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Solanioic Acid, an Antibacterial Degraded Steroid Produced in Culture by the Fungus *Rhizoctonia solani* Isolated from Tubers of the Medicinal Plant *Cyperus rotundus*

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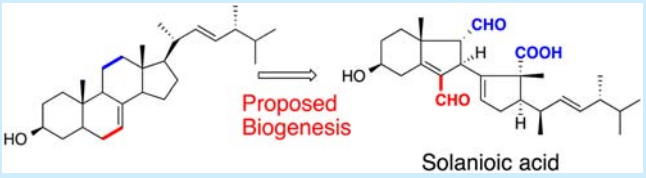
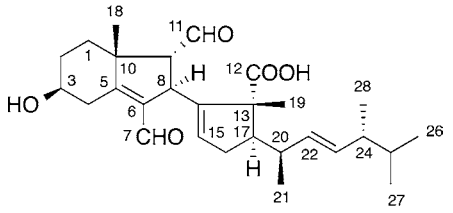
Supporting Information

ABSTRACT: Solanioic acid (**1**), a degraded and rearranged steroid that exhibits *in vitro* antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), has been isolated from laboratory cultures of the fungus *Rhizoctonia solani* obtained from tubers of the plant *Cyperus rotundus* collected in Sri Lanka. The structure of solanioic acid (**1**) was elucidated by detailed analysis of NMR data, a single crystal X-ray diffraction analysis of a reduction product **2**, and Mosher ester analysis on a derivative of the natural product. Solanioic acid (**1**) has an unprecedented carbon skeleton.



Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a serious worldwide medical problem in hospitals, long-term care facilities, and community health settings.[1] The number of deaths due to MRSA infections now exceeds the number of deaths from AIDS each year in the United States, highlighting the urgent need for new antibiotics that are effective against MRSA.[2] Most of the antibiotics currently in clinical use are microbial natural products or semisynthetic analogs of microbial natural products.[3] Penicillin G, the inspiration for the semisynthetic analog methicillin,[4] is a metabolite of the fungus *Penicillium chrysogenum*, and fungi continue to be a resource of interest for new antibiotic discovery.[3,5]

Sri Lanka has a high rate of endemic speciation in both plants and microbes,[6] which offers great potential for the discovery of plant-associated microbes that can produce new natural products with medicinal or agricultural utility.[7−9] As part of an ongoing program aimed at discovering bioactive secondary metabolites of endophytic and epiphytic fungi obtained from Sri Lankan plants,[10−12] we have investigated the antimicrobial extracts of laboratory cultures of the fungus *Rhizoctonia solani* isolated from the tubers of *Cyperus rotundus*, a Sri Lankan weed used in modern ayurvedic medicine for the treatment of fevers, digestive system disorders, and dysmenorrhea.[13,14] Assay guided fractionation of the *R. solani* extracts led to the isolation of the degraded and rearranged steroid solanioic acid (**1**), which shows strong *in vitro* inhibition of MRSA. Details of the isolation, structure elucidation, and biological activity of solanioic acid (**1**), along with a proposed biogenesis, are presented below.



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An isolate of *R. solani* was obtained from surface sterilized[15,16] tubers of *Cyperus rotundus* collected from a home garden in Colombo, Sri Lanka. Laboratory cultures of *R. solani* were grown as lawns on the surface of potato dextrose agar in Petri dishes for 7 days at rt. Mature cultures were cut into small pieces, and the media and fungal mass were coextracted with EtOAc. Evaporation of the EtOAc *in vacuo* gave 280 mg of crude residue that was fractionated by sequential application of Sephadex LH-20 chromatography, Si gel column chromatography, and C_{18} RP-HPLC. The RP-HPLC fractionation gave well-resolved major peak and minor peaks (30:1) that had identical UV spectra indicating they were structurally related. Unexpectedly, the ^{1}H NMR spectra of the pure materials obtained from both the major and minor HPLC

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peaks recorded in MeOH-d_4 were identical and each contained two sets of resonances in a ratio of 2:1. When the MeOH-d_4 solvent was removed from each of the NMR samples *in vacuo* and they were independently redissolved in DMSO-d_6, they again gave identical spectra containing only a single set of well-resolved resonances (Supporting Information). Reinjection of the material collected from the minor RP-HPLC peak described above gave a peak that coeluted with the major peak. The above observations were attributed to the existence of two slowly interconverting rotamers or tautomers of solanioic acid (**1**) in the HPLC elution solvent and MeOH-d_4, which gave resolved C_{18} RP-HPLC peaks and two sets of ^{1}H NMR resonances in MeOH-d_4, but rapidly interconverted or existed as a single form when dissolved in DMSO-d_6.

