Sequoia Sciences

High-throughput Natural Products Chemistry

Approaches to Structure Elucidation

Russell Williams

Successful Structure Elucidation

- Quick
  - You cannot spend days on every structure. If it is a common compound you need to be able to ID it quickly.
- Accurate
  - The time you spend must be spent well. In order to make the most of your time you have to have a plan.

Best Use of Time

- Do you have your own personal NMR?
  - If not then consider the following:
    - Collect and examine the ^{1}H NMR first.
    - If more spectra are needed what should you run?
      - ~~^{13}C~~, COSY, ~~TOCSY~~, HSQC/HMQC, HMBC, ROESY/NOESY, ~~INADEQUATE, etc.~~

Do an NMR database search.

In most cases you only need:

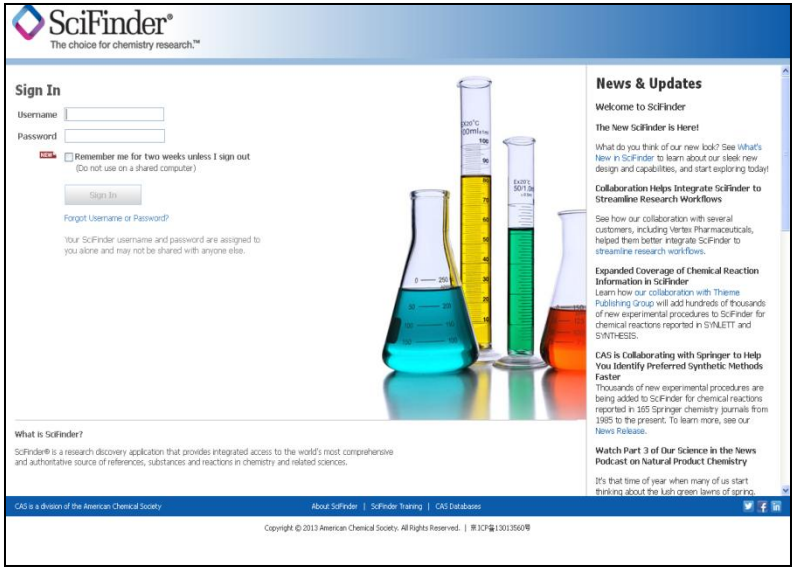
- ^{1}H
- COSY
- HSQC or HMQC
- HMBC
- ROESY or NOESY

Now You Have NMR Spectra, What Next?

- When it comes to structure elucidation you should remember:
  - Your compound is probably known.
  - If your compound is new it is probably related to a known compound.
  - All compounds are made from the same building blocks.

What Are You Working On?

- The first place to go to identify your compound is <u>not your spectra</u>.
- Find out what has been reported from your organism.
  - Everything makes more sense when it is in context.



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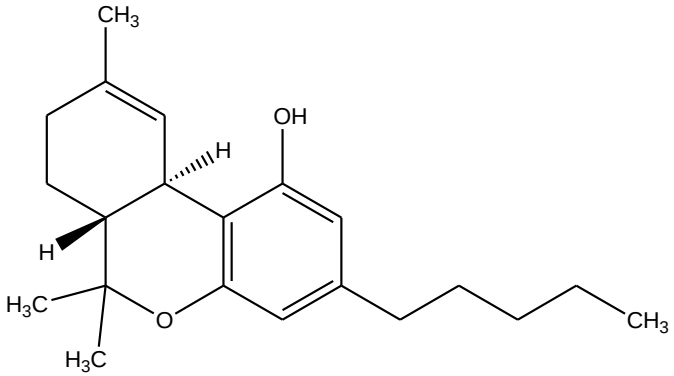
That Strange Plant in Your Roommate's Closet



Tetrahydrocannabinol

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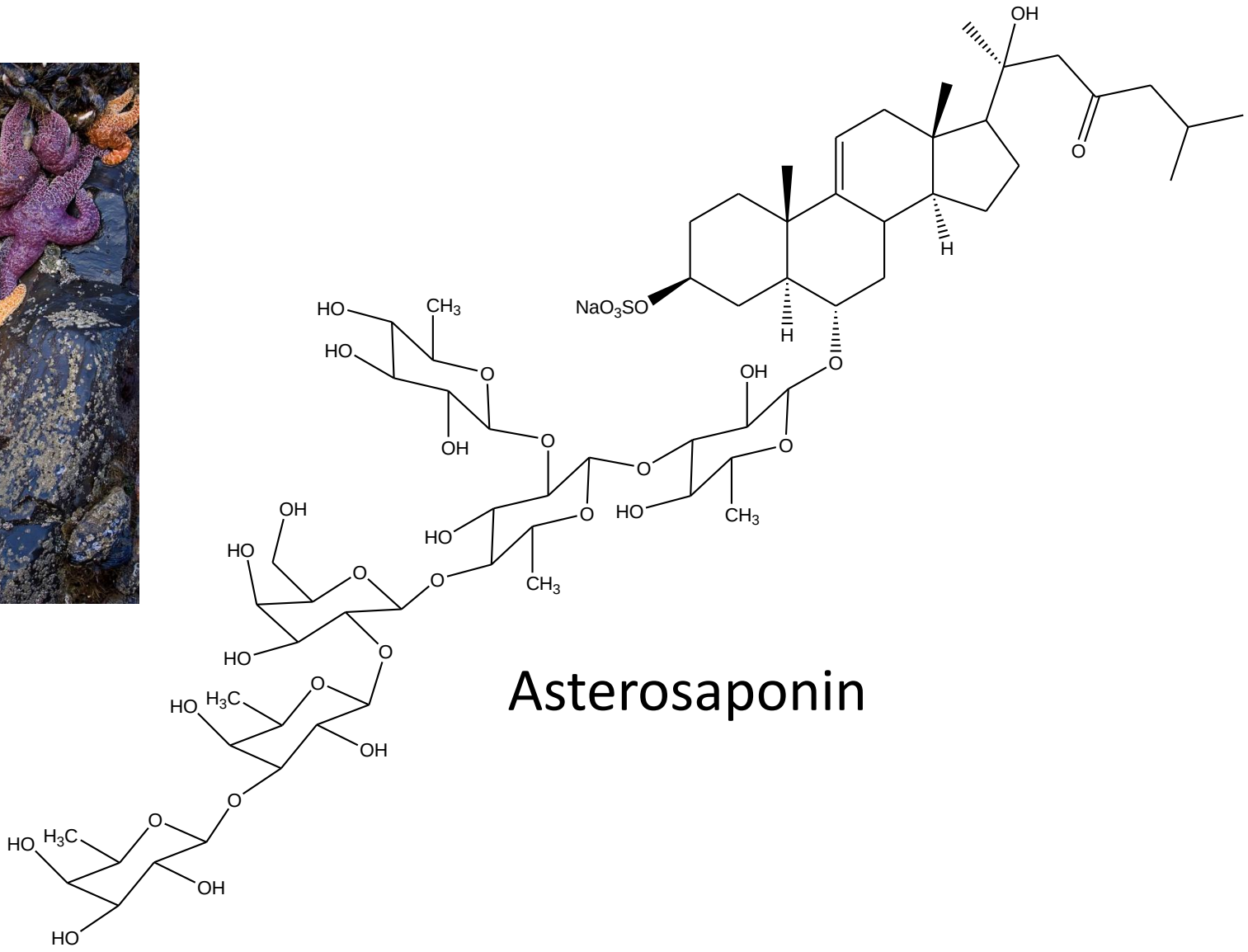
Your Roommate's Aquarium

