rises in going from the liquid to the solid. Further, a discontinuity could be observed both for the *trans*-decalin and cyclohexane during the freezing process. This would suggest some change in structure so as to enable the molecule to adjust itself to the minimum energy state in the crystal lattice. Hence the rigid chain forms of *trans*-cyclohexane and *trans*-decalin (type II of Wightman) must be excluded, in favor of Wightman's B and C for cyclohexane and G or E for *trans*-decahydronaphthalene. Again the value of the dielectric constants of the *cis* and *trans* forms in the solid state appear to be the same as far as experimental results showed. This would indicate the same crystal structure for both and would imply a very close similarity in molecular structure. This can most easily be perceived in Wightman's E and C models. Again, if the excess scattering for the *cis* compound below 50° as observed by Glockler[23] and Tung is due to the formation of cybotactic units then these could most easily arise from Wightman's (F) form.

In conclusion the senior author wishes to express his gratitude for the valuable assistance rendered in the course of this work: *viz.*, George Francis Davies, Donald Ellis McLellan, Marian Robinson, Morton Harold Graham, Stuart Donald Cavers, B. R. Mead and Henry J. Howie; also to Professors Smith and Glockler for their interest in the study of the properties of the two compounds of decahydronaphthalene.

(23) Private communication, Geo. Glockler, University of Iowa.

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[CONTRIBUTION FROM THE BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

On the Crystallization, Structure and Infrared Spectra of Saturated L-α-Lecithins

BY ERICH BAER

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A procedure of crystallization is described by which it is possible to obtain the L-α-(dimyristoyl)-, L-α-(dipalmitoyl)- and L-α-(distearoyl)-lecithins in a distinctly crystalline state. The elementary composition of the crystalline lecithins is in agreement with formula: $RCOOCH_2$–$RCOOCH$–$CH_2OPO(O^-H^+)OCH_2$–$CH_2N^+(CH_3)_3(OH)^-$ but not with $RCOOCH_2$–$RCOOCH$–$CH_2OPO(O)OCH_2$–$CH_2N(CH_3)_3$. The infrared spectra of the pure lecithins are reported.

Various investigators[1,2,3] studying the catalytic reduction of naturally occurring lecithins reported that they had obtained the hydrolecithins in a crystalline state. With the exception of Paal and Oehme,[4] who described their material as cubical crystals, no mention was made of the crystal form. More recently, however, Thannhauser, Benotti and Boncoddo[5] reporting the isolation of dipalmitoyllecithin from beef lung stated that they had obtained the phospholipid in aggregates of small needles by recrystallization from either diisobutyl ketone or a mixture of acetone and acetic acid (40:1).

Three years ago the author and M. Kates described the synthesis of the dimyristoyl-, dipalmitoyl- and distearoyl-L-α-lecithins.[6] To obtain these lecithins in a crystalline form they were reprecipitated from warm (60–80°) diisobutyl ketone. Although the material thus obtained gave distinct X-ray diffraction patterns and exhibited birefringence under polarized light, it did not possess a definite crystal form. In addition it was found that the procedure was not as harmless as had been assumed. The three lecithins after recrystallization from diisobutyl ketone proved to be less stable than either the crude products or lecithins which had been obtained by reprecipitation from chloroform with acetone at room temperature.[7]

The recrystallization of the lecithins from warm dioxane, which yields lecithin crystals large enough to be seen at low magnification[6] may also be hazardous because of the relatively high temperature (65–70°) at which the crystallization has to be carried out.

It seemed therefore desirable to find a method of crystallization which would be non-injurious to the lecithins and which would produce these substances in a distinctly crystalline form. This has now been accomplished by using chloroform as solvent and ether as precipitant, and by conducting the crystallization of the lecithins slowly and in dilute solution at or near room temperature. To obtain well-formed crystals it was found important (1) that the precipitant be added in amounts just sufficient to make the solution saturated with regard to the lecithin, but insufficient to cause their immediate precipitation, (2) that with increasing chain length of the fatty acids the crystallization of the lecithins be carried out in increasingly greater volumes of the chloroform–ether mixture, and (3) that the solutions be kept as undisturbed as possible. In one instance in which the crystallization of the L-α-(dimyristoyl)-lecithin was carried out using twice the volume of chloroform and 2.3 times the volume of ether reported in the experimental part for the recrystallization of this substance, a portion of the lecithin was deposited on the walls of the test tube in the form of rosettes composed of long and narrow prisms which measured up to 5 mm. in length.

