J. Lipid Research, October, 1962
Volume 3, Number 4

The structure of plasmalogens: VI. Configuration of the double bond in the α,β-unsaturated ether linkage of phosphatidal choline*

WILLIAM T. NORTON, EUGENE L. GOTTFRIED, and MAURICE M. RAPPORT †

Departments of Biochemistry, Neurology, and Medicine, Albert Einstein College of Medicine, Yeshiva University, New York 61, N. Y.

[Received for publication June 5, 1962]

SUMMARY

The infrared spectrum of α-1-alkenyl glyceryl ether, prepared from pure phosphatidal choline, shows a singlet at 6.00 μ (1667 cm^{-1}) and a band at 13.55 μ (738 cm^{-1}). These two bands disappear after hydrogenation of the unsaturated ether. No appreciable absorption at 10–11 μ (1000-910 cm^{-1}) was found. This evidence is consistent with the properties of *cis*- and not with those of *trans*-1-alkenyl alkyl ethers, and permits the assignment of the *cis* configuration to the double bond in the α,β-unsaturated ether linkage.

It has been pointed out that the availability of pure phosphatidal choline permits a number of plasmalogen derivatives to be readily prepared (1). These derivatives include the α-1-alkenyl-β-acyl "diglyceride" and the α-1-alkenyl glyceryl ether. In this paper, the preparation and infrared absorption spectrum of the latter compound are described. Absorption bands of the α,β-unsaturated ether group (2–8) can thus be studied without interference from either the phosphate group or the olefinic bonds present in the β-acyl chain. Comparison with infrared spectra of 1-alkenyl ethers of known configuration (2, 3) indicates that the α,β-unsaturated ether double bond of phosphatidal choline has the *cis* configuration.

EXPERIMENTAL METHODS

Infrared Spectra. These were taken with a Perkin-Elmer model 237 double grating instrument. The lipids were examined in both CS_2 and CCl_4 solutions in a cell of 1.0 mm path length with solute concentrations of 8–10 mg/ml, using a matched reference cell.

Analytical Methods. These were the same as cited in earlier papers (1, 9, 10). α,β-Unsaturated ether content was measured by a spectrophotometric iodination method (1).

Preparation of Phosphatidal Choline. This was prepared by selective enzymic hydrolysis of the phosphatidal choline component of beef heart lecithin by *Crotalus atrox* venom (1).

Preparation of α-1-Alkenyl Glyceryl Ether from Phosphatidal Choline. This substance was prepared by alkaline hydrolysis of the product obtained by enzymic removal of the phosphorylcholine residue with *Clostridium perfringens* enzyme (11). A sample of 50 mg (60 μmoles) of phosphatidal choline was suspended in 8.0 ml of 0.01 M aqueous $CaCl_2$. To this suspension was added 6.7 mg of enzyme preparation (dialyzed, lyophilized *C. perfringens* culture filtrate) dissolved in 4.0 ml of 0.01 M $CaCl_2$ and two drops of 0.02% phenol red. Neutrality was maintained by the addition of a total of 1.4 ml of 0.05 N NaOH in increments of 0.10 ml over a period of $3^1/_2$ hr at about 25° (calculated, 1.2 ml). The reaction mixture was extracted with four 10-ml portions of hexane, and the combined extracts were evaporated to dryness. The residue was dried over P_2O_5 *in vacuo* at room temperature to give 35.1 mg (91%) of light yellow oil. This crude product was purified by chromatography on 5 g of Unisil silicic acid, 200–325 mesh (Clarkson Chemical Company). The lipid was placed on the column of adsorbent (12 x 100 mm) in a small volume of hexane, and material eluted with 150 ml of 20% ethanol in hexane was col-

* This work was supported by U. S. Public Health Service grants A-2965, B-1006, B-1570, B-2476, H-3838, M-2562, 2M-6418.

