Glycolipids and Phospholipids of Cured Burley and Flue-Cured Tobacco

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Cured flue-cured tobacco was found to contain about 14% lipid, twice the lipid content of cured burley tobacco. Most of the difference was found to be polar lipids. The major phosphatides of flue-cured tobacco were phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylcholine (PC), and phosphatidylglycerol (PG), with smaller proportions of diphosphatidylglycerol (DPG) and phosphatidylinositol (PI). The major phosphatides of burley tobacco were PI, PE, PA, and PC, with smaller proportions of DPG and PG. The major glycolipids were monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG). The MGDG:DGDG ratio was ca. 1:2 for burley tobacco and 2:1 for flue-cured tobacco. Unidentified glycolipids constituted a third of the lipid carbohydrate in burley tobacco but were negligible in flue-cured tobacco. Stalk position had little effect on lipid composition.

The information available on tobacco lipids is limited to fatty acid analyses of total lipid extracts (Swain and Stedman, 1962; Mold et al., 1966; Tso and Chu, 1970; Chu et al., 1972). The nature of the fatty acids of individual lipid classes is virtually certain to influence the composition of smoke. However, information on the fatty acid composition of the total unseparated lipid extract is likely to be of limited use in predicting fatty acid contributions to smoke chemistry because the chemical properties of the complex lipid probably will influence the reactivity of its component esterified fatty acids during pyrolysis. The nature of the complex lipids of which the fatty acids are components must therefore be known if fatty acid data are to be useful in resolving questions related to smoke chemistry and health.

We have previously reported that flue-cured tobacco contains twice the lipid content of burley but 5 to 10 times as much medium to high polarity lipids (Wassef and Hendrix, 1974a). We report here on the composition of these polar lipid fractions. A preliminary report has been published (Wassef and Hendrix, 1974b).

MATERIALS AND METHODS

Tobacco samples extracted were the following: burley tobacco of unknown cultivar grown in 1971 on the University of Kentucky farms, Lexington, Ky., and stored in bulk on the farm for about 1.5 years; burley (cultivar Burley 21) grown near Lexington in 1972; burley (cultivar Va. 509) grown near Greeneville, Tenn. in 1972; flue-cured (cultivar N.C. 95) grown near Oxford, N.C. in 1972; and flue-cured (cultivars N.C. 2512 and Fla. 105) grown near Tifton, Ga. in 1972.

Procedures for isopropyl alcohol extractions, handling of lipid extracts, removal of nonlipids by Sephadex G-25 column chromatography, and lipid fractionation by silicic acid column chromatography were those used before (Wassef and Hendrix, 1974a). Lipid fractions from the silicic acid columns were separated by three two-dimensional thin-layer chromatographic systems (Rouser et al., 1967) and silicic acid impregnated paper chromatography (Marinetti, 1962, 1964). Specific spray and dip reagents were used according to Kates (1967, 1972). Quantitative analyses of phospholipids were carried out on paper chromatograms (Kates, 1967) and two-dimensional thin-layer chromatograms using the solvent systems chloroform–methanol–28% ammonia (65:25:5) in the first dimension and chloroform–acetone–methanol–glacial acetic acid–water (3:4:1:1:0.5) in the second dimension (Rouser et al., 1969). Glycolipids were separated by TLC by the solvent systems chloroform–methanol–28% ammonia (65:35:5) in the first dimension and chloroform–acetone–methanol–glacial acetic acid–water (5:2:2:1:1:0.5) in the second dimension. Individual glycolipids were made visible by I_2 vapors and circled. After disappearance of I_2, the compounds were aspirated from the plates and subjected to sugar analysis (Dittmer and Wells, 1969).

