ChemPubSoc Europe

DOI: 10.1002/slct.201701870

Chemistry SELECT Communications

Sustainable Chemistry

Mild Thio-Diversification of Bioactive Natural Products. Withaferin A: A Case study.

C. N. Casero,[a] J. N. Garay Novillo,[b] M. E. García,[a] J. C. Oberti,[a] V. E. Nicotra,*[a] A. B. Peñéñory,[c] and F. R. Bisogno*[c]

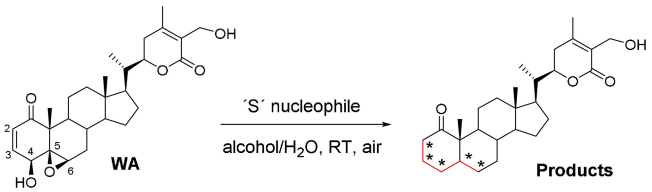
A sustainable strategy was developed to create chemical diversity from bioactive scaffolds. The methodology comprises a set of simple reactions between the parent compound (i.e. withaferin A), and small sulfur nucleophiles in hydroalcoholic media, at room temperature and in open-air atmosphere. Novel steroidal sulfur natural product-analogs were obtained, featuring ring opening, ring formation, new stereocenters and modulation of parameters useful in medicinal chemistry.

Solvent and reagent selection are major issues in drug discovery and lately, sustainability has become a strong 'evolutionary pressure' in global pharma industries,[1] which drives a tendency to reduce waste streams and minimize energy requirements.[2] Nevertheless, in medicinal chemistry, the trend is to minimize the starting material employed in reaction screening, thus allowing a wider area of chemical space to be explored.[3]

Nowadays, new strategies for the design and preparation of chemical libraries present a great challenge in the quest for novel drug candidates in the area of synthetic, medicinal and biological chemistry.[4] Natural products (NPs) have a glorious past as a source of drug candidates[5] and currently attention has been re-focused on these because the expectations created for combinatorial chemistry strategies remain unfulfilled.[6] In recent years, the notion of "biologically relevant chemical space"[7,8] has gained momentum and different approaches have arisen, engendering new chemical entities from privileged scaffolds.[9,10] Likewise, starting from readily available bioactive NPs, (bio)diversification and increasing molecular complexity have provided excellent results in terms of new and more potent bioactive derivatives, often with diminished toxicity.[11] In Nature itself, this strategy has been 'optimized' along with evolution in natural organisms, in which a common intermediate gives rise to a plethora of structure-dissimilar metabolites, merging different biosynthetic pathways and performing key late-stage transformations.[12]

Sulfur-containing natural products are by far less common than their oxygen- and nitrogen- containing counterparts but usually display diverse bioactivity deriving from their ability to chelate metal ions, get involved in (one- and two-electron) redox processes and establish hydrogen-bonding interactions, among others.[13,14] This unique behavior makes sulfur NP-analogs an appealing target to be addressed by thiodiversification strategies onto sulfur-free bioactive scaffolds.[15]

Here, we describe a simple methodology, applied in hydroalcoholic media, at room temperature and in open-air atmosphere to generate several sulfur-containing derivatives of a bioactive NP. In this model case, we use withaferin A (WA), an extremely versatile NP with several reported bioactivities, isolated from plants of the Solanaceae family (Scheme 1). Lately,



Scheme 1. General reaction procedure.

WA has gained much attention in view of its therapeutic potential, seen in *in vitro* and *in vivo* models. In the last three decades, a number of reviews have been published focusing on its structural properties, biosynthesis, synthetic approaches, and biological properties.[16,17] Intriguingly, and despite the presence of several potentially reactive centers in WA,[18,19] just a few reports deal with the diversification of this compound,

[a] *Dr. C. N. Casero, Dr. M. E. García, Prof. Dr. J. C. Oberti, Prof. Dr. V. E. Nicotra*
Departamento de Química Orgánica
Facultad de Ciencias Químicas
Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET)
Universidad Nacional de Córdoba
Medina Allende y Haya de la Torre, Edificio de Ciencias 2, Ciudad Universitaria, 5000, Córdoba, Argentina
E-mail: vnicotra@fcq.unc.edu.ar

[b] *Ph.D. J. N. G. Novillo*
Departamento de Química Orgánica
Facultad de Ciencias Químicas
Universidad Nacional de Córdoba
Medina Allende y Haya de la Torre, Edificio de Ciencias 2, Ciudad Universitaria, 5000, Córdoba, Argentina

