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Letter

Structural Revision of Pseudocerosine and Validation of a Biosynthetic Proposal for E-ring Formation in Pyridoacridine Alkaloids

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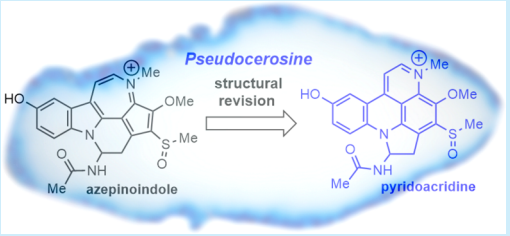
ABSTRACT: Pseudocerosine is the pigment responsible for the bright blue color of the rim on the marine flatworm *Pseudoceros indicus*. Compelling evidence is provided herein that pseudocerosine is actually a pyridoacridine, not an azepinoindole as initially proposed. This study also validates a biosynthesis proposal for E-ring formation in this revered class of alkaloids, and pseudocerosine (along with its intermediates described herein) is a new branch on the pyridoacridine family tree.



The marine flatworm *Pseudoceros indicus* possesses an intensely blue outer rim. The pigment responsible for this color, pseudocerosine (**1**, Figure 1), was reported to contain a structurally unprecedented azepino[4,5-*b*]indole harboring a rare azafulvene unit.[1] During ongoing synthetic studies toward this alkaloid,[2] we have found that several azepinoindoles related to pseudocerosine are unstable, and as a result, it was felt that the structure of the natural product may have been misassigned. This rationale is enforced by the absence of a plausible biosynthesis for the reported azepinoindole structure.[3] During extensive searches of the marine natural product literature, it became apparent that several of the structural subunits present in pseudocerosine are also found in known pyridoacridine alkaloids;[4] an *o*-(methylthio)anisole is present in varamine B,[5] the acetamidyl aminal unit is present in arnoamine D,[6] and the quaternized nitrogen is present in cyclodercitin[7] (Figure 1). As a result, it is plausible that

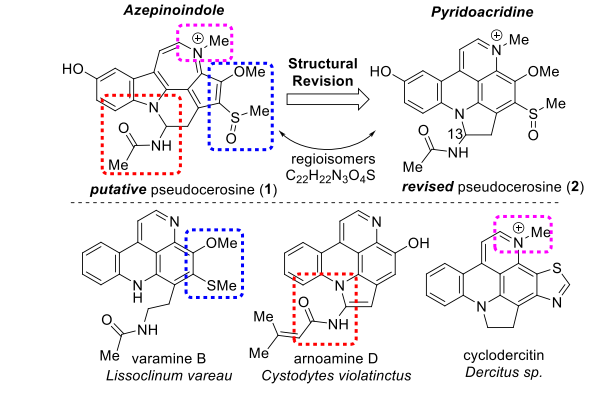
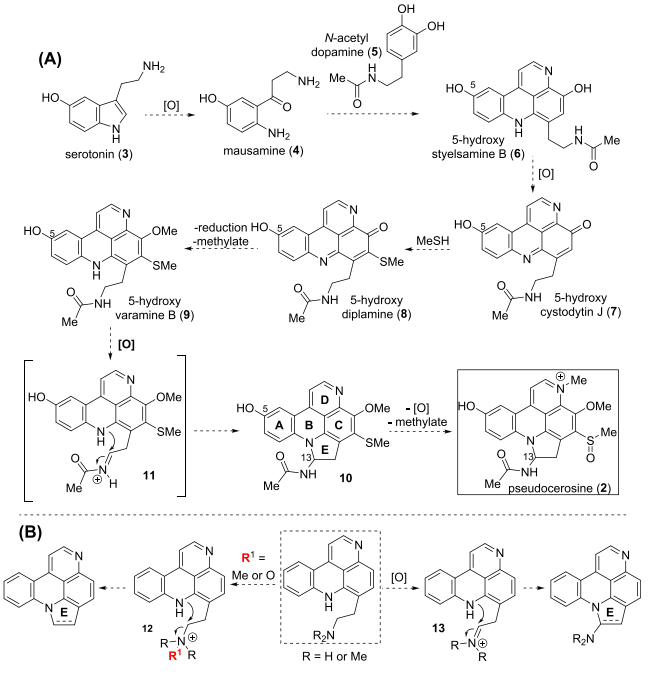


Figure 1. Proposed structural revision of pseudocerosine and pyridoacridines with structural motifs akin to those in pseudocerosine.

