Supplements

Separation of triacylglycerols containing positional isomers of hexadecenoic acids by enantiomeric liquid chromatography-mass spectrometry.

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2.4 Preparation and analysis of methyl and 3-pyridylcarbonyl esters of fatty acids

Preparation of methyl esters. A ~3 mg sample was dissolved in 1 mL diethyl ether and 20 mL methyl acetate, 250 µL of 1 M sodium methoxide in methanol was added. The sample was worked up and left for 50 min at room temperature. Saturated oxalic acid solution (0.5 mL) was added, with brief agitation, to neutralize the solution. The solvent was removed under nitrogen, and an appropriate volume of hexane was added to bring the sample to the concentration required for analysis.

A Perkin-Elmer Model 1310 (Perkin-Elmer, Norwalk, CT) infrared spectrophotometer was used for scanning infrared spectroscopy of methyl esters as neat film.

Splitless injection was at 100 °C, and a fused silica capillary column (Supelcowax 10; 60 m x 0.25 mm i.d., 0.25 mm film thickness; Supelco, Prague) was used. The temperature program was as follows: 100 °C for 1 min, subsequently increasing at 20 °C/min to 180 °C and at 2 °C/min to 280 °C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm/s. All spectra were scanned within the range of *m/z* 50–500. FAMEs were identified according to their mass spectra [34, 38, 39] and using a mixture of chemical standards obtained from Merck.

A solution of potassium tert-butoxide in tetrahydrofuran (0.5 mL, 1.0 M) was added to nicotinyl alcohol (1 mL). After mixing, the appropriate FAME (~0.5 mg) in dry dichloromethane (1 mL) were added, and the mixture was held at 40 °C for 30 min in a closed vial. After cooling to room temperature, water and hexane were added, and the organic phase was collected, dried over anhydrous sodium sulfate, and evaporated.

A fatty acid 3-pyridylcarbonyl ester mixture was analyzed on the instrument described above. Injection temperature (splitless injection) was 100 °C, and an Ultra-1 capillary column (Supelco, Prague, Czech Republic) was used. The temperature program was as follows: 100 °C for 1 min, subsequently increasing at 20 °C/min to 180 °C and at 2 °C/min to 280 °C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm/s. All spectra were scanned within the range of *m/z* 50-500.

2.8. Enzymatic synthesis of TAGs

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (1,2-P/P-PC) was treated with phospholipase C (Type I from *C. perfringens* (*C. welchii*)) as described by Christie [45]. In brief, phospholipase C from *C. welchii* (4 mg) in 0.5 M tris(hydroxymethyl)methylamine (tris) buffer (pH 7.5; 2 mL), 2×10^{-3} M in calcium chloride, was added to phosphatidylcholine (11 mg) in diethyl ether (2 mL). The mixture was shaken at room temperature for 3 hours and extracted three times with diethyl ether (4 mL portions). The ether layer was dried over anhydrous sodium sulfate before being evaporated in a stream of nitrogen at ambient temperature. Pure 1,2- dioleoyl -*sn*-glycerol was obtained by preparative TLC on a layer of silica gel G impregnated with

boric acid (10%, w/w), with hexane-diethyl ether (50:50, v/v) as solvent system. The appropriate band was located under UV light and was eluted from the adsorbent with diethyl ether [45].

The esterification of 1,2-diacyl-*sn*-glycerols with acids was described previously [41, 42]. Briefly, a solution of appropriate 1,2-diacyl-*sn*-glycerol (~6 μM) and fatty acid (28:0) as a free acid in dichloromethane (1.0 mL) was added to DMAP (6.1 mg, 5 μM) and EDCI (18.6 mg, 1.2 μM) in dichloromethane (1.0 mL) under an inert atmosphere (argon). The resulting solution was stirred at room temperature for 15 h. The reaction was stopped by passing the reaction mixture in ether/dichloromethane (10:90) through a short column packed with silica gel. Solvent removal *in vacuo* afforded the pure products as yellowish oil see Table S2. TAGs from 1,2-dipalmitoleoyl-PC (1,2-Po/Po-PC) were prepared similarly.

