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GENERAL, PHYSICAL AND INORGANIC CHEMISTRY

[Contribution from the University of Chicago Toxicity Laboratory[1]]

Study of the Hydrolysis of Several Physostigmine Analogs

By Saul W. Chaikin

Physostigmine (I) and many of its analogs have been the subject of much study with regard to certain physiological properties which they exhibit

I
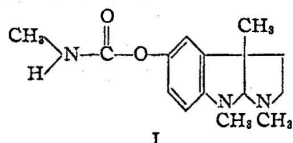
when introduced into the animal body.[2-6] The phenyl carbamate nucleus has been shown to be the active group in the alkaloid.[2] Physostigmine and certain other phenyl carbamate derivatives strongly inhibit choline esterase. The constriction of the pupil of the eye (miosis) of the test animal when the compound is instilled into the eye has been taken as an index of the physiological action and this has been found to depend on variations in the chemical structure of the carbamate used.

Physostigmine is not stable in aqueous solution. A systematic study of analogs of this compound was undertaken by Aeschlimann and Reinert[4] with the object of finding a drug suitable for therapeutic use with a greater degree of stability to hydrolysis. They found the N,N-dialkyl carbamates to be considerably more stable in water solution than the N-monoalkyl compounds and to have a physiological action of the same order of magnitude. The mode of decomposition which they proposed for the N-methyl phenyl carbamates is the formation, in slightly alkaline solution, of the phenol and the isocyanate. The odor of isocyanate was detected when solutions of N-methyl carbamates were boiled.

$$CH_3NHCOOC_6H_4R \longrightarrow CH_3NCO + HOC_6H_4R$$
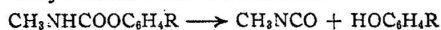
With excess alkali

$$CH_3NCO + 2OH^- \longrightarrow CH_3NH_2 + CO_3^-$$

It was also reported that hydrolysis was greatly suppressed when the pH of the solution was less than 5. P. D. Bartlett[7] observed that the methylamine produced on hydrolysis in a solution at a hydrogen ion concentration of *ca.* pH 9 catalyzed further decomposition of an N-methyl carbamate.

Experiments with this class of compounds were carried out in this Laboratory involving tests of

toxicity following subcutaneous injection into mice.[8] It became of some interest to know the stability *in vitro* of the mono- and di-alkyl carbamates under conditions of hydrogen ion concentration and temperature similar to those in the blood and tissue fluids of animals (pH 7.4 and 38°).

The compounds studied were the N-methyl (M), N,N-dimethyl (DM) and the N,N-pentamethylene (PM) derivatives of 4-dimethylamino-3-isopropylphenyl carbamate methiodide. Since
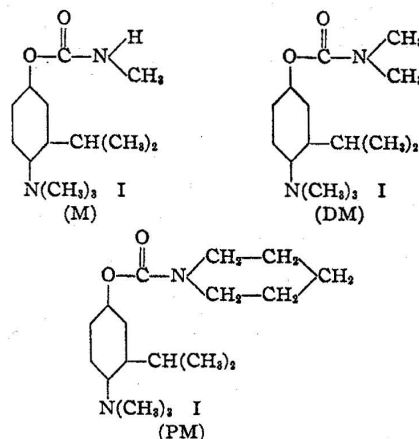
the substituents on the benzene ring are the same in each case, differences in hydrolytic stability of these compounds are due to differences in the carbamate residue.

Experimental

Materials.—The sample of M used was applied by R. L. Shriner, M. E. Synerholm and J. C. Speck, Jr., who were the first synthesizers of this compound. The melting point was given as 184.5° (dec.).

Anal. Calcd. for $C_{14}H_{23}O_2N_2I$: I, 33.55. Found: I, 33.50.

DM was first synthesized by Stevens and Beutel[6] and the sample used in this investigation was submitted by H. Gilman. The melting point was reported as 159° (dec.).[9]

Anal. Calcd. for $C_{15}H_{25}O_2N_2I$: N, 7.15; I, 32.38. Found: N, 7.19 and 7.01; I, 32.40, 32.13 and 32.41.

V. A. Englehardt and L. I. Smith originally prepared PM. The melting point was reported as 161° (dec.).

Anal. Calcd. for $C_{18}H_{29}O_2N_2I$: C, 50.00; H, 6.76. Found: C, 50.21; H, 7.00.

(1) This work was done under contract with the Medical Division of the Chemical Warfare Service.

