Supplements

Enantiomeric separation of triacylglycerols containing acetylenic and allenic 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 [11]. 1,2-Dilinoleyl-sn-glycerol was similarly prepared.

A solution of 1,2-dioleoyl-sn-glycerol (10 μmol) and free acid (laballenic, stearolic, and ximenynic acids) (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 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 [16,17] 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.

2.6. TAG analysis using RP-HPLC/MS-ESI

The LC equipment consisted of a 1090 Win system, PV5 ternary pump and automatic injector (HP 1090 series, Agilent, Santa Clara, CA, USA) and two s Hichrom HIRPB - 250A columns with a 250 x 2.1 mm ID and 5 μm particle size connected in series. This setup was used as a high efficiency column, approximately 105,000 plates/m (see manufacturer's literature), with a flow rate of 0.95 mL/min, an injection volume of 10 μL, and column temperature of 25 °C. TAGs were separated using a solvent gradient program with acetonitrile (ACN) containing 5 mM aqueous ammonium acetate, and 2-propanol (iPrOH) containing 5 mM aqueous ammonium acetate as follows: initial ACN/iPrOH (99:1, vol/vol), a linear change to 25:75 ACN/iPrOH (vol/vol) from 5 min to 100 min, and a hold for 10 min. The composition was returned to the initial conditions over 10 min. The performance was measured with triheptadecenoin as an internal standard under the conditions described above.

An LTQ Orbitrap Velos (Thermo Fisher Scientific, Bremen, Germany) high-resolution hybrid mass spectrometer equipped with a heated electrospray interface (HESI) was used. The ESI-MS analysis was performed in the FT positive ion mode. MS spectra were acquired with a target mass resolution of R = 110,000 at *m/z* 800. The electrospray voltage was set to 3.5 kV, and the scan range of the instruments was set to *m/z* 200–1500. Nitrogen was used as a

nebulizer gas and set to 18 arbitrary units (sheath gas) and 7 arbitrary units (auxiliary gas). Helium was used as a collision gas for CID experiments. A CID normalization energy of 35% was used to fragment the precursor ions. The MS/MS product ions were detected in high-resolution FT mode. Further source conditions in the positive mode included a vaporizer temperature of 250 °C, capillary temperature of 230 °C, capillary voltage of 50 V, and tube lens voltage of 170 V.

Abbreviations:

APCI	atmospheric pressure chemical ionization,
DAG	diacylglycerol,
DB	double bond,
DMAP	4-dimethylaminopyridine
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ESI	electrospray ionization,
FA	fatty acid,
FAME	fatty acid methyl esters,
FID	flame ionization detector,
PC	phosphatidylcholine,
PUFA	polyunsaturated fatty acids,
Rf	retention factor,
TAG	triacylglycerol,
TLC	thin
tR	retention time,
VLCFA	very long chain fatty acid

Shortcuts	Acid
C	crepenynic
Cg	chaulmoogric
Lb	laballenic
L	linoleic
Ln	α-linolenic
O	oleic
P	palmitic
Ph	phlomic
S	stearic
St	sterculic
Xi	ximenynic

Shortcuts of triacylglycerols are formed using three letters, i.e. fatty acids, e.g. OOO - trioleoyl-glycerol, OOC - dioleoyl-crepenynoyl-glycerol, LLLb - dilinoleyl-labalenoyl-glycerol, etc.

Table 1S. Fatty acid composition in *Leucas aspera* seeds.

Systematic name	Trivial name	Shorthand designation	Abbreviation	Content
Hexadecanoic	Palmitic	16:0	P	13.1
9-Hexadecenoic	Palmitoleic	16:1	Po	1.6
Octadecanoic	Stearic	18:0	S	3.6
6-Octadecenoic	Petroselinic	6-18:1	Pe	2.9
9-Octadecenoic	Oleic	9-18:1	O	42.5
9,12-Octadecadienoic	Linoleic	9,12-18:2	L	15.3
5,6-Octadecadienoic	Laballenic	5,6-18:2	Lb	14.7
9,12,15-octadecatrienoic	α-Linolenic	9,12,15-18:3	Ln	3.4
7,8-Eicosadienoic	Phlomic	7,8-20:2	Ph	1.7
9-Eicosenoic	Gadoleic	9-20:1	G	1.2

Table 2S. High-performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS) of the TAGs from the *Leucas aspera* oil, retention time (tR, min), acyl carbon number (ACN) number of double bond(s) (DB), effective carbon number (ECN), and relative quantities (%) of molecular species of TAGs.

