[CONTRIBUTION FROM THE WARNER INSTITUTE FOR THERAPEUTIC RESEARCH]

The Reaction of α-Dimethylaminoacids with Acetic Anhydride

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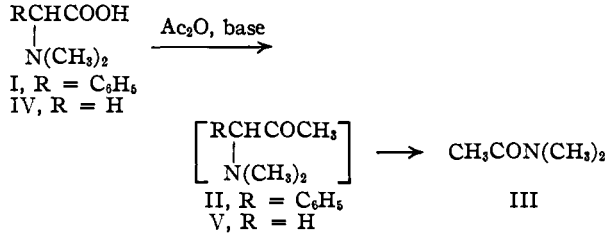
It has been shown that α-dimethylaminophenylacetic acid and dimethylaminoacetic acid on treatment with acetic anhydride and pyridine undergo disruption of the molecules and formation of N,N-dimethylacetamide. Dimethylaminoacetone, the presumed reaction intermediate in the case of dimethylaminoacetic acid, has been shown to yield the same degradation product, and a suggestion is made concerning the course of the reaction.

Although the report by Dakin and West,[1] in 1928, that α-dimethylaminophenylacetic acid underwent no reaction when it was heated on the steam-bath with acetic anhydride and pyridine has weighed heavily in the formulation of several mechanisms which have been proposed[1-6] for the conversion of α-aminoacids to α-aminoketones by means of a mixture of acylating agent and base, this finding has not been verified and its importance in the formulation of a mechanism for this reaction is sufficient to make its verification desirable, particularly since it has been shown[7,8] that merely raising the temperature from 100 to 130° is sufficient to bring about decarboxylation of methylaminoacetic acid in the presence of acetic anhydride and pyridine.

We have found that when α-dimethylaminophenylacetic acid (I) is refluxed for one hour with acetic anhydride and pyridine there is evolved approximately one molar equivalent of carbon dioxide. None of the expected α-dimethylamino-α-phenylacetone (II) could be isolated from the reaction mixture, the products therefrom being N,N-dimethylacetamide (III) and a resinous polymeric material. The same vigorous evolution of carbon dioxide occurred when anhydrous sodium

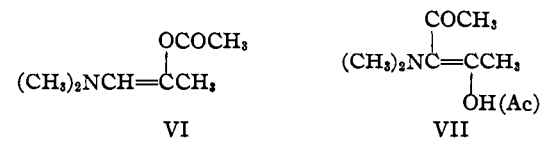


acetate was substituted for pyridine in the reaction mixture, although no attempt was made in this case to isolate the acetamide derivative. Neither boiling pyridine alone nor boiling acetic anhydride alone caused the evolution of carbon dioxide from I.

The same reaction, with refluxing acetic anhydride and pyridine, was carried out on dimethylaminoacetic acid (IV) with the same results; there was vigorous evolution of carbon dioxide but no dimethylaminoacetone (V) could be isolated, the only identifiable product being N,N-dimethylacetamide, produced together with a non-volatile resinous substance. Although boiling pyridine alone had no effect on IV, boiling acetic anhydride alone was as effective in the conversion of IV to III as was the mixture of acetic anhydride and pyridine.

Without entering into speculations concerning the mechanism of the decarboxylative acylation of α-amino acids, we made the assumption that the above-described reactions first took the normal course, whatever that may be, and originally formed α-dimethylaminoacetones; we believe this to be a reasonably justifiable assumption, because the reaction with the N,N-dimethylamino acids is carried out in precisely the same manner, and proceeds with evolution of carbon dioxide in precisely the same manner, as does the reaction with N-monomethylaminoacetic acid.[8] To test our assumption that dimethylaminoacetones were actually formed and then underwent further transformations in the reaction medium we prepared V by another method and subjected it to the reaction conditions. *Dimethylaminoacetone, refluxed one hour with acetic anhydride, gave a 97% yield of N,N-dimethylacetamide and a non-volatile resinous material similar to that which had been obtained from the reaction with dimethylaminoacetic acid.*

After consideration of the structural features of the dimethylaminoacetone molecule it is not difficult to envisage this molecule's disruption by acetic anhydride. Since neither aminoacetone[9] nor monomethylaminoacetone[8] are cleaved by acetic anhydride but only are converted to further monoacetylation products of their N-acetyl derivatives the responsibility for the cleavage of dimethylaminoacetone, *via* its further monoacetylation product, must reside in the more strongly basic dimethylamino group which cannot have its basicity decreased by acetylation, as occurs with the two other acetone derivatives. As far as the ultimate effect is concerned, it is immaterial whether acetylation by an acetic anhydride molecule with liberation of an acetic acid molecule occurs on carbon or on oxygen: the end result, in either case will be a vinylamine type of molecule, VI if acetylation occurs on oxygen and VII (or the enol acetate of the 1,3-diketone) if it occurs on carbon.



