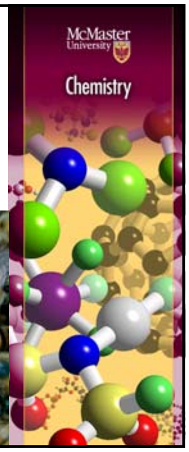
CHEM 1AA3: Intro. Chemistry II

Chemical Biology

Brainwashing Bees,
Drug Discovery, and Aromaticity



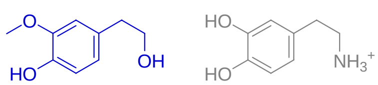
Queen Pheromone Blocks Aversive Learning in Young Worker Bees

- Vergoz *et al. Science* (**2007**) 317:384-386
- queen mandibular pheromone (QMP)
  - causes young workers to feed and groom her
  - suppresses new queens, controls colony behaviour

- read the accompanying file, "2013_Chembio_commentary.pdf"

The Biology of Brainwashing Bees

- QMP contains homovanillyl alcohol (HVA)
  - HVA suppresses bad memories, but not good ones



homovanillyl alcohol (HVA)

dopamine

- Biological role:
  - mitigates unpleasant side effects of QMP (?)

The Biology of Brainwashing Bees

- Significance
  - HVA lowers [dopamine]
  - dopamine is associated with learning
- Therapeutic potential
  - high [dopamine] in some psychoses & schizophrenia
  - HVA-like molecules could be treatments

    BUT

  - need high specificity:
    - attention deficit disorder & Parkinson's disease are linked to low [dopamine]

      ∴ global suppression of [dopamine], or suppression in the wrong parts of the brain, could have undesired side effects

The Biology of Brainwashing Bees

Key concepts

- queen bees control worker bees' memories using HVA
- help understanding human learning
- develop new therapeutics

The Chemical Biology of Brainwashing Bees

- How do we go from a biological phenomenon to the development of a new therapeutic agent?
- 1 new drug requires ~5000 failed compounds

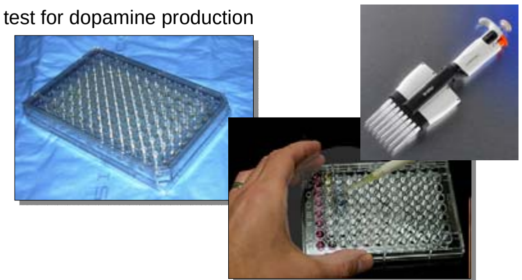
∴ improving synthesis & accelerating assays facilitates drug discovery

High-throughput screening

- high-throughput screening (HTS):
  - fast way to assay for a complex biological response
- 2 components:
  (1) a fast assay
  (2) massively parallel assays
- instead of doing experiments on live bees, use dopamine-producing bee neural cells growing in cell culture
- a fast, colour-producing dopamine assay already exists

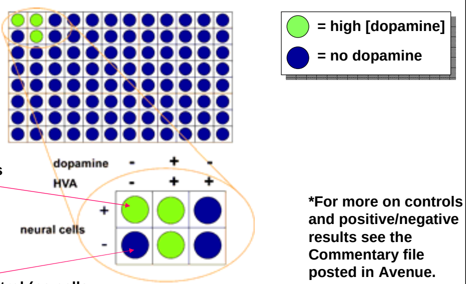
High-throughput screening

- 96 well microtiter plates allow 96 simultaneous assays
- grow bee neural cells in wells of plates
- test for dopamine production



High-throughput screening

- some wells are used for standards and controls, others are for test compounds



Positive control (cells should produce dopamine)*

Negative control (no cells, no dopamine present)*

*For more on controls and positive/negative results see the Commentary file posted in Avenue.

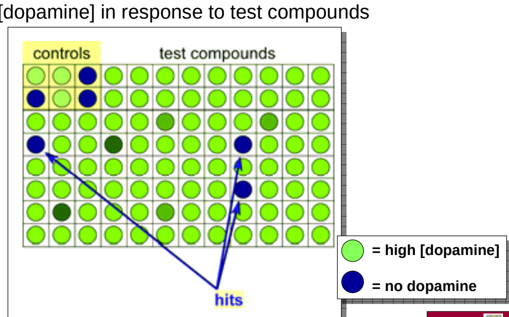
High-throughput screening

- screening must be able to test thousands of compounds per day, therefore it must be automated using robots and microtiter plates

McMaster HTS Facility

High-throughput screening

- grow neural cell cultures in 96-well plates, measure [dopamine] in response to test compounds



