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The Verticillenes. Pivotal Intermediates in the Biosynthesis of the Taxanes and the Phomactins

Matthew J. Palframan and Gerald Pattenden*

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The verticillene family of 6,12-membered ring-fused diterpenes are found in plants, liverworts, corals and insects. Carbocations derived from verticillene hydrocarbons are central intermediates in the biosynthesis of the taxane and the phomactin families of polycyclic natural products. This perspective delineates these unique biosynthetic interrelationships, which are reinforced by recent biomimetic synthesis investigations, alongside quantum chemical calculations and targeted engineering studies of the taxadiene synthase (TXS) cascade.

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1. Introduction

The verticillenes are a family of 6,12-membered ring-fused diterpenes which have structures based on a bicyclo [9.3.1] pentadeca-*E*-3,*E*-7-triene framework. The tertiary alcohol verticillol **1**, found in the evergreen wood of the conifer *Sciandopitys verticillate* (Taxodiaceae) was the first of their number to be described in 1964.¹ Contemporaneously, Lythgoe *et al.*² postulated that the hydrocarbon **2** corresponding to **1**, called “verticillene,” might be a key intermediate in the biosynthesis of the tricyclo[6,9,3.1.0^{3,8}] pentadeca-4,11-diene, *i.e.*, taxadiene, ring system **3** of the taxane alkaloids (Figure 1). Although this postulate is repeated in graduate texts,³ and several naturally occurring alkene isomers of verticillene **2** have been discovered since the 1960s, at this time the particular 3,7,11-triene isomer **2**, has not been

found “free” in Nature. However, in 1985 our research group published a total synthesis of racemic **2** in connection with a program we had designed to examine its possible biomimetic transannular cyclisation into the ring system in taxadiene **3**.⁴ These synthesis and biomimetic studies, alongside later investigations by others, are summarised in Sections 7 and 8.

The absolute stereochemistry of (+)-verticillol isolated from *S. verticillate* was assigned as the enantiomer of **1** in 1978,⁵ but this was revised some twenty five years later to **1** following anomalous dispersion X-ray crystallographic analysis of its *p*-iodobenzoate.⁶ Unfortunately, the absolute configurations of most verticillene derivatives reported in the literature between 1978 and 2005 were assigned on the basis of the incorrect *ent*-stereochemistry originally assigned to natural (+)-verticillol. It follows therefore that most of the absolute stereochemistries given to the verticillenes over the period 1978-2005 have needed to be reversed. Hence, throughout this Report, the verticillene structures in the Schemes and Figures are all drawn with their correct stereochemistries.

A variety of verticillene hydrocarbons and their oxy-derivatives have been found in plants of the genus *Bursera*, endemic to Mexico, and in *Boswellia* species which are trees native to Ethiopia. They have also been isolated from liverworts collected in Japan, from a wide variety of soft corals, and especially from *Taxus* (yew) species. In 2018, a novel verticillene hydrocarbon was reported, for the first time, as a sex-aggregation pheromone in insects. Although some of the plant sources of natural verticillenes have shown biological properties, interest in these compounds has remained largely focussed on the implication of the hydrocarbon verticillene **2**, and carbocation intermediates derived from it, in the biosynthesis of the taxadiene ring system **3**, precursor to the potent antitumor taxoids, *e.g.* taxol (paclitaxel) **6**. The same verticillene hydrocarbon **2** has also been recognised as a key intermediate in the biosynthesis of the closely related bicyclic phomactatriene ring system **4**, precursor to the phomactin family of platelet activating factor (PAF) antagonists, *e.g.*

*School of Chemistry, The University of Nottingham, University Park, Nottingham, NG7 2RD

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Fig. 1 Verticillenes, taxanes and phomactins.⁷

phomactin A (5). Here we summarise the evidence for the biogenetic interrelationships between the verticillenes, the taxanes and phomactins, and show how recent targeted engineering of the taxadiene synthase (TXS) enzyme alongside biomimetic synthesis and biosynthesis investigations have added credence to earlier conclusions.

