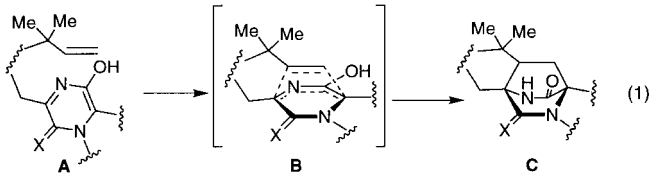
Reverse versus Normal Prenyl Transferases in Paraherquamide Biosynthesis Exhibit Distinct Facial Selectivities**

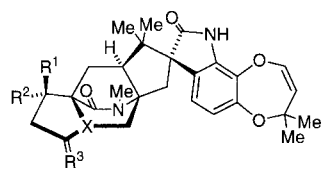
Emily M. Stocking, Juan F. Sanz-Cervera,* and Robert M. Williams*

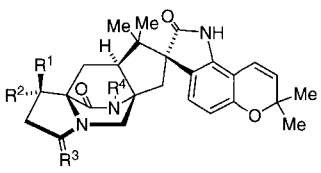
The paraherquamides (**1**–**10**)[1] are a group of fungal metabolites that, together with sclerotamide (**11**),[2] marcfortine (**12**),[3] asperparaline A (**14**, also aspergillimides, **15**),[4] and the brevianamides (**16**, brevianamide A; **17**, brevianamide B),[5] have recently attracted much attention due to the range of interesting biological activities that this family displays, including anthelmintic, paralytic, and insecticidal activities.[1–5] These substances are the consequence of mixed biogenetic origins, being derived from the oxidative polycyclization of amino acids and isoprene units. Most interesting in this regard is the emerging body of evidence that supports the notion that the common bicyclo[2.2.2] core structural motif is formed by a biosynthetic intramolecular [4+2] cycloaddition of the isoprene-derived olefin across an azadiene moiety derived from a preformed, oxidized piperazinedione [**A** →**B** →**C,** Eq. (1)].[6, 7]

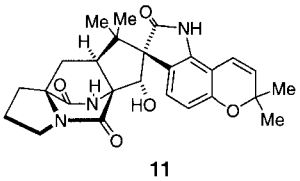


(1)

Although [4+2] cycloadditions are used extensively in synthetic organic chemistry, such pericyclic reactions are quite rare in Nature, and in only a few cases has experimental evidence been obtained to support the intermediacy of a Diels–Alder type of cycloaddition.[7, 8] Our research efforts have thus focused on the biogenesis of these compounds, with particular emphasis on the key cycloaddition step.







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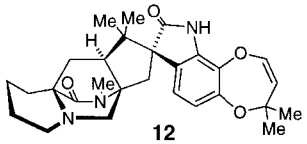
1 A: R^1 = OH, R^2 = Me, R^3 = H_2, X = N
2 B: R^1 = H, R^2 = H, R^3 = H_2, X = N
3 C: R^1 = R^2 = CH_2, R^3 = H_2, X = N
4 D: R^1 = O, R^2 = CH_2, R^3 = H_2, X = N
5 E: R^1 = H, R^2 = Me, R^3 = H_2, X = N
6 VM55596: R^1 = OH, R^2 = Me, R^3 = H_2, X = N^+-O^-
7 VM55597: R^1 = OH, R^2 = Me, R^3 = O, X = N

8 F: R^1 = H, R^2 = Me, R^3 = R^4 = Me
9 G: R^1 = OH, R^2 = Me, R^3 = R^4 = Me
10 VM55595: R^1 = H, R^2 = Me, R^3 = H_2, R^4 = H



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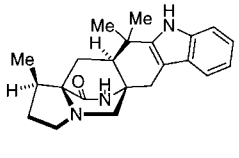
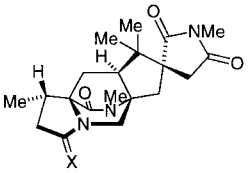
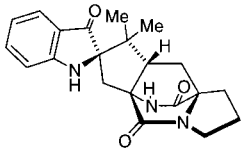


13 VM55599



14 (X = H_2)
15 (X = O)



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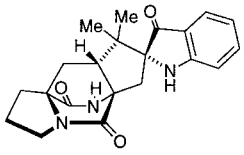

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[**] Financial support from the National Institutes of Health (to R.M.W., Grant CA 70375, Shared Instrumentation Grant GM49631 for mass spectrometer) and the Direccion General de Investigacion Cientifica y Tecnica of Spain (to J.F.S.C., Proyecto PB95-1089) is gratefully acknowledged. NMR spectra were obtained on a Bruker AMX 500 MHz spectrometer at the Los Alamos Stable Isotope Resource courtesy of Dr. Clifford J. Unkefer. We also would like to thank Professor Rodney Croteau and Professor J. Alberto Marco for helpful discussions.

