

[CONTRIBUTION FROM THE PHYSICO-CHEMICAL LABORATORY OF THE NEW YORK STATE EXPERIMENT STATION]

The Isoelectric Point of Asclepain<sup>1,2</sup>

BY D. C. CARPENTER AND F. E. LOVELACE

The separation of a proteolytic enzyme from the latex of the common milk-weed *Asclepasis speciosa* was first recorded by Greenberg and co-workers who named the material asclepain and carried out a number of activation and inactivation experiments.<sup>3</sup> Physico-chemical properties of asclepain which would assist in its preparation or identification, on the whole have been lacking. The present paper deals with the determination of the isoelectric point.

The isoelectric points of the various proteolytic enzymes of plant origin are not known, with the possible exception of papain which is described as being somewhat greater than  $pH\ 8^4$  and more recently as above  $pH\ 8.5.^5$  Many of the proteolytic enzymes are no doubt proteins and as such are of particular interest in the study of protein denaturation by heat and ultraviolet light and the kinetics of proteolysis in general.

## Experimental

**Preparation of Asclepain.**—Greenberg and co-workers used latex obtained by cutting the petioles of the plant

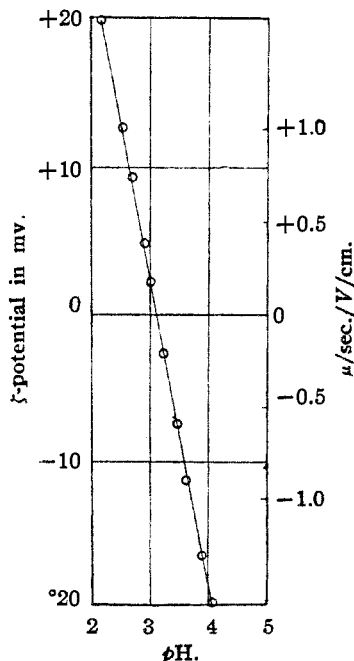


Fig. 1.—Migration velocity and  $\zeta$ -potential of asclepain.

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(2) Our attention was first called to the possible proteolytic properties of milk-weed latex by Dr. A. M. Crance, the latex having been an almost legendary treatment for the removal of warts and other papillary epidermal tissue.

(3) Winnick, Davis and Greenberg. *J. Gen. Physiol.*, **23**, 275, 289, 301 (1940).

(4) Ringer. *Arch. farmacol. sper.*, **48**, 99 (1930).

(5) Balls and Lineweaver. *J. Biol. Chem.*, **130**, 669 (1939).

and collecting the exuded white juice in containers. We were unable to obtain sufficient material in this manner, due to drying of the juice on the cut stem surface. From the stem structure, however, one would expect to find substantially the same juice in the root system. We accordingly used the root of the plant as source material in our experiments.

Three kilos of cleaned milk-weed roots (*Asclepasis syriana*) were ground through a meat grinder and the juice pressed out in a Carver laboratory press. One liter of crude juice was obtained. The juice was first centrifuged to remove cellular debris and then filtered through asbestos and paper-pulp, whereupon 815 ml. of clear brownish-colored filtrate ( $pH\ 6.5$ ) was obtained. The filtrate was half-saturated with solid ammonium sulfate, added in small portions while being mechanically stirred, and set away overnight at  $5^\circ$ . The grayish precipitated material was centrifuged from the mother liquor and the latter treated with a further amount of solid ammonium sulfate as before, until full-saturation with respect to ammonium sulfate was attained. The second precipitate was centrifuged off after cooling and standing at  $5^\circ$  overnight. The first and second precipitates were worked up separately but we could find no difference in the final products and hence one might as well make the initial precipitation at full-saturation with respect to ammonium sulfate. About four times as much material came down in the first fraction as in the second. The first precipitate was dissolved in 155 ml. of water, centrifuged and filtered from a small amount of grayish insoluble material and reprecipitated by half-saturation with solid ammonium sulfate. Separation in the centrifuge was repeated and the precipitate redissolved in 40 ml. of water, filtered through asbestos and paper-pulp, and placed in collodion dialyzer tubes protected with toluene and dialyzed against distilled water for a week, at the end of which time no sulfate could be found in the outer dialyzer liquid. The bulk of the protein was precipitated within the dialyzer tubes and was removed for storage with toluene at  $5^\circ$ . The second fraction was handled in essentially the same way. The yield was in the neighborhood of 5–6 g. from the first fraction. Between 1 and 2 g. was obtained from the second fraction. The enzyme is fairly soluble in water and easily soluble in phosphate buffer solution ( $pH\ 7.0$ ). It crystallizes in microscopic thin rectangular plates having a yellow color, when a solution in phosphate buffer is placed in a collodion dialyzer tube, attached to a stirrer, and suspended in a saturated solution of ammonium sulfate.

Asclepain may be activated by the usual reducing agents, cystein, reduced glutathione, sodium cyanide and hydrogen sulfide, that have been employed for the activation of papain and other proteolytic materials.

