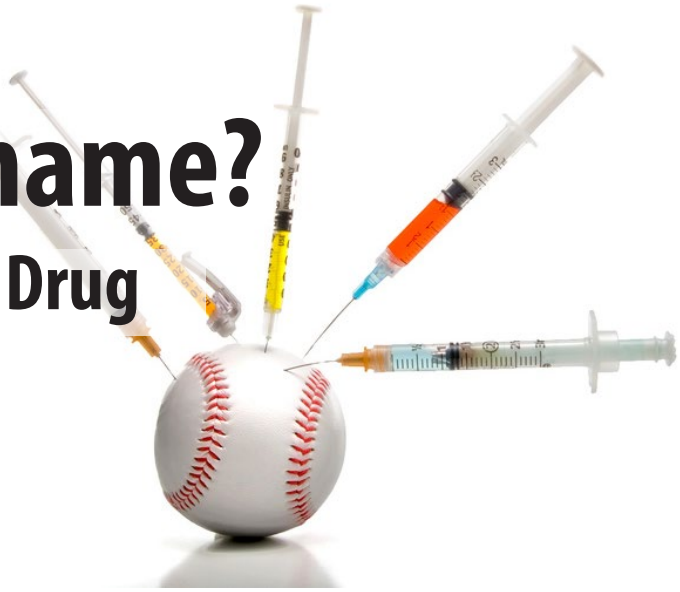
Hall of Fame or Shame?

The Chemistry of a Designer Drug



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Although he's a hero in San Francisco and everyone knows his name, he may be better known for the rules he has allegedly broken than for the sport he once represented. Barry Bonds currently holds the record for hitting the most home runs in baseball history. Nonetheless, his bid for election into the Baseball Hall of Fame was overwhelmingly rejected in 2013 because it was unclear whether talent or chemistry was responsible for the remarkable achievement.

Mr. Bonds was accused of using anabolic steroids to enhance his athletic performance. Investigations into these allegations led to a pharmaceutical company called Bay Area Laboratory Co-Operative (BALCO).* BALCO has been accused of acting as a supplier of steroids to many professional athletes, primarily baseball players. The drugs allegedly distributed by BALCO to athletes included erythropoietin, human growth hormone, modafinil, testosterone cream, and tetrahydrogestrinone (THG). Each of these drugs has different effects, ranging from anabolic effects to sense stimulation. We will focus our attention on THG.

THG is a designer drug engineered by organic chemist Patrick Arnold. Mr. Arnold ultimately pleaded guilty to one count of conspiracy to distribute steroids. Designer drugs are new molecules, which have the same or similar biological properties as existing drugs. These drugs are usually unknown to law enforcement agencies since there are no analytical methods to detect them or their effects, something that works for athletes who want to be on the "clear" while being subjected to rigorous and periodic drug testing. Since THG is derived from similar drugs, it is referred to as a "derivative drug." THG is one of the most effective anabolic steroids developed. The drug can produce the same effects as testosterone (another anabolic steroid) with a much smaller dose. Some studies report that THG is as much as ten times more potent than testosterone.

Read the following article:

> Catlin, D.H., M.H. Sekera, B.D. Ahrens, B. Starcevic, C. Yu-Chen, and C.K. Hatton. 2004. Tetrahydrogestrinone: discovery, synthesis, and detection in urine. *Rapid Communications in Mass Spectrometry* 18(12): 1245–1249. PUBMED: PMCID: 15174177, http://www.ncbi.nlm.nih.gov/pubmed/15174177 (subscription required).

Based on your reading of the article and your own research, answer the following questions.

Questions

1. The UCLA Olympic Analytical Laboratory determined the structure of THG. How did this laboratory obtain the sample?

* For more information about BALCO, see the Bay Area Laboratory Co-Operative (BALCO) Navigator: A list of resources from around the Web about Bay Area Laboratory Co-Operative (BALCO) as selected by researchers and editors of *The New York Times:* http://topics.nytimes.com/top/reference/timestopics/organizations/b/bay_area_laboratory_cooperative/index.html (accessed January 27, 2015).

