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Calcium Pantothenate. Part 2.[1] Optimisation of Oxynitrilase-Catalysed Asymmetric Hydrocyanation of 3-Hydroxy-2,2-dimethylaldehyde: Synthesis of (*R*)-Pantolactone

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Abstract:

The synthesis of (*R*)-pantolactone via oxynitrilase-catalysed asymmetric hydrocyanation of 3-hydroxy-2,2-dimethylaldehyde has been investigated. (*R*)-Oxynitrilases from almonds as well as from apple and plum kernels were employed as catalysts in the form of defatted meal. A number of factors influencing the hydrocyanation process have been studied and the conditions optimised using statistical methods. (*R*)-Pantolactone with 74% yield and 30% ee has been synthesised.

Introduction

The methods of (*R*)-pantolactone ((*R*)-dihydro-3-hydroxy-4,4-dimethyl-2(3*H*)-furanone, (*R*)-**4**) manufacture are still the object of research, despite over 60 years elapsing from its first total synthesis.[2] The reason for that is the importance of (*R*)-pantolactone in calcium (*R*)-pantothenate and (*R*)-panthenol synthesis. Both compounds, as vitamin B_5 precursors, find wide application in food, cosmetics and pharmaceuticals. The existing methods of (*R*)-pantolactone manufacturing are based on the resolution of the racemate,[3,4] or asymmetric hydrogenation of dihydro-4,4-dimethyl-2,3-furandione (**5**).[5] The drawback of both methods are the long and complicated technologies starting from racemic **4** synthesis, which is subsequently resolved into optical isomers or oxidised to ketolactone **5** and reduced back to (*R*)-**4**. However, the methodology of (*R*)-**4** synthesis can be radically shortened and simplified by carrying out an asymmetric hydrocyanation of aldehyde **2** that allows us to obtain (*R*)-pantolactone directly. With such an approach the racemate resolution or the oxidation/reduction processes are no longer necessary (Scheme 1).

The asymmetric hydrocyanation of aldehydes is a well-known reaction, catalysed among others by oxynitrilases, the enzymes present in several plants. A number of reviews over asymmetric hydrocyanation are available.[6] In the case of (*R*)-pantolactone synthesis, the simplest substrate is 3-hydroxy-2,2-dimethylaldehyde (**2**), and a few efforts of its asymmetric hydrocyanation have been reported.[7] In all cases an (*R*)-oxynitrilase from almonds was employed as a catalyst. The isolated and purified enzyme was immobilised on a support or used as a buffered solution, and the syntheses afforded (*R*)-pantolactone in 62−96% yield and 20−89% enantiomeric excess. However, the use of the isolated and purified enzyme is not very practical since an additional stage of enzyme preparation is necessary which obviously prolongs and complicates the whole process and raises its cost. Thus, we attempted to utilise the enzyme without isolating it from the original material by application of defatted meal from almonds as a catalyst in the hydrocyanation of aldehyde **2**. The aim of our work was to maximise both the yield and enantiomeric excess of cyanohydrin (*R*)-**3** and, consequently, those of (*R*)-pantolactone. Since similar application of the defatted almond meal instead of the purified enzyme have been reported in the case of different aldehydes,[8] as well as the possibility of using meals from other sources mentioned,[9] we also tested meals from apple and plum kernels.

