DOI: 10.1002/cbic.200900631

Biomimetic Formation of 2-Tropolones by Dioxygenase-Catalysed Ring Expansion of Substituted 2,4-Cyclohexadienones

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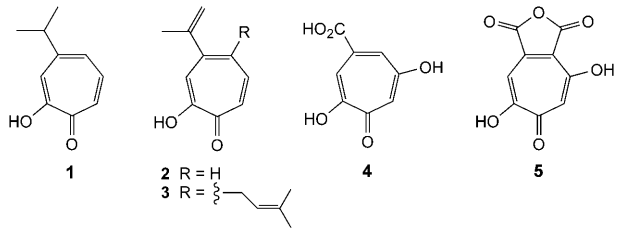
Substituted 2-tropolone natural products are found in plants and fungi. Their biosynthesis is thought to occur by ring expansion from a cyclohexadienone precursor, but this reaction has not previously been demonstrated experimentally. Treatment of 6-hydroxy-6-hydroxymethylcyclohexa-2,4-dienone with the non-haem iron(II)-dependent extradiol catechol dioxygenase MhpB from *Escherichia coli* results in the formation of the 2-tropolone ring-expansion product through a pinacol-type rearrangement. Three further substituted cyclohexa-2,4-dienone analogues were prepared, and treatment of each analogue was found to give the substituted 2-tropolone ring-expansion product. This ring expansion could also be effected nonenzymatically by treatment with 1,4,7-triazacyclononane and $FeCl_2$. This is a novel transformation for non-haem iron-dependent enzymes, and this is the first experimental demonstration of the proposed ring-expansion reaction in tropolone biosynthesis.

Introduction

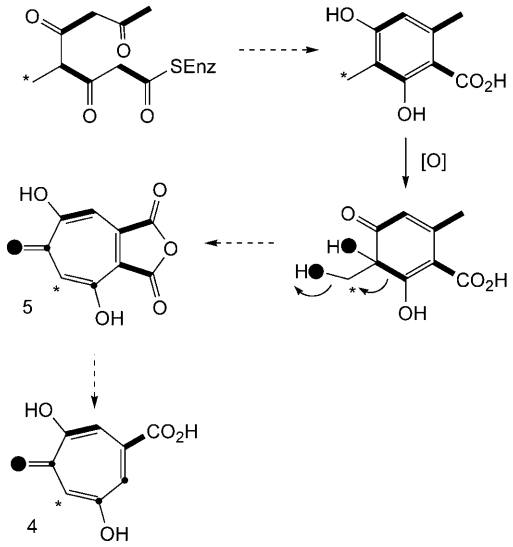
Tropolone natural products are found in plants and fungi,[1] and have attracted interest due to their powerful antibacterial,[2] antifungal[2] and cytotoxic activity.[3] Hinokitiol (β-thujaplicin, **1**; Scheme 1), found in the Chamaecyparis and Thujopsis



Scheme 1. Structures of tropolone natural products.

(hinoki and hiba) evergreen shrubs, shows potent antifungal and insecticidal activity, and is used as a wood preservative.[4] β-Dolabrin (**2**) and procerin (**3**) are further plant tropolones with related structures.[1] Fungal tropolones include stipitatic acid (**4**) and stipitatonic acid (**5**), which are produced by *Talaromyces stipitatus*.[5–8]

Investigation of stipitatic acid biosynthesis by Bentley[5,6] and Scott et al.[7] by using isotope incorporation revealed that C-7 of the tropolone ring (starred atom, Scheme 2) was derived from the one-carbon pool,[5,6] and that the incorporation of isotopically labelled acetate was consistent with ring expansion of a benzenoid precursor.[7] Furthermore, incorporation of one atom of ^{18}O from $^{18}O_2$ into stipitatonic acid was observed in *T. stipitatus*;[8] hence, a ring-expansion mechanism involving 1,2-migration of a 6-hydroxy-2,4-cyclohexadienone intermediate (Scheme 2) was proposed by O'Sullivan and Schwab.[8] Similar ring-expansion mechanisms have been proposed, on the



