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Synthesis of (R)-Pantoyl Lactone by Reduction of Ketopantoate with Formate and *Proteus* species

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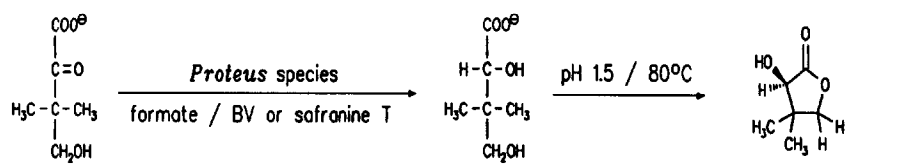
Abstract: (R)-Pantoyl lactone (ee > 97 %) is obtained by stereoselective reduction of ketopantoate with resting cells of anaerobically grown *Proteus vulgaris* or *Proteus mirabilis*. Benzyl-viologen or safranine T have been used as electron mediators.

(R)-Pantoyl lactone **3** is important for the synthesis of (R)-pantothenate and (R)-pantothenol, which are educts for the biological synthesis of coenzym A. (R)-Pantothenate is used in the pharmaceutical and food industry, but particularly as a growth factor in animal food. (R)-Pantothenol is a useful drug for wound and skin disease treatment[1a]. The lactone **3** can also be used for the synthesis of S-*tert*-leucine derivatives[1b] or as a chiral auxiliary in asymmetric rhodium catalyzed cyclopropanations[1c].

The 2-hydroxycarboxylate-viologen-oxidoreductase (HVOR) from *Proteus* (*P.*) species, an unusual molybdo enzyme[2a], reduces reversibly 2-oxocarboxylates with very high stereoselectivity and extremely broad substrate specificity[2b-e]. HVOR shows also an astonishingly broad specificity for artifical mediators (electron carriers). They differ in their standard redox potential $E^{o'}$ from –440 mV to +217 mV. That means, reductions as well as dehydrogenations can be carried out quantitatively[2c,e,3b,c]. The cathode of an electrochemical cell, formate and/or hydrogen gas can be used as electron donors for preparative reductions[2c,e]. It is possible to increase the HVOR and formate dehydrogenase activities in crude extracts by variations of the growth medium of the *Proteus* species to values up to 20-30 U for the pyruvate or phenylpyruvate reduction and 8-18 U for the formate dehydrogenation[3a,b] . Most other 2-oxocarboxylates are reduced with rates of the same order of magnitude. Substrates with a quarternary carbon atom in 3-position show slower reduction rates[2c]. With partially purified HVOR ketopantoate **1** shows in optical tests only an activity of 4-7 % of that observed with pyruvate or phenylpyruvate[2b]. Although **1** is a relatively slow reacting substrate **3** can be prepared with *P. vulgaris* or *P. mirabilis* rather effectively if compared with other procedures.

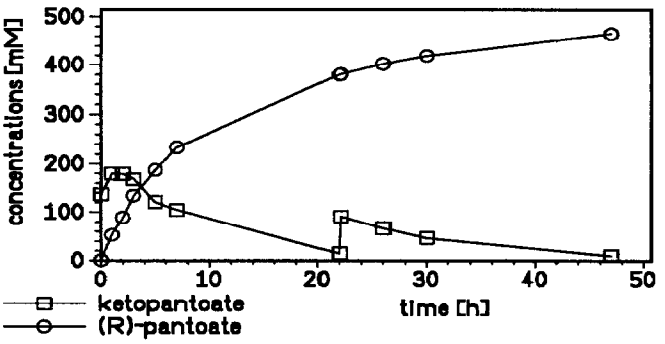
Scheme:



ketopantoate **1** (R)-pantoate **2** (R)-pantoyl lactone **3**

The highest productivity numbers (PN)[5] are reached for the reduction of **1** by *P. vulgaris* or *P. mirabilis* with about 200 mM concentrations of **1**. The time course of such a reduction is shown in the **Figure**. The preparative reduction was carried out in an anaerobic 100 ml bulb and the pH was regulated with 2 N formic acid by an automatic pH control system at pH 7.0[2c]. Three mmol **1** were transformed with 320 mg *P. vulgaris* cells (dry weight) in the presence of 4.5 mmol formate, 240 nmol tetracycline and 24 µmol benzylviologen (BV). A 5 M solution of **1** was automatically added via a syringe with a flow of 200 µl/h for the first 3 h and then with 100 µl/h over a period of additional 7 h holding the concentration of **1** over a period of 6 h at about 150 mM. After 47 h 98.5 % of **1** was reduced with a PN of 740[6] and an ee-value > 97 %[2c].

Figure: Preparative ketopantoate reduction with continuous substrate addition



Instead of BV safranine T (Aldrich) can be used as mediator, too (**Scheme**). As shown in **Table 1** after 3 h a PN is reached with safranine T, which is almost as high as that observed with BV. On a molar basis safranine T is 17fold cheaper than BV. The PNs after 3 h reach their maxima at a safranine T concentration of 100-150 µM. The low solubility of safranine T in water (about 1 mM) is not unfavourable for the reduction of **1**. Already 10 µM safranine T increase the PN from 120 to 910 (**Table 1**).

