the effects measured here are present before the addition of hexamethylene glycol cannot be answered from these data. It is conceivable that the inhibitor produces changes in the polymerized material when it is added to the reaction mixture. The role of hexamethylene glycol in possible dissociation processes may become apparent from similar studies which we are carrying out without the addition of this inhibitor. The effect of *p*H and other variables on the clotting process is also under consideration.

The concentration dependence of the flow birefringence measurements, illustrated in Fig. 5, is different from the linear plots obtained for pure fibrinogen (Fig. 2) and detergent micelles,[9] *i.e.*, the decrease in length is much greater than would be expected from a decrease in solute–solute interaction. We are inclined to attribute this to a dissociation of the polymers into shorter particles upon dilution. Evidence for dissociation has also been obtained by Shulman and Ferry[2] from viscosity studies of inhibited clotting systems.

As the protein concentration decreases, the magnitude of the birefringence (Fig. 4) decreases considerably suggesting a significant decrease in particle length upon dilution.

Thus, the value of the flow birefringence measurements in conjunction with the empirical extrapolation procedure may be seen in that they show the character of the end-to-end aggregation during the course of the clotting reaction and also the reversibility of this aggregation process upon dilution.

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[CONTRIBUTION NO. 1091 FROM STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

The Heat of Denaturation of Pepsin[1]

BY ANNE BUZZELL[2] AND JULIAN M. STURTEVANT

Pepsin undergoes an endothermic reaction, presumably a denaturation, in the same *p*H region as that in which it loses its proteolytic activity. The heat of this reaction increases from a value calorimetrically indistinguishable from zero to a maximum and decreases again to zero within less than one *p*H unit. This behavior is found at both 15 and 35°. The rate of the endothermic reaction is markedly less sensitive to *p*H and temperature changes than is the rate of the inactivation process.

Previous calorimetric study[3] of the denaturation of pepsin by alkali showed that the enthalpy change accompanying this reaction in *p*-nitrophenol buffers is strongly dependent on *p*H. We have now extended these measurements to cover a considerably larger range of *p*H, using an improved apparatus and a new calorimetric method.[4] In the present work, phosphate buffers have been employed.

Experimental

Pepsin.—Five different samples of pepsin were employed. Pepsins A and B were obtained from Armour and Company, and carried their lot numbers 80505 and 80802, respectively. Pepsin C was obtained from the University of Wisconsin. Pepsin D was prepared from Parke–Davis 1:10,000 pepsin essentially as described by Northrop.[5] After three crystallizations the pepsin was dialyzed against distilled water and then lyophilized. Pepsin E was Armour's lot 80802 material carried through three crystallizations from 20% ethanol,[6] followed by washing with *M*/500 HCl and solution in water by careful addition of dilute KOH to *p*H 4. Pepsins A, B, C and D were air-dried (approximately 40% relative humidity) and weighed amounts were dissolved in water (no alkali needed) to prepare solutions for experimentation. Analytical data obtained on solutions of these samples of pepsin are summarized in Table I. The concentrations of these solutions ranged from 0.2 to 2.0%, based on the weight of the air-dried protein used.

Determination of Peptic Activity.—The enzymatic activity of each pepsin stock solution was determined by a modification of the method of Anson.[7] Hemoglobin supplied for this purpose by Armour and Company was used as substrate, in 0.25% solution instead of 2.5% as specified by Anson, in order to obtain a smaller blank. The digestions were run for 10 minutes at 35.5°, and the trichloroacetic acid precipitate was removed by centrifugation rather than filtration. The digestion supernatants were examined without dilution at 280 mμ in a Beckman model DU spectrophotometer. It was found that if the pepsin concentration was adjusted to keep the optical density of the supernatant below 0.4 (1-cm. cell), the digestion rate was accurately zero order with respect to substrate and first order with respect to enzyme. A solution containing HCl, CCl_3COOH and formalin in the same concentrations as the digestion supernatants was used as spectrophotometric blank. Digestion blanks were prepared by adding the pepsin solution to the CCl_3COOH solution before mixing with the substrate solution, and were found to have optical densities close to 0.070.

