The Journal of Antibiotics (2019) 72:246–251
https://doi.org/10.1038/s41429-018-0137-9

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

BRIEF COMMUNICATION

# Stable isotope feeding studies reveal a steroid 5(6→7)*abeo* ring contraction biogenesis for the antibiotic solanioic acid produced by cultures of the fungus *Rhizoctonia solani*

Rukshana Mohamad-Ramshan[1] · Pamoda B. Ratnaweera[2] · David E. Williams[3] · E. Dilip de Silva[1] · Raymond J. Andersen[3]

Received: 30 September 2018 / Revised: 17 November 2018 / Accepted: 13 December 2018 / Published online: 11 January 2019


## Abstract

Culture feeding experiments with [1-$^{13}$C]-acetate, [2-$^{13}$C]- acetate, and [1,2-$^{13}$C]-acetate have shown that the steroid ring B contraction involved in the biogenesis of the unprecedented carbon skeleton of the antibiotic solanioic acid (**1**) by the fungus *Rhizoctonia solani* involves cleavage of the C-5/C-6 bond. The study revealed that 9-*epi*-solanioic acid (**4**), which spontaneously converts to solanioic acid (**1**), is also produced by the cultures and it may be the actual natural product.

It has been estimated that 700,00 people a year die from infections with microbial pathogens that are resistant to antibiotic treatment and the problem continues to grow due to a lack of new antibiotics being developed for clinical use [1]. *Staphylococcus aureus* is the most common cause of human bacterial infections in hospitals, long-term care centers, and community settings [2]. Methicillin-resistant *S. aureus* (MRSA), one of the "ESKAPE" pathogens [3], is resistant to all known β-lactam antibiotics, and clinical isolates of MRSA have shown reduced susceptibility to vancomycin and resistance to linezolid and daptomycin [4]. This perilous resistance landscape has created an urgent need for new antibiotics to treat MRSA infections. The majority of antibiotics available for use by clinicians are microbial natural products or synthetic analogs of microbial natural products [5]. For example, the widely used semi-synthetic β-lactam antibiotic methicillin was inspired by penicillin G, a metabolite of the fungus *Penicillium chrysogenum*. Fungi and other microbial natural product sources continue to be a major focus of new antibiotic discovery efforts in our laboratories [6–8] and elsewhere [5].

In 2015, we reported the production of the new antibiotic solanioic acid (**1**) (Fig. 1) by cultures of the fungus *Rhizoctonia solani* isolated from the tubers of the Sri Lankan weed *Cyperus rotundas* [9]. Solanioic acid (**1**) is a degraded and rearranged steroid with an unprecedented carbon skeleton featuring a highly functionalized conjoint ring system. It showed potent in vitro antimicrobial activity against a small panel of Gram-positive bacteria, including MRSA, with MICs of 1 μg ml$^{-1}$, but it was only weakly active against the yeast *Candida albicans* (MIC 16 μg ml$^{-1}$) and inactive against Gram-negative bacteria at 64 μg ml$^{-1}$. Solanioic acid (**1**) represents a novel antibiotic scaffold with significant in vitro activity against MRSA that warrants further investigation as an antibacterial drug lead.

Steroids with contracted B rings are rarely encountered as natural products, with less than a dozen examples reported from human plasma [10], plants [11, 12], sponges [13, 14], and starfish [15]. Nearly all of these natural products, exemplified by **2** and **3** (Fig. 1) isolated from a plant and a sponge, respectively, have five-membered B rings containing an aldehyde appendage with a β-tertiary alcohol indicating that the ring contraction arose from oxidative

**Supplementary information** The online version of this article (https://doi.org/10.1038/s41429-018-0137-9) contains supplementary material, which is available to authorized users.

