Isolation of 9,10-Dihydroxystearic Acid from Sal (*Shorea robusta*) Fat

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9,10-Dihydroxystearic acid and its triglycerides (DHS-TGs) were isolated from sal fat by silica gel adsorption and solvent and dry fractionation processes, followed by crystallization. Silica gel adsorption gave a higher yield of DHS-TGs than the other two fractionation processes. However, the dry fractionation process was found to be comparatively easy to carry out. 9,10-Dihydroxystearic acid was isolated and identified by TLC, GLC, M.P. and IR-spectrometry. The DHS-TGs were found to contain 30.5% 9,10-dyhydroxystearic, 57.5% stearic, 6.0% palmitic and 5.8% arachidic acids. These processes were found to be useful for recovery of DHS-TGs and also to improve the solidification properties of sal fat required for confectionery.

The search for economical sources of oils/fats containing appreciable amounts of hydroxy acids is stimulated by their potential usefulness in commercial products such as waxes, polishes, cosmetics, paper coatings and multipurpose greases (1). They are also useful as thickeners in puddings and pie fillings (1). Numerous plant sources have been shown to contain hydroxy fatty acids in substantial quantities. Among these are seed oils from *Cardamine impatiens* (2), castor (3), *Strophanthus* (4), *Cephalocroton* (5), etc. The presence of 2-4% of 9,10-dyhydroxystearic (DHS) and 9,10-epoxystearic (ES) acids in the seed fat of sal (*Shorea robusta*), a dipterocarpaceae, has been reported (6). The DHS and ES acids have been shown to be present only at the *Sn2* position and constitute 6-12% of total triglycerides (6). Analyses of these two minor triglycerides by TLC and GLC were reported earlier (7). These minor triglycerides, especially DHS-TGs, also have been found to affect the solidification properties of sal fat (8). The present paper describes simple methods of isolation and identification of DHS-TGs from sal fat.

EXPERIMENTAL PROCEDURES AND RESULTS

Materials. Refined and bleached sal fat was procured from specialty Fats P. Ltd., Khamgaon, India.

Reference compound. Methyl 9,10-dihydroxystearate was procured from Applied Science Laboratories, State College, Pennsylvania.

Chromatographic analyses. Thin layer chromatography (TLC) analyses and separations were carried out on glass plates (20 × 20 cm), spread with 0.25 mm layers of silica gel G, in a mixture of petroleum ether (60-80)/diethyl ether/acetic acid (60:40:1, v/v/v). The spots were visualized by spraying with 50% H_2SO_4 followed by charring at 150 C for 3 hr. For quantitative analyses of individual spots, the chromatoplates were scanned with Camag T scanner equipped with lamp 110-852, primary filter B(7-60), secondary filter 110-823, density 10% and range selector at 3. The relative percentages of individual spots were calculated from the peak areas.

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The normal fatty acid methyl esters and TMS derivatives of hydroxymethyl esters were analyzed in a CIC gas chromatograph, equipped with a flame ionization detector, operating under the following conditions: Column: 2.4 m × 0.3 cm S.S., packed with chromosorb W (60-80 mesh) coated with 15% diethylene glycol succinate; column temperature, 180 C (isothermal); carrier gas, N_2 15 ml/min and H_2 20 ml/min. The peaks were identified by comparing the retention times with those of authentic standards. The results were expressed as proportional wt% of the components in the sample.

The fatty acid methyl esters were prepared by using diazomethane (9). The trimethylsilyl derivative of the methyl ester of hydroxy fatty acid was prepared by the procedure described by Belwadi et al. (7) by reacting 2-3 mg sample in dry pyridine with hexamethyl disilane (0.05 ml) and chlorotrimethyl silane (0.01 ml).

IR spectrometry. IR spectra of methyl 9,10-dihydroxystearate isolated from sal fat and authentic reference compound were recorded in KBr pellets on a Jasco spectrometer.

Cooling curve. The cooling curves were obtained by using Schukoff's flask according to the procedure described by Wilton and Wode (10).

Dilatometry. Dilatation studies were carried out according to the procedure given in British Standard Method 2 (11).

