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# Accessing low-oxidation state taxanes: is taxadiene-4(5)-epoxide on the taxol biosynthetic pathway?<sup>†</sup>

Naomi A. Barton,<sup>a</sup> Benjamin J. Marsh,<sup>a</sup> William Lewis,<sup>a</sup> Nathalie Narraidoo,<sup>b</sup> Graham B. Seymour,<sup>b</sup> Rupert Fray<sup>b</sup> and Christopher J. Hayes<sup>\*a</sup>

We have shown for the first time that taxadiene (**3**) can be epoxidised in a regio- and diastereoselective manner to provide taxadiene-4(5)-epoxide (**12**) as a single diastereoisomer, and that this epoxide can be rearranged to give taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**). Furthermore, the epoxide **12** rearranges under acidic conditions to give taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**), the known bridged ether OCT (**5**) and the new oxacyclotaxane (OCT2) **15**. Contrary to previous speculation, taxadiene-4(5)-epoxide (**12**) is susceptible to rearrangement when exposed to an iron<sup>III</sup> porphyrin, and these observations justify consideration of epoxide **12** as a chemically competent intermediate on the taxol biosynthetic pathway.

Received 14th September 2015

Accepted 26th January 2016

DOI: 10.1039/c5sc03463a

www.rsc.org/chemicalscience

## Introduction

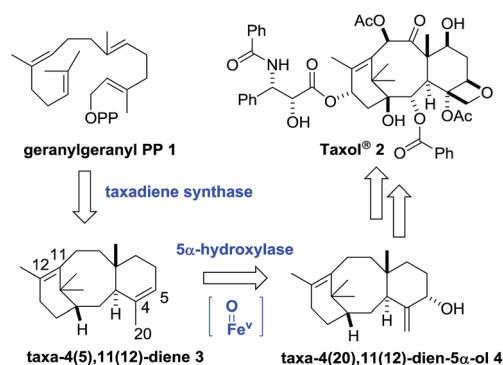
Since its isolation from the pacific yew (*Taxus brevifolia*), and subsequent FDA approval in 1992, taxol and its close derivatives continue to be used as frontline drugs for the treatment of cancer.<sup>1</sup> Its effectiveness in the clinic, coupled with an intriguing tricyclic structure, has ensured that taxol has endured as a molecule of interest to scientists for nearly 50 years.<sup>2</sup> In this paper we show that a combination of metabolic engineering and synthetic chemistry can be used to give ready access to low oxidation state taxanes, giving new insight into the early stages of the 'oxidase-phase' of the taxol biosynthetic pathway.<sup>3</sup>

The first committed step in the taxol biosynthetic pathway (Scheme 1) is the taxadiene synthase-catalysed cyclisation of geranylgeranyl pyrophosphate **1** to produce taxa-4(5),11(12)-diene (**3**).<sup>4</sup> The remaining biosynthetic steps involve a series of oxidation, and functional group interconversion processes, the first of which is the taxadiene-5 $\alpha$ -hydroxylase-mediated oxidation of **3** into taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**).<sup>5</sup>

A number of research groups have reported the overproduction of taxa-4(5),11(12)-diene (**3**) in a variety of chassis organisms (yeast,<sup>6</sup> tobacco,<sup>7</sup> *E. coli*,<sup>8</sup> tomato<sup>9</sup>), and the incorporation of both taxadiene synthase and its 5 $\alpha$ -hydroxylase

(tobacco,<sup>7</sup> *E. coli*<sup>8a</sup>) has also been described. In 2008 Rontein showed that overexpression of both taxadiene synthase and taxa-4(5),11(12)-diene 5-hydroxylase (CYP725A4) in tobacco (*Nicotiana glauca*) did not produce taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**) as expected, but instead led to the production of 5(12)-oxa-3(11)-cyclotaxane (OCT) **5** (Scheme 2).<sup>7</sup>

In 2010 Stephanopoulos reported a significant improvement in this area using *E. coli* as the chassis organism.<sup>8a</sup> Under their optimised conditions, taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**) could be produced, but unfortunately the desired product **4** was obtained as a 1 : 1 mixture with OCT (**5**), thus severely limiting the amount of **4** being produced. These two studies clearly demonstrate that the presence of both taxadiene synthase and taxadiene-5 $\alpha$ -hydroxylase in a metabolically engineered chassis organism does not guarantee satisfactory production of taxadien-5-ol **4**, and the catalytic promiscuity and multispecificity of taxadiene-5 $\alpha$ -hydroxylase has attracted recent attention.<sup>10</sup>