[c] *Prof. Dr. A. B. Peñéñory, Dr. F. R. Bisogno*
Departamento de Química Orgánica
Facultad de Ciencias Químicas
Instituto de Investigaciones en Físico-Química Córdoba (INFIQC-CONICET)
Universidad Nacional de Córdoba
Medina Allende y Haya de la Torre, Edificio de Ciencias 2, Ciudad Universitaria, 5000, Córdoba, Argentina
Tel/Fax: +54-351-535-3867
E-mail: fbisogno@fcq.unc.edu.ar

Supporting information for this article is available on the WWW under https://doi.org/10.1002/slct.201701870

and mainly through Michael-type addition at C3 and O-acylation at C4 and C27 (Figure 1).[20–24]

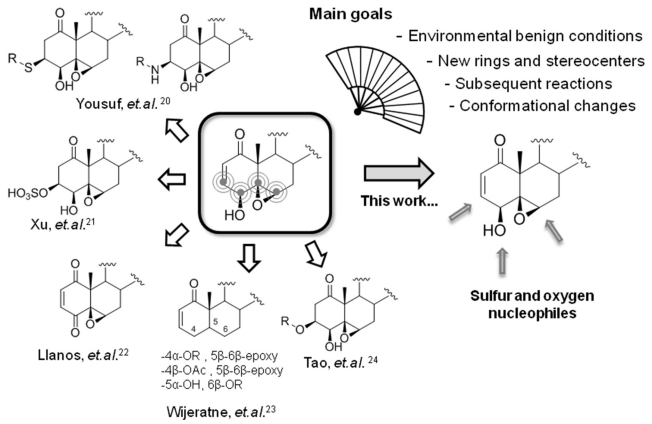


Figure 1. Molecular diversification of withaferin A.

The procedure described here employs small S-nucleophiles, which show different reactivity patterns towards WA depending on the stereoelectronic nature of the nucleophile.

As a first approach, potassium thioacetate (KSAc) was selected as the nucleophile (1.5 equiv.) to react with WA in a 1:1 MeOH/H_2O mixture, stirred at room temperature in air atmosphere.[25,26]

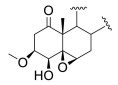
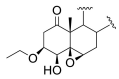
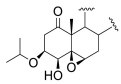
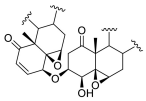
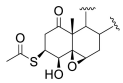
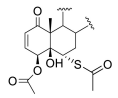
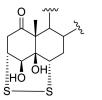
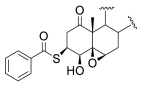
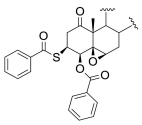
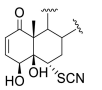
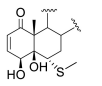
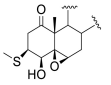
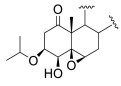
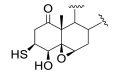
Under these conditions, MeOH competed efficiently as a nucleophile, thus rendering the Michael-adduct 3β-OMe derivative (**1**). This compound was identified as 3β-methoxy-2,3-dihydrowithaferin A by comparison with published spectroscopic and other physical data.[27] The multiplicity and coupling constant value of the signal corresponding to H-4 (d, $J = 3.3$ Hz) in the ^{1}H NMR spectrum of **1** agree with a β-orientation of the methoxy group at C-3, with a H-3/H-4 dihedral angle near 48°.

In order to favor the thiodiversification reaction, a set of 1:1 hydroalcoholic mixtures was screened as reaction media at room temperature for 24 h (Table 1).The reaction between WA and KSAc in EtOH/H_2O mixture yielded compound **2** (entry 2). The ^{1}H and ^{13}C NMR spectra of compound **2** were closely related to that of **1**. The main difference observed between compounds **1** and **2** was the presence of signals corresponding to an ethoxy instead of the methoxy group at C-3 [δ_H 3.49 (m, 2H) and 1.16 t ($J = 6.9$ Hz, 3H), δ_C 64.4 and 15.3]. EtOH showed a similar behavior to that of MeOH (entry 1–2), and thus the 3β-OEt derivative (**2**) was mainly formed.

