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Synthesis of the Pyrazolo[4,3-*e*][1,2,4]triazine Family of Natural Products: Nostocine A, Fluviol A, and Pseudoiodinine

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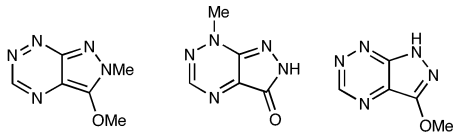
The pyrazolo[4,3-*e*][1,2,4]triazine ring system has the distinction of being one of only two naturally occurring ring skeletons containing more nitrogen than carbon atoms.[1] In the past 30+ years, scientists from Germany, Japan, and Russia have reported the isolation and structural characterization of seven naturally occurring pyrazolo[4,3-*e*][1,2,4]triazines: pseudoiodinine (**1**),[2] nostocine A (**2**),[3] and fluviols A (**3**)−E.[4] In addition to their high nitrogen content, family members are notable for the palette of bright colors they display, including red, violet, purple, and yellow. To date, there has been no independent verification of any of the assigned structures nor reports on activity to achieve the total synthesis of any members of the family. We now record the total synthesis and structure confirmation of nostocine A and fluviol A and a structure reassignment for pseudoiodinine. That structure reassignment is supported by syntheses of both the originally proposed structure and the revised structure.



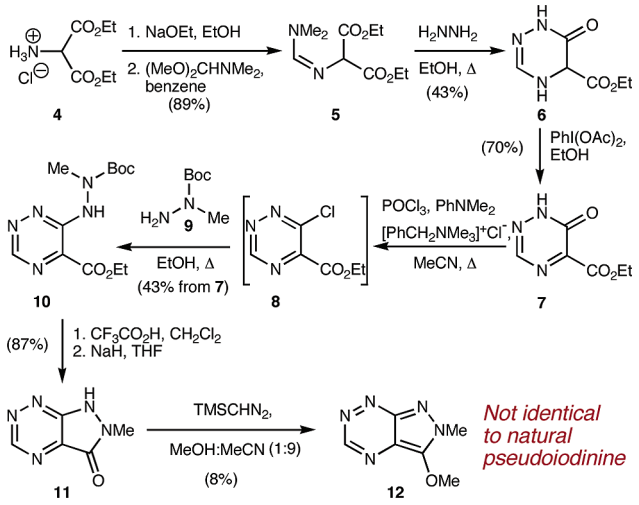
1 (Structure originally assigned to pseudoiodinine) **2** (nostocine A) **3** (fluviol A)

The synthesis of pseudoiodinine was undertaken first, in part because we suspected that the structure was incorrectly assigned. The original structure assignment was based heavily on the X-ray crystallographic characterization of a degradation product of pseudoiodinine named normethylpseudoiodinine and assigned structure **3**. Pseudoiodinine undergoes facile demethylation to give normethylpseudoiodinine.[2] Two or so decades later, a newly isolated natural product, fluviol A, was also assigned structure **3**. Our suspicions regarding the correctness of structure **1** were largely a consequence of our skepticism that **1** would suffer preferential N- (rather than O-) demethylation to give **3**.[5]

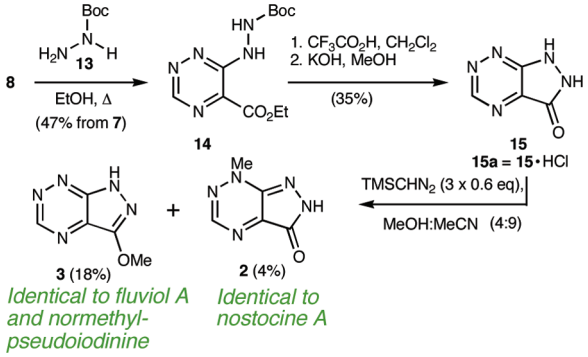
The synthesis of structure **1** is summarized in Scheme 1. Reaction of diethyl aminomalonate with dimethylformamide dimethyl acetal gave amidine **5**;[6] condensation[7] of **5** with hydrazine afforded dihydrotriazinone **6**. The latter could be oxidized to triazinone **7** with iodobenzene diacetate.[8] Treatment of **7** with $POCl_3$ under the conditions of Robins[9] delivered chlorotriazine **8** which was reacted with the Boc-protected methylhydrazine **9**[10] to give **10**. Boc protection of methylhydrazine was necessary because otherwise the methylated nitrogen in methylhydrazine is the more nucleophilic of the two nitrogens[11] (a property of methylhydrazine that is exploited to prepare the Boc derivative **9** itself).

