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REVIEW ARTICLE

Plasmalogens and Related Derivatives: Their Chemistry and Metabolism

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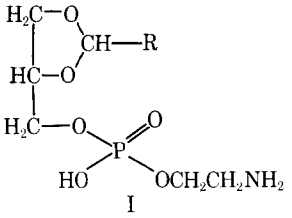
CONTENTS

Plasmalogens are a naturally occurring group of aldehydogenic phosphoglycerolipids widely distributed in plants, animals, and microorganisms but detected in highest concentration in the heart, skeletal muscle, and the myelin of the brain and nerve. Low concentrations of nonphosphatide or "neutral plasmalogens" have also been detected in mammalian tissue as well as in the tissues of different fish. A number of reviews are available on the biochemistry (1–5) and the chemistry and analyses (6–8) of plasmalogens and other ether-linked lipids, and the general reactivity of *O*-alk-1-enyl groupings in organic compounds has just recently been summarized (8).

The presence of plasmalogens in tissues was first noted in 1924 by Feulgen and Rossenbeck (9) in their histochemical studies on the cell nucleus with fuchsin-sulfurous acid. This dye was found to produce a violet stain on both the nuclear and cytoplasmic portions of the cell after mild acid hydrolysis. Because these workers were able to extract the violet color with lipid solvents, they believed that the positive reaction produced in the cytoplasm was due to the presence of lipids rather than sugar aldehydes. Further, when the staining compound was subjected to treatment with acids or mercuric chloride, it liberated a long-chain fatty aldehyde. The cytoplasmic staining was referred to as the plasmal reaction. Feulgen and Voit (10) coined the name "plasmalogen" for the lipoid precursor compound and "plasmal" for the product liberated. The

plasmals appeared to be a mixture of hexadecanal and octadecanal, although other aldehydes might have been present. To demonstrate the presence of plasmals during histochemical studies on tissues, the tissues had to be hydrolyzed in the presence of either HCl or mercuric chloride. Without hydrolysis, Feulgen found that Schiff's test for aldehydes with sulfurous acid and fuchsin dye gave a slow positive reaction with the cytoplasmic lipid-like precursor.

In 1939, Feulgen and Bersin (11) were able to isolate a pure aldehydogenic phospholipid from horse muscle after vigorous alkaline hydrolysis of the ester phosphatides. Apparently, the aliphatic aldehyde formed a cyclic acetal bond with the glycerol skeleton, and the phosphorus and nitrogen base (ethanolamine) was attached by ester bonding to the third hydroxyl group in glycerol. This crystalline ethanolamine plasmalogen (I) was designated "acetal phospholipide." In 1951,

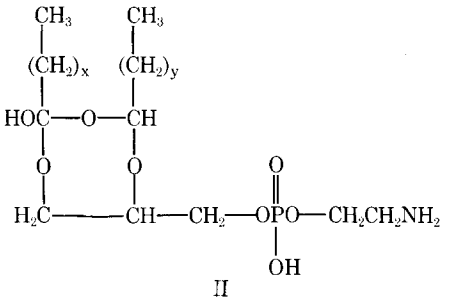


I

R = long-chain alkyl group

Thannhauser *et al.* (12) published a series of papers on the isolation of a crystalline ethanolamine plasmalogen from brain tissue. They used a more prolonged but more gentle alkaline hydrolysis than Feulgen and Bersin (11) had described earlier. The catalytic hydrolysis of their acetal phospholipid was carried out with mercuric chloride with the formation of long-chain fatty aldehydes (hexadecanal and octadecanal) and glycerylphosphorylethanolamine. By periodate titration they demonstrated that the glycerylphosphorylethanolamine derived from the brain acetal phospholipid belonged to the L-α structure.

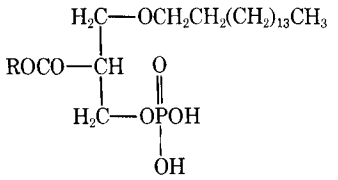
The acetal structure served as a prototype for this class of lipids in earlier years, but its chemical structure was questioned and several alternatives were proposed. Doubt was cast on the correctness of Structure I when it failed to explain the great differences in the reaction rates between synthetically prepared glyceryl acetals and lipid extracts with fuchsin-sulfurous acid. Anchel and Waelsch in 1944 (13) observed that at low temperatures their lipid extracts gave a full color development while the synthetic acetals failed to produce a color; this observation suggested that the aldehyde linkage in the native compound may be more labile than that represented by Structure I. The observations of Schmidt *et al.* (14) also suggested that the native plasmalogens probably contained an alkali-labile residue, because upon hydrolysis of crude brain lipid extracts by the fuchsin-sulfurous acid reagent, no acid-soluble phosphate (glycerylphosphorylethanolamine) was obtained as one would expect from Structure I. However, prior treatment of the crude brain lipid extract with base and then subsequent acid hydrolysis did result in the correct amount of acid-soluble glycerylphosphorylethanolamine. On the basis of these experiments, they assumed that the lipid acetals were derived from other lipid groups during analyses. It was suggested that plasmalogens normally obtained may be artifacts and that rupture of the alkali-labile linkages by which the original compound is bound to other lipid groups occurs during the isolation procedure. Assuming that the native plasmalogens were represented by Structure I, Baer and Stancer (15) proposed Structure II to explain the observations of Schmidt *et al.* (14).



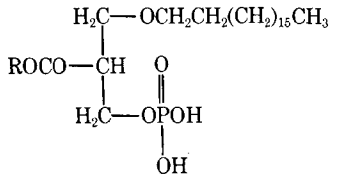
II

The removal of a fatty acid by alkali from Structure II should yield the hemiacetal structure that then could undergo ring closure to form the cyclic acetal (I), and consequently only after pretreatment with alkali would Structure I be expected to give rise to glycerylphosphorylethanolamine when subjected to mild acid hydrolysis.

Further studies have provided strong evidence that the aldehydogenic residue in the native plasmalogens is an alk-1-enyl ether that is not altered by mild alkaline hydrolysis. In 1954, Rapport *et al.* (16) fractionated "acetal" phosphatides from bovine muscle by chromatographic separation on silicic acid. Upon analysis, they showed the presence of two fatty chains per atom of phosphorus, one of which was a fatty acid and the other a fatty aldehyde. Klenk and Debuch (17) found that catalytic reduction of the ethanolamine-plasmalogen led to a complete disappearance of an aldehydic reaction. Subsequent treatment of the reduced compound with base resulted in the isolation of a mixture of *O*-hexadecyl and *O*-octadecyl analogs of phosphatidic acid (III).



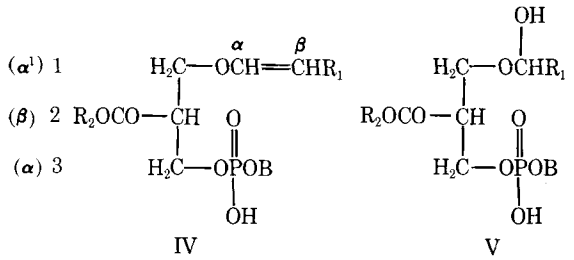
(a) Hexadecyl analog of phosphatidic acid



(b) Octadecyl analog of phosphatidic acid

III

Klenk and Debuch (17) did not investigate the linkage between the aldehyde and glycerol moieties, but they did propose three possible formulas (I, IV, and V) for the plasmalogens. Rapport *et al.* (18) firmly established the *O*-alk-1-enyl linkage in plasmalogens (IV).



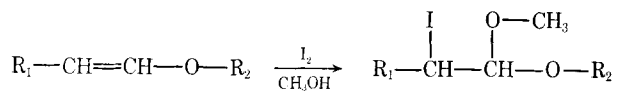
IV V

B = choline, ethanolamine, serine

CHEMISTRY

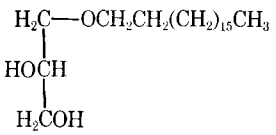
Proof of the Alk-1-enyl Structure—It is now known that the naturally occurring plasmalogens occur as glycerol derivatives of phosphorylcholine, phosphorylethanolamine, or phosphorylserine containing a fatty acid residue and an alk-1-enyl linkage (IV). The terms saturated or unsaturated (19*a*) have been used to refer to the presence or absence of an alk-1-enyl group adjacent to the *O*-ether linkage of the hydrocarbon chain attached to the 1-position of glycerol (1). The α,β-unsaturated glyceryl ethers (enol ethers and vinyl ethers) are alk-1-enyl glyceryl ethers, which are the structural components of plasmalogens; in the earlier literature, the alk-1-enyl types are often referred to as plasmalogens. "Phosphatid*al*" has been proposed to differentiate alk-1-enyl phosphoglycerides from the "phosphatid*yl*" diacyl analogs (19*b*).

