Steroids 172 (2021) 108870

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

An effective synthesis of ursodeoxycholic acid from dehydroepiandrosterone

Wang Chen [a,*], Daihua Hu [a], Zili Feng [a], Zhaopeng Liu [b]

[a] School of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong 723000, China
[b] Key Lab. of Chemical Biology(Ministry of Education), Institute of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China

ARTICLE INFO

Keywords:
Ursodeoxycholic acid
Dehydroepiandrosterone
Synthesis

ABSTRACT

A novel synthetic route of producing ursodeoxycholic acid (UDCA) was developed through multiple reactions from plant-source dehydroepiandrosterone (DHEA), with a Mistunobu reaction and regioselective allyl oxidationat as the key steps. The reaction conditions of the key allyl oxidation reaction were also investigated and optimized, including solvent, oxidant and reaction temperature. In this novel route for the preparation of UDCA, most of the reaction steps have high conversions and overall yield up to 35% for 8 steps. Since all starting materials are cost-effective, commercially available and effectively avoided the risk of animal derived raw materials, this promising synthetic route offers economical and efficient strategies for potential production of UDCA.

1. Introduction

Ursodeoxycholic acid (UDCA) (Fig. 1) is the main component of the bile of black bears which has been widely used in clinics for the treatment of hepatobiliary diseases [1]. As reported [2], UDCA solubilizes cholesterol gallstones, improves the liver function in cholestatic diseases and significantly decreases cholesterol saturation in the bile. Becuase of possessing high efficacy and no side effects, UDCA is the only bile acid drug approved by FDA for the non-surgical treatment of cholesterol gallstone disease [3]. Additionally, UDCA was approved by FDA for the treatment of primary biliary cholangitis [4,5], which is a chronic, autoimmune disease of the liver characterized by destruction of intrahepatic bile ducts, resulting in fibrosis and eventually cirrhosis. UDCA was also reported to exhibit other biological activities such as treatment of cholestasis [6], anti-inflammatory [7], anticancer [8], enhance of insulin sensitivity [9], and improvement mitochondrial function in fibroblasts [10].

UDCA was mainly extracted from natural bear bile. However, the resources of living bears are very limited and the measure violates the animal protection law. With the increase in the need of clinical applications, it is necessary to develop efficient and economical synthetic methods to produce UDCA. At present, UDCA can be commonly produced by transformation of cholic acid(CA), chenodeoxycholic acid (CDCA) or hyodeoxycholic acid(HDCA) [11]. Because CA is the most abundant and least expensive bile acid, it is commonly used as the raw material to produce UDCA through the dihydroxylation at C12 and the epimerization of the 7-OH group. Firstly, the Wolff-Kishner reduction [12–14] and Mozingo reduction [15] methods were applied in the dihydroxylation at C12 of CA. The second step is the epimerization of the 7-OH group as described below. Qiu et al. developed a new route by elimination of C12-mesylate ester group of CA with high yield of 94%, and overall yield up to 65% (7 steps) for preparation of UDCA [16]. CDCA is the most convenient material for the synthesis of UDCA and related steroid compounds, due to the simple steps and high conversion rate. CDCA was first oxidized by NBS [17] (yield 86%), or sodium hypochlorite [18] (yield 89.5%) or IBX [13] (yield 90%) to obtain lithocholic acid. Subsequently, 7-oxo was reduced to 7β-OH to yield UDCA at high yield [11,19] (yield 70%). The enzymatic transformation of CDCA into UDCA can be obtained using 7α-HSDH and 7β-HSDH [20]. Several enzymatic routes for the synthesis of UDCA from bile acids were reported [21]. HDCA is a less expensive and more easily obtained material compared to CDCA, but HDCA was not frequently used because a few extra steps would be required for the conversion between 6-OH and 7-OH. Zhou et al. described a 7-step sequence to synthesize UDCA from HDCA in 15% total yield [22]. More recently, Dou et al. developed a facile route to synthesize UDCA from HDCA with a Shapiro reaction as the key step and in 26% overall yield [23].

However, no matter the CA, CDCA or HDCA, they are all extracted

* Corresponding author at: School of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong 723000, China.
E-mail addresses: chenwang@snut.edu.cn (W. Chen), hudaihua007@163.com (D. Hu), fengzili@snut.edu.cn (Z. Feng), liuzhaop@sdu.edu.cn (Z. Liu).

https://doi.org/10.1016/j.steroids.2021.108870
Received 7 February 2021; Received in revised form 25 April 2021; Accepted 16 May 2021
Available online 24 May 2021