Steven Pavlov



Asterosaponin

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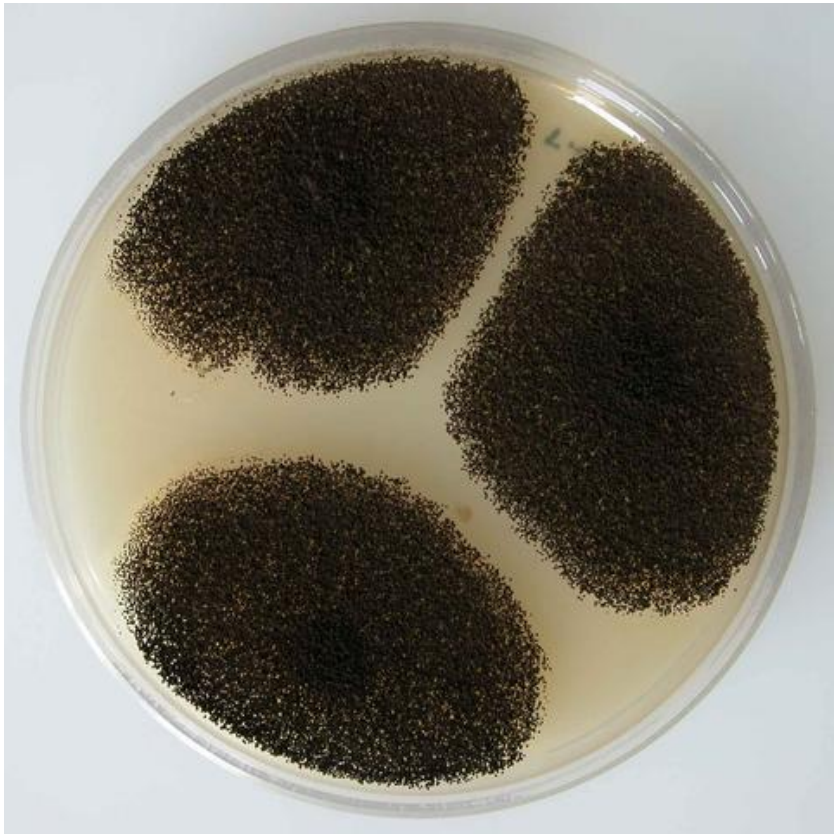
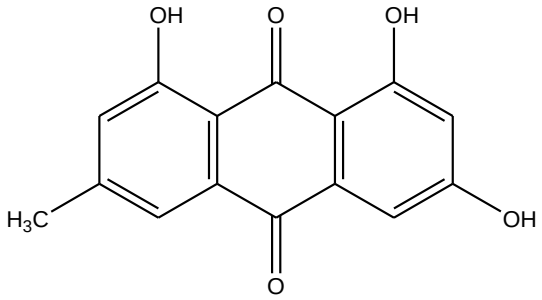
Something Your Roommate Left in the Fridge



