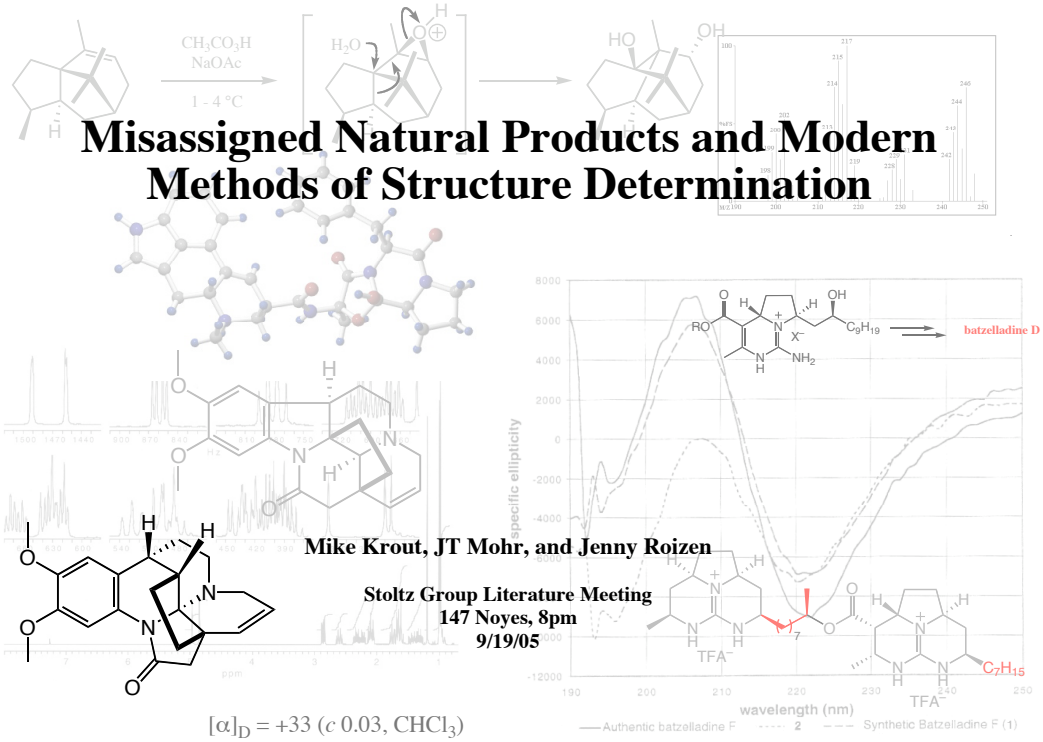
Misassigned Natural Products and Modern Methods of Structure Determination



Mike Krout, JT Mohr, and Jenny Roizen

Stoltz Group Literature Meeting
147 Noyes, 8pm
9/19/05

$[\alpha]_D = +33$ (c 0.03, $CHCl_3$)

Outline

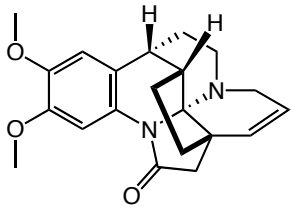
- Brief historical perspective (Quinine)
- Introduction to the basic modern methods of structure elucidation

  1. X-ray analysis
  2. Mass Spectrometry
  3. Optical Rotation
  4. Circular Dichroism
  5. IR
  6. NMR

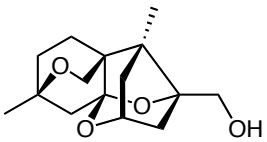
  NOT included: 3D NMR, elemental analysis, UV-vis, and chemical degradation

- Case Studies of misassigned natural products

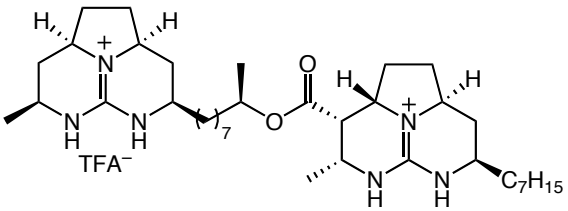
  1. Schizogamine and Isoschizogamine
  2. Batzelladine F
  3. Sporol and Neosporol



Isoschizogamine
(revised)



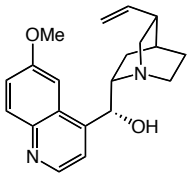
Sporol



batzelladine F
(revised)

Mistaken Identity: Back in the Day

Case Study: Quinine



Quinine

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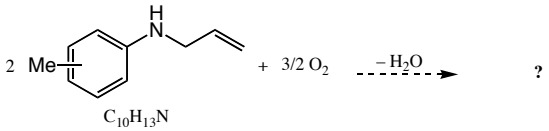
- 1856 Sir Willliam Henry Perkin (age 18) undertook Hofmann's project:



Summary in Nicolaou, K. C. and Snyder, S. A. *Classics in Total Synthesis II*.

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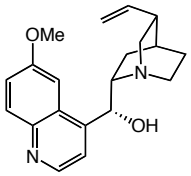

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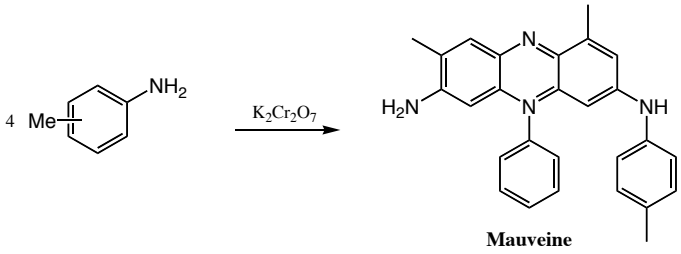

Mauveine

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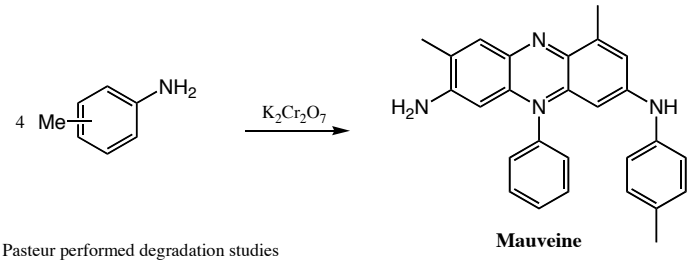
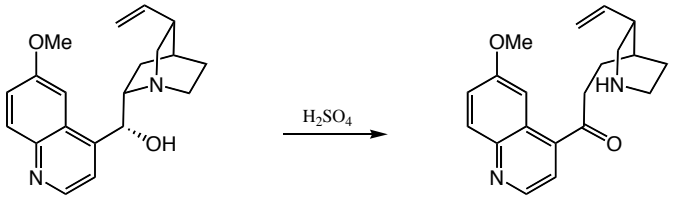


Mauveine

- 1853 Pasteur performed degradation studies



Quinine **Quinotoxine**

- 1908 Paul Rabe reported the correct molecular connectivities of quinine. Of course, no stereochemistry was known.

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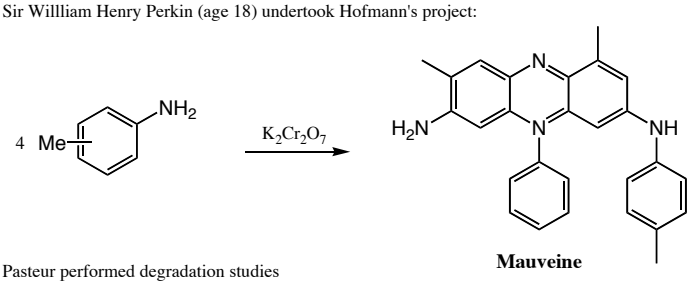

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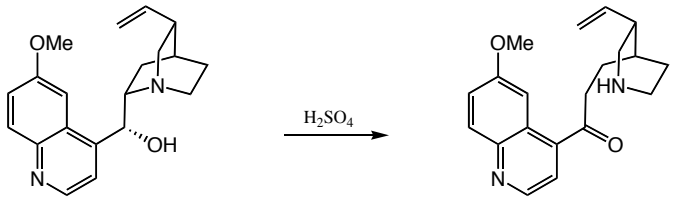

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Mistaken Identity: X-Ray Misassignments

3D map of electron density + math = X-Ray Crystal Structure

- How can we determine the absolute stereochemistry of the small molecule?

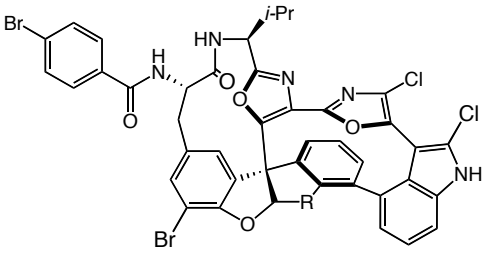
  • stereocenters already known

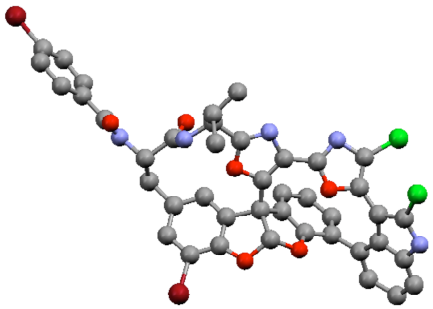
  • heavier element present (heavier than Si)
  - anomalous dispersion occurs when the frequency of the X-ray is near the frequency of electrons within a cloud.
  - This is only large for heavy atoms.
  - With anomalous dispersion, additional calculations gives *absolute stereochemistry*

- How can we confuse atom identities?

  • experimental determination of van der Waals radius

  • electron density integration at a grid point gives ~ atomic number
  - nitrogen and oxygen have similar masses, and hydrogens are difficult to refine





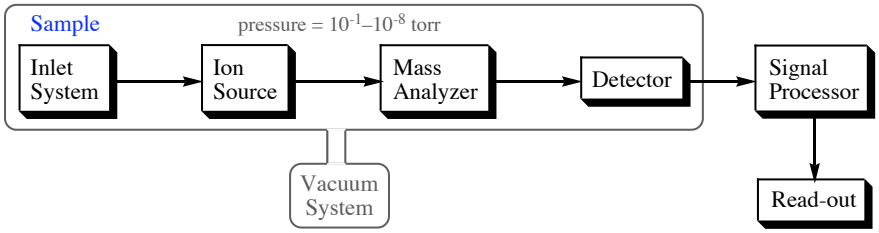
Diazonamide B derivative, R = NH
Original structural assignment, R = O

Fenical, W.; Clardy, J. et al. *JACS* **1991**, *113*(6), 2303-2304;
Stout, Jensen *X-Ray Structure Determination: A Practical Guide*;
Giacovazzo, C. et al. *Fundamentals of Crystallography*

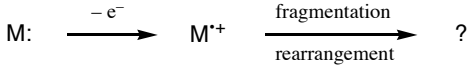
Methods of Structure Determination

Mass Spectral Analysis

- MS analysis is a destructive method of spectroscopy used to determine the molecular formula and identity (through characteristic fragmentations) of molecules of interest.



- Analysis based on the absorption of energy, ionization, and identification of the *m/z* ratio of the resulting molecules/fragments.



- Upon ionization, loss of an electron and generation of a radical cation (molecular ion, $[M]^{\bullet+}$) yields a molecule nearly identical to itself in mass (m_e = 5.49 x 10^{-4} of m_p).

- Different methods of ionization allow the detection of the molecular ion and use on different substrates.

Silversein and Webster. *Spectroscopic Identification of Organic Compounds*; 6th Edition, 1998.
Barker, James. *Mass Spectrometry*; 2nd Edition, 2000.

Methods of Structure Determination

Ionization Methods

Electron Impact (EI): Involves bombarding molecules in gas phase with a high-energy electron beam (50-100 eV). Ionization occurs by the interation of the fields of both the electron and the molecule passing close to or through the molecule. Usually, a good deal of fragmentation occurs... "*hard ionization*"

Chemical Ionization (CI): A sample is ionized in the presence of a large excess of a "reagent" gas (CH_4, isobutane, or NH_3), which is ionized by EI. 1° ions react to form 2° ions that react with the sample. Generates a $[M+1]^+$ ion (quasimolecular ion) cleanly with little fragmentation... "*soft ionization*"

$$CH_5^+ + M \longrightarrow [M+H]^+ + CH_4$$

$$C_2H_5^+ + M \longrightarrow [M+H]^+ + C_2H_4$$

Field Desorption (FD): Method good for solid samples of low volatility or thermal instability. A sample is placed on the anode of a pair of electrodes, and subjected to an intense electric field. Generally see $[M]^{\bullet+}$ and $[M+1]^+$. Inferior to FAB procedures.

Electrospray Ionization (ESI): Ionization involves placing an ionizing voltage at atmoshperic pressure across a nebulizer needle. As the mist of charged droplets evaporate, and coulombic repulsions cause a molecular "explosion." Method can be used for molecules without any ionizable sites through formation of Na^+, K^+, or NH_4^+ adducts. Generally obtain $[M+nH]^{n+}$, and is widely used with HPLC.

Fast-Atom Bombardment (FAB): A sample is adsorbed on a matrix (usually glycerol, *m*-nitrobenzyl alcohol, or diethanolamine) and ionized by a high-energy Xe beam. $[M+H]^+$ usually seen, as well as $[M]^{\bullet+}$ and fragmentations. $[M+Na]^+$ can also be seen with used of salt additives. FAB can achieved up to $>10^4$ Da resolution, but beware of matrix background peaks.

Matrix-Assisted Laser Desorption Ionization (MALDI): The sample in a matrix (2,5-dihydrobenzoic acid or α-cyano-4-hydroxycinnamic acid, both *m/z* <400 and do not form adducts with proteins) is dispersed on a surface and is adsorbed and ionized by a high-energy laser beam (N_2 UV laser beam at 4 ns pulses). MALDI is excellent for large biomolecules and polymers, up to several hundred kDa.

Methods of Structure Determination

Elemental Abundance and Exact Mass

- Higher resolution spectrometers can distinguish very similar molecular formulas.