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In this family of metabolites, the dimethylallyl group involved in forming the bicyclo[2.2.2] nucleus is apparently introduced by reaction of dimethylallyl pyrophosphate (DMAPP) with the tryptophan unit, resulting in a net *reverse prenylation* at the 2-position of the indole ring. In metabolites **1**–**12**, there is a second isoprene fragment that is oxidatively added to a phenolic hydroxy group of the tryptophan core. Until recently, it would have been safe to assume that the isoprene moieties are biosynthesized by the well-known mevalonic acid pathway. However, in the last few years several reports have clearly shown that in some organisms isoprenoids are not formed through this pathway, but rather through the 1-deoxy-D-xylulose pathway. This has been shown not only in bacteria,[9] but also in the green algae *Scenedesmus obliquus*[10] and, somewhat surprisingly, in higher plants like *Taxus chinensis*,[11] barley (*Hordeum vulgare* L.), duckweed (*Lemna giba* L.), carrot (*Daucus carota* L.),[12] and *Ginkgo biloba*.[13] In these cases, it has been shown that the biosynthesis of several cytoplasmic sterols proceeds by the acetate/mevalonate pathway, while the plastidic isoprenoids are synthesized by the new 1-deoxy-D-xylulose pathway.

In the course of isotopic labeling studies aimed at examining the origin of the isoprene units in the paraherquamide structure, we discovered an unexpected stereochemical distribution of the methyl groups derived from DMAPP. We

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carried out feeding experiments with [U-$^{13}C_6$]-glucose and [$^{13}C_2$]-acetate which aid in distinguishing between the two pathways using ^{13}C NMR spectroscopy since all the resonances from the ^{13}C NMR spectrum of paraherquamide A have been unequivocally assigned (with the exception of C27 and C28).[1d] The different labeling patterns to be expected from these two pathways are shown in Figure 1.

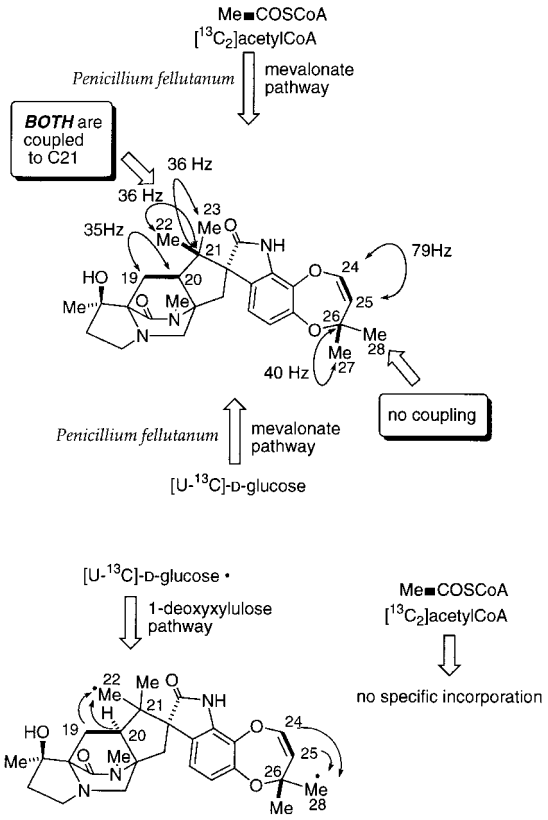

Figure 1. ^{13}C labeling in paraherquamide A corresponding to a feeding experiment with [U-$^{13}C_6$]-D-glucose and [$^{13}C_2$]-acetate by the mevalonate (top) and 1-deoxy-D-xylulose (bottom) pathways. Thick lines represent intact acetate units and arrows represent couplings expected and observed in the ^{13}C NMR spectrum.

