Semisynthesis of *cis*- and *trans*-Solamin by Acidic Opening of Natural Diepomuricanin A − a Mechanistic Investigation

Christophe Gleye,[a] Xavier Franck,[a] Reynald Hocquemiller,[a] Alain Laurens,[a][‡] Olivier Laprévote,[b] Sabine de Barros,[b] and Bruno Figadère*[a]

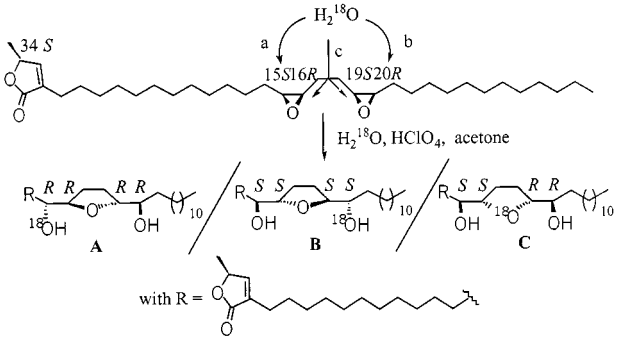
Dedicated to Prof. William H. Okamura on the occasion of his 60th birthday

Keywords: Epoxides / Mass spectrometry / Natural products / ^{18}O labeling / Semisynthesis

MS/MS spectroscopy allowed us to determine the mechanism of the acidic opening of diepomuricanin A, by analysis of the product distribution after treatment with $H_2{}^{18}O$ in the presence of $HClO_4$ in anhydrous acetone.

Introduction

Diepomuricanin A, an annonaceous acetogenin,[1,2] has been isolated from several Annonaceae[3−7] [*Annona muricata* (seeds, roots, stem barks), *A. reticulata* (seeds), *Rollinia membranacea* (seeds), and *R. ulei* (leaves)]. Its total synthesis has been achieved and its absolute configuration was thus assumed to be (15*S*,16*R*,19*S*,20*R*,34*S*).[8] If this absolute configuration is correct as postulated, the opening of the bis(epoxide) unit in the plant thus has to arise from S_N2 attack by H_2O at C-15, with inversion of configuration, followed by opening of the second epoxide ring, by 16-OH at C-19, again with inversion, producing the known natural *trans*-solamin [possessing the (15*R*,16*R*,19*R*,20*R*,34*S*) absolute configurations].[9−12] However, since the absolute configuration of diepomuricanin A was determined by optical means (comparison with the specific rotation of the natural compound, vide infra), some ambiguity remains, due to the strong effect of the absolute configuration of the lactone ring on the specific rotation of the molecule, as shown recently.[13] We thought that, by tagging the water molecule involved in the first opening of the bis(epoxide) and analyzing the relative configuration of the tetrahydrofuran ring thus obtained, we should be able to confirm (or not) the absolute configuration of diepomuricanin A, on the basis of the polyepoxide cascade opening reaction.[14−17] We thus decided to treat diepomuricanin A (isolated in our laboratory from a hexane extract of the seeds of *A. furfuracea*) with $H_2{}^{18}O$ in the presence of $HClO_4$ in anhydrous acetone, as described earlier[18−20] (Scheme 1).



Scheme 1

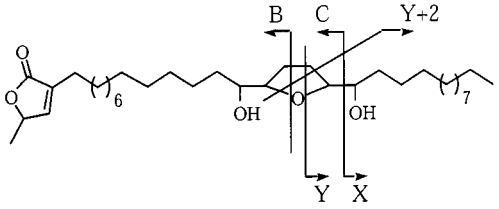
Results and Discussion

The 1.9 M labeled aqueous solution of $HClO_4$ was prepared in situ by mixing anhydrous $KClO_4$ (300 mg) with 95% pure (Prolabo®) H_2SO_4 (45 μL) and $H_2{}^{18}O$ (400 μL). Anhydrous acetone (500 μL) was then added, followed by diepomuricanin A (60 mg, 0.11 mmol) in anhydrous acetone (500 μL) and the reaction mixture was stirred at room temperature for 19 h. Conventional $NaHCO_3/CH_2Cl_2$ workup gave the product mixture, which was analyzed by ^1H NMR and chemical ionization mass spectrometry (CIMS). Careful analysis of the ^1H NMR spectrum showed typical patterns for mono-tetrahydrofuran annonaceous acetogenins with *threo-trans-threo* and *threo-cis-threo* relative configurations. This confirms that the opening had occurred through all pathways a, b, and c, resulting in *threo-trans-threo* compounds **A** and **B**, and *threo-cis-threo* product **C**. Chemical ionization mass spectrometry showed two ion peaks at m/z = 565 [MH^+] and m/z = 567 [MH^+ + 2 amu], in a 30:70 ratio, indicating 70% incorporation of ^{18}O. However, the fragmentations observed did not allow us to