Scheme 1. (A) Proposed Biosynthesis of the Revised Pseudocerosine Structure and (B) Proposed E-ring Formation in Pyridoacridines[8,9]



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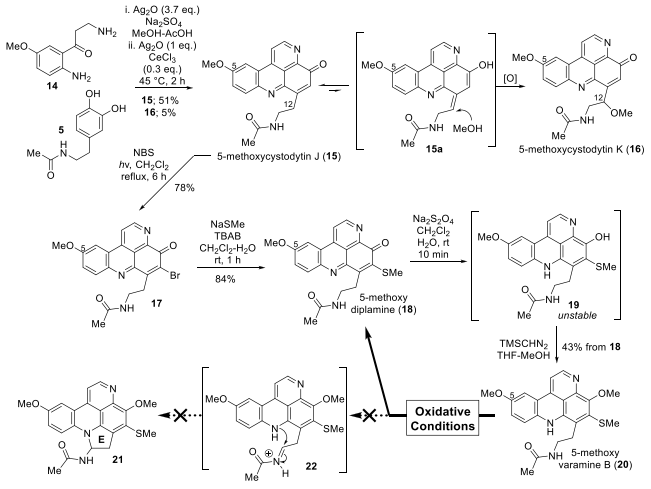
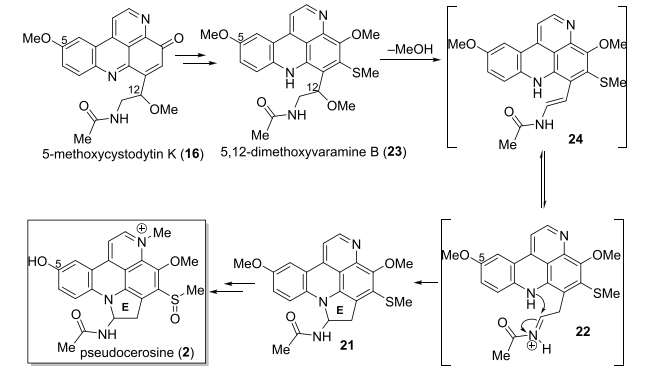
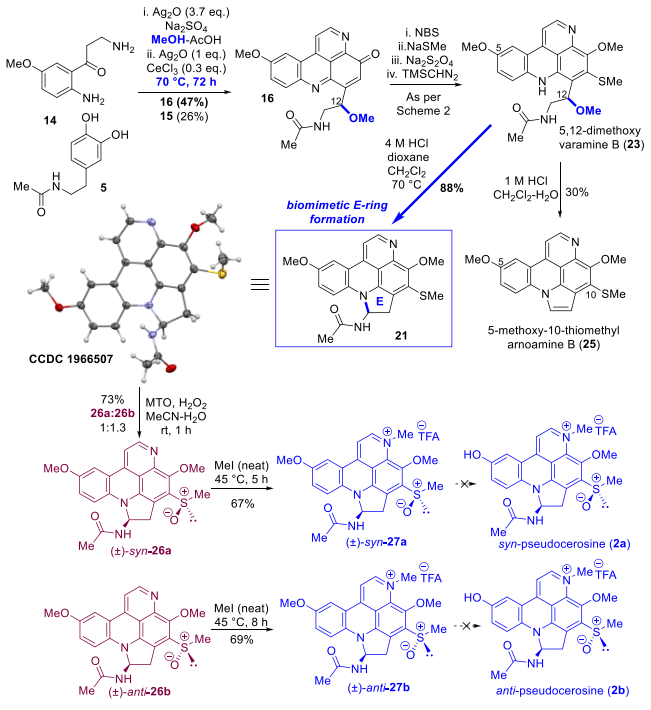
Scheme 2. Synthesis of 5-Methoxyvaramine B (20) and Attempted Biomimetic Cyclization