The first definitive clue to the α,β-unsaturation of the aldehydogenic linkage in plasmalogens was provided by Rapport *et al.* (18) from their studies on lysophosphatid*al* ethanolamine. Alkaline hydrolysis of the ethanolamine-plasmalogen fraction in bovine muscle was used to form the "lyso" compound. The "lyso" derivative exhibited a loss of aldehyde reaction upon hydrogenation, generated one molecule of a long-chain fatty aldehyde, and contained an alk-1-enyl ether group adjacent to the oxygen. Further evidence confirming the structural analogy between α,β-unsaturated ethers and plasmalogens was obtained by Rapport and Franzl (20) on their lyso compound in 1957 by using a modification reported in 1948 by Siggia and Edsberg (21) for the quantitative determination of alk-1-enyl ethers. This method is based on the addition of iodine to alk-1-enyl ether bonds and it is specific for the α,β-unsaturated ethers in the presence of ordinary olefinic unsaturation, alcohols, aldehydes, acetals, or acetylene. An iodo acetal forms during the reaction between methanolic iodine and alk-1-enyl ethers. Other workers (22, 23) have confirmed the specificity of this iodometric procedure. The iodometric reaction was utilized by Rapport and Lerner (24) in conjunction with the procedure of



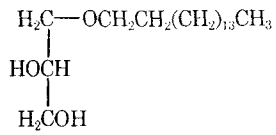
Wittenberg *et al.* (25) based on the formation of a *p*-nitrophenyl hydrazine to measure the plasmalogen content of several normal and neoplastic tissues. Further proof of the alk-1-enyl ether linkage was supplied by Blietz (26), who studied the effect of acid hydrolysis on tissue plasmalogens synthesized in the presence of tritium-labeled water; these experiments demonstrated that the original hemiacetal structure could be formed by the hydrolysis procedure itself (26). Furthermore, tritium-labeled aldehydes were isolated as the semicarbazone (26), which is not in accord with the proposed hemiacetal structure (V) but does support Structure IV.

Position of the Aldehydogenic Residue—The aldehydogenic chain in Structure IV is depicted in the α-position. The other possibility would be for the unsaturated ether chain to be attached to the β-position of the glycerol moiety. Opinions among the early investigators varied considerably with reference to the position of the aldehydogenic chain on the glycerol moiety. Initial studies determining the correct position of the aldehydogenic chain were carried out by Marinetti *et al.* (27, 28) on pig heart lipids. After catalytic reduction and alkali hydrolysis of total pig heart phosphatides, batyl alcohol (VI) (*O*-alkylglycerol) was isolated and indicated that the potential aldehyde group in the pig heart plasmalogens had to be attached to the α-carbon of glycerol. Debuch (29, 30) presented further



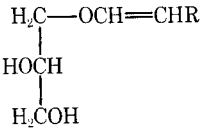
VI

chemical evidence confirming that the aldehydogenic chain is attached to the α-position in the glycerol moiety. This evidence was based on results of hydrogenation studies on brain ethanolamine plasmalogens. The mixture of batyl (VI) and chimyl alcohol (VII), isolated, provided further evidence that the chemical structure of the plasmalogen was a 1-(alk-1-enyl) ether of a 2-acyl-3-glyceryl phosphate (IV).



VII

A number of workers (31, 32) have studied the variations in the aldehydogenic chain by means of gas-liquid chromatography (GLC). A number of branched and unsaturated compounds were isolated in addition to normal C_{12}–C_{18} hydrocarbons. The aldehydogenic chain in Structure IV consists predominantly of 16:0, 18:0, and 18:1 alk-1-enyl chains; the fatty acids are almost completely unsaturated and the alk-1-enyl ether linkage possesses the *cis* configuration (29, 33–36). Norton *et al.* (35), working with pure choline plasmalogen from beef heart, and Warner and Lands (36), working with pig heart lecithin, obtained IR spectra that clearly indicated the configuration of the —OCH=CH— grouping in natural plasmalogens to be wholly *cis*. Enzymatic treatment of phosphorylcholine with phospholipase *C* and hydrolysis of the acyl groups with base resulted in alk-1-enyl glyceryl ether (VIII). There was no absorption in the 930 cm.$^{-1}$ region, which is characteristic of substituted alk-1-enyl ethers possessing a *trans* configuration (37, 38), but the IR spectrum

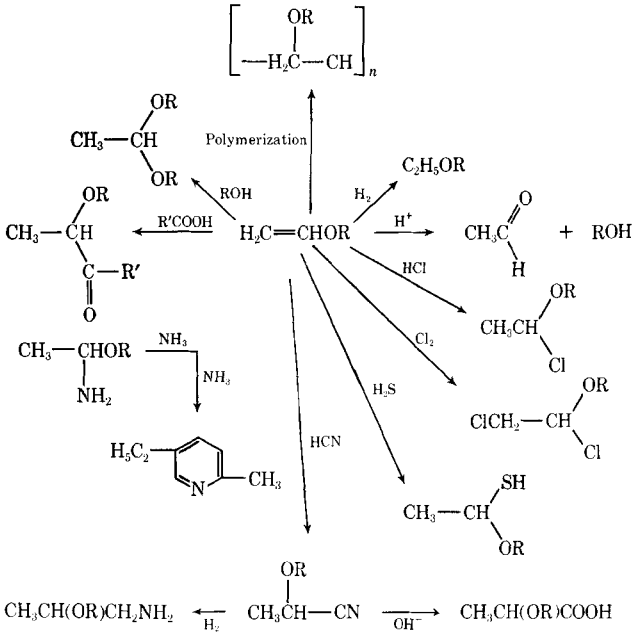


VIII

showed a maximum at 738 cm.$^{-1}$ that is characteristic of the *cis* configuration. Hydrogenation caused complete loss of absorption and resulted in the isolation of an *O*-alkylglycerol. Cymerman-Craig *et al.* (39), by means of optical rotatory dispersion, determined the absolute stereochemical configuration of *cis*-1-(alkenyl-1-enyloxy)-2,3-diacetate obtained from pig heart plasmalogen. The natural plasmalogen had the same configuration as natural chimyl (VII) and batyl alcohols (VI). These data supported the earlier experiments of Thannhauser *et al.* (12) which had established the L-α-structure for plasmalogens by the isolation of L-α-glycerylphosphorylethanolamine from their lyso compound.

Chemical Properties—Little information is available on the chemistry of long-chain α,β-unsaturated ethers, but the —OCH=CH— moiety occurs in other organic compounds on which there are more data available (8). The juxtaposition of the —OCH=CH— moiety relative to the polar glyceryl residue would probably create some chemical differences between the alk-1-enyl linkage as found in plasmalogens and other ethers. Alk-1-enyl ethers are more reactive than other olefinic compounds; they are known to undergo a number of addition reactions as is illustrated in Scheme I. Few of these reactions have been investigated with the native plasmalogens (2). It is known that plasmalogens are stable in an alkaline or neutral medium but very quickly decompose in the presence of trace amounts of acid to form long-chain fatty aldehydes and glycerol derivatives.

The double bond of alk-1-enyl ethers may add halogens, acids of halogens, halides, organic acids (40), alcohols, phenols, and naphthols, and thus gives rise to simple or mixed acetals (41). The addition of alcohols and carboxylic acids is catalyzed by anhydrous acids, such as hydrogen halides, and also by boron fluoride (42, 43). Catalytic amounts of basic compounds cause hydrogen sulfide and mercaptans to add to alk-1-enyl ethers; the addition of water produces a hemiacetal intermediate that forms the aldehyde. An alk-1-enyl transetherification, catalyzed by mercuric sulfate and reported to occur at a temperature range between -70 and $-10°$ (44), illustrates the reactivity of this ether linkage. The addition of iodine to alk-1-enyl ethers produces violent polymerization reactions as do heat and UV light (45). However, methanolic iodine produces a product that has been identified as unsymmetrical methylacetal (21) and which has been used as a sensitive and specific method for estimating plasmalogens and other alk-1-enyl ethers (2, 22). Alk-1-enyl ethers also have a characteristic absorption band in the IR at 8.3 μ (1200 cm.$^{-1}$) which does not appear in native plasmalogens (3, 46). The characteristic absorption band in the infrared of naturally occurring α,β-unsaturated ethers appears at 6.0 μ (1650 cm.$^{-1}$). The plasmalogens with their α,β-unsaturation behave in a manner similar to other alk-1-enyl ethers with reference

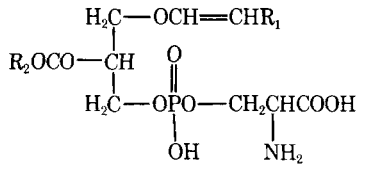


Scheme I
Reactivity of the O*-Alk-1-enyl Linkage in Organic Compounds (also see* Reference 8).

