[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, THE UNIVERSITY OF MICHIGAN, ANN ARBOR, MICH.]

The Configuration of the Double Bond in Naturally-occurring Alkenyl Ethers[1]

BY HUBER R. WARNER[2] AND WILLIAM E. M. LANDS[3]

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Pig heart lecithin was degraded by a series of treatments to yield consecutively, α′-(1-alkenyl)-β-acylglycerol [88% yield], α′-(1-alkenyl)-glycerol [93% yield] and 2-*O*-(1-alkenyl)-glycolaldehyde [77% yield]. Thus the over-all yield of this last product was 63%. The infrared spectra of these derivatives were compared with those of *cis*- and *trans*-methyl 1-dodecenyl ethers. The data indicate that the configuration of the double bond of the alkenyl ethers in these plasmalogens is *cis*. No evidence for the presence of any *trans* compounds was obtained.

Numerous studies[4-6] have indicated that the aldehydogenic property of plasmalogen is due to the presence of an α,β-unsaturated or 1-alkenyl ether group, which is hydrolyzed in acid solution to produce free aldehyde. Alkenyl ether derivatives of α-glycerylphosphorylcholine (GPC) and α-glycerylphosphorylethanolamine (GPE) have been found in various animal tissues.[6-8]

Until recently, there was disagreement as to whether the primary or secondary hydroxyl group of glycerol was involved in the ether bond. Debuch[9] and Marinetti, *et al.*,[10] showed that hydrogenation and hydrolysis of brain alkenyl-acyl-GPE, heart alkenyl-acyl-GPE and heart alkenyl-acyl-GPC yielded exclusively α-glyceryl ethers, as characterized by periodate oxidation and infrared spectra. Furthermore, the action of snake venom phospholipase A, a β-esterase, on heart alkenyl-acyl-GPC also indicates that the primary hydroxyl group is involved in the alkenyl ether linkage.

Studies on the stereochemical configuration of the double bond of these naturally-occurring alkenyl ethers have not been reported. Since compounds containing double bonds of *cis* configuration have characteristically different absorption spectra from those containing double bonds of *trans* configuration, infrared spectra of natural plasmalogens should be useful for distinguishing between these two possibilities. Phosphate esters and unsaturated fatty acids absorb light at wave lengths critical for distinguishing between the *trans* and *cis* configurations (970 and 750 $cm.^{-1}$, respectively) making alkenyl-acyl-GPC unsuitable for these studies. However, the phosphate group can be eliminated by treatment of heart lecithin fraction with phospholipase C, and the acyl chain can be removed by saponification.

This paper describes the preparation of various derivatives of pig heart α′-(1-alkenyl)-β-acyl-GPC and *cis*- and *trans*-methyl 1-alkenyl ethers. The infrared spectra of these compounds were then studied to determine the configuration of the naturally occurring alkenyl ether.

Results and Discussion

Preparation of Plasmologen Derivatives.—A pig heart lecithin fraction, containing about 40% α′-(1-alkenyl)-β-acyl-GPC, was degraded as shown below to yield various other plasmalogens.

Alkenyl-acylglycerol.—Phospholipase C acts faster in the presence of diethyl ether, and some reactions catalyzed by this enzyme have been demonstrated only in the presence of ether.[11] In our experiments, the reaction was carried out in a two-phase, ether–water system, and the extent of reaction was readily measured by the conversion of ether-soluble phosphorus to water-soluble phosphorus (phosphorylcholine).

In several preliminary experiments in which incomplete hydrolysis of the glycerylphosphate bond was obtained, the unreacted lecithin was isolated by silicic acid chromatography and analyzed for phosphorus and alkenyl ether. This lecithin was always richer in plasmalogen than was the starting material, suggesting that the enzyme prefers diacyl-GPC as a substrate over alkenyl-acyl-GPC, under the reaction conditions employed. A similar selectivity has been observed for lecithin hydrolysis using phospholipase A preparations.[12]

The alkenyl-acylglycerol was isolated by chromatography of the chloroform-soluble reaction products on silicic acid. Analyses of the fraction by both the fuchsin (total aldehyde) and iodometric (alkenyl ethers) methods indicate that all of the measured aldehyde was present as 1-alkenyl ether.

Alkenylglycerol.—Silicic acid chromatography of the products obtained after saponification of the alkenyl-acylglycerol preparation indicated that the predominant aldehydogenic product (93%) was eluted in a manner similar to acyl- and alkylglycerols.[13] The remainder (7%) was eluted as free aldehyde. The product was insoluble in hexane and petroleum ether, as are alkylglycerols.

The possibility that the alkaline treatment employed to convert the alkenyl-acylglycerol to alkenylglycerol might alter the configuration of the double bond was checked by treating a mixture of *cis*- and *trans*-methyl 1-dodecenyl ether with methanolic KOH at 70° for 1 hour and a sample of the *cis* isomer alone with methanolic KOH at 37° for 1 hour. No net interconversions were detected by gas–liquid chromatography (G.L.C.) in the former case, or by infrared spectral studies in the latter case, as a result of these treatments.

