acyl diradical in the photochemical decomposition of cyclobutanone in the gas phase. Further investigation in this direction will be reported in more detail.

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The Structure and Configuration of "Neutral Plasmalogens" [1]

Sir:

Previous reports suggested the occurrence of minute amounts of aldehydogenic neutral lipids in various mammalian tissues.[2] Small quantities of "neutral plasmalogens" have also been found in man.[3]

We have detected neutral plasmalogens in flesh, liver, and eggs of the shark *Hydrolagus colliei* ("ratfish") and have separated them by adsorption chromatography from the major lipid constituents of these tissues, *viz.*, O-alkyldiglycerides and triglycerides.[4] The total lipids of ratfish liver contained about 5% of neutral plasmalogens and thus constituted a convenient source. A pure fraction (**1**) was obtained by repeated chromatography of ratfish liver lipids on layers of silicic acid.[5] Fraction **1** yielded O-alkyldiglycerides (**2**) upon catalytic hydrogenation. Treatment with hydrochloric acid[4] did not affect **2**, whereas **1** yielded a mixture of aldehydes and diglycerides. Reaction of **2** with methanolic hydrogen chloride at 80° yielded alkyl glycerol ethers and methyl esters of fatty acids; **1** afforded dimethyl acetals of aldehydes and methyl esters. Upon reaction with lithium aluminum hydride in diethyl ether and subsequent acid-catalyzed hydrolysis, **2** afforded alkyl glycerol ethers and alcohols, **1** afforded aldehydes and alcohols. In contrast, decomposition of the lithium alumino complex obtained from **1**, by water, gave alcohols and alk-1-enyl glycerol ethers. The latter compounds migrated on adsorbent layers slightly ahead of alkyl glycerol ethers and could be cleaved to aldehydes and glycerol by acid-catalyzed hydrolysis.

These findings indicated that the fraction (**1**) isolated from ratfish liver lipids consisted of O-alk-1-enyldiglycerides. The quantity we have prepared was sufficient to determine the position of the alk-1-enyloxy group, the optical configuration of the glycerol moiety, and the geometric configuration of the enolic double bond.

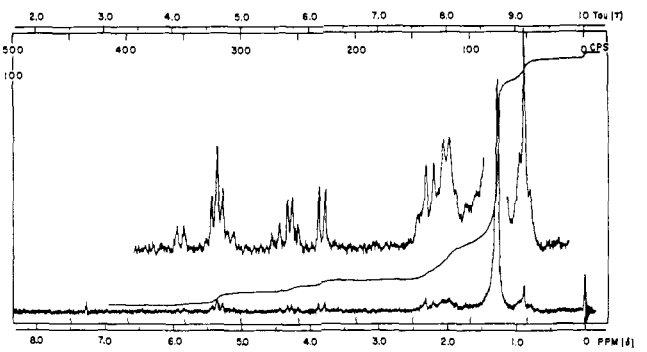


Figure 1.

The alkyl glycerol ethers derived from **1** could be cleaved with sodium metaperiodate and formed isopropylidene ketals with acetone. These reactions and the optical activity of both **1** and **2** proved that these compounds were derivatives of alkyl 1-glycerol ethers, *i.e.*, the asymmetrical isomers. The specific optical rotation[6] of **1** was $[\alpha]^{25}D$ +2.0° (*c* 1.9); that of **2** was $[\alpha]^{25}D$ −2.8° (*c* 1.8), which is in numerical agreement with the specific optical rotation found for a synthetic L-alkyldiglyceride[7] ($[\alpha]^{25}D$ +4.0° (*c* 2.6)). Thus, **1** can be assigned to the D series to which all naturally occurring alkyl 1-glycerol ethers belong.[8,9]

The infrared absorption spectrum[10] of **1** is rather similar to that of **2** and to a synthetic O-alkyldiglyceride.[7] Additional bands associated with the enol ether grouping are those near 1668–1666 and 732–730 cm^{-1}, which are due to a stretching vibration of the carbon double bond in the position α to the ether linkage and to a C–H out-of-plane deformation of the same group, respectively. Both bands were found also in spectra of naturally occurring alk-1-enyl acyl glycerophosphatides and were shown to be strictly associated with the *cis*-alk-1-enyl ether linkage, whereas the spectra of *trans*-alk-1-enyl ethers are known to exhibit doublets near 1670 cm^{-1} and a band near 930 cm^{-1}.[11,12]

The nuclear magnetic resonance spectrum[13] of **1** (see Figure 1) shows a doublet centered at δ 5.89 ppm which accounts for the olefinic hydrogen at the carbon in the position α to the ether linkage. This doublet is associated with the hydrogen at the *cis* enol ether bond, whereas the *trans* isomer would show a doublet near 6.20 ppm (and a pair of triplets near 4.78 ppm).[12] The signal of the olefinic hydrogen at C-2′ near 4.5 ppm is partially embedded in the multiplet centered at 4.30

(1) This investigation was supported in part by Public Health Service Research Grants GM 05817 and HE 08214 from the National Institutes of Health.