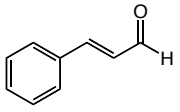
  $C_{16}H_{26}O_2$ and $C_{15}H_{24}NO_2$ with a resolution of 20,000 (*using exact masses of nuclides*)

$$R = \frac{M_n}{M_n - M_m} = \frac{250.9133}{250.1933 - 250.1807} \approx 20{,}000$$

M_n = higher mass number of two adjacent peaks
M_m = lower mass number

- **Index of hydrogen deficiency** (*degree of unsaturation*) helps predict the number of "pairs" of hydrogens to remove from the corresponding "saturated" formula to produce the molecular formula.

$$\omega = \frac{2C - H + 2 - X + N}{2}$$



C_9H_8O

ω = 6

Element	Atomic Weight	Nuclide	Mass	Relative Abundance
Hydrogen	1.00794	^{1}H	1.00783	100
		D (^{2}H)	2.01410	0.0016
Carbon	12.01115	^{12}C	12.00000	100
		^{13}C	13.00336	1.11
Nitrogen	14.0067	^{14}N	14.0031	100
		^{15}N	15.0001	0.38
Oxygen	15.9994	^{16}O	15.9949	100
		^{17}O	16.9991	0.04
		^{18}O	17.9992	0.20
Fluorine	18.9984	^{19}F	18.9984	100
Silicon	28.0855	^{28}Si	27.9769	100
		^{29}Si	28.9765	5.10
		^{30}Si	29.9738	3.35
Phosphorus	30.9738	^{31}P	30.9738	100
Sulfur	32.006	^{32}S	31.9721	100
		^{33}S	32.9715	0.78
		^{34}S	33.9679	4.40
Chlorine	35.4527	^{35}Cl	34.9689	100
		^{37}Cl	36.9659	32.5
Bromine	79.9094	^{79}Br	78.9183	100
		^{81}Br	80.9163	98.0
Iodine	126.9045	^{127}I	126.9045	100

Methods of Structure Determination

Fragmentation and Rearrangement

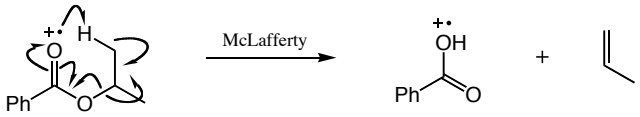
- **Nitrogen Rule:** A $[M]^{\bullet+}$ of *even* weight will contain either *no* or an *even* number of nitrogens.
  A $[M]^{\bullet+}$ of *odd* weight will contain an *odd* number of nitrogens.

- ***Odd/Even* Fragmentation Rule:** An *odd* numbered $[M]^{\bullet+}$ will create *even*-numbered ion fragments.
  An *even* numbered $[M]^{\bullet+}$ will create *odd*-numbered ion fragments.

- Fragmentation occurs in either a *homolytic* or *heterolytic* manner from $[M]^{\bullet+}$; lower lying orbitals (π^* or *n*) typically absorb ionizing radiation, initiating fragmentation.

- Fragmentations eliminating very stable, neutral molecules (H_2O, NH_3, CO) are highly favorable, as well as cleavage of C–C bonds near hetereoatoms.

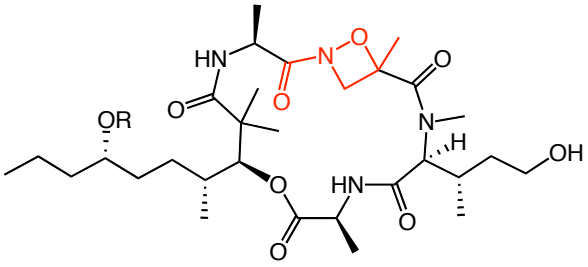
- Rearrangements are not a simply the cleavage of bonds but an intramolecular rearrangement (typically H-atom migration). Characterized by an observation of a fragment that differs by 1 from the *odd/even* fragmentation rule, indicating the retention of H-atom in the rearrangement.



164.2 122.1 42.1

Mistaken Identity: MS Anaylsis

Halipeptins



R= Me, Halipeptin A
R= H, Halipeptin B (Proposed)

Potent anti-inflamatory agent isolated from marine sponge *Haliclona* sp.

Key Characterization Data:

- *NMR*: ^{1}H, ^{13}C, ^{15}N, DEPT, COSY, TOCSY, HMQC, HMBC, ROESY
- ^{15}N shows peak from δ –260 to –270 ppm (similar to N–O bond)
- *MS*: HRFAB *m/z* **627.4073** $[M+H]^+$ calc. $C_{31}H_{54}N_4O_9$ **627.3969**
- Also determined using chemical degradation (MeO^-) and MS analysis, as well as FAB and ESIMS/MS fragmentations.
- Subtracting other characterized motifs leaves $C_4H_5NO_2$, which predicts 2 degrees of unsaturation.
- Data seems to fit oxazetidine motif...

Gomez-Paloma, L. et al. *J. Am. Chem. Soc.* **2001**, *123*, 10870-10876.

Mistaken Identity: MS Anaylsis

Halipeptins

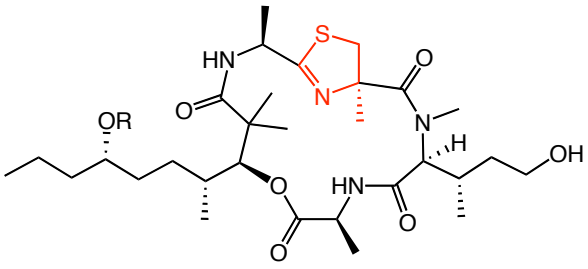
- MS data for recently isolated Halipeptin C suggested a sulfur atom instead of 2 oxygens.
- MS re-evaluation of Halipeptin A and B reveal same trend.
- Molecular foumula left with C_4H_5NS, requiring 2 degrees of unsaturation.

New MS data for Halipeptin A (HRESIMS)

m/z 649.3628 $[M+Na]^+$

calc. for $C_{31}H_{54}N_4NaO_7S$ 649.3611
calc. for $C_{31}H_{54}N_4NaO_9$ 649.3788

"... our first HRMS measurements... were more in agreement with $C_{31}H_{54}N_4O_9$ than with $C_{31}H_{54}N_4O_7S$, leading to ***misinterpretation*** of the NMR data... new data were obtained on superior instrumentation..."



R= Me, Halipeptin A
R= H, Halipeptin B ***(Revised)***

- ^1H and ^{13}C suggest thiazoline moiety, as well as HMBC correlatins between quaternary Me and α-CH_2.
- Reassignment is also supported by NMR calculations of related oxazetidines and thiazolines, as well as ^{13}C comparisons to a synthetic analog.

Gomez-Paloma, L. et al. *Tetrahedron Lett*. **2002**, *43*, 5707-5710.

Methods of Structure Determination

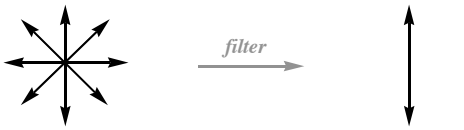
Chirotopical Properties: Optical Activity

Chirotopical properties: Properties of chiral substances arising from their nondestructive interaction with anisotropic radiation, allowing the differentiation between two enantiomers.

Light Polarization: To confine vibrations of the electric vector of light waves to one direction, usually by reflection or transmission through certain substances; "plane polarized light"

Optical Activity/ Rotation: The change of rotation in the plane of polarization that occurs when polarized light is passed through an optically active substance.

Polarizability: The measure of the response of a molecule to an external electric field; when placed in a field, the displacement of electric charge induces a dipole in the molecule.



isotropic *anisotropic*

Technique	Principle	Chromophore Necessary?
Optical Rotation	Refraction	No
Circular Dichroism	Absorption	Yes

optical activity occurs when
$n_R \neq n_L$
(n = index of refraction)

Eliel and Wilen. *Stereochemistry of Organic Compounds*; 1994.
Clayden, Greeves, Warren, and Wothers. *Organic Chemsitry*; 2001.
Lewis, R. J. *Hawley's Condensed Chemical Dictionary*; 14th Edition, 2001.

Methods of Structure Determination

Chirotopical Properties: Optical Activity

- Optical rotation data obtained using polarimeter, and the data is analyzed utilizing Biot's Law:

$$\alpha = [\alpha]_{\lambda}^{T} cl$$

$$[\alpha]_{\lambda}^{T} = \frac{\alpha}{l(\text{dm}) \cdot c(\text{g/mL})} = \frac{100\alpha}{l(\text{dm}) \cdot c'(\text{g/100 mL})}$$

α = observed angle of rotation of the plane of polarization

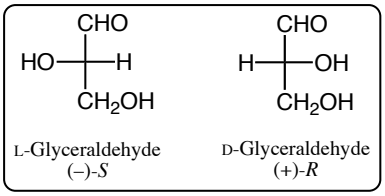
[α] = specific rotation (proportionality constant), dependent upon temperature (**T**) and wavelength (**λ**), usually *Sodium D line* (589.3 nm); also dependent on solvent

l = length of cell, usually in dm

c = concentration of sample, in units of g/mL for neat liquids, but more commonly g/100 mL (wt/V%) for solutions (*see second equation*)

- Molecules characterized by the direction of rotation:
  (+) = dextrorotatory
  (–) = laevorotatory

- Traditional notation:
  d = (+), *l* = (–), and *dl* = (±)
  D = right handed enantiomer, related to D-Glyceraldehyde
  L = left handed enantiomer, related to L-Glyceraldehyde

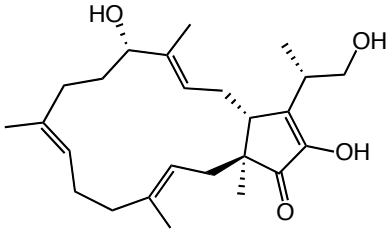


L-Glyceraldehyde (–)-*S* D-Glyceraldehyde (+)-*R*

- The direction of which light is rotated is ***not*** dependent on whether the stereogenic center is *R* or *S*; therefore, D can be (+) or (–). Also, absolute stereochemistry ***can not*** be determined by rotation alone.

Mistaken Identity: Optical Rotation

Terpestacin



Terpestacin

- Isolated in 1993, first asymmetric synthesis in 1998.
- Both reports support same optical rotation values.
- "New" isolate from a different species reported in 2001 identical to Terpestacin isolated in 1993 **except** for the optical rotation; therefore, assigned as its enantiomer.
- Meyers asymmetric synthesis of Terpestacin in 2002 supports the latter optical rotation data...?

What's going on?

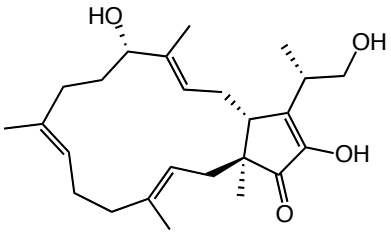
Sample	Specific Rotation ($[\alpha]_D$)	
1993 Isolation	+26	(*c* 0.5, $CHCl_3$)
1998 Synthesis	+27	(*c* 0.22, $CHCl_3$)
2001 Isolation	–16.5	(*c* 0.29, $CDCl_3$)
	–21.5	(*c* 0.32, MeOH)
2002 Synthesis (Meyers)	–17	(*c* 0.58, $CDCl_3$)
	–18	(*c* 0.06, MeOH)

Enantiomers?

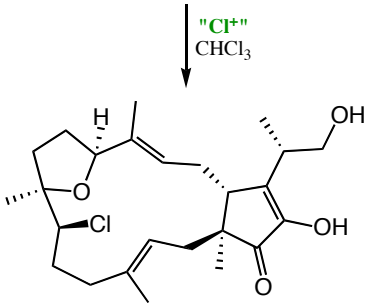
Meyers, A. G. et al. *J. Am. Chem. Soc.* **2002**, *124*, 4230-4232 and references therein.

Mistaken Identity: Optical Rotation

Terpestacin



Terpestacin



$[\alpha]_D$ = +33 (*c* 0.03, $CHCl_3$)

- Depending on the lot and age of $CHCl_3$, varying quantities of Cl-containing product formed.
- $CHCl_3$ stored over K_2CO_3 generates small quantities of Cl_2!
- $CHCl_3$ stored over MS4Å did not test positive for Cl_2 and gave stable solutions and reproducible rotations.

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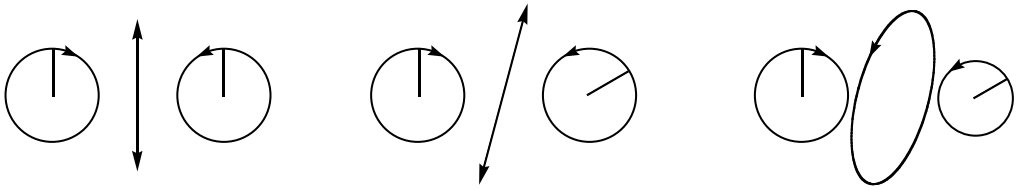
(Meyers)	–18	(*c* 0.06, MeOH)

Meyers, A. G. et al. *J. Am. Chem. Soc.* **2002**, *124*, 4230-4232 and references therein.

Circular Dichroism

- CD arises from unequal absorption of right- and left-handed circularly polarized light (cpl) by chiral chromophores.



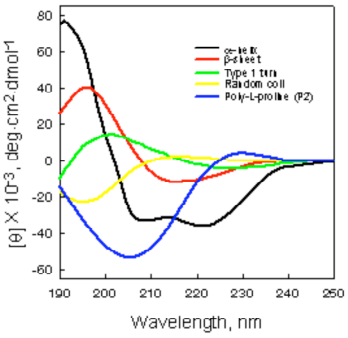
equal velocities of transmission
no rotation

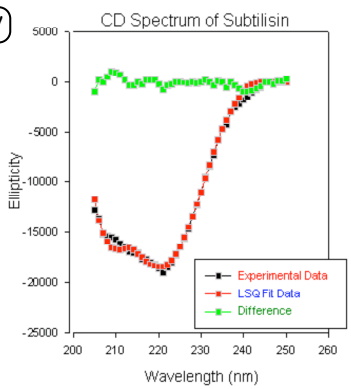
unequal velocities of transmission
rotation

unequal velocities and unequal absorption
rotation and elliptical polarization

CD usually measures the difference in absorbances (ΔA). If molar concentrations are known, then this can be converted to the difference in molar absorption coefficients ($\Delta\varepsilon$) since $\Delta A = \Delta\varepsilon cl$ (where c = concentration and l = path length). CD is also sometimes reported in terms of molar amplitude (a) by the equation $a = 40.28\Delta\varepsilon$ or in terms of molar ellipticity ($[\theta]$) by the equation $[\theta] = 3298.2\Delta\varepsilon$

Useful for identifying protein structures!

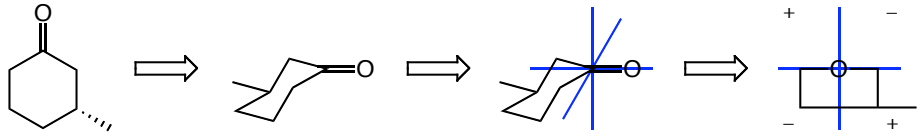




Circular Dichroism

The Octant Rule for Carbonyl Compounds

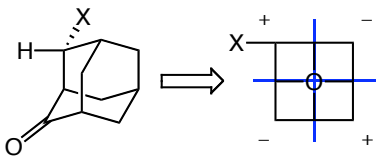
- The Octant Rule can be used to predict the sign of the observed CD based on the absolute structure, or *vice versa*.



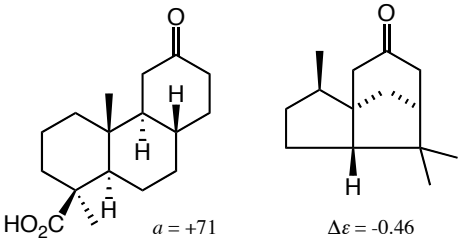
(R)-3-methylcyclohexanone
observed CE: $\Delta\varepsilon$ = +0.56

To apply the octant rule, first superimpose three-dimensional axes over the structure such that the carbonyl bond resides along an axis and the carbonyl carbon resides at the origin. Next, rotate so that you view down the carbonyl bond, with the O atom in front, to produce a 2D picture. The visible octants are labeled as shown. The sign of the octant containing the most polarizable group determines the sign of the CD.