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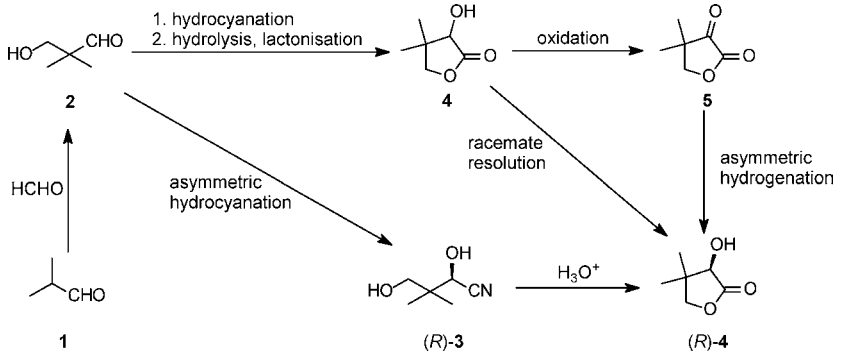
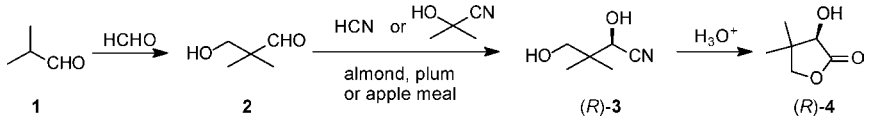
Scheme 1. **(*R*)-Pantolactone manufacturing strategies**



Scheme 2. **Synthesis of (*R*)-pantolactone via asymmetric hydrocyanation of 3-hydroxy-2,2-dimethylaldehyde**



Results and Discussion

(*R*)-Pantolactone synthesis utilizing the asymmetric hydrocyanation consists of three steps: an aldol reaction of 2-methylpropanal (**1**) with formaldehyde that leads to aldehyde (**2**) that undergoes asymmetric hydrocyanation to afford (*R*)-2,4-dihydroxy-3,3-dimethylbutylnitrile (**3**), which is subsequently hydrolysed in acidic conditions with direct formation of (*R*)-**4** (Scheme 2).

The first and the last reaction proceeds without complications with high yields; moreover, acidic conditions during hydrolysis ensures that no racemisation of either the nitrile or the lactone stereogenic centre takes place.[7c] Therefore, the main object of our study was an asymmetric hydrocyanation of aldehyde **2**, as the key step of the whole synthesis, responsible for the optical purity of (*R*)-pantolactone. Among the described syntheses of (*R*)-**3** that employed the isolated enzyme, the best results have been obtained using hydrogen cyanide (HCN).[7b,c] The application of acetone cyanohydrin (ACH) as a HCN source has been reported to give a much lower ee of nitrile (*R*)-**3**;[7a] however, considering safety, we have decided to start with ACH in our work.

We have tested the influence of buffer concentration, z_1, the reaction time, z_2, and temperature, z_3, on the yield, y_1, and enantiomeric excess, y_2, of (*R*)-pantolactone using 2^3 factorial design.[10] The three additional centre point experiments, where the levels for all variables were chosen at an intermediate value, have been set up for checking the experiments' repeatability (0 in Table 1).

Since the nitrile (*R*)-**3** hydrolysis to the lactone (*R*)-**4** proceeds in acidic conditions quantitatively and without any racemisation, we have not measured the respective values for the former but for the latter only. The experiments have been performed at random, and for each one the yield, y_1, and enantiomeric excess, y_2, of (*R*)-pantolactone have been determined by capillary gas chromatography. The results along with the experimental matrix are shown in Table 2.

Table 1. **Factorial design I: variables at maximum and minimum levels[a]**

z_i	natural variable	(−1)	(0)	(+1)
z_1	buffer concentration (% vol)	6	9	12
z_2	reaction time (h)	6	12	18
z_3	temperature (°C)	12	20	28

[a] Enzymatic reaction conditions: 2 mmol of acetone cyanohydrin, 0.12 g of almond meal and 2 mL of diisopropyl ether per mmol of aldehyde **2** respectively. The experiments were performed at a scale of 2 mmol of **2**, variables of the factorial design II were constant ($x_5 = -0.8$, $x_6 = -2.2$).