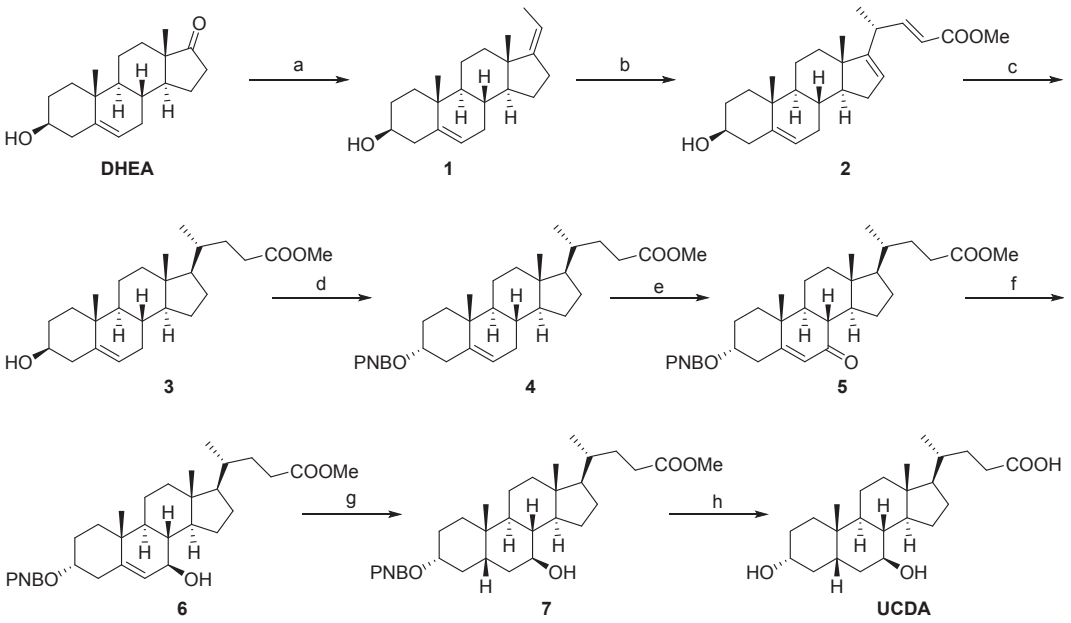


Fig. 1. Structures of bile acids.

from the bile acid and belongs to animal origin. Therefore, developing a plant source compound as the starting material is very valuable. There is only one reliable synthetic route to UDCA based on a plant-source commercial starting material. Qiu et al. reported a novel synthetic route of UDCA from the plant-source bisnoralcohol, with overall yield up to 59% (6 steps) [24]. In this paper, we report an efficiency synthetic route of UDCA from commercial plant-source dehydroepiandrosterone (DHEA).

2. Experimental

2.1. General procedures

All chemicals, unless otherwise noted, were purchased from Energy-Chemical (Shanghai, China) and were used as received without further purification. When needed, the reactions were carried out in oven-dried glassware under a positive pressure of dry nitrogen. Solvents were reagent grade and, when necessary, they were purified and dried by standard methods. All water employed was ultrapure (>18.2 MΩcm^{-1} at 25°C, Milli-Q, Millipore, Billerica, MA). All melting points were determined on a micro melting point apparatus (SGW X4) and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a *Brucker* Avance-600 NMR-spectrometer in the indicated solvents. Chemical shifts are expressed in ppm (δ units) relative to TMS signal as internal reference. The electrospray ionization mass spectrometry (ESI-MS) was carried out on a Thermo Finnigan MSQ 10275. TLC was carried out on Silica Gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd) and spots were visualized by iodine vapors or by irradiation with UV light (254 nm). Flash column chromatography was performed on column packed with Silica Gel 60 (Qingdao Haiyang Chemical Co., Ltd, 200–300 mesh) using the indicated eluents. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure.

2.2. Chemical synthesis

2.2.1. (3β, 17Z)-pregna-5,17(20) -dien-3-ol (1)

A flask was charged with $EtPPh_3Br$ (64.82 g, 174.6 mmol), *t*-BuOK (19.59 g, 174.6 mmol), and a stir bar under nitrogen atmosphere. Anhydrous THF (300 mL) was added via syringe, and the suspension was stirred for 1 h at room temperature. The reaction mixture was cooled to 0 °C, and a solution of the DHEA (16.78 g, 58.2 mmol) in THF (200 mL) was added. The reaction mixture was stirred at 80°C under argon for 4 h. After cooling to 0 °C, water (300 mL) was added slowly, and the product was extracted with EtOAc (150 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. Hexane/EtOAc (v/v = 5:1) (500 mL) was added to the residue to precipitate the byproduct, triphenylphosphine oxide, which was filtered out. The filtrated organic phase was evaporated under reduced pressure to dryness, affording the crude product, which was purified by recrystallization with methanol to give **1** (16.09 g, 92%) as white needles; mp: 119–121 °C [lit. [25] 118–120 °C]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.39–5.34 (m, 1H), 5.18 – 5.10 (m, 1H), 3.57–3.49 (m, 1H), 2.41–2.34 (m, 1H), 2.34–2.28 (m, 2H), 2.27–2.23 (m, 1H), 2.22–2.15 (m, 1H), 2.06–1.99 (m, 1H), 1.89–1.81 (m, 2H), 1.66 (dt, J = 7.2, 1.9 Hz, 3H), 1.64–1.62 (m, 1H), 1.62–1.60 (m, 1H), 1.60–1.56 (m, 1H), 1.55–1.53 (m, 3H), 1.53–1.47 (m, 2H), 1.26–1.15 (m, 1H), 1.17–1.13 (m, 1H), 1.12–1.06 (m, 1H), 1.02 (s, 3H), 1.00–0.95 (td, J = 11.5, 4.8 Hz, 1H), 0.90 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.25, 140.80, 121.58, 113.50, 71.76, 56.54, 50.16, 44.05, 42.30, 37.21, 36.99, 36.57, 31.73, 31.66, 31.46, 31.42, 24.49, 21.24, 19.38, 16.63, 13.14. ESI-MS *m/z*: 301.2 $[M + H]^+$.