Scheme 2. Ring-expansion reaction for the stipitatonic acid biosynthesis proposed in ref. [8], on the basis of isotope incorporation experiments. Bold lines indicate the incorporation of intact acetate units; black circles: incorporation of a single labelled carbon from acetate; *, label incorporated from one-carbon pool; ●: ^{18}O label incorporated from $^{18}O_2$.

basis of isotope-incorporation studies, for the biosynthesis of thiotropocin in *Pseudomonas* CB-104[9] and xenovulene A in *Acremonium strictum*.[10] The biosynthesis of plant tropolone

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*Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.200900631: NMR spectra for the dimerisation of spiroepoxides **7a–d**; GC-MS data for identification of 2-tropolone **10a**, and NMR spectra for 2-tropolone **10c**.*

natural products is less well studied, but it has been proposed that β-thujaplicin (**1**) in *Cupressus lusitanica* is derived from terpenoids containing a cyclohexene skeleton; this would therefore imply a ring-expansion reaction of some kind.[11]

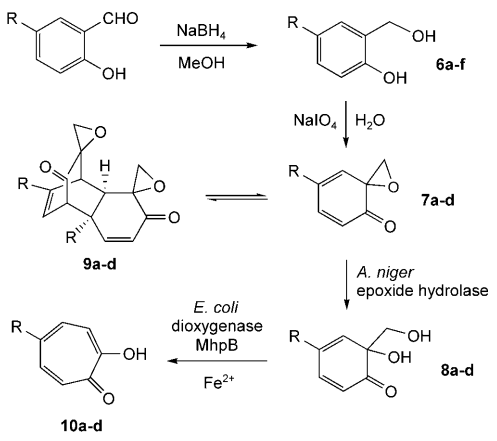
We have recently reported 6-hydroxy-6-hydroxymethylcyclohexa-2,4-dienone as a mechanistic probe for the Criegee rearrangement step of the extradiol and intradiol catechol dioxygenases.[12] Treatment of 6-hydroxy-6-hydroxymethylcyclohexa-2,4-dienone with iron(III)-dependent intradiol dioxygenase CatA gave no reaction, but upon treatment with iron(II)-dependent extradiol dioxygenase MhpB, the formation of 2-tropolone was detected by GC-MS analysis.[12]

In view of the possible relevance to tropolone natural product biosynthesis, we wished to study this transformation in more detail using a range of substrates. Since the tropolone natural products **1**–**5** contain a range of substituents, we wished to investigate the effects of substituents on: 1) the stability of the 6-hydroxycyclohexa2,4-dienone intermediate, 2) the ability of the substrate to undergo ring expansion to a 2-tropolone product and 3) the yield of ring-expansion product. Here we report the preparation of a set of substituted analogues for ring expansion, and observations on their reactivity and their processing to substituted 2-tropolone products.

Results

A series of substituted salicyl alcohols, **6a**–**f**, containing a 4-methyl, 4-chloro, 4-methoxy, 4-nitro or 3,4-fused aryl substituent was prepared by reduction of the corresponding aldehydes by using sodium borohydride in 47–76% yield (see Scheme 3).

Adler et al. have reported the conversion of salicyl alcohol **6a** into spiroepoxide **7a**, by using sodium periodate in water, and they also reported the rapid dimerisation of **7a** through a Diels–Alder reaction to form the cycloadduct **9a**.[13] Upon oxidising **6a** in D_2O at 25 °C, the dimerisation of **7a** to **9a** was observed directly by ^{1}H NMR spectroscopy, with a half-life of approximately 10 min at 25 °C (see the Supporting Information). Only a single major set of NMR signals was observed for cycloadduct **9a**; therefore, cyclisation proceeds to give predominantly one regio- and diastereoisomer. By carrying out the oxidation at 4 °C, it was possible to minimise the dimerisation and obtain **7a** in 33% yield (<5% dimer **9a**).