It is possible that the chromatographic separations might cause isomerization of the double bond involved in the alkenyl ether linkage. However, no conversion of *trans*-methyl 1-dodecenyl ether to the *cis* isomer was noted after 20 hours on silicic acid at room temperature, although about 5% hydrolysis did occur as detected by G.L.C. The infrared data provide no evidence for isomerization occurring in any derivative after the alkenyl-acylglycerol was obtained. Isomerization occurring prior to this time cannot be ruled out.

Periodate Oxidation of Alkenylglycerol.—The extent of oxidation was followed by the decrease in optical density at 300 mμ according to the method used by Marinetti, *et al.*[10] A twofold excess of periodate was used and about four hours was required to decrease the 300 mμ absorption to one-half of its original value.

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(2) National Science Foundation Coöperative Graduate Fellow.

(3) To whom inquiries regarding this article should be sent.

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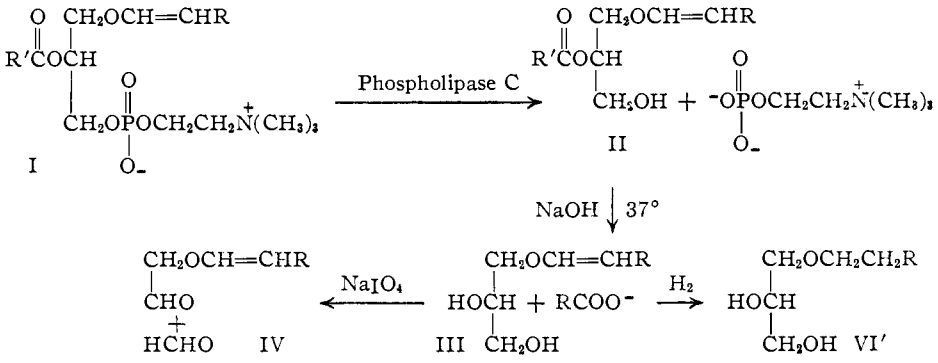
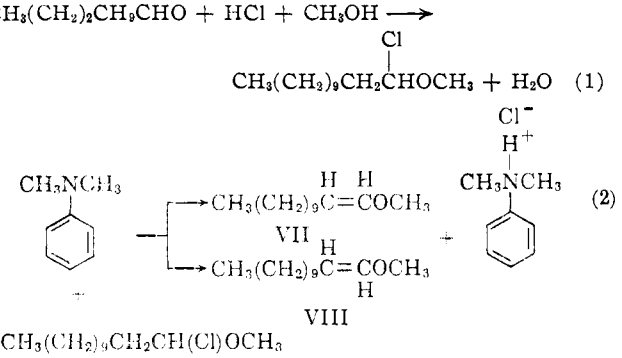
Although glycols are oxidized faster in slightly acid medium,[14] the oxidation was carried out at *p*H 7.0 to prevent hydrolysis of the alkenyl ether. The reaction was carried out in the dark with only a low concentration of periodate in order to diminish the chance of overoxidation. The over-all yield of the alkenylglycolaldehyde based on the original pig heart lecithin was 63%.



Preparation of Methyl 1-Alkenyl Ethers.—The long chain methyl 1-alkenyl ethers were synthesized using a method similar to that used by Böhme and Bentler.[15] The rate of formation of the methyl α-chloroalkyl ether was readily followed by gas–liquid chromatography (G.L.C.) of small aliquots of the reaction mixture. As reaction 1 proceeds the peak due to free



aldehyde (t_R = 1.8 min.) decreases and two new peaks appear (peak a, t_R = 0.85 min.; peak *b*, t_R = 1.1 min.) apparently due to thermal dehydrohalogenation on the column of the α-chloro ether to produce a mixture of *cis* and *trans* isomers. The isomer with the lower retention time (peak a) was subsequently identified as the *cis* isomer by n.m.r. and infrared spectral studies, and appears to be the preferred product of the thermal dehydrohalogenation. The percentage reaction was determined by comparing the sum of the areas of peaks a and b with that of the free aldehyde peak in a series of chromatograms, obtained at different times during the reaction. During one synthesis of methyl α-chloroalkyl ether the reaction proceeded to only 35% completion. Increasing the rate of HCl ebullition caused the reaction to proceed rapidly again, indicating the importance of a high HCl concentration in forcing reaction 1 to completion.

Purification of the *cis* and *trans* Isomers.—Careful fractional distillation of mixtures of the *cis* and *trans* isomers did not result in significant resolution of the two isomers. Some separation could be effected using Florisil or silicic acid chromatographic columns, but alumina was superior to both. Not only were the *cis*- and *trans*-methyl 1-dodecenyl ethers separated from the contaminating dimethylaniline, but also from each other, so that each was obtained in better than 99% purity as indicated by G.L.C. The elution of the two isomers from the alumina column was followed by gas–liquid chromatographic analysis of equal aliquots of each fraction, as shown in Fig. 1.

