24. *Acyl Migration during Dephosphorylation and a Suggested Mechanism.*

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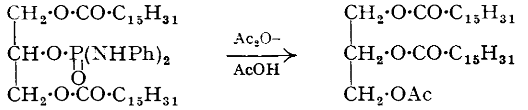
Derivatives of 1 : 3-dipalmitoylphosphatidic acid yield 1-acetyl-2 : 3-dipalmitin when heated with acetic acid–acetic anhydride. For this rearrangement, a Walden inversion mechanism is suggested, in support of which it is found that active 1-methylheptyl diphenyl phosphate undergoes inversion when converted into 1-methylheptyl acetate under the above conditions.

During synthetic work on phosphatides, we had occasion to remove protecting anilido-groups from the dianilide (I) of 1 : 3-dipalmitoylphosphatidic acid. Having found that the method suggested by Zetszche and Buttiker (*Ber.*, 1940, **73**, 47), involving use of acetic acid, was ineffective, we employed more drastic conditions: the dianilido-compound was refluxed with acetic anhydride–acetic acid (1 : 4 by vol.) for several hours, but the phosphate group was entirely removed. The resulting product was an acetyl dipalmitin, which we first assumed to be 2-acetyl-1 : 3-dipalmitin.

In the hope that this reaction might be of value in determining the position of phosphate groups, we submitted the following related compounds to the same treatment: 2′-aminoethyl 1 : 2- and 1 : 3-dipalmitoylphosphatidoate (α- and β-kephalin), 1 : 2-dipalmitoylphosphatidylcholine (α-lecithin), 1 : 2-dipalmitoylphosphatidic acid, and diphenyl 1 : 2-dipalmitoylphosphatidoate. To our surprise, we obtained in all cases the same acetyl dipalmitin as in our original experiment, in practically quantitative yields.

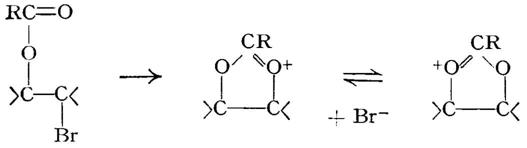
By synthesis of the two acetyl dipalmitins, and *X*-ray and melting-point comparisons, our product was found to be 3-acetyl-1 : 2-dipalmitin. Thus, in two of the above cases, acyl migration takes place during dephosphorylation [see (I) ⟶ (II)].

(I) 

$$\begin{array}{l} CH_2{\cdot}O{\cdot}CO{\cdot}C_{15}H_{31} \\ | \\ CH{\cdot}O{\cdot}\underset{\underset{O}{\|}}{P}(NHPh)_2 \\ | \\ CH_2{\cdot}O{\cdot}CO{\cdot}C_{15}H_{31} \end{array} \xrightarrow[AcOH]{Ac_2O-} \begin{array}{l} CH_2{\cdot}O{\cdot}CO{\cdot}C_{15}H_{31} \\ | \\ CH_2{\cdot}O{\cdot}CO{\cdot}C_{15}H_{31} \\ | \\ CH_2{\cdot}OAc \end{array}$$

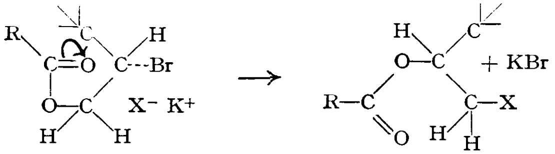
(II)

Fairbourne and Cowdrey (*J.*, 1929, 129) observed a number of similar migrations, but came to no decision concerning the identity of the final product; *e.g.*, the palmitates of 1 : 2- and 1 : 3-dibromohydrins and silver *p*-nitrobenzoate yielded the same di-*p*-nitrobenzoyl-monopalmitin. Winstein and Buckles (*J. Amer. Chem. Soc.*, 1942, **64**, 2780, 2787) postulated the formation of an intermediate resonant ion for certain similar reactions, *viz.*:



