Characterization of Dihydroxystearic Acid from Palm Oleic Acid

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ABSTRACT: Dihydroxystearic acid (DHSA) was prepared from palm oleic acid and characterized by chromatographic and spectroscopic methods (gas chromatography, thin-layer chromatography, Fourier transform infrared and nuclear magnetic resonance spectroscopy) as well as wet chemistry. The crude product has a melting point of 62°C, acid value of 179, saponification value of 178, and hydroxyl value of 196. The yield was about 90% based on unsaturation. The product obtained was found to contain DHSA, saturated fatty acids, and unknown products. DHSA is soluble in alcohol, and its solubility decreased by increasing the alcohol chain length. An irritancy test of DHSA indicated that purified DHSA is nonirritating.

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KEY WORDS: Dihydroxystearic acid, hydroxy fatty acid, irritancy, oleic acid, solubility.

Monohydroxy fatty acids occur in appreciable levels in the seed oils of a few higher plants, for example, *Ricinus communis, Lesquerella fendleri*, and *Wrightia* sp. (1–3). Among the oils from these plants, only castor oil is used commercially in large amounts, and its major constituent is ricinoleic acid. This acid is used industrially for the preparation of a wide variety of technical products, such as sebacic acid, undecylenic acid, polyols for polyurethane, detergents, and lubricants (3). However, the quantities of castor oil (hence ricinoleic acid and its derivatives) available on the market are subject to considerable fluctuations owing to poor harvests in the main growing areas of Brazil and India (4). Accordingly, there is a need for an equivalent and/or a substitute for this chemical.

Hydroxy fatty acids with more than one hydroxyl group can be obtained by functionalization of the alkyl chain of unsaturated fatty acids found in common oils and fats (3). Oleic acid is an important raw material for preparing this type of compound. Oleic acid can be obtained by splitting oils and fats containing the acid, for example, tallow (50% oleic acid), tall oil (45% oleic acid), and palm oil (40% oleic acid) (5). Various procedures have been described for preparation of hydroxylated fatty acids from unsaturated fatty acids using catalysts such as hydrogen peroxide/tungstic acid (6), selenium dioxide/*tert*-butylhydroperoxide (7), and osmium tetroxide (8). Such products are of great current interest in view of their possible use as starting material in various industrial applications. Recent research at the Malaysian Palm Oil Board (MPOB) has successfully produced dihydroxystearic acid (DHSA) from palm oleic acid (9). Therefore, the objective of this study was the characterization of DHSA prepared from palm oleic acid.

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MATERIALS AND METHODS

Materials. Oleic acid (purity, 75%) was purchased from an oleochemical company in Malaysia. DHSA was prepared in the laboratory as described by Awang *et al.* (9). Hydroxy fatty acid standards, i.e., *threo*- and *erythro*-9,10-DHSA (purity, 99%) were purchased from Sigma Chemical Co. (St. Louis, MO). *N*,*O*-Bis-trimethylsilyl acetamide (purity, >98%) was from Merck (Darmstadt, Germany). All other reagents were of analytical grade and used as received. Each experiment was carried out twice to check for the reproducibility of the results.

Thin-layer chromatography (TLC). Sample of DHSA derived from palm oleic acid were dissolved in ethanol and separated on silica gel layers (Merck). The mobile phase of TLC consisted of hexane, ethyl acetate and acetic acid (50:50:1, by vol). *Threo*- and *erythro*-9,10-DHSA were used as standards (Fig. 1). The chromatogram was visualized by charring at 140°C after spraying the plate with sulfuric acid solution (50%).

Gas chromatography (GC) of trimethylsilyl (TMS) derivatives of DHSA. GC analysis was carried out using a Hewlett-Packard HP-6860A *Plus* gas chromatograph (Palo Alto, CA). The TMS derivatives of DHSA were separated on a nonpolar column, HP-5 (Hewlett-Packard, 30 m × 0.25 mm × 0.25 μm) with helium as the carrier gas. The oven was programmed to hold at 150°C for 1 min, followed by ramping from 150 to 290°C at a rate of 10°C/min. The final temperature was held at 290°C for 30 min. The injector and flame-ionization detector were set at 300°C.

