melting range, reagents, *etc.*, has been used wherever feasible.

Chlorphentermine hydrochloride,[1] an anorectic agent indicated for treatment of obesity, is distinguished by a selective pattern of pharmacologic action. This pattern of activity differs qualitatively and quantitatively from the amphetamines, whose indiscriminate effects at anorectic doses on the cardiovascular system and central nervous system are well known. Unlike the amphetamines, chlorphentermine hydrochloride exhibits no adverse effect on blood pressure, heart rate, or blood sugar.

The tablet formulation is a sustained action product so prepared that the medication is released gradually and without interruption over an 8-hr. period. The tablets are labeled to contain 65 mg. of chlorphentermine base which is equivalent to 78 mg. of the hydrochloride salt.

Identity Tests—The chloride test and ultraviolet absorption spectrum are not sufficient for differentiation of chlorphentermine hydrochloride from other sympathomimetic amine hydrochlorides. However, this information together with the infrared spectrum and melting range for chlorphentermine hydrochloride does provide a satisfactory identification. Mason *et al.* (1) have published a comprehensive report dealing with the physical properties of the drug.

Quantitative Methods—The nonaqueous titration for chlorphentermine hydrochloride with perchloric acid using crystal violet T.S. gave an average value of 99.9 ± 0.2%.[2] Analysis of commercial chlorphentermine hydrochloride tablets by the spectrophotometric method gave an average value of 100.5 ± 1.5%.[2] The suitability of the procedure was verified by an average recovery of 100.2 ± 0.3%[2] for a standard chlorphentermine hydrochloride solution carried through the extractive steps as included for the tablets.

[1] Marketed as Pre-Sate by Warner-Chilcott Laboratories, Division of Warner-Lambert Pharmaceutical Co., Inc., Morris Plains, N. J.

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[2] Maximum deviation from the mean value.

Notes

Novel Disubstituted Mannich Product as a Potential Anti-Infective Agent

By ROBERT A. MAGARIAN and W. LEWIS NOBLES

Identification of α,α′-di-{3-(3-azabicyclo[3.2.2]-nonyl)-methyl}-*p*-nitroacetophenone is reported.

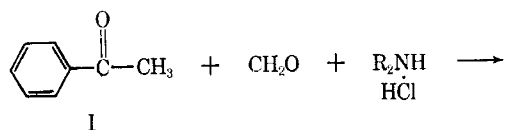
THE ISOLATION of a new disubstituted Mannich product was mentioned in a previous publication (1). The Mannich reaction, involving the reactive hydrogen compound acetophenone (I), is shown and illustrates the replacement of one of the active hydrogen atoms by an aminomethyl group which may arise from formaldehyde and the amine.

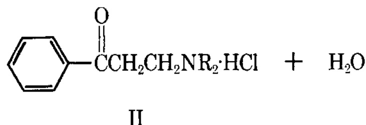
The Mannich product from a methyl ketone (II) contains labile hydrogens, and, in some cases, it is possible to proceed a step further yielding a compound with two basic groups (III) (2).

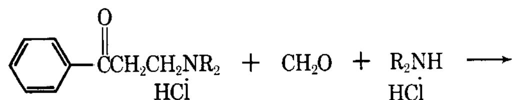
The amine hydrochloride is usually employed to provide the proper acidic environment. Cummings and Shelton (3) and Hellmann and Opitz (4) have carried out kinetic studies of the Mannich reaction and have concluded that the active aminomethylating agent is the electrophilic carbonium-immonium ion ($R_2\ddot{N}-\overset{+}{C}H_2 \leftrightarrow R_2\overset{+}{N}=CH_2$) which arises only under acidic conditions from the aminomethylol (*N*-hydroxymethylamine) derivative R_2NCH_2OH. The nucleophile is thought to be the enolic tautomer of the ketone which condenses with the aminomethylating agent.

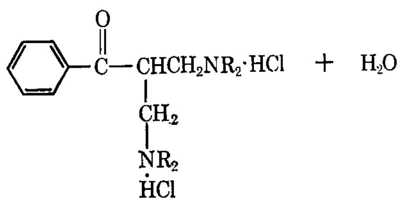
When the free amine is used in equimolar amounts with the other reactants, a dibasic Mannich product may be isolated. The mechanism of the Mannich reaction has not been completely elucidated; it is considered unlikely that a single mechanism can be postulated which will include all known cases involving this reaction. In these studies, the Mannich condensation involving equimolar amounts of *p*-nitroacetophenone, 37% aqueous formaldehyde, and 3-azabicyclo[3.2.2]nonane was first conducted by modifying the conditions set forth by Blanton and Nobles (5); that is, the free base rather than the hydrochloride salt of the amine was employed.