2.2.2. (22E)-3β-hydroxy-Chola-5,16,22-trien-24-oic acid methyl ester (2) [26]

To a solution of **1** (16.0 g, 53.2 mmol) and methyl propiolate (5.37 g, 63.8 mmol) in anhydrous benzene (150 mL) at 0 °C was added dropwise a solution of Et_2AlCl (13 mL, 106 mmol, 1 M in toluene) under nitrogen atmosphere. After being warmed to room temperature and stirred for 24 h, the mixture was quenched with saturated aqueous $NaHCO_3$ solution (150 mL) and extracted with ether (100 mL × 2). The combined organic solution was dried over Na_2SO_4 and concentrated under reduced pressure to dryness, affording the crude product. The crude product was recrystallized in ether yield **2** (19.03 g, 93%) as white powder; no sharp melting point [lit. [26] 80–90 °C]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.94 (dd, J = 15.6, 7.8 Hz, 1H), 5.82 (dd, J = 15.7, 1.0 Hz, 1H), 5.43–5.39 (m, 1H), 5.38–5.34 (m, 1H), 3.73 (s, 3H), 3.56–3.49 (m, 1H), 3.05–2.98 (m, 1H), 2.33–2.28 (m, 1H), 2.27–2.20 (m, 1H), 2.08 (ddd, J = 14.9, 6.4, 3.1 Hz, 1H), 2.04–1.97 (m, 1H), 1.88–1.84 (m, 2H), 1.84–1.80 (m, 3H), 1.80–1.76 (m, 1H), 1.72–1.64 (m, 1H), 1.64–1.59 (m, 2H), 1.56–1.46 (m, 2H), 1.41–1.29 (m, 2H), 1.19 (d, J = 6.9 Hz, 3H), 1.12–1.05 (m, 1H),

1.04 (s, 3H), 1.03–0.98 (m, 1H), 0.79 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.41, 156.97, 153.84, 141.07, 124.03, 121.45, 118.76, 71.69, 57.31, 51.47, 50.66, 47.08, 42.29, 37.20, 36.73, 35.42, 34.82, 31.61, 31.53, 31.15, 30.46, 20.74, 19.58, 19.31, 16.34. ESI-MS *m/z*: 385.3 $[M + H]^+$.

2.2.3. Methyl 3β-hydroxy-5-cholen-24-oate (3)

A flask was charged with **2** (19.0 g, 49.4 mmol), 5% $Pd/CaCO_3$ (1.0 g), ethyl acetate (200 mL) and a magnetic stir bar. The suspension was hydrogenated at room temperature. The hydrogen uptake ceased after consumption of approximate 2.3 L. The catalyst was filtered off, the solvents evaporated under reduced pressure and the residue was purified by recrystallization with aqueous methanol to give **3** (16.09 g, 96%) as white needles; mp: 140–142 °C [lit. [27] 139–141 °C]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.38–5.33 (m, 1H), 3.66 (s, 3H), 3.56–3.48 (m, 1H), 2.39–2.33 (m, 1H), 2.32–2.27 (m, 1H), 2.26–2.19 (m, 2H), 2.02–1.94 (m, 2H), 1.88–1.81 (m, 3H), 1.81–1.76 (m, 1H), 1.66–1.54 (m, 3H), 1.54–1.48 (m, 3H), 1.46–1.39 (m, 3H), 1.36–1.26 (m, 2H), 1.16 (td, J = 12.8, 4.4 Hz, 1H), 1.12–1.04 (m, 3H), 1.01 (s, 3H), 0.95–0.90 (m, 1H), 0.92 (d, J = 6.6 Hz, 3H), 0.68 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.80, 140.75, 121.65, 71.76, 56.70, 55.74, 51.50, 50.05, 42.35, 42.27, 39.72, 37.23, 36.48, 35.36, 31.86, 31.84, 31.62, 31.04, 31.00, 28.10, 24.25, 21.05, 19.39, 18.30, 11.86. ESI-MS *m/z*: 389.3 $[M + H]^+$.