When a *i*PrOH/H_2O mixture was used (entry 3), several interesting products were isolated along with a small amount of the expected 3β-*i*OPr analog **3**: the previously reported 4-acetylwithaferin A **4**[28,29] and two novel derivatives, compounds **5** and **6**.

The ^{1}H and ^{13}C NMR spectra of **3** were characterized by the substitution at C-3, showing signals corresponding to an isopropoxy group [δ_H 3.69 hept ($J = 6.1$, H), 1.10 d ($J = 6.1$ Hz, 3H) and 1.11 d ($J = 6.1$ Hz, 3H), δ_C 69.9, 22.4 and 22.7]. Compound **3** could not be obtained in pure form by normal or

Table 1. Reactivity of Withaferin A under the diversification conditions.

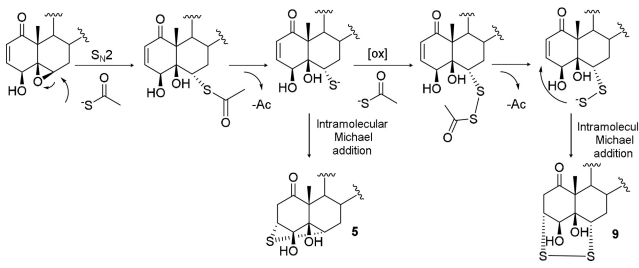
Entry	H_2O-alcohol mixture (1:1)	Sulfur nucleophile (equiv.)	Product (yield %)
1	MeOH/H_2O	KSAc (1.5)	 **1** (55.3)
2	EtOH/H_2O	KSAc (1.5)	 **2** (61.3)
			 **3** (4.0)
			 **4** (3.4)
3	*i*PrOH/H_2O	KSAc (1.5)	 **5** (11.4)
			 **6** (9.5)
4	PEG300/H_2O	KSAc (1.5)	 **7** (22.9)
5	Glycerol/H_2O	KSAc (1.5)	SM[a] -
			 **5** (15.8)
6	*i*PrOH/H_2O	KSAc (3.5)	 **8** (2.6)
			 **9** (12.5)
			 **10** (2.0)
7	*i*PrOH/H_2O	KSBz[b] (3.5)	 **11** (15.5)
8	*i*PrOH/H_2O	NH_4SCN (1.5)	 **12** (16.3)
			 **13** (2.7)
9	*i*PrOH/H_2O	NaSMe (1.5)	 **14** (11.7)
			 **3** (8.1)
			 **15** (8.5)

[a] Starting Material. [b] The nucleophile was formed *in situ* by adding K_2CO_3 (1.1 equiv. based on thiobenzoic acid)

reversed-phase TLC. Since this compound was characterized from a mixture, its structure was tentatively proposed as (17*R*,20*S*,22*R*)-5β,6β-epoxy-4β,27-dihydroxy-1-oxo-3β-isopropoxy-witha-24-enolide.

The HRESITOFMS of compound **5** showed a molecular ion corresponding to a molecular formula of $C_{28}H_{40}O_6S$. Its ^{1}H NMR spectrum showed the absence of olefinic resonances at high-frequency shifts and the presence of two typical thiocarbynolic proton signals at δ 3.37 (td, *J* = 5.1, 1.7 Hz) and 3.53 (dd, *J* = 9.3, 3.2 Hz) assigned to H-3 and H-6, respectively. These observations suggest the opening of the 5β,6β-epoxy group and the formation of a thioether bridge between C-3 and C-6. The location of the thioether bridge was confirmed by the HMBC correlations between the signal of H-3 and C-1 at δ 210.9 and C-4 at δ 79.6, H-4 (δ 4.28 d, *J* = 5.5 Hz) and C-3 at δ 38.9 and C-6 at δ 41.7, and H-6 and C-5 at δ 79.5 and C-10 at δ 55.3. Considering the *cis* A/B ring fusion, the ring strain and an acceptable distance of a thioether linkage, the orientation of the thioether bridge between C-3 and C-6 was rationally assigned to be α relative to the rings and confirmed by comparison of the spectroscopic data of withaperuvins F and G, isolated from *Physalis peruviana*,[30] and (20*S*,22*R*)-3α,6α-epoxy-4β,5β,27-trihydroxy-1-oxowitha-24-enolide, isolated from *Withania somnifera*,[31] which possess an α-oriented ether linkage between C-3 and C-6 positions.

