Analogs of natural lipids. VII.[1] Synthesis of cyclopentanoid analogs of phosphatidylcholine[2]

Anthony J. Hancock,[3] Mark D. Lister, and Henry Z. Sable

Department of Chemistry and School of Medicine, University of Missouri, Kansas City, MO 64110, and Department of Biochemistry, Case Western Reserve University, Cleveland OH 44106[4]

Abstract A series of six analogs of phosphatidylcholine (lecithin) has been synthesized, in which the conformational mobility of the backbone is restricted. The analogs are derivatives of the three diastereoisomeric cyclopentane-1,2,3-triols and were obtained by triisopropylbenzenesulfonyl chloride-mediated condensation of isomers of dipalmitoylcyclopentanetriol phosphate (*cyclopentano*-phosphatidic acid) with choline tosylate. The *cyclopentano*-lecithins obtained include the following: 1,2,3/0-(1P); 1,2,3/0-(2P); 1,2/3-(1P); 1,2/3-(3P); 1,3/2-(1P); 1,3/2-(2P). The two -2P derivatives are *meso*-forms while the other four derivatives are *DL*-pairs. Each lecithin analog has been obtained as a stable microcrystalline solid. Elemental analysis indicates that the compounds are hydrated; the data were consistent with the presence of either one, or in two instances, of one-half molecule of water of hydration. The infrared spectra, melting behavior, and chromatographic mobility of each of the analogs resembled those obtained for dipalmitoyllecithin, but the influence upon physical properties of stereochemical differences among the analogs was observed throughout the series.—**Hancock, A. J., M. D. Lister, and H. Z. Sable.** Analogs of natural lipids. VII. Synthesis of cyclopentanoid analogs of phosphatidylcholine. *J. Lipid Res.* 1982. **23:** 183–189.

Supplementary key words lecithin analogs • *cyclopentano*-lipids • restricted conformational mobility • cyclitols

The two collaborating laboratories have undertaken the systematic synthesis and study of *cyclopentano*-lipids, a new class of conformationally restricted lipids. Previously the synthesis of three homologous series of tris-*homo*acyl lipids derived from each of the three diastereoisomeric cyclopentane-1,2,3-triols (i.e., *cyclopentano*-analogs of triglycerides) has been reported (2). The synthetic program was extended to include the production of cyclopentanoid analogs of diacylglycerophosphoric acid (phosphatidic acid) (3–5) in the belief that the compounds would not only themselves have significant biological properties, but also that they would serve as useful intermediates for the synthesis of conformationally restricted analogs of membrane phospholipids.

Substantial advances in understanding the substrate specificity of phospholipids, as well as the properties of membranes of which they are a part, may be attained by a study of lipid molecular conformation. The relatively free rotation about C-C single bonds in glycerol derivatives allows them to adopt, in principle, a large number of rotameric forms. Although a few of these forms are energetically favored, the actual conformation of the phospholipid molecule is a matter of speculation. However, the three cyclopentane-1,2,3-triols are plausible analogs of glycerol; study of lipids synthesized from them may allow assessment of the rotameric state of the glycerol backbone in natural phospholipids during physiological involvement. Lending credence to this premise is our recent finding[5] that the cyclopentanoid analogs of *sn*-glycerol-3-phosphate vary in substrate activity to rabbit muscle glycerol-3-phosphate dehydrogenase depending on their particular configuration. The enzyme catalyzed the hydrolysis of two isomers [1,2/3-(1P) and 1,2,3/0-(1P)] but was inhibited by the other three isomers. The data allowed definition of the stereochemical requirements for substrate activity in terms of molecular configuration, and thus allowed deduction of the "preferred conformation" of the glycerol backbone in *sn*-glycerol-3-phosphate during the catalytic event. That similar information can be obtained about glycerolipids by means of cyclopentanoid analogs is established by our demonstration that in an isomeric series of dipalmitoylcyclopentanetriol phosphates (*cyclopentano*-PA series) (3, 4), only one isomer [1,3/2-(1P)] was susceptible to a PA-

Abbreviations: PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; HPLC, high pressure liquid chromatography; TLC, thin-layer chromatography; TPS, triisopropylbenzenesulfonyl chloride. Cyclic compounds described in this paper are named according to the tentative Rules for Nomenclature of Cyclitols (1). The names are derived from those of the parent cyclanes of which they are formal derivatives. A summary of these rules has been presented in an earlier communication (2). 1-Phosphates and 2-phosphates are denoted, respectively, by the suffix -1P and -2P.

