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THz and Raman Spectroscopy in Steroid Chemistry

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Abstract

The terahertz time-domain and Raman spectra of steroids in the region of 0.1-3.5 THz have been measured. Steroids have several intense and specific absorption features in the THz frequency region. The features are uniquely determined by a hormone structure. This allows us to analyze steroids in complex samples such as pharmaceuticals, foodstuffs and biological fluids, and in process of the pharmacological and chemical synthesis.

Introduction

Steroids are the organic compounds that contain a characteristic arrangement of four cycloalkane rings that are joined to each other. Examples of steroids include the dietary fat cholesterol, the sex hormones estradiol and testosterone, the anti-inflammatory medicines dexamethasone and acetate cortisone. The core of steroids is composed of twenty carbon atoms bonded together that take the form of four fused rings: three cyclohexane rings (designated as rings A, B, and C in the Fig.1) and one cyclopentane ring (the D ring).

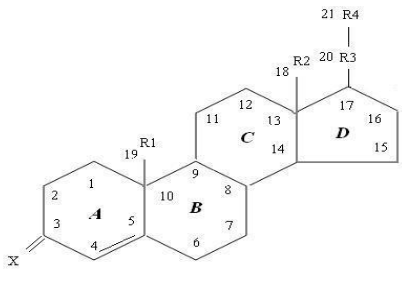

Figure 1: The basic skeleton of a steroid. R1, R2, R3 and R4 can be methyl group, and X - hydroxyl or keto group

The steroids differ only by the position of functional groups attached to this four-ring core and by the oxidation state of the rings. The biological action of steroids is closely related to some features of their chemical structure. The steroids are aggregated into the molecular crystals of two space groups P_{212121} and P_{21}, in general. The molecules could be bound by van der Waals and/or stronger hydrogen forces in molecular crystals. Thus, steroids are convenient object for study of a nature of low-frequency vibrations and effects of hydrogen bonds.

The steroid hormones influence on many physiological processes in humans and animals is well known. Their crucial role in regulating life processes led to a widespread research in steroid chemistry and biochemistry, creating new and fast methods of steroid analysis in biological samples, as well as in food, sewage and pharmaceutical production. Steroids have rather complex molecular structure, show significant reactivity with similar physico-chemical properties and are often located in low quantities in multicomponent matrices. The determination of steroids and their metabolites in biological samples and pharmaceutical drugs can be done by chromatography, mass spectrometry, ELISA and other methods of analysis with high accuracy. However, these methods are time consuming and expensive. In this regard, the development of new operational methods of analysis is highly relevant. The methods of THz-TDS spectroscopy can be helpful for the study of steroids. This type of spectroscopy has a number of advantages such as the possibility to analyze a wide frequency band in a single measurement, to obtain time resolution and phase information, and to measure the complex dielectric permittivity which completely characterizes the optical response of the matter. At the same time, THz-TDS is able to penetrate many opaque materials, but unlike X-rays, it is non-ionizing radiation and can detect the samples without influence or destroys. Moreover, THz spectra of molecular crystals show rich information about collective vibrations and are highly sensitive to the changes of the molecular conformation, structure, environment elements and intermolecular interaction than IR spectra. The aim of our research is to study a wide range of steroids by THz-TDS and Raman spectroscopy.

Materials and methods

Steroids were purchased from Koch-Light Laboratories Ltd, UK, and were used without further purification. For THz-TDS measurements the samples were prepared by pressing the pure polycrystalline powder to disks of thickness 0.4 mm, diameter 5 mm and average density 1.2 mg/mm^3 by applying a pressure about 50 MPa. For the case of thin films and solutions we developed total internal reflection (TIR) scheme using silicon right angle Dowe prism [1, 2]. The THz time-domain spectroscopy apparatus has been reported previously [3]. In brief, The Ti:Sapphire laser (Tsunami, Spectra Physics) pumped by a 532 nm cw Nd:YVO4 (Millennia, Spectra Physics) provides the pulses of 80 fs duration with the repetition rate of 80 MHz. The output laser beam is divided into two parts to illuminate a 1 mm thick ZnTe (110) crystal to generate THz radiation while the other part is used in the fully vacuum electro optical detector based on the 2 mm thick ZnTe crystal.