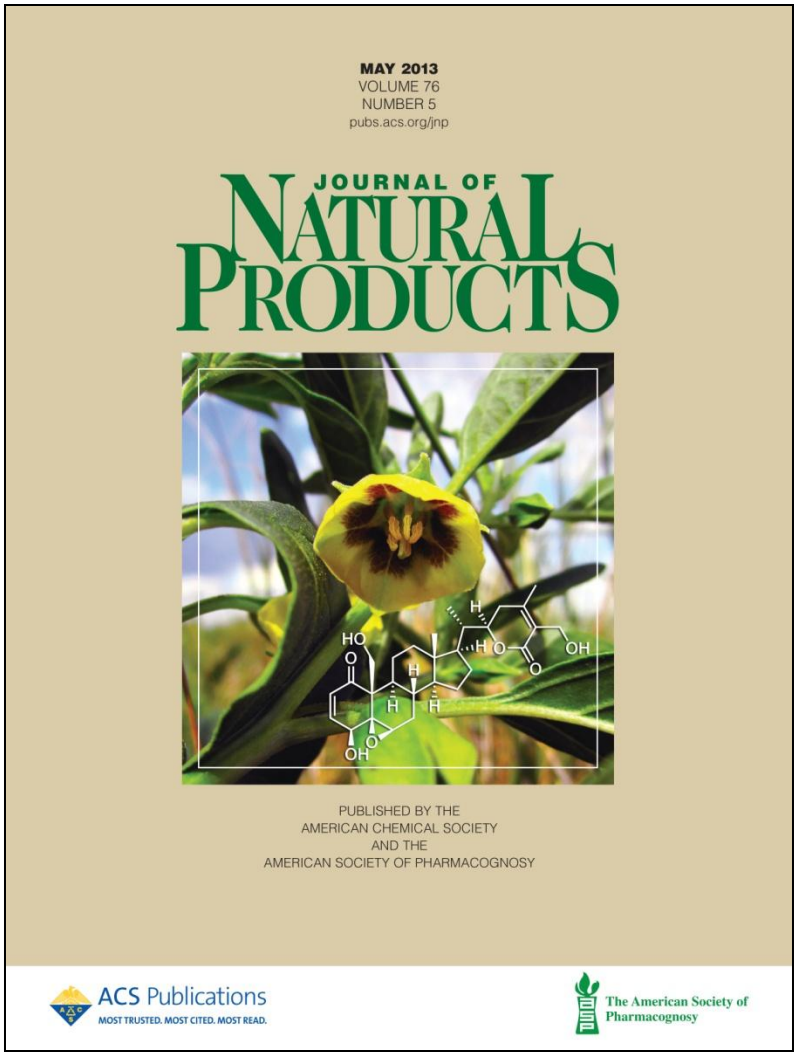
Paul Cannon



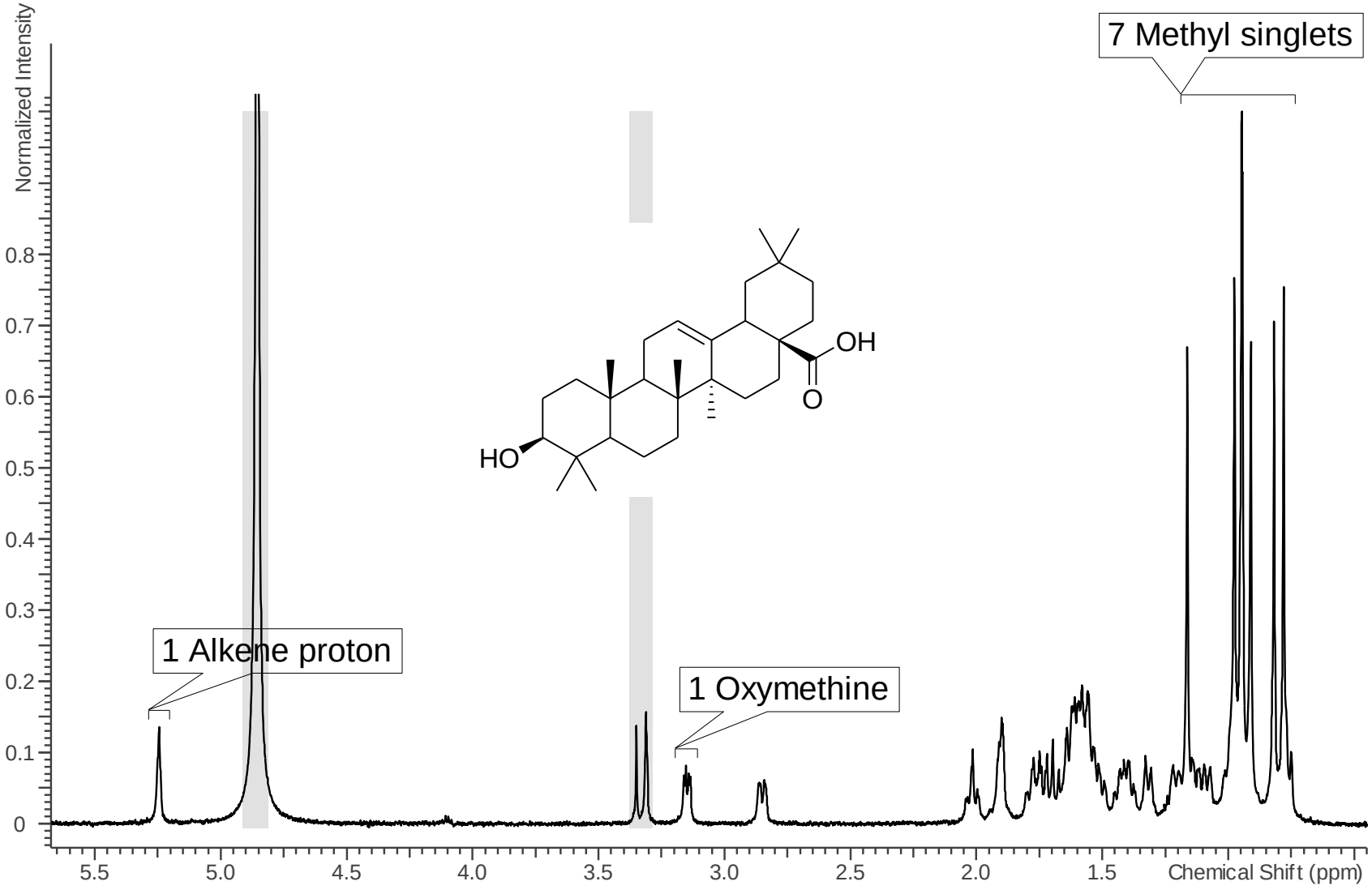
Emodin

Experience

- Can be hard to obtain.
  - Trial and error
  - Borrow spectra from labmates for solved structures
  - Journal reading
    - Supporting Information



Triterpene Spectrum



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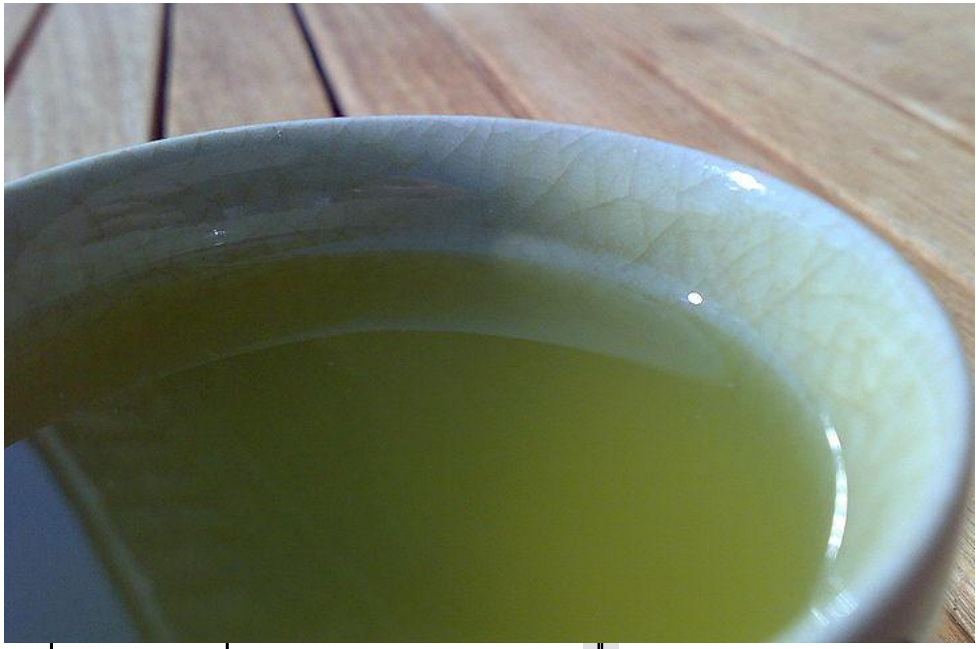
You Recognize the Compound Class.



Green Tea

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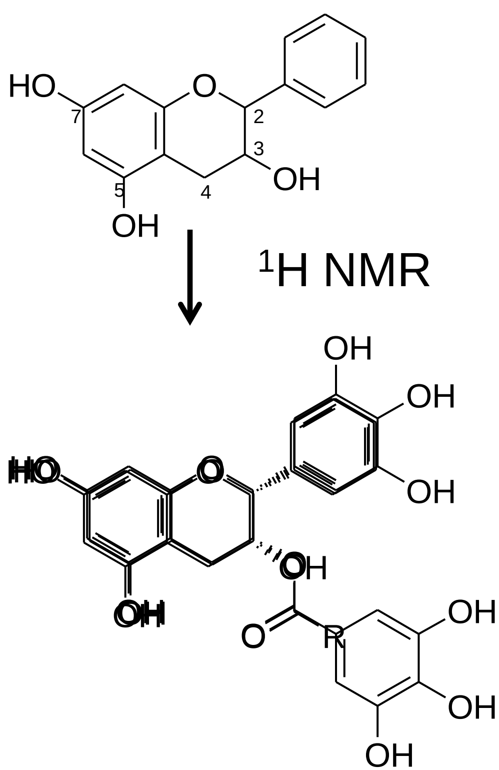
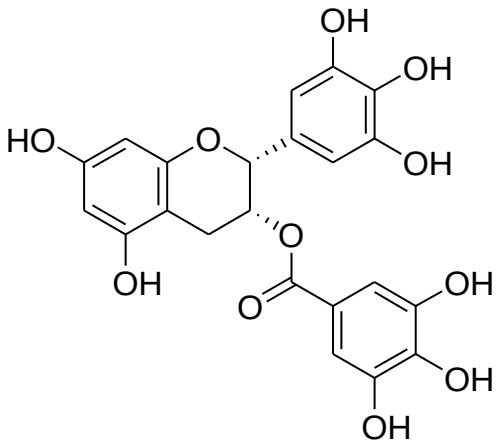
Quick Assignment Based on Compound Class



- Do an NMR database search.
- ^{1}H NMR signal at δ_H 5.96 indicates that the final two protons are equivalent.
- ^{1}H NMR
  - Coupling for H-2 and H-3 indicates the epi
  - This does not seem to match the structure.
  - The chemical shift of H-3 (δ_H 5.53) indicates an ester group.
  - The signals at δ_H 6.95 and δ_H 6.50 indicate galloyl groups.