Solanioic acid (**1**) was obtained as an optically active clear viscous oil that gave an $[M + Na]^+$ ion in the HRESIMS at m/z 479.2780 (calcd 479.2773) appropriate for a molecular formula of $C_{28}H_{40}O_5$ requiring 9 sites of unsaturation. Analysis of the ^{1}H, ^{13}C, gCOSY60, gHSQC, and gHMBC NMR spectra recorded for **1** in DMSO-d_6 (Table 1, Supporting Information) revealed resonances that could be assigned to two aldehydes [^{1}H/^{13}C: δ 9.73 s/188.9 (CH-7); δ 9.64 (d, J = 3.1 Hz)/202.9 (CH-11)], one of which appeared conjugated and lacked vicinal proton−proton coupling; an ester or carboxylic acid carbonyl [^{13}C: δ 177.4 (C-12)]; a conjugated tetrasubstituted alkene [^{13}C: δ 163.8 (C-5); δ 134.3 (C-6)]; one tri- [^{1}H/^{13}C: δ 150.8 (C-14); δ 5.28/126.6 (CH-15)] and one disubstituted [^{1}H/^{13}C: δ 5.18 (d, J = 15.4, 8.0 Hz)/133.8 (CH-22); δ 5.26 (d, J = 15.4, 7.7 Hz)/132.6 (CH-23)] alkene; two quaternary aliphatic methyls [^{1}H/^{13}C: δ 1.34/25.9 (Me-18); δ 0.98/15.6 (Me-19)]; four tertiary aliphatic methyls [^{1}H/^{13}C: δ 0.79/19.8 (Me-26); δ 0.84/19.7 (Me-21); δ 0.88/17.4 (Me-28); δ 0.78/19.5 (Me-27)]; two quaternary sp^3 carbons [^{13}C: δ 52.1 (C-10); δ 57.5 (C-13)]; six sp^3 methines [^{1}H/^{13}C: δ 3.86/40.8 (CH-8); δ 2.66/65.4 (CH-9); δ 2.51/52.4 (CH-17); δ 2.13/37.5 (CH-20); δ 1.85/42.1 (CH-24); δ 1.44/32.5 (CH-25)]; four sp^3 methylenes [(^{1}H/^{13}C: δ 1.25/1.66/32.7 (CH_2−1); δ 1.56/1.72/30.7 (CH_2−2); δ 2.09/3.42/33.3 (CH_2−4); δ 1.84/2.14/34.9 (CH_2−16)]; and one sp^3 oxymethine [^{1}H/^{13}C: δ 3.33/69.9 (CH-3)]. Six of the 9 sites of unsaturation required by the molecular formula of **1** were accounted for by the alkene, aldehyde, and carboxyl functionalities identified in the NMR data, indicating that solanioic acid (**1**) contained three rings. The molecular formula of **1** included 5 oxygen atoms, 2 of which were assigned to aldehydes. There was no NMR evidence for the presence of ester or lactone functionalities, which required the assignment of the remaining 3 oxygen atoms to secondary alcohol and carboxylic acid functionalities.

