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Isolation and Identification of Molecular Species of Phosphatidylcholine and Lysophosphatidylcholine from Jojoba Seed Meal (*Simmondsia chinensis*)

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A mixture of lysophosphatidylcholine (LPC) and phosphatidylcholine (PC) has been isolated by column chromatography from a jojoba meal (*Simmondsia chinensis*) extract. The molecular species of both classes could be separated and isolated by C18 reversed phase HPLC. The two major compounds were identified by 1D and 2D ^{1}H and ^{13}C NMR, by MS, and by GC-MS as 1-oleoyl-3-lysophosphatidylcholine and 1,2-dioleoyl-3-phosphatidylcholine. Eight other molecular species of LPC and four other molecular species of PC could be assigned by comparison of the mass spectra of the isolated compounds with the spectra of the two major compounds. Complete characterization of the individual molecular species was achieved by GC and GC-MS analysis of the fatty acyl composition from the isolated compounds. The PC/LPC proportion in the phospholipid mixture from three different samples is 1.6 ± 0.1. LPC is considered to be an important bioactive compound; the results of this study suggest further research for the evaluation of potential health benefits of jojoba meal phospholipids.

KEYWORDS: ***Simmondsia chinensis*; jojoba seed meal; phospholipids**

INTRODUCTION

The jojoba plant [*Simmondsia chinensis* (Link) Schneider] is a dioecious evergreen shrub native to the Sonora Desert of the southwestern United States and northern Mexico. The plant is now cultivated in many arid and semiarid places around the world because of its oil-containing seeds (*1*). The "wild" plant is, however, today replaced by high-yielding cultivars (*2*, *3*). The oil commonly referred to as jojoba oil is a liquid wax. The wax consists of esters of mono-unsaturated long-chain alcohols and fatty acids of 18−24 carbons; the esters have a chain length of 38−46 carbons (*4*). Only minor quantities of triacylglycerols are present in jojoba oil (*5*). The oil is mainly used in cosmetics and pharmaceuticals; derivatives of the oil are used in the wax and polish industries (*6*).

Jojoba seed meal, the fraction remaining after winning the oil, contains ∼10−20% of a group of unique compounds, known as simmondsins (*7*, *8*). Among them, simmondsin and simmondsin 2′-ferulate exhibit biological activity as food intake inhibitors (*9*, *10*). Three patents have been registered for the use of jojoba meal and for simmondsin as a supplement in pet food (*11*−*13*). Other interesting characteristics of yet unidentified jojoba components are described, as, for example, the influence of jojoba meal on oviduct development in chickens (*14*).

The present study deals with the isolation and identification of biologically active compounds such as phospholipids and lysophospholipids from jojoba seed meal. Phospholipids serve as structural components of cell membranes and subcellular organelles. Lysophospholipids (LPL) are recognized as important cellular signaling molecules and are involved in important processes such as cell proliferation, cell survival, cell migration, diabetes, angiogenesis, inflammation, and cancer, mediated by LPL-specific G-protein-coupled receptors (*15*, *16*).

MATERIALS AND METHODS

Jojoba Seed Meal and Solvents. Jojoba seed meal samples from three different clones were supplied by R. Holser (New Crops and Process Research, USDA-ARS-NCAUR, Peoria, IL) and defatted by continuous extraction with *n*-hexane. All solvents used were of analytical and HPLC grade (Sigma-Aldrich, Bornem, Belgium). Reference fatty acid methylesters were obtained from Merck (Darmstadt, Germany).

Extraction of Jojoba Seed Meal. Defatted jojoba meal samples (50 g) were extracted by means of extraction tubes with 0.5 L of

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methanol during 60 min at room temperature (22 °C). For the calculation of extraction yields, measured volumes of the methanol extracts obtained after centrifugation of the extraction tubes at 1000*g* were submitted to column chromatography for the isolation of the complete phosphoholipid fraction. After evaporation of the solvent under reduced pressure at 60 °C, the weight of the residue obtained was measured. The residual jojoba meal fraction was extracted four times in the same way for the calculation of extraction yields.

