

## Individual Molecular Species of Phospholipids

### VI. Chromatographic Behavior of 1-Oleo-2-stearo-3-acetin and 1-Stearo-2-oleo-3-acetin

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In our previous studies of 1,2-diglyceride acetates derived from natural glycerophosphatides<sup>1,2,3</sup> we have not been able to separate 1-saturated acyl-2-unsaturated acyl-3-acetins from 1-unsaturated acyl-2-saturated acyl-3-acetins by argentation chromatography although the corresponding separation between 2-oleodistearin (SOS) and 1-oleodistearin (OSS) is well known.<sup>4</sup> The present report shows that synthetic 1-stearo-2-oleo-3-acetin (SOAc) and 1-oleo-2-stearo-3-acetin (OSAc) are indeed very similar; they can be separated by thin layer chromatography (TLC) only with difficulties even when the highly efficient technique of multiple development is used.

1-Oleoyl-2-stearin and 1-stearoyl-2-olein were prepared by a procedure described by Mattson and Volpenhein.<sup>5</sup> 2-Mono-glycerides were obtained by acylating 1,3-benzylidene glycerol and removing the protecting group with boric acid in trimethylborate. The products were purified by chromatography on silicic acid which contained boric acid.<sup>6</sup> 2-Mono-olein was acylated with an equivalent amount of stearoyl chloride in the presence of an equivalent amount of pyridine; 2-mono-stearin was treated in the same way with oleoyl chloride. The resulting 1,2-diglycerides were freed from tri- and mono-glycerides by silicic acid chromatography. Acetylation and subsequent chromatography on large columns of silicic acid gave finally 1,2-diglyceride acetates free from contaminating 1,3-diglyceride acetates.<sup>2</sup>

Positional analysis of fatty acids in the two 1,2-diglyceride acetates SOAc and OSAc was carried out with pancreatic lipase; the acids found on C-2 of glycerol in OSAc contained 93.5 % of stearic and 6.0 % palmitic acid; those in SOAc revealed 85 % oleic acid and 4.5 % palmitoleic acid.

The samples of 1-oleo-2-stearin and 1-stearo-2-olein described above were acylated also with hexanoyl (H), decanoyl (D), and tetradecanoyl (T) chlorides to give the following triglycerides: OSH, SOH, OSD, SOD, OST, and SOT.

TLC of the different triglycerides on unmodified silica gel G, with hexane-ether (4:1) as solvent, showed that the two acetates had identical mobilities, and so had the two hexanoates, the decanoates, and the tetradecanoates, respectively. The increase of the acyl groups at C-3 of glycerol caused increased mobility of the triglycerides; this effect was particularly prominent between the acetyl and the hexanoyl derivatives.

TLC on silver nitrate containing silica gel G, with two successive developments by hexane-ether (6:1) showed that SOT ran clearly ahead of OST; similarly SOD was faster than OSD, and also SOH moved ahead of OSH. The differences were large enough for clear separations. This confirms the findings of Barrett *et al.*<sup>4</sup> who separated 2-oleodistearin from the slower 1-oleodistearin. On the other hand the two diglyceride acetates, SOAc and OSAc, had almost identical mobilities in this system and they could not be separated.

The two acetates could be partially separated with argentation chromatography by using multiple development with mixtures of hexane and toluene. Fig. 1 shows that SOAc runs faster than OSAc, and the two

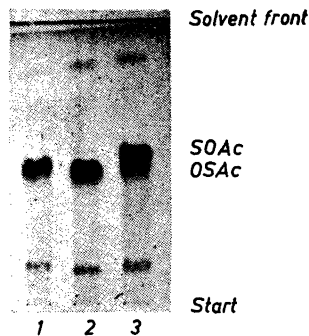


Fig. 1. Argentation TLC of 1-stearo-2-oleo-3-acetin and 1-oleo-2-stearo-3-acetin. Plate: 10 % AgNO<sub>3</sub> in silica gel G; activation 16 h at 140°C. Development: Hexane-toluene (4:6); 12 successive ascents. Samples: 1, 1-stearo-2-oleo-3-acetin; 2, 1-oleo-2-stearo-3-acetin; 3, mixture.

isomers were separated when twelve successive developments with hexane-toluene (4:6) were applied. Thus the order of mobility was the same in the acetates as in the other acylated forms, only the degree of separation was less.