Scheme 3. Preparation of substituted cyclohexa-2,4-dienone spiroepoxides **7a**–**d**, and their conversion by ring expansion to tropolones **10a**–**d**. Substituent R = **a**: H; **b**: CH_3, **c**: Cl, **d**: 3,4-fused aryl, **e**: OCH_3, **f**: NO_2.

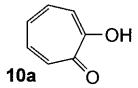
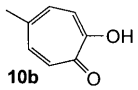
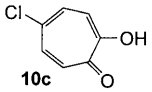
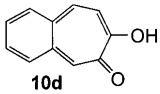
Oxidation of the 4-methyl-substituted analogue (**6b**), 4-chloro-substituted analogue (**6c**) and 3,4-aryl fused analogue (**6d**) with sodium periodate proceeded smoothly to give the substituted spiroepoxides **7b**, **7c** and **7d** in 40–68% yield. The substituted spiroepoxides were found to be more stable towards dimerisation, with half-lives at 25 °C, as measured by ^{1}H NMR spectroscopy, of 40 min for **7c** and 12 h for **7b**, while bicyclic analogue **7d** showed no trace of dimer formation after 24 h. In the case of the 4-methoxy analogue **6e** and 4-nitro analogue **6f**, oxidation with sodium periodate gave a complex mixture of oxidation products, with only a trace of the spiroepoxides **7e** and **7f**, therefore, these two analogues were not investigated further.

We found previously that spiroepoxide **7a** could be converted to the corresponding diol **8a** by treatment with *Aspergillus niger* epoxide hydrolase in 50 mM potassium phosphate buffer (pH 8.0).[12] A peak corresponding to diol **8a** was observed by reversed-phase HPLC at $t_R = 18.5$ min. Analysis of this peak by GC-MS (after silylation) gave a peak at $t_R = 5.69$ min, with mass spectral data consistent with the monosilylated diol (*m/z* 212 $[M]^+$, 197 $[M-CH_3]$). Conversion of **7a** to **8a** in D_2O was monitored by ^{1}H NMR spectroscopy, and gave rise to signals at 3.71 and 3.85 ppm due to a new pair of diastereotopic hydrogens for the CH_2OH group of **8a**. Treatment of **8a** with *Escherichia coli* 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB) led to the formation of a pink colouration.[14] The product was identified by GC-MS (after silylation) as 2-tropolone (**10a**, see Table 1). Formation of 2-tropolone was also observed by GC-MS upon treatment with the iron(II) complex of 1,4,7-triazacyclononane (TACN) in methanol, conditions which we have used previously to mimic the extradiol dioxygenase reaction.[15]

Treatment of the methyl-substituted analogue **7b** with epoxide hydrolase, followed by the addition of dioxygenase MhpB, gave a colour change from yellow to dark green.[14] Analysis of the silylated product by GC-MS showed a mixture of products, with 2-tropolone **10b** as the major product (t_R = 7.19 min, *m/z* 193 $[M-CH_3]^+$). Analysis of the GC-MS data by selected ion monitoring also revealed two isomeric by-products at $t_R = 8.73$ and 10.7 min, which also showed fragment ions at *m/z* 208 ($[M]^+$) and 193 ($[M-CH_3]^+$), but the structures of the by-products could not be unambiguously assigned. Treatment of **7b** with epoxide hydrolase followed by $FeCl_2$/TACN also gave 2-tropolone **10b**, with no by-products detectable by GC-MS analysis. Analysis of the product mixture by ^{1}H NMR spectroscopy showed clean formation of **10b** in the TACN reaction, in >90% purity, whereas the MhpB-catalysed reaction gave a mixture of products, containing ~50% **10b**, some residual diol **8b** and unidentified by-products.

Treatment of the fused aryl analogue **7d** with epoxide hydrolase, followed by addition of dioxygenase MhpB, gave a colour change from yellow to red/brown.[14] The product was derivatised by silylation, and was identified by GC-MS analysis as the 2-tropolone product **10d** (*m/z* 244, $[M]^+$; 229, $[M-CH_3]^+$). Analysis of the reaction product by ^{1}H NMR spectroscopy

Table 1. 2-Tropolone products formed by MhpB- and TACN-catalysed ring expansion.

