Vegetables as biocatalysts in stereoselective hydrolysis of labile organic compounds†

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Hydrolysis of labile esters of β-hydroxyketones has been performed with whole plant tissue from various vegetables. The pheromone 5-hydroxy-4-methyl-3-heptanone (**1**) was used as the model compound. Hydrolysis of acetates and benzoates of **1** was unsuccessful using normal conditions of ester hydrolysis, both by chemical hydrolysis and by the means of commercial lipases. When, however, whole cells of carrot, celery root, eggplant, parsley root, parsnip and potato were used as reagents, hydrolysis of the acetates was successful. At low conversion the hydrolysis was stereoselective and at total conversion virtually no formation of by-products was observed. The selectivity varied among the eight vegetables that were evaluated. Methods of preparation and substrate-to-plant ratio were examined. Furthermore, acetates and benzoates of three analogous compounds [5-hydroxy-3-heptanone (**2**), 5-hydroxy-5-methyl-3-heptanone (**3**) and 5-ethyl-6-hydroxy-4-octanone (**4**)] were hydrolyzed by potato and sweet potato to various degrees, indicating that the method is general for the mild and stereoselective hydrolysis of secondary β-alkoxy- and β-aryloxyketones.

Introduction

In the process of developing environmentally-friendly and sustainable methods for synthesis of chiral compounds, attention has recently turned to plants as reagents. The use of plants in organic synthesis has been reported extensively over the last few years: The common carrot, *Daucus carota*, has proven to be suitable for the asymmetric reduction of prochiral ketones; many authors report enantiomeric excess of over 95% in these reactions.[1-10] The chiral alcohols obtained by plant-mediated reductions of carbonyl groups are in great demand by various industries because they are the precursors of drugs, agrochemicals (pheromones), specialty materials (*e.g.* liquid crystals), flavours and fragrances.[8,9,11,12]

Cell cultures as well as wild tissue have been used, not only for reduction of aldehydes and ketones, but also in other reactions *e.g.* hydrolysis of enol acetates[3] and certain other acetate esters,[13-16] reduction of carboxylic acids[17] and oxidation of alcohols.[15] It is largely unknown which types of enzyme systems are involved in these reactions. One cannot exclude the possibility that microorganisms such as bacteria and yeasts may be involved.[6] Apart from carrots, several vegetables have been reported as active reagents in the above-mentioned reactions, *e.g.* potato,[18] apple,[14] topinambur,[19] sweet potato,[20] burdock,[20] onion,[20] *Manihot* species[21] and more. Considering the wide array of taxonomically different plants and microbes available, it may in future be possible to use biocatalysts for virtually every reaction. General advantages of plants as reagents are their easy disposal after use, because they are biodegradable, the mild reaction conditions, and their wide availability at low cost.

In this paper we have studied the capacity of vegetables to mediate the seemingly trivial hydrolysis reaction, with emphasis on esters of β-hydroxyketones which are very prone to undergo elimination reactions.

The *syn* isomers of 5-hydroxy-4-methyl-3-heptanone (**1**) are known as Sitophilure and are aggregation pheromones of the rice weevil *Sitophilus oryzae* and the maize weevil *S. zeamais*.[22] In attempts to hydrolyze the acetate and benzoate esters of this β-hydroxyketone using standard alkaline or acidic reaction conditions, we failed because of instantaneous elimination. Hydrolysis and alcoholysis attempts using commercial enzymes such as lipases, hydrolases and esterases also failed for this substrate.

However, we found that carrot-mediated hydrolysis of 5-acetoxy-4-methyl-3-heptanone was successful. Encouraged by this, we launched an investigation of the hydrolysis of esters of β-hydroxyketones using 5-acetoxy-4-methyl-3-heptanone as a model compound, and carrot as a model plant. General reaction conditions were evaluated and the rate and selectivity of the hydrolysis reaction were monitored, with the key aim of achieving full conversion within a reasonable timeframe. In this study, the ability to mediate hydrolysis of the acetate ester and benzoate ester of **1** was compared for eight different vegetables. In order to examine the substrate selectivity for these reactions, six analogous β-acyloxyketones were also investigated.