In all measurements the variable delay stage, which provides time delay between the THz pulse and the optical probe pulse, is scanned over a distance of 10 mm providing spectral resolution of 1.5 cm^{-1}. Using a lock-in detection scheme the signal-to-noise ratio achieved in our experiments was more than 10^2.

The Raman spectra were recorded using a triple grating spectrometer T64000 (Horiba Jobin Yvon) in micro-Raman geometry in the range of 14-3200 cm^{-1}, that uses the Ar^+ laser as a light source with the wavelength of 514.5 nm [4]. The spectra were measured at 297 K and 83 K. The polarization of scattered light was detected perpendicular to the polarization of incident light. To minimize laser heating of the sample, an unfocused spot of size 4-6 μm with the power 3-4 mW was used. Various temperatures were measured using a Linkam thermo-cell. Inside the cell nitrogen atmosphere was created to reduce the influence of water vapors.

Results

We have studied different classes of steroids: corticosteroids, androgen and estrogen, and related medicine. Spectral properties of steroids are well described in UV and IR spectral range, but those spectra are similar. Only in THz region substances have several intensive unique features.

The Raman spectra of progesterone were measured at 300 K and 83 K (Fig. 2). As we can see in the range below 40 cm^{-1} (1.2 THz) several strong Raman bands are observed, whereas in THz spectra in this range the bands are weakly defined (Fig. 3). This difference can be explained by the completely different nature of bands on THz and Raman spectra: according to quantum mechanics a vibration is IR-active if the dipole moment is changed during the vibration and is Raman-active if the polarizability is changed during the vibration, so, in general, the vibration can be IR-active, Raman-active, or active in both. The relative intensities in each spectrum are not the same. Upon cooling the vibrational bands in Raman spectra shift to higher frequencies for 2-6 cm^{-1} and the bands sharpen up.

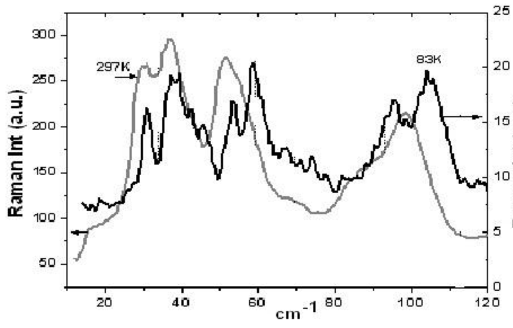

Figure 2: Raman spectra of progesterone at 297 K and 83 K.

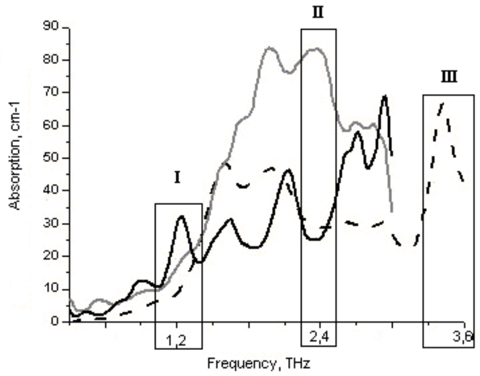

Figure 3: The THz absorption spectra of progesterone (solid black line), 17α-hydroxyprogesterone (dotted black line) and cortisone (gray line). I, II, III - bands of frequencies that can be used to identify these substances in the mixture.

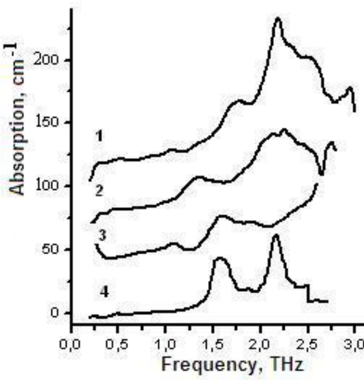

Figure 4: The THz absorption spectra of acetate: corticosterone (1), deoxycorticosterone (2), cortisone (3) and hydrocortisone (4). The spectra are shifted along the vertical axis.