Preparation of TMS derivatives. About 0.01 g of sample was weighed into a vial. Then 2 mL of GC grade *N*,*N*-dimethylformamide and *N*,*O*-bis-trimethylsilyl acetamide were added. The sample was shaken for 15–30 s and warmed at 60°C for 30 min. The sample was allowed to cool for a few minutes before injection.

Nuclear magnetic resonance (NMR) spectroscopy of DHSA. Proton NMR (^{1}H NMR) spectra were obtained on a Hitachi NMR R-1200 spectrometer (60 MHz) in tetradeuterated

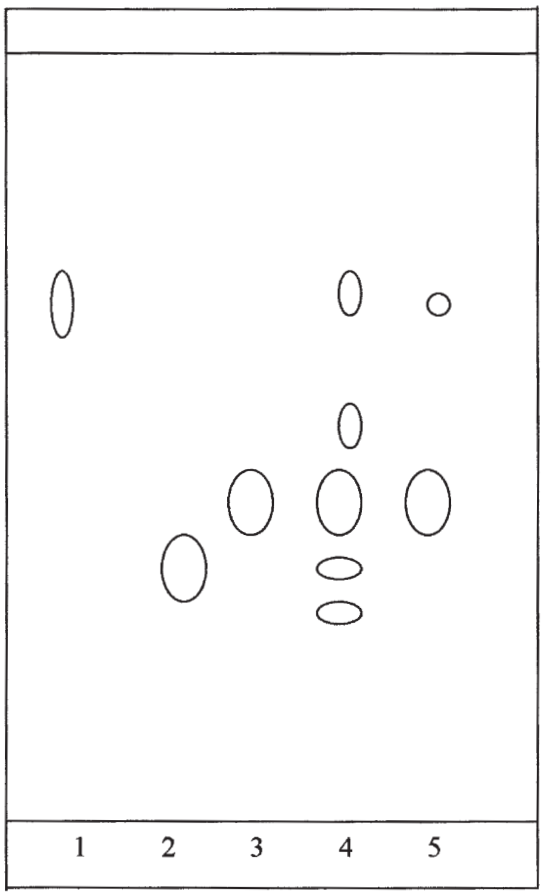

FIG. 1. Thin-layer chromatogram of dihydroxystearic acid (DHSA) derived from palm oleic acid. Developing solvent: Hexane/ethyl acetate/acetic acid (50:50:1, by vol). (1) Oleic acid; (2) *erythro*-9,10-DHSA; (3) *threo*-9,10-DHSA; (4) crude DHSA; (5) purified DHSA

methanol with tetramethylsilane as internal standard. ^{13}C NMR spectra were recorded on a Bruker DRX-300 (Karlsruhe, Germany) spectrometer at 300 MHz. The chemical shifts are expressed in ppm.

Acid value, iodine value, hydroxyl value, and saponification value were determined by standard procedures (AOCS official methods): acid value, Te 1a-64; iodine value, Cd 1d-92; hydroxyl value, Cd 13-60; saponification value TI 1a-64 (10). Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Magna-IR550 (Nicolet, Madison, WI) spectrophotometer.

Solubility test. DHSA (0.1 g) was placed into a conical flask. Solvent was then added dropwise to the flask using a buret. Continuous stirring at 30°C was carried out until no particles were seen. The solution was stirred for 15 min before solubility was determined.

Surface tension. Surface tension measurements (ring method) were conducted with a Krüss (Charlotte, NC) Digital Tensiometer K10T. The required weight of sample was weighed and then dissolved in 1 M sodium hydroxide (NaOH) solution; the original solution was then diluted at various concentrations, and the surface tensions of these solutions were determined. All data points were determined from triplicate measurements.

