Tests of Amino Acids with Alkaloidal Reagents

By CHARLES L. WINEK and THOMAS J. FITZGERALD

THERE ARE MANY references made in the literature to "false" positive tests obtained with alkaloidal precipitating reagents (1–5). These false tests are attributed to several natural constituents, including amino acids. Since at least one reference (2) lists amino acids as giving a positive test with alkaloidal precipitating reagents while other reference books do not mention amino acids as such, it was felt that the matter should be clarified because of the universal presence of amino acids.

In order to determine whether amino acids do or do not give positive tests with common alkaloidal precipitating reagents this investigation was undertaken. Twenty-seven amino acids were tested with four different alkaloidal precipitating reagents in a qualitative manner. A sample of approximately 100 mg. of each amino acid was dissolved in 2 ml. of 5% hydrochloric acid solution. A volume of 0.5 ml. of this solution was placed in each of four test tubes and the alkaloidal reagent added. The 27 amino acids listed in Table I were tested with 1% platinic chloride solution, gold chloride T.S., Valser's reagent U.S.P., and Dragendorff's reagent.

No precipitate resulted with any of the tests performed, indicating that these amino acids are not capable of giving a positive test with these alkaloidal reagents. Color changes were noticed with two of the amino acids. 3-Aminotyrosine hydrochloride gave an immediate purple color with gold chloride T.S. This purple color gradually turned to a ruby-red. D-Methionine decolorized gold chloride T.S.

One can conclude that amino acids are not as likely to be responsible for false alkaloid tests as the literature leads one to believe.

TABLE I.—AMINO ACIDS TESTED WITH ALKALOIDAL PRECIPITATING REAGENTS

DL-Alanine	DL-Homoserine	D-Tryptophane
L-Asparagine	DL-Isoleucine	DL-Phenylalanine
D-Aspartic acid	DL-Homocystine	DL-Proline
L-Arginine	DL-Leucine	DL-Threonine
L-Cysteine	DL-Lysine	DL-Serine
3-Aminotyrosine HCl	DL-Norleucine	DL-Valine
Cystine	DL-Norvaline	β-Alanine
Histidine	D-Methionine	Glycine
Hydroxy-L-proline	DL-Ornithine HCl	DL-Tyrosine

Received September 5, 1961, from the Ohio State University, College of Pharmacy, Columbus 10.
Accepted for publication September 13, 1961.
This investigation was supported in part by Public Health Service Research Grant, RG-5640, National Institutes of Health.
The authors wish to thank Dr. Jack L. Beal, College of Pharmacy, for his suggestions, and Dr. William Boyd, Department of Microbiology, for supplying the amino acids used in this study.

REFERENCES

(1) Fulton, C. C., *Am. J. Pharm.*, 104, 244(1932).
(2) Wilson, C. O., and Gisvold, O., "Textbook of Organic Medicinal and Pharmaceutical Chemistry," 3rd ed., J. B. Lippincott Co., Philadelphia, Pa., 1956, p. 242.
(3) Claus, E. P., "Pharmacognosy," 3rd ed., Lea and Febiger, Philadelphia, Pa., 1956, p. 429.
(4) Pratt, R., and Youngken, H. W., Jr., "Pharmacognosy," J. B. Lippincott Co., Philadelphia, Pa., 1951, p. 129.
(5) Paech, K., and Tracey, M. V., "Modern Methods of Plant Analysis," Vol. 4, Springer-Verlag, Berlin, 1955, p. 373.

Synthesis and Pharmacology of Several Substituted Indole-3-carboxamide Derivatives

By H. C. WORMSER† and S. ELKIN

Several amides have been prepared which showed oxytocic and local anesthetic activity. These compounds were obtained by reacting indole-3-carbonyl chloride with several amines in ethyl acetate. Indole-3-carbonyl chloride was prepared by the decarbonylation of indole-3-glyoxylyl chloride in tetrachloroethane at 115–120°.

