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Flavouring and odorant thiols from renewable natural resources by In^{III}-catalysed hydrothioacetylation and lipase-catalysed solvolysis

Reine-Marie Dia [a,†], Rim Belaqziz [a,b,†], Abderrahmane Romane [b], Sylvain Antoniotti [a,*], Elisabet Duñach [a]

[a] LCMBA UMR 6001, Université de Nice—Sophia Antipolis, CNRS, Institut de Chimie de Nice, UFR Sciences, 28 avenue de Valrose, F-06108 Nice, France
[b] Laboratoire de Chimie Organique Appliquée, Equipe Analyse et Contrôle de Qualité, Université Cadi Ayyad, Marrakech, Morocco

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ABSTRACT

A chemoenzymatic access to thiol compounds, including ethyl 3-thiobutanoate, 3-thio-*p*-menthene and 8-thio-*p*-menthan-2-one, three compounds of interest in flavour and fragrance chemistry presenting various fruity notes, is proposed. It involves an indium(III)-catalysed hydrothioacetylation of renewable precursors followed by an enzymatic solvolysis of the obtained thioesters by lipases in aqueous or organic solvents.

1. Introduction

After years of underestimation, the use of enzymes in organic synthesis has been recognised as a very powerful tool,[1,2] particularly after the discovery of the stability and activity of enzymes in organic solvents.[3–7] With the growing interest for sustainable processes in chemistry,[8–10] the role of biocatalysis is expected to increase in the coming years,[11] with the ease of production of enzymes from recombinant organisms, and the expansion of the substrate scope of enzymes, relying on high throughput selection, directed evolution[12] or catalytic promiscuity.[13]

Lipases belong to the class of hydrolases and are among the most widely used biocatalysts. They typically catalyse the hydrolysis of esters, but they can also be used for acyl group transfer onto alcohols, amines and hydrogen hydroperoxide.[2] Although the presence of thioesterases in fruits (including passion fruit, *Passiflora edulis* Sims)[14] and in other living organisms as a polyketide synthase module[15] has been reported, mainly lipases have been used to hydrolyse thioesters, for example for the specific synthesis of 3-mercaptohexyl derivatives[16,17] 2-methyl-3-furanthiol and 2-furfurylthiol.[18] Interestingly, the chemoselective hydrolysis of a thioester group in the presence of an oxoester has been reported to occur in water in the presence of an esterase isolated from *Pseudomonas fluorescens* MTCC B0015[19] and a thioesterase isolated from a strain of *Alcaligenes sp.* ISH108.[20] Subtilisin Carlsberg, a peptidase from *B. subtilis*, was also shown to catalyse the hydrolysis of amino acid thioesters.[21]

On the other hand, conventional chemical syntheses of thiols require in many instances the action of gaseous H_2S,[22] thiourea or cysteine as sulfur donors across activated olefins,[23] or the radical-based addition across non-activated olefins, the action of NaSH[24,25] or elemental sulfur,[26] generally occurring with selectivity issues. These reactions are quite often followed by treatment of the crude product by metal-based reducing agents to obtain free thiols, resulting in large quantities of metallic waste. In the particular case of fragrant thiols **1** and **2**, the addition of H_2S to ethyl crotonate and pulegone is a common route, but suffers from the technical requirement to handle such reagent and the non-selective formation of rearranged products.

We have been interested in the design of novel methodologies in organic synthesis aimed at providing eco-friendly accesses to flavours and fragrances ingredients, using either enzymatic catalysis[27] or metal-based catalysis.[28–32] In this Letter, we present a novel access to thiol derivatives **1**–**8**, achieved by a chemoenzymatic synthesis involving the addition of thioacetic acid to olefins proceeding in 80–99% yield, followed by enzymatic solvolysis of the obtained thioesters (18–90%). The lipase-catalysed reaction was also efficiently used to prepare thiocitronellol. To our knowledge, this is the first example of such a sequence to obtain thiols from olefins in two steps. It is worth noting that starting materials **9**–**12** may be obtained from renewable natural products, and particularly interesting is the use of pulegone **10**, which accounts for up

* Corresponding author. Tel.: +33 492076172; fax:+33 492076151.
E-mail address: sylvain.antoniotti@unice.fr (S. Antoniotti).
† Contributed equally to this work.