Measurements on standard tyrosine solutions containing HCl, CCl_3COOH and formalin in the same concentrations as the digestion supernatants showed that the number of milliequivalents of tyrosine in 16 ml. (the volume of the digestion supernatants) of solution is obtained by multiplying the optical density by 0.0134. If we use the definition of a pepsin unit (PU) as the amount of enzyme which produces one milliequivalent of "tyrosine" per minute under the stated digestion conditions, it is evident that the number of pepsin units per ml. of enzyme solution is given by 0.00134 times the optical density of the digestion supernatant. The activity data given in Table I have been obtained using this calibration constant.

This definition of the pepsin unit is somewhat different from that employed by Northrop, Kunitz and Herriott,[8] who also examined the digestion product at 280 mμ, in that they referred their data to the original definition of Anson[7] based on the color developed by reaction between tyrosine and Folin phenol reagent.

Determination of *p*H.—All *p*H measurements were made at the temperature of reaction with a Beckman glass elec-

(1) Presented in part at the April, 1950, Meeting of the American Chemical Society, Philadelphia, Pa. The research reported in this paper was supported in part by a grant from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.

(2) Research fellow under a grant from the American Cancer Society.

(3) M. Bender and J. M. Sturtevant, THIS JOURNAL, **69**, 607 (1947).

(4) A. Buzzell and J. M. Sturtevant, *ibid.*, **73**, 2454 (1951).

(5) J. H. Northrop, M. Kunitz and R. M. Herriott, "Crystalline Enzymes," Columbia University Press, New York, N. Y., 1948, p. 255.

(6) Reference 5, p. 257.

(7) M. L. Anson, *J. Gen. Physiology*, **22**, 79 (1938).

(8) Reference 5, p. 305.

TABLE I

SUMMARY OF ANALYTICAL DATA FOR PEPSIN SOLUTIONS

Pepsin sample	Protein nitrogen, mg. per mg. pepsin	Non-protein nitrogen, mg. per mg. total nitrogen	Pepsin unit per mg. pepsin	Pepsin unit per mg. protein nitrogen
A	0.100 ± 0.006[a]	0.231 ± 0.020[a]	0.0447 ± 0.0005[a]	0.447[c]
B	.103 ± .003[a]	.205 ± .020[a,f]	.0391 ± .0021[b]	.380[c,f]
C[d]	.....	.....	.0342	.....
D[d]	.....	.....	.0261	.....
E[e]	.....	.230	.....	.357

[a] Mean value for 4 solutions. [b] Mean value for 18 solutions. [c] Computed from mean values in columns 2 and 4. [d] Protein and non-protein nitrogen not determined. [e] Pepsin E was not obtained in dry form. [f] In an attempt to decrease the non-protein nitrogen content of this material, a sample of Pepsin B was dialyzed against dilute hydrochloric acid (*p*H 4.5) for 70 hr. at 5°. After 16 hours (the period necessary to establish thermal equilibrium in the calorimeters) at 35° and *p*H 4.5, the dialyzed solution was found to contain 0.195 mg. of non-protein nitrogen per mg. of total nitrogen, and 0.378 pepsin units per mg. of protein nitrogen. A calorimetric run with this dialyzed material at *p*H 6.24 and 35° agreed well with the data given in Figs. 1 and 2.

trode *p*H meter, it being assumed that the observed final *p*H was maintained throughout the reaction. The measurements were referred to a buffer 0.1 *M* in acetic acid and 0.1 *M* in sodium acetate to which was assigned the *p*H value[9] 4.66 at 15 and 35°.

Buffer.—All reactions were carried out in potassium phosphate buffers 0.05 *M* in phosphate. Sufficient KCl was added to make the ionic strength 0.175 *M*, no allowance being made for any contribution of the protein to the ionic strength.

Calorimetric Method.—The calorimetric apparatus and method have been described in detail in a previous publication.[4] In each calorimetric experiment, 12.5 ml. of pepsin solution at a *p*H of about 4 was placed in one-half of each calorimeter, and an equal volume of 0.1 *M* phosphate buffer of appropriate *p*H in the other half. After a period of approximately 18 hours, during which thermal equilibrium was established, the reaction was initiated first in one calorimeter, and then, after this reaction was complete, in the other calorimeter.

The inactivation of pepsin is an endothermic process. In the calorimetric method employed here this results in the reacting solution being held accurately isothermal. In the absence of electrical compensation, the calorimeters and solutions would have cooled by 0.7 to 3 millidegrees in the experiments reported.

The two reactions at highest *p*H at 15° were run in the usual way in one calorimeter, but without electrical compensation in the other. A comparison[10] of the results obtained indicates that reactions having half-times less than approximately two minutes may be assigned too small a rate constant on the basis of measurements made with compensation, but that the same heat value is obtained with or without compensation.

