H.I.V. of the Sterculia oil shows that its cyclopropenoid structure is readily saturated without substantial ring-opening. As noted for epoxides and $\alpha$-hydroxy conjugated dienes, other investigators (40) have also found hydrogenolysis of functional groups when cyclopropenoid acids are reduced under usual conditions involving longer times at atmospheric pressure in the presence of Adams catalyst.

Most samples reached a definite pressure end point in 1-5 min in our application of the Brown procedure, dependent in part on experimental conditions such as rate of stirring. Occasionally, however, the end point was not so well defined and the reaction tapered off toward the end, with 15-20 min required until there is no further dropwise introduction from the buret. Although further experience with this analytical method is needed for complete evaluation of its potential, we are impressed with the breadth and accuracy that can be achieved if suitable care is taken in the determination.

REFERENCES


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Determination of Polar Lipids: Quantitative Column and Thin-Layer Chromatography

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Abstract

The structures of the polar lipid classes of plants and animals are presented, their nomenclature discussed, and suggestions are presented for clarification of nomenclature. The three general types of quantitative chromatographic procedures (column chromatography, thin-layer chromatography, and combinations of column and thin-layer chromatography) available for polar lipids are reviewed and a new quantitative two-dimensional thin-layer chromatographic procedure is presented. Useful quantitative procedures employing columns of cellulose, silicic acid, silicic acid mixed with silicate, magnesium silicate, and ion exchange celluloses are presented. New findings with diethylaminoethyl cellulose columns are described. New quantitative procedures employing silicic acid, magnesium silicate, or diethylaminoethyl cellulose column chromatography with quantitative thin-layer chromatography are described.

The purpose of the present communication is to review the general classes of polar lipids and their nomenclature, and to present some of the chromatographic techniques available for their determination. Since the nomenclature of polar lipids is unsatisfactory in several respects, this subject is considered in detail. A new quantitative two-dimensional thin-layer chromatographic procedure is presented and two new procedures employing column chromatography (silicic acid or magnesium silicate) with quantitative thin-layer chromatography (TLC) are presented.

Lipid Classes and Nomenclature

Glycerol Lipids

There are two general groups of polar lipids: the glycerol lipids and the sphingolipids. Each of these main groups can be subdivided into subgroups: phospholipids and lipids without phosphorus. Figures 1–17 show schematically some of the classes of polar lipids. The glycerol lipids are shown in Figures 1–10. Phosphatidic acid, phosphatidyl glycerol, and diphos-
phatidyl glycerol (cardiolipin) shown in Figures 1–3 form an important series of acidic lipids of increasing complexity by addition of glycerol and/or phosphatidic acid units. Phosphatidic acid, a phosphorylated diglyceride, is the parent substance of the glycerol phospholipids and is believed to be an important intermediate in the biosynthesis of phospholipids, although it generally does not occur to any appreciable extent in animal tissues. Phosphatidyl glycerol occurs to a small extent in some animal tissues and is present in much larger amounts in some plant tissues. Diphostatidyl glycerol was originally isolated from heart muscle and named cardiolipin. It has since been found to occur widely in plants and microorganisms and the name diphosphatidyl glycerol is therefore most appropriate, although the term cardiolipin is still used. Phosphatidyl choline (lecithin), phosphatidyl ethanolamine, and phosphatidyl serine (Figs. 4–6) are well-known and widely occurring glycerol phospholipid classes. The well-established term lecithin has not been completely replaced by the more systematic name phosphatidyl choline.

There are at least two relatively well-characterized phosphoinositides. Phosphatidyl inositol (Fig. 7) is the most abundant. It occurs widely in plant and animal tissues and has the structure shown in Fig. 7. Phosphatidyl inositol diphosphate, the triphosphoinoside of brain (Fig. 8), differs from phosphatidyl inositol in having two additional ester-linked phosphoric acid groups. A diphosphoinositide has occasionally been reported, but does not appear to have been isolated and demonstrated to be a native constituent of a tissue.

Recently galactosyglycerides have been isolated and characterized and shown to be important constituents of plants (1). The simplest of these is a monogalactosyldiglyceride (Fig. 9). A digalactosyldiglyceride containing two galactose molecules linked to a diglyceride unit has also been reported. There are interesting plant lipid counterparts to the cerebrosides characteristic of nervous tissue.