Column Chromatography. Methanol extract (5 g) was dissolved in 100 mL of a methanol/chloroform mixture (50:50, v/v), adsorbed on 25 g of silica gel (0.040−0.063 mm), and separated by chromatography on a 15 cm × 5.5 cm i.d. silica gel (0.040−0.063 mm) column. The column was eluted first with chloroform and then with methanol. Column eluates were monitored by TLC for the presence of jojoba oil, simmondsins, carbohydrates, and phosphorus- and nitrogen-containing compounds. The fractions showing positive reactions on TLC for the presence of nitrogen and phosphorus were collected and, after evaporation of the methanol under reduced pressure, weighed and submitted to HPLC for the separation and isolation of pure compounds.

Thin-Layer Chromatography (TLC). TLC was performed by means of 4 × 8 cm Polygram SIL G/UV 254 plates (Machery-Nagel, Dueren, Germany) with two solvent mixtures. Either a mixture of chloroform and methanol (85:15, v/v), for the analysis of methanol extracts from jojoba meal or column eluates, or a mixture of chloroform, methanol, and water (66:25:4, v/v/v), for the analysis of isolated phospholipids, was used. The spots were vizualized by means of different spray reagents.

Sugars and glycosides were visualized by means of the 1-naphthol spray. The spray was prepared by adding 10.5 mL of a 15% ethanolic solution of 1-naphthol to a mixture of 40.5 mL of ethanol and 6.5 mL of sulfuric acid. After spraying with the reagent, the plates were heated in an oven at 110 °C for 5 min.

Nitrogen-containing compounds were visualized by means of the acidified iodoplatinate spray. The spray was prepared by dissolving 0.25 g of hexachloroplatinic(IV) acid and 5 g of potassium iodide in 100 mL of water; the spray was acidified by adding 1 mL of hydrochloric acid (37%) to the solution.

Phosphorus-containing compounds were visualized by means of the ammonium molybdate spray. The spray was prepared by dissolving 0.5 g of ammonium molybdate in a mixture of 5 mL of water, 1.0 mL of HCl (37%), and 2.5 mL of perchloric acid (70%); acetone was added to a total volume of 50 mL. After spraying, the plates were heated in an oven at 110 °C for 5 min.

Iodine fumes were used as a general reagent. Some iodine crystals were allowed to evaporate in a closed glass development tank.

High-Performance Liquid Chromatography (HPLC). Analysis of the fraction reacting positive to the presence of nitrogen and phosphorus on TLC after column chromatography was done by HPLC with a 12.5 cm × 4.6 mm i.d., 5 μm, RP-18 column (Merck) in the isocratic mode with a mixture of methanol and water as the mobile phase. For the more polar fraction a methanol water mixture 85:15 (v/v) was used, and a 94:6 (v/v) composition was used for the separation of the more lipophilic compounds. The flow rate of the eluent was 1.0 mL/min. The eluates were monitored by an L-4250 UV−vis detector (Merck-Hitachi, Darmstadt, Germany) at 205 nm. Eluates containing a single peak were concentrated and the residues used for mass spectrometry (MS), gas chromagtography (GC), and GC-MS of fatty acid methyl esters (FAME) after transmethylation.

Gas Chromatography−Flame Ionization Detection (GC-FID). A Chrompack 9000 gas chromatograph equipped with a flame ionization detector was used for the analysis of FAME after transesterification of isolated HPLC peaks. Separations were made by a 40 m × 0.2 mm i.d., 0.33 μm, film CP Sil-24 capillary column (Chrompack, Antwerp, Belgium). Samples of 1 μL were injected by means of a split injector 1:100. Injector and detector temperatures were set at 320 °C. Helium was used as carrier gas at 25 cm/s (set at 60 °C). Hexane solutions were injected at 100 °C; the oven temperature was kept at 100 °C for 2 min, programmed to 295 °C at 10 °C/min, and kept at 295 °C for 30 min. Data acquisition and processing were done by means of a Merck-Hitachi 2500 chromatointegrator.