† American Cancer Society Professor of Biochemistry.

lected, giving 32.2 mg (84%) of light yellow oil. This material was dissolved in 18 ml of ethanol. To this was added 2.0 ml of 1.0 N NaOH, and the mixture was incubated with shaking at 37° for 90 min. After removal of excess alkali with 0.5 ml of ethyl formate (12), the reaction mixture was evaporated to dryness and taken up in a mixture of 10 ml of water plus 10 ml of hexane. The hexane phase was separated and the aqueous phase successively extracted with 5 ml of hexane and two 5-ml portions of ether. The combined extracts, taken to dryness, gave 34.8 mg of a mixture of desired product and fatty acids. Separation was effected on a Florisil column by the method of Carroll (13). The material was applied to 5 g of Florisil deactivated with 7% water. Three bulk fractions were collected as follows: Fraction I, 25 ml of 50% ether in hexane, 4.0 mg; fraction II, 50 ml of 2% methanol in ether, 13.8 mg; and fraction III, 50 ml of methanol, 19.8 mg.

Fraction II contained the α-1-alkenyl glyceryl ether and had the following analysis: 2.95 μmoles of iodine/mg corresponding to 3.21 μmoles of α,β-unsaturated ether/mg; ester, 0.17 μmoles/mg. This fraction represented 77% recovery based both on weight and on α,β-unsaturated ether content. The molecular weight based on one α,β-unsaturated ether group per mole is 312; the theoretical molecular weight for $C_{19}H_{38}O_3$ is 314.

The infrared spectrum of this material in CCl_4 solution confirmed the presence of a small amount of ester impurity (very weak band at 5.75 μ). In order to purify the compound further, 6.0 mg of Fraction II was resaponified for 45 min exactly as before, and chromatographed on 1 g of Florisil, using the same solvents as before. The second fraction (10 ml of 2% methanol in ether) yielded 4.3 mg of colorless solid. The infrared spectrum of this material in both CCl_4 and CS_2 was the same as that obtained before resaponification with the exception that the ester absorption was reduced to less than 1/3 of that obtained initially, indicating less than 2% ester impurity. This material gave a single spot, R_f about 0.10, by thin-layer silicic acid chromatography using hexane-ether-acetic acid 60:40:1 (14).

Preparation of α-Alkyl Glyceryl Ether. A solution of 4 mg of resaponified α-alkenyl glyceryl ether in 4.0 ml of 95% ethanol was hydrogenated for 40 min at room temperature and atmospheric pressure of hydrogen over 9.8 mg of platinum oxide catalyst. After removal of the catalyst by centrifugation, the supernatant solution was taken to dryness to give 4 mg of colorless product.

Preparation of Batyl Alcohol. Batyl alcohol was obtained in 61% yield by peracetic acid oxidation of allyl-octadecyl ether according to Kornblum and Holmes (15). Three crystallizations from ethanol gave fine colorless crystals, m.p. 70–71° (15).

Preparation of 1-Butenyl Ethyl Ether. Dealcoholization of 1,1-diethoxybutane was carried out in the following manner. To a 1-liter flask fitted with a dropping funnel, stirrer, thermometer, and Vigreux column with still head was added 300 ml of a purified kerosene fraction (b.p. 190–215°), 3.0 g of p-toluene sulfonic acid, and 1.0 ml of 85% H_3PO_4. To the rapidly stirred mixture heated at 190–200°, 200 g of 1,1-diethoxybutane was added dropwise. The product was collected in an ice bath. The still head temperature was kept between 90–100°. The distillate (185 g) was washed twice with ice-cold distilled water. The organic layer (101 g) was dried over K_2CO_3 and distilled through a 30-cm helix-packed column. The distillate boiling at 92–105° (57.5 g, 42%) was 77% pure by α,β-unsaturated ether analysis. The product was freed of carbonyl impurity by refluxing with 10 volumes of 1 N NaOH in 95% ethanol for 60 min, and recovered by steam distillation. The purified material, when carefully redistilled, had a boiling range of 92–96° (b.p. reported in (16) as 93–95° and in (17) as 94.9–95.3°). Fractions taken throughout the boiling range all took up 0.94–0.95 moles of I_2 per mole of compound by α,β-unsaturated ether analysis (18).