Scheme 3. Revised Proposal for Biomimetic E-ring Assembly



Scheme 4. Biomimetic E-ring Assembly and Synthesis of Pseudocerosine Methyl Ethers 27a and 27b



pseudocerosine is not an azepinoindole but instead is pyridoacridine **2** (Figure 1).

The proposed structural revision is supported by a proposed biosynthesis (Scheme 1A). Oxidative cleavage of serotonin (**3**) would give mausamine (**4**) that upon heteroannulation with *N*-acetyldopamine (**5**) would lead to 5-hydroxystyelsamine B (**6**). Oxidation of **6** would give the quinoneimine 5-hydroxycystodytin J (**7**) that upon thiomethylation to 5-hydroxydiplamine (**8**) and reductive methylation would afford 5-hydroxyvaramine B (**9**). The cyclization of the side chain to form the E-ring in **10** would proceed via an *N*-acyliminium ion **11**. Oxidation of the sulfide and *N*-methylation would then afford pseudocerosine (**2**). This structural revision means pseudocerosine would be the first pyridoacridine known to possess an acetamide at C13. Interestingly, the formation of the E-ring in pyridoacridine alkaloids has been proposed to be initiated via two pathways (Scheme 1B):[8,9] (1) *N*-methylation or *N*-oxidation of a tertiary amine (i.e., **12**) or (2) oxidation of the amine to the iminium ion **13**. Neither of these proposals has been validated using biomimetic synthesis.[6]

Setting out to validate our hypothesis, a synthetic route to the revised pyridoacridine structure **2** was developed based on the proposed biosynthesis shown in Scheme 1A. 4-Methoxykynuramine (**14**)[10] and *N*-acetyldopamine (**5**)[11] underwent a biomimetic oxidative heteroannulation[12] to give 5-methoxycystodytin J (**15**) along with 5-methoxycystodytin K (**16**), the latter arising from alkoxylation at C12 (Scheme 2). The formation of **16**, presumably by oxidative alkoxylation of an intermediate like **15a**, is of note, as several pyridoacridines possess a hydroxy/alkoxy substituent at C12,[8] none of which has ever been prepared by synthesis.[6] Bromination of 5-methoxycystodytin J (**15**) gave 5-methoxy-10-bromocystodytin J (**17**), which upon treatment with sodium methanethiolate gave 5-methoxydiplamine (**18**). The reduction of 5-methoxydiplamine (**18**) gave the unstable aminophenol **19** that was immediately subjected to trimethylsilyldiazomethane to give 5-methoxyvaramine B (**20**), setting the stage for the key biomimetic E-ring formation. Unfortunately, subjecting 5-methoxyvaramine B (**20**) to a variety of oxidants known to convert acetamides to the corresponding *N*-acyliminium ion[13] did not trigger cyclization and the formation of the E-ring to give **21**; all of the reaction conditions led to degradation or oxidized the substrate back to 5-methoxydiplamine (**18**).