2. Verticillene vis-à-vis cembrene hydrocarbons

Although the hydrocarbon verticillene **2** has not been reported “free” in Nature, over the past 25 years several research groups have described the presence of a number of alkene regioisomers of (*S*)-verticillene **2**, and its (*R*)-enantiomer, in the essential oil of *Boswellia carterii* olibanum, from various species of the tree *Bursera*, and from the Japanese liverwort *Jackiella javanica*. Thus, Asakawa *et al.*⁸ were the first to describe the presence of the isomeric *ent*-isomers **7** and **8** of *exo*- and *endo*-verticillene respectively in the liverwort *J. javanica* in 1995, and a few years later König *et al.*,⁹ reported the C-4 *exo*-isomer **9** in the essential oil of *Boswellia carterii* (Figure 2). In a comprehensive investigation of verticellene derivatives in *Bursera* species Hernandez *et al.*¹⁰ later established the presence of the two alkene isomers **10** (*ent*-**7**) and **11** (*ent*-**8**) of verticillene, alongside **9**, in *Bursera suntui* and *B. kerberi*, and Marcotullio *et al.*¹¹ reported the presence of **10** in *B. microphylla*. During studies of the chemistry of naturally derived (+)-verticillol **1**, it was shown that the isomeric verticillenes **10** and **11** could be produced following

Fig. 2 Naturally occurring isomeric verticillene hydrocarbons.

dehydration of **1** under acid conditions.⁴ The unusual and interesting Z-8,9-isomer **12** of verticillene **2**, called sobralene, was recently reported to be a sex-aggregation pheromone produced by the sand fly *Lutzomyia longipalpis* from Sorbral (Ceará State) in Brazil.¹² *L. longipalpis* is the main vector of the parasite *Leishmania infantum*, the causative agent of American visceral leishmaniasis (AVL) a potentially fatal human and canine disease in South and Central America.¹³

The verticillene alkene isomers **7-11** have their origin in electrophilic cyclisation of geranylgeranyl diphosphate (GGPP) **13**, which leads initially to the 14-membered macrocyclic carbocation intermediate **15** (Scheme 1). Quantum chemical calculations on the TXS pathway from GGPP to taxadiene **3**, published by Hong and Tantillo in 2011,¹⁴ reinforced an earlier view that the GGPP carbocation species **14** in the cyclisation leading to **15a** is preorganised and that the cyclisation is stereospecific with *re*-face attack of the π -bond and *anti*-displacement of the diphosphate (OPP) group.¹⁵ Correspondingly, *si*-face attack within **14** would lead to the enantiomeric carbocation **15b**. Calculations also showed that the cyclisation process leading to **15a** is likely to be concerted (albeit asynchronous) with loss of OPP. The “cembrenyl” carbocation **15a** in the taxadiene synthase (TXS) cascade is a discrete intermediate, yet it rapidly undergoes transannular cyclisation to the C12 verticillyl carbocation intermediate **16a**. Loss of protons from C13 and C18 in **16a/16b** then lead to the enantiomeric verticillene alkene isomers **7, 8, 10** and **11**. The C-4 *exo*-isomer **9** of verticillene is most likely produced from the C-12 verticillyl carbocation **16a** in a two-step process, involving capture of the proton at C-11 by the C3,C4 double bond leading to the C4 verticillyl carbocation **17**, followed by elimination of a proton from C20 (Scheme 1).

Loss of a proton from one of the methyl groups at C15 in the macrocyclic carbocation intermediate **15** instead delivers

cembrene A (**18**), a member of the “cembrene” family of natural products, *e.g.* hydrocarbons **20-25** (Figure 3), which often occur naturally in both the *R* and *S* enantiomeric series. It is interesting that cembrene A (**18**),¹⁶ as well as the cyclopropane-based diterpene macrocycle casbene **19**,¹⁷ were both once considered to be conceivable intermediates in the biosynthesis of taxadiene from GGPP. Furthermore, in some studies of the cyclisations of analogues of GGPP catalysed by TXS, Scott *et al.*¹⁸ established that 14-membered cembrene hydrocarbons could be major products. Later, during targeted engineering studies of the TXS pathway, Brück *et al.* engineered a TXS mutant incorporating histidine as a specific proton acceptor, and observed the formation of *R*-cembrene A (**18**); they then went on to develop a fermentation system which produced **18** in small quantities.¹⁹ Earlier, Kuzuyama *et al.*²⁰ had described a diterpene synthase produced from *Streptomyces* which also led to the synthesis of cembrene A (**18**) alongside its isopropylidene isomer **20**, and Keasling *et al.*²¹ had cloned cembrene A (**18**) and casbene **19** synthases from Euphorbiaceae plant genes expressed in *Saccharomyces cerevisiae*. Cembrene **21**, which is also called thunbergene or thumbelene, was the first cembrene to be isolated and characterised from pine oleoresins in 1965.²²