Results and discussion

Evaluation of experimental conditions

In order to use this method successfully in practice, some parameters were evaluated in more detail using carrot as the

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model organism, and 5-acetoxy-4-methyl-3-heptanone as the substrate forming the four stereoisomers of 5-hydroxy-4-methyl-3-heptanone (**1**) as the products (Fig. 1).

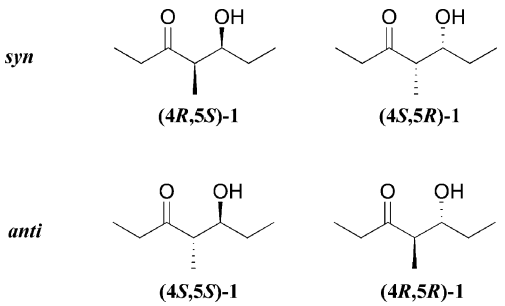


Fig. 1 All four stereoisomers of 5-hydroxy-4-methyl-3-heptanone (**1**).

Under most reaction conditions, one could observe some selectivity for the formation of (4*S*,5*R*)-**1**, and a higher selectivity for the formation of (4*R*,5*R*)-**1**. The enantiomeric/diastereomeric excesses were transient in most cases and usually decreased to zero if the reactions were allowed to go to completion (which usually occurred within 24 h).

It is known that the method of preparation of plants for synthesis is of importance[7,10,23] and in fact, the method of preparing the carrots had great impact on the selectivity and yield. We compared three alternative methods: grating, mincing and slicing. The reaction was most selective when minced carrots were used, but no more than 50% of the ester was hydrolyzed in 48 h, whereafter the reaction stopped. When grated and sliced carrots were used, the reaction was complete within 30 h. In all preparations, the acetate of (4*R*,5*R*)-**1** tended to be consumed the most rapidly and thoroughly.

The reason why minced carrots were least effective in mediating hydrolysis is open to speculation. It may be that the reaction requires live cells in order to proceed, for example because of co-factor recycling,[1] or that proteases were liberated from the cells and degraded relevant enzymes. Microscopy studies of the prepared carrots from the three methods confirmed that far fewer cells remained intact in the minced batch than in the grated batch; most cells were intact in the sliced batch.

To determine the minimum amount of plant tissue required for full turnover of the reaction, a series of experiments with sliced carrots (which proved to be easier for the isolation of the product than grated carrots), and 5-acetoxy-4-methyl-3-heptanone was carried out. The ratio of substrate to plant was altered from 1 : 80 to 1 : 200 to 1 : 800 (w/w). It was found that at 1 : 800 the hydrolysis was fast and that at 1 : 200 full turnover was achieved within 30 h, while at 1 : 80, the rate and turnover decreased rapidly, and the turnover did not reach 50% in 48 h.

Screening of vegetables

The following vegetables were examined for their ability to hydrolyze the acetate and benzoate esters of **1**: celery root (*Apium graveolens*), carrot (*Daucus carota*, blue and yellow cultivars), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), parsnip (*Pastinaca sativa*), parsley root (*Petroselinum crispum*), black salsify (*Scorzonera hispanica*), eggplant (*Solanum melongena*), and potato (*Solanum tuberosum* 'King Edward' cultivar). Vegetables were chosen on the basis of existing studies, many of which employed carrot[1-3,7-10,12,13,15,24-28] but also potato, sweet potato, cassava, eggplant and celery root.[3-5,17,18,20,21,23,29] A variety of plants with edible taproots related to the common carrot (celery root, parsnip and parsley root) were chosen, out of interest in examining how plants within the same botanical family (*Apiaceae*) performed. Other attributes of the chosen plants were their ready availability and low cost.

