

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 Hart⁷ 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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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-dibromoacetone³ (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 P_2O_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 cm^{-1} : 3020 (νaCH_3), 2925 (νaCH_2), 1740 (νCO), 1470 (δaCH_3 ; δsCH_2), 1380 (δsCH_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.⁴ When incubated in a concentration of 5.0×10^{-4} M with the enzyme at 0° for 60 min, 50% inhibition was obtained, and when the concentration was increased to 5.0×10^{-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

human serum at 5.0×10^{-4} M inhibitor using the same incubation conditions as those employed for choline acetylase. Further details on the synthesis of related compounds, as well as the biochemistry and pharmacology, will be reported in a later publication.

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Intramolecular Hydrogen Bonding in Bilirubin

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Fog and associates,^{1,2} after studying infrared spectra of bilirubin, mesobilirubin, and dimethyl-mesobilirubin, have suggested that bilirubin contains intramolecular hydrogen bonds, as shown in formula I, and have pointed out that this structure would explain the relative stability of bilirubin in chloroform solution. This

possibility has been investigated further after exchange of hydrogen with tritium or deuterium in aqueous alkaline solutions of bilirubin sodium salt.

Bilirubin (Sigma, sigma grade) 50 mM dissolved in 0.25 M sodium hydroxide solution at room temperature, with HTO added to 2 mC/ml, was precipitated after one minute by addition of hydrochloric acid. The precipitate was washed twice with HTO in water, dried *in vacuo*, dissolved in toluene, and the radioactivity was measured in a liquid scintillation spectrometer (Packard, Tricarb 2002). The concentration of bilirubin was determined by spectrophotometry. In six experiments it was found that 4.5 – 3.5 – 3.6 – 4.1 – 4.2 and 4.6 atoms of hydrogen had been exchanged. Further drying did not alter the results. The four hydrogen atoms exchanged are presumably two in the carboxyl groups and two connected to nitrogen in the end rings, since all these are acidic protons.

Exchange with deuterium was done under identical conditions with final enrichment 99.3 % D. Infrared spectra (recorded on Beckman IR 7) of the deuterated bilirubin, and of similarly treated bilirubin without deuterium, in KBr discs are seen in Fig. 1. The sharp band at 3420 cm^{-1} has the frequency expected for the NH stretching mode in a cyclic γ -lactam, when this is not hydrogen-bonded.³ In a bonded compound this band would be found at 3175 cm^{-1} . Half of this absorption shifts to 2550 cm^{-1} on exchange with deuterium. This corresponds to the expected shift for deuteration of the NH-groups. The formula⁴

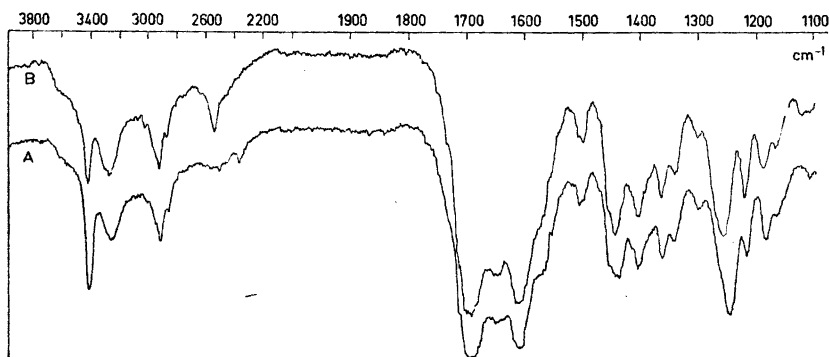


Fig. 1. IR-spectra of bilirubin (A) and deuterated bilirubin (B) in KBr discs.