Contemporaneously, a number of isomeric cembrene hydrocarbons, including isocembrene **22**,²³ were found in

Scheme 1 Origin of the verticillene hydrocarbons **7-11** and the cembrenes **18-23** from GGPP **13**.²⁴

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Fig. 3 Casbene **19** and cembrene diterpene hydrocarbons **20-25**.

other higher plants, including the tobacco plant.²⁵ However, cembrene A (**18**) (also known as neocembrene and neocembrene A) is the most widely distributed cembrene hydrocarbon, and has been isolated, for example, from the Pharaohs ant, *Monomorium pharaonis*,²⁶ and from the Australian subterranean termite, *Nasutitermes exitiosus*,²⁷ where it exhibits pheromone-like activity. It has also been found in paracloacal glands of the Chinese alligator, *Alligator sinensis*,²⁸ and its 3Z-isomer **23** has been isolated from the heads of soldier termites *Cubitermes umbratus*.²⁹ Of some relevance to this discussion, the cembrenes **18**, **22** and **24** have been found to co-occur with the 4-*exo* verticillene **9** in *Boswellia carteri*.⁹

The majority of cembrenes that have been characterised over the past few decades, however, are oxygenated variants (e.g. OH, epoxy, cyclic ether, carbonyl and combinations thereof) of the hydrocarbons **18** and **21-23**, also of cembrene C (**24**) and cembrenene **25**.³⁰ Furthermore, the overwhelming majority of these oxygenated cembrenes are produced by soft corals and gorgonians,^{31,32} where many are precursors to a variety of structurally novel and biologically interesting polycyclic cembranoids.³³

3. Biosynthetic interrelationship between verticillene and taxadiene

The structures and stereochemistries of the taxane family of tricyclic diterpenes (see structures **3** and **6**) were established independently by Lythgoe³⁴ and by Nakanishi³⁵ and their respective collaborators during the 1960s. The later revision of

the absolute configuration of natural (+)-verticillol to **1** with the β -configuration at C-1 in 2005⁶ was therefore consistent with the taxane stereochemistry **3**. In 1966, Lythgoe *et al.*² had postulated that the 6,8,6-ring-fused system in taxadiene **3** could arise in Nature from GGPP (**13**) via verticillene **2** (presumably via the verticillyl carbocation **16a**) followed by a transannulation reaction, implicating the carbocation intermediate **26** (Scheme 2).

Lythgoe's proposal was essentially vindicated some years later during studies of the mechanism of the TXS-catalysed cyclisation of GGPP (**13**) by several groups of researchers during 1995-2005. Thus, early studies by Croteau *et al.*³⁶ showed that when GGPP labelled with deuterium at C10 (see structure **27**) was incubated with taxadiene synthase, the taxadiene isolated contained deuterium located at C7 (see structure **30**). This observation implicated capture of the C11 deuterium in the first-formed verticillyl carbocation **28**, by the C7,8 alkene bond, and its transfer to C7, leading firstly to the isomeric verticillyl carbocation intermediate **29** prior to its cyclisation to the taxadiene **30** (Scheme 3).

In other investigations, Coates *et al.*⁶ showed that when the 6-fluoro analogue **31** of GGPP was similarly incubated with recombinant TXS, only the C12-*exo* **34**, the C-12-*endo* **35** and, significantly, the C-4-*exo* **36** isomeric 7-fluoroverticillenes were produced (88% yield; ratio 3:2:2) (Scheme 4). None of the C-7 fluoro analogue **33** of taxadiene was produced in this incubation. This outcome demonstrated how the inductive effect of the fluoro-substituent at C7 in the fluoroverticillyl carbocation intermediate **32** inhibited the crucial C11 to C7 proton transfer, thereby blocking the cyclisation which would

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Scheme 2 Early outline proposal for the biosynthesis of taxadiene **3** from GGPP (**13**) via verticillene **2** and the verticillyl carbocation intermediates **16a** and **26**.