The vegetables that hydrolyzed practically all 5-acetoxy-4-methyl-3-heptanone (> 90%) within 48 h were carrot, celery root, eggplant, parsley root, parsnip and potato. With the exception of eggplant, which showed selectivity for a (5*S*)-diastereomer, *i.e.* (4*R*,5*S*)-**1**, the catalysis by these vegetables initially resulted in more of (4*S*,5*R*)-**1** and (4*R*,5*R*)-**1** than of the (5*S*)-diastereomers. Carrot and celery root were the most selective catalysts for the preparation of (4*R*,5*R*)-**1** (Table 1).

For the preparation of (4*S*,5*R*)-**1** the best catalysts were cassava, potato and sweet potato. When sweet potato and parsley root were employed, the (4*S*,5*S*)-**1** isomer was formed in < 3% at 50% conversion. At higher conversion, the isomeric purity of the products decreased as the concentration of the favoured substrate decreased in the reaction mixture.

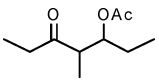
The hydrolysis of the benzoate esters of **1** was more difficult. The most rapid hydrolysis was achieved with potato and required 122 h for 96% conversion (Table 2). Hydrolysis could also be achieved by use of sweet potato and cassava but generally conversions were lower and reaction times longer than with the hydrolysis of the acetate analogues. All vegetables of the family *Apiacea* performed similarly in the hydrolysis reactions of acetates and benzoates, indicating that very similar enzymes are involved in all species.

Investigation of analogues

In order to investigate the general scope of this reaction and to gather clues to the stereochemical prerequisites for the reaction to proceed, a number of analogues were synthesized and tested (Fig. 2). Two tubers found to be successful at hydrolyzing both the acetate ester and benzoate ester of **1** were used: potato and sweet potato. Three analogous β-hydroxyketones were investigated. The compound 5-hydroxy-3-heptanone (**2**) lacks the α-methyl group present in the original compound; in 5-hydroxy-5-methyl-3-heptanone (**3**) the methyl group is moved to position 5, whereby a tertiary alcohol is formed; in the third analogue, 5-ethyl-6-hydroxy-4-octanone (**4**), the methyl group is replaced by an ethyl group and the main chain is one carbon longer.

The racemic acetate ester of **2** was completely hydrolyzed within 24 h (Table 3). Initially slightly more (*S*)-**2** was formed using potato, while more (*R*)-**2** was formed using sweet potato, indicating the action of different enzyme systems in the two tubers. The racemic benzoate ester of **2** was fully hydrolyzed by potato by hour 48, whereas with sweet potato less than 80% was hydrolyzed within 96 h (Table 4). In both experiments, (*R*)-**2** was formed faster, but both enantiomers of the acetate were converted, as the product became virtually racemic at full conversion.

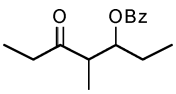
Table 1 Hydrolysis of 5-acetoxy-4-methyl-3-heptanone: Selectivity after 25% conversion and 50% conversion, shown as relative amounts of the four isomers of the product 5-hydroxy-4-methyl-3-heptanone (**1**) and the relative difference in abundance compared with the same isomer of the reagent at t = 0. Additionally, maximal conversion (%) and time for maximal conversion (h) are shown



