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Isolation and Identification of C16 and C18 Fatty Acid Esters of Chloropropanediol in Adulterated Spanish Cooking Oils

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The consumption of cooking oil adulterated with rapeseed oil containing 2% aniline is suspected of causing more than 20,000 illnesses and 300 deaths of people in the vicinity of Madrid, Spain since mid-1981 (PESTAÑA & MUÑOZ 1982). Fourteen samples of cooking oil, four controls and 10 from families affected by the epidemic, were received by the U.S. Food and Drug Administration in July 1981. The samples were analyzed for industrial chemicals, pesticides, and trace metals. Some of these materials were found in the oils, but none at>5 ppm. However, in three of the suspect oils, elevated levels of chlorine, up to 440 ppm, were found by neutron activation analysis (NAA). Of this only a small portion could be accounted for by gas chromatography (GC) with Hall halogen-selective detection. Fatty acid anilides in several suspect oils were subsequently discovered independently by DIACHENKO et al. (1982) and TABUENCA (1981) at levels up to 2,400 ppm. Most of the toxicological investigation of the toxic oil syndrome in Spain has been focused on these anilide contaminants (PESTAÑA & MUÑOZ 1982). This report describes the isolation and identification of C16 and C18 fatty acid monoesters and diesters of chloropropanediol in the three samples of toxic Spanish cooking oil having the highest levels of chlorine (440, 39, and 28 ppm).

MATERIALS AND METHODS

Oil samples were collected on June 16, 1981, in Navas del Mareques, Avila, Spain. Samples were sent to the Centers for Disease Control, Atlanta, GA, who then forwarded portions to us. Oils were diluted to 0.1 g/mL hexane. All organic solvents used, except toluene, were distilled in glass.

Preparative fluorescent silica gel TLC plates 1-2 mm thick were prewashed with acetone. Twenty-five to 50 mg oil was applied to these plates in streaks 1-2 mm wide, which were developed with toluene for 8.2 cm. A band from R_f 0.60 to 0.75 was scraped off. This area was just above an ultraviolet (UV) quenching oil band. Compounds were eluted from the silica gel with ethyl ether or acetone for subsequent analysis by mass spectrometry (MS), NAA, and nuclear magnetic resonance (NMR) spectroscopy.

TLC on 0.25 mm silica gel plates prewashed with 1:1 (v/v) acetone in water was used to detect halogenated contaminants in the oils. Plates were developed with toluene for 10 cm or in a short-bed TLC apparatus (Regis Chemical Co., Morton Grove, IL) with 1% ethyl acetate in hexane for 3 h. They were dried 10 min in a fume hood, sprayed with 1% aqueous $AgNO_3$, and exposed to shortwave UV light (germicidal lamps) for 40 min (GARDNER 1979). Subsequent drying of the plates for 5 min, spraying with water, and re-exposure to UV light for 10 min increased the spot intensity of halogenated substances from toxic Spanish oils about 5-fold. The latter steps did not increase the spot intensity of 200 ng p,p'-dichlorobenzophenone, a standard used to determine the sensitivity of the $AgNO_3$ spray.

Oils were prepared as described in OFFICIAL METHODS OF ANALYSIS (1980). This involved solvent partitioning between acetonitrile and petroleum ether and Florisil column chromatography, taking only the 15 and 50% ethyl ether/petroleum ether eluates for subsequent analysis by GC or MS.

GC was performed on a 6 ft (1.8 m) x 4 mm i.d. glass column packed with 3% OV-101 on 80-100 mesh Chromosorb W(HP) and operated at 230°C with an injector port temperature of 250°C and a helium carrier gas flow rate of 60 mL/min. This column was connected to a model 700A Hall electrolytic conductivity detector operating in the halogen-selective mode. A 25 m capillary OV-1 column was also used, operated at a column temperature of 230°C, an injector port temperature of 250°C, and a nitrogen flow rate of 1 mL/min. This column was connected to a flame ionization detector.

Electron impact (EI) mass spectra were obtained on a Varian MAT CH-5 DF mass spectrometer operating with an emission current of 300 μA, an electron energy of 70 eV, and resolutions of 15,000 and 1,000. TLC oil fractions were introduced by direct probe. Positive ion chemical ionization (CI) mass spectra were obtained on a Finnigan 3300F mass spectrometer at an emission current of 0.5 mA and an electron energy of 140 eV. The ion source temperature was 110-130°C. The methane reagent gas pressure in the ion source was 0.7 torr. Florisil fractions were introduced by direct probe or GC.

