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Tetrahedron: *Asymmetry*

Preparation of (*R*)- and (*S*)-6-hydroxybuspirone by enzymatic resolution or hydroxylation

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Abstract—6-Hydroxybuspirone is an active metabolite of the antianxiety drug buspirone. The (*R*)- and (*S*)-enantiomers of 6-hydroxybuspirone were prepared using an enzymatic resolution process. L-Amino acid acylase from *Aspergillus melleus* (Amano Acylase 30000) was used to hydrolyze racemic 6-acetoxybuspirone to (*S*)-6-hydroxybuspirone in 95% ee after 45% conversion. The remaining (*R*)-6-acetoxybuspirone with 88% ee was converted to (*R*)-6-hydroxybuspirone by acid hydrolysis. The ee of both enantiomers could be improved to 99% by crystallization as a metastable polymorph. (*S*)-6-Hydroxybuspirone was also obtained in 88% ee and 14.5% yield by hydroxylation of buspirone using *Streptomyces antibioticus* ATCC 14890.
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1. Introduction

Buspirone (Buspar®) **1** is a drug used for treatment of anxiety and depression that is thought to produce its effects by binding to the serotonin 5HT1A receptor.[1,2] Mainly as a result of hydroxylation reactions, it is extensively converted to various metabolites[3] and blood concentrations return to low levels a few hours after dosing.[4] A major metabolite, 6-hydroxybuspirone **2**, produced by the action of liver cytochrome P450 CYP3A4 is present at much higher concentrations in human blood than buspirone itself. This metabolite has anxiolytic effects in an anxiety model using rat pups and binds to the human 5-HT1A receptor.[4] Although the metabolite has only about a third of the affinity for the human 5HT1A receptor as buspirone, it is present in human blood at 30–40 times higher concentration than buspirone following a dose of buspirone, and therefore may be be responsible for much of the effectiveness of the drug.[5–7] For the development of 6-hydroxybuspirone as a potential antianxiety drug, the preparation and testing of the two enantiomers as well as the racemate was of interest. Both the (*R*)- and (*S*)-enantiomers, separated by chiral HPLC, were effective in tests using a rat model of anxiety.[5,6] Whereas the (*R*)-enantiomer showed somewhat tighter binding and specificity for the 5HT1A receptor,[5] the (*S*)-enantiomer had the advantage of being cleared more slowly from the blood.[6] Herein, we report an alternative means of preparation of the enantiomers, the enzymatic resolution of 6-acetoxybuspirone **3** and crystallization techniques to enhance the ee of the enzymatic products. Direct hydroxylation of buspirone to (*S*)-6-hydroxybuspirone by *Streptomyces antibioticus* ATCC 14980 is also described.

2. Results and discussion

2.1. Resolution of 6-acetoxybuspirone

Screening of 19 lipases for the resolution of racemic 6-hydroxybuspirone by acetylation with neat vinyl acetate or 5% vinyl acetate in toluene gave no reaction. With succinic anhydride as the acylating agent, some of the lipases were active, but the best ee was only 15%. Resolution by enzymatic hydrolysis of chemically synthesized 6-acetoxybuspirone was more successful.

6-Hydroxybuspirone **2** was found to be unstable in a potassium phosphate buffer (pH 7) while the product appeared by HPLC analysis (by comparison to a

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standard) to be the lactone rearrangement product **4** (Fig. 1). The formation of the lactone under mildly basic conditions has already been described.[3] Therefore, screening reactions were carried out at pH 6, where 6-hydroxybuspirone is more stable. All lipases and proteases in our screening collection were screened for the resolution of racemic 6-hydroxybuspirone by enantioselective hydrolysis in pH 6 phosphate buffer containing 10% toluene. Amano Lipase N from a strain of *Rhizopus* was initially identified as the most selective enzyme. A resolution batch starting with 1 g of 6-acetoxybuspirone gave 395 mg (44% HPLC yield) of (*S*)-6-hydroxybuspirone and 471 mg of (*R*)-6-acetoxybuspirone (47% HPLC yield), both near 100% ee.