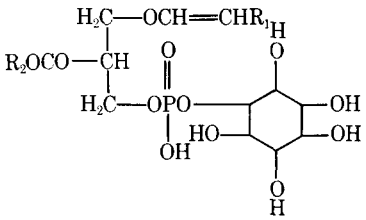
to their alkaline stability, hydrolysis with acids and mercuric ions, reaction with fuchsin-sulfurous acid, addition of hydrogen and methanolic iodine, ozonolysis, and reactions with aldehyde reagents (2, 3, 20).

Related Plasmalogen Structures—Klenk and Böhn (47) and Ansell and Norman (48) have described the isolation and characterization of alk-1-enyl serine phosphatides (IX) in brain tissue. The existence of an alk-1-enyl inositol phosphatide (X) has also been demonstrated (49). Marinetti *et al.* (28) have obtained evidence for the presence of small amounts of diether phosphatides in beef heart, and Marinetti has described reactions involving mild alkaline and acidic hydrolysis (50) of Structures XI, XII, and XIII.

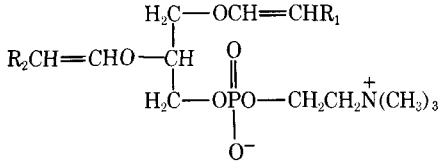
As has been pointed out in this review, the cyclic acetal phospholipid (I) isolated by Feulgen and Bersin (11) and Thannhauser *et al.* (12) from the alkaline hydrolysis of muscle and brain phospholipids is considered an artifact formed by acid hydrolysis of ethanolamine lysoplasmalogen; subsequent studies have proved that the cyclic compounds are mainly *cis*-1-(alk-1-enyl)-2-acylglycerylphosphoryl nitrogenous bases. Further evidence that the cyclic acetal is an artifact of isolation has been presented by Davenport and Dawson (51, 52), who studied the formation of cyclic acetals during the acid hydrolysis of lysoplasmalogens. Evidence was presented to indicate that the mild acid hydrolysis of alk-1-enyl ethers of glycerylphosphorylethanolamine, obtained from alkali treatment of natural plasmalogen, will cause cyclization. The IR spectra were identical with those of a synthetic 2-aminoethyl-2,3-*O*-hexadecylidene-1-glycerophosphate. Mercuric ions prevented this cyclization and liberated the fatty aldehyde. Pietruszko and Gray (53) reported similar results. Piantadosi *et al.* (54) synthesized alk-1-enyl glycerols and studied their conversion to the corresponding cyclic glycerol acetals. The conditions necessary for their



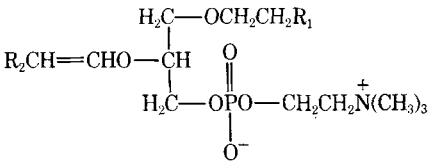
IX



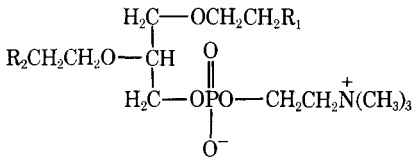
X



XI



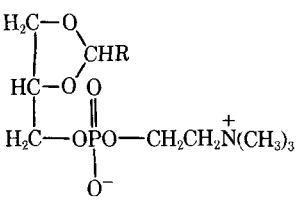
XII



XIII

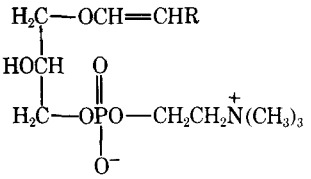
cyclization were studied and no cleavage of the alk-1-enyl linkage was observed. The compounds isolated from the cyclization reaction were identical to the synthetic cyclic glycerol acetals prepared by a different route.

Landowne and Bergmann (55) reported the isolation of a choline plasmalogen (XIV) (1,2-alkylidine glyceryl-3-phosphorylcholine) from the total lipids of the sea anemone, *Anthopleura elegantissima*. Identification of this compound was based on IR data and on the isolation of a long-chain fatty aldehyde on acid hydrolysis. The chemical synthesis of this type of compound (XIV) (R = C_{15}, *i.e.*, 1,2-*O*-hexadecylidene glyceryl-3-phosphorylcholine) has been reported (56). Cyclic acetals of the ethanolamine type (I) have also been established by synthesis (57–59*a*). Recently, Frosolono *et al.* (59*b*) reported a convenient method for the preparation of



XIV

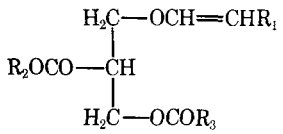
crystalline lysophosphatid*al* choline (XV) in good yields from beef heart lecithin. The plasmalogen was physically



XV

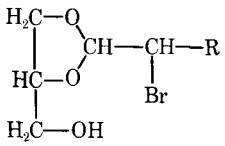
characterized and its chemical properties were compared with other α,β-unsaturated ethers of glycerol. Their (59*b*) evidence indicated that the choline plasmalogens (XIV) isolated from sea anemones were in reality Structure XV, and not a cyclic acetal of glycerol as originally proposed (55). All the evidence available indicates that the cyclic acetal structures probably do not make any significant contribution to the alk-1-enyl glyceryl ethers.

Neutral plasmalogens of nonphosphatide aldehydogenic lipids (XVI) have also been reported (60–62) and have been characterized as alk-1-enyl diacyl glycerols; this subject has been reviewed (5). Bergelson (63) has also presented an extensive review of neutral diol lipids and related compounds.



XVI

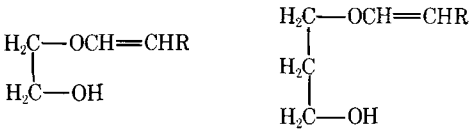
Chemical Synthesis—The synthesis of a *cis*-1-(alk-1-enyl) 2-acyl-3-glycerylphosphate constitutes a chemical problem beset with considerable practical difficulties. In 1961, Piantadosi and Hirsch (64, 65) began the first studies toward the chemical synthesis of plasmalogen. The initial step involved the synthesis of α-bromo cyclic acetal of glycerol, *i.e.*, 2-(1-bromodecyl)-4-hydroxymethyl-1,3-dioxolane (XVII) and its subsequent reaction with sodium (R = C_6–C_{11}). The method used in



XVII

preparing XVII was a transacetalation reaction (66) between glycerol and various 2-bromo-1,1-dimethoxyalkanes derived from long-chain aldehydes. The first step in the transacetalation reaction is acid-catalyzed and pictured as proceeding through a carbonium ion mechanism leading to the formation of the mixed acetal. The second step is characterized by a slow, temperature-dependent inductometric effect that leads to ring closure (67). The dehalogenation of XVII was accomplished with sodium in ether (68, 69) and produced alk-1-enyl ethers with *trans* configuration. The alk-1-enyl ethers were hydrogenated to yield the corresponding saturated ethers. Diol plasmalogens (XVIII and XIX) were prepared in an analogous manner; acylation furnished the neutral-

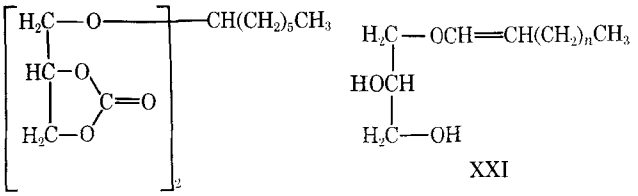
type plasmalogens (64). This synthesis resulted in isomeric products that were about 92% pure, but it was often necessary to fractionate very carefully.



XVIII XIX

Using a modification of the same reactions, Cymerman-Craig *et al.* (70) reported that the debromination of XVII by lithium in dimethoxyethane formed a mixture of four isomers. They later reported a synthesis that yielded solely the 1-(alk-1-enyl) glycerol (71, 72). To avoid the formation of the 2-alk-1-enyl glycerol, 2-benzyloxyglycerol was used in the transacetalation reaction with 2-bromo-1,1-dimethoxyhexadecane. Finally, catalytic debenzylation and debromination afforded a mixture of (±) *cis* and *trans* 3-(*n*-hexadec-1-enyloxy)-1,2-propanediols, whose diacetates could be separated by preparative GLC.