In the presence of the acetic acid formed in the acetylation step the vinylamine might be expected to split[10,11] into dimethylamine and a (substituted)

(1) H. D. Dakin and R. West, *J. Biol. Chem.*, **78**, 91 (1928).
(2) H. D. Dakin and R. West, *ibid.*, **78**, 745 (1928).
(3) G. H. Cleland and C. Niemann, THIS JOURNAL, **71**, 841 (1949).
(4) C. S. Rondestvedt, B. Manning and S. Tabibian, *ibid.*, **72**, 3183 (1950).
(5) S. Searles and G. J. Cvejanovich, *ibid.*, **72**, 3200 (1950).
(6) J. W. Cornforth and D. F. Elliott, *Science*, **112**, 534 (1950). See also J. L. O'Brien and C. Niemann, THIS JOURNAL, **72**, 5348 (1950).
(7) R. H. Wiley, *Science*, **111**, 259 (1950).
(8) R. H. Wiley and O. H. Borum, THIS JOURNAL, **72**, 1626 (1950).
(9) R. H. Wiley and O. H. Borum, *ibid.*, **70**, 2005 (1948).
(10) C. Mannich and H. Davidsen, *Ber.*, **69**, 2106 (1936); C. Mannich, K. Handke and K. Roth, *ibid.*, **69**, 2112 (1936).
(11) R. Adams and J. E. Mahan, THIS JOURNAL, **64**, 2588 (1942).

vinyl acetate; the former gives rise, by acetylation, to the N,N-dimethylacetamide isolated from the reaction mixtures and the latter can account for the non-volatile resinous polymeric accompaniment.

It is worthy of note that the participation of an α-dimethylamino acid in the decarboxylative acylation reaction with acetic anhydride and pyridine is incapable of accommodation by any mechanism for the reaction which requires[3,4,6] intermediate oxazolone formation, although these findings do not invalidate the oxazolone mechanism for the Dakin-West reaction of compounds capable of giving this type of intermediate.

Experimental[12]

N,N-Dimethyl-α-aminophenylacetic Acid.—We verified as reported by Clarke, Gillespie and Weisshaus,[13] that the formaldehyde–formic acid methylation procedure did not work on α-aminophenylacetic acid and the desired methylated acid was alternatively prepared by two different methods: (A) the catalytic alkylation of the amine with formaldehyde, hydrogen and a catalyst activator, by the general method recently described by Stuhmer[14]; and (B) by aminolysis of the chloroester.

Procedure (A).—A solution of 7.6 g. (0.05 mole) of *dl*-α-aminophenylacetic acid in methanol (150 cc.), aqueous formaldehyde (26 cc. of 35% solution) and concd. hydrochloric acid (4.5 cc.) was hydrogenated on a Parr shaker at room temperature in the presence of Adams platinum oxide (1.0 g.); 2400 cc. of hydrogen were taken up in 125 minutes. After removal of the catalyst by filtration the filtrate was taken to dryness under vacuum and the residue (10.7 g.) was refluxed for an hour with dilute hydrochloric acid in order to hydrolyze any methyl ester that might have been formed. After evaporation of this solution to dryness under vacuum and several crystallizations of the residue from absolute alcohol there was obtained 5.57 g. (62% yield) of N,N-dimethyl-α-aminophenylacetic acid hydrochloride, m.p. 230–232° (dec.), which has not been previously described.

Anal. Calcd. for $C_{10}H_{13}NO_2 \cdot HCl$: Cl (ionic), 16.47. Found: Cl (ionic), 16.59.