[‡] Who supplied diepomuricanin A and to whom isolation information should be addressed.
[a] Laboratoire de Pharmacognosie, associé au CNRS (BIOCIS), Université Paris-Sud, Faculté de Pharmacie, Rue Jean-Baptiste Clément, 92296 Châtenay-Malabry, France
Fax: (internat.) + 33-1/46835399
E-mail: Bruno.Figadere@cep.u-psud.fr
[b] ICSN-CNRS, Av. de la Terrasse, 91198 Gif-sur-Yvette, France
Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/eurjoc or from the author.

  1434−193X/01/0816−3161 $ 17.50+.50/0

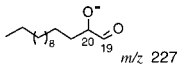
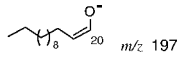
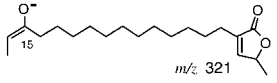
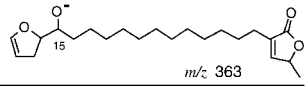
locate the position of the ^{18}O atom unambiguously. The mixture was therefore further purified by HPLC, which afforded two compounds (in approximately 2:1 ratio) with *threo-cis-threo* and *threo-trans-threo* tetrahydrofuran ring relative configurations, on the basis of the previously isolated *cis*-[21] and *trans*-solamin[22] (coinjection with *cis*- and *trans*-solamin confirmed that *cis*- and *trans*-solamin were indeed obtained). It should be noted that the pseudo-enantiomeric compounds **A** and **B** were not separated by HPLC. Furthermore, no other diastereomers (such as *erythro-trans-threo* and *erythro-cis-threo* products) were formed, as judged by NMR and HPLC analyses.[1] This further confirms the S_N2 process of the pattern of opening of the bis(epoxide). In order to locate the position of the ^{18}O atom in the structures of semisynthetic *cis*- and *trans*-solamins, high energy tandem mass spectrometry of $[M - H]^-$ ions was chosen as the investigation method. This technique produces fragment ions containing either the methyl terminal group or the lactone end of the acetogenin molecules, thus providing a simple and reliable method for the identification and localization of the substituents.[23] From now on, the nomenclature of the acetogenin fragment ions will be used.[24] The collision-induced dissociation of $[M - H]^-$ ions generated by LSIMS from ^{18}O-labelled *cis*- and *trans*-solamins (m/z = 565) resulted in the expected diagnostic fragment ions (Scheme 2). Analysis of the daughter ion peaks of the m/z = 565 ion ($[M - H]^-$) of semisynthetic *cis*-solamin showed ion **X** with 8% enrichment of the corresponding fragment + 2 amu (relative to natural *cis*-solamin, see Table 1), in agreement with labeling at C-20. Ion **Y+2** then showed enrichment of 54%, in agreement with labeling at C-19 (identical to C-16) of around 46%. Ion **B** showed enrichment of 45%, in agreement with labeling at C-15. However, analysis of the daughter peaks of the m/z = 565 ion ($[M - H]^-$) of semisynthetic *trans*-solamin showed completely different results: Ion **X** showed 16% enrichment, in agreement with labeling at C-20, and ion **Y+2** showed 89% enrichment, in agreement with labeling at C-16 of around 73%. Ion **B** showed enrichment of 7%, in agreement with labeling at C-15 (whereas 100% enrichment of ion **C** is in agreement with 93% labeling at C-16). These data indicated that opening occurred through all possible pathways a, b, and c, but according to different statistics.



