Oops, I Did It Again: Structural Misassignment of Natural Products

Well that’s not great....

Jeffrey Lipshultz
Group Meeting
MacMillan Group
April 26, 2016
Structural Misassignment of Natural Products

Nobel Prize awarded in 1927, 1928 to Wieland, Windaus

Heinrich Wieland
"for his investigations of the constitution of the bile acids and related substances"

Adolf Windaus
"for the services rendered through his research into the constitution of the sterols and their connection with the vitamins"
Structural Misassignment of Natural Products

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Structural Misassignment of Natural Products

Nobel Prize awarded in 1927, 1928 to Wieland, Windaus

cholesterol
Structural Misassignment of Natural Products

X-ray crystallographic studies call proposed connectivity into question.

"Such measurements are difficult to reconcile with the usually accepted sterol formula..."

"... but agree much better with one where the carbon chain is attached to atom 17 in ring iv..."

"It is clear that the usually accepted sterol structure must be considerably modified."

Structural Misassignment of Natural Products

- X-ray crystallographic studies call proposed connectivity into question

![Diagram of sterol structures]

**general sterol structure**

**newly proposed sterol structure**

**final (correct) general sterol structure**

Structural Misassignment of Natural Products

- Büchi converted α-patchoulene into patchouli alcohol (isolated 1869)

- But, it turns out, there was something else going on...


Butčič, G.; MacLeod, W. D. J. Am. Chem. Soc. 1962, 84, 3205.

Structural Misassignment of Natural Products

Büchi converted \( \alpha \)-patchoulene into patchouli alcohol (isolated 1869)

But, it turns out, there was something else going on...

"Since neither of these rearrangements was recognised, patchouli alcohol and \( \alpha \)-patchoulene were assumed to have identical carbon skeletons."

Structural Misassignment of Natural Products

outline

Better spectroscopic techniques have certainly helped...

Isoschizogamine

Sporol

Sclerophytin

But X-rays are not the panacea...

Diazonamides

Kinamycins

Computational tools can help...

Hexacyclinol

Aquatolide

But sometimes you just need some common sense...

Nomofungin
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Structural Misassignment of Natural Products

C$_7$-epimeric structures of schizogamine and isoschizogamine described in 1965

"Careful analysis of the $^{13}$C NMR spectra indicates, however, a different skeleton..."

Renner U.: personal communication

Structural Misassignment of Natural Products

- C₇-epimeric structures of schizogamine and isoschizogamine described in 1965

\[\text{schizogamine} \quad \text{isoschizogamine}\]

- \(^{13}\text{C} \) NMR and 2D NMR lead to new proposal in 1998

\[\text{CH}_2 84.44 \text{ ppm} \quad \text{CH}_2 173.8 \text{ ppm} \quad \text{CH}_1 35.0, 37.7 \text{ ppm}\]

\[\text{isoschizogamine} \quad \text{isoschizogamine} \text{ (original structure)} \quad \text{isoschizogamine} \text{ (revised structure)}\]

Structural Misassignment of Natural Products

- C7-epimeric structures of schizogamine and isoschizogamine described in 1965

![schizogamine](image1)

![isoschizogamine](image2)

- 13C NMR and 2D NMR lead to new proposal in 1998
  - Rearrangement proposed for schizogamine → isoschizogamine

![isoschizogamine (revised structure)](image3)


Structural Misassignment of Natural Products

Heathcock validated structure via biomimetic total synthesis

-isoschizogamine-

27% yield over 4 steps

Structural Misassignment of Natural Products

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Structural Misassignment of Natural Products

- Novel trichotheceine mycotoxin sporol described with [2.2.2] fused to [2.2.1] in 1986
- Key $C_{15}-C_3$ nOe observed

Structural Misassignment of Natural Products

- Novel trichothecene mycotoxin sporol described with [2.2.2] fused to [2.2.1] in 1986
  - Key C_{15}-C_{3} nOe observed

- Reevaluation of $^1$H NMR and NOESY lead to new structural proposal
  - Connectivity is close! Only one carbon off.

