A favorable characteristic of the analysis is that the absorbance of the product formed is stable and does not fade over a 24-hr. period. This is an advantage over the colorimetric method of Bratton and Marshall. In the latter method, absorbance readings must be made within 15 min. after color development, due to precipitation of the azo dyes in the method (7). The 9-chloroacridine method also does not involve diazotization. Thus, it eliminates the need for freshly prepared sodium nitrite and ammonium sulfamate solutions required with the Bratton-Marshall technique. Control of pH is required in both methods.

The improved method of analysis for local anesthetics by the 9-chloroacridine approach was carried out for some representative local anesthetics, and comparative analysis were performed using the colorimetric procedure of Bratton and Marshall. Assays were performed on procaine, nesacaine, and metabutethamine hydrochlorides.

The procedure outlined by Connors was used for the analysis by the Bratton-Marshall method (8).

Four determinations by each method were performed for each local anesthetic. The mean percent of concentration employed and the percent standard deviation of the mean for each local anesthetic are shown in Table III for both methods (9).

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 2, 1969, from the *Analytical Laboratory, Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30601*

Accepted for publication December 12, 1969.

A portion of this investigation was presented to the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

This work was supported in part by the National Science Foundation Undergraduate Research Grant GY-6087.

Qualitative and Quantitative Determination of 1,2- and 1,3-Diglycerides by Nuclear Magnetic Resonance Spectroscopy

R. J. WARREN and J. E. ZAREMBO

Abstract □ An NMR procedure is presented for the qualitative and quantitative analysis of 1,2- and 1,3-diglycerides alone or in combination. The method provides a rapid, accurate quantitative analysis, as well as serving as a specific identification of the two isomers. The determination can be carried out on sample sizes in the range 20–50 mg.

Keyphrases □ 1,2- and 1,3-Diglycerides—determination □ NMR spectroscopy—analysis

One of the more difficult problems in glyceride analysis is the differentiation and quantitative determination of 1,2- and 1,3-diglycerides in the presence of one another. Chemical methods are tedious and time consuming. IR spectra are of little value when trying to determine low percentages of one isomer in a mixture of the two. Near-IR spectroscopy (1) has been used to differentiate the 1,2- and 1,3-diglycerides and might have some value. The major drawbacks to using this technique are the large amounts of sample required for a determination, overlap of absorption bands, and relatively small differences in absorptivity values.

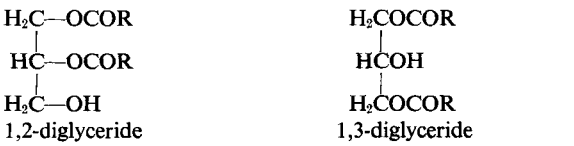
The purpose of this study was to establish the feasibility of using NMR for differentiating between the two isomers and for quantitative analysis of the two isomers.

EXPERIMENTAL

All spectra were recorded on a JEOLCO C60H spectrometer. Deuterated chloroform with 3% $CHCl_3$ added was used as solvent. The spectra were recorded at room temperature at a concentration of 80 mg./ml. Chemical shifts were measured relative to trimethylsilane (TMS).

The 1,2- and 1,3-diglycerides used were 1,2- and 1,3-distearins of reference standard quality.[1]

RESULTS AND DISCUSSION



The NMR spectra of the 1,2- and 1,3-diglyceride isomers differ markedly in the region 220–260 c.p.s. (3.6–4.4 p.p.m.) (Fig. 1). The 1,3-isomer has a singlet absorption at 249 c.p.s. due to the

