JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by American Chemical Society

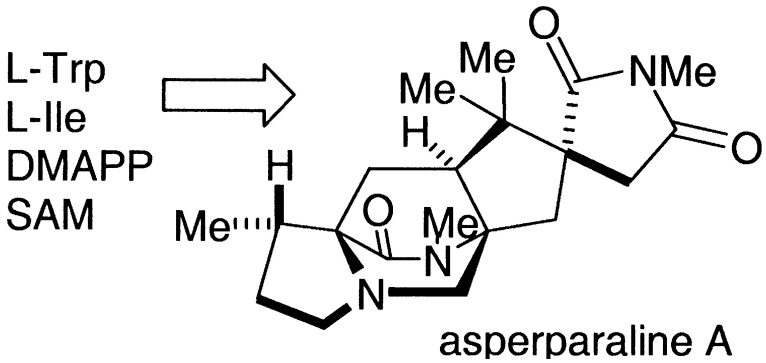
Communication

Studies on the Biosynthesis of Asperparaline A: Origin of the Spirosuccinimde Ring System

Chandele R. Gray, Juan F. Sanz-Cervera, Louis A. Silks, and Robert M. Williams

J. Am. Chem. Soc., **2003**, 125 (48), 14692-14693• DOI: 10.1021/ja037687i • Publication Date (Web): 05 November 2003

Downloaded from http://pubs.acs.org on March 30, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 11/05/2003

Studies on the Biosynthesis of Asperparaline A: Origin of the Spirosuccinimde Ring System

Chandele R. Gray,[†] Juan F. Sanz-Cervera,[‡] Louis A. Silks,[§] and Robert M. Williams*,[†]

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, Departamento de Química Orgánica, Facultad de Química, Universidad de Valencia, Calle Dr. Moliner, 50. E-46100 Burjassot, Spain, and NIH Stable Isotope Resource, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Received July 31, 2003;; E-mail: rmw@chem.colostate.edu

Asperparalines (A−C) are fungal metabolites isolated from *Aspergillus japonicus*[1] JV-23 by Hayashi and co-workers and have been shown to have potent paralytic activities against silkworm. Asperparaline A, also named aspergillimide (VM55598) along with the 16-oxo-derivative were isolated along with several paraherquamide derivatives from *Aspergillus* sp. IMI 337664 by Everett and associates[2] and were reported to display anthelmintic activity. The asperparalines along with the brevianamides[3], paraherquamides,[4] marcfortine A,[5] and sclerotamide[6] comprise an interesting class of structurally related secondary metabolites containing a bicyclo-[2.2.2]diazaoctane core. An emerging body of evidence supports the notion that this structural motif is formed by a biosynthetic intramolecular [4 + 2] cycloaddtion of the isoprene-derived olefin across a preformed azadiene moiety derived from an oxidized piperazinedione (A → B → C) as shown in Scheme 1.[7,8]

Among its interesting structural features, asperparaline A contains an unusual 3-*spiro*-succinimide moiety. A search of the literature revealed that the *spiro*-succinimide ring system has not been reported as constituting part of any known natural products. In addition to sharing the bicyclo[2.2.2]diazaoctane ring, the orientation of the *spiro*-succinimide ring is consistent with the relative configuration of the *spiro*-oxindole ring system observed in the paraherquamides. As part of a program directed primarily at elucidating the biosynthetic mechanism of formation of the bicyclo-[2.2.2] core that is common to all of these alkaloids, we have initiated studies on the biosynthesis of the asperparaline family. Herein, we report the biosynthetic incorporation of primary amino acid building blocks that constitute the core framework of this class of alkaloids.

Earlier studies in these laboratories revealed that the β-methyl proline ring of paraherquamide A is derived from an oxidative cyclization of L-isoleucine.[9] From these results, it seemed plausible that the β-methyl proline ring of asperparaline A might also be derived from L-isoleucine. A simple comparison of the orientation and relative configuration of the *spiro*-succinimide of asperparaline A and the *spiro*-oxindole ring of the paraherquamides suggested that the *spiro*-succinimide could be derived from an oxidative degradation of tryptophan.