(2) M. L. Karnovsky, S. S. Jeffrey, M. S. Thompson, and H. W. Deane, *Biophys. Biochim. Cytol.*, 1, 173 (1955); J. Eichberg, J. R. Gilbertson, and M. L. Karnovsky, *J. Biol. Chem.*, **236**, PC15 (1961); J. C. M. Schogt, P. H. Begemann, and J. Koster, *J. Lipid Res.*, **1**, 446 (1960); J. R. Gilbertson and M. L. Karnovsky, *J. Biol. Chem.*, **238**, 893 (1963).

(3) H. H. O. Schmid, N. Tuna, and H. K. Mangold, *Z. Physiol. Chem.*, **348**, 730 (1967).

(4) H. H. O. Schmid and H. K. Mangold, *Biochim. Biophys. Acta*, **125**, 182 (1966).

(5) Plates coated with layers of silica gel H (Merck), 2 and 0.5 mm thick, were developed twice with hexane–diethyl ether (95:5, v/v). Diethyl ether was used to elute the material from the adsorbent.

(6) Optical rotations were measured at 25° with a Bellingham and Stanley polarimeter, using chloroform as solvent.

(7) W. J. Baumann and H. K. Mangold, *J. Org. Chem.*, **29**, 3055 (1964); W. J. Baumann and H. K. Mangold, *Biochim. Biophys. Acta*, **116**, 570 (1966).

(8) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **140**, 397 (1941).

(9) Alk-1-enyl acyl glycerophosphatides ("plasmalogens") recently were shown to possess the D configuration as well: J. C. Craig, D. P. G. Hamon, K. K. Purushothaman, S. K. Roy, and W. E. M. Lands, *Tetrahedron*, **22**, 175 (1966).

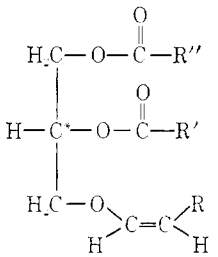
(10) Infrared spectra were recorded with a Perkin-Elmer Model 21 spectrophotometer. Carbon disulfide served as solvent, except in the ranges 2400–2000 and 1650–1400 cm^{-1}, where tetrachloroethylene was used.

(11) W. T. Norton, E. L. Gottfried, and M. M. Rapport, *J. Lipid Res.*, **3**, 456 (1962); H. R. Warner and W. E. M. Lands, *J. Am. Chem. Soc.*, **85**, 60 (1963).

(12) J. C. Craig and D. P. G. Hamon, *J. Org. Chem.*, **30**, 4168 (1965).

(13) Nuclear magnetic resonance spectra were taken in $CDCl_3$ with tetramethylsilane as the internal standard using a Varian A-60A spectrometer.

ppm which is considered to be characteristic of the acylated H_2CO group of glycerol.[14] Additional signals are: a triplet at 0.89 ppm, accounting for the terminal methyl groups; a single peak at 1.28 ppm, associated with the internal methylene groups of the aliphatic chains; a triplet at 5.37 ppm, representing the isolated olefinic groups; and an apparent doublet corresponding to the CH_2 groups in the position α to the carbon double bonds centered at 2.03 ppm. The doublet which appears at 2.27 ppm can be correlated with an α-CH_2 group of the acyl functions. The apparent doublet occurring at 3.83 ppm can be assigned to the CH_2O group of glycerol connected to the ether linkage. The signal of the acylated HCO group of glycerol near 5.17 ppm is partially obscured by the triplet representing the isolated carbon double bonds. A signal near 2.9 ppm, characteristic of a methylene group between two double bonds,[14] is absent. Approximately two out of three aliphatic chains per molecule are monounsaturated.



Chemical reactions, specific optical rotations, and spectroscopic data prove that the fraction (**1**) isolated from the liver of *Hydrolagus colliei* consisted of D(+)-1-O-*cis*-alk-1′-enyldiglycerides.

(14) C. Y. Hopkins in "Progress in the Chemistry of Fats and Other Lipids," Vol. 8, R. T. Holman, Ed., Pergamon Press, New York, N. Y., p 213.

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Oligodeoxynucleotide–Polydeoxynucleotide Interactions. Adenine–Thymine Base Pairs[1]

Sir:

Quantitative data on the interaction of structurally defined oligonucleotides with complementary polynucleotides are of value in practical and theoretical studies on nucleic acid structure. An investigation of oligodeoxynucleotide–polydeoxynucleotide interactions, covering a broad range of chain lengths in both members of the interacting pairs, displays partial confirmation of theory and some anomalous behavior.

