ESTRONE: UV REFLECTION SPECTRUM IN SOLID 1-TETRADECANOL

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Dissolved in 1-tetradecanol, estrone generates a characteristic uv reflection spectrum even in amounts of 0,005 µg (1.89×10^{-11} mole) of estrone per mm^2 of reflecting area. A strong reflectance maximum at $\bar{\nu} = 3.471 \mu m^{-1}$ possesses the molar reflectance of $1,5 \times 10^9$ per mm^2, when smaller quantities than 1×10^{-10} mole of estrone/mm^2 are measured. The application of measurements of uv reflection spectra for the determination of estrone is described in detail. The sensitivity, specificity, accuracy and precision of the method is high.

INTRODUCTION: In the analysis of oral contraceptives ir reflection technique has been used (1) to determine 5 to 50 µg of estrogens with 10-15 % standard deviation. Free estrone in, i.a., plasma, has been estimated using gas-phase chromatography with electron capture detection (2); at least 0,001 µg of the hormone is needed in the original sample for adequate quantitation. Besides contraceptives, some food products, e.g. meat from animals given estrogens, may contain estrone. Studies of uv reflection spectra of estrone under well defined conditions now have been successful.

METHOD: 1-Tetradecanol, prepared by sodium reduction of tetradecanoic acid methyl ester (3), was purified by recrystallisation from ether-alcohol, and distilled 3 times in a semimicro fractionating apparatus at 167°, 15 mm Hg; m.p. 38.0°, mol wt. cryosc. in 1.4-dioxan 214.4. Estrone, prepared for Sigma, was controlled with 0.25 mm silica gel thin-layer chromatograms with ethyl acetate/cyclohexane developing solvent (4).

Estrone, isolated thin-layer chromatographically from sample (1,4) or prepared as above, was dissolved with stirring during 2 hrs in liquid 1-tetra-

decanol at 40°. Estrone dissolves slowly but completely in quantities of interest here, and remains stable for several hours, in liquid 1-tetradecanol at 40°. Thin layers, suitable for reflection spectrophotometry, were prepared by shedding the solution on frosted quartz plates at 40°, which then were rapidly cooled to 20°.

Depending on the construction of the spectro-reflectometer used, areas from 5 mm^2 upwards can be measured. A suitable layer thickness, which always should be kept constant, is about 0,25 mm, corresponding to 0.206 mg 1-tetradecanol/mm^2 at 20°. The measurements reported in this paper were carried out with a doublebeam recording spectroreflectometer and integrating sphere, using 60,0 mg 1-tetradecanol on 315 mm^2 reflecting area (layer thickness 0,231 mm). The appropriate quantity of 1-tetradecanol, and solution of estrone in 1-tetradecanol, respectively, was applied with an automatic pipette. A 5" strip chart linear and log potentiometric recorder was used. Reflection spectra were recorded from 4.46μm^{-1} (217 nm) to $\bar{\nu}$ 1.43μm^{-1} (700 nm). The reflection spectrum of 1-tetradecanol was measured against MgO as reference, fresh prepared by burning of pure Mg and deposited on quartz. The reflection spectrum of MgO is well known (5). Solid 1-tetradecanol on quartz was used as reference for the reflection spectra of estrone. The term reflectivity, used in this paper, is defined as the reflection coefficient at the wave number $\bar{\nu}$ in question, i.e. the ratio of the intensity of the light reflected from the surface to the intensity of the total incident light of the same wave number. Reflectance is defined as $-^{10}$ log reflectivity.

RESULTS: The uv reflection spectrum of estrone is unique (Fig.1). A characteristic, strong reflectance maximum at 288.1 nm, $\bar{\nu}$ =3.471 m^{-1}, which can be exactly measured by quantities of 0.005 - 0.01 µg of estrone/mm^2 (Fig.2 and 3),

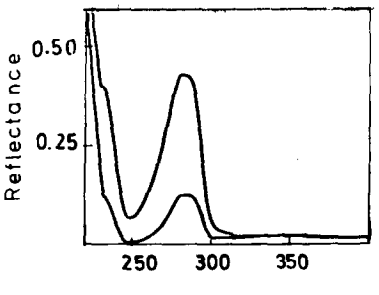

Fig. 1 UV Reflection Spectra of Estrone in solid 1-Tetradecanol at 20°. Upper curve: 0.140 µg (5,18 x 10^{-10} moles) per mm^2 of Estrone in solid 1-Tetradecanol. Lower curve: 0.0222 µg (8.22 x 10^{-11} moles) per mm^2 of reflecting area.

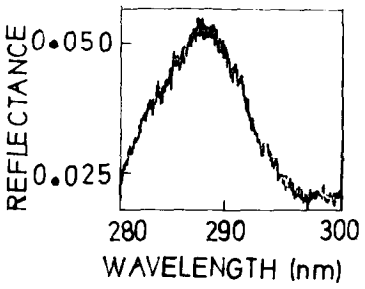

Fig. 2 UV Reflection Spectrum of 0.00831 µg (3.08×10^{-11} moles) of Estrone per mm^2 in solid 1-Tetradecanol at 20°.

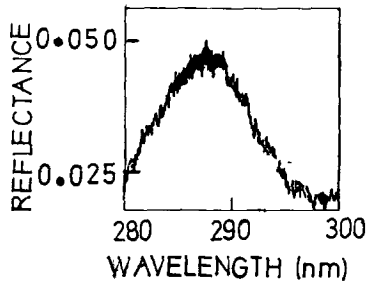

Fig. 3 UV Reflection Spectrum of 0.0051 µg (1.89×10^{-11} moles) of Estrone per mm^2 in solid 1-Tetradecanol at 20°.

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broadens at the very maximum by quantities of 0.02 - 0.1 µg, and more, of estrone/mm^2 (Fig.1). Another strong reflectance maximum at 232 nm, $\bar{\nu}$ 4,31µm^{-1}, is quite near a steep increase of reflectance of estrone at very short wavelengths (Fig.1). 1-Tetradecanol itself has a favourable reflection spectrum. From 650 to 300 nm it is represented by a practically straight line of about 75 % reflectivity in the layer thickness used, when measured against fresh prepared MgO as reference. There is a week reflectivity minimum at 258 nm. With the doublebeam technique described, compensation for differences in reflectivity of 1-tetradecanol at different wave numbers measured, is automatically obtained.

The molar reflectance of estrone per square unit at $\bar{\nu}$ =3.471µm^{-1}, calculated as reflectance/amount of estrone in mols per mm^2 of reflecting area, is about 1.5×10^9, when smaller quantities than 1×10^{-10} moles/mm^2 are measured as described. Bigger amounts of estrone give lower values. This means that the reflectance is not, within wide range, strictly proportional to the amount of estrone in solid 1-tetradecanol. Interpolation is, therefore, only permissible within narrow limits.

Ten different, complete determinations of the amount of estrone in each of 5 samples, containing from 1.5 to 7.0 µg of estrone in all, in the original sample, gave a maximum deviation of 10 % of value. The accuracy of the method is high, even when the content of estrone in sample is substantially lower.

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