To determine the primary amino acid building blocks that comprise asperparaline A, feeding experiments were performed on *A. japonicus* JV-23 using [1,2-$^{13}C_2$]-acetate, [*methyl*-^{13}C]-L-methionine, [1-^{13}C]-L-isoleucine, [1-^{13}C]-L-tryptophan, [*indole*-2-^{13}C]-L-tryptophan, and [3-^{13}C, $^{2}H_2$]-L-serine. The position of the ^{13}C enrichment in asperparaline A was determined by ^{13}C NMR, and the percentage of labeled amino acid enrichment was determined by ^{13}C NMR and electrospray mass spectrometry.[10] The [1,2-$^{13}C_2$]-acetate was incorporated only at isoprene carbons C-19−C-23, indicative of a mevalonate pathway in accordance with the observations we have previously reported for paraherquamide A biosynthesis. Incorporation of [*methyl*-^{13}C]-L-methionine was observed only at C-29, the *N*-methyl position of the monoketopiperazine (25.2%) and at C-30, the *N*-methyl position of the *spiro*-succinimide ring (26.9%), but not as any part of the β-methyl proline ring. [1-^{13}C]-L-Isoleucine was incorporated as expected, showing enrichment (5.8%) at position C-18, analogous to the biosynthesis of paraherquamide A.[9] [1-^{13}C]-L-Tryptophan was incorporated in 6.4−7.2% at C-12, and [*indole*-2-^{13}C]-L-tryptophan was incorporated in 12.2% at position C-2, indicating that tryptophan is indeed the precursor responsible for the construction of the *spiro*-succinimide ring. Incorporation of [*indole*-2-^{13}C]-L-tryptophan was also observed at positions C-29 and C-30 in 1.9 and 1.7%, respectively, probably as a result of catabolism of tryptophan to kynurenine and formate which can be metabolized by tetrahydrofolic acid (THF) into *N*-methyl tetrahydrofolic acid

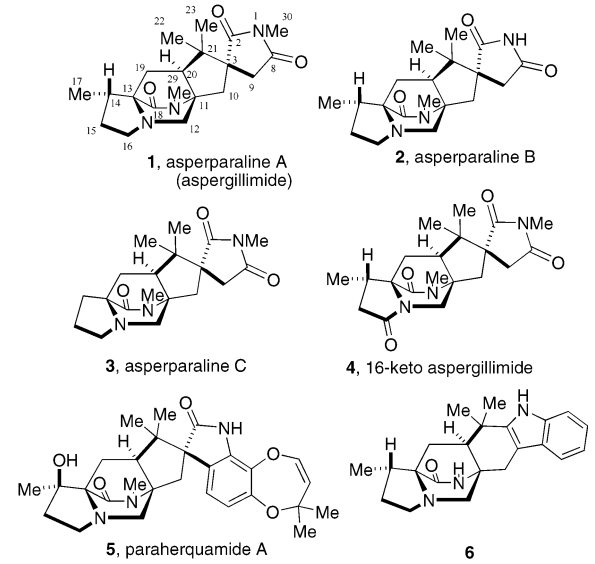
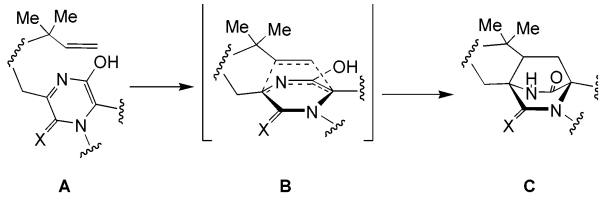


Figure 1. Structures of the asperparalines and related alkaloids.

