Natural *cis*-solamin is a mixture of two tetra-epimeric diastereoisomers: biosynthetic implications for Annonaceous acetogenins†

Yulai Hu,‡[a] Alex R. L. Cecil,[a] Xavier Frank,[b] Christophe Gleye,[b] Bruno Figadère*[b] and Richard C. D. Brown*[a]

Received 9th February 2006, Accepted 10th February 2006
First published as an Advance Article on the web 24th February 2006
DOI: 10.1039/b601943a

The anti-tumour natural product *cis*-solamin has been shown to occur as a mixture two of tetra-epimeric diastereoisomers 1A and 1B, whereas solamin (6) was isolated as a single diastereoisomer; the biosyntheses of 1A/B and 6 are likely to involve enzyme-mediated cyclohydrations of the bis-epoxide acetogenins *anti*-diepomuricanin A2 and *syn*-diepomuricanin A1 respectively, where addition of water occurs regioselectively at either C15 or C20.

Acetogenins isolated from the plant family Annonaceae are the subject of intense interest due to their potent cytotoxic anti-tumour activity.[1] Many acetogenins posses common structural features which include: one or more 2,5-disubstituted tetrahydrofuran (THF) rings; methylene chains attached to the 2- and 5-positions of each THF; and a butenolide moiety attached to the end of one of the methylene chains (Fig. 1). Structural elucidation of acetogenins is typically achieved using mass spectrometry fragmentation data in combination with careful analysis of their ^{1}H and ^{13}C NMR spectra.[1] The latter techniques now permit reliable assignment of the relative stereochemistry around the THF regions of the natural products. Absolute stereochemistry of hydroxyalkyl THF subunits and remote hydroxyl groups have been established *via* synthetic derivatives such as Mosher's esters and 2-naphthylmethoxy acetic esters.[2,3]

The unambiguous determination of stereochemistry in acetogenins is a prerequisite to structure–activity relationship studies, and it also provides important evidence to support biosynthetic hypotheses. Despite advances in characterisation methods, the stereochemical assignment of certain members of this important family of natural products is still incomplete. For example, whilst the stereochemistry within the butenolide ring is known to be *S*, the absolute stereochemistry of the THF regions of certain mono- and bis-THF acetogenins remains undetermined (*e.g. cis*-solamin, *cis*-uvariamycin I and carolins A–C, Fig. 1).[4,5] Here we present results from synthetic and analytical studies that establish the true structure of natural *cis*-solamin, and discuss the implications of our findings in relation to the biosynthetic origin of mono-THF acetogenins.

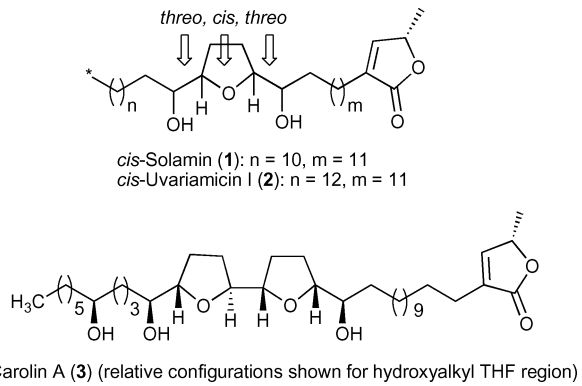


Fig. 1 Structures of selected Annonaceous acetogenins.

The structure of the mono-THF acetogenin *cis*-solamin, isolated from *Annona muricata*, was determined using mass spectroscopy and NMR data.[4] At the time of isolation, the absolute stereochemistry of the THF core was not established, and it was concluded that the likely structure of *cis*-solamin was either **1A** or **1B** (Fig. 2).[4,6] Total syntheses of the diastereoisomers **1A** and **1B** in our laboratory showed that the two compounds displayed practically identical NMR and optical rotation data,[7] although it was discovered that the diastereoisomers were readily differentiated by chiral HPLC.[7]

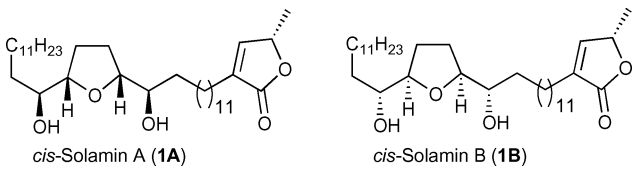


Fig. 2 Possible structures for *cis*-solamin: *cis*-solamin A (**1A**) and *cis*-solamin B (**1B**).