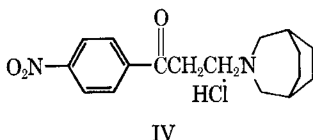
Received December 29, 1967, from the Department of Pharmaceutical Chemistry, School of Pharmacy, The University of Mississippi, University, MS 38677
Accepted for publication May 17, 1967.

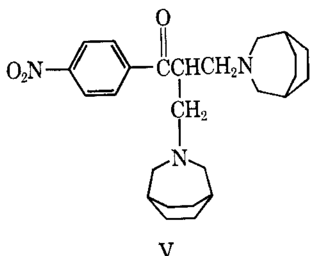












Consequently, the product (IV) as reported by Blanton and Nobles was not isolated. Instead, a bright yellow solid was obtained in 41% yield with a melting point of 148–152° (corrected). Recrystallization several times from absolute ethanol–acetone resulted in a product melting at 158–160° (corrected). The starting ketone melted at 79–82° (corrected) and the melting point of a mixture of the above product and the ketone was 76–79° (corrected). The product was shown to be the disubstituted derivative (V) as evidenced by elemental, infared, and nuclear magnetic resonance analyses.[1]

Anal.—Calcd. for $C_{26}H_{37}N_3O_3$ (mol. wt. 439.6): C, 71.04; H, 8.48; N, 9.56. Found: C, 70.87; H, 8.55; N, 9.46.

The infrared spectrum showed two strong nitro bands at 1520 cm.$^{-1}$ and 1350 cm.$^{-1}$. There was a strong carbonyl band at 1675 cm.$^{-1}$. The relative proton absorption of the NMR spectrum in deuterochloroform was in agreement with the proposed structure. Neither the ketonic nor enolic tautomers seemed to predominate. The phenyl proton pattern might have been partially collapsed by this tautomeric exchange. The NMR spectrum in trifluoroacetic acid showed the low resolution expected from the Stark effect due to a doubly charged ion.

Subsequent to the discovery of the antibacterial activity of the Mannich bases derived from *p*-nitroacetophenone (6), interest was aroused in the reduction of this compound to the corresponding carbinol in order to study the effect of this variation in structure on the biological activity of such a compound when appearing as a side chain in quinoline and acridine derivatives. Consequently, product V was reduced to the corresponding carbinol by the method of Chaikin and Brown (7) as modified by Nobles (8) using sodium borohydride. The reaction proceeded smoothly, and a light yellow solid was isolated in 67% yield with a melting point of 163–165° (corrected). Recrystallization from absolute ethanol resulted in a very light yellow, almost cream-colored, solid with a melting point of 163–164° (corrected). Two additional recrystallizations yielded a white solid with a melting point of 163–164° (corrected). A 2,4-dinitrophenylhydrazine test performed on the product was negative. The melting point of a mixture of the above product and the ketone was 140–144° (corrected).

Anal.—Calcd. for $C_{26}H_{39}N_3O_3$ (mol. wt. 441.6): C, 70.71; H, 8.90; N, 9.52. Found: C, 70.19; H, 8.90; N, 9.19.

Inspection of the infrared spectrum revealed that carbonyl absorption at 1675 cm.$^{-1}$ was absent, and a hydroxy band was present at 3400 cm.$^{-1}$. Also, there were two strong nitro bands at 1520 cm.$^{-1}$ and 1350 cm.$^{-1}$. The relative proton absorption in the NMR spectrum performed in deuterochloroform showed that the disubstituted alcohol was the product. Also, the NMR spectrum indicated a reduction from two electron-withdrawing groups on a benzene ring to only one; *i.e.*, the keto and nitro groups were present in the keto compound, but after keto reduction the only electron-withdrawing group on the benzene ring was the nitro group. The NMR spectrum in trifluoroacetic acid showed low resolution due to the Stark effect from two protonated nitrogens. Due to the optically active carbinol carbon atom, the cyclohexyl ring peaks are separated farther part—103 and 113 c.p.s.

The Mannich reaction involving equimolar amounts of *p*-nitroacetophenone, 37% aqueous formaldehyde, and 3-azabicyclo[3.2.2]nonane was repeated; however, this time the hydrochloride salt of the amine was used and the desired product IV was isolated as reported by Blanton and Nobles (5).

[1] Microanalyses were conducted by Alfred Bernhardt, Microanalytisches Laboratorium, Max-Planck-Institut fur Kohlenforschung, Mulheim (Ruhr), Germany. Infrared spectra were determined on a Perkin-Elmer model 137G Infracord spectrophotometer using potassium bromide disks. The Varian A-60(mc) was employed in obtaining the nuclear magnetic resonance spectra.