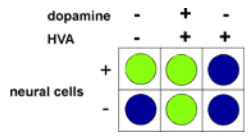
High-throughput screening

Key concepts

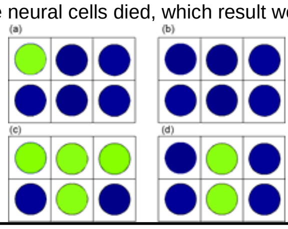
- high throughput assays that replicate complex biological phenomena
- require many compounds (more on this later)
- use microtiter plates
- highly automated

iClicker Question #1

Control wells in the dopamine assay normally look like this, where wells with high [dopamine] are **green:**



If all the bee neural cells died, which result would be observed?

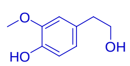


High-throughput screening

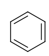
Now we need compounds to feed into our assay…

Aromaticity

- one of the most notable features of HVA is that it is an aromatic compound, like benzene



homovanillyl alcohol (HVA)



benzene

- Ch. 26-6 (10th edition)
- Ch. 26-4 & 26-9, but <u>not</u> "Aromatic substitution reactions" (9th edition)

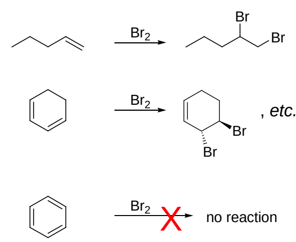
Aromaticity

- benzene looks like "cyclohexatriene", but it does not behave like an alkene

typical alkene

cyclohexadiene

benzene



- aromatic systems are very stable, less reactive than alkenes

Aromaticity

<u>Recognizing aromatic compounds:</u>

- Cyclic
- Planar
- all sp^2 (or sp) in ring
  - all the atoms (C, N, O) in the aromatic ring system must have a p orbital available
- conjugation
  - Often see a pattern of alternating single & double bonds in line drawings
  - not always completely followed for heteroaromatics or ions
- Hückel 4n+2 rule (slide 19)

Aromaticity

- benzene is planar, cyclohexane is bent

benzene (always planar)

cyclohexane (chair conformation) (boat conformation)

Aromaticity

Recognizing aromatic compounds:

- Hückel 4n + 2 rule

- aromatic systems have (4n + 2) π-electrons, n = 0,1,2,3...

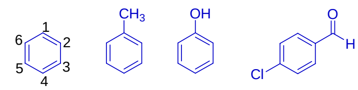
- a π-electron is an electron engaged in a π-bond; there are 2 π-electrons per π-bond

- e.g., benzene has 6 π-electrons, so n = 1

- The p-orbitals of the conjugated atoms are all aligned perpendicular to the ring plane, and the total number of electrons in these p-orbitals meets the Hückel rule

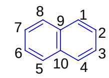
Aromaticity

- other examples of aromatic compounds:



benzene toluene phenol 4-chlorobenzaldehyde (*p*-chlorobenzaldehyde)

6 π-electrons

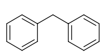


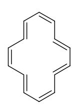
naphthalene (10 π-electrons)

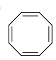
Note: we are <u>not</u> necessarily considering the *total* number of π- electrons in the molecule

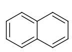
iClicker Question #2

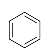
Which one of the following compounds does not satisfy the rules for aromaticity?

(a)


(b)


(c)


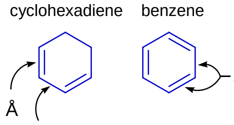
(d)


(e)


Aromaticity

- C-C bonds are all equivalent

cyclohexadiene benzene



1.32 Å

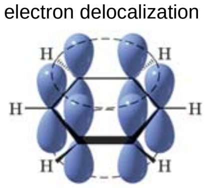
1.53 Å

1.39 Å

Aromaticity

What is special about aromatic compounds?

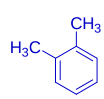
- π-electrons are delocalized
  - electron delocalization is very energetically favourable



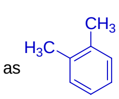
- π-bonds form one big molecular orbital

Aromaticity

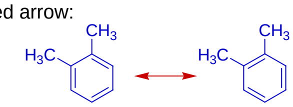
- Kekulé structures:



is the same compound as



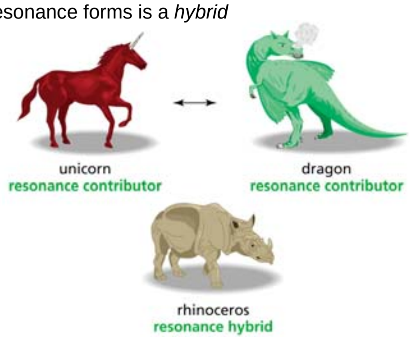
- we represent these two resonance forms with a two-headed arrow:



