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THE MICROBIAL REDUCTIVE SPLITTING OF THE N-O BOND OF DIHYDROOXAZINES; AN ALTERNATIVE TO THE CHEMICAL REDUCTION

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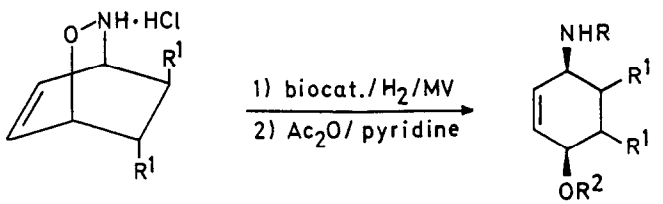
Abstract: In the presence of artificial mediators many bacteria reductively split the N-O bond of dihydrooxazines in a chemoselective way if hydrogen is used as an electron source. The yields as determined by HPLC are almost quantitative i.e. considerably better than by chemical reductions.

During our studies on the synthesis of amino cyclitols as building blocks for the mutasynthesis of aminoglycoside antibiotics /1/ we faced the problem of getting satisfactory yields in the reductive splitting of the N-O bonds of the intermediary dihydrooxazines /2/. So far, only two chemoselective methods have been reported for this purpose: The first /3/ uses zinc dust and acid. Due to the lack of reproducible quality of the reagent the yields vary strongly.
By the second method /4/, reaction with aluminium amalgam (or other amalgams /5/) only a few N-acylated dihydrooxazine derivatives can be reduced. Therefore, we tried the reduction of the N-unsubstituted compounds with suitable microorganisms in the presence of methylviologen and hydrogen gas as an electron donor.



R^1:		
	H	1
	OAc	2
	OMe	3

R^1:	R^2:	
H	Ac	4
OAc	Ac	5
OMe	Ac	6
OMe	H	7

The three dihydrooxazines 1-3 /2,6,7/ have been reduced in the presence of 1 mM methylviologen under an atmosphere of hydrogen gas by various microorganisms. Examples are shown in the Table. The influence of the different residues R is not very marked. The rate of the reaction could easily be followed by the hydrogen uptake measured by mercury filled manometers attached to the reaction vessels. The reaction rate depends on the presence of methylviologen. Without this mediator the rate decreases to about 10 - 20%. There was no spontaneous reduction of the substrates by reduced methylviologen. Also the pH value has a strong effect. The rate for the reduction of 3 at pH 9 is about 3 times higher than at pH 7. At pH 6 the rate was negligible. With the thermophilic organism M. thermoautotrophicum the reduction could be performed at temperatures up to 70°C. There was about a five fold increase of the rate compared to that at 37°C.

The reaction was within the limits of analytical accuracy quantitative. Even after very long reaction times only the N-O bond was reduced. The carbon-carbon double bond of the dihydrooxazines was not attacked. That means, the reduction reveals a complete chemoselectivity. However, a stereoselectivity could not be observed. There was no change in the reduction rate after a 50% conversion of a racemic mixture of the dihydrooxazine derivatives and the rate of the reduction of both enantiomeres having in each case an ee value of 95% showed no difference.

Table: Rates of the reduction of 1 - 3 expressed in productivity numbers[a] by various microorganisms[b].

Organisms	Substrate	PN	Remark
Methanosarcina	1	1850	--
barkeri DSM 800	2	1350	--
	3	160	without MV
Methanobacterium	3	600	--
thermoautotrophicum	3	1300	pH 8.0
DSM 2133	3	1750	pH 9
	3	3100	pH 7.0, 70°C
Clostridium kluyveri	1	920	--
DSM 555	1	50	without MV
	2	600	--
	3	1500	--

a) Productivity number = mmol product/kg catalyst (dry weight) x time (h) /8/.

b) If not stated otherwise the temperature was 36°C, the pH 7.0 and the concentrations of methylviologen 1 mM.

Besides the organisms mentioned in the Table also Thermoproteus tenax DSM 2078 and Clostridium tyrobutyricum (Lal) DSM 1460 catalyzed the reductive splitting of the N-O bond. However, the former organism is difficult to grow and the latter had productivity numbers of only about 50% of the organisms shown in the Table. Usually the reductions were conducted in in 0.1-0.3 mmol scale. In one experiment 6 mmol 3 were reduced in 25 ml buffer by 0.2 g (dry weight) M. thermoautotrophicum. The analytical yield in the reaction mixture was 95%. After isolation and purification the yield was 73%.