Neat oil and selected TLC oil fractions in capped 1 mL polyethylene vials were irradiated 30-60 sec at a neutron flux of 4.9×10^{13} neutrons/cm^2/sec. Irradiated samples were dissolved in ethyl acetate and counted 5-10 with a gamma-ray spectrometer system.

Proton NMR spectra, described by 4,096 data points, were obtained at 80 MHz on a Varian Associates FT-80A NMR spectrometer. Pulse widths of 40 μsec were employed, which correspond to tip angles of ca 80° with conventional 5 mm

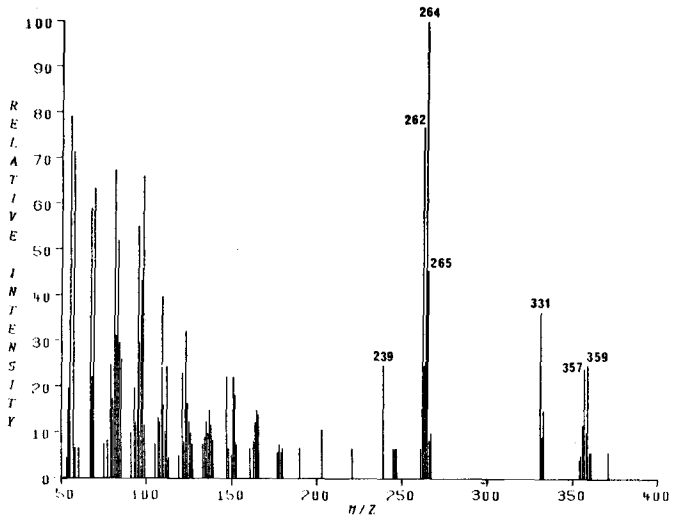

Figure 1. EI mass spectrum of TLC fraction from toxic Spanish oil containing 440 ppm chlorine.

sample tubes. Samples were dissolved in deuterochloroform with tetramethylsilane as an internal reference at δ = 0.0 ppm.

RESULTS AND DISCUSSION

The toxic oil containing the highest level of chlorine, 440 ppm by NAA, was the first to be examined for chlorinated contaminants. A halogenated band at R_f 0.65 was observed in the toxic oil after TLC with toluene. This band was absent in a control oil. The components in this band could be separated into at least four other halogenated bands by short-bed TLC, using 1% ethyl acetate in hexane. NAA confirmed the presence of chlorine in this band, showing that it accounted for 51% of the 440 ppm chlorine.

Figure 1 shows an EI mass spectrum of the chlorinated contaminants contained in the TLC band with R_f 0.65. The mass spectrum was qualitatively identical to the mass spectrum of a mixture of diesters of 3-chloropropanediol (VELIŠEK et al. 1980). Ions at m/z 239, 262, 265, 331, 333, 355, 357, and 359 are diagnostic of the fatty acid components of the diesters (DAVIDEK et al. 1980).

Table 1 lists possible fatty acid components present in the toxic Spanish oil contaminants. MS evidence of chlorinated fatty acid moieties was absent. The presence of chlorine in the $(M-RCO_2)+$ fragment ions at m/z 355, 357, and 359 was confirmed by high resolution EI MS. Exact masses found were: 355.2405 (355.2404 calculated for $C_{21}H_{36}O_2{}^{35}Cl$); 357.2486 (357.2373 calculated for $C_{21}H_{36}O_2{}^{37}Cl$ and 357.2560 calculated for $C_{21}H_{38}O_2{}^{35}Cl$); and 359.2568 (359.2530 calculated for $C_{21}H_{38}O_2{}^{37}Cl$ and 359.2716 for $C_{21}H_{40}O_2{}^{35}Cl$). Overlapping of peaks due to two possible masses for m/z 357 and 359 may account for the discrepancy between calculated and observed values. As further confirmation, the molecular ions $(M+H)+$ of the chloropropanediol diesters were observed in the positive CI mass spectrum, e.g., diesters of oleate/palmitate at m/z 613, oleate/linoleate at m/z 637, and dioleate at m/z 639. A calculation based on the palmitate/oleate diester of chloropropanediol indicated that at least 3,800 ppm of the diesters was present in the contaminated oil sample containing 440 ppm chlorine.

NMR spectroscopy indicated the position of protons attached to the chlorine-bearing carbon in the chloropropanediol diesters. The NMR spectrum showed a doublet at a chemical shift $\delta = 3.65$ ppm (coupling constant, $\underline{J} = 5.5$ Hz). This agreed with values for the doublet representing the protons in the 3-position in synthetic 3-chloropropane-1,2-diol diesters, $\delta = 3.64$ ppm ($\underline{J} = 5.5$ Hz) (DAVIDEK et al. 1980).

