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ULTRAVIOLET SPECTRAL MEASUREMENTS ON ISOLATED DOUBLE BOND SYSTEMS

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RECENTLY, Reichstein and co-workers[1] and Ellington and Meakins[2] reported the ultraviolet absorption spectra of isolated double bonds (particularly in steroids and triterpenoids) measured with nitrogen-flushed, conventional spectrophotometers. The results lacked variation with structure below 195 mμ and appeared in considerable disagreement with Turner's study using a vacuum spectrophotometer.[3] These factors and an interest in the spectra of polycyclic compounds led us to a re-examination of the 175-205 mμ region. This preliminary communication was prompted by recent reports of studies relying on conventional spectrophotometers: (1) an attempt by Chapman and Parker[4] to resolve the above differences, (2) the apparent acceptance by Swiss researchers[5,6] of methods credited to Reichstein and co-workers,[1] and (3) the current interest in interaction studies utilizing measurements in this spectral region.[7] The limitations and some sources of error with such

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[1] K. Stich, G. Rotzler and T. Reichstein, Helv.Chim.Acta 42, 1480 (1959).

[2] P.S. Ellington and G.D. Meakins, J.Chem.Soc. 697 (1960).

[3] D.W. Turner, J.Chem.Soc. 30 (1959).

[4] J.H. Chapman and A.C. Parker, J.Chem.Soc. 2075 (1961).

[5] C.H. Grob and A. Weiss, Helv.Chim.Acta 43, 1390 (1960).

[6] H.P. Sigg and Ch. Tamm, Helv.Chim.Acta 43, 1402 (1960).

[7] S. Winstein, L. De Vries and R. Orloski, J.Amer.Chem.Soc. 83, 2020 (1961).

instruments were recognized but many of the reports[1,2,4] seemed overly optimistic as to the lower limits of reliability. Consequently, structure-spectral relationships based on such results could lead to erroneous conclusions regarding interaction or positional effects. The following discussion stresses some of these discrepancies, suggests probable sources of error, and notes methods of assessing the limitations of the measuring systems.

Our measurements were made using a Beckman "Extended-UV-Range" DK-2 spectrophotometer[8] designed for operation down to 170 mμ.[9] This instrument has excellent stray light characteristics: < 0.5% at 180 mμ with a Corning #7905 Vycor filter with 1% T at 220 mμ; < 2% at 180 mμ with doubly-distilled water (in a special, high transmissivity, 0.1 cm cell provided by the manufacture), if the 2.25 absorbance of the water determined from measurements in a 0.01 cm cell is taken into account. If stray light at 180 mμ is 2%, the maximum error in observed absorbances < 0.5 will not exceed 4%[10] and, since the stray light becomes essentially zero at 185 mμ, this error becomes insignificant. By use of cyclohexane (purified through Davison #923 silica gel) in one-piece stoppered quartz cells (sample 0.011 cm, reference 0.010 cm),[11] errors due to solvent absorbance[12] can be corrected for or are negligible since this cyclohexane has 80% transmittance in 0.01 cm cells at 180 mμ.

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[8] Reference to a company or product name does not imply approval or recommendation of the product by the Department of Agriculture to the exclusion of others that may be suitable.

[9] "Spectroscopy in the Region 175-200 mμ" by Wilbur Kaye, Beckman Instruments, Inc., Reprint R-6150, adequately discusses the special instrumentation and precautions for operation in this region.

[10] K.S. Gibson in M.G. Mellon, *Analytical Absorption Spectroscopy* pp. 247-248. John Wiley, New York (1950).

[11a] American Instrument Co., Silver Springs, Md.; [b] We are indebted to Mr. G.F. Bailey for these measurements.

[12] D.D. Tunnicliff, *Talanta* **2**, 341 (1959), has discussed the types of errors influenced by solvent absorbance.

