glass tubing before reaching the absorber.

A recent report (*1*) has shown that silica gel is effective in retaining a wide variety of nitrogen compounds. The ter Meulen method is capable of determining an equally wide variety of nitrogen types (*4*, *5*). Rather than run experiments on a large number of nitrogen compounds to test the effectiveness of the adsorption–ter Meulen combination, the author evaluated the method with synthetic samples prepared from hydrocarbon solvents and pure nitrogen compounds, with a gas oil containing naturally occurring nitrogen compounds, and with a concentrate of naturally occurring nitrogen compounds obtained from crude oil (Table I). The values given in Table II were obtained on a reformer charge stock and show the precision which can be obtained at this low nitrogen concentration. The absorbance of the blank was about one fifth of that corresponding to the nitrogen in these samples.

ACKNOWLEDGMENT

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Identification and Differentiation of Sympathomimetic Amines

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▶ The reactions of some sympathomimetic amines with benzoyl chloride, *p*-nitrobenzoyl chloride, benzenesulfonyl chloride, picric acid, and ammonium reineckate have been studied. By the use of color and microcrystal tests, and the formation of derivatives, it is possible to differentiate *d*-amphetamine, *dl*-amphetamine, methamphetamine, and ephedrine. Photomicrographs and infrared absorption spectra of the reaction products are presented and their value for characterizing these clinically important drugs is discussed.

MANY techniques for the identification of sympathomimetic amines have appeared in the literature. They include color tests (*3*, *4*, *15*, *18*, *20*, *26*, *31*, *32*, *34*, *35*, *39*, *40*, *42–44*, *46–48*, *52*, *54*, *56*, *59*), precipitation tests by alkaloidal reagents (*21*), crystallographic examination of suitable derivatives (*1*, *2*, *7–9*, *11*, *12*, *16*, *19*, *22*, *27*, *28*, *30*, *38*, *49*, *51*, *55*), spectrophotometric analyses (*10*, *41*), ion exchange (*53*), and chromatographic procedures (*50*, *57*). Reviews on the chemistry, identification, and determination of many sympathomimetic amines have also been published (*17*, *29*).

Although restricted by law to sale on prescription only, these drugs are often obtained illegally; hence reliable criteria for their identification and differentiation are of great importance to the forensic chemist and toxicologist. It is the purpose of this investigation to improve existing procedures and develop new tests for these clinically important products.

EXPERIMENTAL

Reagents. Ephedrine hydrochloride, British Pharmacopoeia.

Amphetamine sulfate, U. S. Pharmacopeia.

d-Amphetamine sulfate, U. S. Pharmacopeia.

Methamphetamine hydrochloride, U. S. Pharmacopeia.

Aqueous 1 and 0.1% solutions of each of the above sympathomimetic amines.

Marquis reagent, 2 drops of 40% formaldehyde in 3 ml. of concentrated sulfuric acid, prepared freshly.

Fröhde reagent, 5 mg. of sodium molybdate per ml. of concentrated sulfuric acid.

Mandelin reagent, 1 gram of ammonium vanadate in 200 grams of concentrated sulfuric acid.

Sanchez reagent, 0.1 gram of dimethylaminobenzaldehyde in 20 ml. of ethyl alcohol and 4 drops of sulfuric acid.

Picric acid solution, 1 to 2 grams of picric acid in 100 ml. of water.

Platinum chloride solution, 1 gram of platinum chloride in 20 ml. of 1*N* hydrochloric acid.

Gold chloride solution, 1 gram of gold chloride in 20 ml. of water.

Ammonium reineckate solution, 1 gram of salt in 100 ml. of water.

RESULTS

Color Tests. The color reactions were carried out on a spot plate using 1 and 0.1% aqueous solutions of the sympathomimetic amines. One drop of reagent was added to 2 drops of solution (approximately 1 and 0.1 mg. of drug, respectively) and the color formations were observed at various time intervals. The experimental data are recorded in Table I.

Microcrystal Tests. One drop of 1 or 0.1% solution of the salt of each sympathomimetic amine was placed on a microscope slide. One drop of reagent was added and the time of crystal formation noted. Photomicrographs were taken before the slide became dry. The results of the tests are listed in Table II and photomicrographs are shown in Figures 1 and 2.