Irritancy test of DHSA. From a preliminary analysis of the structure of DHSA, this compound could be used as thickener in cosmetic formulations. One of the properties required for a cosmetic ingredient is minimal skin irritation. Therefore, it is necessary to evaluate irritancy of this compound. Dermal irritancy of DHSA was conducted using The Irritection Assay System, which consists of a test kit, instrumentation, and computer software (In Vitro International, Irvine, CA). Samples were weighed at five different concentrations—25, 50, 75, 100, and 125 mg—and placed into the membrane discs. Reagent and blanking buffer (1250 μL) were added to a 24-well assay plate. The membrane discs that contained various concentrations of DHSA sample were inserted into the corresponding blank and test sample wells of the plate. The assay plate was then incubated at 25°C for 24 h. After this time the membrane discs were removed from the assay plate, and 250 μL of reagent plus blanking buffer was transferred into the 96-well reading plate. This plate was inserted into the MRX Microplate Reader (Dynex Technologies, Inc., Chantilly, VA).

RESULTS AND DISCUSSION

Analysis of DHSA. The DHSA produced has a high hydroxyl value, low iodine value, satisfactory values for other characteristics listed in Table 1, and satisfactory color. Properties of the starting materials and the products, such as acid value, iodine value, and hydroxyl value, are shown in Table 1. The results showed that the hydroxyl value increased in the conversion from oleic acid to DHSA. This indicates the formation of hydroxyl group(s) in the compound, whereas decreases in iodine value indicate conversion of the double bond carbon of the starting material to a saturated compound.

During the hydroxylation, a secondary hydroxyl group was formed. The formation of the hydroxyl group was observed in the FTIR absorption at 3345 and 3250 cm^{-1} wave numbers (spectrum not shown). The double-bond carbon was no longer detected at 3004 cm^{-1} in the product. A similar observation was reported by Dahlke *et al.* (11) and Rakmi and Sadi (12).

DHSA was also subjected to ^1H and ^{13}C NMR analysis. ^1H NMR of DHSA in CD_3OD exhibited the following peaks: 4.85 (OH), 3.37 (–CH–O–), 2.28 ($–CH_2$ at C2), 1.60–1.29

TABLE 1
Properties of Palm Oleic Acid, Crude DHSA, and Purified DHSA[a,b]

Parameter	Palm oleic acid	Crude DHSA	Purified DHSA
Iodine value (g I_2/100 g)	93.8 ± 1.4	10.2 ± 1.6	1.1 ± 0.2
Acid value (mg KOH/g)	204.0 ± 1.8	179.3 ± 0.4	180.3 ± 1.2
OHV (mg KOH/g)	18.7 ± 0.5	196.0 ± 4.2	309.3 ± 3.9
Sap. value (mg KOH/g)	204.5 ± 2.6	178.0 ± 0.2	178.5 ± 1.0
Melting point (°C)	ND	61.9 ± 1.3	90.6 ± 0.9
Form	Liquid	Semisolid	Solid

[a]Purified DHSA obtained by crystallization of crude DHSA from ethanol (0.3 g/mL) at 5°C.
[b]Average values determined in duplicate and repeated twice. ND, not determined; OHV, hydroxyl value; DHSA, dihydroxystearic acid.

(CH_2), and 0.90 ppm (CH_3). ^{13}C NMR confirmed the presence of the following groups: carbonyl at around 178 ppm, C_9 and C_{10} hydroxyl carbon at 75.6 and 75.5 ppm, $–CH_2–$ carbon over the range from 24.0 to 35.3 ppm, and terminal methyl carbon at 14.8 ppm.

GC analyses of the DHSA produced were compared with standard fatty acids as well as *threo*-9,10-DHSA (data not shown). As shown in Figure 2, the chromatogram of DHSA after conversion to TMS derivatives revealed the presence of other compounds along with DHSA. These are due to the fatty acid compositions of the oleic acid used, which contained saturated fatty acids of various chain lengths (C_{12}, C_{14}, C_{16}, and C_{18}); therefore, the product is not only DHSA but also other side products.