	5-hydroxy-4-methyl-3-heptanone (**1**) formed after 25% conversion:[a] **Relative amount (%)** *(relative change from start composition (%))*.				5-hydroxy-4-methyl-3-heptanone (**1**) formed after 50% conversion:[a] **Relative amount (%)** *(relative change from start composition (%))*.				Max conversion. (%) and time (h) for conversion.	
	(4*R*,5*S*)-**1**	(4*S*,5*R*)-**1**	(4*R*,5*R*)-**1**	(4*S*,5*S*)-**1**	(4*R*,5*S*)-**1**	(4*S*,5*R*)-**1**	(4*R*,5*R*)-**1**	(4*S*,5*S*)-**1**	Conv.	Time
Start composition	33[b]	33[b]	17[b]	17[b]	33[b]	33[b]	17[b]	17[b]	—	—
Plant										
Black salsify	**28** *(-15)*	**45** *(+36)*	**23** *(+35)*	**4** *(-76)*	**28** *(-15)*	**45** *(+36)*	**21** *(+24)*	**6** *(-65)*	53	146
Carrot, blue var.	**22** *(-33)*	**32** *(-3)*	**31** *(+82)*	**15** *(-12)*	**25** *(-24)*	**31** *(-6)*	**28** *(+65)*	**16** *(-6)*	>99	24
Carrot, yellow var.	**22** *(-33)*	**27** *(-18)*	**37***(+118)*	**14** *(-18)*	**26** *(-21)*	**29** *(-12)*	**30** *(+76)*	**15** *(-12)*	>99	24
Cassava	**38** *(+15)*	**47** *(+42)*	**12** *(-29)*	**3** *(-82)*	**33** *(+0)*	**43** *(+30)*	**18** *(+6)*	**6** *(-65)*	95	120
Celery root	**20** *(-39)*	**34** *(+3)*	**37***(+118)*	**9** *(-47)*	**24** *(-27)*	**34** *(+3)*	**30** *(+76)*	**12** *(-29)*	92	48
Eggplant	**54** *(+64)*	**37** *(+12)*	**6** *(-65)*	**3** *(-82)*	**48** *(+45)*	**34** *(+3)*	**8** *(-53)*	**10** *(-41)*	>99	72
Parsley root	**31** *(-6)*	**37** *(+12)*	**29** *(+71)*	**3** *(-82)*	**39** *(+18)*	**37** *(+12)*	**19** *(+12)*	**3** *(-82)*	>99	48
Parsnip	[c]	[c]	[c]	[c]	**27** *(-18)*	**43** *(+30)*	**21** *(+24)*	**9** *(-47)*	>99	8
Potato	**24** *(-27)*	**58** *(+76)*	**15** *(-12)*	**3** *(-82)*	**28** *(-15)*	**50** *(+52)*	**15** *(-12)*	**7** *(-59)*	>99	48
Sweet potato	**12** *(-64)*	**60** *(+82)*	**26** *(+53)*	**2** *(-88)*	**20** *(-39)*	**53** *(+61)*	**25** *(+47)*	**2** *(-88)*	67	96

[a] As measured by GC. [b] The composition of the starting material (5-acetoxy-4-methyl-3-heptanone). [c] Reaction proceeded so fast that >25% conversion occurred before the first measurement.

Table 2 Hydrolysis of benzoate ester of 5-hydroxy-4-methyl-3-heptanone (**1**): Maximal conversion (%) and time for maximal conversion (h) are shown



Plant	Maximal conversion of benzoate ester to 5-hydroxy-4-methyl-3-heptanone (**1**)[a] Conversion (%)	Time (h)
Black salsify	39	120
Carrot, blue var.	<1	96
Carrot, yellow var.	<1	96
Cassava	88	120
Celery root	15	120
Eggplant	58	216
Parsley root	23	118
Parsnip	50	192
Potato	96	122
Sweet potato	65	168

[a] As measured by GC.

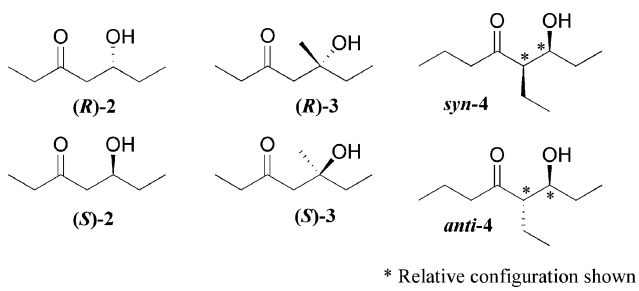


Fig. 2 The various analogous β-hydroxyketones.

Neither the acetate nor the benzoate of the tertiary alcohol **3** was hydrolyzed to any larger extent, regardless of choice of tuber.

