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Electrosynthesis of cholesta-4,6-dien-3-one from cholesterol on a laboratory synthetic scale ☆

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ABSTRACT

Cholesterol has been shown to be electrochemically oxidized at a carbon electrode to form cholesta-4,6-dien-3-one in acetonitrile [Hosokawa, Y. Y.; Hakamata, H.; Murakami, T.; Aoyagi, S.; Kuroda, M.; Mimaki, Y.; Itoh, A.; Morosawa, S.; Kusu, F. *Electrochim. Acta*, **2009**, *54*, 6412–6416]. To further expand on this reaction in the field of organic synthesis, the electrolysis conditions of cholesterol were optimized on a laboratory synthetic scale. Using a flow-through column electrolysis system, cholesta-4,6-dien-3-one was efficiently produced at the applied potential of 1.9 V versus Ag/AgCl with a flow rate of 2.5 mL/min, providing a green tool for the synthesis of cholesta-4,6-dien-3-one.

1. Introduction

Cholesta-4,6-dien-3-one is a biologically important intermediate in the biosynthetic pathway of cholestanol in cerebrotendinous xanthomatosis (CTX), an inherited lipid-storage disease caused by mutation in the CYP27A1 (sterol 27-hydroxylase) gene.[1] It is therefore expected that this compound may be a chemical probe to elucidate the molecular pathophysiology of CTX. Moreover, cholesta-4,6-dien-3-one has been utilized as an intermediate in the further synthesis of steroidal compounds including azasteroids and vitamin D_3 precursors.[2] Thus, a simple and efficient method for preparing cholesta-4,6-dien-3-one would contribute to the research fields of steroid biology and chemistry.

A number of methods for the synthesis of cholesta-4,6-dien-3-one have been reported. Cholesta-4,6-dien-3-one was first synthesized by Dane et al.[3] After that, Wilds and Djerassi developed a more efficient method.[4] In Wilds and Djerassi's method, cholesterol is reacted with quinone and aluminum *tert*-butoxide in toluene for 45 min under reflux. This synthetic procedure is simple and gives cholesta-4,6-dien-3-one in 41% yields, but the purification procedure contains multiple extraction steps which make this method complex.

Cholesta-4,6-dien-3-one has also been synthesized from several steroids other than cholesterol that include 6β-bromocholestenone,[5] 3-acetoxy-3,5-cholestadiene,[6] cholest-4-ene-3-one,[7] and cholesta-4,6-dien-3β-ol.[2b,8] These methods have provided good yields, but the preparation of these precursor compounds is sometimes time consuming. The biological preparation of cholesta-4,6-dien-3-one from cholesterol using Okinawamozuku has also been reported,[9] although the reaction takes one week. Recently, it has been shown that when cholesterol is treated with Magtrieve™ (CrO_2) in acetonitrile for 48 h under reflux, cholesta-4,6-dien-3-one is synthesized in 74% yields.[10] This would be the most efficient and practical method for the preparation of cholesta-4,6-dien-3-one currently available.

Cholesterol used to be regarded as an electrochemically inactive compound[11]; however, recent studies have shown that cholesterol could be electrochemically oxidized under several conditions.[12] A further study has shown that cholesta-4,6-dien-3-one is an electrochemical oxidation product of cholesterol, when cholesterol is oxidized at a carbon electrode in acetonitrile containing 50 mmol/L $LiClO_4$.[13] In the field of organic synthesis, electrochemical reactions often become a powerful tool, because the initial reaction takes place at the electrode surface and the reaction is controllable by electrolysis conditions including an applied potential.[14] For example, Dekeczer et al. have successfully synthesized labeled lanosterol derivatives by electrochemical reduction.[15] In general, electrosynthesis is performed using a beaker-type electrochemical cell. However, the reaction volume is limited to the volume of the beaker-type cell, so that repeated electrolyses are required, if a large amount of the product is necessary. In addition, it is not easy to operate bulk electrolysis using a beaker-type cell. To avoid these issues, electrochemical flow synthesis has been introduced as an

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effective and attractive synthetic system.[16] With this in mind, we employed a flow-through column electrolysis system to perform the electrolysis of cholesterol on a laboratory synthetic scale. Ideally, a continuous-flow system with solvent recycling would be the best synthetic system from a green chemistry point of view. Thus, in the early stages of this study, we tried to develop a continuous-flow system with solvent recycling. However, the optimization of the electrolytic conditions of the continuous-flow system with solvent recycling is difficult, because many conditions (time, volume, concentration, potential, flow rate, etc.) largely affect each other. Thus, in this study, we optimized the electrolytic conditions of the continuous-flow system without solvent recycling in a practical manner.