2.2.4. (3α)-3-[(4-nitrobenzoyl)oxy]-5-cholen-24-oic acid methyl ester (4)

To a solution of **3** (16.0 g, 41.2 mmol), triphenylphosphine (11.93 g, 45.5 mmol) and *p*-nitrobenzoic acid (7.6 g, 45.5 mmol) in anhydrous THF (100 mL), a solution of diisopropyl azodicarboxylate (9.58 g, 47.4 mmol) in anhydrous THF (50 mL) was added dropwise at 0 °C under nitrogen atmosphere. The mixture was warmed up to room temperature and stirred 12 h. The reaction mixture was evaporated to dryness and re-dissolved in EtOAc (200 mL), washed with saturated sodium bicarbonate solution, and brine, then dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash column chromatography on silica gel elution with hexane/EtOAc (v/v = 20:1) to give **4** (19.05 g, 86%) as light yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29 (d, J = 8.8 Hz, 1H), 8.15 (d, J = 8.8 Hz, 1H), 5.34–5.31 (m, 1H), 5.30–5.27 (s, 1H), 3.67 (s, 3H), 2.66–2.61 (m, 1H), 2.40–2.32 (m, 2H), 2.26–2.20 (m, 1H), 2.04–1.93 (m, 2H), 1.92–1.87 (m, 1H), 1.82–1.73 (m, 1H), 1.62–1.55 (m, 3H), 1.51–1.43 (m, 3H), 1.36–1.27 (m, 2H), 1.23–1.17 (m, 1H), 1.14–1.10 (m, 2H), 1.10–1.04 (m, 1H), 1.07 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.87–0.83 (m, 1H), 0.70 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.77, 164.05, 150.39, 138.11, 136.50, 130.60, 123.53, 122.63, 72.50, 56.68, 55.80, 51.51, 50.35, 42.36, 39.69, 37.12, 36.48, 35.38, 33.99, 31.90, 31.81, 31.05, 31.01, 28.11, 26.27, 24.22, 20.80, 18.92, 18.32, 11.87. HRMS(ESI): calcd for $C_{32}H_{43}NO_6$ $[M + H]^+$, 538.3207, found 538.3202.

2.2.5. (3α)-3-[(4-nitrobenzoyl)oxy]-7-oxo-5-cholen-24-oic acid methyl ester (5)

To a mechanically stirred suspension of **4** (14.0 g, 26 mmol), pyridinium dichromate (PDC; 29.34 g, 78 mmol) and celite (30 g) in anhydrous benzene (200 mL), 70% *tert*-butylhydroperoxide (*t*-BuOOH; 22.8 mL, 156 mmol) were added gradually at 0 °C. The whole reaction mixture was stirred at 25 °C for 24 h. After filtration on celite, the organic layer was evaporated under reduced pressure to give a dark brown residue. The crude product was purified by flash column chromatography on silica gel elution with hexane/EtOAc (v/v = 8:1) to give **5** (10.04 g, 70%) as white powder; mp: 157–159 °C [lit. [28] 157–160 °C]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.28 (d, J = 8.8 Hz, 2H), 8.12 (d, J = 8.8 Hz, 2H), 5.73 (d, J = 1.2 Hz, 1H), 5.44 (s, 1H), 3.67 (s, 3H), 2.82–2.74 (m, 1H), 2.64–2.56 (m, 1H), 2.47–2.40 (m, 1H), 2.39–2.35 (m, 1H), 2.29 (t, J = 11.6 Hz, 1H), 2.26–2.20 (m, 1H), 2.12–2.07 (m, 1H), 2.06–2.02 (m, 1H), 1.98–1.92 (m, 1H), 1.90–1.85 (m, 1H), 1.84–1.79 (m, 1H), 1.69–1.57 (m, 5H), 1.46–1.42 (m, 1H), 1.41–1.37 (m, 1H), 1.37–1.34 (m, 1H), 1.33–1.30 (m, 1H), 1.27 (s, 3H), 1.29–1.24 (m, 1H), 1.20–1.14 (m, 1H), 1.11 (q, J = 9.4 Hz, 1H), 0.94 (d, J = 6.5 Hz, 3H), 0.71 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.67, 174.67, 163.82, 163.26, 150.59, 135.64, 130.64, 127.17, 123.65, 71.61, 54.49, 51.52, 49.99, 49.91, 45.49, 43.18, 38.84, 38.57, 36.43, 35.26, 33.34, 31.06, 31.01, 28.41, 26.28, 25.75, 20.90, 18.47, 16.96, 11.98. ESI-MS *m/z*: 552.5 $[M + H]^+$.

2.2.6. (3α,7β)-7-hydroxy-3-[(4-nitrobenzoyl)oxy]-5-cholen-24-oic acid methyl ester (6)