An initial feeding experiment was carried out on *Penicillium fellutanum* with [U-$^{13}C_6$]-glucose; harvesting the resulting paraherquamide A provided a labeling pattern in which most signals appeared split around the singlet corresponding to the uncoupled carbon atom, as one would expect from the catabolism of glucose. In this case we focused our attention on the carbon atoms that form the two C_5 units: C19 to C23, and C24 to C28. The ^{13}C NMR spectrum of paraherquamide A[1d] isolated from a control experiment was used for comparison purposes. Although the specific incorporation was low, the couplings are clearly visible in the ^{13}C NMR spectrum of the resulting paraherquamide A (Table 1).

In order to confirm that acetate, and not 1-deoxy-D-xylulose-5-phosphate, is the key intermediate in the biogenesis of the two C_5 units, a second feeding experiment was carried out, this time using [$^{13}C_2$]-acetate. Specific incorporation of intact C_2 units was observed, in agreement with the mevalonic acid pathway (Figure 1). Regarding the carbon

Table 1. Specific incorporations, chemical shifts, and coupling constants for the C_5 carbon atoms of paraherquamide A in the feeding experiment with [U-$^{13}C_6$]-glucose.

C	δ	$J_{C,C}$ [Hz]	% ^{13}C in each position	% ^{13}C specifically incorporated as intact C_2 units in each positon	% ^{13}C in the [U-$^{13}C_6$] glucose used, specifically incorporated in each position
19	22.17	34	1.4	41	0.0075
20	51.42	34	1.9	32	0.0052
21	46.40	36	1.5	36	0.0061
22	20.47	36	1.8	14	0.0018
23	23.71	36	1.9	21	0.0029
24	138.94	81	2.3	35	0.0059
25	115.05	79	2.0	37	0.0065
26	79.81	40	2.0	37	0.0064
27	29.93	40	2.2	40	0.0075
28	29.80	–	1.7	0	0.0037

atoms that form the two C_5 units, C19 to C23, and C24 to C28, the results of the feeding experiment with [$^{13}C_2$]-acetate were essentially the same as those with [U-^{13}C]-glucose. In both cases, the signal for C-28 at $\delta = 29.80$ showed enhancement with respect to the control spectrum, but no splitting. In the first C_5 fragment, the observed couplings mean that C19 is coupled to C20, while C21 is coupled to C22 or C23, but not to both simultaneously. For the second C_5 unit, the coupling constants show that C24 and C25 are coupled, while C26 is coupled to C27. In this case, C28 shows no coupling.

The non-mevalonic pathway would cause incorporation of [U-$^{13}C_6$]-glucose in such a way that a long-range coupling would be observed between C19 and one of the methyl groups (C22 or C23) as well as C20 and the same methyl group (C22 or C23), due to the fact that in this case three carbon atoms would come from one glucose molecule, while the other two would come from a different one. However, in our feeding experiment with [U-$^{13}C_6$]-glucose no long-range coupling between C22 and C19/C20 or C28 and C24/C25 was observed. This result is consistent with the mevalonic acid pathway, in which two pairs of carbon atoms come from two glucose molecules, while the third carbon atom comes from a third glucose molecule, thus precluding long-range couplings. The lack of observed long-range couplings, together with the observed incorporation of [1,2-$^{13}C_2$]-acetate thus indicate that the operative pathway for the formation of both C_5 units is the mevalonic pathway, as the labeling pattern observed is consistent with that expected for such a mechanism (Figure 1). The electrospray ionization mass spectra of the isolated **1** from both feeding experiments agree with the incorporation of intact C_2 units from acetate.[14]

It is significant that in the C_5 fragment formed by C24 to C28, C28 shows no coupling with C26, while C27 does. This means that the methyl groups in DMAPP are not equivalent in the biosynthesis of this metabolite. In contrast, in the other C_5 fragment, formed by C19 to C23, both methyl groups show coupling with C20, although not simultaneously. This unexpected result must be interpreted to mean that the *reverse* prenyl transferase presents the olefinic π system of DMAPP in a manner in which both faces of the π system are susceptible to attack by the 2-position of the indole moiety.