Use of the chiral HPLC conditions described above would permit for the first time the unambiguous assignment of the structure of *cis*-solamin by analysis of a sample of the authentic natural product. Surprisingly, it was discovered that the chromatogram obtained from natural *cis*-solamin displayed two peaks of approximately equal intensity (**1A** : **1B** = 9 : 8) and with similar retention times to the tetra-epimeric diastereoismers **1A** and **1B**.[8] Subsequent doping experiments using the synthetic samples clearly demonstrated that the natural product is in fact a mixture of two tetra-epimeric diastereoisomers, *cis*-solamin A (**1A**) and B (**1B**).

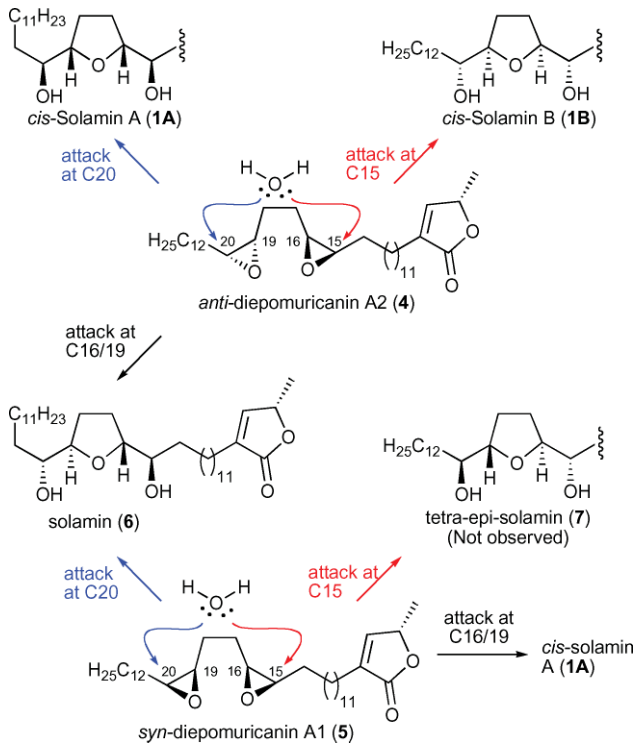
This curious discovery has interesting implications for the biosynthesis of *cis*-solamin.[9] At first inspection one might be tempted to suggest that *cis*-solamin A and B isomers could arise

[a]*School of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK. E-mail: rcb1@soton.ac.uk; Fax: +44 (0)23 8059 6805; Tel: +44 (0)23 8059 4108*
[b]*Laboratoire de Pharmacognosie, associé au CNRS (BioCIS), Université Paris-Sud, Faculté de Pharmacie, Rue J-B Clément, Chatenay-Malabry, 92296, France. E-mail: bruno.figadere@cep.u-psud.fr*

† Electronic Supplementary Information (ESI) available: NMR data for the Mosher's ester derivatives of solamin, and HPLC chromatograms of **1A/B** and natural *cis*-solamin. See DOI: 10.1039/b601943a

‡ Current address: Department of Chemistry, Northwest Normal University, Lanzhou 730070, China.

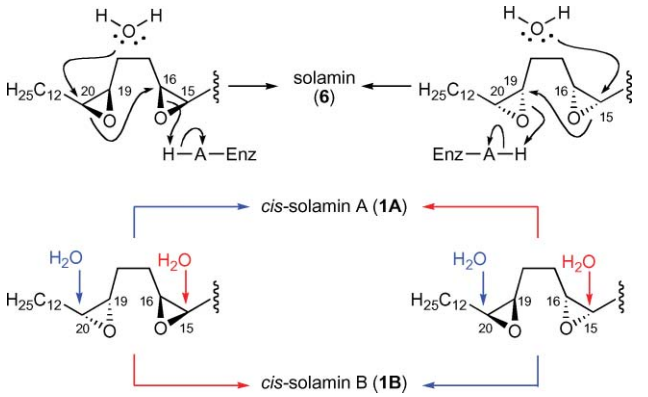
from a non-enzymatic cyclisation of the bis-epoxide acetogenins *syn*-diepomuricanin A1 (**5**) and *anti*-diepomuricanin A2 (**4**).[10,11] Indeed, S_N2 attack by water at C15, C16, C19, or C20 could give rise to a mixture of *cis*-solamins A and B (Scheme 1).[9a] However, the lack of regioselectivity expected from such a process would also lead to the formation of 15,16,19,20-tetra-*epi*-solamin (**7**) along with solamin (**6**). In view of the fact that solamin (**6**) has been isolated and characterised as a single stereoisomer,[12–14] it seems unlikely that *cis*-solamins A and B and solamin arise from a non-enzymatic hydrolytic cyclisation of **4** and **5**.



