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Two-step continuous-flow synthesis of α-terpineol

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Keywords: Continuous-flow, α-terpineol, cascade reactions.

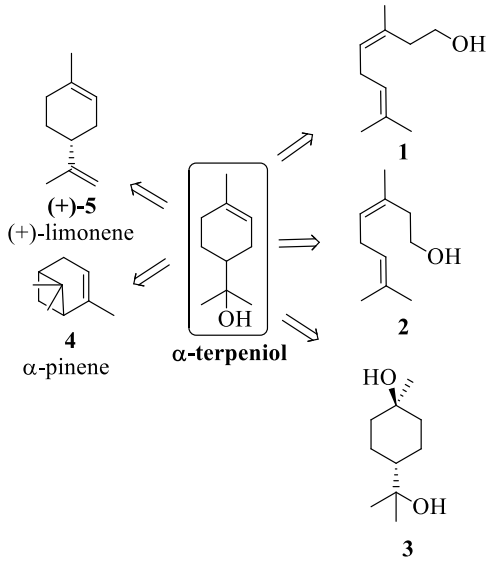
Abstract:

α-Terpineol is a monoterpene naturally present in essential oils, of high value on the market as it is a compound widely used as a flavoring, aromatic substance in the cosmetics and food industry. This study aims to produce α-terpineol by two different synthetic strategies, using both batch and continuous flow systems, focusing on the optimization of the process, improving the reaction conversion and selectivity. The first strategy adopted was a one-stage hydration reaction of α-pinene by an aqueous solution of chloroacetic acid (molar ratio 1:1 between pinene and the acid) in continuous flow conditions. This reaction was carried out at 80 ºC with a residence time of 15 min, obtaining good values of conversion (72 %) and selectivity (76 %), and productivity of 0.67 $Kg.day^{-1}$. The second strategy accomplished was a two-step cascade reaction with limonene as starting material, where the first step is a chemo specific double bond addition using trifluoroacetic acid, and the second step is the basic hydrolysis of the ester promoted by a solution of sodium hydroxide (2.25 M) in methanol (1:1). This reaction was adapted to a continuous flow condition, where all steps happen in a residence time of 40 min, at 25 ºC, with no quenching between steps required, giving a conversion of 97 % and selectivity of 81 %, with productivity of 0.12 $Kg.day^{-1}$.

1. Introduction

Over the years flavor and fragrances sector has been growing in all its applications and nowadays it represents a multi-billionaire global market. The growing global industrialization has led to the massive production of processed food, beverages, personal care products, detergents, cleaning products and soaps, which shows the industry's necessity to produce scented or flavored products. Thereby, such a high demand for natural products in this particular area could be seen as a disadvantage because of the fluctuating prices of raw materials. To outline the problem and continue expanding the market, scientific innovations where needed to deliver synthetic fragrances and flavors.[1–3]

α-Terpineol is a high value monoterpene naturally present in essential oils widely used as a flavoring aromatic substance. Likewise, it is also used as an anti-fungal agent, as a disinfectant in cleaning commodities,[4] as a fine chemical building block,[5] and has antibacterial[6] and antitumoral activities.[7,8] Consequently, there is an intense search for more effective synthetic ways to obtain α-terpineol.[4,9] Different methods have been described in the literature, using both monoterpenes and oxygenated terpenes as starting materials under acidic conditions (Scheme1).[4,9–13]



Scheme 1: Different starting materials for α-terpineol synthesis.

In this context, the development of chemo selective synthesis of α-terpineol has, as main challenge, to avoid degradation and isomerization products which current leads to low yield and selectivities.[14] On the other hand, the adoption of continuous flow technology for the synthesis of natural product on safer and more efficient conditions has become popular. Providing better control on reaction parameters such as mixing, mass and heat transfer and on-line purification, downstream processing help minimize solvent usage, waste and manual handling.[15–17] In this context, our research group[16,18,19] have been involved in the development of flow chemistry methodologies for organic synthesis and biocatalysis[15,16] and here in we report our effort on optimizing α-terpineol synthesis starting from readily available starting materials (limonene and α-pinene) by the use of continuous flow technology.[10,20,21]

2. Material and Methods

2.1. Materials

Reagents were purchased from different sources and used without further purification: Limonene 98 % from ER do Brasil and α-pinene 98 % from Alfa Aesar. Chloroacetic acidfrom Vetec, trifluoracetic acid from Sigma-Aldrich. Cyclohexane and methanol were purchased from Tedia.

