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Lipase-mediated flow synthesis of nature-inspired phenolic carbonates†

Sara Vicinanza, ‡[a] Francesca Annunziata, ‡[a] Desirèe Pecora, [a] Andrea Pinto [b] and Lucia Tamborini *[a]

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A facile and convenient lipase-catalyzed flow approach for the chemoselective synthesis of tyrosol and hydroxytyrosol methyl carbonates has been developed in neat dimethylcarbonate. The products were obtained in quantitative yield with high catalyst productivity. The biocatalytic approach was then exploited for the preparation of value-added symmetrical tyrosol and hydroxytyrosol carbonates.

Introduction

Organic carbonates are important classes of compounds and chemical intermediates, which are renowned for their great versatility, and high biodegradability and, consequently, are widely used for a variety of industrial and synthetic applications.[1,2] A plethora of methods have been reported for synthesizing non-symmetrical carbonates on a variety of substrates such as alcohols,[3] olefins[4,5] and epoxides,[6–8] either relying on metal-based catalysts[9–12] or organic metal-free catalysts.[13] However, carbonating agents such as phosgene and its derivatives, *e.g.*, chloroformates and 1,1-carbonyldiimidazole, are now not recommended because of their inherent toxicity,[14,15] as well as CO_2, whose thermodynamic stability and kinetic inertness limit its use,[16] despite its huge potential as carbonation reagent.

Therefore, the attention for the obtainment of alkyl carbonates is currently focused on the development of eco-compatible methodologies employing non-toxic and biodegradable chemicals.

In the last decades, food-derived phenolic compounds have raised great attention in the food, cosmetic and pharmaceutical sectors for their health-promoting effects such as antioxidant, metal chelator, free radical scavenger, antimicrobial and anti-inflammatory properties.[17,18] However, due to their solubility, metabolic/chemical stability and bioavailability issues, their applicability as active ingredients is still limited. With the aim to overcome this limitation, a growing interest has been devoted to the obtainment of more lipophilic derivatives.[19,20] For example, one of the most common strategies to increase their oil solubility and miscibility in emulsion-based systems is the esterification of phenolic compounds with medium or long aliphatic chains, preserving the –OH phenolic moieties, which are responsible of the desired bioactivity.[21] Other strategies include the incorporation of one or more halogen atoms[22] or the introduction of different aliphatic chains into the molecular skeleton.[23]

In this context, we explored the potential of flow biocatalysis for the sustainable synthesis of tyrosol (Ty) and hydroxytyrosol (HTy) carbonates (Fig. 1) characterized by increased lipophilicity compared to the parent phenolic compounds, which are natural antioxidants present in olive oil and wine. Besides the production of methyl carbonates **1** and **2**, we became particularly intrigued in the obtainment of symmetric carbonated **3** and **4**. Notably, compound **3** has been recently described as monomer of a new class of biodegradable, aromatic polycarbonates deriving from Ty.[24] The synthesis of compound **3** reported until now is far away from being environmentally friendly and sustainable, requiring the use of tyrosol chloroformate and triphosgene under inert atmosphere.[24,25]

Results and discussion

First, the chemoselective carbomethoxylation of the alcoholic function of Ty and HTy was performed under flow conditions using a cheap, non-toxic and green carboxymethylating agent as dimethyl carbonate (DMC),[26,27] exploited also as reaction medium, and Novozyme 435® as biocatalyst (imm-CaLB).[28,29] The carbomethoxylation reaction usually relies on the use of

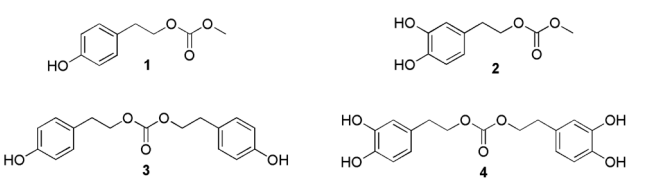

Fig. 1 Structures of the synthesized carbonates of Ty and HTy.