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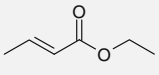
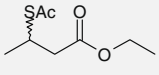
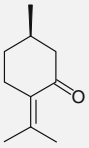
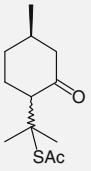
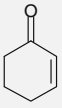
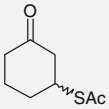
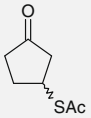
to 83% of the composition of *Mentha pulegium* essential oil,[33–35] and α-pinene **11**, which is a cheap and abundant by-product of the wood industries, and a useful starting material.[36]

The first step involved an In^{III}-catalysed hydrothioacetylation[37] of ethyl *trans*-but-2-enoate **9** and (*R*)-pulegone **10** by thioacetic acid, proceeding through a conjugate addition and yielding only the Michael-type adduct, for example, the corresponding thioesters **15** and **16**, in high yields (Table 1).

In the absence of catalyst (entry 2) or at room temperature (entry 3), the reaction did not proceed. At 60 °C and in the presence of 5 mol % $InCl_3$, α,β-unsaturated carbonyl compounds **9** and **10** were converted into the corresponding thioesters **15** and **16**, with 99% (duplicates, entry 1) and 80% yield (entry 4), respectively. In the case of **16**, a mixture of diastereomers was obtained in an 8:2 ratio (determined by GC/FID). By making a comparison with literature data for *trans*-**2**,[38] we identified *trans*-**16** as the major diastereomer formed in this reaction. Additionally, we engaged simple α,β-unsaturated carbonyl compounds such as cyclohex-2-enone **13** and cyclopent-2-enone **14** in the reaction, which were converted in excellent yields into the corresponding products **19** and **20** (entries 5, 6).

The formation of thioester **17** was achieved using (+)-α-pinene **11** as a substrate under the same reaction conditions. Thioester **17** was obtained in 80% GC yield (42% isolated, 7:3 mixture of diastereomers, hardly separated from mixture of isomers) via a tandem ring-opening/thioacetylation involving the attack of a transient allylic cation[39] by soft *S*-nucleophile, while with hard *O*-nucleophiles such as alcohols, the attack of the terpenyl action is favoured.[28] Thioester **17** was formed along with a mixture of isomerised terpenes and rearranged products.

Table 1
Addition of thioacetic acid to α,β-unsaturated carbonyl compounds **9**, **10**, **13**, **14** catalysed by $InCl_3$ (5 mol %)

Entry[a]	Substrate	*T* (°C)	Time (h)	Product, yield[b]
1	 **9**	60	18	 **15**, 99%[c]
2	**10**	25[d]	24	—
3	**10**	25	24	—
4	 **10**	60	6	 **16**, 80%[e]
5	 **13**[f]	60	10	 **19**, 94%
6	 **14**[f]	60	4	 **20**, 99%

[a] Conditions: substrate (15 mmol), CH_3COSH (30 mmol), $InCl_3$ (0.75 mmol) in dichloroethane (DCE, substrate concn = 10 mg/mL).
[b] Isolated yields after chromatography over silica gel.
[c] Duplicates.
[d] No catalyst was used.
[e] Mixture of *trans*-(1*R*,4*R*)/*cis*-(1*R*,4*S*) diastereomers (8:2).
[f] 1.5 equiv of AcSH was used.

The preparation of thioester (*S*)-**18** was achieved in 66% overall yield in two steps from citronellol (*S*)-**12**, via the formation of the *para*-toluenesulfonate ester followed by the nucleophilic attack of AcSH.

The use of greener solvents such as ethanol, toluene and ethyl acetate with **10** as a model substrate resulted unsatisfactorily in both lower yields and diastereoselectivities, as well as longer reaction times (see Supplementary data).

