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.

REFERENCES

(1) "Tests and Standards for New and Nonofficial Remedies," J. B. Lippincott Co., Philadelphia, Pa., 1953, pp. 131–132.

(2) Vincent, M. C., Krupski, E., and Fischer, L., THIS JOURNAL, **46**, 85(1957).

(3) Varga, E., and Vastagh, G., *Pharm. Zentralhalle*, **96**, 149(1957).

(4) Welsh, L. H., THIS JOURNAL, **36**, 373(1947); *Ibid.*, **417**, 545(1952); *Ibid.*, **44**, 507(1955); *J. Am. Chem. Soc.*, **74**, 496, (1952); *J. Assoc. Offic. Agr. Chemists*, **36**, 714(1952); *Ibid.*, **37**, 685(1954); *Ibid.*, **39**, 639(1956).

(5) Pratt, E. L., THIS JOURNAL, **46**, 505(1957).

(6) Higuchi, T., Sokoloski, T., and Schroeter, L. C., *ibid.*, **48**, 533(1959).

(7) "United States Pharmacopeia," 16th rev., 1st Suppl., Mack Publishing Co., Easton, Pa., 1962, p. 21.

(8) Mannich, C., and Jacobsohn, W., *Ber.*, **43**, 189(1910).

(9) Hoover, F. W., and Hass, H. B., *J. Org. Chem.*, **12**, 501(1947).

(10) "Tests and Standards for New and Nonofficial Remedies," J. B. Lippincott Co., Philadelphia, Pa., 1953, p. 130.

(11) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, p. 338.

(12) Alles, G. A., *J. Am. Chem. Soc.*, **54**, 271(1932).

(13) Woodruff, E. H., and Conger, T. W., *ibid.*, **60**, 465 (1938).

(14) Clemo, G. R., and Duxbury, F. K., *J. Chem. Soc.*, **1952**, 3844.

(15) Fischer, W. H. A., and Plein, E. M., THIS JOURNAL, **44**, 313(1955).

(16) Haley, T. J., *ibid.*, **36**, 301(1947); *Ibid.*, **37**, 378(1948)

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

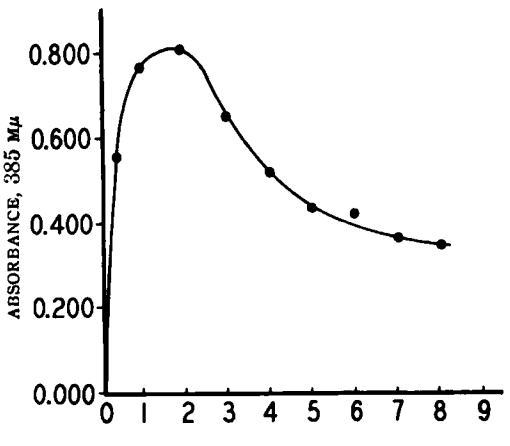

Fig. 1.—Color development of basic hydrolysis of nystatin (5080 units in 5 ml. aliquot).

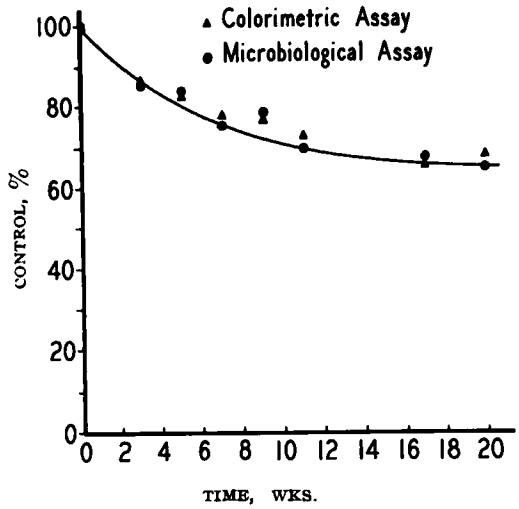

Fig. 2—Degradation of nystatin at 50° C.

(1) Chang, J. C., Honig, A. B., Warren, A. T., and Levine, S., THIS JOURNAL **52**, 536(1963).