✉ E. Dilip de Silva
edilip.desilva@gmail.com

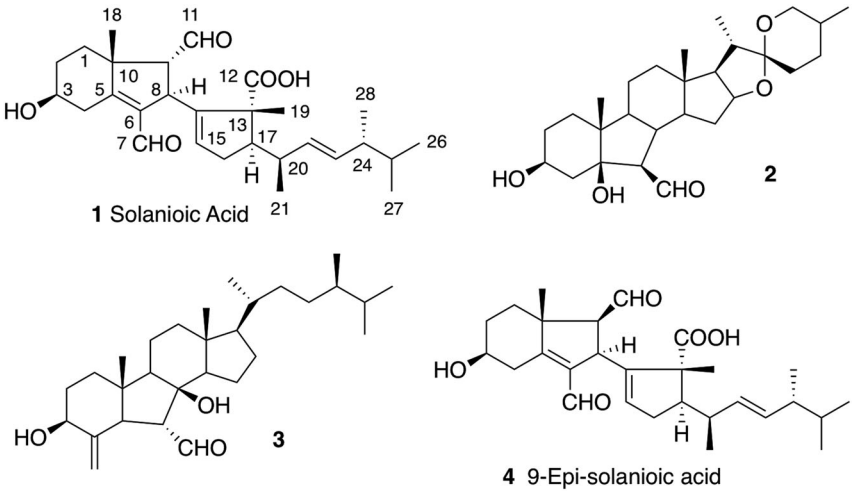
✉ Raymond J. Andersen
raymond.andersen@ubc.ca

[1] Department of Chemistry, University of Colombo, Colombo 00300, Sri Lanka

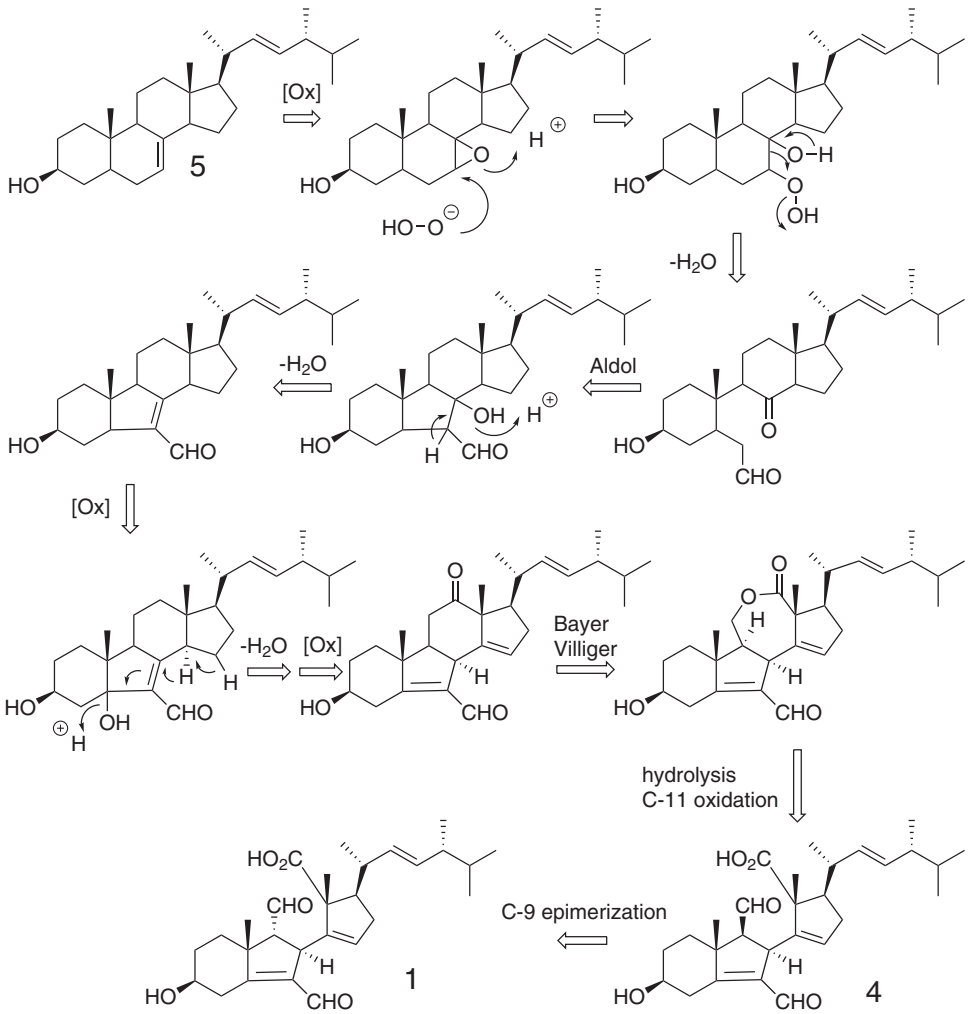
[2] Department of Science and Technology, Uva Wellassa University, Badulla, Sri Lanka