Tropolone product	GC-MS Data (after silylation)	Products observed by ^{1}H NMR spectroscopy	
		MhpB reaction	TACN reaction
 **10a**	t_R = 6.64 min *m/z* 194.1 $[M]^+$	n.a. (rapid dimerisation of **7a**)	n.a.
 **10b**	t_R = 7.19 min *m/z* 193.1 $[M-CH_3]^+$	Mixture containing 50% tropolone **10b**, 30% diol **8b** and other rearrangement products	>90% tropolone **10b**
 **10c**	t_R = 8.01 min *m/z* 213.0 $[M-CH_3]^+$	>95% tropolone **10c**[a]	>95% tropolone **10c**[a]
 **10d**	t_R = 9.32 min *m/z* 229.2 $[M-CH_3]^+$	50% tropolone **10d** 50% epoxide **7d**	50% tropolone **10d** 50% epoxide **7d**

[a] Obtained from a one-pot procedure.

showed a 1:1 mixture of **10d** and residual epoxide **7d**. Treatment of diol **8d** with the iron(II) complex of 1,4,7-triazacyclononane also gave a 1:1 mixture of **10d** and epoxide **7d**. The presence of 50% residual epoxide **7d** in both cases would suggest that, for this more bulky analogue, *A. niger* epoxide hydrolase selectively converts only one enantiomer.

Treatment of the chloro-substituted analogue **7c** with epoxide hydrolase, followed by addition of dioxygenase MhpB, using the same procedure as before, gave none of the 2-tropolone product **10c** by GC-MS analysis. It seemed possible that the chloro substituent might cause the reaction intermediates to be unstable, therefore, a one-pot procedure was devised for conversion of **6c** directly to **10c**. Phenol **6c** was treated with sodium periodate (1 equiv) in water/methanol at 4 °C. After 5 min, either sodium ascorbate or sodium dithionite (1.1 equiv) was added in order to remove remaining periodate oxidant, followed by 50 mM potassium phosphate buffer (pH 8.0) and *A. niger* epoxide hydrolase. After a further 20 min at 4 °C, freshly reactivated dioxygenase MhpB was added to give in each case a rapid colour change from yellow to brown.[14] Analysis of the product by ^{1}H NMR spectroscopy identified the 5-chloro-2-tropolone **10c** as the single product. The same procedure was also used with 1,4,7-triazacyclononane and $FeCl_2$ in place of dioxygenase MhpB, and again the 2-tropolone product **10c** was obtained in high purity.

