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Enzymatic Resolution of the Ethyl Acetals of (*R*)- and (*S*)-4-Hydroxyalk-2-ynals

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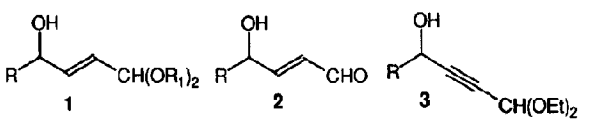
Abstract: 4-hydroxyalk-2-ynals diethylacetals have been efficiently resolved by enatioselective acetylation mediated by *Pseudomonas fluorescens* lipase affording the acetylated (*R*)-enantiomers in preference, independent of the length and bulk of the alkyl substituent at the carbinol group. Reduction with $LiAlH_4$ of unreacted or acetylated isomers affords the corresponding ethylenic acetals with the hydrogen deriving from the hydride in the β position in respect to the allylic hydroxyl.

Recent studies in our laboratories demonstrated[1] that irreversible acylation in organic solvents, mediated by lipase from *Pseudomonas fluorescens* (PFL, SAM-2, Fluka), represents an excellent method to resolve several racemic 4-hydroxy-2-unsaturated acetals of type **1** (R_1= Me) which are easy transformed into the corresponding (*R*)- and (*S*)-(*E*)-4-hydroxyalk-2-enals of type **2**, a group of biologically actives products [2a-d] of lipid peroxidation in cells and organs in response to oxidative stress. For same biological studies we needed to obtain these aldehydes in homochiral ^{3}H-labeled form and realised that, in this respect, the enzymatic resolution of **2** was not of practical utility since it does not permit the introduction of the label in the final step of the preparation.

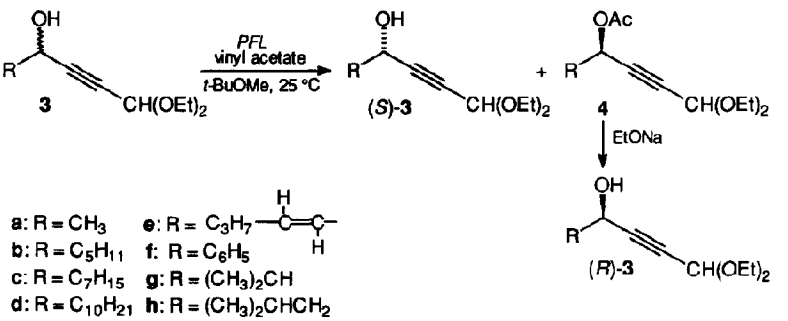


On the contrary the availability of homochiral acetylenic acetals of type **3** which can be easily transformed into the ethylenic analogues **1** (R_1= Et) by $LiAlH_4$ (or isotopically labeled hydride) reduction[3-5] could permit the labelling during the reduction of the triple bond. Thus we decided to search for an enzymatic resolution of acetals of type **3**. Our aim in this research was also influenced by the more general consideration that optically active 1-alkynyl-3-ols are important starting materials for the synthesis of several classes of natural compounds such as prostaglandins,[6] pyrethroids,[7] pheromones,[8] and avenaciolides.[9]

In this paper we report how we obtained the (*R*)- and (*S*)-hydroxy acetals **3a-h** by enantioselective esterification of the 4-hydroxy group of their corresponding racemates with vinyl acetate and PFL in *tert*-butyl methyl ether (*t*-BuOMe)[10]. The results (Table) show that PFL is able to distinguish a propynal diethyl acetal

from a variety of alkyl groups ranging in size from methyl to decyl. In fact for all substrates with a linear saturated alkyl substituent bonded at the carbinol group (entries 1-4) the enantiomeric ratio of the kinetic resolution is excellent (E >25)[11] and the reaction occurs at a reasonable rate and with the same sense of enantioselection, the (*R*)-enantiomers being acetylated in preference, independent of their hydrophobicity and of the length of the alkyl substituent.

Table. Chemoenzymatic obtention of (*R*)- and (*S*)-3a-h



entry	R	convn[a](%) (time, days)	(*S*)-3 ee (%)[b] (yield %)[c]	(*R*)-3 ee (%)[b] (yield %)[d]
1	a	50 (1)	> 95 (44)	> 95 (38)
2	b	48 (3)	88 (46)	95 (37)
3	c	50 (4)	95 (43)	95 (37)
4	d	48 (5)	90 (45)	>95 (36)
5	e	46 (3)	82 (49)	>95 (35)
6	f	48 (7)	88 (46)	95 (37)
7	g	46 (7)	74 (47)	87 (35)
8	h	20 (9)	22 (70)	90 (15)

[a]Monitored by TLC and determined by ^{1}H NMR integrals of crude reaction mixtures. [b]Enantiomeric excesses were determined by ^{1}H NMR analysis of the corresponding Mosher esters. [c]After chromatographic purification. [d]Total yields from starting racemates.

