(3) D. A. Mitchison and C. C. Spicer, *J. Gen. Microbiol.*, **3**, 184(1949).
(4) J. H. Humphrey and J. W. Lightbown, *ibid.*, **7**, 129(1952).
(5) R. K. Finn and F. Deindoerfer, 51st general meeting of the American Society of Bacteriologists, Chicago, Ill., May 1951.
(6) K. E. Cooper, in "Analytical Microbiology," F. Kavanagh, Ed., Academic, New York, N.Y., 1963, chap. 1.
(7) A. H. Linton, *J. Bacteriol.*, **76**, 94(1958).
(8) K. E. Cooper and N. A. Gillespie, *J. Gen. Microbiol.*, **7**, 1(1952).
(9) "Code of Federal Regulations," Title 21, Part 141.110 (1972).
(10) F. Kavanagh, in "Analytical Microbiology," vol. 2, F. Kavanagh, Ed., Academic, New York, N.Y., 1972.
(11) F. Kavanagh, in "Analytical Microbiology," F. Kavanagh, ed., Academic, New York, N.Y., 1963, p. 268.
(12) J. S. Simpson, in "Analytical Microbiology," F. Kavanagh, Ed., Academic, New York, N.Y., 1963, chap. 2.
(13) K. E. Cooper and A. H. Linton, *J. Gen. Microbiol.*, **7**, 8(1952).
(14) K. E. Cooper, A. H. Linton, and S. N. Sehgal, *ibid.*, **18**, 670(1958).
(15) W. Hayes, *J. Pathol. Bacteriol.*, **57**, 457(1945).
(16) J. W. Foster and H. B. Woodruff, *J. Bacteriol.*, **47**, 43(1944).
(17) H. Ericsson, *Scand. J. Clin. Lab. Invest.*, **12**, 423(1960).
(18) R. M. Kline and R. P. Rathmacher, *Develop. Ind. Microbiol.*, **10**, 9(1969).

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Automated System for Analytical Microbiology IV: Accuracy of Measurements

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Abstract □ The automated parts of a system designed for performing microbiological turbidimetric assays were tested for accuracy and precision of photometric and volumetric measurements. The system was tested with potassium ferricyanide solutions. The photometric measurements were accumulated and processed by an on-line computer. They had an average standard deviation of about 0.020. The diluter apparently could contribute an additional small variance to the measurements. The answers had a slight positive bias. Uncorrected multiple reflections in the cell can cause significant errors. The use of a multipoint standard curve makes it unnecessary to correct for multiple reflections in the cell in high precision measurements. Digitizing errors were very small.

Keyphrases □ Automated system—analytical microbiology, studies on precision and accuracy of photometric and volumetric measurements □ Microbiology, automated analytical—determination of accuracy of photometric and volumetric measurements □ Reproducibility—automated system for analytical microbiology, precision and accuracy of photometric and volumetric measurements evaluated

A system[1] for performing turbidimetric microbiological assays was described in detail previously (1, 2). Application of computer technology to the system was shown to improve precision and accuracy by reducing computational errors (3).

The system consists of a diluter, an incubation bath, and a reader. The diluter prepares dilutions of 66-fold and 100-fold in pairs and delivers the diluted sample to an array of test tubes in a carrier. The reader causes solution from the test tubes to flow through a fixed cell in a spectrophotometer and records percentage transmittance of the flowing solutions. The accuracy and precision of the measurements performed by the system are the results of the accuracy and precision of the measurements made by the two modules. The errors and variances were small because the philosophy governing the design was to reduce the electromechanical variations to such an extent that they would not contribute a significant variance to the microbiological assays. The goal was a maximum variation of 0.1% in dilutions or transmittances. Experience with routine measurements of dye solutions indicated probable achievement of the goals. It was also recognized that the accuracies and precisions reported were not necessarily the best values because of the limited resolution of the 3-digit voltmeter used with the spectrophotometer.