The possibility of separating SO and OS type of isomeric molecules is of some analytical importance; both types of monoenoic diglycerides are formed from natural glycerophosphatides. With the two acetates, however, the separation obtained is too poor to be useful for natural lipids; differences in chain length, and also in relative amounts of the two fractions would cause overlapping. These two types of natural diglycerides should probably be separated as decanoates or other suitable long chain derivatives. Lands and Hart<sup>7</sup> have incidentally studied diglyceride heptadecanoates which would seem quite suitable even for this purpose.

The fact that, *e.g.*, OST was more retarded than SOT on argentation chromatograms shows that the olefinic double bond in SOT is slightly hindered in comparison to OST. In SOAc and OSAc, which were much more difficult to separate than the tetradecanoates, the double bonds seem to be more equally exposed to the environment. These findings suggest that under the conditions described the triglyceride molecules prefer conformations where the long chains stick together.

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1. Renkonen, O. *J. Am. Oil Chemists' Soc.* **42** (1965) 298.
2. Renkonen, O. *Biochim. Biophys. Acta* **125** (1966) 288.
3. Renkonen, O. *Advan. Lipid Res.* **5** (1967) 329.
4. Barrett, C. B., Dallas, M. S. J. and Padley, F. B. *Chem. Ind. (London)* **1962** 1050.
5. Mattson, F. H. and Völpenheim, R. A. *J. Lipid Res.* **3** (1962) 281.
6. Thomas, A. E., Scharoun, J. E. and Ralston, H. *J. Am. Oil Chemists' Soc.* **42** (1965) 789.
7. Lands, W. E. M. and Hart, P. *J. Am. Oil Chemists' Soc.* **43** (1966) 290.

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### 3-Bromoacetyltrimethylammonium Bromide, a Choline Acetylase Inhibitor

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In an attempt to develop a specific inhibitor of choline acetylase (acetyl-CoA:choline O-acetyltransferase, EC 2.3.1.6), we decided to synthesize a compound with alkylating properties structurally related to choline (a substrate of this enzyme). Such a compound may be expected to act as an irreversible inhibitor of the enzyme by alkylation after being specifically bound to the active site (*cf.* Refs. 1, 2). A compound which apparently fulfils these requirements, 3-bromoacetyltrimethylammonium bromide  $[(\text{CH}_3)_3\text{NCH}_2\text{COCH}_2\text{Br}]^+\text{Br}^-$ , was prepared as follows: 1,3-dibromoacetone<sup>3</sup> (10 g, 0.046 mole), dissolved in 50 ml of acetone, was treated dropwise with a solution of 4.1 ml of trimethylamine (0.046 mole) in 50 ml of acetone at +4°C while stirring efficiently. The precipitate was filtered off and dissolved in 10 ml of 99.9% ethanol and was reprecipitated with 250 ml of dry ether. The white product was filtered off and dried *in vacuo* over  $\text{P}_2\text{O}_5$ . Yield 6.9 g (54.5%) (Found: C 26.0; H 5.1; N 5.4; Br 56.3. Calc. for  $\text{C}_6\text{H}_{13}\text{Br}_2\text{NO}$ : C 26.2; H 4.8; N 5.1; Br 58.2). The infra-red spectrum of the compound in a KBr pellet showed strong bands at  $\text{cm}^{-1}$ : 3020 ( $\nu\text{aCH}_3$ ), 2925 ( $\nu\text{aCH}_2$ ), 1740 ( $\nu\text{CO}$ ), 1470 ( $\delta\text{aCH}_3$ ;  $\delta\text{sCH}_2$ ), 1380 ( $\delta\text{sCH}_3$ ), in agreement with the expected structure of the compound.

The compound was tested as an inhibitor for choline acetylase prepared from placenta and assayed as previously described.<sup>4</sup> When incubated in a concentration of  $5.0 \times 10^{-4}$  M with the enzyme at 0° for 60 min, 50% inhibition was obtained, and when the concentration was increased to  $5.0 \times 10^{-3}$  M, the inhibition rose to 95%. The inhibited enzyme was not reactivated by removal of free inhibitor on a Sephadex G-25 column or by precipitating the enzyme in 80% ammonium sulphate.

The compound had no inhibitory action on either acetylcholinesterase from human erythrocytes or pseudocholinesterase from