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Regioselective oxidation of cholic acid and its 7β epimer by using *o*-iodoxybenzoic acid

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ABSTRACT

Rational exploration directed by DFT (density functional theory) based atomic Fukui indices, lead to development of regioselective oxidation of cholic acid and its 7β epimer by *o*-iodoxybenzoic acid. In case of cholic acid only, 7α-hydroxyl underwent oxidation, where as in its 7β epimer the selectivity was towards 12α-hydroxy group. Since these oxidations are the key steps in synthesis of ursodeoxycholic acid starting from cholic acid these findings may be useful in devising a protection free synthetic route.

1. Introduction

Interest in steroid chemistry arises as many of these compounds have been accorded in medicine. Steroidal drugs are a niche category and include hormones, such as corticoids, progestins, estrogens, androgens, anabolic agents and others including calciferols, and ursodeoxycholic acid [1]. Cholic acid is a relatively inexpensive bile acid and can be used for synthesis of multi step synthetic product like ursodeoxycholic acid [2], a drug used to solubilise cholesterol gallstones [3]. Regioselective transformations in steroid scaffold are well known including regioselective oxidations of hydroxyl groups. Hypervalent iodine (V) reagents, particularly *o*-iodoxybenzoic acid (IBX) are of current interest [4] and its application and potential in steroid transformations is not fully explored. IBX is a mild, chemo selective, non-toxic, eco-friendly oxidant and our research group is actively engaged in exploring its reactions [5]. In this paper we report our exploration, directed by DFT (density functional theory) based atomic Fukui indices indicating ease of oxidation of hydroxyl groups. It is satisfying to find that oxidations were highly regioselective and were in accordance with the prediction.

2. Experimental

2.1. General

^{1}H NMR spectra were recorded on operating at JEOL-FT-NMR operating at 300 MHz instruments, chemical shifts are expressed in parts per million downfield from TMS in δ units. IR spectra were recorded on FTIR RX1 Perkin–Elmer instrument. Melting points were determined with Veego melting point apparatus having stirred paraffin bath. Silica gel 60–120 was used for column chromatography. Thin layer chromatography (TLC) was performed using Merck Silica gel 60 F254 Plates. Cholic acid **1** was procured from s.d fine chem. Mumbai, India and 3α,7β,12α-trihydroxy-5β-cholane-24-oic acid **4** was prepared by literature procedure [6].

2.2. General experimental procedure for oxidation with IBX

To a suspension of IBX (2.19 g, 14.68 mmol) in 25 ml of *t*-BuOH was added steroid (4 g, 9.7 mmol) and refluxed and reaction progress was monitored by TLC. After completion of reaction (time, 1 h), solids were separated by filtration and washed with dichloromethane. Combined filtrates were concentrated and residue was taken up in dichloromethane and was washed with 10% sodium bisulphite (2 × 15 ml) and brine (2 × 15 ml). Organic layer was dried over sodium sulphate and concentrated under reduced pressure to get crude product, which was then purified by column chromatography (silica gel # 60–120; ethyl acetate–hexane, 40:60).

2.2.1. Oxidation of cholic acid *1*

Cholic acid **1** (4 g, 9.7 mmol) was oxidised following the general procedure and products were separated by column chromatography:

*2.2.1.1. 3α,12α-Dihydroxy-7-keto-5β-cholane-24-oic acid **2**.* Yield: 3.58 g (90%); Mp: 193 °C [lit.[12] 195 °C]; IR (KBr): 3428 (–OH), 2937.1(C—H), 1706(C=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ

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0.77(s, 3H, H-18), 1.06 (d, J = 6.2 Hz, 3H, H-21), 1.26 (s, 3H, H-19), 2.60 (dd, J = 12 Hz, 1H, H-8β), 3.02 (dd, J = 13, 6.6 Hz, 1H, H-6β), 3.58 (br, 1H, H-3β), 4.04 (s, 1H, H-12β).

2.2.1.2. 3α-Hydroxy-7,12-dioxo-5β-cholane-24-oic acid **3.** Yield: 0.29 g (7.5%); Mp: 188 °C [lit.[12] 189 °C]; IR (KBr): 3414.2(–OH), 2957.5, 2916(C—H), 1711(C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.74 (d, J = 6.2 Hz, 3H, H-21), 1.69 (s, 3H, H-19), 1.28 (s, 3H, H-18), 3.57 (br, 1H, H-3β).

2.2.2. 3α,7β,12α-Trihydroxy-5β-cholane-24-oic acid **4**

To a solution of 2.50 g (6.1 mmol) of 3α,12α-dihydroxy-7-keto-5β-cholane-24-oic acid **2** in 20 ml of anhydrous *n*-propanol was added 5.09 g (220.0 mmol) of sodium metal. Reaction mixture was refluxed and progress of reaction was monitored by TLC. Reaction was completed in 3 h. The reaction mixture was cooled to room temperature and gradually diluted with 20 ml of water, acidified with dilute hydrochloric acid, and extracted with (2 × 30 ml) of ethyl acetate. Organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to get residue which was further purified by column chromatography on silica gel using methanol–chloroform (5:95). Yield: 2 g (80%); Mp: 144 °C [lit.[12] 145 °C]; IR (KBr): 1704 (C=0), 3448 (OH), 1042, 1010 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.69 (s, 3H, H-18), 0.91 (s, 3H, H-19), 3.51 (br m, 2H, H-3β and H-7α), 3.91(m, 1H, H-12β).