The factorial design I statistical analysis and the significant coefficients are summarised in Table 3. The coefficients have been calculated using the polynomial function of the experimental coded variables as given in eq 1.

$$y = b_0 + \sum b_i x_i + \sum\sum b_{ij} x_i x_j + e \qquad (1)$$

Mathematical models for the yield, $\hat{y}_1$, and the enantiomeric excess, $\hat{y}_2$, are outlined in eqs 2 and 3.

$$\hat{y}_1 = 5.925 + 1.200 \cdot x_1 + 1.200 \cdot x_2 + 3.825 \cdot x_3 \qquad (2)$$

$$\hat{y}_2 = 5.475 + 1.975 \cdot x_2 - 3.075 \cdot x_3 - 0.775 \cdot x_1 \cdot x_2 + 0.875 \cdot x_1 \cdot x_3 - 2.175 \cdot x_2 \cdot x_3 \qquad (3)$$

The results obtained, both the yield and the ee of (*R*)-pantolactone were rather disappointing. However, the value measured for (*R*)-**4** enantiomeric excess (16.6%) was similar to that reported by Ognyanov et al. (ee = 20% for nitrile (*R*)-**3**).[7a] This unfortunately meant that probably no significant success could be achieved with the acetone cyanohydrin methodology used. Comparison of the reaction conditions applied here and reported by Ognyanov et al. with those reported by Effenberger[7c] to give much better yield and ee of (*R*)-**4** clearly showed that employment of acetone cyanohydrin instead of the free hydrogen cyanide might be the main reason for the poor results of the enzyme-catalysed asymmetric hydrocyanation. Therefore, considering addition-

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Table 2. **Factorial design I: experimental matrix and the results**

	coded variables			yield of (*R*)-**4** (%)		ee of (*R*)-**4** (%)	
trial no.	x_1	x_2	x_3	response y_1	calculated $\hat{y}_1$	response y_2	calculated $\hat{y}_2$
1	−	−	−	0.9	−0.3	3.0	4.5
2	+	−	−	1.3	2.1	5.8	4.3
3	−	+	−	1.5	2.1	16.6	14.4
4	+	+	−	4.7	4.5	8.8	11.1
5	−	−	+	6.4	7.4	3.2	1.0
6	+	−	+	10.3	9.8	2.0	4.3
7	−	+	+	10.1	9.7	0.6	2.1
8	+	+	+	12.2	12.1	3.8	2.3
9	0	0	0	4.5	5.9	5.0	5.5
10	0	0	0	5.8	5.9	5.8	5.5
11	0	0	0	5.1	5.9	5.2	5.5

Table 3. **Factorial design I: influence of variables and statistical analysis**

	values for yfield ($\hat{y}_1$)		values for ee ($\hat{y}_2$)	
significant coefficients b_i	b_i	t_{bi}	b_i	t_{bi}
b_0	5.925	25.757	5.475	37.195
b_1	1.200	5.217	−	−
b_2	1.200	5.217	1.975	13.417
b_3	3.825	16.628	−3.075	20.891
b_{12}	−	−	−0.775	5.265
b_{13}	−	−	0.875	5.944
b_{23}	−	−	−2.175	14.776
t_{bi} critical	4.303		4.303	
level of confidence	0.95		0.95	
degrees of freedom	2		2	
residual variance	0.956		14.625	

Table 4. **Asymmetric hydrocyanation of aldehyde 2 catalysed by isolated (*R*)-oxynitrilase from almonds**

(*R*)-oxynitrilase		(*R*)-**3**[a]		(*R*)-**4**	
amount (U/mmol of **2**)	activity (U/mg)	yield (%)	ee (%)	yield (%)	ee (%)
125[b]	160[b]	−	−	58	62
250[b]	160[b]	−	−	85	80
150	38	55	83	−	−
175	74	84	89	−	−
425	136	77	89	−	−

[a] Reference 7c. [b] According to the supplier: Sigma-Aldrich Chemie GmbH.

ally the statistical analysis of the results obtained (Table 3), we substantially revised our methodology.