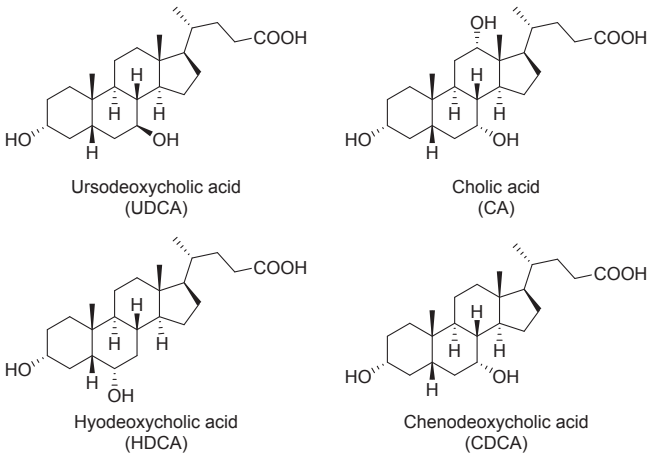
Sodium borohydride (0.78 g, 20.6 mmol) was added in small portions to a clear solution of **5** (9.5 g, 17.2 mmol) and $CeCl_3{\cdot}7H_2O$ (7.69 g, 20.6 mmol) in a mixture of methanol (20 mL) and tetrahydrofuran (80 mL). The reaction mixture was stirred for 4 h at room temperature and then treated with acetone (20 mL). The mixture was filtered to remove the insoluble solids, then the solvents were evaporated under reduced pressure and the residue was purified by flash column chromatography on silica gel elution with hexane/EtOAc (v/v = 4:1) to give **6** (7.62 g, 80%) as white powder; mp: 175–177 °C [lit. [28] 174–177 °C]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29 (d, J = 8.8 Hz, 2H), 8.15 (d, J = 8.8 Hz, 2H), 5.33 (brs, 1H), 5.29 (brs, 1H), 3.90 (dd, J = 7.3, 6.1 Hz, 1H), 3.67 (s, 3H), 2.64 (dd, J = 15.3, 1.8 Hz, 1H), 2.44–2.32 (m, 2H), 2.27–2.20 (m, 2H), 2.06–1.97 (m, 2H), 1.96–1.87 (m, 2H), 1.86–1.80 (m, 2H), 1.78–1.72 (m, 2H), 1.60–1.55 (m, 1H), 1.51–1.42 (m, 6H), 1.36–1.20 (m, 4H), 1.12 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.72 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.71, 163.95, 150.46, 141.03, 136.28, 130.59, 126.52, 123.55, 73.06, 72.12, 55.90, 55.16, 51.51, 48.43, 42.97, 40.74, 39.48, 37.04, 36.08, 35.31, 33.61, 31.06, 31.02, 28.40, 26.28, 26.16, 20.82, 18.71, 18.38, 11.84. ESI-MS *m/z*: 554.5 $[M + H]^+$.

2.2.7. (3α,5β,7β)-7-hydroxy-3-[(4-nitrobenzoyl)oxy]-5-cholen-24-acid methyl ester (7)

A flask was charged with **6** (7.2 g, 13 mmol), $[Ir(cod)pyr(PCy_3)]PF_6$ (Crabtree's catalyst, 0.5 g), anhydrous DCM (100 mL) and a magnetic stir bar. The reaction mixture was hydrogenated at room temperature. The hydrogen uptake ceased after consumption of approximate 0.3 L. The solvent was evaporated to dryness and re-dissolved in ether (200 mL). The catalyst was filtered off, the solvents evaporated under reduced pressure and the residue was purified by recrystallization with aqueous methanol to give **7** (6.86 g, 95%) as white powder; mp: 181–183 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.28 (d, J = 8.9 Hz, 2H), 8.20 (d, J = 8.9 Hz, 2H), 5.00–4.93 (m, 1H), 3.67 (s, 3H), 3.65–3.59 (m, 1H), 2.40–2.33 (m, 1H), 2.27–2.19 (m, 1H), 2.05–2.00 (m, 1H), 1.95–1.88 (m, 2H), 1.87–1.78 (m, 6H), 1.69–1.61 (m, 2H), 1.56–1.41 (m, 7H), 1.38–1.28 (m, 4H), 1.24–1.14 (m, 2H), 1.09 (q, J = 9.6 Hz, 1H), 1.00 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.68, 164.13, 150.47, 136.16, 130.66, 123.47, 75.66, 71.23, 55.76, 54.98, 51.52, 43.79, 43.78, 42.35, 40.11, 39.29, 36.62, 35.25, 34.60, 34.17, 33.18, 31.06, 31.04, 28.59, 26.86, 26.55, 23.36, 21.27, 18.41, 12.15. HRMS(ESI): calcd for $C_{32}H_{45}NO_7$ $[M + H]^+$, 556.3205, found 556.3201.

2.2.8. Ursodeoxycholic acid (UDCA)

To a solution of **7** (6.8 g, 12.2 mmol) in MeOH (100 mL), a solution of 10% NaOH (2.78 mol/L; 11 mL, 30 mmol) was added. The reaction mixture was stirred at room temperature for 6 h, then added 5% HCl (0.13 mol / L) solution, adjust to pH = 3, and remove methanol under reduced pressure. The residues were extracted with EtOAc (100 mL × 2), washed with brine, then dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by recrystallization with aqueous methanol to give UDCA (4.52 g, 94%) as white powder; mp: 198–200 °C [lit. [24] 200–202 °C]. [α]25 D + 60.1 (c 1.0, Ethanol). [lit. [24] [α]25 D + 60.9 (c 1.0, Ethanol]. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.93 (s, 1H), 4.44 (s, 1H), 3.86 (s, 1H), 3.34–3.24 (m, 2H), 2.26–2.20 (m, 1H), 2.13–2.06 (m, 1H), 1.96–1.91 (m, 1H), 1.88–1.81 (m, 1H), 1.77–1.72 (m, 1H), 1.69–1.63 (m, 3H), 1.50–1.44 (m, 3H), 1.42–1.38 (m, 1H), 1.37–1.32 (m, 5H), 1.31–1.27 (m, 1H), 1.23–1.16 (m, 3H), 1.15–1.08 (m, 3H), 1.01 (q, J = 9.6 Hz, 1H), 0.95–0.90 (m, 1H), 0.88 (d, J = 8.5 Hz,



