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Chemoenzymatic Synthesis of Stegobinone and Stegobiol, Components of the Natural Sex Pheromone of the Drugstore Beetle (*Stegobium paniceum L.*)

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NADPH-dependent ketoreductases were used for the chemoenzymatic stereoselective synthesis of the two components of the natural sex pheromone of the drugstore beetle. The key step in the asymmetric synthesis was the enzymatic reduction of an α-methyl-1,3-diketone and an α-methyl-β-keto ester, which finally led to the preparation of crystalline stegobinone and stegobiol.

Introduction

There are many well known species of anobiid beetles that cause extensive damage to wooden materials and stored products. The drugstore beetle, *Stegobium paniceum* (known in the United Kingdom as the biscuit beetle),[1] can cause tremendous damage and economic losses to post-harvest and stored grains and packaged food products. The drugstore beetle is a cosmopolitan species that resembles the cigarette beetle and rivals it as a pantry pest. It is more abundant in warmer climate regions.[1] It feeds on any of the household foods and spices, as well as wool, hair, leather, horn, museum specimens, and drugs. It has been known to perforate books and even tin or aluminum foil and lead.

One of the components of the female-produced sex pheromone of this species was isolated by Kuwahara et al.[2] and called stegobinone. Stegobinone is the major crystalline component of this pheromone and its proposed structure is shown in Figure 1. Stegobinone has also been reported to be an attractant of the furniture beetle, *Anobium punctatum*.[3] The minor component of the pheromone was isolated by Kodama et al.[4] and was shown to be stegobiol (**3**, Figure 1).

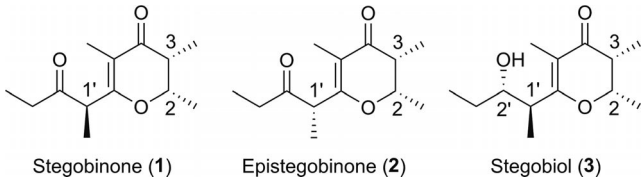

Figure 1. Structures of stegobinone, epistegobinone and stegobiol.

After the establishment of the absolute configuration of these components,[5] several total syntheses were published. The first asymmetric synthesis was reported by Mori et al.[6] This method generated oily stegobinone (**1**), and it was reported that the key cyclization reaction was capricious and difficult to be reproduced. After the development of a more efficient cyclization reaction by Oppolzer and Rodriguez,[7] two asymmetric syntheses of enantiopure stegobinone (**1**) and stegobiol (**3**) were developed.[8] However, one of the two synthetic methods[8b] led to crystalline stegobiol (**3**) and stegobinone (**1**) by using preparative TLC with a low total yield and several synthetic steps. Another synthetic approach, which was accomplished by boronic ester chemistry, was reported by Matteson et al.[9] In this case, stegobiol and stegobinone were produced in pure and crystalline forms; however, many synthetic steps were required. Recently, a new synthetic way for the synthesis of (±)-*epi*-stegobinone was published that reported the use of silacyclopropanes as synthetic intermediates.[10] Presently, the lack of an efficient, high-yielding synthesis of stegobinone requires the development of a practical method to overcome the above-mentioned disadvantages. Such a synthetic method could lead to the highly stereoselective formation of the crystalline stegobinone product, which could be used for the preparation of baited traps for beetles.[11]

Although stegobinone possess a simple structure, a facile synthesis of this molecule seems to be elusive. This is due to the fact that this compound readily epimerizes to 1′-epimer **2** (Figure 1). The 1′-epimer of this compound is strongly repellent to *Stegobium paniceum* even in trace amounts. Racemic **1** is also repellent. Partially crystalline or oily synthetic **1**, which contains an amount of **2** and other diastereomers, is unstable to prolonged storage. Therefore, in order to synthesize stable, crystalline stegobinone, highly

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diastereo- and enantioselective reactions must be accomplished. This crucial observation led us to search for a synthetic approach[7,8b] in which the precursors of stegobinone, **6** and **11** (Figure 2), will be formed in high optical purity and in crystalline form.

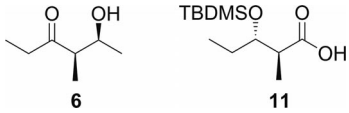


Figure 2. Intermediate compounds for the synthesis of stegobiol and stegobinone.