Formation of Derivatives. All compounds were prepared by standard procedures (Table III).

p-NITROBENZOYL DERIVATIVES were

prepared in accordance with the Schotten-Baumann reaction as outlined by Shriner and Fuson (*45*), recrystallized twice from hot benzene, and dried in vacuo over phosphorus pentoxide. The following compounds have not been reported previously.

p-Nitrobenzoyl *dl*-amphetamine, m.p. 148–9° C. Theoretical: carbon 67.61%, hydrogen 5.63%, nitrogen 9.86%. Found: carbon 68.10%, hydrogen 5.83%, nitrogen 9.59%.

p-Nitrobenzoyl *d*-amphetamine, m.p. 146.8–7.8° C. Theoretical: carbon 67.61%, hydrogen 5.63%, nitrogen 9.86%. Found: carbon 68.25%, hydrogen 5.71%, nitrogen 9.55%.

p-Nitrobenzoyl methamphetamine, m.p. 151.4–1.9° C. Theoretical: carbon 68.46%, hydrogen 6.04%, nitrogen 9.40%. Found: carbon 68.90%, hydrogen 6.31%, nitrogen 9.22%.

BENZENESULFONYL DERIVATIVES were prepared following Hinsberg's procedure for primary amines (*45*), and purified by repeated recrystallizations from Skellysolve C. The following products were isolated for the first time. They were analyzed by nonaqueous titrimetry and the following results obtained:

Benzenesulfonyl *dl*-amphetamine, m.p. 74.7–5.8° C. Theoretical molecular weight, 275.0. Found molecular weight, 274.1.

Benzenesulfonyl *d*-amphetamine, m.p. 64.8–5.6° C. Theoretical molecular weight, 275.0. Found molecular weight, 275.0.

REINECKATES. Excess aqueous ammonium reineckate solution (1%) was added slowly and with constant stirring, to 1% aqueous solutions of the sympathomimetic amine salts. Reaction mixtures were allowed to stand for about 3 hours, and precipitates filtered off, recrystallized from 30% aqueous ethyl alcohol, and dried in vacuo over phosphorus pentoxide. The following products were prepared:

dl-Amphetamine reineckate, m.p. 127.3–30.4° C. Theoretical chromium content, 11.45%; found, 11.45%.

d-Amphetamine reineckate, m.p. 136.8–41.7° C. Theoretical chromium content, 11.45%; found, 11.71%.

Methamphetamine reineckate, m.p. 120.8–3.9° C. Theoretical chromium content, 11.11%; found, 11.44%.

Ephedrine reineckate, m.p. 127.7–31.8° C. Theoretical chromium content, 10.74%; found, 10.86%.

INFRARED SPECTRA. The infrared spectra of the compounds were measured by the potassium bromide pellet technique (*5*).

Table I. Color Reactions of Sympathomimetic Amines with Various Reagents

Reagent	Sympathomimetic Amine	Coloration Observed: 1% solution	Coloration Observed: 0.1% solution
Marquis	Ephedrine hydrochloride	Orange to dark brown	Orange, light brown to pink (no change on standing overnight)
	Amphetamine sulfate *d*-Amphetamine sulfate Methamphetamine hydrochloride	Orange-red, orange-brown, dark brown	Orange-red, orange-brown, gray-green on standing overnight
Mandelin	Ephedrine hydrochloride	Brown to brown-orange on stirring; changes to yellow-light green when heated on water bath	Brown, turning grayish orange on stirring and becoming yellow-green on standing overnight
	Amphetamine sulfate	Green, darkens rapidly. On stirring color passes through several shades to emerald green and dark reddish brown which on heating changes to light red-brown	Green, darkens rapidly. On stirring gradually changes to olive green, yellow-brown, and finally (on standing overnight) gray-yellow
	d-Amphetamine sulfate	Same as for amphetamine sulfate	Green, rapidly darkens, and changes to olive green on stirring and gray-pink on standing overnight
	Methamphetamine hydrochloride	Olive green, turning gray on stirring and becoming gray-green when heated on water bath	Green, rapidly darkens, changing to olive green on stirring, and turns yellow-brown to pale green on standing overnight
Sanchez	Ephedrine hydrochloride	Yellow-brown precipitate, supernatant turning pale yellow-green on addition of water	No reaction
	Amphetamine sulfate *d*-Amphetamine sulfate	Yellow-brown precipitate. Light purple tinge on addition of water	No reaction
	Methamphetamine hydrochloride	Yellow-brown precipitate. Supernatant turning almost colorless on addition of water	No reaction
Fröhde	No reaction obtained with any of the drugs comprised in this study		