[3] Departments of Chemistry and Earth, Ocean and Atmospheric Sciences, University of British Columbia, 2036 Main Mall, Vancouver, BC V6T 1Z6, Canada

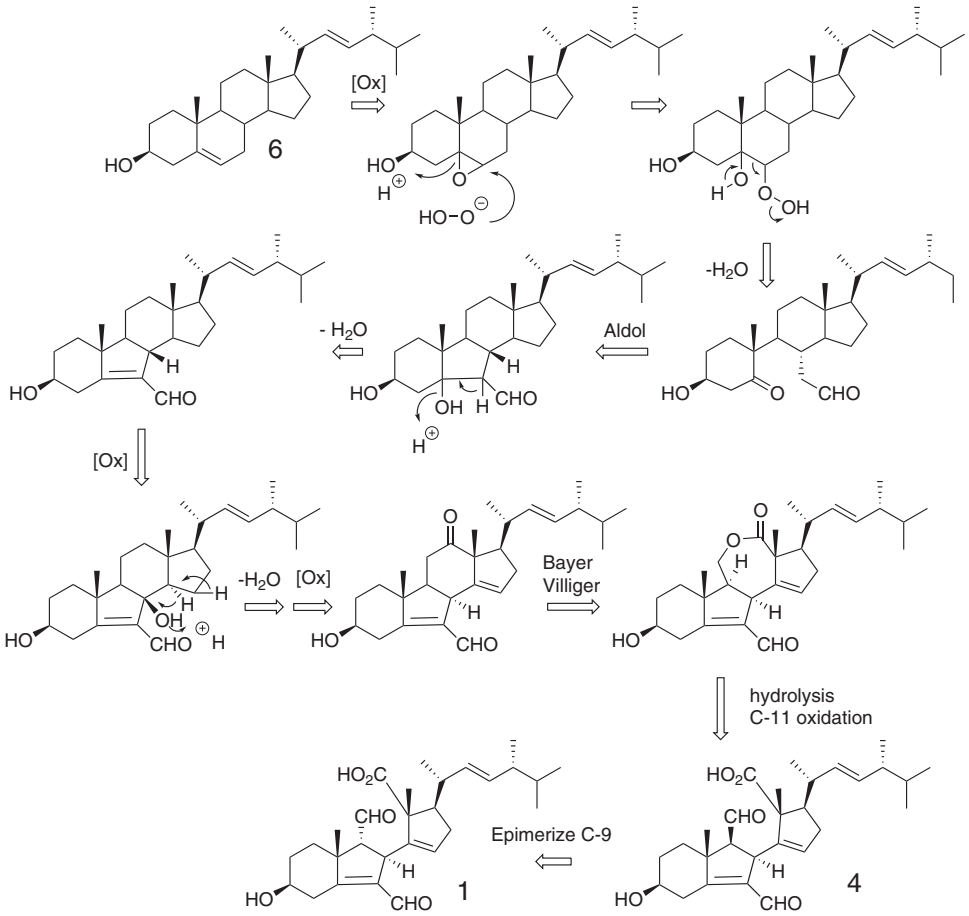
**Fig. 1** Compounds **1** to **4**




**Scheme 1** Proposed biogenesis of solanioic acid (**1**) from fungisterol (**5**) [5].




cleavage of a B ring double bond followed by an aldol condensation to give the new five-membered ring. The location of the tertiary alcohol at C-5 or C-8 presumably predicts whether the contraction was a 5(6→7)*abeo* (e.g. **2**) or 8(7→6)*abeo* (e.g. **3**) process, but there are no reported stable isotope feeding studies that confirm this expectation. Solanioic acid (**1**) appears to be the only B ring contracted steroid isolated to date from a fungus and since it lacks a




**Scheme 2** Proposed biogenesis of solanioic acid (**1**) from ergosta-5,22-dien-3-ol (**6**)

tertiary alcohol at either C-5 or C-8 there is no telltale marker unambiguously predicting whether its biogenesis involved a C-5/C-6 or C7/C-8 alkene bond scission. The position of the alkene in the B ring of solanioic acid (**1**) might suggest a 5(6→7)*abeo* contraction; however, the configuration at C-8 in **1** is the opposite of that found in a normal steroid skeleton indicating that the precursor to **1** may have had a Δ [7, 8] alkene that was cleaved as we originally proposed (Scheme 1) [5].