Analysis of the 2D NMR data as illustrated in Figure 1 established the constitution of solanioic acid (**1**). The gCOSY, gHSQC, and gHMBC data identified the five fragments **A** to **F** that accounted for all of the atoms in **1**. gHMBC correlations observed between Me-18 (δ 1.34) and C-1 (δ 32.7), C-5 (δ 163.8), C-9 (δ 65.4) and C-10 (δ 52.1), and between the aldehyde proton resonating at δ 9.73 (H-7) and C-5 (δ 163.8), C-6 (δ 134.3), and C-8 (δ 40.8) identified an unsaturated 6/5-ring system formed via the combination of fragments **A**, **B**, **D**, and **F**. In addition to the alcohol at C-3, the C-7 and C-11 aldehydes at C-6 and C-9, respectively, and the C-18 bridgehead methyl at C-10, the 6/5 ring system required one additional substituent at C-8 in order to satisfy the fourth valence of this methine carbon. gHMBC correlations between Me-19 (δ 0.98) and C-14 (δ 150.8), C-17 (δ 52.4), C-13 (δ 57.5), and C-12 (δ 177.4), and between the olefinic H-15 resonance (δ 5.28) and C-14 (δ 150.8) and C-23 (δ 57.5), revealed the fusion of fragments **C** and **E** to form a cyclopentene ring, with carboxylic acid and methyl substituents on the C-13 quaternary carbon and one unsatisfied valence at C-14. Since fragments **A** to **F** accounted for all of the atoms in the molecular formula of **1**, linking the unsatisfied valences on C-8 and C-14 provided the final substituent required for both portions of the molecule. gHMBC correlations observed between C-14 (δ 150.8) and both H-8 (δ 3.86) and H-9 (δ 2.66) confirmed the presence of the C-8/C-14 bond and the complete constitution of **1**. A strong J coupling of 15.4 Hz between H-22 (δ 5.18) and H-23 (δ 5.26) demonstrated that the $\Delta^{22,23}$ olefin had the *E* configuration.

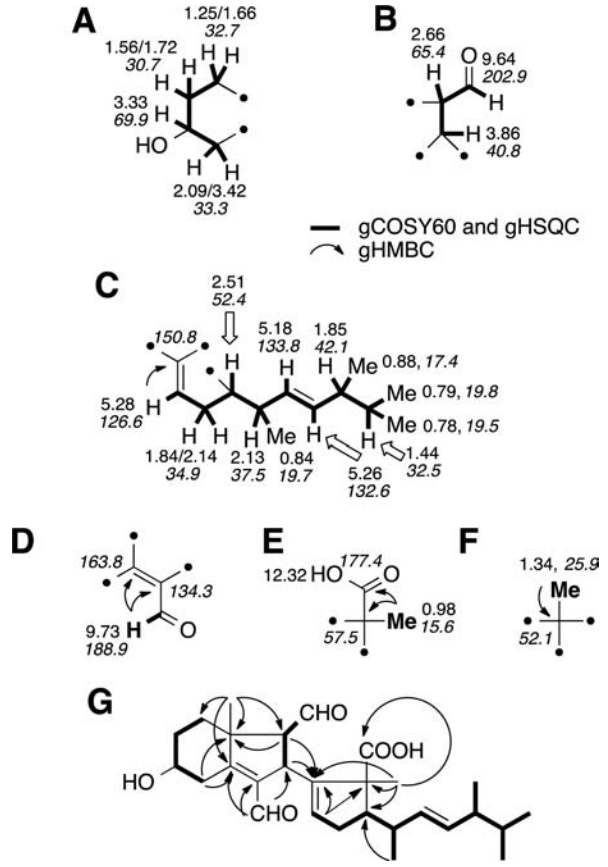

Figure 1. Fragments **A** to **F** and the complete constitution **G** of solanioic acid (**1**) identified by analysis of gCOSY, gHSQC, and gHMBC NMR data.