Scheme 1 Possible pathways for non-enzymatic hydrolytic THF formation from *syn*- and *anti*-diepomuricanins (the absolute stereochemistry of C15, C16, C19 and C20 in **4** and **5** has not been established).

Our observations would be more accurately accounted for by enzyme-catalysed cyclohydration of the pseudo-C_2-symmetric and pseudo-*meso* bis-epoxides **4** and **5**, where attack of water is directed to C15 or C20 (Scheme 2). Accordingly, activation of either epoxide in the pseudo-C_2-symmetrical core of *anti*-diepomuricanin A2 (**4**) would lead to the formation of *cis*-solamins A and B respectively. We suggest that *syn*-diepomuricanin A1 (**5**) would undergo a related cyclisation, except that in this case the two epoxides present in its pseudo-*meso* bis-epoxide core are differentiated by the enzyme, resulting in the formation of solamin (**6**) as a single diastereoisomer. The absolute stereochemistry of the bis-epoxide cores of **4** and **5** have not been unambiguously established,[9a] and it is important to note that similar enzyme-catalysed processes could lead to the same end results commencing from any of four possible diastereoisomeric bis-epoxides (Scheme 2).

In conclusion, we have shown that natural *cis*-solamin isolated from the seeds of *Annona muricata* is a mixture of tetra-epimeric diastereoisomers **1A** and **1B**, whereas natural solamin (**6**) has been



Scheme 2 Possible pathways for the biosynthesis of solamin and *cis*-solamins A and B from diepomuricanins.

isolated as a single diastereoisomer. The bis-epoxide acetogenins *anti*-diepomuricanin A2 (**4**) and *syn*-diepomuricanin A1 (**5**) have also been isolated from the same plant source, and are the likely biosynthetic precursors to *cis*-solamin and solamin. The formation of the three stereoisomers from **4** and **5** may be catalysed by enzymes capable of differentiating the two epoxides present in the pseudo-*meso* bis-epoxide motif, but not in the pseudo-C_2 bis-epoxide. On the basis of our results, we predict that natural *anti*-diepomuricanin A2 (**4**) is a single diastereoisomer, whereas *syn*-diepomuricanin A1 (**5**) could be a mixture of tetra-epimeric diastereoisomers. These remaining questions are under investigation in our laboratories.

Acknowledgements

Support has been provided by The Leverhulme Trust (postdoctoral fellowship to Y.H.), and The Royal Society (University Research Fellowship to R.C.D.B.).