According to our experience anaerobic microorganisms have often rather high reductase activities which are not pyridine nucleotide-dependent. Not seldom such enzymes can be supplied with electrons by artficial mediators such as viologens /8/. Electron donors may be hydrogen gas, formate or the cathode of an electrochemical cell. In the first two cases the presence of a hydrogenase or a viologen-dependent formate dehydrogenase is necessary. With such organisms even in form of resting cells the productivity numbers are usually much higher than those observed with yeasts or the reactions are even an idiosyncracy of such organisms.

This means that relatively small volumes and small amounts of biocatalyst can be used for quantitative conversions leading to a relatively convenient isolation procedure. The cells can be stored at -15°C under the exclusion of oxygen for many months without loss of their activity.

Experimental

All cells were obtained from the Deutsche Sammlung für Mikroorganismen and grown according to the literature. For C. kluyveri see l.c./9/, M. barkeri l.c./10/ and M. thermoautothrophicum l.c. /11/. The cells were harvested and stored without washing under exclusion of oxygen at -15°C. The dry weight of the wet packed sediment is about 20%. Hydrogenations at pH 7 were conducted in 0.1 phosphate buffer, that at higher pH values in 0.1 Tris/HCl buffer.

The hydrogenations were conducted in Warburg vessels shaken at the indicated temperatures in a water bath with about 200 strokes/min. The vessels were equipped with mecury-filled Warburg manometers. All operations were executed under omission of oxygen. After cessation of hydrogen uptake the cells were centrifuged, washed with a little buffer and the combined supernatants evaporated. The residues were ususally acetylated with acetic anhydride in pyridine or the amino alcohol was isolated as such. The products 4-7 could be obtained in pure form as judged by thin layer and gas liquid chromatography. Their structures were established by IR, ^{1}H- and ^{13}-NMR as well as mass spectroscopy. For details see l.c. /2,6,7,12/. Hydrogenation of 3 in a preparative scale: six mmol of the free amine 3 were added to a suspension of 1.0 g (wet packed cells $\hat{=}$ 0.2 g dry weight) in 25 ml 0.1 M Tris/HCl buffer pH 8.5 containing 1.0 mM methylviologen. The vessel was shaken under normal pressure of hydrogen for 10 h. The product could be isolated in a pure form in 73 % yield and characterized as the diacetyl derivative, 1r-acetamido-4c-acetoxy-2c,3c,-dimethoxy-5-cyclohexene, m.p. 111°C. - ^{1}H-NMR: 2.03, 2.11 (2x3H, s, CH_3CO); 3.47, 3.52 (2x3H, s, CH_3O); 3.56 (1H, dd, H-2, $^{3}J_{2,3}$ = 6.2, $^{3}J_{1,2}$ = 4.5);

3.65 (1H, dd, H-3, $^{3}J_{2,3}$ = 6.2, $^{3}J_{3,4}$ = 3.2); 4.86 (1H, br, H-1); 5.25 (1H, mc, H-4); 5.68 (2H, mc, H-5/6); 6.1 (1H, d, NH, $^{3}J_{NH,1}$ = 8.5) - ^{13}C-NMR: 21.1 (CH_3); 45.1 (C-1); 58.6, 58.8 (CH_3O); 68.7 (C-4); 76.2, 76.9 (C-2/3); 124.9, 130.0 (C-5/6); 169.6, 170.2 (C=O).

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References

1. J. Distler, W. Klier, W. Piendl, O. Werbitzky, A. Böck, G. Kresze, W. Piepersberg, FEMS Microbiol. Lett. 30, 145 (1985).
2. H. Braun, K. Klier, G. Kresze, M. Sabuni, O. Werbitzky, J. Winkler, Liebigs Ann. Chem. 1986, 1360 and references cited therein.
3. Review: G. Kresze, J. Firl, Fortschr. Chem. Forsch. 11, 245 (1969).
4. G.E. Keck, S. Flemming, D. Nickell, P. Weider, Synth. Comm. (4) 281 (1972).
5. Chr. Harder, G. Kresze, M. Sabuni, unpublished results.
6. G. Kresze, W. Dittel, Liebigs Ann. Chem. 610 (1981).
7. G. Kresze, E. Kysela, Liebigs Ann. Chem. 202 (1980).
8. H. Simon, J. Bader, H. Günther, S. Neumann, J. Thanos, Angew. Chem. 97, 541 (1985), Angew. Chem. Int. Ed. Engl. 24, 539 (1985) and references cited therein.
9. J. Bader, H. Simon, Arch. Microbiol. 127, 279 (1980).
10. W.E. Bald, G.E. Fox, L.J. Magrum, C.R. Woese, R.S. Wolfe, Microbiol. Rev. 43, 260 (1979).
11. P. Schönheit, J. Moll, R.K. Thauer, Arch. Microbiol. 127, 59 (1980).
12. K. Klier, Dissertation TU-München (1986).

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