Analytical Rate Determinations.—Several determinations of the rate of inactivation were carried out by the usual procedure of measuring the proteolytic activity of samples withdrawn from the reaction mixture at frequent intervals. The samples were "quenched" by being added to a large excess of 0.01 *M* *p*H 4.5 acetate buffer.

Discussion of Results

Heat Data.—The apparent heat of denaturation, expressed in joules per pepsin unit, is shown in Fig. 1 as a function of *p*H and of temperature.[11] In these experiments the pepsin concentrations ranged from 0.2 to 0.5 g. of pepsin per 100 ml. There are included in the figure some determinations made in 1943 with different equipment[3] and in *p*-nitrophenol buffers. The earlier data at 35° have not been previously published. The *p*H scale used in the earlier work has been corrected to coincide with that used in the present experiments. The earlier values agree moderately well with those obtained in our new apparatus, when one considers that a different measure of peptic activity was employed and that the heats are very sensitive functions of *p*H. It should be noted that in our earlier experiments we found an average value of 0.18 PU per mg. PN for the pepsin solutions used, while in the present work this figure is 0.39.

In Table II, the maximum of the heat absorption curve at each temperature, expressed in various units, is listed.

TABLE II

MAXIMUM APPARENT HEAT OF INACTIVATION

Temp., °C.	*p*H$_{max}$	ΔH_{max} Joules per PU	ΔH_{max} Kcal. per mole[a]	ΔH_{max} Kcal. per mole[b]
15	7.16	0.047	15	22
35	6.41	.145	48	69

[a] Calculated on the basis of 0.039 PU per mg. pepsin (Table I, Pepsin B), and mol. wt. 35,000. [b] Calculated on the basis of 0.39 PU per mg. PN (Table I), 14.6% PN (ref. 5, p. 74), and mol. wt. 35,000.

The variation of apparent heat of denaturation with *p*H is surprising. As pointed out earlier, one naturally thinks of ionization effects as a possible source of this variation. Linderstrøm-Lang and Jacobsen[12] have called attention to a somewhat similar variation of the volume change accompanying the hydrolysis of a peptide in a buffered solution. However, we have been unable to find any reasonably simple set of assumptions regarding the ionization equilibria in this system which can account for the observed behavior. We are unable to suggest any convincing explanation for this unusual dependence of heat of reaction on *p*H.

In our first paper[3] on this reaction, we compared our results with those obtained by Conn, Gregg, Kistiakowsky and Roberts[13] by an entirely different method. The remarks made at that time need no significant modification on the basis of the new results reported here, except as will be evident from our discussion below of the difference between the calorimetrically and analytically observed kinetics.

Calorimetric Kinetic Data.—The calorimetrically observed kinetic data are given in Fig. 2, in which the common logarithm of the first order rate con-

(9) R. Bates, *Chem. Revs.*, **42**, 1 (1948).

(10) A discussion of this point is given in ref. 4.

(11) In addition to the data included in Figs. 1 and 2, 13 experiments were discarded because of obvious experimental failure, and 13 because either the rate or heat of reaction, or both, diverged from the body of the data considerably more widely than any of the reported data (except the three values for the heat of reaction obtained with pepsin C.)

(12) K. Linderstrøm-Lang and C. F. Jacobsen, *Compt. rend. trav. Lab. Carlsberg*, **24**, 1 (1941).

(13) J. B. Conn, D. C. Gregg, G. B. Kistiakowsky and R. M. Roberts, THIS JOURNAL, **63**, 2080 (1941).