The validity of our measuring system was established by comparison with published data obtained with vacuum instruments. The ϵ values from the spectrum of cyclohexene are offered as an example (Fig. 1), and our λ_{max} 183.5 mμ, ϵ_{max} 7750 agree with those found using vacuum instruments, i.e. λ_{max} 183 mμ, ϵ_{max} 7500;[3] λ_{max} 183.5, ϵ_{max} 7400[13]; λ_{max} 183, ϵ_{max} 6800 ± 700.[14] That the maximum near the limit of our system cannot be due to stray light is substantiated by the minimum at 183 mμ in the curve for cholesteryl acetate (Fig. 1). As pointed out by Johnson[15], a minimum in the absorbance curve is much more convincing evidence of low stray light than a maximum.

Earlier values[1,2] for cholesteryl acetate (Fig. 1) are in excellent agreement with our values from 210 to 200 mμ, but at about 197 mμ they diverge and indicate spurious maxima at 193 mμ[1] or near 195 mμ.[2] Similarly, the data of Chapman and Parker[4] (λ_{max} 189 mμ, ϵ_{max} 8000), while indicating a maximum at 189 mμ, showed an ϵ value about 18% lower than ours (λ_{max} 187 mμ, ϵ_{max} 9700). Thus, it appears that these results, [1,2,4] while either corrected for [1] or low in[4] far stray light, suffered from errors due to near stray light coupled with insufficient energy at the wavelengths below 195-197 mμ. This latter effect could arise from solvent absorbance in the 0.1 cm cells used,[1,2,4] and from deterioration of instrument components. The errors due to the use of non-transparent solvents, such as ethanol, were discussed[4] in assessing other[2] results. However, unknown variation in path length, uncontrolled solvent loss via evaporation, and neglected errors due to stray light below 220 mμ detract from the significance of the data reported by Chapman and Parker[4].

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[13] H.B. Klevens and J.R. Platt in Chicago University, Dept. of Physics, Laboratory of Molecular Structure and Spectra Technical Report, 1953/54, part 1, p.145, Chicago (1954), ASTIA-AD 53029.

[14] W.J. Potts, J.Chem.Phys. 23, 65 (1955).

[15] E.A. Johnson, Unicam Spectrovision No. 8, 1 (1960).

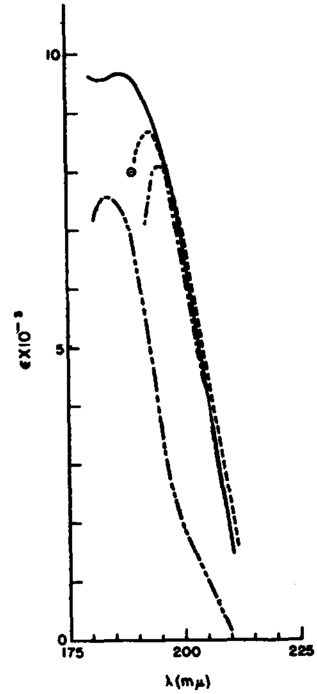

Fig. 1. Absorption spectra of cholesteryl acetate: from data of Stich, Rotzler and Reichstein[1], — — — — ; from data of Ellington and Meakin[2], —— — ——— — ; and from datum of Chapman and Parker[4]. Also shown are the results observed for cholesteryl acetate, ____________ ; and cyclohexene, —— — — —— — — .[*]

Apparently, satisfactory λ_{max} and ϵ_{max} values are not obtainable much below 193-195 mμ with conventional spectrophotometers.[16] We would strongly urge that such ultraviolet instruments be carefully assessed with reference compounds, e.g. cyclohexene, cholesteryl acetate, and Δ^4-cholestene (λ_{max} 193 mμ, ϵ_{max} 10,000). This precaution would permit a resonable and reliable

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[*] All compounds used were purified until they had physical properties comparable to those in standard reference works.

[16] Measurements[11b] on a conventional instrument to 181 mμ with nitrogen-flushing produced divergence of spectral curves at 195-197 mμ much like that noted here.

lower limit to be established. Also of merit is the use of very short paths, e.g. 0.01 cm. The mere observation of low stray light originating above 220 mμ or the appearance of maxima do not necessarily constitute assurance of reliable measurements.

Other observations and correlations of spectra with structure (including ketones) made during this work will be published in a more detailed account of this study.