Stereochemical Studies on the Biosynthesis of Viridicatumtoxin: Evidence for a 1,3-Hydride Shift in the Formation of the Spirobicyclic Ring System

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A study of the fate of the hydrogen atoms in the biosynthesis of viridicatumtoxin using (^{13}C,^{2}H)-labelled mevalonolactones established that a 1,3-hydride shift occurs in the formation of the spirobicyclic ring system.

Stable isotope labelling studies on the biosynthesis of viridicatumtoxin (**1**),[1] a mycotoxin isolated from cultures of *Penicillium expansum* (MRC 97),[2] have indicated a mode of folding of the polyketide progenitor which differs from the one deduced for the tetracyclines.[3] Although the spirobicyclic ring system of viridicatumtoxin is presumably derived from geranyl pyrophosphate the stereochemical course of the cyclisation reaction has remained unknown. We now report results on the incorporation of (^{13}C,^{2}H)-labelled mevalonates, which, in conjunction with the known absolute configuration of viridicatumtoxin,[4] allow us to propose a detailed mechanism for the formation of the spirobicyclic ring system.

The relevant ^{1}H and ^{13}C n.m.r. data for viridicatumtoxin (**1**) are in Table 1. The complete assignments will be described elsewhere. The signals of the proton-bearing carbon atoms were correlated with specific proton resonances in a two-dimensional (2-D) (^{13}C,^{1}H) chemical shift correlation experiment.[5,6] The assignment of the signals at δ_H 0.888 and 0.444 (and thus δ_C 23.97 and 25.48) to the prochiral diastereotopic methyl groups C-21 and C-22, respectively, is based on the

Table 1. N.m.r. data (^{1}H and ^{13}C) for viridicatumtoxin (**1**).[a]

Atom	δ_C	$^{1}J_{CC}$/Hz[b]		δ_H	J_{HH}/Hz
14	41.17 T	33.8	*Re*:	2.904 d	17.3
			Si:	3.455 d	17.3
15	60.15 S	33.7		—	
16	136.58 S	45.4		—	
17	121.40 D	—[c]		5.474 m	5.0, 2.5, 1.0, <0.5
18	22.87 T	33.6	*Re*:	2.179 m	18.0, 11.5, 6.5, 2.5, 2.0
			Si:	1.995 m	18.0, 6.5, 5.0, 2.0, 2.0
19	33.91 T	33.6	*Re*:	1.802 m	13.0, 11.5, 6.5
			Si:	1.312 m	13.0, 6.5, 2.0, <0.5
20	38.54 S	36.0		—	
21	23.97 Q	35.9		0.888 s	
22	25.48 Q	—[c]		0.444 s	
23	20.96 Q	45.0		1.497 m	2.0, 2.0, 1.0

[a] Recorded with a Bruker WM-500 spectrometer; solvent $CDCl_3$. [b] Values obtained from the proton-decoupled ^{13}C n.m.r. spectrum of viridicatumtoxin derived from [1,2-$^{13}C_2$]acetate. [c] Derived from (3*RS*)-[2-^{13}C]mevalonolactone.