Isolation of DHS-TGs. The DHS-TGs were sepa-

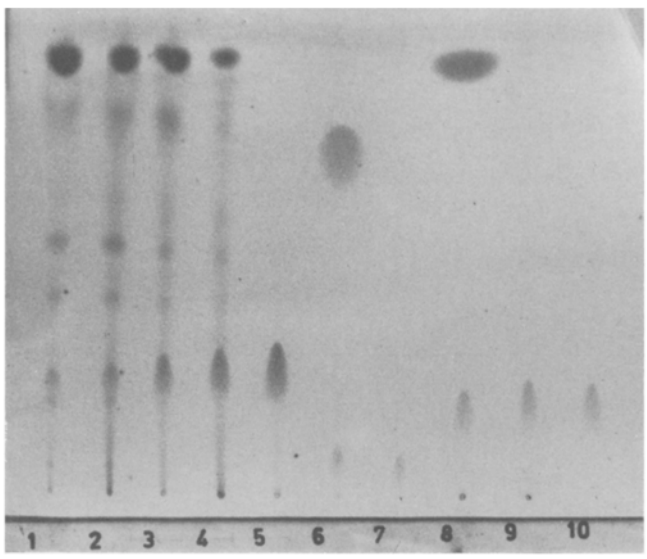

FIG. 1. Thin layer chromatogram of sal fat and its fractions developed with petroleum ether/diethyl ether/acetic acid (60:40:1, v/v/v). 1, sal fat (control); 2, silica gel adsorbed material of sal fat (Fr. 1); 3, solid (Fr. 2) from hexane fractionation; 4, solid (Fr. 3) from dry fractionation; 5, DHS-TGs; 6, DHS-TGs saponified; 7, 9,10-dihydroxystearic acid isolated from sal fat; 8, methyl esters of DHS-TGs; 9, methyl 9,10-dihydroxystearate isolated from sal fat; 10, authentic methyl 9,10-dihydroxystearate.

rated and purified by (i) Silica gel adsorption; (ii) solvent fractionation and (iii) dry fractionation.

For silica adsorption, 100 g of sal fat was dissolved in 200 ml of hexane by warming on a water bath, and 50 g of silica gel (100–200 mesh, activated at 110 C for 3 hr) was added. The mixture was stirred for about 2 hr and filtered. The silica gel residue was washed twice with 25-ml portions of hexane. The filtrate and washings were desolventized to obtain triglycerides containing normal fatty acids (TGs, yield 85 g). Then the residue was shaken with 3 × 100 ml of a mixture of chloroform/methanol (3:1, v/v) and filtered. The extract (Fr. 1) after desolventizing in a rotary flash evaporator (15 g) was dissolved in 20 ml of petroleum ether (60-80) by warming on a waterbath. The solution was allowed to stand at ambient temperature (24-26 C) for about 2 hr, and the separated crystals (Fr. 1a) were removed by filtration and washed with petroleum ether. These crystals (Fr. 1a, yield 2.4 g) showed only one spot on TLC.

In solvent fractionation, 100 g of sal fat was dissolved in 100 ml of hexane (or petroleum ether) by warming to about 45 C. The solution was cooled to ambient temperature (24-26 C) with stirring and held at this temperature for 24 hr. Then the separated crystals (Fr. 2, yield 2.0 g) were removed by filtration and washed with hexane. The solvent from the filtrate was removed to get olein fraction b, (yield, 98 g). Fraction 2 was re-crystallized from hexane to get pure DHS-TGs (Fr. 2a, yield 1.2 to 1.5 g).

For dry fractionation, 100 g of sal fat was heated to about 55 C to get a clear liquid and to destroy all crystal nuclei. The melt was cooled to 35 C with occasional stirring and was held at this temperature for 6 hr. Then the partially crystallized mass was filtered through a Buchner funnel to separate the solid fraction (Fr. 3, yield 12 g) and olein fraction c (yield, 88 g). This solid (Fr. 3) was dissolved in hot petroleum ether (25 ml), and the solution was allowed to stand at ambient temperature (24-26 C) for about 1 hr. The separated crystals (Fr. 3a) were removed by filtration and washed with petroleum ether (60-80) to get pure DHS-TGs (yield 2 g).

The concentration of DHS-TGs in all three fractions, Fr. 1, 2, and 3, and in corresponding olein fractions obtained was estimated by TLC-densitometry.

Characterization of 9,10-dihydroxystearic acid. The mixed fatty acids obtained after saponification of Fr. 1, 2 or 3 showed two spots on TLC, one corresponding to normal fatty acids and the other with low Rf value (Fig. 1). These fatty acids were dissolved in hot diethyl ether and held at about 10 C for 6-8 hr. The separated crystals were removed by filtration and washed with ether. These crystals had m.p. 93 C, identical to that of DL, threo 9,10-dihydroxystearic acid.

Similarly, the mixed methyl esters of Fr. 1, 2 or 3 showed two spots on TLC, one corresponding to normal fatty acid methyl esters and the other having the same Rf as that for authentic methyl threo 9,10-dihydroxystearate (Fig. 1). The mixed methyl esters were dissolved in hot petroleum ether and held at ambient temperature for about 2 hr. The separated crystals were removed by filtration, washed with petroleum ether and recrystallized from the same solvent. These crystals did not show any peaks in GC corresponding to methyl esters of normal fatty acids up to (C 20:0) arachidic acid. TMS derivatives of these crystals showed only one peak with the same retention time as that of TMS derivatives of authentic methyl threo 9,10-dihydroxystearate. The IR spectrum of these crystals was identical to that of authentic methyl threo 9,10-dihydroxystearate. Since the TMS derivatives of methyl 9,10-dihydroxystearate had a retention time similar to that of methyl arachidate, the fatty acid compositions of fractions Fr. 1, 2 or 3 were determined by GC of their methyl esters before and after silylation. These fractions were found to have 6.0% palmitic, 57.5% stearic, 5.8% arachidic and 30.5% 9,10-dihydroxystearic acids.

