

Synthesis and configuration analysis of 9,18- and 10,18-dihydroxystearic acid methyl esters†

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(Received 26 April 1993; revision received 16 July 1993; accepted 9 August 1993)

Abstract

The syntheses of 9,18- and 10,18-dihydroxystearic acid methyl esters, which are components of plant cutin, are described. Conversion of the primary alcohol group of these diols to a t-butyltrimethylsilyl ether followed by reaction of the residual secondary alcohol group with (*S*)-(-)-1-(1-naphthyl)ethylisocyanate produces diastereomeric carbamates that are resolved by silica gel high-performance liquid chromatography and whose configurations may be assigned by the ¹H-NMR shift differences of the carbomethoxy and silylmethyl signals.

Key words: Cutin; Dihydroxystearic acids; High-performance liquid chromatography; Stereochemical analysis

1. Introduction

Structural studies on cutins, polyesters that are the polymeric base of the cuticle matrix of plants, have produced a number of polyhydroxylated fatty acids on depolymerization of the cutins [1]. Among these are 9,18- and 10,18-dihydroxystearic acids, synthetic samples of which were sought by us in connection with ongoing research [2]. We report here the synthesis of the methyl esters of these two acids by a route that is relatively convenient

and can be analogized to other n,ω -dihydroxyacids. We also report a method for determining the configuration of the mid-chain hydroxyl group of such compounds.

2. Results and discussion

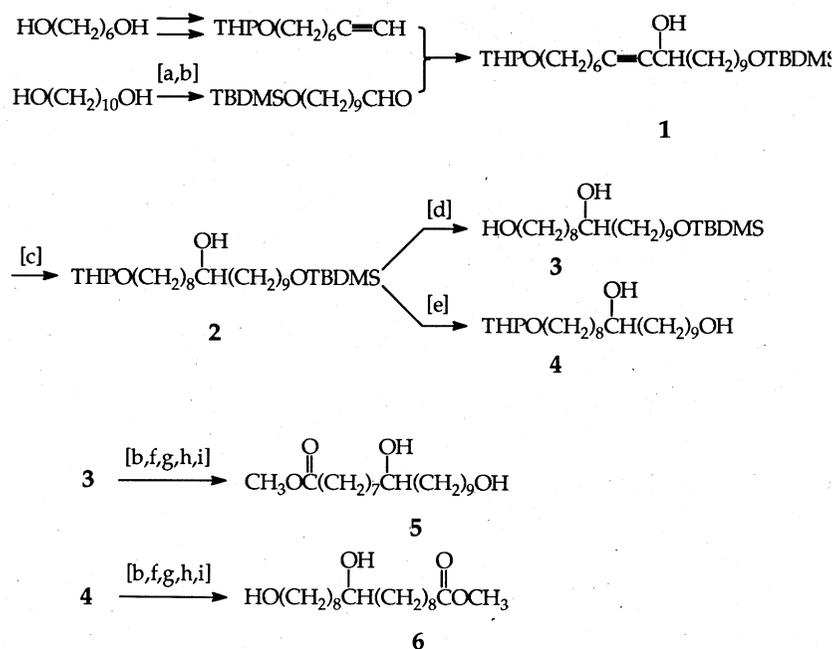
2.1. Synthesis

The synthetic approach we selected generated the secondary hydroxyl group by a condensation of two α,ω -difunctionalized chains from available α,ω -diols (Scheme 1). 1,10-Decanediol was converted to a monosilylated derivative with t-butyltrimethylchlorosilane (TBDMSCl) [3], and the residual primary alcohol function was then oxidized to the corresponding aldehyde with pyridini-

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†Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Abbreviations: PCC, pyridinium chlorochromate; TBDMSCl, t-butyltrimethylchlorosilane; THP, tetrahydropyranyl ether.