We have performed the (*R*)-pantolactone synthesis via aldehyde **2** asymmetric hydrocyanation catalysed by free (*R*)-oxynitrilase from Sigma as described by Effenberger,[7c] additionally followed by direct hydrolysis of nitrile (*R*)-**3** to (*R*)-pantolactone. The yield and enantiomeric excess determined for (*R*)-**4** were substantially similar to that reported for nitrile (*R*)-**3**. Besides proving the positive influence of the free hydrogen cyanide application, this also confirmed our assumption about the nature of the hydrolysis, proceeding in a quantitative manner and without racemisation of either the nitrile or the lactone stereogenic centre (Table 4).

Table 5. **Factorial design II: variables maximum and minimum levels**

z_i	natural variable	(−)	(0)	(+)
z_2	reaction time (h)	2	4	6
z_4	buffer amount (ml/mmol of **2**)	0.1	0.2	0.3
z_5	almond meal amount (g/mmol of **2**)	0.1	0.2	0.3
z_6	solvent amount (ml/mmol of **2**)	5	7.5	10

Therefore, we have performed the new series of asymmetric hydrocyanation experiments using almond meal as catalyst and free hydrogen cyanide as CN^- source. We have decided to use 2.5 molar excess of HCN calculated with respect to aldehyde **2** and, because of the very poor yields obtained at lower temperature in factorial design I, to carry out all the reactions at 28 °C. We have tested the influence of the amounts of buffer, z_4, almonds meal, z_5, and solvent, z_6, as well as the reaction time, z_2, on the yield, y_1, and enantiomeric excess, y_2, of (*R*)-pantolactone using 2^4 factorial design (Table 5).

Again, the experiments have been performed at random, and for each one the yield and enantiomeric excess of (*R*)-pantolactone have been determined by capillary gas chromatography. The polynomial function calculated for the (*R*)-**4** yield from experiments 12−27 using eq 1 did not, however, fit the experimental results. Therefore, we decided to expand our factorial design II to central composite design[10] by carrying out a number of additional experiments (trials 28−38). Complete results along with the experimental matrix are shown in Table 6.

Similarly to the previous design, the experimental coded variables coefficients have been calculated using a polynomial function as given in eq 1, and along with statistical analysis have been summarised in Table 7. An analysis of the variable coefficients indicates a considerable complication level of the reaction model studied. Among four variables, only the reaction time, z_4, has been found insignificant. As expected, we observed relevant interaction coefficient between buffer, x_4, and almonds meal, x_5, amounts (b_{45}).

$$\hat{y}_1 = 31.893 + 6.280 \cdot x_5 - 4.481 \cdot x_2 \cdot x_5 - 2.606 \cdot x_4 \cdot x_5 + 6.139 \cdot x_6^2 \quad (4)$$

$$\hat{y}_2 = 20.747 - 2.766 \cdot x_4 + 4.529 \cdot x_5 + 3.640 \cdot x_6 - 1.797 \cdot x_6^2 \quad (5)$$

