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# Efficient and selective microwave Oppenauer oxidation of sterol derivatives.

Sophie Négrel<sup>1</sup> and Jean Michel Brunel<sup>1\*</sup>

<sup>1</sup> Aix Marseille Univ, INSERM, SSA, MCT, Marseille, France. E-mail : bruneljm@yahoo.fr

## Abstract

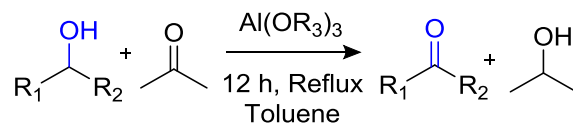
An improved, fast, and accurate procedure for regioselective microwave Oppenauer oxidation of sterol derivatives is reported leading to the expected corresponding ketosterols in moderate to excellent isolated yields ranging from 58 to 81%. The influence of different parameters dealing with the nature of the solvent and of the Lewis acid/ketone couple used, the reaction temperature as well as the hydrolysis conditions were also investigated on the outcome of the reaction.

**Keywords:** Oppenauer reaction – Microwave reaction – Ketosterol derivatives

## Introduction

The oxidation of alcohols to their corresponding carbonyl product is one of the most fundamental and important reactions encountered in organic synthesis. Different methods were described in literature as Jones oxidation with chromium trioxide and sulfuric acid<sup>1</sup> or Swern oxidation involving oxalyl chloride, dimethyl sulfoxide and triethylamine<sup>2</sup>, among others. Nevertheless, these methods lead to considerable drawbacks such as high cost, toxicity, and production of large amounts of waste and the reactions are often performed in environmentally undesirable solvents such as chlorinated hydrocarbons.<sup>3</sup> On the other hand, the selectivity of such methods appears quite limited when several hydroxyl functions are present. In this context, oxidized sterol derivatives are of considerable interest because of their possible involvement in regulation of cholesterol metabolism as well as their use as starting material for the synthesis of more complex compounds. Thus, to date, no efficient, cheap, and totally regioselective methods have been reported for the synthesis of such derivatives. Among them, the oxidation is preferentially achieved with silver carbonate which possesses the advantage to be regioselective but its cost and production of silver waste difficult to be recycled remains detrimental to perform the synthesis in high scale amount. On the other hand, Oppenauer reported in 1937 the oxidation of alcohols in the presence of aluminum

alkoxides and employing ketones as oxidants of primary and secondary alcohols leading to the corresponding aldehydes or ketones (Scheme 1)

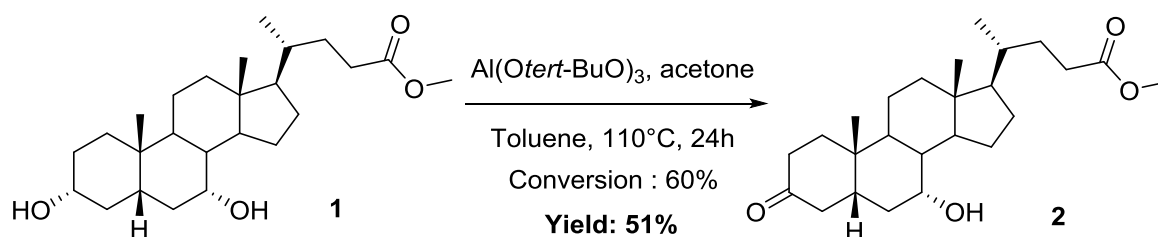


**Scheme 1.** General scheme of Oppenauer oxidation

Since its discovery, Oppenauer oxidation has been widely investigated<sup>4,5</sup> and nowadays this reaction can be performed with Lewis acid derived from aluminum<sup>6</sup>, magnesium<sup>7</sup>, boron<sup>8</sup> or transition metals<sup>9,10</sup>, either catalytically or in stoichiometric way, in solution, on solid support or in flow chemistry<sup>11</sup> and employing aldehydes or ketones as oxidants.<sup>6,8</sup> A great advantage of this reaction over many oxygen-transferring oxidation processes is that functional groups such as carbon-carbon double and triple bonds, aldehydes, amino groups, halogens or sulfur atom-containing groups are not affected.<sup>3</sup> Thus, this oxidation reaction has been successfully used for the oxidation of sterol into their corresponding ketosterol.<sup>12-15</sup> Nevertheless, this process is long (24 h) and costly requiring to be performed at high temperature (100°C) with low to moderate yields. Otherwise, during the last two decades, Microwave Assisted Organic Synthesis (MAOS) has attracted the attention of chemists who have applied this unconventional technique of material heating as a routine in their practice. Microwave irradiation has improved the optimization of synthetic conditions such as reduction reaction times from days and hours to minutes, better selectivity, yield improvements and limitation of side reactions compared to classical process used.<sup>16-20</sup> Despite the number of published work in this area, the exact reasons by which microwave irradiation can enhance chemical processes remain quite unknown.<sup>21-26</sup> Herein, we will report an improved, fast, and accurate procedure for regioselective microwave Oppenauer oxidation of sterol derivatives, and we will study the influence of the experimental conditions on the outcome of the reaction.