Analysis of the scalar couplings and the tROESY data obtained for solanioic acid (**1**) established that the six-membered ring was in a chair conformation with the C-3 hydroxyl equatorial and Me-18 axial as shown in Figure 2. tROESY correlations observed between Me-18 (δ 1.34) and H-9 (δ 2.66), and between H-8 (δ 3.86) and H-1$_{ax}$ (δ 1.25), established that the C-14 substituent at C-8 was *cis* and the aldehyde at C-8 was *trans* relative to Me-18 on the bicyclic ring system (Figure 2). A tROESY correlation observed between Me-19 (δ 0.98) and H-20 (δ 2.13) suggested that C-20 and Me-19 were *cis* relative to each other on the C-13 to C-17 cyclopentene ring. It was not possible to determine the relative configurations at C-20 and C-24, or between the bicyclic and monocyclic fragments of **1**, from the NMR data. However, the presumption of a proposed steroid origin for solanioic acid

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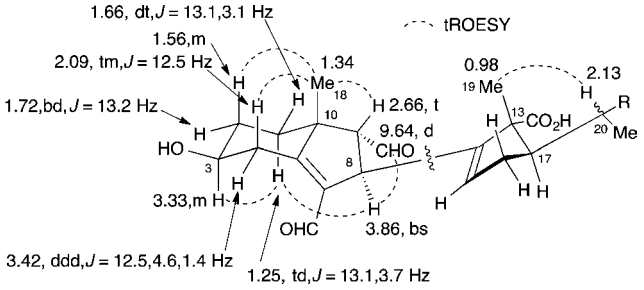
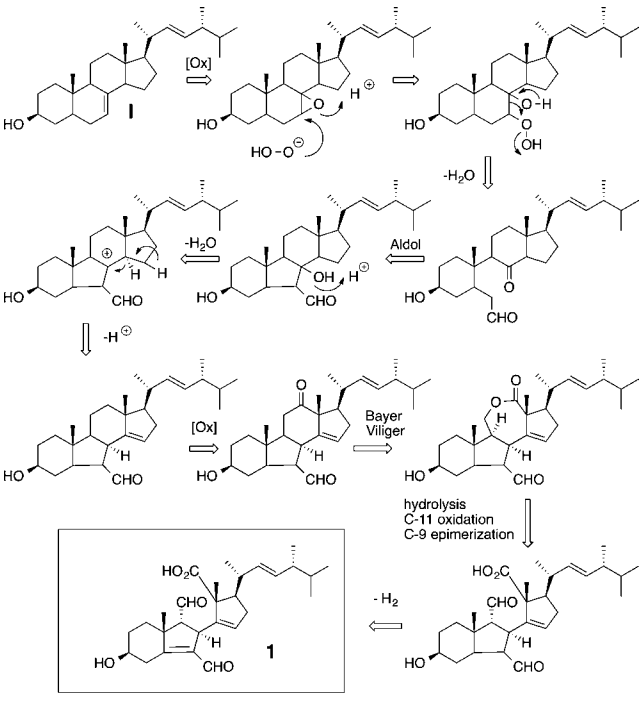


Figure 2. tROESY correlations and J couplings used to assign the relative configuration of the two independent segments of solanioic acid (**1**).

allowed a tentative assignment of the complete absolute configuration of **1** as described below. Examination of the structure of solanioic acid (**1**) suggests that the observation of two slowly interconverting forms in MeOH-d_4 most likely results from hindered rotation about the C-8/C-14 bond linking the two ring systems.

Scheme 1 shows a proposed biogenesis for **1** starting from the common fungal steroid fungisterol (**I**).[17] In this proposal

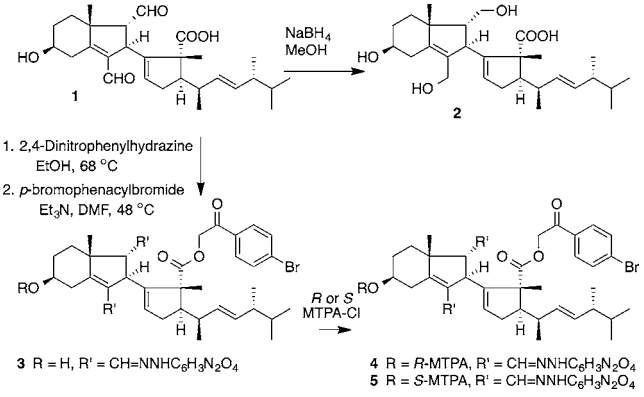
Scheme 1. Proposed Biogenesis of Solanioic Acid (1)



the configuration at C-8 in **1** is established via a suprafacial Wagner Meerwein migration of a hydrogen atom from C-14, and the C-9 configuration results from epimerization α to an aldehyde, which would relieve the steric strain of three bulky groups being *cis* to each other on adjacent carbons of a cyclopentene ring. The degradation and rearrangement transformations proposed in Scheme 1 do not alter the configurations at C-10, C-13, C-20, or C-24. Therefore, the biogenetic proposal, in conjuction with the NMR analysis described in Figure 2, generates a prediction for the entire absolute configuration of solanioic acid as shown in **1**.