[1] Supelco and Applied Science Laboratories, State College, PA 16801

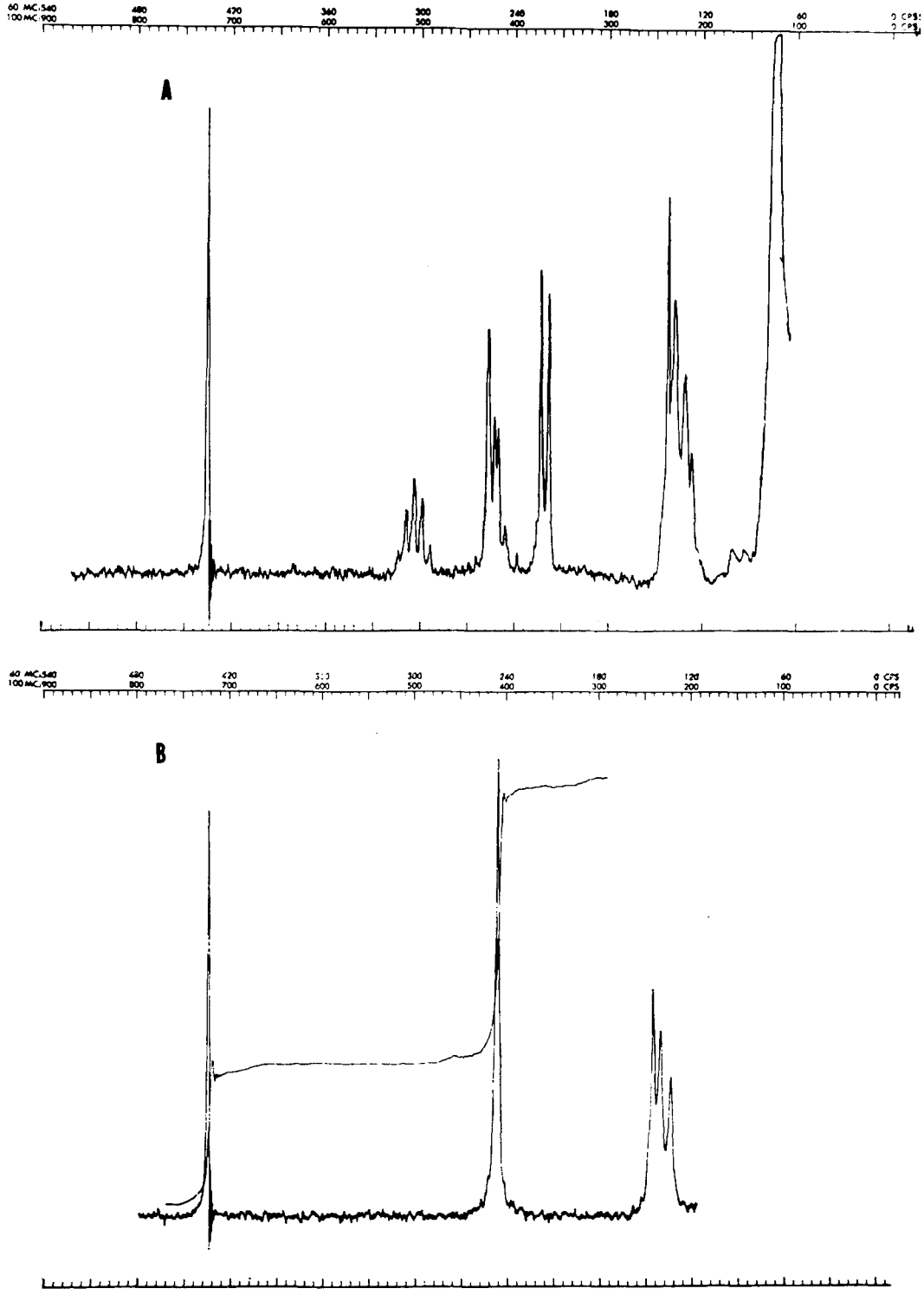


Figure 1—*A, NMR spectra of the 1,2-diglyceride isomer. B, NMR spectra of the 1,3-diglyceride isomer. TMS was used as the internal reference standard.*

five glyceryl protons. The 1,2-isomer has a more complex spectrum consisting of a quartet at 259 c.p.s. from the two mutually non-equivalent 1-protons and a doublet at 225 c.p.s. from the two 3-protons. The quintet of the 2-proton is not used in this analysis. There is no interference from the 1,3-isomer at the first doublet, and this enables one to determine the 1,2-content in a mixture of the two isomers. A series of standards was prepared containing approximately 5, 7.5, 10, and 13 mg. of the 1,2-isomer in 25 mg. of the 1,3-isomer. The materials used for the mixture were reference standard samples. The mixtures were dissolved in $CDCl_3$ containing 3% (v/v) of $CHCl_3$. The single absorption of the $CHCl_3$ at 436 c.p.s. was used as an internal standard in determining the amount of 1,2-isomer present. The NMR spectra of the mixtures were obtained and integrated. Figure 2 shows a spectrum of a representative mixture. The integrated intensity of the 1,2-absorption relative to the internal standard was recorded. The value A 1,2-/A $CHCl_3$ was then plotted *versus* percent of 1,2-diglyceride (Fig. 3). (A 1,2- = integral value of signal at 225 c.p.s. A $CHCl_3$ = integral value of signal at 436 c.p.s.)

Subsequent samples were prepared and run in the same way. By comparison of standards and samples, it was possible to determine the amount of 1,2-isomer present.

