Highly Specific and Convenient Color Reaction for Methylenedioxyamphetamine and Related Drugs Using Chromotropic Acid. Application as a Drug Screening Test

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The color reaction of various drugs with chromotropic acid and sulfuric acid was examined. Methylenedioxyamphetamine (MDMA) exhibited a red-violet color on treatment of an aqueous solution with sulfuric acid and chromotropic acid. The specificity of the reaction was examined using 65 drugs. The results showed that while related drugs with a methylenedioxyphenyl moiety, such as methylenedioxy-amphetamine and N-ethylamphetamine, were all positive, 51 drugs including amphetamine, methamphetamine, opiates, cocaine, and phencyclidine were negative. The drugs exhibiting a positive reaction include tetrahydrocannabinol and related drugs, sulfur-containing drugs such as phenothiazine tranquilizers, and diphenhydramine. These results indicate that the present method is highly specific for MDMA and related drugs although its specificity is not absolute. The detection of MDMA in urine was investigated, and found to be possible using this method.

Key words — methylenedioxyamphetamine, chromotropic acid, color reaction, specificity, drug screening

INTRODUCTION

Abuse of methylenedioxyamphetamine (MDMA), slang name 'Ecstasy,' and related drugs is becoming a serious problem worldwide, especially in Europe and North America. Immunochemical screening using anti-methamphetamine (MP) antibody detects these compounds, but it cannot distinguish between MDMA and MP. The Simon reaction, a color reaction for the secondary amine structure, also cannot distinguish between them. Thus, no specific method for the detection of MDMA and MP has been established yet. This study focused on this problem, and we examined the utility of a color reaction using chromotropic acid for MDMA and related drugs. An early study reported that compounds with the methylenedioxyphenyl structure can be detected by the color developed following reaction with sulfuric acid and chromotropic acid, although MDMA and related drugs were not examined at that time. The reaction involves two steps: 1) release of formaldehyde from the compound by sulfuric acid and 2) formation of a color complex between formaldehyde and chromotropic acid in the presence of sulfuric acid. We examined the utility of this reaction as a screening test for MDMA.

MATERIALS AND METHODS

Materials — MDMA and related drugs having a methylenedioxyphenyl structure were supplied by the Ministry of Health and Welfare, Japan. MP was purchased from the Dainippon Pharmaceutical Co., Osaka, Japan. The following drugs were kindly donated by the sources indicated: amphetamine (AP) from Dr. Yanagita, Central Institute of Experimental Animals, Kawasaki, Japan; benzphetamine from the Upjohn Co., Kalamazoo, MI, U.S.A.; deprenyl from Fujimoto Pharmaceutical Co., Osaka, Japan; and
Table 1. Specificity of Drug Screening Method Using Chromotropic Acid–Sulfuric Acid\(^a\)

<table>
<thead>
<tr>
<th>Result</th>
<th>Drug</th>
<th>Drug</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive(^b)</td>
<td>MDA</td>
<td>MDMA</td>
<td>MDEA</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>THC</td>
<td>Parahexyl</td>
</tr>
<tr>
<td></td>
<td>Promazine</td>
<td>Diphenhydramine</td>
<td>Promazine</td>
</tr>
<tr>
<td></td>
<td>Perphenazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>Amphetamine</td>
<td>Methamphetamine</td>
<td>Ethylamphetamine</td>
</tr>
<tr>
<td></td>
<td>(\rho)-OH-Methamphetamine</td>
<td>PMA</td>
<td>DMA</td>
</tr>
<tr>
<td></td>
<td>DOM (STP)</td>
<td>DOET</td>
<td>TMA</td>
</tr>
<tr>
<td></td>
<td>Phenetermine</td>
<td>Phenylpropanolamine</td>
<td>Ephedrine</td>
</tr>
<tr>
<td></td>
<td>Benzphetamine</td>
<td>Fenethyline</td>
<td>Deprenyl</td>
</tr>
<tr>
<td></td>
<td>Fenproporex</td>
<td>Fenylamine</td>
<td>Cathinone</td>
</tr>
<tr>
<td></td>
<td>N-methylnorex</td>
<td>Phenmetrazine</td>
<td>Phendimetrazine</td>
</tr>
<tr>
<td></td>
<td>Rolicycline</td>
<td>Phenylcyclohexane</td>
<td>DMT</td>
</tr>
<tr>
<td></td>
<td>Psilocine</td>
<td>Atropine</td>
<td>Cocaine</td>
</tr>
<tr>
<td></td>
<td>Codeine</td>
<td>Heroin</td>
<td>Caffeine</td>
</tr>
<tr>
<td></td>
<td>Methaqualone</td>
<td>Mecloqualone</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine</td>
<td>Imipramine</td>
<td>Desipramine</td>
</tr>
<tr>
<td></td>
<td>Amitriptyline</td>
<td>Diazepam</td>
<td>Nortriazepam</td>
</tr>
</tbody>
</table>

\(^a\) Reaction was performed using 0.1 mM and 1 mM drug. \(^b\) The color for methylenedioxyamphetamine (MDA, MDMA, MDEA, N-OH-MDA and MDMA) and tetnyclicidine was red-violet, while the other drugs showed different colors: THC, parahexyl and DMHP, red; diphenhydramine, yellow; promazine, orange; chlorpromazine, promethazine and perphenazine, pink.