TAG	tR	ACN	DB	ECN	content (%)
LnLnLn	44.9	54	9	36	0.60
LnLLn	51.0	54	8	38	0.64
LLLn	57.0	54	7	40	0.82
LnLnO	58.3	54	7	40	0.73
LnLnP	59.7	52	6	40	0.62
LLL	63.1	54	6	42	1.61
LLnO	64.3	54	6	42	1.24
LLnP	65.8	52	5	42	0.77
LnLnS	66.6	54	6	42	0.60
LLLb	67.1	54	6	42	1.57
LLO	70.1	54	5	44	3.45
LLP	70.7	52	4	44	1.46
LnOO	70.9	54	5	44	2.37
LLbLb	71.5	54	6	42	1.55
LLnS	72.2	54	4	46	0.64
LnOP	72.4	52	4	44	1.13
LLbO	74.2	54	4	46	3.34
LOO	76.6	54	4	46	8.56
LLbP	77.0	52	4	44	1.44
LbLbLb	77.6	54	6	42	1.50
LLS	77.8	54	4	46	0.82
LOP	78.2	52	3	46	3.05
LbLbO	78.5	54	5	44	3.23
PPL	79.2	50	2	46	1.35
LbLbP	79.8	52	4	44	1.39
LbOO	80.5	54	4	46	8.25
LLbS	82.0	54	4	46	0.82
GLO	82.2	56	4	48	0.82
OOO	83.1	54	3	48	22.05
PeOO	83.8	54	3	48	2.10
LbOP	84.0	52	3	46	2.94
LOS	84.3	52	3	46	1.26
POO	84.6	52	2	48	7.41
LPS	85.9	52	2	48	0.80
OPP	86.3	52	1	50	2.70
LbOS	88.2	54	3	48	1.24
OOPh	88.6	56	4	48	1.48
OOS	90.4	54	2	50	2.48
OPS	92.0	52	1	50	1.17

Table 3S. Fatty acid composition in *Santalum album* seeds.

Systematic name	Trivial name	Shorthand designation	Abbreviation	Content
Hexadecanoic	Palmitic	16:0	P	6.2
9-Hexadecenoic	Palmitoleic	16:1	Po	1.8
Octadecanoic	Stearic	18:0	S	2.6
9-Octadecenoic	Oleic	9-18:1	O	32.4
9,12-Octadecadienoic	Linoleic	9,12-18:2	L	6.7
9,12,15-octadecatrienoic	α-Linolenic	9,12,15-18:3	Ln	5.3
Octadec-9-ynoic	Stearolic	9a-18:1	St	1.9
11t-octadecen-9-ynoic	Ximenynic	9a,11t-18:2	X	43.1

Table 4S. Fatty acid composition in *Crepis foetida* seeds.

Systematic name	Trivial name	Shorthand designation	Abbreviation	Content
Hexadecanoic	Palmitic	16:0	P	8.5
Octadecanoic	Stearic	18:0	S	3.5
9-Octadecenoic	Oleic	9-18:1	O	4.7
9,12-Octadecadienoic	Linoleic	9,12-18:2	L	22.5
9,12,15-octadecatrienoic	α-Linolenic	9,12,15-18:3	Ln	0.2
Eicosanoic	Arachidic	20:0	A	1.6
9-Octadecen-12-ynoic	Crepenynic	9,12a-18:3	C	58.7
9-Eicosenoic	Gadoleic	9-20:1	G	0.3

Table 5S. High-performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS) of the TAGs from the *Santalum album* oil, retention time (tR, min), acyl carbon number (ACN) number of double bond(s) (DB), effective carbon number (ECN), and relative quantities (%) of molecular species of TAGs.

TAG	tR	ACN	DB	ECN	content (%)
XiXiXi	52.4	54	9	36	12.61
OXiXi	64.2	54	7	40	16.50
LLnO	64.5	54	6	42	0.78
OSeSe	66.2	54	5	44	0.76
LLO	70.1	54	5	44	0.92
LnOO	70.9	54	5	44	1.26
OPoPo	71.2	50	3	44	0.59
LnOP	72.4	52	4	44	0.53
OOXi	73.2	54	5	44	21.62
LnPP	73.9	50	3	44	0.67
LOO	76.6	54	4	46	1.54
OOPo	77.0	52	3	46	1.73
LOP	78.2	52	3	46	0.56
OPPo	78.6	50	2	46	0.59
PPL	79.0	52	2	48	0.87
OOSe	79.3	54	4	46	1.79
OOO	83.1	54	3	48	27.94
POO	84.6	52	2	48	4.42
OPP	86.3	52	1	50	0.98
OOS	90.4	54	2	50	1.43
OPS	92.0	52	1	50	0.62
AOO	96.0	56	2	52	1.31

Table 6S. High-performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/ESI-MS) of the TAGs from the *Crepis foetida* oil, retention time (tR, min), acyl carbon number (ACN) number of double bond(s) (DB), effective carbon number (ECN), and relative quantities (%) of molecular species of TAGs.