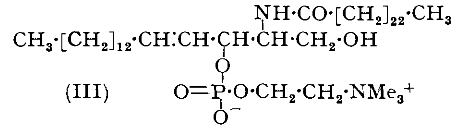
but this implies the possibility of acyl migration from $C_{(1)}$ to $C_{(2)}$ or from $C_{(2)}$ to $C_{(1)}$, whereas only the former occurs in our experiments. This also holds for the quoted dibromohydrin experiment, where we have identified Fairbourne and Cowdrey's final product as 1 : 3-di-*p*-nitrobenzoyl-monopalmitin. It is probable that the course of these reactions is determined by steric influences. When, *e.g.*, the halogen is on $C_{(2)}$, the opposite face of this atom is shielded by the groups on $C_{(1)}$ and $C_{(3)}$ and a normal Walden inversion is prevented. Moreover, as is seen from the following scheme, an acyl group on $C_{(1)}$ or $C_{(3)}$ is favourably placed for combination with $C_{(2)}$. A more obvious example of this shielding effect is the Wagner–Meerwein transformation, for which Bartlett and Pockel (*ibid.*, 1937, **59**, 820) have suggested a Walden mechanism, and there is little doubt that this effect is important in many similar migrations.

A further interesting point arises out of our dephosphorylation experiments. As we have definitely established that migration occurs, we can infer, by reversing our previous argument, that the C–O bond of the phosphate ester is broken, *i.e.*, the removal of the phosphate group should be accompanied by a Walden inversion. This was suggested by Robinson (*Nature*, 1927, **120**, 44) as a mode of biochemical conversion of glucose-4 phosphate into galactose; but, apparently, no case of inversion during enzymic dephosphorylation is known (Raymond in Gilman's "Organic Chemistry," John Wiley and Sons, New York, 1943, p. 1608); nor were Percival and Percival (*J.*, 1945, 874) able to find evidence for inversion during the alkaline hydrolysis of certain sugar phosphates.



On the other hand, Blumenthal and Herbert (*Trans. Faraday Soc.*, 1945, **41**, 611) have shown by the hydrolysis of trimethyl phosphate with water containing ^{18}O that, whilst alkaline hydrolysis cleaves the O–P bond (*i.e.*, there is no inversion), neutral and acid hydrolysis cleaves the O–C bond. In spite, therefore, of the prevalent view that there is no inversion during dephosphorylation, there seemed to be good reason to suppose that inversion should take place during acid hydrolysis, and, on dephosphorylating the diphenyl phosphate of (+)-octan-2-ol, we obtained the (—)-acetate, although, from the rotation value, some racemisation had also occurred.

In order to test the generality of our dephosphorylation method, we extended it to octadecyl phosphate and to sphingomyelin (III); the former was converted quantitatively into octadecyl acetate, and the latter yielded a product which gave correct analyses for *OO*-diacetyl-*N*-lignocerylsphingosine and had a melting point very close to that of the compound obtained from sphingomyelin by Thannhauser and Reichel (*J. Biol. Chem.*, 1940, **135**, 15) by enzymic dephosphorylation followed by acetylation. This change is of special interest because the position of the phosphate group (whether 1 or 3) is not known. Proof of inversion during dephosphorylation would decide in favour of position 3.



(III)

$$\mathrm{CH_3\cdot[CH_2]_{12}\cdot CH{:}CH\cdot \underset{\substack{|\\ O\\ |\\ O{=}P\cdot O\cdot CH_2\cdot CH_2\cdot NMe_3^+\\ |\\ O^-}}{CH}\cdot \overset{\substack{NH\cdot CO\cdot [CH_2]_{22}\cdot CH_3\\ |}}{CH}\cdot CH_2\cdot OH}$$