Dependence on Polarizability



X = F; $\Delta\varepsilon$ = -0.23 (dissignate)
X = Br; $\Delta\varepsilon$ = +7.1 (consignate)



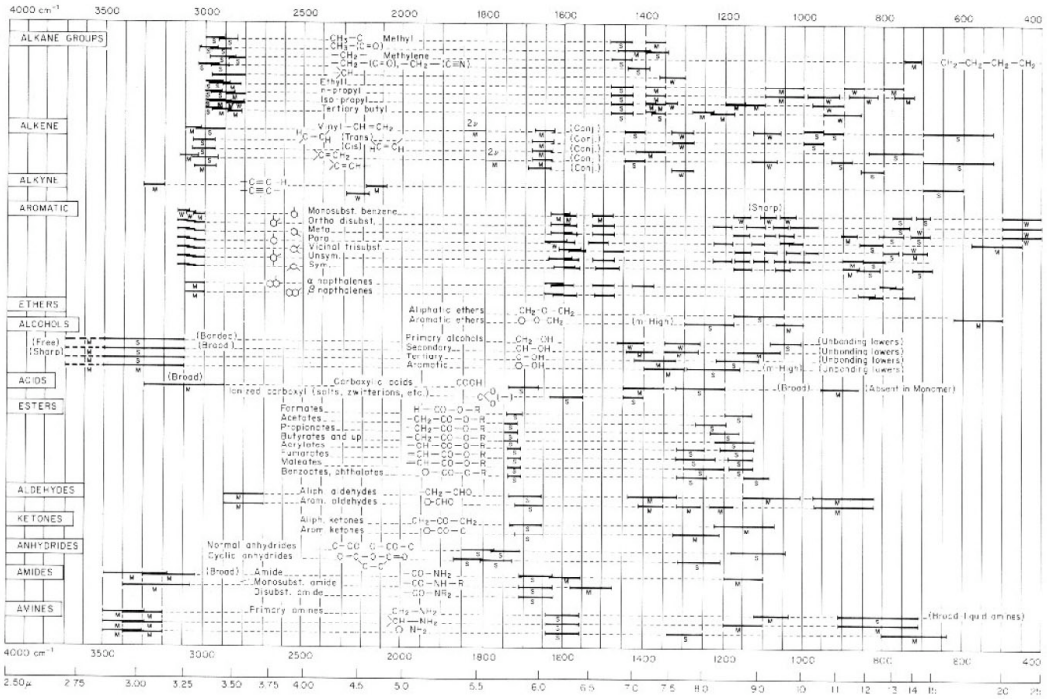
a = +71 Δε = -0.46

- CD results are often solvent dependent, but rarely is there a sign change from one solvent to another.
- Although the Octant Rule applies to many compounds, it is unwise to assign absolute stereochemistry with CD alone.
- Octant predictions can depend on the conformation of the molecule. Small CEs (Δε < 1) are often indicative of this.

Kirk, *Tetrahedron* **1986**, *42*, 777-818.
Eliel, "*Stereochemistry of Organic Compounds*" Ch. 13
Computational predictions (VCD & DFT):
Stephens & Devlin, *Chirality*, **2000**, *12*, 172-179.

IR Spectroscopy

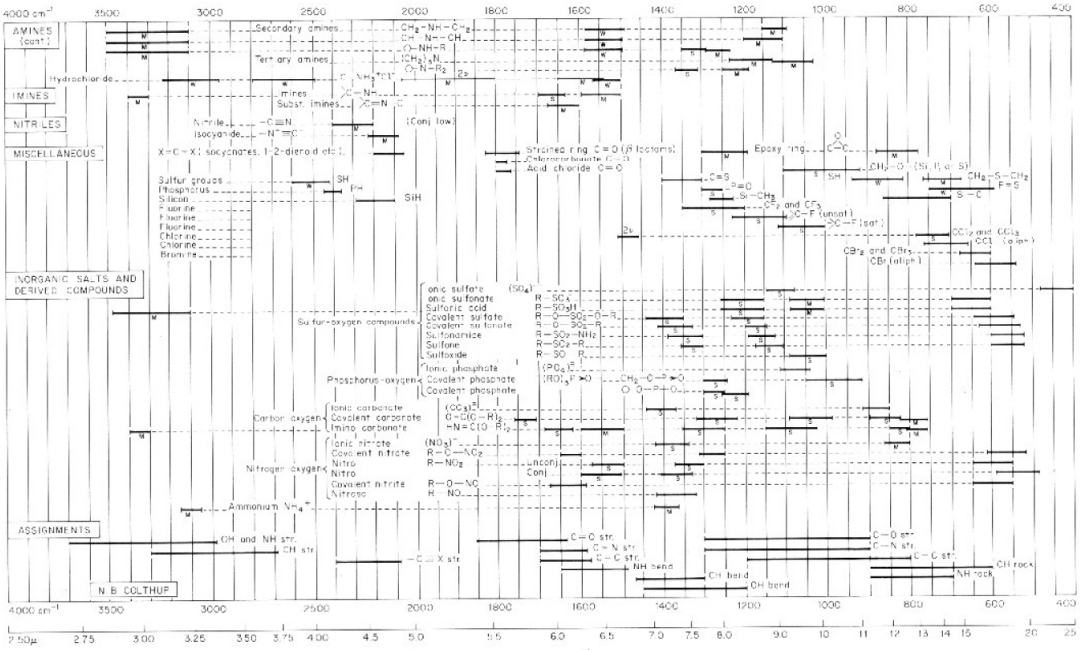
Reference Data



Dyer, "*Applications of Absorption Spectroscopy of Organic Compounds*" 1965.

IR Spectroscopy

Reference Data



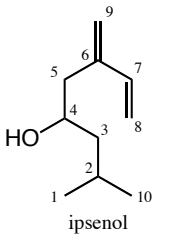
Dyer, "*Applications of Absorption Spectroscopy of Organic Compounds*" 1965.

Overview of Advanced NMR Experiments

*^{13}C DEPT (**D**istortionless **E**nhancement by **P**olarization **T**ransfer)*

- Three experiments which differentiate C, CH, CH_2, CH_3
- Variable proton pulses at 45°, 90°, and 135° affect the carbon types differently

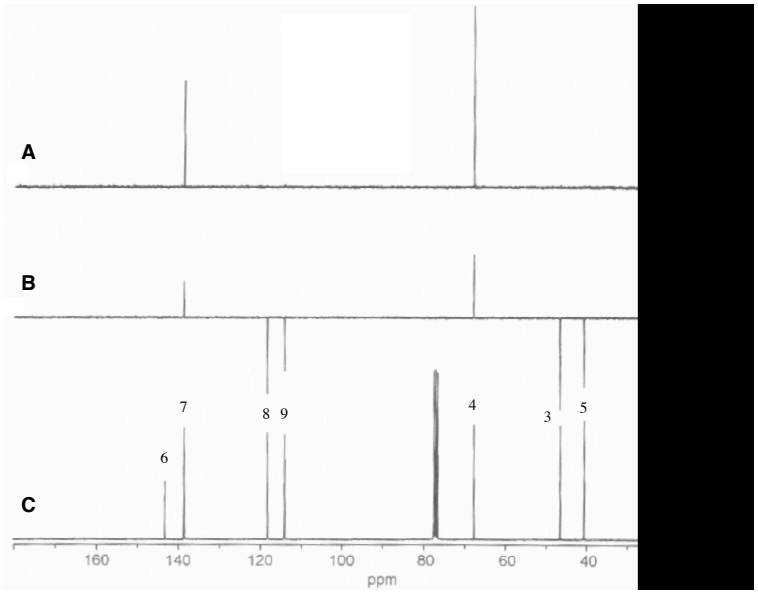
References for NMR experiments:
Silverstein & Webster, "*Spectrometric Identification of Organic Compounds*"
Roberts, "*ABCs of FT-NMR*"
Claridge, "*High-Resolution NMR Techniques in Organic Chemistry*"



ipsenol

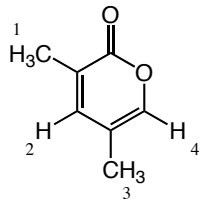
Spectrum A (DEPT 90) - shows CH peaks
Spectrum B (DEPT 135) - shows CH & CH_3 with positive phase and CH_2 with negative phase
Spectrum C is a normal ^{13}C

- DEPT 45 (not shown) shows all protonated carbons



Overview of Advanced NMR Experiments

*1D Difference NOE (**N**uclear **O**verhauser **E**ffect)*

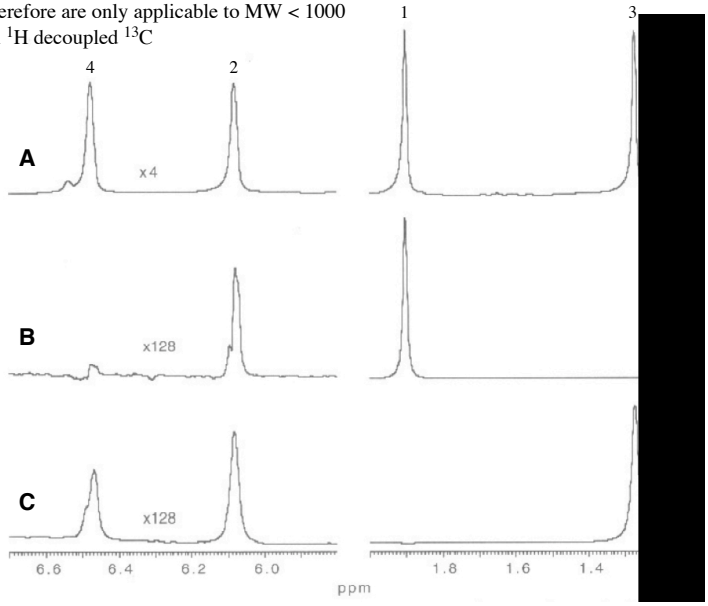


- NOEs arise from dipole-dipole interactions of atoms in close proximity to one another (up to 5 Å). NOE interactions can also be used to determine internuclear separation.
- By irradiating a particular resonance, atoms within range will be affected by either an enhancement or depression of the signal. Subtracting the reference spectrum from the enhanced spectrum reveals the resonances experiencing NOEs.
- 1D NOE detects *steady-state* NOEs and therefore are only applicable to MW < 1000
- NOEs also lead to a signal enhancement in ^{1}H decoupled ^{13}C

Spectrum A - ^{1}H NMR

Spectrum B - Difference NMR from irradiation of protons 1. Proton 2 shows through space signal enhancement.

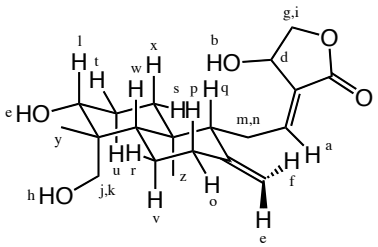
Spectrum C - Difference NMR from irradiation of protons 3. Protons 2 and 4 show through space signal enhancement.



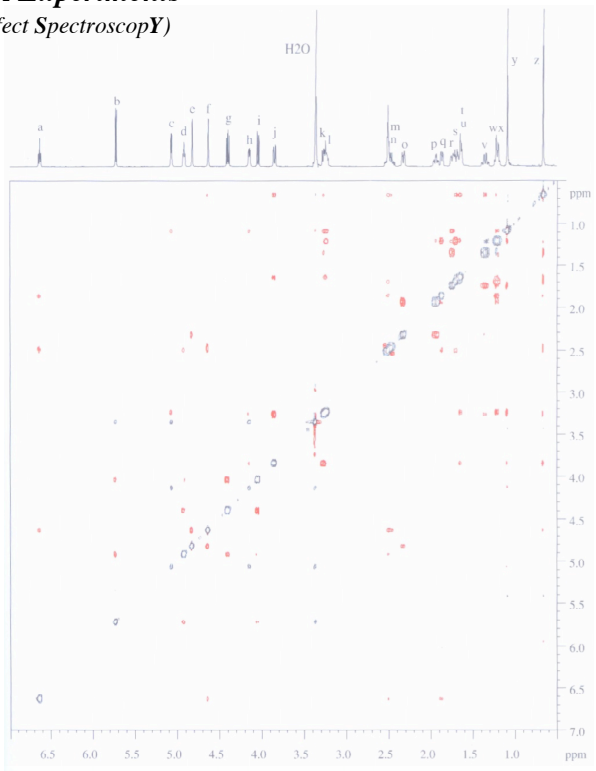
Overview of Advanced NMR Experiments

*2D NOESY (**N**uclear **O**verhauser **E**ffect **S**pectroscop**Y**)*

- Observes the same phenomena as the 1D NOE but *transient* effects. These are dependent on the kinetic rate that these effects develop. As a result, determining internuclear distances from these spectra are not as reliable as in the 1D NOE experiment.
- Transient NOEs are observable for small molecules (MW < 1000) and large molecules (MW > 2000)
- For medium sized molecules a different observation technique is used to detect the same effects (ROESY). ROESY requires more scans and therefore longer acquisition.



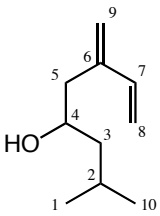
2D NOESY of Andrographolide
peaks in red indicate positive NOE effects



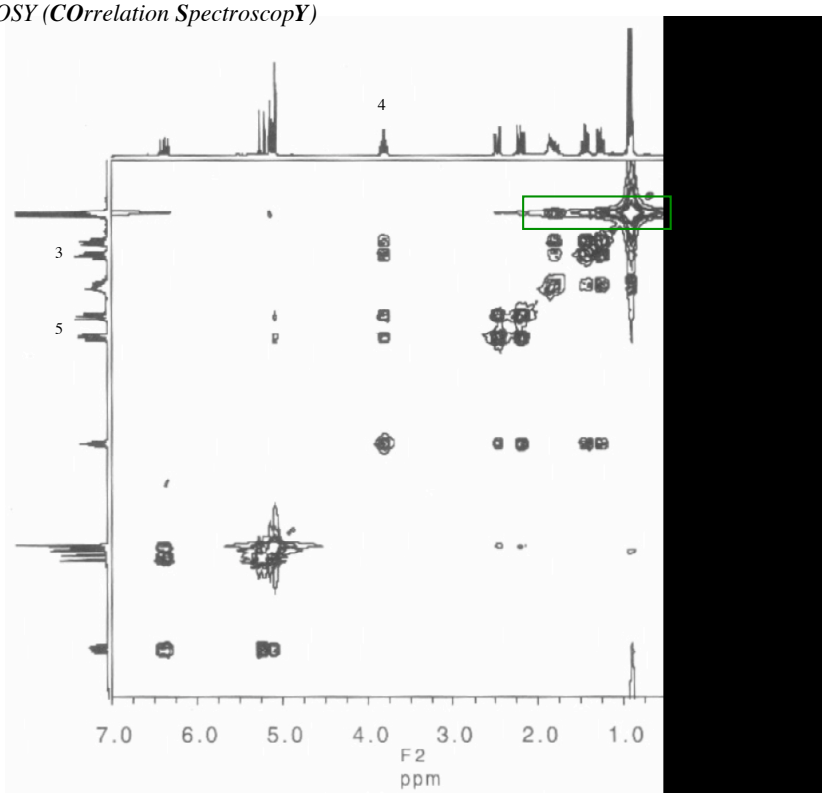
Overview of Advanced NMR Experiments

*2D COSY (**CO**rrelation **S**pectroscop**Y**)*

- 2D COSY reveals scalar, through-bond couplings between protons.
- One ^{1}H spectrum is shown on each axis.
- Start from cross-peaks on the diagonal and trace either vertical or horizontal. Off-diagonal cross-peaks indicate coupled protons.
- The number of protons in a particular spin system is readily determined.