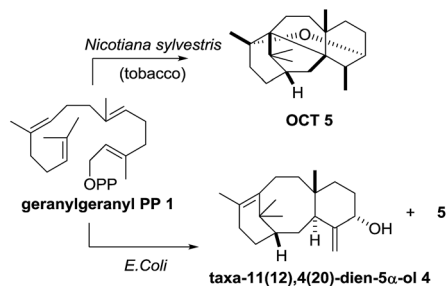
Scheme 1 Biosynthesis of Taxol® from geranylgeranyl-pyrophosphate, via taxadiene.

<sup>a</sup>School of Chemistry, University of Nottingham, University Park, NG7 2RD, Nottingham, UK

<sup>b</sup>Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington, LE12 5RD, Loughborough, UK. E-mail: chris.hayes@nottingham.ac.uk; Fax: +44 (0)115 951 3564; Tel: +44 (0)115 951 3045

<sup>†</sup> Electronic supplementary information (ESI) available: Full experimental procedures and copies of <sup>1</sup>H and <sup>13</sup>C NMR spectra. CCDC 1030909. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c5sc03463a

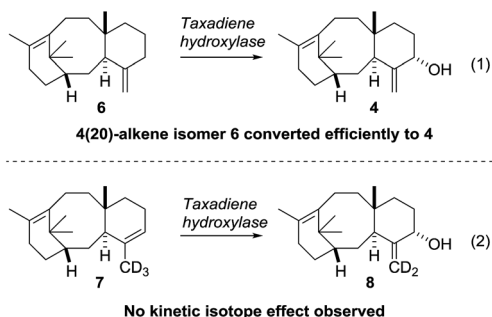




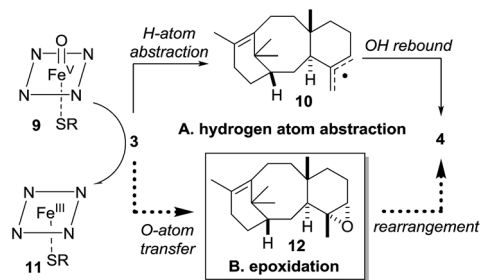
Scheme 2 Production of oxidised taxanes in metabolically engineered tobacco and *E. coli* containing both taxadiene synthase and taxadiene hydroxylase.

Our current understanding of the taxadiene-5 $\alpha$ -hydroxylase oxidation mechanism is derived from experiments performed by Williams and Croteau (Scheme 3).<sup>5</sup> The observation that taxadiene-containing microsomes could convert both the 4(5)-3 (Scheme 1) and the 4(20)-6 alkene isomers of taxadiene to taxadien-5 $\alpha$ -ol 4 with equal efficiency (Scheme 3, eqn (1)), lead Williams and Croteau to suggest an H-atom abstraction/oxygen rebound mechanism, *via* the allylic radical 10, as being the most likely (path A, Scheme 4).

An alternative pathway involving epoxidation of 3 to give 12, followed by rearrangement to give 4 (path B, Scheme 4) was also considered, but was eventually discounted by the fact that the 4(20)-alkene isomer 6 is also converted to 4 by taxadiene hydroxylase (*via* a process unlikely to involve 12).<sup>5</sup> This conclusion was further supported by the fact that the epoxide 12 has not been observed as an oxidation product of 3 in any studies reported thus far. In order to provide further evidence for the H-atom abstraction/oxygen rebound mechanism (path A, Scheme 4), Williams *et al.* prepared deuterium-labelled [C20-<sup>2</sup>H<sub>3</sub>]-taxadiene (7) and subjected this to taxadiene hydroxylase. However, under these conditions, the expected kinetic isotope effect was not observed for the transformation of 7 to 8 (Scheme 3, eqn (2)),<sup>5</sup> which is at odds with the proposed H-atom abstraction process. Furthermore, Williams *et al.* report that their experiment ‘unexpectedly revealed that the deuterated substrate yielded slightly more taxa-4(20),11(12)-dien-5 $\alpha$ -ol than did the unlabeled substrate’,<sup>5b</sup> thus indicating a small inverse isotope effect. This experimental observation actually



Scheme 3 Elucidating the taxadiene hydroxylase mechanism (Williams and Croteau).