^{1}H-NMR was recorded on a Bruker Advance 500 MHz spectrometer. Reported chemical shifts (δ) are expressed in parts per million (ppm) down field from tetra methyl silane (TMS).

2.2. Chromatography Analysis

Samples were prepared by stirring 15 μL of reaction crude and 985 μL of ethyl acetate. Conversion percentages were analyzed by chromatogram areas using the Shimadzu GC2010 GC-MS - SLB-5MS column 30 meters. Injection temperature 250 °C, injection split ratio 50.0, carrier gas was He, pressure 100.0 kPa, column flow 1.0 mL / min. The oven temperature setting

was: 80 °C for 5 min, heated at 10 °C / min to 150 °C and remained for 4 min then heated at 30 ºC / min to 275 ºC and remained for 2 min. Conversion percentages were analyzed by chromatogram area. Mass ion source temperature 250 °C, interface temperature 280 °C, solvent cut time 3 min.

2.3. Experimental Section

2.3.1 Batch synthesis of α-terpineol from α-pinene

To a 4 ml flask was added a mixture of α-pinene (1.58 mL, 10 mmol) in water (0.4 ml), the mixture was stirred and heated to 70 °C. Then, chloroacetic acid (0.94 mL, 10 mmol) was added and the reaction lasted 4 h. The reaction was monitored using thin layer chromatography with a mixture of ethyl acetate/ hexane 30 % as eluent. Then, the reaction mixture was diluted in 10 ml of ethyl acetate, washed with a 20 % K_2CO_3 solution (10 mL). The aqueous phase was re-extracted with ethyl acetate (3 x 10 mL) and the organic phases were combined, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated under reduced pressure. Purification was performed on a flash silica column chromatography using ethyl acetate: hexane 20 % as eluent. The product was obtained as pale yellow oil with 42 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (s, 1H), 2.00 (dd, *J* = 45.7, 17.4 Hz, 3H) 1.87 (m, *J* = 12.3 Hz, 1H), 1.83 – 1.73 (m, 1H), 1.64 (s, 3H), 1.48 (t, *J* = 14.3 Hz, 1H), 1.25 (dd, *J* = 11.8, 5.7 Hz, 2H), 1.17 (d, *J* = 8.4 Hz, 6H). ^{13}C NMR: ($CDCl_3$, 126 MHz) δ 133.9 (C), 120.6 (CH), 72.7 (C-OH), 45.0 (CH), 31.0 (CH_2), 27.4 (CH_3), 26.9 (CH_2), 26.5 (CH_3), 23.9 (CH_2), 23.3 (CH_3).

2.3.2. Continuous flow synthesis of α-terpineol from α-pinene

In a flow line, α-pinene flow through backflow regulator (Swagelok SS-4C-1/3) and is mixed with a second stream of an aqueous solution chloroacetic acid (27 mol. L^{-1}) into a T-mixer. The combined stream then flows through homemade static mixer (stainless column filled

with glass wool 100 mg) and reacted into PTFE reactor coil 16 mL (diameter 0.01 mm) externally heated to 80 ºC during 15 min. The reagents were pumped to maintain a 1:1 molar reaction between acid and substrate the total flow was 1.12 mL/min, being 60 % of the flow rate from the α-pinene (0.68 mL/min) and 40 % of the flow rate from the 27 M acid solution (0.44 mL/min), resulting in conversion values of 72 % and 76 % selectivity.

2.3.3. Batch synthesis of α-terpineol from limonene

The α-terpineol synthesis using limonene as starting material was carried out in two steps.

1º step: α- Terpenyl Trifluoroacetate[22]

To a solution of limonene (1.62 mL, 10 mmol) in cyclohexane (10 mL), under constant stirring, trifluoroacetic acid (10 mmol, 0.76 mL) was added slowly at room temperature. After 1 h, the reaction mixture was diluted in 10 mL of ethyl acetate and washed with a 5 % $NaHCO_3$ solution (10 mL). The organic phase was separated, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated under reduced pressure. Crude α-terpenyl trifluoroacetate was obtained as light brown oil (1.6 g).