Table II. Microcrystal Tests on Sympathomimetic Amines

Reagent	Sympathomimetic Amine	Crystal Formations Observed: 1% solution	Crystal Formations Observed: 0.1% solution
Picric acid	Ephedrine hydrochloride	. . .	. . .
	Amphetamine sulfate *d*-Amphetamine sulfate	Yellow needles start forming in about 5 minutes and continue to grow	. . .
	Methamphetamine hydrochloride	Immediate precipitate becoming crystalline within a few minutes	Crystals appear within a few minutes
Reineckate salt	Ephedrine hydrochloride Amphetamine sulfate *d*-Amphetamine sulfate	Crystals appear within 6–10 minutes	. . .
	Methamphetamine hydrochloride	Immediate amorphous precipitate becoming crystalline on standing	Crystals appear within 20–30 minutes

DISCUSSION

By using the techniques described, it is relatively simple to differentiate the four sympathomimetics. By means of both Marquis and Mandelin reagents, the ephedrine may be distinguished readily from the other three compounds. Mandelin reagent is of value for differentiating methamphetamine from the other three drugs.

However, because color reactions cannot be relied upon as final criteria of identity in drug analyses, precipitation tests were also devised and the crystals examined under the microscope. The results obtained by Haley (*20*) with 5% solutions of chloroauric and chloroplatinic acid were confirmed in this laboratory. He reported that picric acid gives a positive test with methamphetamine only, whereas the