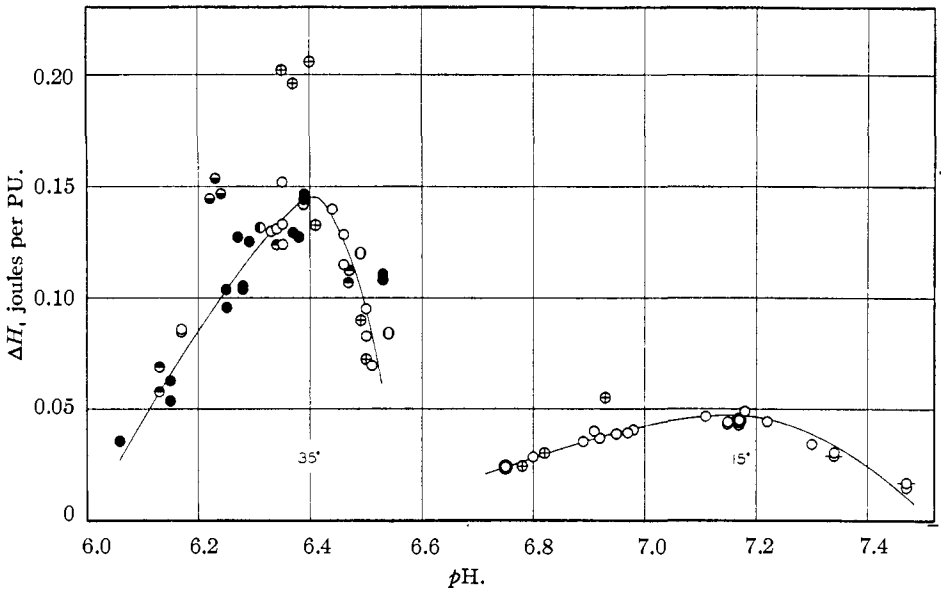

Fig. 1.—The apparent heat of denaturation of pepsin at 15 and 35°: ●, pepsin A; ◒, pepsin C; ◓, pepsin D; O, pepsin B, with compensation; -o-, pepsin B, without compensation; ◐, pepsin E; ⊕, ref. 3 (*p*-nitrophenol buffers).

stant in sec.$^{-1}$ is plotted against *p*H. The lines were obtained by the least squares method.

In every experiment the rate of heat absorption followed closely a first order law from 2 to 4 minutes (readings could not be obtained at smaller times because of the disturbance due to the heat of mixing the reactants) after the start of the reaction to at least 80% completion, usually to more than 90% completion. The experimental points, recorded at one-minute intervals, for each reaction showed an average deviation within this interval from the calculated first order curve of less than ±0.001 joule, which corresponds to about ±10 microdegrees. The heat data cannot be satisfactorily represented by any simple form of rate equation other than the first order.

The average deviation of the rate constants at both 15 and 35° from the least squared lines amounts to ±0.045 *p*H unit or ±16% in rate constant. The lines follow the equations

$$-\log k_{15} = 12.63 - 1.387\ p\mathrm{H}$$
$$-\log k_{35} = 11.66 - 1.398\ p\mathrm{H}$$

and thus have the same slope within experimental error. It is interesting that these lines are separated in *p*H by the same amount as the maxima of the ΔH curves in Fig. 1. Thus, the rate constants at 15 and 35° at the respective *p*H's of maximum heat absorption are both 0.0021 sec.$^{-1}$. This might suggest that the maxima in the heat curves are artifacts resulting from calorimetric lags. However, lags should also affect the rate constants, and there is no indication of any change in the dependence of log *k* on *p*H after the *p*H of maximum heat absorption. Furthermore, as mentioned earlier, our apparatus appears to give reliable heat data for first order reactions considerably faster than this.

The temperature coefficient of the reaction rate at constant *p*H is unusually small for a denaturation process. According to the usual equations of

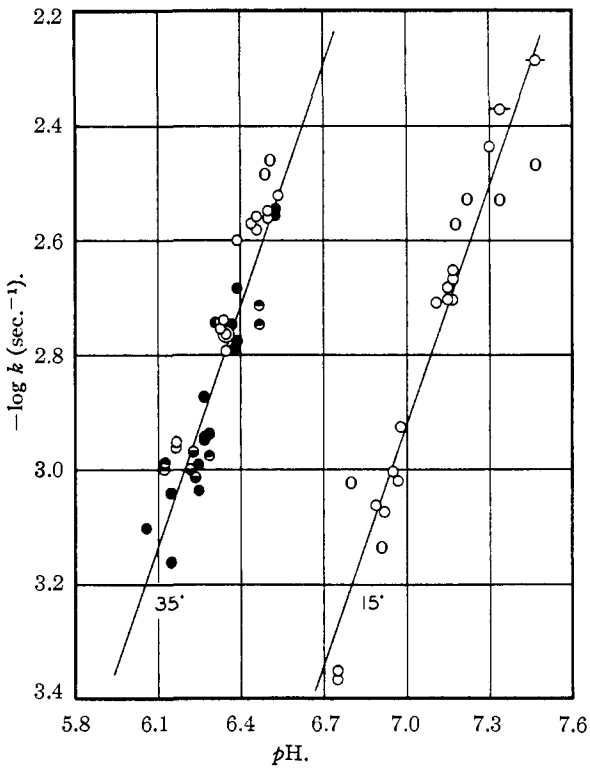

Fig. 2.—The rate of heat absorption during the denaturation of pepsin at 15 and 35°: ●, pepsin A; ◒, pepsin C; ◓, pepsin D; O, pepsin B, with compensation; -o-, pepsin B, without compensation; ◐, pepsin E.