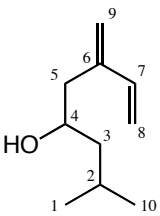
ipsenol



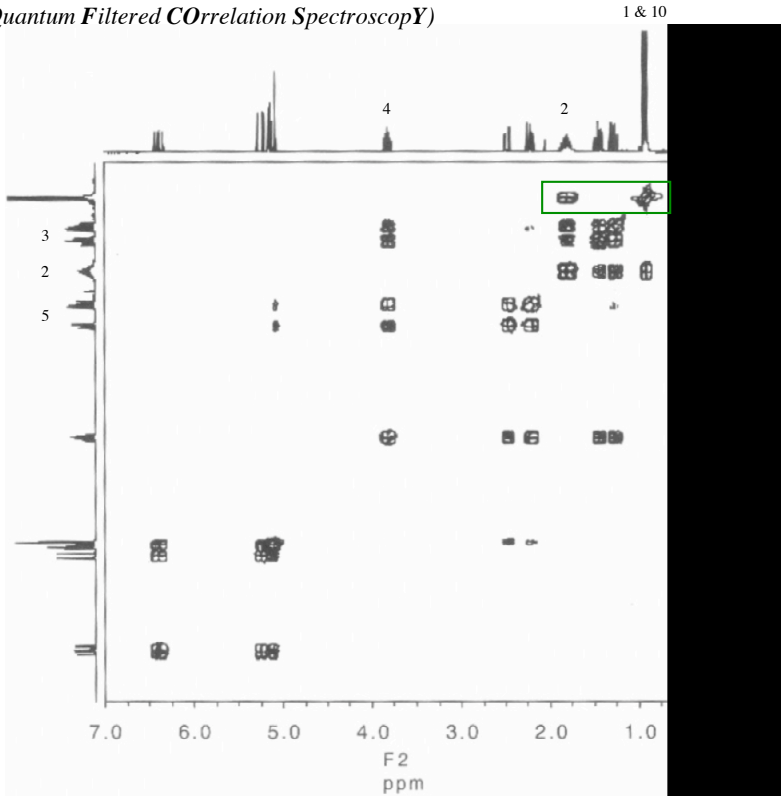
Overview of Advanced NMR Experiments

*2D DQF-COSY (**D**ouble-**Q**uantum **F**iltered **CO**rrelation **S**pectroscop**Y**)*

- Modified 2D-COSY technique
- An additional pulse to the pulse sequence, single quantum transitions are supressed, leaving double quantum and higher transitiuons. As a result, non-coupled resonances like methyl singlets, will be greatly reduced. This allows better resolution of coupled cross-peaks.
- The spectrum on the whole appears "cleaner"



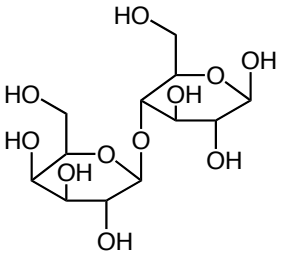
ipsenol



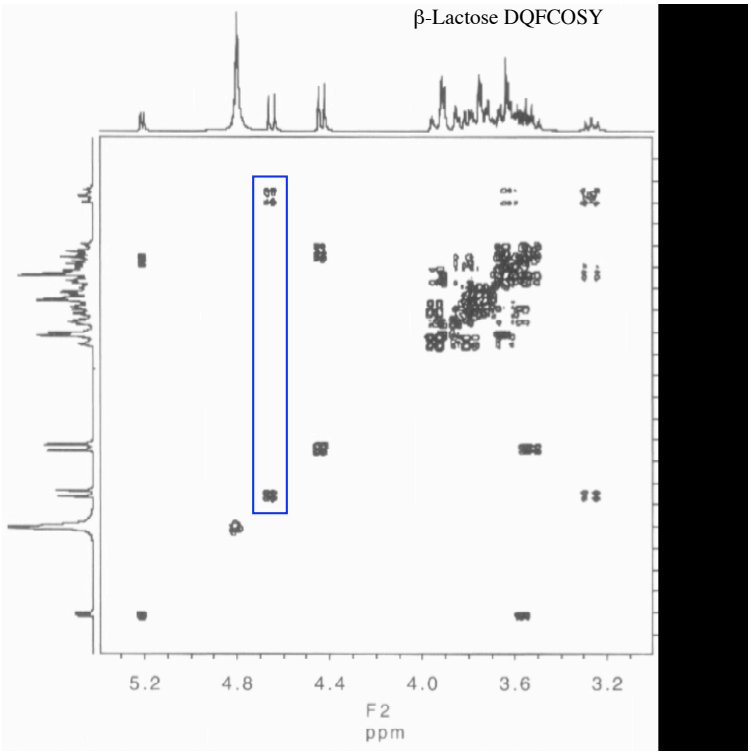
Overview of Advanced NMR Experiments

*TOCSY (**TO**tal **C**orrelation **S**pectroscop**Y**)*

- TOCSY shows correlations between protons in the same spin system even if they aren't directly coupled. DQFCOSY only shows protons directly coupled.



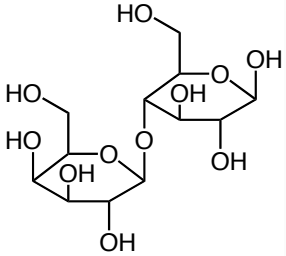
β-lactose



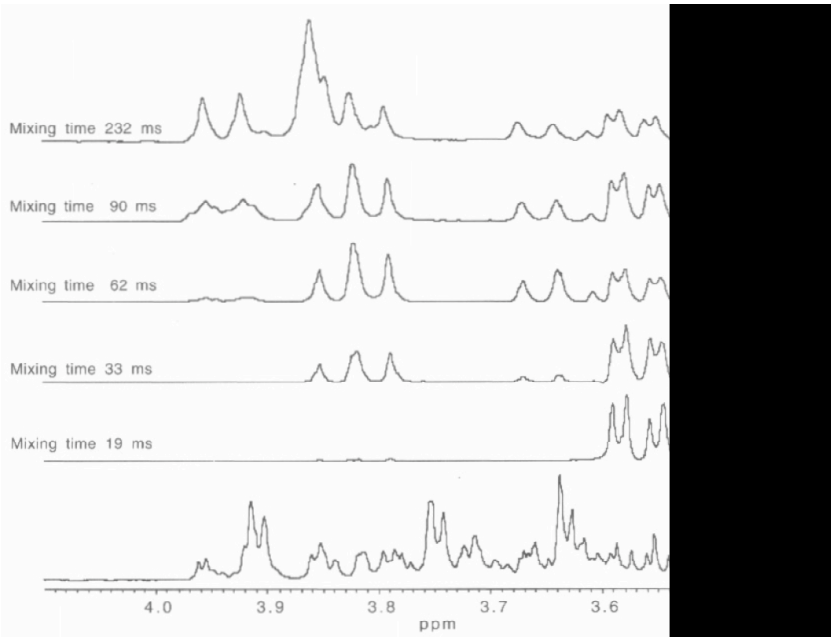
Overview of Advanced NMR Experiments

*1D TOCSY (**TO**tal **C**orrelation **S**pectroscop**Y**)*

- 1D TOCSY shows correlations between protons in the same spin system even if they aren't directly coupled.
- Mixing time is critical, but longer mixing times cause loss of resolution and signal.



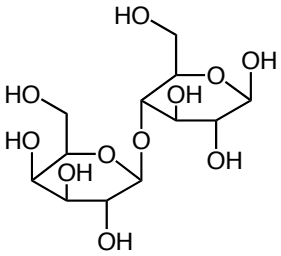
β-lactose



Overview of Advanced NMR Experiments

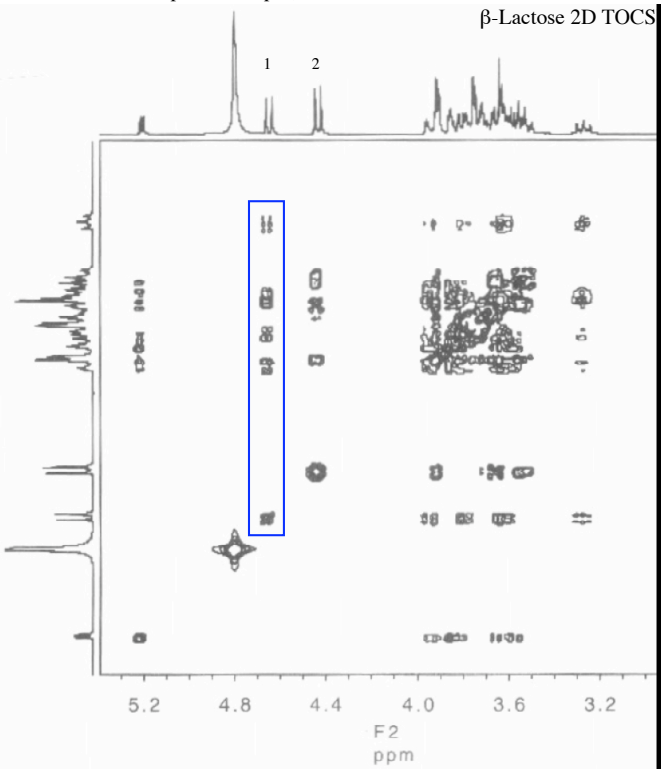
*2D TOCSY (**TO**tal **C**orrelation **S**pectroscop**Y**)*

- 2D TOCSY shows correlations between protons in the same spin system even if they aren't directly coupled.
- Mixing time is critical, but longer mixing times cause loss of resolution and signal.
- TOCSY shows that anomeric protons 1 and 2 are in separate spin systems. Furthermore, the complex multiplet between 3.5 and 4.0 are separated into the two component ring resonances.



β-lactose

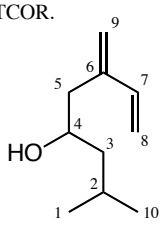
β-Lactose 2D TOCS



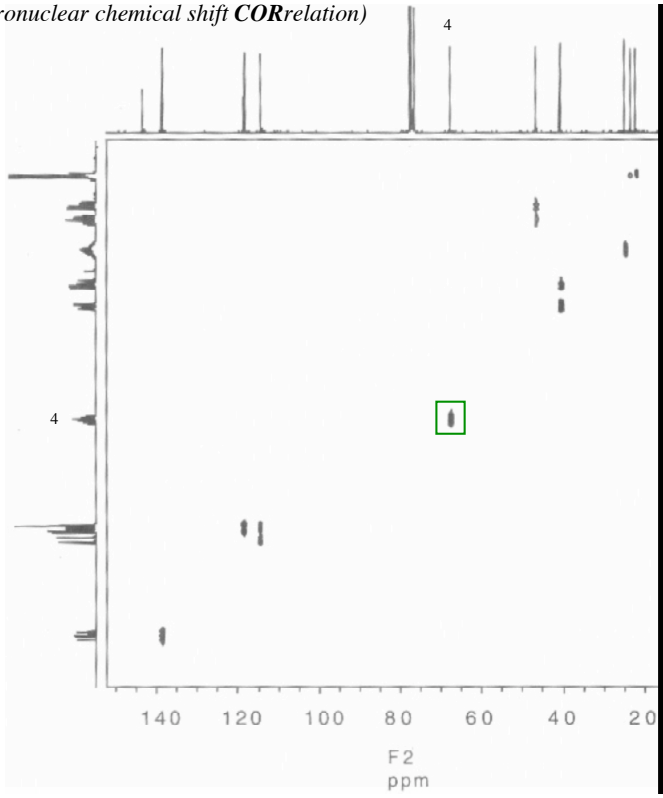
Overview of Advanced NMR Experiments

*2D HETCOR (**HET**eronuclear chemical shift **COR**relation)*

- HETCOR is the ^{1}H-^{13}C COSY. ^{13}C is the detected nucleus
- Shows couplings between carbons and the attached protons.
- Unlike COSY, there is no symmetry to the 2D spectrum. Tracing vertically down from each carbon resonance indicates the number and identity of attached protons
- A related experiment is HMQC (**H**eteronuclear **M**ultiple **Q**uantum **C**oherence) detects ^{1}H instead of ^{13}C. This increases sensitivity, but resolution suffers. This variant is sometimes called inverse-detected HETCOR.