Scheme 1. [a] NaH, THF, reflux 16 h, then TBDMSCl, 25°C, 4 h; [b] PCC, CH₂Cl₂, 25°C, 2 h; [c] H₂, 5% Pd/C, EtOH; [d] MgBr₂, ether, 25°C, 4 h; [e] Bu₄N⁺F⁻, THF, 25°C, 4 h; [f] Ag₂O, aq. EtOH, 25°C, 2 h; [g] CH₂N₂, ether; [h] NaBH₄, MeOH; [i] pTsOH, MeOH, 25°C, 1 h.

um chlorochromate (PCC) [4]. The tetrahydropyranyl ether (THP) of 6-chloro-1-hexanol was treated with lithium acetylide. The alcohol is available commercially but can be prepared from the diol by a general procedure for the preparation of halohydrins of α,ω -diols [5]. The resulting protected 1-octyne-8-ol was condensed with the aldehyde establishing the desired hydrocarbon chain by producing the diprotected 7-octadecyn-1,9,18-triol, **1**. Reduction of the alkyne to give **2** was followed by alternately removing the protecting groups to produce a 1,9,18-triol blocked either at the 18 position as a silyl ether, **3**, or at the 1 position as a THP ether, **4**. Subsequent steps were performed with minimal handling of intermediates and included oxidation to the ketoacid, esterification (CH₂N₂), sodium borohydride reduction of the keto group and removal of the residual protecting group. From **3** was obtained 9,18-dihydroxystearic acid, methyl ester, **5**; and the 10,18-isomer, **6**, was obtained from **4**.

Although the synthesis was relatively uneventful, the following items seem worthy of mention. A variety of silylating agents is available for blocking an alcohol function, of which the TBDMS group is most prominent, offering considerable latitude in the conduct of subsequent synthesis as well as ease of removal [6]. Cleavage of the silyl ether is most often performed with a fluoride salt, and can be accomplished in the presence of a THP ether. However, conditions that are used to reclaim alcohol from a THP block such as dilute acid, aqueous acetic acid and methanolic HCl can cause the silyl ether to cleave. Kim and Park [7] have reported the use of magnesium bromide in anhydrous ether as a selective reagent for cleaving THP ethers in the presence of the TBDMS group. When 1-octanol THP and 1-octanol TBDMS were allowed to react under the reported conditions, we did indeed observe that less than 4% desilylation occurred during the 4 h required to complete the removal of the THP group. Nevertheless, reaction

of the *bis*-protected triol produced additional, less polar, materials. It was necessary, therefore, to purify the intermediate obtained in that instance before proceeding to the oxidation step. Several oxidative procedures were evaluated, including potassium permanganate/benzene [8] and pyridinium dichromate [9,10]. The latter reagent might have avoided oxidation of the secondary alcohol. A two-step procedure that gave best results as judged by overall yields of final product involved oxidation with PCC to the ketoaldehyde followed by oxidation under basic conditions with silver oxide to the ketoacid [11].