Solubility of DHSA. The solubilities (± SD) of DHSA (in mg/mL) in various organic solvents were as follows: methanol, 94.39 ± 0.12; dimethylformamide, 70.01 ± 0.10; acetone, 2.70 ± 0.30; chloroform, 2.56 ± 0.10; toluene, 2.60 ± 0.20; pentane, insoluble; hexane, insoluble. (The solubility test was conducted in triplicate, using 0.1 g DHSA. Maximum volume of solvent used was 40 mL. The test was conducted at 30°C with magnetic stirring.) These results are in agreement with the findings of Hou *et al.* (13). They reported that hydroxy fatty acid is not soluble in nonpolar solvent but is soluble in dimethylsulfoxide and alcohol. The solubility of DHSA in alcohols of various chain lengths was also studied. The result indicated that solubility decreased with increasing alcohol chain length. This is probably due to the increasing solvent viscosity and decreasing polarity (Fig. 3).

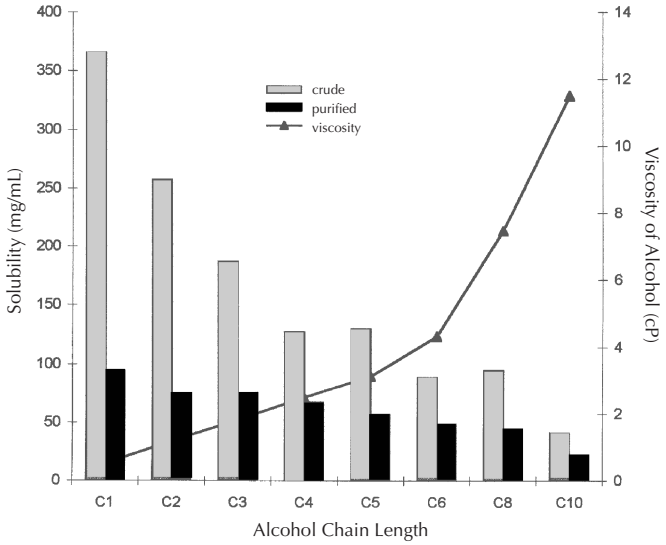

FIG. 3. Effect of alcohol chain length on solubility of DHSA, and relationship between alcohol chain length and viscosity. For abbreviation see Figure 1.

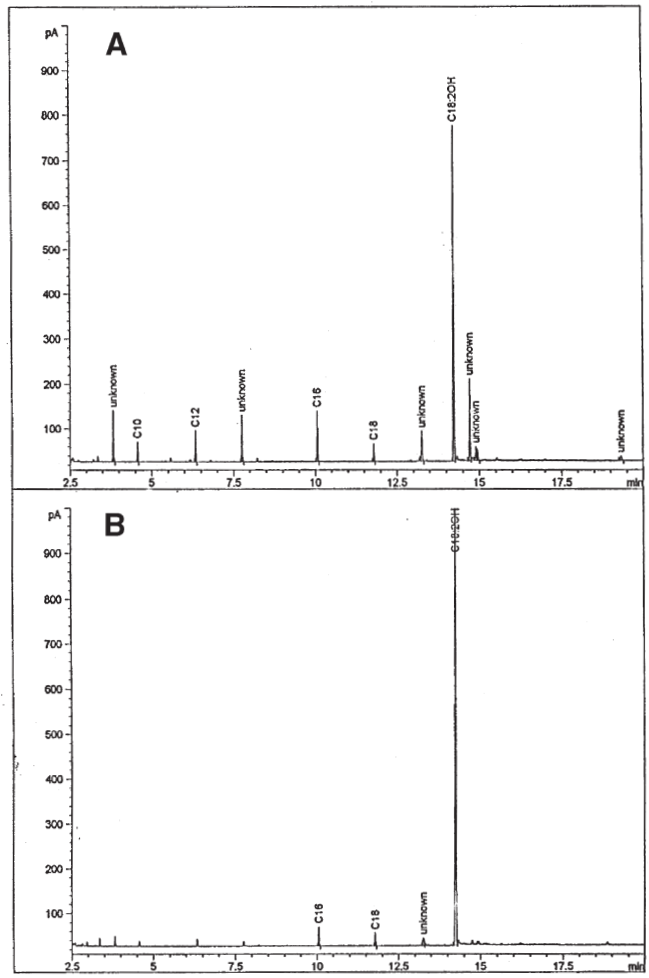

FIG. 2. Gas chromatographic separation of the components of DHSA prepared from palm oleic acid after derivatization with *N,O*-bis trimethylsilyl acetamide. (A) Crude DHSA; (B) purified DHSA, Column: HP-5 [(5%-phenyl)-methylpolysiloxane], 0.25 mm × 30 m × 0.25 µm. Oven: 150°C for 1 min, 150–290°C at 10°C/min, 290°C for 30 min. For abbreviation see Figure 1.