[1] For paper VI, see Gallo, Ref. 9.

[2] The authors dedicate this paper to the memory of their late colleague, Professor Merton F. Utter.

[3] To whom correspondence and reprint requests should be sent.

[4] H. Z. Sable.

[5] Weissman, J. D., et al. 1982. *J. Biol. Chem.* In press.

phosphohydrolase from canine lung surface-active material.[6] The other isomers had little or no activity. These findings, together with the data from physical studies of some of the compounds (6–9) which have led to an increased understanding of the conformational attitudes assumed by the glycerol backbone of natural triglycerides, have prompted us to develop methods for the synthesis of cyclopentanoid analogs of phosphatidylcholine, 4c–9c, (**Fig. 1**), so that studies of the effect of configurational differences in the isomeric series may be assessed. The properties of these compounds approximate more realistically those of a membrane amphiphile than do the *cyclopentano*-PA isomers themselves. The *cyclopentano*-PC isomers described herein have been synthesized from *DL*- or *meso*-PA analogs; no attempt has yet been made to resolve the optical antipodes.

EXPERIMENTAL

Materials and Methods

Melting points were measured on a Thomas Hoover Unimelt capillary melting point apparatus. Infrared spectra were measured for KBr dispersions with a Perkin-Elmer 621 Spectrometer (Perkin-Elmer, Corp., Norwalk, CT) and were calibrated with polystyrene. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Reactions were monitored by thin-layer chromatography on silica gel G (adsorbent thickness, 250 μm; EM Laboratories, Inc., Elmsford, NY). Solvents used were: solvent A, chloroform–methanol–water 65:25:4 (v/v/v); solvent B, chloroform–acetone–methanol–acetic acid–water 6:8:2:2:1 (v/v/v/v/v); solvent C, chloroform–methanol–30% ammonium hydroxide 65:25:4 (v/v/v). Purification of lipid products was by column chromatography on silicic acid buffered with triethylamine, essentially as described by Aneja, Chadha, and Davies (10), or by high pressure liquid chromatography on silicic acid using a Prep LC System 500 liquid chromatograph (Waters Associates, Inc., Milford, MA) according to Patel and Sparrow (11). Phosphates were detected after analytical chromatography by heating with the modified reagent (12) of Dittmer and Lester (13). Amberlite resins (IRA-410 and IR-120-P) used for the purification of the *cyclopentano*-lecithin analogs were obtained from Sigma Chemical Co. (St. Louis, MO). The condensing agent 2,4,6-triisopropylbenzenesulfonyl chloride was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Choline tosylate was prepared from N,N-dimethylethanolamine and methyl *p*-toluenesulfonate as described by Rosenthal (14), recrystallized from dry acetone, and stored in vacuo. The isomeric *cyclopentano*-phosphatidic acids were synthesized as their diphenyl esters as described previously (3–5), and recrystallized twice from methanol to remove traces of pyridine. Pyridine used for condensation reactions generating the *cyclopentano*-lecithins was distilled from barium oxide and stored over molecular sieves (4 Å) and calcium hydride.

[6] Hancock, A. J., and B. J. Benson. Manuscript in preparation.

Cyclopentano-phosphatidic acids

Phenyl groups were removed from the diphenylphosphate esters by hydrogenolysis with platinum catalyst in glacial acetic acid at room temperature as described earlier (3), except that the reactions were done in a Parr apparatus at a hydrogen pressure of 50 p.s.i. The dried *cyclopentano*-phosphatidic acids were freed of traces of cations by a partitioning between chloroform and aqueous methanol according to a modification (15) of the method of Bligh and Dyer (16). The chloroform phase was washed with methanol–water 10:9 (v/v), diluted with pyridine, evaporated to dryness in a rotary evaporator, and dried at room temperature over phosphorus pentoxide in vacuo (0.1 torr). The dried free acid was then used