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The simplest explanation is to invoke binding of the DMAPP in an "upside down" orientation relative to "normal" prenyl transferases which permits a facially nonselective S_N' attack on the π system (Figure 2). In this situation, the pyrophosphate group is likely anchored in the enzyme active site with the hydrophobic isopropenyl moiety being presented in a conformationally flexible (**A** ⇌ **B**) disposition with respect to the tryptophan-derived substrate. This is in contrast to the normal mode of prenyl transfer where, the nucleophilic displacement at the pyrophosphate-bearing methylene carbon atom occurs with inversion of configuration at carbon with the hydrophobic tail of DMAPP buried in the enzyme active site.[15]

In contrast, the methyl groups in the other C_5 unit (dioxepin moiety) are clearly differentiated; therefore, it is quite likely that this C_5 group (C24 to C28) is introduced in the molecule by direct alkylation with DMAPP by a normal prenyl transferase followed by a stereospecific net oxidative addition to the olefinic system. A plausible mechanism, of several possibilities, for the formation of this ring system, is by face-selective epoxidation followed by ring-opening and dehydration. Significantly, since this isoprene unit is introduced without loss of stereochemical integrity, Ockham's razor can be invoked for there not being a mechanism for scrambling the DMAPP via a dimethyl vinyl carbinol-type intermediate which would necessarily provide stereochemically scrambled isoprene equivalents to the cell's cytosolic pool of DMAPP.

The results of these experiments clearly indicate that the C_5 units in **1** are introduced in stereofacially distinct manners. Since it has been established that prenylation of the indole moiety in the biosynthesis of the related brevianamides occurs in a fashion analogous to that postulated for intermediates **19**[7d] (Figure 2) and that this is also a reasonable expectation in the paraherquamide biosynthesis, the prenyl transferase that installs this C_5 unit must display DMAPP to the 2-position of the indole in a π facially indiscriminate manner. The work described herein demonstrates the first case where *both* a non face-selective *and* a face-selective addition to the trisubstituted olefinic portion of a DMAPP-derived moiety has occurred within the same molecule.[16]

Received: September 7, 1998 [Z12390IE]
German version: *Angew. Chem.* **1999**, *111*, 880 – 883

Keywords: biosynthesis · isotopic labeling · paraherquamide

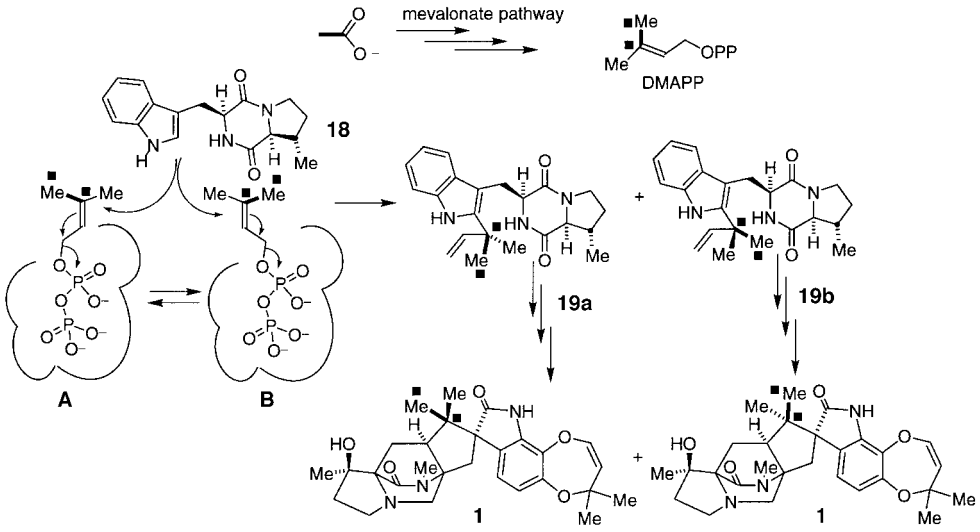

Figure 2. A possible biosynthetic sequence that may explain why C22 and C23 are rendered equivalent in the biosynthesis of paraherquamide A. Thick bonds labeled with ■ represent one intact C2 unit from acetate, incorporated in C3/C5 of individual DMAPP molecules, and in C21, C22, and C23 of **1**. For the sake of clarity and simplicity, the labels that would appear in other positions are not represented (see Figure 1).