Scheme 4 Taxadiene hydroxylase mediated oxidation of taxa-4(5),11(12)-diene (3) to taxa-4(20),11(12)-dien-5 $\alpha$ -ol (4).

supports the epoxide/rearrangement route for the conversion of 3 to 4, as small inverse secondary isotope effects are observed in epoxidation reactions,<sup>11</sup> but no further experiments have been reported to examine this possibility.

The production of OCT 5, along with additional oxidation products, in engineered taxadiene synthase/taxadiene hydroxylase-containing organisms<sup>7,8a</sup> lead us to question whether epoxide 12 could be an intermediate in the taxadiene hydroxylase mechanism as we could envisage viable pathways for the production of both 4 and 5 from epoxide 12. Therefore, we decided to synthesise 12 and study its chemistry in the context of the early stages of the taxol biosynthetic pathway.

## Results and discussion

### Epoxidation of taxadiene

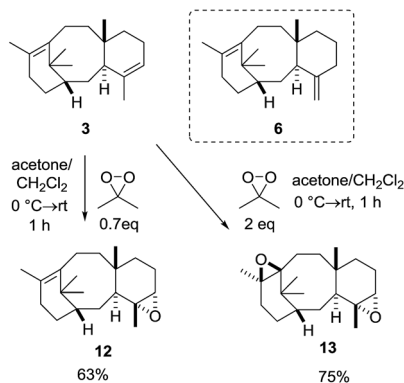
Our studies began by isolating taxadiene from our previously described taxadiene synthase-containing tomatoes,<sup>9</sup> using a slightly modified protocol that allows extraction directly from fresh fruit (see ESI† for details). This procedure afforded taxa-4(5),11(12)-diene (3) and taxa-4(20),11(12)-diene (6) as an inseparable 17 : 1 (3 : 6) mixture. With ready access to taxadiene we next turned our attention to epoxidation of 3, with DMDO being selected as the oxidant due to its ease of use.<sup>12</sup> As we were concerned with the potential over-epoxidation of taxadiene, we performed the reactions with substoichiometric quantities of oxidant. Pleasingly, when taxa-4(5),11(12)-diene (3) was treated with 0.7 equivalents of DMDO, the desired epoxide 12 was obtained as the major new product (95% purity as judged by <sup>1</sup>H NMR; see ESI†) and unreacted taxadiene was recovered (Scheme 5).

Whilst the epoxide derived from 6 was not observed, the recovered taxadiene (20%) was significantly enriched in 6 (1 : 2; 3 : 6) compared to the starting material (17 : 1; 3 : 6), thus indicating that 3 is much more reactive towards epoxidation than 6. Care had to be taken during chromatography on silica gel as the epoxide 12 was acid sensitive (*vide infra*). Treatment of taxadiene (3) with excess DMDO (2 equivalents), produced the bis-epoxide 13 in 75% yield, and this epoxide was found to be much more stable than 12 to chromatography on silica gel (Scheme 5).

### Synthesis of taxa-4(20),11(12)-dien-5 $\alpha$ -ol (4)

With a reliable route to the key epoxide 12 secured, we next wanted to assess its ability to act as a precursor to



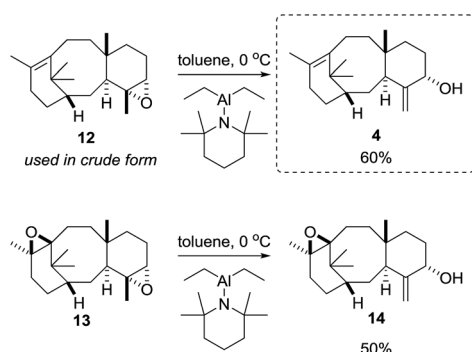
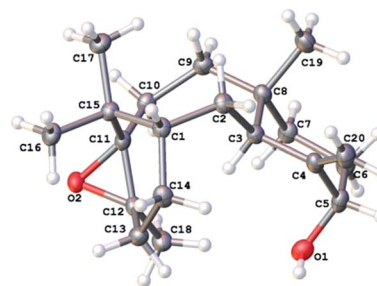


Scheme 5 DMDO epoxidation of taxa-4(5),11(12)-diene.