- resonance forms are the *same molecule* drawn with different bonds: electrons appear to move, but the atoms do not move

Aromaticity

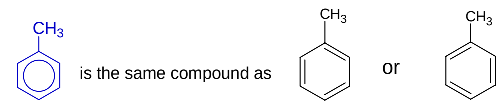
- neither resonance form is “correct”; the sum of resonance forms is a *hybrid*



Aromaticity

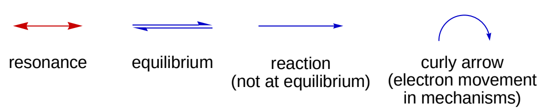
Drawing aromatic compounds

- can draw the resonance hybrid as a circle to represent conjugated π-bonds



Aromaticity

- N.B. - don't confuse different kinds of arrows:



Aromaticity

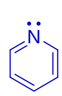
Heterocyclic compounds

- Aromatic compounds can have heteroatoms (e.g. O, N, S) in the ring
- Hückel's rule (4n + 2 π-electrons) still applies
- The heteroatoms contribute electrons to the π-system
  - Sometimes heteroatoms have lone pairs of electrons that contribute to the π-system

Aromaticity

Heterocyclic compounds

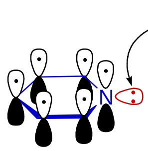
Examples:



pyridine

The ring is aromatic, as in benzene.

The two C-N bond lengths are the same.

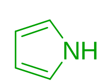


This sp^2-hybridized orbital is perpendicular to the six 2p-orbitals of the π-system.

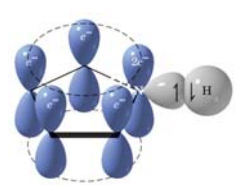
It is not part of the 4n+2 π-electron system; neither are its 2 electrons.

Aromaticity

Heterocyclic compounds



pyrrole



The N atom is **sp^2** hybridized!

The two electrons in the N p-orbital do contribute to the π-system, making the ring aromatic.

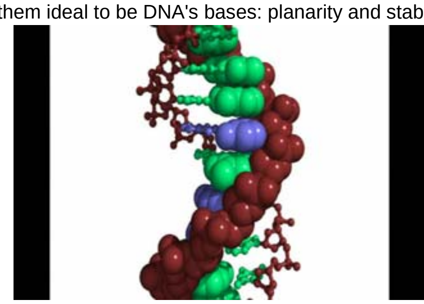
WHY?

- There is a strong driving force toward aromaticity.
- Aromatics are more stable.

Aromaticity

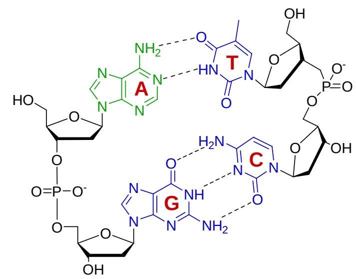
<u>Heterocyclic compounds</u>

- two of the characteristics of aromatic compounds makes them ideal to be DNA's bases: planarity and stability

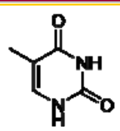


Aromaticity

<u>Nucleic acid bases are aromatic!</u>



Heterocycles – Nucleic acid bases are aromatic!



Thymine

Cytosine

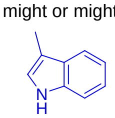
http://en.wikipedia.org/wiki/File:Thymine_chemical_structure.png

http://upload.wikimedia.org/wikipedia/commons/1/10/Cytosine_chemical_structure.png

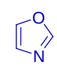
Take-Home Problem: Aromaticity

Are these compounds aromatic?

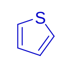
Rationalize whether they might or might not be, based on the criteria for aromaticity.



skatole



oxazole



thiophene

<u>Skatole</u> has 10 π-electrons. The N atom is sp^2-hybridized & contributes 2 π-electrons, as it does in pyrrole.

The N atom in <u>oxazole</u> contributes 1 π-electron, like the N atom in pyridine. The O atom is sp^2-hybridized, with one p-orbital contributing 2 π-electrons, like the pyrrole N atom. The lone pairs of electrons on O and N that are not part of the π-molecular orbital are sp^2-hybrid orbitals, as in pyridine. Overall, there are 6 π-electrons.

In <u>thiophene</u>, we can treat S like an O atom, even though, as a 3rd row element, its electron configuration can sometimes be complicated. It closely resembles the O atom in oxazole. There are 6 p-electrons.

