Commercial Vitamin A Capsules: Is There Any Retinol?

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Abstract: The experiment introduces hands-on 1D and 2D NMR analysis of vitamin A capsules, without the use of any chromatographic purification or extraction step. Among the different species deduced from the NMR spectra, the students conclude with the lackness of any retinol in the tablets analyzed. This laboratory exercise is used to introduce the students to organic spectroscopic techniques and to show them that NMR is not simply a means to elucidate structures of pure compounds but that it can be used to analyze more complex mixtures.

Introduction

Natural products have been, and remain, a rich pool of leads for the pharmaceutical industry and many marketed drugs are directly isolated or are modifications of such substances [1]. Considerable effort is spent in unscrambling and characterizing chemicals from natural sources which can be tested in a variety of biological screens. Vitamin A (retinol) plays an important role in human body as a growth and protection factor that are applied in medicine because of their antioxidative properties [2] and in tumor therapy because of their cytostatic activity [3]. To date, analysis of Vitamin A analogues has been most frequently carried out using methods based on HPLC hyphenated with ultraviolet or fluorescence techniques [4], always involving pre-column saponification steps towards converting all vitamin A forms and possible esters to free retinol. NMR is an emerging valuable alternative for product identification [5], and is one of the most important methods of structure elucidation with an extensive range of biochemical and chemical applications. Retinol **1** belongs to a family of chemical compounds known as retinoids, which includes naturally occurring forms of vitamin A together with the many synthetic analogs.

The basic structure of the retinoid molecule consists of a cyclohexene group, a polyene side chain with alternating double bonds and a polar end group. Variations on side chains and end groups attached to the oxygen create the various classes of retinoids.

In the graduate organic chemistry curriculum still remains a need to learn and to apply more developed NMR experiments such as bidimensional TOCSY, HMQC or HMBC, even though are experiments routinely used in research. As part of our effort to increase exposure to NMR of this kind of students, or even to advanced undergraduate students, i.e. biochemistry lab, we introduce here an experiment based on the direct determination of whether commercially available vitamin A capsules (Auxina A Masiva) do or do not contain retinol **1**.

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With respect to content, the students may pass through the careful analysis of spectral data, with emphasis on the evaluation of NMR signal intensities, integrals, couplings constants and chemical shifts, together with the mandatory phasing and baseline correction. In the classroom the acquired and analyzed NMR data of all students is planned to be eventually pooled together in a particular section, where the students, first as part of a team and subsequently for themselves (for the laboratory report), have to decide, for instance, how many compounds are present, or if the existing retinoid exists as alcohol or as ester. In this section the instructor can provide different structural alternatives to be chosen by the students (see below).

Finally, from a technical and cost savings perspective, NMR spectra are obtained in a non-costly deuterated solvent such as chloroform, and without the need of any tedious step for sample extraction and/or saponification. These particular aspects might be of much broader interest to the graduate education community.

Experimental Section

Schedule. The course is divided in three days (from now on sections). In the first section all of the students attend a two hour pre-lab lecture that covers the basics of preparing the sample, are instructed about the potential components that may be found and how they should proceed to properly structurally assigned them, and taught preliminarily about the main principles and theory behind the NMR experiments that will be run later on. Last but not least, strong instructions will be provided to the students in order to make them run the desired experiments in the NMR instrument.

The second section has a first part of preparation of the sample inside the laboratory (10-20 minutes), and then a second part where the groups of students [7] have at least 2 hours available, previously programmed on the instrument to collect their data, under the supervision of the NMR technician.

The theory behind and interpretation of the spectroscopic techniques applied is reserved to the third section of the course that includes the on-group discussion, always assuming the students have systematically worked through the interpretation.

Data Collection. To each team of students is given a vitamin A capsule, with access to deuterated chloroform and to a 5 mm NMR tube, to prepare the sample.

The operation of each instrument is overseen by either the laboratory assistant or the NMR technician [8]. As each team of