Results and Discussion

Ketoreductases have proved to be very powerful catalysts for the stereoselective reduction of carbonyl compounds.[12] We have recently reported the stereoselective preparation of α-alkyl-β-hydroxy ketones[13] and α-alkyl-β-hydroxy esters by using these enzymes.[14] Two natural pheromones, sitophilure[15] and sitophilate,[16] have also been synthesized chemoenzymatically by using ketoreductases. In this paper, we present the stereoselective synthesis of the female-produced pheromone of *Stegobium paniceum* by utilizing commercially available ketoreductases.

The stereoselective enzymatic reduction of 2-alkyl-1,3-diketones can provide optically pure α-alkyl-β-hydroxy ketones (Figure 3). We applied this biocatalytic method for the stereoselective conversion of 3-methyl-2,4-hexanodione (**5**) into intermediate compound **6**. As published before,[13] compound **6** can be easily produced quantitatively on a small scale with excellent optical purity (Scheme 1).[13] This reduction was carried out in a phosphate buffer (200 mM, pH 6.5) in the presence of ketoreductases, NADPH, and glucose/glucose dehydrogenase (GDH) for cofactor recycling.

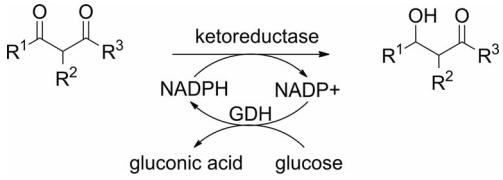
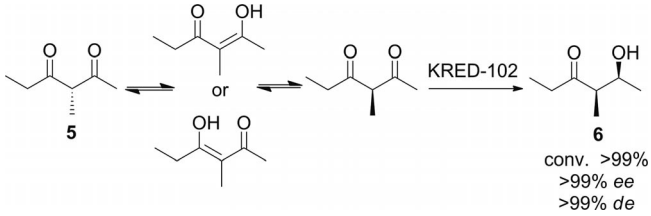
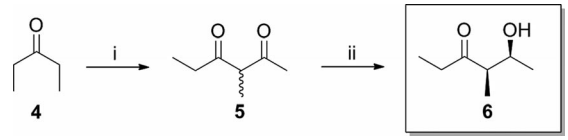


Figure 3. Enzymatic reduction of α-alkyl-1,3-diketones.



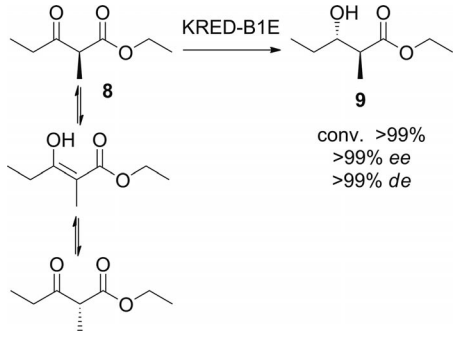
Scheme 1. Enzymatic preparation of hydroxy ketone **6** through dynamic kinetic resolution of diketone **5**.

As shown in Scheme 1, KRED-102 is an excellent catalyst for the regio- and stereoselective transformation of diketone **5**, providing only one of the four stereoisomers of hydroxy ketone **6** without the formation of any byproducts. Furthermore, this reaction was accomplished through dynamic kinetic resolution[17] of chiral diketone **5** to afford intermediate **6** with the desired absolute configuration (2*S*,3*R* enantiomer). Therefore, we applied this efficient biocatalytic procedure on a gram scale (1 g) for the synthesis of hydroxy ketone **6** in high optical purity (Scheme 2). Only two steps were utilized to synthesize **6** starting from commercially available 3-pentanone (**4**). The easy conversion of diketone **4** into diketone **5** followed by enzymatic reduction catalyzed by KRED-102 gave keto alcohol **6** in 78% overall yield and high optical purity (Scheme 2).



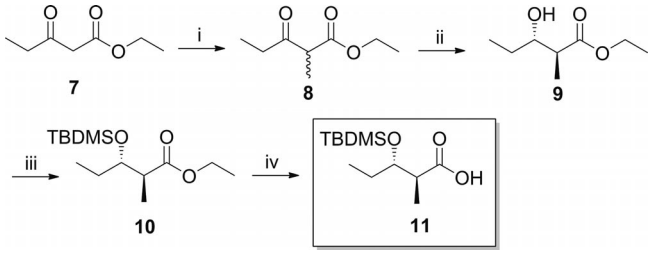
Scheme 2. Chemoenzymatic synthesis of (2*S*,3*R*)-2-hydroxy-3-methyl-4-hexanone. Reagents and conditions: (i)1. LDA, CH_3CHO, dry THF, –78 °C, 99% yield; 2. Jones oxidation, acetone, 0 °C, 90% yield, (89% from 3-pentanone); (ii) KRED-102, NADPH, r.t., 88% yield, >99%*de*, >99%*ee*, overall yield 78%.

