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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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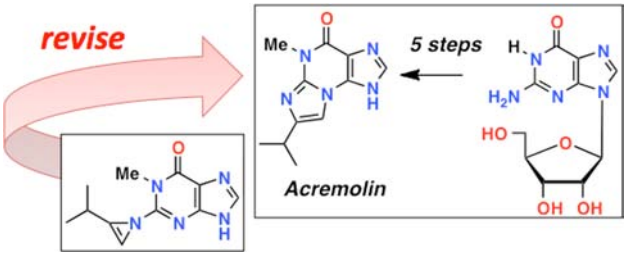
ABSTRACT



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). Additional properties of acremolin are also described including its $^1H-^{15}N$-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.

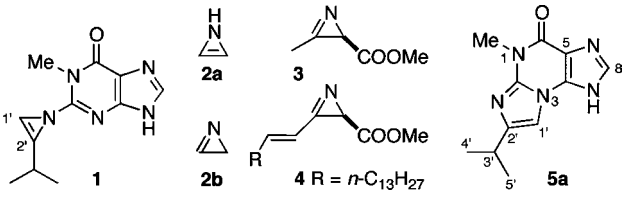


Figure 1. 1*H*-Azirine (**2a**), 2*H*-azirine (**2b**), and natural azirines **3** and **4** (see text). Structure **1** originally assigned to acremolin[2a] and here revised to **5a**.

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

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(1) (a) Nair, V. *The Chemistry of Heterocyclic Compounds*; Hassner, A., Ed.; Wiley: New York, 1983; Vol. 42, part 1, Chapter 2, pp 215. (b) Carter, S. K.; Crooke, S. T.; Alder, N. A. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1979.

(2) (a) Julianti, E.; Oh, H.; Lee, H.-S.; Oh, D.-C.; Oh, K.-B.; Shin, J. *Tetrahedron Lett*. **2012**, *53*, 2885–2886. (b) Hill, R.; Sutherland, A. *Nat. Prod. Rep*. **2012**, *29*, 829–833.

(3) The free energy of the parent **2a** has been calculated to be 140 kJ·mol^{-1} (33.5 kcal·mol^{-1}) higher than that for **2b**. Zavoruev, S. M.; Rakauskas, R-I. I. *Theor. Exp. Chem*. **1990**, *25*, 445–450.

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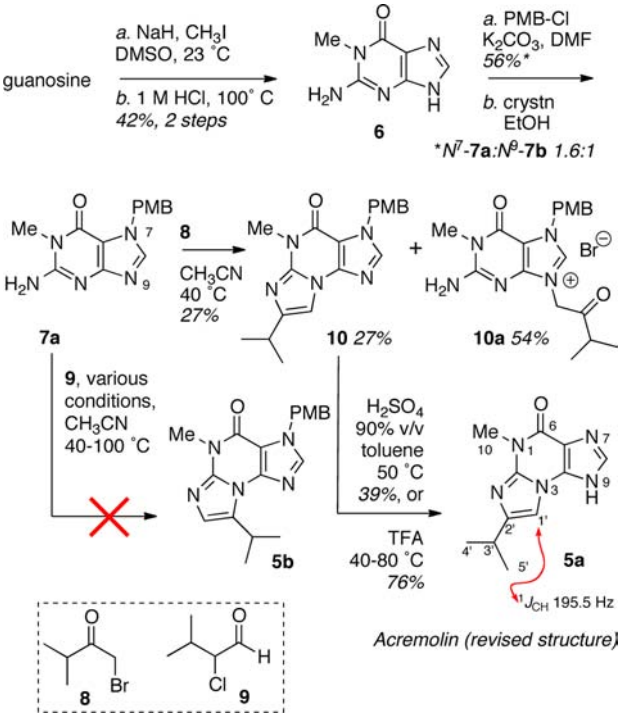
ring-strain energy of the more stable 2*H*-azirine tautomer **2b** has been calculated to be 44.6−48 kcal·mol^{-1} using DFT methods,[5] substituted 2*H*-azirines including azirinomycin (**3**), the long-chain C_{18} base dysidazirine (**4**),[6] and its analogs[7] occur in nature and are remarkably 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.

Upon examination of the spectroscopic evidence presented for **1**[2] we hypothesized two plausible non-azirine structures for acremolin based on the 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 suggested[8] **5a** better fits the NMR data for acremolin based on analysis of predicted ^{13}C NMR chemical shift increments for **5a** and **5b**, although without experimental evidence. Here we describe the first synthesis of **5a** and experimental proof (HRMS, UV−vis, ^{1}H, ^{13}C, and ^{15}N NMR data) that **5a** and acremolin are the same.

The concise preparation 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, attemped 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.

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, CH_3I, NaH)[11] followed by hydrolysis (2 M HCl, 61%). Protection of **6** (PMB-Cl, K_2CO_3, 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 regiosiomers **5a** and **5b**.

