Synthesis of Phenylalanines in High Enantiomeric Excess via Enzymatic Resolution

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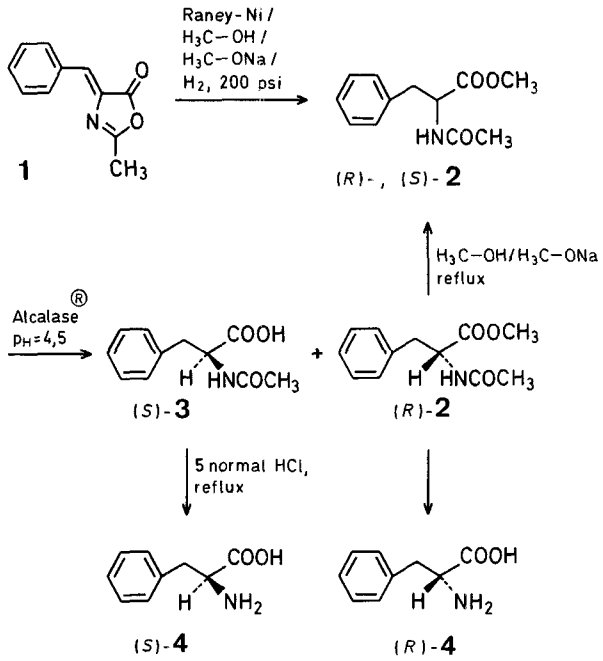
Enzymes offer a convenient, attractive alternative to conventional reagents as a means of expressing chirality in a synthetic scheme. Indeed, selection of the appropriate enzymatic reagent can often afford compounds in higher enantiomeric excess than obtainable by more standard synthetic methods. By utilizing an enzymatic resolution, (*S*)-phenylalanine was prepared in four simple steps. In the procedure described here, derivatized (*R,S*)-phenylalanine was prepared by conventional synthetic procedures after which the racemic mixture was treated with an enzyme which reacted specifically with the (*S*)-isomer. The (*R*)-isomer was unchanged by enzymatic treatment and it was obtained as *N*-acetyl-(*R*)-phenylalanine methyl ester. The enzymatic reaction product, *N*-acetyl-(*S*)-phenylalanine, was isolated by usual techniques and hydrolyzed to yield (*S*)-phenylalanine.

The preparation of (*R,S*)-phenylalanine, suitably derivatized for enzymatic resolution, was accomplished in two steps. In the first step, benzaldehyde and glycine or *N*-acetylglycine were condensed via the Erlenmeyer azlactone synthesis to give azlactone **1** in 67% yield[1]. Hydrogenation of azlactone **1** over Raney nickel in methanol containing sodium methoxide conveniently afforded the racemic *N*-acetyl methyl ester **2** in 95% yield[2]. Addition of sodium methoxide to the hydrogenation medium facilitated opening of the azlactone ring giving *in situ* methyl *N*-acetylaminocinnamate, which is easily hydrogenated. Thus, the synthesis of methyl ester **2** was accomplished directly. Stepwise preparation of this methyl ester would have required hydrolysis of azlactone **1** to *N*-acetylam-

inocinnamic acid, which is subsequently hydrogenated and esterified. Therefore, the simplified procedure employed here gave racemic derivatized phenylalanine in only two steps.



Asymmetry was introduced into the scheme by use of Subtilisn Carlsberg (Subilopeptidase A, E.C. 3.4.21.14), a nonspecific serine proteinase which specifically hydrolyzes the carboxyl group of the (*S*)-isomer of derivatized (*N*-acylated, *C*-esterified) amino acids[3]. Thus, the (*R,S*)-derivative **2** was incubated with Subtilisn in an aqueous medium to give *N*-acetyl acid (*S*)-**3** and unchanged *N*-acetyl ester (*R*)-**2**. Washing the aqueous reaction medium with organic solvent removed the unchanged (*R*)-isomer. Subsequent treatment of *N*-acetyl-(*S*)-phenylalanine with refluxing hydrochloric acid yielded the desired (*S*)-phenylalanine of 98% optical purity. The unchanged (*R*)-isomer (recovered from the organic wash) was epimerized with base to afford a (*R,S*)-mixture **2** suitable for future incubation with Subtilisn.