N.m.r. Spectra of *cis* and *trans*-Methyl 1-Dodecenyl Ether.—The n.m.r. spectra of the two isomers are shown in Fig. 2.[16] The two spectra are readily differentiated from each other by the different chemical shifts and splitting of the proton resonance peaks due

to OC=C (peaks 1) and C=C—C (peaks 2). The shifting to a lower field strength and greater splitting of these peaks are characteristic of *trans* geometrical isomers as compared with *cis* isomers.[17,18] In general

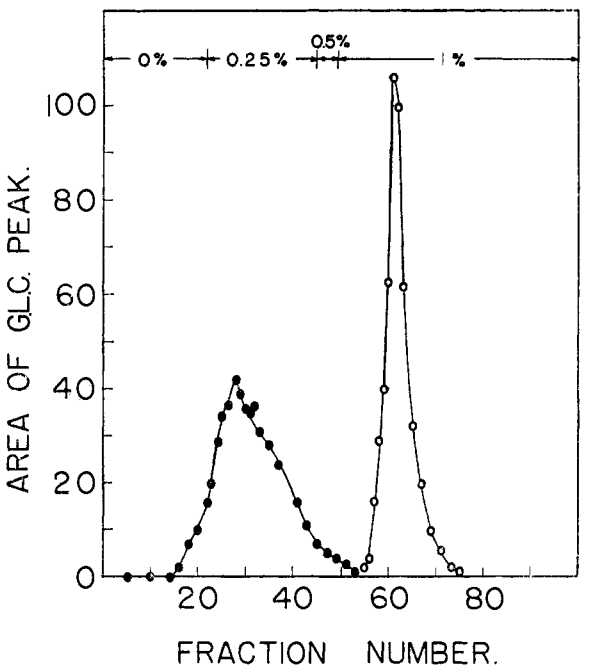


Fig. 1.—Separation of *cis* and *trans* isomers of methyl 1-dodecenyl ether on an alumina column. The ordinate indicates the relative areas of peaks, corresponding to the *cis* isomer (○) and the *trans* isomer (●), in the gas–liquid chromatograms of each eluted fraction. The two isomers were eluted with solutions of petroleum ether containing small amounts of diethyl ether (0%, 0.25%, 0.5%, 1%) indicated at the top of the figure.

the coupling constant, *J*, of the protons of double bonds with the *trans* configuration is $11 < J < 18$ c.p.s. and that of the protons of double bonds of the *cis* configuration is $5 < J < 14$ c.p.s. On the basis of these n.m.r. spectra, we can assign the *cis* configuration to the isomer corresponding to G.L.C. peak a (J = 6.2, 6.7; δ = 0.42, 0.57 for peaks 1 and 2, respectively) and the *trans* configuration to the isomer corresponding to G.L.C. peak b (J = 12.6, 12.4; δ = 0.45, 0.62), since for the latter isomer both J and δ for the alkene proton resonance peaks are larger than those of the former isomer.

(14) J. R. Dyer, in D. Glick (Editor) "Methods in Biochemical Analysis," Vol. 3, Interscience Publishers, Inc., New York, N. Y., 1956, p. 111.

(15) H. Böhme and H. Bentler, *Chem. Ber.*, **89**, 1468 (1956).

(16) We wish to thank Dr. J. C. Martin for his help in obtaining the n.m.r. spectra.

(17) J. D. Roberts, "Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p. 54.

(18) O. Jardetzky and C. D. Jardetzky in D. Glick (Editor), "Methods of Biochemical Analysis," Vol. 9, Interscience Publishers, Inc., New York, N. Y., 1962, p. 235.

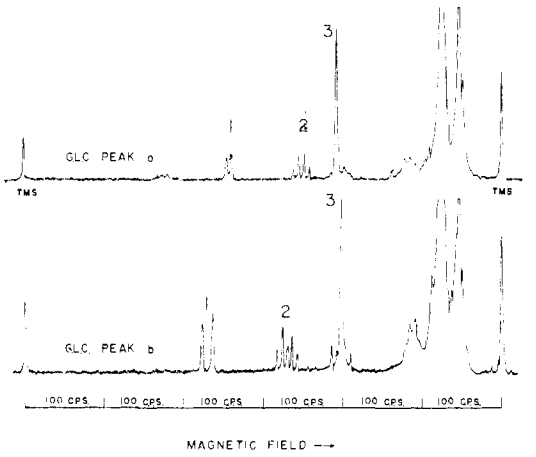


Fig. 2.—N.m.r. spectra of *cis*- and *trans*-methyl 1-dodecenyl ether. The spectra were obtained using a Varian high resolution spectrometer operating at 60 mc, and −28°; 30% solutions in CS_2 were used. Tetramethylsilane (TMS) served as the internal standard and the spectra were calibrated using the sideband at 600 c.p.s. The isomer corresponding to G.L.C. peak a produces the upper spectrum, and that corresponding to G.L.C. peak b produces the lower spectrum.