TABLE 1

Relative Percentages of Minor Components of Sal Fat and its Fractions

Sample	DHS-TGS	DGS	TGS
1) Sal fat	3.4	2.3	92.0
2) Fraction 1	27.6	22.3	31.0
3) Fraction 2	47.2	3.2	32.2
4) Fraction 3	39.0	17.0	38.0
5) Olein fraction b	1.6	5.0	92.0
6) Olein fraction c	1.5	2.4	93.4

DHS-TGS, Triglycerides containing 9,10-dihydroxystearic acid.
DGs, Diglycerides.
TGs, Triglycerides.

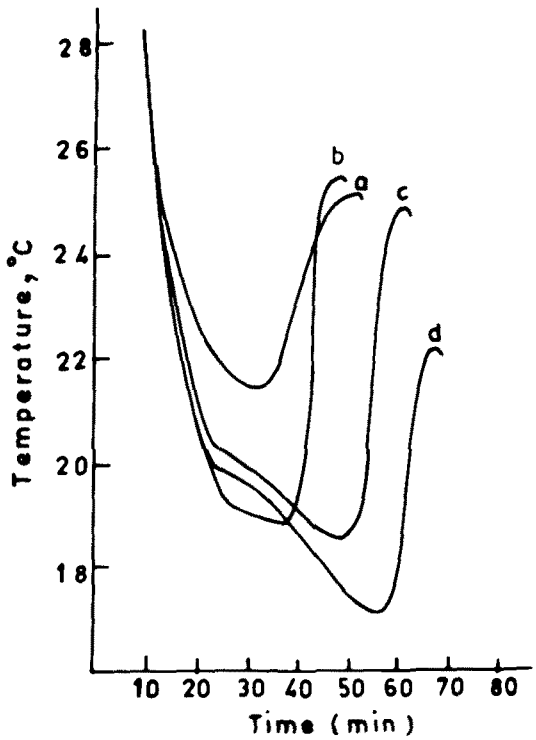

FIG. 2. Cooling curves of: a, sal fat; b, normal triglycerides of sal fat; c, olein fraction from dry fractionation of sal fat; d, olein fraction from hexane fractionation of sal fat.

TABLE 2

Dilatation Values of Sal Fat and its Fractions

Sample	Dilatation (mm^3/25 g) at °C					
	20	25	30	32.5	35	37
1) Sal fat	1800	1790	1530	1220	378	20
2) Normal triglycerides of sal fat	1875	—	1475	1325	490	—
3) Olein fractions (b & c)	1800	1785	1545	1250	520	30

DISCUSSION

The results in Table 1 revealed that the DHS-TGs were concentrated in fractions Fr. 1, 2 and 3. The percentage recovery of DHS-TGs from all three fractions was almost the same. However, the recovery of DHS-TGs from sal fat by the silica gel adsorption process was the highest, followed by dry fractionation and solvent fractionation. This is because in silica gel adsorption all the minor components, includng DHS-TGs, were adsorbed onto the silica gel, whereas in solvent and dry fractionation processes certain quantities of DHS-TGs remained in liquid fractions (Table 1). Although the silica gel adsorption method yielded a higher quantity of DHS-TGs, the other two fractionation processes, especially the dry fractionation process, were found to be comparatively easier to carry out. Also, the fractions (Fr. 2 and 3) Obtained by the latter two fractionation processes contained the major DHS-TGs like Fr. 1 (Table 1). The DHS-TGs isolated from sal fat consisted of 30.5% 9,10-dihydroxystearic acid, 57.5% stearic, 5.8% arachidic and 6.0% palmitic acids. This revealed that DHS-TGs consisted of one mole of 9,10-dihydroxystearic acid and two moles of saturated fatty acids, mostly stearic acid. DHS-TGs have a m.p. of 72 C. Therefore, these DHS-TGs, like castor wax, may be useful in the preparation of waxes, polishes, cosmetics, puddings, pie fillings, etc. (1). The olein fractions obtained after removal of DHS-TGs by all three processes showed improved solidification properties, i.e., higher heat of crystallization, increased supercooling capacity and slightly higher dilatation values at all temperatures compared to those of original fat (Fig. 2 and Table 2). Therefore, these processes are not only useful for recovery of DHS-TGs but also to improve the solidification properties of sal fat required for confectionery.

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