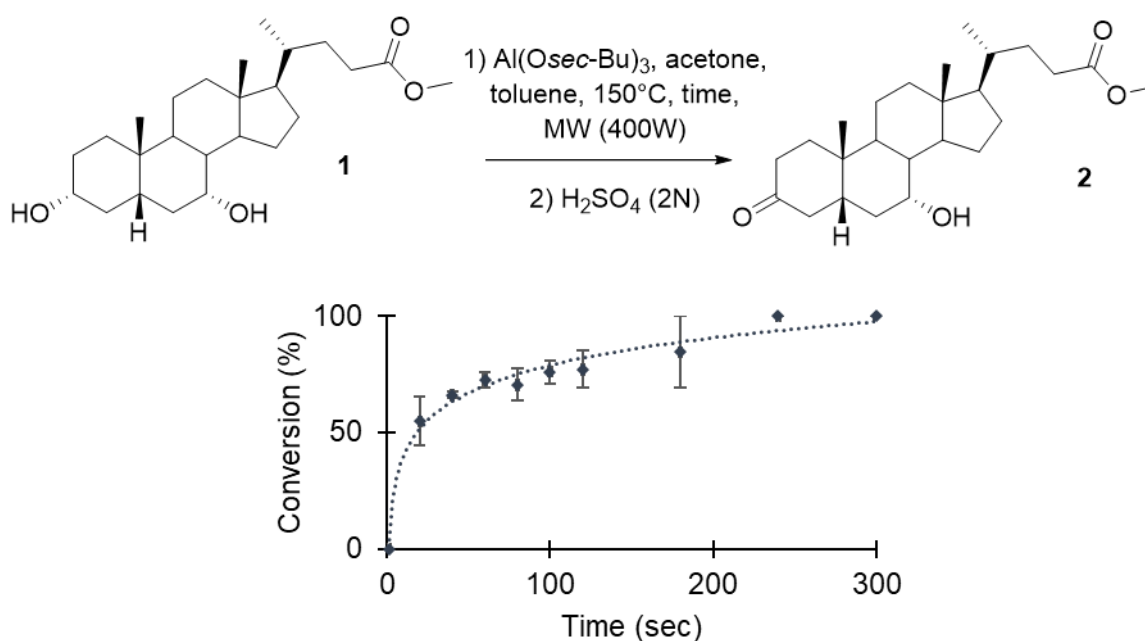
## Results and discussion

In a preliminary attempt, we performed the regioselective but incomplete (60% conversion) oxidation of methyl chenodeoxycholate **1** by using Oppenauer reaction in refluxing toluene for 24 h and affording methyl 3-oxo-chenodeoxycholate **2** in 51% isolated yield (Scheme 2).



**Scheme 2.** Classical Oppenauer oxidation of methyl chenodeoxycholate **1**

Nevertheless, it has been impossible to improve these results and to increase the conversion rate to more than 60% (48% isolated yield) which does not allow us to envision such a reaction for a high scale synthesis and from expensive starting materials. From these disappointing results, we have envisioned to perform such a reaction under microwave irradiation conditions. Thus, our first attempt investigated the oxidation of methyl chenodeoxycholate **1** under microwave irradiation (400W) in toluene and by using  $\text{Al(O}^{\text{sec}}\text{-Bu)}_3$ / acetone as Lewis acid/oxidant couple (Figure 1).

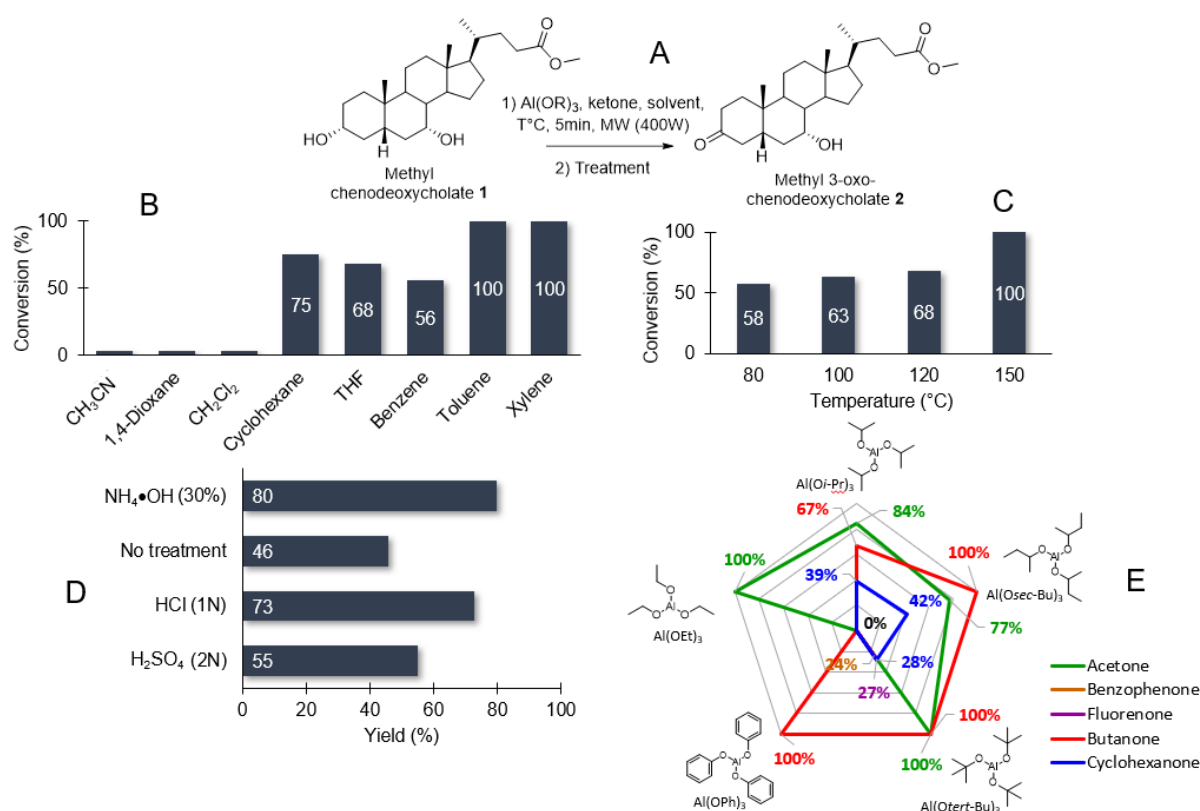


**Figure 1.** Kinetic study of Oppenauer oxidation of methyl chenodeoxycholate **1**

A kinetic study of this reaction indicates that a conversion of 55% is obtained after only 10 seconds demonstrating the efficiency of this methodology compared to classic heating conditions. Furthermore, the expected compound **2** was regioselectively obtained in 80% isolated yield and the reaction occurred with 100% conversion in only 5 minutes. The reasons of such an improvement are the different thermal management between microwave and conduction heating. Indeed, heating in microwaves ovens is based upon the ability of some liquids and solids to absorb and to transform electromagnetic energy into heat, to this end