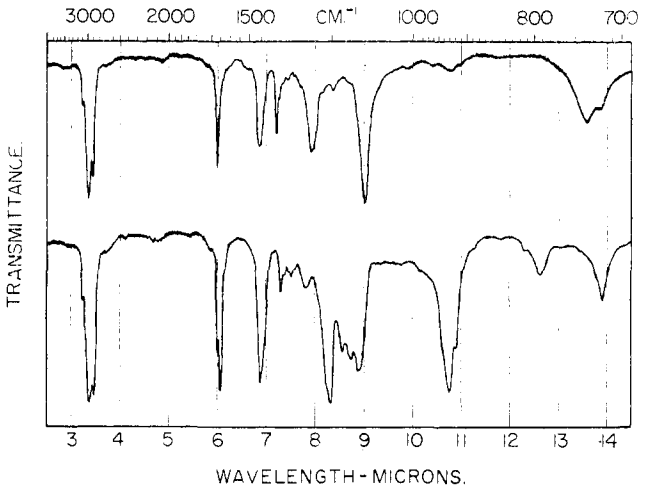


Fig. 3A.—Infrared spectra of *cis*- and *trans*-methyl 1-dodecenyl ethers. The upper spectrum is that of *cis*-methyl 1-dodecenyl ether, and the lower that of *trans*-methyl 1-dodecenyl ether.

The remaining portions of the spectra are essentially identical except the peaks corresponding to the OCH_3 protons (peak 3). In the spectrum of the *cis* isomer this peak occurs at a lower field strength than does the corresponding peak in the spectrum of the *trans* isomer.

Infrared Spectra of *cis*- and *trans*-Methyl 1-Dodecenyl Ether.—The infrared spectra shown in Fig. 3A confirm the assignments made using n.m.r. spectra. The characteristic features are the absorption peaks due to the out-of-plane bending of the alkene C–H bonds and the absorption peaks due to the C–O stretching. The following peak assignments are based on the discussion by Bellamy.[19]

The *trans* isomer has an absorption peak at 930 cm.$^{-1}$ due to the hydrogen atoms which are out-of-plane at the double bond, whereas the corresponding absorption peak occurs near 735 cm.$^{-1}$ in the *cis* isomer.[20] In addition, a sharp, medium absorption peak at 1390 cm.$^{-1}$ occurs in the spectrum of the *cis* isomer, which may be due to some in-plane bending frequencies of the alkene protons.[21] The absorption spectra of both isomers

(19) L. G. Bellamy, "The Infrared Spectra of Complex Molecules, Methuen and Co., Ltd., London, 1954.

(20) J. E. Kilpatrick and K. S. Pitzer, *J. Research Natl. Bur. Standards*, **38**, 191 (1947).

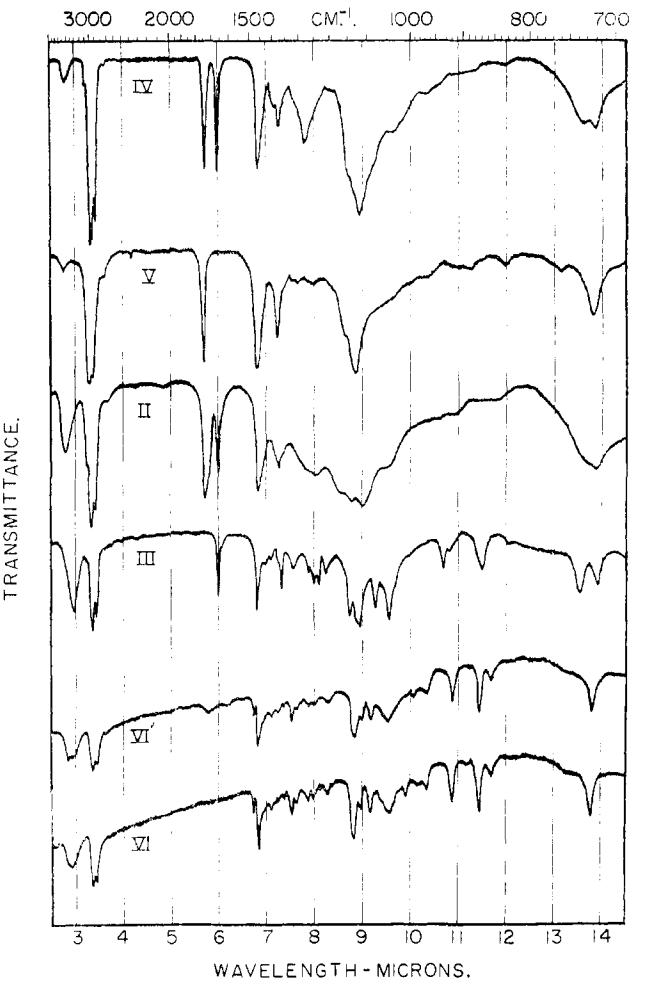


Fig. 3B.—Infrared spectra of alkenylglycolaldehyde IV, hexadecylglycolaldehyde V, alkenyl acylglycerol II, alkenylglycerol III, "Reduced alkenylglycerol" VI′ and hexadecylglycerol VI.