Table S1. List of synthetically prepared TAGs.

starting substance	fatty acid	TAG	optical purity (%)
glycerol	P	PPP	-
glycerol	Po	PoPoPo	-
glycerol	Pv	PvPvPv	-
glycerol	Hy	HyHyHy	-
P/P-PC	Po	*sn*-PPPo	93
P/P-PC	Pv	*sn*-PPPv	93
P/P-PC	Hy	*sn*-PPHy	94
Po/Po-PC	P	*sn*-PoPoP	94
Po/Po-PC	Hy	*sn*-PoPoHy	93
Po/Po-PC	Pv	*sn*-PoPoPv	96

TAGs - triacylglycerols

P – palmitic acid

Po – palmitoleic acid

Pv – palmitvaccenic acid

Hy – hypogeic acid

PC - phosphocholine

Table S2. Relative abundance of $[DAG]^+$ ions, from five analyzes, mean ± SD.

TAG	abundance of $[DAG]^+$ (relative %)	abundance of $[DAG]^+$ (relative %)
sn-PPoP	PP	PPo
	40±5	100
sn-PPPo	PP	PPo
	75±6	100
sn-PPoPo	PPo	PoPo
	100	77±5
sn-PoPPo	PPo	PoPo
	100	37±7
sn-PPvP	PP	PPv
	38±4	100
sn-PPPv	PP	PPv
	78±3	100
sn-PPvPv	PPv	PvPv
	100	74±5
sn-PvPPv	PPv	PvPv
	100	36±5

P – palmitic acid

Po – palmitoleic acid

Pv – palmitvaccenic acid

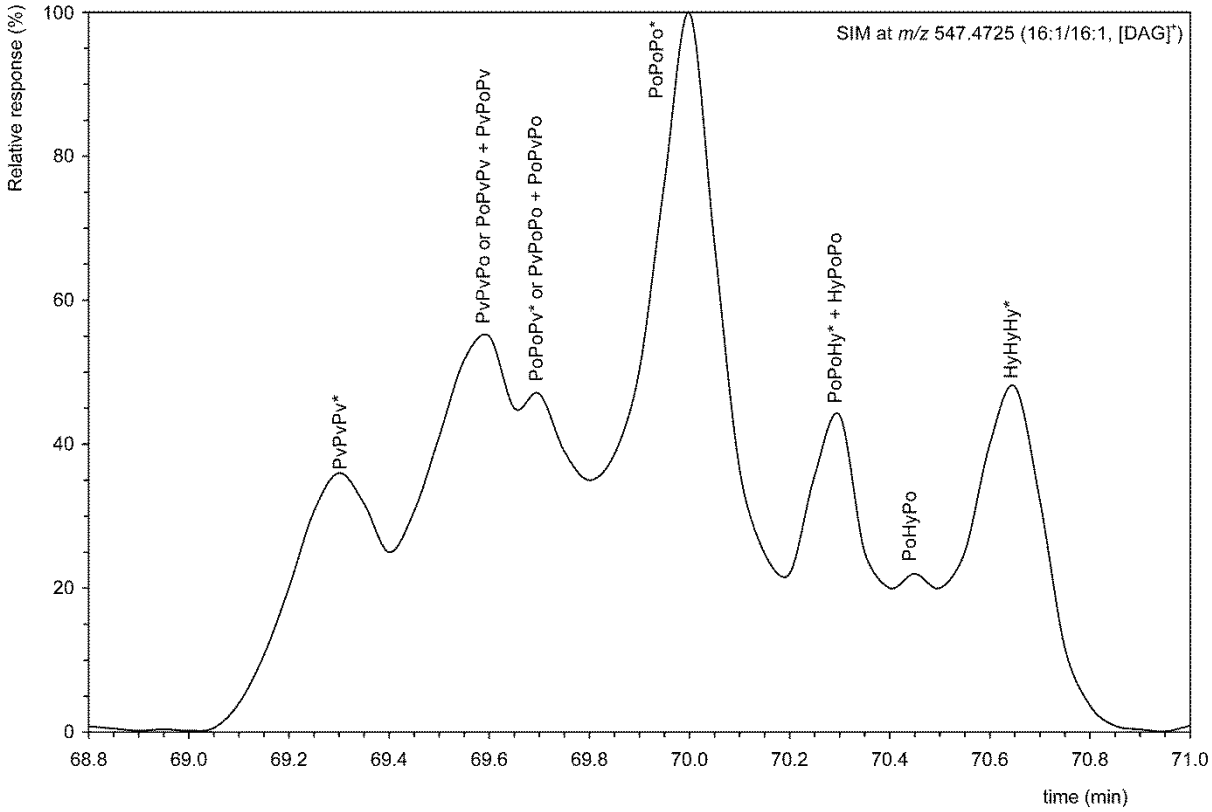

Fig. S1. The reversed phase separation of TAGs mixture containing three different hexadecenoic acids (Hy, Po, and Pv).

