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# Enzymatic Resolutions in 3-amino-1,2-propanediol series

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*Abstract: The resolution of 3-amino-1,2-propanediol derivatives has been carried out by way of enzymatic catalysed hydrolyses or acylations. S substrates are preferentially attacked, and hydrolysis of the diisobutyrate derivative with E.30000 lipase gave the best enantioselectivity.*

3-amino-1,2-propanediol is an interesting synthon since it is the template for a great number of ß-blockers including propranolol, and it has been established that the activity generally resides in the S-isomers.(1) During the last five years several papers have described the enzymatic synthesis of optically active glycerol derivatives useful for the synthesis of enantiomerically pure ß-blockers.(2-14) Curiously no paper deals with the preparation of optically active 3-amino-1,2-propanediol derivatives as starting material for the synthesis of such R and S derivatives.

Our approach was to examine the possibilities for obtaining optically active molecules from simple derivatives of 3-amino-1,2-propanediol which are readily available, and without blocking selectively the primary or the secondary alcohol. As is usual in the enzymatic resolution of alcohols, two routes were studied, the acylation of alcohols (Fig.1), and the hydrolysis of O-acyl derivatives (Fig.2).

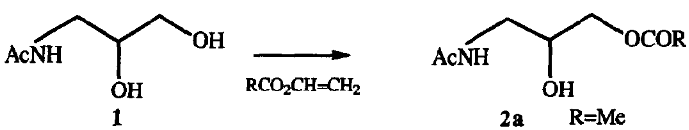



Fig 1 : enzymatic acylation

Enantiomeric excesses were determined for unreacted **1** and **3** in the respective acylation and hydrolysis reactions. Acetalization of racemic **1** with (R)-(+)-3-methylcyclohexanone **6** produced a new stereogenic carbon atom (15) and consequently four diastereomeric dioxolanes (fig.3), (2S,5S,7R)-, (2S,5R,7R)-, (2R,5S,7R)- and (2R,5R,7R)-2-[(acetylamino)-methyl]-7-methyl-1,4-dioxaspiro [4.5] decane **7**, which are cleanly separated by GPC. Of the four peaks, the inner pair correspond to the diastereomers containing the 2S (or 2R) stereogenic center of the aminopropanediol moiety while the outer pair correspond to the diastereomers containing the 2R (or 2S) stereogenic center.

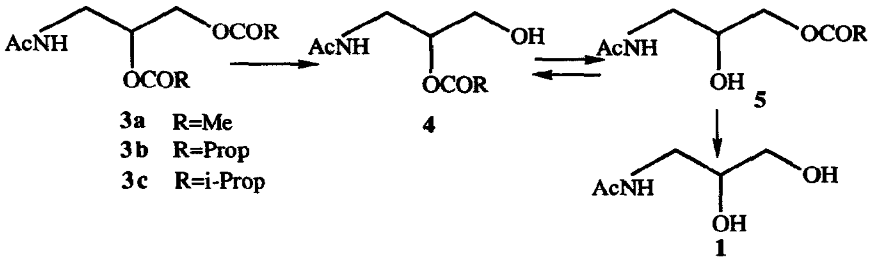
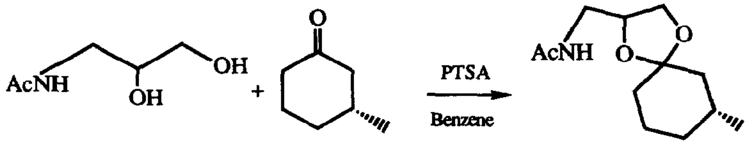



Fig. 2 : enzymatic hydrolysis

For each pair the ratio of the two peaks was 45/55, this is due to the thermodynamic stability of the diastereomers epimeric at carbon 5. The absolute configurations were attributed by determination of the optical rotation of 3-aminopropanediol obtained by total hydrolysis of unreacted 3c (entry 13, table 2), $[\alpha]_{25}^{D}$ +24.5 (c 0.15, HCl 5N)[16]