EXPERIMENTAL

Half-gram amounts of each of the following were separately heated under reflux with 10 c.c. of acetic anhydride–acetic acid (1 : 4 by vol.) for 8 hours; 1 : 3-dipalmitoylphosphatidic dianilide; 2′-aminoethyl 1 : 3-dipalmitoylphosphatidoate; 1 : 2-dipalmitoyl-phosphatidylcholine and -phosphatidic acid, diphenyl 1 : 2-dipalmitoylphosphatidoate. In all cases there separated on cooling, in practically quantitative amount, crystalline 3-acetyldipalmitin, which after one crystallisation from ethanol melted at 51°. Averill, Roche, and King (*J. Amer. Chem. Soc.*, 1929, **51**, 870) give 51—52°, but as the m. p. of the isomeric 2-acetyldipalmitin is 54° (Averill *et al.*) we prepared the two isomers by acylation of 1 : 2- and 1 : 3-dipalmitin, finding m. p.s of 51° and 53° respectively, and determined their short *X*-ray spacings, which were as follows : 3-Acetyldipalmitin : 5·27 moderate; 4·14 strong (very thick; possibly two unresolved lines); 3·77 strong. 2-Acetyldipalmitin : 5·18 weak; 4·54 strong; 3·76 strong. These spacings and mixed m. p.s established the identity of the dephosphorylation product as 3-acetyldipalmitin.

When the isomeric dipalmitins were heated for 8 hours with the dephosphorylating agent, they gave isomeric acetyl derivatives, and hence no migration takes place under these conditions.

Dephosphorylation of 1-Methylheptyl Diphenyl Phosphate.—(+)-Octan-2-ol (3·9 g., 0·03 mol.; $[\alpha]^{20}_{D}$ +9·7°) was added in small portions to a solution of diphenyl chlorophosphonate (16 g., 0·06 mol.) in pyridine (40 c.c.). After 48 hours, the solution was poured into 2N-hydrochloric acid (300 c.c.), and the oil which separated was extracted with ether. The ether was removed after successive treatments with N-hydrochloric acid, sodium hydrogen carbonate solution, and water, and final drying (Na_2SO_4). Residual pyridine was removed at 0·3 mm. There remained (+)-1-methylheptyl diphenyl phosphate (84%) as a viscous oil. This was refluxed for 36 hours with acetic anhydride (10 c.c.) and acetic acid (40 c.c.). After cooling, the solution was poured into an excess of saturated sodium hydrogen carbonate solution, and the precipitated oil extracted with ether. After washing, drying, and removal of ether, the residue was distilled, to yield 1-methylheptyl acetate (2·9 g.), b. p. 84—85°/20 mm., $[\alpha]^{20}_{D}$ −1·9°. Pickard and Kenyon (*J.*, 1914, 830) give $[\alpha]^{20}_{D}$ +6·84°.

In the above experiment, refluxing was for a longer time than usual to ensure complete dephosphorylation, and this may have brought about some racemisation. Alternatively, the replacement itself may involve some racemisation.

Dephosphorylation of Sphingomyelin.—Sphingomyelin (cf. Bevan, Gregory, Poole, and Malkin, *J.*, 1951, 841) (1 g.; m. p. 212°) was heated with the acetic anhydride—acetic acid (1 : 4 by vol.; 10 c.c.) under reflux for 8 hours. On cooling, a solid separated and this was filtered off (0·6 g.; m. p. 68—70°). Three crystallisations from methanol gave *O*-diacetyl-*N*-lignoceryl-sphingosine, m. p. 72—74° (Found : C, 75·3; H, 12·3; N, 1·7. Calc. for $C_{46}H_{87}O_5N$: C, 75·3; H, 11·9; N, 1·9%). Thannhauser and Reichel (*J. Biol. Chem.*, 1940, **135**, 15) give m. p. 70—71°.

Dephosphorylation of Octadecyl Phosphate.—The phosphate (0·5 g.) was heated for 8 hours with the acetic anhydride–acetic acid mixture (5 c.c.). On cooling, octadecyl acetate separated (almost 100%). Recrystallised from ethanol, it had m. p. and mixed m. p. 32°.

1 : 2- *and* 1 : 3-*Di*-p-*nitrobenzoyl-monopalmitins.*—α- and β-Monopalmitins were acylated with *p*-nitrobenzoyl chloride in pyridine, to yield the above isomers respectively. After crystallisation from alcohol they melted at 73° and 106° respectively [Found : N (1 : 2-derivative), 4·5; (1 : 3-derivative), 4·7. Calc. for $C_{33}H_{44}O_{10}N_2$: N, 4·5%]. Fairbourne and Cowdrey give m. p. 102°.

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