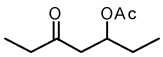
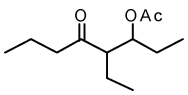
The acetate esters of **4** were hydrolyzed by potato to 97% by hour 48, whereas 92% hydrolysis was achieved by hour 96 using sweet potato (Table 3). With both tubers, the (5*S*,6*S*)-isomer of the alcohol was formed the most slowly. The benzoate esters of **4** was hydrolyzed to 97% in 96 h using potato and to 94% in 72 h using sweet potato (Table 4). Initially, when sweet potato was used, *syn*-**4** was almost exclusively formed. With potato there was also selectivity for *syn*-**4**, although by hour 96 the product mixture approached the expected composition for a non-selective hydrolysis. In line with the results from the previous analogues, the (5*S*,6*S*)-isomer was formed the most slowly. No further conclusions could be drawn because of difficulties in the separation of the *syn* isomers on the enantioselective GC column.

Experimental

General

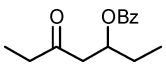
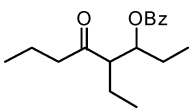
Acetate esters were derived from the corresponding alcohols by treatment with acetic anhydride in pyridine, and benzoate esters by treatment with benzoyl chloride in pyridine according to standard procedures. All substrates were purified by chromatography before use (hence the reported diastereomeric composition of respective alcohols and esters may differ because of partial diastereomeric separation on silica gel). Vegetables were washed under tap water and the outer layer was removed with a potato peeler. Thereafter, they were rinsed in deionized water (MilliQ), dried with tissue paper and cut into thin slices, *ca.* 1.2–3.0 mm thick, depending upon the texture of the plant. Slices were shuffled, so as to make batches as homogenous as possible, and 20 g was transferred to a reaction flask. Plant material was covered in deionized water (MilliQ) and 25 μl of the substrate was added. Flasks were covered with aluminium foil and incubated in a shaking water bath at 25 °C and 120 rpm.

Table 3 Hydrolysis of 5-acetoxy-3-heptanone and 6-acetoxy-5-ethyl-4-octanone: Selectivity after 25% conversion and 50% conversion, shown as relative amounts of the two enantiomers of **2** and pairs of syn/anti diastereomers of **4** respectively, and the relative difference in abundance compared with that of the same isomers of the reagent at t = 0. Additionally, maximum conversion (%) and time for maximal conversion (h) are shown

	5-hydroxy-3-heptanone (**2**) formed after 25% conversion:[a] **Relative amount (%)** *(relative change from start composition (%))*		5-hydroxy-3-heptanone (**2**) formed after 50% conversion:[a] **Relative amount (%)** *(relative change from start composition (%))*		Max conversion (%) and time (h) for conversion	
	(*R*)	(*S*)	(*R*)	(*S*)	Conv.	Time
Start composition	**50**	**50**	**50**	**50**	—	—
Plant						
Potato	**43** *(-14)*	**57** *(+14)*	**47** *(-6)*	**53** *(+6)*	>99	24
Sweet potato	**64** *(+28)*	**36** *(-28)*	**60** *(+20)*	**40** *(-20)*	>99	24
	5-ethyl-6-hydroxy-4-octanone (**4**) formed after 25% conversion: [a]**Relative amount (%)** *(relative change from start composition (%))*.		5-ethyl-6-hydroxy-4-octanone (**4**) formed after 50% conversion: [a]**Relative amount (%)** *(relative change from start composition (%))*		Max conversion (%) and time (h) for conversion	
	Syn	*Anti*	*Syn*	*Anti*	Conv.	Time
Blank, reference	**71**	**29**	**71**	**29**	—	—
Plant						
Potato	**77** *(+8)*	**23** *(-21)*	**75** *(+6)*	**25** *(-14)*	97	48
Sweet potato	**73** *(+3)*	**27** *(-7)*	**74** *(+4)*	**26** *(-9)*	92	96

[a] As measured by GC.