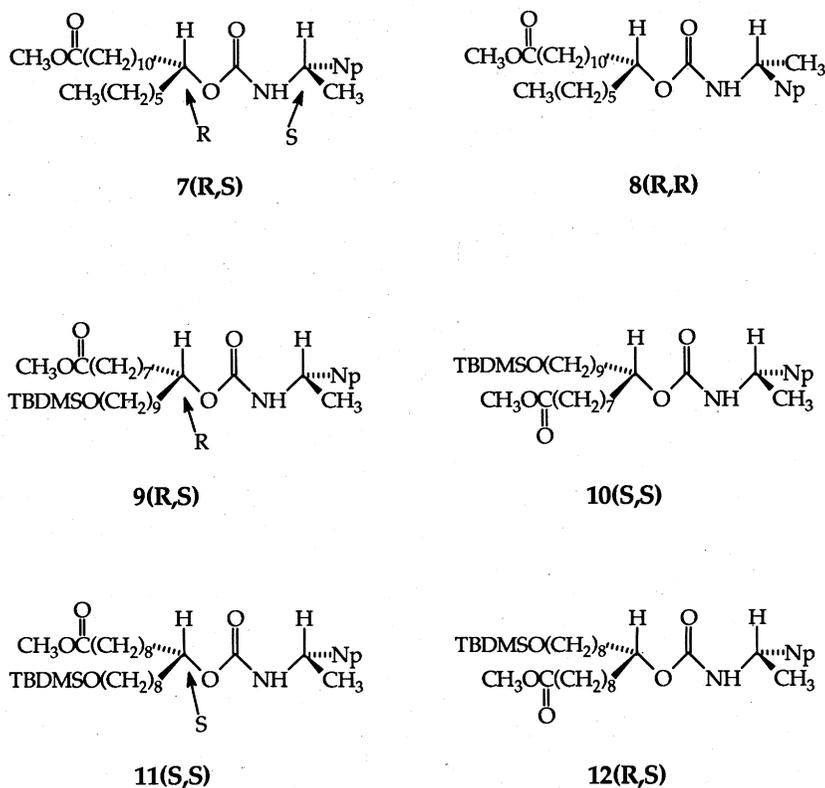
2.2. Configuration analysis

Characterization of the structure of a cutin framework will require knowledge of the configurations of the asymmetric centers in the mix of hydroxylated fatty acids that constitutes the cutin's building blocks. Although chiral GLC and HPLC columns are enjoying expanding applications, the nature of the racemic structures that are most readily resolved is such that the groups attached to the asymmetric center offer useful functionality for non-bonded interactions with the chiral column. Moreover, those structural features that distinguish the substituents are most effective when they are located close to the center of asymmetry. The nature of these dihydroxystearic acids is such that two substituents are long hydrocarbon chains differentiated only at the termini, making resolution by existing chiral columns unlikely.

The reaction of secondary alcohols with enantiomers of either α -methylbenzylisocyanate or 1-(1-naphthyl)ethylisocyanate produces diastereomers whose separation by HPLC and whose $^1\text{H-NMR}$ spectral features have been employed to assign configuration [12,13]. A feature of these adducts is that in solution a low-energy rotational conformer exists that is based on a tendency for the carbamate unit and the attached centers of asymmetry to be coplanar. This will place one of the two alkyl chains bonded to the asymmetric carbon opposite the aryl ring of the chiral derivatizing reagent in one of the diastereomers (Scheme 2). For the other diastereomer, it is the other alkyl chain that will experience proximity to

the aryl ring. The anisotropic magnetic field induced at the aryl ring will cause shielding of protons associated with that particular chain. If the protons are ordinarily clearly visible in the $^1\text{H-NMR}$ spectrum, then the spectra of the diastereomers can be used to assign configuration. The situation is exemplified with a compound of known configuration, namely the hydrogenation product of methyl ricinoleate, (*R*)-12-hydroxystearic acid methyl ester (Scheme 2). Derivatization with a 2:1 mixture of (*R*)- and (*S*)- α -naphthylethylisocyanates provided an identifiable diastereomer mixture with relative shifts of the CO_2CH_3 s that were consistent with the solution conformation model. Specifically, the model suggests that the (*R,S*)-diastereomer, **7**, in which the CO_2CH_3 is placed in greater proximity to the naphthyl ring, would experience greater shielding of that signal. In fact the (*R,S*)-diastereomer exhibited the signal for CO_2CH_3 at slightly higher field (3.645 ppm vs. 3.648 ppm) (Table 1). In this instance, HPLC separation was not achieved, and the degree of resolution by $^1\text{H-NMR}$ was qualitative. One could approximate enantiomeric excess in a mixture of stereoisomers of this particular alcohol. Nevertheless, the relationship of configuration relative to that of the derivatizing isocyanate employed was consistent with that of the previous work [14] wherein we had distinguished enantiomers of methyl ricinoleate and methyl isoricinoleate, compounds in which an internal hydroxyl was part of a homo-, or *bis*-homo-, allylic system, respectively. The diastereomeric carbamates in that case were separable by HPLC, allowing clear definition of diastereomer composition, and configuration assignment (hence enantiomeric excess) could be made using the relative shifts of carbomethoxy methyl groups.

For the dihydroxystearic acid methyl esters, we elected to block the primary alcohol with TBDMSCl to provide the compounds with an additional $^1\text{H-NMR}$ probe for the naphthyl ring of the chiral derivatizing agent, namely SiCH_3 . Accordingly, we expected and found that individual diastereomers of a pair either exhibited shielding of the CO_2CH_3 or of the SiCH_3 . Reaction of the 9,18-isomer, **5**; with (i) TBDMSCl, imidazole, DMF, then (ii) (*S*)-(+)-1-(1-naphthyl)ethyliso-



Scheme 2. Np, 1-naphthyl; TBDMS, tert.-butyldimethylsilyl. The stereochemical designator for the dihydroxy fatty acids changes as the mid-chain hydroxyl is moved from C-9 (**9** and **10**) to C-10 (**11** and **12**).

