Published in final edited form as:

Org Lett. 2013 May 17; 15(10): 2370–2373. doi:10.1021/ol400752s.

Acremolin from *Acremonium strictum* is *N*²,3-Etheno-2'-isopropyl-1-methylguanine, not a 1*H*-Azirine. Synthesis and Structural Revision

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Abstract

The first synthesis of the heterocyclic marine natural product, acremolin, is reported along with the revision of the structure from a 1*H*-azirine to a substituted *N*²,3-ethenoguanine (5-methyl-7-isopropyl-4,5-dihydroimidazo[2,1-*b*]purine). We describe additional properties of acremolin including its ¹H-¹⁵N-HMBC and fluorescence spectra.

Compounds containing aziridine (azacyclopropane) or azirine (azacyclopropene)¹ rings are rare in nature. Fused aziridine rings can be found in the structures of mitomycins,^{1b} potent DNA alkylating antitumor antibiotics, however, 2*H*-azirine-containing natural products are exceptionally uncommon. We were intrigued by the recent report of a modified nucleobase, acremolin, obtained by fermentation of the marine-derived fungus *Acremonium strictum*, and claimed as the “first 1*H*-azirine containing natural product”.² The structure **1** (Figure 1) assigned to acremolin is highly surprising.

It is well-known that the antiaromatic parent 1*H*-azirine (**2a**) is unstable and rapidly tautomerizes to 2*H*-azirine (**2b**); an isomer more stable by at least 30 kcal.mol^{−1}.³ Synthetic efforts to prepare stable substituted 1*H*-azirines have failed although their ephemeral appearance as intermediates in high energy reactions (e.g. *N*-methyl-1*H*-azirines from flash-vacuum pyrolysis of substituted 1*H*-2,3,4-triazoles) has been postulated.⁴ Although the total ring-strain energy of the more stable 2*H*-azirine tautomer **2b** has been calculated to be 44.6–48 kcal.mol^{−1} using DFT methods,⁵ substituted 2*H*-azirines including azirinomycin (**3**), the long-chain C₁₈ base dysidazirine (**4**)⁶ and its analogs,⁷ occur in nature and are remarkably stable. Tautomerism is not possible in the proposed structure **1** due to the tertiary nitrogen, yet skepticism remains that such an unstable compound could exist in nature.

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Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds and synthetic acremolin (**5a**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

Upon examination of the spectroscopic evidence presented for **1**² we hypothesized two plausible non-azirine structures for acremolin based on *N*²,3-ethenoguanine skeleton – **5a** or the regioisomeric **5b** (Scheme 1) would be more compatible with the natural product.^{2a} Independently, Banert proposed the same hypothesis and suggested⁸**5a** better fits the NMR data for acremolin based on analysis of predicted ¹³C NMR chemical shift increments for **5a** and **5b**, although without experimental evidence. Here we describe the first concise preparation of **5a** and experimental proof (HRMS, UV-vis, ¹H, ¹³C and ¹⁵N NMR data) that **5a** and acremolin are the same.

The synthesis of **5a** (Scheme 1) improves on earlier variants of *N*²,3-ethenoguanosine synthesis.^{9,10} Kusmierek reported low yields (~28%) of the latter compound by reaction of 2-chloroacetaldehyde with guanosine. In our hands, attempted reactions of *N*-methylguanosine with α-haloketones or α-haloaldehydes were complicated by mixtures resulting from overalkylation and depurination. LCMS evidence suggested that a dialkylated product arose by initial alkylation at *N*⁷ to give a quaternary adeninium salt which rapidly eliminated ribose, followed by a second alkylation of the liberated free base.

In order to avoid these difficulties, we chose to start with a suitably protected nucleobase. 1-Methylguanine (**6**) was prepared in good yield (69%, Scheme 1) by selective methylation of guanosine (DMSO, CH₃I, NaH)¹¹ followed by hydrolysis (2 M HCl, 61%). Protection of **6** (PMB-Cl, K₂CO₃, DMF)¹² gave a mixture of *N*⁷- and *N*⁹-PMB derivatives **7a,b** (~1.6:1, 56%) from which **7a** could be isolated by fractional crystallization (EtOH).¹³ We explored two complementary electrophiles for condensation with **10** – bromoketone **8**¹⁴ and 2-chloro-3-methylbutanal (**9**)¹⁵ – in attempts to prepare both regioisomers **5a** and **5b**.