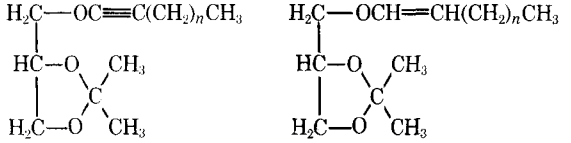
Cunningham and Gigg (73) used heptanal di(glycerol-1,2-carbonate) acetal (XX) as starting material for the synthesis of *cis* and *trans* isomers of ±1-(hept-1-enyl) glycerol (XXI), which was obtained by thermal decomposition of XX followed by alkali hydrolysis (n = 4). The acetal (XX) when treated with acetyl chloride



XX XXI

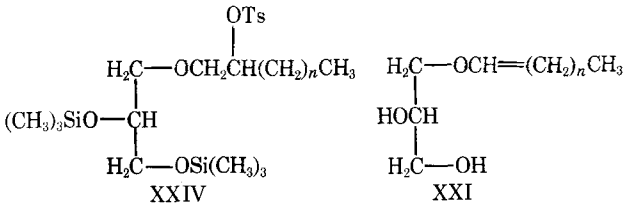
was readily converted into a chloro ether, *i.e.*, 1-chloroheptyl-glycerol-2,3-carbonate, which underwent dehalogenation by the action of triethylamine to yield 1-(hept-1-enyl) glycerol-2,3-carbonate; alkaline hydrolysis formed XXI. The base-catalyzed rearrangement of allyl ethers (74, 75) used earlier by Cunningham *et al.* (76) to prepare 1-(prop-1-enyl) glycerol did not occur with allyl ethers containing a long hydrocarbon chain.

Berezovskaya *et al.* (77, 78) proposed an interesting route leading to the formation of alk-1-enyl ethers of glycerol by the corresponding acetylenic intermediates. These workers reported the synthesis of 1-(alk-1-enyl)-2,3-isopropylideneglycerol (XXII) (n = 3, 11) by reaction of the sodium derivative of 1,2-isopropylideneglycerol with bromo alk-1-ynes. Partial reduction of XXII in the presence of Lindar catalyst yielded the *cis*-1-(alk-1-enyl)-2,3-isopropylideneglycerol (XXIII); however, doubts have been expressed (79) on the validity of this reaction sequence. The results of studies



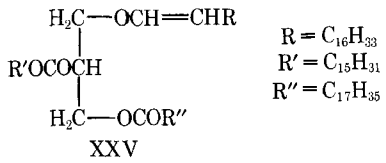
XXII XXIII

using the same method (77, 78) led Chacko *et al.* (79) to suggest that the final product in condensation to form XXII was predominantly an allenic ether rather than the expected acetylenic ether. Oswald *et al.* (80), using the same conditions (77), were unable to obtain the reported alk-1-enyl glyceryl ether. Russian workers (81–83) have further described the synthesis of various derivatives of racemic 1-(alk-1-enyl) glycerols by the elimination of a tosyloxy group from XXIV by potassium *tert*-butoxide (where n = 11, 13). Compound XXIV was prepared by reacting 1,2-epoxyhexadecane with



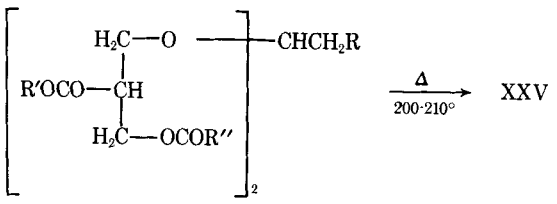
XXIV XXI

isopropylidene to form 1-(2-hydroxyalkyl)-isopropylidene(82). This alcohol was treated with *p*-toluenesulfonyl chloride to give the tosylate. The original ketal group in the isopropylidene was hydrolyzed and subsequently replaced with trimethylsilyl groups. Finally, dehydrotosylation with potassium *tert*-butoxide produced a mixture of *cis* and *trans* isomers of 1-(alk-1-enyl) glycerol (XXI) which was acylated with various long-chain acid chlorides to give a "neutral plasmalogen," 1-(alk-1-enyl) 2,3-diacylglycerol (XXV). The authors did not indicate the presence of any alk-2-enyl ethers, as one might expect. However, under the reaction conditions, the alk-2-enyl ether would decompose, whereas the alk-1-enyl ether would not (73, 85) be affected.



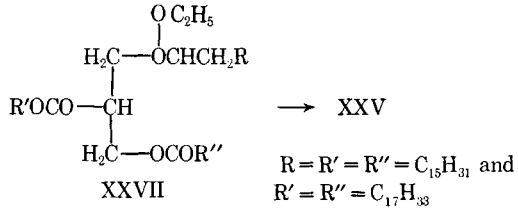
XXV

A similar procedure (82) was investigated for the synthesis of octadecyl alk-1-enyl glyceryl ethers, but results indicated that the desired compound was present in small amounts only (80). A slightly less polar impurity, the major product of the reaction, was difficult to eliminate. Analysis of the fractions obtained after resolution of the total aldehydogenic material on silicic acid columns revealed that 15% of the alk-1-enyl glyceryl ether was present. Other modifications of the dehydrotosylation route have been used to improve the preparation of XXV (85). The application of the transacetalation reaction (66, 67) has been explored and proposed as a possible route to neutral plasmalogens (86–88). The method proposed is based on pyrolytic cleavage of di(α,β-diglyceryl) acetals of long-chain fatty aldehydes, *i.e.*, reactions of diethylacetal of stearaldehyde with the appropriate α,β-diglycerides to form di(α,β-diglyceryl) acetal of stearaldehyde (XXVI) (88). Since an acid is used in the reaction, the possibility of migration of acyl groups in the starting α,β-diglycerates exists, as well as formation of other isomeric products.



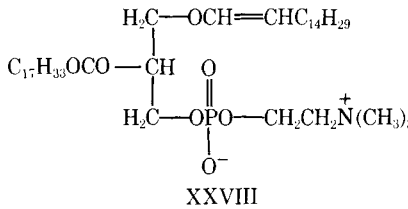
XXVI

A similar method describes the pyrolysis of di-(glycerol-1,2-carbonate) acetal of heptaldehyde (73) as a route to 1-(alk-1-enyl) ethers of glycerol, but this reaction produces a mixture of *cis* and *trans* isomers. The elimination of alcohol from 1-(1-ethoxyalkyl)-2,3-diacylglycerol (XXVII) to form plasmalogens and XXV (87) has also been proposed by other workers (84).

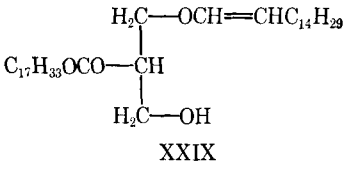


XXVII

Recently, Slotboom *et al.* (89) have described partial chemical synthesis of racemic *trans*-1-(hexadec-1-enyloxy)-2-oleoyl glycerol-3-phosphorylcholine (XXVIII). The synthesis of XXVIII required 1-(1-ethoxyhexadecyl)-2,3-dipalmitoyl glycerol (XXVII) as the intermediate; this was prepared by a modification of the method of Zvonkova *et al.* (87), using ethyl-1-hexadecyl ether and racemic 1,2-dipalmitoyl glycerol. Ethanol was eliminated from XXVII by heating it to 180° in the presence of sulfanilic acids *in vacuo;* this produced Compound XXV, *i.e.*, racemic *trans*-1-(hexadec-1-enyl)-2,3-dipalmitoyl glycerol. This compound was subjected to hydrolysis under alkaline conditions (90) and then reacylated with oleoyl chloride to yield racemic *trans*-1-(hexadec-1-enyl)-2,3-dioleoyl glycerol (XXV) (where R = $C_{15}H_{31}$ and R′ = R″ = $C_{17}H_{33}$). Conversion of Structure XXVII to the monoacyl analog (XXIX) was accomplished enzymatically with pancreatic lipase,



XXVIII



XXIX

which shows specificity for hydrolyzing fatty acyl bonds attached to the primary hydroxyl groups of glycerol. The method of Hirt and Berchtold (91) was used for the synthesis of Structure XXVIII from XXIX.

