Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 24, pp. 2941-2944, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00446-3

ENANTIOSELECTIVE HYDROLYSIS OF (±)-CHLORAMPHENICOL PALMITATE BY HYDROLASES

Robert Chênevert*, Roxane Pouliot and Patrick Bureau.

Département de Chimie, Faculté des Sciences et de Génie, Université Laval, Québec, Canada, G1K 7P4.

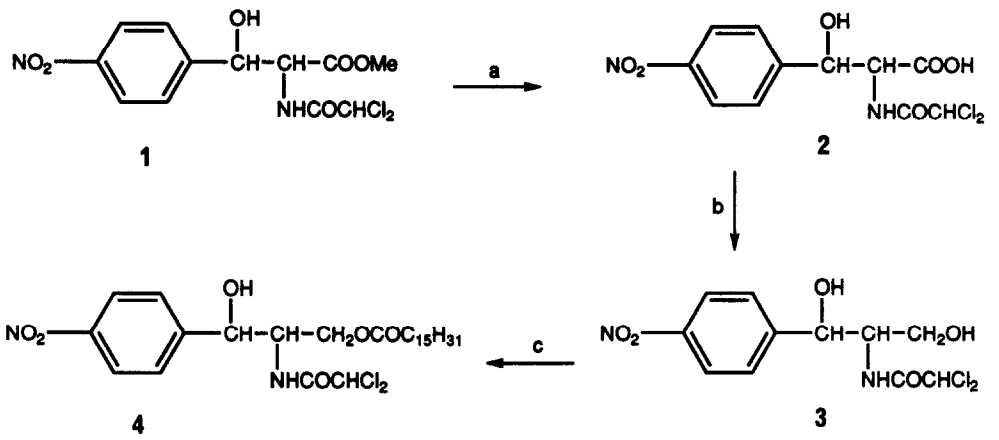
Abstract. (±)–Chloramphenicol palmitate has been efficiently resolved by enantioselective hydrolysis in organic medium in the presence of *Rhizopus* sp. lipase affording the palmitate of RR-chloramphenicol and SS-chloramphenicol in high chemical and enantiomeric yields.

Chloramphenicol, the first of the broad-spectrum antibiotics, was initially isolated from cultures of various *Streptomyces* strains.[1-4] It is also the first antibiotic industrially produced by chemical synthesis rather than by fermentation.[5] Only one of the four possible stereoisomers of chloramphenicol, the D-threo or 2R,3R isomer, has antibacterial activity. Chloramphenicol acts specifically to inhibit the peptidyl transferase centre located on the 50 S ribosomal subunit which is responsible for peptide bond formation during polypeptide chain elongation.[1,6] To mask its bitter taste or improve its physicochemical properties, several esters of chloramphenicol have been prepared (palmitate and hemisuccinate are the more common). These prodrug esters are inactive, but in vivo the parent compound is released by enzymatic hydrolysis.[1,7,8] Also, inactivation of this antibiotic by resistant microorganism involves the acetylation of the primary alcohol by the enzyme chloramphenicol acetyl transferase.[9] We wish to report here the enantioselective hydrolysis of (±)-threo chloramphenicol palmitate by lipases.

The (±)-threo isomer of 4-nitrophenylserinate **1** was first obtained by condensation of 4-nitrobenzaldehyde with methyl glycinate followed by N-acylation with dichloroacetyl chloride according to known procedures (Scheme 1).[10,11] The ester **1** was hydrolysed in aqueous sodium hydroxide to give acid **2**. Reduction of the (±)-threo **2** by borane-methyl sulfide complex gave (±)-threo chloramphenicol **3**. Selective acylation of **3** with palmitoyl chloride in pyridine gave chloramphenicol palmitate **4**.

Initial attempts to resolve chloramphenicol involved enzymatic acylation with several acylating reagents (acetic anhydride, palmitic anhydride, ethyl acetate, isopropenyl acetate), but these attempts gave only moderate chemical and enantiomeric yields.