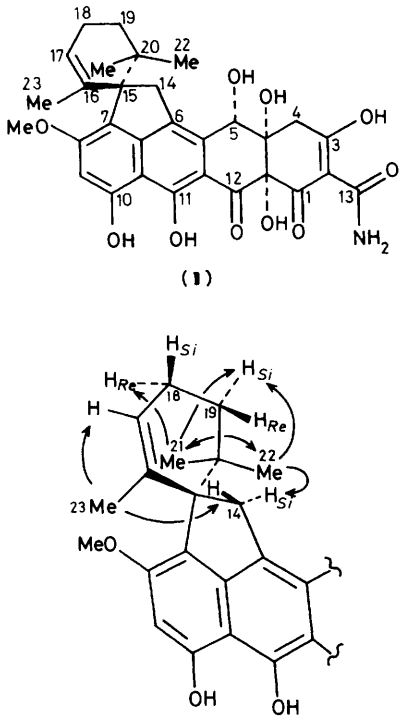


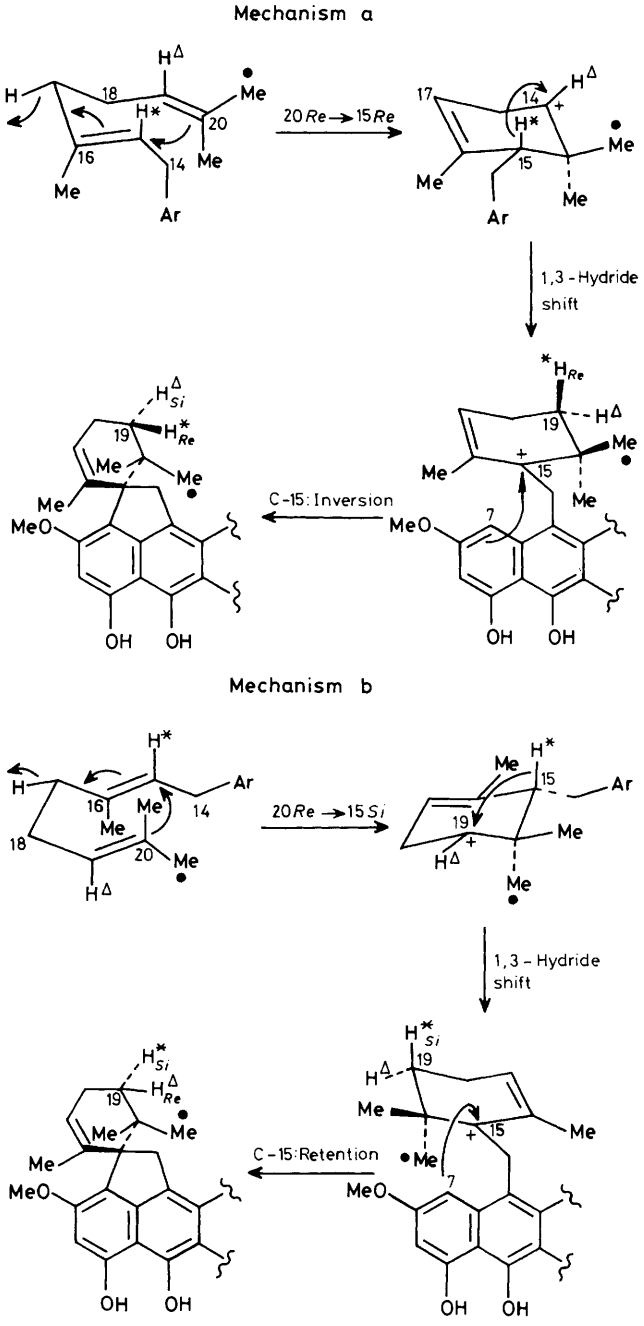
Figure 1. N.O.e. connectivity pattern observed for the spirobicyclic ring system of viridicatumtoxin (**1**).

nuclear Overhauser effects (n.O.e.s) observed in a number of (^{1}H,^{1}H) n.O.e. experiments (see Figure 1) using the C-23 protons as reference point. These results in conjunction with the magnitude of the vicinal (^{1}H,^{1}H) coupling constants also allowed us to assign the signals of the prochiral hydrogen atoms of the C-18 and C-19 methylene protons.

The arrangement of intact acetate units in viridicatumtoxin derived from [1,2-$^{13}C_2$] acetate is based on the measured one-bond (^{13}C,^{13}C) coupling constants. The results show that the 20*Si* methyl group, C-21 forms part of an intact acetate unit and the 20*Re* methyl group is therefore derived from C-2 of mevalonate. This was confirmed by the enhancement of the C-22 signal (enrichment factor 3.3) in the proton-decoupled ^{13}C n.m.r. spectrum of viridicatumtoxin derived from (3*RS*)-[2-^{13}C]mevalonolactone.

The fate of the hydrogen atoms in the biosynthesis of viridicatumtoxin was studied by incorporation of different (^{13}C,^{2}H)-labelled mevalonates and acetate, in which the ^{2}H atoms are two bonds removed from the ^{13}C reporter nucleus. The retention of ^{2}H in such cases can be detected by the characteristic β-shifts in the resonance position of the ^{13}C nucleus.[7,8] The β-isotope shifts of −0.155 and −0.185 p.p.m. observed for the C-15 and C-19 resonances, respectively, indicate that two ^{2}H atoms are retained at both C-14 and C-18 in viridicatumtoxin derived from (3*RS*)-[4-^{13}C,5-$^2H_2$]mevalonolactone.† A mechanism for the formation of the spirobicyclic ring system involving a C-14 sp^2-hybridised intermediate can thus be ruled out. An alternative intermediate involving a C-23 sp^2 centre was excluded by the β-isotope shift of −0.083 p.p.m. observed for C-16 on incorporation of (3*RS*)-[3-^{13}C,6-$^2H_3$]mevalonolactone (99 atom% ^{13}C, >98 atom% ^{2}H), which