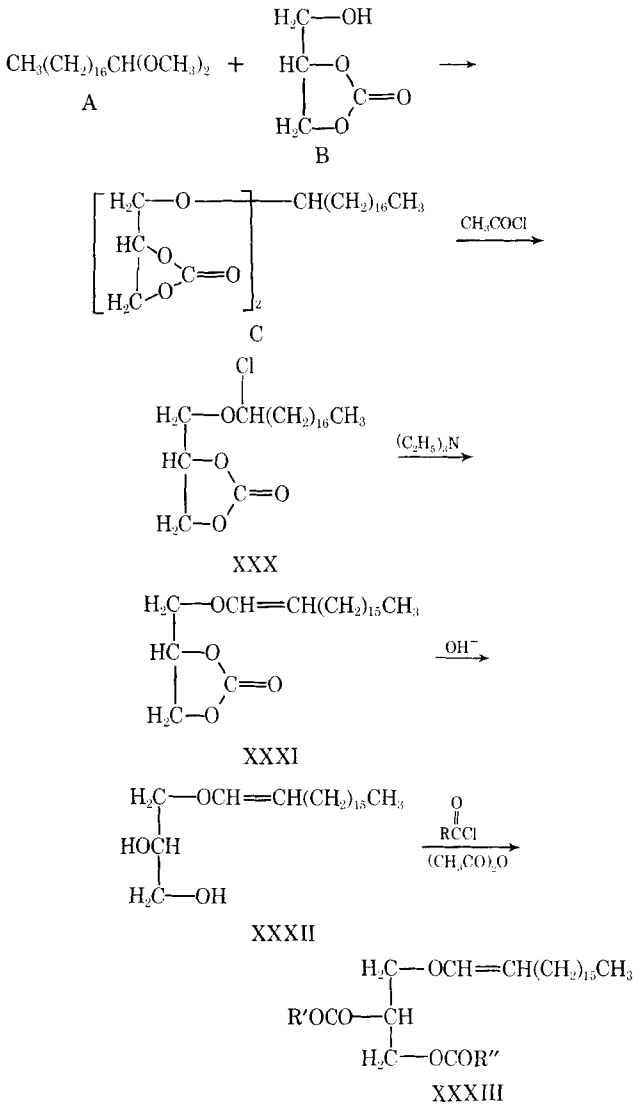
Recently, Gigg and Gigg (92), in an extension of their earlier work on plasmalogens (73), described a chloroether route for the synthesis of racemic and optically active alk-1-enyl glyceryl ethers (neutral plasmalogens) in which the *cis* isomer was the major product. Their use of the pyrolysis method (73) had given a preponderance of the *trans* isomer. Finally, the *cis* and *trans* isomers in the form of their diacetates and dipalmitates were separated by thin-layer chromatography (TLC) on silica gel layers containing silver nitrate. Other workers have also used the dehydrohalogenation reaction to prepare alk-1-enyl ethers of glycerol (93–96).

As shown in Scheme II, Gigg and Gigg (92) used a transacetalation reaction between octadecanal dimethyl acetal [A] and D-(glycerol-1,2-carbonate) [B]. The lengthy sequence required for the synthesis of [B] by degradation (97) has recently been simplified by using a stereoselective route (98). The initial reaction between [A] and [B] produced octadecanal di-D-(glycerol-1,2-carbonate) acetal [C], which was then treated with acetyl chloride to give the chloroether, 3-(1-chlorooctadecyl)-D-glycerol-1,2-carbonate (XXX). The dehydrohalogenation reaction with triethylamine produced a mixture of *cis-trans* isomers of 3-(octadec-1-enyl)-D-glycerol-1,2-carbonate (XXXI); the *cis* isomer was the major product. Alkaline hydrolysis of Structure XXXI gave a mixture of *cis-trans* isomers of 1-(octadec-1-enyl)-L-glycerol (XXXII) that was converted to the diacyl analog by means of palmitoyl chloride and acetic anhydride. The earlier pyrolysis method for preparing the alk-1-enyl ethers (84) produced mainly the *trans* isomer, and the preparation of Slotboom *et al.* (89) contained only the *trans* isomer. At the present time, the most promising route for the synthesis of possible phosphorylated and other derivatives of the naturally occurring *cis* alk-1-enyl glyceryl ethers seems to be the method described by Gigg and Gigg (92). The reaction sequences are illustrated in Scheme II.

METABOLISM

Occurrence—The occurrence of ether bonds in biological compounds is limited in comparison to that of the acetal linkage of sugars, amides of proteins, and acyl groupings of lipids. In nature, ether linkages are found in guaiacol, vanillin, methyl phenyl ethers, enolpyruvylskikimate-5-*P*, muramic acid, thyroxine, diphenyl ethers, and lipids. Lipids are known to contain alkyl and alk-1-enyl hydrocarbon moieties in ether linkage with glycerol or glycol. In general, the more prevalent ether-linked lipids are the alk-1-enyl phospholipids (plasmalogens), but phosphorus-free lipids that contain *O*-alk-1-enyl bonds have also been reported. The ether-linked lipids are found in most living cells, although most commonly in those of animal origin.

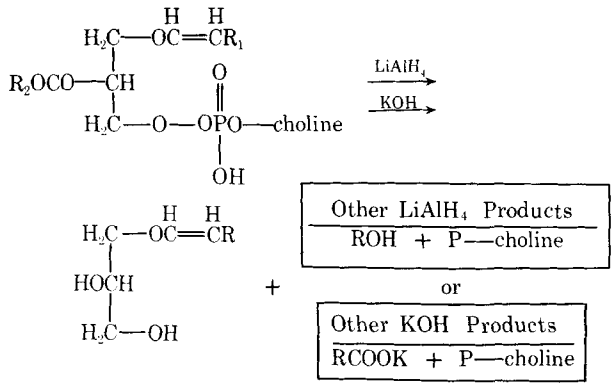
In mammals, the highest concentrations of *O*-alk-1-enyl lipids are found in brain and heart tissues, and plasmalogens appear to be an important constituent of biomembranes. The reader is referred to earlier reviews (1, 2, 5) on the occurrence of plasmalogens, but for convenience a summary of known biological sources of ether-linked lipids is provided in Table I. The table is not meant to be all inclusive but should serve to demonstrate the widespread distribution of lipids with



Scheme II

ether bonds in living cells. Although the close structural and metabolic relationships of the alkyl and alk-1-enyl ether bonds of lipids cannot be ignored (5), the discussion of their biochemistry in this paper will emphasize only the glycerolipids that contain *O*-alk-1-enyl bonds.

Methods of Detection—Methodology currently available for analyses of alk-1-enyl and alkyl ether-linked lipids has recently been reviewed (6, 7, 99). In general, plasmalogens are easily analyzed if the acyl, phosphorus, or phosphorylbase moieties are removed. Alkaline hydrolysis (100–102), enzymatic (103), or $LiAlH_4$ reduction (104–106) of these groups has been successfully used for this purpose. The *O*-alk-1-enyl glycerols formed can be measured quantitatively by photodensitometry or colorimetric reactions. During thin-layer chromatography, the *O*-alk-1-enyl glycerols migrate ahead of the *O*-alkylglycerols on silica gel G layers in solvent systems such as diethyl ether–water (100:0.5, v/v) or chloroform–methanol (98:2, v/v). This R_f behavior is probably explained by differences in the configuration of the alkyl and alk-1-enyl linkages, since the latter has a *cis* bond in the α,β-position (106).

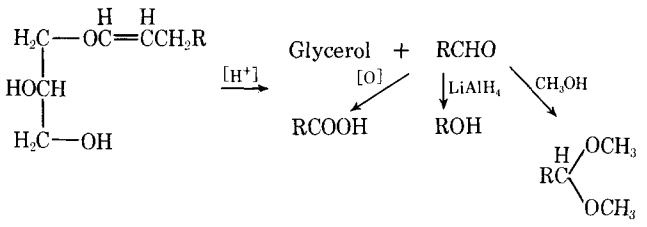


Renkonen (107) has successfully isolated intact plasmalogens from the corresponding diacyl-, alkyl-, or acyl-ethanolamine lipids by TLC after masking the polar base groups as their methylated dinitrophenyl derivatives. However, the resolution of these intact derivatives requires multiple solvent developments. The advantage of this technique is that it permits labeling of all functional groupings in the intact subclasses of *P*-ethanolamine lipids and *P*-choline lipids to be followed in tracer experiments.

The nature of the *O*-alk-1-enyl side chains is best determined by GLC after releasing the long-chain fatty aldehydes by acid treatment (108) or by forming the dimethylacetals (109). Gas-liquid chromatography of long-chain fatty aldehydes and their derivatives has recently been reviewed by Gray (110). The fatty aldehydes can also be converted to fatty alcohols by $LiAlH_4$ reduction (109) or to acids by oxidation (109). The acetate derivatives of the alcohols and the methyl esters of the fatty acids are then analyzed by GLC to determine the lengths of the carbon chains and their degree of unsaturation. Oxidation of aldehydes to acids is only practical with saturated aldehydes, since degradation of unsaturated aldehydes occurs (109).

The variety of techniques used by biological investigators to investigate the quantities and nature of plasmalogens might account for some of the discrepancies that appear in the literature. Furthermore, it is likely that the vulnerability of the *O*-alk-1-enyl linkage to oxidative and acidic alterations would further complicate the meaning of some of the quantitative findings that have been published. Nevertheless, the chemical nature of plasmalogens and their distribution in various lipid classes have been well established by numerous independent investigators.