taxa-4(20),11(12)-dien-5 $\alpha$ -ol (4). Before examining conditions of relevance to the biosynthesis, we first reacted 12 with Yamamoto's aluminium amide reagent (TMPAlEt<sub>2</sub>) to produce 4 as a reference sample (Scheme 6).<sup>13</sup> As the epoxide 12 was prone to decomposition during column chromatography (*vide infra*), we used the epoxide in crude form directly from the DMDO oxidation. Thus, treatment of unpurified 12 with freshly prepared Yamamoto's reagent (BuLi, TMP, ClAlEt<sub>2</sub>, 0 °C, PhMe) gave taxa-4(20),11(12)-dien-5 $\alpha$ -ol (4) in 60% isolated yield over the two steps from taxadiene (3). The spectroscopic data for 4 matched that reported by Williams for the 5 $\alpha$ -stereoisomer,<sup>5</sup> and this enabled us to confirm that epoxidation (3 → 12) must have occurred on the  $\alpha$ -face of taxadiene. Having prepared bis-epoxide 13, we next examined its behaviour under the same rearrangement conditions. Thus, treatment of 13 with Yamamoto's reagent provided epoxy-alcohol 14 as the major isolable product (50%). It is interesting to note that the 11(12)-epoxide moiety is also observed in natural taxanes such as taxinine A 11(12)-epoxide.<sup>14</sup> Fortunately, 14 was obtained as a crystalline solid and we were able to determine an X-ray crystal structure (Fig. 1) to confirm the stereochemistry of the 11(12)-epoxide, and also show that the C5-hydroxyl was on the  $\alpha$ -face.

### Rearrangements of taxadiene-4(5)-epoxide 12

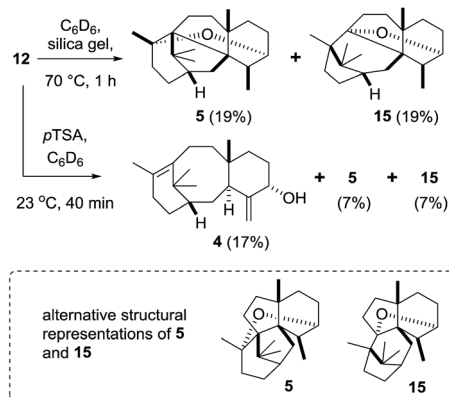
Encouraged by the successful conversion of epoxide 12 to taxa-4(20),11(12)-dien-5 $\alpha$ -ol (4) using Yamamoto's reagent, we next

Scheme 6 Synthesis of taxa-4(20),11(12)-dien-5 $\alpha$ -ol (4) via rearrangement of epoxide 12.Fig. 1 X-ray crystal structure of the taxadiene derived epoxyalcohol 14.<sup>†</sup>

explored the behaviour of 12 under conditions of more relevance to the biosynthesis. We speculated that if taxadiene hydroxylase acts as a monooxygenase and epoxidises taxadiene 3 to produce 12, then this would initially leave a mild Lewis acidic iron centre in close proximity to the epoxide, which could catalyse subsequent rearrangement reactions. Therefore, we decided to examine the behaviour of 12 under a range of acidic conditions.

In order to simulate the acid-mediated decomposition encountered during silica gel chromatography, the epoxide 12 was treated with silica gel in C<sub>6</sub>D<sub>6</sub> at 70 °C. Reaction progress was monitored by <sup>1</sup>H NMR (see ESI<sup>†</sup>), and we determined that 12 converts into OCT (5), the molecule that had previously been produced in metabolically engineered tobacco by Rontein (Scheme 7),<sup>7</sup> and the new isomeric oxacyclotaxane 15 (OCT2). Complete conversion of epoxide 12 was observed, as judged by the loss of the C19 methyl <sup>1</sup>H NMR signal at 0.58 ppm, and the isomeric bridged ethers 5 and 15 were produced in an approximately 3 : 2 ratio (<sup>1</sup>H NMR). Chromatographic separation gave isolated samples of 5 (19%) and 15 (19%), which were then fully characterised.