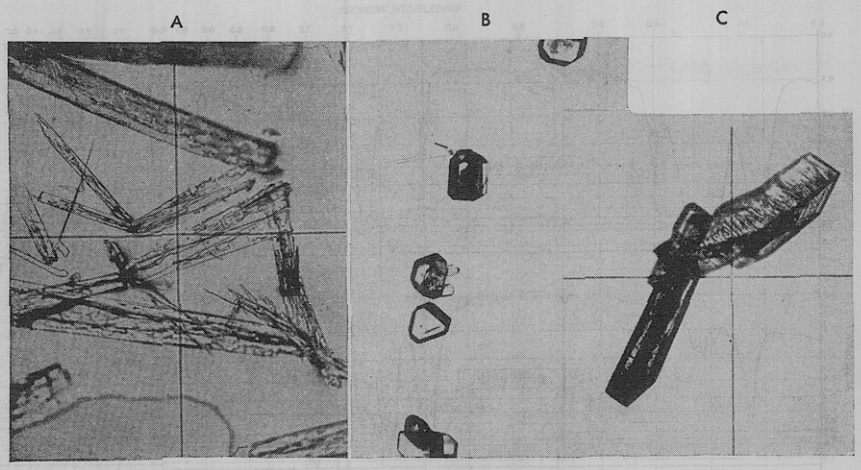


Figure 1. Derivatives of some sympathomimetic amines

A. *dl*-Amphetamine picrate
B. *d*-Amphetamine picrate
C. Methamphetamine picrate

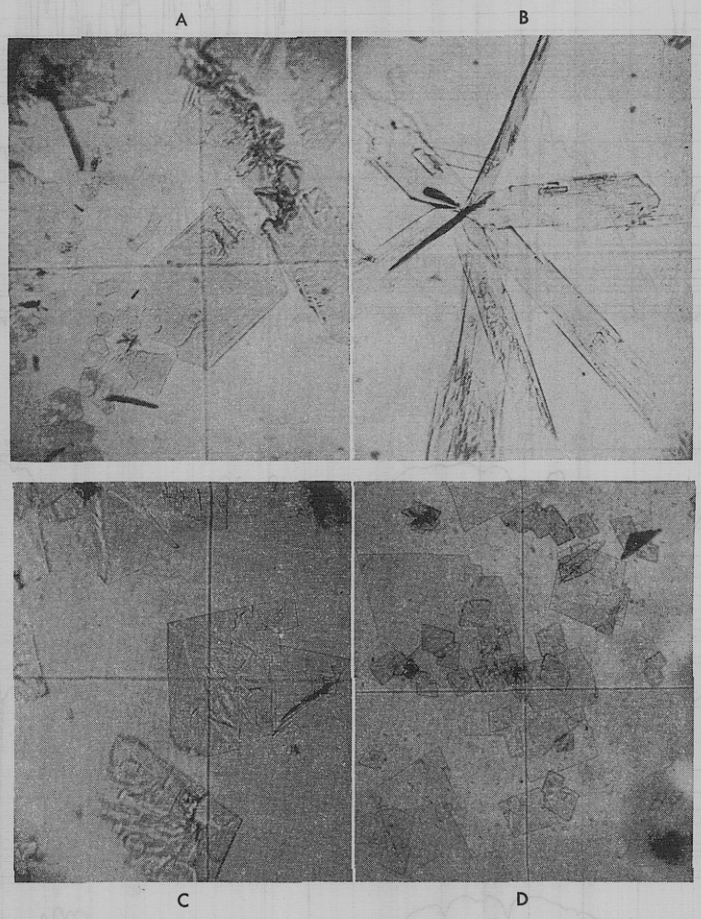


Figure 2. Derivatives of some sympathomimetic amines

A. *dl*-Amphetamine reineckate
B. *d*-Amphetamine reineckate
C. Ephedrine reineckate
D. Methamphetamine reineckate

present study showed that crystal formation occurs also with *d*- and *dl*-amphetamine but that their rate of crystal formation is different from that of methamphetamine. Figure 1 shows the variance in crystalline structures.

Ammonium reineckate is also a useful microanalytical reagent, permitting a ready differentiation of methamphetamine from the other three sympathomimetic amines. A precipitate forms immediately with that compound, whereas the other drugs react more sluggishly. Photomicrographs of the reineckates show that microscopic examination is a valuable tool for differentiating these drugs (Figure 2).

The melting points of the derivatives prepared in this laboratory are compared in Table III with the literature values. The benzoyl derivatives of ephedrine and methamphetamine, ephedrine picrate, and benzenesulfonyl methamphetamine could not be isolated in accordance with the procedures employed in this paper. Although Nagai (*33*) claimed to have prepared *N*-benzoyl ephedrine (melting point 113° C.), he did not describe the procedure he followed. Neither Hun (*24*) nor Ducloux (*9*) recorded the melting points of the reineckates they isolated.

Clark and Wang (*6*) determined milliequivalent weights of aromatic and aliphatic picrates in glacial acetic acid, and their technique, together with previously reported melting ranges, provided satisfactory identification of the picrates prepared in this investigation.

Fritz and Keen (*14*) recommended nonaqueous titration of sulfonamides of primary amines for the determination of equivalent weights of the bases. Using this principle, the benzene sulfonyl derivatives of this investigation were identified. The compounds were dissolved in pyridine and titrations carried out potentiometrically using tetrabutylammonium hydroxide as titrant. The results are shown under the preparation.