Table 1
¹H-NMR and HPLC data for carbamate derivatives.

Compound	HPLC order of elution	CO ₂ CH ₃ (ppm)	SiCH ₃ (ppm)	Configuration
7 ^a	— ^b	3.645	—	<i>R,S</i>
8 ^a	— ^b	3.648	—	<i>R,R</i>
9 ^c	2	3.639	0.031	<i>R,S</i>
10 ^c	1	3.650	0.026	<i>S,S</i>
11 ^c	2	3.644	0.031	<i>S,S</i>
12 ^c	1	3.650	0.024	<i>R,S</i>

^aConfiguration of alcohol known, other assignments were based on shift data. Relative shifts of pairs of diastereomers are accurate to 0.001 ppm.

^bNot resolved.

^cδ (CO₂CH₃) = 0.011 ppm; δ (SiCH₃) = 0.005 ppm.

^dδ (CO₂CH₃) = 0.006 ppm; δ (SiCH₃) = 0.007 ppm.

cyanate, toluene, led to the carbamate diastereomers **9** and **10** (Scheme 2). Likewise, **6** gave a mixture of **11** and **12**. These pairs were cleanly resolved by HPLC (Materials and Methods) (Fig. 1), and their structures were assigned by relative shift data (Table 1; Fig. 2). This method should serve as a useful means by which to assign configuration and configurational purity for the methyl esters of α,ω-dihydroxy fatty acids generally. We also note that the shift differences seem to vary with the position of the mid-chain hydroxyl and may prove useful in locating the position of the hydroxyl group as well.

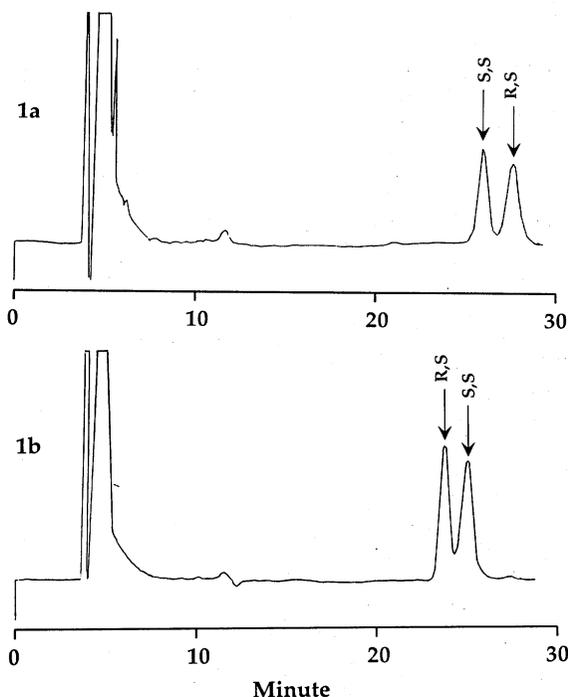


Fig. 1. (a) HPLC separation of **9** and **10** (Materials and Methods); (b) HPLC separation of **11** and **12**.

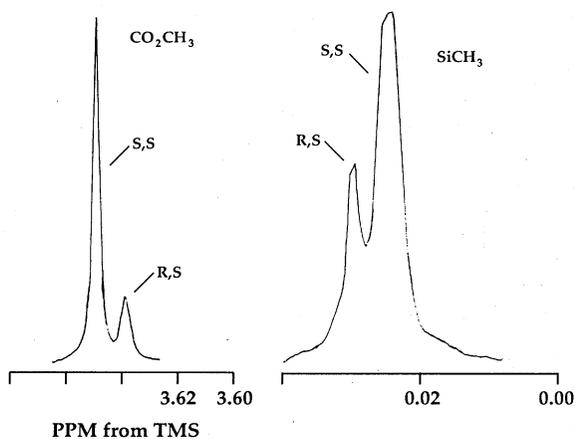


Fig. 2. ¹H-NMR spectrum of **10** containing a smaller amount of **9**, showing the ability to distinguish CO₂CH₃ and SiCH₃ signals.

