Microchemical Identification of Gamma-Hydroxybutyrate (GHB)

Introduction

Gamma-hydroxybutyrate (GHB) was recently made a controlled substance in California, obligating criminalistics laboratories to provide conclusive identification of suspected samples. Unfortunately, the nature of the compound complicates this task. Problems such as impure samples, need for derivatization for chromatography, conversion of the sample to and from the precursor during analysis, and the extremely hygroscopic nature of the drug all make identification by instrumental techniques difficult. The authors have developed a microcrystal reagent that can overcome some of these problems.

GHB SUMMARY

Chemistry

The precursor for the manufacture of gamma-hydroxybutyrate is Gamma-Butyrolactone (GBL), a clear viscous liquid at room temperature with a boiling point of 204°C, used industrially as a solvent. GHB is clandestinely manufactured by the basic hydrolysis of GBL using sodium hydroxide. After a few hours of heating this mixture, the pH is brought down to neutral with hydrochloric acid. GHB in its pure form is a white crystalline solid with a melting point of 145 °C, but it is extremely hygroscopic and if left exposed to air will quickly become colorless as it is saturated with water. It is usually encountered as a concentrated aqueous solution. GHB and GBL exist in equilibrium with each other, with the value of K dependent on the pH (Fig. 1). At pH 14 most of the GBL will be hydrolyzed to GHB, while at pH 0 the reverse is true. Both compounds are stable at neutral pH. There is some indication that buffered acid solutions can still produce GHB from GBL.

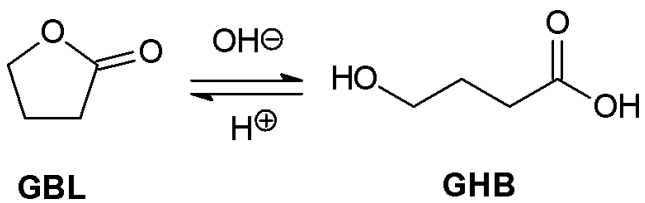

Figure 1.

Scheduling

GHB was made a Schedule II controlled substance in California in July of 1997. It has been made a Schedule I or II drug in several other states, but is not currently a federally scheduled controlled substance. GBL is not a controlled substance anywhere, and in fact is a fairly common industrial chemical. It is important to keep in mind when analyzing GHB that it always exists in equilibrium with its (legal) precursor, and that altering the pH of the sample can create or destroy GHB. This means that pH based extractions cannot be used on GHB samples.

Current Methods of Analysis

There are currently three color tests for GHB. There is a chromic acid color test, but it is a general test for alcohols. The ferric chloride color test for GHB works quite well, but it also gives a reaction with hydroxide ion which may be present in clandestinely manufactured GHB. The cobaltous nitrate color test works well on solid samples, but not on liquids. FTIR can distinguish between GHB and GBL to give a positive identification, but because of the hygroscopic nature of the drug obtaining a good spectrum can be difficult. (Adding a liquid sample directly to ground KBr, drying it in a microwave oven and pressing a pellet works quite well.) Since pH based extractions cannot be used to cleanup GHB samples, spectral interference from other substances (such as the ingredients in a drink that might have had GHB added to it) could be a problem. GC / MS will not give a positive ID without derivitization of the sample; simply shooting GHB will result in it degrading to GBL (probably from the heat of the injection port). BSTFA can be used to make a derivative of GHB that is stable on the column, and can be used for identification. However, the chemicals involved are toxic and water reactive, and the derivitization procedure is time consuming. Additionally, since GHB exists in equilibrium with GBL, even pure GBL will yield a small GHB-BSTFA peak.

MICROCRYSTAL TEST

History

Due to the limitations of other methods for analyzing GHB, we looked for a quick, inexpensive and accurate alternative: microcrystal tests. We began by testing all of the microcrystal tests and color tests in use at the San Bernardino lab with no results. Then Evans found a reference for a crystal test for butyric acid, which is structurally related to GHB. A solution of concentrated cupric nitrate should produce hexagonal crystals with butyric acid, and we hoped that it might give some result with gamma-hydroxybutyric acid. At this point serendipity came into play. There was no cupric nitrate at the lab, so I made some by mixing appropriate amounts of silver nitrate and cupric chloride and then filtering off the silver chloride that precipitated out [$2AgNO_3 + CuCl_2 \rightarrow Cu(NO_3)_2 + 2AgCl_{(s)}$]. This solution grew some very nice rectangular crystals with GHB. However, when we finally obtained some cupric nitrate from Sigma, I was unable to duplicate these crystals with a solution of pure $Cu(NO_3)_2$, while the makeshift reagent still worked. Speculating that there may have been some excess silver ions remaining in the *ad hoc* cupric nitrate solution, I mixed an equal amount of silver nitrate into the pure cupric nitrate solution. This solution grew the same rectangular crystals as the *ad hoc* solution. The final formulation of the crystal reagent was 100 milligrams of $AgNO_3$ and 100 milligrams of $Cu(NO_3)_2$ in 10 milliliters of water. (This is a 1% w / v mixture of silver nitrate and cupric nitrate.) The best technique for growing the crystals is to combine a drop of the reagent and a drop of GHB in aqueous solution via a "neck" without a coverslip. The crystals grow at

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Winner, Most Outstanding Presentation, Spring Seminar, Oakland 1999. Presented by Kevin Andera (left), Orange Co. Sheriff-Coroner Forensic Science Svcs. Based on research by Kevin Andera, Hiram Evans and Cathy Wojcik at the San Bernardino Co. Sheriff Scientific Investigations Division.

the edges of the drop in under 5 minutes (Fig 2). They are best viewed using a polarized light microscope (Fig 3).