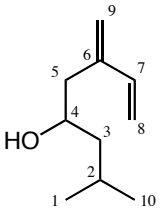
ipsenol



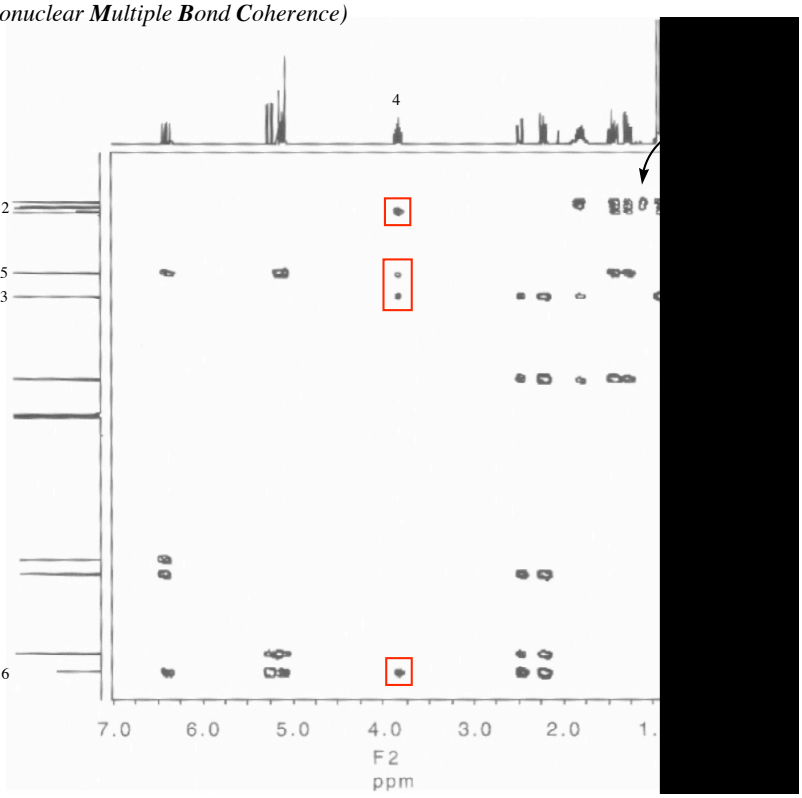
Overview of Advanced NMR Experiments

*2D HMBC (**H**eteronuclear **M**ultiple **B**ond **C**oherence)*

- HMBC shows long-range (2-3 bond) ^{1}H-^{13}C correlations. Couplings to attached carbons are *not* observed. ^{1}H is the detected nucleus.
- Two-bond correlations are almost always found, but three-bond correlations are sometimes absent.
- Artifacts from ^{13}C satelites occassionally appear, especially with methyl carbons. These are easily identified since the apparent cross-peak does not match a resonance in the 1D spectrum.



ipsenol

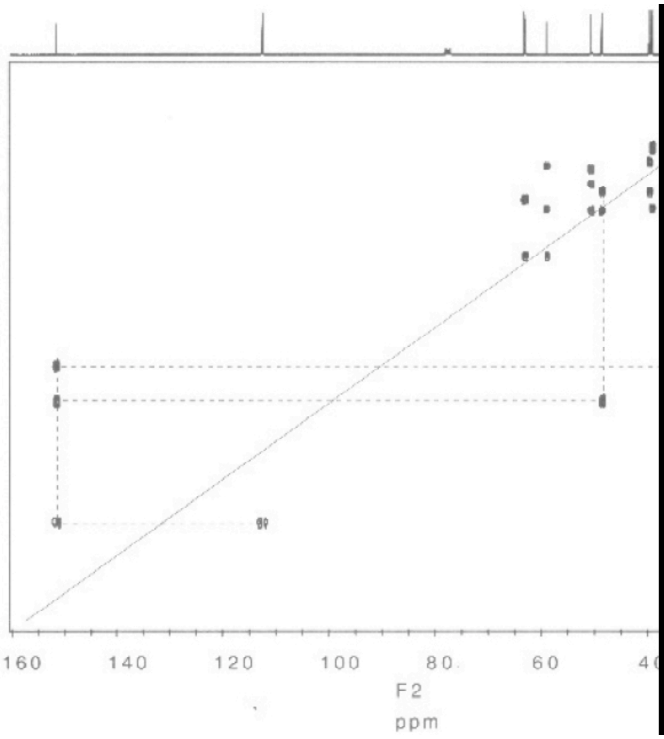


Overview of Advanced NMR Experiments

*INADEQUATE (**I**ncredible **N**atural **A**bundance **D**oubl**E** **QU**A**ntum **T**ransfer **E**xperiment)*

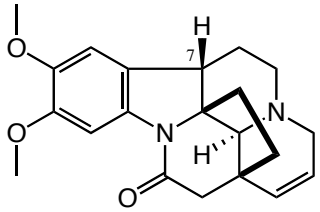
- Similar to COSY, but targeting ^{13}C instead of ^{1}H. Shows ^{13}C-^{13}C couplings.
- Readily reveals C-C connectivity; a cross-peak in the INADEQUATE spectrum indicates the carbons are connected to one another.
- At high resolution, each cross-peak appears as a doublet.

- **Downside:** requires large quantities of material (~250 mg) due to the low natural abundance of ^{13}C



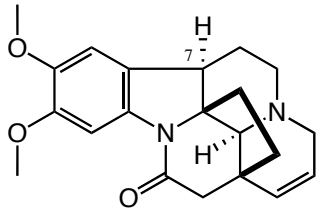
Schizogane Alkaloids

Modern NMR Analysis to the Rescue!



Schizogamine

IR = 6.07 μ (1647 cm^{-1})
λ_{max} = 264 (log ε = 4.09), 302 (log ε = 4.00)
$[\alpha]_D$ = -7.9° ($CHCl_3$, c = 1, 25 °C)
MP 123-125 °C



Isoschizogamine

IR = 5.93 μ (1686 cm^{-1})
λ_{max} = 259 (log ε = 4.12), 290 (log ε = 3.87)
$[\alpha]_D$ = -239° ($CHCl_3$, c = 1, 25 °C)
MP 184-185 °C

Typical IR stretching frequency for tertiary amide carbonyls: 5.99-6.14 μ (1670-1630 cm^{-1})
Typical IR stretching frequency for δ-lactam carbonyls: ~5.95 μ (~1680 cm^{-1})
Typical IR stretching frequency for γ-lactam carbonyls: ~5.88 μ (~1700 cm^{-1})

Isolation:
Renner, *Experientia* **1963**, *19*, 244-246.
Renner, *Lloydia* **1964**, *27*, 267.

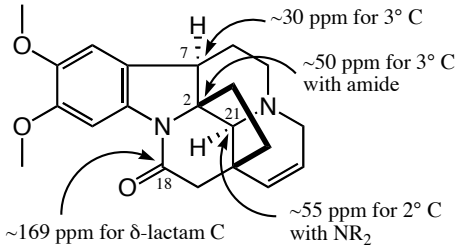
Structure Revision:
Hájicek, *Tetrahedron Lett*. **1998**, *39*, 505-508.

Total Synthesis:
Heathcock, O*rg*. *Lett*. 1**999,** 1, 1315-1317.

Other approaches:
Magomedov, *Org*. *Lett*. **2003**, *5*, 2509-2512.
Padwa, *Org*. *Lett*. **2005**, *7*, 2925-2928.

Isoschizogamine

Problems with NMR Data



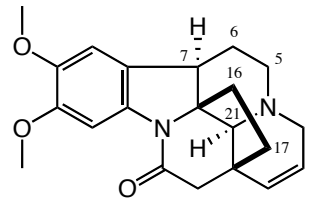
Isoschizogamine
proposed structure

Expected ^{13}C NMR resonances:

C	δ (ppm)	type
18	169	amide
2	50	"4°"
7	30	3°
21	55	"3°"

Observed ^{13}C NMR resonances:

δ (ppm)	type	
173.79	amide	⇨ γ-lactam
84.4	"4°"	⇨ ???
34.95	3°	
37.66	3°	



Isoschizogamine
proposed structure

Expected ^{1}H NMR features:

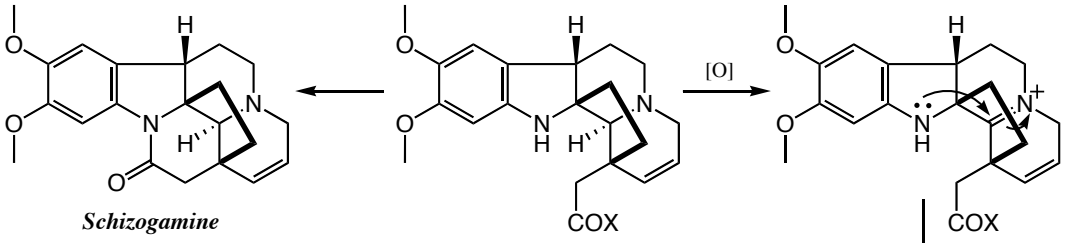
C-H singlet from C21
4 H spin system from C16 & C17
5 H spin system from C5, C6 & C7

Observed ^{1}H NMR resonances:

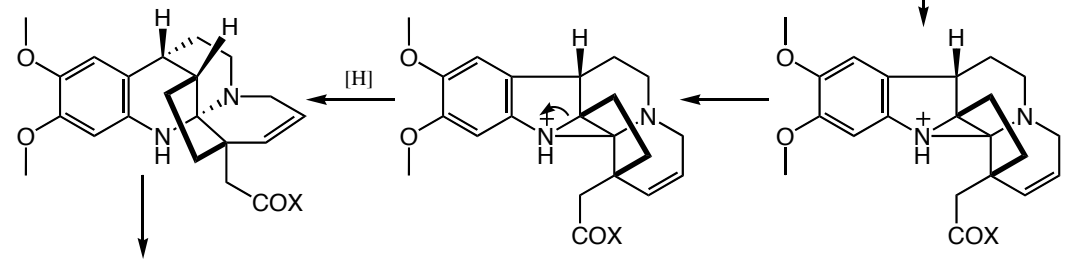
No C-H singlet
10 H spin system (^{1}H-^{1}H 2D-COSY) of the form
N-CH_2-CH_2-CH-CH-CH_2-CH_2

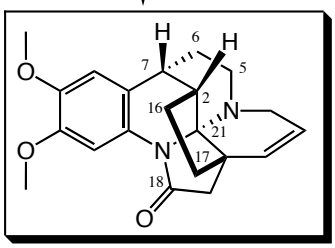
Something is obviously wrong!
Data are okay for Schizogamine, however.

Isoschizogamine - A Biosynthetic Structure Revision



Schizogamine

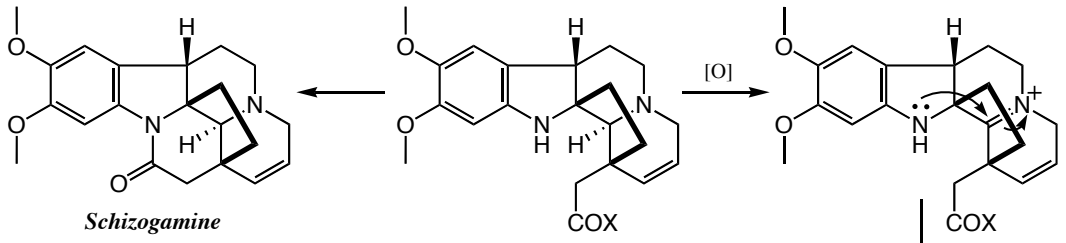




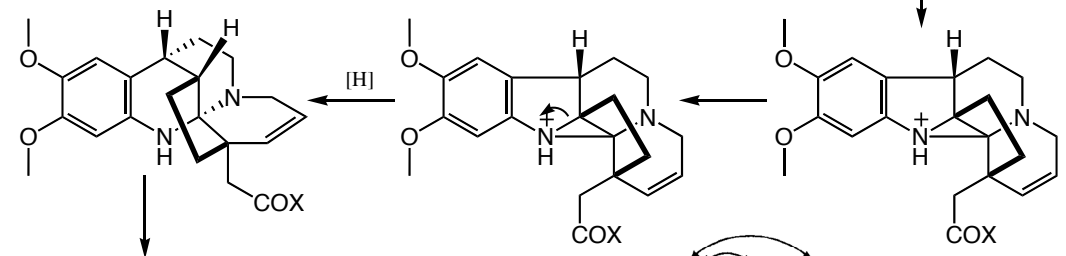
Revised structure

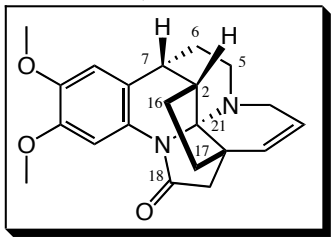
Accounts for observed NMR features:
- C18 is a γ-lactam
- C21 is an aminal
- 10 H spin system from C5, C6, C7, C2, C16, C17

Isoschizogamine - A Biosynthetic Structure Revision



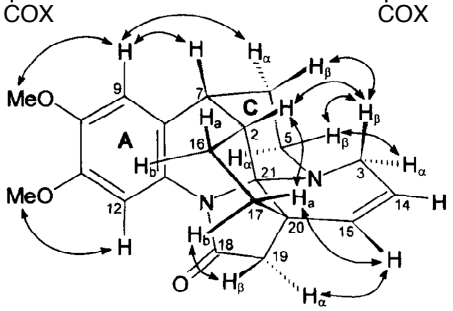
Schizogamine





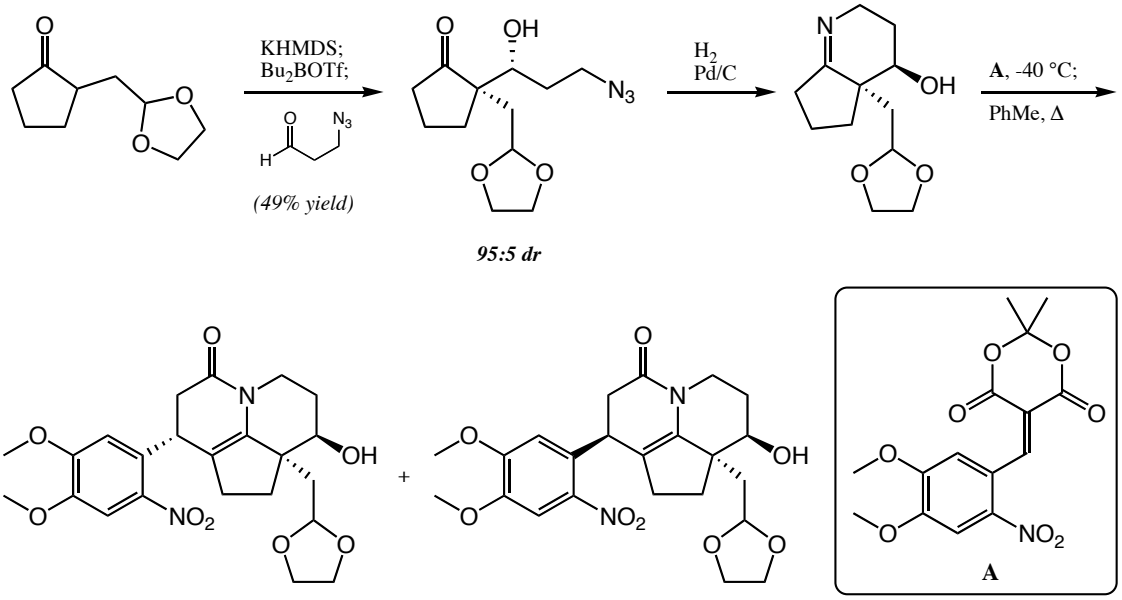
Revised structure

NOE data also agree with this structure assignment



Isoschizogamine

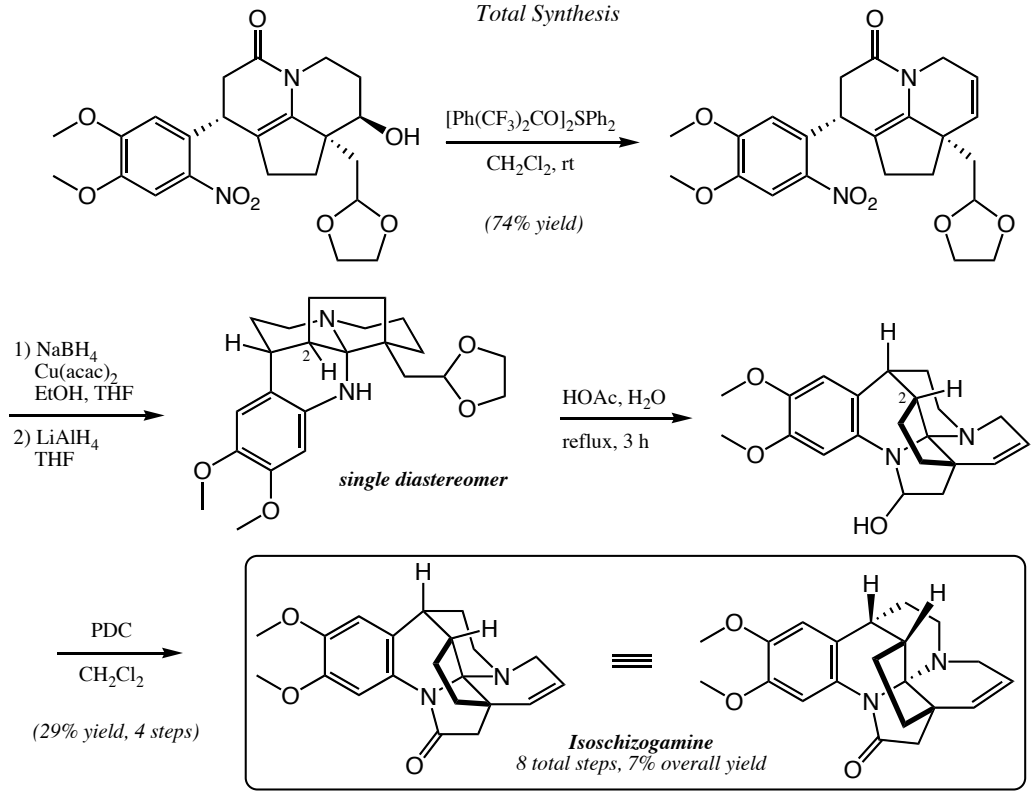
Total Synthesis



(74% yield, 2 steps)

