Highly chemoselective reduction of aromatic nitro compounds to the corresponding hydroxylamines catalysed by plant cells from a grape (*Vitis vinifera* L.)

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Cells from a grape (*Vitis vinifera* L.) reduce aromatic nitro compounds under mild conditions to the corresponding hydroxylamines with unprecedented chemoselectivity.

In the past decades, biotransformation of exogenous substrates by plant cells has becoming increasingly important and has attracted much attention. Plant cells have been widely used as the most promising biocatalysts for various organic reactions such as hydroxylation, glycosylation, hydrolysis, oxidation of alcohol, and reduction of ketone and olefin.[1] However, the synthetic potential of plant cells as a reducing agent of the nitro group has never been discovered to date, despite the fact that the reduction of nitro compounds is one of the most classical reactions in organic synthesis. Very recently, we have reported the first chemoenzymatic method for preparing arylhydroxylamines using bakers' yeast as a biocatalyst.[2] As part of a continued interest in exploring novel biocatalysts for the chemoselective reduction of aromatic nitro compounds, herein we wish report the first example of using plant cells as a reducing agent of the nitro group to prepare arylhydroxylamines.

Our initial investigations focused on the use of various plant cells to reduce 4-nitro-1,8-naphthalic anhydride **1a**. The substrate was selected as a model because it is an important precursor for the synthesis of various naphthalimide derivatives that are potent photonucleases[3] and DNA-targeted antitumor drugs.[4] In a typical experiment,[5] the substrate was reduced by plant cells under conventional conditions and the reaction process was monitored by HPLC.† As shown in Table 1, with the exception of cells from garlic and cactus (entries 10–11), various plant cells were able to reduce **1a** and the reductions afforded hydroxylamine **1c** and amine **1d**, although their ratios varied significantly with plant species. Among the tested plant cells, the grape cells exhibited the highest reactivity and chemoselectivity for hydroxylamine (entry 18). The time-course of this reaction catalyzed by grape cells is shown in Fig. 1. Differing from typical enzyme-catalyzed reactions, the reaction hardly proceeded on the first day, while the conversion increased to 73% after 2 days. When the reaction proceeded for 4 days, the conversion reached 96% and the selectivity was always >98%. It was surprising to find that the selectivity of the reaction remained at 96% after 6 days. The influence of the amount of

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Table 1 Chemoselective reduction of **1a** catalysed by various plant cells[a]

Entry	Plants	Amount of plant/g	Time/d	Conv.[b] (%)	**c/d**[b]
1	Maize (*Zea mays* L.)	20	2	41	17/83
2	White gourd (*Benincasa hispida* Cogn.)	20	2	52	58/42
3	Shallot (*Allium ascalonicum* Hort.)	20	3	20	39/61
4	Onion (*Allium cepa* L.)	20	4	41	80/20
5	Carrot (*Daucus carota* L.)	20	4	81	85/15
6	Tomato (*Lycopersicon esculentum* Mill.)	20	4	78	83/17
7	Potato (*Solanum tuberosum* L.)	20	4	94	13/87
8	Radish (*Raphanus sativus* L.)	20	4	25	60/40
9	Cucumber (*Cucumis sativus* L.)	20	4	82	80/20
10	Garlic (*Allium sativum* L.)	20	5	0	—
11	Cactus (*Opuntia dillenii* (Ker-Gawl.) Haw.)	20	5	0	—
12	Pear (*Pyrus pyrifolia* (Burm.) Nak.)	20	4	67	88/12
13	Banana (*Musa* Spp.)	20	4	85	81/19
14	Peach (*Prunus persica* (L.) Batsch.)	20	4	92	84/16
15	Orange (*Citrus reticulata* Blanco.)	20	4	93	91/9
16	Apple (*Malus pumila* Mill.)	20	4	45	69/31
17	Persimmon (*Diospyros kaki* L.)	20	4	36	58/42
18	Grape (*Vitis vinifera* L.)	20	4	96	>98/2
19	Grape (*Vitis vinifera* L.)	40	4	97	94/6
20	Grape (*Vitis vinifera* L.)	60	4	99	92/8

[a] Reaction conditions: water (100 ml), freshly cut plant, substrate (100 mg), 25 °C. [b] The conversion and the selectivity were determined by ^{1}H NMR.

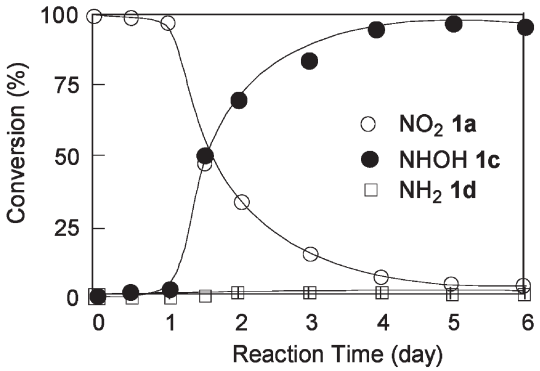


Fig. 1 Chemoselective reduction of **1a** catalysed by grape cells (**1a** 100 mg, grape 20 g, water 100 ml, at 25 °C).

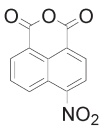
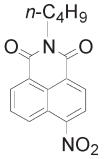
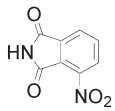
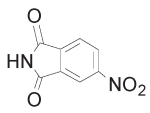
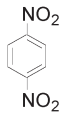
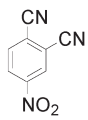
grape on the chemoselectivity was also examined and a little change was observed (entries 19–20). It indicated that the chemoselective reaction catalyzed by grape cells has unique advantages, which did not require strict control of reaction time and amount of catalyst.

