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Synthesis of (−)-lobeline via enzymatic desymmetrization of lobelanidine

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ABSTRACT

The bioactive alkaloid (−)-lobeline was synthesized via the stereoselective acylation (desymmetrization) of meso-lobelanidine by vinyl acetate in the presence of *Candida antarctica* lipase B.

1. Introduction

Lobeline **1** (Fig. 1) is the main alkaloid constituent of *Lobelia inflata*, a plant indigenous to North America. The plant is also known as Indian tobacco because native Americans smoked the dried leaves as a substitute for tobacco. Lobeline has a long history of therapeutic usage and, during the 19th century, was prescribed as an emetic or a respiratory stimulant for the treatment of various ailments.[1]

Several recent studies have shown that lobeline has multiple mechanisms of action,[1] acting as a ligand for nicotinic acetylcholine receptors,[2–4] μ-opioid receptors,[5] and monoamine transporters (dopamine, serotonine, norepinephrine, vesicular monoamine transporter-2).[6,7] Lobeline has been considered as a useful lead for the development of medications to treat psychostimulants addictions (smoking, cocaine, amphetamines)[8–11] and neurological disorders such as Alzheimer's and Parkinson's diseases.[1,12]

To date, a few syntheses of racemic or enantioenriched lobeline have been reported.[1,13,14] Recently, both enantiomers of lobeline were prepared via the enantioselective acylation of lobelanidine **2** with propionic anhydride in the presence of a chiral organocatalyst[15] and Stoltz et al. developed another approach via $PdCl_2$(sparteine)-catalyzed aerobic oxidation of **2**.[16] Herein, we report the synthesis of natural (−)-lobeline **1** via the enzymatic desymmetrization[17] of lobelanidine **2**.

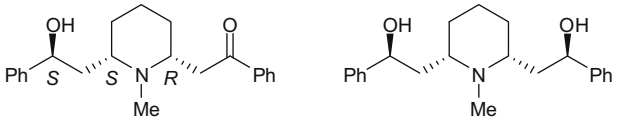

Figure 1. Structure of (−)-lobeline **1** and lobelanidine **2**.

2. Results and discussion

Lobelanidine **2** was prepared in two steps according to literature procedures.[15,18,19] The Mannich condensation of glutaraldehyde, benzoylacetic acid and methylamine hydrochloride provided the diketone lobelanine which was reduced with sodium borohydride to yield a mixture of *cis–trans* diastereomeric diols. The major *cis* isomer, lobelanidine **2**, was purified by recrystallization.

We then completed some screening experiments in order to find lipases with the ability to distinguish the enantiotopic groups of *meso*-lobelanidine **2** (Table 1). The enantiomeric excess (ee) of monoacetate **3** was determined by chiral HPLC. Acylation of **2** with vinyl acetate as the acylation reagent and solvent in the presence of *Candida antarctica* lipase B (CAL-B) provided monoester **3** in fair yield and high enantiomeric excess (ee = 97%) but the reaction was very slow (320 h) at room temperature (entry 1). CAL-B is a thermostable enzyme, allowing operation at temperature up to 90 °C.[20] At 50 °C, the reaction gave a better yield and the same ee in a shorter reaction time (18 h) (entry 2). Substrate **2** is insoluble in solvents of low polarity (hexane, toluene, ether) commonly used in lipase-catalyzed esterification and acetylation in acetonitrile/vinyl acetate (9:1) led to lower enantioselectivity. Acylation of **2** with

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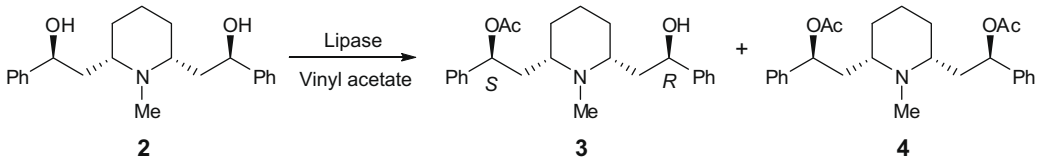
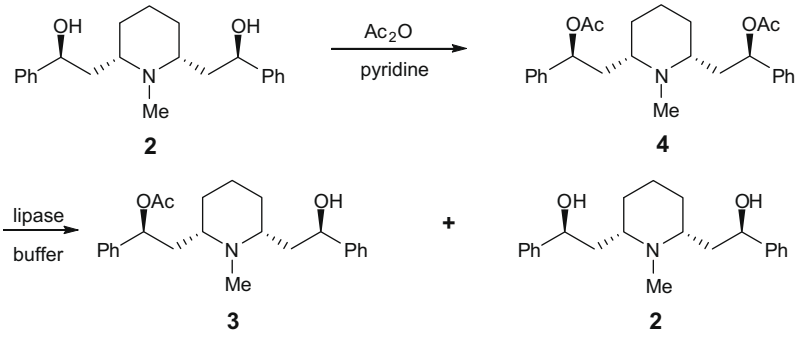
Table 1
Enzymatic acylation (desymmetrization) of lobelanidine **2**