RESULTS

α-1-Alkenyl Glyceryl Ether. The infrared spectrum of this compound (Fig. 1, curve A) has the following main features. The —C=C—stretching absorption is a single band almost exactly at 6.00 μ (1667 cm^{-1}), the same position found for phosphatidal choline (1). The =C—H deformation band is at 13.55 μ (738 cm^{-1}) and is easily distinguishable from the —$(CH_2)_n$—rocking band at 13.8 μ (725 cm^{-1}). There is no appreciable absorption in the 10.7-μ region. These are the principal characteristics of 1-alkenyl ethers of the *cis* configuration (2, 3).

There is a sharp —OH band at 2.78 μ (3597 cm^{-1}) and a weak band at 3.28 μ (3049 cm^{-1}) attributable to the =C—H group (4). The shoulder at 8.8 μ (1136 cm^{-1}) on the strong ether absorption at 9.0 μ (1110 cm^{-1}) may represent the first of two bands that are characteristic of structures of the type "=C—O—C" (8). The strong band at 9.43 μ (1060 cm^{-1}) can be attributed to hydroxyl groups.

α-Alkyl Glyceryl Ether. The bands at 6.0 μ and 13.55 μ assigned to the double bond of the α,β-unsaturated ether disappear in the spectrum of the hydrogenated derivative (Fig. 1, curve B). The shoulder at 8.8 μ also disappears, leaving a single, strong ether band. The remainder of the spectrum is little changed from that of the α-1-alkenyl glyceryl ether. The infrared

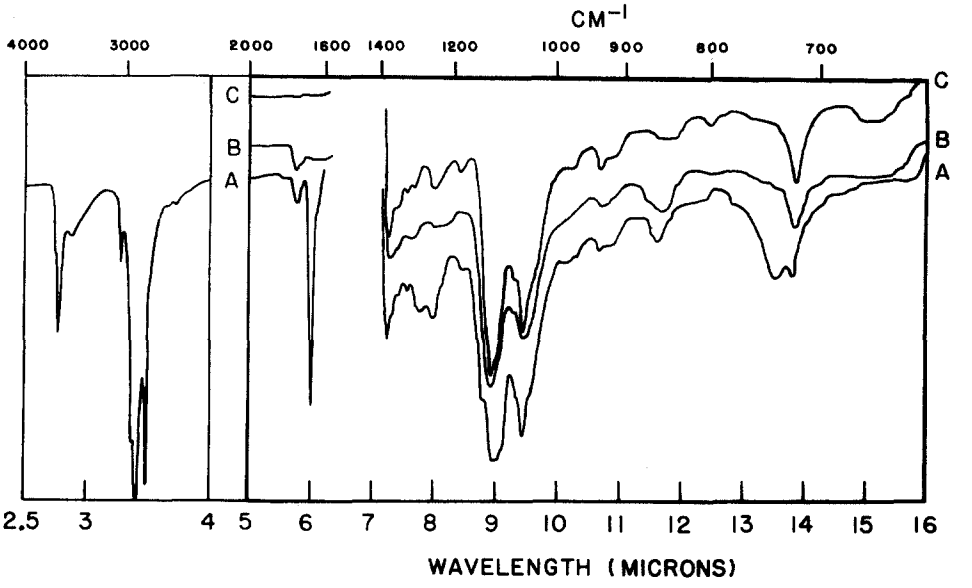

FIG. 1. Infrared spectra in CS_2 solution of (A) α-1-alkenyl glyceryl ether, (B) α-alkyl glyceryl ether, and (C) Synthetic batyl alcohol. Curves A and B have been displaced downward for purposes of comparison. The 2.5- to 4.0-μ regions of B and C have been omitted for clarity of presentation. See text for a discussion of this region of B and C. The region of strong solvent absorption (6.2–7.2 μ) has been omitted.

spectrum of this saturated ether derivative compares well with that of pure synthetic batyl alcohol (curve C) except that it is less well defined, probably because a variety of long-chain alkyl residues are present in the natural product. In the 2.5- to 4.0-μ region (not shown in Fig. 1), the 3.28-μ band is absent. The broad character of the —OH band in this region indicates that the hydroxyl groups of the natural product derivative are more strongly hydrogen bonded than those of batyl alcohol. The reason for this is not understood.

