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Tetrahedron: *Asymmetry* 16 (2005) 3507–3511

Microbial oxidation/amidation of benzhydrylsulfanyl acetic acid. Synthesis of (+)-modafinil

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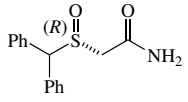
Received 6 July 2005; accepted 18 July 2005
Available online 9 September 2005

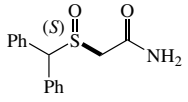
Abstract—A highly enantioselective oxidation of benzhydrylsulfanyl acetic acid to the corresponding (*S*)-sulfinyl carboxylic acid was achieved employing the fungus *Beauveria bassiana* in very good yield. This product was amidated using the bacteria *Bacillus subtilis* to afford (*S*)-modafinil in good yield.

1. Introduction

Modafinil is a psychostimulant agent that has gained a lot of attention because of its recent approval by the FDA for the treatment of excessive daytime sleepiness and because of its lack of abuse liability.[1,2] Recent work suggests that modafinil might also be of utility as a treatment of attention deficit/hyperactivity disorder (ADHD), and in treating opioid-induced sedation.[3] Although modafinil has a stereogenic center at the sulfur atom, the racemic sulfoxide is marketed as Provigil.[4] The exact mechanism of action of modafinil is still unknown. Efforts are directed toward finding their mechanism of action and the physiological differences of its enantiomers.



(-)-modafinil



(+)-modafinil

Several methods have been devised to separate the two enantiomers of modafinil. Cephalon is currently applying chiral stationary phase chromatography to separate the enantiomers on a large scale.[1] The separation of the diastereomeric salts of modafinil acid and the determination of the absolute stereochemistry of (+)- and (−)-modafinil were initially reported by Prisinzano et al.[5] The crystal structures of both enantiomers of modafinil have also been reported.[6] We reported a practical method for the preparation of both enantiomers of modafinil and its analog adrafinil, and proved unequivocally their absolute configuration, via the preparation of a diastereomeric mixture of chiral thiazolidinethione derivatives.[7]

The asymmetric syntheses of dextro- and levo-modafinil should be of great interest because of the importance of studying the biological activity of each enantiomer.[8] Several chemical methods for the enantioselective preparation of sulfoxides are currently available.[9] Chiral sulfoxides can be prepared by the addition of Grignard reagents to chiral sulfinyl menthyl esters,[10] by oxidation with Kagan's[11] and Modena's[12] chiral complexes, and Davis' chiral oxaziridines,[13] amongst others.[14] Enantioselective sulfoxidation can also be carried out utilizing enzymatic and microbial methods.[15] Isolated enzymes, such as pig liver FAD-dependent monooxygenases,[16] chloroperoxidases from *Caldariomyces fumago*,[17] dioxygenases from *Pseudomonas* sp.,[18,19] cyclohexanone monooxygenase from *Acinetobacter calcoaceticus*,[20] and non-redox proteins[21] have been used in the syntheses of chiral sulfoxides. Whole-cell oxidations are generally preferred over the enzymatic oxidations to allow the intracellular recycle of NAD(P)H necessary in the biotransformation.[22] One of the problems frequently encountered in the use of microorganisms is the number of side reactions that might occur because of the

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doi:10.1016/j.tetasy.2005.07.028

presence of several enzymes. However, sometimes several transformations on a substrate are desirable. Herein, we report the synthesis of racemic and enantiomerically enriched (+)-modafinil, via a new microbial oxidation–amidation transformation.

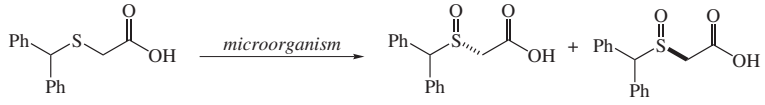
Several wild-type and genetically modified microorganisms containing oxidative enzymes are capable of oxidizing sulfides to chiral sulfoxides with high enantioselectivity.[22] Although better enantioselectivities have been achieved with isolated enzymes, the use of whole-cell microorganisms is more practical in the preparative production of metabolites. Interestingly, some sulfur containing compounds can be oxidized to the opposite enantiomeric sulfoxides by selecting the appropriate enzyme or microorganism.[18,19] Herein, we report the first enantioselective synthesis of (+)-modafinil.