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RESULTS

Separation of Lipids into Polarity Groups. Polarity group distributions for lipids of the Burley 21 and N. C. 95 samples were published previously (Wassef and Hendrix, 1974a). Distributions for the remaining samples are given in Table I. Generally, burley tobacco contained less total lipid when compared to flue-cured tobacco. An exception was the Va. 509 samples, which approached flue-cured tobacco in total lipid content. All samples of the two types were consistent in that neutral lipids (fraction 1) comprised 75% of burley tobacco lipids and 50% of flue-cured tobacco lipids. Va. 509 was similar to the other burley tobacco samples in phospholipid content (fraction 4), while glycolipid contents (fractions 2 and 3) were intermediate between flue-cured and other burley samples. The high lipid content of the Va. 509 samples, when compared with other burley samples, was due primarily to neutral lipids, which were higher in the Va. 509 samples than in the flue-cured samples.

Polar Lipid Analyses. Phospholipids were identified by relative migration in the two-dimensional TLC systems and on silicic acid impregnated paper, reactions to α-naphthol, periodate–Schiff, ninhydrin, and phosphate sprays, and co-chromatography with standards. Quantitative analyses are given in Table II. Most of the lipid phosphorus was accounted for by identifiable compounds. One unknown compound, which migrated above phosphatidylinositol (PI), accounted for 6 or 7% of the lipid phosphorus in both flue-cured and burley tobacco. Material at or very near the origin accounted for 20% of the lipid phosphorus. These materials were also positive for sugars and free amino groups.

The distribution of phosphorus among lipid classes was remarkably uniform in tobacco samples within tobacco types, but the burley and flue-cured types differed (Table II). The major components of burley phospholipids were phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylethanolamine (PE), and phosphatidylcholine (PC), with lesser amounts of diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG). Flue-cured tobacco lipids had much greater PE and slightly greater PG contents than burley, but the PI content was much lower.

The major glycolipids were monogalactosyldiglyceride (MGDG) (in fraction 2, Table I) and digalactosyl digly-

Table I. Separation of Lipids of Burley and Flue-Cured Tobacco into Polarity Groups by Silicic Acid Column Chromatography

	Burley								Flue-cured			
	1971 stored				Va. 509							
	Lugs		Tips		Lugs		Tips		N.C. 2512 leaf		Fla. 105 leaf	
Fraction and eluting solvent	% of lipid	% of tobacco[a]	% of lipid	% of tobacco	% of lipid	% of tobacco	% of lipid	% of tobacco	% of lipid	% of tobacco	% of lipid	% of tobacco
1. Chloroform	72.8	3.94	79.4	2.90	78.3	8.46	75.2	9.31	53.7	7.46	46.6	6.75
2. Chloroform–acetone (1:1)	3.0	0.16	6.6	0.24	10.2	1.10	13.1	1.62	14.9	2.07	13.4	1.94
3. Acetone	6.6	0.36	4.5	0.16	8.6	0.93	8.3	1.03	10.1	1.40	14.3	2.07
4. Methanol	17.6	0.95	9.5	0.35	2.9	0.31	3.5	0.43	21.3	2.96	25.5	3.69
Total		5.41		3.65		10.80		12.38		13.90		14.49

[a] Oven-dry weight of tobacco basis.

Table II. Phospholipid and Glycolipid Composition of Burley and Flue-Cured Tobacco