Improvements accompanying installation of an on-line computer made an evaluation of the system's accuracy and precision worthwhile. The system was tested by processing colored solutions. No assay, not even a high precision one, was suitable because of the introduction of variances external to the system being tested by the microbiological portion of an assay.

EXPERIMENTAL

The purpose was to test the accuracy and precision of the volumetric and photometric measurements of the system. Test solutions were prepared from solutions of potassium ferricyanide dissolved in pH 7 phosphate buffer. These solutions were used because they were shown (4) to follow Beer's law with requisite accuracy (1 part in 10,000). Measurements of transmittance were made at 415 nm by one of two spectrophotometers[2].

Two classes of test solutions were prepared. One was used to test photometric accuracy and the other to test the diluter as well as the photometer. The first class was prepared by diluting a careful-

[1] The AUTOTURB System was obtained from the Elanco Division of Eli Lilly and Co.

[2] Coleman-Hitachi model 101 with Hewlett-Packard 6203B dc power supply for the lamp or a modified Turner model 330 as modified by Arthur H. Thomas Co.

Table I—*Test of Photometric Module*[a]

Sample Number	Sample, % T		Concentration		Standard Deviation × 1000	Mean Responses
1	100	100	0			
	100	100				
2	25.64	25.64	60			
	25.64	25.64				
3	25.59	25.66	60.086	59.966	51	60.013
	25.64	25.64	60.000	60.000		
4	25.64	25.61	60.000	60.052	30	60.026
	25.61	25.64	60.052	60.000		
5	25.64	25.64	60.000	60.000	17	59.991
	25.66	25.64	59.966	60.000		
6	25.64	25.63	60.000	60.017	32	60.021
	25.64	25.60	60.000	60.069		
7	25.63	25.65	60.017	59.983	24	59.983
	25.66	25.66	59.966	59.966		
8	25.66	25.63	59.966	60.017	29	60.009
	25.63	25.62	60.017	60.034		
9	25.63	25.61	60.017	60.052	20	60.034
	25.61	25.63	60.052	60.017		
10	25.63	25.63	60.017	60.017	0	60.017
	25.63	25.63	60.017	60.017		

[a] The first two samples are standards. Samples 3 to 10 are the same solution as Sample 2. Each measurement is from a different tube in the carrier.

ly weighed portion of a stock solution and diluting in a 2-liter volumetric flask. Results of automated photometric measurements of the solution are given in Table I. These measurements tested stability of flow of liquid through the cell, the spectrophotometer, the signal conditioning amplifier and noise filter, the analog–digital converter, and the computer.

The second class of solutions was a series of samples of approximate concentrations ranging from 10 to 60 m*M* processed through the diluter. Relative concentrations of the samples were known to better than 1 part in 10,000. These measurements (Table II), simulating an antibiotic assay, test the entire measuring system.

The system has four channels, and results from each were treated separately, thereby avoiding all assumptions concerning identity of pairs of channels and ratios between the two sets of channels.

The solutions are listed in the tables in the order of measurement. The two values in the column headed 0.10 ml were measured before the two in the column headed 0.15 ml. The order of measurement was down the column. Precision of the set of four measurements on a sample was indicated by the standard deviations.

The dose–response lines (calibration lines) were point to point as absorbance *versus* concentration. The computer calculated four calibration lines for each test, one for each channel. Thus, four responses were obtained for each sample. Even when the colored solutions (Table I) were not processed through the diluter, they were kept in sets of four because the computer was programmed to handle data in such sets and in no other.

