Supplements

Enantiomeric separation of triacylglycerols containing fatty acids with ring (cyclo-fatty acids)

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Preparation of triacylglycerols

In brief, phospholipase C from *C. welchii* (3 mg) in 0.5 M tris(hydroxymethyl)methylamine (tris) buffer (pH 7.5; 2 mL), containing 2 x 10^{-3} M calcium chloride, was added to phosphatidylcholine (15 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 [19]. 1,2-Dipalmitoyl-sn-glycerol, 1-oleoyl-sn-glycerol and 1-palmitoyl-sn-glycerol were similarly prepared.

A solution of 1,2-dipalmitoyl-*sn*-glycerol (10 μmol) and free acid (sterculic or chaulmoogric) (11 μmol) in dichloromethane (1.0 mL) was added to DMAP (10 μmol) and EDCI (12 μmol) 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 product as yellowish oil. The other six TAGs were prepared in a similar manner.

Preparation of methyl esters

A 10 μL 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.

Preparation of 3-pyridylcarbonyl esters

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 drydichloromethane (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.

GC-MS of FAMEs

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 [25,26] and using a mixture of chemical standards obtained from Sigma-Aldrich.

GC-MS of 3-pyridylcarbonyl ester of fatty acids

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.

Table S1. The number of possible TAGs.

Description	Number of Possible TAGs	Number of Possible TAGs for y = 11
without isomers	$x = (y^3 + 3y^2 + 2y)/6$	286
without enantiomers	$x = (y^2 + y^3)/2$	726
all isomers	$x = y^3$	1331

x is the number of TAGs, y is the number of FAs in TAGs

As seen from the Table, for a mere 11 FAs the number of potentially possible TAGs rises to hundreds of molecular species. Let us point out that 11 FAs is the normal number of FAs obtained in the identification of any natural oil or fat. For instance, fish oil contains several dozen FAs and here several hundred molecular species of TAG (exactly 553) were identified, without counting regioisomers and enantiomers [55].

Table S2a. Content of fatty acids from *Sterculia foetida*

Fatty acids	Abbreviations	Trivial name	Content
14:0	myristic	M	0.5
16:0	palmitic	Po	20.5
16:1Δ9c	palmitoleic	Po	0.5
17:0	margaric		0.7
18:0	stearic	S	5.7
18:1Δ9c	oleic	O	20.5
18:1Δ7c	*cis*-vaccenic	V	0.9
18:2Δ9c,12c	linoleic	L	29.8
18:3Δ9c,12c,15c	α-linolenic	Ln	2.1
20:0	arachidic	A	0.5
22:0	behenic	Be	0.3
9,10-cpa-19:0	dihydrosterculic	dSt	0.9
8,9-cpe-18:1	malvalic	Ma	5.8
9,10-cpe-19:1	sterculic	St	11.3

Table S2b. Content of fatty acids from *Hydnocarpus wightiana*

FA	Abbreviations	Trivial name	content
14:0	M	myristic	2.5
16:0	P	palmitic	6.9
16:1Δ9c	Po	palmitoleic	4.7
16:1cy	H	hydnocarpic	27.1
18:0	S	stearic	3.9
18:1Δ9c	O	oleic	5.9
18:2Δ9c,12c	L	linoleic	4.7
18:3Δ9c,12c,15c	Ln	α-linolenic	6.1
18:1cy	C	chaulmoogric	24.2
18:2cyΔ6c	G	gorlic	11.7
20:1cy	Ho	hormelic	1.1
20:2cy	On	oncobic	1.2

Fig. S1. Mass spectrum of 3-pyridylcarbinol esters of sterculic acid.

