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Synthesis and Discovery of Phytofurans: Metabolites of α -Linolenic Acid Peroxidation

 C. Cuyamendous^a, K. S. Leung^b, T. Durand^a, J. C.-Y. Lee^{*b}, C. Oger^{*a}, J.-M. Galano^{*a}

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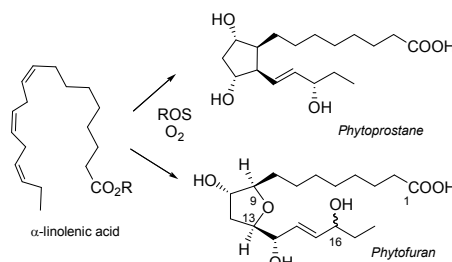
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Phytofurans are novel metabolites produced by non-enzymatic peroxidation of α -linolenic acid. An unprecedented Payne rearrangement-cyclization of a C2-symmetric bisepoxide permitted construction of the core 3-hydroxy-2,5-disubstituted tetrahydrofuran. LC-MS/MS investigation provided evidence for the presence of phytofurans in nuts and seeds for the first time.

Omega 3 polyunsaturated fatty acids (PUFAs) such as α -linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well known for their cardioprotective properties.¹ Under oxidative stress conditions these PUFAs are easily peroxidized to give oxygenated metabolites by reactive oxygen species (ROS),² and we recently showed that some oxygenated metabolites of DHA provide cardioprotection by exerting antiarrhythmic properties.³ In 1998, Muller and co-workers highlighted the formation of ALA enzyme-independent cyclic oxygenated metabolites in plants and expressed them as phytoprostanes (PhytoPs) (Scheme 1).⁴ Currently, PhytoPs stand as biomarkers of oxidative stress in biological systems, but also as protective defence molecules in plants and neurons.⁵ The presence of PhytoPs in plant oil from nuts and seeds is also pertinent in human diet and health.⁶ More recently, a novel non-enzymatic pathway of PUFA was uncovered leading to 3-hydroxy-2,5-disubstituted tetrahydrofuran structures, named Isofurans (IsoFs) from arachidonic acid (AA), Neurofurans (NeuroFs) from DHA and dihom-IsoFs from adrenic acid (AdA). Our explorations in this field prompted us to investigate the occurrence of such mechanism pathways in plants and we successfully identified production of the phytofurans (PhytoFs) (named by analogy to

phytoprostanes and isofurans) from ALA for the first time.

Three strategies have been reported for the synthesis of this family of natural products by the group of Taber (IsoFs),^{7–9} Zannoni-Vidari (NeuroFs)¹⁰ and our group (dihomo-IsoFs and NeuroFs).^{11,12} We report herein on the development of a novel and powerful strategy for the synthesis of all members of this family of targets and their derivatives. We have applied this strategy to the synthesis of PhytoF and to our first target, *ent*-16-(*RS*)-13-*epi*-ST- Δ^{14} -9-PhytoF (**1**) from the enediol family.¹³ The Phytofuranic biosynthetic pathway from ALA can generate two classes of PhytoFs (alkenyl and enediol) each comprising two families, giving a potential total of 128 phytofuran isomers.¹⁴ We made the arbitrary choice to target the enediol metabolite *ent*-16-(*RS*)-13-*epi*-ST- Δ^{14} -9-PhytoF (**1**), knowing that LC-MS/MS analysis will not allow separation of the various stereoisomers in each family of PhytoFs.



Scheme 1. Representative structures of phytoprostane and phytofuran from ALA peroxidation. ROS = reactive oxygen species, R = monogalactosylacylglycerol.

As depicted in Scheme 2, in our synthetic plan for *ent*-16-(*RS*)-13-*epi*-ST- Δ^{14} -9-PhytoF **1** we sought to target tetraol-furanic compound **2** with intrinsic orthogonal protection of the four hydroxyl groups to allow access to alkenyl or enediol PhytoF derivatives at a late stage. While the side chains of **1** could be introduced by Wittig and Horner-Wadsworth-Emmons reactions, the synthesis of enantio-enriched THF **2** would be accomplished via an unprecedented Payne rearrangement-cyclization sequence from C2-symmetric bis-epoxide **3**.¹⁵ Such a bis-epoxide could be accessed in 2 steps from hepta-2,5-diyne-1,7-diol intermediate **4**. These diyne intermediate would