Treatment of epoxide 12 with a stronger acid (*p*TSA, C<sub>6</sub>D<sub>6</sub>) gave taxa-4(20),11(12)-dien-5 $\alpha$ -ol (4) as the major new product, with OCT (5) and OCT2 (15) being produced as minor products (isolated yields: 4 (17%); 5 (7%); 15 (7%)). The formation of 4(20),11(12)-dien-5 $\alpha$ -ol (4) from the epoxide 12 under these



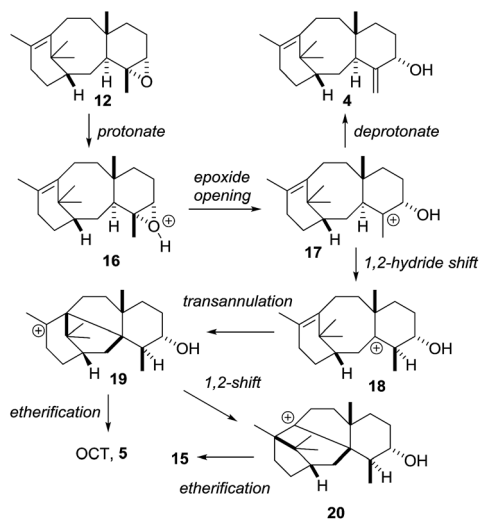
Scheme 7 Rearrangement of taxadiene-4(5)-epoxide (12) under acidic conditions.



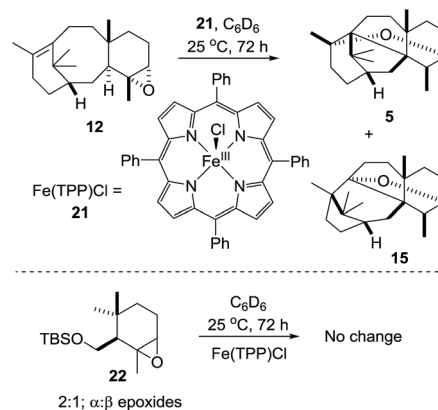
strongly acidic conditions is readily explained by invoking protonation of the epoxide **12** to produce **16** (Scheme 8). Ring-opening then affords the cation **17**, and loss of a proton from the C20 methyl group installs the *exo*-methylene group in **4** (Scheme 8). The formation of OCT (**5**) also implicates the cation **17** as an intermediate. A 1,2-hydride shift first produces the new tertiary cation **18**, which next undergoes transannulation with the C11(12)-alkene leading to the cation **19**. Etherification, involving trapping the cation **19** with the secondary hydroxyl, then gives OCT (**5**). Similarly, the formation of OCT2 (**15**) can be rationalised by invoking a 1,2-alkyl shift of the tertiary cation **19**, leading to the new tertiary cation **20**, which is then trapped as the ether **15** by reacting with the C5-hydroxyl (Scheme 8).

As the biological oxidant (taxadiene hydroxylase<sup>5</sup>) acting upon taxadiene is a cytochrome P450, it is tempting to speculate that the reduced iron<sup>III</sup> porphyrin (**11**, Scheme 4) is capable of facilitating a Lewis acid-catalysed rearrangement of the epoxide *in vivo*. Rontein, however, discounted this proposal<sup>17</sup> on the basis that previous work on very different chemical systems has shown that iron<sup>III</sup> porphyrins are poor catalysts for the rearrangement of epoxides.<sup>15</sup> As we had access to the epoxide **12**, we could test this hypothesis experimentally, and we decided to treat **12** with an iron<sup>III</sup> porphyrin.

Contrary to the literature hypothesis, we were pleased to find that treatment of **12** with Fe<sup>III</sup>(TPP)Cl (2 equiv.) in C<sub>6</sub>D<sub>6</sub> at 25 °C for 72 hours, lead to epoxide rearrangement, with the production of OCT (**5**) and OCT2 (**15**) as the main new products in a 1 : 1 ratio (<sup>1</sup>H NMR). Formation of taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**) was not observed under these Lewis acidic conditions (Scheme 9). As a control experiment, we exposed the similarly-substituted cyclogeraniol-derived epoxide **22**<sup>16</sup> to the same Fe(TPP)Cl rearrangement conditions,<sup>17</sup> and as expected from previous reports,<sup>15</sup> no rearrangement was observed, thus highlighting the propensity of **12** to rearrange.



Scheme 8 Proposed mechanisms for the formation of taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**), OCT (**5**) and OCT2 (**15**) from taxadiene-4(5)-epoxide (**12**).

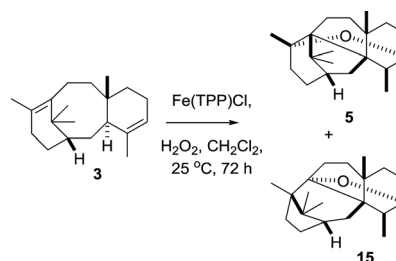


Scheme 9 Iron<sup>III</sup> porphyrin mediated rearrangement of taxadiene-4(5)-epoxide (**12**).