Your Compound Is Known.

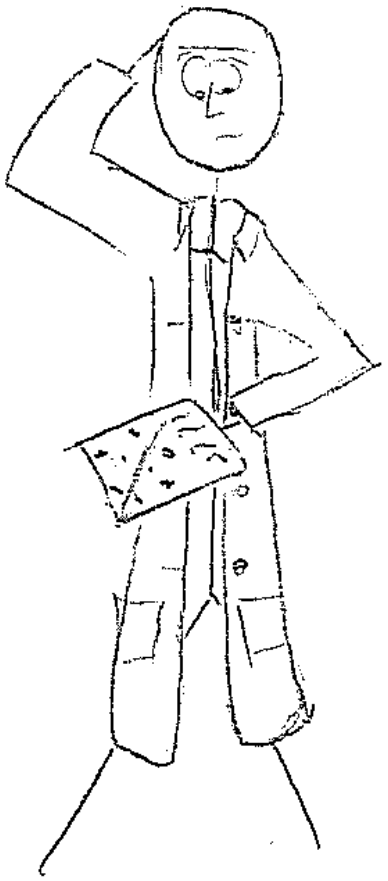
- Refer back to the literature to check your assignments.
- Critically examine the assignments in the literature.



- Chemical shifts agree with literature values.
- Literature assignment is correct.

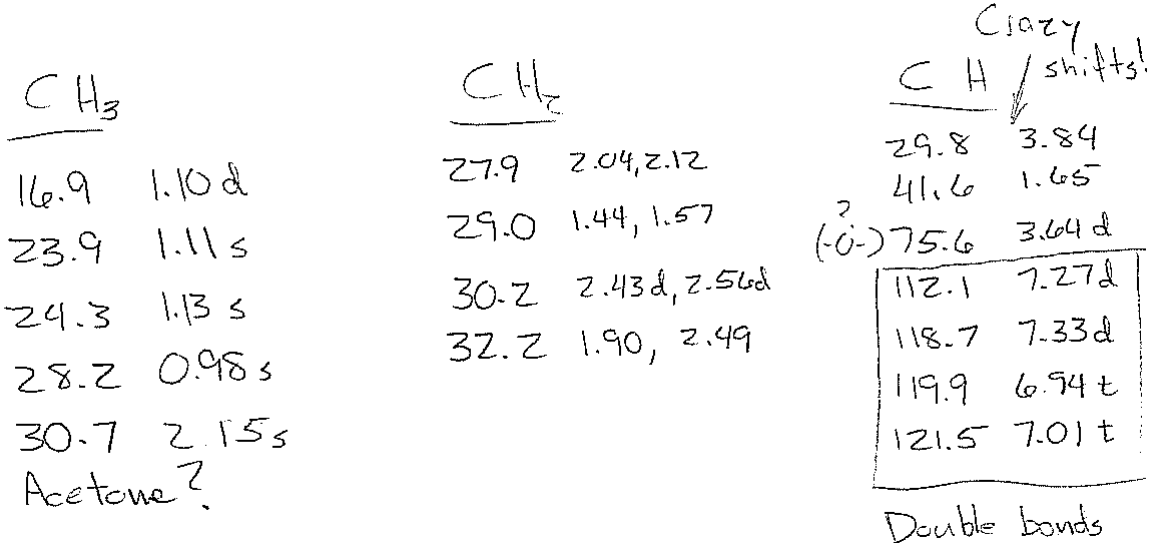
You Still Do Not Know What It Is

- The compound is not obvious.
- Now you must tackle the 2D spectra.
  - Go through the resonances one by one.
  - If you are disorderly you will become confussed.

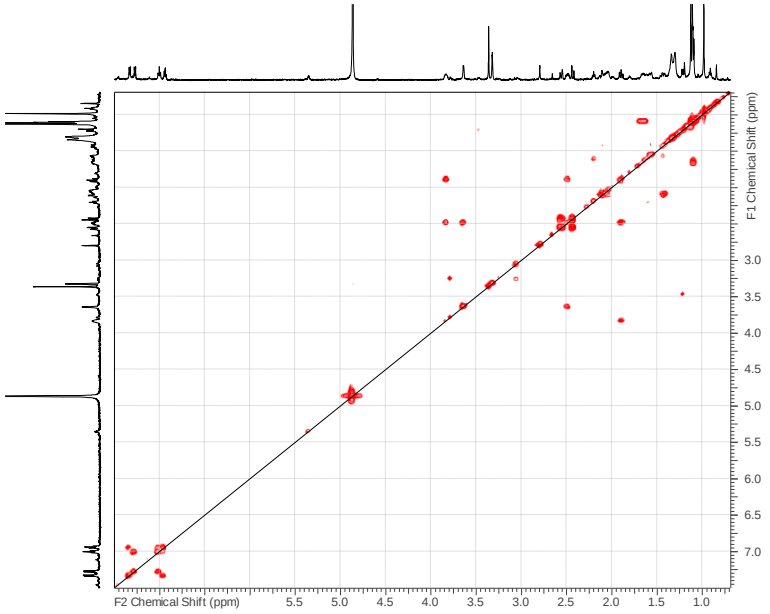


Make a List.

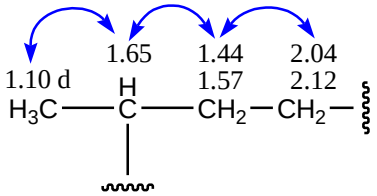
- Use the HSQC or HMQC to make a list of all of the $^{1}H/^{13}C$ groups
  - CH_3, CH_2, CH, and C (if you have a ^{13}C NMR spectrum).
  - Be careful:
    - Impurities can result in extra signals.
    - CH_2 can be tricky, use COSY correlations to help with them.

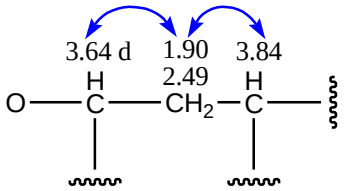


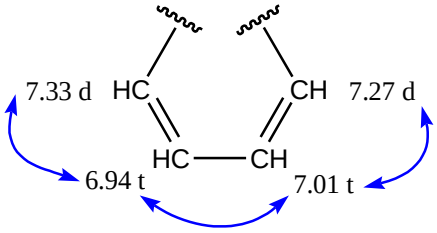
Assign the ^{1}H Spin Systems With COSY



- The COSY data can quickly provide you with large fragments.







CH_3

16.9	1.10 d
23.9	1.11 s
24.3	1.13 s
28.2	0.98 s
~~30.7~~	~~2.15 s~~

~~Acetone?~~

CH_2

27.9	2.04, 2.12
29.0	1.44, 1.57
30.2	2.43 d, 2.56 d
32.2	1.90, 2.49

Crazy shifts!