TAG	tR	ACN	DB	ECN	content (%)
CCC	53.1	54	9	36	31.53
CCL	55.7	54	8	38	23.45
CCO	60.2	54	7	40	2.81
CLL	61.5	54	7	40	15.31
CCP	62.8	52	6	40	5.81
LLL	63.1	54	6	42	4.29
CLO	65.9	54	6	42	3.57
CCG	66.3	56	7	42	0.38
PLC	67.5	52	5	42	4.01
CCS	68.3	54	6	42	1.07
LLP	71.5	52	4	44	2.30
COO	72.6	54	5	44	1.23
SLC	73.8	54	5	44	1.48
COP	74.1	52	4	44	1.33
CCA	74.4	56	6	44	0.51
LLS	77.8	54	4	46	0.92

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

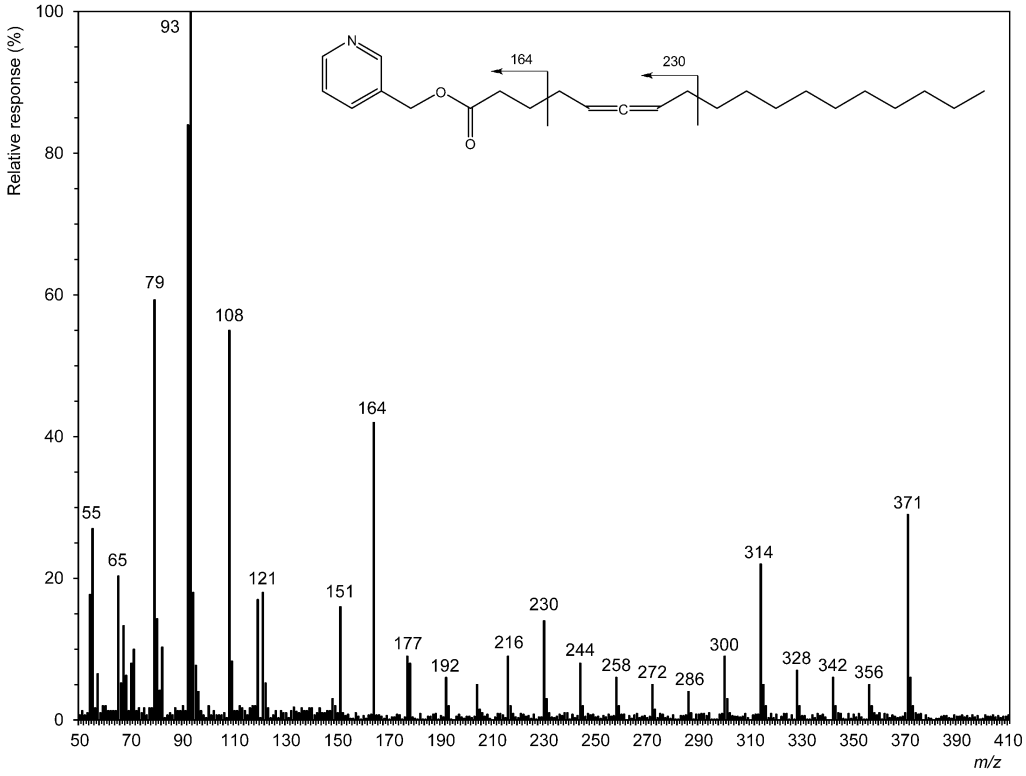

Fig. S2. Mass spectrum of 3-pyridylcarbinol esters of phlomic acid.

