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ENANTIOSELECTIVE HYDROLYSES BY BAKER'S YEAST - III.[1] MICROBIAL RESOLUTION OF ALKYNYL ESTERS USING LYOPHILIZED YEAST

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Abstract - Both enantiomers of optically active 1-alkyn-3-ols were obtained in high optical purity by microbial resolution of their racemic acetates using a stable and ready-to-use lyophilized yeast preparation.

INTRODUCTION

Enzymatic resolution of racemic esters by means of microorganisms has become a frequently used method for the preparation of chiral building blocks during the past decade[2]. Whereas, in general, only more or less sensitive microorganisms have been employed, easy to handle yeast has been examined upon its hydrolytic abilities just on a few selected substrates[3]. To gain more detailed information about this valuable technique we recently started an investigation on this topic[1,4]

RESULTS AND DISCUSSION

Principle of the method

In previous papers we have shown that fermenting yeast (*Saccharomyces cerevisiae Hansen*) is a valuable biocatalyst for the asymmetric hydrolysis of racemic amino acid esters[1,4].

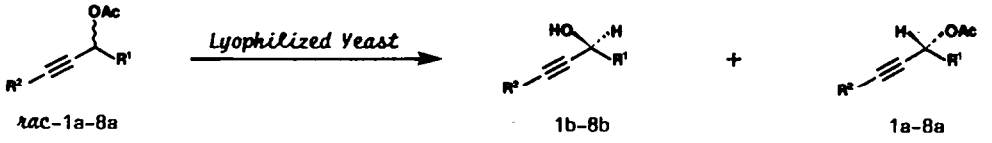
We now tried to employ this method on the microbial resolution of esters bearing their chirality center in the alcohol moiety. In that case, both of the enantiomers can be obtained at an optimum in chemical and optical yield, if the conversion is stopped at the appropriate degree[5]: 40% conversion for the alcohol formed and 60% for the remaining *ent*-ester. This may be monitored by titration of the acid formed during hydrolysis by means of an autoburette. Using *fermenting* yeast this method was not applicable, since carboxylic acids arising from the metabolism of carbohydrates falsified the result of the titration method. The way out of this problem we found in using a *lyophilized* yeast preparation showing the following advantages:

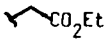
1) No metabolism is detected as long as no carbohydrates are added making the application of a pH-stat feasible.
2) The hydrolytic activity compared to *fermenting* yeast is fully maintained and remains stable for at least three months, when stored at 0-4°C.

Upon examination of the hydrolytic activity of freeze dried yeast towards a variety of structurally different esters[6] we found that *inter alia* 3-acetoxy-1-alkynes were hydrolyzed at a reasonable rate.

Optically active 1-alkyn-3-ols have frequently been used as starting material for the synthesis of several classes of compounds such as: alkaloids[7], prostaglandins[8], pyrethroids[9], pheromones[10], vitamins[11], steroids[12] and antibiotics[13]. For their preparation the asymmetric reduction of 1-alkyn-3-ones[14] and the enantioselective hydrolysis using microorganisms and enzymes[9c,15] are reported. Whereas the first of these methods mentioned requires enantiomerically pure auxiliary reagents which are not always easily accessible, the second method was performed with selected microorganisms only (e.g. *Bacillus subtilis*[9c,15], *Brevibacterium ammoniagenes*[15c] and *Rhizopus nigricans*[16]) which may not be cultivated without sterile fermentation equipment. The use of esterase from *Candida cylindracea* led to products with low enantiomeric excess[15c].

By variation of substituents R^1 and R^2 of our model substrate (see scheme I) we investigated the structural requirements of the enantioselection in asymmetric hydrolysis using lyophilized yeast with acetates of propargylic alcohols applying a strategy previously published[5b,17].



Compound	1	2	3	4	5	6	7	8
R^1			$n\text{-}C_5H_{11}$	$n\text{-}C_7H_{15}$			Ph	
R^2	H	CH_3	H	H	H	H	H	H

SCHEME I: Enantioselective hydrolysis of 3-acetoxy-1-alkynes by lyophilized yeast.

TABLE I: Enantiomeric excess of products.