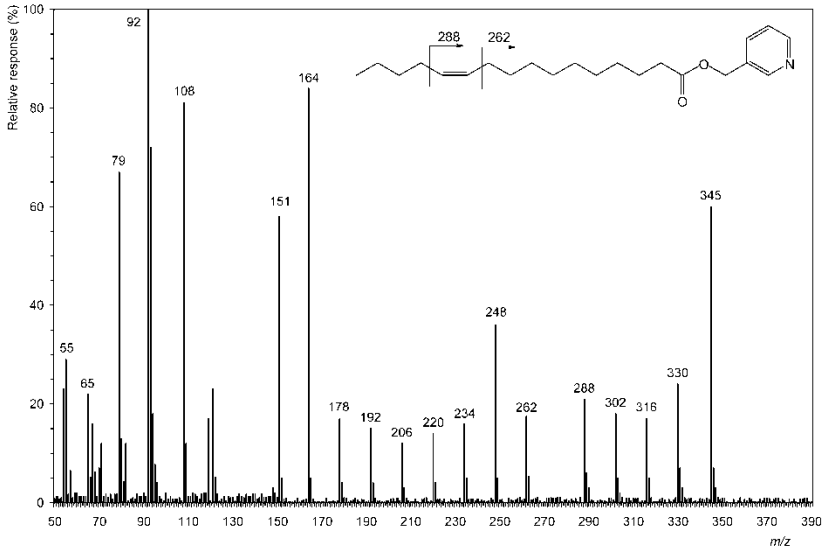

Fig. S2. Mass spectrum of 3-pyridylcarbinol esters of palmitvaccenic acid.

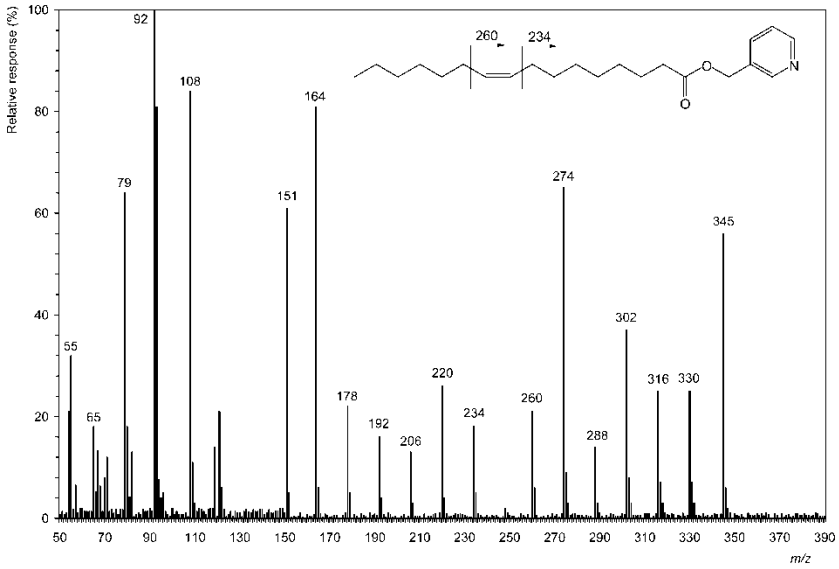

Fig. S3. Mass spectrum of 3-pyridylcarbinol esters of palmitoleic acid.

