Stereochemical Course of Ring Formation in Fumitremorgin B and Verruculogen, Metabolites of *Penicillium verruculosum*: Investigation into the Loss of Stereochemical Integrity of the Geminal Methyl Groups

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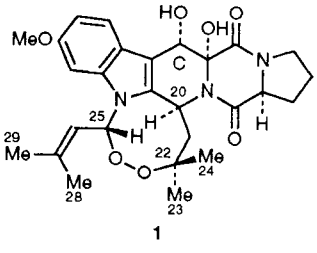
Incorporation studies of [2-$^{2}H_3$,2-^{13}C]acetate and different (^{2}H,^{13}C)-labelled mevalonolactones into verruculogen established the stereochemical course of ring C formation in fumitremorgin B, which results in the loss of stereochemical integrity of the C-22 methyl groups, and of the formation of the eight-membered peroxide ring at C-25 in verruculogen.

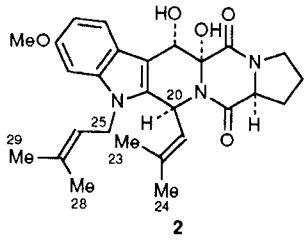
An earlier incorporation study in which (3*RS*)-[2-^{13}C]mevalonolactone was administered to cultures of *Penicillium verruculosum* has established that although C-23 and C-29 in verruculogen **1** are labelled to the same extent, a lower, but significant enrichment is also observed for C-24.[1] A similar phenomenon was observed for fumitremorgin B **2** obtained from the same feeding experiment. As expected (3*RS*)-[2-^{13}C]mevalonolactone labelled the 27-*pro*-*E* methyl group, C-29, of fumitremorgin B but it is C-23, the 22-pro-*Z* methyl group of the 2,2-dimethylvinyl moiety, which is enriched. In addition an enrichment is also observed for the 22-pro-*E* methyl group, C-24.[1] This finding is confirmed in the present study by the one-bond (^{13}C–^{13}C) couplings observed in the ^{13}C{^{1}H} NMR spectra of verruculogen **1** and fumitremorgin B **2** derived from (3*RS*)-[2,3-$^{13}C_2$]mevalonolactone: the enhancement of the C-24 signal was *ca.* 25% of that of the C-23 and C-29 signals. It is evident that the stereochemical integrity of the C-22 diastereotopic methyl groups in verruculogen **1** is lost during the formation of fumitremorgin B **2**. The focus of interest in this paper is the stereochemical course of this process.

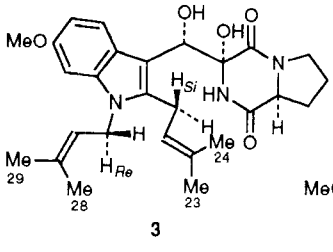
The most likely explanation of this result is that an intermediate, which allows rotation around a C(21)–C(22) single bond, is involved in the formation of ring C in fumitremorgin B. In order to test this hypothesis the fate of the hydrogen atoms in the biosynthesis of verruculogen was studied using ^{2}H in association with ^{13}C as a reporter nucleus and either the α-[2,3] or β-isotope shifts[3,4] in the ^{13}C NMR spectra. Verruculogen was used in these studies as the stereochemical course of the change in hybridisation of the C-22 centre in going from fumitremorgin B to verruculogen is known.[1]

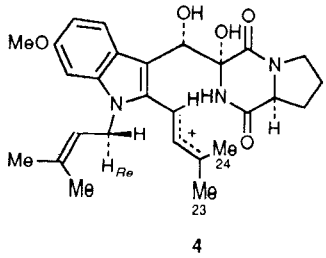
The incorporation of different (^{2}H,^{13}C)-labelled mevalonolactones† into verruculogen established the origin and fate of the C-24 hydrogen atoms. The β-isotope shifts of −0.068 and −0.132 ppm observed for C-27 and C-22, respectively, in the ^{13}C{^{1}H} NMR spectrum of verruculogen derived from (3*RS*)-[6-$^{2}H_3$,3-^{13}C]mevalonolactone (>98 atom% ^{2}H, 99 atom % ^{13}C) indicate that three deuterium atoms are retained at both C-28 and C-24 in verruculogen. The presence of three deuterium atoms at C-28 is based on the established mechanism for the transformation of mevalonolactone to 3,3-dimethylallyl pyrophosphate as well as the β-shift value observed for a similar system in viridicatumtoxin.[5] The single β-shifted ^{13}C signal for C-22 excludes a mechanism involving a C-24 sp^{2}-hybridised intermediate. This finding was corroborated by the observed α-isotope shifts in the ^{13}C{^{2}H,^{1}H} NMR spectrum of verruculogen on incorporation of (3*RS*)-[6-$^{2}H_3$,6-^{13}C]mevalonolactone (99 atom% ^{13}C) containing ^{13}C,$^{2}H_3$-(55 mol%) and ^{13}C,$^{2}H_2$-labelled (45 mol%) species at C-6. The relative intensity of the two α-isotopically shifted signals observed for the C-24 resonance and the magnitude of the α-isotope shifts (Δδ −0.55 and −0.83 ppm) in the major isotopomer were essentially the same as those for the C-28 resonance (Δδ −0.53 and −0.79 ppm), which served as an internal reference as no deuterium loss occurs from this position during the biosynthesis. Similar α-isotopically shifted signals, but with greatly reduced intensity, were evident for the C-23 resonance and suggest that a C-23 sp^{2}-hybridised intermediate can be excluded. This was confirmed by the α-isotope shifts observed in the ^{13}C{^{2}H,^{1}H} NMR spectrum of verruculogen derived from sodium [2-$^{2}H_3$,2-^{13}C]acetate. The observed α-isotopically shifted signals (−0.27 ppm per ^{2}H atom) confirm the expected presence of three and two deuterium atoms at C-28 and C-29, respectively. The intensity and number of the α-shifted signals observed for C-23 (two ^{2}H atoms) and C-24 (three ^{2}H atoms), on comparison with the C-28 and C-29 signals, confirms that none of the C-23 or C-24 hydrogen atoms is lost in the process which results in the loss of the stereochemical integrity of the C-22 methyl groups.