Because the E-ring could not be formed via an *N*-acyliminium ion **22** generated by oxidation, the biomimetic cyclization strategy was revised. Attention turned to the C12-alkoxylated pyridoacridine, 5-methoxycystodytin K (**16**), which formed as a minor product during the initial pyridoacridine construction step shown in Scheme 2. Because ∼10% of pyridoacridines possess a hydroxy or alkoxy substituent at the C-12 site,[8] we propose that they may serve as the precursors to E-ring formation *in vivo* (Scheme 3). The conversion of 5-methoxycystodytin K (**16**) to 5,12-dimethoxyvaramine B (**23**) followed by the loss of methanol would generate enamide **24**; cyclization of the iminium tautomer **22** and attack by the adjacent amine would forge the E-ring in pyridoacridine **21**. Oxidation, *N*-methylation, and demethylation at C5 would then give peudocerosine (**2**). Efforts subsequently focused on the revised biosynthetic proposal (Scheme 4). The yield of 5-methoxycystodytin K (**16**) could be increased by conducting the heteroannulation reaction between **14** and **5** at 70 °C for 3 days.

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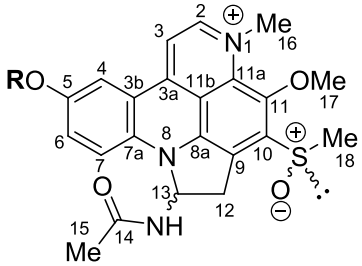
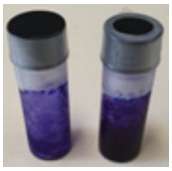
Table 1. NMR Spectroscopic Data for Pseudocerosine (2), 27a, and 27b (CD_3OD)[a]





	pseudocerosine (2)[b] (R = H)		pseudocerosine methyl ether (27a)[c] (R = Me)				pseudocerosine methyl ether (27b)[d] (R = Me)			
atom[a]	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	$\Delta\delta$	δ_C	$\Delta\delta$	δ_H (J in Hz)	$\Delta\delta$	δ_C	$\Delta\delta$
2	8.22, d, 6.8	152.8	8.28, d, 6.8	+0.06	152.9	+0.1	8.25, d, 5.7	+0.03	152.9	+0.1
3	7.32, d, 6.8	106.9	7.61, d, 6.8	+0.29	107.2	+0.3	7.57, d, 5.7	+0.25	107.2	+0.3
3a		148.6			149.4	+0.8			149.4	+0.8
3b		119.5			119.5	+0			119.5	0
4	7.45, d, 2.6	109.9	7.71, d, 2.5	+0.26	107.5	−2.4	7.67, unresolved	+0.22	107.5	−2.4
5		155.9			158.4	+2.5			158.3	+2.4
6	7.33, dd, 9.3/2.6	127.6	7.57, dd, 9.3/2.5	+0.24	128.2	+0.6	7.53, d, 9.2	+0.20	128.2	+0.6
7	7.52, d, 9.3	118.5	7.72, d, 9.3	+0.20	119.1	+0.6	7.70, d, 9.2	+0.18	119.0	+0.5
7a		132.6			134.2	+1.6			134.1	+1.5
11a		119.7			120.2	+0.5			120.3	+0.6
8a		140.2			140.6	+0.4			140.6	+0.4
9		121.0			121.4	+0.4			121.3	+0.3
10		144.6			144.9	+0.3			144.9	+0.3
11		137.0			137.2	+0.2			137.0	0
11a		132.3			132.5	+0.2			132.5	+0.2
12a	4.18, dd, 18.3/9.5	35.8	4.19, dd, 18.5/9.5	+0.01	35.9	+0.1	4.22, dd, 18.3/9.0	+0.04	36.1	+0.3
12b	3.93, dd, 18.3/2.5		3.95, dd, 18.5/2.5	+0.02			3.85, d, 18.3	−0.08		
13	6.92, dd, 9.5/2.5	69.3	7.02, dd, 9.5/2.5	+0.10	69.6	+0.3	6.94, d, 8.4	+0.02	69.8	+0.5
14		172.6			172.4	−0.2			172.6	0
15	1.94, s	22.7	1.95, s	+0.01	22.6	−0.1	1.96, s	+0.02	22.7	0
16	4.28, s	46.4	4.30, s	+0.02	46.2	−0.2	4.29, s	+0.01	46.2	−0.2
17	3.93, s	66.4	3.95, s	+0.02	66.3	−0.1	3.94, s	+0.01	66.3	−0.1
18	3.13, s	42.2	3.08, s	−0.05	42.2	0	3.12, s	−0.01	42.3	+0.1
NH	7.35, d, 8.6									
OMe			3.98, s		56.6		3.96, s		56.6	