AMIDE DERIVATIVES have found extensive use in medicine (1). Bovet (2) reported that relatively simple substituted aminoacetamides exhibited oxytocic activity in both animals and humans. Also several potent amide-type local anesthetics are known. Among these are included dibucaine, lidocaine, and procainamide. The pharmacological potential of an amide-type local anesthetic is important in that the enzyme pseudocholinesterase destroys the ester-type compound, but has no effect on the amide linkage (1).

Since the indole nucleus appears in many naturally occurring compounds, *viz.* ergot alkaloids, serotonin, reserpine, etc., the pharmacological value resulting from the addition of an amide group to the indole moiety is significant.

Received July 24, 1961, from the Department of Chemistry, Temple University, School of Pharmacy, Philadelphia, Pa.
Accepted for publication September 6, 1961.
This work was supported in part by a research grant (B-2102 A) from the USPHS.
Abstracted from a thesis presented by H. C. Wormser to the Graduate School of Temple University in partial fulfillment of the requirements for the degree of Master of Science, 1961.
† Fellow, American Foundation for Pharmaceutical Education.

EXPERIMENTAL

Indole-3-carbonyl Chloride.—Prepared essentially according to the method of Peterson, *et al.* (3), the indole, 20.0 Gm. (0.16 mole), was dissolved in 200 ml. of dry ether and 20 ml. of oxalyl chloride was added slowly to form the corresponding indole-3-glyoxylyl chloride as a bright yellow product. The

latter compound was dissolved in 300 ml. of tetrachloroethane and heated at 115–120° until the rapid evolution of carbon monoxide ceased. The reaction mixture was then allowed to cool rapidly to room temperature and 800 ml. of petroleum ether was added to precipitate the acid chloride. The resulting brown precipitate was filtered, washed with petroleum ether, and dried in a current of dry air. This crude product showed two peaks for the carbonyl grouping in the infrared region (1,750 cm.$^{-1}$ and 1,690 cm.$^{-1}$). It was concluded (3) that two compounds were present in the crude powder obtained: indole-3-carbonyl chloride and a condensation product of the latter, probably a dimer. The product was dissolved in boiling benzene, the solution was filtered, and 50 ml. of petroleum ether was added to the filtrate. The dark brown precipitate which formed was removed by filtration and discarded. Petroleum ether, 150 ml., was added to the now yellow solution and the resulting yellow precipitate collected and dried; m. p. 130° (decompn.). No melting point is given in the literature (3). Yield 5.4 Gm. (24%). This product was identified by hydrolyzing to indole-3-carboxylic acid.

N-(2-Diethylaminoethyl)-indole-3-carboxamide Hydrochloride.—Indole-3-carbonyl chloride, 1.0 Gm. (0.006 mole), was dissolved in 30 ml. of anhydrous ethyl acetate. N,N-Diethylethylenediamine, 1.39 Gm. (0.012 mole), dissolved in 5 ml. of ethyl acetate, was added to the above solution. The reaction mixture was shaken for ten minutes in a separator and then washed several times with water to remove the diamine hydrochloride salt. The ethyl acetate solution was dried over sodium sulfate and the solvent removed under reduced pressure. A light brown oil resulted. After washing this product several times with cold petroleum ether a light tan solid crystallized out slowly. This product was recrystallized from methanol-water to give a white compound having a melting point of 108–109°. The infrared spectrum indicated both N-H and amide structures. A positive Hopkins-Cole test for indole was obtained (propylene glycol). Recrystallization yielded 1.2 Gm. (83.4%). The hydrochloride salt was formed by dissolving the free base in ether and slowly adding, with stirring, a solution of dry hydrochloric acid in cold anhydrous ether. The crude hydrochloride which formed was found to be hygroscopic and had to be recrystallized several times from alcohol-ether to give a pure stable compound, m. p. 146–147°; yield, 0.80 Gm. (48.7%).