Procedure (B).—α-Chlorophenylacetyl chloride (prepared in 73% yield[15]) (45 g., 0.24 mole) was solvolyzed by the gradual addition to ethanol. After the exothermic reaction was ended the solvent was removed under vacuum and the residual ethyl α-chlorophenylacetate was slowly added to a solution of dimethylamine (35 g.) in benzene. After this exothermic reaction subsided the mixture was stirred 2.5 hours at room temperature and then an additional hour under reflux. Working up of the reaction mixture in the usual manner gave 38 g. (77% yield) of ethyl α-dimethylaminophenylacetate, b.p. 71–78° (0.6 mm.); reported,[16] b.p. 135° (13 mm.). Thirty-three grams (0.16 mole) of the aminoester was hydrolyzed by one-hour refluxing with 1:1 hydrochloric acid (300 cc.); after removal of the solvent under vacuum and azeotropic drying of the residue with benzene the dry residue was boiled with dry acetone (300 cc.) and filtered to give 20.5 g. (54% yield) of the same amino acid hydrochloride that was obtained by procedure (A); there was no depression in decomposition point on admixture.

Anal. Calcd. for $C_{10}H_{13}NO_2 \cdot HCl$: Cl (ionic), 16.47. Found: Cl (ionic), 16.29.

3.8 g. (0.0176 mole) of the aminoacid hydrochloride was neutralized with 176 cc. of 0.1 *N* sodium hydroxide and the solution was evaporated to dryness under vacuum. The residue was boiled with absolute alcohol (100 cc.) filtered, then the filtrate was taken to dryness under vacuum to give 3.0 g. (94% yield) of amorphous amino acid, m.p. 240–241° (dec.); the melting point could be raised to 254–255.5° (dec.) by careful recrystallization from aqueous acetone; the reported[17] value is 260–262°.

Reaction of Acetic Anhydride and Pyridine with N,N-Dimethyl-α-aminophenylacetic Acid.—A mixture of N,N-dimethyl-α-aminophenylacetic acid (3.5 g., 0.0195 mole), acetic anhydride (20 cc.) and pyridine (20 cc.) was refluxed one hour, during which time 373 cc. (*not* N.T.P.) of carbon dioxide was evolved. Fractional vacuum distillation of the reaction mixture gave 2.3 g. of material, b.p. 60–72° (5 mm.) and no other volatile material; the resinous residue would not distil at 300° (0.2 mm.). On redistillation the product had b.p. 69–72° (13 mm.) and $n^{25}D$ 1.4240. A small amount of the liquid was refluxed for three hours with concd. hydrochloric acid and the mixture then evaporated to dryness to leave a colorless crystalline residue, m.p. 162–164°; the mixed melting point with dimethylamine hydrochloride (m.p. 168–169°) was 165–167°. Another small portion of the liquid was refluxed 4.5 hours with 10% sodium hydroxide (20 cc.), the solution brought to *p*H 6 with 2 *N* hydrochloric acid, diluted with 15 cc. alcohol and then refluxed 1.5 hours with 1.0 g. of *p*-bromophenacyl bromide; on cooling, the solution deposited a crystalline material which melted, after recrystallization from 50% alcohol, at 80–81.5°; the mixed melting point with *p*-bromophenacyl acetate (m.p. 78–80°) was 79–81°. The boiling points reported for N,N-dimethylacetamide are 165.5° (754 mm.)[18] and 83–84° (32 mm.),[19] and the refractive index is given[19] as $n^{25}D$ 1.4370.

Dimethylaminoacetic Acid.—Dimethylaminoacetonitrile (prepared in 80% yield by the procedure of Turner[20]) was hydrolyzed to the acid by barium hydroxide, as very generally described by Eschweiler.[21] A mixture of dimethylaminoacetonitrile (184 g., 1.00 mole) and barium hydroxide (171 g., 1.00 mole) in 3420 cc. of water (to give a 5% solution of the base) was refluxed 16 hours while protected from atmospheric carbon dioxide by an ascarite tube. The solution was then saturated with carbon dioxide, the precipitated barium carbonate was removed by filtration, and the filtrate was evaporated under vacuum to a crystalline mass that weighed 102.6 g. (99.5% yield) and melted, after recrystallization from alcohol–ether, at 161–166°; the reported[22] value is 157–160°.