temperature inside the crude mixture is homogenic<sup>27</sup> whereas conduction is based on a heating transfer by contact between two areas with different temperatures leading to a non-homogenic temperature of reaction. Thus, microwave interactions lead to thermal effect and “specific microwave effect” which is non-purely thermal effect.<sup>21,22,28,29</sup>

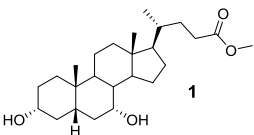
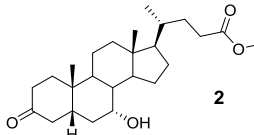
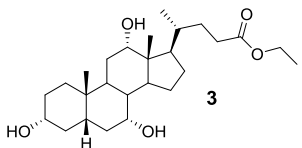
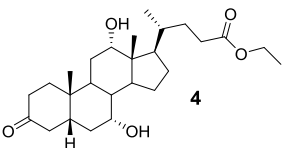
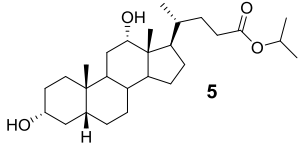
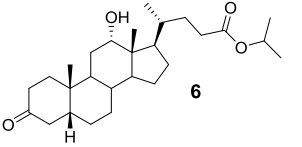
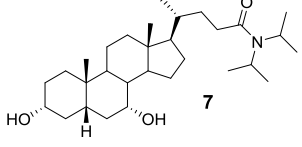
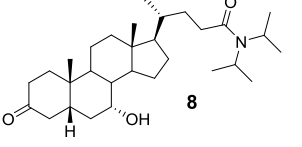
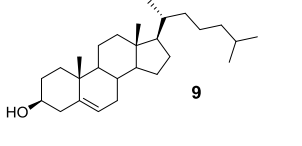
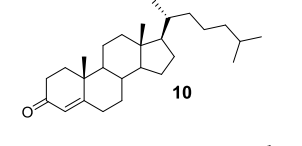
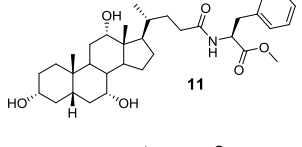
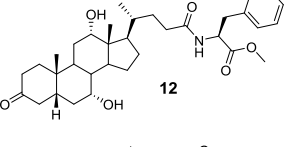
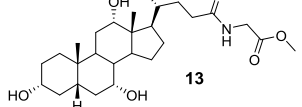
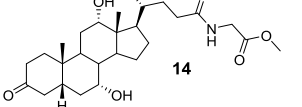
On the other hand, we investigated the influence of different parameters dealing with the nature of the solvent and the Lewis acid/ketone couple used, the reaction temperature as well as the hydrolysis conditions on the outcome of the Oppenauer reaction performed on **1** for 5 minutes (Figure 2).

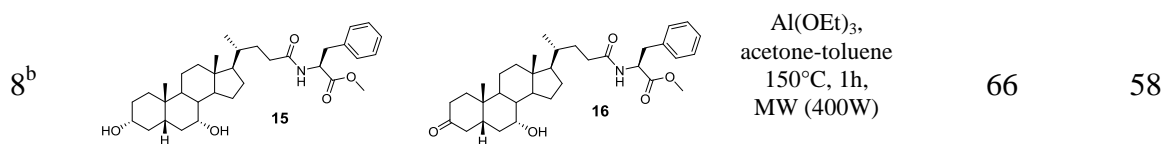


**Figure 2.** (A) Evaluation of the influence of different parameters on the outcome of the Oppenauer oxidation of methyl chenodeoxycholate **1**. (B) Influence of the nature of the solvent at 150°C and of the temperature (C) on the conversion of the reaction performed on 100 mg of **1** with Al(Osec-Bu)<sub>3</sub> and acetone for 5 min. (D) Influence of the nature of the aluminum alkoxides and ketones on the conversion of the reaction (performed on 60 mg of substrate in toluene (5 min reaction time)). (E) Influence of the work-up carried out after the reaction (performed on 100 mg of substrate in xylene with Al(Osec-Bu)<sub>3</sub> and acetone for 5 min).

It clearly appears that the nature of the solvent used has a dramatical effect on the conversion varying from 0 to 100% (Figure 2A). Thus, aromatic solvents such as benzene, toluene, and xylene lead to conversions of 56, 100 and 100%, respectively whereas no conversion was observed by performing the reaction in chlorinated solvents such as dichloromethane or acetonitrile. On the other hand, the influence of the temperature is

important since the better results are obtained at 150°C leading in this case to a 100% optimal conversion (Figure 2B). Furthermore, the nature of the aluminum alkoxide and of the ketone involved demonstrated a strong influence on the conversion as illustrated in Figure 2C. It is noteworthy that the acetone/ Al(OEt)<sub>3</sub> or Al(Osec-Bu)<sub>3</sub> and butanone/ Al(OPh)<sub>3</sub> or Al(Otert-Bu)<sub>3</sub> couples lead to 100% conversion whereas fluorenone, benzophenone or cyclohexanone appeared detrimental to perform this reaction. It is also noteworthy that employing theoretically more efficient oxidants such as benzaldehyde or amyl aldehyde no conversion occurs. Otherwise, the influence of the quenching experimental conditions was evaluated with an increased isolated yield by performing the hydrolysis with NH<sub>4</sub>OH (32%) or HCl (1N). Additionally, performing the reaction with various polyhydroxysterol derivatives afforded in all cases the expected regioselectively oxidized compounds in position C-3 in good to excellent chemical yields varying from 58 to 81% (Table 2).