2. Results and discussion

As described above, for the electrolysis of cholesterol, a flow-through column electrolysis system was employed. Because there were two problems in the original electrolysis system,[13] technical improvements were needed. First, in our experience, repeated electrolyses of cholesterol gradually made the Pt wire counter electrode deteriorate. Thus, the Pt wire had to be changed to another material that could withstand repeated electrolyses and possess a larger surface area. A piece of carbon cloth might be a candidate, because it can satisfy these demands. Among carbon cloths commercially available, our preliminary experiments showed that GF-20-P7 gave the best conductivity and current density. Thus, GF-20-P7 carbon cloth was selected to use as a counter electrode, where it was wound around the Vycor® glass cylinder packing the carbon fiber working electrode (Fig. 1). This change enabled us to conduct continuous electrolyses without frequent maintenance of the electrochemical cells and to make the electrolytic cell more economical. Second, as we previously reported, cholesterol was injected 100 times into our flow-through column electrolysis system.[13] This procedure was simple, but its operation was troublesome. To make the procedure easier, cholesterol was dissolved in the carrier solution, allowing for injection-less electrolysis. In summary, these two technical improvements of the electrolysis system were quite simple but were very helpful in operating the electrolysis.

Next, if it were possible to simultaneously determine cholesta-4,6-dien-3-one and cholesterol in the electrolysis solution, the method would be useful to optimize the electrolysis conditions. For this purpose, a high-performance liquid chromatography with photodiode array detection (HPLC-PDA) method was developed (for apparatus and conditions, see Supplementary data). To examine the performance of the method, a standard mixture of cholesta-4,6-dien-3-one and cholesterol was injected into HPLC-PDA. A chromatogram showed that the retention time of cholesta-4,6-dien-3-one was 24.3 min, while that of cholesterol was 34.5 min, indicating the separation of the two compounds. Next, five-point calibration curves of cholesta-4,6-dien-3-one and cholesterol were drawn. The linear ranges of the two compounds were 10–200 μmol/L with correlation coefficients $r = 0.999$. The relative standard deviation (RSD) was 2.1% for cholesta-4,6-dien-3-one ($n = 10$, 25 μmol/L) and 2.3% for cholesterol ($n = 10$, 25 μmol/L). The detection limits ($S/N = 3$) of cholesta-4,6-dien-3-one (280 nm) and cholesterol (210 nm) were 2.0 μmol/L and 10.0 μmol/L, respectively. These results show that cholesta-4,6-dien-3-one and cholesterol can be determined simultaneously by HPLC-PDA.

Next, to confirm the formation of cholesta-4,6-dien-3-one from the cholesterol, a fraction (20 μL) of the electrolytic solution of cholesterol was injected into HPLC-PDA. Figure S1 (Supplementary data) shows representative chromatograms of the electrolytic solution after flow-through column electrolysis of the cholesterol. The formation of cholesta-4,6-dien-3-one was observed by a clear peak at 24.3 min with a detection wavelength of 280 nm as well as spectroscopic characterization using MS, IR, and NMR as previously shown.[13] A remaining cholesterol peak at 34.5 min was observed with a detection wavelength of 210 nm. These results confirm that the electrolysis of cholesterol produces cholesta-4,6-dien-3-one. In addition, from the HPLC results, it seems that the formation of cholesta-4,6-dien-3-one from cholesterol is selective. However, in addition to cholesta-4,6-dien-3-one and cholesterol peaks, an unknown minor peak appeared at 28 min, suggesting the presence of another electrolysis product. The identification of this peak, which may be a cholesterol oxidation intermediate to cholesta-4,6-dien-3-one and may help clarify the reaction mechanism, is currently under investigation in our laboratory.

Among the conditions affecting electrolysis, the applied potential and flow rate were optimized, because these two conditions are critical for efficient flow electrolysis. First, although the applied potential was optimized in the previous study,[13] only the oxidation current peak height of cholesterol was used for optimization. In this study, for synthetic purposes, the concentration of cholesta-4,6-dien-3-one in the electrolysis solution was determined by HPLC-PDA, and the formation of cholesta-4,6-dien-3-one was used for optimization. As shown in Figure 2A, at the potential of 1.5 V versus Ag/AgCl, about 90% of the cholesterol remained. However, at more positive than 1.9 V versus Ag/AgCl, more than 85% of the cholesterol disappeared, suggesting that the cholesterol was electrolyzed. The formation of cholesta-4,6-dien-3-one reached a maximum at 1.9 V versus Ag/AgCl (Fig. 2B), leading to the determination of the applied potential of 1.9 V versus Ag/AgCl as an optimal condition.