3. Materials and methods

3.1. General procedures and reagents

Melting points are uncorrected. Silica gel TLC plates of 0.25 mm thickness from Analtech, Inc., were used to monitor reactions and purifications with 5% H₂SO₄ in ethanol as a spray followed by briefly heating on a hot plate for visualization. Aldrich silica gel (230–400 mesh) was used for flash chromatography [15]. Solvents were purchased from Burdick and Jackson or were obtained from Aldrich Chemical Co. (THF and HMPT). The THF was dried by distillation from LAH; the HMPT was dried over 13 Å molecular sieves. Reagents were purchased from Aldrich Chemical Co. and were used directly. Methyl ricinoleate was a gift of Dr. R. Benedict of this laboratory and has been used by us in previous work [14]. Its reduction to (*R*)-12-hydroxystearic acid was conducted as were those hydrogenations described below. 1-Octyn-8-ol THP ether was synthesized as previously described, b.p. 80–85°C at 0.2 Torr [16], using 6-chloro-1-hexanol purchased from Aldrich Chemical Co., and lithium acetylide as its complex with ethylenediamine from Ventron Corp. 1,10-Decanediol was converted to a monoether with *t*-butyldimethylsilyl chloride as described, b.p. 132–144°C at 0.5 Torr [17]. Magnesium bromide was prepared from 1,2-dibromoethane and magnesium turnings in anhydrous ether.

Gas-liquid chromatography was performed with a Chrompack-Packard model 438A instrument using a flame ionization detector and a Supelco-wax capillary column (0.25 mm × 30 m) and He carrier set to a 50:1 split ratio. High-performance liquid chromatography was accomplished with a Spectra-Physics SP8800 pump using a Supelcosil LC-SI column (4.6 mm × 25 cm) and a Spectra-Physics SP8480 UV detector at 254 nm. Infrared data were recorded with a Perkin Elmer 1310 spectrophotometer using ca. 1% solutions in CCl₄. NMR spectra (¹³C and ¹H) were obtained with a JEOLJNM-GX400 FT-NMR spectrometer with CDCl₃ solvent and tetramethylsilane as internal standard if it did not obscure a solute signal. Mass spectra were obtained with a Hewlett-Packard HP-5995 GC/MS system employing an OV-1 column

(0.25 mm × 15 m). Combustion analyses were carried out by Micro-Analysis, Inc., Wilmington, DE.

3.2. *7-Octadecyn-1,9,18-triol, 1-tetrahydropyranyl ether, 18-t-butyl dimethylsilyl ether, 1.*

The 1,10-decanediol monosilyl ether (9.94 g, 34.5 mmol) was allowed to react with a suspension of pyridinium chlorochromate (PCC) (11.2 g, 52 mmol) and NaOAc (1.0 g) in 70 ml of methylene chloride at room temperature for 2 h. The reaction mixture was diluted with 5 volumes of ether and filtered through a short bed of Florisil. The solvent was removed by flash evaporation to give the crude aldehyde that was used directly in the condensation step: GLC 4.1 min (240°C); IR 2710, 1725, 1245 and 1090 cm^{-1} ; $^1\text{H-NMR}$ δ 9.74 (t, $J = 1.7$ Hz, HC = O, 1H), 3.57 (t, $J = 6.6$ Hz, CH_2O , 2H), 2.39 (m, $\text{CH}_2\text{CH} = \text{O}$, 2H), 1.2–1.65 (m's, CH_2 , 14H), 0.87 (s, t-Bu, 9H), 0.02 (s, SiCH_3 , 6H) ppm. The crude aldehyde was added to a solution of the lithio derivative of 1-octyn-8-ol THP ether that had been prepared from the acetylene (5.98 g, 28.5 mmol) and butyllithium (11.4 ml, 2.5 M) in 50 ml of THF under nitrogen and cooled to 0–5°C. The reaction mixture was stirred for 1 h without cooling and then was worked up by dilution with water and extraction with hexane. The organic phase was dried (Na_2SO_4) and concentrated. The crude product (13.9 g) was purified using flash chromatography (15% ethyl acetate-hexane) in 3-g lots. The same column could be employed repeatedly, and the yield of **1** was 9.1 g, 72.8% based upon the acetylene: TLC (40% ethyl acetate-hexane) $R_f = 0.63$; IR: 3630, 1250, 1130, 1115, 1090, 1030 and 1020 cm^{-1} ; $^1\text{H-NMR}$ δ 4.55 (m, $\text{HC}[\text{O}]\text{O}$, 1H), 4.30 (m, $\text{HC}(\text{OH})\text{C}\equiv\text{C}$, 1H), 3.3–3.9 (m's, CH_2O , ca. 4H), 3.57 (t, $J = 6.6$ Hz, CH_2OSi , ca. 2H), 2.17 (m, $\text{CH}_2\text{C}\equiv\text{C}$, 2H), 1.3–1.8 (m, CH_2 , ca. 30H), 0.86 (s, t-Bu, 9H), 0.02 (s, SiCH_3 , 6H) ppm; $^{13}\text{C-NMR}$ δ 98.8 (OCO), 95.3 (C-9), 67.5 (C-1), 65.3 (C-8), 63.3 (C-18), 62.7 (C-10), 62.2 (THP C-O), –5.3 (SiCH_3) ppm.