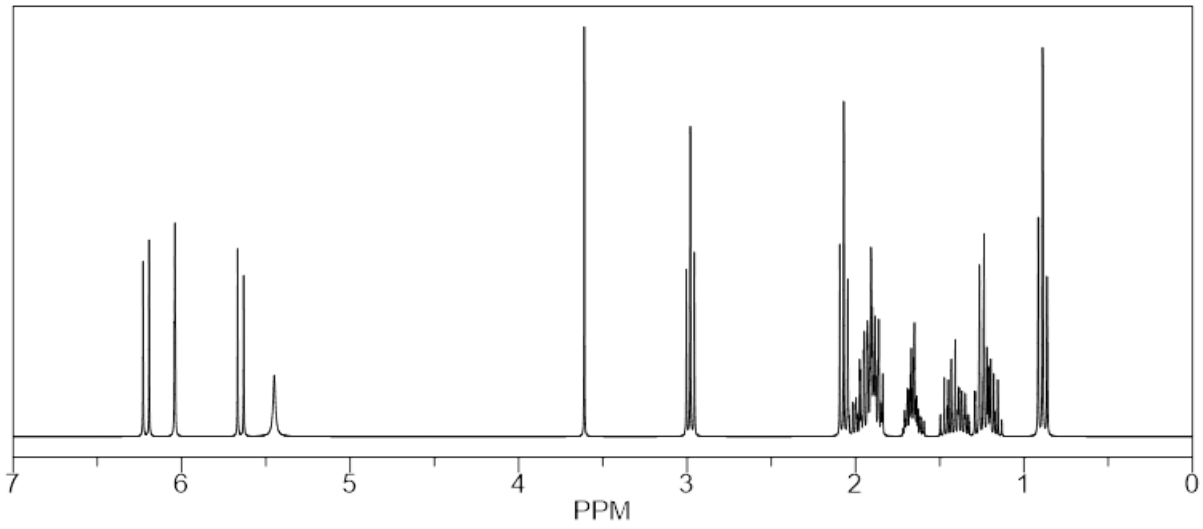
2. The first test was performed by MS and by HRMS (these were coupled either to LC-liquid chromatography or GC-gas chromatography, but don't worry about that just yet). What do MS and HRMS stand for? What information was obtained from these methods?
3. The authors of this paper (Catlin *et al.*, 2004) had some suspicions regarding the structure of the drug based on the HRMS analysis. They had to confirm their suspicions by synthesis. Describe the one-step synthesis that was performed. How would you classify this reaction? How would this change the results of the MS and HRMS analysis?
4. Once the authors synthesized what they believed to be the drug in question, the product had to be fully characterized to confirm the structure. One method of characterization is ^{1}H NMR (or proton NMR). What type of information does this technique provide?
5. Steroids are defined by their characteristic structures. What is the important characteristic that classifies a compound as a steroid?
6. How do the male sex hormones differ from the female sex hormones? Use estradiol and testosterone as examples.
7. Define the meaning of the term *anabolic*. What do we mean when we refer to anabolic steroids?
8. Mr. Bonds was convicted on a single count of obstruction of justice in 2011. After all this time, he never admitted to using steroids, nor was he definitively proven to have used steroids knowingly. Do you think he deserves to be elected to the Baseball Hall of Fame? Explain your reasoning.

Advanced Questions

9. The *nominal* mass of a compound is its molecular weight calculated using the atomic masses of constituent elements taken as integers. Calculate the nominal mass for compounds having molecular formula of:
   a. $C_{21}H_{28}O_2$ (i.e., THG)
   b. $C_{20}H_{24}O_3$
   c. $C_{22}H_{16}O_2$

   The *exact* mass of a compound is calculated from the masses of the most abundant isotopes of each element. For C, H and O these are C = 12.00000, H = 1.007825, and O = 15.994915. Calculate the exact mass (to four decimal places) of the following:
   d. $C_{21}H_{28}O_2$ (i.e., THG)
   e. $C_{20}H_{24}O_3$
   f. $C_{22}H_{16}O_2$

   The resolution of an instrument is defined as $M/\Delta M$. What resolution would be required to distinguish these compounds? What was the resolution of the high resolution instrument described in the paper (Catlin *et al.*, 2004)? What is the advantage of a high resolution instrument?
10. Mass spectrometry not only provides information about the molecular weight of a compound, it also provides structural information from inspection of the fragment ions. Compare the mass spectra of gestrinone and tetrahydrogestrinone in Figure 4 of the paper. Some of the smaller fragments are identical in the two mass spectra and the larger fragments differ by 4 m/z. Explain this observation. The largest fragment in the two spectra which are the same appears to be an m/z of 240. Read the second paragraph under the Discussion section. Suggest a way that these molecules might fragment to produce this ion.
11. The ^{1}H NMR of gestrinone is provided below (next page). Identify the vinyl and acetylinic proton resonances based on their chemical shift and coupling. Identify the protons in gestrinone which should appear as triplets. Try to assign the two triplets at δ0.9 ppm (integrates for 3 H) and δ2.99 ppm (integrates for 2 H), based on their chemical shift. How would you anticipate this ^{1}H NMR spectrum would change upon catalytic hydrogenation to tetrahydrogestrinone? Examine the ^{1}H NMR data for tetrahydrogestrinone given on page 1247 of the paper. Do your predictions match the experimental observation?



12. Three reactions are described in this paper: heterogeneous catalytic hydrogenation, silylation of an alcohol and oxime formation from a ketone and hydroxylamine. Draw the mechanisms for these three reactions.
13. How did performing these reactions support the conclusions regarding the structure of the unknown compound?

Presentation

Prepare a PowerPoint presentation for your classmates. Describe the background information and introduction to the problem faced by the UCLA Olympic Analytical Laboratory. Explain the two techniques of mass spectrometry and proton NMR and the type of data which can be obtained from them. This should be followed by answers and explanation for each of the questions. For this case, explain how the chemists solved the molecular structure of this unknown steroid. In particular, describe their logic and their techniques.