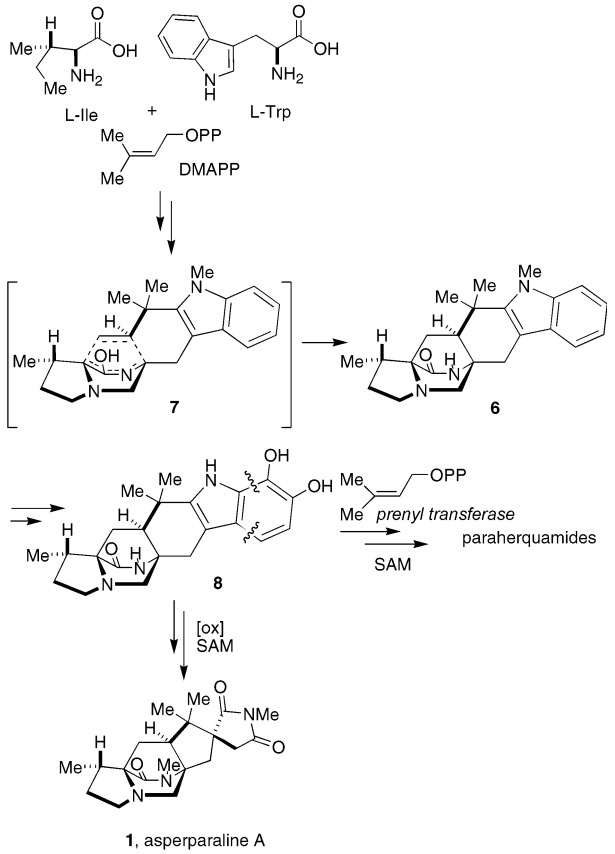
Scheme 1



[†] Colorado State University.
[‡] Universidad de Valencia.
[§] Los Alamos National Laboratory.

10.1021/ja037687i CCC: $25.00 **© 2003 American Chemical Society**

Scheme 2. Possible Biosynthetic Interrelationships between Paraherquamide and Asperparaline



(5-Me-THF) which is responsible for the (*S*)-methyl group of methionine.[11] Incorporation of [3-^{13}C,$^{2}H_2$]-L-serine was not observed at position C-10 in agreement with the proposed biosynthetic mechanism.[11] Curiously, serine incorporation was only observed at positions C-29 and C-30, also as a result of metabolism by THF, suggesting perhaps that *A. japonicus* JV-23 may utilize a non-serine-based tryptophan biosynthesis.

These results suggest that asperparaline A likely shares a common biosynthetic pathway with the paraherquamides (Scheme 2). Prenylation of the *cyclo*-L-tryptophan-L-β-methyl proline and intramolecular [4 + 2] cyclization (via **7**) would provide the putative bicyclo[2.2.2] core (**6**). We have previously demonstrated that compound **6** serves as a biosynthetic precursor to paraherquamide A. Oxidation of the aromatic ring to the catechol derivative (**8**) followed by prenylation, dioxepin formation, and oxidation to the *spiro*-oxindole would yield paraherquamide. Interruption of the prenylation of **8** would provide for a branch point wherein oxidative cleavage of four carbon atoms from the oxygenated aromatic ring could furnish the *spiro*-succinimide ring of asperparaline A. Other sequential oxidative paths of the tryptophan moiety to the *spiro*-succinimide are also possible.

In summary, it has been determined that L-tryptophan, L-isoleucine, and DMAPP are the primary building blocks of asperparaline A, and all indications to date indicate that asperparaline shares a common biogenetic pathway with the paraherquamides. Efforts are underway to investigate the incorporation of (+)-VM55599, an advanced metabolite in the biosynthesis of paraherquamide A, and to determine the timing of the oxidation of the aromatic ring and the oxidative degradation of catechol derivatives.

Acknowledgment. Dedicated to Professor Sir Jack E. Baldwin on the occasion of his 65th birthday. This work was supported by the National Institutes of Health (grant to R.M.W.) and the NIH National Stable Isotopes Resource, NIH Division of Research Resources (NIH/NCRR Grant Number RR02231 to L.A.S). We are indebted to Dr. Hideo Hayashi, of Osaka Prefecture University, for providing us with cultures of *A. japonicus* JV-23. Mass spectra were obtained on instruments supported by the National Institutes of Health Shared Instrumentation Grant GM49631.

