Determination of Trace Amounts of Water in Glycerides by Near-Infrared Spectroscopy

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Abstract □ A rapid accurate method for determining trace amounts of water in glycerides is presented. The method is based on the measurement of the combination band for water which occurs at 1.896 μ in chloroform solution. In addition to the advantage of making it possible to work at low levels, the method is specific for water and free of interferences due to other OH groups. Several commercial samples of glyceryl mono- and distearates were analyzed by this method, and showed water contents of 0.4 to 1.2%.

Keyphrases □ Glycerides—water determination □ Water determination—trace amounts □ Near IR spectrophotometry—analysis

During the authors' investigation of methods for the analysis of mono-, di-, and triglycerides, it became necessary to develop a rapid and accurate method for the analysis of trace amounts of water. Chemical methods, such as the Karl Fischer technique, were tedious, time-consuming, and/or unsatisfactory for these particular needs. Solution spectra on conventional IR instruments were rejected because of the many overlapping absorption bands from the various —OH groups in the glyceride molecule itself. Near-IR spectroscopy was investigated to determine the feasibility of using this technique to determine the OH absorption of water in the presence of OH groups on the glyceride molecule. It was found that OH groups in different molecular environment, such as water or glyceride —OH's, do behave differently, and are amenable to investigation by near-IR spectrophotometry. This has also been shown by others (1). The absorption of water OH, attributable to a combination band of the OH group (2, 3), occurs at 1.896 μ in chloroform solution. The various OH groups of the glyceride molecule are found in the vicinity of 1.430 and 2.100 μ (4). This leaves the area of water absorption free from OH absorptions related to the materials under investigation, and presents us with a method specific for water and free from interfering substances.

EXPERIMENTAL

Glycerides were obtained from the Kessler Chemical Company. The glycerides are commercial samples consisting of a mixture of mono-, di- and tripalmitate, stearate and glyceryl esters. There are also traces of free glycerol, stearic, palmitic, myristic, and other unknown acids in trace amounts. Duplicate analyses of these samples, made using the proposed method, showed 1.2, 1.4, 0.4, 0.5, and 0.8% water.

The near-IR spectra were obtained on a Cary model 14 recording spectrophotometer, using matched fused silica cells of infrared quality having a light path of 5 cm. Chloroform was used as a solvent and as solvent compensator in the reference beam.

RESULTS AND DISCUSSION

The method for the determination of water in glycerides is as follows: transfer about 2.5 g. of sample, accurately weighed, to a 50-ml. volumetric flask; dissolve in, and dilute to volume with chloroform. Record the absorption spectrum of this solution *versus* chloroform in 5-cm. cells from 1.930 to 1.850 μ. Measure the difference (ΔA) between the absorbances at 1.896 and 1.920 μ. From a calibration chart of standards, determine the percent of water present.

$$\text{calculation: } \frac{\text{wt. of water present (from chart)} \times 100}{\text{wt. of glyceride sample}} = \% \text{ water}$$

The calibration or standard curve is prepared as follows: dissolve known amounts of water in 50 ml. of chloroform, and run the absorption spectrum on the Cary Model 14 in 5-cm. cells from 1.930 to 1.850 μ. Measure the difference (ΔA) between the absorbances at 1.896 and 1.920 μ. Plot the absorbance *versus* concentration values to prepare a calibration curve.

The spectra obtained from the calibration curve are shown in Fig. 1. Plotting these values against the concentration or percent water produces the calibration or working plot shown in Fig. 2.