Ziegler was able to confirm structure by total synthesis of sporol

1. Ms₂O; K₂O
2. CSA; H₂O
32% yield

15% yield over 3 steps

**Structural Misassignment of Natural Products**

*outline*

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Structural Misassignment of Natural Products

First isocembrene diterpenes containing two transannular ether linkages

- Note the lack of explicit stereochemistry at the C14 position!

Paquette's interpretation

This is somewhat of a disaster... Paquette does MM3 calculations, the original structure is not good

- "Unfortunately, the intentions behind the use of an 'inverted carbon' symbolism here will never be known."

Paquette shows this is the most likely (thermodynamic) conformer/stereoisomer

Structural Misassignment of Natural Products

- Paquette prepared this corrected structure...

\[
\text{prepared by Danishefsky's diene DA and macrolactonization}
\]

\[
\text{sclerophytin A}
\]

- Overman prepared this corrected structure as well... (hi Dave!)

\[
\text{prepared by Prins-pinacol}
\]

\[
\text{2 steps to 7-deacetoxyalcyonin acetate}
\]

Structural Misassignment of Natural Products

Paquette prepared this corrected structure...


these didn’t match the published data!

prepared by Danishefsky's diene DA

prepared by Prins-pinacol

2 steps to 7-deacetoxyalcyonin acetate

sclerophytin A
Structural Misassignment of Natural Products

- Well, they got the mass incorrect...

- Not only was there a typo (462 instead of 362), but they're missing an equivalent of H₂O!

![Original Sclerophytin B Connectivity](image1)

*original sclerophytin B connectivity*

\[ m/z = 362^*, 302 (-AcOH) \]

![Corrected Sclerophytin B Connectivity](image2)

*corrected sclerophytin B connectivity*

\[ m/z = 381, 321 (-AcOH) \]

- Stereochemistry determined by NOESY

![Sclerophytin B](image3)

*sclerophytin B*

*aka 6-acetyl sclerophytin A*


Structural Misassignment of Natural Products

Once again, Paquette prepared the corrected structure

Overman prepared this corrected structure as well... (again, hi Dave!)

There's been a lot written about it, including 4 separate syntheses and a personal reflection by Paquette


Overman, L. E.; Pennington, L. D. *OL* 2000, 2, 2683.


Structural Misassignment of Natural Products

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Structural Misassignment of Natural Products

- Two complex halogenated, polycyclic, cytotoxic metabolites isolated from marine ascidian
- Diazonamide A is particularly interesting, with IC$_{50}$ vs. HCT-116 (colon cancer) and B-16 (melanoma) < 15 ng/mL

\[
\text{H}_{2}\text{N} \quad \begin{array}{c}
\text{N} \\
\text{O}
\end{array} \\
\text{N}
\]

- Diazonamide A

\[
\text{HN} \\
\text{H}
\]

- Diazonamide B

hemiacetal present!

"3.5 Hz coupling of a D$_2$O-exchangable proton at δ 7.36 to a δ 6.46 proton that was one-bond coupled to the C11 resonance at δ 106.6 in the XHCORR spectrum"

Structural Misassignment of Natural Products

- Two complex halogenated, polycyclic, cytotoxic metabolites isolated from marine ascidians
  - Diazonamide A is particularly interesting, with IC\textsubscript{50} vs. HCT-116 (colon cancer) and B-16 (melanoma) < 15 ng/mL

\[
\begin{align*}
\text{diazonamide A} & \quad \text{diazonamide B}
\end{align*}
\]

**Key differences between B and A**

- A has no Br atom (seen in NMR)
- A has an additional \textsuperscript{1}Pr group (seen in NMR)
- A has m/z of 765 vs 743 for B $\rightarrow$ extra valine residue!
- Valine residue must be attached at indicated N (based on NMR shifts)

Harran prepared the proposed structure of diazonamide A and the diazonamide B derivative.
Harran prepared the proposed structure of diazonamide A and the diazonamide B derivative.