Reaction of **7** with bromoketone **8** ((*i*-Pr)₂NEt, CH₃CN, 40 °C) returned the *N*²,3-ethenoguanine derivative **10** (27%) as a single isomer, along with the guaninium salt **10a** (54%) resulting from mono-alkylation of the imidazole ring.^{16,17} Attempted removal of the PMB group of **10** by hydrogenolysis (H₂, Pd-C, MeOH, 1 atm, 16 h) returned only starting material. Friedel-Crafts type transbenzylation, under the conditions reported by Fujii and coworkers (90% aq H₂SO₄, toluene, 40 °C),¹⁸ smoothly delivered samples of **5a** of high purity, albeit in low yield (39%).¹⁹ Gratifyingly, **5a** was obtained in good yield (76%) by simple treatment of **10** with neat CF₃CO₂H (40–80 °C).f

The ¹H NMR (DMSO-*d*₆, 500 MHz) and ¹³C NMR spectra of **5a** (DMSO-*d*₆, 125 MHz; Table 1) and other data (UV-vis, FTIR, HMBC and HRMS, see Supporting Information) were identical in every respect with those reported by Shin and coworkers for acremolin,^{2a} with one exception: in the ¹H NMR spectrum of **5a** (DMSO-*d*₆), we observed an additional allylic coupling from H-1' to H-3' (⁴*J* = 1.0 Hz). Importantly, the measured one-bond heterocyclic coupling constants of C-1'-H-1'²⁰ in **5a** (¹*J*_{CH} = 195.5 Hz)²¹ were consistent with *N*²,3-ethenoguanosine (¹*J*_{CH} = 180 Hz),^{22,23} imidazole (¹*J*_{CH} = 189 Hz),²⁴ and predicted values,⁸ but incompatible with estimated values for an *N*-substituted 1*H*-azirine (¹*J*_{CH}~230 Hz)⁸

Unlike structure **1**, compound **5a** raises questions of regioisomerism. The location of the isopropyl group in **5a** follows from the mechanism of formation of *N*²,3-ethenoguanine bases. Conceivably, each of the isomers **5a** and **5b** can be formed by reaction of an α-halocarbonyl compound with **6** through two pathways that differ only by the ordering of the elementary steps of alkylation and condensation. Under near neutral or slightly acidic conditions, the most likely order of reactions that gives **5a** is reversible imine formation between the C-2-NH₂ group of **6** and the α-halocarbonyl compound followed by slower irreversible intramolecular alkylation of the weakly nucleophilic *N*³ giving **5a** after tautomerization. Guengerich and Persmark obtained support for the latter mechanism in the

formation of **11** with guanosine in aqueous ethanol (pH = 6.4) through isotopic labeling with ^{13}C -labeled 2-haloacetaldehyde and interpretation of the NMR spectra.²² In our case, the formation of **5a** is also observed under initially basic conditions ($(i\text{-Pr})_2\text{NEt}$, CH_3CN , 45–100 °C), suggesting the order of reactions may be reversed for formation of **5a**, i.e. alkylation first, this time at N^3 , followed by condensation at the C-2 NH_2 group, possibly promoted by the byproduct salt $(i\text{-Pr})_2\text{NEt}\cdot\text{HBr}$, but the outcome is the same. In principle, detection of long-range ^1H - ^{13}C HMBC correlations should resolve this equivocal assignment of **5a** versus **5b**. Unfortunately the key $^3J_{\text{CH}}$ cross peak, H-1' to C-4, was absent in the reported HMBC spectrum of **5a**^{2a} and our own measurements.²⁵