Having in hand the thioester precursors, we studied the enzymatic solvolysis of the thioester group by several hydrolases. In spite of the huge number of papers dealing with lipase-catalysed transformations,[7,40,41] most being oriented towards the resolution of racemates,[42,43] the enzymatic hydrolysis of thioesters remains underexploited and poorly documented. Worth noting are some specific examples for the *Candida antartica* lipase B (CALB)-catalysed resolution of 3-thioacetylhexanal and 3-thioacetylhexanol by hydrolysis,[17] a solvolysis of 2- and 3-thioacetyl methylpropanoate derivatives in propan-1-ol catalysed by *Pseudomonas cepacia* lipase (PCL)[44] and a β-lyase-catalysed cleavage of cysteine conjugates.[16] It is worth noting that CALB also catalysed the addition of sulfides across double bonds of vinyl esters.[45]

A screening of biocatalysts, solvents and reaction conditions was first performed on model substrates 1-thioacetyloctane **21** (non-hindered primary thioester) and 1-thioacetylcyclohexane **22** (non-hindered secondary thioester), focusing at identifying enzymes able to catalyse the hydrolysis of the thioester group (see Supplementary data). Control experiments in the absence of enzyme were performed, confirming that non-enzymatic reactions did not occur under these conditions.

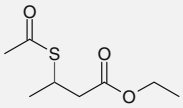
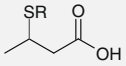
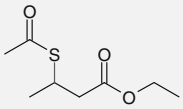
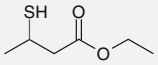
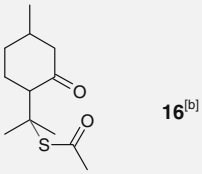
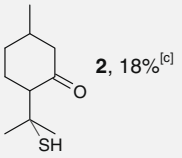
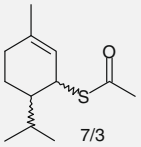
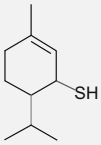
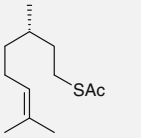
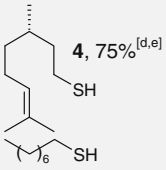
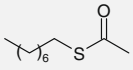
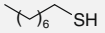
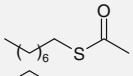
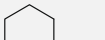
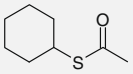
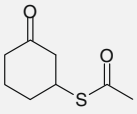
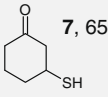
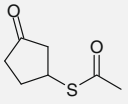
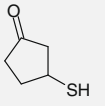
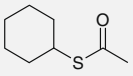
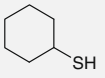
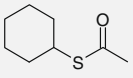
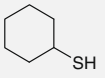
We further extended the enzymatic solvolysis to thioesters **15–22** (Table 2), particularly using the lipase from *Candida rugosa* as a biocatalyst.

With substrate **15**, the question of the chemoselectivity or whether or not the thioester function could react faster than the oxoester one was of concern. Running the enzymatic reactions in aqueous buffer with CRL led mainly to hydrolysis of the ester group with the formation of a mixture of polar products (entry 1).[46] A modified procedure was envisaged by shifting the reaction from aqueous to organic medium. We have thus chosen to run the reaction in a 3:2 mixture of toluene and ethanol, the latter acting as a nucleophile. This modification was successful and the expected thiol **1** was formed with 50% yield and 48% ee (entry 2). In this organic medium, PCL and PPL, although somehow efficient on our model substrates, showed limited activity even after long reaction times and delivered the thiol **1** in 5–10% yields (not shown).

Thioester **16** (1*R*,4*R*)/(1*R*,4*S*) (8:2) was converted to thiol **2** (1*R*,4*R*)/(1*R*,4*S*), (9:1) in 18% yield when treated by CRL in pH 7 aqueous buffer at 40 °C (entry 3). The low yield was probably the result of the steric hindrance at carbon atom bearing the thioester group. Tertiary functions are known to react poorly in such hydrolase-catalysed reactions[27] and need optimised biocatalysts.[47,48] The major diastereomer obtained after AcSH addition across pulegone **10**, (1*R*,4*R*)-**16**, was purified by chromatography over silica gel and engaged separately in enzyme-catalysed solvolysis. (1*R*,4*R*)-**16** reacted poorly and only 11% yield of the fragrant thiol (1*R*,4*R*)-**2**, called mangone and used in flavouring preparations,[49–51] was observed after 6 d (monitored by GC–FID).

