Characterization of Pit Oil from Montmorency Cherry (*Prunus cerasus* L.)

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Triolein, 1,2,3-tri-(*Z*)-9-octadecenoylglycerol, was reported earlier as the constituent of the purified pit oil from Montmorency cherry (*Prunus cerasus* L.). Our findings proved that the clarified kernel oil from Michigan tart cherries, *P. cerasus* L. var. Montmorency, consisted of a single product 1,3-di[(*Z*)-9-octadecenoyl]-2-[(*Z,Z*)-9,12-octadecadienoyl] glycerol (1). Triolein was not present in the Montmorency cherry pit oil in detectable quantities. The structure of 1 is elucidated by chemical and spectral methods and by direct comparison with triolein. The fatty acids present in Montmorency pit oil were identified to be oleic (63.6%) and linoleic (31.5%) acids by spectral and GC analyses. Thermal stability of the Montmorency cherry pit oil is also investigated by thermal gravimetric analysis against apricot, canola, vegetable, olive, and safflower oils and is found to be higher than or equal to that of all of the oils studied.

Most important raw materials for vegetable oils include legume seeds such as soybeans and peanuts and other seeds such as cottonseed, sunflower, rapeseed, coconut, sesame, palm, olive, and castor beans. Scientists around the world are exploring alternate and nontraditional seed sources for oils. Traditional oils and fats are very limited in their chemical constitution as their triglycerides mainly comprise stearic, oleic, and linoleic acids (Klieman et al., 1984). Michigan is the largest producer of tart cherries in the United States. Michigan's total cherry production is about 200 million pounds per year and generates about 16 million pounds of pits. These pits currently contribute to a waste disposal problem. Since the seed oils are chemically diverse, scientifically interesting, and versatile in utility (Wolff, 1966), we have investigated the oil from Montmorency cherry pits for its chemical composition and potential use in cooking.

EXPERIMENTAL PROCEDURES

Instrumentation. NMR spectra were obtained on a Varian VXR 500 spectrometer at 500 MHz for ^{1}H NMR and at 125.6 MHz for ^{13}C NMR. The spectra were recorded at 25 °C in $CDCl_3$. EIMS and FABMS were recorded on JEOL JMS-AX505 and JMS-HX110 mass spectrometers, respectively. IR spectra were recorded on a Nicolet IR/42 FTIR spectrophotometer and the UV on a Shimadzu UV-Visible spectrophotometer, Model UV-260. TLC was carried out on precoated Si gel T-7270 (Sigma) and Si gel Uniplate (1500 μm, Analtech) plates coated with fluorescent indicator. Detection of the spots/bands was achieved by visualizing the spots with a UV lamp at 254 nm. The plates were developed by spraying with 50% H_2SO_4 and heated for 5 min at 120 °C.

Analytical Methods. HPLC analyses of 10-μL solutions of 5.0 mg/mL fatty acid mixtures from the hydrolysis of 1 and oleic and linoleic acid standards (mg/mL) in CH_3CN/H_2O (4:1) were carried out on a Novapak HR C-18 cartridge (8 mm × 100 mm, 6 μm). The mobile phase CH_3CN/H_2O (4:1 v/v) was used under isocratic conditions at a flow rate of 1.5 mL/min. The samples were analyzed at 210 nm (aufs 0.1) on a Waters 490 variable-wavelength UV-visible detector (Waters Associates, Milford, MA). GC analyses were carried out on a Hewlett-Packard Series II GC 5890 using an FID detector and a fused silica DB 1701 column, 30 m × 0.25 mm (J&W Scientific Inc., Rancho Cordova, CA). The initial temperature was 150 °C, held isothermal for 2 min, raised at 5 °C/min to the final temperature 270 °C, and held isothermal for 5 min. Injector and detector temperatures were 250 and 260 °C, respectively. Helium was used as the carrier gas at a split ratio of 1:60. The injection volume was 1.5 μL of milligram per milliliter solutions in hexane. Thermal gravimetric analyses (TGA) were carried out on Thermogravimetric Analysis, Du Pont Model 951 (TA Instruments, New Castle, DE).