Nature of Plasmalogens in Biological Materials—The *O*-alk-1-enyl linkage is found almost entirely in phospholipids, mostly associated with the ethanolamine-containing phosphatides (Table I). In contrast, the *O*-alkyl bonds tend to be associated with the choline-containing phosphatides which have only small quantities of the *O*-alk-1-enyl linkages (99). However, in recent years, trace quantities of *O*-alk-1-enyl glycerolipids have been detected in the neutral lipid fractions of cells (Table I), primarily as 2,3-diacyl-1-*O*-alk-1-enyl



glycerols; such compounds are often referred to as "neutral plasmalogens." The location of the *O*-alk-1-enyl linkage in the 1-position glycerolipids and its *cis* configuration have been firmly established (27–30, 36, 39, 111–114).

The *O*-alk-1-enyl moieties in all glycerolipid classes are primarily 16:0, 18:0, and 18:1 carbon chains (5). Although significant differences in the quantitative distribution of these predominant *O*-alk-1-enyl chains exist, they clearly resemble the *O*-alkyl moieties of various lipid classes. The similarities of *O*-alk-1-enyl and *O*-alkyl moieties in glycerolipids suggest that interconversions might occur. However, no enzymes have yet been found that can catalyze these interconversions.

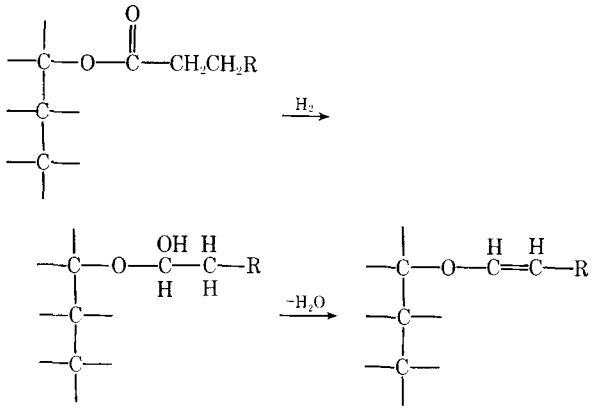
Biological Effects, Properties, and Function of Plasmalogens—Glycerolipids containing alkyl ether bonds have been thought to possess many biologically active properties, such as stimulation of growth, neurogenic activity, and hemopoiesis or inhibition of hemolysis. Bacteriostatic properties have also been associated with the ether-linked lipids. Furthermore, they have been proclaimed as therapeutic agents for radioprotection, wound healing, and bracken poisoning in cattle. These biological effects of alkyl glyceryl ethers have been thoroughly reviewed recently (5). Although many of these studies have been questioned, primarily because of the purity of the preparations or poor statistical comparisons, the fact remains that the listing of beneficial activities associated with *O*-alkyl lipids is overwhelming.

In contrast, no reports have dealt with similar type experiments carried out with plasmalogens. It is, perhaps, important to stress that the *O*-alk-1-enyl grouping in glycerolipids imparts a lower surface potential than that of the corresponding acyl analog (115). This is borne out in a biological system by the significant decrease in the hemolytic activity of lysophosphatidylethanolamine when alk-1-enyl (or alkyl) chains are substituted for an acyl chain (116).

The function of plasmalogens in living cells is not yet known. Their occurrence in biomembranes [organelles (117) and plasma membranes (118, 119)] and especially their high content in nervous tissue (120), suggest that their role is an important one in the structure and function of membranes. The orientation of protein and lipids in biomembranes could probably be a consequence of the ratio of ether- and ester-linked lipids in such residues; this proposition has been made on the basis of experimental data (117, 118). Further speculation on the function of plasmalogens is difficult since their biosynthetic pathway still remains to be elucidated.

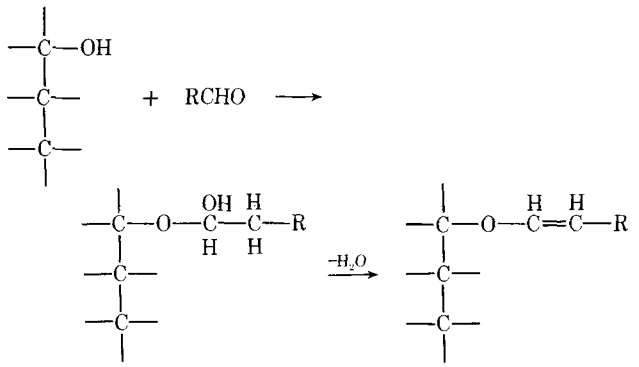
Possible Biochemical Mechanisms for the Biosynthesis of Plasmalogens—Recent experimental work has finally provided an enzymatic system that can synthesize ether-linked glycerolipids (121*a*–121*l*). However, only the alkyl linkage is formed readily in this microsomal system isolated from a number of cells. So far, the biosynthesis of *O*-alk-1-enyl bonds is unknown. In this section, a number of possible mechanisms for the biosynthesis of plasmalogens is summarized; some of these have been discussed previously (3, 5, 122–128).

A. Reduction of Acyl Groups to O*-Alk-1-enyl Groups*—



The reductive step would presumably require a hydrogen donor, such as NADH, NADPH, or FADH.

B. Reaction of a Fatty Aldehyde with a Primary Hydroxyl Group of a Derivative of Glycerol—



The glycerol precursor could be one of many glycolytic products, such as dihydroxyacetone-*P* or even a hexose-*P* that later could be split into a 3-carbon unit.

C. Direct Formation of an Alk-1-enyl Linkage by a Transfer Reaction—

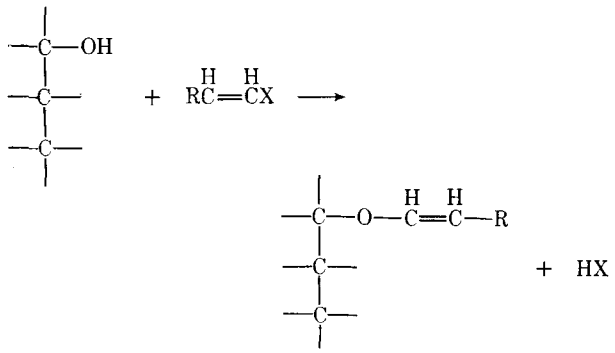
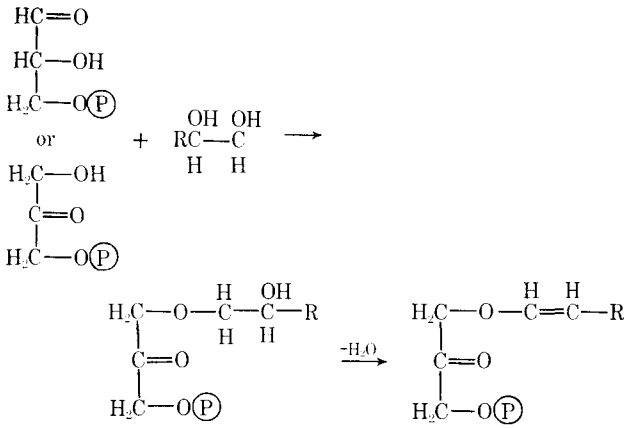


Table I—The Biological Occurrence of Plasmalogens in Phospholipids and Neutral Lipids

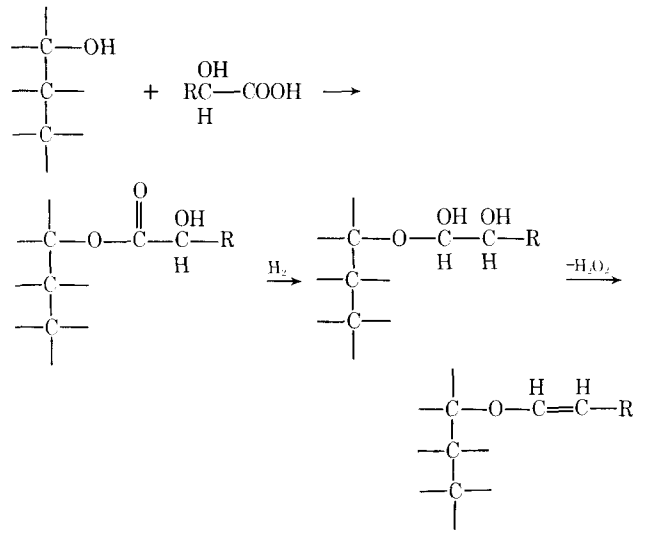
Biological Source	*O*-Alk-1-enyl Phospholipids References[a]	*O*-Alk-1-enyl Neutral Lipids References[a]
Mammals		
Normal tissues	25,106,200	61, 62,106,200,204,205
Neoplastic tissues	25,140,200	149,200
Fishes	139, 201	63,139,201
Invertebrates	61,202	61
Bacteria	149,203	149
Insects	206	

[a] Not a complete listing of references available.