Reaction of Acetic Anhydride and Pyridine with Dimethylaminoacetic Acid.—A mixture of dimethylaminoacetic acid (20 g., 0.194 mole), acetic anhydride (100 cc.) and pyridine (100 cc.) was refluxed three hours; during the first hour 2200 cc. of carbon dioxide was evolved, and only *ca.* 450 cc. more came off during the last two hours. Fractional vacuum distillation of the reaction mixture gave 4.7 g. of relatively pure N,N-dimethylacetamide, b.p. 64–72° (11–12 mm.); 3.5 g. of a less pure fraction; and 14 g. of non-volatile resinous material. On redistillation the N,N-dimethylacetamide boiled at 69–72° (13 mm.), and had $n^{25}D$ 1.4267. Substitution of sodium acetate for pyridine in the reaction mixture apparently had no effect on its course; carbon dioxide evolution was vigorous, although no attempt was made to isolate N,N-dimethylacetamide.

When dimethylaminoacetic acid (5 g.) was refluxed with pyridine (50 cc.) alone no carbon dioxide was evolved and the starting material was recovered unchanged.

However, when the dimethylamino acid (5 g.) was refluxed with acetic anhydride (50 cc.) alone 870 cc. of carbon dioxide was evolved in 80 minutes and on fractional vacuum distillation there was obtained 1.7 g. of N,N-dimethylacetamide, b.p. 70° (16 mm.), $n^{25}D$ 1.4240; the non-volatile residue was the now-expected resinous material.

Action of Acetic Anhydride on Dimethylaminoacetone.—Dimethylaminoacetone was prepared in 71% yield from dimethylamine and chloroacetone in benzene solution, followed by filtration of dimethylamine hydrochloride and two fractional distillations of the filtrate; b.p. 118–124°, reported[23] 123°. A mixture of the aminoketone (10.1 g., 0.10 mole) and acetic anhydride (50 cc.) was refluxed one

(12) All melting points and boiling points herein reported are uncorrected.

(13) H. T. Clarke, H. B. Gillespie and S. Z. Weisshaus, THIS JOURNAL, **55**, 4571 (1933).

(14) W. Stuhmer and W. Neumann, *Chem. Ber.*, **83**, 66 (1950).

(15) J. A. King and F. H. McMillan, THIS JOURNAL, **72**, 833 (1950).

(16) M. Tiffeneau and E. Fourneau, *Bull. soc. chim.*, [4] **13**, 971 (1913), prepared this amino ester by essentially the same procedure from the corresponding bromo ester.

(17) F. Knoop and H. Oesterlin, *Z. physiol. Chem.*, **170**, 186 (1927).

(18) A. P. N. Francimont, *Rec. trav. chim.*, **2**, 329 (1883).

(19) J. W. Bruhl, *Z. physik. Chem.*, **22**, 373 (1897).

(20) R. A. Turner, THIS JOURNAL, **68**, 1607 (1946).

(21) W. Eschweiler, *Ann.*, **278**, 39 (1894).

(22) J. Johnston, *Proc. Roy. Soc.* (*London*), **76A**, 82 (1907).

(23) R. Stoermer and O. Dzimski, *Ber.*, **28**, 2220 (1895), prepared it in aqueous solution, which is considerably less convenient.

hour and then fractionally distilled at atmospheric pressure to give 8.43 g. (97% yield) of N,N-dimethylacetamide, b.p. 163–165°,[18] $n^{25}D$ 1.4234, and a pot-residue of non-volatile resinous material.

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Physical Chemical Studies on Rabbit Papilloma Virus[1]

BY H. K. SCHACHMAN

It has been suggested by Beard and co-workers that rabbit papilloma virus varies in physical properties from preparation to preparation and that there are natural differences in the virus isolated at different times from different source material. In the light of this interpretation of the experimental data several preparations of rabbit papilloma virus were examined in an ultracentrifuge, Tiselius apparatus and viscometer. An impurity not previously reported was observed in sedimentation and electrophoresis patterns. The high intrinsic viscosities observed were attributed to this impurity. As in the studies of Beard and co-workers the sedimentation constant of the principal component varied from preparation to preparation. These variations were explained on the basis of different viscosities of the preparations due to varying amounts of the impurity in the different preparations. Thus, it is not necessary to invoke the hypothesis that the virus particles themselves vary in their physical properties from preparation to preparation.