3.3. *1,9,18-Octadecanetriol, 1-tetrahydropyranyl ether, 18-t-butyl dimethylsilyl ether, 2.*

Compound **1** (9.0 g) was dissolved in 20 ml of abs. ethanol and swirled with 0.2 g of 5% Pd/C.

The mixture was filtered with another 25 ml of ethanol and then hydrogenated over 0.5 g of fresh catalyst at an initial pressure of 30 lb/in^2 . Hydrogenation was complete in 0.5 h, and the product was obtained quantitatively by dilution of the mixture with water and extraction with hexane: TLC and IR data were essentially indistinguishable from those of **1**; $^{13}\text{C-NMR}$ δ 98.8 (OCO), 72.0 (C-10), 67.7 (C-1), 63.3 (C-18), 62.3 (THP C-O), –5.3 (SiCH_3) ppm. This material was employed directly for the subsequent transformations.

3.4. *1,9,18-Octadecanetriol, 18-t-butyl dimethylsilyl ether, 3.*

Compound **2** (5.66 g, 11.4 mmol) was dissolved in 100 ml of anhyd. ether to which MgBr_2 (6.3 g, 34 mmol) was added. The mixture was stirred for 4 h. The product was obtained by dilution with water to which some 2N HCl was added to obtain a clear aqueous phase, and extraction with ether. The ether layer was in turn washed and dried and the ether removed evaporatively. Compound **3** was obtained by flash chromatography (30% ethyl acetate-hexane) to give 2.15 g (45.4%) and was further purified by vacuum sublimation (200–230°C, 0.1 Torr) to a waxy solid: m.p. 31–34°C; TLC (40% ethyl acetate-hexane) $R_f = 0.39$; IR 3630, 1240 and 1090 cm^{-1} ; $^1\text{H-NMR}$ δ 3.5–3.6 (m, HCO, H_2CO , 5H), 1.2–1.6 (m's, CH_2 , ca. 30–31H), 0.86 (s, tBu, 9H), 0.01 (s, SiCH_3 , 6H) ppm; $^{13}\text{C-NMR}$ δ 72.0 (C-9), 63.3 (C-18), 62.9 (C-1), –5.3 (Si-CH_3) ppm.

3.5. *1,9,18-Octadecanetriol, 1-tetrahydropyranyl ether, 4.*

Compound **2** (3.0 g, 6.0 mmol) was dissolved in 12 ml of THF to which was added 12 ml of 1N tetrabutylammonium fluoride. The solution was allowed to stand for 4 h and was then diluted with water and extracted with ether. The organic phase was washed with water, dried (Na_2SO_4) and concentrated to give **4** (1.4 g, 60%): TLC (40% ethyl acetate-hexane) $R_f = 0.28$; m.p. 46–47°C; IR 3620, 1130, 1115, 1070, 1060, 1030 and 1020 cm^{-1} ; $^1\text{H-NMR}$ δ 4.55 (m, $\text{HC}(\text{O})\text{O}$, ca. 1H), 3.3–3.9 (m's, HCO, H_2CO , 7H), 1.2–1.9 (m's, CH_2 , 36H)

ppm; ^{13}C -NMR δ 98.8 (HC[O]O), 72.0 (C-10), 67.7 (C-1), 63.3 (C-18), 62.3 (ring CH_2O) ppm.