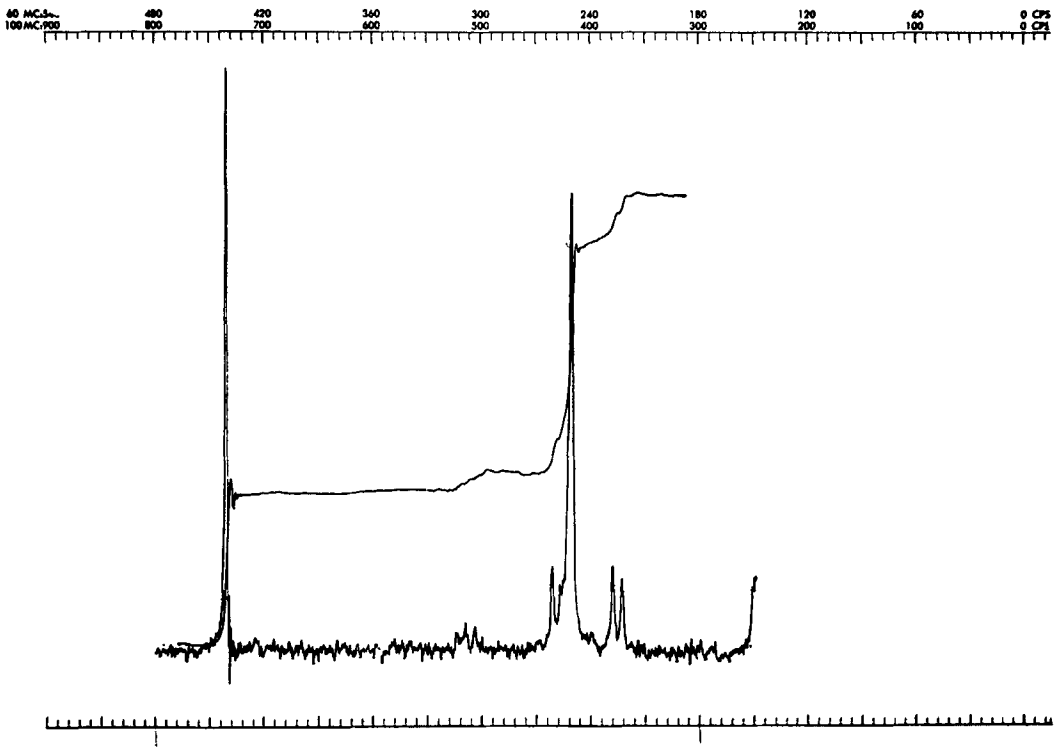


Figure 2—*NMR spectra of a mixture of 1,2- and 1,3-distearin.*

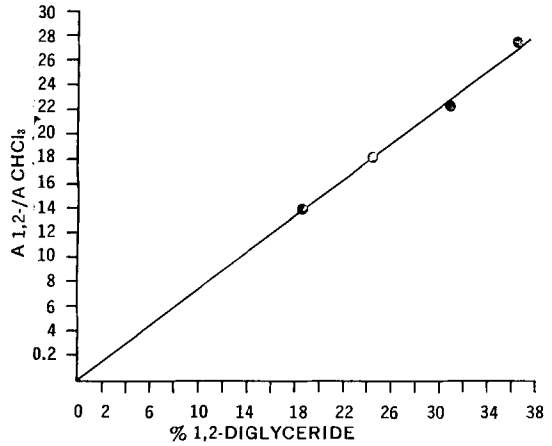


Figure 3—*The value of A 1,2-/A $CHCl_3$ plotted against the percent of 1,2-diglyceride, with A 1,2- = integral value of signal at 225 c.p.s. and A $CHCl_3$ = integral value of signal at 436 c.p.s.*

Some interference could be expected if large amounts of triglycerides were present, but this is an unlikely circumstance. Unsaturated acid moieties would not interfere since they would absorb in the region beyond 275 c.p.s.

The determination can be carried out on samples in the 10–20-mg. range, but the results are best obtained with samples of approximately 50 mg. In addition to the quantitative aspect of the method, the NMR spectra also provide a specific qualitative identification of the isomers.

SUMMARY

A quantitative and qualitative method of analysis for 1,3- and 1,2-diglyceride isomers has been presented. The method can be carried out on sample sizes at the order of 50 mg. The method is fast, accurate, and specific for the two isomers.

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ACKNOWLEDGMENT AND ADDRESSES

Received December 15, 1969, from *Smith Kline & French Laboratories, Philadelphia, PA 19101*

Accepted for publication February 9, 1970.

The authors are grateful to Mr. John A. Messina for technical assistance in obtaining the spectra presented here.