Extraction of Cherry Pits. Dried cherry pits (751 g) were milled and percolated with hexane (3.3 L). The removal of hexane *in vacuo* afforded an oil (47.88 g). This oil was extracted with MeOH (200 mL) to afford a clear yellow methanol-insoluble fraction (46.17 g) and a soluble viscous fraction (1.5 g). The MeOH-insoluble fraction (46.17 g) was dissolved in hexane (500 mL) and decolorized with activated charcoal (5 g) to yield a pale yellow oil (45.36 g), compound 1, and gave a single spot on TLC (hexane/acetone, 4:1; R_f 0.60).

1,3-Di[(*cis*)-9-octadecenoyl]-2-[(*cis,cis*)-9,12-octadecadienoyl]glycerol (1) was obtained as a pale yellow oil, density 0.92; IR ν^{max} (liquid film) 3009, 2926, 2855, 1745, 1466, 1377, 1261, 1161, 1097, 802 cm^{-1}; UV λ^{max} (hexane) 270 nm; EIMS *m/z* (rel intensity) 603 ($M^+ - C_{18}H_{31}O_{89}$, 100.00), 339 (46.00), 281 (6.5), 265 (40.00), 262 (95.00), 249 (7.00), 234 (8.00), 221 (4.00), 207 (3.50), 193 (5.0), 179 (6.5), 165 (12.5), 151 (17.5), 137 (19.0), 123 (25.0), 109 (37.5), 95 (58.00), 81 (55.00), 69 (72.00), 67 (48.00), 55 (55.00); ^{1}H NMR ($CDCl_3$) δ 0.89 [9H, t, *J* = 6.42 Hz, H-18′, 18″], 1.27 [54H, m, H-(12′-17′) × 2, (4′-7′) × 2, 4″-7″ and 15″-17″], 1.59 [6H, m, H-(3′ × 2), 3″], 2.02 [12H, m, H-(8′, 11′) × 2, 8″, 14″], 2.31 [6H, t,b, *J* = 7.82 Hz, H-(2′) × 2, 2″], 2.76 [2H, dd, *J* = 6.14, 5.59 Hz, H -11″], 4.13 [2H, ddd, *J* = 12.01, 5.86, 5.87 Hz, H-1a,b], 4.49 [2H, ddd, *J* = 12.0, 4.19, 4.19 Hz, H-3a,b], 5.26 [1H, m, H-2], 5.31-5.39 [8H, m, H-(9′, 10′) × 2, 9″, 10″, 12″, 13″]; ^{13}C NMR ($CDCl_3$) δ 14.72 [C-18″], 14.77 [C-18′ × 2], 23.22, 23.33, 29.73-30.41, 32.17, 32.55 [C-4′-7′, (12′-17′) × 2, 4″-7″, 15″-17″, interchangeable], 25.49, 25.51, 25.52 [C-(3′) × 2, 3″], 26.27 [C -11″], 27.81, 27.84, 27.86 [C-(8′, 11′) × 2, 8″, 14″], 34.67 [C- (2′) × 2], 34.83 [C-2″], 62.74 [C-1, 3], 69.51 [C-2], 128.52, 128.72, 130.61 [C-9″, 10″, 12″, 13″], 130.35, 130.65 [C-(9′, 10′) × 2], 173.49 [C-1″], 173.89 [C-(1′) × 2].

