NMR Studies on Acyl Migration in Diglyceride Solutions

R. J. WARREN and J. E. ZAREMBO

Abstract □ NMR spectroscopy has been used to study acyl migration in diglycerides. These studies show that chloroform solutions of 1,2-diglycerides give an equilibrium mixture of 1,2- and 1,3-diglycerides. The process is accelerated with increasing temperature. Under the same conditions, 1,3-diglycerides undergo no significant acyl migration. The NMR method has the advantage of providing qualitative and quantitative data on diglycerides as well as on related side-products such as monoglycerides and triglycerides. The data should be of particular interest to those attempting to synthesize pure isomeric forms of the diglycerides.

Keyphrases □ Acyl migration—in diglycerides, effect of temperature □ Diglycerides—acyl migration, using NMR □ NMR spectroscopy—structure, identity

The migration of acyl groups within the diglyceride molecule was reported by Crossley *et al.* (1) who utilized the technique of differential thermal analysis (DTA). The materials they studied were in the solid state and in solution. With the diglyceride solution, a catalyst in the form of hydrogen chloride was passed through ethereal solutions of the 1,2- and 1,3-diglycerides, and the diglycerides were recovered from solution at various time intervals and examined by DTA. The NMR method proposed here allows one to examine the diglycerides while still in solution, thus eliminating the possibility of change or transformation during the recovery process mentioned in the DTA experiments. The NMR spectrum of the solution also enables one to monitor the system for the formation of side-products such as monoglycerides and triglycerides.

