Some Kinetic and Thermodynamic Characteristics of Apomorphine Degradation

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Rates of spontaneous apomorphine degradation have been determined at varying temperature and pH under pseudo first-order conditions. The pH profile, covering a range of pH 5.2 to 6.8 at 30°, describes the influence of the reaction of aqueous media on apomorphine stability. Buffering the medium to lower pH values dramatically decreases the velocity of decomposition. Rate constants have been determined for reactions run at 0.2 pH unit intervals over the range described. Rates calculated at a constant pH 6.0 but at different temperatures (30° and 40°) provide data for the estimation of the heat of activation, which was computed to be 12,057 cal./mole.

FOR MANY YEARS even casual observers have been impressed by the rapid color development in freshly prepared aqueous solutions of apomorphine and its salts and have been misled into believing that this is an invariable reflection of the rapid rate of decomposition of the substance. Although there appears to be little doubt that the greenish coloration that increases in intensity as the solutions age is, in fact, a result of the appearance of apomorphine oxidation product(s), the characteristic color is not a reliable indicator of the degree or extent of degradation. It has been established that only a minute amount of degradation product is sufficient to produce intense discoloration; although such a solution may appear to be most inelegant, it may, nevertheless, contain over 95% of the original apomorphine and retain virtually all of the biological activity of the freshly prepared solution (1).

This report amplifies earlier observations on the stability of apomorphine and presents quantitative information relative to the velocities of degradation and the heat of activation for the decomposition reaction.

EXPERIMENTAL

A 50-mg. quantity of apomorphine hydrochloride ($[\alpha]_D^{25}$ −48°, 1.2% w/v in water) was dissolved in distilled water and made up to 100-ml. volume with McIlvaine buffer (2) of appropriate pH. The initial concentration of all solutions was 0.5 mg./ml. of the hydrochloride salt, equivalent to 3.19×10^{-6} moles of base per milliliter. The buffered solutions were placed in 250-ml. glass-stoppered conical flasks, charged with oxygen, and immersed to the neck in a water bath maintained at either 30 ± 0.1° or 40 ± 0.1°. The flasks were recharged periodically to insure the availability of excess oxygen. pH determinations were made of the contents of each flask at the beginning and end of every run to verify reaction constancy. A 1- or 2-ml. portion of the degrading solution was periodically withdrawn for chemical assay using the spectrophotometric method of Kaul *et al.* (3). The only changes made in the procedure were in the volumes of extraction solvents used to better accommodate the quantities of apomorphine to be measured. The results of the assays were expressed as the quantity of residual unoxidized apomorphine present per unit volume of sample. Incubation periods lasted from 3–12 days, depending upon the rate of disappearance of apomorphine.

pH Profile.—Four to eight replicate 100-ml. samples were run at each pH level, and the curves that described pH and temperature relationships are constructed from data contributed by all samples. Samples incubated at a constant 30° were buffered at 0.2 pH unit intervals over a range of pH 5.2 to 6.8. Half-lives ($t_{1/2}$) and corresponding velocity constants (k) for the degradation reactions were determined from the curves.

Heat of Activation.—One assay, consisting of four replicate samples buffered to a constant pH 6.0, was run at 40°. An identical assay was performed on four samples incubated at 30°. From the velocity of degradation at both temperatures, the heat of activation (ΔH_a) was estimated using the modified Arrhenius expression

$$\log \frac{k_2}{k_1} = \left(\frac{\Delta H_a}{2.303(R)}\right)\left(\frac{T_2 - T_1}{T_2 T_1}\right)$$

where k_1 and k_2 are rate constants at T_1 and T_2, respectively, T_1 and T_2 are absolute temperatures, and R is 1.987 cal. degree^{-1} mole^{-1}.

RESULTS AND DISCUSSION

pH determinations of the contents of all sample flasks verified pH constancy during incubation. Reaction in the flasks at the end of the incubation period did not vary more than 0.01 pH unit from that recorded in the same flask at the start of the period.

The frequent introduction of oxygen into the flasks insured an excess, thereby rendering what was presumed to be a second-order reaction, first order with respect to apomorphine. That first-order conditions prevailed is attested to by the linearity of the curves formed by plotting log concentration against time (Figs. 1 and 3).