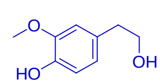
Aromaticity

Key concepts

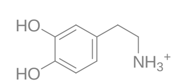
- cyclic, conjugated system (all rings atoms have an available p orbital)
- 4n + 2 π-electrons
- planar
- very stable
- all-carbon rings, or heterocyclic rings

Back to brainwashing bees…

- make many HVA variants, but we want to retain its aromatic character



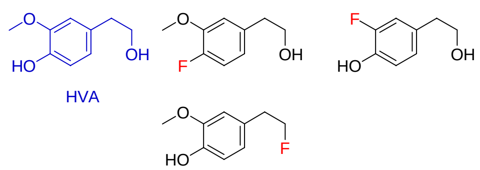
homovanillyl alcohol (HVA)



dopamine

Combinatorial Chemistry

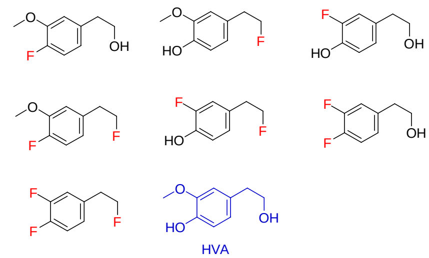
- Combinatorial chemistry... or how do you make 5000 new compounds?



- 4 down (counting HVA), 4996 to go...

Combinatorial Chemistry

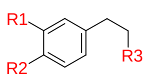
- but if we combine substituents...



- we get 8 compounds by combining the same 3 substituents

Combinatorial Chemistry

- Combinatorial chemistry is mixing-and-matching substituents to create libraries of unique compounds.
- There are 3 diversity sites in HVA, and 2 substituents at each site



R1 = CH_3O, F
R2 = HO, F
R3 = HO, F

- Total number of variants is the product of # substituents at each site:

  2 x 2 x 2 = 8

- OK, 8 down, 4992 to go

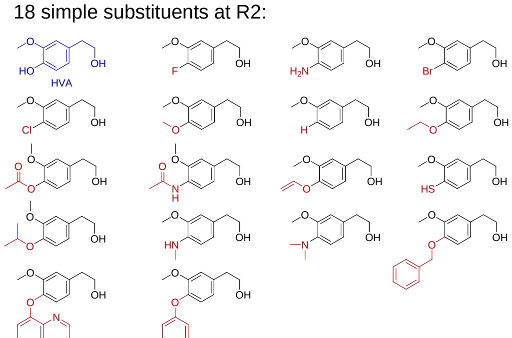
Combinatorial Chemistry

- keep going...
- 18 subsituents at each diversity site will give us:

  18 x 18 x 18 = 5832 unique compounds

Combinatorial Chemistry

- 18 simple substituents at R2:



Combinatorial Chemistry

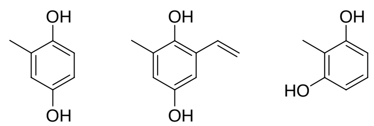
- with 18 substituents each at R1 and R3, we'll have >5800 compounds
- robots do the syntheses

Key concepts

- libraries of compounds
- combinatorial mixing of substituents
- # of compounds = product of # substituents at each diversity site

Diagnostic iClicker Question

How many sites of diversity are there in this combinatorial library?



(A) 1 (B) 2 (C) 3 (D) 4 (E) 5

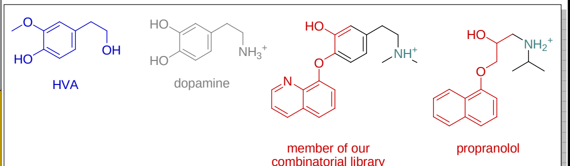
Brainwashing bees, finale

Key concepts for section

- queen bees control workers' memories
- could be useful in understanding memory and/or treating disease
- use chemical biology approaches:
  - high throughput screening - reduce complex behaviour to fast assays
  - use combinatorial chemistry to make many unique compounds
- aromaticity is an important property of organic compounds

Postscript: The Biology of Brainwashing Humans

- We started teaching this section in 2008. A paper in *Nature Neuroscience* in 2009 reported that the hypertension drug propranolol selectively suppresses unpleasant memories (fear) in humans.
- It is not known whether propranolol has any effect on [dopamine] or dopamine responses in the brain.



Kindt *et al.* (2009) *Nature NeuroSci.* **12**:256 - 258. *Beyond extinction: erasing human fear responses and preventing the return of fear*