**Electrophoresis Experiments.**—Solutions of asclepain were prepared by dissolving about 0.5 g. of protein in 100 ml. of each of a series of buffer solutions ( $M/30$  after Sørensen) containing the required amount of the proper citrate, acetate or phosphate, respectively, to give the desired  $pH$  value over the range 1.8 to 8.0. From the published results for papain,<sup>4,5</sup> we had expected asclepain to have a somewhat similar isoelectric point, in the neighborhood of  $pH\ 8$ ; but preliminary results showed that the range  $pH\ 2.0$  to  $4.0$  was the region to investigate. The  $pH$  of the asclepain-buffer solution was determined in each case with a standardized glass electrode against a saturated calomel half-cell and the solutions were stored

with toluene for a few days at 0° until the electrophoresis measurements could be completed.

In our experiments we have employed the electrophoresis cell described by Northrop,<sup>6</sup> making measurements under the microscope of the migration velocity of the particle or an aggregate and reversing the applied potential repeatedly. The measurements at a given pH and recorded in Table I, are the mean of ten or twelve closely agreeing observations taken both at the upper and lower

TABLE I  
ELECTROPHORETIC VELOCITY AND CALCULATED ζ-POTENTIAL OF ASCLEPAIN

pH	Velocity μ/sec./v/cm.	Calcd. ζ-potential, millivolts
1.82	+2.03	+25.6
2.15	+1.58	+19.9
2.50	+1.01	+12.7
2.69	+0.74	+ 9.3
2.90	+ .38	+ 4.8
3.00	+ .18	+ 2.2
3.23	- .21	- 2.6
3.45	- .59	- 7.4
3.60	- .90	-11.3
3.86	-1.31	-16.5
4.05	-1.58	-19.9
4.30	-1.85	-23.3

(6) Northrop, *J. Gen. Physiol.*, **4**, 629 (1922).

stationary levels for the cell. The data are shown graphically in Fig. 1.

From the Helmholtz-Lamb equation  $V = \zeta HD/4\pi h$ , where  $V$  is the velocity of particle,  $\zeta$  is electrokinetic potential,  $H$  is potential gradient per cm.,  $D$  is dielectric constant of the dispersion medium and  $h$  is viscosity of the medium. The values of  $D$  and  $h$  have been assumed to be those for water, namely, 81 and 0.009, respectively, at 25°. All quantities in the above equation are expressed in c. g. s. electrostatic units.

Not until our observations were completed did we note the statement by Winnick, Davis and Greenberg (ref. 3, p. 277) that from the insolubility of their asclepain at pH 3.2 they expected the isoelectric point would lie in this region. That our preparation is the same material employed by Greenberg and co-workers is evident from our value of pH 3.11 for the isoelectric point and from the similar proteolytic properties observed in both laboratories.

### Summary

1. The electrophoretic velocity of asclepain has been measured in citrate, acetate and phosphate buffer solutions in the pH range 1.8 to 8.0 and the electrical charge on the particle calculated.

2. The isoelectric point of asclepain was found to be at pH 3.11 or  $C_H 7.8 \times 10^{-4}$ .

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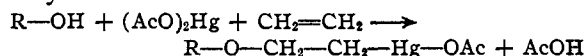
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SCHIEFFELIN & Co.]

## Mercurials from Aliphatic Glycols

BY ANTHONY J. SHUKIS AND RALPH C. TALLMAN

The relationship between the distribution coefficients of compounds which can be distributed between water and a lipid or a lipid solvent and their anti-bacterial activity has attracted considerable attention.<sup>1</sup> It appears to be rather well established that in a given series of compounds with differing distribution coefficients those which are distributed in favor of the lipid or the lipid solvent are the better anti-bacterial agents.<sup>1</sup> In connection with more extensive synthetic work, three series of mercury-containing organic compounds were prepared which afforded the opportunity to test again the reliability of this general concept.

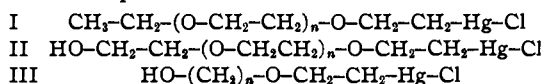
All the syntheses were performed by the well-known reaction<sup>2</sup> whereby an alcoholic hydroxyl group and mercuric compounds such as mercuric acetate add, in effect, to a molecule containing an ethylenic double bond



(1) Harden and Reid, *THIS JOURNAL*, **54**, 4325 (1932); Dunning, Dunning and Reid, *ibid.*, **58**, 1565 (1936); Hurd and Fowler, *ibid.*, **61**, 249 (1939), and others.

(2) Wright, *ibid.*, **57**, 1904 (1935), and others.

In this work, the acetate group in the final product was converted to the chloride. In general the alcohols used were polyethylene glycols, the monoethyl ethers of polyethylene glycols, or polymethylene glycols. The unsaturated compound in every instance was ethylene. Compounds of the following types were produced for the most part



In addition to these general types, several specific derivatives of them were prepared for purposes of comparison. The physical properties of all compounds are included in the table.

It was possible to alter the distribution coefficients of the compounds within the scope of these series by changing the ratio of —OH and —O— to the —CH<sub>2</sub>— groups within a molecule. With all compounds except two (see table) monomerization took place, even where more than one —OH group was present in the starting material. In these instances, with tri- and tetraethylene glycols as starting materials, dimercurials were