2. The kinetics of the base catalyzed hydrolysis of trimethadione (3,5,5-trimethyl-2,4-oxazolidinedione) have been determined for the pH range 8.0 to 10.75 at 30°. Pseudo–first order rate coefficients are reported for the disappearance of trimethadione and for the parallel pseudo–first order appearance of the decomposition products N-methyl-carbamylo-α-hydroxyisobutyric acid and N-methyl-α-hydroxyisobutyramide. The amide is the principal product below pH 8.75, with the acid becoming an increasingly important product above pH 9.50.

3. The overall rate of disappearance of trimethadione in aqueous solution, at constant hydroxide ion concentration, was found to be in agreement with a rate equation of the form

$$\frac{\text{Rate}}{T} = \frac{K(OH^-)\,k_{amide'}}{1 + K(OH^-)} + \frac{(OH^-)^2\,k_{amide''}}{1 + K(OH^-)} + \frac{(OH^-)^2\,k_{acid'}}{1 + K(OH^-)}$$

4. A mechanism was proposed in which there exists a rapidly reversible equilibrium between the cyclic trimethadione and an acyclic structure.

5. Trimethadione is much more sensitive to alkaline hydrolysis than acyclic compounds of similar structure. Trimethadione hydrolyzes approximately 1,000,000 times as fast as ethyl-N-methylcarbamate and approximately 100,000 times as fast as ethyl carbamate (urethan) at pH 10 and 30°.

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Determination and Identification of *p*-Hydroxyamphetamine as the O,N-Diacetyl Derivative

By LLEWELLYN H. WELSH and A. FRANCIS SUMMA

Therapeutic solutions of hydroxyamphetamine hydrobromide, such as the ophthalmic solution U.S.P. and nasal solution, N.N.R. (N.N.D.), may be assayed gravimetrically by treating with acetic anhydride in the presence of bicarbonate and quantitatively isolating the O,N-diacetylhydroxyamphetamine thus formed. The properties of the readily crystallizable derivative serve to identify the parent substance.

TESTS AND STANDARDS for New and Nonofficial Remedies" (1) includes monographs for a 1% nasal solution and a 1% ophthalmic solution of *p*-hydroxyamphetamine hydrobromide. The assay specified for the former solution involves liberation of hydroxyamphetamine base with potassium carbonate, extraction of the base into ether, addition of excess standard acid to the extract, and back-titration after evaporation of the ether. The assay specified for the latter solution is based on measurement of its absorbance at 225 mμ.

Vincent, Krupski, and Fischer (2) have reported an alkalimetric method in which the solution containing a salt of hydroxyamphetamine is passed through a column of Amberlite IR-45. The base so formed is titrated after being eluted with ethanol. Varga and Vastagh (3) have developed a bromometric assay applicable to therapeutic solutions of hydroxyamphetamine.

The alkalimetric methods and the procedure of Varga and Vastagh are relatively nonspecific. In regulatory work it would be necessary to supplement them with experimental data providing some assurance that consumption of reagent is due only to the substance to be quantitated. The N.N.R. (1) alkalimetric method is, in addition, somewhat tedious. Even with the salting-out effect produced by the specified high concentration of potassium carbonate (15 Gm. for a 20-ml. sample), seven extractions are required, and there are other difficulties related to the low specific gravity and high vapor pressure of ether. The N.N.R. spectrophotometric method, although convenient, is applicable only in the laboratory of the manufacturer since it requires employing as a blank the menstruum used in preparing the solution.

In the course of investigating alternative

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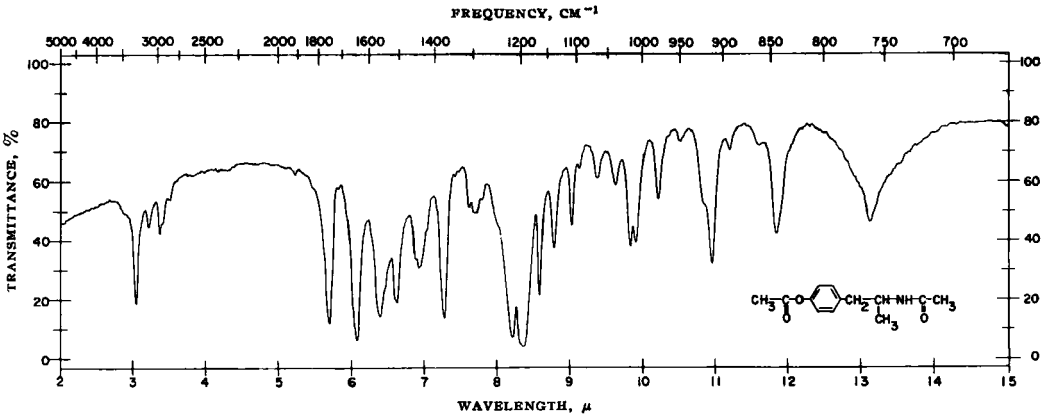

