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Internal Hydrogen Bonding, Rotational Isomerism and Differentiation of 1,2- and 1,3-Diglycerides by Near Infrared Spectroscopy¹

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Abstract

The OH stretching modes of 1,2- and 1,3-diglycerides in dilute carbon tetrachloride solution have been investigated in the fundamental and first overtone region with a spectral resolution of about 1 cm^{-1} . The spectra of both position isomers can be interpreted in terms of three predominant configurations: one internally H-bonded and two involving no H-bonds. The fraction of H-bonded molecules appears to be relatively low. The data suggest that the H-bonded form of 1,3-diglycerides has a *trans,gauche* configuration around the glyceride CC bonds, while the non-H-bonded forms probably have a *trans,trans* configuration around the glyceride backbone, OH being either *trans* or *gauche* with respect to the nearest C-H group. In 1,2-diglycerides H-bonds seem to be predominantly formed between groups attached to the α,γ carbons of the glyceride residue, the configuration around the backbone being *trans,trans*; two stretching bands of non-H-bonded OH groups are associated with rotational isomerism around the alcoholic CO bond. The usefulness of OH stretching modes for analyzing mixtures of 1,2- and 1,3-diglycerides is discussed.

Reasonably accurate results can be obtained by using either the fundamental or the first overtone region.

Introduction

IN A MOLECULE of the general formula $\text{CH}_2\text{XCHYCH}_2\text{Z}$ —which corresponds to a generalized glyceride model where OH and OCOR groups are assumed to be rigid—internal rotation is possible about two carbon to carbon bonds, giving rise to nine rotational isomers. If two or more substituent groups are identical the number of isomers is decreased, but the picture still remains highly complex, as evidenced by recent NMR studies (1). In the case of mono- and diglycerides some isomers are stabilized by internal hydrogen bonding and information regarding rotational isomerism can be obtained by investigating the characteristic OH stretching vibrations which are sensitive to intramolecular environment in general, and hydrogen bonding in particular. The over-all problem is, of course, more complex than the simple model represented by $\text{CH}_2\text{XCHYCH}_2\text{Z}$ suggests, because of hindered rotation around the various C-O bonds and the C-C bonds of hydrocarbon side-chains. The present communication discusses rotational isomerism of 1,2- and of 1,3-diglycerides in dilute CCl_4 solution in the light of observed infrared spectra in the fundamental OH stretching region (around 3500 cm^{-1}) and

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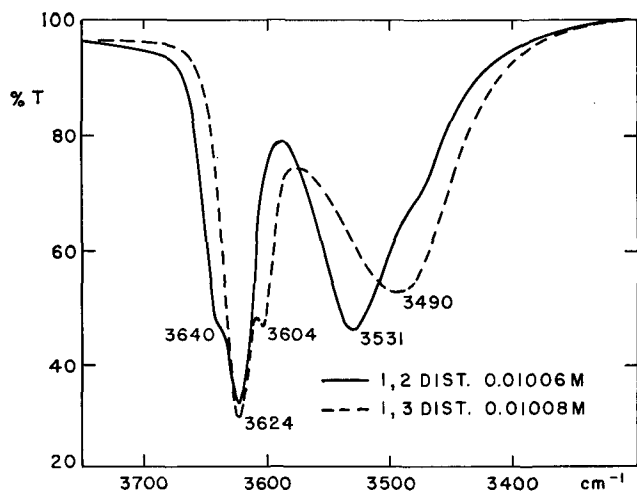


FIG. 1. Transmittance of diglycerides in the fundamental OH stretching region, 1 cm path length.

the first overtone region (around 7000 cm^{-1}). The feasibility of analyzing mixtures of 1,2- and 1,3-diglycerides on the basis of spectral changes caused by environmental differences between OH groups is evaluated.