contain strong absorption peaks near 1670 cm.$^{-1}$, due to C=C stretching.[22] This absorption peak, which is often weak for compounds containing isolated double bonds, is characteristically strong in vinyl ethers. This peak occurs at a slightly lower frequency in the spectrum of the *trans* isomer (1650 cm.$^{-1}$) than in that of the *cis* isomer (1670 cm.$^{-1}$) in contrast to the general rule (not invariable) that the C=C absorption of *cis* isomers is about 17 cm.$^{-1}$ lower than that of the corresponding *trans* isomer.[11]

The absorption peaks due to C–O stretching of the ether bonds are also different in the two isomers. Presumably, at least two peaks would be expected,[23] one from O–CH_3 (aliphatic ethers absorb near 1100 cm.$^{-1}$), and one from O–CH= (aromatic ethers absorb near 1230 cm.$^{-1}$). In the spectrum of *cis*-methyl 1-dodecenyl ether, two strong peaks can be observed, at 1110 cm.$^{-1}$ (O—CH_3) and 1260 cm.$^{-1}$ (O—CH=), the former being the stronger of the two. In the spectrum of the *trans* isomer, however, the strongest peak is observed at 1200 cm.$^{-1}$ (OCH=) with weaker peaks at 1170, 1150 and 1125 cm.$^{-1}$. *cis*- and *trans*-methyl 1-decenyl ethers were prepared similarly and the infrared spectra were identical to those of the dodecenyl derivatives shown in Fig. 3A.

Infrared Spectra of Various Plasmalogen Derivatives.—The infrared spectra of the alkenyl-acylglycerol

(21) N. Sheppard and D. M. Simpson, *Quart. Revs.*, **6**, 1 (1952).

(22) H. W. Thompson and P. Torkington, *Trans. Faraday Soc.*, **42**, 432 (1946).

(23) R. B. Barnes, R. C. Gore, U. Liddel and V. Z. Williams, "Infrared Spectroscopy," Reinhold Publ. Corp., New York, N. Y., 1944.

TABLE I

INFRARED ABSORPTION PEAKS OF PLASMALOGENS

Peak, cm.$^{-1}$	VII	IV	II	III	VIII
(1) —CH=CH—					
(a) 735	M	M	S	M	..
(b) 930	..	..	..	M	S
(c) 1390	M	M	M	M	..
(d) 1670	S	S	S	S	S
(2) —O—C—					
(a) 1110	S	S	S	S	..
(b) 1125	..	..	?	?	SM
(c) 1150	..	W(shoulder)	S(ester)	..	M
(d) 1170	..	..	..	..	M
(e) 1200	..	..	..	W	S
(f) 1260	S	S(1270 cm.$^{-1}$)	M(broad)	M	W
(3) —OH					
(a) 1050 (pr.)	..	W(carbonyl?)	M	S	..
(b) 1080 (sec.)	..	..	..	S	..
(c) 3400	..	W	S	S	..
O ‖ (4) —C—					
(a) 1750	..	S	S	..	..

II, alkenylglycerol III, alkenylglycolaldehyde IV, alkylglycolaldehyde V and alkylglycerol VI and VI′, are shown in Fig. 3B. The spectrum of the alkenylglycolaldehyde is most useful for determining the configuration of the double bond since the only significant difference between this compound and the reference compounds, *cis*- and *trans*-methyl 1-dodecenyl ethers, is the presence of the carbonyl group in the former.

$$OHCCH_2OCH{=}CHR \quad (IV)$$

$CH_3OCH{=}CHR$ *cis* (VII) and *trans* (VIII)

Comparison of the spectrum of alkenylglycolaldehyde (IV) with that of *cis*-methyl 1-dodecenyl ether (VII) indicates that the main difference is the absorption peak at 1750 cm.$^{-1}$, characteristic of a carbonyl group. The spectrum of the alkenylglycolaldehyde also contains peaks at 1670, 1270, 1110 and 735 cm.$^{-1}$ characteristic of a *cis*-alkenyl ether, whereas peaks at 1200 and 930 cm.$^{-1}$, characteristic of a *trans* alkenyl ether, are absent. The characteristic peaks at 1670, 1270 and 735 cm.$^{-1}$, are missing in the spectrum of the alkylglycolaldehyde. These features indicate that the *cis* configuration must be assigned to the double bond in the alkenylglycolaldehyde prepared from the pig heart lecithin fraction as described.