Substrate	Conversion 40%		Conversion 60%	
	Product[a]	e.e.[%]	Product[a]	e.e.[%]
rac-1a	1b	91[b,c]	1a	72[d]
rac-2a	2b	94[b,e]	2a	<10[f]
rac-3a	3b	>97[b]	3a	74[d]
rac-4a	4b	91[b]	4a	18[d,f]
rac-5a	5b	95[b]	5a	35[d,f]
rac-6a	6b	91[b]	6a	>97[g]
rac-7a	7b	86[b,e]	7a	64[d]
rac-8a	8b	87[b]	8a	37[d,f]

[a] For absolute configuration see scheme I. [b] Determined by ^{19}F-NMR spectroscopy of the MTPA-ester using Eu(fod)$_3$. [c] Determined by HPLC-analysis of the MTPA-ester. [d] Determined by ^1H-NMR spectroscopy using Eu(hfc)$_3$. [e] Determined by comparison of the optical rotation value with literature data. [f] Low e.e., since 60% conversion was not achievable within 48 h. [g] Determined by comparison of the optical rotation value with material obtained by acetylation of 6b.

Determination of absolute configuration

Correlation of sense and magnitude of optical rotation with literature data revealed the absolute configuration for compounds 1b[18], 2b[11b,18], 3b[15c], 4b[19], 7b[20] and 8b[9c]. 5b and 6b were hydrogenated to give (*R*)-3-hexanol[21] and (*R*)-3-hydroxypentanoic acid ethyl ester[22], respectively, the absolute configuration of which is well established.

Influence of substituents

Upon examination of the results from table I the following characteristics of the enantioselective hydrolysis by lyophilized yeast were found:

1) All acetates of alcohols 1b-8b, the configuration of which is (*S*) in 1b-6b and (*R*) in 7b and 8b, (the change from (*S*) to (*R*) is only due to the altered priorities of substituents), were cleaved preferentially as compared to the corresponding enantiomers 1a-8a.
2) Independent from the structure of substituent R^1 hydrolysis up to a conversion of 40% proceeded with better enantioselection - yielding alcohols 1b-8b with an e.e. ranging from 87 to >97% - than extended hydrolysis up to 60% conversion which gave products of lower optical purities.
3) Hydrolysis of substrates *rac*-2a, *rac*-4a, *rac*-5a and *rac*-8a ceased beyond about 40% conversion. Therefore, the remaining esters 2a, 4a, 5a and 8a showed very low optical purity. Repeated addition of lyophilized yeast forced the reaction beyond this point, but the large amount of biomass diminished the overall yield of products during extractive workup drastically.
4) Replacement of the acetylenic hydrogen by a methyl group in substrate *rac*-1a (giving *rac*-2a) showed a drastic decrease in the speed of hydrolysis, which made a conversion of 60% unaccomplishable for this substrate.
5) An unsubstituted CH_2-unit adjacent to the acetoxy group was obviously necessary for a high degree of enantioselection. In presence of more sterically hindered groups (substrates *rac*-7a and *rac*-8a) yeast hydrolysis led to products with lower enantiomeric excess.
6) By yeast only hydrolysis of the acetoxy group was catalysed. The ethyl ester moiety of substrate *rac*-6a remained unchanged.

CONCLUSION

By these results it is shown that lyophilized yeast cells present a valuable and practical biocatalyst for enantioselective hydrolysis. Thus, starting from 3-acetoxy-1-alkynes, optically active propargylic alcohols with a variety of structural features were obtained, themselves being useful chiral building blocks for EPC-syntheses. Studies on the hydrolytic enzyme system(s) of yeast and yeast preparations and on its extended use in bioorganic transformations are currently under investigation in this laboratory.

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EXPERIMENTAL

Optical rotations were measured on a Perkin Elmer 141 polarimeter in $CHCl_3$ solution unless otherwise stated. NMR-spectra (^1H, ^{13}C and ^{19}F) were recorded in $CDCl_3$ on a Bruker WH 90 spectrometer. Chemical shifts are reported in δ values from internal standards (TMS, $CDCl_3$ and CCl_3F, resp.). s=singlet, d=dublet, t=triplet, q=quartet and m=multiplet. HPLC-analyses were performed on a RP 18 column (250x4mm) using water/acetonitrile 3:7 as eluent (0.6ml/min, UV-detection at 254nm). Reactions were monitored by TLC using Merck 60 F_{254} silica gel plates and compounds were visualized by spraying with vanillin/sulfuric acid. Elemental analyses (C,H,N) for all novel compounds were within 0.4% of calculated values. Column chromatography was performed on Merck 60 silica gel.

Synthesis of substrates

Propargylic alcohols *rac*-1b[18], *rac*-4b[17] and *rac*-8b[9c] were obtained by ethynylation of the corresponding aldehydes following a well established method[22]. *rac*-3b[19a] and *rac*-7b[22] were purchased from Fluka, *rac*-5b was synthesized according to the literature[24].