1,3-Bis[9,10-epoxyoctadecanoyl]-2-(9,10,12,13-diepoxyoctadecanoyl)glycerol (2). Compound 1 (100 mg) was treated with *m*-chloroperoxybenzoic acid (80 mg) in $CHCl_3$ (20 mL) at ambient temperature (18 h). The reaction mixture (20 mL) was sequentially washed with Na_2SO_3 (saturated solution), Na_2CO_3 (saturated solution), and H_2O (50 mL × 2). The solvent was evaporated *in vacuo*, and the mixture (194.0 mg) on further purification by TLC (hexane/acetone, 8:1, R_f 0.50) afforded colorless viscous oil, 2 (102 mg): ^{1}H NMR ($CDCl_3$) δ 0.87 [9H, t, *J* = 6.42 Hz, H-(18′) × 2, 18″], 1.24-1.74 [74H, m, H-3′- 8′, (11′-17′) × 2, 3″-8″, 11″, 14″-17″], 2.30 [6H, bt, *J* = 7.54 Hz, H-(2′) × 2, 2″], 2.88-3.12 [8H, m, H-(9′, 10′) × 2, 9″, 10″, 12″, 13″], 4.13 [2H, ddd, *J* = 12.0, 5.86, 5.86 Hz, H-1a,b], 4.28 [2H, ddd, *J* = 12.05, 4.2, 4.1 Hz, H-3a,b], 5.24 [1H, m, H-2].

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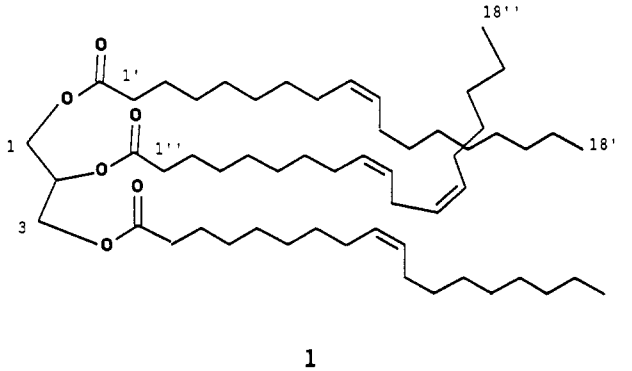

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Figure 1. Compound 1, 1,3-di[(*Z*)-9-octadecenoyl]-2-[(*Z,Z*)-9,12-octadecadienoyl]glycerol.

1,2,3-Tri(octadecanoyl)glycerol (Tristearin, 3). Compound 1 (520 mg) was hydrogenated using Pd/C (15 mg) in THF (50 mL), at ambient temperature (12 h). The reaction mixture was filtered through Celite and washed with hexane (50 mL) and $CHCl_3$ (50 mL). The combined organic extracts were evaporated to dryness and yielded tristearin (517.8 mg), a white amorphous solid (3), which melted at 55–60 °C; MS *m/z* (rel intensity) [M $- C_{18}H_{35}O_2]^+$ 607 (100), 341 (33), 284 (25), 267 (35), 253 (4), 239 (12), 225 (5), 211 (1), 197 (2.5), 183 (3.5), 169 (6), 113 (8), 99 (9), 95 (12), 85 (17), 71 (13), 57 (16); ^1H NMR ($CDCl_3$) δ 0.85 [9H, bt, J = 6.42 Hz, H-(18′) × 3], 1.26 [84H, m, H-(4′–17′) × 3], 1.58 [6H, m, H-(3′) × 3], 2.30 [6H, t, J = 7.54 Hz, H-(2′) × 3], 4.13 [2H, ddd, J = 11.73, 5.86, 5.87 Hz, H-1a,b], 4.29 [2H, ddd, J = 12.01, 4.18, 4.19 Hz, H-3a,b], 5.25 [1H, m, H-2]; ^{13}C NMR ($CDCl_3$) δ 14.09 [C-(18′) × 3], 22.68, 29.11–29.69, 31.91 [C-(4′–17′) × 3, interchangeable], 24.86, 24.90 [C-(3′) × 3], 34.04, 34.21 [C-(2′) × 3], 62.08 [C-1, 3], 68.86 [C-2], 172.82, 173.24 [C- (1′) × 3].