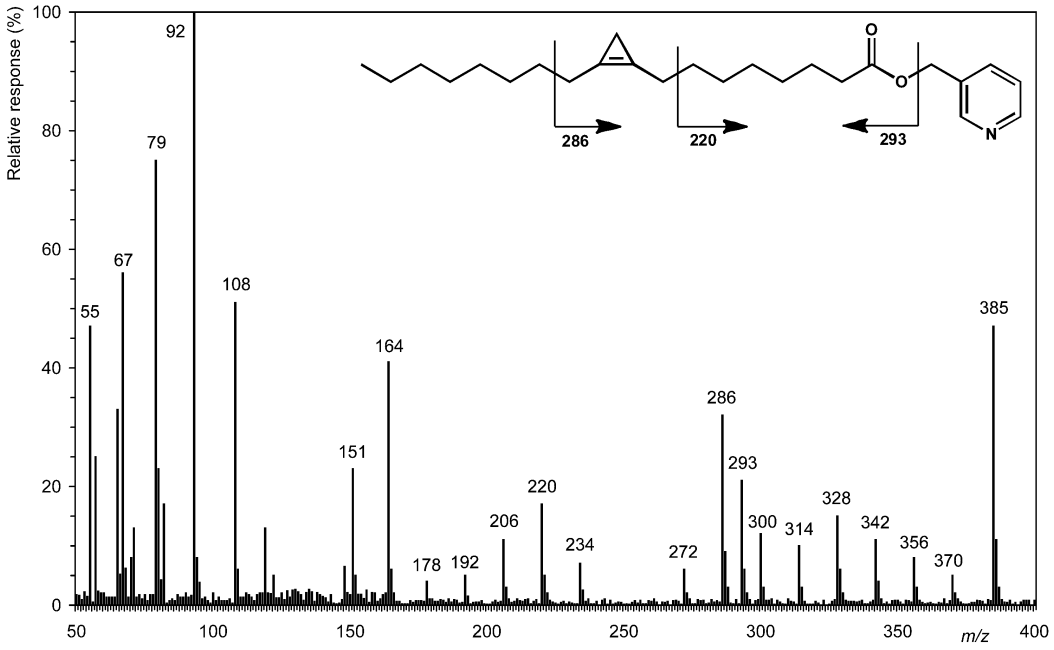

Fig. S2. Mass spectrum of 3-pyridylcarbinol esters of chaulmoogric acid.

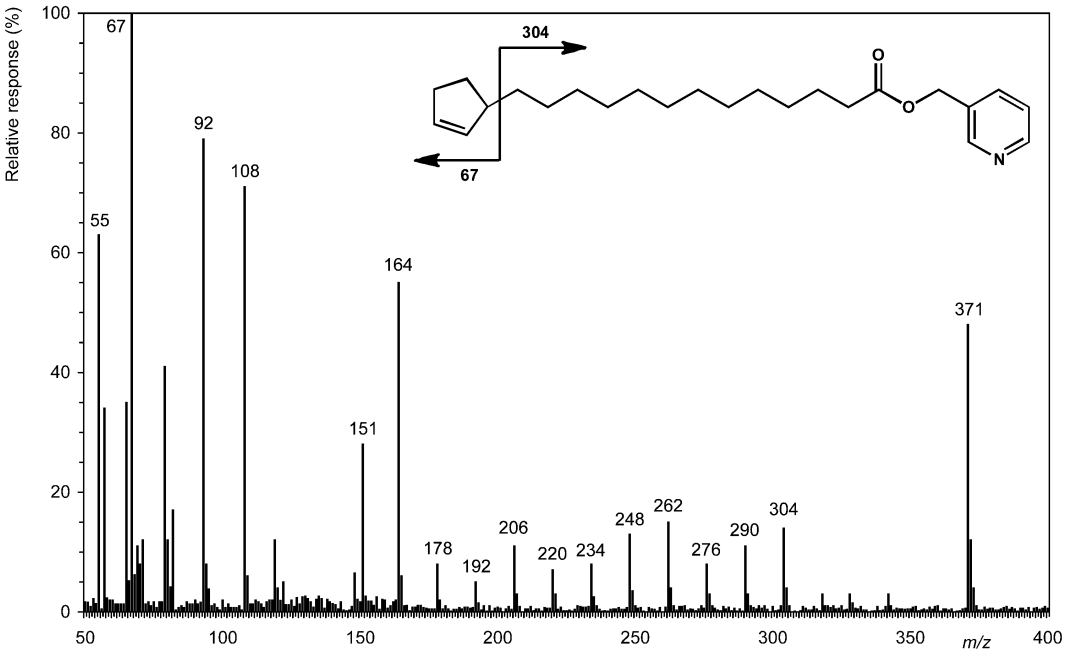

Fig. S3. Organic synthesis of standards.