Plants have been shown to contain a sulfolipid (Fig. 10) that has a sulfonic acid group (2) in contrast to the sulfate ester glycolipid (cerebroside sulfate or sulfatide) of brain (Fig. 14). Roux et al. (3) demonstrated the presence of a direct carbon to phosphorus bond in a sphingolipid of the sea anemone (Fig. 16) and thus C-S and C-P bonds as well as the corresponding C-O-S and C-O-P bonds have been established as occurring in nature in major lipid classes. The first reports of the C-P bond occurring in nature were in crude lipid extracts and as a water-soluble free amino acid (4,5).

The nomenclature of polar lipids is far from uniform and has undergone a series of changes that have not been uniformly accepted. Name changes were proposed as older terms became less precise in the light of new knowledge. The situation is well illustrated by the changes in nomenclature that have been proposed for the diacyl glycerylphosphorylethanolamines (Fig. 5). Originally this lipid class was called cephalin, but in time it became clear that diacyl glycerylphosphorylethanolamine was not the only class of polar lipid in the usual cephalin fraction obtained by precipitation with alcohol. Folch (6) identified diacyl glycerylphosphorylserine and inositol phosphatides in the alcoholic-insoluble fraction, then generally referred to as the cephalin, and suggested that the term cephalin be used to designate the alcohol insoluble fraction and not the glycerylphosphorylethanolamine-based lipids. Folch proposed the term phosphatidyl ethanolamine for the pure glycerylphosphorylethanolamine based lipid from the brain and suggested that corresponding names for the serine, choline, and inositol lipids be used. The terms phosphatidyl serine and phosphatidyl ethanolamine are widely accepted in these lipid classes had not been named before, but phosphatidyl choline has not been as generally accepted in place of the well-established name lecithin. We still frequently use the term lecithin because it is short, well established, and well defined.

Though Folch proposed the name phosphatidyl ethanolamine, the nature of the "ethanolamine plasmalogen" was not defined and in fact the Folch preparation of "phosphatidyl ethanolamine" was largely plasmalogen. Subsequently it was proposed that the term phosphatidyl ethanolamine be reserved for the diacyl glycerylphosphorylethanolamines and "phosphatidyl" for the corresponding monoaeyl derivatives with an $\alpha,\beta$-unsaturated ether linked carbon chain replacing the second acyl group. This proposal has not met with universal acceptance. It is difficult to distinguish the terms phosphatidyl and phosphatidal, particularly when spoken. We prefer to use "phosphatidyl" as a generic term for all glycerylphosphorylethanolamines. "Phosphatidyll" then denotes a glycerol phospholipid. In the past we have distinguished the different glycerylphosphorylethanolamine lipids as diacyl phosphatidyl ethanolamine and phosphatidyl ethanolamine plasmalogen (or more simply ethanolamine plasmalogen). Since glyceryl ethers have recently been isolated from hydrolysatés of ethanolamine lipids (7) and since the vinyl ether linkage has become well established for the plasmalogens (8), we now prefer to designate these forms as diacyl phosphatidyl ethanolamine, vinyl-ether phosphatidyl ethanolamine (plasmalogen), and alkoxy phosphatidyl ethanolamine (glyceryl ether form).

The more general term plasmalogen is still conveniently used when referring to all lipid classes together that possess the vinyl ether linkage. Plasmalogen forms of glycerides have been reported.

Evidence is now available that indicates the natural occurrence of still another form of phosphatidyl ethanolamine. We have isolated a lipid class that gives ethanolamine on acid hydrolysis and is chromatographically indistinguishable from the other forms of phosphatidyl ethanolamine on silicic acid columns or by silicic acid impregnated paper and TLC. This new form is separable from the other three forms by column chromatography on diethylaminoethyl (DEAE) cellulose. We have established that the diacyl, vinyl ether, and alkoxy forms of phosphatidyl ethanolamine are eluted together from DEAE with chloroform/methanol mixtures, but it is eluted with chloroform/methanol mixtures, but it is eluted with acidic or basic solvents and in part with methanol. Since this behavior has been observed for some of the oxidation products of phosphatidyl ethanolamine, we believe this type to be a series of naturally occurring autoxidation products. We have encountered this new type of phosphatidyl ethanolamine in beef heart, beef heart mitochondria, and human brain. We will refer to this new form as altered phosphatidyl ethanolamine until the chemical differences have been defined.

The present proposal appears to be the first to include all of the different forms of glycerol lipids in a convenient, simple, and meaningful group of names. Phosphatidyl choline (lecithin) and other glycerol