[a]NMR spectroscopic data in the isolation report have been assigned to the pyridoacridine numbering system. [b]NMR spectroscopic data for the minor diastereomer were not provided in the isolation report. [c]Minor diastereomer. [d]Major diastereomer.[14]

In this process, TLC and ^{1}H NMR analyses of the reaction mixture confirm that 5-methoxycystodytin (**15**) forms first, which then undergoes oxidative alkoxylation to form 5-methoxycystodytin K (**16**). Following this, the route outlined in Scheme 2 was then effectively replicated to give 5,12-dimethoxyvaramine B (**23**), the key cyclization precursor. Upon subjecting **23** to aqueous acidic conditions, the E-ring did form but was accompanied by concomitant elimination to give 5-methoxy-10-thiomethylarnoamine B (**25**). However, conducting the cyclization under anhydrous acidic conditions led to E-ring formation without the accompanying elimination product **25**, providing **21** bearing the complete skeleton of the revised pseudocerosine structure, as confirmed by X-ray analysis. This high-yielding cyclization provides good evidence that the alkoxy group at C12 plays a crucial role in the assembly of the E-ring and validates the biosynthetic proposal outlined in Scheme 3. With the E-ring assembled and the complete pyridoacridine scaffold in hand, attention turned toward confirming the structural revision. Subjecting pyridoacridine **21** to methylrhenium(VII)-trioxide (MTO)-catalyzed oxidation gave two purple sulfoxides **26a** and **26b** in a 1:1.3 ratio,[14] inferring that there was some slight diastereocontrol during this process. It was not possible to assign the relative stereochemistry of the two diastereomers,[14] an issue that was also encountered by the isolation chemists because pseudocerosine exists as a diastereomeric mixture (5:2).[1] The two diastereomers **26a** and **26b** were separated and individually subjected to methyl iodide to give pseudocerosine methyl ethers **27a** and **27b**. This final *N*-methylation turned the compounds **27a** and **27b** deep blue in color, the same color as the natural product. Attempts to conduct a selective demethylation at C5 were not successful, but the spectroscopic data for the methyl ethers **27a** and **27b** were sufficient to confirm the structural revision (Table 1). The ^{1}H and ^{13}C NMR spectroscopic data of the two ethers **27a** and **27b** were in good agreement with that in the isolation report, with the minor diastereomer **27a** showing slightly better alignment.[1]

In summary, compelling evidence is presented that pseudocerosine, the pigment responsible for the deep blue color of the rim on the flatworm *Pseudoceros indicus*, possesses a pyridoacridine structure and not the azepinoindole initially proposed. A plausible biosynthesis of the revised structure is

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presented, which subsequently inspired a biomimetic synthesis of two pseudocerosine pyridoacridine diastereomers that possess NMR spectroscopic data consistent with the natural product. Moreover, we have validated a biosynthetic proposal for the formation of the E-ring in pyridoacridine alkaloids, and pseudocerosine (including many of its intermediates) is a new branch on the pyridoacridine family tree.[8]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c00953.

Experimental procedures and NMR spectra for all novel compounds (PDF)

Accession Codes

CCDC 1966507 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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(14) We were unable to obtain crystals of **26a**, **26b**, **27a**, and **27b** that were suitable for X-ray analysis. As a result, the relative stereochemistry of each diastereomer could not be determined. The relative relationships between the C−N bond of the C-13 aminal and the S−O bond of the sulfoxide were used to designate the diastereomers as syn and anti. The assignments in Scheme 4 could be inverted (i.e., **26a** and **27a** could be anti; **26b** and **27b** could be syn).