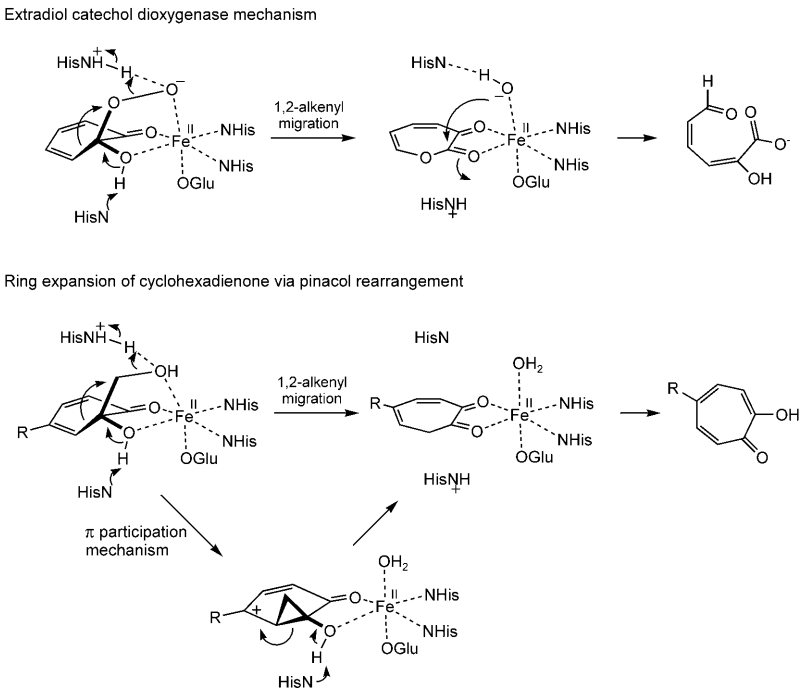
Conclusions

Our recent observation[12] that diol **8a** can be converted by non-haem-iron-dependent dioxygenase MhpB into tropolone **10a** prompted a further investigation into this reaction, as a possible biomimetic reaction for the biosynthesis of substituted tropolone natural products.[1] In this work, we have demonstrated that this reaction is able to successfully convert methyl-, chloro- and aryl-substituted 2,4-cyclohexadienones into the corresponding 2-tropolone products. These observations therefore support the previous proposals that the biosynthesis of fungal tropolone natural products occurs through ring expansion.[8–10] Our observation that either a non-haem-iron(II)-dependent dioxygenase or a non-haem-iron(II) macrocyclic complex is able to catalyse this transformation suggests that a non-haem-iron(II)-dependent enzyme might be responsible for the biosynthetic ring expansion. Perhaps such an enzyme could generate the 6-hydroxycyclohexa-2,4-dienone intermediate by oxidation of a phenol precursor, although such an enzyme has not yet been identified.

The substituted analogues show somewhat different behaviour in the transformation of spiroepoxide **7**, via diol **8**, to tropolone **10**. The substituted 6-spiroepoxy-2,4-cyclohexadienones **7b–d** are more stable with respect to dimerisation than the unsubstituted epoxide **7a**; this can be explained by a slowing of the Diels–Alder dimerisation reaction through steric hindrance (and perhaps also electronic effects for **7c** and **d**). The hydrolysis of epoxide **7** to diol **8** is catalysed in each case by *A. niger* epoxide hydrolase; however, the presence of 50% residual epoxide **7d** in the case of tropolone **10d** suggests that, in this case, only one enantiomer of the more bulky spiroepoxide is converted by epoxide hydrolase. In the case of the chloro-substituted analogue, our observations suggest that one of the reaction intermediates is unstable, due to the presence of the chloro substituent, but we were able to circumvent this problem by means of a one-pot method that converted precursor **6c** directly to **10c**. The methyl-substituted analogue was processed efficiently by the TACN-catalysed reaction, but gave a mixture of products in the MhpB-catalysed reaction. The presence of some residual diol **8b** might indicate that one enantiomer of **8b** is processed more slowly by MhpB, but isomeric rearrangement products are also formed; this suggests that the presence of the methyl substituent can lead to other rearrangements.

The mechanism of ring expansion is presumably via a 1,2-rearrangement, since the extradiol catechol dioxygenase reaction catalysed by MhpB is believed to proceed via a Criegee 1,2-rearrangement.[15] In this case, there would be a 1,2-migration of an alkenyl group onto the hydroxymethyl substituent, as shown in Scheme 4. This rearrangement would therefore require acid catalysis, like the well-known pinacol rearrangement.[16] In addition to Lewis acid catalysis by the non-haem iron centre, we have previously shown that the extradiol dioxygenase reaction mechanism involves concerted acid–base catalysis, which in the TACN model reaction is carried out by pyridine/pyridinium salt,[15] and in the Mhp active site is carried out by two active-site histidine residues, His115 and His179.[17]

An alternative to a direct 1,2-migration step, which is also possible in the extradiol dioxygenase reaction mechanism,[18] is one in which the π system of the diene participates to form a transient cyclopropyl cation (see Scheme 4). One would expect



Scheme 4. Mechanism for the 1,2-rearrangement of diols **8a–d** to 2-tropolones **10a–d**, and comparison with the Criegee 1,2-rearrangement of the extradiol catechol dioxygenase reaction mechanism.

that the carbocation intermediate in this mechanism would be stabilised by a 5-methyl substituent, but destabilised by a 5-chloro substituent. The observation of alternative rearrangement products in the case of the 5-methyl analogue could be explained by the formation of a stable tertiary carbocation in this case, leading to other possible rearrangements; however, the efficient processing of the 5-chloro analogue would argue against such a mechanism.