6-Methyl hept-2-yn-4-ol (*rac*-2b)[11b,18]: A solution of 5-methyl hex-1-yn-3-ol (*rac*-1b)[18] (10.0g, 89mmol), pyridine (14.0g, 178mmol) and trimethylsilyl chloride (10.6g, 98mmol) in CH_2Cl_2 (100ml) was stirred at room temperature overnight. Then the mixture was poured into sat. $NaHCO_3$ solution (100ml). The aqueous phase was separated, extracted with CH_2Cl_2 and the combined organic layer was evaporated to give the crude trimethylsilyl ether (10.1g, 61%). To a cooled (-80°C) solution of this ether (6.0g, 32mmol) in THF (60ml), *n*-Bu Li (20.2ml of a 1.6*N* solution in hexane, 32mmol) was added with stirring. After 20min methyl iodide (4.56g, 32mmol) was added. Then the temperature was allowed to reach 0°C and the reaction was quenched by addition of water (20ml) and K_2CO_3 (2g). After extraction of the mixture with diethyl ether the organic phase was washed with 2*N* HCl for desilylation *in situ*. The volatiles were removed *in vacuo* and the remaining oil was distilled to give 3.9g (96%) of *rac*-2b, bp 60-5°C/10mm (lit.[11b]: 60°C/3mm). ^1H-NMR: 0.97 (d, J=6Hz, 6H), 1.40-1.73 (m, 3H), 1.83 (d, J=2Hz, 3H), 2.33-2.60 (m, 1H), 4.37 (m, 1H).

3-Hydroxy pent-4-ynoic acid ethyl ester (*rac*-6b) was synthesized in analogy to the procedure of Panchenko[25] starting from 3-trimethylsilylprppynal[26] and 2-bromoacetic acid ethyl ester. Yield 71%, bp 65-70°C/0.25mm[27]. ^1H-NMR: 1.30 (t, J=7Hz, 3H), 2.46 (d, J=2Hz, 1H), 2.73 (d, J=6Hz, 2H), 3.23 (d, J=6Hz, 1H), 4.20 (q, J=7Hz, 2H), 4.73 (m, 1H). ^{13}C-NMR: 13.94 (ester-CH_3), 42.09 (C-2), 58.28 (C-3), 60.89 (ester-CH_2), 73.17 (C-5), 83.30 (C-4), 170.9 (C-1).

Acetates *rac*-1a - *rac*-8a were obtained by esterification of the corresponding alcohols *rac*-1b - *rac*-8b using a standard procedure (acetic anhydride/pydidine/4-dimethylaminopyridine, methylene chloride)[28].

5-Methyl-1-hexyn-3-yl acetate (*rac*-1a): yield 58%, bp 70°C/15mm[27]. ^1H-NMR: 0.90 (d, J=6Hz, 6H), 1.65 (m, 3H), 2.00 (s, 3H), 2.48 (d, J=2Hz, 1H), 5.40 (dt, J=2 and 6Hz, 1H).

6-Methyl-2-heptyn-4-yl acetate (*rac*-2a): yield 98%, bp 65-70°C/13mm[27]. ^1H-NMR: 0.78 (d, J=6Hz, 6H), 1.43 (m, 3H), 1.65 (d, J=2Hz, 3H), 2.00 (s, 3H), 5.17 (m, 1H).

1-Octyn-3-yl acetate (*rac*-3a): yield 94%, bp 55-8°C/4.0mm[27]. ^1H-NMR: 0.90 (t, J=7Hz, 3H), 1.30-1.35 (m, 6H), 1.72 (m, 2H), 2.00 (s, 3H), 2.50 (d, J=2Hz, 1H), 5.33 (dt, J=2 and 7Hz, 1H).

1-Decyn-3-yl acetate (*rac*-4a): yield 65%, bp 60-5°C/0.15mm[27]. ^1H-NMR: 8.87 (t, J=7Hz, 3H), 1.05-1.50 (m, 10H), 1.50-2.00 (m, 2H), 2.00 (s, 3H), 2.40 (d, J=2Hz, 1H), 5.30 (dt, J=2 and 6Hz, 1H).

Hex-5-en-1-yn-3-yl acetate (*rac*-5a): yield 83%, bp 45-50°C/15mm[27]. ^1H-NMR: 2.06 (s, 3H), 2.47 (d, J=2Hz, 1H), 2.55 (t, J=7Hz, 2H), 4.94-5.28 (m, 2H), 5.41 (dt, J=2 and 7Hz, 1H), 5.58-6.10 (m, 1H).