Scheme 3 Incubation of C-10 deuterium labelled GGPP (**27**) with taxadiene synthase leading to the C-7 deuterium labelled taxadiene **29**

Scheme 4. Incubation of the fluoro-analogue **31** of GGPP with recombinant TXS leading to the isomeric fluoroverticillenes **34-36** at the expense of the fluorotaxadiene **33**.

have led to the fluorinated taxadiene **33** and allowing the carbocation **32** to undergo alternative reactions leading to the fluoro-verticillenes **34-36**.

Interestingly, the formation of the C-4 *exo*-fluoroverticillene **36** in this incubation, suggested that a two-step proton-transfer process is involved in the conversion of the verticillyl C12 carbocation **16a** into taxadiene **3** (see Scheme 2). Thus, transfer of the proton at C-11 to C-3 in **16a**,

first leads to the verticillyl C-4 carbocation **37**, which, following the transfer of a proton from C-3 to C-7 then leads to the C-8 verticillyl carbocation **39** (Scheme 5). Lastly, transannular cyclisation of **39** produces the tricyclic carbocation intermediate **38** which then loses a proton leading to taxadiene **3** (*cf.* Scheme 2). Indeed, additional quantum chemical calculations by Hong and Tantillo,¹⁴ suggest that the two-step route (**16a**→**37**→**39**) is energetically more favourable

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Scheme 5 Stepwise conversion of the verticillyl C-12 carbocation **16a** into taxadiene **3** via the C-4 and C-8 verticillyl carbocations **37** and **39** and the taxane carbocation **38**.

Fig 4. Different conformations, **40** and **41**, of the verticillyl C-8 carbocation **39** leading to the formation of sobralene **12** and taxadiene **3** respectively.

that the single step route (**16a**→**39**) for transfer of the proton at C11 in **16a** to C7 in **39**. The same calculations also supported an earlier proposal by Coates *et al.*³⁶ that there needs to be a change in conformation of the carbocation **39**, *i.e.* from **40** to **41**, prior to its cyclisation to **38** (energy difference *ca.* 4 kcal/mol; Figure 4). Significantly, although the aforementioned early studies ruled out verticillene **2** as a “free” intermediate they consolidated the view that the verticillyl carbocation **16a** was the central intermediate in the TXS cascade between GGPP (**13**) and taxadiene **3**.

Interestingly, it has been suggested that the biosynthesis of the novel Z-8,9 verticillene isomer sobralene **12**, takes place by elimination of the α -proton at C9 in the high energy conformation **40** of the verticillyl carbocation **39** thereby leading to the Z-configuration of the C8,9 alkene bond in the natural product.¹²

4. Targeted engineering of TXS leading to the production of verticillene intermediates

The crystal structure of a shortened/truncated variant of the TXS enzyme was reported in 2011,³⁷ and this exciting disclosure was a catalyst for others to pursue targeted

engineering of TXS to probe further mechanistic details, as well as accessing and characterising key intermediates, on the pathway from GGPP to taxadiene.³⁸ Although earlier mechanistic studies using deuterium and fluorine-labelled GGPP analogues, and quantum chemical calculations, had clearly established the presence of several verticillyl carbocation intermediates in the TXS cascade (see Section 3) the nature and location of the deprotonating basic sites within the enzyme leading to these reactive species remained a mystery.

Brück *et al.*,¹⁹ constructed a closed TXS-GGPP complex with both TXS and GGPP in their productive conformations. They then used an integrated approach of docking and MM optimisation to model the catalytic sites of TXS and GGPP, and the carbocation intermediates suggested in the cascade mechanism. In this manner Brück and colleagues were able to accurately trace the reaction pathway followed by TXS which, in turn, allowed them to target and then engineer enzymes to generate important verticillene intermediates in the overall TXS cascade. Significantly, Brück *et al.*, demonstrated that particular mutants of TXS gave rise to the production of the verticillenes **2**, **9**, and **11**, as well as the isomer **42** of taxadiene **3** (Scheme 6). Following experiments carried out *in*

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Scheme 6 Overall picture of the TXS cascade from GGPP (**13**) to taxadiene **3**, with the postulated verticillyl carbocation intermediates **16a**, **37** and **39**, the deprotonated verticillene intermediates **2**, **9** and **11**, and the C4-*exo* iso-taxadiene **42**.

vitro, NMR and chiroptical measurements on the isolated verticillene **11** showed that it was identical with, and had the same (+)-*R,R*-configuration as, the same compound isolated earlier from species of *Bursera* and from *Boswellia carterii*.^{9,10}

