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Reaction pathways and kinetics of cholesterol in high-temperature water

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HIGHLIGHTS

- Cholesterol was reacted in high-temperature water at 300, 325, and 350 °C.
- Cholesta-3,5-diene was the sole primary reaction product.
- Rate constants and Arrhenius parameters for cholesterol dehydration were calculated.
- Products consisted nearly entirely of cholestadienes.
- A reaction network for major product pathways was proposed.

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ABSTRACT

We investigated the reaction of cholesterol, a model compound for sterols in microalgae, in high-temperature water at 300, 325, and 350 °C. Products nearly entirely consisted of cholestadienes, with cholesta-2,4-diene, cholesta-3,5-diene, and cholesta-4,6-diene being the most abundant isomers. Cholesta-3,5-diene was the only primary product, formed via dehydration of cholesterol. Cholesta-2,4-diene and cholesta-4,6-diene likely formed from cholesta-3,5-diene by double-bond migration. We report conversion and product molar yields for each reaction condition. The initial rate of disappearance of cholesterol was first-order with an activation energy of $E_a = 127 \pm 12$ kJ mol^{-1} and $A = 10^{8.35\pm2.41}$ s^{-1}. We used a del-plot analysis and mechanistic considerations to develop a reaction network for conditions relevant to hydrothermal liquefaction of microalgae.

1. Introduction

The production of liquid fuels via hydrothermal liquefaction (HTL) of microalgae is of current interest [1–5]. HTL uses the unique properties of high-temperature water (HTW) to convert microalgae into an energy-dense biocrude oil. HTW ($T > 200$ °C, $P > 1.6$ MPa) exhibits properties contrasting with those of liquid water at ambient temperature, including a much higher solubility for organic compounds, lower dielectric constant, higher ion product, and weaker hydrogen bonds [6]. In particular, HTW facilitates acid- and base-catalyzed reactions, such as the hydrolytic decomposition of biomacromolecules in microalgae. Utilizing water as a reaction medium also significantly mitigates the energy requirements of HTL as compared to other processes, including pyrolysis and lipid extraction, that require dry feedstocks [2].

Understanding the reaction kinetics and mechanisms of key microalgal biochemical classes in HTW is imperative to understanding the holistic behavior of microalgae under these conditions. Several previous researchers have used model compounds to understand how various components of microalgae react during HTL. Examples include ethyl oleate [7], phytol [8], cellobiose [9], 1,2-dioleoyl-sn-glycero-3-phosphocoline [10], and various amino acids [11] as model compounds for ester linkages in triglycerides, a component of chlorophyll *a*, cellulose, phospholipids, and polypeptides, respectively. Brown et al. reported that cholesterol and five derivative compounds (cholest-4-ene, cholest-5-ene, cholesta-3,5-diene, cholest-4-en-3-one, and cholest-4,6-diene-3-one) were among the twenty most prevalent products detected by gas chromatography analysis of biocrude oil obtained from the HTL of the alga *Nannochloropsis* sp. at 350 °C for 60 min [1]. Sterols are a subclass of lipids that, to date, have not been extensively

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studied in the HTW environment. The purpose of this study is to understand the behavior of cholesterol, shown in Fig. 1, in HTW as a model for how microalgal sterols behave during HTL.

Sterol content in microalgae varies depending on growth conditions and species type, reaching up to 1.3 dw% [12] (dry weight) of the total algal mass, and on average about 0.5 dw% [12–16]. As a percentage of total lipid content, sterols constitute on average about 4.6 dw% [12,14,17]. Cholesterol typically represents about 75 dw% of sterols in microalgae [12,13,16–22], and is therefore an excellent model compound for studying how sterols react in HTW.

Several previous studies investigated the reaction of cholesterol at elevated temperatures under various conditions. Rushdi et al. examined the hydrous pyrolysis of cholesterol in liquid water both with and without added catalyst at temperatures ranging from 150 to 300 °C for 24 h batch holding times [23]. They identified cholestanes, cholestenes, cholestadienes, diacholestenes, diacholestadienes, sterols, cholestatrienes, cholestenones, and cholestadienones in their products. Additionally, they proposed a reaction network based on their results, albeit without any substantiating kinetic data. Falk et al. examined the pyrolysis of cholesterol at 360 °C in the presence of air, nitrogen, and *en vacuo* at batch times between 30 and 60 min [24]. The authors' focus was to determine reaction products and their carcinogenicity. A reaction network for the decomposition of cholesterol was proposed. Most interestingly, their work suggested that the first step in the reaction network is the dimerization and dehydration of cholesterol to dicholesteryl ether, a claim not present in any other studies. Britt et al. examined the pyrolysis of cholesterol at 700 and 800 °C in a flow reactor at residence times of less than 2 s [25]. Their results show a wide array of polycyclic aromatic hydrocarbons. Meredith et al. studied the hydropyrolysis of cholesterol between 150 and 450 °C in a flow reactor at residence times of about 20 min in the presence of hydrogen and a sulphided molybdenum catalyst [26]. They found that cholesterol hydrogenated to primarily cholestanes, with some alteration to cholestenes and diasterenes. An additional study improved the selectivity of this reaction to favor cholestanes by using a modified procedure and platinum catalyst [27]. Liu et al. and Schüpfer and Gülaçar studied the acid-catalyzed rearrangement of cholesta-6,8(14)-dienes and cholesta-3,5-diene, respectively [28,29]. Their results highlighted various spirosteradienes as the terminal reaction products, and they calculated relative stabilities of intermediate cholestadiene isomers, some of which were hypothesized to exist but not directly observed. Mackenzie et al. summarized geochemical transformations of sterols ($50 < T < 250$ °C) on the timescale of millions of years [30].