(R,S)-1 (R)-6 (2S,5S,7R)-7 (2S,5R,7R)-7 (2R,5S,7R)-7 (2R,5R,7R)-7

Fig. 3

In resolution by the way of acylation it is now well established that enol esters are the best reagents.[17,18]In order to examine the influence of the size of the acyl function on the enantioselectivity of acylation of 3-(acetylamino)-1,2-propanediol 1 (Fig.1), we used vinyl acetate and vinyl butyrate as acylating agents. Our substrate was not soluble in current aprotic solvents, and of the alcohols only t-butanol was convenient for O-acylation since it does not compete with the substrate. For that reason, pyridine and toluene-t-butanol(10/8) were used as reaction mediums. In all cases we noted only the acylation of the primary alcohol. As shown in Table 1, the beef liver acetone powder (entries 1-4) appears as the most efficient catalyst for both reaction velocity and enantioselectivity. The size of the acylating agent has no influence on the enantioselectivity, although pyridine as solvent gave the best values. Finally, except for PGS.L (entries 11,12), acylations occured preferentially for the S alcohols.

Concerning the hydrolysis route, the substrates were the 3-(acetylamino)-1,2-propanediol dialkanoate 3. Our assumption, based on previously described results [18,19],was that the primary alcohol alkanoate should be hydrolysed more rapidly than the secondary alcohol alkanoate. In our early experiments, the monohydrolysis led to a mixture of primary and secondary alcohol acetates in a 77/23 ratio, as evidenced by $^{1}H$ and $^{13}C$ nmr. As chemical preparation of the monoacetate led to a mixture with the same ratio of the two monoacetates and according to observations recently reported,[5,20] we concluded that this mixture resulted from the thermodynamic equilibrium between the two monoacetates upon intramolecular acetyl migration (Fig.2).

| Entry | enzyme* | solvent | R | time (h) | conv % | ee % (unreact 1) (R/S) |
|---|---|---|---|---|---|---|
| 1 | BLAP | tol-tBuOH | Me | 31 | 55 | 24 (R) |
| 2 | id | pyr | Me | 5 | 62 | 64 (R) |
| 3 | id | tol-tBuOH | Prop | 6.3 | 60 | 31 (R) |
| 4 | id | pyr | Prop | 9 | 55 | 64 (R) |
| 5 | PPL | pyr | Me | 23 | 60 | 51 (R) |
| 6 | id | tol-tBuOH | Prop | 78 | 67 | 27 (R) |
| 7 | E.30000 | pyr | Me | 144 | 56 | 15 (R) |
| 8 | id | tol-tBuOH | Prop | 26 | 71 | 8 (R) |
| 9 | *M.miehi* | pyr | Me | 42 | 64 | 15 (R) |
| 10 | id | tol-tBuOH | Prop | 29 | 63 | 9 (R) |
| 11 | PGS.L | pyr | Me | 29 | 63 | 26 (S) |
| 12 | id | tol-tBuOH | Prop | 29 | 53 | 5 (S) |

BLAP: beef liver acetone powder; PPL: pig pancreatic lipase; E.30000: gift of Gist Brocades, France; *M.miehi*: immobilized form from Novo; PGS.L: genetically modified lipase, gift of Plant Genetic System, Belgium.

Table 1: enzymatic acylation of 3-(acetylamino)-1,2-propanediol **1**

| Entry | Enzyme* | Subst | unreacted 3 (%) | diol 1/ tot prod | time (h) | ee (unreact 3) % (R/S) |
|---|---|---|---|---|---|---|
| 13 | E.30000 | 3a | 45 | 15 | 43 | 57 (R) |
| 14 | id | 3b | 37 | 12 | 0.5 | 63 (R) |
| 15 | id | 3c | 44 | 11 | 2.3 | 88 (R) |
| 16 | BLAP | 3a | 46 | 28 | 4.6 | 41 (R) |
| 17 | id | 3b | 44 | 12 | 0.04 | 46 (R) |
| 18 | id | 3c | 41 | 31 | 72 | 71 (R) |
| 19 | PPL | 3a | 51 | 12 | 3.3 | 36 (R) |
| 20 | id | 3b | 31 | 9 | 0.05 | 65 (R) |
| 21 | id | 3c | 39 | 21 | 53 | 76 (R) |
| 22 | WGL | 3a | 41 | | 3.3 | 28 (R) |
| 23 | PFL | 3a | 42 | 31 | 0.7 | 21 (R) |
| 24 | id | 3b | 44 | 16 | 18 | 54 (R) |
| 25 | CCL | 3a | 48 | 40 | 29 | 3 (R) |
| 26 | id | 3b | 44 | 20 | 1.5 | 20 (R) |
| 27 | id | 3c | 45 | 15 | 2.6 | 31 (R) |
| 28 | HLAP | 3a | 49 | 6 | 0.54 | 28 (S) |
| 29 | id | 3b | 43 | 18 | 2.26 | 10 (S) |
| 30 | id | 3c | 37 | 13 | 3.25 | 49 (S) |
| 31 | PLE | 3a | 39 | 16 | 5.9 | 27 (S) |
| 32 | id | 3b | 28 | 51 | 2 | 64 (S) |
| 33 | id | 3c | 31 | 10 | 4.25 | 53 (S) |
| 34 | PGS.L | 3a | 39 | 28 | 0.85 | 18 (S) |
| 35 | id | 3b | 39 | 52 | 0.08 | 9 (S) |
| 36 | id | 3c | 41 | 37 | 0.56 | 17 (S) |