The other substrates (entries 5-8) were tested in order to explore the scope and the limitations of this enzymatic resolution. The results obtained with the hydroxy acetals **3e-g**, show that, despite the different steric requirement of the substituent at the carbinol group, the (*R*)-alcohols were still enantioselectively acetylated, although at a slower rate. A larger difference was observed in the reaction of **3h** (R = *i*-Bu) which was acetylated at a rate of little practical value and with lower (*R*)-enantioselectivity. However, the result shows that, although it is a poor substrate, the isobutyl, which has a branch point at approximately the same distance

from the alcohol centre as does the acetal, is distinguished from this group reasonably well.[12]

These results and our previous observations on the enzymatic resolution of γ-hydroxy acetals **1** (R_1= Me) show the importance of the acetal group to direct the enantioselection of PFL.

The reaction products can be separated in pure form by rapid column chromatography on silica gel,[13] without any hydrolysis of the acetal groups. The (*R*)-4-acetoxyacetals (**4a-h**) were then easily transformed into the corresponding hydroxy acetals (*R*)-**3a-h** by transesterification with sodium ethoxide in ethanol.[14] However, if the final aim is to obtain the (*R*)-enantiomers of the 4-hydroxyalk-2-enals, the (*R*)-4-acetoxy acetals can be directly reduced with $LiAlH_4$ to the corresponding ethylenic acetals **1a-h** (R_1= Et).[3] In fact, the stereogenic centre was not modified by the hydride as shown by reducing the hydroxy acetal (*S*)-**3b** and the acetoxy acetal **4b** into (*S*)- and (*R*)-(*E*)-4-hydroxynon-2-enal (**2**; R = C_5H_{11}), which are very important hydroxy aldehydes isolated from peroxidised microsomes.[15]

This reduction, first reported by Esterbauer[3] for the corresponding racemate was subsequently performed using $LiAlD_4$, but the position of the deuterium atom deriving from deuteride and of the hydrogen deriving from water in the reduced compound was not established.[4,5] Since in the reduction of propargylic alcohols with $LiAlD_4$ the deuteride can be introduced either γ or β to the allylic hydroxyl, depending on the reaction conditions,[16] we decided to establish which was the case for our reduction of (*S*)-**3b** and **4b**. Thus when the reduction was carried out with $LiAlD_4$ and the hydrolysis was performed with water,[17] the product obtained was the monodeuterated acetal with a deuterium atom at position 3 (^{1}H NMR). If the hydrolysis of the intermediate vinyl aluminum derivative was carried out with D_2O, a dideuterated acetal was obtained containing deuterium atoms at positions 2 and 3, as previously reported.[4,5,18]

In conclusion we have developed an efficient enzymatic resolution of 4-hydroxy acetylenic acetals of type **1** (R_1= Et) useful for obtaining in a simple way the (*E*)-4-hydroxyalk-2-enals of type **2** also in isotopically labeled form. In addition the methodology described herein is applicable to a wide variety of synthetic problems in which an homochiral functionalised propargylic alcohol is involved.[19]

REFERENCES AND NOTES

1. Allevi, P.; Anastasia, M.; Cajone, F.; Ciuffreda, P.; Sanvito, A. M. *J. Org. Chem.* **1993**, *58*, 5002.
2. (a) Esterbauer, H.; Zollner, H.; Schaur, R. J. In *Membrane Lipid Oxidation*; Vigo-Pelfrey, C.; Boca Raton, FL: CRC Press; 1990; vol. 1, p 239. (b) Cajone, F.; Salina, M.; Bernelli-Zazzera, A. *Biochem. J.* **1989**, *262*, 977. (c) Allevi, P.; Cajone, F. *Free Radical Res. Commun.* **1992**, 12.13. (d) Allevi, P.; Anastasia, M.; Cajone, F.; Ciuffreda, P.; Sanvito, A. M. Pathophysiology of the Prooxidant / Antioxidant Balance; S.F.R.R. Summer Meeting, Siena (Italy), June 24-26, 1993 Abstr. 2.6.
3. Esterbauer, H.; Weger, W. *Monatsh. Chem.* **1967**, *98*, 1994.
4. Van Kuijk, F. J. G. M.; Thomas, D. W.; Stephens, R. J.; Dratz, E. A. *Meth. in Enzymol.* **1990**, *186*, 399.
5. Sellei, M. L.; Bartlett, M. R.; McGuiness, J. A.; Hapel, A. J.; Ardlie, N. G. *J. Chromatogr.* **1989**, *488*, 329.
6. Elder, J. S.; Mann, J.; Walsh, E. B. *Tetrahedron* **1985**, *41*, 3117.
7. Franck-Newmann, M.; Sedrati, M.; Vigneron, J.-P.; Bloy, V. *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 996.
8. Pirkle, W. H.; Adams, P. E. *J. Org. Chem.* **1979**, *43*, 1571.
9. Mukaiyama, T.; Suzuki, K. *Chem. Lett.* **1980**, 255.
10. Vinyl acetate (2.2 mL, 24 mmol) and *Pseudomonas fluorescens* lipase (500 mg) were added to a solution of the racemic hydroxy acetals **3a-h** (3 mmol) in *t*-BuOMe (10 mL). The resulting suspension was shaken at 25 °C and the progress in the reaction was monitored by TLC.
11. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