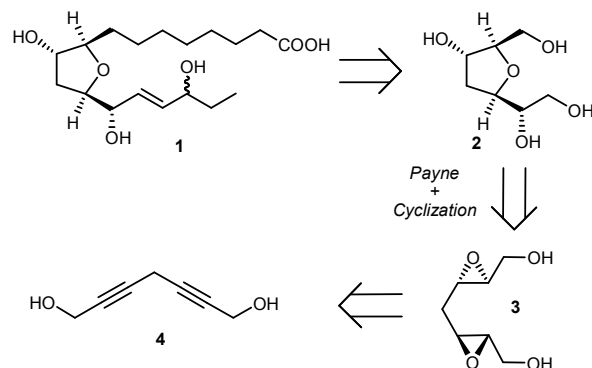
^a Institut des Biomolécules Max Mousseron (IBMM) UMR CNRS 5247 - Université de Montpellier – ENSCM Faculté de Pharmacie 15 avenue Charles Flahault, 34093 Montpellier cedex 05, France E-mail: camille.oger@umontpellier.fr; jean-marie.galano@umontpellier.fr

^b School of Biological Sciences, The University of Hong-Kong, Pokfulam Road, Hong Kong SAR (China) E-mail: jettylee@hku.hk

† Footnotes relating to the title and/or authors should appear here.

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also permit access to various diastereoisomers of **2** by variation of the original alkene stereochemistry (E or Z configurations) before bisepoxide synthesis.

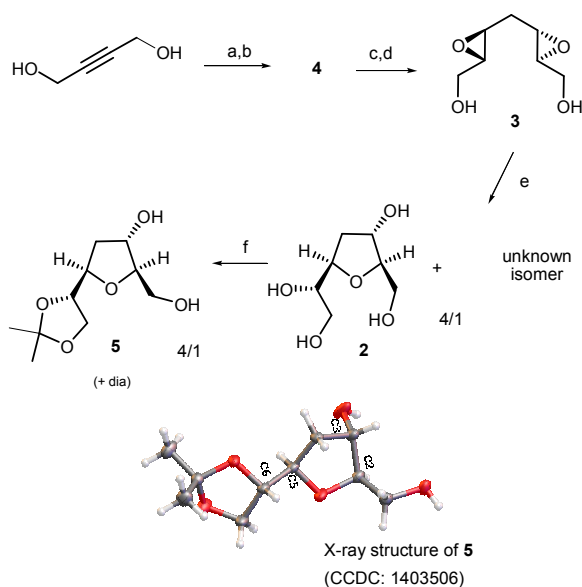


Scheme 2. Retrosynthetic analysis of phytofuran derivatives using an inedit Payne cyclisation sequence of C2-symmetric precursor.

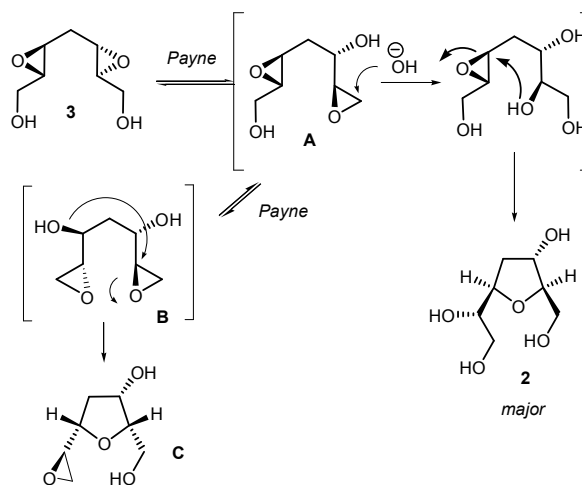
The synthesis of bisepoxide **3** was performed according to the work of Hoffmann et al.,¹⁶ but with slight modification of the literature procedure. It started from the known 4-chlorobut-2-yn-1-ol after SOCl_2 /pyridine chlorination of commercially available but-2-yne-1,4-diol in 46% yield (Scheme 3). A cross coupling reaction between chlorinated alkyne and prop-2-yn-1-ol was performed following Ivanov's procedure,¹⁷ yielding desired hepta-2,5-diyne-1,7-diol **4** in 81% yield. Partial reduction of diyne **4** to give the E/E-diene using freshly prepared Red-Al reagent produced desired hepta-2,5-diene-1,7-diol in 55% yield on a multigram scale. Finally, double Sharpless asymmetric epoxidation (SAE) of the bis-allylic alcohol **3** using L-(+)-diethyl tartrate afforded the desired C2-symmetric (2S,2'S,3S,3'S)-bis-epoxide **3** in 76% yield and with 90% ee on a gram scale quantity and only 4 steps.