WGL: wheat germ lipase; PFL: *Pseudomonas fluorescen* lipase; CCL: *Candida cylindracea* lipase; HLAP: horse liver acetone powder; *PLE: pig liver esterase.

Table 2: enzymatic hydrolysis of 3-(acetylamino)-1,2-diacyloxypropane **3**.

This phenomenon could be troublesome for the enantioselective hydrolysis since the primary alcohol acetate resulting from the first hydrolysis could be itself hydrolysed in a second reaction with a different enantioselectivity. This drawback was avoided by analyzing the unreacted dialkanoate up to more than 50% conversion of the substrate.

In order to evaluate the influence of the size of the acyl groups, on the enantioselectivity of the hydrolysis, acetyl, butyryl and isobutyryl groups were used. The results are summarized in Table 2: the hydrolysis of the diacetate **3a** were performed in water, while the hydrolysis of the dibutyrate **3b** and of the diisobutyrate **3c** were performed in a water-toluene mixture due to the insolubility of these two substrates in water. We observed, as expected, that the dibutyrate **3b** is generally the most rapidly hydrolyzed, but the highest enantiomeric excesses are generally obtained with the diisobutyrate **3c**, and E 30000 showed both rapid hydrolysis and a fairly good enantiomeric excess (entry 15).

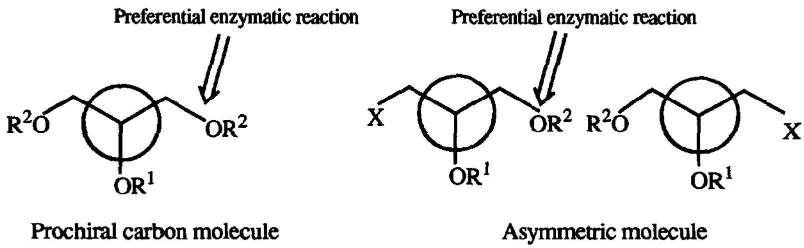



Fig. 4

In order to compare our results with those previously described for the glycerol series it is necessary to consider the substrate structures as shown in figure 4: the hydrogen bound to the secondary carbon atom is situated on the back side of the page and the three other groups on this plane with the $OR^1$ group on the under side.

In all cases described(2-6,14,21) but one(13) it appears that the preferential enzymatic reaction occurs on the $OR^2$ group situated on the right side for the molecules which have a prochiral carbon, or of the enantiomer bearing this group on the right side. Our results are consistent with this observation, acylations and hydrolysis occured preferentially for S substrates. The enantioselectivities of the enzymatic hydrolysis appear to be better than those resulting of the enzymatic acylations. This result can be due to the size of $R^2$: the bigger the $R^2$ group the better the enantioselectivity.

EXPERIMENTAL PART.

***Enzymatic reactions.*** Acylations: 5mmol of substrate, 10mmol of acylating agent and enzyme (BLAP: 0.2g; PPL: 0.25g; E30000: 0.25g; *M. miehi*: 0.95g; PGS.L: 75mg) in $10cm^3$ of solvent are vigourously stirred. Hydrolysis: were performed in solution maintained at pH7 (1N NaOH) and at 37°C in a pHstat, and containing 5mmol of substrate and enzyme (E30000: 0.21g; BLAP: 0.65g; PPL: 0.88g; PFL: 80mg;

CCL: 0.12g; HLAP: 0.8g; PLE: 0.25cm$^3$; PGS.L: 6mg) in 10cm$^3$ of water or 40cm$^3$ (water:toluene, 10:30).