A detailed description of the system was given previously (2). Output of the spectrophotometer as % T was recorded to 3 digits on paper tape. A 1-digit uncertainty was placed in the least-significant (last) digit by the inherent uncertainty of the digital voltmeter. When acquisition and processing of the data by an on-line computer were instituted, resolution of voltage measurement was improved fourfold. As shown here, accuracy and precision were also improved. Output from the spectrophotometer (1 v = 100% T) was conditioned by an amplifier and amplified about 9.5-fold. The amplified and conditioned signal was applied to a 12-bit analog–digital converter and then to the computer for further processing. The computer[3] was programmed to sample the output of the spectrophotometer eight times in 200 μsec, to average the readings, and to store the average. When the data were converted to decimal form for printing, there was some rounding-off during conversion and rounding of potencies to 3 digits to the right of the decimal point of the output format. The analog-digital converter was calibrated at zero and 100% T before each day's measurements. The 100% T reading was equal to about 3400 bits.

The solutions were at the same temperature during measurement. This is important in high precision measurements because absorbance of dilute aqueous solutions will change by about 1 part in 4000 for each degree change in temperature (5) as a result of change in volume of the solution.

RESULTS AND DISCUSSION

The test reported in Table I was designed to measure photometric accuracy and precision. The tubes in the carrier were filled with portions of a 0.6 m*M* solution of potassium ferricyanide. To obtain a 5-digit answer, the standard was assigned a value of 60. One set of the measurements was selected to be the standard. The mean of all of the measurements was 60.019 with a standard deviation of 0.030. The range of individual measurements was from 59.966 to 60.052. These data also showed that any set of measurements (mean response of four individuals) was as good as any other. This is as it should be in a well-designed automated system. These measurements showed that a relatively inexpensive spectrophotometer and a fixed flow cell were capable of photometric accuracy and precision adequate for most analytical purposes.

In the real world of photometric analysis, much more than photometric accuracy is involved in determining the accuracy and precision of an analytical procedure. In this system, dilutions of samples and standards to assay concentration are performed mechanically by the diluter module. The module may be expected to contribute bias and variance to measurements of concentrations of samples. Experience indicated that only small errors were caused by leaking of valves or syringes of a properly maintained diluter. To find the extent of errors and variances of the system (reader and diluter) was the purpose of the experiment reported in Table II. Three samples, processed through the diluter and reader, were measured in terms of a 4-point standard curve. The samples were intermediate in concentration between the bracketing standards. Potency of a sample was obtained by interpolation from the straight line connecting the bracketing standards. The assumption of linearity applied only to the short segment of the calibration line used in obtaining the potency of the sample.

The precision of the four diluting channels was obtained from the data in Table II. The 0.10-ml channels were somewhat more variable than the 0.15-ml channels. The odd-numbered channels were slightly less variable than the even-numbered channels. One syringe of the dual-nozzle filler delivered diluent to the odd-numbered channels, and the other syringe delivered diluent to the even-numbered channels[4]. Since each syringe delivered diluent for both a 0.10- and a 0.15-ml measuring loop, the variance was caused

[3] Hewlett-Packard model 2100A.

[4] Diluent was delivered by a modified model DAB dual-nozzle filler, National Instrument Co., Inc.

Table II—Test of Diluter and Reader Modules as a System[a]