A series of oligodeoxynucleotides, $d(pA)_m$ and $d(pT)_m$[2] with $m = 2$–25, was prepared by DNase I degradation of $d(pA)_n$ and $d(pT)_n$ followed by chromatography on diethylaminoethylcellulose.[3] The melting behavior of the complexes $d(pA)_m + d(pT)_n$, $d(pA)_n + d(pT)_m$, and $d(pA)_n + d(pT)_n$, formed by mixing the constituents in a suitable solvent, was then observed by ultraviolet absorbance changes. The stoichiometry of the interactions was determined from mixing curves or (more directly) by gel filtration on Sephadex G-200.[4]

The interaction of $d(pA)_n$ with $d(pT)_m$ follows the relationship $1000/T_{1/2} = A + B/m$ and the stoichiometry is 1:1 in equimolar mixtures in all solvents used (Table I). This interaction is a multimolecular process

Table I. Melting Transitions for Oligodeoxythymidylate–Polydeoxyadenylate Complexes[a]

Solvent	Oligo-P concn, $M \times 10^5$	A	B
NaCl, 0.15 M; sodium citrate, 0.015 M (SSC)	14.0	2.88	4.57
	55.0	2.88	4.13
Potassium phosphate, 40 mM, pH 7	13.5	2.97	4.78
Potassium phosphate, 40 mM, pH 7, with 8 mM $MgCl_2$	14.5	2.88	4.26

[a] The relationship, $1000\,T_{1/2} = A + B/m$ is observed. The values in the table are A and B for this equation, in different solvents and at different oligodeoxynucleotide concentrations (expressed in nucleotide residues). Polymer is present in an equivalent amount.

and thus $T_{1/2}$ values[5] (Table I) and the slope of the transition are quite sensitive to oligonucleotide concentration. These results are in accord with statistical thermodynamic treatment of oligo and polymer interactions.[6]

The interaction of equimolar amounts of $d(pA)_m$ with $d(pT)_n$ in SSC is complicated by a change in the stoichiometry of the *major* complex present to 1A–2T when $m < 16$. The double-stranded (1:1) complex is not detected in the interaction involving $d(pA)_7$, but it is clearly evident as an early rise in absorbance at 260 mμ (or a decrease at 284 mμ) when $d(pA)_m$, $m = 8$–12, complexes with $d(pT)_n$ are melted. The presence of complexes of different stoichiometry in the mixture could result from a slow approach to equilibrium. Repeated analysis on an equimolar mixture of $d(pA)_8$ and $d(pT)_n$ over a period of 3 months demonstrated that the amounts of double helix and triple helix, detected by melting, remain unchanged. We conclude that an equilibrium mixture of double and triple helices are present in this case. The triple-stranded complexes $d(pA)_m$–$2d(pT)_n$ (with $m < 16$), obtained by mixing the

(1) Supported by Grant CA-08487 from the U. S. Public Health Service.

(2) Abbreviations used: m, oligonucleotide chain length; n, a polymer chain length greater than 300 nucleotides; $T_{1/2}$, temperature at the midpoint of the ultraviolet thermal transition in degrees Kelvin; $d(pA)_m$ and $d(pT)_m$, deoxyadenylate and deoxythymidylate oligonucleotides bearing a 5′-phosphate group and a 3′-hydroxyl group; $d(pA)_n$, polydeoxyadenylate; $d(pT)_n$, polydeoxythymidylate; DNase I, pancreatic deoxyribonuclease; ribonucleotide polymers are prefixed by r.

(3) F. J. Bollum, "Procedures in Nucleic Acid Research," G. Cantoni and D. Davies, Ed., Harper and Row, Inc., New York, N. Y., 1966, pp 577–592.

(4) Separations were made on a 1 × 100 cm column on Sephadex G-200 equilibrated with the appropriate solvent and developed at a temperature about 15° below the $T_{1/2}$ of the complex to be tested. Detailed results will be presented in a future publication.

(5) M. N. Lippsett, L. A. Heppel, and D. F. Bradley, *Biochim. Biophys. Acta* **41**, 175 (1960), have observed the dependence upon the total nucleotide concentration in a study of the interactions of $r(pA)_m$ with poly rU.

(6) (a) W. S. Magee, Jr., J. H. Gibbs, and B. H. Zimm, *Biopolymers*, 1, 133 (1963). Note that the authors have called attention to the dependence on absolute activity of the oligonucleotide in eq 19, and in accordance with their treatment our $B = B'$ in CD, where B' = slope at unit activity, C = absolute activity of the m-mer, and D = the ratio of two internal partition functions. We assume that the absolute activity is essentially constant for all m-mers at the nucleotide residue concentration used (15×10^{-6} M). (b) W. S. Magee, Jr., J. H. Gibbs, and G. F. Newell, *J. Chem. Phys.*, **43**, 2115 (1965).