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



Scheme 1. Stereochemical course for the formation of the spirobicyclic ring system of viridicatumtoxin; ● ≡ derived from (3*RS*)-[2-^{13}C] mevalonolactone.

pointed to the presence of three ^{2}H atoms at C-23.‡ A similar β-isotope shift was observed for C-16 using [1-^{13}C,2-$^2H_3$]acetate as precursor. In addition, the β-isotope shift of −0.104 p.p.m. for the C-18 resonance located a single ^{2}H atom at C-19. The stereospecificity of ^{2}H labelling at C-19 could not be determined by ^{2}H n.m.r. spectroscopy as the relevant resonances are obscured by the broad C-23 resonance. This

‡ The ^{13}C-{^{1}H} n.m.r. spectrum of viridicatumtoxin derived from a sample of (3*RS*)-[3-^{13}C,6-$^2H_{0-3}$]mevalonolactone (99 atom% ^{13}C) containing CD_3- (39.9 mole%), CD_2H- (33.3 mole%), CDH_2- (14.5 mole%), and CH_3-labelled (12.3 mole%) species at C-3, exhibited three isotopically shifted signals for C-16 with the sum of the β-isotope shifts equal to −0.080 p.p.m.

problem was circumvented by the use of (3*RS*)-[5-^{13}C,4-$^{2}H_2$]mevalonolactone as a precursor. The proton-decoupled ^{13}C n.m.r. spectrum of the enriched viridicatumtoxin shows, as expected, a single enhanced signal (enrichment factor 5.2) for C-14. In addition to the natural abundance signal for C-18, two isotopically shifted signals [δ −0.105 p.p.m., enrichment factor 4.1; and $\Delta\delta$ −0.200 p.p.m., enrichment factor 1.1] are also observed and represent species with either one or two ^{2}H atoms at C-19. The ^{2}H n.m.r. spectrum on the other hand provided information on the disposition only of the ^{2}H atoms at C-19 and showed that the 19*Re* (δ_D 1.80 p.p.m.) and 19*Si* (δ_D 1.32 p.p.m.) positions were labelled to the same extent.

The C-2 and C-6 protons of geranyl pyrophosphate are both derived from the 4*Re* position of mevalonate. The presence of two ^{2}H atoms at C-19 of viridicatumtoxin can only be explained by a mechanism involving an intramolecular migration of the original C-2 hydrogen atom of geranyl pyrophosphate to the carbon atom derived from C-6.

The foregoing results afford considerable insight into the stereochemical course of the formation of the spirobicyclic ring system of viridicatumtoxin. The known absolute configuration of viridicatumtoxin[4] and the knowledge on the origin of the carbon and hydrogen atoms requires that formation of the C-20–C-15 bond proceeds by 20*Re*-face attack on the 15*Re*-face as shown in Scheme 1 (mechanism a). It is not yet known which of the two diastereotopic prochiral hydrogen atoms at C-17 is lost in this process. The subsequent suprafacial 1,3-hydride shift of the C-15 proton to the C-19 cationic centre results in the formation of an allylic cation which is then involved in the formation of the C-7–C-15 bond, a process which occurs with overall inversion of configuration at C-15.

The results do not, however, exclude an alternative mechanism (Scheme 1, mechanism b) also involving a suprafacial 1,3-hydride shift, but which requires that C-15–C-20 bond formation is initiated by 20*Re*-face attack on the 15*Si*-face and that the formation of the C-7–C-15 bond occurs with overall retention of configuration at C-15. The two mechanisms can be differentiated by incorporation of either [2-$^{2}H_1$]- or [6-$^{2}H_1$]geranyl pyrophosphate as the origin of the C-19 diastereotopic prochiral hydrogen atoms is dictated by a particular mechanism. Thus, for example, incorporation of [2-$^{2}H_1$]geranyl pyrophosphate according to mechanism a would label the 19*Re* position whereas mechanism b would locate the ^{2}H atom at the 19*Si* position. The synthesis of ^{2}H-labelled geranyl pyrophosphate and their incorporation into viridicatumtoxin is under investigation.

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