Solanioic acid (**1**) failed to give crystals suitable for confirmation of the biogenetic prediction of its absolute configuration via single crystal X-ray diffraction analysis. In an attempt to generate a suitable crystalline derivative, solanioic acid (**1**) was reduced with $NaBH_4$ in MeOH to give the diol **2**, and it was also reacted with 2,4-dinitrophenylhydrazine to give the *bis*-2,4-dinitrophenylhydrazone that was then treated with *p*-bromophenacyl bromide and Et_3N to give the ester **3** (Scheme 2). The ester **3** did not produce adequate crystals, but

Scheme 2. Chemical Transformations of Solanioic Acid (1)



a pure sample of the triol **2** did give crystals that were suitable for X-ray diffraction. The ORTEP diagram in Figure 3 shows the complete relative configuration of **2**, which is in complete agreement with the biogenetic prediction.

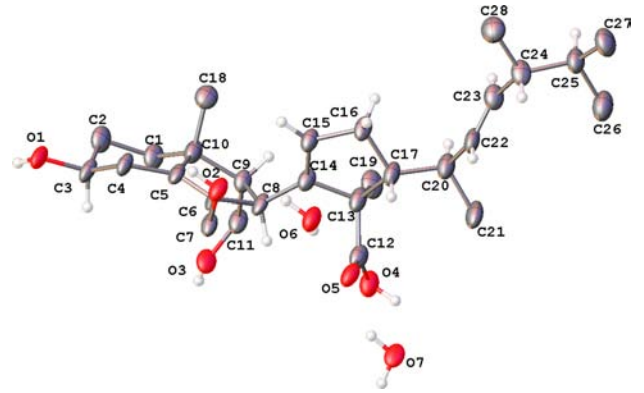


Figure 3. ORTEP diagram for derivative **2**.

While crystals of **2** were marginal, they were of sufficient quality to determine its relative structure. The scattering, however, was too weak, given the material consists solely of light atoms with weak anomalous scattering, to unambiguously determine its absolute configuration. Therefore, as shown in Scheme 2, derivative **3** was reacted with either *R*-MTPA-Cl or *S*-MTPA-Cl to give the Mosher esters **4** and **5**. A standard Mosher ester analysis of the ^{1}H NMR data collected for **4** and **5** (Supporting Information) showed that the absolute configuration at C-3 was *S*. Combining the Mosher ester result for **4** and **5** with the relative configuration determined by X-ray diffraction analysis of **2** confirmed that the complete absolute configuration of **1** was 3*S*,8*S*,9*R*,10*R*,13*R*,17*R*,20*R*,24*R* as predicted by the biogenetic proposal in Scheme 1.

A standard broth microdilution method was used to evaluate the *in vitro* antimicrobial activity of solanioic acid (**1**) against a small panel of human pathogens (Supporting Information). Solanioic acid (**1**) showed *in vitro* MICs against Gram-positive

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bacteria, including *Bacillus subtilis*, *Staphylococcus aureus*, and MRSA, of 1 μg/mL. It showed weaker activity against the yeast *Candida albicans* (MIC 16 μg/mL) and no activity against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* at 64 μg/mL.

Solanioic acid (**1**) has an unprecedented carbon skeleton that features a highly functionalized conjoint ring system.[18] The carbon skeleton in **1** can arise from degradation and rearrangement of a steroidal precursor such as fungisterol as outlined in Scheme 1. Solanioic acid (**1**) represents a new antimicrobial scaffold, with promising *in vitro* activity against the problematic human pathogen MRSA, that merits further biological evaluation as a potential drug lead.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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