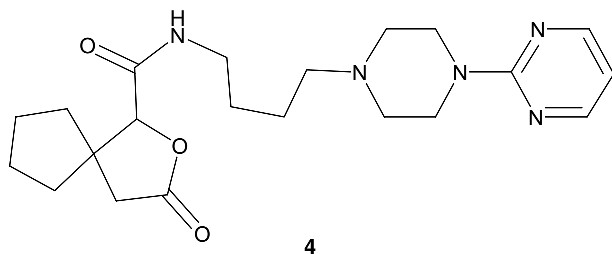

Figure 1. Lactone rearrangement product.

The production of lipase N has now been discontinued by Amano. Old lots from their inventory were less active and less selective. As a result, Amano Acylase 30000 from *Aspergillus melleus* was then chosen for scale-up. The reactions used for the resolution are shown in Figure 2. In preliminary studies, the reaction was tried in a buffer with or without the addition of eight different solvents. The highest *E* value[8] was obtained in a biphasic reaction mixture with 10% toluene. The time course of a 32-g reaction using Acylase 30000 is shown in Figure 3. The *E* value for the process was 121. As usual for this type of resolution, the reaction could be stopped early to give a higher ee of (*S*)-alcohol or run further to improve the ee of the remaining (*R*)-acetate. The enzyme reaction was stopped at high and lower ee of the two products, to determine the limitations of the downstream improvement of ee by crystallization (Table 1).

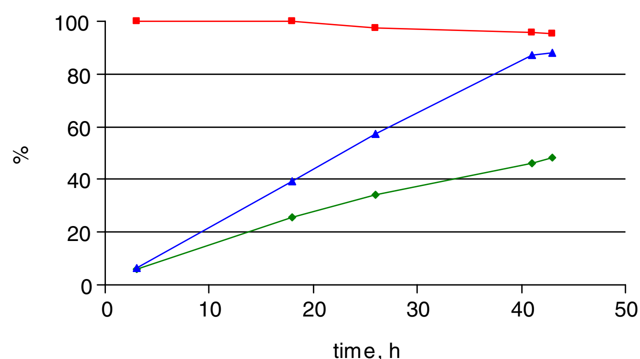

Figure 3. Time course of resolution of 6-acetoxybuspirone **3** using Acylase 30000. Kinetics of a 32.5-g resolution. Reaction conditions: 4.5 g of Amano Acylase 30000 (*Aspergillus melleus*), 540 mL of 50 mM potassium phosphate buffer pH 6 and 60 mL of a toluene solution containing 32.5 g of racemic 6-acetoxybuspirone, stirred at 25 °C. (▲) ee (*R*)-acetate **3b**; (■) ee (*S*)-alcohol **2b**; (◆) conversion.

Batch 1 was stopped after 48% conversion to obtain the (*S*)-alcohol with 95.2% ee. Batch 2 was stopped after 53% conversion to obtain the (*R*)-acetate with 98.1% ee. The reaction could also be stopped early to obtain the (*S*)-alcohol with high ee, and then the isolated (*R*)-acetate could then be subjected to further enzymatic hydrolysis to upgrade the ee. Batch 3 started with the recycled (*R*)-acetate with 83.9% ee and was upgraded to 97.0% ee by a second round of enzymatic hydrolysis.

