Synthesis of heteroatom-substituted analogues of stearic acid

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Summary We report herein the syntheses of several analogues of stearic acid in which oxygen or sulfur atoms or sulfoxide groups have replaced the methylene groups at either position 9 or position 10 of the polymethylene chains. These compounds have been fully characterized by the results of proton and carbon-13 nuclear magnetic resonance, and low and high resolution mass spectral studies. —**Pascal, R. A., Jr., and D. L. Ziering.** Synthesis of heteroatom-substituted analogues of stearic acid. *J. Lipid Res.* 1986. **27:** 221–224.

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As part of a program of research on the enzymatic mechanisms involved in the introduction of olefinic double bonds into fatty acids and their derivatives (and in particular the conversion of stearic acid into oleic acid), it has been necessary for us to synthesize a variety of fatty acid analogues containing unusual functionality at positions 9 and 10 of the polymethylene chain. Although many derivatives of stearic acid are known in which atoms other than hydrogen are attached to carbons 9 and 10, we found no literature examples in which heteroatoms had been substituted for the carbons themselves. Inasmuch as the replacement of these methylene groups by heteroatoms would produce only slight changes in the steric bulk and conformation of the fatty acid chains, such analogues, or the appropriate thioester derivatives, should be excellent candidates for alternate substrates or inhibitors of enzyme systems involved in the oxidation of stearic acid. We report herein short syntheses of several analogues of stearic acid that contain ethers, thioethers, or sulfoxides in positions 9 or 10.

Straightforward nucleophilic substitution reactions were used to assemble the 18-atom-long chain of these substituted fatty acids. The ether-containing analogues of stearic acid were prepared by the condensation of the disodium salts of eight- and nine-carbon ω-hydroxy carboxylic acids with nine- and eight-carbon alkyl tosylates, respectively. The thioether analogues were synthesized by the condensation of the appropriate alkyl thiols with ω-bromo carboxylic acids in the presence of base, and the sulfoxide-containing compounds were prepared by oxidation of the thioethers with hydrogen peroxide.

EXPERIMENTAL PROCEDURES AND RESULTS

General procedures

Melting points, proton nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectroscopy, low and high resolution mass spectrometry, the preparation of trimethylsilyl (TMS) ethers, gas–liquid chromatography (GLC), and high performance liquid chromatography (HPLC) were performed as described previously (1). Columns (6′ × 1/4″) packed with 3% OV-17 on Gas Chrom Q were used for the GLC analyses, and columns (20 × 0.39 cm) of Waters μBondapak C_{18} were used in the HPLC analyses. In the listings of the resonances in the ^{13}C NMR spectra, an asterisk (*) indicates that a peak has twice the usual intensity due to accidental isochrony of the signals from two different carbon atoms.

Materials

8-Bromooctanoic acid, azelaic acid monomethyl ester, 1-octanethiol, 1-nonanethiol, 1-octanol, and 1-nonanol were obtained from Aldrich Chemical Co. 8-Hydroxyoctanoic acid (mp 61–62°C [lit. (2) 58–58.5°C]) was prepared by hydrolysis of 8-bromooctanoic acid with aqueous potassium carbonate. All common solvents and organic and inorganic chemicals were of analytical reagent grade or the highest purity commercially available.

9-Hydroxynonanoic acid

Azelaic acid monomethyl ester (10.9 g, 54.0 mmol) was added to a stirred suspension of sodium hydride (60%, 2.16 g, 54.0 mmol) in dry 2-methoxyethyl ether (150 ml) under argon at 0°C. The mixture was heated to reflux to ensure that the deprotonation of the acid was complete, and then it was cooled to room temperature. Lithium borohydride (2 M solution in tetrahydrofuran, 54 ml, 108 mmol) was added via syringe, and the mixture was refluxed for 8 hr. After cooling and acidification with 1 N HCl, the mixture was thoroughly extracted with ether. The combined ether extracts were washed with brine and dried over sodium sulfate. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane–ether–acetic acid 100:99:1). Recrystallization of the purified product gave 9-hydroxynonanoic acid (7.03 g, 75%), mp 51–52°C (lit. (2) 51–51.5°C); ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.9–1.7 (m, 12H, methylene envelope), 2.31 (t, 2H, J = 7, CH_2COO), 3.61 (t, 2H, J = 7, CH_2O), 6.4 (br s, 2H, OH's).