In the case of thioester **17** (7:3 mixture of diastereomers), the enzymatic hydrolysis was perfomed using 100% w/w CRL in aqueous medium containing 5% v/v DMF. Thiol **3** was formed in 27% yield as a 7:3 mixture of diastereomers (entry 4), while the conversion was 74% after 22 d. Thioester (*S*)-**18** was converted to thiol (*S*)-**4** in 72% yield by 10% w/w of CRL in a mixture aqueous phosphate buffer (pH8) and DMF 95:5 at 40 °C. The presence of DMF was necessary to ensure sufficient solubility of the organic starting

Table 2
Lipase-catalysed hydrolysis of thioesters

Entry	Substrate	Conditions[a]	Conversion (%)	Product, yield
1	 **15**	CRL (100% w/w) aqueous buffer pH 8, 40 °C, 3 h	100	 R=H, Ac, quant.
2	 **15**	CRL (100% w/w) toluene/EtOH (3:2), 40 °C, 1.5 h	50	 **1**, 50%, *ee* 48%
3	 **16**[b]	CRL (100% w/w) aqueous buffer pH 7, 40 °C, 5.5 h	24	 **2**, 18%[c]
4	 **17** 7/3	CRL (100% w/w) aqueous buffer pH 7/DMF (95:5), 40 °C, 22 d	74	 **3**, 27%, dr 7/3
5	 **18**	PCL (40% w/w) aqueous buffer pH 8/DMF (95:5), 40 °C, 4.9 d	100	 **4**, 75%[d,e]
6	 **21**	PPL (100% w/w) aqueous buffer, pH 7, 12 h	98	 **5**, 68%[d]
7	 **21**	PPL (100% w/w) aqueous buffer, pH 8, 12 h	100	 **5**, 62%[d]
8	 **22**	CRL (100% w/w) aqueous buffer, pH 7, 16 h	100	**6**, 68%[d]
9	 **19**	CRL (10% w/w) aqueous buffer pH 6/DMF (95:5), 40 °C, 2 d	99	 **7**, 65%[d]
10	 **20**	CRL (10% w/w) aqueous buffer pH 7/DMF (95:5), 40 °C, 2 d	99	 **8**, 90%[d,f]
11	 **22**	ANL (100% w/w) aqueous buffer, pH 7, 16 h	100	 **6**, 98%[d]
12	 **22**	AAL (100% w/w) aqueous buffer, pH 8, 16 h	100	 **6**, 95%[d]

[a] *Candida rugosa* lipase (CRL), *Aspergillus niger* lipase (ANL), *Pseudomonas cepacia* lipase (PCL), Pig pancreatic lipase (PPL), Amano aspergillus lipase (AAL).
[b] *Trans*/*cis* 8:2.
[c] *Trans*/*cis* 9:1.
[d] Sometimes isolated as disulfides upon thiol oxidative dimerisation under air.
[e] Thiol *S*-**4** could also be formed using CRL and Novozym, but in lower yields (33–68%).
[f] Similar results in terms of yield and reaction time were obtained at pH 6.

material. Maximal conversion was reached within 3 h and thiol **4** was the only detectable product.

Racemic thioesters **19–20, 22** were converted in 62–98% yields into their thiols by CRL, ANL, or AAL in pH 7 and pH 8 aqueous buffer at 40 °C (entries 6–13). No enantioselectivity could be observed since identical reaction rates were measured for both enantiomers of **19** when the course of their reaction was examined by enantioselective-GC/FID (FS-Lipodex® E, 50 m).

In summary, we describe here an eco-compatible chemoenzymatic synthesis of thiols, some of which from renewable sources, involving the sequential intervention of In^{III}-catalysis and lipase-catalysis. The In^{III}-catalysed step provided adducts of olefins and thioacetic acid in good yields, and the lipase-catalysed solvolysis showed the best efficiency with primary thioesters, secondary and tertiary presenting lower yields of thiols. Increased steric hindrance in the vicinity of the thioester function also resulted in lower yields. The influence of the class and the substitution of the substrate suggest that interfacial activation of the lipases by medium engineering[4] could be helpful to overcome this moderate activity, and would deserve substrate specific optimisation. Optimised biocatalyst for improved stereopreference would be the next step forward.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.081.

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