Table 1. Electron Impact Mass Spectrometry Fragment Ions

Ion (m/z)	Fragment	Fatty Acid Indicated
239	$(RCO)+$	palmitic (16:0)
262	$(RCO-H)+$	linoleic (18:2)
264	$(RCO-H)+$	oleic (18:1)
265	$(RCO)+$	oleic (18:1)
267	$(RCO)+$	stearic (18:0)
331	$(M-RCO_2)+$	palmitic (16:0)
357	$(M-RCO_2)+$	oleic (18:1)
359	$(M-RCO_2)+$	stearic (18:0) and oleic with ^{37}Cl

Chloropropanediol diesters were found in TLC fractions of the other two toxic oils with lower levels of chlorine (39 and 28 ppm) by EI MS monitoring of the ions at m/z 355, 357, and 359. Chloropropanediol diesters were not found in a similarly treated control oil.

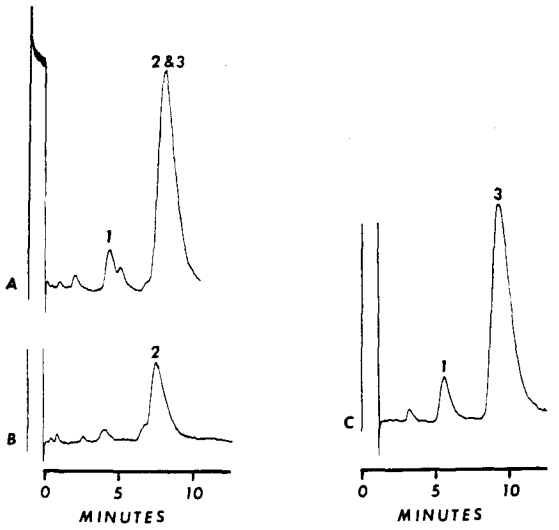

Figure 2. Gas chromatograms, using Hall detector, of extracts of toxic Spanish oil containing 440 ppm chlorine. (A) The extract was cleaned up by acetonitrile/petroleum ether partitioning but not by Florisil column chromatography; (B) the 15% ethyl ether/petroleum ether eluate from Florisil column; (C) the 50% ethyl ether/petroleum ether eluate from Florisil column. Peaks tentatively identified by MS as (1) C16 fatty acid monoester of chloropropanediol, (2) C18 fatty acid ester of dichloropropanol, and (3) C18 fatty acid monoester of chloropropanediol.

GC analyses accounted for less than 3% of the chlorine in the three toxic oil samples. Figure 2 shows gas chromatograms of the toxic oil containing 440 ppm chlorine. Peak 2 in Figure 2B was tentatively identified as a C18:2 fatty acid ester of dichloropropanol by its EI mass spectrum. The spectrum afforded the molecular ion at m/z 390, a chlorine isotope pattern at m/z 390 and m/z 392 indicating the presence of two chlorine atoms, and an $(RCO)^+$ ion at m/z 263. Peaks 1 and 3 in Figure 2C were tentatively identified by capillary GC/CI MS as C16:0 and C18:1 fatty acid monoesters of chloropropanediol. Spectra showed $(M+H)^+$ ions at m/z 349 and 375, $(M+H-HCl)^+$ ions at m/z 313 and 339, and $(RCO)^+$ ions at m/z 239 and 265. GC of the 50% ethyl ether/petroleum ether Florisil eluate on a capillary column produced multiple peaks in peaks 1 and 3, which indicated

the presence of a series of homologs and structural isomers. None of the monoesters were found in similarly treated control oils.

DAVIDEK et al. (1980) have shown that the monoesters and diesters of 3-chloropropanediol may be produced by the hydrolysis of triglycerides with HCl at 110°C. Our discovery of the 3-chloropropanediol diesters and chloropropanediol monoesters in three samples of toxic Spanish oils suggests that these oils, or portions thereof, may have been exposed to HCl during the refining process. HCl may have been added to remove the aniline which was used as a denaturant of the rapeseed oils (WORLD HEALTH ORGANIZATION 1981). To what extent the types of compounds found, the diester of 3-chloro-1,2-propanediol, the monoester of dichloropropanol, and the monoester of chloropropanediol, are implicated in the toxic oil syndrome in Spain is not known. Because these compounds are present as contaminants, their contribution to the toxic oil syndrome should be investigated. Other products that may be generated by HCl treatment of adulterated rapeseed oil should also be investigated as suspected oil contaminants. Our laboratory is continuing to try to account for the remaining chlorine in the toxic oil containing 440 ppm chlorine.

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