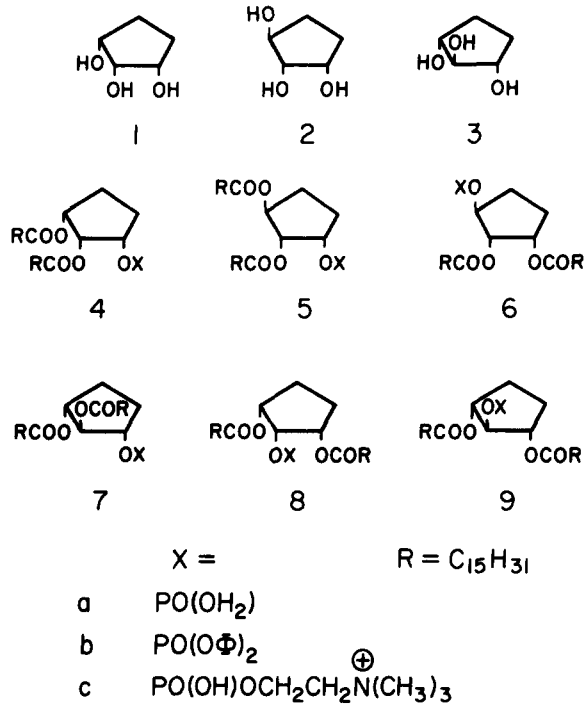

Fig. 1. Configuration of diastereoisomeric cyclopentane triols. 1: (1,2,3/0)-cyclopentane-1,2,3-triol; 2: *DL*-(1,2/3)-cyclopentane-1,2,3-triol; 3: (1,3/2)-cyclopentane-1,2,3-triol. Configuration of *cyclopentano*-phosphatidic acids (4a–9a), *cyclopentano*-phosphatidic acid, diphenyl esters (4b–9b) and *cyclopentano*-lecithins (4c–9c): 4: 1,2,3/0-(1P); 5: 1,2/3-(1P); 6: 1,2/3-(3P); 7: 1,3/2-(1P); 8: 1,2,3/0-(2P); 9: 1,3/2-(2P).

at once for the synthesis of the corresponding *cyclopentano*-lecithin.

Synthesis of (1,3/2)-2,3-dipalmitoylcyclopentano-1-phosphoryl choline (Fig. 1, 7c)[7]

DL-(1,3/2)-2,3-Dipalmitoylcyclopentanetriol-1-phosphate (Fig. 1, 7a) (675 mg; 1 mmol) and choline tosylate (688 mg; 2.5 mmol) were dissolved in anhydrous pyridine (30 ml) with gentle warming (40°C) in a stoppered flask. TPS (1212 mg; 4.0 mmol) was quickly added and the solution was stirred at room temperature for 12 hr. After addition of water (1 ml), the yellow solution was evaporated under reduced pressure to an oil which was then diluted with toluene (25 ml). The solution was evaporated to dryness under reduced pressure, chloroform (250 ml) was added, and the resulting solution was passed through a suspension of Amberlite IR-120-P resin (200 g dry weight) in chloroform. The solvent was removed under reduced pressure to give a tan-colored low melting solid, TLC analysis of which showed a major phosphate-positive spot (R_f 0.23 in solvent A) and a minor unidentified spot at the solvent front. In addition, traces of triisopropylbenzenesulfonic acid (R_f 0.10) were present in the mixture. Small quantities of this mixture (up to 200 mg) were further purified by column chromatography on silicic acid with triethylamine as a buffering agent, as described by Aneja et al. (10) for glycerolipid purification (method 1). The products of larger scale preparations (1–3 g) were purified by high pressure liquid chromatography on silicic acid, essentially as described by Patel and Sparrow (11) (method 2).

Method 1: Silicic acid (50 g) was slurried with chloroform containing 1% freshly distilled triethylamine, poured into a column, and the column was washed with 200 ml of chloroform–triethylamine 200:1 (v/v). A solution of *cyclopentano*-lecithin in $CHCl_3$ (200 mg in 5 ml) was applied to the column, and elution done as follows: chloroform–methanol 95:5 (v/v), 200 ml, and chloroform–methanol 90:10 (v/v); the eluate gave a single spot on TLC in neutral, acidic, and basic solvent systems (see Table 2). The final elution with chloroform–methanol 80:20 (v/v) gave phosphate-positive materials of lower R_f value than the product and which appeared to be degradation products.