[a]*Department of Pharmaceutical Sciences (DISFARM), University of Milan, Via Mangiagalli 25, Milan 20133, Italy. E-mail: lucia.tamborini@unimi.it*

[b]*Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Via Celoria 2, Milan 20133, Italy*

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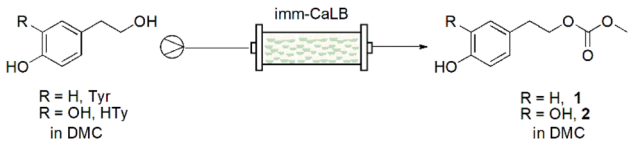
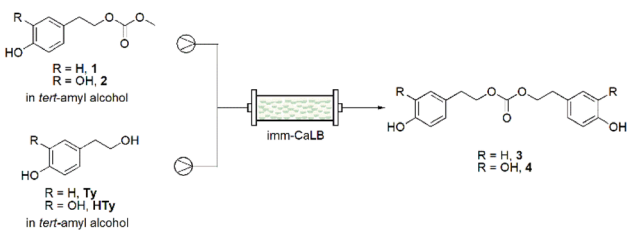
‡ These Authors equally contributed to the work.

Table 1 Biocatalyzed flow synthesis of nature-derived carbonates **1** and **2**



Entry[a]	C_{Ty}[b] (mM)	R_t (min)	T (°C)	c[c] (%)
1	100	15	50	100
2	100	15	40	100
3	100	10	40	74
4	100	10	50	85
5	100	10	60	100
6	100	5	60	58
7	250	10	60	43
8	150	10	60	81
9	250	10	70	57
10	250	10	80	70
11	250	15	80	94

[a] Optimization of reaction parameters to achieve the highest molar conversion was performed using Ty as substrate. [b] Solution in DMC. [c] Molar conversion determined by HPLC analysis.



Scheme 1 Biocatalyzed flow synthesis of symmetric carbonates **3** and **4**.

Table 2 Optimization of reaction parameters to achieve the highest molar conversion was performed using compound **1** as substrate and Ty as nucleophile

Entry	Ratio cpd 1/Ty[a]	R_t (min)	T (°C)	c[b] (%)
1	1 : 1	30	60	15
2	1 : 1	60	60	28
3	1 : 1	120	60	50
4	1 : 2	120	60	65
5	1 : 2	120	70	77
6	1 : 2	120	80	91
7	1 : 2	180	80	92
8	1 : 3	120	80	95

[a] Solutions in *tert*-amyl alcohol. Concentration of compound **1**: 200 mM. [b] Molar conversion determined by HPLC analysis.

organocatalyst under heating[30,31] and, until now, the synthesis of compounds **1** and **2** has been achieved mainly using an acidic catalyst under prolonged heating.[32–34] The immobilized biocatalyst was packed into a glass reactor column and a solution of Ty in DMC was flowed through it (Table 1); temperature (T, range: 40–80 °C), Ty concentration (C_{Ty}, range: 100–250 mM), and residence time (R_t, range: 5–15 min) were varied and conversion (c) was evaluated by HPLC (Table 1).

Using a 100 mM solution of Ty in DMC, the flow biotransformation was complete after only 15 min both at 50 °C and lowering the temperature at 40 °C (Table 1, entries 1 and 2). Keeping constant the temperature at 50 °C and decreasing the residence time at 10 min resulted in only a partial conversion of Ty into product **1** (Table 1, entry 4), but raising the temperature at 60 °C, in only 10 min, full conversion was achieved (Table 1, entry 5). Under these conditions, to improve the productivity of the system, higher concentration of Ty was tested, but the biotransformation was not complete, even increasing temperature up to 80 °C and residence time to 15 min (Table 1, entry 11).

The selected conditions (C_{Ty}: 100 mM in DMC, T = 60 °C, R_t: 10 min) were then successfully applied for the synthesis of compound **2**. Considering the HTy low availability in nature and its price, it was obtained from Ty through a continuous biocatalyzed oxidation using a free tyrosinase.[35] Then, to obtain compounds **3** and **4**, carbonates **1** and **2** were used as substrates and Ty or HTy as nucleophiles. The biotransformation was performed in *tert*-amyl alcohol which was chosen as unconventional medium for its safety profile, low freezing point in comparison to *t*-BuOH, and ability to solubilize polar compounds.[36,37] A solution of compound **1** in *tert*-amyl alcohol was mixed with a solution of Ty and the resulting mixture was flowed through a reactor packed with imm-CaLB (Scheme 1).

