First Direct Evidence in Biological Diels–Alder Reaction of Incorporation of Diene–Dienophile Precursors in the Biosynthesis of Solanapyrones

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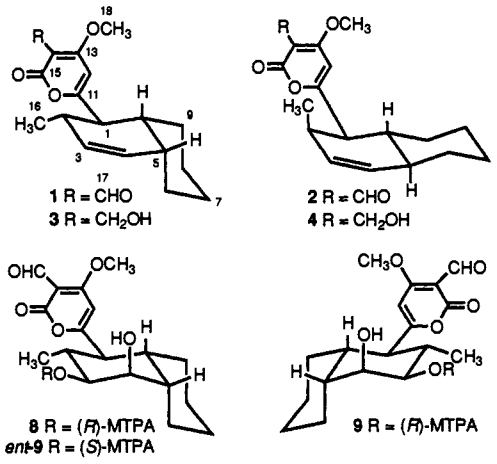
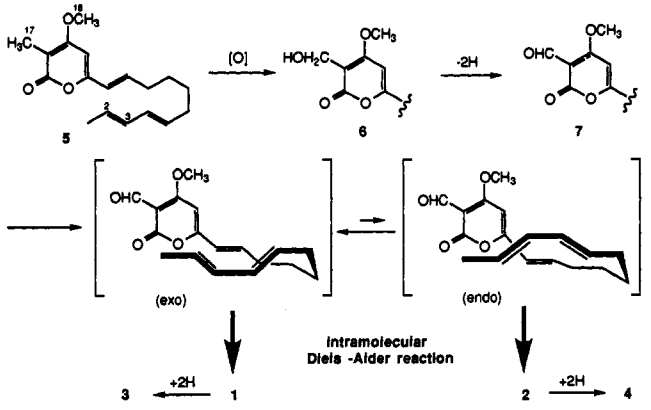
Among natural products, there are a number of metabolites whose parent skeletons are plausibly constructed by biosynthetic versions of pericyclic reactions.[1] In particular, the possible involvement of a biosynthetic Diels–Alder reaction has appeared frequently in the literature.[2] Although there is one reported example of the incorporation of an apparent dienophile precursor into a naturally occurring [4 + 2]-adduct,[3] details of the formation of the presumed diene partner are still unavailable. We report here the first example of the incorporation of precursors containing both diene and dienophile moieties into a group of fungal metabolites, the solanopyrones, thereby providing strong experimental support for the operation of a biosynthetic Diels–Alder reaction.

Previously, we reported the biosynthetic origins of solanapyrone A (**1**),[4] a phytotoxin produced by *Alternaria solani*, the causal fungus of potato early blight disease. At the same time, we found



that this fungus produced a number of probable Diels–Alder adducts, including the minor metabolites solanapyrone D (**2**)[4c] and E (**4**)[5] along with the major metabolites solanapyrones A (**1**) and B (**3**).[4a] Since the presumptive precursor prosolanapyrone III (**7**) would have no chiral center, we proposed the involvement of an enzymatic intramolecular Diels–Alder reaction to account for construction of the characteristic decalin ring of the solanapyrones.