Experimental

Infrared spectra from 3300 cm^{-1} to 3800 cm^{-1} were obtained with a Beckman Model IR-7 prism-grating instrument, from 6600 cm^{-1} to 7400 cm^{-1} (ca. $1.5\text{--}1.35\ \mu$) with a Cary Model 14 instrument. The spectral resolution was better than 1 cm^{-1} at all wavelengths. Quartz cells of 1 cm path length were used for studies in the fundamental region, of 10 cm path length for investigations in the overtone region. Samples were run as dilute solutions in carbon tetrachloride against a solvent blank in an identical quartz cell. The solvent was dried before use by bubbling through it a stream of dry nitrogen. Solutions were prepared, and the absorption cells were filled, in a dry nitrogen atmosphere. Concentrations ranged from 0.005 to 0.030 moles per liter. No external hydrogen bonding was observed under these conditions. Limited solubility prevented investigations at higher sample concentrations.

The following compounds were investigated: 1,3-distearin; 1,3-dipalmitin; 1,3-divernolin; 1,2-distearin; 1,2-dipalmitin; 1-stearoyl, 2 palmitin; 1-palmitoyl, 2 stearin. 1,3-Distearin and 1,3-dipalmitin were prepared from 1-tritylglycerol which was first acylated to

form 1-trityl, 2,3-diglyceride. Detritylation with dry HCl yielded the 1,3-diglyceride (2). A sample of 1,3-divernolin was supplied by C. F. Krewson of this laboratory. 1,2-Distearin was formed by rearrangement of 1,3-distearin according to Crossley et al. (4) and purified by utilizing the greater solubility of the 1,2 isomer in petroleum ether. The synthesis of "mixed" 1,2-diglycerides is illustrated by the example of 1-stearoyl, 2 palmitin. 1-Stearoyl, 3 benzyl ether glyceride was prepared from 1-benzyl ether glycerol and purified by passing through a silica gel chromatographic column. The purified product was treated with palmitoyl chloride and the resulting glyceride hydrogenated to form 1-stearoyl, 2-palmitin.

Assignments in the Fundamental Region and Molecular Configuration

Figure 1 gives the observed spectra of 1,3-distearin and of 1,2-distearin in the fundamental OH stretching region. Although the number of possible rotational isomers is high (even if only rotation around the glyceride CC bonds is considered) only three bands are observed for either compound. This suggests that some isomers give rise to very similar spectra or that some possible configurations have a very low population. The data obtained on other 1,3- and 1,2-diglycerides did not deviate from the ones obtained on distearins, if equimolar concentrations were used.

1,3-Diglycerides exhibit OH stretching bands at 3624 cm^{-1} (sharp), 3604 cm^{-1} (sharp) and 3490 cm^{-1} (broad). The broad 3490 cm^{-1} band must be associated with a configuration permitting strong internal hydrogen bonds, the other two with configurations with non-bonded OH groups. If *trans* and *gauche* are the stable forms around each C—C bond, as commonly assumed, the hydrogen-bonded form must have a *gauche-gauche* or *trans,gauche* configuration. The *trans,gauche* isomer, illustrated schematically in Figure 2-I, appears more probable because of likely repulsion between oxygen atoms. The schematic presentations in Figures 2 and 3 might warrant a brief explanation. The structural formulae on top indicate isomerism around the glyceride CC bonds, i.e., the type of isomerism which determines the possibility of hydrogen bond formation. The figures on the lower right hand side are generally accepted Newman projections which present the rotomers around the C—OH bond (viewed along the O—C axis) of non-hydrogen-bonded species. In these projections C_2 is behind the oxygen atom in Figure 2; C_3 is behind the oxygen atom in Figure 3. It is interesting to note that the population of the hydrogen bonded form must be relatively low, because bands associated with hydrogen bonded OH groups are usually much more intense than "free" OH bands. The conclusion that a relatively low fraction of the molecules is in an internally H-bonded configuration is supported by the observation that the C=O stretching band of 1,3-diglycerides, as observed in dilute CCl_4 solution, is a sharp, almost symmetrical singlet centering at 1745 cm^{-1} , with a very weak shoulder at the low frequency side. The hydrogen bond itself must, however, be strong, as evidenced by the low frequency of the 3490 cm^{-1} band. The observation that the population of the H-bonded *gauche* form is low, although the H-bond itself is strong, supports the view that *gauche* configurations are inherently unstable and that the *trans-gauche* form is the predominant one in internally hydrogen bonded 1,3-diglycerides.