Fig. 1.—Infrared absorption spectrum of crystalline O,N-diacetyl-*p*-hydroxyamphetamine (1 mg.) in 0.5 in. (200 mg.) KBr disk. Recorded with a Perkin-Elmer model 21 spectrophotometer.

methods of assay acceptable for regulatory use, application was made of the general procedure for acetylation which has proved useful in the analysis of other sympathomimetic amines (4–6). Under the conditions of the procedure (dilute solution of the drug in *ca.* 10% aqueous bicarbonate), *p*-hydroxyamphetamine reacts rapidly and quantitatively with acetic anhydride to form the O,N-diacetyl derivative which may be extracted easily into chloroform and weighed after removal of solvent. This is the basis of the assay presently proposed. The method is manipulatively less difficult than the N.N.R. alkalimetric assay, requires one-half as much sample (10 ml.), and is applicable to individual units of the size most commonly marketed (15 ml.).[1] Furthermore, it provides a readily crystallizable derivative, the properties of which serve to identify the drug and give an indication of its purity.[2]

EXPERIMENTAL

Assay.—Pipet 10 ml. of hydroxyamphetamine hydrobromide solution (1%) into a 125-ml. separator. Extract with 15 ml. of chloroform; discard the extract. Rinse the stopper and mouth of the separator with a few drops of water and allow the rinsings to combine with the contents. Add 1.05 Gm. of sodium bicarbonate, preventing it from contacting the mouth of the separator, and swirl until most of the bicarbonate has dissolved. By means of a 1-ml. syringe, rapidly inject 0.50 ml. of acetic anhydride into the contents of the separator, stopper the vessel immediately, and shake it vigorously until the evolution of carbon dioxide has ceased, releasing the pressure as necessary through the stopcock. Allow to stand for 5 minutes and extract the solution with five 10-ml. portions of chloroform; filter each extract through a pledget of cotton (previously washed with chloroform) into a beaker.

Evaporate the combined extracts on a steam bath in a current of air to about 3 ml., completely trans-

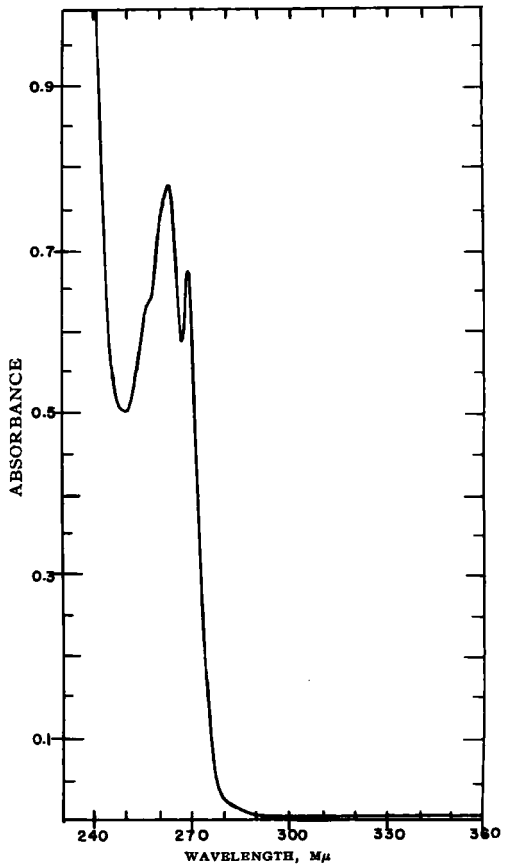

Fig. 2.—Ultraviolet absorption spectrum of O,N-diacetyl-*p*-hydroxyamphetamine in 95% ethanolic solution (400 mg./L., 1-cm. cell). Recorded with a model DK-2 Beckman spectrophotometer: ϵ_{265} = 368, ϵ_{271} = 315.

[1] The assay is now part of the monograph for hydroxyamphetamine hydrobromide ophthalmic solution, recently recognized by the U.S.P. (7).

[2] Identification of the product obtained in the assay does not establish unequivocally the identity of the solute since the same substance would be isolated if the solution originally contained *p*-hydroxyamphetamine, its diacetyl derivative, either of the two possible monoacetyl derivatives, or any combination of the four substances. However, the possibility would appear remote that any one of the relatively inaccessible acetylation products would find its way into a commercial preparation either by accident or design.

fer the concentrate by means of small portions of chloroform to a tared 50-ml. beaker, and continue the evaporation until the solvent is completely removed. Heat the residue of diacetyl derivative at 80° for 90 minutes, cool in a desiccator and weigh. (Weight of the residue × 0.9866 = weight of *p*-hydroxyamphetamine hydrobromide.)