Scheme 1



Incorporation of the acyclic trienes prosolanapyrones I (**5**) and II (**6**) was examined in order to test the formation of the decalin ring system via a [4 + 2]-cycloaddition route (Scheme 1). For this purpose, we prepared multiply labeled prosolanapyrones: [17-$^2H_2$,18-$^2H_3$]prosolanapyrone I (**5**) and [2-^{2}H,3-^{2}H,17-^{2}H,18-$^2H_3$]prosolanapyrone II (**6**)[6] by routes similar to those reported previously[4b] (scheme in supplementary material). Feeding experiments performed with the deuterium-labeled **5** and **6** afforded **1** and **3**. Incorporation of **5** and **6** into **1** was 0.26 and 0.37%, respectively, as determined from the ^{2}H NMR spectra, relative to internal solvent $CHCl_3$ (natural abundance) as a standard. Solanapyrone A (**1**), derived from [$^2H_5$]-**5**, exhibited two signals in its ^{2}H NMR spectrum corresponding to deuterium at C-17 and C-18 (Figure 1c). The integration ratio (1:4.3) for D-17 and D-18 could be compared with the corresponding ratio of 2:3 in the precursor [$^2H_5$]-**5**. A similar ratio (1:5.1) was observed in the ^{2}H NMR spectrum of labeled **3**. These observations indicated that loss of two deuteriums at C-17 occurred at a late stage in the biosynthesis and therefore that **3** was formed by reduction of **1**. Furthermore, labeled **1** derived from deuterated **6** displayed resonances in its ^{2}H NMR spectrum corresponding to deuterium at C-2, C-3, C-17, and C-18 (Figure 1b) in a ratio of 0.7:1:0.4:3, essentially unchanged from that of the labeled precursor prosolanapyrone II (**6**). These results demonstrate intact incorporation of intermediates **5** and **6**.

In order to eliminate the possibility that prosolanapyrone II (**6**) might have cyclized nonenzymatically, the derived solanapyrone A (**1**) was converted to the corresponding diastereomers **8** and **9** in two steps (i, OsO_4, NMO; ii, (*R*)-MTPA, DMAP, DCC). Since the incorporation of **6** was low, we added unlabeled *ent*-**9**

(1) [6 + 4]-cycloaddition: Huff, R. K.; Moppett, C. E.; Sutherland, J. K. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2584–2590. [6 + 6]-cycloaddition: Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1993**, *32*, 1347–1349. [2 + 2]-cycloaddition: Walker, R. P.; Faulkner, D. H.; Engen, D. V.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 6772–6773. [4 + 4]-cycloaddition: Shigemori, H.; Bae, M.-A.; Yazawa, K.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 4317–4320. 6π and 8π electrocyclic reaction: Bandaranayake, W. M.; Banfield, J. E.; Black, D. S. C. *J. Chem. Soc., Chem. Commun.* **1980**, 902–903.

(2) Recent examples of [4 + 2]-cycloaddition: (a) Cane, D. E.; Tan, W.; Ott, W. R. *J. Am. Chem. Soc.* **1993**, *115*, 527–535. (b) Sanz-Cervera, J. F.; Glinka, T.; Williams, R. *J. Am. Chem. Soc.* **1993**, *115*, 347–348. (c) Oikawa, H.; Murakami, Y.; Ichihara, A. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2955–2959. (d) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett.* **1992**, *33*, 2059–2062. (e) Hano, Y.; Ayukawa, A.; Nomura, T.; Ueda, S. *Naturwissenschaften* **1992**, *79*, 180–182. Hano, Y.; Nomura, T.; Ueda, S. *J. Chem. Soc., Chem. Commun.* **1990**, 610–613. (f) Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1988**, 600–602. (g) Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3694–3701.

(3) In ref 2e, the authors studied the biosynthesis of intermolecular Diels–Alder adducts, kuwanons. They succeeded in detecting incorporation of dienophile analogues and cleverly explained formation of the diene by analysis of labeling patterns resulting from feeding of the [2-^{13}C]acetate. However, they have not reported any feeding experiments with intact diene.

(4) (a) Isolation and structure: Ichihara, A.; Tazaki, H.; Sakamura, S. *Tetrahedron Lett.* **1983**, *24*, 5373–5376. Ichihara, A.; Miki, M.; Sakamura, S. *Tetrahedron Lett.* **1985**, *26*, 2453–2454. Alam, S. S.; Bilton, J. M.; Slawin, M. Z.; Williams, D. J.; Sheppard, R. N.; Strange, R. M. *Phytochemistry* **1989**, *28*, 2627–2630. (b) Synthesis: Ichihara, A.; Miki, M.; Tazaki, H.; Sakamura, S. *Tetrahedron Lett.* **1987**, *28*, 1175–1178. (c) Biosynthesis: Oikawa, H.; Yokota, T.; Abe, T.; Ichihara, A.; Sakamura, S.; Yoshizawa, Y.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1989**, 1282–1284. Oikawa, H.; Yokota, T.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1989**, 1284–1285.