In 1926, Grün and Limpächer[8] reported a synthesis of DL-α-(distearoyl)-lecithin. The analysis

(1) F. Ritter, *Ber.*, **47**, 530 (1914).

(2) P. A. Levene and C. J. West, *J. Biol. Chem.*, **33**, 111 (1918); **34**, 175 (1918).

(3) P. A. Levene and J. P. Rolf, *ibid.*, **46**, 353 (1921).

(4) C. Paal and H. Oehme, *Ber.*, **46**, 1297 (1913).

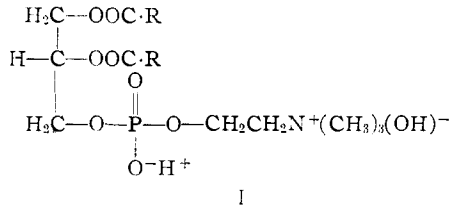
(5) S. J. Thannhauser, J. Benotti and N. F. Boncoddo, *J. Biol. Chem.*, **166**, 669 (1946).

(6) E. Baer and M. Kates, THIS JOURNAL, **72**, 942 (1950).

(7) E. Baer and J. Maurukas, *ibid.*, **74**, 158 (1952).

(8) A. Grün and R. Limpächer, *Ber.*, **59**, 1350 (1926); **60**, 147 (1927).

of this substance gave higher carbon values than required by theory for a (distearoyl)-lecithin possessing the structure shown by formula I (R = $C_{17}H_{35}$). Grün and Limpächer thereupon assumed that their synthetic product had lost one mole of water and had formed the anhydrous inner-salt structure shown by formula II. This anhydro-structure the authors believed to be a characteristic feature of all lecithins. The physical properties of Grün and Limpächer's synthetic product were found, however, to be so different from those of the pure racemic (distearoyl)-lecithin[6] and its response to enzymes was so unlike that of natural lecithin,[9] that their material, it appears, must have been grossly impure. In contrast with Grün's



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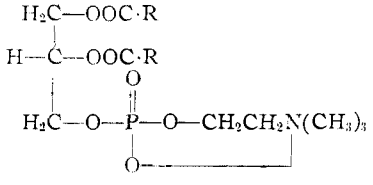

II

product, several independent preparations of each of our three synthetic α-lecithins, including the crystalline forms described below, gave upon analysis carbon values which agreed well with those calculated for structure I.[6,7,10] Consistent also with structure I are the carbon values which have been reported for a pure α-(distearoyl)-lecithin[1,2,3] and α-(dipalmitoyl)-lecithin,[5,11] both of which were obtained from natural sources, and the carbon values of stearoylglycollecithin and palmitoylglycollecithin which were obtained by synthesis.[12]

For the purpose of comparison, the carbon and hydrogen values of the various natural and synthetic α-lecithins and the synthetic glycollecithins, together with the theoretical values for the open and anhydrous forms have been summarized in Table I. It is obvious from these data that the *solid* lecithins do not possess the *anhydrous* inner-salt structure proposed by Grün and Limpächer and that their constitution is best expressed by formula I.

TABLE I

	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Distearoyllecithin				
Anhydro form	66.87		11.22	
Open form	65.38	64.60[6]	11.22	10.84
		65.36[7]		11.14
		65.31[a]		11.32
		65.25[1]		11.39
		65.20[2]		10.89
		65.60[2]		11.03
		65.57[3]		10.92
Dipalmitoyllecithin				
Anhydro form	65.45		10.99	
Open form	63.89	63.9[6]	11.00	11.00
		63.81[7]		11.15
		63.98[a]		11.00
		63.34[11]		11.07
		63.10[4]		10.90
Dimyristoyllecithin				
Anhydro form	63.81		10.70	
Open form	62.13	62.0[6]	10.72	11.00
		62.16[7]		10.81
		62.33[a]		10.70
		62.29[10]		10.82
Stearoylglycollecithin				
Anhydro form	60.82		10.61	
Open form	58.67	58.86	10.63	10.47
Palmitoylglycollecithin				
Anhydro form	59.33		10.39	
Open form	57.12	57.30	10.42	10.27

[a] The values were taken from this paper.