The formation of **5** can be rationalized as follows: KSAc selectively attacked the C6 position leading to oxirane ring-opening with inversion of configuration at the attacked center (C6), followed by thioacetate moiety hydrolysis and further cyclization through an intramolecular thia-Michael reaction at C3 (Scheme 2).[32]



Scheme 2. Proposed mechanism involved in the formation of **5** and **9**.

The molecular formula of compound **6** was determined by HRESIMS as $C_{56}H_{76}O_{12}$, indicating that compound **6** is a dimer, and sulfur does not participate in the linkage. A detailed analysis of the 1D and 2D NMR data enabled the complete assignment of each unit of the dimer. The ^{1}H and ^{13}C NMR data of one of the units (donor) were almost identical to those of WA, while the ^{1}H and ^{13}C NMR data of the second unit (acceptor) were almost identical to those of compounds **1–3**, suggesting the nucleophilic attack of the 4-OH moiety of one unit on the C-3 center of the second unit, in an intermolecular oxa-Michael addition reaction. The positions involved in the link of both units were established by the cross-correlation peaks observed in the HMBC experiment. The key correlations observed in the latter were for H-4 (δ 3.76, d, *J* = 5.8 Hz) with C-5′ (δ 65.1) and for H-2a′ (δ 3.02, dd, *J* = 14.8 and 6.1 Hz) with C-6 (δ 62.8).

When a PEG300/H_2O mixture was used (entry 4), the 3β-thioacetoxy analog (**7**) was isolated as sole product. The identity of the thioacetoxy derivative was suggested by the molecular formula of compound **7**, $C_{30}H_{42}O_7S$, and confirmed by NMR analyses. The ^{1}H NMR and ^{13}C NMR spectra of compound **7** were very similar to those of compounds **1–3**. The most remarkable differences were the upfield shift of the C-3 resonance, which appears at δ 72.7-77.7 in compounds **1–3** and at δ 41.5 in **7**, and the signals corresponding to a thioacetyl group (δ_H 2.35, δ_C 193.7 and 30.8).

In the case of the glycerol/H_2O mixture (entry 5), no reaction was detected under the conditions described and starting material was recovered. From these experiments, it is clear that finding a suitable solvent has a great influence in the reactivity and chemoselectivity of the system. This can be ascribed to nucleophile/substrate/product solubility in the reaction mixture and diverse solvation modes, leading to a different activation energy of the transition states involved. From this set of experiments, it becomes clear that the 1:1 *i*PrOH/H_2O mixture resulted in the solvent of choice attaining diversity with sulfur nucleophiles.

Next, with the aim of increasing the thiodiversification product yield, the amount of KSAc was raised to 3.5 equiv. (entry 6). Under these conditions, compound **5** was again obtained, together with two new thio derivatives, **8** and **9**. Compound **8** had the molecular formula $C_{32}H_{44}O_8S$, as determined by HRESIMS. Inspection of the 1D and 2D NMR spectroscopic data indicated that compound **8** possessed rings C and D, as well as a side-chain closely related to those of WA. Regarding ring A, the ^{1}H NMR spectrum of **8** showed characteristic signals of H-2, H-3, H-4, and acetate group hydrogens for a 1-oxo-2-ene-4β-acetoxy derivative at δ 6.06 (dd, *J* = 10.4,2.4 Hz, H-2), 6.32 (dd, *J* = 10.4,2.0 Hz, H-3), 6.15 (t, *J* = 2.3 Hz, H-4), and δ 2.26 (s, CH_3CO), respectively. As for ring B, the absence of the characteristic signal of an epoxy group at C-5-C-6 and resonances at δ 4.08 (1H, dd, *J* = 13.1, 4.2 Hz) and δ 2.35 (3H, s) were indicative of a 5β-hydroxy,6α-thioacetoxy-substitution pattern. The coupling constants in the H-6 resonance confirmed the α orientation of the 6-thioacetoxy substituent. The ^{13}C NMR spectrum showed the expected chemical shifts for C-5, C-6, and thioacetoxy groups at δ 78.7, 47.3, 194.7, and 31.0, respectively.