Scheme 2. Nomenclature of fragments cited in the text by MS/MS (by analogy with ref.[24])

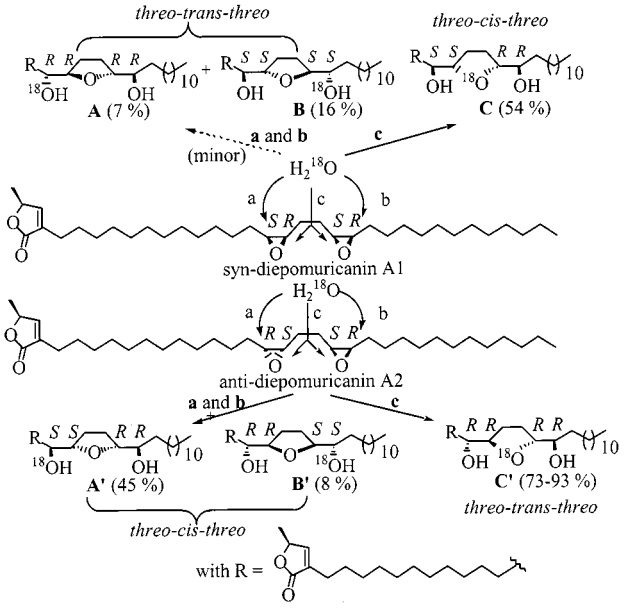
If, however, diepomuricanin A were to possess the absolute configurations as depicted in Scheme 1, *cis*-solamin would have only the C-16 position labeled with ^{18}O, and *trans*-solamin only two positions (C-15 and C-20) labeled. Since both *cis*- and *trans*-solamin showed all three positions labeled, this can only be explained by the presence of a second diepomuricanin A, with an *anti* relationship of the two epoxide rings.

Table 1. LSIMS/MS fragmentations of the $[M - H]^-$ ion at m/z = 565

Ions m/z	Relative abundance *cis*-cpds. ref.[a]	synt. cpd.[b]	(%[c])	*trans*-cpds. ref.[d]	synt. cpd.[e]	(%[c])	Proposed structures
Y+2							
227	10	20		79	10		
229	4	32	(54)	0	77	(89)	*m/z* 227
X							
197	20	22		12	34		
199	0	2	(8)	0	7	(16)	*m/z* 197
B							
321	100	62		100	100		
323	3	53	(45)	0	7	(7)	*m/z* 321
C							
363	Non significant			11	0		
365				0	13	(100)	*m/z* 363

[a] Natural *cis*-solamin. – [b] Synthetic *cis*-solamin. – [c] Enrichment. – [d] Natural *trans*-solamin. – [e] Synthetic *trans*-solamin.

Thus, the mass spectrometry data and the NMR spectroscopic data could only be interpreted by invoking a mixture of two diepomuricanins A (diepomuricanin A1 and diepomuricanin A2), possessing either a *syn* or an *anti* relationship between the two epoxide rings (Scheme 3). It is interesting to note that both diepomuricanin A1 and diepomuricanin A2 have similar ^1H (400 MHz: multiplet, 15-H, 20-H, and 16-H, 19-H at δ = 2.95 and 2.98, respectively) and ^{13}C (50 MHz: C-15, C-16, and C-19, C-20 at δ = 56.4 and 57.3)[3] NMR spectroscopic data, since in one case (the *anti* relationship case) both epoxides are identical, while in the



Scheme 3

syn relationship case they are pseudo enantiomers. It is thus not surprising that they were neither observable by NMR nor separable by classic chromatographic means.

It is also noteworthy that, since the labeling of *trans*-solamin is around 73 and 93% at C-16, the opening should have occurred mainly through an epoxy-diol intermediate (path c), and not through a cascade reaction solely from *anti*-diepomuricanin A2. Furthermore, the *syn*-diepomuricanin A1 was also opened almost exclusively through an epoxy-diol, resulting in the major *cis*-solamin labeled at C-16 (since only 7 and 16% of labeling was observed at C-15 and C-20, respectively). Finally, the observed difference between the labeling at C-15 and C-20 in *cis*-solamin (45 and 8%, respectively) can be explained by taking account of the influence of the *anti*-diepomuricanin A lactone ring on the regioselectivity of the reaction.

Conclusion

In summary, the ^{18}O-labeled acidic opening of the bis(epoxide) pattern of diepomuricanin A, allowed us to show that the natural product is in fact a mixture of two compounds: *syn*-diepomuricanin A1 and *anti*-diepomuricanin A2, in a approximate 1:1 ratio and with incompletely determined absolute configurations. Furthermore, acidic opening of such a bis(epoxide) in solution was shown to occur through all the possible pathways, depending on the relative configuration of the bis(epoxide) unit. This result shows for the first time that the acidic opening of polyepoxides do not occur simply through cascade reactions, but through an epoxy-diol that has not so far been isolated. These results should be of interest in biomimetic total syntheses of Annonaceae acetogenins, for predicting the regioselectivity of the opening reaction.

