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Structural changes of β-carotene and some retinoid pharmaceuticals induced by environmental factors

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HIGHLIGHTS

- We examine structural changes of some retinoids induced by environmental factors.
- Spectroscopic analysis was based on theoretical calculations and vibrational spectra.
- Shifts of the etretinate bands are observed by changing solvents and pH values.
- Raman spectrum of β-carotene shows distinct changes upon the thermal stress.
- The study is important for pharmaceuticals quality control from production to market.

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ABSTRACT

Four pharmaceuticals (β-carotene (**1**), retinoic acid (**2**), isotretinoin (**3**), and etretinate (**4**)) were analyzed using Raman spectroscopy and quantum-chemical calculations followed by potential energy distribution (PED) analysis to gain deeper insight into the experimental vibrational spectra. Small shifts of characteristic bands of (**4**) upon change of solvent and pH were interpreted as a result of molecular aggregation. Temperature-dependent studies on the Raman spectrum of (**1**) were performed in the temperature region of −150 °C to +150 °C. The observed small shifts in the experimental spectra upon heating were explained by increase of the high-energy conformers (of the *trans* type) in the population of (**1**) related to weakening of the intermolecular interactions that enables rotation of the terminal rings with respect to the polyene chain. Deconvolution of the ν_1 band showed changes in intensity and position of the deconvoluted bands with the increase of temperature.

1. Introduction

The natural carotenoids are synthesized in plants in which their primary role is in light-harvesting in chloroplasts, and protecting against the harmful photooxidative effects [1]. They are present in chromoplasts giving plants, algae, fungi and bacteria a characteristic yellow-red-orange color [2]. In flowers and fruits chromoplasts they are mainly secondary metabolites serving as attractants to insects and animals [3]. Antioxidant properties of carotenoids, enhanced by the role of (**1**) as a provitamin A cause that they are considered as beneficial for human health, cancer prevention, cardiovascular diseases, macular degeneration, and stimulation of the immune system [4,5]. Thus, some of them, like (**1**), (**2**), (**3**), and (**4**), are used as drugs in medical treatment. However, despite evidence of their beneficial properties (such as tumor-suppressive capacity) they also exhibit detrimental (e.g. teratogenic) effects [5].

As (**1**) is both a natural antioxidant, vitamin A precursor, and a colorant [6], it is used as an additive in a great number of products, food and pharmaceuticals. Although there is no obvious evidence for its protecting abilities against UV-induced skin cancer [7], (**1**) is an efficient endogenous photo protector preventing UV-induced damage in skin [8]. (**1**) has been used in diet complementation to reduce oxidative cell damage in antibiotic administered patients [9], and to reduce the risk of heart diseases and atherosclerosis [10].

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(**2**) (called also tretinoin) is vitamin A metabolite crucial for vertebrate embryonic development. This very role of (**2**) is the key cause of the high teratogenicity of retinoid pharmaceuticals [11–13]. (**2**) is used to treat acute promyelocytic leukemia [11], but also sun-induced premature skin aging [14]. Therefore, some (**2**) derived pharmaceuticals have been developed to both treat different types of cancer and prevent skin aging and negative effects of UV radiation [12] showing efficiency and good safety in the management of photodamaged skin and as anti-aging agents [13]. (**2**) was also the first retinoid used for the treatment of acne [15], and it is efficient in the treatment of skin wrinkles using cosmetics [7].

Isomerization of (**2**) in the body leads primarily to (**3**) (13-*cis* retinoic acid). Treatment with high doses of (**3**) is effective in preventing cancer recrudescence in patients treated for carcinoma of the head and neck [16]. (**3**) is almost as potent as *all-trans*-(**2**) in many of its actions on epithelial tissues [17], photoaged skin [18], acne [15], and psoriasis [17]. The strong sebostatic activity of (**3**) is surprising, as it exhibits low binding affinities for cellular (**2**)-binding proteins and retinoid receptors [19], responsible for its less irritating effect than that of (**2**) [7].

(**4**) is an aromatic compound that belongs to the second generation of retinoids [12,20] and is FDA approved for the treatment of psoriasis [21]. It is commonly used in dermatology [22], for clinical treatment of actinic keratosis [23] and primary milia [24], in *in vitro* studies of a human skin squamous cell carcinoma cell line [22] and organ transplantation to treat graft-vs.-host disease [25], and has been reported to be extremely effective in oral treatment of rarely occurring types of keratoacanthoma [26].

Carotenoids (in particular (**1**), *all-trans*-(**2**), (**3**), and (**4**)) have various geometrical isomers (*cis–trans*) and conformers, the end groups may usually rotate about a single bond. That is the reason why they are sensitive to solvent and temperature changes [27].