In order to distinguish between the two possible ring B contraction routes, we have carried out feeding experiments with [1-$^{13}$C]-acetate, [2-$^{13}$C]-acetate, and [1,2-$^{13}$C]-acetate. Unexpectedly, workup of one of the feeding study cultures yielded not only solanioic acid (**1**) but also its C-9 epimer **4** (Fig. 1). The details of the isolation and structure elucidation of 9-*epi*-solanioic acid (**4**) and the results of the $^{13}$C labeling studies are described below.

As part of our report of solanioic acid (**1**), we proposed a biogenetic route to its new carbon skeleton that involved an oxidative cleavage of the C-7/C-8 alkene bond in fungisterol (**5**) to give a C-8 ketone and a C-7 aldehyde that underwent an intramolecular C-6/C-8 aldol condensation to give a contracted five-membered ring B with aldehyde and tertiary alcohol substituents (Scheme 1) [5]. An alternative route to the contracted ring B, that was not mentioned in our original report, would involve oxidative cleavage of the C-5/C-6 alkene bond in ergosta-5,22-dien-3-ol (**6**) to give a C-5 ketone and a C-6 aldehyde followed again by an intramolecular C-7/C-5 aldol condensation to give a contracted ring B with aldehyde and tertiary alcohol substituents (Scheme 2). Both ring B contracted intermediates could be elaborated with virtually identical common biosynthetic transformations to solanioic acid (**1**) (Schemes 1 and 2) so that these proposals do not favor one alkene cleavage over the other.

Figure 2 illustrates that the aldehyde substituent at C-7 should be labeled by [1-$^{13}$C]-acetate if the ring B cleavage involves the C-5/C-6 bond and by [2-$^{13}$C]-acetate if the cleavage involves the C-7/C-8 bond. In addition, C-5 and C-6 should be part of the same intact acetate unit only if the cleavage is at C-7/C-8. Therefore, the feeding studies were expected to clearly distinguish between the two pathways.

Stable isotope feeding experiments were carried out by growing cultures of *R. solani* on the surface of solid agar containing potato dextrose media and either [1-$^{13}$C]-acetate, [2-$^{13}$C]-acetate, or [1,2-$^{13}$C]-acetate. The cultures were allowed to grow at RT for 7 days before harvesting by cutting the solid agar and attached fungal mass into small pieces and

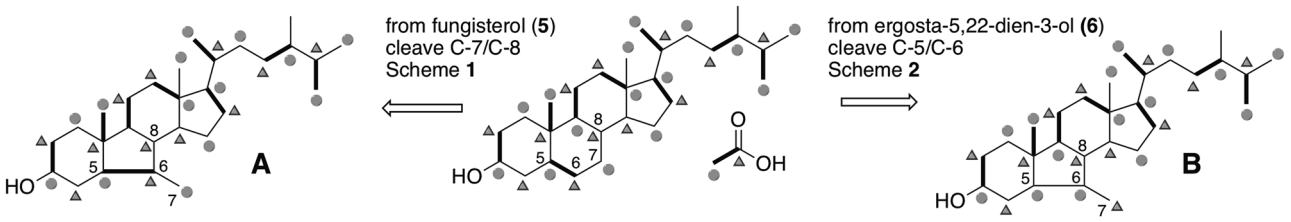



**Fig. 2** Predicted $^{13}$C labeling patterns from feeding [1,2-$^{13}$C]acetate. Panel **A** illustrates labeling resulting from cleavage of C-7/C-8 and Panel **B** illustrates labelling from C-5/C-6 cleavage

**Table 1** NMR data for 9-*epi*-solanioic acid (**4**) and comparison with the NMR data of solanioic acid (**1**) recorded in DMSO-$d_6$