2º step: α-Terpineol[11,23]

A 50 mL flask was added to the crude terpenyl trifluoroacetate, obtained in the first step (1.0 g), and dissolved in methanol (2.5 mL). Then, a 4.5 M, aqueous solution of NaOH (2.5 mL) was added to the methanolic solution and stirred for 1 h. The reaction was monitored using thin layer chromatography with a mixture of ethyl acetate/ hexane 30 % as eluent. Then, an aqueous HCl solution (20 % v / v) was added slowly until the reaction mixture reached pH 8-9. Subsequently, methanol was evaporated under reduced pressure, followed by extraction of the crude solution in the ethyl acetate:hexane 20 % mixture. The organic phase was dried over

anhydrous sodium sulfate, filtered and the solvent was evaporated under reduced pressure. Purification of the product was performed by chromatography on a silica flash column with ethyl acetate: hexane 20 %. The product was obtained as pale yellow oil in 54 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (s, 1H), 1.97 (m, *J* = 5.2 Hz, 3H), 1.81 (m, *J* = 12.5, 5.1, 2.3 Hz, 1H), 1.77 – 1.68 (m, 1H), 1.58 (s, 3H), 1.47 – 1.38 (m, 1H), 1.18 (m, *J* = 24.1, 12.3, 5.8 Hz, 2H), 1.11 (s, 3H), 1.10 (s, 3H). ^{13}C NMR: ($CDCl_3$, 126 MHz) δ 133.0 (C), 120.6 (CH), 72.7 (C-OH), 44.9 (CH), 31.0 (CH_2), 27.4 (CH_3), 26.9 (CH_2), 26.2 (CH_3), 23.9 (CH_2), 23.3 (CH_3).

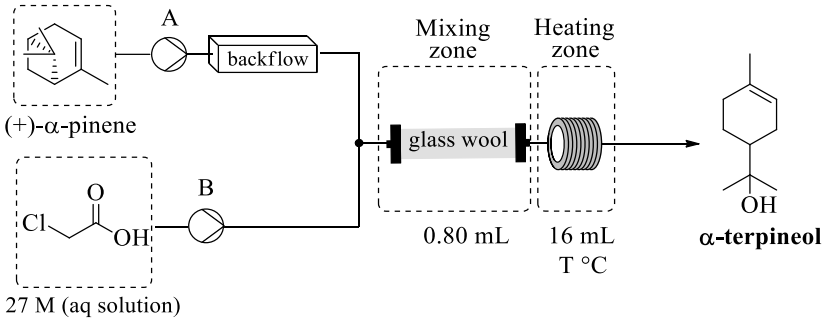
2.3.2. Continuous flow cascade reaction for the synthesis of α-terpineol from limonene

In a flow line, limonene was pumped through a backflow regulator (Swagelok SS-4C-1/3) and mixed with a second stream of trifluoroacetic acid (27 mol. L^{-1}) into a T-mixer. The combined stream then flows through homemade static mixer (stainless column filled with glass wool 100 mg) and reacted into stainless reactor coil 3 mL (diameter 0.1 mm) at room temperature during 8 min. After passing through a second backflow regulator (Swagelok SS-4C-1/3), an aqueous solution of sodium hydroxide (2.25 M) in methanol v/v (1:1) were mixed into a T-mixer integrated with through homemade static mixer (stainless column filled with glass wool 100 mg). The reaction was performed in a PTFA tubular reactor 30 mL (1.5 mm inner diameter) also at room temperature with residence time of 30 min. The entire system had a total flow of 1.06 mL/min, being the flow rate from limonene 0.14 mL/min, the flow rate from the TFA acid solution 0.06 mL/min and 0.86 mL/min from de NaOH/MeOH solution, resulting in conversion values of 97 % and 80 % selectivity.

3. Results and Discussion

We began our work evaluating batch reactions already described in literature for α-terpineol synthesis starting from readily available monoterpenes α-pinene and limonene. The reaction studied for α-terpineol synthesis starting from α-pinene was reproduced according to Román-Aguirre *et al* [4] (23 M Chloroacetic acid aqueous solution, 73 mmol substrate, 4 h at 70 ºC) leading to the desired product in 90 % yield and 58 % of selectivity. Further optimization of reaction parameters (reaction time, temperature, catalyst concentration) leaded us to a slightly reduction on reaction time (3 h) with an increase on selectivity (67 %) without yield reduction, at 70 ºC using a 27 mol. L^{-1} chloroacetic acid stock solution (supporting information, Table S1). After this initial assessment we decided to translated batch protocol to continuous-flow conditions connecting two syringe pumps (A: α-pinene and B: 27 M chloroacetic acid aqueous solution) through a T-mixer into a mixing zone (PBR filled with glass wool) and a reaction zone at temperatures between 70-90 ºC. Flow rates were adjusted in order to have a 1:1 mixture of reagents, according to the desired residence time. Results are found on table 1, and all conversion values were analyzed by a GC/MS considering the substrate, α-pinene, consumption.