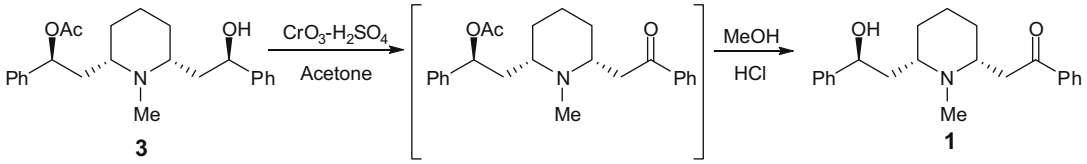


Entry	Lipase	T (°C)	Time (h)	Monoacetate **3**			Diacetate **4**
				Yield[c] (%)	Ee[d] (%)	Absolute configuration[e]	Yield[c] (%)
1	CAL-B[a]	25	320	65	97	*S*	6
2	CAL-B[a]	50	18	70	>98	*S*	3
3	PFL[b]	25	183	71	49	*S*	27

[a] CAL-B: *Candida antarctica* lipase B.
[b] PFL: *Pseudomonas fluorescens* lipase.
[c] Isolated yield.
[d] Measured by HPLC on a chiral phase.
[e] At the CHOAc center.



Scheme 1. Preparation and enzymatic hydrolysis of diacetate **4**.



Scheme 2. Preparation of (−)-lobeline **1** from monoacetate **3**.

Pseudomonas fluorescens lipase (PFL) resulted in a lower ee (49%) (entry 3), and several lipases including *Aspergillus niger* lipase (ANL), *Mucor* sp. lipase (MSL), *Candida rugosa* lipase (CRL), and *Pseudomonas* sp. lipase (PSL) were not active.

Diacetate **4** was prepared by the acetylation of **2** with acetic anhydride in pyridine (Scheme 1). Hydrolysis of diacetate **4** by CAL-B in a phosphate buffer at 50 °C was very slow (several days) and provided enantiomerically pure (ee ⩾ 95%) monoacetate **3** in low yield (23%) and the corresponding achiral diol **2** (74%) indicating that the monoacetate is also a substrate for the lipase (overhydrolysis). Hydrolysis in water-saturated diisopropylether did not improve the rate of conversion or the yield of monoester **3**. Unexpectedly, the enzymatic hydrolysis provided the same monoester as the acylation. In general, the esterification of *meso*-diols and the hydrolysis of the corresponding *meso*-diesters are complementary and give opposite enantiomers but exceptions have been reported.[21,22]

The reactions were monitored by TLC analysis and terminated when all the starting material was consumed (conversion = 100%). The absolute configuration of monoester **3** was determined by transformation into (−)-lobeline **1** (Scheme 2). The Jones oxidation of the alcohol function followed by acidic alcoholysis of the ester function provided (−)-lobeline **1**. Comparison of the optical rotation of this product with literature data revealed its absolute configuration to be identical with that of the natural product. As a result, the absolute configuration of monoacetate **3** was deduced to be *S* at the CHOAc center. Spectroscopic and physical data of synthetic (−)-lobeline were in agreement with literature values; as already noted by others, we observed a *cis–trans* epimerisation in solution.[1,14,16]

3. Experimental

3.1. General

NMR spectra were recorded on a Varian Inova AS 400 spectrometer (400 MHz). Infrared spectra were recorded on a Bomem MB-100 spectrometer. Optical rotations were measured using a JASCO DIP-360 polarimeter (*c* as gram of compound per 100 mL). High resolution mass spectra (HRMS) were recorded on an Agilent

MSTOF-6210 (electrospray) spectrometer. Flash column chromatography was performed on aluminum oxide (activated, neutral, 58 Å, from Aldrich). Thin layer chromatography (TLC) was carried out using aluminum oxide 60 F_{254} (neutral, type E, from Merck). The enantiomeric excess of compound **3** was determined by HPLC with a chiral stationary phase column (Regis Technologies Inc. (*S,S*)-Whelk-O1, 4.6 mm × 250 mm), using *n*-hexane/ethanol (4:1) as the eluent, at a flow rate of 1.5 mL/min with a detection wavelength of 220 and 254 nm, $t_{(S)}$ = 8.25 min, $t_{(R)}$ = 5.55 min. Enzymes were from Sigma-Fluka (PFL, CRL, PSL), Amano International Enzyme (ANL, MSL), and Roche Molecular Biochemicals (CAL-B, Chirazyme L-2, carrier-fixed, C2, from *C. antarctica* fraction B, 4.5 kU/g dry carrier, with tributyrin as the substrate). (−)-Lobeline was purchased from Sigma.