*D. Reaction of an Alkane-1,2-diol with Glyceraldehyde-3-*P—

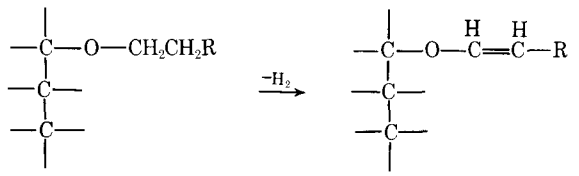


E. Reaction of an α-Hydroxy Fatty Acid and a Primary Hydroxyl Group of a Derivative of Glycerol—



This reaction would require an esterification and a reduction step before the final splitting out of hydrogen peroxide.

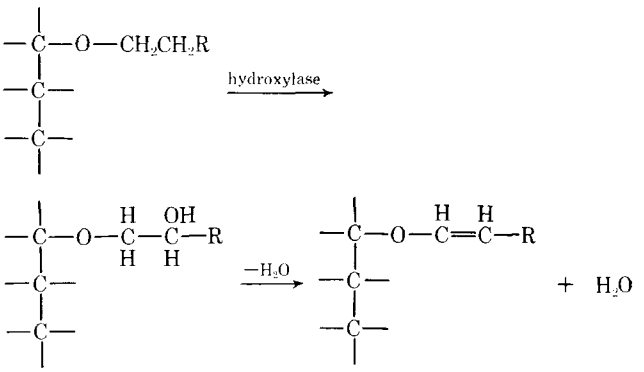
F. Conversion of an O*-Alkyl Linkage to an* O*-Alk-1-enyl Linkage*—



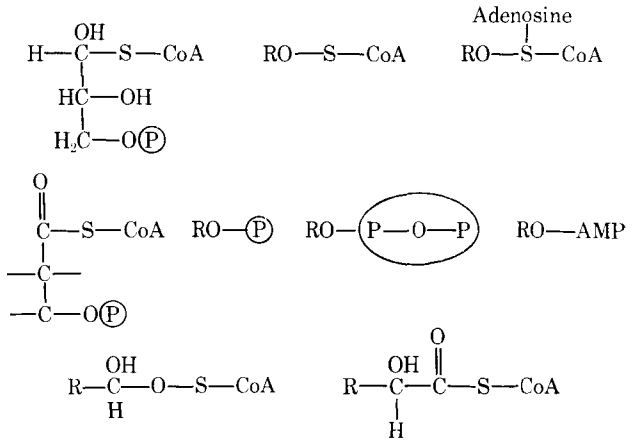
A hydrogen acceptor such as NAD, NADP, or FAD would be an essential cofactor.

Substituted groups such as $-NH_2$, $-OCH_3$, or $-OCH_2CH_3$ on the α- or β-carbons of the alkyl chain of glyceryl ethers could also produce alk-1-enyl ethers by splitting out ammonia, methanol, or ethanol, respectively.

G. Hydroxylation of an O*-Alkyl Lipid*—



H. Involvement of Cofactors—It is presumed that the reaction of functional groups listed in the preceding section would probably be facilitated through CoA or analogous intermediates, since the biosynthesis of the alkyl glyceryl ethers (121*a*) and acyl-substituted lipids (129) have an absolute requirement of coenzyme A, ATP, and Mg^{++}. Possible complexes that can be envisioned for such cofactors are shown below. All are hypothetical, since such complexes have never been isolated from biological materials.



Experimental Studies on the Biosynthesis of Plasmalogens—The reader is referred to an extensive review of this subject recently published (5).

A. Formation of the O*-Alk-1-enyl Linkage*—Cell-free systems that can form *O*-alk-1-enyl linkages in lipids have still not been isolated. Therefore, all data on the biosynthesis of these compounds available at the present time are largely circumstantial. *In vivo* studies have demonstrated that fatty acids (130–144), fatty alcohols (136, 140, 145–147*a* and *b*), fatty aldehydes (130–132, 148–150), and acetate (132, 134, 137, 138, 149, 151–155) can be incorporated into the alk-1-enyl moiety of glyceryl ethers. Experiments with doubly labeled fatty moieties have demonstrated that fatty aldehydes (132, 150) are much better precursors of the alk-1-enyl ethers than fatty acids. Some of the data obtained with fatty alde-

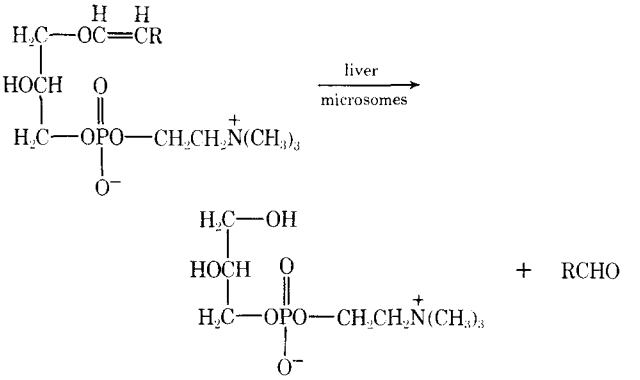
hydes have suggested that they are first oxidized and then incorporated into acyl linkages and that then the ester linkage is reduced (148). Nevertheless, experiments with the doubly labeled aldehydes strongly indicate that they are incorporated into the *O*-alk-1-enyl linkage directly instead of *via* the acyl-reductive mechanism.

Data indicating that alkyl glyceryl ethers can be desaturated in the α,β-position of the alkyl chain to form alk-1-enyl glyceryl ethers in the terrestrial slug, *Arion ater*, have been obtained by Thompson (141–143, 156*a*). Although the early evidence (141–143) for this conversion was based solely on the time course of specific activities, recent experiments with 1-^{14}C-hexadecyl-2-^{3}H-glyceryl ether (156*b*) have demonstrated that the ratio of $^{3}H/^{14}C$ in plasmalogens is identical to that of the alkyl glyceryl ether precursor. Experiments with the doubly labeled alkyl glyceryl ethers in Ehrlich ascites cells (156*b*) agree with the results obtained in slugs. Others (146, 147*a*, 157) have arrived at the same conclusion as Thompson (156*a*) by measuring specific activities of acyl, alkyl, and alk-1-enyl moieties in tracer experiments with glyceryl ether precursors. Some experiments in mammals (158–160) have not confirmed Thompson's thesis on the direct incorporation of ^{14}C-labeled alkyl glyceryl ethers into plasmalogens; radioactivity has been found in the alk-1-enyl chains of brain lipids (160), but this was attributed to the products of ether cleavage. Interconversions between neutral lipid and phospholipid classes that contain ether bonds have not been investigated; ideas about possible relationships have been based on structural studies of lipid classes (161).

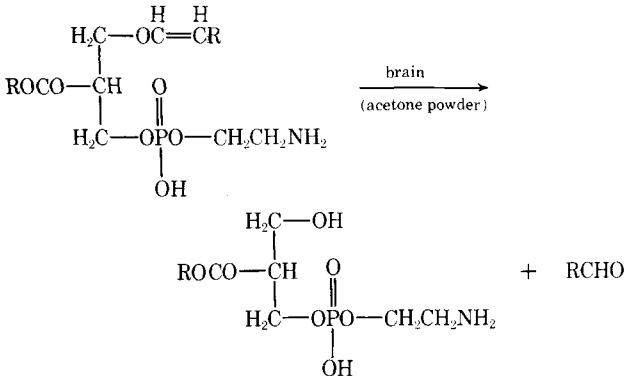
B. Phosphorylbase and Acyl Transferase Reactions Involving Alk-1-enyl Lipids as Substrates—Kiyasu and Kennedy (162) found that a particulate fraction of rat liver could catalyze the transfer of cytidine diphosphate choline or cytidine diphosphate ethanolamine to 1-*O*-alk-1-enyl-2-acyl-glycerols. The formation of the choline and ethanolamine plasmalogens by this reaction indicated that the transferases involved cannot distinguish between acyl and alk-1-enyl substituents on the substrates. Others have confirmed this reaction in homogenates (163–165) or particulate fractions (166) of brain and in a particulate fraction from ox heart (167). However, the importance of these cytidine-catalyzed reactions, at least in the brain, has been questioned by *in vivo* experiments with ^{14}C-ethanolamine (157) that involved the subsequent tracing of acyl, alkyl, and alk-1-enyl groupings in the various phospholipid classes at various times after their administration. Experiments with ^{32}P have also revealed considerable differences in the labeling of alk-1-enyl-acyl and diacyl phosphatides (130, 164, 168–172).