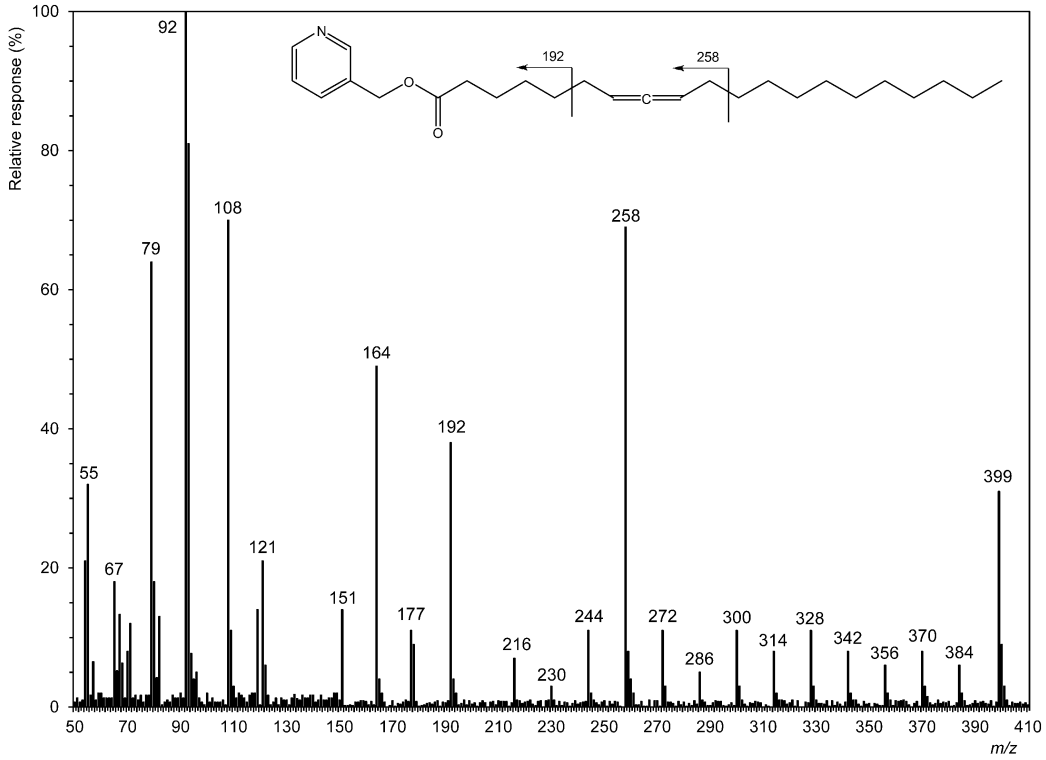

Fig. S3. Mass spectrum of 3-pyridylcarbinol esters of crepenynic acid.

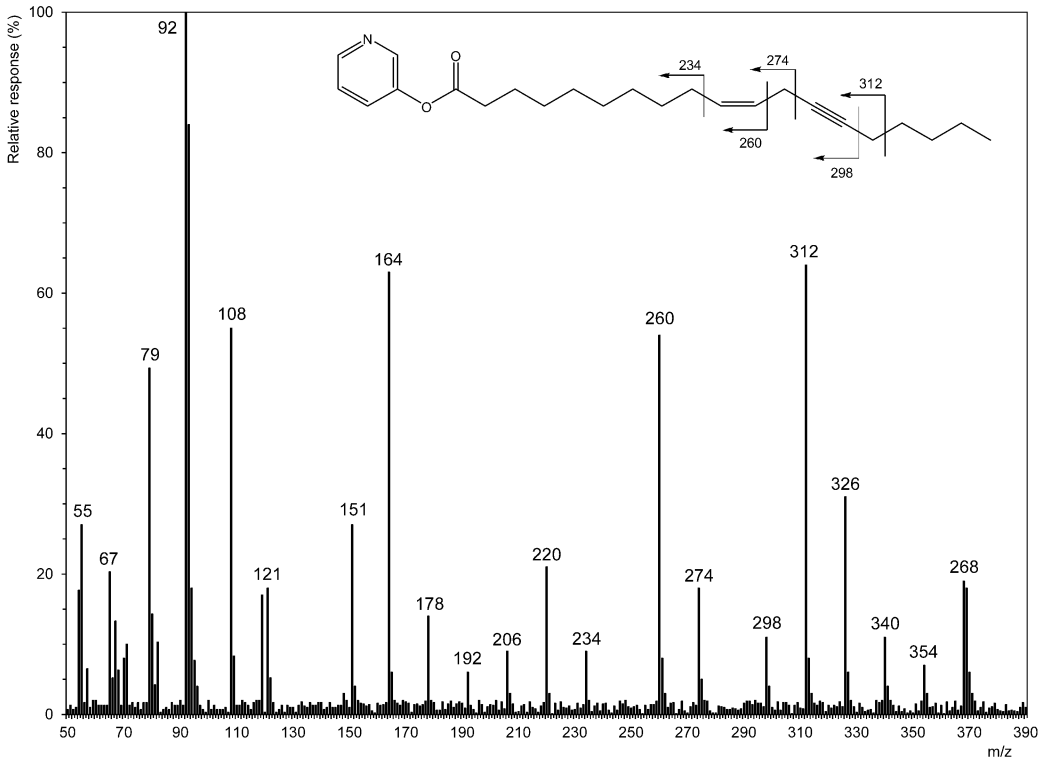

Fig. S4. Mass spectrum of 3-pyridylcarbinol esters of ximenynic (santalbic) acid.