Second, the flow rate of the carrier solution containing cholesterol was determined. For this purpose, fractions (10 mL) of the electrolysis solution were collected. Then, the concentrations of cholesta-4,6-dien-3-one and cholesterol in each fraction were determined by HPLC-PDA. As shown in Figure 3A, when the flow rate was 3.0 mL/min, a portion of the cholesterol remained in the electrolytic solution most likely due to passing through the electrolytic cell without electrolysis. However, when the flow rate was slower than 2.5 mL/min, more than 90% of the cholesterol disappeared. The formation of cholesta-4,6-dien-3-one reached its maximum when the flow rate was 2.5 mL/min (Fig. 3B). Because the formation of cholesta-4,6-dien-3-one slightly increased with time

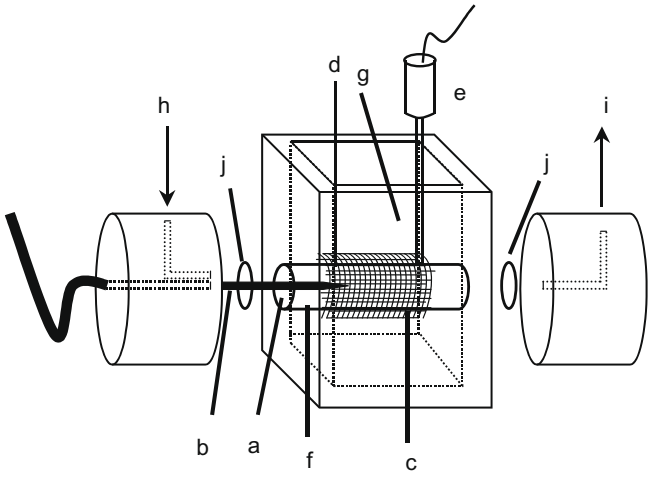

Figure 1. Electrolytic cell with a column electrode: (a) carbon fiber working electrode; (b) contact glassy carbon (GC) rod for working electrode; (c) carbon fiber cloth counter electrode; (d) contact GC rod for counter electrode; (e) Ag/AgCl reference electrode; (f) electrolytic diaphragm, Vycor® glass cylinder; (g) outer solution; (h) sample solution inlet; (i) sample solution outlet; and (j) O-ring. For a cross-section of the cell, please see a previous report.[17]

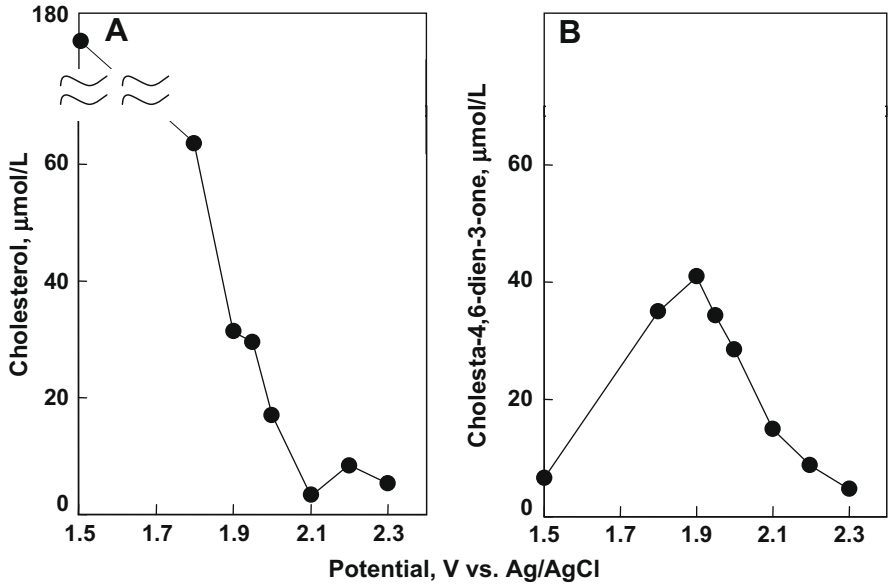

Figure 2. Effect of applied potential to the electrolytic cell on the formation of cholesta-4,6-dien-3-one. After electrolysis of the cholesterol, the remaining cholesterol (A) and formed cholesta-4,6-dien-3-one (B) were determined by HPLC-PDA. The electrolytic conditions were: cholesterol concentration, 200 μmol/L; carrier solution, acetonitrile containing 50 mmol/L $LiClO_4$; flow rate, 1.0 mL/min. The HPLC conditions are the same as in Figure S1 (Supplementary data).