(5) This compound **4** was isolated from a culture of *A. solani*, $[\alpha]^{22.5}_D$ −76.4° (*c* 1.0, $CHCl_3$). The reduction product of solanapyrone D (**2**) was identical to **4** in all respects.

(6) Deuterium enrichments of **5** and **6** were determined by ^{1}H NMR and MS spectroscopy: **5** (^{2}H; 98% for C-17 and C-18), **6** (^{2}H; 96.7, 98, 99, and 99% for C-2, C-3, C-17, and C-18).

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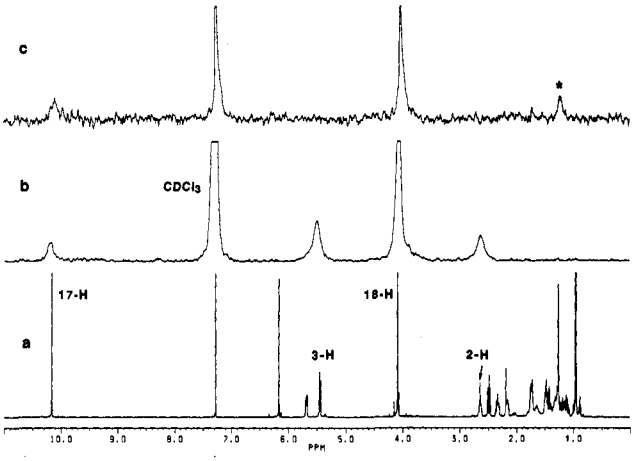

Figure 1. (a) 500-MHz ^1H NMR spectrum of solanapyrone A (**1**) in $CDCl_3$. (b) 76.8-MHz ^2H NMR spectrum of **1** derived from [2-^{2}H,3-^{2}H,17-^{2}H,18-$^2H_3$]prosolanapyrone II (**6**) in $CHCl_3$. (c) ^2H NMR spectrum of **1** derived from [17-$^2H_2$,18-$^2H_3$]prosolanapyrone I (**5**). The asterisk indicates the signal of *t*-BuOH.

to secure complete separation of diastereomers **8** and **9**. The samples obtained by careful chromatography were examined by ^2H NMR spectroscopy. Enriched ^2H NMR signals were found in compound **8** but were absent from **9**. These data show that the deuerated product **1** obtained from incorporation of **6** is not racemic and that **1** is formed by an enzyme-catalyzed Diels–Alder reaction. Direct incorporation of **7**, unfortunately, could not be directly demonstrated because this compound underwent spontaneous cyclization in aqueous solution.[7] Since intramolecular cycloadditions of **5** and **6** are slow in aqueous solution,[7] the fungus apparently converts **5** to **7** for acceleration of [4 + 2]-cycloaddition.[8]

In conclusion, feeding experiments with advanced precursors have elucidated the late stages of solanapyrone biosynthesis and unambiguously established that the decalin system in salanapyrones is formed by an intramolecular Diels–Alder reaction. This is the first direct experimental evidence for a biosynthetic Diels–Alder reaction involving demonstrated diene and dienophile precursors. Currently, we are working on detection of enzymatic activity capable of mediating this unprecedented cyclization.

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Supplementary Material Available: ^2H NMR spectra of **8** and **9** prepared from **1** derived from incorporation of [2-^{2}H,3-^{2}H,17-^{2}H,18-$^2H_3$]prosolanapyrone II (**6**) and scheme for synthesis of labeled precursors **5** and **6** (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(7) In this chemical reaction, the predominant product was not the exo adduct **1** but the endo adduct **2**. Details of the aqueous Diels–Alder reaction for compounds **5**, **6**, and **7** will be reported elsewhere.

(8) Pindur, U.; Lutz, G.; Otto, C. *Chem. Rev.* **1993**, *93*, 741–761.