (4*S*)-pseudo-acaterin (100 mg, 0.37 mmol) in acetic acid (5 ml) and ethyl acetate (5 ml) at –10°C. After the solution turned blue in color, nitrogen was passed into the solution to remove excess ozone. Water (3 ml) and 30% hydrogenperoxide (1 ml) were added to the reaction mixture and the whole was stirred at room temp. overnight. The reaction mixture was poured into water and extracted with ether. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was subjected to preparative silica gel TLC with chloroform / methanol / acetic acid (100 : 10 : 1) and (+)-**10** (30 mg, 37%) was obtained as colorless oil; n_D^{17} = 1.4422, $[\alpha]_D^{19}$ = +17 (*c* = 0.40 in chloroform). IR (CCl_4): ν = 1750, 1725, 1225 cm^{-1}. ^1H NMR: δ = 0.88 (3H, t, *J* = 5.3 Hz,9-H), 1.00 ~ 2.05 (12H, m, 3- 4-, 5-, 6-, 7- and 8-H), 2.14 (3H, s, Ac), 5.01 (1H, t, *J* = 5.8 Hz, 2-H). Anal. Calcd. for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 61.51; H, 9.34.

(R)-2-Acetoxynonanoic acid (authentic sample) [(*R*)-**10**]. — (i) *Ethyl (R)-2-hydroxynonanoate;* A solution of hexylmagnesiumbromide in ether was prepared from hexylbromide (2.5 g, 15 mmol) and magnesium (370 mg, 15 mmol) under argon. To a mixture of this Grignard reagent and copper(I)bromide-dimethyl sulfide complex (70 mg, 0.34 mmol), a solution of (*R*)-**11** (330 mg, 2.8 mmol) in ether (2 ml) was added dropwise under argon at –78°C and the mixture was stirred for 2 h. The reaction mixture was poured into saturated aqueous ammonium chloride solution and extracted with ether. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (20 g). Elution with hexane / ethyl acetate (20 : 1) gave ethyl (*R*)-2-hydroxynonanoate (270 mg, 47%) as colorless oil; n_D^{18} = 1.4343, $[\alpha]_D^{18}$ = +1.93 (*c* = 1.05 in methanol). IR (CCl_4): ν = 3480, 1730, 1200, 1130, 1090, 1020 cm^{-1}. ^1H NMR: δ = 0.88 (3H, t, *J* = 6.6 Hz, 9-H), 1.25 ~ 1.85 (12H, m, 3-, 4-, 5-, 6-, 7- and 8-H), 1.30 (3H, t, *J* = 7.2 Hz, OCH_2-CH_3), 2.72 (1H, br, OH), 4.16 (1H, dd, *J* = 4.1, 7.2 Hz, 2-Me), 4.24 (2H, q, *J* = 7.2 Hz, O-CH_2-CH_3). Anal. Calcd. for $C_{11}H_{22}O_3$: C, 65.31; H, 10.96. Found: C, 64.95 H, 10.96.

(ii) *(R)-2-Acetoxynonanoic acid* [(*R*)-**10**]; Ethyl (*R*)-2-hydroxynonanoate (65 mg, 0.32 mmol) was stirred in 25% aqueous sodium hydroxide solution at room temp. for 30 min. The reaction mixture was adjusted to pH 1 with 1 N hydrochloric acid and extracted with ether. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was dissolved in dichloromethane (2 ml). To this solution, acetic anhydride (70 mg, 0.69 mmol) and 4-dimethylaminopyridine (10 mg, 0.08 mmol) was added and the whole was stirred for 30 min. The reaction mixture was poured into 1 N hydrochloric acid and extracted with ether. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (2 g). Elution with hexane / ethyl acetate (8 : 1) gave (*R*)-**10** (47 mg, 68%) as colorless oil; n_D^{17} = 1.4422, $[\alpha]_D^{19}$ = +22 (*c* = 0.85 in chloroform). IR and ^1H NMR was identical those of (+)-**10** (prepared from (4*S*)-pseudo-acaterin).