Abbreviations: NMR, nuclear magnetic resonance; TMS, trimethylsilyl; GLC, gas–liquid chromatography; HPLC, high performance liquid chromatography.

9-Bromononanoic acid

A mixture of 9-hydroxynonanoic acid (0.800 g, 4.60 mmol), 48% HBr (4 ml), and concentrated sulfuric acid (0.25 ml) was heated at reflux for 6 hr. The dark solution was cooled, diluted with water, and extracted thoroughly with ether. The combined ether extracts were washed with brine and dried over sodium sulfate. The volatiles were removed by rotary evaporation, and the crude product was purified by silica gel column chromatography (hexane–ether–acetic acid 189:20:1) and recrystallized from pentane to yield 9-bromononanoic acid (0.670 g, 65%), mp 32–33°C (lit. (2) 36–36.5°C); ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.9–1.7 (m, 12H, methylene envelope), 2.35 (t, 2H, J = 7, CH_2COO), 3.40 (t, 2H, J = 7, CH_2Br), 12.2 (br s, 1H, COOH).

8-(Nonylthio)octanoic acid (I) (Fig. 1)

A solution of 8-bromooctanoic acid (5.0 g, 22 mmol), nonanethiol (4.0 g, 22 mmol), and potassium hydroxide (2.9 g, 45 mmol) in absolute ethanol (200 ml) was refluxed for 5 hr under an argon atmosphere. After cooling, the solution was acidified, and the solvent was removed under reduced pressure. Water and methylene chloride were mixed with the residue, the organic layer was separated, and it was washed once more with water. The solvent was evaporated to give 6.7 g of solid product. This material was recrystallized from acetone to yield 8-(nonylthio)octanoic acid (5.9 g, 87%), mp 51–52°C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7, CH_3), 1.05–1.75 (m, 24H, methylene envelope), 2.27 (t, 2H, J = 7, CH_2COO), 2.50 (t, 4H, J = 7, CH_2SCH_2), 9.1 (br s, 1H, COOH); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.0, 22.6, 24.6, 28.7, 28.8, 28.9*, 29.2*, 29.5, 29.6, 29.7, 31.8, 32.1, 32.2, 34.0, 179.9; MS, m/z 302 (M^+, 22), 159 ($CH_3(CH_2)_8S^+$, 75), 83 (49), 69 (72), 55 (100). GLC analysis (215°C) of the TMS ester derivative of I indicated a purity in excess of 99%. Exact mass, 302.2273; calcd for $C_{17}H_{34}O_2S$, 302.2279.

9-(Octylthio)nonanoic acid (II)

A solution of 9-bromononanoic acid (1.77 g, 7.86 mmol), 1-octanethiol (1.17 g, 8.02 mmol), and potassium hydroxide (1.01 g, 15.7 mmol) in absolute ethanol (35 ml) was refluxed under argon for 6 hr. The produce was isolated as described for compound I. The yield of recrystallized 9-(octylthio)nonanoic acid was 2.00 g (84%), mp 46–47°C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7, CH_3), 1.05–1.75 (m, 24H, methylene envelope), 2.26 (t, 2H, J = 7, CH_2COO), 2.50 (t, 4H, J = 7, CH_2SCH_2), 8.9 (br s, 1H, COOH); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.0, 22.6, 24.7, 28.8, 28.9, 29.0, 29.1, 29.2*, 29.3, 29.7, 29.8, 31.8, 32.2, 32.3, 33.9, 179.1; MS, m/z 302 (M^+, 42), 145 ($CH_3(CH_2)_7S^+$, 76), 69 (98), 41 (100). GLC analysis (215°C) of the TMS ester derivative of I indicated a purity in excess of 98%. Exact mass, 302.2287; calcd for $C_{17}H_{34}O_2S$, 302.2279.