Sample Number	Sample Volume, ml		Concentration		Standard Deviation × 1000	Mean Responses
	0.10	0.15				
1	100.71	100.59	0			
	100.66	100.63				
2	63.36	49.78	20			
	63.05	49.62				
3	39.68	24.70	40			
	39.38	24.64				
4	25.00	12.36	60			
	24.78	12.39				
5	31.37	17.41	50.174	50.104	45	50.168
	31.10	17.35	50.192	50.205		
6	31.39	17.41	50.146	50.104	38	50.102
	31.20	17.41	50.053	50.104		
7	31.47	17.46	50.036	50.021	18	50.031
	31.23	17.44	50.012	50.054		
8	31.42	17.44	50.105	50.054	25	50.066
	31.20	17.44	50.053	50.054		
9	31.45	17.44	50.063	50.054	25	50.055
	31.18	17.46	50.081	50.021		
10	31.45	17.41	50.063	50.104	47	50.121
	31.12	17.38	50.164	50.154		
11	49.82	34.87	30.275	30.159	76	30.168
	49.66	34.85	30.144	30.095		
12	49.91	34.88	30.197	30.151	42	30.145
	49.67	34.85	30.136	30.095		
13	49.91	34.90	30.197	30.135	45	30.134
	49.70	34.85	30.110	30.095		
14	49.96	34.95	30.155	30.094	63	30.091
	49.70	34.96	30.110	30.005		
15	50.02	34.88	30.103	30.151	30	30.108
	49.73	34.85	30.084	30.095		
16	49.91	34.90	30.197	30.135	47	30.132
	49.70	34.86	30.110	30.087		
17	79.67	70.59	10.114	10.070	35	10.069
	79.55	70.59	10.062	10.029		
18	79.69	70.59	10.103	10.070	27	10.071
	79.53	70.57	10.073	10.037		
19	79.64	70.65	10.130	10.045	39	10.074
	79.53	70.54	10.073	10.049		

[a] The first four samples are standards. Percentage transmittances are given for sample volumes of 0.10 and 0.15 ml.

by the syringe and not by the loops and their attendant valves. Other data indicated that the 0.1-ml channels were slightly less variable than the 0.15-ml channels. When there are no mechanical difficulties with the measuring loops and their valves, variances should be the same. The sudden appearance of obviously erratic measurement in one channel is diagnostic of a mechanical problem associated with the measuring loop. Aberrant measurements associated with a pair of channels indicate difficulty with a syringe of the dual-nozzle filler.

A scan of the mean responses in Table II reveals any measurement of a sample to be as good as any other, the standard deviation to be independent of sample mean, and the bias to be essentially independent of sample. The reason for the biases is unknown.

A rough idea of the contribution of the dilution step to system variance may be obtained by comparing the pooled variances (0.024) of the "60" samples of Table I with the pooled variances of the "50" samples of Table II. The diluter probably is less variable than the reader. This conclusion is not unexpected because there are only four mechanical sources of variance in the diluter whereas there are at least seven electromechanical sources of variance in the reader.

Certain operational details peculiar to this automated system degrade accuracy slightly. One is the slight interaction between successive solutions differing in concentration. Such interaction is caused by the impossibility of completely removing all of the previous sample from the cell by a limited amount of succeeding solution. About 8 ml of solution is used to displace 0.25 ml of the previous sample from the cell by bubble-free, streamline flow. This error resides in the reader. An example of the interaction of successive different concentrations is the first measurement of Sample 11 in Table II. This sample also had the largest standard deviation of the six samples of the "30" concentration. This kind of interaction also occurred in standards. Such bias in the standard caused a corresponding bias in potency of samples for the first measurement of the 0.10-ml pair. Had carryover in the sampling system contributed to the interaction, both 0.10-ml samples would have given answers different from the 0.15-ml samples and from the 0.10-ml sample of succeeding identical solutions. Any cross-contamination from the sampling system caused errors too small to be detected.

The spectrophotometers that formed part of the reader modules achieved stability by being operated at constant voltage. This procedure was considered by Pardue and Rodriguez (6) to be inferior to optical feedback as a means of achieving stability. They reported repeatability of measurements made over 1 hr to be within 0.01% T for their manually operated instrument. The readings in Table I, obtained in about 6 min, had a mean of 25.633% T with a standard deviation of 0.018. Twelve readings differed from the mean by more than 0.01% T, indicating that this system was a little less precise than the spectrophotometer of Pardue and Rodriguez. The instruments developed by Pardue and coworkers (6, 7) probably are more accurate and precise than the system discussed here, but none of them was designed to measure samples at 10-sec intervals and to do it automatically.

A recent commercial instrument[5] and several manually operated ones designed 40 years ago were equal or slightly superior to the

[5] Cary 118 spectrophotometer, Varian Instrument Division.