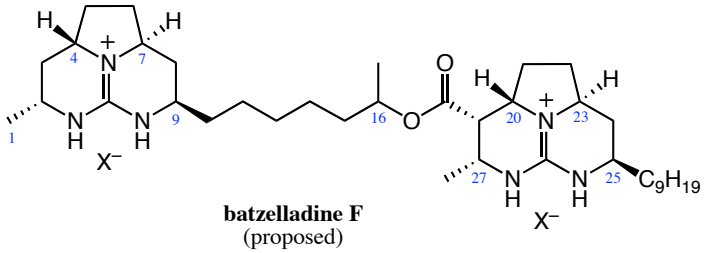
Isoschizogamine

Total Synthesis



Case Study: Batzelladine F

Introduction

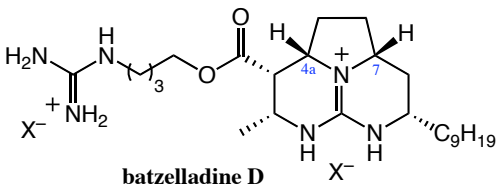


batzelladine F
(proposed)

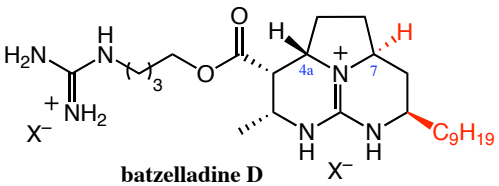
- Potent immunosuppresant isolated in 1997 from *Batzella* sp., member of a family of natural products batzelladines A-I: Faulkner, D. J. *J. Org. Chem.* **1997**, *62*, 1814-1819.
- Relative configuration of the right-hand portion (C17-C20) compared to ^{13}C data from batzelladines A and D: Potts, B. C. M. et al. *J. Org. Chem.* **1995**, *60*, 1182-1188; Snider, B. B. et al. *Tetrahedron Lett.* **1996**, *37*, 6977-6980; Overman, L. E. et al. *Org. Lett.* **1999**, *1*, 2169-2172.
- Relative stereochemistry of C1-C9 based on nOe data, however, no information or analysis was provided in the isolation paper.
- Lengths of connecting chain (C10-C16) and nonyl chain at C25 assigned based on MS fragmentation data, although this was also *not* presented or discussed in the isolation article!
- Relative or absolute configuration at C16 could not be determined.

Case Study: Batzelladine F

Batzelladine D Revises Guandinium Stereochemistry

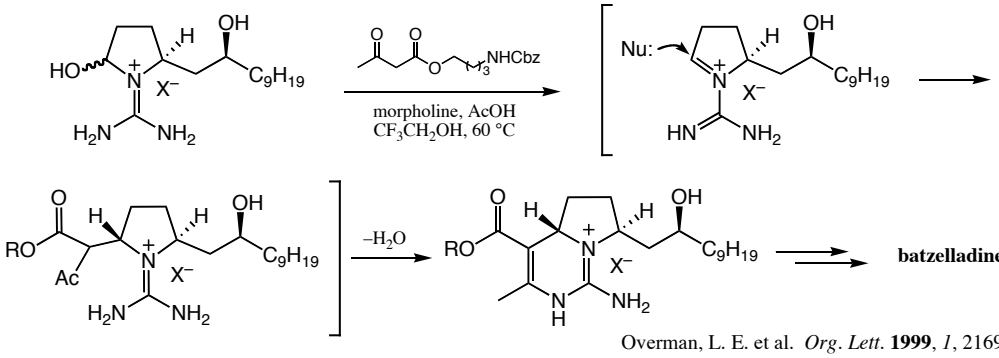


batzelladine D
(propose)



batzelladine D
(revised/ proven)

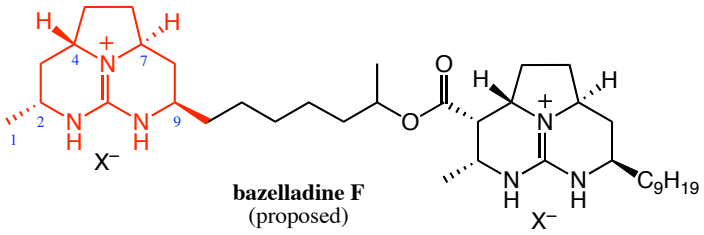
- Snider and Chen were the first to prepare analogues and reassign stereochemistry based on ^{1}H and ^{13}C NMR, as well as chemical degradation (Snider, B. B. et al. *Tetrahedron Lett.* **1996**, *37*, 6977-6980).
- Overman followed with proving the absolute and relative stereochemistries based on Biginelli condensation.



Overman, L. E. et al. *Org. Lett.* **1999**, *1*, 2169-2172.

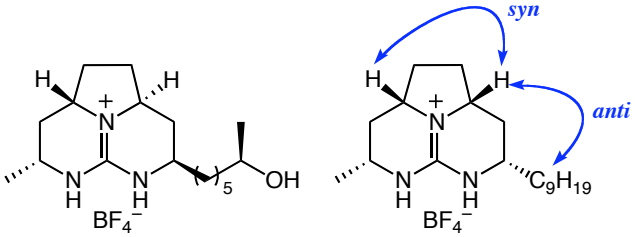
Case Study: Batzelladine F

Detemining the Relative Stereochemistry of the Left-Hand Fragment



bazelladine F
(proposed)

Comparison of synthetic analogues to determine relative configuration of batzelladine F left-hand fragment.



A ***B***

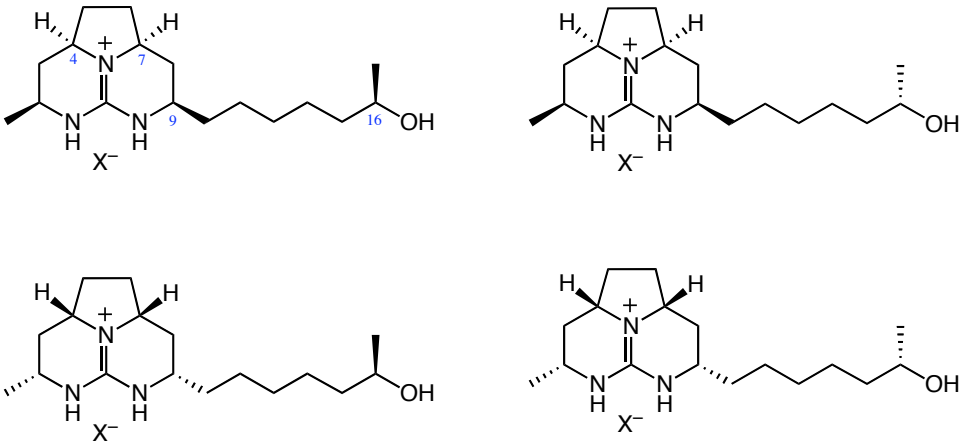
carbon #	A	natural batz. F	B
1	21.7	20.7	20.7
2	48.9	47.2	47.3
4	56.5	57.5	57.5
7	56.4	57.4	57.5
9	53.1	51.6	51.6

Snider, B. B. et al. *Tetrahedron Lett*. **1996**, *37*, 6977-6980.
Cohen, F. Ph.D. thesis, University of California, Irvine, CA, 2001.

Case Study: Batzelladine F

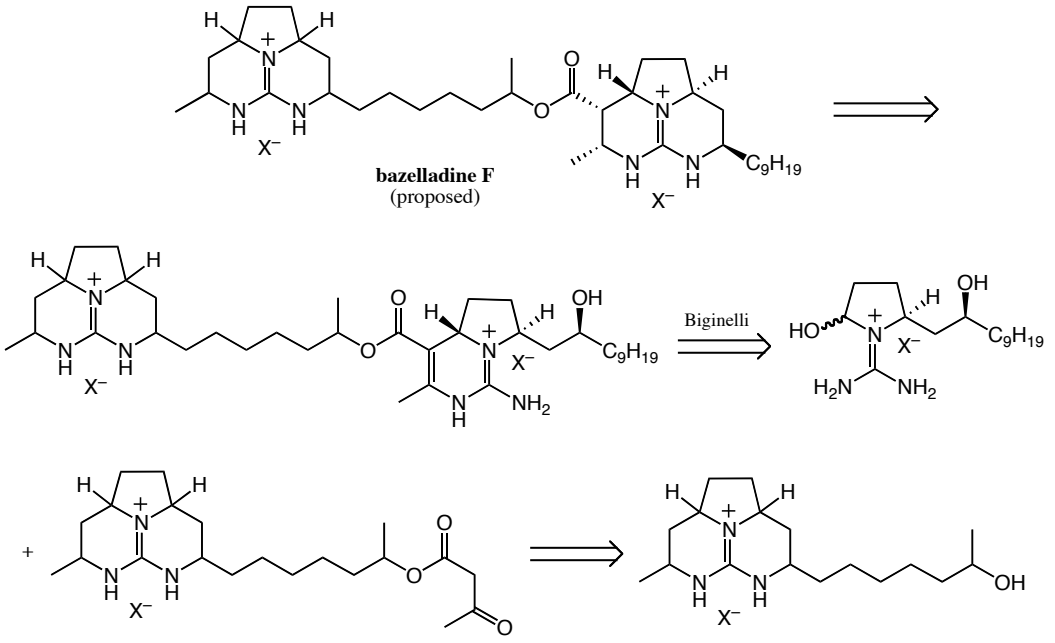
Possible Diastereomers for the Left-Hand Fragment

No information on absolute stereochemisty, therefore, 4 possibilities for left-hand fragment.



Case Study: Batzelladine F

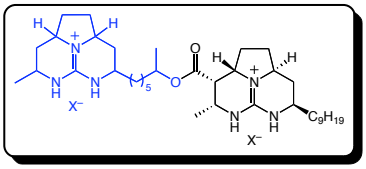
Retrosynthetic Analysis of the Proposed Structure

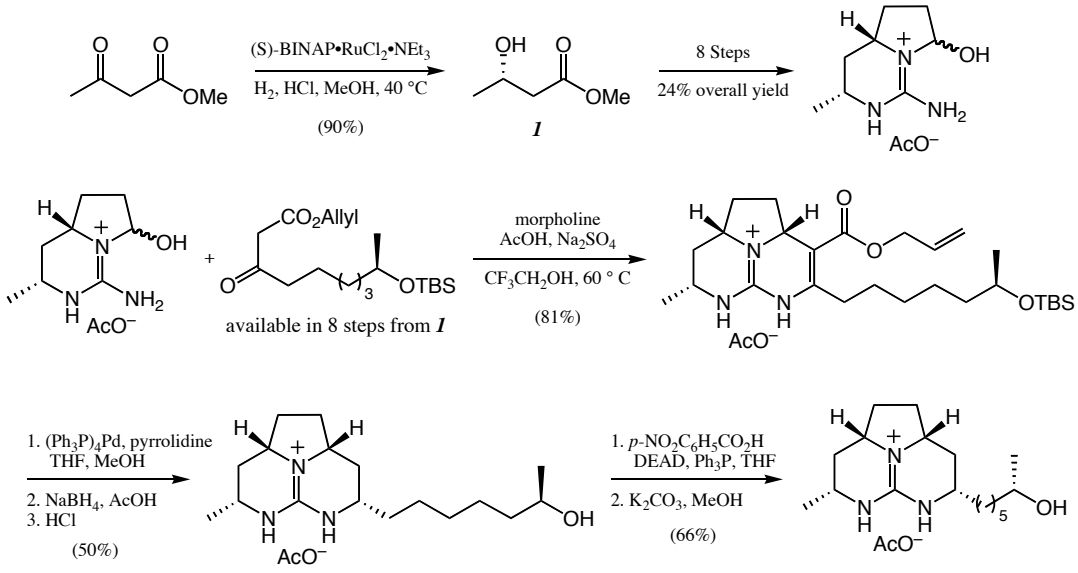


Cohen, F. Ph.D. thesis, University of California, Irvine, CA, 2001.

Case Study: Batzelladine F

Synthesis of the Four Proposed Diastereomers

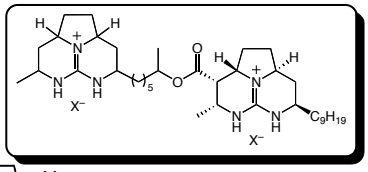


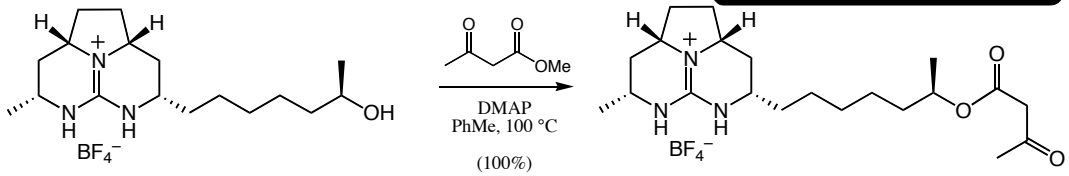


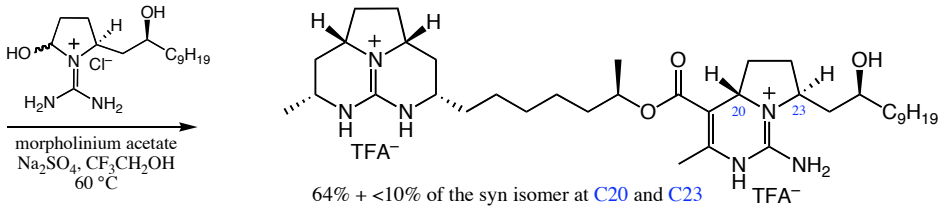
Cohen, F. Ph.D. thesis, University of California, Irvine, CA, 2001.