2. Results and discussion

We selected benzhydrylsulfanyl acetic acid **1**, easily prepared in one step,[5] as our initial substrate for screening several microorganisms known to possess enzymes capable of oxidizing the sulfanyl group.[23] A popular microorganism among synthetic chemists is the fungus *Beauveria bassiana*.[24] This fungus is known to possess oxidative enzymes to hydroxylate unactivated carbons[25] and also oxidize sulfanyl compounds.[26] We were surprised to observe that *B. bassiana* oxidized sulfanyl **1** not only in very good yield but also in high enantioselectivity furnishing the enantiomer (*S*)-**2** (Table 1). *B. bassiana* has been used successfully in several biotransformations, but usually with not very good enantioselectivity with this microorganism. Other fungi employed (entries 2–8) gave poor enantioselectivity, but one of them, *Microsporum gypseum*, gave the sulfinyl product in better yield (entry 3). We also found that some bacteria could give the sulfinyl product, but in low yield and low enantioselectivity (entries 9–12). We then turned our attention to recombinant *Escherichia coli*, expressing naphthalene dioxygenase (NDO) and toluene dioxygenase (TDO) from *Pseudomonas* sp. NCIB 9816-4 and *Pseudomonas putida* F1, respectively. These two dioxygenases have been shown to oxidize sulfides to enantio-complementary sulfoxides.[18,19] Indeed, these recombinant strains gave the expected opposite sulfinyl products, but in poor yields and also poor enantioselectivities. The recombinant *E. coli* CPMO and CHMO, overexpressing cyclopentanone monooxygenase from *Pseudomonas* sp. NCIMB 9872 and cyclohexanone monooxygenase from *Acinetobacter* sp. NCIMB 9871, respectively, also gave the sulfinyl product in 16% and 73% yields with poor enantioselectivity, but with complementary stereochemistry.

In an effort to find microorganisms that could oxidize the sulfanyl group to the complementary enantiomeric sulfoxide of the one obtained with *B. bassiana*, another substrate, benzhydrylsulfanyl acetamide **3**, was then investigated (Table 2). Compound **3** was prepared from benzhydrylsulfanyl acetic acid via acid chloride formation followed by addition to a solution of ammonium hydroxide in THF in 96% yield. Compound **3** was also directly prepared from benzhydrylsulfanyl acetic acid and urea–imidazole under microwave irradiation[27] in 85% yield and also by lipase-mediated ammoniolysis in 91% yield when Novozyme-435 was used.[28] Interestingly, the biotransformation of **3** with *B. bassiana* afforded the (*R*)-isomer of modafinil in good yield, but with low ee. Other microorganisms provided the oxidized (*S*)-isomer also with low yields and enantioselectivities. *M. gypseum* and *Thamnidium elegans* oxidized both the sulfanyl acid **1** and also sulfanyl amide **3** (entries 2 and 3). When sul-

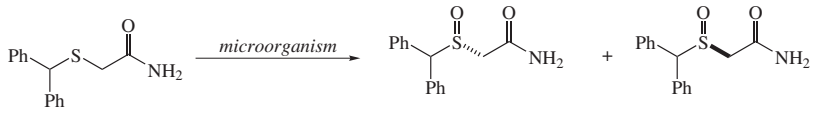
Table 1. Microbial sulfoxidation of benzhydrylsulfanyl acetic acid



1 (*R*)-**2** (*S*)-**2**

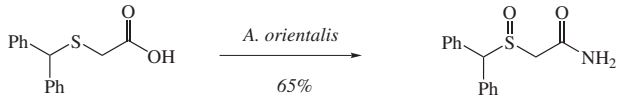
Entry	Microorganism	Major isomer	ee (%)	Yield (%)
1	*Beauveria bassiana* (ATCC-7159)	*S*	99	89
2	*Mortierella isabellina* (ATCC-38063)	*S*	33	66
3	*Microsporum gypseum* (ATCC-11395)	*S*	1	94
4	*Thamnidium elegans* (ATCC-18191)	*S*	3	77
5	*Caldariomyces fumago* (ATCC-16373)	*S*	9	18
6	*Cylindrocarpon radicicola* (ATCC-11011)	*R*	5	6
7	*Cunninghamella echinulata* (ATCC-9244)	*S*	16	38
8	*Mortierella elongata* (NRRL-5513)	*S*	3	19
9	*Sphingomonas* sp. HXN-200	*R*	40	32
10	*Rhodococcus rhodochrous* (ATCC-21197)	*R*	0.6	17
11	*Helminthosporium* sp. (NRRL-4671)	*S*	3	36
12	*Bacillus subtilis* IFO-3108	—	—	2
13	*Escherichia coli* NDO (pDTG141)	*S*	36	16
14	*Escherichia coli* TDO (pDTG601A)	*R*	37	40
15	*Escherichia coli* CPMO	*S*	36	16
16	*Escherichia coli* CHMO	*R*	21	73