Table III—Influence of Digitizing Errors upon Concentration of Samples when the Standard is 60

Concentration of Sample	Change in Absolute Value of Sample Caused by a +1-Bit Change in Measurement of		
	Blank	Sample	Standard
10		−0.016	
20	+0.012	−0.020	+0.017
30		−0.025	
50		−0.041	
60	+0.013	−0.052	+0.052

module used in this work. Both of the early instruments were null balancing types in which the photocells were used only to indicate a null condition and transmittance was read from a rotating sector. Follett (8) reported his measurements to four places. Kortüm (4) recorded transmittances to five places. Variations in measurements of his most favorable samples, gray glass and 2,4-dinitrophenol solutions, occurred mostly in the fifth place.

Mavrodineanu (9) gave details of an elaborate, high accuracy, single-beam spectrophotometer which spanned the spectrum from 275 to 800 nm. Provision was made for acquisition and processing of data by computer. Readings of the photomultiplier current were for 5 sec at the rate of 10/sec. Mean, standard deviation, and relative standard deviation of the 50 readings were printed as the data point for each determination. Samples were glass filters. Transmission of the air path was measured, then the filter, and then the air again. Transmittance of the filter was computed using the mean of the two air measurements. Two successive measurements of air differed by from 1 part in 4000 to 1 in 100,000. The precision of measurement of the glass filters was higher than the precision of measurement of solutions flowing through a cell as reported here.

Burke *et al.* (10) gave an excellent account of the many, and serious, problems associated with selection of substances to be used as light absorbance standards. They discussed chromates, cobalt and nickel salts, phthalate, certain dyes, and the liquid absorbance standard (SRM 931) to be issued by the National Bureau of Standards.

Östling (11) described the application of a discrete analyzer[6], designed for clinical analyses, to colorimetric water analysis. The photometer permitted "measurements with a precision of ±0.001 *A* up to 1.5 *A* with a drift of about 0.001 *A* per hour." The sample was sucked into the cell, measured, returned to the tube, and emptied by a puff of air. Carryover from one aqueous sample to the succeeding one varied between 0.1 and 2%, depending upon the size and construction of the cell.

Efforts to compare the photometric accuracy and precision of the system described here with those in the literature have been frustrating because of the inadequacy of published information. Such statements as "reproducibility of 0.001 *A* at 0.4 *A*" (12) are not exact enough. Usually the mean of some unknown number of measurements is given. A new instrument may be described without any indication of either accuracy or precision. Generally the indications of accuracy and precision of spectrophotometry are imprecisely defined and inadequate in quantity. The ambiguities and imprecision of statistical treatment would be removed by giving original measurements as is done here or by following the admonitions of Eisenhart (13).

The amount of stray light in the spectrophotometer at 415 nm was unknown. Stray light will cause a material not to follow Beer's law, with deviations increasing with the absorbance of the solutions (10). For example, stray light of 1% of that transmitted by a solution of 0.1 absorbance would decrease measured absorbance by 1.1%. That amount of stray light would cause an increase in the measured concentration of the 10 and 30 samples in Table II by about 0.1%. This result illustrates again the advantages of a point-to-point calibration line.

Theoretical treatment is needed of the sources and sizes of errors in a single-beam spectrophotometer containing a fixed cell (flow cell) when it is used as part of an automated data-gathering system. Drift over the extended measuring time and the relatively small number and infrequent measurements of the blank and other calibration points are significant differences from the manual procedures that have been so extensively studied during the last 20 years.

Multiple Reflection in Cell—Light passing through the cell is reflected at each interface. Some light passes through the solution more than once. The result of multiple reflections is an apparent increase in absorbance of the solution in the cell. Size of the increase is a function of absorbance and the relative indexes of refraction of solution and material of the cell faces. Both are functions of wavelength. Multiple reflections cause a high bias in the potency of samples lower in concentration than the standard. The bias decreases with an increase in concentration of the sample. If a single calibrating standard is used, as is the practice when absorbance of the solution follows Beer's law, all samples can have a bias significant in high precision assays. However, if multiple standards are employed as in Table II, the relative error in the sample is small because the sample is measured in terms of two standards bracketing the sample and not of a single standard far removed in concentration.