Cleavage of the Boc group in **10** with trifluoroacetic acid and basification of the crude product leads directly to the bicyclic pyrazolone **11**. O-Methylation with accompanying tautomerization of **11** should produce **1**. The prospects of achieving selective methylation of a single oxygen in the presence of five nitrogens

Scheme 1. Synthesis of Proposed Structure of Pseudoiodinine



Scheme 2. Synthesis of Fluviol A and Nostocine A



might seem dubious, but reaction of **11** with the oxophilic, commercially available (trimethylsilyl)diazomethane afforded **12** in 8% yield (^{13}C NMR clearly distinguishes O- versus N-methylation). Structure **12** is the structure (**1**) previously assigned to pseudoiodinine, but spectra of the yellow synthetic **12** are decidedly different from those of the red natural product. The inescapable conclusion is that **1** is not the structure of pseudoiodinine.

With the demonstration that pseudoiodinine does not possess structure **1**, the next question was whether the pivotal structure (**3**) of normethylpseudoiodinine (supposedly = fluviol A) is correct.[12] To that end, synthesis of **3** was undertaken (Scheme 2). Chlorotriazine **8** was reacted with Boc-hydrazine[13] (**13**) to give **14**. Treatment of **14** with CF_3COOH followed by basification proceeded in analogy to Scheme 1, delivering bicyclic hydrazide **15**, conveniently manipulated as its hydrochloride salt (**15a**). Exposure of **15a** to (trimethylsilyl)diazomethane (3 cycles @ 0.6 equiv[14]) gave primarily two products, an O-methylated and an N-methylated

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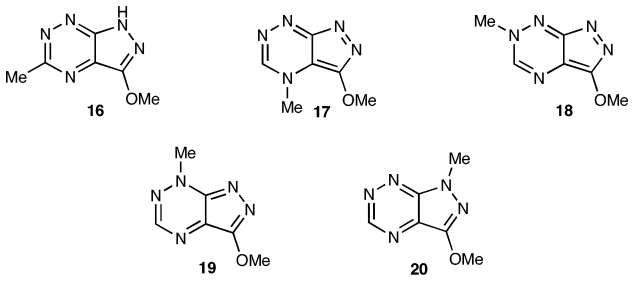


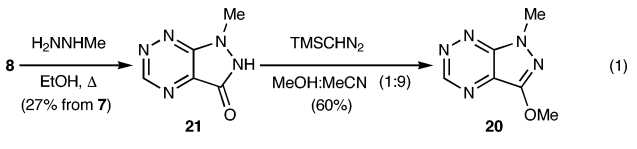
Figure 1. Possible methylation products of **3** (other than **1**).

species. The O-methylated species is identical to normethylpseudoiodinine/fluviol A (**3**), thereby providing independent confirmation of their structures. The N-methylated species was an unplanned bonus since it proved to be identical to nostocine A. The structure of nostocine A (**2**) was originally assigned by X-ray crystallographic analysis, and the location of the methyl group in natural **2** was confirmed by NMR (heteronuclear multiple bond correlation, i.e., HMBC) spectroscopy. HMBC studies[15] on synthetic nostocine A certified the earlier assignments.

While the synthesis of **12** reported above establishes that the structure **1** (= **12**) of pseudoiodinine is wrong, the original workers provided one further piece of information: reaction of normethylpseudoiodinine (**3**) with diazomethane gives pseudoiodinine in approximately 12% yield. With the hope that spectroscopic techniques had advanced sufficiently in the 30+ years since the structure of pseudoiodinine was originally examined and that they might help clarify the situation, we reacted **3** with diazomethane and obtained a 7% yield of synthetic material with properties essentially identical to those reported for pseudoiodinine. It remained only to locate the position of the newly installed methyl group.