(4S)-Acaterin (1S)-camphanate [(4*S*)-**1**]. (1*S*)-Camphanic chloride (100 mg, 0.46 mmol) was added to a solution of (4*S*)-acaterin (50 mg, 0.22 mmol) and 4-dimethylaminopyridine (small amount) in pyridine (1 ml) and stirred at room temp. for 2 h. An excess of 3-N, N-dimethylamino-1-propylamine was added and stirred for 5 min. This was diluted with ether and washed with 1 N hydrochloric acid, water and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (3 g) and elution with hexane / ethyl acetate (1 : 1) gave crude **9** which was recrystallized from hexane / dichloromethane (10 : 1) to give **9** (70 mg, 78 %) as colorless fine needles; m.p. 82.0 ~ 82.3°C, $[\alpha]_D^{22}$ = –6.0 (*c* = 0.59 in chloroform). IR (KBr): ν = 1775, 1740, 1725, 1265, 1210, 1170, 1120, 1090, 1055, 1020 cm^{-1}. ^1H NMR: δ = 0.86 (3H, t, *J* = 6 Hz, 8'-H), 0.98 (3H, s, 10"-Me), 1.07 (3H, s, 8"-Me), 1.11 (3H, s, 9"-H), 1.42 (3H, d, *J* = 6 Hz, 4-Me), 1.1 ~ 2.6 (16H, m, 3'-, 4'-, 5'-, 6'-, 7'-, 3"- and 4"-H), 5.05 (1H, tq, *J* = 1, 6 Hz, 4-H), 5.73 (1H, tt, *J* = 1, 6 Hz, 1'-H), 7.29 (1H, t, *J* = 1 Hz, 3-H).

Reduction of (4R)-7. — (a) *With $Zn(BH_4)_2$:* A solution of zinc borohydride in ether (0.13 M, 4 ml, 0.52 mmol) was added dropwise to a solution of (4*R*)-**7** (50 mg, 0.18 mmol) in toluene (5 ml) at –78°C under argon. Stirred for 3 h, the reaction mixture was quenched with 3% aqueous phosphoric acid and extracted

with ether. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (1 g) and elution with hexane / ethyl acetate (10 : 1) gave a mixture of (4*R*)-**8a** and (4*R*)-**8b** (37 mg, 73.5%) at the ratio 29 : 71 (from ^{1}H NMR) and recovered (4*R*)-**7** (6 mg, 12%). — (b) *With DIBAL-BHT complex:* A solution of diisopropylaluminum hydride in toluene (1.0 M, 1.6 ml, 1.6 mmol) was dropped to a solution of 2, 6-di-*tert*-butyl-4-methylphenol (500 mg, 2.3 mmol) at 0°C under argon and stirred for 1 h. Cooled down to –78°C, a solution of (4*R*)-**7** (54 mg, 0.20 mmol) in toluene (5 ml) was dropped and stirred for 2 h at –78 ~ –65°C. The reaction mixture was poured into 1 N hydrochloric acid and extracted with ether. In the same manner as described above, a mixture of (4*R*)-**8a** and (4*R*)-**8b** (50 mg, 91.9%) was obtained at the ratio 63 : 37 (from ^{1}H NMR). — (c) *With BH_3-THF complex:* A solution of borane-tetrahydrofuran complex (1.0 M, 0.54 ml, 0.54 mmol) was added to a solution of (4*R*)-**7** (50 mg, 0.18 mmol) in tetrahydrofuran (1 ml) at –10°C under argon. Stirred for 4 h at –5°C and for further 4 h at 5°C. The reaction mixture was quenched with acetic acid / H_2O (1 : 1) and extracted with ether. In the same manner as described above, a mixture of (4*R*)-**8a** and (4*R*)-**8b** (29 mg, 57.6%) was obtained at the ratio 65 : 35 (from ^{1}H NMR) and recovered (4*R*)-**7** (7 mg, 14%). — (d) *With $LiAl(O^tBu)_3H$:* A solution of lithium tri-*tert*-butoxyaluminohydride in tetrahydrofuran (1.0 M, 0.56 ml, 0.56 mmol) was added dropwise to a solution of (4*R*)-**7** (50 mg, 0.18 mmol) in tetrahydrofuran (1 ml) at –65°C under argon and stirred for 1 h at –65°C. The reaction mixture was poured into 1 N hydrochloric acid and extracted with ether. In the same manner as described above, a mixture of (4*R*)-**8a** and (4*R*)-**8b** (41 mg, 81.4%) was obtained at the ratio 73 : 27 (from ^{1}H NMR). — (e) *With $LiBH_4$:* A solution of (4*R*)-**7** (50 mg, 0.18 mmol) in tetrahydrofuran (1 ml) was added dropwise to a solution of lithium borohydride (95%, 8 mg, 0.35 mmol) in tetrahydrofuran (1 ml) at –78°C under argon. Stirred for 2 h , the reaction mixture was poured into 1 N hydrochloric acid and extracted with ether. In the same manner as described above, a mixture of (4*R*)-**8a** and (4*R*)-**8b** (42 mg, 83.4%) was obtained at the ratio 25 : 75 (from ^{1}H NMR).

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