the transition state theory, our data give for the heat of activation

$$\Delta H^{\ddagger} = 19.1\ (1 + 0.0117\ p\mathrm{H})\ \text{kcal. per mole}$$

at 25°. The maximum value of ΔH at 35° is about 50 kcal. per mole, so that at *p*H 6.4 the reverse reaction would appear to have a heat of activation of approximately −30 kcal. per mole. That there

TABLE III

THE INACTIVATION OF PEPSIN. ANALYTICAL RATE DETERMINATIONS WITH PEPSIN B AT 15 AND 35°

Run No.	Concn. pepsin, wt. %	Temp., °C.	*p*H	Minutes corresponding to indicated fraction of activity remaining: 0.891	0.794	0.708	0.631	0.501	0.398	0.316	0.224	0.158	0.100	0.056
1	0.25	15	6.77	5	11	19	28	51	83	123	198	294	470	..
2	.25	15	6.79	1.4	3.8	8.6	15.8	36	62	91	...	...	...	..
3	.5	15	6.89	1.3	3.2	5.9	9.8	20.7	35	51	80	113	161	..
4	.25	15	6.93	0.9	3.0	6.9	11.3	21.0	33	47	83	...	...	..
5	.25	15	6.99	.2	0.8	1.6	2.9	6.4	10.9	16.9	28.6	44	74	..
6	.25	15	7.07	.25	.60	.92	1.3	2.1	3.2	4.5	7.0	10.2	15.8	25.3
7[a]	...	15	6.88	5	13	27	43	85	139	207	339	515	...	..
8	.25	35	5.93	21	64	139	233	539	1190	...	...	...	...	..
9	.5	35	6.14	3.2	7.7	15.5	26.3	54	91	143	263	...	...	..
10	.25	35	6.43	0.65	1.25	1.9	2.6	4.3	6.2	8.4	12.3	16.9	24.6	38
11	.25	35	6.62	.18	0.36	0.56	0.8	1.2	1.7	2.3	3.3	4.2	6.3	8.8
12	.005	35	6.49	.24	.49	.75	1.0	1.6	2.4	3.1	4.6	6.7	10.7	..

[a] Pepsin E used in this run.

is nevertheless no indication of any reversal of the reaction, as judged by activity measurements, is to be attributed to a large negative entropy of activation for the reverse reaction. Stated otherwise, since the absence of reversal indicates that ΔF° for the forward reaction is probably more negative than -5 kcal. per mole, we can conclude that at *p*H 6.4 ΔS° is greater than 180 e.u. Since our calorimetric rate data indicate that $\Delta S^\ddagger \lesssim 0$ for the forward reaction at this *p*H, $\Delta S^\ddagger$ for the reverse reaction must be more negative than -180 e.u.

Analytical Kinetic Data.—In our previous publication,[3] we reported that the analytically determined kinetics agreed approximately with the calorimetric kinetic data. This conclusion, which was based on measurements of both types made over a very restricted *p*H range, we now find to be incorrect. Our analytical kinetic data are summarized in Table III; the actual experimental points for each run, 10 to 22 in number, show an average deviation from the curves defined by the figures in the table amounting to 2.5% in the fraction remaining.

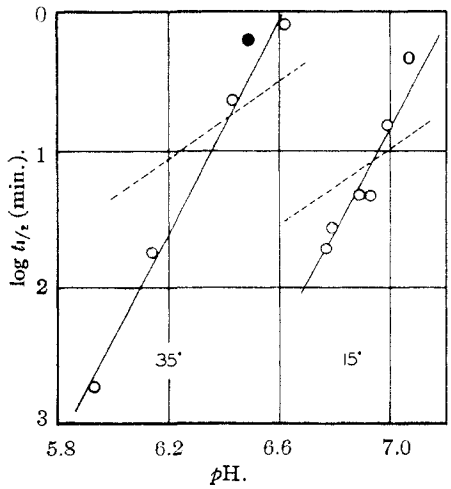

Fig. 3.—Comparison of the times required for half-completion of the inactivation and the heat absorption at 15 and 35°. Analytically determined values at 0.25 to 0.5% initial concentration, O, at 0.005%; ●, calorimetric values, dashed lines.