The HRESIMS of compound **9** showed a quasimolecular ion $[M+Na]^+$ at *m/z* 559.2167, corresponding to a formula of $C_{28}H_{40}O_6S_2Na$. The ^{1}H and ^{13}C NMR spectra of **9** had almost identical signals for all carbons and protons as compound **5**. Spectroscopic and spectrometric data of **9** suggested the presence of an unprecedented disulfide-bridge between the C-3 and C-6. The position of the disulfide-bridge was supported by HMBC correlations from H-3 (δ 3.29 m) to C-1 (δ 212.2), C-2 (δ 38.9), C-4 (δ 79.8), and C-5 (δ 77.4) and from H-6 (δ 3.35 m) to C-7 (δ 34.2) and C-8 (δ 30.7).

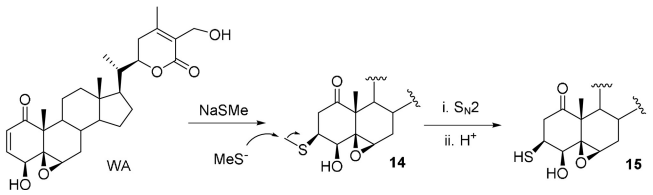
The formation of the latter, as proposed for **5**, would occur as follows: KSAc opened the epoxide and then the SAc-moiety underwent hydrolysis. In the presence of another molecule of KSAc, an oxidation event was able to deliver the acetyl disulfide derivative at C6, which, upon hydrolysis of the SAc group, led to the transient formation of an –SSH group, which immediately attacked C3 (Scheme 2). Similar examples can be found in the biosynthesis of certain cyclic disulfide natural products.[11]

Then, the reactivity of WA toward the bulkier nucleophile, potassium thiobenzoate (KSBz), was explored. The reaction of WA with KSBz (3.5 equiv.) in *i*OPr/H_2O yielded compounds **10** and **11** (entry 7 and 8). Compound **10** had a molecular formula of $C_{35}H_{44}O_7S$ by HRESIMS. The ^1H and ^{13}C NMR spectra of **10** were consistent with the presence of a thiobenzoyl group (δ_H 7.47-7.92, 5H and δ_C 189.9, 136.5, 127.5, and 129.0) assigned to C-3 position. As can be seen (entry 7), 5β,6β-epoxide remained intact and a Michael addition at C3 was the main reaction.

Compound **11** showed a molecular formula of $C_{42}H_{48}O_8S$ by HRESIMS. The NMR data indicated the presence of two benzoyl groups; a thiobenzoyl at C-3 and a benzoyloxy group at C-4. The location of the thiobenzoyl group was determined by a HMBC experiment, showing correlation between the resonances of H-3 (δ 4.34 m) and the carboxyl signal at δ 188.6, while the location of the benzoyloxy group was determined by the correlation between the resonances of H-4 (δ 5.02, d, $J = 2.6$ Hz) with the carboxyl signal at δ 164.9.

Under these conditions, KSBz was unable to open the oxirane ring. This suggests that rearrangements through intramolecular thia-Michael additions may comprise two main features: i) the nucleophile ability to react with the epoxide, thus forming the corresponding thioester, and ii) an ease to undergo acyl cleavage to give rise to the thiolate reactive intermediate (see Scheme 2). Similarly, when NH_4SCN was used as a nucleophile under the same conditions, the 6α-thiocyanate derivative (**12**) was obtained (entry 8). The NMR data of **12** matched the proposed structure, given the following observations: (i) the absence of the typical proton signal of an epoxy group at C-5-C-6 position and the appearance of the proton signal at δ 3.72 (dd, $J = 13.9$, 6.8 Hz) assigned at H-6; (ii) the α orientation of the thiocyanate group at C-6, deduced from the value of the coupling constant between H-6 and H_2-7; and (iii) the signals at δ 78.5, 54.8, and 114.6 in the ^{13}C NMR spectrum assigned to C-5, C-6, and thiocyanate group, respectively. No further rearrangements were noticed, since thiolate formation from SCN moiety cleavage is not favored, compared with its thioester counterpart. Moreover, conducting the reaction with NaSMe as the nucleophile, the 3β-O*i*Pr analog **3** and three novel derivatives, compounds **13**-**15** were obtained (entry 9).