For the elucidation of the structure of new compounds it is desirable to have a detailed knowledge of the infrared spectra of related compounds of established structure. Since the three synthetic L-α-lecithins constitute a homologous series of pure phospholipids of known constitution and configuration the infrared spectra of these biologically interesting compounds are reported. Drs. Stanley F. Kern and Harold Boaz of the Physicochemical Research Division of the Lilly Research Laboratories who had recorded the infrared spectra of the crystalline lecithins described below were kind enough to give the author their permission to include these curves in the present paper (Fig. 1).

Experimental

The lecithins used herein were prepared as described in our earlier publications[6,7] with the exception that they were not recrystallized from diisobutyl ketone or dioxane.

L-α-(**Dimyristoyl**)-**lecithin**.—Three and one-half grams of the amorphous DML was dissolved in 35 ml. of chloroform[13] and the solution after diluting with 85 ml. of ether[13] was centrifuged sharply. The decanted clear supernatant solution was set aside in a closed vessel at room temperature (24–25°) and kept undisturbed until spontaneous crystallization had set in. After a fair amount of crystals had formed, ether was added with gentle swirling in 3-ml. portions at one-hour intervals until a total of 18 ml. had been added. After standing overnight the crystalline lecithin was collected with suction on a buchner funnel and freed

(9) E. J. King, *Biochem. J.*, **28**, 476 (1934).

(10) E. Baer and F. Martin, *J. Biol. Chem.*, **193**, 835 (1951).

(11) A. Lesuk and R. J. Anderson, *ibid.*, **139**, 457 (1941).

(12) The stearoyl- and palmitoylglycollecithins are the first representatives of a new class of phospholipids. The new phospholipids differ from lecithins and lysolecithins in that they contain ethylene glycol instead of glycerol and have the following structure: $CH_3(CH_2)_xCO{-}OCH_2CH_2O{-}PO(O^-H^+){-}OCH_2CH_2N^+(CH_3)_3OH^-$. The synthesis of the glycollecithins was carried out according to the procedure of Baer and Kates for the synthesis of the enantiomeric forms of saturated α-lecithins[6] with the exception that monoacyl glycols were used as starting materials. A detailed description of a generally applicable procedure for the synthesis of glycollecithins will be reported shortly. The glycollecithins were found to possess a strong hemolytic activity towards human red blood cells. This might have been expected since these substances bear close resemblance to the lysolecithins; *i.e.*, the ratios of fatty acid:polyalcohol:phosphoric acid:choline is 1:1:1:1 in both classes of compounds.

(13) Both the chloroform and ether were of U.S.P. grade.

of solvent *in vacuo* (0.01 mm.) at room temperature. The crystalline lecithin weighed 2.75 g. (recovery 73%). If the yield should be lower, the filtrate is cooled to +15°, 50 ml. of ether is added in 10-ml. portions over a period of 30 min., and the mixture is kept at +15° for 2 hours. The lecithin started to sinter at about 105°. On further heating (20° per min. up to 210° and from there on 10° per min.) the droplets became first translucent, then darkened at approx. 200° and finally coalesced suddenly with the formation of a meniscus from 236–237°.[14,15]

Anal. Calcd. for $C_{36}H_{74}O_9NP$ (695.6): C, 62.13; H, 10.72; N, 2.01; P, 4.46. Found: C, 62.33; H, 10.70; N, 1.98; P, 4.54.

L-α-(**Dipalmitoyl**)-**lecithin**.—Three and one-half grams of the amorphous DPL was dissolved in 69 ml. of chloroform and the solution was diluted with 100 ml. of ether. After centrifuging sharply, the decanted solution was set aside at room temperature (24–25°) and left standing undisturbed for a per od of 20 hours. To complete the precipitation 4 ml. of ether was added at the end of the twentieth hour and again at the end of the twenty-second hour. The crystalline DPL was collected with suction on a buchner funnel and after drying *in vacuo* at room temperature, weighed 3.18 g. (recovery 92%). The substance started to sinter at approx. 90° and coalesced suddenly with the formation of a meniscus at 235.5–236.5°.