Table 4 Hydrolysis of the benzoates of 5-hydroxy-3-heptanone (**2**) and 6-hydroxy-5-ethyl-4-octanone (**4**): Maximum conversion (%) and time for maximal conversion (h) are shown

	Maximal conversion and time for conversion of benzoate ester to 5-hydroxy-3-heptanone (**2**)[a]	
Plant	Conversion (%)	Time (h)
Potato	>99	48
Sweet potato	77	96
	Maximal conversion and time for conversion of benzoate ester to 5-ethyl-6-hydroxy-4-octanone (**4**)[a]	
Plant	Conversion (%)	Time (h)
Potato	97	96
Sweet potato	94	72

[a] As measured by GC.

All chemical and enantiomeric purities reported were measured by gas chromatography (GC).

For all synthesized compounds ^{1}H-NMR and ^{13}C-NMR spectra of $CDCl_3$-solutions were recorded at 500 MHz and 125 MHz. Chemical shifts were expressed in ppm in relation to tetramethylsilane, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet), coupling constants (Hz) and number of protons. The starting materials employed were obtained from commercial suppliers and used without further purification. Mass spectra were obtained with an HP 6890 GC interfaced to an HP 5973 mass selective detector, in electron impact mode using 70 eV, with helium as the carrier gas. The GC was equipped with a BPX-70 column (30 m × 0.25 mm i.d. × 0.25 μm, SGE Australia). Enantioselective GC was performed with an HP 5890 GC fitted with a CYCLOSILB column (30 m × 0.25 mm i.d. × 0.25 μm, J & W Scientific, USA) and flame ionization detector (FID). Samples were injected directly into the GC without purification or extraction. Anhydrous solvents were used when appropriate and all moisture sensitive reactions were carried out under nitrogen. The assignments of the absolute configurations of all stereoisomers were based on the well-known stereochemical preference of the Amano-PS lipase.[32] Column chromatography was carried out using a Separo medium pressure liquid chromatography (MPLC) system.[33]

Evaluation of experimental conditions

Preparation methods for vegetables

Carrots were prepared according to the general method. One batch was grated with a manual kitchen grater (coarse); a second batch was minced with an electric osterizer (*ca.* 3 min); the third was sliced with a sharp blade, as described in the general method. The results from the various preparation methods were inspected under a light microscope (400× enlargement). The reactions were monitored by GC at hours 1, 4, 7, 21, 24, 27, 30, 48, 69, 72, and 96. The starting material 5-acetoxy-4-methyl-3-heptanone consisted of 66/34 *syn*/*anti* product of 91% chemical purity and was used as such.

Substrate to plant ratio

Carrots were prepared according to the general method. One batch was prepared with the regular ratio of 1 part substrate (25 µl) to 800 parts of fresh plant material (20 g), one with the ratio 1 : 200 (0.1 ml substrate), and one 1 : 80 (0.25 ml substrate). GC measurements were taken at hours 1, 4, 7, 21, 24, 30 and 48. The reaction with the highest concentration of substrate was further monitored at hours 69, 96, 120, 145 and 169.

Screening of vegetables

The β-hydroxyketone **1** was prepared from 3-pentanone and *n*-propanal in a lithium diisopropylamide (LDA)-mediated condensation reaction.[30] The acetate and benzoate esters of **1** consisted of 66/34 *syn*/*anti* product of 99% purity (acetate) and 77/23 *syn*/*anti* product of 96% purity (benzoate) respectively. GC-measurements were performed at hours 3, 24, 48, 72, and 96, unless the reaction was completed sooner.

Investigation of analogues

The β-hydroxyketone **2** was synthesized by an LDA-mediated aldol reaction between 2-butanone and *n*-propanal yielding a racemic product. The β-hydroxyketone **3** with a tertiary hydroxy group was synthesized by a Grignard reaction between methyl magnesium iodide and 3,5-heptanedione.[31] Compound **4** was synthesized by an LDA-mediated aldol reaction between 4-heptanone and *n*-propanal, analogous to **2**, yielding a product 71/29 *syn*/*anti*. The experiments were carried out according to the general method and the progress of reactions was monitored at 3, 24, 48, 72 and 96 h.