Abbreviations used: MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; MDMA, 5-methoxy-3,4-methylenedioxyamphetamine; THC, tetrahydrocannabinol; DMHP, 3-(1,2-dimethylbicyclo[2,2,1]hept-7-en-2-yl)-7,8,9,10-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran-1-ol; PMA, 4-methoxyamphetamine; DMA, 2,5-dimethoxyamphetamine; DOB, 2,5-dimethoxy-4-bromoamphetamine; DOM, 2,5-dimethoxy-4-methylamphetamine; DOET, 2,5-dimethoxy-4-ethylamphetamine; TMA, trimethoxyamphetamine; DMT, N,N-dimethoxytryptamine; DET, N,N-dimethyltryptamine.

prenylamine from Sanwa Chemical Co., Nagoya, Japan. Morphine and codeine were obtained from Takeda Pharmaceutical Co., Osaka, Japan. Heroin was synthesized in our laboratory by acetylation of morphine. \(p\)-Hydroxy-MP and -AP were prepared by a method described elsewhere.\(^c\) The other drugs listed in Table 1, which are controlled in Japan, were supplied by the Ministry of Health and Welfare, Japan. The other uncontrolled drugs were purchased from commercial sources or supplied by the Laboratory of Pharmacology in our Faculty. Chromotropic acid was purchased from Dojindo, Kumamoto, Japan. Bond Elut Certify was obtained from Varian (Harbor City, CA, U.S.A.).

Color Reaction of Drugs with Chromotropic Acid — In a typical reaction, 1 ml aqueous solution of test drug (0.1 or 1 mM) was mixed with 100 \(\mu\)l 10% (w/v) chromotropic acid, followed by 3 ml sulfuric acid. The red-violet color developed on adding the reagents was examined visually, or the absorbance at 570 nm was measured. Although heating the solution is not always necessary for development of color, the reaction mixture was heated at 100°C for up to 30 minutes in many cases in the present study. In some experiments, MDMA was added to drug-free human urine, and the urine was applied to a Bond Elut Certify column which was pretreated with 2 ml each of methanol and 0.1 M potassium phosphate (pH 6.0). The column was washed with 1 ml 1.0 M acetic acid and 6 ml methanol, and the MDMA retained on the column was eluted with 2 ml 2% (v/v) ammonia in ethyl acetate. The solvent was removed under a stream of nitrogen, and the residue was dissolved in 50 \(\mu\)l water followed by addition of 5 \(\mu\)l 10% chromotropic acid and 150 \(\mu\)l sulfuric acid. The solution was heated in a boiling water bath for 10 min to develop the color.

RESULTS

Color Reaction of MDMA and Optimal Conditions

When MDMA was treated with chromotropic acid and sulfuric acid, a red-violet color developed (Fig. 1) while the solution containing MP showed no color under the same conditions (Fig. 1). When sulfuric acid was added first to MDMA solution followed by addition of chromotropic acid, the color development was much weaker than under the above conditions (data not shown). Therefore, the order of add-
Fig. 1. Color Reaction of MDMA with Chromotropic Acid and Sulfuric Acid

Aqueous MDMA and MP solution (1 ml) at the concentration indicated was treated with 100 μl 10% chromotropic acid, followed by 3 ml sulfuric acid. The photograph was taken after addition of reagents and mixing (no heating).

Fig. 2. The Effect of the Amounts of Chromotropic Acid (A) and Sulfuric Acid (B), and Reaction Time and Temperature (C) on the Color Reaction of MDMA

In these experiments, 0.1 mM MDMA solution (1 ml) was treated in different ways. In experiment A, different volumes of 10% chromotropic acid and 5 ml sulfuric acid were added, and the mixture was heated at 100°C for 30 min, followed by absorbance measurement. In experiment B, 100 μl 10% chromotropic acid and different volumes of sulfuric acid were added, and the mixture treated similarly as above. In experiment C, 100 μl chromotropic acid and 3 ml sulfuric acid were added, and the mixture was reacted for the periods indicated at either 100°C or room temperature.

Each plot is the mean of two analyses.

tation of the reagents is important.

To establish the optimal conditions for the MDMA color reaction, the amounts of chromotropic acid and sulfuric acid, and the reaction time and temperature were examined (Fig. 2). Different volumes ranging from 0.01 to 1 ml 10% chromotropic acid added to the reaction solution gave the same absorbance at 570 nm (Fig. 2A). However, because of the rather low absorbance at 0.01 ml, the volume of chromotropic acid solution was set at 0.1 ml. A volume of sulfuric acid of at least 1.5 ml was needed to get maximal absorbance (Fig. 2B). Heating of the reaction mixture gave a somewhat higher absorbance than was observed without heat (Fig. 2C), but, due to the small difference involved, the reaction could be performed without heating.