Steinhardt,[14] on the basis of accurate measurements made over a wide *p*H range, found the loss of activity in *p*-nitrophenol buffers to follow closely a first order type of equation. Our analytical rate data definitely do not follow a first order law, possibly because the pepsin contained considerably more non-protein impurity than the material used by Steinhardt. Some, but not all, of the runs follow a second order equation with good accuracy, though the fact that the rate is not much affected by decreasing the initial concentration of pepsin to 0.005% (Run 12) indicates that the process is certainly not a true bimolecular reaction. This apparent lack of dependence of rate on initial concentration is in disagreement with the results of Casey and Laidler.[15] These authors found the order of the reaction, in *p*H 4.83 acetate buffer at 50 to 60°, to decrease from 5 at an enzyme concentration of 0.004% to 1 at 0.04 to 1.0%.

Steinhardt found the rate of inactivation to be inversely proportional to the fifth power of the hydrogen ion concentration, and to show a large temperature coefficient. Figure 3, in which the logarithm of the time of half completion of the reaction is plotted against *p*H, shows that our analytical rate determinations, in qualitative agreement with Steinhardt's results, indicate a much greater dependence of rate on *p*H and temperature than do our calorimetric rate determinations. The solid lines in Fig. 3 have a slope of 3.9 as compared with the value 5 reported by Steinhardt.

The marked difference between the calorimetric and analytical kinetics raises the question as to what, if any, connection exists between the process which is accompanied by absorption of heat and which we have chosen, somewhat arbitrarily, to call denaturation, and the reaction which results in the loss of proteolytic activity. Actually, the only connection which has been demonstrated is that the rates of both reactions appear to have measurable values in the same *p*H and temperature ranges. Even this connection may be somewhat illusory in view of the fact that Philpot[16] has shown by means

(14) J. Steinhardt, *Kgl. Danske Videnskab. Selskab. Math.-Fys. Medd.*, **14**, No. 11 (1937).

(15) E. J. Casey and K. J. Laidler, THIS JOURNAL, **73**, 1455 (1951).

(16) J. St. L. Philpot, *Biochem. J.*, **29**, 2458 (1935).

of sedimentation studies that pepsin allowed to stand for brief periods in alkaline solutions remains surprisingly homogeneous, but that drastic changes in sedimentation constant take place when the material is subsequently taken to a lower *p*H. Unfortunately, it is possible to determine the activity of pepsin only in solutions of low *p*H, so that there is some ambiguity as to what *p*H range is pertinent to the observed inactivation process. It is perhaps not surprising that a substance as complicated as pepsin should undergo two or more different and unrelated reactions under similar experimental conditions.

NEW HAVEN, CONNECTICUT RECEIVED JULY 9, 1951

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC., AND THE NOYES CHEMICAL LABORATORY UNIVERSITY OF ILLINOIS]

Plant Insecticides. II.[1] The Alkaloids of *Haplophyton Cimicidum*

BY EDWARD F. ROGERS, H. R. SNYDER AND RUDOLPH F. FISCHER

Two insecticidal alkaloids, "haplophytine," $C_{27}H_{31}O_5N_3$, and cimicidine, $C_{23}H_{28}O_5N_2$, have been isolated from *Haplophyton cimicidum*.

Two crystalline insecticidal alkaloids have been isolated from the Mexican shrub *Haplophyton cimicidum* A.D.C. (*Apocynaceae*), thereby confirming predictions of earlier workers concerning the alkaloidal character of the plant's active principles.[2] The alkaloids, which have been named "haplophytine" and "cimicidine," were secured in yields of 0.007–0.03% and 0.003%, respectively. Haplophytine and cimicidine are amphoteric and this property was utilized in fractionation of the crude alkaloid obtained from the chloroform–methanol extractive of whole plant material.