The bands at 3624 cm^{-1} and 3604 cm^{-1} are undoubt-

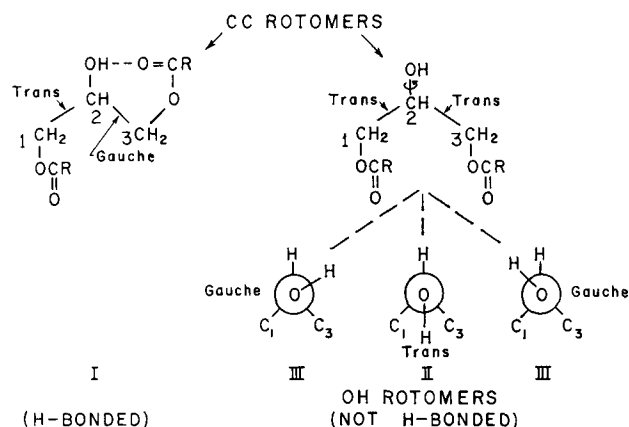


FIG. 2. Schematic presentation of proposed configurations of 1,3-diglycerides.

- I. H-bonded. C_1C_2 *trans*, C_2C_3 *gauche*.
- II. Not H-bonded. C_1C_2 *trans*, C_2C_3 *trans*, HC-OH *trans*.
- III. Not H-bonded. C_1C_2 *trans*, C_2C_3 *trans*, HC-OH *gauche*.

edly associated with free OH groups, the isomerism around the glyceride CC bonds being most probably *trans-trans*, with no hydrogen bonds possible. Comparison with spectra of secondary alcohols (5) strongly suggests that the doublet is caused by hindered rotation around the alcoholic CO bond. Table I compares the frequencies of non-hydrogen-bonded OH groups of 1,3-diglycerides and secondary alcohols. These data suggest that the 3624 cm⁻¹ band is associated with a structure schematically pictured in Figure 2-III, and the 3604 cm⁻¹ band with the structure in Figure 2-II. Form III appears to be more highly populated, as in corresponding alcohols (5).

The observed spectra can thus be interpreted in terms of three predominant configurations: (I) A hydrogen-bonded *trans-gauche* form with strong hydrogen bonds but relatively low population. (II) and (III) Non-hydrogen-bonded *trans-trans* forms distinguished by rotation around the alcoholic CO bond. Isomerism around other bonds does not appear to influence the spectra to any significant degree.

1,2-Diglycerides exhibit three OH stretching bands at 3640 cm⁻¹ (shoulder), 3625 cm⁻¹ (sharp) and 3531 cm⁻¹ (broad) and a very weak shoulder around 3490 cm⁻¹. The 3531 cm⁻¹ band must be associated with an internally hydrogen bonded modification, but the hydrogen bonds appear to be considerably weaker than in the case of 1,3-diglycerides, resulting in a much smaller frequency shift (93 cm⁻¹ as compared to 135 cm⁻¹). The latter observation suggests that in 1,2-diglycerides hydrogen bonds are formed predominantly between groups attached to α,γ carbon atoms and the configuration around the glyceride CC bonds is *trans-trans*. The proposed hydrogen bonded structure is schematically represented in Figure 3-I. It is in agreement with the previous suggestion (see above) that *trans* configurations in glycerides are more stable than *gauche* configurations. The weak shoulder around 3490 cm⁻¹ might be caused by hydrogen bonds between groups attached to α,β carbons, but the population of this configuration must be very low.