In contemporaneous investigations, Edgar *et al.*,³⁹ established a TXS mutant enzyme, expressed in an engineered *E. coli*, and showed that the verticillene **11** was its sole product. The verticillene isomer **11** results from premature deprotonation at C13 in the C12 carbocation intermediate **16a** presumably as a result of the closer orientation of the C13 proton to a basic site in the mutant in comparison with native TXS. The formation of the C4 *exo*-verticillene **9** confirmed the presence of the C4 verticillyl carbocation **37** in the TXS cascade; furthermore, the carbocation **37** is also linked to the verticillene **2** via deprotonation at C3. Overall, the studies of Brück and colleagues supported the earlier proposed two step route, *i.e.* **16a**→**37**→**39** as being more energetically favourable over the alternative single step process **16a**→**39**,⁴⁰ en route to taxadiene.

5. Biosynthetic interrelationship between verticillene and the phomactins

The phomactin family of PAF antagonists have a core bicyclo[9.3.1] pentadecane ring system, and they were first reported in the marine fungus, *Phoma* sp., in 1991.⁴¹ *e.g.* phomactin A (**5**), Sch 49026 (**4**), phomactin B (**43**), phomactin G (**44**) and phomactin K (**45**) (Figure 5). Following their isolation

Fig. 5 Some naturally occurring phomactins

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Scheme 7 Proposed biosynthesis of the phomactatriene **47** from the verticillyl carbocation **16a** via the rearranged carbocation **46**.

Scheme 8 Incubation of the enzyme of recombinant *E. coli* with des-7-methylGGPP (**48**) leading to the des-8-methylphomactatrienes **51** and **52** at the expense of des-8-methyltaxadiene **50**.

it was recognised immediately that the phomactin ring system **4** exhibited a striking structural relationship with that found in the taxanes, *i.e.* **3** which suggested that they probably shared the same verticillene **2** (or verticillyl carbocation intermediate **16a**) in their biosynthesis.⁴² Thus, whereas an enzyme-mediated process featuring transannulation from **16a** produces the C-ring in taxadiene **3**, an alternative sequence of carbocation initiated 1,2-hydrogen and 1,2-methyl group shifts in **16a** leads to the carbocation intermediate **46** instead (Scheme 7). Elimination of a proton in **46** then delivers the natural phomactatriene hydrocarbon **47** which, together with its isomer **4**, are logical precursors to the oxygenated phomactins, *i.e.* **5**, **43**, **44** and **45**. Indeed, several years later the essential ingredients of this biosynthesis proposal were given credence by Oikawa *et al.*⁴³ based on their feeding

experiments with ¹³C-labelled acetates in fermentations of *Phoma* sp. Furthermore, during a study of the hydrocarbon constituents of the phomactin-producing fungus *Phoma* sp the same authors also identified the verticillenes **9**, **10** and **11** together with the phomactins **4** and **47**. These observations reinforced the pivotal role of the verticillyl carbocation **16a** in the biosynthesis of the phomactins and the special relationship they share with the taxanes **3** (see Scheme 7). Furthermore, in contemporaneous studies Scott *et al.*⁴⁴ demonstrated that when des-7-methylGGPP (**48**) was incubated with the enzyme of recombinant *E. coli* the major products were the isomeric des-8-methylphomactatrienes **51** and **52** (Scheme 8). None of the des-8-methyl taxadiene **50** was produced in this incubation. This outcome was rationalised on the basis that the absence of a carbocation

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Fig. 6 Some natural hydroxyverticillenes and their derivatives.

stabilising methyl group at C8 in the verticillol intermediate **49** diverts the pathway away from that leading to the des-methyltaxadiene **50**, allowing time for the carbocation **49** to undergo rearrangement to the desmethylphomactatrienes **51** and **52** instead.