Furthermore, the stereoselective reduction of α-alkyl-β-keto esters can provide optically pure α-alkyl-β-hydroxy esters.[16] This result prompted us to apply this biocatalytic method for the reduction of ester **8** to hydroxy ester **9**, a potential precursor of target intermediate **11**. In particular, KRED-B1E and KRED-B1B catalyzed effectively the reduction of chiral substrate **8** to afford desired product **9** with >99% diastereomeric and enantiomeric excess in only 24 h. This reduction also was accomplished by dynamic kinetic resolution, providing the 2*S*,3*S* stereoisomer[16] with 100% conversion (Scheme 3).



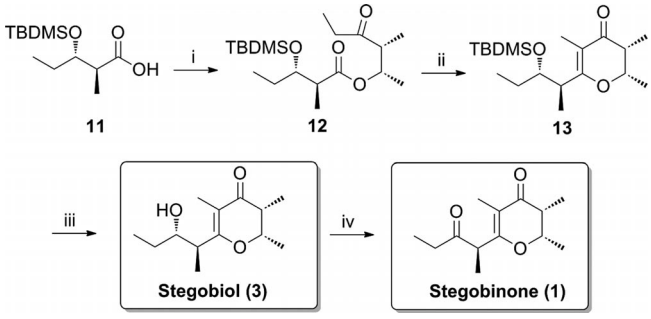
Scheme 3. KRED-B1E-mediated reduction of keto ester **8** by dynamic kinetic resolution.

Therefore, we applied this biocatalytic method to a larger scale (632 mg) to synthesize intermediate **11**. Its synthesis started from commercially available ethyl 3-oxopentanoate (**7**), which was converted quantitatively into keto ester **8**. Hydroxy ester **9** was produced by enzymatic reduction with KRED-B1E. Finally, intermediate **11** was derived by protection with TBDMSCl followed by hydrolysis. The overall yield was 73% starting from keto ester **7** (Scheme 4).



Scheme 4. Chemoenzymatic synthesis of intermediate **11**. Reagents and conditions: (i) K_2CO_3, MeI, dry acetone, reflux, >99% yield; (ii) KRED-B1E, NADPH, r.t., 99% yield, >99% *de*, >99% *ee*; (iii) TBDMSCl, imidazole, DMF, r.t., 85% yield; (iv) NaOH, MeOH/H_2O (1:1), r.t., 87%, overall yield 73%.

In the final step, compounds **6** and **11** were coupled to give ester **12**, which was easily transformed into TBDMS-protected compound **13** by using titanium tetrachloride (5 equiv.) and ethyldiisopropylamine (8 equiv.) in dry CH_2Cl_2. In contrast to the fact that no reproducible results was previously reported,[6] this cyclization was very efficient and easily reproduced and gave 67% yield. Deprotection of compound **13** provided stegobiol (**3**) and after oxidation of **3** with Jones reagent followed by quick purification, crystalline stegobinone (**1**) was isolated in pure form. The absolute configurations of synthesized compounds **1** and **3** were in very good agreement with the previously published data.[6,8b,9] Crystallization was achieved after storage at –20 °C. It is interesting to note that the stegobinone sample, free of byproducts, was crystallized easily because this new synthetic methodology afforded products of high enantio- and diastereoselectivities. The key reaction steps were the high stereoselective transformations provided by the ketoreductases. The overall yield of the final stage was 40% starting from intermediate **11** (Scheme 5).



Scheme 5. Final steps for the synthesis of stegobiol and stegobinone. Reagents and conditions: (i) 1. 2,6-Dichlorobenzoyl chloride, Et_3N, THF, r.t.; 2. **6**, DMAP, C_6H_6, 0 °C to r.t., 83% yield; (ii) $TiCl_4$ (5 equiv.), *i*Pr_2NEt (8 equiv.), CH_2Cl_2, –78 to –15 °C, 67% yield; (iii) HF aq., MeCN, 80% yield; (iv) Jones reagent, acetone, 0 °C, 90% yield.