The bands at 3640 cm⁻¹ and 3625 cm⁻¹ must be associated with non-hydrogen-bonded isomers. Comparison with data on primary alcohols (5), summarized in Table I, suggests that the doublet is again caused by rotation around the alcoholic CO bond. The two configurations are schematically shown in Figure 3-II and 3-III. The configuration around the CC bond next to the OH groups is probably *trans* (to avoid

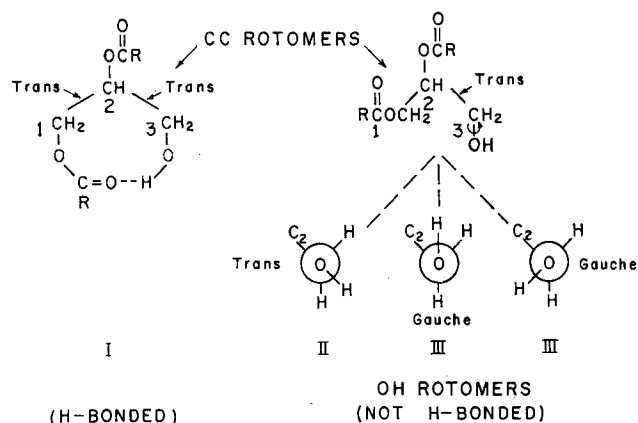


FIG. 3. Schematic presentation of proposed configurations of 1,2-diglycerides.

- I. H-bonded. C₁C₂ *trans*, C₂C₃ *trans*.
 II. Not H-bonded. C₂C₃ *trans*, CC-OH *trans*.
 III. Not H-bonded. C₂C₃ *trans*, CC-OH *gauche*.

TABLE I

Comparison of non-H-Bonded OH Stretching Frequencies (cm⁻¹) in Alcohols and Diglycerides

| 1,3-diglycerides | Sec. alcohols ^a | Orientation of OH ^b |
|------------------|----------------------------|--------------------------------|
| 3624 | 3626-3631 | <i>gauche</i> |
| 3604 | ca. 3600-3610 | <i>trans</i> |
| 1,2-diglycerides | Prim. alcohols | Orientation of OH |
| 3640 | 3634-3640 | <i>trans</i> |
| 3625 | ca. 3625-3630 | <i>gauche</i> |

^a Ref. 5.

^b Cf. Figs. 2 and 3.

hydrogen bonding between neighboring groups) but the arrangement around the other glyceride CC bond is difficult to estimate.

The spectra of 1,2-diglycerides—like the spectra of the 1,3 isomers—are thus most easily interpreted in terms of three predominant configurations: (I) One form where groups attached to the α,γ carbons form hydrogen bonds and the configuration around glyceride CC bonds is *trans-trans*. (II) and (III) Non-hydrogen-bonded forms distinguished by rotation around the alcoholic CO bond.

First Overtones

Figure 4 presents the obtained spectra of 1,2-distearin and 1,3-distearin in the region of the first overtones of OH stretching fundamentals. The assignments are summarized in Table II, together with the first order anharmonicity constants x , as defined by

$$x = \frac{1}{2} \left(1 - \frac{\nu}{2\nu_s} \right)$$

where ν is the observed frequency of the first overtone and ν_s of the corresponding fundamental (6). The first overtones of the stretching fundamentals of non-hydrogen-bonded OH groups can be easily assigned, leading to a reasonable (6,7,8) value of $x = 0.012$. The anharmonicity of the stretching vibrations of hydrogen bonded OH groups appears to be lower, in agreement with the general observation (9) that ν_s is of normal anharmonicity or possibly more harmonic after H-bond formation. The lower anharmonicity accounts for the relatively low intensity of the overtones of the OH stretching vibrations of H-bonded species. Thus, while the stretching fundamentals of H-bonded OH groups are relatively intense, only a weak, broad shoulder is observed in the overtone region (cf. Figs. 1 and 4).

