Lipid Composition of *Calophyllum inophyllum* Kernel

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Total kernel lipids extracted from *Calophyllum inophyllum, Guttifereae* amounted to 60.1% of the dry kernel. The total lipids consisted of 92.0% of neutral lipids, 6.4% glycolipids and 1.6% phospholipids. Neutral lipids consisted of triacylglycerols (82.3%), free fatty acids (7.4%) and small amounts of diacylglycerols, monoacylglycerols and sterols. At least four glycolipids and five phospholipids were identified. Acylmonogalactosyldiacylglycerol and monogalactosylmonoacylglycerol were major glycolipids; while monogalactosyldiacylglycerol and an acylated sterolglucoside were present in small amounts. The phospholipids consisted of phosphatidylethanolamine and phosphatidylcholine as major phospholipids, and minor amounts of phosphatidic acid, phosphatidylserine and lysophosphatidylcholine. The fatty acid composition of these different neutral lipids, glycolipids and phospholipids was determined.

KEY WORDS: Acylmonogalactosyldiacylglycerol *Calophyllum inophyllum, Guttifereae*, lysophosphatidylcholine, monogalactosyldiacylglycerol, monogalactosylmonoacylglycerol, phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine.

Calophyllum inophyllum, Alexandrian Laurel, Guttiferea is distributed in the coastal regions of South India, Andaman Islands, Burma and Ceylon. The fruit of *Calophyllum inophyllum* contains a hard seed which, on decortication, yields a kernel (45%) containing about 60.1% oil (1). This oil is used in soaps, paints and varnishes. Some of the physicochemical characteristics of *Calophyllum inophyllum* kernel have been reported (2), but little information is available in the literature on its lipid composition. An investigation was therefore carried out to determine the composition of *Calophyllum inophyllum* kernel lipids.

MATERIALS AND METHODS

The seeds of *Calophyllum inophyllum* were purchased locally. Authentic neutral lipids, glycolipids, phospholipids and fatty acid methyl esters were purchased from Sigma Chemical Co. (St. Louis, MO) for use as standards. Solvents used were of analytical grade and were distilled before use.

Lipid extraction. The total lipids from triplicate, 10 g samples of *Calophyllum inophyllum* kernels, were extracted and purified following the established procedure of Folch *et al.* (3). A measured portion of the purified lipid extract was used for gravimetric estimation of total lipids. Free fatty acids, refractive index, iodine value, saponification value and unsaponifiable matter of the lipids were determined by AOCS methods Ca 5a-40, Cc 7-25, Cd 1-25, Cd 3-25 and Ca 6a-40, respectively (4).

Lipid classes and fatty acid analysis. The total lipids were fractioned into neutral lipids (NL), glycolipids (GL) and phospholipids (PL) on a silicic acid column (5) with chloroform, acetone and methanol, successively. NL were estimated gravimetrically, GL and PL were quantitated by total sugar estimation (6) and phosphorus estimation (7,8), respectively. NL were separated by thin-layer chromatography (TLC) with hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as solvent system.

Individual components of NL were identified by comparison with standards and quantified by photodensity (9). GL and PL were separated on TLC with chloroform/methanol/acetic acid/water (65:15:10:4, v/v/v/v) as the solvent system. Individual components of GL and PL were identified by comparison with authentic standards and by specific spray reagents (10,11). Quantitation of different components of GL and PL on preparative TLC was effected by estimation of sugar (6) and phosphorus (7,8), respectively.

Fatty acid methyl esters (FAME) were prepared by acid catalyzed transmethylation (12) of the lipids. The FAME were analyzed on a Shimadzu GC 9A chromatograph (Shimadzu Scientific Instruments, Columbia, MD) equipped with flame ionization detector (FID), stainless steel column (152.4 cm × 3.17 mm) packed with 20% diethyleneglycol succinate on 80–100 mesh Chromosorb W support, at a column temperature of 180°C, the injection port and FID at 210°C, under a nitrogen flow rate of 40 mL/min. The peak area and relative percentage of FAME were obtained with a Shimadzu integrator. The components of each peak was identified on the basis of a calibration curve or retention times vs equivalent chain length, and by comparision with those of authentic methyl ester standards. All determinations were performed in triplicate and mean values were reported.

RESULTS AND DISCUSSION

The kernel of *Calophyllum inophyllum* contained 60.1% total lipids (dry basis). The purified lipids had the following physicochemical characteristics: dark green viscous liquid at ambient temperature (25–30°C) with disagreeable odor and taste; refractive index at 15°C, 1.4701; iodine value (Wijs), 82.2; saponification value, 192 mg KOH/g fat; free fatty acids, 7.4 g oleic/100 g fat; and unsaponifiable matter, 1.4%. Fractionation of the lipids by silicic acid column chromatography showed that the total lipids consisted of 92.0% neutral lipids, 6.4% glycolipids and 1.5% phospholipids (Table 1).

TABLE 1

Major Lipid Classes of *Calophyllum inophyllum* Kernel and Their Fatty Acid Composition[a]

		Fatty acid (%)			
Lipid class	Wt (%)	16:0	18:0	18:1	18:2
Total lipids	60.1	17.5	8.3	47.1	27.1
Neutral lipids	92.0	17.5	8.7	50.1	23.7
Glycolipids	6.4	15.3	3.8	8.9	72.0
Phospholipids	1.6	25.1	7.9	25.7	41.3

[a]All values are means of three replicate analysis.