Method 2: The use of preparative HPLC substantially decreased the time required for the purification of the *cyclopentano*-lecithins especially for large scale preparations. The single silica column was pre-equilibrated with chloroform–methanol–water 90:45:10 (v/v/v) and the lipid was dissolved in benzene (1.0 g in 10 ml) for loading. Elution with chloroform–methanol–water 85:25:4 (v/v/v) at a flow rate of 200 ml min^{-1} gave *cyclopentano*-lecithin after approximately two column volumes (1000 ml). The eluate was carefully diluted with water until phase separation occurred, and the (lower) chloroform phase was separated and dried with anhydrous Na_2SO_4.

Removal of solvent from the filtered solutions obtained by either method 1 or method 2 gave a residual gum which was dissolved in the minimum volume of chloroform and diluted with ten volumes of cold (4°C) acetone or acetonitrile. The precipitated *cyclopentano*-lecithin (40–50%) was centrifuged at 4°C and dried in vacuo at room temperature. The supernatant solution could be induced to yield a further crop of solid at a lower temperature (−15°C). The total recovery of microcrystalline lipid obtained by precipitation with acetone or acetonitrile was in the range of 60–70%.

Elemental analyses and chromatographic mobilities for each of the six diastereoisomeric *cyclopentano*-lecithins are given in **Tables 1 and 2.** Melting points are listed in **Table 3.** The yields of chromatographically pure lipid obtained before precipitation from solution were generally between 65–95% based on *cyclopentano*-phosphatidic acid.

RESULTS AND DISCUSSION

Synthesis of *cyclopentano*-lecithins

The *cyclopentano*-lecithins have been synthesized by a method based on that developed by Aneja and co-workers (10, 17) for the synthesis of glycerophospholipids. Each of the *cyclopentano*-phosphatidic acid isomers reacted quickly with choline tosylate in pyridine in the presence of triisopropylbenzenesulfonyl chloride to give yields of *cyclopentano*-PC that were comparable to those obtained by Aneja et al. (10) for the glycerolipids. The method differed from that of Aneja et al. (10) in that choline tosylate was substituted for choline acetate, and the reactions were performed at room temperature. As observed by Rosenthal (14), the tosylate salt of choline is substantially more soluble in pyridine at room temperature than is the acetate. The condensation reactions in the present work were thus complete in a few hours at room temperature and were only marginally accelerated by performing the reaction at 70°C. The least satisfactory yield was that for all-*cis*(1,2,3/0-(2P)) isomer (40%). Little or no unreacted *cyclopentano*-PA could be detected in any of the condensation reaction mixtures after 8 hr. However, a small proportion of material hav-

[7] The experimental conditions described above for the synthesis of the all-*trans* (1,3/2)-isomer (7c) were identical to those used for each of the other isomers (4c–6c, 8c, 9c).

TABLE 1. Analytical data for isomeric *cyclopentano*-lecithins

		C	H	N	P	N/P[a]	H_2O[b]
Theory for							
$C_{42}H_{82}O_8NP \cdot H_2O$ (788.07)		64.83	10.88	1.80	3.98	1.00	2.32
$C_{42}H_{82}O_8NP \cdot \frac{1}{2}H_2O$ (769.12)		65.58	10.88	1.82	4.03	1.00	1.18
$C_{42}H_{82}O_8NP$ (760.06)		66.37	10.88	1.84	4.08	1.00	
Compound	Configuration			Found:[c]			
4c	1,2,3/0-(1P)	64.84	11.05	1.56	3.25	1.06	1.12
5c	1,2/3-(1P)	65.15	11.04	1.78	3.93	1.00	1.94
6c	1,2/3-(3P)	65.46	11.14	1.76	3.81	1.02	1.94
7c	1,3/2-(1P)	65.61	10.74	1.72	3.85	0.99	1.15
8c	1,2,3/0-(2P)	65.13	10.70	1.64	3.82	0.96	2.67
9c	1,3/2-(2P)	65.37	10.63	1.80	4.06	0.98	1.80

[a] N/P is ratio gram-atoms N/gram-atoms P.
[b] Karl Fischer analysis.
[c] Dried over phosphorus pentoxide for 24 hr at room temperature (0.2 torr).