Scheme 1. Synthesis of Acremolin: Revised Structure, **5a**



Reaction of **7** with bromoketone **8** ((*i*-Pr)$_2$NEt, CH_3CN, 40 °C) returned the N^2,3-ethenoguanine derivative **10** (27%) as a single isomer, along with the guaninium salt **10a** (54%) resulting from monoalkylation of the imidazole ring.[16,17] Attempted removal of the PMB group of **10** by hydrogenolysis (H_2, Pd−C, MeOH, 1 atm, 16 h) returned only starting material. Friedel−Crafts type transbenzylation, under the conditions reported by Fujii and co-workers (90% aq H_2SO_4, 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 CF_3CO_2H (40−80 °C).

(4) Gilchrist, T. L.; Gymer, G. E.; Rees, C. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1.

(5) (a) Heimgartner, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 238. (b) Calvo-Losada, S.; Quirante, J. J.; Suárez, D.; Sordo, T. L. *J. Comput. Chem.* **1998**, *19*, 912.

(6) Molinski, T. F.; Ireland, C. M. *J. Org. Chem.* **1988**, *53*, 2103–5.

(7) (a) Salomon, C. E.; Williams, D. H.; Faulkner, D. J. *J. Nat. Prod.* **1995**, *58*, 1463–1466. (b) Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 2592–2597.

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(10) Sattsangi, P. D.; Leonard, N. J.; Frihart, C. R. J. *Org. Chem.* **1977**, *42*, 3292.

(11) Höbartner, C.; Kreutz, C.; Flecker, E.; Ottenschläger, E.; Pils, W.; Grubmayr, K.; Micura, R. *Monatsh. Chem.* **2003**, *134*, 851–873.

(12) Montgomery, J. A.; Hewson, K.; Temple, C. *J. Med. Chem.* **1962**, *5*, 15–24.

(13) The location of the PMB group in **7a** was confirmed at *N*-7 by ^{1}H−^{13}C HMBC (optimized for $^1J_{CH}$ = 8 Hz). Long-range correlations were observed from the ^{1}H NMR signal of the benzylic CH_2 of PMB (δ 5.35, s) to C-5 (δ 107.0, s) and C-8 (δ 143.4, s), but not to C-4 (δ 153.5, s). See Supporting Information for complete HMBC correlations of **7a**.

(14) Gaudry, M.; Marquet, A. *Org. Synth.* **1976**, *55*, 24.

(15) Prepared by chlorination of isobutyraldehyde (NCS, L-proline, CH_2Cl_2). Tessie Borg, T.; Danielsson, J.; Somfai, P. *Chem. Commun.* **2010**, *46*, 1281–1283.

(16) The structure of **10a** was supported by HMBC data (DMSO-d_6) which included correlations from the CH_2(C=O) ^{1}H signal (δ 5.33, s, H-1″) to both C-8 (δ 138.9, d) and C-4 (δ 106.9, s)

(17) Attempts to suppress alkylation of the more nucleophilic imidazole ring by employing the corresponding N^7-Ns and N^7-Ts protected guanines failed to give the product or gave intractable mixtures, respectively.

(18) (a) Leonard, N. J.; Fujii, T.; Saito, T. *Chem. Pharm. Bull.* **1986**, *34*, 2037. (b) Nohara, F.; Nishii, M.; Ogawa, K.; Isono, K.; Ubukata, M.; Fujii, T.; Itaya, T.; Saito, T. *Tetrahedron Lett.* **1987**, *28*, 1287. (c) Ogawa, K.; Nishii, M.; Inagaki, J.-I.; Nohara, J.; Saito, T.; Itaya, T.; Fujii, T. *Chem. Pharm. Bull.* **1992**, *40*, 1315.

(19) The balance of material was mostly a polar product, retained in the H_2O layer after extractive workup. By MS, this appeared to be **10**·SO_3H (*m*/*z* 432, $[M + H]^+$), the product of sulfonation of **10** by H_2SO_4.

The ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C 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 co-workers for acremolin,[2a] with one exception: in the ^{1}H NMR spectrum of **5a** (DMSO-d_6), we observed an additional allylic coupling from H-1′ to H-3′ (^4J = 1.0 Hz). Importantly, the measured one-bond heterocyclic coupling constants of C-1′−H-1′[20] in **5a** ($^1J_{CH}$ = 195.5 Hz)[21] were consistent with N^2,3-ethenoguanosine ($^1J_{CH}$ = 180 Hz),[22,23] imidazole ($^1J_{CH}$ = 189 Hz),[24] and predicted values,[8] but incompatible with estimated values for an *N*-substituted 1*H*-azirine ($^1J_{CH} \approx$ 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-NH$_2$ 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-Pr)$_2$NEt, CH$_3$CN, 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-Pr)$_2$NEt·HBr, but with the same outcome. In principle, detection of long-range ^{1}H−^{13}C HMBC correlations should resolve this equivocal assignment of **5a** vs **5b**. Unfortunately the key $^3J_{CH}$ cross peak, H-1′ to C-4, was absent in the reported HMBC spectrum of **5a**[2a] and our own measurements.[25]

Doubts regarding the structures of **5a** and **5b** were removed upon analysis of the ^{1}H−^{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 ($^3J_{HN}$) 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, $^3J_{HN}$ 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 ($^2J_{HN}$) 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.

