



Direct biocatalytic synthesis of functionalized catechols: a green alternative to traditional methods with high effective mass yield

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Several catechols have been prepared directly from aromatic precursors by treatment with the recombinant organism *Escherichia coli* JM109 (pDTG602), which expresses both toluene dioxygenase (TDO) and dihydrocatechol dehydrogenase (DHCD), the first two enzymes in the natural biodegradation pathway of aromatics by *Pseudomonas* species. The yields and the ease of preparation of these compounds are compared with traditional chemical methods. For three of the products, the *E* value and EMY (effective mass yield, is defined as the percentage of the mass of desired product relative to the mass of all non-benign materials in its synthesis, see ref. 9) are calculated and compared with those obtained by traditional methods to indicate the green component of the preparation. Potential for direct introduction of the catechol unit to various natural product synthons is discussed.

Introduction

The catechol unit, free or alkylated, is a common component of numerous natural products. Among the targets currently pursued by our group several highly oxygenated compounds deserve a mention: morphine (**1**),¹ narciclasine (**2**),² pancratistatin (**3**),³ combretastatins A-1 (**4**)⁴ and B-1 (**5**)⁴ (Fig. 1).

In most synthetic approaches the protected catechol unit of the target is present in the starting material or is introduced at the beginning of the synthesis by oxidation.⁵ However, there

arise occasions where synthesis would be simplified if the catechol could be introduced later in the synthesis by mild and selective procedures. In connection with an approach to morphine,^{1b,6} we required a large amount of bromoguaiacol (**10**), whose non-enzymatic preparation and demethylation to 3-bromocatechol (**8**) are shown in Scheme 1.

3-Bromocatechol is also available by a simple dihydroxylation of bromobenzene with *Escherichia coli* JM109 (pDTG602), an organism developed by David T. Gibson.⁷ As this process and subsequent methylation of **8** to **10** are experimentally superior to conventional synthetic methods, we explored the possibility of direct synthesis of several catechols useful as potential synthons in approaches to the targets mentioned above. The enzymatic route offers clear advantages in environmentally benign manufacturing of such compounds.

Here we report the preparation of several functionalized catechols by direct biooxidation of the corresponding substituted aromatic substrates and compare the effectiveness of this method with traditional procedures for several of the products. The *E* values⁸ and EMY⁹ are reported for these routes. The potential for direct introduction of the catechol unit to more complex substrates that are recognized by TDO and DHCD is indicated as one of the future directions of this research.

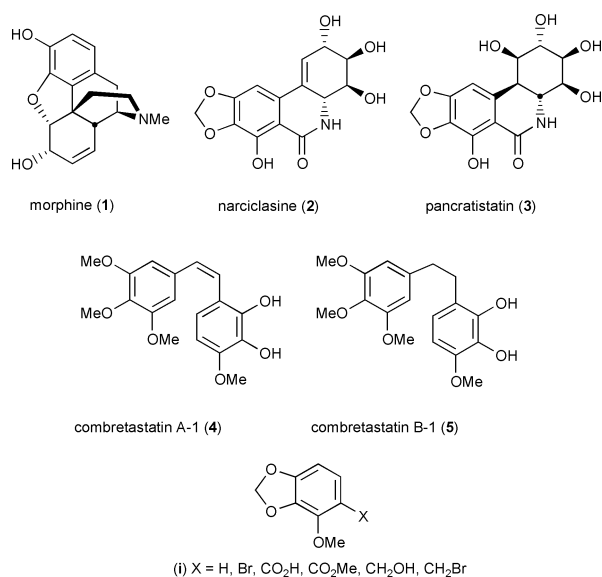


Fig. 1 Highly oxygenated natural products containing a catechol subunit.

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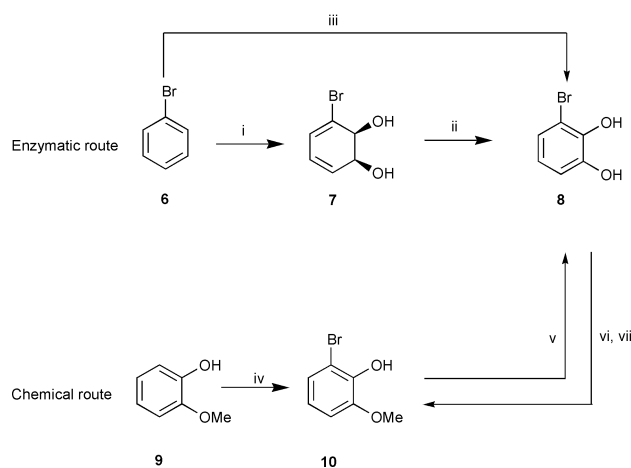
Green Context