Supporting Information Available: (1) Full experimental procedures for the feeding experiments, (2) method for the determination of the percentage of isotopic incorporation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Hayashi, H.; Nishimoto, Y.; Nozaki, H. *Tetrahedron Lett*. **1997**, *38*, 5655−5658. (b) Hayashi, H.; Nishimoto, Y.; Akiyama, K.; Nozaki, H. *Biosci. Biotechnol. Biochem.* **2000**, *64(1)*, 111−115.
(2) Banks, R. M.; Blanchflower, S. E.; Everett, J. R.; Manger, B. R.; Reading, C. *J. Antibiot.* **1997**, *50*, 840−846.
(3) (a) Birch, A. J.; Wright, J. J. *J. Chem Soc. D* **1969**, 644−645. (b) Birch, A. J.; Wright, J. J. *Tetrahedron* **1970**, *26*, 2329−2344. (c) Birch, A. J.; Russell, R. A. *Tetrahedron* **1972**, *28*, 2999−3008. (d) Bird, B. A.; Remaley, A. T.; Campbell, I. M. *Appl. Environ. Microbiol*. **1981**, *42*, 521−525. (e) Bird, B. A.; Campbell, I. M. *Appl. Environ. Microbiol*. **1982**, *43*, 345. (f) Robbers, J. E.; Straus, J. W. *Lloydia* **1975**, *38*, 355. (g) Paterson, R. R. M.; Hawksworth, D. L. *Trans. Br. Mycol. Soc*. **1985**, *85*, 95−100. (h) Wilson, B. J.; Yang, D. T. C.; Harris, T. M. *Appl. Microbiol*. **1973**, *26*, 633−635. (i) Coetzer, J. *Acta Crystallogr*. **1974**, *B30*, 2254−2256.
(4) (a) Yamazaki, M.; Okuyama, E.; Kobayashi, M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135−136. (b) Ondeyka, J. G.; Goegelman, R. T.; Schaffer, J. M.; Kelemen, L.; Zitano, L. *J. Antibiot.* **1990**, *43*, 1375−1376. (c) Liesch, J. M.; Wichman, C. F. *J. Antibiot.* **1990**, *43*, 1380−1386. (d) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Manger, B. R.; Reading, C. *J. Antibiot.* **1991**, *44*, 492−497. (e) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Reading, C. *J. Antibiot.* **1993**, *46*, 1355−1363.
(5) (a) Polonsky, J.; Merrien, M.-A.; Prange, T.; Pascard, C.; Moreau, S. *J. Chem. Soc., Chem. Commun*. **1980**, 601−602. (b) Prange, T.; Bullion, M.-A.; Vuilhorgne, M.; Pascard, C.; Polosky, J. *Tetrahedron Lett.* **1980**, *22*, 1977−1980.
(6) Whyte, A. C.; Gloer, J. B. *J. Nat. Prod.* **1996**, *59*, 1093−1095.
(7) Porter, A. E. A. *J. Chem. Soc., Chem. Commun.* **1970**, 1103.
(8) (a) Williams, R. M.; Kwast, E.; Coffman, H.; Glinka, T. *J. Am. Chem. Soc.* **1989**, *111*, 3064−3065. (b) Williams, R. M.; Kwast, E.; Coffman, H.; Glinka, T.; Stille, J. K. **1990**, *112*, 808−821. (c) Domingo, L. R.; Sanz-Cervera, J. F.; Williams, R. M.; Picher, M. T.; Marco, J. A. *J. Org. Chem.* **1997**, *62*, 1662−1667. (d) Sanz-Cervera, J. F.; Glinka, T.; Williams, R. M. *J. Am. Chem. Soc.* **1993**, *115*, 347−348. (e) Sanz-Cervera, J. F.; Glinka, T.; Williams, R. M. *Tetrahedron* **1993**, *49*, 8471−8472. (f) Williams, R. M.; Sanz-Cervera, J. F.; Sancenon, F.; Marco, J. A.; Halligan, K. *Bioorg. Med. Chem.* **1998**, *6*, 1233−1241.
(9) Stocking, E. M.; Sanz-Cervera, J. F.; Williams, R. M. *J. Am. Chem. Soc.* **1996**, *118*, 7008−7009.
(10) For a leading reference on the method employed, see: Leete, E.; Bodem, G. B. *J. Am. Chem. Soc.* **1976**, *98*, 6321. These values were further corroborated by electrospray mass spectroscopy (see Supporting Information).
(11) Luckner, M., *Secondary Metabolism in Microorganisms, Plants and Animals*, 3rd edition; Springer−Verlag: Berlin, 1990.
(12) Corey, E. J., Xu, F., Noe, M. C. *J. Am. Chem. Soc.* **1997**, *119*, 12414−12415.

JA037687I