6. Natural oxy-substituted verticillenes

The overwhelming majority of verticillenes that have been described accommodate various oxygen substituents, *i.e.* OH, OAc, carbonyl, epoxy, and the major portion of these metabolites have been isolated from soft corals and from *Taxus* species. As a result of their association and structural relationship with taxol several of these oxy-substituted verticillenes have been variously named as bicyclic taxanes,⁴⁵ seco-taxanes,⁴⁶ taxachitrienes,⁴⁷ taxuspines,⁴⁸ and canadenseses,⁴⁹ amongst others. Shi and colleagues have earlier listed the structures of many oxy-substituted verticillenes that have been described in the literature up to about 2005,⁵⁰ and we will not duplicate this listing here. Nevertheless, because it was one of the first oxygenated verticillenes to be characterised in the early 1980s we make special mention of the poly-oxygenated verticillene hypoestoxide **53** (Figure 6).⁵¹ This highly oxygenated verticillene was isolated from the tropical shrub *Hypoestes rosea*, found in the Nigerian rain forests, and extracts of the shrub have been used in folk medicine for generations to treat various skin rashes and fungal infection conditions. Here however, we will focus on the structures of some more recently characterised oxy-substituted verticillenes and particularly those that relate to, or co-occur with, the verticillene hydrocarbons **7–11** of importance in the biosynthesis of the taxanes and the phomactins. Thus, in investigations of the verticillols produced by plants of the genus *Bursera* which grow in parts of Mexico, Hernandez *et al.*¹⁰ showed that although (+)-verticillol **1** is found in *B. suntui*, surprisingly its C-12 epimer **54** is produced by *B. kerberi*. Earlier, Asakawa *et al.*⁵² had reported the presence of the

enantiomers of **1** and **54** in the liverwort *Jackiella jananica*, and in 2017 the same verticillol **54** was found in *B. microphylla*.¹¹ Hence a total of four isomeric verticillols have now been characterised in Nature *i.e.* **1**, *ent*-**1**, **54** and *ent*-**54**. The same liverwort *J. jananica* from which the verticillene hydrocarbons **7** and **8** were isolated is also a source of the interesting hydroxyl-substituted verticillenes, verticillenediol **55**, verticillenediol **56a** and 12-*epi*-verticillenediol **56b**.⁵² Likewise, the shrub *B. suntui* that provides the verticillene hydrocarbons **9**, **10**, and **11** also metabolises the C-20 hydroxy substituted verticillol **57a** and its acetate **57b**,¹⁰ together with the C-7-C-8 epoxides and C7 hydroxy derivatives of **57a** and **57b**.⁵³

7. Some biomimetic conversions of verticillenes into taxadiene and phomactatrienes

We have mentioned earlier that our motivation for synthesising verticillene **2** in the mid-1980s was to establish whether it could be encouraged to undergo a (biomimetic) transannular cyclisation across its 12-membered ring leading to the 6,8,6-tricyclic ring system **3** in the taxanes.⁴ Unfortunately we were frustrated in all of the attempts we made to convert **2** into **3** in the presence of boron trifluoride at 0–50°C, the preferred reagent and conditions for carrying out these types of transannulations at the time; instead verticillene **2** underwent extensive decomposition. We also prepared the C-7,8-epoxide **58** of our synthetic verticillene but this compound only underwent rearrangement to a mixture of the secondary alcohols **59** and **60** on treatment with boron trifluoride etherate. We next prepared the epoxide **61** of natural (+)-verticillol **1**, and also the epoxide **62** produced from the isomeric verticillene **11** which we obtained by dehydration of (+)-verticillol **1** (Scheme 9). Treatment of the epoxide **61** with boron trifluoride etherate or trimethylsilyl triflate under a range of conditions produced largely the corresponding ketone **63** accompanied by small amounts of the product **62** from dehydration of **61**.⁴ In separate experiments and under the

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Scheme 9. Attempted biomimetic conversions of verticillene epoxides

Scheme 10. Biomimetic conversions of (+)-verticillol **1** into the phomactin **47**, taxadiene **3** and the phomactin isomer **64** (Note: yields were calculated from GC analysis).

same boron trifluoride etherate reaction conditions the epoxide **62** underwent extensive decomposition.

Some twenty years after our own work, and following the discovery of the phomactins in the fungus *Phoma* sp in 1991, Oikawa *et al.*⁴³ carried out a more systematic study of the rearrangement of natural verticillol **1** in the presence of boron trifluoride etherate but at various lower temperatures. Thus, they found that when verticillol was treated with boron trifluoride etherate at -78 °C almost equal amounts (*i.e.* 31, 32, and 37% based on peak areas in GC analysis) of the isomeric verticillene hydrocarbons **10** and **11**, and the phomactatriene **47** were obtained (Scheme 10). However, at -30 °C the major product was the phomactatriene **47** (62%)

together with the verticillene **11** (36%), and at -20°C the unusual C-4,5 / C-8,9-positional isomer **64** of **47** predominated (46%). The same phomactatriene positional isomer **64** was also the major product, *i.e.* 65%, when the *exo*-verticillene **10** was separately treated with boron trifluoride etherate at -30 °C and when verticillol was treated with La(OTf)₃ at 40 °C. Interestingly, the same authors also reported the presence of taxadiene **3**, estimated to the extent of 0.004% yield (after repeated HPLC, and detected by co-injection with an authentic sample), when verticillol **1** was treated with boron trifluoride etherate at room temperature. We have already pointed out that, Oikawa *et al.* had simultaneously identified the isomeric verticillenes **9-11** and the phomactatriene **47** in mycelium