Scheme 1. Synthesis of ursodeoxycholic acid from DHEA. Reagents and conditions: (a) $EtPPh_3Br$, *t*-BuOK, anhydrous THF, 80℃, 4 h, under nitrogen atmosphere, 92%; (b) methyl propiolate, Et_2AlCl 1 M in toluene, anhydrous benzene, 0℃ to r.t., 24 h, under nitrogen atmosphere, 93%; (c) 5% $Pd/CaCO_3$, H_2, EtOAc, r.t., 96%; (d) DIAD, PPh_3, p-nitrobenzoic acid, anhydrous THF, 0℃ to r.t., 12 h, under nitrogen atmosphere, 86%; (e) PDC, 70% *t*-BuOOH, celite, anhydrous benzene, r.t., 24 h, 70%; (f) NaBH4, $CeCl_3{\cdot}7H_2O$, THF, MeOH, r.t., 4 h, 80%; (g) Crabtree's catalyst, H_2, anhydrous DCM, r.t., 95%; (h) NaOH, MeOH, then HCl, r.t., 6 h, 94%.

Table 1
Allyl oxidation of compound **4**[a].

Entry	Solvent	Oxidant	RT[b]	Yield[c] (%)
1	DCM	PDC(3 eq)	reflux	N[d]
2	1,2-Dichloroethane	PDC(3 eq)	reflux	N[d]
3	Acetone	PDC(3 eq)	reflux	N[d]
4	Acetone/H_2O (9:1, v/v)	PDC(1.1 eq)/ NHPI(1.1 eq)	25 °C	N[d]
5	Acetone	$Na_2Cr_2O_7$(3 eq)	25 °C	26
6	Acetone	$K_2Cr_2O_7$(3 eq)	25 °C	35
7	Acetone	$K_2Cr_2O_7$(3 eq)/NHPI(1.1 eq)	25 °C	42
8	Acetone	$K_2Cr_2O_7$(3 eq)/NHPI(1.1 eq)/AcOH(1.1 eq)	25 °C	48
9	Acetone	BPO(1.1 eq)/NHPI(1.1 eq)	25 °C	N[d]
10	Pyridine	CrO_3(3 eq)	25 °C	51
11	*t*-BuOH	PDC(3 eq)/TBHP(70%, 6 eq)	25 °C	N[d]
12	Acetone	PDC(3 eq)/TBHP(70%, 6 eq)	25 °C	56
13	MeCN	PDC(3 eq)/TBHP(70%, 6 eq)	25 °C	58
14	Toluene	PDC(3 eq)/TBHP(70%, 6 eq)	25 °C	65
15	DCM	PDC(3 eq)/TBHP(70%, 6 eq)	25 °C	53
16	1,2-Dichloroethane	PDC(3 eq)/TBHP(70%, 6 eq)	25 °C	55
17	Benzene	PDC(3 eq)/TBHP(70%, 6 eq)	25 °C	70
18	Benzene	PDC(3 eq)/TBHP(70%, 6 eq)	50 °C	62
19	Benzene	PDC(3 eq)/TBHP(70%, 6 eq)	reflux	58

[a] All the reactions were performed for 24 h, and the ratio of oxidant/compound **4** was showed.
[b] Reaction temperature.
[c] Isolated yield.
[d] No reaction.

3H), 0.87 (s, 3H), 0.62 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 175.35, 70.19, 69.92, 56.31, 55.15, 43.56, 43.48, 42.65, 39.19, 38.20, 37.74, 35.31, 34.23, 31.24, 30.72, 28.63, 27.18, 23.78, 21.32, 18.78, 12.50. ESI-MS *m/z*: 393.5 $[M + H]^+$.