(*S*)-6-Hydroxybuspirone was isolated from the reaction as a mixture with the (*R*)-acetate by extraction with ethyl acetate followed by crystallization from acetonitrile. Three polymorphic forms of homochiral 6-hydroxybuspirone free base, N-1, N-2, and N-3, have

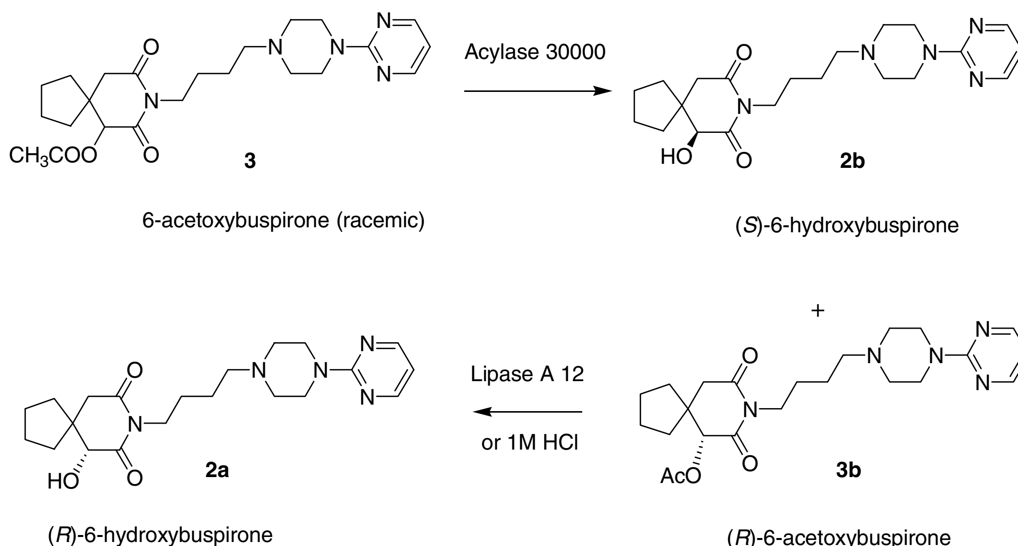

Figure 2. Enzymatic resolution of 6-acetoxybuspirone.

Table 1. Resolution of 6-acetoxybuspirone with Acylase 30000

Batch	Input 6-Acetoxybuspirone (g)	Conversion (%)	(*S*)-Alcohol		(*R*)-Acetate	
			g	ee[a] (%)	g	ee (%)
1	32.5 (racemic)	48.3	13.3	95.2	17.2	88.0
2	34.0 (racemic)	53.3	12.6	90.1	12.2	98.1
3	27.1 (*R*, 83.9% ee)	28.8	10.1	73.1	18.5	97.0

6-Acetoxybuspirone dissolved in toluene (60 mL solution) was treated with Amano Acylase 30000 (4.5 g) in 540 mL 50 mM potassium phosphate buffer, pH 6 at 25 °C.

[a] Ees of alcohol and acetate were determined by chiral HPLC using a Chiralpak AD column.

been characterized. N-1 is the most stable form at room temperature but is enantiotropic with the N-2 form. The N-3 form is the least stable form. Recrystallization of the N-1 form with an ee of <100% yields solid with a decreased ee and mother liquor with an enhanced ee. Clean recrystallization of the N-2 form has been experimentally elusive, so the result of this process is undetermined. In contrast, recrystallization as the N-3 form gives a marked improvement in ee. This metastable form can be obtained by crystallization in the presence of high concentrations of 6-acetoxybuspirone (the chirality is immaterial). Apparently, nucleation of the more stable forms is suppressed under these conditions.

The remaining (*R*)-6-acetoxybuspirone was isolated from the mother liquor/wash following the succinylation of the residual (*S*)-6-hydroxybuspirone as described. This allowed the partition of the (*R*)-6-acetoxybuspirone into ethyl acetate and the succinate derivative into a pH 9 aqueous phase.

Alkaline hydrolysis of the isolated (*R*)-acetate was not practical because of the formation of a lactone impurity (Fig. 1). Lipase A 12 from *Aspergillus niger*, which also preferentially hydrolyzed the (*S*)-acetate but had less selectivity, could be used for hydrolysis of the isolated (*R*)-acetate at pH 6 to give the (*R*)-alcohol as described. However, acid hydrolysis of the isolated (*R*)-acetate was efficient, and enzymatic hydrolysis was not required.