Fatty Acid Analyses. Compound 1 (1.58 g) was stirred with NaOH in MeOH (5% solution, 100 mL) at ambient temperature (1 h). The resulting solution was acidified with 6 N HCl to attain a pH of 5.5, diluted with H_2O (300 mL), and extracted with $CHCl_3$ (200 mL × 2). The combined $CHCl_3$ extract was evaporated *in vacuo*, to afford a mixture of fatty acids (1.22 g) identical to oleic and linoleic acids (Sigma Chemical Co., St. Louis, MO), respectively, by HPLC analysis. This fatty acid mixture (12.0 mg) was methylated with freshly prepared CH_2N_2 in ether (5.0 mL) at room temperature. The methyl esters (12.90 mg) were identical to methyl oleate and methyl linoleate, respectively, by GC analyses.

Thermal Gravimetric Analyses. TGA analyses of 1 (19.45 mg), apricot kernel oil (Bell Flavors and Fragrances; 15.89 mg), canola oil (Puritan; 13.65 mg), vegetable oil (Meijer Michigan brand; 18.63 mg), olive oil (Racconto; 15.43 mg), and safflower oil (Columbus Food Co.; 17.64 mg) were carried out under oxidizing conditions (initial temperature, 150 °C; final temperature, 550 °C; 20 °C/min gradient).

RESULTS AND DISCUSSION

The brown oil obtained by pressing the dried kernels from Montmorency cherry pits was extracted with MeOH and yielded 3.58% by weight of a MeOH-soluble fraction which contained benzaldehyde and several other compounds indicated by TLC analysis. The MeOH-insoluble oil on decolorization with activated charcoal afforded a pale yellow oil. TLC analysis of this oil gave only one spot. MS analysis of 1 (Figure 1) gave a base peak at *m/z* 603 $[M^+ - C_{18}H_{31}O_2]$ similar to the base peak in the MS of triolein. The peak at *m/z* 281 $[C_{18}H_{33}O_2]^+$ in 1, which was absent in the MS of triolein, suggested that 1 is a triglyceride with the molecular formula $C_{57}H_{102}O_6$. The presence of a strong band at 1765 cm^{-1} in the IR spectrum of 1 along with signals at δ 173.69 and 173.89 in its ^{13}C NMR spectrum suggested the presence of two or three ester carbonyls. The ^1H NMR signals at δ 4.13, 4.49, and 5.26 and ^{13}C NMR signals at δ 62.76 and 69.51 along with

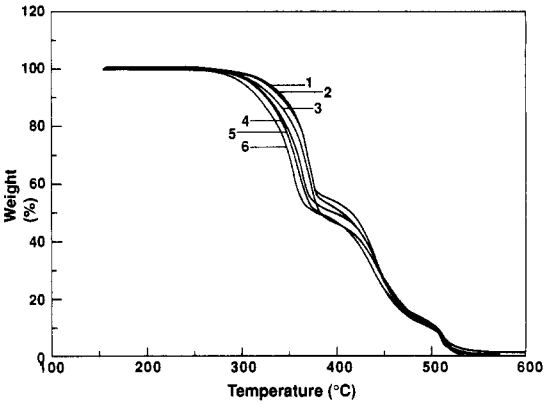

Figure 2. Thermal decomposition profiles and temperatures (TD) of (1) Montmorency cherry pit oil (TD, 352 °C), (2) vegetable oil (TD, 351 °C), (3) apricot kernel oil (TD, 338 °C), (4) canola oil (TD, 337 °C), (5) safflower oil (TD, 330 °C), and (6) olive oil (TD, 330 °C) under oxidizing (air) conditions.