Preparation of compounds in high optical purity is often accomplished by use of chiral reagents rather than resolution methods[4]. Introducing asymmetry into a molecule via use of stoichiometric amounts of a chiral reagent is sometimes impractical and oftentimes gives products of less-than-desired enantiomeric excess. For some substrates such as amino acids, resolution methods, particularly those involving enzymes, offer an attractive alternative to standard chiral reagents. The enzymatic procedure described here provides an efficient method for the production of (*R*)- or (*S*)-phenylalanine from readily available reagents.

^{1}H-N.M.R. spectra were obtained on a Varian EM-390 spectrometer. Optical measurements were performed on a Perkin-Elmer 141 Polarimeter. Melting points were measured on a Thomas-Hoover Unimelt and are uncorrected. The serine proteinase, Subtilisn Carlsberg, was obtained from Novo Laboratories, Wilton, Connecticut under the trade name Alcalase®. It was used as received.

2-Methyl-4-(phenylmethylene)-5(4*H*)-oxazolone (1)[1]:
Glycine (37.5 g, 0.5 mol) and anhydrous sodium acetate (30 g, 0.37 mol) are slurried in a mixture of benzaldehyde (79 g, 0.74 mol) and acetic anhydride (283 ml, 3 mol). The mixture is warmed until dissolution is complete then refluxed for 1 h. Upon cooling, the black reaction mixture affords yellow crystals which are collected, washed with cold water, and dried in vacuo over phosphorus pentoxide to give the azlactone **1**; yield: 62 g (67%); m.p. 147–148°C (Lit.[1], m.p. 148–150°C).

^{1}H-N.M.R. ($CDCl_3$): $\delta = 2.37$ (s, 3 H, CH_3); 7.10 (s, 1 H, vinyl); 7.39 (m, 3 H_{arom}, *m*-H, *p*-H, $J = 6.0$ Hz, 3.0 Hz); 8.05 ppm (dd, 2 H_{arom}, *o*-H, $J = 6.0$ Hz, 3.0 Hz).

N-Acetyl-(*R,S*)-phenylalanine Methyl Ester (2):
A methanol (24 ml) slurry of azlactone **1** (18.7 g, 0.10 mol) is treated with sodium methoxide (0.54 g, 0.01 mol) and the resulting dark solution is stirred for 10 min. The solution is then hydrogenated (200 psi hydrogen) over Raney nickel (5.87 g, 0.10 mol) at 50°C for 2.5 h. After cooling, the reaction mixture is filtered and concentrated in vacuo to afford a red oil which is slurried in diethyl ether (200 ml). To the ethereal slurry, cold hydrochloric acid (0.5 normal, 100 ml) is added. The layers are separated and the aqueous portion is extracted with diethyl ether (100 ml). The combined ether extract is washed with brine (100 ml), dried with anhydrous sodium sulfate, and concentrated in vacuo to afford an oily residue. The residue is triturated in petroleum ether (100 ml) and the solvent is evaporated to give *N*-acetyl-(*R,S*)-phenylalanine methyl ester (**2**) as a light yellow solid, which is recrystallized from ether/petroleum ether to give a colorless solid; yield: 21.0 g (95%); m.p. 62–63°C (Lit.[5], m.p. 60–61°C).

^{1}H-N.M.R. ($CDCl_3$): $\delta = 1.91$ (s, 3 H, $COCH_3$); 3.07 (d, 2 H, CH_2, $J = 6.0$ Hz); 3.69 (s, 3 H, $COOCH_3$); 4.85 (dt, 1 H, CH, $J = 9.0$ Hz, 6.0 Hz); 6.55 (br. d, 1 H, NH, $J = 9.0$ Hz); 7.27 ppm (m, 5 H_{arom}).