Experimental Section

General Remarks: MS and MS/MS spectra were obtained using a ZabSpec-T five-sector tandem mass spectrometer (Micromass, Manchester, UK). $[M - H]^-$ precursor ions were generated by cesium ion bombardment at 30 keV (matrix: *m*-NBA/glycerol, 1:1, v/v). The precursor ions submitted to MS/MS experiments were selected by MS1 set at appropriate *E* and *B* values and then focused in a collision cell located in the fourth field-free region (between E_2 and B_2). Argon was introduced at a pressure producing an attenuation of the precursor ion beam of almost 70%. The collision cell was floated at 4 kV so as to attain a collision energy of 4 keV. Fragment ions detection was achieved by use of the MCAD detector operating with a mass ratio of 1.225:1.0 at an angle of 30° with regard on the ion beam. For each MS/MS acquisition, the mass scale comprised between the precursor ion peak and the lowest mass end (m/z = 50) was covered by successive overlapping exposures of 0.5 s. − HPLC was carried out with a Millipore-Waters (Milford, MA) system equipped with a Waters 484 spectrophotometer. The seeds of *A. furfuracea* were treated as reported in ref.[4] to give a hexane extract (26 g), which after HPLC purification afforded 105 mg of diepomuricanin A. − ^1H and ^{13}C NMR spectra were recorded with a Bruker AM-400 spectrometer (400 MHz) in $CDCl_3$ solution. Chemical shifts (δ) are expressed in ppm with the protonated solvent as reference.

Diepomuricanin A:[3] ^1H NMR (400 MHz): δ = 6.98 (d, J = 1.6 Hz, 1 H), 4.99 (dq, J = 6.8, 1.6 Hz, 1 H), 2.98 (m, 2 H), 2.95 (m, 2 H), 2.25 (t, J = 7.1 Hz, 2 H), 1.68 (m, 4 H), 1.54 (m, 2 H), 1.52 (m, 4 H), 1.41 (d, J = 6.8 Hz, 3 H), 1.25−1.40 (m, 38 H), 0.84 (t, J = 6.7 Hz, 3 H). − ^{13}C NMR (100 MHz): δ = 173.9, 148.8, 134.3, 77.4, 57.3, 56.4, 31.9, 29.5, 27.4, 26.6, 25.2, 25.0, 22.7, 19.2, 14.1.

***cis*-Solamin:**[21] ^1H NMR (400 MHz): δ = 6.99 (d, J = 1.6 Hz, 1 H), 4.99 (dq, J = 6.8, 1.6 Hz, 1 H), 3.81 (m, 2 H), 3.41 (m, 2 H), 2.26 (t, J = 7.1 Hz, 2 H), 1.93 (m, 2 H), 1.74 (m, 2 H), 1.55 (m, 2 H), 1.46 (m, 4 H), 1.41 (d, J = 6.8 Hz, 3 H), 1.24−1.37 (m, 38 H), 0.84 (t, J = 6.7 Hz, 3 H). − ^{13}C NMR (100 MHz): δ = 173.8, 148.8, 134.2, 82.7, 77.4, 74.3, 34.1, 31.9, 29.7, 28.1, 27.4, 25.1, 22.6, 19.2, 14.0.

***trans*-Solamin:**[22] ^1H NMR (400 MHz): δ = 6.99 (d, J = 1.5 Hz, 1 H), 4.98 (dq, J = 6.8, 1.5 Hz, 1 H), 3.80 (m, 2 H), 3.40 (m, 2 H), 2.26 (t, J = 7.1 Hz, 2 H), 1.98 (m, 2 H), 1.68 (m, 2 H), 1.56 (m, 2 H), 1.41 (d, J = 6.8 Hz, 3 H), 1.40 (m, 4 H), 1.25−1.40 (m, 38 H), 0.87 (t, J = 7.0 Hz, 3 H). − ^{13}C NMR (100 MHz): δ = 173.6, 148.8, 134.3, 82.6, 77.4, 74.0, 33.5, 31.9, 29.6, 28.7, 27.4, 25.6, 25.1, 22.7, 19.2, 14.1.