Synthesis and characterization of hydroxyketones

5-Hydroxy-4-methyl-3-heptanone (1). Diisopropylamine (42 ml, 0.30 mol) was dissolved in THF (100 ml). The stirred solution was cooled to 0 °C and butyllithium (2.5 M in hexane, 80 ml, 0.20 mol) was added dropwise over 20 min. After 20 min of stirring at 0 °C the temperature was lowered to −80 °C and 3-pentanone (21.0 ml, 0.20 mol) was added dropwise over 20 min. The mixture was stirred at −70 to −80 °C for 30 min before propanal (14.4 ml, 0.20 mmol) was added dropwise over 30 min and the reaction mixture was kept at the same temperature for 1 h. Then NH_4Cl (400 ml, sat., aq.) was added to the reaction mixture, which was allowed to warm to RT. The aqueous phase was extracted 3 times with diethyl ether and the combined organic phases were washed twice with brine and dried over $MgSO_4$. Concentration *in vacuo* gave a yellow oil of 95% purity (29.13 g, 96%). NMR data corresponded with published data.[30] ^{1}H-NMR δ: 3.82 (m, 1H, *syn*), 3.62 (m, 1H, *anti*), 2.43–2.67 (m, 3H), 1.41–1.57 (m, 2H), 1.13 (d, 3H, J = 7.2 Hz), 1.06 (t, 3H, J = 7.2 Hz), 0.98 (t, 3H, J = 7.4 Hz). ^{13}C-NMR *syn* δ: 217.2, 72.8, 49.5, 35.3, 27.1, 10.6, 10.1, 7.8 ppm; *anti* δ: 217.1, 75.2, 50.8, 36.3, 27.8, 14.5, 10.1, 7.8 ppm. GCMS: 126(15), 97(14), 86(37), 70(18), 69(11), 59(16), 57(100), 55(15).

5-Hydroxy-3-heptanone (2). Diisopropylamine (4.2 ml, 30 mmol) was dissolved in THF (20 ml). The stirred solution was cooled to 0 °C and butyllithium (2.0 M in pentane, 10 ml, 20 mmol) was added dropwise over 15 min. After 20 min of stirring at 0 °C the temperature was lowered to −80 °C and 2-butanone (1.44 g, 20 mmol) was added dropwise. The mixture was stirred at −70 to −80 °C for 1 h before propanal (1.44 ml, 20 mmol) was added dropwise and the reaction mixture was kept at the same temperature for 20 min. Then NH_4Cl (30 ml, sat., aq.) was added to the reaction mixture, which was allowed to warm to RT. The aqueous phase was extracted twice with diethyl ether and the combined organic phases were washed twice with brine and dried over $MgSO_4$. Concentration *in vacuo* gave a yellow oil of 72% purity (2.58 g, 71%). After purification by MPLC a fraction of 93% purity (0.84 g) was used for hydrolysis experiments. NMR data corresponded with published data.[34] ^{1}H-NMR δ: 3.97 (m, 1H), 3.04 (s, 1H), 2.42–2.66 (m, 4H), 1.42–1.53 (m, 2H), 1.07 (t, 3H, J = 7.3 Hz), 0.95 (t, 3H, J = 7.4 Hz). ^{13}C-NMR δ: 69.2, 48.3, 37.0, 29.5, 10.0, 7.8 ppm. GCMS: 112(22), 101(43), 83(28), 72(13), 59(43), 57(100), 56(11), 55(13), 43(41).