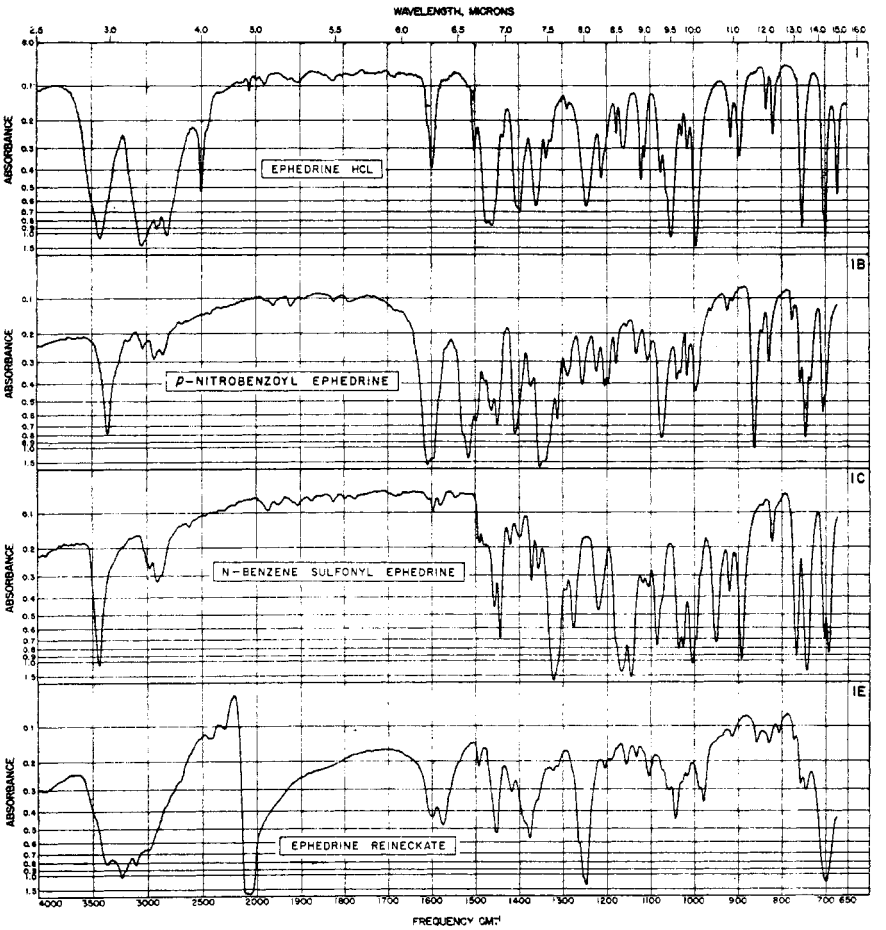
Although Wimer (*58*) titrated a number of amides and acetylated amines in acetic anhydride, it was postulated, in this laboratory, that the inductive effect of the *p*-nitrobenzoyl group would be too great to permit titration of the *p*-nitrobenzamide of amphetamine as a base. This was experimentally demonstrated. It was thought, then, that this same inductive effect might be sufficient to permit titration of these

Table III. Melting Ranges of Derivatives of Sympathomimetic Amines

Derivative	Ephedrine	*dl*-Amphetamine	*d*-Amphetamine	Methamphetamine
Benzoyl	... [113 (*33*)]	134–5 [131–135 (*37*)]	156.5–8.5 [155–158 (*37*)]	...
p-Nitrobenzoyl	188.3–90.2 [188–189 (*25*)]	148.0–9.0	146.8–7.8	151.4–51.9
Benzene sulfonyl	98.5–8.8 [99 (*13*)]	74.7–5.8	64.8–5.6	...
Picrate	...	145.3–6.6 [143 (*23*)]	140.8–1.7 [141–141.5]	145.7–7.1 [144–145 (*36*)]
Reineckate	127.7–31.8	127.3–30.4	136.8–41.7	122.9–5.9

Literature values of previously reported derivatives in brackets.

Figure 3. Infrared spectra of derivatives



compounds as acids. A potentiometric titration was carried out in pyridine with 0.1*N* tetrabutylammonium hydroxide, but the end point could not be selected accurately.

The infrared spectra of the derivatives (shown in Figures 3 to 6) are highly characteristic and should also prove of considerable value for the interpretation of structural features and aid in establishing the identity of these compounds.

Ephedrine hydrochloride (Figure 3) displays sharp absorption in the 3400-cm.$^{-1}$ region (OH stretching vibration) and an intense band at 2500 cm.$^{-1}$ (NH^+ ion frequency) which is also observed in the spectrum of *d*-methamphetamine hydrochloride (Figure 6).

The intense 1350-cm.$^{-1}$ band seen in the spectrum of *p*-nitrobenzoyl ephedrine represents the nitro symmetric stretching vibration. It is also observed in the spectra of the corresponding *dl*- and *d*-amphetamine as well as the *d*-methamphetamine derivative (Figures 4 to 6).

The spectrum of ephedrine reineckate (Figure 3) shows considerably less structural detail than that of the parent compound because of the damping effects exerted by the heavy atoms of the inorganic anion. Two marked absorptions are observed in the 2100- and 1250-cm.$^{-1}$ regions (nitrile stretching vibrations) and these bands are also observed in the spectra of amphetamine reineckate (Figure 5) and *d*-methamphetamine reineckate (Figure 6). Broad, intense absorptions occur throughout the 3500 to 3000 and 700-cm.$^{-1}$ regions. These are also evident in the spectrum of ammonium reineckate and to be considered characteristic spectral contributions by the inorganic moiety of the complex.