Table 6. **Factorial design II: experimental matrix and the results**

	coded variables				yield of (*R*)-**4** (%)		ee of (*R*)-**4** (%)	
trial no.	x_2	x_4	x_5	x_6	response y_1	calculated $\hat{y}_1$	response y_2	calculated $\hat{y}_2$
12	−	−	−	−	20.7	27.5	13.2	13.8
13	−	+	−	−	19.2	27.8	7.0	6.8
14	−	−	+	−	56.9	50.2	22.0	22.7
15	−	+	+	−	43.0	40.0	18.4	18.6
16	−	−	−	+	26.5	23.4	23.0	21.0
17	−	+	−	+	30.3	23.7	14.0	14.0
18	−	−	+	+	49.6	54.2	29.6	29.8
19	−	+	+	+	29.6	44.1	25.6	25.8
20	+	−	−	−	38.8	31.5	15.0	15.1
21	+	+	−	−	47.3	41.7	10.4	8.1
22	+	−	+	−	27.7	36.3	21.2	21.4
23	+	+	+	−	28.8	37.0	17.0	17.3
24	+	−	−	+	31.5	27.5	22.6	22.2
25	+	+	−	+	29.6	37.6	14.4	15.2
26	+	−	+	+	50.7	40.3	28.4	28.6
27	+	+	+	+	50.7	40.0	24.0	24.5
28	−1.547	0	0	0	28.8	30.9	22.0	21.5
29	+1.547	0	0	0	33.4	30.9	20.6	21.5
30	0	−1.547	0	0	22.3	31.9	25.2	24.5
31	0	+1.547	0	0	41.9	31.9	16.6	15.9
32	0	0	−1.547	0	20.4	22.6	7.0	9.1
33	0	0	+1.547	0	44.6	42.0	24.8	23.1
34	0	0	0	−1.547	61.4	52.1	14.7	15.1
35	0	0	0	+1.547	43.3	52.1	26.5	26.4
36	0	0	0	0	35.3	37.5	19.8	20.2
37	0	0	0	0	39.9	37.5	20.8	20.2
38	0	0	0	0	36.5	37.5	19.0	20.2

Table 7. **Factorial design II: influence of variables and statistical analysis, test *F***

	values for the yield ($\hat{y}_1$)		values for the ee ($\hat{y}_2$)	
significant coefficients	b_i	t_{bi}	b_i	t_{bi}
b_0	31.893	44.856	20.747	61.410
b_4	−	−	−2.766	11.125
b_5	6.280	11.999	4.529	18.211
b_6	−	−	3.640	14.636
b_{55}	−	−	−1.797	5.364
b_{66}	6.139	8.706	−	−
b_{25}	−4.481	7.512	−	−
b_{45}	−2.606	4.369	−	−
t_{bi} critical	4.303		4.303	
level of confidence	0.95		0.95	
degrees of freedom	2		2	
residual variance	101.272		1.285	

Mathematical models for the yield, $\hat{y}_1$, and enantiomeric excess, $\hat{y}_2$, are outlined in eqs 4 and 5. Generally, the results achieved with the new methodology utilizing free hydrogen cyanide were much better when compared with those obtained with acetone cyanohydrin. The enantiomeric excess and yield for (*R*)-pantolactone reached 29.6% and 49.6% respectively (trial 18). The optimal conditions inside the design core, calculated with MS Excel Solver were found at the border of the area: $x_2 = -1$, $x_4 = -1$, $x_5 = +1$, $x_6 = +1$ for the yield, $\hat{y}_1$ and $x_4 = -1$, $x_5 = +1$, $x_6 = +1$ for the enantiomeric excess, $\hat{y}_2$ (reaction time, x_2, was not present in eq 5). This corresponded to the best result achieved; however, such values were still not satisfactory, and thus, we have sought the better reaction conditions beyond the investigated range. We have employed a steepest-ascent procedure, as a useful tool for this purpose. Since the equation without the interaction coefficients did not fit the experimental results for the yield (according to the test *F*), we have used the values of the enantiomeric excess to estimate the new reaction conditions (Table 8). The steepest-ascent line has been calculated from the polynomial function for the enantiomeric excess, $\hat{y}_2$, without the interaction coefficients.