O,N - Diacetyl - *p* - hydroxyamphetamine.—The white crystalline substance (m.p. 97.5 to 98.5°) obtained in the assay was recrystallized from carbon tetrachloride (6 ml./Gm.; 95% recovery). Twice-recrystallized material melted at 98 to 98.5° and exhibited the following optical crystallographic properties:[3] (*a*) *habit*–needles, and rods, some in bundles broader at one end than at the other; (*b*) *refractive indices*–α = 1.538, β = 1.548 (common), γ = 1.604 (all ± 0.003); (*c*) *extinction*–parallel and inclined; (*d*) *optic sign*–positive; (*e*) *elongation*–positive and negative; (*f*) 2*V*–moderately small.

The infrared and ultraviolet spectra are shown in Figs. 1 and 2, respectively.

Anal.–Calcd. for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found:[4] C, 66.30; H, 7.47; N, 6.03.

RESULTS AND DISCUSSION

Five assays of a 1% standard solution of hydroxyamphetamine hydrobromide[5] gave recoveries in the range of 100.2 to 100.6% (av. 100.5%). Duplicate assays of a solution prepared to contain 1% of the drug, boric acid (2%), and thimerosal (1:50,000) yielded recoveries of 100.6 and 101.0%, whereas five assays of a commercial ophthalmic solution of the same declared composition afforded results corresponding to 101.1 to 101.4% (av. 101.2%) of the declared amount of active ingredient (m.p. of isolated derivative 97.5 to 98.5°).

Coincidence of the infrared spectrum of the derivative with that of authentic diacetyl-*p*-hydroxyamphetamine is, *per se*, sufficient to identify the parent substance within the limitations discussed.[2] If infrared spectrophotometric equipment is not available, the base (8–10) may be extracted from the solution and identified by the classical methods of qualitative organic analysis. In addition to the hydrobromide (10, 11), the following easily prepared derivatives of *p*-hydroxyamphetamine have been reported in the literature: hydrochloride (9, 12–14), hydriodide (8), 2,4-dinitrobenzoic acid salt, and N-benzoyl derivative (15). Color reactions of *p*-hydroxyamphetamine and its behavior with alkaloidal precipitants have been described by Haley (16).

[3] Determined by Arnold E. Schulze, Division of Microbiology, Food and Drug Administration, U. S. Department of Health, Education, and Welfare.

[4] Microanalyses by Harold G. McCann, National Institutes of Health, U. S. Department of Health, Education, and Welfare.

[5] We are indebted to Smith Kline and French Laboratories, Philadelphia, Pa., for a generous supply of U.S.P. hydroxyamphetamine hydrobromide.

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ERRATUM

In the paper titled "Colorimetric Assay of Nystatin" (1), the ordinate markings for Figs. 1 and 2 were incorrect and are reproduced correctly here

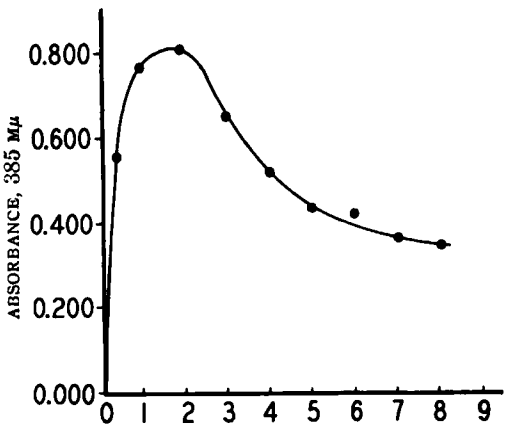

Fig. 1.—Color development of basic hydrolysis of nystatin (5080 units in 5 ml. aliquot).

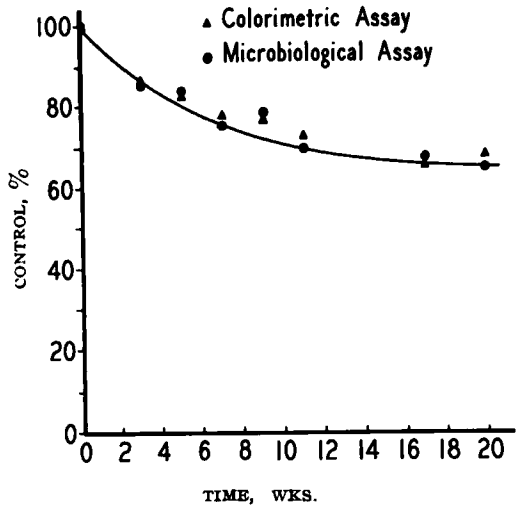

Fig. 2—Degradation of nystatin at 50° C.

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