2.2. Microbial hydroxylation of buspirone

Cultures known to carry out hydroxylation reactions were screened for the hydroxylation of buspirone (Fig. 4) to 6-hydroxybuspirone in shake flasks. More than 20 cultures were found to produce 6-hydroxybuspirone, predominantly the (*S*)-enantiomer, but yields were low due to the production of other metabolites. *S. antibioticus* ATCC 14890 gave the best yield of about 20–25% in flasks, with a 14.5% yield upon scale-up into a bioreactor.

On a 4-L scale, with an input of 4 g buspirone·HCl, ca. 604 mg of 6-hydroxybuspirone was obtained in the whole broth prior to processing. About 32% of the buspirone remained unreacted when the biotransformation was terminated. The majority (>70%) of the buspirone and 6-hydroxybuspirone remained in the supernatant following centrifugation to remove cells. Herein, 430 mg of 6-hydroxybuspirone was recovered in the supernatant following separation from the cell pellet and prior to treatment with resin. Resin adsorption with Amberlite XAD-16 sequestered 91% of the desired product. Elution from the resin with dichloromethane followed by silica gel chromatography to separate residual buspirone gave 274 mg of (*S*)-6-hydroxybuspirone with 88% ee.

3. Conclusion

(*S*)-6-Hydroxybuspirone was prepared by the enantioselective hydrolysis of racemic 6-acetoxybuspirone using L-amino acid acylase. (*R*)-6-Hydroxybuspirone was obtained by hydrolysis of the isolated (*R*)-acetate. Crystallization of either enantiomer in the presence of 6-acetoxybuspirone gave a substantial improvement in ee. (*S*)-6-Hydroxybuspirone was also obtained by hydroxylation of buspirone catalyzed by *S. antibioticus* ATCC 14890.

4. Experimental

4.1. Preparation of racemic 6-acetoxybuspirone from 6-hydroxybuspirone

The preparation of racemic 6-hydroxybuspirone from buspirone has been described previously.[4,9] To a 5-L, three-necked round-bottomed flask was charged 100.5 g (250 mmol, 1.0 equiv) 6-hydroxybuspirone. Two liters of tetrahydrofuran (KF 0.01) was added and stirred at room temperature to dissolve to a slightly hazy solution.

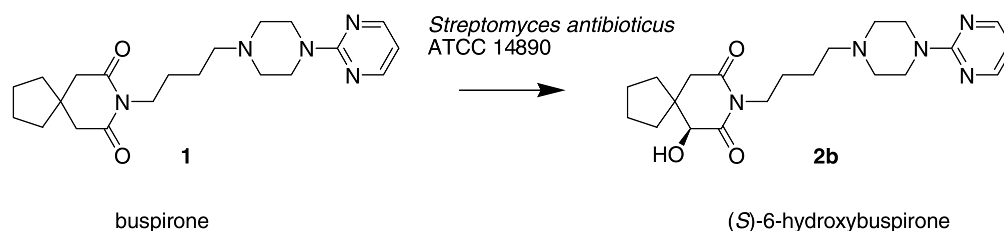

Figure 4. Microbial hydroxylation of buspirone.

To this solution was added 29.11 g (1.15 equiv, 287.7 mmol, 40 mL) of triethylamine and the solution cleared up. 4-Dimethylaminopyridine (1.5 g) was then added to the solution. Acetic anhydride (28.13 g, 1.1 equiv, 275 mmol, 26 mL) was added slowly over 15 min. HPLC assay after 2 h of stirring at room temperature showed some unreacted 6-hydroxy buspirone, so an additional 1 mL of acetic anhydride was added and stirred at room temperature. HPLC after another 2 h showed that the reaction was complete, giving 6-acetoxybuspirone.