This method has the advantages of being rapid, accurate for small

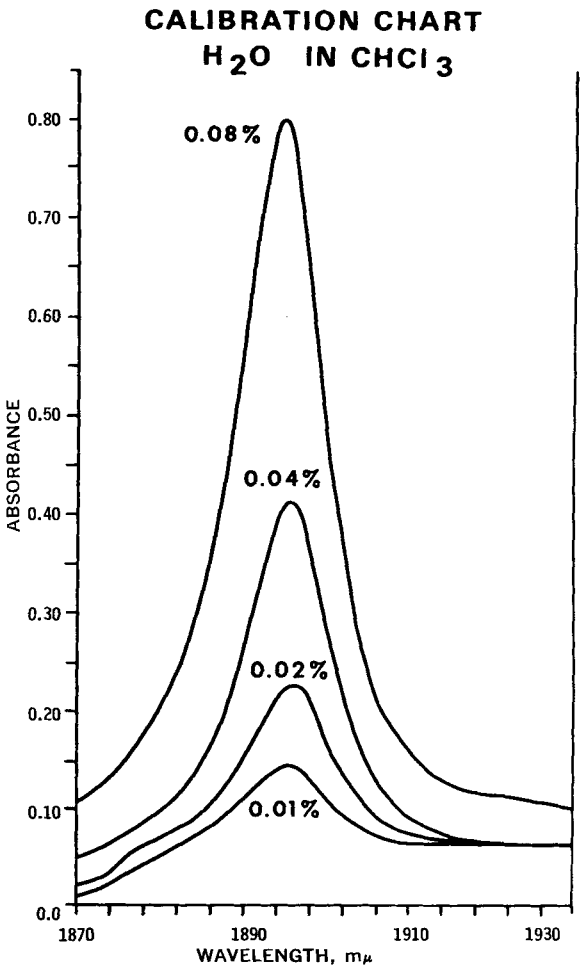

Figure 1—*Spectra obtained from calibration curve (see text for calculation).*

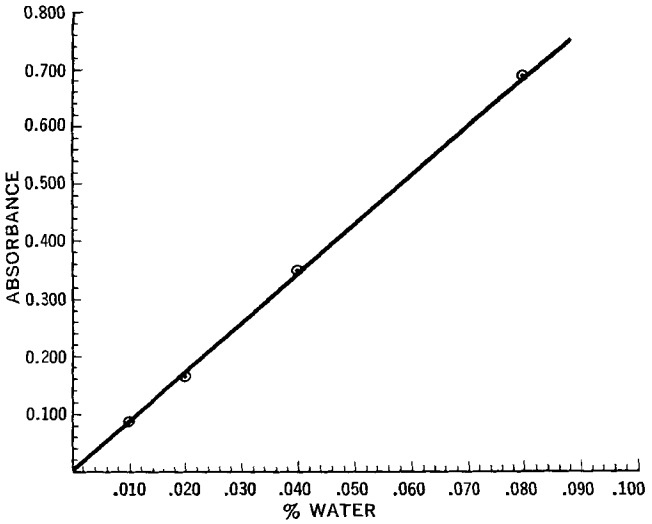

Figure 2—*Calibration or working plot obtained from the values in Fig. 1 plotted against concentration.*

amounts of water, and free from interferences. In addition, it makes it possible to determine water down to the level of 0.05% based on an initial sample weight of 2.5 g.

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Aminosteroids

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Abstract □ Synthesis of 17β-acetamido-6α,16α-dimethylandrost-4-en-3-one and 3-aza-17β-acetamido-6α,16α-dimethylandrost-4a-en-4-one *via* Beckmann and Schmidt rearrangement has been described. Both of these compounds were judged inactive when subjected to Herschberger androgen-anabolic assay.

Keyphrases □ 17β-Acetamido-6α, 16α-dimethylandrost-4-en-3-one—synthesis □ 3-Aza-17β-acetamido-6α, 16α-dimethylandrost-4a-en-4-one—synthesis □ UV spectrophotometry—identity □ IR spectrophotometry—identity

Torizuka *et al.* (1) while investigating the dynamics of protein metabolism in man, found that anabolic steroids such as 19-nor-testosterone phenylpropionate and 4-chlorotestosterone acetate, did not inhibit degradation of protein but exclusively stimulated its synthesis. The primary site of action is possibly at the nuclear level for the production of RNA's essential for protein biosynthesis (2). There are a large number of theoretical possibilities by which an androgen molecule can stimulate this RNA production. One of them suggested by Hübener (3) would be to inhibit a repressor aimed at an operator gene and controlled by a regulator gene. The interaction between androgen molecule and protein has been reported by Westphal (4) and that this interaction is on the β-face of the steroid molecule was suggested by Wolff *et al.* (5).

This work was initiated to investigate this hypothesis and to see if creation of high electron densities in Ring A and D would impart or enhance biological response. 6α,16α-Dimethylprogesterone was chosen for these molecular modifications. Treatment of I with pyrrolidine gave the enamine II which could be converted into 20-oximino Compound III with hydroxylamine hydrochloride. Beckman rearrangement of III using thionyl chloride, followed by acid hydrolysis gave the desired 17β-acetamido-6α,16α-dimethylandrost-4-en-3-one (IV). Compound I when subjected to Schmidt rearrangement gave 3-aza-17β-acetamido-6α,16α-dimethylandrost-4a-en-4-one (V). The spectral data confirmed all the structural assignments. Compounds IV and V were subjected to Herschberger androgen-anabolic assay (6) and judged inactive (see Scheme I).

EXPERIMENTAL

All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. The UV and IR data were obtained on Cary Model 11 and Beckman IR-5 spectrophotometers, respectively. Elemental analyses were performed by Midwest Microlab, Inc., Indianapolis, Ind.

3-[1-Pyrrolidinyl]-6-16α-dimethylpregn-3,5-dien-20-one (II)—Two grams of I was dissolved in 10 ml. of MeOH with heat and treated with 10 drops of pyrrolidine. Heating was continued for an additional 5 min. when copious precipitates of enamine separated. The precipitates were collected by filtration, washed several times with MeOH and dried to give 1.8 g. (77%) of II, m.p. 137–139°; $\lambda_{max.}^{EtOH}$ 278 mμ; $\lambda_{max.}^{KBr}$ 5.88, 6.1, and 6.23μ.

Anal.—Calcd. for $C_{27}H_{41}NO$: N, 3.54. Found: N, 3.23.

3-[1-Pyrrolidinyl]-6,16α-dimethylpregn-3,5-dien-20-one oxime (III)—A mixture containing 1.7 g of II, 500 mg. of $NH_2OH \cdot HCl$ in 5 ml. of pyridine was heated on a steam bath for 1.5 hr. and poured