the MS fragments at *m/z* 603, 262 $[(C_{18}H_{31}O) - H]^+$, and 265 $[C_{18}H_{33}O]^+$ confirmed that compound 1 is a triglyceride with C_{18} fatty acid esters. The overlapping multiplets centered at δ 5.35 for eight protons correlating to signals at δ 128.52 and 130.65 in the HETCOR spectrum indicated the presence of four olefinic bonds in 1. The number of olefinic bonds in 1 was further supported by the integral for an additional eight protons at δ 1.26 in the proton NMR and MS peaks at *m/z* 607 $[C_{39}H_{75}O_4]^+$ and 267 $[C_{18}H_{33}O]^+$ in the hydrogenated product, tristearin (3). The MS fragmentation pattern also suggested the nature of the fatty acid side chains as $C_{18:1}$ at C-1 and C-3, and $C_{18:2}$ at C-2. Two proton dd for H-11″ at δ 2.76 and the signal at δ 26.27 for C-11″ in the ^{13}C NMR spectrum along with the peaks at *m/z* 603, 281 $[(C_{18}H_{33}O_2)H]^+$, and 262 $[(C_{18}H_{31}O) - H]^+$ in the MS confirmed the fatty acid moiety at C-2 as linoleic acid. The DQCOSY spectrum of 1 showed correlation of H-2″ at δ 2.31 to H-3″ multiplets at δ 1.59. The H-2″ proton was also correlated to the multiplet at δ 1.27. The overlapping multiplets of H-8″ and H-14″ centered at δ 2.02 were correlated to the olefinic signals at δ 5.35 (H-9″, 10″, 12″, 13″). This olefinic multiplet was correlated to H-11″ protons at δ 2.76. Similarly, respective correlations were also obtained for oleic acid moeity at the C-1 and C-3 positions in compound 1. Complete proton and carbon assignments of 1 were obtained by DEPT, DQCOSY, and HETCOR experiments. Treatment of 1 with *m*-chloroperoxybenzoic acid in $CHCl_3$ resulted in the epoxidation of all four double bonds. The ^1H NMR of this epoxide (2) gave no olefinic signals but gave an 8H multiplet at δ 3.0 for four epoxide CH_2 protons.

Alkaline hydrolysis of 1 yielded two fatty acids, which were identical to the authentic samples of (*Z*)-9-octadecenoic acid (*Z*-oleic acid) and (*Z,Z*)-9,12-octadecadienoic acid (*Z,Z*-linoleic acid), respectively. Also, the GC analyses of the methyl esters of the fatty acids from 1 were identical to those of the methyl esters of oleic and linoleic acids, respectively. The ratio of the oleic and linoleic acids composition in 1 was 2:1, respectively. Compound 1 has been reported earlier from various sources such as Egyptian cottonseed and seed oils from sunflower, safflower, and pumpkin (Manganaro et al., 1981; Wada et al., 1985; Cheon and Park, 1987a,b; Oboh and Oderinde, 1988; Tsuyuki, 1988; Palmer and Palmer, 1989; Fiad, 1991).

TD under oxidizing conditions for some of the commercially available cooking oils were as follows: canola oil, 337 °C, vegetable oil, 351 °C; olive oil, 330 °C; safflower oil, 330 °C; and apricot kernel oil, 338 °C (Figure 2). The TD value of Montmorency pit oil, 352 °C, was similar or

slightly superior to that of most of the oils studied and, therefore, suggested its potential for cooking or frying food.

Analysis of Montmorency cherry pits has shown that the dried pits contained 22.7% kernel which produced 31.8% oil (Lin et al., 1990). The total weight of compound 1 could be approximately 0.7 million pounds per 200 million pounds of tart cherries. Triolein was reported to be the only triglyceride present in the tart cherry pit oil (Cruess, 1938). Our results confirmed that the seed oil from Montmorency cherry pits contained only compound 1 and not triolein as reported by Cruess (1938). Also, the oil contains only unsaturated fatty acids, 63.6% oleic and 31.5% linoleic acids, as compared to olive, safflower, sesame, soybean, peanut, and sunflower oils (Pryde, 1979). Since the oil from Montmorency cherry pits is free from saturated fat and possesses a TD value of 352 °C, it could be used in food preparations along with olive or canola oils.

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