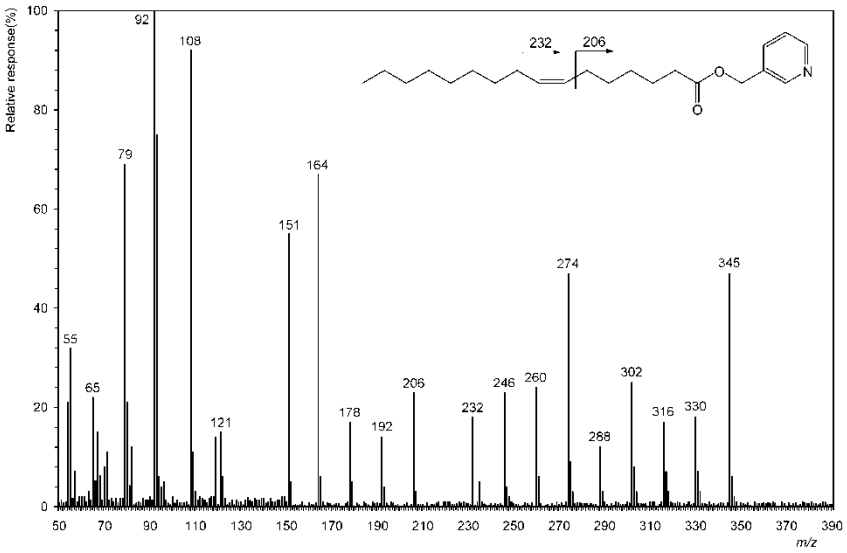

Fig. S4. Mass spectrum of 3-pyridylcarbinol esters of hypogeic acid.

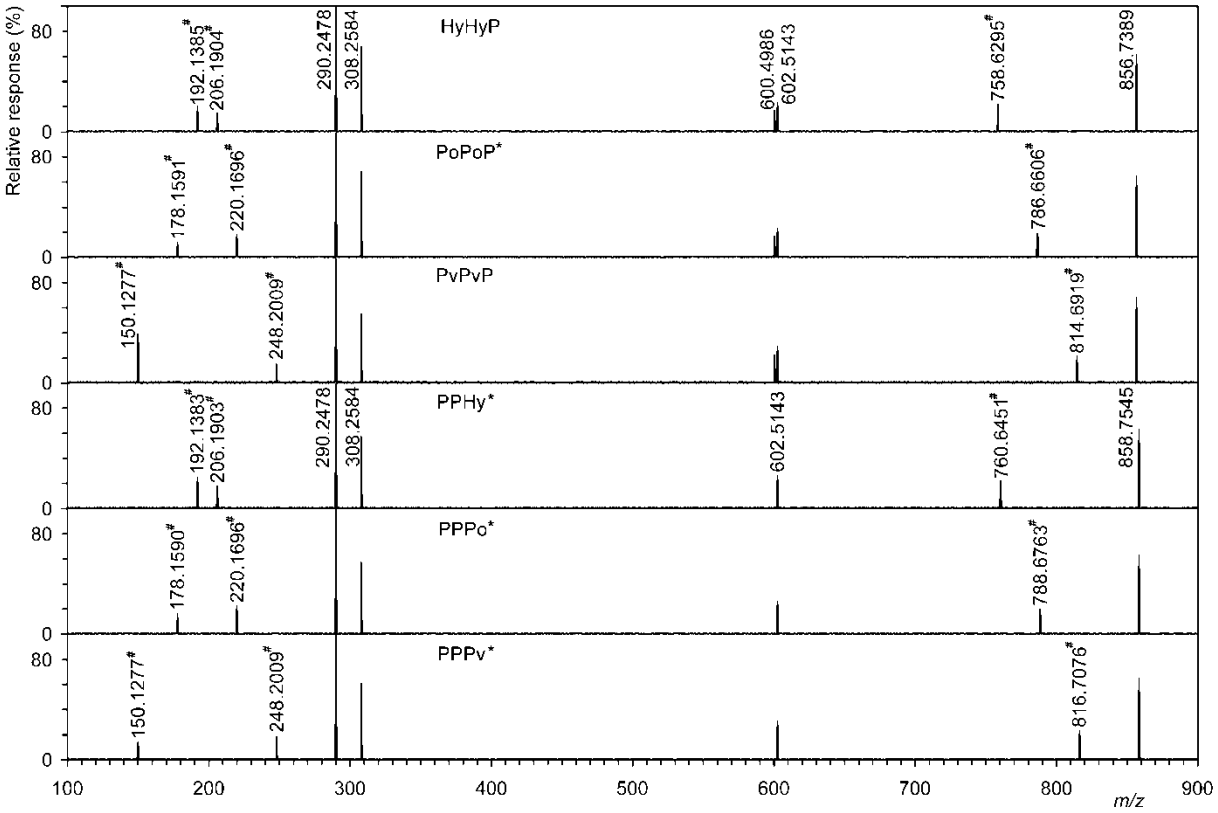

Fig. S5. Tandem mass spectra of six molecular species, obtained both synthetically (marked with an asterisk) and from natural sources. Tandem mass spectra were obtained from the $[M+54]^+$ ion (ion formed by the addition of 1-methyleneimino)-1-ethenyl ion to the fatty acid acyl double bond).