Compounds **3**, **13** and **14** could not be obtained in a pure form by normal or reversed-phase chromatography techniques. Consequently, these compounds were characterized from 6.9 mg of a mixture in a 3:1:3 ratio. The ^1H NMR data of compound **13** were similar to that of **12**. The differences observed between **12** and **13** were the upfield shift of the thiocarbynolic proton from δ 3.72 for compound **12** to δ 3.08 for compound **13**, assigned to C-6, and the presence of the typical SCH_3 methyl signal at δ 2.34 s, indicating the opening of the 5β,6β-epoxy group and the formation of the 5β-hydroxy,6α-thiomethyl derivative. The α orientation of the SMe group was determined by the multiplicity and coupling constant value of the signal corresponding to H-6 (dd, $J = 13.7$, 4.4 Hz). Compound **14** showed a molecular formula of $C_{29}H_{42}O_6S$ by HRESIMS and the ^1H and ^{13}NMR data showed the signals corresponding to a SMe group (δ_H 2.15 s and δ_C 14.9). These data indicated a 3β-thiomethyl derivative. As compounds **13** and **14** were characterized from a mixture, their structures were tentatively proposed as (17*R*,20*S*,22*R*)-4β,5β,27-trihydroxy-1-oxo-6α-thiomethyl-witha-2,24-dienolide and (17*R*,20*S*,22*R*)-5β,6β-epoxy-4β,27-dihydroxy-1-oxo-3β-thiomethyl-witha-24-enolide, respectively. Finally, compound **15** revealed a molecular formula of $C_{28}H_{40}O_6S$ by HRESIMS. The ^1H and ^{13}C NMR spectra of compound **15** were closely related to that of **14**. The main differences observed between compounds **15** and **14** were the absence of the SMe signals and the downfield shift of the H-3 and C-3 resonances, which appear at δ_H 3.23 and δ_C 44.4 in compounds **14** and at δ_H 4.33 and δ_C 68.7 in **15**. The formation of this 3β-SH derivative can be rationalized by a S_N2 reaction of a second equivalent of NaSMe in the 3β-SMe group, followed by protonation of the thiolate leaving group,[33] as depicted in Scheme 3. It is worth noting that C-3 and C-6 are



Scheme 3. Proposed mechanism involved in the formation of **14** and **15**.

accessible to this nucleophile, but no cyclization was detected, as thiolate formation was involved only in the formation of derivative **15,** in which the β-stereochemistry at C-3 precludes the intramolecular nucleophilic attack on oxiranic C6 and therefore thiolate protonation takes place.

As discussed above, not only does the size of the sulfur nucleophile play a crucial role but also its ability, once attached to the substrate, to generate a thiolate group that may undergo further reactions.[34]

For all Michael-type derivatives at C-3, the orientation of the substituent was beta, based on the multiplicity and coupling constant value of the signal corresponding to H-4. The strict stereocontrol exerted by the *cis* A–B ring-fusion is remarkable, since the 3α-epimer was not detected.[20,24,35]

Energy minimizations were performed for WA derivatives. The results showed that subtle structural changes drastically distort the A–B ring system, not only by adding new rings but also through simple modifications such as substitution or hybridization change. For instance, compound **5** and **9** have an additional 5- and 6-membered ring, respectively, and showed a completely different conformation than **14** or **12** (for more details see figure SI-1 in the Supplementary material).

Prediction of molecular properties and drug-likeness were calculated [log P and molecular polar surface area (TPSA)]. The results showed that creating structurally more complex entities led to a significant impact on log P [ranging from 3.5 (**4**) to 8.0 (**11**)] and TPSA [ranging from 96 (**15**) to 181 (**6**)], which may influence solubility, hydrophobicity, permeability, among other parameters (see Table S4 for more details).

Summarizing, in this study we have established a straightforward methodology for the thiodiversification of a bioactive compound, WA, under environmentally friendly conditions by using a small set of commercially available sulfur nucleophiles in water:alcohol mixtures.

In this study, from ca. 300 mg of WA as starting compound, it was possible to obtain and characterize fifteen derivatives, thirteen of which are novel chemical entities, thus showing the productivity and effectiveness of the methodology. In this context, from a screening and optimization point of view, the global efficiency of the reaction in terms of chemical yield becomes less important than the exploration of chemical space. From the fifteen derivatives obtained, ten possess sulfur in their structure and two of them feature thiacycles of five or six members, the latter forming an interesting disulfide bridge. This protocol enables the formation of new sp^3 carbons at the expense of sp^2, with a concomitant rise in stereocenters, strain release and the formation of new cyclic motifs, along with discrete changes in the log P and TPSA values. Taken together, these features make the proposed thiodiversification a powerful tool for the creation of biologically relevant chemical diversity from privileged scaffolds, such as NPs, under benign conditions.

