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PARTIAL SYNTHESIS OF "MIXED-ACID" L-α-LECITHINS

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IN a previous paper[1] we have reported the preparation of ten L-α-lecithins, each containing two identical acyl groups, by acylation of L-α-glyceryl-phosphorylcholine. As by-products the corresponding monoacyl derivatives ("lysolecithins") were isolated in rather larger amounts.

If it should be possible to acylate these "lysolecithins" with a different fatty acid, "mixed-acid" L-α-lecithins could be obtained in a way less elaborate than through total synthesis.[2,3] The "lysolecithins" under consideration, however, resisted under various conditions acylation towards the required "mixed-acid" lecithins. Paper chromatography showed that these "lysolecithins" were not identical* with snake venom-formed lysolecithins.

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* Initially the fatty acid moiety of the "lysolecithins", obtained as by-products, was supposed to be at the γ-position. This wrong assignation was based on the supposed γ-specificity of lecithinase A, which until recently was generally admitted. A definite elucidation of the structure of these "lysolecithins", however, requires further investigation.

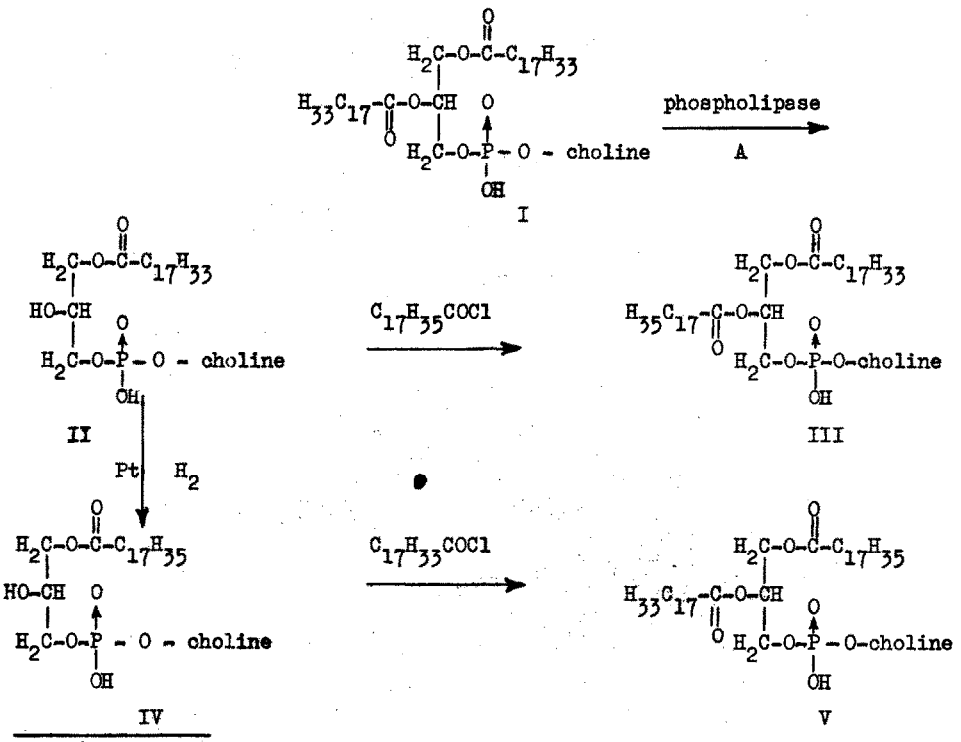
1 F. Kögl †, G.H. de Haas and L.L.M. van Deenen, Rec.Trav.Chim. 79, 661 (1960).

2 G.H. de Haas and L.L.M. van Deenen, Tetrahedron Letters No. 9, 1 (1960).

3 G.H. de Haas and L.L.M. van Deenen. Unpublished Work.

Therefore the conversion was tried of lysolecithins prepared by phospholipase A (lecithinase A) degradation of L-α-lecithins containing two identical fatty acids. As demonstrated by degradation experiments on fully synthetic "mixed-acid" L-α-lecithins, lecithinase A liberates only the β-attached fatty acid,[4] thus yielding γ-lysolecithins. Actually upon acylation of these γ-lysolecithins we did obtain "mixed-acid" lecithins in satisfactory yields.

An example is presented of the preparation of the two structural isomeric L-α-lecithins containing one stearic and one oleic acid chain:



[4] G.H. de Haas and L.L.M. van Deenen, Vth Conference on biochemical Problems of Lipids, Marseille, 21-23, July 1960; Cf. also: N.H. Tattrie, J.Lipid.Res. 1, 61 (1959); D.J. Hanahan, H. Brockerhaff and E.J. Barron, J.Biol.Chem. 235, 1917 (1960).