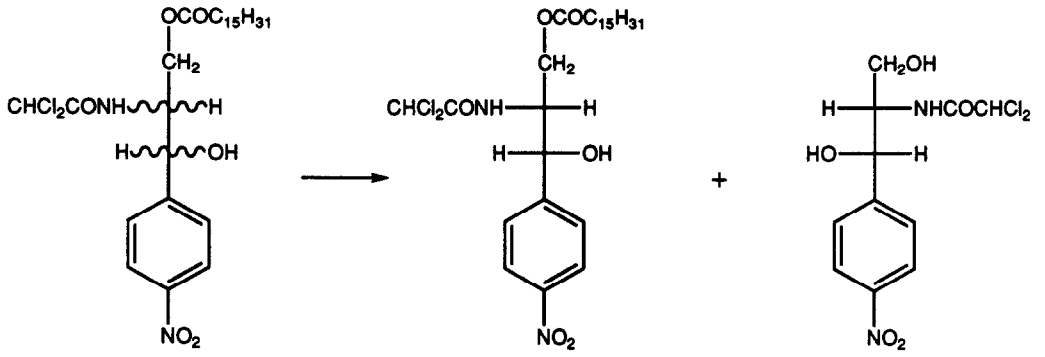
The poor solubility of compound **4** in water led us to study the hydrolysis in water-saturated organic solvents. The enantioselectivity of enzymatic hydrolysis of **4** was investigated in diisopropylether saturated with an aqueous buffer. A number of proteases, lipases and esterases[12] have been tested (Table 1), and the best enantioselectivity was obtained with lipase from *Rhizopus* sp. which gave SS-chloramphenicol and unreacted palmitate of RR-chloramphenicol[13] in high chemical and enantiomeric yields (Scheme 2).



Scheme 1

Reagents and conditions :

a) NaOH, 0.1 M, THF, 94% b) $Me_2S \cdot BH_3$, THF, 98% c) $C_{15}H_{31}COCl$, pyridine, 91%



Racemic-4 Palmitate of RR-chloramphenicol-4 (SS) -3

Scheme 2

In a typical experiment, racemic palmitate **4** (50 mg), *Rhizopus* sp. lipase (100 mg) and Celite (100 mg) were added to diisopropyl ether (20 mL) saturated with 0.05 N phosphate buffer (pH 7.0), and the reaction mixture was stirred at room temperature. The reaction was monitored by HPLC analysis and stopped at 50% of conversion (24 h). The solid enzyme preparation was filtered and washed with ethyl acetate, and the organic layer was concentrated in vacuo. Flash chromatography (silica gel, dichloromethane - ethyl acetate, gradient 20:1 to 5:2) afforded SS-chloramphenicol and unreacted palmitate of RR-chloramphenicol.

The enantiomeric excess (ee) of the chloramphenicol produced was determined by ^{19}F and ^{1}H NMR analysis of the corresponding Mosher's esters (ester of α-methoxy-α-trifluoromethylphenylacetic acid). The ee values of the remaining ester substrate were determined by ^{1}H NMR analysis in the presence of tris[(heptafluoropropyl-hydroxymethylene)-d-camphorato] Europium (III) as a chiral shift reagent.[14] We have always found that the enzymes preferentially utilizes the SS enantiomer. Consequently, the remaining palmitate contains the RR-chloramphenicol and the alcohol product (chloramphenicol) has the SS configuration. The absolute configurations were determined by comparison with authentic samples.

Table 1

Enzymatic hydrolysis of (±)-threo-chloramphenicol in organic medium

Enzyme	Reaction[a] Time (h)	Recovered ester[b] 2R,3R/2S,3S	Alcohol (chloramphenicol)[b] 2S,3S/2R,3R
Esterase from			
Aspergillus niger	15	75/25	77/23
Penicillium sp.	50	65/35	66/34
Lipase from			
Porcine pancreas	9	50/50	50/50
Pancreatin	100	78/22	75/25
Geotricum candidum	18	87/13	89/11
Candida cylindracea	9	85/15	84/16
Pseudomonas sp.	12	95/5	90/10
Rhizopus sp.	24	97/3	99/1

a) Percentage of hydrolysis (conversion) was always 50%.

b) All chemical yields were above 90%.