The acylation of alkyl glyceryl ethers occurs *in vivo* (158, 173–177) and *in vitro* (178–181). Cell-free systems isolated from numerous tissues have been shown to acylate 1-*O*-alkyl glycerols only in the 3-position (181). Presumably, acylation of the 2-position on the 1-isomer of alkyl glyceryl ethers requires the phosphorylated alkyl glyceryl ether as a substrate. Limited information on acylation reactions is available for the *O*-alk-1-enyl-linked lipids. Although rat liver microsomes (182) were unable to utilize 1-*O*-alk-1-enyl glycero-3-phosphorylcholine as a substrate for acyl-CoA–phospholipid transferase, this acylation reaction did occur in erythrocytes from humans and in sarcoplasmic reticulum from rabbits (183). Endogenous phospholipids have been shown to be important factors in the reactivation of alk-1-enyl-GPC hydrolase (184).

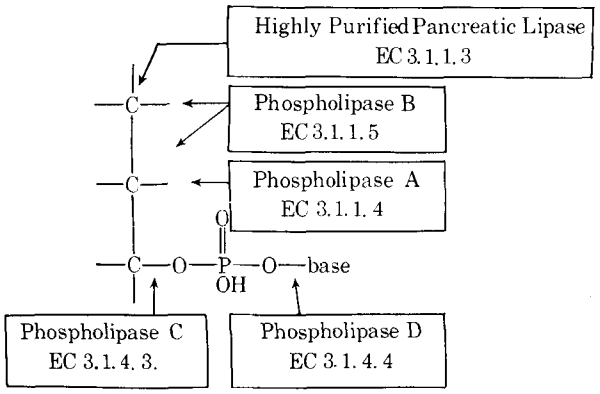
Experimental Studies on the Enzymatic Degradation of Plasmalogens—*A. Enzymatic Cleavage of the O-Alk-1-enyl Linkage*—Enzymes from liver (185) and brain (186) have been described that can catalyze the cleavage of the *O*-alk-1-enyl bond in plasmalogens. The liver enzyme, located in microsomes, can only utilize 1-*O*-alk-1-enyl glycerylphosphorylcholine as a substrate. The enzyme is specific for the lysocholine compound and no other cofactors are required.



The enzyme isolated from acetone powders of brain yields products similar to those found with the liver preparation, but it preferentially hydrolyzes the *O*-alk-1-enyl bond of 1-*O*-alk-1-enyl-2-acyl-glycerylphosphorylethanolamine, and Mg^{++} was required as a cofactor. The enzyme was also capable of cleaving the ether linkage of the lyso derivative, but the cleavage did not equal that obtained with the native plasmalogen substrate.



Other nonspecific reactions involving the enzymatic cleavage of the *O*-alk-1-enyl moieties of plasmalogens have been documented (187–190), but cleavage reactions of this type have not been reported for *O*-alk-1-enyl bonds found in neutral lipids. Considerable efforts have centered on the enzymatic cleavage of *O*-alkyl bonds in neutral lipids (191, 192). Enzymes for the cleavage of *O*-alkylglycerols have been found in rat liver that require tetrahydropteridine and NADPH as cofactors; the initial products formed during this cleavage are fatty aldehydes and glycerol. Similar systems have been found in other cells (192).



B. Removal of Acyl and Phosphorylbase Moieties from Plasmalogens by Phospholipases—Phospholipases are defined as A, B, C, or D, depending on the enzymatic specificity for the groups attached to the three positions of glycerol in phosphatides (103, 193). None of the lipase or phospholipase enzymes attacks ether bonds in lipids. Phospholipase A catalyzes the hydrolysis of fatty acids from the 2-position of phospholipids, whereas phospholipase B is thought to catalyze the hydrolysis of fatty acids from positions 1 and 2 or only position 1. Phospholipase B activity is the least understood since it is unknown whether its dual specificity is due merely to contamination with phospholipase A. Phospholipase C catalyzes the hydrolysis of the entire phosphorylbase moiety and phospholipase D catalyzes the hydrolysis of the base (choline or ethanolamine) portion only. It has also been shown that electrophoretically purified pancreatic lipase will catalyze the hydrolysis of acyl moieties in the 1-position of glycerophosphatides (194).

All of the phospholipases are capable of utilizing *O*-alk-1-enyl phosphatides as substrates under certain conditions, but generally the reaction rates are somewhat slower than with the diacyl analogs. Marinetti *et al.* (195) found that phospholipase A required several days to remove the acyl moiety from alk-1-enyl-acyl-choline phosphatides in yields equivalent to those obtained with phosphatidyl choline. The sluggishness of this reaction was successfully applied to the purification of native plasmalogens (removal of contaminating phosphatidyl choline) by Gottfried and Rapport (33). However, the source of the enzyme is thought to be important, since Hartree and Mann (196) found essentially the opposite results in similar studies of phosphatides isolated from the sperm of rams. Phospholipase C from *Clostridium welchii* has also been found to hydrolyze *O*-alk-1-enyl-acyl-choline phosphatides more slowly than the diacyl analog (197); *O*-alk-1-enyl ethanolamine phosphatides are also hydrolyzed by phospholipase C obtained from *Bacillus cereus* (198). In contrast, phospholipase D isolated from cabbage was unable to hydrolyze the choline moiety from the *O*-alk-1-enyl-acyl-choline phosphatides (182). Investigations of lipases that catalyze the removal of acyl groups in neutral lipid classes containing *O*-alk-1-enyl linkages has recently been reported by Slotboom (199).

CONCLUSIONS

Even after 40-odd years of research, the biosynthesis and function of the alk-1-enyl linkage in plasmalogens remain obscure although the structures, chemistry, and occurrence of plasmalogens in living cells are now firmly established. The striking similarities of chain lengths and degree of unsaturation between *O*-alkyl and *O*-alk-1-enyl moieties in glycerolipids suggest a close metabolic relationship and that they might perhaps originate from identical precursors. The requirements for the biosynthesis of alkyl ether bonds are known, and a clear understanding of the mechanism of this new pathway could elucidate fresh approaches to solving the riddle of plasmalogen biosynthesis. The biosynthetic problem is mirrored by the poor yields of plasmalogens synthesized in the organic laboratory. Certainly, the presence of *O*-alk-1-enyl bonds in biomembranes hints at their significance in the structural orientation of the lipid and protein in these structures. It is hoped that this review will serve to acquaint other chemists with both the past and present scope of research involving the *O*-alk-1-enyl linkages of plasmalogens.

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RESEARCH ARTICLES

Effect of Certain Additives on the Photochemistry of Riboflavin

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Abstract □ The quantum efficiency of riboflavin under aerobic conditions was determined by using a microirradiation method. It was found that the initial quantum efficiency was constant and independent of intensity of light, wavelength of light, and concentrations employed. The quantum efficiency of riboflavin in the presence of phenols and other compounds was also determined. Only in the presence of phenols was the quantum efficiency decreased yielding a linear relationship between the Hammett's sigma values and the rate of photodecomposition. Benzyl alcohol and benzoic acid were found to be relatively ineffective as photochemical stabilizers compared to phenols. Cinnamyl alcohol, as an electron donor, enhanced the photodecomposition of riboflavin. It appears, from the compounds tested, that the hydroxyl group should be either attached to the benzene ring or be in conjugation with the benzene ring in order to be an effective photochemical stabilizer. The effects of temperature and pH on the system were also determined. Kinetic studies were made to elucidate the reaction mechanism.

Keyphrases □ Riboflavin, photochemistry—additives, temperature, pH effects □ Microirradiation—riboflavin, quantum efficiency □ Kinetic studies—riboflavin degradation □ Quantum riboflavin, efficiency—equations derived □ Spectrophotometry—analysis

The photosensitivity of riboflavin was first observed in 1932 by Warburg and Christian (1). Since then its photochemistry has been the subject of extensive investigation, and can be followed by recent reviews (2, 3). Although the fact that riboflavin will undergo photoreduction in the absence of an electron donor has been generally accepted, there have been conflicting views concerning the actual mechanism of the reaction. However, the presently accepted theory for the photodecomposition of riboflavin proposes that the reaction proceeds from the lowest triplet state of the flavin and involves intramolecular hydrogen-transfer from the ribityl sidechain with the subsequent formation of lumichrome and/or lumiflavin depending on basicity of the solution (4–6). The photolysis of riboflavin and several other flavins in acid or neutral solution is subject to general acid and base catalysis (6).

The formation of molecular complexes between riboflavin and various compounds has also been observed (7–11). Particularly, studies of the charge-transfer complexes between riboflavin and phenol derivatives have received considerable attention, because interactions of donor-acceptor type may be quite common in biological systems (12). Thus, an understanding of the correlation of these properties with the photochemical behavior of riboflavin might provide an insight into some of the energy transfer and storage mechanisms of living organisms.

Stabilization of riboflavin to light in the presence of additives is pharmaceutically important, since certain additives were observed to have considerable influence on the light stability of the system (13–22). Despite this importance, very few photochemical kinetic studies have been published for riboflavin in the presence of complexing agents. Therefore, the present study was undertaken