3-Acetoxy pent-4-ynoic acid ethyl ester (*rac*-6a): yield 93%, bp 80-5°C/0.55mm[27]. ^1H-NMR: 1.21 (t, J=7Hz, 3H), 2.00 (s, 3H), 2.48 (d, J=2Hz, 1H), 2.83 (dt, J=2 and 7Hz, 2H), 4.17 (q, J=7Hz, 2H), 5.74 (dt, J=2 and 7Hz, 1H).

1-Phenyl prop-2-yn-1-yl acetate (*rac*-7a): yield 97%, bp 80-5°C/0.8mm[27]. ^1H-NMR: 2.00 (s, 3H), 2.67 (d, J=2Hz, 1H), 6.36 (d, J=2Hz, 1H), 7.13-7.53 (m, 5H).

(*E*)-4-Methyl hept-4-en-1-yn-3-yl acetate (*rac*-8a): yield 94%, bp 70-3°C/13mm[27]. ^1H-NMR: 1.00 (t, J=7Hz, 3H), 1.74 (s, 3H), 1.80-2.00 (m, 2H), 2.08 (s, 3H), 2.50 (d, J=2Hz, 1H), 5.78 (m, 2H).

Preparation of lyophilised yeast

Fresh yeast [*Saccharomyces cerevisiae Hansen*, 600ml of 17% (w/w) suspension] from the late exponential growth phase was incubated with saccharose (60g). After stirring the culture at 34°C for 2 h - saccharose was consumed at this point - the cells were isolated by centrifugation (3000xg), resuspended in dist. water (500ml) and centrifuged again. The pellet obtained was lyophilized at -20°C to give about 80g of a yellowish powder. No significant decrease in hydrolytic activity was observed during a period of 3 months when stored at 0-4°C.

General procedure for the asymmetric hydrolysis using lyophilised yeast

Lyophilized yeast (150% of substrate weight) was suspended in phosphate buffer (0.1*N*, pH 7.0, 70ml). The mixture was equilibrated for 20 min at r.t. Then substrate ester *rac*-1a - *rac*-8a (10mmol) was added at once. While vigorous stirring was maintained the pH was kept constant at 7.0 by addition of *N* NaOH from an autoburette. When the appropriate degree of conversion was accomplished (40% for the alcohol formed and 60% for the remaining ester), the cells were removed by centrifugation and the products were extracted from the supernatant with CH_2Cl_2 or petroleum ether (3x50ml). The pellet was resuspended in ethanol (20ml), stirred for 20 min, the cells were filtered and the filtrate was evaporated. The residue was combined with the organic phase. After removal of the volatiles the alcohols formed (1a-8a) were separated from the remaining esters 1b-8b by column chromatography and distilled. Depending on the volatility of the compounds the yields were in a range of 40-80% for alcohols and 70-90% for esters.

TABLE II: Optical rotation values.

Compound[a]	$[\alpha]_D^{20}$ [°]	c [g/100ml]	e.e. [%]
1a	+72.9	4.9[b]	72
1b	-18.9	3.3[b]	91
2a	+14.7	1.7[b]	6
2b	-14.8	1.3[b]	94
3a	+61.0	2.7[b]	74
3b	-7.0	5.4[b]	>97
4a	+12.1	2.7[b]	18
4b	-3.9	3.4[b]	91
5a	+24.2	3.3[b]	35
5b	-39.1	2.5[b]	95
6a	+82.3	3.7[b]	>97
6b	-22.1	3.6[b]	91
7a	-2.8	7.9[b]	64
7b	-26.8	3.5[b]	86
	-17.4	3.3[c]	86
8a	-16.1	4.9[d]	37
8b	+31.7	4.1[d]	87

[a] For absolute configuration and determination of e.e. see scheme I and table I. [b] $CHCl_3$ solution. [c] 1,4-Dioxane solution, see ref. 20. [d] Diethyl ether solution, see ref. 9c.

REFERENCES AND NOTES

1 For part II see: B.I.Glänzer, K.Faber and H.Griengl, *Tetrahedron* 43, 771 (1987).

2 Leading references are cited in ref.1; recent publications: G.M.R.Tombo, H.-P.Schär, W.Zimmermann and O.Ghisalba, *Chimia* 39, 313 (1985). H.Ohta, Y.Miyamae and G.Tsuchihashi, *Agric.Biol.Chem.* 50, 3181 (1986).

3a W.J.Marsheck and M.Miyano, *Biochim.Biophys.Acta* 316, 363 (1973).

b S.Miura, S.Kurozumi, T.Toru, T.Tanaka, M.Kobayashi, S.Matsubara and S.Ishimoto, *Tetrahedron* 32, 1893 (1976).

c K.Laumen and M.Schneider, *Tetrahedron Lett.*5875, 1984.