(2) E. Stedman, *Biochem. J.*, **20**, 719–734 (1926).

(3) E. Stedman, *ibid.*, **23**, 17–24 (1929).

(4) Aeschlimann and Reinert, *J. Pharm. Exp. Therap.*, **43**, 413–444 (1931).

(5) White and Stedman, *ibid.*, **41**, 259 (1931).

(6) Stevens and Beutel, This Journal, **63**, 308–311 (1941).

(7) P. D. Bartlett. *et al.*, unpublished data.

(8) C. F. Failey, W. Elder and B. Ginsburg, unpublished data, University of Chicago Toxicity Laboratory.

(9) The following is a note from a letter from H. Gilman giving information about the compound DM: "The melting point reported by Stevens and Beutel, This Journal, **63**, 310 (1941) was 170°. Subsequently, we learned by private communication that the corrected melting point was 165.5°. A melting point determination of their sample taken in the same bath and at the same time as ours was 158° (ours melting at 159°)."

The sample of P (identified later) was synthesized by H. Gilman and D. A. Shirley. The melting point was given as 205° (dec.).

Anal. Calcd. for $C_{12}H_{20}ONI$: I, 39.5. Found: I, 39.6 and 39.3.

Procedure.—The course of the hydrolysis was followed by measuring the change in absorption of light by solutions of carbamate using wave lengths at which the difference in absorption by the carbamate and phenol was great. The Beckman Quartz Spectrophotometer Model DU was used after having been calibrated against solutions of known absorption maxima. The carbamates were all found to be stable in acid solution so that a standard procedure was followed involving removing aliquots from the buffered hydrolyzing solution at intervals and adding them to an acid solution to stop the hydrolysis. Spectral absorption curves were prepared (Fig. 1) for M, DM, PM and P (a pure sample of the phenol which is produced on hydrolysis of the carbamates). For purposes of comparison all spectrophotometric data have been corrected to a concentration of 0.15 mM per liter. In all cases the concentrations of the solutions prepared were within 5% of this value. The curve for P is useful in determining completeness of hydrolysis and in ascertaining the effect of hydrolysis products other than phenol on the absorption curve of the completely hydrolyzed carbamate.

The hydrolysis rate was measured as follows. A 250-ml. volumetric flask containing phosphate buffer (NaH_2PO_4 0.002 M, Na_2HPO_4 0.01 M) adjusted to pH 7.4 was immersed in a water-bath maintained at 38° until thermal equilibrium was attained. The flask was removed and a sample of carbamate weighing about 15 mg. was introduced. The solution was quickly mixed and replaced in the bath. At intervals, 10-ml. aliquots were withdrawn and added to 0.17 ml. of 6 N hydrochloric acid which so acidified the solution as to stop hydrolysis. Absorption of light of 275 mμ wave length by the solution was then measured. Quartz cuvettes of 1.00-cm. optical depth were used.
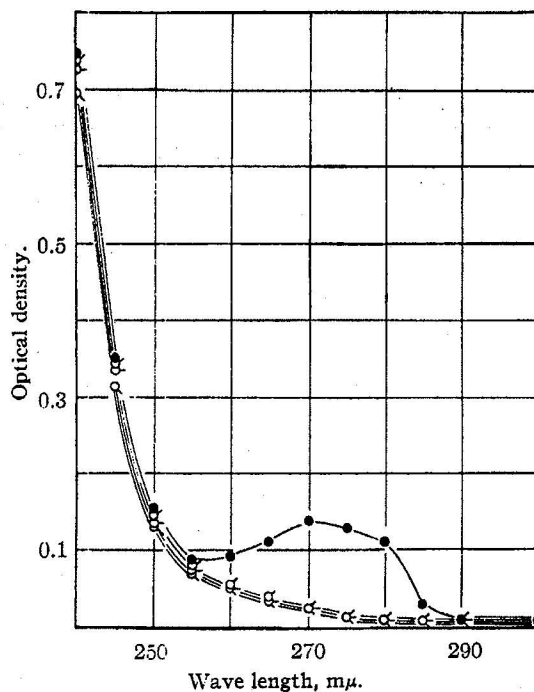
The solutions used in preparing the curves in Fig. 1 contained 0.04 mM. of compound in a 250-ml. volumetric flask containing phosphate buffer and hydrochloric acid of the same concentration as given above. The blank used for the readings was a solution of buffer and acid, also of the above concentrations.