Figures 1 and 2 graphically describe the influence of pH on the velocity of apomorphine degradation at 30°. Over the range pH 5.2–5.8, the rate of decomposition is increased dramatically as the pH is elevated. From pH 5.8–6.8, the rate of degradation continues to increase, though less markedly. The over-all effect of pH on apomorphine stability was observed and commented on by several investigators (1, 3–5), although an accurate profile had never been constructed. The velocity constants and the corresponding half-lives are presented in Table I. On the basis of these data, it is estimated that the first visually distinct appearance of green color in the apomorphine solutions can be attributed to the

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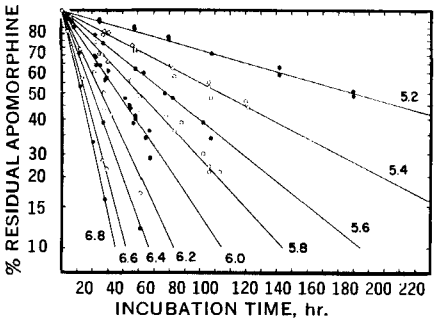

Fig. 1.—pH profile of apomorphine degradation at 30°.

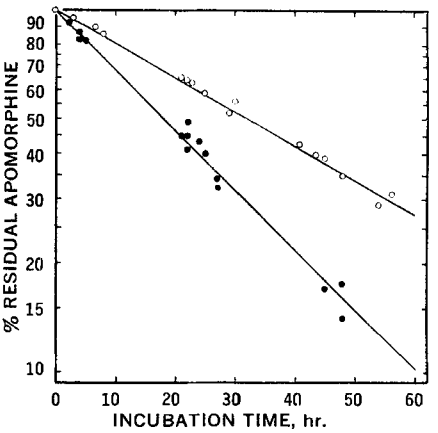

Fig. 3.—Decomposition of apomorphine at pH 6.0. Key: -O-, 30°; -●-, 40°.

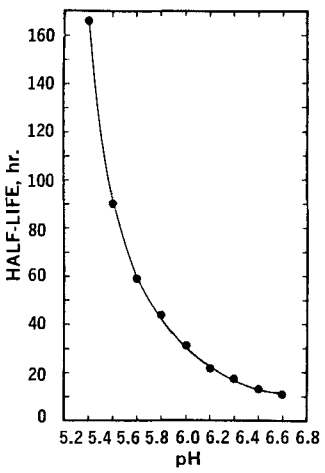

Fig. 2.—Exponential relationship between the rate of degradation of apomorphine at 30° and the pH of the aqueous medium in which decomposition took place.

TABLE I.—INFLUENCE OF pH ON THE RATE OF APORMORPHINE DEGRADATION AT 30°

pH	Half-Life, hr.	Velocity Constant, hr.$^{-1}$
5.2	166	0.0041
5.4	90	0.0077
5.6	59	0.0118
5.8	44	0.0157
6.0	32	0.0217
6.2	22	0.0318
6.4	17	0.0391
6.6	13	0.0533
6.8	11	0.0630

decomposition of approximately 0.1–0.2% of the original apomorphine HCl. Thus, in the 100-ml. samples, the degradation of about 50–100 mcg. was responsible for the obvious coloration. It is also estimated that the degradation of about 2% (1 mg. in 100 ml. of solution) of the available apomorphine produced a very dark green color. Therefore, it is possible that a deeply pigmented preparation of apomorphine may contain up to 98% of the original drug.

Attention is drawn to the velocity constant for the decomposition at pH 5.4: 0.0077 hr.$^{-1}$, indicating that apomorphine was disappearing at a rate of about 0.8% per hour. An unbuffered solution containing identical quantities of apomorphine HCl (0.5 mg./ml. initially) has a pH of approximately 5.5; this preparation, like all of the buffered solutions, becomes intensely colored within the first few hours. Yet the unbuffered solution undergoes degradation at a rate lower than the buffered solution since it has been observed that the pH of unbuffered solutions progressively decreases. After 60 days, the pH was about 3.6, and at this point 70% of the original apomorphine was still present (1). In contrast, solutions buffered to pH 5.4 lost all but about 20% of the original apomorphine within 9 days (Fig. 1). The *relative* stability of the unbuffered solutions is supported by biological and chemical analyses (1).

Velocity constants for degradation at a fixed pH 6.0 but at two temperatures (30° and 40°) were 0.0215 hr.$^{-1}$ ($t_{1/2}$ = 32.5 hr.) and 0.0408 hr.$^{-1}$ ($t_{1/2}$ = 17 hr.), respectively. A 10° rise in temperature therefore increased the rate of decomposition by a factor of 1.9. From these data, the heat of activation of the degradation was estimated to be 12,057 cal. mole^{-1}.

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