To our knowledge, the only other enzymatic ring expansion that has been demonstrated biochemically is that involved in cephalosporin biosynthesis, which is also catalysed by a non-haem-iron-dependent oxygenase, deacetoxycephalosporin C synthetase.[19] This unusual transformation thus provides a rationalisation for the biosynthesis of tropolone natural products, and offers another example of the novel chemistry that can be catalysed by non-haem-iron-dependent enzymes in biology.

Experimental Section

Materials: Substituted salicyl alcohols **6a–f** were prepared in 47–76% yield by reduction of commercially available substituted benzaldehydes by using sodium borohydride in methanol. *Aspergillus niger* epoxide hydrolase was purchased from Fluka. *Escherichia coli* 2,3-dihydroxyphenylpropionate 1,2-dioxygenase (MhpB) was overexpressed from a recombinant plasmid pIPB containing the *mhpB* gene, as previously described,[12] and was purified to near homogeneity by hydrophobic interaction chromatography, as previously described,[20] by using potassium phosphate buffer (50 mM, pH 7.0) containing 0.07% (*v/v*) 2-mercaptoethanol as buffer. Other chemicals and biochemicals were purchased from Sigma–Aldrich.

Preparation of spiroepoxides 7a–d: Spiroepoxides **7a–d** were prepared by using a procedure adapted from Adler et al.[13] In order to minimise the dimerisation of the spiroepoxides, all procedures were carried out at 4 °C in flasks wrapped with Al foil. A solution of sodium periodate (2.41 g, 11 nmol) in water (50 mL) was added dropwise to a stirred solution of substituted salicyl alcohol **6a–d** (10 mmol) in water (75 mL). Ethyl acetate (50 mL) was then added to the resulting yellow solution, and the reaction was stirred at 4 °C for 1 h. The organic layer was separated, and solvent was removed at reduced pressure (without heating) to give spiroepoxides **7a–d**. The rate of self-dimerisation of each spiroepoxide was assessed by NMR spectroscopy, in D_2O at 25 °C, with sodium *p*-toluenesulfonate as internal standard (see the Supporting Information).

*Cyclohexa-2,4-dien-1-one 6-oxamethylene spiroepoxide (**7a**) (prepared in ref. [13]):* Yield 33%; dimerisation $t_{1/2}=10$ min; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.43$ (ddd, $J=10.5$, 6.0, 1.4 Hz, 1H; H-3), 6.66 (dd, $J=10.2$, 6.0 Hz, 1H; H-4), 6.27 (dd, $J=10.2$, 1.4 Hz, 1H; H-5), 6.24 (d, $J=10.5$ Hz, 1H; H-2), 3.39, 3.25 ppm (d, $J=7.5$ Hz, 2×1H; CH_2O).

*4-Methyl-cyclohexa-2,4-dien-1-one 6-oxamethylene spiroepoxide (**7b**):* Yield 40%; dimerisation $t_{1/2}=12$ h; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=6.98$ (d, $J=10.0$ Hz, 1H; H-3), 6.13 (d, $J=10.0$ Hz, 1H; H-2), 5.74 (s, 1H; H-5), 3.22, 3.05 (d, $J=8.2$ Hz, 2×1H; CH_2O), 2.03 ppm (s, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=195.3$, 146.1, 136.0, 133.2, 125.8, 57.8, 56.5, 21.2 ppm; MS (ES +ve ion) *m/z*: 137 $[M+H]^+$, 159 $[M+Na]^+$; HRMS obsd for $C_8H_8O_2Na$: 159.0410, calcd 159.0417.