DISCUSSION

Vinyl ethers (4–7, 19) and substituted vinyl ethers (2, 3, 20) may be characterized by several absorption bands in the infrared spectrum. Very strong absorptions of the C—O stretching frequency between 8 and 9 μ (1250–1100 cm^{-1}) differ from those of normal ethers, and absorptions characteristic of the double bond adjacent to oxygen are different in both intensity and frequency from those obtained with normal olefins.

The 6-μ (1650 cm^{-1}) —C=C—Stretching Region. This region is the most useful for detecting the presence of the α,β-unsaturated ether group. When double bonds are conjugated with phenyl, carbonyl, or other olefinic groups, the absorption intensity of this band is markedly increased (21). The effect of an ether linkage is similar to that of conjugation. An examination of the published spectra of vinyl acetate and allyl acetate indicates an eightfold increase in intensity of the 6.06-μ (1650 cm^{-1}) band in vinyl acetate (6). Some extinction coefficients obtained for a series of allyl and vinyl ethers show a six- to twentyfold increase in the vinyl ether absorption intensity in this region (5). Our own experience with *substituted* vinyl ethers (1-butenyl ethyl ether, methyl-1-octadecenyl ether, and various plasmalogens and their derivatives) show that the intensity of this band in these compounds is 8 to 12 times higher than that observed in methyl oleate and triolein.

Whereas vinyl ethers have two strong bands in this region (2, 4, 5, 7, 19), the nature of the absorption of *β-alkyl substituted* vinyl ethers depends on the configuration of the double bond. Hall *et al.* (2) showed that *trans*-1-butenyl butyl ether has a strong doublet at about 6.02 μ (1660 cm^{-1}) and *cis*-1-butenyl butyl ether has a single band at the same wavelength. Dege, Harris, and MacKenzie (3) found that *trans*-1-propenyl butyl ether has a strong doublet at 5.95 μ (1680 cm^{-1}) and 6.02 μ (1660 cm^{-1}) while the *cis* isomer has only one band at 5.98 μ (1672 cm^{-1}).

The α-1-alkenyl glyceryl ether and its parent compound, phosphatidal choline (1), have only a single sharp band at 6.00 μ (1667 cm^{-1}), which indicates that the double bond of the enol-ether has a *cis* configuration.

The 10- to 15-μ Region (1,000–670 cm^{-1}). The out-of-plane deformation vibrations of the hydrogens attached to the double bond occur in this region. With

compounds containing *trans* double bonds isolated from the influences of polar groups, the characteristic band near 10.3 μ (970 cm^{-1}) is observed. Normally *cis* double bonds have a variable absorption band near 14.0 μ (700 cm^{-1}) (21). In substituted vinyl ethers, however, this band has more useful diagnostic value. In both *trans*-1-butenyl butyl ether (2) and *trans*-1-propenyl butyl ether (3), the 10.3-μ band is shifted to 10.72 μ (933 cm^{-1}). *cis*-1-Butenyl butyl ether has a strong band at 13.64 μ (733 cm^{-1}) and in *cis*-1-propenyl butyl ether this band occurs at 13.83 μ (723 cm^{-1}). Neither of the *cis* isomers has a strong band in the 9.7–11.7-μ (1030–850 cm^{-1}) region; similarly, the *trans* isomers have no strong bands in the 12–14-μ (830–700 cm^{-1}) region. Hydrogenation of either *cis* or *trans* isomers results in the disappearance of all bands characteristic of the double bond.

The infrared spectrum of the α-1-alkenyl glyceryl ether has the features one would expect for an α,β-unsaturated ether of the *cis* configuration: a band at 13.55 μ (738 cm^{-1}), no strong absorption in the 10.7-μ region, and a singlet at 6.00 μ. Further confirmation of this assignment is the fact that both bands disappear on hydrogenation.