Ethyl acetate (500 mL) was added to the reaction mixture and the organic phase was washed with 400 mL of saturated sodium bicarbonate. This addition was exothermic with the temperature rising by a couple of degrees. The phases were separated and the organic phase washed with deionized water and brine and dried over sodium sulfate. The organic phase was then concentrated on a rotary evaporator to give 111 g of an oil. The oil was dissolved in 111 mL of toluene for the resolution step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (m, 2H), 6.58 (m, 1H), 4.87 (s, 1H), 3.46 (m, 2H), 3.16 (m, 4H), 2.54 (m, 4H), 2.36 (m, 2H), 2.1 (s, 2H), 2.01 (s, 3H), 1.53 (m, 6H), 1.41 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.02, 171.27, 170.663, 163.20, 159.31, 111.41, 76.24, 59.82, 54.68, 51.66, 45.90, 45.22, 44.88, 44.81, 40.67, 37.46, 33.153, 27.482, 27.21, 27.03, 25.77, 23.11. IR [neat] 3027, 2960, 2868, 2811, 2771, 1757, 1734, 1716, 1690, 1582, 1547, 1501, 1444, 1357, 1224 cm^{-1}.

HRMS m/z (positive ion ES), $C_{23}H_{34}N_5O_4$ $(M + H^+)$: calcd 444.2611; found 444.2625.

4.2. Screening for the hydrolysis of racemic 6-acetoxybuspirone

Initial screening reactions contained 2 mL 50 mM potassium phosphate buffer, pH 6, 10 mg enzyme, and 10 mg racemic 6-acetoxybuspirone in 0.2 mL toluene. Samples were stirred at room temperature with a magnetic stirrer bar for 15 h. Samples were extracted with 4 mL of ethyl acetate and the extracts dried and redissolved in 2 mL of methanol for HPLC.

4.3. Hydrolysis of racemic 6-acetoxybuspirone using Amano Lipase N from *Rhizopus*

Lipase N (1 g, 92,800 u/g in manufacturer's assay) was added to 18 mL of 50 mM potassium phosphate buffer (pH 6) in a jacketed reactor maintained at 25 °C with a circulating water bath and stirred with a magnetic stirrer. After the enzyme had dispersed, 2 mL of a toluene solution containing 1 g racemic 6-acetoxybuspirone was added to start the reaction. After 6 h, 0.057 mL of 1 M NaOH was added to bring the pH from 5.97 back to 6.0, and the pH remained at 6.0 with no further addition of NaOH. The reaction was stopped when the ee of (*R*)-6-acetoxybuspirone reached 100% (27 h). NaOH (1 M, 1.4 mL) was added to bring the pH to 6.7 and the reaction mixture was extracted immediately with three 20-mL portions of toluene. The pooled toluene extract contained 395 mg (43.6% yield) of (S)-6-hydroxybuspirone and 471 mg of (*R*)-6-acetoxybuspirone (47.1% yield), both near 100% ee.

4.4. Hydrolysis of racemic 6-acetoxybuspirone using Amano Acylase 30000 from *Aspergillus melleus*

Amano Acylase 30000 (4.5 g, 30,000 u/g in manufacturer's *N*-acetyl-D,L-methionine hydrolysis assay) was dissolved in 20 mL of 50 mM potassium phosphate buffer (pH 6) and then added to 520 mL of the same buffer in a 1-L jacketed flask maintained at 25 °C and stirred magnetically. After the enzyme was dispersed, 60 mL of a toluene solution containing 32.5 g of racemic 6-acetoxybuspirone was added. Periodic additions of 1 M NaOH (1, 8.4, and 2.5 mL at 3, 18, and 26 h, respectively), were used to maintain the pH between 5.8 and 6.0. Samples (10 µL) were taken periodically, diluted with 0.99 mL methanol, and analyzed by chiral HPLC to monitor the ee of the alcohol and acetate. After 43 h, the reaction was terminated by addition of 85 mL of 1 M HCl to bring the pH to 3. (After acidification of the reaction mixtures to pH 3 there was no change in the amount of acetate or alcohol or the ee at room temperature or 4 °C over 6 days.) The reaction mixture contained 13.3 g of (*S*)-6-hydroxybuspirone (45.4% yield, 95.2% ee) and 17.2 g of (*R*)-6-acetoxybuspirone (53.0% yield, 88.0% ee).