Conclusions

In conclusion, commercially available ketoreductases were used for the synthesis of the female-produced sex pheromone of the drugstore beetle, *Stegobium paniceum*. Thus, crystalline stegobinone was obtained without detection of the 1′-epimer of this compound. It was very stable after storage at –20 °C for more than one year. The key step for this synthesis was the high stereoselective reduction of α-alkyl-1,3-diketones and α-alkyl-β-keto esters catalyzed by ketoreductases.

Experimental Section

(2*S*,3*R*)-2,3-Dihydro-6-[(1′*S*,2′*S*)-2′-hydroxy-1′-methylbutyl]-2,3,5-trimethyl-4*H*-pyran-4-one (3): To a solution of **13** (248 mg, 0.72 mmol) in CH_3CN was added 40% HF (0.1 mL, 1.8 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 h, then diluted with Et_2O and a satd. $NaHCO_3$ solution and separated. The aqueous layer was extracted with Et_2O. The combined organic layers were washed with a satd. $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc, 5:1). Yield: 80%, 130 mg (oil). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.46–4.53 (m, 1 H), 3.54–3.61 (m, 1 H), 2.82–2.88 (m, 1 H), 2.35–2.42 (m, 1 H), 1.90 (d, *J* = 7.0 Hz, 1 H), 1.75 (s, 3 H), 1.53–1.63 (m, 1 H), 1.37–1.46 (m, 1 H), 1.32 (d, *J* = 6.5 Hz, 3 H), 1.18 (d, *J* = 7.0 Hz, 3 H), 1.04 (d, *J* = 7.5 Hz, 3 H), 1.00 (t, *J* = 7.5 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 9.2, 9.4, 10.0, 14.6, 15.8, 28.2, 40.9, 43.6, 75.2, 76.6, 109.2, 172.7, 197.0 ppm. MS: *m*/*z* (%) = 226.34 (11.1), 168 (72.1), 141 (20.6), 139 (13.4), 125 (9.7), 124 (9.3), 112 (100). $[a]_D^{25}$ = –112 ± 3 (*c* = 0.26, $CHCl_3$) {ref.[6b] $[a]_D^{19}$ = –110 ± 6 (*c* = 0.42, $CHCl_3$)}.

(2*S*,3*R*)-2,3-Dihydro-6-[(1′*R*)-1′-methyl-2′-oxobutyl]-2,3,5-trimethyl-4*H*-pyran-4-one (1): To a stirred and cooled solution of **3** (130 mg, 0.57 mmol) in acetone (15 mL) was slowly added Jones reagent at 0 °C over 40 s. Then the mixture was diluted with Et_2O and a satd. aqueous sodium thiosulfate, stirred over 5 min and the organic layer was separated. The aqueous layer was extracted with Et_2O. The combined organic layers were washed with water, a satd. $NaHCO_3$ solution, and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc, 4:1) to give pure and crystalline stegobinone. Yield: 90%, 115 mg. Stegobionone was crystallized when left overnight in a refrigerator at –20 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.43–4.47 (m, 1 H), 3.63 (q, *J* = 7.0 Hz, 1 H), 2.35–2.52 (m, 3 H), 1.79 (s, 1 H), 1.30 (d, *J* = 7.0 Hz, 3 H), 1.28 (d, *J* = 7.0 Hz, 3 H), 1.06 (t, *J* = 7.0 Hz, 3 H), 1.03 (d, *J* = 7.5 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 7.9, 9.36, 9.39, 12.8, 15.7, 33.9, 43.7, 49.2, 77.2, 109.4, 169.0, 197.0, 207.5 ppm. MS: *m*/*z* (%) = 224.18 (8.6), 168 (44.5), 113 (28.4), 83 (22.9), 57 (100). M.p. 52.0–53.0 °C (ref.[2b] 52.5–53.5 °C). $[a]_D^{25}$ = –285 ± 6 (*c* = 0.21 $CHCl_3$) {ref.[8b] $[a]_D^{24}$ = –283 (*c* = 0.07 $CHCl_3$)}.

Supporting Information (see footnote on the first page of this article): Additional experimental procedures and characterization data, GC traces, and copies of selected ^{1}H and ^{13}C NMR spectra.

Acknowledgments

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