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Scheme 11. The first total synthesis of (±)-verticillene **2**.

extracts of the fungus. These studies clearly reinforced the central role that the verticillyl carbocation **16a** plays in linking the biosynthetic interrelationships within the verticillene, the taxane and the phomactane families of diterpenes

8. Synthetic studies towards the verticillenes

Synthetic studies within the verticillene family of natural products have been relatively sparse, although two reports of the total synthesis of verticillene **2** have been presented. The first synthesis of racemic verticillene **2** was described by ourselves in 1985,⁴ and was based on an intramolecular McMurray reductive coupling reaction with the *bis*-aldehyde **72** leading to the macrocycle **73** as the key step (Scheme 10).⁴ The synthesis of **72** started from the 3-*tert*-butoxycyclohexenone **65a** which was first alkylated with geranyl bromide leading to **65b**. Addition of methyllithium to **65b** followed by hydrolysis next gave the cyclohexenone **66**, which was then added to lithium dimethylcuprate leading to an enolate which could be trapped with TMS chloride producing the silyl enol ether **67**. The silyl enol ether **67** was

next alkylated with trimethyl orthoformate in the presence of TiCl_4 , and chromatography separated the *cis*-ketoacetal **68** in 58% yield. The ketoacetal **68** was next converted into the unsaturated aldehyde **70** in two steps, following treatment with methylmagnesium iodide, producing **69**, and concomitant hydrolysis and dehydration of **60**, using POCl_3 in pyridine. Regioselective allylic oxidation of **70**, using catalytic selenium dioxide/*tert*-butyl hydroperoxide next gave the *E*-allylic alcohol **71a** which underwent smooth oxidation using MnO_2 producing the central intermediate *bis*-aldehyde **72**. In one of the first reported demonstrations of the scope of the intramolecular McMurray reductive coupling reaction, at the time, treatment of **72** with TiCl_4 and Zn-Cu couple gave a mixture of the macrocyclic tetraene **73** and the isomer **74** which resulted from concurrent 1,5-sigmatropic rearrangement in **73**. 1,4-Reduction of the conjugated 1,3-diene unit in **74** using sodium in liquid ammonia then led to *E,E*-verticillene **2**.

Interestingly, a few years later, Croteau *et al.*³⁶ used a modification of the above synthetic approach to prepare C-10 deuterium-labelled verticillene **77** in connection with their investigations of the mechanism of taxadiene synthase (*cf.*

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i) NaBD₄, CeCl₃; ii) NH₄F, THF; iii) PBr₃, Et₂O; iv) Ni(CO)₄, NMP

Scheme 12. Synthesis of C-10-deuterium labelled verticillene **77**.

i) SnCl₄ at -90 °C; ii) SnCl₄ at -30 °C; iii) LiCl, 100 °C; iv) ZnCl₂, RT then 2-methyl-2-butene; v) LiAlH₄, ZnCl₂, -78 °C; vi) PPh₃, CCl₄, reflux; vii) n-BuLi, (TMS)₂NH, 60 °C; viii) DIBALH, -78 °C; ix) RhCl(PPh₃)₃, PhCN, 150 °C

Scheme 13. Kato's synthesis of (±)-verticillene **2**.

Scheme 3). Thus, reduction of the aldehyde group in **71b** using NaBD₄ in the presence of CeCl₃, followed by deprotection of the silyl ether protecting group in the D-labelled product, first gave the corresponding *bis*-allylic alcohol **75** (Scheme 12). Bromination of **75** using PBr₃, followed by intramolecular reductive coupling of the resulting *bis*-allylic bromide **76** in the presence of Ni(CO)₄ then gave C-10 deuterium-labelled verticillene **77**.