Notes and references

1 For recent reviews: (*a*) A. Bermejo, B. Figadère, M. C. Zafra-Polo, I. Barrachina, E. Estornell and D. Cortes, *Nat. Prod. Rep.*, 2005, **22**, 269; (*b*) F. Q. Alali, X. X. Liu and J. L. McLaughlin, *J. Nat. Prod.*, 1999, **62**, 504.
2 M. J. Rieser, Y. H. Hui, J. K. Rupprecht, J. F. Kozlowski, K. V. Wood, J. L. McLaughlin, P. R. Hanson, Z. Zhuang and T. R. Hoye, *J. Am. Chem. Soc.*, 1992, **114**, 10203.
3 P. Duret, A. I. Waechter, B. Figadère, R. Hocquemiller and A. Cavé, *J. Org. Chem.*, 1998, **63**, 4717.
4 Isolation of *cis*-solamin and *cis*-uvariamicin: C. Gleye, P. Duret, A. Laurens, R. Hocquemiller and A. Cavé, *J. Nat. Prod.*, 1998, **61**, 576.
5 Isolation of carolins A–C: E. F. Queiroz, F. Roblot, B. Figadère, A. Laurens, P. Duret, R. Hocquemiller, A. Cavé, L. Serani, O. Laprévote, J. Cotte-Laffitte and A.-M. Quéro, *J. Nat. Prod.*, 1998, **61**, 34.
6 The first reported total synthesis of the two possible proposed structures for *cis*-solamin concluded that the structure of natural *cis*-solamin was likely to be **1A** (15*R*,16*R*,19*S*,20*S*) on the basis of optical rotation data: (*a*) H. Makabe, Y. Hattori, Y. Kimura, H. Konno, M. Abe, H. Miyoshi, A. Tanaka and T. Oritani, *Tetrahedron*, 2004, **60**, 10651; (*b*) H. Makabe, Y. Hattori, A. Tanaka and T. Oritani, *Org. Lett.*, 2002, **4**, 1083.
7 (*a*) A. R. L. Cecil, Y. Hu, M. J. Vicent, R. Duncan and R. C. D. Brown, *J. Org. Chem.*, 2004, **69**, 3368; (*b*) A. R. L. Cecil and R. C. D. Brown, *Org. Lett.*, 2002, **4**, 3715.
8 For chromatograms of tetra-epimers **1A** and **1B** and natural *cis*-solamin, see the ESI†.
9 For biosynthetic hypotheses: (*a*) C. Gleye, X. Franck, R. Hocquemiller, A. Laurens, O. Laprévote, S. de Barros and B. Figadère, *Eur. J. Org.*

Chem., 2001, 3161; (*b*) T. Colman-Saizarbitoria, H. A. Johnson, F. Q. Alali, D. C. Hopp, L. L. Rogers and J. L. McLaughlin, *Phytochemistry*, 1998, **49**, 1610; (*c*) D. Gromek, B. Figadère, R. Hocquemiller, A. Cavé and D. Cortes, *Tetrahedron*, 1993, **49**, 5247; (*d*) A. Hisham, U. Sreekala, L. Pieters, T. Debruyne, H. Vandenheuvel and M. Claeys, *Tetrahedron*, 1993, **49**, 6913; (*e*) F. Roblot, T. Laugel, M. Leboeuf, A. Cavé and O. Laprévote, *Phytochemistry*, 1993, **34**, 281; (*f*) S. D. Jolad, J. J. Hoffmann, K. H. Schram, J. R. Cole, M. S. Tempesta, G. R. Kriek and R. B. Bates, *J. Org. Chem.*, 1982, **47**, 3151.

10 Diepomuricanin A has been isolated from the seeds of *Annona muricata*, and was recently shown to occur as a mixture of *syn*- and *anti*-tetra-epimers.[9a] See also refs. 9*d*,*e* and: O. Laprévote, C. Girard, B. C. Das, T. Laugel, F. Roblot, M. Leboeuf and A. Cavé, *Rapid Commun. Mass Spectrom.*, 1992, **6**, 352.

11 For the asymmetric synthesis of (15*S*,16*R*,19*S*,20*R*,34*S*)-diepomuricanin: H. Konno, H. Makabe, A. Tanaka and T. Oritani, *Tetrahedron Lett.*, 1996, **37**, 5393.

12 Isolation of solamin from *Annona muricata*: S. H. Myint, D. Cortes, A. Laurens, R. Hocquemiller, M. Leboeuf, A. Cavé, J. Cotte and A.-M. Quéro, *Phytochemistry*, 1991, **30**, 3335.

13 The *R* and *S* Mosher's ester derivatives of solamin indicated that solamin was isolated as a single diastereoisomer. See the ESI† and: C. Gleye, *Ph.D. Thesis*, Université Paris-Sud, 1998.

14 The structure of solamin has also been confirmed by total synthesis. For early examples: (*a*) S. C. Sinha and E. Keinan, *J. Am. Chem. Soc.*, 1993, **115**, 4891; (*b*) H. Makabe, A. Tanaka and T. Oritani, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1975; (*c*) B. M. Trost and Z. P. Shi, *J. Am. Chem. Soc.*, 1994, **116**, 7459.