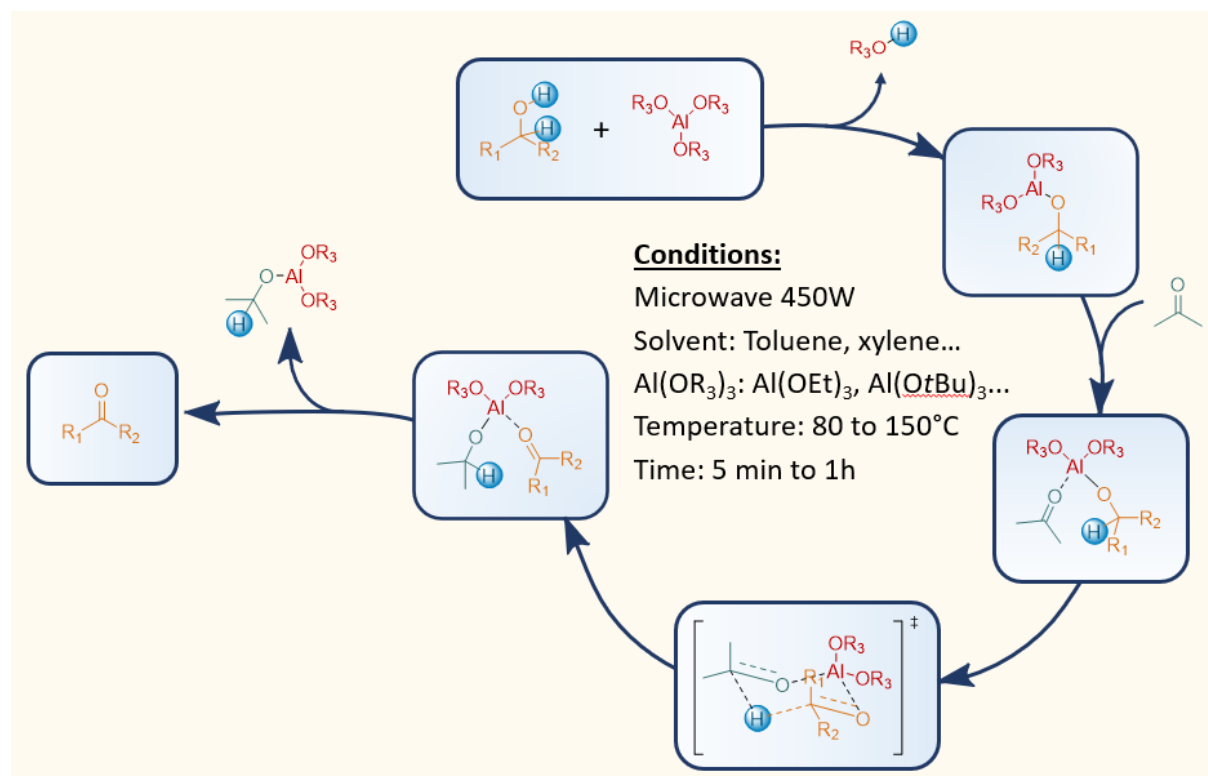
| Entry          | Starting material   | Product   | Reaction conditions   | Conversion (%) | Yield (%) |
|----------------|---|---|---|----------------|-----------|
| 1 <sup>a</sup> |   |   | Al(OEt) <sub>3</sub> ,<br>acetone-toluene<br>150°C, 1h,<br>MW(400W)         | 100            | 80        |
| 2 <sup>b</sup> |  |  | Al(Osec-Bu) <sub>3</sub> ,<br>acetone- xylene<br>150°C, 1h,<br>MW(400W)     | 100            | 59        |
| 3 <sup>b</sup> |  |  | Al(Osec-Bu) <sub>3</sub> ,<br>acetone- xylene<br>150°C, 1h,<br>MW(400W)     | 80             | 59        |
| 4 <sup>b</sup> |  |  | Al(Osec-Bu) <sub>3</sub> ,<br>acetone- xylene<br>150°C, 1h,<br>MW(400W)     | 100            | 72        |
| 5 <sup>c</sup> |  |  | Al(Osec-Bu) <sub>3</sub> ,<br>acetone- xylene<br>150°C, 1h,<br>MW(400W)     | 70             | 64        |
| 6 <sup>a</sup> |  |  | Al(Osec-Bu) <sub>3</sub> ,<br>acetone-toluene<br>150°C, 10 min,<br>MW(400W) | 100            | 81        |
| 7 <sup>a</sup> |  |  | Al(Osec-Bu) <sub>3</sub> ,<br>acetone-toluene<br>150°C, 10 min,<br>MW(400W) | 100            | N. D.     |



**Table 2.** Microwave Oppenauer oxidation performed on several sterols. Hydrolysis conditions (a) NH<sub>4</sub>•OH (30%); (b) H<sub>2</sub>SO<sub>4</sub> (2N), NaHCO<sub>3</sub> (10%); (c) H<sub>2</sub>SO<sub>4</sub>, EtOAc.