| Position | 1 | | 4 | |
|---|---|---|---|---|
| | $^{13}$C ($\delta$) | $^{1}$H ($\delta$) | $^{13}$C ($\delta$) | $^{1}$H ($\delta$) |
| $1_{ax}$<br>$1_{eq}$ | 32.7 | 1.25 td $J = 13.1$, 3.7 Hz 1.66 dt $J = 13.1$, 3.1 Hz | 37.8 | 1.25 td $J = 12.6$, 3.0 Hz<br>1.67[a] |
| $2_{ax}$<br>$2_{eq}$ | 30.7 | 1.56 m<br>1.72 bd $J = 13.2$ Hz | 30.3 | 1.56 bddd $J = 12.6$, 12.6, 12.6 Hz<br>1.67[a] |
| 3 | 69.9 | 3.33 m | 70.4 | 3.35 m |
| $4_{ax}$<br>$4_{eq}$ | 33.3 | 2.09 tm $J = 12.5$ Hz<br>3.42 ddd $J = 12.5$, 4.6, 1.4 Hz | 33.4 | 2.13[a]<br>3.44 bdd $J = 12.8$, 4.1 Hz |
| 5 | 163.8 | / | 162.5 | / |
| 6 | 134.3 | / | 134.7 | / |
| 7 | 188.9 | 9.73 s | 188.9 | 9.61 s |
| 8 | 40.8 | 3.86 bs | 43.0 | 3.73 d $J = 9.4$ Hz |
| 9 | 65.4 | 2.66 t $J = 3.1$ Hz | 60.1 | 2.51 dd $J = 9.4$, 5.8 Hz |
| 10 | 52.1 | / | 51.5 | / |
| 11 | 202.9 | 9.64 d $J = 3.1$ Hz | 206.0 | 9.56 d $J = 5.8$ Hz |
| 12 | 177.4 | / | 177.4 | / |
| 13 | 57.5 | / | 58.1 | / |
| 14 | 150.8 | / | 145.1 | / |
| 15 | 126.6 | 5.28 m | 130.9 | 5.61 m |
| 16a<br>16b | 34.9 | 1.84<br>2.14 | 35.4 | 1.95 dd $J = 16.1$, 8.6 Hz<br>2.21 ddd $J = 16.1$, 8.6, 2.52 Hz |
| 17 | 52.4 | 2.51 | 51.5 | 2.55 ddd $J = 8.6$, 8.6, 8.6 Hz |
| 18 | 25.9 | 1.34 s | 19.6 | 1.19 s |
| 19 | 15.6 | 0.98 s | 14.8 | 0.92 s |
| 20 | 37.5 | 2.13 | 37.6 | 2.12[a] |
| 21 | 19.7 | 0.84 d $J = 6.8$ Hz | 19.8 | 0.86 d $J = 6.5$ Hz |
| 22 | 133.8 | 5.18 dd $J = 15.4$, 8.0 Hz | 132.7 | 5.18 dd $J = 15.3$, 8.4 Hz |
| 23 | 132.6 | 5.26 dd $J = 15.4$, 7.7 Hz | 132.7 | 5.26 dd $J = 15.3$, 7.0 Hz |
| 24 | 42.1 | 1.85 | 42.1 | 1.85 qdd $J = 7.0$, 7.0, 7.0 Hz |
| 25 | 32.5 | 1.44 m | 32.5 | 1.45 m |
| 26 | 19.8 | 0.79 d $J = 7.0$ Hz | 20.0 | 0.79 d $J = 6.7$ Hz |
| 27 | 19.5 | 0.78 d $J = 6.8$ Hz | 19.5 | 0.78 d $J = 7.0$ Hz |
| 28 | 17.4 | 0.88 d $J = 6.8$ Hz | 17.3 | 0.88 d $J = 7.0$ Hz |
| 12-COO<u>H</u> | / | 12.32 bs | / | 12.46 bs |

[a]Multiplicity not determined due to overlapping signals/chemical shifts determined from 2D data.

extracting them by soaking in ethyl acetate. Crude ethyl acetate extracts were concentrated in vacuo and fractionated using gradient Si-gel and Sephadex LH20 chromatography, and reversed phase HPLC, to give pure $^{13}$C-labeled solanioic acid (**1**) from all three cultures and 9-*epi*-solanioic acid (**4**) only from the [1-$^{13}$C]-acetate feeding experiment.