The weak band at 7190 cm⁻¹, which appears in the spectra of both position isomers, is not easily explained as a second harmonic. It could be due to a ternary combination involving fundamentals other than OH stretching modes.

TABLE II

Assignment of Observed Bands

| cm ⁻¹ | Assignment ^a | $x = 1/2 (1 - \nu/2\nu_s)$ |
|------------------|-------------------------|----------------------------|
| 3624 | 1,3 diglycerides | |
| 3604 | Form III | |
| 3490 | Form II | |
| | Form I | |
| 7190 | | |
| 7074 | 2 × 3624 = 7248 | 0.012 |
| 7035 | 2 × 3604 = 7208 | 0.012 |
| (6945) | 2 × 3490 = 6980 | (0.002) |
| 3640 | 1,2-diglycerides | |
| 3625 | Form II | |
| 3531 | Form III | |
| | Form I | |
| 7190 | | |
| 7107 | 2 × 3640 = 7280 | 0.012 |
| 7074 | 2 × 3625 = 7250 | 0.012 |
| (6945) | 2 × 3531 = 7062 | (0.008) |

^a Cf. Figs. 2 and 3. Approximate centers of very shallow bands are given in parentheses.

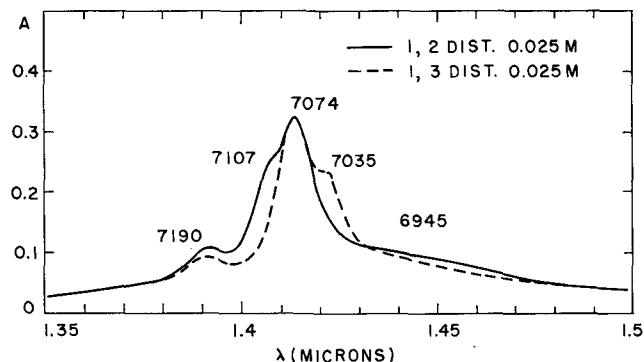


FIG. 4. Absorbance of diglycerides in the first overtone region. 10 cm path length. The numbers within the figure indicate frequencies in cm^{-1} .

The overtone region adds little to interpretation in terms of rotational isomerism. It might be of some importance in analytical work because of high performance characteristics of various commercial instruments available for absorption measurements in this spectral region.

Analytical Application

Analysis of mixtures of 1,2- and 1,3-diglycerides by chemical methods is at best a tedious and time consuming process. The near infrared spectra pictured in Figures 1 and 4 can be utilized for rapid identification and for reasonably accurate analyses of mix-

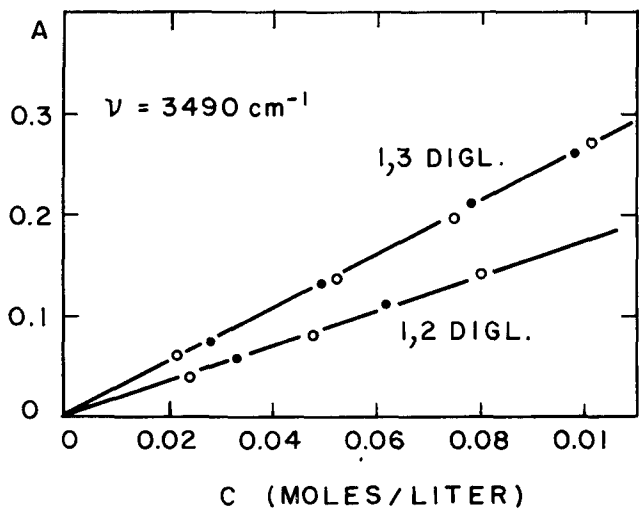
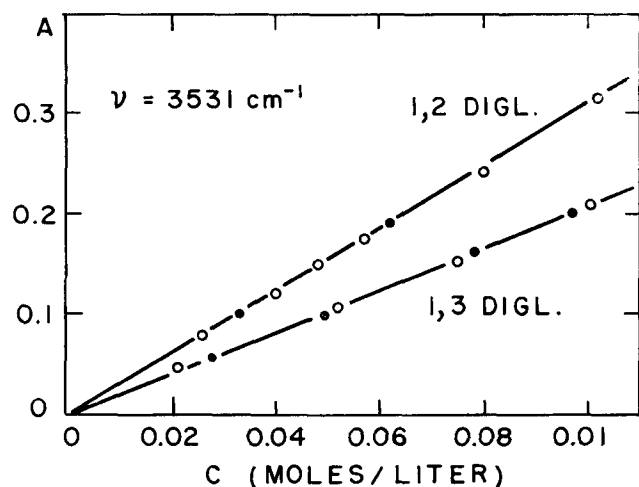