Recently, antibodies elicited against a phosphonate transition-state analogue have been found to catalyze the hydrolysis of chloramphenicol monoesters (inactive prodrug) to generate chloramphenicol (bioactive parent

drug).[15] Also, Carrea *et al.*[16] reported the regioselective esterification of chloramphenicol and its synthetic analogue thiamphenicol by the action of lipases in the presence of various methyl carboxylates. In both studies, the substrate was a single enantiomer and the enantioselectivity of the reactions was not investigated.

In summary, we found that the hydrolase catalyzed hydrolysis of chloramphenicol palmitate was enantioselective, and the fast-reacting enantiomer was always the biologically inactive 2S,3S enantiomer. Chloramphenicol palmitate was conveniently resolved by hydrolysis in organic medium in the presence of *Rhizopus* sp. lipase.

Acknowledgement. We acknowledge the financial support of this work by the National Sciences and Engineering Research Council of Canada.

References and Notes

1. a) *Comprehensive Medicinal Chemistry*; Hansch, C.; Sammes, P.G.; Taylor, J.B. Eds.; Pergamon Press, New-York, 1990. b) Ehrhart, *G. Chem. Rev.* **1953**, *86*, 483.
2. Hahn, F.E., *Antiobiotics*; Gottlieb, D.; Shaw, P.D. Eds.; Springer-Verlag, Heidelberg, 1967, Vol. 1, p. 308.
3. Pestka, S., *Antibiotics*; Corcoran, J.W.; Hahn, F.E. Eds.; Springer-Verlag, Heidelberg, 1975, Vol. 3, p. 370.
4. Pongs, O., *Antibiotics*; Hahn, F.E. Ed.; Springer-Verlag, Heidelberg, 1979, Vol. 5, Part 1, p. 26.
5. Johnson, F., *The Total Synthesis of Natural Products*; Apsimon, J. Ed.; Wiley, New York, 1973, Vol. 1, p. 457.
6. Traut, R.R.; Monro, R.E. *J. Mol. Biol.*; **1964**, *10*, 63.
7. Shaw, W.V.; Unowsky, J. *J. Bacteriol.*; **1968**, *95*, 1976.
8. Goodman and Gilman's *The Pharmacological Basis of Therapeutics*; 6th ed.; MacMillan Publishing Co., New York, 1980, pp. 1191-1198.
9. Shaw, W.V.; Leslie, A.G. *G. Annu. Rev. Biophys. Chem.*; **1991**, *20*, 363.
10. Chênevert, R.; Thiboutot, S. *Synthesis*; **1989**, 444.
11. Ehrhart, G.; Siedel, W.; Nahm, H. *Chem. Ber.*; **1957**, *90*, 2088.
12. Pancreatin, lipase from *Candida cylindracea* and porcine pancreatic lipase were purchased from Sigma. All other enzymes were from Amano.
13. Because of the sequence-rule procedure of the Cahn-Ingold-Prelog nomenclature the palmitate of the 2R,3R chloramphenicol must be denoted 2S,3R.
14. *Asymmetric Synthesis*; Morrison, J.D. Ed.; Academic Press, New York, 1983, Vol. 1.
15. Miyashita, H.; Karaki, Y.; Kikuchi, M.; Fujii, I. *Proc. Natl. Acad. Sci. U.S.A.*; **1993**, *90*.
16. Ottolina, G.; Carrea, G.; Riva, S. *J. Org. Chem*; **1990**, *55*, 2366.

(*Received in USA* 12 *October* 1994; *accepted* 18 *November* 1994)