

*POLAR LIPID COMPOSITION OF THE LEAVES AND SEEDS FROM THE TEA PLANT (*CAMELLIA SINENSIS* L)

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Thin-layer chromatography has been used to separate, identify and quantitatively estimate the polar lipids in tea leaves and seeds. Young shoots have been found to contain a higher concentration of phosphatidyl ethanolamine and phosphatidyl choline than older leaves, which have a high concentration of galactolipids. Seeds contain a high concentration of phosphatidyl choline.

INTRODUCTION

The chemical constitution of the tea leaf has been the subject of a number of investigations because the young shoots of the tea plant are used for the manufacture of black tea of commerce. These investigations together with studies on the biochemical changes taking place during the manufacture of tea have resulted in a partial elucidation of the importance of chemical leaf constituents to the properties of made tea.¹ The relationship of the chemical constituents of tea leaves to their susceptibility or resistance to disease and pests is also of increasing interest.

Polar lipids have been recognised to play an important role in regulating many biochemical reactions and membrane phenomena.^{2,3} The extraction, separation and estimation of this group of compounds in tea leaves and seeds, described in this paper, is a further contribution towards understanding the biochemistry of the tea plant and the chemical basis for the properties of black tea. The chemistry of tea seeds has become important with the recent finding that these could be used as starting material for the extraction of tea-seed oil and saponins.

EXPERIMENTAL

Plant material

Plant material was collected from a field near the laboratory and analysed within 3 h. Young shoots were made up of the bud and first two leaves. Coarse leaves consisted of the sixth of leaf downwards from the tip of the shoot. The mature seed kernels were analysed after removal of the hard testa.

Extraction

The extraction was carried out using the method described by Nichols.⁷

Chromatography

The crude lipid extract after partitioning with 0.7% NaCl was streaked on plates coated with silica gel and the thin-layer chromatograms were developed in chloroform: methanol: acetic acid: water (85:15:10:7.5). A number of other solvents examined were found to be less effective. The following reagents described by

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Randerath⁸ were used for the location and preliminary identification of the compounds: (1) iodine vapour; (2) 25% sulphuric acid followed by heating for 10 min; (3) bromothymol blue; (4) molybdate-perchloric acid; (5) modified Dragendorf reagent; (6) diphenylamine reagent.

Quantitative analysis

The lipids were located on the thin-layer plates by exposing to iodine vapour, the dark brown areas were marked out carefully and the iodine allowed to volatilise off at room temperature. The areas on the thin-layer plate with adsorbed lipids were scraped out into tubes for analysis.

Phospholipids

The phospholipids were estimated by a modification of a method described by Randerath.⁹ The sample of silica with the adsorbed lipid was treated with 0.4 ml of 70% perchloric acid saturated with ammonium molybdate and digested at 180°C for 4 h. The tubes were then cooled and treated with 2 ml of water and again heated for 10 min. The cooled solution was finally treated with 0.3 ml each of 2.5% ammonium molybdate and 10% ascorbic acid. The solutions were allowed to stand for 45 min at 38 to 40°C in a bath and made up to a suitable volume, centrifuged and the absorbance measured in a Unicam SP600 spectrophotometer at 820 nm. The standard graph was prepared with inorganic phosphate.

Glycolipids and sulpholipids

Glycolipids and sulpholipids were estimated using a method described by Roughan and Batt.¹⁰ Analar glucose was used as a standard. Suitable blanks were run for all determinations.

RESULTS

The polar lipids of tea leaves were separated into six spots by thin-layer chromatography. The separated compounds were identified to be monogalactosyl diglyceride (positive reaction with diphenylamine), phosphatidyl ethanolamine (positive reaction with ninhydrin), digalactosyl diglyceride (positive reaction with diphenylamine), phosphatidyl glycerol, phosphatidyl choline (orange colour with Dragendorf reagent), sulpholipid and phosphatidyl inositol.

Digalactosyl diglyceride and phosphatidyl glycerol co-chromatographed in the solvent system used but this spot can be separated in chloroform: methanol: 7 N-ammonia (65:24:4). The seeds contain only five spots because sulpholipids are absent in seeds. The identification of the above compounds was based on their relative R_f values (compared to egg yolk lecithin) and by their reaction with specific spray reagents as indicated above.

Polar lipids of leaves and seeds

The phospholipids and glycolipids were analysed by separate methods and the results of analysis are presented in Table 1.

TABLE 1 — *The concentration of polar lipids in parts of the tea plant μ mol lipid/g fresh weight)*

	Compound	Young shoots	Coarse leaf	Seed kernel
1	MGG	2.78 (18)	7.62 (34)	1.39 (16)
2	PE	2.16 (14)	1.72 (8)	1.66 (20)
3	DGG	1.58 (10)	3.45 (16)	0.97 (11)
	PG	1.33 (8)	1.87 (9)	0.26 (3)
4	PC	4.66 (31)	3.34 (15)	3.77 (45)
5	SL	1.38 (9)	2.71 (12)	0
6	PI	1.40 (9)	1.17 (5)	0.41 (5)

Figures in parentheses represent the percent of total polar lipid content in that tissue.

*MGG, Monogalactosyl diglyceride; PE, phosphatidyl ethanolamine; DGG, digalactosyl diglyceride; PG, phosphatidyl glycerol; PC, phosphatidyl choline; SL sulpholipid; PI, phosphatidyl inositol.

CONCLUSIONS

It can be concluded from the results of analysis presented in Table 1 that young shoots contain a higher concentration of phosphatidyl ethanolamine and phosphatidyl choline than coarse leaves. The coarse leaves have a high concentration of monogalactosyl diglyceride and digalactosyl diglyceride. The distribution of lipids in the seeds is different from the leaves in that nearly half the polar lipids of seeds is made up of phosphatidyl choline. The galactolipids are present in low concentration and sulpholipids are absent. The seeds contain about 18 to 22% by weight of non-polar lipids (triglyceride) on a dry weight basis.

The conclusion that galactolipids and sulpholipids are predominantly present in the leaves is in general agreement with earlier reports^{3,6} that these compounds are mainly present in tissues capable of photosynthesis.

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