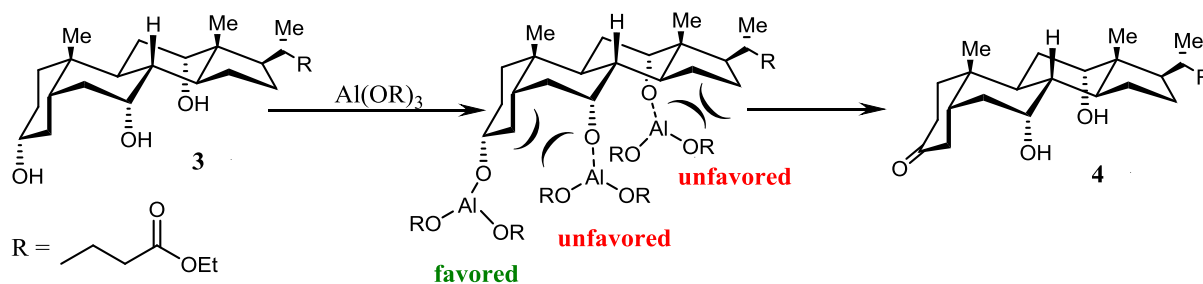
To demonstrate that this reaction occurred in a total regioselective manner a <sup>13</sup>C NMR spectrum analysis of a crude mixture of a microwave Oppenauer reaction performed on ethyl ester cholic acid **3** as substrate test was realized leading to the conclusion that exclusive formation of ethyl-3-oxo-cholate **4** was encountered without the formation of any detectable side product (see supplementary information, Figure S1).

Finally, a mechanism of reaction proceeding via a complex in which both the hybrid-acceptor carbonyl group and the alcohol are bounds to the metal ion was postulated to account for the observed regioselectivity of the oxidation. To conclude on the real nature of the species involved in such a reaction, <sup>1</sup>H and <sup>13</sup>C NMR experiments were attempted. Nevertheless, despite all our efforts, we have been unable to notice the formation of the postulated aluminum complexes and demonstrate the existence of intermediates such as those postulated in Figure 3.



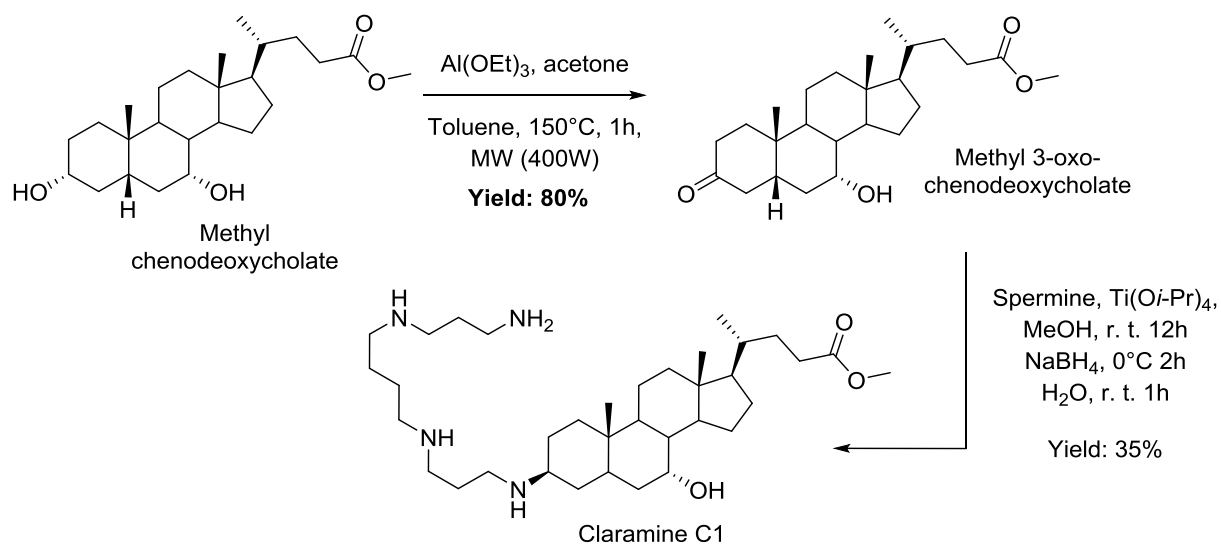
**Figure 3.** Possible mechanism involved for the Oppenauer oxidation

Nevertheless, the encountered regioselectivity of the reaction could be explained by considering ethyl ester cholic acid **3** as substrate test for which it clearly appears that oxidation of the hydroxyl group at position C7 and C12 is highly unfavored due to important steric hindrance by contrast to the position C3 easier to access as illustrated in Figure 4. Indeed, this reaction is under fast equilibrium and under thermodynamic control; hence the repartition of product is only dictated by the relative stability of products which can be justified looking at the conformation of the oxidized product.



**Figure 4.** Supposed reaction intermediates rationalizing the preferred formation of **4**

Then, in all our examples the thermodynamic product is favored under microwave heating and the microwave processing seems to be easily scaled and reproducible as exemplified for the synthesis of the antimicrobial agent Claramine C1<sup>30</sup> involving an oxidation key step of methyl chenodeoxychola-*L*-phenylalaninate **15** in 80% isolated yield. As outlined in Figure 5 Claramine C1 is obtained in a 3 steps synthesis and is active against both Gram-positive and Gram-negative bacteria with Minimum Inhibitory Concentrations (MIC) ranging from 4 to 32  $\mu\text{g/mL}$ .



| MIC ( $\mu\text{g/mL}$ ) | IC <sub>50</sub> ( $\mu\text{M}$ ) |
|--------------------------|------------------------------------|
|--------------------------|------------------------------------|

|                               |                                 |                             |                                   |     |
|-------------------------------|---------------------------------|-----------------------------|-----------------------------------|-----|
| <i>S. aureus</i><br>ATCC25923 | <i>E. faecalis</i><br>ATCC29212 | <i>E. coli</i><br>ATCC25922 | <i>P. aeruginosa</i><br>ATCC27853 | CHO |
| 4                             | 8                               | 32                          | 32                                | 41  |

**Figure 5.** Chemical synthesis of Claramine C1 using microwave irradiation. Minimal Concentration Inhibition of Claramine C1 against Gram-positive and Gram-negative bacteria as well as half-maximal Inhibitory Concentration against Chinese Hamster Ovary cells

## Conclusion

The microwave Oppenauer system reported here is simple to prepare and easy to use for the efficient oxidation of various sterol derivatives. This system is also unique as it succeeds to oxidize selectively an alcohol in position 3 of a sterol compound. To the best of our knowledge, there is no such report on a simple microwave aluminum system that alone can differentiate different types of alcohols bounded to a sterol moiety and afford in quantitative yields exclusively the expected C-3 oxidized compounds.