GC-MS. Mass spectra were obtained with a Hewlett-Packard 5890 series II gas chromatograph equipped with a Hewlett-Packard 5971A mass selective detector and with an electron impact ion source (70 eV), a quadrupole mass filter, an electron multiplier detector, and a 30 m × 0.25 mm i.d., 0.25 μm, HP-50 column. The carrier gas was He set at 20 cm/s. Extracts were introduced by splitless injection at 70 °C. The oven temperature was kept at 70 °C for 2 min and programmed to 100 °C at 35 °C/min and from 100 to 280 °C at 10 °C/min. The oven temperature was then maintained at 280 °C for 30 min.

Preparation of FAME. Methyl esters were prepared according to the transmethylation method of Christie (*18*). Samples of ∼1 mg were dissolved in 0.5 mL of anhydrous diethyl ether and 20 μL of methyl acetate. Transesterification proceeded with the addition of 50 μL of 2 N NaOMe in methanol. After 30 min of reaction time at 20 °C, the reaction was stopped by the addition of 2 μL of acetic acid. The solvent was removed under a stream of nitrogen; the residue was dissolved in 1 mL of hexane, sonicated, and centrifuged at 1000*g* for 2 min. The supernatant was used for GC or GC-MS analysis.

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded at 499.3 and 125.6 MHz, respectively, with a Varian Unity 500 spectrometer. Samples were dissolved in $CDCl_3$ or DMSO and measured at 22 °C. Chemical shifts are reported in parts per million (ppm) using the residual HOD signal set at 4.70 ppm as reference for the ^{1}H spectra and external dioxane set at 67.4 ppm for the ^{13}C NMR spectra. The coupling patterns in the ^{1}H NMR spectra were elucidated by homonuclear decoupling techniques and especially by 1D TOCSY spectra (total correlation spectroscopy). Peak assignments were based on 2D correlation experiments such as $^{1}H-^{13}C$ COSY spectra or hetereonuclear multiple quantum coherence spectra (HMQC), inverse detected GHSQC (gradient heteronuclear single quantum coherence), and GHMBC (gradient heteronuclear multiple bond coherence

MS. High- and low-resolution mass spectrometry was performed with a qTof 2 quadrupole/orthogonal acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a mixture of 2-propanol and water (1:1, v/v) at 3 μL/min.

RESULTS AND DISCUSSION

Column Chromatography of Jojoba Seed Meal Methanol Extract. Traces of residual jojoba oil were eluted with 150 mL of chloroform; simmondsin ferulates, simmondsins, and carbohydrates, in that order, were eluted with 300 mL of methanol. Further elution with methanol (1.0 L) allowed us to isolate fractions that showed a positive reaction to nitrogen and phosphorus on TLC; those fractions were collected and concentrated by evaporation of the methanol under reduced pressure and submitted to HPLC. The isolated phospholipid fraction on a weight basis represented 2.5 ± 0.2% ($n = 3$) of the defatted jojoba meal; this fraction contained however, a small quantity of an as yet unidentified compound.

Extraction Yields. The methanol extraction of the meal and isolation of the phospholipid fraction by column chromatography resulted in yields of 79 ± 3% phospholipids after one extraction, 94 ± 4% after two extractions, and 97 ± 4% after three extractions. No measured quantities of phospholipids could be detected in the methanol fraction obtained after the fourth extraction. The quantity of phospholipids obtained after three extractions was used for the calculation of extraction yields.

The method described allows for an easy isolation of the total phospholipid fraction from jojoba seed meal.

HPLC. The isocratic system making use of a mixture of methanol and water (94:6, v/v) for the separation of the more lipophilic components of the extract showed the presence of five baseline separated peaks. The isocratic system with the same solvents with a 85:15 (v/v) composition for the separation of

Table 1. TLC, MS, and GC-MS Results for 1-Oleoyl-3-lysophosphatidylcholine and 1,2-Dioleoyl-3-phosphatidylcholine and for Their Isolated Molecular Species