The spectra of the alkenyl-acylglycerol further confirms this assignment. Although the 1100–1300 cm.$^{-1}$ region is not sharp in the spectrum of alkenyl-acylglycerol, certain important features are evident. There is considerable absorption near 1260 cm.$^{-1}$ and strong absorption at 1110 cm.$^{-1}$ characteristic of *cis*-alkenyl ethers, but absorption peaks at 1200 and 930 cm.$^{-1}$, characteristic of a *trans*-alkenyl ether, are absent. Absorption near 735 cm.$^{-1}$ is characteristic of double bonds with the *cis* configuration, but cannot be used as proof for a *cis*-alkenyl ether in this compound since the fatty acids in this molecule are unsaturated. The remaining absorption peaks can be attributed to the primary hydroxyl group and the ester group. The spectra of the alkenylglycerol and alkylglycerol are less satisfactory, but certain features are worth notice. The absorption peaks near 1670, 1390, 1250 and 735 cm.$^{-1}$ in the spectrum of the alkenylglycerol disappear after hydrogenation, whereas the peak near 930 cm.$^{-1}$ does not. The strong peak near 1110 cm.$^{-1}$ is shifted to a slightly higher frequency following hydrogenation. These features suggest that peaks typical for *cis*-alkenyl ethers disappear following hydrogenation, whereas any that might correspond to the *trans* isomer do not, and therefore must be caused by some other type of absorption. The vicinal hydroxyl groups appear to be in some way responsible for the two peaks in the 850–950 cm.$^{-1}$ region since these peaks are absent in the alkenyl acylglycerol and the periodate-treated compounds. Table I summarizes the infrared data used to indicate the *cis* configuration for pig heart plasmalogen. Similar results were obtained using plasmalogen derivatives from beef heart lecithin fractions.

Experimental

Infrared Spectra.—All spectra were obtained with a Perkin–Elmer Infracord 137, using NaCl plates. In some cases it was possible to use a thin liquid film of the pure material. In the case of the alkyl- or alkenylglycerols, a thin solid film was obtained by evaporating a concentrated diethyl ether solution of the sample directly on the plates. Spectra obtained in this way were less satisfactory than those obtained using liquid films, but were not improved significantly by using Nujol mulls.

Gas–Liquid Chromatography.—All gas–liquid chromatograms were obtained using an 8-ft column packed with ethylene glycol–succinate polymer on an acid-washed Celite base. Argon (at 15 p.s.i.) was used as the carrier gas, and detection was accomplished using a Barber–Colman model 10 ionization detector. The column was operated isothermally at 175° and retention times, t_R, were calculated assuming $t_R = 0$ for the initial air peak, which barely precedes the solvent peak.

Silicic Acid.—Mallinckrodt silicic acid was sieved to obtain the 100–200 mesh fraction and activated by heating for at least 12 hours at 100°. Used silicic acid was regenerated by washing with methanol, 1:1 water–methanol, and finally deionized water, and then reactivated by heating.

Chemical Analyses.—The lipid fractions were characterized by determining the content of total phosphorus,[24] ester,[25] alkenyl ether,[26] amino nitrogen[27] and total aldehyde.[28]

Alkenyl-GPC, prepared by treatment of beef heart lecithin fraction with 0.2 *N* methanolic NaOH for 15 minutes at 37° and purified by chromatography on silicic acid, was used as an aldehyde standard. Alkenylglycerol and alkenyl-acylglycerol developed color with the fuchsin reagent more slowly than did either alkenyl-acyl-GPC or alkenyl-GPC when they were analyzed for aldehyde by the above procedure. To ensure complete hydrolysis of these alkenyl ethers to free aldehyde, the acetic acid incubation was carried out at 70°. This treatment did not alter the optical density obtained with the standard.

Preparation of Heart Lecithin Fraction.—The lecithin fraction prepared as described earlier[28] was rechromatographed on 200 g. of silicic acid using chloroform–methanol eluting mixtures. The lecithin was put on the column in chloroform–methanol (9:1) and then eluted with chloroform–methanol (1:1). The first 1.3 l. of eluate gave negative results when tested for aldehyde and were discarded. The lecithin fraction was then eluted from the column with the next 2 l. of chloroform–methanol (1:1). The eluates containing lecithin were combined, evaporated to dryness, taken up in a small volume of chloroform–methanol (1:1) and stored at 5°.

Anal. (average of 6 samples): ester/P = 1.54 (1.32–1.65); alkenyl ether/p = 0.45 (0.44–0.46); total aldehyde/p = 0.45 (0.44–0.57). Composition of aldehydes released by acid hydrolysis and analyzed by G.L.C.: mole %: *n*-C_{14}, 3%; *n*-C_{15}, 1%; *n*-C_{16}, 70%, *n*-C_{17}, 1%; *n*-C_{18}, 14%; *n*-C_{19}, 3%.