Only the first experiment carried out along the path of steepest ascent brought some improvement of the yield, y_1, and the determined ee, y_2, has been in compliance with the expected value. For the next steps (trials 40, 41) the enantiomeric excess of (*R*)-pantolactone rapidly decreased and similarly did the yield. An analysis of the reaction conditions showed that this was not as surprising as it seemed at the first glance. The buffer amount coefficient for the enantiomeric excess had a negative sign (Table 7, b_1), thus indicating that inside the factorial design II a decrease in the buffer amount had a positive influence on the ee of (*R*)-**4**. However, the steepest-ascent line going beyond the range of factorial design II brought this value almost to zero, that made a qualitative change in the reaction conditions. It is well-known that the enzyme requires small amounts of water to retain its catalytic activity;[11] therefore, the insufficient amount of buffer had certainly suppressed the enzymatic reaction in steps 2 and 3 of the steepest-ascent procedure. This not only caused rapid ee decrease but also significantly

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Table 8. **Reaction conditions[a] and the results of the steepest-ascent procedure**

			step 1	step 2	step 3
natural variable/result		z_i or y_i	trial 39	trial 40	trial 41
buffer amount (ml/mmol of **2**)		z_4	0.134	0.068	0.002
almond meal amount (g/mmol of **2**)		z_5	0.305	0.411	0.511
solvent amount (ml/mmol of **2**)		z_6	9.71	11.93	14.14
yield (%)	response	y_1	74.1	47.3	20.0
enantiomeric excess (%)	response	y_2	29.4	12.1	3.4
	calculated	y_2	28.2	37.2	46.2

[a] Reaction time, z_2, of all experiments was 4 h.

Table 9. **Comparison of the catalytic properties of meals from different sources[a]**

no.	meal source	yield of (*R*)-**4** (%)	ee of (*R*)-**4** (%)
18	almonds	49.6	29.6
43	apple kernels	32.7	11.5
44	plum kernels	36.6	19.6

[a] Reactions carried out at optimal conditions from factorial design II ($x_2 = -1$, $x_4 = -1$, $x_5 = +1$, $x_6 = +1$).

lowered the yield, and the residual yield was the result of the noncatalysed reaction.

Taking into account the literature data reporting good results of asymmetric hydrocyanation of a number of aliphatic aldehydes with meals from various plants,[9] we have decided to investigate the reaction catalysed by defatted meals from kernels of plums and apples. The experiments were performed at the optimum conditions calculated from the factorial design II ($x_2 = -1$, $x_4 = -1$, $x_5 = +1$, $x_6 = +1$), and the results comparing catalytic properties of kernel meal from almonds, apples, and plums are shown in Table 9.

Application of meals from apple and plum kernels brought no improvement either of the yield or the enentiomeric excess of synthesised (*R*)-**4**. The best results remained the ee's of 29.4% and 29.6% and yields 74.1% and 49.6% obtained in trials 39 and 18, respectively.

Conclusions

In summary, we have completed the synthesis of (*R*)-pantolactone (*R*)-**4** with the key step of asymmetric hydrocyanation of 3-hydroxy-2,2-dimethylaldehyde (**2**) catalysed by defatted meal from almonds as well as from kernels of apples and plums. We showed that acetone cyanohydrin is not a satisfactory cyanide source for a meal-catalysed aldehyde **2** hydrocyanation. By employing free hydrogen cyanide as the CN^- source we have studied the influence of several factors on the yield and enantiomeric excess of the product with the aid of DOE, finding the buffer, solvent, and meal amounts to be most important. We have synthesised (*R*)-**4** with 74.1% yield and 29.4% enantiomeric excess using the defatted meal from almonds. An attempt to apply meals from plum and apple kernels brought no further improvment.

Experimental Section

Commercially available solvents and reagents were used without further purification. Defatted meals were prepared from almonds and kernels of mature garden plums and apples by triple extraction with ethyl acetate and drying under reduced pressure (300 hPa). Meals were refrigerated and stored under argon. 3-Hydroxy-2,2-dimethylaldehyde (**2**) was distilled under reduced pressure and was melted under argon atmosphere prior to reaction. Pantolactone yield was determined by capillary GC chromatography: HP 6890 apparatus, HP-1 column 30 m × 0.32 mm, temperature program 100−160 °C at 10 °C/min, injector 250 °C, detector 250 °C, carrier gas He 2.0 mL/min. GC analysis of pantolactone enantiomers: HP 6890 chromatograph, capillary column with chiral stationary phase Supelco 2-4304 (*beta dex 120*) 30 m × 25 mm, temperature program 140−160 °C at 4 °C/min, 160 °C 2 min, injector 235 °C, FID detector 235 °C, carrier gas He 1.0 mL/min. Retention times: (*S*)-**4**, 7.0 min and (*R*)-**4**, 7.1 min.