Doubts about the structures **5a** and **5b** were removed upon analysis of the ^1H - ^{15}N HMBC spectrum of synthetic **5a** (Figure 2);²⁶ six long-range correlations were observed for **5a** which served to assign the ^{15}N NMR chemical shifts. Two three-bond correlations ($^3J_{\text{H-N}}$) were observed from H-8 (δ 8.16, s) to the expected low-field imidazole N-7 (δ 232.6 ppm) and N-9 (166.1 ppm) of the purine ring. Most importantly, $^3J_{\text{H-N}}$ correlations were observed from H-3' (δ 2.88, sept d, J = 6.9, 1.0 Hz) and H-1' (δ 7.38, d, J = 1.0 Hz) to the low-field imidazole N-11 (δ 221.9), and a two-bond correlation ($^2J_{\text{H-N}}$) from H-1' to N-3 (δ 156.1). These data support only regioisomer **5a** and exclude **5b**; thus, acremolin is identical to synthetic **5a** and its structural revision from **1** to **5a** is confirmed.

Attempted reactions of **6** with **9** to obtain the complementary 'endo' cyclized regioisomer **5b** (CH_3CN , 45–100 °C, with or without added base [$(i\text{-Pr})_2\text{NEt}$] or acid (NH_4OAc , AcOH) or nucleophilic catalyst ($n\text{-Bu}_4\text{NBr}$) were unsuccessful, presumably due to steric hindrance, and returned only starting material. Similarly, attempts to effect reductive alkylation of **6** or **7a** (**9**, NaCNBH_3 , MeOH , 23–80 °C), in anticipation of subsequent cyclization-autoxidation, failed to deliver the expected secondary amine.

Additional spectroscopic data were measured for **5a**. The UV-spectrum of synthetic **5a** [MeOH , pH 7, λ_{max} 223 nm (4.49), 269 (4.05)] was essentially identical to that reported for acremolin [MeOH , λ_{max} 224 (4.27), 269 nm (3.89)]. The chromophore of the proposed structure of **1** would be expected to show UV properties similar to those of **6** (λ_{max} 249, 273 nm) but acremolin shows the short-wavelength band red-shifted to λ 269 nm (log ϵ 3.89). In addition, we found dilute solutions of **5a** exhibited visible blue fluorescence (MeOH , λ_{ex} 296 nm; λ_{em} 420 nm; Stokes shift = 124 nm), a property shared by $N^2,3$ -ethenoguanosine (**11**, H_2O λ_{ex} 264; λ_{em} 400, Stokes shift = 136 nm)⁹ but not **6** or isomers **7a** and **7b**.

Etheno-bridged guanine bases have a rich history. In 1957, Dunn²⁷ and Hoard²⁸ independently reported a 'fluorescent compound' formed as an artifact during acid-promoted hydrolysis of DNA and condensation of guanine with deoxyribose degradation products, but earlier mistaken as a constituent of native DNA. A decade later, Howard and Dekker provided full characterization of the fluorescent compound as 1'-acetyl- $N^2,3$ -ethenoguanine (**12**) (Figure 3).²⁹ The chemistry and biology of 1, N^2 - and $N^2,3$ -ethenoguanines have been extensively studied,³⁰ most importantly in the context of exocyclic base modifications of DNA and RNA that arise from environmental exposure to toxicants (e.g. vinyl chloride), lipid peroxidation and tobacco smoke.^{30a,b} Exocyclic bases, notably $N^2,3$ -ethenoguanine (**11**) and its linearly fused regioisomer, 1, N^2 -ethenoguanine (**13**), have been detected in the DNA isolated from livers of rats subjected to inhalation exposure to high concentrations of vinyl chloride,³¹ a carcinogenic compound that is transformed by liver cytochrome P₄₅₀ enzymes to the genotoxic metabolite, chloroacetaldehyde. Compound **11**, and its corresponding riboside and 5'- O -phosphate ester, have been prepared by direct condensation of 2-bromoacetaldehyde with 6- O -methylguanosine, followed by depurination or enzymatic 5'- O -phosphorylation, albeit in lower yield⁹ than reported for **10** (Scheme 1).