Apart from **1**, there are five possible methylation products of **3**, **16**−**20** (Figure 1). The ^{1}H NMR spectrum of synthetic pseudoiodinine contains a resonance for an aromatic hydrogen, eliminating **16** as a possible structure. HMBC spectroscopy reveals that the hydrogens on the two methyl groups in pseudoiodinine each correlate with only one—but different—ring carbon, neither of which bears a hydrogen. That finding excludes **17** and **18** as possible structures for pseudoiodinine.

To determine whether **19** or **20** is the correct structure for pseudoiodinine, we again turned to synthesis. The synthesis of **20** (eq 1) was begun by reaction of chloro ester **8** with methylhydrazine to give the bicyclic **21** directly (shown to be different from its previously synthesized regioisomer **11**). Reaction of **21** with (trimethylsilyl)diazomethane is capable of delivering only one O-methylated product (**20**), which it does in 60% yield. The spectra of yellow synthetic **20** show that it is not pseudoiodinine.



(1)

By process of elimination, **19** must be the structure of pseudoiodinine. Nonetheless, it was desirable to positively affirm the identity. That was done: O-methylation of nostocine A (**2**), which we had already synthesized, with ethereal diazomethane gives pseudoiodinine, identical with the material produced (as described above) by reaction of **3** with diazomethane. The structure of pseudoiodinine is thus revised from **1** to **19** and confirmed by total synthesis.[16]

In conclusion, these first-reported syntheses of any members of the naturally occurring pyrazolo[4,3-*e*][1,2,4]triazine family verify the structures of nostocine A and fluviol A as **2** and **3**, respectively, and lead to the revision of the structure of pseudoiodinine from **1** to **19**. Structures **1** and **19** were also established by total synthesis.

Acknowledgment. J. P. acknowledges the Ministerio de Educación y Ciencia (Madrid) for a postdoctoral fellowship. We are extremely grateful to Dr. Svetlana A. Dovzhenko for helpful exchange of information, and Drs. Elena A. Kiprianova and Elena P. Ivanova for assistance. We thank Professor John Frost (Michigan State University) for bringing ref 18 to our attention.

Supporting Information Available: Experimental and characterization data for compounds **2**, **3**, **5**−**12**, **14**, **15**, and **19**−**21**; reproductions of selected NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) That assertion is based on a thorough search of the "Dictionary of Natural Products"[17] and an informal polling of numerous members of the organic community. To our knowledge, one 1,2,4-triazole has been found in nature.[18] The two most likely exceptions to the generalization, 1,2,3-triazoles[19] and azapurines,[20] have both been stated by others not to occur naturally.
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(12) The structure of normethylpseudoiodinine was determined by X-ray crystallography, which is regarded as an absolute method, but the literature is replete with erroneous X-ray structures: See, for example: (a) Jones, P. G. *Chem. Soc. Rev.* **1984**, *13*, 157−172. (b) Parkin, G. *Acc. Chem. Res.* **1992**, *25*, 455−460. (c) Marsh, R. E.; Kapon, M.; Hu, S.; Herbstein, F. H. *Acta Crystallogr., Sect. B: Struct. Sci.* **2002**, *B58*, 62−77. (d) Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1012−1044.
(13) Hydrazine itself is symmetrical. In contrast to the situation with methylhydrazine, the reason for the Boc protection in the present case is to suppress reaction of hydrazine with two molecules of **8**, which was a significant side reaction.
(14) Less than 1 equiv per cycle was used to suppress dimethylation.
(15) See Supporting Information.
(16) That **19** is the structure of pseudoiodinine means that our previously mentioned skepticism about selective N- (rather than O-) demethylation is misguided in the case of **19**. The explanation may be related to the finding that, unlike 2-pyridones,[5] 3-hydroxypyrazoles exist primarily as the hydroxy rather than oxo tautomer in nonpolar media: Katritzky, A. R.; Maine, F. W. *Tetrahedron* **1964**, *20*, 315−322.
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