Table 1: Results on continuous flow protocol using α-pinene as substrate.



Entry	T (ºC)	Res. time (min)	Conv. (%) ± SD*	Select. (%) ± SD*

1	70	60	48 ± 1.63	65 ± 0.94
2	70	30	60 ± 0.94	81 ± 5.91
3	80	60	68 ± 10.96	57 ± 2.45
4	80	30	73 ± 5.79	71 ± 0.82
5	80	15	72 ± 2.45	76 ± 1.25
6	90	30	84 ± 1.63	75 ± 2.16
7	90	15	72 ± 4.19	76 ± 1.70

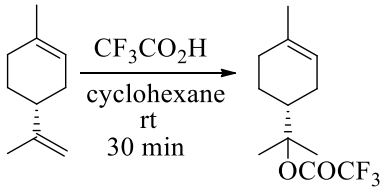
Reaction using α-pinene 98 %, chloroacetic acid aqueous solution (27 M). *All conversion and selectivity values were determined by GC/MS considering the substrate, α-pinene, consumption. Values were measured in triplicate; the medium value is reported, as well as the standard deviation (SD).

Under the conditions outlined on table 1, first experiments at 70 ^{o}C have shown that long residence time led to lower selectivity towards the desired product (Entries 1 and 2, Table 1). At this point, 30 min (Entry 2, Table 1) residence time already gave us moderate conversion (60 %) with high selectivity (81 %) and increasing reaction temperature to 80 and 90 ^{o}C, keeping residence time on 15 min, allowed a slight increase on reaction conversion with similar selectivity (Entries 5 and 7, respectively, Table 1). Under the best conditions found for α-terpineol synthesis (Entry 5, Table 1) a space-time-yield of 0.67 Kg. day^{-1} can be obtained.

As a second strategy we decided to evaluate the approach of starting from limonene for α-terpineol synthesis, which requires a two-step methodology consisted in an oxidation reaction mediated by trifluoroacetic acid followed by hydrolysis of the intermediate ester **6**. We have used the work of Mattos *et al*[22] as a starting point where the reaction was performed at room temperature for 1 h in cyclohexane. It was performed a previous optimization in batch condition evaluating the conversion and selectivity values in function of reaction time (supporting information, Table S2). These results showed that after 30 min of reaction time no further

enhancement on conversion and selectivity values were observed. Moreover, other experiments evaluating molar ratio condition of limonene and trifluoroacetic acid were also tested on batch condition (Table 2).

Table 2: Limonene as starting material for intermediate ester **6** synthesis.



(+)-5 **6**

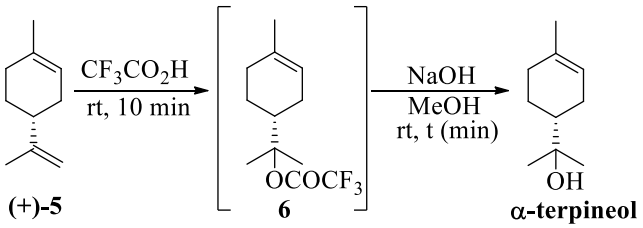
Entry	Molar ratio (5:acid)	Conv. (%)*	Select. (%)*
1	1:1	88	87
2	1:1.2	90	90
3	1:2	81	75
4	1:1 (no-solvent)	93	80

Reaction using limonene (10 mmol) and CF_3CO_2H 10 mmol (1:1), at room temperature, for 30 min. *All conversion and selectivity values were determined by a GC/MS considering the substrate, limonene, consumption.

In our experiments a small decrease on selectivity was detected when 1:2 molar ratio (limonene: trifluoroacetic acid) was used (Entry 3, Table 1). It is important to highlight that with the aim of finding a better condition to continuous-flow process, we decided to evaluate a solvent free reaction, giving excellent conversions and good selectivity's (Entry 4, Table 2). Considering preliminary results, this reactional condition has become a very interesting protocol for process intensification. Therefore, a study monitoring the reaction time on solvent-free condition allowed us to observe that after only 5 min 93 % of conversion was already achieved with very good selectivity, 89 % (supporting information, Table S3).