Table 2. Microbial sulfoxidation of benzhydrylsulfanyl acetamide



3 (*R*)-modafinil (*S*)-modafinil

Entry	Microorganism	Major isomer	ee (%)	Yield (%)
1	*Beauveria bassiana* (ATCC-7159)	*R*	22	67
2	*Microsporum gypseum* (ATCC-11395)	*S*	49	37
3	*Thamnidium elegans* (ATCC-18191)	*S*	15	42
4	*Amycolatopsis orientalis* (ATCC-19795)	*S*	5	91
5	*Escherichia coli* CPMO	*S*	47	6
6	*Escherichia coli* CHMO	*R*	25	31
7	*Escherichia coli* NDO (pDTG141)	—	—	2
8	*Escherichia coli* TDO (pDTG601A)	—	—	2



1 (±)-modafinil

Scheme 1. Biotransformation of **1** with *Amycolatopsis orientalis*.

fanyl acetamide **3** was biotransformed with *Amycolatopsis orientalis*, modafinil was obtained in very good yield but with almost no enantioselectivity (entry 4).

Strikingly, when benzhydrylsulfanyl acetic acid **1** was fermented with the fungus *A. orientalis*, both amidation of the carboxylic acid and oxidation of the sulfanyl to the sulfoxide group took place, albeit with no enantioselectivity, furnishing racemic modafinil (Scheme 1). Interestingly, only a few isolated cases of direct amidation of carboxylic acids employing microorganisms can be found in the literature.[29] A lot of work has been devoted to the enzymatic hydrolysis of nitriles to furnish amides with high enantioselectivity.[30]

These results directed us to study the biotransformation of racemic and enantiomerically pure β-sulfinyl carboxylic acids **2** with *A. orientalis* (Table 3). It is noteworthy that lipase-mediated ammoniolysis[28] and microwave accelerated amidation[27] reactions were unsuccessful with compound **2**. When racemic **2** was used as substrate with *A. orientalis*, the (*S*)-modafinil was obtained in low yield and low enantioselectivity (entry 1). When enantiopure (*R*)-**2** and (*S*)-**2** were used as substrates, prepared by hydrolysis of chiral thiazolidinethione diastereomers,[7] (*R*)- and (*S*)-modafinil were obtained in low yields without racemization (entries 2 and 3). Several *Bacillus* sp. were also screened for the amidation of sulfinyl carboxylic acid **2**. A better yield was obtained for the amidation of racemic-**2** with *B. subtilis* var. *niger*, but no selectivity was observed (entries 2 and 3). Also, better yields were obtained in the amidation of enantiopure **2** with *B. subtilis* (entries 4–6).

3. Conclusion

To the best of our knowledge, this is the first report of a biocatalytic one-flask oxidation/amidation utilizing a whole-cell transformation. The synthesis of racemic modafinil described here, was accomplished utilizing two strategies: (a) one chemical and one-flask–two microbial transformations (65% overall yield) and (b) one chemical, one chemo-enzymatic and one microbial transformations (81% overall yield). The enantioselective synthesis of (*S*)-modafinil was accomplished in three steps, one chemical step and two microbial

Table 3. Microbial amidation of benzhydrylsulfinyl acetic acid

2 modafinil

Entry	Microorganism	**2**	Modafinil (%)	ee (%)
1	*Amycolatopsis orientalis* (ATCC-19795)	*R*,*S*	43 (*S*)	38
2	*Amycolatopsis orientalis* (ATCC-19795)	*R*	100 (*R*)	22
3	*Amycolatopsis orientalis* (ATCC-19795)	*S*	100 (*S*)	28
4	*Bacillus subtilis* var.*niger* (IFO-3180)	*R*,*S*	—	81
5	*Bacillus subtilis* var.*niger* (IFO-3180)	*R*	100 (*R*)	60
6	*Bacillus subtilis* var.*niger* (IFO-3180)	*S*	100 (*S*)	68

transformations (60% overall yield). These syntheses of modafinil are extremely short and represent low environmental impact chemical processes.