FIG. 5. Absorbance of 1,2- and 1,3-diglycerides at 3531 and 3490 cm^{-1} as a function of concentration. Open circles—distearins. Closed circles—dipalmitins.

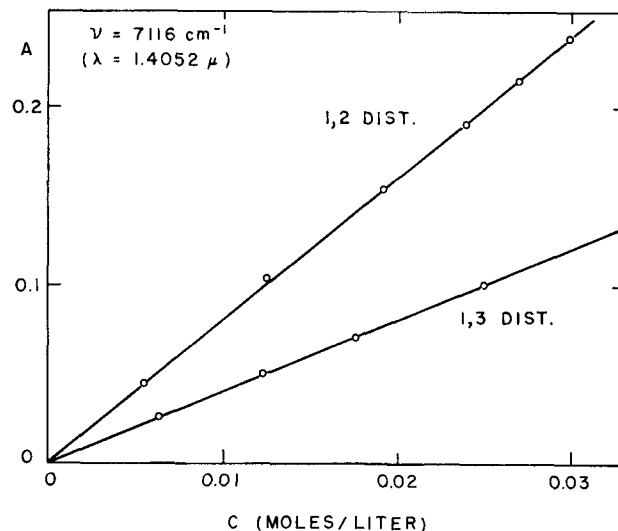


FIG. 6. Absorbance of 1,2- and 1,3-distearin at 7116 cm^{-1} as a function of concentration.

tures, although optimum differences in absorptivity values are relatively small, and sample concentrations are limited by solubility and by intermolecular hydrogen bonding. Relatively low signal to noise ratios must therefore be expected. In Figures 5 and 6 concentration vs. absorbance is plotted at two wavelengths in the fundamental OH stretching region, and at one wavelength in the overtone region for representative 1,2- and 1,3-diglycerides (cf. Figs. 1 and 4). A "base line" technique was employed, with reference points at 3300 cm^{-1} and 3750 cm^{-1} in the fundamental region, and at 6660 cm^{-1} and 7410 cm^{-1} in the overtone region.

To evaluate the obtainable accuracy, mixtures containing 10%, 30%, 50%, 70%, and 90% of 1,2-distearin in 1,3-distearin were analyzed 1) by measuring base line absorptivity at 3531 cm^{-1} (1 cm quartz cells of 2.5 cm diam, total concn 0.01 moles/liter, amount of sample ca. 0.05 g), and 2) by measuring base line absorptivity at 7116 cm^{-1} (10 cm quartz cells, total concn 0.025 moles/liter, amount of sample ca. 0.8 g). The procedure was closely analogous to the one previously described for mixtures of 1- and 2-monoglycerides (10) and the results were of comparable accuracy. Maximum deviation between measured and known composition was around 2%. This represents satisfactory agreement considering the small absorbance differences between the two classes of compounds, the low concentrations which have to be used and the resulting low signal to noise ratio.

The fundamental region is more convenient for such analytical measurements because of the higher intensity of the bands and consequently the smaller amount of sample required. On the other hand, commercial instruments designed for absorption measurements in the 1–2 μ region frequently offer a higher signal to noise ratio and higher photometric reproducibility.

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