The existing literature sheds light on the thermal stability of cholesterol under elevated temperatures under a variety of conditions. Rushdi et al. is the only previous study to examine cholesterol in HTW, but these authors considered just a single, long reaction time of 24 h. No previous work has obtained quantitative data for the hydrothermal treatment of cholesterol on timescales relevant to HTL. Additionally, no prior work has reported kinetics for cholesterol disappearance in HTW. Herein we report on the reaction of cholesterol in HTW from 300 to 350 °C with detailed quantitative product compositions and reaction kinetics for the rate of disappearance of cholesterol. The results outlined herein provide new understanding of how sterols behave during the HTL of microalgae.

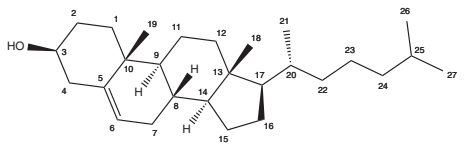

Fig. 1. Chemical structure of cholesterol.

2. Materials and experimental methods

2.1. Apparatus and procedure

All reactions occurred in 1.67 mL (internal volume) batch reactors constructed from 3/8 in 316 stainless steel Swagelok tube fittings (one port connector and two caps). Prior to use, we conditioned all reactors by filling them with deionized water and placing them in a fluidized sandbath (Techne IFB-51 with a Eurotherm 3216 PID controller) preheated to 350 ± 1 °C, for 1 h. This conditioning exposes the interior of the reactors to the HTW environment, purging any residual materials that may reside inside. We rinsed all reactors thoroughly with a 1:1 (v/v) solution of dichloromethane (Fisher Scientific, ⩾99.9%) and methanol (Fisher Scientific, ⩾99.9%) before use.

We loaded reactors with 10 mg of cholesterol (Sigma–Aldrich, ⩾99%) and enough deionized water to occupy 95% of the internal volume at reaction conditions, based on the density of pure, saturated liquid water at these conditions. For the control experiment at 23 °C, we loaded enough water to fill the uncapped portion of the reactor completely. Specifically, 1.20, 1.13, 1.04, and 0.91 mL of water were loaded for reactions at 23, 300, 325, and 350 °C, respectively. The headspace in each reactor contained ambient air. We sealed loaded reactors with 45 ft-lb of torque and placed them in the sandbath preheated to the desired reaction temperature (23, 300, 325, or 350 °C) held for various batch times. Reactions were conducted in at least triplicate at each temperature and time point. Pressures at reaction conditions, as reported in the saturated steam tables, were 8.6, 12.1, and 16.5 MPa at 300, 325, and 350 °C, respectively. The reactor heat-up time is about 3 min [3], which is short relative to the batch holding times employed in this study. Once each batch holding time was reached, reactors were removed from the sandbath and quenched in a water bath at room temperature for 5 min, then allowed to equilibrate with ambient air for 15 min. Upon cooling, we opened the reactors and collected their contents using 9 mL of a 1:1 (v/v) solution of dichloromethane and methanol. This solution dissolved all reactants and products formed, with the exception of small amounts of char, producing a clear homogeneous liquid for further analysis.

2.2. Analysis

We used an Agilent Technologies model 6890 GC-FID with an HP-5 fused silica, non-polar capillary column (50 m length × 0.20 mm inner diameter × 0.33 μm film thickness) to quantify products. We used an Agilent Technologies model 6890N GC-MSD with an HP-5MS fused silica, non-polar capillary column (50 m length × 0.20 mm inner diameter × 0.33 μm film thickness) to identify products. We employed a Wiley mass spectral library for compound identification by cross-referencing mass fragment patterns of products with those in the library. GC analyses used an inlet temperature of 260 °C, a split ratio of 10:1, and an injection volume of 1 μL. The temperature program consisted of an initial oven temperature of 245 °C (isothermal for 5 min) followed by a 1 °C/min ramp to 300 °C, for a total runtime of 60 min. Helium served as the carrier gas at a flow rate of 1 mL/min.

We created a linear calibration curve for cholesterol by preparing standard solutions of known concentrations spanning two orders of magnitude over the range of interest and measuring their peak responses using GC-FID. Compounds were identified either from matches with the Wiley mass spectral library, comparison with mass spectra reported in the literature, or by interpreting mass fragmentation patterns. Compounds with low-quality library matches were categorized based on the parent ion (M^+)