CH

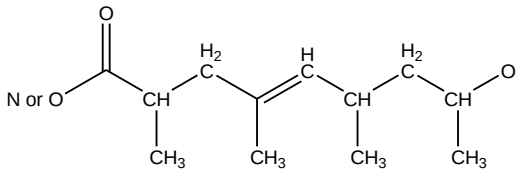
29.8	3.84
41.6	1.65
(-O-) 75.6	3.64 d

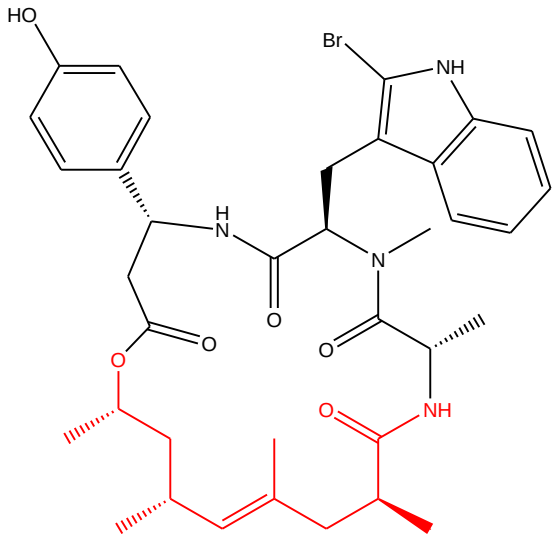
112.1	7.27 d
118.7	7.33 d
119.9	6.94 t
121.5	7.01 t

Double bonds

Substructure Search

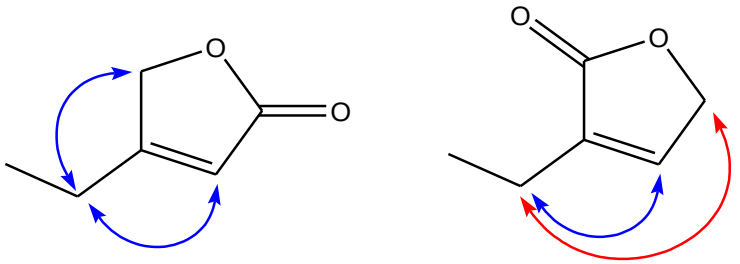
- If you get a nice large fragment that looks unique, go ahead and search for it.
  - 113 related structures.
  - Most common is jasplakinolide.





Be Cautious

- There are some potential traps with COSY spectra.
  - Long range correlations are possible. Especially around double bonds.
  - Some expected correlations are not seen.
  - Overlap



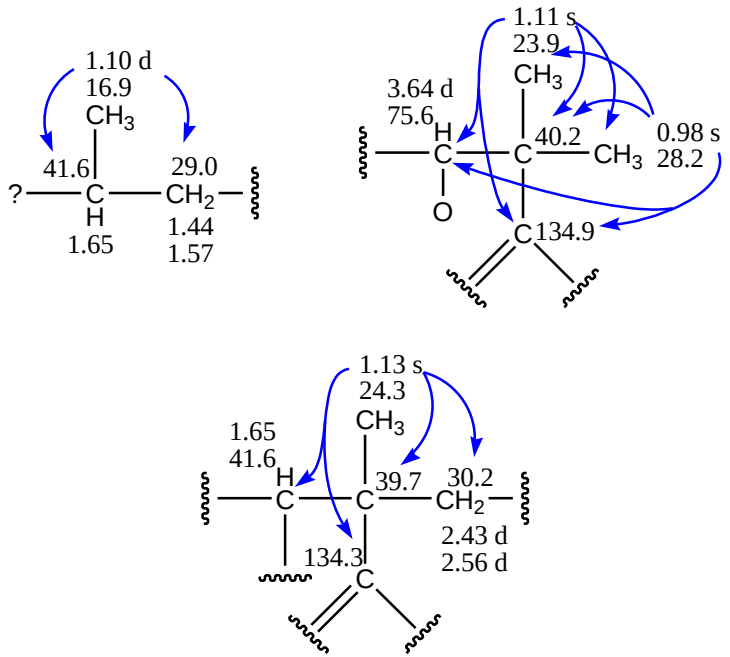
4-bond and 5-bond correlations

If *J* is very small you may not see a correlation.

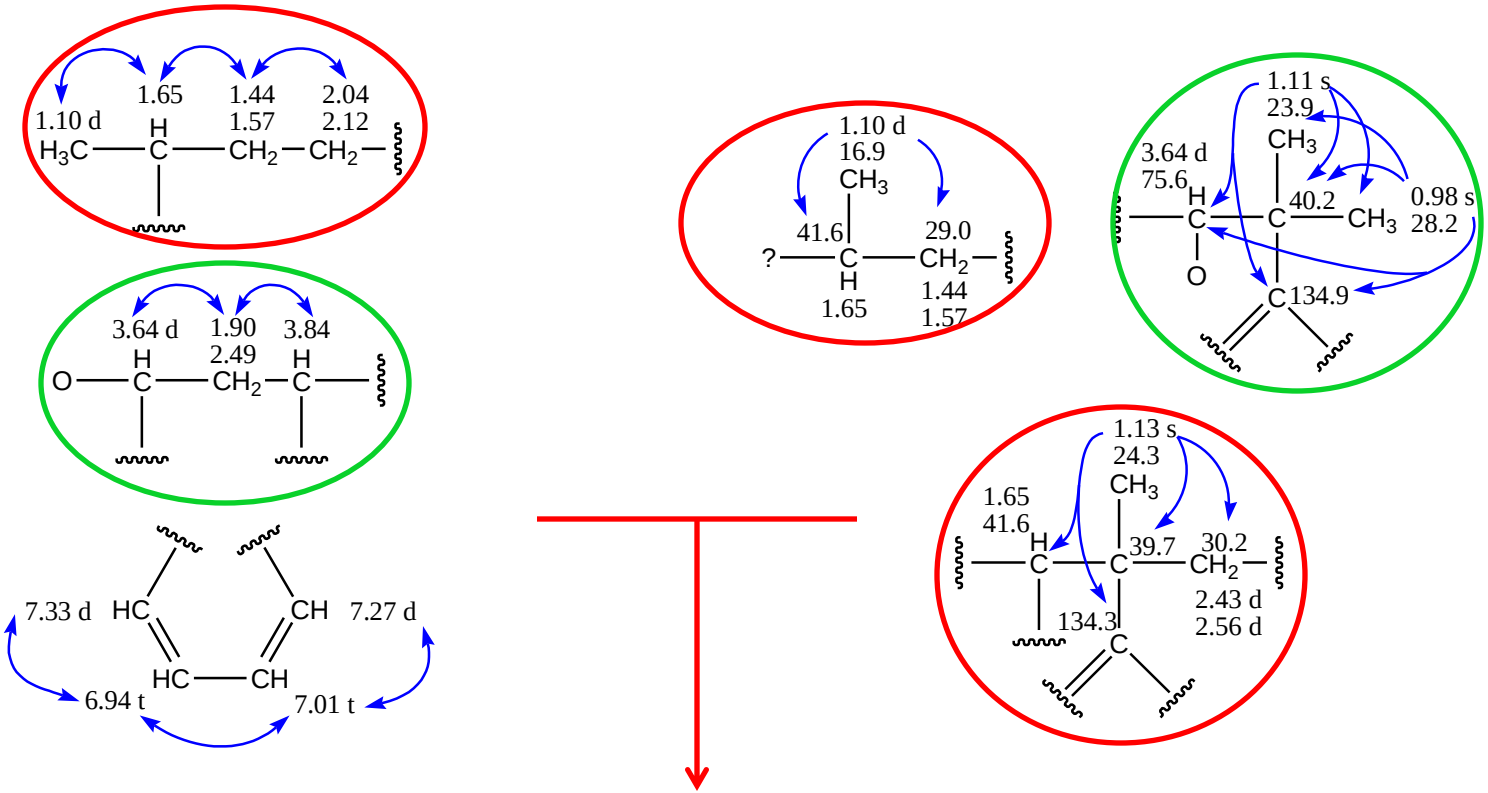
If your ^{1}H NMR contains a lump of overlapping proton signals you should not use those correlations.