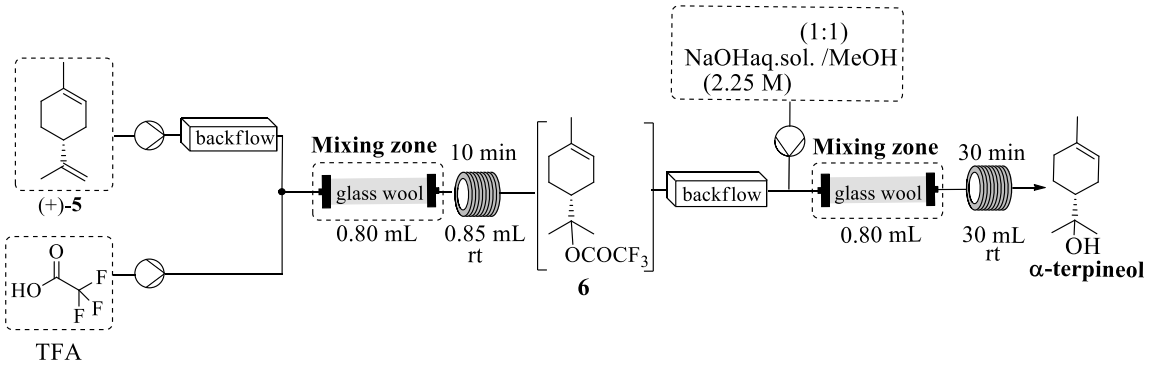
As mentioned before, in order to arrive at the desired product, we need to run a two-step reaction. Since the ester intermediate is very unstable in acidic media, a cascade batch process is needed to fully understand the potential of this solvent free approach (Scheme 2). Therefore,

the methanolic sodium hydroxide solution was added directly to the reaction media after the first step reaction time and samples were taken to follow product formation. After 40 min of total reaction time, the reaction has already reached maximum conversion (97 %) and excellent selectivity (93 %).



Scheme 2: Two-step cascade batch reaction of the α-terpineol synthesis.

With this results in hands we decided to move forward in order to translate batch protocol to a continuous-flow cascade process. Firstly, the reaction first step was study in flow conditions (supporting information, Table S4) and later on, the second step was assembled. The complete continuous-flow setup is shown on Scheme 3 and it is composed of three syringe pumps, two mixing zones and two reaction zones, both at room temperature. Residence time was also adjusted in order to fit equipment requirements. The conversion and selectivity values were determined by GC/MS considering the substrate limonene (**(+)-5**) consumption. Those values were measured in triplicate so we could obtain standard deviation values.



Scheme 3: Cascade continuous-flow setup for the synthesis of α-terpineol.

The continuous-flow cascade system starting from (+)-limonene (**(+)-5**) could reproduce similar conversions to the batch system (97 %, SD = 0.47 %) with a slightly decrease on selectivity (80 %, SD = 1.25), where changes on residence time could not allow better results. Residence time on the first step had a small change compared to the optimization protocol in order to have a flow rate where we could meet the second step requirements of residence time. For the second reaction, mixing is a crucial step, so we decided to have an extended residence time in order to accomplish the hydrolysis reaction. Space time yield obtained for this cascade process is 0.12 $Kg.day^{-1}$, lower than the one obtained for the continuous-flow strategy starting from α-pinene (**4**). The final compound can be easily purified by distillation from reaction crude mixture.

4. Conclusions

Based on the results presented, it was possible to develop two processes for the synthesis of α-terpineol in continuous flow. It was possible to carry out the synthesis of a-terpineol in continuous flow using α-pinene as starting material and chloroacetic acid in molar ratio 1:1, at 80 ºC with a total residence time of 15 min, obtaining good conversion values (72 % ± 2.45) and selectivity (76 % ± 1.25). These results proved to be much more interesting than those obtained in batch, where the reactions were carried out at 70 ºC for 4 h resulting in 88 % conversion and 67 % selectivity. Although the conversion value was higher for the batch reaction, in the continuous flow system the reaction time was reduced in 94 %, providing a huge increase in the efficiency of the reaction, resulting in a productivity of 0.67 $Kg.day^{-1}$ under the best conditions found.

For the two-step cascade reaction to the obtainment of α-terpineol starting from limonene, excellent conversion (97 % ± 0.47) and selectivity (80 % ± 1.25) results were presented. The

advantages of this reaction system were: the first step was carried out without solvent, the second was carried in aqueous solution, and the hole processes could be done at room temperature, and the total residence time was of 40 min. As described, in batch, the total reaction time was of 2.5 h and resulted in 56 % conversion and 81 % selectivity. The productivity of this flow system was 0.12 $Kg.day^{-1}$.

5. Acknowledgment

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