4.5. Isolation of (*S*)-6-hydroxybuspirone

The reaction mixture [680 mL, containing 13.3 g of (*S*)-6-hydroxybuspirone and 17.2 g of (*R*)-6-acetoxybuspirone] was mixed with diatomaceous earth and filtered, washing with 68 mL of water. The filtrate (pH 3.1) was washed with 205 mL of ethyl acetate and the organic phase discarded. The rich lower phase was extracted at pH 7 (adjusted with 2 M K_3PO_4) with 3×170 mL of ethyl acetate and the combined extract washed with water (3×34 mL). The extract was concentrated in vacuo to a syrup and three 100-mL portions of acetonitrile were added and evaporated to remove the residual ethyl acetate. The residue was dissolved in 51 mL of acetonitrile and warmed in an 80 °C bath to dissolve 6-hydroxybuspirone that started to crystallize. The solution was cooled to 35 °C, stirred with a paddle stirrer, and seeded with 13 mg of form N-3 (*S*)-6-hydroxybuspirone. This form crystallizes spontaneously when most of the solvent is removed from the crude mixture of (*S*)-6-hydroxybuspirone and (*R*)-6-acetoxybuspirone. Seeds of this metastable form can initially be obtained in this way. The stirred mixture was cooled to 0 °C over 1.5 h and filtered, washing the solid with 127 mL of cold (~5 °C) heptane–EtOAc, 4:1. The solid was dried in vacuo at room temperature, to give 9.53 g of (*S*)-6-hydroxybuspirone, ee 99.6%.

4.6. Isolation of (*R*)-6-acetoxybuspirone

The mother liquor from Section 4.5 was concentrated in vacuo. A solution of the residue, which contained 2.59 g (6.45 mmol) of (*S*)-6-hydroxybuspirone and 12.06 g of (*R*)-6-acetoxybuspirone in 26 mL of dry THF, was mixed with 1.8 mL (12.9 mmol) of triethylamine,

0.29 g polyvinyl DMAP, and 1.39 g (13.9 mmol) of succinic anhydride. The solution was stirred and heated at 62 °C, monitoring the progress of the reaction by HPLC. An additional 0.9 g (9.0 mmol) of succinic anhydride was added to complete the succinylation of the 6-hydroxybuspirone. After 33 h, the solution was cooled to room temperature, diluted with 100 mL of methanol, and filtered to remove the polyvinyl DMAP. Concentration of the filtrate followed by partition of the residue between 220 mL of ethyl acetate, 70 mL of water, and 2 mL of 10 M sodium hydroxide (pH 9.0) gave 10.5 g of (*R*)-6-acetoxybuspirone in the organic phase by HPLC assay.

4.7. Hydrolysis of (*R*)-6-acetoxybuspirone using Amano lipase A 12 from *Aspergillus niger*

Amano lipase A 12, 60 mg (120,000 u/g in manufacturer's assay), was added to a tube containing 1.8 mL of 50 mM potassium phosphate buffer, pH 6, and 56.9 mg of (*R*)-6-acetoxybuspirone (near 100% ee) dissolved in 0.2 mL toluene. The biphasic mixture was stirred at ambient temperature (about 22 °C) with a magnetic stirrer. Samples (10 μL) were taken periodically, dried, and dissolved in 1 mL of methanol for HPLC analysis. After 87 h, the reaction mixture contained 56 mg of (*R*)-6-hydroxybuspirone (109% yield, 97.6% ee) with only a trace of (*R*)-6-acetoxybuspirone.