Use HMBC to tie everything together

- HMBC can now be used to connect the COSY fragments and other pieces together.
  - HMBC can be confusing due to the presence of both 2 and 3 bond correlations.
    - Start with a group you already know something about.

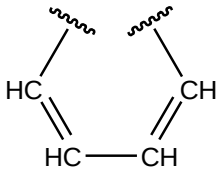


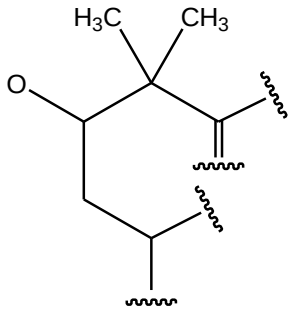
Combine Fragments that Overlap

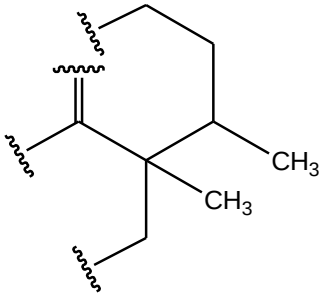


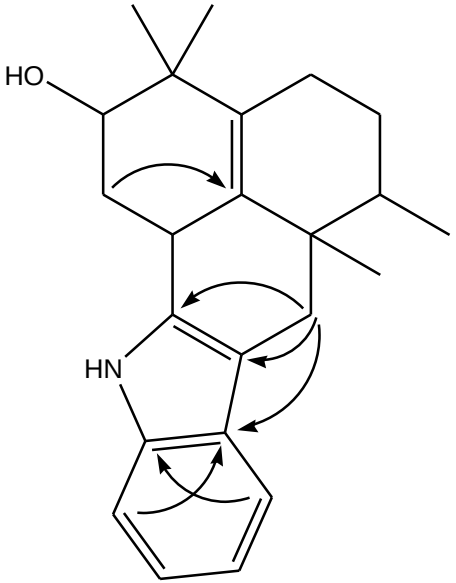
Look for a Few Additional Correlations

- From these large fragments only a few additional HMBC correlations are needed to finish the structure.



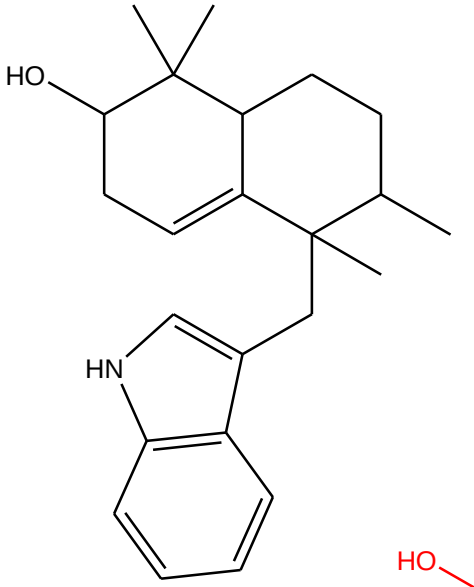


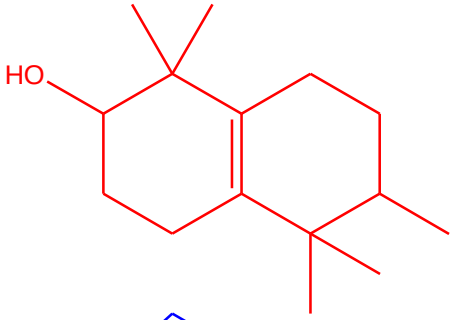


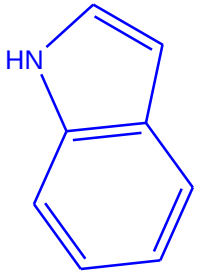


Check Your Work

- At this point you should have most if not all of the structure assembled.
- If your compound is new, look for similar compounds to compare chemical shifts.



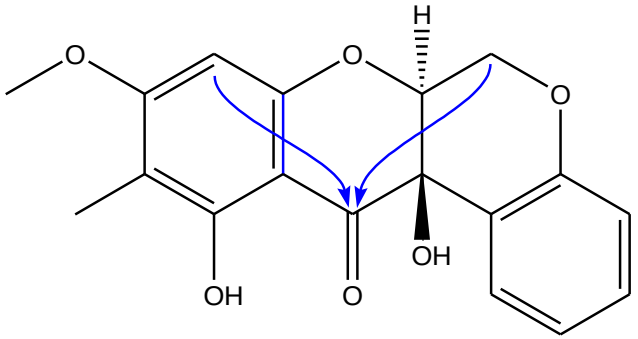




Be Cautious

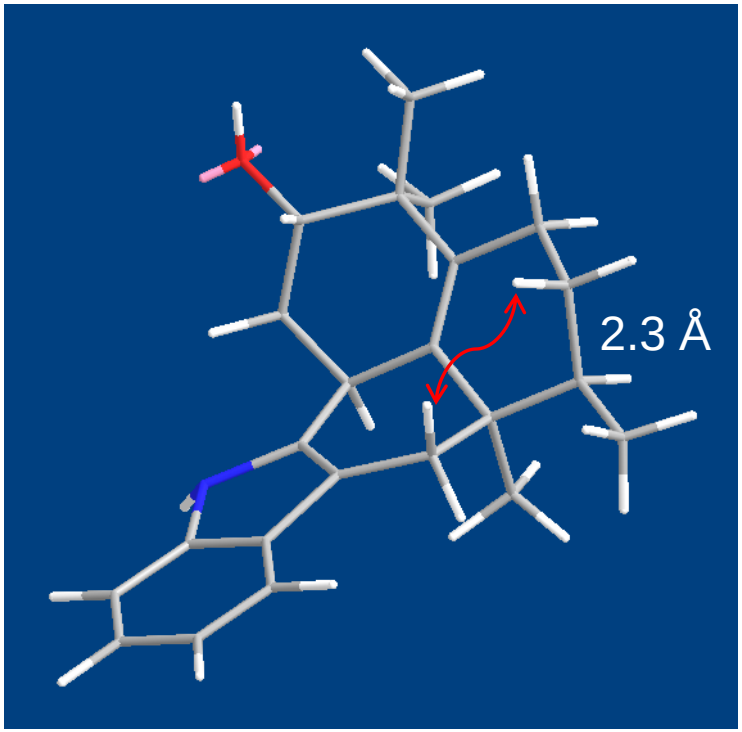
- Do not jump into the middle of the vast unknown, chip away at the edges.
- A note of caution, for rigid compounds, such as fused aromatic rings, 4 and 5 bond correlations are possible.
- Try not to base your structure on any one single correlation.