Anal. Calcd. for $C_{40}H_{82}O_9NP$ (752): C, 63.89; H, 11.00; N, 1.86; P, 4.12. Found: C, 63.98; H, 10.91; N, 1.88; P, 4.23.

L-α-(**Distearoyl**)-**lecithin**.—Three and one-half grams of crude distearoyl lecithin was dissolved in 105 ml. of chloroform. The solution was filtered, diluted with 212 ml. of ether and the turbid mixture (stoppered vessel) was placed in a water-bath of 36 ± 0.5°. After the solution had become clear, 41 ml. of ether was added. When the turbidity again had disappeared the temperature of the water-bath was gradually lowered to 34° and kept at this temperature throughout the crystallization. To induce crystallization the solution was seeded with crystalline material obtained in previous experiments. Two hours after seeding, 16 ml. of ether was added, followed by a second 16-ml. portion and four 33-ml. portions of ether at 45-min. intervals. One hour after the addition of the last portion of ether, the still warm solution was filtered with suction and the lecithin was dried *in vacuo* at room temperature. The yield of crystalline lecithin was 2.14 g. (recovery 61%). The distearoyllecithin started to sinter at approx. 90° and on further heating (20° per min. up to 200° and from there on 7–10° per min.) coalesced suddenly with the formation of a meniscus from 233 to 234°.

Anal. Calcd. for $C_{44}H_{90}O_9NP$ (808.2): C, 65.38; H, 11.22; N, 1.73; P, 3.83. Found: C, 65.31; H, 11.32; N, 1.81; P, 3.88.

(14) All melting points reported in this paper were carried out in capillary tubes using an electrically heated bath of *n*-butyl phthalate and short-stem thermometers with a range of 50°.

(15) Photomicrographs of the six crystalline lecithins accompanied the original manuscript, but their publication was not approved by the editors.

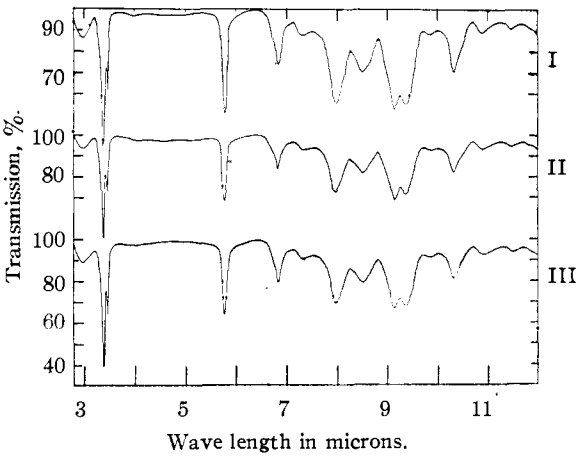

Fig. 1.—Beckman IR-2T spectrophotometer, 0.092 mm. cell path, chloroform solution: I, L-α-(dimyristoyl)-lecithin, *c* 3.6 (w./v.); II, L-α-(dipalmitoyl)-lecithin, *c* 2.1; III, L-α-(distearoyl)-lecithin, *c* 2.4. Band positions and relative intensities of (I): 2.94 μ (7), 3.41 (10), 3.49 (3), 5.66 (5), 6.80 (3), 7.25 (1), 7.98 (6), 8.51 (4), 9.15 (5), 9.35 (5), 9.86 (1), 10.31 (3), 10.87 (1), 11.48 (1). Probable assignment of band positions: 2.94 μ (OH, hydrogen bonded), 3.41 (CH_2, unsym. stretching), 3.49 (CH_2, sym. stretching), 5.66 (C=O), 6.80 (CH_2, CH_3 bending), 7.25 (CH_3 bending), 7.98 (C—OH), 8.51 (C—OR, ester).

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TORONTO, CANADA