4. Experimental

4.1. General information

The microorganisms used were obtained from the American Type Culture Collection (ATCC), except for the strains obtained from the research groups cited in the Acknowledgments section. Enantiomeric excesses were determined by chiral HPLC using a Cyclobond I-200 RSP column (250 × 4.6 mm).

4.2. Benzhydrylsulfanyl acetic acid 1

This compound was prepared according to the procedure of Prisinzano et al.[5] The synthesis employed benzhydrol (50.0 g, 271.4 mmol) and thioglycolic acid (25.0 g, 271.4 mmol) to give the title compound as a white solid: 69.2 g (99% yield); mp 126–129 °C. Spectroscopic data were identical to the lit. data.[5]

4.3. 2-Benzhydrylsulfanyl acetamide 3

4.3.1. Chemical method. This compound was obtained from the corresponding acid chloride. To a solution of acid **1** (777 mg, 3 mmol) in benzene was added $SOCl_2$ (833 mg, 7 mmol). The solution was heated to reflux for 1 h. The solvent was evaporated to give a yellow oil: 832.1 mg (99.9%). A solution of benzhydrylsulfanyl acetyl chloride (1.089 g, 4.21 mmol) in CH_2Cl_2 (10 mL) was added to a solution of NH_4OH–THF (3:2, 30 mL) at 0 °C. The reaction was stirred for 1 h. The reaction mixture was then treated with H_2O (20 mL) and extracted with CH_2Cl_2 (2 × 30 mL). The organic layer was washed with saturated $NaHCO_3$ (20 mL) and H_2O (20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (3 × 10 cm). Elution with 9:1 $CHCl_3$–MeOH gave a light yellow solid: 1.044 g (96%).

4.3.2. Chemo-enzymatic method. To a solution of benzhydrylsulfanyl acetic acid (259 mg, 1.0 mmol) in *tert*-butanol (28 mL) was added ammonium carbamate (79 mg, 1.0 mmol) and Novozyme-435 (100 mg). The reaction flask was closed tightly and the reaction stirred at 60 °C for 7 days. The reaction mixture was filtered through a cotton plug and concentrated under vacuum to give a colorless turbid oil. The crude oil was purified by flash column chromatography on silica gel (2 × 9 cm). Elution with hexanes–ethyl acetate (3:2) furnished the sulfanyl amide as a light yellow solid: 232 mg (90% yield). Silica gel TLC R_f 0.26 (1:1 hexanes–ethyl acetate); silica gel TLC R_f 0.60 (9:1 chloroform–methanol); mp 109–110 °C; ^{1}H NMR ($CDCl_3$) δ 7.43–7.31 (10H, m), 6.52 (1H, br s), 5.87 (1H, br s) 5.17 (1H, s), 3.08 (2H, s); ^{13}C NMR ($CDCl_3$) δ 171.5 (CO), 140.4 (2C), 128.9 (4CH), 128.4 (4CH), 127.8 (2CH), 54.9 (CH), 35.7 (CH_2).

4.4. (+)-(*S*)-(Diphenylmethanesulfinyl)acetic acid (+)-2

A culture of *B. bassiana* (ATCC-7159) was obtained from ATCC and transferred onto potato dextrose agar slants using techniques described by ATCC and those provided with the culture. The culture was grown at 28 °C for 7 days, sealed, and stored at 4 °C. Subculturing was performed every two weeks, with cultures ready for use after 5 days of growth at 28 °C. Stage I cultures were grown from potato dextrose agar slants in 25 mL of Iowa medium in 125 mL DeLong flask. Flasks were shaken at 250 rpm and 28 °C for 72 h. Stage II cultures were grown from Stage I cultures in 200 mL of Iowa medium in 1 L DeLong flasks. After 24 h of growth, substrate (200 mg, 0.77 mmol) was added as a solution in dimethylformamide (1 mL). The reaction was monitored by thin-layer chromatography by taking samples at 24, 48, 72, and 144 h. After 5–7 days, the fermentation broth was filtered through a cheesecloth. The filtrate was loaded into a glass column containing a Dowex 1X2-200 ion-exchange resin (previously washed with 0.5 M NaOH solution (50 mL) and deionized water (100 mL)). The crude sulfinyl-acid was eluted with 0.5 M HCl solution. The fractions containing the sulfinyl-acid were extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated to give the title product as a white solid: 189 mg (89%). Silica gel TLC R_f 0.36 (7:3 chloroform–methanol); mp 118–120 °C; $[\alpha]_D^{22} = +38.3$ (*c* 1.0, CH_3OH); ee 99.1%, Chiral HPLC analysis. Column: Cyclobond I-200 RSP: UV detector: λ 225 nm; solvent: 0.02 M phosphate buffer (pH 3)-acetonitrile, 85:15; flow rate: 0.6 mL/min; retention time: (+)-**2**, 34.8 min; (−)-**2**, 36.8 min.