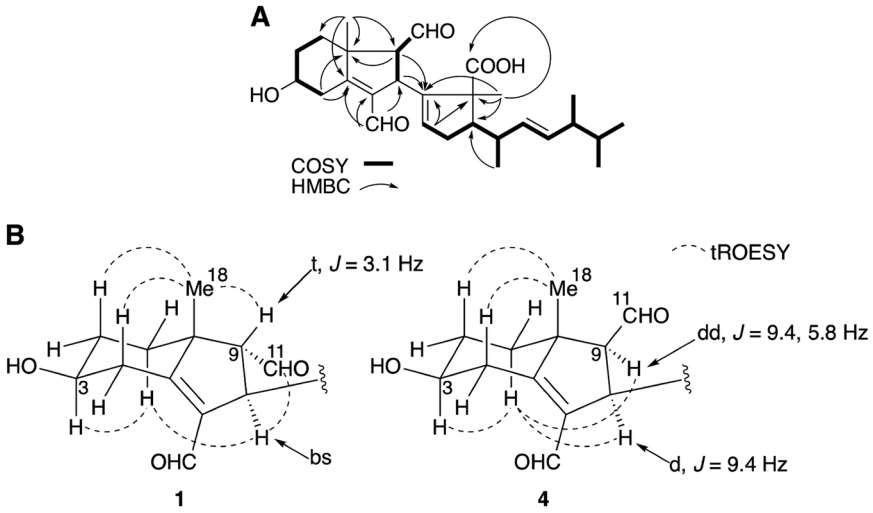



**Fig. 3** COSY (Panel **A**), HMBC (Panel **A**), tROESY correlations (Panel **B**), and *J* couplings (Panel **B**) for H-8 and H-9 of **1** and **4** used to assign the planar structure and configuration of 9-*epi*-solanioic acid (**4**)

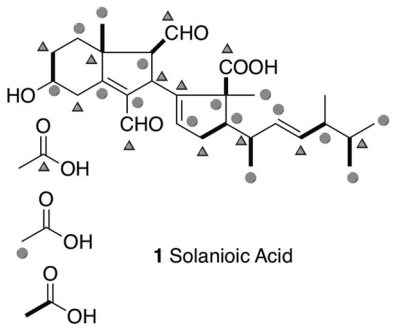



**Fig. 4** Labeling pattern observed in solanioic acid (**1**) resulting from separate feeding experiments with [1-$^{13}$C]-acetate, [2-$^{13}$C]-acetate, and [1,2-$^{13}$C]-acetate

9-*Epi*-solanioic acid (**4**) was isolated as a clear viscous oil that gave an $[M + Na]^+$ ion at *m/z* 479.2765 (calcd for $C_{28}H_{40}O_5Na$, 479.2773) in the HRTOFESIMS appropriate for a molecular formula of $C_{28}H_{40}O_5$ identical to that of solanioic acid (**1**). The 1D $^{13}$C and $^1$H NMR data obtained for **4** showed a close resemblance to the data obtained for solanioic acid (**1**) (Table 1) and analysis of the 2D NMR data obtained for **4** (Supporting Information) showed that it had the same planar structure as **2** (Fig. 3a). Therefore, **4** was presumed to be a stereoisomer of **2**. The largest deviations in the NMR data for **1** and **4** were observed in the $^1$H and $^{13}$C chemical shifts for H-7 ($\delta$ **1**: 9.73; **4**: 9.61), H-8 ($\delta$ **1**: 3.86; **4**: 3.73), and H-9 ($\delta$ **1**: 2.66; **4**: 2.51) and C-8 ($\delta$ **1**: 40.8; **4**: 43) and C-9 ($\delta$ **1**: 65.4; **4**: 60.1) indicating that the configurational differences were located in this region of the stereoisomers. ROESY correlations were observed between the H-9 ($\delta$ 2.66) and Me-18 ($\delta$ 2.51) resonances in **1** consistent with H-9 being *cis* to Me-18, while ROESY correlations were observed between the H-9 and H-$1_{ax}$ ($\delta$ 1.25) resonances in **4** consistent with H-9 being *cis* to H-$1_{ax}$ and *trans* to Me-18 (Fig. 3b). The NMR data described above were consistent with **4** being the C-9 epimer of **1**. Upon sitting in the NMR tube in DMSO-$d_6$, **4** spontaneously and quantitatively converted to **1** confirming our structure assignment.