Anal.—Calcd. for $C_{15}H_{22}ClN_3O$: N, 14.21; C, 60.92; H, 7.50. Found: N, 14.36; C, 61.61; H, 7.94.

N-(3-Dimethylaminopropyl)-indole-3-carboxamide Hydrochloride.—Prepared in a similar manner to the foregoing, the free base, obtained by reacting 3.0 Gm. (0.018 mole) of indole-3-carbonyl chloride and 3.67 Gm. (0.036 mole) of N,N-dimethylpropylenediamine, melted at 119–122°; yield, 1.42 Gm. (34.6%). The hydrochloride, upon recrystallization from an ethanol-ether mixture, melted at 233–235°; yield, 0.85 Gm. (18.1%).

Anal.—Calcd. for $C_{13}H_{19}ClN_3O$: N, 14.91; C, 59.69; H, 7.15. Found: N, 15.39; C, 60.35; H, 7.23.

1-(3-Indolyl)-4-methylpiperazine Hydrochloride.—Prepared in an analogous manner from 3.0 Gm. (0.018 mole) of indole-3-carbonyl chloride and 2.32 (0.036 mole) of N-methylpiperazine, the free base was recrystallized from aqueous ethanol; m. p. 194°. The hydrochloride melted at 268–270°; yield, 0.96 Gm. (20.6%).

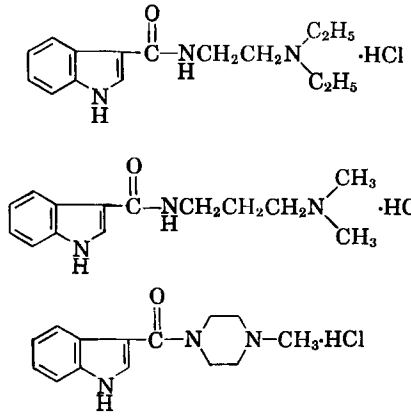
Anal.— Calcd. for $C_{14}H_{18}ClN_3O$: N, 15.02; C, 60.11; H, 6.48. Found: N, 14.55; C, 59.54; H, 6.35.

Testing Procedures.—*Local Anesthetic Activity.*— The winking reflex of the rabbit's eye was tested by touching the cornea gently with a von Frey hair. Several drops of the compound were instilled into the conjunctival sac and the time for the winking reflex to return to normal was noted.

Oxytocic Activity.—Five female rats were injected in the inguinal muscle 24 hours prior to the experiment with 5.5 mcg. of estradiol propionate in peanut oil. The uterus was excised and a strip (approximately two-thirds of the total horn in length) was suspended in a 100-ml. beaker and filled with Tyrode's solution at 30°. This temperature was maintained throughout the experiment. The results were recorded on a revolving smoked drum.

RESULTS

The following substituted amide derivatives of indole have been prepared



All three compounds showed local anesthetic activity. In all cases, the optimum concentration was 2%, lower concentrations had a shorter duration of action, and higher concentrations caused hyperemia. The most effective local anesthetic was N-(2-diethylaminoethyl)-indole-3-carboxamide hydrochloride.

The three compounds, in concentrations of 1 to 2 mg. per 100 ml. of solution, had a pronounced stimulating action on the estrous uterus causing the isolated segment to contract and increase in tone and motility. Epinephrine was added to determine whether the action of the compounds tested was specific and, in each case, a relaxation of the uterine muscle occurred. N-(3-Dimethylaminopropyl)-indole-3-carboxamide hydrochloride showed the most potent oxytocic activity.

REFERENCES

(1) Burger, A., "Medicinal Chemistry," 2nd ed., Interscience Publishers, Inc., New York, N. Y., 1960, pp. 37, 622.
(2) Bovet, D., Nitto, F., and Marini-Bettolo, G. B., *XIX International Physiological Congress, Abstracts of Communications*, 1953, 224.
(3) Peterson, P. A., Wolf, J. P., and Niemann, C., *J. Org. Chem.*, 23, 303(1958).