Results and Discussion

It was found that the solutions of DM and PM at 38° and pH 7.4 showed no significant hydrolysis during the period of observation of four days. M was found to be unstable under these conditions. Since the hydrolysis of M is more rapid in alkaline solution it is undoubtedly second order, but the hydroxyl ion concentration being held constant in these experiments, the rate depends only on the carbamate concentration. A plot of the log of the fraction hydrolyzed against time results in a straight line (Fig. 2). Calculation of reaction constants can therefore be made by methods ordinarily applied to first order reactions. When complete hydrolysis of M is effected spectral absorption data for the region from 255 to 300 mμ are identical, within experimental error, with those of curve P. At shorter wave lengths the deviation gradually increases until at 240 mμ it is 5% higher than curve P. This deviation may be caused by other products of hydrolysis but since it occurs outside the region taken for hydrolysis measurements it is of no consequence in this connection. The half life for M at 38° and pH 7.4 is seen from Fig. 2 to be fifty-two minutes and the velocity constant 0.0133.

Fig. 1.—Spectral absorption of P, M, DM and PM, concn. of reagents: NaH_2PO_4, 0.002 M; Na_2HPO_4, 0.01 M; HCl, 0.1 M; compound, 0.15 mM; ●, P; ◖, M; ○⁻, DM; ◒, PM.
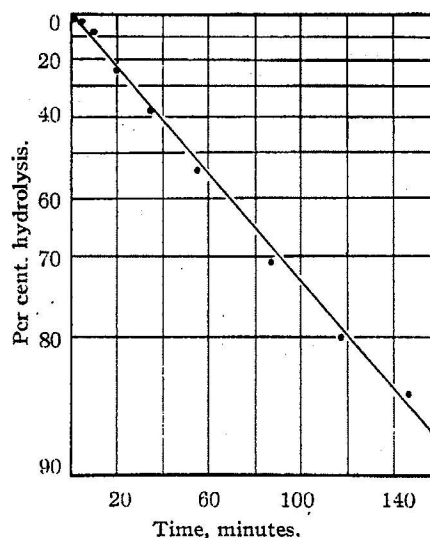

Fig. 2.—Hydrolysis–time curve for M at pH 7.4 and 38°.

This study is concerned only with the rate of disappearance of the carbamate (or the rate of appearance of the phenol) and not with the route of mechanism by which such a transformation occurs. The work of Bartlett,[7] mentioned earlier, indicates that the hydrolysis reaction is probably

specific amine or general base catalyzed[10] in which case variation of reaction rate with buffer concentration (at pH 7.4) may be expected.

Summary

Hydrolytic stability of three physostigmine

(10) L. P. Hammett, "Physical Organic Chemistry," 1st ed., McGraw-Hill Book Company, Inc., New York, N. Y., 1940, pp. 344-345 and 215-218.

analogs at 38° and pH 7.4 was investigated by measurement of light absorption of the phenolic hydrolysis product at a wave length of 275 mμ. The two N,N-dialkyl carbamates were stable under these conditions during a four day observation period. The N-methyl carbamate was found to be unstable and to have a half life of 52 minutes and a velocity constant of 0.0133.

Chicago, Illinois   Received December 12, 1946

The Pigments of Cottonseed. III.[2] Gossyfulvin, a Native Cottonseed Pigment Related to Gossypol[3]

By Charlotte H. Boatner, Robert T. O'Connor, Maizie C. Curet and Carolyn S. Samuels

An orange colored pigment, gossyfulvin, has previously been detected[4] in cottonseed. Although gossyfulvin has been shown to differ from gossypol in many of its properties it can be readily converted into the latter pigment. Since the work on gossyfulvin was reported, larger quantities of the pigment have been prepared by the procedure outlined in the accompanying diagram and it has therefore been possible to obtain further insight into its structure and its relationship to gossypol.

Gossyfulvin forms rather large orange-colored rhombohedra (Fig. 1), changing at 212° to a more deeply colored form which melts with decomposition at 238–239° (cor.). The crystalline form and habit of gossyfulvin differentiate it sharply from gossypol, which latter pigment, upon recrystallization from diethyl ether and petroleum naphtha, forms clusters of dog-toothed prisms (Fig. 2), m. p. 182.5–183.5°.