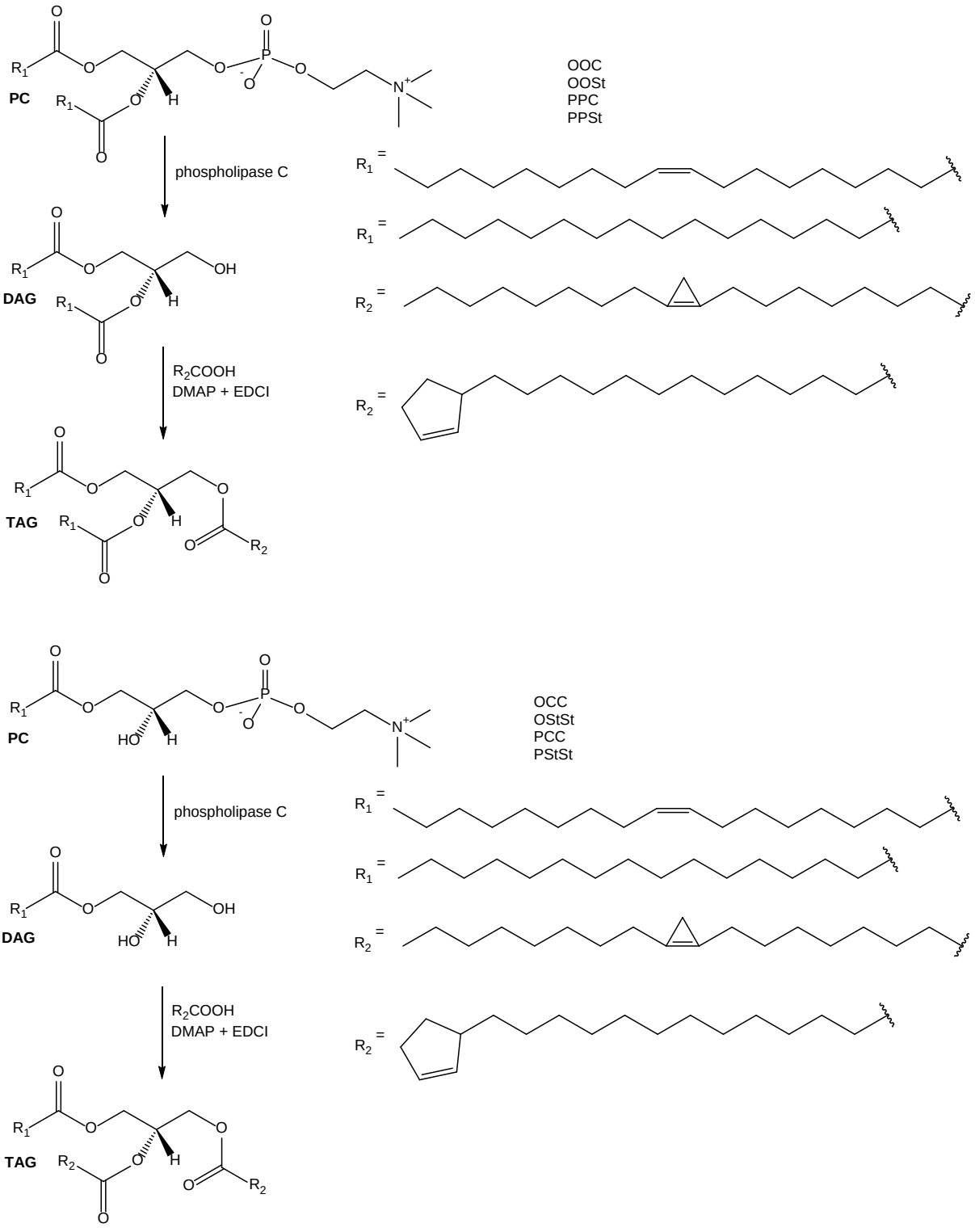

Fig. S4. Models of fatty acids