Resolution of *N*-Acetyl-(*R,S*)-phenylalanine Methyl Ester (2):
N-Acetyl-(*R,S*)-phenylalanine methyl ester (**2**; 2.21 g, 0.01 mol) is slurried in water and the pH adjusted to 7.5 with sodium hydroxide (0.02 normal). Purified Subtilism Carlsberg (10 mg; 0.05 wt% based on the aqueous solution) is added with stirring. The pH of the solution immediately decreases and is readjusted to pH 7.5 and maintained at that value until enzymatic activity ceases after about 45 min (a total of 24.3 ml of sodium hydroxide is required). The aqueous reaction mixture is extracted with dichloromethane (2 × 100 ml). The combined organic extract is dried with anhydrous magnesium sulfate and concentrated to afford *N*-acetyl-(*R*)-phenylalanine methyl ester [(*R*)-**2**] as a colorless solid; yield: 1.08 g (98%); m.p. 84–85°C (Lit.[6], m.p. 86–87°C).

^{1}H-N.M.R. ($CDCl_3$): $\delta = 1.97$ (s, 3 H, OCH_3); 3.10 (d, 2 H, CH_2, $J = 6.0$ Hz); 3.72 (s, 3 H, $COCH_3$); 4.89 (dt, 1 H, CH, $J = 6.6$ Hz, 6.0 Hz); 6.18 (br. d, 1 H, NH, $J = 6.6$ Hz); 7.27 ppm (m, 5 H_{arom}).

The aqueous portion of the reaction mixture is acidified to pH 1 by dropwise addition of concentrated sulfuric acid; then extracted with ethyl acetate (2 × 100 ml). The combined organic extracts are dried with anhydrous magnesium sulfate and concentrated in vacuo to afford a colorless solid, which is recrystallized from water to give *N*-acetyl-(*S*)-phenylalanine [(*S*)-**3**]; yield: 1.0 g (96%); m.p. 171–172°C (Lit.[7], m.p. 171–172°C); $[\alpha]_D^{26}$: 50.4° (*c* 0.5, C_2H_5OH); optical purity: 98%.

^{1}H-N.M.R. ($CDCl_3$ + DMSO-d_6): $\delta = 1.90$ (s, 3 H, CO—CH_3); 3.11 (m, 2 H, CH_2); 4.77 (m, 1 H, CH, $J_{HCNH} = 7.5$ Hz); 6.89 (br. d, 1 H, NH, $J_{HCNH} = 7.5$ Hz); 7.28 (m, 5 H_{arom}); 9.73 ppm (br. s, 1 H, COOH).

Racemization of *N*-Acetyl-(*R*)-phenylalanine Methyl Ester [(*R*)-2]:
A methanol (5 ml) solution of *N*-acetyl-(*R*)-phenylalanine methyl ester (1.75 g, 7.9 mmol) is treated with sodium methoxide (43 mg, 0.79 mmol), then refluxed for 3 h. The mixture is concentrated in vacuo to afford an oil which later solidifies.

(*S*)-Phenylalanine [(*S*)-4]:
N-Acetyl-(*S*)-phenylalanine (1.40 g, 6.7 mmol) is slurried in 5 normal hydrochloric acid (10 ml) and subsequently heated to reflux. The solution turns light yellow and is refluxed for 1.25 h. The aqueous mixture is then concentrated to dryness in vacuo to yield a light yellow residue. While chilling in an ice bath, the residue is treated with 95% ethanol saturated with ammonia (10 ml). The resultant slurry is heated to reflux, then water is added until dissolution is complete. An additional volume of 95% ethanol (5 ml) is added. After cooling overnight, (*S*)-phenylalanine is obtained as a crystalline, colorless solid; yield: 0.86 g

(78%); m.p. 283–284°C (Lit.[8], m.p. 283–284°C); $[\alpha]_D^{20}$: −34.3° (*c* 1.0, water); optical purity: 98%.

^{1}H-N.M.R. (D_2O/DCl/TSP): $\delta = 3.29$ (dd, 2H, CH_2, $J = 7.5$ Hz, 3.0 Hz); 4.40 (t, 1H, CH, $J = 7.5$ Hz); 7.41 ppm (m, 5 H_{arom}).

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