Examination of the $^{13}$C NMR spectrum of **1** and **4** isolated from the [1-$^{13}$C]-acetate and [2-$^{13}$C]-acetate feeding experiments showed that C-7 (**1**: $\delta$ 188.9), C-11 (**1**: $\delta$ 202.9), and C-12 (**1**: $\delta$ 177.4) were labeled by [1-$^{13}$C]-acetate and not labeled by [2-$^{13}$C]-acetate as shown in Fig. 4 and Supporting Information Table SI-1. The $^{13}$C NMR of solanioic acid (**1**) isolated from the [1,2-$^{13}$C]-acetate feeding experiment showed intense flanking doublets for C-11 and C-12 indicative of these carbons coming from intact acetate units, while the C-5 ($\delta$ 163.8) resonance showed no strong flanking doublet but rather just an enriched natural abundance singlet and a series of weak unresolved doublets at its base indicating that it was not part of an intact acetate unit but had multiple long-range couplings to other acetate units in the same molecules resulting from very high incorporation of [1,2-$^{13}$C]-acetate. The complete incorporation pattern resulting from the three feeding experiments is shown in Fig. 4. This labeling pattern is exactly what is expected for cleavage of a C-5/C-6 alkene bond followed by an intramolecular aldol condensation between C-7 and C-5 to give the contracted ring B as outlined in Scheme 2 and Fig. 2b. The isolation of 9-*epi*-solanioic acid (**4**) suggests that first natural product formed by this pathway is **4**, that has the normal C-9 steroid configuration. 9-*epi*-solanioic acid (**4**) is then either biosynthetically or spontaneously epimerized to solanioic acid (**1**), which is the thermodynamically most stable epimer.

**Acknowledgements** M. Leblanc provided technical assistance with fungal culturing. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Publisher's note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References


1. O'Neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. 2014. https://amr-review.org/.
2. Klein E, Smith DL, Laxminarayan R. Community-associated methicillin-resistant *Staphylococcus aureus* in outpatients, United States, 1999–2006. Emerg Infect Dis. 2009;15:1925–30.
3. Brown D. Antibiotic resistance breakers: can repurposed drugs fill the antibiotic discovery void? Nat Rev Drug Discov. 2015;14:821–32.
4. Lehar SM, Pillow T, Xu M, et al. Novel antibody–antibiotic conjugate eliminates intracellular *S. aureus*. Nature. 2015;527:323–8.
5. Demain AL, Sanchez S. Microbial drug discovery: 80 years of progress. J Antibiot. 2009;62:5–16.
6. Barsby T, Kelly MT, Gagne S, Andersen RJ. Bogorol A produced in culture by a marine *Bacillus* sp. reveals a novel template for cationic peptide antibiotics. Org Lett. 2001;3:437–40.
7. Williams DE, Dalisay DS, Chen J, Polishchuk E, Patrick BO, Narula G, et al. Aminorifamycins and sporalactams produced in culture by a *Micromonospora* sp. isolated from a Northeastern-Pacific marine sediment are potent antibiotics. Org Lett. 2017;19:766–9.
8. Gerard J, Lloyd R, Barsby T, Haden P, Kelly MT, Andersen RJ. Massetolides A to H, antimycobacterial cyclic depsipeptides produced by two marine pseudomonads. J Nat Prod. 1997;60:223–9.
9. Ratnaweera PB, Williams DE, Patrick BO, de Silva ED, Andersen RJ. Solanioic acid, an antibacterial degraded steroid produced in culture by the fungus *Rhizoctonia solani* isolated from tubers of the medicinal plant *Cyperus rotundus*. Org Lett. 2015;17:2074–7.
10. Wentworth P, Nieva J, Takeuchi C, et al. Evidence for ozone formation in human atherosclerotic arteries. Science. 2003;302:1053–6.
11. Abdel-Azziz A, Brain K, Shatalebi MA, Blunden G, Patel A, Crabb T, et al. A B-ring contracted spirostane from *Tacca leontopetaloides*. Phytochemistry. 1990;29:2623–7.
12. Liu J, Yang C, Zhang J, Wu J, Chen Y. A New 5(6→7)abeo-sterol from the twigs of *Podocarpus fleuryi*. Nat Prod Res. 2017;31:175–80.
13. Miyamoto T, Kodama K, Aramaki Y, Higuchi R, Van Soest RWM. Orostanal, a novel abeo-sterol inducing apoptosis in leukemia cell from a marine sponge, *Stelletta hiwasaensis*. Tetrahedron Lett. 2001;42:6349–51.
14. Zhang H-J, Yi Y-H, Liu H-W. Oxygenated 4-methylidene sterols from the South China Sea sponge *Theonella swinhoei*. Helv Chim Acta. 2010;93:1120–6.
15. Thao NP, Cuong NX, Luyen BTT, et al. Steroidal constituents from the starfish *Astropecten polyacanthus* and their anticancer effects. Chem Pharm Bull. 2013;61:1044–51.