4 B.I.Glänzer, K.Faber and H.Griengl, *Tetrahedron Lett.* 4293, 1986.

5a C.-S.Chen, Y.Fujimoto, G.Girdaukas and C.J.Sih, *J.Am.Chem.Soc.*104, 7294 (1982).

b Th.Oberhauser, M.Bodenteich, K.Faber, G.Penn and H.Griengl, *Tetrahedron* submitted (1987).

6 To be published in a forthcoming paper.

7 L.E.Overman and K.L.Bell, *J.Am.Chem.Soc.*103, 1851 (1981).

8a J.Fried and J.C.Sih, *Tetrahedron Lett.* 3899, 1973.

b J.S.Elder, J.Mann and E.B.Walsh, *Tetrahedron* 41, 3117 (1985).

9a T.Matsuo, T.Nishioka, M.Hirano, Y.Suzuki, K.Tsushima, N.Itaya and H.Yoshioka, *Pestic.Sci.*11, 202 (1980).

b M.Franck-Neumann, M.Sedrati, J.-P.Vigneron and V.Bloy, *Angew.Chem.*97, 995 (1985); *Int.Ed.Engl.*24, 996 (1985).

c T.Sugai, S.Kuwahara, C.Hishino, N.Matsuo and K.Mori, *Agric.Biol.Chem.*46, 2579 (1982).

10a S.Kuroda, T.Katsuki and M.Yamaguchi, *Tetrahedron Lett.* 803, 1987.
b K.Sato, T.Nakayama and K.Mori, *Agric.Biol.Chem.*43, 1571 (1979).
c W.H.Pirkle and P.E.Adams, *J.Org.Chem.*44, 2169 (1979).
11a K.-K.Chan, A.C.Specian Jr. and G.Saucy, *J.Org.Chem.*43, 3435 (1978).
b K.-K.Chan, N.Cohen, J.P.De Noble, A.C.Specian Jr. and G.Saucy, *J.Org.Chem.*41, 3497 (1976).
12 W.S.Johnson, B.Frei and A.S.Gopalan, *J.Org.Chem.*46, 1512 (1981).
13a J.L.Herrmann, M.H.Berger and R.H.Schlessinger, *J.Am.Chem.Soc.*101, 1544 (1979).
b J.D.Buynak, J.Mathew and M.N.Rao, *J.Chem.Soc., Chem.Commun.*941, 1986.
14 M.M.Midland, A.Tramontano, A.Kazubski, R.S.Graham, D.J.S.Tsai and D.B.Cardin, *Tetrahedron* 40, 1371 (1984).
15a K.Mori and H.Akao, *Tetrahedron Lett.* 4127, 1978.
b K.Mori and H.Akao, *Tetrahedron* 36, 91 (1980).
c T.Oritani and K.Yamashita, *Agric.Biol.Chem.*44, 2407 (1980).
16 H.Ziffer, K.Kawai, M.Kasai, M.Imuta and C.Froussios, *J.Org.Chem.*48, 3017 (1983).
17 G.Eichberger, G.Penn, K.Faber and H.Griengl, *Tetrahedron Lett.*2843, 1986.
18 J.-P.Vigneron and V.Bloy, *Tetrahedron Lett.*2683, 1979.
19 M.D.De Marquez, P.J.Rowland, P.M.Scopes and V.Thaller, *J.Chem.Res. (M)* 1348, 1986.
20 G.Tadema, R.H.Everhardus, H.Westmijze and P.Vermeer, *Tetrahedron Lett.*3935, 1978.
21 H.C.Brown, M.C.Desai and P.K.Jadhav, *J.Org.Chem.*47, 5065 (1982).
22 D.Seebach, F.Giovannini and B.Lamatsch, *Helv.Chim.Acta* 68, 958 (1985).
23 L.Skattebol, E.R.H.Jones and M.C.Whiting, *Org.Synth., Coll.Vol.*IV, 792 (1963).
24 A.Viola and J.H.MacMillan, *J.Am.Chem.Soc.*92, 2404 (1970).
25 S.I.Panchenko, N.A.Bychkova, G.P.Yurevich, N.V.Zotchik and I.A.Rubtsov, *Zh.Org.Khim.*18, 2197 (1983).
26 K.J.H.Kruithof, R.F.Schmitz and G.W.Klumpp, *Tetrahedron* 39, 3073 (1983).
27 Kugelrohr distillation.
28 G.Höfle, W.Steglich and H.Vorbrüggen, *Angew.Chem.*90, 602 (1978); *Int.Ed.Engl.*17, 569 (1978).
29 Omitting this fermentation step led to a preparation showing only about half of the hydrolytic activity.