Having shown that the two step epoxidation/Fe<sup>III</sup> induced rearrangement mimics that seen *in vivo* (tobacco) mediated by taxa-4(5),11(12)-diene 5-hydroxylase (CYP725A4), we wondered if the initial oxidation of taxadiene could also be achieved using the Fe<sup>III</sup>(TPP)Cl catalyst and a suitable stoichiometric oxidant (Scheme 10). Thus, treatment of taxadiene (**3**) with Fe<sup>III</sup>(TPP)Cl (10 mol%) and hydrogen peroxide (1 equiv.)<sup>18</sup> lead to complete consumption of starting material (as judged by t.l.c. and <sup>1</sup>H NMR), and the subsequent production of oxidation products. Although the isolated yields were low, <sup>1</sup>H NMR of the crude reaction mixture showed that the two major products were OCT (**5**) and the OCT2 (**15**). The production of taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**) was not observed under these conditions (Scheme 10).

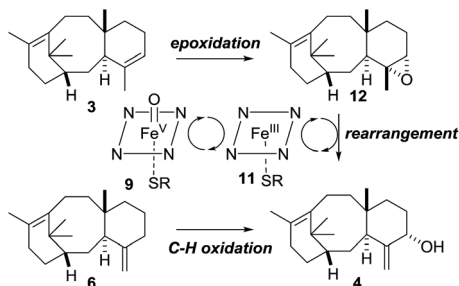
### Implications for the taxol biosynthetic pathway

As discussed in the introduction (Scheme 2), the current proposal for the biosynthesis of **4** from taxadiene **3** is that taxadiene hydroxylase performs an H-atom abstraction from the C20 methyl group of the 4(5)-alkene isomer of **3** to form the allyl radical **10**, and involvement of the epoxide **12** was rejected. Further support for the involvement of a common allyl radical **10** came from the fact that the 4(20)-alkene isomer **6** was also converted to taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**) by taxadiene hydroxylase. However, our experiments, coupled with the previously published kinetic isotope effect data,<sup>5</sup> demonstrate that the epoxide **12** cannot be discounted as an intermediate on



Scheme 10 Iron<sup>III</sup> porphyrin mediated oxidation of taxa-4(5),11(12)-diene (**3**).





Scheme 11 Proposal for the role of epoxide **12** in the biosynthesis of taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**).

the taxol biosynthetic pathway. We have shown that the major, naturally occurring, 4(5)-alkene isomer of taxadiene **3** can be converted to taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**) via the epoxide **12**, and this suggests that the 4(5)-**3** and 4(20)-**6** alkene isomers of taxadiene are processed differently by taxadiene hydroxylase (Scheme 11).<sup>19</sup>

It is possible that 4(5)-alkene isomer **3** is epoxidised to produce **12**, which is then rearranged to **4**, by the action of the reduced form of the hydroxylase **11**. In contrast, the 4(20)-alkene isomer **6** could be converted directly to **4** via the accepted H-atom abstraction mechanism. The involvement of epoxide **12** in the pathway provides an explanation for the lack of a significant primary kinetic isotope effect and the presence of an inverse secondary isotope effect when deuterium labelled [C20-<sup>2</sup>H<sub>3</sub>]-taxadiene (**7**) was oxidized by taxadiene hydroxylase. The labelled C20 methyl likely plays only a small role in the epoxidation process (*i.e.* leads to small inverse isotope effect), and loss of a proton from C20 in an intermediate such as **19** (Scheme 8) is unlikely to be rate-limiting.

## Conclusions

In this study, we have shown that taxa-4(5),11(12)-diene (**3**) can be isolated from the fruit of metabolically engineered tomatoes using our new optimised procedure. Furthermore, we have shown that taxadiene (**3**) can be epoxidised in a regio- and diastereoselective manner to provide taxadiene-4(5)-epoxide (**12**), and that this epoxide can be rearranged to give taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**) in 60% over the two chemical steps. We have shown that the epoxide **12** is sensitive to acids, and that both taxa-4(20),11(12)-dien-5 $\alpha$ -ol (**4**), the known bridged ether OCT (**5**) and the new oxacyclotaxane (OCT2) **15** can be obtained from this material. We have shown that contrary to previous speculation, taxadiene-4(5)-epoxide (**12**) is susceptible to rearrangement when exposed to an iron<sup>III</sup> porphyrin, and these observations combine to warrant reconsideration of the epoxide **12** as a chemically competent intermediate on the taxol biosynthetic pathway.