L-α-dioleoyllecithin (I) was prepared according to known methods by acylation of either free L-α-glycerylphosphorylcholine[1] (L-α-GPC) or its amorphous cadmium chloride adduct.[5] Baer and Buchnea[5] pointed out that deacylation of L-α-lecithins with mercuric chloride yields partially racemized L-α-GPC, but that neither racemization nor a migration of the phosphorylcholine moiety occurs during the acylation of synthetic L-α-GPC. Actually in our previous preparation of lecithins[1] using L-α-GPC prepared by mercuric chloride hydrolysis of egg lecithin we obtained lecithins with somewhat too low optical rotations, thus confirming the statements of Baer and Buchnea. Using, however, L-α-glycerylphosphorylcholine prepared by hydrogenolysis of egg lecithin with lithiumaluminium hydride according to the method of Urakami and Okura,[6] optically pure I was obtained (α_D^{20} = + 6.0°; C 9.0 in chloroform). Since lecithinase A does not hydrolyse D-α-lecithins and degradates only the L-isomers to yield the corresponding lysolecithins,[4] it is even possible to prepare by the present method optically pure "mixed-acid" L-α-lecithins starting from optically impure lecithins containing two similar fatty acids.

Degradation of I with snake venom lecithinase A under conditions outlined by Hanahan[7] yielded the corresponding γ-oleoyl-L-α-lysolecithin (II). [Yield: 90-95%; m.p. 240°; α_D^{20} = -3.2°; C 12.0 in chloroform: ethanol (5:1 v/v)]. Analysed as the cadmium chloride adduct. (Found: N 1.64, P 3.65. $(C_{26}H_{54}O_8NP)_2(CdCl_2)_3$ requires N 1.66, P 3.80).

The conversion of this lysolecithin to γ-octadecenoyl-β-octadecanoyl-L-α-lecithin (III) appeared to be possible by acylation of the free product

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[5] E. Baer and D. Buchnea, Canad.J.Biochem.Physiol. 37, 953 (1959).

[6] C. Urakami and H. Okura, Bull.Chem.Soc.Japan 31, 779 (1958).

[7] D. J. Hanahan, J.Biol.Chem. 195, 199 (1952).

or of the cadmium chloride adduct.

In both cases the crude "mixed-acid" lecithin had to be purified by silica chromatography. Crystallization from chloroform-acetone yielded the wanted lecithin (III) as a colourless, very hygroscopic powder (m.p. 233-235°; α_D^{20} = + 6.0°; C 10.0 in chloroform; yield 43 %, calculated upon the amount of lysolecithin). (Found: C 64.77, H 11.44, N 1.72, P 3.71. $C_{44}H_{88}O_9NP$ requires C 65.39, H 11.22, N 1.73, P 3.83).

For the preparation of the structural isomeric L-α-lecithin (V) II was converted by catalytic hydrogenation (Adams Pt-catalyst) in absolute ethanol into γ-stearoyl-L-α-lysolecithin (IV). (m.p. 235-237°; α_D^{20} = -3.0°; C 5.0 in chloroform-methanol 1:1 v/v; yield 96%). Analysed as the cadmium chloride adduct. (Found: C 38.20, H 7.06, N 1.66, P 3.73. $(C_{26}H_{56}O_8NP)_2(CdCl_2)_3$ requires C 38.23, H 6.91, N 1.71, P. 3.79).

The cadmium chloride adduct of IV was allowed to react with oleoyl chloride according to the method of Baer.[5] After chromatography on silica and crystallization from chloroform-acetone the lecithin (V) was obtained as a colourless, very hygroscopic powder. (m.p. 237°; α_D^{20} = + 6.2°; C 10.0 in chloroform). (Found N 1.81; P 3.97. $C_{44}H_{88}O_9NP$ requires N 1.73, P 3.83).

Both isomeric L-α-lecithins (III and V) were compared using physical and biochemical methods with γ-stearoyl-β-oleoyl-L-α-lecithin obtained by total synthesis.[3] Both isomers exhibit the same molecular orientation at the air/water interface, as demonstrated by the fully identical force-area curves of unimolecular films in the Langmuir-Adams trough. Debye-Scherrer diagrams did not give satisfactory results so far, probably because of lack of crystallinity of these oleic acid containing lecithins

Lecithinase A degradation, however, supplied confirmatory evidence on

the dissimilar position of the fatty acids in both isomers. As in the case of the fully synthetic γ-stearoyl-β-oleoyl-L-α-lecithin, the enzymatic hydrolysis of the lecithin with formula (V) yielded only oleic acid, whereas from the lecithin (III) exclusively stearic acid was released.

This partial synthesis appears to offer vast opportunities for the preparation of many types of "mixed-acid" lecithins, especially those containing highly unsaturated fatty acids. The preparation of other types of phosphatides, e.g. "mixed-acid" cephalins by the outlined procedure, is in progress.

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