In our original plan, the epoxydiol THF **C** was to be obtained via two consecutive Payne epoxide rearrangement reactions (**A** to **B**) of **3** followed by cyclization (Scheme 4). However, it soon became apparent that the poor solubility of **3** in almost any organic solvent compatible with a Payne rearrangement reaction tempered this design process (a non-nucleophilic base in an aprotic media was necessary to avoid hydrolysis of the newly formed terminal epoxides from the Payne reactions).¹⁸ Switching to water or MeOH as the solvent resulted in complete dissolution of **3**, and a screening of bases (NaOH, LiOH, KOH, MeONa) and temperature conditions (rt, 80 °C) was performed to focus on the formation of the THF derivatives. Indeed, treating **3** with KOH (5 eq.) in water at 80 °C for 2 h furnished a 4/1 mixture of inseparable tetraol THF derivatives such as **2** in 65% yield (Scheme 3).

The other alkaline bases also furnished **2** but with lower yields, and reaction at rt required more than 4 days to proceed to completion with lower yields. Intensive NMR investigations did not secure the relative configuration of the two THF compounds recovered. Gratifyingly, treatment of that mixture of THF derivatives with 2,2-dimethoxypropane and a catalytic amount of PTSA in boiling acetone gave the corresponding protected inseparable diols in 79% yield. The crystalline nature



Scheme 3. Reagents and conditions: a) SOCl_2 , Pyridine, Benzene, RT, 46%; b) prop-2-yn-1-ol, K_2CO_3 , CuI, NaI, DMF, RT, 81%; c) LiAlH_4 , 2-methoxyethanol, THF, -40 °C to RT, 55%; d) L-(+)-DET, $\text{Ti}(\text{O}i\text{-Pr})_4$, *t*-BuOOH, $\text{CH}_2\text{Cl}_2/\text{CHCl}_3$, -30 °C, 76%, 90% ee; e) KOH, H_2O , 80 °C, 65%; f) 2,2-dimethoxypropane, PTSA, acetone, reflux, 79%. DET = diethyltartrate, PTSA = *para*-toluenesulfonic acid.

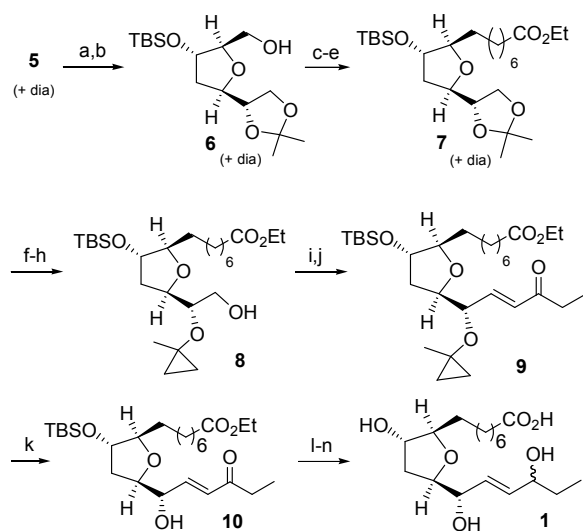


Scheme 4. Proposed mechanisms for the major diastereomer **2** and double Payne sequence.

of major diastereoisomer **5** meant that it could be crystallised to give crystals suitable for X-ray diffraction, therefore allowing its configuration to be assigned.¹⁴