A possible mechanism for this process, which must occur during the formation of ring C in fumitremorgin B, is as follows. The introduction of the 3,3-dimethylallyl moiety at N-1 and C-2 of the indole nucleus in the putative precursor **3** occurs with inversion of configuration. The loss of one of the C-20 diastereotopic methylene protons generates an allylic carbocation **4** which allows rotation around the C(20)–C(21) bond and concomitant loss of the stereochemical integrity of the C-22 methyl groups. Attack of N-19 on the 20*Si* face of the allylic carbocation generates the correct stereochemistry at C-20 in fumitremorgin B. The process could proceed by either an ionic or radical mechanism.









† The details on the synthesis of the (^{2}H, ^{13}C)-labelled mevalonolactones used in this study will be reported in full elsewhere.

Earlier studies on the incorporation of (3*RS*)-[5-$^2H_2$]-mevalonolactone into verruculogen established that a single deuterium atom is retained at both C-20 and C-25.[1] The question as to which of the two diastereotopic C-5 protons of mevalonate is retained in each case, is answered in the present study by incorporation of stereospecifically labelled (3*RS*,5*S*)- and (3*RS*,5*R*)-[5-^{2}H,4-^{13}C]mevalonolactone into verruculogen. The retention of deuterium at C-20 in verruculogen derived from the 5*S* stereoisomer was evident from the β-isotope shift of −0.094 ppm observed for the C-21 resonance in the $^{13}C\{^1H\}$ NMR spectrum. No β-shifted signal was observed for the C-26 resonance. This pattern was reversed in the spectrum of verruculogen derived from the 5*R* stereoisomer: a β-shifted signal (Δδ −0.077 ppm) was observed for the C-26 resonance but none for the C-21 resonance. The results establish that the 5*Si* proton of mevalonate is retained at C-20 of verruculogen whereas the 5*Re* proton is retained at C-25.

The authors thank Professor B. Frank, Westfalischen Wilhems-Universität, Münster, for a culture of *P. verruculosum*, the Los Alamos Stable Isotope Resource (NIH Grant P41 RR 02231) for ^{13}C-labelled material, Professor J. C. Vederas, University of Alberta, Edmonton for recording the $^{13}C\{^2H,^1H\}$ NMR spectra, and the Foundation for Research and Development, Pretoria, for financial support (to R. V.)

Received, 4th September 1992; Com. 2/04786A

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A Novel Stereoselective Synthesis of the Macrocycle of Haem d_1 that establishes its Absolute Configuration as 2*R*,7*R*

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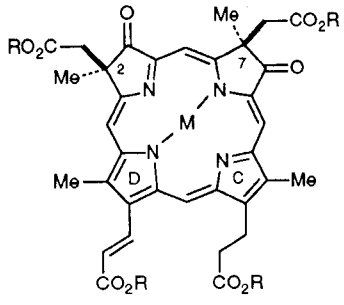
A novel route to isobacteriochlorins is developed that allows the stereoselective synthesis of the macrocycle of haem d_1 and so establishes its absolute configuration 2*R*,7*R*.

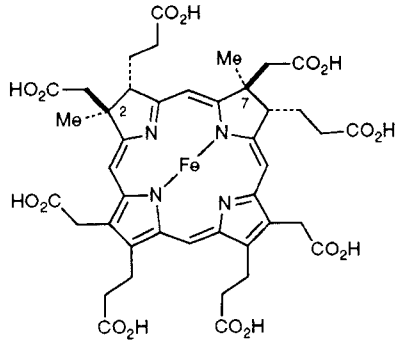
Haem d_1 is the iron-containing prosthetic group of bacterial reductase–cytochrome oxidase enzymes which carry out the reduction of nitrite. It was isolated by Timkovich *et al.*[1] and Chang[2] suggested that the ligand holding the iron is a dioxoisobacteriochlorin, see **2**. That the ester of the ligand has the gross structure **2**, without definition of the stereochemistry, was established by Wu and Chang's synthesis[3] of a mixture of racemic diastereoisomers corresponding to **2**, one of which was identical, apart from being a racemate, with the esterified metal-free ligand from haem d_1. Then Montforts *et al.*[4] showed that the *C*-methyl groups at C-2 and C-7 are *syn*-oriented by a partial synthesis yielding all the diastereoisomers of a related macrocycle (as **25**) followed by X-ray analysis of the racemic diastereoisomer known[3] to correspond to haem d_1. Thus haem d_1 has the absolute configuration **1** or it is the corresponding enantiomer.

It is important to determine which is the true configuration in order to know whether haem d_1 is related stereochemically, and so probably biosynthetically also, to sirohaem (the cofactor for sulfite reductases) and F-430 (the cofactor for methane production). Sirohaem,[5] which is related stereochemically to F-430,[6] has the absolute configuration **3**.

Our plan was to achieve a stereochemically controlled synthesis of the macrocycle of haem d_1, as its ester **2**, by the photochemical approach developed in Cambridge.[7,8] The initial target was the isobacteriochlorin **24**, which requires the synthesis of **23** from the lactams **15** and **19**, Scheme 2. Previously,[7,8] the best way to build **15** and **19** was *via* the nitriles **16** and **20** with subsequent removal of the cyano group by difficult chemistry. The present synthesis eliminates this



1; R = H, M = Fe^{II}
2; R = Me, M = H,H



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