5-Hydroxy-5-methyl-3-heptanone (3). Magnesium was added to a flask containing iodine (*ca.* 5 mg) which was heated and then cooled before diethyl ether (50 ml) was added, followed by iodomethane (13.72 g, 88 mmol) in diethyl ether (10 ml) at a rate that maintained reflux. Reflux was continued for 20 min and the stirred reaction mixture was cooled to 0 °C before 3,5-heptadione (5.12 g, 40 mmol) in diethyl ether (10 ml) was added. After 1 h at 0 °C and 1 h at RT, ice-water and HCl (10%, aq.) were added subsequently, until all solids were dissolved. The reaction mixture was extracted five times with diethyl ether, and the combined organic phases were washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo* to yield a crude product of 29% purity as a yellow oil (4.73 g, 24%). After purification by MPLC, a fraction of 1.29 g was collected (99% purity). ^{1}H-NMR δ: 3.85 (s, 1H), 2.60 (d, 1H, J = 17.0 Hz), 2.54 (d, 1H, J = 17.0 Hz) 2.45 (q, 2H, J = 7.3Hz), 1.52 (m, 2H), 1.06 (t, 3H, J = 7.3Hz), 0.90 (t, 3H, J = 7.5Hz). ^{13}C-NMR δ: 72.1, 50.8, 38.1, 34.9, 26.4, 8.5, 7.7 ppm. GCMS: 129(7), 126(6), 115(42), 97(12), 73(53), 72(19), 58(5), 57(100), 55(14), 43(49).

5-Ethyl-6-hydroxy-4-octanone (4). Compound **4** was synthesized analogously to **2** starting from 4-heptanone and *n*-propanal. After purification by MPLC 3.29 g (96%) product was obtained. Because of the apparent poor stability of the product for GC analysis, no purity was obtained by GC; by NMR the purity was estimated to be > 95%; 65/35 *syn*/*anti*. ^{1}H-NMR δ: 3.67 (m, 1H, *syn*), 3.63 (m, 1H, *anti*), 2.39–2.56 (m, 3H), 1.37–1.78 (m, 6H), 0.87–0.98 (m, 9H). Because of

overlap between the two diastereomers, NMR data were difficult to interpret fully. The diastereomeric identification was based on predicted selectivity of the synthesis,[30] previously published data for analogous compounds[30] and the *syn* : *anti* ratio determined by GCMS. All signals correlated well with previously published data.[35] ^{13}C-NMR δ: 73.8 (*anti*), 73.2 (*syn*), 57.8 (*syn*), 57.7 (*anti*), 47.1 (*anti*), 46.8 (*syn*), 28.7 (*anti*), 27.6 (*syn*), 22.7 (*anti*), 19.8 (*syn*), 16.9 (*syn*), 16.8 (*anti*), 13.9 (*syn* and *anti*), 12.7 (*syn*), 12.1 (*anti*), 10.7 (*syn*), 10.5 (*anti*) ppm. The diastereomeric identification is tentative, but based on Heathcock's empirical rule[36] in addition to relative intensities of peaks in ^{1}H-NMR spectrum in relation to relative areas of GC chromatograms. GCMS: 154(11), 143(18), 114(26), 99(29), 86(15), 84(14), 83(13), 71(100), 69(13), 59(19), 58(11), 57(16), 56(13), 55(20), 43(47), 41(18).

Conclusions

Hydrolysis of esters is usually a trivial and simple reaction that can be catalyzed efficiently by a number of abiotic and biotic catalysts, but this reaction mediated by whole tissue of vegetables seems to have been more or less overlooked by the scientific community. We believe that the use of plants in organic synthesis is a promising new field of science and we have demonstrated that vegetables can be used as biocatalysts in a simple and robust method for hydrolysis of elimination-prone β-acyloxyketones. The method is straightforward, with most of the benefits of using isolated enzymes, but without the need for additional methods of isolation or rigorous control of reaction conditions. Most reactions proceeded quick enough for practical use for the preparation of chiral alcohols, although the mechanism for the reaction has not been established. The prospect of environmentally friendly and easy preparation of chiral compounds with simple recovery of products is very appealing and we are first in the field to apply this method for hydrolysis of β-hydroxyketones in the practical synthesis of an insect pheromone, with the aim of reducing pesticide protection of crops. We have utilized the method on a preparative scale in the synthesis of all individual enantiomers of 5-hydroxy-4-methyl-3-heptanone (**1**) and this work is to be reported elsewhere. Many more applications of vegetables in organic synthesis remain to be discovered, and in the future this area should develop quickly as the method becomes more recognized.

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