	TLC			GC	MS	fatty acid composition	
peak	P	N	R_f value	retention time FAME (min)	$[M + H]^+$	shorthand designation	common name
1	+	+	0.24	24.23	538.4	12-(OH)18:1(*n*–9)	ricinoleic acid
2	+	+	0.24	21.67	518.4	18:3(*n*–6)	γ-linolenic acid
3	+	+	0.24	18.90	494.4	16:1(*n*–7)	palmitoleic acid
4	+	+	0.24	21.35	520.4	18:2(*n*–6)	linoleic acid
5	+	+	0.24	19.01	496.4	16:0	palmitic acid
6	+	+	0.24	21.14	522.4	18:1(*n*–9)	oleic acid
7	+	+	0.24	21.09	524.4	18:0	stearic acid
8	+	+	0.24	23.23	550.5	20:1(*n*–9)	gondoic acid
9	+	+	0.24	25.91	578.2	22:1(*n*–9)	erucic acid
10	–	–	0.32	21.14	410.5	18:1(*n*–9)	oleic acid
11	+	+	0.64	21.14/21.67	782.5	18:1/18:3	oleic/γ-linolenic acid
12	+	+	0.64	21.14/21.35	784.5	18:1/18:2	oleic/linoleic acid
13	+	+	0.64	19.01/21.14	760.5	16:0/18:1	palmitic/oleic acid
14	+	+	0.64	21.14	786.5	18:1/18:1	oleic/oleic acid
15	+	+	0.64	21.14/23.23	812.5	18:1/20:1	oleic/gondoic acid

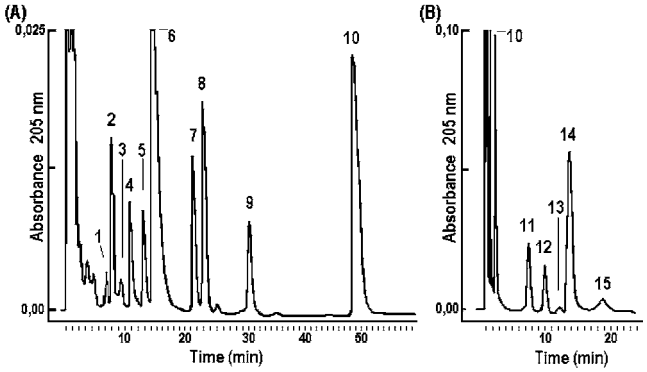

Figure 1. Typical HPLC chromatograms from lysophospholipid (**A**) and phospholipid (**B**) fractions isolated from jojoba seed meal: column, RP-C18, 12.5 cm × 0.4 cm i.d.; detector, UV at 205 nm; mobile phases, methanol, water (85:15, v/v) for (**A**) and methanol/water (94:6, v/v) for (**B**).

the more polar fraction of the extract showed the presence of 10 baseline separated peaks. Typical chromatograms for the separation of both groups are represented in **Figure 1**.

Repeated injections of the isolated fraction and evaporation of the solvents under a stream of nitrogen at 50 °C allowed the isolation of ~10 mg of peaks 6 and 14, sufficient for MS and NMR analysis. The other baseline-separated peaks were collected and, after elimination of the solvents, used for TLC, MS, GC, and GC-MS.

TLC. Analysis of the 15 isolated peaks by TLC with a mixture of chloroform, methanol, and water (66:25:4, v/v/v) as eluent and three spray reagents (N, P, and general reagent) showed the presence of spots with three different R_f values. Nine spots with R_f 0.24 for peaks 1−9, one spot with R_f 0.32 for peak 10, and five spots with R_f 0.64 for peaks 11−15 were seen. Peak 10 reacted negatively to the reagents for P and for N and reacted positively only to the iodine fumes. The other 14 isolated peaks reacted positively both with the molybdate reagent (blue spots) and with the iodoplatinate reagent (purple-brown spots).

The presence of both phosphorus and nitrogen in the fractions mentioned suggests the presence of phospholipids; the two different R_f values indicate the presence of two classes of phopholipids.

The TLC results obtained are represented in **Table 1**.

Identification of Peak 6 as 1-Oleoyl-3-lysophosphatidylcholine. *NMR.* The different NMR assignments for the present product are summarized in **Table 2**.