Introduction

Among the animal viruses isolated thus far, rabbit papilloma virus is one of the few obtained in relatively pure form. In fact, the homogeneity of the virus preparations, as judged by several criteria, has been such as to justify favorable comparison of the virus with many so-called pure proteins.

The results of early studies by Beard and co-workers[2a,b] on twelve different preparations of purified virus, using the ultracentrifuge, the Tiselius electrophoresis apparatus, diffusion measurements, and the electron microscope, were interpreted by them as indicating homogeneity of the virus particles. All of the data fit into a unified picture indicating a uniformity of the virus particles with respect to size, shape, density and electrical properties.

In a later paper, Sharp, Taylor and Beard[3] reported on the density of the virus particles in solution. From sedimentation studies in solutions of varying density, they calculated that the virus particles contained about 58% water by volume. At the same time, they pointed out that the variations in sedimentation constant found earlier for different preparations of the virus were far beyond the experimental error. In a re-evaluation of the data, they attributed these variations in sedimentation constant in different preparations to natural differences in the virus particles, probably in their water content.

Inasmuch as the particles within each preparation appeared to have essentially the same physical properties and, furthermore, since each preparation was isolated from a pool of warts obtained at random from many rabbits, it seemed unlikely that the virus particles themselves had different physical properties in the various preparations. Therefore, an attempt was made to find an alternative explanation for the variations in sedimentation constant from preparation to preparation. Through the kindness of Dr. C. A. Knight of this Laboratory a limited amount of purified rabbit papilloma virus was made available. This communication presents the results of some physical chemical measurements made on this material and an alternative explanation for the discrepancies observed in the earlier sedimentation studies.

Materials and Methods

Several preparations of rabbit papilloma virus were used for these studies. All of the virus was obtained from warty growths of cottontail rabbits trapped in Kansas in different years. Details of the method of purification are given in a paper by Knight.[4] In general, the method is similar to that used by Beard and associates[5] and consists of a series of four or five cycles of alternate high- and low-speed centrifugation. M/15 phosphate buffer at *p*H 6.6 was used as a solvent during the isolation and for many of the physical chemical measurements. Ultracentrifuge studies were performed in both an air-driven ultracentrifuge of the Bauer–Pickels type and an electrically driven Spinco ultracentrifuge. Both ultracentrifuges were equipped with a Philpot–Svensson optical system. Electrophoresis experiments were conducted in a Perkin–Elmer Tiselius apparatus using a microcell and the Longsworth scanning optical system. An Ostwald type viscometer, specially designed for a low average shear gradient, was used for viscosity studies.

Ultracentrifuge and Viscosity Studies

All preparations examined in the ultracentrifuge showed a principal, sharp boundary with a sedimentation constant for the different samples between 269 and 290 *S*. In addition, most of the samples showed a faster and a slower component whose boundaries were much more diffuse than the main component. The faster component, which has a sedimentation constant of about 390 *S*, is probably similar to that observed in the early studies of Beard and Wyckoff.[6] The sedimentation constant of the slower component, whose presence has not been reported previously, is between 170 and 190 *S*.

Figure 1 shows the ultracentrifuge patterns and the sedimentation constants of the principal component of two different samples of rabbit papilloma virus. The amount of the slow moving component, sedimentation constant about 181 *S*, relative to the main one varies from one sample to another. Corresponding to the variation in the amount of the trailing component there is a variation in the sedimen-

(1) Presented before the Division of Biological Chemistry at the 116th National Meeting, American Chemical Society, Atlantic City, N. J., September 20, 1949.

(2) (a) H. Neurath, G. R. Cooper, D. G. Sharp, A. R. Taylor, D. Beard and J. W. Beard, *J. Biol. Chem.*, **140**, 293 (1941); (b) D. G. Sharp, A. R. Taylor, D. Beard and J. W. Beard, *Proc. Soc. Exp. Biol. Med.*, **50**, 205 (1942).

(3) D. G. Sharp, A. R. Taylor and J. W. Beard, *J. Biol. Chem.*, **163**, 289 (1946).

(4) C. A. Knight, *Proc. Soc. Exp. Biol. Med.*, **75**, 843 (1950).

(5) J. W. Beard, W. R. Bryan and R. W. G. Wyckoff, *J. Infect. Dis.*, **65**, 43 (1939).

(6) J. W. Beard and R. W. G. Wyckoff, *J. Biol. Chem.*, **123**, 461 (1938).