Acknowledgments

The authors express their thanks to Dr. Valérie Geertsen (CEA, Saclay, France) for a generous gift of $H_2{}^{18}O$, Samara Gonçalvez-Pires for technical assistance, and Nathalie Hue (ICSN, Gif-sur-Yvette, France) for acquisition of the MS/MS spectra.

[1] A. Cavé, D. Cortes, B. Figadère, A. Laurens in *Progress in the Chemistry of Organic Natural Products* (Eds.: W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, C. Tamm), Springer, New York, **1997**, vol. 70, pp. 81−288.
[2] F. Q. Alali, X. X. Liu, J. L. McLaughlin, *J. Nat. Prod.* **1999**, *62*, 504−540.
[3] O. Laprévote, C. Girard, B. C. Das, T. Laugel, F. Roblot, M. Lebœuf, A. Cavé, *Rapid Commun. Mass Spectrom.* **1992**, *6*, 352−355.
[4] F. Roblot, T. Laugel, M. Lebœuf, A. Cavé, O. Laprévote, *Phytochemistry* **1993**, *34*, 281−285.
[5] A. Hisham, U. Sreekala, L. Pieters, T. De Bruyne, H. Van den Heuvel, M. Claeys, *Tetrahedron* **1993**, *49*, 6913−6920.
[6] Vu Thi Tam, Phan Quan Chi Hieu, B. Chappe, F. Roblot, O. Laprévote, B. Figadère, A. Cavé, *Nat. Prod. Lett.* **1994**, *4*, 255−262.
[7] O. Laprévote, F. Roblot, R. Hocquemiller, A. Cavé, *Tetrahedron Lett.* **1990**, *31*, 2283−2286.
[8] H. Konno, H. Makabe, A. Tanaka, T. Oritani, *Tetrahedron Lett.* **1996**, *37*, 5393−5396.
[9] H. Makabe, A. Tanaka, T. Oritani, *J. Chem. Soc., Perkin Trans. 1* **1994**, 1975−1981.
[10] S. C. Sinha, E. Keinan, *J. Am. Chem. Soc.* **1993**, *115*, 4891−4892.
[11] B. M. Trost, Z. Shi, *J. Am. Chem. Soc.* **1994**, *116*, 7459−7460.
[12] C. Gleye, Ph. D. Dissertation, University of Paris-Sud, **1998**.
[13] P. Duret, B. Figadère, R. Hocquemiller, A. Cavé, *Tetrahedron Lett.* **1997**, *38*, 8849−8852.
[14] T. R. Hoye, J. C. Suhadolnik, *J. Am. Chem. Soc.* **1985**, *107*, 5312−5313.
[15] T. R. Hoye, J. C. Suhadolnik, *J. Am. Chem. Soc.* **1987**, *109*, 4402−4403.

[16] T. R. Hoye, J. C. Suhadolnik, *Tetrahedron* **1986**, *42*, 2855.
[17] T. R. Hoye, Z. Zhuang, *J. Org. Chem.* **1988**, *53*, 5578−5580.
[18] D. Gromek, B. Figadère, R. Hocquemiller, A. Cavé, D. Cortes, *Tetrahedron* **1993**, *49*, 5247−5252.
[19] A. V. Rama Rao, S. Krishnappa, K. L. Narasimba Reddy, K. Ashok Reddy, *Synth. Commun.* **1986**, *16*, 1141−1148.
[20] L. F. Fieser, M. Fieser in *Reagents for Organic Chemistry*, Wiley, New York, **1967**, vol. 1, pp. 796.
[21] C. Gleye, P. Duret, A. Laurens, R. Hocquemiller, A. Cavé, *J. Nat. Prod.* **1998**, *61*, 576−579.
[22] S. H. Myint, D. Cortes, A. Laurens, R. Hocquemiller, M. Lebœuf, A. Cavé, J. Cotte, A. M. Quéro, *Phytochemistry* **1991**, *30*, 3335−3338.
[23] C. Gleye, A. Laurens, R. Hocquemiller, N. Faucheur, L. Serani, O. Laprévote, *Rapid Commun. Mass Spectrom.* **1998**, *12*, 1051−1056.
[24] O. Laprévote, B. C. Das, *Tetrahedron* **1994**, *28*, 8479−8490.

Received February 7, 2001
[O01062]