## Acknowledgements

We thank the EPSRC for providing DTG studentships for NAB and BJM, and the University of Nottingham for additional financial support of this work.

## References

- (a) M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon and A. T. McPhail, *J. Am. Chem. Soc.*, 1971, **93**, 2325; (b) G. M. Cragg, *Med. Res. Rev.*, 1998, **18**, 315; (c) D. G. I. Kingston, *Chem. Commun.*, 2001, 867.
- (a) R. A. Holton, C. Somoza, H. B. Kim, F. Liang, R. J. Biediger, P. D. Boatman, M. Shindo, C. C. Smith and S. Kim, *J. Am. Chem. Soc.*, 1994, **116**, 1597; (b) R. A. Holton, H. B. Kim, C. Somoza, F. Liang, R. J. Biediger, P. D. Boatman, M. Shindo, C. C. Smith and S. Kim, *J. Am. Chem. Soc.*, 1994, **116**, 1599; (c) K. C. Nicolaou, Z. Yang, J. J. Liu, H. Ueno, P. G. Nantermet, R. K. Guy, C. F. Claiborne, J. Renaud, E. A. Couladouros, K. Paulvannan and E. J. Sorensen, *Nature*, 1994, **367**, 630; (d) S. J. Danishefsky, J. J. Masters, W. B. Young, J. T. Link, L. B. Snyder, T. V. Magee, D. K. Jung, R. C. A. Isaacs, W. G. Bornmann, C. A. Alaimo, C. A. Coburn and M. J. Di Grandi, *J. Am. Chem. Soc.*, 1996, **118**, 2843; (e) P. A. Wender, N. F. Badham, S. P. Conway, P. E. Floreancig, T. E. Glass, J. B. Houze, N. E. Krauss, D. Lee, D. G. Marquess, P. L. McGrane, W. Meng, M. G. Natchus, A. J. Shuker, J. C. Sutton and R. E. Taylor, *J. Am. Chem. Soc.*, 1997, **119**, 2757; (f) K. Morihira, R. Hara, S. Kawahara, T. Nishimori, N. Nakamura, H. Kusama and I. Kuwajima, *J. Am. Chem. Soc.*, 1998, **120**, 12980; (g) T. Mukaiyama, I. Shiina, H. Iwadare, M. Saitoh, T. Nishimura, N. Ohkawa, H. Sakoh, K. Nishimura, Y. Tani, M. Hasegawa, K. Yamada and K. Saitoh, *Chem.-Eur. J.*, 1999, **5**, 121; (h) T. Doi, S. Fuse, S. Miyamoto, K. Nakai, D. Sasuga and T. Takahashi, *Chem.-Asian J.*, 2006, **1**, 370.
- (a) R. A. Holton, R. R. Juo, H. B. Kim, A. D. Williams, S. Harusawa, R. E. Lowenthal and S. Yogai, *J. Am. Chem. Soc.*, 1988, **110**, 6558; (b) S. M. Rubenstein and R. M. Williams, *J. Org. Chem.*, 1995, **60**, 7215; (c) Q. Huang, J. D. Pennington, H. J. Williams and A. I. Scott, *Synth. Commun.*, 2006, **36**, 2577; (d) A. Mendoza, Y. Ishihara and P. S. Baran, *Nat. Chem.*, 2012, **4**, 21; (e) Y. Ishihara, A. Mendoza and P. S. Baran, *Tetrahedron*, 2013, **69**, 5685; (f) N. C. Wilde, M. Isomura, A. Mendoza and P. S. Baran, *J. Am. Chem. Soc.*, 2014, **136**, 4909.
- (a) A. E. Koeppe, M. Hezari, J. Zajicek, B. S. Vogel, R. E. LaFever, N. G. Lewis and R. Croteau, *J. Biol. Chem.*, 1995, **270**, 8686; (b) M. Köksal, Y. Jin, R. M. Coates, R. Croteau and D. W. Christianson, *Nature*, 2011, **469**, 116.
- (a) S. Jennewein, R. M. Long, R. M. Williams and R. Croteau, *Chem. Biol.*, 2004, **11**, 379; (b) J. Hefner, S. M. Rubenstein, R. E. B. Ketchum, D. M. Gibson, R. M. Williams and R. Croteau, *Chem. Biol.*, 1996, **3**, 479.
- (a) J. DeJong, Y. Liu, A. P. Bollon, R. M. Long, S. Jennewein, D. Williams and R. B. Croteau, *Biotechnol. Bioeng.*, 2006, **93**, 212; (b) B. Engels, P. Dahm and S. Jennewein, *Metab. Eng.*, 2008, **10**, 201.
- D. Rontein, S. Onillon, G. Herbette, A. Lesot, D. Werck-Reichhart, C. Sallaud and A. Tissier, *J. Biol. Chem.*, 2008, **283**, 6067.