2.2.3. Oxidation of 7β epimer **4**

7β Epimer **4** (4 g, 9.7 mmol) was oxidised following the general procedure.

2.2.3.1. 3α,7β-Dihydroxy-12-oxo-5β-cholane-24-oic acid **5**. Yield: 3.5 g (90%); Mp: 137 °C [lit.[12] 136 °C]; IR (KBr): 1740, 1670 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 6H, H-18 and H-19), 3.58 (br m, 2H, H-3β and H-7α).

2.2.2.2. 3α,7β-Dihydroxy-5β-cholane-24-oic acid **6**. Wolf–Kishner reduction of 3α,7β,12-oxo-5β-cholane-24-oic acid **5** was carried out by using reported literature procedure and isolated after chromatography:

To a suspension of 0.5 g (1.23 mmol) 3α,7β-dihydroxy-12-oxo-5β-cholane-24-oic acid 5 and 99% hydrazine hydrate 0.21 g (4.31 mmol) with 6 ml of triethylene glycol, was added potassium hydroxide 0.24 g (4.18 mmol). The reaction mixture was heated to 110 °C for 3 h and distilled until the temperature of the reaction was raised to 135 °C and maintained this temperature till the reaction gets completed. Then reaction mixture was cooled to room temperature and acidified with dilute hydrochloric acid to get white residue after filtration. Which was further purified by column chromatography on silica gel using methanol–chloroform (3:97) Yield: 0.39 g (82%); Mp 196 °C [lit.[12] 198 °C]; IR (KBr) 3518, 3552 (O—H), 2937(C—H), 1716, 1654 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.76 (s, 3H, H-18), 1.00 (d, J = 6.2 Hz, 3H, H-21), 1.00 (s, 3H, H-19), 3.55 (br, 2H, H-3β and H-7α). $[\alpha]_D^{25}$ = +59 (c = 0.2 ethanol).

3. Results and discussion

Cholic acid **1** and its 7β epimer **4** have three hydroxyl groups present at different positions and study of their oxidation would give some insights into the chemistry of IBX and outcome may also have some practical utility. Before investigations of oxidation some theoretical calculation were performed to understand the preponderance of hydroxyl group towards oxidation. To visualise reactivity pattern of hydroxyl groups and scope for regioselective oxidation in substrates for cholic acid **1** and 7β epimer **4,** DFT (density functional theory) based atomic Fukui indices were calculated computationally. Fukui indices have been used for prediction of reactivities [7]. The Fukui function is defined as the differential change in electron density due to an infinitesimal change in the number of electrons. They were calculated by using software Jaguar [8]. Methodology employed for calculation is as follows. Conformation search was performed using Macro Model [9], to determine lower energy conformers. Conformer with lowest energy was selected for further calculations and was subjected to DFT optimization using basis set B3LYP/6-31G** with single point solvation from Poisson–Boltzmann quantum theory (t-BuOH solvent, ε = 12.4, r_{probe} = 2.6 Å) and values are given in Table 1. Atomic Fukui index f_NN HOMO indicates.

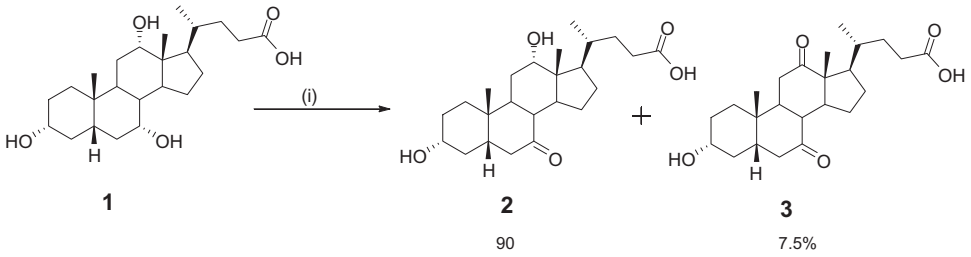
Preferred sites of electrophilic attack and susceptibility when its value is large [10]. Cholic acid **1** shows highest f_NN HOMO for C-7 followed by C-12 and C-3 hydroxy sites. Therefore order of reactivity of hydroxy groups would be: C-7 > C-12 > C-3. Similarly for 7β epimer **4** highest f_NN HOMO was observed for C-12 followed by C-7 and C-3 hydroxy sites. Therefore order of reactivity of hydroxy groups would be: C-12 ≫ C-7 > C-3. The oxidation of cholic acid **1** and its 7β epimer **4** using reagents such as chromic acid, bromine in aqueous alkali was found to show very poor regioselectivity indicating that they are not mild enough to distinguish these fine differences in reactivity pattern of hydroxyl groups [11]. However oxidation with NBS shows high regioselectivity and follows this trend with cholic acid **1** but no data is available for oxidation of 7β epimer **4**. IBX is a mild oxidant and it is expected to distinguish this reactivity pattern and show high regioselectivity. Oxidation experiments were carried out on cholic acid **1** under different conditions using t-BuOH as solvent and with 1.5 equivalent of IBX under reflux, as less than 1.5 equivalents were found to be insufficient to take the reaction to completion. Both 3α,12α-dihydroxy-7-oxo-5β-cholan-24-oic acid **2** and 3α-hydroxy-7, 12-dioxo-5β-cholan-24-oic acid **3** were isolated in the yields of 90% and 7.5%, respectively (Scheme 1). Thus showing very high regioselectivity towards 7α-hydroxy group.