With regard to the stereochemistry observed for **2**, it became evident that the expected double Payne rearrangement/cyclization process (from **A** to **C**) did not occur but and that a single Payne rearrangement/ bimolecular epoxide-opening/ cyclization cascade was likely (Scheme 4). Such a conclusion also resulted from the isolation of a small amount of compound **B** from the reaction with KOH showing the feasibility of a double Payne reaction. Interestingly treatment of **B** under the same Payne conditions did not produce compound **C** and only starting material and

unidentified polar compounds were isolated from the reaction. Finally, a greater mechanistic understanding of the reaction sequence was obtained by treatment of **3** with MeONa in MeOH at 80 °C. This reaction afforded a mixture of THF compounds (7:3 ratio) bearing a methoxy substituent (the major compound having the MeO group in place of the nucleophilic hydroxide following scheme 4).¹⁴ Following the discovery of a reliable and easy route to supply the functionalized THF core of PhytoFs, we continued our synthetic venture. The poor solubility and handling of **2** meant that acetone THF **5** (together with minor unknown diastereoisomer) was used for the synthesis of the monoprotected TBS-ether **6** via a bisprotection-regioselective deprotection sequence in an overall 64% yield (Scheme 5). The primary alcohol **6** was oxidized using Dess-Martin periodinane followed by Wittig reaction with previously described $\text{BrPh}_3\text{P}(\text{CH}_2)_6\text{CO}_2\text{Et}$ and NaHMDS in THF at -78°C in 75% overall yield. The Z-double bond formed was then subjected to catalytic hydrogenation with palladium over charcoal to complete the first side chain of compound **7** in 87% yield. To introduce the lipidic second side chain, functionalization of the acetone moiety was required.



Scheme 5. Reagents and conditions: a) TBSCl, imidazole, DMAP, CH_2Cl_2 , RT, 88%; b) PPTS, EtOH, 0°C, 73%; c) DMP, NaHCO_3 , CH_2Cl_2 , RT; d) $\text{BrPh}_3\text{P}(\text{CH}_2)_6\text{CO}_2\text{Et}$, NaHMDS, THF, -78°C to RT, 75% over 2 steps; e) H_2 , Pd/C, EtOH, RT, 87%; f); i -PrNEt, TMSOTf, CH_2Cl_2 , 0°C to RT; g) Et_2Zn , CH_2Cl_2 , Et_2O , RT; h) K_2CO_3 , MeOH, RT, 62% over 3 steps; i) DMP, CH_2Cl_2 , 0°C to RT; j) 1-(triphenylphosphoranylidene)butan-2-one, CH_2Cl_2 , 51% over 2 steps; k) NBS, THF/ H_2O , RT, 85%; l) $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, NaBH₄, MeOH, 0°C RT; m) TBAF, THF, RT; n) LiOH, THF/ H_2O , RT, 67% over 3 steps. TES = triethylsilyl, Tf = trifluoromethanesulfonyl, NBS = *N*-bromosuccinimide, TBAF = tetrabutylammonium fluoride. TBS = *tert*-butyldimethylsilyl, DMAP = 4-(*N,N*-dimethylamino)pyridine, PPTS = pyridinium *para*-toluenesulfonate, DMP = Dess Martin Periodinane, NaHMDS = sodium *bis*(trimethylsilyl)amide.

The best compromise was a three-step sequence developed by Rychnovsky and co-workers¹⁹ which permitted transformation of the acetone **7**, through an enol intermediate, into the free primary alcohol 1-methyl-1-cyclopropyl hydroxyl derivative **8** in 62% yield. Interestingly, flash chromatography purification of **8** permitted removal of the minor isomer obtained from the Payne sequence.

Oxidation of the alcohol with Dess-Martin periodinane followed by the coupling with 1-(triphenylphosphoranylidene)butan-2-one gave enone **9** in 51% over 2 steps. The cyclopropyl group of **9** was removed thereafter using *N*-bromosuccinimide to give **10** in 85% yield.¹⁹ Luche reduction of the enone function gave a 1:1 mixture of epimers at C16 (epimers were chosen for mass spectrometry identification), followed by TBS deprotection, before hydrolysis using LiOH to give the first synthesis of PhytoF, *ent*-16-(*R,S*)-13-*epi*-ST- Δ^{14} -9-PhytoF **1**. Eight milligrams of **1** was obtained using this 20-step sequence with an overall yield of 0.8%. Identification of the mass fragmentations from the MS/MS of the synthesized PhytoFs and evaluation by LC-MS/MS in plant foodstuffs will corroborate its presence without ambiguity.