The spectra of *dl*- and *d*-amphetamine sulfate (Figures 4 and 5) show sharp and intense bands throughout the 750-to 700-cm.$^{-1}$ as well as the 1500- to 1400-cm.$^{-1}$ regions (phenyl ring frequencies) and broad absorptions throughout the 3000- and 1100-cm.$^{-1}$ wave length range (sulfate ion). In spite of these similarities, however, the spectra may also differentiate both isomers. Thus, *dl*-amphetamine sulfate shows three distinct bands in the 750- to 700-cm.$^{-1}$ region, whereas the *d*-compound displays but two absorptions in this wave length range. Similarly the *p*-nitrobenzoyl

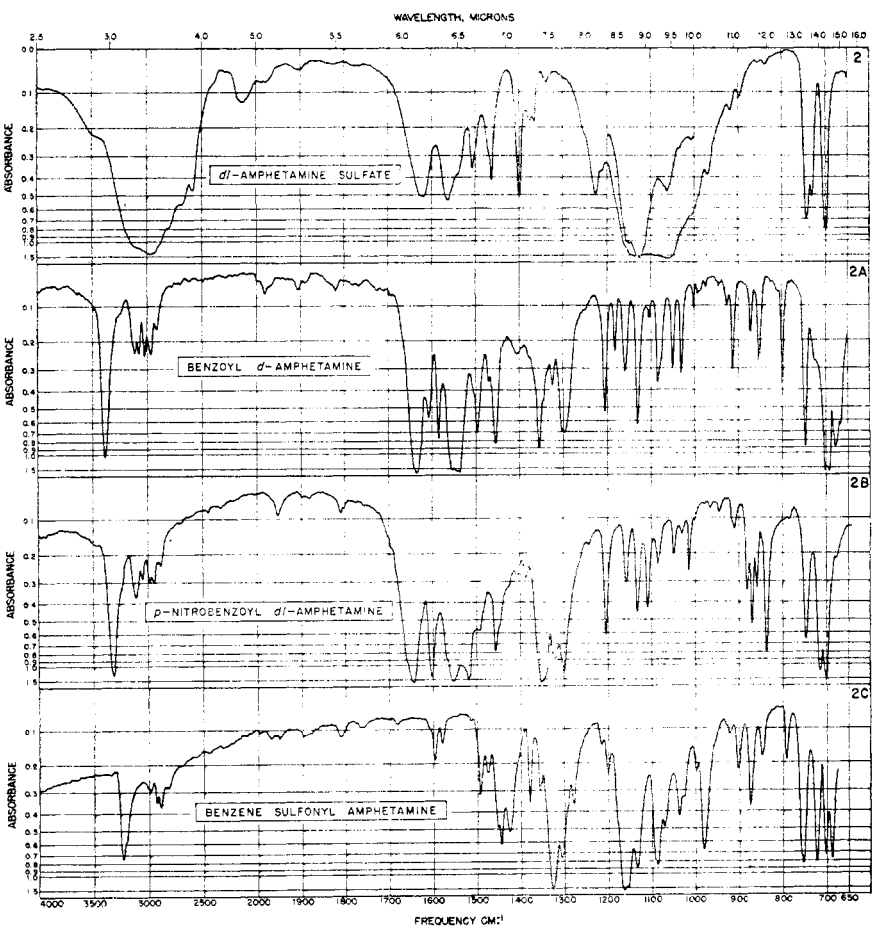
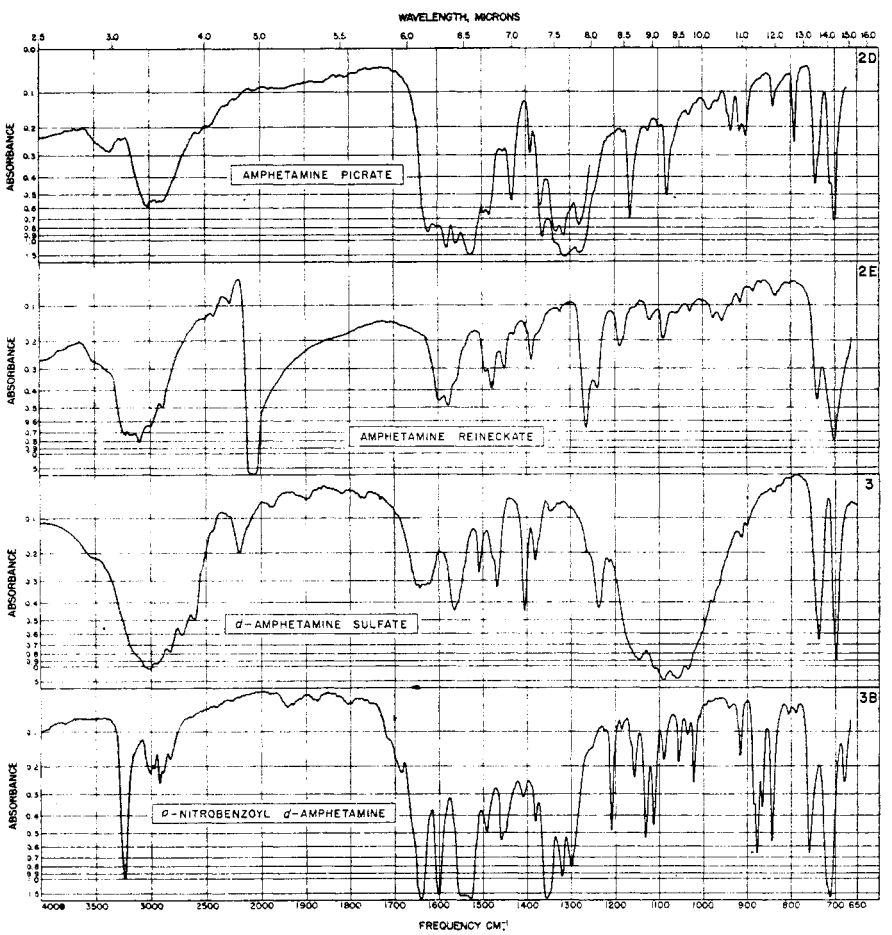