*4-Chloro-cyclohexa-2,4-dien-1-one 6-oxamethylene spiroepoxide (**7c**):* Yield 50%; dimerisation $t_{1/2}=40$ min; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 7.27 (d, $J=10.0$ Hz, 1H; H-3), 6.31 (s, 1H; H-5), 6.29 (d, $J=10.0$ Hz, 1H; H-2), 3.38, 3.26 ppm (d, $J=7.5$ Hz, 2×1H; CH_2O); MS (ES −ve ion) *m/z*: 155 $[M-H]^-$; HRMS obsd for $C_7H_4ClO_2$: 154.9911, calcd 154.9905.

*2-Oxo-1,2-dihydronaphthalene 1-oxamethylene spiroepoxide (**7d**):* Yield 68%; only monomer observed; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 7.53 (d, $J=10.0$ Hz, 1H; H-4), 7.6–7.3 (m, 3H), 7.19 (d, $J=7.0$ Hz, 1H), 6.27 (d, $J=10.0$ Hz, 1H; H-3), 3.32, 3.05 ppm (d, $J=8.2$ Hz, 2×1H; CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=197.3$, 145.9, 133.6, 131.9, 130.5, 129.7, 128.7, 125.5, 123.6, 72.4, 66.5 ppm; MS (ES +ve ion) *m/z*: 173 $[M+H]^+$, 195 $[M+Na]^+$; HRMS obsd for $C_{11}H_8O_2Na$: 195.0434, calcd 195.0417.

Conversion of spiroepoxides to substituted tropolones

Using dioxygenase MhpB: Spiroepoxide **7a, 7b** or **7d** (1 mmol, 10 mM) was dissolved in potassium phosphate buffer (50 mM, 100 mL, pH 8.0), to which was then added *Aspergillus niger* epoxide hydrolase (5 mg), and the mixture was stirred at 4 °C for 30 min. To each incubation was added MhpB enzyme (400 μL, 30 units), which had been pre-activated for 1 min at 0 °C by the addition of ammonium iron(II) sulfate (5 mM) and sodium ascorbate (5 mM).[20] Aliquots (5 mL) were removed after 10, 60, 90, 180 min and 48 h, and the reaction was stopped by adding 100% trichloroacetic acid (TCA; 300 μL). The samples were centrifuged for 10 min at 13000 rpm, and then analysed by HPLC and GC-MS. From the remaining reaction mixture, products were extracted into ethyl ace-

tate (2×20 mL), which was dried ($MgSO_4$) and evaporated under reduced pressure to give the substituted tropolone products.

One-pot method: The above procedure gave no tropolone product from **7c**. In this case, 2-hydroxymethyl-4-chlorophenol (**6c**, 0.19 mmol) was dissolved in water/methanol (1:1, 40 mL), to which was then added sodium periodate (0.2 mmol), and the mixture was stirred for 5 min at 4 °C. Next sodium dithionite (0.24 mmol) was added, and the reaction mixture was stirred for 2 min at 4 °C. Potassium phosphate buffer (50 mM, pH 8.0, 80 mL) was then added, followed by *A. niger* epoxide hydrolase (3 mg), and the mixture was stirred for 20 min at 4 °C. Freshly activated *E. coli* MhpB (30 units, 1 mg) was then added, and the reaction mixture was stirred for 1 h at 4 °C. The products were extracted into ethyl acetate (2×20 mL), which was dried ($MgSO_4$) and evaporated under reduced pressure to give the substituted tropolone product **10c** in 55% yield.