ing chromatographic mobilities similar to those of the corresponding *cyclopentano*-PA was occasionally detected during subsequent purification by silicic acid chromatography. Such decomposition on silicic acid was minimized by the addition of triethylamine (0.5%) to the eluting solvents during prolonged chromatographic separations, as described by Aneja et al. (10) for the acid-labile trityl derivative of phosphatidylethanolamine. It appears thus, that the *cyclopentano*-lecithins are more acid-sensitive than their glycero-counterparts. However, each of the isomeric lecithin analogs was stable in the dry state at 4°C or in hexane solution at 4°C. Slight decomposition occurred at room temperature in either of these states as evidenced by the appearance of a minor phosphate-positive spot on TLC (R_f 0.05 in solvent A). Solutions of the lipid in chloroform were still less stable when stored at room temperature, giving rise within a few days to impurities detectable by TLC.

TABLE 2. Chromatographic mobilities of isomeric *cyclopentano*-lecithins

		R_f in Solvent[a]		
Compound	Configuration	A	B	C
4c	1,2,3/0-(1P)	0.15	0.10	0.15
5c	1,2/3-(1P)	0.17	0.12	0.18
6c	1,2/3-(3P)	0.17	0.13	0.17
7c	1,3/2-(1P)	0.23	0.19	0.24
8c	1,2,3/0-(2P)	0.14	0.11	0.14
9c	1,3/2-(2P)	0.19	0.16	0.19
	DPPC	0.15	0.11	0.15

[a] Solvent A: chloroform–methanol–water 65:25:4 (v/v/v); solvent B: chloroform–acetone–methanol–acetic acid–water 6:8:2:2:1 (by volume); solvent C: chloroform–methanol–30% ammonium hydroxide 65:25:4 (v/v/v).

Thin-layer chromatography

A dependence of chromatographic mobility on the configuration of the cyclopentanoid backbone was marginally evident in acidic, basic, and neutral solvent systems. For a given *cyclopentano*-lecithin, the R_f values were identical in the neutral system (solvent A) and the basic system (solvent C), and greater than the R_f value in the acidic system (solvent B). Four of the *cyclopentano*-lecithins (1,2/3-(3P), 1,2/3-(1P), 1,3/2-(1P), 1,3/2-(2P)) migrated faster than DPPC, while the other two (1,2,3/0-(1P), 1,2,3/0-(2P)) were indistinguishable from DPPC in each of the three solvent systems. Significant difference in chromatographic mobility is evident between the all-*trans* 1-phosphate and the all-*trans* 2-phosphate (1,3/2-(1P), 1,3/2-(2P)). The high chromatographic mobility of the all-*trans* 1-phosphate (1,3/2-(1P)) is striking since the corresponding (1,3/2-(1P)) *cyclopentano*-PA did not have a high R_f value relative to the other members of the series of *cyclopentano*-PA isomers (3).

Analytical data

Analytical data for the *cyclopentano*-lecithins were consistent with those expected for hydrated zwitterions. Vacuum treatment of the analogs (0.1 torr, 56°C, 48 hr) failed to render them anhydrous. After this treatment, Karl Fischer analysis for water indicated the presence of one molecule of water per molecule of *cyclopentano*-lipid in all but two compounds. These two compounds (1,2,3/0-(1P), 1,3/2-(1P)) gave analytical data corresponding to one-half molecule of water per molecule lipid (Table 1). Comparative data from the literature for synthetic DPPC include those for an anhydrous compound after recrystallization from dioxane (17), a hydrated compound from diisobutylketone (18), while data for a

TABLE 3. Melting points of isomeric *cyclopentano*-lecithins

Compound	Configuration	MP Range[a] °C
4c	1,2,3/0-(1P)	225–226
5c	1,2/3-(1P)	163–166
6c	1,2/3-(3P)	203–206
7c	1,3/2-(1P)	189–199
8c	1,2,3/0-(2P)	122–125
9c	1,3/2-(2P)	153–154

[a] DL-DPPC literature mp 227–229° (18).

monohydrated synthetic sulfonium analog of lecithin have recently been reported (15).