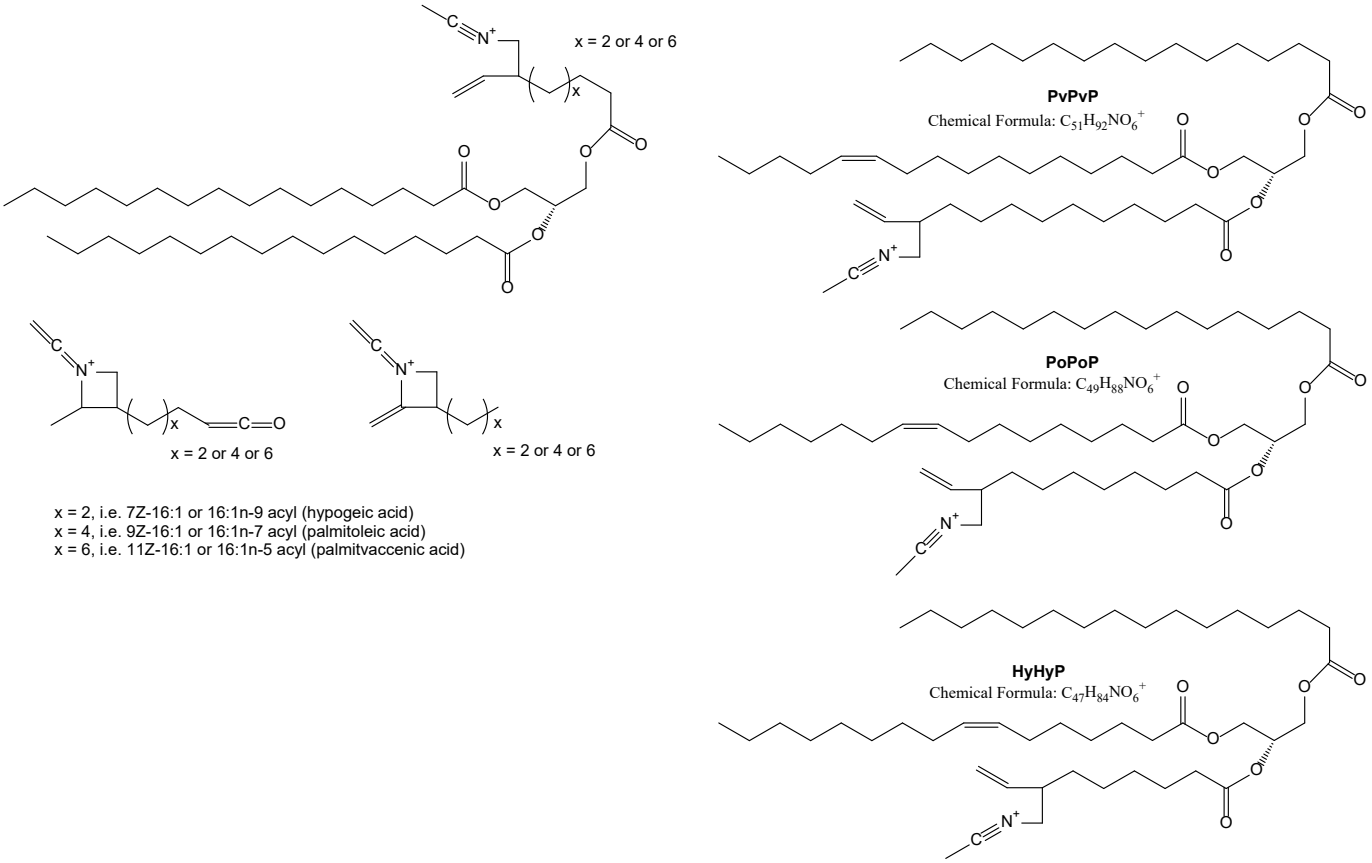

Fig. S6. Structure of diagnostic ions was based on the www page "https://lipidlibrary.aocs.org/lipid-analysis/selected-topics-in-the-analysis-of-lipids/identification-of-fame-double-bond-location-by-covalent-adduct-chemical-ionization-(caci)-tandem-mass-spectrometry", where J. Thomas Brenna and Cynthia Tyburczy published the ion structure on their Figure 7, entitled "Reaction of MIE with a monoene, with subsequent dissociation to form the α-diagnostic ion". We also used the results published in Table 3 of the paper Van Pelt C.K. and Brenna J.T. (DOI: 10.1021/ac981387f).