3. Results and discussion

The synthesis of UDCA is shown in Scheme 1. Wittig olefination of DHEA as starting material gave compound **1** stereoselectively in 92% yield. The witting reaction with a resonance stabilized is known to predominantly deliver cis- stereoisomer, which can be proved by the ^1H and ^{13}C NMR spectra. Compound **3** was obtained by stereo-selective introduction of side chains at C (17) and C (20) in 93% yield. This approach could set the stereochemistry of the C-20 carbon in the natural configuration [26]. The triene intermediate **2** was selectively hydrogenated with palladium on calcium carbonate in ethyl acetate to **3** in 96% yield. The 3β-OH of **3** was converted to 3α-(4-nitrobenzoyl)oxyl group by Mitsunobu reaction. The inversion of stereochemistry from the 3β-OH could up to 100% configuration reversal and give **4** in 86% yield. The yield of oxidation at the C-7 position of **4** to afford compound **5** was relatively low. Therefore, a model system was employed to find an ideal solvent and optimal reaction conditions (Table 1). After screening various oxidants, we found that the oxidant of PDC/ TBHP was the optimal oxidant for this reaction. Then we examined several different solvents, and the results showed that benzene is the optimal solvent. We also investigated the reaction temperature, and discovered that 25 °C was the most suitable temperature, and the yield was up to 70% for the oxidation reaction. Increasing the temperature was beneficial to the rapid completion of the reaction. But, the oxidation of compound **4** under higher temperature produced more by-products. Regioselective reduction of compound **5** was performed following the Luche reduction procedure with sodium borohydride in THF/MeOH to obtain 7β epimer **6** after isolation by column chromatography in 80% yield. Selective hydrogen addition of **6** in the presence of Crabtree's catalyst given 5β epimer **7** in 95% yield. The addition of hydrogen on one face of a cyclic molecule can be directed by the presence of a hydroxyl group on that face using Crabtree's catalyst[29], however using Pd/C as catalyst given 5α epimer [28]. Lastly, hydrolysis of the C-24 methyl ester of **7** under NaOH in MeOH provided ursodeoxycholic acid in 94% yield. In this novel route for the preparation of UDCA from DHEA, most of the conversions are very efficient in 8 steps and overall yield up to 35%.

4. Conclusion

In summary, we designed an efficient and economical method for the synthesis of UDCA from plant-source DHEA with the Mistunobu reaction and regioselective oxidationat C7 as the key steps. The final product was obtained through the 7β-OH and 5β-H inversion employing Luche reduction and Crabtree's catalyst. Since all starting materials are cost-effective, commercially available and most of the conversions are efficiently and the overall yield is high, this synthetic route can effectively avoid the risk of animal derived raw materials. The key reaction conditions were investigated and the optimal solvent, oxidant and reaction temperature were determined. However, the yield of intermediate **5** and **6** are low, which is 70% and 80% respectively, and the conditions of allylic oxidation need further optimization. Further studies on improved routes and higher yield are underway in our laboratory.

Acknowledgments

This work was supported by Key laboratory project of Shaanxi Provincial Department of Education (20JS023) and Key projects of science and Technology Department of Shaanxi Province (2018ZDXM-SF-083).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2021.108870.

References

[1] G. Paumgartner, U. Beuers, Ursodeoxycholic acid in cholestatic liver disease: Mechanisms of action and therapeutic use revisited, Hepatology 36 (3) (2002) 525–531.
[2] T. Ikegami, Y. Matsuzaki, Ursodeoxycholic acid: Mechanism of action and novel clinical applications, Hepatology Res. 38 (2) (2008) 123–131.
[3] E. Roda, F. Bazzoli, A.M.M. Labate, G. Mazzella, A. Roda, C. Sama, D. Festi, R. Aldini, F. Taroni, L. Barbara, Ursodeoxycholic acid vs. chenodeoxycholic acid as