Infrared spectroscopy

The infrared spectra of KBr dispersions of the *cyclopentano*-lecithins are shown in **Fig. 2.** Each spectrum is consistent with that expected for a hydrated zwitterionic phospholipid (19). Absorption bands observed in all spectra measured include those for P=O (1255–1280 cm^{-1}), P-O-C (1065 cm^{-1}), $P\text{-}O^-$ (1110 cm^{-1}), $-N^+(CH_3)_3$ (970 cm^{-1}) (20), in addition to those characteristic of esterified fatty acid. The strong $P\text{-}O^-$ absorption band (1110 cm^{-1}) and the absence of P-OH absorption in the 2700 cm^{-1} region suggest that, at least in the microcrystalline state, the *cyclopentano*-lecithins exist as zwitterions. The broad absorption centered at 3450 cm^{-1} may indicate the presence of strongly-bonded water in the *cyclopentano*-phospholipid. This absorption is consistent with the analytic data (Table 1) which are themselves in accord with a hydrated zwitterionic structure.

Melting behavior

The *cyclopentano*-lecithins exhibited marked differences in melting point. These differences appear to originate from two distinct kinds of structural feature, although the melting points observed may also reflect a contribution due to the degree of hydration of the lipid. The structural features are *1*) the configurational ge-

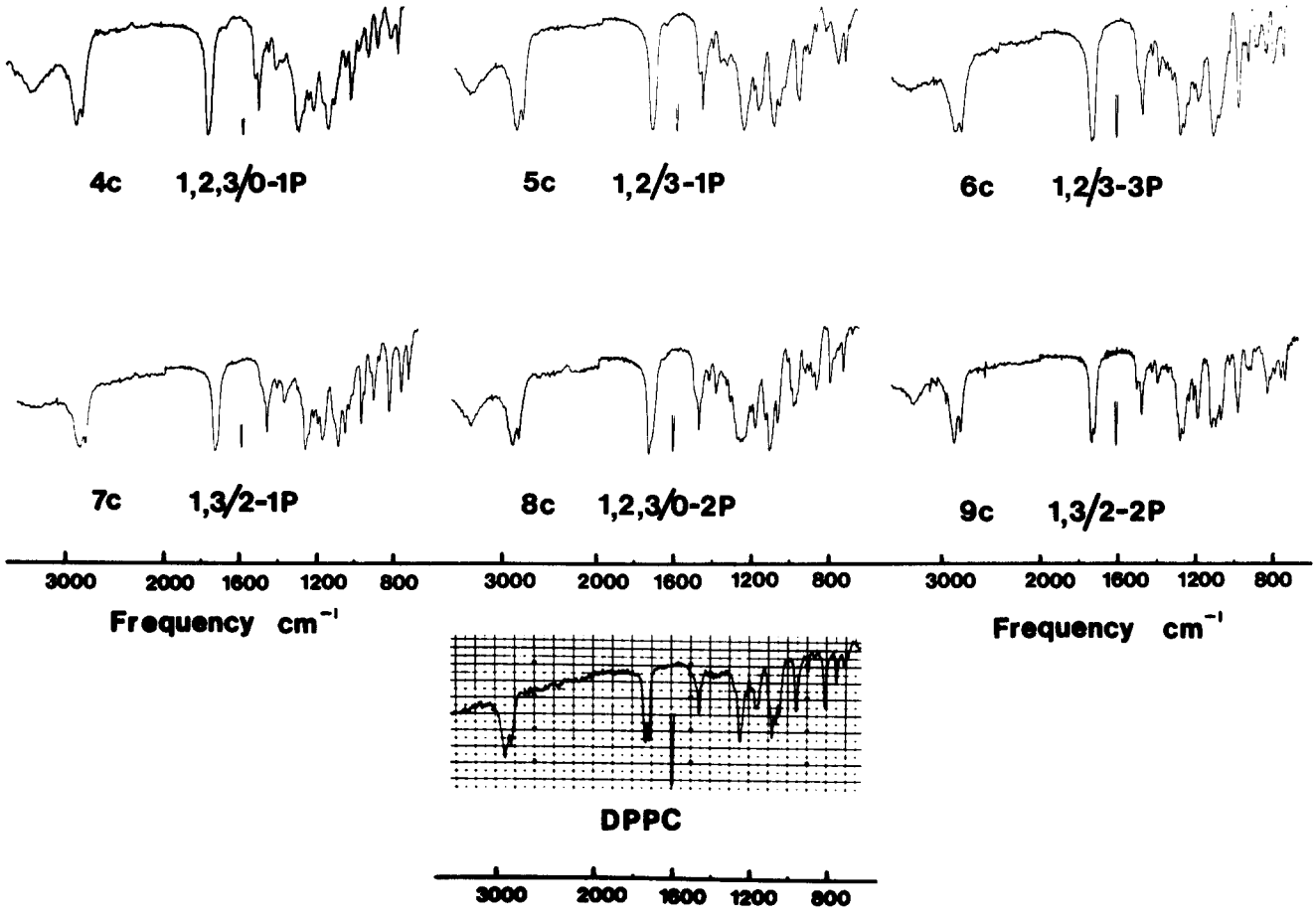

Fig. 2. Infrared spectra of KBr dispersions of the diastereoisomeric *cyclopentano*-lecithins (dipalmitoyl esters) and DPPC.

ometry of the cyclopentane ring, and *2*) the position of substitution of the polar head groups relative to the two acyl groups. The effect of ring configuration in these isomers is shown by the progressive decrease in melting point in the series: 1,2,3/0-(1P) > 1,2/3-(3P) > 1,3/2-(1P). The difference in melting temperature (Δt_f) between the all-*cis* isomer (1,2,3/0-(1P)) and the all-*trans* isomer (1,3/2-(1P)) is 36°C. Configurational effects shown here for the polar *cyclopentano*-lipids contrast with the relative insensitivity of the melting behavior of the neutral *cyclopentano*-lipids (tris-*homo*acyl cyclopentane-1,2,3-triols) (2) to differences in ring configuration. The effect of changing the position of the polar head group from C-1 (or C-3) to the C-2 position is considerable. The melting point difference of the two all-*cis* isomers (1,2,3/0-(1P) and 1,2,3/0-(2P)), Δt_f, is 100°C. In each case, the interpolation of the polar head group between the acyl substituents appears to exert a powerful influence on the acyl chain packing in the crystal. It is also evident