A different synthetic approach towards verticillene **2** was used by Kato *et al.*⁵⁴ whereby the cyclohexene ring in the substituted verticillene intermediate **82** was produced via an intramolecular alkylation and elimination process, reminiscent of a Friedel-Crafts reaction, from the chloride intermediate **80**. Thus, the substituted C10-geranyl units **78** and **79** were first

coupled in the presence of SnCl₄ at -90 °C leading to **80** which on further reaction with SnCl₄ at -30 °C led to a mixture of the diastereoisomeric substituted cyclohexanes **81a** and **81b** (Scheme 13). Then diastereoisomer **81a** was treated with LiCl at 100 °C which resulted in dehydrochlorination leading to the substituted cyclohexene **82**, while diastereoisomer **81b** was treated with ZnCl₂ leading to cyclohexene **82**. In several steps the ester group in **82** was then converted into the corresponding alkyl chloride **83**. An intramolecular alkylation in **83** in the presence of lithium *bis*-(trimethylsilyl) amide at 60 °C next gave the macrocycle **84** as a single diastereoisomer in 73% yield. Reduction of the nitrile group in **84** followed by decarbonylation of the resulting aldehyde **85** using Wilkinson's catalyst finally gave racemic verticillene **2**.

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i) LDA, (*E*)-1-bromohex-2-ene, $-78\text{ }^{\circ}\text{C}$ to $0\text{ }^{\circ}\text{C}$; ii) MeLi, then HCl, $-78\text{ }^{\circ}\text{C}$ to $23\text{ }^{\circ}\text{C}$; iii) MeLi, CuI, then TMSCl; iv) MeLi, then ZnCl_2 , then (3*E*,8*E*)-3,8-dimethyltetradeca-3,8,13-trienal, $-45\text{ }^{\circ}\text{C}$; v) TMSCl, Et_3N , $0\text{ }^{\circ}\text{C}$ to $23\text{ }^{\circ}\text{C}$; vi) MeMgBr, $0\text{ }^{\circ}\text{C}$; vii) triphosgene, pyridine $23\text{ }^{\circ}\text{C}$; viii) Grubbs II catalyst $110\text{ }^{\circ}\text{C}$; ix) NaOH, dioxane, $23\text{ }^{\circ}\text{C}$; x) MsCl, DMAP, pyridine, $23\text{ }^{\circ}\text{C}$; xi) lithium naphthalenide, $78\text{ }^{\circ}\text{C}$.

Scheme 14 Synthesis of the incorrect atropisomer of *Z*-3,4-verticillol **90**.

Scheme 15. Relationships between cembrenes, phomactanes, taxanes, verticillenes and sobralene derived from GGPP.

More recently, during their synthetic efforts directed towards hypostoxide **53** Njardarson *et al.*,⁵⁵ succeeded in preparing the *Z*-4,5-isomer of verticillol **1**. Their synthesis started with

the *tert*-butoxycyclohex-2-enone **65a** which was first converted into the substituted cyclohexanone **87** in five straightforward steps (Scheme 14) *via* **86**, using essentially the

same strategy used earlier to convert **65a** into the cyclohexanone **69** in Scheme 10. Treatment of **87** with methylmagnesium bromide next led to the corresponding 1,3-diol which was protected as the cyclic carbonate **88**. Ring-closure metathesis of **88** using Grubb's II catalyst in toluene under reflux, followed by carbonate deprotection resulted in the formation of the verticillane diol **89** in 40% yield, but unfortunately it had the undesired Z-3,4- stereochemistry and was also the incorrect atropisomer. Mesylation of the secondary hydroxyl group in **89** followed by reduction of the resulting mesylate using sodium naphthalenide then gave the atropisomer **90** of the Z-4,5-isomer of verticillol (cf. natural verticillol **1**).

9. Concluding remarks

We now have a clear insight into the origins and biosynthetic interrelationships within the taxane and phomactin families of natural products. This insight has emerged through concise studies of taxadiene synthase(TXS)-catalysed cyclisations with deuterium and fluorine-labelled GGPP and its analogues, alongside feeding experiments with ¹³C-labelled acetates in fermentations of *Phoma* sp, and detailed biomimetic interconversions with key verticillyl carbocation intermediates. These relationships, (summarised in Scheme 15), have been significantly reinforced following recent quantum chemical calculations and targeted engineering of the TXS enzyme.

10. Conflicts of interest

"There are no conflicts to declare".

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