The table of Burnett (14) was used to calculate errors in samples for the two conditions. If a single standard of 60 is employed and no corrections are made for multiple reflections, the errors in samples of concentrations 10, 30, and 50 would be +0.135, +0.060, and +0.015%, respectively. When the calibration is multipoint, as in Table II, the errors caused by uncorrected multiple reflections become +0.044% for the 10 and +0.01% for the 50 concentrations. These results show that a multiple-point calibration line can greatly reduce the errors caused by uncorrected multiple reflections in the cell.

Digitizing Errors—The output voltage of the spectrophotometer had to be translated into a language understood by the computer. The analog signal from the spectrophotometer was transformed into binary numerical representation by an analog–digital converter. The numbers obtained from the analog–digital converter were linearly related to the voltage from the spectrophotometer and, therefore, to percent transmittance. The 12-bit analog–digital converter provided 4095 discrete values from its full-scale input. However, in this application, about 3400–3600 bits represented 100% *T*. Therefore, resolution of the spectrophotometer was 1 in 3400.

Two kinds of digitizing errors occur. One is the inherent 1-bit uncertainty of the digitizing process. The other is that of unknown size and frequency caused by noise and drift in the analog–digital converter. The random 1-bit uncertainty occurs in all digitized numbers and is independent of the size. Maximum deviation in a reading caused by inherent uncertainty of the digitizing process would be 2-bits: one from the sample and one from the standard of opposite sign. Digitizing error also could be zero; it is a chance event.

Influences of a 1-bit error on the computer concentration of samples are given in Table III using data from Table II. The influence of a fixed digitizing error depended upon whether it occurred in the blank, the standard, or the sample as well as upon the relative concentrations of the standard and the sample. The percentage error decreased somewhat with an increase in the concentration of the sample.

The 60 set of values in Table I changed by multiples of 0.017, a number one-third of that caused by a 1-bit change in the digitized value of transmittance. This small number showed that averaging the eight readings reduced the variation; otherwise, the concentrations would have changed by steps of 0.052. Only one of the 32 concentrations differed from 60 by more than 0.052.

The measuring system would be improved by using a more stable and less noisy signal conditioning amplifier and a 13-bit analog–digital converter. The increased stability and resolution would be of marginal value in most microbiological assays performed with the system.

[6] Autolab system, Linson Instrument AB, Stockholm, Sweden.

REFERENCES

(1) N. R. Kuzel and F. W. Kavanagh, *J. Pharm. Sci.*, **60,** 764(1971).

(2) *Ibid.*, **60,** 767(1971).

(3) F. Kavanagh, *J. Pharm. Sci.*, **60,** 1858(1971).

(4) G. Kortüm, *Angew. Chem.*, **50,** 193(1937).

(5) C. F. Hiskey, *Anal. Chem.*, **21**, 1440(1949).
(6) H. L. Pardue and P. A. Rodriguez, *ibid.*, **39**, 901(1967).
(7) H. L. Pardue and S. N. Deming, *ibid.*, **41**, 986(1969).
(8) D. H. Follett, *Proc. Phys. Soc.*, **46**, 499(1934).
(9) R. Mavrodineanu, *J. Res. Nat. Bur. Stand.*, **76A**, 405(1972).
(10) R. W. Burke, E. R. Deardorff, and O. Menis, *ibid.*, **76A**, 469(1972).
(11) G. Östling, *Chem. Instrum.*, **5**, 1(1973).
(12) B. Gabaric, I. Piljac, and I. Filipovic, *Anal. Chem.*, **45**, 1932(1973).
(13) C. Eisenhart, *Science*, **160**, 1201(1968).
(14) R. W. Burnett, *Anal. Chem.*, **45**, 383(1973).