For the first time, we established the presence of the natural PhytoFs in nuts and seeds, the progenitors of plants. The consumption of nuts and seeds has increased in recent years due to the popularity of the vegetarian and Mediterranean diets. ALA found in these nuts and seeds is the precursor of essential fatty acids, mainly EPA and DHA for the vegetarians, which are important components for the brain development and central nervous system, as well as in prevention of metabolic disorders. However, the conversion of dietary plant ALA to EPA and DHA *in vivo* is poor. Nevertheless, there are reports concerning the benefits of dietary nuts and seeds in prevention of cognitive dysfunction and cardiovascular diseases.^{20,21} When all facts are considered, it is possible that the beneficial *in vivo* effect of ALA from plants is due to other bioactive components. PhytoPs from ALA peroxidation have emerged as potential active components in plants and humans, and several isomers have been identified previously in plant oils.^{22,23} In human cells, some PhytoPs were found to display anti-inflammatory properties, and protect against oxidant-induced neuronal injuries.⁶

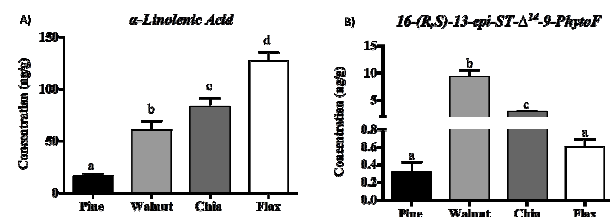


Figure 1. Concentration of α -linolenic acid and *ent*-16-(*R,S*)-13-*epi*-ST- Δ^{14} -9-PhytoF in nuts and seeds. The lipid component was isolated by Soxhlet extraction and cleaned by SPE prior to LC-MS/MS measurement (refer to SI). Values of the column in graphical display is annotated as mean \pm SD, n=4. Columns sharing different alphabetical superscripts in each graph are significantly different at $p < 0.05$.

As depicted in Figure 1, we quantified the levels of ALA in nuts (pine nuts and walnuts) and seeds (flaxseeds and chia seeds) using the LC-MS/MS.¹⁴ We also identified and quantified one particular family of PhytoF for the first time with the help of our synthetic *ent*-16-(*R,S*)-13-*epi*-ST- Δ^{14} -9-PhytoF **1** and the use of MS/MS.¹⁴ It should be noted that such analyses quantify the overall sum of all possible stereoisomers of 16-(*R,S*)-13-*epi*-ST- Δ^{14} -9-PhytoF (because secondary MS fragmentation is likely to be similar for all isomers) which can be formed following the

biosynthetic pathways. Surprisingly, large variations in ALA levels were observed in various samples (Figure 1A), and seeds generally had higher concentrations than the nuts. In addition, the *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* concentration was successfully quantified in the same samples (Figure 1B). Notably, flaxseeds showed the highest ALA level but had a relatively low *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* level compared to walnuts and chia seeds, that contained less ALA. However, pine nuts had the lowest ALA level and the lowest *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* level, and the concentration of *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* in walnuts and chia seeds was approximately 10- to 20-fold higher than in flaxseeds and pine nuts.¹⁴ Our observations also indicate that the relative concentrations of the *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* do not necessarily mirror the relative ALA concentrations in the nuts and seeds.

Nuts and seeds consumed whole or in the form of an oil extract are known to have health benefits; to date, some of this bioactivity has been attributed to oxygenated ALA and potentially to phytoprostanes. However, the concentrations of *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* found in the nuts and seeds are comparable to those of phytoprostanes reported previously²³ and so could also have a functional role in human health. Currently our group is conducting *in vivo* studies to validate the potency of *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* in biological systems.

In conclusion, the total synthesis of a phytofuran natural product, *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* (**1**) was accomplished using an innovative synthetic strategy involving a Payne rearrangement-cyclization sequence of a C2-symmetric intermediate. We also quantified levels of *ent-16-(RS)-13-epi-ST- Δ^{14} -9-PhytoF* in plant foodstuffs for the first time. Further studies are on-going to achieve the synthesis of the three remaining types of PhytoFs using our novel and flexible approach and to further establish their presence in dietary plants and their role in human health.

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Notes and references

‡ Relative concentration of ALA and PhytoF in seeds and nuts can be subject to variation depending on the source and storage conditions.

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