***3-(acetylamino)-1,2-propanediol*** **1**. A solution of 3-amino-1,2-propanediol (9.8g, 0.11mol) and acetic anhydride (51cm$^3$, 0.5mol) in methanol (100cm$^3$) was stirred for 4h at room temperature. After evaporation of solvent and excess anhydride, the residue was put on a cations ($H^+$) exchange resin column, and the product **1** eluted with water. Evaporation of water yielded **1** (13.8g, 83%) as a viscous liquid (Found: C, 40.1; H, 8.8; N, 9.5. $C_5H_{11}NO_3$ $H_2O$ requires C, 39.7; H, 8.8; N, 9.3%); $\delta_H$ (200 MHz; $CDCl_3$) 1.78 (3H, s)), 2.97 (1H, dd, *J* 15, *J* 6), 3.12 (1H, dd, *J* 15, *J* 6), 3.28 (1H, dd, *J* 13, *J* 10), 3.38 (1H, dd, *J* 13, *J* 10), 3.58 (1H, m).

***3-(acetylamino)-1,2-propanediol diacetate*** **3a**. A solution of 3-amino-1,2-propanediol (10.3g, 0.113mol) and acetic anhydride (160cm$^3$, 1.5mol) in pyridine (100cm$^3$) was stirred at room temperature for 4h. Evaporation of pyridine and excess anhydride, and distillation yielded **3a** ($E_1$ 162°C, F 69°C) (2.07g, 84%) (Found 49.8; H, 6.75; N, 6.55. $C_9H_{19}NO_5$ requires C, 49.75; H, 6.95; N, 6.45%); $\delta_H$ (200MHz, $CDCl_3$) 2.06 (3H, s), 2.17 (6H,s), 3.53 (1H, ddd,*J* 14, *J* 7, *J* 5.5) 3.66 (1H, ddd, *J* 14, *J* 4, J 4.5), 4.18 (1H, dd, *J* 11, *J* 6), 4.34 (1H, dd, *J* 11, *J* 4), 5.17 (1H, m), 6.7 (1H, m).

***3-(acetylamino)-1,2-propanediol dibutyrate*** **3b**. Purification by liquid column chromatography yielded viscous **3b** (1.53g, 33%) (Found: C, 56.85; H, 8.5; N, 5.5. $C_{13}H_{23}NO_2$ requires C, 57.1; H, 8.45; N, 5.1%); $\delta_H$ (200MHz, $CDCl_3$) 0.65 (6H, t, *J* 7.4), 1.33 (4H, m), 1.79 (3H, s), 2.01 (2H, t, J 5.5), 2.03 (3H, s), 3.13 (1H, ddd, J 14.5, *J* 7.5, *J* 5.5), 3.28 (1H, ddd, *J* 14.5, *J* 7, *J* 5.5), 3.83 (1H, dd, *J* 13, *J* 7.5), 4.87 (1H, m), 7.56 (1H, m).

***3-(acetylamino)-1,2-propanediol diisobutyrate*** **3c**. (88% yield) (Found, C, 56.6; H, 8.65; N, 5.45. $C_{13}H_{23}NO_2$ requires C, 57.1; H, 8.45; N, 5.1%); $\delta_H$ (200MHz, $CDCl_3$) O.86 (12H, d, *J* 7), 2.26 (2H, m), 3.17 (2H, m), 3.83 (1H, dd, *J* 12, *J* 6.5), 4.0 ( 1H, dd, *J* 12, *J* 3.5), 4.83 (1H, m), 6.51 (1H, m).