Table 1: Productivity number after 3 h of the ketopantoate reduction with *P. mirabilis* in the absence and presence of mediators

mediator	PN after 3 h	relative rates (%)
without artificial mediator	120	8
safranine T (10 µM)	910	57
safranine T (20 µM)	1170	73
safranine T (100 µM)	1420	88
safranine T (150 µM)	1430	89
safranine T (250 µM)	1430	90
benzylviologen (1 mM)	1610	100

Experiments with only 30 mg cells (dry weight) in the presence of safranine T showed no decrease of the reaction rate for about 100 h. Safranine T is not as efficient as BV as mediator for the reduction of 2-oxo-

carboxylates, which are reduced 10-100 times faster than **1**, because the electron transport or the formate dehydrogenase is then the reaction limiting step.

The advantages of the here described (R)-pantoyl lactone synthesis can be realized by comparision with other methods (**Table 2**).

Table 2: Literature survey for the synthesis of (R)-pantoyl lactone

method (mM)*)	**ee-value**	**yield**)**	**catalysts, chiral educt or microorganisms***)**
chemical	56.0 %	93 %	[Rh(bppm)(1,5-hexadien)Cl] [7a]
chemical	80.5 %	95 %	[Rh(bppm)(cod)Cl] [7b]
chemical	86.7 %	93 %	[Rh(bppm)(cod)Cl] [7c]
chemical	50.6 %	n.b.	(R,R)-DMPNPH [7d]
chemical	> 99 %	40.0 %	Dimethyl (R)-malate [7e]
chemical	26.4 %	37.5 %	Deguphos® [7f]
microbial (40)	> 98 %	(> 90 %)	*Saccharomyces cerevisiae* (30) [7g]
microbial (n.g.)	95-100 %	(> 90 %)	*Byssochamys fulva* [7h]
microbial (390)	> 98 %	(> 90 %)	*Nocardia asteroides/ Candida parapsilosis* (130) [7i]
microbial (700)	94-98 %	(> 90 %)	*Candida parapsilosis* [7j]
microbial (140)	94.4 %	(90.5 %)	*Rhodococcus erythropolis* [7k]
microbial (380)	99.7 %	(80.3 %)	*Rhodotorula minuta* [7l]
microbial (910)	> 98 %	(90.0 %)	*Agrobacterium sp. S-246* (120) [7m]
microbial (620)	82.8 %	(89.0 %)	*Nocardia asteroides/ Agrobacterium radiobacter* (130) [7n]
microbial (530)	96.0 %	(90.0 %)	*Fusarium oxysporum* [7o]
microbial (20)	93.0 %	39.0 %	*Saccharomyces cerevisiae* [7p]
microbial (460)	> 97 %	(98.5 %)	*Proteus vulgaris* (740, this work)

*) Final product concentration; **) Yields of isolated product; the conversion yields are given in parenthesis;***) The PNs are noted in parenthesis, if they could be calculated from the literature; n.g.: not given.
bppm = (2S,4S)-N-*tert*-butoxycarbonyl-4-diphenylphosphino-2-diphenylphosphinomethylpyrrolodine; cod = 1,5-cyclooctadien; DMPNPH = N-α-methylbenzyl-1-propyl-2,4-dimethyl-1,4-dihydronicotinamide.

Chemical methods deliver high yields, but the ee-values are low to moderate with the exception of the method of Wasmuth et al.[7e], which has however a low yield. The microbial methods used the reduction of **1**[7k,m,n] or ketopantoyl lactone[7g,h,i,j,l,p], but also the stereoselective dehydrogenation of (S)-pantoyl lactone[7i,k,n] or the stereospecific hydrolysis of **3** from the racemate[7o]. It is possible to convert (R,S)-pantoyl lactone with one[6k] or two[6i,n] microorganisms to (R)-pantoyl lactone. These microbial methods also led to mostly excellent ee-values and yields, but the PNs are low (30 to 130). So the reduction of **1** with the HVOR in *Proteus* species is an efficient alternative for the production of **3**. The formation of the product in form of **3** in a concentration of ≥ 460 mM is rather simple and effective. For the isolation of **3** the solution was acidified with conc. hydrochloric acid to pH 1.5, centrifugated with 38000 g for 20 min, the supernatant solution was heated at 80°C for 2 h and then continuously extracted with diethyl ether. The enantiomeric excess was determined after evaporation of the diethyl ether. Afterwards **3** was recrystallized from toluol[6].

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(5) PN = product [mmol]/ dry weight of the cells [kg] x time [h]

(6) The (R)-pantoyl lactone showed a m.p.: 87-89°C; ^{1}H-NMR ($CDCl_3$): δ 4.16 (s, 1H), 4.03 (d, 1H, J = 9.85 Hz), 3.96 (d, 1H, J = 9.85 Hz), 1.23 (s, 3H), 1.09 (s, 3H); ^{13}C-NMR ($CDCl_3$): δ 177.9 (C1), 76.5 (C4), 75.7 (C2), 40.8 (C3), 22.9 and 18.8 (C5 and C6); Anal. calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75; Found: C, 55.31; H, 7 64.

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