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[14] For the control paraherquamide A ($M_W = 493$), the isotopic peaks for the protonated molecular ion appear at *m*/*z* (%): 494.30 (100), 495.30 (32.65), 496.30 (6.49), and 497.27 (1.13). In contrast, for **1** isolated from the [U-$^{13}C_6$]-glucose feeding experiment, the molecular ion peaks appear at: *m*/*z* (%): 494.27 (100), 495.27 (45.27), 496.27 (18.04), 497.27 (6.87), 498.26 (2.94), 499.25 (1.45), and 500.22 (0.79). For **1** isolated from the [$^{13}C_2$]-acetate feeding experiment, the molecular ion peaks appear at: *m*/*z* (%): 494.25 (100), 495.24 (47.42), 496.24 (17.76), 497.23 (6.66), and 498.19 (3.38). The increased intensities of the protonated $M+2$ (*m*/*z* 496) and $M+4$ (*m*/*z* 498) peaks agree with the incorporation of the acetate units.
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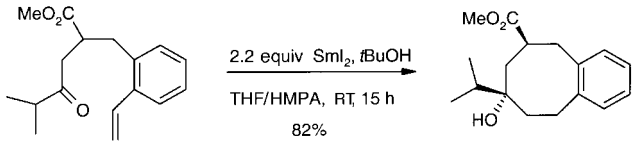
A New Samarium Diiodide Induced Reaction: Intramolecular Attack of Ketyl Radical Anions on Aryl Substituents with Formation of 1,4-Cyclohexadiene Derivatives**

Chimmanamada U. Dinesh and Hans-Ulrich Reissig*

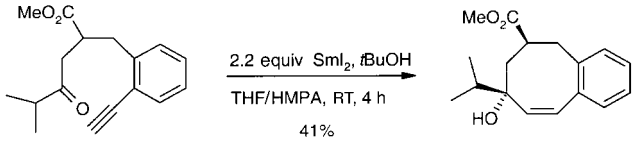
Dedicated to Professor Edward Piers on the occasion of his 60th birthday

Samarium diiodide was introduced by Kagan et al.[1] as a selective one electron transfer reagent in organic chemistry. Several variants of SmI_2-induced cyclization reactions[2] have attained synthetic importance owing to their high stereoselectivity and ability to undergo sequential reactions.[3] Frequently, ketyl radical anions generated by the electron transfer are the reactive species which add to a multiple bond offered at an appropriate distance. We now report that these ketyl radical anions can attack an aryl moiety in an intramolecular fashion and, after a second electron transfer, lead to 1,4-cyclohexadiene derivatives. This reaction has not yet been observed in samarium chemistry to the best of our knowledge, and it should gain considerable synthetic importance owing to its high diastereoselectivity.

Motivated by the examples reported by Molander and McKie,[4] we recently developed a synthesis of benzannulated cyclooctane derivatives.[5] Our previously unpublished example **1**→**2** (HMPA = hexamethyl phosphoramide) demonstrates that this samarium diiodide promoted transformation proceeds with high diastereoselectivity and in very good yield in spite of the sterically demanding isopropyl group. To explore further mechanistic details of the 8-*endo* cyclization and to obtain new options for functionalizations of the newly generated eight-membered ring, alkyne derivatives such as **3** were subjected to the general reaction conditions. The expected benzannulated cyclooctene **4** was isolated in 41% yield as a single diastereomer. To our knowledge this is the first successful 8-*endo-dig* cyclization of a samarium ketyl.[6]



1 **2**



3 **4**

Since they were more readily accessible,[7] the disubstituted alkynes **5**–**7** were first subjected to samarium diiodide cyclization conditions. However, we obtained products derived from neither an 8-*endo-dig* cyclization nor from the more likely 7-*exo-dig*-reaction, but surprisingly cyclohexadienes **11**–**13** which were formed by attack on aryl substituents. Examples **8** and **9** demonstrate that the alkyne units are not required for the cyclization, as samarium diiodide now affords cyclization products **14** and **15** or the lactone **16**. The cyclohexanone moiety in **6**–**8** allows smooth cyclization;[8] however, the steric hindrance in isopropyl derivative **10** seems to be too high. Under the conditions applied mainly starting material was recovered and no product was formed. The fact that **1** and **3** were transformed into bicyclic **2** and **4**, and that the precursor **10** was essentially inert, supports the reversibility of the first electron transfer to the carbonyl group. The subsequent steps are responsible for the productivity of the sequence.

Particularly remarkable is the high diastereoselectivity of the reaction, since in all examples only one diastereomer could be detected. The relative configuration at the two newly generated asymmetric centers of **14** was proven by X-ray analysis.[9] This showed not only the *cis* arrangement of the bridgehead hydrogen atom with respect to the hydroxyl group, but also the location of R^2 and of the methoxycarbonyl

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[**] We are most grateful to the Alexander-von-Humboldt-Stiftung for a research fellowship (C.U.D.). The financial assistance from the Volkswagen-Stiftung and the Fonds der Chemischen Industrie is gratefully acknowledged. We also thank Dr. R. Zimmer for useful discussions and R. Czerwonka for experimental assistance.

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