TECHNICAL NOTE

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Microcrystalloscopic Differentiation of 3,4-Methylenedioxyamphetamine and Related Amphetamine Derivatives

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ABSTRACT: Commonly used gold chloride and its diluted forms are compared with gold bromide for potential microcrystalloscopic differentiation of 3,4-methylenedioxyamphetamine (MDA) and related amphetamine derivatives. The crystal formation characteristics of MDA, mescaline, and 2,5-dimethoxy-4-ethylamphetamine (DOET) with diluted and undiluted gold chloride allow differentiation of these drugs, while 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA) form similar crystals and are not easily differentiated. The compounds 2,5-dimethoxy-4-methylamphetamine (DOM) and *N*-hydroxy-MDA were soluble in the gold chloride reagents, and no crystals were formed.

KEYWORDS: toxicology, analogs, 3,4-methylenedioxyamphetamine, crystallography, microcrystalline test

Of the various drug testing approaches introduced prior to the routine use of mass spectrometry, microcrystalline tests are considered reasonably sensitive and specific and continue to enjoy popularity in laboratories where the acquisition of major instrumentation is difficult. In these tests, related compounds tend to form similar crystals with the same reagent [*1*]. Microcrystalline tests are, at present, used mostly as preliminary measures, requiring additional confirmatory testing procedures, such as mass spectrometry methods of analysis. With the limited availability of training programs on microscopic methods of analysis, the easy access to various instrumentation, and the emphasis on "instrumental" methods, developments in methods based on microcrystalline tests are scarce and rarely reported. However, in the hands of properly trained personnel, microcrystalline tests are ideal for providing quick, effective, economical, and reliable screens for a limited number of samples. Thus, they have been utilized in the analysis of a variety of drugs [*2–4*].[2]

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This project explores the possibility of utilizing commonly used gold chloride and closely related reagents for the differentiation of 3,4-methylenedioxyamphetamine (MDA) and related amphetamine derivatives. Since many of these compounds are only now being encountered by crime laboratories, dissemination of the characteristics of their crystal formations will aid forensic drug chemists in analyzing unknown drug samples and interpreting test results.

Materials and Methods

Reagents and Drug Standards

The sources of the reagents and drugs used were the following: for gold chloride, Sigma Chemical Co. (St. Louis, Missouri) and Fisher Scientific (Atlanta, Georgia); for gold bromide, Fluka Chemical (New York, New York).

The compounds 2,5-dimethoxy-4-ethylamphetamine (DOET) and 2,5-dimethoxy-4-methylamphetamine (DOM) were purchased from Supelco (Bellefonte, Pennsylvania). All other drug standards, including MDA, *N*-hydroxy-MDA, mescaline, 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), were obtained from the Forensic Science Program, University of Alabama at Birmingham (UAB) (Birmingham, Alabama), Dr. John Beaton of the UAB Neuropsychiatry Program, and F. Taylor Noggle, Jr., of the Alabama Department of Forensic Sciences (Birmingham, Alabama).

An Olympus BH-2 polarizing microscope, with a Nikon 35-mm camera with a macro lens attachment was used for microcrystalline observation and recording. For this study, however, a polarizing microscope was not necessary.

Procedure

The gold chloride reagent was prepared by dissolving 1 g of chloroauric acid in 20 mL of dilute phosphoric acid (H_3PO_4). (The H_3PO_4 was diluted 1:3 in water.) This solution was then used to prepare working solutions in water and H_3PO_4 media at both 1:5 and 1:10 dilution levels.

The gold bromide reagent was prepared by dissolving 1 g of gold bromide in 28.5 mL of dilute sulfuric acid (H_2SO_4). (The H_2SO_4 was diluted 2:5 in water.) This solution was further diluted 1:3 in H_3PO_4 to obtain the working solution.

The crystal formations were obtained by placing a small amount of the sample on a slide and adding one drop of the selected reagent. The slide was immediately examined microscopically for any crystal formation under polarized light using $\times 10$ magnification with a dark field. A strong light source was used for the analysis.

Analyses were done using both undiluted and diluted gold chloride reagent and undiluted gold bromide reagent solutions. Duplicate analyses were performed to assure the identification and reproducibility of the crystal formation.