- 8 (a) P. K. Ajikumar, W.-H. Xiao, K. E. J. Tyo, Y. Wang, F. Simeon, E. Leonard, O. Mucha, T. H. Phon, B. Pfeifer and G. Stephanopoulos, *Science*, 2010, **330**, 70; (b) Q. Huang, C. A. Roessner, R. Croteau and A. I. Scott, *Bioorg. Med. Chem.*, 2001, **9**, 2237; (c) K. Huang, Q. Huang, M. R. Wildung, R. Croteau and A. I. Scott, *Protein Expression Purif.*, 1998, **13**, 90.
- 9 K. Kovacs, L. Zhang, R. S. T. Linforth, B. Whittaker, C. J. Hayes and R. G. Fray, *Transgenic Res.*, 2007, **16**, 121.
- 10 V. G. Yadav, *J. Mol. Catal. B: Enzym.*, 2014, **110**, 154.
- 11 (a) Y. S. Angelis and M. Orfanopoulos, *J. Org. Chem.*, 1997, **62**, 6083; (b) R. P. Hanzlik and G. O. Shearer, *Biochem. Pharmacol.*, 1978, **27**, 1441.
- 12 W. Adam, J. Bialas and L. Hadjiarapoglou, *Chem. Ber.*, 1991, **124**, 2377.
- 13 A. Yasuda, H. Yamamoto and H. Nozaki, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1705.
- 14 (a) R. Murakami, Q. Shi and T. Oritani, *Phytochemistry*, 1999, **52**, 1577; (b) Y.-F. Wang, Q.-W. Shi, M. Dong, H. Kiyota, Y.-C. Gu and B. Cong, *Chem. Rev.*, 2011, **111**, 7652.
- 15 D. C. Liebler and F. P. Guengerich, *Biochemistry*, 1983, **22**, 5482.
- 16 For the synthesis of epoxide 22 see: M. Uroos and C. J. Hayes, *Org. Lett.*, 2010, **12**, 5294.
- 17 For Lewis acid-mediated rearrangements of cyclohexene oxides see: (a) E. A. Braude, A. A. Webb and M. U. S. Sultanbawa, *J. Chem. Soc.*, 1958, 3328; (b) R. E. Parker and N. S. Isaacs, *Chem. Rev.*, 1959, **59**, 737; (c) K. Maruoka, T. Ooi and H. Yamamoto, *J. Am. Chem. Soc.*, 1989, **111**, 6431.
- 18 (a) J. T. Groves, T. E. Nemo and R. S. Myers, *J. Am. Chem. Soc.*, 1979, **101**, 1032; (b) T. G. Traylor, S. Tsuchiya, Y.-S. Byun and C. Kim, *J. Am. Chem. Soc.*, 1993, **115**, 2775; (c) D. P. Barbosa Sousa, A. T. Fricks, H. M. Alvarez, G. C. Salomao, M. H. Neves Olsen, L. Cardozo Filho, C. Fernandes and O. A. C. Antunes, *Catal. Commun.*, 2007, **8**, 1041.
- 19 Whilst this manuscript was under review, a complementary study by Stephanopoulos *et al.* has been reported that also proposes taxadiene epoxidation by taxadiene-5 $\alpha$ -hydroxylase as being a step on the taxol biosynthetic pathway. Please see: S. Edgar, K. Zhou, K. Qiao, J. R. King, J. H. Simpson and G. Stephanopoulos, *ACS Chem. Biol.*, 2016, DOI: 10.1021/acscchembio.5b00767.