3.6. 9,18-Dihydroxystearic acid, methyl ester, 5.

Compound 3 (2.15 g, 5.2 mmol) was oxidized with PCC (3.4 g, 16 mmol) in 30 ml of CH_2Cl_2 and worked up in the manner described above. The product was then stirred for 2 h in a suspension of silver oxide prepared from 28 ml of 1.1 M KOH and 2.8 ml of 4.5 M AgNO_3 in 28 ml of ethanol. The mixture was acidified with 2N HCl, filtered, and extracted with ether. The organic phase was washed with water, dried (MgSO_4) and concentrated. The oily residue, a ketoacid, was treated with excess ethereal diazomethane to form the methyl ester that was subsequently reduced by dissolving in methanol and adding small portions of NaBH_4 . The silyl group was removed by dissolving the material in 10 ml of methanol containing 0.1 g of p-toluenesulfonic acid. After 1 h the solution was diluted and extracted with ether to give 5. The compound was purified by flash chromatography (50% ethyl acetate-hexane) yielding 0.34 g, 20% TLC (40% ethyl acetate-hexane) R_f = 0.26; m.p. 58–59°C; IR 3630 and 1735 cm^{-1} ; ^1H -NMR δ 3.64 (s, $\text{CH}_3\text{O}_2\text{C}$, 3H), 3.61 (t, J = 6.8 Hz, H_2CO , 2H), 3.56 (m, HCO, 1H), 2.27 (t, J = 7.5 Hz, CH_2CO_2 , 2H), 1.2–1.7 (m, CH_2 ca. 28H) ppm; ^{13}C -NMR δ 174.3 (C = O), 71.9 (C-10), 63.0 (C-18), 51.4 ($\text{CH}_3\text{O}_2\text{C}$) ppm; GC-MS was performed with the trimethylsilylated dihydroxyacid, namely the *bis*-trimethylsilyloxytrimethylsilylester, and the synthetic material was identical with the corresponding derivative of the naturally occurring material. The major ions that arise from fragmentation on either side of the secondary alcohol are m/e : 259, 317.

3.7. 10,18-Dihydroxystearic acid, methyl ester, 6.

Compound 4 (1.31 g, 3.4 mmol) was relayed through the sequence of reactions as described for 5, yielding 0.32 g, 62.5% m.p. 52.5–55°C; TLC and spectral data were essentially the same as those for 5. GC-MS comparisons showed the trimethylsilyl ether to be identical to that of the naturally occurring compound with major ions resulting from fragmentation on either side of the secondary alco-

hol; m/e 273, 303. Anal. calcd. for $\text{C}_{19}\text{H}_{38}\text{O}_4$: C, 69.05; H, 11.59. Found: C, 69.93; H, 11.25.

3.8. Synthesis of derivatives 9–12.

The dihydroxystearic acid methyl ester (33 mg, 0.10 mmol) was added to 70 μl of DMF with ca. 20 mg of imidazole. TBDMSCl (18 mg, 0.12 mmol) was added and the vial was capped and vortexed. The mixture was allowed to stand at ambient temperature overnight, then was diluted with water and extracted several times with ether. The ether extract was washed thoroughly with water, dried (MgSO_4) and concentrated. Toluene, 0.5 ml, was added to the 18-silylated ester, and, after adding ca. 50 μl of (*S*)- α -naphthylethylisocyanate, the mixture was heated under N_2 for 1 h at 90°C. The adducts were purified by HPLC using 95:5:1 hexane/ethyl acetate/THF: 9 and 10 (k' = 6.24, 5.80; α = 1.076); 11 and 12 (k' = 5.46, 5.05; α = 1.081); ^1H -NMR (see Table 1 for distinctions between pairs of diastereomers; other data identical) δ 7.2–8.2 (m, aryl H, 7H), 3.64 (s, CO_2CH_3 , 3H), 3.58 (m, HCO, 1H), 2.27 (m, $\text{CH}_2\text{CO}_2\text{CH}_3$, 2H), 1.2–1.8 (m's, CH_2 , ca. 32H), 0.03 (s, SiCH_3 6H) ppm.

4. Acknowledgment

The authors express their gratitude to Mrs. G. Brouillette for her operation of the NMR spectrometer.

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