***2-[(acetylamino)methyl]-7-methyl-1,4-dioxaspiro [4.5] decane*** 7. From **1**: in a Dean Stark equipped round bottom flask containing **1** (1.09g, 7.2mmol) in anhydrous benzene-methanol 25/5 (50cm$^3$) were added (R)-3-methylcyclohexanone (2cm$^3$, 1.64mmol) and p-toluene sulfonic acid (0.1g). The mixture was refluxed for 2h, evaporated and the residue dissolved in $CH_2Cl_2$, washed (water, concentrated aqueous sodium carbonate) and dried ($MgSO_4$). The solution was concentrated and liquid chromatography (basic alumina, ethyl acetate) yielded **7** (1.02g, 62%). Mass: 227, 184, 170, 155; $\delta_H$ (200MHz, $CDCl_3$) 0.61 (4H, m), 1.3 (2H, m), 1.65 (6H, m), 1.9 (3H, s), 3.25 (1H, m), 3.55 (3H, m), 4.05 (1H, m), 4.25 (1H, m).From unreacted **3**: a solution of **3** (1mmol) and 1N sodium methylate (1.25cm$^3$) in methanol (25cm$^3$) and dioxanne (25cm$^3$) was stirred for 10mn before synthesis.

REFERENCES


1. a) Karow, A.M.,Jr.; Riley, M.W.; Alquist, R.P.; *Arzneimtell Forsch.*(Engl) **1971**, 15, 103; b) Biel, J.H.; Lum, B.K.B.; *Prog. Drug Res.*(Engl) **1966**, 10, 46; c) Ariens, E.J.; *Eur. J. Clin. Pharmacol.* **1984**, 26; 663.
2. Ghisalba, O.; Lattmann, R.; Gygax, D.; *Recl. Trav. Chim. Pays-bas* **1991**, 110, 263-264.
3. Wirz, B.; Schmid, R.; Foricher, J.; *Tetrahedron: Asymmetry* **1992**, 3, 137-142.
4. Kerscher, V.; Kreiser, W.; *Tetrahedron Lett.* **1987**, 28, 531-534.
5. Iriuchijima, S.; Kojima, N.; *Agric. Biol. Chem.* **1982**, 46, 1153-1157.
6. Breitgoff, D.; Laumen, K.; Schneider, M.P.; *J. Chem. Soc., Chem. Commun.* **1986**, 1523-1524.
7. Ader, U.; Schneider, M.P.; *Tetrahedron: Asymmetry* **1992**, 3, 205-208.
8. Hamaguchi, S.; Ohashi, T.; Watanabe, K.; *Agric. Biol. Chem.* **1986**, 50, 375-380.
9. Hsu, S.-H.; Wu, S.-S.; Wang, Y.-F.; Wong, C.-H.; *Tetrahedron Lett.* **1990**, 31, 6403-6406.
10. Hamaguchi, S.; Hasegawa, J.; Kawaharada, H.; Watanabe, K.; *Agric. Biol. Chem.* **1984**, 48, 2055-2059.
11. Hamaguchi, S.; Asada, M.; Hasegawa, J.; Watanabe,K.; *Agric. Biol. Chem.* **1984**, 48, 2331-2337.
12. Ader, U.; Schneider, M.P.; *Tetrahedron: Asymmetry* **1992**, 3, 201-204.
13. Kan, K.; Miyama, A.; Hamaguchi, S.; Ohashi,T.; Watanabe, K; *Agric. Biol. Chem.* **1982**, 46, 1593-1597.
14. Iriuchijima, S.; Keiyu, A.; Kojima, N.; *Agric. Biol. Chem.* **1982**, 46, 1593-1597.
15. Hiemstra, H.; Wynberg, H.; *Tetrahedron Lett.* **1977**,25, 2183.
16. Pitré, D.; Fedeli, F.; *Arch. Pharm.* **1986**, 319, 193.
17. Degueil-Castaing, M.; De Jeso, B.; Drouillard, M.; Maillard, B.; *Tetrahedron Lett.* **1987**, 28, 953.
18. Wang, Y.-F.; Lalonde, J.J.; Momongan, M.; Bergbreiter, D.E.; Wong, C.-H.; *J. Amer. Chem. Soc.* **1988**, 110, 7200.
19. a) Theil, F.; Ballschuh, S.; Kunath, A.; Schik, H.; *Tetrahedron: Asymmetry* **1991**, 2, 1031-1034; b) Therisod, M.; Klibanov, A.M.; *J. Amer. Chem. Soc.* **1986**, 108, 5638.
20. Liu, K.K.-C.; Nozaki, K.; Wong, C.-H.; *Biocatalysis* **1990**, 3, 169-177.
21. Baba, N.; Tahara, S.; Yoneda, K.; *Chemistry Express* **1991**, 6, 423-426.