Shin and co-workers inferred the involvement of an 'isoprene unit' in biosynthesis of acremolin.² In view of the known origin of **11–13**, it appears more likely that **5a** arises from condensation of 1-methylguanine (or 1-methylguanosine and accompanying hydrolysis) with an isovaleraldehyde equivalent – possibly derived from 2-oxo-isocaproic acid originating from transamination of leucine – followed by oxidative cyclodehydration-decarboxylation.³²

In summary, acremolin has been synthesized and the structure revised from the substituted 1*H*-azirine **1** to *N*²,3-etheno-2'-isopropyl-1-methylguanine (**5a**); a structure fully consistent with the published spectroscopic properties^{2a} of the natural product, and the ¹H-¹⁵N HMBC data, reported here. The one-bond homonuclear coupling constants (¹*J*_{CH}) and fluorescence properties of **5a** are described. In all likelihood, 1*H*-azirines remain “unprecedented among natural products”.^{2a}

Supplementary Material

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Acknowledgments

We thank B. Duggan and A. Mrse for assistance with NMR measurements. The 500 MHz NMR spectrometer and the HPLC TOFMS were purchased with funding from the NSF (Chemical Research Instrument Fund, CHE0741968) and the NIH Shared Instrument Grant (S10RR025636) programs, respectively. We are grateful for generous research support from the NIH (AI039987, AI100776).

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- (19). The balance of material was mostly a polar product, retained in the H₂O layer after extractive workup. By MS, this appeared to be **10**•SO₃H (*m/z* 432, [M+H]⁺), the product of sulfonation of **10** by H₂SO₄.
- (20). For ease of comparison, we have retained the numbering scheme of the isopropyl-substituted ring in acremolin used by Shin and coworkers. See Ref. 2. The systematic name for **5a**, 5-methyl-7-isopropyl-4,5-dihydroimidazo[2,1-*b*]purine, reflects differences between IUPAC and conventional purine numbering.
- (21). ¹J_{CH} values were conveniently measured in ¹H NMR spectrum of **5a** (DMSO-*d*₆) from the '13C satellites' of the H-1' doublet unambiguously identified by allylic coupling to H-3' (⁴J_{HH} = 1.0 Hz). The H-8 ¹J_{CH} value is significantly larger (¹J_{CH} = 212.7 Hz) than H-1'.
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Figure 1. 1*H*-Azirine (**2a**), 2*H*-azirine (**2b**), natural azirines **3** and **4** (see text). Structure **1** originally assigned to acremolin,^{2a} and here revised to **5a**.

Org Lett. Author manuscript; available in PMC 2014 May 17.

Figure 3.
Etheno-bridged guanines.

Scheme 1.
Synthesis of acremolin: revised structure, **5a**.

Table 1

Comparison of ^1H and ^{13}C NMR data for natural acremolin, synthetic **5a** ($(\text{CD}_3)_2\text{SO}$) and estimated ^{13}C δ values.

no. ^b	acremolin ^b		synthetic 5a ^c		est.
	δ_{H} m, J (Hz)	δ_{C} ^a	δ_{H} m, J (Hz) ^{c,e}	δ_{C} ^{c,e}	δ_{C} ^d
2		142.5		142.3	153.4
4		141.6		141.6	141.7
5		108.3		108.9	108.9
6		152.6		152.8	153.4
8	8.16, s	140.3	8.16, s	140.5	140.0
9-NH	13.85, br s		13.87, br s		
10	3.57, s	28.9	3.57, s	28.9	–
1'	7.37, s	103.1	738, d (1.0 ^f)	103.2	105.7
2'		148.0		148.0	147.2
3'	2.88, sept (6.8)	27.6	2.88, sept d (6.9, 1.0 ^f)	27.7	–
4', 5'	1.25 (d, 6.8)	22.0	1.25 d (6.9)	22.1	–

^a See footnote 20.

^b Data from ref 2a.

^c This work.

^d See ref 8.

^e Assignments confirmed by HMBC, DEPT, HSQC and 1D-NOESY (500 MHz).

^f Allylic coupling, $^4J_{\text{H-H}}$.