Different experiments were performed changing residence time, stoichiometry and temperature and the outcome was monitored by HPLC (Table 2). Unsatisfactory conversions were achieved using a stoichiometric ratio between compound **1** and Ty, even after 120 min of residence time (Table 2, entries 1–3). So, a 1 : 2 ratio was tested (Table 2, entries 4–7). The reaction did not reach completion even after 180 min of residence time at 80 °C and a significant hydrolysis of carbonate **2** was observed, leading to the formation of Ty that was then recovered by column chromatography obtaining the desired product in 25% yield. It was not possible to avoid or reduce this side reaction even reducing the temperature or the residence time, changing the solvent, or adding molecular sieves to produced a mixed bed reactor.[38] Since the differences in the reaction outcome between 120 min and 180 min were not significant, the final set-up was settled for a residence time of 120 min which allowed to obtain a conversion of 91% and an isolated yield of 23%. It is worth noting that the same results were reached in batch after 4 days.

Conclusions

Alkyl carbonates are an important class of compounds widely used for a variety of industrial and synthetic applications, including as protecting groups for the alcoholic function. In this work, the high-yield biocatalyzed chemoselective carbomethoxylation of the alcoholic chain of natural phenolic compounds was performed in flow conditions, without

affecting the phenolic moiety. The obtained methyl carbonates **1** and **2** were then reacted to obtain the symmetric carbonates **3** and **4**. The biotransformations were carried out in DMC or *tert*-amyl alcohol as the solvents and the biocatalyst (Novozyme 435®) showed very good stability in these unconventional media. Concerning the synthesis of methyl carbonates derivatives **1** and **2**, the switch from a batch traditional set up to a continuous allowed a reduction of the reaction time, an easier work-up and a higher yield: in fact, the batch reaction needed 6 h to bring the reaction to completeness followed by a filtration and washing under pressure and by a flash chromatography purification to isolate the pure product **1** in 93% yield; the flow biotransformation required only 10 min of residence time to reach the completeness, did not require downstream processes giving quantitative yield. Using a packed bed reactor with a volume of 1.6 mL (545 mg of imm-CaLB), a multi-gram amount of compounds **1** (4.5 g day^{-1}) and **2** (4.8 g day^{-1}) were produced in 24 h, that means a biocatalyst productivity of more that 8 $g_{product}\ g_{imm\text{-}CaLB}{}^{-1}\ day^{-1}$. Moreover, DMC was recovered by evaporation and reused. Carbonates **3** and **4** were obtained in moderate yield but the present protocol represents the first biocatalyzed approach towards these symmetric scaffolds, that were synthesised without the need of protecting the phenolic group(s).

Experimental

Materials and methods

Reagents and solvents were obtained from commercial suppliers and were used without further purification. NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer using the residual signal of the deuterated solvent as internal standard. ^{1}H chemical shifts (δ) are expressed in ppm, and coupling constants (J) in hertz (Hz). Continuous flow biotransformations were performed using a R4 Vapourtec flow reactor and Asia Flow Chemistry Syringe pumps (Syrris) equipped with an Omnifit® glass column (i.d. 6.6 mm × 100 mm length). The temperature sensor sits on the wall of the reactors. Immobilized lipase from *Candida antarctica* B (CaLB, Novozyme®) was purchased from Merk (Milan, Italy). HTy was synthetized from Ty as previously reported.[35] Analytical thin layer chromatography (TLC) was carried out on TLC plates precoated with silica gel 60 F_{254}. Spots were further evidenced using a dilute alkaline solution of $KMnO_4$ or an acidic solution of vanillin in ethanol. HPLC analyses were performed using a Waters 1525 Binary HPLC Pump, equipped with a Waters 2489 UV-vis detector (Waters, Milford, MA).

HPLC method

For the analysis of tyrosol, hydroxytyrosol and compounds **1–4** an isocratic method H_2O/acetonitrile (6 : 4) was used. Waters C18 column μBondapack (10 μm, 125 Å), 210 nm (λ). Injection volume: 10 μL. Flow rate: 1 mL min^{-1}. Retention times: Ty = 3.4 min, HTy = 3.1 min, **1** = 5.1 min, **2** = 4.2 min, **3** = 6.8 min, **4** = 4.5 min.