cholesterol gallstone-dissolving agents: A comparative randomized study, Hepatology 2 (6) (1982) 804–810.
[4] R. Sahu, R. Mishra, C. Majee, An insight into primary biliary cholangitis and its recent advances in treatment: semi-synthetic analogs to combat ursodeoxycholic-acid resistance, Expert. Rev. Gastrooent. 14 (10) (2020) 985–998.
[5] T. Namisaki, Y. Fujinaga, K. Moriya, H. Yoshiji, The association of histological progression with biochemical response to ursodeoxycholic acid in primary biliary cholangitis, Hepatology Res. 51 (1) (2021) 31–38.
[6] U. Beuers, J.L. Boyer, G. Paumgartner, Ursodeoxycholic acid in cholestasis: Potential mechanisms of action and therapeutic applications, Hepatology 28 (6) (1998) 1449–1453.
[7] S.J. Keely, N.-K.-L.-M. Ginley, C.J. Steer, Ursodeoxycholic acid: A promising therapeutic target for inflammatory bowel diseases? Am. J. Physiol. Gastrointest. Liver. *Physio*l. 317 (6) (2019) 872–881.
[8] J.F. Goossens, C. Bailly, Ursodeoxycholic acid and cancer: From chemoprevention to chemotherapy, Pharmacol. Ther. 203 (2019), 107396.
[9] T. Tsuchida, M. Shiraishi, T. Ohta, K. Sakai, S. Ishii, Ursodeoxycholic acid improves insulin sensitivity and hepatic steatosis by inducing the excretion of hepatic lipids in high-fat diet–fed KK-Ay mice, Metabolism 61 (7) (2012) 944–953.
[10] S.M. Bell, K. Barnes, H. Clemmens, A.R. Al-Rafiah, E.A. Al-ofi, V. Leech, O. Bandmann, P.J. Shaw, D.J. Blackburn, L. Ferraiuolo, H. Mortiboys, Ursodeoxycholic Acid Improves Mitochondrial Function and Redistributes Drp1 in Fibroblasts from Patients with Either Sporadic or Familial Alzheimer's Disease, J. Mol. Biol. 430 (21) (2018) 3942–3953.
[11] F. Tonin, I. Arends, Latest development in the synthesis of ursodeoxycholic acid (UDCA): a critical review, Beilstein. J Org. Chem. 14 (2018) 470–483.
[12] A.F. Hofmann, G. Lundgren, O. Theander, J.S. Brimacombe, M.C. Cook, The Preparation of Chenodeoxycholic Acid and Its Glycine and Taurine Conjugates, Acta. Chem. Scand. 17 (1963) 173–186.
[13] P.S. Dangate, C.L. Salunke, K.G. Akamanchi, Regioselective oxidation of cholic acid and its 7β epimer by using o-iodoxybenzoic acid, Steroids 76 (12) (2011) 1397–1399.
[14] M. Ferrari, F. Zinetti, Process for preparing high purity ursodeoxycholic acid, United States (2014).
[15] Y. Sato, N. Ikekawa, Notes: Preparation of Chenodeoxycholic Acid, J Org. Chem. 24 (9) (1959) 1367–1368.
[16] X.-L. He, L.-T. Wang, X.-Z. Gu, J.-X. Xiao, W.-W. Qiu, A facile synthesis of ursodeoxycholic acid and obeticholic acid from cholic acid, Steroids 140 (2018) 173–178.
[17] G.M. Zhang, X.L. Yu, C.P. Ya, Improved preperation of 7-oxolitnocholic acid, Chin. J. Pharm. 27(5) (1996) 245-245.
[18] M.F. Lu, W.C. Yin, F.D. Wang, D.M. Peng, Improved Synthesis of Ursodeoxycholic Acid, Chin. J. Pharm. 46 (010) (2015) 1058–1059.
[19] S.J. Lee, Y.R. Malpani, I.W. Kim, Development of a packed-bed flow process for the production scale hydrogenation of 7-oxo-lithocholic acid to ursodeoxycholic acid, J. Flow. Chem. 10 (4) (2020) 637–646.
[20] X. Zhang, D. Fan, X. Hua, T. Zhang, Large-scale production of ursodeoxycholic acid from chenodeoxycholic acid by engineering 7α- and 7β-hydroxysteroid dehydrogenase, Bioprocess. Biosyst. Eng. 42 (9) (2019) 1537–1545.
[21] T. Eggert, D. Bakonyi, W. Hummel, Enzymatic routes for the synthesis of ursodeoxycholic acid, J. Biotechnol. 191 (2014) 11–21.
[22] W.S. Zhou, Z.Q. Wang, B. Jiang, Stereocontrolled conversion of hyodeoxycholic acid into chenodeoxycholic acid and ursodeoxycholic acid, J. Chem. Soc., Perkin Trans. 1 (1) (1990) 1–3.
[23] Q. Dou, Z. Jiang, A Facile Route to Ursodeoxycholic Acid Based on Stereocontrolled Conversion and Aggregation Behavior Research, Synthesis 48 (04) (2016) 588–594.
[24] J. Wang, X.Z. Gu, L.M. He, C.C. Li, W.W. Qiu, Synthesis of ursodeoxycholic acid from plant-source (20S)-21-hydroxy-20-methylpregn-4-en-3-one, Steroids 157 (2020), 108600.
[25] R.P. Litvinovskaya, S.V. Drach, V.A. Khripach, Synthesis of 16-Substituted 23,24-Dinorcholane Derivatives, Russ. J. Org. Chem. 42 (1) (2006) 27–35.
[26] W.G. Dauben, T. Brookhart, Stereocontrolled synthesis of steroidal side chains, J. Am. Chem. Soc. 103 (1) (1981) 237–238.
[27] T. Iida, G. Kakiyama, Y. Hibiya, S. Miyata, T. Inoue, K. Ohno, T. Goto, N. Mano, J. Goto, T. Nambara, A.F. Hofmann, Chemical synthesis of the 3-sulfooxy-7-N-acetylglucosaminyl-24-amidated conjugates of 3β,7β-dihydroxy-5-cholen-24-oic acid, and related compounds: Unusual, major metabolites of bile acid in a patient with Niemann-Pick disease type C1, Steroids 71 (1) (2006) 18–29.
[28] S. Liu, G.Q. Wang, Z.K. Liu, Q.A. Wang, A Novel Synthetic Approach of Alloursodeoxycholic Acid from Hydeoxycholic Acid, Chin. J. Org. Chem. 33 (10) (2013) 2216–2219.
[29] J.J. Verendel, O. Pàmies, M. Diéguez, P.G. Andersson, Asymmetric Hydrogenation of Olefins Using Chiral Crabtree-type Catalysts: Scope and Limitations, Chem. Rev. 114 (4) (2014) 2130–2169.