The progesterone, 17α-hydroxyprogesterone and cortisone room temperature THz absorption spectra are shown on Figure 3. The substances under study have several intense spectral features in the frequency range 0.1-3.6 THz. The features are uniquely determined by a hormone structure. This allows one to analyze a complex mixture on specific frequencies and to obtain information about individual components.

The steroid acetates room temperature THz absorption spectra are shown on Figure 4. These substances are often used as anti-inflammatory drugs. The steroid acetates have spectral features, which can be used to analyze these substances in pills, ointments and during chemical synthesis.

We have studied the THz absorption spectra of all classes of steroids. The results are presented in Table 1.

Table 1: The positions of the resonances in the THz absorption spectra of steroids

Steroids	THz
Progestines progesterone	0,89; 1,24; 1,68
17a-hydroxyprogesterone	1,57; 2,01; 3,45
Glucocorticoids: cortisol	1,29; 2,71
cortisone	1,94; 2,43
11-deoxycortisol	1,51; 2,28; 3,39
Mineralocorticoids: corticosterone	1,59; 1,95; 2,56
deoxycorticosterone	1,45; 1,97
aldosterone	1,27; 1,78
Androgens: dehydroepiandrosterone	1,39; 1,95
androstenedione	1,15; 1,88; 2,42
testosterone	1,42; 1,88; 2,4
5α-dihydrotestosterone	1,09; 1,45; 2,39
Estrogens: estradiol	2,29; 2,68
estron	1,27; 1,45; 1,74; 2,51
estriol	1,31; 1,74; 2,23; 2,83
Drugs: cortisol acetate	1,57;1,82
cortisone acetate	1,55; 2,17
corticosterone acetate	1,73; 2,17; 2,49; 2,94
deoxycorticosterone acetate	1,32; 2,25

For the study of thin films of steroids we used total internal reflection scheme. Figure 5 shows the THz absorption spectrum of polycrystalline powder of cortisone acetate and THz reflection spectrum of film this substance on quartz. We can see that the basic shape of the spectra is coinciding. Spectrum TIR of the film is characterized by narrower lines probably due to partial orientation of the molecules near the surface of quartz and therefore better resolution. Amount of the substance to be analyzed in this way is less than 30 times compared with an analysis of the dry product. Thus, the use of TIR spectroscopy allows analyzing thin films of steroids in the production of drugs and in the study of biological material.

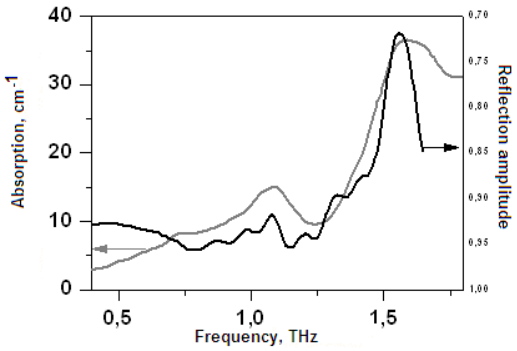

Figure 5: The THz absorption and reflection spectra of cortisone acetate pellet (gray line) and film (solid black line).

Thus, different classes of steroids have several intense features in the region of 0.1-3.5 THz. The features are uniquely determined by a hormone structure. This allows one to analyze a complex mixture on specific frequencies and to obtain information about individual components. THz-TDS can be used to analyze steroids in complex samples such as pharmaceuticals, foodstuffs and biological fluids, and in process of the pharmacological and chemical synthesis. A number of diseases accompanied by overproduction of steroid hormones in the adrenal glands and other organs and the results of this work will be applicable in the THz imaging of biological tissues and find direct use in medical diagnosis.

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