Table 2. ^{1}H and ^{13}C NMR Assignments for 1-Oleoyl-3-lysophosphatidylcholine (Peak 6) and 1,2-Dioleoyl-3-phosphatidylcholine (Peak 14) Dissolved in $CDCl_3$

	1-oleoyl-3-lyso-phosphatidylcholine		1,2-dioleoyl-3-phosphatidylcholine	
position	^{13}C ppm	^{1}H ppm	^{13}C ppm	^{1}H ppm
18′	14.1 (CH_3)	0.88 (t)	14.0 (CH_3)	0.86 (t)
17′	22.6 (CH_2)	1.28 (m)	22.6 (CH_2)	1.29 (m)
16′	31.9 (CH_2)	1.28 (m)	31.8 (CH_2)	1.27 (m)
12–15′	29.5 (CH_2)	1.28 (m)	29.5 (CH_2)	1.27 (m)
11′	27.2 (CH_2)	2.01 (q)	27.2 (CH_2)	2.02 (q)
10′	129.6 (CH)	5.33 (m)	129.6 (CH)	5.34 (m)
9′	129.9 (CH)	5.33 (m)	129.9 (CH)	5.34 (m)
8′	27.2 (CH_2)	2.01 (q)	27.2 (CH_2)	2.02 (q)
4–7′	29.5 (CH_2)	1.28 (m)	29.5 (CH_2)	1.27 (m)
3′	24.9 (CH_2)	1.57 (m)	24.8 (CH_2)	1.58 (m)
2′	34.1 (CH_2)	2.3 (t)	34.3 (CH_2)	2.29 (m)
1′	173.9 (C)		173.5 (C)	
1	65.2 (CH_2)	4.06 (d)	63.0 (CH_2)	4.11 + 4.39 (dd)
2	68.6 (CH)	3.94 (m)	70.5 (CH)	5.19 (m)
3	67.1 (CH_2)	3.93 (m) + 3.81 (m)	63.4 (CH_2)	3.96 (m)
4	59.4 (CH_2)	4.27 (m)	59.3 (CH_2)	4.26 (m)
5	66.2 (CH_2)	3.72 (m)	66.2 (CH_2)	3.71 (m)
N Me	54.2 (3 Me)	3.24 (s, 9H)	54.3 (3 Me)	3.30 (s, 9H)
1″			173.1 (C)	
2″			34.1 (CH_2)	2.29 (m)

^{1}H and ^{13}C NMR spectra clearly showed proton and carbon signals and a ^{31}P signal (0.13 ppm) specific to a glycerophosphocholine (*19*) and to a monounsaturated fatty acid moiety.

The choline-containing part of the molecule was indicated by the characteristic ^{1}H singlet at 3.24 ppm corresponding to nine protons of three methyl groups correlated with the ^{13}C singlet at 54.2 ppm. The DEPT spectrum of this compound showed the presence of two methyl signals: the signal for the three mentioned choline methyls at 54.2 ppm and another methyl at 14.1 ppm, for the presence of an aliphatic methyl group. 2D experiments such as COSY-45 and especially GHSQC spectra allowed the assignment of the signals at 4.27 and 3.72 ppm to the H4 and the H5 choline methylene protons; the corresponding ^{13}C signals were seen at 59.4 ppm for C4 and at 66.2 ppm for C5. The multiplets at 3.81, 3.93, and 3.94 and the doublet at 4.06 could be assigned to glycerol methylene and glycerol methine protons. The multiplets at 3.81 ppm and at 3.93 ppm were assigned to the glycerol H3 methylene protons correlated with the ^{13}C3 absorption at 67.1 ppm; this was confirmed by the observation of a coupling between the doublet at 4.06 ppm

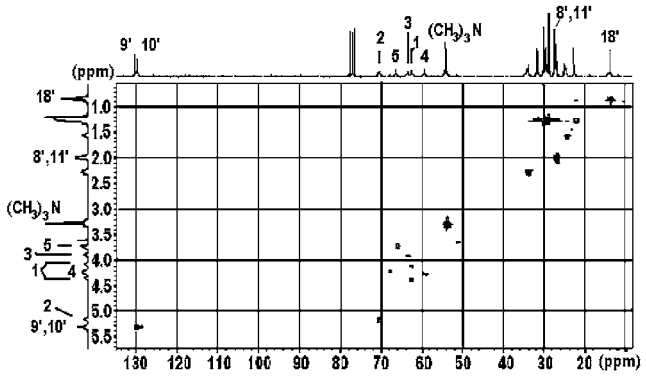

Figure 2. GHSQC spectrum of 1,2-dioleoyl-3-phosphatidylcholine (peak 14).