Preparation of Alkenyl-acylglycerol.—Pig heart lecithin (630 μmoles P, 250 μmoles plasmalogen) was dissolved in 30 ml. of diethyl ether. After the addition of 0.15 ml. of EtOH and 0.15 ml. of 0.1 *M* $CaCl_2$, 2 ml. of 0.1 *M* Tris-chloride buffer, *p*H 8.3, containing 12 mg. of phospholipase C (Worthington, *Cl. welchii* α-toxin), were added and the mixture was stirred at room temperature in a stoppered flask. The extent of reaction was followed by measuring the decrease in ether-soluble phosphorus. After 15 hours of incubation (96% reaction), the reaction was stopped by the addition of 100 ml. of chloroform–methanol (2:1), and 30 ml. of water. The aqueous layer was analyzed for total phosphorus (606 μmoles) and discarded. The chloroform layer was evaporated to dryness, the residue dissolved in a small volume of 5% (v./v.) diethyl ether in petroleum ether and put on a 30-g. silicic acid column (diameter, 2 cm.). The column was washed with 200 ml. of 5% diethyl ether, 100 ml. of 10% diethyl ether, and the alkenyl-acylglycerol was then eluted slowly with 15% diethyl ether before the bulk of the diglyceride. About 2 l. of 15% diethyl ether was required for

(24) G. R. Bartlett, *J. Biol. Chem.*, **234**, 466 (1959).
(25) W. E. M. Lands, *ibid.*, **231**, 883 (1958).
(26) W. T. Norton, *Biochem. et Biophys. Acta*, **38**, 340 (1960).
(27) C. H. Lea and D. N. Rhodes, *Biochem. J.*, **56**, 613 (1954).
(28) H. R. Warner and W. E. M. Lands, *J. Biol. Chem.*, **236**, 2404 (1961).

complete elution of the diglyceride fraction. The alkenylacylglycerol fraction contained about 220 μmoles of alkenyl ether (88% yield) and 360 μmoles of ester suggesting that it was 75% pure, the other 25% being diglyceride that was not removed by chromatography.

Preparation of Alkenylglycerol.—To 15 ml. of methanol–chloroform (4:1) containing 198 μmoles of alkenyl-acylglycerol, 1 ml. of water and 1 ml. of 1 *N* NaOH in methanol were added. This mixture was incubated for 1 hour at 37°; then 25 ml. of chloroform and 7 ml. of water were added. The water layer was discarded. The chloroform layer was evaporated to dryness under nitrogen, the residue dissolved in 10% diethyl ether in petroleum ether, and put on a 20-g. silicic acid column (diameter, = 2 cm.). The column was washed with 100 ml. of 10% diethyl ether, 100 ml. of 25% diethyl ether, 100 ml. of 40% diethyl ether, and the alkenylglycerol was then eluted with about 300 ml. of 60% diethyl ether until the fractions gave a negative test for total aldehyde. The alkenylglycerol fractions were combined, evaporated to dryness, and the residue washed three times with cold hexane, yielding 185 μmoles of alkenylglycerol (93% yield).

Preparation of Alkenylglycolaldehyde and Hexadecylglycolaldehyde.—To 88 ml. of ethanol, containing 99 μmoles of alkenylglycerol, were added 20 ml. of 0.01 *M* $NaIO_4$ and 2 ml. of 0.1 *M* potassium phosphate buffer, *p*H 7.0. The mixture was let stand at room temperature in the dark. The extent of the reaction was determined by the decrease in optical density of the reaction mixture at 300 mμ. When the reaction was 95% complete on this basis (about 4 hours), 160 ml. of chloroform and 30 ml. of water were added. The water layer was discarded. The chloroform layer was evaporated to dryness, the residue dissolved in petroleum ether and put on a 10-g. silicic acid column (diameter, 1.5 cm.). The column was washed with 100 ml. of 1% diethyl ether in petroleum ether and then the alkenylglycolaldehyde was eluted with 100 ml. of 4% diethyl ether. The elution of the alkenylglycolaldehyde was rapidly completed as the column was washed with 100 ml. of 10% diethyl ether. Unreacted alkenylglycerol (20 μmoles) was then eluted from the column with diethyl ether. The elution of both these compounds was readily followed by analyzing the tubes for total aldehyde. The yield of alkenylglycolaldehyde was 76 μmoles.

In a similar manner, 16.2 mg. of hexadecylglycerol (51 μmoles) was treated with periodate and isolated by silicic acid chromatography. Although this compound contains no alkenyl ether group, the compound reacts with the fuchsin reagent for aldehydes because of the periodate-produced aldehyde group. About 53 μmoles of hexadecylglycolaldehyde was eluted from the column, based on the total aldehyde analysis.

Preparation of Methyl α-Chlorododecyl Ether.—Dodecanal (56.5 g., 0.307 mole) was dissolved in 60 ml. of hexane in a two-necked flask equipped with a magnetic stirrer. Gaseous HCl was bubbled through this mixture for 1 minute; then 9.8 g. of methanol (0.307 mole) was added dropwise over the next 3 minutes. The HCl ebullition was continued during this addition and for the remaining course of the reaction. No attempt was made to control the temperature during the reaction. Aliquots were removed at various times and analyzed by G.L.C. to determine the extent of reaction. After 40 minutes, 90% of the aldehyde had been converted to the chloro ether which was present in the upper phase of the reaction mixture.