	Burley						Flue-cured			
	1971 (stored)		Burley 21		Va. 509		N.C. 95			
Lipid class[a]	Lugs	Tips	Lugs	Tips	Lugs	Tips	Cutters	Tips	N.C. 2512, leaf	Fla. 105, leaf
	% of Phosphatides ± Standard Errors									
DPG	9.0 ± 0.1	7.9 ± 0.2	7.9 ± 0.3	8.1 ± 0.4	7.3 ± 0.3	8.3 ± 0.1	7.4 ± 0.2	6.6 ± 0.4	6.7 ± 0.4	7.7 ± 0.2
PE	14.4 ± 0.3	14.9 ± 0.6	15.0 ± 0.5	14.7 ± 1.2	14.5 ± 0.6	15.1 ± 0.4	21.6 ± 0.4	19.8 ± 0.2	20.4 ± 0.5	21.1 ± 0.3
PG	8.1 ± 0.1	9.4 ± 0.2	7.0 ± 0.5	6.8 ± 0.9	7.0 ± 0.2	6.9 ± 0.1	10.5 ± 0.3	11.0 ± 0.4	11.3 ± 0.4	11.4 ± 0.7
PC	12.3 ± 0.7	12.0 ± 0.5	11.2 ± 0.5	10.6 ± 0.7	10.3 ± 0.5	11.0 ± 0.4	12.5 ± 0.4	12.8 ± 0.4	12.4 ± 0.6	12.0 ± 0.5
Unknown[b]	6.1 ± 0.1	5.8 ± 0.4	6.5 ± 0.3	6.5 ± 0.3	6.7 ± 0.3	6.4 ± 0.1	7.5 ± 0.3	7.0 ± 0.2	6.8 ± 0.4	6.9 ± 0.2
PI	16.6 ± 0.2	16.6 ± 0.2	17.7 ± 0.4	17.0 ± 0.7	19.8 ± 1.0	16.0 ± 0.5	6.8 ± 0.4	7.3 ± 0.3	7.7 ± 0.3	7.7 ± 0.2
PA	13.4 ± 0.2	12.6 ± 0.5	14.2 ± 0.6	14.8 ± 1.1	13.9 ± 0.6	14.2 ± 0.3	14.7 ± 0.3	14.9 ± 0.2	13.5 ± 0.8	14.1 ± 0.4
Origin	20.2 ± 0.4	20.9 ± 0.6	21.0 ± 1.2	21.4 ± 0.2	20.5 ± 0.6	22.1 ± 0.5	19.1 ± 0.9	20.8 ± 0.6	21.3 ± 0.6	19.1 ± 0.4
	% of Glycolipids ± Standard Errors									
MGDG	27.4 ± 1.3	25.1 ± 1.1	22.3 ± 0.9	24.5 ± 1.0	25.1 ± 2.6	23.9 ± 2.3	67.4 ± 1.6	63.8 ± 1.8	62.5 ± 2.8	70.1 ± 1.2
DGDG	40.6 ± 1.5	49.5 ± 2.7	56.4 ± 2.9	58.9 ± 2.5	41.8 ± 3.5	44.2 ± 2.7	30.8 ± 1.3	34.1 ± 1.5	32.4 ± 2.0	30.1 ± 2.4
Unknowns	33.0	26.0	21.3	16.6	33.2	32.7	1.8	2.1	5.1	0

[a] Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; MGDG, monogalactosyl diglyceride; DGDG, digalactosyl diglyceride. [b] Migrates just above PI in the first dimension and with PI in the second dimension.

ceride (DGDG) (in fraction 3), as shown in Table II. The ratio of MGDG to DGDG was ca. 1:2 in burley tobacco and 2:1 in flue-cured tobacco. Unidentified substances comprised up to a third of the glycolipid in burley tobacco but were negligible in flue-cured tobacco.

DISCUSSION

Burley and flue-cured tobacco differ greatly in lipid content. Flue-cured tobacco contains twice the lipid content of burley, and three-fourths of the increased lipid content is accounted for by polar lipids. Glycolipids are three- to fourfold higher, and the phospholipid-containing fraction sixfold higher, in flue-cured than burley tobacco. These differences are consistent with our earlier conclusions (Wassef and Hendrix, 1974a).

The lower polar lipid content of burley tobacco should be borne in mind when interpreting the phospholipid and glycolipid data. While it appears that the PI content of burley tobacco is higher than in flue-cured, actually the PI content of burley is only 40% of that of flue-cured on a percent tobacco basis. The levels of other phosphatides are considerably lower still, i.e., burley tobacco contains 11% as much PE, 14% as much PC, 16% as much PA, and 19% as much DPG as flue-cured tobacco. Similarly, in considering the reversal of the MGDG:DGDG ratio in flue-cured vs. burley tobacco, it must be remembered that burley contains 10% as much MGDG and about half as much DGDG as flue-cured tobacco.