Continuous synthesis of 4-hydroxyphenethyl methyl carbonate (1) and 3,4-dihydroxyphenetyl methyl carbonate (2). A 0.1 M solution of Ty or HTy in DMC (4.2 mmol, 42 mL) was pumped through a column reactor packed with 545 mg of imm-CaLB (volume: 1.6 mL) with a flow rate of 0.16 mL min^{-1} (residence time 10 min) at 60 °C. DMC was employed as flow stream. The resulting crude was collected, the solvent was evaporated under reduced pressure to give the desired product that was used without further purifications.

*4-Hydroxyphenethyl methyl carbonate (**1**).* Yield: quantitative; white solid; Rf = 0.66 (cyclohexane/ethyl acetate 6 : 4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 4.98 (s, 1H, OH), 4.30 (t, J = 7.2 Hz, 2H), 3.77 (s, 3H), 2.90 (t, J = 7.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.8, 154.3, 130.1, 129.3, 115.4, 68.7, 54.6, 34.2.[39]

*3,4-Dihydroxyphenetyl methyl carbonate (**2**).* Yield: quantitative; colourless oil; Rf = 0.52 (cyclohexane/ethyl acetate 6 : 4); ^{1}H NMR (300 MHz, CD_3OD) δ 6.69–6.66 (m, 2H), 6.55–6.51 (m, 1H), 4.22 (t, J = 7.1 Hz, 2H), 3.71 (s, 3H), 2.78 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 155.9, 144.9, 143.8, 128.8, 119.7, 115.5, 114.9, 68.5, 53.7, 34.0.[40]

Continuous synthesis bis(4-hydroxyphenethyl) carbonate (3) and bis(3,4-dihydroxyphenethyl) carbonate (4). A solution of compound **1** or **2** (2.9 mmol, 1 eq.) in *tert*-amyl alcohol (58 mL, 0.2 M), and a solution of tyrosol (822 mg, 5.9 mmol, 2 eq.) in *tert*-amyl alcohol (58 mL, 0.2 M) were prepared. The two solutions were pumped through a column reactor packed with 1.3 g imm-CaLB (volume: 3.0 mL), with a total flow rate of 25 μL min^{-1} (residence time 2 h) at 80 °C. *tert*-Amyl alcohol was used as flow stream. The resulting crude was collected and the solvent was evaporated under reduced pressure. The crude was purified by flash column chromatography (cyclohexane/ethyl acetate 8 : 2 for compound **3**, dichloromethane/methanol 98 : 2–95 : 5 for compound **4**).

*Bis(4-hydroxyphenethyl) carbonate (**3**).* Yield: 23%; white solid; Rf = 0.45 (cyclohexane/ethyl acetate 6 : 4); ^{1}H NMR (300 MHz, CD_3OD) δ 7.02 (d, J = 8.5 Hz, 4H), 6.70 (d, J = 8.5 Hz, 4H), 4.21 (t, J = 7.1 Hz, 4H), 2.82 (t, J = 7.1 Hz, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ 155.7, 155.2, 129.5, 128.1, 114.8, 68.3, 33.8.

*Bis(3,4-dihydroxyphenethyl) carbonate (**4**).* Yield: 25%; white solid; Rf = 0.58 (dichloromethane/methanol 9 : 1); ^{1}H NMR (300 MHz, CD_3OD) δ 6.70–6.66 (m, 4H), 6.54–6.51 (m, 2H), 4.20 (t, J = 7.1 Hz, 4H), 2.77 (t, J = 7.1 Hz, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ 155.7, 144.8, 143.5, 128.9, 119.8, 115.6, 114.9, 68.4, 29.1.

Author contributions

Conceptualization, L. T. and A. P.; methodology, S. V., F. A. and D. P.; investigation, S. V., F. A. and D. P.; resources, L. T. and A. P.; data curation, S. V. and F. A.; writing—original draft preparation, L. T., S. V. and F. A.; writing—review and editing, all authors; project administration, L. T. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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