## Acknowledgments

S. N. thanks Biosqual company for a CIFRE PhD grant.

## Experimental section

All solvents were purified according to reported procedures, and reagents were used as commercially available. Methanol, ethyl acetate, dichloromethane, ammonia, and petroleum ether (35–60°C) were purchased from Sigma-Aldrich and used without further purification. Column chromatography was performed on Macherey-Nagel silica gel (70–230 mesh). <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker AC400 spectrometers working at 400 MHz and 100 MHz, respectively (the usual abbreviations are used: s: singlet, d: doublet, t: triplet, q: quadruplet, m: multiplet). The chemical shifts (ppm) were determined relative to Me<sub>4</sub>Si (<sup>1</sup>H and <sup>13</sup>C). Microwave reactions were performed by using a Biotage Initiator + apparatus.

## Kinetic study of Oppenauer oxidation of methyl chenodeoxycholate 1

In a 0.5-2 mL microwave reaction vial were introduced in 1 mL of toluene and 0.6 mL of acetone, 20 mg of methyl chenodeoxycholate **1** (0.049 mmol) and 2 equivalents of aluminum *sec*-butoxide (24 mg, 0.098 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (400 Watt) at 150 °C, during

various times (20 seconds, 40 seconds, 60 seconds, 80 seconds, 100 seconds, 120 seconds, 180 seconds, 240 seconds, and 300 seconds), using a normal mode and a pre-stirring of 20 seconds. 0.8 mL of  $\text{NH}_4\bullet\text{OH}$  (30%) solution was added, and the mixture was stirred for 15 min. The reaction mixture was filtered over a pad of Celite, rinsed with  $\text{CH}_2\text{Cl}_2$  then concentrated *in vacuo*. The  $^{13}\text{C}$  NMR spectra of the crude reaction were recorded, and the conversion was estimated by integrating the area under the  $\text{C}=\text{O}$  and  $\text{CHOH}$  signals.

### Synthesis of methyl 3-oxo-chenodeoxycholate 2

In a 10-20 mL microwave reaction vial were introduced in 10 mL of toluene and 6 mL of acetone, 200mg of methyl chenodeoxycholate **1** (0.49 mmol) and 2 equivalents of aluminum ethoxide (160 mg, 0.98 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (400 Watt) at 150 °C for 1 hour using a normal mode and a pre-stirring of 20 seconds. 2 mL of  $\text{NH}_4\bullet\text{OH}$  (30%) solution was added, and the mixture was stirred for 15 min. The reaction mixture was filtered over a pad of Celite, rinsed with  $\text{CH}_2\text{Cl}_2$  then concentrated *in vacuo*. The expected methyl 3-oxo-chenodeoxycholate **2** was successfully obtained as yellow oil in 80% yield after filtration.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 4.00 (m, 1H), 3.75 (s, 3H), 2.26-1.02 (m, 33H), 0.79 (s, 3 H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) :  $\delta$  (ppm) = 213.33, 174.63, 68.18, 55.68, 51.38, 50.17, 45.51, 43.12, 42.57, 39.40, 39.24, 36.83, 36.71, 35.23, 35.19, 33.80, 33.13, 30.85, 28.03, 23.53, 21.82, 20.86, 18.15, 11.67. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{NH}_4]^+$ ) for  $\text{C}_{25}\text{H}_{44}\text{NO}_4^+$  : calcd 422.3265, found 422.3263.

### General procedure applied for the synthesis of derivatives 4, 6, 8

#### Synthesis of ethyl 3-oxo-cholate 4

In a 10-20 mL microwave reaction vial were introduced in 11 mL of xylene and 6 mL of acetone, 175mg of ethyl cholate **3** (0.40 mmol) and 2 equivalents of aluminum *sec*-butoxide (230 mg, 0.93 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (400 Watt) at 150 °C for 1 hour using a normal mode and a pre-stirring of 20 seconds. 5 mL of  $\text{H}_2\text{SO}_4$  (2N) solution was added and the mixture was stirred for 15 min. The organic phase was washed with 5mL of  $\text{NaHCO}_3$  (10%). The aqueous phases were extracted twice with 5mL of dichloromethane and the combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated *in vacuo* to afford the expected product ethyl 3-oxo-cholate **4** as a yellow oil in 59% yield. NMR  $^1\text{H}$  ( $\text{CDCl}_3$ ):  $\delta$

(ppm) = 4.92 (m, 1H), 4.05-3.78 (m, 2H), 3.36-3.18 (m, 4H), 2.26-0.81 (m, 34H), 0.60 (m, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) :  $\delta$  (ppm) = 213.76, 174.51, 73.00, 68.34, 60.33, 47.32, 46.66, 45.60, 43.09, 41.80, 39.53, 36.85, 36.69, 35.32, 34.99, 33.99, 31.44, 30.91, 28.65, 27.55, 27.18, 23.25, 21.69, 17.40, 14.32, 12.60. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{NH}_4]^+$ ) for  $\text{C}_{26}\text{H}_{46}\text{NO}_4^+$  : calcd 422.3371, found 422.3368.