Phosphatidic acid is an enzymatic hydrolysis product of other phosphatides. The high PA content of both burley and flue-cured tobacco suggests that considerable enzymatic hydrolysis has occurred, and the relatively low polar lipid content of burley tobacco suggests that hydrolysis has been more extensive in burley than in flue-cured tobacco. Apparently PI, among phosphatides, and DGDG, among glycolipids, are relatively resistant to autolysis. The nature of the neutral lipids has not been investigated, but the neutral lipid content of burley tobacco apparently does not decline to the extent of the polar lipids.

These differences in lipid content between the two tobacco types may be explained by the curing procedures. Burley tobacco cures slowly at ambient temperatures over a period of several weeks, while flue-cured tobacco is cured over a period of only a few days. The longer curing time at ambient temperatures for burley permits a more extensive enzymatic hydrolysis of the polar lipids and may account for the lower polar lipid content.

There are other factors than curing method which could

be involved, however. Flue-cured samples from two locations and three cultivars were relatively uniform. However, the Va. 509 sample differed from other burley samples, primarily in neutral lipid content. Thus, cultivars, location of production, or local curing conditions may be involved in the differences among burley samples, and location and cultivar differences may be involved in the differences in lipid composition between burley and flue-cured tobacco.

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Protein Composition and Classification of Tobacco

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Protein composition and classification of flue-cured tobacco were studied during maturation and flue curing, and flue-cured leaf was contrasted with that of air-cured Burley and Maryland types. Most of the N in immature leaves was proteinaceous (77% PN:23% NPN) and composition was 23% simple and 77% residual proteins. Upon maturity PN had declined (53% PN:47% NPN) and protein composition for mature leaf was 36% simple:64% residual proteins. After flue curing PN had further declined (38% PN:62% NPN) as simple proteins increased (49% simple:51% residual protein). Simple proteins made up about half the total protein for all three types of cured tobacco, although Maryland had a higher PN ratio (51% PN:49% NPN) than flue cured or Burley (38% PN:62% NPN). More of the simple proteins for all three types of cured tobacco were glutelins. The enzyme proteins (albumin and globulin) increased slightly during flue curing. The data suggest that fraction I protein was located in the residual protein fraction and was rapidly broken down during flue curing.

Few significant studies have appeared in the recent literature on the protein composition of tobacco leaf (Stedman, 1968). Yet the degradation of leaf proteins is an extremely important aim of tobacco processing. It is believed that these compounds are associated with poor smoking quality (Pogel et al., 1957; Johnstone and Plimmer, 1959). Shmuk (1953) demonstrated that tobacco quality is positively correlated with soluble sugars and negatively correlated with proteins. Increased alkalinity of cigarette smoke caused by a preponderance of ammonia and other alkaline products of partial combustion of proteins causes a harsher and more disagreeable smoke and increases irritation of mucous membranes. Abdallah (1970) stated that a decrease in protein content always increased taste quality but some amount of protein is needed to enhance taste sensation.

Studies on green tobacco leaf have shown the presence of a soluble cytoplasmic protein termed "fraction I" and a minor heterogenous protein component termed "fraction II" (Wildman et al., 1949). Recently fraction I protein, once thought to be ribulose diphosphate carboxylase until Anderson et al. (1968) showed it was significantly different in molecular weight in some microorganisms, has been designated by Kawashima and Wildman (1970) as a particular high molecular weight protein found wherever chlorophyll a is present. Fraction I protein is rapidly broken down during curing; however, appreciable amounts of fraction II protein remain after curing (Pogel et al., 1957). Kawashima et al. (1967) reported a marked increase of smaller molecular weight proteins during curing. Extensive studies reveal that many of the proteins possessing much enzymatic activity remain relatively stable during curing (Johnstone and Plimmer, 1959). Rapid proteolysis occurs during curing with liberation of amino acids from hydrolyzed proteins.

Proteins from fresh leaves are usually grouped into the three categories of chloroplastic, cytoplasmic, and nuclear proteins (Miller, 1957). The classical Osborne method of classifying proteins according to solubility has traditionally been used on storage proteins, namely seeds. Commercial tobacco, although a leaf, is bought and sold in a dried state (not fresh leaf). Chemical analysis of commercial tobacco is normally preceded by drying the leaf to a moisture-free state and mill grinding the leaf into a fine dry powder.

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