Figure 4. Infrared spectra of derivatives



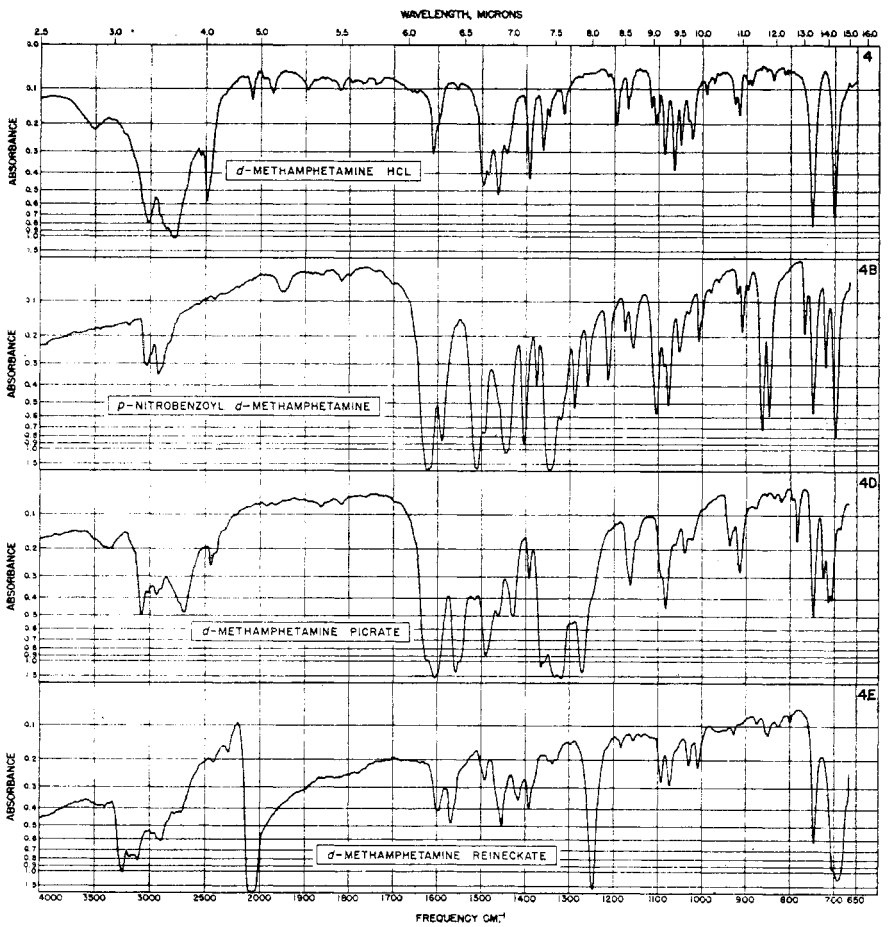


Figure 5. Infrared spectra of derivatives

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derivatives may also be used for identification. *p*-Nitrobenzoyl *dl*-amphetamine shows a triplet in the 875-cm.$^{-1}$ region, followed by a strong band at 830 cm.$^{-1}$. *p*-Nitrobenzoyl *d*-amphetamine displays a doublet in the 875-cm.$^{-1}$ region which is preceded by a shoulder and followed by a weak band at 850 cm.$^{-1}$

Inspection of Figures 3 to 6 thus reveals that in addition to spectral similarities which reflect common structural features of these compounds there exist marked spectral differences, particularly at longer wave lengths where molecular rotation effects become more marked. It is evident that infrared measurements may serve to differentiate the sympathomimetic amines comprised in this study and positively establish their identity.

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Figure 6. Infrared spectra of derivatives

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Stable Apparatus for High-Frequency Analysis

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▶A general study of high-frequency analysis was undertaken and an apparatus was constructed. The responses of the instrument measured as the current and the shift of the tuning frequency were interpreted mathematically as the functions of the dielectric and the resistive characters of the sample contained in the condenser of the tuning circuit. Because the method of analysis is general and applicable to any kind of sample, the photochemical reactions in the leaves of waterpot plants were investigated. The reactions in soybean and buckwheat leaves illuminated by an electric lamp with and without a blue filter are accompanied by a change in resistance. Reactions of leaves illuminated by the lamp covered by a red filter are intermediate between those of capacitive and resistive natures.

IN high-frequency analysis, changes in the sample are detected by the detector circuit as measurable responses of current and frequency shift. In the experiments reported here, the following two points must be considered: The loading effect of the sample on the oscillating circuit should be kept as small as possible, and the responses should be linearly proportional to the magnitude of the change in the sample. The