\[
\begin{align*}
&\text{CbzHN$_2$} \quad \text{AcO} \\
&\text{Me} \quad \text{Me} \\
&\text{HN} \quad \text{N} \\
&\text{O} \quad \text{O} \\
&\text{O} \\
&\text{N} \\
&\text{Br} \\
&\text{h} \quad \nu \\
&\text{AcO} \\
&\text{NH} \\
&\text{OBn} \\
&\text{O} \\
&\text{Me} \quad \text{Me} \\
&\text{Me} \\
&\text{AcO} \\
&\text{O} \\
&\text{O} \\
&\text{O} \\
&\text{Cl} \\
&\text{Cl} \\
&\text{OH} \\
&\text{OH} \\
&\text{HN} \\
&\text{H} \\
&\text{Me} \quad \text{Me} \\
&\text{Me} \\
&\text{AcO} \\
&\text{AcO} \\
&\text{OBn} \\
&\text{O} \\
&\text{Me} \quad \text{Me} \\
&\text{Me} \\
&\text{CBzHN$_2$} \\
&\text{Me} \quad \text{Me} \\
&\text{Me} \\
&\text{H$_2$N} \quad \text{CONH$_2$} \\
&\text{Me} \quad \text{Me} \\
&\text{Me} \\
\end{align*}
\]

\textit{diazonamide A}

\textit{44\% yield over 5 steps}

\textit{does not match natural material!}

\textit{32-40\% yield}

Harran prepared the proposed structure of diazonamide A and the diazonamide B derivative.

Structural Misassignment of Natural Products

Harran proposes a new, corrected structure

Isolation chemists actually had the data to show they were wrong, but ignored it!

"In retrospect, evidence argued against this [structure]. However, in the excitement of uncovering such a remarkable new class of materials... certain details were inadvertently overlooked."


Structural Misassignment of Natural Products

Harran proposes a new, corrected structure

Isolation chemists actually had the data to show they were wrong, but ignored it!

"Acetylation of the amine group of the... valine residue was also indicated by the shift of the C37 α-proton to δ 5.11. This proton did not couple to an amide proton suggesting the presence of a secondary amine, but no spectral feature... suggested the nature of the additional substituent."

"In a triacetate derivative, the C37 proton shifts downfield to δ 5.11, although it now appears as a doublet rather than the more complex pattern one might expect for a C37 acetamide... We believe these observations are consistent with the C37 substituent in natural diazonamide A being an alcohol rather than an amine."

Structural Misassignment of Natural Products

Harran proposes a new, corrected structure

$^1$H/$^{15}$N-HSQC and COSY unequivocally show that the heteroatom attached to C11 is N, not O

"[The $^1$H/$^{15}$N-HSQC] indicates... the $\delta = 7.16$ (N2H) resonance is coupled to C11H (DQF-COSY) and was originally assigned as O7H... the exchangeable one-proton doublet at $\delta = 5.46$, first identified as N7H$_2$, is not coupled to $^{15}$N — consistent with our C37 hydroxy model."


Structural Misassignment of Natural Products

- Harran proposes a new, corrected structure
- Isolation chemists actually had the data to show they were wrong, but ignored it!

"However, the C17-O3 bond... is measured at 1.433(16) Å. This is 0.048 Å longer than the mean and, notably, exceeds the maximal value (1.409 Å) observed for a bond of this type... This indicates that the O3 assignment should be changed to an element with fewer electrons and a larger covalent radius... these data are consistent with the electron density assigned as O3 being a protonated nitrogen atom..."

**Structural Misassignment of Natural Products**

  - (S)-isovaleric acid was confirmed as sidechain stereochemistry
  - Synthetic details are not super important— a lot has been written, go check it out if you're interested

![diazonamide A](image)

---


Structural Misassignment of Natural Products

outline

Better spectroscopic techniques have certainly helped...

- Isoschizogamine
- Sporol
- Sclerophytin

But X-rays are not the panacea...

- Diazonamides
- Kinamycins

Computational tools can help...