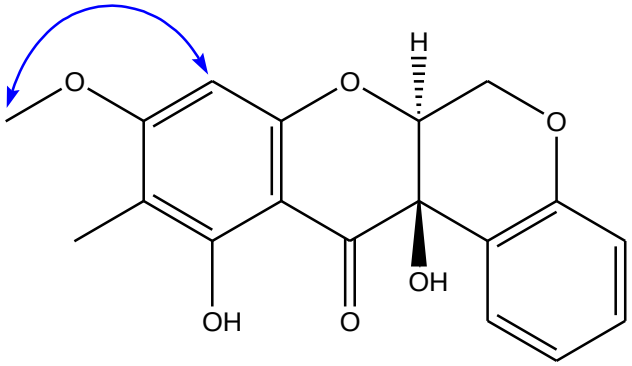
Start with Me groups and by trying to expand COSY fragments.



Relative Configuration

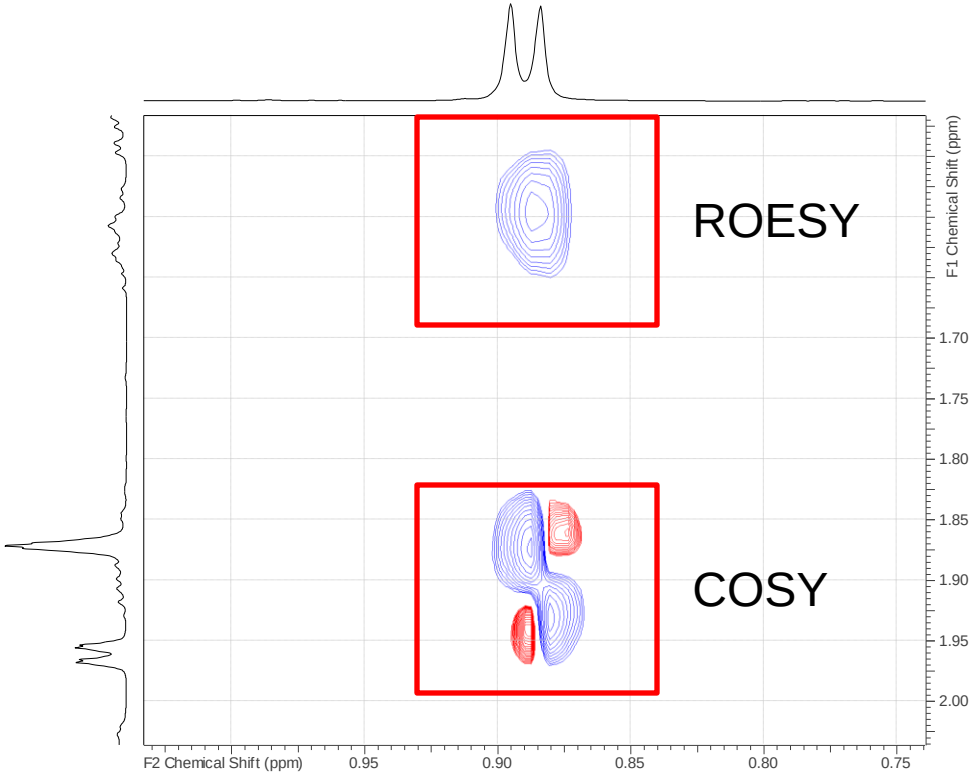
- If you have a chiral compound you should use ROESY or NOESY to assign the relative configuration.
  - This is not always straightforward. Use modeling software to minimize energies and verify interatomic distances.
  - ROESY and NOESY is also very helpful in assigning methoxy groups.





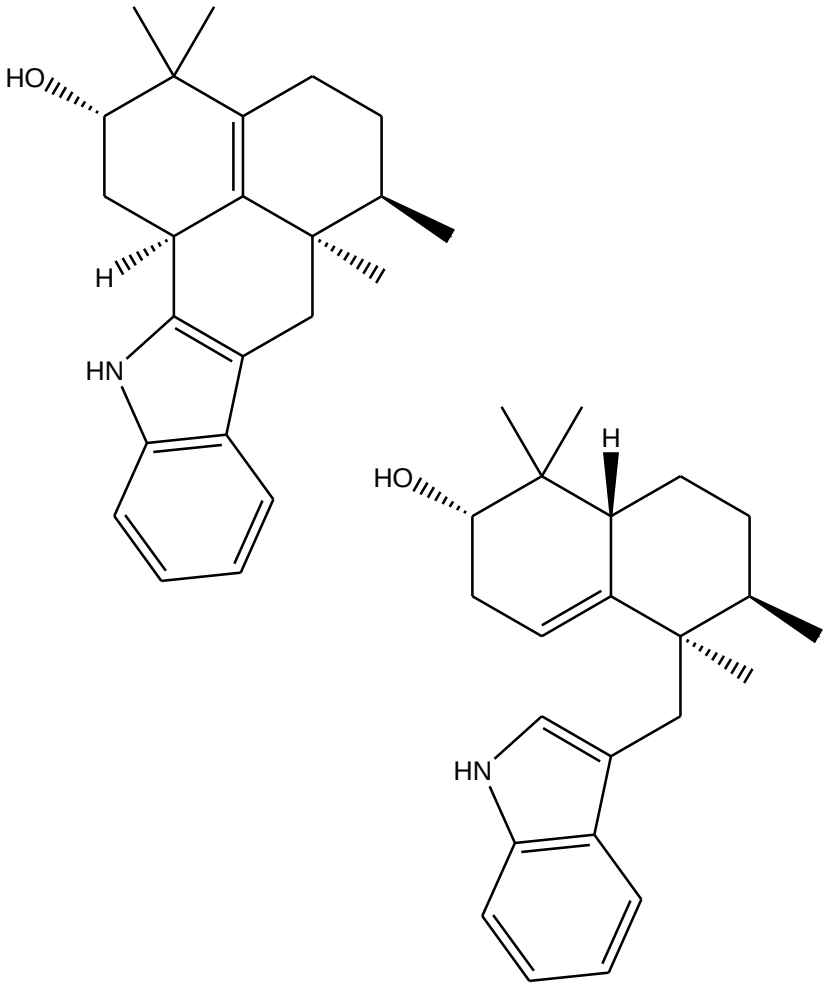
ROESY/NOESY Traps

- Be careful avoid COSY correlations that are often present in ROESY and NOESY spectra.
  - They are usually out of phase, but over processing can obscure that.