(J = 5.2 Hz), assigned to the H1 methylene protons corresponding to the ^{13}C1 signal at 65.2 ppm; the multiplet at 3.93 ppm was assigned to the methine H2 proton, which is correlated with the ^{13}C2 absorption at 68.6 ppm. The DEPT spectrum confirmed the presence of this methine carbon at 68.6 ppm. The C2 hydroxyl function was noticed only in the spectrum obtained in DMSO as solvent, at 6.16 ppm. Multibond correlation between the carbonyl at 173.9 ppm and the H1 doublet at 4.06 ppm allowed the assignment of the position of a fatty acid side chain. Correlations of the C1′ carbonyl at 173.9 ppm with the multiplets at 2.30 and 1.57 ppm allowed assignment of the αH2′ and βH3′ protons. A relatively narrow ($\Delta\nu$ = 13) multiplet at 5.33 ppm, integrating for two protons, was correlated with signals at 129.9 and 129.6 ppm and suggests the presence of two cis double-bond protons (H9′ and H10′). Integration of the acyl peaks compared to the nine trimethyl protons indicated the presence of an oleoyl residue

MS. The $[M + Na]^+$ mass obtained for the isolated compound was 544.3368, corresponding with the elemental composition of $C_{26}H_{52}NO_7PNa$ (calculated 544.3397) or 1-oleoyl-3-lysophosphatidylcholine, sodium adduct. The protonated molecular ion $[M + H]^+$ was seen at *m*/*z* 522.3555 (calculated 522.3559). This structure is confirmed by the fragmentation pattern of the molecule. A fragment at *m*/*z* 504.3 corresponded with the loss of one molecule of water from the molecular ion. The important fragment at *m*/*z* 445.3 corresponded with the loss of water together with loss of the $N(CH_3)_3$ group. The fragment ion at *m*/*z* 184.1 in the isolated product was related to the presence of the phosphocholine moiety $(H_3C)_3N^+CH_2CH_2O-PO(OH)_2$; this fragment ion is specific for phosphocholine-containing phospholipids (*17*). The fragment at *m*/*z* 264.2 is related to the oleoyl fatty acyl chain. According to the identity of the fatty acid chain in the phospholipids, this fragment will have a specific value. The characteristics mentioned confirm the structure elucidated by NMR.

GC and GC-MS of FAME. GC of FAME obtained after transmethylation of the mentioned compound showed the presence of a peak with the retention time of oleic acid methyl ester. The identity of this methyl ester was confirmed by GC-MS and is additional proof for the structure of the isolated lysophospholipid.

Identification of Peak 14 as 1,2-Dioleoyl-3-phosphatidylcholine. *NMR.* The NMR assignments of fraction 14 are represented in **Table 2**. As can be seen from this table, there is a high similarity with the spectrum of 1-oleoyl-3-lysophosphatidylcholine; only characteristics indicating the presence of a second fatty acid chain will be discussed. The presence of two oleoyl side chains is proved by the presence of two carbonyl absorptions at 173.1 and 173.5 ppm for C1′ and C1″ respectively, and the integration of the fatty acid proton peaks. The multiplet at 3.94 ppm for the H2 proton in the mono-oleoyl derivative is now replaced by a multiplet at 5.19 ppm, confirming the presence of a fatty acid substituent in this position. The ^{13}C signals at δ 34.3 and at δ 34.1 could be assigned to C2′ and C2″ by correlation with the two carbonyls mentioned. The GHSQC spectrum of 1,2-dioleoyl-3-phosphatidylcholine is represented in **Figure 2**.