On the basis of analytical results reported in this communication and corroborative unpublished data, the following empirical formulas are proposed: haplophytine, $C_{27}H_{31}O_5N_3$; cimicidine, $C_{23}H_{28}O_5N_2$. A close relation between the two compounds is indicated by similar ultraviolet and infrared absorption spectra. Of special interest in the infrared spectra (see Fig. 1) are: (a) the complete absence of absorption in the region 3700–3000 cm.$^{-1}$ (OH and NH) and (b) the complex absorption in the region 1850–1620 cm.$^{-1}$ (carbonyl); it is probable that two carbonyl groups are present and that OH and NH groupings are absent.[3]

Both alkaloids are toxic to German roaches on contact, ingestion and injection. The LD/50 dosage of haplophytine is 18 γ/g. (contact, 48 hr.) and for cimicidine is about 60 γ/g. It was observed that haplophytine caused prolonged paralysis at dosage levels far below the LD/50 value. The toxicity data on the various fractions (see Table I) indicate that approximately one-fourth of the toxic principles present in the crude alkaloid are recoverable as pure haplophytine and cimicidine. Some of the losses are due to incompleteness of precipitation and others are ascribable to degradation during separation; alkaline or acidic solutions of haplophytine darken more or less rapidly when exposed to the air. While not remarkably low, the LD/50 dosage of haplophytine compares favorably with that of several widely used insecticides and most of the toxicity of Haplophyton is probably due to this alkaloid. The total crude alkaloid has been found to be toxic to a wide range of insects including European corn borers, Mexican bean beetle larvae, Colorado potato beetle larvae and adults, grasshoppers, egg-plant lace bugs and codling moths.

Experimental[4]

Preliminary Treatment and Extraction of Plant.—Authentic *Haplophyton cimicidum*, dried whole plant, was ground in a fourteen-inch hammermill (30 H.P. motor) to pass through a one-half inch screen. Most of the material thus obtained was very finely divided and was extracted without further treatment. Samples of plant of two degrees of maturity were employed. The comparative extractions were run on young, relatively immature plants, but several extractions and much of the entomological work were conducted on rather mature plants (few leaves, many seeds, larger size).

An extraction container was prepared by cutting the bottom out of an ordinary five-gallon chloroform can. This was inverted, and the spout was connected to a 12-liter flask, which in turn was attached to a vacuum line. Seven to eight kilograms of ground plant was tamped into this container and covered with 12–13 liters of a mixture of 80–90% chloroform and 10–20% methanol. The can was covered and allowed to stand 2 or 3 days, after which it was drained, the vacuum line being used to complete the solvent removal. This process was repeated six times (only 5–6 liters came through each time), with concentration of the extracts as the volume reached 10–11 liters. The final solvent pass was pure chloroform, and the final volume of concentrate was about one liter of chloroform solution.

The above procedure represents the best of several tried on the younger plant material; chloroform and methanol were about equally effective as solvents, and a mixture of the two was somewhat better than either alone. Cold percolation with this mixture gave about the same yields as continuous hot extraction; water was about one-third as efficient as chloroform, and benzene was only one-tenth as effective a solvent.

Isolation of Haplophytine.—The concentrate was extracted four times with 200-ml. portions of 2 *N* hydrochloric acid, and the *p*H of the extracts was quickly adjusted to 8 with solid sodium carbonate; the resulting suspension was then extracted with four 200-ml. portions of chloroform. To determine the "total crude alkaloid" at this point, the

(1) For the preceding paper in this series see E. F. Rogers, F. R. Koniuszy, J. Shavel, Jr., and K. Folkers, THIS JOURNAL, **70**, 3085 (1948).

(2) (Mexico) Comision de Parisitologia Agricola 1.00-01; El Gusano de la Fruta (*Instrypetas ludens* I.D.B.); *Bol. Com. Parisitologia*, **1**, 21, 28–30, 45, 46, 49–51, 54, 55, 90, 108, 188; L. Flores, Datos para la Materia Medica Mexicana, Secretaria de Fomento (Mexico); *Instituto Mexico Nacional*, **4**, 93 (1907); C. C. Plummer, Toxicity of *Haplophyton cimicidum* to Fruit Flies, U.S.D.A. Circ. No. 455, April, 1938; R. E. Heal, E. F. Rogers, R. T. Wallace and O. Starnes, *Lloydia*, **13**, 89 (1950).

(3) L. Marion, D. A. Ramsay and R. N. Jones, THIS JOURNAL, **73**, 305 (1951).

(4) Unless otherwise noted, all decomposition points were determined on a calibrated Fisher block.