Supporting Information Summary

Additional supporting information with complete experimental procedures, minimum energy structures, ^{1}H and ^{13}C-NMR assignment, 1D and 2D spectra of new derivatives and other relevant information may be found in the online version of this article at the publisher's web site.

Acknowledgments

Authors acknowledge IMBIV-CONICET, INFIQC-CONICET and Universidad Nacional de Córdoba (UNC). This work was partly supported by CONICET, SECyT-UNC and FONCyT. C.N.C. and M.G. acknowledge CONICET for the receipt of a fellowship. J. G-N acknowledge CIN for the receipt of a fellowship (Beca de Estímulo a las Vocaciones Científicas). We are grateful to Dr. G. Bonetto for NMR assistance and Prof. G. Barboza for the plant identification and Joss Heywood for English language editing.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: natural products · sulfur heterocycles · sulfur nucleophiles · sustainable chemistry · withaferin A

[1] M. Kopach, D. Leahy, J. Manley, *Innovations in Pharmaceutical Technology* **2012**, *43*, 42–75. http://www.iptonline.com/articles/public/ACSGreenChemistry.pdf
[2] R. J. Giraud, P. A. Williams, A. Sehgal, E. Ponnusamy, A. K. Phillips, J. B. Manley, *ACS Sustainable Chem. Eng.* **2014**, *2*, 2237–2242.
[3] P. Richardson in *Green Chemistry and High Throughput Screening. Green Chemistry Strategies for Drug Discovery.* RSC Drug Discovery Series N° 46, (Eds.: E. A. Peterson, J. B. Manley), Royal Society of Chemistry, **2015**, pp. 94–126.
[4] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2012**, *75*, 311–335.
[5] M. S. Butler, *J. Nat. Prod.* **2004**, *67*, 2141–2153.
[6] A. L. Harvey, R. Edrada-Ebel, R. J. Quinn, *Nat. Rev Drug Discov.* **2015**, *14*, 111–129.
[7] M. A. Koch, A. Schuffenhauer, M. Scheck, S. Wetzel, M. Casaulta, A. Odermatt, P. Ertl, H. Waldmann, *Proc. Natl. Acad. Sci. U S A*, **2005**, *102*, 17272–17277.
[8] J. Reymond, *Acc. Chem. Res.* **2015**, *48*, 722–730.
[9] P. Ertl, S. Jelfs, J. Mühlbacher, A. Schuffenhauer, P. Selzer, *J. Med. Chem.* **2006**, *49*, 4568–4573.
[10] H. Lachance, S. Wetzel, K. Kumar, H. Waldmann, *J. Med. Chem.* **2012**, *55*, 5989–6001.
[11] K. C. Nicolaou, M. Lu, S. Totokotsopoulos, P. M. Heretsch, D. Giguere, Y. Sun, D. Sarlah, T. H. Nguyen, I. C. Wolf, D. F. Smee, C. W. Day, S. Bopp, E. A. Winzeler, *J. Am. Chem. Soc.* **2012**, *134*, 17320–17332.
[12] P. M. Dewick in *Medicinal Natural Products: A Biosynthetic Approach*, 2rd Edition, Wiley& Sons Ltd, **2002**, pp. 1–485.
[13] C. Jacob, *Nat. Prod. Rep.* **2006**, *23*, 851–863.
[14] M. D. Pluth, T. S. Bailey, M. D. Hammers, M. D. Hartle, H. A. Henthorn, A. K. Steiger, *Synlett* **2015**, *26*, 2633–2643.
[15] N. Boyer, K. C. Morrison, J. Kim, P. J. Hergenrother, M. Movassaghi, *Chem. Sci.* **2013**,*4*, 1646–1657.
[16] R. Misico, V. Nicotra, J. Oberti, G. Barboza, R. Gil, G. Burton in *Withanolides and related steroids. Progress in the Chemistry of Organic Natural Products*, Ed. Springer-Wien-NewYork, **2011**, pp. 127–229.
[17] P. Bargagna-Mohan, A. Hamza, Y–E. Kim, Y. KhuanHo, N. Mor-Vaknin, N. Wendschlag, J. Liu, R. M. Evans, D. M. Markovitz, C.-G. Zhan, K. Bo Kim, R. Mohan, *Chem. Biol.* **2007**, *14*, 623–634.
[18] S. Amslinger, *ChemMedChem* **2010**, *5*, 351–356.
[19] L. Misra, P. Lal, N. D. Chaurasia, R. S. Sangwan, S. Sinha, R. Tuli, *Steroids* **2008**, *73*, 245–251.
[20] S. K. Yousuf, R. Majeed, M. Ahmad, P. Sangwan, B. Purnima, A. K. Saxsena, K. A. Suria, D. Mukherjee, S. C. Taneja, *Steroids* **2011**, *76*,1213-1222.
[21] Y. Xu, M. T. Marron, E. Seddon, S. P. McLaughlin, D. T. Ray, L. Whitesell, A. A. L. Gunatilaka, *Bioorg. Med. Chem.* **2009**, *17*, 2210–2214.
[22] G. G. Llanos, L. M. Araujo, I. A. Jiménez, L. M. Moujir, I. L. Bazzocchi, *Eur. J. Med. Chem.* **2012**, *54*, 499–511.
[23] E. M. Kithsiri Wijeratne, Y. Xu, R. Scherz-Shouval, M. T. Marron, D. D. Rocha, M. X. Liu, L. V. Costa-Lotufo, S. Santagata, S. Lindquist, L. Whitesell, A. A. L. Gunatilaka, *J. Med. Chem.* **2014**, *57*, 2851–2863.
[24] S. Tao, J. Tillotson, E. M. Wijeratne, Y. Xu, M. Kang, T. Wu, E. C. Lau, C. Mesa, D. J. Mason, R. V. Brown, J. J. La Clair, A. A. Gunatilaka, D. D. Zhang, E. Chapman, *ACS Chem. Biol.* **2015**, *10*, 1916–1924.
[25] F. R. Bisogno, A. Cuetos, I. Lavandera, V. Gotor, *Green Chem.* **2009**, *11*, 452–454.
[26] C. Capello, U. Fischera, K. Hungerbühler, *Green Chem.* **2007**, *9*, 927–934.
[27] D. Lavie, E. Glotter, Y. Sho, *J. Chem. Soc.* **1965**, 7517–7531.
[28] S. S. Nittala, D. Lavie, *J. Chem. Soc., Perkin Trans.* **1982**, *1*, 2835–2839.
[29] Selective acylation of *sec*-alcohol in the presence of a *prim*-alcohol can be explained by assuming that the actual acyl donor is an *in situ* formed thioester moiety pending either on WA C3 or C6, thus favoring the approach of the acyl group to the *sec*-alcohol over the *prim*-alcohol. Similar *sec*-selective acylation can be found in enzymatic (see for instance: F. Moris, V. Gotor, *J. Org. Chem.* **1993**, *58*, 653–660) and organocatalized (see for instance: T. Kawabata, W. Muramatsu, T. Nishio, T. Shibata, H. Schede *J. Am. Chem. Soc.*, **2007**, *129*, 12890–12895) reactions.
[30] P. Neogi, M. Sahai, A. B. Ray, *Phytochemistry* **1987**, *26*, 243–247.