Moreover, a conjugated double bond system makes them responsive to oxidation and heat, as the potential difference between the first oxidation and first reduction potential is a linear function of the reciprocal conjugated double bond chain length and the radical cation formation is in principle determined by the chain length [28]. This is exceptionally important when a carotenoid is applied as a medicine because its change into one of its geometrical isomers (e.g., *all-trans*-(**2**) into (**3**) or oppositely) may change its pharmacological activity and be detrimental to patient. Furthermore, degradation or transformation of retinoid medicines caused by light (leading to possible isomers or photodecomposition product) [29], temperature or improper storage conditions can result in both decreasing pharmaceutical dosage reaching the drug target and exposure to side effects caused by decomposition side-products. Thus quality control of carotenoid drugs, at any stage from production to market and patient, is of particular importance [30–32].

The pharmaceutical analysis is dominated by variety of chromatographic techniques and methods, which, although being very sensitive and powerful [33], require some preliminary preparations such as dissolution, pulverization, heating and extraction, and cannot provide insight into a chemical structure comparable with that offered by vibrational spectroscopy methods. In addition, chemical liability of some carotenoids increases requirements to an analytical technique to not disturb the chemical state of the analyzed compound. This is why some spectroscopic techniques (such as Raman spectroscopy) are considered as a possible complimentary approach that can be performed without preliminary preparation [34,35]. There are numerous examples of successful pharmaceutical application of Raman methods and techniques [36–38]. This paper shows a potential in application of Raman spectroscopy and imaging in retinoid drugs.

2. Experimental and theoretical methods

2.1. Raman spectroscopy

Raman spectra were recorded using a Bruker MultiRAM FT-Raman spectrometer equipped with a Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. Solid samples were mounted directly under the laser beam in the metal rings without any preparation. Liquid samples and solutions were settled in test tubes. Spectra of pharmaceuticals were collected with laser power of 50 mW and 128 scans, whereas spectra of solutions were measured with laser power of 200 mW and 256 scans. All spectra were obtained with a spectral resolution of 4 cm^{-1} in the wavenumber range from 100 to 4000 cm^{-1}.

A Linkam THMS600 stage was used to heat the sample of (**1**) standard in the temperature range maximally from −150 to 150 °C. Spectra were measured every 10 °C. The heating ratio was set on 10° per min and 32 scans were accumulated and averaged. (**1**) did not decompose during heating in the investigated range of temperatures.

2.2. Samples

Liquids containing (**1**) and (**3**) were taken from the inner part of widely accessible medicines (namely *Betakaroten* and *Curacne*, 5 mg). Synthetic (**2**) (Sigma, ⩾98.0% (HPLC), mp. 180–181 °C) and synthetic (**1**) (Fluka, ⩾97.0% (UV), mp. 176–184 °C) were used without any further purification, while yellow powder of (**4**) was taken from the inner part of *Tigason* (25 mg) capsules (provided by the University Hospital of Krakow). (**1**) taken from the medicine was used only for spectroscopic studies, whereas synthetic (**1**) was applied for a temperature experiment. Solutions of (**4**), made from powder taken from the medicine, (ca. 0.1 M) were prepared in water, propan-2-ol, *n*-hexane, methanol, toluene, acetone, chloroform, and ethyl acetate, and were kept for 1 day in room temperature to avoid a residual insolubility. Solvents were pure enough for spectroscopic usage. The pH was varied by adding the appropriate amounts of HCl or NaOH and was checked using litmus papers. All samples were stored in dark to avoid photo-degradation and decomposition. In the case of three used pharmaceuticals, i.e. *Betakaroten, Curacne, and Tigason*, other components such as soybean oil, yellow wax, hydrogenated vegetable oil, lecithin, and sodium ascorbate (characteristic bands in the region of 1400–1500 and 1600–1700 cm^{-1}) [39,40], did not contribute to the Raman spectra in the same region as the fundamental bands of the retinoids and (**1**).

2.3. Calculations

All calculations were performed using the DFT/B3LYP method [41] and the 6-31G(d,p) basis set using the Gaussian 09 program [42]. To find the most stable conformers the energy profiles resulted from rotation of the terminal ring by a constant angle were obtained. Then, all structures corresponding to energy minima were optimized with no constraints to get real conformers. It was confirmed that all studied stable structures exhibit all positive frequencies. The calculated harmonic frequencies were multiplied by factor of 0.960 to compensate for anharmonicity shifts [43] and the limitations of a basis set. Potential energy distributions (PED) of the normal modes were computed in terms of natural internal coordinates [44] with the Gar2ped program [45].