Gossyfulvin reacts with strong mineral acids when its chloroform solutions are treated with concentrated aqueous solutions of these acids yielding gossypol in amounts equal to as much as 86.8% of the weight of gossyfulvin treated. On the basis of the experimentally determined elementary composition, $C_{34}H_{34}N_2O_8$, the molecular weight of gossyfulvin is 598.

Gossypol obtained after recrystallization from diethyl ether and petroleum naphtha at low temperatures and dried without elevation of temperature yields analytical values which agree with those calculated for $C_{30}H_{32}O_9$.
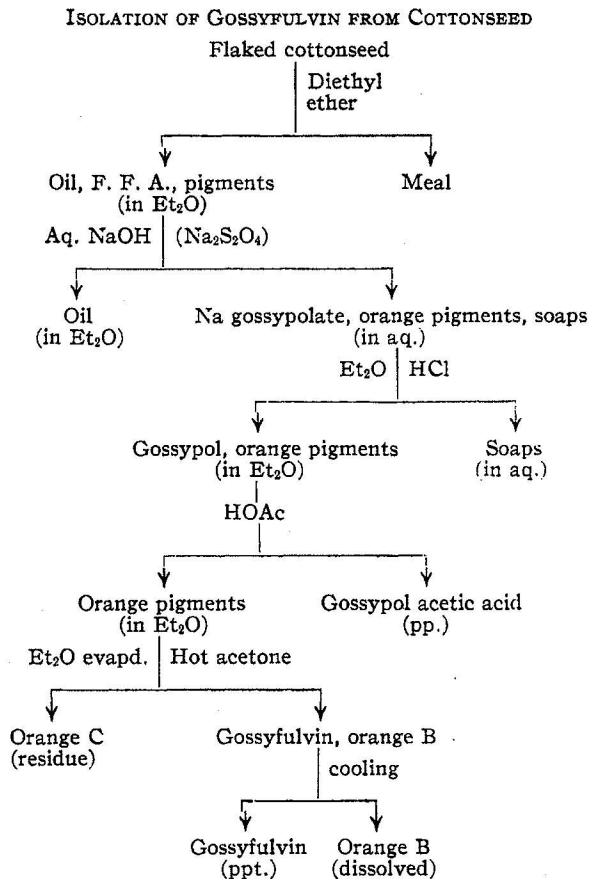
Comparison of gossyfulvin with such nitrogen derivatives of gossypol as diamino- and dianilinogossypol, reveals several significant differences,

(1) One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

(2) For previous paper of this series see Boatner, Samuels, Hall and Curet, This Journal, 69, 668–672 (1947).

(3) Presented before the 109th Meeting of the American Chemical Society, Atlantic City, New Jersey, April 8 to 12, 1946.

(4) Boatner, Caravella and Samuels, This Journal, 66, 838 (1944).

Isolation of Gossyfulvin from Cottonseed

```
                    Flaked cottonseed
                          │ Diethyl
                          │ ether
          ┌───────────────┴───────────────┐
          ▼                               ▼
   Oil, F. F. A., pigments              Meal
      (in Et₂O)
   Aq. NaOH │ (Na₂S₂O₄)
      ┌─────┴─────────────────────────┐
      ▼                               ▼
     Oil                 Na gossypolate, orange pigments, soaps
   (in Et₂O)                        (in aq.)
                            Et₂O │ HCl
                    ┌─────────────┴─────────────┐
                    ▼                           ▼
         Gossypol, orange pigments           Soaps
              (in Et₂O)                     (in aq.)
                HOAc │
          ┌──────────┴──────────┐
          ▼                     ▼
   Orange pigments        Gossypol acetic acid
      (in Et₂O)                  (pp.)
  Et₂O evapd. │ Hot acetone
      ┌────────┴────────┐
      ▼                 ▼
   Orange C        Gossyfulvin, orange B
   (residue)              │ cooling
                  ┌────────┴────────┐
                  ▼                 ▼
             Gossyfulvin        Orange B
               (ppt.)          (dissolved)
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e. g., the melting point of gossyfulvin lies between those of the other nitrogen derivatives. The compounds differ with respect to solubility and stability. Diaminogossypol dissolved in diethyl ether or warmed in acetic acid is reported[5] to evolve ammonia and revert to gossypol. Dianilinogossypol, on the other hand, is one of the most stable of the compounds formed from gossypol, and is hydrolyzed to gossypol only upon re-

(5) Miller and Adams, *ibid.*, 1736–1738 (1937).