Table 1. Comparison of ^{1}H and ^{13}C NMR Data for Natural Acremolin, Synthetic **5a** ((CD$_3$)$_2$SO) and Estimated ^{13}C δ Values

	acremolin[b]		synthetic **5a**[c]		est.
no.[b]	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	141.7
4		141.6		141.6	141.0
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	7.38, 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 ref 20. [b] Data from ref 2a. [c] This work. [d] See ref 8. [e] Assignments confirmed by HMBC, DEPT, and HSQC (500 MHz). [f] Allylic coupling, $^4J_{HH}$.

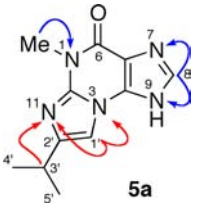

Figure 2. ^{1}H−^{15}N HMBC correlations of **5a** (600 MHz, DMSO-d_6, optimized for J = 8 Hz). Critical correlations are highlighted in red.

Attempted reactions of **6** with **9** to obtain the complementary 'endo' cyclized regioisomer **5b** (CH$_3$CN, 45−100 °C, with or without added base [(i-Pr)$_2$NEt] or acid (NH$_4$OAc, AcOH) or a nucleophilic catalyst (n-Bu$_4$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$_3$,

(20) For ease of comparison, we have retained the numbering scheme of the isopropyl-substituted ring in acremolin used by Shin et al. 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 ^{1}H NMR spectrum of **5a** (DMSO-d_6) from the '^{13}C 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′.

(22) Guengerich, F. P.; Persmark, M. *Chem. Res. Toxicol.* **1994**, *7*, 205–208.

(23) Coincidently, the $^1J_{CH}$ value for C-1′ in **5a** is similar to that of the sp^3 carbon (C-3−H-3) in 2*H*-azirines, e.g. **4** ($^1J_{CH}$ = 189.5 Hz). See ref 6.

(24) Pretsch, E.; Bühlmann, P.; Badertscher, M. *Structure Determination of Organic Compounds*, 4th ed.; Springer-Verlag: Berlin, 2009; p 108.

(25) Our ^{1}H−^{13}C HMBC measurements of **5a** (optimized both for ^3J = 4 and 8 Hz) also failed to detect this cross peak; see Supporting Information, Table S2.

(26) The ^{15}N NMR spectra of guanosine and related purine bases have been assigned previously. (a) Glemarec, C.; Besidsky, Y.; Chattopadhyaya, J.; Kusmierek, J.; Lahti, M.; Oivanen, M.; Lönnberg, H. *Tetrahedron* **1991**, *47*, 6689–6704. (b) Cho, B. P.; Kadlubar, F. F.; Culp, S. J.; Evans, F. E. *Chem. Res. Toxicol.* **1990**, *3*, 445–452.

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 idenitical 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, Dunn[27] and Hoard[28] 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′-acetonyl-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 P_{450} 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-bromoacetaldehye with 6-*O*-methylguanosine, followed by depurination or enzymatic 5′-*O*-phosphorylation, albeit in lower yield[9] than reported for **10** (Scheme 1).

Shin et al. inferred the involvement of an 'isoprene unit' in the 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]

Figure 3. Etheno-bridged guanines.

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 $^1H-^{15}N$ HMBC data, is reported here. The one-bond homonuclear coupling constants ($^1J_{CH}$) and fluorescence properties of **5a** are described. In all likelihood, 1*H*-azirines remain "unprecedented among natural products".[2a]

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Note Added after ASAP Publication. Table 1 contained errors in the version published ASAP on May 1, 2013; the correct version reposted on May 17, 2013.

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.

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(30) (a) Medeiros, M. H. G. *Chem. Res. Toxicol.* **2009**, *22*, 419–425. (b) Marnett, L. J.; Burcham, P. C. *Chem. Res. Toxicol.* **1993**, *6*, 771–785. (c) Leonard, N. J. *CRC Crit. Rev. Biochem.* **1984**, *15*, 125–199.
(31) Swenberg, J. A.; Fedtke, N.; Ciroussel, F.; Barbin, A.; Bartsch, H. *Carcinogenesis* **1992**, *13*, 727–729.

(32) Ethenoguanine adducts have been detected during cell incubations of guanosine under various conditions; therefore, we cannot rule out the possibility that acremolin is an artifact of the fermentation of *Acremonium strictum*.[2a]

The authors declare no competing financial interest.