12. For all hydroxy acetals (*S*)-**3a-h** and (*R*)-**3a-h** (obtained by ethanolysis of **4a-h**) the enantiomeric excess and the configurational assignment was done by analysis of the ^{1}H NMR data of their (*R*)- and (*S*)-Mosher esters (Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543). The configurational assignment was done according to the Mosher's modified method: Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092. Diagnostic for the assignment were the chemical shifts of the protons at positions 1, 4, and 5 (if present). In fact, in the (*S*)-**3a-h**, the first two protons of the corresponding (*R*)-MTPA esters appear significantly shielded with respect to those of the (*S*)-MTPA diastereomers, the proton(s) at C-5 is (are), on the contrary, deshielded in (*R*)-MTPA esters relative to (*S*)-MTPA ones. Specular results were obtained for the Mosher esters of the hydroxy acetals (*R*)-**3a-h**.
13. Still, W. C.; Kahn, M.; Mirta, A. *J. Org. Chem.* **1978**, *43*, 2923. The unreacted hydroxy acetals (*S*)-**3a-h** showed correct ^{1}H NMR spectra identical to those of the starting racemates. Optical rotations, $[\alpha]_D^{25}$ ($CHCl_3$, *c* 1), were: -10.3 for (*S*)-**3a**, -1.4 for (*S*)-**3b**, +0.7 for (*S*)-**3c**, +0.8 for (*S*)-**3d**, +33.3 for (*S*)-**3e**, -7.9 for (*S*)-**3f**, -0.8 for (*S*)-**3g**, and -2.5 for (*S*)-**3h**. The acetoxy acetals **4a-h** showed ^{1}H NMR spectra with appropriate proton signals. Optical rotations, $[\alpha]_D^{25}$ ($CHCl_3$, *c* 1), were: +85.9 for (*R*)-**4a**, +55.8 for (*R*)-**4b**, +56.2 for (*R*)- **4c**, +42.5 for (*R*)-**4d**, -19.4 for (*R*)-**4e**, +11.8 for (*R*)-**4f**, +65.0 for (*R*)-**4g**, and +77.6 for (*R*)-**4h**.
14. A solution of **4a-h** (1 mmol) in ethanol (5 mL) was treated with sodium ethoxide (0.2 mmol) at room temperature for 1 h. Column chromatography purification (hexane-EtOAc, 80:20 v/v), afforded the (*R*)-**3a-h** (in 84-87% yields), showing physicochemical properties identical to those observed for (*S*)-enantiomers, apart from the optical rotations, $[\alpha]_D^{25}$ ($CHCl_3$, *c* 1), which were: +10.4 for (*R*)-**3a**, +1.5 for (*R*)-**3b**, -0.7 for (*R*)-**3c**, -0.9 for (*R*)-**3d**, -38.8 for (*R*)-**3e**, +8.5 for (*R*)-**3f**, +0.9 for (*R*)-**3g**, and +10.2 for (*R*)-**3h**.
15. Benedetti, A.; Comporti, M.; Esterbauer, H. *Biochim. Biophys. Acta* **1980**, *620*, 281.
16. Corey, E. J.; Katzenellenbogen, J. A.; Posner, G. H. *J. Am. Chem. Soc.* **1967**, *89*, 4245.
17. All reductions were performed using the conditions reported by Esterbauer for racemic 4-hydroxyalk-2-ynals.[3] The acetals (1 mmol) was treated with $LiAlH_4$ or $LiAlD_4$ (2 mmol) in diethyl ether (10 mL) at -25 °C for 5 h. Hydrolysis was performed with H_2O or D_2O and the product was purified on TLC (hexane-EtOAc, 70:30 v/v). The enantiomeric excess was evaluated by HPLC after treatment of the crude reaction product with (*R*)-1-(1-naphthyl)ethyl isocyanate to afford the corresponding Pirkle derivative (Pirkle, W. H.; Hoekstra, M. S. *J. Org. Chem.* **1974**, *39*, 3904) and hydrolysis of the acetal group with acid ion exchange resin (Dowex 50 x 8-200, Coppola, G. M. *Synthesis* **1984**, 1021).
18. The reaction was also satisfactory performed with $LiAlH_4$ and quenched with $[^3H]_2O$. The results will be reported with biological experiments.
19. This work was supported by Italian MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica) and by Regione Lombardia (Piano di Ricerche Finalizzate per il Settore Sanitario).

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