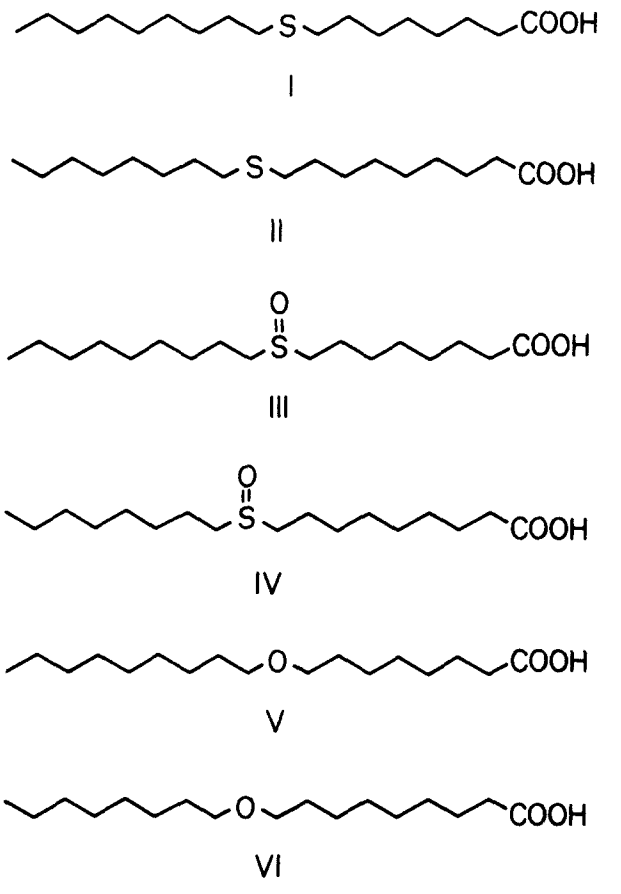

Fig. 1. Heteroatom-substituted analogues of stearic acid: I, 8-(nonylthio)octanoic acid; II, 9-(octylthio)nonanoic acid; III, 8-(nonylsulfinyl)-octanoic acid; IV, 9-(octylsulfinyl)nonanoic acid; V, 8-(nonyloxy)octanoic acid; VI, 9-(octyloxy)nonanoic acid.

8-(Nonylsulfinyl)octanoic acid (III)

8-(Nonylthio)octanoic acid (906 mg, 3.00 mmol), 30% hydrogen peroxide (0.39 ml, 3.6 mmol), and acetone (10 ml) were stirred overnight at room temperature. Evaporation of the solvent and recrystallization of the residue from acetone yielded 8-(nonylsulfinyl)octanoic acid (860 mg, 90%), mp 78–80°C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7, CH_3), 1.05–1.75 (m, 24H, methylene envelope), 2.33 (t, 2H, J = 7, CH_2COO), 2.70 (m, 4H, CH_2SOCH_2), 5.5 (br s, 1H, COOH); ^{13}C NMR (62.9 MHz, $CDCl_3$), δ 14.0, 22.6*, 22.71, 22.74, 28.7, 28.8*, 28.9, 29.2*, 29.4, 31.1, 34.0, 52.2, 52.4, 177.4; MS, m/z 318 (M^+, 1), 301 (M–OH, 59), 174 (M–OH–$CH_3(CH_2)_8$, 35), 159 ($CH_3(CH_2)_8S^+$, 42), 69 (44), 55 (100). HPLC analysis (MeOH–0.1% aqueous H_3PO_4 6:4) indicated a purity in excess of 98%. Exact mass, 318.2206; calcd for $C_{17}H_{34}O_3S$, 318.2229.