Photomicrographs of the crystal formations were taken using a 35-mm camera attached to the microscope. Ektachrome slide film (ASA 160) was used at automatic exposure settings and at ± 1 f-stop settings.

Results and Discussion

In recent years, MDA and its derivatives, particularly MDMA (Ecstasy) and MDEA (Eve), have been encountered by crime laboratories in increasing numbers. Numerous studies on the synthetic characteristics and the analytical approaches to these "amphetamines" have been reported [*5–10*].

Gold chloride reagent is the preferred reagent [*6,11*] for microcrystalloscopic identification of amphetamines and related compounds. This study explores the potential differences in the crystal formations of various amphetamines when using gold bromide and gold chloride at two dilution levels in water and H_3PO_4.

The chemical structures and most identifiable crystal formations of the drugs studied are shown in Figs. 1 and 2 and in Tables 1 and 2. Photomicrographs of the crystals described in these tables are further shown in Fig. 1.

Of the seven compounds tested, two gave negative results with all of the reagents because of their solubility in these reagents. DOM and *N*-hydroxy-MDA dissolved in the test reagent, while the other MDA and the MDA derivatives formed crystals. This allows quick exclusion of *N*-hydroxy MDA and DOM because of their solubility. No further conclusion was drawn from this phenomenon because of the many factors that affect crystal formation. The remaining four compounds gave positive results with at least one of the reagents used.

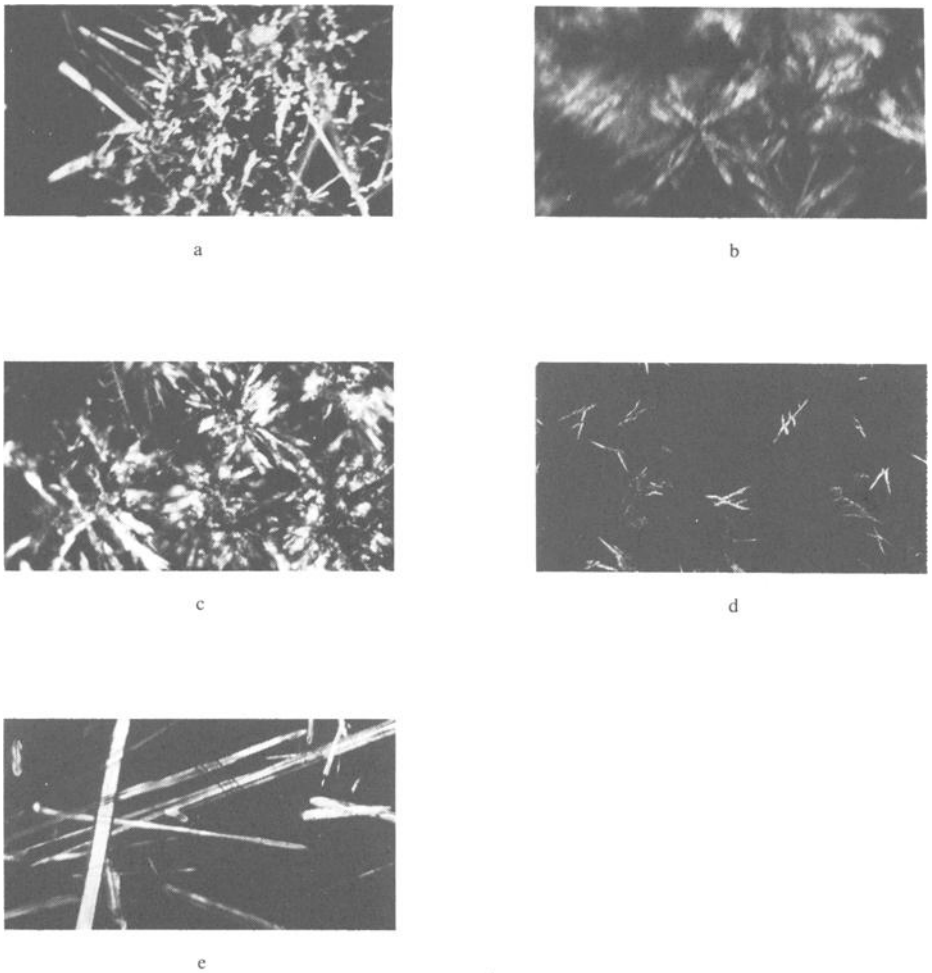

FIG. 1—*Most identifiable crystal formations for* (a) *3,4-methylenedioxyamphetamine,* (b) *3,4-methylenedioxymethamphetamine,* (c) *3,4-methylenedioxy-*N*-ethylamphetamine,* (d) *mescaline, and* (e) *2,5-dimethoxy-4-ethylamphetamine.*