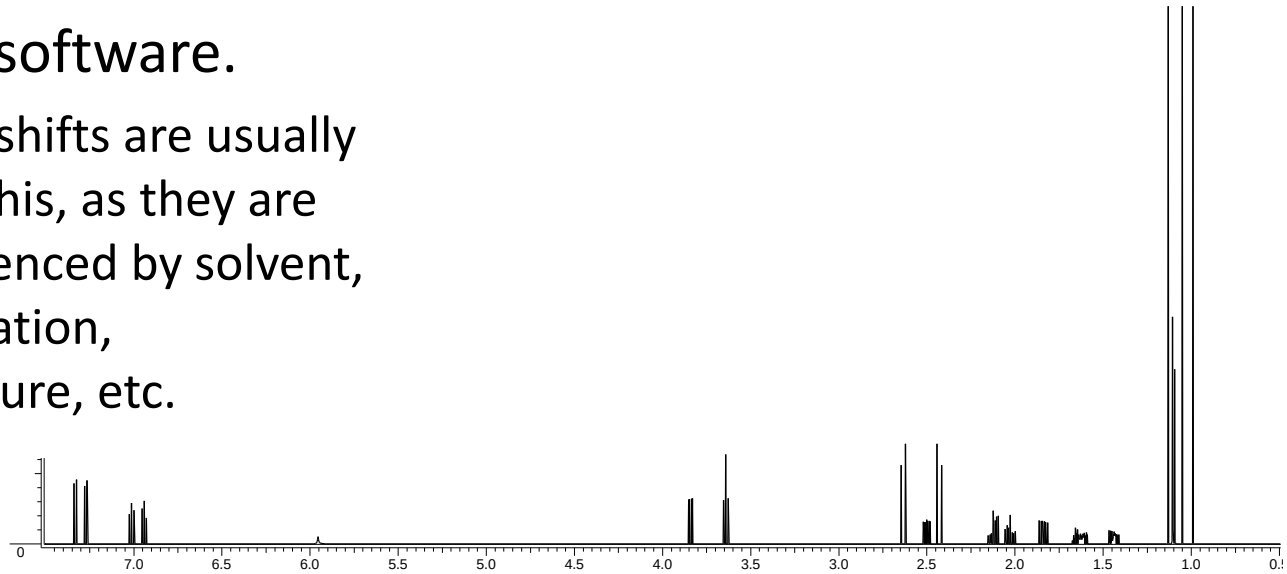
Check Your Work

- Is your relative configuration reasonable compared to known compounds?
  - Differences must be supported.



Do You Have a New Compound?

- If your compound is new:
  - Compare shifts to similar compounds.
  - Check shifts with NMR prediction software.
    - ^{13}C NMR shifts are usually best for this, as they are less influenced by solvent, concentration, temperature, etc.



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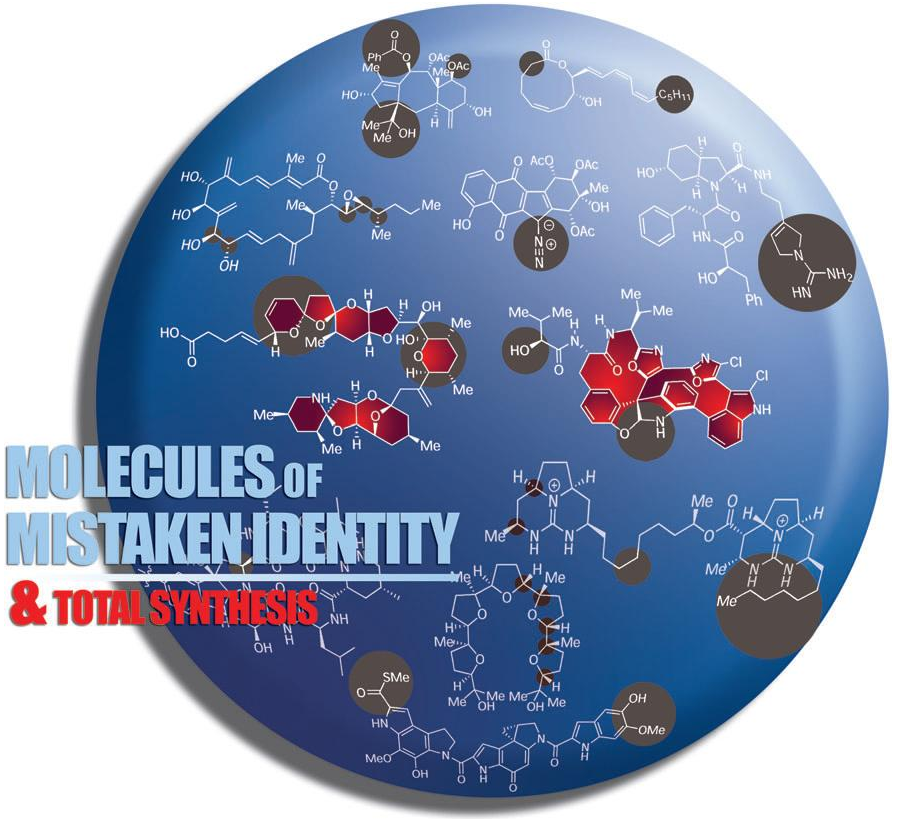
Is Your Compound "Novel"

- If you are proposing an unprecedented structure, consider:
  - Is your structure chemically stable?
  - Is your structure biosynthetically possible?
- If not, expect to be challenged.

Chasing Molecules That Were Never There: Misassigned Natural Products and the Role of Chemical Synthesis in Modern Structure Elucidation

K. C. Nicolaou* and Scott A. Snyder



Angew. Chem. Int. Ed. **2005**, *44*, 1012 – 1044.

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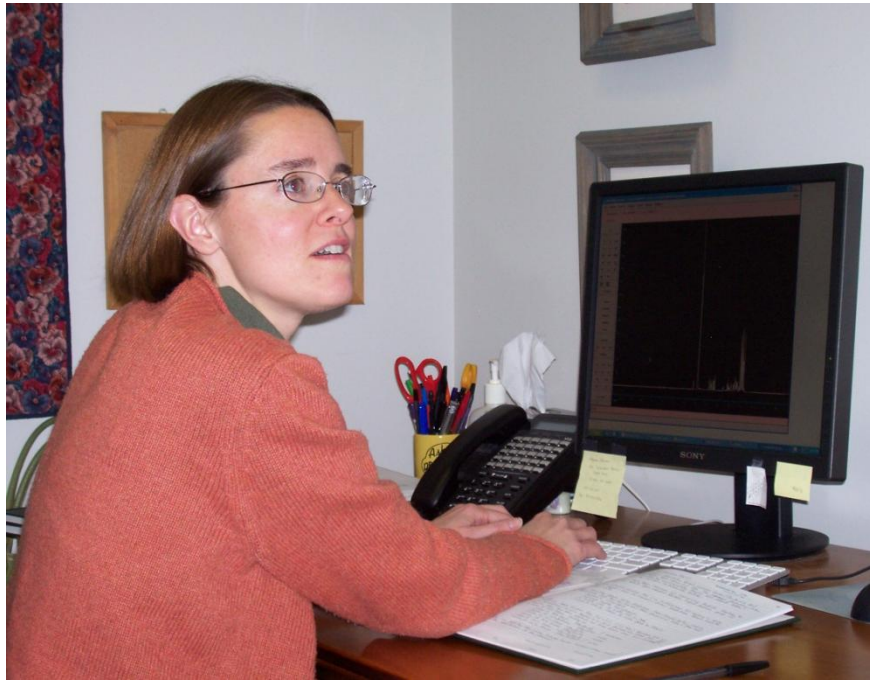
Independent Review

- After you have completed your assignment:
  - Ask one of your fellow group members to check your work.
    - It would be best if they did this without looking at your assignment to avoid bias.



Summary

- Reduce time spent on common known compounds.
- Quickly ID minor analogues of known compounds.
- Ease into complex spectra.
- Watch out for traps.
- Evaluate, evaluate, evaluate.