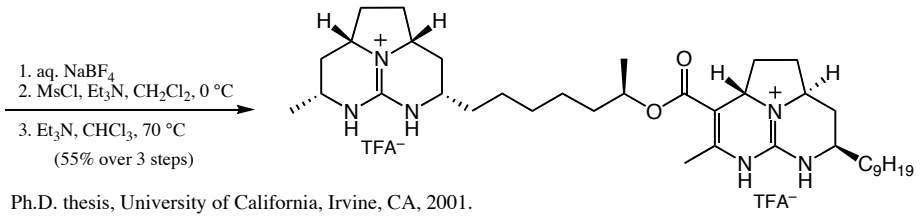
Case Study: Batzelladine F

Synthesis of the Four Proposed Diastereomers





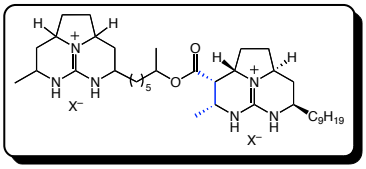


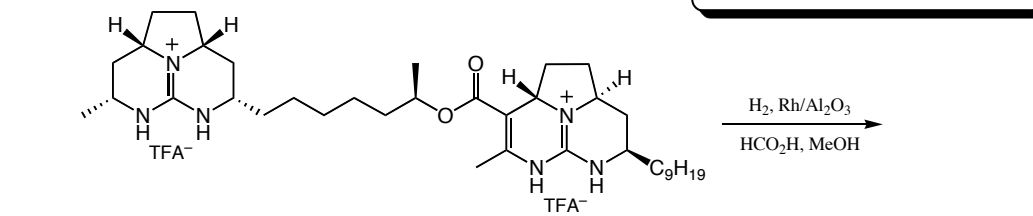


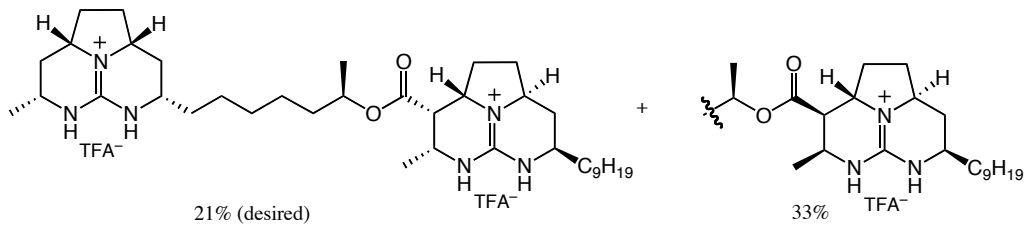
Cohen, F. Ph.D. thesis, University of California, Irvine, CA, 2001.

Case Study: Batzelladine F

Synthesis of the Four Proposed Diastereomers





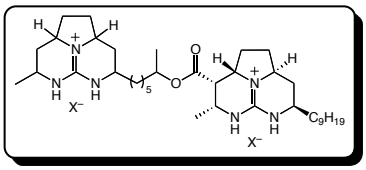


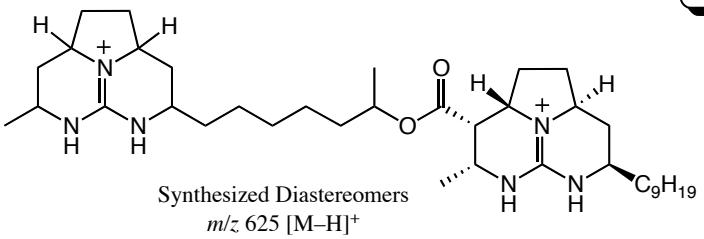
- All four diastereomers prepared in same manner, however **NONE** matched the HPLC trace of natural batzelladine F (co-injection).
- What is in error? NMR is not useful due to essentially identical spectra, but what about MS?

Cohen, F. Ph.D. thesis, University of California, Irvine, CA, 2001.

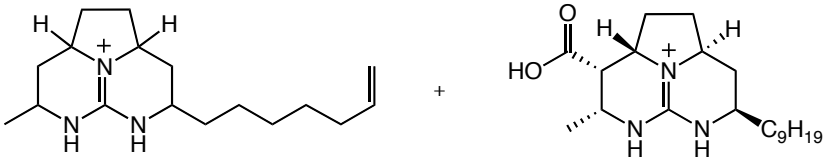
Case Study: Batzelladine F

MS Analysis of Prepared Diastereomers vs. Natural





Synthesized Diastereomers
m/z 625 $[M–H]^+$



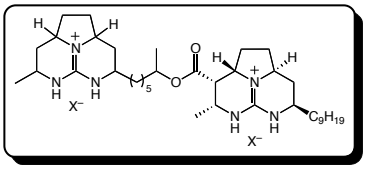
m/z 276 *m/z* 350

- MS analysis for synthetic material yields two major peaks, *m/z* = 276 and 350.
- MS anaylsis of natural material yields two different major peaks, *m/z* = 304 and 322.

Cohen, F. Ph.D. thesis, University of California, Irvine, CA, 2001.

Case Study: Batzelladine F

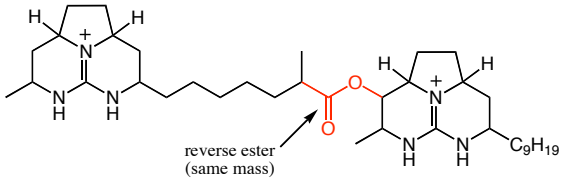
MS Analysis of Prepared Diastereomers vs. Natural



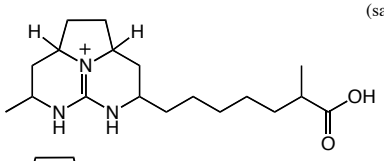
batzelladine F (authentic) —MS→ *m/z* = 322 + *m/z* = 304

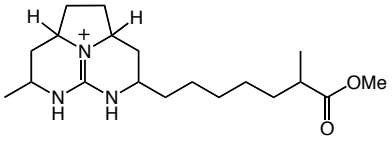
batzelladine F (authentic) —NaOMe, MeOH, 110 °C→ Exact Mass: 336.26651, $C_{19}H_{34}N_3O_2$ + Exact Mass: 322.2858, $C_{19}H_{36}N_3O$

Fragmentation of Synthetic material gave fragments that are ± 28 amu than expected.

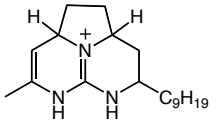


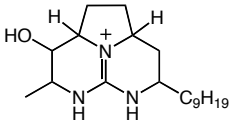
Not Consistent with 2D NMR Data!





m/z	
322	304
336	322





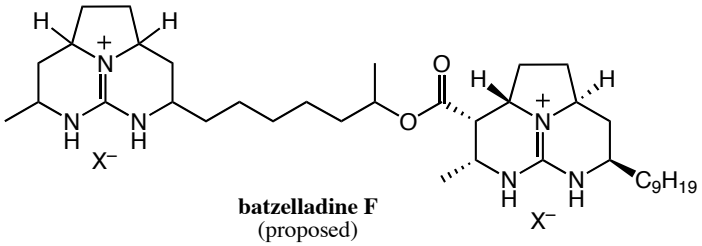
276 synthetic 350

Case Study: Batzelladine F

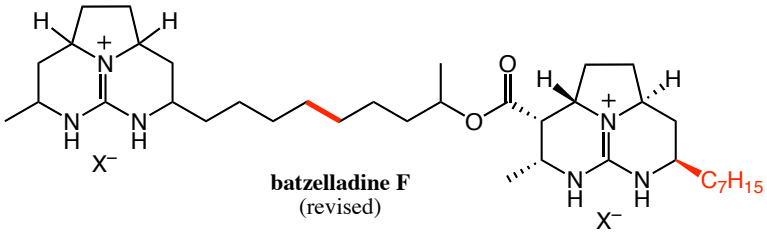
MS Analysis of Prepared Diastereomers vs. Natural

What could account for an exchange of 28 amu on either side, yet still match 2D NMR data?

$-CH_2CH_2- = 28$



batzelladine F
(proposed)



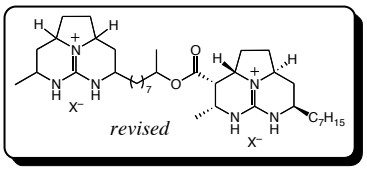
batzelladine F
(revised)

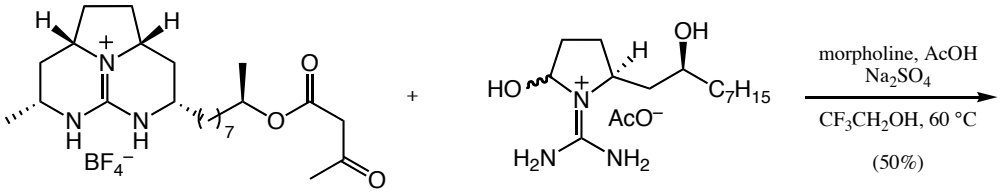
The previous synthetic routes are still applicable, however, there are still ***four*** diastereomers possible for left-hand fragment.

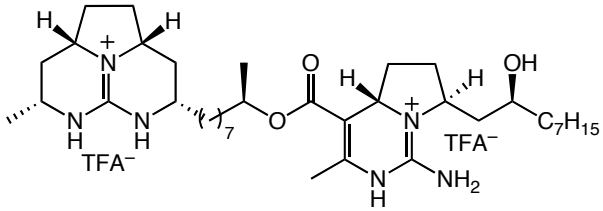
Cohen, F. Ph.D. thesis, University of California, Irvine, CA, 2001.
Cohen, F.; Overman, L. E. *J. Am. Chem. Soc.* **2001**, *123*, 10782-10783.

Case Study: Batzelladine F

Synthesis of Revised Structure and Diastereomers







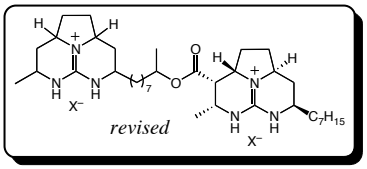
synthetic batzelladine F
(revised)

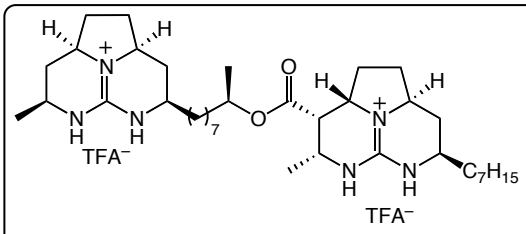
All four possible diastereomers prepared for comparison.

Cohen, F.; Overman, L. E. *J. Am. Chem. Soc.* **2001**, *123*, 10782-10783.

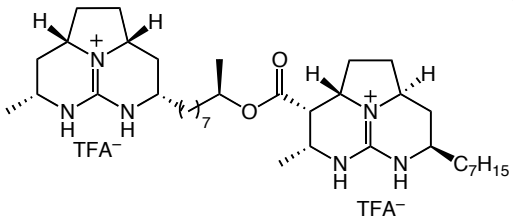
Case Study: Batzelladine F

HPLC Analysis of Four Diastereomers of Revised Structure



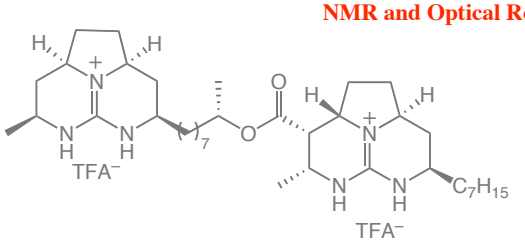


1
co-elute with ***2*** and authentic **batzelladine F**

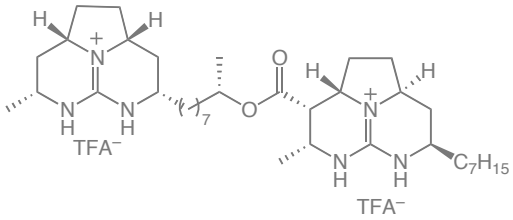


2
co-elute with ***1*** and authentic **batzelladine F**

NMR and Optical Rotation Not Useful...



3
NOT batzelladine F by HPLC

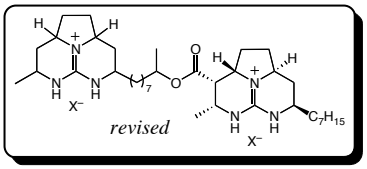


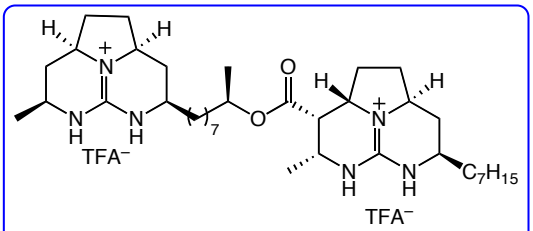
4
NOT batzelladine F by HPLC

Cohen, F.; Overman, L. E. *J. Am. Chem. Soc.* **2001**, *123*, 10782-10783.

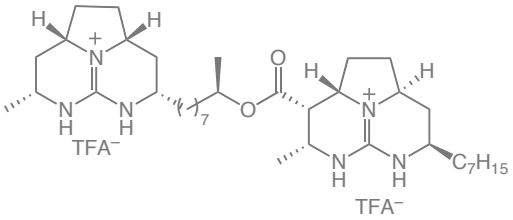
Case Study: Batzelladine F

Circular Dichroism Saves the Day!

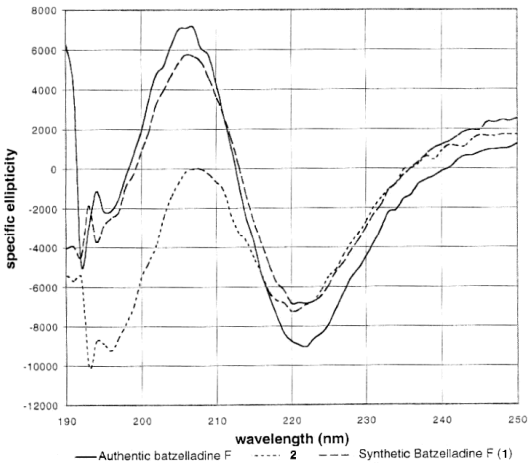




1
HPLC co-elute with ***2*** and authentic **batzelladine F**



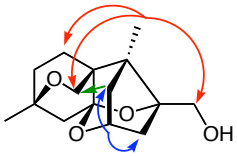
2
HPLC co-elute with ***1*** and authentic **batzelladine F**



Cohen, F.; Overman, L. E. *J. Am. Chem. Soc.* **2001**, *123*, 10782-10783.