### Synthesis of isopropyl 3-oxo-deoxycholate **6**

Performed according the same procedure than involved for the synthesis for **4**. The crude mixture was purified by chromatography on silica gel using petroleum ether-ethylacetate (1:1) as eluent. The expected isopropyl 3-oxo-deoxycholate **6** was obtained as a yellow oil in 59% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 5.00 (m, 1H), 4.04 (m, 1H), 2.46-2.12 (m, 4H), 2.06-0.96 (m, 35H), 0.71 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) :  $\delta$  (ppm) = 212.74, 173.57, 72.98, 67.34, 48.22, 47.59, 46.70, 44.30, 42.33, 37.04, 36.92, 35.87, 35.05, 34.45, 34.11, 31.76, 31.00, 29.06, 27.40, 26.64, 25.55, 23.58, 22.38, 21.8. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{NH}_4]^+$ ) for  $\text{C}_{27}\text{H}_{48}\text{NO}_4^+$  : calcd 450.3578, found 450.3575.

### Synthesis of N, N-diisopropyl-3-oxo-chenodeoxycholamide **8**

Performed according the same procedure than involved for the synthesis for **4**. The crude mixture was purified by chromatography on silica gel using petroleum ether-ethylacetate (1:1) as eluent. The expected N, N-diisopropyl-3-oxo-chenodeoxycholamide **8** was obtained as a yellow oil in 72% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 4.08-3.88 (m, 2H), 3.43 (m, 1H), 2.41-0.90 (m, 45H), 0.65 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) :  $\delta$  (ppm) = 213.54, 172.60, 67.94, 55.72, 50.14, 45.55, 45.47, 43.25, 42.51, 39.37, 39.26, 36.84, 36.74, 35.52, 35.17, 33.81, 33.05, 32.03, 31.41, 28.12, 23.51, 21.81, 20.87, 20.53, 18.40, 11.64. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{30}\text{H}_{52}\text{NO}_3^+$  : calcd 474.3942, found 474.3942.

### Synthesis of cholest-4-ene-3-one **10**

In a 10-20 mL microwave reaction vial were introduced in 12 mL of xylene and 6 mL of acetone, 211mg of cholesterol **9** (0.54 mmol) and 2 equivalents of aluminum *sec*-butoxide (258 mg, 1.0 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (400 Watt) at 150 °C for 1 hour using a normal mode and a pre-stirring of 20 seconds. 3 mL of  $\text{H}_2\text{SO}_4$  (2N) solution was added and the mixture was stirred for 15 min. The aqueous phases were extracted with 3mL of ethyl acetate

and the combined organic phases were dried over  $\text{Na}_2\text{SO}_4$  and filtered. The mixture was concentrated *in vacuo* to afford the expected crude compound which was purified by chromatography on silica gel using petroleum ether-ethylacetate (9:1) as eluent. The expected cholest-4-ene-3-one **10** was obtained as a white solid in 64% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 5.71 (s, 1H), 2.41-2.27 (m, 4H), 2.04-1.99 (m, 2H), 1.84-1.80 (m, 2H), 1.67-1.28 (m, 11H), 1.17-0.97 (m, 12H), 0.91-0.83 (m, 10H), 0.70 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 199.76, 171.81, 123.86, 56.23, 56.00, 53.94, 42.51, 39.75, 39.62, 38.72, 36.24, 35.87, 35.82, 35.74, 34.11, 33.08, 32.17, 28.30, 28.27, 28.13, 24.30, 23.94, 22.94, 22.91, 22.68, 21.15, 18.77, 17.51, 12.08. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{27}\text{H}_{45}\text{O}^+$ : calcd 385.3465, found 385.3466.

### General procedure for the synthesis of derivatives **12** and **14**

#### Synthesis of methyl 3-oxo-chola-*L*-phenylalalaninate **12**

In a 10-20 mL microwave reaction vial were introduced in 10 mL of toluene and 6 mL of acetone, 299mg of methyl chola-*L*-phenylalalaninate **11** (0.52 mmol) and 2 equivalents of aluminum *sec*-butoxide (274 mg, 1.1 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (400 Watt) at 150 °C for 10 minutes using a normal mode and a pre-stirring of 20 seconds. 5 mL of  $\text{NH}_4\bullet\text{OH}$  (30%) solution was added, and the mixture was stirred for 15 min. Reaction mixture was filtered over a pad of Celite which was rinsed with  $\text{CH}_2\text{Cl}_2$ . The mixture was concentrated *in vacuo* to afford the expected methyl 3-oxo-chola-*L*-phenylalalaninate **12** obtained as a pale yellow oil in 81% yield after filtration.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 7.23-6.88 (m, 3H), 6.80-6.57 (m, 2H), 4.95 (s, 1H), 4.79 (s, 1H), 4.57 (q, 1H), 4.24- 3.74 (m, 4H), 3.65 (s, 2H), 3.31 (s, 4H), 3.24-2.98 (m, 2H), 2.85-2.35 (m, 6H), 2.24-1.45 (m, 20H), 0.68 (t, 3H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 216.26, 176.49, 173.69, 157.32, 131.10, 128.66, 116.23, 73.73, 68.70, 55.32, 52.60, 47.49, 46.55, 44.71, 42.89, 40.87, 37.74, 37.58, 36.94, 36.71, 35.96, 35.80, 35.01, 33.73, 33.07, 29.82, 29.57, 28.33, 26.25, 24.12, 22.15, 17.72, 13.07, 9.75. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{NH}_4]^+$ ) for  $\text{C}_{34}\text{H}_{50}\text{N}_2\text{O}_6^+$ : calcd 585.3898, found 585.3898.

#### Synthesis of methyl 3-oxo-chola-*L*-glycinate **14**

Performed according the same procedure than involved for the synthesis for **12**. The expected product methyl 3-oxo-chola-*L*-glycinate **14** was obtained as yellow oil in a quantitative yield.