Testing

The most important aspect of testing was selectivity, to see if this reagent actually could distinguish GHB from other substances. First we made sure that the crystals obtained on drying the reagent (Fig 4) are different from the crystals obtained from GHB. Next the reagent was tested by Wojcik against 22 controlled substances. These included commonly encountered drugs (e.g. methamphetamine, cocaine, etc.), drugs similar to GHB in effect (e.g. flunitrazepam, barbiturates, etc.), two structural isomers of GHB (alpha-and beta-hydroxybutyrate), and the GBL precursor. (Note that the barbiturates are sodium salts of acidic drugs, as was the GHB we used.) Most of these substances gave no crystals at all, and none of them produced crystals similar to those grown with GHB. The reagent is specific to GHB, doesn't react with GBL, and the reagent crystals are not easily mistaken for a positive result. The next step was to set up a blind test to see how useful the reagent would be for unknown samples. Ten samples, including controlled and non-controlled chemicals, were prepared by Andera and tested by Wojcik. There were two positive identifications, one false negative and no false positives. Since no samples were mistakenly identified as GHB, and two of the three GHB samples were correctly identified, we concluded that the reagent is selective for GHB. (The missed GHB sample may have been due to an interfering precipitate.) The final stage of testing was determining the sensitivity of the reagent. Using a GHB standard obtained from Sigma, we prepared serial dilutions ranging in concentration from 125 mg / ml down to 2 mg / ml. Crystals formed in under 5 minutes with concentrations down to 4 mg / ml, and no crystals were observed after 10 minutes at 2 mg / ml. Note that 125 mg / ml is about a 1 molar solution, while most clandestine recipes for GHB yield a 6 to 12 molar concentration. Sensitivity can also be affected by the presence of negative ions or the precursor, GBL. Silver ions form precipitates with Cl^- and OH^-, both of which might be present from the manufacturing process. These precipitates may interfere with or mask the crystal growth, and are not easily removed. While GBL does not form crystals or precipitates with the reagent, its presence does inhibit crystal formation. However, it can be removed from solution by a few simple washes with toluene or chloroform.

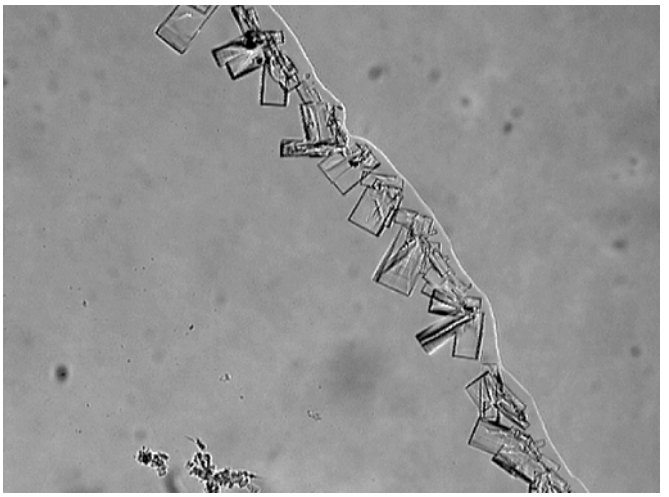

Figure 2.

Figure 3.

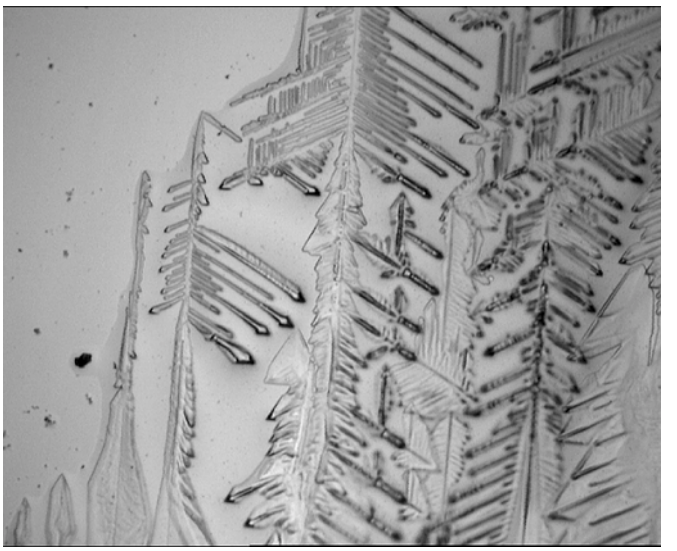

Figure 4.

CONCLUSION

The crystal test described here is a fast, accurate and inexpensive method of testing for GHB. If the limitations of interference and sensitivity are kept in mind, it should provide a positive identification of GHB, especially if used in combination with one of the instrumental methods described above. Additional testing of this reagent will include its utility on GHB in ethanol solutions, non-neutral solutions, and on any other analogs of GHB that come to the attention of law enforcement in the future. A more detailed version of the information presented here will be published in the *Journal of Forensic Sciences*, tentatively scheduled for May 2000.

Endnotes:

1. As presented at the 93rd Semi-Annual CAC Seminar, Oakland, CA; May 1999
2. Photomicrographs by Wayne Moorehead, Orange County Sheriff-Coroner.