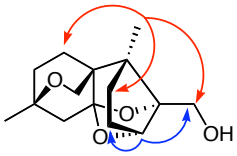
Case Study: Sporol and Neosporol

NMR Comparisons



Sporol

- 1 methine (next to ether)
- 7 methylenes
  - 1 next to alcohol
  - 1 next to ether
- 2 methyls



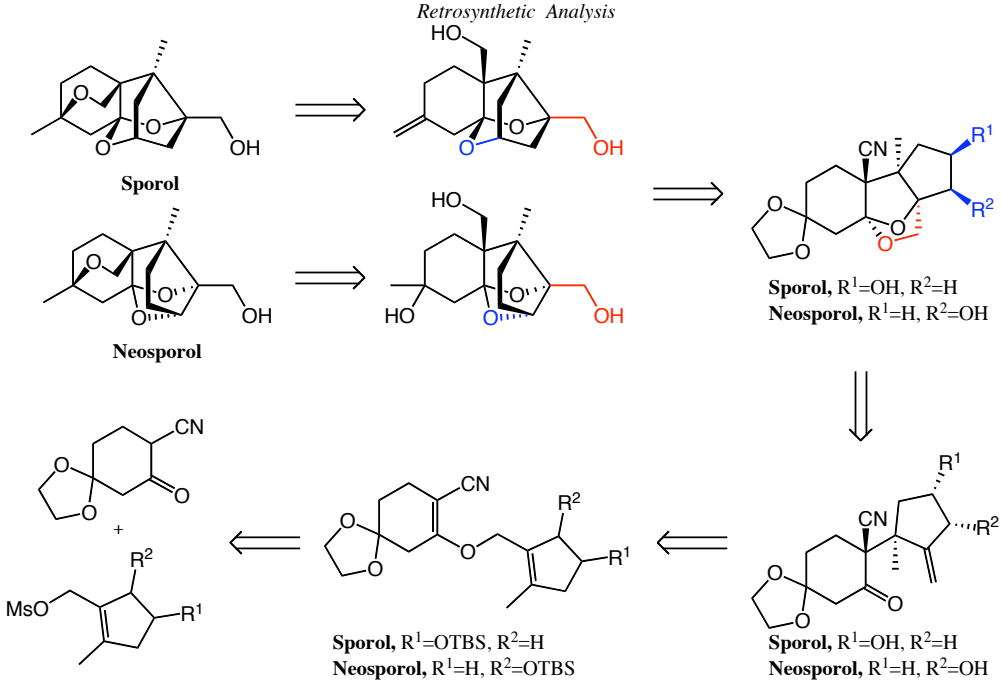
Neosporol

- 1 methine (next to ether)
- 7 methylenes
  - 1 next to alcohol
  - 1 next to ether
- 2 methyls

Ziegler, F. E. *et al. Tetrahedron Lett.* **1988**, *29*(14), 1665-1668.
Ziegler, F. E. *et al. JACS* **1993**, *115* (7), 2581-2589.

Case Study: Sporol and Neosporol

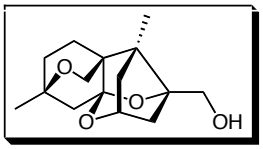
Retrosynthetic Analysis

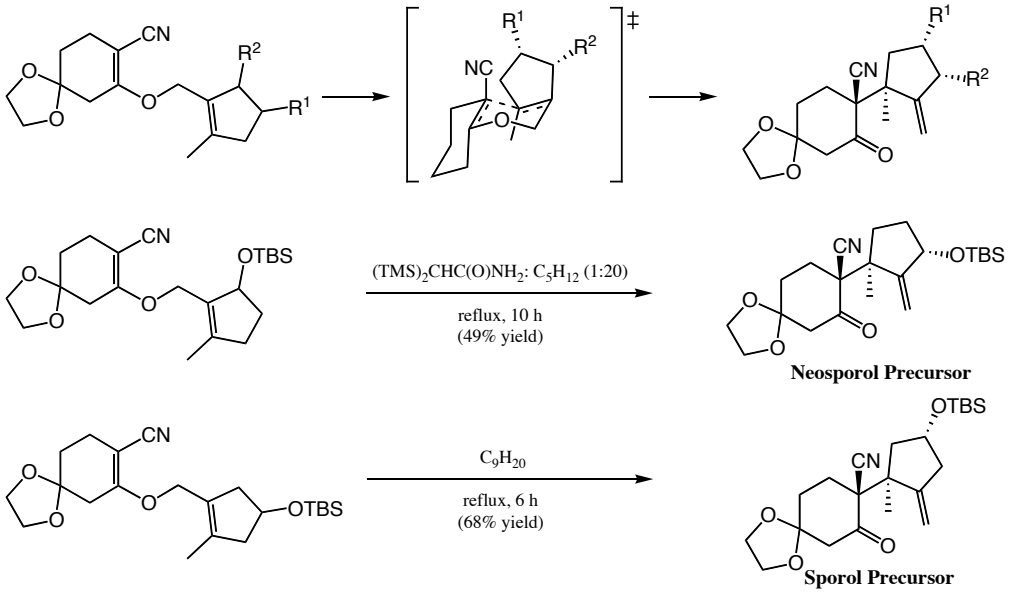


Ziegler, F. E. et al. *JACS* **1993**, *115* (7), 2581-2589.

Case Study: Sporol

Claisen Rearrangement : Enol Ethers with Controlled Geometry

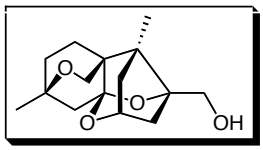


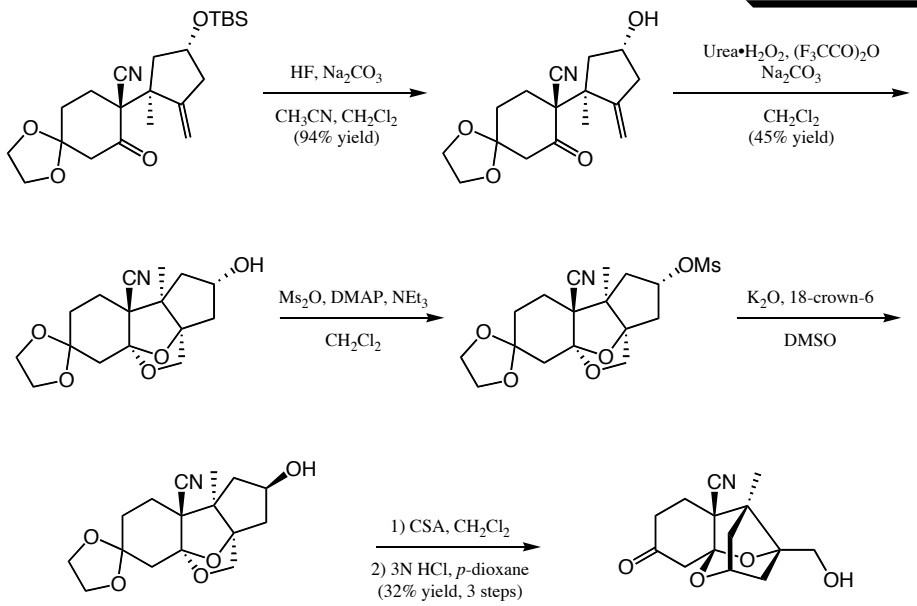


Ziegler, F. E. et al. *JACS* **1993**, *115* (7), 2581-2589;
Ziegler, F. E. *et al.* *JACS* **1987**, *109* (13), 3987-3991.

Case Study: Sporol

Preparation of the Dioxane

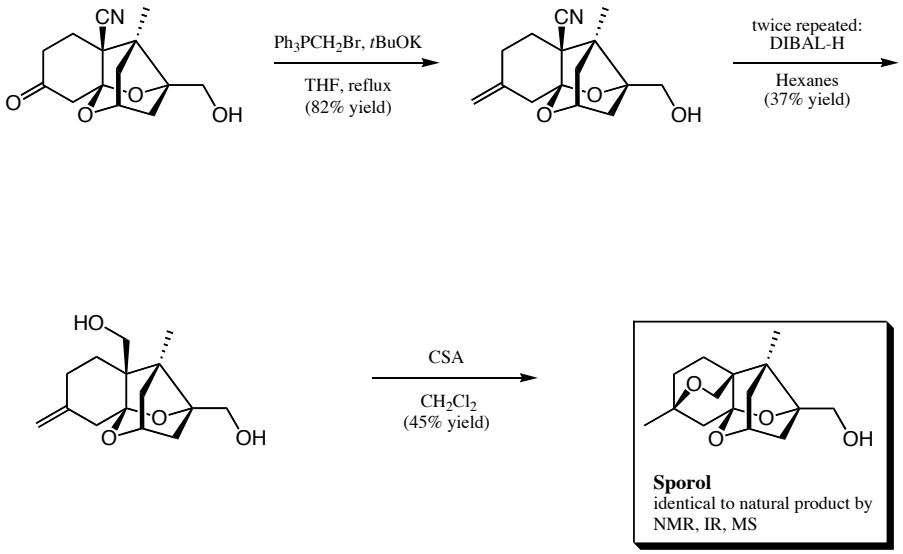




Ziegler, F. E. et al. *JACS* **1993**, *115* (7), 2581-2589.

Case Study: Sporol

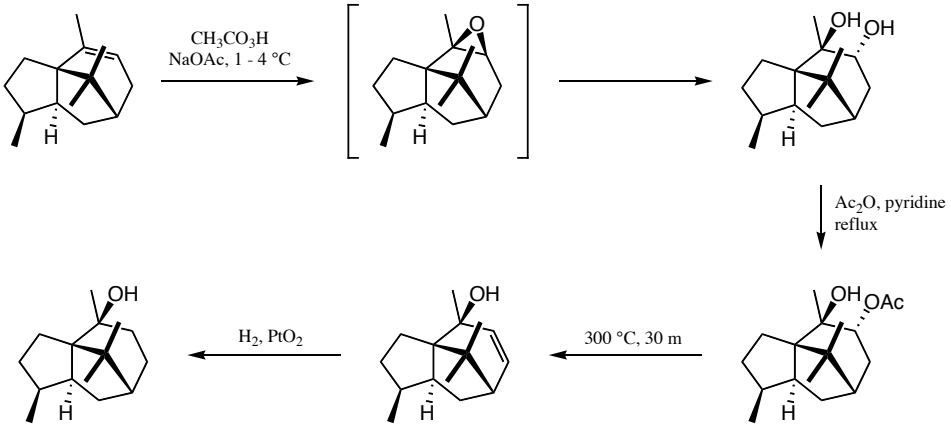
End Game



Ziegler, F. E. et al. *JACS* **1993**, *115* (7), 2581-2589.

Mistaken Identity: Organic Synthesis

Assigned Patchouli Structure

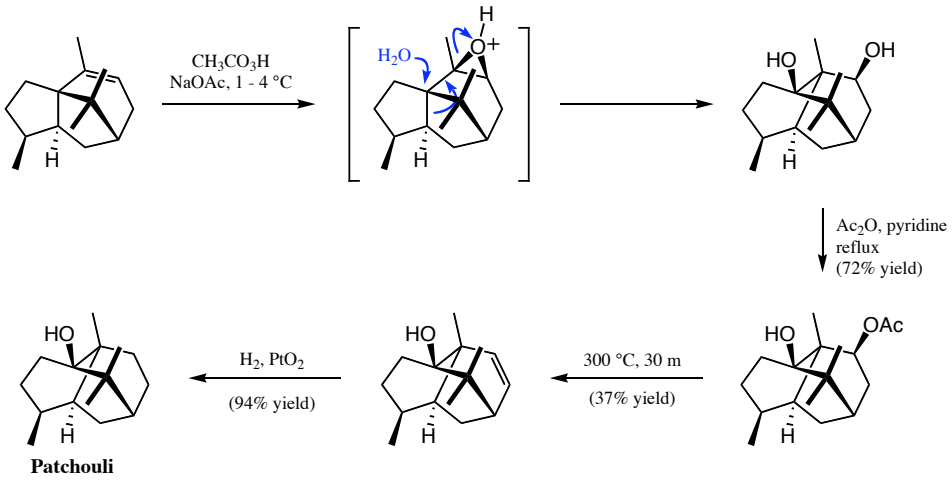


Is this patchouli?

Büchi, G. *et al. JACS* **1964**, *86*, 4438-4444.

Mistaken Identity: Organic Synthesis

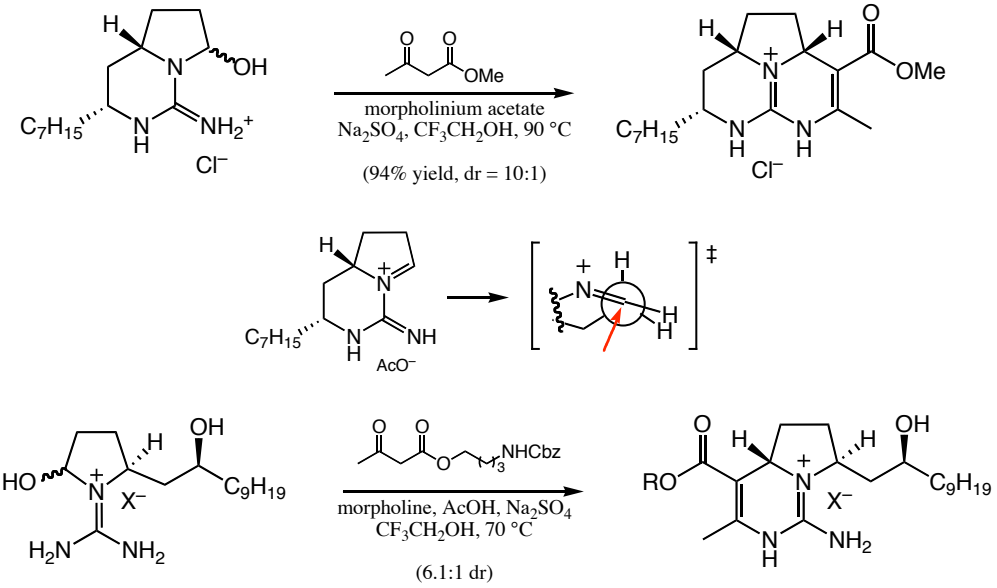
Assigned Patchouli Structure



Büchi, G. *et al. JACS* **1961**, *83*, 927-938;
Büchi, G. *et al. JACS* **1964**, *86*, 4438-4444

Biginelli Condensation

Defining Syn and Anti Stereochemistries



McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520-1528.
Cohen, F. et al. *Org. Lett.* **1999**, *1*, 2169-2172.
Aron, Z. D.; Overman, L. E. *Chem. Commun.* **2004**, 253-265.