Immobilised (*R*)-Oxynitrilase-Catalysed Hydrocyanation of 3-Hydroxy-2,2-dimethylaldehyde (2). (*R*)-Oxynitrilase from Sigma-Aldrich Chemie GmbH was immobilised on Celite (250 U/300 mg or 500 U/600 mg) and inoculated for 15 min. with 0.3 mL of 0.02 M acetate buffer pH = 3.3. Then 0.21 g (2 mmol) of freshly melted aldehyde **2** in diisopropyl ether was added, followed by 4 mmol of hydrogen cyanide (2.94 M solution in diisopropyl ether; CAUTION: TOXIC, all operations with HCN and its solutions should be carried out under a ventilation hood, using appropriate protective clothes). The reaction was maintained for 2 h at 20 °C; the immobilised enzyme was filtered off and washed with diethyl ether. Combined ether fractions were evaporated, affording nitrile (*R*)-**3** as a colourless oil, which was immediately hydrolysed to (*R*)-**4**.

Almond Meal-Catalysed Hydrocyanation of 3-Hydroxy-2,2-dimethylaldehyde (2) with Acetone Cyanohydrin. Defatted almond meal (0.6 g) was inoculated for 15 min with 0.02 M citrate buffer (pH 5.5, for amounts see Table 1). Then 0.21 g (2 mmol) of freshly melted aldehyde **2** in diisopropyl ether was added, followed by 2 equiv of acetone cyanohydrin. The reaction was maintained for given times and conditions (Table 1, Table 2); the almond meal was then filtered off and washed with diethyl ether. Combined ether fractions were evaporated, affording nitrile (*R*)-**3** as a colourless oil, which was immediately hydrolysed to (*R*)-**4**.

Defatted Plant Meal-Catalysed Hydrocyanation of 3-Hydroxy-2,2-dimethylaldehyde (2) with Hydrogen Cyanide: General Procedure. Defatted meal from almonds as well as apple and plum kernels was inoculated for 15 min. with 0.02 M citrate buffer (pH 5.5, for buffer amount see

Tables 5, 8−10). Then, freshly melted aldehyde **2** in diisopropyl ether was added, followed by 2.5 equiv. of hydrogen cyanide (2.94 M solution in diisopropyl ether; CAUTION: TOXIC, all operations with HCN and its solutions should be carried out under a ventilation hood, using appropriate protective clothes). The reaction was maintained for given times and conditions (Tables 5, 8−10); the meal was then filtered off and washed four times with diethyl ether. Combined ether fractions were evaporated, affording nitrile (*R*)-**3** as a colourless to pale-yellow oil, which was immediately hydrolysed to (*R*)-**4**.

Nitrile (*R*)-3 Hydrolysis to (*R*)-Pantolactone ((*R*)-4): General Procedure. Concentrated hydrochloric acid (1.5 equiv) was immediately added to the crude nitrile (*R*)-**3** followed by 5−10 mL of methanol. The reaction mixture was then maintained for 12−16 h at room temperature and 3−4 h at reflux. After cooling, the pH was adjusted to 4.3−4.6 with sodium carbonate, and methanol was evaporated off. The residue was dissolved in diethyl ether and dried with magnesium sulphate. The solids were filtered off and washed with diethyl ether, and the combined filtrates were evaporated to give a colourless to pale-yellow crystallising syrup of (*R*)-pantolactone. The yield and enantiomeric excess of (*R*)-**4** was then determined by capillary gas chromatography.

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