To investigate the scope of the chemoselective reaction catalyzed by grape cells, a series of aromatic nitro compounds bearing electron-withdrawing groups was examined under the optimized reaction conditions, and the results are summarized in Table 2. Reduction of 4-nitro-substituted naphthalimides **2a** gave the corresponding hydroxylamines **2c** with 100% selectivity, though the conversion is moderate (entry 2).‡ The chemoselective reduction was also successfully applied to nitro-substituted phthalimides **3a** and **4a**, affording the desired hydroxylamines **3c** and **4c** with >98 and 96% selectivities, respectively (entries 3–4). However, no reaction took place in the reduction of **5a** and **6a** under the same conditions, although both substrates also possess strong electron-withdrawing groups (entries 5–6). Similar to the reduction of nitroarenes using bakers' yeast,[2] the nitroso compounds **b** were not detected. In all cases, no by-products such as hydrazines, azoarenes and azoxyarenes were detected by the ^{1}H NMR spectrum of the mixture of crude products.

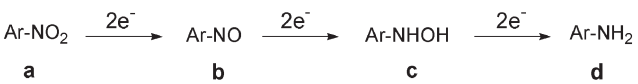
It should be noted that the very similar polarity makes it extremely difficult to separate hydroxylamine and amine even though the reaction has very high chemoselectivity. The desired hydroxylamines were easily purified by flash chromatography owing to nearly complete chemoselectivity for a chemoselective reaction catalyzed by grape cells.

Based on the experimental results and the known reductive metabolism of nitroaromatics catalyzed by nitroreductases (Scheme 1),[6] a possible explanation is proposed to account for this chemoselective reaction catalyzed by grape cells. The polycyclic π-conjugated system with a severely deficient electron causes the nitrogen atom to carry more positive charge than the nucleophilic attack of nitroreductase required, so that the nitro group of substrates **1**–**4a** could be reduced. When the hydroxylamine formed, the molecule became a relatively stable electron donor–acceptor system and it is difficult to reduce the hydroxylamine further by grape cells. Compared with the catalytic activity of bakers' yeast toward the substrates,[2] the activity of grape cells is much weaker and thus this reaction exhibits higher chemoselectivity for the arylhydroxyamine.

Table 2 Chemoselective reduction of various aromatic nitro compounds catalysed by plant cells from a grape (*Vitis vinifera* L.)[a]

Entry	Substrate	Grape (g)	Time (d)	Conv.[b] (%)	**c**/**d**[b]	Yield (%)[c]
1		20	4	96	>98/2	81
2		40	5	50	100/0	43
3		20	3	97	>98/2	82
4		40	5	95	96/4	79
5		40	5	0	—	—
6		40	5	0	—	—

[a] Reaction conditions: water (100 ml), freshly cut grape, substrate (100 mg), 25 °C. [b] The conversion and the ratio were determined by ^{1}H NMR. [c] Isolated yield of the corresponding hydroxylamines.



Scheme 1

In summary, we have developed a novel and highly chemoselective method for the reduction of aromatic nitro compounds to the corresponding hydroxylamines using grape cells. Though currently limited in scope, the procedure is remarkably simple, convenient and efficient. This research not only opens up the use of plant cells as potent reducing agents of nitro groups in the field of organic synthesis, but will also facilitate the progress of biodegradation of nitroaromatic compounds. Efforts to elucidate the exact mechanism of this reaction and to expand the reaction scope, as well as to design and synthesize new biologically active compounds *via* arylhydroxylamine intermediates are currently under way.

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Notes and references

† Experimental procedure: the substrate was added to a suspension of freshly cut plant in 100 ml of water, and the mixture was stirred at 25 °C. The process of the reaction was monitored by HPLC. After completion of the reaction, the suspension was filtered off and the filtrate was extracted with ethyl acetate (3 × 80 ml). Then the combined organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude mixture was purified by flash chromatography (silica gel, hexanes : ethyl acetate = 5 : 1, v/v) to give the pure hydroxylamine.

‡ Spectral data of the new compound **2c**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, –NH), 9.31 (s, –OH), 8.43 (d, J = 7.0 Hz, 1H), 8.42 (d, J = 7.8 Hz, 1H), 8.34 (d, J = 8.4 Hz, 1H), 7.90 (dd, J = 7.8, 7.0 Hz, 1H), 7.20 (d, J = 8.4 Hz), 4.01 (t, J = 7.3), 1.58 (qui, J = 7.3 Hz), 1.35 (sxt, J = 7.3 Hz), 0.91 (t, J = 7.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.55, 162.85, 152.16, 133.82, 130.55, 128.08, 124.71, 121.79, 117.94, 117.55, 109.09, 104.63, 29.87, 29.31, 19.91, 13.84; IR (neat, cm^{-1}) 3390.1, 1728.5; HRMS-EI (70 eV) *m/z* calcd for $C_{16}H_{16}N_2O_3$ 283.1083, found 283.1079; mp 229–230 °C. HPLC data for **2a–c**: Agilent 1100 HPLC-DAD, column: Hiq Sil C18 4.6 mm × 250 mm, 5 μm (Japan), eluent: citric acid (1 g L^{-1}) in water (solvent A) + citric acid (1 g L^{-1}) in methanol (solvent B), a linear gradient of 60% of B to 100% over 25 min, UV detection at 254 nm, flow 0.80 ml min^{-1}, retention time: **2a**, 19.7 min; **2c**, 12.1 min. The spectral data of **1c**, **3c**, **4c**: see electronic supplementary information (ESI) of ref. 2.

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