[31] J. Zhao, N. Nakamura, M. Hattori, T. Kuboyama, C. Tohda, K. Komatzsu, *Chem. Pharm. Bull.* **2002**, *50*, 760–765.
[32] For a similar thioester cleavage/thia-Michael addition in natural products synthesis, see: H. Tohma, Y. Harayama, M. Hashizume, M. Iwata, Y. Kiyono, M. Egi, Y. Kita, *J. Am. Chem. Soc.* **2003**, *125*, 11235–11240.
[33] L. Testaferri, M. Tingoli, M. Tiecco. *Tetrahedron Lett.* **1980**, *21*, 3099–3100.
[34] A. Heredia, S. M. Soria-Castro, L. M. Bouchet, G. Oksdath-Mansilla, C. A. Barrionuevo, D. A. Caminos, F. R. Bisogno, J. E. Argüello, A. B. Peñéñory, *Org. Biomol. Chem.* **2014**, *12*, 6516–6526.
[35] M. E. García, G. E. Barboza, J. C. Oberti, C. Ríos-Luci, J. M. Padrón, V. E. Nicotra, A. Estévez-Braun, A. G. Ravelo, *Phytochemistry*, **2012**, *76*, 150–157.

Submitted: August 16, 2017
Revised: November 1, 2017
Accepted: November 2, 2017