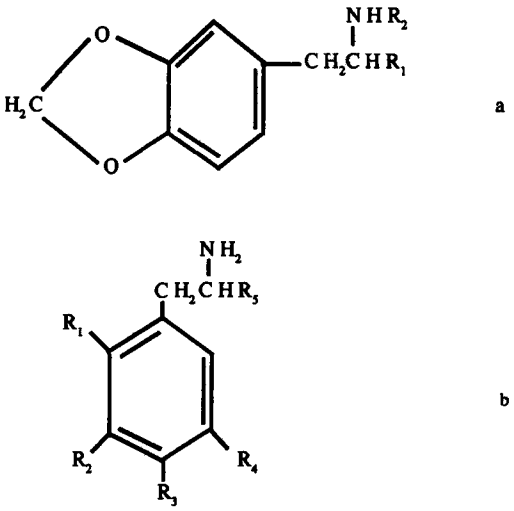

FIG. 2—*Structural frameworks for* (a) *methylenedioxyamphetamines (Table 1) and* (b) *amphetamines (Table 2).*

MDA formed crystals only with undiluted gold chloride, while MDMA and MDEA formed similar crystals with all three concentration levels (in both water and H_3PO_4) of gold chloride. Mescaline and DOET formed crystals with undiluted gold chloride, gold bromide, and H_3PO_4-diluted gold chloride reagents. Only the most identifiable crystal formations are listed in Tables 1 and 2.

Conclusions

These results indicate that it is possible to differentiate MDA, mescaline, and DOET based on their crystal formations in diluted reagents. DOM and *N*-hydroxy-MDA are easily distinguished from the other derivatives because of their solubility in both gold chloride and gold bromide. MDMA and MDEA could be distinguished from the other compounds; however, their similar crystal formations left them indistinguishable from each other. It should be noted that the results obtained from microcrystal tests are subjective in the description of the crystal formations.

Microcrystalline tests have been known to be pH sensitive. At different pH values, the reaction between the sample and the test reagent yields different crystal formations. This study shows that, by utilizing the reagents at various dilutions in water and acid, improvement in differentiation can be achieved.

Basic guidelines for crystal shape descriptions have been set forth by Stewart and Stolman [*13*] and by Fulton [*6*]; however, experience is an important factor in distinguishing these crystal formations and in the subsequential identification of the drugs tested.

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TABLE 1—*Chemical structures and crystal formations of 3,4-methylenedioxyamphetamine derivatives.*

Name	R_1	R_2	Most identifiable crystal and reagent	Figure	Drawing
3,4-Methylenedioxy-amphetamine (MDA)	CH_3	H	Yellow wheat sheaths; gold chloride;	1a	
3,4-Methylenedioxy-methamphetamine (MDMA)	CH_3	CH_3	Yellow burrs; gold chloride;	1b	
3,4-Methylenedioxy-N-ethyl-amphetamine (MDEA)	CH_3	CH_2CH_3	Yellow rosettes; gold chloride;	1c	
N-Hydroxy-MDA	CH_3	OH	------		

TABLE 2—*Chemical structures and crystal formations of amphetamine derivatives.*

Name	R_1	R_2	R_3	R_4	R_5	Most identifiable crystal and reagent	Figure	Drawing
Mescaline	H	CH_3O	CH_3O	CH_3O	H	Yellow cross; gold chloride (1:5 in phosphoric acid)	1d	
2,5-Dimethoxy-4-methyl-amphetamine	CH_3	H	CH_3	CH_3O	CH_3	-----		
2,5-Dimethoxy-4-ethyl-amphetamine	CH_3	H	CH_2CH_3	CH_3O	CH_3	Yellow needles; gold bromide	1e	

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