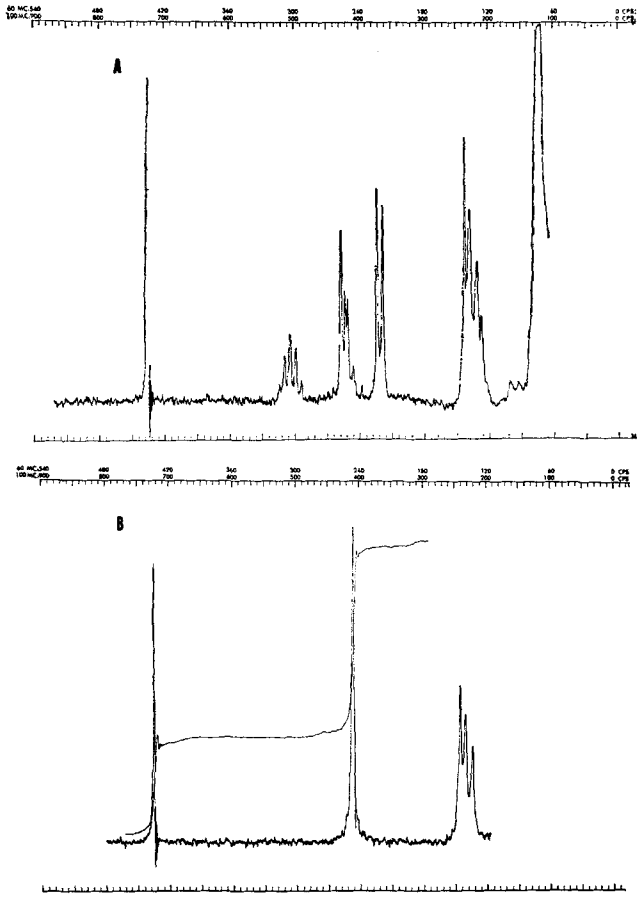

Figure 1—*NMR spectra of: A, 1,2-distearin; and B, 1,3-distearin.*

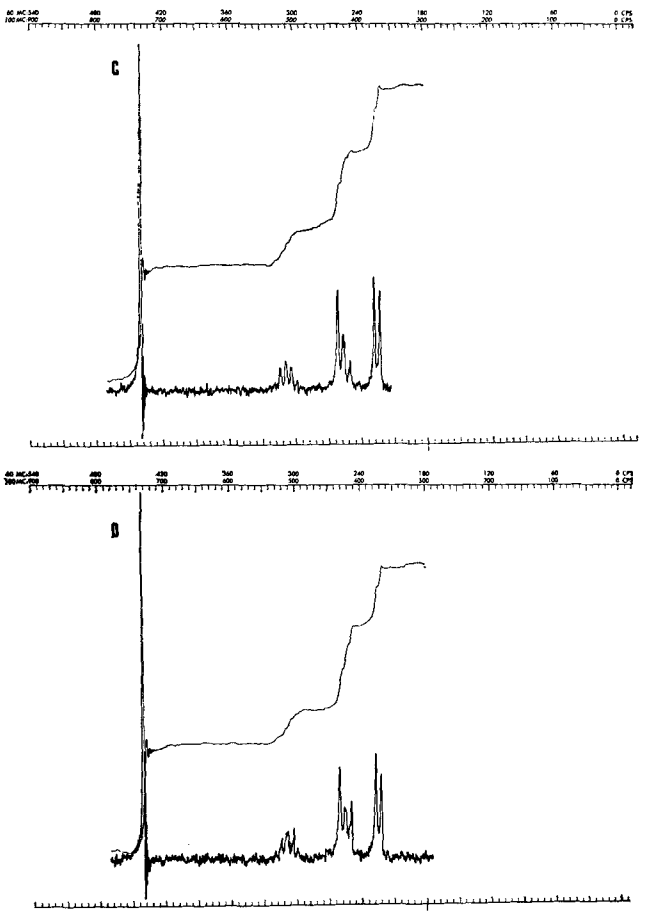

Figure 2—*NMR spectra of 1,2-distearin after being at 60° for several hours.*

In addition, the acyl migration observed in the present experiments took place without the addition of HCl catalyst, and the standard samples used in this study were examined by gas chromatography[1] and found to have no detectable free stearic acid present. These data should be important in the selection of experimental conditions for the synthesis of 1,2- and 1,3-diglycerides.

EXPERIMENTAL

All measurements were carried out with a Jeolco C60H NMR spectrometer equipped with a variable temperature probe.

Deuterated chloroform, with 3% $CHCl_3$ added, was used as

[1] Method to be published separately.

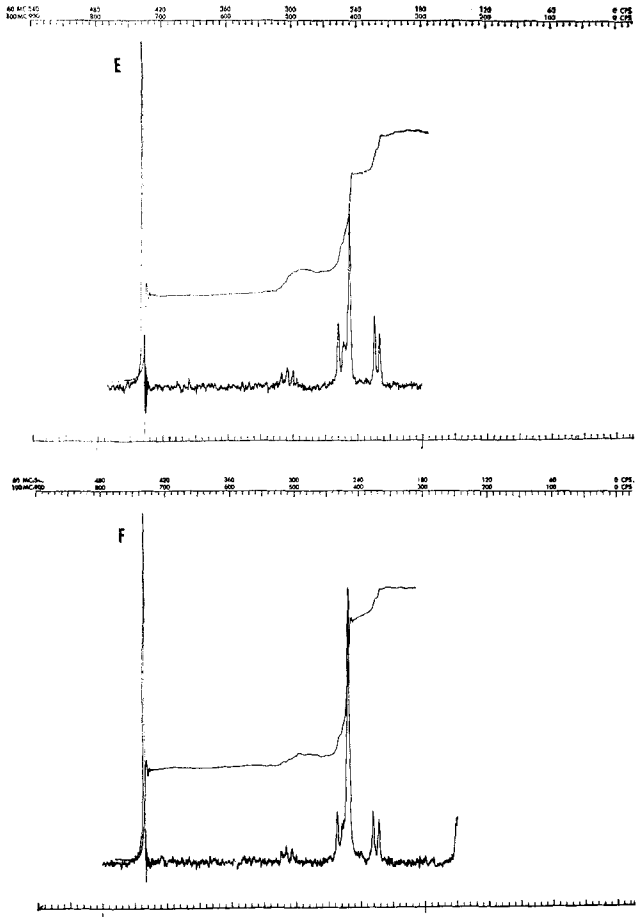

Figure 3—*NMR spectra of: E, 1,2-distearin after storage at 60°; and F, equilibrium mixture of 1,2- and 1,3-distearin.*

solvent. The spectra were recorded on solutions at concentrations of 80 mg./ml.

Samples of 1,2- and 1,3-diglycerides[2] were prepared for NMR analysis by dissolving 80 mg. of the diglycerides in 1 ml. of $CDCl_3$ solution. The spectra of the individual isomers were taken immediately after the samples were dissolved (Fig. 1). The integrated intensity of the band at 259 Hz. relative to the $CHCl_3$ internal standard was recorded. The NMR sample tube containing the 1,2-diglyceride in $CDCl_3$ solution was then transferred to a 60° constant-temperature bath. The sample was withdrawn at selected intervals, and the NMR spectrum was run and integrated.