Oxyfunctionalisation of aromatics leads to a wide range of useful synthetic intermediates. However, traditional catalysis has had limited (but nonetheless significant) success in this area. This paper describes the use of whole-cell organisms to oxygenate aromatics directly to catechols and discusses the use of such products in synthetic applications. An *E. coli* strain which expresses two enzymes capable of aromatic degradations is shown to be useful in this respect. Chemical yields, effective mass yields and *E*-factors compare very favourably to traditional routes. *DJM*

Results and discussion

Syntheses of simple catechols have been reviewed.⁵ For the most part, methods involve the cleavage of mono- or dialkylated catechols by strong protic or Lewis acids under relatively harsh conditions.¹⁰ Other methods of catechol synthesis require strong oxidants¹² or cleavage of mono- and diacetates.¹¹ Despite the great potential that catechols offer as synthetic intermediates, their preparation is usually arduous, non-selective and low-yielding.¹³ Only three enzymatic methods of catechol synthesis have been reported.¹⁴ Our synthesis of Amaryllidaceae alkaloids would be greatly simplified by an efficient preparation of the oxygenated aromatic fragment.¹⁵

Our goal was to explore the practical preparation of (especially) 3-bromocatechol (**8**) and other catechols by treatment with whole-cell *E. coli* JM109 (pDTG602), an organism developed by D. T. Gibson. The procedure for growing the cells was identical to that reported for *E. coli* JM109 (pDTG601), which expresses toluene dioxygenase.^{16,17} Single colonies of cells are grown for 12 h in a shake flask containing 300 mL of preculture solution. The inoculum is then



Scheme 1 Enzymatic and non-enzymatic preparation of 3-bromocatechol **8**. Reagents: i, *E. coli* JM109 (pDTG601); ii, [O]; iii, *E. coli* JM109 (pDTG602); iv, Br₂, Bu^tNH₂; v, BBr₃; vi, K₂CO₃, MeI; vii, TMSI.

transferred to a 12-L fermentor (8-L working volume), induced with isopropyl thiogalactopyranoside (IPTG), and the culture grown for 24 h to an optical density (OD) of *ca.* 50. Substrates are generally added over a period of 4 h, with the progress of the biooxidation monitored by UV or TLC. In the case of 3-bromocatechol (**8**), the substrate (bromobenzene) was added at a rate of 0.3 g min⁻¹ until 12 g had been added, at which point foaming (indicative of cell death) began, and the process was halted. The product was isolated by centrifugation, followed by extraction and distillation. The results for several catechols are shown in Table 1.

A comparison of the efficiency of biocatalytic *vs.* traditional synthesis of three catechols is shown in Table 2. The overall chemical yields are indicated as reported in the literature. The *E* value is calculated by dividing the weight of all materials used in synthesis by the weight of product. EMY is calculated by expressing the weight of product as a percentage of all non-benign mass used in the manufacturing. Solvents used for extractions are not taken into account in either calculation as they are recycled during manufacturing. It is obvious that the EMY are more informative of the efficiency of the process than the overall yields, because the latter do not provide any information about the process itself. It is also evident that biooxidation in a single step is far more efficient than traditional preparation, even though the yield is limited by the concentration of product at 1–2 g L⁻¹, beyond which it is toxic to the organism.^{14b,c}

Table 2 Comparison of efficiency of the preparation of 3-bromocatechol (**8**), 3-iodocatechol (**12**) and 3-methylcatechol (**13**) by traditional and enzymatic methods

Catechol	Non-enzymatic			Enzymatic		
	Overall yield	<i>E</i> value	EMY	Overall yield	<i>E</i> value	EMY
8	35% ^a 52% ^b	47.5 ^a 36.3 ^b	2.1% ^a 2.8% ^b	63%	1.25	75.0%
12	No chemical synthesis			34%	2.0	38%
13	1% ^c	63.5 ^c	1.6% ^c	45%	2.25	60.6%

^a Ref. 13l (repetition of procedure from ref. 13k). ^b Ref. 13j. ^c Ref. 13h.

Table 1 Catechols prepared by biooxidation of corresponding arenes with *E. coli* JM109 (pDTG602)

	[Yield (g L ⁻¹)] ^a		[Yield (g L ⁻¹)] ^a		[Yield (g L ⁻¹)] ^a
	[1.0]		[0.5]		[0.5]
	[2.0]		R = H ^b R = Me [0.65]		[0.4]

^a Yields are not optimized. ^b This experiment was done on shake-flask scale only.

Conclusions

Functionalized catechols can be prepared directly from mono- or di-substituted aromatic precursors by biooxidation on a preparative scale. These are important starting materials for synthetic routes to many natural products and are often unattainable by traditional methods, e.g. iodocatechol (**12**). Halogenated catechols are useful for the introduction of this unit into intermediates that rely on Heck cyclization^{1a} or tethered radical^{1b} cyclization for C–C bond formation. In the near future we intend to screen a broad range of substrates for the recombinant organism and establish substitution limits for recognition by the enzymes. We will report on these findings in due course.

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