Surface tension. In this study, surface tension was measured in 1 M NaOH solutions. Surface tension for 1 M NaOH was 61.7 mN/m. This value served as a reference for the initial

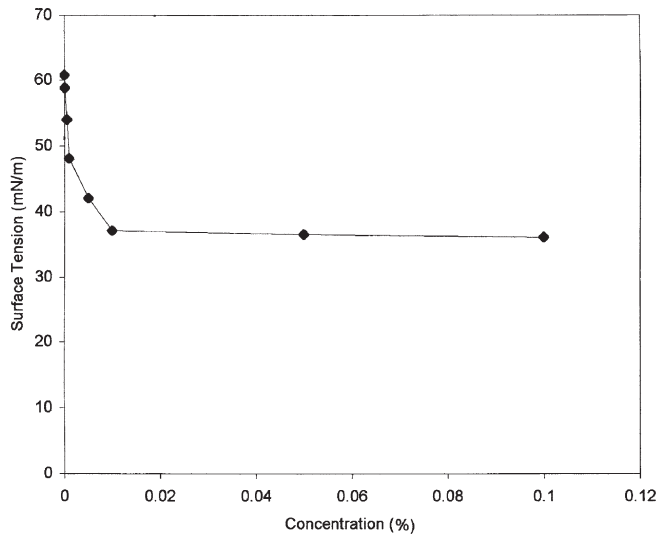

FIG. 4. Plot of surface tension vs. concentration of DHSA (in 1 M NaOH). For abbreviation see Figure 1.

TABLE 2
Irritancy Test of Crude and Purified DHSA[a,b]

Sample	Dose (mg)	Irritancy score	Irritancy classification
Crude DHSA	25	0.62	Nonirritant
	50	3.08	Irritant
	75	3.81	Irritant
	100	>4.00	Irritant
	125	>4.00	Irritant
Purified DHSA	25	0.25	Nonirritant
	50	0.40	Nonirritant
	75	0.30	Nonirritant
	100	0.21	Nonirritant
	125	0.29	Nonirritant

[a]Data were from single analyses.
[b]Data were obtained using The Irritection Assay System (In Vitro International, Irvine, CA) as described in the Materials and Methods section of the text. For abbreviation see Table 1.

surface tension before adding the product. Critical micelle concentration (CMC) of the product was determined graphically by plotting surface tension against concentration of DHSA in 1 M NaOH solutions. The results showed that the CMC of the product was about 0.01% with a surface tension of about 37.0 mN/m. Therefore, DHSA can be used as a surfactant. Figure 4 shows the surface tension of the product in 1 M NaOH. Knothe *et al.* (14) also reported similar surface tension values for dihydroxy fatty acids in their work on surface tension studies of novel allylic mono- and dihydroxy fatty compounds. It has been reported that hydroxy compounds may be suitable for use in microemulsion or co-solvency or as additives in commercial products (14).

Irritancy test of DHSA. As shown in Table 2, for the crude DHSA the maximal qualified score is more than 4.0, which is irritating to the skin, whereas for purified DHSA, the maximal qualified score is 0.4, which is nonirritating. The irritancy of crude DHSA may be due to the presence of acetic acid. However, this chemical was removed during the purification process. This result showed the skin compatibility of purified DHSA. Therefore, it has potential for use in cosmetics and personal-care products. It was reported that the Dermal Irritection Assay method could be employed to evaluate multiple samples at varying concentrations readily. Thus the test serves as a useful screening tool that facilitates all stages of raw material selection, formulation development, and final product selection (15)

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