Thin-layer chromatograms were made using 0.4 *M* boric acid/silica gel GF plates. The solvent system was chloroform–acetone–formic acid (96:4:0.4). The plate was equilibrated in the tank for 15 min. before development. For detection, the plate was sprayed with a 95:5 H_2SO_4–HNO_3 solution and then heated on a hot plate until black spots appeared.

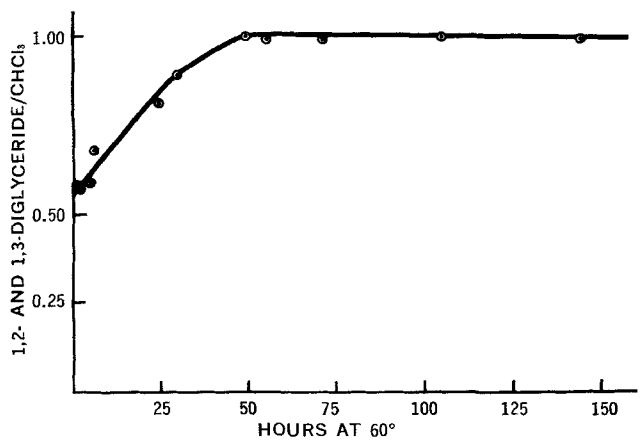

Figure 4—*Plot of amount of diglyceride* versus *time.*

[2] Obtained from the Supelco and Applied Science Laboratories.

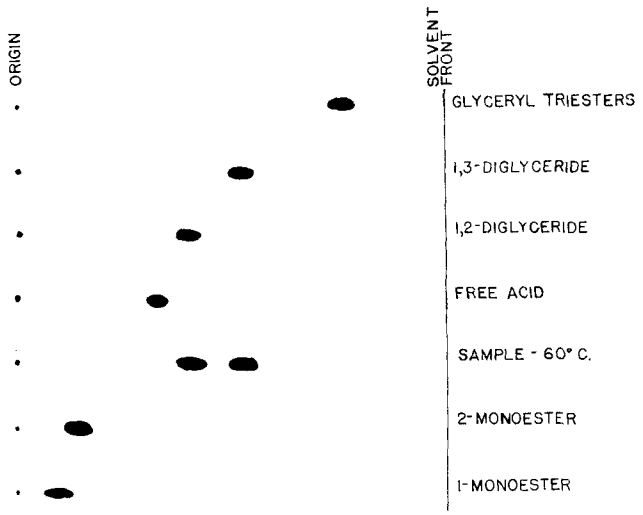

Figure 5—*TLC of glyceride esters.*

RESULTS AND DISCUSSION

The NMR technique has been shown to be applicable in the differentiation and quantitative analyses of 1,2- and 1,3-diglycerides (2). The region of the spectrum used for qualitative and quantitative analysis is 220 to 260 Hz. (3.6 to 4.4 p.p.m.) (Fig. 1). This area contains the absorption signals assignable to the five glyceryl protons in the diglyceride molecules (2). These protons are sensitive to changes within the molecule, such as acyl migration and isomer formation. Therefore, the NMR signals due to these protons afford the basis of a method for following acyl migration in the diglyceride molecules. The technique is quantitative as well as qualitative, and it can be used to calculate the amount of either isomer present at any given time.

The results of these experiments are shown in Figs. 2 and 3. A plot of the amount of 1,2-diglyceride *versus* time shows that, after approximately 50 hr., the solution reaches an equilibrium mixture (Fig. 4). The mixture contains 55% 1,3-distearin and 45% 1,2-distearin. There is no evidence for the formation of any side-products. A thin-layer chromatogram of the equilibrium mixture (Fig. 5) also shows the presence of only two components, namely, the 1,2- and 1,3-distearins. The same experiments at room temperature showed a very slow rate of acyl migration in the same system. The 1,3-diglyceride was found to undergo no significant acyl migration under these conditions.

CONCLUSION

NMR can be used to monitor acyl migration in diglyceride solutions under various conditions. The amount of migration at room temperature is small. Increasing temperature speeds up the migration, and at 60° an equilibrium mixture is attained in approximately 50 hr. The equilibrium mixture contains 55% 1,3-diglycerides and 45% 1,2-diglycerides. No side-products formed under these conditions.

REFERENCES

(1) A. Crossley, I. P. Freeman, B. J. F. Hudson, and J. H. Pierce, *J. Chem. Soc.*, **1959**, 760.

(2) R. J. Warren and J. E. Zarembo, *J. Pharm Sci.*, **59**, 840(1970).

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