9-(Octylsulfinyl)nonanoic acid (IV)

9-(Octylthio)nonanoic acid (165 mg, 0.55 mmol), 30% hydrogen peroxide (0.081 ml, 0.75 mmol), and acetone (10 ml) were stirred overnight at room temperature. Evaporation of the solvent and recrystallization of the residue from acetone yielded 9-(octylsulfinyl)nonanoic acid (160 mg, 92%), mp 77–79°C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7, CH_3), 1.05–1.75 (m, 24H, methylene envelope), 2.33 (t, 2H, J = 7, CH_2COO), 2.70 (m, 4H, CH_2SOCH_2), 6.2 (br s, 1H, COOH); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.5, 22.6, 22.7, 24.7, 28.8*, 28.9*, 29.1, 29.2, 29.3, 31.8, 33.9, 52.2, 52.8, 177.4; MS, m/z 318 (M^+, 0.2), 301 (M–OH, 28), 188 (M–OH–$CH_3(CH_2)_7$, 23), 145 ($CH_3(CH_2)_7S^+$, 79), 69 (95), 55 (100). HPLC analysis (MeOH–0.1% aqueous H_3PO_4 6:4) indicated a purity in excess of 98%. Exact mass, 318.2218; calcd for $C_{17}H_{34}O_3S$, 318.2229.

8-(Nonyloxy)octanoic acid (V)

Nonyl *p*-toluenesulfonate [^{1}H NMR (80 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 7, alkyl CH_3), 1.05–1.75 (m, 14H, methylene envelope), 2.44 (s, 3H, aryl CH_3), 4.02 (t, 2H, J = 7, CH_2SO_2), 7.33 and 7.83 (AA'BB' system, 4H, aryl-H_4)] was prepared from 1-nonanol and *p*-toluenesulfonyl chloride according to a standard procedure (3). 8-Hydroxyoctanoic acid (1.00 g, 6.25 mmol) was added to a suspension of sodium hydride (60%, 1.18 g, 17.2 mmol) in dry dimethylformamide (30 ml). The mixture was heated at 60°C for 1 hr with rapid stirring. After cooling to 15°C, nonyl *p*-toluenesulfonate (4.85 g, 17.2 mmol) was added, and the mixture was heated for 36 hr at 80°C. After cooling, the reaction mixture was poured into a mixture of 1 N HCl and ether. The ether layer was separated and washed with brine. The solvent was evaporated, and the residue was refluxed for 4 hr with KOH (4.0 g) in 95% ethanol (100 ml). The solvent was evaporated, and the residue was dissolved in water. The solution was acidified and extracted with ether. The ether extract was washed with water and brine, and it was dried over $MgSO_4$. Evaporation of the solvent gave the crude product which was purified by silica gel column chromatography (hexane–ether–acetic acid 80:20:0.5). The product so obtained was recrystallized from acetone to yield 8-(nonyloxy)octanoic acid (235 mg, 13%), mp 33–35°C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7, CH_3), 1.05–1.75 (m, 24H, methylene envelope), 2.35 (t, 2H, J = 7, CH_2COO), 3.39 (t, 4H, J = 7, CH_2OCH_2); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.0, 22.7, 24.7, 26.1, 26.2, 29.05, 29.09, 29.3, 29.53, 29.59, 29.7, 29.8, 31.9, 33.9, 70.9, 71.0, 178.8; MS, m/z 286 (M^+, 2), 227 (M–CH_2COOH, 8), 159 (M–C_9H_{19}, 32), 141 (M–H_2O–C_9H_{19}, 84), 41 (100). GLC analysis (200°C) of the TMS ester of V indicated a purity of 96%. Exact mass, 286.2506; calcd for $C_{17}H_{34}O_3$, 286.2508.