The structure of 1,2-dioleoyl-3-phosphatidylcholine is represented in **Figure 3**.

MS. The $[M + Na]^+$ mass obtained for the isolated compound was 808.5842, corresponding with an elemental composition of $C_{44}H_{84}NO_8PNa$ (calculated 808.5832) or 1,2-dioleoyl-3-phosphatidylcholine, sodium adduct.

The protonated molecular ion $[M + H]^+$ corresponds with the ion at *m*/*z* 786.6018 (calculated 786.6012). The fragmentation pattern confirms the mentioned structure. The fragments at *m*/*z* 560.3 and 544.3 correspond to mono-oleoylphosphatidylcholine as potassium and sodium salts, respectively. The *m*/*z* 522.3 ion corresponds with the mono-oleoylphosphatidylcholine fragment. The fragment ion at *m*/*z* 280.1 corresponds to the sodium adduct of the glycerophosphocholine part. The fragment ion at *m*/*z* 184.1 in the isolated product is related to the presence of the phosphocholine moiety. The identity of the specific acyl side chain is confirmed by GC and GC-MS.

GC of FAME obtained after transmethylation of the mentioned compound showed the presence of one peak with the retention time of oleic acid methyl ester. The identity of the peak was confirmed by GC-MS and is additional proof that the molecule species contains only oleic acid as fatty acyl groups.

Identification of Molecular Species of Lysophosphatidylcholine (LPC) and Phosphatidylcholine (PC). MS of the mentioned isolated compounds in relation to the two identified reference components allowed the assignment of the different peaks as molecular species of LPC and PC. In the different mass spectra molecular ion adducts $[M + H]^+$ or $[M + Na]^+$ were seen, corresponding to different molecular species with different acylation patterns. The identity of the fatty acids present in the present molecular species was confirmed by means of GC and GC-MS of FAME after transmethylation of the isolated substances and comparison with the data obtained from reference fatty acid methyl esters.

The relatively most important esterified fatty acid in both classes is oleic acid as in the triglycerides present in minor quantities in the oil (*5*); jojoba wax esters contain only a minor

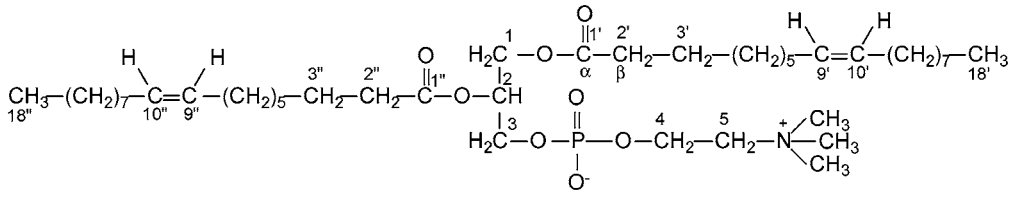

Figure 3. Structure of 1,2-dioleoyl-3-phosphatidylcholine.

amount of esterified oleic acid (*4*). The molecular weights of the isolated molecular species along with the identity of the esterified fatty acids in the different isolated molecular species from LPC and PC are represented in **Table 1**. The mass spectra, however, do not allow us to determine the relative position of the two different acyl groups in the PC molecular species. Positional distribution of fatty acids in phosphocholines is usually determined by enzymatic hydrolysis of the *sn*-1 fatty acid ester with a lipase from *Rhizopus arrhizus delemar* (*20*) and GC-MS analysis of the released fatty acid and the fatty acid of the formed lyso derivative.

The proportion of the sum of the peak areas from the phosphatidyl molecular species to the lysophosphatidyl molecular species for three different jojoba meal samples was 1.6 ± 0.1. The reason for this relatively high concentration of LPC is not yet completely understood. Enzymatic as well as chemical hydrolysis of PC can result, of course, in LPC as an artifact. However, because comparable concentrations are found in samples from three different clones, we assume that LPC is, at least for the most important part, present in jojoba nuts. This relatively high concentration of biologically active LPC in the meal phospholipid fraction suggests further research for an evaluation of potential health effects.

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