that in the group of 1- (or 3-) phosphoryl compounds, those that have two adjacent and "parallel" acyl chains have higher melting points than the compounds in which the chains are in an *anti* disposition. Comparison of the melting points of the *cyclopentano*-lecithins with that of DL-DPPC (Table 3) shows that, as was found for the tris-*homo*acyl cyclopentane-1,2,3-triols (2), each of the isomers melts at a temperature lower than that of the glycerolipid counterpart. This suggests that the overall molecular packing in the *cyclopentano*-lecithins gives rise to crystal structures that are less stable than those found for the glycerolipid. The low lattice stability may indeed reflect the degree of conformational restraint in each of the *cyclopentano*-lecithin isomers. Work is in progress to examine and identify by diffraction techniques the effect of these configurational and positional differences on the molecular packing in the crystalline *cyclopentano*-lecithins.

We should point out that the overall melting behavior of phospholipids involves not only the associations within acyl chain aggregates, but also interactions at the polar head groups, so the melting point differences observed in our work may reflect the degree of hydration of the lipids. Indeed, comparison of the analytical and melting point data (Tables 1 and 3) does suggest the possibility that isomers analyzing for >1 mole water per mole lipid melt at a lower temperature than those analyzing for 0.5 mole water per mole lipid (compare 1,2,3/0-(2P), 8c and 1,2,3/0-(1P), 4c). That this correlation is not a consistent one is shown by comparison of isomers 5c and 6c (1,2/3-(1P) and 1,2/3-(3P)), the melting points of these isomers differing by 40°C in spite of identical molar water content. In order to investigate in a biologically relevant way the possibility of a relationship between the configuration of the analogs and hydration characteristics, we plan to study ^2H_2O dispersions of the analogs by deuteron magnetic resonance spectroscopy. This study may provide information about the water binding energies and the degrees of anisotropy of motion of the polar head groups in these compounds (21).

The authors thank Linda R. Berger, Patrick Vano, Henry J. Kaminski, and Everett O. Lewis for the synthesis of intermediates used in this work. The work was supported by grants from the National Institutes of Health (Grants AM-7719 and GM-21047-06) and from the Missouri Heart Association.

Manuscript received 23 March 1981 and in revised form 10 August 1981.