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 5.11-4.60 (m, 11H), 4.06-3.68 (m, 5H), 2.63-1.49 (m, 24H), 0.73 (t, 3H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) :  $\delta$  (ppm) = 216.57, 177.31, 171.16, 73.85, 68.79, 48.06, 47.60, 46.62, 44.92, 43.02, 42.25, 40.98, 36.89, 36.08, 35.05, 33.79, 33.14, 29.81, 28.71, 28.38, 24.16, 22.13, 22.00, 19.70, 17.70, 13.03, 9.98. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{27}\text{H}_{45}\text{NO}_6^+$  : calcd 478.3124, found 478.3162.

### Synthesis of methyl 3-oxo-chenodeoxychola-*L*-phenylalalinate **16**

In a 10-20 mL microwave reaction vial were introduced in 10 mL of toluene and 6 mL of acetone, 250 mg of methyl chenodeoxychola-*L*-phenylalalinate **15** (0.45 mmol) and 4 equivalents of aluminum *sec*-butoxide (291 mg, 1.8 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (400 Watt) at 150 °C for 1 hour using a normal mode and a pre-stirring of 20 seconds. 5 mL of  $\text{H}_2\text{SO}_4$  (2N) solution was added and the mixture was stirred for 15 min. The organic phase was washed with 5mL of  $\text{NaHCO}_3$  (10%). The aqueous phases were extracted twice with 5mL of dichloromethane and the combined organic phases were dried over  $\text{Na}_2\text{SO}_4$  and filtered. The mixture was concentrated *in vacuo* to afford the expected crude compound which was purified by chromatography on silica gel using petroleum ether-ethylacetate (1:1) as eluent. The expected product methyl 3-oxo-chenodeoxychola-*L*-phenylalalinate **16** was obtained as a yellow oil in 58% yield.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 7.49-7.37 (m, 5H), 5.61 (s, 1H), 3.95-3.93 (m, 1H), 3.77 (s, 3H), 3.60 (q, 1H), 2.73-0.94 (m, 35H), 0.82-0.75 (m, 3H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) :  $\delta$  (ppm) = 215.95, 176.04, 172.52, 137.45, 129.81, 129.43, 128.69, 68.65, 57.97, 57.22, 52.95, 51.34, 46.52, 44.66, 43.59, 42.12, 40.82, 40.60, 37.87, 37.66, 36.68, 36.31, 35.05, 34.42, 33.43, 29.53, 29.15, 24.52, 22.42, 22.09, 18.98, 18.15, 15.11, 12.34. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{34}\text{H}_{50}\text{N}_2\text{O}_5^+$  : calcd 569.3949, found 569.3953.

### Synthesis of 3-spermino methyl chenodeoxycholate (Claramine C1)

In a 100 mL two necked round flask was introduced methyl 3-oxo-chenodeoxycholate **2** (3.5 g, 8.6 mmol) dissolved into 50 mL of methanol. 4 equivalents of titanium (IV) isopropoxide (10 mL, 34 mmol), then 3 equivalents of spermine (5.3 g, 26 mmol) were subsequently introduced. The mixture was stirred at room temperature for 24 hours. After cooling at 0°C, 4 equivalents of sodium borohydride (1.3 g, 34 mmol) were then added, and the resulting mixture was stirred for additional 2 hours. The reaction was then quenched by adding water (4 mL). Stirring was continued at room temperature for 1 hour then the reaction mixture was

filtrated over a pad of Celite which was subsequently rinsed with  $\text{NH}_4\bullet\text{OH}$  (32%) and methanol. The mixture was concentrated *in vacuo* to afford the expected crude compound which was purified by flash chromatography on silica gel using  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\bullet\text{OH}$  (32%) 7:3:1 as eluent. The expected Claramine C1 was obtained as a viscous yellow oil in 35% yield.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 3.84 (m, 1H), 3.69 (s, 2H), 3.02-3.44 (m, 3H), 2.92-2.35 (m, 14H), 2.03-2.31 (m, 3H), 0.98-1.98 (m, 40H), 0.74 (s, 3H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) :  $\delta$  (ppm) = 176.53, 69.02, 59.43, 577.66, 52.06, 51.08, 48.28, 45.83, 45.72, 43.90, 43.64, 41.28, 41.07, 40.70, 40.64, 36.82, 36.72, 36.02, 34.35, 32.46, 32.15, 31.70, 29.27, 27.92, 24.73, 23.56, 21.96, 18.98, 12.37. HRMS (ESI-TOF):  $m/z$  ( $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{35}\text{H}_{67}\text{N}_4\text{O}_3^+$  : calcd 591.5168, found 591.5169.

### **Determination of minimal inhibitory concentrations**

Antimicrobial activity of the compounds was studied by determination of minimal inhibitory concentrations (MIC) according to the NCCLS guidelines M7-A2 using the microbroth dilution methods. The bacteria strains were grown on trypticase soy agar (Becton Dickinson) at 37 °C for 24 h. Inocula were prepared in TCE (tryptone 0.1%, NaCl 8%, wt/vol) by adjusting the turbidity at 623 nm to obtain  $1-3 \times 10^5$  CFU/mL. Antimicrobial activities of the Claramine C1 were determined by using a broth microdilution method performed in sterile 96-well microplates. The compound was solubilized in water at a concentration of 10 mM and were transferred to each microplate well in order to obtain a two-fold serial dilution in 100  $\mu\text{L}$  of broth and 100  $\mu\text{L}$  of inoculum containing  $2-6 \times 10^5$  CFU of each bacterium were added to each well. Some wells were reserved for positive controls and inoculum viability. After 24 h incubation, MIC was defined for each agent from duplicate observations as the lowest concentration of compound allowing no visible growth.

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