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NOTES

Amphetamine Derivatives: 10(e)- and 10(a)-Amino-1,2,3,4,4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrene

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Abstract □ Amphetamine analogs 10(e)- and 10(a)-amino-1,2,3,4,4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrene, I and II, respectively, were prepared. Hydrogenolysis (methanolic hydrochloric acid) of 9(a)-hydroxy-10(e)-amino-1,2,3,4,4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrene afforded I. A similar procedure for the preparation of II from 9(a)-hydroxy-10(a)-amino-1,2,3,4,4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrene was followed, except the more drastic conditions of a mixture of acetic and perchloric acids were necessary. The compounds were inactive when assayed for amphetamine behavioral and hyperthermia effects.

Keyphrases □ 10(e)- and 10(a)-Amino-1,2,3,4,4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrene—synthesized and evaluated for amphetamine behavioral and hyperthermia effects □ Amphetamine derivatives—synthesis of 10(e)- and 10(a)-amino-1,2,3,4,4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrene, evaluated for amphetamine behavioral and hyperthermia effects

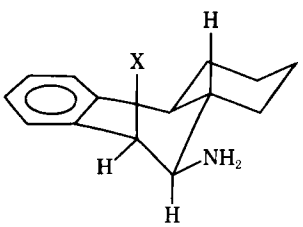
Conformationally rigid analogs have been used to study drug receptor systems, but few studies have been reported (1–5) with respect to amphetamines. In the cyclopropane series, the *trans*-isomer shows equivalent peripheral amphetamine-like effects, *e.g.*, pressor response, moderate central nervous system stimulation, and increased monoamine oxidase inhibition when compared to amphetamine. Some differences were noted in other rigid analogs, although only changes in motor activity and LD_{50} data have been reported (3, 4). The norephedrine analogs in the octahydrophenanthrene system were reported previously (6), in which a potentiation and blockade of the effects of norepinephrine were noted; a logical extension of this work was the preparation of these analogous amphetamine derivatives.

DISCUSSION

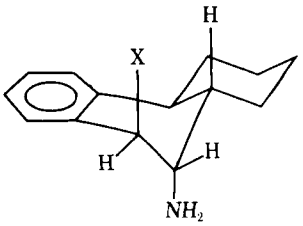
In a related study, the isomeric 9-hydroxy-10-amino-1,2,3,4,-4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrenes were prepared (6). The alcohols[1] III and IV were subjected to hydrogenolysis conditions. The conversion of III-HCl occurred smoothly using palladium-on-carbon in methanol containing hydrochloric acid. Similar results were obtained in a related tricyclic system (7). Attempted hydrogenolysis of the IV-HCl under these conditions failed. More rigorous conditions, acetic acid–hydrochloric acid, also failed. However, when using acetic acid–perchloric acid, hydrogenolysis did occur.

The mass spectra of 10(e)- and 10(a)-amino-1,2,3,4,4a,9,10,10a-(*trans*-4a,10a)-octahydrophenanthrene, I and II, respectively, showed parent peaks at *m/e* 201.1494 and 201.1502, respectively (calculated 201.1518). The NMR spectra were not useful in assigning the relative stereochemistry because of similar chemical shifts of several protons in each spectrum. The small quantities of compound precluded preparation of suitable derivatives. When using a TLC developing system of ether–methanol–aqueous ammonia (90:8:2) and silica gel plates, R_f values of 0.46 and 0.28 were found for I and II, respectively, demonstrating that the potential hazard of dehydration to the intermediate enamine is not a likely process during the hydrogenolysis step.

The behavioral effects of amphetamine in mice (25 mg/kg), *e.g.*,



I: X = H
III: X = OH



II: X = H
IV: X = OH

[1] All materials are racemic, although only a single isomer is shown.