9-(Octyloxy)nonanoic acid (VI)

Octyl *p*-toluenesulfonate [^{1}H NMR (80 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 7, alkyl CH_3), 1.05–1.75 (m, 12H, methylene envelope), 2.44 (s, 3H, aryl CH_3), 4.09 (t, 2H, J = 7, CH_2SO_2), 7.33 and 7.83 (AA'BB' system, 4H, aryl-H_4)] was prepared from 1-octanol and *p*-toluenesulfonyl chloride according to a standard procedure (3). 9-Hydroxynonanoic acid (500 mg, 2.87 mmol) was added to a suspension of sodium hydride (60%, 0.30 g, 7.5 mmol) in dry dimethylformamide (10 ml). The mixture was heated at 60°C for 1 hr with rapid stirring. After cooling to 15°C, octyl *p*-toluenesulfonate (2.01 g, 7.5 mmol) and additional dimethylformamide (10 ml) were added. The mixture was heated overnight at 70°C. After cooling, the product was isolated and purified as described for compound V. The yield of 9-(octyloxy)nonanoic acid was 50 mg (6%), mp 34–35°C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7, CH_3), 1.05–1.75 (m, 24H, methylene envelope), 2.34 (t, 2H, J = 7, CH_2COO), 3.39 (t, 4H, J = 7, CH_2OCH_2); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 14.0, 22.6, 24.7, 26.2, 26.3, 29.0, 29.1, 29.2, 29.3, 29.5, 29.80, 29.83, 31.9, 33.9, 70.9, 71.0, 178.6; MS, m/z 286 (M^+, 0.5), 268 (M–H_2O, 0.5), 173 (M–C_8H_{17}, 4), 155 (M–H_2O–C_8H_{17}, 29), 41 (100). GLC analysis (200°C) of the TMS ester of VI indicated a purity in excess of 99%. Exact mass, 286.2505; calcd for $C_{17}H_{34}O_3$, 286.2508.

DISCUSSION

The syntheses of the sulfur-containing analogues of stearic acid (I–IV, see Fig. 1) were straightforward. Condensation of the appropriate alkyl thiols and ω-bromo carboxylic acids in the presence of base yielded the thioethers I and II in good yields (87% and 84%, respectively). Oxidation of the thioethers with hydrogen peroxide in acetone gave high yields of the corresponding sulfoxides III and IV (90% and 92%, respectively). No difficulties were encountered in the preparation of these compounds, and their spectroscopic characteristics were fully consistent with the assigned structures.

The preparation of the oxygen-substituted analogs was more difficult. We chose the ω-hydroxy acids, rather than ω-bromo acids, as starting materials to remove the possibilities of lactone or oligomer formation by intramolecular or intermolecular displacements, respectively, of terminal halides by the carboxyl groups. In the syntheses of acids V and VI described above, the appropriate alkyl *p*-toluenesulfonates were condensed with the disodium salts of the ω-hydroxy carboxylic acids in dimethylformamide solution. At least two equivalents of the alkyl *p*-toluenesulfonates are required in such reactions, since alkylation of both the hydroxyl and carboxyl group of the hydroxyacid takes

place under these conditions. We used 2.7 equivalents, a convenient excess, but larger excesses of alkylating agent did not improve the yield. Alkaline hydrolysis of the resulting esters yielded the desired ether-containing carboxylic acids, which were purified by column chromatography. However, although the product acids were of high quality, the yields in these reactions were quite low (compound V, 13%; compound VI, 6%). A variety of modifications of the synthetic method were tried, including the use of longer reaction times and higher temperatures, the use of alkyl bromides and iodides in place of the *p*-toluenesulfonates, the use of dipotassium salts of the hydroxyacids in the presence of 18-crown-6 (4), the use of thallium salts of the hydroxyacids (5), and the use of benzene and acetonitrile as solvents, as well as some combinations of these variants. Unfortunately, none of these methods gave improved yields. Although the exact reasons for the difficulties encountered in the syntheses of the ethers remain unclear, the presence of olefins in the crude reaction mixtures (as judged by proton NMR) suggests that base-catalyzed elimination of the alkyl tosylates or halides competes effectively with the desired nucleophilic substitution process. Elimination reactions were not a problem in the thioether series because of the extremely high nucleophilicity but relatively weak basicity of thiolate anions.

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