4.8. Acid hydrolysis of (*R*)-6-acetoxybuspirone

A solution of 5 g of (*R*)-6-acetoxybuspirone (11.3 mmol, 99.4% ee) in 120 mL of 1 M HCl was refluxed for 20 min, cooled to room temperature, stirred with 32 mL of EtOAc and 10 mL of 2 M K_3PO_4, and sufficient 10 M NaOH was added to bring the pH to 7.0. The organic phase was separated and the aqueous phase extracted with two additional 32-mL portions of EtOAc. The combined organic extract was washed with three small portions of water and concentrated in vacuo, to give 3.97 g of (*R*)-6-hydroxybuspirone, 98.6% ee, 87.9% yield.

4.9. Recrystallization of (*R*)-6-hydroxybuspirone to improve enantiomeric purity

Crude (*R*)-6-hydroxybuspirone, 10.8 g, 87.8% ee, was mixed with 10.8 g of racemic 6-acetoxybuspirone and refluxed briefly in 43 mL of 2-propanol to dissolve any solid. The solution was cooled to 22 °C and seeded with 10 mg of the N-3 form of (*R*)-6-hydroxybuspirone. The mixture was stirred for 4 h at room temperature and then with cooling in an ice bath for 2 h. The product was filtered out, washed with 110 mL of cold heptane–EtOAc, 4:1, and dried in vacuo at room temperature to give 8.62 g of (*R*)-6-hydroxybuspirone, purity 96.3%, 98.6% ee, residual 6-acetoxybuspirone 2%.

4.10. Hydroxylation screening procedures

Test cultures were grown at 28 °C, 200 rpm, on a medium consisting of 0.5% toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K_2HPO_4, 0.5% NaCl, adjusted to pH 7 with HCl.[10] Bacterial strains were started with a 1% inoculum from a frozen vial on a 10 or 100 mL medium for 3 days; then a 10% inoculum from this flask was used to inoculate a 10 mL or 100 mL medium. Filamentous fungi were started from a 1-mL spore suspension on 100 mL medium and used in the first stage. Buspirone·HCl dissolved in water (0.3 mg/mL final concentration) was added after 24 h growth of the second stage bacterial cultures or first stage fungal cultures. Samples were diluted with an equal volume of acetonitrile for initial HPLC analysis. For chiral HPLC analysis, the broth was extracted with two volumes of ethyl acetate, the extract was dried under a nitrogen stream at 40 °C, and the residue was dissolved in 1.5 mL methanol.

4.11. Hydroxylation by *Streptomyces antibioticus* SC 16282 (ATCC 14890)

Hydroxylation of buspirone to 6-hydroxybuspirone was conducted on a 4-L scale in a 5-L fermentor, using *S. antibioticus* SC 16282 (ATCC 14890). The medium described in Section 4.10 for screening was also used for scale-up. Inoculum was prepared by transferring 1-mL aliquots from vials into 500-mL flasks containing 100 mL of the above medium. The cultures were incubated at 28 °C and 200 rpm for 3 days. Flasks were pooled to generate 200 mL (5%) of the inoculum, which was then transferred to a sterile fermentor containing 4 L of the same medium with an additional 0.02% SAG5693 antifoam to control foaming. The fermentor was run at 28 °C and 500 rpm, with 4 L/min aeration. After 20 h, a filter-sterilized substrate solution containing 4.0 g of buspirone·HCl dissolved in 100 mL of deionized water was added. Fermentation was continued at 28 °C, 500 rpm, and 4 L/min aeration, while pH 6.5 was maintained by the automatic addition of 5% H_2SO_4 or 5% NaOH. Periodically, aseptic samples were taken, diluted with an equal volume of acetonitrile, and analyzed by HPLC for buspirone and 6-hydroxybuspirone concentration. The same sample preparation method was also used for chiral HPLC analysis.