3.2. Enzymatic acylation of lobelanidine 2

To a solution of lobelanidine **2** (100 mg, 0.27 mmol) in vinyl acetate (10 mL) was added *C. antarctica* lipase B (3700 units). The mixture was stirred at 50 °C for 18 h. The enzyme was removed by filtration, and the filtrate was evaporated to dryness. The crude product was purified by flash chromatography (neutral alumina, CH_2Cl_2 containing 1% EtOH) to provide lobelanidine monoacetate **3** (72 mg, 70%) as a colorless oil and lobelanidine diacetate **4** (3.6 mg, 3%) as a white solid. Lobelanidine monoacetate **3**: $[\alpha]_D^{23}$ −2.7 (*c* 0.57, $CHCl_3$); IR (NaCl) 3380, 2940, 1730, 1237, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ *1.39–1.80* (m, 8H), 2.04 (s, 3H), 2.17–2.24 (m, 1H), 2.51 (s, 3H), 2.54–2.61 (m, 1H), 3.27–3.35 (m, 1H), 3.76 (br s, 1H), 4.83 (dd, *J* = 10.8 and 1.6 Hz, 1H), 5.80 (dd, *J* = 9.8 and 4.0 Hz, 1H), 7.22–7.40 (m, 10H), 8.2 (br s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 23.2, 23.7, 39.8, 41.8, 60.1, 63.1, 72.9, 125.7, 126.8, 127.4, 128.6, 128.9, 139.9, 145.1, 170.8; HRMS (ES) *m/z* calcd for $C_{24}H_{32}NO_3$ $(MH)^+$: 382.2377. Found 382.2389.

3.3. Lobelanidine diacetate 4

To a solution of lobelanidine **2** (200 mg, 0.53 mmol) in pyridine (5 mL) was added acetic anhydride (1 mL, 10.5 mmol) and the mixture was stirred overnight at room temperature. The solvent was evaporated and the crude product was purified by flash chromatography (neutral alumina, CH_2Cl_2) to give lobelanidine diacetate **4** (173 mg, 77%) as a white solid: mp: 205–210 °C (dec.); IR (KBr) 3038, 2934, 1737, 1521, 1457, 1243 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ *1.54–1.69* (m, 3H), 1.85–2.10 (m, 5H), 2.12 (s, 6H), 2.28–2.36 (m, 2H), 2.70 (br s, 3H), 3.47–3.54 (m, 2H), 5.79 (dd, *J* = 13.4 and 4.9 Hz, 2H), 7.31–7.40 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.4, 21.6, 22.7, 38.9, 53.7, 72.3, 126.7, 128.7, 129.1, 139.5, 170.8, 175.8; HRMS (ES) *m/z* calcd for $C_{26}H_{34}NO_4$ $(MH)^+$: 424.2482. Found 424.2487.

3.4. Enzymatic hydrolysis of lobelanidine diacetate 4

To a solution of lobelanidine diacetate **4** (50 mg, 0.118 mmol) in diethyl ether (1 mL) were added a phosphate buffer (pH 7.1, 9 mL) and CAL-B (1000 units). The mixture was stirred at 50 °C. The reaction was monitored by TLC (neutral alumina, CH_2Cl_2/2% EtOH) and quenched by filtration of the enzyme when all the starting material was consumed. The solvents were evaporated (water–MeOH azeotrope) and the crude product was purified by flash chromatography (neutral alumina, CH_2Cl_2/1% EtOH) to give monoacetate **3** (10.6 mg, 23%, ee ⩾ 95%) and lobelanidine **2** (29.9 mg, 74%). Spectral data as above.

3.5. Synthesis of (−)-lobeline 1 from lobelanidine monoacetate 3

To a solution of lobelanidine monoacetate **3** (78 mg, 0.204 mmol) in acetone (5 mL) cooled to 0 °C, was added Jones reagent (25 mg of CrO_3, 20 μL of H_2SO_4 and 70 μL of H_2O). The mixture was stirred at room temperature for 1 h. The mixture was diluted with chloroform (30 mL) and saturated aqueous $NaHCO_3$ was slowly added until the organic phase became colorless. The organic phase was separated, dried, and evaporated to yield the crude intermediate keto-ester as an oil which was dissolved in methanol (15 mL) and 3 N HCl (8 mL). The mixture was stirred at reflux overnight. The solvent was then evaporated and the crude product was purified by recrystallization (43 mg, 57% for two steps). All characterization data were identical to those of a commercial sample of (−)-lobeline hydrochloride.

Acknowledgments

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