first requirement is due to the fact that the sample forms a part of the oscillator and competes with the sensitivity of the apparatus. The use of the infinite impedance detector system and the mechanism of high-frequency titration have been discussed before (*2*, *3*). This article presents some results of a general consideration of high-frequency analysis and shows its application.

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APPARATUS

The block diagram of the apparatus is shown in Figure 1. The oscillator, V_0, is the Franklin type which has high stability of the oscillating frequency against the fluctuations of the constants of the oscillating tubes. It is suitable for measurements of long duration.

A pentode tube, V_1, is used as the buffer amplifier. The sample is contained in cell, C, of the tuning circuit, C_v and L_2, connected to the plate of V_1.

The detector circuit and the measuring system consist of V_2 and the meter, M. The detector circuit is the infinite impedance type which works with good linearity when the input voltage to V_2 is appropriate (higher than 6 volts). The disadvantage of this detector is loss of sensitivity with use of the cathode-follower scheme. A parallel triode tube, V_2, is therefore used as the detector to eliminate this difficulty; the signal voltage, e_{out}, the output voltage from the cathode of the left half of V_2, is put in parallel to the reference voltage, e_r, which is the output of the cathode of the other half of V_2, and the difference between e_r and e_{out} is applied to the meter.

The function of each component of the apparatus may be explained by Figure 2, where e_g is the input voltage to the grid of V_1 and is assumed constant so long as the oscillating voltage is kept constant.

Then, the input voltage to the grid of V_2, e_{in}, may be written as

$$e_{in} = \frac{1}{\frac{1}{\rho} + \frac{1}{Z}} (g_m e_g)$$

where ρ, g_m, and Z are the plate resistance, mutual conductance of V_1, and the total impedance of the tuning circuit, respectively. Because a pentode is used for V_1, $\rho \gg Z$ and $e_{in} = g_m e_g Z$. As mentioned above, e_{in} is linearly proportional to e_g, and hence, $e_{out} = k Z$, where $k = c g_m e_g$ and c is a constant.

The current to be measured at the final stage of the measuring system, i, is given by

$$i = (e_{out} - e_r)/(r + r_o)$$
$$= A Z + B$$
$$A = g_m e_g [c/(r + r_o)]$$