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Structural Misassignment of Natural Products

Kinamycins A, B, C, and D first described in 1970

- At first, simple common structural fragments were described, but no full structure

"The kinamycins have an infrared absorption maximum at 2155 cm\(^{-1}\), assignable to a nitrile or isonitrile group..."

NMR analysis and derivatization of deacetylkinamycin C lead to a proposed structure

- Hydrolysis of deacetylkinamycin C with refluxing 30% KOH liberated ammonia, but not formic acid → nitrile!

Location of the unprecedented N-cyano group confirmed by x-ray crystal structure! In 1972!

Even in the crystal structure paper, they cite the NH$_3$ liberation result to confirm nitrile vs. isonitrile.

$N$-C bond length is $1.34$ Å
$C\equiv N$ bond length is $1.14$ Å


Structural Misassignment of Natural Products

- By fermenting bacteria with $^{15}\text{NH}_4\text{SO}_4$, able to obtain $^{15}\text{N}_2\text{kinamycin D}$
  - Observed the nitrile C by $^{13}\text{C}$ NMR for the first time! Except...

  "Although we cannot unequivocally explain the large upfield shift (ca. 30 ppm)..."

- Synthetic work calls the N-cyano pyrrole into question...

  does not match natural (fermented) sample ($^1\text{H}$, IR)

Structural Misassignment of Natural Products

Gould, who had done most of the biosynthetic work, realized there were too many inconsistencies.

Reconsideration included crystal structure of different ester derivative.

"the diazo alternative was clearly possible: $^{13}\text{C}$ NMR resonances of a number of diazo compounds have been observed in the $\delta$ 60-80 region, including 9-diazofluorene at $\delta$ 63.2."

More complete synthetic work confirms that N-cyano can not be correct.

IR $\nu_{\text{max}}$ = 2155 cm$^{-1}$


Structural Misassignment of Natural Products

Gould, who had done most of the biosynthetic work, realized there were too many inconsistencies. Reconsideration included crystal structure of different ester derivative.

"Revised Structure for the Kinamycin Antibiotics: 5-Diazobenzo[b]fluorenes Rather Than Benzo[b]carbazole Cyanamides"

More complete synthetic work confirms that N-cyano can not be correct.

"The Kinamycins are Diazofluorenes and Not Cyanocarbazoles"

Structural Mis assignment of Natural Products

There's quite a bit more... including recent total synthesis efforts and medicinal chem investigations


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**Structural Misassignment of Natural Products**

- Hexacyclinol is probably the most absurd chemical structure story
  - First described in 2002, isolated from Siberian fungus

```
H O O Me
H H Me Me
H H Me OMe
```

- I'm not going to get into La Clair’s retracted synthesis...

"Occasionally, blatantly wrong science is published, and to the credit of synthetic chemistry, the corrections usually come quickly and cleanly," comments Harvard University chemistry professor E. J. Corey.

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**Hexacyclinol Report Retracted**

*Organic Synthesis: Paper pulled for lack of sufficient supporting information*

By Bethany Halford

---

**Hexacyclinol? Or Not?**

By Derek Lowe | June 5, 2006

*Second of two total syntheses casts doubt on earlier structure, synthesis*

By Bethany Halford

---

**Retraction**

Retraction: Total Syntheses of Hexacyclinol, 5-epi-Hexacyclinol, and Desoxohexacyclinol Unveil an Antimalarial Prodrug Motif

James J. La Clair Dr.

---

**Hexacyclinol Retracted. It Only Took Six Years.**

By Derek Lowe | November 14, 2012


Structural Misassignment of Natural Products

- Rychnovsky, spurred on by La Clair’s "provocative synthesis" decides to "reexamine the original structure"

- Computational methods can allow for highly accurate NMR predictions ($^{13}$C shifts within average $|\Delta \delta|$ < 2 ppm)

**elisapterosin B**
- average $|\Delta \delta|$ = 1.9 ppm
- max $|\Delta \delta|$ = 3.8 ppm

**maoecrystal V**
- average $|\Delta \delta|$ = 1.2 ppm
- max $|\Delta \delta|$ = 3.7 ppm

**elisabethin A**
- average $|\Delta \delta|$ = 1.4 ppm
- max $|\Delta \delta|$ = 3.8 ppm

- Polycyclic, unsaturated, oxygenated molecules can be accurately predicted... how does hexacyclinol do?