Specificity of the Color Reaction

Increasing absorbance was observed on increasing the concentration of MDMA up to 500 μM (Fig. 3). At concentrations of MDMA over 500 μM, the absorbance reached a plateau. The limit for visual detection of the MDMA color reaction was about 50 μM (final concentration: about 12.2 μM). MP did not show any color at concentrations ranging from 0.01 to 10 mM (Fig. 3).

To assess the specificity in more detail, 65 drugs were treated with chromotropic acid/sulfuric acid and heated at 100°C for 10 min to develop the color (Table 1). Each drug was tested using 0.1 and 1.0 mM aqueous solutions, but no drug showed a different result when the two concentra-
Fig. 3. Effect of Drug Concentration on the Color Reaction of MDMA

MDMA and MP solution (1 ml) at the concentration indicated was treated with 100 μl 10% chromotropic acid and 3 ml sulfuric acid. Following treatment, the reaction mixture was heated at 100°C for 30 min.

Each plot is the mean of two analyses.

Fig. 4. Detection of MDMA in Urine with Chromotropic Acid/Sulfuric Acid

The urine from healthy human volunteers, to which MDMA was added, was partially purified using Bond Elut Certify and the extract was treated with chromotropic acid/sulfuric acid (see Experimental section). Urine of control 1–3 was supplied by volunteers not receiving any drugs.

Sample subjected to this cleaning procedure obtained from urine containing 100 μM MDMA showed the red-violet color on adding it to a solution of chromotropic acid/sulfuric acid and heating (Fig. 4). Urine containing 10 μM, but not 1 μM, also exhibited a positive color, although the
intensity was weak.

**DISCUSSION**

In this study, we investigated the formaldehyde released from MDMA and related drugs. Sulfuric acid-mediated release of formaldehyde was shown to be effective for MDMA's, and increased the specificity of the present method, although it is not completely specific for MDMA's. There are classical color tests such as the Marquis test and Mandel's test for screening of drugs in solid form,\(^9\) however, these methods have lower specificity than the present method. For example, the Marquis reagent detects not only MDMA's, but also a number of other drugs including AP, MP, morphine and cocaine, which test negative in our method.

It is possible that MDMA's can be detected by analyzing the catechol derivatives of these drugs. Analytical methods for catechols using color or fluorescence detection have been reported.\(^5\)\(^-\)\(^10\) However, these methods cannot be applied to MDMA screening until the problem of how to release the catechol from its substrate, without interfering with the derivatization reaction to give the colored/fluorescent product, is solved. Furthermore, the reported methods for catechol analysis have the following disadvantages: 1) the reagent is unavailable commercially; 2) the reaction has to be carried out in organic solvent; 3) the fluorescent product cannot be detected visually due to its short emission wavelength.

As listed in Fig. 5, the present method is positive for phenothiazine tranquilizers, THC and related drugs, diphenhydramine and tenocyclidine as well as MDMA's. Of these drugs, phenothiazines give a color with sulfuric acid alone. The observation that MDMA's did not

![Chemical structures of drugs](image)

**Fig. 5.** Drugs Showing Positive in the Colored Reaction with Chromotropic Acid/Sulfuric Acid

The drugs with underline were negative, but listed for comparison with tenocyclidine, a positive drug.
exhibit any color on adding sulfuric acid suggests a method for distinguishing MDMAs from phenothiazines and other drugs: namely, if the sample produces a color on adding sulfuric acid alone, it is not an MDMA. Since tenocyclidine, a sulfur-containing drug, but not other related drugs such as phencyclidine, gave a positive reaction (see also Fig. 5), the present method detects sulfur-containing drugs non-specifically. The mechanism by which sulfur-containing compounds give color remains to be clarified. Positive reaction of only tenocyclidine may suggest that the reaction for sulfur-containing compounds is due to reaction of chromotropic acid with the sulfur atom and not with formaldehyde released. Another interesting aspect of the present method is that THC and derivatives tested positive, although the color was different from that for MDMAs. Therefore, this method may be used for the screening of cannabinoids although this cannot be finally concluded until we know whether non-cannabinoid compounds in the extracts from cannabis leaves give a positive reaction by this method.

Urinary MDMAs were also detectable by the method reported here, if urinary formaldehyde is removed prior to analysis. The limit of detection was about 10 μM (about 2 μg free base/ml urine). Kunsman et al. have reported that when 34 urine specimens from MDMA users were analyzed, the concentration of MDMA ranged from 0.38 to 96.2 μg/ml (mean 1.6 μg/ml). Thus, the present method seems applicable to the screening of MDMA in human urine. However, the detection sensitivity of the present method is too low to analyse low concentrations of urinary MDMA. More sensitive detection of the formaldehyde released from MDMA, for example by fluorescence detection, may make this method more valid.

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REFERENCES