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Estimation of Amphetamine in Urine of Race Horses

By M. S. KARAWYA, M. A. EL-KIEY, S. K. WAHBA, and A. R. KOZMAN

Separation of amphetamine from urine of doped horses was achieved on anion-exchange resin (Na^+) and Silica Gel G plates. A micro method for the estimation of the free base was performed by measuring the violet color developed after nitration of the amphetamine eluted from either an ion-exchange resin column or Silica Gel G plates.

IN EGYPT in 1961, the menace of doping with amphetamine hovered over the horseraces and seriously threatened the results of the race. Since then, the race authorities have decided to stand against this new doping invader.

The method of analysis of amphetamine adopted by the B.P. (1) depends on steam distillation, while that of the E.P. (2) makes use of the direct extraction with ether.

The biological fluids contain normally some steam volatile and ether extractable bases (3) which may interfere in the determination of amphetamine. This fact renders the official methods not applicable for the separation and determination of amphetamine in urine of doped animals.

In an attempt to eliminate these interfering substances, ion-exchange and thin-layer chromatographic techniques were adopted.

Tampsett (4) was able to elute amphetamine on a cationic ion-exchange resin[1] column. He used 400 ml. of 8.0 *N* hydrochloric acid to achieve complete recovery. However, on trying this method, the eluate was found to be highly colored, and the colorimetric assay of the eluted amphetamine was almost impossible.

Materials—The materials used in this experiment were horse urine containing amphetamine (100 mcg./100 ml.); a column of ion-exchange resin[2] (Na^+) (1 × 15 cm.); and eluents used in the following succession: (*a*) 0.5% ammonium hydroxide, (*b*) acetone–water (3:7), (*c*) distilled water, (*d*) 10% aqueous solution of potassium chloride–1 *N* hydrochloric acid (1:1).

EXPERIMENTAL

Separation of Amphetamine on Ion-Exchange Resin (Na^+)

In the present work an anion-exchange resin[2] (Na^+) was chosen for the quantitative elution of amphetamine from horse urine.

Procedure—Add the urine sample to the resin column. Remove the impurities by washing successively with 50 ml. of each of the above-mentioned eluents (*a*), (*b*), and (*c*), and then elute the amphetamine with 150 ml. of eluent (*d*). Render the eluate alkaline with ammonium hydroxide T.S., extract the free base with chloroform (4 × 25 ml.), evaporate the chloroform, and keep the residue for the colorimetric estimation as described later. The results in Table I show the following observations.

A—The successive washing of the column with 0.5% ammonium hydroxide, acetone–water (3:7), and distilled water achieves the removal of most of the urine pigments and other impurities but not amphetamine.

B—Complete recovery of amphetamine is effected by washing the ion-exchange resin column with 150 ml. of 10% potassium chloride–1 *N* hydrochloric acid (1:1).

Separation of Amphetamine on Silica Gel G Plates

Separation of amphetamine from the saliva on silica gel plates was performed by Baeumler *et al.* (5) using triethanolamine–acetone–methanol (0.03:1:1). However, it was found difficult to separate amphetamine from urine on adopting the same procedure.

Other systems were tried and the system *n*-butanol–glacial acetic acid–water (4:1:5) proved to effect good separation of amphetamine from urine pigments, R_f values being 0.65 and 0.95, respectively.

Amphetamine is located with bromocresol blue T.S. when a blue color is obtained.

Procedure—Amphetamine is first extracted from urine before its application on the plate as follows.

TABLE I—RECOVERY OF AMPHETAMINE FROM HORSE URINE BY COLUMNS OF ION-EXCHANGE RESIN (Na^+)

Fraction of Effluent	100 mcg./100 ml. Urine: Ion-Exchange Resin	Color of Effluent
Eluent (*a*) (50 ml.)	...	Yellow
Eluent (*b*) (50 ml.)	...	Yellow
Eluent (*c*) (50 ml.)	...	Yellow
Eluent (*d*) (50 ml.)	58.20	Colorless
Eluent (*d*) (50 ml.)	30.15	Colorless
Eluent (*d*) (50 ml.)	13.20	Colorless
8 *N* HCl (400 ml.)	...	Colorless
Total	101.55	

Received January 9, 1967, from the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt, U. A. R.
Accepted for publication March 30, 1967.

[1] Dowex-50, Dow Chemical Co., Midland, Mich.
[2] Amberlite IRC-50, Rohm & Haas, Philadelphia, Pa.