Our studies of a mixed isomer preparation of 1-butenyl ethyl ether further indicate the usefulness of the correlations established from the studies of 1-butenyl ether (2) and 1-propenyl butyl ether (3). Lower boiling fractions of this compound (enriched in the *cis* isomer) show a strong band at 13.55 μ (738 cm^{-1}) and a weak band at 10.65 μ (939 cm^{-1}), whereas the opposite is found in higher boiling cuts enriched in the *trans* isomer. The fractions having high *trans* content also have a shoulder at 5.98 μ (1,672 cm^{-1}) as well as the strong band at 6.02 μ (1661 cm^{-1}).

In order to study the spectral properties of the α,β-unsaturated ether bond of phosphatidal choline, it was necessary to prepare the simplest derivative possible. The phosphorylcholine group had to be removed because the strong band at 10.3 μ (970 cm^{-1}), characteristic of choline phosphatides, interfered with the 10.7-μ region sufficiently to introduced ambiguity into the interpretation. The finding that the alkenyl chain in the α-position of phosphatidal choline is predominantly saturated (with the exception of the enol-ether double bond) and that the normal olefinic unsaturation is restricted to the β-acyl chain (1) makes this study of configuration unambiguous. The degradation product, α-1-alkenyl glyceryl ether, is thus essentially free of olefinic groups other than the one under consideration.

A similar attempt to deduce configuration directly from the infrared spectra of crystalline lysophosphatidalethanolamine (22) and its hydrogenated derivative was frustrated by complications attributable to the presence of the phosphorylethanolamine residue.

REFERENCES

1. Gottfried, E. L., and M. M. Rapport. *J. Biol. Chem.* **237:** 329, 1962.
2. Hall, R. H., A. R. Philpotts, E. S. Stern, and W. Thain. *J. Chem. Soc.* 3341, 1951.
3. Dege, G. J., R. L. Harris, and J. S. MacKenzie. *J. Am. Chem. Soc.* **81:** 3374, 1959.
4. Meakins, G. D. *J. Chem. Soc.* 4170, 1953.
5. Davison, W. H. T., and G. R. Bates. *J. Chem. Soc.* 2607, 1953.
6. Rosenkrantz, H., and M. Gut. *Helv. Chim. Acta* **36:** 1000, 1953.
7. Teeter, H. M., E. J. Dufek, C. B. Coleman, C. A. Glass, E. H. Melvin, and J. C. Cowan. *J. Am. Oil Chemists' Soc.* **33:** 399, 1956.
8. Tschamler, H., and R. Leutner. *Monatsh. Chem.* **83:** 1502, 1952.
9. Rapport, M. M., and N. F. Alonzo. *J. Biol. Chem.* **235:** 1953, 1960.
10. Rapport, M. M., and N. Alonzo. *J. Biol. Chem.* **217:** 199, 1955.
11. Hanahan, D. J., and R. Vercamer. *J. Am. Chem. Soc.* **76:** 1804, 1954.
12. Dawson, R. M. C. *Biochem. J.* **75:** 45, 1960.
13. Carroll, K. K. *J. Lipid Research* **2:** 135, 1961.
14. Vogel, W. C., W. M. Doizaki, and L. Zieve. *J. Lipid Research* **3:** 138, 1962.
15. Kornblum, N., and H. N. Holmes. *J. Am. Chem. Soc.* **64:** 3045, 1942.
16. Deschamps, J., M. Doty, and P. Pineau. *Compt. Rend.* **238:** 2006, 1954.
17. Voronkov, M. G. *J. Gen. Chem. USSR* **20:** 2060, 1950.
18. Siggia, S., and R. L. Edsberg. *Anal. Chem.* **20:** 762, 1948.
19. Butler, G. B. *J. Am. Chem. Soc.* **77:** 482, 1955.
20. Erickson, J. L. E., and M. Z. Woskow. *J. Org. Chem.* **23:** 670, 1958.
21. Bellamy, L. J. *The Infra-red Spectra of Complex Molecules.* New York, John Wiley & Sons, Inc., 2nd ed., 1958, pp. 34–56.
22. Rapport, M. M., B. Lerner, N. Alonzo, and R. E. Franzl. *J. Biol. Chem.* **225:** 859, 1957.