Preparation of Methyl 1-Dodecenyl Ether.—The upper phase from the above reaction was added to 64 g. (0.528 mole) of dimethylaniline and this mixture was heated on the steam-bath for 60 minutes. The mixture was allowed to cool for 2.5 hours, and the upper layer was decanted and filtered. The filtrate was then distilled under a pressure of about 1 mm. Two fractions were collected: fraction 1, 49–60°, was mostly dimethylaniline; fraction 2, 60–100° (90 ml. of distillate, mostly collected between 80–100°) contained *cis*- and *trans*-methyl 1-dodecenyl ether, dodecanal, dimethylaniline, and dimethylaniline hydrochloride. An air condenser was used during the collection of fraction 2 to permit operation of the condenser at a higher temperature and thereby avoid troublesome gels due to dimethylaniline·HCl which tends to sublime at that temperature.

Separation and Purification of *cis* (VII) and *trans* (VIII) Methyl 1-Dodecenyl Ethers.—Twenty ml. of fraction 2 was put on a column containing 1600 g. of alumina (column diameter, 4.5 cm.). Elution was carried out as shown in Fig. 1. Fractions 16–52 were combined, concentrated under nitrogen and distilled at 55° under a pressure of 0.045 mm. to obtain the *cis* isomer. Fractions 57–75 were treated similarly to obtain the *trans* isomer. The yields were 3.0 g. of VII and 2.9 g. of VIII. Had all of the original distillate, fraction 2, been processed in this way, the over-all yield would be 43%.

Anal.[29] Calcd. for $C_{13}H_{26}O$: C, 78.68; H, 13.21; OCH_3, 15.67; iodine no., 127.9, Found: for VII: C, 79.0; H, 13.6; OCH_3, 13.2; iodine no., 128. For VIII: C, 79.3; H, 13.7; OCH_3, 13.7; iodine no., 129.[30]

(29) Elemental analyses were performed by Weiler and Strauss Microanalytical Laboratory, Oxford, Eng.

(30) NOTE ADDED IN PROOF.—Additional evidence for the *cis*-alkenyl configuration of beef heart plasmalogen has been provided recently by Norton, *et al.*, *J. Lipid Res.*, **3**, 456 (1962).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FACULTY OF PURE AND APPLIED SCIENCE, UNIVERSITY OF OTTAWA, OTTAWA, ONTARIO, CAN.]

Stereospecific Total Synthesis of Two 5-Amino-5,6-dideoxy-DL-hexonic Acids, a Novel Class of Aminosugar Related Compounds[1]

BY B. BELLEAU AND YUM-KIN AU-YOUNG

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The stereospecific total synthesis of 5-amino-5,6-dideoxy-DL-allonic acid XVII and 5-amino-5,6-dideoxy-DL-gulonic acid XXVI is described. The reaction sequence involves a Diels–Alder condensation between methyl sorbate and 1-chloro-1-nitrosocyclohexane V to give the adduct VI. The structure and configuration of the latter was established by its conversion to the new aminoacid XI and to 6-methyl-3-piperidinol XIII, which was shown to possess the *trans* configuration by n.m.r. spectroscopy. Hydroxylation of the N-benzoyl-adduct IX with osmium tetroxide followed by hydrolysis and catalytic hydrogenolysis afforded the aminoacid XVII of the allose series. Epoxidation of the N-benzoyladduct IX with peroxytrifluoroacetic acid gave in high yield a 1:1 mixture of the epoxides XIX and XX which were easily separated by crystallization. Reaction of these epoxides with hydriodic gave the pure iodohydrins XXX and XXXI. Structural and configurational assignments to these iodohydrins and the precursor epoxides were made on the basis of the course of the methanolysis of the iodohydrins. Reaction of the epoxides with formic acid gave the diol monoformates XXI and XXII which underwent ready methanolysis to XXIII and XXIV and hydrolysis to a single tetrahydro-1,2-oxazinecarboxylic acid XXV. Hydrogenolysis of the methanolysis products gave an aminoacid XXVI of the gulose series. Treatment of the epoxide XIX with hydrogen chloride in methanol gave the chlorohydrin XXVII, whereas the oxide XX afforded a mixture of the N-debenzoylated chlorohydrin XXVIII and the corresponding lactone XXIX. Spectroscopic properties (infrared and n.m.r.) of several intermediates are reported.

Introduction.—A variety of aminosugars are known to occur in antibiotics,[2] antigenic polysaccharides,[3] cell wall constituents,[4] etc. The major efforts in their synthesis has dealt with alteration of naturally occurring sugars. Their possible availability by total synthesis appears more remote and constitutes a prob-

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(2) E. E. van Tamelen, *Fortschr. Chem. Org. Naturstoffe*, **16**, 90 (1958); A. B. Foster and D. Horton, *Adv. Carbohydrate Chem.*, **4**, 213 (1953); W. G. Overend, *Ann. Rep. Chem. Soc. (London)*, **56**, 286 (1959).

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(4) R. E. Strange and L. H. Kent, *Biochem. J.*, **71**, 333 (1959).