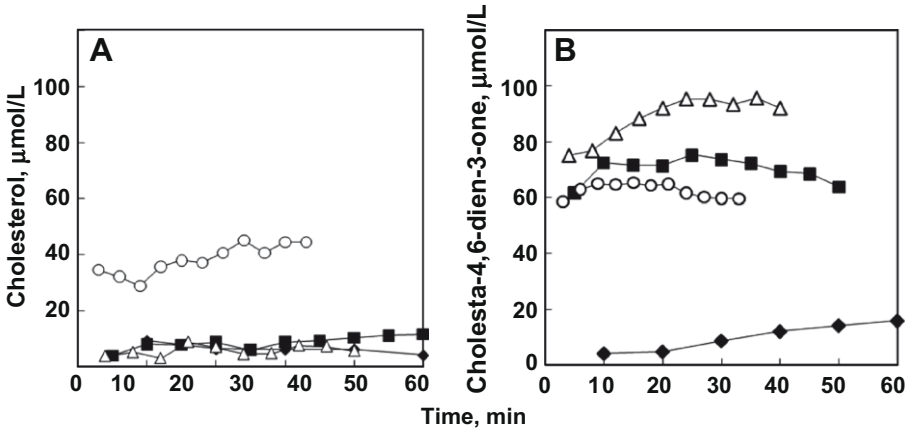

Figure 3. Effect of flow rate on the formation of cholesta-4,6-dien-3-one. After electrolysis of the cholesterol, the remaining cholesterol (A) and formed cholesta-4,6-dien-3-one (B) were determined by HPLC-PDA. The electrolytic conditions were: cholesterol concentration, 200 μmol/L; carrier solution, acetonitrile containing 50 mmol/L $LiClO_4$; applied potential, 1.9 V versus Ag/AgCl. The HPLC conditions are the same as in Figure S1 (Supplementary data). Flow rate: 1.0 mL/min (♦), 2.0 mL/min (■), 2.5 mL/min (△), 3.0 mL/min (○).

and reached a plateau, the electrode is expected to take some time to become stable. As such, we set the flow rate at 2.5 mL/min as an optimal condition. Because the cell volume of the column electrode was 1.5 mL, the residence time of the carrier solution at a flow rate of 2.5 mL/min was calculated to be 0.6 min.

The optimized electrolytic conditions determined were: carrier solution, acetonitrile containing 50 mmol/L $LiClO_4$; applied potential, 1.9 V versus Ag/AgCl; flow rate 2.5 mL/min. Under these conditions, 100 mL of 200 μmol/L cholesterol were electrolyzed at room temperature (25 °C). The amount of cholesta-4,6-dien-3-one obtained was 3.3 mg, providing a large enough amount for the analytical as well as cell biological purposes within a laboratory setting. In spite of the optimization of the electrolysis conditions, the yield was 43%. Although there are several reasons for this limited yield, one of the reasons could be that cholesta-4,6-dien-3-one was not the only electrolytic product of the cholesterol. This was supported by the presence of the unknown peak that appeared at around 28 min (Fig. S1, Supplementary data). Another reason could be that the column electrode trapped a part of the cholesterol and/or electrolytic products. So, the electrolytic solution might be enriched in cholesta-4,6-dien-3-one. In fact, despite the yield, it was easy to purify cholesta-4,6-dien-3-one by silica gel column chromatography (Kanto Silica Gel 60N, Kanto Chemical Co., Inc., Tokyo, Japan) using a hexane–acetone mixture (6:1, v/v) as a mobile phase. Thus, from the point of view of organic synthesis, we believe that this electrosynthesis method is simple, fast, and environmentally friendly among the various known methods for the synthesis of cholesta-4,6-dien-3-one at least on a laboratory synthetic scale.

3. Conclusions

In this study, a simple method for the electrosynthesis of cholesta-4,6-dien-3-one from cholesterol has been developed by improving on a previously reported flow-though column electroly-

sis system. The electrolysis takes only 40 min to electrolyze 100 mL of 200 μmol/L cholesterol (7.7 mg) in acetonitrile containing 50 mmol/L $LiClO_4$, giving 3.3 mg of cholesta-4,6-dien-3-one. Because the reaction does not require multi-steps, but consists of just one step, the operation of the reaction is very simple. Moreover, although the synthetic scale is limited on a laboratory scale, we were able to obtain cholesta-4,6-dien-3-one (mg level) using an appropriate amount of organic solvent and salt. The amount of the product was enough for structure elucidation by spectroscopic and spectrometric analyses and for in vitro biological experiments. In addition, this method does not require any hazardous metal catalysts. Thus, when compared to available methods of the chemical synthesis of cholesta-4,6-dien-3-one on a laboratory scale, the present method is simple, fast, and green.

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Supplementary data

Supplementary data (experimental procedure and additional result) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.10.106.

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