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The fatty acid composition of total lipids showed that oleic acid (47.1) and linoleic acid (27.1) were the predominant acids, followed by palmitic acid (17.1), while stearic acid was present to the extent of 8.3% (Table 1). The fatty acid profile of NL largely reflected that of TL, while GL and PL fractions had high amounts of linoleic acid as compared to TL and NL.

In regard to the neutral lipid fraction, triacylglycerols and free fatty acids were found to be the major components (Table 2). The amount of *sn*-1,2(2,3)-diacylglycerols was slightly higher than *sn*-1,3-diacylglycerols, and was similar to that observed in peanut oil by Sanders (13). The fatty acid composition of different components of NL (except for sterols, sterol esters and hydrocarbons) is presented in Table 2. Palmitic, oleic and linoleic acids together constituted 89.6–94.0% of the total fatty acids, while stearic acid was present in small quantities (< 10.4%) in all the components of neutral lipid fraction.

TABLE 2

Neutral Lipids of *Calophyllum inophyllum* Kernel and Their Fatty Acid Composition[a]

		Fatty acid (%)			
Neutral lipids	Wt (%)	16:0	18:0	18:1	18:2
Monoacylglcerols	1.8	17.5	9.0	49.9	23.6
sn-1, 3-Diaclyglycerols	2.4	16.3	6.0	46.1	31.6
sn-1, 2 (2, 3)-Diacylglycerols	2.6	20.0	10.4	52.7	17.9
Free fatty acids	7.4	15.1	6.8	48.3	29.8
Triacylglycerols	82.3	20.8	8.0	60.2	11.0
Sterols, sterolesters and hydrocarbons	3.5	—	—	—	—

[a]All values are means of three replicate analysis.

The glycolipid fraction was resolved into acylmonogalactosyldiacylglycerol (AMGDG), monogalactosylmonoacylglycerol (MGMG), acylated sterolglucoside (ASG) and monogalactosyldiacylglycerol (MGDG) by TLC (Table 3). AMGDG and MGMG were the major glycolipids present at 53.3% and 22.3%, respectively, while ASG and MGDG were present in small quantities (< 13.1%). The fatty acid composition of these glycolipids (Table 3) showed that AMGDG and ASG contained a large amount of linoleic acid (86.1%, 85.5%) as compared to MGMG (45.4%) and MGDG (43.1%). Palmitic and oleic acids were present in high amounts in MGDG and MGMG (28.0%, 20.0%, and 21.0%, 26.4%) as compared to AMGDG and ASG. Stearic acid was present to the extent of 7.6–8.2% in ASG, MGDG and MGMG, while in AMGDG it was present in a small quantity (0.1%).

TABLE 3

Glycolipids of *Calophyllum inophyllum* Kernel and Their Fatty Acid Composition[a]

		Fatty acid (%)			
Glycolipids	Wt (%)	16:0	18:0	18:1	18:2
Monogalactosyldiacylglycerol	11.4	28.0	7.9	21.0	43.1
Acylated sterolglucoside	13.1	5.6	7.6	1.3	85.5
Monogalactosylmonoacylglcerol	22.2	20.0	8.2	26.4	45.4
Acylmonogalactosyldiacylglycerol	53.3	12.9	0.1	0.9	86.1

[a]All values are means of three replicate analysis.

The phospholipid fraction was resolved into five components by TLC (Table 4). The major phospholipids were phosphatidylethanolamine (PE) and phosphatidylcholine (PC), present to the extent of 46.3% and 33.8%, while phosphatidic acid (PA), phosphatidylserine (PS) and lysophosphatidylcholine (LPE) were present at small quantities (< 8.1%). The fatty acid composition of individual phospholipids (Table 4) showed that the palmitic was the major fatty acid present to the extent of 60.4% and 50.3% in LPE and PC, while in other phospholipids it was present to the extent of < 25.5%. Oleic acid was present to the extent of 13.5-37.6% in PC, PS, PA and PE, while in LPC it was present in small quantitites (7.9%). Linoleic acid was found to the extent of 22.1–50.1% in all the different phospholipids. Similarly, stearic acid was found to the extent of 6.0–9.6% in all of the five phospholipids.

TABLE 4

Phospholipids of *Calophyllum inophyllum* Kernel and Their Fatty Acid Composition[a]

		Fatty acid (%)			
Phospholipids	Wt (%)	16:0	18:0	18:1	18:2
Lysophosphatidylcholine	5.7	60.4	9.6	7.9	22.1
Phosphatidylserine	6.1	25.5	7.7	23.7	43.1
Phosphatidic acid	8.1	10.0	6.0	33.9	50.1
Phosphatidylcholine	33.8	50.3	9.4	13.5	36.8
Phosphatidylethanolamine	46.3	6.9	7.3	37.6	48.2

[a]All values are means of three replicate analysis.

This preliminary investigation indicated that the glycolipids and phospholipids of *Calophyllum inophyllum* kernel and their fatty acid composition are similar to other seed glycolipids and phospholipids. The composition of lipids of *Calophyllum inophyllum* kernel is reported for the first time in this study.

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