IR ν 2925, 2778, 2527, 1716, 1500, 1451, 1287, 1187, 1016, 750, 704 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.56–7.51 (4H, m), 7.44–7.32 (6H, m), 5.40 (1H, s), 3.41 (1H, d, $J = 13.7$ Hz), 3.23 (1H, d, $J = 13.7$ Hz); ^{1}H NMR (acetone-d_6) δ 7.63–7.57 (4H, m), 7.47–7.33 (6H, m), 5.35 (1H, s), 3.62 (1H, d, $J = 14.2$ Hz), 3.41 (1H, d, $J = 14.2$ Hz); ^{13}C NMR ($CDCl_3$) δ 166.6 (CO), 134.4 (C), 133.9 (C), 129.8 (2CH), 129.6 (2CH), 129.2 (2CH and CH), 129.0 (CH), 128.9 (2CH), 71.5 (CH), 50.9 (CH_2); ^{13}C NMR (acetone-d_6) δ 167.3 (CO), 137.6 (C), 135.8 (C), 129.8 (2CH), 129.1 (2CH), 128.7 (2CH), 128.5 (2CH), 128.2 (CH), 128.1 (CH), 71.4 (CH), 55.4 (CH_2).

4.5. (+)-(*S*)-(Diphenylmethanesulfinyl)acetamide (+)-modafinil

Stage I cultures of *B. subtilis* were grown from nutrient agar slants in 25 mL of Iowa medium in 125 mL DeLong flask. Flasks were shaken at 250 rpm and 28 °C for 72 h. Stage II cultures were grown from stage I cultures in 200 mL of Iowa medium in 1 L DeLong flasks. After 24 h of growth, (+)-(diphenylmethanesulfinyl)acetic acid (200 mg, 0.77 mmol, ee 100%) was added as a solution in dimethylformamide (1 mL). The reaction was monitored by thin-layer chromatography by taking samples at 24, 48, 72, and 144 h. After 7 days, the cells were separated by centrifugation at 4 °C. The decanted

fermented broth was extracted with chloroform in a liquid–liquid continuous extractor. The organic layer was concentrated under vacuum to give a light yellow oil. The oil residue was purified by flash column chromatography on silica gel (2 cm × 10 cm). Elution with gradient 1–5% MeOH–$CHCl_3$ afforded the modafinil as white solid 135 mg (68%). Silica gel TLC R_f 0.48 (9:1 chloroform–methanol); mp 158–159 °C; $[\alpha]_D^{22} = +79$ (*c* 1.0, $CHCl_3$); ee 100%, Chiral HPLC analysis. Column: Cyclobond I-200 RSP: UV detector: λ 225 nm; solvent: 0.02 M phosphate buffer (pH 3)-acetonitrile, 85:15; flow rate: 0.6 mL/min; retention time: (+)-**1**, 23.0 min; (−)-**1**, 25.1 min. IR *v* 3383, 3314, 3257, 3191, 1690, 1617, 1495, 1376, 1027, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51–7.48 (2H, m), 7.45–7.32 (8H, m), 7.07 (1H, br s), 5.88 (1H, br s), 5.24 (1H, s), 3.47 (1H, d, $J = 14.2$ Hz), 3.14 (1H, d, $J = 14.2$ Hz); ^{13}C NMR ($CDCl_3$) δ 166.5 (CO), 134.7 (C), 134.3 (C), 129.62 (2CH), 129.58 (2CH), 129.1 (2CH), 128.98 (3CH), 128.8 (CH), 71.6 (CH), 52.0 (CH_2).

Acknowledgments

This research was supported by the National Science Foundation (EEC-0310689). We thank Prof. Jack Rosazza for his invaluable advice and encouragement. We thank Proffers. Rebecca Parales (U California-Davis) and David T. Gibson (U Iowa) for the *E. coli* NDO and TDO, Prof. Marko Mihovilovic (U Austria) for the *E. coli* CHMO and CPMO, Prof. Karl-H. Engesser (U Stuttgart) for *Sphingomonas* sp. HXN-200, and Dr. Kerry O'Donnell (USDA) for *Helminthosporium* strains.

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