REFERENCES

1. IUBAC-IUB Commission on Biochemical Nomenclature. 1968. Tentative rules for the nomenclature of cyclitols. *Arch. Biochem. Biophys.* **128:** 269–279.
2. Hancock A. J., S. M. Greenwald, and H. Z. Sable. 1975. Analogs of natural lipids. I. Synthesis and properties of tris-*homo*acyl derivatives of cyclopentane-1,2,3-triols. *J. Lipid Res.* **16:** 300–307.
3. Hancock, A. J., M. H. Stokes, and H. Z. Sable. 1977. Analogs of natural lipids. IV. Synthesis and properties of cyclopentanoid analogs of phosphatidic acid. *J. Lipid Res.* **18:** 81–92.
4. Hancock, A. J., and M. D. Lister. 1979. Synthesis of all-*cis*-1,3-diacylcyclopentane-1,2,3-triol-2-phosphate via acyl group migration in a cyclic diglyceride analog. *J. Lipid Res.* **20:** 271–273.
5. Hancock, A. J. 1981. Synthesis of cyclopentanoid analogs of diacylglycerophosphate. *Methods Enzymol.* **72:** 640–672.
6. Dorset, D. L., and A. J. Hancock. 1977. Glycerol conformation and the crystal structure of lipids. I. An electron diffraction study of tripalmitin and conformationally fixed analogs. *Z. Naturforsch.* **32C:** 573–580.
7. Dorset, D. L., W. A. Pangborn, A. J. Hancock, and I. S. Lee. 1978. Influence of molecular conformation on the solid state packing of 1,2-diglycerides. Study of 1,2-dipalmitin and some structural analogs by electron diffraction, X-ray diffraction, and infrared spectroscopy. *Z. Naturforsch.* **33C:** 39–49.
8. Dorset, D. L., W. A. Pangborn, A. J. Hancock, T. C. van Soest, and S. M. Greenwald. 1978. Glycerol conformation and the crystal structure of lipids. II. A further study of tripalmitin and conformationally fixed analogs by small angle X-ray diffraction. *Z. Naturforsch.* **33C:** 50–55.
9. Gallo, A. A. 1977. ^{13}C NMR Relaxation of trilauroin and its cyclopentanoid analogs. *FEBS Lett.* **81:** 97–100.
10. Aneja, R., J. S. Chadha, and A. P. Davies. 1970. A general synthesis of glycerophospholipids. *Biochim. Biophys. Acta.* **218:** 102–111.
11. Patel, K. M., and J. T. Sparrow. 1978. Rapid, large scale purification of crude egg phospholipids using radially compressed silica gel columns. *J. Chromatogr.* **150:** 542–547.
12. Ryu, E. K., and M. MacCoss. 1979. Modification of the

Dittmer-Lester reagent for the detection of phospholipids on thin-layer chromatograms. *J. Lipid Res.* **20:** 561–563.

13. Dittmer, J. C., and R. L. Lester. 1964. A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. *J. Lipid Res.* **5:** 126–127.
14. Rosenthal, A. F. 1966. New, partially hydrolyzable synthetic analogues of lecithin, phosphatidylethanolamine, and phosphatidic acid. *J. Lipid Res.* **7:** 779–785.
15. Tremblay, P. A., and M. Kates. 1979. Chemical synthesis of *sn*-3-phosphatidyl sulfocholine, a sulfonium analog of lecithin. *Can. J. Biochem.* **57:** 595–604.
16. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
17. Aneja, R., and J. S. Chadha. 1971. A facile synthesis of phosphatidylcholines. *Biochim. Biophys. Acta.* **248:** 455–457.
18. Baer, E., and M. Kates. 1950. Synthesis of enantiomeric α-lecithins. *J. Am. Chem. Soc.* **72:** 942–949.
19. Abramson, M. G., W. T. Norton, and R. Katzman. 1965. Study of ionic structures in phospholipids by infrared spectra. *J. Biol. Chem.* **240:** 2389–2395.
20. Nelson, G. J. 1968. Differentiation of nitrogenous phospholipids by infrared absorption between 9 and 11 microns. *Lipids.* **3:** 104.
21. Finer, E. G., and A. Darke. 1974. Phospholipid hydration studies by deuteron magnetic resonance spectroscopy. *Chem. Phys. Lipids.* **12:** 1–16.