Using TACN/$FeCl_2$: Spiroepoxide **7a, 7b** or **7d** (0.2 mmol, 10 mM concentration) was dissolved in potassium phosphate buffer (50 mM, 20 mL, pH 8.0), to which was then added *Aspergillus niger* epoxide hydrolase (5 mg), and the mixture was stirred at 4 °C for 30 min. $FeCl_2$ (0.2 mM), TACN (0.2 mM) and pyridine (0.6 mM) were dissolved in methanol (20 mL), the mixture was added to the ring-opened spiroepoxide, and the solution was stirred for 48 h at room temperature. The products were analysed by HPLC or GC-MS, as described below. From the remaining reaction mixture, products were extracted into ethyl acetate (2×20 mL), which was dried ($MgSO_4$) and evaporated under reduced pressure to give the substituted tropolone products. A one-pot procedure, as described above, was used to convert **6c**, except that the mixture of $FeCl_2$ (0.2 mM), TACN (0.2 mM) and pyridine (0.6 mM) dissolved in methanol (20 mL) was added to the ring-opened spiroepoxide, and the solution was stirred for 48 h at room temperature, prior to work-up.

Analysis by HPLC and GC-MS: HPLC analysis was carried out on a Phenomenex Luna C_{18} reversed-phase column. The mobile phase consisted of A (100% water) and B (100% methanol) delivered at a flow rate of 0.8 mL min^{-1}. The gradient programme was 5% B (5 min); 5–100% B (15 min); 100% B (3 min); 100–5% B (7 min). Samples for GC-MS analysis were extracted into HPLC-grade dichloromethane, and the organic layer was dried with Na_2SO_4. A portion of the product was treated with *N,O*-bis(trimethylsilyl)acetamide (200 μL) and chlorotrimethylsilane (10 μL) overnight, then diluted with HPLC-grade dichloromethane. It was then analysed by GC-MS in electron-impact mode, with a Micromass Autospec GC-MS system Varian 4000 (at 70 eV) on a DB5 silica capillary column by using the following temperature gradient: 50 °C for 1 min, 50–140 °C at 25 °C min^{-1} and 140–250 °C at 5 °C min^{-1}.

*2-Tropolone (**10a**):* GC-MS (silylated) t_R = 6.64 min, *m/z*: 194.1 $[M]^+$, 180.1. NMR spectrum is identical to commercially available 2-tropolone.

*5-Methyl-2-tropolone (**10b**):* ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.93 (d, *J* = 8.0 Hz, 1H; H-6), 6.76 (d, *J* = 8.0 Hz, 1H; H-7), 6.69 (d, *J* = 8.0 Hz, 1H; H-4), 6.28 (d, *J* = 8.0 Hz, 1H; H-3), 2.19 ppm (s, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.5, 171.9, 148.8, 137.4, 129.8, 128.7, 116.3, 19.8 ppm; GC-MS (silylated) t_R = 7.19 min, *m/z*: 193.1 $[M-CH_3]^+$.

*5-Chloro-2-tropolone (**10c**):* ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.06 (dd, *J* = 8.5, 2.4 Hz, 1H; H-6), 6.94 (dd, *J* = 8.0, 2.4 Hz, 1H; H-4), 6.71 (d, *J* = 8.5 Hz, 1H; H-7), 6.22 ppm (d, *J* = 8.0 Hz, 1H; H-3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.1, 154.6, 150.1, 129.7, 127.5, 126.3, 117.8 ppm; GC-MS (silylated) t_R = 8.01 min, *m/z*: 213.0 $[M-CH_3]^+$.

*5,6-Benzene fused 2-tropolone (**10d**):* ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.55 (d, *J* = 8.0, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H; H-4), 7.23 (s, H-7, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.00 (t, *J* = 8.0 Hz, 1H), 6.20 ppm (d, *J* = 8.0 Hz, 1H; H-3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 195.1, 151.9, 146.3, 137.4, 130.6, 129.8, 128.7, 127.6, 125.4, 123.4, 118.2 ppm; GC-MS (silylated) t_R = 9.32 min, *m/z*: 229.2 $[M-CH_3]^+$.

Acknowledgement

We thank Dr. John Bickerton (University of Warwick) for assistance with the GC-MS experiments.

Keywords: biosynthesis · cyclohexadienones · dioxygenases · pinacol rearrangement · ring expansion · tropolones

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Received: October 14, 2009
Published online on December 10, 2009