After 102 h from the time of substrate addition, HPLC assays indicated 0.15 mg/mL of 6-hydroxybuspirone in the fermentation broth, and the buspirone concentration had declined from 0.82 mg/mL at the time of substrate addition (broth volume had increased to >4.0 L by this time) to 0.32 mg/mL. Chiral HPLC analysis indicated that the (*S*)-enantiomer was formed predominantly.

4.12. Solid phase extraction of 6-hydroxybuspirone by resin adsorption

Following scale-up into the 5-L bioreactor, 4 L of broth were harvested 102 h after substrate addition and centrifuged at 5000*g* for 15 min. Recovery of supernatant was 3.6 L, to which 1% w/v washed Amberlite XAD-16 resin was added. The resin/supernatant mixture, which was still pH 6.5, was then agitated in a 4-L flask on a shaker at 170 rpm and 28 °C. Samples were taken every hour, and after 7 h of mixing, an additional 1% w/v resin was added. Mixing was halted 2 h later, for a total of 9 h. Adsorption on the resin was monitored by HPLC

analysis of the supernatant, with the recovery yield calculation based on the difference between product level in the supernatant before and after resin binding. A total of 80 g of resin (wet weight) was recovered.

4.13. Isolation of 6-hydroxybuspirone from resin

The loaded resin (80 g) was batch-extracted twice with mixtures of dichloromethane (DCM, 100 mL) and water (50 mL) and then eluted on a column with DCM until the effluent contained negligible 6-hydroxybuspirone. The combined DCM solution, which contained 289 mg of 6-hydroxybuspirone and 721 mg of buspirone, was concentrated to give 2.6 g of residue. This was chromatographed on a 100-mL column of silica gel eluted with a gradient from DCM–HOAc–EtOH–water, 900:100:0:8 to 818:91:91:22, over 1500 mL. Buspirone eluted between 460 and 820 mL and 6-hydroxybuspirone between 780 and 1040 mL with only a little overlap. Fractions rich in 6-hydroxybuspirone (800–1040 mL) were combined and concentrated, giving 0.46 g of a strongly colored residue. This was dissolved in 20 mL of EtOAc and washed with two 20-mL portions of 1/2 saturated sodium bicarbonate (which removed most of the color) and several small portions of water. Concentration of the organic phase in vacuo followed by chasing with acetonitrile gave 274 mg of (*S*)-6-hydroxybuspirone as a slightly amber crystalline solid, 88.3% ee by chiral HPLC. The potency relative to a reference sample of 6-hydroxybuspirone was 98.9% and the level of residual buspirone was 2.1% (w/w).

4.14. HPLC analysis

Quantification was carried out using a YMC ODS-A column (C18, 150 × 6 mm, 3 μ particle size). For hydrolysis of 6-acetoxybuspirone, the mobile phase was 35% acetonitrile/65% 0.1% trifluoroacetic acid in water, flow rate was 1 mL/min, temperature was 40 °C, detection was at 243 nm, and injection volume was 10 μL. Retention times were: 6-acetoxybuspirone, 8.52 min and 6-hydroxybuspirone, 4.84 min. For the hydroxylation of buspirone the mobile phase was 30% acetonitrile/70% 0.1% trifluoroacetic acid in water, which gave retention times: buspirone, 8.54 min and 6-hydroxybuspirone, 5.33 min.

Chiral HPLC was carried out with a Chiralpak AD column (250 × 4.6 mm, 10 μ particle size).

The mobile phase was 85% ethanol/15% acetonitrile/0.06% triethylamine, flow rate was 1 mL/min, temperature was 18 °C, detection was at 243 nm and injection volume was 10 μL. Retention times were: 6-acetoxybuspirone, (*S*)-enantiomer 5.7 min, (*R*)-enantiomer 4.6 min; 6-hydroxybuspirone (*S*)-enantiomer 12.4 min, (*R*)-enantiomer 8.2 min.

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