**hexacyclinol**
- average $|\Delta \delta|$ = 6.8 ppm
- max $|\Delta \delta|$ = 22.0 ppm

So, that's probably not hexacyclinol... what is?

Rychnovsky proposes a related compound to panepophenanthrin, from the same fungus... isolation artifact?

Rychnovsky's hexacyclinol
average $|\Delta \delta| = 1.8$ ppm
$\max |\Delta \delta| = 5.8$ ppm

Porco prepares Rychnovsky's proposed structure...

La Clair claimed the structures could have the same $^1$H NMRs, so of course people had to show otherwise.

Applying Computer-Assisted Structure Elucidation Algorithms for the Purpose of Structure Validation: Revisiting the NMR Assignments of Hexacyclinol

...a more in-depth analysis, concluding "we accept the conclusion [Rychnovsky's assignment] as being accurate and appropriate."

Can Two Molecules Have the Same NMR Spectrum? Hexacyclinol Revisited

..."The structure of hexacyclinol is confirmed to be [Rychnovsky's]. If [La Clair's structure] had been synthesized... its NMR spectra are sufficiently different... as to guarantee their distinction."
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Structural Misassignment of Natural Products


- Related compounds isolated from same source allow for some idea of biosynthesis

Structural Misassignment of Natural Products

- Biosynthesis of aquatolide seems to be an intramolecular [2+2] of asteriscunolide C

\[ \text{asteriscunolide C} \] \[ \rightarrow \text{aqutolide} \]

- Tantillo wanted to study the biosynthesis in detail, but wanted to confirm structure... guess how that went?

\[ \text{aqutolide} \]

\[ ^{13}C \text{ NMR} \]
- average $|\Delta \delta|$ = 7.23 ppm
- max $|\Delta \delta|$ = 24.33 ppm

\[ ^{1}H \text{ NMR} \]
- average $|\Delta \delta|$ = 0.35 ppm
- max $|\Delta \delta|$ = 1.31 ppm

Structural Misassignment of Natural Products

- Try computational analysis of all the possible [2+2] products of all the asteriscunolides...

- The originally discounted [2+2] shown delivered an excellent agreement with observed NMR

Tantillo and coworkers re-isolated aquatolide and reexamined $^1$H NMR coupling. Computations predict an unusually large $^2J = 6.8$ Hz between the highlighted atoms.

COSY, HSQC, and NOESY further strengthened the assignment. The new assignment agrees with observed nOe much better.

Structural Misassignment of Natural Products

- Photochemical [2+2] allows for rapid construction of [2.1.1] core

Structural Misassignment of Natural Products

- Computational prediction of NMR has been proven to be a powerful tool


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### Structural Misassignment of Natural Products

- **Interesting new analogue to the communesins described in 2001**
  - Isolated from a fungus that then died, couldn't be recovered, thus "nomofungin"

- **One problem... the reported NMRs were strikingly similar...** (select comparisons below)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nomofungin</strong></td>
<td></td>
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<tr>
<td>$^13$C</td>
<td>82.4</td>
<td>29.2</td>
<td>142.6</td>
<td>51.4</td>
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<td>2.84</td>
<td>--</td>
<td>--</td>
<td>5.10</td>
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<td><strong>Communesin B</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$^13$C</td>
<td>82.39</td>
<td>29.60</td>
<td>142.65</td>
<td>51.40</td>
<td>79.00</td>
</tr>
<tr>
<td>$^1$H</td>
<td>4.70</td>
<td>2.85</td>
<td>--</td>
<td>--</td>
<td>5.11</td>
</tr>
</tbody>
</table>

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Stoltz postulated that, given biosynthetic pathway, nomofungin couldn't be made in nature.
Funk took a fully synthetic approach by preparing the N,O and N,N cores of communesin/nomofungin.
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There's a few good reviews and group meetings, check them out below.