Reduction of compound **2** was performed following the literature procedure with sodium in anhydrous *n*-propanol to obtain 80% yield of 7β epimer **4** and 10% of cholic acid **1** after isolation by column chromatography. When 7β epimer **4** was subjected oxidation under the same condition almost exclusively 3α,7β-dihydroxy-12-oxo-5β-cholane-24-oic acid **5** was obtained 90%, with no detectable amount of diketone **3** (Scheme 2). Again showing very high regioselectivity but towards 12α hydroxy group. These results are consistent with the reactivity as predicted by atomic Fukui indices. Lastly dihydroxy-ketone **5** was subjected to Wolf–Kishner reduction by known process [12]e and isolated ursodeoxycholic acid **6** in 82% yields.

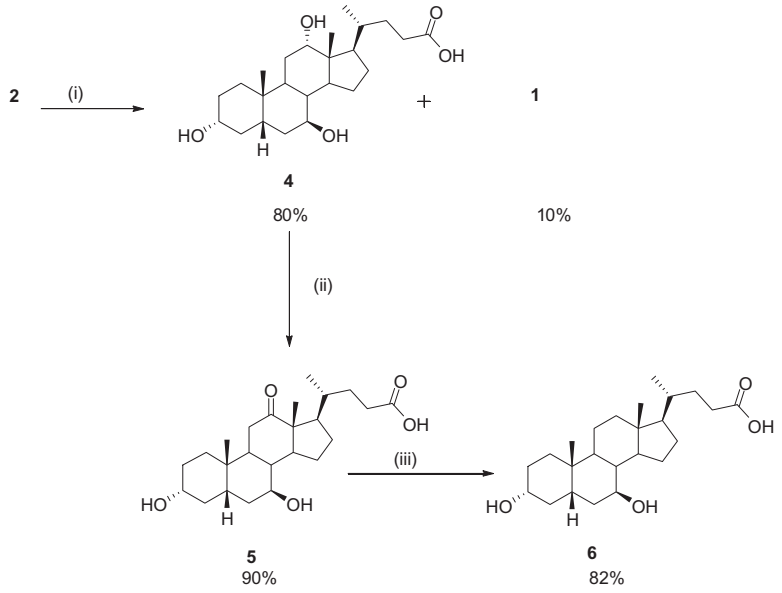
Many procedures are reported for synthesis of ursodeoxycholic acid **6** from cholic acid **1** [12] and all the chemical synthesis routes require at least one protection–deprotection step to overcome the problem of chemoselectivity with various oxidising agents employed, however, recently a protection-group-free chemoenzymatic route is disclosed by Giovannini et al. [12e] in which they obtained 70% overall yield of ursodeoxycholic acid **6**. With

Table 1
Calculated values of atomic Fukui indices for cholic acid **1** and 7β epimer **4**.

Substrate	Atoms (hydroxy sites)	$f k$ f_NN HOMO for reactive sites (t-BuOH)
Cholic acid **1**	O_3	0.0048
	O_7	0.2093
	O_{12}	0.0728
7β Epimer **4**	O_3	0.0021
	O_7	0.0168
	O_{12}	0.3264



Scheme 1. Reagents and conditions: (i) IBX, *t*-BuOH, 1 h, **2,** 90%; **3,** 7.5%.



Scheme 2. (i) Na/*n*-propanol, 3 h reflux, **4,** 80%; **1**, 10%, (ii) IBX, *t*-BuOH, 1 h reflux, 90%, (iii) $NH_2NH_2{\cdot}H_20$, KOH, trigol, reflux, 82%.

the kind of selectivity observed with IBX oxidation, we succeeded to envisage a protection free route with overall yield of 53%.

4. Conclusion

A very high regioselective oxidation of hydroxyl groups in cholic acid and its 7β epimer has been observed with *o*-iodoxybenzoic acid in *t*-BuOH as solvent and observed selectivities were consistent with the reactivity pattern predicted by Fukui indices. With these results a protection-free route for synthesis of ursodeoxycholic acid from cholic acid can be envisaged.

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